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**UNITED STATES DISTRICT COURT  
DISTRICT OF NEW JERSEY**

PUMA BIOTECHNOLOGY, INC. and  
WYETH LLC,

Plaintiffs,

v.

SANDOZ INC.,

Defendant.

Civil Action No. 21-\_\_\_

(Filed Electronically)

**COMPLAINT FOR PATENT INFRINGEMENT**

Plaintiffs Puma Biotechnology, Inc. (“Puma”) and Wyeth LLC (“Wyeth”) (collectively, “Plaintiffs”), by their attorneys, bring this complaint against Defendant Sandoz Inc. (“Sandoz”) and hereby allege as follows:

### **NATURE OF THE ACTION**

1. This is an action for patent infringement under the patent laws of the United States, 35 U.S.C. § 100, *et seq.*, including 35 U.S.C. § 271(a)–(c), (e)(2), the Drug Price Competition and Patent Term Restoration Act of 1984, 21 U.S.C. § 355(j) (the “Hatch-Waxman Act”), and the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202, that arises out of Sandoz’s submission of an Abbreviated New Drug Application (“ANDA”) to the U.S. Food and Drug Administration (“FDA”) seeking approval to commercially manufacture, use, offer for sale, sell, and/or import a purported generic version of Nerlynx® (neratinib) Tablets, 40 mg (“Nerlynx®”) prior to the expiration of U.S. Patent No. 7,399,865 B2 (“the ’865 patent” or “the patent-in-suit”).

### **PARTIES**

2. Plaintiff Puma is a corporation organized and existing under the laws of the State of Delaware, having a principal place of business at 10880 Wilshire Boulevard, Suite 2150, Los Angeles, California 90024. Puma is the exclusive licensee of the patent-in-suit under a license agreement with Wyeth. The compound claimed in the ’865 patent, neratinib, was approved by the FDA in 2017 for the extended adjuvant treatment of adult patients with early-stage human epidermal growth factor 2 (“HER2”) overexpressed/amplified breast cancer, following adjuvant trastuzumab-based therapy. Puma commenced commercial sales of the drug in 2017, and it is marketed in the United States as Nerlynx® tablets. In February 2020, Nerlynx® was also approved by the FDA in combination with capecitabine for the treatment of adult patients with advanced or metastatic HER2-positive breast cancer who have received two or more prior anti-HER2-based regimens in the metastatic setting.

3. Plaintiff Wyeth is a limited liability company organized and existing under the laws of the State of Delaware with offices at 235 East 42nd Street, New York, NY 10017. Wyeth is a wholly owned subsidiary of Pfizer Inc. Wyeth owns the patent-in-suit.

4. On information and belief, Defendant Sandoz is a corporation organized and existing under the laws of the State of Colorado, having a principal place of business at 100 College Road West, Princeton, New Jersey 08540. On information and belief, Sandoz is in the business of, among other things, manufacturing, promoting, marketing, selling, offering for sale, using, distributing, and importing into the United States, generic versions of branded pharmaceutical drugs for the U.S. market.

5. Upon information and belief, Sandoz has several places of business in the State of New Jersey, including, but not limited to, at the following business address: One Health Plaza, Bldg. 435, East Hanover, New Jersey 07936.

6. By a letter dated September 29, 2021 (“Sandoz Notice Letter”), Sandoz notified Plaintiffs that Sandoz had submitted to the FDA ANDA No. 216236 (“Sandoz’s ANDA”) describing a purported generic version of neratinib tablets in a 40 mg dosage strength (“Sandoz ANDA Product”). Sandoz seeks FDA approval to engage in the commercial manufacture, use, sale, offer for sale, and/or importation of the Sandoz ANDA Product in or into the United States, including in New Jersey, prior to the expiration of the ’865 patent.

7. On information and belief, Sandoz knows and intends that upon approval of Sandoz’s ANDA, Sandoz will manufacture, market, sell, offer for sale, import, use, and/or distribute the Sandoz ANDA Product throughout the United States, including in New Jersey.

#### **JURISDICTION AND VENUE**

8. Plaintiffs incorporate each of the preceding paragraphs as if fully set forth herein.

9. This is a civil action for patent infringement arising under the patent laws of the United States, 35 U.S.C. § 100, *et seq.*, including 35 U.S.C. § 271, for infringement of the patent-in-suit.

10. This Court has jurisdiction over the subject matter of this action pursuant to 28 U.S.C. §§ 1331, 1338(a), 2201, and 2202.

11. This Court has personal jurisdiction over Sandoz because Sandoz is a corporation having a principal place of business and at least one other regular and established place of business in New Jersey.

12. Sandoz is also subject to personal jurisdiction in New Jersey because, among other things, Sandoz has purposely availed itself of the benefits and protections of New Jersey's laws such that it should reasonably anticipate being sued in this Court. On information and belief, Sandoz develops, manufactures, imports, markets, distributes, uses, offers to sell, and/or sells generic drugs throughout the United States, including in the State of New Jersey, and therefore transacts business within the State of New Jersey related to Plaintiffs' claims, and/or has engaged in systematic and continuous business contacts with the State of New Jersey.

13. Sandoz has committed an act of infringement in this Judicial District by filing ANDA No. 216236 with the intent to make, use, sell, offer for sale, and/or import the Sandoz ANDA Product in or into this Judicial District, prior to the expiration of the patent-in-suit.

14. Sandoz has taken the costly, significant step of applying to the FDA for approval to engage in future activities, including the marketing of the Sandoz ANDA Product, that will be purposefully directed to New Jersey and elsewhere in the United States.

15. On information and belief, Sandoz has systematic and continuous contacts with New Jersey; has established distribution channels for drug products in New Jersey; regularly and continuously conducts business in New Jersey, including by selling drug products in New Jersey, either directly or indirectly through its subsidiaries, agents, or affiliates; has purposefully

availed itself of the privilege of doing business in New Jersey; and derives substantial revenue from the sale of drug products in New Jersey.

16. On information and belief, if Sandoz's ANDA is approved, Sandoz will manufacture, market, sell, offer for sale, import, use, and/or distribute the Sandoz ANDA Product within the United States, including in New Jersey, consistent with Sandoz's practices for the marketing and distribution of other generic pharmaceutical products. On information and belief, Sandoz regularly does business in New Jersey, and its practices with other generic pharmaceutical products have involved placing those products into the stream of commerce for distribution throughout the United States, including in New Jersey. On information and belief, Sandoz's generic pharmaceutical products are used and/or consumed within and throughout the United States, including in New Jersey. On information and belief, the Sandoz ANDA Product will be prescribed by physicians practicing in New Jersey, dispensed by pharmacies located within New Jersey, and used by patients in New Jersey. Each of these activities would have a substantial effect within New Jersey and would constitute infringement of the patent-in-suit in the event that the Sandoz ANDA Product is approved before the patent-in-suit expires.

17. Sandoz is registered as "Manufacturer and Wholesale" with the State of New Jersey's Department of Health under Registration No. 5003732.

18. Sandoz is registered with the State of New Jersey's Division of Revenue and Enterprise Services as a business operating in New Jersey under Business ID No. 010097265.

19. On information and belief, Sandoz derives substantial revenue from generic pharmaceutical products that are used and/or consumed within New Jersey, and that are manufactured by Sandoz and/or for which Sandoz is the named applicant(s) on approved ANDAs.

On information and belief, various products for which Sandoz is the named applicant(s) on approved ANDAs are available at retail pharmacies in New Jersey.

20. On information and belief, Sandoz has consented to jurisdiction in New Jersey in one or more prior cases arising out of the filing of its ANDAs, and/or have filed counterclaims in such cases. *See, e.g., Merck Sharp & Dohme B.V., et al. v. Sandoz, Inc., et al.*, No. 2:20-cv-03117 (D.N.J. Mar. 20, 2020); *Vifor (Int'l) AG, et al. v. Sandoz, Inc.*, No. 3:19-cv-16305-FLW-DEA (D.N.J. Aug. 2, 2019); *Adamas Pharma, LLC v. Sandoz Inc.*, No. 3:18-cv-09032-BRM-TJB (D.N.J. May 10, 2018); *Par Pharm., Inc., et al. v. Sandoz Inc.*, No. 3:18-cv-14895-BRM-DEA (D.N.J. Oct. 11, 2018).

21. Venue is proper in this Court pursuant to 28 U.S.C. § 1400(b) as to Sandoz because, on information and belief, Sandoz maintains its principal place of business in New Jersey, and because, on information and belief, Sandoz has committed or aided, abetted, contributed to, and/or participated in the commission of, acts of infringement of the asserted patent that will lead to foreseeable harm and injury to Plaintiffs by preparing or assisting in preparing Sandoz's ANDA in New Jersey and/or with the intention of seeking to market the Sandoz ANDA Product nationwide, including within New Jersey.

#### **THE PATENT-IN-SUIT**

22. The '865 patent, titled, "Protein Tyrosine Kinase Enzyme Inhibitors," was duly and legally issued on July 15, 2008 (attached as Exhibit A). Plaintiff Wyeth owns, Plaintiff Puma is the exclusive licensee of, and, together, they have all the rights to enforce, the '865 patent.

23. The '865 patent includes claims that recite the compound (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide or a pharmaceutically acceptable salt thereof, and pharmaceutical compositions comprising (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-

4-(dimethylamino)-2-butenamide or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier. That compound claimed in the '865 patent is also referred to as neratinib, and it is the active ingredient in Puma's Nerlynx® cancer therapy product. The '865 patent is listed in FDA's Approved Drug Products with Therapeutic Equivalence Evaluations ("Orange Book") in conjunction with Nerlynx®. Although the Orange Book currently lists the expiration of the '865 patent as December 29, 2025, the patent will not actually expire until several years later. On February 22, 2021, the United States Patent and Trademark Office ("PTO") issued a Notice Of Final Determination and Requirement for Election on the patent term extension ("PTE") application for the '865 patent, wherein the PTO determined that the '865 patent is eligible for 5 years of PTE. On February 25, 2021, Puma elected the '865 patent for PTE. Therefore, once the PTE certificate is issued, the expiration of the '865 patent will be December 29, 2030, and the Orange Book will be corrected accordingly.

