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IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF NEW JERSEY

GENZYME CORPORATION, SOUTHERN RESEARCH INSTITUTE, and SANOFI-AVENTIS U.S. LLC, Plaintiffs, v. MYLAN LABORATORIES LIMITED, MYLAN PHARMACEUTICALS INC., and MYLAN INC., Defendants.	Civil Action No. **COMPLAINT** *Electronically Filed*

Plaintiffs Genzyme Corporation ("Genzyme"), Southern Research Institute ("Southern Research"), and sanofi-aventis U.S. LLC ("Sanofi") (collectively, "Plaintiffs"), by their

attorneys, for their Complaint against Mylan Laboratories Limited, Mylan Pharmaceuticals Inc., and Mylan Inc. (collectively, "Mylan" or "Defendants"), allege as follows:

NATURE OF THE ACTION

1. This is an action for infringement of United States Patent No. 5,661,136 ("'136 patent," a true and accurate copy of which is attached hereto as Exhibit A) arising under the Patent Laws of the United States, Title 35, United States Code, Sections 100 *et seq.* This action relates to Abbreviated New Drug Application ("ANDA") No. 208860, filed by Mylan Laboratories Limited with the United States Food and Drug Administration ("FDA") for approval to market a proposed generic version of the Clolar® (clofarabine) injection drug product.

THE PARTIES

2. Genzyme is a corporation organized and existing under the laws of the State of Massachusetts, having its principal place of business at 500 Kendall Street, Cambridge, Massachusetts 02142.

3. Southern Research is a corporation organized and existing under the laws of the State of Alabama, having its principal place of business at 2000 Ninth Avenue South, Birmingham, Alabama 35205-5305.

4. Sanofi is a limited liability company organized and existing under the laws of the State of Delaware with its principal place of business at 55 Corporate Drive, Bridgewater, New Jersey 08807.

5. On information and belief, Defendant Mylan Inc. is a company organized and existing under the laws of the Commonwealth of Pennsylvania with its principal place of business at 1000 Mylan Boulevard, Canonsburg, Pennsylvania 15317. On information and

belief, Mylan Inc. is in the business of, among other things, marketing and selling generic versions of branded pharmaceutical products for the United States market, alone and/or through its wholly-owned subsidiaries and agents.

6. On information and belief, Defendant Mylan Laboratories Limited ("Mylan Labs") is a company organized and existing under the laws of India, with a principal place of business at Plot No. 564/A/22, Road No. 92, Jubilee Hills 500034, Hyderabad, India. On information and belief, Mylan Labs is in the business of manufacturing and selling generic versions of pharmaceutical products for the United States market. On information and belief, Mylan Labs is a wholly-owned subsidiary of Mylan Inc., is controlled by Mylan Inc., and is an agent or affiliate of Mylan Pharmaceuticals Inc.

7. On information and belief, Defendant Mylan Pharmaceuticals Inc. ("Mylan Pharma") is a company 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 26505-4310. On information and belief, Mylan Pharma is in the business of manufacturing and selling generic versions of pharmaceutical products for the United States market. On information and belief, Mylan Pharma is wholly-owned subsidiary of Mylan Inc., is controlled by Mylan Inc., and is an agent or affiliate of Mylan Labs.

8. On information and belief, Mylan Labs operates in the United States through Mylan Inc. and Mylan Pharma.

9. On information and belief, the acts of Mylan Labs complained of herein were done at the direction or, with the authorization of, and/or with the cooperation, participation, and assistance of Mylan Inc. and Mylan Pharma. On information and belief, the acts of Mylan Labs complained of herein were done at least in part for the benefit of Mylan Inc. and Mylan Pharma.

MYLAN LABS' ANDA

10. On information and belief and as stated in the letter dated August 8, 2016, and received by Plaintiffs on or about August 9, 2016, purporting to be a notice pursuant to Section 505(j)(2)(B)(ii) and (iv) of the Federal Food Drug and Cosmetic Act (21 U.S.C. § 355(j)) and 21 C.F.R. § 314.95(c) (the "Notice Letter"), Mylan Labs submitted ANDA No. 208860 to the FDA under Section 505(j) of the Federal Food Drug and Cosmetic Act (21 U.S.C. § 355(j)) seeking approval to engage in the commercial manufacture, use, sale and/or importation of a clofarabine injection, 1mg/mL, 20mL in single-dose vials ("Clofarabine ANDA Injection") as a generic version of the Clolar® (clofarabine) injection drug product throughout the United States, including within the State of New Jersey, prior to the expiration of the '136 patent.

11. On information and belief, Mylan Labs is the holder of ANDA No. 208860.

JURISDICTION AND VENUE

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

13. On information and belief, Mylan Labs, with the assistance and/or direction of Mylan Inc. and/or Mylan Pharma, develops, formulates, manufactures, imports, offers for sale, sells, commercializes, markets, and/or distributes generic versions of branded pharmaceutical products in/into the United States, including in the State of New Jersey.

14. On information and belief, Mylan Inc. and Mylan Pharma acted in concert with Mylan Labs to develop Mylan Labs' generic copy of Clolar®.

15. On information and belief, Mylan Labs prepared and filed ANDA No. 208860, seeking approval from the FDA to sell Clofarabine ANDA Injection throughout the United States, including within the State of New Jersey.

16. On information and belief, Mylan Inc. and/or Mylan Pharma participated in the preparation and/or filing of ANDA No. 208860, seeking approval from the FDA to sell Clofarabine ANDA Injection throughout the United States, including within the State of New Jersey.

17. This Court has personal jurisdiction over Defendants because, *inter alia*, they have committed an act of patent infringement under 35 U.S.C. § 271 (e)(2), and intend a future course of conduct that includes acts of patent infringement in New Jersey. These acts have led and will lead to foreseeable harm and injury to Plaintiffs in New Jersey. For example, on information and belief, following approval of ANDA No. 208860, Defendants will work in concert to make, use, import, sell, and/or offer for sale Clofarabine ANDA Injection in/into the United States, including in this State, prior to the expiration of the '136 patent.

18. This Court has personal jurisdiction over Mylan Labs because, *inter alia*, Mylan Labs, on information and belief: (1) maintains substantial, systemic, and continuous contacts with the State of New Jersey; (2) regularly transacts and/or solicits business in the State of New Jersey; (3) continuously and systematically places its products into the stream of commerce for distribution and consumption in the State of New Jersey and throughout the United States; (4) engages in regular conduct of business within this judicial district; (5) derives substantial revenue and income from sales of its generic versions of branded pharmaceutical products throughout the United States, including in the State of New Jersey; (6) is registered with the State of New Jersey's Department of Health as a drug wholesaler; (7) maintains a broad distributorship network within this State; (8) intends to manufacture for distribution, market, sell, or distribute Clofarabine ANDA Injection to residents of this State, which is confirmed by the

filing of ANDA No. 208860 (*see Acorda Therapeutics Inc. v. Mylan Pharmaceuticals Inc.*, 817 F.3d 755 (Fed. Cir. 2016)).

19. Additionally, personal jurisdiction over Mylan Labs is also proper because Mylan Labs has previously consented to this Court's jurisdiction and has availed itself of the benefits and protections afforded by the Court by, at a minimum, asserting counterclaims against plaintiffs in this judicial district. *See, e.g., Horizon Pharma, Inc. et al. v. Mylan Pharmaceuticals, Inc., Mylan Laboratories Limited, and Mylan Inc.*, Civil Action No. 3:15-cv-03327-MLC-DEA (D.N.J.); *AstraZeneca Pharmaceuticals LP et al. v. Mylan Pharmaceuticals, Inc., Mylan Laboratories Limited, and Mylan Inc.*, Civil Action No. 1-15-cv-07009-RMB-KMW (D.N.J.); *AstraZeneca Pharmaceuticals LP et al. v. Mylan Pharmaceuticals, Inc., Mylan Laboratories Limited, and Mylan Inc. et al.*, Civil Action No. 1:15-cv-06039-RMB-KMW.

