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(54) Title: INTRACELLULAR KINASE INHIBITORS

(57) Abstract: Intracellular kinase inhibitors and their therapeutic uses for patients with T cell malignancies, B cell malignancies, autoimmune disorders, and transplanted organs.

INTRACELLULAR KINASE INHIBITORS

[01] This application claims the benefit of and incorporates by reference co-pending provisional application Serial No. 60/801,074 filed May 18, 2006 and Serial No. 60/869,664 filed December 12, 2006.

FIELD OF THE INVENTION

[02] The invention relates to intracellular kinase inhibitors and their therapeutic uses.

BACKGROUND OF THE INVENTION

[03] Intracellular kinases play important functions in cells of the immune system. For example, interleukin-2 inducible tyrosine kinase (ITK) plays a key role in T cell development and differentiation; it regulates IL-2 production via phospholipase C γ1 (PLCγ1) and nuclear factor of activated T cells (NFAT); it mediates Th2 cell differentiation; and it regulates T cell migration and recruitment to lymphatic organs. Bruton's tyrosine kinase (BTK) is involved in signal transduction pathways which regulate growth and differentiation of B lymphoid cells. BTK also is involved in platelet physiology by regulating the glycoprotein VI/Fc receptor γ chain (GPVI-FcRγ)-coupled collagen receptor signaling pathway. For these reasons, inhibitors of intracellular kinases are useful for treating blood cell malignancies, solid tumors and for suppressing the immune system, for example in patients with autoimmune disorders or organ transplants. Intracellular kinase inhibitors also are useful for preventing or reducing the risk of thromboembolism.

BRIEF DESCRIPTION OF THE DRAWING

[04] FIG. 1. Results of a BIACORE® experiment in which the ITK kinase domain was immobilized on a biosensor and evaluated for its ability to bind and dissociate from a small molecule.

[05] FIG. 2. Alignment of human ITK (SEQ ID NO:1) and BTK (SEQ ID NO:2).

[06] FIG. 3. Alignment of kinase domains. Bolded amino acids, hinge; bolded and underlined amino acids, gatekeeper; italicized and bolded amino acids, Cys442 equivalents.

DETAILED DESCRIPTION OF THE INVENTION

[07] The invention provides compounds which inhibit intracellular kinases, particularly ITK and BTK, with an IC_{50} of 1 μM or below in an *in vitro* kinase assay as disclosed herein. The invention also provides pharmaceutical compositions and methods of using the compounds therapeutically. Patients who can be treated include those with blood cell malignancies, solid tumors, autoimmune disorders, and transplanted organs.

[08] A review of the literature and patent database revealed the existence of compounds that inhibit ITK or BTK kinases. However, these compounds differ significantly from the compounds disclosed herein. In several instances, the compounds are pyrrolopyridines (*e.g.*, US 2005/0215582). In other instances, the compounds are methyl dimethylbenzoates that belong thiazolyl family of compounds (*e.g.*, US 2004/0077695). In all cases, these published compounds differ from the compounds disclosed herein based on the following parameters: the compounds do not correspond to the general structure shown in this application, do not require the amino acid triad DKC found in the kinase binding site and necessary for optimal compound inhibitory capability described herein, do not undergo elimination, and do not bind covalently to the kinase binding pocket.

[09] Compounds of the invention which inhibit ITK can be used, *e.g.*, to treat T cell malignancies. Preferred compounds of the invention inhibit both ITK and BTK with an IC_{50} of 1 μM or below for each enzyme. Such compounds can be used, *e.g.*, to treat both T and B cell malignancies, as well as EGFR or HER positive tumors.

[10] The Tec family of kinases share a common subunit structure composed of a Src homology domain 2 (SH_2), an SH_3 and a catalytic kinase domain. Further, they are uniquely identified by the presence of a Tec homology region (TH) and a pleckstrin homology (PH) domain. There are four known crystallographic structures described for

the Tec family of kinases. These include (a) two structures representing the phosphorylated and unphosphorylated staurosporine-bound ITK (PDB codes 1SM2, 1SNU); (b) one structure of the unphosphorylated apo-form of ITK (PDB code 1SNX), and (c) one structure for the unphosphorylated apo-form of BTK (Mao et al. *J. Biol. Chem.* **2001,** *276*, 41435-41443). For the purpose of clarity of explanation, this disclosure will represent these kinase structures with those of the nearly identical ITK structures in (a) and (b) incorporated herein by reference (Brown et al. *J. Biol. Chem.* **2004,** *279*, 18727-18732) focusing attention on the ATP binding site. For the sake of uniformity, the residue numbering in these kinase structures as represented in the Protein Data Bank have been incorporated throughout this document to describe the kinase domain. The amino acid sequence of human ITK is shown in SEQ ID NO:1. The amino acid sequence of human BTK is shown in SEQ ID NO:2. Homologous residues in the other kinases and sequences from other sources may be numbered differently.

[11] Referring to Figure 2, The ITK kinase domain (residues 357-620) can be broken down into two components: the N-terminal lobe (residues 357-437) and the C-terminal lobe (residues 438-620). Like most kinases, the connecting region between the two lobes is a flexible hinge region described below, that forms part of the catalytic active site. The ordered nature of the C-helix places the catalytically important residues of Glu406, Lys391 and Asp500 in an orientation typical of the active form of a protein kinase. The Gly-rich loop (residues 362-378), commonly observed in kinases, assumes an extended and open conformation typical of an active kinase.

[12] The boundaries of the ATP binding site are demarcated by the following residues: (a) the glycine-rich loop (Gly370, Ser371, Gly372, Gln373, Phe374 and Gly375); (b) the hinge region (Phe435, Glu436, Phe437, Met438, Glu439, His440, Gly441 and Cys442); and (c) the catalytic residues Lys391 and Asp500. Additionally, the active site also comprises several other hydrophobic residues including Ala389, Ile369, Val377, Val419, and Leu 489 as well as the hydrophilic residue Ser499.

[13] Similar to other kinases, the hinge region of ITK contains two backbone carbonyls and one backbone amino group as potential hydrogen bond acceptor and donor sites

respectively. Similar backbone interactions have been observed in the interaction of kinases with the adenine base of ATP and several competitive inhibitors have been designed pursuing this concept. At the N-terminal end of the hinge region lies the "gatekeeper" residue, Phe435. This residue blocks access to an internal hydrophobic pocket, and, at the same time, provides a potential site of interaction for aromatic or hydrophobic groups. This "gatekeeper" residue is a significant difference between ITK and BTK. Despite the strong overall sequence identity between BTK and ITK, the presence of the smaller threonine residue as a gatekeeper in the active site of BTK justifies a key similarity of the latter to the active site of several kinases such as Src/Abl/EGFR. The absence of the bulkier Phe gatekeeper allows access to an internal hydrophobic pocket for these kinases, a fact that has been exploited for the design of allosteric inhibitors, and to improve the affinity of ATP-competitive inhibitors through the addition of a hydrophobic pharmacophore.

Definitions

[14] "Alkyl" is a monovalent linear or branched saturated hydrocarbon radical and can be substituted or unsubstituted. Linear or branched alkyls typically have between 1 and 12 carbon atoms (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12). Lower alkyls, or "C_1-C_6 alkyls," have between 1 and 6 carbon atoms (*e.g.*, 1, 2, 3, 4, 5, or 6). Optional substitutents include halogen, hydroxyl, alkoxy, aryloxy, amino, N-alkylamino, N,N-dialkylamino, alkylcarbamoyl, arylcarbamoyl, aminocarbamoyl, N-alkylaminocarbamoyl, N,N-dialkylaminocarbamoyl, alkylsulfonylamino, arylsulfonylamino, carboxy, carboxyalkyl, N-alkylcarboxamido, N,N-dialkylcarboxamido, alkylthio, alkylsulfinyl, alkylsulfonyl, trihaloalkylsulfonylamino (*e.g.*, trifluoromethylsulfonylamino), arylthio, arylsulfinyl, arylsulfonyl, and heterocyclyl. Examples of linear or branched C_1-C_6 alkyl are methyl, ethyl, propyl, isopropyl, sec-butyl, tert-butyl, n-butyl, n-pentyl, sec-pentyl, tert-pentyl, n-hexyl, isopentyl, fluoromethyl, trifluoromethyl, hydroxybutyl, dimethylcarboxyalkyl, aminoalkyl, and benzylpropyl.

[15] "Acyl" (or "alkylcarbonyl") is the radical $-C(O)R^8$, wherein R^8 is an optionally substituted lower alkyl. Examples of acyl include, but are not limited to, acetyl, propionyl, n-butyryl, sec-butyryl, t-butyryl, iodoacetyl, and benzylacetyl.

[16] "Acyloxy" is the radical $-OC(O)R^8$, wherein R^8 is an optionally substituted lower alkyl. Examples of acyloxy include, but are not limited to, acetoxy, propionyloxy, butyryloxy, trifluoroacetoxy, and diiodobutyryloxy.

[17] "Alkoxy" is the radical $-OR^8$, wherein R^8 is an optionally substituted lower alkyl. Examples of alkoxy include methoxy, ethoxy, propoxy, 2-propoxy, butoxy, sec-butoxy, tert-butoxy, pentyloxy, hexyloxy, fluoromethoxy, and iodoethoxy.

[18] "Alkylamino" is the radical $-NR^7R^8$, wherein R^7 is hydrogen or an optionally substituted lower alkyl and R^8 is an optionally substituted lower alkyl. Examples of alkylamino groups are methylamino, ethylamino, isopropylamino, dimethylamino, diethylamino, and trifluoromethylamino.

[19] "Alkylaminocarbonyl" (or "alkylcarbamoyl") is the radical $-C(O)NR^7R^8$, wherein R^7 is hydrogen or an optionally substituted lower alkyl and R^8 is an optionally substituted lower alkyl. Examples of alkylaminocarbonyl include, but are not limited to, methylaminocarbonyl, dimethylaminocarbonyl, t-butylaminocarbonyl, n-butylaminocarbonyl, iso-propylaminocarbonyl, and trifluoromethylaminocarbonyl.

[20] "Alkylaminosulfonyl" is the radical $-S(O)_2NR^7R^8$, wherein R^7 is hydrogen or an optionally substituted lower alkyl and R^8 is an optionally substituted lower alkyl. Examples of alkylaminosulfonyl include, but are not limited to, methylaminosulfonyl, dimethylaminosulfonyl, and triiodomethylaminosulfonyl.

[21] "Alkoxycarbonyl" or "alkyl ester" is the radical $-C(O)OR^8$, wherein R^8 is an optionally substituted lower alkyl. Examples of alkoxycarbonyl radicals include, but are not limited to, methoxycarbonyl, ethoxycarbonyl, sec-butoxycarbonyl, isopropyloxycarbonyl, and difluoromethoxycarbonyl.

[22] "Alkylcarbonylamino" is the radical $-NR^7C(O)R^8$, wherein R^7 is hydrogen or an optionally substituted lower alkyl and R^8 is an optionally substituted lower alkyl. Examples of alkylcarbonylamino include, but are not limited to, methylcarbonylamino, iso-propylcarbonylamino, and t-butylcarbonylamino.

[23] "Alkylcarboxamido" is the radical $-C(O)NR^7R^8$, wherein R^7 is hydrogen or an optionally substituted lower alkyl and R^8 is an optionally substituted lower alkyl. Examples of alkylcarboxamidos are methylcarboxamido, ethylcarboxamido, isopropylcarboxamido, and n-propylcarboxamido.

[24] "Alkylsulfonyl" is the radical $-S(O)_2R^8$, wherein R^8 is an optionally substituted lower alkyl. Examples of alkylsulfonyl include, but are not limited to, methylsulfonyl, trifluoromethylsulfonyl, and propylsulfonyl.

[25] "Alkylsulfonylamino" is the radical $-NR^7S(O)_2R^8$, wherein R^7 is hydrogen or an optionally substituted lower alkyl and R^8 is an optionally substituted lower alkyl. Examples of alkylsulfonylamino include, but are not limited to, methylsulfonylamino, propylsulfonylamino, and trifluoromethylsulfonylamino.

[26] "Aryl" is the monovalent aromatic carbocyclic radical of one individual aromatic ring or two or three fused rings in which at least one of the fused rings is aromatic. Aryls can be optionally substituted on one or more rings with one or more of halogen, hydroxyl, alkoxy, aryloxy, amino, N-alkylamino, N,N-dialkylamino, alkylcarbamoyl, arylcarbamoyl, aminocarbamoyl, N-alkylaminocarbamoyl, N,N-dialkylaminocarbamoyl, alkylsulfonylamino, arylsulfonylamino, carboxy, carboxyalkyl, N-alkylcarboxamido, N,N-dialkylcarboxamido, alkylthio, alkylsulfinyl, alkylsulfonyl, trifluoromethylsulfonylamino, arylthio, arylsulfinyl, arylsulfonyl, hydroxyalkyl, alkoxyalkyl, aryloxalkyl, aminoalkyl, N-alkylaminoalkyl, N,N-dialkylaminoalkyl, alkylcarbamoylalkyl, arylcarbamoylalkyl, aminocarbamoylalkyl, N-alkylaminocarbamoylalkyl N,N-dialkylaminocarbamoylalkyl, alkylsulfonylaminoalkyl, arylsulfonylaminoalkyl, alkylcarboxy, alkylcarboxyalkyl, N-alkylcarboxamindoalkyl, N,N-dialkylcarboxamindoalkyl, alkylthioalkyl, alkylsulfinylalkyl, alkylsulfonylalkyl,

trifluoromethylsulfonylaminoalkyl, arylthioalkyl, arylsulfinylalkyl, and arylsulfonylalkyl. Examples of aryls are phenyl, naphthyl, tetrahydronaphthyl, indanyl, indanonyl, tetralinyl, tetralonyl, fluorenonyl, phenanthryl, anthryl, and acenaphthyl.

[27] "Arylalkoxycarbonyl" or "arylalkyl ester" is the radical $-C(O)OR^8X$, wherein R^8 is an optionally substituted lower alkyl and X is an optionally substituted aryl. Examples of aryloxycarbonyl radicals include, but are not limited to, benzyl ester, phenyl ethyl ester, and dimethylphenyl ester.

[28] "Arylalkylcarbamoyl" is the radical $-C(O)NHR^8X$, wherein R^8 is an optionally substituted lower alkyl and X is an optionally substituted aryl. Examples of arylalkylcarbamoyl include, but are not limited to, benzylcarbamoyl, phenylethylcarbamoyl, and cyanophenylcarbamoyl.

[29] "Arylalkylcarbonyl" (or "aralkylcarbonyl") is the radical $-C(O)R^8X$, wherein R^8 is an optionally substituted lower alkyl and X is an optionally substituted aryl. Examples of arylalkylcarbonyl radicals include, but are not limited to, phenylacetyl and fluorophenylacetyl.

[30] "Arylaminocarbonyl" (or "arylcarbamoyl") is the radical $-C(O)NXX'$, wherein X is an optionally substituted aryl and X′ is hydrogen or an optionally substituted aryl. Examples of arylaminocarbonyl include, but are not limited to, phenylaminocarbonyl, methoxyphenylaminocarbonyl, diphenylaminocarbonyl, and dimethoxyphenyl-aminocarbonyl.

[31] "Arylaminosulfonyl" is the radical $-S(O)_2NXX'$, wherein X is an optionally substituted aryl and X′ is hydrogen or an optionally substituted aryl. Examples of arylaminosulfonyl include, but are not limited to, phenylaminosulfonyl, methoxyphenylaminosulfonyl, and triiodomethylaminosulfonyl.

[32] "Arylcarbonyl" is the radical $-C(O)X$, wherein X is an optionally substituted aryl. Examples of arylcarbonyl radicals include, but are not limited to, benzoyl, naphthoyl, and difluorophenylcarbonyl.

[33] "Arylcarbonylamino" is the radical –NHC(O)X, wherein X is an optionally substituted aryl. Examples of arylcarbonylamino include, but are not limited to, phenylcarbonylamino, tosylcarbonylamino, and cyanophenylcarbonylamino.

[34] "Aryloxy" is –OX, wherein X is an optionally substituted aryl. Examples of aryloxys include phenyloxy, naphthyloxy, tetrahydronaphthyloxy, indanyloxy, indanonyloxy, biphenyloxy, tetralinyloxy, tetralonyloxy, fluorenonyloxy, phenanthryloxy, anthryloxy, and acenaphthyloxy.

[35] "Aryloxycarbonyl" or "aryl ester" is the radical –C(O)OX, wherein X is an optionally substituted aryl. Examples of aryloxycarbonyl radicals include, but are not limited to, phenyl ester, naphthyl ester, dimethylphenyl ester, and trifluorophenyl ester.

[36] "Arylsulfonyl" is the radical $-S(O)_2X$, wherein X is an optionally substituted aryl. Examples of arylsulfonyl include, but are not limited to, phenylsulfonyl, nitrophenylsulfonyl, methoxyphenylsulfonyl, and 3,4,5-trimethoxyphenylsulfonyl.

[37] "Arylsulfonylamino" is the radical $-NS(O)_2X$, wherein X is an optionally substituted aryl. Examples of arylsulfonylamino include, but are not limited to, phenylsulfonylamino, naphthylsulfonylamino, 2-butoxyphenylsulfonylamino, 4-chlorophenylsulfonylamino, 2,5-diethoxysulfonylamino, 4-hexyloxyphenylsulfonylamino, 4-methylphenylsulfonyl-amino, naphtylsulfonylamino, 4-methoxyphenylsulfonylamino, N-methylphenylsulfonyl-amino, and 4-cyanophenylsulfonylamino, phenylsulfonylamino, 4-methylphenylsulfonyl-amino, naphtylsulfonylamino. phenylsulfonylamino, and 4-metylphenylsulfonylamino.

[38] "Arylsulfonyloxy" is the radical $-OS(O)_2X$, wherein X is an optionally substituted aryl. Examples of arylsulfonyloxy include, but are not limited to, benzenesulfonyloxy and 4-chloro-benzenesulfonyloxy.

[39] "Cycloalkyl" is a monovalent saturated carbocyclic radical consisting of one or more rings, preferably one, of three to seven carbons per ring and can be optionally substituted with one or more of hydroxyl, alkoxy, aryloxy, amino, N-alkylamino, N,N-dialkylamino, alkylcarbamoyl, arylcarbamoyl, aminocarbamoyl, N-alkylaminocarbamoyl, N,N-

dialkylaminocarbamoyl, alkylsulfonylamino, arylsulfonylamino, carboxy, carboxyalkyl, N-alkylcarboxamido, N,N-dialkylcarboxamido, alkylthio, alkylsulfinyl, alkylsulfonyl, trifluoromethylsulfonylamino, arylthio, arylsulfinyl, arylsulfonyl, hydroxyalkyl, alkoxyalkyl, aryloxalkyl, aminoalkyl, N-alkylaminoalkyl, N,N-dialkylaminoalkyl, alkylcarbamoylalkyl, arylcarbamoylalkyl, aminocarbamoylalkyl, N-alkylaminocarbamoylalkyl N,N-dialkylaminocarbamoylalkyl, alkylsulfonylaminoalkyl, arylsulfonylaminoalkyl, alkylcarboxy, alkylcarboxyalkyl, N-alkylcarboxamindoalkyl, N,N-dialkylcarboxamindoalkyl, alkylthioalkyl, alkylsulfinylalkyl, alkylsulfonylalkyl, trifluoromethylsulfonylaminoalkyl, arylthioalkyl, arylsulfinylalkyl, and arylsulfonylalkyl. Examples of cycloalkyls are cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, adamantyl, cyclooctyl, cycloheptyl, tetrahydro-naphthalene, methylenecylohexyl, indanyl, indenyl, and fluorenyl.

[40] "Cycloalkylcarbonyl" is the radical –C(O)R, wherein R is an optionally substituted cycloalkyl radical. Examples of cycloalkylcarbonyl radicals include, but are not limited to, cyclobutanoyl, cyclopentanoyl, cyclohexanoyl, and trifluorocyclopentanoyl.

[41] "Halogen" includes fluorine, chlorine, bromine, and iodine.

[42] "Heteroaryl" is a monovalent aromatic cyclic radical having one or more rings, preferably one to three rings, of four to eight atoms per ring, incorporating one or more heteroatoms selected independently from nitrogen, oxygen, silicon, and sulfur. Heteroaryls can be optionally substituted on one or more rings with one or more of halogen, hydroxyl, alkoxy, aryloxy, amino, N-alkylamino, N,N-dialkylamino, alkylcarbamoyl, arylcarbamoyl, aminocarbamoyl, N-alkylaminocarbamoyl, N,N-dialkylaminocarbamoyl, alkylsulfonylamino, arylsulfonylamino, carboxy, carboxyalkyl, N-alkylcarboxamido, N,N-dialkylcarboxamido, alkylthio, alkylsulfinyl, alkylsulfonyl, trifluoromethylsulfonylamino, arylthio, arylsulfinyl, arylsulfonyl, hydroxyalkyl, alkoxyalkyl, aryloxalkyl, aminoalkyl, N-alkylaminoalkyl, N,N-dialkylaminoalkyl, alkylcarbamoylalkyl, arylcarbamoylalkyl, aminocarbamoylalkyl, N-alkylaminocarbamoylalkyl N,N-dialkylaminocarbamoylalkyl, alkylsulfonylaminoalkyl, arylsulfonylaminoalkyl, alkylcarboxy, alkylcarboxyalkyl, N-alkylcarboxamindoalkyl,

N,N-dialkylcarboxamindoalkyl, alkylthioalkyl, alkylsulfinylalkyl, alkylsulfonylalkyl, trifluoromethylsulfonylaminoalkyl, arylthioalkyl, arylsulfinylalkyl, and arylsulfonylalkyl.

[43] Representative examples of monocyclic ring system heteroaryls include, but are not limited to, azetidinyl, azepinyl, aziridinyl, diazepinyl, 1,3-dioxolanyl, dioxanyl, dithianyl, furyl, imidazolyl, imidazolinyl, imidazolidinyl, isothiazolyl, isothiazolinyl, isothiazolidinyl, isoxazolyl, isoxazolinyl, isoxazolidinyl, morpholinyl, oxadiazolyl, oxadiazolinyl, oxadiazolidinyl, oxazolyl, oxazolinyl, oxazolidinyl, piperazinyl, piperidinyl, pyranyl, pyrazinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, pyridyl, pyrimidinyl, pyridazinyl, pyrrolyl, pyrrolinyl, pyrrolidinyl, tetrahydrofuranyl, tetrahydrothiophenyl, tetrazinyl, tetrazolyl, thiadiazolyl, thiadiazolinyl, thiadiazolidinyl, thiazolyl, thiazolinyl, thiazolidinyl, thiophenyl, thiomorpholinyl, 1,1-dioxidothiomorpholinyl, thiopyranyl, triazinyl, triazolyl, and trithianyl.

[44] Bicyclic ring systems include any of the above monocyclic ring systems fused to an aryl group, a cycloalkyl group, or another heteroaryl monocyclic ring system. Representative examples of bicyclic ring systems include but are not limited to, benzimidazolyl, benzothiazolyl, benzothiophenyl, benzoxazolyl, benzofuranyl, benzopyranyl, benzothiopyranyl, benzodioxinyl, 1,3-benzodioxolyl, cinnolinyl, indazolyl, indolyl, indolinyl, indolizinyl, naphthyridinyl, isobenzofuranyl, isobenzothiophenyl, isoindolyl, isoindolinyl, isoquinolyl, phthalazinyl, pyranopyridyl, quinolyl, quinolizinyl, quinoxalinyl, quinazolinyl, tetrahydroisoquinolyl, tetrahydroquinolyl, and thiopyranopyridyl.

[45] Tricyclic rings systems include any of the above bicyclic ring systems fused to an aryl group, a cycloalkyl group, or a heteroaryl monocyclic ring system. Representative examples of tricyclic ring systems include, but are not limited to, acridinyl, carbazolyl, carbolinyl, dibenzofuranyl, dibenzothiophenyl, naphthofuranyl, naphthothiophenyl, oxanthrenyl, phenazinyl, phenoxathiinyl, phenoxazinyl, phenothiazinyl, thianthrenyl, thioxanthenyl, and xanthenyl.

[46] "Heteroarylaminocarbonyl" is the radical –C(O)NZZ′, wherein Z is an optionally substituted heteroaryl and Z′ is hydrogen or an optionally substituted heteroaryl. Examples of heteroarylaminocarbonyl include, but are not limited to, pyridinylaminocarbonyl, and thienylaminocarbonyl, furanylaminocarbonyl.

[47] "Heteroarylaminosulfonyl" is the radical $-S(O)_2N$ ZZ′, wherein Z is an optionally substituted heteroaryl and Z′ is hydrogen or an optionally substituted heteroaryl. Examples of heteroarylaminosulfonyl include, but are not limited to, thienylaminosulfonyl, piperidinylaminosulfonyl, furanylaminosulfonyl, and imidazolylaminosulfonyl.

[48] "Heteroarylcarbonyl" is the radical –C(O)Z, wherein Z is an optionally substituted heteroaryl. Examples of heteroarylcarbonyl radicals include, but are not limited to, pyridinoyl, 3-methylisoxazoloyl, isoxazoloyl, thienoyl, and furoyl.

[49] "Heteroarylsulfonyl" is the radical $-S(O)_2Z$, wherein Z is an optionally substituted heteroaryl. Examples of heteroarylsulfonyl include, but are not limited to, thienylsulfonyl, furanylsulfonyl, imidazolylsulfonyl, and N-methylimidazolylsulfonyl.

[50] "Heteroarylsulfonyloxy" is the radical $-OS(O)_2Z$, wherein Z is an optionally substituted heteroaryl. An examples of hetroarylsulfonyloxy is thienylsulfonyloxy.

[51] "Heterocycle" is a saturated or partially unsaturated carbocyclic radical having one, two, or three rings each containing one or more heteroatoms selected independently from nitrogen, oxygen, silicon, and sulfur. A heterocycle can be unsubstituted or substituted on any or all of the rings with one or more of halogen, aryl, heteroaryl, hydroxy, alkoxy, aryloxy, amino, N-alkylamino, N,N-dialkylamino, alkylsulfonylamino, arylsulfonylamino, alkylcarbamoyl, arylcarbamoyl, aminocarbamoyl, N-alkylaminocarbamoyl, N,N-dialkylaminocarbamoyl, carboxy, alkylcarboxy, N-alkylcarboxamido, N,N-dialkylcarboxamido, alkylthio, alkylsulfinyl, alkylsulfonyl, trifluoromethylsulfonylamino, arylthio, arylsulfinyl, arylsulfonyl, carboxyalkyl, hydroxyalkyl, alkoxyalkyl, aryloxalkyl, aminoalkyl, N-alkylaminoalkyl, N,N-dialkylaminoalkyl, alkylcarbamoylalkyl, arylcarbamoylalkyl, aminocarbamoylalkyl, N-

alkylaminocarbamoylalkyl N,N-dialkylaminocarbamoylalkyl, alkylsulfonylaminoalkyl, arylsulfonylaminoalkyl, alkylcarboxyalkyl, N-alkylcarboxamindoalkyl, N,N-dialkylcarboxamindoalkyl, alkylthioalkyl, alkylsulfinylalkyl, alkylsulfonylalkyl, trihaloalkylsulfonylaminoalkyl, arylthioalkyl, arylsulfinylalkyl, and arylsulfonylalkyl. Examples of heterocycles include piperazinyl, piperidinyl, pyrrolidinyl, morpholinyl, thiamorpholinyl, pyrrolyl, phthalamide, succinamide, and maleimide.

[52] "Heterocyclylcarbonyl" (or "heterocyclocarbonyl") is the radical –C(O)M′, wherein M′ is an optionally substituted heterocycle.. Examples of heterocyclylcarbonyl include, but are not limited to, piperazinoyl, morpholinoyl, and pyrrolindinoyl.

[53] "Heterocyclylsulfonyl" is the radical $-S(O)_2Z'$, wherein M′ is an optionally substituted heterocycle. Examples of heterocyclylsulfonyl include, but are not limited to, piperidinylsulfonyl and piperazinylsulfonyl.

[54] "Heterocyclylsulfonyloxy" is the radical $-\ OS(O)_2M'$, wherein M′ is an optionally substituted heterocycle. Examples of heterocyclylsulfohyloxy include, but are not limited to, 3,5,dimethyl-isoxazolesulfonyloxy and pyrrolidinylsulfonyloxy.

Compounds

[55] This invention provides compounds which inhibit tyrosine kinases, particularly Tec (*e.g.*, ITK, BTK), Src (Src, Lck, etc.) and EGFR kinases (*e.g.*, EGFR1, Her 2, Her 4), and Jak kinase (*e.g.*, Jak3), having structures that exploit a discrete mechanistic rationale described herein. This mechanism provides for the utilization of the kinase catalytic machinery, described in the ITK crystallographic structures as the acid-base pair residues Lys391 and Asp500 (herein referred to as the "catalytic dyad"), to trigger a transformation that activates the proposed inhibitory compounds within the enzyme active site. This transformation involves the elimination of a leaving group, resulting in the *in situ* formation of an electrophilic intermediate capable of forming a covalent adduct with an active site cysteine residue thereby irreversibly inhibiting the function of the target enzyme. This cysteine residue is identifiable as Cys442 in the ITK crystallographic structure. The group of kinases with the above described triad, including

ITK, BTK, BMX, Tec, TXK, BLK, EGFr, Her 2, Her 4 and JAK3, will be referred to as the DKC triad kinases. Various embodiments of the invention relate to this group, its possible sub-groupings, and to its individual members.

[56] It is known that several compounds, typically containing electrophilic Michael acceptors, form covalent adducts with enzymatic nucleophiles present in the active site to irreversibly inhibit the target enzyme (Slichenmeyer, W.J.; Elliott, W.C.; Fry, D.W. *Semin. Oncol.* **2001,** *28*, 80-85; Shimamura, T.; Ji, H.; Minami, Y.; Thomas, R. K.; Lowell, A.M.; Sha, K.; Greulich, H.; Glatt, K.A.; Meyerson, M.; Shapiro, I.; Wong, K.-K. *Cancer Res.* **2006,** *66*, 6487-6491). However, the compounds described in this invention are unique in that the transformation that forms the electrophilic intermediate takes place preferentially *in situ,* i.e. within the enzyme active site. Outside of an appropriate active site, these compounds are much less likely to undergo beta-elimination and form adducts with other proteins. The compounds described within must first bind in the active site of the target kinase and achieve a specific conformational geometry with respect to the relevant catalytic residues in order to effectively trigger elimination of the leaving group, thereby unmasking the adduct-forming intermediate. This intermediate forms a covalent, irreversible adduct with the proximal active site cysteine residue. In some embodiments the reaction proceeds stepwise; in other embodiments it is concerted. In preferred embodiments additional portions of the inhibitor molecule interact with other portions of the kinase, particularly in the active site, to promote favorable binding affinity and positioning. Such interactions contribute to the specificity of various inhibitors so that some inhibitors are inhibit a single kinase whereas others inhibit multiple kinases with similar or different IC_{50}s. To our knowledge, this is the first example of an *in situ* formation of an active inhibitor in a kinase active site.

Compound interaction with the kinase domain

[57] Without specifying the kinetics of the reaction, the inhibition of the target kinase goes through the following sequence of steps to form the adduct with the inhibitory compounds:

(1) The catalytic lysine N-H is positioned within hydrogen bonding distance (approximately 1.8 – 4.0 Angstroms) of a hydrogen bond acceptor Y in the compound that exists in the form of a C=Y (Y=O, S, NOR) functionality. Polarization of the C=Y bond results in increasing the acidity of the proton (H_A) at a carbon atom alpha to the C=Y group.

(2) Acting as a base, the aspartate of the catalytic dyad extracts the acidic proton H_A, leaving behind a conjugated carbanion that forms for Y = O, an enol, H-bonded enolate through standard electronic rearrangement. For Y = S, it would form a thioenol or H-bonded thioenolate, and for Y = NOR, it would form an alkoxy (R = alkyl), aryloxy (R=aryl) or hydroxy (R=H) enamine.

