

(51) International Patent Classification:
C07D 209/42 (2006.01)

(21) International Application Number:

PCT/GB2011/052386

(22) International Filing Date:

2 December 2011 (02.12.2011)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

1020397.4 2 December 2010 (02.12.2010) GB

(71) Applicant (for all designated States except US): **THE UNIVERSITY OF NOTTINGHAM** [GB/GB]; University Park, Nottingham, Nottinghamshire NG7 2RD (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **MADHUSUDAN, Srinivasan** [GB/GB]; c/o The University of Nottingham, University Park, Nottingham, Nottinghamshire NG7 2RD (GB). **FISCHER, Peter** [CH/GB]; c/o The University of Nottingham, University Park, Nottingham, Nottingham-shire NG7 2RD (GB). **LAUGHTON, Charles** [GB/GB]; c/o The University of Nottingham, University Park, Nottingham, Nottinghamshire NG7 2RD (GB).(74) Agent: **BARKER BRETTELL LLP**; 100 Hagley Road, Edgbaston, Birmingham B16 8QQ (GB).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE,

[Continued on next page]

(54) Title: COMPOUNDS

2nd Generation APE1 inhibitors (IC₅₀ 500nM - 20μM)

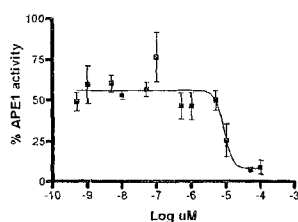


Fig 1a

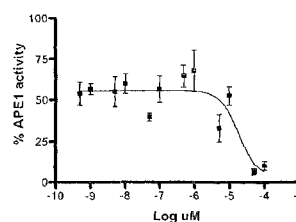


Fig 1b

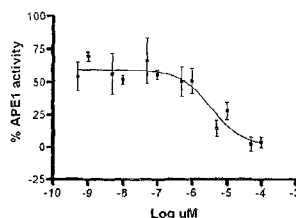


Fig 1c

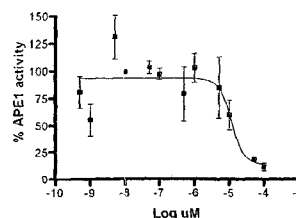


Fig 1d

(57) Abstract: The invention provides a compound with the structural formula of any one of Formula 1 to Formula 18 or a pharmaceutically acceptable salt thereof, and uses thereof.



DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

Compounds

Field of the Invention

5 The field of the invention is generally related to new compounds, the new compounds for use in a method for treatment of the human or animal body by therapy, and, in particular, their use in a method of treating cancer. Specifically, there is provided a method of treating cancer involving the inhibition of the cancer cell DNA base excision repair (BER) pathway through
10 the inhibition of apurinic/apyrimidinic endonuclease (APE1) activity. Inhibition of APE1 activity results in an accumulation of unrepaired apurinic/apyrimidinic (AP) sites in the cancer cell. AP sites are cytotoxic and can induce a breakdown in cell integrity and promote cell apoptosis.

15 Background of the Invention

BER is required for the accurate removal of bases that have been damaged by alkylation, oxidation or ring-saturation. This pathway also handles a variety of other lesions including deaminated bases and DNA single strand breaks.
20 BER is performed by at least two major sub-pathways. These sub-pathways differ from each other in the length of the repair patch and in the subsets of enzymes involved. However, both pathways are initiated by a damage specific DNA glycosylase, which removes the damaged base creating an abasic (AP) site. AP sites are obligatory intermediates in the BER pathway.

25 AP sites are cytotoxic and represent a major threat to the integrity and survival of a cell. Inactivation of APE1 leads to an accumulation of AP sites, retards cell proliferation and activates apoptosis. APE1 is a critical enzyme in the recognition and processing of AP sites in the BER pathway. APE1 accounts
30 for over 95% of the total AP endonuclease activity in human cell lines. AP sites can arise spontaneously at a rate of about 10,000 AP sites per day in each human cell. In addition the activity of DNA glycosylases that process damaged DNA bases generate AP sites that would certainly add to this burden. Indeed, the steady state level of AP lesions is estimated in some

studies to be much higher approaching 50,000 or more per cell depending on age and tissue source. Inactivating APE1 and hence the BER pathway inhibits the ability of the cell to repair itself and thus maximises the cytotoxicity of chemotherapeutic agents which induce genomic DNA damage.

5

Chemotherapy and radiation are the two main treatments currently available to improve outcomes in patients with advanced cancer. The cytotoxicity of many of these agents is directly related to their propensity to induce genomic DNA damage. However, the ability of cancer cells to recognise this damage and initiate DNA repair is an important mechanism for therapeutic resistance that negatively impacts upon therapeutic efficacy. Pharmacological inhibition of the BER pathway of DNA repair, therefore, has the potential to maximise the cytotoxicity of a diverse range of anticancer agents.

10

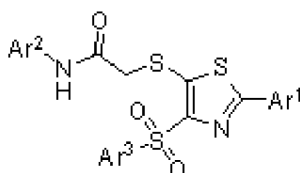
15 **Summary of the Invention**

The present invention is defined by the claims.

According to a first aspect of the present invention there is provided a compound having the structure of Formula 1 and its pharmaceutically acceptable salts:

20

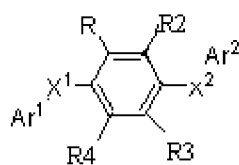
Formula 1



25 wherein Ar₁, Ar₂ and Ar₃ are as defined below.

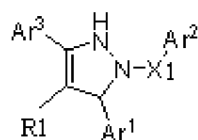
According to a further aspect of the present invention there is provided a method of treating cancer in a patient comprising the step of administering a therapeutically effective amount of a compound of any one of Formulae 1, 2, 3, 4, 5 and 6 or a pharmaceutically acceptable salt thereof.

30

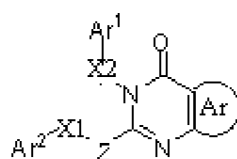


Formula 2

5

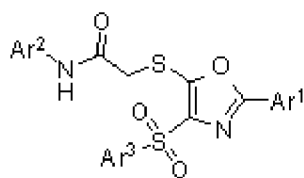


Formula 3

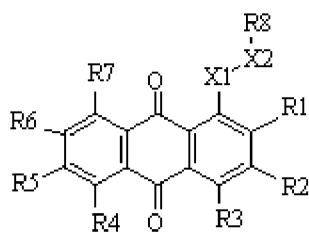


Formula 4

10



Formula 5



15 Formula 6

In the formulae above,

Ar, Ar1, Ar2, Ar3 are each independently aryl or heteroaryl groups which may be substituted or unsubstituted with one or more R9 group.

5

X1 and X2 are each independently alkyl or heteroalkyl groups, typically lower alkyl or heteroalkyl groups comprising a carbon backbone of 1 to 10 carbon atoms optionally including one or more heteroatoms, suitably 1 to 6, more suitably 2 to 4 carbon atoms optionally including one or more heteroatoms.

10 The alkyl or heteroalkyl groups may be mono- or poly- substituted typically with one or more R10 group.

Z is S or NH.

15 R, R1, R2, R3, R4, R5, R6, R7, R8, R9 and R10 are each independently H, alkyl, heteroalkyl, aryl, heteroaryl and combinations of two or more thereof or R11, R11 being selected from O-, N-, NH-, CO, COO, CON, CONH, SO2, SO2N and SO2NH group linking one or more alkyl, heteroalkyl, aryl or heteroaryl group.

20

The alkyl, heteroalkyl, aryl and heteroaryl groups may be substituted or unsubstituted, typically being substituted with one or more halogeno, NH2, NO2, CN, OH, COOH, CONH2, C(=NH)NH2, SO3H, SO2NH2, SO2CH3, OCH3 or CF3 group

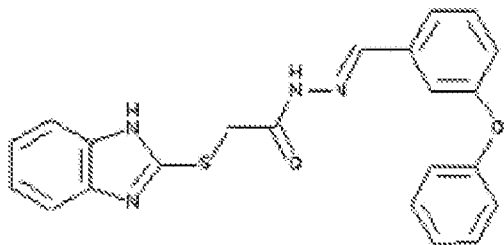
25

Two of R1 to R11 may be linked to form a cyclic group, such as a cycloalkyl, cycloheteroalkyl, polycyclic, cyclic ketone, cyclic ketone, cyclic alcohol, cyclic ester and cyclic ether group.

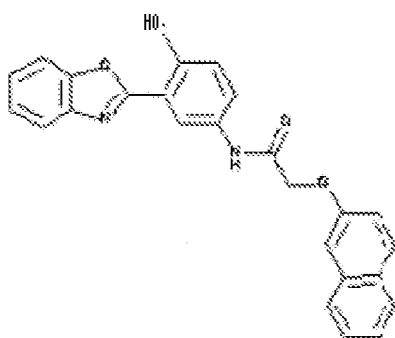
30 Suitable heteroatoms include S, O, N and Se.

According to a further aspect of the present invention there is provided a method of treating cancer in a patient comprising the step of administering a

therapeutically effective amount of a compound of any one of Formulae 7 to 18 or a pharmaceutically acceptable salt thereof.

