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Title: 2-AMINOTHIOPHENECARBOXAMIDES USEFUL AS CANCER CHEMOTHERAPEUTIC AGENTS

Abstract: This invention relates to novel 2-aminothiophenecarboxamide compounds, pharmaceutical compositions containing such compounds, and the use of those compounds or compositions as cancer chemotherapeutic agents.
2-Aminothiophenecarboxamides Useful as Cancer Chemotherapeutic Agents

This application claims benefit of U.S. Provisional Application Serial No.60/603172; filed on August 20, 2004, the contents of which are incorporated herein by reference in their entirety.

This invention relates to novel 2-aminothiophenecarboxamide compounds, pharmaceutical compositions containing such compounds, and the use of those compounds or compositions as cancer chemotherapeutic agents.

Many disease conditions are known to be associated with deregulated angiogenesis. Among these are retinopathies; chronic inflammatory disorders including arthritis; arteriosclerosis; atherosclerosis; macular degeneration; and neoplastic diseases such as cancer. In recent years, much work has been carried out to find inhibitors of angiogenesis, in hopes of developing treatments for such disorders.


US patent 6,448,277 (Novartis) discloses and claims certain benzamide derivatives for inhibition of VEGF receptor tyrosine kinase, tumor growth, and VEGF-dependent cell proliferation.

Published PCT application WO 02/066470 (Amgen) broadly discloses heterocycles containing amido and amino substituent groups, for prophylaxis and treatment of angiogenesis-mediated diseases. Published PCT application WO 2004/005279 (Amgen) discloses certain substituted anthranilic amide derivatives for the prophylaxis and treatment of angiogenesis-mediated diseases. Published PCT application WO 2004/007458 (Amgen) relates to substituted 2-alkylamine nicotinic amide derivatives and their uses in treatment of cancer and other disorders.

Published PCT application WO 00/27819 (Schering) discloses certain anthranilic acid amides for treatment of diseases that are triggered by angiogenesis. Published PCT
application WO 02/090352 (Schering) relates to selective anthranilamide pyridine amides as inhibitors of VEGFR-2 and VEGFR-3. Published PCT application WO 01/81311 (Schering) relates to substituted benzoic acid amides and use thereof for the inhibition of angiogenesis.


The present invention relates to a compound of formula (I) or formula (II)

![Chemical Structures](image)

wherein

$R^1$ represents $-C_{1-4}$ alkyl, or halogen; and the subscript $a$, which represents the number of substituents $R^1$, is 0, 1, or 2;

$R^2$ represents $-C_{1-4}$ alkyl, $-C_{1-4}$ alkoxy, or halogen; and the subscript $b$, which represents the number of substituents $R^2$, is 0, 1, or 2;

$R^3$ represents $-\text{C(O)NR}^{3-1}R^{3-1}$, $-\text{NHC(O)R}^{3-3}$, $-\text{OR}^{3-4}$, or $-\text{CN}$; $-\text{halogen}$; or $-C_{1-4}$ alkoxy; and the subscript $d$, which represents the number of substituents $R^3$, is 0, 1, or 2.
2;

wherein

substituents $R^{3-1}$ each independently represent hydrogen, -C$_{1-4}$ alkyl, -C$_{3-6}$ cycloalkyl, or hydroxy-C$_{1-4}$-alkyl; and

substituents $R^{3-2}$ each independently represents hydrogen or -C$_{1-4}$ alkyl, wherein -C$_{1-4}$ alkyl can optionally be substituted with a substituent selected from the group consisting of hydroxy, -C$_{1-4}$ alkoxy, carboxy, -C$_{1-4}$ alkoxy carbonyl, morpholinyl and N-methylpiperazinyl;

substituent $R^{3-3}$ represents hydrogen or -C$_{1-4}$ alkyl;

substituent $R^{3-4}$ represents hydrogen or -C$_{1-4}$ alkyl; or

$R^3$ represents

$$\begin{array}{c}
\text{N} \quad \text{N} \\
\text{CH}_3 \\
\text{H}_2\text{C} \\
\text{N} \quad \text{N}
\end{array}$$

; and

$X$ represents N or CH;

or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates to a compound of formula (I-1) or formula (II-1),
wherein

$R^3$ represents $-C(O)NR^{3-1}R^{3-1};$ $-NR^{3-2}R^{3-2};$ $-NHC(O)R^{3-3};$ or $-C(O)OR^{3-4};$ and the subscript $d$, which represents the number of substituents $R^3$, is 1, or 2;

wherein

substituents $R^{3-1}$ each independently represent hydrogen, $-C_{1-4}$ alkyl, $-C_{3-6}$ cycloalkyl, or hydroxy-$C_{1-4}$-alkyl; and

substituents $R^{3-2}$ each independently represents hydrogen or $-C_{1-4}$ alkyl, wherein $-C_{1-4}$ alkyl can optionally be substituted with a substituent selected from the group consisting of hydroxy, $-C_{1-4}$ alkoxy, carboxy, $-C_{1-4}$ alkoxy carbonyl, morpholinyl and N-methylpiperazinyl;

substituent $R^{3-3}$ represents hydrogen or $-C_{1-4}$ alkyl;

substituent $R^{3-4}$ represents hydrogen or $-C_{1-4}$ alkyl; or

$R^3$ represents

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*  
\[\text{H}_3\text{C} - \text{CH}_3\]
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; and

$X$ represents $N$ or $\text{CH}$;

or a pharmaceutically acceptable salt thereof.
In another embodiment, the present invention relates to a compound of formula (I-2) or formula (II-2), wherein

![Chemical Structures](image)

wherein

R³ represents -C(O)NR³-1R³-1; -NR³-2R³-2; or -NHC(O)R³-3;

wherein

substituents R³-1 each independently represent hydrogen, -C1-4 alkyl, -C3-6 cycloalkyl, or hydroxy-C1-4-alkyl; and

substituents R³-2 each independently represent hydrogen or -C1-4 alkyl, wherein -C1-4 alkyl can optionally be substituted with a substituent selected from the group consisting of hydroxy, -C1-4 alkoxy, carboxy, -C1-4 alkoxy carbonyl, morpholinyl and N-methylpiperazinyl;

substituent R³-3 represents -C1-4 alkyl; or

R³ represents

![Chemical Structure](image)

or a pharmaceutically acceptable salt thereof.
The invention also relates to pharmaceutical compositions which comprise a compound of formula (I) or formula (II) as defined above plus a pharmaceutically acceptable carrier.

In addition, the invention relates to a method of treating cancer comprising administering to a subject in need thereof an effective amount of a compound of formula (I) or formula (II) as defined above.

Pharmaceutically acceptable salts of the compounds (I) include acid addition salts of mineral acids, carboxylic acids and sulphonic acids, for example salts of hydrochloric acid, hydrobromic acid, sulphuric acid, phosphoric acid, methanesulphonic acid, ethanesulphonic acid, toluenesulphonic acid, benzenesulphonic acid, naphthalenesulphonic acid, acetic acid, propionic acid, lactic acid, tartaric acid, malic acid, citric acid, fumaric acid, maleic acid and benzoic acid.

Pharmaceutically acceptable salts of the compounds (I) also include salts of customary bases, such as for example and preferably alkali metal salts (for example sodium and potassium salts, alkaline earth metal salts (for example calcium and magnesium salts) and ammonium salts derived from ammonia or organic amines having 1 to 16 carbon atoms, such as illustratively and preferably ethylamine, diethylamine, triethylamine, ethyldiisopropylamine, monoethanolamine, diethanolamine, triethanolamine, dicyclohexylamine, dimethylaminoethanol, procaine, dibenzylation, N-methylmorpholine, dihydroabietylamine, arginine, lysine, ethylenediamine and methylpiperidine.

Solvates for the purposes of the invention are those forms of the compounds that coordinate with solvent molecules to form a complex in the solid or liquid state. Hydrates are a specific form of solvates, where the coordination is with water.

For the purposes of the present invention, the substituents have the following meanings, unless otherwise specified:

The terms “halogen” and “halo” mean fluoro, chloro, bromo and iodo, wherein fluoro, chloro, and bromo are preferred.
The term "-C<sub>1,4</sub> alkyl" means a linear or branched saturated carbon group having from 1 to 4 carbon atoms. Such groups include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl.

The terms "-C<sub>1-2</sub> alkoxy and -C<sub>1,4</sub> alkoxy" mean a linear or branched saturated carbon group having from 1 to 2, or from 1 to 4 carbon atoms, said carbon group being attached to an oxygen atom. The oxygen atom is the point of attachment of the alkoxy substituent to the rest of the molecule. Such groups include but are not limited to methoxy, ethoxy, n-propoxy, isopropoxy, and the like.

The term "hydroxy-C<sub>1,4</sub>-alkyl" means an -C<sub>1,4</sub> alkyl in which a hydrogen atom on any carbon atom in the group is replaced by a hydroxy group. Such groups include but are not limited to hydroxymethyl, hydroxyethyl, and the like.

The term "-C<sub>1,4</sub> alkyl-C<sub>1-2</sub> alkoxy" means an -C<sub>1,4</sub> alkyl in which a hydrogen atom on any carbon atom in the group is replaced by a -C<sub>1,2</sub> alkoxy group. Such groups include but are not limited to methoxymethyl, ethoxymethyl, 2-methoxyethyl, 4-ethoxybutyl and the like.

A * symbol next to a bond denotes the point of attachment in the molecule.

When a substituent is attached to its cyclic structure by a 'floating' bond, i.e. a bond which is not attached to a particular atom in the cyclic structure, that substituent is meant to be possible to attach at any atom of the cyclic structure. For example, R<sup>3</sup> is meant to be possible to attach at any carbon atom of the pyridine or pyrimidine ring of the cyclic structure, also at the carbon atom of CH if X represents CH, substituting the hydrogen atom; likewise R<sup>2</sup> is meant to be possible to attach at any carbon atom of the thiophene ring of the cyclic structure.

The compounds of this invention may contain one or more asymmetric centers, depending upon the location and nature of the various substituents desired. Asymmetric carbon atoms may be present in the (R) or (S) configuration. It is intended that all possible stereoisomers (including enantiomers and diastereomers) are included within the scope of the present invention. Preferred compounds are those with the absolute configuration of
the compound of this invention which exhibits the more desirable biological activity. Separated, pure or partially purified stereoisomers or racemic mixtures of the compounds of this invention are also included within the scope of the present invention. The purification of said isomers and the separation of said stereoisomeric mixtures can be accomplished by standard techniques known in the art.

In another embodiment, the present invention relates to a compound of formula (I) or formula (II), wherein

\[ R^1 \text{ represents } -C_{1-4} \text{ alkyl, or halogen; and the subscript } a, \text{ which represents the number of substituents } R^1, \text{ is 0, 1, or 2;} \]

\[ R^2 \text{ represents } -C_{1-4} \text{ alkyl, } -C_{1-4} \text{ alkoxy, or halogen; and the subscript } b, \text{ which represents the number of substituents } R^2, \text{ is 0, 1, or 2;} \]

\[ R^3 \text{ represents } -C(O)NR^{3-1}_{1}R^{3-1}; -NR^{3-2}_{1}R^{3-2}; -CN; \text{-halogen; or } -C_{1-4} \text{ alkyl; and the subscript } d, \text{ which represents the number of substituents } R^3, \text{ is 0, 1, or 2;} \]

wherein

substituents \( R^{3-1} \) each independently represent hydrogen, \(-C_{1-4} \text{ alkyl, } -C_{3-6} \text{ cycloalkyl, or } -C_{1-4}-\text{alkyl-C}_{1-2}-\text{alkoxy;} \) and

substituents \( R^{3-2} \) each independently represent hydrogen or \(-C_{1-4} \text{ alkyl;} \)

or a pharmaceutically acceptable salt, solvate, or a solvate of a salt thereof.

In another embodiment, the present invention relates to a compound of formula (I-1) or formula (II-1), wherein

\[ R^2 \text{ represents methyl; and the subscript } b, \text{ which represents the number of substituents } R^2, \text{ is 0 or 1;} \]
R³ represents -C(O)NHCH₃; -C(O)NHCH₂CH₃; -C(O)NHCH₃H₅; -NHCH₃; -CN; or chloro; and the subscript d, which represents the number of substituents R³, is 0 or 1;

or a pharmaceutically acceptable salt, solvate, or a solvate of a salt thereof.

General Methods of Preparation

Compounds of formulae (I), (II), (I-1), (I-2), (II-1) or (II-2) may be prepared by synthetic procedures known to those skilled in the art or by methods analogous thereto.

Suitable for use as solvents for processes of this invention are the customary organic solvents which do not change under the reaction conditions and are able to dissolve the reactants of a given reaction. In some cases, as described in the process for Intermediate H, Step 2, the preferable solvent is a protic solvent which include alcohols, such as methanol, ethanol, propanol, isopropanol, or even water. In other cases such as the process for Intermediate A, the preferable solvent is an inert aprotic solvent which include ethers, such as diethyl ether, dioxane, tetrahydrofuran, glycol dimethyl ether or butyl methyl ether. In yet other cases, ketones, such as acetone or butanone, or amides, such as dimethylformamide or hexamethylphosphoric triamide can be used. Carboxylic acids, such as acetic acid or propionic acid, or dimethyl sulphoxide, acetonitrile, ethyl acetate, or halogenated hydrocarbons, such as methylene chloride, chloroform or carbon tetrachloride, or pyridine, picoline or N-methylpiperidine may be used. It is also possible to use mixtures of the solvents mentioned. It is well known to one skilled in the art that many reactions, such as the process for Example 1, Step 1 require the use of anhydrous solvents while some require only solvents of ordinary purity.

In all processes, the reaction temperatures can be varied within a relatively wide range. In general, the reactions are carried out between -78°C and +200°C, preferably between +20°C and +100°C, in particular at the boiling point of the solvent in question.

The reactions can be carried out under atmospheric pressure or else under elevated or
reduced pressure. In general, the reactions are carried out under atmospheric pressure. Most reaction processes of this invention were conducted under an inert atmosphere of dry nitrogen or argon. For example, it is well known to those skilled in the art that some processes, such as Step 1 of Example 1 are best done using such an inert gas, while others, such as Step 2 of Example 1 probably do not require use of an inert gas, however.

