UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

THE UNITED STATES OF AMERICA,
Plaintiff–Counterclaim Defendant,

v.

GILEAD SCIENCES, INC.,
Defendant–Counterclaim Plaintiff,

AND GILEAD SCIENCES IRELAND UC,
Defendant.

Civil No. 1:19-cv-02103-MN

UNITED STATES' MOTION TO DISMISS

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Plaintiff United States of America (Government or United States) submits this Motion to Dismiss Defendant Gilead Sciences, Inc.'s, (Gilead) counterclaims of noninfringement and invalidity raised in its Second Amended Answer and Counterclaims (D.I. 21) pursuant to Federal Rule of Civil Procedure 12(b), and states as follows:

INTRODUCTION AND BACKGROUND

This is an action brought by the Government against Gilead seeking money damages for infringement of U.S. Pat. Nos. 9,044,509; 9,579,333; 9,937,191; and 10,335,423 (collectively, the Patents-in-Suit). The Government filed its Complaint on November 6, 2019. D.I. 1.

Gilead filed its original Answer and Counterclaims on January 23, 2020. D.I. 7. Gilead pled twelve counterclaims: four invalidity claims, four non-infringement claims, and four inequitable conduct claims. *Id.* at 87–96. Gilead did not directly address sovereign immunity and cited only 35 U.S.C. §§ 1 *et seq.*, 28 U.S.C. §§ 1331, 1338, 2201, and 2202 in its statement of jurisdiction. *Id.* at 70 ¶¶ 3–4.

The Government informed Gilead of the Counterclaims' failure to plead a waiver of sovereign immunity, *see* Exhibit 1, and, through a joint stipulation, Gilead filed its First Amended Answer and Counterclaims on March 27, 2020. D.I. 13. These Counterclaims omitted inequitable conduct counterclaims, but preserved counterclaims of noninfringement and invalidity for each of the four Patents-in-Suit. *Id*. at 92–98. In addition, for the first time, Gilead addressed the issue of sovereign immunity, but asserted only that "[t]he Government's sovereign immunity does not bar GSI's counterclaims." *Id.* ¶ 4 (citing *U.S. Dep't of Health & Human Servs. v. Mylan Pharms., Inc.*, No. 10-cv-5956-WHW, 2011 WL 13238650 (D.N.J. July 13, 2011); *Delano Farms Co. v. California Table Grape Comm'n*, 655 F.3d 1337, 1343-1344, 1349 & n.6 (Fed. Cir. 2011)). *Id.* Gilead made no other revisions to its counterclaims. *See* D.I. 13-1 (redline) at 95–108.

The Government subsequently filed a Motion to Dismiss, D.I. 17, for failure to state a claim and lack of subject matter jurisdiction regarding Gilead's remaining counterclaims as well as a Motion to Strike, D.I. 16, Gilead's equitable affirmative defenses. In response, Gilead, consistent with another stipulation of the parties, filed its Second Amended Answer and Counterclaims on May 29, 2020. D.I. 21. Its Second Amended Counterclaims added additional allegations denying the Government's sovereign immunity to Gilead's counterclaims. *Id*. (Counterclaims) at ¶¶ 5–6. It also added two new sections, "Invalidity of the HHS Patents," *id.* at ¶¶ 19–24, and "Non-infringement of the HHS Patents," *id*. at ¶¶ 25–35. For the first time, Gilead explicitly asserts that sovereign immunity is waived for three reasons: (1) that the "Government waived its sovereign immunity to Gilead's counterclaims by bringing this action;" (2) that "Gilead's counterclaims are [] proper recoupment claims that may be asserted against the Government notwithstanding its general sovereign immunity;" and (3) that the "Government has [] expressly and unequivocally waived its sovereign immunity through 5 U.S.C. § 702." *Id*. at ¶¶ 5–6

SUMMARY OF THE ARGUMENT

Gilead has failed to establish a legally sufficient waiver of sovereign immunity for its remaining Counterclaims of noninfringement and invalidity for each of the four Patents-in-Suit.

Though Gilead argues that there is a proper waiver based on (1) Federal Rule of Civil Procedure 13, (2) recoupment, and (3) section 702 of the Administrative Procedure Act (APA), each of these theories fails under prevailing law. Rule 13 by itself provides no waiver of sovereign immunity. Gilead's recoupment allegations fail to account for clear precedent that mandates that its recoupment claims must be monetary. The recoupment allegations also fail as they seek affirmative, declaratory judgments, prohibited relief that is different in kind from the damages sought by the Government. And section 702 does not provide the requisite waiver as Gilead's allegations challenge the infringement and validity of the Patents-in-Suit (and, secondarily, the

Government's actions in enforcing them) rather than an unlawful agency action, as required by the statute. The discretion of agency actions in the protection of federal "inventions" is broad and not subject to review.

For these reasons, Gilead fails to plead adequate grounds for establishing an express and unequivocal waiver of sovereign immunity for its twice-amended Counterclaims and they should be dismissed under Rule 12(b)(1).

ARGUMENT

I. GILEAD'S COUNTERCLAIMS SHOULD BE DISMISSED FOR FAILURE TO ESTABLISH A WIAVER OF SOVEREIGN IMMUNITY

"It is elementary that the 'United States, as sovereign, is immune from suit save as it consents to be sued . . . , and the terms of its consent to be sued in any court define that court's jurisdiction to entertain the suit.'" *United States v. Mitchell*, 445 U.S. 535, 538 (1980) (quoting *United States v. Sherwood*, 312 U.S. 584, 586 (1941)). A waiver of sovereign immunity "cannot be implied but must be unequivocally expressed," *United States v. King*, 395 U.S. 1, 4 (1969), and "must be strictly construed in favor of the United States," *Clinton Cty. Comm'rs v. United States Envtl. Protection Agency*, 116 F.3d 1018, 1021 (3d Cir. 1997).

"Absent a waiver, sovereign immunity shields the Federal Government and its agencies from suit." *FDIC v. Meyer*, 510 U.S. 471, 475 (1994) (citing *Loeffler v. Frank*, 486 U.S. 549, 554 (1988)). Accordingly, Gilead bears the burden of pleading an applicable waiver of sovereign immunity as a prerequisite for jurisdiction. *Green v. Locke*, CIV. A. No. 10-707 MLC, 2010 WL 3614216, at *4 (D.N.J. Sept. 8, 2010) (citing *In re Univ. Med. Ctr.*, 973 F.2d 1065, 1085 (3d Cir. 1992)); *United States v. Park Place Assocs., Ltd.*, 563 F.3d 907, 924 (9th Cir. 2009) ("As the party asserting a claim against the United States, [the plaintiff] has the burden of 'demonstrating an unequivocal waiver of immunity.'") (citation omitted). Specifically, Gilead is "required to set

forth in [its pleading] the specific statute containing a waiver of the government's immunity from suit." *Warminster Twp. Mun. Auth. v. United States*, 903 F. Supp. 847, 849 (E.D. Pa. 1995).

A. Gilead Incorrectly Asserts That the United States Waives Its Immunity Simply by Bringing This Suit.

Gilead leads with the assertion that "[t]he Government waived its sovereign immunity to Gilead's counterclaims by bringing this action," D.I. 21 at 92 (¶ 5), because Gilead's "counterclaims are compelled by Federal Rule of Civil Procedure 13(a) and are therefore adverse claims that have arisen out of the same transaction which gave rise to the Government's suit." *Id.* This view of waiver is directly contrary to controlling precedent and Rule 13 itself.

"[A sovereign] does not waive its sovereign immunity from actions that could not otherwise be brought against it merely because those actions were pleaded in a counterclaim to an action filed by the [sovereign]." *Okla. Tax Comm'n v. Citizen Band Potawatomi Indian Tribe*, 498 U.S. 505, 509 (1991).[1] Where a sovereign "[p]ossess[es] immunity from direct suit, we are of the opinion it possesses a similar immunity from cross-suits." *United States v. U.S. Fid. & Guar. Co.*, 309 U.S. 506, 513 (1940). Indeed, the premise that "the bare act of filing suit does not operate as a complete, automatic waiver that subjects a [sovereign] to any counterclaims filed by the defendant" is "unremarkable." *Quinault Indian Nation v. Pearson*, 868 F.3d 1093, 1097 (9th Cir. 2017).

While Gilead cites Federal Rule of Civil Procedure 13(a) in support of its waiver claim, D.I. 21 at 92 (¶ 5), it fails to cite the relevant provision in the Rule. Rule 13(d) explicitly states

[1] The Government notes that the immunity of the United State is broader even than the immunity of the sovereign Indian Nations as it is the "superior sovereign," who "enjoys absolute sovereign immunity from unconsented suits." *United States v. Yakima Tribal Court*, 806 F.2d 853, 861, 858 (9th Cir. 1986) (emphasis added).

that "[t]hese rules do not expand the right to assert a counterclaim—or to claim a credit—against the United States or a United States officer or agency." (Emphasis added). Further, "Supreme Court precedent couldn't be clearer on this point: a [sovereign's] decision to go to court doesn't automatically open it up to counterclaims — even compulsory ones." *Ute Indian Tribe of the Uintah & Ouray Reservation v. Utah*, 790 F.3d 1000, 1011 (10th Cir. 2015) (Gorsuch, J.). Gilead's Rule 13 argument should be dismissed as wholly without merit.

B. Gilead Fails to Correctly Apply the Test for Recoupment.

Gilead's secondary assertion of waiver alleges that its Counterclaims are "proper recoupment claims that may be asserted against the Government, notwithstanding its general sovereign immunity." D.I. 21 at 92 (¶ 5). In support, Gilead pleads that its Counterclaims (1) "arise from the same transactions that gave rise to the Government's suit," (2) "seek relief of the same kind or nature of as the Government's suit (i.e., a finding on whether the claims of the HHS Patents are valid and infringed)," and (3) "do not seek an amount of relief that is in excess of the Government's claims (i.e., because they seek only declaratory relief)." *Id.* This view of waiver is similarly flawed.

As set forth by numerous circuit courts, including the Third Circuit, a recoupment claim must (i) "arise from the same transaction" that gave rise to the Government's suit, (ii) "seek relief of the same kind or nature as the plaintiff's suit" and (iii) "seek an amount not in excess of the plaintiff's claim." *E.g., United States v. Washington*, 853 F.3d 946, 968 (9th Cir. 2017) (citation omitted).[2] While it is not disputed that Gilead's claims arise from the same transaction or occurrence, its Counterclaims nonetheless do not meet the other two elements of recoupment.

[2] *See also United States v. CITGO Asphalt Ref. Co.* (*In re Frescati Shipping Co.*), 886 F.3d 291, 311 (3d Cir. 2018); *Berrey v. Asarco*, 439 F.3d 636, 645 (10th Cir. 2006); *FDIC v. Hulsey*, 22 F.3d

In particular, Gilead's citation to *U.S. Dep't of Health & Human Servs. v. Mylan Pharms., Inc.*, No. 10-cv-5956, 2011 WL 13238650 (D.N.J. July 13, 2011) in support of its positions, D.I. 21 at 92 (¶ 5), conflicts with Supreme Court and circuit court precedent and is, in any event, inapplicable here.

1. Gilead's Counterclaims Are Not Monetary.

"It is implicit in the use of the word 'amount' in [the] third criterion that a recoupment claim is a monetary claim." *Washington*, 853 F.3d at 968; *see also United States v. U.S. Fid. & Guar. Co.*, 309 U.S. 506, 511 (1940) ("[A] defendant may, without statutory authority, recoup on a counterclaim *an amount* equal to the principal claim.") (emphasis added). Indeed, the Ninth Circuit has explained that "recoupment claims must be monetary, not injunctive or declaratory." *Quinault*, 868 F.3d at 1100.

Gilead submits that the requirement that a recoupment claim must "seek an amount not in excess of the plaintiff's claim" is met in every, and any, declaratory judgment counterclaim. D.I. 21 at 92 (¶ 5). In Gilead's view, because it seeks only declaratory relief—specifically, "a finding on whether the claims of the HHS Patents are valid and infringed," *id.*—it necessarily does not seek an "amount of relief that is in excess of the Government's claims." *Id.*

But these statements demonstrate the impropriety of Gilead's claim of recoupment. Gilead seeks an affirmative, declaratory "finding," not a reduction or other diminishment of the Government's claim to monetary damages. "[R]ecoupment is permitted only to reduce or

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1472, 1487 (10th Cir. 1994); *Frederick v. United States*, 386 F.2d 481, 488 (5th Cir. 1967); *United States v. Brotech Corp.*, Civil Action No. 00-2428, 2000 U.S. Dist. LEXIS 13859, at *15 (E.D. Pa. Sep. 19, 2000); *United States v. Atlas Minerals & Chems., Inc.*, 797 F. Supp. 411, 421 (E.D. Pa. 1992).

eliminate damages, not to gain some other form of relief." *Bolduc v. Beal Bank, SSB*, 167 F.3d 667, 672 n.4 (1st Cir. 1999). Again, there is no question that Gilead's Counterclaims, which seek affirmative declaratory relief, are not monetary claims, and thus not claims for recoupment.[3] *See, e.g.*, *United States v. Levering*, 446 F. Supp. 977, 978 (D. Del. 1978) ("Counterclaims seeking affirmative relief against the United States, whether compulsory or permissive, cannot be maintained unless the United States has statutorily consented to be sued on the claim.").

2. Gilead's Counterclaims Seek Relief that Is Different in Kind from the Relief the Government Seeks.

For similar reasons, Gilead's Counterclaims do not "seek relief of the same kind or nature as the plaintiff's suit," and therefore do not meet the second requirement for a recoupment claim. Here, the Government seeks monetary damages for Gilead's willful infringement of federally owned patents while Gilead brings counterclaims seeking affirmative, declaratory relief of invalidity or non-infringement.

"The requirement that a defendant seek the same kind of relief as has been sought in the plaintiff's claim is a fundamental requisite for recoupment. The defense is not intended to be a catch-all to allow any claims otherwise barred by time, settlement, or statute to be heard as equity seems to require." *CITGO Asphalt*, 886 F.3d at 312. And "'recoupment is in the nature of a defense' to defeat a plaintiff's claims, not a vehicle for pursuing an affirmative judgment." *Ute Indian Tribe*, 790 F.3d at 1011 (Gorsuch, J.) (quoting *Bull v. United States*, 295 U.S. 247, 262 (1935); *see also United States v. Agnew*, 423 F.2d 513, 514 (9th Cir. 1970); *Levering*, 446 F. Supp. at 978 (D. Del. 1978). Gilead seeks such an impermissible affirmative judgment here.

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[3] While the Government has raised this point repeatedly to Gilead, both in correspondence, *e.g.*, Exhibits 1–4**,** and in its prior motion, D.I. 17 at 12–14, Gilead continues to plead nonmonetary claims for affirmative declaratory relief.

3. The Unpublished *Mylan* Decision Was Wrongly Decided and Is Inapplicable.

Gilead's recoupment theory relies almost entirely on *Mylan*, an unpublished ANDA decision where declaratory judgment claims of invalidity and noninfringement were found to be permissible under a theory of recoupment. 2011 WL 13238650, at *3. But that decision was contrary to controlling law. As set forth by Supreme Court and circuit court precedential decisions, the only claims that the doctrine of recoupment permits to be brought against the United States are monetary and, thus, claims seeking declaratory relief, like Gilead's, do not seek recoupment. On that point, the Government specifically disagrees with the finding in *Mylan* that because both parties' claims there were for declaratory (nonmonetary) judgments, the third, "amount" criterion was a "non-issue." *Id.* That ruling effectively eliminated the third required element of a recoupment claim.

Additionally, regarding the second criterion of recoupment, *Mylan* related to an artificial act of infringement under 35 U.S.C. § 271(e)(2), where the act of submitting the application to the FDA creates a statutory act of infringement. Consequently, monetary damages were not at issue and the only remedies were the determination of liability and an injunction to prohibit actual future sales of a pharmaceutical product. *U.S. Dep't of Health & Human Servs. v. Mylan Pharms., Inc.*, No. 10-cv-5956-WHW, Compl. (D.I. 1) at 6 (Exhibit 5). Here, the Government seeks money damages for Gilead's infringement of the Patents-in-Suit. Gilead's Counterclaims do not seek monetary relief in the form of a recoupment of monies owed, and are not, accordingly, of the same

kind as the Government's claims as discussed above.[4] This portion of the *Mylan* decision is simply inapplicable to Gilead's claims here.

C. Gilead's Assertion of Waiver under 5 U.S.C. § 702 Is Unsupported by Gilead's Superficial Pleadings of an APA Claim.

Gilead's third assertion of waiver references the Administrative Procedure Act (APA) and the Federal Circuit's decision in *Delano Farms*, 655 F.3d at 1343–1344, 1349 & n.6. Gilead generally alleges that:

> The Government has also expressly and unequivocally waived its sovereign immunity through 5 U.S.C. § 702. . . . Gilead's counterclaims are within the scope of § 702's waiver because they seek only declaratory relief, not money damages; because HHS, CDC, and/or their employees acted unlawfully in their official capacities in obtaining the HHS Patents, which claim subject matter that is not patentable; and because HHS and/or its employees acted unlawfully in their official capacities in asserting that Gilead infringes the claims of the HHS Patents and demanding that Gilead license the HHS Patents.

D.I. 21 at 93 (¶ 6). Gilead's cursory allegations are insufficient and of a different kind than addressed in *Delano Farms*.

Section 702 does provide a waiver of sovereign immunity, for an action "seeking relief other than monetary damages and stating a claim that an agency or an officer or employee thereof acted or failed to act in an official capacity or under color of legal authority shall not be dismissed . . . on the ground that it is against the United States . . . ." (Emphasis added). But the pleadings that established such a waiver in *Delano Farms* were far different substantively from those offered by Gilead in its second attempt to amend its counterclaims.

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[4] The Government has previously set forth its disagreement with the *Mylan* decision in correspondence with Gilead that prompted the amendments to its original answer and counterclaims. Exs. 1–4.

As noted in the Government's previous motion, D.I. 17 at 14–15, the *Delano Farms* plaintiff explicitly alleged that the U.S. Department of Agriculture (USDA) "acted unlawfully in obtaining the patents [in suit] and entering into a license agreement with the [California Table Grape] Commission." 655 F.3d at 1341. More specifically, the plaintiff alleged that a USDA employee (and named inventor) had committed an invalidating prior use of the patented grape varieties and subsequently failed to inform the Patent Office of the alleged use. *Id.* at 1340–41. In this context, the Federal Circuit stated "USDA's act of obtaining ownership of the patents makes it subject to declaratory judgment action seeking to invalidate the patents or hold them unenforceable . . . under the Patent Act" in view of "section 702['s] waive[r of] the agency's sovereign immunity." *Id.* at 1350 n.6.

While the Federal Circuit did not agree with a number of other circuits[5] that "agency action" and "final agency action," as set forth in the APA itself, must be applied in evaluating a waiver, it nonetheless acknowledges section 702's waiver of sovereign immunity requires, at least, that the claims challenge some unlawful act or unlawful failure to act by an agency or its officers.

[5] The Second and Sixth Circuit have ruled that section 702 only applies when there has been an "agency action," as defined by the APA. *SEC v. Credit Bancorp, Ltd.*, 297 F.3d 127, 141 (2d Cir. 2002); *Blakely v. United States*, 276 F.3d 853, 860, 870 (6th Cir. 2002). The Fourth, Fifth, and Ninth Circuits have similarly held that section 702 is subject to 5 U.S.C. § 704's requirement of "final agency action." *Gallo Cattle Co. v. USDA*, 159 F.3d 1194, 198–99 (9th Cir. 1998); *Beamon v. Brown*, 125 F.3d 965,967 (6th Cir. 1997); *Taylor-Callahan-Coleman Counties Dist. Adult Probation Dep't* v. *Dole*, 948 F.2d 953, 956 (5th Cir. 1991); *Food Town Stores, Inc. v. EEOC*, 708 F.2d 920, 922 (4th Cir. 1983). The Federal Circuit, however, sided with the position of the Eighth Circuit and D.C. Circuit that section 702 "is not limited to 'agency action' or 'final agency action,' as those terms are defined in the APA." *Delano*, 655 F.3d at 1344; *compare with Trudeau v. FTC*, 456 F.3d 178, 180 (D.C. Cir. 2006); *Red Lake Band of Chippewa Indians v. Barlow*, 846 F.2d 474, 476 (8th Cir. 1988)).

The alleged agency action was articulated extensively in Delano Farm's first amended complaint, which presented the declaratory judgment claims subject to the Federal Circuit ruling. *See Delano Farms Co. v. Cal. Table Grape Comm'n*, No. 1:07-CV-1610, 2009 U.S. Dist. LEXIS 100093, at *1 (E.D. Cal. July 26, 2010), *rev'd*, 655 F.3d 1377, 1350 & n.6 (Fed. Cir. 2011); Exhibit 6 (D.I. 50, First Amended Complaint). Plaintiff Delano Farms specifically detailed that "[w]hile delaying the decision to seek patent protection, and failing to implement security measures at its facilities, the USDA knew that public use of new varieties more than one year before applying for a patent would bar later filing for patent protection. Indeed, the Commission and [USDA researcher and inventor] Dr. [David] Ramming discussed the fact that public uses and sales of new varieties prior to seeking patent protection could jeopardize the Commission's patenting program." Ex. 6 at 13 (¶ 57). In turn, these alleged public uses (and the associated unlawful acts by the USDA) form the entire basis of Delano's invalidity claims. *Id.* at 19-25 (¶¶ 103–38).

Thus, the USDA allegedly undertook the actions that establish the invalidity of the Patents-in-Suit rather than simply alleging the validity of the patents. Similarly, Delano Farms based its inequitable conduct claim entirely on the failure of the USDA and its agents to properly notify the U.S. Patent and Trademark office of the USDA's prior use. *Id.* at 25-27 (¶¶ 139–52).

Unlike Delano's claims, replete with direct, specific allegations of unlawful agency action, Gilead's Counterclaims lack such a basis or such allegations. Gilead's four noninfringement claims (Counts 1, 3, 5, and 7) never allege that the claimed noninfringement is due to unlawful Government action, only that the "Government bears the burden of proving infringement by GSI, and it is unable to meet that burden," D.I. 21 at 101 (¶ 25). Likewise, Gilead's general allegation that "HHS and/or its employees acted unlawfully in their official capacities in asserting that Gilead infringes," D.I. 21 at 93 (¶ 6) (emphasis added), fails to establish a noninfringement claim based

on unlawful agency action. Thus, Gilead's noninfringement claims challenge the Government's infringement allegations, not any act or failure to act by HHS. Accordingly, section 702 does not apply to Gilead's noninfringement claims.

Gilead's four invalidity claims (Counts 2, 4, 6, and 8) all rely on essentially the same set of allegations set forth in its Counterclaims. D.I. 21 at 95–101 (¶¶ 19–24). No allegations are plausibly connected to any agency action and only cite to Government research, publications, or statements by Government employees that Gilead contends supports the invalidity of the Patents-in-Suit. *Id.* The Counterclaims do not challenge the lawfulness of any act or failure to act by HHS or other agencies. Rather, Gilead's claims simply raise statutory challenges to thevalidity of the Patents-in-Suit.

Gilead's general statement that the Government has waived sovereign immunity under section 702 "because HHS, CDC, and/or their employees acted unlawfully in their official capacities in obtaining the HHS Patents, which claim subject matter that is not patentable," D.I. 21 at 93 (¶ 6), demonstrates the flaw in Gilead's arguments. Gilead is simply equating unlawful agency action in obtaining these patents with the invalidity of the patents themselves—not challenging actual agency actions that themselves invalidate the patents. Accordingly, as with its noninfringement claims, Gilead cannot establish a waiver under section 702 for its invalidity claims.

D. Section 701(a)(2) Precludes Review of Agency Actions at Issue.

Moreover, an "agency action" is not subject to review under section 702 where 5 U.S.C. § 701(a)(2) precludes the APA's applicability to that action—specifically where the agency "action is committed to agency discretion by law." While the Supreme Court applies a "strong presumption" in favor of judicial review, *Mach Mining, LLC v. EEOC*, 135 S. Ct. 1645, 1651 (2015), that presumption is rebutted—and action is considered unreviewable under section

701(a)(2)—"in those rare instances where statutes are drawn in such broad terms that in a given case there is no law to apply." *Cnty. of Westchester v. U.S. Dep't of Housing and Urban Dev.*, 778 F.3d 412, 419 (2d Cir. 2015) (internal quotation omitted). Other circuit courts have similarly found that where statutory authority for agency action is so broad that it is viewed as "permissive," the agency's action is unreviewable because the statute does not impose any standard for determining how or when the official must act. *See, e.g.*, *Ferry v. Udall*, 336 F.2d 706, 712 (9th Cir. 1964).

While agency actions in obtaining and maintaining patents—authorized under section 207(a)(1)—were relied upon by the Federal Circuit in *Delano* to find that the waiver of section 702 made the Government subject to the declaratory judgment actions, the Federal Circuit did not consider or address whether section 701(a)(2) precluded review. 655 F.3d at 1350 n.6. [6] At least one other circuit, however, has found section 701(a)(2) to preclude review of agency action under the Bayh-Dole Act and to other governmental actions related to patent rights. *See S. Research Inst. v. Griffin Corp.*, 938 F.2d 1249, 1254–55 (11th Cir. 1991).

Specifically, the *Southern Research* court found an agency's discretion in applying for, obtaining, and maintaining Government patents (under 35 U.S.C. § 207(a)(1) and (2)) to be broad and an agency's discretion to assign or transfer federal patent rights (under 35 U.S.C. § 202(e)) to be not only equally broad, but, as a result, unreviewable. *Id.* at 1254 n.9. This is consistent with the language of the statute, which expressly "authorize[s]" government agencies to "apply for, obtain, and maintain patents . . . on inventions in which the Federal Government owns a right, title, or interest," 35 U.S.C. § 207(a)(1) (emphasis added), where an "invention" is defined as "any

[6] This could be because the Federal Circuit found that section 702 "is not limited to 'agency action' or 'final agency action,' as those terms are defined in the APA." *Delano*, 655 F.3d at 1344. But there is no explicit finding to that effect in the *Delano* decision.

invention or discovery which is or may be patentable," *id*. § 201(d) (emphasis added). *Southern Research* thus stands squarely against permitting a counterclaim plaintiff to use *Delano* to reflexively subject the Government to the waiver of section 702.

The same rationale should apply to the Government's efforts "to undertake all other suitable and necessary steps to protect and administer rights to federally owned inventions" under section 207(a)(3), preventing Gilead's Counterclaims from relying on the Government's actions in the protection of federal inventions to qualify as a the waiver under section 702. HHS actions in seeking and protecting a patent are not contrary to law *even if* the patent does not issue or should not have been issued, and accordingly, such decisions are committed to the broad discretion of HHS by law. *Westchester*, 778 F.3d at 419. Gilead thus cannot plead an unlawful agency action sufficient to avail itself of section 702.

E. Gilead's Other Citations Are Not Expressly Invoked and, Regardless, Are Not "Unequivocal Waivers" of the Government's Sovereign Immunity.

To the extent that Gilead asserts reliance on citations related to subject matter jurisdiction, the Declaratory Judgment Act, and the "Patent Laws of the United States" in support of its arguments of waiver, they are inapplicable and may be quickly dismissed. None of the cited statutory references provide an "unequivocal expression" of a waiver of sovereign immunity.

First, the "Patent Laws of the United States," 35 U.S.C. §§ 1, *et seq*., (Counterclaims at ¶ 3), contain no relevant express waiver of sovereign immunity.[7] Accordingly, the Patent Laws

[7] There is a limited waiver of sovereign immunity set forth in 35 U.S.C. § 183, a provision of the Invention Secrecy Act, but it is not relevant to the issue discussed here. *Horton v. United States*, No. C-13-4912 MMC, 2014 U.S. Dist. LEXIS 34629, at *3–12 (N.D. Cal. Mar. 14, 2014). And 28 U.S.C. § 1498 waives sovereign immunity for claims of manufacture or use of a patented invention by or for the United States in the Court of Federal Claims.

do not operate to subject the United States to counterclaims of noninfringement or invalidity, as asserted here. This is not to say, of course, that defendants in actions brought by the United States are left defenseless. Such defendants may "assert affirmative defenses against [a sovereign's] claims, but [can]not bring counterclaims absent [a] waiver of sovereign immunity." *Quinault*, 868 F.3d at 1098.[8] As discussed previously, Counterclaims are significantly different from affirmative defenses in that the counterclaims survive dismissal of the original claims.[9] Accordingly, "the mere fact that the [United States] initiated this action is not enough for [Gilead] to assert its barrage of counterclaims without offending the [United States'] sovereign immunity." *Id.* at 1097.

Second, any reliance on the Declaratory Judgment Act, 28 U.S.C. §§ 2201–2202, is similarly unavailing. "The Declaratory Judgment Act is not an independent ground for jurisdiction; it permits the award of declaratory relief only when other bases for jurisdiction are present." *Jones v. Alexander*, 609 F.2d 778, 781 (5th Cir. 1980) (citation omitted). In other words, "[t]he operation of the Declaratory Judgment Act is procedural only," and does not extend the jurisdiction of federal courts. *Aetna Life Ins. Co. of Hartford, Conn. v. Haworth*, 300 U.S. 227, 240 (1937). *Accord Abdul Qadir v. Gonzales*, Civil Action No. 07-3741 (FLW), 2008 U.S. Dist. LEXIS 50355, at *16–17 (D.N.J. June 26, 2008) (citing *Ragoni v. United States*, 424 F.2d 261, 264 (3d Cir. 1970)).

---

[8] Not all affirmative defenses are available to every defendant in cases brought by the United States. Specifically, Gilead's affirmative defenses that sound in equity are unavailable for the reasons discussed in the Government's corresponding Motion to Strike.

[9] Indeed, Gilead's Counterclaims may serve only to keep the United States in court against its will and, crucially, without its consent. This is improper as a matter of law. *Quinault*, 868 F.3d at 1099 (finding a counterclaim-plaintiff's "asserted ability to drag out the proceedings and hold the Nation hostage in its own litigation is a direct affront to the Nation's sovereign immunity when there has been no unequivocal waiver.").

Finally, neither 28 U.S.C. § 1331 nor § 1338 waives the sovereign immunity of the United States. Section 1331 is the general federal-question jurisdiction statute, but "do[es] not operate as waivers of sovereign immunity" for claims against the United States. *Munaco v. United States*, 522 F.3d 651, 653 n.3 (6th Cir. 2008); *accord Mitchell*, 445 U.S. at 538. Indeed, "when [a] plaintiff seeks to sue the United States or an instrumentality thereof, he may not rely on the general federal question jurisdiction of 28 U.S.C. § 1331, but must identify a specific statutory provision that waives the government's sovereign immunity from suit." *Clinton Cty. Comm'rs*, 116 F.3d at 1021 (emphasis added); *see also Harbert v. United States*, 206 F. App'x 903, 907 (11th Cir. 2006).

Similarly, section 1338 establishes the jurisdiction of the district courts over patent cases. But, like section 1331, this section is "merely a general provision vesting jurisdiction over certain types of actions in the district counts. It does not contain any consent by the United States, express or implied, permitting the institution of suits against it for [] infringement." *Turton v. United States*, 212 F.2d 354, 355 (6th Cir. 1954). Indeed, the existence of 28 U.S.C. § 1498, which grants exclusive jurisdiction over patent infringement claims to the Court of Federal Claims, makes clear that Congress did not intend to waive sovereign immunity for patent claims through general jurisdictional statutes, such as sections 1331 and 1338.

Accordingly, none of these cited statutes constitute waivers of immunity upon which Gilead may rely.

CONCLUSION

Gilead's failure to identify and plead a sufficient express and unequivocal waiver of sovereign immunity warrants dismissal of its Counterclaims under Rule 12(b)(1).

Respectfully submitted,

JOSEPH H. HUNT
Assistant Attorney General

GARY L. HAUSKEN
Director

/s/ Walter W. Brown

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Dated: June 26, 2020

Exhibit 1

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March 12, 2020

VIA E-MAIL AND FEDERAL EXPRESS

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7 World Trade Center
250 Greenwich Street
New York, NY 10007 USA
Email: david.bassett@wilmerhale.com

Re: ***United States v. Gilead Sciences, Inc. et al.,***
D. Del. (Civil No. 1:19-cv-02103-MN)

Dear Mr. Bassett:

Gilead Sciences, Inc. (GSI) asserts twelve declaratory judgment counterclaims relating to the infringement, validity, and enforceability of the four patents-in-suit. According to GSI, those counterclaims are brought "under the Patent Laws of the United States, 35 U.S.C. §§ 1 et seq., for a declaratory judgment pursuant to 28 U.S.C. §§ 2201-2202" and that the "Court has subject matter jurisdiction over Defendant's Counterclaims under 28 U.S.C. §§ 1331 and 1338." No other statutory bases of jurisdiction are identified.

As a general matter, the Government enjoys sovereign immunity from counterclaims and does not waive its immunity by bringing suit against a private party to seek redress for civil wrongs committed against the United States. *See, e.g., United States v. Mitchell,* 445 U.S. 535, 538 (1980). In turn, any waiver of sovereign immunity "must be unequivocally expressed," *United States v. King*, 395 U.S. 1, 4 (1969), and "must be strictly construed in favor of the United States." *Clinton Cty. Comm'rs v. United States Envtl. Protection Agency*, 116 F.3d 1018, 1021 (3d Cir. 1997). "Absent a waiver, sovereign immunity shields the [] Government and its agencies from suit." *FDIC v. Meyer*, 510 U.S. 471, 475 (1994) (citation omitted).

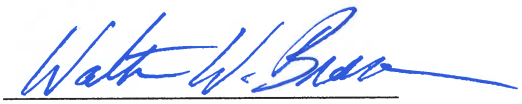
Further, "it is clear that the United States, by filing [an] original complaint . . . [does] not thereby consent to be sued on a counterclaim based upon a cause of action as to which it had not otherwise given its consent to be sued." *United States v. Forma*, 42 F.3d 759, 764 (2d Cir. 1994) (quoting *United States v. Silverton*, 200 F.2d 824, 826 (1st Cir. 1952)). The District of Delaware has long agreed with this law. *United States v. Levering*, 446 F. Supp. 977, 978 (D. Del. 1978).

Accordingly, GSI bears the burden of pleading an applicable waiver of sovereign immunity as a prerequisite for jurisdiction. *Green v. Locke*, No. CIV. A. 10-707 MLC, 2010 WL 3614216, at *4 (D.N.J. Sept. 8, 2010) (citing *In re Univ. Med. Ctr.*, 973 F.2d 1065, 1085 (3d Cir. 1992)); *see also* 5 Wright & Miller, *Fed. Prac. & Proc. Civ.* § 1212 (3d ed.). The statutes identified by GSI, however, do not operate to waive sovereign immunity for its Counterclaims. Section 1331 confers federal-question jurisdiction on the district courts; but "jurisdictional statutes, such as this statute giving federal district courts original jurisdiction of civil actions arising under Constitution, laws, or treaties of United States, do not operate as waivers of sovereign immunity." *Munaco v. United States*, 522 F.3d 651, 653 n.3 (6th Cir. 2008); *accord Mitchell*, 445 U.S. at 538. Indeed, "when [a] plaintiff seeks to sue the United States or an instrumentality thereof, he may not rely on . . . 28 U.S.C. § 1331, but must identify a specific statutory provision that waives the government's sovereign immunity from suit." *Clinton Cty. Comm'rs*, 116 F.3d at 1021 (emphasis added); *see also Harbert v. United States*, 206 F. App'x 903, 907 (11th Cir. 2006). Similarly, Section 1338 is "merely a general provision vesting jurisdiction over certain types of actions in the district counts." *Turton v. United States*, 212 F.2d 354, 355 (6th Cir. 1954). It does not provide a waiver to filing suit against the government. *Id.*

Further, neither the cited patent statutes nor the Declaratory Judgment Act underlying GSI's Counterclaims provide the requisite waiver. The Declaratory Judgment Act does not operate on its own to waive sovereign immunity. *Jones v. Alexander*, 609 F.2d 778, 781 (5th Cir. 1980). And the patent statutes contain no relevant waiver of sovereign immunity. The lone patent-specific waiver of sovereign immunity, 28 U.S.C. § 1498, only permits parties to bring patent infringement suits in the Court of Federal Claims for monetary damages.

Thus, given that GSI's Counterclaims fail to properly plead or identify a specific waiver of sovereign immunity, we request that GSI voluntarily withdraw its claims prior to the Government's filing of any responsive pleading. Alternatively, if GSI believes it can now identify a specific waiver, the Government is amenable to discussing such authority as well as any proposal by GSI to amend its Counterclaims. The Government further requests that Gilead respond by Tuesday, March 17, 2020 so that the Government can timely request an extension of its current filing deadline, if needed, to facilitate such a discussion.

Very truly yours,



Walter W. Brown
Senior Litigation Counsel
Commercial Litigation Branch

Exhibit 2

WILMERHALE

March 15, 2020

David B. Bassett

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By E-mail

Walter Brown, Esq.
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Re: *United States v. Gilead Scis., Inc.*, C.A. No. 19-2103-MN (D. Del.)

Dear Wally:

We write in response to the Government's March 12, 2020 letter, which misstates the law of sovereign immunity. Your letter asserts that "the Government enjoys sovereign immunity from counterclaims and does not waive its immunity by bringing suit against a private party to seek redress for civil wrongs committed against the United States." That statement—while true "[a]s a general matter"—has a long-held exception: A party may also assert a claim against the Government when the Government initiates the suit if the claim arises out of the same transaction or occurrence as the Government's claim and would reduce or defeat the government's recovery. *See United States v. Forma*, 42 F.3d 759, 764 (2d Cir. 1994) (noting the "significant limitation to the general bar of sovereign immunity in the context of counterclaims"). As the Third Circuit has explained:

> It is, of course, true that when the United States ... initiates a suit, it thereby submits itself to the jurisdiction of the court, but, as to claims against the sovereign, the latter's submission to a court's jurisdiction, because of its suit, draws in only such adverse claims as have arisen out of the same transaction which gave rise to the sovereign's suit.

In re Monogahela Rye Liquors, 141 F.2d 864, 869 (3d Cir. 1944); *see also* Wright & Miller, 6 Fed. Prac. & Proc. Civ. § 1427 (3d ed.) ("[W]hen the United States institutes an action, defendant may assert by way of recoupment any claim arising out of the same transaction or occurrence as the original claim in order to reduce or defeat the government's recovery."); *cf. United States v. Bankers Ins. Co.*, 245 F.3d 315, 319-20 (4th Cir. 2001) ("Sovereign immunity is not a sword, but a shield....").

The Government's letter cites no case holding that counterclaims like Gilead's—i.e., declaratory-judgment counterclaims of invalidity, noninfringement, and unenforceability for patents that the Government has asserted—are barred by sovereign immunity. We are aware of only one decision addressing sovereign immunity in that context, and in that case, the court

rejected the same arguments that the Government made in its letter. In *U.S. Department of Health & Human Services v. Mylan Pharmaceuticals, Inc.*, No. 10-cv-5956-WHW, 2011 WL 13238650 (D.N.J. July 13, 2011), the Government asserted a patent-infringement claim against Mylan, which counterclaimed seeking a declaratory judgment of invalidity and non-infringement. *Id.* at *1. The Government moved to dismiss, alleging that Mylan's counterclaims were barred by sovereign immunity. *Id.* at *2. The court—following Third Circuit law—denied the Government's motion, holding that "[b]y initiating suit in federal court, the Government waives its sovereign immunity in regard to claims that arise out of the same transaction which gave rise to the original suit." *Id.* The court noted that:

> It is clear from the facts of this case that defendants' counterclaims arose out of the same transaction or occurrence as the Government's claim. Both the Government and defendants seek a declaratory judgment determining the validity/invalidity and infringement/non-infringement of the [asserted] patent. The issue on which both parties request relief arose from the same occurrence: namely, defendants' [alleged act of infringement]. Defendants' counterclaims are also of the same kind or nature as the Government's claims, as the declaratory judgment sought by defendants is a mirror-image of the judgment sought by the Government.

Id. at *3. "Because the Government waived its sovereign immunity when it initiated suit in federal court," the court held, "it is subject to adjudication of defendants' compulsory counterclaims properly sounding in recoupment." *Id.* at *4.

Mylan squarely addresses Gilead's mirror-image declaratory-judgment counterclaims for invalidity and non-infringement against the Government, so we will not agree to drop those counterclaims. But, to avoid unnecessary motion practice, if the Government agrees that Gilead may file an amended answer before the Government files a responsive pleading—which Gilead could do as of right anyway after the Government files that responsive pleading—Gilead will withdraw its four unenforceability counterclaims and agree to stipulate to extend the 14-day period in Rule 15(a)(3) for the Government to respond to the amended answer and counterclaims to 28 days. Please let us know if the Government agrees or if it would be helpful to discuss.

Best regards,

David B. Bassett

cc: All Counsel of Record

Exhibit 3

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March 17, 2020

VIA E-MAIL

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Re: ***United States v. Gilead Sciences, Inc. et al.*,**
D. Del. (Civil No. 1:19-cv-02103-MN)

Dear Mr. Bassett:

I write in response to your March, 15, 2020 letter, in which Gilead Sciences, Inc. (GSI) offers to amend its counterclaims (prior to a Government responsive pleading) by: "withdrawing its four unenforceability counterclaims," and extending the Government's date for a responsive pleading to 28 days. In the interest of avoiding unnecessary motion practice, the Government can agree to this approach. Presently, we believe that 28 days will be sufficient to respond to GSI's amended counterclaims, assuming that GSI will not make any substantive amendments other than those disclosed in your letter. While coronavirus-related issues may ultimately lead the Government to ask for additional time, we can take up that issue at a later date.

That being said, we disagree with GSI's understanding of the scope of waiver provided by the recoupment exception. To constitute a recognized waiver of sovereign immunity, GSI must establish more than merely that (i) its counterclaims "arise out of the same transaction" that gave rise to the Government's suit. As set forth by a number of Circuit courts, a recoupment claim must also (ii) "seek relief of the same kind or nature as the plaintiff's suit" and (iii) "seek an amount not in excess of the plaintiff's claim." *United States v. Washington*, 853 F.3d 946, 968 (9th Cir. 2017) (citation omitted); *see also Berrey v. Asarco*, 439 F.3d 636, 645 (10th Cir. 2006); *FDIC v. Hulsey*, 22 F.3d 1472, 1487 (10th Cir. 1994); *Frederick v. United States*, 386 F.2d 481, 488 (5th Cir. 1967). Further, courts have "ma[d]e explicit that the remedy (the 'amount') sought by the United States and by the defendant in recoupment *must be monetary*." *Washington*, 853 F.3d at 968 (emphasis added); *Quinault Indian Nation v. Pearson*, 868 F.3d 1093, 1100 (9th Cir. 2017) ("recoupment claims must be monetary, not injunctive or declaratory."). Courts in the Third Circuit have

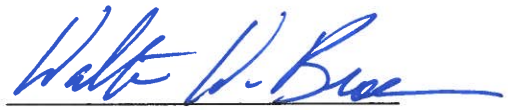
followed this three-pronged test. *See United States v. Brotech Corp.*, CIVIL ACTION NO. 00-2428, 2000 U.S. Dist. LEXIS 13859, at *15 (E.D. Pa. Sep. 19, 2000); *United States v. Atlas Minerals & Chems., Inc.*, 797 F. Supp. 411, 421 (E.D. Pa. 1992). And the Third Circuit has recently confirmed the propriety of this approach. *United States v. CITGO Asphalt Ref. Co. (In re Frescati Shipping Co.)*, 886 F.3d 291, 311 (3d Cir. 2018).