20. This Court has personal jurisdiction over Mylan Inc. because, *inter alia*, Mylan Inc., on information and belief: (1) is registered to do business in New Jersey under entity ID No. 0100971292 and has appointed a registered agent in New Jersey (*see Otsuka Pharm. Co., Ltd. v. Mylan Inc.*, 106 F.Supp.3d 456 (D.N.J. 2015)); (2) maintains substantial, systemic, and continuous contacts with the State of New Jersey; (3) regularly transacts and/or solicits business in the State of New Jersey; (4) continuously and systematically places its products into the stream of commerce for distribution and consumption in the State of New Jersey and throughout the United States; (5) engages in regular conduct of business within this judicial district; (6) derives substantial revenue and income from sales of its generic versions of branded pharmaceutical products throughout the United States, including in the State of New Jersey; (7) is registered with the State of New Jersey's Department of Health as a drug manufacturer and wholesaler; (8) maintains a broad distributorship network within this State; (9) intends to

manufacture for distribution, market, sell, or distribute Clofarabine ANDA Injection to residents of this State, which is confirmed by the filing of ANDA No. 208860 (*see Acorda Therapeutics Inc. v. Mylan Pharmaceuticals Inc.*, 817 F.3d 755 (Fed. Cir. 2016)).

21. Additionally, personal jurisdiction over Mylan Inc. is also proper because Mylan Inc. has previously consented to this Court's jurisdiction and has availed itself of the benefits and protections afforded by the Court by, at a minimum, asserting counterclaims against plaintiffs in this judicial district. *See, e.g., Horizon Pharma, Inc. et al. v. Mylan Pharmaceuticals, Inc., Mylan Laboratories Limited, and Mylan Inc.*, Civil Action No. 3:15-cv-03327-MLC-DEA (D.N.J.); *AstraZeneca Pharmaceuticals LP et al. v. Mylan Pharmaceuticals, Inc., Mylan Laboratories Limited, and Mylan Inc.*, Civil Action No. 1-15-cv-07009-RMB-KMW (D.N.J.); *AstraZeneca Pharmaceuticals LP et al. v. Mylan Pharmaceuticals, Inc., Mylan Laboratories Limited, and Mylan Inc. et al.*, Civil Action No. 1:15-cv-06039-RMB-KMW.

22. This Court has personal jurisdiction over Mylan Pharma because, *inter alia*, Mylan Pharma, on information and belief: (1) is registered to do business in New Jersey under entity ID No. 0100214277 and has appointed a registered agent in New Jersey (*see Otsuka Pharm. Co., Ltd. v. Mylan Inc.*, 106 F.Supp.3d 456 (D.N.J. 2015)); (2) maintains substantial, systemic, and continuous contacts with the State of New Jersey; (3) regularly transacts and/or solicits business in the State of New Jersey; (4) continuously and systematically places its products into the stream of commerce for distribution and consumption in the State of New Jersey and throughout the United States; (5) engages in regular conduct of business within this judicial district; (6) derives substantial revenue and income from sales of its generic versions of branded pharmaceutical products throughout the United States, including in the State of New Jersey; (7) is registered with the State of New Jersey's Department of Health as a drug

manufacturer and wholesaler; (8) maintains a broad distributorship network within this State; (9) intends to manufacture for distribution, market, sell, or distribute Clofarabine ANDA Injection to residents of this State, which is confirmed by the filing of ANDA No. 208860 (*see Acorda Therapeutics Inc. v. Mylan Pharmaceuticals Inc.*, 817 F.3d 755 (Fed. Cir. 2016)).

23. Additionally, personal jurisdiction over Mylan Inc. is also proper because Mylan Inc. has previously consented to this Court's jurisdiction and has availed itself of the benefits and protections afforded by the Court by, at a minimum, asserting counterclaims against plaintiffs in this judicial district. *See, e.g., Horizon Pharma, Inc. et al. v. Mylan Pharmaceuticals, Inc., Mylan Laboratories Limited, and Mylan Inc.*, Civil Action No. 3:15-cv-03327-MLC-DEA (D.N.J.); *AstraZeneca Pharmaceuticals LP et al. v. Mylan Pharmaceuticals, Inc., Mylan Laboratories Limited, and Mylan Inc.*, Civil Action No. 1-15-cv-07009-RMB-KMW (D.N.J.); *AstraZeneca Pharmaceuticals LP et al. v. Mylan Pharmaceuticals, Inc., Mylan Laboratories Limited, and Mylan Inc. et al.*, Civil Action No. 1:15-cv-06039-RMB-KMW.

24. Venue is proper in this judicial district pursuant to 28 U.S.C. §§ 1391 and 1400(b).

THE PATENT

25. The '136 patent was duly and legally issued on August 26, 1997 to inventors Drs. John A. Montgomery and John A. Secrist, III. The '136 patent was assigned to Southern Research. With patent term extension, the '136 patent will expire on January 14, 2018. Pediatric exclusivity extends the expiration of the '136 patent by six months to July 14, 2018. At all times from the issuance of the '136 patent to the present, Southern Research has been the owner of the '136 patent. Genzyme is Southern Research's exclusive licensee under the '136 patent. Sanofi is Genzyme's exclusive sub-licensee under the '136 patent.

ACTS GIVING RISE TO THIS ACTION

26. Genzyme is the holder of the approved New Drug Application ("NDA") No. 021673 for the Clolar® (clofarabine) injection drug product ("Clolar® NDA"). Southern Research, Genzyme, and Sanofi all share in the revenue generated from the sale of Clolar®.

27. Clolar® is indicated for the treatment of pediatric patients 1 to 21 years old with relapsed or refractory acute lymphoblastic leukemia after at least two prior regimens ("Approved Indication"). Usage of Clolar® and the Approved Indication are described in the Clolar® Prescribing Information, which also states that a mechanism of action of the clofarabine in Clolar® is inhibiting DNA synthesis through an inhibitory action on ribonucleotide reductase and by competitive inhibition of DNA polymerases.

28. The '136 patent is listed in the FDA publication entitled "Approved Drug Products with Therapeutic Equivalence Evaluation" (the Orange Book) as being applicable to Clolar®.

29. The '136 patent covers the use of Clolar® according to its Approved Indication, which occurs via a mechanism of action as described in the Clolar® Prescribing Information.

30. Defendants have knowledge of the '136 patent.

31. By the Notice Letter, Mylan Labs notified Plaintiffs that Mylan Labs had submitted ANDA No. 208860 to the FDA seeking approval to engage in the commercial manufacture, importation, use, and/or sale of Clofarabine ANDA Injection prior to the expiration of the '136 patent.

32. Mylan Labs' ANDA was submitted to obtain FDA approval to engage in the commercial manufacture, importation, use, and/or sale of Clofarabine ANDA Injection prior to the expiration of the '136 patent.

33. On information and belief, Defendants intend to engage in the commercial manufacture, importation, use, and/or sale of Clofarabine ANDA Injection in/into the United States and/or induce or contribute to such acts promptly upon receiving FDA approval to do so and during the term of the '136 patent.

34. In the Notice Letter, Mylan Labs notified Plaintiffs that ANDA No. 208860 contained a certification pursuant to 21 U.S.C. § 355(j)(2)(A)(vii)(IV) that, in Mylan Labs' opinion, the '136 patent is invalid, unenforceable, and/or will not be infringed by the manufacture, use, sale, offer for sale, and/or importation of Clofarabine ANDA Injection in/into the United States ("Paragraph IV Certification").

35. On information and belief, the active ingredient of Clofarabine ANDA Injection is clofarabine, which is the same active ingredient in Clolar® and the same active ingredient used in the methods of one or more claims of the '136 patent, including but not limited to Claims 1 and 5.