(3) The formation of the enol/thioenol/enamine facilitates the elimination of the leaving group attached at a carbon beta to C=Y, through a process known as "β-elimination." The leaving group, attached to the compound through protonatable heteroatom Z, may optionally be additionally tethered to the rest of the compound.

(4) Being a strong nucleophilic species, the sulfhydryl group of the neighboring cysteine residue reacts with the newly formed electrophilic elimination product. This addition reaction (thioalkylation) forms the covalent adduct to the kinase resulting in its irreversible inhibition and abrogation of activity.

[58] The inhibitory activity of this class of compounds toward select kinases is dependent on their ability to bind effectively in proximity to the appropriate calalytic environment, the existence of a polarizable C=Y group (C=O in formula (I), below) with appropriate reactivity and an adjacent alpha proton to allow elimination of the beta leaving group.

[59] In turn, the elimination process that generates a reactive electrophilic species requires removal of the abstractable alpha proton that is facilitiated by adequate positioning of the C=Y group in the catalytic environment. The generated electrophilic Michael acceptor, in turn is required to be positioned within reactive distance of the key cysteine residue.

The appropriate positioning of the abstractable proton in the kinase binding site is achieved through pharmacophoric elements that include:

(i) a C=Y moiety that serves the dual purpose of polarizing the proximal C-H bond of the abstractable proton, and hydrogen bonding to the lysine residue of the catalytic pair;

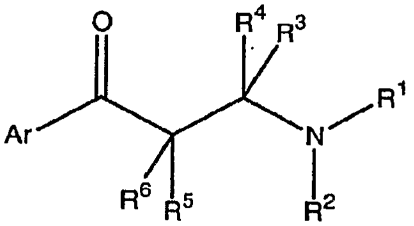
(ii) a hydrophobic aryl or heteroaryl group that interacts with specific hydrophobic residues in the binding site at an approximate distance of 3-5 Å from Y,

(iii) several (one to 3) hydrophilic pharmacophores that interact with the backbone in the hinge region,

(vi) a carbon atom in the beta position from the C=Y carbon atom, that is positioned within reactive distance of the sulfhydryl group of the relevant cysteine as explained below.

[60] The effective "reactive distance" to the cysteine sulfhydryl group as stated above is observed in the range of about 3-10 Å using computational design methods that test the binding of inhibitors to the ITK ATP binding site, wherein the enzyme is maintained in a fixed conformation. While a distance of 10 Å in a rigid system would be too far to effect a chemical reaction, the enzymatic nucleophilic moiety and the inhibitor's electrophilic moiety can readily be brought together through a series of low energy barrier rotations around the flexible inhibitor bonds as well as the cysteinyl side chain. Overall global conformational changes, common to kinase systems, cannot be ruled out either but are not readily measurable. Such conformational changes, which can be envisioned by computational predictions, are adequate in bringing the two reactive pieces in close enough proximity to effect covalent bond formation.

[61] Compounds according to the invention have the structural formula:



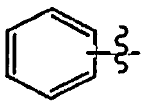
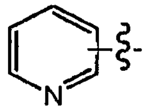
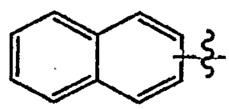
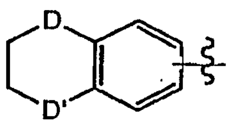
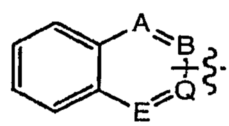
(I)

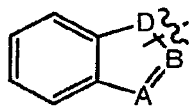
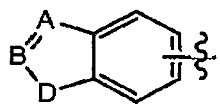
wherein:

Ar is optionally substituted aryl or optionally substituted heteroaryl;

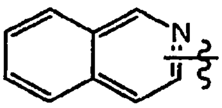
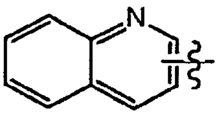
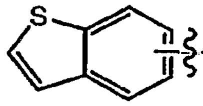
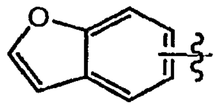
R^3, R^4, R^5, and R^6 are independently hydrogen or optionally substituted C_1-C_6 alkyl; and R^1 and R^2 (a) are independently hydrogen, optionally substituted C_1-C_6 alkyl, piperidine, or furanyl; or (b) are taken together with the nitrogen atom to which they are attached to form (i) a 5- to 7-membered optionally substituted aryl, (ii) a 5- to 7-membered optionally substituted heteroaryl, or (iii) a 5- to 7-membered optionally substituted heterocycle which may be unfused or fused to an optionally substituted aryl.

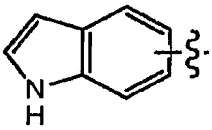
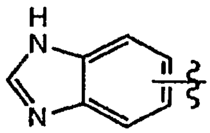
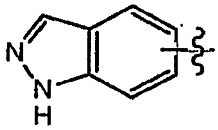
[62] In some embodiments Ar is selected from the group consisting of:

, , , , ;

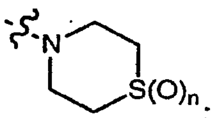
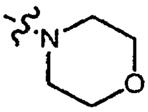
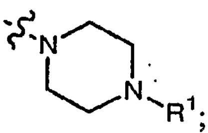
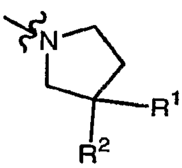
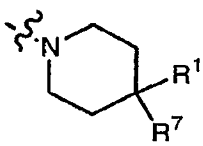
; and , wherein A, B, E, and Q are independently CH, O, or N; and D and D' are independently CH_2, NH, O, or S.

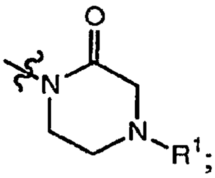
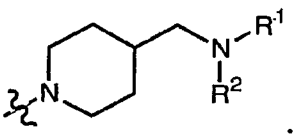
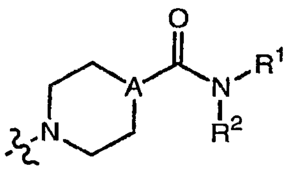
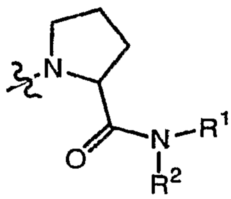
[63] In other embodiments Ar is selected from the group consisting of:

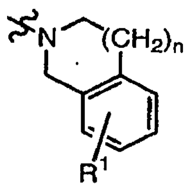
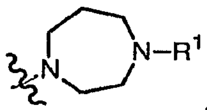
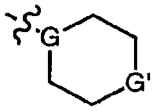
 ;  ;  ;  ;

 ;  ; and  .

[64] Examples of 5- to 7-membered heterocycles include:

 ;  ;  ;  ;  ;

 ;  ;  ;  ;

 ;  ; and 

wherein:

G is N, CH, or S;

G' is NH, CH, or S;

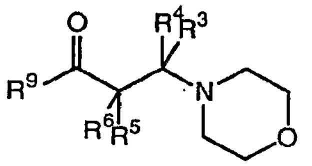
n = 0-2;

R^1 and R^2 are as defined above; and

R^7 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted aryl, or optionally substituted heteroaryl.

[65] Preferred 5- to 7-membered heterocycles are piperazinyl, piperidinyl, pyrrolidinyl, and morpholinyl. Preferred substituents for piperazinyl are C_1-C_6 alkyl, dialkyl C_1-C_6 aminoalkyl, aryl, aralkyl, cycloalkyl, and cycloalkyl-alkyl. Preferred substituents for piperidinyl are C_1-C_6 alkyl and aralkyl. In some embodiments piperidinyl is benzofused to form isoquinolinyl. Preferred substituents for pyrrolidinyl are C_1-C_6 alkyl, aryl, and aralkyl. In some embodiments pyrrolidinyl is benzofused to form isoindolyl. Preferred substituents for morpholinyl are C_1-C_6 alkyl and arylalkyl.

[66] Some compounds have the structural formula:

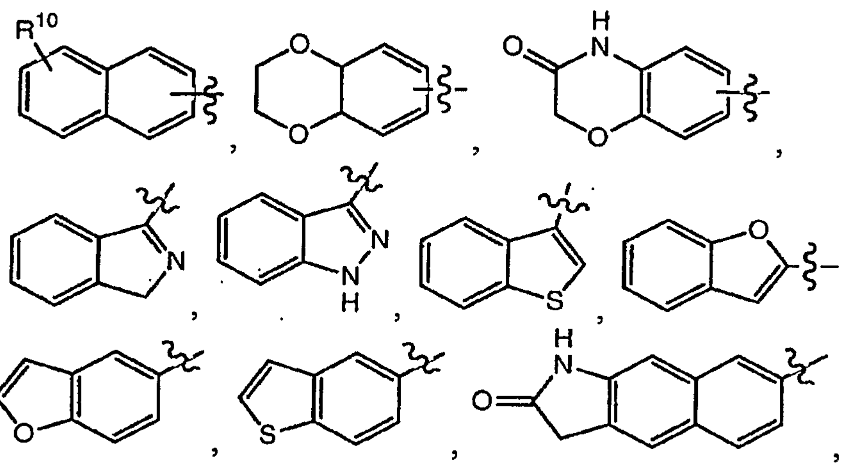


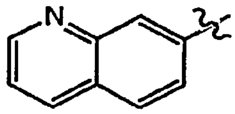
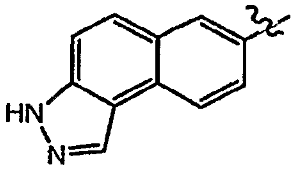
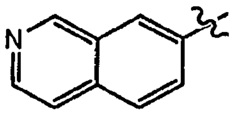
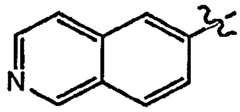
(II)

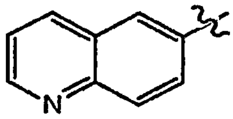
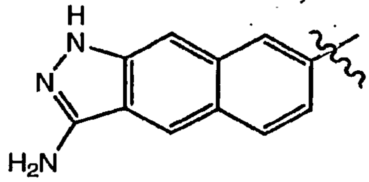
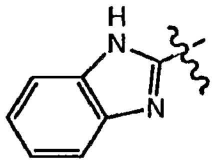
wherein:

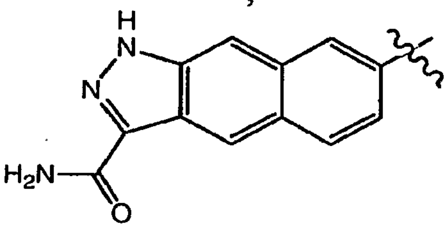
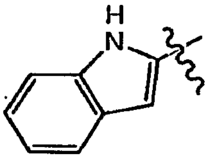
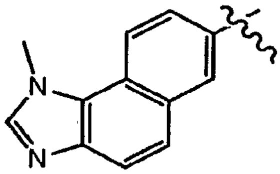
R^3, R^4, R^5, and R^6 are as defined above;

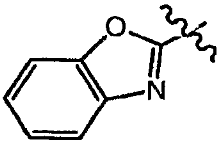
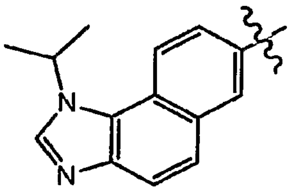
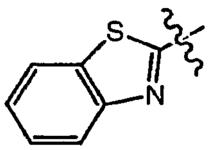
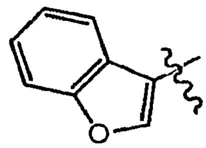
R^9 is selected from,

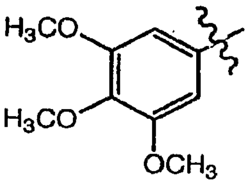
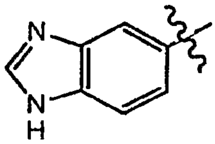
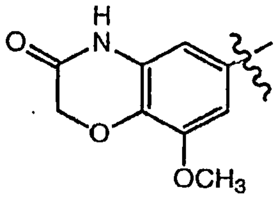
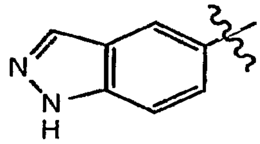


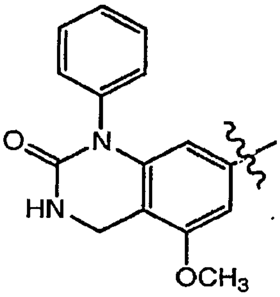
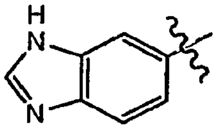
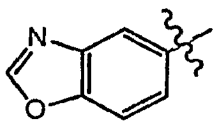
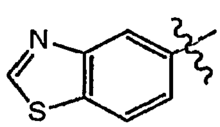
, , , ,

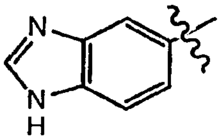
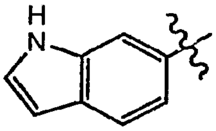
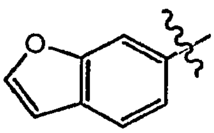
, , ,

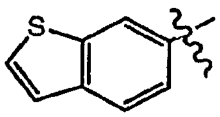
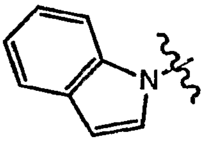
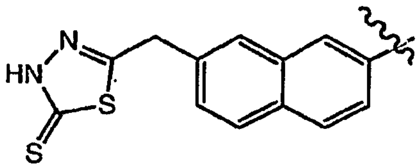
, , ,

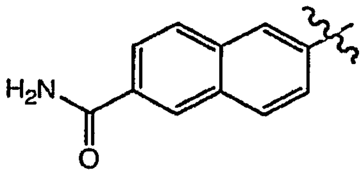
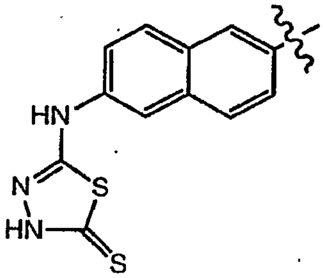
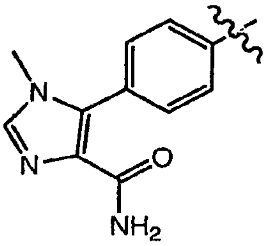
, , , ,

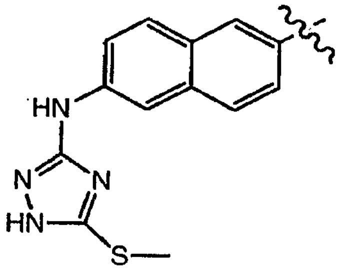
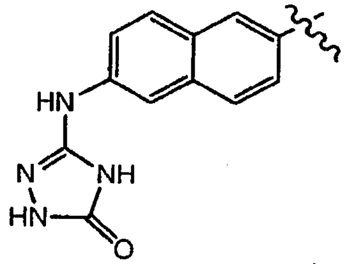
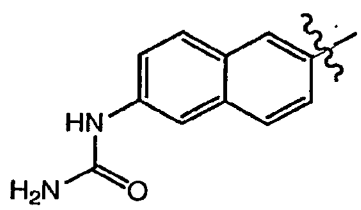
, , , ,

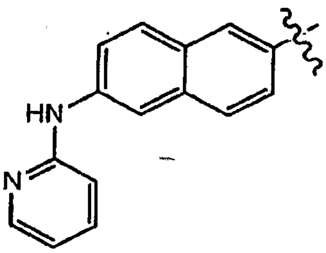
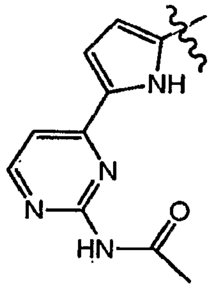
, , , ,

, , ,

, , ,

, , ,

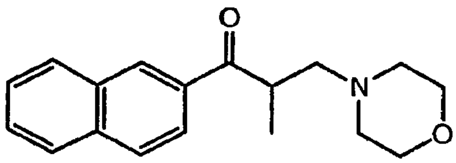
, , ,

, and ; and

R^{10} is hydrogen, –OH, –COOH, $-CONH_2$, or –NCO,

wherein if R^9 is naphthyl, then R^5 and R^6 are not both methyl.

[67] Examples of these compounds include: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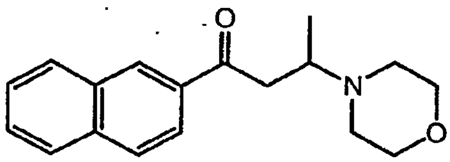
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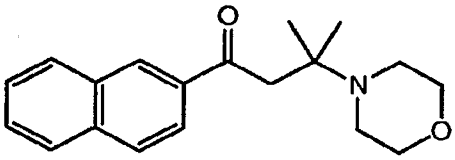
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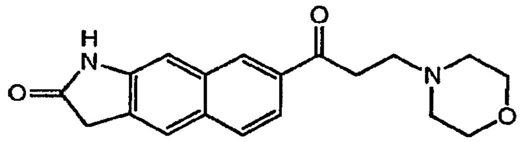
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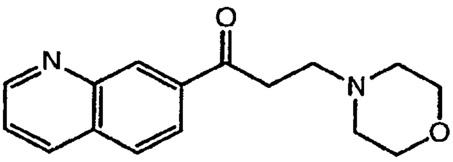
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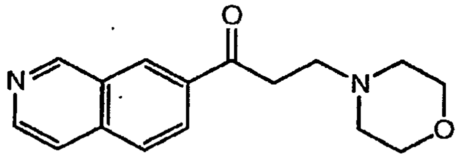
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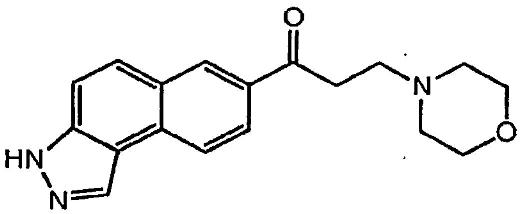
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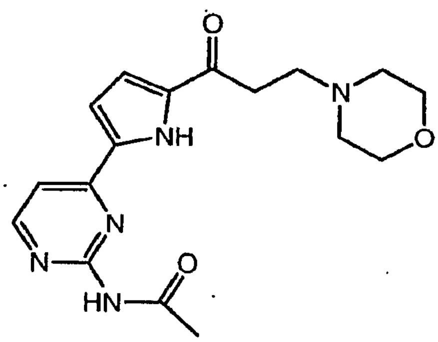
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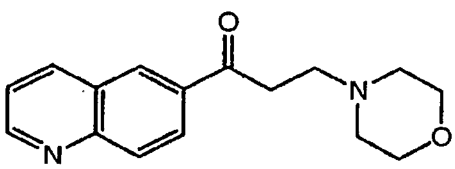
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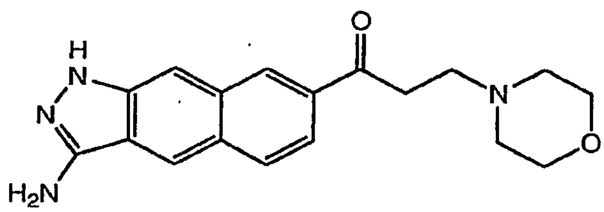
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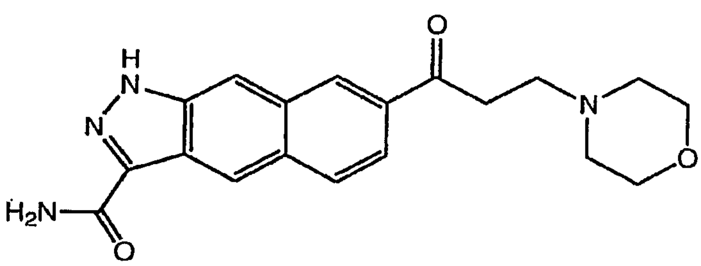
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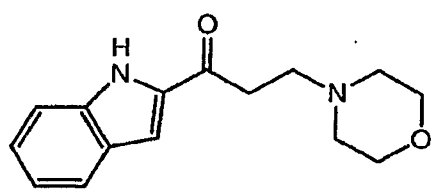
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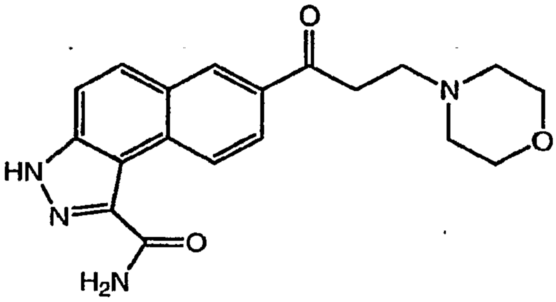
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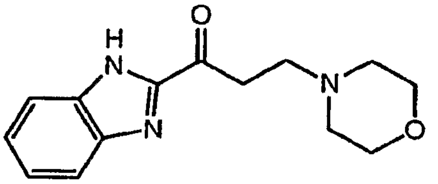
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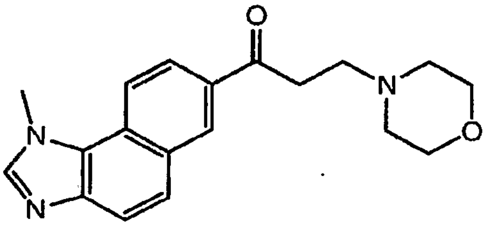
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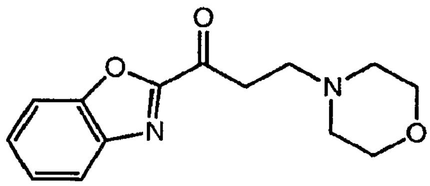
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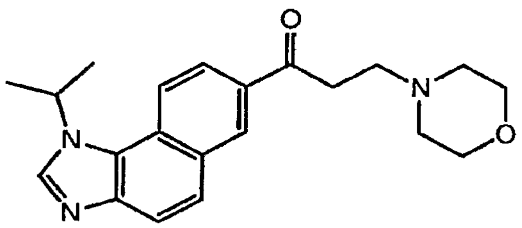
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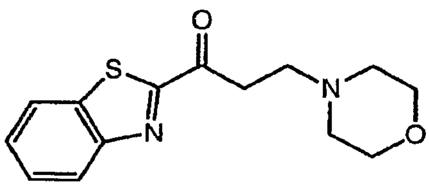
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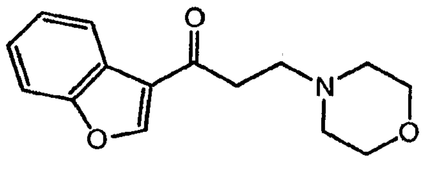
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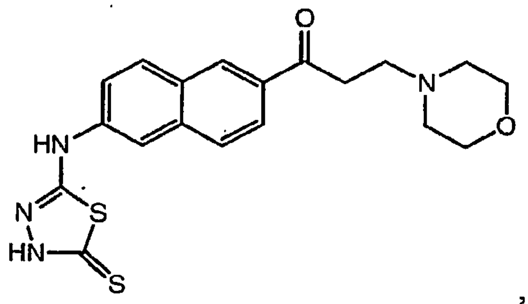
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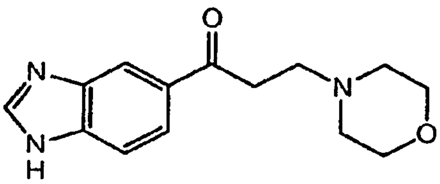
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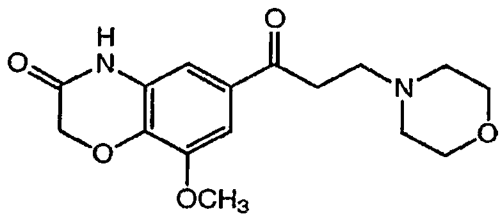
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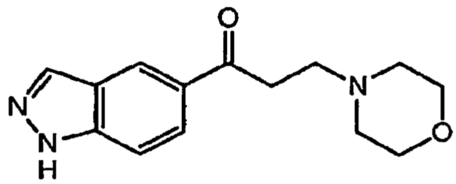
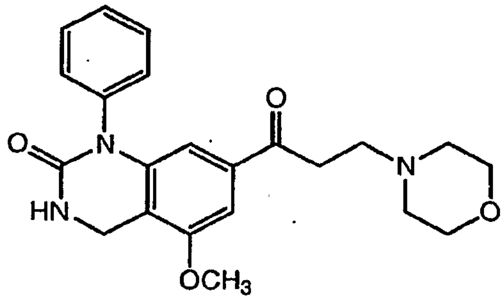
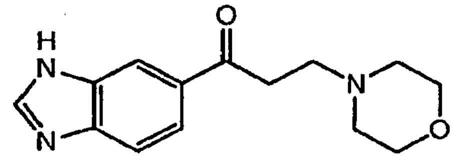
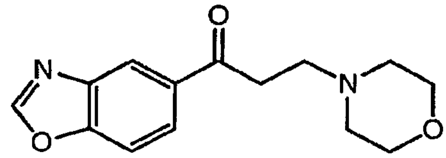
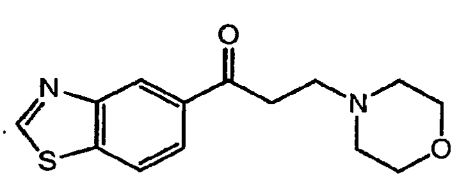
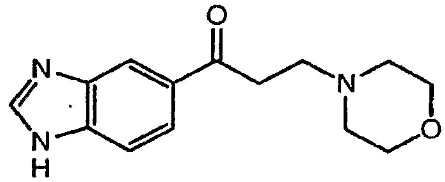
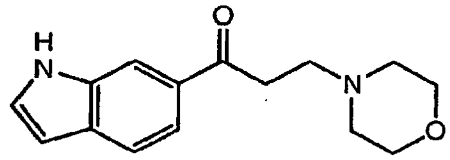
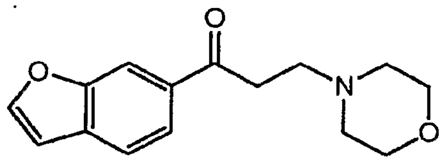
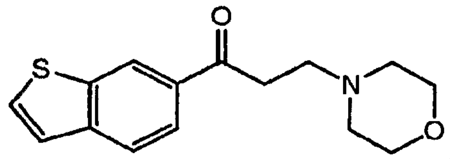
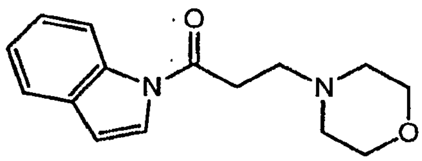
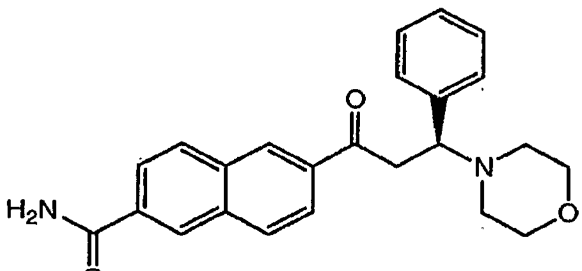
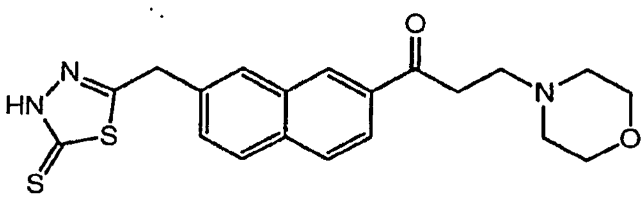
,
,

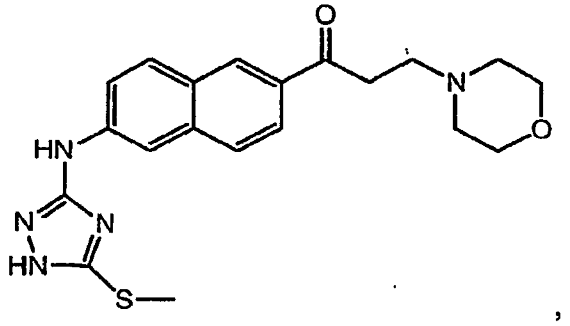
,
,

,
,

,
,

,
,

,
,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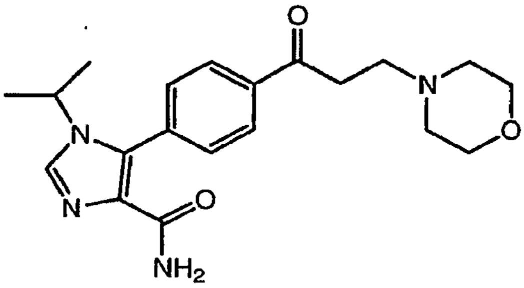
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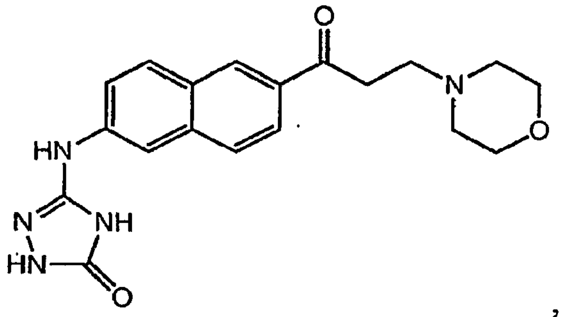
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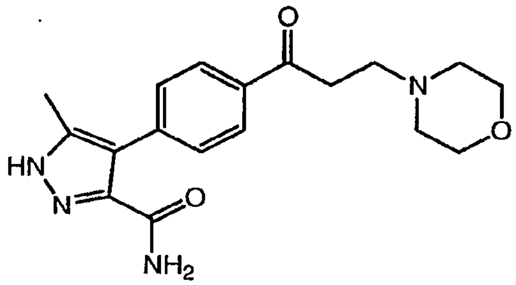
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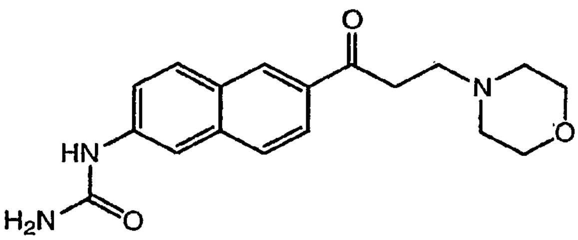
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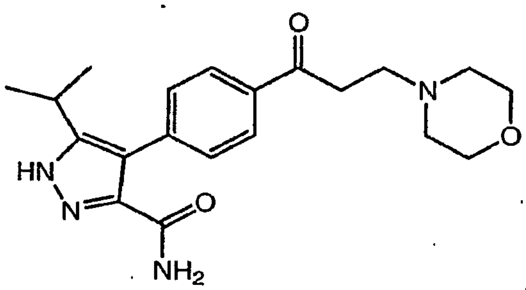
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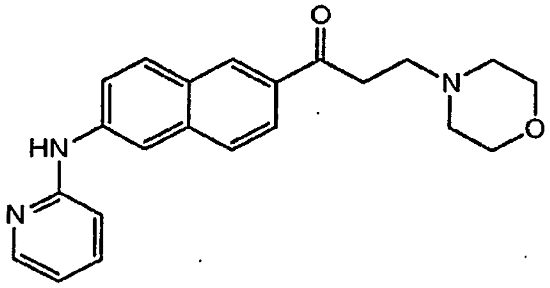
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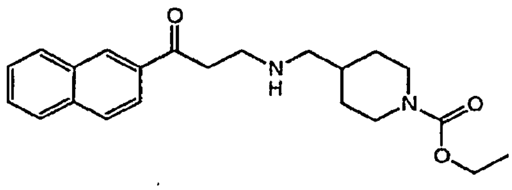
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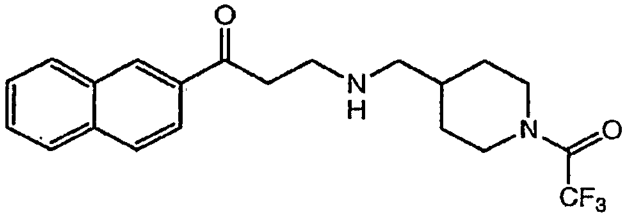
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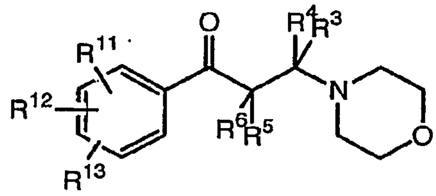
[68] Other compounds have the structural formula:

