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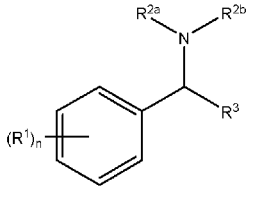

(I)

(57) Abstract: The invention relates to compounds of general formula (I): wherein R^1, n, R^{2a}, R^{2b}, and R^3 are as defined herein. The compounds are inhibitors of Bcl-2-associated death promoter (BAD) phosphorylation and have anti-apoptotic activity and are useful in the treatment of cancer, particularly breast cancer, endometrial cancer, ovarian cancer, liver cancer, colon cancer, prostate cancer or pancreatic cancer.

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SMALL MOLECULE INHIBITORS OF BCL-2-ASSOCIATED DEATH PROMOTER (BAD) PHOSPHORYLATION

The present invention relates to novel compounds, and in particular to compounds which are inhibitors of Bcl-2-associated death promoter (BAD) in cancer cells, to the compounds for use in the treatment or prevention of cancer and to pharmaceutical compositions comprising the compounds.

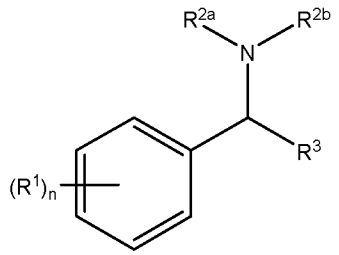
Apoptosis or programmed cell death is a regulatory mechanism pivotally involved in elimination of auto-reactive immune cells, aged cells and cells with irreparable DNA damage [1]. Apoptosis is executed by two distinct mechanisms in vertebrates – the extrinsic and the intrinsic pathway [2]. The intrinsic pathway is activated under hypoxic conditions, after severe genomic damage, oxidative stress or after growth factor depletion [3]. BCL-2 family proteins possess a pivotal role in regulating the intrinsic apoptotic pathway [4]. More than 20 BCL-2 family members have been identified and broadly classified into anti-apoptotic, pro-apoptotic, and BH3-only proteins. BCL-2, BCL-xL and BCL-w are anti-apoptotic; BAK and BAX are pro-apoptotic; and BAD, BIM, PUMA, NOXA are the predominant pro-apoptotic BH3 only proteins [5-7]. Hence , the members of the BCL-2 family serve both pro- and anti-apoptotic functions with an equilibrium maintained between them, and pro-apoptotic BH3 only proteins are normally repressed to ensure homeostasis [8].

Suppression of apoptosis contributes to inappropriate cell survival and cancer development and/or progression [9-11]. In most malignancies, expression of anti-apoptotic proteins is increased leading to the development of resistance to apoptosis. Bcl-2 associated death promoter (BAD) plays a critical role in regulating apoptosis by interacting with Bcl-2, Bcl-xL and Bcl-w [12]. Human BAD is phosphorylated at Ser75 (equivalent murine residue is Ser112) by p44/42 MAP kinase and Ser99 (equivalent murine residue is Ser136) by AKT/p70S6K [13]. Both serine residues are also phosphorylated by Pim family kinases to prevent apoptosis [14]. Phosphorylation of BAD at either of these residues results in loss of the ability of hBAD to heterodimerize with BCL-xL or BCL-2 [15]. Phosphorylated BAD protein is heterodimerized with 14-3-3 protein and sequestered in the cytoplasm [16]. Upon initiation of apoptosis, BAD undergoes dephosphorylation and heterodimerizes with BCL-2, BCL-xL or BCL-w which allows BAK and BAX to promote the release of cytochrome C to the cytoplasm with subsequent promotion of the intrinsic apoptotic pathway [17]. BAD has also been reported to possess other protein and functional interactions within the cell. For example, BAD expression is regulated by p53 and BAD complexes with p53 to promote apoptosis [18]. Bad is transcriptionally up-

regulated by p53 and forms a Bad/p53 complex at the mitochondria to induce apoptosis [18]. Clinically, high BAD expression is associated with high Gleason scores in prostate cancer [15] and BAD phosphorylation has been reported to predict poor overall survival in ovarian cancer [19] and to be associated with resistance to cisplatin [19]. Furthermore, phosphorylated BAD is observed in more than 80% of the CD44 positive cancer stem cell (CSC) population in breast cancer and BAD phosphorylation has been reported to be essential for CSC survival [20].

Numerous small molecule modulators of programmed cell death have been developed, targeting BCL-2 family proteins for therapeutic use against various human malignancies [21-23]. To date there has been no reported small molecule modulators of BH3-only proteins such as BAD. Thus, there is a necessity to develop better and efficient compounds/therapies for managing cancer by inhibiting BAD phosphorylation without affecting the upstream events.

In the present invention there is provided a compound of general formula (I):



(I)

wherein:

each R^1 is independently halo, OH, cyano, nitro, $NR^{10}R^{11}$, $C(O)R^{10}$, $C(O)OR^{10}$, $C(O)NR^{10}R^{11}$, $-S(O)_qNR^{10}R^{11}$, C_{1-6} alkyl, C_{1-6} haloalkyl, $-O(C_{1-6}$ alkyl), $-O(C_{1-6}$ haloalkyl), aryl, heteroaryl, -O-aryl or -O-heteroaryl,

wherein

each R^{10} and R^{11} is independently selected from H or C_{1-6} alkyl optionally substituted with one or more substituents selected from OH, halo, cyano, NH_2, aryl or heteroaryl;

alkyl and haloalkyl groups R^1 are optionally substituted with one or more substitutents selected from OH, cyano, $-S(O)_pNR^4R^5$, $-C(O)NR^4R^5$, aryl, heteroaryl, -O-aryl or -O-heteroaryl $-O(C_{1-6}$ alkyl) optionally substituted with aryl or $-O(C_{1-6}$ haloalkyl);

aryl or heteroaryl groups R^1 are optionally substituted with one or more substituents selected from halo, OH, cyano, nitro, $-NR^4R^5$, $-S(O)_pNR^4R^5$, $-C(O)NR^4R^5$, $-C(O)R^4$,

-C(O)OR^4 or -C$_{1-6}$ alkyl or -O(C$_{1-6}$ alkyl) , either of which is optionally substituted with one or more substituents selected from OH, halo, aryl, heteroaryl, -O(C$_{1-6}$ alkyl), O(C$_{1-6}$ haloalkyl), -O-aryl or –O-heteroaryl);

p is 1 or 2;

each R^4 and R^5 is independently selected from H or C$_{1-4}$ alkyl or R^4 and R^5 together with a nitrogen atom to which they are attached may form a 3- or 8-membered heterocyclic ring, optionally containing one or more additional heteroatoms selected from O, N and S;

n is 0, 1, 2, 3 4, or 5;

R^{2a} and R^{2b} are each independently C$_{1-6}$ alkyl optionally substituted with one or more substituents selected from halo, OH, aryl or heteroaryl; or

R^{2a} and R^{2b} together with the nitrogen atom to which they are attached form a 5- or 6-membered heterocyclic ring optionally containing one or more further heteroatoms selected from O, N or S and optionally substituted with one or more substituents R^6;

each R^6 is independently selected from aryl, heteroaryl, -O-aryl, -O-heteroaryl, carbocyclyl, heterocyclyl, -O-carbocyclyl, -O-heterocyclyl, R^{12}, OR^{12}, C(O)R^{12}, C(O)OR^{11}, C(O)NR^{11}R^{12}, CN,OH,

each R^{11} and R^{12} is independently H or C$_{1-4}$ alkyl, either or which may be substituted with one or more aryl or heteroaryl groups, wherein aryl and heteroaryl groups are substituted with one or more substituent selected from halo, OH, cyano, nitro, -NR^{4}R^5, -S(O)$_p$NR^{4}R^5, -C(O)NR^{4}R^5, -C(O)R^4, -C(O)OR^4 or -C$_{1-6}$ alkyl or -O(C$_{1-6}$ alkyl) , either of which is optionally substituted with one or more substituents selected from OH, halo, aryl, heteroaryl, -O(C$_{1-6}$ alkyl), O(C$_{1-6}$ haloalkyl), -O-aryl or –O-heteroaryl);

wherein R^4 and R^5 are as defined above; or R^{11} and R^{12} may combine with a nitrogen atom to which they are attached to form a 3 to 8-membered heterocyclic ring optionally containing one or more further heteroatoms selected from N, O and S and optionally substitututed with C$_{1-4}$ alkyl, C$_{1-4}$ haloalkyl or halo;

R^3 is aryl, heteroaryl, carbocyclyl or heterocyclyl any of which is optionally substituted with one or more substituents R^7 selected from halo, -C$_{1-4}$ alkyl optionally substituted with aryl, -O(C$_{1-4}$ alkyl) optionally substituted with aryl, -C$_{1-4}$ haloalkyl, -O(C$_{1-4}$ haloalkyl) or -C(O)NR^{8}R^9;

each R^8 and R^9 is independently selected from H, C_{1-4} alkyl or C_{3-6} cycloalkyl or R^8 and R^9 together with the nitrogen atom to which they are attached may form a 5- or 6-membered heterocyclic ring, optionally containing one or more additional heteroatoms selected from O, N and S;

or a pharmaceutically acceptable salt, solvate or hydrate thereof or a deuterated or tritiated variant thereof, including all stereoisomers.

The compounds of general formula (I) inhibit of site-specific BAD phosphorylation without affecting its upstream kinase and are therefore of use in promoting apoptosis in multiple cancer-derived cells.

In the present specification, except where the context requires otherwise due to express language or necessary implication, the word “comprises”, or variations such as “comprises” or “comprising” is used in an inclusive sense i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

In the present specification the term “C_{1-6} alkyl” refers to a fully saturated straight or branched hydrocarbon chain having from 1 to 6 carbon atoms. Examples include methyl, ethyl, n-propyl, isopropyl, t-butyl, n-hexyl.

The term “C_{1-4} alkyl” has a similar meaning but refers to alkyl groups with between 1 and 4 carbon atoms.

The term “C_{1-6} haloalkyl” refers to a C_{1-6} alkyl group as defined above in which one or more hydrogen atoms are replaced by halo atoms. Haloalkyl groups may have any number of halo substituents from 1 to perhalosubstituted. Examples inlcude chloromethyl, trifluoromethyl, 1-bromoethyl, 1,1,2,2-tetrafluoroethyl etc.

The term “carbocyclyl” refers to a non-aromatic hydrocarbon ring having from 3 to 7 carbon atoms and optionally containing one or more carbon-carbon double bond. Examples include C_{3-7} cycloalkyl groups such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl and cycloalkenyl groups such as cyclohexenyl.

The term “heterocyclyl” refers to a non-aromatic ring having from 5 to 7 carbon atoms and at least one ring heteroatom selected from N, O and S. Examples include

piperazinyl, piperidinyl, morpholinyl, pyrrolidinyl and imidazolinyl.

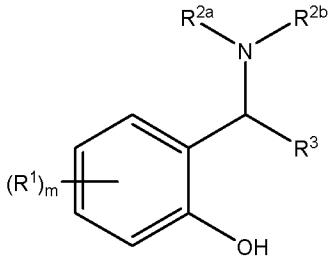
In the present specification, "halo" refers to fluoro, chloro, bromo or iodo.

The term "aryl" in the context of the present specification refer to a ring system with aromatic character having from 5 to 14 ring carbon atoms unless specified otherwise, and containing up to three rings. Where an aryl group contains more than one ring, not all rings must be fully aromatic in character. Examples of aromatic moieties are benzene, naphthalene, indane and indene.

The term "heteroaryl" in the context of the specification refer to a ring system with aromatic character having from 5 to 14 ring atoms (unless specified otherwise) at least one of which is a heteroatom selected from N, O and S, and containing up to three rings. Where a heteroaryl group contains more than one ring, not all rings must be fully aromatic in character. Examples of heteroaryl groups include pyridine, pyrimidine, indole, isoindole, benzofuran, benzimidazole, benzoxazole, benzisoxazole and indolene.

In the compounds of general formula (I), n is suitably 2 or 3, for example 2. At least one R^1 group may be OH. In this case, the OH group is suitably at the position adjacent to the $-CH(R^3)NR^{2a}R^{2b}$ group.

Suitably, the compound of general formula (I) is a compound of general formula general formula (IA):



(IA)

wherein R^1, R^{2a}, R^{2b} and R^3 are as defined above and m is 0, 1, 2, 3 or 4.

Suitably, in the compound of general formulae (I) and (IA):

each R^1 is independently halo, C_{1-6} alkyl, C_{1-6} haloalkyl, aryl or heteroaryl, wherein aryl or heteroaryl groups are optionally substituted with one or more substituents selected from halo, OH, cyano, nitro, $-S(O)_pNR^4R^5$, $-C(O)NR^4R^5$, $-C_{1-6}$ alkyl optionally substituted with aryl, $-C_{1-6}$ haloalkyl, $-O(C_{1-6}$ alkyl) optionally substituted with aryl or $-O(C_{1-6}$ haloalkyl);

p is 0, 1 or 2;

each R^4 and R^5 is independently selected from H or C_{1-4} alkyl or R^4 and R^5 together with the nitrogen atom to which they are attached may form a 5- or 6-membered heterocyclic ring, optionally containing one or more additional heteroatoms selected from O, N and S;

R^{2a} and R^{2b} are each independently C_{1-6} alkyl optionally substituted with one or more substituents selected from halo, OH, aryl or heteroaryl; or

R^{2a} and R^{2b} together with the nitrogen atom to which they are attached form a 5- or 6-membered heterocyclic ring optionally containing one or more further heteroatoms selected from O, N or S and optionally substituted with one or more substituents R^6;

each R^6 is independently selected from aryl, heteroaryl, -O-aryl, -O-heteroaryl or C_{1-4} alkyl substituted with one or more aryl or heteroaryl groups, wherein aryl and heteroaryl groups are substituted with one or more substituent selected from halo, C_{1-4} alkyl, C_{1-4} haloalkyl, -O(C_{1-4} alkyl) or –O(C_{1-4} haloalkyl);

R^3 is aryl, heteroaryl, carbocyclyl or heterocyclyl any of which is optionally substituted with one or more substituents R^7 selected from halo, -C_{1-4} alkyl optionally substituted with aryl, -O(C_{1-4} alkyl) optionally substituted with aryl, -C_{1-4} haloalkyl, -O(C_{1-4} haloalkyl) or -C(O)NR^8R^9;

each R^8 and R^9 is independently selected from H, C_{1-4} alkyl or C_{3-6} cycloalkyl or R^8 and R^9 together with the nitrogen atom to which they are attached may form a 5- or 6-membered heterocyclic ring, optionally containing one or more additional heteroatoms selected from O, N and S;

or a pharmaceutically acceptable salt, solvate or hydrate thereof or a deuterated or tritiated variant thereof, including all stereoisomers.

In some suitable compounds of general formula (IA), m is 0 so that the group R^1 is not present.

In other suitable compounds m is 1 or 2, but more usually 1.

In such cases, R^1 is suitably halo, particularly chloro or fluoro; or alternatively R^1 is an aryl or heteroaryl group optionally substituted as described above. More usually, R^1 is chloro, fluoro or aryl optionally substituted as described above.

When R^1 is an aryl or heteroaryl group it is suitably at the 4-position of the phenyl ring (where the carbon substituted with the OH group is designated as position 1).

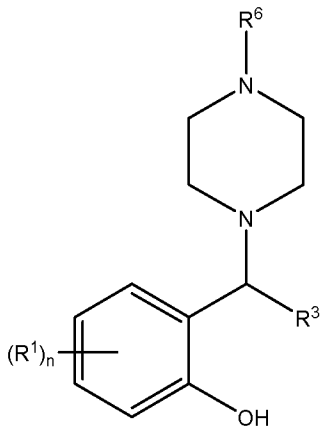
Suitable substituents for aryl or heteroaryl groups R^1 include halo, OH, cyano, nitro, $-SO_2NH_2$, $-C(O)NR^4R^5$, $-C_{1-4}$ alkyl optionally substituted with aryl, $-C_{1-4}$ haloalkyl, $-O(C_{1-4}$ alkyl) optionally substituted with aryl or $-O(C_{1-4}$ haloalkyl), where R^4 and R^5 together with the nitrogen atom to which they are attached form a piperidine or pyrrolidine ring.

More suitable substituents for aryl or heteroaryl groups R^1 include chloro, fluoro, methyl, ethyl, trifluoromethyl, benzyl, methoxy, ethoxy, benzyloxy, trifluoromethoxy and piperidine-1-carbonyl.

In some suitable compounds of the invention, R^{2a} and R^{2b} together with the nitrogen atom to which they are attached form a 5- or 6-membered heterocyclic ring optionally containing one or more further heteroatoms selected from O, N or S and optionally substituted with one or more substituents R^6, wherein R^6 is as defined above.

More suitably, R^{2a} and R^{2b} together with the nitrogen atom to which they are attached form a 6-membered heterocyclic ring optionally substituted with one or more substituents R^6.

Still more suitably, the 6-membered ring formed by R^{2a} and R^{2b} is a piperidine or piperazine ring, most suitably a piperazine ring. When R^{2a} and R^{2b} form a piperazine ring, it may optionally be substituted by one or more substituents R^6 as defined above but is suitably substituted by a single R^6 substituent at the piperazine 4-position such that the compound of general formula (I) is a compound of general formula (IB):



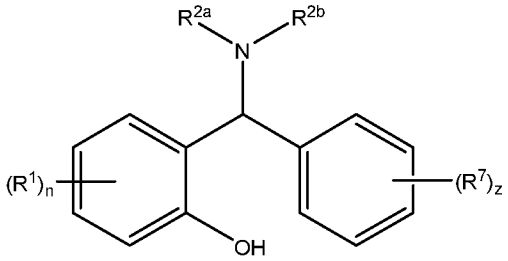
(IB)

wherein R^1, n, R^3 and R^6 are as defined for general formulae (I) and (IA).

Particularly suitable substituents R^6 include aryl, heteroaryl, -O-aryl, -O-heteroaryl or methyl substituted with one or two aryl or heteroaryl groups; wherein aryl and heteroaryl groups are substituted with one or more substituents selected from halo, methyl, ethyl, trifluoromethyl, methoxy, ethoxy or trifluoromethoxy.

Still more suitable substituents R^6 include phenyl, heteroaryl, -O-phenyl, -O-heteroaryl, benzyl, -CH(phenyl)$_2$, -CH_2-heteroaryl and –CH(heteroaryl)$_2$, where the heteroaryl group is selected from pyridinyl, indolyl, isoindolyl, benzoxazolyl and benzisoxazolyl and wherein any of the above R^6 groups may be substituted as described above, but more suitably with one or more substituents selected from halo, methyl ethyl and trifluoromethyl.

In more suitable compounds of general formula (I) and (IA), R^3 is aryl or heteroaryl optionally substituted with one or more substituents R^7 as described above.
Most suitably, R^3 is phenyl optionally substituted with one or more substituents R^7 such that the compound of general formula (I) is a compound of general formula (IC):



(IC)

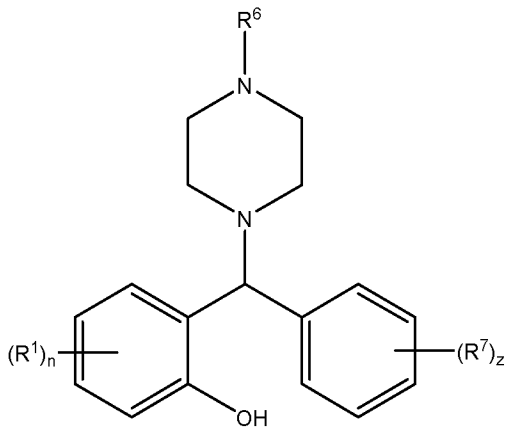
wherein R^1, n, R^{2a}, R^{2b} and R^7 are as defined for general formula (I) or (IA) and z is 0 to 5.

More suitably, z is 0, 1, 2 or 3, most suitably 0, 1 or 2.

R^7 is as defined above but in some suitable compounds R^7 not present (i.e. z is 0), or R^7 is halo, -C_{1-4} alkyl, benzyl, -O(C_{1-4} alkyl) benzyloxy, -C_{1-4} haloalkyl, -O(C_{1-4} haloalkyl) or -C(O)NR^8R^9, where R^8 and R^9 together with the nitrogen atom to which they are attached form a piperidinyl ring or wherein R^8 is H and R^9 is C_{3-7} cycloalkyl.

More suitably, R^7 not present or each R^7 is independently halo, especially chloro or fluoro, methyl, ethyl, benzyl, methoxy, ethoxy, benzyloxy, -C(O)-piperidinyl or -C(O)NH-C_{3-7} cycloalkyl.

Some particularly suitable compounds of the invention are compounds of general formula (ID):



(ID)

wherein R^1, n, R^6, R^7 and z are as defined above for general formulae (I), (IA), (IB) and (IC).

Example compounds of general formula (I) include:

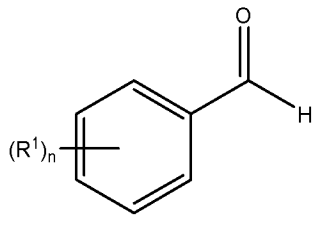
2-((2-chlorophenyl)(4-(4-methoxyphenyl)piperazin-1-yl)methyl)phenol (Compound 1);
2-((4-chlorophenyl)(4-(4-methoxyphenyl)piperazin-1-yl)methyl)phenol (Compound 2);
2-((4-(benzyloxy)-3-fluorophenyl)(4-(4-methoxyphenyl)piperazin-1-yl)methyl)phenol (Compound 3);
(4-((2-hydroxyphenyl)(4-(4-Methoxyphenyl)piperazinyl)methyl)phenyl)(piperidin-1-yl)methanone (Compound 4);
3-((5-chloro-2-hydroxyphenyl)(4-(4-methoxyphenyl)piperazin-1-yl)methyl)-N-cyclopentylbenzamide (Compound 5);
2-((4-(benzyloxy)-3-fluorophenyl)(4-(4-methoxyphenyl)piperazin-1-yl)methyl)-4-chlorophenol (Compound 6);
2-((4-(benzyloxy)-3-fluorophenyl)(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)phenol (Compound 7);
2-((4-(2, 3-dichlorophenyl)piperazin-1-yl)(o-tolyl)methyl)phenol (Compound 8);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(2-hydroxyphenyl)methyl) benzamide (Compound 9, NPB);
2-((4-(benzyloxy)-3-fluorophenyl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)phenol (Compound 10);
2-((4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)(phenyl)methyl)phenol (Compound 11);
2-((4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)(p-tolyl)methyl)phenol (Compound

12);
2-((4-chlorophenyl)(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)methyl)phenol (Compound 13);
2-((4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)(4-ethylphenyl)methyl)phenol (Compound 14);
(4-((4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)(2-hydroxyphenyl)methyl)phenyl)(piperidin-1-yl)methanone (Compound 15);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 16);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-2'-methyl-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 17);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-3'-methyl-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 18);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-4'-methyl-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 19);
3-((2'-chloro-4-hydroxy-[1,1'-biphenyl]-3-yl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-N-cyclopentylbenzamide (Compound 20);
3-((3'-chloro-4-hydroxy-[1,1'-biphenyl]-3-yl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-N-cyclopentylbenzamide (Compound 21);
3-((4'-chloro-4-hydroxy-[1,1'-biphenyl]-3-yl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-N-cyclopentylbenzamide (Compound 22);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4'-ethyl-4-hydroxy-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 23);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-4'-(piperidine-1-carbonyl)-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 24);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-4'-methoxy-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 25);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(2'-ethyl-4-hydroxy-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 26);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(2'-fluoro-4-hydroxy-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 27);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(3'-fluoro-4-hydroxy-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 28);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4'-fluoro-4-hydroxy-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 29);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-3'-nitro-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 30);

N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-3'-sulfamoyl-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 31);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-2'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 32);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-3'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 33);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-4'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 34);
3-((2'-cyano-4-hydroxy-[1,1'-biphenyl]-3-yl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-N-cyclopentylbenzamide (Compound 35);
3-((3'-cyano-4-hydroxy-[1,1'-biphenyl]-3-yl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-N-cyclopentylbenzamide (Compound 36)
3-((4'-cyano-4-hydroxy-[1,1'-biphenyl]-3-yl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-N-cyclopentylbenzamide (Compound 37);
3-((2'-chloro-4-hydroxy-4'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl)(4-(2,3-dichlorophenyl) piperazin-1-yl)methyl)-N-cyclopentylbenzamide (Compound 38);
N-cyclopentyl-3-((2',4'-dichloro-4-hydroxy-[1,1'-biphenyl]-3-yl)(4-(2,3-dichlorophenyl) piperazin-1-yl)methyl)benzamide (Compound 39);
3-((4'-chloro-2',4-dihydroxy-[1,1'-biphenyl]-3-yl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-N-cyclopentylbenzamide (Compound 40);
3-((4-(4-chlorophenyl)piperazin-1-yl)(2-hydroxyphenyl)methyl)-N-cyclopentylbenzamide (Compound 41, NCK1);
2-((4-chlorophenyl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)phenol (Compound 42, NCK2);
2-((4-(2,3-dichlorophenyl)piperazin-1-yl)(3-methoxyphenyl)methyl)phenol (Compound 43, NCK3);
1-(5-((4-(2,3-dichlorophenyl)piperazin-1-yl)(2-hydroxyphenyl)methyl)thiophen-2-yl)ethanone (Compound 44, NCK4);
2-((4-(2,3-dichlorophenyl)piperazin-1-yl)(naphthalen-1-yl)methyl)phenol (Compound 45, NCK5);
5-((4-(2,3-dichlorophenyl)piperazin-1-yl)(2-hydroxyphenyl)methyl)furan-2-carbaldehyde (Compound 46, NCK6);
2-((4-(5,6-dichlorocyclohexa-1,5-dien-1-yl)piperazin-1-yl)(2-fluoro-3-methylpyridin-4-yl)methyl)phenol (Compound 47, NCK7);
2-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-(trifluoromethyl)phenyl)methyl)phenol (Compound 48, NCK8);
2-((6-chloro-5-methylpyridin-3-yl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)phenol

(Compound 49, NCK9);
2-((4-(2,3-dichlorophenyl)piperazin-1-yl)(pyridin-3-yl)methyl)phenol (Compound 50, NCK10);
1-(5-((4-(4-chlorophenyl)piperazin-1-yl)(2-hydroxyphenyl)methyl)thiophen-2-yl)ethanone (Compound 51, NCK14);
3-((4-(4-chlorophenyl)piperazin-1-yl)(4-(diethylamino)-2-hydroxyphenyl)methyl)-N-cyclopentylbenzamide(Compound 52, NCK16);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-(diethylamino)-2-hydroxyphenyl)methyl)benzamide (Compound 53, NCK18);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(2-hydroxy-4,6-dimethoxyphenyl)methyl)benzamide (Compound 54, NCK19);
2-((4-chlorophenyl)(4-(4-chlorophenyl)piperazin-1-yl)methyl)phenol (Compound 55, NCK20);
2-((4-(4-chlorophenyl)piperazin-1-yl)(6-methylpyridin-3-yl)methyl)phenol (Compound 56, NCK21);
2-(o-tolyl(4-(p-tolyl)piperazin-1-yl)methyl)phenol (Compound 57, SG1);
2-((4-(p-tolyl)piperazin-1-yl)(4-(trifluoromethyl)phenyl)methyl)phenol (Compound 58, SG2);
N-cyclopentyl-4-((2-hydroxyphenyl)(4-(p-tolyl)piperazin-1-yl)methyl)benzamide (Compound 59, SG3);
2-((4-chlorophenyl)(4-(p-tolyl)piperazin-1-yl)methyl)phenol (Compound 60, SG4);
2-((3-methoxyphenyl)(4-(p-tolyl)piperazin-1-yl)methyl)phenol (Compound 61, SG5);
5-((2-hydroxyphenyl)(4-(p-tolyl)piperazin-1-yl)methyl)furan-2-carbaldehyde (Compound 62, SG6); and
2-((6-methylpyridin-3-yl)(4-(p-tolyl)piperazin-1-yl)methyl)phenol (Compound 63, SG7).

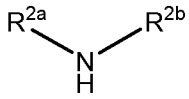
Compounds of general formula (I) may be prepared by reacting an aldehyde of general formula (II):



(II)

wherein R^1 and n are as defined for general formula (I);

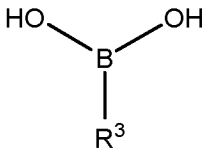
with a compound of general formula (III):



(III)

wherein R^{2a} and R^{2b} are as defined for general formula (I);

and a boronic acid of general formula (IV):



(IV)

wherein R^3 is as defined for general formula (I).

The reaction is a Petasis reaction, which is a 3-component boronic mannich- type reaction which uses boronic acids of general formula (IV) as a potential nucleophilic species to react with the salicylaldehyde derivative of general formula (II) and the amine of general formula (III) to form a new carbon-carbon bond. The Petasis reaction proceeds via the formation of an iminium species, which reacts with the boronic acid to yield tertiary amines.

Suitable reaction solvents include organic solvents, for example cyclic ethers such as dioxane.

Suitably, the process comprises:

i. reacting the compound of general formula (II) with the compound of general formula (III) to form a mixture comprising intermediate iminium species;

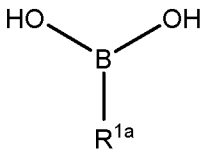
ii. reacting the product of step (i) with the compound of general formula (IV).

Suitably step (ii) is carried out at elevated temperature, for example 50 to 100°C, for example at the reflux temperature of the solvent. When the solvent is dioxane, this is about 85 to 95°C, typically about 90°C.

The product may be extracted into a second solvent such as ethyl acetate, dried and purified using standard methods such as described below in the examples.

Compounds of general formulae (II), (III) and (IV) are known and are commercially available or may be prepared using known methods.

Compounds of general formula (I) may also be prepared from other compounds of general formula (I). For example, a compound of general formula (I) in which R^1 is optionally substituted aryl or heteroaryl may be prepared from compounds of general formula (I) in which R^1 is halo by reaction with a compound of general formula (V):



(V)

wherein R^{1a} is aryl or heteroaryl optionally substituted as defined for general formula (I).

The reaction may be carried out using a Suzuki coupling reaction over a palladium catalyst.

Compounds of general formula (V) are known and are commercially available or may be prepared using known methods.

The methods for preparing compounds of general formula (I) represent a further aspect of the present invention.