36. On information and belief, Mylan Labs asserted in ANDA No. 208860 that Clofarabine ANDA Injection is bioequivalent to Clolar®.

37. On information and belief, Mylan Labs' ANDA No. 208860 refers to and relies upon the Clolar® NDA and contains data that, according to Mylan Labs, demonstrate the bioequivalence of Clofarabine ANDA Injection and Clolar®.

38. On information and belief, Mylan Labs is seeking approval to market Clofarabine ANDA Injection for the same Approved Indication as Clolar®.

39. On information and belief, Mylan Inc. and Mylan Pharma were actively involved in the preparation and/or submission of ANDA No. 208860 including the Paragraph IV certification against the '136 patent.

40. On information and belief, Mylan Inc. and Mylan Pharma actively and knowingly provided Mylan Labs with material information and support in preparing and submitting ANDA No. 208860 and have therefore aided and/or abetted in the filing of ANDA No. 208860.

41. On information and belief, Defendants will work in concert with one another to commercially manufacture, use, offer for sale, and/or sell Clofarabine ANDA Injection throughout the United States, import Clofarabine ANDA Injection into the United States, and/or induce to such acts promptly upon receiving FDA approval to do so and during the term of the '136 patent.

42. On information and belief, Defendants will knowingly accompany Clofarabine ANDA Injection with prescribing information that will contain instructions for use that substantially copy the instructions for Clolar®, including instructions for administering Clofarabine ANDA Injection as claimed in the '136 patent, including but not limited to Claims 1 and 5.

43. On information and belief, Defendants' prescribing information for Clofarabine ANDA Injection will instruct users to administer Clofarabine ANDA Injection to bring about a cytotoxic effect in a mammalian cancerous cell.

44. On information and belief, Defendants' prescribing information for Clofarabine ANDA Injection will instruct users to administer Clofarabine ANDA Injection to inhibit ribonucleotide reductase and DNA polymerase α in a mammalian cell.

45. On information and belief, Defendants have knowledge and/or an expectation that Clofarabine ANDA Injection will be used in accordance with its prescribing information.

46. On information and belief, Defendants know that the prescribing information that will accompany Clofarabine ANDA Injection will induce and/or contribute to others using Clofarabine ANDA Injection in the manner set forth in the prescribing information.

47. On information and belief, physicians, health care providers, and/or patients will directly infringe one or more claims of the '136 patent, including but not limited to Claims 1 and 5, by using Clofarabine ANDA Injection in accordance with the prescribing information provided by Defendants after the FDA approves ANDA No. 208860.

48. On information and belief, Defendants specifically intend that physicians, health care providers, and/or patients will use Clofarabine ANDA Injection in accordance with the prescribing information provided by Defendants to directly infringe one or more claims of the '136 patent, including but not limited to Claims 1 and 5.

49. On information and belief, Defendants designed Clofarabine ANDA Injection for use in a way that would infringe the '136 patent and will instruct users of Clofarabine ANDA Injection to use Clofarabine ANDA Injection in a way that would infringe one or more claims of the '136 patent.

50. On information and belief, Clofarabine ANDA Injection is not a staple article or commodity of commerce suitable for substantial non-infringing use.

51. On information and belief, Defendants knowingly have taken and intend to take active steps to induce and/or contribute to physicians, health care providers, and/or patients using Clofarabine ANDA Injection in a manner that directly infringes one or more claims of the '136 patent, including but not limited to by providing prescribing information with instructions for administering Clofarabine ANDA Injection as claimed in one or more claims of the '136 patent, including but not limited to Claims 1 and 5.

52. On information and belief, Defendants are aware of the decision issued on August 22, 2013 in *Southern Research Institute et al. v. Abon Pharmaceuticals LLC,* 1:12-cv-04709, construing phrases from the claims of the '136 patent to include "cells in, or derived from, a mammal (such as a human)."

53. Plaintiffs commenced this action within 45 days of receiving the Notice Letter.

COUNT I
INFRINGEMENT OF U.S. PATENT NO. 5,661,136

54. Plaintiffs repeat and reallege the allegations of paragraphs 1-53 as if fully set forth herein.

55. Mylan Labs' submission of ANDA No. 208860 containing the Paragraph IV Certification to obtain approval from the FDA to engage in the commercial manufacture, importation, use, or sale of Clofarabine ANDA Injection in/into the United States prior to the expiration of the '136 patent constitutes infringement of at least one claim of the '136 patent, including but not limited to Claims 1 and 5, under 35 U.S.C. § 271 (e)(2)(A).

56. Mylan Inc. and Mylan Pharma actively and knowingly aided, abetted, and induced Mylan Labs to submit ANDA No. 208860 containing the Paragraph IV Certification before the expiration of the '136 patent.

57. Defendants had notice of the '136 patent at the time of their infringement. Defendants' infringement has been, and continues to be, deliberate.

58. Plaintiffs will be substantially and irreparably harmed if Defendants' infringement of the '136 patent is not enjoined. Plaintiffs do not have an adequate remedy at law.

59. This is an exceptional case within the meaning of 35 U.S.C. § 285, which warrants reimbursement of Plaintiffs' reasonable attorney fees.

COUNT II
DECLARATORY JUDGMENT OF INFRINGEMENT
OF U.S. PATENT NO. 5,661,136

60. Plaintiffs repeat and reallege the allegations of paragraphs 1-59 as if fully set forth herein.

61. This claim arises under the Patent Laws, 35 U.S.C. § 1 *et seq.*, and the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202, based upon an actual controversy between the parties. Defendants have taken immediate and active steps, through the submission of ANDA No. 208860, to obtain approval from the FDA to commercially manufacture, import, use, or sell Clofarabine ANDA Injection prior to the expiration of the '136 patent.

62. After obtaining FDA approval, Defendants plan to act in concert with each other to commercially manufacture, use, offer for sale, and/or sell Clofarabine ANDA Injection in the United States, import Clofarabine ANDA Injection into the United States, and/or induce such acts prior to the expiration of the '136 patent.

63. Upon FDA approval of ANDA No. 208860, Defendants will infringe one or more of the claims of the '136 patent, including but not limited to Claims 1 and 5, under §§ 271 (a), (b), or (c) by making, using, selling, offering for sale, or importing Clofarabine ANDA Injection in/into the United States prior to the expiration of '136 patent, unless enjoined by this Court. Accordingly, an actual and immediate controversy exists between the parties regarding infringement of the '136 patent under 35 U.S.C. §§ 271 (a), (b), and/or (c).

64. Upon FDA approval of ANDA No. 208860, use of Clofarabine ANDA Injection as directed by the instructions to be included with Clofarabine ANDA Injection will directly infringe at least one of the claims of the '136 patent, including but not limited to Claims 1 and 5,

either literally or under the doctrine of equivalents, under 35 U.S.C. § 271 (a), unless enjoined by this Court.

65. Defendants have taken and intend to take active steps to induce or contribute to the direct infringement of one or more claims of the '136 patent, including but not limited to Claims 1 and 5, under 35 U.S.C. § 271 (b) and/or § 271 (c) after ANDA No. 208860 is approved, unless enjoined by this Court.

66. Defendants have knowledge of the '136 patent and, by the prescribing information that will be included with Clofarabine ANDA Injection, know or should know that they will aid and abet another's direct infringement of at least one of the claims of the '136 patent, including but not limited to Claims 1 and 5, either literally or under the doctrine of equivalents.

67. Defendants' offering for sale, sale, and/or importation of Clofarabine ANDA Injection in/into the United States with the prescribing information for Clofarabine ANDA Injection will actively induce infringement of at least one of the claims of the '136 patent, including but not limited to Claims 1 and 5, either literally or under the doctrine of equivalents, under 35 U.S.C. § 271 (b).