The Government's position also comports with the Supreme Court's understanding of recoupment claims, *Bull v. United States*, 295 U.S. 247, 261–62 (1935), and is consistent with well-established law that claims for recoupment "defeat or diminish the sovereign's recovery" but provide no "affirmative relief." *United States v. Agnew*, 423 F.2d 513, 514 (9th Cir. 1970).

For these reasons, we disagree that the unpublished decision in *U.S. Department of Health & Human Services v. Mylan Pharmaceuticals, Inc.*, No. 10-cv-5956-WHW, 2011 WL 13238650 (D.N.J. July 13, 2011), correctly states the law. Recoupment must be monetary. Likewise, *Mylan* involved an ANDA suit that sought only a finding of liability, whereas the current suit seeks monetary damages—relief of a different kind.

We therefore reserve the right to assert these arguments (or any others) in response to GSI's anticipated amended counterclaims. As you are aware, the Government's current deadline to respond to GSI's counterclaims is March 23, 2020. Please let us know how you propose proceeding with respect to moving the Court to amend GSI's pleading. Given the ongoing coronavirus-related issues, we will work with GSI on any extensions the parties may need to facilitate GSI's anticipated amendment.

Very truly yours,



Walter W. Brown
Senior Litigation Counsel
Commercial Litigation Branch

Exhibit 4

Holvey, Patrick C. (CIV)

From: Bassett, David <David.Bassett@wilmerhale.com>
Sent: Wednesday, March 18, 2020 6:05 PM
To: Brown, Walter (CIV)
Cc: Machen, Ronald C.; WH Gilead - HHS Patent Litigation; cottrell@rlf.com; farnan@rlf.com; Ewing, Alexandra M.; Anis, Shamoor (USADE); Hatcher, Laura (USADE); Sternhell, Philip C. (CIV); Holvey, Patrick C. (CIV); Kim, Nicholas J. (CIV)
Subject: RE: Correspondence in United States v Gilead Sciences, Inc. et al. case
Attachments: Stipulation to Amend Answer_(179226860)_(4).DOCX

Wally:

We continue to disagree that GSI's counterclaims seeking declaratory judgments of invalidity and noninfringement are improper, but we can discuss that further after Gilead's amended answer is filed if necessary.

Attached is a proposed stipulation. Please let us know if we have your consent to sign and file.

Best regards,

Dave

From: Brown, Walter (CIV) <Walter.Brown2@usdoj.gov>
Sent: Tuesday, March 17, 2020 4:27 PM
To: Bassett, David <David.Bassett@wilmerhale.com>
Cc: Machen, Ronald C. <Ronald.Machen@wilmerhale.com>; WH Gilead - HHS Patent Litigation <WHGilead-HHSPatentLitigation@wilmerhale.com>; cottrell@rlf.com; farnan@rlf.com; Ewing, Alexandra M. <Ewing@rlf.com>; Anis, Shamoor (USADE) <Shamoor.Anis@usdoj.gov>; Hatcher, Laura (USADE) <Laura.Hatcher@usdoj.gov>; Sternhell, Philip C. (CIV) <Philip.C.Sternhell@usdoj.gov>; Holvey, Patrick C. (CIV) <Patrick.C.Holvey@usdoj.gov>; Kim, Nicholas J. (CIV) <Nicholas.J.Kim@usdoj.gov>
Subject: RE: Correspondence in United States v Gilead Sciences, Inc. et al. case

EXTERNAL SENDER

Dave,

See attached letter, responding to you March 15th letter.

-Wally

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From: Bassett, David <David.Bassett@wilmerhale.com>
Sent: Sunday, March 15, 2020 2:58 PM
To: Brown, Walter (CIV) <wabrown@CIV.USDOJ.GOV>
Cc: Machen, Ronald C. <Ronald.Machen@wilmerhale.com>; WH Gilead - HHS Patent Litigation <WHGilead-HHSPatentLitigation@wilmerhale.com>; cottrell@rlf.com; farnan@rlf.com; Ewing, Alexandra M. <Ewing@rlf.com>; Anis, Shamoor (USADE) <SAnis@usa.doj.gov>; Hatcher, Laura (USADE) <lhatcher@usa.doj.gov>; Sternhell, Philip C. (CIV) <psternhe@CIV.USDOJ.GOV>; Holvey, Patrick C. (CIV) <pholvey@CIV.USDOJ.GOV>; Kim, Nicholas J. (CIV) <nikim@CIV.USDOJ.GOV>
Subject: RE: Correspondence in United States v Gilead Sciences, Inc. et al. case

Wally:

Please see that attached letter responding to your March 12 letter.

Best regards,

Dave

From: Brown, Walter (CIV) <Walter.Brown2@usdoj.gov>
Sent: Thursday, March 12, 2020 6:54 PM
To: Bassett, David <David.Bassett@wilmerhale.com>
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Subject: Correspondence in United States v Gilead Sciences, Inc. et al. case

EXTERNAL SENDER

Mr. Bassett,

Please see attached correspondence.

-Wally

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Exhibit 5

UNITED STATES DISTRICT COURT
DISTRICT OF NEW JERSEY

THE UNITED STATES OF AMERICA and THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS, Plaintiffs, v. MYLAN PHARMACEUTICALS INC. and LUPIN PHARMACEUTICALS, INC., and LUPIN LIMITED, Defendants.	Civil Action No._____ **COMPLAINT FOR PATENT INFRINGEMENT** **(Filed Electronically)**

Plaintiffs the United States of America ("government") and the Board of Trustees of the University of Illinois ("University of Illinois") (together, "Plaintiffs"), by their undersigned attorneys, for their Complaint against defendants Mylan Pharmaceuticals Inc. ("Mylan"), Lupin Pharmaceuticals, Inc., and Lupin Limited (together, "Lupin" and, collectively, "Defendants") herein allege:

NATURE OF THE ACTION

1. This is an action for patent infringement under the patent laws of the United States, Title 35 of the United States Code, arising from the Defendants' filing of Abbreviated New Drug Applications ("ANDAs") with the United States Food and Drug Administration (the "FDA") seeking approval to commercially manufacture and market generic versions of the pharmaceutical drug product Prezista® prior to the expiration of United States Patent No. 7,470,506 B1 (the "'506 patent"), which covers methods of using Prezista®.

THE PARTIES

2. Plaintiff the United States of America is the government of the United States of America, which acts through its Department of Health and Human Services, National Institutes of Health, located in Bethesda, Maryland.

3. Plaintiff Board of Trustees of the University of Illinois is a body corporate and politic of the State of Illinois, having a place of business in Urbana, Illinois.

4. On information and belief, Defendant Mylan Pharmaceuticals Inc. is a corporation organized and existing under the laws of the State of West Virginia, having a principal place of business at 781 Chestnut Ridge Road, Morgantown, West Virginia, 26504.

5. On information and belief, Lupin Pharmaceuticals, Inc. ("LPI") is a corporation organized and existing under the laws of the State of Virginia, having a principal place of business at Harborplace Tower, 111 South Calvert Street 21st floor Baltimore MD 21202. On information and belief, LPI is a wholly-owned subsidiary of Defendant Lupin Limited.

6. On information and belief, Defendant Lupin Limited is a corporation organized and existing under the laws of India, having a principal place of business at B/4 Laxmi Towers, Bandra Kurla Complex, Bandra (E), Mumbai 400 051, India. On information and belief, Lupin Limited, by itself and through its wholly-owned subsidiary, LPI, is in the business of making and selling generic pharmaceutical products, which it distributes in the State of New Jersey and throughout the United States. Lupin Ltd. has previously submitted to the jurisdiction of this Court, and has availed itself of the jurisdiction of this Court by filing lawsuits and asserting counterclaims in lawsuits filed in the United States Court for the District of New Jersey.

JURISDICTION AND VENUE

7. This Court has subject matter jurisdiction over this action, pursuant to 28 U.S.C. §§ 1331 and 1338(a).

8. This Court has personal jurisdiction over Mylan by virtue of, *inter alia*, its presence in New Jersey, having conducted business in New Jersey, having availed itself of the rights and benefits of New Jersey law, previously consenting to personal jurisdiction in this Court, availing itself of the jurisdiction of this Court, and having engaged in systematic and continuous contacts with the State of New Jersey.

9. This Court has personal jurisdiction over Lupin by virtue of, *inter alia*, its having conducted business in New Jersey, having availed itself of the rights and benefits of New Jersey law, previously consenting to personal jurisdiction in this Court, availing itself of the jurisdiction of this Court, and having engaged in systematic and continuous contacts with the State of New Jersey.

10. Venue is proper in this District pursuant to 28 U.S.C. §§1391 and 1400(b).

THE PATENT-IN-SUIT

11. On December 30, 2008, the United States Patent and Trademark Office issued the '506 patent, entitled "Fitness Assay and Associated Methods." At the time of its issue, the '506 patent was assigned to the Plaintiffs, and the Plaintiffs currently hold title to the '506 patent. A copy of the '506 patent is attached hereto as Exhibit A.

12. As authorized by a license agreement with the University of Illinois, the government granted a non-exclusive license of the '506 patent to Tibotec Pharmaceuticals, (formerly known as Tibotec Pharmaceuticals Ltd.) is an Irish corporation having its principal place of business as Eastgate Village, Eastgate, Little Island, County Cork, Ireland. ("Tibotec").

PREZISTA®

13. Tibotec holds approved New Drug Application No. 21-976 for Duranavir Ethanolate Tablets, 75 mg, 150 mg, 300 mg, 400 mg, and 600 mg dosage strengths, which are sold by Tibotec under the trade name Prezista®.

14. Pursuant to 21 U.S.C. § 355(b)(1) and attendant FDA regulations, the '506 patent is listed in the FDA publication "Approved Drug Products with Therapeutic Equivalence Evaluations" (the "Orange Book") with respect to Prezista®.

DEFENDANTS' ANDAs

15. On information and belief, Mylan submitted ANDA No. 202-136 to the FDA pursuant to 12 U.S.C. § 355(j), seeking approval to commercially manufacture, use, and market Darunavir Ethanolate Tablets, 75 mg, 150 mg, 300 mg, 400 mg, and 600 mg dosage strengths.

16. On information and belief, Lupin submitted ANDA No. 202-073 to the FDA pursuant to 12 U.S.C. § 355(j), seeking approval to commercially manufacture, use, and market Darunavir Ethanolate Tablets, 400 mg and 600 mg dosage strengths. The Darunavir Ethanolate Tablets described in Mylan's ANDA No. 202-136 and Lupin's ANDA No. 202-073 (collectively, the "Defendants' ANDAs") are herein referred to as "Defendants' Products."

17. The Defendants' ANDAs refer to, and rely upon, the Prezista® NDA and contain data that, according to the Defendants, demonstrate the bioequivalence of the Defendants' Products to Prezista®.

18. The government and the University of Illinois received letters from each of the Defendants, dated October 1, 2010, and attached memoranda (collectively, the "Defendants' Notifications"), stating that the Defendants had included certifications in their respective ANDAs, pursuant to 21 U.S.C. § 355(j)(2)(A)(vii)(IV), that the '506 patent is invalid,

unenforceable, and/or will not be infringed by the commercial manufacture, use, or sale of the Defendants' Products (the Paragraph IV certifications). The Plaintiffs received notice of the Defendants' ANDA on October 1, 2010 and are filing this complaint within the 45 day interval specified by 21 U.S.C. § 355(c)(3)(C).

COUNT ONE: INFRINGEMENT OF THE '506 PATENT

19. Plaintiffs reallege and incorporate by reference the allegations of paragraphs 1-17 of this Complaint.

20. Defendants have infringed the '506 patent, pursuant to 35 U.S.C. § 271(e)(2)(A), by submitting the Defendants' ANDAs, by which the Defendants' seek approval from the FDA to engage in the commercial manufacture, use, offer to sell, sale, or importation of the Defendants' Products prior to the expiration of the '506 patent.

21. Defendants' commercial manufacture, use, offer to sell, or sale of the Defendants' Products within the United States, or importation of the Defendants' Products into the United States, during the term of the '506 patent would further infringe the '506 patent under 35 U.S.C. §§ 271(a), (b), and/or (c).

22. The Plaintiffs will be substantially and irreparably harmed if the Defendants are not enjoined from infringing the '506 patent.

23. The Plaintiffs have no adequate remedy at law.

24. This case is an exceptional one, and Plaintiffs are entitled to an award of attorneys' fees under 35 U.S.C. § 285.

COUNT TWO: INDUCEMENT OF INFRNGEMENT OF THE '506 PATENT

25. Under 35 USC 271(b), "[w]hoever actively induces infringement of a patent shall be liable as an infringer."

26. The proposed generic versions of Prezista as described in ANDA Nos. 202-136 and 202-073, if utilized in treatment according to their proposed indications, will infringe every limitation of at least one claim of the '506 patent.

27. Defendants are thus knowingly, intentionally, and deliberately seeking approval of a product that, if used according to its indications, will infringe the '506 patent.

28. In addition, if ANDA Nos. 202-136 and 202-073 are approved, Defendants will be knowingly, intentionally, deliberately and actively involved in inducing treating physicians, among others, to utilize Defendants' Products in a manner that infringes the '506 patent.

29. Defendants are therefore liable under 35 U.S.C. 271(e)(2) for inducement of infringement of the '506 patent.

PRAYER FOR RELIEF

Wherefore, the government and the University of Illinois pray for a Judgment in their favor and against Defendants Mylan, LPI, and Lupin, and respectfully request the following relief:

A. A Judgment that Defendants have infringed U.S. Patent No. 7,470,506 B1;

B. A Judgment pursuant to 35 U.S.C. § 271(e)(4)(B) preliminarily and permanently enjoining the Defendants, their officers, agents, servants, employees, and those persons in active concert or participation with any of them, from commercially manufacturing, using, offering to sell, or selling the Defendants' Products within the United States, or importing the Defendants' Products into the United States, prior to the expiration of the '506 patent;

C. A Judgment ordering that, pursuant to 35 U.S.C. § 271(e)(4)(A), the effective date of any approval of ANDA Nos. 202-136 and 202-073 under § 505(j) of the Federal Food, Drug

and Cosmetic Act (21 U.S.C. § 355(j)) shall not be any earlier than the expiration date of the '506 patent, including any extensions;

D. If Defendants commercially manufacture, use, offer to sell, or sell the Defendants' Products within the United States, or import the Defendants' Products into the United States, prior to the expiration of the '506 patent, including any extensions, a Judgment awarding Plaintiffs monetary relief together with interest;

E. Attorneys' fees in this action as an exceptional case pursuant to 35 U.S.C. § 285;

F. Costs and expenses in this action; and

G. Such other relief as the Court deems just and proper.

Dated: November 15, 2010

Respectfully submitted,

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LOCAL CIVIL RULE 11.2 CERTIFICATION

I hereby certify that the matter captioned TIBOTEC INC. and TIBOTEC PHARMACEUTICALS v. LUPIN LIMITED, LUPIN PHARMACEUTICALS INC., MYLAN PHARMACEUTICALS INC. and MYLAN INC., is a related patent infringement case because the matter involves all of the same defendants and the same Abbreviated New Drug Application seeking FDA approval to market a generic version of the same drug product, Prezista®.

I further certify that, to the best of my knowledge, the matter in controversy is not the subject of any other action pending in any court, or of any pending arbitration or administrative proceeding.

Dated: November 15, 2010

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EXH A
10-5956 (w HW)

Exhibit A

(12) **United States Patent**
Erickson et al.

(10) **Patent No.: US 7,470,506 B1**
(45) **Date of Patent: Dec. 30, 2008**

(54) **FITNESS ASSAY AND ASSOCIATED METHODS**

(75) Inventors: **John W. Erickson**, Frederick, MD (US); **Sergei V. Gulnik**, Frederick, MD (US); **Hiroaki Mitsuya**, Chevy Chase, MD (US); **Arun K. Ghosh**, River Forest, IL (US)

(73) Assignees: **The United States of America as represented by the Department of Health and Human Services**, Washington, DC (US); **Board of Trustees of the University of Illinois**, Urbana, IL (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/720,276**

(22) PCT Filed: **Jun. 23, 1999**

(86) PCT No.: **PCT/US99/14119**

§ 371 (c)(1),
(2), (4) Date: **Mar. 7, 2001**

(87) PCT Pub. No.: **WO99/67417**

PCT Pub. Date: **Dec. 29, 1999**

Related U.S. Application Data

(60) Provisional application No. 60/090,393, filed on Jun. 23, 1998.

(51) **Int. Cl.**
C12Q 1/70 (2006.01)

(52) **U.S. Cl.** **435/5**

(58) **Field of Classification Search** 435/5; 514/357, 332, 478, 482, 228.2
See application file for complete search history.

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Primary Examiner—Emily M. Le
(74) *Attorney, Agent, or Firm*—Laydig, Voit & Mayer, Ltd.

(57) **ABSTRACT**

The present invention provides an assay for determining the biochemical fitness of a biochemical species in a mutant replicating biological entity relative to its predecessor. The present invention further provides a continuous fluorogenic assay for measuring the anti-HIV protease activity of protease inhibitor. The present invention also provides a method of administering a therapeutic compound that reduces the chances of the emergence of drug resistance in therapy. The present invention also provides a compound of formula (I) or a pharmaceutically acceptable salt, a prodrug, a composition, or an ester thereof, wherein A is a group of formulas (A), (B), (C) or (D); R^1, R^2, R^3, R^5 or R^6 is H, or an optionally substituted and/or heteroatom-bearing alkyl, alkenyl, alkynyl, or cyclic group; Y and/or Z are CH_2, O, S, SO, SO_2, amino, amides, carbamates, ureas, or thiocarbonyl derivatives thereof, optionally substituted with an alkyl, alkenyl, or alkynyl group; n is from 1 to 5; X is a bond, an optionally substituted methylene or ethylene, an amino, O or S; Q is C(O), C(S), or SO_2; m is from 0 to 6; R^4 is OH, =O (keto), NH_2, or alkylamino, including esters, amides, and salts thereof; and W is C(O), C(S), S(O), or SO_2. Optionally, R^5 and R^6, together with the N—W bond of formula (I), comprises a macrocyclic ring.

9 Claims, 5 Drawing Sheets

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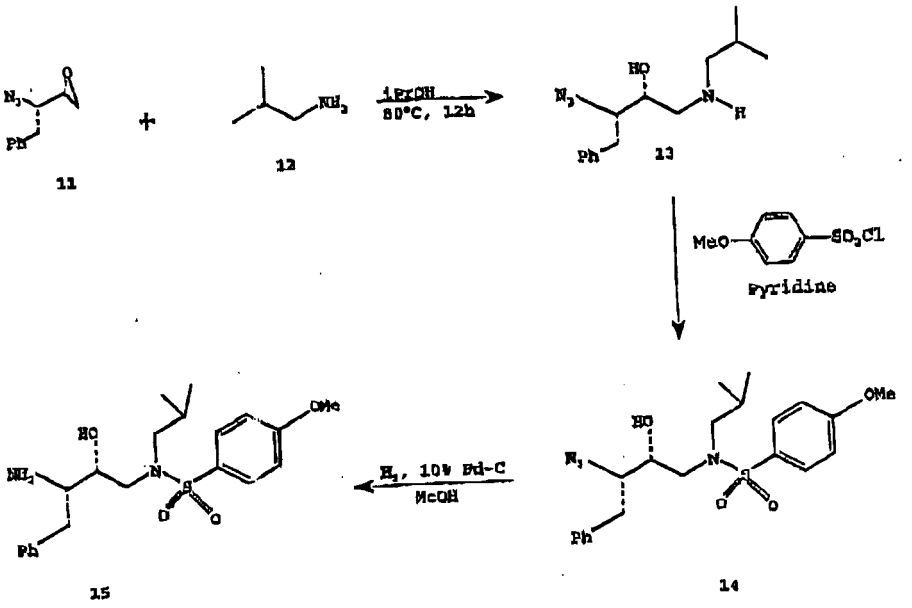

Fig. 1

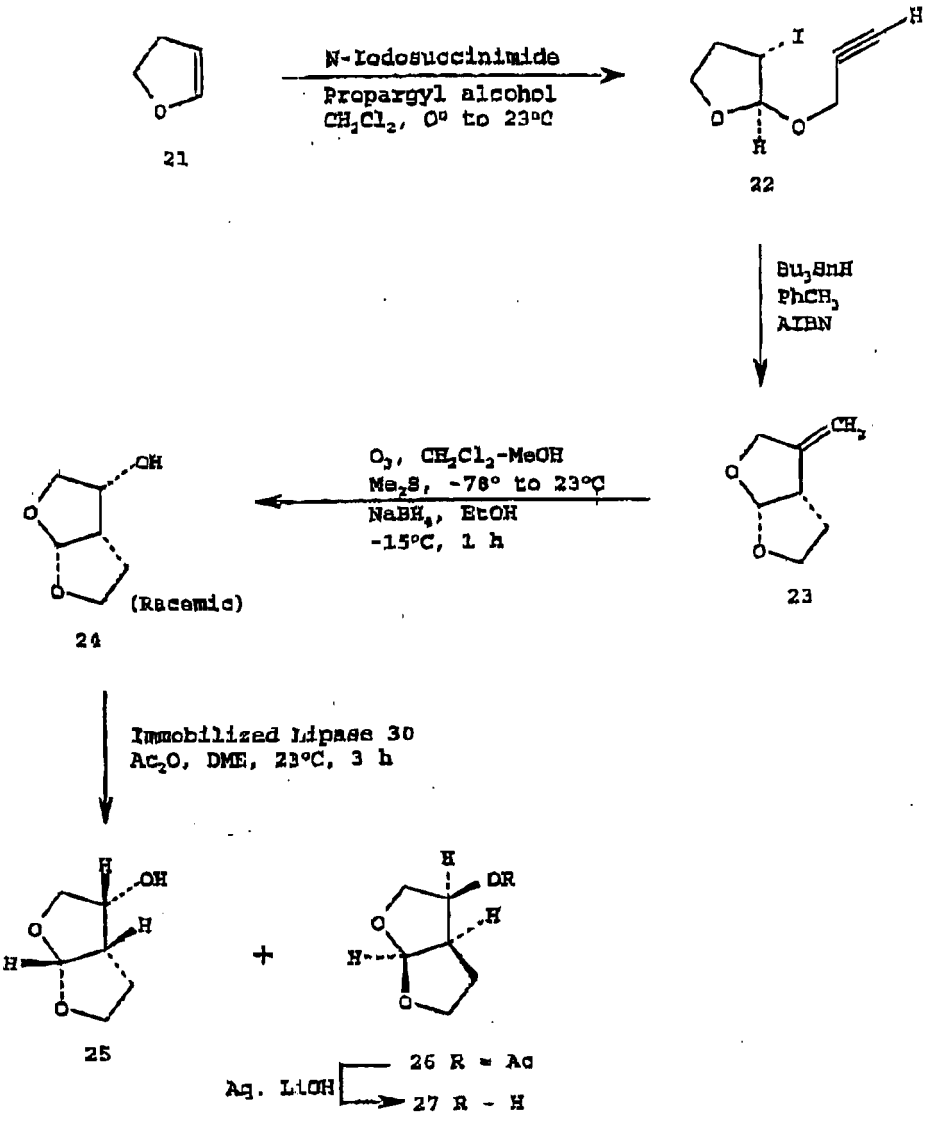

Fig. 2

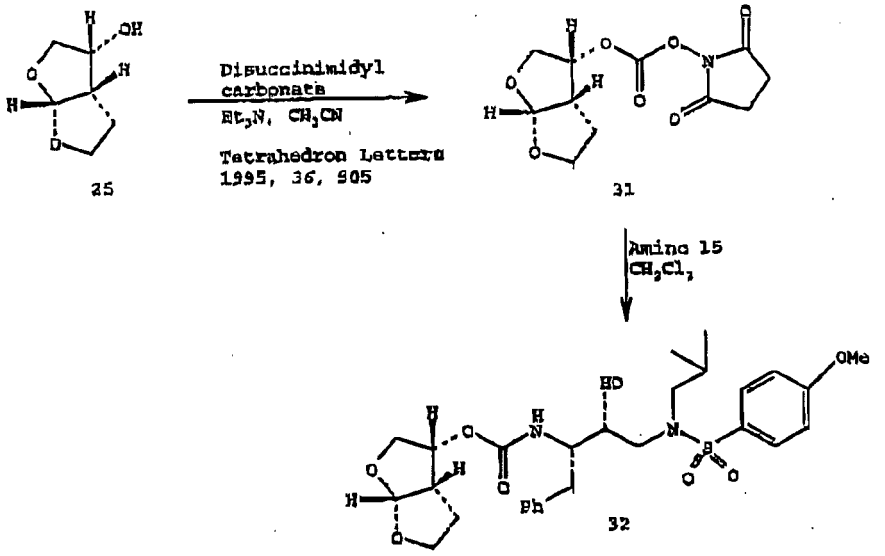

Fig. 3A

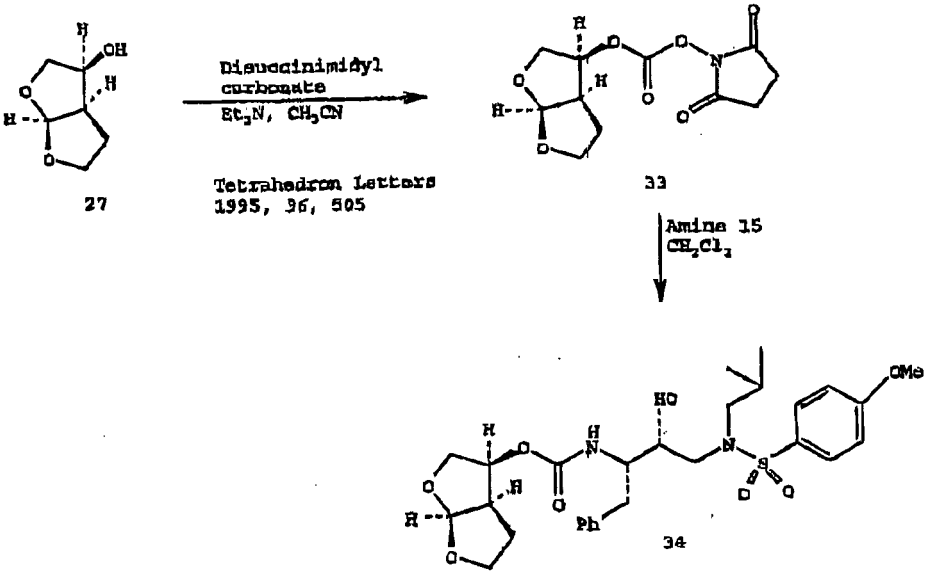

Fig. 3B

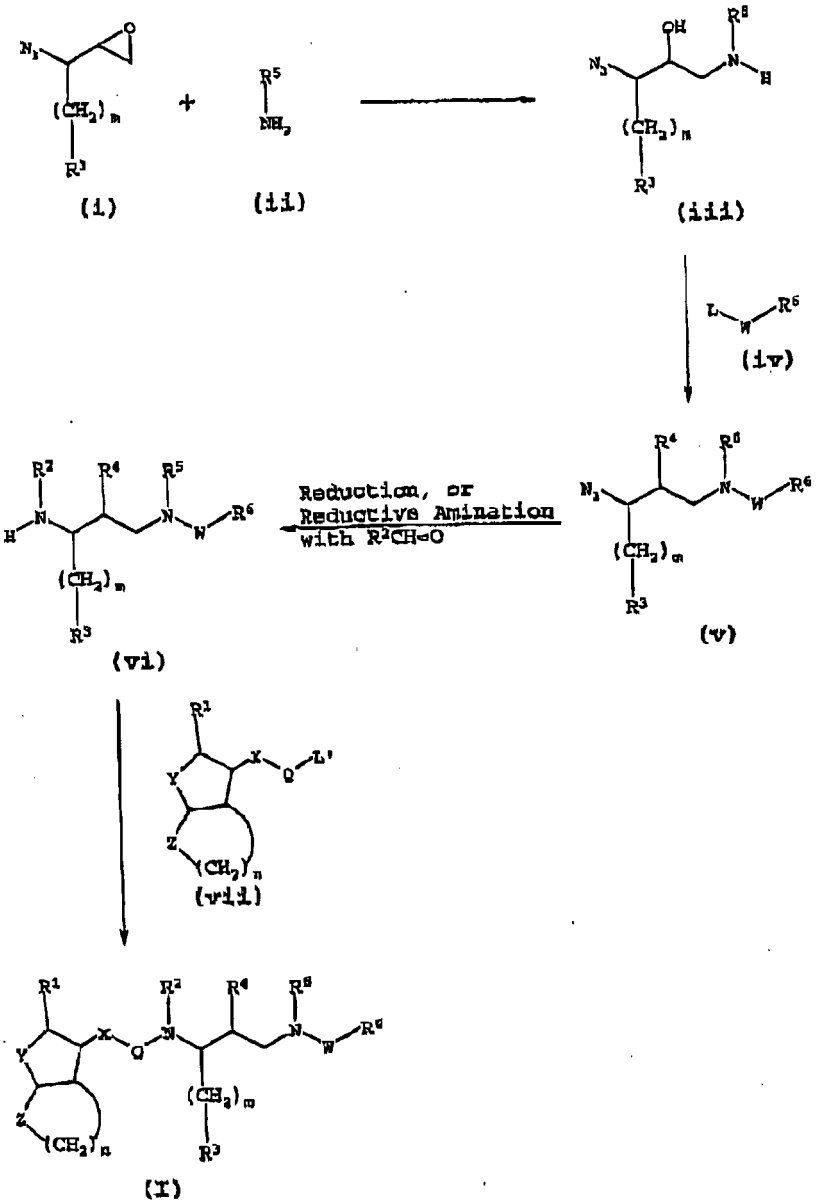

Fig. 4

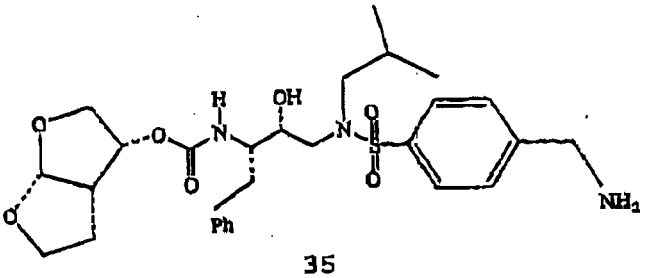

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Fig. 5A

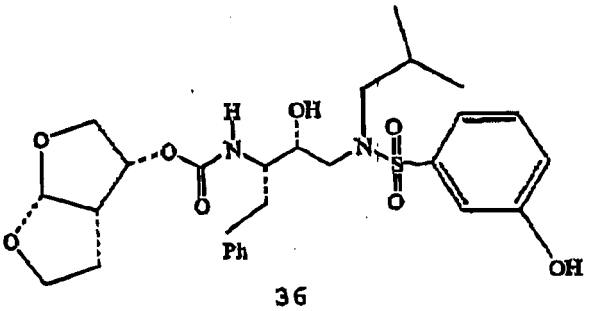

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Fig. 5B

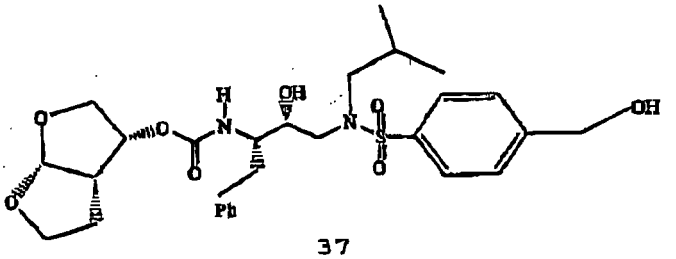

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Fig. 5C

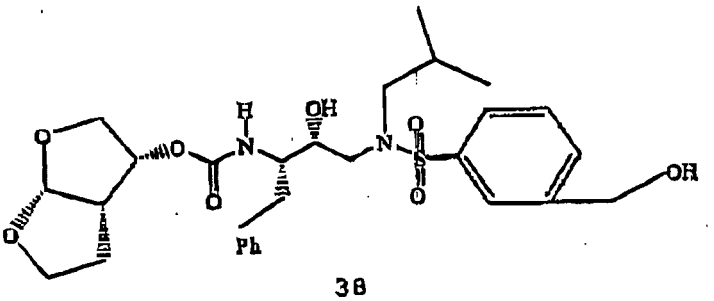

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Fig. 5D

FITNESS ASSAY AND ASSOCIATED METHODS

TECHNICAL FIELD OF THE INVENTION

The present invention relates to a biochemical fitness assay and related methods.

BACKGROUND OF THE INVENTION

The development of drug resistance is one of the most perplexing challenges in the field of medicine. One of the most common causes of drug failure in the treatment of diseases involving replicating biological entities, for example, cancer and infectious diseases, is the emergence of drug resistance. One of the most dramatic and tragic examples of drug resistance can be found in connection with the antiviral therapy of acquired immune deficiency syndrome (AIDS).

AIDS is a fatal disease, reported cases of which have increased dramatically within the past several years. Estimates of reported cases in the very near future also continue to rise dramatically.

The AIDS virus was first identified in 1983. It has been known by several names and acronyms. It is the third known T-lymphocyte virus (HTLV-III), and it has the capacity to replicate within cells of the immune system, causing profound cell destruction. The AIDS virus is a retrovirus, a virus that uses reverse transcriptase during replication. This particular retrovirus is also known as lymphadenopathy-associated virus (LAV), AIDS-related virus (ARV) and, most recently, as human immunodeficiency virus (HIV). Two distinct families of HIV have been described to date, namely HIV-1 and HIV-2. The acronym HIV will be used herein to refer to HIV viruses generically.

Specifically, HIV is known to exert a profound cytopathic effect on the CD4+ helper/inducer T-cells, thereby severely compromising the immune system. HIV infection also results in neurological deterioration and, ultimately, in the death of the infected individual.

The field of viral chemotherapeutics has developed in response to the need for agents effective against retroviruses, in particular HIV. For example anti-retroviral agents, such as 3'-azido-2',3'-dideoxythymidine (AZT), 2'3'-dideoxycytidine (ddC), and 2'3'-dideoxyinosine (ddI) are known to inhibit reverse transcriptase. There also exist antiviral agents that inhibit transactivator protein. Nucleoside analogs, such as AZT, are currently available for antiviral therapy. Although very useful, the utility of AZT and related compounds is limited by toxicity and insufficient therapeutic indices for fully adequate therapy.

Retroviral protease inhibitors also have been identified as a class of anti-retroviral agents. Retroviral proteases processes polyprotein precursors into viral structural proteins and replicative enzymes. This processing is essential for the assembly and maturation of fully infectious virions. Accordingly, the design of protease inhibitors remains an important therapeutic goal in the treatment of AIDS.

The use of HIV protease inhibitors, in combination with agents that have different antiretroviral mechanisms (e.g., AZT, ddI and ddT), also has been described. For example, synergism against HIV-1 has been observed between certain C_2 symmetric HIV inhibitors and AZT (Kageyama et al., *Antimicrob. Agents Chemother.*, 36, 926-933 (1992)).

Numerous classes of potent peptidic inhibitors of proteases have been designed using the natural cleavage site of the precursor polyproteins as a starting point. These inhibitors typically are peptide substrate analogs in which the scissile

P_1-P_1' amide bond has been replaced by a non-hydrolyzable isostere with tetrahedral geometry (Moore et al, *Perspect. Drug Dis. Design*, 1, 85 (1993); Tomasselli et al., *Int. J. Chem. Biotechnology*, 6 (1991); Huff, *J. Med. Chem.*, 34, 2305 (1991); Norbeck et al., *Ann. Reports Med. Chem.*, 26, 141 (1991); and Meek, *J. Enzyme Inhibition*, 6, 65 (1992)). Although these inhibitors are effective in preventing the retroviral protease from functioning, the inhibitors suffer from some distinct disadvantages. Generally, peptidomimetics often make poor drugs, due to their potential adverse pharmacological properties, i.e., poor oral absorption, poor stability and rapid metabolism (Plattner et al, *Drug Discovery Technologies*, Clark et al., eds., Ellish Horwood, Chichester, England (1990)).

The design of the HIV-1 protease inhibitors based on the transition state mimetic concept has led to the generation of a variety of peptide analogs highly active against viral replication in vitro (Erickson et al, *Science*, 249, 527-533 (1990); Kramer et al., *Science*, 231, 1580-1584 (1986); McQuade et al., *Science*, 247, 454-456 (1990); Meek et al., *Nature* (London), 343, 90-92 (1990); and Roberts et al., *Science*, 248, 358-361 (1990)). These active agents contain a non-hydrolyzable, dipeptidic isostere, such as hydroxyethylene (McQuade et al., supra; Meek et al., *Nature* (London), 343, 90-92 (1990); and Vacca et al., *J. Med. Chem.*, 34, 1225-1228 (1991)) or hydroxyethylamine (Ghosh et al., *Bioorg. Med. Chem. Lett.*, 8, 687-690 (1998); Ghosh et al., *J. Med. Chem.*, 36, 292-295 (1993)); Rich et al., *J. Med. Chem.*, 33, 1285-1288 (1990); and Roberts et al., *Science*, 248, 358-361 (1990)) as an active moiety that mimics the putative transition state of the aspartic protease-catalyzed reaction.

Two-fold (C_2) symmetric inhibitors of HIV protease represent another class of potent HIV protease inhibitors, which were created by Erickson et al., on the basis of the three-dimensional symmetry of the enzyme active site (Erickson et al. (1990), supra). Typically, however, the usefulness of currently available HIV protease inhibitors in the treatment of AIDS has been limited by relatively short plasma half-life, poor oral bioavailability, and the technical difficulty of scale-up synthesis (Meek et al. (1992), supra).

In a continuing effort to address the problem of short plasma half-life and poor bioavailability, new HIV protease inhibitors have been identified. For example, HIV protease inhibitors incorporating the 2,5-diamino-3,4-disubstituted-1,6-diphenylhexane isostere are described in Ghosh et al., *Bioorg. Med. Chem. Lett.*, 8, 687-690 (1998) and U.S. Pat. Nos. 5,728,718 (Randad et al.). HIV protease inhibitors, which incorporate the hydroxyethylamine isostere, are described in U.S. Pat. Nos. 5,502,060 (Thompson et al.), 5,703,076 (Talley et al.), and 5,475,027 (Talley et al.).

Recent studies, however, have revealed the emergence of mutant strains of HIV, in which the protease is resistant to the C_2 symmetric inhibitors (Otto et al., *PNAS USA*, 90, 7543 (1993); Ho et al., *J. Virology*, 68, 2016-2020 (1994); and Kaplan et al., *PNAS USA*, 91, 5597-5601 (1994)). In one study, the most abundant mutation found in response to a C_2 symmetry based inhibitor was Arg to Gln at position 8 (R8Q), which strongly affects the S_3/S_3' subsite of the protease binding domain. In this study, the shortening of the P_3/P_3' residues resulted in inhibitors that were equipotent towards both wild-type and R8Q mutant proteases (Majer et al., 13th *American Peptide Symposium*, Edmonton, Canada (1993)). Inhibitors have been truncated to P2/P2' without significant loss of activity (Lyle et al., *J. Med. Chem.*, 34, 1230 (1991)); and Bone et al., *J. Am. Chem. Soc.*, 113, 9382 (1991)). These results suggest that inhibitors can be truncated and yet maintain the crucial interactions necessary for strong binding. The benefits

of such an approach include the elimination of two or more peptide bonds, the reduction of molecular weight, and the diminishment of the potential for recognition by degradative enzymes.

More recently, new mutant strains of HIV have emerged that are resistant to multiple, structurally diverse, experimental and chemotherapeutic retroviral protease inhibitors. Such multidrug-resistant HIV strains are typically found in infected patients, who had undergone treatment with a combination of HIV protease inhibitors or a series of different HIV protease inhibitors. The number of reported cases of patients infected with multidrug-resistant HIV is rising dramatically. Tragically for these patients, the available options for AIDS chemotherapy and/or HIV management is severely limited or is, otherwise, completely nonexistent.

Drug resistance is unfortunately the most common reason for drug failures generally. One of the most dramatic examples of drug failure due to resistance is in HIV therapy. Once HIV resistance is obtained to first-line therapy, the chances of future success are greatly diminished because of the development of multidrug cross resistance. Other diseases involving infectious agents (e.g., viruses, bacteria, protozoa, and prions) or other disease-causing cells (e.g., tumor cells) present similar challenges in that drug resistance is a primary cause of drug failure.

In view of the foregoing problems, there exists a need to determine whether a mutant will be capable of replicating in the presence of a drug. There also exists a need for a method of predicting whether drug resistance is likely to emerge in a disease involving a replicating biological entity. There is also a need for a method of devising a long-term therapeutic regimen that minimizes the likelihood that resistance will occur in a disease involving a replicating biological entity. Moreover, there is a need for a method of preventing or inhibiting the development of drug resistance in such diseases.

The present invention provides such methods. These and other advantages of the present invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

BRIEF SUMMARY OF THE INVENTION

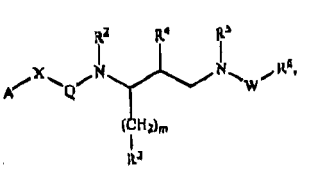
The present invention is predicated on the surprising and unexpected discovery that biochemical "vitality," as described below, can be used to determine the biological fitness of a mutant replicating biological entity relative to its predecessor under the selection pressure of an inhibitor. The present invention provides an assay for determining the biochemical fitness of a biochemical target (i.e., a biomolecule having a biochemical function), of a mutant replicating biological entity relative to its predecessor's biochemical target, in the presence of a compound that acts upon the biochemical target. The assay method of the present invention includes obtaining the predecessor, determining the biochemical vitality of the biochemical target of both the predecessor and the mutant in the presence of a compound that acts upon the biochemical target of the predecessor, and comparing the vitality of the mutant's biochemical target relative to the vitality of the predecessor's biochemical target. Where the biochemical vitality of the mutant is greater than the biochemical fitness of the predecessor, the mutant is predicted to be more biologically fit in the presence of the compound. The assay method can thus be used to predict the emergence of drug resistance for a particular replicating biological entity (e.g., a disease-causing cell) in the presence a drug (e.g., an inhibitor). Utilization of the assay in accordance with the present invention permits the administration of an inhibitor or combination of inhibitors to treat a disease in a way that decreases the likelihood that drug resistance will develop.

The present invention further provides a continuous fluorogenic assay for measuring the anti-HIV protease activity of a protease inhibitor. The continuous fluorogenic assay of the present invention utilizes a substrate of the formula Ala-Arg-Val-Tyr-Phe(NO_2)-Glu-Ala-Nle-NH_2. The continuous fluorogenic assay of the present invention is highly sensitive and particularly useful for the prediction of the antiviral inhibitory activity of a compound against mutant HIV.

The present invention further provides a method of administering a therapeutic compound that inhibits a biochemical target of a disease-causing replicating biological entity. The therapeutic compound, when administered in accordance with the method of the present invention, minimizes the chances that the disease-causing entity will develop drug resistance. As such, the method of administering a therapeutic compound in accordance with the present invention improves the chances of long-term success in therapy.