#### **THE NERLYNX® DRUG PRODUCT**

24. Puma is the holder of New Drug Application ("NDA") No. 208051, under which the FDA approved the commercial marketing of Nerlynx® on July 17, 2017, under Section 505(b) of the Federal Food, Drug, and Cosmetic Act ("FDCA"), 21 U.S.C. § 355(a). As a single agent, Nerlynx® is indicated for the extended adjuvant treatment of adult patients with early-stage HER2 positive breast cancer, to follow adjuvant trastuzumab-based therapy. In combination with capecitabine, Nerlynx® is indicated for the treatment of adult patients with advanced or metastatic HER2-positive breast cancer who have received two or more prior anti-HER2 based regimens in the metastatic setting. Nerlynx® is distributed in the United States by Puma in 40 mg tablets.

25. Nerlynx® is covered by one or more claims of the patent-in-suit. Pursuant to 21 U.S.C. § 355(b)(1) and attendant FDA regulations, the patent-in-suit has been listed in connection with NDA No. 208051 in the FDA's Orange Book.

**SANDOZ'S ANDA AND NOTICE OF PARAGRAPH IV CERTIFICATION**

26. On information and belief, Sandoz has submitted or caused the submission of Sandoz's ANDA to the FDA under 21 U.S.C. § 355(j), to obtain approval to engage in the commercial manufacture, use, offer to sell, or sale within the United States or importation into the United States of the Sandoz ANDA Product, as a purported generic version of Nerlynx®, prior to the expiration of the patent-in-suit.

27. On information and belief, the Sandoz ANDA relies upon the Nerlynx® NDA and, according to Sandoz, contains the required data with respect to the bioavailability or bioequivalence of the Sandoz ANDA Product to Nerlynx®.

28. In the Sandoz Notice Letter, Sandoz notified Plaintiffs of the submission of Sandoz's ANDA to the FDA. The purpose of this submission was to obtain approval under the FDCA to engage in the commercial manufacture, use, offer for sale, sale, and/or importation of the Sandoz ANDA Product prior to the expiration of the '865 patent.

29. In the Sandoz Notice Letter, Sandoz acknowledged that the Reference Listed Drug for Sandoz's ANDA is Nerlynx®.

30. In the Sandoz Notice Letter, Sandoz also notified Plaintiffs that, as part of its ANDA, Sandoz had filed a purported Paragraph IV Certification pursuant to 21 U.S.C. § 355(j)(2)(B)(iv)(II), with respect to the '865 patent.

31. On information and belief, Sandoz submitted its ANDA to the FDA containing certifications pursuant to 21 U.S.C. § 355(j)(2)(A)(vii)(IV) asserting that the '865 patent is invalid, unenforceable, and/or will not be infringed by the manufacture, use, offer for sale, sale, and/or importation of the Sandoz ANDA Product. In the Sandoz Notice Letter, Sandoz stated that the Sandoz ANDA Product contains neratinib as the active ingredient.



32. On information and belief, Sandoz, through its own actions and through the actions of its agents, affiliates, and subsidiaries, prepared and submitted Sandoz's ANDA, and intends to further prosecute Sandoz's ANDA. On information and belief, if the FDA approves Sandoz's ANDA, Sandoz will manufacture, distribute, market, offer for sale, or sell the Sandoz ANDA Product within the United States, or will import the Sandoz ANDA Product into the United States. On information and belief, if the FDA approves Sandoz's ANDA, Sandoz, through its own actions and through the actions of its agents, affiliates, and subsidiaries, will actively induce or contribute to the manufacture, use, offer for sale, sale, or importation of the Sandoz ANDA Product in or into the United States.

33. Plaintiffs bring this action within forty-five days of receipt of the Sandoz Notice Letter. Accordingly, Plaintiffs are entitled to a stay of FDA approval pursuant to 21 U.S.C. § 355(j)(5)(B)(iii).

**COUNT I – INFRINGEMENT OF THE '865 PATENT**

34. Plaintiffs incorporate each of the preceding paragraphs as if fully set forth herein.

35. The Sandoz ANDA Product, and the use of the Sandoz ANDA Product, are covered by one or more claims of the '865 patent, including at least claims 2 and 7 of the '865 patent, because claims 2 and 7 of the '865 patent encompass the neratinib utilized in the Sandoz ANDA Product.

36. In the Sandoz Notice Letter, Sandoz did not contest infringement of claims 2 and 7 of the '865 patent. If Sandoz had a factual or legal basis to contest infringement of those claims of the '865 patent, then it was required by applicable regulations to state such a basis in the Sandoz Notice Letter. *See* 21 CFR 314.52 (requiring paragraph IV notice letter to include a detailed, claim-by-claim analysis of its bases for claiming non-infringement or invalidity of any

patent that is listed in the Orange Book in conjunction with the reference listed drug as to which the applicant has submitted a Paragraph IV Certification).

37. Sandoz's submission of Sandoz's ANDA with a Paragraph IV Certification for the purpose of obtaining approval to engage in the commercial manufacture, use, offer for sale, sale, and/or importation of the Sandoz ANDA Product in or into the United States before the expiration of the '865 patent is an act of infringement of the '865 patent under 35 U.S.C. § 271(e)(2)(A).

38. If approved by the FDA, Sandoz's commercial manufacture, use, importation, sale, and/or offer for sale of the Sandoz ANDA Product in or into the United States will directly infringe, contribute to the infringement of, and/or actively induce the infringement of one or more claims of the '865 patent under 35 U.S.C. § 271(a)-(c).

39. On information and belief, Sandoz will engage in the commercial manufacture, use, offer for sale, sale, marketing, distribution, and/or importation of the Sandoz ANDA Product in or into the United States immediately and imminently upon approval of Sandoz's ANDA.

40. The commercial manufacture, use, sale, offer for sale, or importation of the Sandoz ANDA Product in or into the United States would infringe one or more claims of the '865 patent.

41. On information and belief, upon FDA approval of Sandoz's ANDA, Sandoz will, through its own actions or through the actions of its agents, affiliates, and subsidiaries, market and/or distribute the Sandoz ANDA Product to resellers, pharmacies, hospitals, clinics, healthcare professionals, and other end users. On information and belief, Sandoz will knowingly and intentionally accompany the Sandoz ANDA Product with a product label or product insert that

will include instructions for using or administering the Sandoz ANDA Product, which are substantially similar to the instructions in the prescribing information for Nerlynx®. Use of Nerlynx® in accordance with the product label or the sale of that product will infringe the '865 patent. Accordingly, Sandoz will induce resellers, pharmacies, hospitals, clinics, healthcare professionals, and other sellers and end users of the Sandoz ANDA Product to directly infringe the '865 patent. On information and belief, upon FDA approval of Sandoz's ANDA, Sandoz will intentionally encourage acts of direct infringement with knowledge of the '865 patent and knowledge that its acts are encouraging infringement.

42. On information and belief, Sandoz plans and intends to, and will, actively induce infringement of the '865 patent when Sandoz's ANDA is approved, and plans, intends to, and will do so immediately and imminently upon approval. Sandoz's activities will be done with knowledge of the '865 patent and specific intent to infringe that patent.

43. On information and belief, Sandoz knows that the Sandoz ANDA Product and proposed labeling are especially made or adapted for use in infringing the '865 patent, that the Sandoz ANDA Product is not a staple article or commodity of commerce, and that the Sandoz ANDA Product and accompanying proposed labeling are not suitable for a substantial noninfringing use. On information and belief, Sandoz plans, intends to, and will contribute to infringement of the '865 patent immediately and imminently upon approval of Sandoz's ANDA.

44. Notwithstanding Sandoz's knowledge of the claims of the '865 patent, Sandoz has continued to assert its intent to manufacture, use, offer for sale, sell, distribute, and/or import the Sandoz ANDA Product with its product labeling in or into the United States following FDA approval of Sandoz's ANDA prior to the expiration of the '865 patent.

45. The foregoing actions by Sandoz constitute and/or will constitute direct infringement of the '865 patent; active inducement of infringement by others of the '865 patent; and contribution to the infringement by others of the '865 patent.

46. On information and belief, Sandoz, in concert with its agents, affiliates, and subsidiaries, submitted Sandoz's ANDA with a Paragraph IV Certification without adequate justification for asserting that the '865 patent is invalid, unenforceable, and/or not infringed by the commercial manufacture, use, offer for sale, sale, or importation in or into the United States of the Sandoz ANDA Product. On information and belief, Sandoz has acted with full knowledge of the '865 patent and without a reasonable basis for believing that it would not be liable for direct infringement of the '865 patent; active inducement of infringement by others of the '865 patent; and/or contribution to the infringement by others of the '865 patent. On information and belief, the direct and indirect infringement by Sandoz of the '865 patent was and is willful. Sandoz's conduct renders this case "exceptional" under 35 U.S.C. § 285.

47. Plaintiffs will be substantially and irreparably damaged by infringement of the '865 patent.

**COUNT II – DECLARATORY JUDGMENT OF INFRINGEMENT OF THE '865  
PATENT**

48. Plaintiffs incorporate each of the preceding paragraphs as if fully set forth herein.

49. There is a substantial and immediate controversy between Plaintiffs and Sandoz concerning the '865 patent. Plaintiffs are entitled to a declaratory judgment under 28 U.S.C. §§ 2201 and 2202 that Sandoz will infringe, actively induce infringement of, and/or contribute to the infringement of the '865 patent upon approval of Sandoz's ANDA.

50. The Sandoz ANDA Product, and the use of the Sandoz ANDA Product, are covered by one or more claims of the '865 patent, including at least claims 2 and 7 of the '865 patent, because claims 2 and 7 of the '865 patent encompass the neratinib utilized in the Sandoz ANDA Product.

51. In the Sandoz Notice Letter, Sandoz did not contest infringement of claims 2 and 7 of the '865 patent. If Sandoz had a factual or legal basis to contest infringement of those claims of the '865 patent, then it was required by applicable regulations to state such a basis in the Sandoz Notice Letter. *See* 21 CFR 314.52 (requiring paragraph IV notice letter to include a detailed, claim-by-claim analysis of its bases for claiming non-infringement or invalidity of any patent that is listed in the Orange Book in conjunction with the reference listed drug as to which the applicant has submitted a Paragraph IV Certification).

52. If approved by the FDA, Sandoz's commercial manufacture, use, importation, sale, and/or offer for sale of the Sandoz ANDA Product in or into the United States will directly infringe, contribute to the infringement of, and/or actively induce the infringement of one or more claims of the '865 patent under 35 U.S.C. § 271(a)-(c).