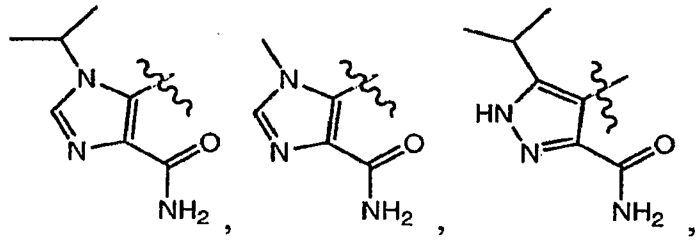


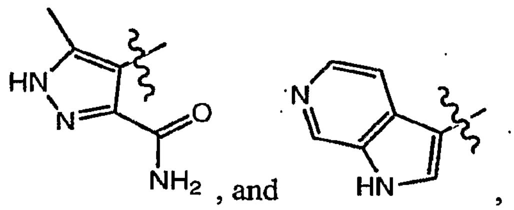
(III)

wherein:

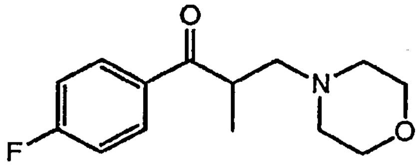
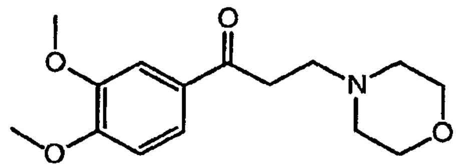
R^3, R^4, R^5, and R^6 are as defined above;

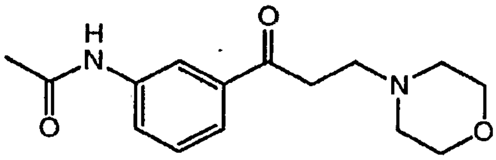
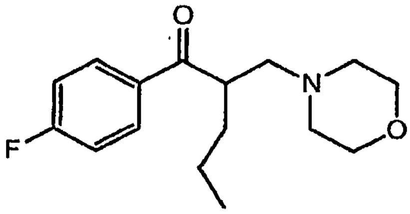
R^{11} and R^{12} are independently selected from hydrogen, $-OCH_3$, halogen, $-NO_2$, $-CN$, $-CF_3$, $-NCOR'$ (wherein R' is hydrogen or C_1-C_4 alkyl), phenyloxy, $-OCF_3$, $-NR'R''$ (wherein R' and R'' are independently hydrogen or C_1-C_4 alkyl), C_1-C_4 alkyl, C_1-C_4 alkoxy, and $-SO_2R'$ (wherein R' is hydrogen or C_1-C_4 alkyl); and

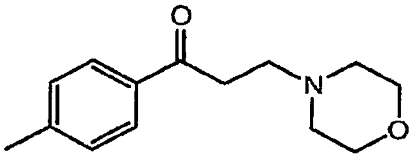
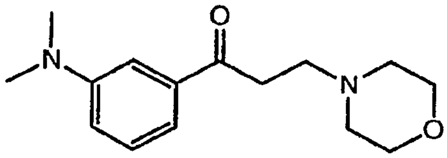
R^{13} is hydrogen, C_1-C_4 alkyl, 

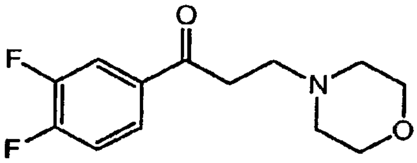
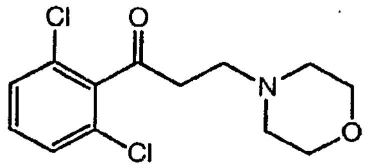


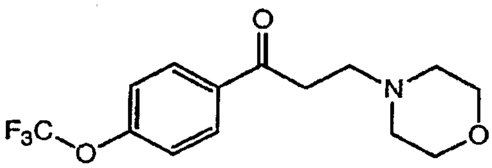
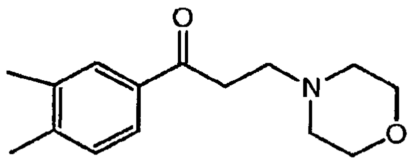
with the proviso that formula (III) does not include the following compounds:

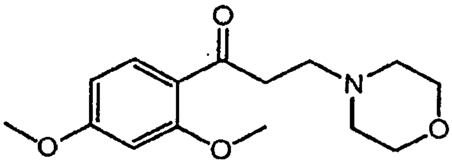
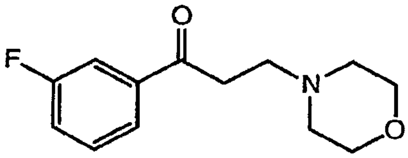
,
,

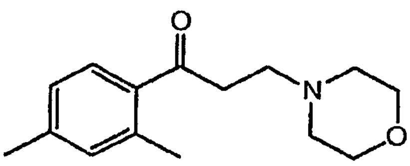
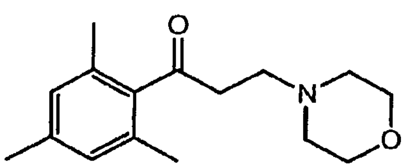
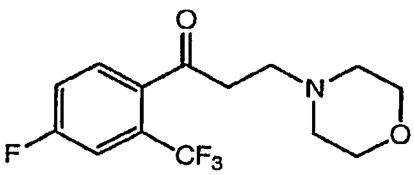
,
,

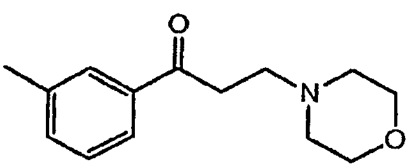
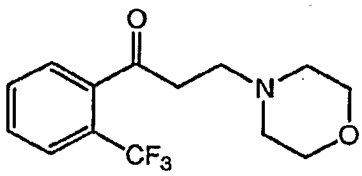
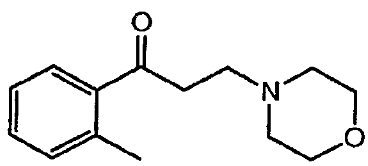
,
,

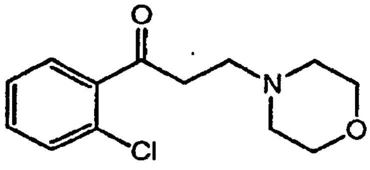
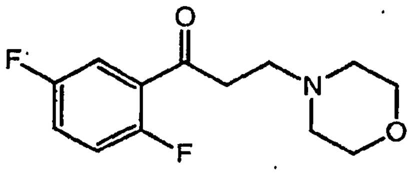
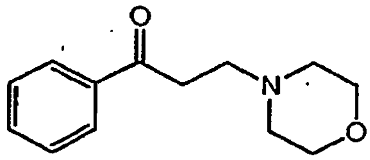
,
,

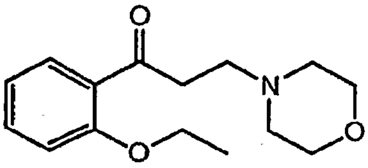
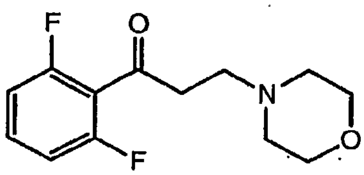
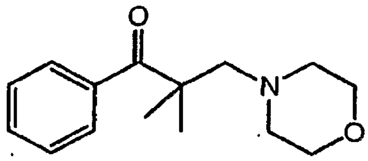
,
,

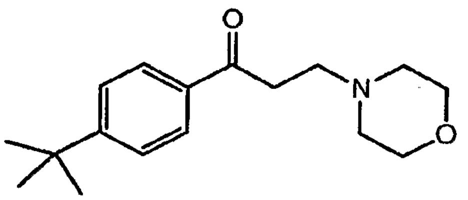
,
,

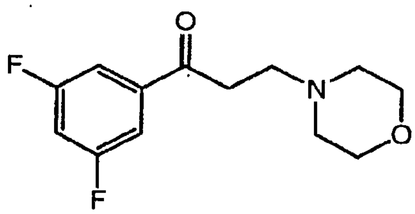
,
,
,

,
,
,

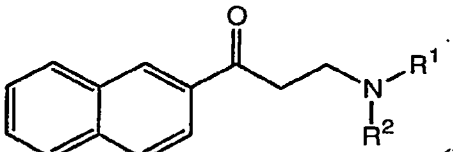
, , ,

 , , and

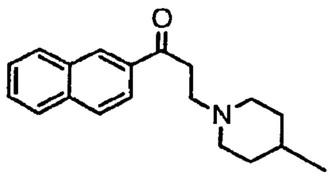
.

One example of a compound of formula (III) is .

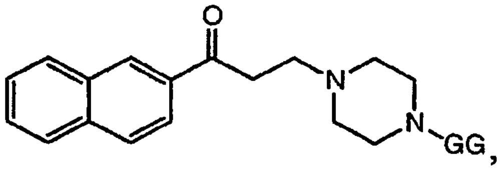
[69] Some compounds of the invention have the structural formula:

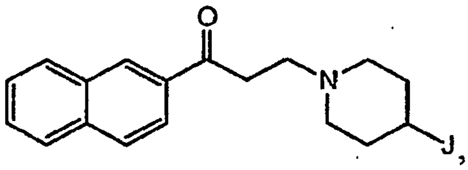


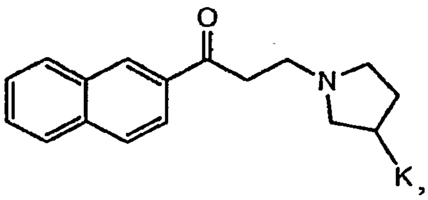
(IV)

wherein R^1 and R^2 are as defined above, with the exception of .

[70] Examples of such compounds include those of with the following structural formulae:



,


,


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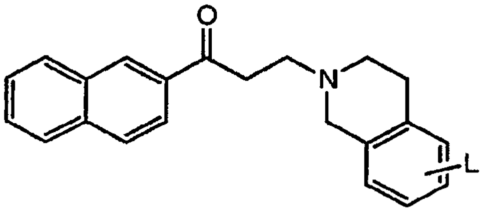
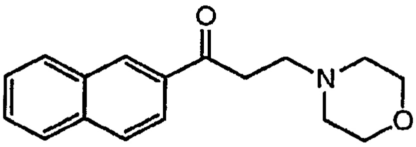
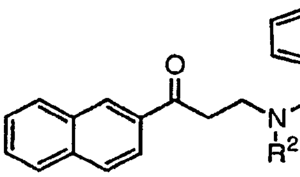
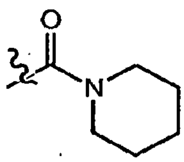


,


, and


,

in which:

GG is hydrogen, dimethylaminoalkyl, aryl, C_1-C_6 alkyl, cyclohexylalkyl, pyridine, $-COCF_3$; $-CONR'R''$, or 

;

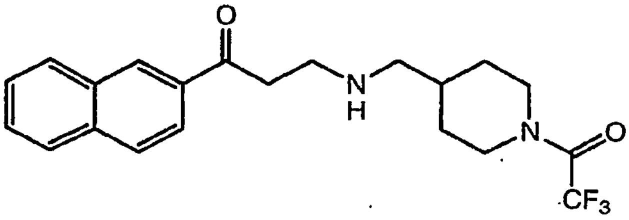
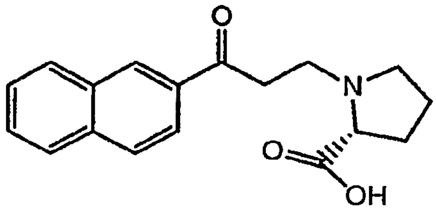
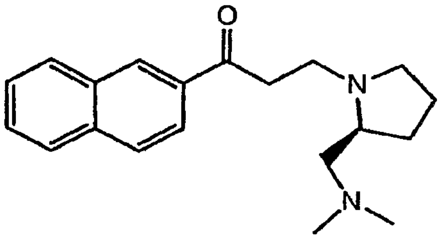
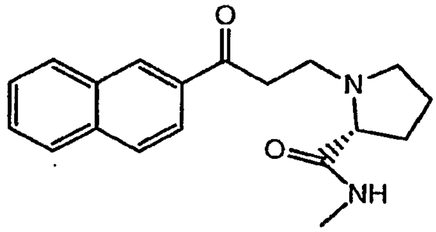
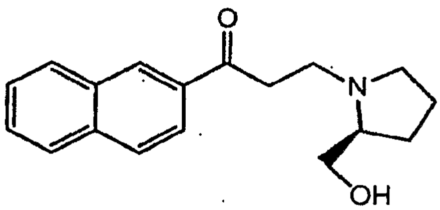
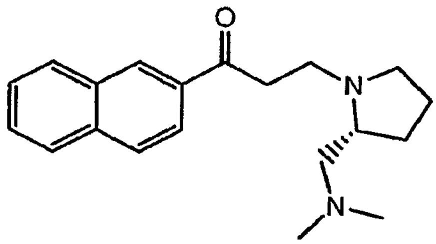
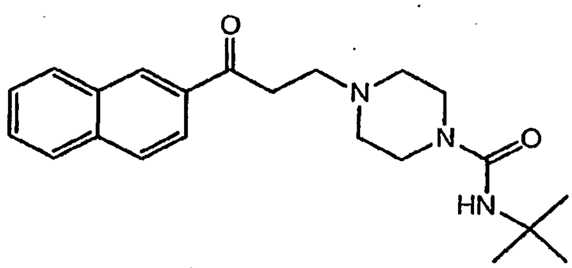
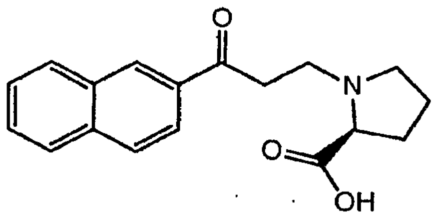
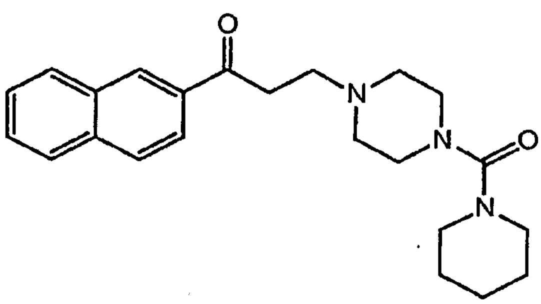
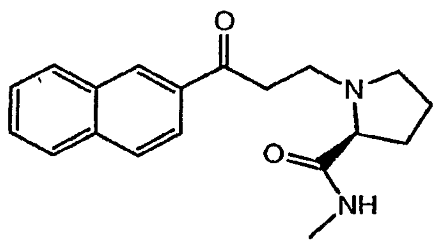
J is hydrogen, aralkyl, C_1-C_6 alkyl, $-CNHCOOR'$, or $NR'R''$;

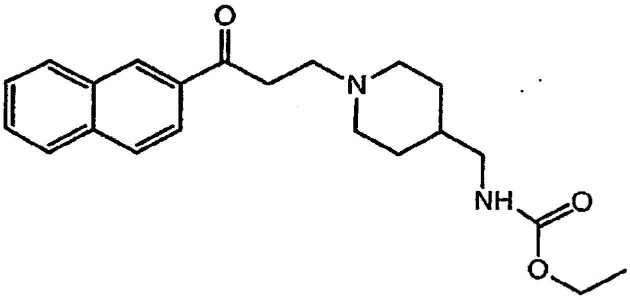
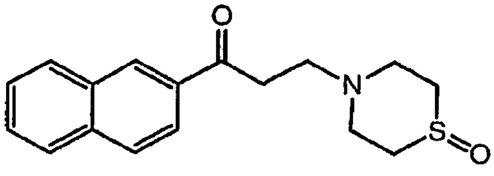
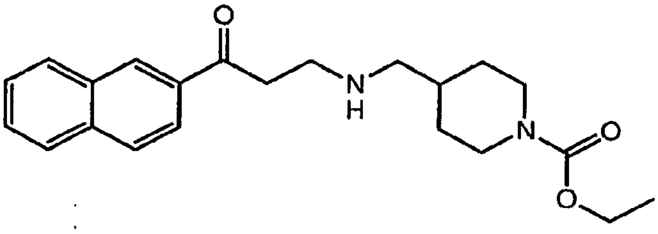
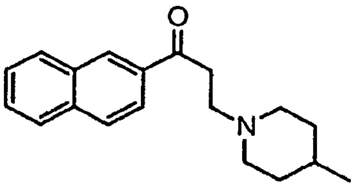
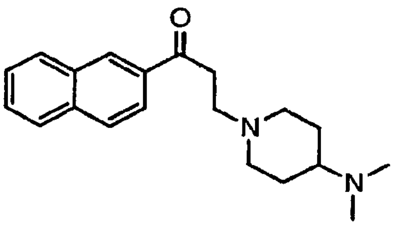
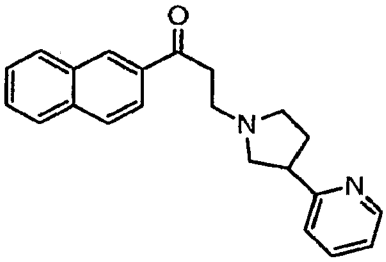
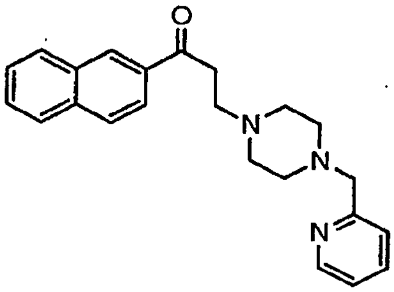
K is hydrogen, pyridine, aryl, --COOH, $-CONR'R''$, $-COH$, or $-CNR'R''$;

L is hydrogen or alkyloxy; and

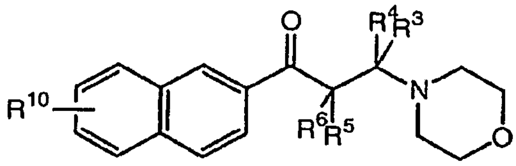
R^2 is as defined above.

[71] Other compounds of formula (IV) include:

,
,
,
,
,
,
,
,
,
,

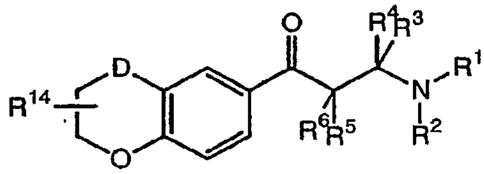
,
,
,
,
,
, and
.

[72] Other compounds have the structural formula:


(V)

wherein R^3, R^4, R^5, R^6, and R^{10} are as defined above.

[73] Other compounds have the structural formula:



(VI)

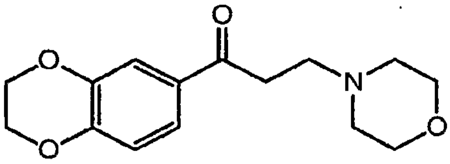
wherein:

R^1, R^2, R^3, R^4, R^5, and R^6 are as defined above; and

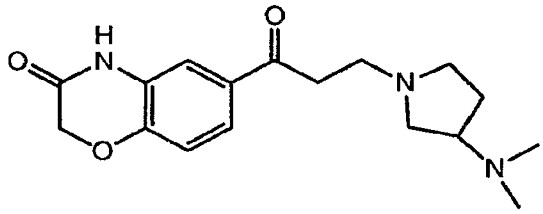
R^{14} is hydrogen or =O;

and D is CH or NH,

with the exception of:

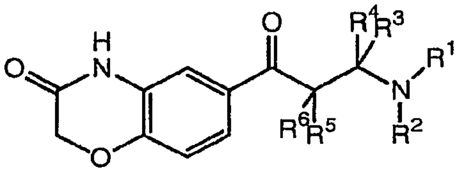


and



.

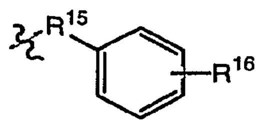
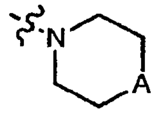
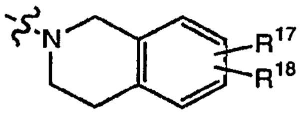
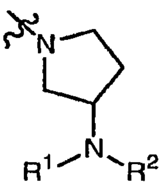
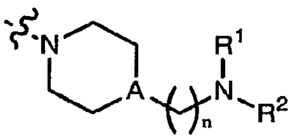
[74] Other compounds have the structural formula:

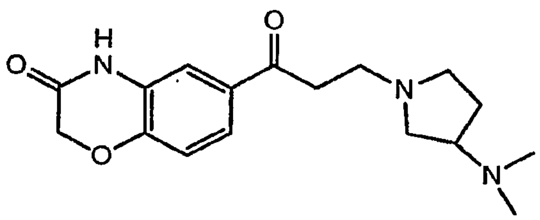


(VII)

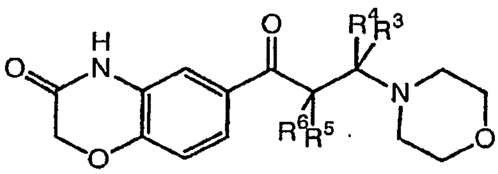
wherein:

R^3, R^4, R^5, and R^6 are as defined above; and

R^1 and R^2 are independently hydrogen, C_1-C_4 alkyl,  (wherein R^{15} is halogen or C_1-C_4 alkyl and R^{16} is C_1-C_4 alkyl), or R^1 and R^2 together with the nitrogen to which they are attached form an aryl group selected from ,  (wherein R^{17} and R^{18} are independently hydrogen or $-OCH_3$),  (wherein R^1 and R^2 are independently hydrogen or C_1-C_4 alkyl),  (n = 1-4), phenyl-C_1–C_4 alkyl (optionally substituted with halogen),

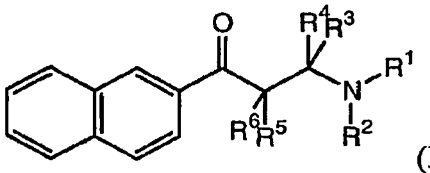
with the exception of .

[75] Other compounds have the structural formula:

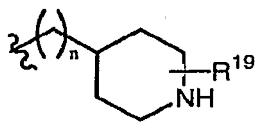
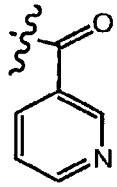
 (VIII)

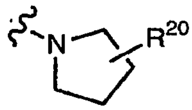
wherein R^3, R^4, R^5, and R^6 are as defined above.

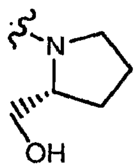
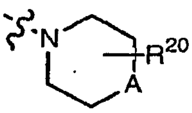
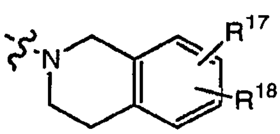
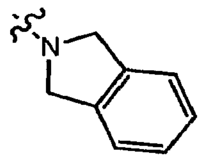
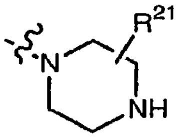
[76] Other compounds have the structural formula:

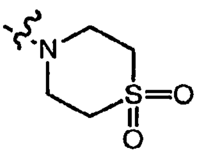
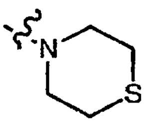
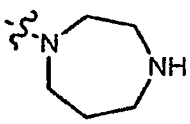

(IX)

wherein R^3, R^4, R^5, and R^6 are as defined above and wherein R^1 is hydrogen and R^2 is

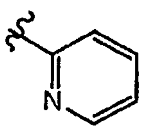
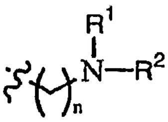
, wherein R^{19} is selected from hydrogen and ; or

R^1 and R^2 together with the nitrogen to which they are attached are ,

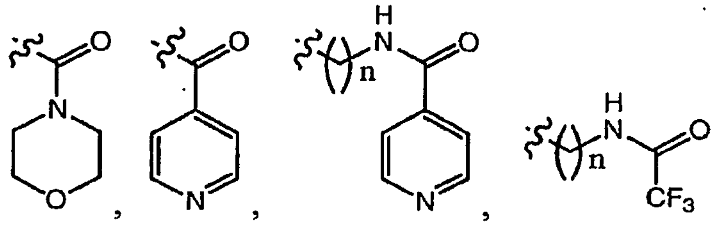
, , , , ,

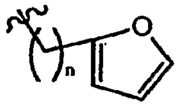
, , and ,

A is N or O;

R^{20} is phenyl-C_1-C_4 alkyl optionally substituted with one or more halogens, hydrogen, C_1-C_4 alkyl, amino- C_1-C_4 alkyl, , ;

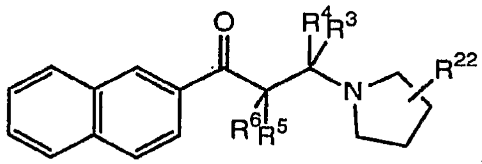
R^{17} and R^{18} are independently hydrogen or $-OCH_3$;

R^{21} is $-CONR'R''$, $-COR'$,  , and

 ; and

R′ and R″ are independently selected from hydrogen and C_1-C_4 alkyl.

[77] In other embodiments compounds have the structural formula:

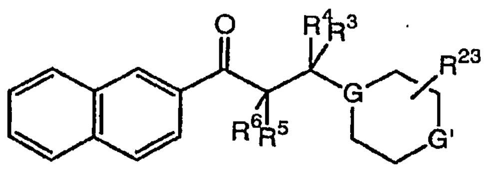


(X)

wherein R^3, R^4, R^5, and R^6 are as defined above and wherein R^{22} is selected from hydrogen, C_1-C_4 alkyl, $-NR'R''$, $-COH$, $-COOH$, $-CNR'R''$, and $-CONHR'$,

wherein R′ and R″ are as defined above.

[78] In other embodiments compounds have the structural formula:

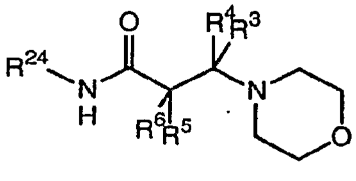


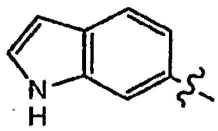
(XI)

wherein R^3, R^4, R^5, R^6, G, and G′ are as defined above; and R^{23} is hydrogen, $-NR'R''$ C_1-C_4 linear alkyl, C_1-C_4 alkyl, phenyl-C_1-C_4 alkyl, $-CONH_2$, and $-CO$ R′R″,

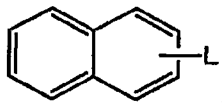
wherein R′ and R″ are as defined above.

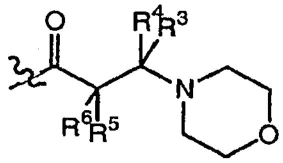
[79] In other embodiments compounds have the structural formula:


(XII)

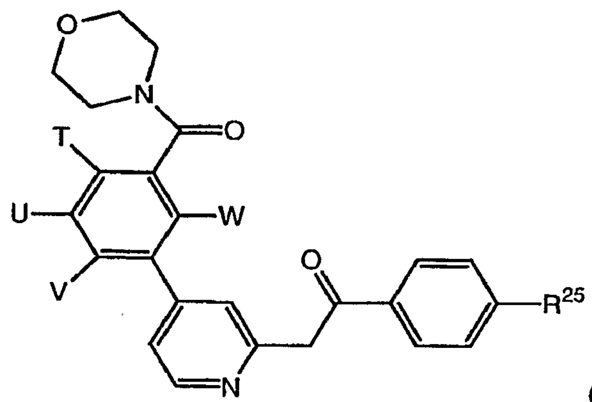
R^3, R^4, R^5, and R^6 are as defined above and wherein R^{24} is 

[80] In other embodiments compounds have the structural formula:


(XIII)

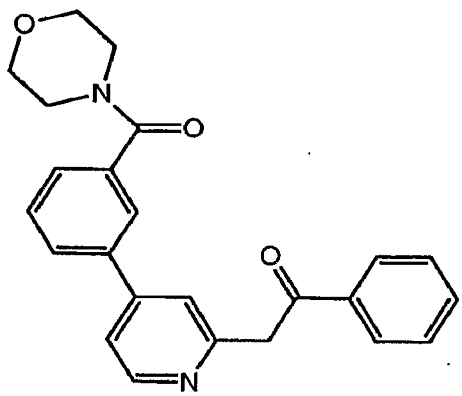
wherein L is , and wherein R^3, R^4, R^5, and R^6 are as defined above.

[81] Some compounds have the structural formula:

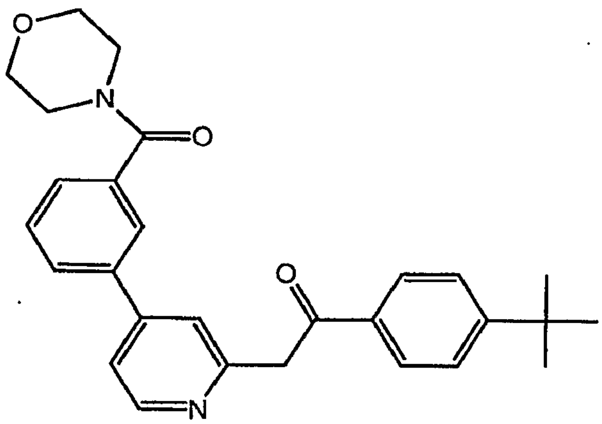


(XIV)

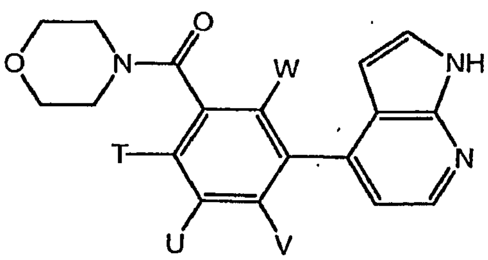
wherein T, U, V, and W independently are selected from hydrogen; halogen; −O; C_1-C_3 alkyl; and C_1-C_3 alkyloxy; and wherein R^{25} is hydrogen or C_1-C_3 alkyl. Representative compounds include:



and



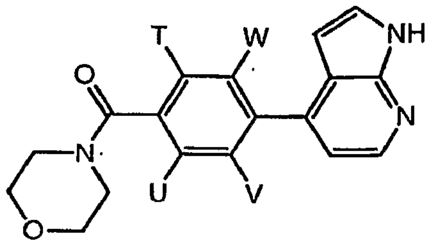
[82] Other compounds have the structural formula:



(XV),

wherein T, U, V, and W independently are selected from hydrogen; halogen; –O; C_1-C_3 alkyl; and C_1-C_3 alkyloxy; and wherein R^8 is hydrogen or C_1-C_3 alkyl.

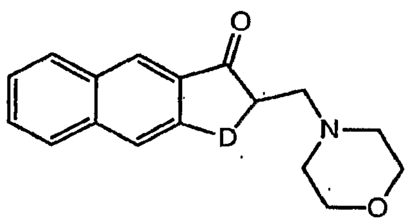
[83] Still other compounds have the structural formula:



(XVI),

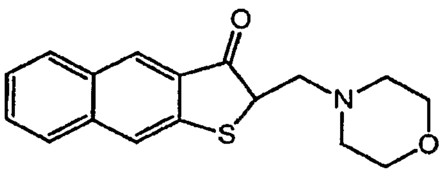
wherein T, U, V, and W independently are selected from hydrogen; halogen; –O; C_1-C_3 alkyl; and C_1-C_3 alkyloxy; and wherein R^8 is hydrogen or C_1-C_3 alkyl.