68. The use of Clofarabine ANDA Injection constitutes a material part of at least one of the claims of the '136 patent; Defendants know that Clofarabine ANDA Injection is especially made or adapted for use in infringing at least one of the claims of the '136 patent, including but not limited to Claims 1 and 5, either literally or under the doctrine of equivalents; and Defendants know that Clofarabine ANDA Injection is not a staple article of commerce or commodity of commerce suitable for substantial noninfringing use.

69. Defendants' manufacture, use, offering for sale, sale, and/or importation of Clofarabine ANDA Injection in/into the United States will contributorily infringe at least one of the claims of the '136 patent, including but not limited to Claims 1 and 5, either literally or under the doctrine of equivalents, under 35 U.S.C. § 271 (c).

70. Defendants will have notice of the '136 patent at the time of their infringement. Defendants' infringement of the '136 patent will be deliberate.

71. Plaintiffs will be substantially and irreparably harmed if Defendants' infringement is not enjoined. Plaintiffs do not have an adequate remedy at law.

72. This is an exceptional case within the meaning of 35 U.S.C. § 285, which warrants reimbursement of Plaintiffs' reasonable attorney fees.

PRAYER FOR RELIEF

WHEREFORE, Plaintiffs respectfully request the following relief:

(a) A judgment declaring that Defendants have infringed one or more claims of the '136 patent by the filing of ANDA No. 208860;

(b) A judgment declaring that Defendants' manufacturing, using, selling, offering for sale, or importing Clofarabine ANDA Injection in/into the United States will infringe one or more claims of the '136 patent;

(c) A judgment under 35 U.S.C. § 271 (e)(4)(A) providing that the effective date of any FDA approval of ANDA No. 208860 under Section 505(j) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 355(j)) be a date no earlier than July 14, 2018, the date on which the '136 patent expires, or the expiration of any other exclusivity to which Plaintiffs are or become entitled;

(d) Injunctive relief under 35 U.S.C. § 271 (e)(4)(B) preliminarily and permanently enjoining Defendants from making, using, selling, offering for sale, or importing Clofarabine ANDA Injection in/into the United States until after expiration of the '136 patent or the expiration of any other exclusivity to which Plaintiffs are or become entitled;

(e) A permanent injunction pursuant to 35 U.S.C. § 271 (e)(4)(B) restraining and enjoining Defendants from practicing any methods as claimed in the '136 patent, or from actively inducing or contributing to the infringement of any claim of the '136 patent, until after the expiration of the '136 patent or the expiration of any other exclusivity to which Plaintiffs are or become entitled;

(f) A Declaration that the commercial manufacture, use, sale, offer for sale, and importation in/into the United States of Clofarabine ANDA Injection will directly infringe, induce, and/or contribute to infringement of the '136 patent;

(g) Damages under 35 U.S.C. § 271 (e)(4)(C), which this Court should treble pursuant to 35 U.S.C. § 284, if Defendants infringe the '136 patent by engaging in the commercial manufacture, importation, use, sale, offer for sale, or import the Clofarabine ANDA Injection in/into the United States prior to the expiration of the '136 patent or the expiration of any other exclusivity to which Plaintiffs are or become entitled;

(h) An award of reasonable attorney fees in this action 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: September 19, 2016

WALSH PIZZI O'REILLY FALANGA LLP

s/Liza M. Walsh

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RULE 11.2 CERTIFICATION

We hereby certify that the matter in controversy is related to the following action pending before the Honorable Stanley R. Chesler, U.S.D.J. in the United States District Court, District of New Jersey: *Southern Research Institute and Genzyme Corporation v. Amneal Pharmaceuticals LLC*, Civil Action No. 2:16-cv-03892-SRC-CLW.

We certify that, to the best of our knowledge, the matter in controversy is not the subject of any other pending or anticipated litigation in any court or arbitration proceeding other than the above referenced matter, nor are there any non-parties known to Plaintiffs that should be joined to this action. In addition, I recognize a continuing obligation during the course of this litigation to file and to serve on all other parties and with the Court an amended certification if there is a change in the facts stated in this original certification.

DATED: September 19, 2016

WALSH PIZZI O'REILLY FALANGA LLP

s/Liza M. Walsh

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RULE 201.1 CERTIFICATION

We hereby certify that the above-captioned matter is not subject to compulsory arbitration in that the Plaintiffs seek, *inter alia*, injunctive relief.

DATED: September 19, 2016

WALSH PIZZI O'REILLY FALANGA LLP

s/Liza M. Walsh

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EXHIBIT A

US005661136A

United States Patent [19]

Montgomery et al.

[11] Patent Number: 5,661,136

[45] Date of Patent: Aug. 26, 1997

[54] **2-HALO-2'-FLUORO ARA ADENOSINES AS ANTINOPLASTIC AGENTS**

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[63] Continuation of Ser. No. 693,646, May 10, 1991, Pat. No. 5,384,310, which is a continuation-in-part of Ser. No. 355, 358, May 23, 1989, Pat. No. 5,034,518.

[51] **Int. Cl.**[6] **A61K 31/70**

[52] **U.S. Cl.** **514/46**; 536/27.4; 536/27.63

[58] **Field of Search** 514/46, 27.4; 536/27.63

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[57] **ABSTRACT**

The present invention is directed to certain 2'-fluoro, 2-substituted purine nucleosides which are toxic to cancerous cell lines.

12 Claims, No Drawings

2-HALO-2'-FLUORO ARA ADENOSINES AS ANTINOPLASTIC AGENTS

The application is a continuation of application Ser. No. 07/693,646, filed May. 10, 1991, now U.S. Pat. No. 5,384, 310 which is a continuation-in-part of application Ser. No. 07/355,358, filed May 23, 1989now U.S. Pat. No. 5,034,518.

The research leading to the discovery of the present invention was funded, in part, by funds from the United Stated Department of Health and Human Services. Accordingly, the United States government has certain statutory rights to the present invention under 37 USC 200 et seq.

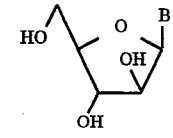
The development of effective anticancer agents is a complex problem for a number of reasons, but primarily because of the lack of an identifiable, exploitable biochemical difference between normal and malignant tumor cells, be they of animal or human origin.

The simplest and most used strategy for the discovery of new anticancer agents is by empirical search, which has been most successful in identifying useful antitumor antibiotics. The search for lead compounds among synthetics is somewhat different, since few clinically useful agents have resulted from strictly random screening, which in fact is not a truly random search since it reflects the status of organic chemistry and, largely, what synthetic chemists have found of interest for whatever reason. In fact, most synthetics found to have clinical activity were screened for a reason. A prime example is one of the first clinically useful agents, nitrogen mustard, which was tested because of its effects on the blood elements discovered in the chemical warfare program. Regardless of the method of discovery, anticancer agents can be classified in five broad groupings:

A. Antimetabolites
Glutamine antagonists
Inhibitors of dihydrofolic reductase
Purine and pyrimidine analogs
Nucleoside diphosphate inhibitors
B. Nucleic acid complexors
Actinomycins
Anthracyclines
Bleomycins
Mitomycins
Mithramycin
Neocarcinostatin
Anthramycins
C. Chemically reactive compounds
Nitrogen mustards
Aziridines
Sulfonates
Triazenes
Nitrosoureas
Procarbazine
cis-Platinum
D. Mitotic inhibitors
Vinca alkaloids
Podophyllum derivatives
E. Hormones
Estrogens
Androgens
Progestogens
Glucocorticoids
Miscellaneous synthetics

From these groupings, it is clear that anticancer agents with proven utility interfere one way or another with cell division and, since cancer cells must divide or eventually die, they are cytotoxic agents with some degree of specificity for neoplastic cells. Thus it would seem logical that the search for new lead compounds should focus on new structural types that will also interfere with one or another of the processes of cell division. The most approachable of these is the design of enzyme inhibitors. There are at least 85 enzymatic reactions involved in the de novo synthesis of purine and pyrimidine nucleotides, in their interconversion, in their polymerization to nucleic acids, and in the so called salvage pathways. Of these 85 enzymes, approximately 14 are known to be inhibited by metabolic analogs or anabolites thereof. These inhibitions are thought to be responsible for, or at least contribute to, the anticancer activity of these compounds.

