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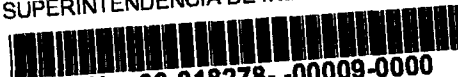
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AMINOQUINOLINA Y SU USO COMO LIGANDO A₃ DE
DE ADENOSINA**
Solicitante : SANOFI-AVENTIS
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Fecha solicitud: 23 de febrero de 2006

1. Yo, Emilio FERRERO, obrando como apoderado del solicitante en el asunto antes identificado, me refiero al Oficio No. **5706** notificado por fijación en lista del 15 de mayo de 2009 y atentamente presento junto con este escrito los siguientes documentos:

1.1 J. MED. CHEM., vol. 27, No. 2. 1984, pp 232-235, title: IMPROVED SYNTHESIS AND ANTITUMOR EVALUATION OF 5,8-DIDEAZAISOFOLIC ACID AND CLOSELY RELATED ANALOGUES

1.2 Bioinorganic & Medicinal Chemistry, title: INHIBITORS OF THE TISSUE FACTOR/FACTOR VIIa-INDUCED COAGULATION: SYNTHESIS AND IN VITRO EVALUATION OF NOVEL SPECIFIC 2-ARYL SUBSTITUTED 4H-3,1-BENZOAXIN-4-ONES. Elsevier Science LTD, GB, vol. 8, no. 8, 2000, pp 2095-2103.

1.3 DATABASE BEILSTEIN INSTITUTE FOR ORGANIC CHEMISTRY, FRANKFURT-MAIN, DE; XP002299336 & CARBONI: GAZZ. CHIM. ITAL., vol. 83, 1953, pp 637-640

and GM 29291 from the National Institute of General Medical Science. We are grateful to Dr. G. R. Stark of Stanford University for the kind gift of the PALA-resistant hamster cells. We thank Carla K. Robertson for excellent technical assistance.

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87862-96-2; 10, 34234-57-6; 11, 87862-97-3; 15, 87862-98-4; 18, 34017-27-1; 19, 76646-28-1; 20 [(R)-sulfoxide], 87862-99-5; 20 [(S)-sulfoxide], 87863-00-1; 21, 87863-02-3; 22, 87863-03-4; 23, 62305-89-9; 24, 20263-06-3; 1,1'-carbonyldiimidazole, 530-62-1; DBU, 6674-22-2; S-methylcysteine, 1187-84-4; benzyl chloroformate, 501-53-1; O-(mesitylenesulfonyl)hydroxylamine, 36016-40-7; dihydroorotase, 9024-93-5.

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P: 232-235

(4)

Improved Synthesis and Antitumor Evaluation of 5,8-Dideazaisofolic Acid and Closely Related Analogues¹

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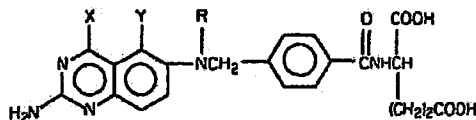
Department of Pharmaceutical Sciences, College of Pharmacy, Medical University of South Carolina, Charleston, South Carolina 29425, and Department of Pharmacology, Northwestern University Medical School, Chicago, Illinois 60611. Received May 13, 1983

A new synthetic route to 5,8-dideazaisofolic acid (IAHQ) is described which precludes the possibility of contamination due to its 4-amino counterpart 5,8-dideazaisoaminopterin. Substitution of D-glutamic acid in this synthetic scheme gave D-IAHQ. The 9-formyl, 9-methyl, 6-methyl, and 5,9-dimethyl modifications of IAHQ were also prepared. These compounds, together with several structurally related or isomeric analogues, were studied for inhibitory effects upon the growth of four human gastrointestinal adenocarcinoma cell lines in vitro. In general, the compounds having a normal folate configuration at positions 9 and 10 are more active than their reversed bridge isomers. The lack of antitumor activity of D-IAHQ provides indirect evidence concerning the mechanism of action of IAHQ.

5,8-Dideazaisofolic acid (IAHQ, 1a) was first described in 1975 as part of an ongoing synthetic program concerned with quinazoline analogues of folic acid.² Earlier studies had shown that its isomer 5,8-dideazafolic acid (AHQ), which has a normal folate configuration at positions 9 and 10, and the 10-CH₃ analogue (10-CH₃-AHQ) were effective inhibitors of thymidylate synthase from several different sources.³⁻⁵ In vitro, 10-CH₃-AHQ inhibited the growth of mouse neuroblastoma cells, although not nearly as effectively as certain structurally related 2,4-diaminoquinazolines, which were shown to be potent inhibitors of dihydrofolate reductase (DHFR).⁶

Preliminary studies with IAHQ showed that at low doses using a single dose regimen on day 1 after tumor inoculation, the compound was ineffective against L1210 leukemia in mice.² IAHQ was a moderately effective inhibitor of rat liver DHFR, being some 10-fold less inhibitory than AHQ.⁶ Subsequently, a large series of quinazoline analogues of folic acid was evaluated as inhibitors of thymidylate synthase from *Lactobacillus casei* and from L1210 leukemia cells.⁷ IAHQ was found to be an effective inhibitor of the L1210 enzyme; however, AHQ and 10-CH₃-AHQ were significantly more inhibitory toward this enzyme. More recent studies revealed that IAHQ was an effective inhibitor of the growth of human colon adenocarcinoma cells (HCT-8) in vitro, thus generating interest in this compound for potential use in the treatment of methotrexate (MTX) unresponsive tumors.⁸ Significant activity against colon tumor 38 in mice was also demonstrated, and when a regimen of 85 mg/kg on days 2 and 10 following tumor inoculation was used, there were 6 of 20 tumor-free animals after 90 days.⁸ MTX was not effective in this model.⁹ It was also found that IAHQ protected newborn hamsters from mortality due to transplantable human osteosarcoma cells, whereas MTX had no effect against this xenograph at the maximally tolerated dose.¹⁰

Chemistry. This paper describes a new unequivocal synthetic route to 1a, which precludes the possibility of



1a, X = OH; Y = R = H 1d, X = OH; Y = H; R = CHO
b, X = NH₂; Y = R = H e, X = OH; Y = H; R = CH₃
c, X = OH; Y = CH₃; R = H f, X = OH; Y = CH₃; R = CH₃

trace contamination due to its 4-amino counterpart, 5,8-dideazaisoaminopterin (1b). The latter compound was shown to be a reasonably potent inhibitor of DHFR from rat liver.⁶ Numerous earlier synthetic efforts employed diethyl esters of glutamate, which were removed in the final step under hydrolysis conditions using sodium hydroxide. During this study, the carboxyl groups of glutamic acid were protected by the use of *tert*-butyl esters, which are removed readily at ambient temperature under mildly acidic conditions. This modification yields final

(1) This paper has been presented in part. See: "Abstracts of Papers", 183rd National Meeting of the American Chemical Society, Las Vegas, NV, Mar 28-Apr 2, 1982; American Chemical Society: Washington, DC, 1982; Abstr MEDI 69.

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Scheme I. Improved Synthetic Route to IAHQ and Analogues

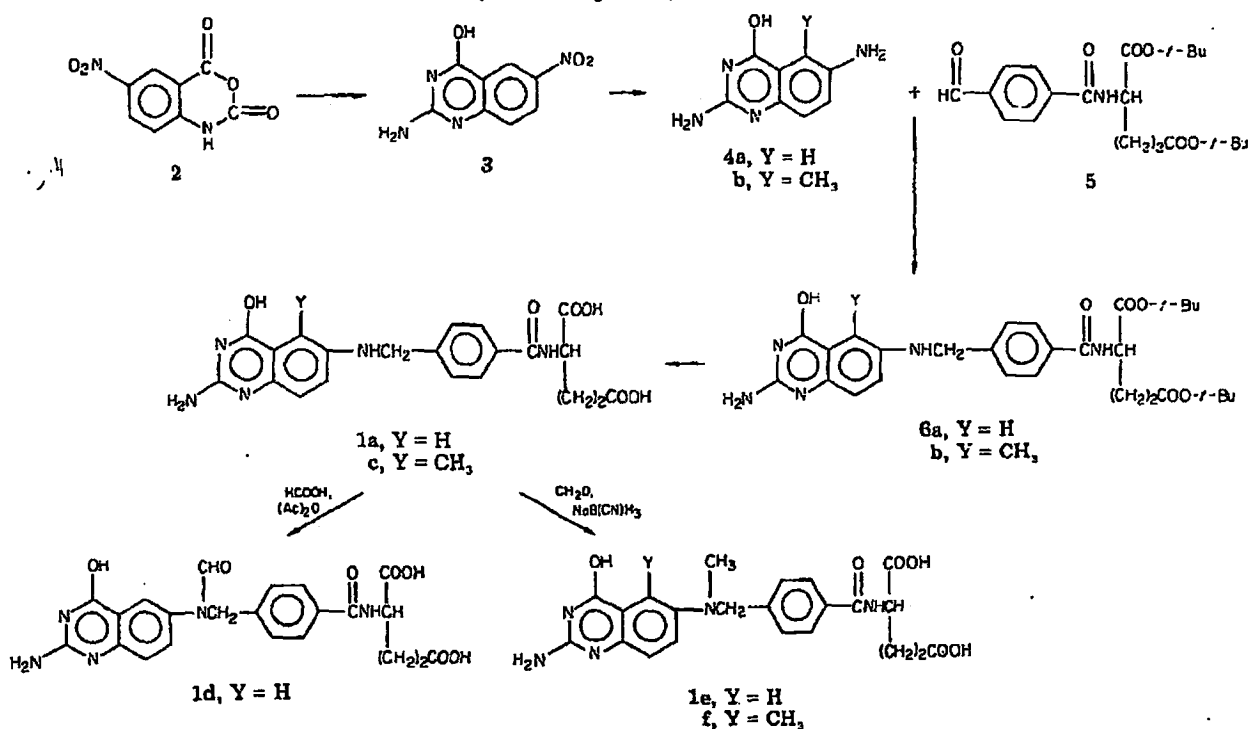


Table I. Inhibition of Growth of Human Gastrointestinal Adenocarcinoma Cells in Vitro by Selected Quinazoline Analogues of Folic Acid

cell	growth inhibition: IC ₅₀ , μM										MTX
	IAHQ	D-IAHQ	AHQ	9-CHO-IAHQ	10-CHO-AHQ	9-CH ₃ -IAHQ	10-CH ₃ -AHQ	5-CH ₃ -IAHQ	5,9-(CH ₃) ₂ -IAHQ	1b	
HuTu80	4.6	>300	0.7	95	46	4.8	1.9	4.0	3.6	0.15	0.009
HT29	1.7	>300	0.6	100	38	3.2	1.2	1.1	2.7	0.20	0.018
SW480	4.5	>300	1.1	150	84	5.0	1.6	10	4.8	0.20	0.015
WIDR	4.2	>300	0.6	195	41	6.3	1.3	4.0	4.8	0.20	0.026

products having a higher degree of purity and rules out the possibility of racemization occurring during the deprotection step. The steps employed are outlined in Scheme I. The key intermediate, 2,6-diamino-4-hydroxyquinazoline (4), was prepared in two steps from the commercially available 5-nitroisatoic anhydride [6-nitro-2H-3,1-benzoxazine-2,4(1H)-dione (2)]. Cyclization with guanidine carbonate, followed by reduction of the resulting nitroquinazoline using catalytic hydrogenation, yielded 4. Compound 4 had formerly been derived by the acid hydrolysis of 2,4,6-triaminoquinazoline.² Di-*tert*-butyl L-glutamate was prepared by the acid-catalyzed reaction of L-glutamic acid with excess isobutylene under low pressure.¹¹ Subsequent coupling to 4-formylbenzoic acid using the mixed anhydride method gave di-*tert*-butyl *N*-(4-formylbenzoyl)-L-glutamate (5), which was obtained as a viscous oil. This was employed in succeeding transformations without further purification, since attempts to obtain 5 in solid form were unsuccessful. The reductive condensation of 4 with 5 in the presence of Raney nickel gave the di-*tert*-butyl ester of 1a, 6. After purification using silica gel chromatography, 6 was deprotected with anhydrous trifluoroacetic acid, affording 1a in good yield. Substitution of D-glutamic for the natural isomer in the aforementioned sequence gave the D isomer, D-IAHQ. The

reductive condensation of 2,6-diamino-4-hydroxy-5-methylquinazoline (4b) with 5 yielded the di-*tert*-butyl ester of the 5-methyl compound 6b. Treatment with anhydrous trifluoroacetic acid then gave 5-CH₃-IAHQ (1c), which was found to have a greater degree of purity than that prepared earlier from diethyl L-glutamate, as determined by reverse-phase high-performance liquid chromatography.²

The reaction of 1a with formic acid in the presence of acetic anhydride gave 9-CHO-IAHQ (1d) in respectable yield. The introduction of the 9-methyl group was accomplished according to the procedure employed for the methylation of folic acid, yielding 9-CH₃-IAHQ (1e).¹² Purification of this product required ion-exchange chromatography using DEAE-cellulose. The methylation of 1c was effected in an analogous manner, giving 5,9-(CH₃)₂-IAHQ (1f). Isomeric compounds having a normal folate configuration at positions 9 and 10 were prepared as described elsewhere,¹³ with the exception of 10-CH₃-IAHQ. This compound was more readily prepared by direct

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until H_2 uptake ceased. After the addition of charcoal, the catalyst was removed by filtration, and the filtrate was basified to pH 8 with NH_4OH . The precipitate was collected by filtration, washed with H_2O and then hexane, and dried in vacuo over P_2O_5 . The product was dissolved in 100 mL of $CHCl_3$ and washed with 2 × 100 mL of 10% $NaHSO_4$, 1 × 100 mL of H_2O , 2 × 100 mL of 5% citric acid, and 2 × 100 mL of H_2O and then dried over $MgSO_4$. The solvent was removed under vacuum, and the product was washed with hexane and then dried in vacuo over P_2O_5 . The product was purified by flash chromatography over Baker silica gel (40 μm), column 50 × 175 mm, using a stepwise gradient of $CHCl_3$ to $CHCl_3/MeOH$, 9:1: yield 5.5 g (52%); mp 145–147 °C; TLC ($CHCl_3$ - $MeOH$, 85:15, Whatman KC_{18} F RPTLC, $MeOH/H_2O$, 80:20). Anal. ($C_{22}H_{21}N_5O_6 \cdot 0.75H_2O$) C, H, N.

5,8-Dideazaaisofolic Acid (1a). A 5.5 g (9.8 mmol) sample of 6a was stirred in 80 mL of CF_3COOH for 1 h at ambient temperature. The product was precipitated by the addition of excess Et_2O and collected by centrifugation. The Et_2O - CF_3COOH supernatant was decanted, and the product was washed 4 times with Et_2O and then 2 times with H_2O . The product was suspended in H_2O , basified to pH 8.5 with NH_4OH , and then acidified to pH 4.0 with 0.5 N HCl to effect precipitation. The product was collected by filtration, washed with copious amounts of cold H_2O and then $(Me)_2CO$, and finally dried in vacuo at 100 °C to yield 3.5 g (81.5%); mp >195 °C dec; TLC (0.1 M $NaHPO_4$, pH 7.5, cellulose); HPLC; UV λ_{max} (0.1 M $NaHPO_4$, pH 7.0), 235 nm (ϵ 33.8×10^3); NMR (CF_3COOD) δ 8.4–7.5 (m, 7, aromatic), 5.2–4.85 (m, 3, $NCH_2 + NCH$), 2.9–2.3 [m, 4, $(CH_2)_2$]. Anal. ($C_{21}H_{21}N_5O_6 \cdot 0.5H_2O$) C, H, N.

D-5,8-Dideazaaisofolic Acid (D-IAHQ). Di-*tert*-butyl *N*-(4-formylbenzoyl)-D-glutamate was prepared in a manner identical with the preparation of its L isomer, 5. The reductive condensation with 4, followed by deblocking with CF_3COOH , then gave D-IAHQ. NMR, TLC, HPLC, and mp were identical with 1a. Anal. ($C_{21}H_{21}N_5O_6 \cdot CF_3COOH \cdot 0.5H_2O$) C, H, N.