The compounds of general formula (I) are capable of inhibiting/downregulating/suppressing Bcl-2 associated death promoter (BAD) protein, particularly inhibiting/downregulating/suppressing phosphorylation of Bcl-2 associated death promoter (BAD) protein in a cancer cell. The inhibition/downregulation/suppression of BAD occurs without affecting the upstream Ser/Thr kinase called Akt/protein kinase B (PKB). Therefore, the compounds of general formula (I) preferentially inhibit/downregulate/suppresses phosphorylation of BAD.

The inhibition/downregulation of BAD is carried out by the compound of general formula (I) occupying the hydrophobic groove within the protein-protein interface of Bcl-2/BAD. Additionally, the compound of general formula (I) occupies an additional hydrophobic side pocket within the interface formed by the side-chains of Leu-97, Trp-144, and Phe-198 of the human protein with a dichlorophenyl moiety.

The compounds of formula (I) are potent inhibitors/downregulators of BAD in cancer cells, particularly breast cancer, endometrial cancer, ovarian cancer, liver cancer, colon cancer, prostate cancer and pancreatic cancer cells and induce apoptosis due to the downregulation/inhibition of BAD phosphorylation. Said compounds enhance cytotoxicity

of cancer cells and carry out target specific downregulation/inhibition of BAD phosphorylation without affecting the upstream kinases.

The compounds of general formula (I) were screened for their anticancer activity against MCF7 cells and NPB (Compound 9) was found to be the most potent compound. NPB was subsequently evaluated against panel of normal immortalized mammary epithelial cells (MCF10A and MCF12A), normal hepatocytes (LO2), mammary carcinoma (BT549, MDA-MB-231, MCF7, T47D, BT474), endometrial cancer (Ishikawa, Ecc1, RL95-2, AN3), ovarian cancer (SK-OV-3, OVCAR-2, Caov-3, HEY C2, Ovca433), liver cancer (Hep3B, H2P, H2M), colon cancer (HCT116, DLD-1, Caco-2), prostate cancer (PC3, LNCaP, DU145) and pancreatic cancer (AsPC-1, BxPC-3) cells. NPB significantly enhanced apoptosis in all the tested cancer cell lines and NPB exhibited no substantial activity against normal hepatocytes and immortalized mammary epithelial cells. Using a Laplacian-modified Naïve Bayes classifier, NPB is subjected to in silico analysis which anticipates the human target of NPB as BAD protein.

NPB was found to induce apoptotic cell death in a range of carcinoma cells but did not have cytotoxic effect on normal (*immortalized*) epithelial cells.

The hallmark features of cells undergoing apoptosis are phosphatidylserine externalization, caspase activation and genomic DNA fragmentation. In an embodiment, NPB exhibits apoptosis against MCF7 cells using FITC-annexin-V and propidium iodide staining. FITC-annexin-V staining confirms the loss of membrane integrity and PI staining confirms the late apoptotic events and these results are correlated with caspase 3/7 activity.

Therefore in a further aspect of the invention, there is provided a compound of general formula (I) for use in medicine.

The compounds of general formula (I) are of particular use in the treatment of cancer, suitably, a cancer in which there is BAD phosphorylation..

In a further aspect, therefore, there is provided a compound of general formula (I) for use in the treatment of cancer.

Further, there is provided the use of a compound of general formula (I) in the preparation of an agent for the treatment of cancer.

There is also provided a method for the treatment of cancer, the method comprising administering to a patient in need of such treatment an effective amount of a compound of general formula (I).

The compounds are particularly useful for treating cancer in which there is BAD phosphorylation and, for example, the cancer may be breast cancer, endometrial cancer, ovarian cancer, liver cancer, colon cancer, prostate cancer or pancreatic cancer or other epithelial-derived cancers in which BAD is phosphorylated.

The compounds of general formula (I) are suitably administered in a pharmaceutical composition and therefore in a further aspect of the invention there is provided a pharmaceutical composition comprising a compound of general formula (I) and a pharmaceutically acceptable excipient.

The pharmaceutically acceptable excipient may be an adjuvant, diluent, carrier, granulating agent, binding agent, lubricating agent, disintegrating agent, sweetening agent, glidant, anti-adherent, anti-static agent, surfactant, anti-oxidant, gum, coating agent, coloring agent, flavouring agent, coating agent, plasticizer, preservative, suspending agent, emulsifying agent, plant cellulosic material, spheronization agent, other conventionally known pharmaceutically acceptable excipient or any combination of excipients thereof.

In another embodiment, the pharmaceutical composition of the present invention is formulated for intraperitoneal administration, hepatoportal administration, intravenous administration, intra articular administration, pancreatic duodenal artery administration or intramuscular administration, or any combination thereof.

The invention will now be described in greater detail with reference to the examples and to the drawings in which:

Figures 1(a) and 1(b) depict the ^{1}H-NMR and ^{13}C-NMR spectra of Compound 1.

Figure 2 depicts the ^{13}C-NMR spectra of compound 2.

Figures 3(a) and 3(b) depict the ^{1}H-NMR and ^{13}C-NMR spectra of Compound 3.

Figures 4(a) and 4(b) depict the ^{1}H-NMR and ^{13}C-NMR spectra of Compound 4.

Figures 5(a) and 5(b) depict the ^{1}H-NMR and ^{13}C-NMR spectra of Compound 5.

Figure 6 depicts the ^{1}H-NMR spectra of compound 6.

Figures 7(a) and 7(b) depict the ^{1}H-NMR and ^{13}C-NMR spectra of Compound 7.

Figure 8 depicts the ^{13}C-NMR spectra of Compound 8.

Figures 9(a) and 9(b) depict the ^{1}H-NMR and LCMS spectra of Compound 9.

Figure 10 depicts the ^{1}H-NMR spectra of Compound 10.

Figure 11: IC50 values of NPB in a range of carcinoma cell lines.

Figure 12: NPB suppresses cell viability and promote apoptosis in carcinoma cell lines.

Effect of NPB (5μM) on carcinoma cell viability including, mammary, endometrial, ovarian, liver, colon, prostate, and pancreatic carcinoma cell lines. (**A**) Cell viability (**B**) caspase 3/7 activities and (**C**) cytotoxicity were evaluated using ApoTox-Glo™ Triplex Assay Kit as described in methodology. Statistical significance was assessed by an unpaired two-tailed *Student's t-test* using GraphPad Prism5. The column represents mean of triplicate determinations; bars, ±*SD*. ***P* < *0.001*, **P* < *0.05*. **Note**: RFU, relative fluorescence unit; RLU, relative luminescence unit, #; non-transformed, *immortalized* epithelial cells; MB-231, MDA-MB-231.

Figure 13: NPB stimulates apoptotic cell death in MCF7 cells.

(**A**) Apoptotic cell death of MCF7 cells measured after treatment with 10μM NPB using flow cytometry analysis. Annexin V-FITC staining is indicated on the X-axis and PI staining on the Y-axis. The lower left quadrant represents live cells, the lower right quadrant represents early apoptotic cells, the upper left quadrant represents necrotic cells, and the upper right quadrants display late apoptotic cells. Acquisition of Annexin V and PI data were represented as a percentage (%) in each quadrant. (**B**) Cell cycle analysis of MCF7 cells measured after treatment with 10μM NPB using flow cytometry analysis. (**C**) Cell

viability of performed colonies generated by MCF7 cell after exposure to NPB or DMSO cultured 14 days in 3D Matrigel using AlamarBlue® viability assay. Microscopic visualization (*below*) of Calcein AM stained colonies generated by MCF7 cells after exposure to NPB or DMSO cultured in 3D Matrigel. (**D**) Cell viability in colonies generated by MCF7 cell after exposure to NPB or DMSO cultured in Soft agar using AlamarBlue® viability assay. (**E**) Crystal Violet staining of foci colonies generated by MCF7 cells after exposure to NPB or DMSO. All assays performed as described in methodology. Statistical significance was assessed by an unpaired two-tailed *Student's t-test* using GraphPad Prism5. The column represents mean of triplicate determinations; bars, ±*SD*. $^{**}P < 0.001$, $^{*}P < 0.05$.

Figure 14: Cheminformatics and surface plasmon resonance (SPR) analysis predicts an interaction of NPB compound to BAD protein.

(**A**) Sensorgrams obtained by SPR analysis of NPB with the BAD protein subunit. The BAD protein subunit was immobilized onto the surface of a CM5 sensor chip. A solution of NPB at variable concentrations (20-100µM) was injected to generate result binding responses (RU) recorded as a function of time (sec). The results were analyzed using BIA evaluation 3.1. (**B**) Western blot (WB) analysis was used to assess the level of Ser99 phosphorylation of BAD in MCF7 cells after treatment with NPB. *(Below) Calculated* IC_{50} *of NPB from dose-response for BAD phosphorylation (Ser99), BAD and β-ACTIN as in shown above by use of ImageJ software from NIH, USA (http://imagej.nih.gov/ij/).* (**C**) WB analysis was used to assess the level of a multiple proteins involved upstream of BAD in MCF7 cells after treatment with NPB. (**D**) WB analysis was used to assess the level of a multiple protein involved cell survival and cell proliferation in MCF7 cells after treatment with NPB. For WB analysis, soluble whole cell extracts were run on an SDS-PAGE and immunoblotted as described in methodology. β-ACTIN (ACTB) was used as an input control for cell lysate. The sizes of detected protein bands in kDa are shown on the *left side.*

Figure 15: NPB specifically inhibits phosphorylation of BAD (at Ser99) in carcinoma cell lines independent of AKT signalling

(A) WB analysis was used to assess the levels of phosphorylated human BAD (at Ser75 and Ser99) and BAD protein in the range of carcinoma cell lines, including mammary, ovarian, pancreatic, endometrial, hepatocellular, colon and prostate cancer after treatment with NPB (5µM). Total BAD was used as an input control for cell lysate. **(B)** WB analysis was used to assess the levels of pBAD (Ser99) and pAKT (Ser473), AKT and BAD in MCF7, Caov-3, Ishikawa, and AsPC-1 cells. 5µM each of AKT inhibitor (IV) and NPB was

used to treat cells. Depletion of *AKT* expression was achieved using transient-transfection of *short hairpin* (*sh*)-*RNA* (*1&2*) directed to *AKT* transcript as described in methodology. β-ACTIN was used as an input control for cell lysate. For WB analysis, soluble whole cell extracts were run on an SDS-PAGE and immunoblotted as described in materials methodology. The sizes of detected protein bands in kDa are shown on the *left side.* **Note**: #; non-transformed *immortalized*-cell line.

Figure 16: *siRNA-mediated* depletion of *BAD* expression prevents the effect of NPB in carcinoma cell lines.

(A) WB analysis was used to assess the levels of pBAD (Ser99) activity and BAD protein in MCF7, BT474, Caov-3, Ishikawa, AsPC-1, and DLD-1 cells after treatment with 5μM NPB. Depletion of *BAD* expression was achieved using transient-transfection of *small interfering* (*si*)-*RNA* directed to the *BAD* transcript. Soluble whole cell extracts were run on an SDS-PAGE and immunoblotted as described in materials and methods. β-ACTIN was used as input control. Effects of NPB (5μM) in MCF7, BT474, Caov-3, Ishikawa, AsPC-1, and DLD-1 cells. **(B)** Cell viability and **(C)** caspase 3/7 activities were evaluated using the ApoTox-Glo™ Triplex Assay Kit. All assays performed as described in methodology. Statistical significance was assessed by an unpaired two-tailed *Student's t-test* (*P<0.05* was considered as significant) using GraphPad Prism5. The column represents mean of triplicate determinations; bars, ±*SD*. ***P < 0.001*, **P < 0.05*. **Note** RFU, relative fluorescence unit; RLU, relative luminescence unit.

Figure 17: NPB inhibits phosphorylation of BAD Ser99 in mammary carcinoma and inhibits tumour growth

(A) Measurement of tumour volume in BALB/c-nu female mice *as described in materials and methods. Animals (n=5 each group)* were treated with vehicle, 5 mg/kg NPB or 20mg/kg NPB, and relative tumour burden was recorded. Animal weight was measured daily for the duration of the experiment. **(B)** Tumours were excised after the NPB treatment regime and weighed. Representative resected tumours are shown in the *right side*. **(C)** WB of tumour tissue to determine levels of p-BAD (Ser99) and BAD. Soluble whole cell extracts were run on an SDS-PAGE and immunoblotted as described in methodology. β-ACTIN was used as an input control. The sizes of detected protein bands in kDa are shown on the *left side.* **(D)** Histological analyses of phospho-BAD, BAD, Ki67 and TUNEL staining. Tumour tissue sections were immunolabeled with goat anti-pBAD (Ser 136) polyclonal antibody (Santa Cruz Biotechnology), mouse anti-BAD monoclonal (Santa Cruz Biotechnology) and anti-Ki67 antibody (Abcam, ab15580) and stained with hematoxylin. Apoptotic DNA fragmentation was detected using TUNEL Apoptosis Detection Kit (Gen

Script USA Inc.) as described in methodology. Statistical significance was assessed by an unpaired two-tailed *Student's t-test* (*P<0.05* was considered as significant) using GraphPad Prism5. The point represents mean of triplicate experiments; bars, ±*SD*. ***P* < *0.001*, **P* < *0.05*.

Figure 18:

(**A**) Western blot analysis was used to assess the level of BAD Ser99 phosphorylation of pBAD, BAD, pAKT, and AKT in MCF7 cells after an increasing period of treatment with NPB (10μM). Soluble whole cell extracts were run on an SDS-PAGE and immunoblotted as described in methodology. The sizes of detected protein bands in kDa are shown on the *left side.* (**B**) Kinases and phosphorylated substrates were detected using a Western Blot array (Proteome Profiler Human Phospho-Kinase Array Kit. MCF7 cells treated with NPB (10μM) or DMSO for 12h at 37^0C before preparation of cell lysate. Mean pixel density was analysed using ImageJ software and is represented *below*.

Figure 19 depicts the ^{1}H-NMR spectra of Compound NCK5.

Figure 20 depicts the ^{1}H-NMR spectra of Compound NCK16.

Figure 21 depicts the ^{1}H-NMR spectra of Compound NCK18.

Figures 22A, 22B, 22C, 22D and 22E: IC_{50} values of NPB structure-based analogues in a carcinoma cell lines.

Note: NV, no value

<u>Materials employed to arrive at the Examples of the present disclosure</u>

Cell culture and Reagents –

The human *immortalized* mammary epithelial cell lines, MCF10A, and MCF12A; and immortalized hepatocellular epithelial cell line, LO2 were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and were cultured as per ATCC propagation instructions. MC cell lines, MCF7, T47D, BT474, BT549, and MDA-MB-231 (denoted as MB-231); endometrial carcinoma cell lines, Ishikawa, ECC1, RL95-2 and AN3; hepatocellular carcinoma cell lines, Hep3B, H2P, and H2M; colon carcinoma cell lines, HCT116, DLD-1, and Caco-2; and prostate carcinoma cell lines, PC3, LNCaP, DU145 were obtained from the American Type Culture Collection (ATCC, Rockville, MD). Ovarian carcinoma cell lines, SK-OV-3, OVCAR-2, Caov-3, HEY C2, and Ovca433 were obtained from Dr Ruby Huang's laboratory at The Cancer Science Institute of Singapore, National

University of Singapore (NUS). Pancreatic carcinoma cell lines were obtained from Prof. H. Phillip Koeffler's laboratory at The Cancer Science Institute of Singapore, National University of Singapore (NUS). All carcinoma cell lines were cultured as per ATCC propagation instructions. AKT inhibitor IV was purchased from Calbiochem (San Diego, CA, USA). BAD directed stealth (sh)-RNA-BAD (shRNA-BAD1, 5'-GCUCCGCACCAUGAGUGACGAGUUU-3' and shRNA-BAD2, 5'AAACUCGUCACUCAUCCUCCGGAGC3') was purchased from Life Technologies (Singapore). AKT directed shRNA (shRNA-AKT1, 5'-CCGGCGCGTGACC ATGAACGAGTTTCTCGAGAAACTCGTTCATGGTCACGCGTTTTTG-3' and shRNA2-AKT, 5'-CCGGGGACTACCTGCACTCGGAGAACTCGAGTTCTCCGAGTGCAGGTAG TCCTTTTTG-3') was purchased from Life Technologies (Singapore), and cloned in to PLKO.1 vector (Sigma, Singapore). Cells were transiently-transfected with 20nM shRNA (AKT or BAD) or universal negative control (Invitrogen, Carlsbad, CA, USA) using FuGENE HD (Promega) for 24h and further assays performed. Alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), blood urea nitrogen (BUN) commercial kits were purchased from AGAPPE Diagnostics Ltd, Kerala, India.

Example 1

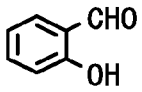
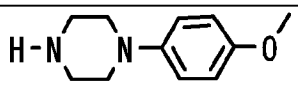
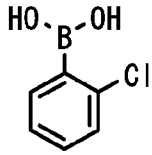
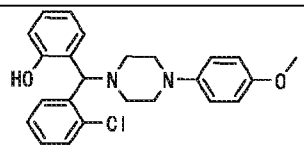
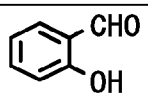
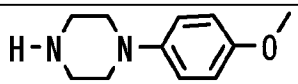
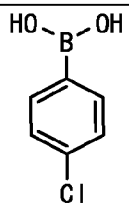
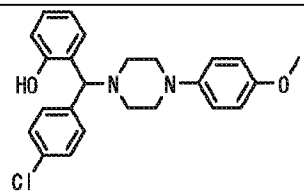
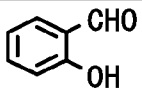
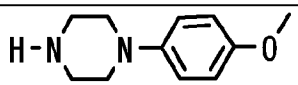
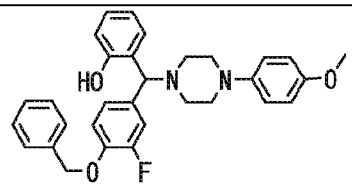
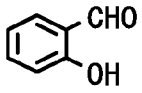
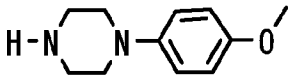
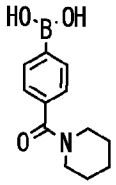
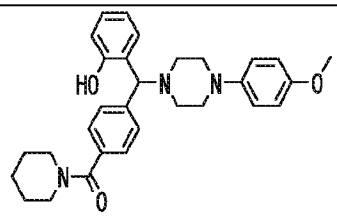
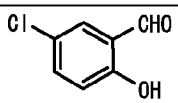
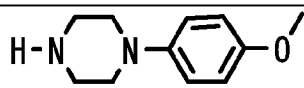
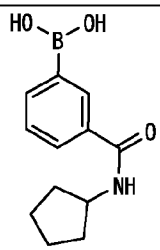
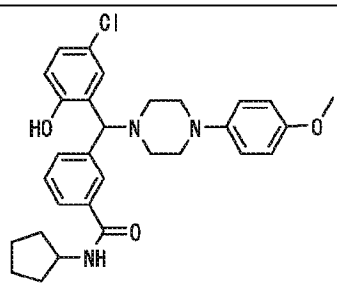
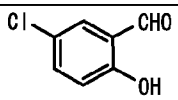
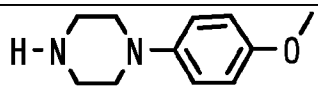
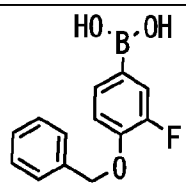
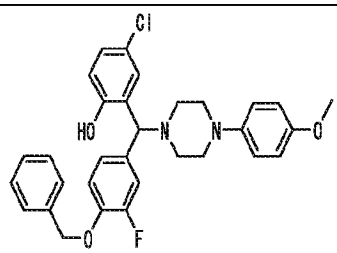
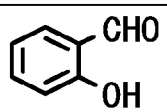
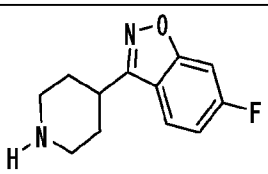
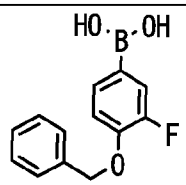
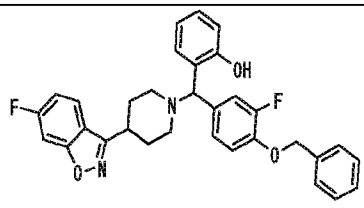
Synthesis and Characterization of Formula I compounds

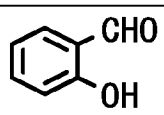
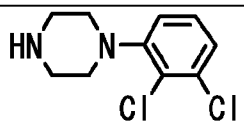
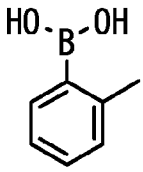
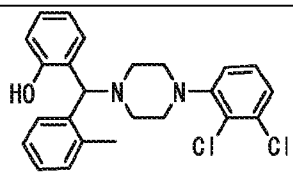
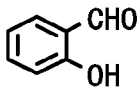
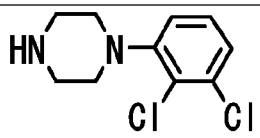
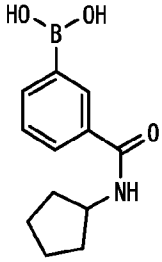
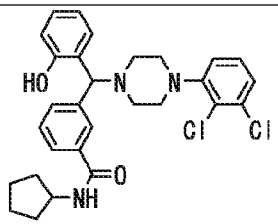
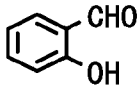
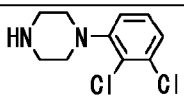
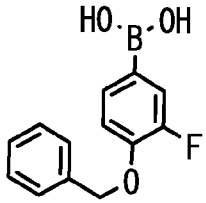
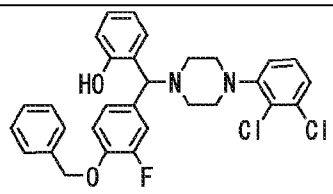
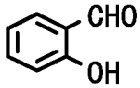
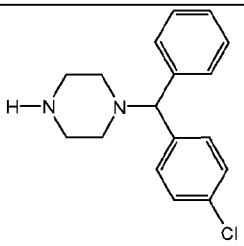
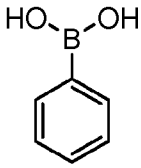
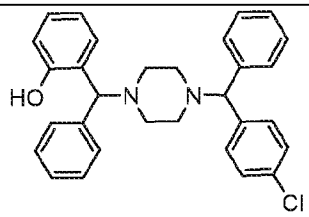
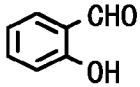
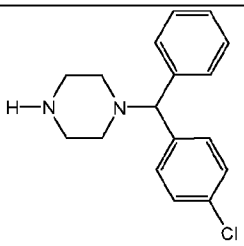
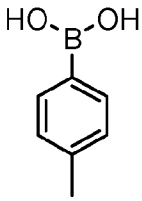
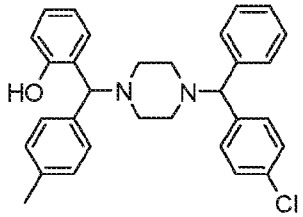
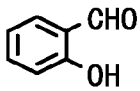
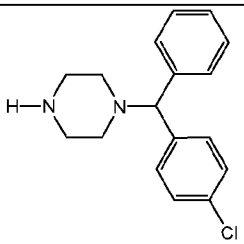
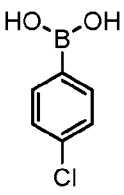
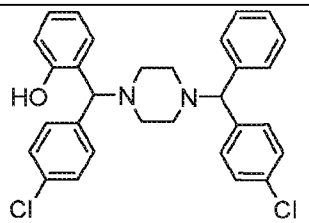
General Synthesis of Compound of Formula I

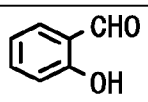
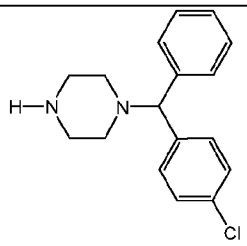
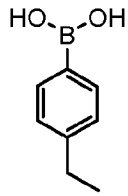
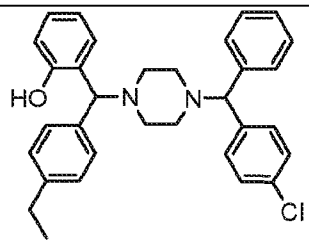
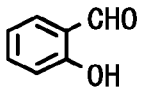
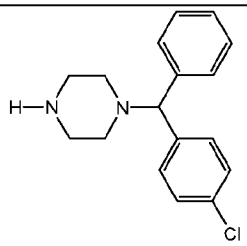
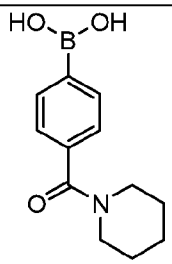
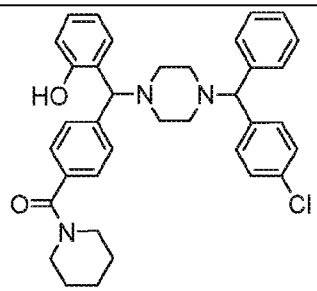
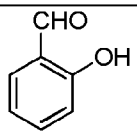
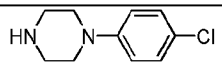
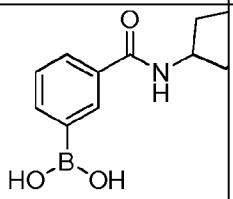
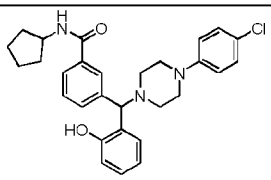
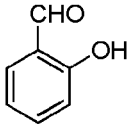
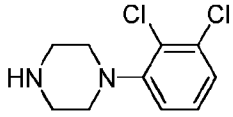
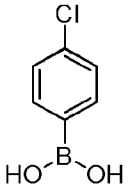
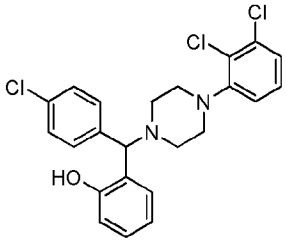
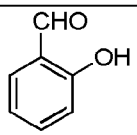
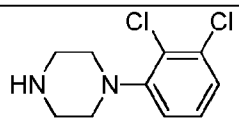
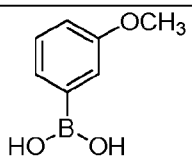
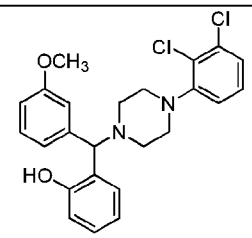
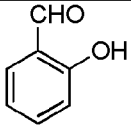
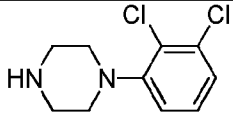
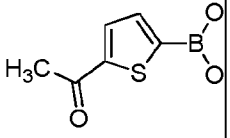
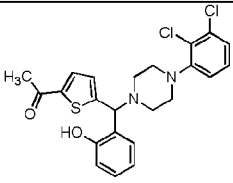
Piperazines (0.8mmol) and salicylaldehyde (0.8mmol) are taken in an RBF and stirred for about 10 minutes using Dioxane as solvent. After about 10 minutes, Aryl boronic acid (0.8mmol) is added to the mixture and refluxed with continuous stirring for about 8 hours using Dioxane as solvent on a hot plate maintained at about 90ºC. After about 8 hours, ethyl acetate and water are added to the reaction mixture and the ethyl acetate layer is separated using separating funnel and dried over anhydrous sodium sulfate. Ethyl acetate is evaporated to obtain the product. The desired phenolic compound product is obtained by separation using column chromatography.

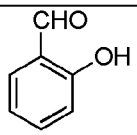
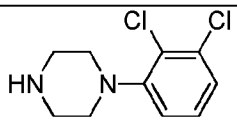
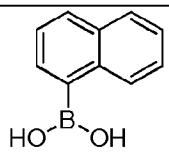
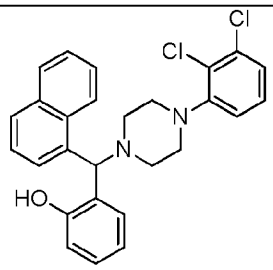
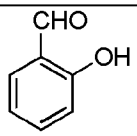
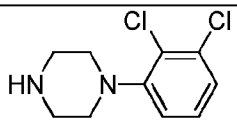
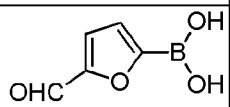
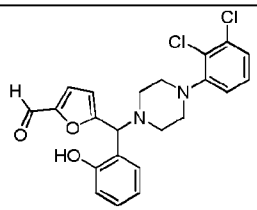
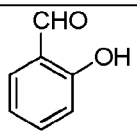
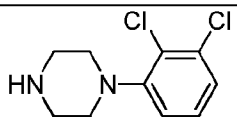
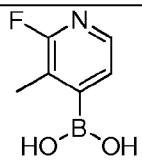
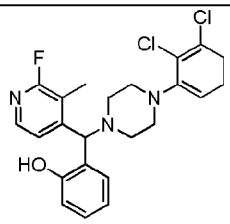
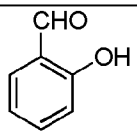
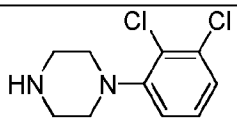
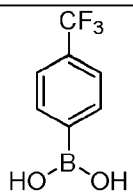
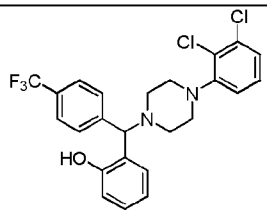
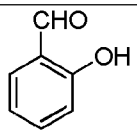
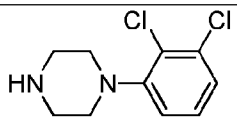
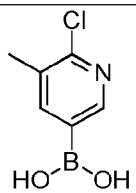
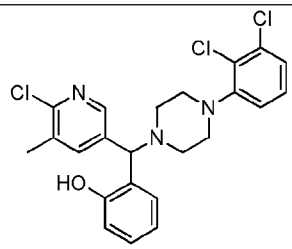
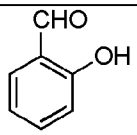
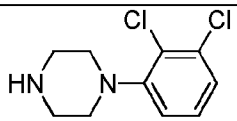
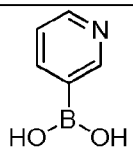
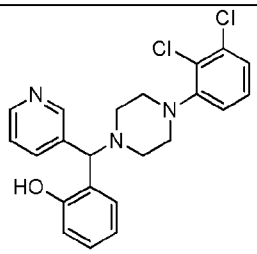
The specific reagents used to obtain Compounds 1 to 15 and 41 to 63 are provided in Table 1 below.