When carrying out processes of this invention, any ratio of the substances involved in the reaction may be used. However, in general, the reactants are used in molar amounts. Isolation and purification of the substances according to the invention can be carried out by removing the solvent by distillation under reduced pressure and recrystallizing the residue, which may be obtained in crystalline form after cooling with ice, from a suitable solvent. In many cases, it may be necessary to purify the compounds of the formula (I) or formula (II) by chromatography. Typically, the chromatography is conducted using silica gel and a gradient from hexane to ethyl acetate or a gradient from methylene chloride to 10% methanol. Alternatively, reverse phase (RP) chromatography is used with C-18 bonded packing material and a solvent gradient from, for example, 10% to 60% acetonitrile in water. Such preparative RP chromatography is usually done with an additive such as 0.05% TFA in the solvent to sharpen peak shape. It is well known to those skilled in the art that other modes of chromatography such as preparative TLC, ion exchange or Florosil can be used for normal phase chromatography. Other bonded phases, for reverse phase chromatography, can also be used to purify products.

Suitable bases are the customary inorganic or organic bases. These preferably include alkali metal hydroxides, such as, for example, sodium hydroxide, lithium hydroxide or potassium hydroxide. Alkali metal carbonate, such as sodium carbonate or potassium carbonate, or alkali metal alkoxides, such as, for example, sodium methoxide or potassium methoxide, or sodium ethoxide or potassium ethoxide can be used. Organic amines, such as triethylamine, picoline or N-methylpiperidine, or amides, such as sodium amide, lithium amide, lithium isopropylamidine, or organometallic compounds, such as butyl lithium or phenyl lithium can also be used.

These methods used to synthesize compounds of formulae (I), (II), (I-1), (I-2), (II-1) or (II-2) are summarized below in Reaction Scheme 1, where
means either formula (I), (II), (I-1), (I-2), (II-1) or (II-2). Unless otherwise specifically defined, \( R^1 - R^3 \), a, b and d have the same meanings as defined herein above.

The compounds of formula (I) and (II) are generally prepared starting from the compound of formula (III), where formula (III) means either formula (IIIa) or (IIIb).

Specifically the compound of formula (I) is prepared starting from the compound of formula (IIIa), and the compound of formula (II) is prepared starting from the compound of formula (IIIb) using chemical methodology outlined in Reaction Scheme 1.
As illustrated in Reaction Scheme 1, three synthetic routes can be used to prepare the compounds of formulae (I) and (II).
In one route, the amino group of the compound of formula (III) [i.e., formula (IIIA) or formula (IIIB)] is subjected to either reductive amination using a pyridine carboxaldehyde of formula (IV) and a reducing agent, such as sodium cyanoborohydride, or to direct N-alkylation using a pyridine methyl halide, tosylate or mesylate of formula (V) and a base such as K$_2$CO$_3$ or using NaI. The product formed, formula (VI), is then allowed to react with an aromatic amine of formula (VIII), in the presence of a coupling agent such as PyBOP (when R' = H) or Al(Me)$_3$ (when R' = alkyl) giving the compound of either formula (I) or formula (II).

In the second route, the compound of formula (III) compound is first converted to the aminoamide of formula (IX) by reaction with an aromatic amine of formula (VIII), and the appropriate coupling agent as described above. The formula (IX) compound is then converted to the formula (I) or (II) compound using either the reductive amination method direct N-alkylation as described above for preparation of (VI).

The third route that is described in Reaction scheme 1 starts with the protection of the amino group of formula (III) to give a compound of formula (VII) using a reagent such as (BOC)$_2$. Amide formation by reaction with an aromatic amine of formula (VIII), in the presence of a coupling agent such as PyBOP (when R' = H) or Al(Me)$_3$ (when R' = alkyl) giving the compound of formula (IX). The formula (IX) compound is then converted to the formula (I) or (II) compound using either the reductive amination method direct N-alkylation as described above for preparation of (VI).

Starting materials of Formulae (IV), (V) and (VIII) are commercially available (e.g., Lanxess, Germany) or may be prepared by standard means well known in the art, or as described in Reaction Schemes 2-7.

![Reaction Scheme 2](image)

Compounds of Formula (Va) [Formula (V) where R$^3$ is and Y is Cl], may be prepared as shown in Reaction Scheme 2 by reaction of an acid chloride with a
chloromethyl heteroarylamine of Formula (X), generally in the presence of a base such as triethylamine.

**Reaction Scheme 3**

\[
\text{(XI)} \quad \begin{array}{c}
\text{OH} \\
\text{X} \\
\text{NH}_2
\end{array} \quad \rightarrow \quad \begin{array}{c}
\text{Opg} \\
\text{X} \\
\text{NH}_2
\end{array} \quad \rightarrow \quad \begin{array}{c}
\text{Opg} \\
\text{X} \\
\text{NHBOC}
\end{array} \quad \rightarrow \quad \begin{array}{c}
\text{R}^3 \\
\text{X}
\end{array} \quad \text{lg}
\]

\[
\text{(XIV)} \quad \begin{array}{c}
\text{Opg} \\
\text{X} \\
\text{NBOC}
\end{array} \quad \rightarrow \quad \begin{array}{c}
\text{OH} \\
\text{X} \\
\text{NH}
\end{array} \quad \rightarrow \quad \begin{array}{c}
\text{Y} \\
\text{X}
\end{array} \quad \begin{array}{c}
\text{R}^3 \\
\text{H}
\end{array}
\]

\[\text{pg = protecting group, e.g., BOC}\]
\[\text{Y, lg = leaving group, e.g., halo, MsO, etc.}\]

Compounds of Formula (Vb) [Formula (V) where \(R^3\) is \(\text{H}\)], can be prepared as shown in Reaction Scheme 3 from hydroxymethylheteroaryl amines of Formula (XI). Protection of the alcohol and conversion to the BOC-derivative of Formula (XIII) is followed by N-alkylation to give the intermediate of Formula (XIV).

Deprotection of the alcohol and amine, followed by conversion of the hydroxy group to a leaving group (for example, using \(\text{SOCl}_2\), when \(Y\) is Cl), gives the intermediate of Formula (Vb).
Reaction Scheme 4

Compounds of Formula (Vc) [Formula (V) where $R^3$ is $N^2-R^3$] can be prepared by the route illustrated in Reaction Scheme 4. The chloroheteroarylcarboxylic acid derivative of Formula (XVI) is reduced to the chloroheteroaryl alcohol of Formula (XVII) with a standard reagent such as lithium borohydride. Reaction of the chloro compound with an amine of Formula $(R^{3-2})(R^{3-2})NH$ gives the intermediate alcohol of Formula (XVIII). Conversion of this alcohol to a leaving group, e.g. mesylate, completes the synthesis of the compound of Formula (Vc)

Reaction Scheme 5
Compounds of Formula (Vd) [Formula (V) where $R^3$ is $R^3$], can be prepared as shown in Reaction Scheme 5 from the dicarboxylic acid of Formula (XIX) by conversion through the half acid ester (XX) to the acid amide of Formula (XXI). Esterification of (XXI) provides (XXII) which can be reduced with sodium borohydride to the alcohol (XXIII) and then converted to the Formula (Vd) compound, using for example MsCl and a base such as triethylamine.

**Reaction Scheme 6**

\[
\begin{align*}
\text{O} & \quad \text{Et} \\
\text{O} & \quad \text{Et} \\
\text{N} & \quad \text{O} & \quad \text{NH}_2
\end{align*}
\]

(XXIV) (XXIIb)

An alternate method of preparing the pyridine amide ester of Formula (XXII) is via the Minisci reaction shown in Reaction Scheme 6 in which the pyridine carboxylic acid ester is stirred in formamide with cooling to 10 °C in the presence of an equivalent of concentrated $H_2SO_4$, $FeSO_4$ and $H_2O_2$.

**Reaction Scheme 7**

\[
\begin{align*}
\text{O} & \quad \text{N} & \quad \text{NH} \\
\text{O} & \quad \text{N} & \quad \text{H}
\end{align*}
\]

(XXV) (Ve)

Compounds of Formula (Ve) [Formula (V) where $R^3$ is Cl], can be prepared as shown in Reaction Scheme 7 from 6-chloromethyluracil of Formula (XXV) by treatment with $POCl_3$ as described in *Biorg. Med. Chem.* 2002, 10, 525.

A variety of compounds of Formula (I) can be prepared by elaboration of
compounds, also of Formula (I), prepared by the above schemes. These elaboration methods are illustrated below in Reaction Schemes 8-13.

**Reaction Scheme 8**

![Reaction Scheme 8](image)

For example, the amino compound of Formula (Ia) can be converted to the amide compound of Formula (Ib) as shown in Reaction Scheme 8, by reaction with an acid chloride.

**Reaction Scheme 9**

![Reaction Scheme 9](image)

Additionally, the chloro compound of Formula (Ic) can be converted to the substituted amino compound of Formula (Id) by reaction with an amine and a base such as pyridine in a sealed tube at elevated temperatures. (Scheme 9)

**Reaction Scheme 10**

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Esters of Formula (Ie) and substituted amides of Formula (Ig) may be prepared from the unsubstituted amide of Formula (Ie) by the sequence illustrated in Reaction Scheme 10. Reaction of the amide (Ie) with dimethylformamide-dimethylacetal in methanol provides the ester of Formula (If); reaction of the ester with a substituted amine gives the amide of Formula (Ig).

Reaction Scheme 11

Amino pyrimidines of Formula (2-Amino Li, Im) (6-Amino Ij, In) and unsubstituted pyrimidines of Formula (Ik) may be prepared from the 2, 6-dichloro...
pyrimidine of Formula (Ih) by the sequence illustrated in Reaction Scheme 11. Reaction of the 2, 6-dichloro pyrimidine of Formula (Ih) with a substituted amine (\((R^{3,2}) (R^{3,2})\) NH) gives a mixture of aminochloro pyrimidines of Formula (Ii and Ij) which can be reduced using palladium hydroxide and ammonium formate, to give amino pyrimidines of Formula (Im and In). The starting material of Formula (Ih) can also be reduced using palladium hydroxide and ammonium formate to give the unsubstituted pyrimidine of Formula (Ik).

**Reaction Scheme 12**

![Chemical structures](image)

Diamino pyrimidines of Formula (Io and Ip) can be prepared from aminochloro pyrimidines of Formula (2-Amino Ii) (6-Amino Ij) by the sequence illustrated in Reaction Scheme 12. Treatment of the aminochloro pyrimidines of Formula (2-Amino Ii) (6-Amino Ij) with a substituted amine (\((R^{3,2}) (R^{3,2})\) NH) gives the diamino pyrimidines of Formula (Io and Ip).

**Reaction Scheme 13**

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The alkoxyamino pyrimidine of Formula (Iq) can be prepared from aminochloro pyrimidines of Formula (2-Amino II) by the sequence illustrated in Reaction Scheme 13. Treatment of the aminochloro pyrimidines of Formula (2-Amino II) with a base such as LiOH and an alcohol such as ROH gives alkoxyamino pyrimidine of Formula (Iq).

Generally, a desired salt of a compound of this invention can be prepared in situ during the final isolation and purification of a compound by means well known in the art. Or, a desired salt can be prepared by separately reacting the purified compound in its free base form with a suitable organic or inorganic acid and isolating the salt thus formed. These methods are conventional and would be readily apparent to one skilled in the art.

Additionally, sensitive or reactive groups on the compound of this invention may need to be protected and deprotected during any of the above methods. Protecting groups in general may be added and removed by conventional methods well known in the art (see, for example, T. W. Greene and P.G.M. Wuts, *Protective Groups in Organic Synthesis*; Wiley: New York, (1999)).

By using the above illustrated general schemes and choosing the appropriate starting materials the compounds of the invention may be prepared. To further illustrate the invention, the following specific examples are provided, but are not meant to limit the scope of the invention in any way.
A. Examples

Abbreviations and Acronyms

When the following abbreviations are used throughout the disclosure, they have the following meaning:

- AcOH: acetic acid
- Biotage®: registered trademark of Biotage Corp. brand of MPLC
- CDCl₃-d: chloroform-d
- CD₂Cl₂-d₂: methylene chloride-d₂
- CD₃CN-d₃: acetonitrile-d₃
- CH₂Cl₂: dichloromethane
- DCM: dichloromethane
- DMAP: N,N-dimethylpyridin-4-amine
- DMF: N,N-dimethylformamide
- DMF-DMA: 1,1-dimethoxy-N,N-dimethylmethanamine
- DMSO-d₆: dimethylsulfoxide-d₆
- EtOAc: ethyl acetate
- h: hour(s)
- ¹H NMR: proton nuclear magnetic resonance
- H₂SO₄: sulfuric acid
- HCl: hydrochloric acid
- Hex: hexanes
- HPLC: high performance liquid chromatography
- LCMS: liquid chromatography / mass spectroscopy
- LiOH: lithium hydroxide
- MeOD-d₄: methanol-d₄
- MeOH: methanol
- NaHCO₃: sodium bicarbonate
- NaOH: sodium hydroxide
- Na₂SO₄: sodium sulfate
- min: minute(s)
- POCl₃: phosphoric trichloride
PyBOP (1H-1,2,3-benzotriazol-1-yloxy)(tripyrrolidin-1-yl) phosphonium hexafluorophosphate
rt room temperature
RT retention time (HPLC or LCMS)
R_f TLC retention factor
TFA trifluoroacetic acid
THF tetrahydrofuran
TLC thin layer chromatography

General Analytical Procedures

The structures of representative compounds of this invention were confirmed using the following procedures.
Electron impact mass spectra (EI-MS) were obtained with a Hewlett Packard 5989A mass spectrometer equipped with a Hewlett Packard 5890 Gas Chromatograph with a J & W DB-5 column (0.25 μM coating; 30 m x 0.25 mm). The ion source is maintained at 250 °C and spectra were scanned from 50-800 amu at 2 sec per scan.
High pressure liquid chromatography-electrospray mass spectra (LC-MS) were obtained using either a:
(A) Hewlett-Packard 1100 HPLC equipped with a quaternary pump, a variable wavelength detector set at 254 nm, a YMC pro C-18 column (2 x 23 mm, 120A), and a Finnigan LCQ ion trap mass spectrometer with electrospray ionization. Spectra were scanned from 120-1200 amu using a variable ion time according to the number of ions in the source. The eluents were A: 2% acetonitrile in water with 0.02% TFA and B: 2% water in acetonitrile with 0.018% TFA. Gradient elution from 10% B to 95% over 3.5 minutes at a flowrate of 1.0 mL/min is used with an initial hold of 0.5 minutes and a final hold at 95% B of 0.5 minutes. Total run time is 6.5 minutes.

or
(B) Gilson HPLC system equipped with two Gilson 306 pumps, a Gilson 215 Autosampler, a Gilson diode array detector, a YMC Pro C-18 column (2 x 23 mm, 120 A), and a Micromass LCZ single quadrupole mass spectrometer with z-spray electrospray ionization. Spectra were scanned from 120-800 amu over 1.5 seconds. ELSD
(Evaporative Light Scattering Detector) data is also acquired as an analog channel. The eluents were A: 2% acetonitrile in water with 0.02% TFA and B: 2% water in acetonitrile with 0.018% TFA. Gradient elution from 10% B to 90% over 3.5 minutes at a flowrate of 1.5 mL/min is used with an initial hold of 0.5 minutes and a final hold at 90% B of 0.5 minutes. Total run time is 4.8 minutes. An extra switching valve is used for column switching and regeneration.