53. On information and belief, Sandoz will engage in the commercial manufacture, use, offer for sale, sale, marketing, distribution, and/or importation of the Sandoz ANDA Product in or into the United States immediately and imminently upon approval of Sandoz's ANDA.

54. The commercial manufacture, use, sale, offer for sale, or importation of the Sandoz ANDA Product in or into the United States would infringe one or more claims of the '865 patent.

55. On information and belief, upon FDA approval of Sandoz's ANDA, Sandoz will, through its own actions or through the actions of its agents, affiliates, and subsidiaries, market and/or distribute the Sandoz ANDA Product to resellers, pharmacies, hospitals, clinics, healthcare professionals, and other end users. On information and belief, Sandoz will knowingly and intentionally accompany the Sandoz ANDA Product with a product label or product insert that will include instructions for using or administering the Sandoz ANDA Product, which are substantially similar to the instructions in the prescribing information for Nerlynx®. Use of Nerlynx® in accordance with the product label or the sale of that product will infringe the '865 patent. Accordingly, Sandoz will induce resellers, pharmacies, hospitals, clinics, healthcare professionals, and other sellers and end users of the Sandoz ANDA Product to directly infringe the '865 patent. On information and belief, upon FDA approval of Sandoz's ANDA, Sandoz will intentionally encourage acts of direct infringement with knowledge of the '865 patent and knowledge that its acts are encouraging infringement.

56. On information and belief, Sandoz plans, intends to, and will actively induce infringement of the '865 patent when Sandoz's ANDA is approved, and plans, intends to, and will do so immediately and imminently upon approval. Sandoz's activities will be done with knowledge of the '865 patent and specific intent to infringe that patent.

57. On information and belief, Sandoz knows that the Sandoz ANDA Product and proposed labeling are especially made or adapted for use in infringing the '865 patent, that the Sandoz ANDA Product is not a staple article or commodity of commerce, and that the Sandoz ANDA Product and accompanying proposed labeling are not suitable for a substantial noninfringing use. On information and belief, Sandoz plans, intends to, and will contribute to infringement of the '865 patent immediately and imminently upon approval of Sandoz's ANDA.

58. Notwithstanding Sandoz's knowledge of the claims of the '865 patent, Sandoz has continued to assert its intent to manufacture, use, offer for sale, sell, distribute, and/or import the Sandoz ANDA Product with its product labeling in or into the United States following FDA approval of Sandoz's ANDA prior to the expiration of the '865 patent.

59. The foregoing actions by Sandoz constitute and/or will constitute direct infringement of the '865 patent; active inducement of infringement by others of the '865 patent; and contribution to the infringement by others of the '865 patent.

60. On information and belief, Sandoz, in concert with its agents, affiliates, and subsidiaries, submitted Sandoz's ANDA with a Paragraph IV Certification without adequate justification for asserting that the '865 patent is invalid, unenforceable, and/or not infringed by the commercial manufacture, use, offer for sale, sale, or importation in or into the United States of the Sandoz ANDA Product. On information and belief, Sandoz has acted with full knowledge of the '865 patent and without a reasonable basis for believing that it would not be liable for direct infringement of the '865 patent; active inducement of infringement by others of the '865 patent; and/or contribution to the infringement by others of the '865 patent. On information and belief, the direct and indirect infringement by Sandoz of the '865 patent was and is willful. Sandoz's conduct renders this case "exceptional" under 35 U.S.C. § 285.

61. Plaintiffs will be substantially and irreparably damaged by infringement of the '865 patent.

62. Plaintiffs have no adequate remedy at law.

63. The Court should declare that the commercial manufacture, use, sale, offer for sale, and/or importation of the Sandoz ANDA Product in or into the United States, with its proposed labeling, will infringe the '865 patent under 35 U.S.C. §§ 271(a), (b), and/or (c).

**PRAYER FOR RELIEF**

WHEREFORE, Plaintiffs request the following relief:

(a) A Judgment that the '865 patent has been infringed under 35 U.S.C. § 271(e)(2) by Sandoz's submission to the FDA of Sandoz's ANDA;

(b) A Judgment, pursuant to 35 U.S.C. § 271(e)(4)(A), ordering that the effective date of any FDA approval of the commercial manufacture, use, or sale of the Sandoz ANDA Product, or any other drug product that infringes or the use of which infringes the '865 patent, be not earlier than the expiration date of the '865 patent, inclusive of any extension(s) and additional period(s) of exclusivity;

(c) A preliminary and permanent injunction, pursuant to 35 U.S.C. §§ 271(e)(4)(B) and 283 and Rule 65, Fed. R. Civ. P., enjoining Sandoz, and all persons acting in concert with Sandoz, from the commercial manufacture, use, offer for sale, or sale in the United States, or importation into the United States, of the Sandoz ANDA Product, or any other drug product covered by or whose use is covered by the '865 patent, prior to the expiration of the '865 patent, inclusive of any extension(s) and additional period(s) of exclusivity;

(d) A Judgment declaring that the commercial manufacture, use, offer for sale, or sale in the United States, or importation into the United States, of the Sandoz ANDA Product, or any other drug product that is covered by or whose use is covered by the patent-in-suit, prior to the expiration of the patent-in-suit, inclusive of any extension(s) and additional period(s) of exclusivity, will infringe, induce the infringement of, and contribute to the infringement by others of the patent-in-suit;

(e) A Judgment declaring that Sandoz's commercial manufacture, use, offer for sale, or sale in the United States, or importation into the United States, of the Sandoz ANDA Product,



or inducing or contributing to such conduct, would constitute infringement of one or more claims of the the patent-in-suit by Sandoz under one or more of 35 U.S.C. § 271(a), (b), and (c);

(f) An award of damages or other relief, pursuant to 35 U.S.C. § 271(e)(4)(C), if Sandoz engages in the commercial manufacture, use, offer for sale, or sale in the United States, or importation into the United States, of the Sandoz ANDA Product, or any product that infringes the '865 patent, or induces or contributes to such conduct, prior to the expiration of the '865 patent, inclusive of any extension(s) and additional period(s) of exclusivity;

(g) A Judgment that Sandoz willfully and deliberately infringed the patent-in-suit;

(h) A declaration that this is an exceptional case and an award of attorneys' fees pursuant to 35 U.S.C. § 285;

(i) Costs and expenses in this action; and

(j) Such further and other relief as this Court may deem just and proper.

Dated: November 10, 2021

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**CERTIFICATION PURSUANT TO LOCAL CIVIL RULES 11.2 AND 40.1**

I hereby 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 10, 2021

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# **EXHIBIT A**



US007399865B2

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

(10) **Patent No.:** **US 7,399,865 B2**  
(45) **Date of Patent:** **Jul. 15, 2008**

(54) **PROTEIN TYROSINE KINASE ENZYME INHIBITORS**

WO WO 2000/018761 \* 4/2000  
 WO WO 03/050090 A1 6/2003  
 WO WO 2004/096224 A2 11/2004

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(73) Assignee: **Wyeth**, Madison, NJ (US)

(\* ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 475 days.

\* cited by examiner

(21) Appl. No.: **10/939,007**

(22) Filed: **Sep. 10, 2004**

*Primary Examiner*—D. Margaret Seaman  
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(65) **Prior Publication Data**

US 2005/0059678 A1 Mar. 17, 2005

**Related U.S. Application Data**

(60) Provisional application No. 60/560,724, filed on Sep. 15, 2003.

(57) **ABSTRACT**

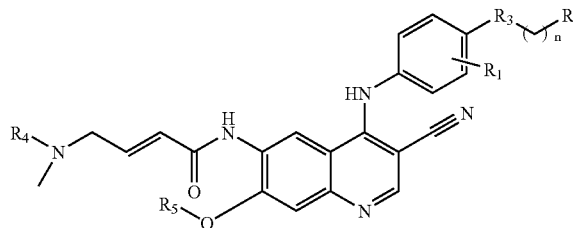
This invention provides compounds of formula 1, having the structure

(51) **Int. Cl.**

**C07D 215/38** (2006.01)

(52) **U.S. Cl.** ..... **546/160**; 546/153; 546/152

(58) **Field of Classification Search** ..... 514/313, 514/312, 311; 546/160, 153, 152  
 See application file for complete search history.



wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, and R<sub>5</sub> are described within the specification.

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WO WO 98/43960 10/1998

**10 Claims, No Drawings**



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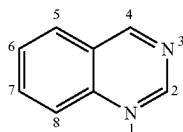
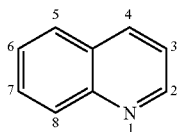
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- (E)-N-{4-[4-(benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide;
- (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide;
- (E)-N-(4-{3-chloro-4-[(3-fluorobenzyl)oxy]anilino}-3-cyano-7-ethoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide;
- (2E)-N-(4-{[3-chloro-4-(1,3-thiazol-2-ylsulfanyl)phenyl]amino}-3-cyano-7-methoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide;
- (E)-N-(4-{3-chloro-4-[(4,6-di-methyl-2-pyrimidinyl)sulfanyl]anilino}-3-cyano-7-ethoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide;
- (E)-N-{4-[3-chloro-4-(1,3-thiazol-2-ylsulfanyl)anilino]-3-cyano-7-methoxy-6-quinolinyl}-4-[(2-methoxyethyl)(methyl)amino]-2-butenamide;

The following experimental details are set forth to aid in an understanding of the invention, and are not intended, and should not be construed, to limit in any way the invention set forth in the claims that follow thereafter.

#### DETAILED DESCRIPTION OF THE INVENTION

The compounds of this invention are certain substituted 3-cyano quinolines. Throughout this patent application, the quinoline ring system will be numbered as indicated in the formula below; the numbering for the quinazoline ring system is also shown:



The pharmaceutically acceptable salts of the compounds of this invention are those derived from such organic and inorganic acids as: acetic, lactic, citric, tartaric, succinic, maleic, malonic, gluconic, hydrochloric, hydrobromic, phosphoric, nitric, sulfuric, methanesulfonic, and similarly known acceptable acids.

For purposes of this invention "halogen" is F, Cl, Br, or I.

Where a group is referred to as "substituted", preferred substituents are selected from alkyl of up to six carbon atoms, alkoxy, of up to six carbon atoms and halogen. A particularly preferred substituent is methyl.

The compounds of this invention may contain one or more asymmetric carbons atoms; in such cases, the compounds of this invention include the individual diastereomers, the racemates, and the individual R and S enantiomers thereof. Some of the compounds of this invention may contain one or more double bonds; in such cases, the compounds of this invention include each of the possible configurational isomers as well as mixtures of these isomers.