Di-*tert*-butyl 6-Methyl-5,8-dideazaaisofolate (6b). A mixture of 9.57 g (24.5 mmol) of 5 and 4.65 g (24.5 mmol) of 2,6-diamino-4-hydroxy-5-methylquinazoline (4b)² in 200 mL of 70% HOAc was hydrogenated in the presence of Raney Ni (~500 mg) until H_2 uptake ceased. After addition of charcoal, the catalyst was removed by filtration, and the filtrate was basified to pH 8.5 with NH_4OH . The precipitate was collected by centrifugation, washed with 4 × 200 mL of H_2O , and then lyophilized. After drying over P_2O_5 , there was obtained 7.74 g (54%); mp 139–141 °C; TLC ($DMF-H_2O$, 1:2, cellulose). Anal. ($C_{20}H_{23}N_5O_6 \cdot 2.25H_2O$) C, H, N.

6-Methyl-5,8-dideazaaisofolic Acid (1c). A 5.6 g (9.2 mmol) sample of 6b was treated with 80 mL of CF_3COOH for 1 h, and the product was obtained as described for 1a. There was obtained 2.45 g (56%); mp 253–254 °C (lit.² mp 220–225 °C); TLC ($DMF-H_2O$, 1:2, cellulose); HPLC; UV λ_{max} (0.1 M $NaHPO_4$, pH 7.0) 240 nm (ϵ 40.0×10^3); NMR (CF_3COOD) δ 8.08–7.42 (m, 6, aromatic), 5.17–4.80 (m, 3, $NCH_2 + NCH$), 2.98–2.30 [m, 7, $CCH_3 + (CH_2)_2$]. Anal. ($C_{22}H_{23}N_5O_6 \cdot 1.5H_2O$) C, H, N.

9-Formyl-5,8-dideazaaisofolic Acid (1d). A 0.224 g (0.5 mmol) sample of 1a was dissolved in a solution of 10 mL of $HCOOH$ (97%), and 5 mL of $(Ac)_2O$ was added with stirring at room temperature. After 1 h, the product was precipitated by the addition of excess Et_2O , followed by chilling in an ice bath for 20 min. The precipitate was collected by filtration and washed with H_2O , then $(Me)_2CO$, and finally Et_2O . The product was dried at 100 °C in vacuo, yielding 0.182 g (68.5%); mp >210 °C dec; TLC (0.1 M $NaHPO_4$, pH 7.5, cellulose); HPLC; UV λ_{max} (0.1 M $NaHPO_4$, pH 7.0) 230 nm (ϵ 44.5×10^3); NMR (CF_3COOD) δ 8.3 (s, 1, CHO), 8.1–7.15 (m, 7, aromatic), 5.50–4.80 (m, 3, $NCH_2 + NCH$), 2.93–2.23 [m, 4, $(CH_2)_2$]. Anal. ($C_{22}H_{21}N_5O_7 \cdot 2H_2O$) C, H, N.

9-Methyl-5,8-dideazaaisofolic Acid (1e). A 1.0 g (2.2 mmol) sample of 1a was dissolved in 55 mL of 0.1 N NaOH and then neutralized to pH 6.4 with 1 N HCl. To this was added 1.0 g (12.7 mmol) of 38% H_2CO , followed by 0.20 g (3.18 mmol) of $NaB(C_6H_5)_4$. The pH of the reaction mixture was maintained at 6.5 for 45 min by the occasional addition of 1 N HCl, and the reaction was continued for 20 h. The solution was then adjusted to pH 11 with 1 N NaOH, with stirring, and held at that point for approximately 10 min to allow HCN gas to escape. The solution

was acidified to pH 4 with 1 N HCl to precipitate the product, which was collected by filtration, washed with H_2O and then Et_2O , and dried at 100 °C in vacuo: yield 1.01 g (95%); mp >235 °C dec. Purification was effected by anion-exchange chromatography using a 2 × 20 cm column containing Cellex D (Bio-Rad). A linear gradient of 0.02–0.35 M NH_4HCO_3 , pH 8.5, was used to elute the product. After lyophilization and neutralization to pH 4, the solid had mp >235 °C after vacuum drying at 100 °C: TLC (0.1 M $NaHPO_4$, pH 7.5, cellulose); HPLC; UV λ_{max} (0.1 M $NaHPO_4$, pH 7.0) 235 nm (ϵ 38.6×10^3); NMR (CF_3COOD) δ 8.54–7.48 (m, 7, aromatic), 5.13–4.83 (m, 3, $NCH_2 + NCH$), 3.6 (s, 3, NCH_3), 2.9–2.3 [m, 4, $(CH_2)_2$]. Anal. ($C_{22}H_{23}N_5O_6 \cdot 2.75H_2O$) C, H, N.

5,8-Dimethyl-5,8-dideazaaisofolic Acid (1f). A 0.88 g (1.83 mmol) sample of 1c was methylated and purified according to the procedures employed for 1e. Following anion-exchange chromatography, a 69.5% yield was obtained: mp 223–225 °C; TLC (0.1 M $NaHPO_4$, pH 7.5, cellulose); HPLC; UV λ_{max} (0.1 M $NaHPO_4$, pH 7.0) 235 nm (ϵ 39.2×10^3); NMR (CF_3COOD) δ 8.32–7.30 (m, 6, aromatic), 5.25–4.80 (m, 3, $NCH_2 + NCH$), 3.65 (s, 3, NCH_3), 2.95–2.20 [m, 7, $CCH_3 + (CH_2)_2$]. Anal. ($C_{23}H_{25}N_5O_6 \cdot 1.5H_2O$) C, H, N.

10-Methyl-5,8-dideazaaisofolic Acid (10- CH_3 -AHQ). A 0.70 g (1.5 mmol) sample of 5,8-dideazaaisofolic acid¹⁴ was methylated by the same method described for 1e: yield 0.59 g (84%); mp >220 °C dec (lit.¹⁴ mp 220–225 °C dec); TLC (0.1 M $NaHPO_4$, pH 7.5, cellulose); HPLC; UV λ_{max} (0.1 M $NaHPO_4$, pH 7.0) 230 nm (ϵ 44.5×10^3); NMR (CF_3COOD) δ 8.35–7.45 (m, 7, aromatic), 5.22–4.90 (m, 3, $NCH_2 + NCH$), 3.62 (s, 3, NCH_3), 2.9–2.3 [m, 4, $(CH_2)_2$].

Biological Evaluation. Determinations of the growth of tumor cells in the presence of drugs were conducted in CoStar (Cambridge, MA) 24-well plates. Experiments were begun by the addition of 1 mL of media containing $0.7-1 \times 10^5$ monodispersed growing cells to wells in which 0–50 μL of solution of the particular drug had been placed. Compounds were dissolved just prior to assay in 0.5 mL of 0.1 M NaOH and then diluted with 4.5 mL of phosphate buffer (pH 7.0). The cells were incubated at 37 °C in a humidified 8% CO_2 atmosphere for 72 to 96 h. The monolayers of cells were then removed by trypsinization and counted in suspension. Cell number was determined on a Coulter counter and maximally increased 10- to 15-fold over that inoculated.²⁰ IC_{50} values refer to that concentration of inhibitor necessary to inhibit cell growth by 50% compared to control cells grown under identical conditions, except that the inhibitor was omitted. Standard deviations for all values presented in Table I, based upon four determinations, were less than 5%. The establishment of the cell lines employed in this study has been described previously: HuTu90,²¹ HT29,²² SW480,²³ and WIDR.²⁴

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Registry No. 1a, 56239-21-5; 1b, 56239-22-6; 1c, 56277-35-1; 1d, 87539-56-8; 1e, 87614-69-5; 1f, 87597-82-8; 2, 4693-02-1; 3, 87597-83-9; 4a, 53745-23-6; 4b, 56239-17-9; 5, 87597-84-0; 6a, 87597-85-1; 6b, 87597-86-2; guanidine carbonate, 593-85-1; 4-formylbenzoic acid, 619-68-9; di-*tert*-butyl glutamate, 16874-06-9; di-*tert*-butyl *N*-(4-formylbenzoyl)-D-glutamate, 87597-87-3; D-5,8-dideazaaisofolic acid, 87597-88-4; 5,8-dideazaaisofolic acid, 5854-11-5; 10-methyl-5,8-dideazaaisofolic acid, 5854-12-6.

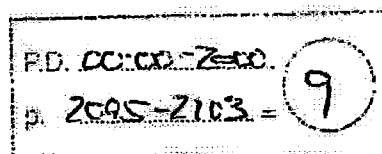
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Pergamon

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03

Inhibitors of the Tissue Factor/Factor VIIa-Induced Coagulation: Synthesis and In Vitro Evaluation of Novel Specific 2-Aryl Substituted 4*H*-3,1-Benzoxazin-4-ones

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Abstract—The synthesis of a series of novel 2-aryl substituted 4*H*-3,1-benzoxazin-4-ones and their evaluation as specific inhibitors of the Tissue Factor (TF)/Factor VIIa (FVIIa)-induced pathway of coagulation is reported. Inhibitory activities (IC₅₀ values) in the range 0.17 to >40 μM on the activation of Factor X (FX) by the TF/FVIIa complex were found for compounds having one or two electronegative substituents such as F, Cl and NO₂ in the 2-aryl substituent. Different substitutions both electron-attracting and donating groups were allowed in the 5, 6, 7 and 8 positions. Several of the compounds showed a selectivity ratio towards FX and thrombin of >50, thus being the first small molecules described as potential drugs for oral antithrombotic treatment without side effects such as bleeding which is observed especially with thrombin inhibitors. The best substituent pattern being the 2-aryl group substituted with: 2-F; 2,6-F₂; or 2-FX; 6-Cl; together with electronegative substitution in the 5, 6, 7, or 8 positions. 2-Heteroaryl substituents like thienyl and furanyl were of low activity while some 2-(2-chloro-3-pyridyl) derivatives had inhibitory activity <10 μM and a good selectivity. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Blood coagulation is a complex process involving various blood components or factors that eventually give rise to a fibrin clot. Activated Factor X (FXa) is required to convert prothrombin to thrombin which then converts fibrinogen to fibrin as a final stage in forming a fibrin clot.

There are two pathways that promote the activation of Factor X (FX). The 'intrinsic pathway' refers to those reactions leading to thrombin formation through utilisation of factors present only in blood. A series of protease-mediated activations generate Factor IXa which, in conjunction with Factor VIIIa, cleaves FX into FXa.

In the 'extrinsic pathway' Factor VIIa (FVIIa) and its cofactor, Tissue Factor (TF), exert the same proteolysis. TF is a membrane-bound protein and does not normally circulate in plasma. Upon vessel disruption, however, it is exposed and forms a complex with FVIIa to catalyse FX or Factor IX activation in the presence of Ca²⁺ and phospholipid (1). While the relative importance of the two coagulation pathways in hemostasis is unclear,

FVIIa and TF appear to be the principal initiators of blood coagulation *in vivo*.¹

It is often desirable to inhibit the coagulation cascade in a patient. Anticoagulants such as heparin, coumarin and its derivatives, indandione derivatives, low-molecular-weight thrombin or FXa inhibitors, or other agents may be used. Treatment with heparin and other anticoagulants may, however, have undesirable side effects, for example, bleedings. Inhibition at the initial stage of blood coagulation, i.e. of the FVIIa/TF activity, results in significantly less bleeding.² In addition, clinically available anticoagulants act throughout the body, rather than acting specifically at the site of injury where the coagulation cascade is active. Other known anticoagulants comprise thrombin and Factor Xa inhibitors derived from blood-sucking organisms. Antithrombins, hirudin, hirulog and hirugen are recombinant proteins or peptides derived from the leach *Hirudo medicinalis*, whereas the Factor Xa inhibitor antistatin and the recombinant derivative rTAP are tick-derived proteins. Inhibitors of platelet aggregation such as monoclonal antibodies or synthetic peptides, which interfere with the platelet receptor gpIIb/IIIa, are also effective as anticoagulants. These agents are, however, not suitable for oral administration. Specific protein inhibitors of the FVIIa/TF activity are the

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physiological inhibitor TFPI (Tissue Factor Pathway Inhibitor) and the inactivated FVII (FVIIa) which binds to TF and compete with FVIIa

There is still a need for improved compositions having anticoagulant activity and which can be administered orally or otherwise non-intravenously at relatively low doses without producing undesirable side effects. We found that some 2-arylsubstituted 4*H*-3,1-benzoxazin-4-ones showed specific inhibitory activity on FVIIa-TF in in vitro assays, and therefore seem to be potential drug candidates for blood coagulation regulation, suitable for oral administration and presumably without the above mentioned side effects. We report here the synthesis and SAR of some 2-arylsubstituted 4*H*-3,1-benzoxazin-4-ones in in vitro systems of TF/FVIIa-catalyzed activation of FX, as well as their effects on the amidolytic activity of FXa and thrombin and the prolongation of clotting time caused by selected compounds. 4*H*-3,1-Benzoxazin-4-one-derivatives are known to act as serine protease inhibitors, but very few of the reported compounds contain an aryl group connected directly to the ring system together with substituents in the 5, 6, 7, or 8 positions.^{3–9} None of the previously described compounds have been reported to specifically inhibit the TF/FVIIa-induced coagulation pathway, but rather to act as thrombin and FXa inhibitors. Similar structures, often containing a 2-amino substituent instead of the 2-aryl substituent, are reported to act as inhibitors of HSV-1 protease,¹⁰ Cl[–] serine protease,¹¹ and human cytomegalovirus protease.¹² A solid-phase synthetic approach towards 2-amino substituted 4*H*-3,1-benzoxazin-4-ones has been reported.¹³

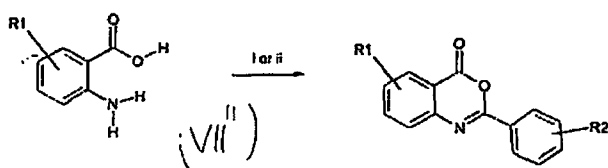
DMF using triethylamine or pyridine as base. However, the yields varied considerably (0% to 100%), depending on the substitution pattern in both the anthranilic acid and the aryl carboxylic acid or acid chloride. Syntheses using an aromatic *ortho* aminocarboxylic acid ester and an aryl carboxylic acid chloride as starting materials performing the ring closure with sulphuric acid³ gave very low yields, and the same was found for syntheses using an anthranilic acid derivative and an arylcarboxylic acid performing the reaction with HOBT and EDAC, probably due to the presence of two carboxylic acids which could be activated by HOBT.^{4–9} The reaction is depicted in Scheme 1.

In order to conveniently prepare a series of analogues for the SAR investigation, a solution phase parallel synthesis procedure was developed (Scheme 2).

Appropriately substituted 2-aminobenzoic acids were dissolved in toluene, triethylamine was added and the mixture shaken for 10 min at room temperature. Subsequently, substituted benzoic acid chloride was added and the mixture shaken overnight at room temperature. Ethyl acetate and HCl (0.1*N*) were added followed by shaking. The organic layer was separated, saturated NaHCO₃ added, and the mixture shaken for 2 min. The organic layer was separated and filtered through a silica-gel column (Sep-Pack plus), the column washed with dichloromethane and the collected organic phases evaporated. The identity and purity were checked by LC-MS, >90% of the experiments gave >75% yield as estimated from the ELS measurement.

Chemistry

2-Aryl substituted 4*H*-3,1-benzoxazin-4-ones were conveniently prepared from optionally substituted anthranilic acids and an aryl carboxylic acid chloride in toluene or

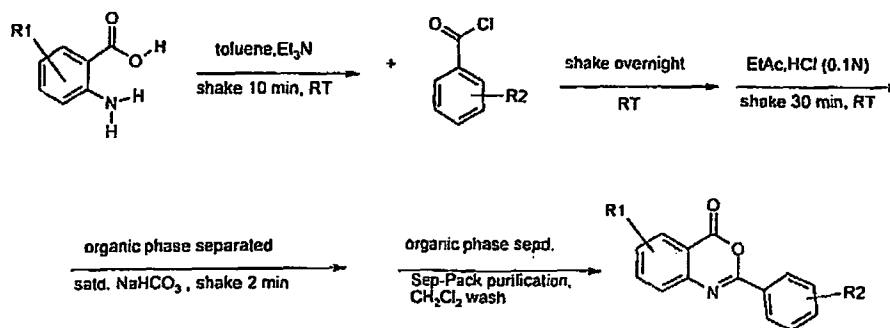


Scheme 1. Synthetic pathway used for the preparation of compounds 2, 5, 17–20, 22, 24, 27, 28, 30, 32, 33, 36, 38, 44, 59 and 60. i: Ar(R2)-COCl, Et₃N, Toluene; ii: Ar(R2)COOH, HOBT, EDAC, DMF.