Routine one-dimensional NMR spectroscopy is performed on 400 MHz Varian Mercury-plus spectrometers. The samples were dissolved in deuterated solvents obtained from Cambridge Isotope Labs, and transferred to 5 mm ID Wilmad NMR tubes. The spectra were acquired at 293 K. The chemical shifts were recorded on the ppm scale and were referenced to the appropriate solvent signals, such as 2.49 ppm for DMSO-\(d_6\), 1.93 ppm for CD₃CN-\(d_3\), 3.30 ppm for MeOD-\(d_4\), 5.32 ppm for CD₂Cl₂-\(d_2\) and 7.26 ppm for CDCl₃-\(d_3\) for \(^1\)H spectra.

Table 1

REFERENCE TABLE FOR SOURCES AND PREPARATIVE METHODS OF STARTING MATERIALS

<table>
<thead>
<tr>
<th>Intermediate</th>
<th>Source</th>
</tr>
</thead>
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<td>Commerially available from Lancaster, USA.</td>
</tr>
<tr>
<td><img src="image" alt="Image" /></td>
<td>Reference: Schirrmacher, R.; Wangler, B.; Schirrmacher, E.; August, T.; Rosch, F. Dimethylpyridin-4-ylamine-Catalyzed Alcoholysis of 2-Amino-N,N,N-trimethyl-9H-purine-6-</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Source</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>![Chemical Structure]</td>
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<td>![Chemical Structure]</td>
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<tr>
<td>![Chemical Structure]</td>
<td>Commercially available from Aldrich, USA.</td>
</tr>
<tr>
<td>![Chemical Structure]</td>
<td>Commercially available from Maybridge, UK.</td>
</tr>
</tbody>
</table>

**Preparation of Intermediates**

**Intermediate A:** Preparation of \(2-[(\text{methylamino})\text{carbonyl}]\text{pyridin-4-yl})\text{methyl methanesulfonate}
To a solution of 4-(hydroxymethyl)-N-methylpyridine-2-carboxamide (9.78 g, 58.9 mmol) in THF (250 mL) was added triethyl amine (12.3 mL, 88.3 mmol). The reaction was cooled to 0 °C and methanesulfonyl chloride (5.5 mL, 70.6 mmol) was added dropwise over 15 min. The reaction was allowed to slowly come to room temperature and then stirred an additional 3 h. The resulting solution was concentrated, re-dissolved in EtOAc (200 mL), transferred into a separatory funnel and extracted with EtOAc (2 x 200 mL). The combined organic layers were washed with cold satd. NaHCO₃ (2 x 200 mL). The organic layer was dried (MgSO₄), filtered and concentrated to afford 1.16 g of the above compound as a solid (4.75 mmol, yield 81%). ¹H-NMR (CD₂Cl₂-d₂) δ 8.59 (d, J = 4.88 Hz, 1H), 8.15 to 8.16 (m, 1H), 7.48 to 7.50 (m, 1H), 5.31 (s, 2H), 3.10 (s, 3H), 3.01 (d, J = 5.08 Hz, 3H); LCMS: 245 [M+H]⁺, RT 1.24 min.

**Intermediate B:** Preparation of (2-cyanopyridin-4-yl)methyl methanesulfonate

Same procedure as in Intermediate A except 4-(hydroxymethyl)pyridine-2-carbonitrile was used in place of 4-(hydroxymethyl)-N-methylpyridine-2-carboxamide.

¹H-NMR (CDCl₃-d₅) 8.42 (d, J = 5.02 Hz, 1H), 7.46 to 7.47 (m, 1H), 7.23 to 7.24 (m, 1H), 4.53 (s, 2H), 1.52 (s, 3H); LCMS: 213.1 [M+H]⁺, RT 1.59 min.

**Intermediate C:** Preparation of 4-(bromomethyl)-N-methylpyridine-2-carboxamide

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To a solution of 4-(hydroxymethyl)-N-methylpyridine-2-carboxamide (533 mg, 3.21 mmol) in THF (15 mL) was added triphenylphosphine (883.3 mg, 3.37 mmol) and carbon tetrabromide (1.1 g, 3.37 mmol). A white precipitate began to crash out of solution upon addition of the carbon tetrabromide. The solution was allowed to stir at room temperature for 16 h. The mixture was then filtered to remove the precipitate and the filtrate was concentrated to oil. The crude product was purified via flash silica chromatography (40:60 → 60:40, EtOAc:Hex) to yield 345 mg (47%) of the product as a clear oil. The product did not appear to be stable as determined by rapid color change and was thus quickly used in the next step. LCMS: 229.1, 231.0 [M+H]^+.

**Intermediate D: Preparation of dimethyl pyridine-2,4-dicarboxylate**

To a solution of 2,4-pyridinecarboxylic acid hydrate (505 mg, 2.73 mmol) in MeOH (5 mL) was added conc. H₂SO₄ (0.29 mL, 5.46 mmol). The solution was stirred until clear and trimethylorthoformate (1.2 mL, 10.9 mmol) was added to the reaction flask. The reaction was stirred at reflux for 16 h until complete. The resulting solution was concentrated *in vacuo* to afford 336 mg of the above compound as a solid (1.72 mmol, yield 63%). The crude material was used in further reactions without purification. "H NMR (CDCl₃-d) δ 8.90 (d, J = 4.89 Hz, 1H), 8.65 to 8.66 (m, 1H), 8.03 to 8.04 (m, 1H), 4.05 (s, 3H), 4.01 (s, 3H); LCMS: 196 [M+H]^+. 
Intermediate E: Preparation of [2-[(dimethylamino)carbonyl]pyridin-4-yl]methyl methanesulfonate

Step 1: Preparation of methyl 2-[(dimethylamino)carbonyl]isonicotinate

Intermediate D (560 mg, 2.87 mmol) was taken up with dichloromethane (6 mL) and allowed to stir for 15 min until all of the diester was in solution. The solution was then cooled to 0 °C and magnesium chloride (174.84 mg, 1.84 mmol) was added. This was allowed to stir for 30 min at 0 °C. To the reaction vessel was then added dimethylamine (2M, 2.15 mL) over the course of 3 h. The reaction mixture was allowed to stir 16 h at rt. The reaction was then quenched with water (5 mL) and aqueous monobasic potassium phosphate (1M, 5 mL). The solution was extracted using CH$_2$Cl$_2$ (3 X 10 mL) and the organic fractions were combined, dried and concentrated to a white solid (525 mg, 88%). $^1$H NMR showed the solid to be the correct compound in roughly 90% purity. The crude product was used without further purification. $^1$H NMR (DMSO- $d_6$) δ 8.77 (d, J= 5 Hz, 1H) 7.91 (s, 1H), 7.27, (d, J= 5Hz, 1H), 3.88 (s, 3H), 3.99 (s, 3H), 2.91 (s, 3H); LCMS: 209 [M+H]$^+$, RT 1.13 min.

Step 2: Preparation of 4-(hydroxymethyl)-N,N-dimethylpyridine-2-carboxamide
2-Dimethylcarbamoyl-isonicotinic acid methyl ester (140.00 mg, 0.67 mmol) was dissolved into 1,4-dioxane (1.16 mL). MeOH (0.18 mL) and water (0.01 mL) were then added and the solution was allowed to stir 15 minutes. The solution was then cooled to 0 °C and sodium borohydride (31.80 mg, 0.84 mmol) was added portion-wise over the course of 1 h. The mixture was allowed to stir for 16 h. The crude reaction mixture was then added directly to a Biotage® silica samplet cartridge and dried under vacuum for 3 h. The sample was then chromatographed (5% MeOH in EtOAc) to yield 79.2 mg (64.8%) of the product as an oil. $^1$H NMR (CD$_2$Cl$_2$-d$_2$) δ 8.49 (d, J = 7 Hz, 1H) 7.41 (d, J = 7 Hz, 2H), 7.79, (d, J = 5 Hz, 2H), 3.09 (s, 3H), 2.76 (s, 3H); LCMS: 181 [M+H]$^+$, RT 0.95 min.

Step 3: Preparation of [2-[(dimethylamino)carbonyl]pyridin-4-yl]methyl methanesulfonate

Same procedure as in Intermediate A except 4-(hydroxymethyl)-N,N-dimethylpyridine-2-carboxamide was used in place of 4-(hydroxymethyl)-N-methylpyridine-2-carboxamide.

Intermediate F: Preparation of [2-[(ethylamino)carbonyl]pyridin-4-yl]methyl methanesulfonate

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Same procedure as in Intermediate E except in Step 1, ethylamine was used in place of dimethylamine.

**Intermediate G: Preparation of [2-[(cyclopropylamino)carbonyl]pyridin-4-yl]methyl methanesulfonate**

Same procedure as in Intermediate E except in Step 1, cyclopropylamine was used in place of dimethylamine.

**Intermediate H: Preparation of [2-(aminocarbonyl)pyridin-4-yl]methyl methanesulfonate**

Step 1: Preparation of ethyl 2-(aminocarbonyl)isonicotinate
A solution of ethyl isonicotinate (25.2 mL, 165 mmol) in formamide (200 mL) was stirred with ice/methanol bath cooling as concentrated sulfuric acid (8.80 mL, 165 mmol) was added. Ferric sulfate heptahydrate (69 g, 248 mmol) and hydrogen peroxide (25.6 mL of 30% in water) were added slowly over 25 min in alternating portions such that the temperature of the mixture was kept between 8-10.5 °C. During this addition small pieces of dry ice were added to the bath to keep the reaction temperature in the desired range. After addition was complete, the ice bath was removed and the dark mixture was stirred for 2 h without cooling and then poured into a solution of trisodium citrate dihydrate (80.6 g) in water (700 mL) and then residues left in the reaction flask were washed out with a little methanol and water. The resulting mixture was rapidly stirred in a large flask as solid NaHCO₃ was added slowly, portion-wise, until the mixture was basic. Some saturated aqueous NaHCO₃ was added to make the mixture more basic and then it was vacuum filtered through Celite® and the solids were washed down with three 200 mL portions of dichloromethane. The phases of the filtrate were separated and the aqueous layer was extracted twice with dichloromethane. The combined extract was dried (Na₂SO₄) and evaporated in vacuo. The resulting solid residue was washed with ether/hexane (200 mL, 1:30) twice with warming and sonication followed by cooling and filtration to yield 13.9 g (44%) of pure title compound. The wash solutions, which contained some highly contaminated desired product, were discarded.

1H NMR (300 MHz, DMSO-d₆) 8 8.83 (d, 1H), 8.39 (d, 1H, meta coupling), 8.24 (bs, 1H), 8.00 (d, 1H), 7.81 (bs, 1H), 4.39 (q, 2H) and 1.37 ppm (t, 3H); ES-MS m/z 195.0 [M+H]⁺, HPLC RT (min) 1.83.

Step 2: Preparation of 4-(hydroxymethyl)pyridine-2-carboxamide

A slurry of ethyl 2-(aminocarbonyl)isonicotinate (5.00 g, 25.8 mmol) in absolute ethanol (150 mL) was stirred under nitrogen as sodium borohydride (2.92 g, 77.2 mmol) was added. After 22 h stirring at ambient temperature, the reaction was carefully quenched by addition of 17 mL of saturated aqueous ammonium chloride followed by stirring until the bubbling stopped and then evaporation in vacuo to leave a white solid residue. Saturated aqueous sodium chloride (80 mL) was added followed by five extractions with 200 mL portions of ethyl acetate. Combined extracts were dried
(Na₂SO₄) and evaporated in vacuo to yield 3.85 g (98%) of pure title compound as a white solid.

¹H NMR (300 MHz, DMSO-d₆) δ 8.52 (d, 1H), 8.00 (s, 1H), 8.07 (bs, 1H), 7.46 (d, 1H), 7.60 (bs, 1H), 5.54 (t, 1H) and 4.60 ppm (d, 2H); ES-MS m/z 154.0 [M+H, weak signal]⁺, HPLC RT (min) 1.05.

Step 3: Preparation of [2-(aminocarbonyl)pyridin-4-yl]methyl methanesulfonate

4-(hydroxymethyl)pyridine-2-carboxamide (1.00 g, 6.57 mmol) was dissolved in ethyl acetate (80 mL) and then cooled to 0 °C with stirring under nitrogen in an ice bath before triethylamine (1.37 mL, 9.86 mmol) was added, followed by methanesulfonyl chloride (0.66 mL, 8.54 mmol, added dropwise over 7 min). The ice bath was removed and the resulting suspension was stirred 2 h, and then the reaction mixture was poured into 60 mL water and stirred rapidly for 10 min. The phases were separated and the aqueous was extracted twice more with ethyl acetate. Each extract was washed with brine and the combined extracts were dried (Na₂SO₄) and evaporated in vacuo to yield 1.50 g (99%) of pure product as a fine white solid which turned pink on storage. Re-assay by NMR after such color change did not show significant decomposition.

¹H NMR (300 MHz, DMSO-d₆) δ 8.64 (d, 1H), 8.06 (s, 1H), 8.14 (bs, 1H), 7.6 (d, 1H), 7.70 (bs, 1H), 5.41 (s, 2H) and 3.33 ppm (s, overlaps with water in solvent).