[84] Other compounds have the structural formula:

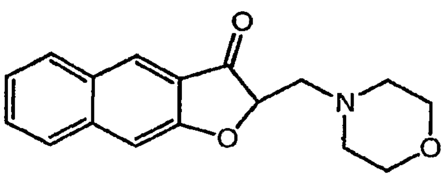


(XVII)

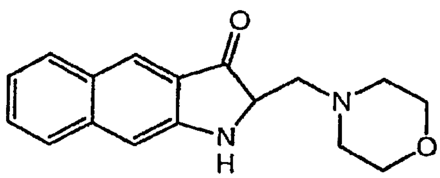
wherein D is S, O, or NH; *i.e.*,



,

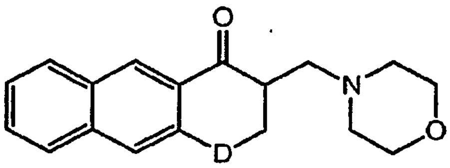


, and

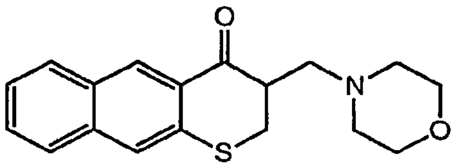


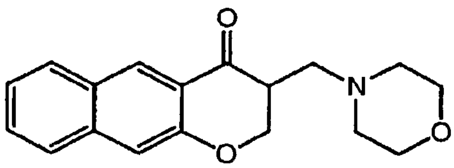
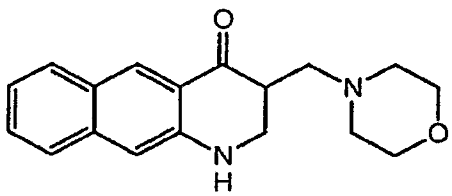
.

[85] Other compounds of the invention include those with the structural formula:

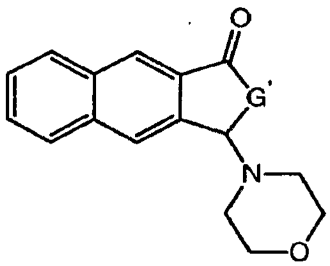


(XVIII),

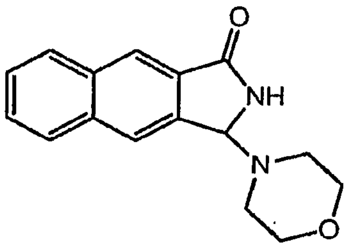
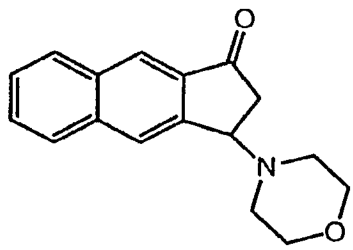
wherein D is defined above; *i.e.*,  ,

 , and  .

[86] Other compounds of the invention include those with the structural formula:



(XIX),

wherein G′ is NH or CH; *i.e.*,  or  .

[87] The invention also includes the compounds identified in Examples 15 and 16.

[88] The compounds of the present invention may have asymmetric centers and may occur as racemates, stereoisomers, and tautomers. The invention includes all possible racemates, tautomers, stereoisomers, and mixtures thereof.

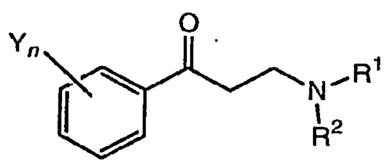
[89] Suitable methods of preparing compounds of the invention are illustrated by the representative examples provided below. Starting materials are known compounds and can be obtained by standard procedures of organic chemistry.

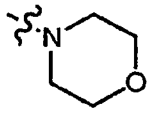
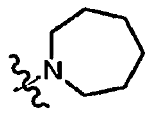
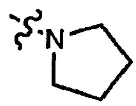
Provisos for compound claims

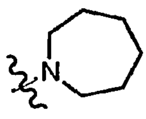
[90] Compounds of the invention preferably do not have one or more of the following activities: vasodilator, hypotensive, bradycardiac, anti-depressant, anti-arrhythmic, anti-arteriosclerotic, serum cholesterol lowering, triglyceride level lowering, neuroleptic, anti-inflammatory, tranquilizing, anti-convulsant, anesthetic, muscle relaxing, anti-fungal, anti-bacterial, insecticidal, fumigant, anti-parasitic, central nervous system depressant, antagonization of sedation, antipollakiurea, antihistamine, anti-allergy, bronchodilating, analgesic, spasmolytic, muscarinic antagonist, preventing or decreasing production of abnormally phosphorylated paired helical filament epitopes associated with Alzheimer's Disease, hypolipidemic, male anti-fertility, anti-sporicidal, inhibition of nitric oxide production, or central nervous system stimulant activities.

[91] To the extent any of the following compounds are not novel, Applicants reserve the right to present compound and/or composition claims which include a proviso excluding the compounds and/or their pharmaceutically acceptable salts from the scope of the claims:

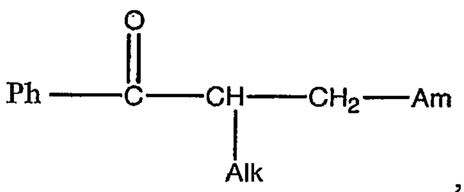
a. compounds having the structural formula:



wherein *n* is 0, 1, 2, or 3 and R^1 and R^2 together with the nitrogen atom to which they are attached are , , or  and Y is alkyl, halogen, halogenoalkyl, alkyoxy, akylthio, halogenoalkyloxy, halogenoalkylthio, cycloalkyl, or a cyane radical;

b. compounds of formula (I) in which Ar is phenyl, if R^3, R^4, R^5, and R^6 are each hydrogen, and R^1 and R^2 together form a ring with the nitrogen atom to which they are attached ;

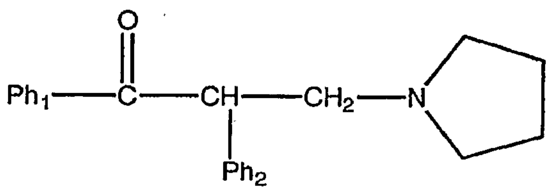
c. compounds having the structural fotmula formula:



,

in which Ph is an optionally substituted monocyclic carbocyclic aryl radical, Alk is C_1-C_3 lower alkyl, and Am is a tertiary amino group, salts, N-oxides, or quaternary ammonium derivatives thereof;

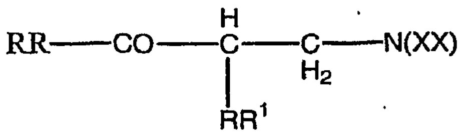
d. compounds having the structural formula:



,

in which Ph_1 and Ph_2 are monocyclic carboxylic aryl radicals and the acid addition salts thereof;

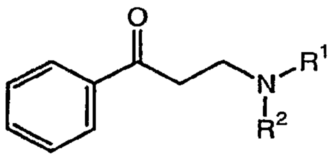
e. compounds having the structural formula:



,

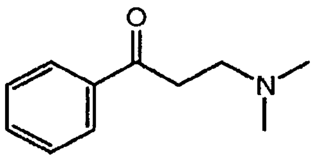
in which RR is selected from the group consisting of aliphatic, aromatic, and araliphatic radicals; RR^1 is selected from the group consisting of hydrogen, aliphatic, aromatic, and araliphatic radicals; –N(XX) is the residue of a secondary amine selected from the group consisting of dialkylamine and dialkylamines;

f. compounds having the structural formula:



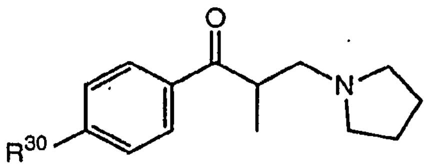
,

wherein R^1 and R^2 are as defined in formula (I), including the compound



;

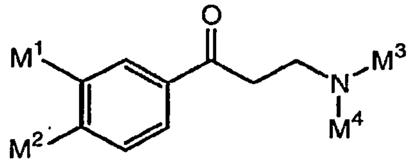
g. compounds having the structural formula:



,

wherein R^{30} is an ethyl-, propyl-, isopropyl-, butyl-, or isobutyl group or a cycloalkyl group having 5-7 carbon atoms;

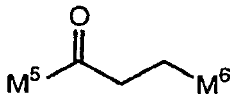
h. compounds having the structural formula:



,

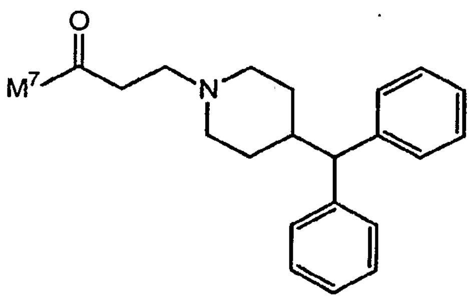
in which M^2 is hydrogen, halogen, or C_1-C_{12} alkoxy, M^1 is hydrogen or halogen, and M^3 and M^4 are lower alkyl or, taken together with the nitrogen atom to which they are attached, (a) are a heterocyclic amino group or an N-lower alkyl quaternary heterocyclic ammonium group or (b) a tri-lower alkyl-ammonium;

i. compounds having the structural formula:



or a picrate salt thereof, wherein M^5 is a simple or substituted aryl group and M^6 is a simple or substituted amino group;

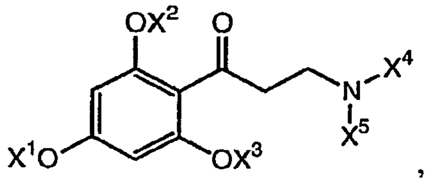
j. compounds having the structural formula:



,

in which M^7 is thienyl, phenyl or substituted phenyl;

k. compounds having the structural formula:

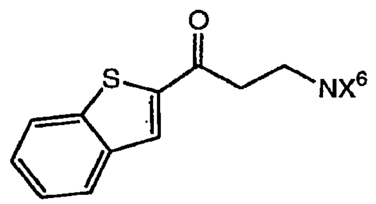


,

in which each of X^1, X^2, and X^3 are independently hydrogen or an alkyl group, and each of X^5 and X^4 are independently hydrogen or an alkyl group or, together with the nitrogen atom to which they are attached, form a heterocyclic group with 5, 6, or 7 ring atoms, optionally containing, in addition to N, a further heteroatom selected from N, S, and O;

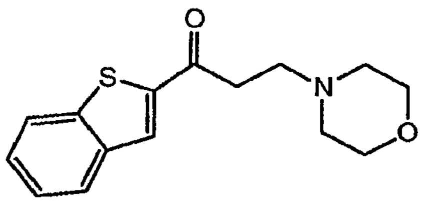
l. compounds of formula (II) in which R^9 is phenyl and R^3, R^4, R^5, and R^6 are each hydrogen;

m. compounds having the structural formula:

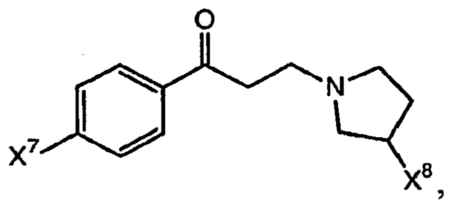


,

in which X^6 forms with the nitrogen atom pyrrolidine, piperidine, morpholine, hexamethyleneimine, or 3-azabicyclo-3,2,2 nonane, including the compound

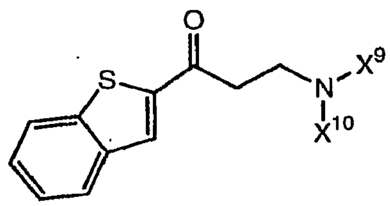


n. compounds having the structural formula:



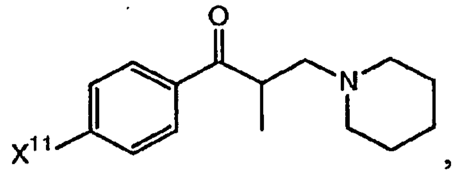
in which X^7 is hydrogen or fluorine; X^8 is $N(X^9)$phenyl (wherein the phenyl is optionally monosubstituted with C_1-C_8 alkoxy, C_1-C_8 alkyl, trifluoromethyl, or halogen), $-C(OH)(X^9)$ phenyl (wherein the phenyl is optionally monosubstituted with C_1-C_8 alkoxy, C_1-C_8 alkyl, trifluoromethyl, or halogen), or phenyl (wherein the phenyl is optionally monosubstituted with C_1-C_8 alkoxy, C_1-C_8 alkyl, trifluoromethyl, or halogen); and X^9 is hydrogen, C_1-C_8 alkyl, or lower alkanoyl;

o. compounds having the structural formula:



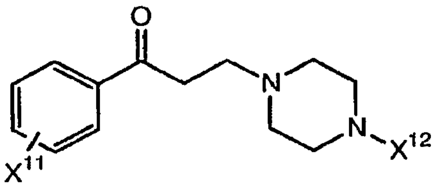
wherein X^9 and X^{10} each designate a saturated or unsaturated aliphatic hydrocarbon having 1 to 4 carbon atoms or, together with the nitrogen to which they are attached, form a heterocyclic radical selected from pyrrolidino, piperidine, perhydroazepino, and morpholino;

p. compounds having the structural formula:



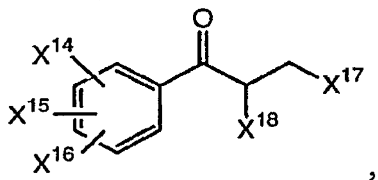
in which X^{11} is C_2-C_3 alkyl;

q. compounds having the structural formula:



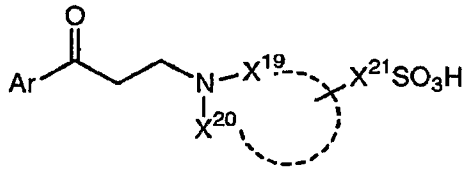
in which X^{11} is hydrogen, halogen, C_1-C_4 alkoxy, nitro, or C_1-C_4 secondary amine; X^{12} is $(CH_2)_nOX^{13}$; n is 2 or 3; and X^{13} is C_1-C_4 alkoxyphenyl, nitrophenyl, trifluoromethylphenyl, or phenyl disubstituted with two halogens, two C_1-C_4 alkyls, halogen and nitro, halogen and C_1-C_4 alkyl, halogen and C_1-C_4 alkoxy, or C_1-C_4 alkoxy and C_1-C_4 alkoyl;

r. compounds having the structural formula:



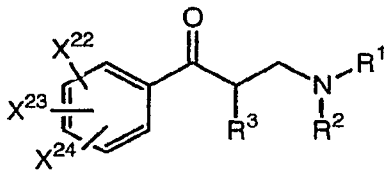
in which X^{14}, X^{15}, and X^{16} are independently hydrogen, halogen, C_1-C_4 alkyl, halogeno- C_1-C_4 alkyl, C_1-C_4 alkoxy, or a cycloalkyl group having 3-8 carbon atoms and two of X^{14}, X^{15}, and X^{16} may combine to form methylenedioxy or ethyleneoxy; X^{18} is hydrogen or C_1–C_4 alkyl; and X^{17} is pyrrolidinyl-, piperidinyl, morpholinyl-, or azepinyl;

s. compounds having the structural formula:



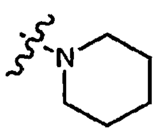
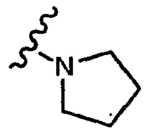
Ar denotes an aryl radical; and X^{19} and X^{20} (a) are both C_1-C_6 alkyl or (b) together with the N atom form the remaining members of a saturated heterocyclic radical and X^{21} is –OH, C_1-C_6 alkyl, or aryl;

t. compounds having the structural formula:

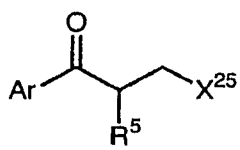


,

wherein R^1 and R^2 independently represent an alkyl radical; or R^1 and R^2, together with the nitrogen atom to which they are bonded complete an optionally substituted heterocyclic radical of the formula

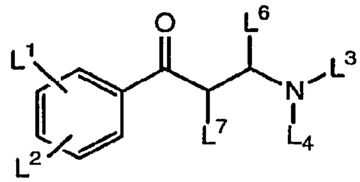
 or  ; R^3 is hydrogen or C_1-C_4 alkyl; and X^{22}, X^{23}, and X^{24} are independently C_1-C_4 alkyl, halogen, or a halogeno- C_1-C_4 alkyl, C_1-C_4 alkoxy, alkylthio, halogeno- C_1-C_4 alkoxy, halogeno- C_1-C_4 alkylthio, cycloalkyl 3 to 7 carbon atoms, or cyano;

u. compounds having the structural formula:



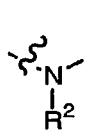
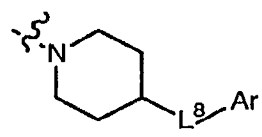
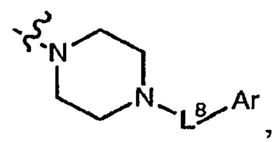
wherein Ar is non-substituted aryl or aryl substituted with a hydroxyl group, lower alkoxy group or halogen, or non-substituted benzo[b]thienyl group or benzo[b]thienyl group substituted by hydroxyl group, lower alkyl group, lower alkoxy group, aryl group or halogen; R^5 is hydrogen or C_1-C_4 alkyl; and X^{25} is a group other than piperidine;

v. compounds having the structural formula:

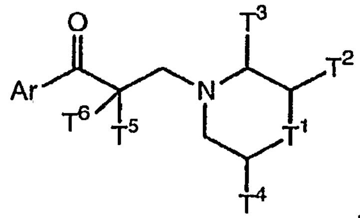


,

wherein L^1 and L^2 are independently halogen or alkyl; L^6 and L^7 are independently hydrogen or alkyl; and L^3 and L^4 are independently hydrogen or an aliphatic group or combine together with the nitrogen to which they are attached to form a ring;

w. compounds of formula (I), (IV), (VI), (VII), (IX), and (XI) in which if R^3 and R^4 are hydrogen, then  is not  or , wherein L^8 is a carbonyl, sulfonyl, methylene, or methylene substituted with optionally substituted phenyl; and Ar is an aryl group;

x. compounds having the structural formula:



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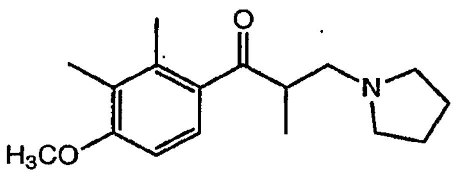
in which T^1 is O, S, or NT^7; T^7 is hydrogen, C_1-C_4 alkyl, and $CH_2CH_2COAr_1$; T^6 is hydrogen, C_1-C_6 alkyl, or T^6 and a substituent on the aryl group together represent CH_2, CH_2CH_2, CH_2O, or CH_2S to form a five or six membered ring where the ring is optionally substituted with C_1-C_6 alkyl or phenyl; T^5 is hydrogen, C_1-C_6 alkyl, or optionally substituted phenyl; T^2, T^3, and T^4 are independently hydrogen or C_1-C_6 alkyl; and Ar and Ar_1 are aryl or optionally substituted phenyl; and

y. the following compounds:



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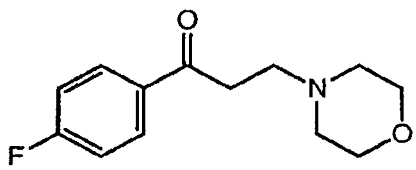

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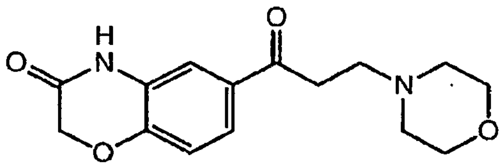

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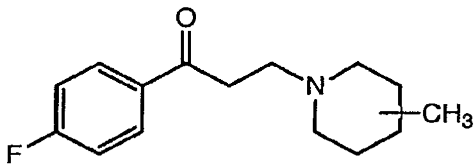

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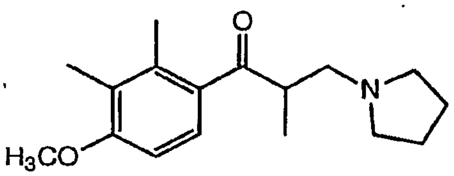

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Pharmaceutical Preparations

[92] Compounds of the invention can be formulated as pharmaceuticals using methods well known in the art. Pharmaceutical formulations of the invention typically comprise at least one compound of the invention mixed with a carrier, diluted with a diluent, and/or enclosed or encapsulated by an ingestible carrier in the form of a capsule, sachet, cachet, paper or other container or by a disposable container such as an ampoule.

[93] A carrier or diluent can be a solid, semi-solid or liquid material. Some examples of diluents or carriers which may be employed in the pharmaceutical compositions of the present invention are lactose, dextrose, sucrose, sorbitol, mannitol, propylene glycol, liquid paraffin, white soft paraffin, kaolin, microcrystalline cellulose, calcium silicate, silica polyvinylpyrrolidone, cetostearyl alcohol, starch, gum acacia, calcium phosphate, cocoa butter, oil of theobroma, arachis oil, alginates, tragacanth, gelatin, methyl cellulose, polyoxyethylene sorbitan monolaurate, ethyl lactate, propylhydroxybenzoate, sorbitan trioleate, sorbitan sesquioleate and oleyl alcohol.

[94] Pharmaceutical compositions of the invention can be manufactured by methods well known in the art, including conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

[95] For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as acetate, Hanks's solution, Ringer's solution, or physiological saline buffer. Preferably the solutions are sterile and non-pyrogenic. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[96] For oral administration, the active compound(s) can be combined with pharmaceutically acceptable carriers which enable the compound(s) to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like. Fillers can be used, such as gelatin, sugars (*e.g.*, lactose, sucrose, mannitol, or sorbitol); cellulose preparations (*e.g.*, maize starch, wheat starch, rice starch, potato starch, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose); and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[97] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions,

and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

[98] Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compound(s) may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration preferably are in dosages suitable for such administration.

[99] For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

[100] For administration by inhalation, pharmaceutical preparations of the invention can be delivered in the form of an aerosol sprays from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide, or other suitable gas. If desired, a valve can be used to deliver a metered amount. Capsules and cartridges of *e.g.*, gelatin for use in an inhaler or insufflator, may be formulated containing a powder mix of a compound and a suitable powder base such as lactose or starch.

[101] Compounds of the invention can be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

[102] Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds. Additionally, suspensions of the active compounds may be

prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

[103] Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

[104] The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

[105] Compounds of the invention typically are soluble and stable in 50 mM acetate at a concentration of 10 mg/ml or above, and can be delivered intraperitoneally and orally in this buffer. Some compounds are soluble in hydroxypropyl-b-cyclodextrin (HBPCD, 3-5%), and can be delivered intraperitoneally and orally in this solvent. For intravenous delivery, compounds can be suspended or dissolved in 5% mannitol.

[106] In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[107] The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

[108] In addition to the common dosage forms set out above, the compounds of the present invention may also be administered by controlled release means and/or delivery devices including ALZET® osmotic pumps which are available from Alza Corporation. Suitable delivery devices are described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; 3,944,064 and 4,008,719.

Therapeutic Methods

[109] The identified compounds can be administered to a human patient, either alone or in pharmaceutical compositions where they are mixed with suitable carriers or excipient(s) at doses to treat or ameliorate blood-related cancers (*e.g.*, lymphomas and leukemias) and autoimmune disorders. Reduction of intracellular kinase activity also is useful to suppress the immune system of transplant patients prior to, during, and/or after transplant.

[110] Lymphomas are malignant growths of B or T cells in the lymphatic system, including Hodgkin's lymphoma and non-Hodgkin's lymphoma. Non-Hodgkin's lymphomas include cutaneous T cell lymphomas (*e.g.*, Sezary syndrome and Mycosis fungoides), diffuse large cell lymphoma, HTLV-1 associated T cell lymphoma, nodal peripheral T cell lymphoma, extranodal peripheral T cell lymphoma, central nervous system lymphoma, and AIDS-related lymphoma.

[111] Leukemias include acute and chronic types of both lymphocytic and myelogenous leukemia (*e.g*, acute lymphocytic or lymphoblastic leukemia, acute myelogenous leukemia, acute myeloid leukemia, chronic myelogenous leukemia, chronic lymphocytic leukemia, T cell prolymphocytic leukemia, adult T cell leukemia, and hairy cell leukemia).

[112] Autoimmune disorders include systemic lupus erythematosus, anti-phospholipid antibody syndrome, multiple sclerosis, ulcerative colitis, Crohn's disease, rheumatoid arthritis, asthma, Hashimoto's thyroiditis, Reiter's syndrome, Sjögren's syndrome, Guillain-Barré syndrome, myasthenia gravis, large vessel vasculitis, medium vessel vasculitis,

polyarteritis nodosa, pemphigus vulgaris, scleroderma, Goodpasture's syndrome, glomerulonephritis, primary biliary cirrhosis, Grave's disease, membranous nephropathy, autoimmune hepatitis, celiac sprue, Addison's disease, polymyositis, dermatomyositis, monoclonal gammopathy, Factor VIII deficiency, cryoglobulinemia, peripheral neuropathy, IgM polyneuropathy, chronic neuropathy, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, pernicious anemia, ankylosing spondylitis, vasculitis, inflammatory bowel disease, and type I diabetes mellitus. The autoimmune disease may involve a secretory cell, such as a T lymphocyte, B lymphocyte, Mast cell, or dendritic cell. Compounds of the invention also can be used to treat patients who undergo protein replacement therapies and who develop antibodies to the replacement.

Routes of administration

[113] Pharmaceutical preparations of the invention can be administered locally or systemically. Suitable routes of administration include oral, pulmonary, rectal, transmucosal, intestinal, parenteral (including intramuscular, subcutaneous, intramedullary routes), intranodal, intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, intraocular, transdermal, topical, and vaginal routes. As described in more detail above, dosage forms include, but are not limited to, tablets, troches, dispersions, suspensions, suppositories, solutions, capsules, creams, patches, minipumps and the like. Targeted delivery systems also can be used (for example, a liposome coated with target-specific antibody).

Dosage

[114] A pharmaceutical composition of the invention comprises at least one active ingredient in a therapeutically effective amount. A "therapeutically effective dose" is the amount of an active agent which, when administered to a patient, results in a measurable improvement in a characteristic of the disease being treated (*e.g.*, improved laboratory values, retarded development of a symptom, reduced severity of a symptom, improved levels of a biological marker such as CD25a or IL2). The improvement can be evident after a single administration of the therapeutically effective dose. More usually multiple administrations are utilized in order to achieve or maintain optimal effect. In preferred

embodiments frequency of administration can range from twice a month to once a week to several times a day, for example 1-4 times a day. In alternative embodiments administration can be by time-release formulations, or extended or continuous infusions. The frequency of administration can be selected to achieve a systemic or local concentration at or above some predetermined level for a period of time. The period of time can be all or a substantial portion of the interval between administrations or comprise the period of time-release or infusion. In some embodiments, the treatment schedule can require that a concentration of the compound be maintained for a period of time (*e.g.*, several days or a week) and then allowed to decay by ceasing administration for a period of time (*e.g.*, 1, 2, 3, or 4 weeks).

[115] Determination of therapeutically effective amounts is well within the capability of those skilled in the art. A therapeutically effective dose initially can be estimated from *in vitro* enzyme assays, cell culture assays and/or animal models. For example, a dose can be formulated in an animal model to achieve a circulating concentration range that includes the IC_{50} as determined in an *in vitro* enzyme assay or in a cell culture (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of ITK or BTK activity). Such information can be used to more accurately determine useful doses in humans.

[116] Appropriate animal models for the relevant diseases are known in the art. *See, e.g., Exp Hematol. 34*, 284-88, 2006 (aggressive systemic mastocytosis and mast cell leukemia); *Leuk. Lymphoma. 47*, 521-29, 2006 (acute myeloid leukemia); *Leuk. Lymphoma. 7*, 79-86, 1992 (disseminated human B-lineage acute lymphoblastic leukemia and non-Hodgkins lymphoma); *J. Virol. 79*, 9449-57, 2006 (adult T-cell leukemia); *Neoplasia 7*, 984-91, 2005 (lymphoma); *Oligonucleotides 15*, 85-93, 005 (lymphoma); *Transfus. Apher. Sci. 32*, 197-203, 2005 (cutaneous T cell lymphoma); *Nature 17*, 254-56, 1991 (follicular lymphoma and diffuse large cell lymphoma); *Cell. Mol. Immunol. 2*, 461-65, 2005 (myasthenia gravis); *Proc. Natl. Acad. Sci. USA 102*, 11823-28, 2005 (type I diabetes); *Arthritis Rheum. 50*, 3250-59, 2004 (lupus erythymatosus); *Clin. Exp. Immunol. 99*, 294-302, 1995 (Grave's disease); *J. Clin. Invest. 116*, 905-15, 2006 (multiple sclerosis); *Pharmacol Res.* e-published Feb. 1, 2006 (ulcerative colitis); *J. Pathol.* e-published March 21, 2006 (Crohn's disease); *J. Clin. Invest. 116*, 961-973, 2006 (rheumatoid arthritis); *Endocrinol. 147*, 754-61, 2006 (asthma); *Exp Mol Pathol. 77*, 161-67, 2004 (Hashimoto's thyroiditis); *J. Rheumatol. Suppl. 11*, 114-17, 1983 (Reiter's syndrome); *Rheumatol. 32*, 1071-75, 2005 (Sjögren's syndrome); *Brain Pathol. 12*, 420-29, 2002 (Guillain-Barré syndrome); *J. Clin. Invest.* 110, 955-63, 2002 (vessel vasculitis); *Vet. Pathol. 32*, 337-45, 1995 (polyarteritis nodosa); *Immunol. Invest. 3*,47-61, 2006 (pemphigus vulgaris); *Arch. Dermatol. Res. 297*, 333-44, 2006 (scleroderma); *J. Exp. Med. 191*, 899-906, 2000 (Goodpasture's syndrome); *J. Vet. Med. Sci. 68*, 65-68, 2006 (glomerulonephritis); *Liver Int. 25*, 595-603, 2005 (primary biliary cirrhosis); *Clin. Exp. Immunol. 99*, 294-302, 1995 (Grave's disease); *J. Clin. Invest. 91*, 1507-15, 1993 (membranous nephropathy); *J. Immunol. 169*, 4889-96, 2002 (autoimmune hepatitis); *Isr. J. Med. Sci. 15*, 348-55, 1979 (celiac sprue); *Surgery 128*, 999-1006, 2000 (Addison's disease); *J. Neuroimmunol. 98*, 130-35, 1999 (polymyositis); *Am. J. Pathol. 120*, 323-25, 1985 (dermatomyositis); *Bone 20*, 515-20, 1997 (monoclonal gammopathy); *Haemophilia 11*, 227-32, 2005 (Factor VIII deficiency); *Proc. Natl. Acad. Sci. USA 94*, 233-36, 1997 (cryoglobulinemia); *Pain 110*, 56-63, 2004 (peripheral neuropathy); *Ann. Neurol. 49*, 712-20, 2001 (IgM polyneuropathy); *J. Neurosci. Res: 44*, 58-65, 1996 (chronic neuropathy); *Eur. J. Immunol. 32*, 1147-56, 2002 (autoimmune hemolytic anemia); *Haematologica 88*, 679-87, 2003 (autoimmune thrombocytopenic purpura); *Curr. Top. Microbiol. Immunol. 293*, 153-77, 2005 (pernicious anemia); *J. Immunol. 175*, 2475-83, 2005 (ankylosing spondylitis); *Inflamm. Res. 53*, 72-77, 2004 (vasculitis); *Vet. Pathol. 43*, 2-14, 2006 (inflammatory bowel disease); and *J. Biol. Chem. 276*, -13821, 2001 (anti-phospholipid antibody syndrome).