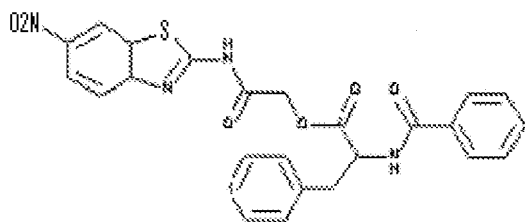


5 Formula 7



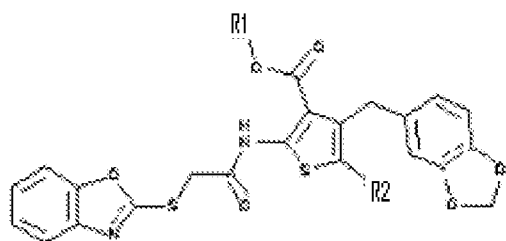
10

Formula 8



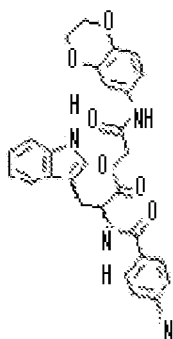
15

Formula 9

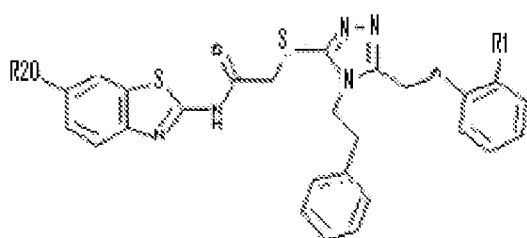


Formula 10

5



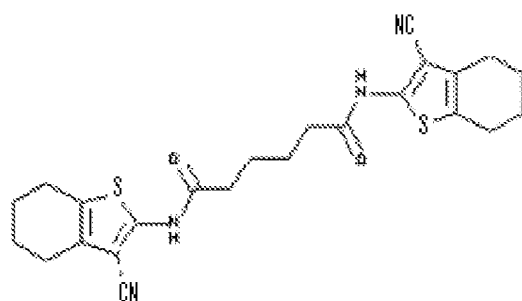
Formula 11



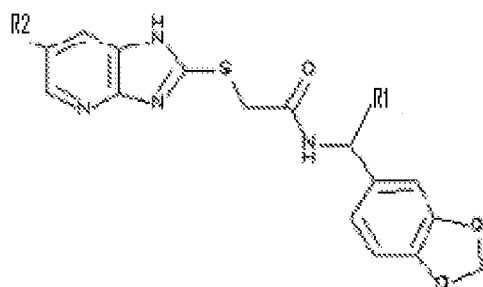
10

Formula 12

15



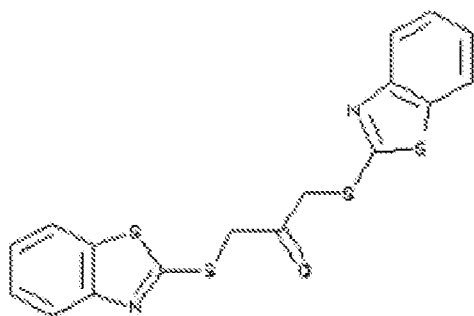
Formula 13



5

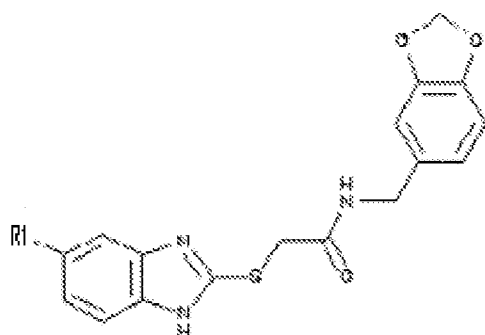
Formula 14

10



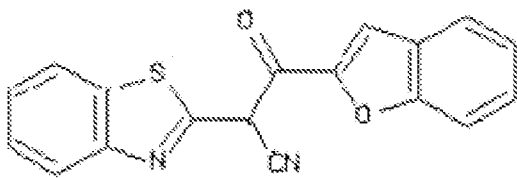
Formula 15

15



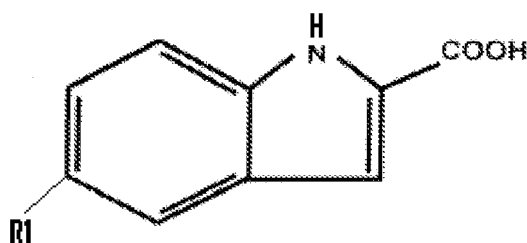
Formula 16

20



Formula 17

5



Formula 18

10

In the formulae above, all groups are defined as above. Formulae 7 to 18 may include one or more substituent, defined as R above. Where heteroatoms are specified in Formulae 7 to 18, these may be the heteroatoms specified, or may be substituted for another heteroatom. Suitable heteroatoms include S,
O, N and Se.

15

In Formula 10, R1 and R2 generally represent alkyl groups, in particular 1 to 5 atom alkyl groups. In particular R1 may represent a methyl group and R2 may represent an ethyl group.

20

In Formula 12, R1 and R2 generally represent alkyl groups, in particular 1 to 5 atom alkyl groups. In particular R1 and R2 may represent methyl groups.

25

In Formula 14, R1 generally represents an alkyl group, in particular a 1 to 5 atom alkyl group. In particular R1 may represent a methyl group. R2 generally represents a halo group, in particular Cl.

In Formula 16, R1 is generally an ether group, in particular MeO.

In Formula 18, R1 generally represents a halo group, in particular F.

5 A further aspect of the present invention relates to a compound of any one of Formulae 1 to 18, or a pharmaceutically acceptable salt thereof, for use in a method for treatment of the human or animal body by therapy.

10 A further aspect of the present invention relates to a compound of any one of Formulae 1 to 18, or a pharmaceutically acceptable salt thereof, for use in the treatment of cancer.

15 A further aspect of the present invention relates to a pharmaceutical composition comprising a compound of any one of Formulae 1 to 18 or a pharmaceutically acceptable salt thereof and one or more diluents, carriers or excipients.

20 A further aspect of the present invention relates to a pharmaceutical composition comprising a compound of any one of Formulae 1 to 18 or a pharmaceutically acceptable salt thereof and one or more diluents, carriers or excipients for use in a method for treatment of the human or animal body by therapy.

25 A further aspect of the present invention relates to a pharmaceutical composition comprising a compound of any one of Formulae 1 to 18 or a pharmaceutically acceptable salt thereof and one or more diluents, carriers or excipients for use in a method for use in the treatment of cancer.

30 A further aspect of the present invention relates to a process for the preparation of a compound of Formulae 1 to 18 as hereinbefore defined.

The present invention also provides a method for increasing, preferably maximising, the cytotoxicity of a chemotherapeutic agent comprising the steps of administering the chemotherapeutic agent and a compound of any one of Formulae 1 to 18

A further aspect of the present invention relates to a compound of any one of Formulae 1 to 18, or a pharmaceutically acceptable salt thereof, in combination with a chemotherapeutic agent for use in a method for treatment of the human or animal body by therapy; preferably, for use in the treatment of cancer; and, more preferably, for use in increasing the cytotoxicity of the chemotherapeutic agent.

Detailed Description

As used herein the term "alkyl" includes both straight chain and branched alkyl groups. The alkyl group may be substituted (mono- or poly-) or unsubstituted. Suitable substituents include, for example, halo, CF₃, OH, CN, NO₂, SO₃H, SO₂NH₂, SO₂Me, NH₂, COOH, CONH₂, alkyl and alkoxy. Typically, the alkyl group is a C1-20 alkyl group, generally a C1-15 alkyl group. The alkyl group may be a C1-12 alkyl group, suitably a C1-6 alkyl group, advantageously a C1-3 alkyl group. Particularly preferred alkyl groups include, for example, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl and hexyl.

As used herein, the term "heteroalkyl" includes an alkyl group as defined above which comprises one or more heteroatoms.

As used herein, the term "cycloalkyl" refers to a cyclic alkyl group which may be substituted (mono- or poly-) or unsubstituted. Suitable substituents include, for example, halo, CF₃, OH, CN, NO₂, SO₃H, SO₂NH₂, SO₂Me, NH₂, COOH, CONH₂ and alkoxy.

Likewise, the term "cycloheteroalkyl" refers to a cyclic heteroalkyl group which may be substituted (mono- or poly-) or unsubstituted. Suitable substituents include, for example, halo, CF₃, OH, CN, NO₂, SO₃H, SO₂NH₂, SO₂Me,

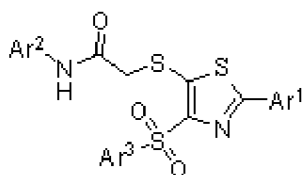
NH₂, COOH, CONH₂ and alkoxy. Preferred cycloheteroalkyl groups include morpholino, piperaziny and piperidiny groups.

As used herein, the term "aryl" refers to an aromatic, substituted (mono- or poly-) or unsubstituted group, and includes, for example, phenyl, naphthyl etc. Again, suitable substituents include, for example, halo, CF₃, OH, CN, NO₂, SO₃H, SO₂NH₂, SO₂Me, NH₂, COOH, CONH₂ and alkoxy.

As used herein, the term "heteroaryl" refers to an aromatic, substituted (mono- or poly-) or unsubstituted group, which comprises one or more heteroatoms. Preferred heteroatoms include N, S, O. Preferred heteroaryl groups include pyrrole, pyrazole, pyrimidine, pyrazine, pyridine, quinoline, triazine, triazole, thiophene, selenazol, thiazole and furan. Again, suitable substituents include, for example, halo, CF₃, OH, CN, NO₂, SO₃H, SO₂NH₂, SO₂Me, NH₂, COOH, CONH₂ and alkoxy.

As used herein the term "halo" or "halogeno" refers to F, Cl, Br or I.

According to an aspect of the present invention there is provided a compound of Formula 1 and its pharmaceutically acceptable salts:



wherein Ar₁, Ar₂ and Ar₃ may each independently represent a substituted or unsubstituted aryl or heteroaryl group. Typically Ar₁, Ar₂ and Ar₃ each independently represent 6-membered aryl rings which may be substituted or unsubstituted. Preferred substituents include one or more halo group, in particular fluoro group and/or one or more alkyl group, typically a lower alkyl group having a carbon backbone of one to six C atoms.

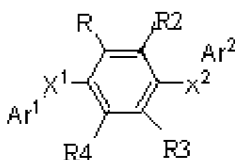
Advantageously Ar₁, Ar₂ and Ar₃ are each independently phenyl groups unsubstituted or substituted with one or more halo or methyl group.

According to one embodiment Ar1 is a toluyyl group, in particular a 4-toluyyl group, Ar2 is a fluorophenyl group, in particular a 4-fluorophenyl group and Ar3 is an unsubstituted phenyl group.