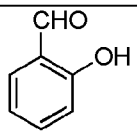
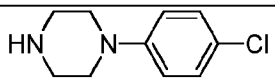
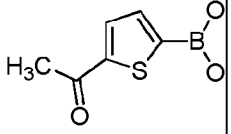
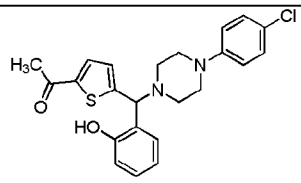
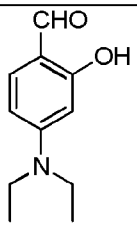
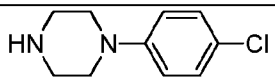
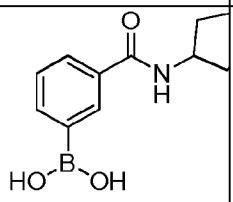
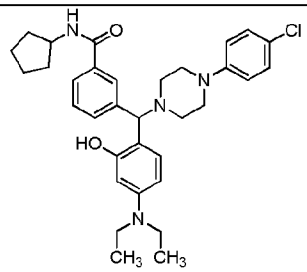
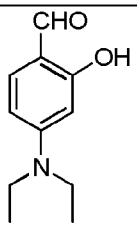
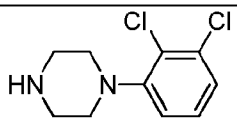
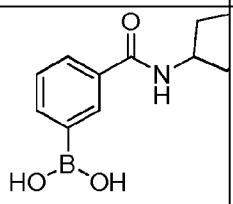
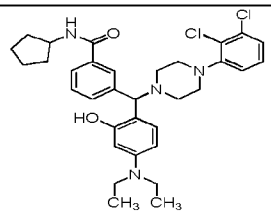
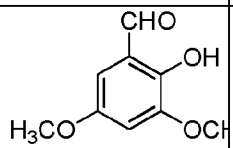
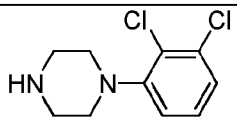
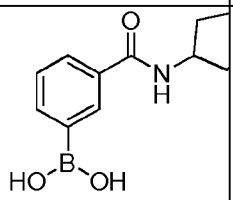
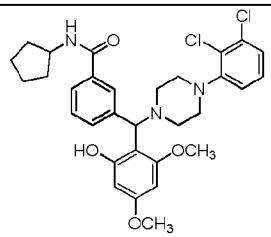
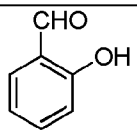
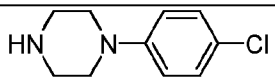
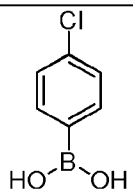
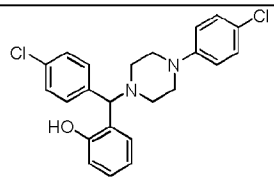
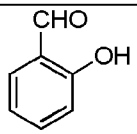
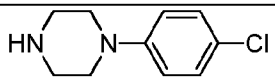
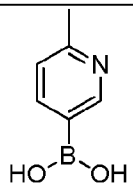
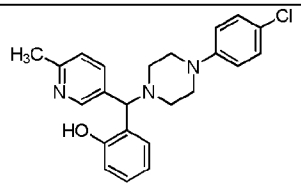
TABLE 1

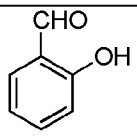
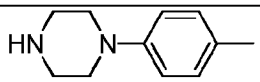
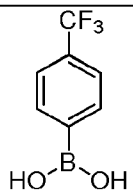
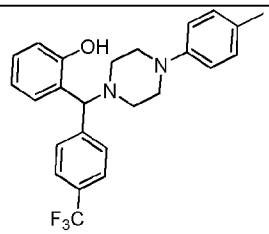
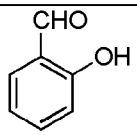
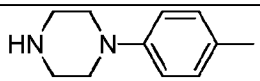
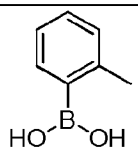
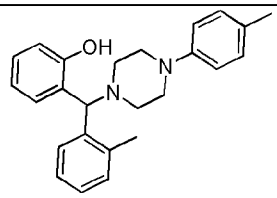
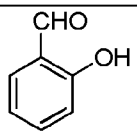
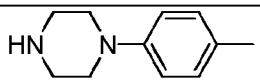
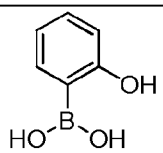
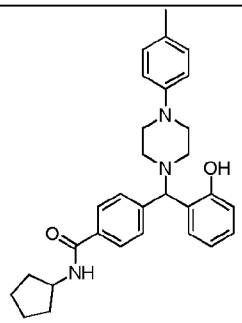
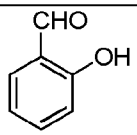
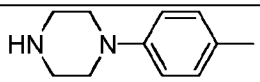
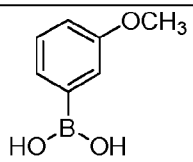
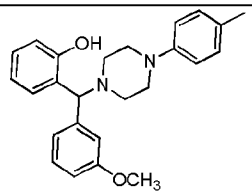
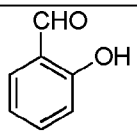
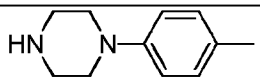
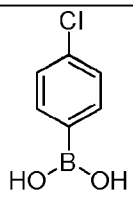
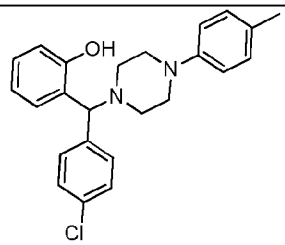
No	Salicylaldehyde	Piperazine	Aryl boronic acid	Product
1				
2				
3				
4				
5				
6				
7				

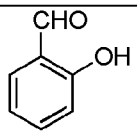
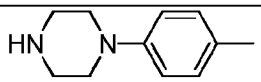
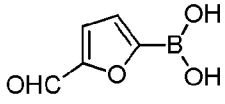
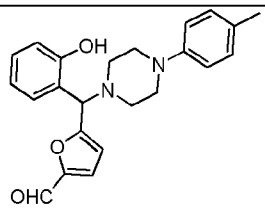
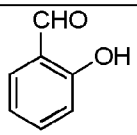
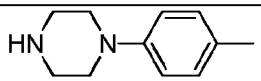
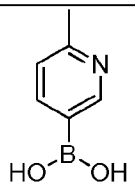
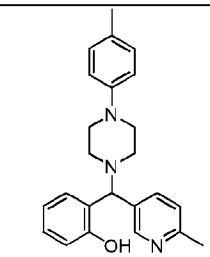
No	Salicylaldehyde	Piperazine	Aryl boronic acid	Product
8				
9				
10				
11				
12				
13				

No	Salicylaldehyde	Piperazine	Aryl boronic acid	Product
14				
15				
41				 Molecular Weight: 490.0363
42				 Molecular Weight: 447.7846
43				 Molecular Weight: 443.3656
44				 Molecular Weight: 461.4040

No	Salicylaldehyde	Piperazine	Aryl boronic acid	Product
45				 Molecular Weight: 463.3983
46				 Molecular Weight: 431.3118
47				 Molecular Weight: 448.3606
48				 Molecular Weight: 481.338
49				 Molecular Weight: 462.7993
50				 Molecular Weight: 414.3276

No	Salicylaldehyde	Piperazine	Aryl boronic acid	Product
51				 Molecular Weight: 426.9589
52				 Molecular Weight: 561.16
53				 Molecular Weight: 595.60
54				 Molecular Weight: 584.53
55				 Molecular Weight: 413.34
56				

No	Salicylaldehyde	Piperazine	Aryl boronic acid	Product
58				 Molecular Weight: 426.4740
57				 Molecular Weight: 372.5026
59				
61				 Molecular Weight: 388.5020
60				 Molecular Weight: 392.9211

No	Salicylaldehyde	Piperazine	Aryl boronic acid	Product
62				 Molecular Weight: 364.4806
63				 Molecular Weight: 373.4907

The compounds shown in Table 2 were obtained by reacting NPB bromide (see column 1 of Table 2) with a boronic acid using a palladium catalysed Suzuki coupling reaction as shown in Scheme 1.

Scheme 1

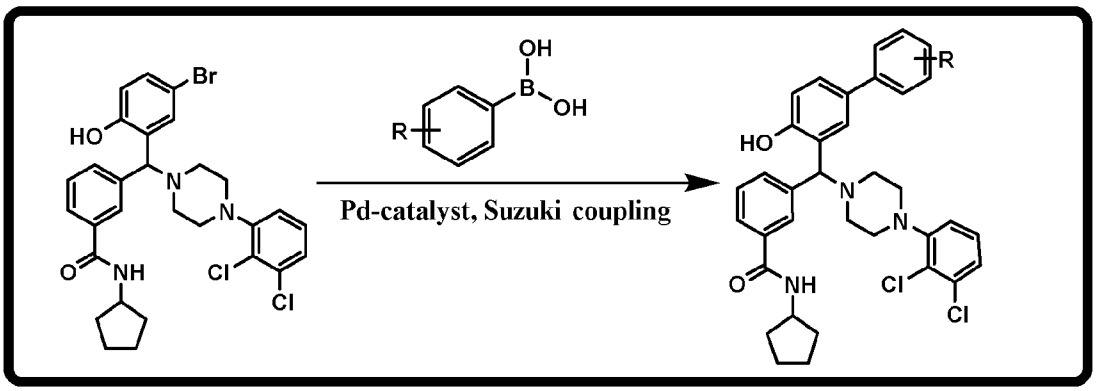
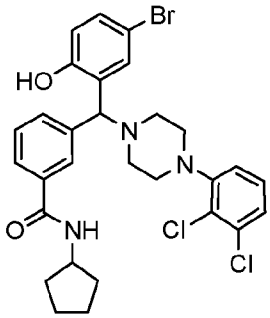
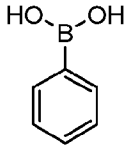
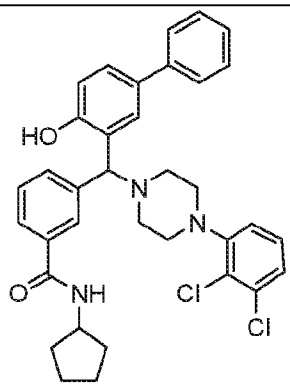
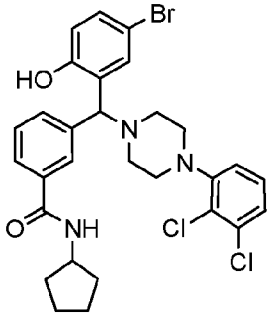
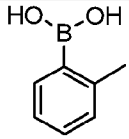
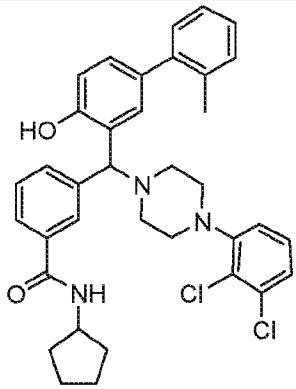
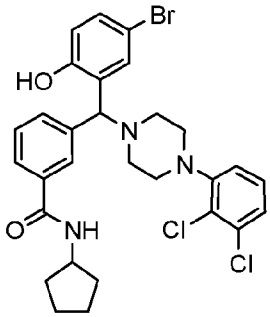
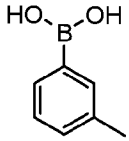
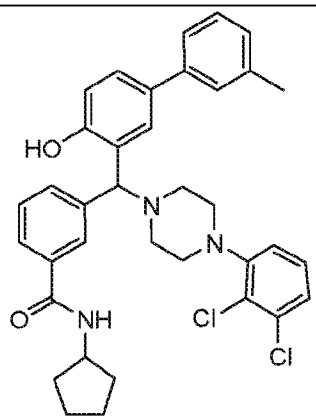
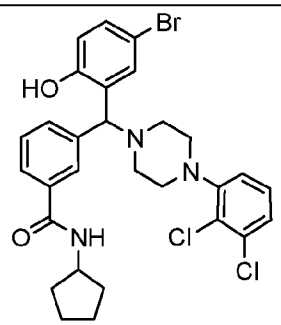
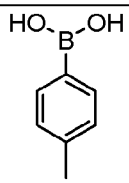
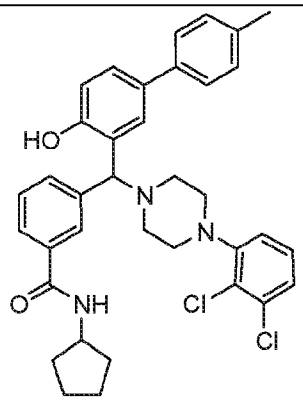
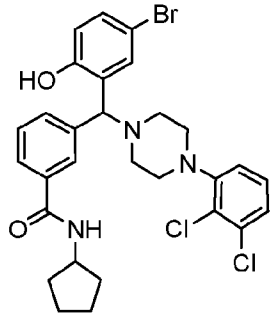
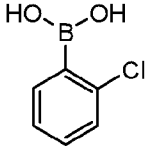
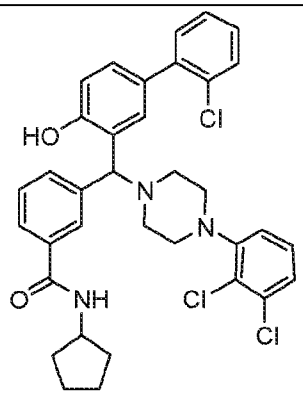
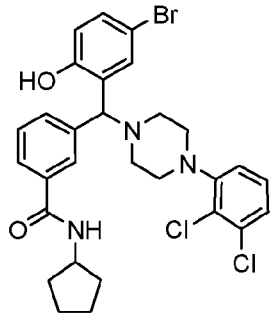
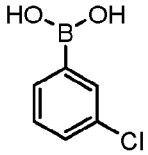
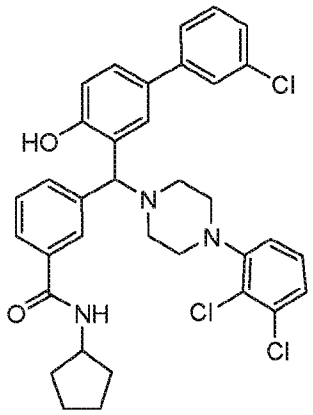
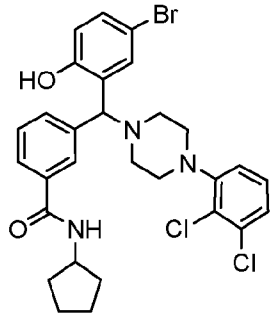
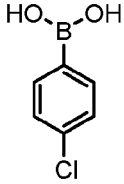
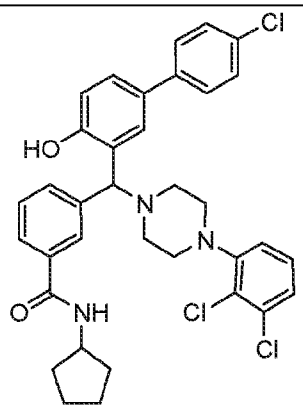
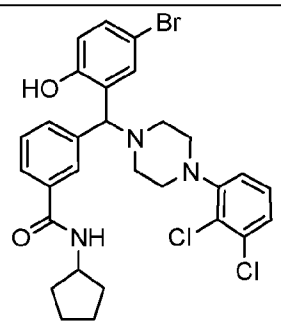
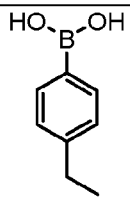
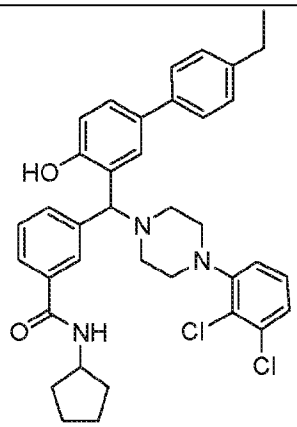
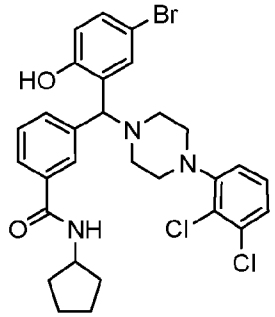
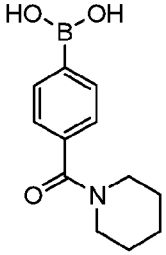
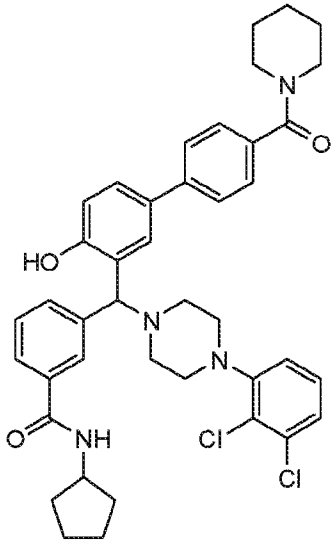
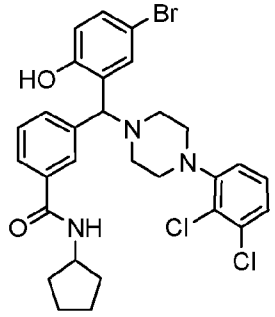
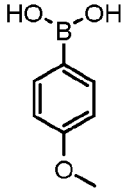
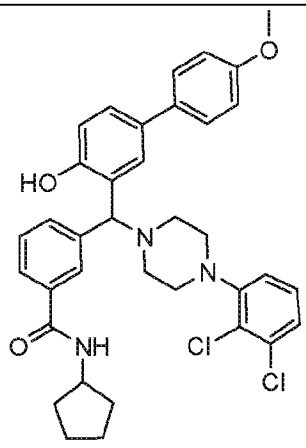
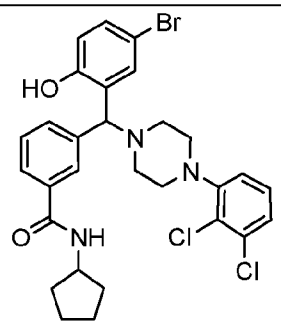
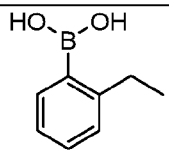
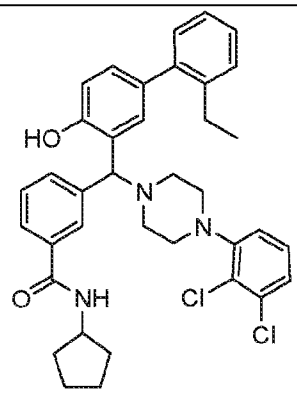
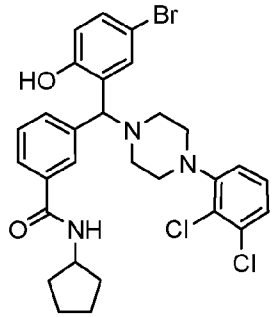
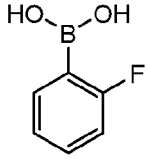
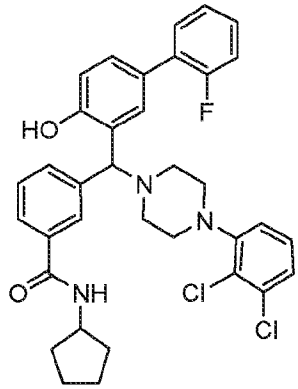
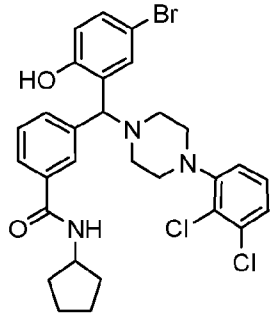
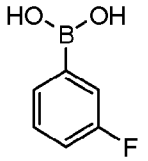
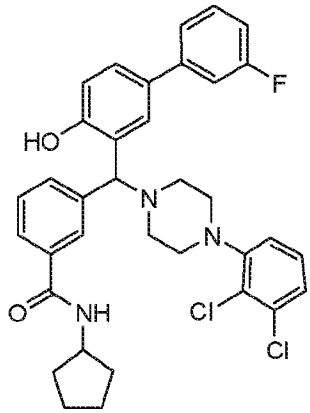
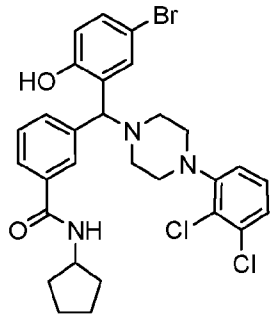
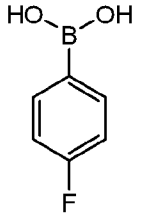
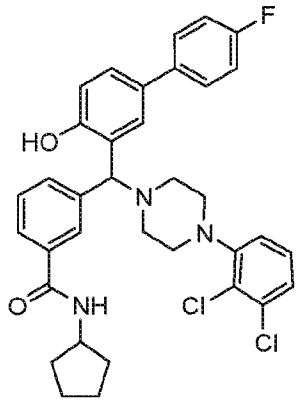


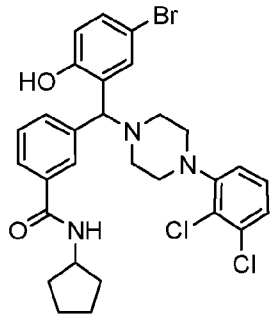
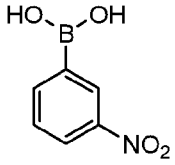
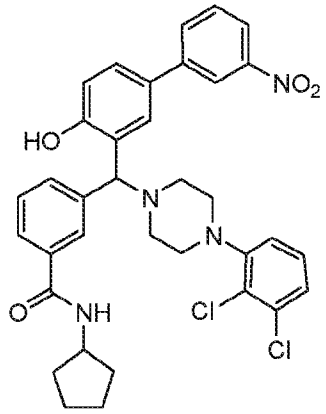
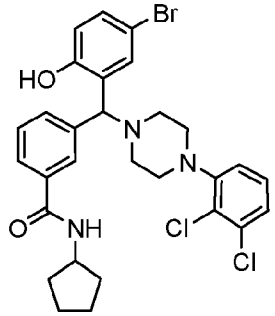
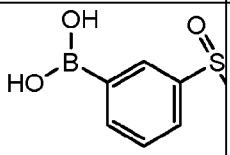
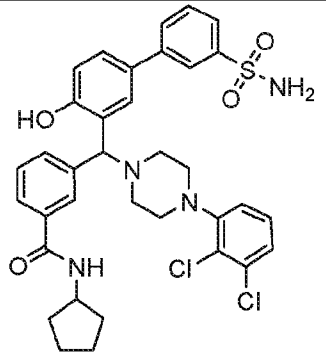
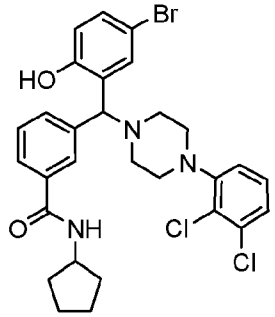
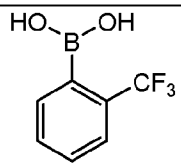
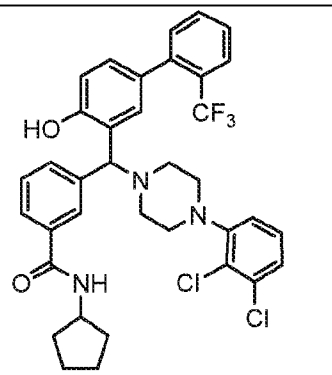
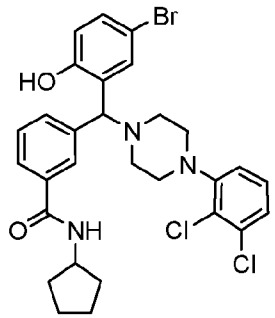
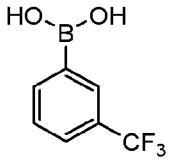
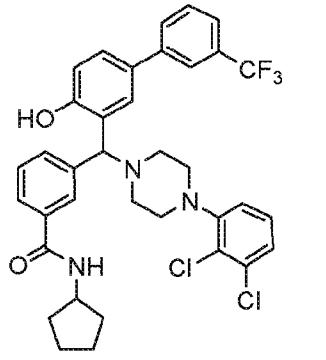
Table 2

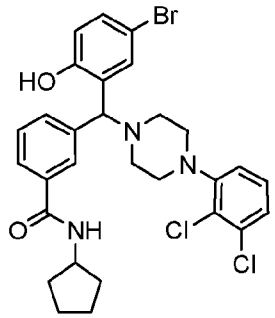
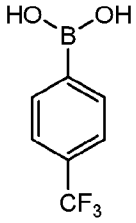
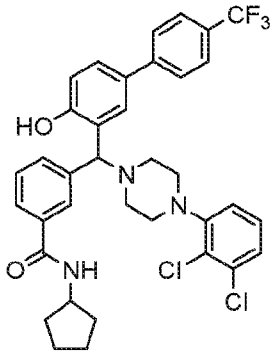
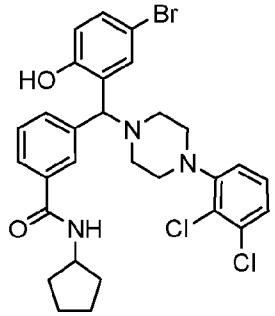
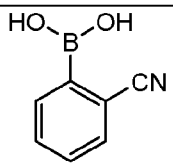
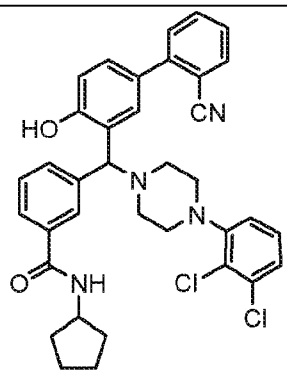
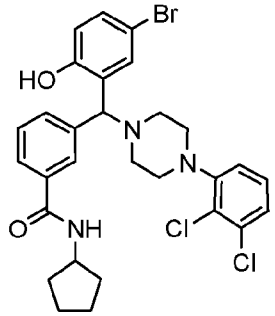
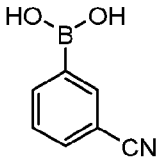
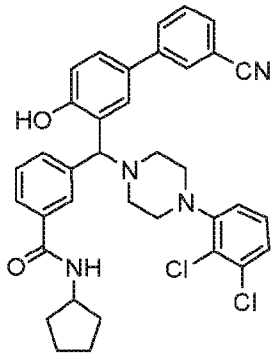
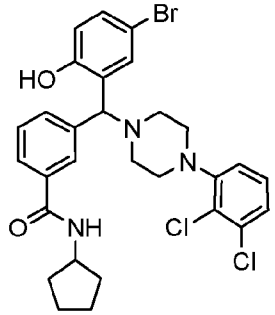
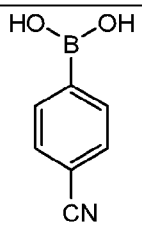
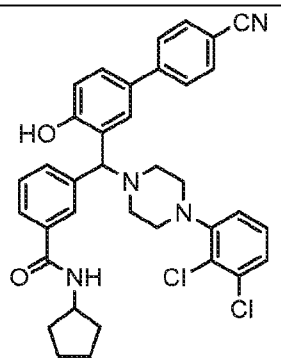
	NPB-Br	Boronic acid	Product
16			
17			
18			

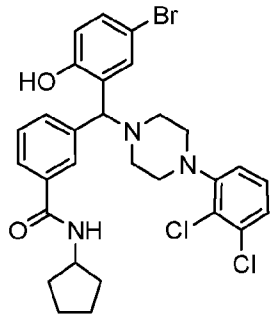
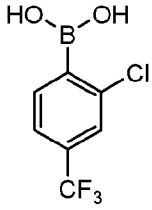
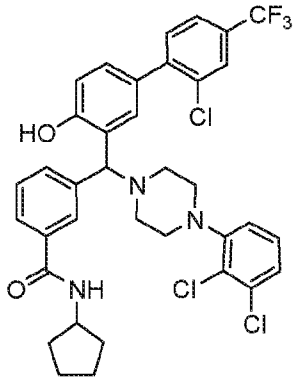
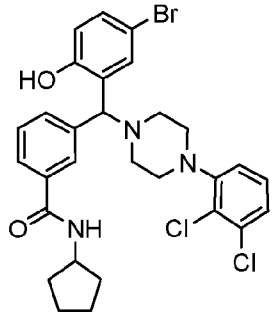
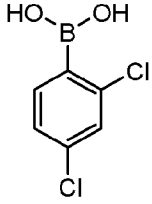
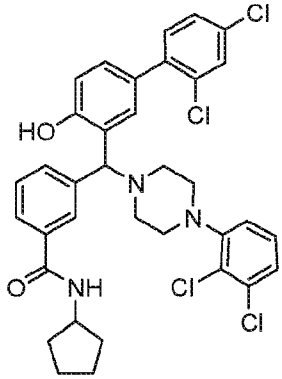
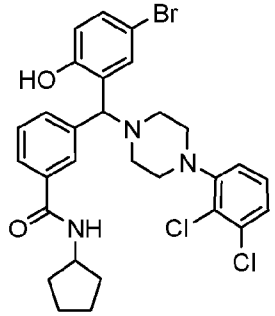
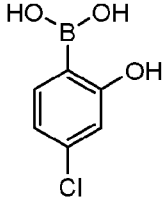
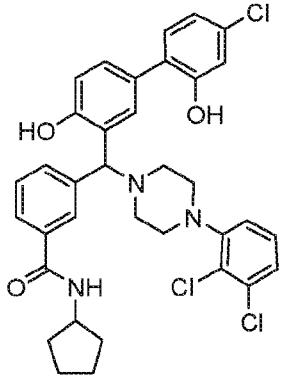
19			
20			
21			
22			

23			
24			
25			

26			
27			
28			
29			

30			
31			
32			
33			

34			
35			
36			
37			

38			
39			
40			

Characterization of Compound 1:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.691(s, 3H), 5.303(s, 1H,C-H), 1.184-3.691(m, 8H-piperazine protons), 7.597-7.615(d, 1H, J=7.2 Hz), 7.341-7.323(d,1H, J=7.2 Hz), 7.060-7.189(m, 4H-ArH), 6.555-6.815(m,5H-ArH), 6.956-6.974, (d,1H, J=7.2Hz)11.85(s, 1H-OH brd peak);^{13}C NMR (400 MHz, CDCl3)δ: 50.854, 55.521, 69.50, 114.45, 117.17, 118.44, 119.547, 122.05, 127.78, 128.83, 129.01, 129.19, 129.84, 129.93, 133.89, 145.11, 156.60; Melting point 120-124^{0}C. **(FIGURE 1)**