For purposes of this invention a "neoplasm" is defined as cells selected from the breast, kidney, bladder, mouth, larynx, esophagus, stomach, colon, ovary, pancreas, brain, prostate and lung having a morphology not found in the majority of the cells of a mammal.

In one embodiment, the present invention provides for a method of inhibiting the neoplasm. The method comprises contacting a cell with an amount of a compound effective to decrease or prevent HER-2 function. The cell may be a mammalian cell and more specifically a human cell. The cell may

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also be a bacterial cell such as for example *E. Coli*. The cell may include but is not limited to, a neuronal cell, an endothelial cell, a glial cell, a microglial cell, a smooth muscle cell, a somatic cell, a bone marrow cell, a liver cell, an intestinal cell, a germ cell, a myocyte, a mononuclear phagocyte, an endothelial cell, a tumor cell, a lymphocyte cell, a mesangial cell, a retinal epithelial cell, a retinal vascular cell, a ganglion cell or a stem cell. The cell may be a normal cell, an activated cell, a neoplastic cell, a diseased cell, or an infected cell.

In another embodiment, the present invention provides a method for the treatment or prevention of a neoplasm in a mammal. The present invention accordingly provides to a mammal, a pharmaceutical composition that comprises a compound of this invention in combination or association with a pharmaceutically acceptable carrier. The compound of this invention may be administered alone or in combination with other therapeutically effective compounds or therapies for the treatment or prevention of the neoplasm.

The compounds may be provided orally, by intralesional, intraperitoneal, intramuscular or intravenous injection; infusion; liposome-mediated delivery; topical, nasal, anal, vaginal, sublingual, urethral, transdermal, intrathecal, ocular or otic delivery. In order to obtain consistency in providing the compound of this invention it is preferred that a compound of the invention is in the form of a unit dose. Suitable unit dose forms include tablets, capsules and powders in sachets or vials. Such unit dose forms may contain from 0.1 to 300 mg of a compound of the invention and preferably from 2 to 100 mg. Still further preferred unit dosage forms contain 5 to 50 mg of a compound of the present invention. The compounds of the present invention can be administered orally at a dose range of about 0.01 to 100 mg/kg or preferably at a dose range of 0.1 to 10 mg/kg. Such compounds may be administered from 1 to 6 times a day, more usually from 1 to 4 times a day. The effective amount will be known to one of skill in the art; it will also be dependent upon the form of the compound. One of skill in the art could routinely perform empirical activity tests to determine the bioactivity of the compound in bioassays and thus determine what dosage to administer.

The compounds of the invention may be formulated with conventional excipients, such as a filler, a disintegrating agent, a binder, a lubricant, a flavoring agent, a color additive, or a carrier. The carrier may be for example a diluent, an aerosol, a topical carrier, an aqueous solution, a nonaqueous solution or a solid carrier. The carrier may be a polymer or a toothpaste. A carrier in this invention encompasses any of the standard pharmaceutically accepted carriers, such as phosphate buffered saline solution, acetate buffered saline solution, water, emulsions such as an oil/water emulsion or a triglyceride emulsion, various types of wetting agents, tablets, coated tablets and capsules.

When provided orally or topically, such compounds would be provided to a subject by delivery in different carriers. Typically, such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid, talc, vegetable fats or oils, gums, or glycols. The specific carrier would need to be selected based upon the desired method of delivery, for example, phosphate buffered saline (PBS) could be used for intravenous or systemic delivery and vegetable fats, creams, salves, ointments or gels may be used for topical delivery.



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The compounds of the present invention may be delivered together with suitable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers useful in treatment or prevention of neoplasm. Such compositions are liquids or lyophilized or otherwise dried formulations and include diluents of various buffer content (for example, Tris-HCl, acetate, phosphate), pH and ionic strength, additives such as albumins or gelatin to prevent absorption to surfaces, detergents (for example, TWEEN 20, TWEEN 80, PLURONIC F68, bile acid salts), solubilizing agents (for example, glycerol, polyethylene glycerol), anti-oxidants (for example, ascorbic acid, sodium metabisulfate), preservatives (for example, thimerosal, benzyl alcohol, parabens), bulking substances or tonicity modifiers (for example, lactose, mannitol), covalent attachment of polymers such as polyethylene glycol, complexation with metal ions, or incorporation of the compound into or onto particulate preparations of hydrogels or liposomes, micro-emulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroblasts. Such compositions will influence the physical state, solubility, stability, rate of in vivo release, and rate of in vivo clearance of the compound or composition. The choice of compositions will depend on the physical and chemical properties of the compound capable of treating or preventing a neoplasm.

The compound of the present invention may be delivered locally via a capsule that allows a sustained release of the compound over a period of time. Controlled or sustained release compositions include formulation in lipophilic depots (for example, fatty acids, waxes, oils).

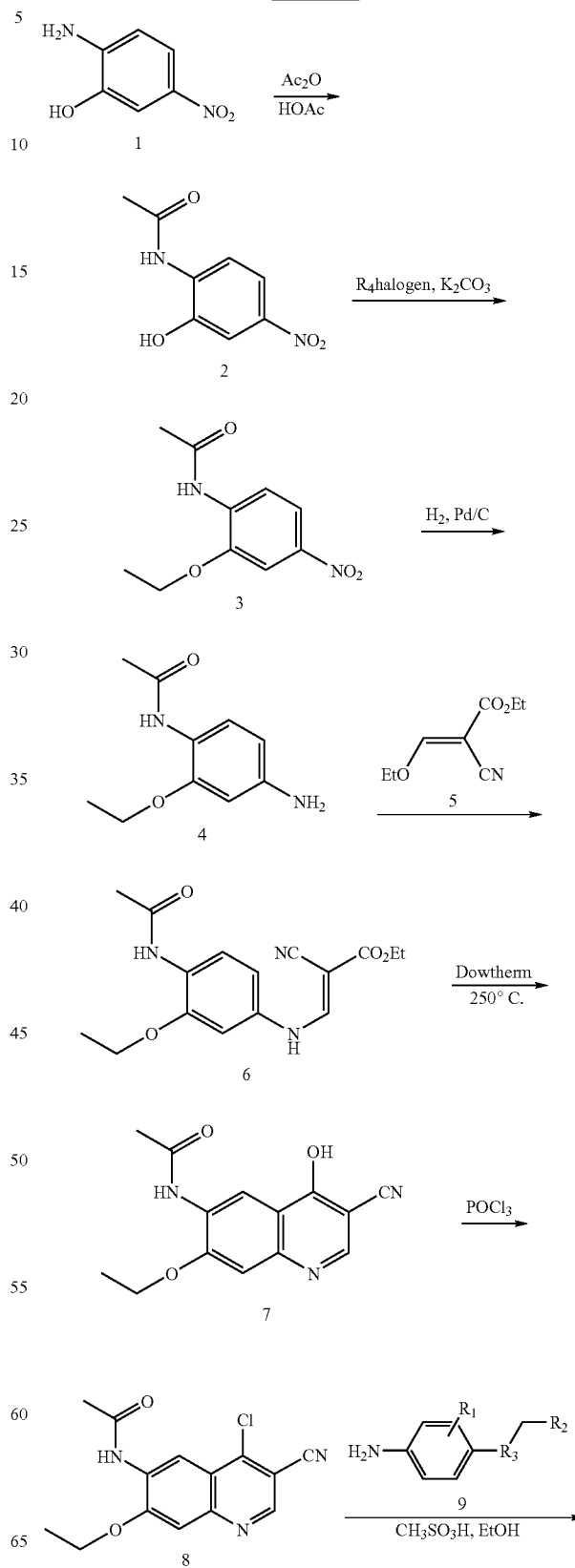
The present invention further provides a compound of the invention for use as an active therapeutic substance for preventing neoplasm.

The present invention further provides a method of treating neoplasm in humans, which comprises administering to the infected individual an effective amount of a compound or a pharmaceutical composition of the invention.

The compounds of this invention can be prepared as outlined in Flowsheet 1 wherein  $R_1$ ,  $R_2$ , and  $R_3$  are as described above. The amino group of compound 1 can be protected as an amide group by acetylation using acetic anhydride in a solvent such as acetic acid. The hydroxyl group of 2 can be alkylated with an alkyl bromide, iodide, tosylate, or mesylate using potassium carbonate in a refluxing solvent such as acetone. The nitro group of 3 can be reduced using catalytic hydrogenation to give the substituted aniline 4. Heating of 4 with reagent 5 with or without a solvent gives the intermediate 6. Refluxing 6 in a high boiling solvent such as Dowtherm results in cyclization to the hydroxy quinoline 7. This can be chlorinated by heating in phosphorous oxychloride to give the chloro derivative 8. Condensation of 8 with an aniline of formula 9 in a refluxing solvent such as ethanol in the presence of a catalytic amount of acid yields the intermediate 10. The acetate group of 10 can be removed by hydrolysis using acidic or basic conditions followed by neutralization to give 11. The intermediate 11 can be acylated with an amino acid chloride 12 (as the hydrochloride salt) to give the compounds of this invention of formula 13. Methods used to prepare the compounds in U.S. Pat. No. 6,288,082, WO-9633978 and WO-9633980 can also be used to prepare the compounds of this invention and are hereby incorporated by reference.