5

According to a further aspect of the present invention there is provided a method of treating cancer in a patient comprising the step of administering a therapeutically effective amount of a compound of Formulae 2 or a pharmaceutically acceptable salt thereof:

10



15 wherein Ar1 and Ar2 are typically each independently aryl or heteroaryl groups which may be substituted or unsubstituted. Typical substituents include one or more halo, COO-, SO2, =O, NO2, ether, amine or alkyl groups (optionally substituted by, for instance, one or more OH, COO- or halo groups).

20

According to one embodiment, Ar1 and Ar2 independently represent a fused multi ring aryl or heteroaryl group, such as a 5- or 6-membered aryl or heteroaryl ring fused to a 5- or 6-membered aryl or heteroaryl ring. Suitable heteroatoms include N, S and O, more suitably N and O. Advantageously Ar1 and Ar2 independently represent a 5- membered aryl or heteroaryl ring fused to a 6-membered aryl or heteroaryl ring. Suitably Ar1 and Ar2 independently represent a 5-membered heteroaryl ring fused to a 6-membered aryl ring.

25

Ar1 and Ar2 may be the same.

Alternatively A1 and/or A2 may represent a 5- or 6- membered aryl group, suitably a 6- membered aryl group, typically substituted with a COO- group.

- 5 According to one embodiment, Ar1 and/or Ar2 represents benzoxazol-2-yl, or benzimidazol-2-yl.

Alternatively Ar1 may represent 3-chlorobenzothien-2-yl and Ar2 may represent 4-benzoate.

10

- X1 and X2 are independently alkyl or heteroalkyl linker groups having a backbone of 1 to 10 atoms, typically 1 to 6 atoms. According to one embodiment X1 and X2 have a backbone of 2 atoms. Typical heteroatoms include S, O and N. The alkyl or heteroalkyl groups may be substituted or
15 unsubstituted. Suitable substituents include =O, OH, halo, amine, ether, alkyl.

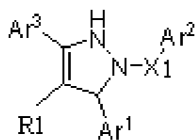
X1 and X2 may be the same.

- According to an aspect of the present invention X1 and/or X2 represent
20 CH₂S. Alternatively X1 may represent C(=O) and X2 may represent CH=N.

According to one embodiment, R1, R2, R3 and R4 represent H. Alternatively R1 may represent an alkyl or heteroalkyl group, advantageously OMe.

- 25 According to a further aspect of the present invention there is provided a method of treating cancer in a patient comprising the step of administering a therapeutically effective amount of a compound of Formulae 3 or a pharmaceutically acceptable salt thereof:

30



wherein Ar1, Ar2 and Ar3 are typically each independently aryl or heteroaryl groups which may be substituted or unsubstituted. Typical substituents include one or more halo, COO-, SO2, =O, NO2, ether amine or alkyl groups (optionally substituted by for instance one or more OH, COO- or halo groups), in particular methoxy.

Suitably Ar1 and Ar2 independently represent a 5- or 6- membered aryl or heteroaryl group. Suitable heteroatoms include N, S and O, more suitably N and S. According to one embodiment, Ar1 is a phenyl group and Ar2 thienyl or a pyrimidine group. Typically Ar1 and Ar2 are unsubstituted or substituted with one or more =O, NO2 or lower alkyl group.

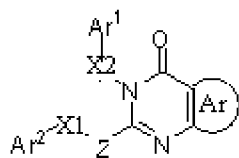
Ar3 may represent a fused multi ring aryl or heteroaryl group, such as a 5- or 6-membered aryl or heteroaryl ring fused to a 5- or 6-membered aryl or heteroaryl ring. Such aryl or heteroaryl groups may be substituted or unsubstituted. According to one embodiment, Ar3 represents a naphthyl group.

X1 represents an alkyl or heteroalkyl linker group having a backbone of 1 to 10 atoms, typically 2 to 6 atoms. Typical heteroatoms include S, O and N, in particular O. The alkyl or heteroalkyl groups may be substituted or unsubstituted. Suitable substituents include =O, OH, halo, amine, ether, alkyl. According to one embodiment X1 represents COCH2OCO.

Typically R1 represents H.

According to a further aspect of the present invention there is provided a method of treating cancer in a patient comprising the step of administering a

therapeutically effective amount of a compound of Formulae 4 or a pharmaceutically acceptable salt thereof:



5

wherein Ar, Ar1 and Ar2 are typically each independently aryl or heteroaryl groups which may be substituted or unsubstituted. Typical substituents include one or more halo, COO-, SO2, =O, NO2, ether, amine or alkyl groups (optionally substituted by for instance one or more OH, COO- or halo groups), in particular methoxy.

Suitably Ar1, Ar2 and Ar3 independently represent a 5- or 6-membered aryl or heteroaryl group. Suitable heteroatoms include N, S and O.

Alternatively Ar1, Ar2 and Ar3 may independently represent a fused multi ring aryl or heteroaryl group, such as a 5- or 6-membered aryl or heteroaryl ring fused to a 5- or 6-membered aryl or heteroaryl ring. Suitable heteroatoms include N, S and O, in particular O. Suitably Ar1, Ar2 and Ar3 independently represent a 5-membered heteroaryl ring fused to a 6-membered aryl ring.

According to one embodiment, Ar1 represents a benzodioxole group, Ar2 represents a pyrazole or a thiazole group and Ar3 represents a benzodioxole or a thiophene group.

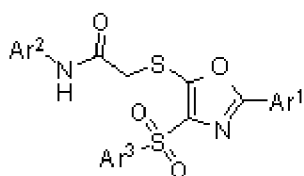
X1 and X2 are independently alkyl or heteroalkyl linker groups having a backbone of 1 to 10 atoms, typically 1 to 6 atoms. Typical heteroatoms include S, O and N, in particular O and N. The alkyl or heteroalkyl groups may

be substituted or unsubstituted. Suitable substituents include =O, OH, halo, amine, ether, alkyl.

According to one embodiment, X1 represents CH₂CONH and X2 represents
5 (CH₂)₃CONHCH₂.

Z represents S or NH, preferably S.

According to a further aspect of the present invention there is provided a
10 method of treating cancer in a patient comprising the step of administering a therapeutically effective amount of a compound of Formulae 5 or a pharmaceutically acceptable salt thereof:



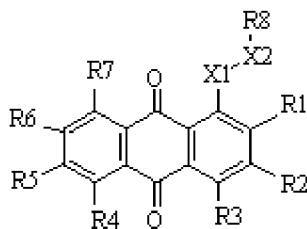
15 wherein Ar₁, Ar₂ and Ar₃ may each independently represent substituted or unsubstituted aryl or heteroaryl group. Typically Ar₁, Ar₂ and Ar₃ independently represent 6-membered aryl rings which may be substituted or unsubstituted. Preferred substituents include one or more halo group, in particular fluoro group and/or one or more alkyl group, typically a lower alkyl
20 group having a carbon backbone of one to six C atoms.

Advantageously Ar₁, Ar₂ and Ar₃ are each independently phenyl groups unsubstituted or substituted with one or more halo or methyl group.

25 According to one embodiment Ar₁ is a toluyl group, in particular a 4-toluyl group, Ar₂ is a fluorophenyl group, in particular a 4-fluorophenyl group and Ar₃ is an unsubstituted phenyl group.

According to a further aspect of the present invention there is provided a
30 method of treating cancer in a patient comprising the step of administering a

therapeutically effective amount of a compound of Formulae 5 or a pharmaceutically acceptable salt thereof:



X1 and X2 are independently alkyl or heteroalkyl linker groups having a backbone of 1 to 10 atoms, typically 1 to 6 atoms. According to one embodiment X1 and X2 have a backbone of 1 to 3 atoms. Typical heteroatoms include S, O and N, in particular N and O. X1 and X2 may be cyclo-alkyl or heteroalkyl groups. The alkyl or heteroalkyl groups may be substituted or unsubstituted. Suitable substituents include =O, OH, halo, amine, ether, alkyl.

According to an aspect of the present invention X1 represents NHC=O , OC=O or C=OO .

X2 may typically represent CH_2S , cyclopropyl, $\text{CH}_2\text{OC=O}$ or CH_2 .

According to one embodiment, R1 to R7 represent H.

R8 typically represents an alkyl, heteroalkyl, aryl or heteroaryl group which may be substituted or unsubstituted. Typical substituents include one or more halo, COO^- , SO_2 , =O, NO_2 , ether, amine or alkyl groups (optionally substituted by for instance one or more OH, COO^- or halo groups), in particular methyl. Suitable heteroatoms include N, S and O, more suitably N.

According to an aspect of the present invention R8 represents a 5-membered heteroaryl group, especially a thiazole group.

According to one embodiment R8 is a 1 to 6 carbon alkyl chain, in particular a methyl, ethyl, propyl or butyl group. In particular R8 is a butyl group.

Alternatively, R8 is a 5- or 6- membered aryl ring, in particular a phenyl group.

R8 may represent a substituted or unsubstituted phenyl group, such as a

5 methoxyphenyl group.

The compounds of Formulae 1 to 18 react with APE1 meaning that APE1 is unable to react with unrepaired AP sites. Through the reaction with the compounds of the present invention the activity of APE1 is greatly inhibited.

10 As noted above, APE1 is a critical enzyme in the BER pathway, and this inactivation of APE1 leads to a corresponding depletion in the repair of DNA through the BER pathway. In addition, the depletion of APE1 leads to an accumulation of unrepaired AP sites. Such AP sites are cytotoxic, and their accumulation is associated with decreased cell viability and increased

15 apoptosis. The cell proliferation is also reduced.

Typically the method of the present invention involves a reduction in the activity of APE1 in a cancerous cell of at least about 50%, suitably at least about 70%, advantageously at least about 90%. Generally the method of the

20 present invention involves a reduction in the activity of APE1 in a cancerous tumour of at least about 50%, suitably at least about 70%, advantageously at least about 90%.

Generally the method of the present invention involves an increase in the

25 amount of AP sites in cancerous cells of at least about 50%, suitably at least about 70%, advantageously at least about 90%.

According to one embodiment, the method of the present invention results in a reduction of the repair of DNA of cancerous cells through the BER mechanism

30 of at least about 50%, generally at least about 70%, preferably at least about 90%. Such a reduction in the repair of cancerous cell DNA through the BER mechanism would result in a reduction of total repair of cancerous cell DNA of at least about 30%.