Two such compounds are the arabinofuranosyl nucleosides, 9-β-D-arabinofuranosyladenine and 1-β-D-arabinofuranosylcytosine, of the formula:



wherein B is adenine or cytosine, have well-known antiviral (B=adenine) and anticancer (B=cytosine) activity. In addition, other arabinofuranosyl nucleosides with 2'-substituents other than hydroxyl have also exhibited useful biological effects. All of these nucleosides require activation (phosphorylation) to be effective, and generally this is accomplished by different enzymes than the corresponding ribofuranosyl nucleosides.

In addition, a number of 2'-substituted-9-1β-D-arabinofuranosyl-2-haloadenines [see J. Med. Chem. 31:405 (1988), and J. Med. Chem. 29:2389 (1986)] have also been developed along this general design. 9-β-D-arabinofuranosyl-2-fluoroadenine monophosphate is, for example, is a drug of choice against chronic lymphocytic leukemia; and 2-chloro-2'deoxyadenosine has shown some promise in a phase I trial against T-cell neoplasms and in phase II trials against chromic lymphocytic leukemia of B-cell origin that is refractory to conventional therapy, and against hairy-cell leukemia. However, the search for better and more effective, anticancer compounds continues.

Thus, in accordance with the present invention it has now been found that the incorporation of a 2-halo substituent onto the purine ring of these prior compounds significantly alters the metabolism of adenine nucleosides, specifically by reducing the ability of the compound to serve as a substrate for adenosine deaminase; that substituting a fluorine in the arabino configuration at C-2' makes these derivatives highly resistant to phosphorolytic cleavage; and that the combination of these two changes in the same molecule provide enhanced biological and anti cancer activity of the resulting compound.

The present invention relates to a family of novel nucleoside compounds, and pharmaceutically acceptable salts thereof, represented by the general formula:

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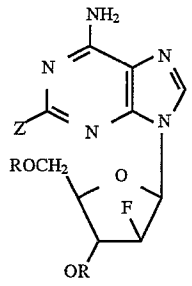
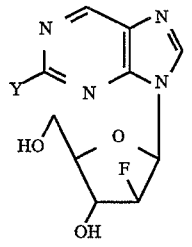


in which R, which may be the same or different, is a hydrogen or acyl protecting group such as an alkanoyl protecting or blocking group such as benzoyl, and wherein Z is a halogen of the group F, Cl, and Br. In accordance with one aspect of the present invention, where R is acyl, the nucleoside compound acts as a prodrug in prolonging the in vivo life of the compound.

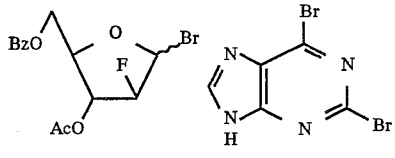
More specifically, the most preferred compounds of the present invention are those of formula:



wherein Y is F, Cl or Br, or the pharmaceutically acceptable salts thereof.

This and other aspects of the present invention will become clearer in the following discussion and description, both provided for purposes of clarification and not limitation as to the scope of the present invention.

In its broadest description, 2'-substituted purine arabinonucleosides are prepared from 2-haloadenosines via their 3',5'-O-(tetraisopropyldisiloxane-2'-0-triflate derivatives according to the process discussed in J. Med. Chem. 31:405 (1988). Since this prior approaches failed to provide the 2'-fluoroarabinonucleosides in reasonable yields, these compounds had to be prepared by reaction of the appropriately blocked 2'-fluoro sugar (compound 1) with 2,6-dichloropurine followed by modification of the purine [see J. Med. Chem. 29:2389 (1986)].



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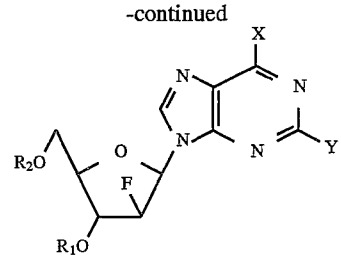

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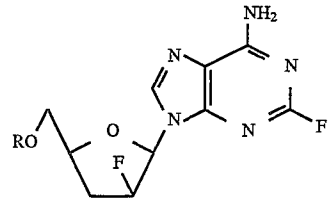

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a) X = Y = Br, R_1 = Ac, R_2 = Bz a) R = Bz

b) X = NH_2, Y = Br, R_1 = R_2 = H b) R = H

c) X = Y = Cl, R_1 = Ac, R_2 = Bz
d) X = NH_2, Y = Cl, R_1 = R_2 = H
e) X = Y = NH_2, R_1 = Ac, R_2 = Bz
f) X = NH_2, Y = F, R_1 = Ac, R_2 = Bz
g) X = NH_2, Y = F, R_1 = R_2 = H
h) X = NH_2, Y = F, R_1 = H, R_2 = Bz

The same sequence was also applied to 2,6-dibromopurine (compound 2) for the preparation of the 2-bromoadenine nucleoside. The blocked 2'-fluoro sugar was condensed with 2,6-dibromopurine in refluxing 1,2-dichloroethane in the presence of 4A molecular sieves. The anomeric configuration and substitution positions for compound 3a were confirmed by ^{1}H NMR comparisons with compound 3c. Animation and deprotection of compound 3a or 3c done in ethanolic ammonia yielded a mixture of the desired product and the 5'-benzoyl protected compound. This residual blocking group may be removed if desired by treating the mixture with LiOH in MeCN—H_2O to give either compound 3b or 3d.

Non-aqueous diazotization of compound 3e with tert-butylnitrite in 60% hydrogen fluoride/pyridine at −20° C. produced the 2-fluoro compound 3f. Deacylation of compound 3f was accomplished with LiOH in MeCN—H_2O, allowing a reasonable yield of compound 3g, free of any side products.

In order to prepare the dideoxy compound 4b, the 3'-acetyl of compound 3f was first selectively removed with $NaHCO_3$ in MeOH. The resulting product, compound 3h, was then treated with thiocarbonyldiimidazole followed by reduction with tri-n-butyltin hydride to give compound 4a. The 5'-benzoyl protecting group of compound 4a was then removed with LiOH to produce compound 4b.

The following examples, given for purposes of clarity in more fully demonstrating the methods by which the compounds of the present invention may be prepared, are provided. However, these examples are not meant to be limiting in any manner, and modifications and adaptions may be made to provide other routes, which are to be considered to be within the scope of the present invention, for the synthesis of the desired compounds.

EXAMPLE I

2,6-Dibromo-9-(3-O-acetyl-5-O-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-9H-purine

(compound 3a)

A solution of 3-acetyl-5-benzoyl-2-deoxy-2-fluoroarabinofuranosylbromide (33.2 mmol) in 400 mL of dry dichloroethane was stirred for 10 min with 4A molecular sieves (250 mL) before the addition of (9.3 g. 33.5 mmol) 2,6-dibromopurine. The mixture was vigorously stirred with an overhead stirrer and placed in a preheated 100° C. oil bath. Heating was continued for 32 h until all the bromosugar was consumed. (TLC 2:1 cyclohexane-ethyl acetate, using 4-(4-nitrobenzyl) pyridine spray for detection.) After the mixture had cooled to room temperature, it was filtered through Celite. The solids were washed with dichloroethane, and the combined filtrates were evaporated to dryness in vacuo. The residue (16.5 g) was a mixture of three nucleosides which were separated by flash chromatography on 150 g of silica gel (230–400 mesh) using 2:1 cyclohexane-ethyl acetate as the eluting solvent. By combining pure fractions, the desired compound was obtained as a glass 3.64 g (19.7%) which was chromatographically homogeneous but would not crystallize. A second column run on impure fractions gave 2.21 g (11.9%) more pure product for a total yield of 31.6%.

