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(54) **Title:** THERAMUTEIN MODULATORS

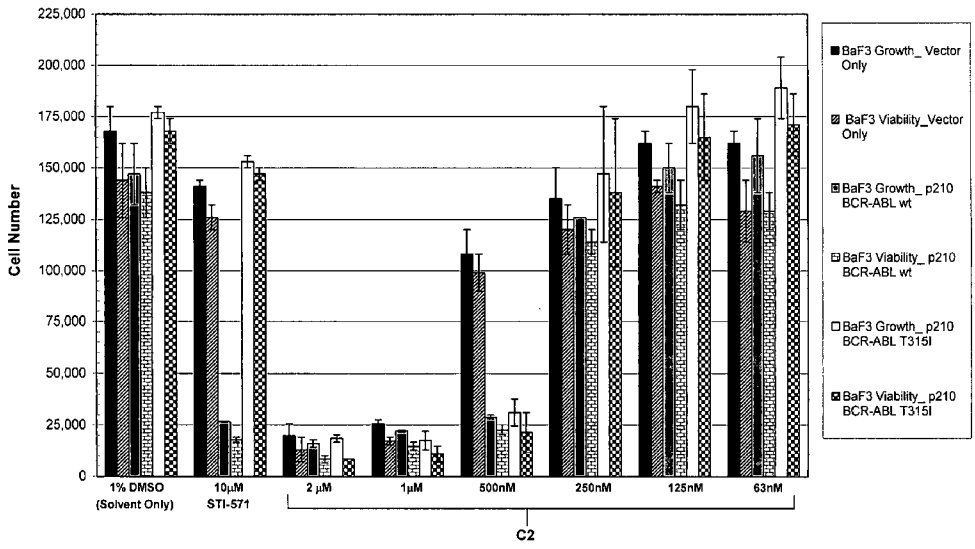

Fig. 1

(57) **Abstract:** This invention relates to agents that are inhibitors or activators of variant forms of endogenous proteins and novel methods of identifying such variants. Of particular interest are inhibitors and activators of endogenous protein variants, encoded by genes which have mutated, which variants often arise or are at least first identified as having arisen following exposure to a chemical agent which is known to be an inhibitor or activator of the corresponding unmutated endogenous protein.

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THERAMUTEIN MODULATORS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Ser. No. 60/904,115, filed February 27, 2007, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] This invention relates to agents that are inhibitors or activators of variant forms of endogenous proteins and novel methods of identifying such variants. Of particular interest are inhibitors and activators of endogenous protein variants, encoded by genes which have mutated, which variants often arise or are at least first identified as having arisen following exposure to a chemical agent which is known to be an inhibitor or activator of the corresponding unmutated endogenous protein.

BACKGROUND OF THE INVENTION

[0003] The progressive development of drug resistance in a patient is the hallmark of chronic treatment with many classes of drugs, especially in the therapeutic areas of cancer and infectious diseases. Molecular mechanisms have been identified which mediate certain types of drug resistance phenomena, whereas in other cases the mechanisms of acquired as well as *de novo* resistance remain unknown today.

[0004] One mechanism of induced (acquired) drug resistance originally thought to be relevant in the area of cancer therapy involves increased expression of a protein known as P-glycoprotein (P-gp). P-gp is located in the cell membrane and functions as a drug efflux pump. The protein is capable of pumping toxic chemical agents, including many classical anti-cancer drugs, out of the cell. Consequently, upregulation of P-glycoprotein usually results in resistance to multiple drugs. Upregulation of P-glycoprotein in tumor cells may represent a defense mechanism which has evolved in mammalian cells to prevent damage from toxic chemical agents. Other related drug resistance proteins have now been identified with similar functions to P-gp, including multidrug-resistance-associated protein family members such as MRP1 and ABCG2. In any event, with the advent of the development of compounds that are specific for a given target protein, and less toxic, the importance of

P-glycoprotein and related ATP-binding cassette (ABC) transporter proteins in clinically significant drug resistance has lessened.

[0005] Another possible molecular mechanism of acquired drug resistance is that alternative signal pathways are responsible for continued survival and metabolism of cells, even though the original drug is still effective against its target. Furthermore, alterations in intracellular metabolism of the drug can lead to loss of therapeutic efficacy as well. In addition, changes in gene expression as well as gene amplification events can occur, resulting in increased or decreased expression of a given target protein and frequently requiring increasing dosages of the drug to maintain the same effects. (Adcock and Lane, 2003)

[0006] Mutation induced drug resistance is a frequently occurring event in the infectious disease area. For example, several drugs have been developed that inhibit either the viral reverse transcriptase or the viral protease encoded in the human immunodeficiency (HIV) viral genome. It is well established in the literature that repeated treatment of HIV-infected AIDS patients using, for example, a reverse transcriptase inhibitor eventually gives rise to mutant forms of the virus that have reduced sensitivity to the drug. Mutations that have arisen in the gene encoding reverse transcriptase render the mutant form of the enzyme less affected by the drug.

[0007] The appearance of drug resistance during the course of HIV treatment is not surprising considering the rate at which errors are introduced into the HIV genome. The HIV reverse transcriptase enzyme is known to be particularly error prone, with a forward mutation rate of about 3.4×10^{-5} mutations per base pair per replication cycle (Mansky et al., *J. Virol.* 69:5087-94 (1995)). However, analogous mutation rates for endogenous genes encoded in mammalian cells are more than an order of magnitude lower.

[0008] New evidence shows that drug resistance can also arise from a mutational event involving the gene encoding the drug target (Gorre et al., Science, 2001; PCT/US02/18729). In this case, exposure of the patient to a specific therapeutic substance such as a given cancer drug that targets a specific ***protein-of-interest*** (POI, or "target" protein) may be followed by the outgrowth of a group of cells harboring a mutation occurring in the gene encoding the protein that is the target of the therapeutic substance. Whether the outgrowth of this population of cells results from a small percentage of pre-existing cells in the patient which already harbor a mutation which gives rise to a drug-resistant POI, or

whether such mutations arise *de novo* during or following exposure of the animal or human being to a therapeutic agent capable of activating or inhibiting said POI, is presently unknown. In either case, such mutation events may result in a mutated protein (defined below as a ***theramutein***) which is less affected, or perhaps completely unaffected, by said therapeutic substance.

[0009] Chronic myelogenous leukemia (CML) is characterized by excess proliferation of myeloid progenitors that retain the capacity for differentiation during the stable or chronic phase of the disease. Multiple lines of evidence have established deregulation of the Abl tyrosine kinase as the causative oncogene in certain forms of CML. The deregulation is commonly associated with a chromosomal translocation known as the Philadelphia chromosome (Ph), which results in expression of a fusion protein comprised of the *BCR* gene product fused to the Abelson tyrosine kinase, thus forming $p210^{Bcr\text{-}Abl}$ which has tyrosine kinase activity. A related fusion protein, termed $p190^{Bcr\text{-}Abl}$, that arises from a different breakpoint in the *BCR* gene, has been shown to occur in patients with Philadelphia chromosome positive (Ph+) Acute Lymphoblastic Leukemia (ALL) (Melo, 1994; Ravandi et al., 1999). Transformation appears to result from activation of multiple signal pathways including those involving RAS, MYC, and JUN. Imatinib mesylate ("STI-571" or "Gleevec®") is a 2-phenylamino pyrimidine that targets the ATP binding site of the kinase domain of Abl (Druker et al, NEJM 2001, p. 1038). Subsequently it has also been found by other methods to be an inhibitor of platelet-derived growth factor (PDGF) β receptor, and the Kit tyrosine kinase, the latter of which is involved in the development of gastrointestinal stromal tumors (see below).

[0010] Until recently, it had not been observed that during the course of treatment with a specific inhibitor of a given endogenous cellular protein that a mutation in its corresponding endogenous gene could lead to the expression of protein variants whose cellular functioning was resistant to the inhibitor. Work by Charles Sawyers and colleagues (Gorre et al., Science 293:876-80 (2001); PCT/US02/18729) demonstrated for the first time that treatment of a patient with a drug capable of inhibiting the $p210^{Bcr\text{-}Abl}$ tyrosine kinase (i.e., STI-571) could be followed by the emergence of a clinically significant population of cells within said patient harboring a mutation in the gene encoding the $p210^{Bcr\text{-}Abl}$ cancer causing target protein which contains the Abelson tyrosine kinase domain. Various such

mutations gave rise to mutant forms of $p210^{Bcr\text{-}Abl}$ which were less responsive to Gleevec treatment than was the original cancer causing version. Notably, the mutations that emerged conferred upon the mutant protein a relative resistance to the effects of the protein kinase inhibitor drug, while maintaining a certain degree of the original substrate specificity of the mutant protein kinase. Prior to the work of Gorre et al., it was generally believed by those skilled in the art that the types of resistance that would be observed in patients exposed to a compound which inhibited the Abelson protein kinase, such as STI-571, would have resulted from one or more of the other mechanisms of drug resistance listed above, or by some other as yet unknown mechanism, but that in any event said resistance would involve a target (protein or otherwise) which was distinct from the drug's target POI.

[0011] Accordingly, the ability to treat clinically relevant resistant mutant forms of proteins that are otherwise the targets of an existing therapy would be extremely useful. Such mutated proteins (theramuteins as defined below) are beginning to be recognized and understood to be important targets in recurring cancers, and will become important in other diseases as well. There exists a need for therapeutic agents that are active against such drug resistant variant forms of cellular proteins that may arise before, during or following normally effective drug therapies. A key purpose of this invention is to provide compounds that may serve as potential therapeutic agents useful in overcoming mutation-induced drug resistance in endogenously occurring proteins.

BRIEF SUMMARY OF THE INVENTION

[0012] This invention relates to agents that are inhibitors or activators of variant forms of proteins. The invention also relates to agents that are inhibitors or activators of certain variant forms of endogenous proteins. Of particular interest are inhibitors and activators of endogenous protein variants, encoded by genes which have mutated, which variants often arise or are at least first identified as having arisen following exposure to a chemical agent which is known to be an inhibitor or activator of the corresponding unmutated endogenous protein. Such protein variants (mutant proteins) are herein termed "theramuteins," and may occur either spontaneously in an organism (and be pre-existing mutations in some cases), or said mutants may arise as a result of selective pressure which results when the organism is treated with a given chemical agent capable of inhibiting the non-mutated form of said theramutein (herein termed a "prototheramutein"). It will be

understood that in some cases a prototheramutein may be a "wild type" form of a POI (*e.g.*, a protein that gives rise to a disease due to disregulation). In other cases, the prototheramutein will be a disease causing variant of a "wild type" protein, having already mutated and thereby contributing to the development of the diseased state as a result of said prior mutation. One example of the latter type of prototheramutein is the $P210^{BCR-ABL}$ oncoprotein, and a mutant form of this protein harboring a threonine (T) to isoleucine (I) mutation at position 315 is termed $P210^{BCR-ABL-T315I}$ and is one example of a theramutein. As used herein, the designation "$P210^{BCR-ABL}$" is synonymous with the term "$p210^{Bcr-Abl}$", the "wild-type Bcr-Abl protein", and the like.

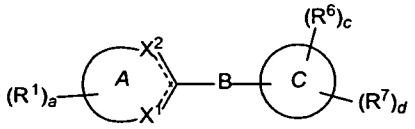
[0013] Theramuteins are a rare class of endogenous proteins that harbor mutations that render said proteins resistant to drugs that are known to inhibit or activate in a therapeutically effective manner their non-mutated counterparts. The endogenous genes encoding a few such proteins are presently known to exhibit such mutations under certain circumstances. In one embodiment, this invention is directed toward compositions that inhibit certain drug-resistant mutants (theramuteins) of the Abelson tyrosine kinase protein, originally termed P210-Bcr-Abl in the literature, that is involved in the development of chronic myelogenous leukemia.

[0014] The present invention is particularly directed toward the identification of *specific* inhibitors or *specific* activators of proteins. Use of the term "specific" in the context of the terms "inhibitor" or "activator" (see definitions below) means that said inhibitor or activator binds to the protein and inhibits or activates the cellular functioning of the protein without also binding to and activating or inhibiting a wide variety of other proteins or non-protein targets in the cell. The skilled investigator is well aware that there is a certain degree of variability in the medical literature with respect to the concept of a *specific inhibitor* or a *specific activator*, and of the related concept of target protein "specificity" when discussing the actions of inhibitors or activators of a protein. Accordingly, for the purposes of this invention, a substance is a specific inhibitor or a specific activator of a given protein if said substance is capable of inhibiting or activating said protein at a given concentration such that a corresponding phenoresponse is modulated in the appropriate manner, without having an appreciable effect at the same given concentration upon the phenoresponse (if any) of a corresponding control cell that essentially does not express the protein.

[0015] In certain embodiments, a substance may be a modulator of two closely related proteins such as a prototheramutein and one of its corresponding theramuteins. In other embodiments, in addition to being a modulator of the prototheramutein and theramutein, a substance may also modulate the activities of proteins that have similar functions. As discussed above, in addition to inhibiting the $p210^{Bcr\ Abl}$ tyrosine kinase, imatinib mesylate is also capable of inhibiting the c-kit oncogene product (also a tyrosine kinase) which is overexpressed in certain gastrointestinal stromal tumors, as well as the PDGF β receptor (also a tyrosine kinase), which is expressed in certain chronic myelomonocytic leukemias (CMML). Such a compound is sometimes termed a "moderately specific" inhibitor.

[0016] The invention also provides a general method that can be used to identify substances that will activate or inhibit a theramutein, to the same extent, and preferably to an even greater extent than a known drug substance is capable of inhibiting the corresponding "wild type" form of that protein. (The skilled artisan is well aware, however, that said "wild type" forms of such proteins may have already mutated in the course of giving rise to the corresponding disease in which said protein participates.)

[0017] In a preferred embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula I



(I)

wherein:

ring *A* is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

X^1 is selected from N, N-R^0 or C-R^1;

X^2 is selected from N, N-R^0 or C-R^1;

each R^1 is independently selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^{11}, $-(CH_2)_pC(O)(CH_2)_qR^{11}$, $-(CH_2)_pC(O)N(R^{12})(R^{13})$, $-(CH_2)_pC(O)O(CH_2)_qR^{11}$, $-(CH_2)_pN(R^{11})(CH_2)_qC(O)R^{11}$, $-(CH_2)_pN(R^{12})(R^{13})$, $-(CH_2)_pN(R^{11})(CH_2)_qR^{11}$, $-N(R^{11})SO_2R^{11}$, $-OC(O)N(R^{12})(R^{13})$, $-SO_2N(R^{12})(R^{13})$, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

a is 0 to 4;

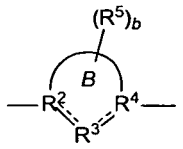
each R^{11} is independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

each R^{12} and R^{13} are independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring; or R^{12} and R^{13} may be taken together with the nitrogen to which they are attached form a 5- to 7- membered ring which may optionally contain a further heteroatom; wherein the 5- to 7-membered ring may optionally be substituted with one to three substituents that are independently selected from alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, CO_2R^0, $C(O)R^0$, halo, aryl, and a heterocyclic ring;

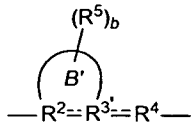
p is 0 to 4;

q is 0 to 4;

B is selected from a group having the formula:



and



;

Ring *B* and Ring *B'* are a 5-, or 6- membered ring;

R^2 is selected from N, C and CH;

R^3 is selected from N, NR^{31}, O, S, CR^{31} and CHR^{31};

R^{31} is selected from H, and alkyl;

$R^{3'}$ is selected from N, C and CH;

R^4 is selected from N, C or CH;

each R^5 is independently selected from OH, O-alkyl, =O, SH, S-alkyl, =S, halo, alkyl, CF_3, NH_2, NHalkyl, and $N(alkyl)_2$;

b is 0, 1, or 2;

dashed lines represent optional double bonds;

Ring *C* is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

each R^6 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, NH_2, halo, aryl, and a heterocyclic ring;

additionally or alternatively an R^6 and an R^5 may be taken together to form a 5-, to 7-membered ring that is fused to Ring B or Ring B' and to Ring C;

R^7 is -Q–R^9;

Q is selected from a chemical bond or a group having the formula -O-, $-(CH_2)_i-$, $-(CH_2)_iC(O)(CH_2)_j-$, $-(CH_2)_i-N(R^{71})-(CH_2)_j-$, $-(CH_2)_iC(O)-N(R^{71})-(CH_2)_j-$,

$-(CH_2)_iC(O)O(CH_2)_j-$, $-(CH_2)_iN(R^{71})C(O)-(CH_2)_j-$, $-(CH_2)_iOC(O)N(R^{71})-(CH_2)_j-$, and $-O-(CH_2)_i-C(O)N(R^{71})-(CH_2)_j-$;

R^{71} is selected from H, alkyl, aryl, and a heterocyclic ring;

R^9 is selected from an aryl and a heterocyclic ring;

i is 0 to 4;

j is 0 to 4;

each R^0 is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring;

c is 0 to 3; and

d is 0 or 1.

[0018] The invention provides for a fundamentally new way of treating cancer and other diseases where treatment with an existing drug compound, by whatever mechanism, is followed by identifiable (clinically significant) theramutein-mediated drug resistance, by providing alternative drugs that can be administered as theramuteins arise and are identified as such (Wakai et al., 2004, reports an example wherein a theramutein may arise during the course of an on-going treatment regimen), or preemptively before the outgrowth of clinically significant populations of theramutein expressing cells. Further, where a drug treatment for a particular disease is less effective in a subset of individuals that express a certain theramutein of a protein that the drug targets, the invention enables the tailoring of treatments for those subjects by providing alternative drug substances that will be effective against said theramutein.

[0019] The invention provides a method of determining whether a chemical agent is at least as effective a modulator of a theramutein in a cell as a known substance is a modulator of a corresponding prototheramutein. One embodiment of the method involves contacting a control cell that expresses the prototheramutein and is capable of exhibiting a responsive phenotypic characteristic (linked to the functioning of the prototheramutein in the cell) with the known modulator of the prototheramutein, contacting a test cell that expresses the theramutein and is also capable of exhibiting the responsive phenotypic characteristic (linked to the functioning of the theramutein in the cell) with the chemical agent, and comparing the response of the treated test cell with the response of the treated control cell; to

determine that the chemical agent is at least as effective a modulator of the theramutein as the known substance is a modulator of the prototheramutein. In certain other embodiments, one type of control cell may not express the prototheramutein at all. In other embodiments, the control cell may express about the same amount of the prototheramutein as the test cell expresses of the theramutein. In still other embodiments, the control cell may be capable of exhibiting the responsive phenotypic characteristic to about the same extent as the test cell under certain conditions. In additional embodiments, the test cell may express a given protein, whereas the control cell expresses little or essentially none of the protein.

[0020] Proteins of the invention that are of particular interest are those involved in regulatory function, such as enzymes, protein kinases, tyrosine kinases, receptor tyrosine kinases, serine threonine protein kinases, dual specificity protein kinases, proteases, matrix metalloproteinases, phosphatases, cell cycle control proteins, docking proteins such as the IRS family members, cell-surface receptors, G-proteins, ion channels, DNA- and RNA-binding proteins, polymerases, and the like. No limitation is intended on the type of theramutein or other protein that may be used in the invention. At the present time, three theramuteins are known: BCR-ABL, c-Kit, and EGFR.

[0021] Any responsive phenotypic characteristic that can be linked to the presence of the protein (including, *e.g.*, a theramutein or prototheramutein) in the cell can be employed for use in the method, including, for example, growth or culture properties, the phosphorylation state (or other modification) of a substrate of the theramutein, and any type of transient characteristic of the cell, as will be defined and discussed in detail.

DESCRIPTION OF THE FIGURES

[0022] Figure 1 shows the effect on growth and viability of different concentrations of Compound 2 (C2) for non-transformed vector control Ba/F3 cells (which are IL-3 dependent) as well as Ba/F3 cells expressing the "wild type" $p210^{Bcr\text{-}Abl}$ (designated $p210^{Bcr\text{-}Abl\text{-}wt}$), and Ba/F3 cells expressing the $p210^{Bcr\text{-}Abl\text{-}T315I}$ drug resistant mutant. Cell counts and viability were determined on an automated cell counter as discussed in detail in the specification. Cell counts are shown by the solid color bars; cell viability is shown by the hashed bars. Note that STI-571 potently inhibits growth of the P210 cell line (grey bar) whereas it is unable to inhibit the growth of the T315I cell line (white bar) even at 10 μM

concentration. 500 nM C2 shows the largest specificity gap within this dose-response series. Compare STI-571 at 10 μM to C2 at 500 nM on the T315I cell line (white bars). Abbreviations: DMSO: dimethylsulfoxide (solvent used for drug dissolution).

[0023] Figure 2 shows the effect on growth and viability of different concentrations of Compound 6 (C6) for non-transformed vector control Ba/F3 cells as well as Ba/F3 cells expressing the $p210^{Bcr\text{-}Abl\text{-}T315I}$ drug resistant mutant. All other details are as per Fig. 1.

[0024] Figure 3 shows various determinations of the specificity gap by comparing the effects of various compounds identified in the screen in terms of their effects on the prototheramutein- and theramutein-expressing cell lines. Compound 3 (C3) shows the best example of the ability of the method to identify a compound that exerts an even greater effect on the theramutein than on its corresponding prototheramutein. (Panel E). Panel A: control DMSO treatments; B: negative heterologous specificity gap; C: slightly positive heterologous specificity gap; D: large positive homologous specificity gap; E: positive heterologous specificity gap. See text for explanations.

DETAILED DESCRIPTION OF THE INVENTION

[0025] The term "halo" or "halogen" as used herein includes fluorine, chlorine, bromine and iodine.

[0026] The term "alkyl" as used herein contemplates substituted and unsubstituted, straight and branched chain alkyl radicals having from 1 to 6 carbon atoms. Preferred alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *tert*-butyl, and the like. Additionally, the alkyl group may be optionally substituted with one or more substituents selected from halo, CN, CO_2R, C(O)R, $C(O)NR_2$, NR_2, cyclic-amino, NO_2, and OR.

[0027] The term "cycloalkyl" as used herein contemplates substituted and unsubstituted cyclic alkyl radicals. Preferred cycloalkyl groups are those with a single ring containing 3 to 7 carbon atoms and include cyclopropyl, cyclopentyl, cyclohexyl, and the like. Other cycloalkyl groups may be selected from C_7 to C_{10} bicyclic systems or from C_9 to C_{14} tricyclic systems. Additionally, the cycloalkyl group may be optionally substituted with one or more substituents selected from halo, CN, CO_2R, C(O)R, $C(O)NR_2$, NR_2, cyclic-amino, NO_2, and OR.

[0028] The term "alkenyl" as used herein contemplates substituted and unsubstituted, straight and branched chain alkene radicals. Preferred alkenyl groups are those containing two to six carbon atoms. Additionally, the alkenyl group may be optionally substituted with one or more substituents selected from halo, CN, CO_2R, C(O)R, $C(O)NR_2$, NR_2, cyclic-amino, NO_2, and OR.

[0029] The term "alkynyl" as used herein contemplates substituted and unsubstituted, straight and branched chain alkyne radicals. Preferred alkynyl groups are those containing two to six carbon atoms. Additionally, the alkynyl group may be optionally substituted with one or more substituents selected from halo, CN, CO_2R, C(O)R, $C(O)NR_2$, NR_2, cyclic-amino, NO_2, and OR.

[0030] The term "aralkyl" as used herein contemplates an alkyl group which has as a substituent an aromatic group, which aromatic group may be substituted and unsubstituted. The aralkyl group may be optionally substituted on the aryl with one or more substituents selected from halo, CN, CF_3, NR_2, cyclic-amino, NO_2, OR, CF_3, $-(CH_2)_xR$, $-(CH_2)_xC(O)(CH_2)_yR$, $-(CH_2)_xC(O)N(R')(R'')$, $-(CH_2)_xC(O)O(CH_2)_yR$, $-(CH_2)_xN(R')(R'')$, $-N(R)SO_2R$, $-O(CH_2)_xC(O)N(R')(R'')$, $-SO_2N(R')(R'')$, $-(CH_2)_xN(R)-(CH_2)_y-R$, $-(CH_2)_xN(R)-C(O)-(CH_2)_y-R$, $-(CH_2)_xN(R)-C(O)-O-(CH_2)_y-R$, $-(CH_2)_x-C(O)-N(R)-(CH_2)_y-R$, $-(CH_2)_xC(O)N(R)-(CH_2)_y-R$, $-O-(CH_2)_x-C(O)-N(R)-(CH_2)_y-R$, substituted and unsubstituted alkyl, substituted and unsubstituted cycloalkyl, substituted and unsubstituted aralkyl, substituted and unsubstituted alkenyl, substituted and unsubstituted alkynyl, substituted and unsubstituted aryl, and a substituted and unsubstituted heterocyclic ring, wherein the substituted alkyl, substituted cycloalkyl, substituted aralkyl, substituted alkenyl, substituted alkynyl, substituted aryl, and substituted heterocyclic ring may be substituted with one of more halo, CN, CF_3, CO_2R, C(O)R, $C(O)NR_2$, NR_2, cyclic-amino, NO_2, and OR.

[0031] The term "heterocyclic group" or "heterocyclic ring" as used herein contemplates aromatic and non-aromatic cyclic radicals having at least one heteroatom as a ring member. Preferred heterocyclic groups are those containing 5 or 6 ring atoms which includes at least one hetero atom, and includes cyclic amines such as morpholino, piperidino, pyrrolidino, and the like, and cyclic ethers, such as tetrahydrofuran, tetrahydropyran, and the like. Aromatic heterocyclic groups, also termed "heteroaryl" groups contemplates single-ring hetero-aromatic groups that may include from one to three heteroatoms, for example, pyrrole,

furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine, pyrimidine, and the like. The term heteroaryl also includes polycyclic hetero-aromatic systems having two or more rings in which two atoms are common to two adjoining rings (the rings are "fused") wherein at least one of the rings is a heteroaryl, e.g., the other rings can be cycloalkyls, cycloalkenyls, aryl, heterocycles and/or heteroaryls. Examples of polycyclic heteroaromatic systems include quinoline, isoquinoline, tetrahydroisoquinoline, quinoxaline, quinaxoline, benzimidazole, benzofuran, purine, imidazopyridine, benzotriazole, and the like. Additionally, the heterocyclic groups may be optionally substituted with halo, CN, CF_3, NR_2, cyclic-amino, NO_2, OR, CF_3, $-(CH_2)_xC(O)(CH_2)_yR$, $-(CH_2)_xC(O)N(R')(R'')$, $-(CH_2)_xC(O)O(CH_2)_yR$, $-(CH_2)_xN(R')(R'')$, $-N(R)SO_2R$, $-O(CH_2)_xC(O)N(R')(R'')$, $-SO_2N(R')(R'')$, $-(CH_2)_xN(R)-(CH_2)_y-R$, $-(CH_2)_xN(R)-C(O)-(CH_2)_y-R$, $-(CH_2)_xN(R)-C(O)-O-(CH_2)_y-R$, $-(CH_2)_x-C(O)-N(R)-(CH_2)_y-R$, $-(CH_2)_xC(O)N(R)-(CH_2)_y-R$, $-O-(CH_2)_x-C(O)-N(R)-(CH_2)_y-R$, substituted and unsubstituted alkyl, substituted and unsubstituted cycloalkyl, substituted and unsubstituted aralkyl, substituted and unsubstituted alkenyl, substituted and unsubstituted alkynyl, substituted and unsubstituted aryl, and a substituted and unsubstituted heterocyclic ring, wherein the substituted alkyl, substituted cycloalkyl, substituted aralkyl, substituted alkenyl, substituted alkynyl, substituted aryl, and substituted heterocyclic ring may be substituted with one of more halo, CN, CF_3, CO_2R, C(O)R, $C(O)NR_2$, NR_2, cyclic-amino, NO_2, and OR.

[0032] The term "cyclic-amino" as used herein contemplates aromatic and non-aromatic cyclic radicals having at least one nitrogen as a ring member. Preferred cyclic amino groups are those containing 5 or 6 ring atoms, which includes at least one nitrogen, and includes morpholino, piperidino, pyrrolidino, piperazino, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine, pyrimidine and the like. Additionally, the cyclic-amino may be optionally substituted with halo, CN, CF_3, NR_2, NO_2, OR, CF_3, substituted and unsubstituted alkyl, substituted and unsubstituted cycloalkyl, substituted and unsubstituted aralkyl, substituted and unsubstituted alkenyl, substituted and unsubstituted alkynyl, substituted and unsubstituted aryl, and a substituted and unsubstituted heterocyclic ring, wherein the substituted alkyl, substituted cycloalkyl, substituted aralkyl, substituted alkenyl, substituted alkynyl, substituted aryl, and substituted heterocyclic ring may be substituted with one or more of halo, CN, CF_3, CO_2R, C(O)R, $C(O)NR_2$, NR_2, NO_2, and OR.

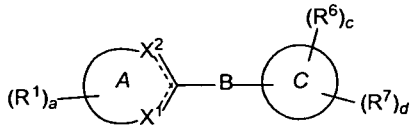
[0033] The term "aryl" or "aromatic group" as used herein contemplates single-ring aromatic groups (for example, phenyl, pyridyl, pyrazole, etc.) and polycyclic ring systems (naphthyl, quinoline, etc.). The polycyclic rings may have two or more rings in which two atoms are common to two adjoining rings (the rings are "fused") wherein at least one of the rings is aromatic, e.g., the other rings can be cycloalkyls, cycloalkenyls, aryl, heterocycles and/or heteroaryls. Additionally, the aryl groups may be optionally substituted with one or more substituents selected from halo, CN, CF_3, NR_2, cyclic-amino, NO_2, OR, CF_3, $-(CH_2)_xC(O)(CH_2)_yR$, $-(CH_2)_xC(O)N(R')(R'')$, $-(CH_2)_xC(O)O(CH_2)_yR$, $-(CH_2)_xN(R')(R'')$, $-N(R)SO_2R$, $-O(CH_2)_xC(O)N(R')(R'')$, $-SO_2N(R')(R'')$, $-(CH_2)_xN(R)-(CH_2)_y-R$, $-(CH_2)_xN(R)-C(O)-(CH_2)_y-R$, $-(CH_2)_xN(R)-C(O)-O-(CH_2)_y-R$, $-(CH_2)_x-C(O)-N(R)-(CH_2)_y-R$, $-CH_2)_xC(O)N(R)-(CH_2)_y-R$, $-O-(CH_2)_x-C(O)-N(R)-(CH_2)_y-R$, substituted and unsubstituted alkyl, substituted and unsubstituted cycloalkyl, substituted and unsubstituted aralkyl, substituted and unsubstituted alkenyl, substituted and unsubstituted alkynyl, substituted and unsubstituted aryl, and a substituted and unsubstituted heterocyclic ring, wherein the substituted alkyl, substituted cycloalkyl, substituted aralkyl, substituted alkenyl, substituted alkynyl, substituted aryl, and substituted heterocyclic ring may be substituted with one of more halo, CN, CF_3, CO_2R, C(O)R, $C(O)NR_2$, NR_2, cyclic-amino, NO_2, and OR.

[0034] The term "heteroatom", particularly as a ring heteroatom, refers to N, O, and S.

[0035] Each R is independently selected from H, substituted and unsubstituted alkyl, substituted and unsubstituted cycloalkyl, substituted and unsubstituted aralkyl, substituted and unsubstituted aryl and a substituted and unsubstituted heterocyclic ring, wherein the substituted alkyl, substituted cycloalkyl, substituted aralkyl, substituted aryl and substituted heterocyclic ring may be substituted with one or more halo, CN, CF_3, OH, CO_2H, NO_2, $C_{1\text{-}6}$alkyl, $-O-(C_{1\text{-}6}\text{alkyl})$, $-NH_2$, $-NH(C_{1\text{-}6}\text{alkyl})$ and $-N(C_{1\text{-}6}\text{alkyl})_2$. Each R' and R" are independently selected from H, or substituted and unsubstituted alkyl, substituted and unsubstituted cycloalkyl, substituted and unsubstituted aralkyl, substituted and unsubstituted aryl and a substituted and unsubstituted heterocyclic ring, wherein the substituted alkyl, substituted cycloalkyl, substituted aralkyl, substituted aryl and substituted heterocyclic ring may be substituted with one or more halo, CN, CF_3, OH, CO_2H, NO_2, $C_{1\text{-}6}$alkyl, $-O-(C_{1\text{-}6}\text{alkyl})$, $-NH_2$, $-NH(C_{1\text{-}6}\text{alkyl})$ and $-N(C_{1\text{-}6}\text{alkyl})_2$; or R' and R" may be taken together

with the nitrogen to which they are attached form a 5- to 7- membered ring which may optionally contain up to three further heteroatoms, which heteroatoms may be substituted by C_{1-6}alkyl. Each *x* and each *y* are independently selected from 0 to 4.

[0036] In a preferred embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula I



(I)

wherein:

ring *A* is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

X^1 is selected from N, N-R^0 or C-R^1;

X^2 is selected from N, N-R^0 or C-R^1;

each R^1 is independently selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^{11}, $-(CH_2)_pC(O)(CH_2)_qR^{11}$, $-(CH_2)_pC(O)N(R^{12})(R^{13})$, $-(CH_2)_pC(O)O(CH_2)_qR^{11}$, $-(CH_2)_pN(R^{11})(CH_2)_qC(O)R^{11}$, $-(CH_2)_pN(R^{12})(R^{13})$, $-(CH_2)_pN(R^{11})(CH_2)_qR^{11}$, $-N(R^{11})SO_2R^{11}$, $-OC(O)N(R^{12})(R^{13})$, $-SO_2N(R^{12})(R^{13})$, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

a is 0 to 4;

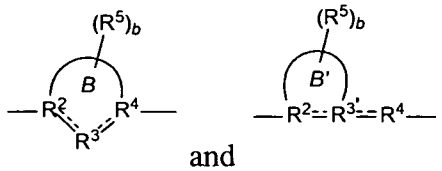
each R^{11} is independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

each R^{12} and R^{13} are independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring; or R^{12} and R^{13} may be taken together with the nitrogen to which they are attached form a 5- to 7- membered ring which may optionally contain a further heteroatom; wherein the 5- to 7-membered ring may optionally be substituted with one to three substituents that are independently selected from alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, CO_2R^0, $C(O)R^0$, halo, aryl, and a heterocyclic ring;

p is 0 to 4;

q is 0 to 4;

B is selected from a group having the formula:



and ;

Ring *B* and Ring *B'* are a 5-, or 6- membered ring;

R^2 is selected from N, C and CH;

R^3 is selected from N, NR^{31}, O, S, CR^{31} and CHR^{31};

R^{31} is selected from H, and alkyl;

$R^{3'}$ is selected from N, C and CH;

R^4 is selected from N, C or CH;

each R^5 is independently selected from OH, O-alkyl, =O, SH, S-alkyl, =S, halo, alkyl, CF_3, NH_2, NHalkyl, and $N(alkyl)_2$;

b is 0, 1, or 2;

dashed lines represent optional double bonds;

Ring *C* is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

each R^6 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, NH_2, halo, aryl, and a heterocyclic ring;

additionally or alternatively an R^6 and an R^5 may be taken together to form a 5-, to 7-membered ring that is fused to Ring B or Ring B' and to Ring C;

R^7 is -Q–R^9;

Q is selected from a chemical bond or a group having the formula -O-, $-(CH_2)_i-$, $-(CH_2)_iC(O)(CH_2)_j-$, $-(CH_2)_i-N(R^{71})-(CH_2)_j-$, $-(CH_2)_iC(O)-N(R^{71})-(CH_2)_j-$, $-(CH_2)_iC(O)O(CH_2)_j-$, $-(CH_2)_iN(R^{71})C(O)-(CH_2)_j-$, $-(CH_2)_iOC(O)N(R^{71})-(CH_2)_j-$, and $-O-(CH_2)_i-C(O)N(R^{71})-(CH_2)_j-$;

R^{71} is selected from H, alkyl, aryl, and a heterocyclic ring;

R^9 is selected from an aryl and a heterocyclic ring;

i is 0 to 4;

j is 0 to 4;

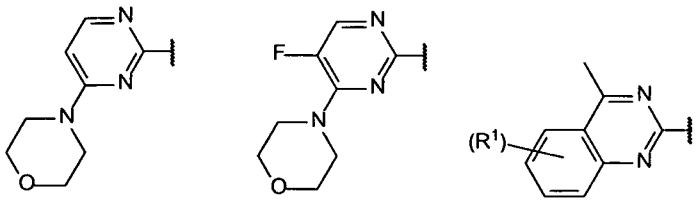
each R^0 is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring;

c is 0 to 3; and

d is 0 or 1.