Intermediate I: Preparation of 2,4-dichloro-6-(chloromethyl)pyrimidine

This product was prepared similarly to the 5-methyl substituted analog described in *Biorg. Med. Chem.*, 2002, 10, 525, which is hereby incorporated by reference. A stirred suspension of 6-(chloromethyl)pyrimidine-2,4(1H,3H)-dione (5.2 g, 32.6 mmol) in POCl₃ (9.1 mL, 97.9 mmol) was refluxed for 16 h under nitrogen. The mixture was cooled and evaporated to leave a dark colored oil. Ice water was slowly added and the product was
extracted into dichloromethane. The organic layer was washed with brine, dried over MgSO₄, and concentrated under reduced pressure to give 2,4-dichloro-6-(chloromethyl)pyrimidine (5 g) as a yellow oil. Though this product could be used in the next step without purification, another batch prepared in the same way was further purified by chromatography to show the following NMR.

\[^1\text{H NMR (DMSO-}d_6\text{)} \delta 7.90 \text{ (s, 1H) and 4.78 ppm (s, 2H).}\]

**Intermediate J: Preparation of N-[4-(chloromethyl)pyridin-2-yl]acetamide**

![Chemical Structure](image)

**Step 1: Preparation of 4-(chloromethyl)pyridin-2-amine**

![Chemical Structure](image)

(2-Aminopyridin-4-yl)methanol (11.2 g, 90 mmol) was stirred in a flask with ice bath cooling as thionyl chloride (65.8 mL, 902 mmol) was slowly added. After about 10 mL was added, the temperature increased suddenly to about 50 °C and addition was halted as the mixture was broken up so that stirring could continue as the rest of the thionyl chloride was added. The cooling bath was then removed and the reaction was stirred for 2 h at ambient temperature before it was evaporated in vacuo and then toluene was added twice and evaporated each time in vacuo to yield the hydrochloride salt of the title compound. A suspension of this material in dichloromethane (150 mL) was stirred with saturated aqueous sodium bicarbonate (150 mL) for 1.5 h. The phases were separated and the organic extract was washed twice with water, once with brine and then dried (Na₂SO₄) and evaporated in vacuo to yield 10.71 g (83%) of pure title compound.

\[^1\text{H NMR (300 MHz, DMSO-}d_6\text{)} \delta 7.87 \text{ (d, 1H), 6.48 (d, 1H), 6.45 (s, 1H), 6.04 (s, 2H) and 4.60 ppm (s, 2H); ES-MS } m/z \text{ 143.2 [M+H]}^+, \text{ HPLC RT (min) 1.34.}\]

**Step 2: Preparation of N-[4-(chloromethyl)pyridin-2-yl]acetamide**

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A suspension of 4-(chloromethyl)pyridin-2-amine (2.30 g, 16 mmol) and triethylamine (8.9 mL) in dichloroethane (10 mL) was stirred under nitrogen with ice bath cooling as acetyl chloride (1.4 mL, 19 mmol) was added slowly over 10 min. After 2 h stirring with cooling, TLC showed no starting material. The mixture was diluted with dichloromethane and washed with water and then brine. It was dried (Na$_2$SO$_4$) and evaporated in vacuo. The residue was purified by chromatography on silica gel using a gradient from 0-3% methanol in dichloromethane to yield 2 g (67%) of pure title compound contaminated with diacylated (N-acetyl-N-[4-(chloromethyl) pyridin-2-yl]acetamide) product in about a 1:1 product ratio. The mixture was used as is in the next reaction and side products were separated by chromatography after the subsequent reaction.

$^1$H NMR (300 MHz, CD$_2$Cl$_2$) δ 8.33 ( bs, 1H), 7.41 ( d, 1H), 7.30 (s, 1H), 7.10 (d, 1H), 4.65 (s, 2H) and 2.20 ppm (s, 3H); ES-MS $m/z$ 185.0 [M+H]$^+$, HPLC RT (min) 1.16. Signals for the contaminating diacyl compound show at $^1$H NMR (300 MHz, CD$_2$Cl$_2$) δ 8.56 (d, 1H), 8.18 (s, 1H), 7.82 (d, 1H), 4.75 (s, 2H) and 2.25 ppm (s, 6H); ES-MS $m/z$ no significant M+H$^+$ ion, HPLC RT (min) 0.97. Because of the closeness of the % content of the two compounds, it is possible that some of the NMR peak assignments have been switched between the desired material and the contaminant.

**Intermediate K: Preparation of 3-amino-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide**

Step 1: Preparation of methyl 3-[(tert-butoxycarbonyl)amino]thiophene-2-carboxylate
Methyl 3-aminothiophene-2-carboxylate (23g, 146.3 mmol), di-tert-butyl dicarbonate (41 g, 190.2 mmol) and DMAP (1.788 g, 14.63 mmol) were dissolved in DCM (230 mL) and the reaction mixture was stirred for 16 h. Sodium 2-methylpropan-2-olate (11.25 g, 117.1 mmol) was added and the mixture was stirred for 2 h. The mixture was evaporated and the product was purified by a silica gel column chromatography using 10% EtOAc in hexanes as eluent to give the desired product (32 g, 85%).

Step 2: Preparation of 3-[(tert-butoxycarbonyl)amino]thiophene-2-carboxylic acid

Methyl 3-[(tert-butoxycarbonyl)amino]thiophene-2-carboxylate (32 g, 124.4 mmol) (step 1) was dissolved with methanol (140 mL) and sodium hydroxide was added (1N, 190 mL). The mixture was stirred at 50 °C for 16 h. Some starting material was remaining by TLC and so additional sodium hydroxide was added (1.7N, 275 mL) and the mixture was stirred at 50 °C for 2 h. The mixture was cooled to rt and acidified to pH 5 with HCl (1N). Solids were filtered out and combined with the solid remaining after extraction with EtOAc, drying with Na₂SO₄, and evaporation. The product was used without further purification.

Step 3: Preparation of tert-butyl (2-[(2,2-difluoro-1,3-benzodioxol-5-yl) amino]carbonyl]-3-thienyl)carbamate
3-[(Tert-butoxycarbonyl)amino]thiophene-2-carboxylic acid (7.3 g, 30.3 mmol) (step 2) was dissolved in DCM (155 mL), triethyl amine (12 mL), DMF (31 mL) and 2,2-difluoro-1,3-benzodioxol-5-amine (5 g, 29 mmol) was added. PyBOP (16.5 g, 32 mmol) was added and the mixture was stirred at 60 °C for 16 h. The reaction mixture was cooled and diluted with water and EtOAc. The organic layer was washed with water, dried with Na₂SO₄ and evaporated. The crude residue was purified using silica gel chromatography to yield the desired product (7.7 g, 67 %).

¹H NMR (DMSO-δ₆) δ 9.95 (bs, 1H), 7.95 (d, 1H), 7.65, (s, 1H), 7.45 (m, 2H), 7.10 (s, 2H), 1.50 (s, 9H); LCMS: 398.9 [M+H]^+, RT 4.12 min.

Step 4: Preparation of 3-amino-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide

Tert-butyl(2-[(2,2-difluoro-1,3-benzodioxol-5-yl)amino]carbonyl)-3-thienyl)carbamate (31 g, 78 mmol) (step 3) was dissolved in DCM (433 mL), and TFA (60 mL). The mixture was stirred and rt for 3 h. The mixture was evaporated and then EtOAc was added which was washed with water, dried with Na₂SO₄ and evaporated. The crude residue was purified using silica gel chromatography and 35% EtOAc in hexanes as eluent to provide the desired product (23 g, 99%).

¹H NMR (DMSO-δ₆) δ 9.43 (s, 1H), 7.75 (s, 1H), 7.50 (s, 1H), 7.40 (s, 1H), 7.30 (s, 1H), 6.63 (m, 3H); LCMS: 299.0 [M+H]^+, RT 3.12 min.

Preparative Examples of the Invention Compounds

Example 1:  4-[[2-[(2,2-difluoro-1,3-benzodioxol-5-yl)amino]carbonyl]-3-
thienyl]amino[methyl]-N-methylpyridine-2-carboxamide

Step 1: Preparation of methyl 3-[(2-[(methylamino)carbonyl]pyridin-4-yl]methyl]amino]thiophene-2-carboxylate

Intermediate A (3.10 g, 12.7 mmol) and sodium iodide (2.86g, 19.1mmol) were added into a dry round bottom flask. DMF (70 mL) was added and the solution was allowed to stir for 2 minutes at rt. A solution of 3-Amino-thiophene-2-carboxylic acid methyl ester (4.00 g, 25.4 mmol) in DMF (30 mL) was added via syringe and the mixture was heated to 60 °C for 16 h. The reaction mixture was cooled to rt and concentrated in vacuo to a dark oil. The oil was taken up in EtOAc (200 mL) and washed with water (3 x 200 mL) and brine (100 mL). The organic solution was dried with sodium sulfate and re-concentrated to an oil. The crude oil was purified via flash chromatography (4:6, EtOAc:Hex) to yield 1.70 g (43.8%) of methyl 3-[(2-[(methylamino)carbonyl]pyridin-4-yl]methyl]amino]thiophene-2-carboxylate as an off-white solid. $^1$H NMR (DMSO-$d_6$) δ 8.73 (d, $J = 6$ Hz, 1H), 8.53 (d, $J = 5$ Hz, 1H), 7.95 (s, 1H), 7.60 (d, $J = 5$ Hz, 1H), 7.53 - 7.45 (m, 2H), 6.64 (d, $J = 5$ Hz, 1H), 4.63 (d, $J = 8$ Hz, 2H), 3.74 (s, 3H), 2.77 (d, $J = 6$ Hz, 3H); LCMS 306 [M+H$^+$], RT 2.51 min.

Methyl 3-[(2-[(methylamino)carbonyl]pyridin-4-yl)methyl]amino]thiophene-2-carboxylate (100 mg, 0.33 mmol) was added to ethanol (1.5 mL) and heated to 60°C. The mixture was stirred for 15 min until all solids had dissolved into solution. Water was added (1.5 mL) followed by the addition of LiOH (41.23 mg, 0.98 mmol). The solution was allowed to stir for 6 h at 60°C until all of the starting material had been consumed. The crude reaction mixture was then concentrated to an oil and taken up in EtOAc. The organic was added to a separatory funnel and water was added. An aqueous solution of 1M potassium dihydrogen phosphate was added until the aqueous layer was pH 6. This was extracted (3x, 50 mL) with EtOAc. The organics were combined, dried with sodium sulfate and concentrated to an amorphous solid (90 mg, 94.3%) which was taken directly to the next step. LCMS: 292 [M+H]^+, RT 2.46 min.


3-[(2-[(methylamino)carbonyl]pyridin-4-yl)methyl]amino]thiophene-2-carboxylic acid (90 mg, 0.31 mmol) was dissolved into dichloromethane (1 mL) and 2,2-Difluoro-Benzo[1,3]dioxol-5-ylamine (59.4 mg, 0.31 mmol) was added followed by the addition of triethylamine (0.05 mL, 0.31 mmol). To the solution was added pyBOP (178.6 mg, 0.31 mmol). The solution was allowed to stir for 16 h at room temperature. The solution was
made dilute with dichloromethane (15 mL) and washed with water. The mixture was
neutralized with a 1M solution of potassium dihydrogen phosphate. The mixture was
extracted with dichloromethane (3x, 15 mL). The organic fractions were combined, dried
with sodium sulfate, and concentrated to an oil. The oil was purified via flash
chromatography (35% EtOAc in Hex) to yield 21 mg (15%) of the final product as a white
solid. $^1$H NMR (DMSO-$d_6$) $\delta$ 9.58 (s, 1H), 8.73 (d, $J = 5.5$ Hz, 1H), 8.55 (d, $J = 5.0$ Hz, 
1H), 8.09, (bs, 1H), 7.98 (s, 1H), 7.80 (s, 1H), 7.59 (d, $J = 8.4$ Hz, 1H), 7.45 (d, $J = 7.2$
Hz, 1H), 7.40 (d, $J = 8.4$ Hz, 1H), 7.37 (d, $J = 8.8$ Hz, 1H), 6.69 (d, $J = 8.8$ Hz, 1H), 4.63
(d, $J = 8.4$ Hz, 2H), 2.79 (d, $J = 8.4$ Hz, 3H); LCMS 447.1 [M+H$^+$], RT 3.59 min.

The following compounds (Examples 2-4) were synthesized using the same synthetic
method as Example 1 but with the specific starting materials mentioned below:

**Example 2:** 4-([(2-[(2,2-difluoro-1,3-benzodioxol-5-yl)amino]carbonyl)-3-thienyl]amino)[methyl]-N-ethylpyridine-2-carboxamide

![Chemical Structure]

In Step 1, Intermediate F was used in place of Intermediate A.

$^1$H NMR (DMSO-$d_6$) $\delta$ 9.57 (s, 1H), 8.77 (t, $J = 6$ Hz, 1H), 8.53 (d, $J = 5$ Hz, 1H), 8.05 (t, $J = 7$Hz, 1H), 7.96 (s, 1H), 7.79 (s, 1H), 7.58 (d, $J = 6$Hz, 1H), 7.47 (d, $J = 5$ Hz, 1H), 7.40 (d, $J = 10$ Hz, 1H), 7.32 (d, $J = 8$Hz, 1H), 6.68 (d, $J = 8$ Hz, 1H), 4.62 (d, $J = 6$ Hz, 2H), 3.31-3.22 (m, 2H), 1.07 (t, $J = 8$Hz, 3H); LCMS: 461 [M+H$^+$], RT 3.39 min.

**Example 3:** N-cyclopropyl-4-([(2-[(2,2-difluoro-1,3-benzodioxol-5-yl)amino]carbonyl)-3-thienyl]amino)[methyl]pyridine-2-carboxamide

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In Step 1, Intermediate G was used in place of Intermediate A.

$^1$H NMR (DMSO-$d_6$) δ 9.57 (s, 1H), 8.68 (d, $J$ = 6 Hz, 1H), 8.51 (d, $J$ = 5 Hz, 1H), 8.04 (t, $J$ = 7Hz, 1H), 7.96 (s, 1H), 7.78 (s, 1H), 7.57 (d, $J$ = 6, 1H), 7.47 (d, $J$ = 5 Hz, 1H), 7.41 (d, $J$ = 10 Hz, 1H), 7.31 (d, $J$ = 8Hz, 1H), 6.68 (d, $J$ = 8 Hz, 1H), 4.62 (d, $J$ = 6 Hz, 2H), 2.88-2.81 (m, 1H), 0.65-0.60 (m, 4H); LCMS: 473 [M+H]$^+$, RT 3.43.