Results

The compounds were evaluated for inhibitory activity on the TF/FVIIa-induced activation of FX using a two-step amidolytic assay. Compounds showing good inhibitory activity in that assay were further tested for activity on FXa and thrombin in order to sort out structures with the best selectivity. Selected compounds were further tested for their ability to prolong clotting time in a standard in vitro clotting assay. Selected data for 2-aryl substituted 4*H*-3,1-benzoxazin-4-ones are depicted in Table 1.

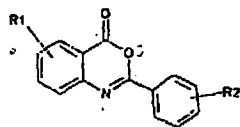
The data revealed a broad variety of selectivity, ranging from compounds having a similar inhibitory effect on



Scheme 2. Solution phase parallel synthesis approach for the preparation of compounds 3, 4, 6–9, 12–16, 21, 25, 26, 29, 31, 39, 40, 42, 43, 46–50 and 61.

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Table 1. IC₅₀ values (μM) for the inhibition of TF/FVIIa-catalyzed activation of FX and the inhibition of FXa and thrombin amidolytic activity



Compound no.	R1	R2	FX activation IC ₅₀ μM	FXa IC ₅₀ μM	Thrombin IC ₅₀ μM
1	H	2-F	4.7	138	56
2	6-Me	2-F	3.6	> 200	145
3	6-CF ₃	2-F	3.1	37	34
4	6-OMe	2-F	3.0	> 200	40
5	5-Cl,8-Cl	2-F	1.2	180	> 200
6	5-COOMe	2-F	5.6	138	56
7	6-F,7-F	2-F	7.2	> 200	> 200
8	5-NO ₂	2-F	17	26	17
9	6-F,7-F	2-F,3-F	18	> 200	105
10	6-Br,8-Br	2-F	25	> 200	> 200
11	7-Cl	2-F	30	> 200	199
12	6-Me	2-F,3-F	32	> 200	> 200
13	6-NO ₂	2-F,3-F	40	NT	NT
14	5-CF ₃	2-F	> 40	NT	NT
15	8-CF ₃	2-F	> 40	NT	NT
16	5-COOEt	2-F	> 40	NT	NT
17	5-Cl	2-F,6-F	0.17	4.9	> 200
18	5-NO ₂	2-F,6-F	0.35	6.3	> 200
19	5-Cl,8-Cl	2-F,6-F	0.36	6.3	> 200
20	6-Me	2-F,6-F	0.60	22	8
21	6-CF ₃	2-F,6-F	0.63	16	98
22	7-NO ₂	2-F,6-F	0.82	112	> 200
23	5-F	2-F,6-F	0.90	6	NT
24	6-NO ₂	2-F,6-F	1.0	19	> 200
25	7-CF ₃	2-F,6-F	1.4	174	134
26	6-OMe	2-F,6-F	1.5	154	30
27	6-NHAc	2-F,6-F	1.6	35	> 200
28	6-NH ₂	2-F,6-F	3.2	154	149
29	5-COOMe	2-F,6-F	4.1	112	156
30	5-Me	2-F,6-F	4.3	89	> 200
31	8-CF ₃	2-F,6-F	4.5	80	> 200
32	7-NH ₂	2-F,6-F	22	> 200	> 200
33	5-NH ₂	2-F,6-F	30	> 200	> 200
34	6-Me	2-F,6-Cl	0.9	10	NT
35	5-F	2-F,6-Cl	1.2	6.0	NT
36	6-Me	2-Cl,3-Cl	1.2	110	36
37	6-I	2-Cl	2.2	15	NT
38	6-Me	2-Cl,6-Cl	2.8	63	> 40
39	5-NO ₂	2-NO ₂	0.32	< 1.6	97
40	5-NO ₂	2-OMe	3.9	5.8	19
41	H	2-OMe,5-Cl	6.8	30	NT
42	5-NO ₂	2-OCOMe	7.3	26	> 200
43	6-NO ₂	2-OCOMe	10	49	> 200
44	6-Me	2-OCF ₃	12	> 200	NT
45	6-Cl	2-Br	1.5	6	NT
46	2-Me	2-OCOMe	33	> 200	> 200

all three enzymes to compounds active only in the FX activation assay. Compounds with a detectable inhibitory effect on FXa will also appear to effect FX activation and FVIIa/TF-initiated clot formation, without necessarily inhibiting FVIIa directly. Thus true IC₅₀-values for the FVIIa inhibition as measured by the FX activation assay are obtained only for compounds which do not inhibit Fxa.

The best compounds regarding inhibitory activity and selectivity were 5, 22 and 25 with 5,8-dichloro, 7-nitro,

and 7-trifluoromethyl substitution in the benzoxazine ring, respectively, and 2-fluoro or 2,6-difluoro substitution in the 2-phenyl substituent. These compounds also prolonged the TF-induced clotting time, the ratios being 1.32, 1.31, and 1.51 times the control clotting time. The best inhibitors of the TF/FVIIa-induced activation of FX were substituted with electronegative substituents like Cl, NO₂, CF₃, F in the 5, 6, or 7 positions in the benzoxazine ring together with a 2,6-difluoro substitution in the 2-phenyl group. However, these compounds also possessed good inhibitory effects on the FXa amidolytic activity resulting in a specificity factor below 50, while their activities at the active site of thrombin were low. Also a 6-methyl substituent (compound 20) inhibited the FX activation at sub-μM concentrations, however, the inhibitory effect on thrombin was also good (8 μM). Compounds carrying-NH₂,-OMe or-COOMe in the benzoxazine ring (compounds 4, 26, 28, 32 and 33) gave a 3-fold to 10-fold higher IC₅₀ in the FX activation assay than found for the electronegative substituents, while the IC₅₀ for FXa and thrombin was often found to be > 200 μM (i.e. no effect).

Selected data for the inhibitory effect of 2-heteroaryl substituted 4H-3,1-benzoxazin-4-ones is presented in Table 2.

The compounds having a heteroaromatic substituent in position 2 by and large showed a 10-fold higher IC₅₀ value than found with a substituted phenyl group in position 2. This probably reflects the difference in electronegativity for the two substituent groups.

Compound 47, however, had a good profile in agreement with the 6,7-halogen disubstituted 2-phenyl compounds 7, 9 and 10.

The compounds giving the best prolongation in the clotting time (17, 18, 20, 28 and 47) were substituted in the 2-position with a 2,6-difluorophenyl group (or a 2-chloro-3-pyridyl for compound 47), while the substitution on the benzoxazin ring was Cl, NO₂, Me, NH₂ and F (Table 3).

Discussion

The expected mechanism of action for the serine protease inhibition by benzoxazinone-type of compounds is deactivation by means of reaction of the benzoxazinone lactone with the active site serine, probably under the formation of a drug-enzyme ester followed by its hydrolysis.^{7,14} This mechanism was supported by data obtained for activity in a TF/FVII assay and in a FVII amidolytic assay in which we found that the benzoxazinones gave inhibition of the same order of magnitude as found in the FX activation assay (not shown).

The corresponding open anthranilic acid amide derivatives (the 'open form' of the benzoxazinone ring) were found without inhibitory effects. These compounds could be prepared from the corresponding benzoxazinones by hydrolysis with NaOH in methanol followed by careful acidification with an equimolar amount of HCl at room

Table 2. IC₅₀ values (μM) for the inhibition of TF/FVIIa-catalyzed activation of FX, FXa and thrombin

Compound no.	R1	R3	FX-activation IC ₅₀ μM	FX amidolytic IC ₅₀ μM	Thrombin IC ₅₀ μM
47	6-F,7-F	2-Cl-3-pyridyl	5.0	> 200	> 200
48	5-Me	2-Cl-3-pyridyl	7.1	> 200	30
49	5-NO ₂	2-Cl-3-pyridyl	14	98	9.7
50	6-NO ₂	2-Cl-3-pyridyl	21	140	> 200
51	6-Br6-OMe	4-pyridyl	27	> 200	NT
52	7-OMe	3-Cl,5-CF ₃ -2-pyridyl	33	> 200	NT
53	6-Br	3-Cl,5-CF ₃ -2-pyridyl	33	> 200	NT
54	7-Cl6-OMe	5-NO ₂ -2-furanyl	16	> 200	NT
55	7-OMe	2-furanyl	25	> 200	> 200
56	6-Br	2-furanyl	34	> 200	NT
57	6-Me	5-NO ₂ -2-furanyl	> 40	NT	NT
58	H	2-thienyl	27	> 200	NT
59	6-Me	2-thienyl	35	> 200	> 200
60	6-Me	3-Br-2-thienyl	40	NT	> 200
61	6-F,7-F	2-thienyl	> 40	NT	NT

temperature. Prolonged standing or higher temperatures gave a mixture of the 'open form' and the benzoxazinone. The 'open form' was also accessible from the reaction of anthranilic acid and arylcarboxylic acid chloride in pyridine at -30°C . However, also this method gave mixtures of the 'open' and ring-closed forms (data not shown). Taking this expected mechanism into account, it seems reasonable to expect that electronegative substituents on the 2-aryl group as well as on the 5, 6, 7, or 8 positions of the benzoxazinone ring will make the ring carbonyl more reactive towards the serine OH-group of the enzyme.

Compounds without substitution in the 2 or 6-positions in the 2-aryl substituent did not inhibit the TF/FVIIa-catalyzed FX activation at concentrations below 40 μM (data not shown).

Introduction of an ester functionality at the 5-position (compounds 6, 16 and 29) or at the 2-position in the 2-phenyl substituent (compounds 42, 43 and 46) was performed in order to clarify if this group might, in some way, compete with the ester formation between the serine-OH and the benzoxazinone ring, either by steric hindrance of the drug-enzyme reaction or by participating in the ester formation with the enzyme. These compounds, however, gave results of the same magnitude and selectivity as found for both electronegative and electron-donating substituents at the same position. Consequently, no proof in favour of that hypothesis was obtained.

Compounds substituted with 2-thienyl, 2-furanyl or 2-, 3- or 4-pyridyl in the 2-position of the benzoxazine ring (compounds 47–61) showed the same pattern as found for the 2-phenyl substituted compounds. IC₅₀ values of < 10 μM were observed only for 47 and 48 carrying an electronegative *ortho*-substituent in the 2-substituent together with 6,7-difluoro or 5-Me substitution. Compound 49 had a surprisingly good effect on thrombin (9.7 μM), i.e. equipotent for thrombin and FVIIa/TF,

Table 3. Effects on TF/FVIIa-induced clotting. The ratio of the clotting times with (330 μM) and without test compound is given

Compound no.	Clotting ratio
1	1.45
2	1.47
3	1.59
5	1.32
7	1.60
17	> 3
18	2.58
19	1.68
20	> 3
22	1.31
25	1.51
28	> 3
33	1.06
38	1.88
40	1.25
42	1.14
43	1.2
47	2.32
49	1.52
50	1.16
55	1.35
59	1.76

while the IC₅₀ for inhibition of FXa was 10-fold higher. This is in contrast to the findings for compounds 18, 39 and 40 for which the inhibitory effects were best in the TF/FVIIa-catalyzed FX activation assay.

2-Pyridyl and 4-pyridyl substituents were found to be of low activity in the activation assay, in the former case probably because the ring nitrogen occupied an *ortho* position and no electron-attracting groups were positioned in an *ortho* position, and in the latter because no *ortho* electronegative substituent was present. For the thienyl and furanyl substituted compounds, only compound 60 had an electronegative substituent in the *ortho* position, however, in this case a bromo substituent was no good compared to compound 45 (IC₅₀ values in the FX activation assay being 40 and 1.5 μM, respectively).

Table 4. Analytical data for compounds prepared by solution phase parallel synthesis

No.	Compound	Analytical details ^a
3	2-(2-Fluoro-phenyl)-6-trifluoromethyl-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> ^a : 310 RT: 15.48, ELS: 1%
4	2-(2-Fluoro-phenyl)-6-methoxy-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> : 272 RT: 14.10, ELS: 8%
6	2-(2-Fluoro-phenyl)-4- <i>H</i> -3,1-benzoxazin-4-oxo-5-carboxylic acid methyl ester	<i>m/z</i> : 300 RT: 12.97, ELS: 00%
7	6,7-Difluoro-2-(2-fluoro-phenyl)-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> : 278 RT: 14.78, ELS: 97%
8	2-(2,3-Difluoro-phenyl)-5-nitro-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> : 305 RT: 13.70, ELS: 95%
9	2-(2,3-Difluoro-phenyl)-6,7-difluoro-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> : 296 RT: 14.91, ELS: 98%
12	2-(2,3-Difluoro-phenyl)-5-methyl-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> : 274 RT: 15.1, ELS: 82%
13	2-(2,3-Difluoro-phenyl)-6-nitro-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> : 305 RT: 14.26, ELS: 80%
14	2-(2-Fluoro-phenyl)-5-trifluoromethyl-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> : 310 RT: 15.47, ELS: 81%
15	2-(2-Fluoro-phenyl)-8-trifluoromethyl-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> : 310 RT: 15.15, ELS: 99%
16	2-(2-Fluoro-phenyl)-4-oxo-4- <i>H</i> -3,1-benzoxazin-5-carboxylic acid ethyl ester	<i>m/z</i> : 314 RT: 13.73, ELS: 95%
21	2-(2,6-Difluoro-phenyl)-6-trifluoromethyl-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> : 328 RT: 15.26, ELS: 82%
25	2-(2,6-Difluoro-phenyl)-7-trifluoromethyl-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> : 328 RT: 15.51, ELS: 91%
26	2-(2,6-Difluoro-phenyl)-6-methoxy-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> : 290 RT: 13.8/11.45, ELS: 80%
29	2-(2,6-Difluoro-phenyl)-4-oxo-4- <i>H</i> -3,1-benzoxazin-5-carboxylic acid methyl ester	<i>m/z</i> : 318 RT: 12.82, ELS: 67%
31	2-(2,6-Difluoro-phenyl)-8-trifluoromethyl-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> : 328 RT: 14.73, ELS: 94%
39	5-Nitro-2-(2-nitro-phenyl)-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> : 314 RT: 13.11, ELS: 81%
40	2-(2-Methoxy-phenyl)-5-nitro-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> : 299 RT: 12.81, ELS: 61%
42	Acetic acid 2-(5-nitro-4-oxo-4- <i>H</i> -3,1-benzoxazin-2-yl)-phenyl ester	<i>m/z</i> : 327 RT: 12.88, ELS: 84%
43	Acetic acid 2-(6-nitro-4-oxo-4- <i>H</i> -3,1-benzoxazin-2-yl)-phenyl ester	<i>m/z</i> : 327 RT: 13.37, ELS: 54%
46	Acetic acid 2-(5-methyl-4-oxo-4- <i>H</i> -3,1-benzoxazin-2-yl)-phenyl ester	<i>m/z</i> : 296 RT: 14.11, ELS: 54%
47	2-(2-Chloro-pyridin-3-yl)-6,7-difluoro-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> : 295 RT: 12.75, ELS: 96%
48	2-(2-Chloro-pyridin-3-yl)-5-methyl-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> : 273 RT: 12.61, ELS: 100%
49	2-(2-Chloro-pyridin-3-yl)-5-nitro-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> : 304 RT: 11.64, ELS: 82%
50	2-(2-Chloro-pyridin-3-yl)-6-nitro-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> : 304 RT: 11.94, ELS: 88%
61	6,7-Difluoro-2-thiophen-2-yl-4- <i>H</i> -3,1-benzoxazin-4-one	<i>m/z</i> : 266 RT: 14.68, ELS: 98%

^a*m/z*: The molecular ion (*M* + 1) from the LC-MS investigation; RT: Retention time (min); ELS: The purity estimated from the electrospray (positive ion) measurement.