[0037] In preferred embodiments of the invention, Ring *A* is an aromatic ring.

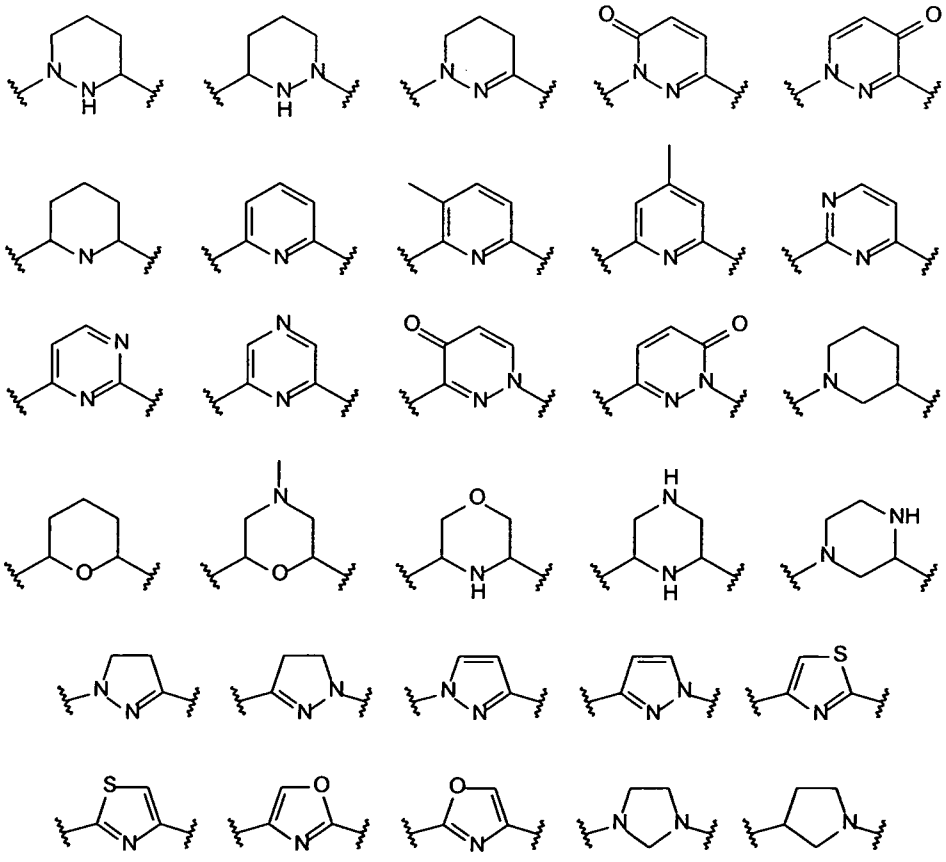
[0038] In preferred embodiments of the invention, X^1 or X^2 is N. In another preferred embodiment, both X^1 and X^2 are N. In particularly preferred embodiments of the invention, Ring *A* is a pyridine ring or a pyrimidine ring. In still further preferred embodiments, Ring *A* is selected from the structures provided below:

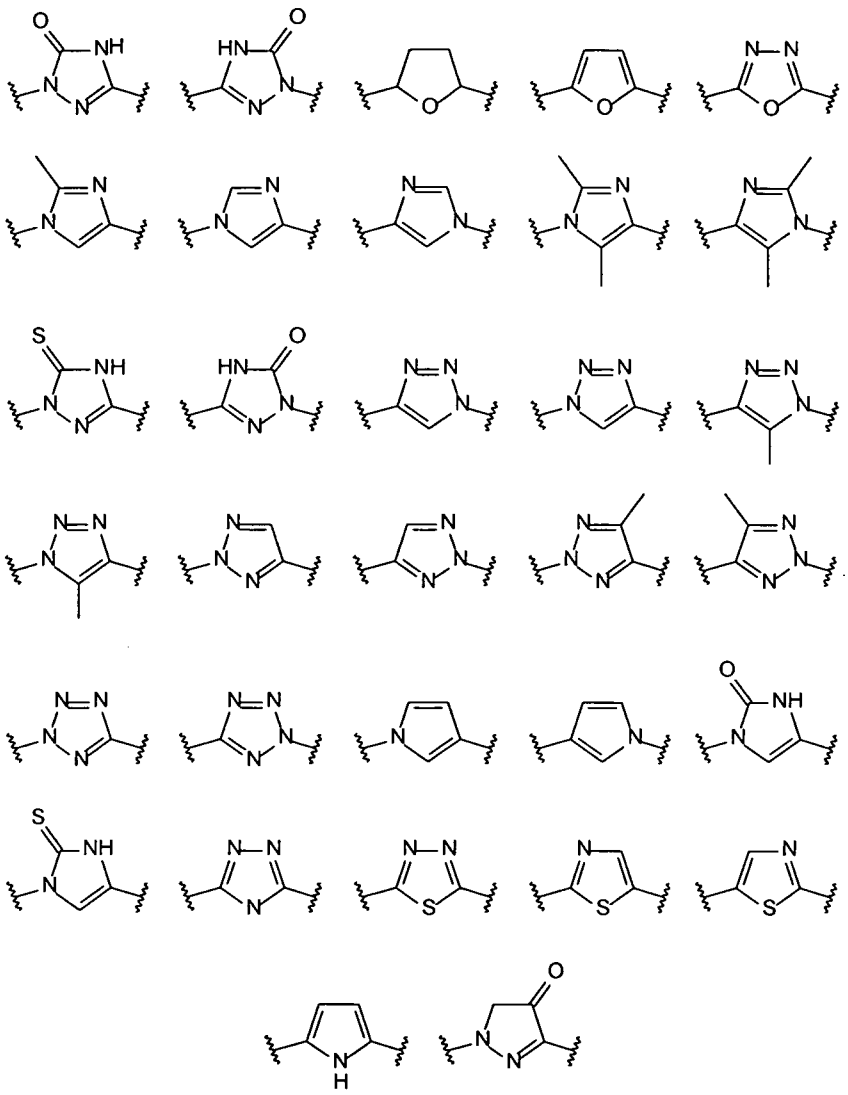


[0039] In preferred embodiments of the invention, Ring *C* is a 6-membered aryl group, including phenyl, pyridyl, and pyrimidyl. Pyridyl is particularly preferred.

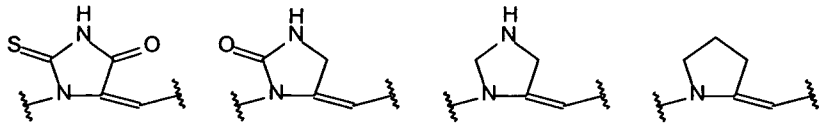
[0040] In other preferred embodiments, Ring *C* is a 10-membered bicyclic aryl group, including naphthyl, quinoline, isoquinoline, quinazoline, and quinoxaline.

[0041] In preferred embodiments of the invention, Ring *B* is selected from the following chemical groups:

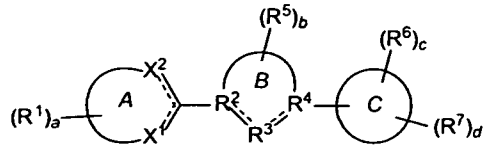




[0042] In preferred embodiments of the invention, Ring *B'* is selected from the following chemical groups:



[0043] In a further preferred embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula II



(II)

wherein

Ring *A* is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

X^1 is selected from N, N-R^0 or C-R^1;

X^2 is selected from N, N-R^0 or C-R^1;

each R^1 is independently selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^{11}, $-(CH_2)_pC(O)(CH_2)_qR^{11}$, $-(CH_2)_pC(O)N(R^{12})(R^{13})$, $-(CH_2)_pC(O)O(CH_2)_qR^{11}$, $-(CH_2)_pN(R^{11})(CH_2)_qC(O)R^{11}$, $-(CH_2)_pN(R^{12})(R^{13})$, $-(CH_2)_pN(R^{11})(CH_2)_qR^{11}$, $-N(R^{11})SO_2R^{11}$, $-OC(O)N(R^{12})(R^{13})$, $-SO_2N(R^{12})(R^{13})$, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

a is 0 to 4;

each R^{11} is independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

each R^{12} and R^{13} are independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring; or R^{12} and R^{13} may be taken together with the nitrogen to which they are attached form a 5- to 7- membered ring which may optionally contain a further heteroatom; wherein the 5- to 7-membered ring may optionally be substituted with one to three substituents that are independently selected from alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, CO_2R^0, $C(O)R^0$, halo, aryl, and a heterocyclic ring;

p is 0 to 4;

q is 0 to 4;

Ring *B* is a 5-, or 6- membered ring;

R^2 is selected from N, C and CH;

R^3 is selected from N, NR^{31}, O, S, CR^{31} and CHR^{31};

R^{31} is selected from H, and alkyl;

R^4 is selected from N, C or CH;

each R^5 is independently selected from OH, O-alkyl, =O, SH, S-alkyl, =S, halo, alkyl, CF_3, NH_2, NHalkyl, and $N(alkyl)_2$;

b is 0, 1, or 2;

dashed lines represent optional double bonds;

Ring *C* is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

each R^6 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, NH_2, halo, aryl, and a heterocyclic ring;

additionally or alternatively an R^6 and an R^5 may be taken together to form a 5-, to 7-membered ring that is fused to Ring B and to Ring C;

R^7 is -Q–R^9;

Q is selected from a chemical bond or a group having the formula -O-, $-(CH_2)_i-$, $-(CH_2)_iC(O)(CH_2)_j-$, $-(CH_2)_i-N(R^{71})-(CH_2)_j-$, $-(CH_2)_iC(O)-N(R^{71})-(CH_2)_j-$, $-(CH_2)_iC(O)O(CH_2)_j-$, $-(CH_2)_iN(R^{71})C(O)-(CH_2)_j-$, $-(CH_2)_iOC(O)N(R^{71})-(CH_2)_j-$, and $-O-(CH_2)_i-C(O)N(R^{71})-(CH_2)_j-$;

R^{71} is selected from H, alkyl, aryl, and a heterocyclic ring;

R^9 is selected from an aryl and a heterocyclic ring;

i is 0 to 4;

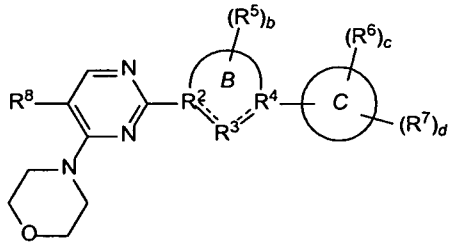
j is 0 to 4;

each R^0 is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring;

c is 0 to 3; and

d is 0 or 1.

[0044] In a further preferred embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula III:



(III)

wherein:

Ring *B* is a 5-, or 6- membered ring;

R^2 is selected from N, C and CH;

R^3 is selected from N, NR^{31}, O, S, CR^{31} and CHR^{31};

R^{31} is selected from H, and alkyl;

R^4 is selected from N, C or CH;

each R^5 is independently selected from OH, O-alkyl, =O, SH, S-alkyl, =S, halo, alkyl, CF_3, NH_2, NHalkyl, and $N(alkyl)_2$;

b is 0, 1, or 2;

dashed lines represent optional double bonds;

Ring *C* is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

each R^6 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, NH_2, halo, aryl, and a heterocyclic ring;

additionally or alternatively an R^6 and an R^5 may be taken together to form a 5-, to 7-membered ring that is fused to Ring B and to Ring C;

R^7 is -Q–R^9;

Q is selected from a chemical bond or a group having the formula -O-, $-(CH_2)_i-$, $-(CH_2)_iC(O)(CH_2)_j-$, $-(CH_2)_i-N(R^{71})-(CH_2)_j-$, $-(CH_2)_iC(O)-N(R^{71})-(CH_2)_j-$, $-(CH_2)_iC(O)O(CH_2)_j-$, $-(CH_2)_iN(R^{71})C(O)-(CH_2)_j-$, $-(CH_2)_iOC(O)N(R^{71})-(CH_2)_j-$, and $-O-(CH_2)_i-C(O)N(R^{71})-(CH_2)_j-$;

R^{71} is selected from H, alkyl, aryl, and a heterocyclic ring;

R^9 is selected from an aryl and a heterocyclic ring;

i is 0 to 4;

j is 0 to 4;

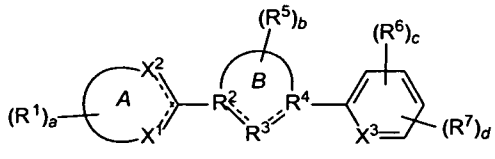
R^8 is selected from H and F;

each R^0 is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring;

c is 0 to 3; and

d is 0 or 1.

[0045] In a further preferred embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula IV



(IV)

wherein

Ring *A* is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

X^1 is selected from N, N-R^0 or C-R^1;

X^2 is selected from N, N-R^0 or C-R^1;

each R^1 is independently selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^{11}, $-(CH_2)_pC(O)(CH_2)_qR^{11}$, $-(CH_2)_pC(O)N(R^{12})(R^{13})$, $-(CH_2)_pC(O)O(CH_2)_qR^{11}$, $-(CH_2)_pN(R^{11})(CH_2)_qC(O)R^{11}$, $-(CH_2)_pN(R^{12})(R^{13})$,

$-(CH_2)_pN(R^{11})(CH_2)_qR^{11}$, $-N(R^{11})SO_2R^{11}$, $-OC(O)N(R^{12})(R^{13})$, $-SO_2N(R^{12})(R^{13})$, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

a is 0 to 4;

each R^{11} is independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

each R^{12} and R^{13} are independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring; or R^{12} and R^{13} may be taken together with the nitrogen to which they are attached form a 5- to 7- membered ring which may optionally contain a further heteroatom; wherein the 5- to 7-membered ring may optionally be substituted with one to three substituents that are independently selected from alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, CO_2R^0, $C(O)R^0$, halo, aryl, and a heterocyclic ring;

p is 0 to 4;

q is 0 to 4;

Ring *B* is a 5-, or 6- membered ring;

R^2 is selected from N, C and CH;

R^3 is selected from N, NR^{31}, O, S, CR^{31} and CHR^{31};

R^{31} is selected from H, and alkyl;

R^4 is selected from N, C or CH;

each R^5 is independently selected from OH, O-alkyl, =O, SH, S-alkyl, =S, halo, alkyl, CF_3, NH_2, NHalkyl, and $N(alkyl)_2$;

b is 0, 1, or 2;

dashed lines represent optional double bonds;

X^3 is selected from N, CH, and $C\text{-}R^6$;

each R^6 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, NH_2, halo, aryl, and a heterocyclic ring;

additionally or alternatively an R^6 and an R^5 may be taken together to form a 5-, to 7-membered ring that is fused to Ring B and to Ring C;

R^7 is $-Q\text{–}R^9$;

Q is selected from a chemical bond or a group having the formula -O-, $-(CH_2)_i$-, $-(CH_2)_iC(O)(CH_2)_j$-, $-(CH_2)_i$-$N(R^{71})$-$(CH_2)_j$-, $-(CH_2)_iC(O)$-$N(R^{71})$-$(CH_2)_j$-, $-(CH_2)_iC(O)O(CH_2)_j$-, $-(CH_2)_iN(R^{71})C(O)$-$(CH_2)_j$-, $-(CH_2)_iOC(O)N(R^{71})$-$(CH_2)_j$-, and $-O$-$(CH_2)_i$-$C(O)N(R^{71})$-$(CH_2)_j$-;

R^{71} is selected from H, alkyl, aryl, and a heterocyclic ring;

R^9 is selected from an aryl and a heterocyclic ring;

i is 0 to 4;

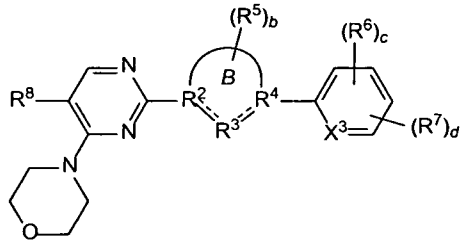
j is 0 to 4;

each R^0 is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring;

c is 0 to 3; and

d is 0 or 1.

[0046] In a further preferred embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula V



(V)

wherein:

Ring B is a 5-, or 6- membered ring;

R^2 is selected from N, C and CH;

R^3 is selected from N, NR^{31}, O, S, CR^{31} and CHR^{31};

R^{31} is selected from H, and alkyl;

R^4 is selected from N, C or CH;

each R^5 is independently selected from OH, O-alkyl, =O, SH, S-alkyl, =S, halo, alkyl, CF_3, NH_2, NHalkyl, and $N(alkyl)_2$;

b is 0, 1, or 2;

dashed lines represent optional double bonds;

X^3 is selected from N, CH, and C-R^6;

each R^6 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, NH_2, halo, aryl, and a heterocyclic ring;

additionally or alternatively an R^6 and an R^5 may be taken together to form a 5-, to 7-membered ring that is fused to Ring B and to Ring C;

R^7 is -Q–R^9;

Q is selected from a chemical bond or a group having the formula -O-, -$(CH_2)_i$-, -$(CH_2)_iC(O)(CH_2)_j$-, -$(CH_2)_i$-$N(R^{71})$-$(CH_2)_j$-, -$(CH_2)_iC(O)$-$N(R^{71})$-$(CH_2)_j$-, -$(CH_2)_iC(O)O(CH_2)_j$-, -$(CH_2)_iN(R^{71})C(O)$-$(CH_2)_j$-, -$(CH_2)_iOC(O)N(R^{71})$-$(CH_2)_j$-, and -O-$(CH_2)_i$-$C(O)N(R^{71})$-$(CH_2)_j$-;

R^{71} is selected from H, alkyl, aryl, and a heterocyclic ring;

R^9 is selected from an aryl and a heterocyclic ring;

i is 0 to 4;

j is 0 to 4;

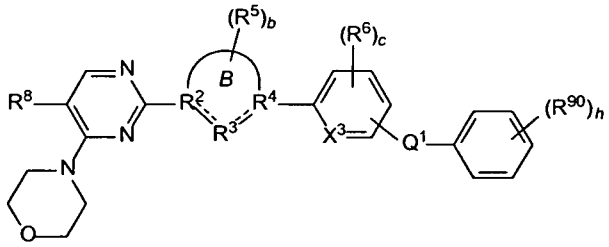
R^8 is selected from H and F;

each R^0 is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring;

c is 0 to 3; and

d is 0 or 1.

[0047] In a further preferred embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula VI:



(VI)

wherein:

Ring B is a 5-, or 6- membered ring;

R^2 is selected from N, C and CH;

R^3 is selected from N, NR^{31}, O, S, CR^{31} and CHR^{31};

R^{31} is selected from H, and alkyl;

R^4 is selected from N, C or CH;

each R^5 is independently selected from OH, O-alkyl, =O, SH, S-alkyl, =S, halo, alkyl, CF_3, NH_2, NHalkyl, and $N(alkyl)_2$;

b is 0, 1, or 2;

dashed lines represent optional double bonds;

each R^6 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, NH_2, halo, aryl, and a heterocyclic ring;

additionally or alternatively an R^6 and an R^5 may be taken together to form a 5-, to 7-membered ring that is fused to Ring B and to Ring C;

Q^1 is selected from a chemical bond or a group having the formula -O-, $-CH_2-$, -NH-, -C(O)-NH-, -C(O)O-, -NH-C(O)-, -OC(O)NH-, and -O-C(O)NH-;

each R^{90} is selected from halo, alkyl, CN, $N(R^{92})_2$, cyclic-amino, NO_2, OR^{92}, and CF_3;

h is 0 to 5;

each R^{92} is selected from H, alkyl, aryl, aralkyl and a heterocyclic ring;

c is 0 to 3; and

R^8 is selected from H and F.

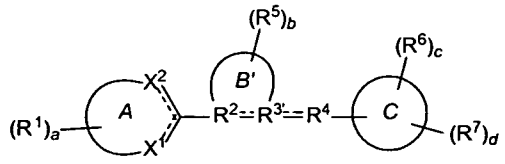
[0048] In preferred embodiments, R^{90} is selected from $N(R^{92})_2$, CF_3, and OH.

[0049] In preferred embodiments, Q is selected to be $-(CH_2)_i-N(R^{71})-(CH_2)_j-$.

[0050] In further preferred embodiments,Q^1 is -NH-.

[0051] In preferred embodiments, X^3 is N.

[0052] In a further preferred embodiment, the present invention provides inhibitors of the $P210^{BCR-ABL-T315I}$ theramutein having the formula VII:



(VII)

wherein:

ring *A* is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

X^1 is selected from N, N-R^0 or C-R^1;

X^2 is selected from N, N-R^0 or C-R^1;

the dotted lines represent optional double bonds;

each R^1 is independently selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^{11}, $-(CH_2)_pC(O)(CH_2)_qR^{11}$, $-(CH_2)_pC(O)N(R^{12})(R^{13})$, $-(CH_2)_pC(O)O(CH_2)_qR^{11}$,$-(CH_2)_pN(R^{11})(CH_2)_qC(O)R^{11}$, $-(CH_2)_pN(R^{12})(R^{13})$, $-(CH_2)_pN(R^{11})(CH_2)_qR^{11}$, $-N(R^{11})SO_2R^{11}$, $-OC(O)N(R^{12})(R^{13})$, $-SO_2N(R^{12})(R^{13})$, halo,

aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

a is 0 to 4;

each R^{11} is independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

each R^{12} and R^{13} are independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring; or R^{12} and R^{13} may be taken together with the nitrogen to which they are attached form a 5- to 7- membered ring which may optionally contain a further heteroatom; wherein the 5- to 7-membered ring may optionally be substituted with one to three substituents that are independently selected from alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, CO_2R^0, $C(O)R^0$, halo, aryl, and a heterocyclic ring;

p is 0 to 4;

q is 0 to 4;

Ring B' is a 5-, or 6- membered ring:

R^2 is selected from N, C and CH;

$R^{3'}$ is selected from N, C and CH;

R^4 is selected from N, C or CH;

each R^5 is independently selected from OH, O-alkyl, =O, SH, S-alkyl, =S, halo, alkyl, CF_3, NH_2, NHalkyl, and $N(alkyl)_2$;

b is 0, 1, or 2;

dashed lines represent optional double bonds;

Ring *C* is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

each R^6 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, NH_2, halo, aryl, and a heterocyclic ring;

additionally or alternatively an R^6 and an R^5 may be taken together to form a 5-, to 7-membered ring that is fused to Ring B or Ring B' and to Ring C;

R^7 is -Q–R^9;

Q is selected from a chemical bond or a group having the formula -O-, $-(CH_2)_i-$, $-(CH_2)_iC(O)(CH_2)_j-$, $-(CH_2)_i-N(R^{71})-(CH_2)_j-$, $-(CH_2)_iC(O)-N(R^{71})-(CH_2)_j-$, $-(CH_2)_iC(O)O(CH_2)_j-$, $-(CH_2)_iN(R^{71})C(O)-(CH_2)_j-$, $-(CH_2)_iOC(O)N(R^{71})-(CH_2)_j-$, and $-O-(CH_2)_i-C(O)N(R^{71})-(CH_2)_j-$;

R^{71} is selected from H, alkyl, aryl, and a heterocyclic ring;

R^9 is selected from an aryl and a heterocyclic ring;

i is 0 to 4;

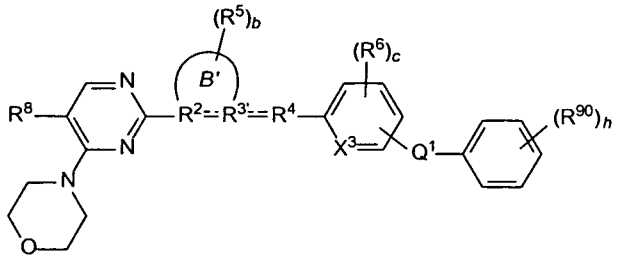
j is 0 to 4;

each R^0 is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring;

c is 0 to 3; and

d is 0 or 1.

[0053] In a further preferred embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula VIII:



(VIII)

wherein:

Ring B' is a 5-, or 6- membered ring:

R^2 is selected from N, C and CH;

$R^{3'}$ is selected from N, C and CH;

R^4 is selected from N, C or CH;

each R^5 is independently selected from OH, O-alkyl, =O, SH, S-alkyl, =S, halo, alkyl, CF_3, NH_2, NHalkyl, and $N(alkyl)_2$;

b is 0, 1, or 2;

dashed lines represent optional double bonds;

each R^6 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, NH_2, halo, aryl, and a heterocyclic ring;

additionally or alternatively an R^6 and an R^5 may be taken together to form a 5-, to 7-membered ring that is fused to Ring B' and to Ring C;

c is 0 to 3;

Q^1 is selected from a chemical bond or a group having the formula -O-, $-CH_2-$, -NH-, -C(O)-NH-, -C(O)O-, -NH-C(O)-, -OC(O)NH-, and -O-C(O)NH-;

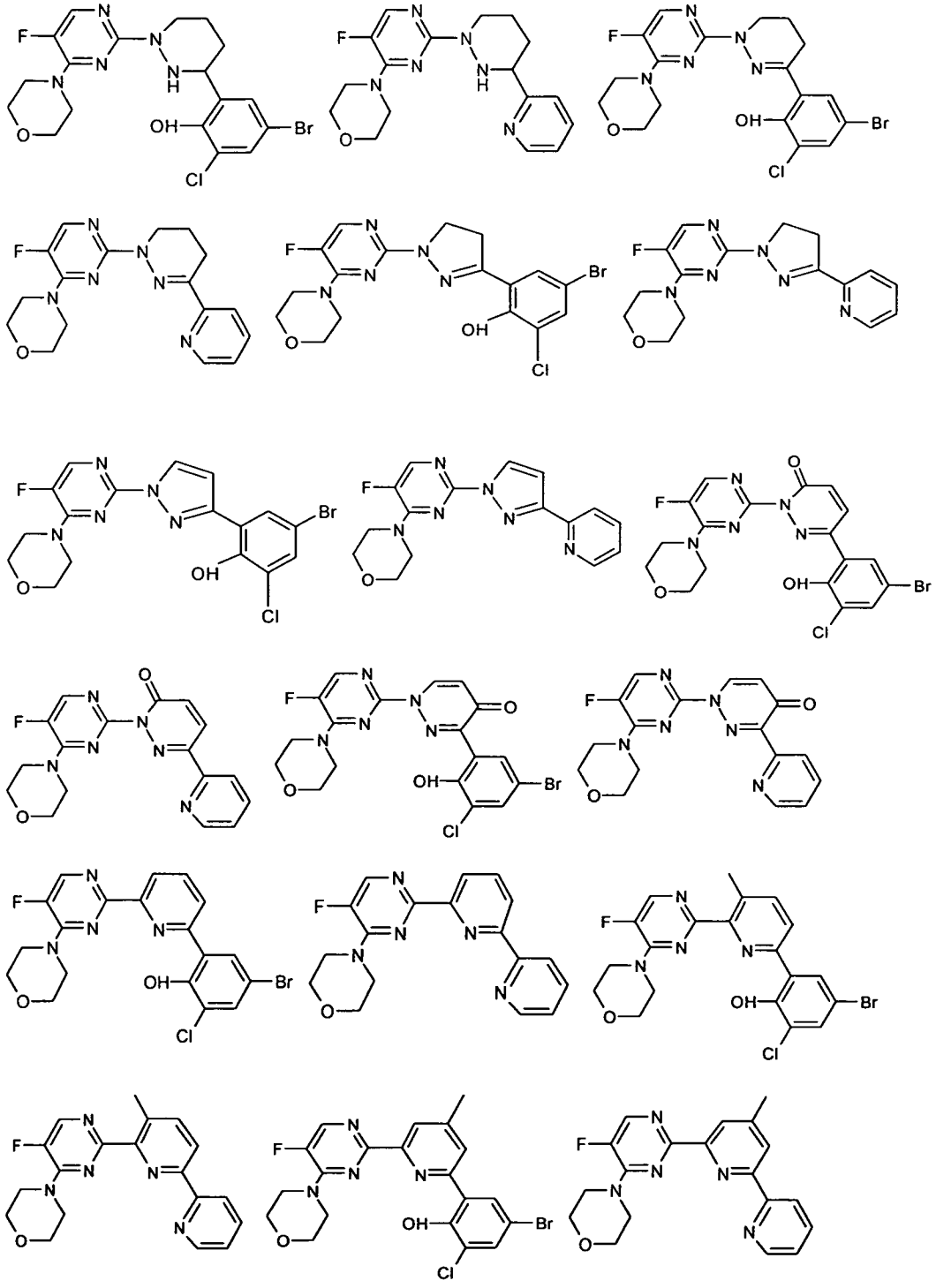
each R^{90} is selected from halo, alkyl, CN, $N(R^{92})_2$, cyclic-amino, NO_2, OR^{92}, and CF_3;

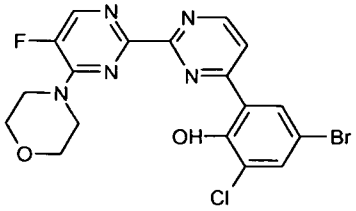
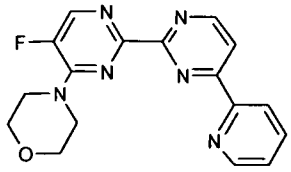
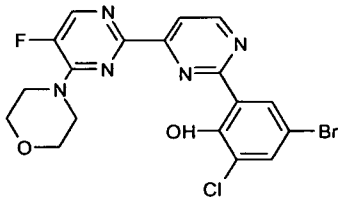
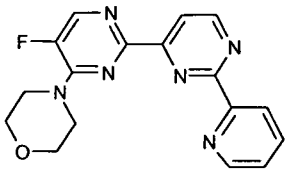
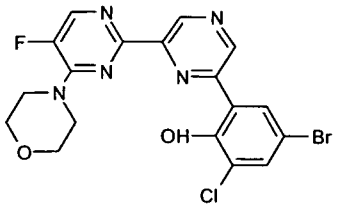
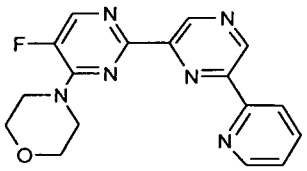
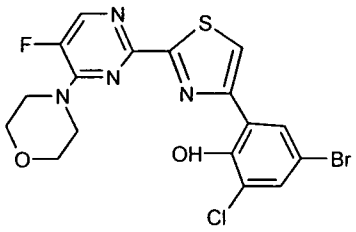
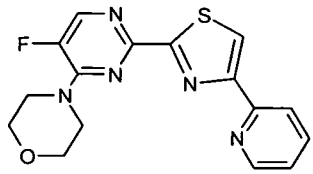
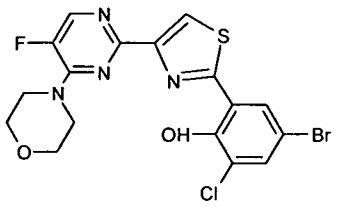
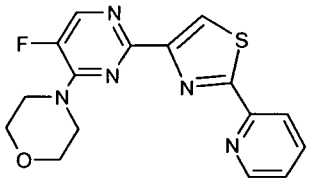
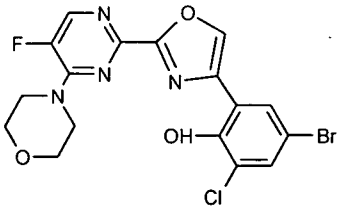
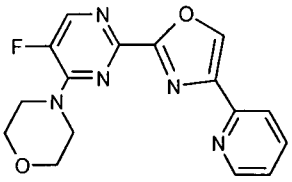
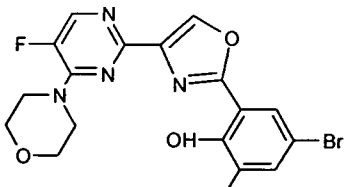
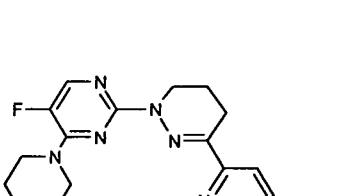
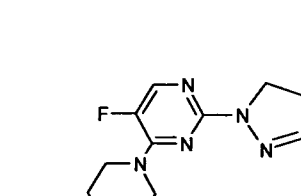
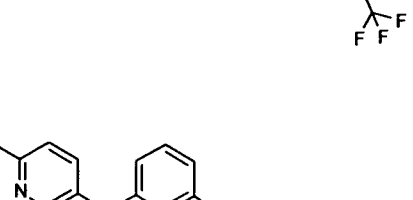
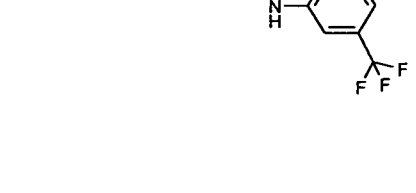
h is 0 to 5;

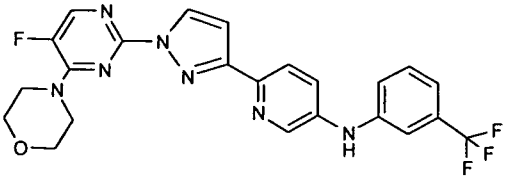
each R^{92} is selected from H, alkyl, aryl, aralkyl and a heterocyclic ring; and

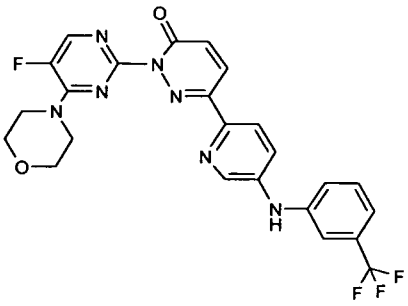
R^8 is selected from H and F.