**Example 4:** 4-[[((2,2-difluoro-1,3-benzodioxol-5-yl)amino]carbonyl]-3-thienyl]amino]methyl]-N-methylpyridine-2-carboxamide

In Step 1, methyl 4-aminothiophene-3-carboxylate was used in place of methyl 4-aminothiophene-3-carboxylate.

$^1$H NMR (MeOD-$d_4$) δ 8.50 (d, $J$ = 5 Hz 1H), 8.09-8.11(m, 2H), 7.76 (d, $J$ = 2 Hz, 1H), 7.52-7.54 (m, 1H), 7.34 (d, $J$ = 2 Hz, 1H), 7.32 (d, $J$ = 2 Hz, 1H), 7.16 (s, 1H), 7.14(s, 1H), 5.84 (d, $J$ = 4 Hz, 1H), 4.49 (s, 2H), 2.95(s, 3H); LCMS: 469.1 [M+H]$^+$, RT 3.22 min.
Example 5: N-(2,2-difluoro-1,3-benzodioxol-5-yl)-4-[(pyridin-4-ylmethyl)amino]thiophene-3-carboxamide

Step 1: Preparation of methyl 4-[(pyridin-4-ylmethyl)amino]thiophene-3-carboxylate

To a solution of methyl 4-aminothiophene-3-carboxylate (1.02g, 6.42mmol) in THF (40 ml) was added 4-pyridinecarboxaldehyde (0.80g, 7.46mmol), followed by addition of AcOH (0.45ml, 7.8mmol) and sodium triacetoxyborohydride (NaBH(OAc)_3) (1.93 g, 9.08 mmol). The mixture was stirred 48 h at rt. The reaction was added slowly with 10 ml saturated aq. NaHCO_3 and stirred at rt for 15 min. The resulting crude was extracted with EtOAc (3 x 100 ml). The combined extract was washed with brine, dried over Na_2SO_4 and the solvent was evaporated to give a yellow crude oil. The resulting crude was purified on Biotage® column with 5% MeOH/CH_2Cl_2 to yield 800 mg of methyl 4-[(pyridin-4-ylmethyl)amino]thiophene-3-carboxylate with a purity ca. 70%. The product was subject to next step reaction without further purification.

Step 2: Preparation of 4-[(pyridin-4-ylmethyl)amino]thiophene-3-carboxylic acid
The procedure for Example 1, Step 2 was used.

Step 3: Preparation of N-(2,2-difluoro-1,3-benzodioxol-5-yl)-4-[(pyridin-4-ylmethyl)amino]thiophene-3-carboxamide

The procedure for Example 1, Step 3 was used.

$^1$H NMR (CD$_2$Cl$_2$-d$_2$) δ 8.50 (d, $J = 2$ Hz, 2H), 8.38 (broad, 1H), 7.8 (d, $J = 3$Hz, 1H), 7.68 (d, $J = 2$ Hz, 1H), 7.3 (d, $J = 4$Hz, 1H), 7.06-7.13 (m, 2H), 7.03 (broad, 1H), 5.79 (d, $J = 3$ Hz, 1H), 4.38 (s, 2H); LCMS: 390.1 [M+H]$^+$, RT 2.27 min.

The following compounds (Examples 6-8) can be synthesized using the same synthetic route as in Example 5 but with the specific starting materials mentioned below:

Example 6:  N-(2,2-difluoro-1,3-benzodioxol-5-yl)-3-[(pyridin-4-ylmethyl)amino]thiophene-2-carboxamide
In Step 1, methyl 3-aminothiophene-2-carboxylate is used in place of methyl 4-aminothiophene-3-carboxylate.

**Example 7:**  
N-(2,2-difluoro-1,3-benzodioxol-5-yl)-5-methyl-3-[(pyridin-4-yImethyl)amino]thiophene-2-carboxamide

In Step 1, methyl 3-amino-5-methylthiophene-2-carboxylate is used in place of methyl 4-aminothiophene-3-carboxylate.

**Example 8:**  
N-(2,2-difluoro-1,3-benzodioxol-5-yl)-4-methyl-3-[(pyridin-4-yImethyl)amino]thiophene-2-carboxamide
In Step 1, methyl 3-amino-4-methylthiophene-2-carboxylate is used in place of methyl 4-aminothiophene-3-carboxylate.

The following compounds (Examples 9-16) can be synthesized using the same synthetic route as in Example 1 but with the specific starting materials mentioned below:

**Example 9:** 4-[[2-[[2,2-difluoro-1,3-benzodioxol-5-y]l]amino]carbonyl]-4-methyl-3-thienyl]amino)methyl]-N-methylpyridine-2-carboxamide

![Chemical Structure Image]

In Step 1, methyl 3-amino-4-methylthiophene-2-carboxylate is used in place of methyl 3-aminothiophene-2-carboxylate.

**Example 10:** 4-[[2-[[2,2-difluoro-1,3-benzodioxol-5-y]l]amino]carbonyl]-5-methyl-3-thienyl]amino)methyl]-N-methylpyridine-2-carboxamide

![Chemical Structure Image]

In Step 1, methyl 3-amino-5-methylthiophene-2-carboxylate is used in place of methyl 3-aminothiophene-2-carboxylate.

**Example 11:** 4-[[2-[[2,2-difluoro-1,3-benzodioxol-5-y]l]amino]carbonyl]-3-thienyl]amino)methyl]-N,N-dimethylpyridine-2-carboxamide

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In Step 1, Intermediate E is used in place of Intermediate A.

**Example 12:** 4-[[4-[[2,2-difluoro-1,3-benzodioxol-5-yI]amino]carbonyl]-3-thienyl]amino]methyl]-N-ethylpyridine-2-carboxamide

In Step 1, methyl 4-aminothiophene-3-carboxylate is used in place of methyl 3-aminothiophene-2-carboxylate and Intermediate F is used in place of Intermediate A.


In Step 1, methyl 4-aminothiophene-3-carboxylate is used in place of methyl 3-aminothiophene-2-carboxylate and Intermediate G is used in place of Intermediate A.
Example 14: 3-{[(2-cyanopyridin-4-yl)methyl]amino}-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide

In Step 1, Intermediate B is used in place of Intermediate A.

Example 15: 4-{[(2-cyanopyridin-4-yl)methyl]amino}-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-3-carboxamide

In Step 1, methyl 4-aminothiophene-3-carboxylate is used in place of methyl 3-aminothiophene-2-carboxylate and Intermediate B is used in place of Intermediate A.

Example 16: 3-{[(2-chloropyridin-4-yl)methyl]amino}-N-(2,2-difluoro-1,3-benzodioxol-5-yl)-4-methylthiophene-2-carboxamide
In Step 1, methyl 3-amino-4-methylthiophene-2-carboxylate is used in place of methyl 3-aminothiophene-2-carboxylate and (2-chloropyridin-4-yl)methyl methanesulfonate is used in place of Intermediate A. (2-chloropyridin-4-yl)methyl methanesulfonate is generated from the commercially available starting (2-chloropyridin-4-yl)methanol and mesylating the alcohol using a procedure such as that for intermediate A.

**Example 17: N-(2,2-difluoro-1,3-benzodioxol-5-yl)-3-((2-(methylamino)pyridin-4-yl)methyl)amino)thiophene-2-carboxamide**

![Chemical Structure](image.png)

**Step 1: Preparation of 3-{{(2-chloropyridin-4-yl)methyl}amino}-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide**

![Chemical Structure](image.png)

There are three steps used in the preparation of this compound that are similar to that of Example 16. The first step of the procedure is the same as that of Example 16 except in step 1 methyl 3-aminothiophene-2-carboxylate can be used instead of methyl 3-amino-4-methylthiophene-2-carboxylate. 3-{{(2-chloropyridin-4-yl)methyl}amino}-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide is made after the first 3 steps of Example 16.

**Step 4: Preparation of N-(2,2-difluoro-1,3-benzodioxol-5-yl)-3-{{2-...
(methylamino)pyridin-4-yl]methyl]amino)thiophene-2-carboxamide

3-[[2-chloropyridin-4-yl]methyl]amino]-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide is dissolved in pyridine and methyl amine (40% solution in water) and added to a sealed tube. The sealed tube is heated to 200 °C to form N-(2,2-difluoro-1,3-benzodioxol-5-yl)-3-[[2-(methylamino)pyridin-4-yl]methyl]amino)thiophene-2-carboxamide.

Example 18: N-(2,2-difluoro-1,3-benzodioxol-5-yl)-4-[[2-(methylamino)pyridin-4-yl]methyl]amino)thiophene-3-carboxamide

The procedure for the preparation of this compound is the same as that of Example 17 except in step 1, methyl 4-aminothiophene-3-carboxylate is used in place of methyl 3-aminothiophene-2-carboxylate.

Example 19: 3-[[2-(acetylamino)pyridin-4-yl]methyl]amino)-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide
A solution containing N-[4-(chloromethyl)pyridin-2-yl]acetamide (464 mg, 2.5 mmol) (Intermediate J), 3-amino-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide (500 mg, 1.7 mmol) (Intermediate K) and Sodium iodide (377 mg, 2.5 mmol) in DMF (5 mL) was heated to 90 °C for 2h. The reaction was diluted with ethyl acetate and extracted with water (3X). The organic layer was separated, dried with sodium sulfate, and evaporated under vacuum. The residue was purified by HPLC to obtain 3-([2-(acetylamino)pyridin-4-yl]methyl)amino)-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide in 53% yield (400 mg).

¹H NMR (CD3OD-d4) δ 8.24 (d, 1H), 7.68 (d, 1H), 7.47 (m, 3H), 7.28 (dd, 1H), 7.14 (d, 1H), 6.63 (d, 1H), 4.75 (s, 2H), 2.24 (s, 3H); LCMS: 447.0 [M+H]⁺, RT 2.66 min.

**Example 20: 3-([2-aminopyridin-4-yl]methyl)amino)-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide**

1N aqueous solution of NaOH (3.4 mL, 134 mmol) was added to a solution containing 3-(([2-(acetylamino)pyridin-4-yl]methyl)amino)-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide (75 mg, 0.17 mmol) (Example 19) in THF (3 mL) and methanol (1 mL). The reaction was stirred at room temperature for 2h and then it was heated to 50 °C for 16 h. The organic solvents were evaporated under vacuum and the aqueous layer was extracted with a mixture of dichloromethane/isopropanol (5:1) and the
organic layer was separated, dried with sodium sulfate and filtered. The filtrate was evaporated under vacuum and the residue was purified by biotage (0-70% ethyl acetate in hexanes) to obtain 3-\{[(2-aminopyridin-4-yl)methyl]amino\}-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide (40 mg) in 59% yield. $^1$H NMR (CD3OD-$d_4$) $\delta$ 7.75 (d, 1H), 7.67 (d, 1H), 7.47 (d, 1H), 7.27 (dd, 1H), 7.13 (d, 1H), 6.92 (bm, 1H), 6.84 (dd, 1H), 6.63 (d, 1H), 4.60 (s, 2H); LCMS: 405.0 [M+H]$^+$, RT 2.55 min.

**Example 21: Preparation of 4-\{[(2,2-difluoro-1,3-benzodioxol-5-yl)amino]carbonyl\}-3-thienyl)amino]methyl\}pyridine-2-carboxamide**

![Chemical Structure]

[2-(Aminocarbonyl)pyridin-4-yl]methyl methanesulfonate (Intermediate H) (1.87 g, 6.2 mmol) was dissolved in DMF (100mL) and sodium iodide (846 mg, 5.64 mmol) was added and the mixture was stirred for 1h. 3-Amino-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide (Intermediate K) (1.3 g, 5.6 mmol) was added and the mixture was stirred at 30 °C for 2 h. Water and EtOAc was added and the organic phase was dried with Na$_2$SO$_4$ and evaporated. The residue was purified using silica gel chromatography to give the desired product (50 mg, 1.84%) and a large amount of starting material (Intermediate K).

$^1$H NMR (CD3OD-$d_4$) $\delta$ 8.57 (d, 1H), 8.14 (s, 1H), 7.65 (s, 1H), 7.59 (d, 1H), 7.40 (d, 1H), 7.27 (d, 1H), 7.13 (d, 1H), 6.60 (d, 1H), 4.68 (s, 2H); LCMS: 455.1 [M+Na]$^+$, RT 3.44 min.

**Example 22: methyl 4-\{[(2-[2,2-difluoro-1,3-benzodioxol-5-yl)amino]carbonyl\}-3-thienyl)amino]methyl\}pyridine-2-carboxylate**
4-[[2-[[2,2-Difluoro-1,3-benzodioxol-5-yl]amino]carbonyl]-3-thienyl]amino]methyl]pyridine-2-carboxamide (60 mg, 0.14 mmol) (previous example) was dissolved in methanol (10 mL) in a sealed tube and DMF-DMA (0.059 mL, 0.444 mmol) was added. The mixture was stirred at 50 °C for 3 h at which time the mixture was stirred for 14 h at rt. The mixture was purified by silica gel chromatography to give the desired product (46 mg, 74%).

\(^1\)H NMR (CD\textsubscript{3}OD-\textsubscript{d}_4) \delta 8.59 (d, 1H), 8.13 (s, 1H), 7.66 (s, 1H), 7.62 (d, 1H), 7.43 (d, 1H), 7.30 (d, 1H), 7.13 (d, 1H), 6.62 (d, 1H), 4.70 (s, 2H), 3.94 (s, 3H); LCMS: 470.1 [M+Na]^+, RT 3.28 min.

**Example 23:** 4-[[2-[[2,2-difluoro-1,3-benzodioxol-5-yl]amino]carbonyl]-3-thienyl]amino]methyl]-N-(2-hydroxyethyl)pyridine-2-carboxamide

Methyl 4-[[2-[[2,2-difluoro-1,3-benzodioxol-5-yl]amino]carbonyl]-3-thienyl]amino]methyl]pyridine-2-carboxylate (50 mg, 0.112 mmol) was dissolved in methanol (10 mL) in a sealed tube and 2-aminoethanol (0.015 mL, 0.224 mmol) was added and the mixture was stirred at 60 °C for 16 h. The mixture was purified by HPLC to give the desired product (39 mg, 73%).