Conclusion

The synthetic approach described easily yielded a series of 2-aryl substituted 4*H*-3,1-benzoxazin-4-ones in fair to good yields by standard synthetic methods and through a solution-phase parallel fashion. The 2-aryl substituted 4*H*-3,1-benzoxazin-4-ones described comprise several compounds with excellent activity and selectivity thus being the first small molecules described having that profile. Good inhibition requires the presence of at least one electron-attracting substituent in the *ortho* position of the 2-aryl or heteroaryl substituent, the best structures also bearing one or two electronegative substituents in the 5, 6, 7 or 8 positions. The best compounds as regards both inhibitory activity of the TF/FVIIa-catalyzed FX activation and best selectivity (5, 22 and 25) were all found within this pattern. Simple heterocycles like thienyl, furanyl and 2- and 4-pyridyl as the 2-substituents gave compounds of low activity while 2-(3-pyridyl) substituents were found to be of similar activity as the phenyl substituents (47).

Experimental

General procedures. Melting points (uncorrected) were measured on a Büchi 535. NMR spectra were recorded on a Bruker AVANCE DPX 200 Mhz or 300 MHz instrument operating at room temperature. Mass spectra were obtained on a Finnigan Mat TSQ 70 apparatus using a direct inlet system. The HPLC-MS analyses were performed on a PE Sciex API 100 LC/MS System using a Waters™ 3 mm×150 mm, 3.5 μ, C-18 Symmetry column and positive ion spray with a flow rate at 20 μL/min. The

column was eluted with a linear gradient of 5–90% A, 85–0% B and 10% C for 15 min at a flow rate of 1 mL/min (solvent A=acetonitrile, solvent B=water and solvent, C=0.1% trifluoroacetic acid in water). Microanalyses were performed by Novo Nordisk Analytical Department.

FX activation assay

The compounds were dissolved in DMSO and mixed with FVIIa (produced in-house) in 50 mM Hepes, pH 7.4, containing 0.1 M NaCl, 5 mM CaCl₂, and 1 mg/mL bovine serum albumin (1 + 5). 30 μL of this mixture was then mixed with 45 μL TF (American Diagnostica, relipidated in PC/PS vesicles) and 25 μL of FX (Enzyme Research Laboratories), all in a Ca²⁺-containing buffer. This gave final concentrations of 100 pM FVIIa, 5 pM TF, 175 nM FX, and various concentrations of the compounds. After a 5 min incubation, the FVIIa/TF-catalyzed activation of FX was terminated by an addition of a 50 μL buffer containing enough EDTA to give an excess over the Ca²⁺ ions present. 50 μL of a 2-mM solution of S-2765 (Chromogenix, 2 mM in water) was then added and the FXa formed was allowed to hydrolyze the substrate for 10 min during which the absorbance at 405 nm was continuously monitored in a SPEC-TRAMax™ 340 plate reader. The slope of the absorbance curve was compared to that of a control where only DMSO was added to FVIIa/TF/FX.

Thrombin activity assay

170 μL thrombin (Boehringer Mannheim, 3.5 nM) in the buffer described above, 10 μL test compound in DMSO (or DMSO alone in the control) and 20 μL

methylation of AHQ rather than using the multistep sequence employed earlier.¹⁴

Biological Evaluation. Each of the target compounds, together with three isomeric analogues, having a normal folic acid configuration were evaluated for inhibitory activity upon the growth of four human gastrointestinal cell lines in vitro, and the results are presented in Table I. The 4-aminofolate analogues MTX and 1b were included in order to compare relative antitumor potencies. It will be seen that each cell line was quite sensitive to MTX and that compound 1b was approximately one order of magnitude less inhibitory. This correlates with the relative abilities of these two compounds to inhibit mammalian DHFR.⁶ It is interesting that IAHQ, as well as its 5-CH₃, 9-CH₃, and 5,9-(CH₃)₂ derivatives, has approximately the same modest level of inhibitory activity toward these four cell lines. The 9-CHO derivative was considerably less active, while D-IAHQ was at least 100-fold less inhibitory than its L isomer. The di-*tert*-butyl ester 6a was inactive within solubility limits. Both AHQ and its 10-CH₃ derivative were more cytotoxic than IAHQ. Since each of these was a better inhibitor of DHFR,⁶ as well as of thymidylate synthase,⁷ no conclusion can be drawn to explain this enhanced activity. As was the case for the formyl derivative of IAHQ, 10-CHO-AHQ was dramatically less inhibitory than AHQ. Since the latter compound was shown to be a good inhibitor of both DHFR⁶ and thymidylate synthase,⁷ it is suggested that the formyl modifications are not effectively transported into these cell lines.

Discussion

Recent studies with IAHQ have indicated that its cytotoxicity may be related to intracellular conversion to poly(γ -glutamyl) metabolites, which are significantly more inhibitory toward thymidylate synthase.⁸ This inhibition was augmented by higher concentrations of 2'-deoxyuridine monophosphate, which accumulate as the result of inhibition of thymidylate synthase.⁸ The formation of γ -L-glutamate metabolites would also be expected to result in enhanced retention of IAHQ within tumor cells, as has been observed for MTX in cultured human cells.¹⁵ The inactivity of D-IAHQ may be attributable to the fact that it is not a substrate for folylpolyglutamate synthetase, as was the case for D-folic acid.¹⁶ On the other hand, D-IAHQ may not be as effectively transported into these cells as IAHQ. Earlier studies with structurally related 2,4-diaminoquinazolines showed that a D-glutamic acid derivative had a much slower rate of influx into L1210 leukemia cells than its L isomer.¹⁷ The wide divergence in activity between the two stereoisomers provides indirect evidence that racemization does not occur during the deblocking procedure. Additional studies using MTX-insensitive tumor cells both in vitro and in vivo will be required to determine whether IAHQ or one of its analogues will be of value in treating human neoplastic diseases.

Experimental Section

Melting points were determined on a Mel-temp apparatus and are uncorrected. Elemental analyses were performed by Galbraith

Laboratories, Knoxville, TN. All analytical samples were dried under vacuum at 100 °C unless stated otherwise and gave combustion values for C, H, and N within $\pm 0.4\%$ of the theoretical values. All intermediates were free of significant impurities on TLC using silica gel media (Gelman SAF or Baker 1B2-F). Target compounds (free glutamates) were analyzed by HPLC on a Micromeritics Model 7000B liquid chromatograph containing a Partisil PSX 10/25 ODS-2 column (4.6 $\times$ 25 cm) and a UV (254 nm) detector. The workup procedures described below were developed in such a manner that the final products were free of significant impurities using linear gradient elution (0–20% v/v) of MeCN in H₂O at pH 4.0 and a flow rate of 2 mL/min. Samples for HPLC were dissolved in Me₂SO just prior to injection. The UV spectra were determined with a Cary 219 spectrophotometer. The ¹H NMR spectra were determined with a Varian T-80 spectrometer operating at 60 MHz or a Varian EM 390 spectrometer operating at 90 MHz. Values for chemical shifts are presented in parts per million downfield from Me₄Si as the internal standard. The relative peak areas are given to the nearest whole number, and chemical shifts in the case of multiplets are measured from the approximate center. Di-*tert*-butyl L-glutamate was prepared according to the literature method but was maintained in oil form rather than being converted into a salt derivative.¹¹ The free ester was found to be stable when stored at 4 °C: TLC (CHCl₃-MeOH-HOAc, 85:10:5). 5-Nitroisatoic anhydride was obtained from Sherwin Williams Chemicals and was recrystallized from EtOH-H₂O, 95:5, prior to use.

2-Amino-4-hydroxy-6-nitroquinazoline (3). A mixture of 16 g (0.077 mol) of 2 and 13.9 g (0.077 mol) of guanidine carbonate in 250 mL of DMF was heated at reflux with stirring for 48 h. The solution was added to 500 mL of H₂O to give a solid, which was separated by filtration and washed with (Me)₂CO and hexane to give 3: yield 12.8 g (75%); TLC (DMF-EtOAc, 1:2). For analysis, a sample was recrystallized from Me₂NAC-H₂O to yield a yellow powder: mp >350 °C dec; TLC (DMF-H₂O, 1:2); HPLC; NMR (CF₃COOD) δ 9.25 (d, 1, H₈, $J_{8,7} = 2$ Hz), 8.33 (dd, 1, H₇, $J_{7,8} = 9$ Hz, $J_{7,6} = 2$ Hz), 8.03 (d, 1, H₆, $J_{7,6} = 9$ Hz). Anal. (C₈H₆N₄O₃·0.1C₄H₉NO·0.8H₂O) C, H, N: calcd, 25.05; found, 24.58. The presence of approximately 10% of Me₂NAC was confirmed by NMR.

2,6-Diamino-4-hydroxyquinazoline (4). This compound was prepared by the literature method for the preparation of 2,4,6-triaminoquinazoline.¹⁸ A 8.71 g (42.3 mmol) sample of crude 3 dissolved in 200 mL of DMF containing 2.6 mL (46.5 mmol) of glacial HOAc and 1 g of 10% Pd/C was hydrogenated at low pressure until H₂ uptake ceased. After filtration, the solution was poured into 1.4 L of EtOAc, and the resulting solid was collected by filtration and washed with H₂O and (Me)₂CO. After drying in vacuo at 100 °C there was obtained 4.0 g (54%) of tan crystalline solid: mp 330–335 °C dec (lit.¹⁸ mp >300 °C dec); TLC (DMF-H₂O, 1:2).

Di-*tert*-butyl N-(4-Formylbenzoyl)-L-glutamate (5). A solution of 20.3 g (0.135 mol) of 4-formylbenzoic acid and 13.6 g (0.135 mol) of 4-methylmorpholine in 200 mL of DMF was cooled to –15 °C, and the mixed anhydride was formed by the addition of 18.37 g (0.135 mol) of isobutyl chloroformate. After 2 min, a precooled (–15 °C) solution of 35.0 g (0.135 mol) of di-*tert*-butyl glutamate and 13.65 g (0.135 mol) of 4-methylmorpholine in 100 mL of DMF was added at one time. The mixture was stirred at –15 °C for 45 min and then allowed to warm to room temperature and stirred for 18 h. The solvent was removed at reduced pressure, and the residue was dissolved in 150 mL of EtOAc. This solution was washed with 3 $\times$ 50 mL of 5% NaHCO₃, 3 $\times$ 50 mL of 1 N H₂SO₄, and 3 $\times$ 50 mL of saturated NaCl. After the solution was dried over Na₂SO₄, the solvent was removed under vacuum, and the resulting oil was dried under vacuum until a constant weight of 45.7 g (81%) was obtained: TLC (EtOAc-MeOH, 98:2). Purification by silica gel column chromatography using CHCl₃ as the eluent yielded a pure fraction (TLC), which became contaminated, presumably due to oxidation during the solvent-removal step, and failed to crystallize: NMR (CHCl₃) δ 10.1 (s, 1, CHO), 7.92 (s, 4, aromatic), 4.67 (m, 1, NCH), 2.6–1.8 [m, 4, (CH₂)₂], 1.52 [s, 9, OC(CH₃)₃], 1.43 [s, 9, OC(CH₃)₃].

Di-*tert*-butyl 5,8-Dideazaiofolate (6a). A mixture of 8.0 g (19.0 mmol) of 5 and 3.75 g (19.1 mmol) of 4 in 160 mL of 70% HOAc was hydrogenated in the presence of Raney Ni (~500 mg)

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S-2238 (Chromogenix, 10 mM in water) were mixed. The hydrolysis of S-2238 was monitored as described for the FX activation assay.

FXa activity assay

170 μ L FXa (Enzyme Research Laboratories, 1.2 nM in buffer) in the buffer described above, 10 μ L test compound and 20 μ L S-2765 (Chromogenix, 10 mM in water) were mixed. The hydrolysis of S-2765 was monitored as described for the FX activation assay.

FVIIa/TF-initiated clotting assay

The test compounds, 20 mM in DMSO, were diluted in citrated normal human plasma just before the analysis (1 + 19) and placed in the sample carousel of an ACL 300 Research coagulometer. 55 μ L sample (compound in plasma) was mixed with 55 μ L of thromboplastin (Innovin, Dade; diluted 1:500 after dissolution) and incubated for 5 min. The clotting reaction was started by adding 55 μ L of a 25 mM CaCl_2 solution, yielding a final compound concentration of 0.33 mM. The ratio between the clotting time in the presence and absence of test compounds was used to quantify the anticoagulant effect.

The following compounds were commercially available from Maybridge (compounds 1, 10, 11, 23, 34, 35, 37, 51, 52, 53, 54, 57 and 58), CSC (compounds 41, 55 and 56), and SPECS (compound 45).

2-(2-Fluoro-phenyl)-6-methyl-4-*H*-3,1-benzoxazin-4-one (2). 2-Fluorobenzoic acid (3.3 mmol, 0.52 g) was mixed with HOBT (3.3 mmol, 0.44 g) and EDAC (6.6 mmol, 1.26 g) in CH_2Cl_2 (5 mL). The mixture was stirred for 1 h and subsequently 2-amino-5-methylbenzoic acid (3.3 mmol, 0.5 g) in DMF (5 mL) was added dropwise (10 min). After stirring for 3 h, HCl (4N, 10 mL) was added and the mixture extracted with CH_2Cl_2 (3 $\times$ 10 mL). The collected organic phases were extracted with HCl (4N, 10 mL) and then twice with water (10 mL). The organic phases were dried (MgSO_4), filtered and evaporated to dryness. The crude product was purified on silica-gel using CH_2Cl_2 as eluent. Yield of 2 (30%); LC-MS 256 ($M+1$); RT 14.61 min; ELS-purity 100%; mp 144 °C. Calculated for $\text{C}_{15}\text{H}_{10}\text{FNO}_2$: C, 70.58%; H, 3.95%; N, 5.49%. Found: C, 70.41%; H, 3.98%; N, 5.39%. ^1H NMR (CDCl_3 , ppm) 8.08 (1H, d t), 7.98 (1H, d), 7.82–7.57 (3H, m), 7.57–7.32 (2H, m), 2.45 (3H, s).

5,8-Dichloro-2-(2-fluoro-phenyl)-4-*H*-3,1-benzoxazin-4-one (5). 2-Amino-3,6-dichlorobenzoic acid (0.5 g) and triethyl:toluene (1:1) (20 mL) were mixed. 2-Fluorobenzoyl chloride (0.77 g) was added dropwise under cooling and stirring. The mixture was subsequently heated to room temperature and stirred until the disappearance of the starting material was observed on TLC (silica-gel) using heptane:ethyl acetate (4:1) as eluent. After cooling on ice, the precipitate was filtered off and the filtrate evaporated to dryness. The residue was partitioned between methylene chloride and potassium carbonate (2N), the organic layers separated, dried over magnesium sulfate and evaporated to dryness. The raw product was

purified by column chromatography on silica-gel using heptane:ethyl acetate (4:1) as eluent. Yield 0.31 g (42%); mp 140 °C; MS 311/309 (M^+) (CI isotope pattern). Calculated for $\text{C}_{14}\text{H}_6\text{Cl}_2\text{FNO}_2$: C, 54.22%; H, 1.95%; N, 4.52%. Found: C, 53.53%; H, 1.91%; N, 4.38%. ^1H NMR (CDCl_3 , ppm) 8.20 (1H, double t); 7.78 (1H, d); 7.65–7.52 (1H, m); 7.48 (1H, d); 7.35–7.15 (2H, m).

5-Chloro-2-(2,6-difluoro-phenyl)-4-*H*-3,1-benzoxazin-4-one (17). 6-Chloroanthranilic acid (0.566 g), 2,6-difluorobenzoyl chloride (0.93 mL) and triethyl amine:toluene (1/1) (18 mL) were reacted by heating to 50 °C for 2 days. Extraction between ethyl acetate (100 mL) and HCl (2N, 100 mL), followed by separation of the organic layer, drying over MgSO_4 , and finally filtering and evaporation gave a crude product that was dissolved in warm THF (20 mL) and precipitated with hexane. The resulting mixture was further purified on a silica-gel column using dichloromethane as eluent. The isolated fraction was dissolved in warm THF (20 mL) and precipitated with hexane, yielding 17 (0.65 g, 70%); mp. 176 °C; MS m/e 329/295 (M^+). Calculated for $\text{C}_{14}\text{H}_6\text{Cl}_2\text{FNO}_2$: C, 57.62%; H, 2.06%; N, 4.77%. Found: C, 57.35%; H, 2.06%; N, 4.60%. ^1H NMR (DMSO, ppm) 7.93 (1H, t); 7.82–7.65 (3H, m); 7.38 (1H, d); 7.32 (1H, d).