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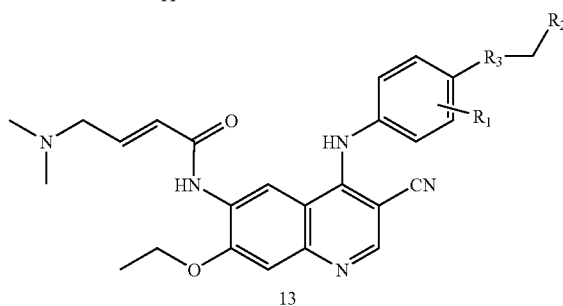
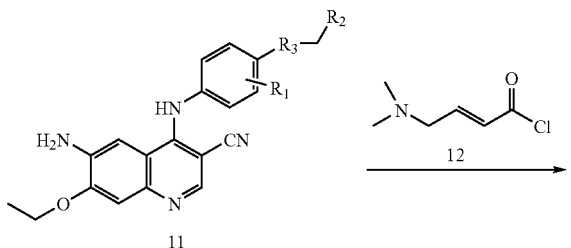
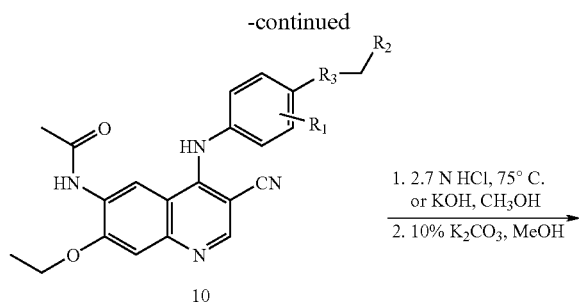
Flowsheet 1





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In addition to the method described herein above, there are a number of patent applications that describe methods that are useful for the preparation of the compounds of this invention. Although these methods describe the preparation of certain quinazolines, they are also applicable to the preparation of correspondingly substituted 3-cyanoquinolines and are hereby incorporated by reference. The chemical procedures described in the application WO-9633980 can be used to prepare the 3-cyanoquinoline intermediates used in this invention wherein the substitution at position 6 is an aminoalkylalkoxy group. The chemical procedures described in the application WO-9633978 can be used to prepare the 3-cyanoquinoline intermediates used in this invention wherein the substitution at position 6 is an aminoalkylamino group.

## EXAMPLE 1

(E)-N-{4-[4-(benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide

A 1.74 ml (2.54 g, 0.02 moles) portion of oxalyl chloride was added to 3.31 grams (0.02 moles) of (E)-4-(dimethylamino)-2-butenic acid hydrochloride in 75 ml of acetonitrile. To this was added a small drop of dimethylformamide. The reaction was heated and stirred in an oil bath at 63° for 20 minutes, giving an orange solution. This solution as concentrated in vacuo without the application of heat to about half its original volume. This solution was cooled in an ice bath and a solution of 4.45 g (0.01 moles) of the 6-amino-4-[4-(benzyloxy)-3-chloroanilino]-7-ethoxy-3-quinolinecarbonitrile in 50 ml of N-methylpyrrolidone was added in a stream. The

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reaction was cooled and stirred for 2 hours. The reaction was poured onto 100 ml of saturated aqueous sodium bicarbonate in ice. On standing the resulting gum solidified and the solid was filtered. This solid was chromatographed on silica gel. The column was washed with 3 liters of 1:19 methanol-ethyl acetate, then the product was eluted with 3 liters of 1:5:94 triethylamine-methanol-ethyl acetate. Concentration of the eluate gave a solid, which was filtered to give 2.96 grams of the title compound. From the filtrate was obtained an additional 1.0 grams of product. Total: 3.96 g.

## EXAMPLE 2

(E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide

A solution of (E)-4-(dimethylamino)-2-butenic acid hydrochloride in 1.2 L of tetrahydrofuran (THF) and a catalytic amount of dimethylformide (DMF) (1.2 ml) was cooled to 0-5° C. Oxalyl chloride (0.95 eq) was added dropwise and the mixture was warmed to 25-30° C. and stirred for 2 hours. The orange suspension was checked for complete consumption of oxalyl chloride by HPLC then cooled to 0-5° C. A solution of 111 g of 4-[4-(2-pyridylmethoxy)-3-chloro]amino-6-amino-3-cyano-7-ethoxyquinoline in 1.47 L of 1-methyl-2-pyrrolidinone was added dropwise and the mixture was stirred until  $\leq 1.0\%$  of the starting aniline remained (3-16 hours). The reaction was quenched with water and the mixture was warmed to 40° C. Aqueous sodium hydroxide was added to bring the pH to 10-11. The resulting precipitates were filtered hot and washed with water. The wet solids were heated to reflux (70-75° C.) in acetonitrile:THF (1.5:1) and the solution cooled over 3 hours to room temperature. The product was filtered and washed with acetonitrile:THF. The product was dried (50° C., 10 mm Hg, 24 hours) to give 80-85% yield. Melting point of maleate salt 178-183° C.

## EXAMPLE 3

(E)-N-(4-{3-chloro-4-[3-fluorobenzyl]oxy}anilino)-3-cyano-7-ethoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide

A solution of 108 g (E)-4-(dimethylamino)-2-butenic acid hydrochloride in 1.1 L of tetrahydrofuran (THF) and a catalytic amount of dimethylformide (DMF) (1.2 ml) was cooled to 0-5° C. Oxalyl chloride (0.95 eq) was added dropwise and the mixture was warmed to 25-30° C. and stirred for 2 hours. The orange suspension was checked for complete consumption of oxalyl chloride by HPLC then cooled to 0-5° C. A solution of 150 g of 6-amino-4-[3-chloro-4-(3-fluorobenzyl)oxy]anilino-3-cyano-7-ethoxy quinoline in 1.5 L of 1-methyl-2-pyrrolidinone was added dropwise and the mixture was stirred until  $\leq 1.0\%$  of the starting aniline remained (3-16 hours). The reaction was quenched with water and the mixture was warmed to 40° C. Aqueous sodium hydroxide (101 g in 750 ml) was added to bring the pH to 10-11. The resulting precipitates were filtered hot and washed with water. The wet solids were heated to reflux (70-75° C.) in acetonitrile:THF (1.5:1) and the solution was cooled over 3 hours to room temperature. The product was filtered and washed with acetonitrile:THF. The product was dried (50° C., 10 mm Hg, 24 h) and obtained in 80-85% yield. Melting Point 165-167° C.

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## EXAMPLE 4

## 4-Benzyloxy-3-chloro-nitrobenzene

A 15.43 g (0.275 moles) portion of solid (pellets) potassium hydroxide was added to a solution of 43.89 g (0.25 moles) of 3-chloro-4-fluoro nitrobenzene and 32.34 ml (33.79 grams, 0.373 moles) of benzyl alcohol in 220 ml of acetonitrile. The reaction was vigorously stirred with a mechanical stirrer overnight. The resulting solid was filtered. Concentration of the filtrate gave a second crop, which was also filtered. On standing more solid came out of this filtrate. This mixture was treated with ether, and the solid filtered. All solids were washed thoroughly with water, and combined to give 49.71 g of the title compound.

## EXAMPLE 5

## 4-Benzyloxy-3-chloro-phenylamine

A mixture of 6.59 g (0.025 moles) of the 4-benzyloxy-3-chloro nitrobenzene (example 4), 4.19 g (0.075 moles) of iron powder, and 12.04 g (0.225 moles) of ammonium chloride in 100 ml of ethanol and 25 ml of water was stirred mechanically and refluxed for half an hour. The reaction was allowed to cool and stir for 1 hour. The mixture was filtered and solids were washed with ethanol. The combined filtrates were taken to dryness in vacuo. This solid was dissolved in methylene chloride and passed through Magnesol. Removal of the solvent from the filtrate in vacuo gave 5.60 g of title compound.

## EXAMPLE 6

## N-{4-[4-(Benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}acetamide

A mixture of 4.17 g (0.0149 moles) of the N-(4-chloro-3-cyano-7-ethoxy-6-quinolinyl)acetamide, 4.04 g (0.0173 moles) of 4-benzyloxy-3-chloro-phenylamine (example 5), and 2.0 g (0.017 moles) of pyridine hydrochloride in 85 ml of isopropanol was stirred and refluxed in an oil bath for 30 minutes. The reaction was cooled in an ice bath, and the solid was collected by filtration and washed with isopropanol, and then with ether yielding 7.26 g of crude product as the hydrochloride salt. This material was purified by chromatography of the free base on silica gel by elution with 1:39 methanol-methylene chloride.

## EXAMPLE 7

## 6-Amino-4-[4-(benzyloxy)-3-chloroanilino]-7-ethoxy-3-quinolinecarbonitrile

A solution of 298 mg (0.612 mmoles) of the purified N-{4-[4-(benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinolinyl}acetamide (example 6) and 97 mg (1.73 mmoles) of potassium hydroxide in 10 ml of methanol was stirred and refluxed for 60 hours. On cooling a solid formed. This mixture was poured onto ice, and the resulting solid was filtered and washed with water. On drying, 242 mg of the title compound was obtained.

## EXAMPLE 8

## 2-Acetamido-5-nitrophenol

To 400 g of 2-amino-5-nitrophenol in a 5-L multi-necked flask equipped with a mechanical stirrer, reflux condenser, nitrogen inlet, 500-mL addition funnel, heating mantle, and a

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thermocouple attached to a temperature controller was added 1.6 L of acetic acid. The mixture was stirred at 60° C. as 398 g of acetic anhydride was added over 1.5 hours. After 1 hour, another 37 g of acetic anhydride was added. After another 1 hour, the mixture was cooled and diluted with 2 L of water. Solid was collected by filtration and washed with water and heptane. The solid was dried in a vacuum oven to give 509 g of the title compound.

## EXAMPLE 9

## 4-Acetamido-3-ethoxynitrobenzene

To 400 g of 2-acetamido-5-nitrophenol in a 12-L, 4-necked flask equipped with a reflux condenser, nitrogen inlet, thermocouple, addition funnel, and mechanical stirrer was added 790 g of potassium carbonate and 2.0 L of dimethylformamide. The mixture was stirred at 60° C. as 294 g of ethyl bromide was added over 30 minutes. After 1 hour, an additional 27 g of ethyl bromide was added and the mixture was stirred at 60° C. for another hour. The mixture was cooled to room temperature and poured into 4 L of water. After 30 minutes, the product was collected by filtration and washed with water and heptane. The product was dried in a vacuum oven at 60° C. to give 457 g of the title compound.

## EXAMPLE 10

## 3-(4-Acetamido-3-ethoxyaniline)-2-cyanopropenoic acid ethyl ester

A suspension of 4-acetamido-3-ethoxynitrobenzene compound in tetrahydrofuran (10 parts) was reduced to the aniline derivative using 10% Pd/C wet at 50 psi hydrogen and 30° C. for 2 hours. The resulting solution was filtered and concentrated to 2 parts of tetrahydrofuran. The concentrate was diluted with toluene and allowed to react with commercially available ethyl (ethoxymethylene)cynoacetate at reflux for 16 hours. After reaction completion, the mixture was cooled. The precipitated product was collected by filtration, washed and dried. The product was obtained in 90% yield.