\(^1\)H NMR (CD\textsubscript{3}OD-\textsubscript{d}_4) \delta 8.52 (d, 1H), 8.10 (s, 1H), 7.68 (s, 1H), 7.50 (bm, 1H), 7.40 (d, 1H), 7.28 (d, 1H), 7.12 (d, 1H), 6.60 (d, 1H), 4.65 (s, 2H), 3.70 (t, 2H), 3.52 (t, 2H); LCMS: 477.0 [M+H]^+, RT 3.07 min.
Example 24: 3-[[2,6-dichloropyrimidin-4-yl]methyl]amino]-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide

2,4-Dichloro-6-(chloromethyl)pyrimidine (Intermediate I) (3.4 g, 17.6 mmol) was dissolved in DMF (140 mL). Sodium iodide (2.6 g, 17.6 mmol) and 2,6-di-tert-butyl-4-methylphenol (100 mg) was added. 3-Amino-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide (Intermediate K) (3.5 g, 11.7 mmol) was added and the mixture was stirred at 60 °C for 2 h. Water and EtOAc was added and the organic phase was dried with Na₂SO₄ and evaporated. The residue was purified using silica gel chromatography to give the desired product (2.6 g, 48%).

Example 25: N-(2,2-difluoro-1,3-benzodioxol-5-yl)-3-[[pyrimidin-4-ylmethyl]amino]thiophene-2-carboxamide

3-[[2,6-Dichloropyrimidin-4-yl]methyl]amino]-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide (40 mg, 0.087 mmol) was dissolved in methanol (2 mL) and EtOAc (2 mL). Palladium hydroxide (50 mg, 0.356 mmol) and ammonium formate (55 mg, 0.87 mmol) was added. The mixture was refluxed for 10 h and evaporated. The residue was purified by HPLC using 5-45% acetonitrile in water (0.1% TFA) as eluent to give the desired product (20 mg, 60%).
\(^1\)H NMR (CD3OD-d4) δ 9.12 (s, 1H), 8.69 (d, 1H), 7.69 (s, 1H), 7.52 (d, 1H), 7.46 (d, 1H), 7.32 (d, 1H), 7.13 (d, 1H), 6.67 (d, 1H), 4.65 (s, 2H); LCMS: 391.0 [M+H]+, RT 3.20 min.

**Example 26:** 3-\{[(2-aminopyrimidin-4-yl)methyl]amino\}-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide

![Chemical Structure](image)

Step 1: Preparation of 3-\{[(2-amino-6-chloropyrimidin-4-yl)methyl]amino\}-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide

![Chemical Structure](image)

3-\{[(2,6-Dichloropyrimidin-4-yl)methyl]amino\}-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide (Example 24) (3 g, 6.53 mmol) was dissolved in ammonia in ethanol (2M, 32 mL) and stirred until the TLC showed no starting material remaining. The mixture was evaporated and the residue was purified by HPLC to give two regioisomers of which the desired product was the higher Rf spot by TLC.

Step 2: Preparation of 3-\{[(2-aminopyrimidin-4-yl)methyl]amino\}-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide

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The crude product mixture from step 1 (250 mg, 0.568 mmol), which was a mixture of the two isomers was dissolved in methanol (2 mL) and EtOAc (5mL). Palladium hydroxide (50 mg, 0.356 mmol) and ammonium formate (358 mg, 5.68 mmol) was added. The mixture was refluxed for 10 h and then evaporated. The residue was purified by HPLC using 5-45% acetonitrile in water (0.1% TFA) as eluent to separate the desired isomer (100 mg, 32%, less polar) to the undesired isomer (more polar).

$^1$H NMR (CD$_3$OD-$d_6$) δ 8.19 (d, 1H), 7.62 (m, 1H), 7.50 (d, 1H), 7.26 (dd, 1H), 7.14 (d, 1H), 6.91 (d, 1H), 6.73 (d, 1H), 4.61 (s, 2H); LCMS: 406.3 [M+H]$^+$, RT 2.73 min.

**Example 27:** 3-[[6-amino-2-chloropyrimidin-4-yl)methyl]amino]-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide

The by-product of the preparation of Example 26 step 1 was the desired product shown above and was the lower Rf spot by TLC.

$^1$H NMR (DMSO-$d_6$) δ 9.57 (s, 1H), 7.92 (t, 1H), 7.79 (s, 1H), 7.63 (d, 1H), 7.37 (m, 4H), 6.69 (d, 1H), 6.25 (s, 1H), 4.34 (bd, 2H).

**Example 28:** 3-[[6-amino-2-[[3-hydroxypropyl]amino]pyrimidin-4-yl]methyl]amino]-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide
3-[(6-Amino-2-chloropyrimidin-4-yl)methyl]amino]-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide (Example 27) (50 mg, 0.114 mmol) and 3-aminopropan-1-ol (9 mg, 0.11 mmol) was added to a sealed tube and the mixture was heated to 100 °C for 10 min. The mixture was diluted with methanol and the solution was purified by HPLC to give the desired product (10 mg, 19%).

$^1$H NMR (DMSO-$d_6$) δ 12.07 (bs, 1H), 9.62 (s, 1H), 8.12 (bs, 2H), 7.85 (m, 1H), 7.80 (d, 1H), 7.60 (d, 1H), 7.41 (d, 1H), 7.32 (d, 1H), 6.79 (d, 1H), 5.90 (s, 1H), 4.58 (bs, 1H), 4.37 (m, 2H), 3.44 (m, 4H), 1.65 (m, 2H); LCMS: 479.1 [M+Na]$^+$, RT 2.39 min.


3-[(2,6-Dichloropyrimidin-4-yl)methyl]amino]-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide (Example 24) (90 mg, 0.196 mmol) was dissolved in ethanol (2 mL) and ethyl 4-aminobutanoate (51 mg, 0.392 mmol) was added and the mixture was stirred at rt until the TLC showed no starting material remaining. The mixture was evaporated and the residue was purified by HPLC to give two regioisomers of which the product used for the next step was the higher Rf spot (20 mg, 16%) and the other regioisomer (lower Rf spot) was obtained also (59 mg, 54%).

Step 2: Preparation of methyl 4-[(4-[(2,2-difluoro-1,3-benzodioxol-5-yl)amino]carbonyl)-3-thienyl]amino[methyl]-6-methoxypyrimidin-2-yl]amino]butanoate

Ethyl 4-[(4-chloro-6-[(2-[(2,2-difluoro-1,3-benzodioxol-5-yl)amino]carbonyl]-3-thienyl]amino[methyl]pyrimidin-2-yl]amino]butanoate (Step 1 above) (109 mg, 0.196 mmol) was dissolved in methanol (1mL), water (2 mL), THF (1mL) and lithium hydroxide (47 mg, 1.965 mmol) was added. The mixture was heated to 50 °C for 16 h. The crude reaction mixture was acidified to pH 5 using HCl (1N) and EtOAc was added. The organic layer was dried with Na₂SO₄ and concentrated to give the crude product, which was purified by HPLC to give the desired product (4.8 mg, 5%).

¹H NMR (CD3OD-d4) δ 7.67 (s, 1H), 7.52 (d, 1H), 7.28 (d, 1H), 7.12 (d, 1H), 6.75 (bd,
1H), 6.29 (bs, 1H), 4.50 (bs, 2H), 4.02 (bs, 3H), 3.65 (s, 3H), 3.53 (bm, 2H), 2.22 (t, 2H), 1.83 (p, 2H); LCMS: 536.6 [M+H]+, RT 2.94 min.

Example 30: N-(2,2-difluoro-1,3-benzodioxol-5-yl)-3-([(6-(methylamino) pyrimidin-4-yl)methyl]amino)thiophene-2-carboxamide

![Chemical Structure](image)

Step 1: Preparation of 3-([(2-chloro-6-(methylamino)pyrimidin-4-yl)methyl]amino)-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide

![Chemical Structure](image)

3-[(2,6-Dichloropyrimidin-4-yl)methyl]amino)-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide (1.5 g, 3.27 mmol) was dissolved in 2M methylamine in methanol (17 mL) and the mixture was stirred until the reaction was complete by TLC. The resulting mixture was purified by HPLC to give two regioisomers of which the lower retention time product was the desired product (1.10 g, 74%) and the higher retention time product was the undesired product (400 mg, 27%).

Step 2: Preparation of N-(2,2-difluoro-1,3-benzodioxol-5-yl)-3-([(6-(methylamino) pyrimidin-4-yl)methyl]amino)thiophene-2-carboxamide
3-((2-Chloro-6-(methylamino)pyrimidin-4-yl)methyl}amino)-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide (80 mg, 0.176 mmol) (step 1) was dissolved in methanol (2 mL) and EtOAc (2mL). Palladium hydroxide (50 mg, 0.356 mmol) and ammonium formate (111 mg, 1.76 mmol) was added. The mixture was refluxed for 16 h, filtered through celite, and the filtrate evaporated. The residue was purified by HPLC using 5-45% acetonitrile in water (0.1% TFA) as eluent to give the desired product (46 mg, 62%).

$^1$H NMR (CD3OD-$d_4$) δ 8.35 (s, 1H), 7.65 (s, 1H), 7.44 (d, 1H), 7.29 (d, 1H), 7.10 (d, 1H), 6.61 (d, 1H), 6.45 (s, 1H), 4.38 (bs, 2H), 2.82 (bs, 3H); LCMS: 420.2 [M+H]$^+$, RT 2.78 min.

**Example 31:** 4-[[4-[(2,2-difluoro-1,3-benzodioxol-5-yl)amino|carbonyl}-3-thienyl]amino]methyl]-6-(methylamino)pyrimidin-2-yl]amino]butanoic acid

3-((2-Chloro-6-(methylamino)pyrimidin-4-yl)methyl}amino)-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide (Product of step 1 of preparation of Example 30) (50 mg, 0.11 mmol) was dissolved in n-butanol (1 mL), N-ethyl-N-isopropylpropan-2-amine (0.038 mL) and ethyl 4-aminobutanoate (92 mg, 0.551 mmol) was added and the mixture was heated in a sealed tube to 110 °C for 20 min. The mixture was evaporated
and the residue was purified by HPLC to give the desired product (14 mg, 24%).

\[^1^H\text{NMR (CD3OD-}d_4\text{)} \delta 7.68 \text{ (s, 1H), 7.52 \text{ (d, 1H), 7.28 \text{ (d, 1H), 7.16 \text{ (d, 1H), 6.75 \text{ (d, 1H), 6.00 \text{ (s, 1H), 4.40 \text{ (s, 2H}, 3.53 \text{ (bm, 2H), 3.00 \text{ (s, 3H), 2.40 (t, 2H), 1.82 (p, 2H);}}

\text{LCMS: 521.3 [M+H]^+, RT 2.63 min.}}

\text{Example 32: 3-[[6-chloro-2-(methylamino)pyrimidin-4-yl]methyl]amino]-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide}

\[
\begin{align*}
\text{N} & \quad \text{O} \\
\text{NH} & \quad \text{C} \\
\text{N} & \quad \text{NH} \\
\text{Cl} & \quad \text{N} \\
\end{align*}
\]

The procedure used for the preparation of this compound is the same as that for the preparation of Example 30 Step 1 and the higher Rf compound is the desired product shown above which was used in the preparation of Example 33.

\text{LCMS: 453.9 [M+Na]^+, RT 3.64 min.}

\text{Example 33: N-(2,2-difluoro-1,3-benzodioxol-5-yl)-3-[[6-[[3-methoxypropyl]amino]-2-(methylamino)pyrimidin-4-yl]methyl]amino)thiophene-2-carboxamide}

\[
\begin{align*}
\text{O} & \quad \text{C} \\
\text{NH} & \quad \text{C} \\
\text{NH} & \quad \text{NH} \\
\text{H}_3\text{C} & \quad \text{O} \\
\end{align*}
\]

\text{3-[[6-Chloro-2-(methylamino)pyrimidin-4-yl]methyl]amino)-N-(2,2-difluoro-1,3-benzodioxol-5-yl)thiophene-2-carboxamide (Example 32) (100 mg, 0.22 mmol) and 3-methoxypropan-1-amine (196 mg, 2.203 mmol) was added to a sealed tube and the mixture was heated to 110 °C for 10 min. The mixture was diluted with methanol and the}
solution was purified by HPLC to give the desired product (91 mg, 82%).

$^1$H NMR (CD3OD-$d_4$) δ 7.69 (s, 1H), 7.52 (d, 1H), 7.29 (d, 1H), 7.13 (d, 1H), 6.74 (s, 1H), 6.00 (s, 1H), 4.40 (bs, 2H), 3.52 (bt, 2H), 3.40 (t, 2H), 3.33 (s, 3H), 2.98 (s, 3H), 1.83 (p, 2H); LCMS: 507.2 [M+H]$^+$, RT 3.03 min.

**Example 34**: 4-[(2-chloro-6-[[2-[[2,2-difluoro-1,3-benzodioxol-5-yl]amino]carbonyl]-3-thienyl]amino][methyl]pyrimidin-4-yl]amino]butanoic acid

![Chemical Structure]

*Step 1*: Preparation of ethyl 4-[(2-chloro-6-[[2-[[2,2-difluoro-1,3-benzodioxol-5-yl]amino]carbonyl]-3-thienyl]amino][methyl]pyrimidin-4-yl]amino]butanoate

![Chemical Structure]

This compound was prepared using the procedure for the preparation of Example 29 step 1 and the lower Rf spot was the desired product shown above which was used in step 2.

Ethyl 4-[(2-chloro-6-[(2-[[2,2-difluoro-1,3-benzodioxol-5-yl]amino]carbonyl]-3-thienyl)amino]methyl]pyrimidin-4-yl]amino]butanoate (from step 1 above) (108 mg, 0.196 mmol) was dissolved in ethanol (1 mL) and THF (1 mL). LiOH was added (47 mg, 1.965 mmol) followed by water (2 mL). The mixture was warmed to 50 °C for 16 h. The mixture was cooled to rt and acidified to pH 5 and extracted with EtOAc. The organic phase was washed with brine, dried with Na₂SO₄, and evaporated. The residue was purified using HPLC to yield the desired product (15 mg, 15%).

¹H NMR (CD3OD-d₄) δ 7.67 (s, 1H), 7.48 (d, 1H), 7.28 (d, 1H), 7.13 (d, 1H), 6.65 (bm, 1H), 6.40 (bs, 1H), 4.39 (bs, 2H), 3.40 (bm, 2H), 2.33 (bt, 2H), 1.83 (bm, 2H); LCMS: 526.0 [M+H]⁺, RT 3.28 min.