2-(2,6-Difluoro-phenyl)-5-nitro-4-*H*-3,1-benzoxazin-4-one (18). 6-Nitroanthranilic acid (0.6 g), 2,6-difluorobenzoyl chloride (0.93 mL) and triethyl amine:toluene (1:1) (18 mL) were reacted by heating to 50 °C for 2 days. Extraction between ethyl acetate (100 mL) and HCl (2N, 100 mL), followed by separation of the organic layer, drying over MgSO_4 , and finally filtering and evaporation gave a crude product that was dissolved in warm THF (20 mL) and precipitated with hexane. The resulting mixture was further purified on a silica-gel column using dichloromethane as eluent. The isolated fraction was dissolved in warm THF (20 mL) and precipitated with hexane, yielding 18 (0.475 g, 40%); mp 172 °C; MS m/e 304 (M^+); LC-MS m/e 305 ($M+1$); ELS-purity 100%. $\text{C}_{14}\text{H}_6\text{F}_2\text{N}_2\text{O}_4$: C, 56.25%; H, 2.02%; N, 7.03%. Found: C, 55.27%; H, 1.99%; N, 8.96%. ^1H NMR (DMSO, ppm) 8.22–8.05 (2H, m); 7.97 (1H, dd); 7.85–7.68 (1H, m); 7.36 (2H, t).

5,8-Dichloro-2-(2,6-difluoro-phenyl)-4-*H*-3,1-benzoxazin-4-one (19). 2-Amino-3,6-dichlorobenzoic acid (0.125 g), 2,6-difluorobenzoyl chloride (0.168 mL) and triethyl amine:toluene (1:1) (1.5 mL) were reacted by heating to 50 °C for 2 h. HCl (0.2N, 1 mL) was added and the organic layer separated and rinsed by pressing through a silica-gel column (Sep Pack). Evaporation resulted in the isolation of 19 (0.20 g, 10%); mp 190 °C; MS m/e 328 (M^+); LC-MS 329 ($M+1$); RT 14.85; ELS-purity 99.1%. $\text{C}_{14}\text{H}_5\text{Cl}_2\text{F}_2\text{NO}_2$: C, 51.25%; H, 1.54%; N, 4.27%. Found: C, 51.69%; H, 1.95%; N, 4.02%. ^1H NMR (DMSO, ppm) 8.08 (1H, d); 7.88–7.68 (2H, m); 7.35 (2H, t).

2-(2,6-Difluoro-phenyl)-6-methyl-4-*H*-3,1-benzoxazin-4-one (20). 2-Amino-5-methylbenzoic acid (0.5 g) and triethyl amine (10 mL) were mixed in dry toluene (20 mL). 2,6-Difluorobenzoyl chloride (1.28 g) was added dropwise

under cooling and stirring. The mixture was subsequently heated to room temperature for 2 days. After cooling on ice, the precipitate was filtered off and the filtrate evaporated to dryness. The residue was partitioned between methylene chloride and potassium carbonate (2N), the organic layers separated, dried over magnesium sulfate and evaporated to dryness. The raw product was purified by column chromatography on silica-gel using heptane/ethyl acetate as eluent. Yield of 20 (0.85 g, 95%); mp 156 °C; LC-MS 274 (M + 1); RT 14.22 min; ELS-purity 100%. C₁₅H₉F₂NO₂: C, 65.69%; H, 3.30%; N, 5.15%. Found: C, 66.18%; H, 3.33%; N, 5.06%. ¹H NMR (DMSO, ppm) 8.03 (1H, d); 7.90–7.60 (3H, m); 7.35 (2H, t); 2.50 (3H, s).

2-(2,6-Difluoro-phenyl)-7-nitro-4-*H*-3,1-benzoxazin-4-one (22). 4-Nitroanthranilic acid (0.6 g), 2,6-difluorobenzoyl chloride (0.93 mL) and triethyl amine:toluene (1:1) (18 mL) were reacted by heating to 50 °C for 2 days. Extraction between ethyl acetate (100 mL) and HCl (2N, 100 mL), followed by separation of the organic layer, drying over MgSO₄, and finally filtering and evaporation gave a crude product which was dissolved in warm THF (20 mL) and precipitated with hexane twice to get the pure product 22 (0.42 g, 41%); mp 187 °C; MS 304 (M⁺). C₁₄H₆F₂N₂O₄: C, 55.28%; H, 1.99%; N, 9.21%. Found: C, 54.85%; H, 2.07%; N, 9.39%. ¹H NMR (DMSO, ppm) 8.38 (3H, s, broad); 7.88–7.68 (1H, m); 7.35 (2H, t).

2-(2,6-Difluoro-phenyl)-6-nitro-4-*H*-3,1-benzoxazin-4-one (24). 5-Nitroanthranilic acid (0.6 g) was dissolved in triethyl amine:toluene (1:1) (18 mL) and stirred for 10 min. 2,6-Difluorobenzoyl chloride (1.3 g) was slowly added under stirring resulting in the formation of a precipitate. The reaction was performed in an N₂-atmosphere. After stirring at room temperature for 24 h, the mixture was extracted with saturated NaHCO₃ and ethyl acetate (20 mL), and the organic layer was separated and evaporated. The crude product was rinsed by precipitation from hot dioxane, the resulting mass transferred to a silica-gel column and eluted with dichloromethane, the isolated fraction was dissolved in hot toluene (2 mL) and precipitated with hexane, resulting in 24 (0.13 g, 13%); mp 188 °C; MS 304 (M⁺). C₁₄H₆F₂N₂O₄: C, 55.28%; H, 1.99%; N, 9.21%. Found: C, 52.41%; H, 1.91%; N, 8.55%. ¹H NMR (DMSO, ppm) 8.81 (1H, dd); 8.70 (1H, dd); 7.95 (1H, d); 7.86–7.68 (1H, m); 7.35 (2H, t).

6-Acetamido-(2,6-difluoro-phenyl)-4-*H*-3,1-benzoxazin-4-one (27). 5-Acetamidoanthranilic acid (0.64 g) and 2,6-difluorobenzoyl chloride (1.3 g) were reacted in triethyl amine:toluene (1:1) (20 mL) by heating to 50 °C for 1 h. Ethyl acetate (50 mL) and HCl-solution (1 mL 4N in 50 mL water) were added resulting in the formation of a precipitate. The mixture was filtered and the residue dissolved in THF followed by evaporation to dryness, subsequent dissolution in hot dioxane followed by precipitation with hexane resulted in colourless crystals of 27 (0.98 g, 95%); mp 246 °C; MS *m/e* 316 (M⁺); LC-MS 317 (M + 1); RT 10.32; ELS-purity 100%. C₁₆H₁₀F₂N₂O₃: C, 60.76%; H, 3.19%; N, 8.86%. Found: C, 60.31%; H, 3.19%; N, 8.44%. ¹H NMR (DMSO, ppm) 8.53 (1H, dd); 8.04 (1H, dd); 7.72 (2H, dd); 7.35 (2H, t).

6-Amino-2-(2,6-difluoro-phenyl)-4-*H*-3,1-benzoxazin-4-one (28). 6-Nitro-2-(2,6-difluoro-phenyl)-4-*H*-3,1-benzoxazin-4-one 24 (50 mg) was dissolved in acetic acid (5 mL) under N₂, PtO₂ (2.5 mg) was added and the mixture was hydrogenated with H₂. Reaction time 2 h. The reaction mixture was filtered through Hyflo[®] which was rinsed afterwards with ethyl acetate. The combined organic phases were evaporated to dryness and subsequently treated three times with toluene followed by evaporation. The resulting mixture was dissolved in THF and precipitated with hexane resulting in 28 (12 mg, 20%); LC-MS 275 (M + 1); RT 10.28 min; ELS-purity 96%. ¹H NMR (MeOH, ppm) 7.70–7.50 (1H, m); 7.45 (1H, d); 7.36 (1H, d); 7.26–7.08 (3H, m).

2-(2,6-Difluoro-phenyl)-5-methyl-4-*H*-3,1-benzoxazin-4-one (30). 2-Amino-6-methylbenzoic acid (0.498 g), 2,6-difluorobenzoyl chloride (0.93 mL) and triethyl amine:toluene (1:1) (20 mL) were reacted by heating to 50 °C for 2 h. Extraction between ethyl acetate (100 mL) and HCl (2N, 100 mL), followed by separation of the organic layer, drying over MgSO₄, and finally filtering and evaporation gave a crude product which was re-dissolved in toluene (10 mL) and precipitated with hexane (5 mL) resulting in 30 (0.45 g, 50%); mp 156 °C; MS 273 (M⁺); LC-MS 274 (M + 1); RT 14.34 min; ELS-purity 100%. C₁₅H₉F₂NO₂: C, 65.94%; H, 3.32%; N, 5.13%. Found: C, 65.24%; H, 3.27%; N, 4.85%. ¹H NMR (DMSO, ppm) 7.90–7.67 (2H, m); 7.55 (2H, t); 7.35 (2H, t).

7-Amino-2-(2,6-difluoro-phenyl)-4-*H*-3,1-benzoxazin-4-one (32). 7-Nitro-2-(2,6-difluoro-phenyl)-4-*H*-3,1-benzoxazin-4-one 22 (0.10 g) was dissolved in acetic acid (10 mL) under N₂, PtO₂ (5 mg) was added and the mixture was hydrogenated with H₂ gas. Reaction time day. The reaction mixture was filtered through Hyflo[®], which was rinsed afterwards with ethyl acetate. The combined organic phases were evaporated to dryness and subsequently treated three times with toluene followed by evaporation. The resulting mixture was purified on a silica-gel column using dichloromethane as eluent. One fraction was collected and identified as 32 (15 mg, 20%); mp 196 °C; MS 274 (M⁺). C₁₄H₈F₂N₂O₂: C, 61.32%; H, 2.94%; N, 10.22%. Found: C, 61.44%; H, 3.45%; N, 8.91%. ¹H NMR (DMSO, ppm) 7.82 (1H, d); 7.70–7.58 (1H, m); 7.35 (2H, t); 6.85 (1H, dd); 6.75 (2H, s, broad, NH); 6.68 (1H, d).

5-Amino-2-(2,6-difluoro-phenyl)-4-*H*-3,1-benzoxazin-4-one (33). 5-Nitro-2-(2,6-difluoro-phenyl)-4-*H*-3,1-benzoxazin-4-one 18 (0.4 g) was dissolved in acetic acid (30 mL) under N₂, PtO₂ (20 mg) was added and the mixture was hydrogenated with H₂. Reaction time 1 day. The reaction mixture was filtered through Hyflo[®], which was rinsed afterwards with ethyl acetate. The combined organic phases were evaporated to dryness and subsequently treated 3 times with toluene followed by evaporation. The resulting mixture was dissolved in THF and precipitated with hexane giving a crude product that was purified on a silica-gel column using dichloromethane as eluent. One fraction was collected

(51 mg), identified as 33; LC-MS 275 ($M+1$); -RT 12.45 min; ELS-purity 100%; mp 197 °C. $C_{14}H_8F_2N_2O_2$: C, 61.32%; H, 2.94%; N, 10.22%. Found: C, 61.55%; H, 3.04%; N, 9.73%. ^1H NMR ($CDCl_3$, ppm) 7.55–7.38 (2H, m); 7.05 (2H, t); 6.90 (1H, d); 6.70 (1H, d); 6.08 (2H, s, broad, NH).

2-(2,3-Dichloro-phenyl)-6-methyl-4-*H*-3,1-benzoxazin-4-one (36). 2-Amino-5-methylbenzoic acid (0.5 g) and dry pyridine (20 mL) were mixed with 2,3-dichlorobenzoyl chloride (1.52 g) in dry toluene (20 mL) at room temperature. Subsequently, the mixture was heated to 80 °C for 1 h. The mixture was evaporated to dryness, and the residue was partitioned between methylene chloride and potassium carbonate (2N), the organic layers separated, dried over magnesium sulfate and evaporated to dryness. The raw product was recrystallised from toluene yielding 36 (1.0 g, 99%); mp 166 °C. $C_{15}H_9Cl_2NO_2$: C, 58.85%; H, 2.96%; N, 4.58%. Found: C, 58.88%; H, 2.96%; N, 4.45%. ^1H NMR ($CDCl_3$, ppm) 7.55–7.38 (2H, m); 7.05 (2H, t); 6.90 (1H, d); 6.70 (1H, d); 6.08 (2H, s, broad, NH).

2-(2,6-Dichloro-phenyl)-6-methyl-4-*H*-3,1-benzoxazin-4-one (38). 2-Amino-5-methylbenzoic acid (0.5 g) and triethyl amine (10 mL) were mixed in dry toluene (20 mL). 2,6-Dichlorobenzoyl chloride (1.52 g) was added dropwise under cooling and stirring. The mixture was subsequently heated to room temperature for 24 h, then heated to 80 °C for 1 h. After cooling on ice, the precipitate was filtered off and the filtrate evaporated to dryness. The residue was partitioned between methylene chloride and potassium carbonate (2N), the organic layers separated, dried over magnesium sulfate and evaporated to dryness. The raw product was purified by column chromatography on silica-gel using heptane/ethyl acetate as eluent giving 38 (0.12 g, 11%); mp 175 °C; MS 305, 307 (M^+) (Cl isotope pattern); LC-MS 306, 308 ($M+1$); RT 15.75 min; ELS-purity 98%. $C_{15}H_9Cl_2NO_2$: C, 58.85%; H, 2.96%; N, 4.58%. Found: C, 57.96%; H, 2.90%; N, 4.09%. ^1H NMR ($CDCl_3$, ppm) 8.10 (1H, s, broad); 7.72–7.58 (2H, m); 7.48–7.31 (3H, m); 2.54 (3H, s).

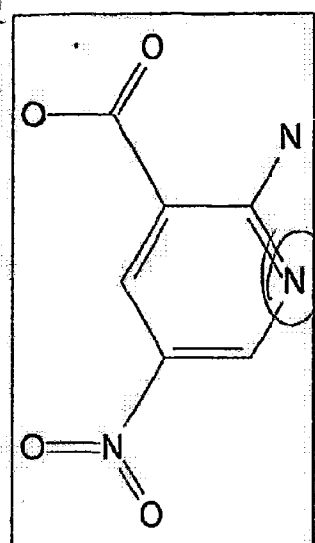
6-Methyl-2-(2-trifluoromethoxy-phenyl)-4-*H*-3,1-benzoxazin-4-one (44). 2-Amino-5-methylbenzoic acid (0.5 g) and triethyl amine (10 mL) were mixed in dry toluene (20 mL). 2-Trifluoromethoxybenzoyl chloride (1.64 g) was added dropwise under cooling and stirring. The mixture was subsequently heated to room temperature for 2 days. After cooling on ice, the precipitate was filtered off and the filtrate evaporated to dryness. The residue was partitioned between methylene chloride and potassium carbonate (2N), the organic layers separated, dried over magnesium sulfate and evaporated to dryness. The raw product was purified by column chromatography on silica-gel using heptane/ethyl acetate as eluent giving 44 (1.0 g, 98%); mp 70–80 °C; MS 321 (M^+). $C_{16}H_{10}F_3NO_3$: C, 59.82%; H, 3.14%; N, 4.36%. Found: C, 61.12%; H, 3.53%; N, 4.18%. ^1H NMR ($CDCl_3$, ppm) 8.10 (1H, dd); 8.04 (1H, d, broad); 7.70–7.52 (3H, m); 7.49–7.36 (2H, m); 2.50 (3H, s).