The present method of administering a therapeutic compound involves the identification of at least one mutant replicating biological entity (the mutant) capable of evolving from the disease-causing replicating biological entity (the predecessor). Biochemical fitness is determined by comparing the biochemical vitality of the mutant's biochemical target with the biochemical vitality of the predecessor's biochemical target. Biochemical fitness is determined in the presence of a drug (e.g, an inhibitor). The biochemical vitality of the mutant's biochemical target is compared to biochemical vitality of the predecessor's biochemical target in the presence of the drug. When there are two or more drugs available for treatment, biochemical fitness can be determined for each drug in accordance with the present invention. A therapeutic compound is then administered from among one of the compounds that produces a lower value for biochemical fitness with respect to one or more mutants. Administration of a therapeutic compound producing a lower fitness value for a particular mutant indicates that the predecessor is less likely to develop resistance in the presence of that compound.

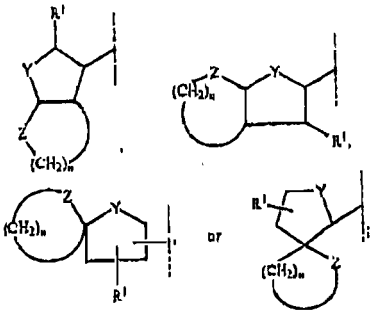
The present invention also provides a method of preventing the development of drug resistance of HIV in an HIV-infected mammal by the administration of a drug resistance-inhibiting effective amount of a compound of the formula:



(I)

or a pharmaceutically acceptable salt, a prodrug, or an ester thereof, or a pharmaceutical composition thereof, wherein:

A is a group of the formula:



R^1 is H or an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a cycloalkylalkyl, an aryl, an aralkyl, a heterocycloalkyl, a heterocycloalkylalkyl, a heteroaryl, or a heteroaralkyl radical, which unsubstituted or substituted;

Y and Z are the same or different and are each selected from the group consisting of CH_2, O, S, SO, SO_2, NR^9, $R^9C(O)N$, $R^9C(S)N$, $R^9OC(O)N$, $R^9OC(S)N$, $R^9SC(O)N$, $R^9R^9NC(O)N$, and $R^9R^2NC(S)N$, wherein R^9 and R^2 are each H, an alkyl, an alkenyl, or an alkynyl;

n is an integer from 1 to 5;

X is a covalent bond, CHR^{10}, $CHR^{10}CH_2$, CH_2CHR^{10}, O, NR^{10}, or S, wherein R^{10} is H, an alkyl, an alkenyl, or an alkynyl;

Q is C(O), C(S), or SO_2;

R^2 is H, an alkyl, an alkenyl, or an alkynyl;

m is an integer from 0 to 6;

R^3 is a cycloalkyl, a heterocycloalkyl, an aryl, or a heteroaryl which is unsubstituted or substituted;

R^4 is OH, =O (keto), NH_2, or a derivative thereof;

R^5 is H, a C_1-C_6 alkyl radical, a C_2-C_6 alkenyl radical, or $(CH_2)_qR^{14}$, wherein q is an integer form 0 to 5, and R^{14} is a cycloalkyl, a heterocycloalkyl, an aryl, or a heteroaryl which is unsubstituted or substituted;

W is C(O), C(S), S(O), or SO_2; and

R^9 is a cycloalkyl, heterocycloalkyl, aryl, or heteroaryl which is unsubstituted or substituted.

Optionally, R^5 and R^6, together with the N—W bond of formula (I), comprise a macrocycle ring which can contain at least one additional heteroatom in the ring skeleton.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates the synthesis of a particular sulfonamide isostere core of a compound of the present invention.

FIG. 2 illustrates the synthesis of a bis-tetrahydrofuran ligand and the optical resolution thereof.

FIG. 3A illustrates the synthesis of a compound of the present invention via coupling of a bis-tetrahydrofuran ligand to a sulfonamide isostere of the present invention.

FIG. 3B illustrates the synthesis of a compound of the present invention via coupling of a bis-tetrahydrofuran ligand to a sulfonamide isostere of the present invention.

FIG. 4 illustrates generally the present method of synthesizing a compound of the present invention.

FIGS. 5A-5D illustrate the structures of particular compounds that were tested against various drug resistant HIV mutants.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is predicated on the surprising and unexpected discovery to that the "vitality" of a biochemical target of a mutant replicating biological entity relative to that of its predecessor's biochemical target can be used to predict the biological fitness of the mutant under the selection pressure of an inhibitor of the biochemical target. The "vitality" of a biochemical target of a mutant replicating biological entity relative to the "vitality" of its predecessor's biochemical target is defined herein as the "biochemical fitness."

"Vitality" as utilized herein describes the ability of a particular biomolecular "target" (i.e., a biochemical species intended to be inhibited by a particular inhibitor) to perform its biochemical function in the presence of the inhibitor. Biochemical vitality is a function of at least two variables: the ability of a particular inhibitor to inhibit a biochemical target of the replicating biological entity in question, and the ability of the cell's biochemical target to inherently perform its biochemical function (irrespective of an inhibitor). Biochemical vitality also can include other factors that effect the ability of a biochemical target to perform its biochemical function in the presence of the inhibitor.

The biochemical target in question can include, for example, a biochemical species with one or more known or unknown biological functions. The biochemical target can be, for example, a biochemical species having one or more specific biochemical function, or it can be a biochemical species that effects or influences a biochemical function directly or indirectly. Suitable biochemical targets include, for example, enzymes, proteins, oligomers, receptors, and the like. Suitable enzymes include, for example, reverse transcriptases, proteases (e.g., retroviral proteases, plasmepsins, and the like), methylases, oxidases, esterases, acyl transferases, and the like. Suitable enzymes also include, for example, viral and non-viral helicases, topoisomerases, DNA gyrases, DNA and RNA polymerases, parasite-encoded proteases, and the like.

Suitable proteins include, for example, proteins that incorporate a conformational change as a major functional requirement, and the like. Examples of such proteins include HIV gp41 and other fusogenic viral proteins and peptides, topoisomerases, and all DNA enzymes, and the like.

Suitable oligomers include, for example, oligomers that require oligomerization in order to perform their biochemical function. Examples of such oligomers include HIV protease, retroviral fusion proteins, peptides, HIV gp 41, viral and non-viral membrane fusion proteins, tumor suppressor proteins (e.g., p53, and the like) prions, ribosomes, and the like.

The ability of a particular inhibitor to inhibit a biochemical target of a particular replicating biological entity can be determined by any suitable method and/or can be obtained from any suitable source. The ability of a particular inhibitor to inhibit a biochemical function of a replicating biological entity can be determined, for example, on the basis of a measurable property, or a measurable relationship of properties, that correlate with the ability of the inhibitor to inhibit the target. Suitable methods for determining the ability of the inhibitor to inhibit the target include, for example, assays, and the like. In some instances, the ability of the inhibitor to inhibit the target can be obtained from one or more suitable sources, for example, assay data from a database, a textbook, or the literature.

When the biochemical target is a protein, the ability of an inhibitor to inhibit the protein can be determined, for example, by obtaining the equilibrium dissociation constant

(K_d) of drug binding to the target where drug binding interferes with the function of the protein.

When the biochemical target is an enzyme, the ability of an inhibitor to inhibit the enzyme can be determined, for example, by obtaining the inhibition constant (K_{inh}), or the like. The inhibition constant can be in terms of drug inhibition constant for the effect of the drug on substrate catalysis (e.g., K_i) or dissociation constant for drug binding (e.g., K_d) where drug binding correlates with inhibition of enzyme function.

When the biochemical target is an oligomer, the ability of an inhibitor to inhibit the oligomer can be determined, for example, by obtaining the equilibrium dissociation constant (K_d) for drug binding where drug binding interferes with oligomerization of the target.

Where the biochemical target is a protein that requires a conformational change for its function, the ability of an inhibitor to inhibit the conformational change can be determined, for example, by obtaining the equilibrium dissociation constant (K_d) for drug binding where drug binding interferes with the conformational change of the target.

When the biochemical target is a protein that is required to bind to a ligand, macromolecule, or macromolecular complex to perform its biochemical function, the ability of an inhibitor to inhibit the protein function can be determined by obtaining the equilibrium dissociation constant (K_d) for drug binding where drug binding interferes with ligand binding, macromolecule binding, or macromolecular complex binding.

When the biochemical target is a nucleic acid binding protein, the ability of an inhibitor to inhibit the nucleic acid binding protein's function can be determined by obtaining the equilibrium dissociation constant (K_d) for drug binding where drug binding interferes with nucleic acid binding.

Vitality also is a function of the biochemical target's ability to inherently perform its biochemical function (irrespective of an inhibitor). The biochemical target's ability to inherently perform its biochemical function can be determined by any suitable method and/or can be obtained from any suitable source. The biochemical target's ability to inherently perform its biochemical function can be determined, for example, on the basis of a measurable property, or measurable relationship of properties, that correlate with the ability of the biochemical target's ability to inherently perform its biochemical function. Suitable methods for determining the biochemical target's ability to inherently perform its biochemical function include, for example, biochemical assays, and the like. In some instances, the ability of a cell's biochemical target to inherently perform its biochemical function can be obtained from one or more suitable sources, for example, assay data from a database, a textbook, or the literature.

When the biochemical target is an enzyme, the ability of the enzyme to inherently perform its biochemical function can be determined, for example, by determining the catalytic efficiency of the enzyme. For example, the catalytic efficiency for enzymes that exhibit Michaelis-Menton kinetics can be determined by obtaining the k_{cat}/K_M ratio, or by a similar method, wherein k_{cat} is the catalytic rate and K_M is the Michaelis constant.

When the biochemical target is a protein, the ability of the protein to inherently perform its biochemical function can be determined, for example, by obtaining the equilibrium constant (K_{eq}) for the biochemical function of the protein, or the like.

When the biochemical target is an oligomer, the ability of an inhibitor to perform its biological function can be determined, for example, by obtaining the equilibrium constant (K_{eq}) that is associated with oligomerization.

Where the biochemical target is a protein that requires a conformational change for its function, the ability of the target to perform its function can be determined, for example, by obtaining the equilibrium constant (K_{eq}) associated with conformational change.

When the biochemical target is a protein that is required to bind to a ligand to perform its function, the ability of the target to perform its function can be determined, for example, by obtaining the equilibrium dissociation constant (K_d) for ligand binding.

When the biochemical target is a nucleic acid binding protein, the ability of an inhibitor to perform its function can be determined by obtaining the equilibrium dissociation constant (K_d) for nucleic acid binding.

It will be appreciated that vitality also can be a function of other factors that effect the ability of a biochemical target to perform its biochemical function in the presence of the inhibitor. If the biochemical target is a dimeric species, for example, other factors that influence biochemical vitality might include the ability of the species to dimerize in the presence and/or in the absence of the inhibitor. If, by way of example, a mutation causes the dimerization rate to become a factor in the biochemical function of the biochemical target of the mutant relative to its predecessor's, then dimerization rate can be included in the vitality determination.

The biochemical vitalities of a mutant replicating biological entity and its predecessor, when compared, describes the biochemical fitness of the target of the mutant cell. In keeping with the invention, it has been found that the biochemical fitness relates to the biological fitness of the mutant in the presence of the inhibitor. When the value for the biochemical vitality of the target of the mutant exceeds the value for the biochemical vitality of the target of a predecessor of the mutant, the target of the mutant has greater biochemical fitness in the presence of the inhibitor. In such cases, the mutant replicating biological entity is favored over the predecessor and resistance to the inhibitor that is used to treat the predecessor is likely to develop.

Biochemical vitality can be determined in many different ways that suitably relate the various factors relating to the biochemical vitality of the target. For example, a mathematical function may be used to relate the various factors. By way of illustration, when the biochemical target is an enzyme, the vitality can be determined as a function of K_{inh} (e.g., K_i or K_d) and enzymatic or catalytic efficiency (e.g., K_{cat}/K_M) vitality can be determined as the product of K_{inh} and enzymatic efficiency, for example, (K_{inh})×(catalytic efficiency), or (K_i)×(catalytic efficiency) or (K_d) (catalytic efficiency). Alternatively, vitality can be determined, for example, as the log of the product of K_{inh} and enzymatic efficiency, for example, log [(K_{inh})×(catalytic efficiency)], or log [(K_i)×(catalytic efficiency)] or log [(K_d)×(catalytic efficiency)]. Similarly, for enzymes that exhibit Michaelis-Menton kinetics, vitality can be determined as a function of K_{inh} (e.g., K_i or K_d) and the k_{cat}/K_M ratio. For example, vitality can be determined as the product of K_{inh} and k_{cat}/K_M, e.g., (K_{inh})×(k_{cat}/K_M), wherein K_{inh} is K_i or K_d. Alternatively, vitality can be determined, for example, as the log of the product of K_{inh} and k_{cat}/K_M, e.g., log [(K_{inh})×(k_{cat}/K_M)], wherein K_{inh} is K_i or K_d. In a preferred embodiment, the biochemical target is an enzyme and the vitality is (K_i)×(k_{cat}/K_M), or log [(K_i)×(k_{cat}/K_M)].

"Fitness," unless otherwise indicated, means biochemical fitness. "Biochemical fitness" as utilized herein is a value that represents the vitality of a biochemical target of a mutant replicating biological entity relative to the vitality the biochemical target of its predecessor. Biochemical fitness is determined by comparing the vitality of a biochemical target

of a mutant replicating biological entity relative to that of its predecessor. Any suitable comparison of the vitality of a biochemical target of a mutant replicating biological entity relative to that of its predecessor can be used in the determination of fitness. For example, biochemical fitness can be determined as the difference between the biochemical vitality of a biochemical target of a predecessor (biochemical vitality$_{pred}$) and the biochemical vitality of the biochemical target of a particular mutant replicating biological entity that can evolve from the predecessor (biochemical vitality$_{mut}$), e.g., (biochemical vitality$_{mut}$)−(biochemical vitality$_{pred}$). If biochemical fitness is determined on the basis of this difference, then a positive value indicates that the mutant has a higher fitness relative to its predecessor in the presence of the inhibitor, whereas a negative value indicates that the mutant is less fit relative to its predecessor. A value of zero indicates that the fitness of the mutant and the predecessor are equal. A higher positive value indicates a greater chance that resistance to the inhibitor will emerge, whereas a higher negative value indicates a lower chance that resistance to the inhibitor will emerge.

Alternatively, and preferably, fitness can be determined as the quotient of two biochemical vitalities, for example, as the quotient of a biochemical target of a particular mutant replicating biological entity and the biochemical vitality of the biochemical target of a predecessor, e.g.,

$$\text{fitness} = \frac{\text{vitality}_{mut}}{\text{vitality}_{pred}}.$$

If fitness is determined on the basis of this quotient, then a value greater than one indicates that the mutant has a higher fitness relative to its predecessor, in the presence of the inhibitor. A value of one indicates that the fitness of the mutant and the predecessor are equal. A value less than one indicates that the mutant is less fit relative to its predecessor. A higher value indicates a greater chance that resistance to the inhibitor/drug will emerge, whereas a lower value indicates a lower chance that resistance to the inhibitor/drug will emerge. A value less than one indicates that the mutant will not emerge in the presence of the inhibitor/drug.

Alternatively, fitness can be determined as the log of the quotient of two biochemical vitalities, for example, as the log of the quotient of a biochemical target of a particular mutant replicating biological entity and the biochemical vitality of the biochemical target of a predecessor, e.g.,

$$\text{fitness} = \log\left[\frac{\text{vitality}_{mut}}{\text{vitality}_{pred}}\right].$$

If fitness is determined on the basis of this log, then a value greater than zero indicates that the mutant has a higher fitness relative to its predecessor, in the presence of the inhibitor. A negative value indicates that the mutant is less fit relative to its predecessor. A value of zero indicates that the fitness of the mutant and the predecessor are equal. A higher positive value indicates a greater chance that resistance to the inhibitor/drug will emerge, whereas a lower positive value indicates a lower chance that resistance to the inhibitor/drug will emerge. A negative value indicates that the mutant will not emerge in the presence of the inhibitor/drug.

Fitness can be determined in the presence of any suitable compound that inhibits a biochemical target from performing its biological function. The inhibitor, for example, can be a compound that inhibits an enzyme. Suitable enzyme inhibitors include, for example, protease inhibitors, reverse transcriptase inhibitors, DNA polymerase inhibitors, methylase inhibitors, oxidase inhibitors, esterase inhibitors, acyl transferase inhibitors, and the like.

Suitable protease inhibitors include, for example, viral protease inhibitors, plasmepsin inhibitors, and cathepsin D inhibitors. In a preferred embodiment, the inhibitor is a viral protease inhibitor, more preferably a retroviral protease inhibitor, still more preferably an HIV-1 or an HIV-2 protease inhibitor, and most preferably and HIV-1 protease inhibitor. Exemplary HIV-1 protease inhibitors include, for example, saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, and HIV-1 protease inhibitors that are undergoing clinical trials, e.g., tipranavir (PNU-140690).

Suitable plasmepsin inhibitors include, for example, inhibitors of plasmepsin I or II, including inhibitors of plasmepsin I or II that have antimalarial activity. Suitable inhibitors of cathepsin D include, for example, cathepsin D inhibitors that inhibit cathepsin D in primary breast cancer tissues, including cathepsin D inhibitors that inhibit cathepsin D in primary breast cancer tissues and would be expected to lower the risk of metastasis and/or shorter relapse-free survival in breast cancer patients. See, e.g., Gulnik et al., *J. Mol. Biol.*, 227, 265-270 (1992).

Suitable reverse transcriptase inhibitors include, for example, retroviral reverse transcriptase inhibitors, e.g., AZT, 3TC, ddI, ddC, D4T, and the like.

Suitable protein inhibitors include, for example, compounds that inhibit a conformational change in a protein, and the like. Suitable oligomerization inhibitors include, for example, T-20 peptide inhibitor of HIV-1 fusion and other compounds that inhibit oligomers from oligomerizing on a cell surface or within a cell membrane.

In accordance with the present invention, fitness in the presence of an inhibitor can be determined for a biological entity that produces or includes a biological target of the inhibitor. The biological entity is preferably a replicating biological entity, for example, a virus, a parasite, or a cell, preferably a disease-causing cell. Disease-causing replicating biological entities include, for example, tumor cells, cancer cells, and infectious organisms (e.g., fungi, protozoa, bacteria, and the like) and prions.

Cancer cells include, for example, cells associated with breast cancer, colon cancer, lung cancer, and the like. Fitness can be determined for a rapidly growing tumor cell.

Fungi include, for example, *candida albicans*, and the like. Protozoa include, for example, trypanosome species, schistosomal species, malarial protozoa, e.g., *Plasmodium* species. *Plasmodium* species include, for example, *Plasmodium Falciparum*, *Plasmodium ovale*, *Plasmodium vivax*, *Plasmodium malariae*, and the like. Bacteria include, for example, *Helicobacter pylori*, *Escherichia coli*, *Salmonella*, *Streptococcus pyogenes*, *Staphylococcus aureas*, *Bacillus anthrax*, *Mycobacterium tuberculosis*, *Hemophilus influenza*, and the like. Viruses include, for example, retroviruses (e.g., HIV-1 and HIV-2), herpes viruses, cytomegaloviruses, influenza viruses, epstein-barr virus (EBV), Kaposi's sarcoma herpes virus (KSHV), varicella-zoster virus (VZV), human papillomavirus (HPV), echovirus, picornaviruses, rhinoviruses, poliovirus, coxsackie virus, measles, mumps, human T-cell leukemia virus (HTLV-1), rubella, rotaviruses, yellow fever virus, ebola virus, and other pathogenic viruses, and the like.

Replicating biological entities also include multicellular organisms, for example, infectious microorganisms, e.g., helminths. Helminths include, for example, hookworms (e.g.,

ancylostoma duodenale) strongyloides stercoralis, fasciola hepatica, *trichuris trichiura*, *trichinella spiralis*, *taenia solium*, *taenia saginata*, and the like.

It is believed that drug resistance is the evolutionary result of fitness-based selection of mutant cells/microorganisms in the presence of a drug (or any compound that has biological activity). In accordance with the present invention, the emergence (or non-emergence) of drug resistance in a disease caused by a disease-causing replicating biological entity can be predicted by determining the fitness of a biochemical target of a mutant in the presence of the drug. Thus, the emergence (or non-emergence) of drug resistance can be predicted on the basis of biochemical fitness. While resistance profiles may, in some instances, reflect fitness, it cannot be assumed that the emergence of drug resistance for a particular mutant can be directly predicted on the basis of its resistance profile alone.

The present invention thus provides an assay that can be used to predict the biological fitness of a replicating biological entity in the presence of a particular inhibitor. In a preferred embodiment, an assay is provided for determining the biochemical fitness of a biochemical target of a mutant replicating biological entity relative to its predecessor. In accordance with the assay of the present invention, a predecessor to the mutant is obtained, the biochemical vitality of the biochemical target of the predecessor in the presence of a compound capable of inhibiting the biochemical target of the predecessor is determined, the biochemical vitality of the biochemical target of the mutant in the presence of the compound is determined, and the biochemical vitality of the biochemical target of the mutant relative to the biochemical vitality of the biochemical target of the predecessor are compared.

The assay can be used with a wide variety of infectious microorganisms, as described above, including, for example, a virus, a fungus, a protozoa, or bacterium, a retrovirus, including HIV-1 or HIV-2, and cancer cells. When the infectious microorganism is a protozoa, it is preferably a malarial parasite, which is more preferably a *plasmodium* species.

In another embodiment, the predecessor is a cancer cell, which is preferably a rapidly growing tumor cell, for example, a rapidly growing cancer cell found in breast cancer, colon cancer, lung cancer, a tumor cell of a lymphoid origin, a tumor-derived cell with a high metastatic potential, or the like.

The assay of the present invention can be applied to any suitable biochemical target, preferably a biochemical target whose biochemical vitality can be determined using measurable properties that can be obtained by assay. Desirably, the biochemical target is one that plays an important role in the replication and growth of the entity. By way of example, the biochemical target of the predecessor (and the mutant) can be an enzyme and the compound can be an inhibitor of the enzyme of the predecessor.

The enzyme can be a viral enzyme. Illustrative of viral enzymes are a viral protease enzyme, a viral reverse transcriptase, a viral integrase, a viral polymerase, a viral protein with enzymatic activity, or a retroviral enzyme, including an HIV-1 or an HIV-2 enzyme. Viral protease enzymes, include a retroviral protease, such as an HIV-1 protease or an HIV-2 protease. Viral integrase enzymes include, for example, HIV-1 integrase, HIV-2 integrase, and the like. Viral polymerase can be a retroviral polymerase, including an HIV-1 polymerase or an HIV-2 polymerase. A viral protein with enzymatic activity can be a retroviral protein, such as an HIV-1 protein or an HIV-2 protein.

The enzyme also can be a protozoal enzyme, including a protozoal protease enzyme. The protozoal protease can be a malarial protease. The malarial protease can be a plasmepsin, including plasmepsin I or plasmepsin II. The malarial enzyme can also be a plasmodial enzyme or a protein with enzymatic activity.

In yet another embodiment, the biochemical target of the predecessor is an oligomer and the compound inhibits the oligomerization of the oligomer of the predecessor. In yet another embodiment, the biochemical target of the predecessor is a protein and the compound inhibits a conformational change in the protein of the predecessor.

The biochemical vitality determination can also take into account other factors, preferably measurable factors, that affect the ability of a biochemical target to perform its biochemical function in the presence of the inhibitor. When the biochemical target is an enzyme and the compound is an enzyme inhibitor, the biochemical vitality of the enzyme of the mutant replicating biological entity preferably corresponds to $K_{inh\text{-}mut}$, $k_{cat\text{-}mut}$, $K_{M\text{-}mut}$, and the biochemical vitality of the enzyme of the predecessor preferably corresponds to $K_{inh\text{-}prod}$, $k_{cat\text{-}prod}$, and $K_{M\text{-}prod}$. K_{inh} is an inhibition constant of the compound, k_{cat} is the biochemical catalytic rate, and K_M is the Michaelis constant. More preferably, the vitality of the enzyme corresponds to K_{inh}, k_{cat}, and K_M, and the biochemical vitality of the enzyme of the mutant replicating biological entity is defined by the relationship $K_{inh\text{-}mut}(k_{cat\text{-}mut}/K_{M\text{-}mut})$ (i.e., $(K_{inh\text{-}mut})\times(K_{cat\text{-}mut}/K_{M\text{-}mut})$) and the biochemical vitality of the enzyme of the predecessor is defined by the relationship $K_{inh\text{-}prod}(k_{cat\text{-}prod}/K_{M\text{-}prod})$. The variables $K_{inh\text{-}mut}$, $K_{inh\text{-}prod}$, $k_{cat\text{-}mut}$, $k_{cat\text{-}prod}$, $K_{M\text{-}mut}$, and $K_{M\text{-}prod}$ can be obtained by any suitable means, and are preferably obtained by measurement (e.g., from an assay). When vitality is determined on the basis of these relationships, biochemical fitness in the presence of a given inhibitor/drug preferably is defined by the equation:

$$\frac{K_{inh\text{-}mut}(k_{cat\text{-}mut}/K_{M\text{-}mut})}{K_{inh\text{-}prod}(k_{cat\text{-}prod}/K_{M\text{-}prod})}, \text{ or } \log\left[\frac{K_{inh\text{-}mut}(k_{cat\text{-}mut}/K_{M\text{-}mut})}{K_{inh\text{-}prod}(k_{cat\text{-}prod}/K_{M\text{-}prod})}\right].$$

K_{inh} can be determined by any suitable means, but typically is determined on the basis of K_i or K_d.

The present invention also provides a method of administering a therapeutic compound, which method increases the chances of successful long-term therapy. In a preferred embodiment, the present invention provides a method of administering a therapeutic compound that inhibits a biochemical target of a replicating disease-causing replicating biological entity (disease causing predecessor), including identifying at least one mutant capable of evolving from the disease-causing predecessor. A first biochemical vitality of the biochemical target of the disease-causing predecessor in the presence of a first compound capable of inhibiting the biochemical target of the disease-causing predecessor, and a first biochemical vitality of the biochemical target of the mutant in the presence of the first compound, are determined.

Additional biochemical vitalities of the biochemical target of the disease-causing replicating biological entity in the presence of additional compounds capable of inhibiting the biochemical target of the disease-causing cell, and additional biochemical vitalities of the biochemical target of the mutant in the presence of the additional compounds, are also determined.

Fitnesses in the presence of different inhibitors/drugs can be compared and a therapeutic compound administered on

the basis of the comparison. A first biochemical fitness of the biochemical target of the mutant relative to the disease-causing predecessor is determined by comparing the first biochemical vitality of the biochemical target of the mutant with the first biochemical vitality of the biochemical target of the disease-causing predecessor, and a second biochemical fitness of the biochemical target of the mutant relative to the disease-causing replicating biological entity is determined by comparing the second biochemical vitality of the biochemical target of the mutant with the second biochemical vitality of the biochemical target of the disease-causing replicating biological entity. Additional biochemical fitness determinations can be made in the presence of additional compounds. The biochemical fitness values for one or more mutants in the presence of each compound are compared. A therapeutic compound is then administered from among the first and the additional compound(s), which therapeutic compound produces the lowest biochemical fitness values.

In accordance with the method of the present invention, the replicating disease-causing replicating biological entity is less likely to develop resistance in the presence of the therapeutic compound. The therapeutic compound can be administered from among any particular set of compounds, which can have the same biochemical target or different biochemical targets with respect to each other. The method of administering a compound in accordance with the present invention is, therefore, not limited to comparing fitness in the presence of compounds that act on the same biochemical target.

In one embodiment, the disease-causing replicating biological entity is an infectious microorganism, for example, a virus, a fungus, a protozoa, or a bacterium, more preferably a virus or a protozoa. When the infectious microorganism is a virus, it is preferably a retrovirus, which is more preferably HIV-1 or HIV-2, and most preferably HIV-1. When the infectious microorganism is a protozoa, it is preferably a malarial parasite, which is more preferably a *plasmodium* species.

In another embodiment, the disease-causing replicating biological entity is a cancer cell, which is preferably a rapidly growing tumor cell, for example, a rapidly growing cancer cell found in breast cancer, colon cancer, lung cancer, or the like.

The method of administering a compound in accordance with the present invention can be applied to any suitable biochemical target, preferably a biochemical target whose biochemical vitality can be determined using measurable properties that can be obtained by assay. In one embodiment, the biochemical target of the predecessor (and the mutant) is an enzyme and the compound inhibits an enzyme of the predecessor. The enzyme can be any enzyme whose biochemical vitality can be measured including, for example, an enzyme described herein in connection with the fitness assay of the present invention.

In another embodiment, the biochemical target of the disease-causing replicating biological entity is an oligomer and the compound inhibits the oligomerization of the oligomer of the predecessor. In yet another embodiment, the biochemical target of the disease-causing replicating biological entity is a protein and the compound inhibits a conformational change in the protein of the predecessor.

The biochemical vitality can be determined in any suitable manner. For example, vitality can be determined as described herein, e.g., as described in connection with the assay of the present invention.

When an infectious microorganism is tested in accordance with the assay of the present invention, the predecessor can be a wild-type species, or the predecessor can itself be a mutant species. In a particularly preferred embodiment, the predecessor is a retrovirus, which is more preferably a wild-type HIV-1 or HIV-2 strain, most preferably HIV-1. When the predecessor is a wild-type HIV strain, the mutant replicating biological entity preferably has at least one mutation in the biochemical target thereof. When the predecessor has at least one mutation in the biochemical target thereof, the mutant preferably has at least two mutations in the biochemical target thereof.

Similarly, when the method of administering a therapeutic compound in accordance with the present invention is used in connection with an infectious microorganism, the disease-causing replicating biological entity can be a wild-type species, or the disease-causing entity can itself be a mutant species. In a particularly preferred embodiment, the disease-causing replicating biological entity is a retrovirus, which is more preferably a wild-type HIV-1 or HIV-2 strain, most preferably HIV-1. When the disease-causing replicating biological entity is a wild-type HIV strain, the mutant preferably has at least one mutation in the biochemical target thereof. When the disease-causing replicating biological entity has at least one mutation in the biochemical target thereof, the mutant preferably has at least two mutations in the biochemical target thereof.

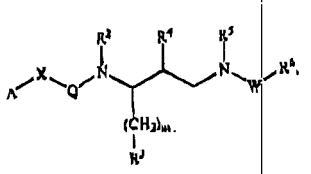
When the predecessor or the disease-causing replicating biological entity in the assay of the present invention, or in the method of administering a compound in accordance with the present invention, is a wild-type HIV strain, the biochemical target of the mutant preferably has at least one active site mutation. When the predecessor in the assay of the present invention has at least one mutation, and the mutant replicating biological entity has at least two mutations, the biochemical target of the predecessor or of the mutant preferably has at least one active site mutation. When the disease-causing replicating biological entity in the method of the present invention has at least one mutation in the biochemical target thereof, and the mutant has at least two mutations in the biochemical target thereof, the biochemical target of the disease-causing entity or of the mutant preferably has at least one active site mutation.

The present invention further provides a continuous fluorogenic assay for measuring the anti-HIV protease activity of a protease inhibitor, which method comprises adding a solution of HIV protease to a substrate stock solution, in which the substrate has the formula Ala-Arg-Val-Tyr-Phe(NO_2)-Glu-Ala-Nle-NH_2, to provide a substrate reaction solution. The fluorescence of the substrate reaction solution is then measured at specified time intervals. The solution of HIV protease is then added to a solution of the protease inhibitor and the substrate stock solution, to provide an inhibitor-substrate reaction solution. The fluorescence of the inhibitor-substrate reaction solution is then measured at specified time intervals. The initial velocity of the inhibitor-substrate reaction solution is then calculated by applying the equation: $V=V_o/2E_t(([K_i(1+S/K_m)+I_t-E_t)^2+4K_i(1+S/K_m)E_t]^{1/2}-(K_i((1+S/K_m)+I_t-E_t))$, wherein V is the initial velocity of the inhibitor reaction solution, V_o is the initial velocity of the substrate reaction solution, K_m is the Michaelis-Menten constant, S is the substrate concentration, E_t is the protease concentration, and I_t is the inhibitor concentration.

The assay method described herein is highly sensitive and particularly useful for the prediction of the antiviral inhibitory activity of a compound against mutant HIV, more particularly multiple mutant HIV, specifically multidrug-resistant human immunodeficiency viruses. The continuous fluorogenic assay of the present invention is distinctly advantageous in that it is more sensitive than standard assays in determining the activity of protease inhibitors against multidrug-resistant HIV. The

continuous fluorogenic assay of the present invention is disclosed in more detail in the examples that follow. The inhibitory data obtained in accordance with this continuous fluorogenic assay can be used to determine vitality and fitness for HIV-1 protease in the presence of a protease inhibitor, in accordance with the present invention.

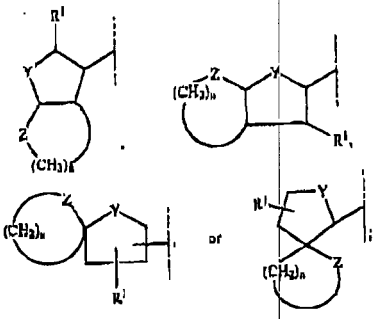
The present invention also provides a method of preventing the emergence of drug resistance in an HIV-infected mammal that includes the administration of a drug resistance-inhibiting effective amount of a compound represented by the formula:



(I)

or a pharmaceutically acceptable salt, a prodrug, or an ester thereof, or a pharmaceutical composition thereof, wherein:

A is a group of the formula:



R^1 is H or an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a cycloalkylalkyl, an aryl, an aralkyl, a heterocycloalkyl, a heterocycloalkylalkyl, a heteroaryl, or a heteroaralkyl radical, in which at least one hydrogen atom is optionally substituted with a substituent independently selected from the group consisting of OR^7, SR^7, CN, NO_2, N_3, and a halogen, wherein R^7 is H, an alkyl, an alkenyl, or an alkynyl;

Y and Z are the same or different and are independently selected from the group consisting of CH_2, O, S, SO, SO_2, NR^8, $R^8C(O)N$, $R^8C(S)N$, $R^8OC(O)N$, $R^8OC(S)N$, $R^8SC(O)N$, $R^8R^9NC(O)N$, and $R^8R^9NC(S)N$, wherein R^8 and R^9 are independently selected from the group consisting of H, an alkyl, an alkenyl, and an alkynyl;

n is an integer from 1 to 5;

X is a covalent bond, CHR^{10}, $CHR^{10}CH_2$, CH_2CHR^{10}, O, NR^{10}, or S, wherein R^{10} is H, an alkyl, an alkenyl, or an alkynyl;

Q is C(O), C(S), or SO_2;

R^2 is H, an alkyl, an alkenyl, or an alkynyl;

m is an integer from 0 to 6;

R^3 is a cycloalkyl, a heterocycloalkyl, an aryl, or a heteroaryl in which at least one hydrogen atom is optionally substituted with a substituent independently selected from the group consisting of H, alkyl, $(CH_2)_pR^{11}$, OR^{12}, SR^{12}, CN, N_3, NO_2, $NR^{12}R^{13}$, $C(O)R^{12}$, $C(S)R^{12}$, CO_2R^{12}, $C(O)SR^{12}$, $C(O)NR^{12}R^{13}$, $C(S)NR^{12}R^{13}$, $NR^{12}C(O)R^{13}$, $NR^{12}C(S)R^{13}$, $NR^{12}CO_2R^{13}$, $NR^{12}C(O)SR^{13}$, and a halogen, wherein:

p is an integer from 0 to 5;

R^{11} is a cycloalkyl, a heterocycloalkyl, an aryl, or a heteroaryl in which at least one hydrogen atom is optionally substituted with a substituent independently selected from the group consisting of a halogen, OH, OCH_3, NH_2, NO_2, SH, and CN; and

R^{12} and R^{13} are independently selected from the group consisting of H, an alkyl, an alkenyl, and an alkynyl;

R^4 is OH, =O (keto), or NH_2, wherein, when R^4 is OH, it is optionally in the form of a pharmaceutically acceptable ester or prodrug, and when R^4 is NH_2, it is optionally an amide, a hydroxylamino, a carbamate, a urea, an alkylamino, a dialkylamino, a protic salt, or a tetraalkylammonium salt;

R^5 is H, a C_1-C_6 alkyl radical, a C_2-C_6 alkenyl radical, or $(CH_2)_qR^{14}$, wherein q is an integer form 0 to 5, and R^{14} is a cycloalkyl, a heterocycloalkyl, an aryl, or a heteroaryl radical in which at least one hydrogen atom is optionally substituted with a substituent independently selected from the group consisting of a halogen, OH, OCH_3, NH_2, NO_2, SH, and CN;

W is C(O), C(S), S(O), or SO_2; and

R^6 is a cycloalkyl, heterocycloalkyl, aryl, or heteroaryl radical in which at least one hydrogen atom is optionally substituted with a substituent independently selected from the group consisting of a halogen, OR^{15}, SR^{15}, $S(O)R^{15}$, SO_2R^{15}, $SO_2NR^{15}R^{16}$, $SO_2N(OH)R^{15}CN$, $CR^{15}=NR^{16}$, $CR^{15}=N(OR^{16})$, N_3, NO_2, $NR^{15}R^{16}$, $N(OH)R^{15}$, $C(O)R^{15}$, $C(S)R^{15}$, CO_2R^{15}, $C(O)SR^{15}$, $C(O)NR^{15}R^{16}$, $C(S)NR^{15}R^{16}$, $C(O)N(OH)R^{15}$, $C(S)N(OH)R^{15}$, $NR^{15}C(O)R^{16}$, $NR^{15}C(S)R^{16}$, $N(OH)C(O)R^{15}$, $N(OH)C(S)R^{15}$, $NR^{15}CO_2R^{16}$, $N(OH)CO_2R^{15}$, $NR^{15}C(O)SR^{16}$, $NR^{15}C(O)NR^{16}R^{17}$, $NR^{15}C(S)NR^{16}R^{17}$, $N(OH)C(O)NR^{15}R^{16}$, $N(OH)C(S)NR^{15}R^{16}$, $NR^{15}C(O)N(OH)R^{16}$, $NR^{15}SO_2R^{15}$, $NHSO_2NR^{15}R^{16}$, $NR^{15}SO_2NHR^{16}$, $P(O)(OR^{15})(OR^{16})$, an alkyl, an alkoxy, an alkylthio, an alkylamino, a cycloalkyl, a cycloalkylalkyl, a heterocycloalkyl, a heterocycloalkylalkyl, an aryl, an aryloxy, an arylamino, an arylthio, an aralkyl, an aryloxyalkyl, an arylaminoalkyl, an aralkoxy, an (aryloxy)alkoxy, an (arylamino)alkoxy, an (arylthio)alkoxy, an aralkylamino, an (aryloxy)alkylamino, an (arylamino)alkylamino, an (arylthio)alkylamino, an aralkylthio, an (aryloxy)alkylthio, an (arylamino)alkylthio, an (arylthio)alkylthio, a heteroaryl, a heteroaryloxy, a heteroarylamino, a heteroarylthio, a heteroaralkyl, a heteroaralkoxy, a heteroaralkylamino, and a heteroaralkylthio,

wherein R^{15}, R^{16}, and R^{17} are H, an unsubstituted alkyl, and an unsubstituted alkenyl,

wherein, when at least one hydrogen atom of R^6 is optionally substituted with a substituent other than a halogen, OR^{15}, SR^{15}, $S(O)R^{15}$, SO_2R^{15}, $SO_2NR^{15}R^{16}$, $SO_2N(OH)R^{15}$, CN, $CR^{15}=NR^{16}$, $CR^{15}=N(OR^{16})$, N_3, NO_2, $NR^{15}R^{16}$, $N(OH)R^{15}$, $C(O)R^{15}$, $C(S)R^{15}$, CO_2R^{15}, $C(O)SR^{15}$, $C(O)NR^{15}R^{16}$, $C(S)NR^{15}R^{16}$, $C(O)N(OH)R^{15}$, $C(S)N(OH)R^{15}$, $NR^{15}C(O)R^{16}$, $NR^{15}C(S)R^{16}$, $N(OH)C(O)R^{15}$, $N(OH)C(S)R^{15}$, $NR^{15}CO_2R^{16}$, $N(OH)CO_2R^{15}$, $NR^{15}C(O)SR^{16}$, $NR^{15}C(O)NR^{16}R^{17}$, $NR^{15}C(S)NR^{16}R^{17}$, $N(OH)C(O)NR^{15}R^{16}$, $N(OH)C(S)NR^{15}R^{16}$, $NR^{15}C(O)N(OH)R^{16}$, $NR^{15}C(S)N(OH)R^{16}$, $NR^{15}SO_2R^{16}$, $NHSO_2NR^{15}R^{16}$, $NR^{15}SO_2NHR^{16}$, or $P(O)(OR^{15})(OR^{16})$, then at least one hydrogen atom on said substituent is optionally substituted with a halogen, OR^{15}, SR^{15}, $S(O)R^{15}$, SO_2R^{15}, $SO_2NR^{15}R^{16}$, $SO_2N(OH)R^{15}$, CN, $CR^{15}=NR^{16}$, $CR^{15}=N(OR^{16})$, N_3, NO_2, $NR^{15}R^{16}$, $N(OH)R^{15}$, $C(O)R^{15}$, $C(S)R^{15}$, CO_2R^{15}, $C(O)SR^{15}$, $C(O)NR^{15}R^{16}$, $C(S)NR^{15}R^{16}$, $C(O)N(OH)R^{15}$, $C(S)N(OH)R^{15}$, $NR^{15}C(O)$

R^{16}, $NR^{15}C(S)R^{16}$, $N(OH)C(O)R^{15}$, $N(OH)C(S)R^{15}$, $NR^{15}CO_2R^{16}$, $N(OH)CO_2R^{15}$, $NR^{15}C(O)SR^{16}$, $NR^{15}C(O)NR^{16}R^{17}$, $NR^{15}C(S)NR^{16}R^{17}$, $N(OH)C(O)NR^{15}R^{16}$, $N(OH)C(S)NR^{15}R^{16}$, $NR^{15}C(O)N(OH)R^{16}$, $NR^{15}C(S)N(OH)R^{16}$, $NR^{15}SO_2R^{16}$, $NHSO_2NR^{15}R^{16}$, $NR^{15}SO_2NHR^{16}$, or $P(O)(OR^{15})(OR^{16})$.

Optionally, R^5 and R^6 are covalently bonded such that R^5 and R^6, together with the N—W bond of formula (I), comprise a 12 to 18 membered ring. The 12 to 18 membered ring can comprise at least one additional heteroatom in the ring skeleton other than the nitrogen of the N—W bond (e.g., N, O, or S) within the ring. In the practice of the method of preventing the emergence of drug resistance in an HIV-infected mammal, it is preferable that a mutant virus that is capable of evolving from the infection has low fitness, relative to the infecting virus, in the presence of the compound or combination of compounds that are administered.

As utilized herein, the term "alkyl" means a straight-chain or branched alkyl radical containing from about 1 to about 20 carbon atoms chain, preferably from about 1 to about 10 carbon atoms, more preferably from about 1 to about 8 carbon atoms, still more preferably from about 1 to about 6 carbon atoms. Examples of such substituents include methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, pentyl, isoamyl, hexyl, octyl, dodecanyl, and the like.