The method of the present invention may result in an increase in cancer cell apoptosis of about 20% or more. A reduction in cancer cell proliferation of about 10% or more may also be observed.

- 5 The size of cancerous tumours may be reduced by about 10% or more through the method of the present invention.

Advantageously, the method of the present invention results in the effective prevention of the BER mechanism in cancerous cells. According to one
10 embodiment, the method of the present invention results in the effective prevention of the BER mechanism in cancerous tumours.

Preferably, the method of the present invention specifically targets APE1, and does not react with or inhibit any other molecules.

15

Typically the compounds of Formulae 1 to 18 have an IC₅₀ of less than about 30µM; suitably less than about 10µM; preferably less than about 5µM.

Generally the compounds of the present invention have a relatively high
20 aqueous solubility. Typically the compounds of the present invention have a favourable solubility profile with xLogP ranging from about 1.0 to about 5.0 or at least. The compounds of Formulae 1 to 18 have been found to be effective *in vitro*, in whole cell extracts as well as in connection with purified APE1. They are associated with good membrane permeability.

25

The method of the present invention may be used to treat all cancers. However, the method of the present invention is particularly effective in treating cancers associated with an aberrant expression of APE1, typically an elevated expression of APE1. Suitable cancers include breast cancer, lung
30 cancer, in particular non-small cell lung cancer, bone cancer, head-and-neck cancer, ovarian cancer, pancreatic cancer, gastro-oesophageal cancer, melanoma and brain cancer. Moreover the compounds of the present invention may be used in homologous recombination repair deficit, including BRCA-deficient breast and ovarian tumours.

The dosage of compounds required is dependent on the specific compound administered. However, in general the method of the present invention may involve the administration of compounds at less than about 30 μ M, suitably
5 less than about 10 μ M, more suitably less than about 5 μ M.

Typically the method of the present invention involves the administration of the compounds of the present invention at regular intervals, for instance once every week or more, potentially three times a week to daily administrations
10 are envisaged.

More than one compound of the present invention may be administered.

The method of the present invention may involve the administration of one or
15 more chemotherapeutic agent which does not fall into the scope of Formulae 1 to 18. In particular, the method of the present invention may involve the administration of one or more chemotherapeutic agents which cause damage to cancer cell DNA, generally through alkylation, oxidation or ring-saturation of cancer cell DNA, or through instigation of damage such as deamination of
20 DNA bases and DNA single strand breaks.

Surprisingly it has been found that the administration of the compounds of the present invention with such chemotherapeutic agents results in a synergistic increase in cancer cell apoptosis and reduction in cancer cell proliferation.
25

According to one embodiment, the method of the present invention prevents or greatly reduces the proliferation of cancerous cells. Typically the proliferation of cancerous cells is reduced by at least about 10%, generally about 20% or more, preferably about 50% or more. In particular the spread of
30 cancerous cells to different types of tissue is greatly reduced.

As noted above, the method of the present invention inhibits the activity of APE1, and this leads to an increase in the number of AP sites. AP sites are

cytotoxic and their accumulation increases cancer cell apoptosis as well as reducing proliferation of cancer cells.

According to a further aspect of the present invention there is provided the
5 use of a compound of any one of Formulae 1 to 18 in the treatment of cancer.

According to a further aspect of the present invention there is provided the use of a compound of any one of Formulae 1 to 18 in the manufacture of a medicament for the treatment of cancer.

10

According to a further aspect of the present invention there is provided a method of preventing cancer, or preventing the proliferation of cancer involving the administration of one or more compounds of Formulae 1 to 18.

15 The present invention also relates to compounds of Formulae 1 to 18, or pharmaceutically acceptable salts thereof, for use in any of the methods disclosed herein.

The reaction of the compounds of the present invention with APE1 prevents
20 APE1 from reacting with AP sites. This leads to an inhibition of cancer cell DNA repair through the BER pathway resulting in an accumulation of AP sites. Cell proliferation is reduced or prevented accordingly. The rate of apoptosis is also increased accordingly. These effects lead to a decreased chance of cancer cell formation. The progression of the disease is slowed or halted
25 accordingly.

In particular, the risk of cancer progressing to different types of tissue is reduced.

30 According to a further aspect of the present invention there is provided a composition comprising a compound of Formulae 1 to 18.

Typically the composition comprises pharmaceutically acceptable excipients.

The composition may comprise more than one of the compounds of Formulae 1 to 18.

5 The composition may comprise a chemotherapeutic agent which does not fall into the scope of Formulae 1 to 18.

The composition may be in the form of a tablet, a capsule, a solution, a suspension an elixir or any other standard pharmaceutical preparation.

10 A further aspect of the invention provides the compositions comprising a compound of Formulae 1 to 18 for use in any one of the methods disclosed herein.

15 According to a further aspect of the present invention there is provided a method of increasing, preferably maximising, the cytotoxicity of a chemotherapeutic agent comprising the steps of administering a compound of any one of Formulae 1 to 18 and the chemotherapeutic agent.

20 Several known chemotherapeutic agents and ionising radiation act through damaging cancer cell DNA. In particular, through alkylation, oxidation or ring saturation of cancer cell DNA, or through instigation of damage such as deamination o DNA bases and DNA single strand breaks. Such damage is generally repaired through the DNA base excision repair (BER) pathway. The therapeutic activity of such chemotherapeutic agents is necessarily limited by
25 the contemporaneous repair of such damage tthrough the BER mechanism. The compounds of the present invention inactivate APE1, inhibiting its ability to react with AP sites, and thus at least partially blocking the BER pathway. This reduces the rate of repair of DNA damage resulting in an associated increase in the effectiveness of such chemotherapeutic agents.

30

The chemotherapeutic agents are generally those which act through damaging cancer cell DNA, such as alkylating agents (including temozolomide and dacarbazine), bleomycin, gencitabine, 5-fluorouracil and analogues,

platinum compounds (such as cisplatin, oxaliplatin, carboplatin and others), 6-Thioguanine and other nucleoside analogues.

- According to one aspect of the present invention, the method involves the
- 5 steps of administering a compound of any one of Formulae 1 to 18 and one or more of the group consisting of temozolomide, gemcitabine, 5-fluorouracil and analogues thereof, ionizing radiation, platinum compounds and nucleoside analogues.
- 10 The compound of the present invention may be administered concomitantly or sequentially to the chemotherapeutic agent. The compound of the present invention and the chemotherapeutic agent may be in a single dosage form or multiple dosage forms.
- 15 The compound of the present invention is advantageously administered as a single agent therapy in the treatment of breast cancer and ovarian cancer with homologous recombination repair deficit, including BRCA-deficient ovarian and breast cancer tumours.
- 20 Typically the method of the present invention results in an increase in cytotoxicity of known chemotherapeutic agents of about 20% or more, generally about 30% or more, advantageously about 50% or more. Generally there is a correlation in the increase in the number of AP sites and the increase in the cytotoxicity of known chemotherapeutic agents.
- 25 Surprisingly, when the compounds of the present invention are administered together with chemotherapeutic agents that act through damaging cancer cell DNA, a synergistic effect is exhibited, in that the cytotoxicity of the chemotherapeutic agents is surprisingly great and the associated damage to
- 30 cancer cells is surprisingly great.

The present invention will now be described by way of example only with reference to the accompanying Figures in which:

Figures 1a to 1d illustrate the inhibition of APE1 upon contact with the compounds of the present invention;

Figure 2 details the structures of preferred compounds of the present invention;

- 5 Figure 3 details the inhibitory profile of various compounds of the present invention with respect to APE1 and Endonuclease IV.

Example 1

10 **MATERIALS AND METHODS**

Enzymes, oligonucleotides and chemicals Human APE1, uracil-DNA glycosylase and E.coli endonuclease IV were obtained from New England Biolabs. Methyl methane sulfonate (MMS) was purchased from Sigma-Aldrich. Stock solutions of test compounds were dissolved in DMSO. MMS was
15 dissolved in phosphate buffered saline.

The oligonucleotides; 5' F-GCCCCCXGGGGACGTACGATATCCCGCTCC 3' and 3' Q-CGGGGGCCCCCTGCATGCTATAGGGCGAGG 5' [where F = fluorescein, Q = dabcyI and X is 3-hydroxy-2-(hydroxymethyl)-tetrahydrofuran
20 (abasic site analogue)] were custom-made by Eurogentec Ltd. The oligonucleotides for the radiolabeled DNA substrates for HeLa WCE assays – 18FNMR 5'GTCACCGTGFTACGACTC 3'(F = tetrahydrofuran) and 18GNMR 5' GAGTCGTAGCACGGTGAC 3' – were obtained from Trilink Biotechnologies, Inc and Midland certified reagent company, respectively.

25 Potential APE1 inhibitors were purchased from Maybridge Chemicals (Tintagel, UK), ChemBridge Corporation (CA, USA), ASINEX intelligent chemistry (Laan van Vredenoord, Netherlands), Life Chemicals (Braunschweig, GERMANY), Enamine Ltd (Kiev, Ukraine), Specs Chemicals
30 (Delft, Netherlands), ChemDiv Inc. (CA, USA), Ukrorgsynthesis Ltd (Kiev, Ukraine) and Sigma-Aldrich.

The MeWo melanoma cancer cell line was a gift from Dr Andrew Jackson, University of Nottingham, UK. U89MG The glioma cancer cell line was a gift

from Dr Tracey Bradshaw, University of Nottingham, UK. CellTiter 96 [®] AQueous Non-Radiative Cell Proliferation Assay (MTS) Kit was purchased from Promega.