6-Methyl-2-thiophen-2-yl-4-*H*-3,1-benzoxazin-4-one (5). 2-Amino-5-methylbenzoic acid (1.5 g) and triethyl amine:toluene (1:1) (80 mL) were mixed with 2-thienylcarbonyl chloride (3.18 g). The mixture was stirred at room temperature for 5 h and the precipitate was filtered off and the filtrate evaporated to dryness. The residue was partitioned between methylene chloride and potassium carbonate (2N), the organic layers separated, dried over magnesium sulfate and evaporated to dryness. The raw product was purified by dissolution in dioxan followed by precipitation with hexane resulting in 59 (2.0 g, 85%); mp 156 °C; MS 243 (M^+); LC-MS 244 ($M+1$); RT 1.61 min; ELS-purity 100%. $C_{13}H_9NO_2S$: C, 64.18%; H, 3.73%; N, 5.76%. Found: C, 64.20%; H, 3.72%; N, 5.56%. ^1H NMR (DMSO, ppm) 7.95 (1H, dd); 7.86 (2H, s, broad); 7.70 (1H, dd); 7.50 (1H, dd); 7.27 (1H, t); 2.42 (3H, s). ^{13}C NMR (DMSO, ppm) 159.24, 153.28, 145.02, 139.08, 138.73, 134.65, 134.11, 132.21, 129.58, 128.53, 127.12, 117.12, 21.54.

2-(3-Bromothiophen-2-yl)-6-methyl-4-*H*-3,1-benzoxazin-4-one (60). 2-Amino-5-methylbenzoic acid (0.5 g) and triethyl amine (10 mL) were mixed in dry toluene (10 mL). 3-Bromo-2-thienylcarbonyl chloride (0.72 g) was added dropwise under cooling and stirring, the mixture was further stirred at room temperature for 2 days and subsequently the mixture was kept at 4 °C for 30 days. The precipitate was filtered off and the filtrate evaporated to dryness. The residue was partitioned between methylene chloride and potassium carbonate (2N), the organic layers separated, dried over magnesium sulfate and evaporated to dryness. The raw product was purified by column chromatography on silica-gel using toluene as eluent. Yield of 60 (0.45 g, 48%); mp 159 °C; MS 321, 323 (M^+) (Br-isotope pattern). NMR ($CDCl_3$, ppm) 7.99 (1H, dd); 7.66–7.50 (2H, m); 7.49 (1H, d); 7.12 (1H, d); 2.47 (3H, s). ^{13}C NMR ($CDCl_3$, ppm) 159.13, 152.37, 144.93, 139.31, 138.28, 134.15, 131.18, 128.88, 128.74, 127.36, 116.91, 115.14, 21.75.

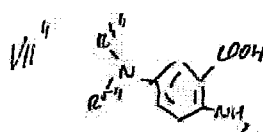
The following compounds were prepared using solution phase parallel synthesis following the general description below:

The appropriately substituted 2-aminobenzoic acid (30 mg) was dissolved in toluene (300 μ L), triethyl amine (300 μ L) was added and the mixture shaken for 10 min at room temperature. Appropriately substituted benzoic acid chloride (2.2 equivalent) was added and the mixture shaken overnight at room temperature. Ethyl acetate (1 mL) and HCl (0.1N, 1 mL) were added followed by shaking for 30 min. The organic layer was separated and saturated, $NaHCO_3$ (0.5 mL) added followed by shaking for 2 min. The organic layer was separated and filtered through a silica-gel column (Sep-Pack plus), the column was washed with dichloromethane (3 mL) and the collected organic phases evaporated. The identity and purity was checked by LC-MS using a PE Sciex API 100 LC/MS system using Waters 3 mm $\times$ 150 mm 3.5 μ C-18 symmetry column and positive ion-spray with a flow rate at 20 μ L/min (Table 4).



XP-002299336

246
Cafe P91/PCI
D4



DO: DO: DO

1-6

6

Substance

Beilstein Registry Number	181275
Beilstein Preferred RN	6760-14-1
CAS Registry Number	6760-14-1
Chemical Name	2-amino-5-nitro-nicotinic acid
	2-Amino-5-nitro-nicotinsaeure
	2-amino-5-nitro-3-pyridinecarboxylic acid
Autoname	2-amino-5-nitro-nicotinic acid
Molecular Formula	C ₆ H ₅ N ₃ O ₄
Molecular Weight	183.12
Lawson Number	27806
Compound Type	heterocyclic
Constitution ID	143144
Tautomer ID	178764
Beilstein Reference	4-22-00-06725, 5-22-13-00610, 6-22
Entry Date	1988/06/27
Update Date	1993/05/05

Field Availability List 1-10 of 10

Code	Field Name	Occ.
<u>RX</u>	Reaction	9
<u>XREF</u>	Crossfile Reference	1
<u>RSTR</u>	Related Structure	2
<u>CDER</u>	Derivative	3
<u>CPD</u>	Crystal Property Description	1
<u>MP</u>	Melting Point	6
<u>UV</u>	Ultraviolet Spectra	1
<u>SLB</u>	Solubility (MCS)	1
<u>FINFO</u>	Further Information	1
<u>CNR</u>	Reference	8

Reaction 1 of 9

Reaction ID 28439

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Reactant BRN
Product BRN
No. of Reaction Details
Reaction Classification
Reagent

Temperature
Other Conditions

Note 1
Ref. 1

119031 2-amino-nicotinic acid
181275 2-amino-5-nitro-nicotinic acid
1
Preparation
concentrated aqueous HNO₃
H₂SO₄
60 C
Erwaermen des Reaktionsprodukts mit Aethanol

Handbook
705353; Journal; Carboni; GCITA9; Gazz.Chim.Ital.; 83; 1953;
637, 640; GCITA9; Gazz.Chim.Ital.; 85; 1955; 1201, 1202.

Reaction 2 of 9

Reaction ID
Reactant BRN
Product BRN
No. of Reaction Details
Reaction Classification
Reagent
Temperature

Note 1
Ref. 1

260329
181272 2-nitroamino-nicotinic acid
181275 2-amino-5-nitro-nicotinic acid
4
Preparation
concentrated aqueous HNO₃
40 C

Handbook
705353; Journal; Carboni; GCITA9; Gazz.Chim.Ital.; 83; 1953;
637, 640; GCITA9; Gazz.Chim.Ital.; 85; 1955; 1201, 1202.

Reaction 3 of 9

Reaction ID
Reactant BRN
Product BRN
No. of Reaction Details
Reaction Classification
Temperature
Pressure

Note 1
Ref. 1

260336
181275 2-amino-5-nitro-nicotinic acid
120353 5-nitro-pyridin-2-ylamine
1
Preparation
300 - 305 C
2 Torr

Handbook
1091785; Journal; Bojarska-Dahlig; ROCHAC; Rocz.Chem.;
30; 1956; 493, 497; Chem.Abstr.; 1957; 14726.

Reaction 4 of 9

Reaction ID
Reactant BRN
Product BRN
No. of Reaction Details
Reaction Classification
Reagent

Note 1
Ref. 1

260337
181275 2-amino-5-nitro-nicotinic acid
181276 2-hydroxy-5-nitro-nicotinic acid
2
Preparation
aqueous NaNO₂
aqueous H₂SO₄

Handbook
705353; Journal; Carboni; GCITA9; Gazz.Chim.Ital.; 83; 1953;
637, 640; GCITA9; Gazz.Chim.Ital.; 85; 1955; 1201, 1202.

Reaction 5 of 9

Reaction ID
Reactant BRN
No. of Reaction Details
Reaction Classification

5372786

181275 2-amino-5-nitro-nicotinic acid

1

Chemical behaviour (half reaction)

Ref. 1

2531379; Journal; Collins; JCSOA9; J.Chem.Soc.; 1963; 1337,1338.

Ref. 2

2583594; Journal; La Manna et al.; BCFAAI; Boll.Chim.Farm.; 112; 1973; 22,23.

Reaction 6 of 9

Reaction ID
Product BRN
No. of Reaction Details
Reaction Classification

5469013

181275 2-amino-5-nitro-nicotinic acid

1

Preparation (half reaction)

Ref. 1

2531379; Journal; Collins; JCSOA9; J.Chem.Soc.; 1963; 1337,1338.

Ref. 2

2583594; Journal; La Manna et al.; BCFAAI; Boll.Chim.Farm.; 112; 1973; 22,23.

Reaction 7 of 9

Reaction ID
Reactant BRN

Product BRN
No. of Reaction Details
Reaction Classification
Temperature

5469014

2037554 sulfuric acid

181272 2-nitroamino-nicotinic acid

181275 2-amino-5-nitro-nicotinic acid

1

Chemical behaviour

100 C

Note 1

Handbook

Ref. 1

705353; Journal; Carboni; GCITA9; Gazz.Chim.Ital.; 83; 1953; 637, 640; GCITA9; Gazz.Chim.Ital.; 85; 1955; 1201, 1202.

Ref. 2

1091785; Journal; Bojarska-Dahlig; ROCHAC; Roczn.Chem.; 30; 1956; 493, 497; Chem.Abstr.; 1957; 14726.

Reaction 8 of 9

Reaction ID
Reactant BRN

Product BRN
No. of Reaction Details
Reaction Classification
Temperature

5469015

3587310 Nitric acid

181272 2-nitroamino-nicotinic acid

181275 2-amino-5-nitro-nicotinic acid

1

Chemical behaviour

40 C

Note 1

Handbook

Ref. 1

705353; Journal; Carboni; GCITA9; Gazz.Chim.Ital.; 83; 1953; 637, 640; GCITA9; Gazz.Chim.Ital.; 85; 1955; 1201, 1202.

Reaction 9 of 9

Reaction ID

5469016

Reactant HNO₃+H₂SO₄
Reactant BRN **119031** 2-amino-nicotinic acid
Product BRN **181275** 2-amino-5-nitro-nicotinic acid
No. of Reaction Details 1
Reaction Classification Chemical behaviour

Note 1 Handbook
Ref. 1 **1055351**; Journal; Philips; CHBEAM; Chem.Ber.; 27; 1894;
840; JLACBF; Justus Liebig's Ann. Chem.; 288; 1895; 259.

Crossfile Reference

Name 3-Pyridinecarboxylic acid, 2-amino-5-nitro-
Crossfile Source CRC Handbook of Chemistry and Physics
External Access ID 22840

Related Structure 1 of 2

Ref. 1 **2592605**; Journal; Carboni; Da Settimo; RSRAAQ; Ric.Sci.
Parte 2 Sez.A.; 8; 1965; 6,1531,1532; Chem.Abstr.; 65;
7137e; 1966.

Related Structure 2 of 2

Related Structure Die fruher (s. H 22 542) mit Vorbehalt unter dieser
Konstitution beschriebene Verbindung ist wahrscheinlich als 2-
Nitroamino-nicotinsaeure (S. 7227) zu formulieren.

Note 1 Handbook
Ref. 1 **1091785**; Journal; Bojarska-Dahlig; ROCHAC; Rocz.Chem.;
30; 1956; 493, 497; Chem.Abstr.; 1957; 14726.

Derivative 1 of 3

Note 1 Sulfat: C₆H₅N₃O₄*H₂SO₄: F.: 185grad (Zers.); B.: 5-Nitro-2-
amino-nicotinsaeure-ethylester, sd. 50percentig. H₂SO₄ (30
min)
Ref. 1 **2531379**; Journal; Collins; JCSEA9; J.Chem.Soc.; 1963;
1337,1338.

Derivative 2 of 3

Note 1 K-Salz: gelb; wenig loesl. in sd. A.
Ref. 1 **2531379**; Journal; Collins; JCSEA9; J.Chem.Soc.; 1963;
1337,1338.

Derivative 3 of 3

Note 1 Sulfat: C₆H₅N₃O₄*H₂SO₄: F.: 185grad (Zers.), aus W.; C.:
kurzes Kochen m. Cyclohexanon (Decarboxylierung) -> 5-
Nitro-2-amino-pyridin
Ref. 1 **2531379**; Journal; Collins; JCSEA9; J.Chem.Soc.; 1963;
1337,1338.

250
150

Crystal Property Description

Ref. 1 2531379; Journal; Collins; JCSOA9; J.Chem.Soc.; 1963; 1337,1338.

Melting Point 1-6 of 6

VALUE (MP) C	Solvent (.SOL)	Note	Ref.
318			1
318			2-3
318		1	4
318 - 319			5
318 - 319	nitrobenzene	2,3	6
240			7

Note 1	Handbook
Note 2	Decomp.
Note 3	Handbook
Ref. 1	<u>5620170</u> ; Journal; Sepiol, Jadwiga; Tomasik, Piotr; ACHUDC; Acta Chim.Hung.; EN; 121; 4; 1986; 333-338.
Ref. 2	<u>2592605</u> ; Journal; Carboni; Da Settimo; RSRAAQ; Ric.Sci. Parte 2 Sez.A.; 8; 1965; 6,1531,1532; Chem.Abstr.; 65; 7137e; 1966.
Ref. 3	<u>2592904</u> ; Journal; Carboni; Da Settimo; RSRAAQ; Ric.Sci. Parte 2 Sez.A.; 8; 1965; 6,1531; Chem.Abstr.; 65; 7137e; 1966.
Ref. 4	<u>705353</u> ; Journal; Carboni; GCITA9; Gazz.Chim.Ital.; 83; 1953; 637, 640; GCITA9; Gazz.Chim.Ital.; 85; 1955; 1201, 1202.
Ref. 5	<u>2583594</u> ; Journal; La Manna et al.; BCFAAI; Boll.Chim.Farm.; 112; 1973; 22,23.
Ref. 6	<u>1091785</u> ; Journal; Bojarska-Dahlig; ROCHAC; Roczn.Chem.; 30; 1956; 493, 497; Chem.Abstr.; 1957; 14726.
Ref. 7	<u>2531379</u> ; Journal; Collins; JCSOA9; J.Chem.Soc.; 1963; 1337,1338.

Ultraviolet Spectra

Description	Absorption maxima
Absorption Maxima	285 nm
Ext./Abs. Coefficient	65 l*mol ⁻¹ *cm ⁻¹

Ref. 1 5620170; Journal; Sepiol, Jadwiga; Tomasik, Piotr; ACHUDC; Acta Chim.Hung.; EN; 121; 4; 1986; 333-338.

Solubility (MCS)

Ref. 1 2531379; Journal; Collins; JCSOA9; J.Chem.Soc.; 1963; 1337,1338.

Further Information

Ref. 1

2531379; Journal; Collins; JCSOA9; J.Chem.Soc.; 1963; 1337,1338.

Reference 1 of 8

705353; Journal; Carboni; GCITA9; Gazz.Chim.Ital.; 83; 1953; 637, 640; GCITA9; Gazz.Chim.Ital.; 85; 1955; 1201, 1202.

Reference 2 of 8

1065351; Journal; Philips; CHBEAM; Chem.Ber.; 27; 1894; 840; JLACBF; Justus Liebigs Ann. Chem.; 288; 1895; 259.

Reference 3 of 8

1091785; Journal; Bojarska-Dahlig; ROCHAC; Roczn.Chem.; 30; 1956; 493, 497; Chem.Abstr.; 1957; 14726.

Reference 4 of 8

2531379; Journal; Collins; JCSOA9; J.Chem.Soc.; 1963; 1337,1338.

Reference 5 of 8

2583594; Journal; La Manna et al.; BCFAAI; Boll.Chim.Farm.; 112; 1973; 22,23.

Reference 6 of 8

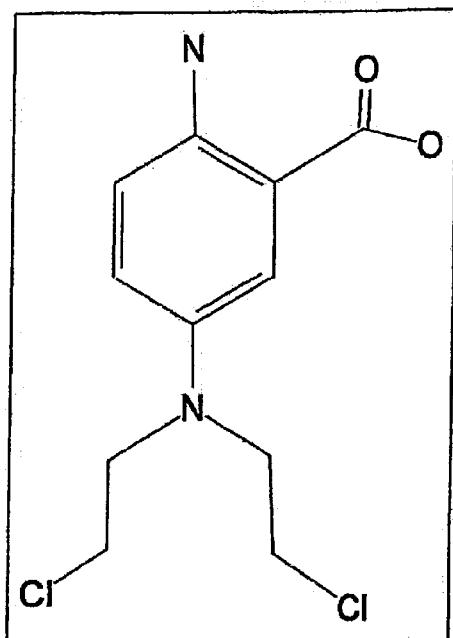
2592605; Journal; Carboni; Da Settimo; RSRAAQ; Ric.Sci. Parte 2 Sez.A.; 8; 1965; 6,1531,1532; Chem.Abstr.; 65; 7137e; 1966.