The term "alkenyl" means a straight-chain or branched-chain alkenyl radical having one or more double bonds and containing from about 2 to about 20 carbon atoms chain, preferably from about 2 to about 10 carbon atoms, more preferably from about 0.2 to about 8 carbon atoms, still more preferably from about 2 to about 6 carbon atoms. Examples of such substituents include vinyl, allyl, 1,4-butadienyl, isopropenyl, and the like.

The term "alkynyl" means a straight-chain or branched-chain alkynyl radical having one or more triple bonds and containing from about 2 to about 20 carbon atoms chain, preferably from about 2 to about 10 carbon atoms, more preferably from about 2 to about 8 carbon atoms, still more preferably from about 2 to about 6 carbon atoms. Examples of such radicals include ethynyl, propynyl (propargyl), butynyl, and the like.

The term "alkoxy" means an alkyl ether radical, wherein the term "alkyl" is defined as above. Examples of alkoxy radicals include methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, sec-butoxy, tert-butoxy, hexanoxy, and the like.

The term "alkylthio" means an alkyl thioether radical, wherein the term "alkyl" is defined as above. Examples of alkylthio radicals include methylthio (SCH_3), ethylthio (SCH_2CH_3), n-propylthio, isopropylthio, n-butylthio, isobutylthio, sec-butylthio, tert-butylthio, n-hexylthio, and the like.

The term "alkylamino" means an alkyl amine radical, wherein the term "alkyl" is defined as above. Examples of alkylamino radicals include methylamino ($NHCH_3$), ethylamino ($NHCH_2CH_3$), n-propylamino, isopropylamino, n-butylamino, isobutylamino, sec-butylamino, tert-butylamino, n-hexylamino, and the like.

The term "cycloalkyl" means a monocyclic or a polycyclic alkyl radical defined by one or more alkyl carbocyclic rings, which can be the same or different when the cycloalkyl is a polycyclic radical having 3 to about 10 carbon atoms in the carbocyclic skeleton in each ring, preferably about 4 to about 7 carbon atoms, more preferably 5 to 6 carbon atoms. Examples of monocyclic cycloalkyl radicals include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, and the like. Examples of polycyclic cycloalkyl radicals include decahydronaphthyl, bicyclo[5.4.0]undecyl, adamantyl, and the like.

The term "cycloalkylalkyl" means an alkyl radical as defined herein, in which at least one hydrogen atom on the alkyl radical is replaced by a cycloalkyl radical as defined herein. Examples of cycloalkylalkyl radicals include cyclohexylmethyl, 3-cyclopentylbutyl, and the like.

The term "heterocycloalkyl" means a cycloalkyl radical as defined herein (including polycycles), wherein at least one carbon which defines the carbocyclic skeleton is substituted with a heteroatom such as, for example, O, N, or S, optionally comprising one or more double bond within the ring, provided the ring is not heteroaryl as defined herein. The heterocycloalkyl preferably has 3 to about 10 atoms (members) in the carbocyclic skeleton of each ring, preferably about 4 to about 7 atoms, more preferably 5 to 6 atoms. Examples of heterocycloalkyl radicals include epoxy, aziridyl, oxetanyl, tetrahydrofuranyl, dihydrofuranyl, piperadyl, piperidinyl, pyperazyl, piperazinyl, pyranyl, morpholinyl, and the like.

The term "heterocycloalkylalkyl" means an alkyl radical as defined herein, in which at least one hydrogen atom on the alkyl radical is replace by a heterocycloalkyl radical as defined herein. Examples of heterocycloalkylalkyl radicals include 2-morpholinomethyl, 3-(4-morpholino)-propyl, 4-(2-tetrahydrofuranyl)-butyl, and the like.

The term "aryl" refers to an aromatic carbocyclic radical, as commonly understood in the art, and includes monocyclic and polycyclic aromatics such as, for example, phenyl and naphthyl radicals, optionally substituted with one or more substituents selected from the group consisting of a halogen, an alkyl, alkoxy, amino, cyano, nitro, and the like.

The term "aryloxy" means aryl as defined herein, wherein a hydrogen atom is replaced by an oxygen. Examples of aryloxy radicals include phenoxy, naphthoxy, 4-fluorophenoxy, and the like.

The term "arylamino" means aryl as defined herein, wherein a hydrogen atom is replaced by an amine. Examples of arylamino radicals include phenylamino, naphthylamino, 3-nitrophenylamino, 4-aminophenylamino, and the like.

The term "arylthio" means aryl as defined herein, wherein a hydrogen atom is replaced by a sulfur atom. Examples of arylthio radicals include phenylthio, naphthylthio, 3-nitrophenylthio, 4-thiophenylthio, and the like.

The term "aralkyl" means alkyl as defined herein, wherein an alkyl hydrogen atom is replaced by an aryl as defined herein. Examples of aralkyl radicals include benzyl, phenethyl, 3-(2-naphthyl)-butyl, and the like.

The term "aryloxyalkyl" means alkyl as defined herein, wherein an alkyl hydrogen atom is replaced by an aryloxy as defined herein. Examples of aryloxyalkyl radicals include phenoxyethyl, 4-(3-aminophenoxy)-1-butyl, and the like.

The term "arylaminoalkyl" means alkyl as defined herein, wherein an alkyl hydrogen atom is replaced by an arylamino as defined herein. Examples of arylaminoalkyl radicals include phenylaminoethyl, 4-(3-methoxyphenylamino)-1-butyl, and the like.

The term "aralkoxy" means alkoxy as defined herein, wherein an alkyl hydrogen atom is replaced by an aryl as defined herein. Examples of aralkoxy radicals include 2-phenylethoxy, 2-phenyl-1-propoxy, and the like.

The term "(aryloxy)alkoxy" means alkoxy as defined herein, wherein an alkyl hydrogen atom is replaced by an aryloxy as defined herein. Examples of (aryloxy)alkoxy radicals include 2-phenoxyethoxy, 4-(3-aminophenoxy)-1-butoxy, and the like.

The term "(arylamino)alkoxy" means alkoxy as defined herein, wherein an alkyl hydrogen atom is replaced by an arylamino as defined herein. Examples of (arylamino)alkoxy radicals include 2-(phenylamino)-ethoxy, 2-(2-naphthylamino)-1-butoxy, and the like.

The term "(arylthio)alkoxy" means alkoxy as defined herein, wherein an alkyl hydrogen atom is replaced by an arylthio as defined herein. Examples of (arylthio)alkoxy radicals include 2-(phenylthio)-ethoxy, and the like.

The term "aralkylamino" means alkylamino as defined herein, wherein an alkyl hydrogen atom is replaced by an aryl as defined herein. Examples of aralkylamino radicals include 2-phenethylamino, 4-phenyl-n-butylamino, and the like.

The term "(aryloxy)alkylamino" means alkylamino as defined herein, wherein an alkyl hydrogen atom is replaced by an aryloxy as defined herein. Examples of (aryloxy)alkylamino radicals include 3-phenoxy-n-propylamino, 4-phenoxybutylamino, and the like.

The term "(arylamino)alkylamino" means alkylamino as defined herein, wherein an alkyl hydrogen atom is replaced by an arylamino as defined herein. Examples of (arylamino) alkylamino radicals include 3-(naphthylamino)-1-propylamino, 4-(phenylamino)-1-butylamino, and the like.

The term "(arylthio)alkylamino" means alkylamino as defined herein, wherein an alkyl hydrogen atom is replaced by an arylthio as defined herein. Examples of (arylthio) alkylamino radicals include 2-(phenylthio)-ethylamino, and the like.

The term "aralkylthio" means alkylthio as defined herein, wherein an alkyl hydrogen atom is replaced by an aryl as defined herein. Examples of aralkylthio radicals include 3-phenyl-2-propylthio, 2-(2-naphthyl)-ethylthio, and the like.

The term "(aryloxy)alkylthio" means alkylthio as defined herein, wherein an alkyl hydrogen atom is replaced by an aryloxy as defined herein. Examples of (aryloxy)alkylthio radicals include 3-phenoxypropylthio, 4-(2-fluorophenoxy)-butylthio, and the like.

The term "(arylamino)alkylthio" means alkylthio as defined herein, wherein an alkyl hydrogen atom is replaced by an arylamino as defined herein. Examples of (arylamino) alkylthio radicals include 2-(phenylamino)-ethylthio, 3-(2-naphthylamino)-n-propylthio, and the like.

The term "(arylthio)alkylthio" means alkylthio as defined herein, wherein an alkyl hydrogen atom is replaced by an arylthio as defined herein. Examples of (arylthio)alkylthio radicals include 2-(naphthylthio)-ethylthio, 3-(phenylthio)-propylthio, and the like.

The term "heteroaryl" means a radical defined by an aromatic heterocyclic ring as commonly understood in the art, including monocyclic radicals such as, for example, imidazole, thiazole, pyrazole, pyrrole, furane, pyrazoline, thiophene, oxazole, isoxazol, pyridine, pyridone, pyrimidine, pyrazine, and triazine radicals, and also including polycyclics such as, for example, quinoline, isoquinoline, indole, and benzothiazole radicals, which heteroaryl radicals are optionally substituted with one or more substituents selected from the group consisting of a halogen, an alkyl, alkoxy, amino, cyano, nitro, and the like. It will be appreciated that the heterocycloalkyl and heteroaryl substituents can be coupled to the compounds of the present invention via a heteroatom, such as nitrogen (e.g., 1-imidazolyl);

The term "heteroaryloxy" means heteroaryl as defined herein, wherein a hydrogen atom on the heteroaryl ring is replaced by an oxygen. Heteroaryloxy radicals include, for example, 4-pyridyloxy, 5-quinolyloxy, and the like.

The term "heteroarylamino" means heteroaryl as defined herein, wherein a hydrogen atom on the heteroaryl ring is replaced by an nitrogen. Heteroarylamino radicals include, for example, 4-thiazolylamino, 2-pyridylamino, and the like.

The term "heteroarylthio" means heteroaryl as defined herein, wherein a hydrogen atom on the heteroaryl ring is replaced by a sulfur. Heteroarylthio radicals include, for example, 3-pyridylthio, 3-quinolylthio, 4-imidazolylthio, and the like.

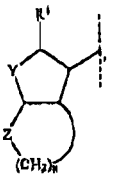
The term "heteroaralkyl" means alkyl as defined herein, wherein an alkyl hydrogen atom is replaced by a heteroaryl as defined herein. Examples of heteroaralkyl radicals include 2-pyridylmethyl, 3-(4-thiazolyl)-propyl, and the like.

The term "heteroaralkoxy" means alkoxy as defined herein, wherein an alkyl hydrogen atom is replaced by a heteroaryl as defined herein. Examples of heteroaralkoxy radicals include 2-pyridylmethoxy, 4-(1-imidazolyl)-butoxy, and the like.

The term "heteroarylalkylamino" means alkylamino as defined herein, wherein an alkyl hydrogen atom is replaced by a heteroaryl as defined herein. Examples of heteroaralkylamino radicals include 4-pyridylmethylamino, 3-(2-furanyl)-propylamino, and the like.

The term "heteroaralkylthio" means alkylthio as defined herein, wherein an alkyl hydrogen atom is replaced by a heteroaryl as defined herein. Examples of heteroaralkylthio radicals include 3-pyridylmethylthio, 3-(4-thiazolyl)-propylthio, and the like.

In the compound of Formula I, A is preferably a group of the formula:

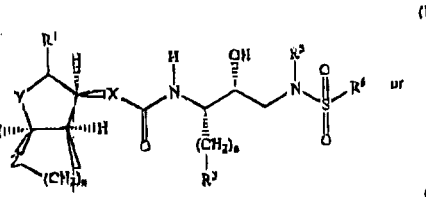


R^1 is H or an alkyl, an alkenyl, a cycloalkyl, a cycloalkylalkyl, an aryl, an aralkyl, a heterocycloalkyl, a heterocycloalkylalkyl, a heteroaryl, or a heteroaralkyl radical, in which at least one hydrogen atom is optionally substituted with a substituent independently selected from the group consisting of OR^7, SR^7, CN, NO_2, N_3, and a halogen, wherein R^7 is H, an unsubstituted alkyl, or an unsubstituted alkenyl; Y and Z are the same or different and are independently selected from the group consisting of CH_2, O, S, SO, SO_2, NR^8, $R^8C(O)N$, $R^8C(S)N$, $R^8OC(O)N$, $R^8OC(S)N$, $R^8SC(O)N$, $R^8R^9NC(O)N$, and $R^8R^9NC(S)N$, wherein R^8 and R^9 are independently selected from the group consisting of H, an unsubstituted alkyl, and an unsubstituted alkenyl; X is a covalent bond, CHR^{10}, $CHR^{10}CH_2$, CH_2CHR^{10}, O, NR^{10}, or S, wherein R^{10} is H, an unsubstituted alkyl, or an unsubstituted alkenyl; R^3 is H, a C_1-C_6 alkyl radical, or a C_2-C_6 alkenyl radical; R^{12} and R^{13}, as defined with respect to R^3, are independently selected from the group consisting of H, an unsubstituted alkyl, and an unsubstituted alkenyl radical; R^4 is OH, NH_2, or $NHCH_3$; W is C(O), C(S), or SO_2; and R^6 is a cycloalkyl, heterocycloalkyl, aryl, or heteroaryl radical in which at least one hydrogen atom is optionally substituted with a substituent independently selected from the group consisting of a halogen, OR^{15}, SR^{15}, CN, N_3, NO_2, $NR^{15}R^{16}$, $C(O)R^{15}$, $C(S)R^{15}$, CO_2R^{15}, $C(O)SR^{15}$, $C(O)NR^{15}R^{16}$, $C(S)NR^{15}R^{16}$, $NR^{15}C$

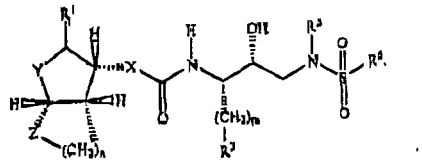
$(O)R^{16}$, $NR^{15}C(S)R^{16}$, $NR^{15}CO_2R^{16}$, $NR^{15}C(O)SR^{16}$, $NR^{15}C(O)NR^{16}R^{17}$, and $NR^{15}C(S)NR^{16}R^{17}$, an alkyl, an alkoxy, an alkylthio, an alkylamino, a cycloalkyl, a cycloalkylalkyl, a heterocycloalkyl, a heterocycloalkylalkyl, an aryl, an aryloxy, an arylamino, an arylthio, an aralkyl, an aryloxyalkyl, an arylaminoalkyl, an aralkoxy, an (aryloxy)alkoxy, an (arylamino)alkoxy, an (arylthio)alkoxy, an aralkylamino, an (aryloxy)alkylamino, an (arylamino)alkylamino, an (arylthio)alkylamino, an aralkylthio, an (aryloxy)alkylthio, an (arylamino)alkylthio, an (arylthio)alkylthio, a heteroaryl, a heteroaryloxy, a heteroarylamino, a heteroarylthio, a heteroaralkyl, a heteroaralkoxy, a heteroaralkylamino, and a heteroaralkylthio, wherein R^{15}, R^{16}, and R^{17} are H, an unsubstituted alkyl, and an unsubstituted alkenyl, such that when at least one hydrogen atom of R^6 is optionally substituted with a substituent other than a halogen, OR^{15}, SR^{15}, CN, N_3, NO_2, $NR^{15}R^{16}$, $C(O)R^{15}$, $C(S)R^{15}$, CO_2R^{15}, $C(O)SR^{15}$, $C(O)NR^{15}R^{16}$, $C(S)NR^{15}R^{16}$, $NR^{15}C(O)R^{16}$, $NR^{15}C(S)R^{16}$, $NR^{15}CO_2R^{16}$, $NR^{15}C(O)SR^{16}$, $NR^{15}C(O)NR^{16}R^{17}$, or $NR^{15}C(S)NR^{16}R^{17}$, at least one hydrogen atom on said substituent attached to R^6 is optionally substituted with a halogen, OR^{15}, SR^{15}, CN, N_3, NO_2, $NR^{15}R^{16}C(O)R^{15}$, $C(S)R^{15}$, CO_2R^{15}, $C(O)SR^{15}$, $C(O)NR^{15}R^{16}$, $C(S)NR^{15}R^{16}$, $NR^{15}C(O)R^{16}$, $NR^{15}C(S)R^{16}$, $NR^{15}CO_2R^{16}$, $NR^{15}C(O)SR^{16}$, $NR^{15}C(O)NR^{16}R^{17}$, or $NR^{15}C(S)NR^{16}R^{17}$.

It is further preferred that when R^1 is an alkyl or an alkenyl radical (i.e., an alkyl or an alkenyl substituent), then it is a C_1-C_6 alkyl or, in the case when R^1 is an alkenyl, it is a C_2-C_6 alkenyl. When R^1 is a monocyclic substituent such as, for example, a cycloalkyl, a heterocycloalkyl, an aryl, or a heteroaryl, it preferably comprises 4-7 members in the ring that defines the monocyclic skeleton. When R^7, R^8 or R^9 is an unsubstituted alkyl, it is preferably a C_1-C_6 unsubstituted alkyl; and when R^7, R^1 or R^9 is an unsubstituted alkenyl, it is preferably a C_2-C_6 unsubstituted alkenyl. The ring defined by R^3 preferably comprises 4-7 members or, in the case of polycyclics, each ring comprises 4-7 members. When R^3 is $(CH_2)_m$ R^{11}, the ring defined by R^{11} preferably comprises 4-7 members, or, in the case of polycyclics, each ring comprises 4-7 members. When either of R^{12} or R^{13} is an unsubstituted alkyl, it is preferably a C_1-C_6 unsubstituted alkyl, and when either of R^{12} or R^{13} is an unsubstituted alkenyl, it is a C_2-C_6 unsubstituted alkyl. When R^{14} is a cycloalkyl, a heterocycloalkyl, an aryl, or a heteroaryl, the ring defined by R^{14} preferably comprises 4-7 members, or, in the case of polycyclics, each ring comprises 4-7 members. When R^6 is a cycloalkyl, a heterocycloalkyl, aryl, or a heteroaryl, the ring defined by R^6 preferably comprises 4-7 members, or, in the case of polycyclics, each ring comprises 4-7 members, and when R^6 is substituted with a substituent that is an alkyl, an alkylthio, or an alkylamino, it is preferred that the substituent comprises from one to six carbon atoms, and when R^6 is substituted with a substituent that is a cycloalkyl, a heterocycloalkyl, an aryl, or a heteroaryl, the ring defined by the substituent preferably comprises 4-7 members or, in the case of polycyclics, each ring comprises 4-7 members.

In a preferred embodiment, the method of preventing the emergence of resistance in accordance with the present invention includes administering a compound of Formula (I), wherein Q is C(O), R^2 is H, and W is C(O) or SO_2. In a further preferred embodiment, Q is C(O), R^2 is H, R^4 is OH, W is SO_2, and the stereochemical orientation of the asymmetric centers is represented by formula (IA) or (IB) below:

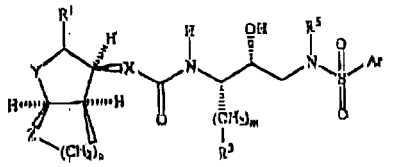


(IA)

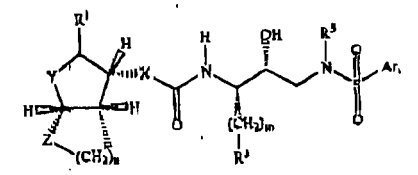


(IB)

It is further preferred that R^6 is a monocyclic substituent, preferably an aromatic ring, which is preferably a substituted benzene ring, as illustrated by the formula:



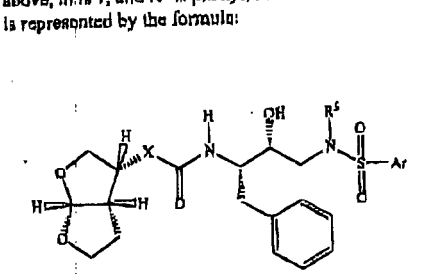
(IC)



(ID)

wherein Ar is a phenyl which is optionally substituted with a substituent selected from the group consisting of methyl, amino, hydroxy, methoxy, methylthio, hydroxymethyl, aminomethyl, and methoxymethyl.

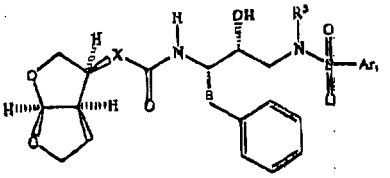
In a preferred series, Y and Z are oxygen atoms, n is 2, the resulting bis-tetrahydrofuranyl ring system has the stereochemical orientations illustrated in Formulae (IC) and (ID) above, m is 1, and R^3 is phenyl, in which case the compound is represented by the formula:



(IE)

-continued

(IF)



wherein Ar is a phenyl which is optionally substituted with a substituent selected from the group consisting of methyl, amino, hydroxy, methoxy, methylthio, hydroxymethyl, aminomethyl, and methoxymethyl. When the compound is a compound of Formula (IE) or (IF), wherein at least one hydrogen atom on Ar substituted with a substituent selected from the group consisting of methyl, amino, hydroxy, methoxy, methylthio, hydroxymethyl, and methoxymethyl, it is further preferred that X is an oxygen. Still more preferably, X is an oxygen and R^5 is isobutyl. Suitable Ar substituents include phenyl groups that are substituted at the para position, the meta position, and/or the ortho position. Examples of suitable Ar substituents are shown in Table 4, and in FIGS. 3 and 5A-5D.

A resistance-inhibiting effective amount is an amount sufficient to produce an in vivo drug concentration or level in which the biochemical vitality of a mutant HIV is lower than the biochemical vitality of the HIV (predecessor) infecting the HIV-infected mammal. For example, a resistance-inhibiting effective amount is an amount sufficient to produce an in vivo drug concentration or level where the value for biochemical fitness is less than one, when determined by the ratio of the biochemical vitality of the mutant to the biochemical vitality of the predecessor. The compound can be administered to a wild-type HIV-infected mammal to prevent the emergence of first line resistance, or it can be administered to a mammal infected with a mutant-HIV to prevent the emergence of drug resistance due to further mutations.

The compound is preferably administered in the form of a pharmaceutical composition. The pharmaceutical composition preferably includes a pharmaceutically acceptable carrier and a resistance-inhibiting effective amount of at least one of the aforesaid compound, alone or in combination with another antiretroviral compound such as, for example, a wild-type HIV protease inhibitor, a mutant HIV retroviral protease inhibitor, or a reverse transcriptase inhibitor. Generally, the pharmaceutical composition of the present invention comprises a resistance-inhibiting effective amount of at least one compound of Formula (I), as disclosed herein, and a pharmaceutically acceptable carrier.

In a preferred embodiment, a pharmaceutical composition is administered that comprises a resistance-inhibiting effective amount of at least one compound of Formula (IA) or Formula (IB), or a pharmaceutically acceptable salt, prodrug, or ester thereof, and a pharmaceutically acceptable carrier. In a further preferred embodiment, the pharmaceutical composition comprises a resistance-inhibiting effective amount of at least one compound of Formula (IC) or Formula (ID), or a pharmaceutically acceptable salt, prodrug, or ester thereof, and a pharmaceutically acceptable carrier. In a highly preferred embodiment, the pharmaceutical composition comprises a resistance-inhibiting effective amount of at least one compound of Formula (IE), and pharmaceutically acceptable salts, prodrugs, and esters thereof, and a pharmaceutically acceptable carrier.

Pharmaceutically acceptable carriers are well-known to those of skill in the art. The choice of a carrier will be determined in part by the particular composition, as well as by the particular mode of administration. Accordingly, there are a wide variety of suitable formulations for administration in accordance the present invention.

The pharmaceutical composition may be administered in a form suitable for oral use such as, for example, tablets, troches, lozenges, aqueous or oily suspensions or solutions, dispersible powders or granules, emulsions, hard or soft capsules, syrups or elixirs. Compositions intended for oral use may be prepared according to any method known in the art form the manufacture of pharmaceutical compositions, and such compositions can contain one or more agents such as, for example, sweetening agents, flavoring agents, coloring agents, and preserving agents in order to provide a pharmaceutically elegant and/or palatable preparation. Tablets can contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for manufacture of tablets. Such excipients can be, for example, inert diluents such as, for example, calcium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents such as, for example, maize starch or alginic acid; binding agents such as, for example, starch, gelatine or acacia, and lubricating agents such as, for example, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate alone or with a wax may be employed.

Formulations for oral use also can be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example arachis oil, peanut oil, liquid paraffin or olive oil.

Aqueous suspensions typically contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example, sodium carboxymethyl cellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a natural-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyoxyethylene sorbitan mono-oleate. The aqueous suspensions also can contain one or more preservatives, for example, ethyl or n-propyl p-hydroxy benzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents such as, for example, sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oil suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol.

Sweetening agents, such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions can be preserved by the addition of an antioxidant such as, for example, ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, also may be present.

The pharmaceutical composition also can be administered in the form of oil-in-water emulsions. The oily phase can be a vegetable oil, for example, olive oil or arachis oils, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soya bean lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan mono-oleate, and condensation products of the said partial esters and ethylene oxide, for example polyoxyethylene sorbitan mono-oleate. The emulsions also can contain sweetening and flavoring agents.

The pharmaceutical composition also can be administered in the form of syrups and elixirs, which are typically formulated with sweetening agents such as, for example, glycerol, sorbitol or sucrose. Such formulations also can contain a demulcent, a preservative and flavoring and coloring agents.

Further, the pharmaceutical composition can be administered in the form of a sterile injectable preparation, for example, as a sterile injectable aqueous or oleaginous suspension. Suitable suspensions for parenteral administration can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. Formulations suitable for parenteral administration include, for example, aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostates, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The sterile injectable preparation can be a solution or a suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in water or 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed, for example, are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as, for example, oleic acid find use in the preparation of injectables.

Further, the compound can be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include, for example, cocoa butter and polyethylene glycols. Formulations suitable for vaginal administration can be presented as pessaries, tampons, creams, gels, pastes, and foams.

Formulations suitable for topical administration may be presented as creams, gels, pastes, or foams, containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

The composition can be made into an aerosol formulation to be administered via inhalation. Such aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also can be formulated as pharmaceuticals for non-pressured preparations such as in a nebulizer or an atomizer.

The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

Any suitable dosage level can be employed in the pharmaceutical compositions of the present invention. The dose administered to an animal, particularly a human, in the context of the present invention should be sufficient to effect a prophylactic or therapeutic response in the animal over a reasonable time frame. The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects that might accompany the administration of a particular composition. Suitable doses and dosage regimens for the prevention of drug resistance can be determined by comparisons to antiretroviral chemotherapeutic agents that are known to inhibit the proliferation of a retrovirus in an infected individual. The preferred dosage is the amount that results in the inhibition of the emergence of mutant drug-resistant retroviruses, particularly the emergence of multi-drug-resistant retroviral HIV, without significant side effects. In proper doses and with suitable administration of certain compounds, a wide range of antiretroviral chemotherapeutic compositions are possible. A suitable dose includes a dose or dosage which would be insufficient to completely suppress the growth of a wild-type or predecessor virus, but would be sufficient to inhibit or effectively suppress the growth of a mutant.

In accordance with the present invention, the compound or composition can be administered in combination with other antiretroviral compounds such as, for example, ritonavir, amprenavir, saquinavir, indinavir, AZT, ddI, ddC, D4T, lamivudine, 3TC, and the like, as well as admixtures and combinations thereof, in a pharmaceutically acceptable carrier. The individual daily dosages for these combinations can range from about one-fifth of the minimally recommended clinical dosages to the maximum recommended levels for the entities when they are given singly.

The present invention also provides a method of preventing the emergence of multidrug-resistant retroviruses in an HIV-infected mammal, which method comprises administering to the mammal a multidrug resistance-inhibiting effective amount of a compound of the present invention, so as to inhibit the emergence of a multidrug-resistant retrovirus in the mammal. The dose administered to an animal, particularly a human in the context of the present invention, should be sufficient to effect a therapeutic response in the animal over a reasonable time frame. The dose will be determined by the strength of the particular composition employed and the condition of the animal, as well as the body weight of the animal to be treated. The size of the dose will also be determined by the existence, nature, and extent of any adverse side-effects that might accompany the administration of a particular compound. Other factors which effect the specific dosage include, for example, bioavailability, metabolic pro-

file, and the pharmacodynamics associated with the particular compound to be administered in a particular patient. One skilled in the art will recognize that the specific dosage level for any particular patient will depend upon a variety of factors including, for example, the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination, CD4 count, the potency of the active compound with respect to the particular mutant retroviral strain to be inhibited, and the severity of the symptoms presented prior to or during the course of therapy. What constitutes a resistance-inhibiting effective amount can be determined, in part, by use of one or more of the assays described herein, particularly the fitness assay of the present invention.

One skilled in the art will appreciate that suitable methods of administering compounds and pharmaceutical compositions are available, and, although more than one route can be used to administer a particular composition, a particular route can provide a more immediate and/or more effective reaction than another route.

Numerous compounds have been identified that exhibit potent antiretroviral activity, in particular retroviral protease activity, against wild-type HIV. However, among the fifteen currently FDA-approved antiretroviral agents which are all known potent inhibitors of wild-type HIV, five of which are potent inhibitors of wild-type HIV protease, none of those compounds have the ability to prevent the emergence of drug-resistance mutations that are associated with high level cross resistance. Thus, these inhibitors do not have the ability to suppress the sufficiently fit mutant retroviruses that can (and almost certainly will) emerge under the selection pressure of these inhibitors.

Surprisingly, it has been discovered that compound 32 (shown in FIG. 3A), which is a potent wild-type HIV inhibitor, possesses remarkably potent and unprecedented broad-spectrum inhibitory activity against a panel of recombinant mutant HIV protease targets. These enzymes represent the key or primary resistance mutations, most of which occur in the active site region. Based on this finding, the compound was tested against a panel of drug resistant mutant patient isolates of HIV and was found to possess broad spectrum antiviral activity against a wide range of clinically isolated, multiply drug-resistant, human immunodeficiency viruses. Other compounds described herein showed similar activity. The mutant viruses were obtained from infected humans who had received several antiviral drugs. Although applicants do not wish to abound by any one particular theory, it is believed that the combination of the bicyclic ligand (vii) with isostere (vi) gives the antiretroviral compounds of the present invention the unique ability to bind to the active site of the mutant proteases of multiply drug-resistant human immunodeficiency viruses generally, which fruit has heretofore not been reported with respect to any known chemotherapeutic and/or experimental HIV protease inhibitor. A wild-type preliminary screen was utilized to determine the antiretroviral activity of analogs against wild-type HIV. It is predicted that compounds of Formula (I), which have potent antiretroviral or protease-inhibitory activity against wild-type HIV, also will be potent inhibitors of drug-resistance, even multiple drug-resistance, in wild-type HIV, or even a mutant thereof.

The resistance-inhibiting compounds of the present invention can be synthesized by any suitable method known in the art. The preferred synthetic method is generally illustrated in FIG. 4, which is an representation of the synthetic approach to preparing a preferred series of compounds, wherein a compound of Formula (I) is synthesized in several steps starting from azidoepoxide (i), wherein R^1-R^{17}, m, n, p, Q, W, X, y, and z are defined as above. Referring to FIG. 4, amine (ii) is nucleophilically added to azidoepoxide (i), providing aminoalcohol (iii). The amine functional group of aminoalcohol (iii) is then reacted with intermediate (iv), wherein L represents a leaving group (e.g., halogen, N-oxysuccinimide), which can be displaced by the amine of aminoalcohol (iii), to provide azide (v). Reduction of azide (v), or, when R^5 is not hydrogen, reductive amination with aldehyde $R^5CH{=}O$, provides intermediate (vi), which is subsequently coupled with activated bicyclic ligand (vii), to provide compounds of Formula I. Of course, it will be appreciated by a person of ordinary skill in the art that there are combinations of substituents, functional groups, R-groups, and the like, which are reactive under particular reaction conditions, and require the utilization of an appropriate protecting group or groups, which are known in the art, to ensure that the desired synthetic transformation will take place without the occurrence of undesired side reactions. For example, possible substituents at R^5 (e.g., NH_2) can be competitive nucleophiles requiring the attachment of an appropriate protecting group thereon (e.g., benzyloxycarbonyl, tert-butoxycarbonyl) in order obtain proper selectivity in the ring opening of epoxide (i) with amine (ii).

FIGS. 1-3B illustrate the synthesis of a preferred series of compounds for use in the method of preventing the emergence of resistance in accordance with the present invention. FIG. 1, which is a synthetic scheme for the synthesis of a particular sulfonamide, illustrates the synthesis of a preferred isosteric core, particularly, the sulfonamide isosteric core represented by aminosulfonamide 15. With reference to FIG. 1, aminosulfonamide core 15 can be synthesized by initially providing azidoepoxide 11 and subjecting it to nucleophilic addition with amine 12 to give aminoalcohol 13, which is subsequently converted to sulfonamide 14 by reaction with 4-methoxybenzenesulfonylchloride. The azide group of 14 is then reduced to provide aminosulfonamide 15, which can be used as a core for synthesizing numerous multidrug-resistant retroviral protease inhibitors of the present invention.

FIG. 2, which is a reaction scheme detailing the preparation of bicyclic alcohols, illustrates the synthesis of a preferred series of bicyclic ligands, particularly bis-tetrahydrofurans 25 and 26. With reference to FIG. 2, dihydrofuran 21 is treated with N-iodosuccinimide in the presence of propargyl alcohol to give iodoether 22, which is cyclized to methylene-substituted bis-tetrahydrofuran 23. Ozonolysis of the oxomethylene residue of 23, followed by reduction, provides bicyclic racemic alcohol 24, which is resolved to give, separately, bicyclic alcohol 25 and its enantiomeric acetate ester 26, which ester group of 26 is subsequently hydrolyzed to afford enantiomer 27.

FIGS. 3A and 3B, which are reaction schemes describing the preparation of two protease inhibitors, illustrate the preparation of two preferred multidrug-resistant HIV protease inhibitors of the present invention. With reference to FIG. 3A, compound 32 was synthesized by coupling succinimidocarbonate 31 with aminosulfonamide 15. Succinimidocarbonate 31 was prepared by reacting optically pure bicyclic alcohol 25 with disuccinimidyl carbonate in the presence of triethylamine. Inhibitor 34, which possesses the enantiomeric bis-tetrahydrofuranyl ligand (relative to inhibitor 32), was prepared in the same fashion, except that the enantiomeric bicyclic alcohol 27 was used instead of alcohol 25, as illustrated in FIG. 3B.

The following examples further illustrate the present invention but, of course, should not be construed as in any way limiting its scope.

Example 1

This example describes the synthesis of exemplary epoxide 11 (FIG. 1), which is used as an intermediate in the synthesis of a particular series of compounds within the scope of the present invention.

Anhydrous CuCN (4.86 g, 54 mmol) was added to a solution of butadiene monoxide (38 g, 540 mmol) in anhydrous tetrahydrofuran (1.2 L) and the resulting mixture was stirred at −78° C. Commercial phenyl magnesium bromide solution (Aldrich) in ether (65 mmol) was added dropwise over a period of 10 min. The resulting reaction mixture was then allowed to warm to 0° C. and it was continued to stir until the reaction mixture was homogeneous. After this period, the reaction mixture was cooled to −78° C. and 0.5M mole of phenylmagnesium bromide solution in ether was added dropwise for 30 min. The reaction mixture was allowed to warm to 23° C. for 1 h. The reaction was quenched by slow addition of saturated aqueous NH_4Cl (120 mL) followed by NH_4OH (70 mL), saturated NH_4Cl (500 mL) and then H_2O (300 mL). The aqueous layer was thoroughly extracted with ethyl acetate (2×300 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was distilled under vacuum (0.12 torr) at 95° C. to give trans-4-phenyl-2-butene-1-ol (75.6 g).

To a suspension of powdered 4 Å molecular sieves (6.6 g) in anhydrous methylene chloride (750 mL), titanium tetraisopropoxide (Aldrich, 3.2 mL) and then diethyl D-tartrate (2.3 mL) were added. The resulting mixture was cooled to −22° C. and tert-butylhydroperoxide solution in isooctane (Aldrich, 430 mmol) was added over a period of 10 min. The mixture was stirred an additional 30 min and then a solution of trans-4-phenyl-2-butene-1-ol (32.6 g, 213 mmol), in anhydrous methylene chloride (120 mL), was added dropwise over a period of 40 min at −22° C. The reaction mixture was then aged in a freezer at −22° C. for 24 h. After this period, water (100 mL) was added to the reaction mixture at −22° C. and the mixture was allowed to warm to 0° C. After stirring at 0° C. for 45 min, 20% NaOH in brine (20 mL) was added. The resulting mixture was then allowed to warm to 23° C. and was stirred at that temperature for 1 h. After this period, the layers were separated and the aqueous layer was extracted with methylene chloride (2×200 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was diluted with toluene (800 mL) and then evaporated under reduced pressure. The residue was chromatographed over silica gel (35% ethyl acetate in hexane as eluent) to provide (2R,3R)-epoxy-4-phenylbutan-1-ol (21.8 g).

To a solution of titanium isopropoxide (12 mL) in anhydrous benzene (250 mL) was added azidotrimethylsilane (11 mL) and the resulting mixture was refluxed for 6 h. A solution of (2R,3R)-epoxy-4-phenylbutan-1-ol (5.32 g) in anhydrous benzene (25 mL) was added to the above refluxing mixture. The resulting mixture was refluxed for addition 25 min. After this period, the reaction mixture was cooled to 23° C. and the reaction was quenched with aqueous 5% H_2SO_4 (400 mL). The resulting mixture was stirred for 1 h and the layers were separated and the aqueous layer was extracted with ethyl acetate (2×300 mL). The combined organic layers were washed with saturated $NaHCO_3$ (200 mL), dried over Na_2SO_4 and concentrated under reduced pressure to afford the (2S,3S)-2-hydroxy-3-azido-4-phenyl-butan-12-ol (5.1 g) as a white solid (mp 81-82° C.).

To a stirred solution of the azidodiol (5.1 g) in chloroform (100 mL) at 23° C., 2-acetoxyisobutyryl chloride (Aldrich, 5 mL) was added. The resulting reaction mixture was stirred at 23° C. for 8 h. The reaction was quenched by addition of saturated sodium bicarbonate (100 mL) and the resulting mixture was stirred 30 min. The layers were separated and the aqueous layer was extracted with chloroform (2×200 mL). The combined organic layer was extracted with chloroform (2×200 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The resulting residue was dissolved in anhydrous THF (50 mL) and solid NaOMe (2.1 g) was added. The mixture was stirred for 4 h at 23° C. and after this period, the reaction was quenched with saturated NH_4Cl (50 mL). The resulting mixture was extracted with ethyl acetate (2×200 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a residue, which was chromatographed over silica gel (10% ethyl acetate in hexanes) to afford the 3(S)-azido-(1,2R)-epoxy-4-phenylbutane 11 (3.3 g) as an oil: ^1H NMR (300 MHz): $CDCl_3$; δ 7.4-7.2 (m, 5H,), 3.6 (m, 1H), 3.1 (m, 1H), 2.95 (dd, 1H, J=4.6, 13.9 Hz), 2.8 (m, 3H).

Example 2

This example illustrates the synthesis of azidoalcohol 13 (FIG. 1), which can be used as an intermediate in the synthesis of a preferred series of the compounds of the present invention.

To a stirred solution of above azidoepoxide 11 (700 mg, 3.7 mmol) in isopropanol (70 mL) was added isobutyl amine (Aldrich, 0.74 mL 7.4 mmol) and the resulting mixture was heated at 80° C. for 12 h. After this period, the reaction mixture was concentrated under reduced pressure and the residue was chromatographed over silica gel to provide azidoalcohol 13 (800 mg) as an oil.

Example 3

This example illustrates the synthesis of azidosulfonamide 14, the structure of which is shown in FIG. 1.

To a stirred solution of 13 (600 mg, 2.28 mmol) in CH_2Cl_2 (20 mL) was added 4-methoxybenzenesulfonyl chloride (Aldrich, 530 mg, 2.52 mmol) and saturated aqueous $NaHCO_3$ (6 mL). The resulting heterogeneous mixture was stirred at 23° C. for 12 h. The reaction was diluted with CH_2Cl_2 and the layers were separated. The organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated to dryness. The residue was chromatographed over silica gel (25% ethyl acetate/hexane) to provide 900 mg of azidosulfonamide 14.

Example 4

This example illustrates the preparation of aminosulfonamide 15 via reduction of azidosulfonamide 14, as shown in FIG. 1.

A solution of 14 (1.53 g) in THF (45 mL), MeOH (10 mL) and acetic acid (0.5 mL), was shaken with 10% palladium on carbon catalyst (200 mg) at 50 psi hydrogen pressure for 2 h. Removal of the catalyst by filtration over celite and concentration under reduced pressure gave a crude residue, which was diluted with CH_2Cl_2 (100 mL), and was washed successively with saturated aqueous $NaHCO_3$ and brine. The

organic layer was dried over $MgSO_4$ and concentrated to give the corresponding aminosulfonamide 15 (1.2 g).

Example 5

This example demonstrates the synthesis of trans-2-(propargyloxy)-3-iodotetrahydrofuran 22 (FIG. 2).

To a stirred, ice-cold suspension of 15 g (66.6 mmol) of N-iodosuccinimide in 150 mL of CH_2Cl_2 was added a mixture of dihydrofuran 21 (66.6 mmol, 4.67 g, 5.1 mL) and propargyl alcohol (100 mmol, 5.0 g, 5.2 mL) of in 50 mL of CH_2Cl_2 over 20 min. After warming to 24° C. with stirring over 2 h, 200 mL of water were added and the stirring continued for 1 h. The layers were separated and the aqueous layer was extracted with 2×100 mL of CH_2Cl_2. The combined organic extracts were washed with brine solution containing small amount of $Na_2S_2O_3$ (70 mg), dried over anhydrous Na_2SO_4, filtered, and concentrated. Chromatography over silica gel using 30% ethyl acetate in hexane afforded (15.4 g, 92%) the title iodoether 22 as an oil.

Example 6

This example illustrates the synthesis of (O)-(3aR, 6aS) and (3aS, 6aR)-3-methylene-4H-hexahydrofuro-[2,3-b]furan 23, as shown in FIG. 2.

To a refluxing solution of (20.7 mL, 77 mmol) tributyltin hydride containing AIBN (100 mg) in toluene (200 mL) was added dropwise a solution of 15.4 g (61 mmol) of iodotetrahydrofuran 22 in toluene (50 mL) over a period of 1 h. The resulting mixture was stirred at reflux for an additional 4 h (monitored by TLC). The mixture was then cooled to 23° C. and concentrated under reduced pressure. The residue was partitioned between petroleum ether and acetonitrile (200 mL of each) and the acetonitrile (lower) layer was concentrated. The residue was purified by chromatography on silica gel, using 10% ethyl acetate in hexane as the eluent to provide the title product 23 (5.84 g, 76%) as an oil.

Example 7

This example demonstrates the synthesis of (+)-(3SR, 3aRS, 6aS) and (3R,3aS, 6aR)-3-hydroxy-4H-hexahydrofuro [2,3-b]furan 24, as shown in FIG. 2.