Virtual screening strategy

5

Publicly available coordinates of the high resolution crystal structure of APE1 (PDB accession code 1BIX) , was downloaded from the protein data bank (www.pdb.org). The crystal structure was then visualised using Visual Molecular Dynamics 1.8.6 (VMD1.8.6). Molecular modelling was

10 accomplished using Sybyl8.0, a computational tool kit for molecular design and analyses. Sybyl8.0 was used to build inhibitor templates based on three previously reported APE1 inhibitors: lucanthone:, 1-[[2-(diethylamino)ethyl]amino]-4-methyl-9H-thioxanthen-9-one. In addition, based on the structural features of the active site of APE1, three new

15 pharmacophore templates were designed in silico (M1, M2 and M3). Using these six templates, a shape-based similarity searching strategy using the programme Rapid Overlay of Chemical Structures 2.3 (ROCS 2.3; OpenEye Scientific) was applied to extract pharmacophorically related subsets of compounds from the ZINC database (<http://zinc.docking.org/>; 2008 version

20 with ca. 2.5 million drug-like compounds). A Tanimoto similarity cut-off was set at 0.75 for all queries with the exception of the template M3, where the cut-off was set at 0.6. Hits from each of the six templates were saved in *.sdf format and subjected to docking against the APE1 active site model. The Docking runs were performed using a UNIX supercomputer system. Briefly,

25 the structures were energy minimised and stored in .mol2 format. Gene Optimisation for Ligand Docking 2.7 (GOLD2.7) was used for calculating the docked poses of chemical structures in the APE1 active site pocket. Predicted ligand poses were ranked on the basis of two fitness scoring functions: GOLDScore and ChemScore. One hundred docking runs were performed for

30 each ligand.

Fluorescence-based AP site cleavage assay

A fluorescence based AP site cleavage assay was developed. Briefly, APE1 enzyme (80 ng, New England Biolabs)) was incubated in a buffer system comprising of 50 mM Tris-HCl, pH 8.0, 1 mM MgCl₂, 50 mM NaCl, 2 mM DTT at 37°C for 10 minutes. 5' F-GCCCCCXGGGGACGTACGATATCCCGCTCC 3' and its complementary oligonucleotide 3' Q-CGGGGGCCCCCTGCATGCTATAGGGCGAGG 5' were annealed in a buffer containing 100 µM Tris-HCl, 50 mM NaCl and 1 µM EDTA. AP site cleavage was initiated by addition of the annealed substrate (25 nM) to the reaction mix. Fluorescence readings were taken at 5 minute intervals during a 30 minute incubation at 37°C using an Envision® Multilabel reader from Perkins Elmer with a 495 nM excitation and a 512 nM emission filter. If the DNA is cleaved at the abasic site at position 7 from the 5' end by APE1, the 6-mer fluorescein-containing molecule can dissociate from its complement by thermal melting. As a result, the quenching effect of the 3' dabcyI label (which absorbs fluorescein fluorescence when in close proximity) is lost, and APE1 activity can be measured indirectly as an increase in fluorescence signal. Similar assays were developed for monitoring the AP endonuclease activity of endonuclease IV using a buffering system containing 10 mM HEPES-KOH, pH 7.4, 100 mM KCl and 60 ng of endonuclease IV (Trevigen). The final DMSO concentration was maintained at 1.2% in all assays.

Screening of virtual APE1 inhibitor candidates

APE1 was incubated with the candidate inhibitors at 100 µM prior to initiating the AP site cleavage assay described in the previous section. Those candidates that showed complete or more than 90% inhibition of APE1 activity were subjected to serial dilution experiments for IC₅₀ calculations. In addition, counter screening of potential inhibitors (at 100 µM concentration) was performed using endonuclease IV cleavage assays.

Fluorescence quenching assay

To investigate the possibility that compounds might possess intrinsic quenching activity, fluorescence quenching assays were performed. Briefly,

the oligonucleotides 5'-F-GCCCCCXGGGGACGTACGATATCCCGCTCC-3' and 3'CGGGGGCCCCCTGCATGCTATAGGGCGAGG-5' were annealed as described previously. The double stranded oligonucleotide (5 nM) was incubated with 100 μ M of potential APE1 inhibitors in a buffer consisting of 50 μ M Tris-HCl, Ph 8.0, 1 mM MgCl₂, 50 mM NaCl, and 2 mM DTT at 37°C for 30 minutes. Fluorescence intensity was measured every 5 minutes. Any hits that showed a decrease of more than 50% in the fluorescence intensity were considered as quenchers.

10 HeLa whole cell extract AP site cleavage assay

HeLa cells, maintained in DMEM with 10% fetal bovine serum and 1% penicillin-streptomycin, were harvested, washed with PBS, resuspended in cold 222 mM KCl plus protease inhibitors (0.5 mM PMSF, 1 μ g/mL each of leupeptin and pepstatin A) incubated on ice for 30 min, and clarified by centrifugation at 12,000 x g for 15 min at 4°C. The supernatant (whole cell extract) was retained, the protein concentration determined using the Bio-Rad Bradford reagent, and aliquots were stored at -80°C. AP endonuclease activity assays using radiolabelled oligonucleotide substrates (see above) were performed. In brief, potential APE1 inhibitors identified by the fluorescence assay were incubated at 100 μ M concentrations with 30 ng of HeLa whole cell extract at room temperature for 15 min in incision buffer consisting of 50 mM Tris-HCl pH 8, 1 mM MgCl₂, 50 mM NaCl, and 2mM DTT. After incubation, 0.5 pmol 32P-radiolabeled tetrahydrofuran-containing 18mer double-stranded DNA was added. Incision reactions were then carried out immediately at 37 °C for 5 min in a final volume of 10 μ L, after which the reaction was terminated by the addition of an equal volume of stop buffer (0.05% bromophenol blue and xylene cynol, 20 mM EDTA, 95% formamide) followed by denaturation of samples at 95°C for 10 min. The radiolabelled substrate and product were separated on a standard polyacrylamide denaturing gel and quantified by phosphorimager analysis.

Cell lines and culture media

MeWo melanoma cancer cell line was grown in RPMI culture medium [supplemented with penicillin 0.06 g/L, streptomycin 0.1 g/L pH 7.0, 10%

foetal bovine serum (FBS, PAA Cell Culture Company). U89MG was grown in DMEM culture medium [supplemented with penicillin 0.06 g/L, streptomycin 0.1 g/L, pH 7.0, 10% foetal bovine serum (FBS, PAA Cell Culture Company). Only cultures with a plating efficiency of over 70% were used for the following

5 analysis.

96[®] AQueous Non-Radioactive Cell Proliferation Assay

To evaluate intrinsic cytotoxicity and to evaluate the potentiation of cytotoxicity of alkylating agents by APE1 inhibitors, CellTiter 96[®] AQueous Non-

10 Radioactive Cell Proliferation Assays (MTS assay) were performed as per manufacturer's recommendation (Promega). Briefly, 2,000 cells per well (in 200 μ L of medium) were seeded into a 96-well plate. For intrinsic cytotoxicity assessments, cells were incubated with varying concentrations of APE1 inhibitors and MTS assay was performed on day 5. For potentiation
15 experiments, cells were pre-incubated with APE1 inhibitor for 24 hours and then exposed to alkylating agents (temozolomide or MMS) and MTS assay was conducted as described previously

RESULTS

Virtual screening

The publicly available coordinates of the high resolution crystal structure of APE1 (1BIX) were

downloaded from the protein data bank and the DNA repair domain localised based on the previously reported ten critical amino acid residues that are essential for the AP endonuclease activity of APE1 (D70, D90, E96, Y171, D210, N212, D219, D283, D308, and H309). The active site of APE1 was well defined and the Mg²⁺ ion involved in the APE1 catalytic mechanism was observed to occupy the centre of the cleft.

Three new pharmacophore templates (M1, M2 and M3) were designed and visualised on the APE1 model. The structures were then energy minimised and subjected to docking against the active site of the APE1 model. Predicted ligand poses were ranked on the basis of two fitness scoring functions: GOLDScore and ChemScore. One hundred docking runs were performed for each ligand and the docking programme was set to assign the best fitness score in a total of hundred runs.

Shape-based similarity searching was applied to extract pharmacophorically related subsets of compounds from the ZINC database of commercially available small molecule compounds. A total of 2,533 virtual hits with similarities to the templates were identified (C= 359, A=371, L= 485, M1 = 373, M2 = 459, M3=488). The conformations of these compounds were then energy minimised and subjected to docking against the active site of the APE1 model. The top ranking 25% of the compounds with favourable GOLDScore and ChemScores were shortlisted for further analyses.

Presented below are in silico, biochemical, and cytotoxicity analyses of five representative compounds as described below. 5-Fluoro-1H-indole-2-carboxylic acid (compound 1) belongs to the "CRT0044876 (C)" template (see Figure 2).

The compounds N-(3-benzooxazol-2-yl-4-hydroxy-phenyl)-2-(2-naphthyloxy)acetamide (compound 2), [3-(2-naphthyl)-5-phenyl-2,5-dihydropyrazol-1-yl]carbonylmethyl 5-nitrothiophene-2-carboxylate (compound 3) and N-(4-fluorophenyl)-2-[4-phenylsulfonyl-2-(p-tolyl)oxazol-5-yl]sulfanylacetamide (compound 4) belong to the M3 template (see Figure 2) and (9,10-dioxo-1-anthryl)carbamoymethyl pentanoate (compound 5) belongs to the "lucanthone (L)" template (see Figure 2).

Biochemical screening (Figure 3)

Compounds 1-5 were tested in the fluorescence APE1 cleavage assay. CRT0044876, our previously described inhibitor, was used as a positive control in these studies. The IC₅₀ for APE1 inhibition ranged between 3 μ M to 26 μ M. We then counter-screened the compounds against endonuclease IV, an E.coli orthologue of APE1 that perform AP site cleavage in a way similar to APE1 but has a structurally different active site. We found that these compounds had no inhibitory activity against endonuclease IV implying that they are specific to the exonuclease III family of AP endonucleases to which APE1 belongs. We then tested if the compounds possessed any intrinsic fluorescence quenching activity, which was not the case. We next confirmed APE1 inhibition in a radiolabelled oligonucleotide assay. In order to determine potency, the compounds were tested in the HeLa whole cell extract assay. Compounds 1, 2, and 4 showed significant inhibition of AP site cleavage.