EXAMPLE II

2-Bromo-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-9H-purin-6-amine

(compound 3b)

A solution of the example I product (5.84 g, 10.5 mmol) in 400 mL of ethanolic ammonia (saturated at 0° C.) was sealed in a glass-lined stainless steel bomb and left at room temperature for 3 days. The solution was evaporated to dryness and evaporated with ethanol to remove ammonia. The residue, containing the desired product and 5'-benzoyl compound, was dissolved in 440 mL of acetonitrile and 120 mL of water. Lithium hydroxide monohydrate (881 mg, 21 mmol) was added, and the solution was stirred for 16 h at room temperature. Thin-layer chromatography (5:1 $CHCL_3$—MeOH) indicated complete reaction. The chilled solution was carefully neutralized with glacial acetic acid and evaporated to dryness. The white solid residue was recrystallized from water. The product was dried in vacuo at room temperature at 100° C. for 2 h: 2.15 g (59.2%); Mp 209–210° C.

EXAMPLE III

2-Chloro-9-(2-deoxy-2-fluoro-B-D-arabinofuranosyl)-2,0 9H-purin-6-amine

(compound 3d)

A solution of the compound 3c [see J. Med. Chem. 29:2389 (1986)] (5.1 g, 10.9 mmol) in ethanol saturated (0° C.) with anhydrous ammonia (100 mL) was placed in a glass-lined stainless steel bomb and left at room temperature for three days. Thin layer chromatography (2:1 cyclohexane-ethyl acetate and 5:1 $CHCl_3$—MeOH) indicated the absence of starting material. However, two major products were present: the desired compound and its 5'-benzoyl analog. The solution was evaporated to dryness and co-evaporated with acetonitrile. The residue was dissolved in acetonitrile (100 mL) and diluted with water (60 mL) before the addition of lithium hydroxide monohydrate (915 mg, 21.8 mmol). The solution was stirred at room temperature for 3 h, at which time thin layer chromatography (5:1 $CHCl_3$—MeOH) indicated the reaction had gone to completion. The solution was cooled, neutralized with acetic acid, and evaporated to dryness. Three recrystallizations from water gave the pure compound: 1.4 g (42.3%); Mp 225°–226° C.

EXAMPLE IV

2-Fluoro-9-(3-O-acetyl-5-O-benzoyl-2-deoxy-2-fluoro-β-arabinofuranosyl)-9H-purin -6-amine

(compound 3f)

Diamino compound 3e [see J. Med. Chem. 29:2389 (1986)] (700 mg, 1.63 mmol) was dissolved in 3:2 HF-pyridine (15 mL) at –25° C. and treated with tert-butylnitrite (271 μL, 2.28 mmol). After 1 h at –20° C., the reaction was incomplete as indicated by thin layer chromatography. Additional tert-butylnitrite (70 μL, 0.59 mmol) was added, and the reaction was held at –20° C. for an additional 2 h. The cold reaction solution was added dropwise to saturated aqueous $NaHCO_3$ (1 L) containing ice. The foaming mixture was stirred vigorously for 20 min, then diluted with $CHCl_3$ (300 mL). The solution was allowed to layer, and the layers were separated, and the aqueous layer was extracted with additional $CHCl_3$ (2×175 mL). The combined organic extracts were washed with water (3×175 mL), dried (over $MgSO_4$), and evaporated to dryness. The resulting residue, in $CHCl_3$, was applied to a flash column containing 50 g of silica gel (230–400 mesh) with $CHCl_3$ as eluant. Fractions were combined to give essentially pure product (500 mg, 70%). Crystallization of a small sample from EtOH gave pure product: Mp 208°–209° C.

EXAMPLE V

2-Fluoro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) -9H-purin-6-amine

(compound 3g)

A suspension of the example IV product (430 mg, 0.99 mmol) in 1:1 MeCN—H_2O (40 mL) was treated in one portion with solid lithium hydroxide monohydrate (125 mg, 2.97 mmol). The reaction became a clear solution after being stirred at room temperature for 20 min. A 3 h thin layer chromatography aliquot showed the deblocking to be complete. Glacial acetic acid (57 μL) was added, and the solution was evaporated until a white solid was deposited. After being chilled, the solid was collected, washed with cold water, and dried in vacuo at room temperature to give a crude solid (252 mg). This solid was dissolved in 40 mL of water and applied to a water-equilibrated SM-4 Bio-Bead column (1.5×32 cm). After initial elution with water, the product was eluted with a step-wise gradient, 5%→20% EtOH in water. The residue from the combined evaporated column fractions was crystallized from 25 mL of boiling water with charcoal treatment, and dried in vacuo at 56° C. for 16 h to yield a pure product: 178 mg (59%); Mp 207–209° C.

EXAMPLE VI

2-Fluoro-9-(5-O-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-9H-purin-6-amine

(compound 3h)

A suspension of the example IV product (312 mg, 0.72 mmol) in MeOH (25 mL) at 10° C. was treated with solid

$NaHCO_3$ (181 mg, 2.16 mmol). After being stirred at room temperature for 2.5 h, the reaction was quenched by the addition of glacial acetic acid (170 μL) and evaporated to dryness. This residue in hot EtOH was applied to two silica gel thick plates (Analtech, GF, 2000 μm) and subsequently developed in 9:1 $CHCl_3$—MeOH. The product was extracted with hot EtOH and evaporated to dryness to give essentially pure product: 208 mg (74%).

EXAMPLE VII

2-Fluoro-9-(5-O-benzoyl-2,3-dideoxy-2-fluoro-8-β-arabinofuranosyl)-9H-purin-6-amine

(compound 4a)

191 mg (0.49 mmol) of the compound made in accordance with example VI was dissolved in dry acetonitrile (20 mL) at 45° C., and then treated with 1,1'-thiocarbonyldiimidazole (339 mg, 1.7 mmol). The resulting cloudy yellow solution was stirred under N_2 at 45° C. for 24 h at which time thin layer chromatography analysis (EtOAc) showed one major product. The reaction was evaporated to dryness, and the residue was dissolved in dry toluene (15 mL). Treatment with AIBN (13.7 mg, 0.08 mmol) and tri-n-butyltin hydride (1.3 mL, 4.7 mmol) produced a yellow mixture that was placed directly in a 120° C. bath. A clear solution was observed after a 5 min reflux, and at 1 h the reaction was complete as indicated by thin layer chromatography. The solvent was then removed in vacuo, and the resulting syrup was coevaporated once with EtOH. Trituration of this residue with petroleum ether (50 mL) produced a white solid that was collected and washed with fresh solvent to give 214 mg of crude solid. This material in hot EtOH was applied to two Analtech (GF, 2000 μm) layer plates. After three developments in 9:1 $CHCl_3$—MeOH, the product band was extracted with boiling EtOH. The residue from evaporation of the combined extracts was crystallized from boiling EtOH to yield sufficiently pure product 160 mg (87%); Mp 215°–217° C. Without any further purification, this material was used in the deprotection step of example VIII.