Characterization of Compound 2:

^{1}H NMR ($CDCl_3$, 400 MHz) δ:2.539-3.065(m, 8H), 3.674(s, 3H), 4.359(s, 1H), 6.651(m, 1H), 6.740-6.804(m, 5H), 6.855-6.872(d, 1H, J=6.8Hz), 7.086(m, 1H), 7.165(m, 2H),

7.280(m, 1H), 7.361(m, 1H); ^{13}C NMR (400 MHz, CDCl3)δ: 50.74, 51.76, 55.52, 75.86, 114.49, 117.24, 118.43, 119.62, 124.41, 126.58, 128.28, 128.54, 128.92, 129.20, 130.28, 134.66, 141.63, 145.07, 154, 156.18; Melting point 85-89^{0}C. **(FIGURE 2)**

Characterization of Compound 3:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.194-3.079(m,8H), 3.689(s, 3H), 4.336(s, 1H), 5.041(s, 2H), 6.672-6.702(m, 1H), 6.781-6.819(m, 5H), 6.863-6.867(m, 2H), 7.026-7.082(m, 2H), 7.196(m, 1H), 7.279-7.348(m, 5H); ^{13}C NMR (400 MHz, CDCl3)δ: 50.770, 55.52, 71.26, 75.36, 114.46, 115.46, 117.16, 118.42, 119.54, 124.82, 127.34, 128.14, 128.62, 128.76, 129.14, 145.04, 156.14; Melting point 72-76^{0}C. **(FIGURE 3)**

Characterization of Compound 4:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.183-2.469(m, 10H), 2.557-3.684(m, 8H), 3.684(s, 3H), 4.412(s, 1H), 6.631(m, 2H), 6.745-6.813(m, 4H), 7.059-7.095(m, 1H), 7.191-7.216(m, 1H), 7.261-7.269(m, 2H), 7.351-7.409(m, 2H); ^{13}C NMR (400 MHz, CDCl3)δ: 24.51, 26.525, 29.68, 43.60, 48.73, 50.71, 51.80, 55.51, 76.04, 114.45, 117.134, 118.366, 119.198, 119.52, 124.75, 126.74, 127.50, 128.04, 128.50, 128.79, 129.29, 136.18, 141.02, 145, 154, 156.2, 169.79(C=O); Melting point 78-82^{0}C. **(FIGURE 4)**

Characterization of Compound 5:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.186-1.650(m, 8H), 1.978-3.633(m, 8H), 3.689(s, 3H), 4.298-4.345(m, 1H), 4.421(s, 1H), 5.979-5.992(s, 1H) 6.736-6.803(m, 5H), 6.860(m, 1H), 7.192(s, 1H), 7.737(s, 1H), 7.304-7.339(m, 1H), 7.540-7.557(m, 2H); ^{13}C NMR (400 MHz, CDCl3)δ: 23.79, 33.18, 50.67, 51.83, 55.51, 67.06, 75.06, 114.46, 118.45, 118.58, 124.02, 126.11, 126.42, 128.72, 128.87, 129.39, 139.39, 144.89, 154.19, 154.95, 166.68; Melting point 102-106^{0}C. **(FIGURE 5)**

Characterization of Compound 6:

^{1}H NMR ($CDCl_3$, 400 MHz) δ:1.179-3.620(m, 8H), 3.676(s, 3H), 4.261(s, 1H), 5.029(s, 2H), 6.716-6.791(m, 5H), 6.823(m, 1H), 6.862-6.882(m, 1H), 6.973-7.015(m, 2H), 7.110-7.139(m, 1H), 7.243-7.258(m, 2H), 7.280-7.317(m, 1H), 7.331-7.350(m, 2H); ^{13}C NMR (400 MHz, CDCl3)δ: 50.70, 51.59, 55.52, 71.27, 74.93, 114.48, 115.52, 118.58, 123.97, 124.39, 126.3, 127.3, 128.2, 128.8, 132.1, 136.27, 144.96, 146.74, 154.19, 154.93; Melting point 60-64^{0}C. **(FIGURE 6)**

Characterization of Compound 7:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.648-3.820 (m, 8H) 4.245(s, 1H), 5.009(s, 1H), 5.651(s, 2H), 7.276(s, 1H), 7.436-7.485(m, 3H), 7.606-7.682(m, 3H), 7.797(m, 2H), 7.876-7.954(m, 5H), 8.185(s, 1H); ^{13}C NMR (400 MHz, CDCl3)δ: 30.972, 50.76, 67.60, 71.79, 75.85, 98.16, 100.9, 104.9, 113.16, 115.97, 117.65, 119.96, 122.84, 125.4, 127.8, 128.6, 129.1, 129.6, 133.2, 136.8, 157.06, 160.87, 164.51, 165.86; Melting point 58-62^{0}C. **(FIGURE 7)**

Characterization of Compound 8:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.479(s, 3H), 4.927(s, 1H), 2.260-3.063(m, 8H), 6.533-6.551(d, 1H, J=7.2Hz), 6.631-6.692(m, 2H), 6.739-6.758(d, 1H, J=7.6Hz), 6.789-6.809(d, 1H ,J=8Hz), 6.864(m, 3H), 7.092-7.183(m, 1H), 7.281-7.297(m, 1H), 7.537-7.552(d, 1H, J=6Hz); ^{13}C NMR (400 MHz, CDCl3) δ: 20.92, 51.16, 51.42, 73.44, 116.07, 116.96, 117.14, 118.716, 119.27, 119.83, 124.965, 125.24, 126.445, 127.12, 127.545, 128.266, 128.729, 129.260, 130.869, 134.072, 138.171, 150.600, 156.443 Melting point 108-112^{0}C. **(FIGURE 8)**

Characterization of Compound 9 (**NPB**):

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.183-1.647(m, 8H), 2.019-3.067(m, 8H), 4.509(s, 1H), 4.312-4.327(m, 1H, NH), 5.965(s, 1H), 6.668(m, 1H), 6.801-6.896(m, 3H), 7.073-7.190(m, 3H), 7.305(m, 1H), 7.527-7.542(m, 2H), 7.770(s, 1H); ^{13}C NMR (400MHz, CDCl3)δ: 23.78, 33.18, 51.22, 51.78, 76.10, 117.14, 118.59, 119.67, 124.69, 124.98, 126.22, 127.53, 128.85, 129.29, 131.08, 134.08, 135.53, 140.28, 150.5, 156.1, 166.73;m/z (M+2 ,526.2,527.2) Melting point 174-178^{0}C. **(FIGURE 9)**

Characterization of Compound 10:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.183-3.074(m, 8H), 4.361(s, 1H), 5.034(s, 2H), 6.658-6.694(t, 1H, J=7,2Hz), 6.768(m, 1H), 6.856-6.873(m, 3H), 7.023(m, 1H), 7.055-7.094(m, 3H), 7.164(m, 1H), 7.231(m, 1H), 7.266(m, 1H), 7.304(m, 1H), 7.322-7.355(m, 2H); ^{13}C NMR (400 MHz, CDCl3)δ: 51.264, 71.260, 75.434, 117.15, 118.6, 119.6, 124.8, 124.9, 127.3, 127.5, 128.1, 128.6, 128.8, 129.18, 150.5, 156.09; Melting point 75-80^{0}C. (**FIGURE 10)**

Example 2

Compound of formula I decreases the cell viability of a range of carcinoma cells

Oncogenicity Assay

We initially investigated the effect of newly synthesized small molecule compounds against MCF7 cells (ER+ MC cells) using an AlamarBlue® cell viability assay. Among the series

of novel small molecule compounds, NPB was identified as an efficacious small molecule compound reducing viability of MCF7 cells compared to vehicle (DMSO) treated cells. Among the compounds, Compound 9 N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(2-hydroxyphenyl)methyl)benzamide (NPB) is identified as the most potent antiproliferative compound with the IC_{50} of 6.5 μM. We next determined the inhibitory concentration 50% (IC_{50}) of NPB in wide-range of carcinoma cell lines including those derived from ER- mammary (BT549, MDA-MB-231), ER+ mammary (MCF7, T47D, and BT474), endometrial (Ishikawa, ECC-1, RL95-2, and AN3), ovarian (SK-OV-3, OVCAR-2, Caov-3, HEY C2, and OVCA433), hepatocellular (Hep3B, H2P, and H2M), colon (HCT116, DLD-1, and Caco-2), prostate (PC3, LNCaP, and DU145) and pancreatic (AsPC-1, BxPC-3) carcinoma. As normal cell controls, we also included *immortalized* mammary epithelial cells (MCF10A and MCF12A), and *immortalized* hepatocytes (LO2) in the panel of cell lines. The IC_{50} values for NPB in the carcinoma cell lines are tabulated in Figure 11.

Example 3

NPB induces apoptotic cell death in range of carcinoma cell lines

In addition, NPB is evaluated against several cancer-derived cell lines to determine effect on whole cell viability, apoptosis, and cytotoxicity using ApoTox-Glo™ Triplex Assay Kit, Promega (Singapore) according to manufacturer's instructions (Figure 12). In brief, cells are seeded in black opaque 96-well plates (Corning®, Singapore). After an overnight incubation of cells, the medium is changed to the indicated NPB concentration. After about 48 hours of incubation, the viability/cytotoxicity reagent containing both GF-AFC substrate and bis-AAF-R110 substrate are added to the cells as suggested by the supplier. After about 45 minutes incubation at about 37 °C, fluorescence is recorded at 400 nm excitation/505 nm emission for viability and 485 nm excitation/520 nm emission for cytotoxicity using a Tecan microplate reader for fluorescence (Tecan, Singapore). Caspase-Glo 3/7 Reagent is further added to the cells and after about 25 minutes of incubation at room temperature, luminescence is recorded using Tecan microplate reader. Numbers of viable, cytotoxic, and apoptotic cells are measured in triplicates.

Annexin V and propidium iodide (Annexin V–PI) apoptosis assay

Phosphatidylserine exposure and cell death are assessed by FACS analysis using Annexin-V-FLUOS Staining Kit (Life Technologies, Singapore) and PI-stained cells. Briefly, 1×10^5 MCF cells/well (190μL/well) are seeded in 6-well plates and incubated with different concentrations of NPB for about 24 hours and DMSO treated samples are used

as control. Cells are then washed with Annexin V binding buffer (10mM HEPES/NaOH, pH7.4, 140 mM NaCl, 2.5 mM $CaCl_2$), stained with Annexin V FITC for about 30 minutes at room temperature in the dark, then washed again and re-suspended in Annexin V binding buffer containing PI. Samples are analyzed immediately on a BD FACSAria Cell Sorter (BD Biosciences, San Jose, CA).

Loss of membrane integrity and translocation of phosphatidylserine to outer leaflet of plasma membrane are the early events of apoptosis which can be detected using FITC conjugated annexin-V and propidium iodide staining. It is observed that NPB induces apoptosis in MCF7 cells using FITC-annexin V and propidium iodide. On treatment with NPB, increase in both early (PI negative, FITC-Annexin V positive) and late apoptotic cells (PI positive, FITC-Annexin V positive) is observed in a dose dependent manner as shown in Figure 13A.

Example 4

Molecular interaction of NPB with recombinant BAD protein

To provide molecular interaction of the most promising candidate, NPB binding affinity to BAD, we performed surface plasmon resonance (SPR) measurements with immobilized BAD subunit using NPB as the analyte. We know that BAD could bind to BAD subunit *in vitro*. Hence, we analyzed the interaction using the BIAcore system. The recombinant BAD was immobilized on CM5 sensor chip. To determine the association and dissociation curves, various concentrations of NPB were injected individually onto the surface of a sensor chip coated with BAD. The overlaid sensorgrams shown in Figure 14 were analyzed collectively. The direct binding of BAD to NPB was demonstrated (Figure 14A). The calculation of kinetic parameters for the interaction of NPB with BAD revealed the association rate constant of $(1.4\pm.4)x10^3$ $M^{-1}S^{-1}$ and dissociation rate constant of $(5.4\pm0.38)x10^3$ S^{-1} of binding affinity, which yielded dissociation equilibrium constants (Kd) of 37.12 µM. These kinetic parameters shows affinity support for the interaction of BAD with NPB structure.

Surface plasmon resonance analysis

Molecular interactions were analyzed based on surface plasmon resonance using a BIAcore-2000 system (BIAcore AB, Uppsala, Sweden). Human recombinant BAD protein (Catalog No.MBS143012, MyBiosource, USA) was immobilized on a sensor chip as described by the manufacturer protocol. To examine the interaction of BAD with NPB, various concentrations of NPB (20 to 100 µM) in the running buffer (HBS-EP, pH 7.4, BIAcore AB) were injected onto the surface of the BAD-immobilized sensor chip with a flow rate of 15 µl/min as per the manufacturer's directions. NPB was allowed to interact

with BAD subunit for 2 min for association and dissociation, respectively, after which the sensor chip was regenerated by injecting 1 M NaCl for 2 min before the next injection. Using BIA evaluation software 4.1 (BIAcore AB), the kinetic parameters were such as association and dissociation rate constants (ka and kd), dissociation equilibrium constants (Kd) using a 1:1 binding model with mass transfer. Sensograms obtained were overlaid using BIA evaluation software.

Example 5

Effect of NPB on BAD phosphorylation is specific for Ser99 (human)

Phosphorylation of hBAD at residues Ser-75 (mouse BAD serine residue 112) and Ser-99 (mouse BAD serine residue 136) are crucial in regulating the activity of the BCL-2 family of anti-apoptotic proteins [15]. hBAD phosphorylation either at Ser-75 or Ser99 (or the corresponding residues in mouse bad) results in loss of the ability of hBAD to heterodimerize with BCL-xL or BCL-2 [15]. To further validate the predicted target, we first analyzed the effect of NPB on phosphorylation of hBAD at Ser99 by western blot analysis. Treatment of MCF7 cells with NPB produced a dose dependent decrease in phosphorylation of hBAD Ser99 without a significant change in total hBAD protein (Figure 14B). The calculated EC_{50} for inhibition of BAD Ser99 phosphorylation by NPB was 0.41 $\pm$0.21μM.

We next analyzed the effect of NPB on phosphorylation of hBAD at both Ser75 and Ser99 by western blot analysis in 25 carcinoma cell lines derived seven different types of cancer. It was observed that NPB largely inhibits the phosphorylation of BAD at the Ser99 site in all the tested carcinoma cell lines; however, NPB demonstrated no effect on the phosphorylation of hBAD at the Ser75 site in the same cells indicating that NPB specifically inhibited phosphorylation at Ser99 of hBAD (Figure 15).

***siRNA-mediated* depletion of *BAD* expression revert effect of NPB in carcinoma cell lines.**

To confirm the functional specificity of NPB directed to the BAD protein, we further examined the effect of NPB exposure after *siRNA-mediated* depletion of *BAD* expression in 6 carcinoma cell lines (MCF7, BT474, Caov-3, Ishikawa, AsPC-1 and DLD-1). Transient-transfection of the different carcinoma cells with *siRNA* directed to the *BAD* transcript decreased BAD expression and also decreased levels of phosphor-Ser99 BAD compared to their control cells (transfected with *scrambled oligo*) as observed by western blot analysis (Figure 16A). Cell viability nor apoptosis were significantly altered upon siRNA mediated depletion of BAD as previously reported [26]. As described above, NPB treatment of the control transfected carcinoma cell lines decreased BAD phosphorylation

(Ser99) compared to vehicle-treated cells. Concomitantly, exposure of the same carcinoma cell lines to NPB decreased cell viability and increased caspase 3/7 activity compared to vehicle-exposed cells. In contrast, NPB did not affect cell viability nor caspase3/7 activity in carcinoma cell lines with depleted expression of *BAD* (Figure 16B&C).

Example 6

NPB inhibits BAD phosphorylation independent of AKT signaling in carcinoma cell lines

The upstream AKT Ser/Thr kinase regulates the phosphorylation of hBAD at Ser99 [13]. We therefore determined whether NPB inhibits the phosphorylation of hBAD (Ser99) via modulation of the activity of AKT (as indicated by phosphorylation of Ser473) using western blot analysis. We observed no change in the levels of pAKT or levels of total AKT protein after exposure of four different carcinoma cells lines (MCF7, Caov-3, Ishikawa, and AsPC-1) to 10μM NPB. However, all NPB treated carcinoma cell lines (MCF7, Caov-3, Ishikawa, and AsPC-1) exhibited inhibition of BAD phosphorylation at the Ser99 site and with no change in the level of total BAD protein (Figure 15B). Additionally, we examined BAD phosphorylation after depletion of AKT using two independent *shRNA* targeting *AKT* expression or inhibition of AKT activity with AKT inhibitor IV as a positive control in the different carcinoma cell lines. We observed that depleted expression of AKT in the carcinoma cell lines was associated with a concomitant decrease in pAKT (Ser474) and pBAD (Ser99) levels compared to control cells; indicative that BAD Ser99 phosphorylation is AKT dependent in all tested cancer-derived cell lines and as previously published by others [13, 15, 27-29]. NPB therefore specifically inhibits BAD phosphorylation at Ser99 without affecting the activity of the upstream kinase (AKT) [29]. These results are concordant with the *in silico* target prediction and NPB binding to BAD observed by SPR. Hence, NPB specifically inhibits phosphorylation of BAD at Ser99 independent of the upstream (AKT) kinase.

Example 7:

5- to 6-week-old BALB/c-nu female mice were subcutaneously implanted with 17β-estradiol pellets (Innovative Research of America) at 0.72mg/pellet with a 60-day release in the scruff of the neck after three days mice were injected subcutaneously with 100μl of cell suspension ($1x10^7$ cells) in right flanks. Tumour growth was monitored by measuring the tumour size using callipers. About 12 days after implantation, mice were randomized and divided into three groups (each group, n=8), according to treatments administered

200µl of NPB (dissolved in 5% DMSO, 50% PEG400 and 45% water pH 5.0) by intraperitoneal injection every day for seven days. The first group of mice was treated with vehicle, the second with 5mg/kg dose of NPB, and the third with 20mg/kg dose of NPB. Animal weight and tumour volumes were measured daily. After completion tumours were excised, photographed, weighed, and fixed or stored in liquid nitrogen for later analysis. Histological analysis was performed as previously described (30-32).

We examined the i*n vivo* efficacy of NPB in a xenograft (MCF7) of MC. Randomly grouped mice with preformed tumours (volume ~150cm^3) were injected intraperitoneally with vehicle or NPB at 5mg/kg or 20mg/kg. A significant reduction in tumour volume was observed in NPB-treated mice as compared to their vehicle-treated counterparts (Figure 17A). During this period, animal weight was not significantly different between the groups (Figure 17A, below). However, the tumour weight of NPB-treated animals was reduced compared to vehicle-treated mice and in a dose-dependent manner (Figure 17B). We further analysed the effect of NPB on *h*BAD Ser99 phosphorylation levels in tumour tissue using WB analysis (Figure 17C). NPB treatment significantly inhibited phosphorylation of BAD (at Ser99) in a tumour compared to control specimens. No change was observed in total levels of BAD protein between NPB treated, and the control treated tumours.

Histological analyses of tumour specimens resected from the animals treated with NPB showed significantly reduced p-BAD (Ser99) compared to vehicle-treated tumours (Figure 17D), whereas BAD protein was not significantly different between the groups. Animals treated with NPB exhibited a significantly decreased percentage of Ki67 positive cells in tumours and a significantly increased TUNEL positivity compared to vehicle-treated (Figure 17D).

Example 8:

To elucidate the possibility that NPB decreased hBAD Ser99 phosphorylation by modulation of kinase activity, we assessed the effects of NPB on various kinases using Human Phospho-Kinase Antibody Array Kit from R&D Systems. No significant changes in kinase activity or phosphorylated substrates were observed in MCF7 cells exposed to NPB compared to DMSO exposed cells despite NPB inhibition of hBAD Ser99 phosphorylation in the same extract (Figure 18).

Example 9:

We also generated further analogues of NPB (Figures 19,20,21) according to the claimed chemical template and which may exhibit better pharmacokinetic profiles than NPB. Their structure and in vitro efficacy as determined by IC50 is shown in Figure 22.

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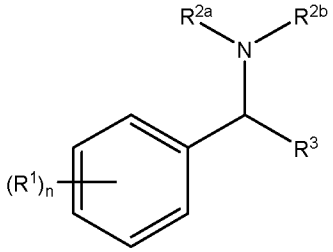
CLAIMS

1. A compound of general formula (I):



(I)

wherein:

each R^1 is independently halo, OH, cyano, nitro, $NR^{10}R^{11}$, $C(O)R^{10}$, $C(O)OR^{10}$, $C(O)NR^{10}R^{11}$, $-S(O)_qNR^{10}R^{11}$, C_{1-6} alkyl, C_{1-6} haloalkyl, $-O(C_{1-6}$ alkyl), $-O(C_{1-6}$ haloalkyl), aryl, heteroaryl, -O-aryl or -O-heteroaryl,

wherein

each R^{10} and R^{11} is independently selected from H or C_{1-6} alkyl optionally substituted with one or more substituents selected from OH, halo, cyano, NH_2, aryl or heteroaryl;

alkyl and haloalkyl groups R^1 are optionally substituted with one or more substitutents selected from OH, cyano, $-S(O)_pNR^4R^5$, $-C(O)NR^4R^5$, aryl, heteroaryl, -O-aryl or -O-heteroaryl $-O(C_{1-6}$ alkyl) optionally substituted with aryl or $-O(C_{1-6}$ haloalkyl);

aryl or heteroaryl groups R^1 are optionally substituted with one or more substituents selected from halo, OH, cyano, nitro, $-NR^4R^5$, $-S(O)_pNR^4R^5$, $-C(O)NR^4R^5$, $-C(O)R^4$, $-C(O)OR^4$ or $-C_{1-6}$ alkyl or $-O(C_{1-6}$ alkyl) , either of which is optionally substituted with one or more substituents selected from OH, halo, aryl, heteroaryl, $-O(C_{1-6}$ alkyl), $O(C_{1-6}$ haloalkyl), -O-aryl or –O-heteroaryl);

p is 1 or 2;

each R^4 and R^5 is independently selected from H or C_{1-4} alkyl or R^4 and R^5 together with a nitrogen atom to which they are attached may form a 3- or 8-membered heterocyclic ring, optionally containing one or more additional heteroatoms selected from O, N and S;

n is 0, 1, 2, 3 4, or 5;

R^{2a} and R^{2b} are each independently C_{1-6} alkyl optionally substituted with one or more substituents selected from halo, OH, aryl or heteroaryl; or

R^{2a} and R^{2b} together with the nitrogen atom to which they are attached form a 5- or 6-membered heterocyclic ring optionally containing one or more further heteroatoms selected from O, N or S and optionally substituted with one or more substituents R^6;

each R^6 is independently selected from aryl, heteroaryl, -O-aryl, -O-heteroaryl, carbocyclyl, heterocyclyl, -O-carbocyclyl, -O-heterocyclyl, R^{12}, OR^{12}, $C(O)R^{12}$, $C(O)OR^{11}$, $C(O)NR^{11}R^{12}$, CN,OH,

each R^{11} and R^{12} is independently H or C_{1-4} alkyl, either or which may be substituted with one or more aryl or heteroaryl groups, wherein aryl and heteroaryl groups are substituted with one or more substituent selected from halo, OH, cyano, nitro, $-NR^4R^5$, $-S(O)_pNR^4R^5$, $-C(O)NR^4R^5$, $-C(O)R^4$, $-C(O)OR^4$ or $-C_{1-6}$ alkyl or $-O(C_{1-6}$ alkyl) , either of which is optionally substituted with one or more substituents selected from OH, halo, aryl, heteroaryl, $-O(C_{1-6}$ alkyl), $O(C_{1-6}$ haloalkyl), -O-aryl or –O-heteroaryl);

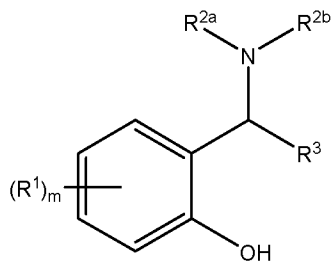
wherein R^4 and R^5 are as defined above; or R^{11} and R^{12} may combine with a nitrogen atom to which they are attached to form a 3 to 8-membered heterocyclic ring optionally containing one or more further heteroatoms selected from N, O and S and optionally substitututed with C_{1-4} alkyl, C_{1-4} haloalkyl or halo;

R^3 is aryl, heteroaryl, carbocyclyl or heterocyclyl any of which is optionally substituted with one or more substituents R^7 selected from halo, $-C_{1-4}$ alkyl optionally substituted with aryl, $-O(C_{1-4}$ alkyl) optionally substituted with aryl, $-C_{1-4}$ haloalkyl, $-O(C_{1-4}$ haloalkyl) or $-C(O)NR^8R^9$;

each R^8 and R^9 is independently selected from H, C_{1-4} alkyl or C_{3-6} cycloalkyl or R^8 and R^9 together with the nitrogen atom to which they are attached may form a 5- or 6-membered heterocyclic ring, optionally containing one or more additional heteroatoms selected from O, N and S;

or a pharmaceutically acceptable salt, solvate or hydrate thereof or a deuterated or tritiated variant thereof, including all stereoisomers.

2. compound of general formula general formula (IA):



(IA)

wherein:

each R^1 is independently halo, C_{1-6} alkyl, C_{1-6} haloalkyl, aryl or heteroaryl, wherein aryl or heteroaryl groups are optionally substituted with one or more substituents selected from halo, OH, cyano, nitro, $-S(O)_pNR^4R^5$, $-C(O)NR^4R^5$, $-C_{1-6}$ alkyl optionally substituted with aryl, $-C_{1-6}$ haloalkyl, $-O(C_{1-6}$ alkyl) optionally substituted with aryl or $-O(C_{1-6}$ haloalkyl);

p is 0, 1 or 2;

each R^4 and R^5 is independently selected from H or C_{1-4} alkyl or R^4 and R^5 together with the nitrogen atom to which they are attached may form a 5- or 6-membered heterocyclic ring, optionally containing one or more additional heteroatoms selected from O, N and S;

m is 0, 1, 2, 3 or 4;

R^{2a} and R^{2b} are each independently C_{1-6} alkyl optionally substituted with one or more substituents selected from halo, OH, aryl or heteroaryl; or

R^{2a} and R^{2b} together with the nitrogen atom to which they are attached form a 5- or 6-membered heterocyclic ring optionally containing one or more further heteroatoms selected from O, N or S and optionally substituted with one or more substituents R^6;

each R^6 is independently selected from aryl, heteroaryl, -O-aryl, -O-heteroaryl or C_{1-4} alkyl substituted with one or more aryl or heteroaryl groups, wherein aryl and heteroaryl groups are substituted with one or more substituent selected from halo, C_{1-4} alkyl, C_{1-4} haloalkyl, $-O(C_{1-4}$ alkyl) or $–O(C_{1-4}$ haloalkyl);

R^3 is aryl, heteroaryl, carbocyclyl or heterocyclyl any of which is optionally substituted with one or more substituents R^7 selected from halo, $-C_{1-4}$ alkyl optionally substituted with aryl, $-O(C_{1-4}$ alkyl) optionally substituted with aryl, $-C_{1-4}$ haloalkyl, $-O(C_{1-4}$ haloalkyl) or $-C(O)NR^8R^9$;

each R^8 and R^9 is independently selected from H, C_{1-4} alkyl or C_{3-6} cycloalkyl or R^8 and R^9 together with the nitrogen atom to which they are attached may form a 5- or 6-membered heterocyclic ring, optionally containing one or more additional heteroatoms selected from O, N and S;

or a pharmaceutically acceptable salt, solvate or hydrate thereof or a deuterated or tritiated variant thereof, including all stereoisomers.

3. A compound according to claim 1 wherein n is 1 or 2 and at least one R^1 group is OH; or a compound according to claim 2 wherein m is 0 or 1.

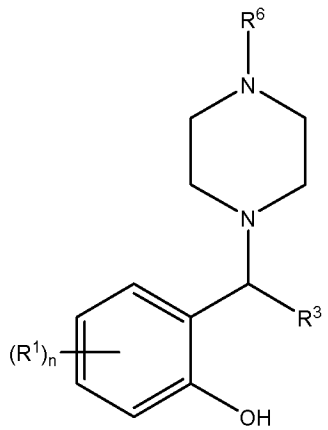
4. A compound according to claim 2 or claim 3 wherein m is other than 0 and R^1 is halo or an aryl or heteroaryl group optionally substituted as defined in claim 2.

5. A compound according to claim 4 wherein R^1 is an aryl or heteroaryl group optionally substituted with halo, OH, cyano, nitro, $-SO_2NH_2$, $-C(O)NR^4R^5$, $-C_{1-4}$ alkyl optionally substituted with aryl, $-C_{1-4}$ haloalkyl, $-O(C_{1-4}$ alkyl) optionally substituted with aryl or $-O(C_{1-4}$ haloalkyl), where R^4 and R^5 together with the nitrogen atom to which they are attached form a piperidine or pyrrolidine ring.

6. A compound according to claim 5 wherein R^1 is an aryl or heteroaryl group optionally substituted with chloro, fluoro, methyl, ethyl, trifluoromethyl, benzyl, methoxy, ethoxy, benzyloxy, trifluoromethoxy and piperidine-1-carbonyl.

7. A compound according to any one of claims 1 to 6 wherein R^{2a} and R^{2b} together with the nitrogen atom to which they are attached form a 6-membered heterocyclic ring optionally substituted with one or more substituents R^6.

8. A compound according to claim 7 wherein R^{2a} and R^{2b} together with the nitrogen atom to which they are attached form a a piperazine ring substituted by a single R^6 substituent at the piperazine 4-position such that the compound of general formula (I) is a compound of general formula (IB):

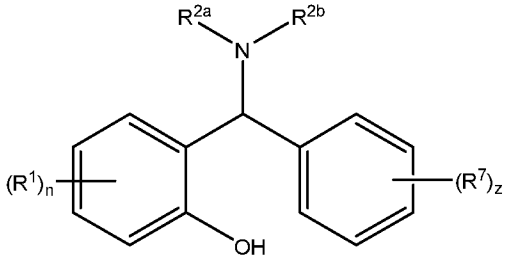


(IB)

wherein R^1, n, R^3 and R^6 are as defined in claim 2.

9. A compound according to any one of claims 1 to 8 wherein R^6 is phenyl, heteroaryl, -O-phenyl, -O-heteroaryl, benzyl, -CH(phenyl)$_2$, -CH_2-heteroaryl and –CH(heteroaryl)$_2$, where the heteroaryl group is selected from pyridinyl, indolyl, isoindolyl, benzoxazolyl and benzisoxazolyl and wherein any of the above R^6 groups may be substituted as defined in claim 1.