## EXAMPLE 11

## 3-Cyano-7-ethoxy-4-hydroxy-6-N-acetylquinoline

A solution of 210 g of 3-(4-acetamido-3-ethoxyaniline)-2-cyanopropenoic acid ethyl ester in 12 L of Dowtherm was stirred under nitrogen at 250° C. for 15 to 20 hours. The mixture was cooled to room temperature and solid was collected by filtration. The solid was washed with toluene and mixed with 1.2 L of tetrahydrofuran. The mixture was refluxed for 30 minutes and then cooled to room temperature. The solid was collected and washed with tetrahydrofuran. After drying 179.4 g of the title compound was obtained.

## EXAMPLE 12

## 4-Chloro-3-cyano-7-ethoxy-6-nitro quinoline

A stirred mixture of 300 g 3-cyano-7-ethoxy-4-hydroxy-6-N-acetylquinoline in 2.53 L of 1,2-diethoxyethane was heated to 80-85° C. To this was added 224 ml of phosphorus oxychloride over 30-40 minutes. The mixture was stirred at 80-85° C. for 2-4 hours. The mixture was cooled, filtered over a celite pad and washed with 1,2-diethoxyethane. The filtrates were added over 1.5 hours to a cooled (0-10° C.) potassium carbonate (537 g in 1.5 L water) solution. The resulting yellow mixture was stirred for a minimum of 12 hours. The mixture was filtered and washed with hot water. The solids

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were dried (50° C., 10 mm Hg, 24 h) to give the title compound in 30-50% yield. The material was used directly in the next step.

## EXAMPLE 13

## 3-Chloro-4-(2-pyridylmethoxy)nitrobenzene

A mixture of 160 g of potassium hydroxide and 2-pyridylcarbinol in 8 L acetonitrile was stirred for 20-30 minutes. To this was added 400 g of 3-chloro-4-fluoronitrobenzene and the mixture was stirred at 40° C. for a minimum of 18 hours until the reaction was complete. Water was added and the precipitated yellow solids were filtered and washed with water. The product was dried (40-50° C., 10 mm Hg, 24 h) to the product in 85-95% yield.

## EXAMPLE 14

## 3-Chloro-4-(3-fluorobenzoyloxy)nitrobenzene

This compound was prepared from 3-chloro-4-fluoronitrobenzene and 3-fluorobenzyl alcohol using the method described above in Example 13.

## EXAMPLE 15

## 6-Amino-4-(3-chloro-4-(3-fluorobenzoyloxy))anilino-3-cyano-7-ethoxy quinoline

To a mixture of 400 g of 3-chloro-4-(3-fluorobenzoyloxy)nitrobenzene (example 14) and 464 g zinc dust in 4 L of ethanol at 40-50° C. was added aqueous ammonium chloride (152 g in 800 ml water). After stirring a minimum of 2 hours, the reaction mixture was filtered hot through a celite pad and washed with hot ethanol. The filtrate was evaporated and 1.72 L of 2-methyl THF, water and brine were added. The organic layer was separated and washed with water. The organic layer was evaporated and replaced with 3.8 L of ethanol. 4-Chloro-3-cyano-7-ethoxy-6-N-acetylamino-quinoline was added with a catalytic amount of methane sulfonic acid and the mixture was heated at 70-75° C. for a minimum of 2 hours until reaction completion. Concentrated 1.69 L HCl was added at 70-75° C. and held for a minimum of 2 hours until complete hydrolysis. Water was added and the mixture was cooled to 40° C., solid was collected and washed with water. The wet cake was slurried in 5.4 L of methanol, 10% aqueous potassium carbonate (315 g in 2.8 L water) was added and the mixture was stirred for 2.5 hours. The mixture was filtered and washed with 1:1 methanol:water. The product was dried (50° C., 10 mm Hg, 24 hours) to give the title compound in 80-90% yield.

## EXAMPLE 16

## 6-Amino-4-(4-(2-pyridylmethoxy)-3-chloro)anilino-3-cyano-7-ethoxyquinoline

The above-identified compound was prepared from 3-chloro-4-(2-pyridylmethoxy)nitrobenzene and 4-chloro-3-cyano-7-ethoxy-6-N-acetylamino-quinoline using the method described above in Example 15.

## EXAMPLE 20

## 4-Dimethyl-but-2-enoic acid [4-(3-chloro-4-fluorophenylamino)-3-cyano-7-ethoxy-quinolin-6-yl]-amide

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## EXAMPLE 21

## N-{4-[3-Chloro-4-(1-methyl-1H-imidazol-2-ylsulfanyl)-phenylamino]-3-cyano-quinolin-6-yl}-acrylamide

## EXAMPLE 22

## 6,7-Diethoxy-4-(1H-indol-6-ylamino)-quinoline-3-carbonitrile

## EXAMPLE 23

## 4-(2,3-Dihydro-benzo[1,4]dioxin-6-ylamino)-6,7-diethoxy-quinoline-3-carbonitrile

## EXAMPLE 24

## 4-(1H-Indazol-6-ylamino)-6,7-bis-(2-methoxyethoxy)-quinoline-3-carbonitrile

## EXAMPLE 25

## 4-(1,4-Dioxo-1,2,3,4-tetrahydro-phthalazin-6-ylamino)-6,7-diethoxy-quinoline-3-carbonitrile

## EXAMPLE 26

## 6,7-Diethoxy-4-(indan-5-ylamino)-quinoline-3-carbonitrile

## EXAMPLE 27

## 4-(2,4-Dioxo-1,4-dihydro-2H-benzo[d][1,3]oxazin-6-ylamino)-6,7-diethoxy-quinoline-3-carbonitrile

## EXAMPLE 28

## 6,7-Diethoxy-4-(1-oxo-indan-5-ylamino)-quinoline-3-carbonitrile

## EXAMPLE 29

## 6,7-Diethoxy-4-(3-oxo-1,3-dihydro-isobenzofuran-5-ylamino)-quinoline-3-carbonitrile

## EXAMPLE 30

## 4-(1,1-Dioxo-1H-1-benzo[b]thiophen-6-ylamino)-6,7-diethoxy-quinoline-3-carbonitrile

## EXAMPLE 31

## 7-Ethoxy-4-(1H-indazol-6-ylamino)-6-methoxy-quinoline-3-carbonitrile

## EXAMPLE 32

## 6-Ethoxy-4-(1H-indazol-6-ylamino)-7-methoxy-quinoline-3-carbonitrile

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EXAMPLE 33	EXAMPLE 44
6,7-Diethoxy-4-(1-methyl-2,5-dioxo-2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepin-7-ylamino)-quinoline-3-carbonitrile	4-{3-chloro-4-[(4-oxo-3,4-dihydro-2-quinazoliny]sulfanyl]anilino}-7-methoxy-6-hydro-2-quinazoliny]sulfanyl]anilino}-7-methoxy-6-nitro-3-quinolin-ecarbonitrile
EXAMPLE 34	EXAMPLE 45
4-(1H-Indazol-6-ylamino)-6-methoxy-7-(3-morpholin-4-yl-propoxy)-quinoline-3-carbonitrile	EXAMPLE 46
EXAMPLE 35	6-amino-4-{3-chloro-4-[(4-oxo-3,4-dihydro-2-quinazoliny]sulfanyl]anilino}-7-3,4-dihydro-2-quinazoliny]sulfanyl]anilino}-7-methoxy-3-quinolin-ecarbonitrile
4-({3-chloro-4-[(1-methyl-1H-imidazol-2-yl)sulfanyl]phenyl}amino)-7-methoxy-6-nitro-3-quinolin-ecarbonitrile	EXAMPLE 47
EXAMPLE 36	(E)-N-(4-{3-chloro-4-[(4-oxo-3,4-dihydro-2-quinazoliny]sulfanyl]anilino}-3-fanyl]anilino)-3-cyano-7-methoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide
6-amino-4-({3-chloro-4-[(1-methyl-1H-imidazol-2-yl)sulfanyl]phenyl}amino)-7-methoxy-3-quinolin-ecarbonitrile	EXAMPLE 48
EXAMPLE 37	(E)-N-(4-{4-[acetyl(3-pyridinylmethyl)amino]-3-chloroanilino≡-3-cyano-7-methoxy-]-3-chloroanilino}-3-cyano-7-methoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide
(2E)-N-[4-({3-chloro-4-[(1-methyl-1H-imidazol-2-yl)sulfanyl]phenyl}amino)-3-cyano-7-methoxy-6-quinolinyl]-4-(dimethylamino)-2-butenamide	EXAMPLE 49
EXAMPLE 38	N-{2-chloro-4-[(3-cyano-7-methoxy-6-nitro-4-quinolinyl)amino]phenyl}-N-(3-7-methoxy-6-nitro-4-quinolinyl)amino]phenyl}-N-(3-pyridinylmethyl)acetamide
4-{{3-chloro-4-(1,3-thiazol-2-yl)sulfanyl}phenyl}amino}-7-methoxy-6-nitro-3-quinolin-ecarbonitrile	EXAMPLE 50
EXAMPLE 39	N-(4-{{6-(acetylamino)-3-cyano-7-methoxy-4-quinolinyl]amino]-2-chlorophenyl)-N-(3-methoxy-4-quinolinyl)amino]-2-chlorophenyl}-N-(3-pyridinylmethyl)acetamide
6-amino-4-{{3-chloro-4-(1,3-thiazol-2-yl)sulfanyl}phenyl}amino}-7-methoxy-3-quinolin-ecarbonitrile	EXAMPLE 51
EXAMPLE 40	4-{3-chloro-4-[(1-methyl-1H-imidazol-2-yl)sulfanyl]anilino}-6-methoxy-7-[3-(4-morpholinyl)propoxy]-3-quinolin-ecarbonitrile
(2E)-N-(4-{{3-chloro-4-(1,3-thiazol-2-yl)sulfanyl}phenyl}amino)-3-cyano-7-methoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide	EXAMPLE 52
EXAMPLE 41	4-(3-chloro-4-{{5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl}amino}anilino)-7-ethoxy-6-nitro-3-quinolin-ecarbonitrile
4-{{3-chloro-4-(1H-imidazol-1-yl)anilino]-7-methoxy-6-nitro-3-quinolin-ecarbonitrile	
EXAMPLE 42	
6-amino-4-{{3-chloro-4-(1H-imidazol-1-yl)anilino]-7-methoxy-3-4-(1H-imidazol-1-yl)anilino]-7-methoxy-3-quinolin-ecarbonitrile	
EXAMPLE 43	
(E)-N-{{4-{{3-chloro-4-(1H-imidazol-1-yl)anilino]-3-cyano-7-methoxy-6-quinolinyl}-azol-1-yl)anilino]-4-(dimethylamino)-2-}anilino-2-butenamide	