Example VIII

2-Fluoro-9-(2-3-dideoxy-2-fluoro-β-D-arabinofuranosyl)-9H-purin-6-amine

(compound 4b)

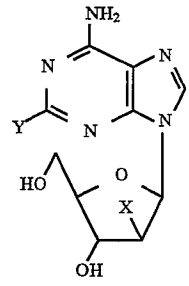
A suspension of the example VII compound (135 mg, 0.36 mmol) in 3:1 MeCN-H_2O was treated in one portion at room temperature with solid $LiOH \cdot H_2O$ (38 mg, 0.9 mmol). The stirred mixture became a clear solution after ½h. At 7 h an aliquot examined by TLC (5:1 $CHCl_3$—MeOH) showed the absence of the example VII compound. Glacial acetic acid (35 μL) was added, and the reaction was evaporated to dryness. This residue in hot acetonitrile was applied to one silica gel thick plate (Analtech, GF, 2000 μm). After the plate was developed three times in 5:1 $CHCl_3$—MeOH, the product band was extracted with boiling MeCN. Evaporation of this extract gave slightly impure material that was chromatographed as above on three prep plates (Analtech, GF, 1000 pm). The resulting residue was crystallized from boiling H_2O (25 mL) containing EtOH (0.5 mL). After being chilled, the white solid was collected, washed with cold H_2O and dried in vacuo at 56° C. for 16 h to give pure product, 71 mg (73%); Mp 249°–250° C.

In contrast to the previously reported 2'-substituted 9-β-D-arabinofuranosyl-2-haloadenines, the 2'-fluoro compounds were quite cytotoxic to three human cell lines, H.Ep.-2, CCRF-CEM, and K562, and the murine lukemia line, L1210. They, in fact, are significantly more cytotoxic than the corresponding 9-β-D-arabinofuranosyl-2-haloadenines, resembling more closely the 2'-deoxy-2-halodenosines (see Table 1).

Obviously, to be useful as anticancer agents, the nucleosides of the present invention must show the ability to kill cells in vitro. The results in Table 1, indicating the concentration required to inhibit cell proliferation to 50% of untreated controls, show that these nucleosides can, at reasonable concentrations, kill cells. One cell line (L1210) is a murine lukemia, whereas the other three are human neoplasms. Based on many years of experience, we believe that compounds that do not require activation by the liver must have an IC_{50} of about 1–10 μM or less to show useful activity in the in vivo animal models—and in man. Many people today emphasize the importance of toxicity to human cell lines.

TABLE I

Cytotoxicity [as IC_{50} (μM)] of 2-Haloadenine Nucleosides



Compound	H.Ep.-2	L1210	CCRF-CEM	K562
when Y = F				
X = OH	9	3	0.4	0.15
X = H	0.2	0.9	0.2	
X = F	0.34	0.38	0.14	0.3
when Y = Cl				
X = OH	3	<3	10	
X = H	0.03	0.07	0.003	
X = F	0.012	0.23	0.05	0.003
when Y = Br				
X = OH	4	3		
X = H	0.02	0.9	0.02	
X = F	0.22	0.26	0.02	0.05

The data in Table I (given in μM amounts) clearly establishes the ability of the compounds according to the present invention to kill neoplastic cells.

Subsequently, the phosphorolysis of these compounds were compared by *E. coli* purine nucleoside phosphorylase. The arabino and 2'-deoxyribonucleosides are rapidly cleaved by this enzyme, whereas the arabino nucleosides substituted at 2' by Cl, N_3, or NH_2 are almost completely resistant. The 2'-fluoro compounds are less resistant to cleavage, being cleaved at roughly one-third the rate of the arabino and 2'-deoxynucleosides. This reduction in cleavage rate may be acceptable for pharmaceutical purposes as phosphorylation in mammalian cells is quite rapid.

More specifically, an enzyme reaction mixture consisting of 0.5 mM nucleoside substrate, 50 mM pH 8.0 phosphate buffer and purine nucleoside phosphorylase in a final vol-

ume of 1.0 mL was allowed to incubate for 30, 60, 120, 180 and 240 minutes, and the amounts of nucleoside and substrate remaining were determined by HPLC. The results of this experiment are tabulated in the following Table II.

TABLE II

Phosphorolysis of Nucleosides

Compound	% Cleavage
3b	45
3d	39
3g	10
2-fluoro-9-β-D-arabinofuranosyladenine	99
2-chloro-2'-deoxyadenosine	>99
2-fluoro-2'-deoxyadenosine	>99

A recent report from our laboratory [see Cancer Research 51:2386 (May 1st 1991) which is incorporated in toto herein] indicates that the compound 3d of the present invention inhibits DNA synthesis due to the inhibition of ribonucleotide reductase activity and inhibition of chain elongation by DNA polymerase α. These inhibitions of the ribonucleotide reductase and DNA polymerase α enzymes by compound 3d were important to the development of the cancerous K562 cells. Although this finding is similar to observations with 9-β-D-arabinofuranosyl-2-fluoroadenine and 2-chloro-2'-deoxyadenosine, the degree of inhibition of these enzymes by the 5'-triphosphate of these nucleoside analogues is quite different. The inhibition of ribonucleoside reductase by 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine 5'triphosphate was the same as that seen with 2-chloro-2'-deoxyadenosine 5'-triphosphate, and the inhibition of DNA polymerase α by 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) adenine 5' triphosphate was similar to that seen with 9-13-D-arabinofuranosyl-2-fluoroadenine 5'triphosphate. In contrast, 9-β-D-arabinofuranosyl-2-fluoroadenine 5'triphosphate was a much less potent inhibitor of ribonucleotide reductase than either 2-chloro-2'-deoxyadenosine 5'-triphosphate or 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine 5'triphosphate, and although all of the 2'-deoxyadenosine nucleotide analogues inhibit the incorporation of 2'-deoxyadenosine 5'triphosphate by DNA polymerase α into the DNA and were more efficient substrates for the polymerase, the incorporation of 2-chloro-2'-deoxyadenosine 5'monophosphate into DNA by DNA polymerase α did not inhibit the further elongation of the DNA chain to the degree that was seen with the incorporation of either 9-β-D-arabinofuranosyl-2-fluoroadenine 5'monophosphate or 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) adenine 5'monophosphate. These results indicated that the 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine (compound 3d) incorporates properties of both 9-β-D-arabinofuranosyl-2-fluoroadenine and 2-chloro-2'-deoxyadenosine into one compound. Furthermore, in the cell the inhibition of DNA polymerase α by these nucleoside analogues is a function of the ratio of [analogue nucleoside triphosphate] to [2'-deoxyadenosine 5'triphosphate]. Because 9-β-D-arabinofuranosyl-2-fluoroadenine 5'triphosphate inhibits ribonucleotide reductase at a 10-fold higher concentration than that required with 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) adenine 5' triphosphate, the 2° -deoxyadenosine 5'triphosphate pool should be lower and the inhibition of DNA polymerase α should be greater, in cells treated with 2-chloro-9-(2-deoxy-2-fluoro-8-D-arabinofuranosyl) adenine than in cells treated with equimolar concentrations of 9-β-D-arabinofuranosyl-2-fluoroadenine, assuming equal conversion to the triphosphate. These metabolic features may contribute to the potent inhibition of K562 cell growth with compound 3d [2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) adenine] of the present invention. In addition, the solubility problems associated with the administration of 9-β-D-arabinofuranosyl-2-fluoroadenine should not occur with this compound because of its greater solubility and high potency.

The reason that the 2'-fluorine atom disrupts chain extension is not obvious because the 2'-carbon is not involved in the reaction and a fluorine has an atomic radius slightly larger than a hydrogen atom. Steric hindrance would be expected to be less than is believed to be in the case of arabinofuranosyl nucleotides. It is possible that the electron-withdrawing properties of fluorine may affect the reactivity of the 3'-hydroxyl and/or the three dimensional structure of the DNA chain such that extension of a DNA chain terminated with a 2'-fluoronucleoside by the polymerase is inhibited.

Studies with the P388 leukemia cell line in mice (see Table III) indicate that the most effective compound of the present invention is the compound according to general formula 3d, that is the 2-chloro-2'-fluoro substituted nucleoside. This, coupled with the lower toxicity of the cleavage product, 2-chloroadenine, relative to 2-fluoroadenine, make this compound a preferred compound of the present invention. The following Table III provides a summary of the in vivo activity of the 9-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)-2-haloadenines vs P388 leukemia cell line in which CD2F1 mice were implanted ip with 10^6 P388 leukemia cells on day 0 in accordance with the protocol of Waud et al [*Cancer Res.* 50:3232 (1990)].