A stream of ozone was dispersed into a solution of 15 (5.84 g, 46.4 mmol) at −78° C. in 150 mL of methanol and 150 mL of CH_2Cl_2 for 30 min. The resulting blue solution was purged with nitrogen until colorless, then quenched with 20 mL of dimethyl sulfide and the resulting mixture was allowed to warm to 23° C. The mixture was concentrated under reduced pressure to afford the crude ketone. The resulting crude ketone was dissolved in ethanol (50 mL) and the solution was cooled to 0° C. and sodium borohydride (2.1 g, 55.6 mmol) was added. The reaction mixture was stirred for an additional 2 h at 0° C. and then quenched with 10% aqueous citric acid (10 mL). The resulting mixture was concentrated under reduced pressure and the reside was partitioned between ethyl acetate and brine. The layers were separated and the aqueous layer was extracted with ethyl acetate (2×100 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated carefully under reduced pressure. The resulting residue was chromatographed over silica gel using 30% ethyl acetate in hexane as the eluent to furnish (4.52 g, 75%) the title racemic alcohol 24 as an oil.

Example 8

This example illustrates the preparation of immobilized Amano Lipase 30, which was used to resolve racemic aminoalcohol 24 (FIG. 2).

Commercially available 4 g of Celite® 521 (Aldrich) was loaded on a buchner funnel and washed successively with 50 mL of deionized water and 50 mL of 0.05 N phosphate buffer (pH=7.0; Fisher Scientific). The washed celite was then added to a suspension of 1 g of Amano lipase 30 in 20 mL of 0.05 N phosphate buffer. The resulting slurry was spread on a glass dish and allowed to dry in the air at 23° C. for 48 h (weight 5.4 g; water content about 2% by Fisher method).

Example 9

This example demonstrates the synthesis of (3R,3aS, 6aR) 3-hydroxyhexahydrofuro[2,3-b]furan 25 by immobilized lipase catalyzed acylation, as illustrated in FIG. 2.

To a stirred solution of racemic alcohol 24 (2 g, 15.4 mmol) and acetic anhydride (4 g, 42.4 mmol) in 100 mL of DME was added 2.7 g (about 25% by weight of lipase PS30) of immobilized Amano lipase and the resulting suspension was stirred at 23° C. The reaction was monitored by TLC and ^1H NMR analysis until 50% conversion was reached. The reaction mixture was filtered and the filter cake was washed repeatedly with ethyl acetate. The combined filtrate was carefully concentrated in a rotary evaporator, keeping the bath temperature below 15° C. The residue was chromatographed over silica gel to provide 843 mg (42%) of 25 (95% ee; $[\alpha]_D^{25}$-11.9°, MeOH); ^1H-NMR ($CDCl_3$,) d 1.85 (m, 2H), 2.3 (m, 1H), 2.9 (m, 1H), 3.65 (dd, J=7.0, 9.1, 1H), 3.85-4.0(m, 3H), 4.45 (dd, J=6.8, 14.6, 1H), 5.7 (d, J=5.1, 1H); also, 1.21 g of 26 after washing with 5% aqueous sodium carbonate (45%, $[\alpha]_D^{25}$+31.8°, MeOH); ^1H-NMR ($CDCl_3$,)d 1.85-2.1 (m, 2H), 2.1 (s, 3H), 3.1 (m, 1H), 3.75(dd, J=6.6, 9.2, 1H), 3.8-4.1 (m, 3H), 5.2 (dd, J=6.4, 14.5, 1H), 5.7 (d, J=5.2, 1H). Acetate 26 was dissolved in THF (5 mL) and 1 M aqueous LiOH solution (20 mL) was added to it. The resulting mixture was stirred at 23° C. for 3 h and the reaction was extracted with chloroform (3×25 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was chromatographed over silica gel to provide 733 mg of 27 (97% ee; $[\alpha]_D^{25}$-12.5°, MeOH).

Example 10

This example demonstrates the synthesis of activated carbonates 31 and 33, as illustrated in FIGS. 3A and 3B.

To a stirred solution of [3R,3aS, 6aS]-3-hydroxyhexahydrofuro[2,3-b]furan 25 (65 mg, 0.5 mmol) in dry CH_3CN (5 mL) at 23° C. were added disuccinimidyl carbonate (192 mg, 0.75 mmol) and triethylamine (0.25 mL). The resulting mixture was stirred at 23° C. for 12 h. The reaction was quenched with saturated aqueous $NaHCO_3$ (10 mL) and the mixture was concentrated under reduced pressure. The residue was extracted with CH_2Cl_2 (2×25 mL) and the combined organic

layers were washed with brine (10 mL) and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure gave a residue, which was chromatographed over silica gel (50% ethyl acetate/hexane) to furnish (3R,3aS, 6aR) 3-hydroxyhexahydrofuro[2,3-b]furanyl-succinimidyl carbonate 31 (70 mg) as a brown oil. Carbonate 33 (65 mg) was prepared from 60 mg of alcohol 27 by following a similar procedure.

Example 11

This example illustrates the preparation of multidrug-resistant HIV inhibitor 32, as illustrated in FIG. 3A.

To a stirred solution of amine 15 (82 mg, 0.2 mmol) in dry CH_2Cl_2 (5 mL) was added succinimidyl carbonate 31 (55 mg, 0.18 mmol). The resulting solution was stirred at 23° C. for 12 h. After this period, the reaction was quenched with saturated aqueous $NaHCO_3$ (10 mL) and diluted with CH_2Cl_2 (25 mL). The layers were separated and the organic layer was washed with brine (15 mL) and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure afforded a residue, which was purified by silica gel chromatography (75% ethyl acetate/hexane) to furnish compound 32 (85 mg) as a white solid (m.p 55-58° C.). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.71 (d, 2H, J=8.8 Hz), 7.29-7.20 (m, 5H), 6.99 (d, 2H, J=7.0 Hz), 5.65 (d, 1H, J=5.19), 5.01 (m, 2H), 3.95-3.82 (m, 7H), 3.69 (m, 2H), 3.0-2.7 (m, 6H), 1.85 (m, 1H), 1.64-1.45 (m, 3H), 0.90 (two d, 6H, J=6.5 Hz, 6.6 Hz).

Example 12

This example illustrates the preparation of multidrug-resistant HIV inhibitor 33, as illustrated in FIG. 3B.

Carbonate 33 (55 mg) was reacted with amine 15 (82 mg, 0.2 mmol) according to the procedure mentioned above to provide compound 34 (81 mg). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.69(d, 2H, J=8.8 Hz), 7.28-7.21 (m, 5H), 6.87 (d, 2H, J=5.84 Hz), 5.67 (d, 1H, J=5.46 Hz), 5.0 (m, 2H), 3.86-3.81 (m, 7H), 3.58 (dd, 2H, J=6.6 Hz, 3.6 Hz), 3.17-2.73 (m, 6H), 2.17-1.83 (m, 4H), 0.90 (two d, 6H, J=6.5 Hz, 6.6 Hz).

Example 13

This example describes the protocol for the sensitive continuous fluorogenic assay for HIV protease of the present invention and its application. Using this assay, the inhibitory activity of compound 32 (FIG. 3A) was tested against the proteases of wild-type HIV-1 (WT) and various mutant enzymes: D30N, V32I, I84V, V32I/I84V, M46F/V82A, G48V/L90M, V82F/I84V, V82T/I84V, V32I/K45I/F53L/A71V/I84V/L89M, V32I/L33F/K45I/F53L/A71V/I84V, and 20R/36I/54V/71V/82T, which protease enzymes are available from Dr. John W. Erickson, Structural Biochemistry Program, SAIC Frederick, P.O. Box B, Frederick, Md. 21702-1201, upon written request. The inhibition constant for wild-type HIV-1, $K_{i\,mut}/K_{i\,wt}$ ratio, and vitality were measured. (See Gulnik et al., *Biochemistry*, 34, 9282-9287 (1995). Protease activity was measured using the fluorogenic substrate Lys-Ala-Arg-Val-Tyr-Phe (NO$_2$)-Glu-Ala-Nle-NH$_2$ (Bachem Bioscience, Inc.). (See Peranteau et al., D. H. (1995) *Anal. Biochem.*).

Typically, 490 μl of 0.125 M ACES-NaOH buffer, pH 6.2, containing 1.25 M $(NH_4)_2SO_4$, 6.25 mM DTT and 0.1% PEG-8000 was mixed with 5 μl of titrated protease (final concentration 1-5 nM) and incubated 3 min at 37° C. The reaction was initiated by the addition of 5 μl of substrate stock solution in water. Increase in fluorescence intensity at the

emission maximum of 306 nm (excitation wavelength was 277 nm) was monitored as a function of time using Aminco Bowman-2 luminescence spectrometer (SLM Instruments, Inc.). The initial rate of hydrolysis was calculated by second degree polynomial fit using SLM AB2 2.0 operating software. Kinetic parameters were determined by nonlinear regression-fitting of initial rate versus substrate concentration data to the Michaelis-Menten equation using program Enzfiter version 1.05.

For inhibition studies, inhibitors were prepared as stock solutions at different concentrations in dimethylsulfoxide. In a typical experiment 485 μl of 0.125 M ACES-NaOH buffer, pH 6.2, containing 1.25 M $(NH_4)_2SO_4$, 6.25 mM DTT AND 0.1% PEG-8000, was mixed with 5 μl of inhibitor stock solution and 5 μl of titrated protease (final concentration of 1-5 nM) and preincubated 3 min at 37° C. The reaction was initiated by the addition of 5 μl of substrate stock solution in water. For data analysis, the mathematical model for tight-binding inhibitors was used. (See Williams and Morrison (1979), In: Methods of Enzymol. 63, (ed. D. L. Purich), 437-467, Academic Press, NY, London). The data were fitted by nonlinear regression analysis to the equation: $V=V_0/2E_t(\{[K_i(1+S/K_m)+I_t-E_t]^2+4K_i(1+S/K_m)E_t\}^{1/2}-[K_i((1+S/K_m)+I_t-E_t])$ with the program Enzfiter (version 1.05), where V and V_0 are initial velocities with and without inhibitor, respectively, K_m is a Michaelis-Menten constant, and S, E_t and I_t are the concentrations of substrate, active enzyme, and inhibitor, respectively. Biochemical fitness for each mutant was determined by comparing the biochemical vitality of each mutant ($vitality_{mut}$) with the biochemical vitality of the wild-type reference ($vitality_{wt}$), according to the formula

$$(vitality_{mut})/(vitality_{wt}),$$

wherein vitality is $(K_i)(k_{cat}/K_M)$. The results are shown below in Table 1.

TABLE 1

Compound 32			
Enzyme	K_i (pM)	$K_{i\text{-}mut}/K_{i\text{-}wt}$	Biochemical Fitness
WT	14	1	1
D30N	<5	0.33	0.3
V32I	8	0.57	0.5
I84V	40	2.85	1
V32I/I84V	70	5	0.7
M46F/V82A	<5	0.33	0.1
G48V/L90M	<5	0.33	0.1
V82F/I84V	7	0.5	0.1
V82T/I84V	23	1.57	0.1
V32I/K45I/F53L/A71V/I84V/L89M	31	2.2	0.1
V32I/L33F/K45I/F53L/A71V/I84V	46	3.3	0.1
20R/36I/54V/71V/82T	31	2.2	0.1

The above results demonstrate that compound 32 is a potent inhibitor of multiple HIV protease mutants that contain the primary or key drug resistance mutations. These data predict that compound 32 will have potent and broad-spectrum multidrug-resistant antiretroviral activity. Moreover, the biochemical fitness of each mutant relative to wild type is equal to or less than one in the presence of compound 32.

Based on this fitness profile, it is believed that drug resistant viruses containing the characteristic mutations assayed herein will not emerge from the wild-type in the presence of compound 32.

Example 14

This example illustrates the potent and broad-spectrum multidrug-resistant antiretroviral activity of an exemplary compound of the present invention.

Compound 32, shown in FIG. 3A, was tested side-by-side with four other known HIV-1 protease inhibitors against various wild-type HIV-1 strains (HIV-$1_{SARS104pre}$, HIV-1_{LAI}, and HIV-1_{MAL}), and mutant multidrug-resistant HIV-1 strains clinically isolated from eight different patients who had received numerous antiviral drugs, either singly or in combination. The patients from which the mutant strains were isolated had a history of anti-HIV therapy with a variety of different drugs such as, for example, ritonavir, saquinavir, indinavir, amprenavir, AZT, ddI, ddC, d4T, 3TC, ABV (abacavir), DLV (delaviridine), and PFA (foscarnet). The patient profiles are shown below in Table 2.

TABLE 2

Patient/ Isolate Code	$CD4^+$ ($/mm^3$)	HIV-1 RNA level (copies/mL)	Months on Antiviral Therapy	Prior and Present Anti-HIV Therapy
1	281	246,700	64	AZT, ddI, ddC, d4T, 3TC, ABV, IDV, RTV, SQV, AMV, DLV
2	3	353,700	46	AZT, ddI, ddC, d4T, 3TC, ABV, IDV, SQV, AMV
3	108	42,610	30	AZT, ddI, ddC, d4T, 3TC, ABV, IDV, SQV, AMV
4	360	60,000	81	AZT, ddI, ddC, D90, d4T, 3TC, ABV, IDV, SQV, AMV
5	—	—	32	AZT, ddI, ddC, d4T, 3TC, ABV, IDV, SQV, AMV
6	—	—	34	AZT, ddI, ddC, d4T, 3TC, ABV, IDV, SQV, AMV
7	—	—	83	AZT, ddI, ddC, d4T, 3TC, ABV, IDV, SQV, RTV, AMV
8	—	—	60	AZT, ddI, ddC, d4T, 3TC, PFA, ABV, IDV, SQV, AMV

The four known chemotherapeutic HIV protease inhibitors used for comparative purposes in this example have been utilized in actual human HIV chemotherapy, and are: Ritonavir ("RTV," Abbott Laboratories); Indinavir ("IDV," Merck Research Laboratories); Amprenavir (AMV, See Ghosh et al., *Bioorg. Med. Chem. Lett.*, 8, 687-690 (1998)); and Saquinavir ("SAQ", Roche Research Centre). The IC_{50} values (μM) for all five compounds were determined with respect to wild-type and multidrug-resistant HIV-1.

To determine protease inhibitory activity against multidrug resistant HIV, the IC_{50}'s were measured against a panel of clinically isolated mutant HIV isolates. The IC_{50}'s were determined by utilizing the PHA-PBMC exposed to HIV-1 (50 $TCID_{50}$ dose/1×10^6 PBMC) as target cells and using the inhibition of p24 Gag protein production as an endpoint.

The IC_{50}'s were determined by utilizing the PHA-PBMC assay in which target cells are exposed to HIV-1 (50 $TCID_{50}$ dose/1×10^6 PBMC) and inhibition of p24 Gag protein production is used as an endpoint. All drug sensitivities were performed in triplicate. In order to determine whether the HIV isolates were syncitium inducing (SI) or non-syncitium

inducing (NSI), an aliquot of viral stock supernatant, containing 100 $TCID_{50}$, was cultured with 1×10^5 MT-2 cells in a 12-well plate. Cultures were maintained for four weeks and were examined for syncytium formation twice a week. The results are shown below in Table 3.

TABLE 3

		IC_{50} (μM)				
Phenotype	Patient/ Isolate code (See Table 2)	RTV	IDV	AMV	SAQ	Compound 32
SI	HIV-$1_{SARS104pre}$	0.055	0.013	0.021	0.01	<0.001
SI	HIV-1_{LAI}	0.0047	0.019	0.019	0.0054	0.0004
NSI	HIV-1_{MAL}	0.018	0.0056	0.014	0.0057	0.0004
	1	>1	>1	0.29	0.29	0.002
	2	>1	0.24	0.24	0.035	<0.001
	3	>1	0.46	0.33	0.036	<0.001
	4	>1	0.24	0.4	0.033	0.001
	5	>1	0.8	0.28	0.24	0.002
	6	>1	0.37	0.11	0.19	<0.001
	7	>1	>1	0.42	0.13	0.004
	8	>1	>1	0.22	0.009	0.001

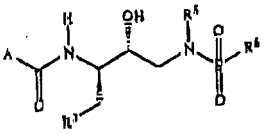
The above IC_{50}'s clearly demonstrate the broad-spectrum and extraordinarily potent activity of compound 32 against wild-type HIV-1 and the eight different multidrug-resistant clinical isolates tested as was predicted from the biochemical fitness profiles in Example 13. For example, compound 32 exhibits nanomolar and sub-nanomolar potency against all the multidrug-resistant strains tested, whereas Ritonavir, a reasonably potent wild-type inhibitor, is virtually inactive toward the resistant viruses. Moreover, compound 32 is about 9 to about 150 times more potent against the multidrug-resistant viruses than Saquinavir, one of the most potent known compounds against known multidrug-resistant strains of HIV-1. Patients with viral plasma loads greater than 10,000 RNA copies/mm^3 are at risk for developing fatal AIDS complications. There are no effective therapeutic options currently available for these patients infected with these multidrug resistant viruses. Compound 32 and analogs thereof are predicted to be potent in preventing the selection of these viral strains in vivo.

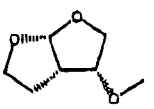
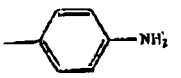
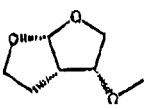
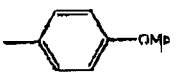
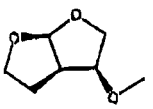
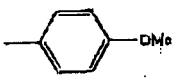
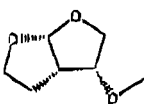
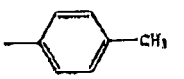
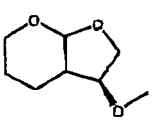
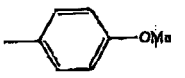
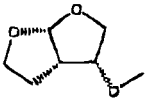
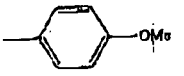
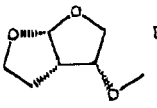
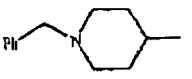
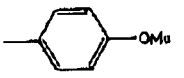
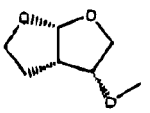
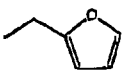
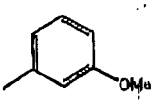
Example 15

This example demonstrates the wild-type antiretroviral activity of the compounds of the present invention.

It is predicted that the activity of the present inventive compounds against wild-type HIV protease correlates with of antiretroviral activity against multidrug-resistant HIV. Numerous compounds of the present invention were tested against wild-type HIV (See, Ghosh et al., *J. Bioorg. Med. Chem. Lett.*, 8, 6870690 (1998)). Exemplary compounds, which demonstrate potent wild-type HIV protease activity, are shown below in Table 4.

TABLE 4



A	R^2	R^3	R^6	Ki (nM)	ID_{50} (nM)	Comments
	Ph			2.1	4.5	
	Ph			1.1	1.4	Compound 32 (FIG. 3A)
	Ph					Compound 34 (FIG. 3B)
	Ph			1.2	3.5	
	Ph			2.2	4.5	
	Ph					
						
	Ph					

It is believed that the above compounds in Table 4 will prevent the emergence of resistance in an HIV-infected human.

Example 16

This example demonstrates the oral absorption of compound 32 in an in vivo experimental model.

Compound 32 was orally administered to a rat at a dose of about 40 mg per kg body mass, using a PEG 300 vehicle as a carrier. The plasma blood levels of compound 32 were measured over a 24 h period after oral administration. The results are shown in Table 5 below.

TABLE 5

Time After Administration		Plasma Concentration	
Hours	Minutes	(nM)	(ng/mL)
0.25	17	1598	898
1.00	60	878	493
2.07	124	626	352
4.01	240	670	377
6.01	360	594	334
8.05	483	1115	627
12.04	722	246	138
14.08	845	102	57
24.00	1440	82	46

These results demonstrate that compound 32 maintains high blood levels (e.g., nearly 0.6 uM after 6 hours) long after oral administration. Although applicants do not wish to abound by any one particular theory, it is believed that the non-peptide structure of the compounds of the present invention make them less prone to biological (e.g., enzymatic) degradation, and thereby contribute to their prolonged blood levels after oral administration. From these data, the compounds of the present invention are predicted to have excellent oral bioavailability in humans, and maintain therapeutically significant blood levels over prolonged periods after oral administration.

Example 17

This example demonstrates the influence of human protein binding on the antiviral activity of compound 32. Several potent and orally bioavailable HIV protease inhibitors failed to have in vivo antiviral efficacy. These failures have been ascribed, but not definitively proven, to be due to excessive binding to human plasma proteins, particularly serum albumin and AAG. The protein binding against human alpha acid glycoprotein (AAG, 10 μM) and against human serum albumin (HAS, 300 μM) were compared for compound 32 and amprenavir, a structurally related analog that is an FDA approved drug. The results are shown in Table 6.

TABLE 6

	IC_{50} (μM)		
Compound	(-)	AAG	AH
32	0.0015 (1X)	0.0022 (1.5X)	0.003 (2X)
amprenavir	0.020 (1X)	0.18 (6X)	0.021 (1X)

These data demonstrate that the presence of AAG and HAS in physiologically excessive amounts does not adversely affect the antiviral activity of compound 32. From these data, the affinity of compound 32 for human AAG and HSA is predicted to be actually lower than that for amprenavir, a known drug. From these data, the compounds of the present invention are expected to have excellent in vivo efficacy in humans, and maintain therapeutically significant levels over prolonged periods of time.

Example 18

This example describes the inhibitory activity of compounds 35 (FIG. 5A), 36 (FIG. 5B), 37 (FIG. 5C) and 38 (FIG. 5D), in accordance with the technique disclosed in Example 13 above, the inhibitory activity of these compounds was tested against proteases of the wild-type HIV-1. Compound 36, 37 and 38 were also tested against proteases containing the deleterious drug resistance associated mutations V82F/I84V and G48V/V82A. Fitness was determined in accordance with Example 13. The results of these experiments are shown below in Table 7.

TABLE 7

COMPOUND	ENZYME	K_i (pM)	$K_{i\text{-}mt}/K_{i\text{-}wt}$	Fitness
35	WT	81	1	
36	WT	5<		
	V82F/I84V	24.4	>4.9	>0.8
	G48V/V82A	15.3	>3.0	>0.8
37	WT	12	1	
	V82F/I84V	25.7	2.1	0.3
	G48V/V82A	64	5.3	1.4
38	WT	>5		
	V82F/I84V	66.8	>13	>2.1
	G84V/V82A	34	>6.8	>1.8

These results further demonstrate compounds of the present invention that are potent inhibitors against mutant proteases. Based on the fitness profile, it is believed that drug resistant viruses containing the characteristic mutations assayed herein will not emerge from the wild-type in the presence of compound 37.

Example 19

This example further demonstrates the broad-spectrum and potent activity of exemplary compounds of the present invention against multidrug-resistant clinical isolates.

The IC_{50} values (μM) for all compounds 32, 35, 36, 37, and 38 were determined with respect to wild type clinical isolates HIV-1_{LAI} and HIV-1_{BaL}. The latter is a monocytotropic strain of HIV.

The IC_{50}'s for isolates HIV-1_{LAI} and HIV-1_{BaL} were determined by exposing the PHA-stimulated PBMC to HIV-1 (50 $TCID_{50}$ dpse/1×10^6 PBMC), in the presence of various concentrations of compounds 32, 35, 36, 37 and 38, and using the inhibition of p24 Gag protein production as an endpoint on day 7 of culture ("p24 assay"). All drug sensitivities were performed in triplicate. The IC_{50}'s for isolate HIV-1_{LAI} were also determined by exposing MT-2 cells (2×10^3) to 100

$TCID_{50}$s of HIV-1_{LAI} cultured in the presence of various concentrations of compounds **32**, **35**, **36**, **37** and **38**. The IC_{50}'s were determined using the MTT assay on day 7 of culture. All sensitivities were determined in duplicate. The results are shown below in Table 8.

TABLE 8

Virus	Cell Type/ Assay	Comp. 32 IC_{50} (μM)	Comp. 35 IC_{50} (μM)	Comp. 36 IC_{50} (μM)	Comp. 37 IC_{50} (μM)	Comp. 38 IC_{50} (μM)
HIV-1_{LAI}	MT-2/MTT	0.00022	0.028	0.017	0.0053	0.028
HIV-1_{LAI}	PBMC/p24	0.00022	0.020	0.034	0.0027	0.0080
HIV-1_{RF-L}	PBMC/p24	0.00033	0.013	0.038	0.0030	0.0093

These results demonstrate the potent antiretroviral activity of particular compounds of the present invention.

Example 20

This example further illustrates the potent and broad-spectrum multidrug-resistant antiretroviral activity of an exemplary compound of the present invention.

Compound 32, shown in FIG. 3A, was tested against various mutant multidrug-resistant HIV-1 strains clinically isolated from patients. These isolates were all taken from patients who failed therapy on one or more HIV protease inhibitors due to high level clinical resistance. All of these isolates exhibit high level phenotypic resistance in antiviral assays against many of the commonly use HIV protease inhibitor drugs. Compound 32 was tested against these multidrug-resistant clinical isolates side-by-side with known drugs that are commonly used in HIV antiviral therapy, including reverse transcriptase inhibitors such as AZT, 3TC, DDI, DDC, and D4T, and protease inhibitors such as Indinavir (Ind.), Nelfinavir (Nel.), Ritonavir (Rit.), and Saquinavir (Saq.). The IC_{50}'s for compound 32 and the comparative drugs against the multidrug-resistant HIV-1 clinical isolates, and against wild-type HIV-1 (WT), are shown in Table 9a.

The mutant multidrug-resistant HIV-1 strains corresponding to each patient, numbered 9-35, were genetically analyzed in terms of the nucleic acid sequences of the protease (PR) and a portion of the reverse transcriptase (RT) genes from which mutations in those enzymes were determined. The mutations in the protease and reverse transcriptase of the multidrug-resistant viruses isolated from each patient are shown below in Table 9b.

TABLE 9a

	IC_{50} (μM)									
Patient Isolate	AZT	3TC	DDI	DDC	D4T	Ind.	Nel.	Rit.	Saq.	Comp. 32
9	0.01	0.30	0.7	0.15	0.01	1.087	0.98	0.53	>0.3125	0.0003
10	0.02	1.35	1.7	0.37	1.29	>1.25	>1.25	2.03	>0.3125	0.0017
11	0.11	23.61	2.4	0.18	3.10	0.012	0.03	0.01	0.001	0.0004
12	0.07	0.78	0.9	0.20	1.23	>1.25	>1.25	2.47	>0.3125	0.0010
13	0.17	1.04	0.5	<0.1221	0.78	>1.25	0.47	1.64	>0.3125	0.0004
14	0.64		2.4	<0.1221	1.10	0.089	0.01	0.04	0.040	0.0003
15	0.20	>31.25	2.2	0.32	1.10	0.265	0.47	1.14	>0.3125	0.0011
16	0.97	37.98	3.5	0.57	1.81	0.384	0.86	1.34	>0.3125	0.0021
17	>1.25	26.05		0.63	4.28	0.502	0.53	0.87	0.107	0.0022
18	0.55	>31.25	2.2	0.48	2.08	0.369	0.60	3.02	0.039	0.0019
19	>1.25	>31.25	36.6	6.80	35.63	0.784	0.50	2.94	0.055	0.0005
20	1.25	3.21	7.1	0.57	22.54	0.501	0.58	1.90	0.032	
21	>1.25	1.69	1	0.38	3.38	1.250	>1.25	2.18	0.21	0.0023
22	1.02	>31.25	3.7	0.63	4.68	0.173	0.10	0.36	0.003	
23	0.19	>31.25	1.8	0.28	1.00	0.461	0.28	1.82	0.008	0.0004
24										0.0004
25										0.0019
26										0.0019
27	0.03	1.72	2.4	0.41	4.00	>1.25	>1.25	2.97	>0.3125	0.0009
28	>1.25	2.08	2.8	0.36	5.44	1.040	>1.25	2.66	>0.3125	
29	>1.25	2.24	3.8	0.34	5.29	0.569	0.67	0.36	0.050	0.0000
30	0.16	>31.25	2.8	0.24	2.92	0.270	0.52	1.03	0.191	0.0019
31		>31.25	2.6	<0.1221	3.11	0.251	0.24	0.85	0.074	0.0010
32	0.32	>31.25	8.4	0.91	2.41	0.223	0.22	0.37	>0.3125	
33	0.51	>31.25	2.0	0.28	2.73	0.133	0.35	0.18	0.059	0.0005
34	>1.25	>31.25	9.1	1.13	7.71	0.595	0.24	3.38	0.063	0.0024
35	0.98	>31.25	17.0	2.46	18.13	0.500	0.48	2.69	0.0616	0.0012
(WT)	0.022	0.264	0.895	0.243	1.050	0.02	0.031	0.019	0.007	0.0007

TABLE 9b

Isolate		Mutations						
9	PR	V0031	L010I	S037N	R041K	G048V	I054S	I062V
	RT	P004S	V060I	V090I	E122K	I135V	Q174K	Y181C
		E207R	L301L/I					
10	PR	V003I	L010I.	S037N	R041K	G048V	I054S	I062V
	RT	P004S	V060I	V090I	E122K	I135V	T165A/T	Q174K
		V245M	R277K					

TABLE 9b-continued

11	PR	V003I	L010I	I015V	M036I	S037N	R041K	L063T
	RT	K020R/K	M041L	K043Q	E044D	V060I	D067N	T069D
		L210W	R211K					
12	PR	V003I	L010I	I015V	K020R	M036I	S037N	R041K
		I093L						
	RT	M041L	K043Q	E044D	V060I	D067N	T069D	L074L/I
		L201W	R211K					
13	PR	V003I	L010I	I015V	K020R/K	M036I	S037N	R041K
		I072T/I	T074A/T	V082A	I093L			
	RT	M041L	K043Q	E044D	V060I	D067N	T069D	L074L/I
		L210W	R211K					
14	PR	V003I	L010I	K020R	E035D	M036I	S037D	R041K
	RT	M041L	T069T/N	L074L/V	E122K	D123E	Y181C	Q207E
		R277K	E297K					
15	PR	V003I	L010I	E035D	R041K	L063P	A071A/V	I072V/I
	RT	D067N	T069D	I142V	E169D	Y181C	M184V	Q207E
		L283I	I293V					
16	PR	V003I	L010I	I015V	E035D	S037A	R041K	L063P
	RT	K020R	M041L	K043N	D067N	D123N	D177E	I178M/I
		R277K	G333E					
17	PR	V003I	L010I	I015V	E035D	S037A	R041K	L063P
	RT	K020R	M041L	K043N	D067N	D123N	D177E	I178M/I
		G333D	A360T					
18	PR	V003I	L010V	S037N	K043T	I054V	L063P	A071V
	RT	K020R	V035M	K064H	D067G	T069N	K070R	K102R/K
		D128E	K219Q					
19	PR	V003I	L010I	L010I	S037Q	M046L	I054V	R057K
	RT	K020R	T058N	A063V	S068G	T069T/I	V075I	F077L
		Y181C	M184V					
20	PR	V003I	L010I	T012P	K014R	I015V/I	G016D	S037N
		V077I	V082A	I085V	L090M			
	RT	K020R	V035I	T039A	M041L	K043E	E044A	D067N
		L210W	R211K					
21	PR	V003I	L010I	I015V	K020R	E035D	M036I	S037K
		T074S	V082F	N088D	L090M	I093L		
	RT	K020R	V035T	T039R	M041L	K043E	E044D	V060I
		I135T/I	I142V					
22	PR	V003I	L010I	E034E/Q	S037N	M046I	I054V	I062V
	RT	K020R/K	T039A/T	M041L	K043E	E044D	D067N	V118I
		L214P	T215Y					
23	PR	V003I	L010I	I015V	K020I	L024I	M036I	S037N
	RT	K011R	D067N	K070R	I135T	Y181V/D	M184V	D218E/D
		M357T/M	Q359G/E					
24	PR	V003I	I015V	D030N	E035D	S037D	L063P	V077I
	RT	K064R	E122K	D123E	D177E	M184V	Q196R	R211Q
		N248I	R258K					
25	PR	V003I	K020I	T026T/I	S037N	M046I	L063P	A071V
	RT	V035M	D067N	T069D	K070R	E122P	D177E	M184V
		E224K	R277K					
26	PR	V003I	L010I	S037N	K043K	G048V	I054S	I062V
	RT	P004S	V060I	V090I	E122K	I135Y	T135A/T	Q174K
		V246M	R277K					
27	PR	V003I	L010I	I015V	K020R	M036I	S037N	R041K
		I093L						
	RT	M041L	K043Q	E044D	V060I	D067N	T069D	L074L/I
		H208Y	L210W					
28	PR	V003I	L010I	I015V	M036I	S037D	G048V	I054V
		L090M	I093L					
	RT	P004S	M041L	D067N	T069D	K070R	V090I	K103N
		L214P	T215P					
29	PR	V003I	L010I	K020I	S037N	M046M/I	L063P	I072I/K
	RT	V035I	T039A/E	M041L	E044D	L074L/V	R083K	K102Q
		L214P	T215Y					
30	PR	V003I	L010I	E035D	R041K	L063P	A071A/V	I072V/I
	RT	D067N	T069D	I142V	E169D	Y181C	M184V	Q207E
		L283I	I293V					
31	PR	V003I	L010L/I	E035D	M036M/I	S037N	M046K	I054V
	RT	K032R/K	K064R	D067N	K070R	K103N/K	E122K	Y181F/C
		T286A	I293V					
32	PR	V003I	L010I	S037N	G048V	I054V	I062V/I	L063P
	RT	K020R	M041L	D123N	I178L	M184V	T200A/T	E203D
		Q334L/Q	T338S/T					
33	PR	V003I	L010I	E035D	M036I	S037D	D060E	L063P
	RT	M041L/M	D067N	T069T/N	K070R	D177D/E	M184V	I202V
		V245T	P272A					
34	PR	V003I	L010V	S037N	K043T	I054V	L063P	A071V
	RT	K020R	V035M	K064H	D067G	T069N	K070R	K102R/K
		D218G	K219Q					

TABLE 9b-continued

35	PR	V003I	L010I	L010I	S037Q	M046L	I054V	R057K
	RT	K020R	T039N	A062V	S068G	T069T/I	V075I	F077L
		Y181C	M184V					

Isolate		Mutations						
9	PR	L063S	I064L	I064L	A071V	V082A	I093L	
	RT	E194E/K	G196E	R211K	L214F	V245M	R227K	
10	PR	L063S	I064L	I064L	A071V	V082A	I093L	
	RT	Y181C	E194K	G196E	R211K	L214F	H221H/Y	
11	PR	I093L						
	RT	E122E/K	D123E	Y181C/Y	M184V	G196E	H208Y	
12	PR	Q048V	I054T/I	L063T	A071V	T074A	V082A/V	
	RT	K103N	D123E	H135T	Y181C	G196E	H208Y	
13	PR	Q048V/Q	I054T/I	Q058E/Q	Q061R/Q	L063T	A071A/V	
	RT	K103N	D123E	H135T/I	Y181C	G196E	H208Y	
14	PR	Q048V	L063C	A071V	I073T	V082A/V	I093L	
	RT	L210W	R211K	L214F	T215Y	L228R	E248D	
15	PR	G073R/C	V077I	I084V	L090M	I093L		
	RT	R211K	L214F	T215Y	D250E	P272A	Q278E	
16	PR	A071V	G073E	I084V	L090M			
	RT	M184V	G196E	E203D	L214F	T215Y	K219Q	
17	PR	A071V	G073G/S	I084V	L090M			
	RT	M184V	G196E	E203D	L214F	T215Y	R277K	
18	PR	V082A	L090M					
	RT	V118I	E122K	H135T	S162A	M184V	T215S	
19	PR	L063P	A071V	V082A	L090M			
	RT	A098S	K103N	F116Y	H135T	I142M	Q151M	
20	PR	M046I	I054V	K055R	I062V	L063N	A071T	
	RT	V075A	K103N	V118I	H135M	Y181C	H208Y	
21	PR	R041N	K043T/K	M041I	L063P	H069K	A071V	
	RT	I063M/I	D067N	T069D	A098G	V118I	D121N	
22	PR	L063S	V082A	L089L/M				
	RT	M184V	E203E/K	Q207E	H208Y	L210W	R211K	
23	PR	I054V	R057K	L063P	A071V	V082A		
	RT	K219Q	P272A	R277K	R284I/K	I293V	E297V	
24	PR	N088D						
	RT	L214F	V245T/M	E207A	D126V	D129L	T139S	
25	PR	G073S	V077I	I084V	L090M	I093L		
	RT	I202V	Q207E	R211K	L214F	T215F	K219Q	
26	PR	L063S	I064L	A071V	V082A	I093L		
	RT	Y181C	E194K	G196E	R211K	L214F	H221H/Y	
27	PR	Q048V	I054T/I	L063T	A071A/V	T074A	V082A	
	RT	K103N	F116F/L	D123E	H135T	Y181C	G196E	
28	PR	D060E	Q061E	I062V	I064V	A071V	V082A	
	RT	H135T	S162A	V179I	Y181C	G196E	Q207E	
29	PR	G073C	V077I	L090M				
	RT	S162C	H178L	E203K	H208Y	L210W	R211K	
30	PR	G073G/S	V077I	I084V/I	L090M	I093L		
	RT	R211K	L214F	T215Y	D250E	P272A	Q278E	
31	PR	L063P	I066F	A071V	V082A/T	I084V/I		
	RT	M184V	R211K	L214F	D218E	K219Q	E248D	
32	PR	A071A/T	V077I	V082A	I093L			
	RT	Q207E	L210L/W	L214F	T215Y	R277K	T286A	
33	PR	I064V	I084V	L090M				
	RT	Q207E	L210W	R211K	L214F	T215Y	K219Q	
34	PR	V082A	L090M					
	RT	V118I	E122K	H135T	S162A	M184V	T215S	
35	PR	L063P	A071V	V082A	L090M			
	RT	A098S	K103N	F116Y	H135T	I142M	Q151M	

The results of this experiment further show the effectiveness of an exemplary compound of the present invention against a wide range of viral mutants compared to other well-known inhibitors. These mutant viruses represent a panel of the most broadly cross resistant clinical isolates known to date based on their resistance to therapeutically used HIV protease inhibitors. Compound 32 was consistently potent against all of the clinically isolated mutant viruses tested, and was significantly more potent against these multidrug resistant viruses than the comparative drugs which are currently used in human HIV-1 therapy. Compound 32 was ten to one-thousand times more potent against these viruses than even saquinavir, one of the most potent known compounds against multidrug-resistant HIV-1. Based on the high potency, it is believed that these mutants will not only be inhibited, but also that these mutants would not be able to emerge if the compound is administered to a patient infected with a predecessor virus.

All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties by reference.

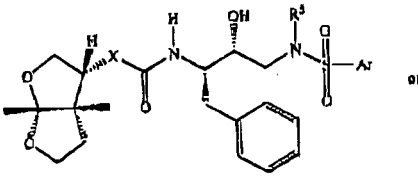
While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiments may be used and that it is intended that the invention may be practiced otherwise than as specifically

described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

What is claimed is:

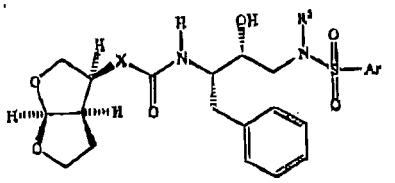
1. A method of treating a HIV-infected mammal who has developed resistance to HIV treatments, the method comprising (i) determining whether the mammal has developed resistance to HIV treatments; (ii) administering to the HIV-infected mammal an effective amount of a compound of the formula:

(II)



or

(III)



wherein X is oxygen, R^3 is isobutyl, and Ar is substituted phenyl; and

(iii) administering at least one antiviral agent selected from the group consisting of ritonavir, indinavir, amprenavir and saquinavir; whereby the HIV-infected mammal is treated.

2. The method of claim 1, wherein Ar is a phenyl substituted at the para-position.

3. The method of claim 1, wherein Ar is a phenyl substituted at the meta-position.

4. The method of claim 1, wherein Ar is a phenyl substituted at the ortho-position.

5. The method of claim 1, wherein Ar is selected from the group consisting of para-aminophenyl, para-toluoyl, para-methoxyphenyl, meta-methoxyphenyl, and meta-hydroxymethylphenyl.

6. The method of claim 1, wherein the HIV-infected mammal is infected with a wild-type HIV.

7. The method of claim 1, wherein the HIV-infected mammal is infected by a mutant HIV with least one protease mutation.

8. The method of claim 1, wherein the HIV-infected mammal is infected by a mutant HIV having at least one reverse transcriptase mutation.

9. The method of claim 1, wherein the at least one antiviral agent is ritonavir.

* * * * *

Exh A
10-5956 (WHW)

UNITED STATES DEPARTMENT OF JUSTICE

COMMERCIAL LITIGATION BRANCH-CIVIL DIVISION

FACSIMILE TRANSMITTAL

TO: Diane Richards
973.645.6689 6142

FROM: Donna G. Hall

TELE NO. 202-514-4325

FAX NO: 973.645.6689

DATE: 11.17.2010

NUMBER OF PAGES INCLUDING COVER SHEET

COMMENTS: For Case 10-cv-5956, Civil Case Sheet
- Exhibit "A".
Thanks!
Donna G. Hall

CALL TO VERIFY RECEIPT

10-5956 (JHW)

CIVIL COVER SHEET

JS 44 (Rev. 12/07, NJ 5/08)

The JS 44 civil cover sheet and the information contained herein neither replace nor supplement the filing and service of pleadings or other papers as required by law, except as provided by local rules of court. This form, approved by the Judicial Conference of the United States in September 1974, is required for the use of the Clerk of Court for the purpose of initiating the civil docket sheet. (SEE INSTRUCTIONS ON THE REVERSE OF THE FORM.)

I. (a) PLAINTIFFS

The United States of America and The Board of Trustees of the University of Illinois.

(b) County of Residence of First Listed Plaintiff: Washington, D.C.

(c) Attorney's (Firm Name, Address, Telephone Number and Email Address)

Daniel Gibbons Daniel.Gibbons@usdoj.gov
Office of the U.S. Attorney
970 Broad Street, 7th Floor
Newark, NJ 07102 973.645.2700

DEFENDANTS

Mylan Pharmaceuticals Inc. and Lupin Pharmaceuticals, Inc. and Lupin Limited.

County of Residence of First Listed Defendant: Monongalia, WV

NOTE: IN LAND CONDEMNATION CASES, USE THE LOCATION OF THE LAND INVOLVED.