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## EXAMPLE 53

(E)-N-[4-(3-chloro-4-{[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]amino}anilino)-3-cyano-7-ethoxy-6-quinoliny]-4-(dimethylamino)-2-butenamide

## EXAMPLE 54

4-[3-chloro-4-(4-pyridinyloxy)anilino]-7-ethoxy-6-nitro-3-quinolinecarbonitrile

## EXAMPLE 55

6-amino-4-[3-chloro-4-(4-pyridinyloxy)anilino]-7-ethoxy-3-quinolinecarbonitrile

## EXAMPLE 56

(E)-N-{4-[3-chloro-4-(4-pyridinyloxy)anilino]-3-cyano-7-ethoxy-6-quinoliny]-4-(dimethylamino)-2-butenamide

## EXAMPLE 57

4-{3-chloro-4-[(3-pyridinylmethyl)amino]anilino}-7-methoxy-6-nitro-3-quinolinecarbonitrile

## EXAMPLE 58

(E)-N-(4-{3-chloro-4-[(4-phenyl-1,3-thiazol-2-yl)sulfanyl]anilino}-3-cyano-7-methoxy-6-quinoliny)-4-(dimethylamino)-2-butenamide

## EXAMPLE 59

6-amino-4-(3-chloro-4-{[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]amino}anilino)-7-ethoxy-3-quinolinecarbonitrile

## EXAMPLE 60

4-[3-chloro-4-(1H-imidazol-1-ylmethyl)anilino]-7-ethoxy-6-nitro-3-quinolinecarbonitrile

## EXAMPLE 61

6-amino-4-[3-chloro-4-(1H-imidazol-1-ylmethyl)anilino]-7-ethoxy-3-quinolinecarbonitrile

## EXAMPLE 62

(E)-N-{4-[3-chloro-4-(1H-imidazol-1-ylmethyl)anilino]-3-cyano-7-ethoxy-6-quinoliny]-4-(dimethylamino)-2-butenamide

## EXAMPLE 63

4-{3-chloro-4-[(4-methyl-2-pyrimidinyl)sulfanyl]anilino}-7-ethoxy-6-nitro-3-quinolinecarbonitrile

## EXAMPLE 64

6-amino-4-{3-chloro-4-[(4-methyl-2-pyrimidinyl)sulfanyl]anilino}-7-ethoxy-3-quinolinecarbonitrile

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## EXAMPLE 65

(E)-N-(4-{3-chloro-4-[(4-methyl-2-pyrimidinyl)sulfanyl]anilino}-3-cyano-7-ethoxy-6-quinoliny)-4-(dimethylamino)-2-butenamide

## EXAMPLE 66

(E)-N-(4-{3-chloro-4-[(4,6-dimethyl-2-pyrimidinyl)sulfanyl]anilino}-3-cyano-7-ethoxy-6-quinoliny)-4-(dimethylamino)-2-butenamide

## EXAMPLE 67

7-ethoxy-6-nitro-4-[4-[(4-phenyl-1,3-thiazol-2-yl)sulfanyl]-3-(trifluoromethyl)anilino]-3-quinolinecarbonitrile

## EXAMPLE 68

6-Amino-7-ethoxy-4-[4-(4-phenylthiazol-2-ylsulfanyl)-3-trifluoromethyl-phenylamino]-quinoline-3-carbonitrile

## EXAMPLE 69

(E)-N-{3-cyano-7-ethoxy-4-[4-[(4-phenyl-1,3-thiazol-2-yl)sulfanyl]-3-(trifluoromethyl)anilino]-6-quinoliny]-4-(dimethylamino)-2-butenamide

## EXAMPLE 70

(E)-N-(4-{3-chloro-4-[(5-phenyl-1,3-thiazol-2-yl)sulfanyl]anilino}-3-cyano-7-methoxy-6-quinoliny)-4-(dimethylamino)-2-butenamide

## EXAMPLE 71

(E)-N-{4-[3-chloro-4-(1,3-thiazol-2-ylsulfanyl)anilino]-3-cyano-7-methoxy-6-quinoliny]-4-[(2-methoxyethyl)(methyl)amino]-2-butenamide

(E)-N-{4-[3-chloro-4-(1,3-thiazol-2-ylsulfanyl)anilino]-3-cyano-7-methoxy-6-quinoliny]-4-[(2-methoxyethyl)(methyl)amino]-2-butenamide was prepared by adding dropwise, 3.43 g (18.71 mmol, 1.95 mL) 4-bromocrotonyl chloride in 12 mL THF over 45 minutes to a stirred solution of 4.7 g (10.69 mmol) 6-amino-4-[3-chloro-4-(thiazol-2-ylsulfanyl)-phenylamino]-7-methoxy-quinolin-3-carbonitrile in 588 mL THF containing 3.73 mL (21.36 mmol) diisopropylethylamine, at 0° C. under nitrogen. The reaction produced a mixture of 4-bromo-(and chloro)-but-2-enoic acid {4-[3-chloro-4-(thiazol-2-ylsulfanyl)-phenylamino]-3-cyano-7-methoxy-quinolin-6-yl}-amide. A 300 mL portion of the solution was cooled to 0° C. and 2.38 g (26.7 mmol) (2-methoxyethyl)-methylamine in 11 mL THF was added dropwise. After the reaction had warmed to room temperature, 401 mg (0.5 eq) of sodium iodide was added and the solution was stirred overnight. The solvents were evaporated to leave a red gum, which was partitioned between EtOAc and saturated NaHCO<sub>3</sub>. After standing overnight, the layers were separated and the organic layer was dried and evaporated. Chromatography of the residue on a short column of Kieselgel 60, eluting with EtOAc, then EtOAc/15% MeOH, and finally EtOAc/15% MeOH/1% Et<sub>3</sub>N yielded 1.3 g (41%) of the product as a yellow glass; HRMS (ESI) m/z 595.13338 (M)<sup>+</sup>, Δ=-2.28 mmu.



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## EXAMPLE 72

(E)-N-(4-{3-chloro-4-[(4-phenyl-1,3-thiazol-2-yl)sulfanyl]anilino}-3-cyano-7-ethoxy-6-quinolinyl)-4-(dimethylamino)-2-butenamide

## EXAMPLE 73

4-{3-chloro-4-[(1-methyl-1H-imidazol-2-yl)sulfanyl]anilino}-6-methoxy-7-[3-triazol-1-yl]propoxy]-3-quinolinecarbonitrile

## EXAMPLE 74

4-{3-chloro-4-[(4,6-dimethyl-2-pyrimidinyl)sulfanyl]anilino}-7-ethoxy-6-nitro-3-quinolinecarbonitrile

## EXAMPLE 75

6-amino-4-{3-chloro-4-[(4,6-dimethyl-2-pyrimidinyl)sulfanyl]anilino}-7-ethoxy-3-quinolinecarbonitrile

## EXAMPLE 76

(2E)-N-{4-[3-chloro-4-(2-thienylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide

Representative compounds of this invention were evaluated in several standard pharmacological test procedures that showed that the compounds of this invention possess significant activity as inhibitors of HER-2 and are antiproliferative agents. Based on the activity shown in the standard pharmacological test procedures, the compounds of this invention are therefore useful as antineoplastic agents. The test procedures used and results obtained are shown below.

Kinase Assays: example 1, example 2 and example 3 are potent inhibitors of the HER-2 enzyme, example 20 is not. Purified recombinant C-terminal fragment of each enzyme is incubated with ATP in the absence or presence of a range of compound concentrations. Autophosphorylation of the receptors was evaluated with phosphotyrosine antibodies in an ELISA format. In a cell-free autophosphorylation assay

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using the recombinant cytoplasmic domain of HER-2, all three inhibitors reduced enzyme activity by 50% (IC<sub>50</sub>) at concentrations between 33-65 nM (Table 1).

TABLE 1

Compound	Enzyme IC <sub>50</sub> (μg/mL)	
	HER-2	EGFR
Example 1	0.036	0.028
Example 2	0.033	0.051
Example 3	0.019	0.019
Example 20	0.58	0.02

They also inhibited EGFR under similar assay conditions at 33-92 nM.

Cell Proliferation Assays: example 1, example 2, and example 3 repressed the proliferation of a mouse fibroblast cell line transfected with the HER-2 oncogene (3T31neu) by 50% (IC<sub>50</sub>) at 3-5 nM (Table 2). This value was substantially lower than that obtained with the isogenic untransfected cells (3T3; IC<sub>50</sub> 683-906 nM), indicating a high degree of selectivity for this oncogenic pathway. Cells were incubated with various concentrations of compound for 2 days (6 days for BT474 cells). Cell survival was determined using a protein binding dye assay (SRB), (Rubinstein LV, Shoemaker R H, Paull K D, Simon R M, Tosini S, Skehan P, Scudiero D A, Monks A, Boyd MR. Comparison of in vitro anticancer-drug-screening data generated with a tetrazolium assay versus a protein assay against a diverse panel of human tumor cell lines. J. Natl. Cancer Inst. 82(13):1113-8, 1990, Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst. 82(13):1107-12, 1990).

The concentration of drug (nM) which inhibits enzyme activity or cell proliferation by 50% is shown. The three inhibitors also inhibited two other HER-2 overexpressing breast cancer cell lines, SK-Br-3 and BT474 (IC<sub>50</sub> 2-4 nM), but were much less active on MDA-MB-435 and SW620 cells (a breast cancer and a colon cancer cell line, respectively), that are EGFR— and HER-2-negative. The compounds repressed the epidermal carcinoma cell line, A431, that overexpresses EGFR (IC<sub>50</sub> 81-120 nM) (Table 2).