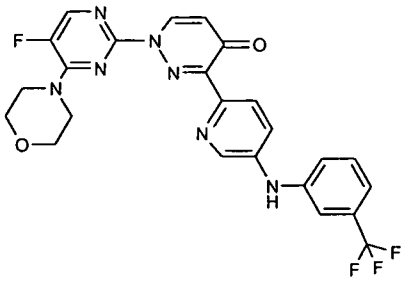
[0054] Exemplary compounds of the formula I-VIII include the following structures:

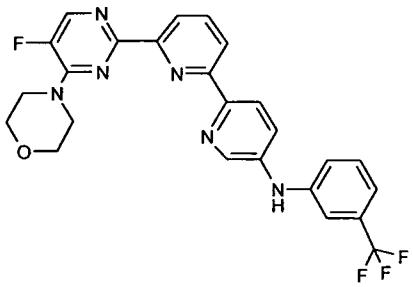


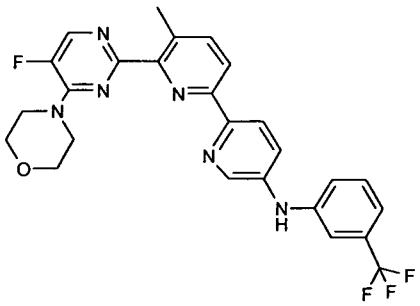


















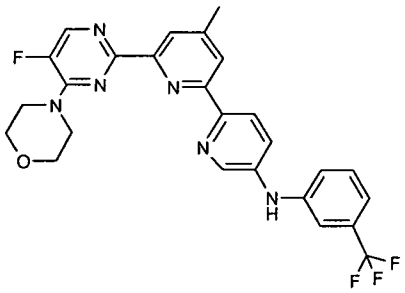


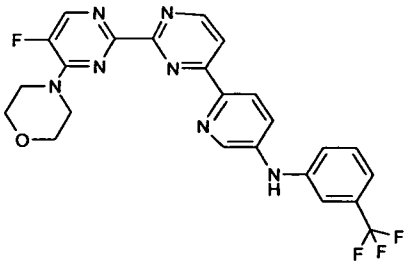


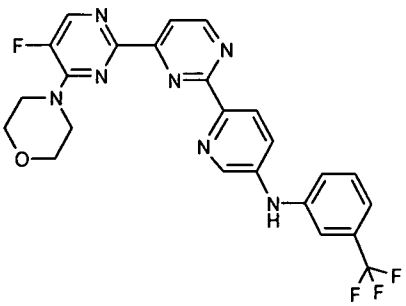


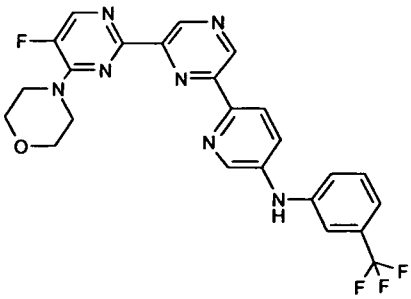


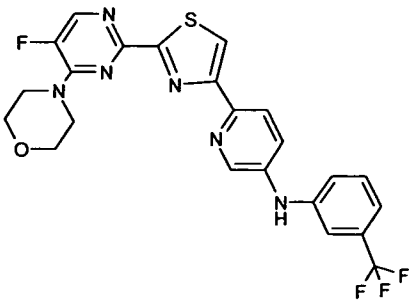


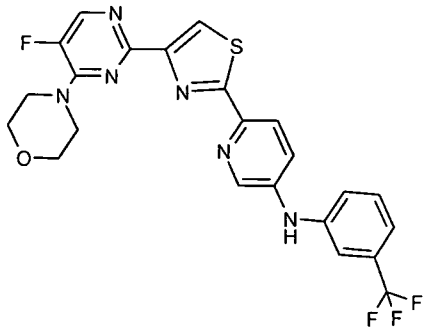


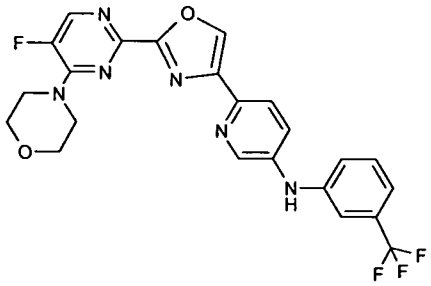


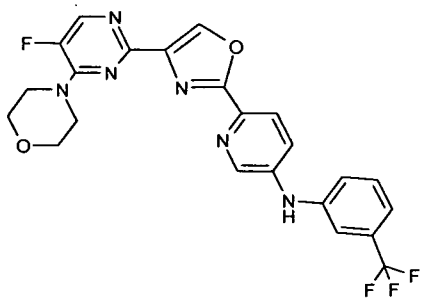


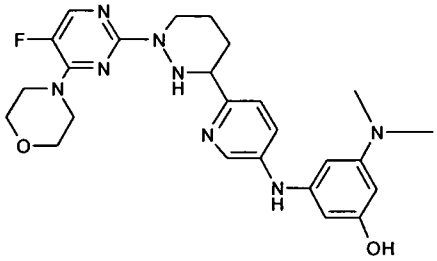


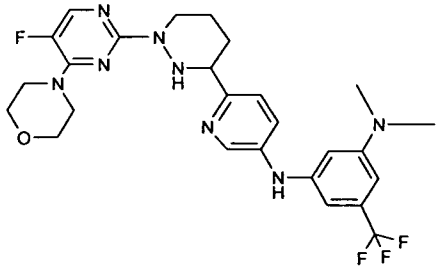


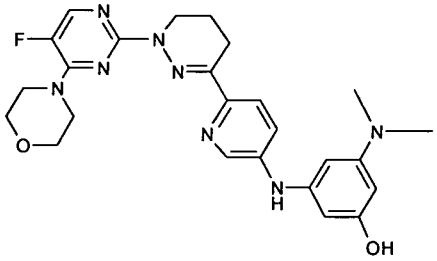


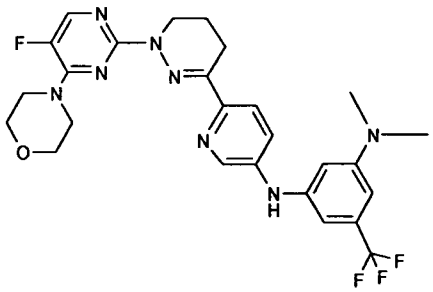


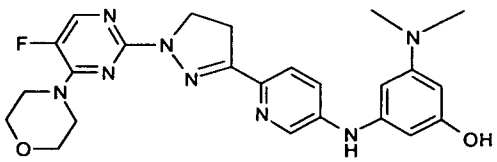


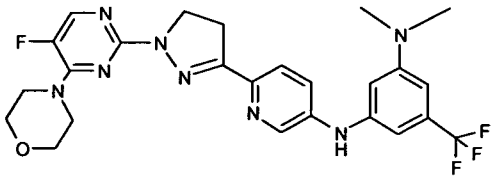


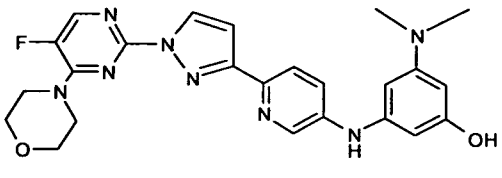


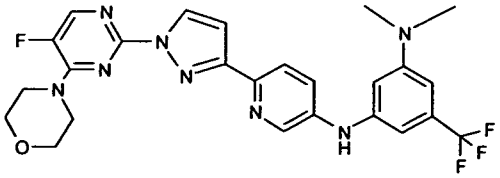


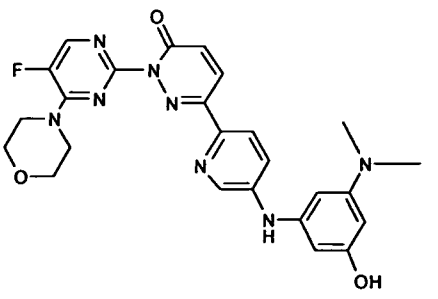


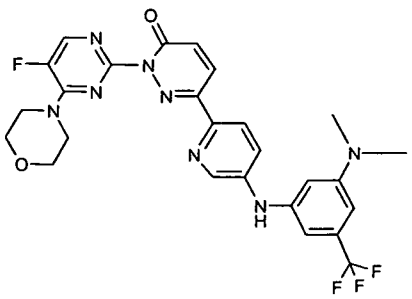


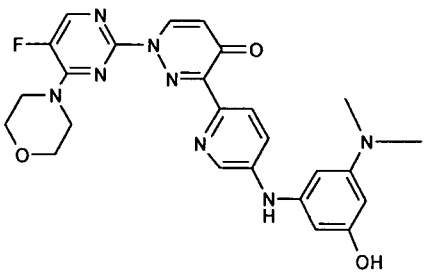


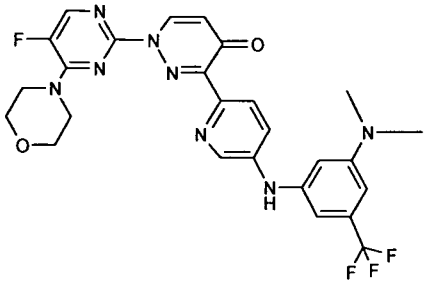


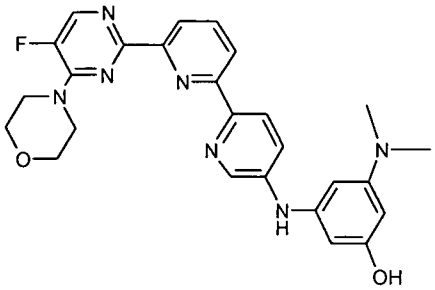


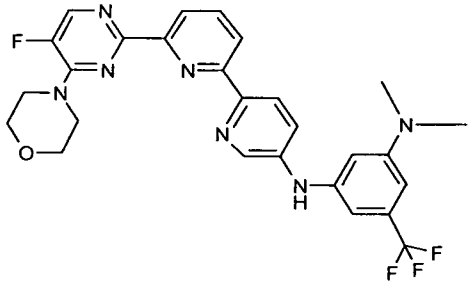


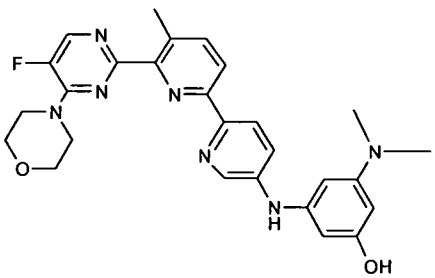


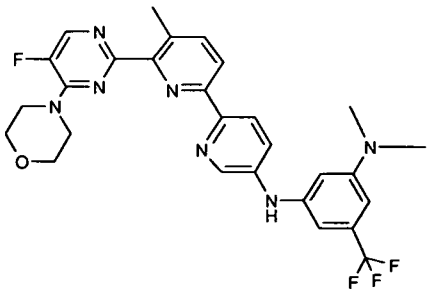


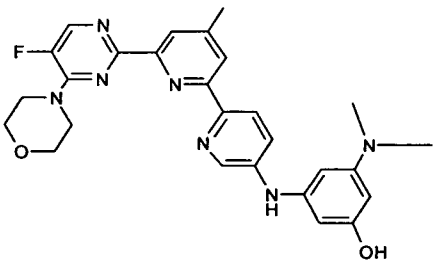


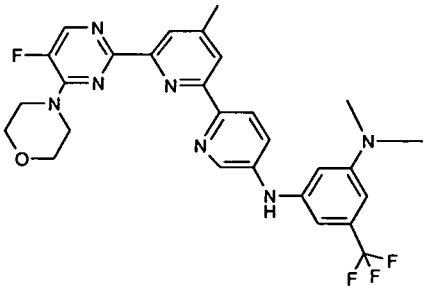


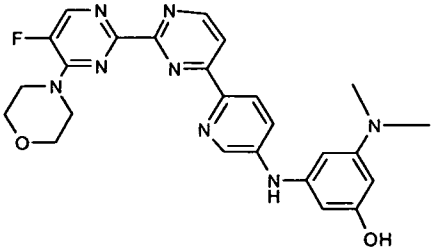


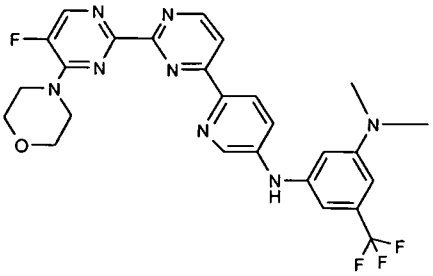


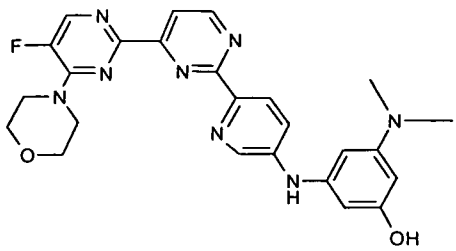


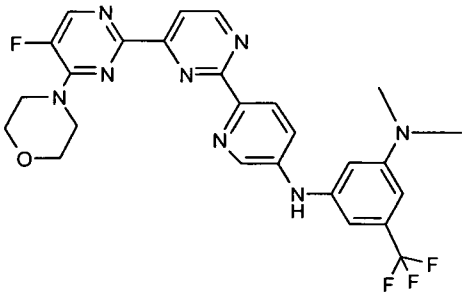


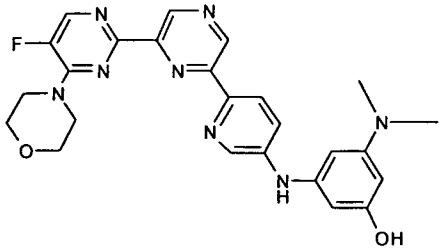


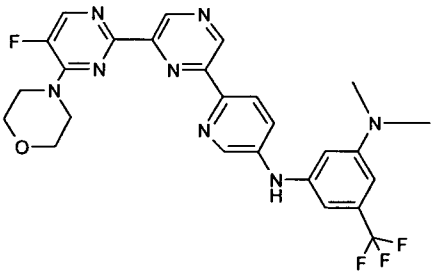


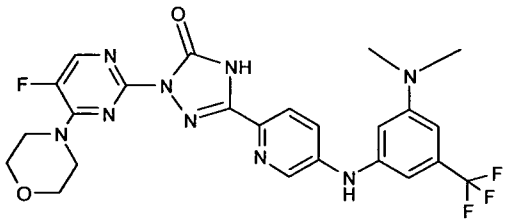


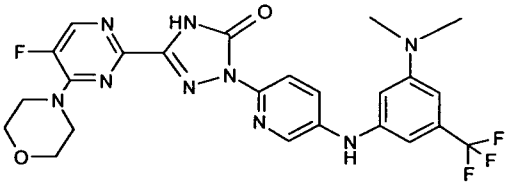


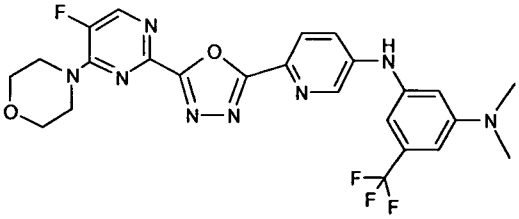


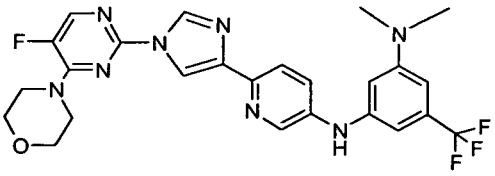


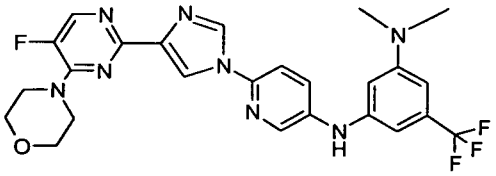


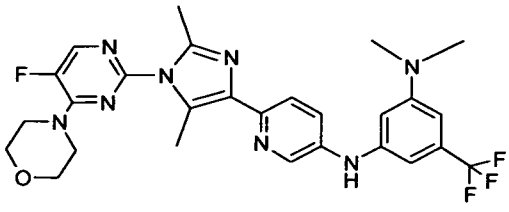


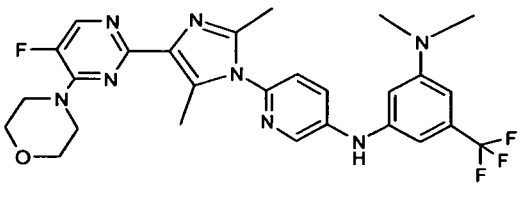


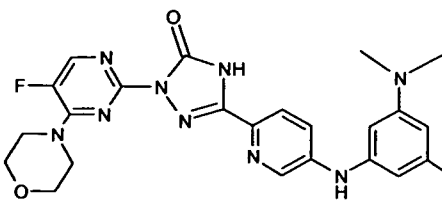


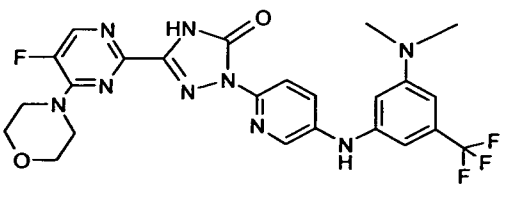


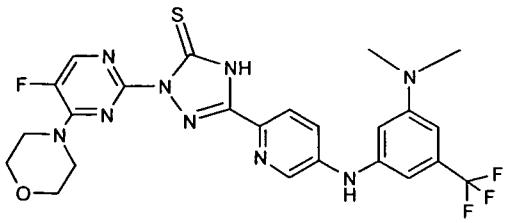
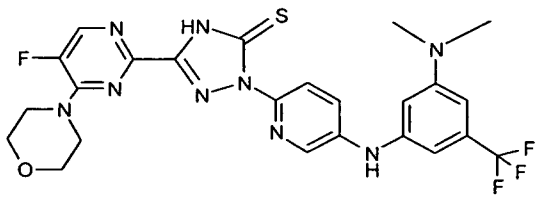
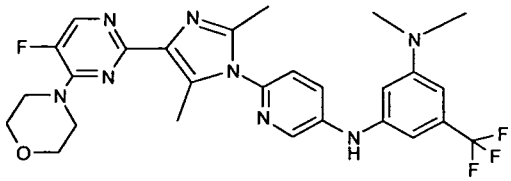
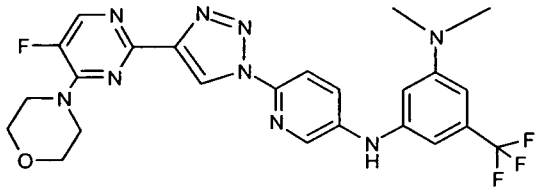
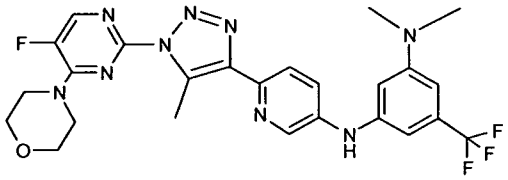
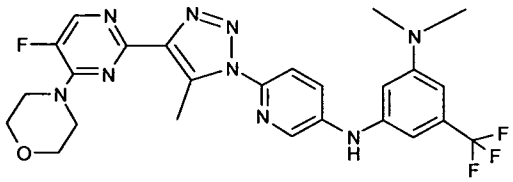
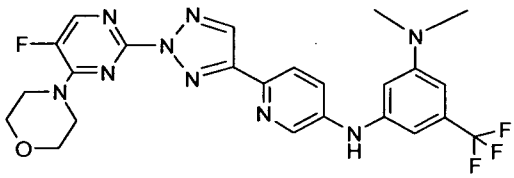
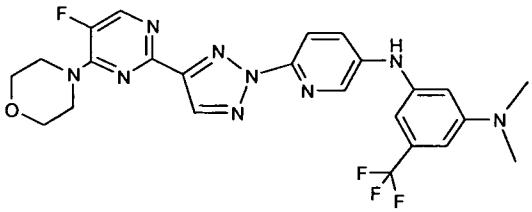
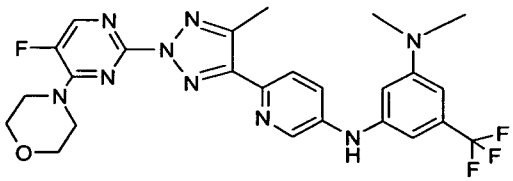
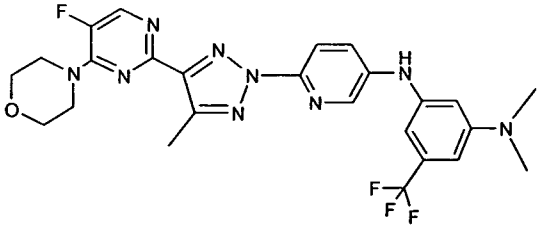
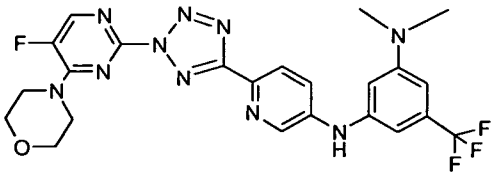
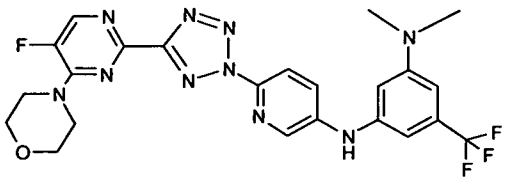
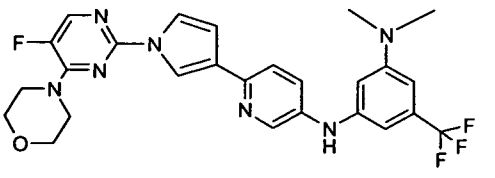
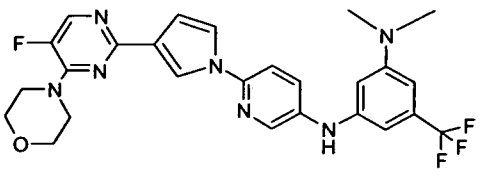
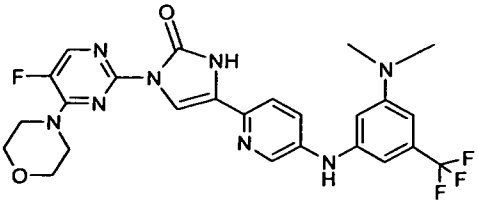
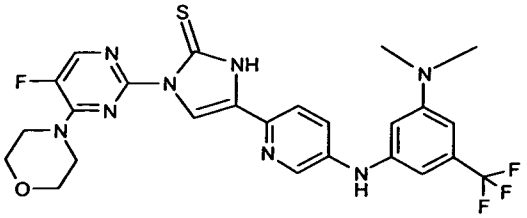


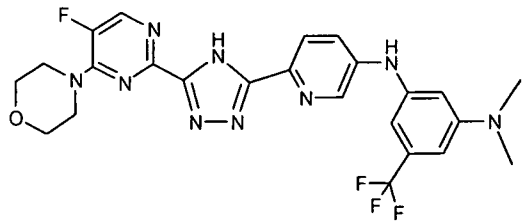


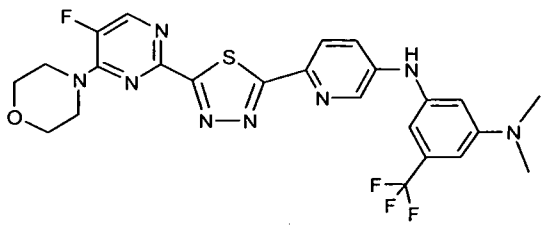


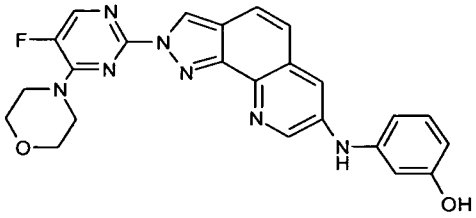


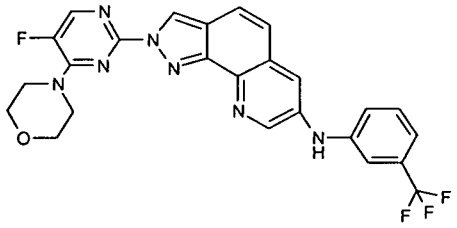


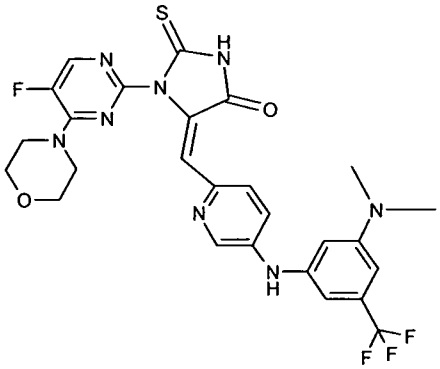

















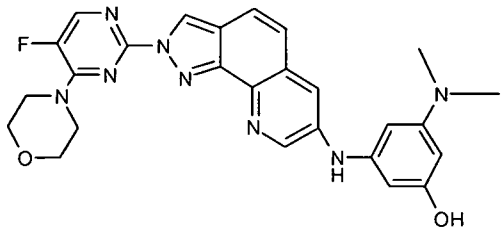


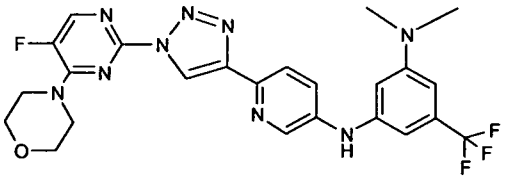


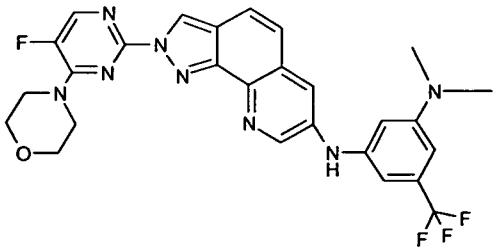


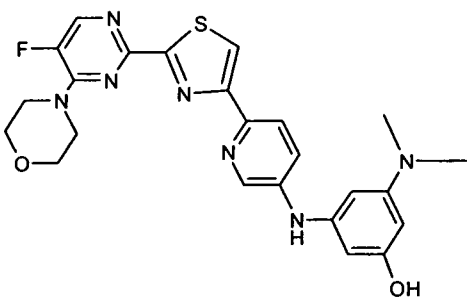


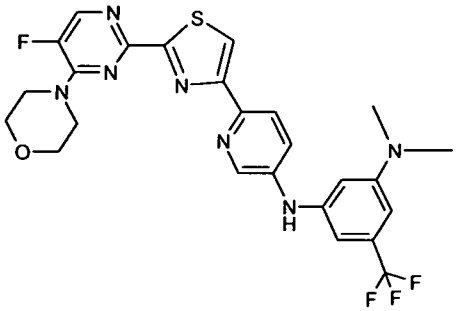


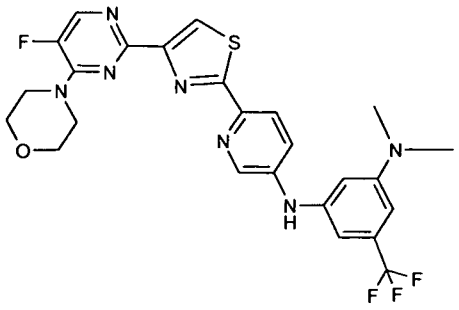


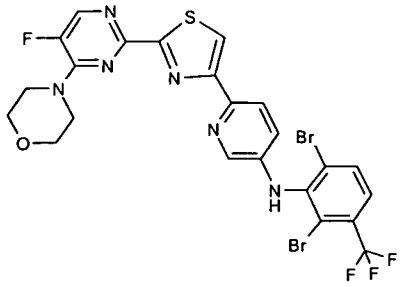












[0055] As used herein, the definition of each expression, e.g. alkyl, *a*, *b*, R, R', R^1, etc., when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

[0056] For each of the above descriptions of compounds, each recitation of the terms halo, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, heterocyclic group or heterocyclic ring, are independently selected from the definitions of these terms as provided in the beginning of this section.

[0057] It will be understood that chemical structures provided herein include the implicit proviso that substitution is in accordance with permitted valence of the substituted atom and the substituent(s), and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

[0058] When one or more chiral centers are present in the compounds of the present invention, the individual isomers and mixtures thereof (e.g., racemates, etc.) are intended to be encompassed by the formulae depicted herein.

[0059] When one or more double bonds are present in the compounds of the present invention, both the cis- and trans- isomers are intended to be encompassed by the formulae depicted herein. Although chemical structures may be depicted herein in either cis or trans configuration, both configurations are meant to be encompassed by the each of the formulae.

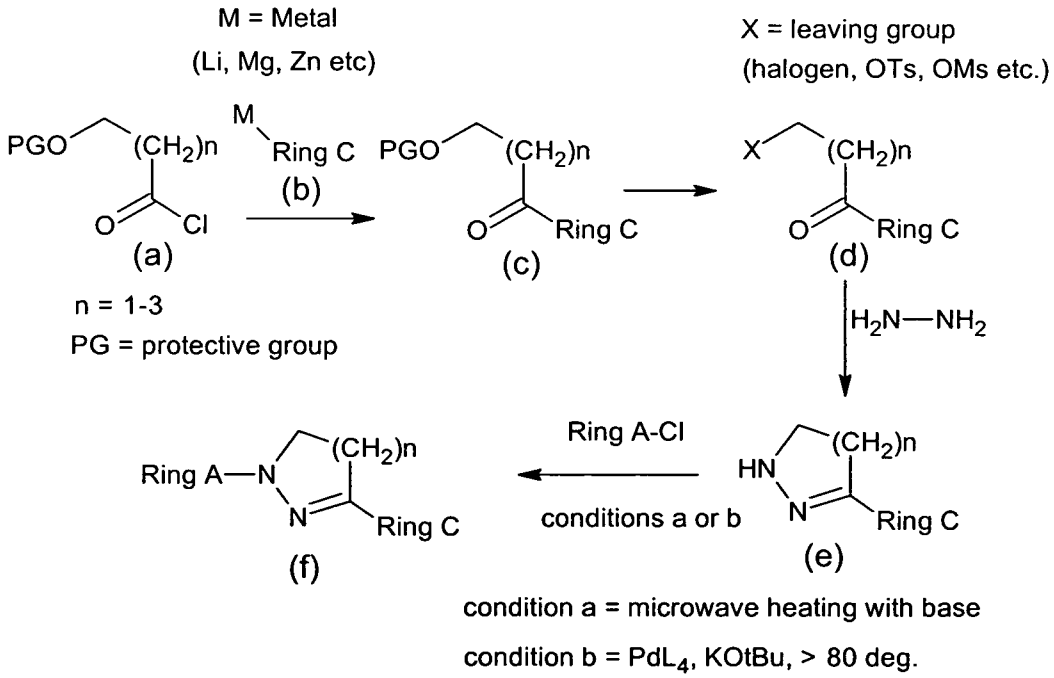
[0060] In certain embodiments, compounds of the invention may exist in several tautomeric forms. Accordingly, the chemical structures depicted herein encompass all possible tautomeric forms of the illustrated compounds.

[0061] The compounds of the invention may generally be prepared from commercially available starting materials and known chemical techniques. Embodiments of the invention may be synthesized as follows. One of skill in the art of medicinal or synthetic chemistry would be readily familiar with the procedures and techniques necessary to accomplish the synthetic approaches given below.

[0062] The compounds exemplified by Formulas I-VIII may be prepared by conventional synthetic techniques well known to one of ordinary skill in organic chemistry.

[0063] For example, compounds in which Ring *B* is a cyclic hydrazone, or a derivative thereof, may be prepared according to the following general Scheme 1.

Scheme 1

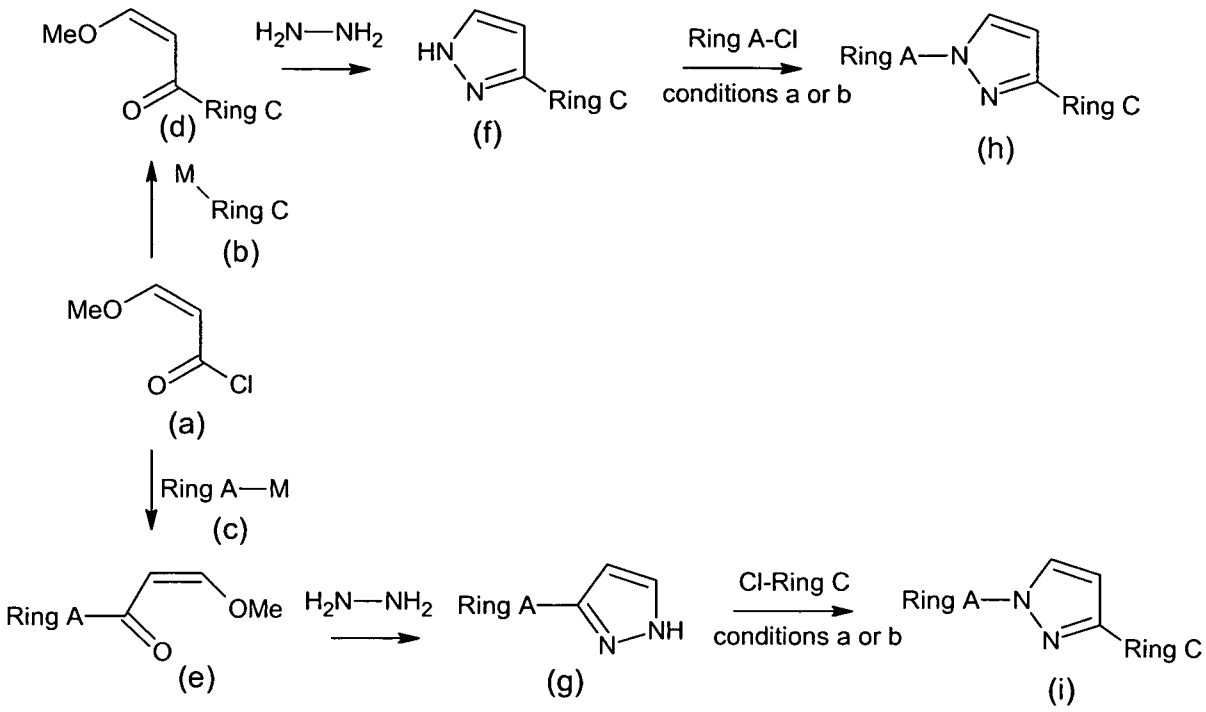


[0064] Reaction of an appropriately protected β, γ or δ alkoxy acid chloride (a) with a suitable nucleophile (b) will prepare the ketone (c). M refers to one of the electropositive elements including for example Li, Mg, and Zn. It will be understood be the skilled practitioner that for bivalent metals such as Mg or Zn, that an additional chemical moiety such as a halogen will be bound to the metal. Suitable protective groups (PG) may include silyl ethers, acetals, esters and other groups well known to persons skilled in the art. Removal of the protective group and conversion to a suitable leaving group to give intermediate (d) may be carried out under conditions compatible with each individual Ring C. Cyclization with hydrazine will give the hydrazone (e), which may be coupled with Ring A

under either basic or organometallic conditions to give the product (f). The coupling may be carried out in microwave reactor (condition a). Alternatively, the coupling may be performed using an organometallic catalyst such as a palladium catalyst with a strong, non-nucleophilic base and heat. Conditions for the coupling may be adapted from the processes disclosed in the art, for example, "Efficient nucleophilic substitution reactions of pyrimidyl and pyrazyl halides with nucleophiles under focused microwave irradiation" Y-J. Cherng *Tetrahedron* **2002**, *58*, 887-890; "Copper fluorapatite catalyzed *N*-arylation of heterocycles with bromo and iodoarenes" M. Lakshmi Kantam, G.T. Venkanna, Ch. Sridhar and K.B. Shiva Kumar *Tetrahedron Lett* **2006**, 47, 3897-3899; "Design and Evolution of Copper Apatite Catalysts for N-Arylation of Heterocycles with Chloro- and Fluoroarenes" B. M. Choudary, M. Boyapati; C. Sridhar; M. L. Kantam; G. T. Venkanna; B. Sreedhar, J. Am. Chem. Soc. **2005**, *127*, 9948-9949; "The Synthesis of Aminopyridines: A Method Employing Palladium-Catalyzed Carbon-Nitrogen Bond Formation" S. Wagaw; S. L. Buchwald, *J. Org. Chem.* **1996**, *61*, 7240-7241; "Synthesis of some compounds derived from ω-chlorobutyrophenones" W. Schliemann; A. Buege; L. Reppel, *Pharmazie* **1980**, *35*, 140-3; "The reaction of 4-alkyl-3-thiosemicarbazides with β-halo ketones" W. D. Jones, J. M. Kane; A. D. Sill, *J. Heterocyclic Chem.* **1983**, *20*, 1359-61; "Experiments on halogenated ketones. The conversion of 1-bromo-1,3-diphenylpropan-2-one into 1-phenylindan-2-one" A. C. B. Smith; W. Wilson, *J. Chem. Soc.* **1955**, 1342-7; "Insecticidal anthranilic diamides: A new class of potent ryanodine receptor activators" G. P. Lahm; T. P. Selby; J. H. Freudenberger; T. M. Stevenson; B. J. Myers; G. Seburyamo; B. K. Smith; L. Flexner; C. E. Clark; D. Cordova, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4898-4906.