10. A compound according to any one of claims 1 to 9 wherein R^3 is phenyl optionally substituted with one or more substituents R^7 such that the compound of general formula (I) is a compound of general formula (IC):

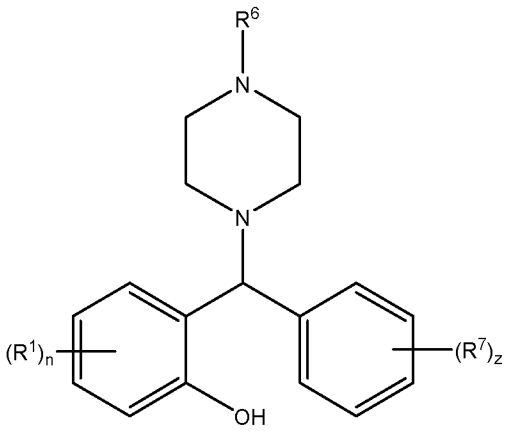


(IC)

wherein R^1, n, R^{2a}, R^{2b} and R^7 are as defined for general formula (I) and z is 0 to 5.

11. A compound according to claim 10 wherein z is 0, 1 or 2 R^7 not present (i.e. z is 0), or R^7 is halo, -C_{1-4} alkyl, benzyl, -O(C_{1-4} alkyl) benzyloxy, -C_{1-4} haloalkyl, -O(C_{1-4} haloalkyl) or -C(O)NR^8R^9, where R^8 and R^9 together with the nitrogen atom to which they are attached form a piperidinyl ring or wherein R^8 is H and R^9 is C_{3-7} cycloalkyl.

11. A compound according to any one of claims 1 to 10 which is a compound of general formula (ID):



(ID)

wherein R^1, n, R^6, R^7 and z are as defined above.

12. A compound according to claim 1 selected from:

2-((2-chlorophenyl)(4-(4-methoxyphenyl)piperazin-1-yl)methyl)phenol (Compound 1);

2-((4-chlorophenyl)(4-(4-methoxyphenyl)piperazin-1-yl)methyl)phenol (Compound 2);

2-((4-(benzyloxy)-3-fluorophenyl)(4-(4-methoxyphenyl)piperazin-1-yl)methyl)phenol (Compound 3);

(4-((2-hydroxyphenyl)(4-(4-Methoxyphenyl)piperazinyl)methyl)phenyl)(piperidin-1-yl)methanone (Compound 4);

3-((5-chloro-2-hydroxyphenyl)(4-(4-methoxyphenyl)piperazin-1-yl)methyl)-N-cyclopentylbenzamide (Compound 5);

2-((4-(benzyloxy)-3-fluorophenyl)(4-(4-methoxyphenyl)piperazin-1-yl)methyl)-4-chlorophenol (Compound 6);

2-((4-(benzyloxy)-3-fluorophenyl)(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)methyl)phenol (Compound 7);

2-((4-(2, 3-dichlorophenyl)piperazin-1-yl)(o-tolyl)methyl)phenol (Compound 8);

N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(2-hydroxyphenyl)methyl) benzamide (Compound 9, NPB);

2-((4-(benzyloxy)-3-fluorophenyl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)phenol (Compound 10);

2-((4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)(phenyl)methyl)phenol (Compound 11);

2-((4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)(p-tolyl)methyl)phenol (Compound 12);

2-((4-chlorophenyl)(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)methyl)phenol (Compound 13);

2-((4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)(4-ethylphenyl)methyl)phenol (Compound 14);

(4-((4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)(2-hydroxyphenyl)methyl)phenyl)(piperidin-1-yl)methanone (Compound 15);

N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 16);

N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-2'-methyl-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 17);

N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-3'-methyl-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 18);

N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-4'-methyl-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 19);

3-((2'-chloro-4-hydroxy-[1,1'-biphenyl]-3-yl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-

N-cyclopentylbenzamide (Compound 20);
3-((3'-chloro-4-hydroxy-[1,1'-biphenyl]-3-yl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-N-cyclopentylbenzamide (Compound 21);
3-((4'-chloro-4-hydroxy-[1,1'-biphenyl]-3-yl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-N-cyclopentylbenzamide (Compound 22);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4'-ethyl-4-hydroxy-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 23);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-4'-(piperidine-1-carbonyl)-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 24);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-4'-methoxy-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 25);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(2'-ethyl-4-hydroxy-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 26);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(2'-fluoro-4-hydroxy-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 27);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(3'-fluoro-4-hydroxy-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 28);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4'-fluoro-4-hydroxy-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 29);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-3'-nitro-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 30);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-3'-sulfamoyl-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 31);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-2'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 32);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-3'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 33);
N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-hydroxy-4'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl)methyl)benzamide (Compound 34);
3-((2'-cyano-4-hydroxy-[1,1'-biphenyl]-3-yl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-N-cyclopentylbenzamide (Compound 35);
3-((3'-cyano-4-hydroxy-[1,1'-biphenyl]-3-yl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-N-cyclopentylbenzamide (Compound 36)
3-((4'-cyano-4-hydroxy-[1,1'-biphenyl]-3-yl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-N-cyclopentylbenzamide (Compound 37);
3-((2'-chloro-4-hydroxy-4'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl)(4-(2,3-dichlorophenyl) piperazin-1-yl)methyl)-N-cyclopentylbenzamide (Compound 38);

N-cyclopentyl-3-((2',4'-dichloro-4-hydroxy-[1,1'-biphenyl]-3-yl)(4-(2,3-dichlorophenyl) piperazin-1-yl)methyl)benzamide (Compound 39);

3-((4'-chloro-2',4-dihydroxy-[1,1'-biphenyl]-3-yl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-N-cyclopentylbenzamide (Compound 40);

3-((4-(4-chlorophenyl)piperazin-1-yl)(2-hydroxyphenyl)methyl)-N-cyclopentylbenzamide (Compound 41, NCK1)

2-((4-chlorophenyl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)phenol (Compound 42, NCK2)

2-((4-(2,3-dichlorophenyl)piperazin-1-yl)(3-methoxyphenyl)methyl)phenol (Compound 43, NCK3)

1-(5-((4-(2,3-dichlorophenyl)piperazin-1-yl)(2-hydroxyphenyl)methyl)thiophen-2-yl)ethanone (Compound 44, NCK4)

2-((4-(2,3-dichlorophenyl)piperazin-1-yl)(naphthalen-1-yl)methyl)phenol (Compound 45, NCK5)

5-((4-(2,3-dichlorophenyl)piperazin-1-yl)(2-hydroxyphenyl)methyl)furan-2-carbaldehyde (Compound 46, NCK6)

2-((4-(5,6-dichlorocyclohexa-1,5-dien-1-yl)piperazin-1-yl)(2-fluoro-3-methylpyridin-4-yl)methyl)phenol (Compound 47, NCK7)

2-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-(trifluoromethyl)phenyl)methyl)phenol (Compound 48, NCK8)

2-((6-chloro-5-methylpyridin-3-yl)(4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)phenol (Compound 49, NCK9)

2-((4-(2,3-dichlorophenyl)piperazin-1-yl)(pyridin-3-yl)methyl)phenol (Compound 50, NCK10)

1-(5-((4-(4-chlorophenyl)piperazin-1-yl)(2-hydroxyphenyl)methyl)thiophen-2-yl)ethanone (Compound 51, NCK14)

3-((4-(4-chlorophenyl)piperazin-1-yl)(4-(diethylamino)-2-hydroxyphenyl)methyl)-N-cyclopentylbenzamide(Compound 52, NCK16)

N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(4-(diethylamino)-2-hydroxyphenyl)methyl)benzamide (Compound 53, NCK18)

N-cyclopentyl-3-((4-(2,3-dichlorophenyl)piperazin-1-yl)(2-hydroxy-4,6-dimethoxyphenyl)methyl)benzamide (Compound 54, NCK19)

2-((4-chlorophenyl)(4-(4-chlorophenyl)piperazin-1-yl)methyl)phenol (Compound 55, NCK20)

2-((4-(4-chlorophenyl)piperazin-1-yl)(6-methylpyridin-3-yl)methyl)phenol (Compound 56, NCK21)

2-(o-tolyl(4-(p-tolyl)piperazin-1-yl)methyl)phenol (Compound 57, SG1)

2-((4-(p-tolyl)piperazin-1-yl)(4-(trifluoromethyl)phenyl)methyl)phenol (Compound 58, SG2)

N-cyclopentyl-4-((2-hydroxyphenyl)(4-(p-tolyl)piperazin-1-yl)methyl)benzamide (Compound 59, SG3)

2-((4-chlorophenyl)(4-(p-tolyl)piperazin-1-yl)methyl)phenol (Compound 60, SG4)

2-((3-methoxyphenyl)(4-(p-tolyl)piperazin-1-yl)methyl)phenol (Compound 61, SG5)

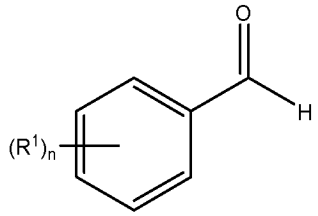
5-((2-hydroxyphenyl)(4-(p-tolyl)piperazin-1-yl)methyl)furan-2-carbaldehyde (Compound 62, SG6)

2-((6-methylpyridin-3-yl)(4-(p-tolyl)piperazin-1-yl)methyl)phenol (Compound 63, SG7);

or a pharmaceutically acceptable salt, solvate or hydrate thereof or a deuterated or tritiated variant thereof, including all stereoisomers.

14. A process for the preparation of a compound according to any one of claims 1 to 13 which comprises either:

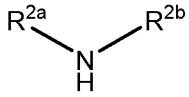
i. reacting an aldehyde of general formula (II):



(II)

wherein R^1 and n are as defined in claim 1;

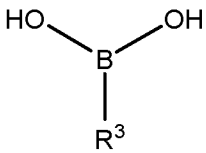
with a compound of general formula (III):



(III)

wherein R^{2a} and R^{2b} are as defined in claim 1;

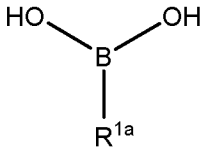
and a boronic acid of general formula (IV):



(IV)

wherein R^3 is as defined in claim 1; or

ii. reacting a compound of general formula (I) in which R^1 is halo with a compound of general formula (V):



(V)

wherein R^{1a} is aryl or heteroaryl optionally substituted as defined for R^1 in claim 1; via a Suzuki coupling reaction in the presence of a palladium catalyst to obtain a compound of general formula (I) in which R^1 is R^{1a}.

15. A compound according to any one of claims 1 to 13 for use in medicine.

16. A compound according to any one of claims 1 to 13 for use in the treatment of cancer.

17. The use of a compound according to any one of claims 1 to 13 in the preparation of an agent for the treatment of cancer.

18. A method for the treatment of cancer, the method comprising administering to a patient in need of such treatment an effective amount of a compound according to any one of claims 1 to 13.

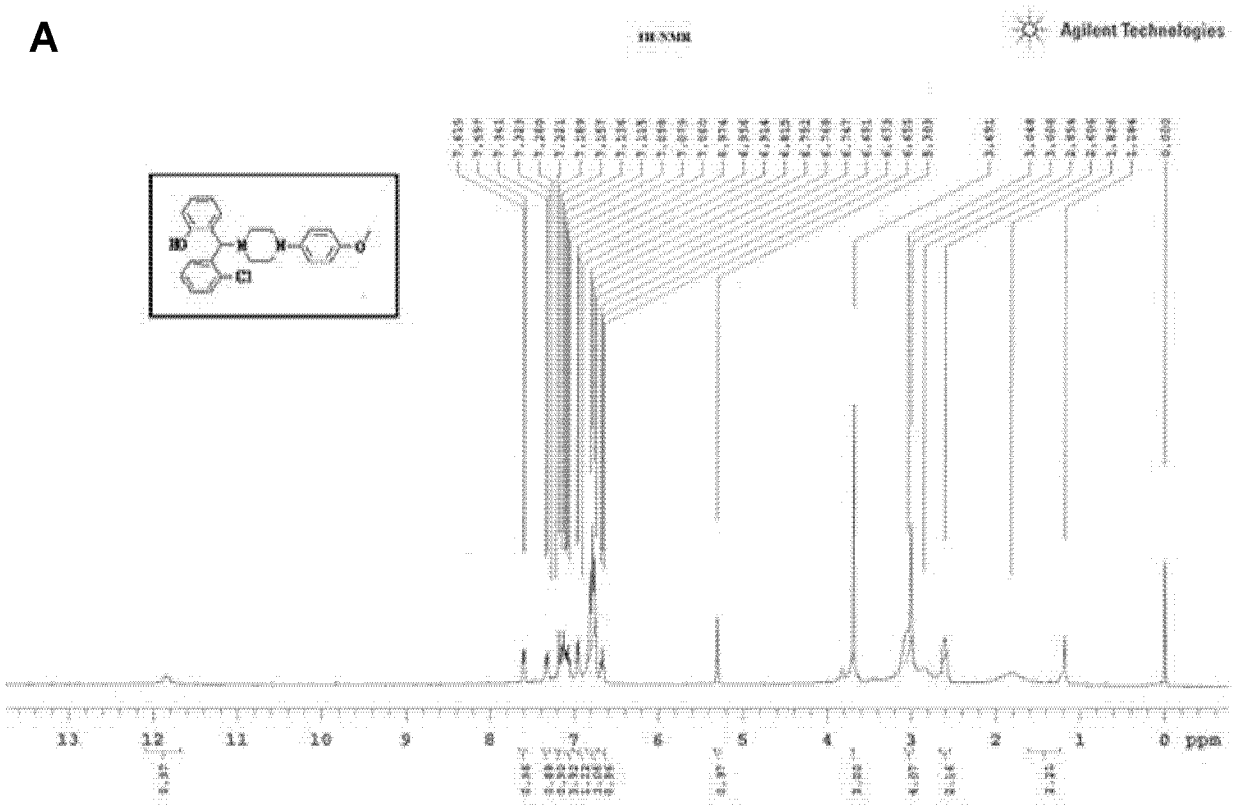
19. A compound for use, use or method according to any one of claims 16 to 18 wherein the cancer is a cancer in which there is BAD phosphorylation, for example breast cancer, endometrial cancer, ovarian cancer, liver cancer, colon cancer, prostate cancer or pancreatic cancer or any other epithelial derived cancer in which BAD is phosphorylated.

20. A pharmaceutical composition comprising a compound according to any one of claims 1 to 13 and a pharmaceutically acceptable excipient.

21. A pharmaceutical composition according to claim 20 which is formulated for intraperitoneal administration, hepatoportal administration, intravenous administration, intra articular administration, pancreatic duodenal artery administration or intramuscular administration, or any combination thereof.

Figure 1

A


Agilent Technologies
HO
N
H
O
Cl
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm

B

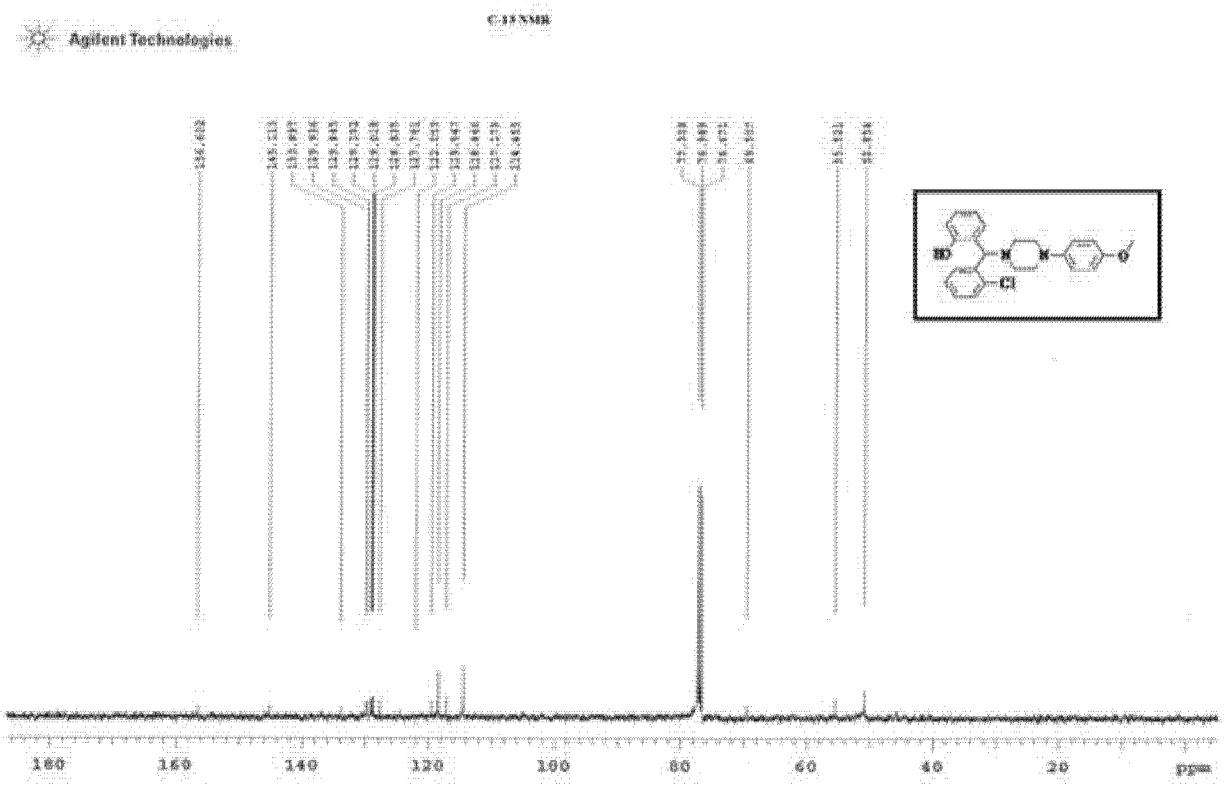

Agilent Technologies
HO
N
H
O
Cl
180
160
140
120
100
80
60
40
20
ppm

Figure 2

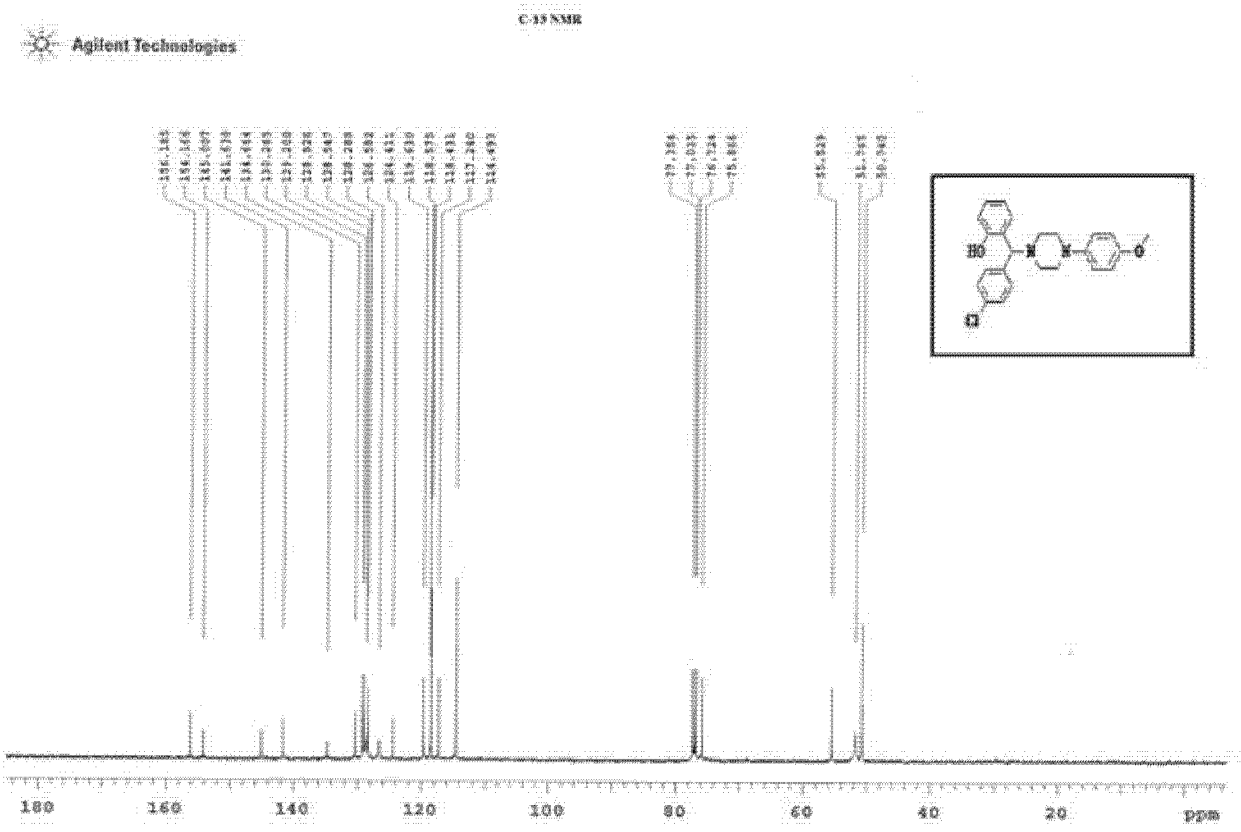
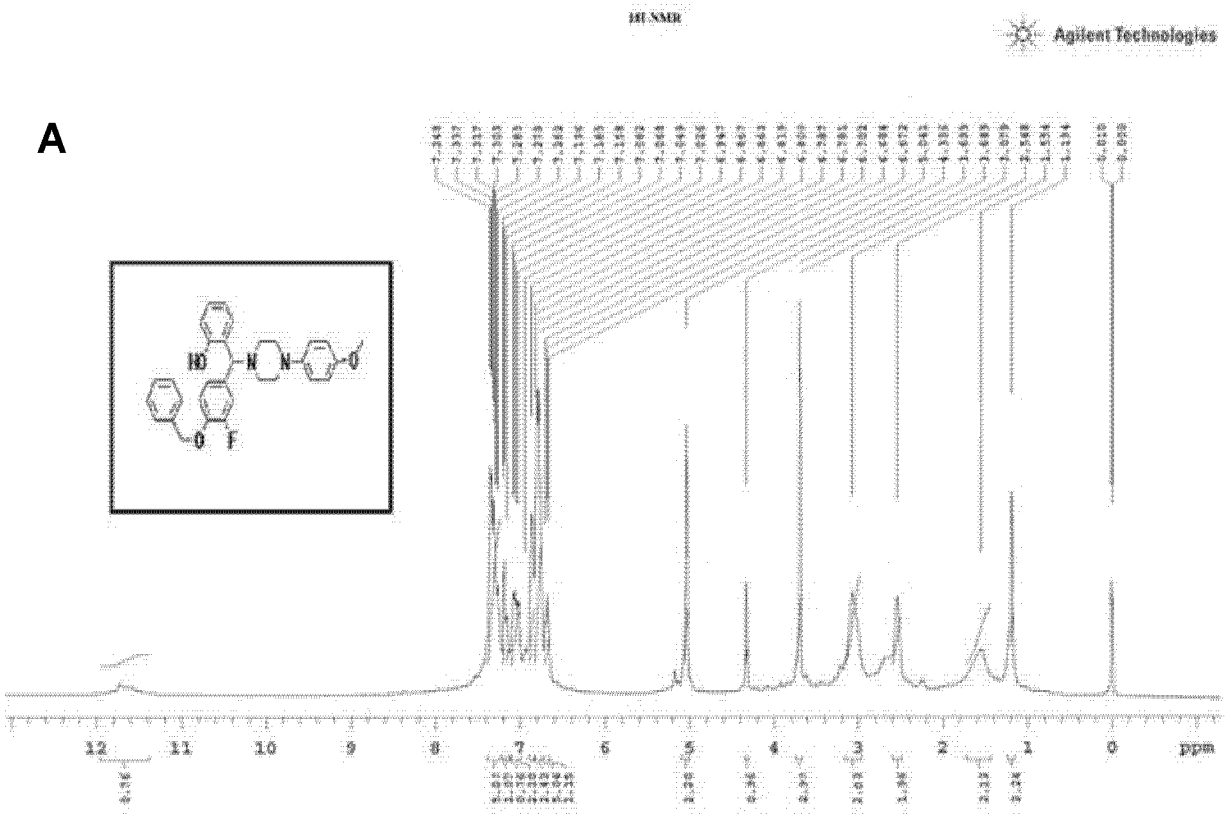

C-13 NMR
Agilent Technologies
180
160
140
120
100
80
60
40
20
ppm

Figure 3


A
1H NMR
Agilent Technologies
HO
N
N
O
O
F
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm

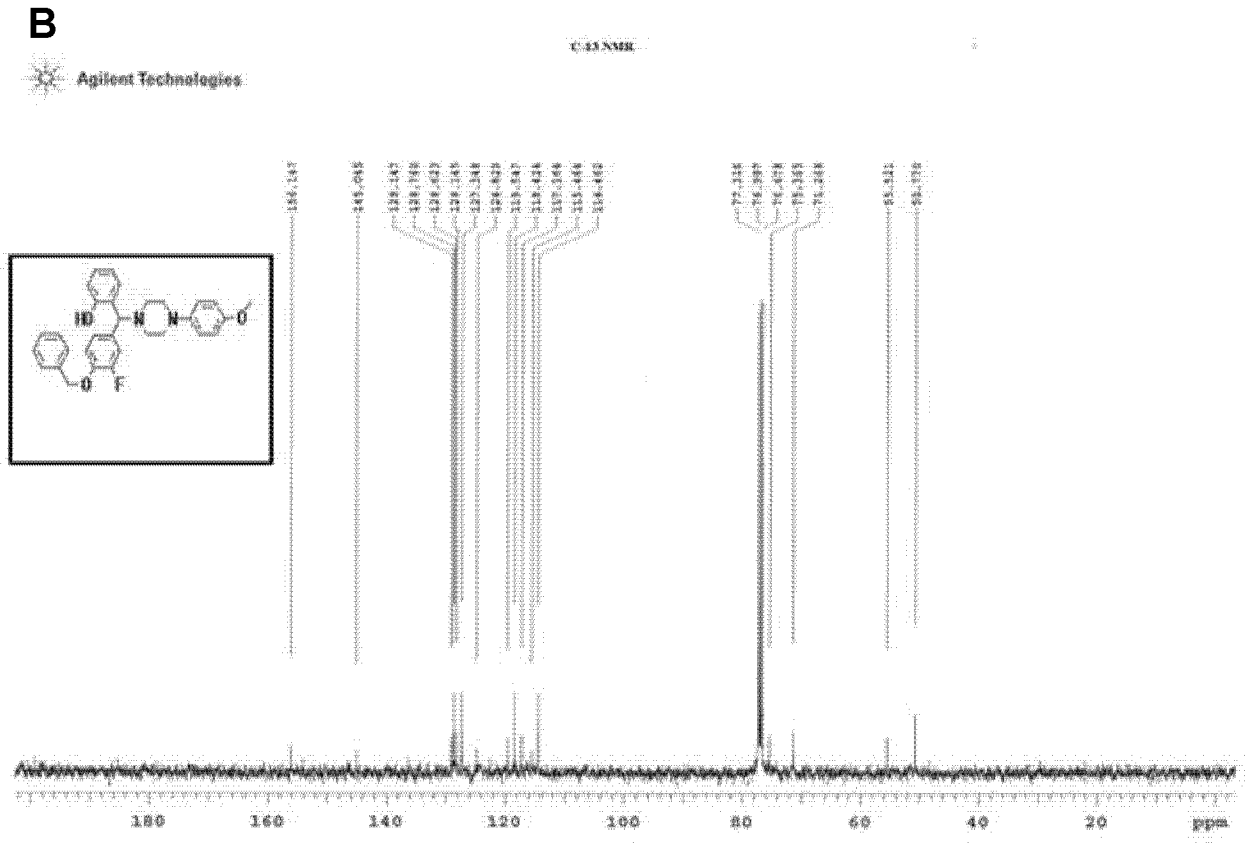
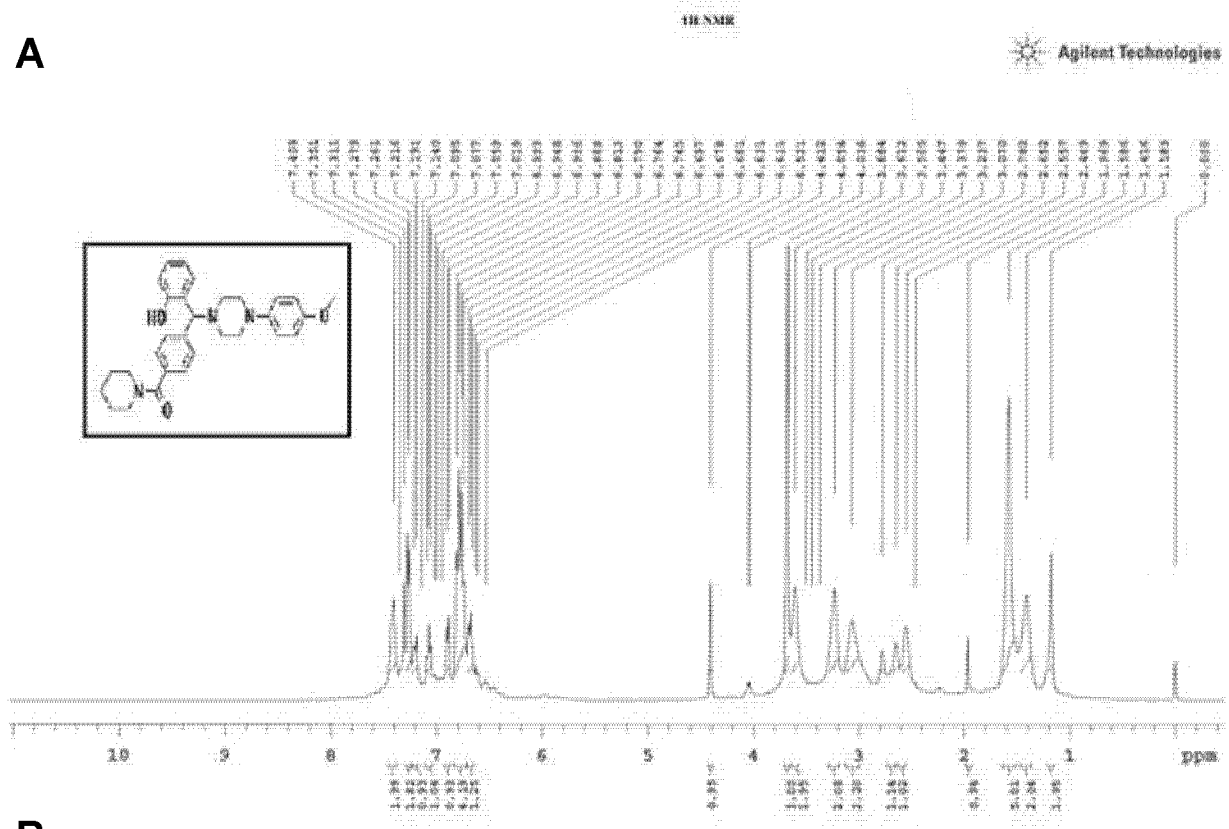