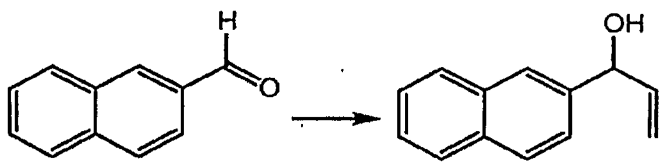
[117] LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population) can be determined by standard pharmaceutical procedures in cell cultures and/or experimental animals. Data obtained from cell culture assays or animal studies can be used to determine initial human doses. As is known in the art, the dosage may vary depending upon the dosage form and route of administration used.

[118] As is well known, the FDA guidance document "Guidance for Industry and Reviewers Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers" (HFA-305) provides an equation for use in calculating a human equivalent dose (HED) based on *in vivo* animal studies. Based on the studies described in Example 16, below, the human equivalent dose ranges between 1.5 mg/kg and 8 mg/kg, with some compounds showing considerable efficacy at lower or higher doses than those estimated by the HED. Thus, human dosages for systemic administration can range from, *e.g.*, 1.5 mg/kg to 3 mg/kg; 2 mg/kg to 4 mg/kg; 5 mg/kg to 7 mg/kg; and 4 mg/kg to 8 mg/kg. The amount of composition administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the disorder, the manner of administration and the judgment of the prescribing physician.

[119] All patents, patent applications, and references cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples, which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

Preparation of 1-naphthalen-2-yl-prop-2-en-1-ol

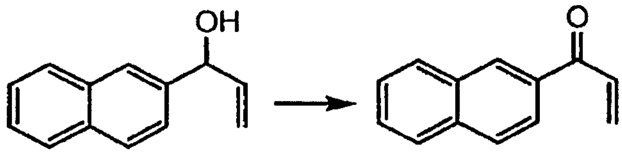


[120] Naphthaldehyde (5.0 g, 32.0 mmole) was dissolved in anhydrous tetrohydrofuran and stirred at -78 °C under N_2 (g) atmosphere. To the mixture was added vinyl magnesium bromide (50 ml, 1 M solution in THF) and the reaction was warmed to room temperature and stirred overnight. The reaction was quenched with water and partitioned between EtOAc and water. The organic layer was washed with brine, dried over sodium sulfate,

filtered, and concentrated under vacuum to give the desired product as yellow oil (5.0 g, 85%). ESI-MS m/z 185 $(M+H)^+$.

EXAMPLE 2

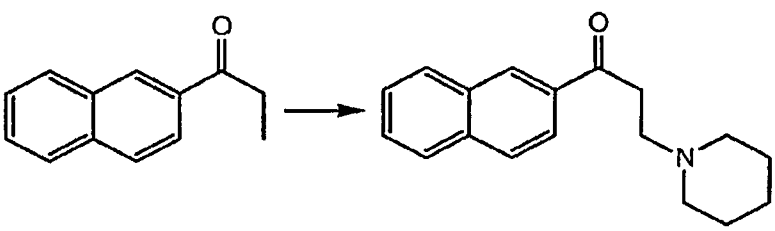
Preparation of 1-naphthalen-2-yl-propenone



[121] To a solution of 1-naphthalen-2-yl-prop-2-en-1-ol (1.3 g, 7.0 mmole) in 30 ml of dichloromethane was added pyridinium chlorochromate (1.5 g, 7.0 mmole). The mixture was stirred at room temperature until oxidation was complete. The solution was filtered through celite and the solvent was concentrated under vacuum. The residue was re-dissolved in EtOAc and washed with water and brine, dried over sodium sulfate, filtered, and concentrated under vacuum. The residue was purified by HPLC using a 0-100% EtOAc-Hx gradient to give the desired product as yellow oil (280 mg, 22%). ESI-MS m/z 183 $(M+H)^+$.

EXAMPLE 3

Preparation of 1-naphthalen-2-yl-3-piperidin-1-yl-propan-1-one

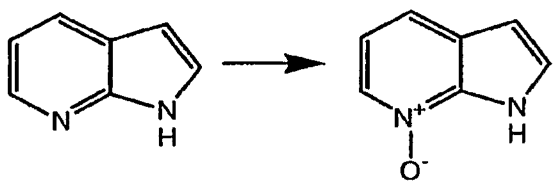


[122] 1-Naphthalen-2-yl-propenone (10 mg, 0.05 mmole) was dissolved in 100 µl of DMSO in one well of a 96 well polypropylene plate. To the mixture was added piperidine (12 µl, 0.10 mmole) and diisopropylethyl amine (17 µl, 0.1mmole). After completion, the

product was purified using HPLC to give the desired product (50 mm x 10 mm Phenomenex GEMINI™ column using a 30-100% acetonitrile-water gradient). ESI-MS m/z 268 $(M+H)^+$.

EXAMPLE 4

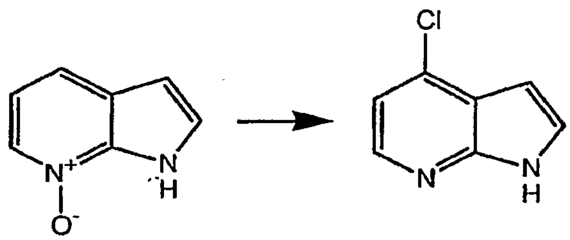
Preparation of 1H-Pyrrolo[2,3-b]pyridine 7-oxide



[123] 7-Azaindole (10g, 84.7 mmol) was dissolved in ether (300 mL) at room temperature. M-CPBA (29.1 g, 1.5 eq.) was added in portions and stirred by manual agitation. After all oxidant was added, the mixture was stirred at room temperature for a further 3 hours. LC/MS showed complete conversion. The mixture was filtered, and the solid was washed with ether (40 mL X 3) and air-dried. NMR analysis of this solid in d6-DMSO obtained showed the product as mostly the *meta*-Chloro benzoic acid salt of 1H-Pyrrolo[2,3-b]pyridine 7-oxide (off white, 17.9 g); MS: *m/z* 135.3 $[MH^+]$.

EXAMPLE 5

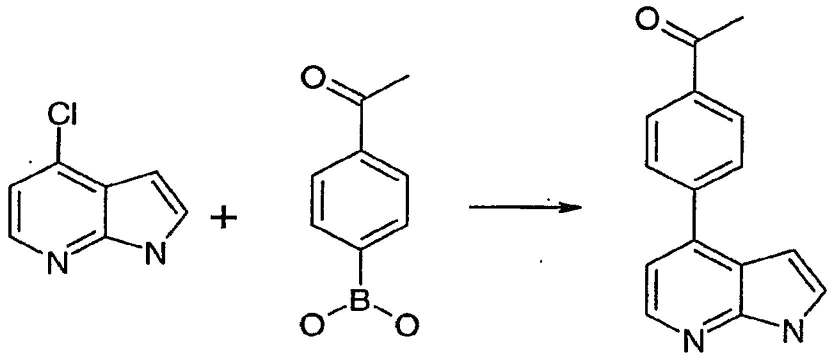
Preparation of 4-Chloro-1H-pyrrolo[2,3-b]pyridine



[124] The *m*-CBA salt of 1H-Pyrrolo[2,3-b]pyridine 7-oxide (9 g) was taken into $POCl_3$ (46 mL, 7.5 eq.). The mixture was heated at 90 °C for 15 hours and to 106 °C for another 4 hours. The mixture was cooled to room temperature, and most of the $POCl_3$ was distilled off under high vacuum. The residue was dissolved in CH_3CN (10 mL). Water (20 mL) was added slowly to quench the reaction. The resulted mixture was adjusted to pH ~ 9 using 10 N NaOH. The solid was filtered. The crude solid was redissolved in several ml of THF and combiflashed using 0-10% MeOH in DCM to give 4-Chloro-1H-pyrrolo[2,3-b]pyridine as a slightly yellowish solid. (4 g). MS: *m/z* 154.9 [MH^+].

EXAMPLE 6

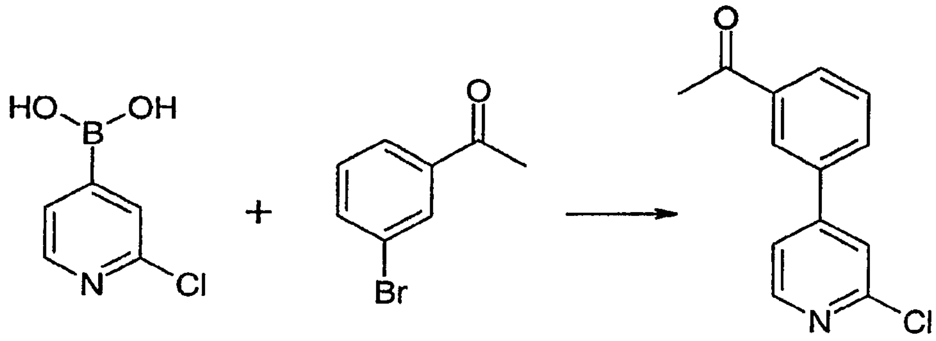
Preparation of 1-[4-(1H-Pyrrolo[2,3-b]pyridin-4-yl)-phenyl]-ethanone



[125] 4-Chloro-1H-pyrrolo[2,3-b]pyridine (500 mg, 3.27 mmol) was dissolved in dioxane (11 mL). 4-Acetyl phenylboronic acid (802 mg, 4.9 mmol, 1.5 eq), $dppfPdCl_2$ (41 mg, 0.03mmol, 0.01 eq) and Na_2CO_3 (2 N aq., 8.6 mL) were charged. The mixture was vacuumed and flushed with N_2 and microwaved at 160 °C for 15 minutes. Six batches of this same reaction were carried out. The crude mixture was pooled and partitioned between DCM (40 mL) and water (20 mL). Combi-flash of the residue using hexane/EtOAc (0% to 100%) gave the free base azaindole derivative as a slightly yellowish solid. The solid was redissolved in DCM (20 mL) and stirred in an ice bath. A 2M HCl solution in ether (10 mL) was added dropwise. The precipitate was filtered and dried to give 1-[4-(1H-Pyrrolo[2,3-b]pyridin-4-yl)-phenyl]-ethanone. (2.5g, 48%). MS: *m/z* 237.3 [MH^+].

EXAMPLE 7

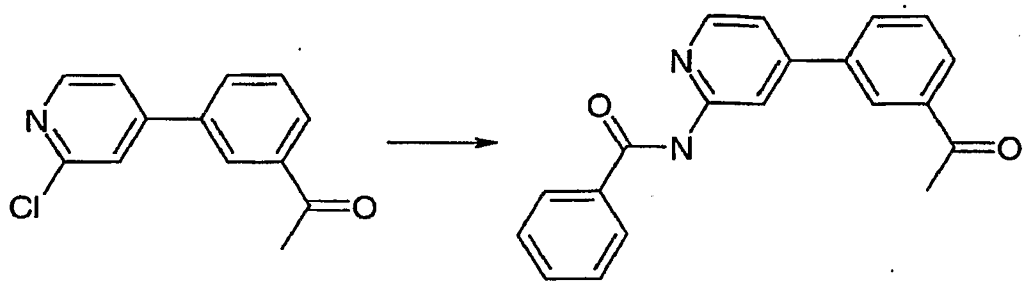
Preparation of 1-[3-(2-Chloro-pyridin-4-yl)-phenyl]-ethanone



[126] 2-Chloropyridine-4-boronic acid (11.0 g, 69.9 mmol), 3-Bromoacetophenone (11.2 mL, 83.9 mmol, 1.2 eq.), Na_2CO_3 (35 mL, 244.65 mmol, 3.5 eq.) and dppf$PdCl_2$ (572 mg, 0.07 mmol, 0.01 eq.) were mixed in THF (200 mL). The mixture was heated to reflux and continued at this temperature for 6 hours. It was then cooled and concentrated *in vacuo*. The residue was partitioned between DCM and water (100 mL/40 mL). The layers were separated and the aqueous layer was washed further with DCM (2 x 40 mL). The combined organic layer was dried (Na_2SO_4) and filtered. The filtrate was concentrated, and the residue was chromatographed using 1/1 hexane/EtOAc to give 1-[3-(2-Chloro-pyridin-4-yl)-phenyl]-ethanone as a white solid (9.5 g, 58%). MS: *m/z* 232.1 [MH^+].

EXAMPLE 8

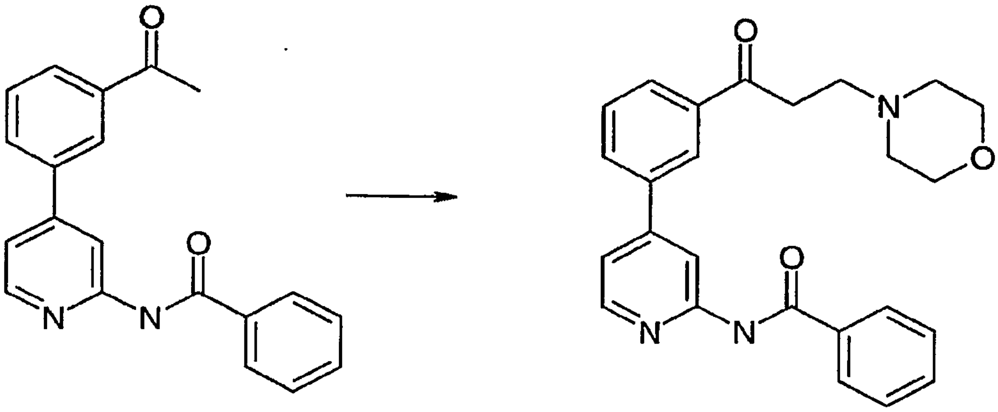
Preparation of N-[4-(3-Acetyl-phenyl)-pyridin-2-yl]-benzamide



[127] A degassed mixture of 1-[3-(2-Chloro-pyridin-4-yl)-phenyl]-ethanone (500mg, 2.16 mmol), benzamide (523mg, 4.32 mmol, 2 eq.), Xantphos (120 mg, 0.21 mmol, 0.1 eq.), $Pd(OAc)_2$ (24 mg, 0.10 mmol, 0.05 eq.), K_2CO_3 (448 mg, 3.24 mmol, 1.5 eq.) in dioxane (12 mL) was irradiated with microwaves at 150 °C for 1 hour. LC/MS control. Conversion was mostly 100% based on disappearance of starting material. Dimer (M+: 392) being the major by-product. If any starting material is unreacted at this point, another portion of Xantphos and $Pd(OAc)_2$ may be added and the mixture microwaved for another 30 minutes. The mixture was then partitioned between DCM and water (20 mL/10 mL). The layers were separated and the aqueous layer was washed further with DCM (2 x 20 mL). The combined organic layer was dried (Na_2SO_4) and filtered. The filtrate was concentrated and the residue was chromatographed using 1/1 Hexane/EtOAc to give N-[4-(3-Acetyl-phenyl)-pyridin-2-yl]-benzamide as a white solid (375 mg, 55%). MS: *m/z* 317.1 [MH^+].

EXAMPLE 9

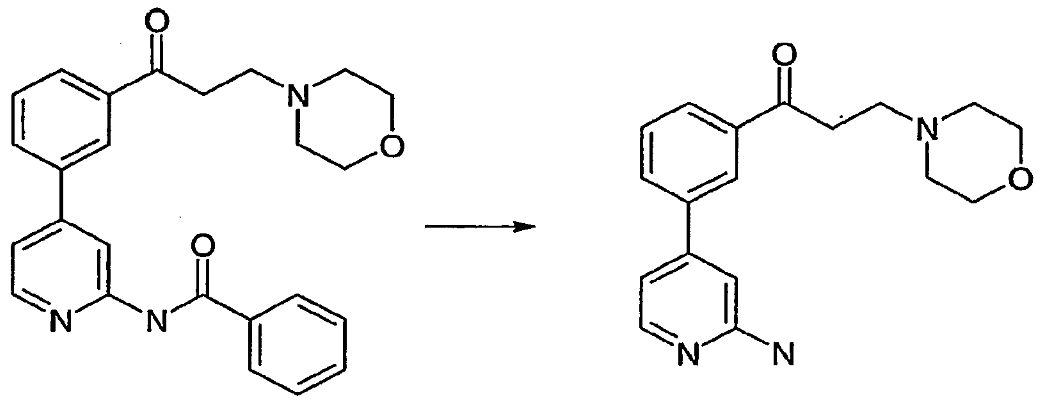
Preparation of N-{4-[3-(3-Morpholin-4-yl-propionyl)-phenyl]-pyridin-2-yl}-benzamide



[128] N-[4-(3-Acetyl-phenyl)-pyridin-2-yl]-benzamide (200mg, 0.632 mmol), morpholine HCl salt (78 mg, 0.632 mmol, 1 eq.) and paraformaldehyde (19 mg, 0.632 mmol, 1 eq.) were mixed with dioxane (2 mL) in a microwave tube. It was irradiated at 180 °C for 15 minutes. The mixture was partitioned between DCM/water (10 mL/5 mL). The aqueous layer was washed further with DCM (2 x 10 mL). The combined organic layer was dried (Na_2SO_4) and filtered. The filtrate was concentrated and the residue was chromatographed using 20/1 DCM/MeOH to give N-{4-[3-(3-Morpholin-4-yl-propionyl)-phenyl]-pyridin-2-yl}-benzamide as a slightly yellow solid (100 mg, 38%). MS: *m/z* 416.3 [MH^+].

EXAMPLE 10

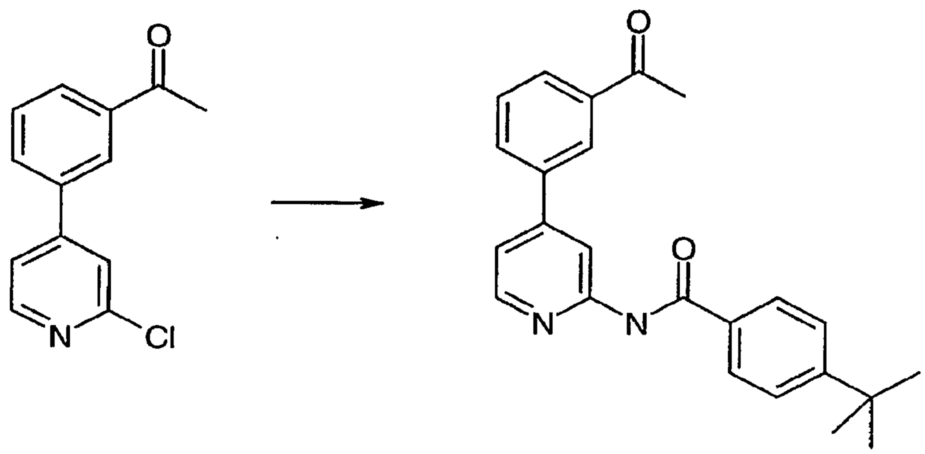
Preparation of 1-[3-(2-Amino-pyridin-4-yl)-phenyl]-3-morpholin-4-yl-propan-1-one



[129] N-{4-[3-(3-Morpholin-4-yl-propionyl)-phenyl]-pyridin-2-yl}-benzamide (100 mg, 0.32 mmol) was dissolved in HCl (2 mL, 6 N). The mixture was irradiated with microwaves at 140 °C for 30 minutes. The mixture was diluted with DCM (20 mL) and neutralized with NaOH to pH ~ 9. The layers were separated and the aqueous layer was washed further with DCM (2 X 15 mL). The combined organic layer was dried (Na_2SO_4) and filtered. The filtrate was concentrated and the residue was purified to give 1-[3-(2-Amino-pyridin-4-yl)-phenyl]-3-morpholin-4-yl-propan-1-one (TFA salt) as a white solid (84 mg, 78%). MS: *m/z* 312.3 [MH^+].

EXAMPLE 11

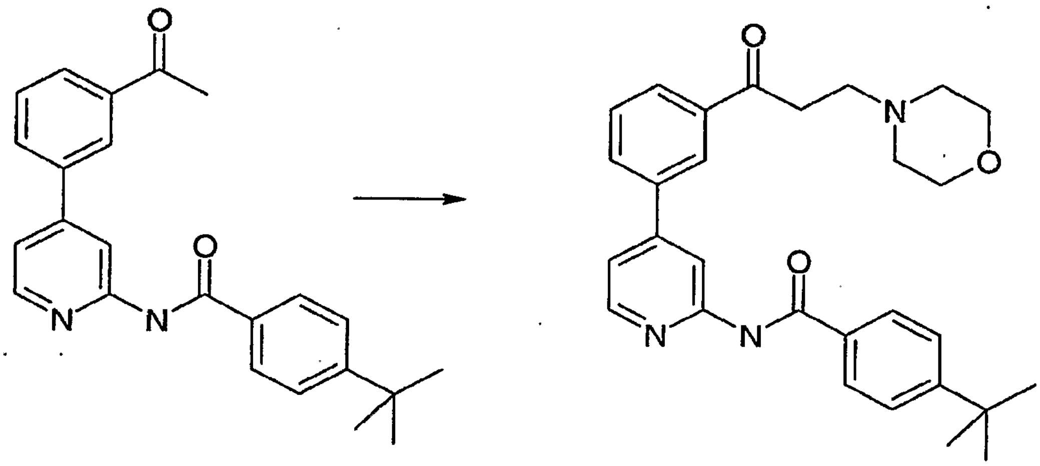
Preparation of N-[4-(3-Acetyl-phenyl)-pyridin-2-yl]-4-tert-butyl-benzamide



[130] According the same procedure for the preparation of N-[4-(3-Acetyl-phenyl)-pyridin-2-yl]-benzamide, N-[4-(3-Acetyl-phenyl)-pyridin-2-yl]-4-tert-butyl-benzamide (130 mg, 81%, slight impurity) was obtained from 1-[3-(2-Chloro-pyridin-4-yl)-phenyl]-ethanone (100 mg, 0.43 mmol) and 4-tert-butylbenzamide (153 mg, 0.86 mmol). MS: *m/z* 373.1 [MH$^+$].

EXAMPLE 12

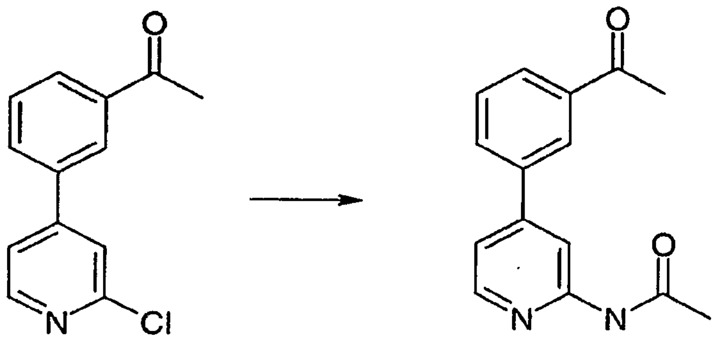
Preparation of 4-tert-Butyl-N-{4-3-(3-morpholin-4-yl-propionyl)-phenyl]-pyridin-2-yl}-benzamide



[131] According to the same procedure for the preparation of 1-[3-(2-Amino-pyridin-4-yl)-phenyl]-3-morpholin-4-yl-propan-1-one, 4-tert-Butyl-N-{4-3-(3-morpholin-4-yl-propionyl)-phenyl]-pyridin-2-yl}-benzamide (12 mg, 30%) was obtained from N-[4-(3-Acetyl-phenyl)-pyridin-2-yl]-4-tert-butyl-benzamide (36 mg, 0.1 mmol). MS: *m/z* 472.3 [MH^+].

EXAMPLE 13

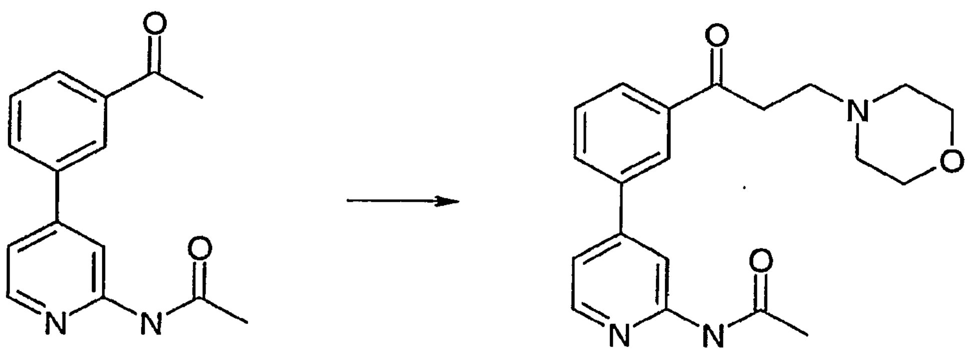
Preparation of N-[4-(3-Acetyl-phenyl)-pyridin-2-yl]-acetamide



[132] According the same procedure for the preparation of N-[4-(3-Acetyl-phenyl)-pyridin-2-yl]-benzamide, N-[4-(3-Acetyl-phenyl)-pyridin-2-yl]-acetamide (50 mg, 50%, slight impurity) was obtained from 1-[3-(2-Chloro-pyridin-4-yl)-phenyl]-ethanone (100 mg, 0.43 mmol) and acetamide (26 mg, 0.86 mmol). MS: *m/z* 255.1 [MH$^+$].

EXAMPLE 14

Preparation of N-{4-[3-(3-Morpholin-4-yl-propionyl)-phenyl]-pyridin-2-yl}-acetamide



[133] According to the same procedure for the preparation of 1-[3-(2-Amino-pyridin-4-yl)-phenyl]-3-morpholin-4-yl-propan-1-one, N-{4-[3-(3-Morpholin-4-yl-propionyl)-phenyl]-pyridin-2-yl}-acetamide (10 mg, 20%) was obtained from N-[4-(3-Acetyl-phenyl)-pyridin-2-yl]-acetamide (50 mg, 0.2 mmol). MS: *m/z* 354.3 [MH^+].

EXAMPLE 15

In vitro assays

Measurement of IL-2 Production

[134] Human T cell lines were plated in 96 well plates pre-coated with anti-CD3 monoclonal antibodies. Wells were either left untreated or treated with anti-CD28 for 2 days. The supernatant was collected and tested for IL-2 production in the presence or absence of a test compound using a human IL-2 ELISA assay.

T Cell Proliferation Assay

[135] Human T cell lines were plated in 96 well plates pre-coated with anti-CD3 monoclonal antibodies. Wells were either left untreated or treated with anti-CD28 for 2 days. Cell proliferation was measured in the presence or absence of a test compound using a commercially available CELLTITER-GLO™ assay (Promega).

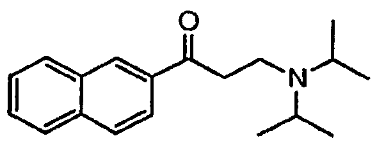
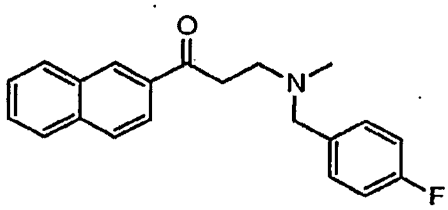
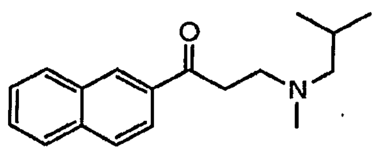
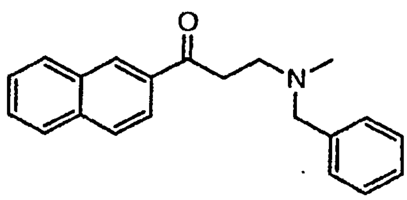
In vitro Kinase Assays

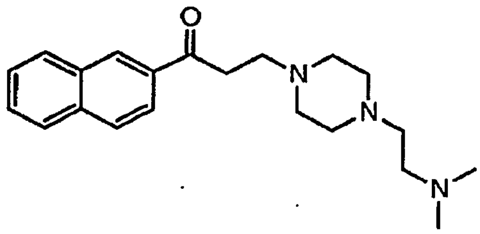
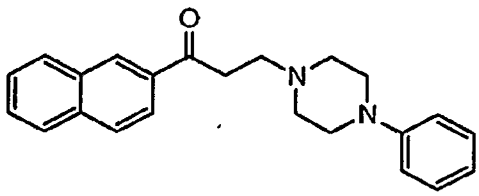
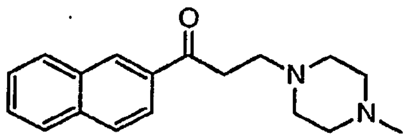
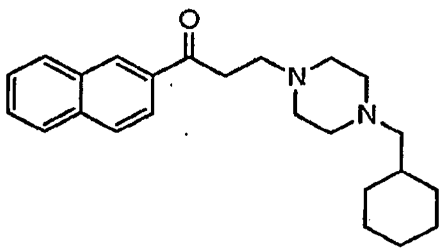
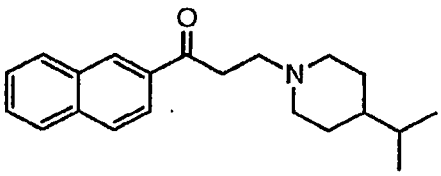
[136] Compounds were screened using the HITHUNTER™ enzyme fragment complementation method (Discoverx). Briefly, a recombinantly produced, N-terminally His-tagged ITK kinase domain (amino acids 352-617) was incubated with various concentrations of individual compounds. ATP and substrate were added, and the kinase reaction was allowed to proceed for 2-16 hours. Commercially available detection reagents were added and allowed to react for 2-4 hours. The reaction was evaluated by luminescence. Initial results were confirmed using full-length recombinant ITK protein.

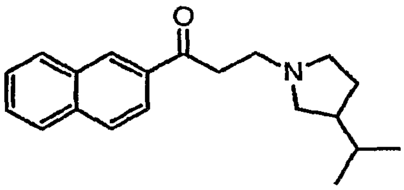
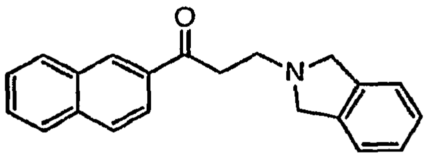
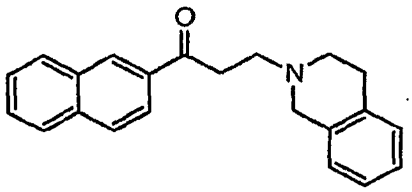
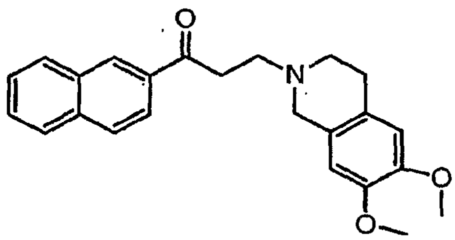
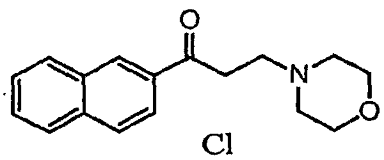
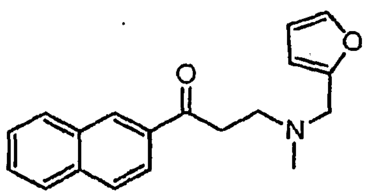
[137] Similarly, commercially available reagents such as HITHUNTER™ were used to evaluate the effect of compounds on the activity of additional kinases. The kinase domains of BTK, LCK and ERK were expressed as recombinant purified proteins were used for these studies.

[138] The compounds in Table 1 were tested and shown to inhibit IL-2 production, to inhibit T cell proliferation, and to inhibit ITK with an IC_{50} of less than 1 μM.