Attorneys (If Known)

William A. Rakoczy

II. BASIS OF JURISDICTION (Place an "X" in One Box Only)

- ☒ 1 U.S. Government Plaintiff
- ☐ 2 U.S. Government Defendant
- ☐ 3 Federal Question (U.S. Government Not a Party)
- ☐ 4 Diversity (Indicate Citizenship of Parties in Item III)

III. CITIZENSHIP OF PRINCIPAL PARTIES (Place an "X" in One Box for Plaintiff and One Box for Defendant)
(For Diversity Cases Only)

	PTF	DEF		PTF	DEF
Citizen of This State	☐ 1	☐ 1	Incorporated or Principal Place of Business In This State	☐ 4	☐ 4
Citizen of Another State	☐ 2	☐ 2	Incorporated and Principal Place of Business In Another State	☐ 5	☐ 5
Citizen or Subject of a Foreign Country	☐ 3	☐ 3	Foreign Nation	☐ 6	☐ 6

IV. NATURE OF SUIT (Place an "X" in One Box Only)

CONTRACT
- ☐ 110 Insurance
- ☐ 120 Marine
- ☐ 130 Miller Act
- ☐ 140 Negotiable Instrument
- ☐ 150 Recovery of Overpayment & Enforcement of Judgment
- ☐ 151 Medicare Act
- ☐ 152 Recovery of Defaulted Student Loans (Excl. Veterans)
- ☐ 153 Recovery of Overpayment of Veteran's Benefits
- ☐ 160 Stockholders' Suits
- ☐ 190 Other Contract
- ☐ 195 Contract Product Liability
- ☐ 196 Franchise

REAL PROPERTY
- ☐ 210 Land Condemnation
- ☐ 220 Foreclosure
- ☐ 230 Rent Lease & Ejectment
- ☐ 240 Torts to Land
- ☐ 245 Tort Product Liability
- ☐ 290 All Other Real Property

TORTS

PERSONAL INJURY
- ☐ 310 Airplane
- ☐ 315 Airplane Product Liability
- ☐ 320 Assault, Libel & Slander
- ☐ 330 Federal Employers' Liability
- ☐ 340 Marine
- ☐ 345 Marine Product Liability
- ☐ 350 Motor Vehicle
- ☐ 355 Motor Vehicle Product Liability
- ☐ 360 Other Personal Injury

PERSONAL INJURY
- ☐ 362 Personal Injury - Med. Malpractice
- ☐ 365 Personal Injury - Product Liability
- ☐ 368 Asbestos Personal Injury Product Liability

PERSONAL PROPERTY
- ☐ 370 Other Fraud
- ☐ 371 Truth in Lending
- ☐ 380 Other Personal Property Damage
- ☐ 385 Property Damage Product Liability

CIVIL RIGHTS
- ☐ 441 Voting
- ☐ 442 Employment
- ☐ 443 Housing/ Accommodations
- ☐ 444 Welfare
- ☐ 445 Amer. w/Disabilities - Employment
- ☐ 446 Amer. w/Disabilities - Other
- ☐ 440 Other Civil Rights

PRISONER PETITIONS
- ☐ 510 Motions to Vacate Sentence
- Habeas Corpus:
- ☐ 530 General
- ☐ 535 Death Penalty
- ☐ 540 Mandamus & Other
- ☐ 550 Civil Rights
- ☐ 555 Prison Condition

FORFEITURE/PENALTY
- ☐ 610 Agriculture
- ☐ 620 Other Food & Drug
- ☐ 625 Drug Related Seizure of Property 21 USC 881
- ☐ 630 Liquor Laws
- ☐ 640 R.R. & Truck
- ☐ 650 Airline Regs.
- ☐ 660 Occupational Safety/Health
- ☐ 690 Other

LABOR
- ☐ 710 Fair Labor Standards Act
- ☐ 720 Labor/Mgmt. Relations
- ☐ 730 Labor/Mgmt.Reporting & Disclosure Act
- ☐ 740 Railway Labor Act
- ☐ 790 Other Labor Litigation
- ☐ 791 Empl. Ret. Inc. Security Act

IMMIGRATION
- ☐ 462 Naturalization Application
- ☐ 463 Habeas Corpus - Alien Detainee
- ☐ 465 Other Immigration Actions

BANKRUPTCY
- ☐ 422 Appeal 28 USC 158
- ☐ 423 Withdrawal 28 USC 157

PROPERTY RIGHTS
- ☐ 820 Copyrights
- ☒ 830 Patent
- ☐ 840 Trademark

SOCIAL SECURITY
- ☐ 861 HIA (1395ff)
- ☐ 862 Black Lung (923)
- ☐ 863 DIWC/DIWW (405(g))
- ☐ 864 SSID Title XVI
- ☐ 865 RSI (405(g))

FEDERAL TAX SUITS
- ☐ 870 Taxes (U.S. Plaintiff or Defendant)
- ☐ 871 IRS—Third Party 26 USC 7609

OTHER STATUTES
- ☐ 400 State Reapportionment
- ☐ 410 Antitrust
- ☐ 430 Banks and Banking
- ☐ 450 Commerce
- ☐ 460 Deportation
- ☐ 470 Racketeer Influenced and Corrupt Organizations
- ☐ 480 Consumer Credit
- ☐ 490 Cable/Sat TV
- ☐ 810 Selective Service
- ☐ 850 Securities/Commodities/ Exchange
- ☐ 875 Customer Challenge 12 USC 3410
- ☐ 890 Other Statutory Actions
- ☐ 891 Agricultural Acts
- ☐ 892 Economic Stabilization Act
- ☐ 893 Environmental Matters
- ☐ 894 Energy Allocation Act
- ☐ 895 Freedom of Information Act
- ☐ 900Appeal of Fee Determination Under Equal Access to Justice
- ☐ 950 Constitutionality of State Statutes

V. ORIGIN (Place an "X" in One Box Only)

☒ 1 Original Proceeding ☐ 2 Removed from State Court ☐ 3 Remanded from Appellate Court ☐ 4 Reinstated or Reopened ☐ 5 Transferred from another district (specify) ☐ 6 Multidistrict Litigation ☐ 7 Appeal to District Judge from Magistrate Judgement

VI. CAUSE OF ACTION

Cite the U.S. Civil Statute under which you are filing (Do not cite jurisdictional statutes unless diversity): 35 U.S.C. § 271

Brief description of cause: Patent Infringement

VII. REQUESTED IN COMPLAINT:

☐ CHECK IF THIS IS A CLASS ACTION UNDER F.R.C.P. 23

DEMAND $

CHECK YES only if demanded in complaint:
JURY DEMAND: ☐ Yes ☐ No

VIII. RELATED CASE(S) (See instructions): JUDGE DOCKET NUMBER

Explanation: Case name TIBOTEC INC. and TIBOTEC PHARMACEUTICALS v. LUPIN LIMITED, LUPIN PHARMACEUTICALS INC., MYLAN PHARMACEUTICALS INC. and MYLAN INC. filed 11/15/2010

DATE: 11/15/2010

SIGNATURE OF ATTORNEY OF RECORD

Exhibit 6

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Attorneys for Plaintiffs,
DELANO FARMS COMPANY; FOUR STAR FRUIT, INC.;
GERAWAN FARMING, INC.

UNITED STATES DISTRICT COURT

EASTERN DISTRICT OF CALIFORNIA

DELANO FARMS COMPANY; FOUR STAR FRUIT, INC.; GERAWAN FARMING, INC. Plaintiffs, vs. THE CALIFORNIA TABLE GRAPE COMMISSION; UNITED STATES OF AMERICA; UNITED STATES DEPARTMENT OF AGRICULTURE; TOM VILSACK, SECRETARY OF THE UNITED STATES DEPARTMENT OF AGRICULTURE (IN HIS OFFICIAL CAPACITY) Defendants.	CASE NO. 1:07-CV-1610 OWW (SMS) **FIRST AMENDED COMPLAINT** **DEMAND FOR JURY TRIAL**

For their first amended complaint against Defendant The California Table Grape Commission ("the Commission") and Defendants the United States of America ("U.S."), the United States Department of Agriculture ("USDA") and Tom Vilsack, Secretary of the United States Department of Agriculture (in his official capacity) ("Secretary Vilsack"), Plaintiffs Delano Farms Company; Four Star Fruit, Inc.; and Gerawan Farming, Inc. (collectively "Plaintiffs") alleges as follows:

JURISDICTION AND VENUE

1. This is a civil action arising in part under laws of the United States relating to patents (35 U.S.C. §§271, 281, 283, 284 and 285) and the Sherman Act and Clayton Act (15 U.S.C. §1, *et seq*.). This Court has federal jurisdiction of such federal question claims pursuant to 28 U.S.C. §§ 1331, 1337 and 1338(a). Additionally, this Court has jurisdiction over the claims for declaratory judgment of invalidity pursuant to the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202. This Court also has jurisdiction over the claims for declaratory judgment against the United States of America, the United States Department of Agriculture and Tom Vilsack, Secretary of the United States Department of Agriculture, pursuant to the Administrative Procedure Act, 5 U.S.C. §§ 701, *et seq*.

2. This Court has supplemental jurisdiction over the claims in this Complaint that arise under statutory and common law of the State of California pursuant to 28 U.S.C §1367(a), because the state law claims are so related to the federal claims that they form part of the same case or controversy and derive from a common nucleus of operative facts.

3. The acts and transactions complained of herein were conceived, carried out, made effective, and had effect within the State of California and within this district, among other places. Venue is proper under 28 U.S.C. §§1391(b), 1391(c) and 1400(b), in that Plaintiffs reside in this District, Defendant the California Table Grape Commission resides in this District and has a regular and established place of business in this District and in that a substantial part of the events or omissions giving rise to the claims and the threatened and actual harm to Plaintiffs occurred in this District by reason of Defendants' conduct as alleged below.

THE PARTIES

4. Plaintiff Delano Farms Company is a corporation duly organized and existing under the laws of the State of Washington, with its principle place of business at 815 Eighth Street, P.O. Box 240, Hoquiam, Washington 98550.

5. Plaintiff Four Star Fruit, Inc. is a corporation duly organized and existing under the laws of the State of California, with its principle place of business at 13830 Avenue 24, Delano, California 93215.

6. Plaintiff Gerawan Farming, Inc. is a corporation duly organized and existing under the laws of the State of California, with its principle place of business at 15749 East Ventura, Sanger, California 93657.

7. Plaintiffs are engaged in the business, *inter alia*, of growing, harvesting and selling table grapes.

8. Defendant The California Table Grape Commission is a corporation of the State of California, established by the 1967 Ketchum Act. Cal. Food & Agric. Code §§65550 – 65551. Defendant's principle place of business is at 392 W. Fallbrook, Suite 101, Fresno, California 93711.

9. The stated purpose of the Commission is to expand and maintain the market for California table grapes for the benefit of the State of California as well as the State's over five hundred California table grape growers. The Commission is funded primarily by assessments levied on each shipment of California table grapes and paid by the State's table grape shippers. No general revenues of the State fund the Commission.

10. Defendant the United States of America is a sovereign nation organized and existing under the Constitution of the United States. Pursuant to Fed. R. Civ. P. 4(i), the United States may be served through the United States Attorney for the Eastern District of California – Lawrence Brown, United States Attorney's Office, 501 I Street, Suite 10-100, Sacramento, CA 95814. Additionally, under Rule 4(i), a copy of the summons and complaint must be sent by registered or certified mail to the Attorney General of the United States, Office of the Attorney General, U.S. Department of Justice, 950 Pennsylvania Avenue, NW, Washington, DC 20530-0001.

11. Defendant the United States of America and its representative, the United States

Department of Agriculture, reside and conduct business within the territorial jurisdiction of this Court.

12. Defendant United States Department of Agriculture is a United States federal executive department, established by 7 U.S.C. § 2201. Defendant USDA's headquarters are located at 1301 Independence Avenue, S.W., Washington, D.C. 20004.

13. The stated purpose of the USDA is to provide leadership on food, agriculture, natural resources, and related issues based on sound public policy, the best available science, and efficient management.

14. Defendant Tom Vilsack, Secretary of the United States Department of Agriculture, has overall responsibility for the United States Department of Agriculture. He is named in his official capacity and may be served at the Department of Agriculture at 1301 Independence Avenue, S.W., Washington, D.C. 20004.

FACTS

Overview

15. For years, California table grape growers and shippers have funded a research program under the USDA to develop new table grape varieties. Growers and shippers fund the USDA research program through the Commission by an assessment on each box of table grapes shipped in California. Prior to 2002, the USDA provided the new varieties under development to area growers for evaluation of growing potential and commercial marketability. Once new varieties appeared commercially viable, the USDA "released" the variety, and distributed plant material of the variety to area growers free-of-charge. The USDA did not charge California growers for the new varieties since California growers and shippers already paid for a large portion of the development.

16. In the late 1990s, the Commission developed a scheme by which it and a few select nurseries could profit from the new varieties that the USDA distributed for free. At the urging of the Commission, the USDA agreed to begin patenting new table grape varieties. Although California shippers already funded much of the development, the USDA agreed to give the Commission an exclusive license to all new patented varieties, and to allow the Commission to charge royalties

when growers wished to obtain the new varieties. The USDA also agreed to give the Commission exclusive enforcement powers over its new patent rights.

17. Under the Commission's "patent and licensing" scheme, the Commission hand-selected three nurseries to exclusively sell all new patented table grape varieties. Unlike the prior free distribution, the nurseries would be allowed to sell new varieties to growers. Additionally, the nurseries would be required to pay a royalty to the Commission for each plant sold, which the nursery could pass onto the growers. One of the hand-selected nurseries now able to profit from newly developed varieties previously distributed without charge is owned by the son of a long-standing Commission member.

18. When a grower seeks to obtain a new variety from a nursery, it is required to enter a "Domestic Grower License Agreement" with the Commission. Under the terms of the Agreement, the grower cannot propagate the variety beyond the plant purchased. Moreover, if the Commission believes the grower has violated the License Agreement, it can void the Agreement and order that all purchased plants be destroyed.

19. The first three varieties that the Commission identified to the USDA for patenting had been under development for years. Indeed, at least one of the varieties had been distributed to growers for wide-scale commercial evaluation and sale.

20. Recognizing that at least one of the new varieties identified for patenting (and perhaps all three) had been previously in public use and/or sold commercially, the Commission created a so-called "amnesty program" designed to hide the fact that valid patents could not be obtained, and to extort funds from growers already in possession of the varieties. Under the amnesty program, the Commission widely disseminated notices to growers and shippers stating that they were in violation of the law if they possessed the varieties intended for patenting. The notices also offered confidential "settlements" to any growers who, within a narrow window, agreed to license the varieties, pay a "penalty" to the Commission, and accept the Commission's license restrictions on further propagation. Growers and shippers who refused the "amnesty" were threatened with lawsuits, including money damages and injunctions. On information and belief, the U.S, through the USDA, knew of and authorized the amnesty program as a way of concealing the fact that valid

patents could not be obtained on the grapevine varieties identified by the Commission for patenting.

21. Confirming the Commission's expectation that varieties identified for patenting were in public use, at least 17 growers confirmed possession of the varieties and agreed to pay the penalties demanded by the Commission. Astonishingly, the Commission threatened growers and demanded penalties even before the USDA had been issued patents on the new varieties. Even more troublesome, the USDA and inventor of the new varieties breached their duty of candor to the United States Patent & Trademark Office ("USPTO") by not reporting these prior public uses and sales when applying for patents on the new varieties. Under Patent Law, public use or sale of an invention more than one year prior filing a patent application bars patentability.

22. Based on these facts, none of the patents on the new varieties are valid. Moreover, the USDA and inventor committed inequitable conduct before the USPTO. In demanding licenses and accepting royalties on knowingly invalid patents, the Commission violated federal antitrust laws, and committed other violations of federal and state law. This action seeks to remedy these wrongs, by (among other things) obtaining a judicial declaration that the U.S. and USDA acted in an arbitrary, capricious manner, and in a manner not in accordance with applicable law, including the legal standards for obtaining a valid patent, obtaining a judicial declaration that the U.S. and USDA acted in a manner not in accordance with applicable law by granting the Commission an exclusive license with the Commission and allowing the Commission to collect royalties on invalid patents, by obtaining a judicial declaration that the Commission cannot enforce invalid patents, by obtaining judgment that the Commission's actions in enforcing patents obtained though inequitable conduct violates the antitrust laws of the United States, and by obtaining a judgment that the Commission's actions in connection with its licensing program constitutes unfair competition with Plaintiffs. Plaintiffs further seek to obtain the return of royalty payments illegally collected from growers and shippers, and to stop the Commission from engaging in further illegal activities through the use of patents so the varieties at issue can be freely distributed.

The Patents at Issue

Sweet Scarlet

23. On February 20, 2003, the USDA filed patent application No. 371,512 (the "'512

Application") on a grapevine denominated "Sweet Scarlet." The application listed David W. Ramming and Ronald E. Tarallo as co-inventors. On July 26, 2005, the '512 Application issued as U.S. Patent No. PP15,891, entitled "Grapevine Denominated Sweet Scarlet" (the "'891 patent"). A true copy of the '891 patent is attached as Exhibit A.

24. The United States of America, as represented by the Secretary of Agriculture, is the owner by assignment of the '891 patent.

25. On information and belief, the Commission is the exclusive licensee of the '891 patent pursuant to a license agreement entered into between the United States Government, as represented by the United States Department of Agriculture, Agricultural Research Services, and the Commission. The exclusive license includes the right to license the '891 patent and to enforce the '891 patent against alleged infringers.

Autumn King

26. On September 28, 2004, the USDA filed patent application No. 953,387 (the "'387 Application") on a grapevine denominated "Autumn King." The application listed David W. Ramming and Ronald E. Tarallo as co-inventors. On February 21, 2006, the '387 Application issued as U.S. Patent No. PP16,284, entitled "Grapevine Denominated Autumn King" (the "Autumn King or '284 patent"). A true and correct copy of the '284 patent is attached as Exhibit B.

27. The United States of America, as represented by the Secretary of Agriculture, is the owner by assignment of the '284 patent.

28. On information and belief, the Commission is the exclusive licensee of the '284 patent pursuant to a license agreement entered into between the United States Government, as represented by the United States Department of Agriculture, Agricultural Research Services, and the Commission. The exclusive license includes the right to license the '284 patent and to enforce the '284 patent against alleged infringers.

Scarlet Royal

29. On September 28, 2004, the USDA filed patent application No. 953,124 (the "'124 Application") on a grapevine denominated "Scarlet Royal." The application listed David W. Ramming and Ronald E. Tarallo as co-inventors. On January 31, 2006, the '124 Application issued

as U.S. Patent No. PP16,229, entitled "Grapevine Denominated Scarlet Royal" (the "Scarlet Royal or '229 patent"). A true and correct copy of the '229 patent is attached as Exhibit C.

30. The United States of America, as represented by the Secretary of Agriculture, is the owner by assignment of the '229 patent.

31. On information and belief, the Commission is the exclusive licensee of the '229 patent pursuant to a License Agreement entered into between the United States Government, as represented by the United States Department of Agriculture, Agricultural Research Services, and the Commission. The exclusive license includes the right to license the '229 patent and to enforce the '229 patent against alleged infringers.

The Commission's Licenses to Nurseries and Plaintiffs

32. The Commission has sublicensed its right to grow and propagate the Sweet Scarlet variety described in the '891 patent, the Autumn King variety described in the '284 patent, and the Scarlet Royal variety described in the '229 patent (collectively, the "Patented Varieties") to three privately-owned nurseries – Sunridge Nurseries, Vintage Nurseries, and Casa Crystal Nurseries (the "Licensed Nurseries"). Each Licensed Nursery is authorized to sell Autumn King, Sweet Scarlet and Scarlet Royal vines to growers who execute a "Domestic Grower License Agreement" with the Commission. The Domestic Grower Agreement prohibits growers who purchase the Patented Varieties from propagating the vines or distributing the vines to third parties. Under the Domestic License Agreement, the Commission may revoke the license for any grower who violates the terms, and require the grower to destroy all Patented Varieties purchased.

33. The Commission receives a royalty on each Patented Variety sold. The Licensed Nurseries are responsible for paying the royalty, but the Licensed Nurseries are allowed to pass the royalty amount onto the purchasing growers, which they do and have done. The Commission pays a portion of the royalty to the USDA.

34. Casa Crystal Nursery is owned by Andrew Zaninovich. Andrew's father is Marco Zaninovich, who has been a long-time and active board member of the Commission.

35. Plaintiffs are in possession of the Autumn King, Sweet Scarlet and Scarlet Royal varieties, which they purchased through Licensed Nurseries. Plaintiffs paid the royalties on each

purchased plant imposed by the Commission.

36. Plaintiffs have entered into a Domestic Grower License Agreement with the Commission for the '891 patent. In consideration for this limited, nonexclusive license, Plaintiffs have paid a license fee to a Licensed Nursery. Under the terms of this Agreement, Plaintiffs have a limited, nonexclusive license to the '891 patent to grow the Sweet Scarlet variety and sell the fruit produced. Plaintiffs cannot propagate the grapevines or distribute the vines to third parties. Furthermore, Plaintiffs are obligated to destroy all Sweet Scarlet plant material upon termination of the agreement.

37. Plaintiffs have entered into a Domestic Grower License Agreement with respect to the '284 patent. In consideration for this limited, nonexclusive license, Plaintiffs have paid a license fee to a Licensed Nursery. Under the terms of this Agreement, Plaintiffs have a limited, nonexclusive license to the '284 patent to grow the Autumn King variety and sell the fruit produced. Plaintiffs cannot propagate the grapevines or distribute the vines to third parties. Furthermore, Plaintiffs are obligated to destroy all Autumn King plant material upon termination of the agreement.

38. Plaintiffs have entered into a Domestic Grower License Agreement with respect to the '229 patent. In consideration for this limited, nonexclusive license, Plaintiffs have paid a license fee to a Licensed Nursery. Under the terms of this Agreement, Plaintiffs have a limited, nonexclusive license to the '229 patent to grow the Scarlet Royal variety and sell the fruit produced. Plaintiffs cannot propagate the grapevines or distribute the vines to third parties. Furthermore, Plaintiffs are obligated to destroy all Scarlet Royal plant material upon termination of the agreement.

The Commission's Patent and Licensing Program

39. The Commission requires that California grape shippers pay an assessment of approximately $0.13 per box of table grapes. The Commission operates at an annual surplus from these assessments, but does not return any of the assessment money back to the California growers or shippers.

40. Dr. Ramming, the co-inventor of the patented Autumn King, Sweet Scarlet and Scarlet Royal varieties, is a researcher at the Agriculture Research Center ("ARC") of the USDA

located in Fresno, California. For at least 20 years, Dr. Ramming has operated a research program at the ARC relating to the development of new table grape varieties. Since the early 1980s, the Commission has funded a portion of Dr. Ramming's grapevine breading program with funds collected through the shipper assessments. In many years, the Commission's funding has amounted to over one-third of the total table grape research budget at the ARC, excluding employee salaries.

41. Prior to 2003, the USDA had never sought patent protection for any new table grape variety developed at the ARC. Instead, for nearly two decades, new grape varieties developed by the ARC with California table grape shipper assessment revenues were distributed freely to California growers by the USDA.

42. In the late 1990s, the Commission adopted a plan to request that the USDA seek patent protection on all new table grape varieties developed prior to general release. Initially, the patents were intended to control competition from foreign growers. In the past, foreign growers frequently obtained new table grape varieties developed through assessments on California shippers, then competed with California growers in markets for the new varieties. Under the Commission's plan, California growers would be provided patented varieties free of charge, and patented varieties would either be excluded from foreign growers or provided to foreign growers at high royalty rates. After several meetings between the Commission and the USDA, the USDA ultimately agreed to seek patent protection as requested by the Commission. The USDA further agreed that the Commission could serve as the exclusive licensee for patented varieties in the collection of royalties and enforcement against infringers. However, the USDA prohibited the Commission from excluding foreign growers from receiving patented varieties, blocking imports from foreign growers, or otherwise discriminating against foreign growers.

43. Although the USDA barred the Commission from implementing the original goals behind the patenting program – namely, the control of foreign competition – the Commission agreed to proceed with the patent and licensing plan. The Commission recognized that the patenting program could have several benefits. First, the Commission recognized that patenting would give it an important new revenue source through licensing fees. Second, the Commission, which is controlled by a number of large growers, recognized that it could limit access to new varieties,

particularly by smaller growers, through the amount of royalties it charged for obtaining the patented varieties and through the terms of its sublicense agreements. Third, the Commission recognized that nurseries, who are also represented through the Commission, could profit by selling new grapevines, which the USDA previously gave away for free.

44. In exchange for seeking patent protection, and providing an exclusive license to the Commission, the Commission and the USDA agreed that revenues from the patent licensing program would be shared between the USDA and the Commission. However, the USDA indicated that it was not interested in profiting from the patenting program. Additionally, Dr. Ramming received no extra compensation from the patenting of varieties he developed.

45. In accordance with the agreement between the Commission and the USDA, the Commission charges nurseries who distribute patented varieties a $5,000 participation fee per patented variety and an additional $1 per production unit royalty. These costs are then passed on by the nurseries to the California grape growers who purchase the patented plant material from the nurseries, including Plaintiffs who purchased the Patented Varieties.

46. The California grape growers who bear the ultimate costs of the royalty fees imposed by the Commission are the same California grape growers who bear the cost of the per box assessment charged by the Commission, which funds much of Dr. Ramming's breeding program. Thus, California table grape growers essentially pay for the development of patented varieties, then pay again to obtain the varieties.

47. The Commission's Research Committee and Board oversee and administer the patent and licensing program. Specifically, the Board sets the royalty rates on patented plants, determines penalties for infringement, and establishes enforcement policy. The Research Committee oversees Dr. Ramming's breeding program and makes recommendations regarding which new varieties should be patented and released. To date, the USDA has only patented new varieties that the Commission has recommended for patenting, and has only applied for patents once receiving a recommendation from the Commission to do so.

Prior Uses and Sales of the Patented Varieties

48. Prior to the Commission's patent and licensing program, the USDA provided

varieties under development to certain growers for large-scale evaluation. Growers participating in these "trials" were allowed to grow the new varieties, sell the fruit produced, and retain any profits. While certain growers were selected for the trials, the USDA provided no mechanism for preventing other growers from accessing and reproducing the varieties as well. Accordingly, when a variety under development appeared commercially successful, it was not uncommon for many growers to have reproduced and commercially sold the variety prior to an official "release" by the USDA. For example, when the USDA developed the "Princess" variety, it was well known that growers throughout the central valley had been reproducing Princess grapevines and selling Princess fruit for years.

49. Neither the USDA nor the Commission had any incentive to restrict the distribution of new grapevines prior to a release. Widespread reproduction and sales of new varieties allowed greater customer acceptance prior to release. Additionally, growers and shippers contributed substantially to the development of new varieties in Dr. Ramming's grapevine breeding program. Moreover, even after release, the USDA provided plant material from which growers could reproduce the variety free of charge.

50. The USDA, Dr. Ramming, and the Commission knew that plant material for varieties under development frequently entered the public domain prior to release.

51. Development of the Patented Varieties began in about 1993. Prior to 2003, the Dr. Ramming had reproduced each of the Patented Varieties, produced fruit from each of the Patent Varieties, and had evaluated the potential commercialization of each Patented Variety.

52. Dr. Ramming did not keep the development of the Patented Varieties secret. To the contrary, Dr. Ramming discussed each of the Patented Varieties with the Commission over many years, including between 2001 and 2003. Dr. Ramming discussed the Patented Varieties during public meetings of the Commission's Research Committee. Additionally, prior to 2003, Dr. Ramming displayed fruit from the Patented Varieties at Commission meetings, which area growers and shippers attended. Attendees were allowed to take samples of fruit from the three Patented Varieties.

53. By 2001, the Commission's Research Committee was actively evaluating the Sweet

Scarlet, Autumn King, and Scarlet Royal varieties (among others) for USDA release. In 2002, the Commission recommended that Sweet Scarlet be released. The Commission also recommended that the USDA seek patent protection on Sweet Scarlet as the first variety for patenting under the Commission's new patent and licensing program. After receiving the Commission's recommendation, the UDSA proceeded with the release of Sweet Scarlet and filed a patent application on the Sweet Scarlet variety in February 2003.

54. Although the Commission recommended proceeding with the release of Sweet Scarlet, the Commission decided to delay any release and patenting of Autumn King and Scarlet Royal. Instead, the Commission recommended that Autumn King and Sweet Scarlet undergo further evaluation prior to release. Approximately two years later, in 2004, the Commission finally recommended release of Autumn King and Scarlet Royal. At that time, the Commission further recommended that the UDSA seek patent protection for Autumn King and Scarlet Royal.

55. Despite waiting to seek patent protection until the Commission recommended "release" of the new varieties, Dr. Ramming could have filed patent applications much earlier. All three Patented Varieties had been reproduced and undergone several growing cycles well before 2002 when the Commission recommended the release of Sweet Scarlet, and well before 2004 when the Commission recommended the release of Autumn King and Scarlet Royal. By 2001-2002 (if not before), all three varieties had been developed to a point at which they were ready for patenting. The Commission's recommendations regarding continued evaluation of the Autumn King and Sweet Scarlet varieties prior to release did not prevent the USDA from seeking patent protection on these varieties long before receiving a recommendation regarding release.

56. Despite delaying the decision to apply for patents on Autumn King and Scarlet Royal, the USDA made little effort to prevent these varieties from entering the public domain. The USDA did not conceal the varieties. To the contrary, prior to seeking patent protection, the USDA displayed and discussed the varieties at public meetings. Moreover, the USDA kept its fully-developed Autumn King and Scarlet Royal plants at unsecured facilities at California State University at Fresno ("Fresno State"), which could be accessed though the Fresno State grounds. The USDA considered placing a fence around its facilities adjacent to the Fresno State campus, but

declined to do so. Although the USDA purportedly told employees that they were not to take or distribute plant materials from new varieties, the USDA made no efforts to examine materials removed from the USDA facility to ensure that persons entering the facility did not remove plant material for these varieties. Finally, the USDA never made efforts to secure plant materials sent to other facilities for testing.

57. While delaying the decision to seek patent protection, and failing to implement security measures at its facilities, the USDA knew that public use of new varieties more than one year before applying for a patent would bar later filing for patent protection. Indeed, the Commission and Dr. Ramming discussed the fact that public uses and sales of new varieties prior to seeking patent protection could jeopardize the Commission's patenting program.

58. Not surprisingly, all three Patented Varieties entered the public domain more than one year before the USDA sought patent protection on each respective variety.

59. The Sweet Scarlet variety and its fruit was publicly used, distributed, offered for sale and sold by growers and shippers prior to February 20, 2002 – more than one year prior to the filing of the '512 Application on the Sweet Scarlet variety. Specifically, approximately nine growers received Sweet Scarlet from Dr. Ramming for trials in 1999 and 2000. At least three of these growers sold fruit produced into commercial markets before 2002.

60. Additionally, at least 17 other growers, who were not part of trials, received and reproduced the Sweet Scarlet variety. On information and belief, these reproductions took place prior to 2002. Neither the USDA nor Dr. Ramming oversaw or controlled the reproductions created by these 17 growers.

61. In early 2002, more than two years before filing the patent applications for Autumn King and Scarlet Royal, a grower in Delano, California (J&J Farms owned by Jim and Jack Ludy) obtained "sticks" of several new varieties, including Autumn King and Scarlet Royal. Jim and Jack Ludy provided some of the plant material for the new varieties (including Autumn King and Scarlet Royal) to their cousin (Lawrence Ludy) who owned and operated an adjacent farm. With these sticks, both J&J Farms and Lawrence Ludy reproduced Autumn King and Scarlet Royal grapevines on their farms in 2002. Lawrence Ludy reproduced additional Autumn King on his farm in mid-

2003. In total, J&J Farms and Lawrence Ludy Farms publicly reproduced more than five-hundred Autumn King and Scarlet Royal plants before September 2003.

62. J&J Farms and Lawrence Ludy Farms received the Autumn King and Scarlet Royal plant material without any written or verbal agreement or restrictions on disclosure or use. Neither the USDA nor Dr. Ramming oversaw or controlled the reproductions that occurred on the Ludys' farms. Although both Ludy farms were privately owned, they placed no special restrictions such as fences or gates limiting public access to their fields and the location of the Autumn King and Scarlet Royal plants. Not did the Ludy Farms place any confidentiality restrictions on employees who viewed the reproduced new varieties. Finally, prior to September 2003, both J&J Farms and Lawrence Ludy Farms showed the reproduced varieties to members of the public, including neighboring farmers, without any confidentiality restrictions.

The Commission's Efforts to Hide Prior Public Uses and Sales

63. On information and belief, both the USDA and the Commission knew, before the respective patents issued on the Patented Varieties, that (i) Sweet Scarlet had been in the public domain since before February 2002, and (ii) either knew or suspected that Autumn King and/or Scarlet Royal had been in the public domain since before September 2003.

64. Because the public use and sale of the Patented Varieties more than one year before the patent application filing dates would prevent issuance of valid patents, the Commission (with the USDA's knowledge and approval), created a scheme to prevent challenges to patentability based on these prior uses and sales.

65. In May 2004, the Commission sent a notice to all California table grape growers and shippers stating that the UDSA had applied for a patent on the Sweet Scarlet variety. Although no enforceable patent had yet issued, the Commission offered "amnesty" for any grower who had previously reproduced Sweet Scarlet. Under its so-called "amnesty" program, a grower with Sweet Scarlet could keep the vines reproduced, so long as the grower (i) admitted to possession prior to July 2004, (ii) paid $2 per vine reproduced, (iii) paid $2 per box of Sweet Scarlet grapes previously shipped, and (iv) agreed to no further propagation of the Sweet Scarlet variety from the plants possessed.

66. In July 2004, the Commission sent another notice to all California table grape growers and shippers extending the "amnesty" time period for one month, and extending the "amnesty" to include Autumn King and Scarlet Royal varieties.

67. In both notices, the Commission threatened to sue growers who did not come forward, and to seek money damages and injunctions. Yet, at the time of the second notice, the USDA patent application on Sweet Scarlet not only remained un-issued, but had been rejected by the USPTO. Moreover, the USDA had not even applied for a patent on either Autumn King or Scarlet Royal. Thus, the USDA had no patent rights, and the Commission lacked any enforcement rights.

68. On information and belief, at the time the Commission sent the "amnesty" notices in May and June, 2004, the USDA, and Dr. Ramming knew of the public use and sale with respect to the Sweet Scarlet that occurred prior to February 20, 2002 – more than one year prior to the filing of the '512 Application on the Sweet Scarlet variety.

69. On information and belief, the Commission, the USDA, and Dr. Ramming learned (prior to the July 25, 2005 issue date for Sweet Scarlet) that at least some of the 17 growers who agreed to the Commission's "amnesty" program for that variety had possessed and reproduced Sweet Scarlet prior to February 2002. Additionally, on information and belief, the Commission, the USDA, and Dr. Ramming knew that such information was material to the patentability of the Sweet Scarlet variety.

70. The USDA and Dr. Ramming did not disclose to the USPTO the information regarding all the growers who possessed and reproduced Sweet Scarlet prior to February 20, 2002, which the Commission learned though its "amnesty" program.

FIRST CLAIM FOR RELIEF

(Declaratory Relief Under 5 U.S.C. §702 Against Defendants U.S., USDA and Secretary Vilsack)

71. Plaintiffs reallege and incorporate by reference all paragraph 1-70 as if fully set forth herein.

72. This is an action for declaratory and injunctive relief brought, in part, under the

Administrative Procedure Act, 5 U.S.C. §§701, *et seq.* with respect to final agency actions of the USDA and its officers. The discrete acts complained of herein constitute "agency action" as defined by 5 U.S.C. §551(13) and are reviewable by this Court pursuant to 5 U.S.C. §704.

73. The U.S.'s, USDA's and Secretary Vilsack's actions with respect to the Patented Varieties is subject to the procedural requirements of the Administrative Procedure Act ("APA"), at 5 U.S.C. §§ 551, *et seq.*

74. The USDA engaged in a discrete and final agency action under 5 U.S.C. §702 by deciding and agreeing to engage in a patenting program with the Commission with respect to the Patented Varieties and in cooperating with the Commission in connection with that patenting program.

75. The USDA engaged in a discrete and final agency action under 5 U.S.C. §702 by deciding, approving and cooperating in the filing and prosecution of patent applications for the Patented Varieties.

76. The USDA engaged in a discrete and final agency action under 5 U.S.C. §702 by engaging in inequitable conduct before the USPTO with respect to the application for the '891 patent.

77. The USDA engaged in a discrete and final agency action under 5 U.S.C. §702 by procuring, accepting the issuance, and maintaining the '284, '891 and '229 patents.

78. The USDA engaged in a discrete and final agency action under 5 U.S.C. §702 by granting the Commission an exclusive license in the '284, '891 and '229 patents.

79. The USDA engaged in a discrete and final agency action under 5 U.S.C. §702 by approving, allowing and cooperating with the Commission's amnesty program, licensing program and enforcement program with respect to the Patented Varieties.

80. The USDA engaged in a discrete and final agency action under 5 U.S.C. §702 by allowing the Commission to collect royalties for the Patented Varieties from farmers who had funded the USDA's research program that led to the development of the Patented Varieties, where prior USDA policy was to allow such farmers to benefit from the USDA's research free of charge. The USDA further engaged in a discrete and final agency action under 5 U.S.C. §702 by

cooperating, encouraging and acting in concert with the Commission in the collection of royalties for the Patented Varieties and by receiving a portion of those royalties from the Commission.

81. The acts complained of herein constitute final agency actions by the U.S., USDA and Secretary Vilsack subject to review under the APA.

82. The acts complained of herein mark the consummation of the U.S.'s, USDA's and Secretary Vilsack's decision-making process.

83. The acts complained of herein are not interlocutory, interim or tentative in nature.

84. The acts complained of herein are acts by which rights or obligations have been determined and from which legal consequences have flowed, continue to flow and will flow. Specifically, the acts complained of herein have directly determined the rights and obligations of Plaintiffs and the Commission with respect to the growing, distribution, sale, propagation, reproduction, and otherwise free use of the plant material of the Patented Varieties, and the right to collect royalties and the right to exclude thereof.

85. The acts complained of herein by the U.S., USDA and Secretary Vilsack are arbitrary, capricious, and otherwise not in accordance with applicable laws and regulations.

86. The U.S.'s, USDA's and Secretary Vilsack's actions with respect to the Patented Varieties and '284, '891 and '229 patents are arbitrary, capricious, and otherwise not in accordance with applicable laws and regulations, because no such patents could issue under 35 U.S.C. §102(b).

87. The U.S.'s, USDA's and Secretary Vilsack's actions with respect to the Patented Varieties and the '284, '891 and '229 patents are arbitrary, capricious, and otherwise not in accordance with applicable laws and regulations, because the Commission's enforcement of these patents violate anti-trust and unfair competition laws.

88. The U.S.'s, USDA's and Secretary Vilsack's actions in prosecuting and procuring the '891 patent are arbitrary, capricious, and otherwise not in accordance with applicable laws and regulations, because that patent is unenforceable, as it was obtained through inequitable conduct committed by the USDA and/or its employees before the USPTO.

89. The U.S.'s, USDA's and Secretary Vilsack's action in granting the Commission an exclusive license to the '284, '891, and '229 patents is arbitrary, capricious, and otherwise not in

accordance with applicable laws and regulations, because those patents are invalid under 35 U.S.C. §102(b).

90. The U.S.'s, USDA's and Secretary Vilsack's action in granting the Commission an exclusive license to the '284, '891, and '229 patents is arbitrary, capricious, and otherwise not in accordance with applicable laws and regulations, because, among other things, the exclusive license is in violation of 35 U.S.C § 209(a), and in particular section 209(a)(4), in that the exclusive license substantially lessens competition in the distribution, production, and reproduction of the Patented Varieties and either creates or maintains a violation of the Federal Antitrust laws as alleged in the Sixth and Ninth Claims for Relief.

91. The U.S.'s, USDA's and Secretary Vilsack's action in granting the Commission an exclusive license to the '891 patent is arbitrary, capricious, and otherwise not in accordance with applicable laws and regulations, because that patent is unenforceable.

92. The U.S.'s, USDA's and Secretary Vilsack's actions conferred important rights from which significant legal consequences have flown, including rights to the Commission regarding the enforcement of the '284, '891, and '229 patents, collection of royalties with respect to the Patented Varieties and the right to restrict the sale, growing, distribution, propagation and use of the Patented Varieties.

93. Plaintiffs have been harmed directly by the U.S.'s, USDA's and Secretary Vilsack's actions, have suffered a legal wrong because of the U.S.'s, USDA's and Secretary Vilsack's actions and have been adversely affected and aggrieved by the U.S.'s, USDA's and Secretary Vilsack's actions.

94. The U.S.'s, USDA's and Secretary Vilsack's actions have directly impacted Plaintiffs' day-to day business, including Plaintiffs' ability to sell, grow, distribute, propagate and use the Patented Varieties.

95. As a result of the U.S.'s, USDA's and Secretary Vilsack's actions, Plaintiffs have been (1) restricted from growing, selling, distributing, reproducing, propagating or otherwise freely using plant material for Patented Varieties, (2) restricted from growing, selling, reproducing, distributing, propagating or otherwise freely using the fruit for the Patented Varieties, and (3) forced

to license and pay royalties to the Commission and its agents with respect to the Patented Varieties. This has harmed and damaged Plaintiffs directly.

96. Plaintiffs are entitled to a declaration from the Court that the U.S.'s, USDA's and Secretary Vilsack's actions in connection with the Patented Varieties and the '284, '891 and '229 patents are unlawful and invalid.

97. Plaintiffs are entitled to a declaration from the Court that the U.S.'s, USDA's and Secretary Vilsack's actions in procuring the '284, '891 and '229 patents were unlawful and invalid under 35 U.S.C. §102(b).

98. Plaintiffs are entitled to a declaration from the Court that the U.S.'s, USDA's and Secretary Vilsack's prosecution of the '891 patent was unlawful and renders the '891 patent unenforceable because the duty of candor before the USPTO was breached during the prosecution of the '891 patent.

99. Plaintiffs are entitled to a declaration from the Court that the U.S.'s, USDA's and Secretary Vilsack's approval and cooperation in the patent program with the Commission, is unlawful with respect to the Patented Varieties, including the USDA's approval and cooperation in (1) the enforcement of the '284, '891 and '229 patents, (2) the collection of royalties for the Patented Varieties, and (3) the amnesty program for the Patented Varieties.

100. Plaintiffs are entitled to a declaration from the Court that the U.S.'s, USDA's and Secretary Vilsack's granting of an exclusive license to the Commission in the Patented Varieties was unlawful and invalid.

101. This Court may and should hold unlawful and set aside the U.S's, USDA's and Secretary Vilsack's actions giving rise to this First Claim for Relief pursuant to 5 U.S.C. §706(2)(A), (C), and (D).

102. This Court may and should compel the U.S., USDA and Secretary Vilsack to comply with their obligations under the Patent Act pursuant to 5 U.S.C. §706(1).

SECOND CLAIM FOR RELIEF

(Declaration of Invalidity of '891 Patent Against All Defendants)

103. Plaintiffs reallege and incorporate by reference all paragraphs 1-102 as if fully set

forth herein.

104. This claim arises under the patent laws of the United States, Title 35 of the United States Code, and the Declaratory Judgment provisions of §§ 2201 and 2202 of Title 28 of the United States Code and the APA at 5 U.S.C. §§702, *et seq*.

105. The U.S., USDA, and Secretary Vilsack engaged in a discrete and final agency actions by applying for, prosecuting, procuring, accepting issuance, maintaining and exclusively licensing to the Commission the '891 patent. The acts complained of herein constitute final agency actions by the U.S., USDA and Secretary Vilsack subject to review under the APA. These acts mark the consummation of the U.S.'s, USDA's and Secretary Vilsack's decision-making process. These acts are not interlocutory, interim or tentative in nature. These acts are those by which rights or obligations have been determined or from which legal consequences will flow. Specifically, the acts complained of herein have directly determined the rights and obligations of Plaintiffs and the Commission with respect to the growing, distribution, sale, reproduction, propagation and otherwise free use of the plant material and fruit of the Sweet Scarlet grapevine, and the right to collect royalties and the right to exclude thereof. These acts are arbitrary, capricious, and otherwise not in accordance with applicable laws and regulations, including 35 U.S.C. §102(b).

106. The Commission, as the exclusive licensee of the '891 patent, has demanded that Plaintiffs enter a license agreement to plant and harvest fruit from Sweet Scarlet grapevines.