[0065] Compounds in which Ring *B* is a pyrazole or a derivative thereof may be prepared according to the following general Scheme 2.

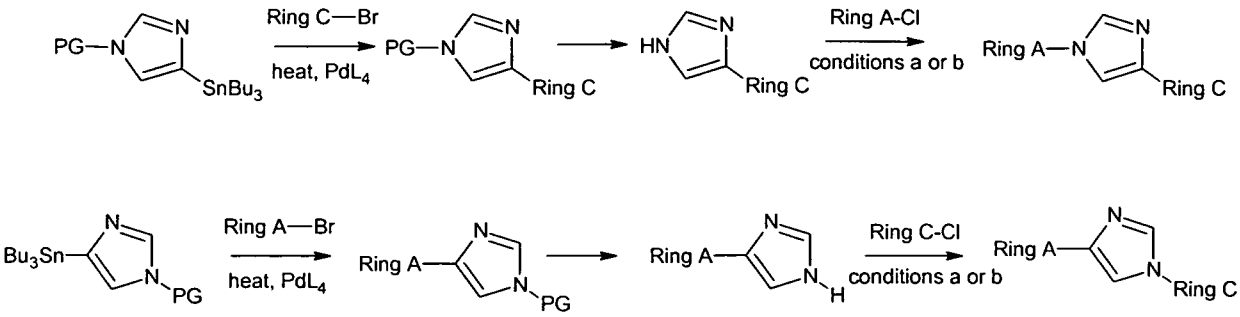
Scheme 2



[0066] Reaction of methoxyl acrylic acid chloride (a) with a nucleophilic Ring A (c) or Ring C (b) provides the intermediates (e) and (d), respectively. M is as described above for Scheme 1. The intermediates (e) and (d) are treated with hydrazine to give the pyrazoles (g) and (f). Reaction with a halogenated Ring A or Ring C under the same conditions described above will produce the disubstituted pyrazole (i) and (h).
Reaction conditions may be adapted from the processes disclosed in the art, for example, "Insecticidal anthranilic diamides: A new class of potent ryanodine receptor activators" G. P. Lahm; T. P. Selby; J. H. Freudenberger; T. M. Stevenson; B. J. Myers; G. Seburyamo; B. K. Smith; L. Flexner; C. E. Clark; D. Cordova, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4898-4906.

[0067] Compounds in which Ring *B* is an imidazole, or a derivative thereof, may be prepared from the appropriately protected tributyl tin imidazole according to general Scheme 3.

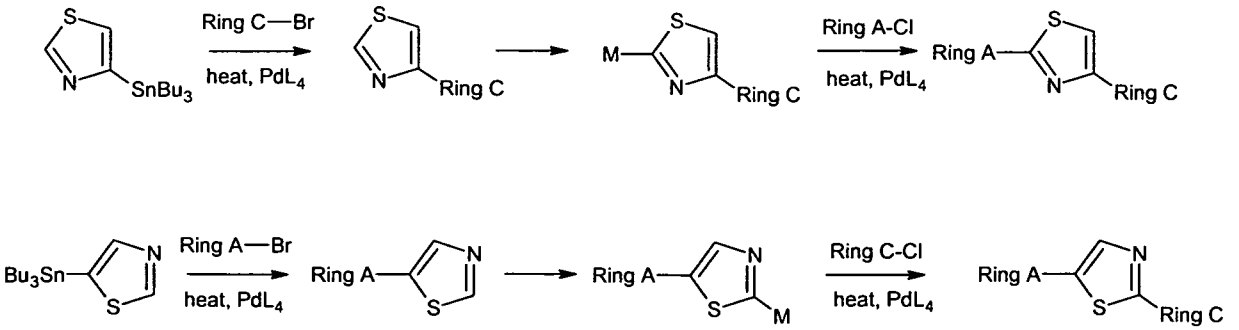
Scheme 3



[0068] PG is an appropriate protecting group. Organometallic coupling with a halogenated Ring A or C will produce the monosubstitued imidazoles. The reaction may be carried out using a palladium catalyst. The disubstituted imidazole may be produced by reaction with halogenated Ring A or C using conditions described above. "Comparative study on the reactivity of 6-haloimidazo[1,2-a]pyridine derivatives towards Negishi- and Stille-coupling reactions." Reaction conditions may be adapted from the processes disclosed in the art, for example, M. Hervet; I. Thery; A. Gueiffier; C. Enguehard-gueiffier, *Helv. Chim. Acta* **2003**, *86*, 3461-3469; "Synthesis of the heterocyclic core of the GE2270 antibiotics and structure elucidation of a major degradation product." G. Heckmann; T. Bach *Angew. Chemie, Internat. Ed.* **2005**, *44*, 1199-1201.

[0069] Compounds in which Ring *B* is a thiazole, or a derivative thereof, may be produced according to the general Scheme 4.

Scheme 4

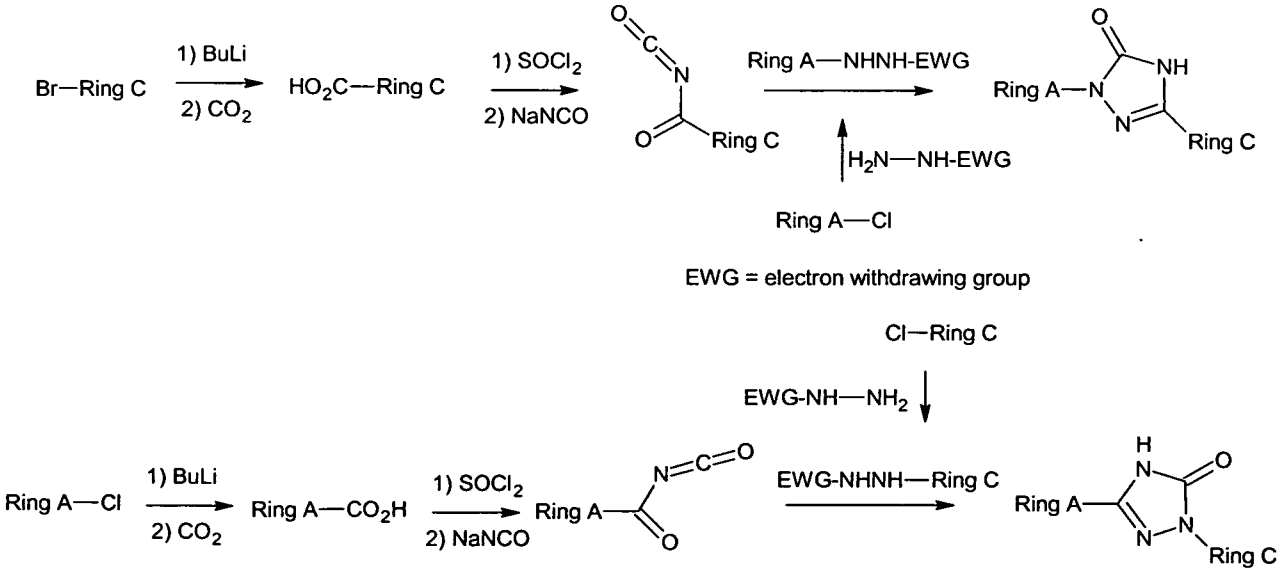


[0070] An appropriate halogenated Ring A or C is reacted with a 2 or 5-tributyltin thiazole. The monosubstituted thiazole ring may be metallated using n-butyl lithium or similar strong base, with or without transmetallation, or by other means well known to those skilled in the art. The metallated thiazole may be coupled with the appropriately halogenated

Ring A or C derivative under organometallic conditions. Such conditions include heat and a palladium catalyst. Reaction conditions may be adapted from the processes disclosed in the art, for example, "Synthesis of the heterocyclic core of the GE2270 antibiotics and structure elucidation of a major degradation product." G. Heckmann; T. Bach, *Angew. Chemie, Internat. Ed.* **2005**, *44*, 1199-1201; "Synthesis of 6-allyl- and 6-heteroarylindoles by palladium catalyzed Stille cross-coupling reaction" R. Benhida; F. Lecubin; J.-L. Fourrey; L. R. Castellanos; L. Quintero, *Tetrahedron Lett.* **1999**, *40*, 5701-5703; "Scavenging and Reclaiming Phosphines Associated with Group 10 Metal-Mediated Couplings" B. H. Lipshutz; B. Frieman; H. Birkedal, *Org. Lett.* **2004**, *6*, 2305-2308; "An Improved Method for the Palladium Cross-Coupling Reaction of Oxazol-2-ylzinc Derivatives with Aryl Bromides" M. R. Reeder; H. E. Gleaves; S. A. Hoover; R. J. Imbordino; J. J. Pangborn, *Org. Proc. Res. & Dev.* **2003**, *7*, 696-699.

[0071] Compounds in which Ring *B* is a triazolone, or a derivative thereof, may be prepared according to Scheme 5.

Scheme 5

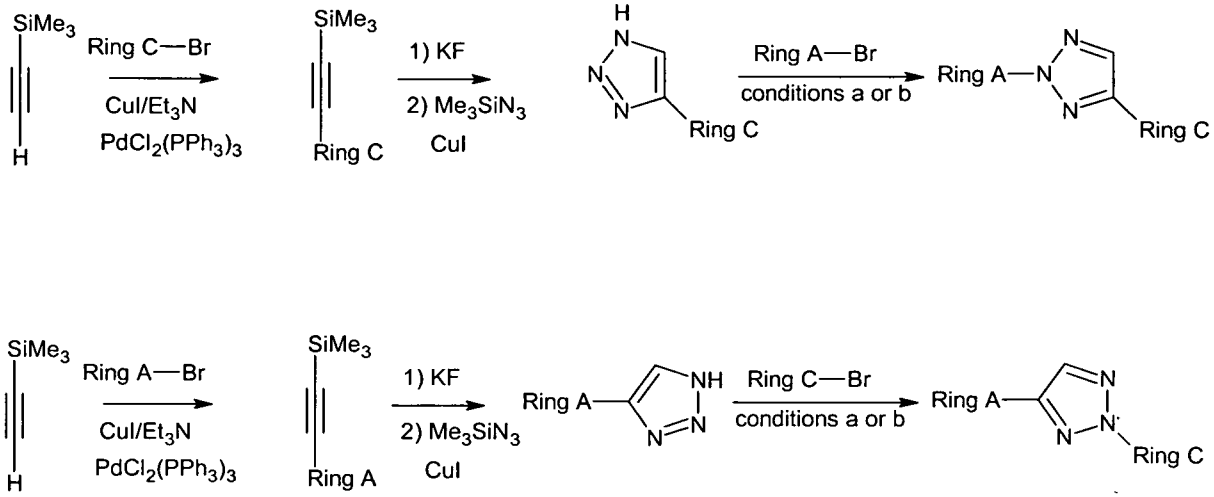


[0072] An appropriate Ring A or C carboxylic acid is prepared by reaction of a metallated Ring A or Ring C compound with carbon dioxide. The metalation may be carried out using n-butyl lithium or equivalent thereof. The carboxylic acid may be converted to the acid chloride using conventional reactions, including thionyl chloride or equivalent thereof. Conversion of the acid chloride to the keto-isocyanate and condensation with an Ring A or C hydrazone derivative will produce the desired triazolone. Reaction conditions may be

adapted from the processes disclosed in the art, for example, "Heterocyclic Tautomerism. IV. The Solution and Crystal Structures of 1-Aryl-3-phenyl-1,2,4-triazol-5-ones," A. D. Rae; C. G. Ramsay; P. J. Steel, *Australian J. Chem.* **1988**, *41*, 419-428.

[0073] Compounds in which Ring *B* is a triazoles, or derivatives thereof, may be prepared according to the general Scheme 6.

Scheme 6



[0074] Cycloaddition of an appropriate Ring A or Ring C alkyne with azide results in the respective triazole. The alkyne may be prepared using a Sonagashira or other organometallic coupling reaction. The other carbon atom of the alkyne may be protected with a silyl protective group, which is removed with fluoride prior to cycloaddition. The monosubstituted triazole may be reacted with an appropriately halogenated Ring A or Ring C compound under conditions previously described to produce the desired triazole-bridged product. Reaction conditions may be adapted from the processes disclosed in the art, for example, "Table annelated chiral NADH models with a rigidified amide part in the quinoline series: synthesis, reactivity and grafting on a Merrifield resin," C. Vitry, J.-L. Vasse; G. Dupas; V. Levacher; G. Quéguiner; J. Bourguignon, *Tetrahedron* **2001**, *57*, 3087-3098; "Copper-Catalyzed Synthesis of N-Unxubstituted 1,2,3-Triazoles from Nonactivated Terminal Alkynes," T. Jin; S. Kamijo; Y. Yamamoto, *Eur. J. Chem.* **2004**, 3789-3791; "A One-Pot Procedure for the Regiocontrolled Synthesis of Allyltriazoles via the Pd-Cu Bimetallic Catalyzed Three-Component Coupling Reaction of Nonactivated Terminal Alkynes, Allyl Carbonate, and Trimethylsilyl Azide," S. Kamijo; T. Jin, Z. Huo;Y. Yamamoto, *J. Org. Chem.* **2004**, 69, 2386-2393; "The development of new triazole based

inhibitors of tumor necrosis factor-α (TNF-α) production," J. S. Tullis; J. C. VanRens; M; G. Natchus; M. P. Clark; B. De; L. C. Hsieh; M. J. Janusz, *Bioorg. & Med. Chem. Lett.* **2003**, 13, 1665-1668.

[0075] It will be apparent to a practitioner skilled in the art of organic molecule synthesis that the reaction processes illustrated above are representative of a broader set of methods that are logical extensions of the illustrated processes. Thus, additional embodiments of the invention that incorporate additional variants in R^2, R^3, R^4, and R^5 claimed by this invention are prepared by obvious modifications of the above processes.

[0076] As would be recognized by a person of ordinary skill, it may be advantageous to employ a temporary protecting group in achieving the final product. The phrase "protecting group" as used herein means temporary modifications of a potentially reactive functional group which protect it from undesired chemical transformations. Examples of such protecting groups include esters of carboxylic acids, silyl ethers of alcohols, and acetals and ketals of aldehydes and ketones, respectively. The field of protecting group chemistry has been reviewed (Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 2nd ed.; Wiley: New York, 1991).

[0077] One embodiment of this invention is directed to any endogenously occurring mammalian target protein selected by the skilled investigator to be of interest for the identification and/or optimization of a compound as an inhibitor or activator of said protein. In general such selected proteins will already be known to be involved in the etiology or pathogenesis of a human disease. In another embodiment, the invention is also directed toward mutant forms of such mammalian proteins. A "mutein" is a protein having an amino acid sequence that is altered as a result of a mutation that has occurred in its corresponding gene (Weigel et al, 1989). Such mutations may result in changes in one or more of the characteristics of the encoded protein. For example, an enzyme variant that has modified catalytic activity resulting from a change in one or more amino acids is a mutein.

[0078] This invention is concerned with proteins harboring an alteration of at least one amino acid residue (the terms "amino acid sequence change" or "amino acid sequence alteration" include changes, deletions, or additions, of at least one amino acid residue, or any combination of deletions, additions, changes) such that the resulting mutein has become (as a result of the mutation) resistant to a known therapeutic agent relative to the sensitivity of the

non-mutated version of said protein to the therapeutic agent. This specialized class of muteins is hereinafter referred to as a ***theramutein***, and the corresponding protein lacking the mutation is referred to herein as a ***prototheramutein***.

[0079] As used herein, "prototheramutein" refers to an endogenously occurring protein in a cell that is susceptible to mutation that confers relative insensitivity (i.e. resistance) to a therapeutic compound which otherwise inhibits or activates the protein. Accordingly, "theramutein" refers to an endogenously occurring protein or portion of a protein in a cell that contains at least one amino acid sequence alteration relative to an endogenous form of the protein, wherein the amino acid sequence change is or was identified or becomes identifiable, and is or has been shown to be clinically significant for the development or progression of a given disease, *following exposure of at least one human being to a substance that is known to inhibit or activate the prototheramutein*. Solely for the purposes of defining the preceding sentence, a substance need not be limited to a chemical agent for the purposes of first defining the existence of a theramutein. Thus, ***by definition***, a theramutein is a protein which harbors a mutation in its corresponding endogenous gene, wherein said mutation is associated with the development of clinical resistance in a patient to a drug that is normally able to activate or inhibit the non-mutated protein. With respect to a given theramutein, the term "corresponding prototheramutein" refers to the prototheramutein which, through mutation, gives rise to said theramutein. Similarly, with respect to a given prototheramutein, the "corresponding theramutein" refers to the theramutein which has arisen by mutation from said prototheramutein.

[0080] Accordingly, it is apparent to a skilled artisan that, as the genes which encode theramuteins are limited to endogenously occurring genes, the definition of a theramutein excludes proteins encoded by disease-causing infectious agents such as viruses and bacteria. As used herein, the term "endogenous gene" refers to a gene that has been present in the chromosomes of the organism at least in its unmutated form, since inception. The term "cell" as used herein refers to a living eukaryotic cell whether in an organism or maintained under appropriate laboratory tissue or organ culture conditions outside of an organism.

[0081] In one embodiment of the invention, the target protein (POI) may be any endogenously encoded mammalian protein. In another aspect of the invention, the POI is a theramutein, which is a protein that is altered for the first time with respect to a commonly

occurring "wild type" form of the protein (*i.e.*, a wild type protein is the prototheramutein from which the theramutein arises). In yet another aspect of the invention, a theramutein is a variant of a protein that is, itself, already a mutein (*i.e.*, a mutein is the prototheramutein from which the theramutein arises). In still another embodiment, a theramutein may be further mutated as compared to a previously existing theramutein. In such instances, the first theramutein (such as the T315I mutant of p210 BCR-ABL (see below), may be thought of as a "primary" theramutein, whereas subsequent mutations of the (already mutated) T315I variant may be termed a secondary theramutein, tertiary theramutein, etc. As exemplified below, a mutein of the invention is a variant of Bcr-Abl tyrosine kinase that escapes inhibition by an inhibitor of the "wild type" Bcr-Abl. Such a Bcr-Abl mutein is altered with respect to a more common or "wild type" form of Bcr-Abl (which is also a mutein as well) in such a way that a property of the protein is altered.

[0082] It is understood that a protein of interest (POI) is an endogenously encoded mammalian protein. It will also be understood that a mutein of primary interest is a theramutein that may have the same, increased, or decreased specific activity relative to its prototheramutein, and that it is not inhibited or is poorly inhibited by an agent that is used to inhibit the prototheramutein. Likewise, another theramutein of primary interest is one that has the same, increased or decreased specific activity (relative to its prototheramutein) and that is not activated or is poorly activated by an agent that is used to activate the prototheramutein. Other variations are obvious to the skilled artisan. It will be further appreciated that theramuteins can include naturally occurring or commonly observed variants of a protein, for example, variants that are expressed from different alleles of a particular gene. In some cases such variants may be unremarkable with respect to their normal cellular function, with functional differences becoming apparent only in the presence of agents that differentially inhibit or activate the cellular function of the variants. For example, naturally occurring variants of a particular enzyme may have activity profiles that are not substantially different, but a therapeutic agent that modulates one may be ineffective in modulating the other.

[0083] It will be appreciated that one aspect of the invention is the identification of an agent that is active against a selected POI whose cellular function contributes to a given disease state such that activators or inhibitors of said POI would be expected to be

therapeutically effective during the course of treatment for the disease. No limitation of any kind or nature is intended on the type of disease that may be treated, nor on the type of protein that may be targeted for modulation according to the teachings herein, provided that all other limitations stated herein are met, including the fact that any such protein that is selected for targeting must be an endogenous protein. Obviously, the skilled investigator may use non-endogenously occurring nucleic acids such as cDNAs in order to practice the method taught herein provided that the amino acid sequence corresponds to an endogenously occurring POI.

[0084] It will also be appreciated that, whereas one aspect of the invention is the identification of an agent that is active against a protein or theramutein that arises or becomes dominant (by any mechanism) prior to or during the course of a treatment for a given disease, another aspect is the identification of an agent that is active against a mutein that is common within a population of unafflicted individuals, but wherein said mutein is less susceptible to modulation by an approved drug, and where the variation in the activity profile of the mutein becomes important (and is therefore first identified as being a theramutein) in a disease state such as where it is overexpressed or participates in a signaling process which has otherwise become abnormally regulated. For example, a neoplastic disease may be caused by abnormal regulation of a cellular component other than the theramutein or its prototheramutein, and still be treatable with an inhibitor of the prototheramutein, whereas the same treatment would be less effective or ineffective where the theramutein was present. This can be an issue where it is observed that the response of a particular tumor type to an anticancer agent varies among individuals that express different variants of an enzyme against which the anticancer agent is directed (Lynch et al., 2004). Here, the variants would not have arisen or become predominant during the course of treatment of the disease, but are preexisting in the healthy population and are detected only by their altered responsiveness to a particular course of established therapeutic treatment.

[0085] As used herein, the terms “agonist” and “activator” of a protein are used interchangeably. An activator (agonist) is limited to a substance that binds to and activates the functioning of a given protein. Unless explicitly stated otherwise, an "activator", an "agonist", and an "activator of a protein" are identical in meaning. The activation by an activator may be partial or complete. Likewise, as used herein, the terms “antagonist” and

"inhibitor" of a protein are used interchangeably. An inhibitor (antagonist) is limited to a substance that binds to and inhibits the functioning of a given protein. To state that a substance "inhibit(s)" a protein means the substance binds to the protein and reduce(s) the protein's activity in the cell without materially reducing the amount of the protein in the cell. Similarly, to state that a substance "activate(s)" a protein, such as a prototheramutein or theramutein, is to state that the substance increases the defined function of the protein in the cell without substantially altering the level of the protein in the cell. Unless explicitly stated otherwise, an "inhibitor", an "antagonist" and an "inhibitor of a protein" are also synonymous. The inhibition by an inhibitor may be partial or complete. A modulator is an activator or an inhibitor. By way of example, an "activator of $PKC_{\beta I}$" should be construed to mean a substance that binds to and activates $PKC_{\beta I}$. Similarly, an "inhibitor of $p210^{Bcr\text{-}Abl}$" is a substance that binds to and inhibits the functioning of $p210^{Bcr\text{-}Abl}$. To state that a substance "inhibits a protein" requires that the substance bind to the protein in order to exert its inhibitory effect. Similarly, to state that a substance "activates protein X" is to state that the substance binds to and activates protein X. The terms "bind(s)," "binding," and "binds to" have their ordinary meanings in the field of biochemistry in terms of describing the interaction between two substances (*e.g.*, enzyme-substrate, protein-DNA, receptor-ligand, etc.). As used herein, the term "binds to" is synonymous with "interacts with" in the context of discussing the relationship between a substance and its corresponding target protein. As used herein, to state that a substance "acts on" a protein, "affects" a protein, "exerts its effect on" a protein, etc., and all such related terms uniformly mean (as the skilled investigator is well aware) that said substance activates or inhibits said protein.

[0086] The concept of inhibition or activation of a mutated form of an endogenous protein to a greater extent than the corresponding non-mutated counterpart protein is defined for the first time and referred to herein as a positive "***specificity gap***." In general terms, *and using an inhibitor case as an example*, the ***specificity gap*** refers to the difference between the ability of a given substance, under comparable conditions to inhibit the theramutein in a cell-based assay system of the invention as compared to either:

a) the ability of the same substance under comparable conditions to inhibit the prototheramutein; or

b) the ability of a second substance (usually a known inhibitor of the prototheramutein) to inhibit the theramutein under comparable conditions; or

c) the ability of the second substance to inhibit the prototheramutein under comparable conditions.

[0087] When the comparison is made between the effects of two distinct substances (tested individually) on the theramutein alone, the result is termed a ***homologous specificity gap*** determination.

[0088] Alternatively, when a comparison is made between the effects of two distinct substances (generally, but not always), one of which is tested on the theramutein and the other on the prototheramutein, respectively, the result is termed a ***heterologous specificity gap*** (SG) determination. Thus, (a) and (c) as given above are examples of heterologous specificity gap (SG) determinations (although (a) uses the same substance in both instances), whereas (b) is an example of a homologous specificity gap determination.

[0089] Analogous issues apply when the case concerns an activator. It will be immediately obvious to the skilled artisan that the term "comparable conditions" includes testing two different compounds, for example, at the same concentration (such as comparing two closely related compounds to determine relative potency), or by comparing the effects of two different compounds tested at their respective IC_{50} values on the corresponding prototheramutein and theramutein. The skilled investigator will easily recognize other useful variations and comparable conditions.

[0090] Thus, in one embodiment of the application of this approach, substances that are more effective against a theramutein have a "positive specificity gap." A "zero, null or no" specificity gap indicates that there is no significant measurable difference between the effect of a substance on the theramutein as compared to its effect on the prototheramutein (however such compounds may be quite useful in their ability to inhibit or activate both a theramutein and its corresponding prototheramutein), and a "negative specificity gap" indicates a substance that at a given concentration is less effective against the given theramutein than against a form of the corresponding prototheramutein or other comparative form of the theramutein (such as one that may harbor a different mutation). The latter category is generally of lesser interest than the former categories of compounds, except in the case where the compound is so potent that its relatively lesser effect on the theramutein is of no real concern from the perspective of therapeutic efficacy. The skilled investigator can easily recognize a variety of approaches to quantifying the specificity gap assessment in a

manner tailored to his or her needs. Such an analysis may assist the skilled investigator in classifying various compounds into discrete categories that may be helpful in guiding further lead optimization or biological profiling studies on such compounds.

[0091] The invention also provides a means for identifying compounds that exhibit a desired specificity gap. Such compounds can be identified and their ability to inhibit or activate the theramutein determined using an *in vitro* cell-based assay system where the effect of a substance on the cellular functioning of the mutated endogenous form of the protein is compared to the effect of the same drug on the cellular functioning of a non-mutated endogenous form of the protein.

[0092] Thus, the system enables the discovery of compounds capable of binding to a theramutein and exerting a greater modulatory effect on the cellular functioning of said theramutein than on its corresponding prototheramutein. Further, the system enables the discovery of compounds capable of binding to a theramutein and exerting at least as great or greater modulatory effect on the cellular functioning of a theramutein than previously known compounds are able to exert on the corresponding prototheramutein. In a particular embodiment of the invention, a compound may be screened for and identified that 1) is at least as effective against the theramutein as the original drug is against the prototheramutein, and/or 2) is similarly effective against the prototheramutein as against the theramutein (*i.e.*, displays a small or essentially zero specificity gap).

[0093] In an embodiment of the invention, cells that overexpress a theramutein of interest are used to identify chemical agents that are inhibitors or activators of (*i.e.*, that bind to and inhibit or that bind to and activate) at least the selected theramutein. The chemical agents may also be inhibitors or activators of the prototheramutein or even other theramuteins of the same prototheramutein. As used herein, the terms "chemical agent" and "compound" are used interchangeably, and both terms refer exclusively to substances that have a molecular weight up to, but not including, 2000 atomic mass units (Daltons). Such substances are sometimes referred to as "small molecules." Unless otherwise stated herein, the term substance as used herein refers exclusively to chemical agents/compounds, and does not refer to ***biological agents***. As used herein, "***biological agents,***" are molecules which include proteins, polypeptides, and nucleic acids, and have molecular weights equal to or greater than 2000 atomic mass units (Daltons).

[0094] In one embodiment of the invention, a theramutein is selected and used in a phenoresponse-based cellular assay system of the present invention designed to identify agents that are inhibitors or activators of the theramutein. Where two or more distinct theramuteins originating from the same prototheramutein are known, it is preferable to select the most resistant theramutein available for use in the assay system. In general, the degree of resistance of a theramutein to a given chemical agent is determined relative to its non-mutated counterpart (prototheramutein) using the drug that was first administered and known to inhibit or activate the prototheramutein and against which the theramutein "arose." The methods of determining the degree of such resistance, for example by analysis of IC_{50} or AC_{50} values, are well known and standard in the art and will not be reiterated herein. However, no causal relationship is necessary or should be inferred between the treatment of the patient with a given therapeutic agent *per se* and the subsequent appearance of a theramutein. Rather, what is required in order to practice the invention as it pertains to theramuteins is that a true theramutein be properly selected according to the teachings herein.

[0095] Thus, for example, randomly generated site directed mutants of known proteins that are created in the laboratory but that have ***not*** been shown to be clinically relevant are not appropriate muteins for use within the scope of this invention. Such muteins would not, of course, be properly classified as theramuteins either.

[0096] For example, in an effort to obtain potential inhibitors of mutants of $p210^{Bcr\text{-}Abl}$, Huron et al. (2003) used a recombinant c-abl preparation and screened a series of compounds known to inhibit c-src tyrosine kinase activity. The authors performed c-abl kinase assays on their compounds and identified the most potent compound as an 8 nM inhibitor against c-abl. When this compound (PD166326) was tested against various $p210^{Bcr\text{-}Abl}$ theramuteins, however, it showed activity against some of the mutants such as $p210^{Bcr\text{-}Abl\text{-}E255K}$, but the $p210^{Bcr\text{-}Abl\text{-}T315I}$ theramutein was found to remain 10 fold more resistant (Huron et al. 2003, Table 3). Furthermore, in each case the compound was still markedly ***less effective*** on the $p210^{Bcr\text{-}Abl}$ theramuteins than it was against the wild-type $p210^{Bcr\text{-}Abl}$. When the compound was tested against $p210^{Bcr\text{-}Abl\text{-}T315I}$ mutant activity, it was unable to inhibit the activity to any appreciable extent (p. 1270, left hand column, second paragraph; see also Fig. 4.). Thus, the disclosed compound was able to inhibit a theramutein that is partially resistant to STI-571, but had no activity against the T315I mutant of Bcr-Abl.,

which was already known at that time to be the theramutein that exhibited the most resistance to STI-571. Hence, the Huron methodology failed to identify an effective inhibitor of the $p210^{Bcr\text{-}Abl\text{-}T315I}$ theramutein.

[0097] It cannot be overemphasized that such compounds would be immensely useful, because at the present time there is no alternative for patients who progress to $p210^{Bcr\text{-}Abl\text{-}T315I}$ theramutein-mediated imatinib mesylate-resistant status. Once patients develop such resistance, there is no other effective alternative treatment available, and death is certain. The method described herein provides the first reported approach to identify, pharmacologically characterize and chemically synthesize effective inhibitors of the $p210^{Bcr\text{-}Abl\text{-}T315I}$ theramutein. Moreover, the skilled investigator will immediately recognize the applicability and generalizability of this approach to any highly drug-resistant theramutein. Finally, the skilled investigator will further recognize that linking a phenoresponse as defined herein to the increased presence and functional activity of a particular POI in the cell under appropriate conditions allows one to utilize the method with any given endogenous target protein for which a therapeutically effective compound is sought.

[0098] In the present invention, a test cell is used that displays a carefully selected phenotypic characteristic (as defined below) which is linked to the presence and functional activity of the particular protein-of-interest (POI) or theramutein-of-interest (TOI) in the cell under appropriate conditions. With respect to a theramutein, this should be qualitatively the same as the phenotypic characteristic displayed by a cell that expresses the prototheramutein. A phenotypic characteristic (i.e. a non-genotypic characteristic of the cell) is a property which is observed (measured), selected and/or defined for subsequent use in an assay method as described herein. Expression of the phenotypic characteristic is responsive to the total activity of the protein in the cell, and is a result of the absolute amount of the protein and its specific activity. Often, the phenotypic characteristic is observable as a result of elevated levels of protein activity and is not apparent in cells that express low amounts of the protein, or if the protein is also a theramutein, then the phenotypic characteristic will often not be apparent in cells expressing low amounts of either the theramutein or its corresponding prototheramutein. Further, it can often be demonstrated that the phenotypic characteristic is modulated by modulating the specific activity of the protein with an inhibitor or activator of the theramutein, although this is not always the case since an inhibitor or activator of the TOI

may not always be available at the time the skilled investigator undertakes such a project. (However, clearly a known inhibitor or activator of a given prototheramutein will always exist as a result of the intrinsic definition of the nature of a theramutein itself.) Thus, for the purpose of defining the phenotypic characteristic to be subsequently used with a given test cell for assay purposes, the skilled investigator may also use a substance capable of increasing or decreasing the expression of the gene encoding a given POI (such as a theragene in the case of a theramutein), which will in turn lead to increases or decreases of the level of the corresponding theramutein. This allows the skilled investigator to simulate the effects of certain types of activators or inhibitors of the theramutein (such as a suicide inhibitor of the theramutein, which is a class of chemical agent which binds irreversibly and covalently modifies the TOI, rendering it permanently inactive), without actually having access to such a compound, for the purposes of refining the appropriate phenotypic characteristic for subsequently establishing a useful cellular assay system. Examples known to one of ordinary skill that would be helpful for such purposes include the use of anti-sense DNA oligonucleotides, small interfering RNAs, other RNA interference-based methodologies, and vector constructs containing inducible promoter systems. In this manner, the selected phenotypic characteristic is linked to the activity of the theramutein in the test cell. Notably for theramuteins, the selected phenotypic characteristic is usually also displayed by a cell that overexpresses the prototheramutein and in which the phenotypic characteristic is modulated by known inhibitors or activators of the prototheramutein.