Table 1

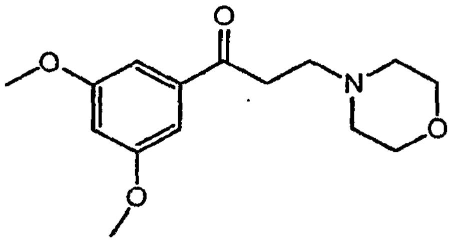
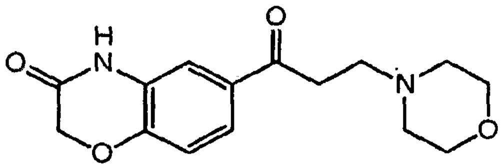
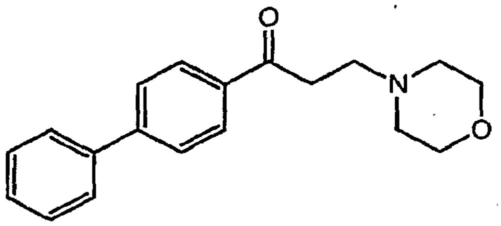
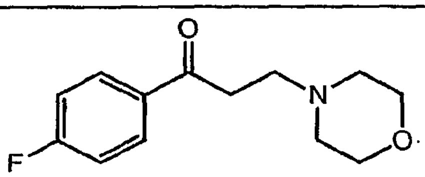
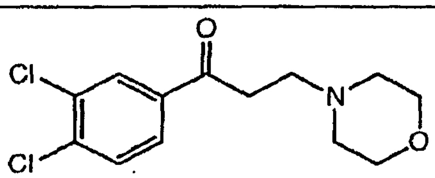
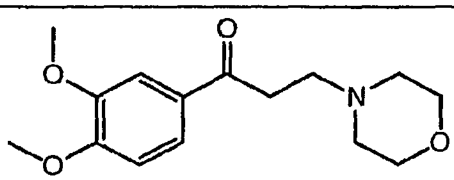
Compound	**IC_{50} (μM)**
	0.01807
	0.00954
	0.01355
	0.02851

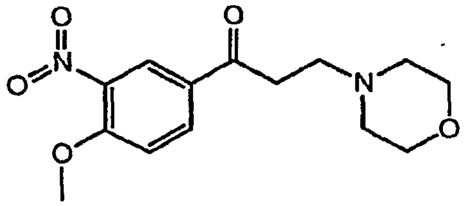
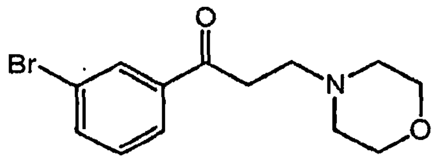
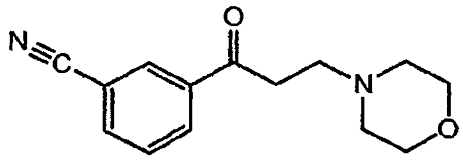
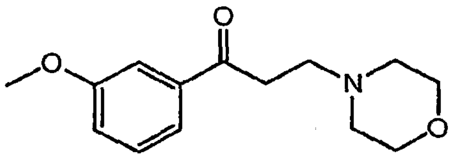
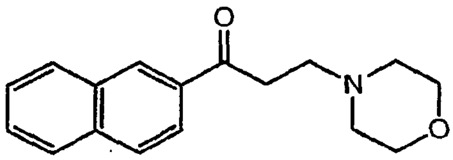
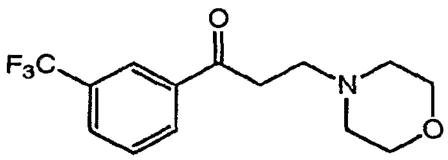
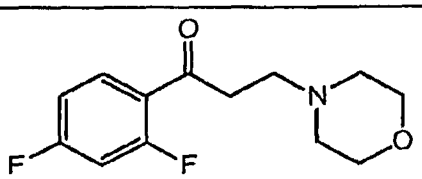
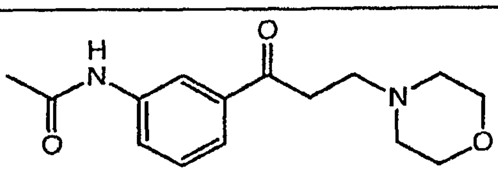
	0.00533
	0.00426
	0.05043
	0.0114
	0.01327

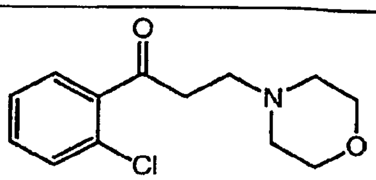
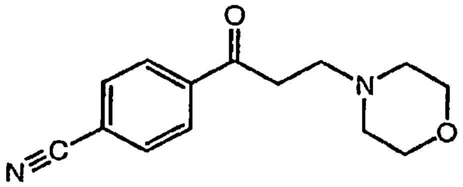
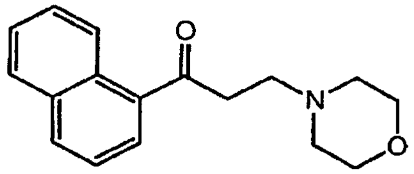
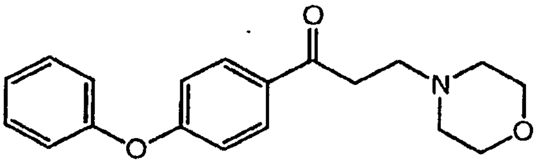
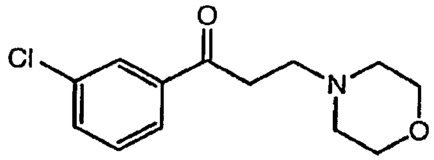
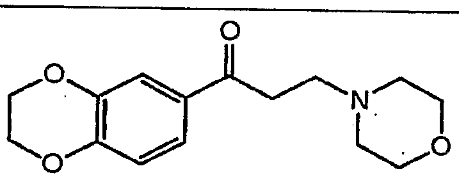
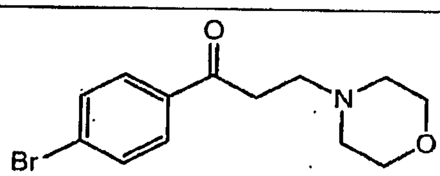
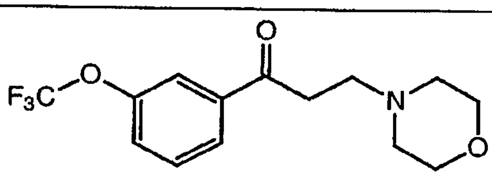
	0.00686
	0.02855
	0.01825
	0.00085
	0.07194
	0.01964

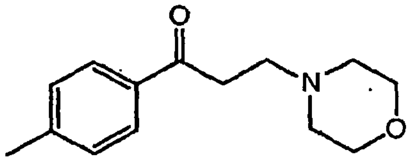
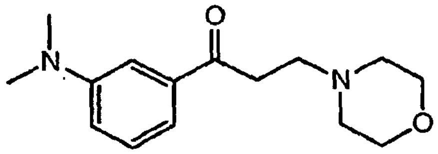
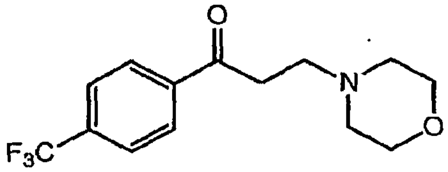
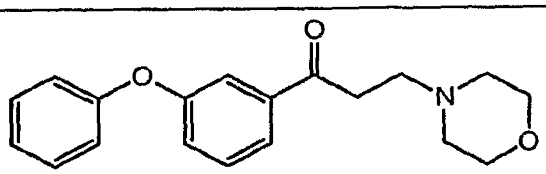
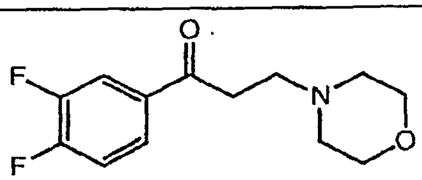
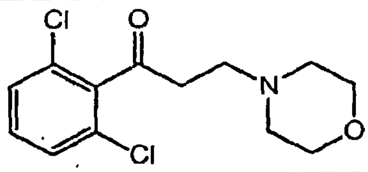
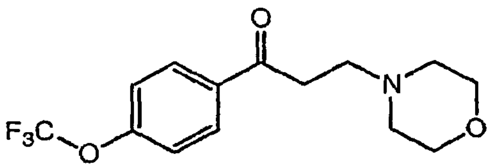
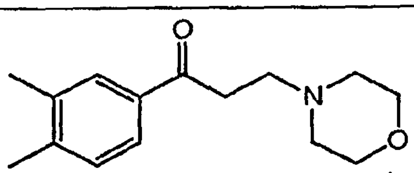
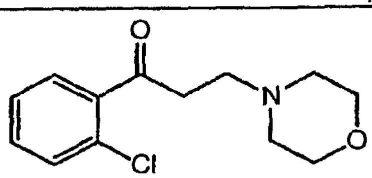
[139] The compounds in Tables 2-5 were tested in *in vitro* kinase assays:

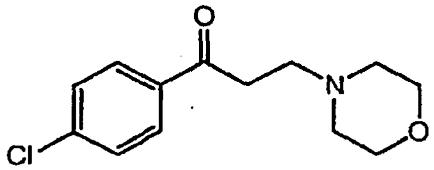
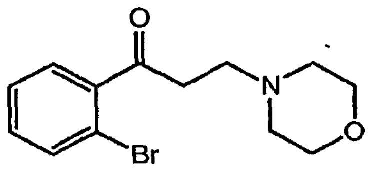
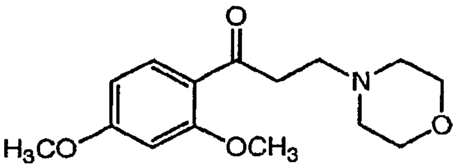
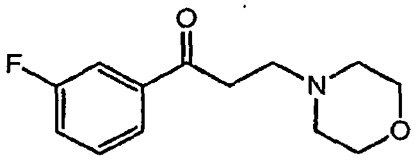
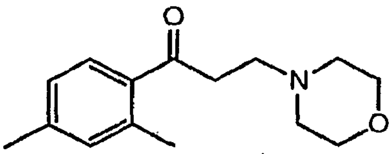
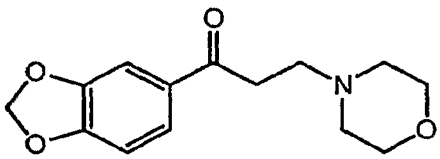
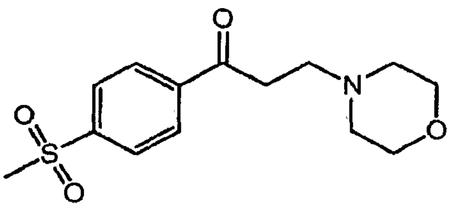
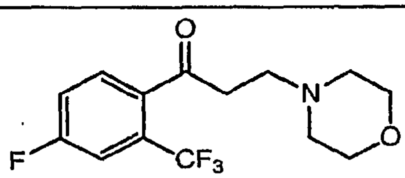
Table 2

Compound	IC_{50} ITK (µM)	IC_{50} BTK (µM)	IC_{50} LCK (µM)
	0.005	0.42482	12.55299
	0.040	0.27584	2.89341
	0.04022	0.0369	8.03843
	0.013	1.41274	27.7419
	0.013	0.10223	34.05941
	0.014	1.83528	23.89837

	0.020		
	0.025	0.36501	NO IC50
	0.029	0.64341	413.06105
	0.035	0.94241	16.4214
	0.036	0.039	19.969
	0.043	0.6561	27.11277
	0.056	0.86517	NO IC50
	0.065	1.24489	18.43928

	0.067	0.12463	28.09552
	0.072	0.7368	NO IC50
	0.065	0.39763	23.16665
	0.091	0.09415	18.46087
	0.077	0.77538	47.61179
	0.096	1.53948	20.8277
	0.104	0.23242	NO IC50
	0.148	0.77352	28.01341

	0.180	1.52018	163.63704
	0.186	3.67569	20.64831
	0.199	0.4735	NO IC50
	0.207	0.09415	18.46087
	0.208	2.89272	33.1157
	0.207	0.08071	NO IC50
	0.219	1.30729	NO IC50
	0.223	1.47599	21.15799
	0.241	0.81405	NO IC50

	0.290	0.68214	25.86619
	0.305	0.74064	NO IC50
	0.345	3.1355	21.10834
	0.381	3.03351	32.57859
	0.385	1.47531	25.34326
	0.385	3.92321	23.25
	0.385	0.75252	23.94596
	0.468	1.21899	NO IC50

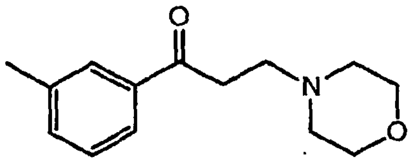
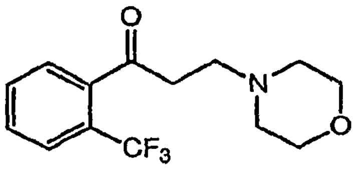
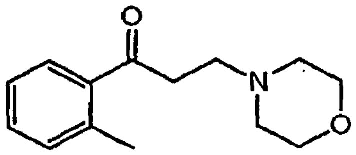
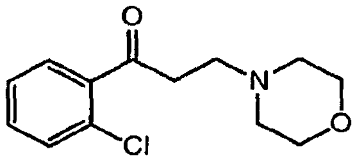
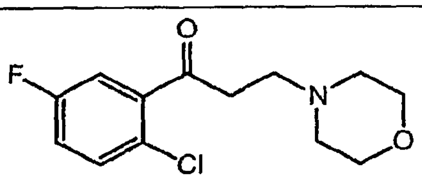
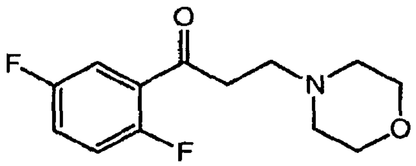
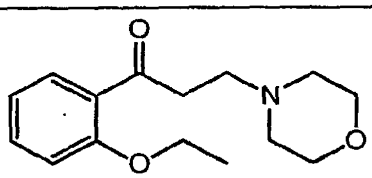
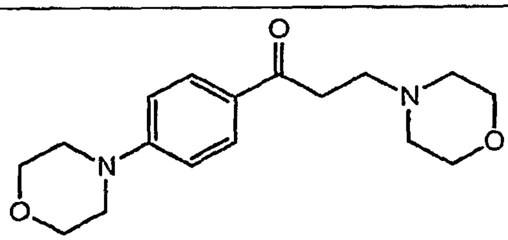
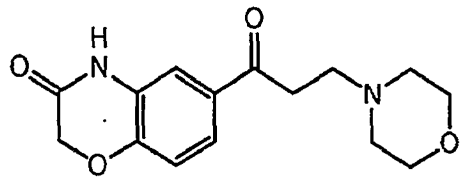
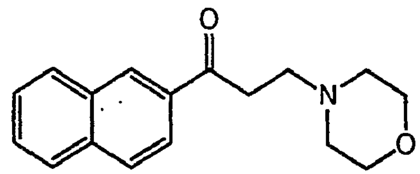
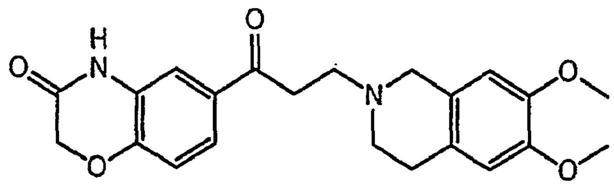
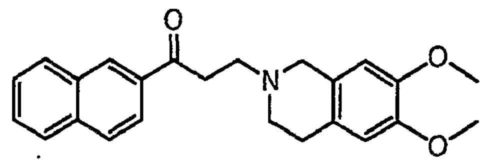
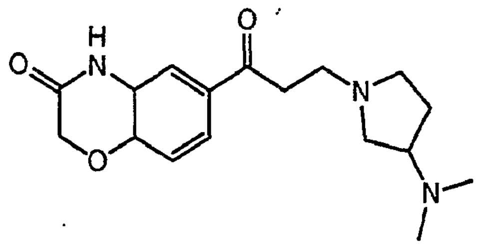
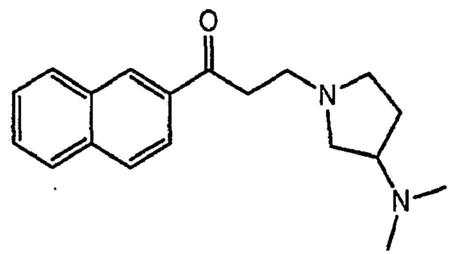
	0.560	3.06627	24.36134
	0.569	1.01979	NO IC50
	0.611	2.31114	NO IC50
	0.797	3.62429	NO IC50
	0.935	0.99267	29.52378
	0.874	2.57662	NO IC50
	1.279	0.55617	704.77096
	1.406	4.1378	27.08267

Table 3

Compound	IC_{50} ITK (μM)	IC_{50} BTK (μM)	naphthyl analog	IC_{50} ITK (μM)	IC_{50} BTK (μM)
	0.0059	0.7810		0.020	0.03947
	0.030	0.276		0.007	0.01276
	0.072	1.894		0.007	0.00215

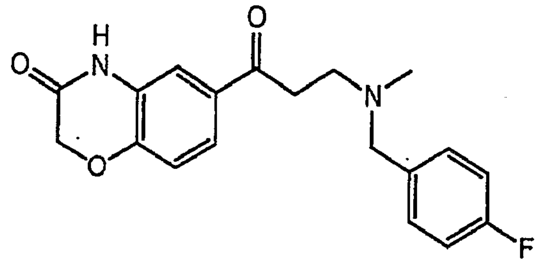
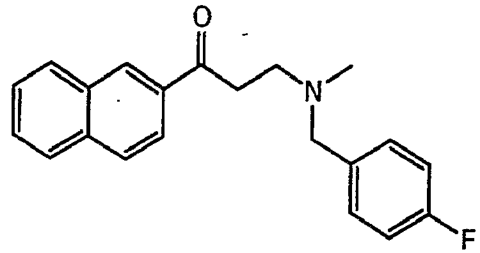
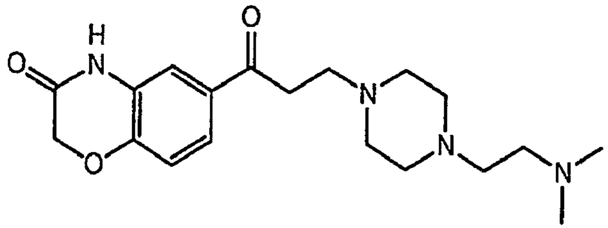
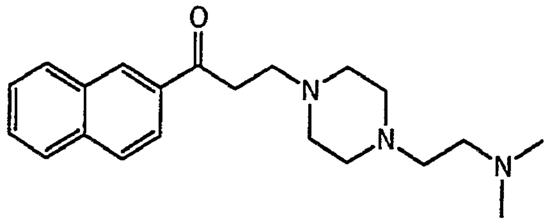
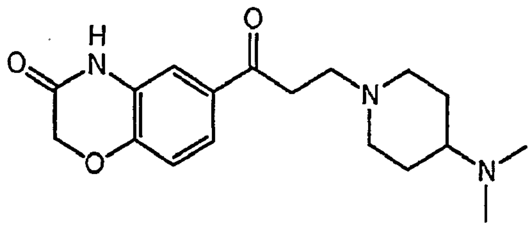
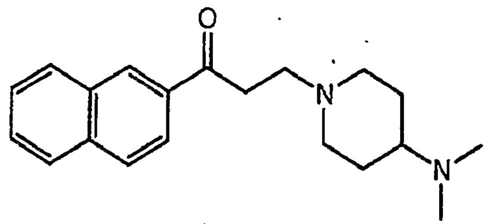
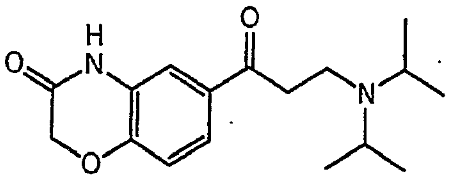
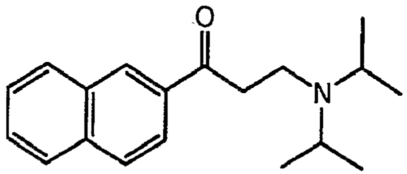
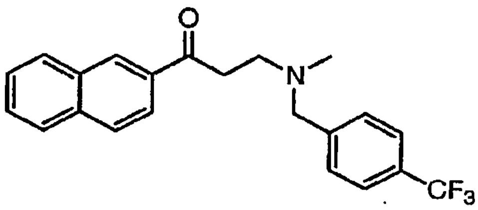
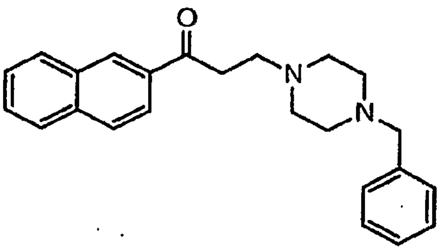
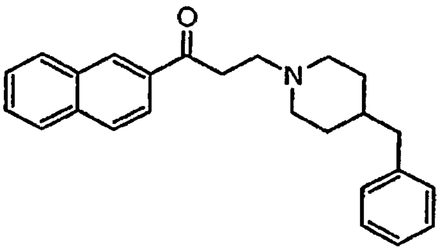
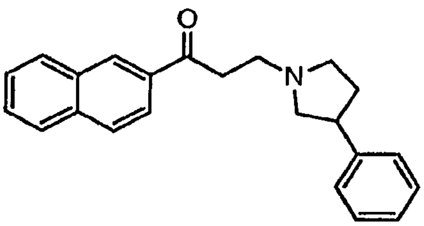
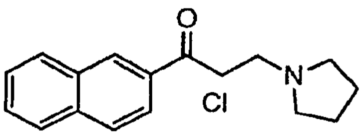
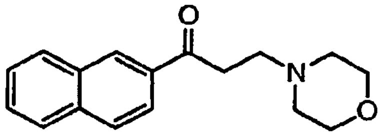
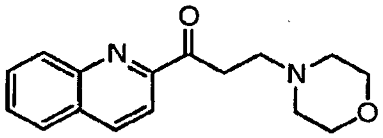
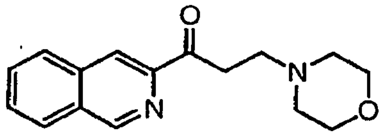
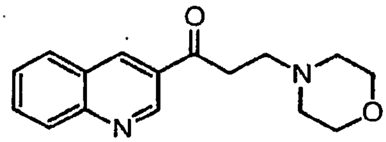
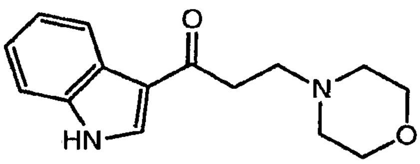
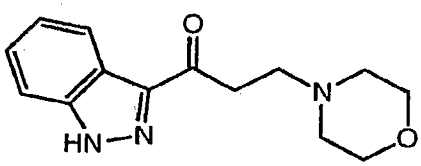
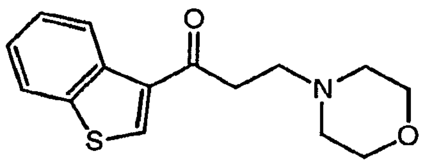
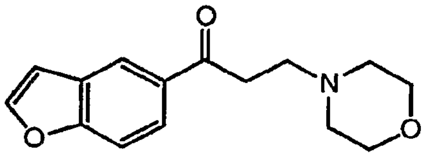
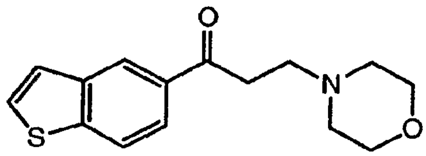
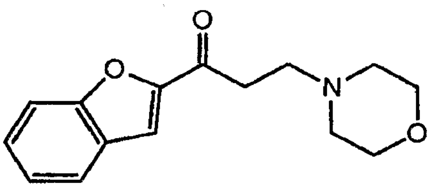
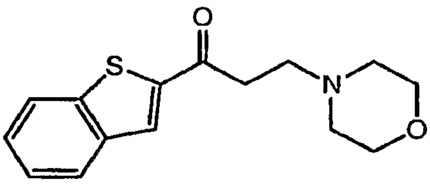
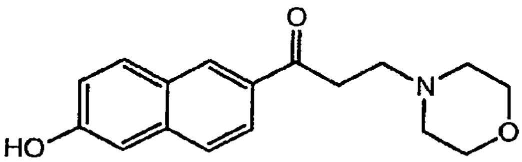
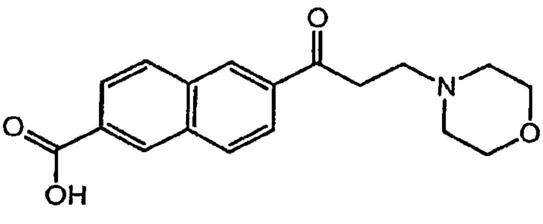
	0.010	0.300		0.006	0.00338
	0.018	0.481		0.008	0.00152
	0.013	0.150		0.009	0.00654
	0.023	0.544		0.009	0.00072

Table 4

Compound	IC_{50} ITK (μM)	IC_{50} BTK (μM)
	0.05666	
	0.03034	
	0.09281	
	0.02285	
	0.07489	

	0.020	0.039
	0.048	5.001
	1.684	no IC_{50}
	0.189	0.100
	0.176	8.174
	0.049	0.296
	0.134	0.611

	0.075	2.038
	0.060	0.334
	56.392	no IC_{50}
	0.010	0.017
	0.030	0.006
	3.339	2.364

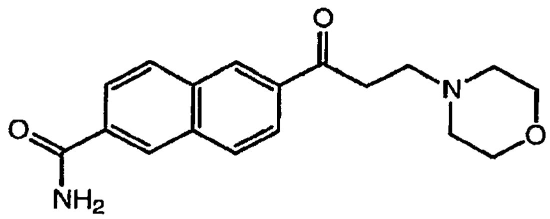
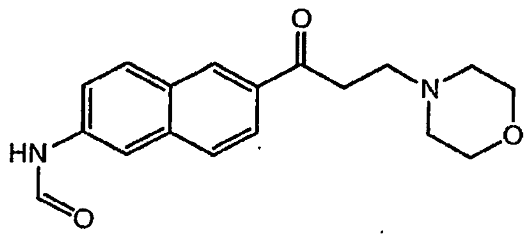
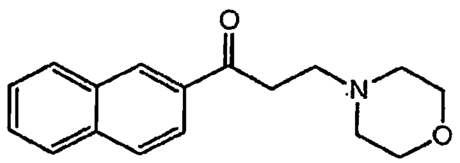
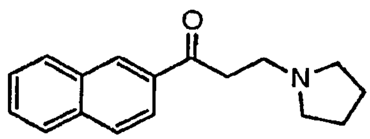
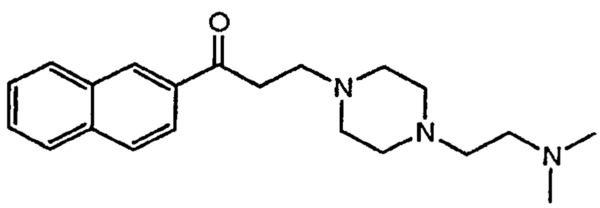
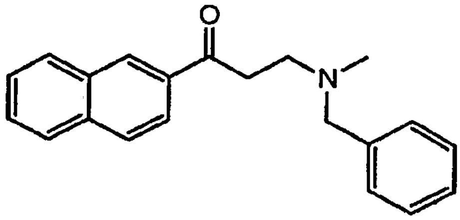
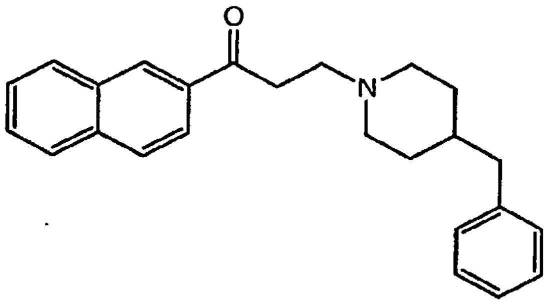
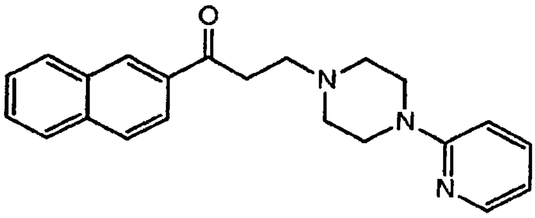
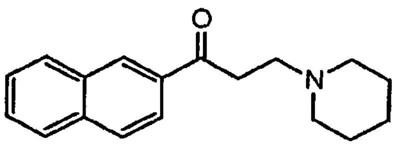
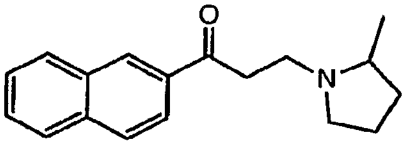
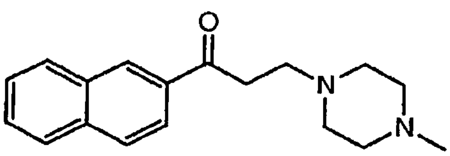
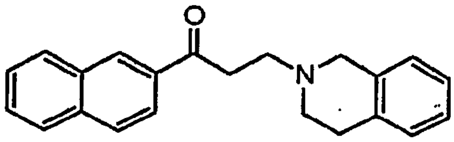
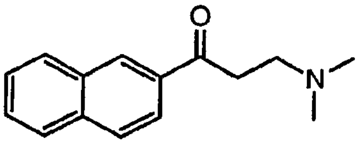
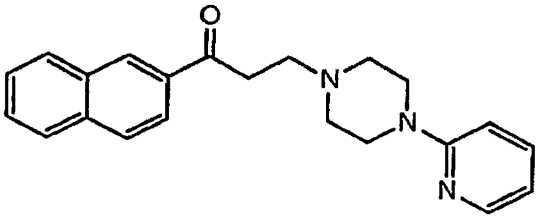
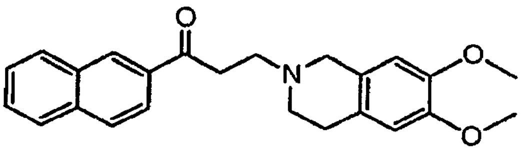
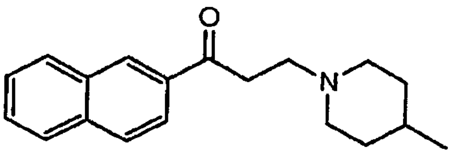
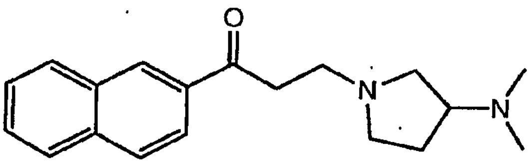
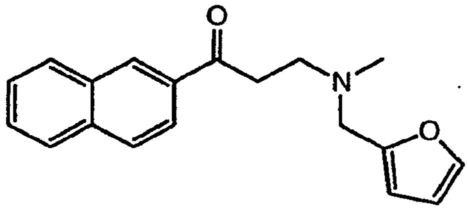
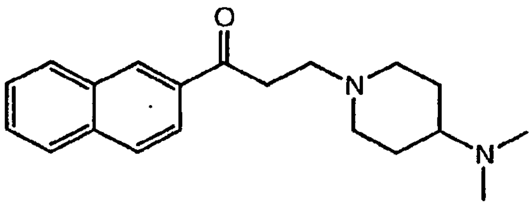
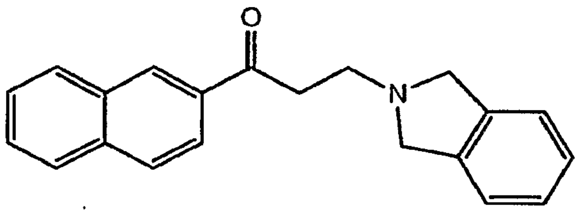
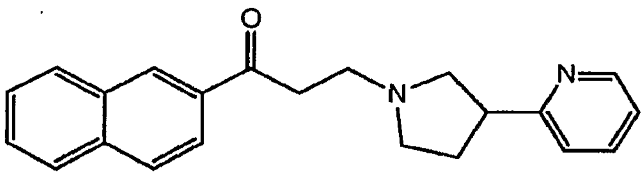
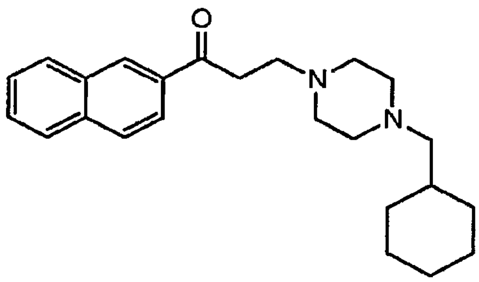
	0.005	0.001
	0.042	0.002