107. Plaintiffs have paid for and obtained a license to the '891 patent, and possess Sweet Scarlet grapevines.

108. Plaintiffs assert that the '891 patent is invalid under at least 35 U.S.C. §102(b), and that they should not be required to license the '891 patent from the Commission or pay royalties therefor.

109. An actual case and controversy exists between Plaintiffs and the Commission, the U.S., the USDA and Secretary Vilsack concerning the '891 patent.

110. The action of the U.S., USDA and Secretary Vilsack in procuring the '891 patent was unlawful under 35 U.S.C. §102(b).

111. Plaintiffs have been harmed directly by the U.S.'s, USDA's and Secretary Vilsack's

actions, have suffered a legal wrong because of the U.S.'s, USDA's and Secretary Vilsack's actions and have been adversely affected and aggrieved by the U.S.'s, USDA's and Secretary Vilsack's actions. The U.S.'s, USDA's and Secretary Vilsack's actions have directly impacted Plaintiffs' day-to day business. As a result of the U.S.'s, USDA's and Secretary Vilsack's actions, Plaintiffs have been (1) restricted from growing, selling, distributing, reproducing, propagating or otherwise freely using plant material for the Sweet Scarlet grapevine, (2) restricted from growing, selling, reproducing, distributing, propagating or otherwise freely using the fruit for the Sweet Scarlet grapevine, and (3) forced to license and pay royalties to the Commission and its agents with respect to the Sweet Scarlet grapevine plant material.

112. Plaintiffs are entitled to declaratory judgment from this Court that the '891 patent is invalid pursuant to at least 35 U.S.C. §102(b) and that the U.S's, USDA's and Secretary Vilsack's actions in applying for, prosecuting, procuring, accepting issuance, maintaining and exclusively licensing to the Commission the '891 patent are final agency actions that are arbitrary, capricious and not otherwise in accordance with applicable laws and regulations at least under 35 U.S.C. §102(b).

113. This Court may and should hold unlawful and invalid the '891 patent pursuant to 5 U.S.C. §706(2)(A), (C), and (D).

114. This Court may and should compel the U.S., USDA and Secretary Vilsack to comply with their obligations under the Patent Act pursuant to 5 U.S.C. §706(1) with respect to the '891 patent.

THIRD CLAIM FOR RELIEF

(Declaration of Invalidity of '284 Patent Against All Defendants)

115. Plaintiffs reallege and incorporate by reference all paragraphs 1-114 as if fully set forth herein.

116. This claim arises under the patent laws of the United States, Title 35 of the United States Code, and the Declaratory Judgment provisions of §§ 2201 and 2202 of Title 28 of the United States Code and the APA at 5 U.S.C. §§702, et seq.

117. The acts complained of herein constitute final agency actions by the U.S., USDA and

Secretary Vilsack subject to review under the APA. These acts mark the consummation of the U.S.'s, USDA's and Secretary Vilsack's decision-making process. These acts are not interlocutory, interim or tentative in nature. These acts are those by which rights or obligations have been determined or from which legal consequences will flow. Specifically, the acts complained of herein have directly determined the rights and obligations of Plaintiffs and the Commission with respect to the growing, distribution, sale, reproduction, propagation, and otherwise free use of the plant material and fruit of the Autumn King grapevine, and the right to collect royalties and the right to exclude thereof. These acts are arbitrary, capricious, and otherwise not in accordance with applicable laws and regulations, including 35 U.S.C. §102(b).

118. The Commission, as the exclusive licensee of the '284 patent, has demanded that Plaintiffs enter a license agreement to plant and harvest fruit from Autumn King grapevines.

119. Plaintiffs have paid for and obtained a license to the '284 patent, and possess Autumn King grapevines.

120. Plaintiffs assert that the '284 patent is invalid under at least 35 U.S.C. §102(b), and that they should not be required to license the '284 patent from the Commission or pay royalties therefor.

121. An actual case and controversy exists between Plaintiffs and the Commission, the U.S., the USDA and Secretary Vilsack concerning the '284 patent.

122. The action of the U.S., USDA and Secretary Vilsack in procuring the '284 patent was unlawful under 35 U.S.C. §102(b).

123. Plaintiffs have been harmed directly by the U.S.'s, USDA's and Secretary Vilsack's actions, have suffered a legal wrong because of the U.S.'s, USDA's and Secretary Vilsack's actions and have been adversely affected and aggrieved by the U.S.'s, USDA's and Secretary Vilsack's actions. The U.S.'s, USDA's and Secretary Vilsack's actions have directly impacted Plaintiffs' day-to day business. As a result of the U.S.'s, USDA's and Secretary Vilsack's actions, Plaintiffs have been (1) restricted from growing, selling, distributing, reproducing, propagating or otherwise freely using plant material for the Autumn King grapevine, (2) restricted from growing, selling, reproducing, distributing, propagating or otherwise freely using the fruit for the Autumn King

grapevine, and (3) forced to license and pay royalties to the Commission and its agents with respect to the Autumn King grapevine plant material.

124. Plaintiffs are entitled to declaratory judgment from this Court that the '284 patent is invalid pursuant to at least 35 U.S.C. §102(b) and that the U.S's, USDA's and Secretary Vilsack's actions in applying for, prosecuting, procuring, accepting issuance, maintaining and exclusively licensing to the Commission the '284 patent are final agency actions that are arbitrary, capricious and not otherwise in accordance with applicable laws and regulations at least under 35 U.S.C §102(b).

125. This Court may and should hold unlawful and invalid the '284 patent pursuant to 5 U.S.C. §706(2)(A), (C), and (D).

126. This Court may and should compel the U.S., USDA and Secretary Vilsack to comply with their obligations under the Patent Act pursuant to 5 U.S.C. §706(1) with respect to the '284 patent.

FOURTH CLAIM FOR RELIEF

(Declaration of Invalidity of '229 Patent Against All Defendants)

127. Plaintiffs reallege and incorporate by reference all paragraphs 1-126 as if fully set forth herein.

128. This claim arises under the patent laws of the United States, Title 35 of the United States Code, and the Declaratory Judgment provisions of §§ 2201 and 2202 of Title 28 of the United States Code and the APA at 5 U.S.C. §§702, et seq.

129. The U.S., USDA, and Secretary Vilsack engaged in a discrete and final agency actions by applying for, prosecuting, procuring, accepting issuance, maintaining and exclusively licensing to the Commission the '229 patent. The acts complained of herein constitute final agency actions by the U.S., USDA and Secretary Vilsack subject to review under the APA. These acts mark the consummation of the U.S.'s, USDA's and Secretary Vilsack's decision-making process. These acts are not interlocutory, interim or tentative in nature. These acts are those by which rights or obligations have been determined or from which legal consequences will flow. Specifically, the acts complained of herein have directly determined the rights and obligations of Plaintiffs and the

Commission with respect to the growing, distribution, sale, reproduction, propagation and otherwise free use of the plant material and fruit of the Scarlet Royal grapevine, and the right to collect royalties and the right to exclude thereof. These acts are arbitrary, capricious, and otherwise not in accordance with applicable laws and regulations, including 35 U.S.C. §102(b).

130. The Commission, as the exclusive licensee of the '229 patent, has demanded that Plaintiffs enter a license agreement to plant and harvest fruit from Scarlet Royal grapevines.

131. Plaintiffs have paid for and obtained a license to the '229 patent, and possess Scarlet Royal grapevines.

132. Plaintiffs assert that the '229 patent is invalid under at least 35 U.S.C. §102(b), and that they should not be required to license the '229 patent from the Commission or pay royalties therefor.

133. An actual case and controversy exists between Plaintiffs and the Commission, the U.S., the USDA and Secretary Vilsack concerning the '229 patent.

134. The action of the U.S., USDA and Secretary Vilsack in procuring the '229 patent was unlawful under 35 U.S.C. §102(b).

135. Plaintiffs have been harmed directly by the U.S.'s, USDA's and Secretary Vilsack's actions, have suffered a legal wrong because of the U.S.'s, USDA's and Secretary Vilsack's actions and have been adversely affected and aggrieved by the U.S.'s, USDA's and Secretary Vilsack's actions. The U.S.'s, USDA's and Secretary Vilsack's actions have directly impacted Plaintiffs' day-to day business. As a result of the U.S.'s, USDA's and Secretary Vilsack's actions, Plaintiffs have been (1) restricted from growing, selling, distributing, reproducing, propagating, or otherwise freely using plant material for the Scarlet Royal grapevine, (2) restricted from growing, selling, reproducing, distributing, propagating, or otherwise freely using the fruit for the Scarlet Royal grapevine, and (3) forced to license and pay royalties to the Commission and its agents with respect to the Scarlet Royal grapevine plant material.

136. Plaintiffs are entitled to judgment from this Court that the '229 patent is invalid pursuant to at least 35 U.S.C. §102(b) and that the U.S's, USDA's and Secretary Vilsack's actions in applying for, prosecuting, procuring, accepting issuance, maintaining and exclusively licensing to

the Commission the '229 patent are final agency actions that are arbitrary, capricious and not otherwise in accordance with applicable laws and regulations at least under 35 U.S.C §102(b).

137. This Court may and should hold unlawful and invalid the '229 patent pursuant to 5 U.S.C. §706(2)(A), (C), and (D).

138. This Court may and should compel the U.S., USDA and Secretary Vilsack to comply with their obligations under the Patent Act pursuant to 5 U.S.C. §706(1) with respect to the '229 patent.

FIFTH CLAIM FOR RELIEF

(Declaration of Unenforceability for Inequitable Conduct Regarding '891 Patent Against All Defendants)

139. Plaintiffs reallege and incorporate by reference all paragraphs 1-138 as if fully set forth herein.

140. The Sweet Scarlet variety was in public use through reproduction, and fruit from the Sweet Scarlet variety was publicly sold, prior to February 2002.

141. The prior possession and reproduction of the Sweet Scarlet variety, particularly by growers who admitted to such possession and reproduction under the Commission's "amnesty" program, was material to the patentability of the Sweet Scarlet variety.

142. On information and belief, the USDA and Dr. Ramming knew of the prior possession and reproduction of the Sweet Scarlet variety by growers who admitted to such possession and reproduction under the Commission's "amnesty" program. Additionally, on information and belief, Margaret A. Conner, John D. Fado, and/or Lesley Shaw, who prosecuted the application on the Sweet Scarlet variety before the USPTO, knew of the prior possession and reproduction of the Sweet Scarlet variety by growers who admitted to such possession and reproduction under the Commission's "amnesty" program.

143. Prior to issuance of the '891 patent, neither the USDA, nor Dr. Ramming, nor Ms. Conner, Mr. Fado, and/or Ms. Shaw notified the USPTO of the prior possession and reproduction of the Sweet Scarlet variety by growers who admitted to such possession and reproduction under the Commission's "amnesty" program.

144. The failure of the USDA, Dr. Ramming, and Ms. Conner, Mr. Fado, and/or Ms. Shaw to fully disclose to the USPTO the prior possession and reproduction of the Sweet Scarlet variety by growers who admitted to such possession and reproduction under the Commission's "amnesty" program breached the duty of candor owed to the USPTO in applying for a patent on the Sweet Scarlet variety. On information and belief, this breach of the duty of candor was done with an intent to deceive the USPTO and constitutes inequitable conduct.

145. The inequitable conduct described in the above paragraphs renders the '891 unenforceable.

146. The U.S., USDA, and Secretary Vilsack engaged in a discrete and final agency actions by prosecuting, procuring, accepting issuance, maintaining and exclusively licensing to the Commission the '891 patent. The acts complained of herein constitute final agency actions by the U.S., USDA and Secretary Vilsack subject to review under the APA. These acts mark the consummation of the U.S.'s, USDA's and Secretary Vilsack's decision-making process. These acts are not interlocutory, interim or tentative in nature. These acts are those by which rights or obligations have been determined or from which legal consequences will flow. Specifically, the acts complained of herein have directly determined the rights and obligations of Plaintiffs and the Commission with respect to the growing, distribution, sale, reproduction, propagation, and otherwise free use of the plant material and fruit of the Sweet Scarlet grapevine, and the right to collect royalties and the right to exclude thereof. These acts are arbitrary, capricious, and otherwise not in accordance with applicable laws and regulations, including the duty of candor before the USPTO.

147. The action of the U.S., USDA and Secretary Vilsack in procuring the '891 patent was arbitrary, capricious and otherwise not in accordance with applicable laws and regulations, because in doing so the U.S., USDA and Secretary Vilsack breached the duty of candor before the USPTO.

148. Plaintiffs have been harmed directly by the U.S.'s, USDA's and Secretary Vilsack's actions, have suffered a legal wrong because of the U.S.'s, USDA's and Secretary Vilsack's actions and have been adversely affected and aggrieved by the U.S.'s, USDA's and Secretary Vilsack's actions. The U.S.'s, USDA's and Secretary Vilsack's actions have directly impacted Plaintiffs' day-

to day business. As a result of the U.S.'s, USDA's and Secretary Vilsack's actions, Plaintiffs have been (1) restricted from growing, selling, distributing, reproducing, propagating, or otherwise freely using plant material for the Sweet Scarlet grapevine, (2) restricted from growing, selling, reproducing, distributing, propagating or otherwise freely using the fruit for the Sweet Scarlet grapevine, and (3) forced to license and pay royalties to the Commission and its agents with respect to the Sweet Scarlet grapevine plant material.

149. An actual case and controversy exists between Plaintiffs and the Commission, the U.S., the USDA and Secretary Vilsack concerning enforceability of the '891 patent.

150. Plaintiffs are entitled to judgment from this Court that the '891 patent is unenforceable for inequitable conduct during the prosecution of the '891 patent application and that the U.S's, USDA's and Secretary Vilsack's actions in prosecuting, procuring, accepting issuance, maintaining and exclusively licensing to the Commission the '891 patent are final agency actions that are arbitrary, capricious and not otherwise in accordance with applicable laws and regulations at least under the duty of candor before the USPTO.

151. This Court may and should hold unenforceable the '891 patent pursuant to 5 U.S.C. §706(2)(A), (C), and (D).

152. This Court may and should compel the U.S., USDA and Secretary Vilsack to comply with their obligations under the Patent Act pursuant to 5 U.S.C. §706(1) with respect to the '891 patent.

SIXTH CLAIM FOR RELIEF

(Sherman Act and Clayton Act Against The Commission)

153. Plaintiffs reallege and incorporate by reference all paragraphs 1-152 as if fully set forth herein.

154. On information and belief, the Commission knew that the prior uses and sales of Sweet Scarlet, particularly the uses reported to the Commission under its "amnesty" program, were not disclosed to the USPTO prior to the issuance of the '891 patent. On information and belief, the Commission further knew that this failure to disclose material information rendered the '891 patent unenforceable once issued.

155. On information and belief, the Commission has illegally monopolized interstate and foreign commerce in bad faith by (i) enforcing alleged patent rights and collecting royalty fees on the Patented Varieties under its "amnesty" program prior to issuance of a valid United States patents, and (ii) enforcing patent rights (including demanding the removal of Patented Varieties) and collecting royalties on the Sweet Scarlet variety while knowing that the patent on Sweet Scarlet could not be enforced due to prior public use and inequitable conduct.

156. On information and belief, the Commission has illegally monopolized interstate and foreign commerce by misusing the Patented Varieties though its enforcement and royalty collection activities.

157. On information and belief, the Commission is the exclusive licensee of the Patented Varieties and has abused its patent position and attempted to extend its patent monopoly by threatening new entrants and potential new entrants into the table grape market with patent infringement litigation and injunctions.

158. The existence and misuse of the patents on the Patented Varieties has deprived Plaintiffs of revenues and profits that it would have otherwise enjoyed absent the Commission's anticompetitive activities and patent misuse.

159. The acts of the Commission constitute monopolization, attempts to monopolize, and a conspiracy to monopolize the United States market for grapevine plant material producing table grapes having the characteristics of late season ripening, seedless fruit, attractive pale green coloration, cylindrical to ovoid fruit shape, firm fruit texture with neutral sweet flavor, and medium to tight cluster. The scope of the market constitutes all grapevine plant material producing table grapes having the described characteristics. The Commission holds market power in this market. The market for grapevine plant material producing table grapes having these characteristics is distinct within the grapevine market, because these unique characteristics set these grapevine plant materials apart from other grapevine plant material producing table grapes with other characteristics. Grapevine plant material producing table grapes having characteristics different than those described herein are not reasonably interchangeable and do not provide effective substitutes to the grapevine plant material producing table grapes having the characteristics identified herein, because the

characteristics described herein are uniquely valuable and distinct from other grapevine plant materials. As a direct result of the Commission's monopolistic activities, Plaintiffs have been harmed by being (1) restricted and excluded from growing, selling, distributing, propagating or using plant material in this market, (2) restricted and excluded from growing, selling, growing, propagating or distributing fruit from such plant material, and (3) forced to license and pay a royalty for plant material in these markets.

160. The acts of the Commission constitute monopolization, attempts to monopolize, and a conspiracy to monopolize the United States market for grapevine plant material producing table grapes having the characteristics of midseason ripening, seedless fruit, attractive raspberry red coloration, ovoid fruit shape, firm fruit texture with a light muscat flavor, and medium to tight cluster. The scope of the market constitutes all grapevine plant material producing table grapes having the described characteristics. The Commission holds market power in this market. The market for grapevine patent material producing table grapes having these characteristics is distinct within the grapevine market, because these unique characteristics set these grapevine plant materials apart from other grapevine plant material producing table grapes with other characteristics. Grapevine plant material producing table grapes having characteristics different than those described herein are not reasonably interchangeable and do not provide effective substitutes to the grapevine plant material producing table grapes having the characteristics identified herein, because the characteristics described herein are uniquely valuable and distinct from other grapevine plant materials. As a direct result of the Commission's monopolistic activities, Plaintiffs have been harmed by being (1) restricted and excluded from growing, selling, distributing, propagating or using plant material in this market, (2) restricted and excluded from growing, selling, growing, propagating or distributing fruit from such plant material, and (3) forced to license and pay a royalty for plant material in these markets.

161. The acts of the Commission constitute monopolization, attempts to monopolize, and a conspiracy to monopolize the United States market for grapevine plant material producing table grapes having the characteristics of midseason ripening, seedless fruit, attractive dark red coloration, oval fruit shape, firm fruit texture with neutral sweet flavor, and medium dense cluster. The scope

of the market constitutes all grapevine plant material producing table grapes having the described characteristics. The Commission holds market power in this market. The market for grapevine patent material producing table grapes having these characteristics is distinct within the grapevine market, because these unique characteristics set these grapevine plant materials apart from other grapevine plant material producing table grapes with other characteristics. Grapevine plant material producing table grapes having characteristics different than those described herein are not reasonably interchangeable and do not provide effective substitutes to the grapevine plant material producing table grapes having the characteristics identified herein, because the characteristics described herein are uniquely valuable and distinct from other grapevine plant materials. As a direct result of the Commission's monopolistic activities, Plaintiffs have been harmed by being (1) restricted and excluded from growing, selling, distributing, propagating or using plant material in this market, (2) restricted and excluded from growing, selling, growing, propagating or distributing fruit from such plant material, and (3) forced to license and pay a royalty for plant material in these markets.

162. Plaintiffs have suffered damages as a direct result of the Commissions monopolistic activities from having been unable to propagate or distribute their own grapevines or grapevine plant material within the relevant markets.

163. The Commission has monopoly power in the alleged relevant markets, including the power to control prices and exclude competition. Specifically, the Commission sub-licenses grapevine varieties within the relevant markets to nurseries and the nurseries sell the grapevine plant material with restrictions, imposed, monitored and enforced by the Commission, on use, re-sale ability and propagation. The Commission also sets the prices for the plant material within the relevant markets. Further, the Commission is the primary party enforcing the licenses to the grapevine plant material within the relevant markets. The nurseries do not have the power to lessen or destroy competition without the consent of the Commission. The nurseries only sell the grapevines falling within the relevant markets based on the restrictions placed by the Commission. The Commission is the primary actor dictating the terms of marketing and use of the grapevine plant material within the relevant markets.

164. The Commission's monopolistic activities are in violation of the Sherman and Clayton Acts.

165. Plaintiffs are entitled to judgment from this Court that the Commission's conduct is a violation of the Sherman Act (15 U.S.C. § 2) and that Plaintiffs are entitled to recovery, including damages, reasonable attorneys' fees, restitution, disgorgement of all royalty fees obtained by the Commission through its "patent licensing" and "amnesty" programs, and treble damages under the Clayton Act (15 U.S.C. § 15).

SEVENTH CLAIM FOR RELIEF

(Declaration That Exclusive License Agreements For Patented Varieties Are Void And Unlawful Against the Commission Under Federal Law)

166. Plaintiffs reallege and incorporate by reference all paragraphs 1-165 as if fully set forth herein.

167. This claim arises under the patent laws of the United States, Title 35 of the United States Code, and the Declaratory Judgment provisions of §§ 2201 and 2202 of Title 28 of the United States Code and the APA at 5 U.S.C. §§702, *et seq.*

168. The '284, '891 and '229 patents are invalid under 35 U.S.C. §102(b).

169. The '891 patent in unenforceable because it was obtained through inequitable conduct before the USPTO.

170. The USDA's action of granting the Commission an exclusive license in the invalid '284, '891 and '229 patents is contrary to law.

171. The exclusive license between the Commission and the USDA for the Patented Varieties is void and invalid, because the '284, '891 and '229 patents are invalid under 35 U.S.C. §102(b).

172. The exclusive license between the Commission and the USDA for the '891 patent is void and invalid, because the '891 patent is unenforceable.

173. Plaintiffs have directly suffered harm as a result of the USDA and the Commission entering into an unlawful and void exclusive license agreement for the '284, '891 and '229 patents, including without limitation, Plaintiffs being (1) restricted from growing, selling, distributing,

propagating or using plant material for Patented Varieties, (2) restricted from selling, growing, propagating or distributing fruit for the Patented Varieties, and (3) forced to pay license fees for the Patented Varieties.

174. An actual case and controversy exists between Plaintiffs and the Commission and the USDA concerning the viability of the exclusive license between the Commission and the USDA for the '284, '891 and '229 patents.

175. Plaintiffs are entitled to judgment from this Court that the exclusive license between the USDA and the Commission for the '284, '891 and '229 patents is void and unlawful.

176. Plaintiffs are entitled to judgment from this Court that the USDA's action in granting the Commission an exclusive license to the '284, 891 and '229 patents was unlawful under 5 U.S.C. §702, *et seq.*

EIGHTH CLAIM FOR RELIEF

(Declaration That Exclusive License For Patented Varieties Are Void And Unlawful Against The Commission Under State Law)

177. Plaintiffs reallege and incorporated by reference all paragraphs 1-176 as if fully set forth herein.

178. This claim arises under the patent laws of the United States, Title 35 of the United States Code, and California Code of Civil Procedure §1060 for declaratory relief.

179. The '284, '891 and '229 patents are invalid under 35 U.S.C. §102(b).

180. The '891 patent in unenforceable because it was obtained through inequitable conduct before the USPTO.

181. The exclusive license between the Commission and the USDA for the Patented Varieties is void and invalid, because the '284, '891 and '229 patents are invalid under 35 U.S.C. §102(b).

182. The exclusive license between the Commission and the USDA for the '891 patent is void and invalid, because the '891 patent is unenforceable.

183. Plaintiffs have directly suffered harm as a result of the USDA and the Commission entering into an unlawful and void exclusive license agreement for the '284, '891 and '229 patents,

including without limitation, Plaintiffs being (1) restricted from growing, selling, distributing, propagating or using plant material for Patented Varieties, (2) restricted from selling, growing, propagating or distributing fruit for the Patented Varieties, and (3) forced to pay license fees for the Patented Varieties.

184. An actual case and controversy exists between Plaintiffs and the Commission and the USDA concerning the viability of the exclusive license between the Commission and the USDA for the '284, '891 and '229 patents.

185. Plaintiffs are entitled to judgment from this Court that the exclusive license between the USDA and the Commission for the '284, '891 and '229 patents is void and unlawful.

NINTH CLAIM FOR RELIEF

(Unfair Competition Under California Business and Professions Code §17200 and California Common Law Against The Commission)

186. Plaintiffs reallege and incorporate by reference all paragraphs 1-185 as if fully set forth herein.

187. The Commission has unlawfully and unfairly exploited the '284, '891 and '229 patents in a manner that violates antitrust laws and in a manner that attempts to extend these patents beyond their lawful scope. Such acts constitute unfair trade practices and unfair competition under California Business and Professions Code §17200, *et seq.*, and under the common law of the State of California, entitling Plaintiffs to relief.

188. Pursuant to California Business and Professions Code §17203, Defendant is required to disgorge and restore to Plaintiffs all profits and property acquired by means of Defendant's unfair competition.

189. Due to the conduct of Defendant, Plaintiffs have suffered irreparable harm, have suffered injury in fact and have lost money or property as a result of Defendant's acts of unfair business practices alleged herein. It would be difficult to ascertain the amount of money damages that would afford Plaintiffs adequate relief at law for Defendant's acts. Plaintiffs' remedy at law is not adequate to compensate Plaintiffs for the injuries inflicted by Defendant. Accordingly, Plaintiffs are entitled to preliminary and permanent injunctive relief pursuant to California Business and

Professions Code §17203.

190. Plaintiffs are informed and believe and on that basis allege that Defendant's conduct has been intentional and willful and in conscious disregard of Plaintiffs' rights and, therefore, Plaintiffs are entitled to its attorneys' fees.

TENTH CLAIM FOR RELIEF

(Unjust Enrichment Against The Commission)

191. Plaintiffs reallege and incorporate by reference all paragraphs 1-190 as if fully set forth herein.

192. By acquiring licensing revenue for the '891 patent, which was fraudulently procured and maintained, the Commission has received significant benefits. In addition, by acquiring licensing revenue for the '284, '891 and '229 patents in a manner that expands these patents beyond their lawful scope, the Commission has received significant benefits.

193. The Commission's unjust receipt and retention of such benefits has unjustly enriched them at the expense of the Plaintiffs and all California table grape growers who have paid the Commission license fees.

ELEVENTH CLAIM FOR RELIEF

(Constructive Trust Against The Commission)

194. Plaintiffs reallege and incorporate by reference all paragraphs 1-193 as if fully set forth herein.

195. California table grape growers have transferred to the Commission licensing fees, being induced by fraud, duress or undue influence of the Commission.

196. The Commission is subject to an equitable duty to convey the license fees it has received from Plaintiffs and other California table grape growers on the ground that the Commission would be unjustly enriched if the Commission were permitted to retain those license fees.

197. The licensing fees acquired by the Commission for the Patented Varieties should be held in constructive trust for the benefit of California table grape growers who have paid the Commission license fees with respect to the Patented Varieties.

PRAYER FOR RELIEF

Wherefore, Plaintiffs prays for the following relief against Defendant:

A. Declaring that the U.S., USDA and Secretary Vilsack acted arbitrarily, capriciously, and not in accordance with law under the APA by engaging in the following final agency actions:

a. Entering into the patenting program with the Commission with respect to the Patented Varieties;

b. Filing the applications for the '284, '891 and '229 patents;

c. Prosecuting the '284, '891 and '229 patents;

d. Accepting issuance of the '284, '891 and '229 patents;

e. Procuring the '284, '891 and '229 patents;

f. Maintaining the '284, '891 and '229 patents;

g. Granting the Commission an exclusive license to the '294, '891 and '229 patents;

B. Declaring that the '284, '891 and '229 patents are invalid;

C. Declaring that the '891 patent is unenforceable;

D. Declaring that the Commission and the USDA committed inequitable conduct in the prosecution and procurement of the '891 patent;

E. That the Court adjudge and decree that the Commission has violated the Sherman and Clayton Acts;

F. That the Court adjudge and decree that the Commission has violated the antitrust laws of the State of California;

G. That the Court adjudge and decree that the Commission's exclusive license in the '284, '891 and '229 patents is void and invalid;

H. That the Court adjudge and decree that the Commission has violated California statutory and common law unfair competition laws;

I. That the Court adjudge and decree that the Commission has been unjustly enriched;

J. That the Court compel the U.S., USDA and Secretary Vilsack to act in accordance with law, including applicable patent laws, pursuant to 5 U.S.C. §706;

K. That the Court set aside the following agency actions under the APA, including 5 U.S.C. §706:

a. Entering into the patenting program with the Commission with respect to the Patented Varieties;

b. Filing the applications for the '284, '891 and '229 patents;

c. Prosecuting the '284, '891 and '229 patents;

d. Accepting issuance of the '284, '891 and '229 patents;

e. Procuring the '284, '891 and '229 patents;

f. Maintaining the '284, '891 and '229 patents;

g. Granting the Commission an exclusive license to the '294, '891 and '229 patents;

L. A preliminary and permanent injunction enjoining the Commission, its officers, servants, commissioners, employees, attorneys, all parent and subsidiary corporations, all assignees and successors in interest, and those persons in active concert or participation with the Commission, including distributors and nurseries from enforcing or threatening to enforce the '284, '891 and '229 patents;

M. That the Court order the Commission to dedicate to the public patents regarding the Autumn King, Sweet Scarlet and Scarlet Royal varieties, both domestic and foreign; or alternatively grant all requesting persons royalty-free licenses to propagate, grow, harvest and sell Autumn King, Sweet Scarlet and Scarlet Royal varieties and their fruit which are claimed in the '284, '891 and '229 patents.

N. An award of damages against the Commission, together with pre-judgment and post-judgment interest;

O. Disgorgement of all royalty fees obtained by the Commission in connection with the Patented Varieties;

P. That the licensing fees acquired by the Commission for the Patented Varieties be held in constructive trust for the benefit of California table grape growers who have paid the Commission license fees with respect to the Patented Varieties;

Q. A trebling of said damages awarded against the Commission;

R. That Plaintiffs recover the costs of this suit together with reasonable attorneys' fees;

S. That Plaintiffs have such other and further relief as the nature of this case may require and as the Court may deem just and proper.

DATED: March 19, 2008

HENNIGAN BENNETT & DORMAN LLP

By /s/ Lawrence M. Hadley
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DEMAND FOR JURY TRIAL

Plaintiffs hereby demand a trial by jury as to all issues so triable.

DATED: March 19, 2008

HENNIGAN BENNETT & DORMAN LLP

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US00PP15891P3

(12) **United States Plant Patent**
Ramming et al.

(10) **Patent No.: US PP15,891 P3**
(45) **Date of Patent: Jul. 26, 2005**

(54) **GRAPEVINE PLANT DENOMINATED 'SWEET SCARLET'**

(50) Latin Name: ***Vitis vinifera L.***
Varietal Denomination: **Sweet Scarlet**

(75) Inventors: **David W. Ramming**, Fresno, CA (US); **Ronald E. Tarailo**, Fresno, CA (US)

(73) Assignee: **The United States of America as represented by the Secretary of Agriculture**, Washington, DC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 170 days.

(21) Appl. No.: **10/371,512**

(22) Filed: **Feb. 20, 2003**

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(51) **Int. Cl.**[7] **A01H 5/00**
(52) **U.S. Cl.** **Plt./205**
(58) **Field of Search** Plt./205

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Primary Examiner—Kent Bell
(74) *Attorney, Agent, or Firm*—Margaret A. Connor; John D. Fado; Lesley Shaw

(57) **ABSTRACT**

A new and distinct variety of grapevine denominated 'Sweet Scarlet' which is characterized by its midseason ripening seedless fruit, attractive raspberry red coloration, its ovoid fruit shape, its firm fruit texture with a light muscat flavor, and its medium to tight cluster.

2 Drawing Sheets

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

The new variety was developed by the United States Department of Agriculture of the Agricultural Research Service, Postharvest Quality and Genetics Research Unit in Parlier, Calif.

Latin name of the genus and species of the plant claimed: *Vitis vinifera* L.

Variety denominated: 'Sweet Scarlet'.

BACKGROUND OF THE INVENTION

The present invention relates to a new and distinct variety of grapevine, *Vitis vinifera* L., which will hereinafter be

denominated varietally as the 'Sweet Scarlet' grapevine, and, more particularly, to a grapevine which has fruit maturing for commercial harvesting and shipment approximately August 23 in the San Joaquin Valley of central California. The fruit has an attractive red skin coloration at maturity with a muscat flavored flesh and outstanding fruit quality.

The grapevine of the present invention originated from a hand-pollinated cross of United States Department of Agriculture selection 'C33-30' (unpatented) and the United States Department of Agriculture selection 'C103-141' (unpatented) made in 1989 at the United States Department of Agriculture, Agricultural Research Service, Postharvest Quality and Genetics Research Unit plots at California State University, Fresno, in Fresno Calif. The female was 'C33-30' a seedless, red-fruited grapevine having reflex anthers in

the flower, large oval berries with firm flesh and medium skin, and a neutral flavor. The fruit of the 'C33-30' ripen about one week before the instant variety. The pollen parent was 'C103-141' a seedless red-purple fruited grape with medium size, oval to round berries with medium skin and firm flesh. The fruit of the 'C103-141' grapevine ripen at the same time as the variety of the subject invention. Both of the parents of the instant cultivar are hybrids of the grapevine genus and species *Vitis vinifera* L.

Aborted seeds resulting from this controlled hybridization were developed further through invitro tissue culture and germinated in the laboratory during the fall of 1989. The resulting seedlings were planted in the spring of 1990 in a vineyard at the United States Department of Agriculture, Agricultural Research Service plots on the California State University, Fresno, campus in Fresno, Calif. The seedlings fruited in the summer of 1992 and one, the grapevine of the present invention, was selected for its attractive red seedless firm muscat flavored fruit, medium berry size, and outstanding fruit quality.

In 1993 at the inventors' direction, the grapevine of the subject invention was propagated asexually by rooting hardwood cuttings at Fresno, Calif. and a test planting of five grapevines of the subject invention was established in the United States Department of Agriculture, Agricultural Research Service plots on the California State University, Fresno campus. Subsequently, larger test plantings have been established with asexually multiplied grapevines of the instant invention. When hardwood cuttings were used for propagation, the instant cultivar rooted readily therefrom. All grapevines of the new variety planted from hardwood cutting propagation, fruited in the third season of growth after planting. All propagules, or resulting plants, of the present invention have been observed by the inventors to be true to type in that all asexual reproduced grapevines of the variety possessed the characteristics identical to those of the originally discovered grapevine.

SUMMARY OF THE INVENTION

The grapevines of the subject invention possess medium vigor and have produced fruit well both as own-rooted and grafted grapevines. The size of the grapevines was determined by growing the grapevines on a three cross arm 'T' type trellis structure with a top cross arm of 122 cm in length set 189 cm above the ground; a second cross arm of 102 cm in length set 156 cm above the ground; and a third cross arm 91 cm in length set 125 cm above the ground. The trellis structure had two wires per cross arm and indicted a grapevine height of 216 cm and a grapevine spread of 218 cm.

The fruit of the new variety ripens in midseason, about the same time as the 'Ruby Seedless' grapevine (unpatented). The average ripening date in Fresno, Calif. is August 23. Berries adhere well to the fruit pedicel and have minimal shatter from the clusters during storage. The fruit is raspberry red in color at maturity. The fruit shape is ovoid. Fruit skins are medium thick and similar to the 'Ruby Seedless' grapevine. 'Sweet Scarlet' differs from 'Ruby Seedless' by having a light muscat flavor. The pulp of the fruit adheres to the skins of the berry and the fruit texture is firm and meaty. The berries are medium in size, or 3.6 grams. The flavor of the fruit is sweet and has a light muscat flavor when ripe. The flavor has been rated high. Soluble solids concentration of the juice at fruit maturity averages 21.6% with titratable acid of 0.47 grams/100 milliliters of juice. The fruit is of the stenospermocarpic type of seedlessness and contains small, aborted seed traces that are not noticeable when eaten. The fruit clusters are usually borne on the average of 0.53 per shoot on cane pruned vines. The fruit clusters are conical and are large in size, or 1681 grams, medium to slightly tight and attractive. The fruit cluster peduncles are medium in length.

The grapevine and fruit of the new variety are susceptible to powdery mildew disease of grape plants. A spray program for powdery mildew disease control is required.

BRIEF DESCRIPTION OF THE DRAWINGS

The drawings of the grapevine of the present invention are color photographs showing in FIG. **1** a typical specimen of the fruit and in FIG. **2** a shoot with leaves and a flower cluster all of the new variety of the present invention. The color of the photographs is as nearly true as it is reasonably possible to provide in such color photographs. Description of the new invention applies to vines of 'Sweet Scarlet' grown on its own roots at a density of 1,119 vines per hectare in Fresno County, Calif. in 2002. These vines were in their fourth year of full production having been planted in 1996.

DETAILED BOTANICAL DESCRIPTION

The new variety cv. 'Sweet Scarlet' may be distinguished from other commercial grape cultivars known to us by a combination of characteristics, including its midseason ripening seedless fruit with attractive raspberry red coloration, its firm fruit texture with a light muscat flavor, its ovoid fruit shape and its medium to tight cluster.

The new variety of grapevine is most similar to its pollen parent 'C103-141' by having similar dates of harvest and similar berry size. It is distinguished therefrom and an improvement thereon in a number of fruit characteristics. The flesh of the new variety is firmer, the skin color is an attractive raspberry red not a purple red coloration. The berry shape is ovoid compared to the oval to round berries of 'C103-141'. The most distinguishing difference is the light muscat flavor that is not present in the pollen parent. The new grapevine is also similar to the commercial variety 'Ruby Seedless' (unpatented) in that they ripen at a similar time, both have large clusters and similar size and shape berries. It is distinguished therefrom and an improvement thereon in that the flesh of the new variety is firmer, the color is more attractive being a raspberry red compared to a dull purple red of 'Ruby Seedless'. The most distinguishing difference is the light muscat flavor of the new variety.

The new variety also differs substantially from its mother parent 'C33-30'. The new variety has perfect flowers with functional male and female parts while 'C33-30' has only functional female parts. The new variety has smaller aborted seeds. The most distinguishing difference is the light muscat flavor of the new variety, while 'C33-30' has none.

Referring more specifically to the botanical details of this new and distinct variety of grapevine, the following has been observed under the ecological conditions prevailing at the orchard of origin which is located in Fresno in the San Joaquin Valley of central California. All major color code designations are by reference to the *Dictionary of Color,* by Maerz and Paul, First Edition, 1930. Common color names are also occasionally employed. Where dimensions, sizes, colors and other characteristics are given, it is to be understood that such characteristics are approximations of averages set forth as accurately as practicable. The description hereof was taken from specimens grown in Fresno, Calif. The grapevines used for measurement were grown in a fine sandy loam soil and the grapevines were irrigated using trickle, or drip irrigation. In a substantial part, the data hereof was from grapevines that were six (6) years old.

VINE

Generally:

Size.—Medium. Grapevine size as determined on grapevines growing on a three cross arm 'T' trellis with the top cross arm 122 cm (47.58 inches) long set 189 cm (73.71 inches) above the ground; the second cross arm 102 cm (39.78 inches) long set 156 cm (60.84 inches) above the ground; and the third cross arm 91 cm (35.49 inches) long set 125 cm (48.75 inches) above the ground. There were two wires per cross arm and was trained to produce a grapevine height of 216 cm (84.24 inches) and a grapevine spread of 218 cm (85.02 inches).

Vigor.—Medium vigor. Vigor as measured by weighing prunings at dormant pruning for cane pruned grapevines (with 5 canes and an average of 13 buds per cane) was 3.0 Kg.

Productivity.—Productive, 15.89 Kg per grapevine as compared to the Ruby Seedless grapevine which produces 16.8 Kg per grapevine on grapevines spaced 8 ft. (243.84 cm) by 12 ft. (365.76 cm).

Regularity of bearing.—Regular. Annual pruning of canes is required for reliable production.

CANES

Size:

Diameter — mature canes.—Medium diameter, medium vigor, upright in growth habit.

Mature canes:

Diameter — internode base.—11.4 mm (0.456 inches).

Diameter — internode midpoint.—9.1 mm (0.364 inches).

Diameter — internode tip.—5.4 mm (0.216 inches).

Diameter — node base.—12.7 mm (0.508 inches).

Diameter — node midpoint.—10.5 mm (0.42 inches).

Diameter — node tip.—7.5 mm (0.3 inches).

Internode length:

Base.—10.0 cm (3.9 inches).

Midpoint.—10.1 cm (3.939 inches).

Tip.—9.1 cm (3.549 inches).

Average length of canes.—240.8 cm (93.912 inches).

Surface texture.—Smooth.

Color of mature cane.—Brown (plate 11 H6). No anthocyanin observed on mature canes.

Buds:

Color.—Brown (plate 13 F8).

Texture.—Smooth.

Dormant bud (compound bud or eye):

Width.—At base of cane 5.3 mm (0.212 inches); at midpoint of cane 5.4 mm (0.216 inches) and at tip of cane 4.4 mm (0.176 inches). The average number of buds on a current, single-season growth cane is 26.

Date of bud break.—March 3rd, midseason.

Young shoots.—Young shoots have cobwebby indument.

Diameter of young shoots in spring (measured when shoots are 24 inches).—At base 8.5 mm (0.34 inches), at midpoint 7.4 mm (0.296 inches) and at tip 3.1 mm (0.124 inches).

Internode length.—11.2 cm (4.368 inches) at 4th internode from base.

Young shoots:

Color.—Pale yellow green (plate 18 L7) with slight copper on edge.

Stem of shoot tip:

Color.—Green (plate 20 L6) with a slight copper tint in sun.

Shoot:

Shape.—Straight to slightly curved.

Shoot tip:

Form.—Open.

Tendrils:

Size.—Length — 25.2 cm (9.828 inches).

Size.—Diameter — 2.45 mm (0.098 inches).

Shape.—Usually triforcated or quadforcated and curled on distal end.

Pattern.—Found beginning opposite node 6 and 7, then again at nodes 9, 10, 12, 13, 15, 16 with this repeating intermittent pattern to the distal end of the cane.

Tendril:

Color immature growth.—Yellow green (plate 21 L6) with slight copper on tip.

Disease resistance: Susceptible to powdery mildew, and fungicides were applied to the grapevines under evaluation to control powdery mildew.

Insect resistance: Insecticides were applied to the grapevines under evaluation to control grapevine leafhoppers and variegated leafhoppers. No resistances to these pests were determined in these evaluations due to chemical control of these pests.

LEAVES

Size:

Generally.—Leaves simple and alternate. The mid vein (L1) is 14.9 cm (5.811 inches) long, vein L2 is 12.2 cm (4.758 inches) long and vein L3 is 8.9 cm (3.471 inches) long. The angle between the mid vein L1 and L3 is 55.1 degrees and between L1 and the 1st vein off L3 is 148.8 degrees.

Average length.—20.6 cm (8.034 inches).

Average width.—18.9 cm (7.371 inches).

Shape.—Orbicular.

Lobes:

Number.—Five (5).

Color:

Upwardly disposed surface.—Dark green (plate 23 H9). Upward surface is glabrous, flat and smooth to slightly bullate.

Downwardly disposed surface.—Green (plate 22 I6). Lower surface is glabrous with short hairs along the main midrib vein.

Leaf vein.—Light green (plate 19 I6) with occasional red (plate 6 I4) on main veins near center of leaf.

Leaf vein — thickness.—Thickness of mid vein at center of leaf is 1.9 mm (0.076 inches).

Leaf margin.—Serrated with shape of teeth pointed and medium in size.

Petiole sinus.—Lyre shape and usually petiole lobes overlap causing a closed petiole sinus. On mature leaf is 4.4 cm (1.72 inches) deep and 1.3 cm (0.507 inches) wide at widest point.