[0099] A phenotypic characteristic is simply a characteristic of a cell other than a genotypic characteristic of the cell. Except for the specific requirements of a properly defined phenotypic characteristic as disclosed herein for the purposes of creating useful cellular assay systems according to the teachings of certain of the embodiments of the invention, no other limitation of the term phenotypic characteristic of any kind or nature is intended or appropriate in order to properly and effectively practice the invention. Indeed, the skilled artisan must be able to select any characteristic of the cell that maximizes the utility of establishing the proper cell-based assay for his or her needs. The phenotypic characteristic can be quantitative or qualitative and be observable or measurable directly (*e.g.*, observable with the naked eye or with a microscope), but most commonly the characteristic is measured indirectly using standard automated laboratory equipment and assay procedures which are known to those of skill in the art. The term "observable" means

that a characteristic may be measured or is otherwise detectable under appropriate conditions by any means whatsoever, including the use of any type of laboratory instrumentation available. The term "detectable" is not the same as "detected." A characteristic may be detectable to a skilled artisan without being detected at any given time, depending upon how the investigator chooses to design the assay system. For example, in searching for activators of a POI such as a prototheramutein (or theramutein), it may be desirable to have the relevant phenotypic characteristic detected only after the addition of a known activator or test substance capable of activating the POI. This provides the ability to maximize the intensity of the signal that is generated by the test cell in the assay.

[0100] Phenotypic characteristics include but are not limited to growth characteristics, transformation state, differentiation state, substrate phosphorylation state, catalytic activity, ion flux across the cell membrane (calcium, sodium, chloride, potassium, hydrogen ions, etc.), pH changes, fluctuations of second messenger molecules or other intracellular chemical species such as cAMP, phosphoinositides, cyclic nucleotides, modulations of gene expression, and the like. The characteristic of the cell may be observable or measurable continuously (*e.g.*, growth rate of a cell), or after a period of time (*e.g.*, terminal density of a cell culture), or transiently (*e.g.*, modulation of a protein causes a transient change in phosphorylation of a substrate of the protein, or a transient flux in ion flow across the membrane, or elevations or reductions in intracellular cAMP levels). In certain embodiments, a selected phenotypic characteristic may be detected only in the presence of a modulator of the protein. No limitations are intended with respect to a characteristic that may be selected for measurement. As used herein, the terms "characteristic of a cell" and "phenotypic characteristic", and simply "characteristic", when used to refer to the particular measurable property of the intact cell or a subcellular fraction of the cell following the treatment of a test cell with a substance, are identical. For example, a phenotypic characteristic can be focus formation that becomes observable when a cell that over expresses a selected protein is cultured in the presence of an activator of the protein, or it may be a transient increase or decrease in the level of an intracellular metabolite or ion, such as cAMP, calcium, sodium, chloride, potassium, lithium, phosphatidylinositol, cGMP, bicarbonate, etc. It is obvious to one of ordinary skill in the art that after a cell is exposed to a test substance, the characteristic so measured (assayed) may be determined on a sub-cellular fraction of the cell. However, the initial treatment of the cell with a substance, which thereby causes the

substance to come into contact with the cell, must be performed on the intact cell, not a sub-cellular fraction.

[0101] The characteristic selected for measurement within the cell must not be an intrinsic physical or chemical property of the protein (or theramutein or prototheramutein) itself (such as the mere amount (mass) of the protein inside the cell), but rather must be a characteristic that results from the activity of the protein (or theramutein or prototheramutein) inside the cell, thus affecting a characteristic of the cell which is distinct from the theramutein itself, as discussed in detail above. For example, where the theramutein is a protein kinase that is capable of undergoing autophosphorylation, a process whereby the enzyme is capable of catalyzing the phosphorylation of itself by transferring a terminal phosphate group from ATP onto itself, it would NOT be appropriate to select the phosphorylation state of the TOI as an appropriate phenotypic characteristic of the cell for measurement. This is because such a characteristic does not reflect the activity of the TOI on other cellular components. As the skilled investigator knows, autophosphorylation is not necessarily reflective of the activity of a protein kinase in a cell, since mutants of protein kinases are known that retain enzymatic activity sufficient to undergo autophosphorylation, yet have lost the capability to engage in signal transduction events within the cell. The classic paper by White et al. (1988) is both educational and noteworthy in this respect.

[0102] The term "responsive phenotypic characteristic" means a characteristic of the cell which is responsive to inhibitors or activators of a given protein (including, *e.g.*, a prototheramutein or theramutein). The term "known therapeutic agent" is defined as any agent that has been administered to a human being for the treatment of a disease in a country of the world.

[0103] A useful phenotypic characteristic, as exemplified herein in association with $p210^{Bcr\text{-}Abl}$ and theramuteins thereof, is disregulation of cell growth and proliferation. It is noted that the same or similar assay may be appropriate for use with many different proteins of interest. For example, disregulations of growth, proliferation, and/or differentiation are common phenotypic characteristics that may result from overexpression of a variety of different cellular proteins. It is an important teaching of this invention that by overexpressing a selected protein in order to cause the appearance of such a phenotypic characteristic, the characteristic becomes linked to the presence, amount, and specific activity of that selected

protein under suitable conditions, and this linkage allows the skilled investigator to identify inhibitors or activators of a protein of interest (POI) as desired. Accordingly, the phenotypic characteristic is responsive to changes in the level and/or specific activity of the selected protein. Such a responsive phenotypic characteristic, when also demonstrated to be responsive to a known modulator of the POI is referred to herein as a "***phenoresponse***." In the special case of a theramutein which has no known modulator, a modulator of the prototheramutein must be utilized to establish a phenorespone to be used with the theramutein. The conception and recognition of this highly useful property of a cell represents one of the substantial advances of this invention over the prior art, including Applicant's own prior original work in the general area of cell-based assays (U.S. Pat. Nos. 4,980,281; 5,266,464; 5,688,655; 5,877,007). The identification and selection of the phenoresponse provides the skilled investigator with a cellular assay system that is extremely sensitive in terms of its ability to identify inhibitors or activators of the POI, and therefore identifies such chemical agents with a much higher degree of assurance than any other related assay method disclosed in the prior art.

[0104] Though not always necessary, it will often be advantageous to employ cells that express high levels of the POI, and to select a phenotypic characteristic that results from overexpression of the POI. This is because phenotypic characteristics linked to the functioning of the POI generally become more distinguishable (easier to measure) as a POI is overexpressed to a greater extent. Further, phenoresponses that are observed in response to modulators of the POI are often amplified as the functional level of the POI is increased. Expressed another way, the selected phenoresponse observed in cells that overexpress the protein (or theramutein) is particularly sensitive to modulators of the protein (or theramutein).

[0105] Preferably, the protein is stably expressed in a test cell. Stable expression results in a level of the protein in the cell that remains relatively unchanged during the course of an assay. For example, stimulation or activation of a component of a signaling pathway may be followed by a refractory period during which signaling is inhibited due to down-regulation of the component. For proteins of the invention, such down-regulation is usually sufficiently overcome by artificially overexpressing the protein. Expressed another way, the expression is sufficiently maintained that changes in a phenotypic characteristic that are observed during the course of an assay are due primarily to inhibition or activation of the

protein, rather than a change in its level, even if down-modulation of the protein subsequently occurs. For these reasons, although stable expression of the protein is preferred, transfection followed by transient expression of the protein may be employed provided that the selected phenotypic characteristic is measurable and the duration of the assay system is short relative to the progressive decline in the levels of the transiently expressed protein that is to be expected in such systems over time. For these reasons, stably expressing cell lines are preferred (U.S. Patent No. 4,980,281).

[0106] The term "cellular specificity" means the ability of a compound, at a given concentration, to modulate a selected phenoresponse of the Test cell without affecting the Control Cell to the same extent, if at all. The term "cellular specificity gap" ("CSG") means a measurement of the ability of a selected compound to modulate the selected phenoresponse corresponding to a given target protein (not limited to a theramutein) in a test cell relative to the ability of said compound to modulate the same phenoresponse in a corresponding control cell. For the purposes of applying the CSG technique to non-theramutein endogenous target proteins, the selected phenoresponse must have been previously defined using a known inhibitor or activator of the target protein.

[0107] Determination of the CSG provides the skilled investigator with a method of comparing the relative potential therapeutic value of different compounds within a group of compounds (two or more) by comparing their relative cross-reactivity with control cells irrespective of the potency against the target protein of any given compound within the group. Compounds that exhibit the greatest "specificity" in their activity against test cells relative to control cells are generally the most desirable compounds, since a "wide" CSG will assist in selecting a compound that may reasonably be assumed to have minimal potential side effects in patients as compared to other compounds within the aforementioned group that have "narrow" CSGs. The effects of the CSG measurement are seen most easily when comparing cell-based assay generated dose-response curves in their entirety, however the following hypothetical example is also instructive.

[0108] Consider the following table of hypothetical compounds and their corresponding IC_{50} values using a cell-free assay system. This example uses a protein kinase as the target protein. This is the sort of situation that investigators skilled in the art are faced with on a daily basis when dealing with the problem of trying to perform lead optimization on

a selected compound or group of compounds for the purpose of identifying a potential optimized lead candidate compound for subsequent pre-clinical (animal) and clinical studies.

Table 1. Cell-Free Purified Protein Kinase Inhibition Assay.			
Compound	**IC_{50} against Target Protein Kinase (nM)**	**IC_{50} against a Non-Target Protein Kinase (nM)**	**IC_{50} Ratio**
A	0.2	10,000	50,000
B	3	10,000	3,333
C	250	10,000	40
D	500	10,000	20

[0109] A standard approach in the art at the present time is to identify compounds that exhibit a high degree of *potency* with respect to inhibition of the target protein kinase's enzymatic activity in a cell-free assay system without showing significant inhibitory activity against a distinct but closely related protein kinase. As the results of the cell-free assay system shown above in Table 1 indicate, compound A is the most potent of the series of compounds (A, B, C, D) and also shows the largest difference between its IC_{50} against the target protein relative to its effect on the non-target protein. For example, if one were interested in identifying inhibitors of the Abl kinase, one might use another protein kinase, such as the EGF receptor, c-kit, or c-Src, as a "negative" control kinase in such an assay. As with c-Abl, all of these latter enzymes are tyrosine protein kinases. Indeed, it is commonplace in the field at the present time to use so-called "panels" of protein kinases, including serine/threonine kinases, tyrosine kinases, and dual-specificity kinases, in order to identify compounds that inhibit as few protein kinases as possible (other than the target protein kinase itself). The reasoning behind this approach is that the fewer the number of kinases that are inhibited in a cell-free system by a given compound, the less likely the compound is to have untoward side effects in the patient. However, there is very little clinical evidence that actually supports this view.

[0110] Furthermore, in some cases it has been argued by others that compounds that target more than one kinase may have additional therapeutic effects as compared to those compounds that are highly specific for only a single target protein. There is some evidence for this being true in the case of imatinib, whose cross-reactivity with c-kit has resulted in

beneficial effects for patients with certain histologic types of carcinoma of the small intestine, as discussed previously herein. Despite this cross-reactivity with c-kit, however, imatinib displays a high degree of cellular specificity in the assay systems of the present invention, which is consistent with its high degree of clinical efficacy and relatively modest side effect profile within the first three years of treatment. However, as the specificity of a given compound drops *in the cellular systems of the present invention*, the increased cross-reactivity with other targets such as (in this example) other protein kinase family members may result in untoward side effects in the patient. This is discussed in further detail below.

Table 2. Results from Applying the Method(s) of the Invention by Utilizing a *Phenoresponse-Based* Cellular Assay System and Measuring the *Cellular Specificity Gap (CSG)*

Compound	IC_{50} against Test Cells (nM)	IC_{50} against Control Cells (nM)	CSG
A	1	1	1
B	10	100	10
C	500	20,000	40
D	10	200	20

[0111] The conclusion that would be drawn by the investigator from the results of the cell-free assay shown in Table 1 above is that compound A is the most potent compound, showing a 50% inhibitory concentration (IC_{50}) value of 0.2 nanomolar (0.2 nM). As shown in Table 2, however, this compound, shows no specificity for the Test cells relative to the Control Cells, since it's IC_{50} for the Control cells is also 1 nanomolar. Similarly, compounds B and D are still quite potent, with both having IC_{50}'s of 10 nM against the Test cells, whereas compound D is more specific than B since its IC_{50} for the Control cells is higher (200 nM). The CSG measurements of compounds B and D immediately reflect that compound D would be the preferred compound between these two, all other considerations being equal. Most importantly, however, is that compound C is shown in this example to be the best compound of the group in terms of its CSG, at 40 (Table 2). This means that compound C shows the greatest specificity for the Test cells relative the Control cells, and would be expected to have the lowest incidence of inducing unwanted side effects in patients, since this finding is derived from a direct testing of the compound in a living cellular system, the ultimate point of pharmacological action for the overwhelming majority of medicines.

The important point in this example is that the CSG measurement allows the skilled investigator to rank order the potential therapeutic value of a series of compounds *independently* of their potency in cell-free systems. Thus, although compound C is the least potent compound, it is the most specific in its ability to inhibit the Test cells while leaving the Control cells relatively unaffected over wide concentration range. This is reflected in its CSG of 40, and demonstrates that compound C, rather than compound A, is the compound that should be given the highest priority for further pre-clinical and clinical development efforts.

[0112] Thus, one can rank order and prioritize the pharmaceutical discovery and development process. Through iterative application of the approach given above, the skilled investigator working together with medicinal chemists synthesizes analogs of compounds that score positively in assay systems such as described herein, tests such compounds in the assay methods of the invention, rank orders the compounds according to their CSG values, selects the best ones for further development, and repeats the process as many times as necessary in order to fully develop and optimize compounds that exhibit a high degree of specificity against a given Test cell relative to its corresponding Control cell. Once a given compound has been optimized using the system described herein, which generally means that the CSG between Control and Test cells (if measured using the cellular IC_{50} ratio method described above) is at least three to five fold, the skilled investigator can then proceed to complete the lead optimization process through additional chemical modifications of the compound selected in this way and tested for properties such as plasma half-life, oral bioavailability, and related parameters using appropriate animal models. Of course, the likelihood of such compounds having the desired effects on the target protein in the cellular environment is virtually assured as a result of the process of optimizing said compounds according to the methods described herein, rather than with older, cell-free methods.

[0113] It will be immediately apparent to the skilled investigator that analogous approaches may also be utilized with activators of a given target protein. It will also be apparent to the skilled investigator that there are other ways of determining the CSG. For example, using the inhibitor example given above, another useful approach to determine the CSG is to measure the ratio of the highest concentration of a compound which results in 50% growth inhibition of the control cell line, divided by the lowest concentration of the

compound at which at least 90% of the test cell line is inhibited. Other obvious modifications of this approach may be utilized as well, including computing the logarithm of the concentrations at which compounds show a given percentage of activity, normalization of either the control or test cell responses relative to one another, etc. No limitation is intended on the nature of the computed or observed comparison of the control cell responsiveness to the test cell responsiveness for the purposes of determining the CSG.

[0114] If one uses the IC_{50}-ratio of control cells/test cells method as described above, then compounds with CSG values less than or equal to 1 would not generally be considered to be good clinical candidate compounds, whereas compounds with CSG values of greater than approximately 10 would be quite promising and worthy of further consideration.

[0115] This example also highlights the distinctions between the effects of a given compound in a cell-free system versus the more medically and physiologically relevant cell-based system of the present invention.

[0116] In an embodiment of the invention, compound profiling is used to identify and/or minimize side effects associated with administration of a compound to a patient. The cell-based lead optimization method allows early identification of potential side effects as compared to the cell-free approach. For example, imatinib shows a wide cellular specificity gap according to the methods of the invention described herein. This is consistent with imatinib's significant advance in the area of anti-cancer drugs. However, it is not without side effects. Recent evidence demonstrates that imatinib is associated with cardiac toxicity in a small percentage of patients (Kerkelä et al., 2006). This group may increase over time as patients take imatinib for longer periods of time.

[0117] Imatinib tested at various concentrations on the wild type Ba/F3 cell line shows a slight but significant growth inhibitory effect at concentrations that are substantially below the apparent IC_{50} for cellular toxicity (about 10 μM) on the control cell line that imatinib exhibits at markedly higher concentrations. Such results become even more evident when the comparison of the effects of other compounds on the Control cell line are compared to those of imatinib.

[0118] Compounds which may show promising activity in a cell-free system but have small CSG values (as discussed above) would be expected to have higher potential side

effects in patients, especially over longer treatment periods. Compounds having low CSG values have been reported by others with respect to certain targets such at $p210^{Bcr\text{-}Abl\text{-}T315I}$ mutant (Carter et al., 2005), and still other groups have even entered such compounds into clinical trials. However, based upon the teachings of this invention it may be expected that such compounds will have an increased incidence of untoward side effects in patients.

[0119] A preferred drug screening method for identification and optimization of compounds of the present invention involves the following:

[0120] 1) Identification of a protein of interest (POI), such as a theramutein for which a novel inhibitor or activator is desired. Often, the POI is implicated or suspected to be implicated in establishment or maintenance of a disease state, perhaps due to inappropriate expression or a mutation-induced change in specific activity. Identification of an appropriate theramutein, for example, may be performed using standard techniques (See, Gorre et al., Science, 2001; see also PCT/US02/18729). Briefly, patients that have been given a course of a therapeutically effective treatment using an activator or inhibitor of a known or suspected prototheramutein and have subsequently shown clinical signs and symptoms consistent with disease relapse are identified, and cells or tissue samples derived from such patients are obtained. Using standard laboratory techniques such as RT-PCR, the sequence of the prototheramutein is determined and compared to the previously determined nucleic acid sequence of the known prototheramutein gene or cDNA sequence. Mutations, if present, are identified and are correlated with functional resistance of the prototheramutein's function either in cell-based or, more commonly, cell-free assay systems, again using standard methodology. Once resistance-inducing mutations are confirmed, then said one or more confirmed mutants comprise a defined theramutein which may be used in the subsequent methods as described herein. For the compounds disclosed herein, the theramutein of particular interest is $p210^{Bcr\text{-}Abl\text{-}T315I}$, although the reactivities of the compounds with other theramuteins of $p210^{Bcr\text{-}Abl}$ are also of interest.

[0121] 2) Provision of a test cell that expresses the POI and displays an observable (measurable) phenotypic characteristic that is linked to expression of the POI. In the case of a theramutein, the phenotypic characteristic is usually one which has been previously shown to be responsive to inhibitors or activators of the theramutein or, more commonly, the corresponding prototheramutein. Such a phenotypic characteristic that is linked to expression

of the POI and has been previously shown to be responsive to inhibitors or activators of the POI (or the prototheramutein-of-interest (pTOI)) is defined herein as a "phenoresponse." One embodiment of this invention is the definitive use of the phenoresponse for the purpose of identifying compounds that are likely to be inhibitors or activators of the TOI. This may be accomplished through the use of a high-throughput screen using a cell line overproducing a given TOI and for which an appropriate phenoresponse has been identified and characterized. Alternatively, one may utilize a high-throughput primary screen using a more generic phenotypic characteristic of a cell line (that does not qualify as a phenoresponse according to the teachings herein) and then utilize a secondary screen according to the teachings herein to distinguish between compounds that are true positive "hits", i.e. inhibitors or activators of the theramutein of interest, from false positive compounds that are not inhibitors or activators of the theramutein of interest. In one embodiment, a cell is selected that naturally expresses the theramutein such that a responsive phenotypic characteristic is present under suitable culture conditions which are obvious to one of ordinary skill in the art. In other embodiments, the theramutein is overexpressed, in some instances in a host cell that does not otherwise express the theramutein at all. This usually involves construction of an expression vector from which the theramutein can be introduced into a suitable host cell and overexpressed using standard vector systems and methodology. (Gorre et al., 2001; Housey et al., 1988). In one embodiment, overexpression results in a level of the theramutein that is at least about 3 times the amount of the protein usually present in a cell. Alternatively, the amount is at least about 10 times the amount usually present in a cell. In another embodiment, the amount is at least about 20 times or more preferably at least about 50 times the amount usually present in a cell.

[0122] 3) Provision of a control cell that expresses the POI to a lesser extent or not at all (*e.g.*, an unmodified host cell or host cell harboring an expression vector that does not express the POI). In the case of a theramutein of interest, the control cell can also be a cell expressing the prototheramutein corresponding to the theramutein of interest.

[0123] As some of the muteins that are described herein are also enzymes, they usually retain catalytic activity, and therefore the control cell usually displays substantially the same phenotypic characteristic as the test cell. The phenotypic characteristic need not be quantitatively alike in both cells, however. For example, a mutation that leads to reactivation

of the prototheramutein may also increase, decrease, or otherwise affect its specific activity with respect to one or more of its substrates in the cell. As a result, it may exhibit the selected phenotypic characteristic to a greater or lesser extent. Accordingly, it may be desirable in some cases to adjust expression of either or both of the prototheramutein and the theramutein such that test and control cells exhibit the phenotypic characteristic to approximately the same degree. This may be done, for example, by expressing the proteins from promoters whose activity can be adjusted by adjusting the amount of inducer present, all using standard methodology (see, for example, Sambrook et al. 1989 and 2001).

[0124] It will be obvious to one of ordinary skill in the art that a properly defined phenoresponse may be *quantitatively* different between the prototheramutein- and the theramutein-expressing cell lines as a result of differences in the specific activity (if any) between the theramutein and its corresponding prototheramutein. Theramutein-inducing mutations may increase or decrease the specific activity of said theramutein relative to the corresponding prototheramutein. When comparing a theramutein expressing cell line with a prototheramutein expressing cell line, it is preferable that the selected phenoresponse is qualitatively the same in both cell types. Thus, the skilled investigator may choose to normalize the activity of the theramutein-expressing cell line to that of the prototheramutein-expressing cell line, or vice versa. Such normalization methods are standard in the art. See, for example, Bolstad et al. (2003).

[0125] Alternatively, the skilled investigator may also wish to use unmodified host cells or host cells harboring the expression vector only as control cells for certain experimental procedures. (The host cells are the cells into which an expression vector encoding the theramutein was introduced in order to generate the test cells.) This may be the case where the investigator is only interested in identifying a specific inhibitor or activator of the theramutein of interest, irrespective of whether or not said compound is also effective against the prototheramutein of interest (pTOI).

[0126] 4) The test and control cells are then maintained or propagated (although not necessarily at the same time) in growth media (or even in intact animals) under suitable conditions such that the phenoresponse may be expressed and assayed. Control cells that are expressing the prototheramutein may be treated with a known modulator of the prototheramutein, or with a test substance, and test cells are treated with test compounds to

determine whether they are active against the theramutein, as measured by the ability of said substances to modulate the phenoresponse in the expected manner. Alternatively, control cells not expressing the prototheramutein may also be substituted, depending upon the particular phenoresponse that the skilled investigator has chosen for study. Substances may then be assayed on the test cells and, optionally, on the control cells at the same time, or at another time, and the results compared.

[0127] In one embodiment of the invention, substances that are active with regard to the test cells can be rapidly identified by their ability to modulate the phenoresponse of the test cells in the same manner as, for example, the known modulator of the prototheramutein alters the phenoresponse of prototheramutein-expressing control cells. In another embodiment, active substances may be identified by their ability to modulate the activity of the theramutein in the test cells while having little or no effect on the unmodified (prototheramutein and/or theramutein non-expressing) control cells. The skilled investigator will readily appreciate the many variations of this approach that may be utilized to identify, for example, modulators that are more effective against the theramutein, or that are equally effective against both the prototheramutein and one or more corresponding specific theramuteins.

[0128] Other phenoresponses can be observed and/or measured and include, for example, detection of substrates of the prototheramutein, and detection of gene expression changes that are regulated by the activity of the theramutein. In the simplest terms, any characteristic of the cell that the skilled investigator has previously correlated with the functional activity of the theramutein may be suitable for use with such methods. However, in selecting a given characteristic, the skilled investigator must first verify that said characteristic fulfills the criteria of being a phenoresponse according the teachings as given in detail herein. The skilled investigator may also wish to normalize the phenoresponse with the theramutein expressing cells to that of the prototheramutein expressing cells.

[0129] Characteristics suitable for detection may be measured by a variety of methods very well known to those of skill in the art. Such methods include, but are not limited to, detection of fluorescence of suitably labeled proteins (FACS), immunohistochemistry (IHC) for detection of protein expression, competitive radioligand binding assays, solid matrix blotting techniques, such as Northern, Southern, and Western blots of cell extracts, reverse

transcriptase polymerase chain reaction (RT-PCR), enzyme linked immunosorbent assays (ELISA), phosphorylation assays, gel retardation assays, membrane potential perturbations, and the like. The relevant phenotypic characteristic may be detected either on the intact cell after treatment with a test substance or, alternatively, on a subcellular fraction of the cell after treatment of the intact cell with a test substance.

[0130] Once compounds are identified that have the desired effect on the theramutein expressing test cells, it may be desirable (but not necessary) to independently verify that the compounds identified are exerting their effects on the theramutein through a direct binding mechanism, i.e. that the compounds fulfill the criteria of being inhibitors or activators (as desired) of the theramutein according to the teachings of the invention (the reader is referred to the definitions of the terms "activator" and "inhibitor" as given above). This may be accomplished with numerous standard binding assays that are known to one of ordinary skill in the art, involving either purified protein samples or intact cellular binding assays using cells transfected with the appropriate prototheramutein or theramutein together with appropriate controls as dictated by sound scientific methods. Since such methods are well established in the art they will not be reiterated here. Numerous reference texts comprehensively discuss such techniques (see, for example, Foreman and Johansen, 2002; Enna S.J. et al. (1991) Current Protocols in Pharmacology, Wiley & Sons, Incorporated; Bonifacino, J.S. et al. (1999) Current Protocols in Cell Biology, Wiley & Sons, Incorporated). See also Housey, G.M. 1988, Chapter 4, and references therein; see also Horowitz et al., 1981.

[0131] The $p210^{Bcr\text{-}Abl}$ prototheramutein and $p210^{Bcr\text{-}Abl\text{-}T315I}$ (or other) theramutein can each be expressed in Ba/F3 (murine) cells using standard methodology and the phenoresponses that are observed are growth characteristics (terminal cell density for a carefully defined cell culture, and growth in the absence of Interleukin-3 (IL-3). Unmodified host cells, or host cells containing the expression vector only or both, may optionally also be used. In still another embodiment, the test cells alone may be used with or without reference to a known inhibitor or activator.

[0132] Another useful assay is the determination of the state of phosphorylation of a direct substrate of $p210^{Bcr\text{-}Abl\text{-}T315I}$. One such substrate is Crkl (Gorre et al., *Science* 293:876-80 (2001)), an adapter protein which mediates the connection between Bcr-Abl and Ras. The

phosphorylation state of CRKL is representative of the signaling activity of $p210^{Bcr\text{-}Abl}$ in a cell. Another downstream substrate is p62DOK. Any such substrate would suffice for these purposes, provided of course that phosphorylation of said substrate has been shown to occur inside the cell, and is not simply an autophosphorylation event of the TOI or PTOI as discussed above. Other signal transduction cascade components may also be monitored, including src family kinases, STAT5, PI3 Kinase, raf kinase, RAS, MEK, ERK1 and ERK2, JNK1, 2 and 3, MLK1, 2 and 3, MKK4, MKK7, AKT, mTOR, HSP90, and others.

[0133] According to the present invention, a therapeutically effective amount of one or more compounds that modulate the functional activity of a $p210^{Bcr\text{-}Abl}$ theramutein is administered to a mammal in need thereof. The term "administering" as used herein means delivering the compounds of the present invention to a mammal by any method that may achieve the result sought. They may be administered, for example, orally, parenterally (intravenously or intramuscularly), topically, transdermally or by inhalation. The term "mammal" as used herein is intended to include, but is not limited to, humans, laboratory animals, domestic pets and farm animals. "Therapeutically effective amount" means an amount of a compound that, when administered to a mammal, is effective in producing the desired therapeutic effect, such as inhibiting kinase activity, inhibiting cancer cell growth and division, etc.

[0134] The invention provides a method of treating disease in a mammal by administering to the mammal an effective amount of a modulator of a theramutein. Suitable diseases to be treated according to the present invention include, but are not limited to, relapsing neoplastic or other proliferative disorders that have become resistant to previously administered drugs. The method is also useful for overcoming variation among individuals with respect to susceptibility to drug treatment that results from allelic differences among therapy targets. For example, the role of $p210^{Bcr\text{-}Abl}$ tyrosine kinase signaling in CML has been extensively demonstrated, as has the role of theramuteins of $p210^{Bcr\text{-}Abl}$ in drug resistant recurrence of CML. Further, different muteins of $p210^{Bcr\text{-}Abl}$ exhibit varying sensitivity to inhibitors of $p210^{Bcr\text{-}Abl}$. Although some theramuteins arise during drug therapy, others may preexist in the population. These latter examples will not be recognized as theramuteins until such time as the disease state ensues and is followed by treatment with a known class of therapeutic agents. Only after said treatment will such preexisting theramuteins reveal

themselves as being clinically significant in terms of relative non-responsiveness leading to the progression of the disease in the patient harboring the theramutein.

[0135] In an embodiment of the invention, theramutein modulators are administered in combination with one or more other anti-neoplastic agents. Any suitable anti-neoplastic agent can be used, such as a chemotherapeutic agent, radiation or combinations thereof. The anti-neoplastic agent can be an alkylating agent or an anti-metabolite. Examples of alkylating agents include, but are not limited to, cisplatin, cyclophosphamide, melphalan, and dacarbazine. Examples of anti-metabolites include, but not limited to, doxorubicin, daunorubicin, and paclitaxel, gemcitabine, and topoisomerase inhibitors irinotecan (CPT-11), aminocamptothecin, camptothecin, DX-8951f, topotecan (topoisomerase I inhibitor), and etoposide (VP-16; topoisomerase II inhibitor) and teniposide (VM-26; topoisomerase II inhibitor). When the anti-neoplastic agent is radiation, the source of the radiation can be either external (external beam radiation therapy – EBRT) or internal (brachytherapy – BT) to the patient being treated. The dose of anti-neoplastic agent administered depends on numerous factors, including, for example, the type of agent, the type and severity of the tumor being treated and the route of administration of the agent. It should be emphasized, however, that the present invention is not limited to any particular dose, route of administration, or combination of chemotherapeutic agents or other therapeutic regimens that are combined with the administration of protein modulators.

[0136] Anti-neoplastic agents which are presently known in the art or being evaluated can be grouped into a variety of classes including, for example, mitotic inhibitors, alkylating agents, anti-metabolites, intercalating antibiotics, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, anti survival agents, biological response modifiers, anti-hormones, and anti-angiogenesis agents, all of which can be administered with inhibitors or activators of theramuteins.

[0137] A modulator of a theramutein can be administered with antibodies that neutralize other receptors involved in tumor growth. Further, a modulator of a theramutein can be administered with a compound that otherwise modulates a component of a signal transduction pathway, preferably a component of the signal transduction pathway in which the theramutein is active and which is common to one or more other signal transduction pathways. In an embodiment of the invention, a theramutein modulator is used in

combination with a receptor antagonist that binds specifically to the Epidermal Growth Factor Receptor (EGFR). Particularly preferred are antigen-binding proteins that bind to the extracellular domain of EGFR and block binding of one or more of its ligands and/or neutralize ligand-induced activation of EGFR. An EGFR antagonist can be an antibody that binds to EGFR or a ligand of EGFR and inhibits binding of EGFR to its ligand. Ligands for EGFR include, for example, EGF, TGF-α, amphiregulin, heparin-binding EGF (HB-EGF) and betacellulin. EGF and TGF-α are thought to be the main endogenous ligands that result in EGFR-mediated stimulation, although TGF-α has been shown to be more potent in promoting angiogenesis. It should be appreciated that the EGFR antagonist can bind externally to the extracellular portion of EGFR, which can or can not inhibit binding of the ligand, or internally to the tyrosine kinase domain in the case of chemical agents. Examples of EGFR antagonists that bind EGFR include, without limitation, biological agents such as antibodies (and functional equivalents thereof) specific for EGFR, and chemical agents (small molecules), such as synthetic kinase inhibitors that act directly on the cytoplasmic domain of EGFR.

[0138] Other examples of growth factor receptors involved in tumorigenesis are the receptors for vascular endothelial growth factor (VEGFR-1 and VEGFR-2), platelet-derived growth factor (PDGFR), nerve growth factor (NGFR), fibroblast growth factor (FGFR), and others.

[0139] In a combination therapy, the theramutein inhibitor is administered before, during, or after commencing therapy with another agent, as well as any combination thereof, *i.e.*, before and during, before and after, during and after, or before, during and after commencing the anti-neoplastic agent therapy. For example, the theramutein inhibitor can be administered between 1 and 30 days, preferably 3 and 20 days, more preferably between 5 and 12 days before commencing radiation therapy. In a preferred embodiment of the invention, chemotherapy is administered prior to, concurrently with or, more preferably, subsequent to antibody therapy.

[0140] In the present invention, any suitable method or route can be used to administer theramutein inhibitors of the invention, and optionally, to co-administer anti-neoplastic agents and/or antagonists of other receptors. The anti-neoplastic agent regimens utilized according to the invention, include any regimen believed to be optimally suitable for

the treatment of the patient's neoplastic condition. Different malignancies can require use of specific anti-tumor antibodies and specific anti-neoplastic agents, which will be determined on a patient to patient basis. Routes of administration include, for example, oral, intravenous, intraperitoneal, subcutaneous, or intramuscular administration. The dose of antagonist administered depends on numerous factors, including, for example, the type of antagonists, the type and severity of the tumor being treated and the route of administration of the antagonists. It should be emphasized, however, that the present invention is not limited to any particular method or route of administration.

[0141] Suitable carriers include, for example, one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. Carriers can further comprise minor amounts of auxiliary substances, such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the theramutein modulator as the active ingredient. The compositions can, as is well known in the art, be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the mammal.

[0142] The compositions of this invention can be in a variety of forms. These include, for example, solid, semi-solid and liquid dosage forms, such as tablets, pills, powders, liquid solutions, dispersions or suspensions, liposomes, suppositories, injectable and infusible solutions. The preferred form depends on the intended mode of administration and therapeutic application.

[0143] Such compositions of the present invention are prepared in a manner well known in the pharmaceutical art. In making the composition the active ingredient will usually be mixed with a carrier, or diluted by a carrier and/or enclosed within a carrier which can, for example, be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, excipient or medium for the active ingredient. Thus, the composition can be in the form of tablets, lozenges, sachets, cachets, elixirs, suspensions, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, injection solutions, suspensions, sterile packaged powders and as a topical patch.

[0144] It should be appreciated that the methods and compositions of the present invention can be administered to any suitable mammal, such as a rabbit, rat, or mouse. More preferably, the mammal is a human.