B
C-13 NMR
Agilent Technologies
HO
N
N
O
O
F
180
160
140
120
100
80
60
40
20
ppm

Figure 4


A
Agilent Technologies
10
9
8
7
6
5
4
3
2
1
ppm

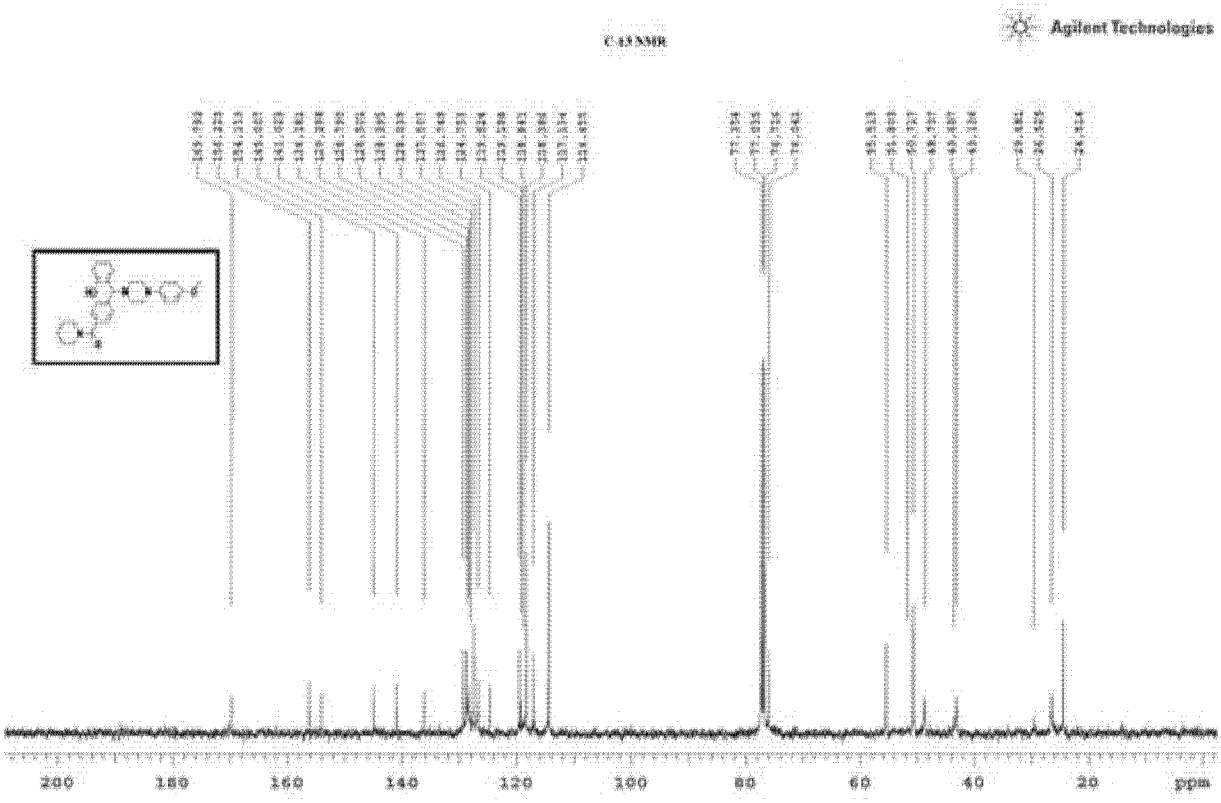
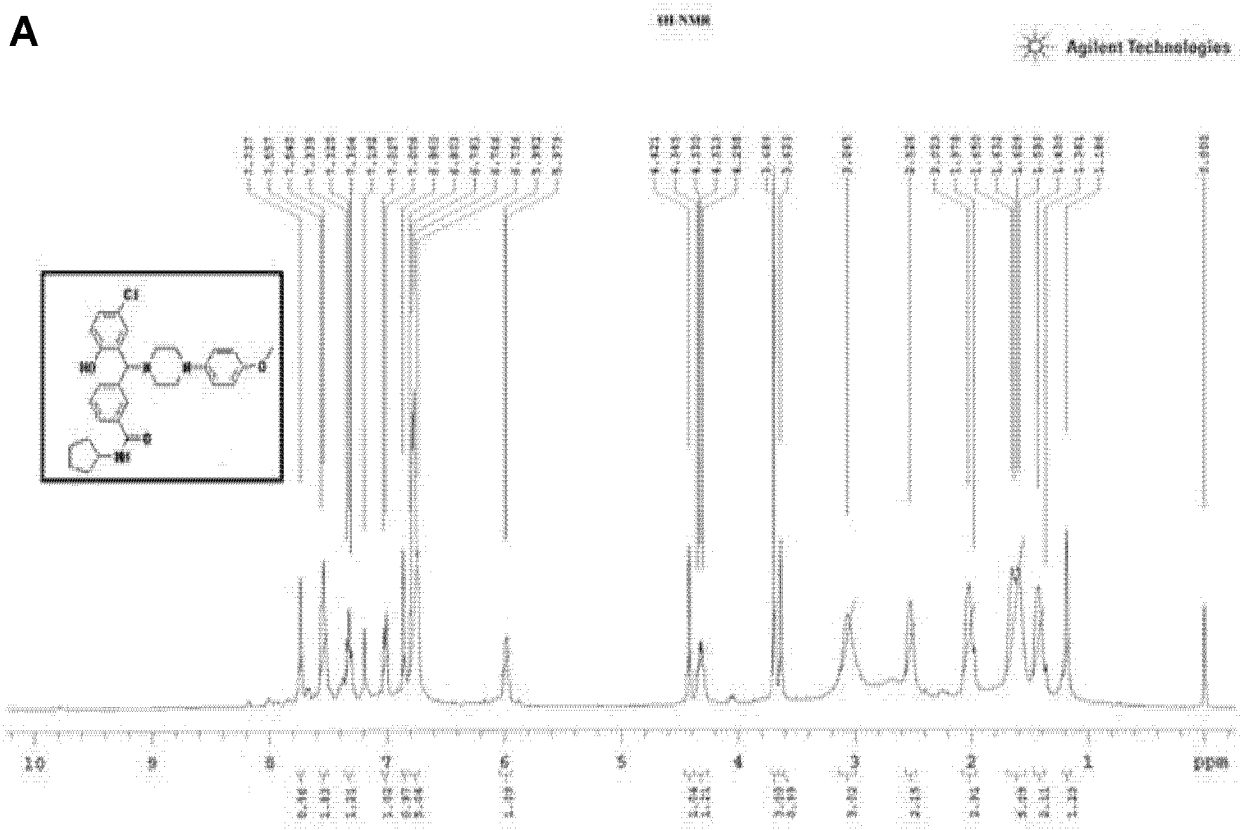

B
Agilent Technologies
200
180
160
140
120
100
80
60
40
20
ppm

Figure 5


A
Agilent Technologies
10
9
8
7
6
5
4
3
2
1
ppm

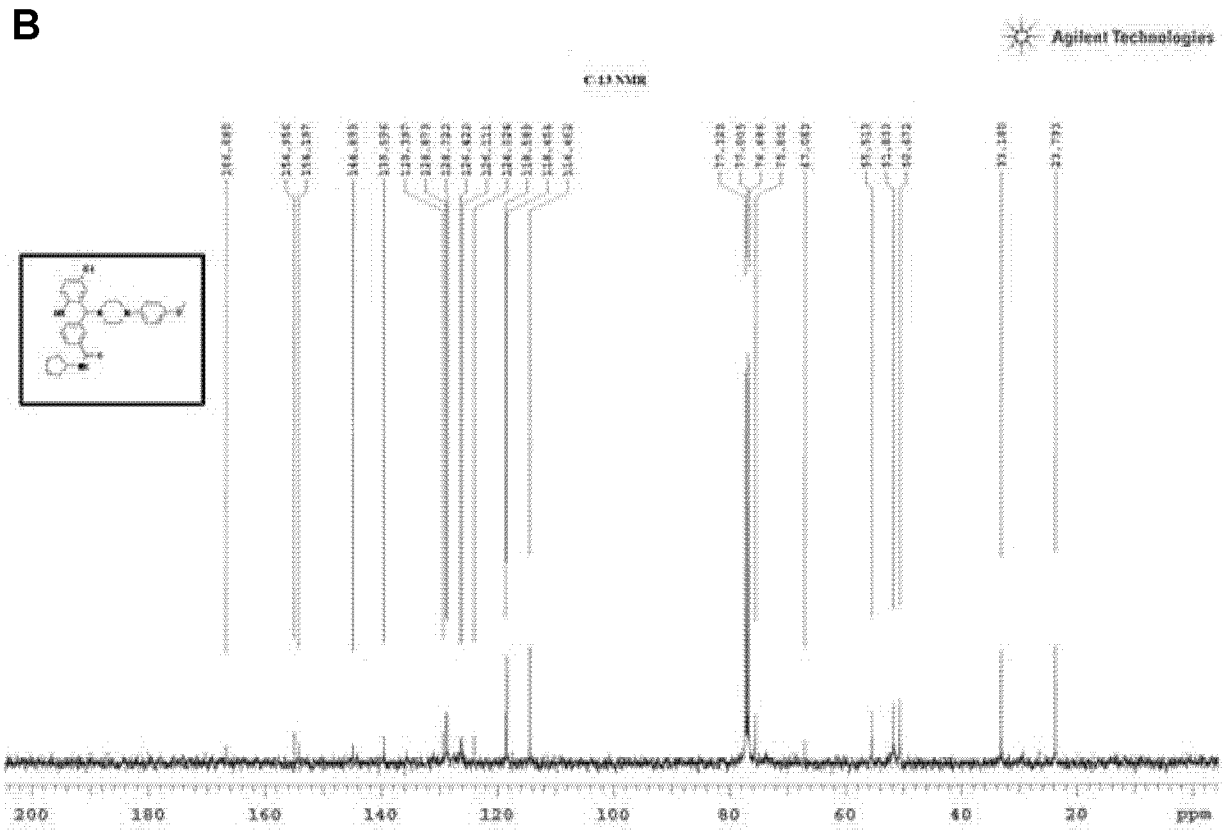

B
Agilent Technologies
200
180
160
140
120
100
80
60
40
20
ppm

Figure 6

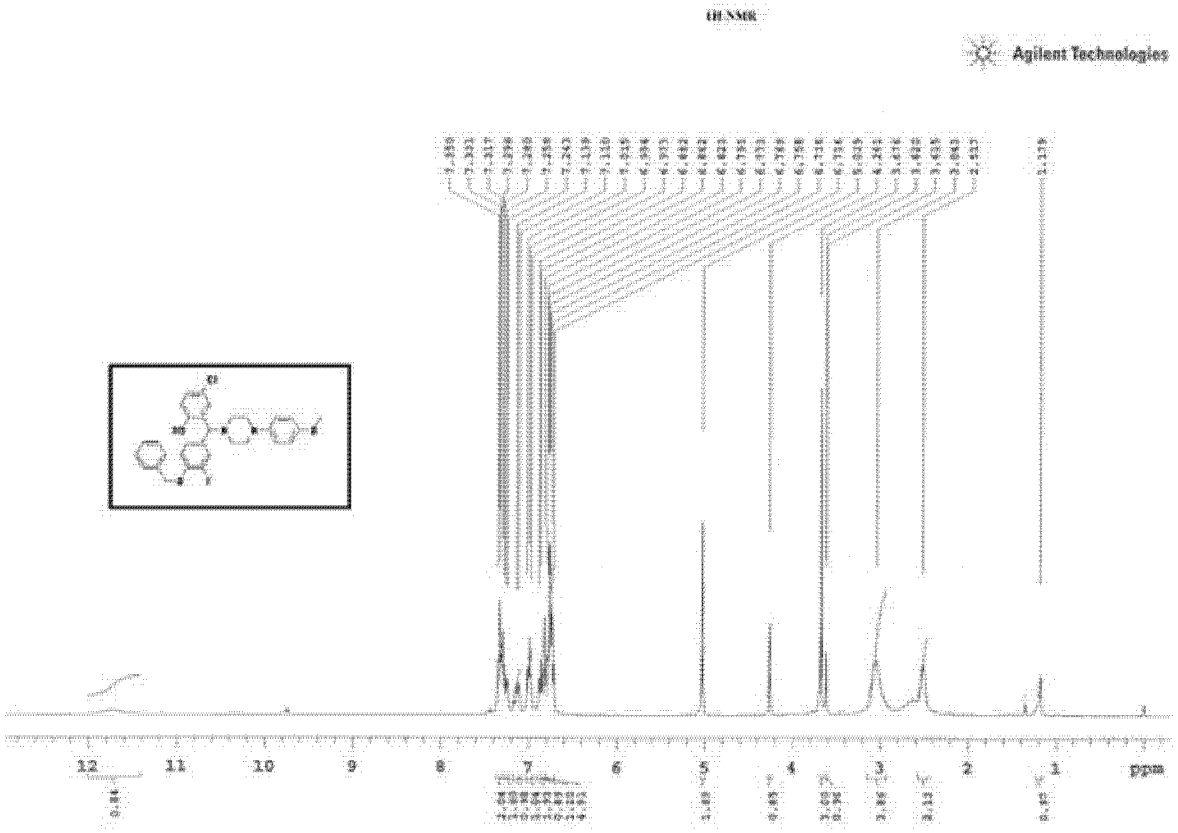
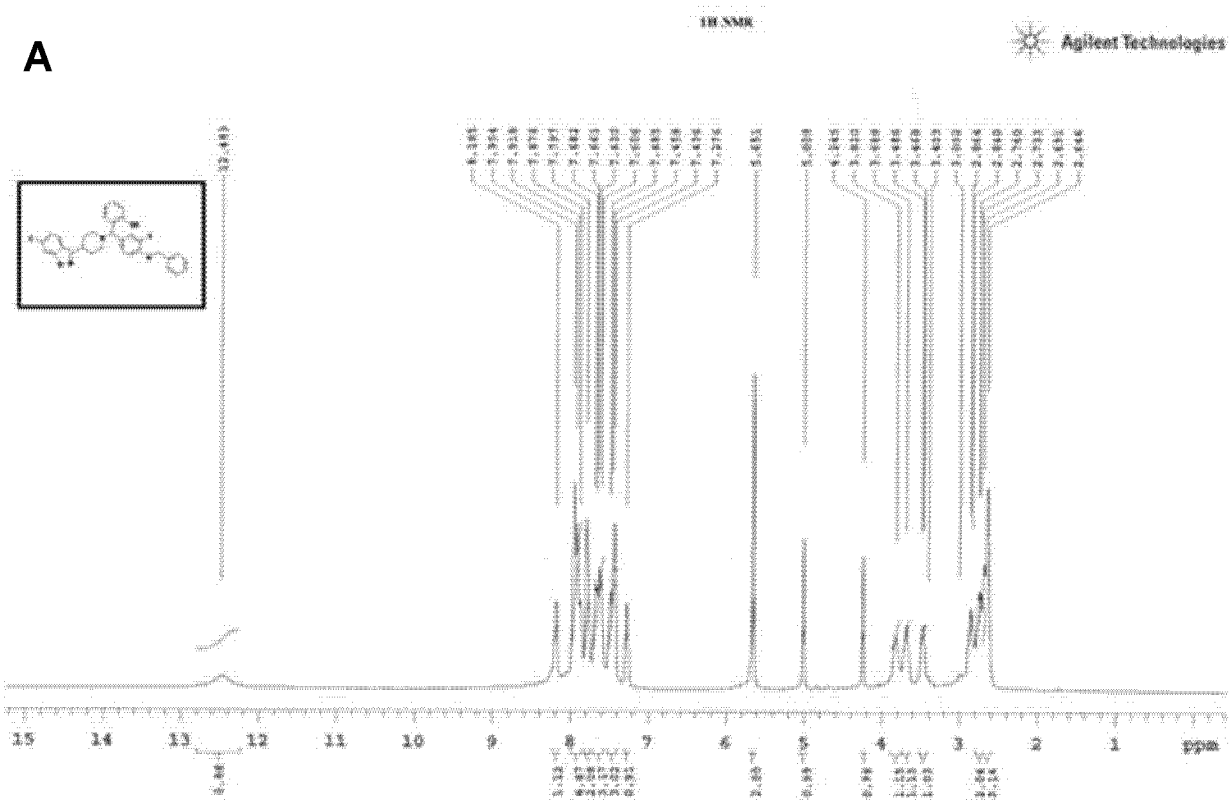


Figure 7


A
Agilent Technologies
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm

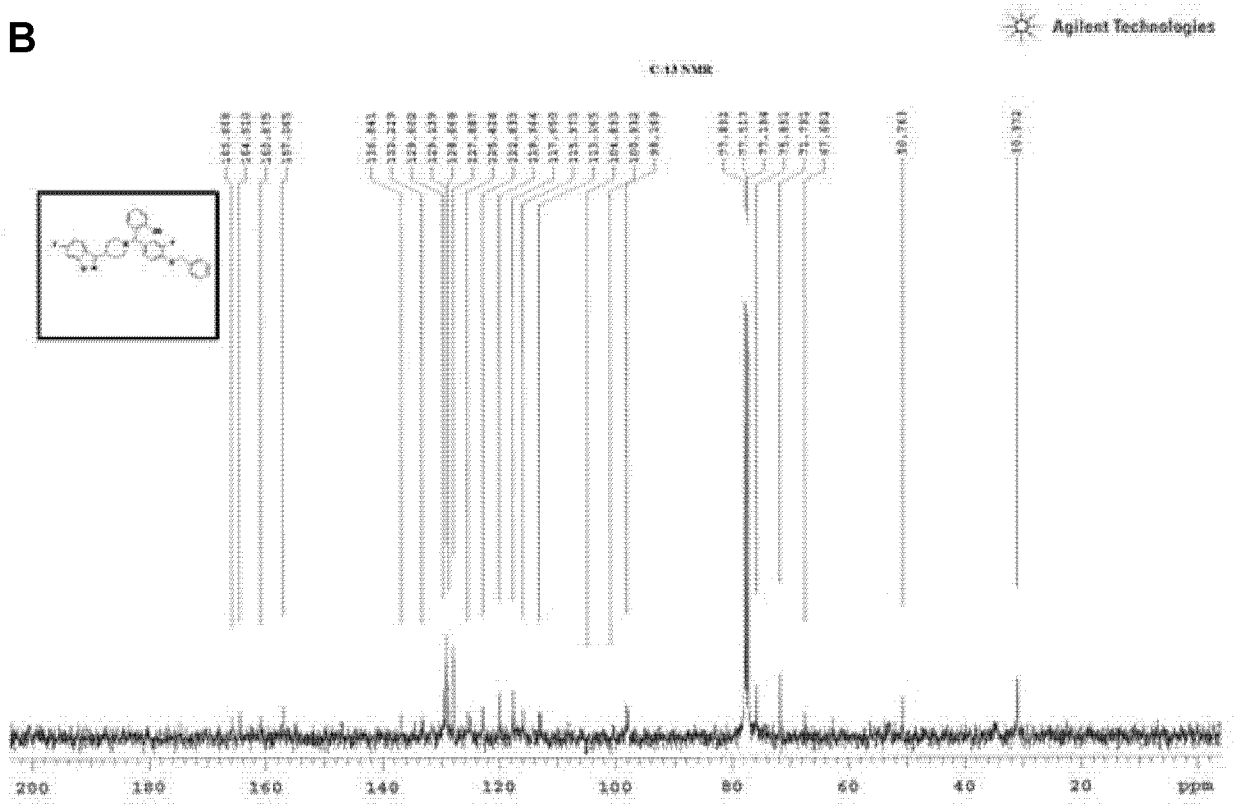

B
Agilent Technologies
200 180 160 140 120 100 80 60 40 20 ppm

Figure 8

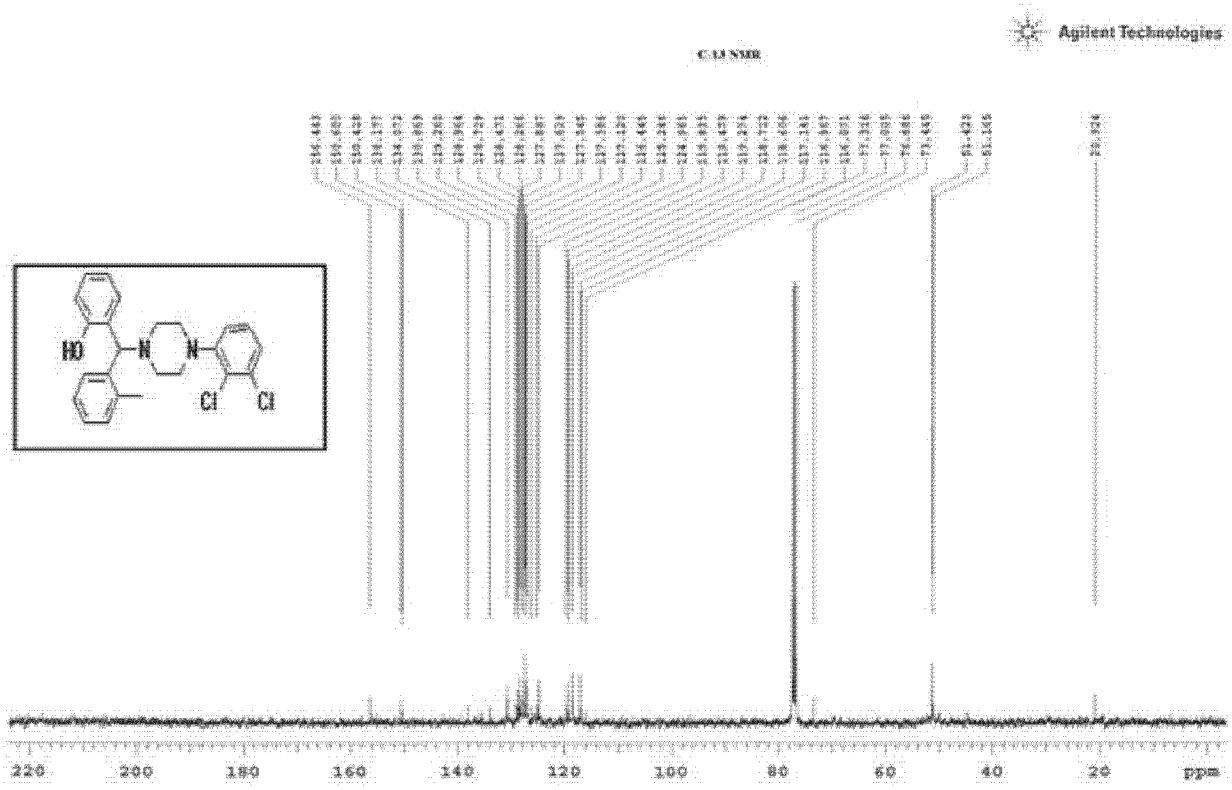
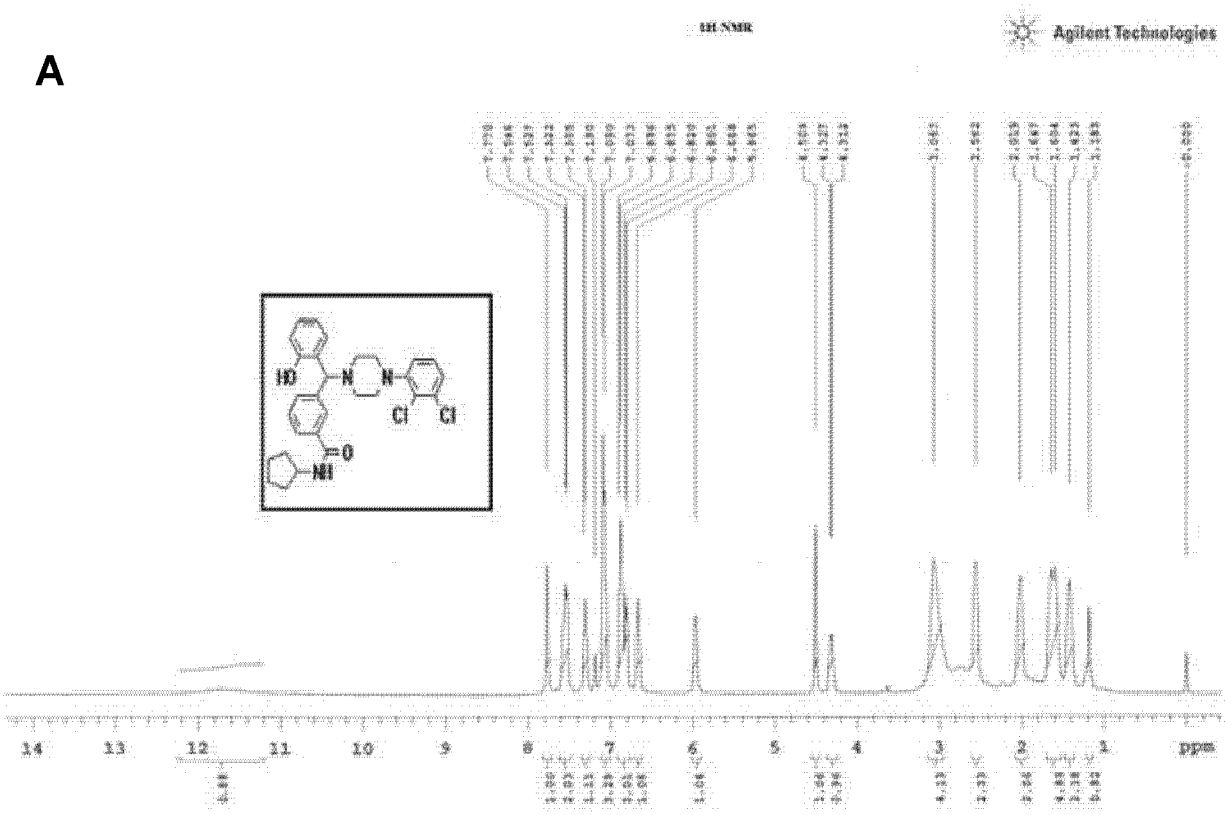
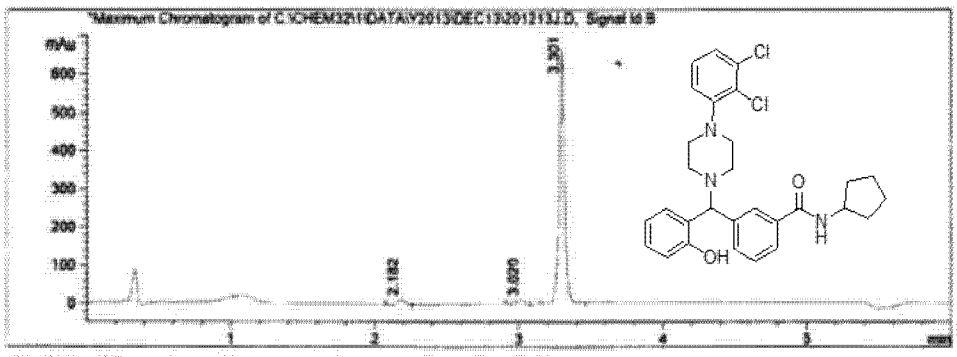

Agilent Technologies
C 13 NMR
HO
N
N
Cl
Cl
220
200
180
160
140
120
100
80
60
40
20
ppm

Figure 9

A



B



Peak No	RT min	Area	Area %
1	2.182	89.369	4.949
2	3.020	53.555	2.966
3	3.301	1662.783	92.085

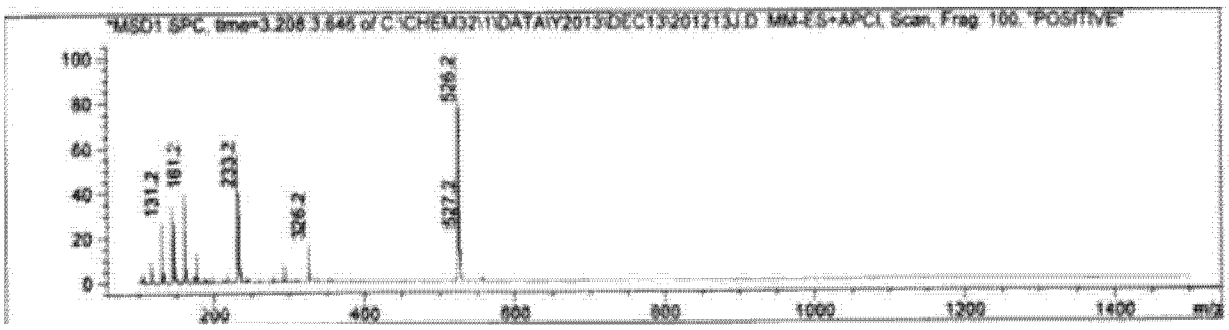


Figure 10

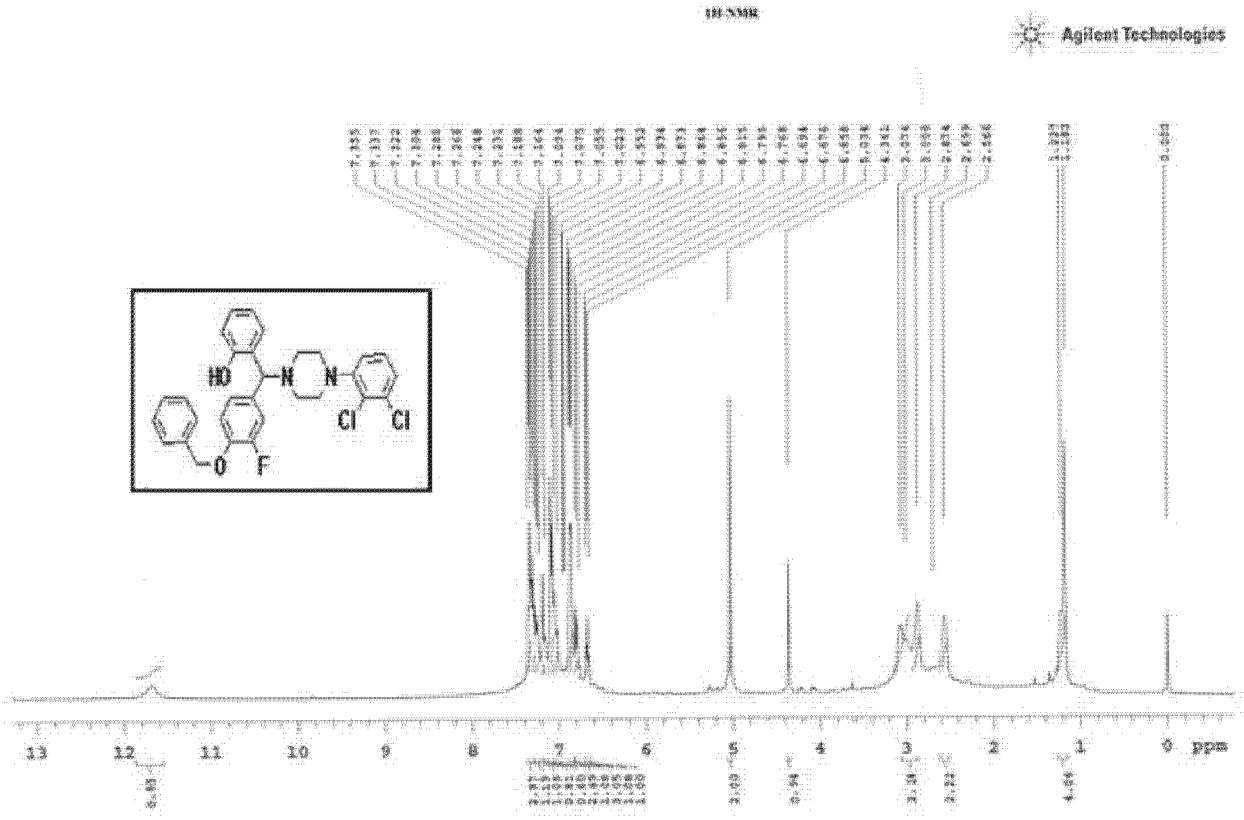

Agilent Technologies
HO
N
N
Cl
Cl
O
F
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm