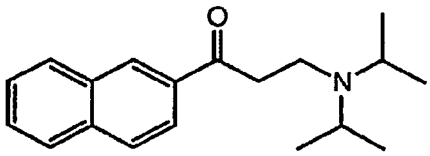
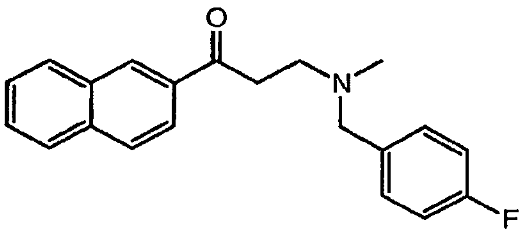
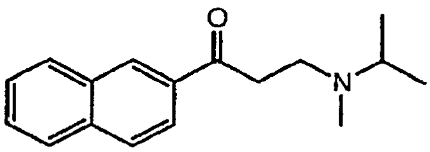
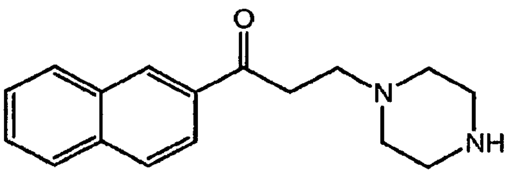
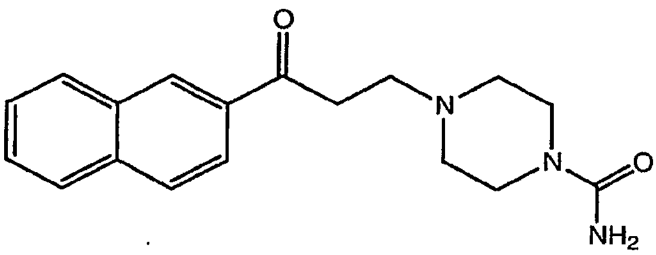
Table 5

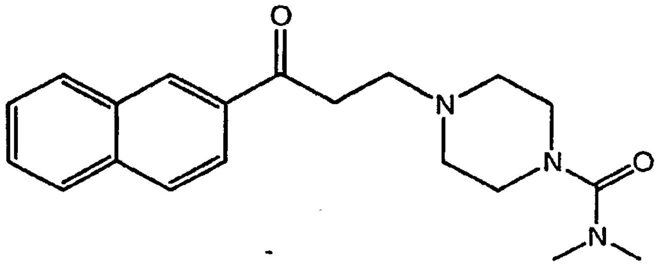
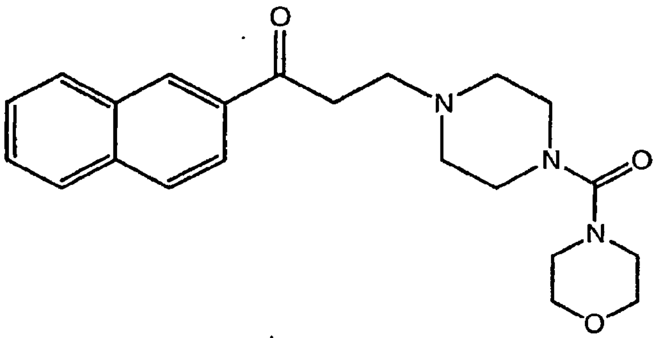
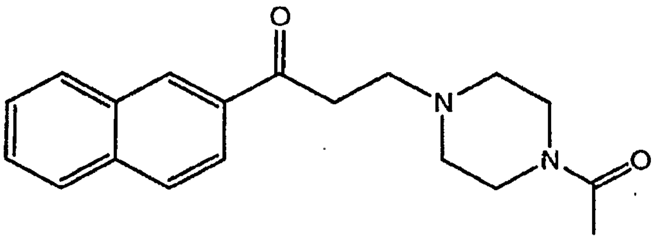
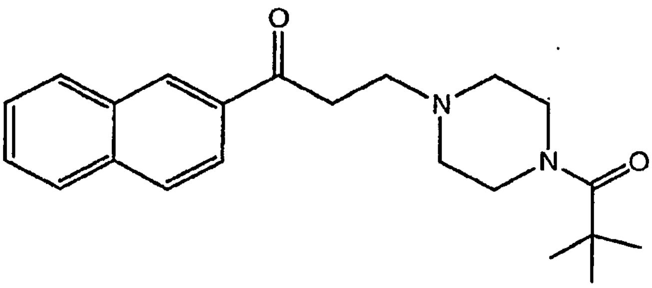
Compound	IC_{50} ITK (μM)	IC_{50} BTK (μM)
	0.0205	0.0395
	0.1369	8.21849
	0.0080	0.06706

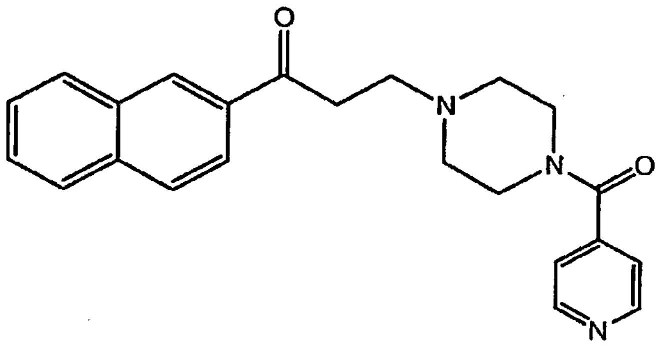
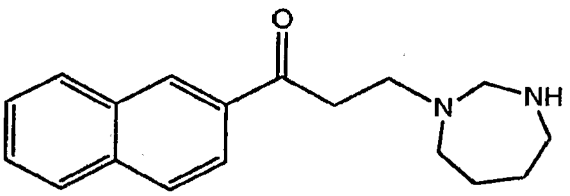
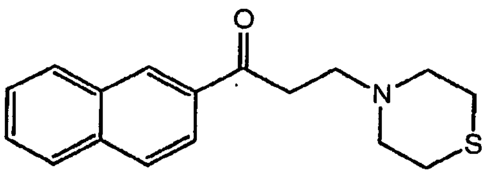
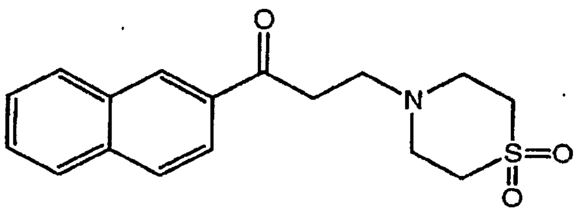
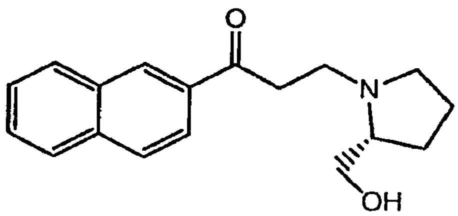
	0.0169	
	0.0602	0.12799
	0.0148	
	0.5375	
	0.8516	
	0.0309	

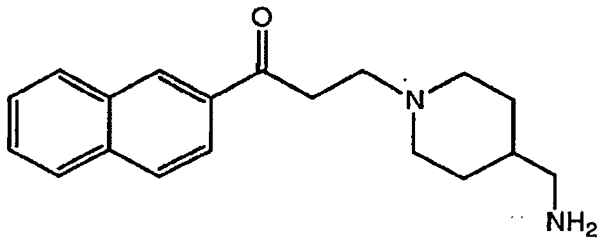
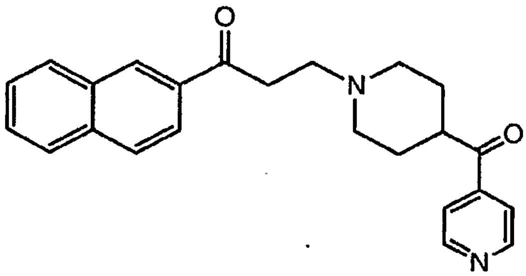
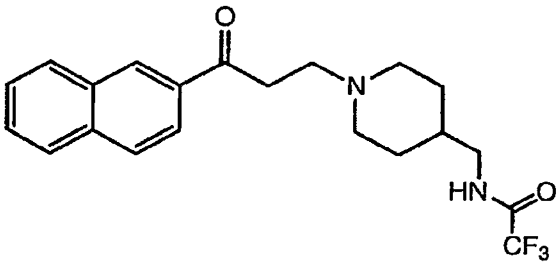
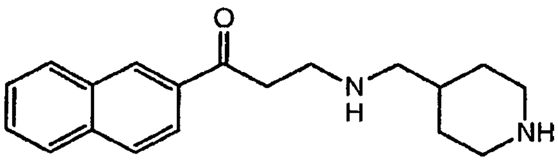
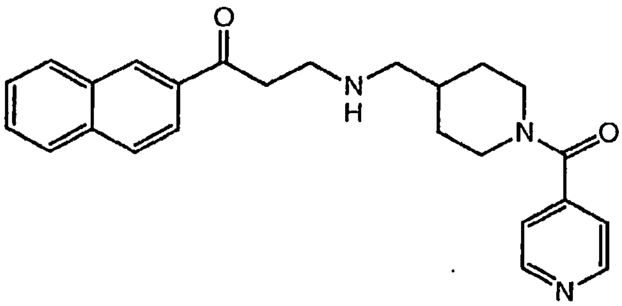
	0.0212	
	0.1609	
	0.0242	
	0.0072	0.11532
	0.3096	
	0.0069	0.0492

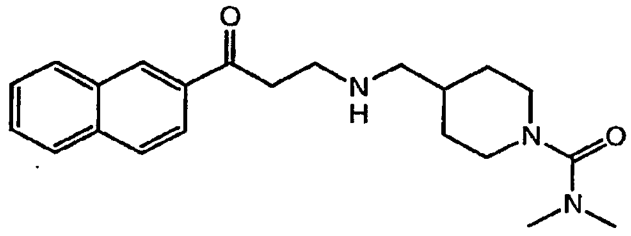
	0.0187	
	0.0095	
	0.0162	
	0.0359	
	0.0147	

	0.0092	
	0.0062	0.16054
	0.0163	
	0.117	0.410
	0.023	0.153

	0.056	0.452
	0.060	0.242
	0.066	0.089
	0.064	0.360

	0.054	0.018
	0.087	0.051
	0.031	0.071
	0.066	0.117
	0.049	0.123

	0.086	0.084
	0.284	0.486
	0.217	0.266
	0.163	0.100
	0.036	0.004

	0.737	0.373

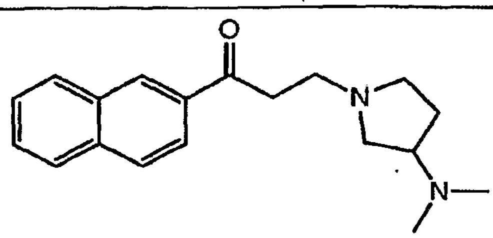
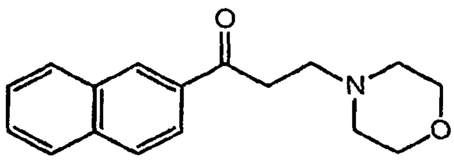
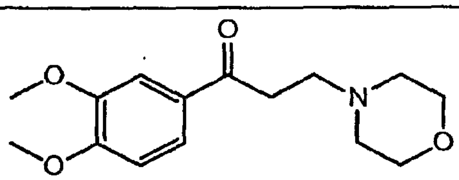
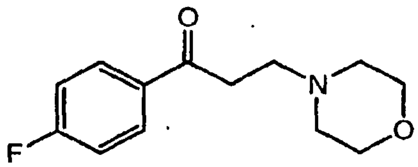
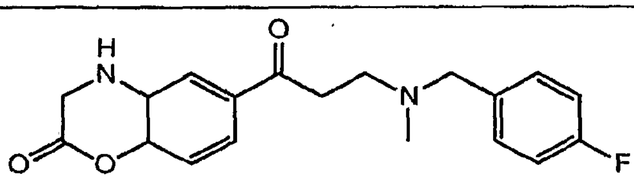
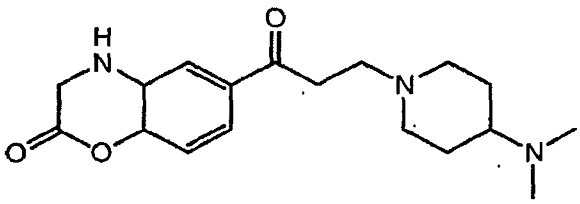
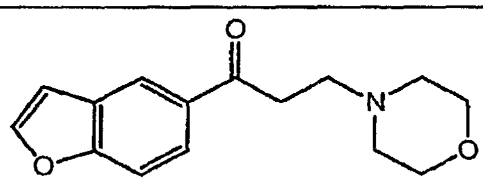
EXAMPLE 16

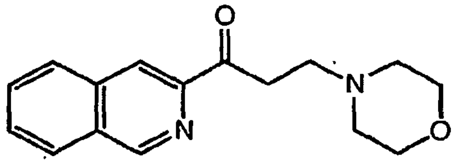
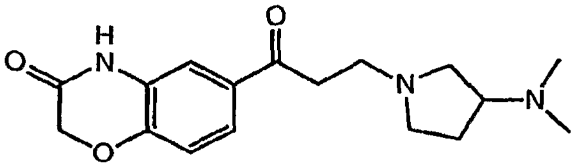
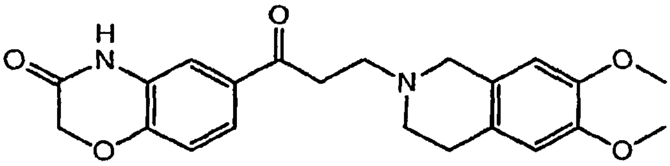
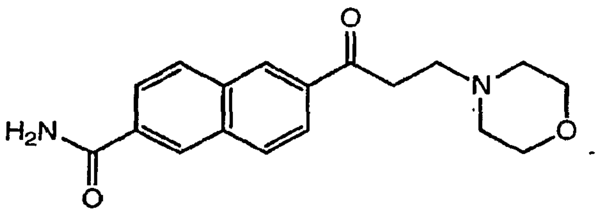
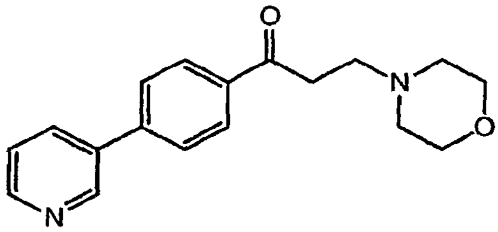
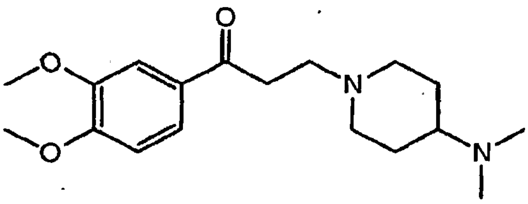
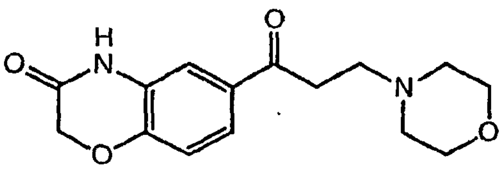
In vivo studies

[140] Several representative compounds were evaluated for efficacy in mouse *in vivo* tumor models. NOD/SCID mice were implanted intraperitoneally with T cell leukemia/lymphoma cells. One group was treated with vehicle alone (mock treatment) while the other groups were treated with several small molecule inhibitors via intraperitoneal route. Tumor growth was evaluated by peritoneal lavage and FACS analysis. Table 6 summarizes percent inhibition of tumor growth relative to a mock group treated with vehicle alone. Doses of compounds evaluated in this study were below the maximal tolerated dose, and showed minimal toxicity.

[141] The compounds in Table 6 were tested and inhibited tumor growth by at least 50% at the concentrations shown.

Table 6

Compound	mg/kg	% inhibition tumor growth
	100	70-90
	100	85-98
	80	92-99
	80	50-80
	80	90-99
	80	99
	80	67-81

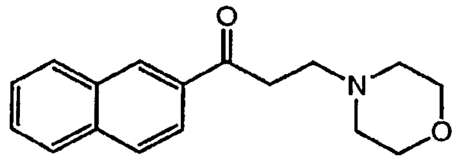
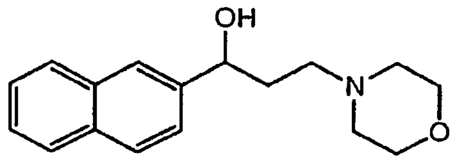
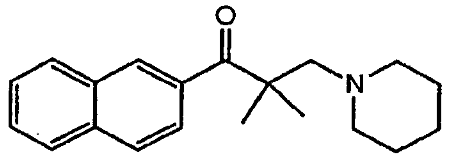
	80	92-99
	100	99
	80	97-99
	30	99
	100	99
	80	81-95
	20	40
vehicle	-	0

EXAMPLE 17

Compound activity mechanism

[142] The compound class interacts selectively with kinase domains of such kinase families as Tec and EGFR, as well as a few additional kinases. There is evidence indicating that this class of compounds reacts irreversibly at the ATP binding site of the kinase binding domain, through a mechanism that involves the exposure of a reactive aminoethyl C=Y warhead through the *in situ* elimination of a leaving group. The compounds contain an abstractable proton adjacent to the C=Y group, which upon exposure to an appropriate catalytic environment in the active site of a kinase of interest will promote elimination of the beta-amino functionality. This elimination thus generates a reactive electrophilic species (commonly termed a Michael acceptor moiety) which, due to the existence of a proximal cysteine residue in the kinase active site, rapidly forms a covalent adduct between this cysteine residue and the *in situ* generated electrophylic species. The combination of a kinase with the catalytic environment in close proximity to a nucleophilic cysteine, is a vital and unique requirement that describes this mechanism of action. The data below support that in situ elimination promotes the inhibitory activity of compounds in depicted in this invention. When a compound is modified in a manner that prevents elimination, the compound fails to exhibit inhibitory activity.

Table 7

compound	IC_{50} ITK
	0.0446
	no IC_{50}
	no IC_{50}

EXAMPLE 18

Covalent binding to Select Kinases

[143] As a result of elimination in proximity of a relevant cysteine, a covalent adduct is formed between the compound and the kinase domain. The irreversible binding that ensues can be demonstrated by several methods, including surface plasmon resonance (SPR) and co-precipitation of the compound with the kinase.

[144] BIACORE® is a SPR-based protein interaction approach, whereby the kinase is immobilized on the sensor chip, and a small molecule solution allowed to interact with the kinase. Detection of small molecule/kinase interaction occurs in real time, and is detected as a difference in SPR response. Figure 1 shows a BIACORE®experiment in which the ITK kinase domain was immobilized on a biosensor, and evaluated for its ability to bind and dissociate form a small molecule. The data indicates that compounds depicted in this application bind to the ITK kinase domain irreversibly.

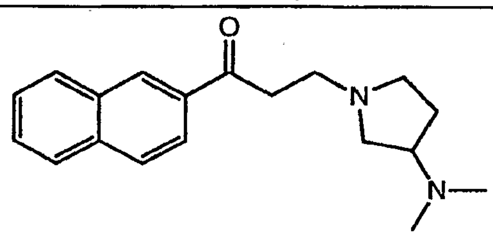
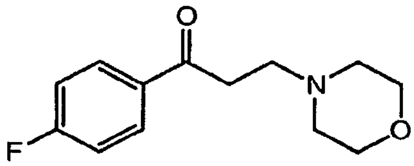
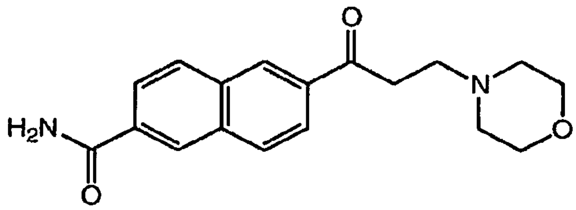
[145] In the co-precipitation assay, 1-10 mM labeled compound is incubated with cell lysates from either kinase expressing or kinase lacking cells. The label is then used to precipitate the compound and any bound proteins. The mixture is separated by SDS-PAGE and proteins are identified by western blotting and/or Mass spectrophotometry.

EXAMPLE 19

Contribution of Cysteine 442 to adduct formation

[146] In order to confirm the mechanism by which compounds depicted herein interact with the kinase domain of Tec and EGFR kinases, we created a point mutant of the ITK kinase domain, whereby the key amino acid, namely C442 was mutated to alanine. The protein was expressed in a commercial baculovirus expression system using the manufacturer's general protocol (Invitrogen, pBlueBac). Protein was expressed and purified using standard techniques. Both wild type (WT) ITK kinase domain and C442A kinase domain exhibited kinase activity. While the activity of WT-ITK was inhibited by compounds depicted in this application, the same compounds had no activity towards the C442A mutant kinase domain.

Table 8

compound	IC_{50} (μM)	
	wild-type ITK	C442A-ITK
control (BMS-488516)	0.0392	0.0532
	0.011	>10
	0.0496	>10
	0.0111	>10

CLAIMS

1. A protein kinase inhibitor which binds to a DKC triad kinase active site, comprising:

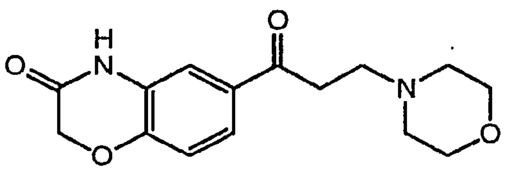
(a) a proton acceptor placed in a kinase active site within hydrogen-bonding distance to an amino group of a lysine of a catalytic dyad in the kinase active site;

(b) an abstractable proton in hydrogen bonding proximity to an aspartate of the catalytic dyad in the kinase active site, wherein removal of the abstractable proton creates a conjugated system capable of electronic rearrangement to an enol/enolate or thiol/thiolate or enamine;

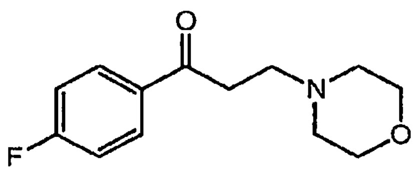
(c) a leaving group wherein further electronic rearrangement leads to the β-elimination of the leaving group, whereby a Michael acceptor in the inhibitor is created, and wherein in at least one conformation of the inhibitor and the kinase is such that the Michael acceptor moiety is located within a distance of 3-10 Å from a cysteinyl nucleophile, causing a reaction to form a Michael adduct of the enzyme; and

(d) a kinase binding moiety with affinity for a portion of the ATP binding site selected from the group consisting of the hinge region of the kinase, several hydrophobic residues, hydrophilic residues, and a combination thereof,

with the proviso that the protein kinase inhibitor is not



or



, and wherein the protein kinase inhibitor inhibits ITK with an IC_{50} of 0.00085 μM - 1 μM and/or which inhibits BTK with an IC_{50} of 0.00072 μM - 1 μM in an *in vitro* kinase assay.

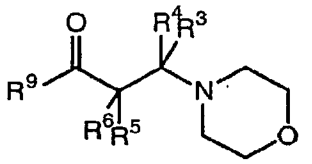
2. The protein kinase inhibitor of claim 1 wherein the kinase binding moiety contacts the kinase at a gatekeeper residue with a suitable hydrophobic aryl, heteroaryl or alkyl group.

3. The protein kinase inhibitor of claim 1 wherein the kinase binding moiety bypasses a gatekeeper residue with a hydrophobic aryl, heteroaryl or alkyl group to access an internal hydrophobic site.

4. The protein kinase inhibitor of claim 1 wherein the kinase binding moiety contacts a kinase at a hinge region through hydrogen bond(s) to backbone amide moieties through hydrogen bond acceptors and donors present in an aryl or heteroaryl group of the inhibitor.

5. The protein kinase inhibitor of claim 1 wherein the kinase binding moiety interacts with at least one active site hydrophobic residues, whereby overall binding energy is increased.

6. The protein kinase inhibitor of claim 1 which has the structural formula

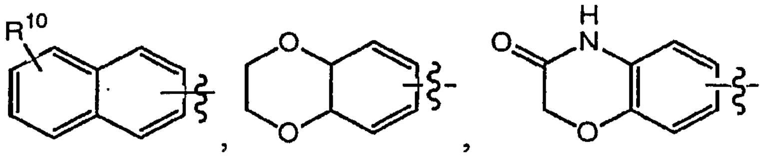


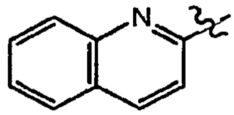
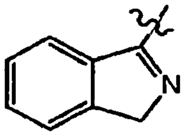
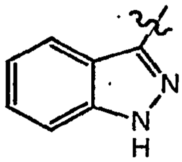
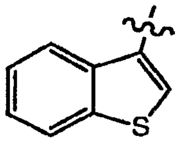
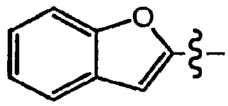
(II)

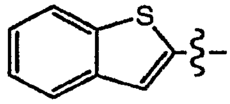
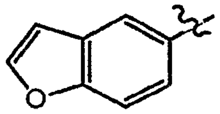
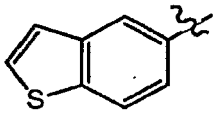
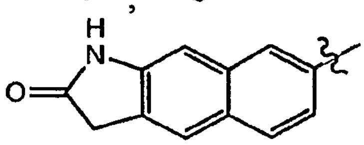
or a pharmaceutically acceptable salt thereof, wherein:

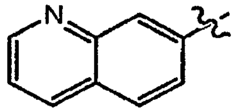
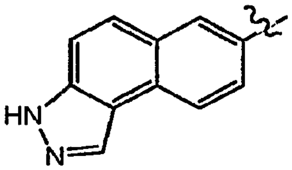
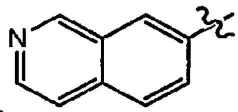
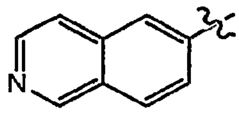
R^3, R^4, R^5, and R^6 are independently hydrogen or optionally substituted C_1-C_6 alkyl;

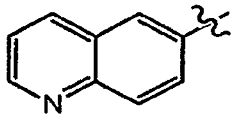
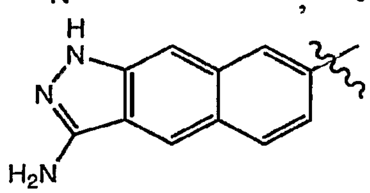
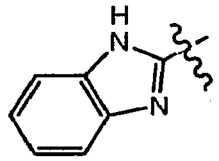
R^9 is selected from,

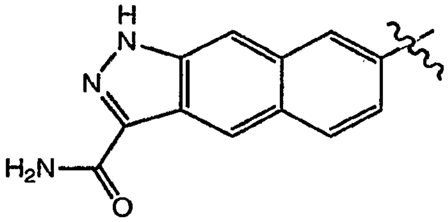
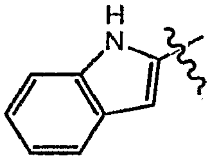
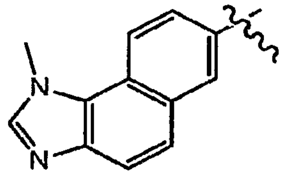


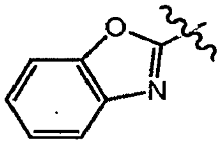
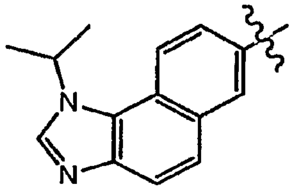
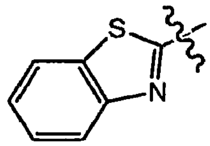
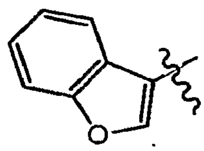
, , , , ,

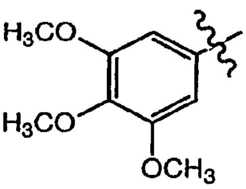
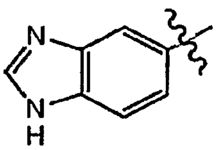
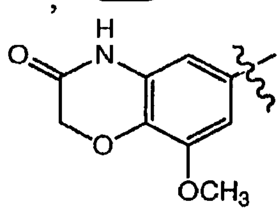
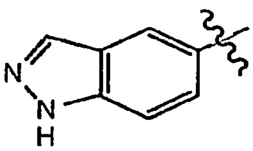
, , , ,

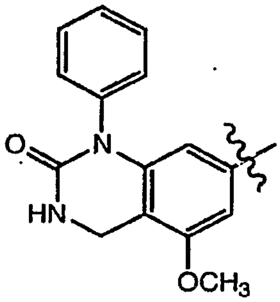
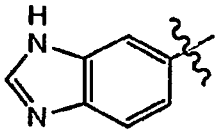
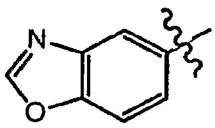
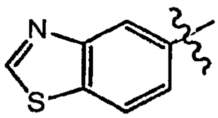
, , , ,

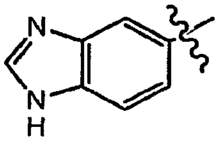
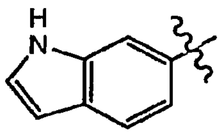
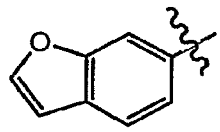
, , ,

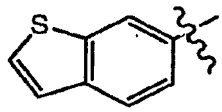
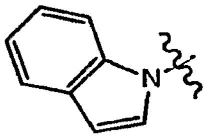
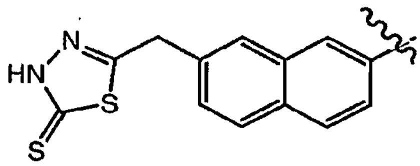
, , ,

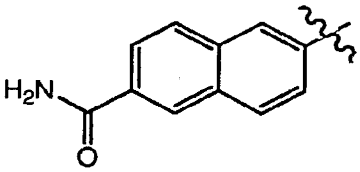
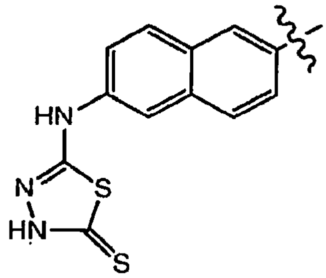
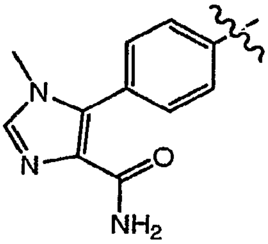
, , , ,

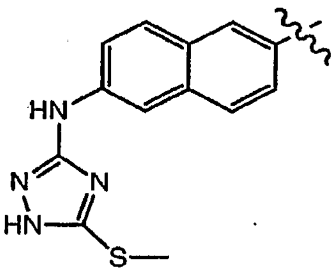
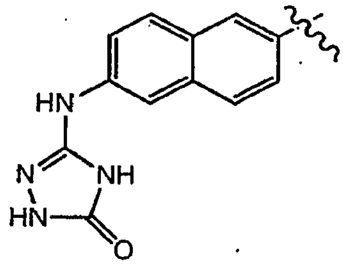
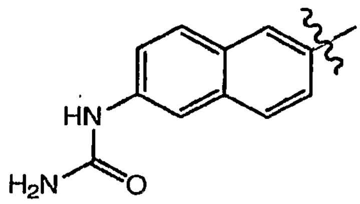
, , , ,

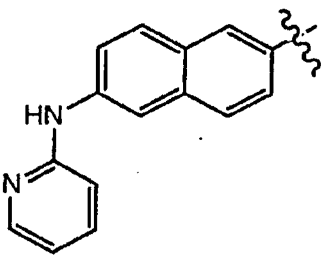
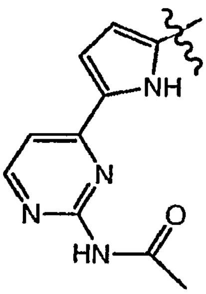
 ,  ,  ,  ,

 ,  ,  ,

 ,  ,  ,

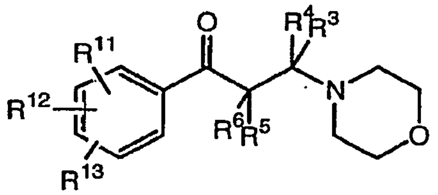
 ,  ,  ,

 ,  ,  ,

 , and  ; and

R^{10} is hydrogen, –OH, –COOH, $–CONH_2$, or –NCO.