TABLE 2

	CELL IC <sub>50</sub> (μg/mL)						
	EGFR						
	-	-	+++	-	+	-	-
	Her-2						
	-	+++	+	+++	+++	-	-
	COMPOUND						
	3T3	3T3/NEU	A431	SKBr3	BT474	MDA-MB-435	SW620
Example 1	0.38	0.0029	0.062	0.0015	0.0014	0.47	0.24
Example 2	0.39	0.0018	0.045	0.001	0.0013	0.44	0.44
Example 3	0.52	0.0023	0.069	0.0015	0.0024	0.51	0.29
Example 20	0.26	0.0230	0.030	0.0071	0.020	0.34	0.32
Example 21		0.463	0.62	0.01		4.57	1.84
Example 22		0.933	0.123	0.0374		0.365	0.286
Example 23		0.375	0.27	0.281		0.235	0.411
Example 24		>5	1.961	>5		2.045	>5
Example 25		>5	>5	>5		>5	>5
Example 26		0.0198	0.342	0.294		0.352	0.294

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TABLE 2-continued

	CELL IC <sub>50</sub> (µg/mL)					
	EGFR					
	-	-	+++	-	+	-
	Her-2					
-	+++	+	+++	+++	-	
COMPOUND						
3T3	3T3/NEU	A431	SKBr3	BT474	MDA-MB-435	SW620
Example 27	4.616	>5	>5		>5	2.922
Example 28	0.0311	0.0181	0.0281		0.028	0.0244
Example 29	3.301	>5	>5		3.404	1.565
Example 30	0.251	0.257	0.336		0.00328	0.146
Example 31	0.0267	0.0368	0.022		0.0359	0.0212
Example 32	4.801	0.786	2.094		2.626	4.313
Example 33	>5	>5	>5		>5	>5
Example 34	>5	>5	>5		>5	>5
Example 35	4.09	2.88	0.669		1	1.55
Example 36	1.06	3.04	0.011		0.39	3.16
Example 37	0.02	0.02	0.0004		0.43	0.43
Example 38	0.262	0.148	0.124		0.35	0.15
Example 39	0.333	0.663	0.236		0.65	0.55
Example 40	0.002	0.017	0.0007		0.33	1.13
Example 41	1.09	1.79	1.48		0.95	1.66
Example 42	0.53	1.63	1.73		1.27	5.99
Example 43	1.46	0.51	0.32		0.57	1.45
Example 44	4.54	2.28	4.54		>5	1.96
Example 45	1.88	1.22	2.15		4.58	4.66
Example 46	0.15	0.34	0.06		>5	>5
Example 47	>5	0.646	>5		1.16	1.64
Example 48	1.79	1.6	0.68		2.61	2.57
Example 49	2.4	>5	3.41		3.76	>5
Example 50	>5	3.68	3.94		>5	>5
Example 51	0.196	0.775	0.32			2.18
Example 52	1.89	1.79	1.22		1.84	2.54
Example 53	0.89	0.728	0.179		0.95	1.05
Example 54	>5	>5	>5		2.54	>5
Example 55	>5	>5	>5		1.61	>5
Example 56	>5	3.27	1.51		2.06	>5
Example 57	4.03	1.6	0.726		1.87	3.21
Example 58	0.028	0.162	0.005		0.23	0.57
Example 59	>5	>5	0.551		0.91	1.38
Example 60	>5	>5	2.44		>5	>5
Example 61	>5	>5	0.75		>5	>5
Example 62	0.99	0.95	0.045		2.1	3.8
Example 63	1.49	1.2	0.45		1.3	0.9
Example 64	3.03	1.53	>5		1.8	2
Example 65	0.003	0.12	0.001		0.3	0.2
Example 66	0.01	0.24	0.006		0.2	0.32
Example 67	0.68	0.76	0.268		0.4	0.4
Example 68	2.52	>5	0.943		2.5	3.1
Example 69	0.42	0.3	>5		0.3	0.6
Example 70	0.12	0.22	0.01		0.08	0.5
Example 71	0.002	0.03	0.002		0.09	0.4
Example 72	0.02	0.24	0.006		0.18	0.54
Example 73	0.973	1.83	0.104			3.69
Example 74	>5	>5	>5		4.1	>5
Example 75	2.12	0.76	0.98		1.3	1.36
Example 76	0.41	0.0039	0.066	0.004	0.003	0.77
					0.77	0.25

Receptor Phosphorylation: Compounds that repressed the proliferation of a mouse fibroblast cell line transfected with the HER-2 oncogene (3T3/neu) by 50% (IC<sub>50</sub>) <0.05 µg/ml in Table 2 above were tested for in vitro phosphorylation. For Her-2 and EGFR phosphorylation assays, cells (BT474 and A431, respectively) were incubated with various concentrations of compound for 3 hours at 37° C. Protein extracts were analyzed by immunoblotting using phospho-tyrosine antibodies. Blots were quantified by densitometric scanning. Concentration of compound (nM) which inhibits phosphorylation by 50% was determined. Example 1, Example 3 and HKI-272 decreased ligand-independent receptor phosphory-

55 lation by 50% (IC<sub>50</sub>) at 5-23 nM in BT474 cells (Table 3). They also repressed EGF-dependent phosphorylation of EGFR in A431 cells at a comparable dose (IC<sub>50</sub> 3-7 nM).

TABLE 3

Compound	IC50 (µg/mL)	
	BT474	A431
Example 1	0.0075	0.0031
Example 2	0.0026	0.0014

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TABLE 3-continued

Compound	IC50 (µg/mL)	
	BT474	A431
Example 3	0.013	0.0042
Example 20	0.080	0.0031
Example 37	<1	
Example 40	10-50	
Example 58	50-500	
Example 76	0.0015	0.0025

IN VIVO: The in vivo antitumor activity of example 3 was evaluated in tumor xenograft models. Tumor cells (grown in tissue culture) or tumor fragments were implanted subcutaneously in female nude mice. Treatment was initiated after tumors had reached a size of 90-200 mg, following random assignment of the animals to different treatment groups (staging). Alternatively (3T3/neu), treatment was initiated the day after tumor implantation, due to the rapid outgrowth of these tumors. Compounds were formulated in 0.5% Methocel-0.4% polysorbate-80 (Tween-80) and administered daily, PO, by gavage. Tumor mass  $[(L \times W^2)/2]$  was determined every 7 days. Statistical significance of compound effects was evaluated using Student's t-test.

The activity of example 3 was first evaluated in xenografts of 3T3/neu cells example 3 inhibited tumor growth when administered to animals at 20 mg/kg/day (65% inhibition, day 21), 40 mg/kg/day (97% inhibition), and 80 mg/kg/day (99% inhibition). These results were almost identical to those obtained with example 2 treatment (53%, 95%, and 98% inhibition, respectively at 20, 40 and 80 mg/kg/day). In two other independent tests, EXAMPLE 3 treatment produced a statistically-significant inhibition of tumor growth (21-33%) at a dose of 10 mg/kg/day. Based on these studies, the minimum efficacious dose (MED) was estimated to be 10 mg/kg/day. This is the smallest dose that produces a sustained, statistically-significant ( $p < 0.05$ ) reduction of tumor growth.

The effect of example 3 was next studied in xenografts of HER-2-dependent human tumor cell lines. In animals bearing BT474 xenografts, example 3 treatment reduced tumor growth when dosed between 10 mg/kg/day and 40 mg/kg/day. Maximum inhibition was observed on day 21, and ranged from 59% (10 mg/kg/day) to 96% (40 mg/kg/day). For example 2, inhibition ranged from 76% (10 mg/kg/day) to 95% (40 mg/kg/day). Similar results were obtained in two other independent experiments. In animals bearing xenografts of SUM-190 (a second HER-2-dependent breast cancer cell line), example 3 treatment resulted in substantial repression of tumor growth when dosed at 40 mg/kg/day (94% inhibition, day 28). Example 3 was also effective against xenografts of SK-OV-3 (a HER-2-dependent human ovarian carcinoma cell line). Here, example 3 was active between 20 mg/kg/day (86% inhibition, day 35) and 60 mg/kg/day (91% inhibition). The MED in the HER-2 over-expressing human xenograft models was estimated at 10

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mg/kg/day, similar to example 2. In these studies, there was no decrease in tumor size below the initial size at the start of dosing. Furthermore, tumors showed evidence of re-growth when treatment was completed, which is consistent with a non-cytotoxic mode of action for example 3.

What is claimed is:

1. A compound:

(E)-N-{4-[4-(benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinoliny]}-4-(dimethylamino)-2-butenamide or a pharmaceutically acceptable salt thereof.

2. A compound: (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinoliny]}-4-(dimethylamino)-2-butenamide or a pharmaceutically acceptable salt thereof.

3. A compound: (E)-N-(4-{3-chloro-4-[(3-fluorobenzyl)oxy]anilino}-3-cyano-7-ethoxy-6-quinoliny)-4-(dimethylamino)-2-butenamide or a pharmaceutically acceptable salt thereof.

4. A compound: (2E)-N-(4-{[3-chloro-4-(1,3-thiazol-2-ylsulfanyl)phenyl]amino}-3-cyano-7-methoxy-6-quinoliny)-4-(dimethylamino)-2-butenamide or A compound: (E)-N-(4-{3-chloro-4-[(4,6-di-methyl-2-pyrimidinyl)sulfanyl]anilino}-3-cyano-7-ethoxy-6-quinoliny)-4-(dimethylamino)-2-butenamide or a pharmaceutically acceptable salt thereof.

5. A compound (E)-N-{4-[3-chloro -4-(1,3-thiazol-2-ylsulfanyl )anilino]-3-cyano-7-methoxy-6-quinoliny]}-4-[(2-methoxyethyl)(methyl)amino]-2-butenamide or a pharmaceutically acceptable salt thereof.

6. A pharmaceutical composition comprising: (E)-N-{4-[4-(benzyloxy)-3-chloroanilino]-3-cyano-7-ethoxy-6-quinoliny]}-4-(dimethylamino)-2-butenamide or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

7. A pharmaceutical composition comprising: (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinoliny]}-4-(dimethylamino)-2-butenamide or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

8. A pharmaceutical composition comprising: (E)-N-(4-{3-chloro-4-[(3-fluorobenzyl)oxy]anilino}-3-cyano-7-ethoxy-6-quinoliny)-4-(dimethylamino)-2-butenamide or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

9. A pharmaceutical composition comprising: (E)-N-(4-{3-chloro-4-[(4-methyl-2-pyrimidinyl)sulfanyl]anilino}-3-cyano -7-ethoxy-6-quinoliny)-4-(dimethylamino)-2-butenamide or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

10. A pharmaceutical composition comprising (E)-N-{4-[3-chloro-4-(1,3-thiazol-2-ylsulfanyl )anilino]-3-cyano-7-methoxy-6-quinoliny]}-4-[(2-methoxyethyl)(methyl)amino]-2-butenamide or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

\* \* \* \* \*