Cytotoxicity analysis

In order to test the biological activity of APE1 inhibitors under physiological conditions, survival analysis was undertaken in MeWo melanoma and U89MG glioma cell lines. Initially compounds were tested for their inherent toxicity. Whilst compound 1 was non-toxic at up to 100 μ M, the GI₅₀ (cell growth inhibition) ranged between 400 nM and 50 μ M for other APE1 inhibitors. We then investigated if our newly identified inhibitors at relatively non-toxic concentrations would potentiate the cytotoxicity of MMS. Compound 4 (at 20 μ M)

significantly potentiated the cytotoxicity of MMS in the MeWo melanoma cell line. Similar results were also observed in the U89MG glioma cell line.

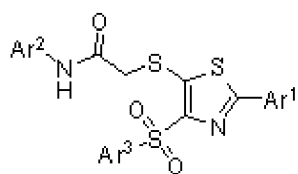
Example 2

The compounds summarised in Figure 2 were prepared in accordance with the methods summarised above. Their specificity for the inhibition of AP endonuclease was tested and is detailed in Figure 3.

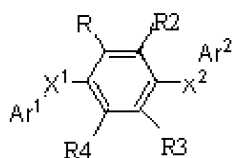
While the present invention has been illustrated by the description of one or more embodiments thereof, and while the embodiments have been described in considerable detail, they are not intended to restrict or in any way limit the scope of the appended claims to such detail. Additional advantages and modifications will readily appear to those skilled in the art. The invention in its broader aspects is therefore not limited to the specific details, representative apparatus and method and illustrative examples shown and described. Accordingly, departures may be made from such details without departing from the scope or spirit of the general inventive concept.

What is claimed:

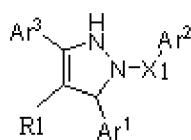
1. A compound with the structural formula



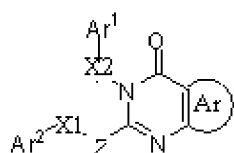
Formula 1



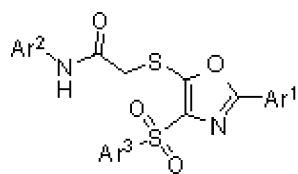
Formula 2



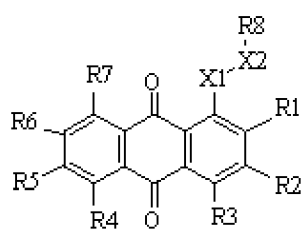
Formula 3



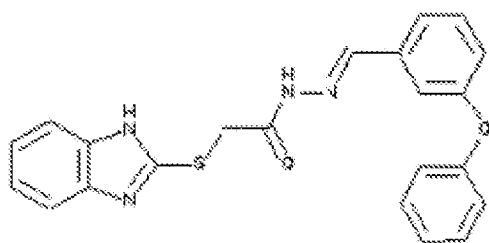
Formula 4



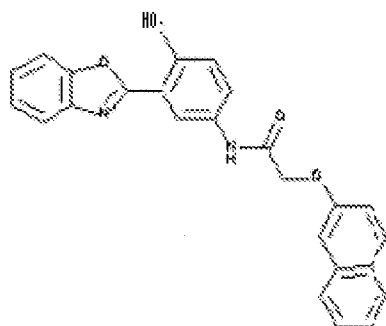
Formula 5



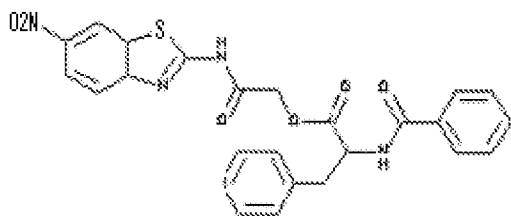
Formula 6



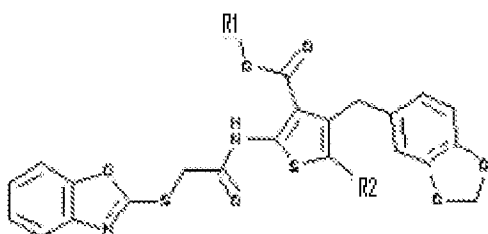
Formula 7



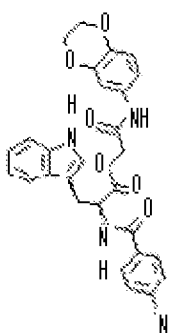
Formula 8



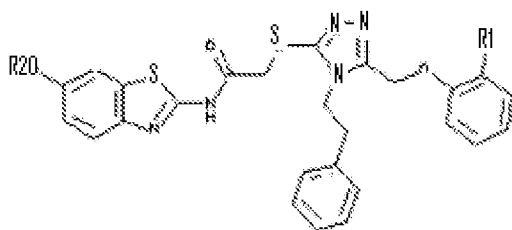
Formula 9



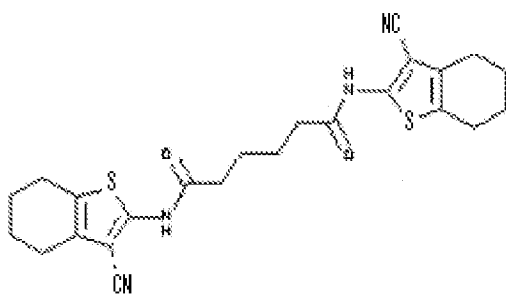
Formula 10



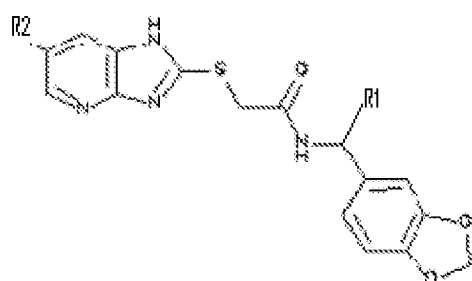
Formula 11



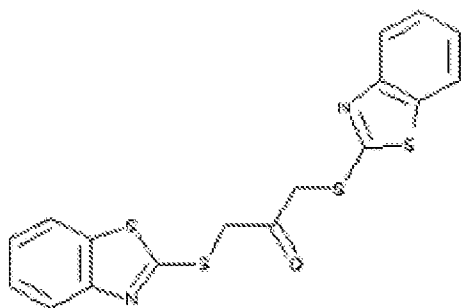
Formula 12



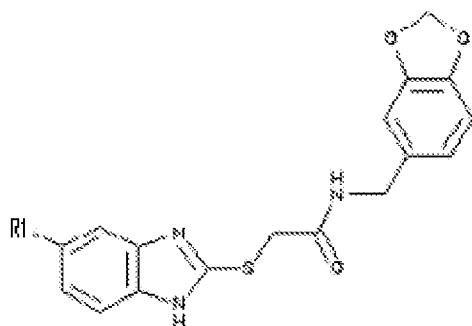
Formula 13



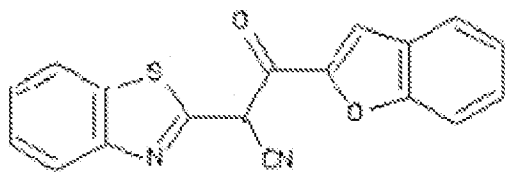
Formula 14



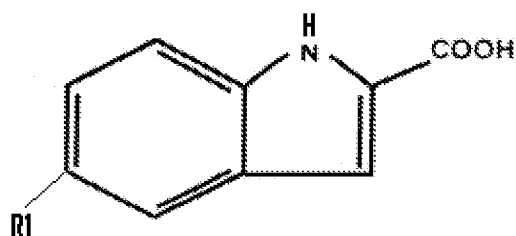
Formula 15



Formula 16



Formula 17 or



Formula 18

or a pharmaceutically acceptable salt thereof;

wherein Ar, Ar1, Ar2, Ar3 are each independently aryl or heteroaryl groups which may be substituted or unsubstituted with one or more R9 group;

wherein X1 and X2 are each independently alkyl or heteroalkyl groups;

wherein Z is S or NH;

wherein R, R1, R2, R3, R4, R5, R6, R7, R8, R9 and R10 are each independently H, alkyl, heteroalkyl, aryl, heteroaryl and combinations of two or more thereof or R11, R11 being selected from O-, N-, NH-, CO, COO, CON, CONH, SO2, SO2N and SO2NH group linking one or more alkyl, heteroalkyl, aryl or heteroaryl group; and

wherein alkyl, heteroalkyl, aryl and heteroaryl groups may be substituted or unsubstituted,

2. A compound according to claim 1 for use in a method for treatment of the human or animal body by therapy.
3. A compound according to claim 1 for use in a method of treating cancer.
4. A compound according to claim 1 for use in a method for increasing the cytotoxicity of a chemotherapeutic agent comprising the steps of administering the chemotherapeutic agent and said compound.
5. A compound according to claim 4 wherein said compound is administered concomitantly or sequentially to the chemotherapeutic agent.
6. A compound according to claim 1 for use in a method of treating cancer wherein said compound is administered in combination with a treatment comprising the use of ionizing radiation.
7. A pharmaceutical composition comprising at least one compound according to claim 1 and one or more diluents, carriers or excipients.
8. The composition according to claim 7 further comprising a chemotherapeutic agent which does not fall into the scope of Formulae 1 to 18.
9. The composition according to claim 8 or the compound according to claim 4 wherein the the chemotherapeutic agents in use damages cancer cell DNA, preferably through alkylation, oxidation or ring saturation.
10. The composition according to claim 8 or the compound according to claim 4 wherein the chemotherapeutic agent is selected from the group consisting of

alkylating agents, including temozolomide and dacarbazine; bleomycin; gencitabine; 5-fluorouracil and analogues; platinum compounds, such as cisplatin, oxaliplatin and carboplati; and 6-Thioguanine.

11. The compound according to claim 4 or 5 or composition according to claim 8 to 10 wherein said compound and said chemotherapeutic agent are in a single dosage form or multiple dosage form.

12. The composition according to any of claims 7 to 11 in the form of a tablet, a capsule, a solution, a suspension, an elixir or any other standard pharmaceutical preparation.

13. The composition according to any of claim 7 to 12 for use in a method for treatment of the human or animal body by therapy.

14. The composition according to any of claim 7 to 12 for use in a method of treating cancer.

15. The compound according to claim 3 or composition according to claim 14 wherein said cancer comprises an aberrant expression of APE1, typically an elevated expression of APE1.