TABLE III

Compound	Optimal IP Dose	Schedule	Median % ILS (dying mice only)	Log_{10} change	Tumor-free Survivors
3d	100	qd 1–5	+38	−0.3	0/5
	200	qd 1–5	+59	−1.6	0/3
	20	q 3 h × 8 (Days 1, 5, 9)	+220	−6.6	1/6
	25	q 3 h × 8 (Days 1, 5, 9)	+118	−2.8	0/5
3g	100	qd 1–5	+63	−1.8	0/3
	25	q 3 h × 8 (Days 1, 5, 9)	+81	−0.1	0/6
3b	30	q 3 h × 8 (Days 1, 5, 9)	+100	−1.0	0/6
	50	q 3 h × 8 (Days 1, 5, 9)	+41	+1.6	0/6
	200	qd 1–5	+33	+0.1	0/6

In the above table, the optimal dose refers to mg/kg/dose ($\leqq LD_{50}$); ILS refers to the increase in life span; and the log change refers to the change in viable tumor cell population at the end of therapy compared to that at the start of therapy, based on the median day of death among the animals that died. The data in this table is presented in accordance with the National Cancer Institute activity criteria for drug testing in which an ILS of 20–74% is considered moderate activity, and an ILS of 75% or more is considered good activity.

In addition to the above, the 2,3-dideoxynucleoside depicted as compound 4b showed slight activity against HIV (strain IIIB) in either CEM or MT cell lines in culture.

In a similar test, compound 3d was administered orally and evaluated for antitumor activity against ip P388 leuke-

mia cells. As the data in Table III indicates, the optimal regimen for the compound, administered ip, is in divided doses five on days 1, 5, and 9, a similar schedule was selected for the oral administration of this compound. In this set of experiments, an oral dosage of 67 mg/kg/dose, given q 6 h×4 on days 1, 5, and 9, effected a reduction in tumor burden of 1.7 $\log_{10}$ units, a figure which is approximately 2.5 $\log_{10}$ units less that that obtained in studies using ip drug administration.

The compounds according to the present invention are useful for their cytotoxic effects, and thus are useful as anticancer compounds in the treatment of cancerous cells in mammals when administered in an amount sufficient to bring about their cytotoxic effect to the desired cancerous cell. The compounds may be administered in a wide range of regimens ranging from about 10 mg to about 1000 mg per day. These regimens may be designed to give the compounds as a single dose or as multiple doses over extended periods of time, and the regimen may be adapted to provide the optimum therapeutic response. For example, several divided doses may be administered daily, or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. The compounds according to the present invention may be administered in the form of the free purine nucleoside or as a nontoxic pharmaceutically acceptable salt thereof, and may be administered either alone or in combination with one or more compounds of the present invention or with additional pharmaceutically active compounds.

The active compounds of the present invention may be administered parenterally, e.g. by subcutaneous, intramuscular, or intravenous injection. Solutions or suspensions of the active compound as a pharmaceutically acceptable salt can be prepared in water or saline containing the appropriate buffers and additives for administration. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability is provided; it must be stable under the conditions of manufacture and storage, and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier may be a solvent or dispersion medium containing, for example, water, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures thereof. Compositions suitable for intramuscular or subcutaneous injection may also contain minor amounts of salts, acids, and bases to adjust tonicity and buffer the pH.

The compounds according to the present invention may also be suitable for oral administration, for example with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard or soft shell gelatin capsules, or compressed into tablets. For oral therapeutic administration, the compounds may be incorporated with excipients commonly used in the formulation of oral pharmaceutical preparations as, for example, sweetening agents, and preservatives.

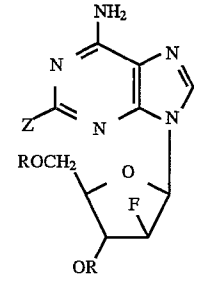
In addition, the compounds of the present invention may be formulated in accordance with acceptable pharmaceutical formulation techniques for administration by other routes such as administration within topical ointments, creams or salves, as suppositories, or as lozenges.

Thus, while we have illustrated and described the preferred embodiment of our invention, it is to be understood that this invention is capable of variation and modification and we therefore do not wish to be limited to the precise terms set forth, but desire to avail ourselves of such changes and alterations which may be made for adapting the invention to various usages and conditions. Among such variations and modifications are, for example, and without limitation, the use of pharmaceutically acceptable salts of the disclosed purine nucleosides which may be designed for providing the purine nucleosides according to the present invention to a cell susceptible to cytotoxicity, to any minor substitution on the active nucleoside according to the present invention which results in no untoward effects upon the activity of the modified nucleoside from that of the depicted purine nucleosides or which results in the same or substantially the same activity as that found in the purine nucleosides depicted in accordance with the preceding disclosure; changes in formulation made due to the specific route of administration of the nucleosides according to the present invention; and changes made to the nucleoside molecule or composition formulation because of a specific salt form of the nucleoside according to the present invention. Accordingly, such changes, alterations and modifications are properly intended to be within the full range of equivalents, and therefore within the purview of the following claims.

Having thus described our invention and the manner and process of making and using the same in such full, clear, concise, and exact terms so as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same,

We claim:

1. A method for bringing about a cytotoxic effect in a mammalian cancerous cell which comprises contacting said cancerous cell with an effective amount of a cytotoxic compound having the formula



wherein R, each which may be the same or different, is hydrogen or a protecting group; wherein Z is a selected from the group consisting of F, Cl, and Br; and pharmaceutically acceptable salts thereof.

2. A method according to claim **1** which comprises contacting said cancerous cell with a compound wherein R is a protecting group.

3. A method according to claim **1** which comprises contacting said cancerous cell with compound wherein R is hydrogen.

4. A method according to claim **1** which comprises contacting said cancerous cell with a compound wherein Z is Cl.

5. A method according to claim **1** which comprises contacting said cancerous cell with a compound wherein the compound is 2-Chloro-9-(2-deoxy-2- fluoro-β-D-arabinofuranosyl)-9H-purin-6-amine.

6. A method for inhibiting ribonucleotide reductase and DNA polymerase α in a mammalian cell which comprises contacting said mammalian cell with of a cytotoxic compound having the formula

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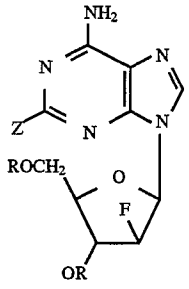

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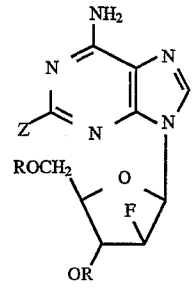

wherein R, each which may be the same or different, is hydrogen or a protecting group; wherein Z is a halogen selected from the group consisting of F, Cl, and Br; and pharmaceutically acceptable salts thereof.

7. A method according to claim **6** wherein R of said compound is a protecting group.

8. A method according to claim **6** wherein R of said a compound is hydrogen.

9. A method for inhibiting ribonucleotide reductase and DNA polymerase α in a mammalian cell which comprises contacting said mammalian cell with of a cytotoxic compound having the formula

wherein R, each which may be the same or different, is hydrogen or a protecting group; wherein Z is a halogen selected from the group consisting of F, Cl, and Br; and the pharmaceutically acceptable salts thereof.

10. A method according to claim **9** wherein R of said compound is a protecting group.

11. A method according to claim **9** wherein R of said compound is hydrogen.

12. A method according claim **9** wherein said compound is 2- Chloro-9-(2-deoxy-2-fluoro-β-D arabinofuranosyl)-9H-purin-6-amine.

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