Figure 11

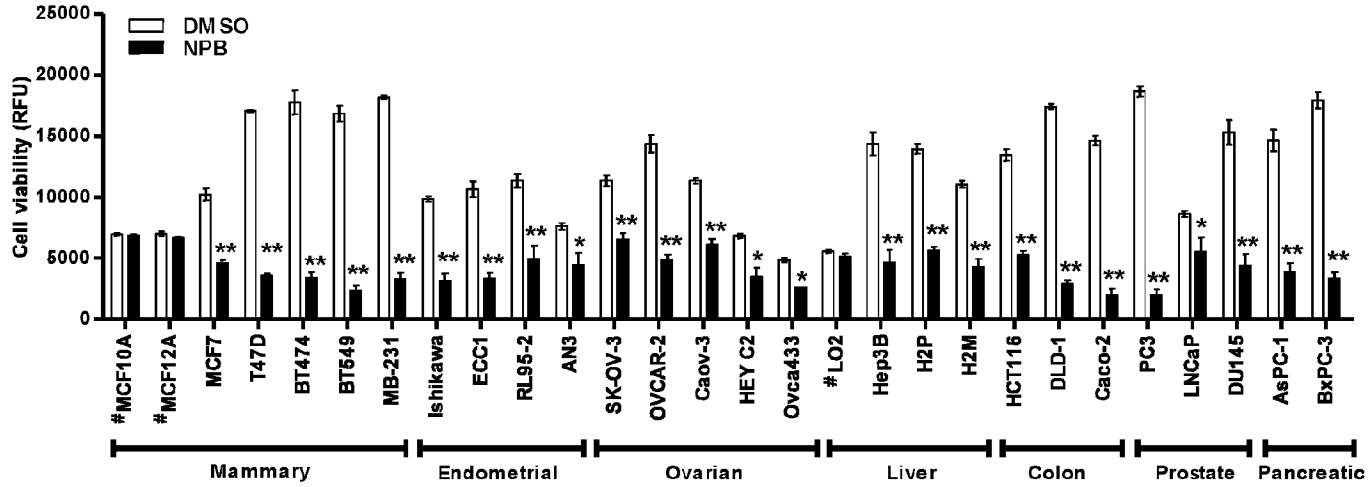
IC_{50} values of NPB in a range of carcinoma cell lines

Panel of cell lines		NPB (μM)	
Tissue	Cells	IC_{50}^	±SD
Mammary	#MCF10A	NV	NV
	#MCF12A	NV	NV
	MCF7	6.5	1.06
	T47D	7.24	1.91
	BT474	5.31	2.04
	BT549	4.88	1.31
	MDA-MB-231	6.94	1.86
Endometrial	Ishikawa	7.51	2.08
	ECC1	2.61	0.97
	RL95-2	6.38	1.85
	AN3	11.37	2.61
Ovarian	SK-OV-3	7.34	2.03
	OVCAR-2	4.21	1.74
	Caov-3	3.95	0.93
	HEY C2	6.82	1.94
	Ovca433	9.79	2.48
Hepatocellular	#LO2	NV	NV
	Hep3B	6.94	1.07
	H2P	4.18	0.83
	H2M	5.57	2.61
Colon	HCT116	7.29	2.02
	DLD-1	2.46	0.91
	Caco-2	3.08	0.76
Prostate	PC3	3.77	1.51
	LNCaP	8.02	3.3
	DU145	6.99	1.72
Pancreatic	AsPC-1	3.83	1.14
	BxPC-3	7.25	2.09

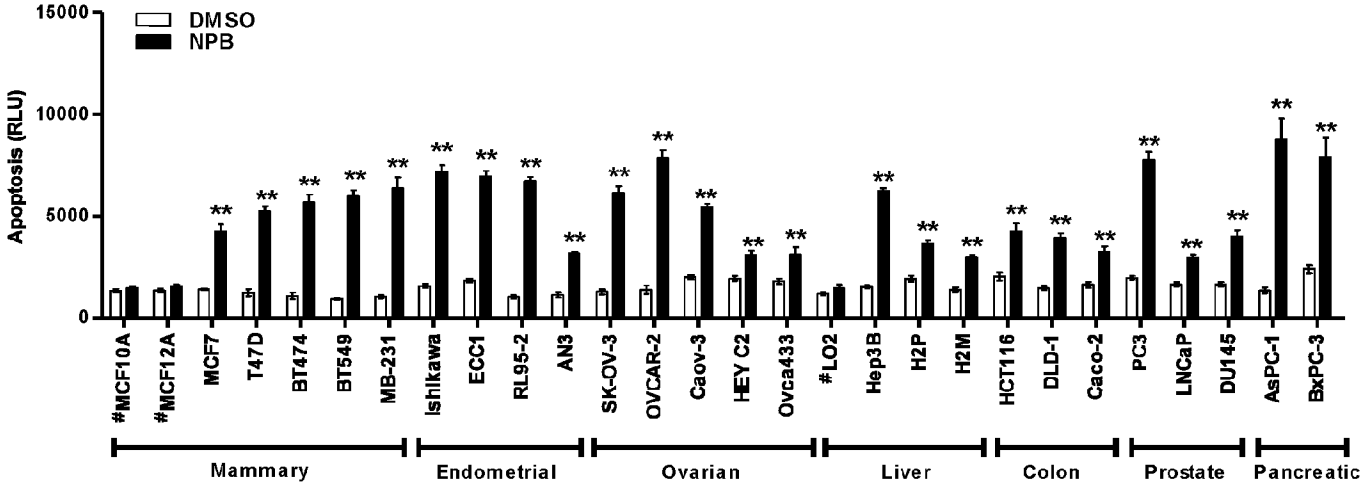
Note: NV, No value; #, Normal cells;
^IC50 values calculated using GraphPad Prism software (Version 5.0). Cell viability measured Using AlamarBlue® cell viability assay.

Figure 12

A. Cell viability



B. Caspase 3/7 activity(Apoptosis)



C. Cytotoxicity

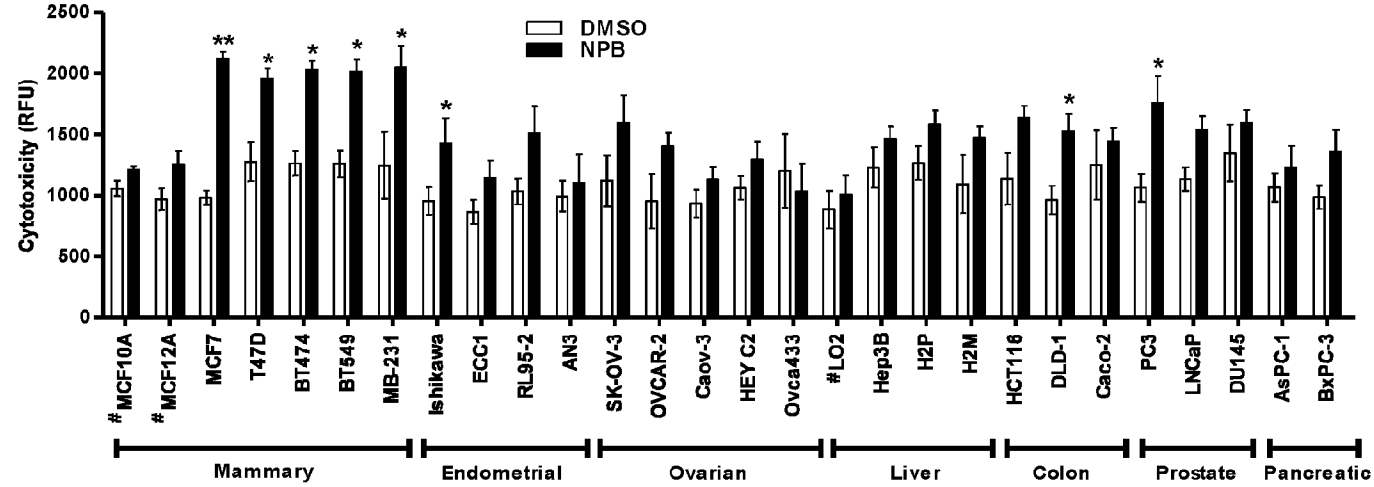
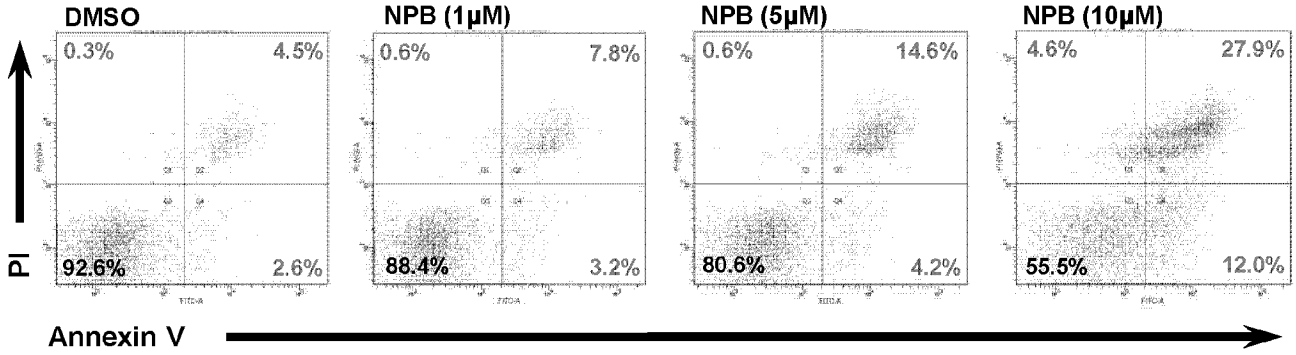
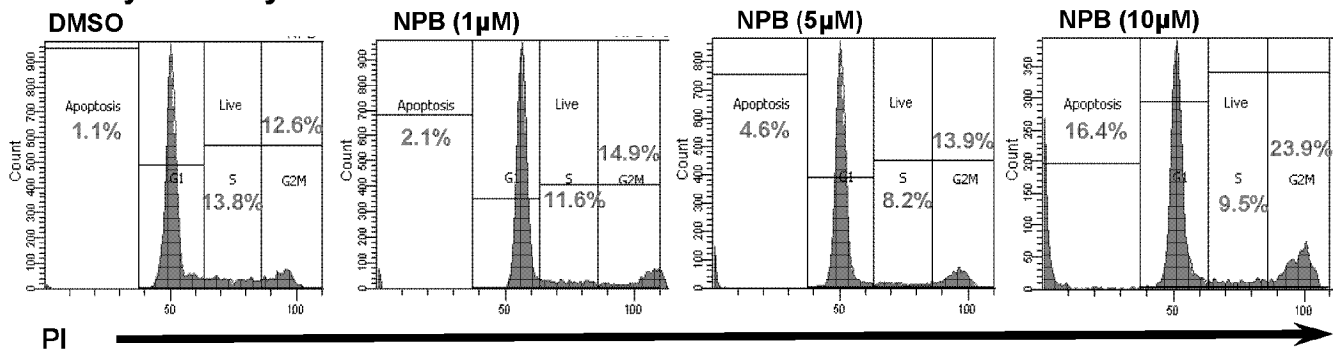


Figure 13:

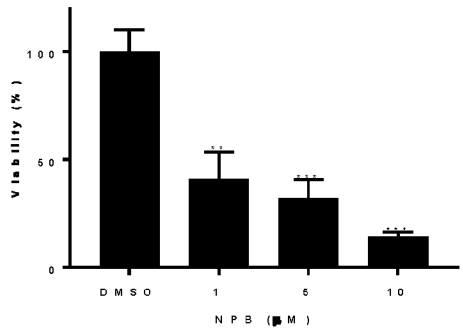
A. Flow cytometry



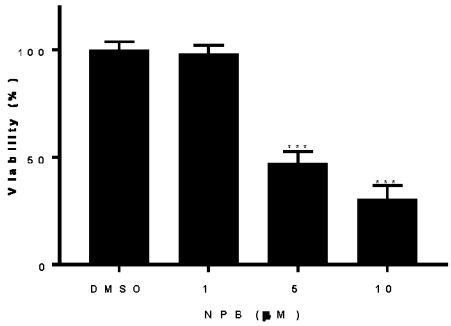
B. Cell cycle analysis

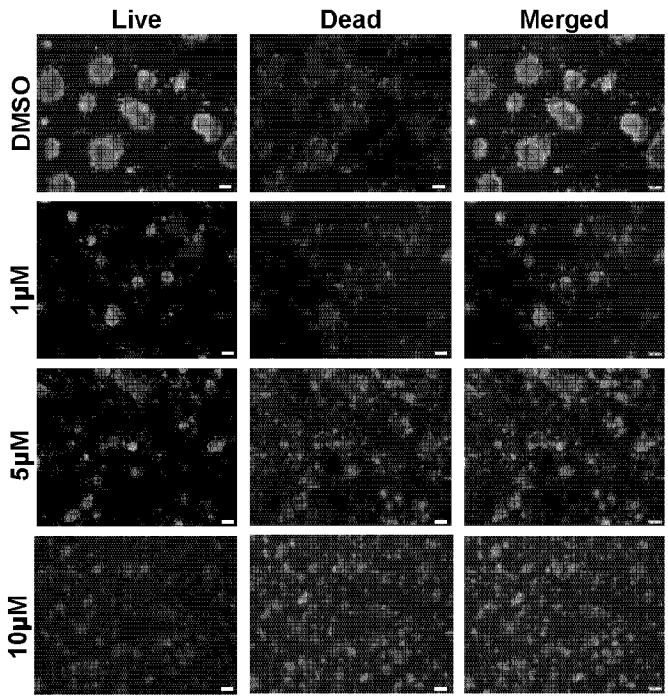


C. 3D matrigel growth



D. Soft agar colony formation





E. Foci formation

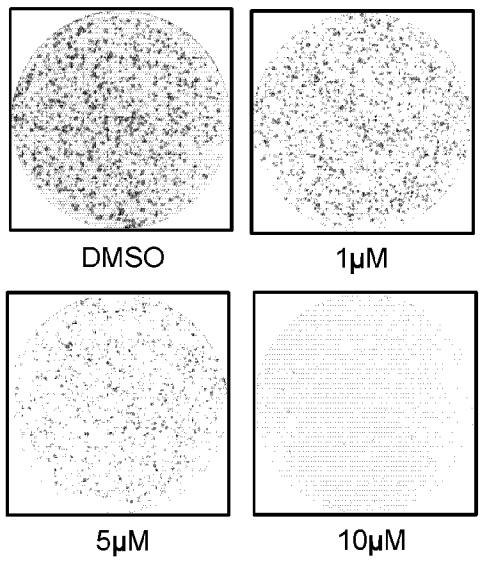
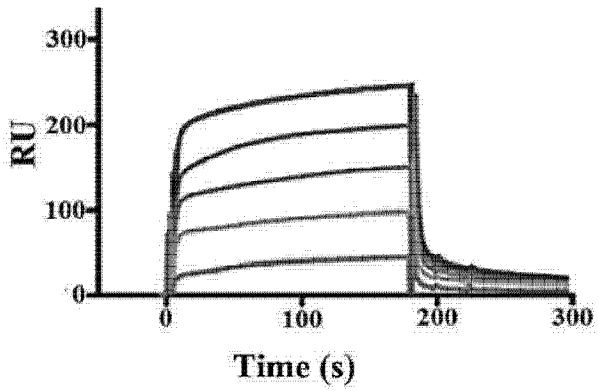


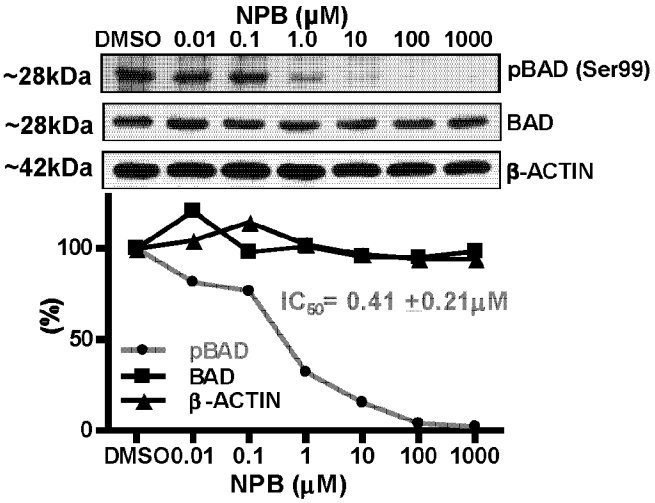
Figure 14:

A. Surface Plasmon Resonance (SPR)

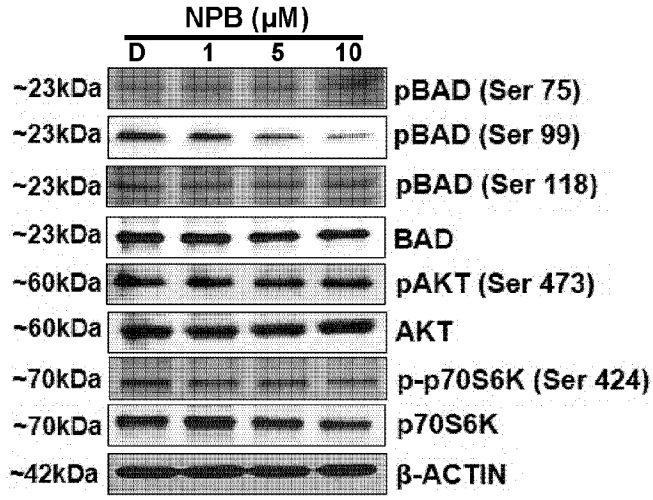
Association (k_a): 1.4 ±0.4 x10^3 $M^{-1}S^{-1}$
Dissociation (k_d): 5.4 ±0.38 x10^3 S^{-1}
Affinity (K_D): 37.12 µM



B. Western blot



C. Western blot



D. Western blot

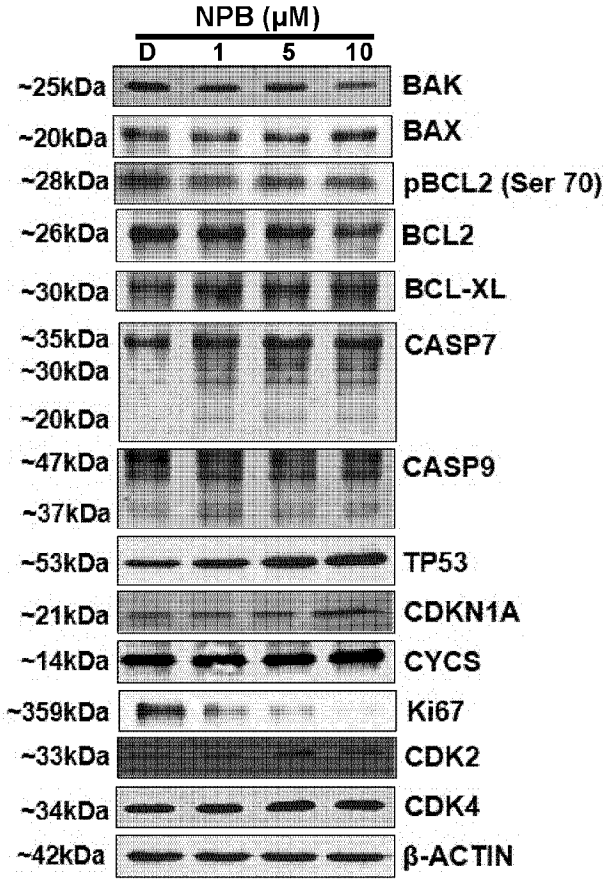
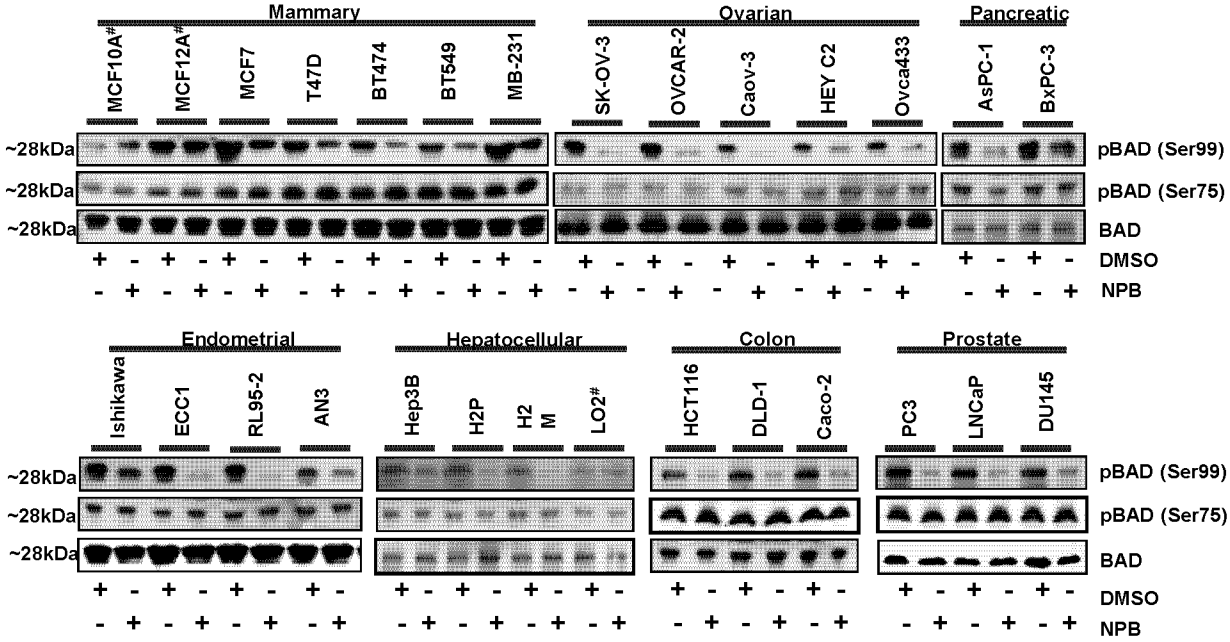


Figure 15:

A. Western blot



B. Western blot

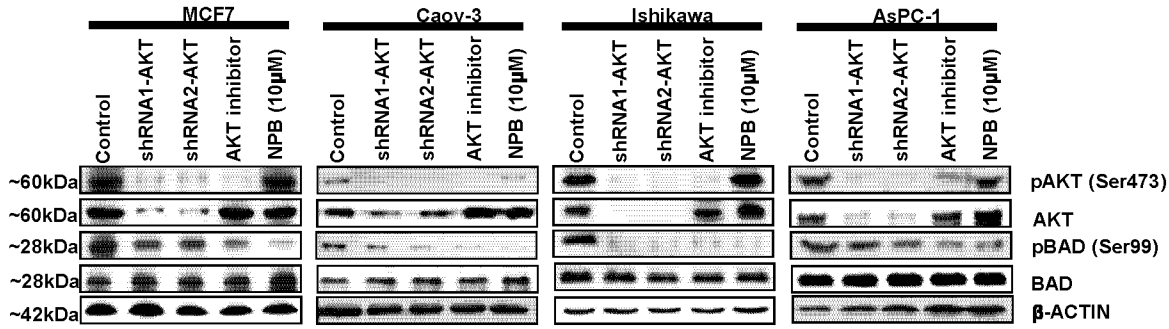


Figure 16:

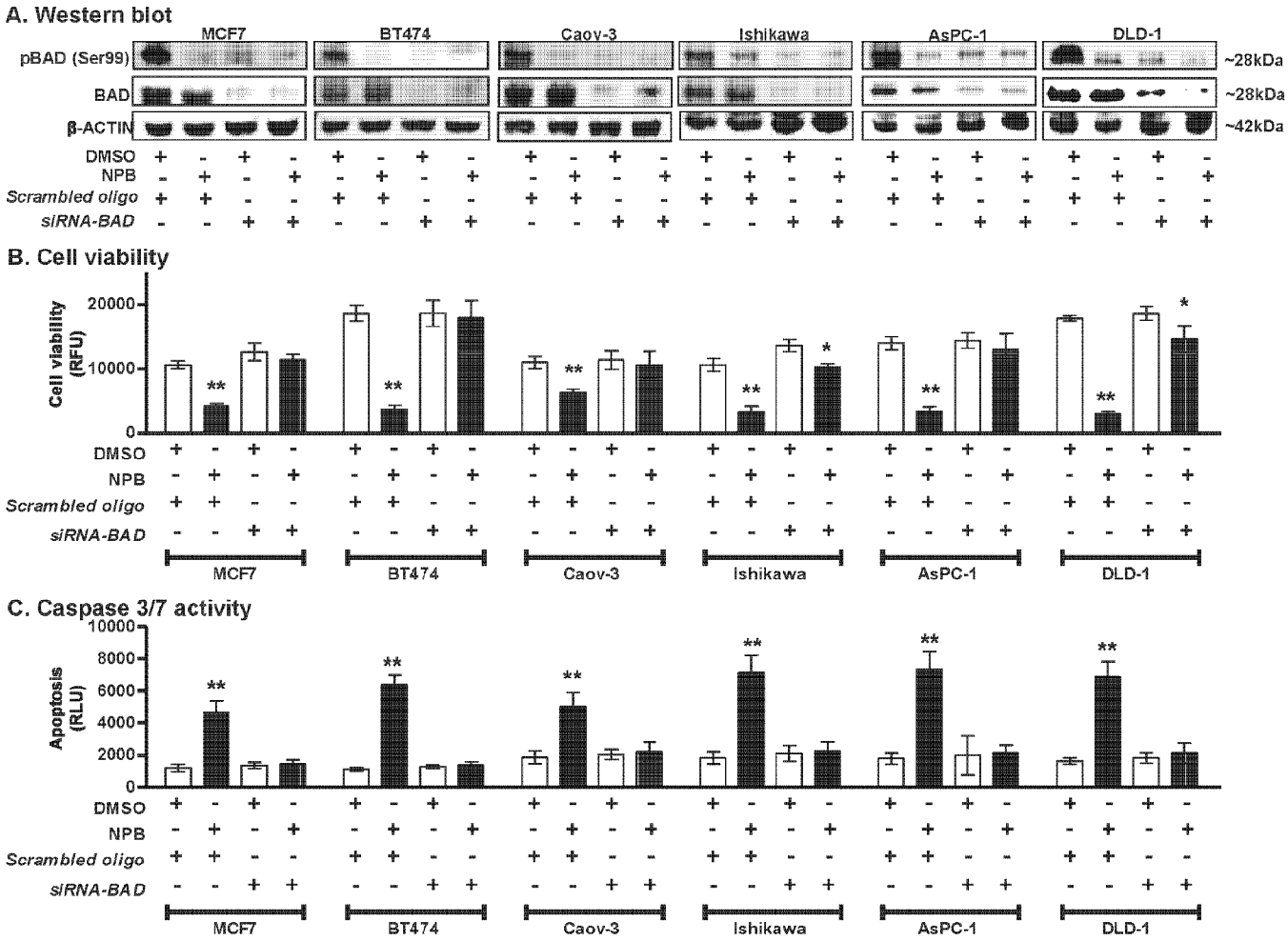

A. Western blot
MCF7
BT474
Caov-3
Ishikawa
AsPC-1
DLD-1
pBAD (Ser99)
BAD
β-ACTIN
~28kDa
~28kDa
~42kDa
DMSO
NPB
Scrambled oligo
siRNA-BAD
B. Cell viability
Cell viability (RFU)
20000
10000
0
C. Caspase 3/7 activity
Apoptosis (RLU)
10000
8000
6000
4000
2000
0