[0145] The compounds according to the invention may also be present as salts. In the context of the invention, preference is given to pharmaceutically acceptable salts. Pharmaceutically acceptable salts refers to an acid addition salt or a basic addition salt of a compound of the invention in which the resulting counter ion is understood in the art to be generally acceptable for pharmaceutical uses. Pharmaceutically acceptable salts can be salts of the compounds according to the invention with inorganic or organic acids. Preference is given to salts with inorganic acids, such as, for example, hydrochloric acid, hydrobromic acid, phosphoric acid or sulfuric acid, or to salts with organic carboxylic or sulfonic acids, such as, for example, acetic acid, maleic acid, fumaric acid, malic acid, citric acid, tartaric acid, lactic acid, benzoic acid, or methanesulfonic acid, ethanesulfonic acid, phenylsulfonic acid, toluenesulfonic acid or naphthalenedisulfonic acid. Pharmaceutically acceptable salts can also be metal or ammonium salts of the compounds according to the invention. Particular preference is given to, for example, sodium, potassium, magnesium or calcium salts, and also to ammonium salts which are derived from ammonia or organic amines, such as, for example, ethylamine, di- or triethylamine, di- or triethanolamine, dicyclohexylamine, dimethylaminoethanol, arginine, lysine, ethylenediamine or 2-phenylethylamine. (see, Berge et al. *J. Pharm. Sci.* 1977, *66*, 1-19).

[0146] Throughout this application, various publications, reference texts, textbooks, technical manuals, patents, and patent applications have been referred to. The teachings and disclosures of these publications, patents, patent applications and other documents in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which the present invention pertains.

[0147] Detailed descriptions of conventional methods, such as those employed in the construction of vectors and plasmids, the insertion of genes encoding polypeptides into such vectors and plasmids, the introduction of plasmids into host cells, and the expression and determination thereof of genes and gene products can be obtained from numerous publications, including Sambrook, J et al., (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press; Coligan, J. et al. (1994) Current Protocols in

Immunology, Wiley & Sons, Incorporated; Enna, S.J. et al. (1991) Current Protocols in Pharmacology, Wiley & Sons, Bonifacino, J.S. et al. (1999) Current Protocols in Cell Biology, Wiley & Sons, and U.S. Patent 4,980,281. All references mentioned herein are incorporated by reference in their entirety.

EXAMPLES

[0148] It is to be understood and expected that variations in the principles of the invention herein disclosed may be made by one skilled in the art and it is intended that such modifications are to be included within the scope of the present invention. Examples of the invention which follow are set forth to further illustrate the invention and should not be construed to limit the invention in any way.

EXAMPLE 1: IDENTIFICATION OF A PROTEIN MODULATOR

[0149] **p210Bcr-Abl-T315I is a theramutein of the p210Bcr-Abl protein (p210Bcr-Abl) that is** resistant to inhibition by imatinib mesylate (Gleevec, STI-571). The mutation at position 315 converts a threonine to an isoleucine residue and is one of several mutations that are observed among resistant or relapsed patients. This particular mutant, however, is the most resistant such theramutein yet identified.

[0150] A phenoresponse was determined for a Ba/F3 cell line engineered to overexpress the $p210^{Bcr\text{-}Abl\text{-}T315I}$ theramutein. The phenoresponse was determined relative to non-transformed Ba/F3 cells and Ba/F3 cells that express the $p210^{Bcr\text{-}Abl\text{-}wt}$ prototheramutein. The phenoresponse was the ability of the T315I mutants to grow to a higher cell saturation density under analogous culture conditions as compared to the control non-transformed Ba/F3 cell line, and to grow in the absence of interleukin 3 (IL-3), which is required for maintenance of the control non-transformed Ba/F3 cell line. The phenoresponse was defined and characterized according to the teachings given above.

[0151] The detection system utilized was a high speed cell imaging and counting system in which 3 μl sample volumes of cells were sequentially injected through a 5 μl optical microcell, digitally imaged and electronically stored, scanned, and then counted, all under a microcomputer-based control system. The system has the capacity to perform direct cell counts on samples from cultures as small as 500 μl and provides statistically significant total cell counts from culture samples containing as few as 12,500 cells. All of the figures

displaying cell count and viability assays utilized this system for data acquisition and analysis. Simultaneously with the cell count performed, the system is also capable of determining overall cell viability by distinguishing counted, imaged cells that have excluded trypan blue (counted as "viable" cells) from cells which have taken up the trypan blue dye (counted as "non-viable" cells). Injection of trypan blue into the cell sample occurs immediately prior to the sample being sequentially injected into the microcell for simultaneous cell counting and imaging.

[0152] The system may be integrated into the workflow of high-throughput screening devices to provide a sensitive and precise cell counting and cell viability assay system that is more reliable and less prone to confounding effects of metabolic viability-based cellular assays such as XTT or Alamar blue.

[0153] Initially, approximately 113,000 compounds were screened at concentrations generally ranging from 10 to 20μM to identify a subset that was capable of affecting growth of Ba/F3 cells (Ba/F3 T315I cells) overexpressing the $p210^{Bcr\text{-}Abl\text{-}T315I}$ theramutein by any means.

[0154] A total of approximately 11,760 compounds showed greater than 50% growth inhibition, which were thought to correspond to approximately 4500 distinct chemical classes. Retesting of these compounds with the same cell line yielded a database of compound responsiveness which was then sorted and rank ordered according to those compounds exhibiting the highest overall growth inhibition. From this rank ordered database, the highest scoring 130 compounds (based upon the greatest degree of growth inhibition observed at the lowest concentrations that compounds were tested) were then rescreened in a defined cell-based assay system using Ba/F3 T315I as test cells and wild type Ba/F3 as control cells according to the methods of the present invention. Compounds of interest were those that differentially inhibited growth of Ba/F3 cells expressing the $p210^{Bcr\text{-}Abl\text{-}T315I}$ theramutein relative to non-transformed wild type Ba/F3 cells. Six compounds were identified that fulfilled the desired criteria, and some of these compounds were analyzed in further detail using the Ba/F3 $p210^{Bcr\text{-}Abl\text{-}wt}$ cells line (Ba/F3 P210 cells) as well. One compound was unavailable for further testing due to lack of availability of additional material from the chemical supplier. The remaining five compounds were independently evaluated in additional cell-based assays using the aforementioned cell lines as well as in a cell-free

purified protein kinase assay using human recombinantly produced 120 Kd kinase domain fragments isolated from both wild type P210 Bcr-Abl as well as P210 T315I mutant kinase domain.

[0155] All five compounds inhibited $p210^{Bcr\text{-}Abl\text{-}T315I}$ 120 Kd activity as measured by inhibition of autophosphorylation activity. Thus, of the 6 highest scoring compounds out of more than 113,000 compounds screened, at least 5 of the six directly inhibited the $p210^{Bcr\text{-}AblT315I}$ mutant directly. One compound appeared to spread the recombinant protein band out on the SDS page gel. This was also evident on the silver-stained gel (data not shown). It is possible that this compound may actually be a "suicide" inhibitor that is able to covalently cross-link the POI in order to permanently inhibit its activity, but this will require further study.

[0156] Taken together, the teachings and the results described herein provide conclusive proof that the system is capable of identifying inhibitors or activators of the selected theramutein, and the skilled investigator will immediately recognize that such a system may be easily applied to any other theramutein or other protein with only obvious, minor modifications.

[0157] Representative examples of the cell-based assay results demonstrating selective inhibition of growth of the Ba/F3 T315I cell line relative to the wild type non-transformed Ba/F3 cells are shown in Figures 1 and 2. The compounds inhibited growth and reduced the viability of cells expressing the T315I theramutein at concentrations under which the growth and viability of the wild type Ba/F3 non-transformed cells (not expressing either $p210^{Bcr\text{-}Abl\text{-}wt}$ or $p210^{Bcr\text{-}Abl\text{-}T315I}$) were relatively unaffected, whereas cells expressing both the prototheramutein as well as the theramutein were substantially inhibited. In some instances, the T315I expressing cells were inhibited to an even greater extent than the P210 prototheramutein expressing cells. (See, for example, Figure 3, right hand side, Compound 3 results against P210 and T315I cells.

[0158] In summary, the methods presented herein provide a fundamental advance in the form of a generalizable approach for creating or identifying modulators of any given theramutein. The results demonstrate conclusively the power of the method to identify critically needed compounds to overcome a specific type of acquired drug resistance that is uniformly fatal in certain patient populations and is presently untreatable. Furthermore, it is

evident to one of skill in this art that the techniques and methods described herein may, using obvious modifications, be straighforwardly generalized to any potential theramutein or other disease associated protein of clinical significance.

[0159] It is remarkable that out of a primary screen of more than 100,000 compounds where approximately 10,000 compounds exhibited some degree of growth inhibition, when the most potent growth inhibitory substances were rescreened using the Method described in detail herein, 6 distinct compounds were identified and all of the compounds that were subsequently tested exhibited inhibitory activity in a cell-free purified protein kinase assay using the T315I mutant (one compound was unavailable for further testing). Based upon such remarkable results, it becomes immediately clear to the skilled artisan that *the method may be effectively applied toward the identification of inhibitors or activators of any protein* based upon the proper selection and definition of the phenoresponse according to the teachings in the sections given above and the documents incorporated by reference herein. For example, with knowledge of the foregoing, one of ordinary skill in the art could easily design an assay system to identify inhibitors of theramuteins derived from other prototheramuteins known to exhibit mutations that confer drug resistance such as the c-kit gene product or the Epidermal Growth Factor (EGF) Receptor (EGFR), or the Platelet Derived Growth Factor (PDGF) Receptor α and β. No limitation should be inferred upon the utility of the method with respect to its ability to be utilized with any given protein, including theramuteins and protothermuteins, expressed in any mammalian cell type for which a corresponding phenoresponse is detectable.

EXAMPLE 2: PHENORESPONSE-BASED OPTIMIZATION OF A PROTEIN MODULATOR

[0160] Compound C2 which was originally identified as an inhibitor of the T315I theramutein was subjected to a novel lead optimization program as follows. Various chemical modifications were introduced into the basic scaffold structure of compound C2 using standard medicinal chemistry synthetic methods. Once synthesized, the various analogues (chemical variants) were tested using the phenoresponse-based cellular assay system described above in Example 1.

EXAMPLE 3: PHENORESPONSE-BASED PROFILING OF A PROTEIN MODULATOR

[0161] The phenoresponse-based assay system of the invention can be used to profile the biological activity of a given compound with respect to its ability to inhibit or activate multiple distinct protein targets to differing extents. For example, in certain instances the skilled artisan may be interested in identifying or optimizing modulators of a given target protein where additional proteins are known that are distinct but highly related to the target POI. Such protein families may consist of two or more members that share a high degree of homology at both the DNA and amino acid sequence levels, yet the family members may have distinct functions within the cell. Through iterative application of the phenoresponse-based system described herein, one could create individual Test Cells expressing each of the distinct family members and then utilize three or four or more distinct Test Cell lines with corresponding defined phenoresponses to identify or optimize compounds that are selective for one particular family member.

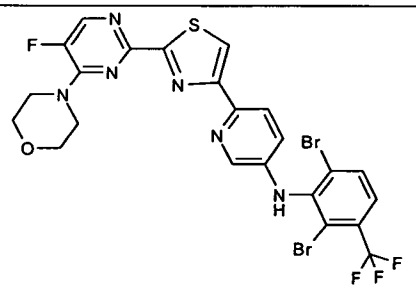
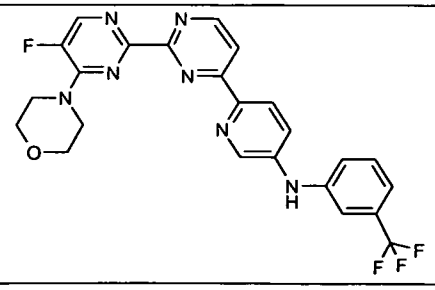
[0162] In yet another embodiment of the present invention, the skilled artisan may also choose to express two or three or even four distinct protein targets in a single Test Cell (or Test Cell line) and create a phenoresponse-based assay system useful for identifying compounds that are NOT selective among individual isozymes of a given protein family. In certain therapeutic situations, lack of selectivity among individual family members may be preferable. Ibuprofen, for example, is an established, low-cost safe and effective non-steroidal anti-inflammatory drug that does not significantly discriminate between the cyclooxygenase type 1 (COX-1) and COX-2 family members. Such lack of discrimination may in some instances be beneficial and may reduce the likelihood of certain unwanted side effects that may occur with an overly selective chemical agent.

[0163] Profiling the biological effects of a given compound with respect to its ability to inhibit or activate certain related protein targets, whether or not such targets are members of the same protein family, also has substantial value from the perspective of understanding the molecular and cellular mechanism(s) of action of a given chemical agent. For example, in the case of imatinib, not only does the compound inhibit the wild type version of the P210 Bcr/Abl protein ($p210^{Bcr\text{-}Abl\text{-}wt}$), it also cross reacts with and is capable of inhibiting the c-kit oncoprotein as well. As discussed above in the background of the invention, this cross-reactive inhibition of the kit oncoprotein is serendipitous, because gastrointestinal stromal

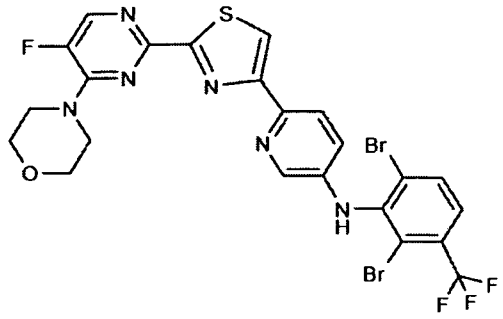
tumors (GIST), a type of tumor arising in the small intestine, are driven by kit activity and are thus responsive to imatinib treatment as well (Druker et al, NEJM, 2001, 344, 1031-1037). Thus, such cross reactivity with other related proteins need not always be associated with toxicity of a drug. In some instances such cross-reactivities can be therapeutically effective.

[0164] Representative Compounds of the Invention corresponding to the various chemical formulae given above were tested in the cellular assay system described elsewhere herein (see Example 1) and assigned activity categories as shown in Table 3. The assigned activity categories are represented by the following designations, wherein the IC_{50} for a given cell line is the concentration at which a given compound inhibits the growth of that cell line by 50% in the cellular assay system. Compounds tested on a given cell line that exhibited an IC_{50} value that was < 300 nM (less than 300 nanomolar) were designated as Category "A" compounds. Compounds tested on a given cell line that exhibited an IC_{50} value that was <1μM (less than 1micromolar) were designated as Category "B" compounds. Compounds tested on a given cell line that exhibited an IC_{50} value that was < 10μM (less than 10 micromolar) were designated as Category "C" compounds. Compounds tested on a given cell line that exhibited an IC_{50} value that was ≥ 10μM (greater than or equal to 10 micromolar) were designated as Category "D" compounds.

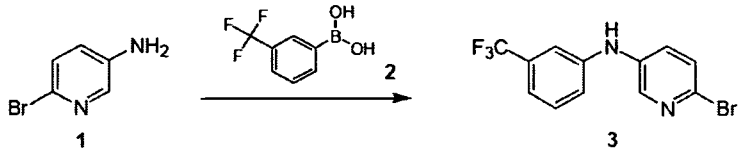
Table 3.

Structure:	wt BaF3	T315I
	C	C
	B	B

EXAMPLE 4:

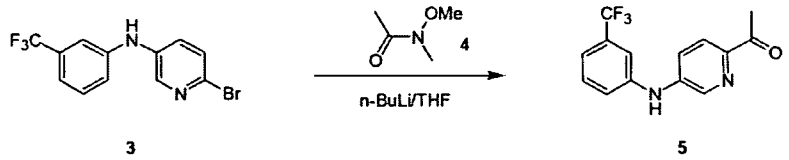


[0165] **1. Reaction Scheme:**



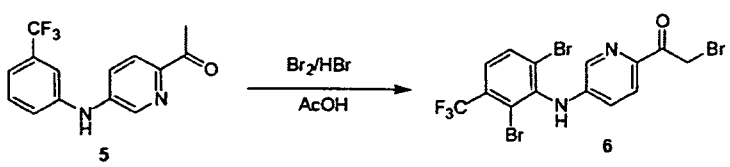
[0166] **Experimental Details:** To a solution of **1** (2.0 g, 14 mmol) in dichloromethane (20 mL) were sequentially added **2** (6.65 g, 35 mmol), Et_3N (3.5 g, 35 mmol), and copper (II) acetate (8.17 g, 45 mmol). The mixture was stirred for 18 hours and then filtered. The cake was washed with MeOH and evaporated to dryness. The crude product was purified by column chromatography on silica gel to give compound **3** as a solid.

[0167] **2. Reaction Scheme:**



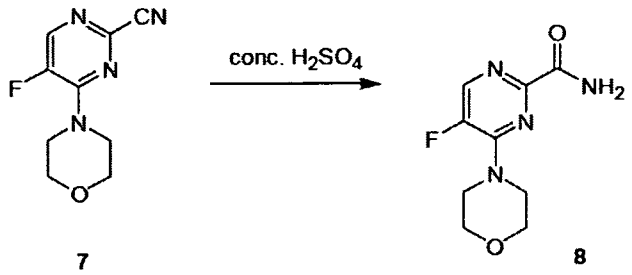
[0168] **Experimental Details:** A solution of n-BuLi (2.5 M, 11 mL) was added dropwise to a solution of **3** (4 g, 12.6 mmol) in THF (150 mL) at -78 °C. The resulting mixture was stirred for 90 minutes before the dropwise addition of **4** (2.6 g, 25.2 mmol). After stirring for additional 6 hours, the mixture was allowed to warm to room temperature, diluted with aqueous $NaHCO_3$ (30 mL) and extracted with Et_2O (100 mL). The organic layers were combined, dried over Na_2SO_4. After filtrating off the solid, the filtrate was concentrated to dryness. The residue was purified by column to give the product **5**.

[0169] **3. Reaction Scheme:**



[0170] **Experimental Details:** To a solution of **5** (2.8 g, 0.01 mol) in acetic acid (15 ml) were sequentially added dropwise hydrobromic acid (10 ml), and bromine (1 g, 15 mmol) while the temperature was maintained at 15°C. The resulting mixture was stirred for 30 minutes at 15°C before it was diluted with saturated aqueous $NaHCO_3$ (30 mL), extracted with Et_2O (20 mL × 3). The extracts were combined, dried over Na_2SO_4. After filtrating off the solid, the filtrate was concentrated. The residue was purified by column chromatography to give product **6**.

[0171] **4. Reaction Scheme:**



7 **8**

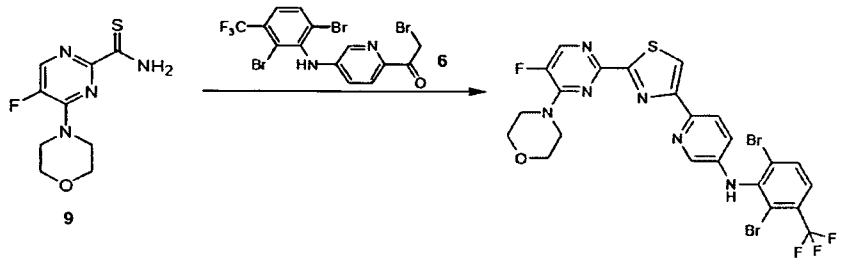
[0172] **Experimental Details:** To concentrated H_2SO_4 (10 mL) was added **7** (0.9 g,4.32 mmol) at 0°C with stirring. The reaction mixture was stirred at room temperature for 16 hours before it was poured into ice-water and filtered. The solids were dried under reduced pressure to give product **8**.

[0173] **5. Reaction Scheme:**

8 **9**

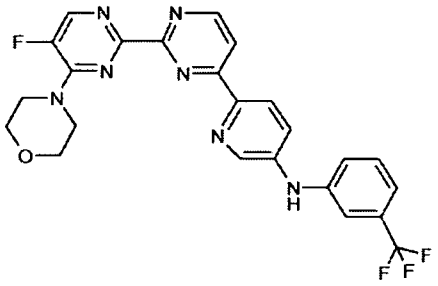
[0174] **Experimental Details:** To a solution of **8** (1.5 g, 6.64 mmol) in THF (25 mL) was added Lawesson's reagent (1.35 g, 3.32 mmol). The mixture was heated at reflux for 4 hours before it was cooled to room temperature and concentrated. The residue was purified by column chromatography to give the product **9**.

[0175] **6. Reaction Scheme:**

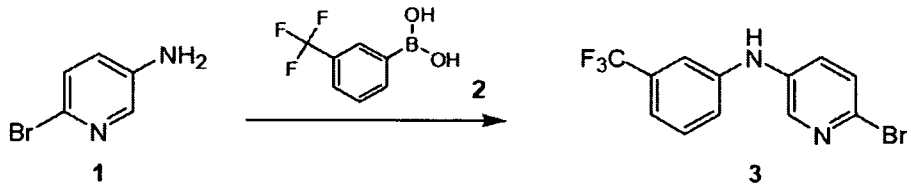


[0176] **Experimental Details:** To a solution of **9** (242 mg, 1 mmol) in EtOH (15 ml) was added **6** (570 mg, 1.2 mmol) and the mixture was heated at reflux for 6 h. The reaction was concentrated and purified by preparative HPLC to give the desired compound.

EXAMPLE 5.

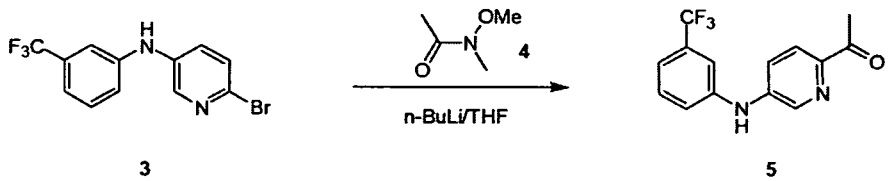


[0177] **1. Reaction Scheme:**



[0178] **Experimental Details:** To a solution of **1** (2.0 g, 14 mmol) in dichloromethane (20 mL) were sequentially added **2** (6.65 g, 35 mmol), Et_3N (3.5 g, 35 mmol), and copper (II) acetate (8.17 g, 45 mmol). The reaction mixture was stirred for 18 hours under N_2 atmosphere. The precipitate was filtered, washed with MeOH. The filtrate was evaporated to dryness. The residue was purified by column chromatography on silica gel to give compound **3** as a solid.

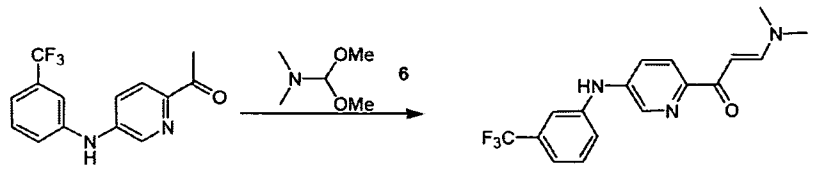
[0179] **2. Reaction Scheme:**



[0180] **Experimental Details:** A solution of n-BuLi (2.5 M, 11 mL) was added dropwise to a solution of **3** (4 g, 12.6 mmol) in THF (150 mL) at -78 °C. The resulting mixture was stirred for 90 minutes before the dropwise addition of **4** (2.6 g, 25.2 mmol).

After stirring for additional 6 hours, the mixture was allowed to warm to room temperature, diluted with aqueous $NaHCO_3$ (30 mL), and extracted with Et_2O (100 mL). The organic layers were combined, dried over Na_2SO_4. After filtrating off the solid, the filtrate was concentrated to dryness. The residue was purified by column to give the product **5**.

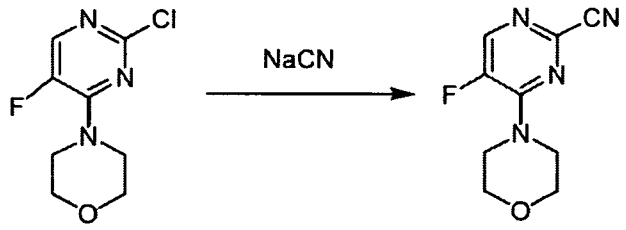
[0181] **3. Reaction Scheme:**



5 7

[0182] **Experimental Details:** A solution of **5** (0.5 g, 1.78 mmol) in dimethoxymethyl-dimethyl amine (60 mL) was heated at reflux for 2 h. The solvent was removed under reduced pressure to give compound **7**, which was used in the next step directly.

[0183] **4. Reaction Scheme:**



8 9

[0184] **Experimental Details:** A mixture of **8** (17.2 g, 79.3 mmol), DABCO (9.43 g, 79.5 mmol), DMSO (100 mL), and water (10 mL) was stirred 80°C for 18 h before it was partitioned between water, and ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to give the crude product. It was purified by column chromatography on silica gel to give compound **9**.

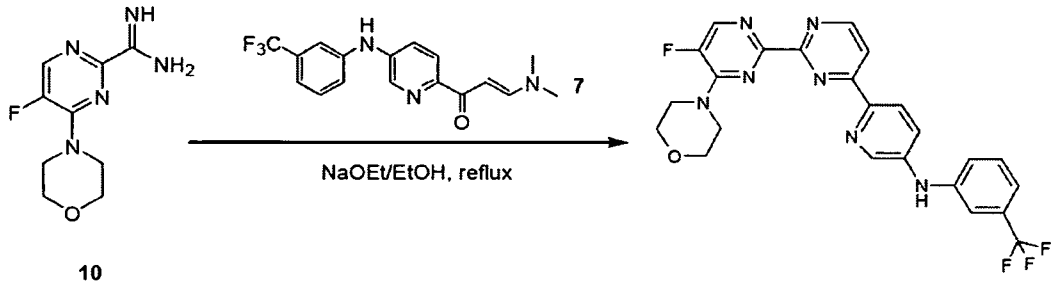
[0185] **5. Reaction Scheme:**

9 10

[0186] **Experimental Details:** Ammonium chloride (179.4 mg, 3.37 mmol) was suspended in dry toluene (10 mL) under N_2 atmosphere and the mixture was cooled to 0°C. A

solution of trimethlaluminium in hexanes (1.12 mL, 3 M) was added dropwise and the resulting mixture was stirred at room temperature until gas evolution ceased. Following the addition of **9** (350 mg, 1.68 mmol), the mixture was stirred at 80°C overnight, cooled down to 0°C. Methanol (2 mL) was then added and the mixture was stirred for 1 h at room temperature, then filtered, the solids washed with methanol several times. The filtrate was evaporated to dryness in *vacuo* and the residue was washed with dichloromethane to give **10** as a solid.

[0187] **6. Reaction Scheme:**



[0188] **Experimental Details:** A solution of **10** (100 mg, 0.44 mmol) in absolute EtOH (15 mL) was added to a stirred solution of **7** (128 mg, 0.38 mmol) in boiling absolute EtOH (10 mL). The mixture was stirred for 20 min before the addition of Na (20 mg, 0.76 mmol) in absolute EtOH (10 mL). The reaction mixture was heated at reflux for 2 h, allowed to cool to room temperature. The precipitate was removed by filtration followed by concentration of the filtrate to give the crude product, which was purified by preparative HPLC to give the desired compound as a yellow solid.

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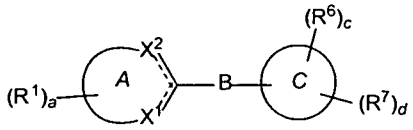
WE CLAIM:

1. A compound having the formula I:



(I)

wherein:

ring *A* is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

X^1 is selected from N, N-R^0 or C-R^1;

X^2 is selected from N, N-R^0 or C-R^1;

each R^1 is independently selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^{11}, $-(CH_2)_pC(O)(CH_2)_qR^{11}$, $-(CH_2)_pC(O)N(R^{12})(R^{13})$, $-(CH_2)_pC(O)O(CH_2)_qR^{11}$, $-(CH_2)_pN(R^{11})(CH_2)_qC(O)R^{11}$, $-(CH_2)_pN(R^{12})(R^{13})$, $-(CH_2)_pN(R^{11})(CH_2)_qR^{11}$, $-N(R^{11})SO_2R^{11}$, $-OC(O)N(R^{12})(R^{13})$, $-SO_2N(R^{12})(R^{13})$, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

a is 0 to 4;

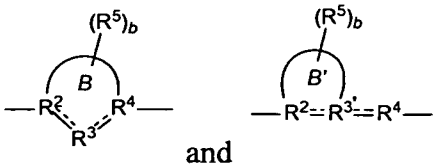
each R^{11} is independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

each R^{12} and R^{13} are independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring; or R^{12} and R^{13} may be taken together with the nitrogen to which they are attached form a 5- to 7- membered ring which may optionally contain a further heteroatom; wherein the 5- to 7-membered ring may optionally be substituted with one to three substituents that are independently selected from alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, CO_2R^0, $C(O)R^0$, halo, aryl, and a heterocyclic ring;

p is 0 to 4;

q is 0 to 4;

B is selected from a group having the formula:



and ;

Ring *B* and Ring *B'* are a 5-, or 6- membered ring;

R^2 is selected from N, C and CH;

R^3 is selected from N, NR^{31}, O, S, CR^{31} and CHR^{31};

R^{31} is selected from H, and alkyl;

$R^{3'}$ is selected from N, C and CH;

R^4 is selected from N, C or CH;

each R^5 is independently selected from OH, O-alkyl, =O, SH, S-alkyl, =S, halo, alkyl, CF_3, NH_2, NHalkyl, and $N(alkyl)_2$;

b is 0, 1, or 2;

dashed lines represent optional double bonds;

Ring *C* is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

each R^6 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, NH_2, halo, aryl, and a heterocyclic ring;

additionally or alternatively an R^6 and an R^5 may be taken together to form a 5-, to 7-membered ring that is fused to Ring B or Ring B' and to Ring C;

R^7 is -Q–R^9;

Q is selected from a chemical bond or a group having the formula -O-, $-(CH_2)_i-$, $-(CH_2)_iC(O)(CH_2)_j-$, $-(CH_2)_i-N(R^{71})-(CH_2)_j-$, $-(CH_2)_iC(O)-N(R^{71})-(CH_2)_j-$, $-(CH_2)_iC(O)O(CH_2)_j-$, $-(CH_2)_iN(R^{71})C(O)-(CH_2)_j-$, $-(CH_2)_iOC(O)N(R^{71})-(CH_2)_j-$, and $-O-(CH_2)_i-C(O)N(R^{71})-(CH_2)_j-$;

R^{71} is selected from H, alkyl, aryl, and a heterocyclic ring;

R^9 is selected from an aryl and a heterocyclic ring;

i is 0 to 4;

j is 0 to 4;

each R^0 is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring;

c is 0 to 3; and

d is 0 or 1.

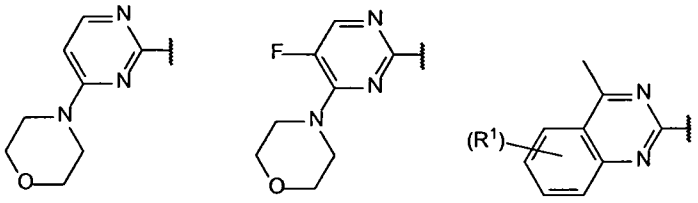
2. The compound of claim 1, wherein Ring *A* is an aromatic ring.

3. The compound of claim 2, wherein one of X^1 or X^2 is N.

4. The compound of claim 3, wherein X^1 and X^2 are N.

5. The compound of claim 1, wherien Ring *A* is a pyridine ring or a pyrimidine ring.

6. The compound of claim 1, wherien Ring *A* is selected from the structures provided below:



7. The compound of claim 1, wherein Ring *C* is a 6-membered aryl group.

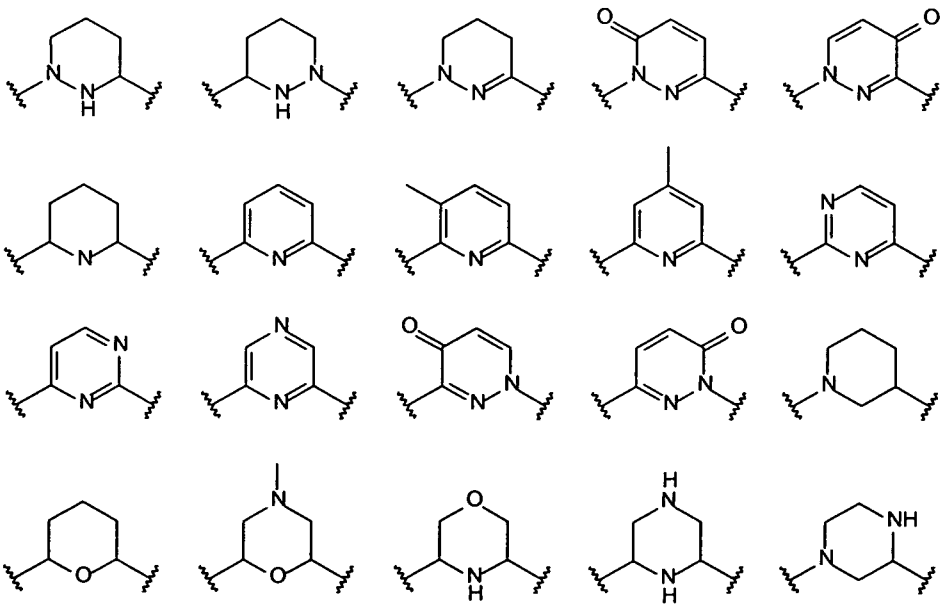
8. The compound of claim 7, wherein Ring *C* is selected from a phenyl, pyridyl, and pyrimidyl group.

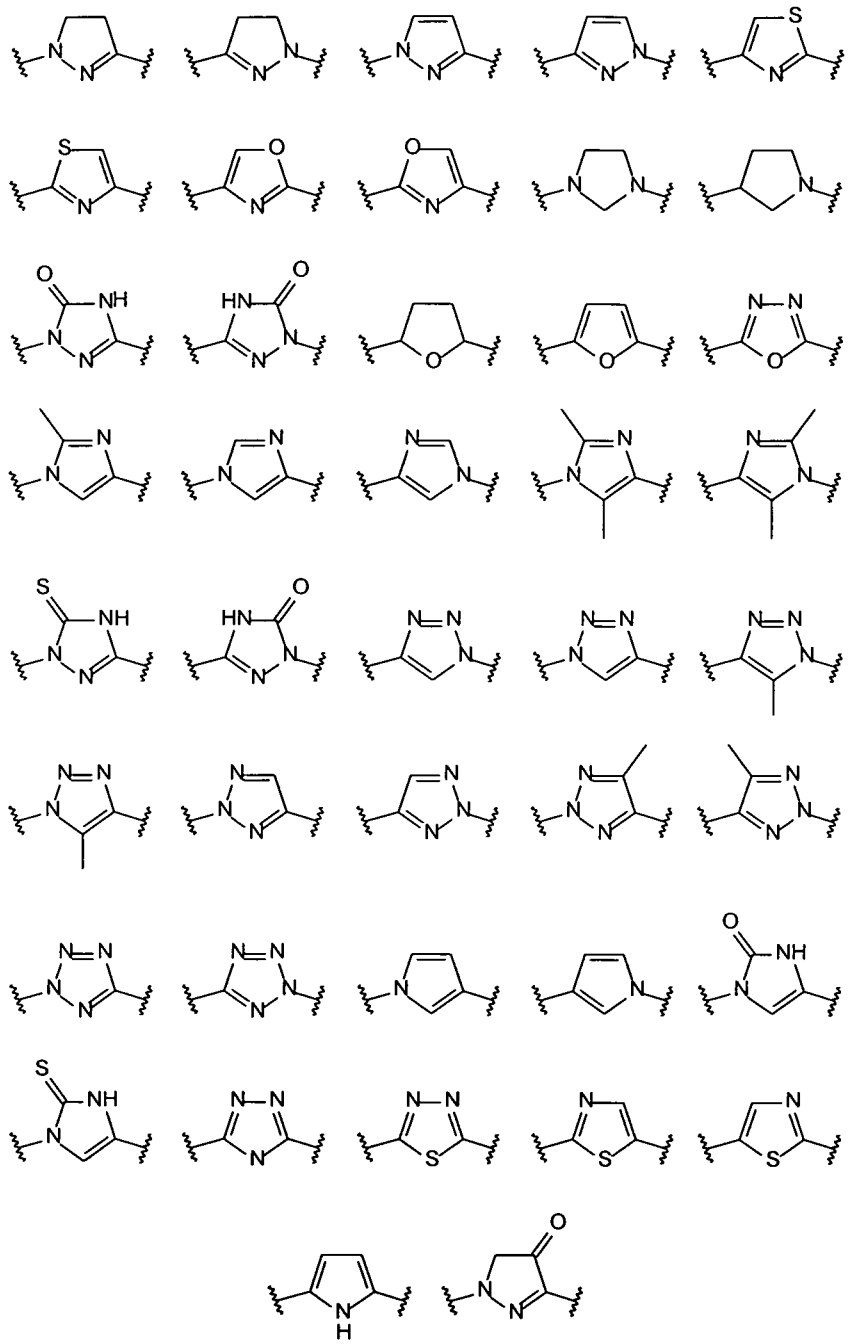
9. The compound of claim 7, wherein Ring *C* is Pyridyl.