Reference 7 of 8

2592904; Journal; Carboni; Da Settimo; RSRAAQ; Ric.Sci. Parte 2 Sez.A.; 8; 1965; 6,1531; Chem.Abstr.; 65; 7137e; 1966.

Reference 8 of 8

5620170; Journal; Sepiol, Jadwiga; Tomasik, Piotr; ACHUDC; Acta Chim.Hung.; EN; 121; 4; 1986; 333-338.



XP-002299337

252
Case 291 PCT
DS 252

00 20 00
1-2 2

Substance

Beilstein Registry Number	2736699
Beilstein Preferred RN	50891-49-1
CAS Registry Number	50891-49-1
Chemical Name	2-amino-5-[bis-(2-chloro-ethyl)-amino]-benzoic acid
Autoname	2-amino-5-[bis-(2-chloro-ethyl)-amino]-benzoic acid
Molecular Formula	C ₁₁ H ₁₄ Cl ₂ N ₂ O ₂
Molecular Weight	277.15
Lawson Number	16039, 2826
Compound Type	isocyclic
Constitution ID	2481440
Tautomer ID	2627529
Beilstein Reference	5-14
Entry Date	1989/07/11
Update Date	1992/04/28

Field Availability List 1-3 of 3

Code	Field Name	Occ.
<u>RX</u>	Reaction	1
<u>MP</u>	Melting Point	1
<u>CNR</u>	Reference	1

Reaction

Reaction ID	7745412
Product BRN	2736699 2-amino-5-[bis-(2-chloro-ethyl)-amino]-benzoic acid
No. of Reaction Details	1
Reaction Classification	Preparation (half reaction)

Ref. 1 2968014; Journal; Burdulene et al.; LMDBAL; Liet.TSR Mokslu Akad.Darb.Ser.B; 4; 1973; 55,58; Chem.Abstr.; 80; 36800b; 1974.

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1 25

**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES**

202012

Bogotá, D.C.: 01 septiembre de 2009

Apoderado: Emilio Ferrero

ASUNTO

Radicación 06-018278
Trámite 002
Evento 001
Actuación 430
Oficio
Folios 10580

Patente de Invención



Vía PCT (fase nacional)



Cap. I



Cap. II



No. Sol. Internacional

PCT/HU2004/000080

Fecha de Presentación Internal.

23-jul-2004

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

Documentos estudiados

Descripción:

Folios: 52 a 108

Como se radicó inicialmente.

Reivindicaciones:

Folios 109 a 123

Como se radicó inicialmente.

Folios 179 a 189

Modificadas.

Folios 196 a 206

Modificadas nuevamente.

Folios 219 a 230

Modificadas nuevamente.

Prioridad:

Folio: 1 , HU P0302440, 31/jul/2003

HU P0401467, 21/jul/2004

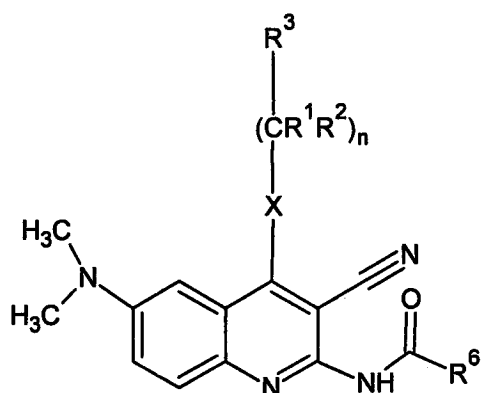
HU P0401468, 21/jul/2004

Objeto de la invención

Título de la solicitud: DERIVADOS AMINOQUINOLINA Y SU USO COMO LIGANDOS A₃ DE ADENOSINA

Definir el objeto de la invención son los compuestos que caen bajo el alcance de la fórmula:

255
2



Reivindicaciones 1-5: Compuestos de la fórmulas I y II"
Reivindicación 5: Procedimientos de síntesis para obtener los compuestos.
Reivindicación 6 y 7: Composiciones que los contienen

Debe especificarse en qué clasificación se catalogó la invención, según la Clasificación Internacional de Patentes : C07D215/54, C07C229/60, A61K31/47, A61P11/00, 9/00

Determinación del Estado de la Técnica

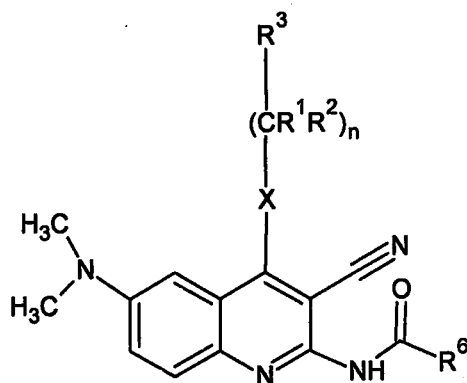
La fecha que se ha tenido en cuenta para determinar el estado de la técnica teniendo en cuenta será la de la fecha de presentación de la solicitud de la cual se reclama prioridad, 31/jul/2003

Teniendo en cuenta la fecha indicada se realizó la búsqueda en el estado de la técnica en las bases de datos a que esta oficina tiene acceso, encontrándose los siguientes documentos más relevantes:

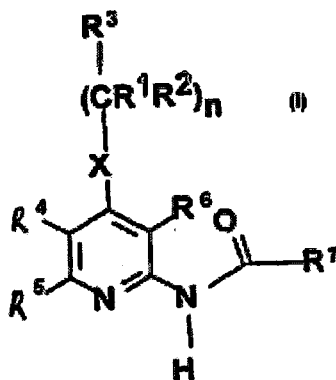
Nº	Documento	Título	Fecha de publicación*
D1	WO02096879	Aminoquinoline and aminopyridine derivatives and their use as adenosine A ₃ ligands	02-dic-2002
D2	WO0018761	Substituted 3-cyanoquinolinones as protein tyrosinekinases inhibitors	06-abr-2000

Análisis de patentabilidad (según requisito) (artículo 14 D486 de la C.AN.)

La solicitud en estudio reclama derivados 3-cianoquinolinonas de fórmulas (I) y (II")



-D1 [WO 02096879] describe derivados 3-cianoquinolina útiles como ligandos del receptor de adenosina A_3 .



R^6 stands for hydrogen atom or a cyano group.

R^4 and R^5 stand for hydrogen atom or form together an 1,3-butadienyl group, optionally substituted by a methylenedioxy group or one or more straight or branched C_{1-4} alkyl group, straight or branched C_{1-4} alkoxy group, hydroxy group or halogen atom;

The present invention relates to adenosine A_3 receptor ligands of the general formula (I), within those preferably antagonists, as well as their salts, solvates and isomers, and the pharmaceutical compositions containing them, to the use of the compounds of the general formula (I), as well as their salts, solvates and isomers, to the preparation of the compounds of the general formula (I) and their salts, solvates and isomers, furthermore to the new intermediates of the general formulae (II) (III) and (IV) and to the preparation thereof.

+

N-(4-benzylamino-3-cyanoquinolin-2-yl)thiophene-2-carboxamide;

N-(4-[2-thienylmethylamino]-3-cyanoquinolin-2-yl)thiophene-3-carboxamide;
4-methoxy-N-(4-[2-thienylmethylamino]-3-cyanoquinolin-2-yl)benzamide;

3,4-methylenedioxy-N-(4-[2-thienylmethylamino]-3-cyanoquinolin-2-yl)-benzamide;

N-(4-[2-furylmethylamino]-3-cyanoquinolin-2-yl)furan-2-carboxamide;

N-(4-[2-furylmethylamino]-3-cyanoquinolin-2-yl)thiophene-3-carboxamide,

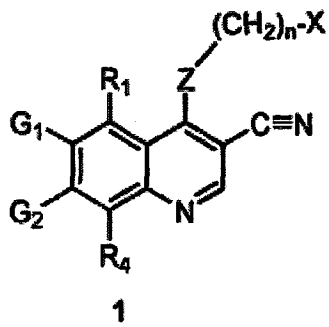
Vease toda la solicitud especialmente folios 6, tabla I, tabla II, tabla III y esquemas de reacción, que demuestran que la materia reclamada en la presente solicitud no es nueva.

Nivel inventivo (artículo 18 D486 de la C.A.N.)

257
4 257

D2 [WO 0018761] enseña derivados de 3-cianoquinolinas

This invention provides a compound of formula 1:



wherein:
X is a bicyclic aryl or bicyclic heteroaryl ring system of 8 to 12 atoms where the bicyclic heteroaryl ring contains 1 to 4 heteroatoms selected from N, O, and S with the proviso that the bicyclic heteroaryl ring does not contain O-O, S-S, G1, G2, R1, and R4 are each, independently, hydrogen, phenyl, thiophenoxy, benzyl, amino, hydroxyamino, alkoxyamino of 1-4 carbon atoms, alkylamino of 1-6 carbon atoms, dialkylamino of 2 to 12 carbon atoms, N-alkylcarbamoyl, N,N-dialkylcarbamoyl, N-alkyl-N-
Vease todo D2 especialmente folios 7-15, esquemas de reacción donde se observa que la materia reclamada en la solicirud en estudio presenta una diferencia en el sustituyente en la posición 2. En D2 no aparece ese sustituyente amido, pero para una persona normalmente versada en la materia es posible hacer esa sutitución ya que los compuestos de la solicitud no muestran un avance técnico real respecto a D2. No se muestra un salto cualitativo, o una regla técnica diferente, por lo tanto la solicitud en estudio se considera que no tiene nivel inventivo.

Conclusión:

Los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:
Una solicitud que tiene afectada la novedad obviamente no posee nivel inventivo.

N°	Documento N°	Reivindicaciones afectadas	Requisito que afecta
D1	WO 02096879	1-8	Novedad y nivel inventivo.
D2	WO 0018761	1-8	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de 60 días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
5 December 2002 (05.12.2002)

PCT

(10) International Publication Number
WO 02/096879 A1(51) International Patent Classification⁷: C07D 215/48,
A61K 31/4706, A61P 43/00, C07D 405/12, 409/12,
409/14, 405/14, 215/46, 401/12, 213/85

(21) International Application Number: PCT/HU02/00048

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(25) Filing Language: English

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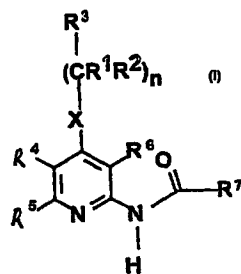
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ning of each regular issue of the PCT Gazette.

(54) Title: AMINOQUINOLINE AND AMINOPYRIDINE DERIVATIVES AND THEIR USE AS ADENOSINE A3 LIGANDS

(57) Abstract: Compounds of general formula (I), wherein R⁴ and R⁵ stand for hy-
drogen atom or form together an 1,3-butadienyl group, optionally substituted by a
methylenedioxy group or one or more straight or branched C₁₋₄ alkyl group, straight
or branched C₁₋₄ alkoxy group, hydroxy group or halogen atom; are strong adenosine
A₃ receptor ligands preferably antagonists.A favourable group of the compounds of the general formula (IA) is
formed by the compounds whereinR¹ stands for hydrogen atom, or methyl group;R² stands for hydrogen atom, or methyl group;5 R³ stands for phenyl- or thienyl- or furyl group;R⁹, R¹⁰, R¹¹, and R¹² mean independently hydrogen atom or straight or branched C₁₋₄ alkyl
group, or straight or branched C₁₋₄ alkoxy group, or hydroxy group or halogen atom,
orR⁹ and R¹² stand for hydrogen atom and R¹⁰ and R¹¹ form together a methylenedioxy
group;R⁶ stands for hydrogen atom, or cyano group;R⁷ stands for 4-methoxyphenyl-, 3-methylphenyl-, 3-methoxyphenyl-, 3-thienyl-, or 3-
furyl-group,

X stands for -NH-group or for oxygen atom and

15 n stands for 1 -

and their salts, solvates, optically active isomers and the salts, solvates thereof.

Especially favourable are the following compounds complying with the above
criteria:

3-methyl-N-(4-benzylamino-3-cyanoquinolin-2-yl)benzamide;

20 4-methoxy-N-(4-benzylamino-3-cyanoquinolin-2-yl)benzamide;

3-methoxy-N-(4-benzylamino-3-cyanoquinolin-2-yl)benzamide;

3,4-methylenedioxy-N-(4-benzylamino-3-cyanoquinolin-2-yl)benzamide;

N-(4-benzylamino-3-cyanoquinolin-2-yl)thiophene-2-carboxamide;

25 N-(4-[2-thienylmethylamino]-3-cyanoquinolin-2-yl)thiophene-3-carboxamide;

4-methoxy-N-(4-[2-thienylmethylamino]-3-cyanoquinolin-2-yl)benzamide;

3,4-methylenedioxy-N-(4-[2-thienylmethylamino]-3-cyanoquinolin-2-yl)-benzamide;

N-(4-[2-furylmethylamino]-3-cyanoquinolin-2-yl)furan-2-carboxamide;

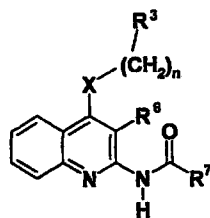
N-(4-[2-furylmethylamino]-3-cyanoquinolin-2-yl)thiophene-3-carboxamide,

30 and their salts, solvates, optically active isomers and the salts, solvates thereof.

According to another of its aspects, the present invention also relates to
pharmaceutical compositions containing as active principles the compounds of the general
formula (I) or their isomers, salts and solvates, which are preferably oral compositions, but

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TABLE I



5

No.:	X	R ³	R ⁶	R ⁷	n	Mp [°C]
11.	NH		CN		1	237
12.	NH		CN		1	128
13.	NH		CN		1	116
14.	NH		CN		1	100,5
15.	NH		CN		1	223

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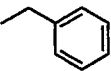
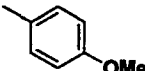
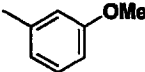
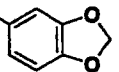
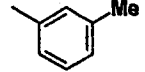
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17.	NH		CN		1	193
18.	NH		CN		1	208
19.	NH		CN		1	215
20.	NH		CN		1	250
21.	NH		CN		1	205
22.	NH		CN		1	238
23.	NH		CN		1	212
24.	NH		CN		1	215
25.	NH		CN		1	234

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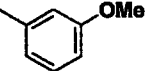
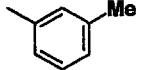
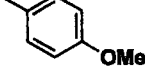
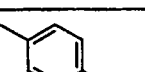
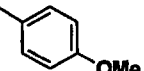
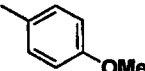
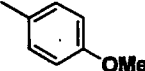
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26.	NH		CN		1	160,5
27.	NH		CN		1	184
28.	NH		CN		1	141,5
29.	NH		CN		1	194
30.	NH		CN		1	203
31.	NH		CN		1	152
32.	NH		CN		1	190
33.	NH		CN		1	202
34.	NH		CN		1	207
35.	NH		CN		1	159
36.	NH		CN		1	200

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37.	NH		CN		1	206
38.	NH		CN		1	221
39.	NH		CN		1	198
40.	NH		CN		1	158
41.	NH		CN		1	178
42.	NH		CN		1	198,5
43.	NH		CN		1	197,5
44.	NH		CN		1	191
45.	NH		CN		1	168,5
46.	N-Me		CN		1	155

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47.	NH		H		1	172
48.	NH		H		1	250
49.	NH		H		1	264
50.	NH		H		1	265
51.	NH		H		1	163
52.	O		CN		1	157
53.	S		CN		1	196
54.	S=O		CN		1	205
55.	NH	H	CN		0	266
56.	NH		CN		1	154

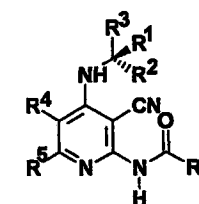
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57	NH		CN		1	145
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TABLE II.