Figure 17

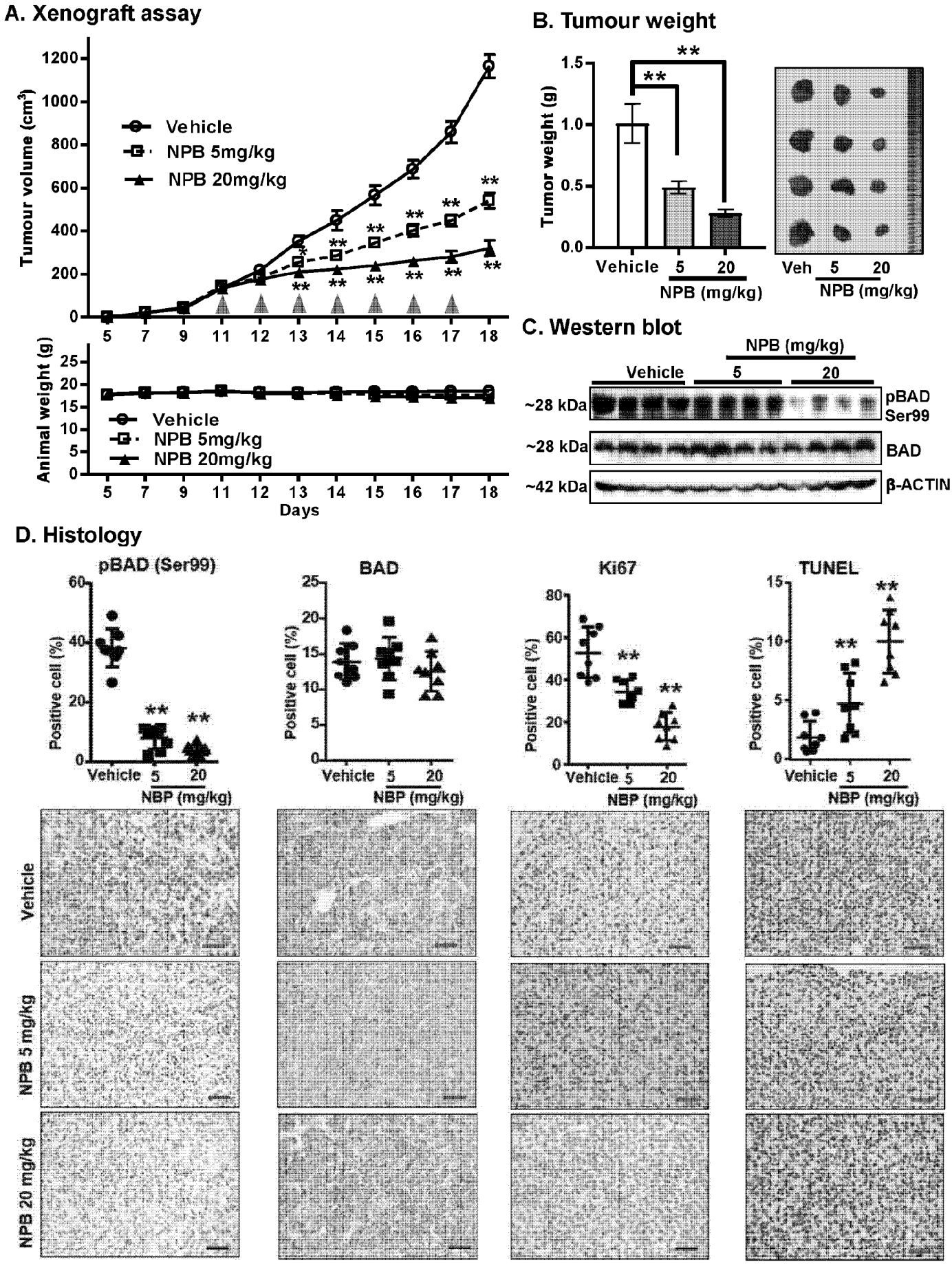

A. Xenograft assay
Tumour volume (cm3)
Vehicle
NPB 5mg/kg
NPB 20mg/kg
Animal weight (g)
Days
B. Tumour weight
Tumor weight (g)
Vehicle
NPB (mg/kg)
Veh
C. Western blot
~28 kDa
pBAD Ser99
BAD
~42 kDa
β-ACTIN
D. Histology
pBAD (Ser99)
BAD
Ki67
TUNEL
Positive cell (%)
NBP (mg/kg)
Vehicle
NPB 5 mg/kg
NPB 20 mg/kg

Figure 18:

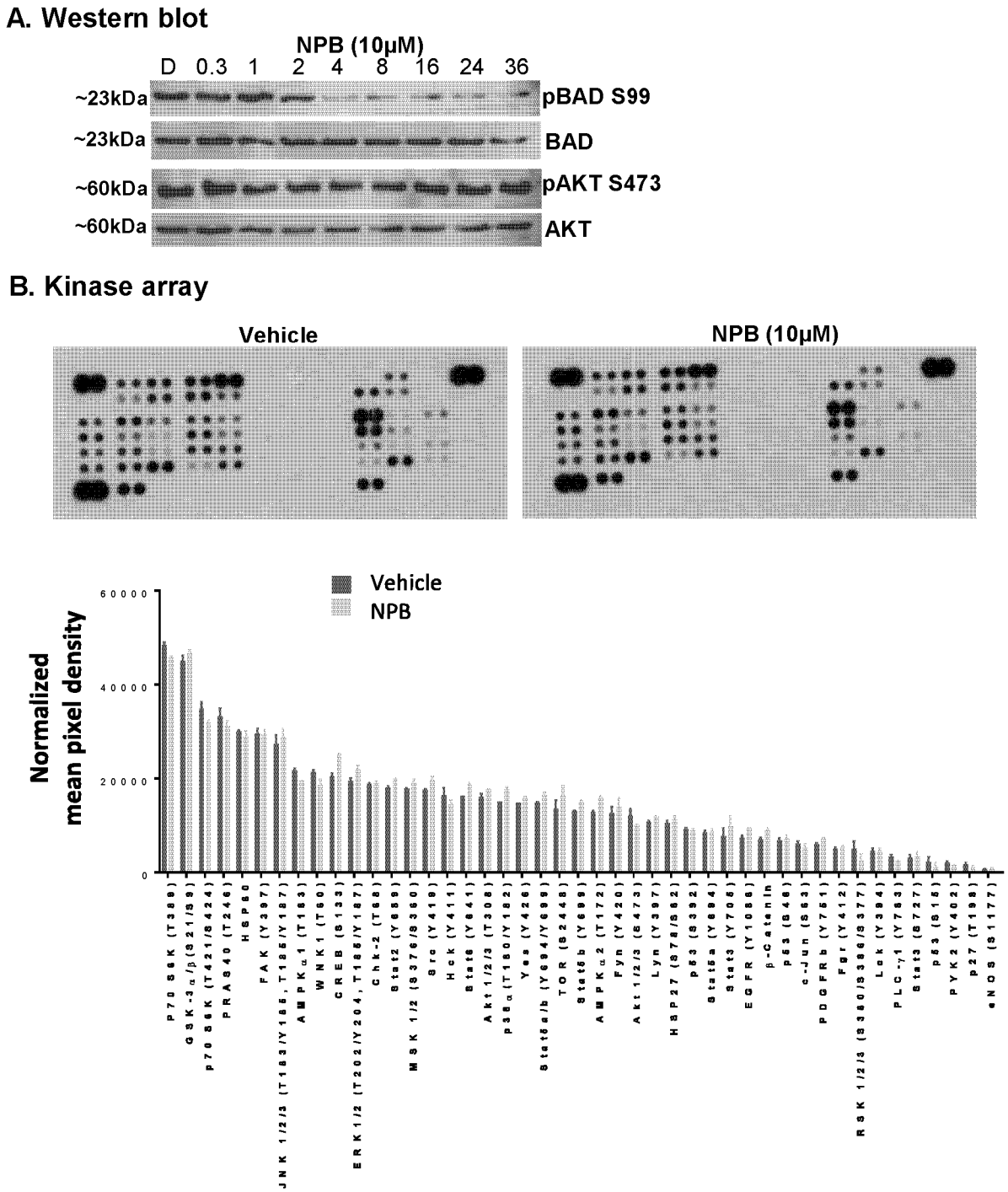

A. Western blot
NPB (10μM)
D 0.3 1 2 4 8 16 24 36
~23kDa
pBAD S99
~23kDa
BAD
~60kDa
pAKT S473
~60kDa
AKT
B. Kinase array
Vehicle
NPB (10μM)
Vehicle
NPB
Normalized mean pixel density
60000
40000
20000
0
P70 S6K (T389)
GSK-3α/β(S21/S9)
p70 S6K (T421/S424)
PRAS40 (T246)
HSP60
FAK (Y397)
JNK 1/2/3 (T183/Y185, T185/Y187)
AMPKα1 (T183)
WNK1 (T60)
CREB (S133)
ERK1/2 (T202/Y204, T185/Y187)
Chk-2 (T68)
Stat2 (Y689)
MSK 1/2 (S376/S360)
Src (Y418)
Hck (Y411)
Stat6 (Y641)
Akt1/2/3 (T308)
p38α(T180/Y182)
Yes (Y426)
Stat6a/b (Y694/Y698)
TOR (S2448)
Stat6b (Y698)
AMPKα2 (T172)
Fyn (Y420)
Akt1/2/3 (S473)
Lyn (Y397)
HSP27 (S78/S82)
p53 (S392)
Stat5a (Y694)
Stat3 (Y705)
EGFR (Y1068)
β-Catenin
p53 (S46)
c-Jun (S63)
PDGFRb (Y751)
Fgr (Y412)
RSK 1/2/3 (S380/S386/S377)
Lck (Y394)
PLC-γ1 (Y783)
Stat3 (S727)
p53 (S15)
PYK2 (Y402)
p27 (T198)
eNOS (S1177)

Figure 19

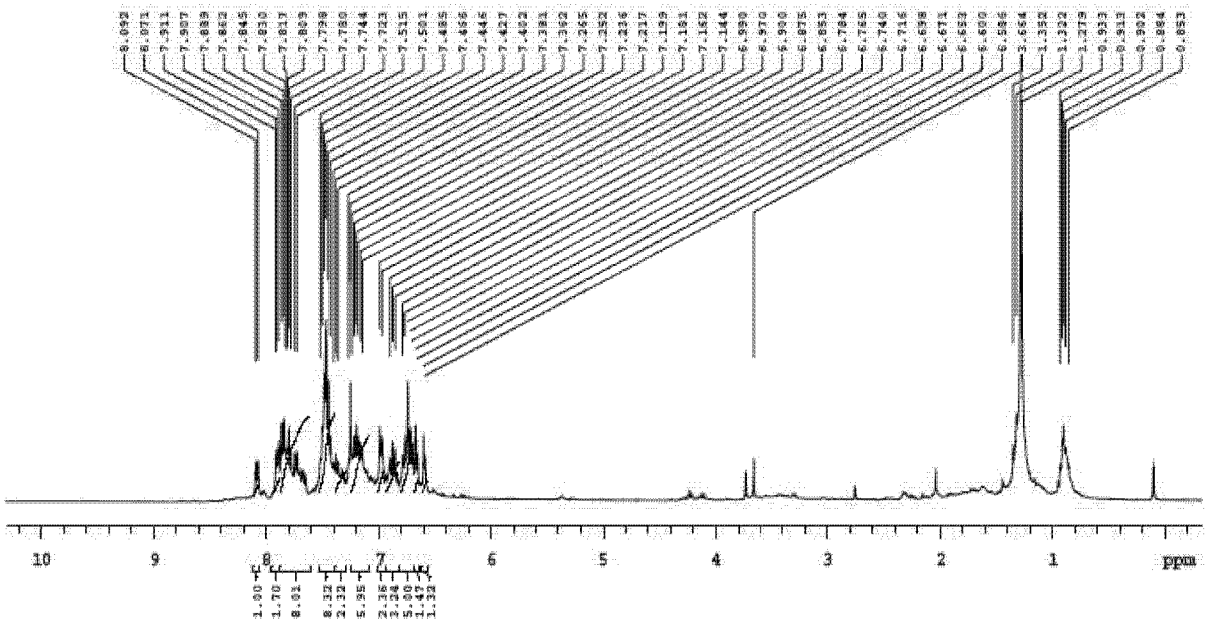

8.093
8.071
7.911
7.907
7.889
7.862
7.845
7.830
7.817
7.809
7.798
7.780
7.744
7.723
7.515
7.501
7.485
7.456
7.445
7.427
7.402
7.381
7.362
7.265
7.252
7.236
7.217
7.199
7.151
7.142
7.144
6.990
6.970
6.900
6.875
6.853
6.784
6.765
6.740
6.716
6.698
6.671
6.653
6.600
6.586
3.654
1.332
1.322
1.279
0.933
0.913
0.902
0.884
0.853
10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.70
8.01
8.32
2.32
5.95
2.36
3.38
5.00
1.45
1.32

Figure 20

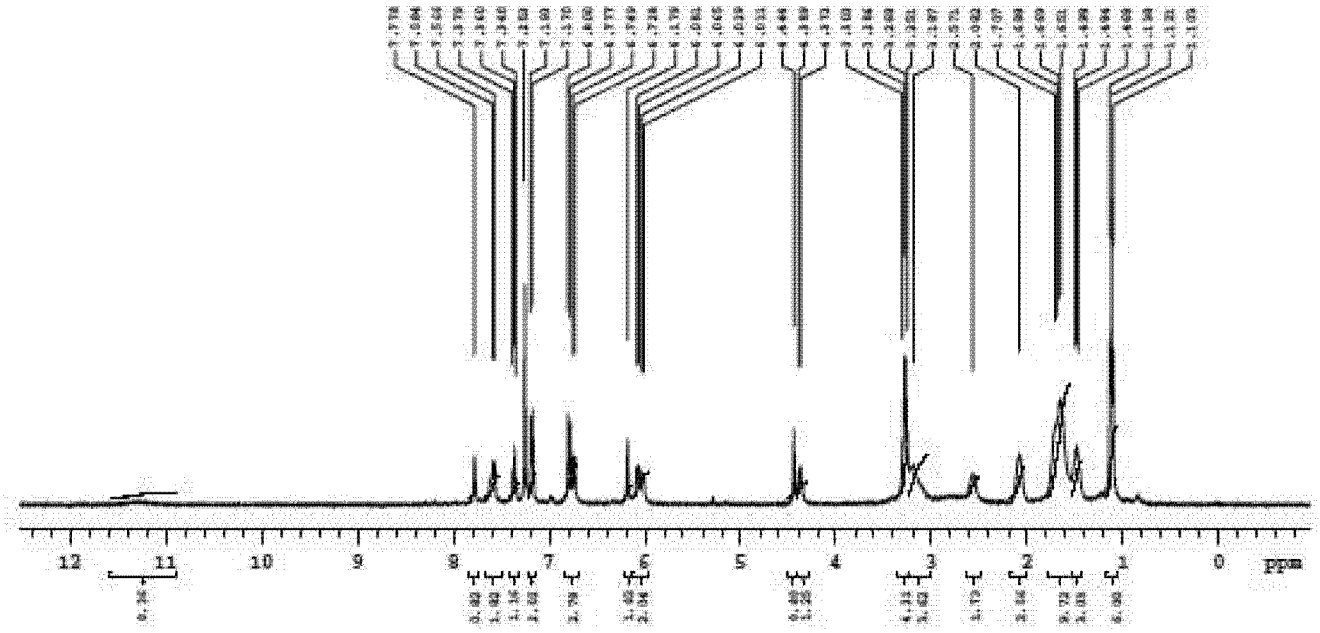


Figure 21

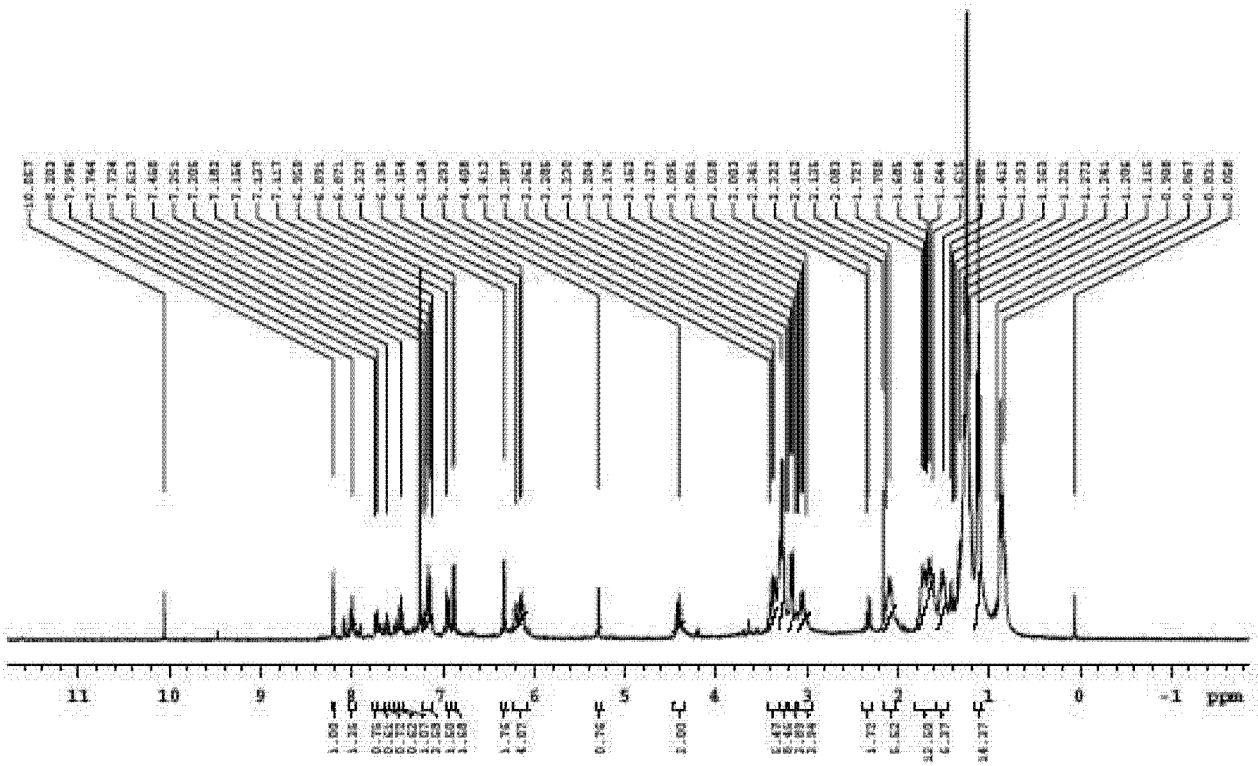


Figure 22

A

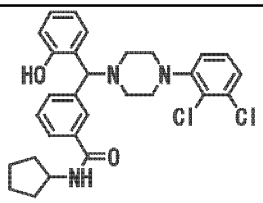
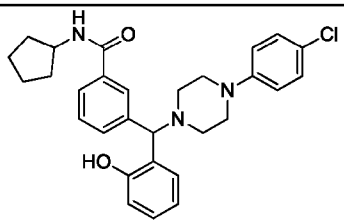
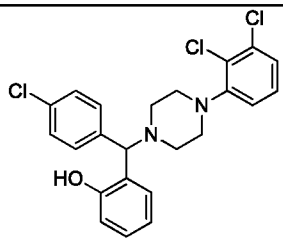
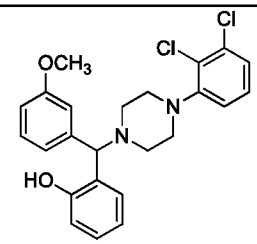
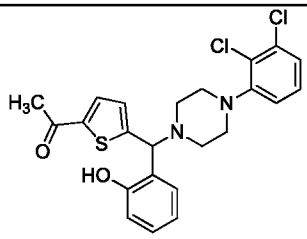
Analogues	Structure	MCF7 IC_{50}	A2780 IC_{50}
NPB	 MW: 523	9.52	5.33
NCK1	 Molecular Weight: 490.0363	NV	26.63
NCK2	 Molecular Weight: 447.7846	NV	19.74
NCK3	 Molecular Weight: 443.3656	NV	50.82
NCK4	 Molecular Weight: 461.4040	NV	NV

Figure 22

B

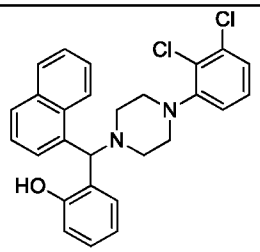
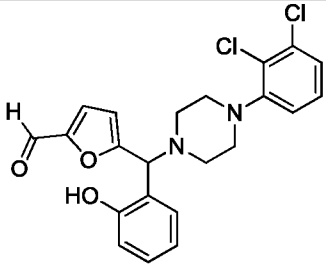
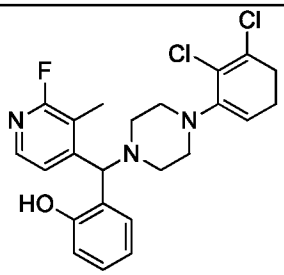
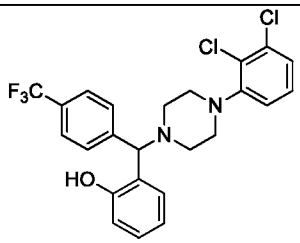
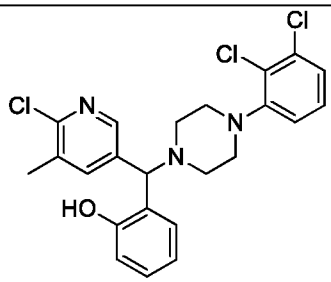
NCK5	 Molecular Weight: 463.3983	5.907	27.39
NCK6	 Molecular Weight: 431.3118	3.114	6.68
NCK7	 Molecular Weight: 448.3606	47.28	38.74
NCK8	 Molecular Weight: 481.338	59.52	23.66
NCK9	 Molecular Weight: 462.7993	NV	16.06

Figure 22

C

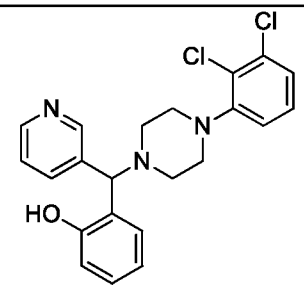
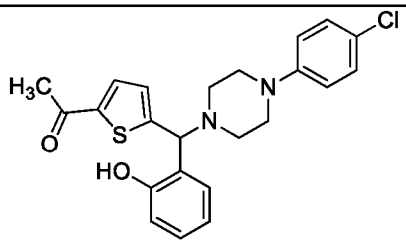
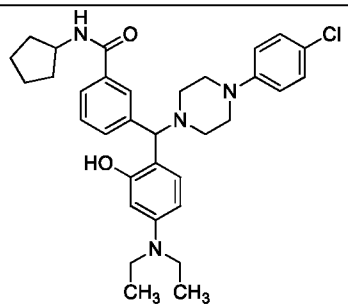
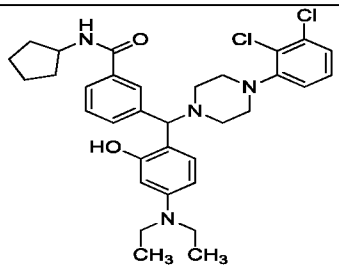
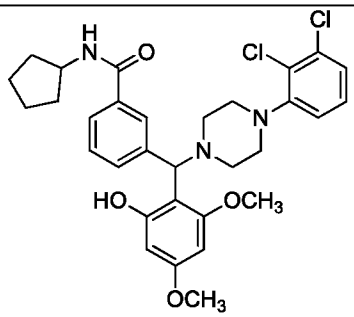
NCK10	 Molecular Weight: 414.3276	24.83	35.86
NCK14	 Molecular Weight: 426.9589	NV	23.26
NCK16	 Molecular Weight: 561.16	23.35	9.53
NCK18	 Molecular Weight: 595.60	20.93	2.97
NCK19	 Molecular Weight: 584.53	NV	48.02

Figure 22

D

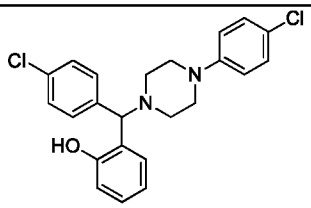
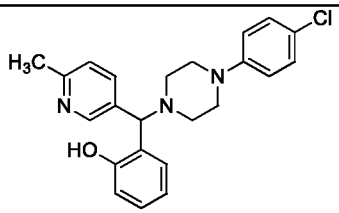
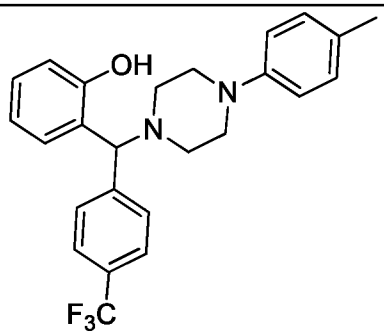
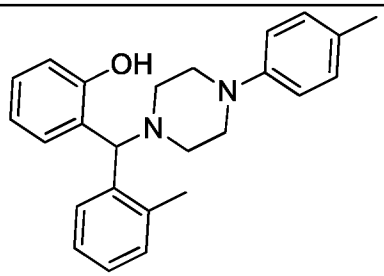
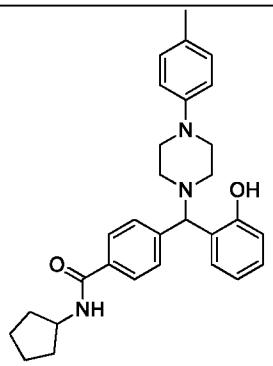
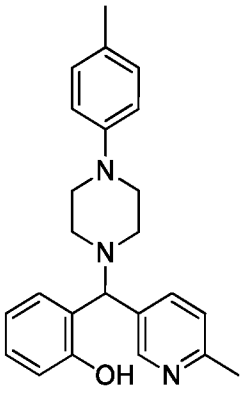
NCK20	 Molecular Weight: 413.34	253.8	8.395
NCK21		20.91	21.90
SG2	 Molecular Weight: 426.4740	155.6	89.69
SG1	 Molecular Weight: 372.5026	NV	NV
SG3	 Molecular Weight: 469.6178	56.47	20.69

Figure 22

E

SG7		49.87	41.61
SG 4, 5, and 6	NOT TESTED		

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2018/050194

A. CLASSIFICATION OF SUBJECT MATTER

See Supplemental Box

According to International Patent Classification (IPC)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Registry, CAplus, Biosis, Embase, Medline (structure search, keywords such as apoptosis, programmed cell death, caspase-mediated cell death, Bcl-2-associated death promoter (BAD), tumour, cancer, proliferation, neoplasm and the like)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	HOSSEINZADEH R. ET AL., A green protocol for the one-pot multicomponent Petasis boronic Mannich reaction using ball milling. *J. Iran. Chem. Soc.*, 4 October 2016, Vol. 14, No. 2, pages 347-355 [Retrieved on 2018-06-13] <DOI: 10.1007/S13738-016-0983-Y> Scheme 1 and 3; table 2 compounds 4a-4e, 4g-4k	1-3, 7-12, 14 4-6, 13
X A	Compound with Registry No. 1088182-85-7. 22 December 2008 [Retrieved on 2018-06-13 from CAS Registry]	1-3, 7-12 4-6, 13
X A	CHRISTOV K. ET. AL., Short-term Modulation of Cell Proliferation and Apoptosis and Preventive/Therapeutic Efficacy of Various Agents in a Mammary Cancer Model. *Clin. Cancer Res.*, 15 September 2007, Vol. 13, No. 18, pages 5488-5496 [Retrieved on 2018-06-13] <DOI: 10.1158/1078-0432.CCR-07-0404> Abstract; Figure 1; Table 1 and 2; page 5490-5492	1, 7, 15-21 4-6, 13

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

*Special categories of cited documents:

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"O" document referring to an oral disclosure, use, exhibition or other means

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Date of the actual completion of the international search	Date of mailing of the international search report
15/06/2018 *(day/month/year)*	04/07/2018 *(day/month/year)*

Name and mailing address of the ISA/SG

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Jenefer Alam (Dr)

IPOS Customer Service Tel. No.: (+65) 6339 8616

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2018/050194

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	LIN J.-C. ET AL., Induction of apoptosis and cell-cycle arrest in human colon cancer cells by meclizine. *Food Chem. Toxicol.*, 9 December 2006, Vol. 45, No. 6, pages 935-944 [Retrieved on 2018-06-13] <DOI: 10.1016/J.FCT.2006.11.016> Abstract; Results; 3.6-3.7; Fig 1, 8-9	1, 7, 15-21 4-6, 13
X A	RICHARD D. J. ET AL., Hydroxyquinoline-derived compounds and analoguing of selective Mcl-1 inhibitors using a functional biomarker. *Bioorg Med Chem.*, 15 August 2013, Vol. 21, No. 21, pages 6642-6649 [Retrieved on 2018-06-15] <DOI: 10.1016/J.BMC.2013.08.017.> Abstract; page 2, 3rd paragraph; Section 2.5; page 5-6; Section 3.2 and 3.4; Discussion; compound 9; fig 1 and 3; tables 1 and 2	1, 7, 15-21 4-6, 13
X A	THANKRAN A. K. ET AL., Synthesis and Pharmacological Evaluation of 1-Benz-Hydryl Piperazine Derivatives. *Int. J. Pharm. Sci. Res.*, 1 January 2012, Vol. 3, No. 1, pages 213-217 [Retrieved on 2018-06-13] <DOI: 10.13040/IJPSR.0975-8232.3(1).213-17 > Abstract; Compounds AT-2 to AT-4; Tables 1-4; Biological studies; figures 1 and 2	1, 7, 9 4-6, 13
X A	WO 01/07050 A1 (SCHERING CORPORATION) 1 February 2001 example 1-3, 9-10; Tables 1, 2, and 4; example 12	1, 7, 9 4-6, 13
X A	KARUBE K. ET AL., Comprehensive gene expression profiles of NK cell neoplasms identify vorinostat as an effective drug candidate. *Cancer Lett.*, 21 January 2013, Vol. 333, No. 1, pages 47-55 [Retrieved on 2018-06-13] <DOI: 10.1016/J.CANLET.2012.12.022> Section 3.2; Table 2	1, 7 4-6, 13
P,X	JP 2017128541 A (TOKYO WOMEN'S MEDICAL COLLEGE ET AL.) 27 July 2017 whole document of the machine translation	1, 7, 15-16, 19-21

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SG2018/050194

Note: This Annex lists known patent family members relating to the patent documents cited in this International Search Report. This Authority is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 01/07050 A1	01/02/2001	US 2001/0011092 A1	02/08/2001
		US 6262066 B1	17/07/2001
		AU 2629800 A	13/02/2001
		CA 2379398 A1	01/02/2001
		JP 2003505420 A	12/02/2003
		EP 1200087 A1	02/05/2002
		US 2004/0152707 A1	05/08/2004
		US 2003/0073690 A1	17/04/2003
		CN 1374865 A	16/10/2002
JP 2017128541 A	27/07/2017	NONE	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2018/050194

Supplemental Box
(Classification of Subject Matter)

Int. Cl.
C07D 211/06 (2006.01)
C07D 241/04 (2006.01)
A61K 31/445 (2006.01)
A61K 31/496 (2006.01)
A61P 35/00 (2006.01)