Anthocyanin:

Main veins — location.—With occasional red (plate 6 I4) on main veins near center of leaf.

Petiole:

Size.—Medium.

Length.—11.7 cm (4.563 inches).

Diameter.—3.3 mm (0.132 inches).

Color.—Green (plate 20 L4) with occasional red (plate 6 I4) covering.

Color:
Young leaf — upper surface.—Pale green (plate 21 L8) with light copper and cobwebby indument on upper surface.
Young leaf — lower surface.—Pale green (plate 22 K7).
Shape unfolded — young leaf.—Concave to flat.
Petiole of young leaf — color.—Medium green (plate 21 L8).
Stipules.—Onion skin.

TRUNK

Size: Large.
Height.—Approximately 104 cm (40.56 inches) above the vineyard floor.
Diameter.—7.41 cm (2.8899 inches) as measured just below the cordon or head point at 81.28 cm (31.6992 inches) above vineyard floor; and 6.81 cm (2.6559 inches) at 15.2 cm (5.928 inches) above the vineyard floor.
Bark — color.—(plate 16 A2).

FLOWERS

Flower:
Size — generally.—Medium.
Unopened — diameter.—2.8 mm (0.112 inches).
Unopened — length.—2.2 mm (0.088 inches).
Unopened — surface texture.—Smooth.
Date of bloom.—First bloom May 10.
Date of full bloom.—May 14 at 90%.
Inflorescence.—Panicle.
Cluster size:
At bloom.—Generally, large to very large.
Cluster — length.—27.3 cm (10.647 inches).
Width.—24.6 cm (9.594 inches).
Peduncle:
Length.—3.3 cm (1.287 inches).
Shape of cluster.—Conical with shoulder well developed.
Calyptra:
Color.—Green (plate 20 L6).
Stamens.—Five (5) and erect.
Pistil.—Well developed.
Ovary:
Color.—Green (plate 20 L8).
Pollen.—Normal, fertile, abundant.
Anthers:
Color.—Straw (plate 10 G2).

FRUIT

Maturity when descried: Ripe for commercial harvesting and shipment approximately August 23 in Fresno, Calif. Midseason or with the 'Ruby Seedless' grapevine.
Cluster:
Size — cane pruned vines.—1,681 grams (58.835 oz).
Length.—31.0 cm (12.09 inches).
Width.—23.0 cm (8.97 inches).
Shape.—Conical.
Density.—Medium to tight, on average has 460 berries per cluster.
Clusters per vine.—25.
Clusters per shoot.—0.53 clusters per shoot.
Peduncle:
Size:
Length.—Medium, 5.0 cm (1.95 inches).
Diameter.—Medium, 6.3 mm (0.252 inches).

Color.—Green (plate 20 K6).
Texture.—Smooth, glabrous.
Pedicel:
Generally.—There is a medium to good attachment between the berry and the pedicel.
Size — length.—7.4 mm (0.296 inches).
Size — diameter. —0.95 mm (0.038 inches).
Color.—Green (plate 20 H7).
Texture.—Glabrous with a few lenticels.
Brush:
Length.— 2.6 mm (0.104 inches).
Brush color.—Green (plate 20 D2).
Berry:
Size.—Medium, avg. 3.6 grams (0.126 oz).
Shape.—Ovoid 2.07 cm (0.8073 inches) long and 1.70 cm (0.663 inches) wide.
Length.—2.07 cm (0.8073 inches).
Width.—1.70 cm (0.663 inches).
Color.—Raspberry red (plate 6 I15).
Bloom.—Light.
Skin:
Generally.—The skin adheres to the flesh.
Thickness.—Medium in thickness.
Texture.—Smooth.
Tendency to crack.—None.
Flesh:
Flesh color.—Translucent and very pale yellow green (plate 18 B1).
Texture.—Firm, meaty.
Juice production.—Medium.
Color of juice.—Clear.
Flavor.—Sweet and sub acid, light muscat flavor.
Soluble solids.—21.6%.
Titratable acid.—0.47 g/100 ml juice.
Aroma.—None.
Ripening.—Uniform.
Eating quality.—Very good, sweet.
Character of seeds: Stenospermocarpic seedless, small aborted seed traces that are not noticeable when eaten. Average aborted seed trace when present are 11.5 mg fresh weight, 4.85 mm (0.194 inches) long and 2.51 mm (0.1004 inches) wide. Seed color is auburn (plate 7 C11).
Use: Fresh market. No wine nor raisin evaluations have been done.
Keeping quality: Good.
Resistance to disease: No resistance to powdery mildew.
Shipping and handling qualities: Berries ship and handle similar to 'Ruby Seedless' except the pedicel dries somewhat quicker.

Although the new variety of grapevine possesses the described characteristics noted above as a result of the growing conditions prevailing in Fresno, Calif. in the central San Joaquin Valley of California, United States of America, it is to be understood that variations of the usual magnitude and characteristics incident to changes in growing conditions, training, irrigation, fertilization, pruning, pest control, climatic variation and the like are to be expected.

Having thus described and illustrated our new variety of grapevine, what we claim as new and desire to be secured by Plant Letters Patent is:

1. A new and distinct variety of grapevine plant, 'Sweet Scarlet', substantially as illustrated and described, characterized by its attractive raspberry red fruit color, ovoid fruit shape, and firm flesh texture with a light muscat flavor.

* * * * *

FIG. 1

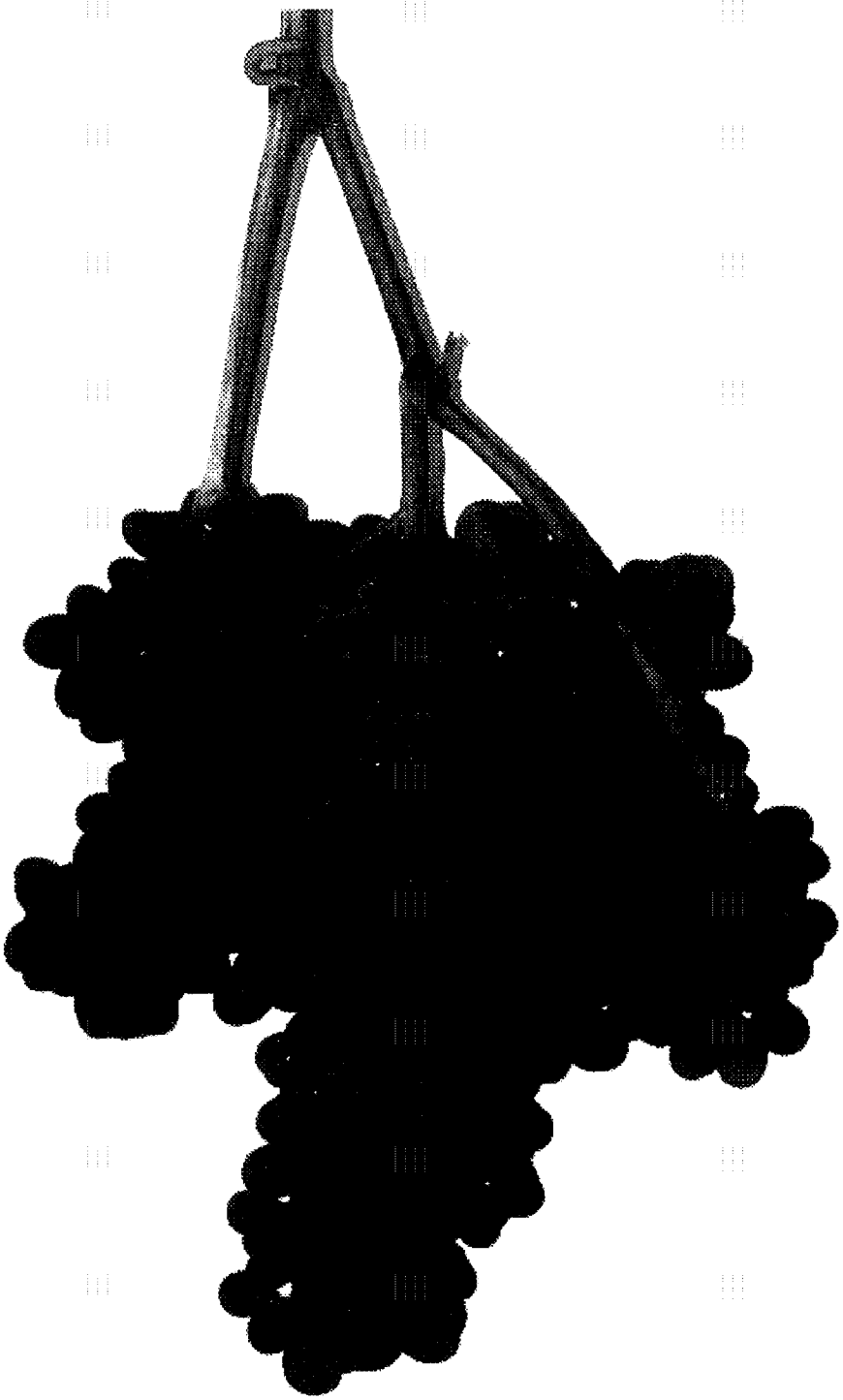

FIG. 2

US00PP16284P2

(12) **United States Plant Patent**
Ramming et al.

(10) **Patent No.: US PP16,284 P2**
(45) **Date of Patent: Feb. 21, 2006**

(54) **GRAPEVINE DENOMINATED 'AUTUMN KING'**

(50) Latin Name: ***Vitis vinifera L.***
Varietal Denomination: **Autumn King**

(75) Inventors: **David W. Ramming**, Fresno, CA (US); **Ronald E. Tarailo**, Fresno, CA (US)

(73) Assignee: **The United States of America as represented by the Secretary of Agriculture**, Washington, DC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 161 days.

(21) Appl. No.: **10/953,387**

(22) Filed: **Sep. 28, 2004**

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A01H 5/00 (2006.01)

(52) **U.S. Cl.** **Plt./207**
(58) **Field of Classification Search** Plt./207
See application file for complete search history.

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(74) *Attorney, Agent, or Firm*—Margaret A. Connor; John D. Fado; Leslie Shaw

(57) **ABSTRACT**

A new and distinct variety of grapevine denominated 'Autumn King' which is characterized by its late season ripening seedless fruit, attractive pale green coloration, its cylindrical to ovoid fruit shape, its firm fruit texture with neutral sweet flavor, and its medium to tight cluster.

2 Drawing Sheets

BACKGROUND OF THE INVENTION

The present invention relates to a new and distinct variety of grapevine, *Vitis vinifera* L., which will hereinafter be denominated varietally as the 'Autumn King' grapevine, and, more particularly, to a grapevine which has fruit maturing for commercial harvesting and shipment approximately October 23 in the San Joaquin Valley of central California. The fruit has an attractive pale green skin coloration at maturity with large cylindrical to ovoid shape seedless berries.

The grapevine of the present invention originated from a hand-pollinated cross of United States Department of Agriculture selection 'A61-20' (unpatented) and the United States Department of Agriculture selection 'B99-131' (unpatented) made in 1993 at the United States Department of Agriculture, Agricultural Research Service, Postharvest Quality and Genetics Research Unit plots at California State University, Fresno, in Fresno Calif. The female was 'A61-20', a seeded white-fruited grapevine having large size, ovoid berries with firm flesh and good skin, and a neutral flavor. The fruit of the 'A61-20' ripen about two weeks before the instant variety. The pollen parent was 'B99-131', a seedless white fruited grape with very large size, oval berries with medium skin and medium firm flesh. The fruit

of the 'B99-131' grapevine ripen one month before the variety of the subject invention. Both of the parents of the instant cultivar are hybrids of the grapevine genus and species *Vitis vinifera* L.

The seeds resulting from this controlled hybridization were germinated in the greenhouse during the winter and spring of 1994. The resulting seedling population totaled 534 individual plants. All seedlings were planted in the spring of 1994 in a vineyard at the United States Department of Agriculture, Agricultural Research Service plots on the California State University, Fresno, campus in Fresno, Calif. The seedlings fruited in the summer of 1996 and one, the grapevine of the present invention, was designated as 'C67-120' and selected for its attractive pale green seedless, medium firm, large berry size, good fruit quality and late maturity.

In 1997 at the inventors' direction, the grapevine of the subject invention was propagated asexually by rooting hardwood cuttings at Fresno, Calif. and a test planting of two grapevines of the subject invention was established in the United States Department of Agriculture, Agricultural Research Service plots on the California State University, Fresno campus. Subsequently in 1998 a larger test planting of 24 vines was established with rooted hardwood cuttings of the instant invention. The instant cultivar rooted readily from hardwood cuttings. All grapevines of the new variety

planted from hardwood cutting propagation, fruited in the third season of growth after planting. All propagules, or resulting plants, of the present invention have been observed by the inventors to be true to type in that all asexual reproduced grapevines of the variety possessed the characteristics identical to those of the original parent grapevine.

SUMMARY OF THE INVENTION

The grapevines of the subject invention possess medium vigor and have produced fruit as own-rooted grapevines. The size of the grapevines was determined by growing the grapevines on a three cross arm 'T' type trellis structure with a top cross arm of 122 cm in length set 189 cm above the ground; a second cross arm of 102 cm in length set 156 cm above the ground; and a third cross arm 91 cm in length set 125 cm above the ground. The trellis structure had two wires per cross arm and indicted a grapevine height of 200 cm and a grapevine spread of 199 cm.

The fruit of the new variety ripens late, about 8 weeks after the 'Thompson Seedless' (non-patented) and 4 weeks after 'Autumn Seedless' (non-patented). The average ripening date in Fresno, Calif. is October 23. Berries adhere medium well to the fruit pedicel and have minimal shatter from the clusters during storage. The fruit is pale green in color at maturity. The fruit shape is cylindrical to ovoid. Fruit skins are medium thick and similar to the 'Thompson Seedless' grapevine. 'Autumn King' differs from 'Thompson Seedless', ripening 8 weeks later. The pulp of the fruit adheres to the skins of the berry and the fruit texture is firm and meaty. The berries are large to very large in size, or 9.8 grams. The flavor of the fruit is sweet and has been rated good. Soluble solids concentration of the juice at fruit maturity averages 18.6% with titratable acid of 0.31 grams/100 milliliters of juice. The fruit is of the stenospermocarpic type of seedlessness and contains small, aborted seed traces that are not noticeable when eaten. The fruit clusters are usually borne on the average of 1.02 per shoot on cane pruned vines. The fruit clusters are conical and are medium in size, or 539 grams, medium to slightly tight and attractive. The fruit cluster peduncles are medium in length.

The grapevine and fruit of the new variety are susceptible to powdery mildew disease of grape plants. A spray program for powdery mildew disease control is required.

BRIEF DESCRIPTION OF THE DRAWINGS

The drawings of the grapevine of the present invention are color photographs showing in FIG. **1** a typical specimen of the fruit and in FIG. **2** a shoot with leaves and a flower cluster all of the new variety of the present invention.

The color of the phtographs is as nearly true as it is reasonably possible to provide in such color photographs. Description of the new invention applies to vines of 'Autumn King' grown on its own roots at a density of 1,119 vines per hectare in Fresno County, Calif. in 2002. These vines were in their second year of full production having been planted in 1998.

DETAILED BOTANICAL DESCRIPTION

The new variety cv. 'Autumn King' may be distinguished from other commercial grape cultivars known to us by a combination of characteristics, including its late season ripening seedless fruit with attractive pale green coloration, its medium firm fruit texture with a neutral sweet flavor, its cylindrical to ovoid fruit shape and its medium to tight cluster.

The new variety of grapevine is most similar to its pollen parent 'B99-131' by having similar berry size and pale green fruit. It is distinguished therefrom and an improvement thereon in a number of fruit characteristics. The flesh of the new variety is firmer, the skin color is more attractive not showing veins. The berry shape is cylindrical to ovoid compared to the oval to round berries of 'B99-131'. The most distinguishing difference is the maturity time, being 4 weeks later thatn the pollen parent. The new grapevine is also similar to the commercial varieties 'Thompson Seedless' and 'Autumn Seedless' in that they have pale green seedless neutral flavored fruit. It is distinguished therefrom and an improvement thereon in that the berries of tne new variety are larger that those of 'Thompson Seedless' and 'Autumn Seedless'. It is also distinguished from 'Thompson Seedless' and 'Autumn Seedless' by ripening 8 and 4 weeks later, respectively.

The new variety also differs substantially from its mother parent 'A61-20'. It is distinguished therefrom and an improvement thereon in that the new variety is seedless, having small aborted (stenospermocarpic) seeds, while 'A61-20' is seeded with functional seeds. The berries of the new variety are substantially larger, being on average about 9.8 grams, while the berries of 'A61-20' are about 6.8 grams.

Referring more specifically to the botanical details of this new and distinct variety of grapevine, the following has been observed under the ecological conditions prevailing at the orchard or origin which is located in Fresno in the San Joaquin Valley of central California. All major color code designations are by reference to the *Dictionary of Color*, by Maerz and Paul, First Edition, 1930. Common color names are also occasionally employed. Where dimensions, sizes, colors and other characteristics are given, it is to be understood that such characteristics are approximations of averages set forth as accurately as practicable. The description hereof was taken from specimens grown in Fresno, Calif. The grapevines used for measurement were grown in a fine sandy loam soil and the grapevines were irrigated using trickle, or drip irrigation. In a substantial part, the data hereof was from grapevines that were five (5) years old.

VINE

Generally:

Size.—Medium. Grapevine size as determined on grapevines growing on a three cross arm 'T' trellis with the top cross arm 122 cm long set 189 cm above the ground; the second cross arm 102 cm long set 156 cm above the ground; and the third cross arm 91 cm long set 125 cm above the ground. There were two wires per cross arm and was trained to produce a grapevine height of 200 cm and a grapevine spread of 199 cm.

Vigor.—Medium vigor. Vigor as measured by weighing prunings at dormant pruning for cane pruned grapevines (with 6 canes and an average of 15 buds per cane) was 4.5 Kg.

Productivity.—Productive, 18.5 Kg per grapevine on grapevines spaced 8 ft. (243.84 cm) by 12 ft. (365.76 cm).

Regularity of bearing.—Regular. Annual pruning of canes is required for reliable production.

CANES

Size.—Diameter — Mature Canes — Medium diameter, medium vigor, upright in growth habit.

Mature canes.—Diameter — Internode Base — 11.8 mm.

Mature canes.—Diameter — Internode Midpoint — 9.5 mm.
Mature canes.—Diameter — Internode Tip — 4.2 mm.
Mature canes.—Diameter — Node Base — 13.4 mm.
Mature canes.—Diameter — Node Midpoint — 11.6 mm.
Mature canes.—Diameter — Node Tip — 5.5 mm.
Internode length.—Base — 5.9 cm.
Internode length.—Midpoint — 7.4 cm.
Internode length.—Tip — 4.1 cm.
Average length of canes.—263.3 cm.
Surface texture.—Smooth.
Color of mature cane.—Brown (plate 14 D8). No anthocyanin observed on mature canes.
Buds.—Color — Brown (plate 15 A10).
Buds.—Texture — Smooth.
Dormant bud (*compound bud or eye*).—Width — At base of cane 5.3 mm; at midpoint of cane 6.6 mm and at tip of cane 4.1 mm. The average number of buds on a current, single-season growth cane is 44.
Date of bud break.—March 29, late season.
Young shoots.—Young shoots have cobwebby indument.
Diameter of young shoots in spring (*measured when shoots are 24 inches*).—At base 8.3 mm, at midpoint 5.8 mm and at tip 2.8 mm.
Internode length.—7.2 cm at 4^{th} internode from base.
Young shoots.—Color — Light yellow green (plate 20 J6) with slight bronze on edge.
Stem of shoot tip.—Color — Yellow green (plate 20 K7) with occasional red on the sun exposed side.
Shoot.—Shape — Straight.
Shoot tip.—Form — Open.
Tendrils.—Size — Length — 18.6 cm.
Tendrils.—Size — Diameter — 2.21 mm.
Tendrils.—Shape — Usually biforcated or triforcated and curled on distal end.
Tendrils.—Pattern — Found beginning opposite node 8, then again at nodes 10, 11, 13, 14, 16, 17 with this repeating intermittent pattern to the distal end of the cane.
Tendril.—Color Immature Growth — Yellow green (plate 20 L6).
Disease resistance.—Susceptible to powdery mildew, and fungicides were applied to the grapevines under evaluation to control powdery mildew.
Insect resistance.—Insecticides were applied to the grapevines under evaulation to control grapevine leafhoppers and variegated leafhoppers. No resistances to these pests were determined in these evaluations due to chemical control of these pests.

LEAVES

Size:
Generally.—Leaves simple and alternate. The mid vein (L1) is 14.2 cm long, vein L2 is 11.1 cm long and vein L3 is 7.8 cm long. The angle between the mid vein L1 and L3 is 106 degrees and between L1 and the 1st vein off L3 is 165 degrees.
Average length.—19.5 cm.
Average width.—17.5 cm.
Shape.—Orbicular
Lobes.—Number — Five (5).
Color.—Upwardly Disposed Surface — Dark green (plate 23 L12). Upward surface is glabrous, flat and smooth to bullate.

Color.—Downwardly Disposed Surface — Green (plate 22 K9). Lower surface is glabrous with very few short hairs along the main midrib vein.
Color.—Leaf Vein — Light green (plate 19 I5) with occasional red (plate 6 I4) on main veins near center of leaf.
Leaf vein.—Thickness — Thickness of mid vein at center of leaf is 1.5 mm.
Main veins.—Anthocycanin — Location — With occasional red (plate 6 I4) on main veins near center of leaf.
Leaf margin.—Serrated with shape of teeth pointed and medium in size.
Petiole sinus.—Lyre shape and usually petiole lobes are half overlapped causing a closed petiole sinus. On mature leaf is 3.4 cm deep and 1.2 cm wide at widest point.
Petiole.—Size — Medium.
Petiole.—Length — 11.5 cm.
Petiole.—Diameter — 2.8 mm.
Petiole.—Color — Yellow green (plate 20 L7) with 50% red (plate 45 J3) covering. Young leaf — Upper Surface — Color — Green (plate 22 L7) with very light cobwebby indument on upper surface.
Young leaf.—Lower Surface — Color — Pale green (plate 21 L8).
Young leaf.—Shape unfolded — Concave to flat.
Petiole of young leaf.—Color — Green (plate 20 L7).
Stipules.—Onion skin.

TRUNK

Size.—Large.
Size.—Height — Approximately 104 cm above the vineyard floor.
Size.—*Diameter*.—6.47 cm as measured just below the cordon or head point at 81.28 cm above vineyard floor; and 6.63 cm at 15.2 cm above the vineyard floor.
Bark.—Color — (plate 16 C7).

FLOWERS

Flower.—Size — Generally — Medium.
Flower.—Unopened — Diameter — 2.1 mm.
Flower.—Unopened — Length — 2.9 mm.
Flower.—Unopened — Surface Texture — Smooth.
Date of bloom.—First bloom May 7, 2002.
Date of full bloom.—May 16, 2002 at 90%.
Inflorescence.—Panicle.
Cluster size.—At Bloom — Generally, medium.
Cluster.—Length — 17.0 cm.
Cluster.—Width — 11.0 cm.
Peduncle.—Length — 3.9 cm.
Shape of cluster.—Conical with short shoulders.
Calyptra.—Color — Green (plate 20 J7).
Stamens.—Five (5) and erect.
Pistil.—Well developed.
Ovary.—Color — Dark green (plate 22 L9).
Pollen.—Normal, fertile, abundant.
Anthers.—Color — Light yellow (plate 10 J1).

FRUIT

Maturity when described: Ripe for commercial harvesting and shipment approximately October 23 in Fresno, Calif. Late season or 8 weeks after 'Thompson Seedless' grapevine or 4 weeks after 'Autumn Seedless' grapevine.

Cluster:

Size. —Cane Pruned Vines — 539 grams.
Length.—28.5 cm.
Width.—16.3 cm.
Shape.—Conical to cylindrical.
Density.—Medium to tight, on average has 55 berries per cluster.
Clusters per vine.—56, cane pruned.
Clusters per shoot.—1.02 clusters per shoot.

Peduncle:

Size.—Length — Medium, 5.0 cm.
Size.—Diameter — Medium, 6.0 mm.
Color.—Green (plate 21 L7).
Texture.—Smooth, glabrous.

Pedicel:

Generally.—There is a medium to good attachment between the berry and the pedicel.
Size.—Length — 8.1 mm.
Size.—Diameter — 1.6 mm.
Color.—Green (plate 21 I5).
Texture.—Glabrous with a few lenticels.
Brush.—Length — 2.8 mm.
Brush color.—Green (plate 19 F1).

Berry:

Size.—Large, avg. 9.8 grams.
Shape.—Cylindrical to ovoid.
Length.—3.1 cm.
Width.—2.3 cm.
Color.—Pale green (plate 19 E1).
Bloom.—Light.
Skin: Generally.—The skin adheres to the flesh.
Thickness.—Medium in thickness.
Texture.—Smooth.
Tendency to crack.—None.

Flesh:

Flesh color.—Translucent and very pale yellow green (plate 19 I1).
Texture.—Firm, meaty.

Juice production.—Medium.
Color of juice.—Clear.
Flavor.—Sweet and sub acid, neutral flavor.
Soluble solids.—18.6%.
Titratable acid.—0.31 g/100 ml juice.
Aroma.—None.
Ripening.—Uniform.
Eating quality.—Very good, sweet.

Character of seeds: Stenospermocarpic seedless, small aborted seed traces that are not noticeable when eaten. Average aborted seed trace when present are 6.4 mg fresh weight, 3.8 mm long and 1.8 mm wide. Seed color is light brown (plate 13 C8).

Use: Fresh market. No wine nor raisin evaluations have been done.

Keeping quality: Very good.

Resistance to disease: No resistance to powdery mildew.

Shipping and handling qualities: Berries ship and handle similar to 'Thompson Seedless' except there is less berry shatter.

Although the new variety of grapevine possesses the described characteristics noted above as a result of the growing conditions prevailing in Fresno, Calif. in the central San Joaquin Valley of California, United States of America, it is to be understood that variations of the usual magnitude and characteristics incident to changes in growing conditions, training, irrigation, fertilization, pruning, pest control, climatic variation and the like are to be expected.

Having thus described and illustrated our new variety of grapevine, what we claim as new and desire to be secured by Plant Letters Patent is:

1. A new and distinct variety of grapevine plant, 'Autumn King', substantially as illustrated and described, characterized by its attractive pale green fruit color, cylindrical to ovoid fruit shape, and firm flesh texture with a neutral sweet flavor.

* * * * *

FIG. 1

FIG. 2

US00PP16229P2

(12) United States Plant Patent

Ramming et al.

(10) Patent No.: US PP16,229 P2
(45) Date of Patent: Jan. 31, 2006

(54) **GRAPEVINE DENOMINATED 'SCARLET ROYAL'**

(50) Latin Name: *Vitis vinifera*
Varietal Denomination: **Scarlet Royal**

(75) Inventors: **David W. Ramming**, Fresno, CA (US); **Ronald E. Tarailo**, Fresno, CA (US)

(73) Assignee: **The United States of America as represented by the Secretary of Agriculture**, Washington, DC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 140 days.

(21) Appl. No.: **10/953,124**

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A01H 5/00 (2006.01)

(52) **U.S. Cl.** **Plt./205**
(58) **Field of Classification Search** Plt./205
See application file for complete search history.

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(57) **ABSTRACT**

A new and distinct variety of grapevine denominated 'Scarlet Royal' which is characterized by its mid-season ripening seedless fruit, attractive dark red coloration, its oval fruit shape, its firm fruit texture with neutral sweet flavor, and its medium dense cluster.

2 Drawing Sheets

Latin name of the genus and species of the plant claimed: *Vitis vinifera* L.

Variety denominated: 'Scarlet Royal'.

BACKGROUND OF THE INVENTION

The present invention relates to a new and distinct variety of grapevine, *Vitis vinifera* L., which will hereinafter be denominated varietally as the 'Scarlet Royal' grapevine, and, more particularly, to a grapevine which has fruit maturing for commercial harvesting and shipment approximately August 15 in the San Joaquin Valley of central California. The fruit has an attractive dark red skin coloration at maturity with oval shape seedless berries.

The grapevine of the present invention originated from a hand-pollinated cross of United States Department of Agriculture selection 'C33-30' (unpatented) and the United States Department of Agriculture selection 'C51-63' (unpatented) made in 1992 at the United States Department of Agriculture, Agricultural Research Service, Postharvest Quality and Genetics Research Unit plots at California State University, Fresno, in Fresno, Calif. The female was 'C33-30', a seedless, red-fruited grapevine with reflex anthers in

the flower, large oval berries with firm flesh and medium skin, and a neutral flavor. The fruit of the 'C33-30' ripen about two weeks after the instant variety. The pollen parent was 'C51-63' a seedless red-purple fruited grape with medium size, oval to elliptical berries with good skin and firm flesh. The fruit of the 'C51-63' grapevine ripen four weeks after the variety of the subject invention. Both of the parents of the instant cultivar are hybrids of the grapevine genus and species *Vitis vinifera* L.

The aborted seeds resulting from this controlled hybridization were developed further through in vitro tissue culture and germinated in the laboratory during the fall of 1992. The resulting seedling population totaled 21 individual plants. All seedlings were planted in the spring of 1993 in a vineyard at the United States Department of Agriculture, Agricultural Research Service plots on the California State University, Fresno, campus in Fresno, Calif. The seedlings fruited in the summer of 1995 and one, the grapevine of the present invention, was designated as 'B34-82' and selected for its attractive dark red seedless, firm, large berry size, and outstanding fruit quality.

In 1996 at the inventors' direction, the grapevine of the subject invention was propagated asexually by rooting hard-

wood cuttings at Fresno, Calif. and a test planting of two grapevines of the subject invention was established in the United States Department of Agriculture, Agricultural Research Service plots on the California State University, Fresno campus. Subsequently in 1997 a larger test planting of 24 vines was established with rooted hardwood cuttings of the instant invention. The instant cultivar rooted readily from hardwood cuttings. All grapevines of the new variety planted from hardwood cutting propagation, fruited in the third season of growth after planting. All propagules, or resulting plants, of the present invention have been observed by the inventors to be true to type in that all asexual reproduced grapevines of the variety possessed the characteristics identical to those of the original parent grapevine.

SUMMARY OF THE INVENTION

The grapevines of the subject invention possess medium vigor and have produced fruit as own-rooted grapevines. The size of the grapevines was determined by growing the grapevines on a three cross arm 'T' type trellis structure with a top cross arm of 122 cm in length set 189 cm above the ground; a second cross arm of 102 cm in length set 156 cm above the ground; and a third cross arm 91 cm in length set 125 cm above the ground. The trellis structure had two wires per cross arm and indicated a grapevine height of 199 cm and a grapevine spread of 179 cm.

The fruit of the new variety ripens in midseason, about the same time as the 'Ruby Seedless' grapevine (unpatented). The average ripening date in Fresno, Calif. is August 15. Berries adhere very well to the fruit pedicel and have minimal shatter from the clusters during storage. The fruit is dark red in color at maturity. The fruit shape is oval. Fruit skins are thick compared to medium thick skins for 'Ruby Seedless' grapevine. The pulp of the fruit adheres to the skins of the berry and the fruit texture is firm and meaty. The berries are medium in size, or 5.8 grams. The flavor of the fruit is sweet and has been rated high. Soluble solids concentration of the juice at fruit maturity averages 22.0% with titratable acid of 0.55 grams/100 milliliters of juice. The fruit is of the stenospermocarpic type of seedlessness and contains small, aborted seed traces that are not noticeable when eaten. The fruit clusters are usually borne on the average of 0.56 per shoot on spur pruned vines. The fruit clusters are conical and are large in size, or 835 grams, medium density and attractive. The fruit cluster peduncles are medium in length.

The grapevine and fruit of the new variety are susceptible to powdery mildew disease of grape plants. A spray program for powdery mildew disease control is required.

BRIEF DESCRIPTION OF THE DRAWINGS

The drawings of the grapevine of the present invention are color photographs showing in

FIG. **1** a typical specimen of the fruit and in

FIG. **2** a shoot with leaves and a flower cluster all of the new variety of the present invention.

The color of the photographs is as nearly true as it is reasonably possible to provide in such color photographs. Description of the new invention applies to vines of 'Scarlet Royal' grown on its own roots at a density of 1,119 vines per hectare in Fresno County, Calif. in 2002. These vines were in their third year of full production having been planted in 1997.

DETAILED BOTANICAL DESCRIPTION

The new variety cv. 'Scarlet Royal' may be distinguished from other commercial grape cultivars known to us by a combination of characteristics, including its mid-season ripening seedless fruit with easy to develop attractive dark red coloration, its firm fruit texture with a neutral sweet flavor, its oval fruit shape, large berry size and its medium density cluster.

The new variety of grapevine is most similar to its pollen parent 'C51-63' by having dark red, firm texture fruit. It is distinguished therefrom and an improvement thereon in a number of fruit characteristics. The berry size is larger, and the dark red color is easier to develop. The fruit ripens 4 weeks before 'C51-63'. The new grapevine is also similar to the commercial variety 'Ruby Seedless' in that they ripen at the same time and have red seedless berries. It is distinguished therefrom and an improvement thereon in that the berries of the new variety are larger and firmer than those of 'Ruby Seedless'. It is also similar to the commercial variety 'Crimson Seedless' (unpatented), in that they have firm red seedless fruit. It is distinguished therefrom and an improvement thereon in that the berries of the new variety are larger and develop the dark red color easier than does 'Crimson Seedless'.

The new variety also differs substantially from its mother parent 'C33-30'. The new variety has perfect flowers with functional male and female parts while 'C33-30' has only functional female parts. The most distinguishing difference is the thicker skin, firmer berries that develop dark red color easily, while 'C33-30' has thin skin, medium firm berries that do not develop red color easily.

Referring more specifically to the botanical details of this new and distinct variety of grapevine, the following has been observed under the ecological conditions prevailing at the orchard of origin which is located in Fresno in the San Joaquin Valley of central California. All major color code designations are by reference to the *Dictionary of Color,* by Maerz and Paul, First Edition, 1930. Common color names are also occasionally employed. Where dimensions, sizes, colors and other characteristics are given, it is to be understood that such characteristics are approximations of averages set forth as accurately as practicable. The description hereof was taken from specimens grown in Fresno, Calif. The grapevines used for measurement were grown in a fine sandy loam soil and the grapevines were irrigated using trickle, or drip irrigation. In a substantial part, the data hereof was from grapevines that were six (6) years old.

VINE

Generally:

Size.—Medium. Grapevine size as determined on grapevines growing on a three cross arm 'T' trellis with the top cross arm 122 cm long set 189 cm above the ground; the second cross arm 102 cm long set 156 cm above the ground; and the third cross arm 91 cm long set 125 cm above the ground. There were two wires per cross arm and was trained to produce a grapevine height of 200 cm and a grapevine spread of 199 cm.

Vigor.—Medium vigor. Vigor as measured by weighing prunings at dormant pruning for spur pruned grapevines (with 34 spurs and 2 buds per spur) was 5.96 Kg.

Productivity.—Productive, 31.2 Kg per grapevine on grapevines spaced 8 ft. (243.84 cm) by 12 ft. (365.76 cm).

Regularity of bearing.—Regular. Annual pruning is required for reliable production.

CANES

Size.—Diameter — Mature Canes — Medium diameter, medium vigor, upright in growth habit. Mature Canes — Diameter — Internode Base — 10.4 mm. Mature Canes — Diameter — Internode Midpoint — 8.9 mm. Mature Canes — Diameter — Internode Tip — 4.5 mm. Mature Canes — Diameter — Node Base — 12.5 mm. Mature Canes — Diameter — Node Midpoint — 10.4 mm. Mature Canes — Diameter — Node Tip — 6.1 mm.

Internode length.—Base — 8.5 cm. Internode Length — Midpoint — 9.4 cm. Internode Length — Tip — 6.4 cm.

Average length of canes.—233.6 cm.

Surface texture.—Smooth.

Color of mature cane.—Orange brown (plant 14 E7). No anthocyanin observed on mature canes.

Buds.—Color — Brown (plate 8 L6). Buds — Texture — Smooth.

Dormant bud (compound bud or eye).—Width — At base of cane 5.1 mm; at midpoint of cane 6.1 mm and at tip of cane 4.3 mm. The average number of buds on a current, single-season growth cane is 29.

Date of bud break.—March 29, late season.

Young shoots.—Young shoots have cobwebby indument.

Diameter of young shoots in spring (measured when shoots are 24 inches).—At base 8.3 mm, at midpoint 7.1 mm and at tip 4.5 mm.

Internode length.—5.3 cm at 4th internode from base.

Young shoots.—Color — Pale green (plate 21 L6) with very slight red on edge.

Stem of shoot tip.—Color — Green (plate 21 L9) with ½ to ¾ covered with red streaks (plate 46 L6) on the sun exposed side.

Shoot.—Shape — Straight to slightly curved.

Shoot tip.—Form — Open.

Tendrils.—Size — Length — 22.5 cm. Tendrils — Size — Diameter — 2.39 mm. Tendrils — Shape — Usually biforcated or triforcated and curled on distal end. Tendrils — Pattern — Found beginning opposite node 7 and 8, then again at nodes 10, 11, 13, 14, 16, 17 with this repeating intermittent pattern to the distal end of the cane. Tendril — Color Immature Growth — Yellow green (plate 20 L7) with dark red streaks (plate 46 L6).

Disease resistance.—Susceptible to powdery mildew, and fungicides were applied to the grapevines under evaluation to control powdery mildew.

Insect resistance.—Insecticides were applied to the grapevines under evaluation to control grapevine leafhoppers and variegated leafhoppers. No resistances to these pests were determined in these evaluations due to chemical control of these pests.

LEAVES

Size.—Generally — Leaves simple and alternate. The mid vein (L1) is 13.3 cm long, vein L2 is 12.0 cm long and vein L3 is 9.2 cm long. The angle between

the mid vein L1 and L3 is 106 degrees and between L1 and the 1st vein off L3 is 151 degrees.

Average length.—18.7 cm.

Average width.—18.2 cm.

Shape.—Orbicular.

Lobes.—Number — Five (5).

Color.—Upwardly Disposed Surface — Dark green (plate 23 L7). Upward surface is glabrous, flat and smooth to slightly bullate. Color — Downwardly Disposed Surface — Green (plate 23 L6). Lower surface is glabrous with medium amount of very few short erect hairs along the main midrib vein.

Leaf vein.—Color — Light green (plate 19 K3) with no red pigment on veins of leaf. Leaf Vein — Thickness — Thickness of mid vein at center of leaf is 1.7 mm.

Leaf margin.—Serrated with shape of teeth pointed and medium to large in size.

Petiole sinus.—Lyre shape and usually petiole lobes are half open. On mature leaf is 3.5 cm deep and 1.47 cm wide at widest point.

Petiole.—Size — Medium. Petiole — Length — 16.3 cm. Petiole — Diameter — 2.8 mm. Petiole — Color — Green (plate 20 J5) with 50% to 90% red (plate 4 G2) covering.

Young leaf.—Color — Upper Surface — Green (plate 21 L6) with copper over color and cobwebby indument on upper surface. Young leaf — Color — Lower Surface — Green (plate 20 I6). Young leaf — Shape unfolded — Concave.

Petiole of young leaf.—Color — Green (plate 22 I8).

Stipules.—Onion skin.

TRUNK

Size.—Medium. Size — Height — Approximately 104 cm above the vineyard floor. Size — Diameter — 6.7 cm as measured just below the cordon or head point at 81.28 cm above vineyard floor; and 6.8 cm at 15.2 cm above the vineyard floor.

Bark.—Color — (plate 15 C4).

FLOWERS

Flower.—Size — Generally — Medium. Flower — Unopened — Diameter — 2.2 mm. Flower — Unopened — Length — 3.1 mm. Flower — Unopened — Surface Texture — Smooth.

Date of bloom.—First bloom May 6, 2002.

Date of full bloom.—May 13, 2002 at 90%.

Inflorescence.—Panicle.

Cluster size.—At Bloom — Generally, medium. Cluster — Length — 15.3 cm. Cluster — Width — 13.7 cm.

Peduncle.—Length — 4.1 cm.

Shape of cluster.—Conical with well developed shoulders.

Calyptra.—Color — Green (plate 20 K7).

Stamens.—Five (5) and erect.

Pistil.—Well developed.

Ovary.—Color — Green (plate 20 L7).

Pollen.—Normal, fertile, abundant.

Anthers.—Color — Light yellow (plate 9 J1).

FRUIT

Maturity when described: Ripe for commercial harvesting and shipment approximately August 15 in Fresno, Calif. Mid-season with 'Ruby Seedless' grapevine.

Cluster:
Size.—Spur Pruned Vines — 835 grams.
Length.—23.8 cm.
Width.—18.5 cm.
Shape.—Conical.
Density.—Medium, on average has 144 berries per cluster.
Clusters per vine.—48, spur pruned.
Clusters per shoot.—0.56 clusters per shoot.
Peduncle:
Size.—Length — Medium, 5.5 cm. Size — Diameter — Medium, 5.8 mm.
Color.—Green (plate 20 H6).
Texture.—Smooth, glabrous.
Pedicel: Generally — There is good attachment between the berry and the pedicel.
Size.—Length — 8.1 mm. Size — Diameter — 2.0 mm.
Color.—Green (plate 20 F7).
Texture.—Glabrous with a few lenticels.
Brush.—Length — 3.0 mm. Brush color — Green (plate 20 D3).
Berry:
Size.—Medium, avg. 5.8 grams.
Shape.—Oval.
Length.—2.43 cm.
Width.—1.79 cm.
Color.—Dark red (plate 6 L6).
Bloom.—Medium.
Skin: Generally — The skin adheres to the flesh.
Thickness.—Medium to thick in thickness.
Texture.—Smooth.
Tendency to crack.—None.
Flesh:
Flesh color.—Translucent and very pale yellow green (plate 19 I2).
Texture.—Firm, meaty.
Juice production.—Medium.

Color of juice.—Clear.
Flavor.—Sweet and acid, neutral flavor.
Soluble solids.—22.0%.
Titratable acid.—0.55 g/100 ml juice.
Aroma.—None.
Ripening.—Uniform.
Eating quality.—Very good, sweet.
Character of seeds: Stenospermocarpic seedless, small aborted seed traces that are not noticeable when eaten. Average aborted seed trace when present are 12.5 mg fresh weight, 5.6 mm long and 1.9 mm wide. Seed color is gray (plate 7 A1).
Use: Fresh market. No wine nor raisin evaluations have been done.
Keeping quality: Very good.
Resistance to disease: No resistance to powdery mildew.
Shipping and handling qualities: Berries ship and handle similar to Thompson Seedless except there is less berry shatter.

Although the new variety of grapevine possesses the described characteristics noted above as a result of the growing conditions prevailing in Fresno, Calif. in the central San Joaquin Valley of California, United States of America, it is to be understood that variations of the usual magnitude and characteristics incident to changes in growing conditions, training, irrigation, fertilization, pruning, pest control, climatic variation and the like are to be expected.

Having thus described and illustrated our new variety of grapevine, what we claim as new and desire to be secured by Plant Letters Patent is:

1. A new and distinct variety of grapevine plant, 'Scarlet Royal', substantially as illustrated and described, characterized by its attractive dark red fruit color, oval fruit shape, and firm flesh texture with a neutral sweet flavor.

* * * * *

FIG. 1

FIG. 2