Example 35: 3-[[2-Chloro-6-(3-morpholin-4-yl-propylamino)-pyrimidin-4-ylmethyl]-amino]-thiophene-2-carboxylic acid (2,2-difluoro-benzo[1,3]dioxol-5-yl)-amide.

3-[(2,6-Dichloro-pyrimidin-4-ylmethyl)-amino]-thiophene-2-carboxylic acid (2,2-difluoro-benzo[1,3]dioxol-5-yl)-amide (Example 24) (40 mg, 0.087 mmol) was dissolved in 2-propanol (3 ml). 3-Morpholin-4-yl-propylamine (41 mg, 0.26 mmol) was added followed by the addition of 2 drops of 1N HCl. The mixture was stirred at room temperature overnight. After the reaction, the mixture was purified by preparative HPLC.
to give the title compound. Two isomers were obtained of which the desired was the more polar: 1H NMR (300 MHz, DMSO-d6) δ ppm 9.56 (s, 1 H), 7.99 (s, 2 H), 7.76 (m, 2 H), 7.63 (m, 1 H), 7.38 (d, 2 H), 7.32 (m, 1 H), 6.78 (s, 1 H), 6.68 (s, 1 H), 4.34 (s, 2 H), 4.25 (s, 1 H), 3.91 (s, 6 H), 3.70 (s, 1 H), 1.83 (s, 4 H); LCMS 567.1 [M+H+], RT = 3.12 min.

Example 36: 3-[[6-Chloro-2-{3-[4-(3-dimethylamino-propyl)piperazin-1-yl]-propylamino}-pyrimidin-4-ylmethyl]-amino]-thiophene-2-carboxylic acid (2,2-difluoro-benzo[1,3]dioxol-5-yl)-amide.

![Chemical Structure](image)

3-[[2,6-Dichloro-pyrimidin-4-ylmethyl]-amino]-thiophene-2-carboxylic acid (2,2-difluoro-benzo[1,3]dioxol-5-yl)-amide (40 mg, 0.087 mmol) (Example 24) was dissolved in 2-propanol (3 ml). N-dimethyl-3-piperazin-1-ylpropan-1-amine (45 mg, 0.26 mmol) was added followed by the addition of 2 drops of 1N HCl. The mixture was stirred at room temperature overnight. After the reaction, the mixture was purified by preparative HPLC to give the title compound. Two isomers were obtained of which the desired was the less polar: 1H NMR (300 MHz, DMSO-d6) δ ppm 9.58 (s, 1 H), 7.78 (s, 5 H), 7.38 (s, 2 H), 7.33 (s, 2 H), 7.31 (s, 1 H), 6.76 (s, 1 H), 6.62 (s, 1 H), 2.76 (s, 6 H), 2.69 (s, 6 H), some aliphatics obscured by solvent; LCMS 594.18 [M+H+], RT = 3 min.

The following examples were made by the method indicated in the table.
<table>
<thead>
<tr>
<th>Ex.-No.</th>
<th>Structure</th>
<th>Chemical Name</th>
<th>LC-MS [M + H] / RT (min)</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td><img src="" alt="Structure Image" /></td>
<td>N-(2-chloro-6-[[2-[[2,2-difluoro-1,3-benzodioxol-5-yl]amino]carbonyl]-3-thienyl]amino]methyl]pyrimidin-4-yl)glycine;</td>
<td>497.8 / 3.54</td>
<td>Preparation of Example 29, Step 1</td>
</tr>
<tr>
<td>39</td>
<td><img src="" alt="Structure Image" /></td>
<td>N-(4-chloro-6-[[2-[[2,2-difluoro-1,3-benzodioxol-5-yl]amino]carbonyl]-3-thienyl]amino]methyl]pyrimidin-2-yl)glycine;</td>
<td>498.0 / 3.7</td>
<td>Preparation of Example 29, Step 1</td>
</tr>
<tr>
<td>40</td>
<td><img src="" alt="Structure Image" /></td>
<td>N-(2,2-difluoro-1,3-benzodioxol-5-yl)-3-[[2-(methylamino)-6-[[3-(4-methylpiperazin-1-yl)propyl]amino]pyrimidine-4-yl]methyl]thiophene-2-carboxamide;</td>
<td>575.3 / 2.45</td>
<td>Preparation of Example 33</td>
</tr>
<tr>
<td></td>
<td>Chemical Structure</td>
<td>Chemical Formula</td>
<td>Molecular Weight</td>
<td>Preparation Conditions</td>
</tr>
<tr>
<td>---</td>
<td>--------------------</td>
<td>------------------</td>
<td>-------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>41</td>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>N-(2,2-difluoro-1,3-benzodioxol-5-yl)-3-[(2-[3-(3-methoxypropyl)amino]-6-(methylamino)pyrimidin-4-yl)methyl]amino)thiophene-2-carboxamide;</td>
<td>507.3 / 3.02</td>
<td>Preparation of Example 31 except it was run neat at 110°C for 10 min</td>
</tr>
<tr>
<td>42</td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>N-(2,2-difluoro-1,3-benzodioxol-5-yl)-3-[(6-(methylamino)-2-[(3-morpholin-4-yl)propyl]amino]pyrimidin-4-yl)methyl]amino)thiophene-2-carboxamide;</td>
<td>562.3 / 2.61</td>
<td>Preparation of Example 31 except it was run neat at 80°C for 6 h</td>
</tr>
</tbody>
</table>

Generally, a desired salt of a compound of this invention can be prepared in situ during the final isolation and purification of a compound by means well known in the art. Or, a desired salt can be prepared by separately reacting the purified compound in its free base form with a suitable organic or inorganic acid and isolating the salt thus formed. These methods are conventional and would be readily apparent to one skilled in the art. Additionally, sensitive or reactive groups on the compound of this invention may need to be protected and deprotected during any of the above methods. Protecting groups in general may be added and removed by conventional methods well known in the art (see, for example, T. W. Greene and P.G.M. Wuts, *Protective Groups in Organic Synthesis*; Wiley: New York, (1999).
B. Evaluation of physiological activity

The utility of the compounds of the present invention can be illustrated, for example, by their activity in the P-AKT/PKB Cytoblot Assay described below. The involvement of the P-AKT/PKB [PI3K/AKt] pathway as a target for cancer chemotherapy has been recognized in the art. For example, see F. Chang et al, Involvement of PI3K/Akt pathway in cell cycle progression, apoptosis, and neoplastic transformation: a target for cancer chemotherapy, Leukemia, 2003, 17: p. 590-603; K. A. West et al, Activation of the PI3K/Akt pathway and chemotherapeutic resistance, Drug Resistance Updates, 2002, 5: p. 234-248; and P. Sen et al, Involvement of the Akt/PKB signaling pathway with disease processes, Molecular and Cellular Biochemistry, 2003, 253: p. 241-246.

P-AKT/PKB Cytoblot Assay Protocol with H209 Cells

H209 small cell lung carcinoma cells in log phase were plated at 50,000 cells/well in 96-well poly-lysine coated, clear bottom/ black-sided plates (Becton-Dickinson, USA Cat # 354640) in 100 µl RPMI medium containing 0.1% (w/v) BSA, and incubated overnight at 37°C in 5% CO₂ incubator. The following day, compounds (10 mM stock solutions in DMSO) were added to the plates to generate final concentrations of 0.0, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0 and 10 µM for IC₅₀ determinations and incubated for 1 hour at 37°C. Cells were then left untreated or stimulated with Stem Cell Factor (SCF: Biosource Cat # PHC2116) at a final concentration of 25 ng/mL for 5 minutes at 37°C in 5% CO₂ incubator. The media was then removed using a vacuum manifold and the cells were washed once with Tris Buffered Saline (TBS). Cells were then fixed by adding 200 µl of cold 3.7% (v/v) formaldehyde in TBS to each well for 15 minutes at 4°C. After removal of the formaldehyde, the cells were treated with the addition of 50 µl of methanol (at –20°C) to each well for 5 minutes. After removal of the methanol, 200 µl of 1% (w/v) BSA in TBS was added to each well to block non-specific antibody binding sites and the plate was incubated at room temperature for 30 minutes. After removal of the blocking buffer, 50 µl of p-(S473) AKT rabbit polyclonal antibody (Cell Signaling, USA Cat # 9277S) was added at a dilution of 1:250 in 0.1% (w/v) BSA in TBS, and the plate was incubated at room temperature for 1 hour. Plates were then washed
3 times with cold TBS containing 0.05% (v/v) Tween 20 (TBS-T) and 100 μl of Horseradish peroxidase (HRP)-conjugated goat-anti-rabbit antibody (Amersham, USA Cat # NA934V) at a dilution of 1:250 in TBS-T was added and the plate was incubated at room temperature for 1h. After washing with ice-cold TBS-T four times, 100 μl of Enhanced Chemiluminescence (ECL) reagent (Amersham, USA Cat# RPN2209) was added to each well and mixed on a mini-orbital shaker for 1 min. The plate was then read on a Perkin Elmer Victor 5 Multilabel Counter (#1420-0421).

Compounds of examples 1-5, 19-23, 25-28, 30, 35-36, were tested in the above P-AKT/PKB Cytoblot assay, with the result that these examples exhibited IC$_{50}$ values of less than or equal to 3 μM. In one embodiment, the present invention relates to a compound which exhibits an IC$_{50}$ value of less than or equal to 3 μM in this assay.

The utility of the compounds of the present invention can also be illustrated, for example, by their activity in the phospho-ERK Assay described below.


Phospho-ERK Cytoblot Assay Protocol with MDA-MB 231 Cells

MDA-MB-231 cells in log phase were plated at 25,000 cells/well in 96-well opaque plates (Falcon, USA Cat # 353296) in 100 μL RPMI medium containing 10% (w/v) FBS, and incubated overnight at 37°C in 5% CO₂ incubator. The following day, the growth medium
was removed from the plate by aspiration and replaced with RPMI medium containing 0.1% BSA and example compounds diluted to generate final concentrations of 0.0, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1 and 3 μM. Cells were incubated with compound for 1 hour at 37°C in a 5% CO₂ incubator. The media was then removed from the plate by aspiration and the cells were washed once with 180 μL/well cold Tris Buffered Saline (TBS). After removal of the wash buffer, the cells were fixed by adding 180 μL of cold 3.7% (v/v) formaldehyde in TBS to each well for 1 hour at 4°C. After removal of the formaldehyde, the cells were treated with the addition of 60 μL of -20°C methanol to each well for 5 minutes at 4°C. The methanol was removed and the cells were washed with 180 μL/well of 5% (w/v) BSA in TBS. To block non-specific antibody binding sites, each well was treated with 180 μL/well 5% BSA (w/v) in TBS for thirty minutes at room temperature. After removal of the blocking buffer, 50 μL of an anti-phospho-p44/42 MAP kinase (Thr202/Tyr204) rabbit polyclonal antibody (Cell Signaling, USA Cat # 9101) was added to each well at a dilution of 1:1000 in 5% (w/v) BSA in TBS, and the plate was incubated at 4°C overnight. Plates were then washed three times with 300 μL/well TBS at room temperature. The plates were then incubated with 50 μL of Horseradish peroxidase (HRP)-conjugated goat-anti-rabbit antibody (Amersham, USA Cat. # NA934V) at a dilution of 1:1000 in 5% BSA-TBS at room temperature for 1 hr. After washing the plate three times with 300 μL/well TBS, 60 μL of Enhanced Chemiluminescence (ECL) reagent (Amersham, USA Cat# RPN2209) was added to each well and incubated at room temperature for five minutes. The plate was then read on a Perkin Elmer Victor 5 Multilabel Counter (#1420-0421).

The compounds of examples 1-4, 19-21, 29, 32, were tested and showed an IC₅₀ value of less than or equal to 3 μM in this assay. In one embodiment, the present invention relates to a compound which exhibits an IC₅₀ value of less than or equal to 3 μM.

The utility of the compounds of the present invention can also be illustrated, for example, by their activity in the flk-1(murine VEGFR2) Assay described below.

The VEGF-VEGFR2 signaling pathway has been extensively characterized as an important regulator of angiogenesis and tumor angiogenesis (See G. Yancopoulos et al, Vascular-specific growth factors and blood vessel formation, Nature, 2000, 407: p.. 242-248; D. Shweiki et al, Induction of vascular endothelial growth factor expression by