16. The compound according to claim 3 or 15 or composition according to claim 14 or wherein said cancer is selected from the group consisting of breast cancer; lung cancer, in particular non-small cell lung cancer; bone cancer; head-and-neck cancer; ovarian cancer; pancreatic cancer; gastro-oesophageal cancer; melanoma; and brain cancer.

17. The compound according to claim 1 or composition according to claim 7 to 12 for use in the treatment of homologous recombination repair deficit; including BRCA-deficient breast and ovarian tumours.

18. A method for treating cancer in a patient comprising the step of administering a therapeutically effective amount of a compound according to claim 1.

19. A compound, composition or method according to any preceding claim wherein alkyl, heteroalkyl, aryl and heteroaryl groups are substituted with one or more halogeno, NH₂, NO₂, CN, OH, COOH, CONH₂, C(=NH)NH₂, SO₃H, SO₂NH₂, SO₂CH₃, OCH₃ or CF₃ group;

20. A compound, composition or method according to any preceding claim wherein two of R₁ to R₁₁ may be linked to form a cyclic group, such as a cycloalkyl, cycloheteroalkyl, polycyclic, cyclic ketone, cyclic ketone, cyclic alcohol, cyclic ester and cyclic ether group.

2nd Generation APE1 inhibitors (IC₅₀ 500nM - 20µM)

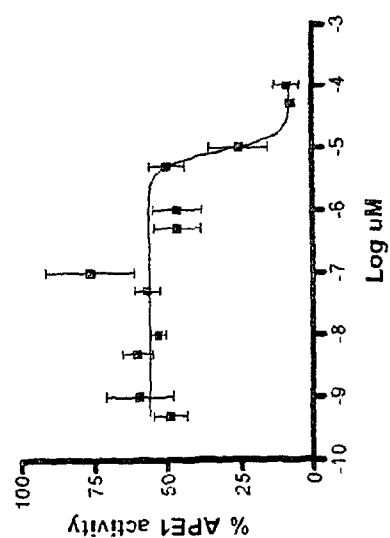


Fig 1a

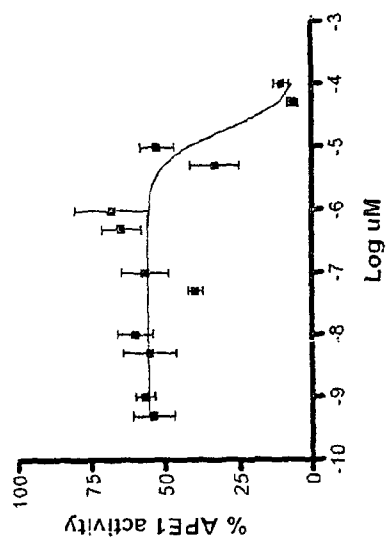


Fig 1b

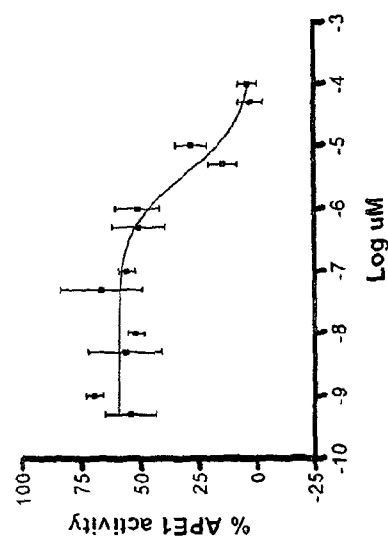


Fig 1c

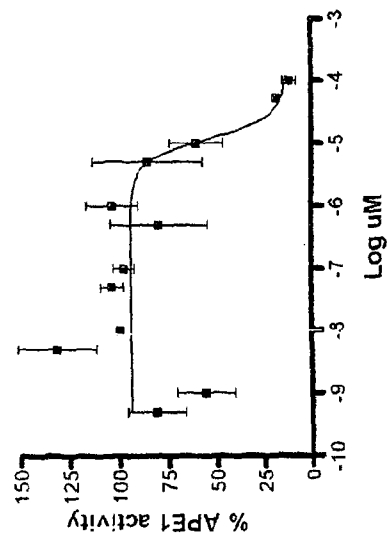
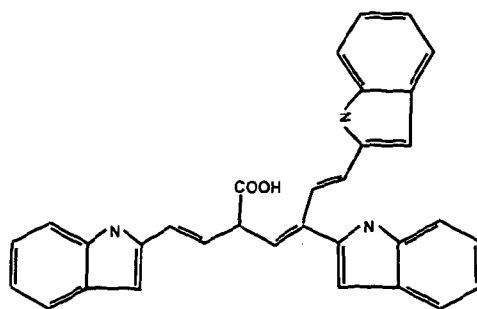


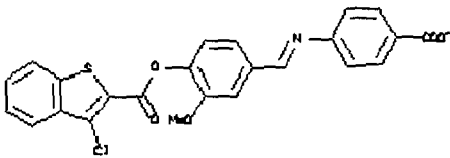
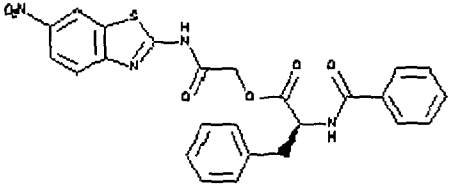
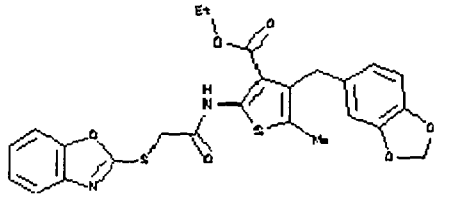
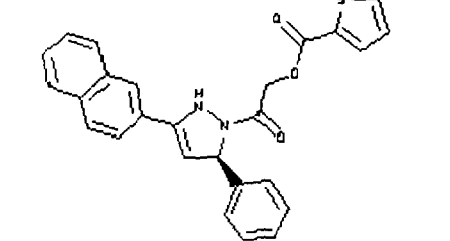
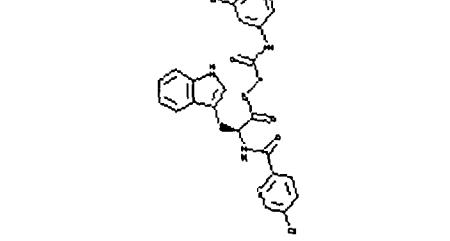
Fig 1d

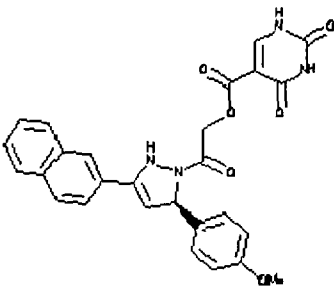
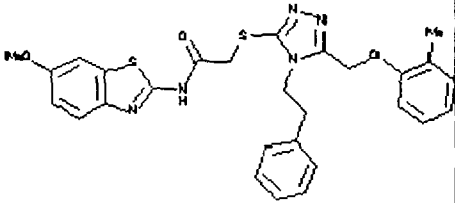
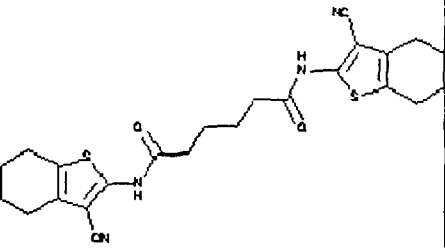
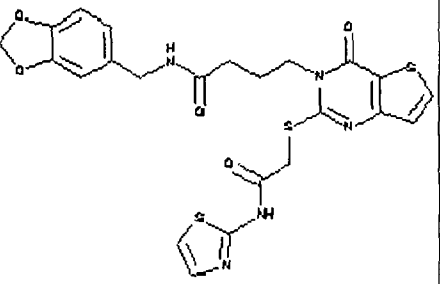
Figure 2.

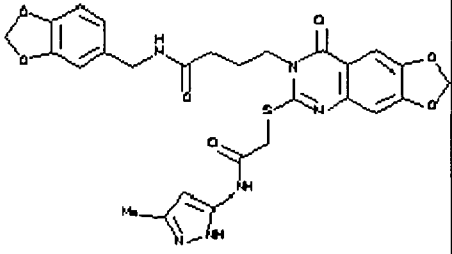
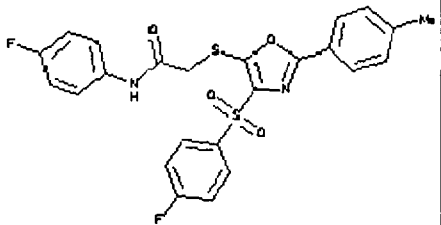
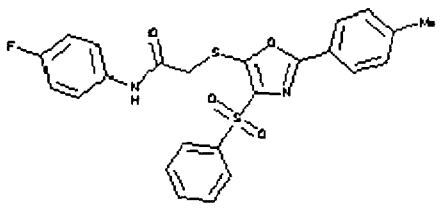
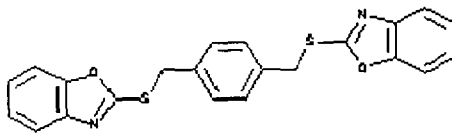
M3 Template Hits.