10. The compound of claim 1, wherein Ring *C* is a 10-membered bicyclic aryl group.

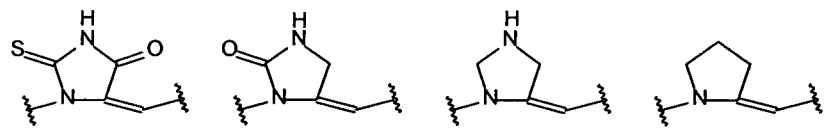
11. The compound of claim 10, wherein Ring *C* is selected from naphthyl, quinoline, isoquinoline, quinazoline, and quinoxaline.

12. The compound of claim 1, wherein Ring *B* is selected from the following chemical groups:

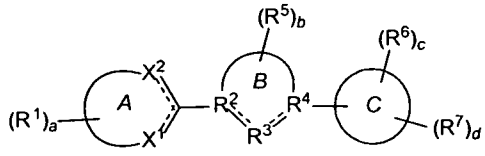




13. The compound of claim 1, wherein Ring *B'* is selected from the following chemical groups:



14. The compound of claim 1, having the formula II



(II)

wherein

Ring *A* is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

X^1 is selected from N, N-R^0 or C-R^1;

X^2 is selected from N, N-R^0 or C-R^1;

each R^1 is independently selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^{11}, $-(CH_2)_pC(O)(CH_2)_qR^{11}$, $-(CH_2)_pC(O)N(R^{12})(R^{13})$, $-(CH_2)_pC(O)O(CH_2)_qR^{11}$, $-(CH_2)_pN(R^{11})(CH_2)_qC(O)R^{11}$, $-(CH_2)_pN(R^{12})(R^{13})$, $-(CH_2)_pN(R^{11})(CH_2)_qR^{11}$, $-N(R^{11})SO_2R^{11}$, $-OC(O)N(R^{12})(R^{13})$, $-SO_2N(R^{12})(R^{13})$, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

a is 0 to 4;

each R^{11} is independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

each R^{12} and R^{13} are independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring; or R^{12} and R^{13} may be taken together with the nitrogen to which they are attached form a 5- to 7- membered ring which may optionally contain a further heteroatom; wherein the 5- to 7-membered ring may optionally be substituted with one to three substituents that are independently selected from alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, CO_2R^0, $C(O)R^0$, halo, aryl, and a heterocyclic ring;

p is 0 to 4;

q is 0 to 4;

Ring *B* is a 5-, or 6- membered ring;

R^2 is selected from N, C and CH;

R^3 is selected from N, NR^{31}, O, S, CR^{31} and CHR^{31};

R^{31} is selected from H, and alkyl;

R^4 is selected from N, C or CH;

each R^5 is independently selected from OH, O-alkyl, =O, SH, S-alkyl, =S, halo, alkyl, CF_3, NH_2, NHalkyl, and $N(alkyl)_2$;

b is 0, 1, or 2;

dashed lines represent optional double bonds;

Ring *C* is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

each R^6 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, NH_2, halo, aryl, and a heterocyclic ring;

additionally or alternatively an R^6 and an R^5 may be taken together to form a 5-, to 7-membered ring that is fused to Ring B and to Ring C;

R^7 is -Q–R^9;

Q is selected from a chemical bond or a group having the formula -O-, $-(CH_2)_i-$, $-(CH_2)_iC(O)(CH_2)_j-$, $-(CH_2)_i-N(R^{71})-(CH_2)_j-$, $-(CH_2)_iC(O)-N(R^{71})-(CH_2)_j-$, $-(CH_2)_iC(O)O(CH_2)_j-$, $-(CH_2)_iN(R^{71})C(O)-(CH_2)_j-$, $-(CH_2)_iOC(O)N(R^{71})-(CH_2)_j-$, and $-O-(CH_2)_i-C(O)N(R^{71})-(CH_2)_j-$;

R^{71} is selected from H, alkyl, aryl, and a heterocyclic ring;

R^9 is selected from an aryl and a heterocyclic ring;

i is 0 to 4;

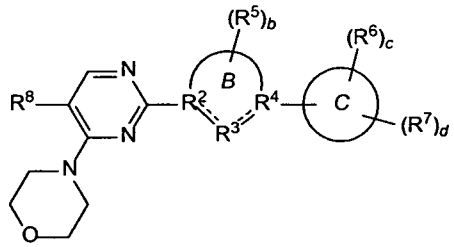
j is 0 to 4;

each R^0 is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring;

c is 0 to 3; and

d is 0 or 1.

15. The compound of claim 1 having the formula III:



(III)

wherein:

Ring *B* is a 5-, or 6- membered ring;

R^2 is selected from N, C and CH;

R^3 is selected from N, NR^{31}, O, S, CR^{31} and CHR^{31};

R^{31} is selected from H, and alkyl;

R^4 is selected from N, C or CH;

each R^5 is independently selected from OH, O-alkyl, =O, SH, S-alkyl, =S, halo, alkyl, CF_3, NH_2, NHalkyl, and $N(alkyl)_2$;

b is 0, 1, or 2;

dashed lines represent optional double bonds;

Ring *C* is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

each R^6 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, NH_2, halo, aryl, and a heterocyclic ring;

additionally or alternatively an R^6 and an R^5 may be taken together to form a 5-, to 7-membered ring that is fused to Ring B and to Ring C;

R^7 is -Q–R^9;

Q is selected from a chemical bond or a group having the formula -O-, $-(CH_2)_i-$, $-(CH_2)_iC(O)(CH_2)_j-$, $-(CH_2)_i-N(R^{71})-(CH_2)_j-$, $-(CH_2)_iC(O)-N(R^{71})-(CH_2)_j-$, $-(CH_2)_iC(O)O(CH_2)_j-$, $-(CH_2)_iN(R^{71})C(O)-(CH_2)_j-$, $-(CH_2)_iOC(O)N(R^{71})-(CH_2)_j-$, and $-O-(CH_2)_i-C(O)N(R^{71})-(CH_2)_j-$;

R^{71} is selected from H, alkyl, aryl, and a heterocyclic ring;

R^9 is selected from an aryl and a heterocyclic ring;

i is 0 to 4;

j is 0 to 4;

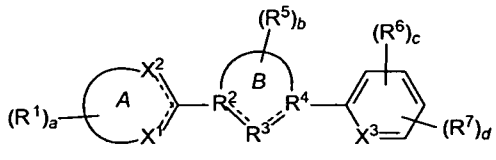
R^8 is selected from H and F;

each R^0 is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring;

c is 0 to 3; and

d is 0 or 1.

16. The compound of claim 1 having the formula IV:



(IV)

wherein

Ring *A* is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

X^1 is selected from N, N-R^0 or C-R^1;

X^2 is selected from N, N-R^0 or C-R^1;

each R^1 is independently selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^{11}, $-(CH_2)_pC(O)(CH_2)_qR^{11}$, $-(CH_2)_pC(O)N(R^{12})(R^{13})$, $-(CH_2)_pC(O)O(CH_2)_qR^{11}$, $-(CH_2)_pN(R^{11})(CH_2)_qC(O)R^{11}$, $-(CH_2)_pN(R^{12})(R^{13})$, $-(CH_2)_pN(R^{11})(CH_2)_qR^{11}$, $-N(R^{11})SO_2R^{11}$, $-OC(O)N(R^{12})(R^{13})$, $-SO_2N(R^{12})(R^{13})$, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

a is 0 to 4;

each R^{11} is independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

each R^{12} and R^{13} are independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring; or R^{12} and R^{13} may be taken together with the nitrogen to which they are attached form a 5- to 7- membered ring which may optionally contain a further heteroatom; wherein the 5- to 7-membered ring may optionally be substituted with one to three substituents that are independently selected from alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, CO_2R^0, $C(O)R^0$, halo, aryl, and a heterocyclic ring;

p is 0 to 4;

q is 0 to 4;

Ring *B* is a 5-, or 6- membered ring;

R^2 is selected from N, C and CH;

R^3 is selected from N, NR^{31}, O, S, CR^{31} and CHR^{31};

R^{31} is selected from H, and alkyl;

R^4 is selected from N, C or CH;

each R^5 is independently selected from OH, O-alkyl, =O, SH, S-alkyl, =S, halo, alkyl, CF_3, NH_2, NHalkyl, and $N(alkyl)_2$;

b is 0, 1, or 2;

dashed lines represent optional double bonds;

X^3 is selected from N, CH, and $C\text{-}R^6$;

each R^6 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, NH_2, halo, aryl, and a heterocyclic ring;

additionally or alternatively an R^6 and an R^5 may be taken together to form a 5-, to 7-membered ring that is fused to Ring B and to Ring C;

R^7 is $-Q\text{–}R^9$;

Q is selected from a chemical bond or a group having the formula -O-, -$(CH_2)_i$-, -$(CH_2)_iC(O)(CH_2)_j$-, -$(CH_2)_i$-$N(R^{71})$-$(CH_2)_j$-, -$(CH_2)_iC(O)$-$N(R^{71})$-$(CH_2)_j$-, -$(CH_2)_iC(O)O(CH_2)_j$-, -$(CH_2)_iN(R^{71})C(O)$-$(CH_2)_j$-, -$(CH_2)_iOC(O)N(R^{71})$-$(CH_2)_j$-, and -O-$(CH_2)_i$-$C(O)N(R^{71})$-$(CH_2)_j$-;

R^{71} is selected from H, alkyl, aryl, and a heterocyclic ring;

R^9 is selected from an aryl and a heterocyclic ring;

i is 0 to 4;

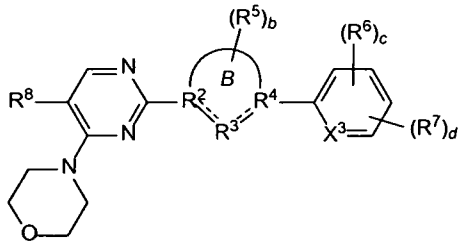
j is 0 to 4;

each R^0 is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring;

c is 0 to 3; and

d is 0 or 1.

17. The compound of claim 1 having the formula V:



(V)

wherein:

Ring B is a 5-, or 6- membered ring;

R^2 is selected from N, C and CH;

R^3 is selected from N, NR^{31}, O, S, CR^{31} and CHR^{31};

R^{31} is selected from H, and alkyl;

R^4 is selected from N, C or CH;

each R^5 is independently selected from OH, O-alkyl, =O, SH, S-alkyl, =S, halo, alkyl, CF_3, NH_2, NHalkyl, and $N(alkyl)_2$;

b is 0, 1, or 2;

dashed lines represent optional double bonds;

X^3 is selected from N, CH, and C-R^6;

each R^6 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, NH_2, halo, aryl, and a heterocyclic ring;

additionally or alternatively an R^6 and an R^5 may be taken together to form a 5-, to 7-membered ring that is fused to Ring B and to Ring C;

R^7 is -Q–R^9;

Q is selected from a chemical bond or a group having the formula -O-, -$(CH_2)_i$-, -$(CH_2)_i$C(O)$(CH_2)_j$-, -$(CH_2)_i$-N(R^{71})-$(CH_2)_j$-, -$(CH_2)_i$C(O)-N(R^{71})-$(CH_2)_j$-, -$(CH_2)_i$C(O)O$(CH_2)_j$-, -$(CH_2)_i$N(R^{71})C(O)-$(CH_2)_j$-, -$(CH_2)_i$OC(O)N(R^{71})-$(CH_2)_j$-, and -O-$(CH_2)_i$-C(O)N(R^{71})-$(CH_2)_j$-;

R^{71} is selected from H, alkyl, aryl, and a heterocyclic ring;

R^9 is selected from an aryl and a heterocyclic ring;

i is 0 to 4;

j is 0 to 4;

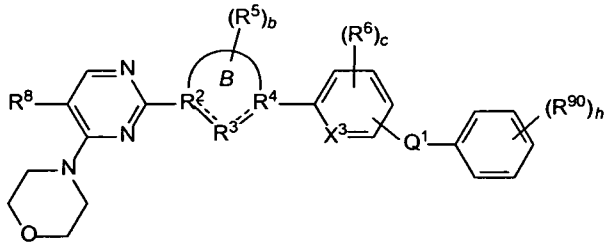
R^8 is selected from H and F;

each R^0 is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring;

c is 0 to 3; and

d is 0 or 1.

18. The compound of claim 1 having the formula VI:



(VI)

wherein:

Ring B is a 5-, or 6- membered ring;

R^2 is selected from N, C and CH;

R^3 is selected from N, NR^{31}, O, S, CR^{31} and CHR^{31};

R^{31} is selected from H, and alkyl;

R^4 is selected from N, C or CH;

each R^5 is independently selected from OH, O-alkyl, =O, SH, S-alkyl, =S, halo, alkyl, CF_3, NH_2, NHalkyl, and N(alkyl)$_2$;

b is 0, 1, or 2;

dashed lines represent optional double bonds;

each R^6 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, NH_2, halo, aryl, and a heterocyclic ring;

additionally or alternatively an R^6 and an R^5 may be taken together to form a 5-, to 7-membered ring that is fused to Ring B and to Ring C;

Q^1 is selected from a chemical bond or a group having the formula -O-, $-CH_2-$, -NH-, -C(O)-NH-, -C(O)O-, -NH-C(O)-, -OC(O)NH-, and -O-C(O)NH-;

each R^{90} is selected from halo, alkyl, CN, $N(R^{92})_2$, cyclic-amino, NO_2, OR^{92}, and CF_3;

h is 0 to 5;

each R^{92} is selected from H, alkyl, aryl, aralkyl and a heterocyclic ring;

c is 0 to 3; and

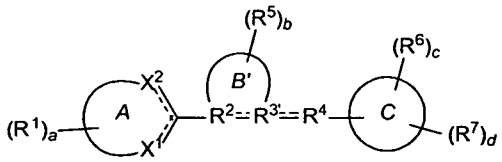
R^8 is selected from H and F.

19. The compound of claim 18, wherein R^{90} is selected from $N(R^{92})_2$, CF_3, and OH.

20. The compound of claims 18 and 19, wherein,Q^1 is -NH-.

21. The compound of claims 18-20, wherein X^3 is N.

22. The compound of claim 1 having the formula VII:



(VII)

wherein:

ring *A* is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

X^1 is selected from N, N-R^0 or C-R^1;

X^2 is selected from N, N-R^0 or C-R^1;

the dotted lines represent optional double bonds;

each R^1 is independently selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^{11}, $-(CH_2)_pC(O)(CH_2)_qR^{11}$, $-(CH_2)_pC(O)N(R^{12})(R^{13})$, $-(CH_2)_pC(O)O(CH_2)_qR^{11}$,$-(CH_2)_pN(R^{11})(CH_2)_qC(O)R^{11}$, $-(CH_2)_pN(R^{12})(R^{13})$, $-(CH_2)_pN(R^{11})(CH_2)_qR^{11}$, $-N(R^{11})SO_2R^{11}$, $-OC(O)N(R^{12})(R^{13})$, $-SO_2N(R^{12})(R^{13})$, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

a is 0 to 4;

each R^{11} is independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

each R^{12} and R^{13} are independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring; or R^{12} and R^{13} may be taken together with the nitrogen to which they are attached form a 5- to 7- membered ring which may optionally contain a further heteroatom; wherein the 5- to 7-membered ring may optionally be substituted with one to three substituents that are independently selected from alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, CO_2R^0, $C(O)R^0$, halo, aryl, and a heterocyclic ring;

p is 0 to 4;

q is 0 to 4;

Ring B' is a 5-, or 6- membered ring:

R^2 is selected from N, C and CH;

$R^{3'}$ is selected from N, C and CH;

R^4 is selected from N, C or CH;

each R^5 is independently selected from OH, O-alkyl, =O, SH, S-alkyl, =S, halo, alkyl, CF_3, NH_2, NHalkyl, and $N(alkyl)_2$;

b is 0, 1, or 2;

dashed lines represent optional double bonds;

Ring *C* is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

each R^6 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, NH_2, halo, aryl, and a heterocyclic ring;

additionally or alternatively an R^6 and an R^5 may be taken together to form a 5-, to 7-membered ring that is fused to Ring B or Ring B' and to Ring C;

R^7 is -Q–R^9;

Q is selected from a chemical bond or a group having the formula -O-, $-(CH_2)_i-$, $-(CH_2)_iC(O)(CH_2)_j-$, $-(CH_2)_i-N(R^{71})-(CH_2)_j-$, $-(CH_2)_iC(O)-N(R^{71})-(CH_2)_j-$, $-(CH_2)_iC(O)O(CH_2)_j-$, $-(CH_2)_iN(R^{71})C(O)-(CH_2)_j-$, $-(CH_2)_iOC(O)N(R^{71})-(CH_2)_j-$, and $-O-(CH_2)_i-C(O)N(R^{71})-(CH_2)_j-$;

R^{71} is selected from H, alkyl, aryl, and a heterocyclic ring;

R^9 is selected from an aryl and a heterocyclic ring;

i is 0 to 4;

j is 0 to 4;

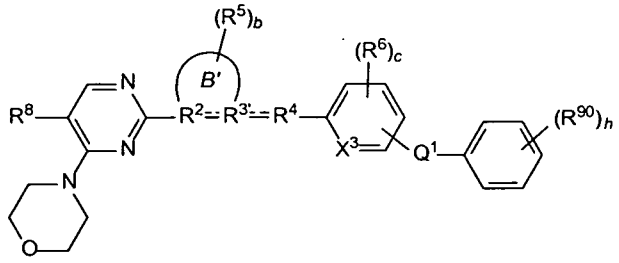
each R^0 is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic

ring;

c is 0 to 3; and

d is 0 or 1.

23. The compound of claim 1 having the formula VIII:



(VIII)

wherein:

Ring B' is a 5-, or 6- membered ring:

R^2 is selected from N, C and CH;

$R^{3'}$ is selected from N, C and CH;

R^4 is selected from N, C or CH;

each R^5 is independently selected from OH, O-alkyl, =O, SH, S-alkyl, =S, halo, alkyl, CF_3, NH_2, NHalkyl, and $N(alkyl)_2$;

b is 0, 1, or 2;

dashed lines represent optional double bonds;

each R^6 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^0, NH_2, halo, aryl, and a heterocyclic ring;

additionally or alternatively an R^6 and an R^5 may be taken together to form a 5-, to 7-membered ring that is fused to Ring B' and to Ring C;

c is 0 to 3;

Q^1 is selected from a chemical bond or a group having the formula -O-, $-CH_2-$, -NH-, -C(O)-NH-, -C(O)O-, -NH-C(O)-, -OC(O)NH-, and -O-C(O)NH-;

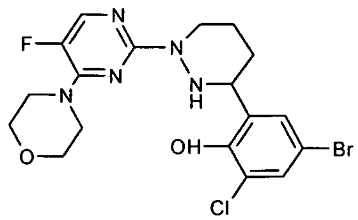
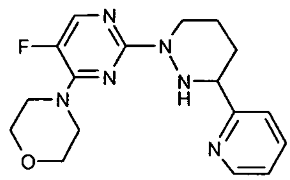
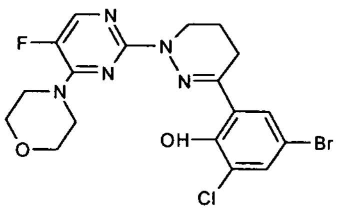
each R^{90} is selected from halo, alkyl, CN, $N(R^{92})_2$, cyclic-amino, NO_2, OR^{92}, and CF_3;

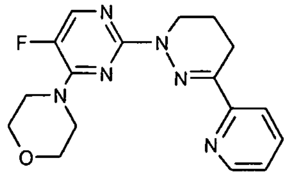
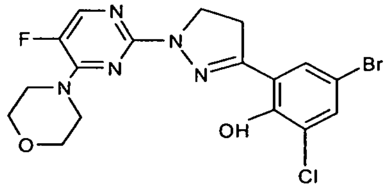
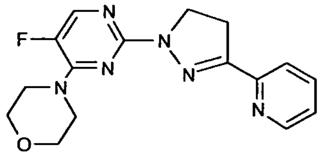
h is 0 to 5;

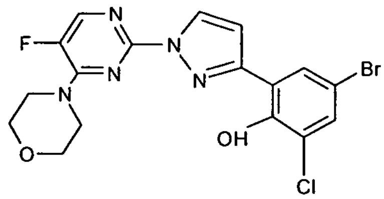
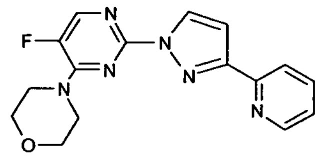
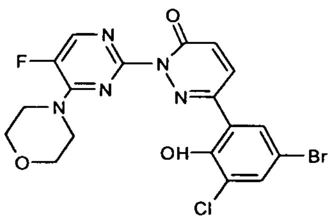
each R^{92} is selected from H, alkyl, aryl, aralkyl and a heterocyclic ring; and

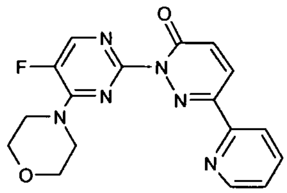
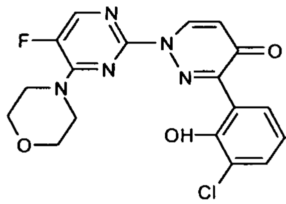
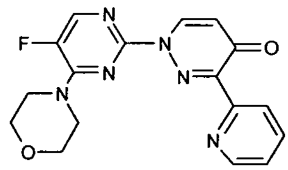
R^8 is selected from H and F.

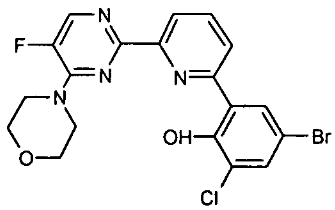
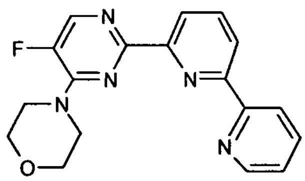
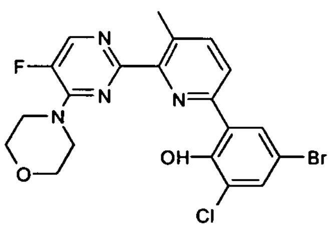
24. The compound of claim 1 having a formula selected from the following structures:

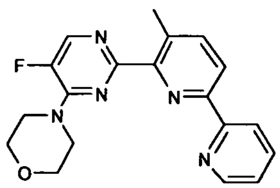
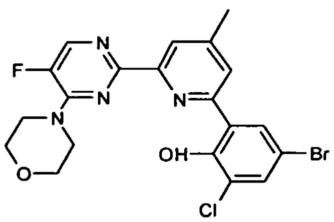
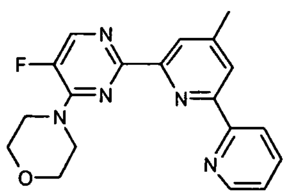




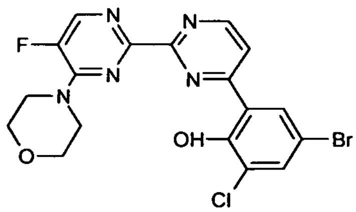
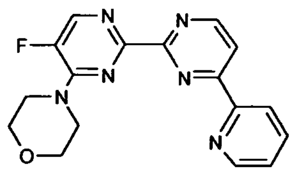
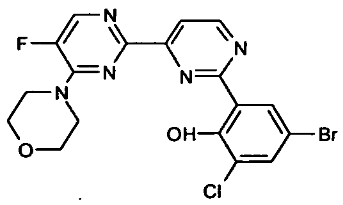




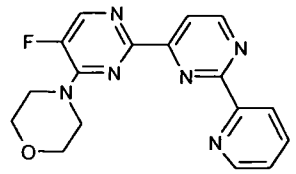
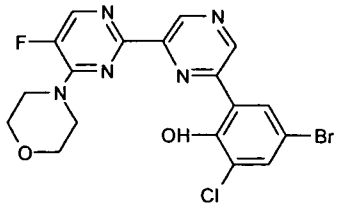
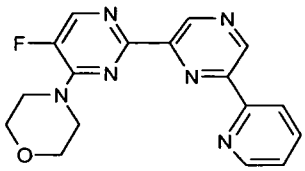
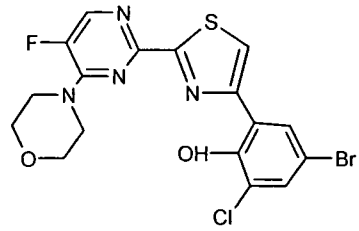
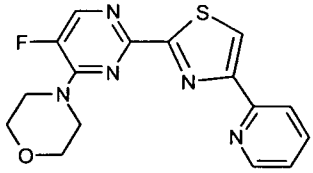
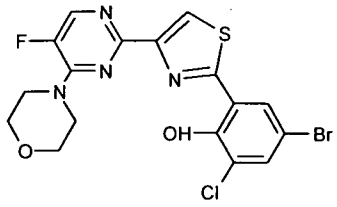
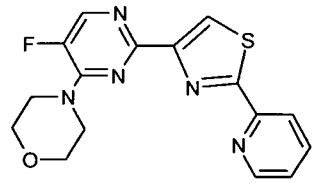
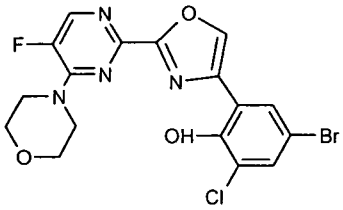
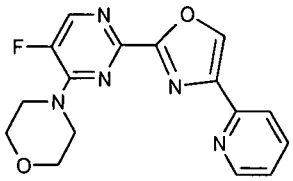
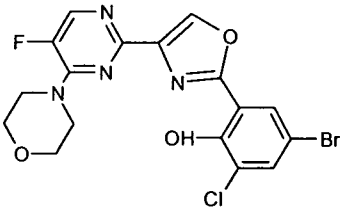
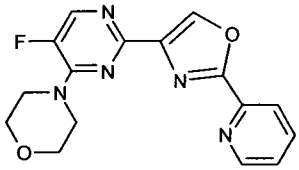
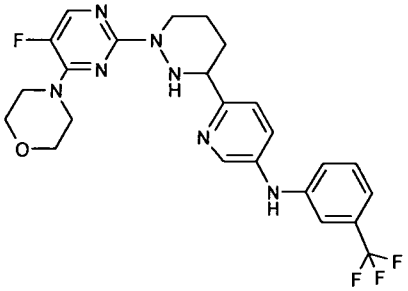
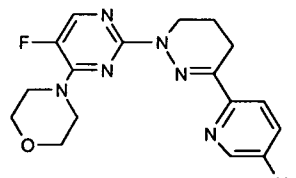
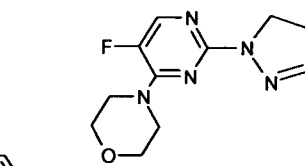
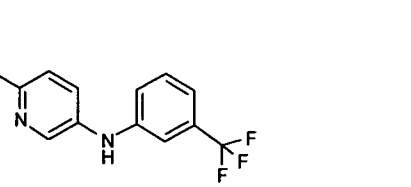
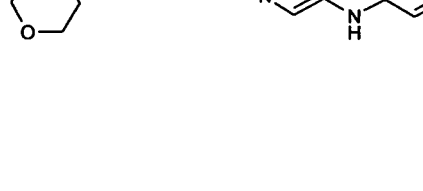
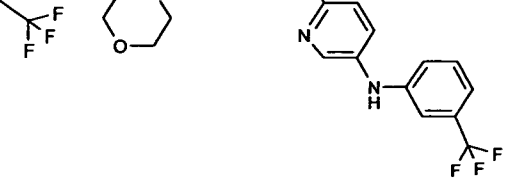