Flk-1 (murine VEGFR-2) Biochemical Assay
This assay was performed in 96-well opaque plates (Costar, USA Cat #3915) in the TR-FRET format. Reaction conditions were as follows: 10 µM ATP, 25 nM poly (Glu,Tyr)-biotin (CIS BIO International, USA Cat#61GT0BLD), 2 nM Eu-labelled phospho-Tyr Ab (Perkin Elmer, USA Cat#AD0067), 10 nM Strepavidin-APC (Perkin Elmer, USA Cat#CR130-100), 7 nM Flk-1 (kinase domain), 1% DMSO, 50 mM HEPES pH 7.5, 10 mM MgCl₂, 0.1 mM EDTA, 0.015% BRIJ, 0.1 mg/mL BSA, 0.1% mercapto-ethanol. Prior to the addition of enzyme, compounds were added to final concentrations ranging from 10 µM to 4.56 nM in 1% DMSO. The reaction was initiated upon addition of enzyme. Final reaction volume in each well was 100µL. Time-resolved fluorescence was read after excitation at 340 nM. Emission readings were taken at both 665 and 615 nM on a Perkin Elmer Victor V Multilabel counter at 1.5 - 2.0 hrs after reaction initiation. Signal was calculated as follows: Emission 665 nm/ Emission 615 nM x 10000 for each well. The compounds of examples 1 and 5 were tested and showed an IC₅₀ value of less than 3 µM in this assay. In one embodiment, the present invention relates to a compound which exhibits an IC₅₀ value of less than or equal to 3 µM.
Method of Treating
Another embodiment of the present invention thus relates to a method of using the compounds described above, including salts thereof and corresponding compositions thereof, as cancer chemotherapeutic agents. This method comprises administering to a patient an amount of a compound of this invention, or a pharmaceutically acceptable salt thereof, which is effective to treat the patient's cancer. A patient, for the purpose of this invention, is a mammal, including a human, in need of treatment for a particular cancer. Cancers include but are not limited to solid tumors, such as cancers of the breast, respiratory tract, brain, reproductive organs, digestive tract, urinary tract, eye, liver, skin, head and neck, thyroid, parathyroid and their distant metastases. Those disorders also include lymphomas, sarcomas, and leukemias.
Examples of breast cancer include, but are not limited to invasive ductal carcinoma, invasive lobular carcinoma, ductal carcinoma in situ, and lobular carcinoma in situ.
Examples of cancers of the respiratory tract include, but are not limited to small-cell and non-small-cell lung carcinoma, as well as bronchial adenoma and pleuropulmonary blastoma.
Examples of brain cancers include, but are not limited to brain stem and hypophtalmic glioma, cerebellar and cerebral astrocytoma, medulloblastoma, ependymoma, as well as neuroectodermal and pineal tumor.
Tumors of the male reproductive organs include, but are not limited to prostate and testicular cancer. Tumors of the female reproductive organs include, but are not limited to endometrial, cervical, ovarian, vaginal, and vulvar cancer, as well as sarcoma of the uterus.
Tumors of the digestive tract include, but are not limited to anal, colon, colorectal, esophageal, gallbladder, gastric, pancreatic, rectal, small-intestine, and salivary gland cancers.
Tumors of the urinary tract include, but are not limited to bladder, penile, kidney, renal pelvis, ureter, and urethral cancers.
Eye cancers include, but are not limited to intraocular melanoma and retinoblastoma.
Examples of liver cancers include, but are not limited to hepatocellular carcinoma (liver cell carcinomas with or without fibrolamellar variant), cholangiocarcinoma (intrahepatic bile duct carcinoma), and mixed hepatocellular cholangiocarcinoma.
Skin cancers include, but are not limited to squamous cell carcinoma, Kaposi’s sarcoma,
malignant melanoma, Merkel cell skin cancer, and non-melanoma skin cancer.
Head-and-neck cancers include, but are not limited to laryngeal / hypopharyngeal / nasopharyngeal / oropharyngeal cancer, and lip and oral cavity cancer.
Lymphomas include, but are not limited to AIDS-related lymphoma, non-Hodgkin’s lymphoma, cutaneous T-cell lymphoma, Hodgkin’s disease, and lymphoma of the central nervous system.
Sarcomas include, but are not limited to sarcoma of the soft tissue, osteosarcoma, malignant fibrous histiocytoma, lymphosarcoma, and rhabdomyosarcoma.
Leukemias include, but are not limited to acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, and hairy cell leukemia.
These disorders have been well characterized in humans, but also exist with a similar etiology in other mammals, and can be treated by administering pharmaceutical compositions of the present invention.

The compounds of this invention can be administered as the sole pharmaceutical agent or in combination with one or more other pharmaceutical agents where the combination causes no unacceptable adverse effects. For example, the compounds of this invention can be combined with known anti-hyper-proliferative, chemotherapeutic, or other indication agents, and the like, as well as with admixtures and combinations thereof.
Optional anti-hyper-proliferative agents which can be added to the composition include but are not limited to compounds listed on the cancer chemotherapy drug regimens in the 11th Edition of the Merck Index, (1996), such as cisplatin.
Other anti-hyper-proliferative agents suitable for use with this invention include but are not limited to those compounds acknowledged to be used in the treatment of neoplastic diseases in Goodman and Gilman’s The Pharmacological Basis of Therapeutics (Ninth Edition), editor Molinoff et al., publ. by McGraw-Hill, pages 1225-1287, (1996) such as idarubicin.
C.  Operative examples relating to pharmaceutical compositions

The active compound can act systemically and/or locally. For this purpose it can be administered in a suitable manner, such as for example by oral, parenteral, pulmonary, nasal, sublingual, lingual, buccal, rectal, dermal, transdermal, ophtalmic or otic administration or in the form of an implant or stent. The active compound can be administered in forms suitable for these modes of administration.

Suitable forms of oral administration are those according to the prior art which function by releasing the active compound rapidly and/or in a modified or controlled manner and which contain the active compound in a crystalline and/or amorphous and/or dissolved form, such as for example tablets (which are uncoated or coated, for example with enteric coatings or coatings which dissolve after a delay in time or insoluble coatings which control the release of the active compound), tablets or films/wafers which disintegrate rapidly in the oral cavity or films/lyophilisates, capsules (e.g. hard or soft gelatin capsules), dragées, pellets, powders, emulsions, suspensions and solutions.

Parenteral administration can be carried out by avoiding an absorption step (e.g. by intravenous, intraarterial, intracardial, intraspinal or intralumbar administration) or by including absorption (e.g. by intramuscular, subcutaneous, intracutaneous or intraperitoneal administration). Suitable parenteral administration forms are for example injection and infusion formulations in the form of solutions, suspensions, emulsions, lyophilisates and sterile powders.

Suitable forms of administration for the other modes of administration are for example inhalation devices (such as for example powder inhalers, nebulizers), nasal drops, solutions and sprays; tablets or films/wafers for lingual, sublingual or buccal administration or capsules, suppositories, ear and eye preparations, vaginal capsules, aqueous suspensions (lotions or shaking mixtures), lipophilic suspensions, ointments, creams, transdermal therapeutic systems, milky lotions, pastes, foams, dusting powders, implants or stents.

The active compounds can be converted into the abovementioned forms of administration
in a manner known to the skilled man and in accordance with the prior art using inert, non-toxic, pharmaceutically suitable auxiliaries. The latter include for example excipients (e.g. microcrystalline cellulose, lactose, mannitol, etc.), solvents (e.g. liquid polyethylene glycols), emulsifiers and dispersants or wetting agents (e.g. sodium dodecyl sulphate, polyoxysorbitan oleate etc.), binders (e.g. polyvinyl pyrrolidone), synthetic and/or natural polymers (e.g. albumin), stabilizers (e.g. antioxidants, such as, for example, ascorbic acid), dyes (e.g. inorganic pigments such as iron oxides) or taste- and/or odour-corrective agents.

The total amount of the active ingredient to be administered will generally range from about 0.01 mg/kg to about 200 mg/kg, and preferably from about 0.1 mg/kg to about 20 mg/kg body weight per day. A unit dosage may contain from about 0.5 mg to about 1500 mg of active ingredient, and can be administered one or more times per day. The daily dosage for administration by injection, including intravenous, intramuscular, subcutaneous and parenteral injections, and use of infusion techniques will preferably be from 0.01 to 200 mg/kg of total body weight. The daily rectal dosage regimen will preferably be from 0.01 to 200 mg/kg of total body weight. The daily vaginal dosage regimen will preferably be from 0.01 to 200 mg/kg of total body weight. The daily topical dosage regimen will preferably be from 0.1 to 200 mg administered between one to four times daily. The transdermal concentration will preferably be that required to maintain a daily dose of from 0.01 to 200 mg/kg. The daily inhalation dosage regimen will preferably be from 0.01 to 100 mg/kg of total body weight.

It may however be necessary to deviate from the abovementioned quantities, depending on the body weight, mode of administration, the individual patient response to the active compound, the type of preparation and the time or interval of administration.

The compounds of this invention can be administered as the sole pharmaceutical agent or in combination with one or more other pharmaceutical agents where the combination causes no unacceptable adverse effects. For example, the compounds of this invention can be combined with known anti-hyper-proliferative, chemotherapeutic, or other indication agents, and the like, as well as with admixtures and combinations thereof. Optional anti-hyper-proliferative agents which can be added to the composition include but are not limited to compounds listed on the cancer chemotherapy drug regimens in the
11th Edition of the *Merck Index*, (1996), such as cisplatin. Other anti-hyper-proliferative agents suitable for use with this invention include but are not limited to those compounds acknowledged to be used in the treatment of neoplastic diseases in *Goodman and Gilman's The Pharmacological Basis of Therapeutics* (Ninth Edition), editor Molinoff et al., publ. by McGraw-Hill, pages 1225-1287, (1996) such as idarubicin.

The compounds according to the invention can be converted into pharmaceutical preparations as follows:

**Tablet:**
Composition:
100 mg of the compound of Example 1, 50 mg of lactose (monohydrate), 50 mg of maize starch (native), 10 mg of polyvinylpyrrolidone (PVP 25) (from BASF, Ludwigshafen, Germany) and 2 mg of magnesium stearate.
Tablet weight 212 mg, diameter 8 mm, curvature radius 12 mm.

Preparation:
The mixture of active component, lactose and starch is granulated with a 5% solution (m/m) of the PVP in water. After drying, the granules are mixed with magnesium stearate for 5 min. This mixture is moulded using a customary tablet press (tablet format, see above). The moulding force applied is typically 15 kN.

**Suspension for oral administration:**
Composition:
1000 mg of the compound of Example 1, 1000 mg of ethanol (96%), 400 mg of Rhodigel (xanthan gum from FMC, Pennsylvania, USA) and 99 g of water.
A single dose of 100 mg of the compound according to the invention is provided by 10 ml of oral suspension.

Preparation:
The Rhodigel is suspended in ethanol and the active component is added to the suspension. The water is added with stirring. Stirring is continued for about 6h until the
swelling of the Rhodigel is complete.

It is believed that one skilled in the art, using the preceding information, can utilize the present invention to its fullest extent. It should be apparent to one of ordinary skill in the art that changes and modifications can be made to this invention without departing from the spirit or scope of the invention as it is set forth herein. Other embodiments of the invention will be apparent to the skilled in the art from a consideration of this specification or practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.
We claim:

1. A compound of formula (I) or formula (II)

wherein

$R^1$ represents $-C_{1-4}$ alkyl, or halogen; and the subscript a, which represents the number of substituents $R^1$, is 0, 1, or 2;

$R^2$ represents $-C_{1-4}$ alkyl, $-C_{1-4}$ alkoxy, or halogen; and the subscript b, which represents the number of substituents $R^2$, is 0, 1, or 2;

$R^3$ represents $-C(O)NR^{3-1}$, $-NR^{3-2}R^{3-2}$, $-NHC(O)R^{3-3}$, $-C(O)OR^{3-4}$, $-CN$; -halogen; or $-C_{1-4}$ alkoxy; and the subscript d, which represents the number of substituents $R^3$, is 0, 1, or 2;

wherein

substituents $R^{3-1}$ each independently represent hydrogen, $-C_{1-4}$ alkyl, $-C_{3-6}$ cycloalkyl, or hydroxy-$C_{1-4}$-alkyl; and

substituents $R^{3-2}$ each independently represents hydrogen or $-C_{1-4}$ alkyl, wherein $-C_{1-4}$ alkyl can optionally be substituted with a substituent selected
from the group consisting of hydroxy, -C<sub>1-4</sub> alkoxy, carboxy, -C<sub>1-4</sub> alkoxy carbonyl, morpholinyl and N-methylpiperazinyl;

substituent R<sup>3-3</sup> represents hydrogen or -C<sub>1-4</sub> alkyl;

substituent R<sup>3-4</sup> represents hydrogen or -C<sub>1-4</sub> alkyl; or

R<sup>3</sup> represents

![Chemical structure](image)

; and

X represents N or CH;

or a pharmaceutically acceptable salt thereof.

2. A compound of formula (I-1) or formula (II-1),

![Chemical structures](image)

wherein

R<sup>3</sup> represents -C(O)NR<sup>3-1</sup>R<sup>3-1</sup>; -NR<sup>3-2</sup>R<sup>3-2</sup>; -NHC(O)R<sup>3-3</sup>; or -C(O)OR<sup>3-4</sup>; and the subscript d, which represents the number of substituents R<sup>3</sup>, is 1, or 2;

wherein
substituents R\(^3\) each independently represent hydrogen, -C\(_{1-4}\) alkyl, -C\(_{3-6}\) cycloalkyl, or hydroxy-C\(_{1-4}\)-alkyl; and

substituents R\(^3\)-2 each independently represents hydrogen or -C\(_{1-4}\) alkyl, wherein -C\(_{1-4}\) alkyl can optionally be substituted with a substituent selected from the group consisting of hydroxy, -C\(_{1-4}\) alkoxy, carboxy, -C\(_{1-4}\) alkoxy carbonyl, morpholinyl and N-methylpiperazinyl;

substituent R\(^3\)-3 represents hydrogen or -C\(_{1-4}\) alkyl;

substituent R\(^3\)-4 represents hydrogen or -C\(_{1-4}\) alkyl; or

R\(^3\) represents

\[ \text{structure image} \] ; and

X represents N or CH;

or a pharmaceutically acceptable salt thereof.

3. A compound of formula (I-2) or formula (II-2), wherein

\[ \text{formula images} \]

wherein

R\(^3\) represents -C(O)NR\(^3\)-1R\(^3\)-1; -NR\(^3\)-2R\(^3\)-2; or -NHC(O)R\(^3\)-3;

wherein
substituents $R^{3-1}$ each independently represent hydrogen, -C$_{1-4}$ alkyl, -C$_{3-6}$ cycloalkyl, or hydroxy-C$_{1-4}$-alkyl; and

substituents $R^{3-2}$ each independently represents hydrogen or -C$_{1-4}$ alkyl, wherein -C$_{1-4}$ alkyl can optionally be substituted with a substituent selected from the group consisting of hydroxy, -C$_{1-4}$ alkoxy, carboxy, -C$_{1-4}$ alkoxy carbonyl, morpholiny1 and N-methylpiperazinyl;

substituent $R^{3-3}$ represents -C$_{1-4}$ alkyl; or

$R^3$ represents

```
\*----N----N----N
 | \      | \\
| \   \  | \\
| H3C  | CH3
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or a pharmaceutically acceptable salt thereof.

4. A compound of claim 1 for the treatment or prevention of disorders.

5. A pharmaceutical composition comprising the compound of claim 1.

6. The pharmaceutical composition of claim 5, additionally comprising at least one pharmaceutically acceptable carrier or excipient.

7. The pharmaceutical composition of claim 7 for the treatment or prevention of cancer.

8. A process for preparing the pharmaceutical composition of claim 7, comprising combining at least one compound according to claim 1 with at least one pharmaceutically acceptable carrier or excipient and bringing the resulting combination into a form suitable for said pharmaceutical composition.
9. A use of a compound of claim 1 for manufacturing a pharmaceutical composition for the treatment or prevention of a disease.

10. The use of claim 9, wherein the disease is cancer.

11. A method of treating a disease or condition in a mammal, comprising administering to a mammal in need thereof an effective amount of a compound of claim 1.

12. The method of claim 11, wherein the disease or condition is cancer.

13. A packaged pharmaceutical composition comprising a container comprising the pharmaceutical composition of claim 7 and instructions for using the pharmaceutical composition to treat a disease or condition in a mammal.