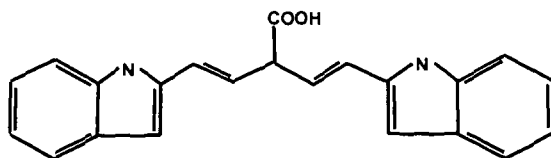
M3 template

<u>ZINC ID</u> #P.I codes Specificity for inhibition of AP endonucleasae	Properties: xLogP, ap & p desolvation, HBD, HBA, Charge, Mwt, NRB Annotations; Similarity Chemical name	Structure Click for quick 3D display
<u>1211006</u> #4 Specific	ref: 5.15,-2.39,-14.76,2,6,0, 402.479, 7 2-(1H-benzoimidazol-2-ylsulfanyl)-N-[(3-phenoxyphenyl)methyleneamino]acetamide IC₅₀ = 0.4uM	
<u>1215578</u> #46 Specific	ref: 5.40,-2.66,-25.72,2,6,0, 410.429, 5 N-(3-benzooxazol-2-yl-4-hydroxy-phenyl)-2-(2-naphthyloxy)acetamide IC₅₀ = 25uM	
<u>1102617</u> #65 Specific	ref: 5.95,-3.58,-12.33,2,4,0, 402.548, 6 2-[[4-(1H-benzoimidazol-2-ylsulfanylmethyl)phenyl]methylsulfanyl]-1H-benzimidazole IC₅₀ = 1.6uM	

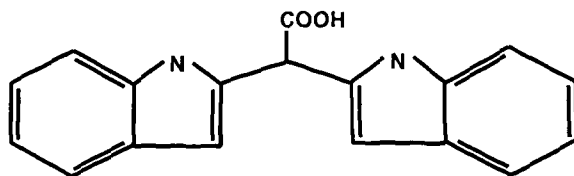
<u>2903501</u> #73 Specific	ref: 6.31,1.49,-58.49,0,6,-1, 464.906, 7 4-[[4-(3-chlorobenzothiophen-2-yl)carbonyloxy-3-methoxy-phenyl]methyleneamino]benzoic acid IC₅₀ >100uM	
<u>3434925</u> #88 Non-Specific	ref: 4.08,-0.63,-19.7,2,10,0, 504.524, 10 (6-nitrobenzothiazol-2-yl)carbamoymethyl 2-benzoylamino-3-phenyl-propanoate IC₅₀ = 0.7uM	
<u>3257904</u> #91 Non-Specific	ref: 5.17,-2.26,-16.37,1,8,0, 510.593, 9 ethyl 4-(benzo[1,3]dioxol-5-ylmethyl)-2-(2-benzooxazol-2-ylsulfanylacetyl)amino-5-methylthiophene-3-carboxylate IC₅₀ = 3.2uM	
<u>3277194</u> #93 Specific	ref: 5.33,1.32,-14.03,1,8,0, 485.521, 7 [3-(2-naphthyl)-5-phenyl-2,5-dihydropyrazol-1-yl]carbonylmethyl 5-nitrothiophene-2-carboxylate IC₅₀ = 3uM	
<u>2649134</u> #95 Specific	ref: 3.92,-1.93,-16.81,3,9,0, 533.968, 9 2,5-dioxabicyclo[4.4.0]deca-7,9,11-trien-8-ylcarbamoymethyl 2-(4-chlorobenzoyl)amino-3-(1H-indol-3-yl)propanoate IC₅₀ = 0.5uM	

<u>2638090</u> #96 Specific	ref: 2.55,-1.42,-26.01,3,10,0, 498.495, 7 [5-(4-methoxyphenyl)-3-(2-naphthyl)-2,5-dihydropyrazol-1-yl]carbonylmethyl 2,4-dioxo-1H-pyrimidine-5-carboxylate IC₅₀ = 0.5uM	
<u>904848</u> #101 Specific	ref: 5.73,-0.57,-18.85,1,8,0, 545.69, 11 N-(6-methoxybenzothiazol-2-yl)-2-[[5-[(2-methylphenoxy)methyl]-4-phenethyl-1,2,4-triazol-3-yl]sulfanyl]acetamide IC₅₀ = 4uM	
<u>4195895</u> #103 Non-Specific	ref: 4.75,0.1,-13.43,2,6,0, 466.632, 7 N,N'-bis(3-cyano-4,5,6,7-tetrahydrobenzothiophen-2-yl)hexanediamide IC₅₀ =25uM	
<u>2925395</u> #115 Specific	ref: 2.52,-5.41,-28.67,2,10,0, 543.652, 10 N-(benzo[1,3]dioxol-5-ylmethyl)-4-[2-oxo-4-(thiazol-2-yl)carbamoylmethylsulfanyl]-9-thia-3,5-diazabicyclo[4.3.0]nona-4,7,10-trien-3-yl]-butanamide IC₅₀ = 12.5uM	

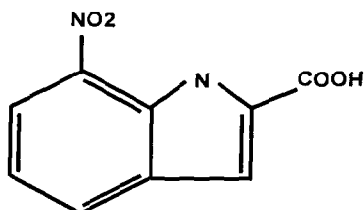
<u>2975647</u> #116 Specific	ref: 2.30,-6.18,-25.6,3,13,0, 578.607, 10 N-(benzo[1,3]dioxol-5-ylmethyl)-4-[(5-methyl-2H-pyrazol-3-yl)carbamoylmethylsulfanyl-oxo-BLAHyl]-butanamide IC₅₀ = 15uM	
<u>2721681</u> #117 Non-Specific	ref: 5.58,-3.6,-18.17,1,6,0, 500.548, 7 N-(4-fluorophenyl)-2-[4-(4-fluorophenyl)sulfonyl-2-(p-tolyl)oxazol-5-yl]sulfanyl-acetamide IC₅₀ = 10uM	
<u>2721656</u> #118 Specific	ref: 5.41,-4.34,-20.29,1,6,0, 482.558, 7 N-(4-fluorophenyl)-2-[4-phenylsulfonyl-2-(p-tolyl)oxazol-5-yl]sulfanyl-acetamide IC₅₀ = 11.6uM	
<u>2322284</u> #126 Specific	ref: 6.15,-3.82,-15.48,0,4,0, 404.516, 6 2-[[4-(benzooxazol-2-ylsulfanylmethyl)phenyl]methylsulfanyl]benzooxazole IC₅₀ = 15uM	

M2 Template Hits

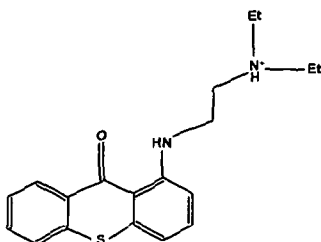
<u>ZINC ID</u> #P.I codes Specificity for inhibition of AP endonucleasae	Properties:xLogP, ap & p desolvation, HBD,HBA,Charge,Mwt,NRB Annotations; Similarity Cemical name	Structure Click for quick 3D display
<u>9367024</u> #97 Non-Specific	ref: 3.23,5.91,-14.43,2,7,0,390.852, 5 N-[(1S)-1-(1,3-benzodioxol-5-yl)ethyl]-2-[(6-chloro-1H-imidazo[4,5-b]pyridin-2-yl)sulfanyl]acetamide IC₅₀ = 15uM	
<u>1029672</u> #111 Specific	ref: 5.04,9.62,-17.55,0,3,0,388.564, 6 1,3-bis(1,3-benzothiazol-2-ylthio)acetone IC₅₀ = 3uM	
<u>8685966</u> #125 Specific	ref: 2.78,-4.23,-14.46,2,7,0,371.418, 6 N-(benzo[1,3]dioxol-5-ylmethyl)-2-[(5-methoxy-1H-benzimidazol-2-yl)sulfanyl]acetamide IC₅₀ = 3uM	

M1 Template hits

<u>ZINC ID</u> #P.I codes Specificity for inhibition of AP endonucleasae	Properties:xLogP, ap & p desolvation, HBD,HBA,Charge,Mwt,NRB Annotations; Similarity Cemical name	Structure Click for quick 3D display
<u>3196143</u> #34 Non-Specific	ref: 3.57,1.45,-11.11,0,4,0, 318.357, 3 3-benzofuran-2-yl-2-benzothiazol-2-yl-3-oxo-propanenitrile IC₅₀ = 2.5uM	<chem>O=C(O=C(c1c[nH]c2ccccc12)C#N)c3cc4ccccc4o3</chem>

CRT0044876 Template hits

<u>ZINC ID</u> #P.I codes Specificity for inhibition of AP endonucleasae	Properties:xLogP, ap & p desolvation, HBD,HBA,Charge,Mwt,NRB Annotations; Similarity Cemical name	Structure Click for quick 3D display
<u>83028</u> #22 Specific	ref: 1.14,4.07,-44.07,1,3,-1, 178.142, 1 5-fluoro-1H-indole-2-carboxylic acid IC₅₀ =8.3uM	<chem>O=C(O)c1c[nH]c2cc(F)ccc12</chem>

Lucanthone Template hits

ZINC ID #P.I codes Specificity for inhibition of AP endonucleasae	Properties: xLogP, ap & p desolvation, HBD,HBA,Charge,Mwt,NRB Annotations; Similarity Chemical name	Structure Click for quick 3D display
<u>4977184</u> #30 Specific	ref: 2.80,-0.43,-14.37,2,7,0, 364.386, 4 N-(9,10-dioxo-1-anthryl)-2-(4H-1,2,4-triazol-3-ylsulfanyl)acetamide IC₅₀ = 4uM	
<u>5003653</u> #69 Non-Specific	ref: 4.90,1.92,-14.93,1,5,0, 384.387, 4 (8-hydroxy-9,10-dioxo-1-anthryl) 2-phenylcyclopropane-1-carboxylate IC₅₀ = 1.6uM	
<u>5781327</u> #128 Specific	ref: 4.30,2.19,-11.74,1,6,0, 365.385, 7 (9,10-dioxo-1-anthryl)carbamoylmethyl pentanoate IC₅₀ = 16uM	

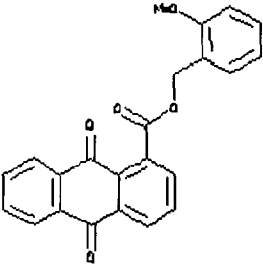
<u>6428148</u> #133 Specific	ref: 5.06,3,-16.07,0,5,0, 372.376, 5 2-methoxyphenyl)methyl 9,10-dioxoanthracene-1- carboxylate IC₅₀ = 3uM	
---	---	--

Figure 3. Inhibitory profile for compounds 1-4. (-) = no inhibition, (+) = inhibition

APE1 inhibitors	APE1 (IC₅₀)	Endonuclease IV	Quencher	WCE	xLogP	Mol.Wt
Compound 1	10µM	-	-	+	1.14	178.14
Compound 2	25µM	-	-	+	5.4	410.43
Compound 3	3µM	-	-	+	5.33	485.52
Compound 4	20µM	-	-	+	5.41	482.56
Compound 5	16µM	-	-	+	4.3	365.39