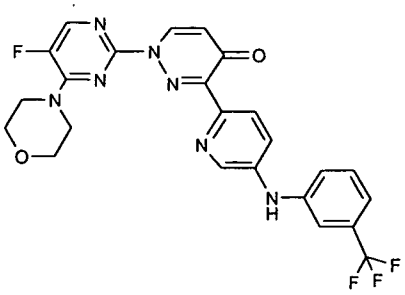




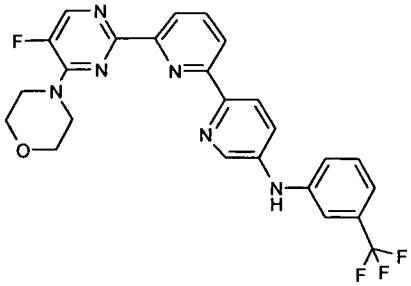




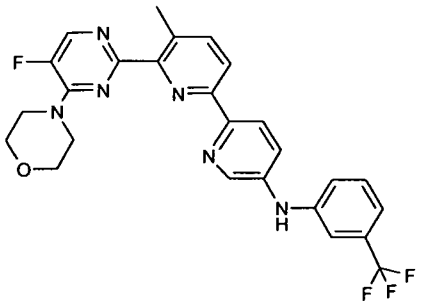




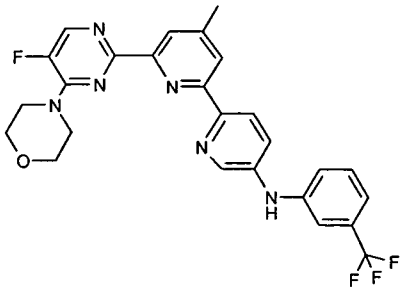




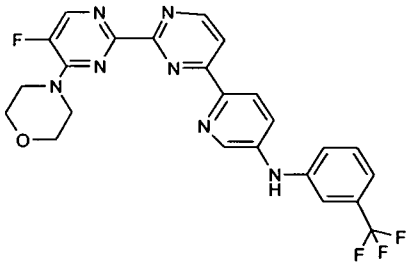


















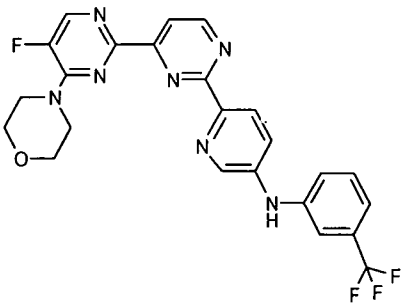


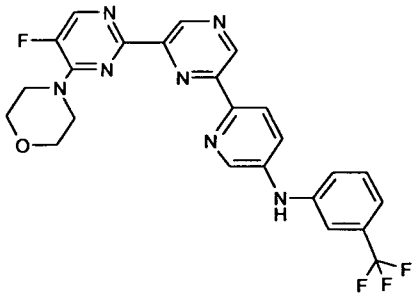


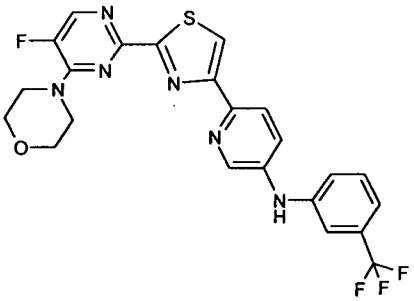


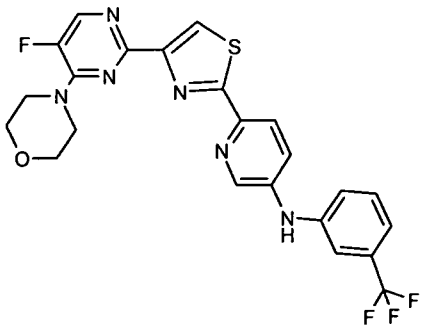


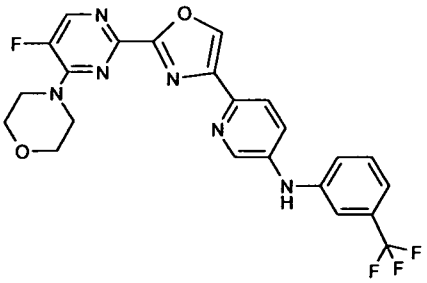


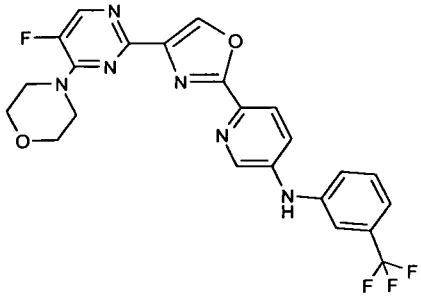


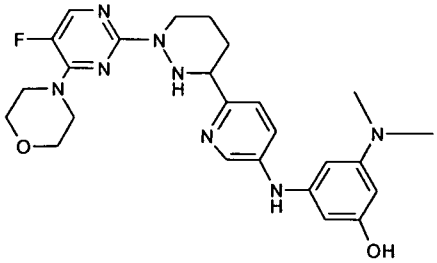


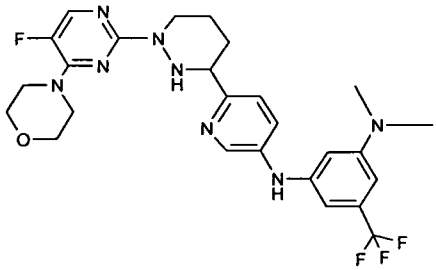


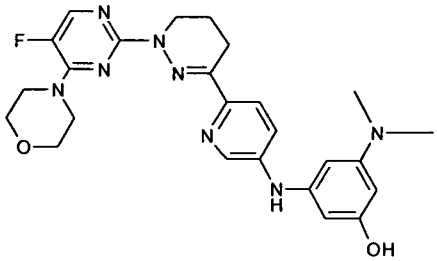


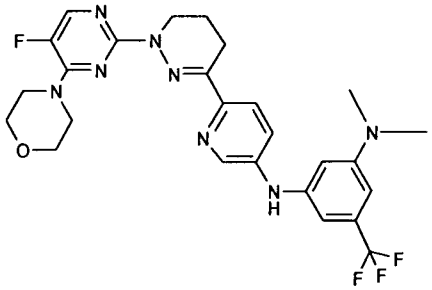


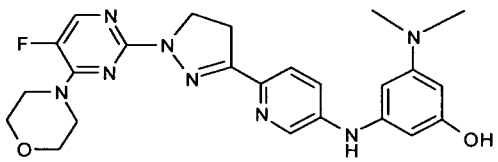


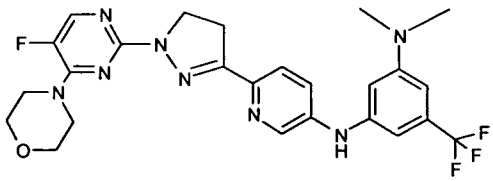


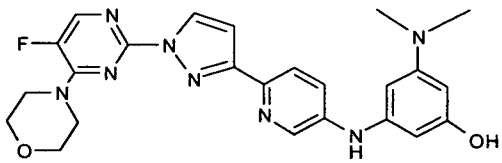


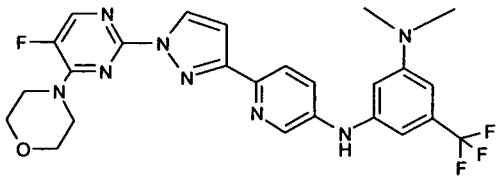


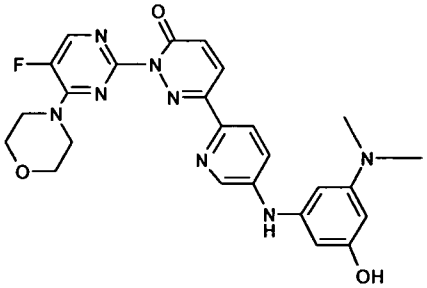


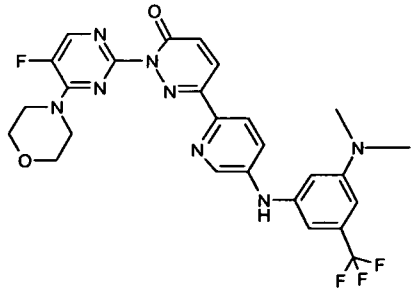


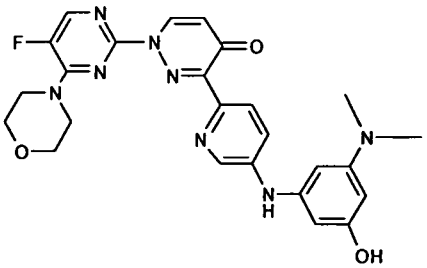


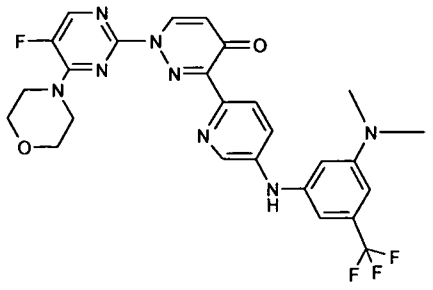


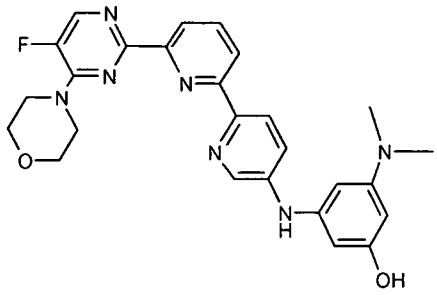


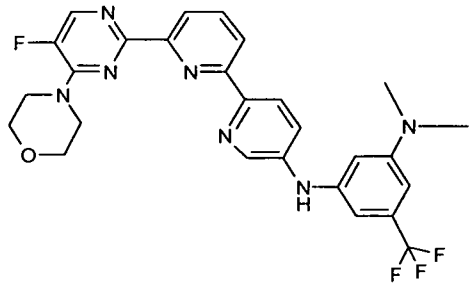


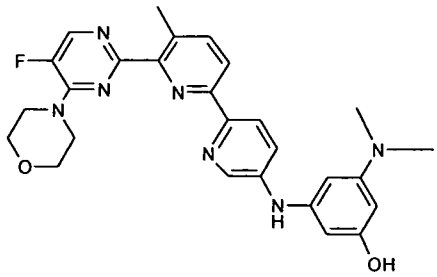


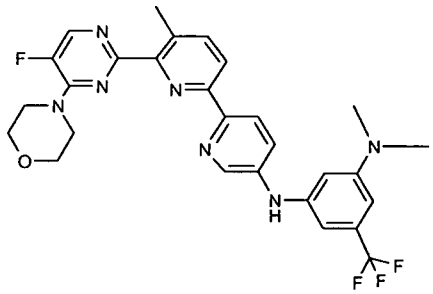


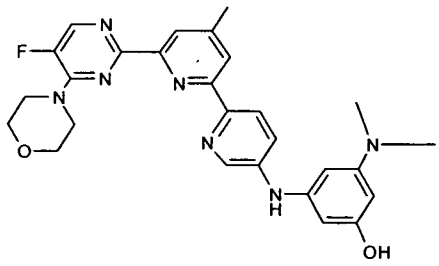


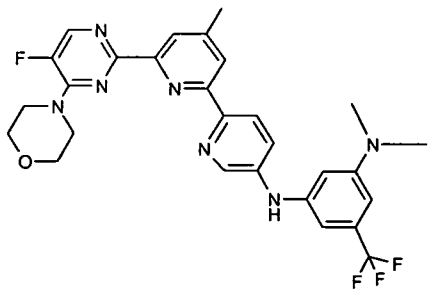


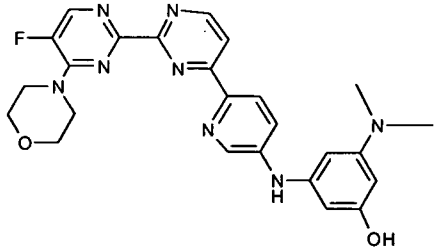


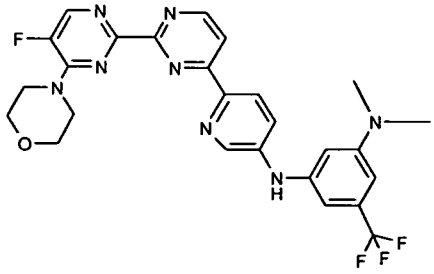


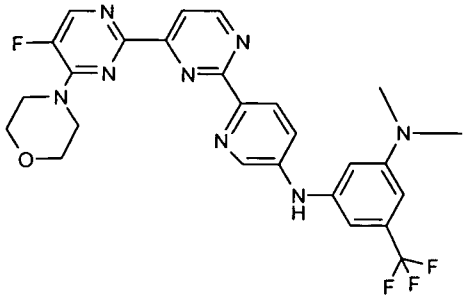


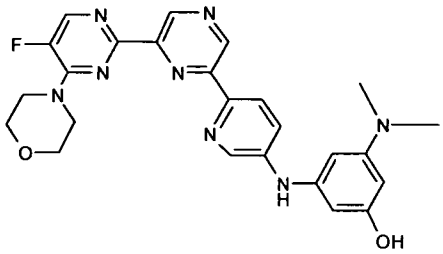


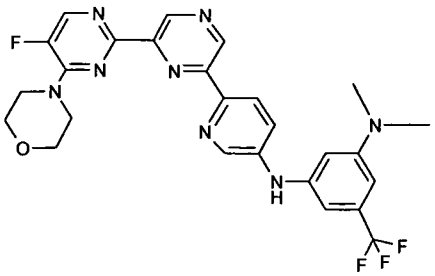


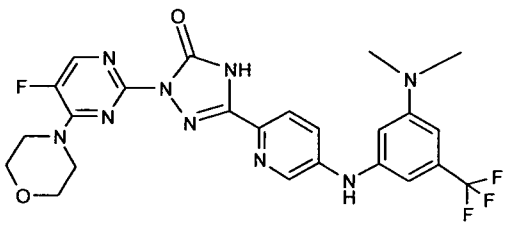


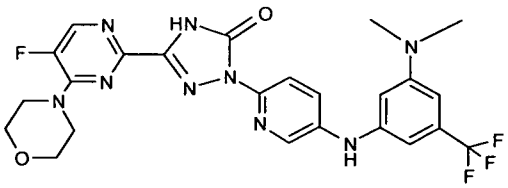


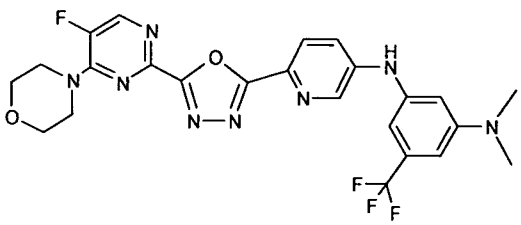


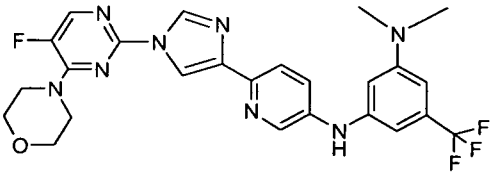


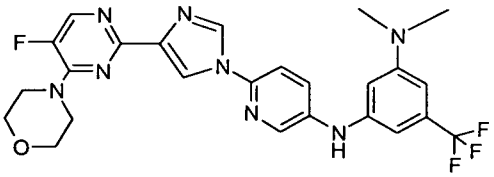


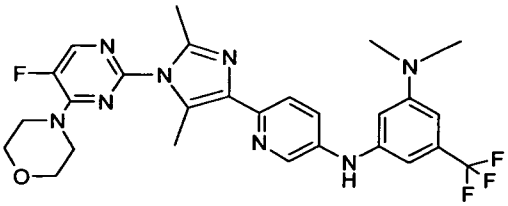


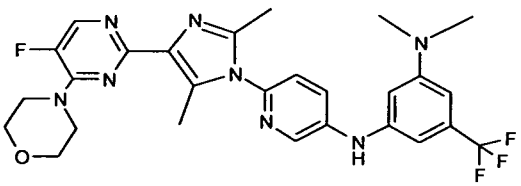


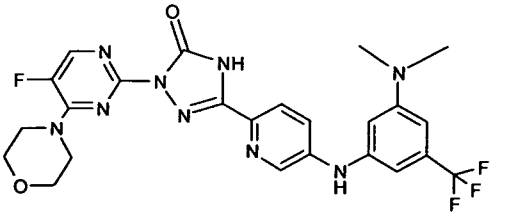


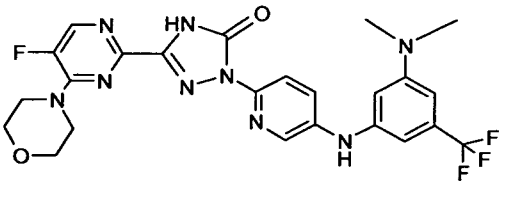


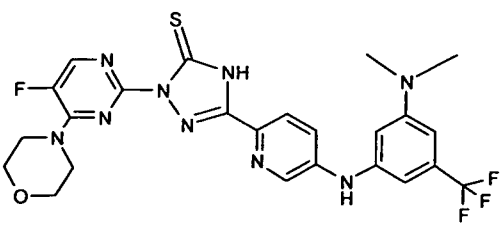


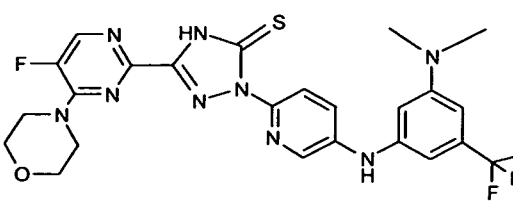


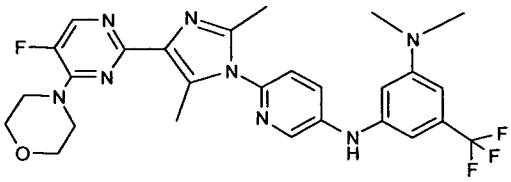
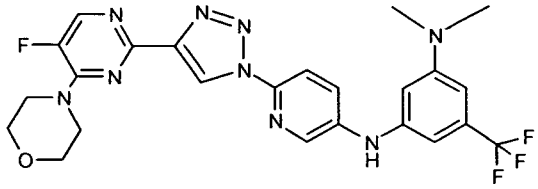
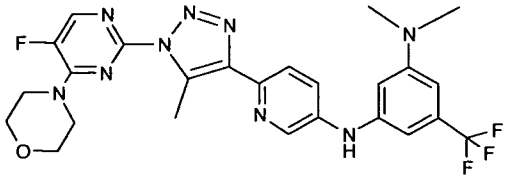
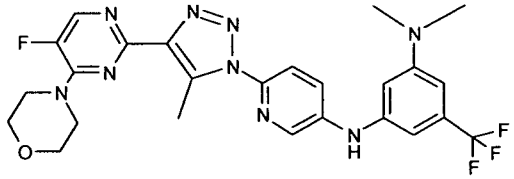
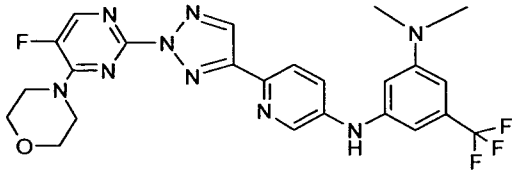
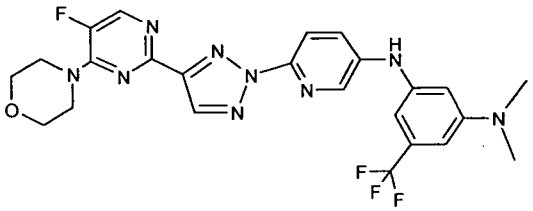
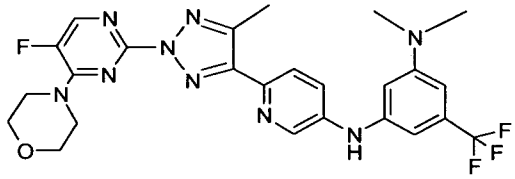
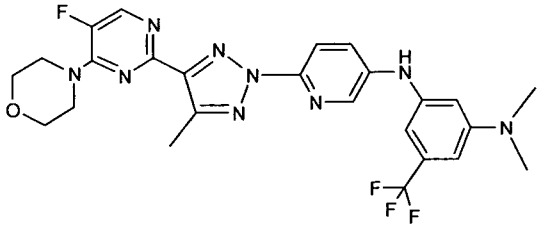
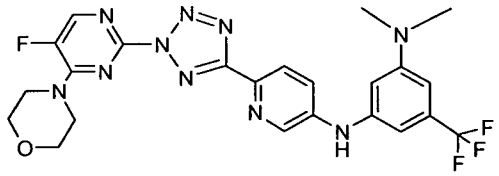
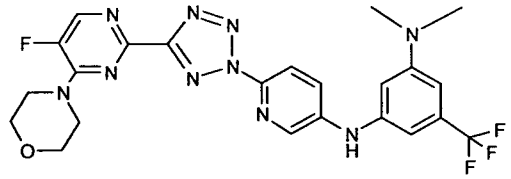
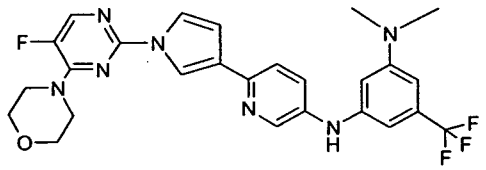
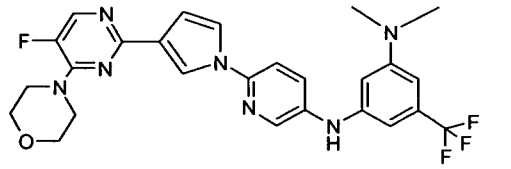
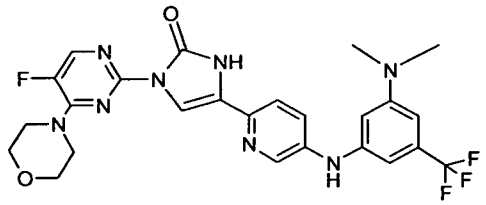
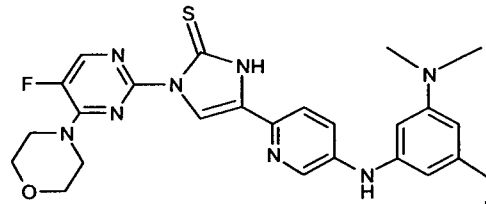


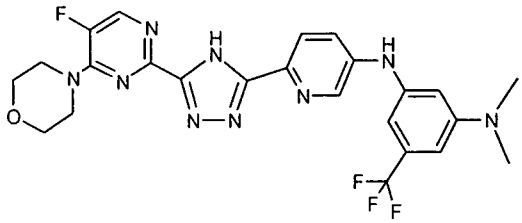


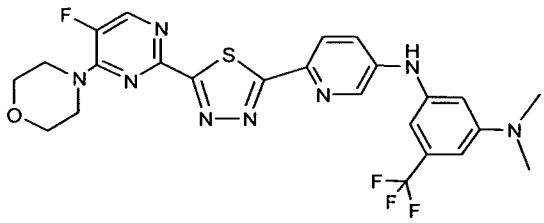


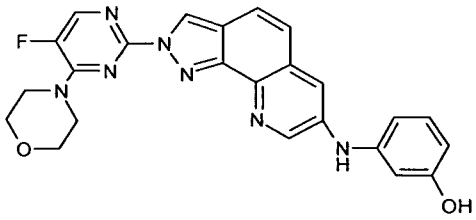


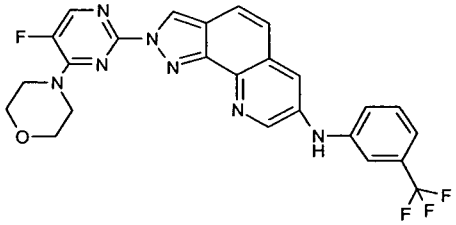


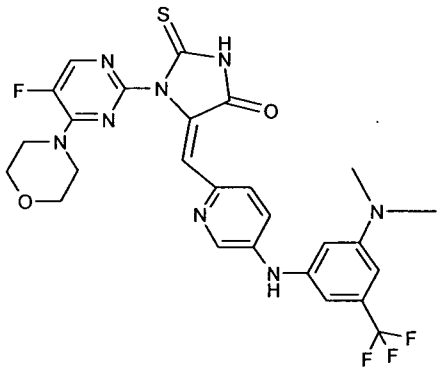















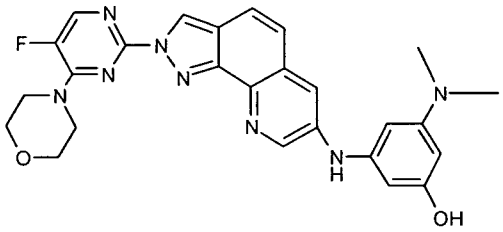


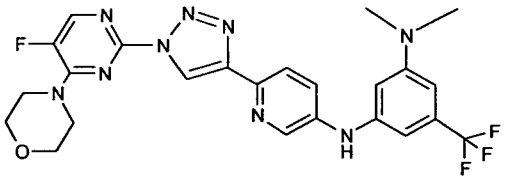


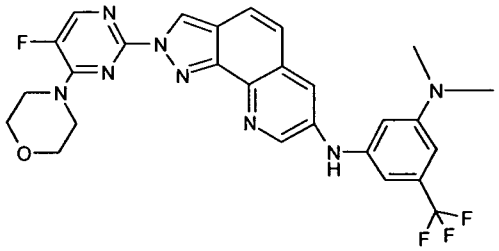


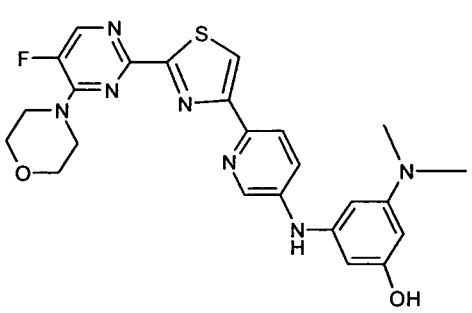


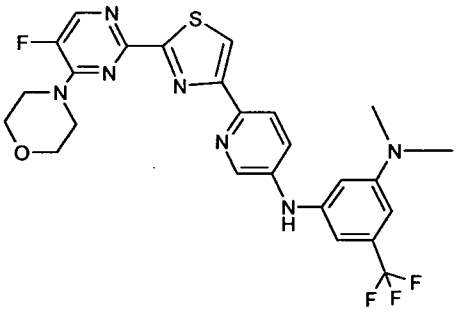


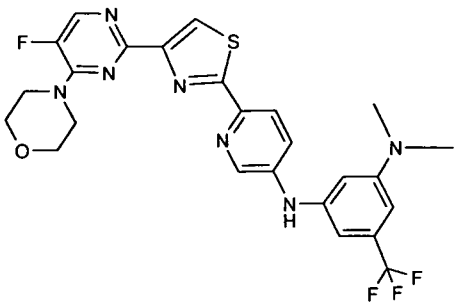


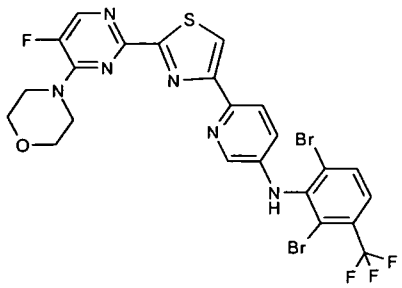






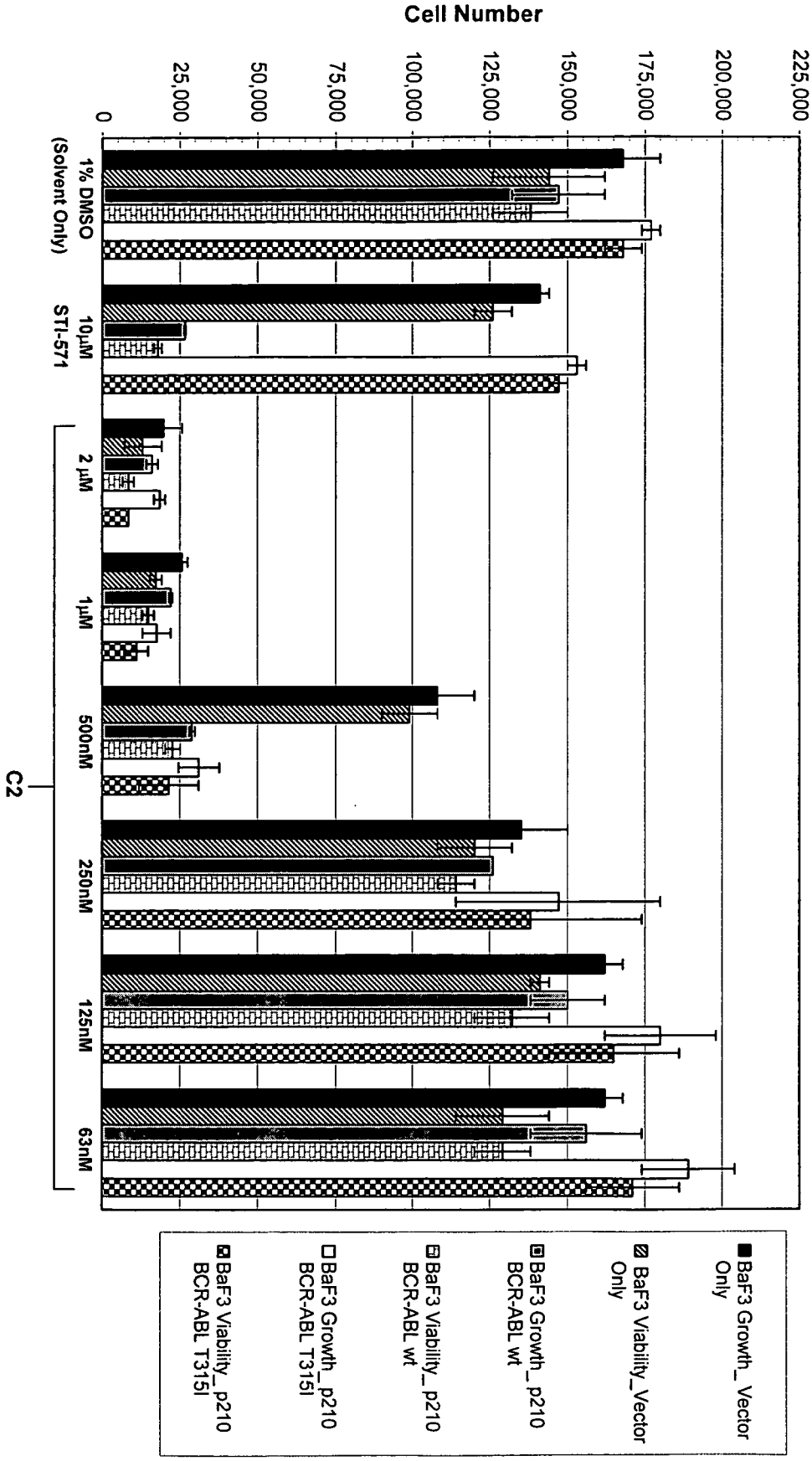






25. A method of inhibiting $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein in a cell comprising administering to a human a compound according to any one of claims 1-24.

26. A method of treating a neoplastic disease or a proliferative disorder in a human comprising administering a therapeutically effective amount of a compound according to any one of claims 1-24.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25,000
50,000
75,000
100,000
125,000
150,000
175,000
200,000
225,000
1% DMSO (Solvent Only)
10µM STI-571
2 µM
1µM
500nM
250nM
125nM
63nM
C2
BaF3 Growth_ Vector Only
BaF3 Viability_ Vector Only
BaF3 Growth_ p210 BCR-ABL wt
BaF3 Viability_ p210 BCR-ABL wt
BaF3 Growth_ p210 BCR-ABL T315I
BaF3 Viability_ p210 BCR-ABL T315I

Fig. 1

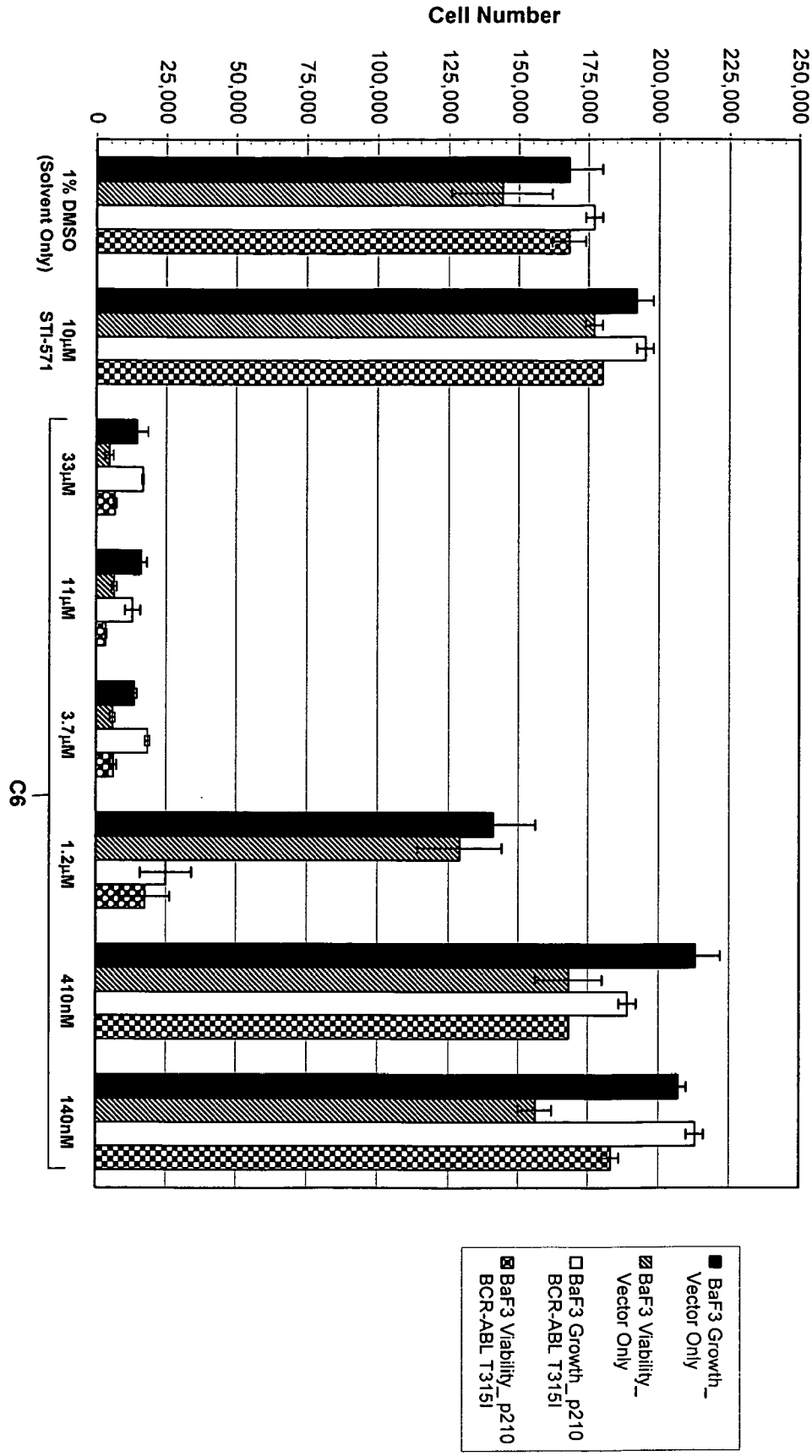
Cell Number
250,000
225,000
200,000
175,000
150,000
125,000
100,000
75,000
50,000
25,000
0
1% DMSO (Solvent Only)
10μM STI-571
33μM
11μM
3.7μM
1.2μM
410nM
140nM
C6
BaF3 Growth_ Vector Only
BaF3 Viability_ Vector Only
BaF3 Growth_ p210 BCR-ABL T315I
BaF3 Viability_ p210 BCR-ABL T315I

Fig. 2

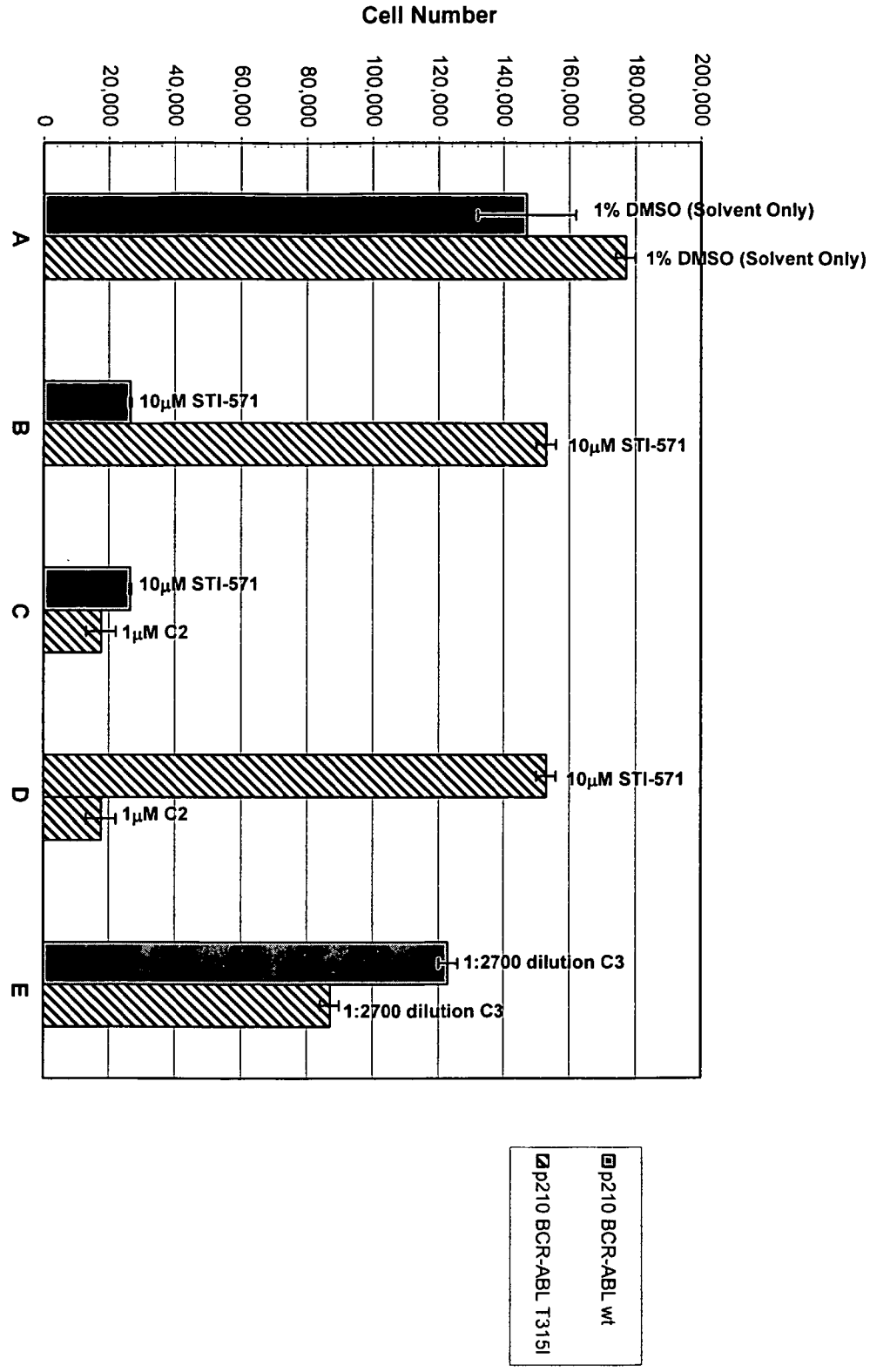
Cell Number
0
20,000
40,000
60,000
80,000
100,000
120,000
140,000
160,000
180,000
200,000
A
1% DMSO (Solvent Only)
1% DMSO (Solvent Only)
B
10μM STI-571
10μM STI-571
C
10μM STI-571
1μM C2
D
10μM STI-571
1μM C2
E
1:2700 dilution C3
1:2700 dilution C3
p210 BCR-ABL wt
p210 BCR-ABL T315I

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 08/02656

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 43/54; A61K 31/505 (2008.04)
USPC - 514/273
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A01N 43/54; A61K 31/505 (2008.04)
USPC - 514/273

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8) - A01N 43/04, 43/90; A61K 31/70, 31/519; C07D 239/42, 401/04, 471/04, 471/22, 487/04 (2008.04)
USPC - 514/49, 256-257, 259.5, 262.1, 269, 272, 275

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(USPT,PGPB,EPAB,JPAB); Google
Search Terms Used:
theramutein modulators, amine, drug resistance, glycoprotein, pentacyclic glycoprotein inhibitor, pyridyl

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2005/115992 A1 (HOUSEY) 08 December 2005 (08.12.2005), entire doc. esp. pages 7-28	1-26
Y	WO 1997/041102 A1 (COSTANZO et al.) 06 November 1997 (06.11.1997), page 3	13
Y	WO 2006/047631 A2 (WELSH et al.) 04 May 2006 (04.05.2006), page 12	1-26

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 May 2008 (22.05.2008)	26 JUN 2008
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (April 2007)