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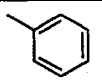
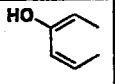
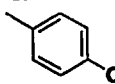
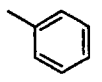
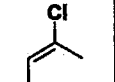
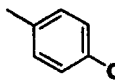
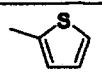
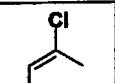
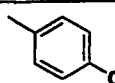
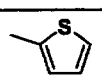
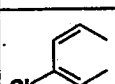
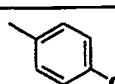
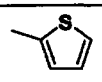
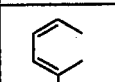
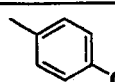
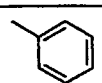
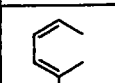
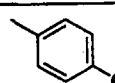
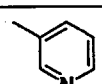
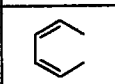
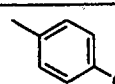
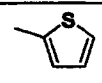
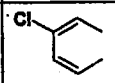
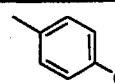
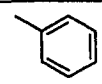
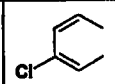
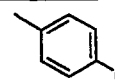
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59.	H	Me					145
60.	H	H					119
61.	H	H					119

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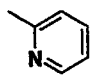
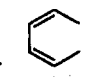
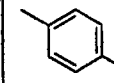
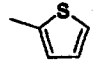
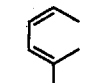
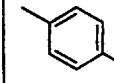
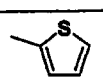
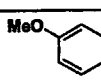
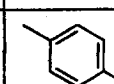
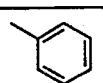
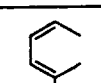
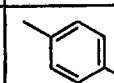
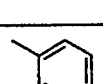
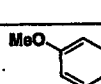
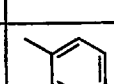
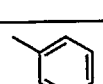
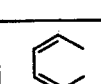
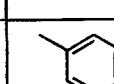
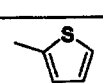
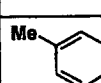
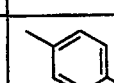
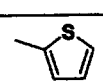
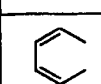
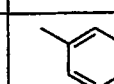
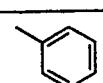
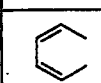
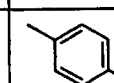
262

22

62.	H	H				243
63.	H	H				176
64.	H	H				171
65.	H	H				199
66.	H	H				203
67.	H	H				180
68.	H	H				117
70.	H	H				153
71.	H	H				215

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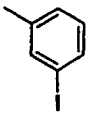
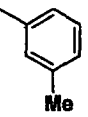
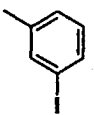
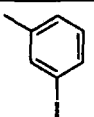
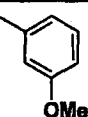
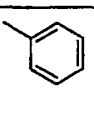
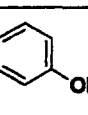
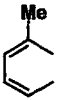
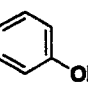
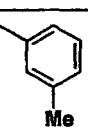
72.	H	H				237
73.	H	H				275
74.	H	H				245
75.	H	H				247
76.	H	H				222
77.	H	H				218
78.	H	H				214
79.	H	H				252
80.	H	H				178

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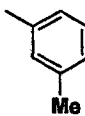
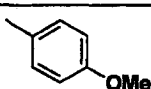
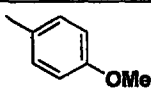
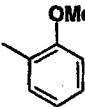
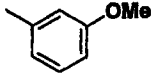
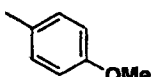
263

24

81.	H	H				173
82.	H	H				212
83.	H	H				184
84.	H	H				150
85.	H	H				195
86.	H	H				171
87.	H	H				217
88.	H	H				149
89.	H	H				135

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90.	H	H				12	
91.	H	H				25	
92.	H	H				26	
93.	H	H				15	
94.	H	H				14	
95.	H	H				21	
96.	H	H				18	
97.	H	H		H	H		16
98.	H	H		H	H		16
99.	H	H		H	H		18

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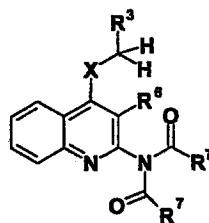
26

100	H	H		H	H		216
101	H	H		H	H		206

Structure and physical characteristics of intermediates of general formula (II) prepared by the method described in Example 1. are shown in Table III.

5

TABLE III.



10

No.:	X	R ³	R ⁶	R ⁷	Mp [°C]
102	NH		CN		213
103.	NH		CN		208

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104	NH		CN		178s
105	NH		CN		158
106	NH		CN		210
107	NH		CN		223
108	NH		CN		224
109	NH		CN		212
110	NH		CN		198
111	NH		CN		208
112	NH		CN		168
113	NH		CN		168

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114	NH		CN		225
115	NH		CN	Me	152
116	NH		CN	Et	192
117	NH		CN		177
118	NH		CN		169
119	NH		CN		151
120	NH		CN		218
121	NH		CN		194
122	NH		CN		188
123	NH		CN		179

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124	NH		CN		239
125	NH		H		162
126	NH		H		262
127	S		CN		170
128			CN		228

Structure and physical characteristics of intermediates of general formula (III) and (IIIa) prepared by the method described in Example 1. are shown in Table IV.

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Substituted 3-cyanoquinolines as protein tyrosine
kinase inhibitors

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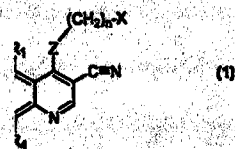
A1	(11) International Publication Number	WO 00/18761
	(43) International Publication Date	6 April 2000 (06.04.00)

IS99/22054 (22.09.99) (74) Agents: MILOWSKY, Arnold, S.; American Home Products Corporation, Patent Law Dept. 2B, One Campus Drive, Parsippany, NJ 07054 (US) et al.

(58) US (81) Designated States: AB, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

AS PROTEIN TYROSINE KINASE INHIBITORS



1) wherein R₁, G₁, G₂, R₄, Z, X and n are defined herein, or a pharmaceutically acceptable salt and in the treatment of polycystic kidney disease.

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PCT/US99/22054

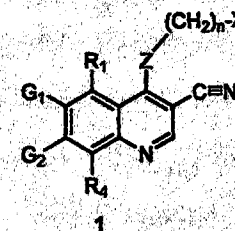
- 7 -

the substituents at position 6 and a halogen atom at position 7. The application WO-9519774 describes compounds where one or more of the carbon atoms at positions 5-8 can be replaced with heteroatoms resulting in a large variety of bicyclic systems where the left-hand ring is a 5 and 6-membered heterocyclic ring; in addition, a variety of substituents are allowed on the left-hand ring. The application EP-682027-A1 describes certain pyrrolopyrimidine inhibitors of PTKs. The application WO-9519970 describes compounds in which the left-hand aromatic ring of the basic quinazoline structure has been replaced with a wide variety of different heterocyclic rings so that the resulting inhibitors are tricyclic. The application EP-635507 describes quinazolines where an additional 5 or 6-membered heterocyclic ring with optional substitution is fused at positions 5 and 6.

In addition to the aforementioned patent applications, a number of publications describe 4-anilinoquinazolines: Fry, D.W., et al., *Science*, 265, 1093 (1994), Rewcastle G.W., et al., *J. Med. Chem.*, 38, 3482 (1995), and Bridges, A.J., et al., *J. Med. Chem.*, 39, 267, (1996). There are no publications that describe 3-cyano quinolines as PTK inhibitors.

DESCRIPTION OF THE INVENTION

This invention provides a compound of formula 1:



wherein:

X is a bicyclic aryl or bicyclic heteroaryl ring system of 8 to 12 atoms where the bicyclic heteroaryl ring contains 1 to 4 heteroatoms selected from N, O, and S with the proviso that the bicyclic heteroaryl ring does not contain O-O, S-S,

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he bicyclic aryl or bicyclic heteroaryl ring may be
 r tetra-substituted with a substituent selected from
 ogen, oxo, thio, alkyl of 1-6 carbon atoms, alkenyl
 yl of 2-6 carbon atoms, azido, hydroxyalkyl of 1-6
 hyl, alkoxymethyl of 2-7 carbon atoms,
 7 carbon atoms, alkoxy of 1-6 carbon atoms,
 atoms, hydroxy, trifluoromethyl, cyano, nitro,
 -7 carbon atoms, carboalkyl of 2-7 carbon atoms,
 oxy, benzoyl, benzyl, amino, alkylamino of 1-6
 ino of 2 to 12 carbon atoms, phenylamino,
 no of 1-6 carbon atoms, alkenoylamino of 3-8
 ino of 3-8 carbon atoms, carboxyalkyl of 2-7
 alky of 3-8 carbon atoms, aminoalkyl of 1-5 carbon
 of 2-9 carbon atoms, N,N-dialkylaminoalkyl of 3-
 kylaminoalkoxy of 2-9 carbon atoms, N,N-
) carbon atoms, mercapto, and benzoylamino; or

pyrimidinyl, or phenyl ring; wherein the pyridinyl,
 ; may be optionally mono- or di-substituted with a
 ie group consisting of halogen, alkyl of 1-6 carbon
 bon atoms, alkynyl of 2-6 carbon atoms, azido,
 n atoms, halomethyl, alkoxymethyl of 2-7 carbon
 of 2-7 carbon atoms, alkoxy of 1-6 carbon atoms,
 atoms, hydroxy, trifluoromethyl, cyano, nitro,
 -7 carbon atoms, carboalkyl of 2-7 carbon atoms,
 oxy, benzoyl, benzyl, amino, alkylamino of 1-6
 ino of 2 to 12 carbon atoms, phenylamino,
 no of 1-6 carbon atoms, alkenoylamino of 3-8
 ino of 3-8 carbon atoms, carboxyalkyl of 2-7

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carbon atoms, carboalkoxyalkyl of 3-8 carbon atoms, aminoalkyl of 1-5 carbon
 atoms, N-alkylaminoalkyl of 2-9 carbon atoms, N,N-dialkylaminoalkyl of 3-
 10 carbon atoms, N-alkylaminoalkoxy of 2-9 carbon atoms, N,N-
 dialkylaminoalkoxy of 3-10 carbon atoms, mercapto, and benzoylamino;

5 T is bonded to a carbon of A and is:

-NH(CH₂)_m-, -O(CH₂)_m-, -S(CH₂)_m-, -NR(CH₂)_m-, -(CH₂)_m-
 -(CH₂)_mNH-, -(CH₂)_mO-, -(CH₂)_mS-, or -(CH₂)_mNR-;

L is an unsubstituted phenyl ring or a phenyl ring mono-, di-, or tri-substituted with a
 substituent selected from the group consisting of halogen, alkyl of 1-6 carbon
 10 atoms, alkenyl of 2-6 carbon atoms, alkynyl of 2-6 carbon atoms, azido,
 hydroxyalkyl of 1-6 carbon atoms, halomethyl, alkoxymethyl of 2-7 carbon
 atoms, alkanoyloxymethyl of 2-7 carbon atoms, alkoxy of 1-6 carbon atoms,
 alkylthio of 1-6 carbon atoms, hydroxy, trifluoromethyl, cyano, nitro,
 15 carboxy, carboalkoxy of 2-7 carbon atoms, carboalkyl of 2-7 carbon atoms,
 phenoxy, phenyl, thiophenoxy, benzoyl, benzyl, amino, alkylamino of 1-6
 carbon atoms, dialkylamino of 2 to 12 carbon atoms, phenylamino,
 benzylamino, alkanoylamino of 1-6 carbon atoms, alkenoylamino of 3-8
 carbon atoms, alkynoylamino of 3-8 carbon atoms, carboxyalkyl of 2-7
 carbon atoms, carboalkoxyalkyl of 3-8 carbon atoms, aminoalkyl of 1-5 carbon
 20 atoms, N-alkylaminoalkyl of 2-9 carbon atoms, N,N-dialkylaminoalkyl of 3-
 10 carbon atoms, N-alkylaminoalkoxy of 2-9 carbon atoms, N,N-
 dialkylaminoalkoxy of 3-10 carbon atoms, mercapto, and benzoylamino;
 provided that L can be an unsubstituted phenyl ring only when m > 0 and T is
 not -CH₂NH- or -CH₂O-; or

25 L is a 5- or 6-membered heteroaryl ring where the heteroaryl ring contains 1 to 3
 heteroatoms selected from N, O, and S, with the proviso that the heteroaryl
 ring does not contain O-O, S-S, or S-O bonds, and where the heteroaryl ring
 is optionally mono- or di-substituted with a substituent selected from the
 group consisting of halogen, oxo, thio, alkyl of 1-6 carbon atoms, alkenyl of
 30 2-6 carbon atoms, alkynyl of 2-6 carbon atoms, azido, hydroxyalkyl of 1-6

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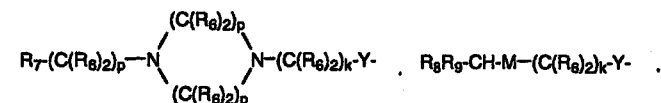
- 10 -

, alkoxyethyl of 2-7 carbon atoms, alkanoyloxy-
ms, alkoxy of 1-6 carbon atoms, alkylthio of 1-6
fluoromethyl, cyano, nitro, carboxy, carboalkoxy of
alkyl of 2-7 carbon atoms, phenoxy, phenyl,
nzy, amino, alkylamino of 1-6 carbon atoms,
12 carbon atoms, phenylamino, benzylamino,
bon atoms, alkenoylamino of 3-8 carbon atoms,
rbon atoms, carboxyalkyl of 2-7 carbon atoms,
rbon atoms, aminoalkyl of 1-5 carbon atoms, N-
rbon atoms, N,N-dialkylaminoalkyl of 3-10 carbon
y of 2-9 carbon atoms, N,N-dialkylaminoalkoxy of
to, and benzoylamino;

carboalkyl of 2-7 carbon atoms;
ependently, hydrogen, halogen, alkyl of 1-6 carbon
on atoms, alkynyl of 2-6 carbon atoms, alkenyloxy
kynyloxy of 2-6 carbon atoms, hydroxymethyl,
of 1-6 carbon atoms, alkenoyloxy of 3-8 carbon
3 carbon atoms, alkanoyloxymethyl of 2-7 carbon
of 4-9 carbon atoms, alkynoyloxymethyl of 4-9
yl of 2-7 carbon atoms, alkoxy of 1-6 carbon atoms,
atoms, alkylsulphinyl of 1-6 carbon atoms,
on atoms, alkylsulfonamido of 1-6 carbon atoms,
i carbon atoms, alkynylsulfonamido of 2-6 carbon
methyl, trifluoromethoxy, cyano, nitro, carboxy,
atoms, carboalkyl of 2-7 carbon atoms, phenoxy,
zyl, amino, hydroxyamino, alkoxyamino of 1-4
of 1-6 carbon atoms, dialkylamino of 2 to 12
lcarbamoyl, N,N-dialkylcarbamoyl, N-alkyl-N-

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alkenylamino of 4 to 12 carbon atoms, N,N-dialkenylamino of 6-12 carbon
atoms, phenylamino, benzylamino,



5

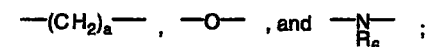
$R_7-(C(R_6)_2)_q-Y$, $R_7-(C(R_6)_2)_p-M-(C(R_6)_2)_k-Y$, or $Het-(C(R_6)_2)_q-W-(C(R_6)_2)_k-Y$;

or R_1 and R_4 are as defined above and G_1 or G_2 or both are R_2-NH- ;

10

or if any of the substituents R_1 , G_2 , G_3 , or R_4 are located on contiguous
carbon atoms then they may be taken together as the divalent radical
 $-O-C(R_6)_2-O-$;

Y is a divalent radical selected from the group consisting of

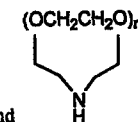


15 R_7 is $-NR_6R_6$, $-OR_6$, $-J$, $-N(R_6)_3$, or $-NR_6(OR_6)$;

M is $>NR_6$, $-O-$, $>N-(C(R_6)_2)_pNR_6R_6$, or $>N-(C(R_6)_2)_p-OR_6$;

W is $>NR_6$, $-O-$ or is a bond;

Het is selected from the group consisting of morpholine, thiomorpholine,
thiomorpholine S-oxide, thiomorpholine S,S-dioxide, piperidine, pyrrolidine,
20 aziridine, pyridine, imidazole, 1,2,3-triazole, 1,2,4-triazole, thiazole,
thiazolidine, tetrazole, piperazine, furan, thiophene, tetrahydrothiophene,



tetrahydrofuran, dioxane, 1,3-dioxolane, tetrahydropyran, and

wherein Het is optionally mono- or di-substituted on carbon or nitrogen with
25 R_6 , optionally mono- or di-substituted on carbon with hydroxy, $-N(R_6)_2$, or

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