7. The protein kinase inhibitor of claim 1 which has the structural formula

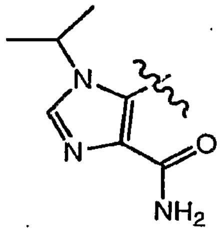
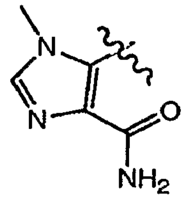
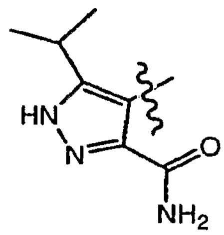


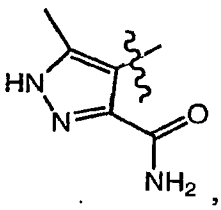
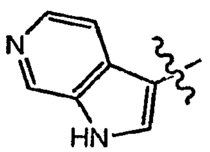
(III)

or a pharmaceutically acceptable salt thereof, wherein:

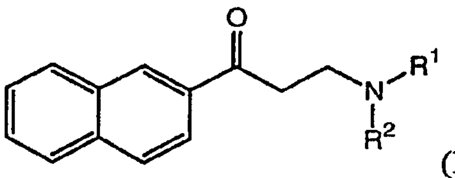
R^3, R^4, R^5, and R^6 are as defined above;

R^{11} and R^{12} are independently selected from hydrogen, $-OCH_3$, halogen, $-NO_2$, $-CN$, $-CF_3$, $-NCOR'$ (wherein R' is hydrogen or C_1-C_4 alkyl), phenyloxy, $-OCF_3$, $-NR'R''$ (wherein R' and R'' are independently hydrogen or C_1-C_4 alkyl), C_1-C_4 alkyl, C_1-C_4 alkoxy, and $-SO_2R'$ (wherein R' is hydrogen or C_1-C_4 alkyl); and

R^{13} is hydrogen, C_1-C_4 alkyl,  ,  ,  ,

 , or  .

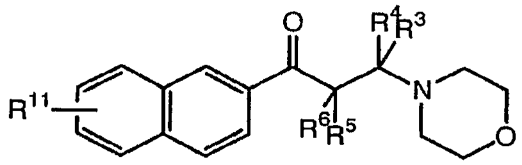
8. The protein kinase inhibitor of claim 1 which has the structural formula



(IV)

or a pharmaceutically acceptable salt thereof, wherein R^1 and R^2 (a) are independently hydrogen, optionally substituted C_1-C_6 alkyl, piperidine, or furanyl; or (b) are taken together with the nitrogen atom to which they are attached to form (i) a 5- to 7-membered optionally substituted aryl, (ii) a 5- to 7-membered optionally substituted heteroaryl, or (iii) a 5- to 7-membered optionally substituted heterocycle which may be unfused or fused to an optionally substituted aryl.

9. The protein kinase inhibitor of claim 1 which has the structural formula:

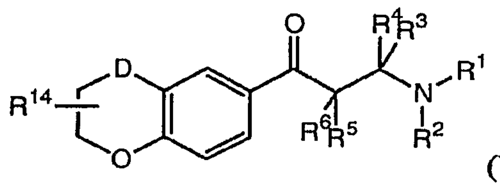


(V)

wherein:

R^3, R^4, R^5, R^6, and R^{11} are as defined above.

10. The protein kinase inhibitor of claim 1 which has the structural formula



(VI)

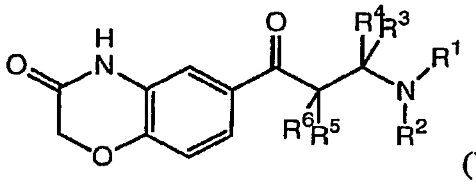
or a pharmaceutically acceptable salt thereof, wherein:

R^1, R^2, R^3, R^4, R^5, and R^6 are as defined above;

R^{14} is hydrogen or =O; and

D is CH or NH.

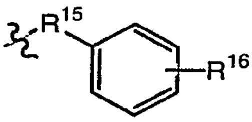
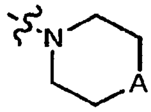
11. The protein kinase inhibitor of claim 1 which has the structural formula

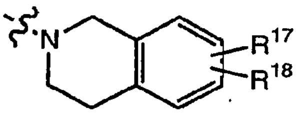


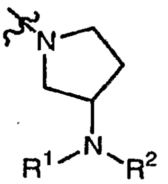
(VII)

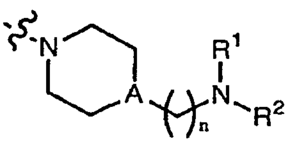
or a pharmaceutically acceptable salt thereof, wherein:

R^3, R^4, R^5, and R^6 are as defined above; and

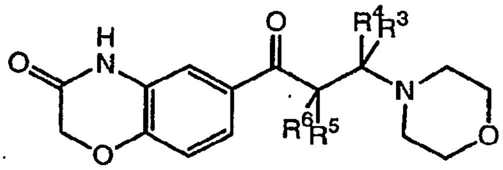
R^1 and R^2 are independently hydrogen, C_1-C_4 alkyl,  (wherein R^{15} is halogen or C_1-C_4 alkyl and R^{16} is C_1-C_4 alkyl), or R^1 and R^2 together with the nitrogen to which they are attached form an aryl group selected from ,

 (wherein R^{17} and R^{18} are independently hydrogen or $-OCH_3$,)

 (wherein R^1 and R^2 are independently hydrogen or C_1-C_4 alkyl),

 (wherein n = 1-4), and phenyl-C_1–C_4 alkyl, optionally substituted with halogen.

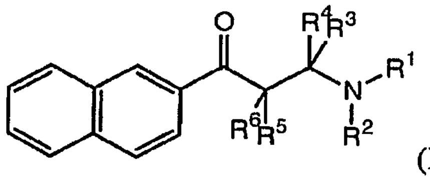
12. The protein kinase inhibitor of claim 1 which has the structural formula



(VIII)

or a pharmaceutically acceptable salt thereof, wherein R^3, R^4, R^5, and R^6 are as defined above.

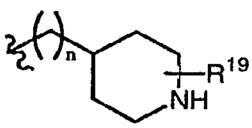
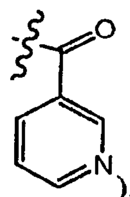
13. The protein kinase inhibitor of claim 1 which has the structural formula



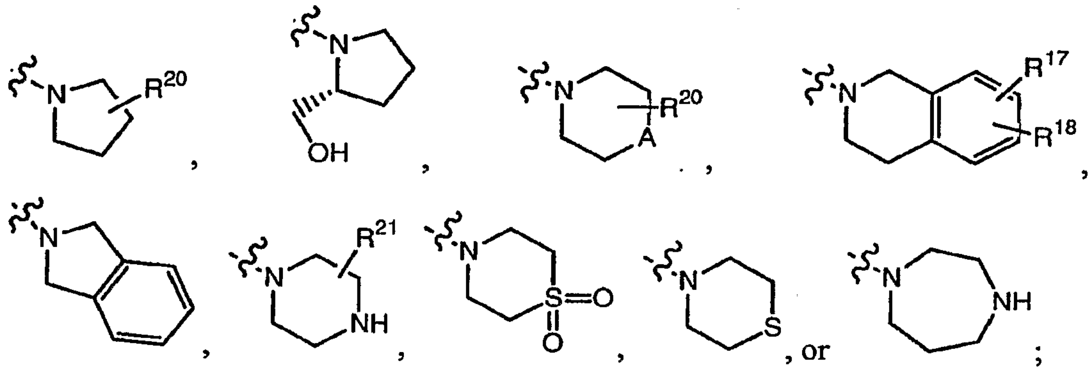
(IX)

or a pharmaceutically acceptable salt thereof, wherein:

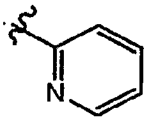
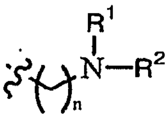
R^3, R^4, R^5, and R^6 are as defined above; and either

R^1 is hydrogen and R^2 is  (wherein R^{19} is selected from hydrogen and ); or

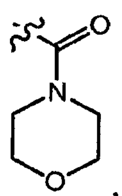
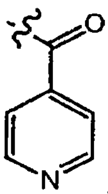
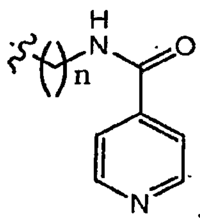
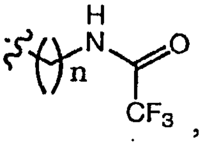
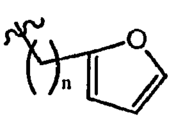
R^1 and R^2 together with the nitrogen to which they are attached are



A is N or O;

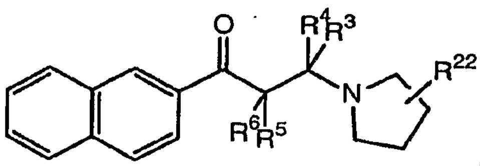
R^{20} is phenyl-C_1-C_4 alkyl optionally substituted with one or more halogens, hydrogen, C_1-C_4 alkyl, amino- C_1-C_4 alkyl, , or ;

R^{17} and R^{18} are independently hydrogen or $-OCH_3$;

R^{21} is $-CONR'R''$, $-COR'$, , , , , or ; and

R′ and R″ are independently selected from hydrogen and C_1-C_4 alkyl.

14. The protein kinase inhibitor of claim 1 which has the structural formula



(X)

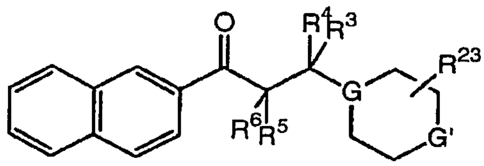
or a pharmaceutically acceptable salt thereof, wherein:

R^3, R^4, R^5, and R^6 are as defined above;

R^{22} is selected from hydrogen, C_1-C_4 alkyl, –NR′R″, –COH, –COOH, –CNR′R″, and –CONHR′; and

R′ and R″ are as defined above.

15. The protein kinase inhibitor of claim 1 which has the structural formula



(XI)

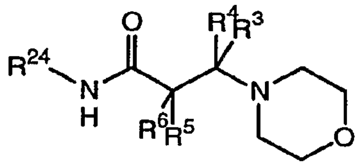
or a pharmaceutically acceptable salt thereof, wherein:

R^3, R^4, R^5, R^6, G, and G′ are as defined above;

R^{23} is hydrogen, –NR′R″ C_1-C_4 linear alkyl, C_1-C_4 alkyl, phenyl-C_1-C_4 alkyl, –$CONH_2$, or –CO R′R″; and

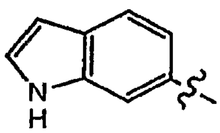
R′ and R″ are as defined above.

16. The protein kinase inhibitor of claim 1 which has the structural formula

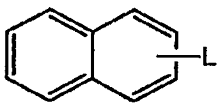


(XII)

or a pharmaceutically acceptable salt thereof, wherein R^3, R^4, R^5, and R^6 are as defined above

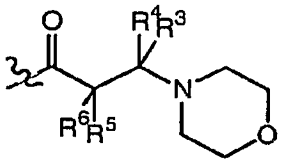
and wherein R^{24} is .

17. The protein kinase inhibitor of claim 1 which has the structural formula

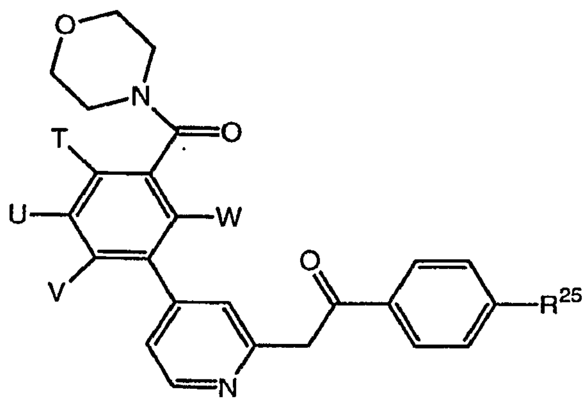


(XIII)

or a pharmaceutically acceptable salt thereof, wherein:

L is , and wherein R^3, R^4, R^5, and R^6 are as defined above.

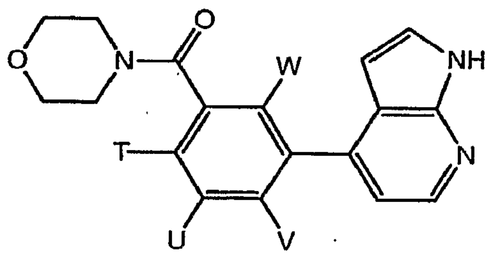
18. The protein kinase inhibitor of claim 1 which has the structural formula



(XIV)

or a pharmaceutically acceptable salt thereof, wherein T, U, V, and W independently are selected from hydrogen; halogen; –O; C_1-C_3 alkyl; and C_1-C_3 alkyloxy; and wherein R^{25} is hydrogen or C_1-C_3 alkyl.

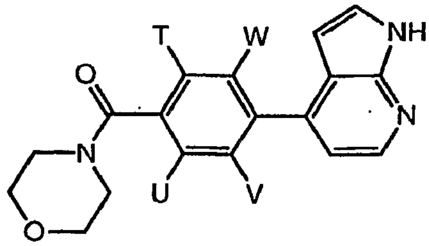
19. The protein kinase inhibitor of claim 1 which has the structural formula



(XV),

or a pharmaceutically acceptable salt thereof, wherein T, U, V, and W independently are selected from hydrogen; halogen; –O; C_1-C_3 alkyl; and C_1-C_3 alkyloxy; and wherein R^8 is hydrogen or C_1-C_3 alkyl.

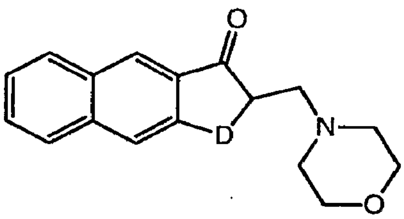
20. The protein kinase inhibitor of claim 1 which has the structural formula



(XVI),

or a pharmaceutically acceptable salt thereof, wherein T, U, V, and W independently are selected from hydrogen; halogen; –O; C_1-C_3 alkyl; and C_1-C_3 alkyloxy; and wherein R^8 is hydrogen or C_1-C_3 alkyl.

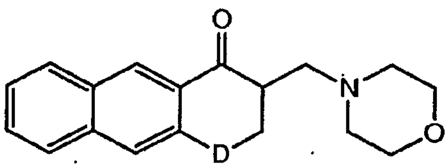
21. The protein kinase inhibitor of claim 1 which has the structural formula



(XVII)

or a pharmaceutically acceptable salt thereof, wherein D is S, O, or NH.

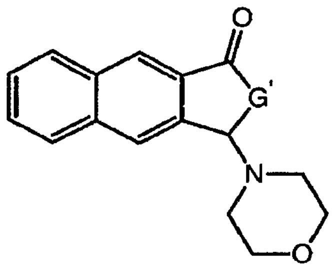
22. The protein kinase inhibitor of claim 1 which has the structural formula



(XVIII)

or a pharmaceutically acceptable salt thereof, wherein D is as defined above.

23. The protein kinase inhibitor of claim 1 which has the structural formula

 (XIX),

or a pharmaceutically acceptable salt thereof, wherein G′ is NH or CH.

24. A composition comprising:

(a) a pharmaceutically acceptable vehicle; and

(b) a compound of claim 1.

25. An adduct comprising:

(a) a compound of claim 1; and

(b) a DKC triad kinase domain.

26. A method of inhibiting kinase activity, comprising contacting a DKC triad kinase with a compound of claim 1 or a pharmaceutically acceptable salt thereof, whereby kinase activity of the DKC triad kinase is inhibited.

27. The method of claim 26 wherein the contacting occurs in a cell-free system.

28. The method of claim 26 wherein the contacting occurs in a cell.

29. The method of claim 28 wherein the cell is *in vitro*.

30. The method of claim 29 wherein the cell is in a patient.

31. The method of claim 30 wherein patient has an organ transplant, an autoimmune disease, or a blood cell malignancy.

32. A complex comprising the protein kinase inhibitor of claim 1 which is bound to a DKC triad kinase.

33. The complex of claim 32 wherein the DKC triad kinase is ITK or BTK.

34. The complex of claim 32 which consists of the protein kinase inhibitor of claim 1 bound to the DKC triad kinase.

35. The complex of claim 34 wherein the DKC triad kinase is ITK or BTK.

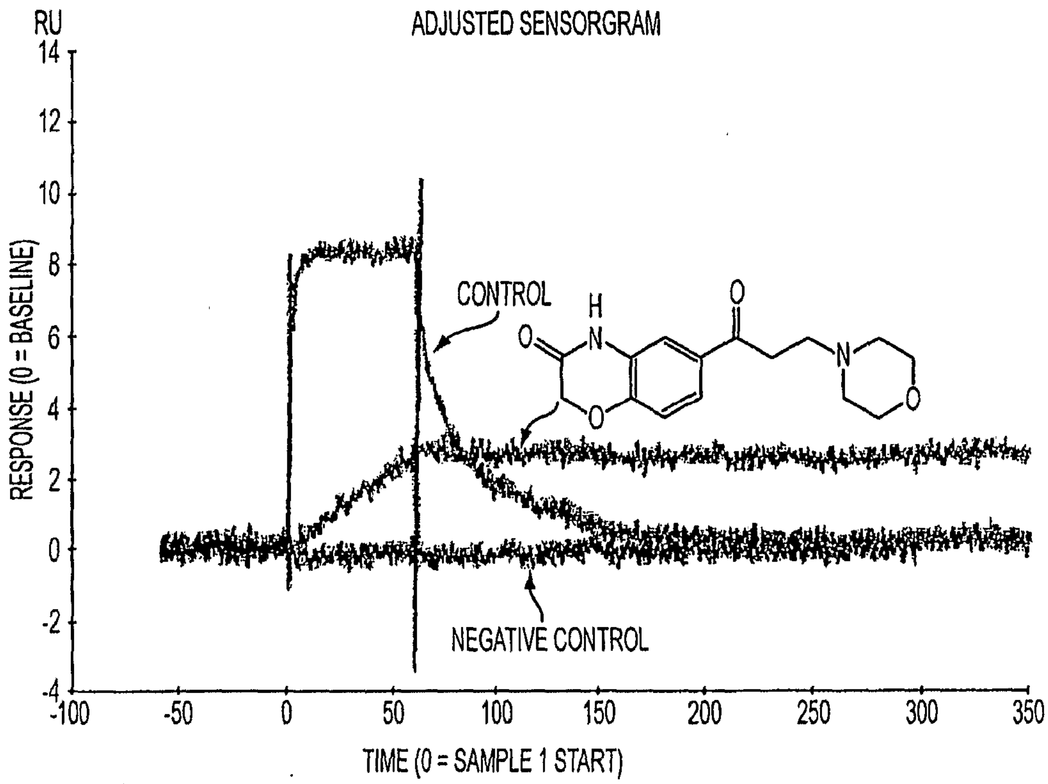
36. The protein kinase inhibitor of claim 1 wherein the DKC triad kinase is ITK or BTK.

37. The adduct of claim 25 wherein the DKC triad kinase domain is an ITK or BTK kinase domain.

38. The method of claim 26 wherein the DKC triad kinase is ITK or BTK.

39. Use of a compound of any of claims 1-23 in the preparation of a medicament for inhibiting a DKC triad kinase.

40. The use of claim 39 wherein the DKC triad kinase is ITK or BTK.



ADJUSTED SENSORGRAM
RU
14
12
10
8
6
4
2
0
-2
-4
RESPONSE (0 = BASELINE)
CONTROL
NEGATIVE CONTROL
H
O
N
O
O
N
O
-100
-50
0
50
100
150
200
250
300
350
TIME (0 = SAMPLE 1 START)

FIG. 1

```
huITK  5     ILLEEQLIKKSQQKRRTSPSNFKVRFFVLTKASLAYFED--RHGKKRTLKGSIELSRIKC  62
             ++LE   +K+SQQK++TSP NFK R F+LT   L+Y+E     G++ + KGSI++ +I C
huBTK  4     VILESIFLKRSQQKKKTSPLNFKKRLFLLTVHKLSYYEYDFERGRRGSKKGSIDVEKITC  63

Query  63    VEIVKSD---------------------ISIPCHYKYPFQVVHDNYLLYVFAPDRESRQR  101
             VE V  +                     ISI   + YPFQVV+D   LYVF+P  E R+R
Sbjct  64    VETVVPEKNPPPERQIPRRGEESSEMEQISIIERFPYPFQVVYDEGPLYVFSPTEELRKR  123

Query  102   WVLALKEETRNNNSLVPKYHPNFWMDGKWRCCSQLEKLATGCAQYDPTKNAS--------  153
             W+  LK   R N+ LV KYHP FW+DG++ CCSQ  K A GC Q    +N S
Sbjct  124   WIHQLKNVIRYNSDLVQKYHPCFWIDGQYLCCSQTAKNAMGC-QILENRNGSLKPGSSHR  182

Query  154   --KKPLPPTPEDNR------------RPLWEPEETVVIALYDYQTNDPQELALRRNEEYC  199
               KKPLPPTPE+++             P+   E   V+ALYDY   +  +L LR+ +EY
Sbjct  183   KTKKPLPPTPEEDQILKKPLPPEPAAAPVSTSELKKVVALYDYMPMNANDLQLRKGDEYF  242

Query  200   LLDSSEIHWWRVQDRNGHEGYVPSSYLVEKSPNNLETYEWYNKSISRDKAEKLLLDTGKE  259
             +L+ S + WWR +D+NG EGY+PS+Y+ E + +++E YEWY+K ++R +AE+LL   GKE
Sbjct  243   ILEESNLPWWRARDKNGQEGYIPSNYVTE-AEDSIEMYEWYSKHMTRSQAEQLLKQEGKE  301

Query  260   GAFMVRDSRTAGTYTVSVFTKAVVSENNPCIKHYHIKETNDNPKRYYVAEKYVFDSIPLL  319
             G F+VRDS  AG YTVSVF K+   +    I+HY +  T +   +YY+AEK++F +IP L
Sbjct  302   GGFIVRDSSKAGKYTVSVFAKST-GDPQGVIRHYVVCSTPQS--QYYLAEKHLFSTIPEL  358

Query  320   INYHQHNGGGLVTRLRYPVCFGRQKAPVTAGLRYGKWVIDPSELTFVQEIGSGQFGLVHL  379
             INYHQHN  GL++RL+YPV    + AP TAGL YG W IDP +LTF++E+G+GQFG+V
Sbjct  359   INYHQHNSAGLISRLKYPVSQQNKNAPSTAGLGYGSWEIDPKDLTFLKELGTGQFGVVKY  418

Query  380   GYWLNKDKVAIKTIREGAMSEEDFIEEAEVMMKLSHPKLVQLYGVCLEQAPICLVFEFME  439
             G W  +  VAIK I+EG+MSE++FIEEA+VMM LSH KLVQLYGVC +Q PI ++ E+M
Sbjct  419   GKWRGQYDVAIKMIKEGSMSEDEFIEEAKVMMNLSHEKLVQLYGVCTKQRPIFIITEYMA  478

huITK  440   HGCLSDYLRTQRGLFAAETLLGMCLDVCEGMAYLEEACVIHRDLAARNCLVGENQVIKVS  499
             +GCL +YLR  R  F  + LL MC DVCE M YLE    +HRDLAARNCLV +  V+KVS
huBTK  479   NGCLLNYLREMRHRFQTQQLLEMCKDVCEAMEYLESKQFLHRDLAARNCLVNDQGVVKVS  538

Query  500   DFGMTRFVLDDQYTSSTGTKFPVKWASPEVFSFSRYSSKSDVWSFGVLMWEVFSEGKIPY  559
             DFG++R+VLDD+YTSS G+KFPV+W+ PEV  +S++SSKSD+W+FGVLMWE++S GK+PY
Sbjct  539   DFGLSRYVLDDEYTSSVGSKFPVRWSPPEVLMYSKFSSKSDIWAFGVLMWEIYSLGKMPY  598

Query  560   ENRSNSEVVEDISTGFRLYKPRLASTHVYQIMNHCWKERPEDRPAFSRLLRQLAEIAE  617
             E  +NSE  E I+ G RLY+P LAS  VY IM  CW E+ ++RP F  LL  + ++ +
Sbjct  599   ERFTNSETAEHIAQGLRLYRPHLASEKVYTIMYSCWHEKADERPTFKILLSNILDVMD  656
```

FIG. 2

TK_Tec__BTK	LTFLKELGTGQFGVVKYGKWRG-----QYDVAIKMIKEGS	(SEQ ID NO:3)
TK_Tec__BMX	ITLLKELGSGQFGVVQLGKWKG-----QYDVAVKMIKEGS	(SEQ ID NO:4)
TK_Tec__TEC	LTFMRELGSGLFGVVRLGKWRA-----QYKVAIKAIREGA	(SEQ ID NO:5)
TK_Tec__TXK	LAFIKEIGSGQFGVVHLGEWRS-----HIQVAIKAINEGS	(SEQ ID NO:6)
TK_Tec__ITK	LTFVQEIGSGQFGLVHLGYWLN-----KDKVAIKTIREGA	(SEQ ID NO:7)
TK_Src__BLK	LRLVRKLGSGQFGEVWMGYYKN-----NMKVAIKTLKEGT	(SEQ ID NO:8)
TK_EGFR__EGFR	FKKIKVLGSGAFGTVYKGLWIPEGEKVKIPVAIKELREAT	(SEQ ID NO:9)
TK_EGFR__HER2_ErbB2	LRKVKVLGSGAFGTVYKGIWIPDGENVKIPVAIKVLRENT	(SEQ ID NO:10)
TK_EGFR__HER4_ErbB4	LKRVKVLGSGAFGTVYKGIWVPEGETVKIPVAIKILNETT	(SEQ ID NO:11)
TK_JakA__JAK3	LKYISQLGKGNFGSVELCRYDPLAHNTGALVAVKQLQHSG	(SEQ ID NO:12)
TK_Tec__BTK	--MSEDEFIEEAKVMMNLSHEKLVQLYGVCTKQR-PIFI	(SEQ ID NO:13)
TK_Tec__BMX	--MSEDEFFQEAQTMMKLSHPKLVKFYGVCSKEY-PIYI	(SEQ ID NO:14)
TK_Tec__TEC	--MCEEDFIEEAKVMMKLTHPKLVQLYGVCTQQK-PIYI	(SEQ ID NO:15)
TK_Tec__TXK	--MSEEDFIEEAKVMMKLSHSKLVQLYGVCIQRK-PLYI	(SEQ ID NO:16)
TK_Tec__ITK	--MSEEDFIEEAEVMMKLSHPKLVQLYGVCLEQA-PICL	(SEQ ID NO:17)
TK_Src__BLK	--MSPEAFLGEANVMKALQHERLVRLYAVVTKE---PIYI	(SEQ ID NO:18)
TK_EGFR__EGFR	SPKANKEILDEAYVMASVDNPHVCRLLGICLTS---TVQL	(SEQ ID NO:19)
TK_EGFR__HER2_ErbB2	SPKANKEILDEAYVMAGVGSPYVSRLLGICLTS---TVQL	(SEQ ID NO:20)
TK_EGFR__HER4_ErbB4	GPKANVEFMDEALIMASMDHPHLVRLLGVCLSP---TIQL	(SEQ ID NO:21)
TK_JakA__JAK3	-PDQQRDFQREIQILKALHSDFIVKYRGVSYGPGRPELRL	(SEQ ID NO:22)
TK_Tec__BTK	**ITEYM**ANG**C**LLNYLREMRH-RFQTQQLLEMCKDV------	(SEQ ID NO:23)
TK_Tec__BMX	**VTEYI**SNG**C**LLNYLRSHGK-GLEPSQLLEMCYDV------	(SEQ ID NO:24)
TK_Tec__TEC	**VTEFM**ERG**C**LLNFLRQRQG-HFSRDVLLSMCQDV------	(SEQ ID NO:25)
TK_Tec__TXK	**VTEFM**ENG**C**LLNYLRENKG-KLRKEMLLSVCQDI------	(SEQ ID NO:26)
TK_Tec__ITK	**VFEFM**EHG**C**LSDYLRTQRG-LFAAETLLGMCLDV------	(SEQ ID NO:27)
TK_Src__BLK	**VTEYM**ARG**C**LLDFLKTDEGSRLSLPRLIDMSAQIAEGMAY	(SEQ ID NO:28)
TK_EGFR__EGFR	**ITQLM**PFG**C**LLDYVREHKD-NIGSQYLLNWCVQIAKGMNY	(SEQ ID NO:29)
TK_EGFR__HER2_ErbB2	**VTQLM**PYG**C**LLDHVRENRG-RLGSQDLLNWCMQIAKGMSY	(SEQ ID NO:30)
TK_EGFR__HER4_ErbB4	**VTQLM**PHG**C**LLEYVHEHKD-NIGSQLLLNWCVQIAKGMMY	(SEQ ID NO:31)
TK_JakA__JAK3	**VMEYL**PSG**C**LRDFLQRHRA-RLDASRLLLYSSQICKGMEY	(SEQ ID NO:32)
TK_Tec__BTK	-----FLHRDLAARNCLVNDQGVVKVS**DFG**LSRYVLDDEY	(SEQ ID NO:33)
TK_Tec__BMX	-----FIHRDLAARNCLVDRDLCVKVS**DFG**MTRYVLDDQY	(SEQ ID NO:34)
TK_Tec__TEC	-----FIHRDLAARNCLVSEAGVVKVS**DFG**MARYFLDDQY	(SEQ ID NO:35)
TK_Tec__TXK	-----YIHRDLAARNCLVSSTCIVKIS**DFG**MTRYVLDDEY	(SEQ ID NO:36)
TK_Tec__ITK	-----VIHRDLAARNCLVGENQVIKVS**DFG**MTRFVLDDQY	(SEQ ID NO:37)
TK_Src__BLK	IERMNSIHRDLRAANILVSEALCCKIA**DFG**LAR-IIDSEY	(SEQ ID NO:38)
TK_EGFR__EGFR	LEDRRLVHRDLAARNVLVKTPQHVKIT**DFG**LAKLLGAEEK	(SEQ ID NO:39)
TK_EGFR__HER2_ErbB2	LEDVRLVHRDLAARNVLVKSPNHVKIT**DFG**LARLLDIDET	(SEQ ID NO:40)
TK_EGFR__HER4_ErbB4	LEERRLVHRDLAARNVLVKSPNHVKIT**DFG**LARLLEGDEK	(SEQ ID NO:41)
TK_JakA__JAK3	LGSRRCVHRDLAARNILVESEAHVKIA**DFG**LAKLLPLDKD	(SEQ ID NO:42)

FIG. 3