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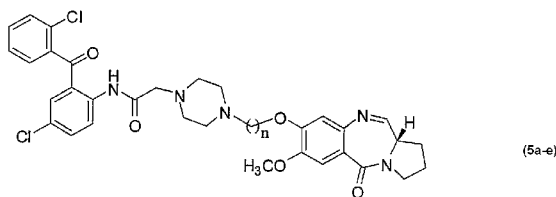
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(54) **Title:** BENZOPHENONE-PIPERAZINE LINKED PYRROLO[2,1-C][1,4]BENZODIAZEPINE HYBRIDS AS POTENTIAL ANTICANCER AGENTS AND PROCESS FOR THE PREPARATION THEREOF

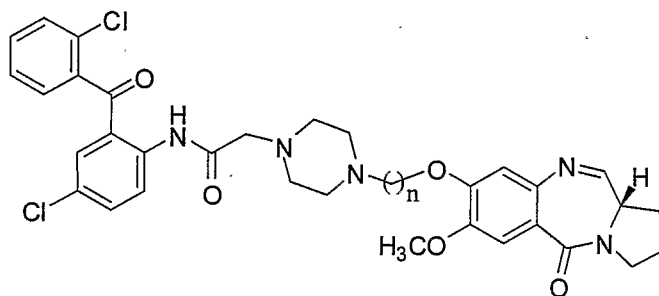


n = 3, 4, 5, 6 or 8

(57) **Abstract:** The present invention provides benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrids of general formula 5a-e, useful as potential antitumour agents against human cancer cell lines. The present invention further provides a process for the preparation of pyrrolo[2,1-c][1,4]benzodiazepine hybrids of general formula (5a-e).

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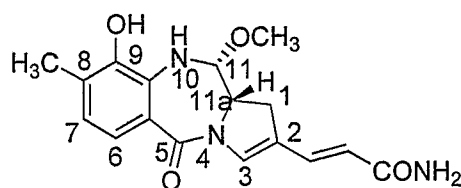
The present invention relates to benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrids and a process for the preparation thereof. More particularly, it relates to 7-Methoxy-8-[n-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazino-acetamide}alkoxy]-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4] benzodiazepine-5-one with aliphatic chain length variations useful as anticancer (antitumour) agent. The structural formula of these benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrids is given below.



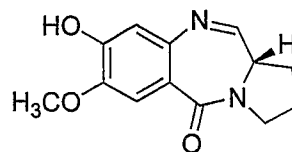
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Pyrrolo[2,1-c][1,4]benzodiazepine antitumour antibiotics are commonly known as anthramycin class of compounds. In the last few years, a growing interest has been shown in the development of new pyrrolo [2,1-c][1,4]benzodiazepines (PBDs). These antibiotics react covalently with DNA to form an N2-guanine adduct that lies within the minor groove of duplex DNA *via* an acid-labile aminal bond to the electrophilic imine at the N10-C11 position (Kunimoto, S.; Masuda, T.; Kanbayashi, N.; Hamada, M.; Naganawa, H.; Miyamoto, M.; Takeuchi, T.; Unezawa, H. *J. Antibiot.*, **1980**, 33, 665.; Kohn, K. W. and Speous, C. L. *J. Mol. Biol.*, **1970**, 51, 551.; Hurley, L. H.; Gairpla, C. and Zmijewski, M. *Biochem. Biophys. Acta.*, **1977**, 475, 521.; Kaplan, D. J. and Hurley, L. H. *Biochemistry*, **1981**, 20, 7572). The molecules have a right-handed twist, which allows them to follow the curvature of the minor groove of B-form double-stranded DNA spanning three base pairs. A recent development has been the linking of two PBD units through their C-8 positions to give bisfunctional-alkylating agents capable of cross-linking DNA (Thurston, D. E.; Bose, D. S.; Thomson, A.

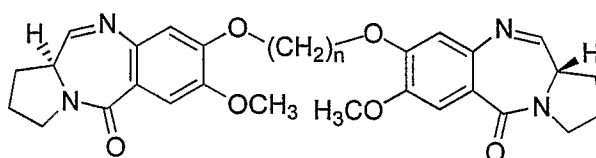
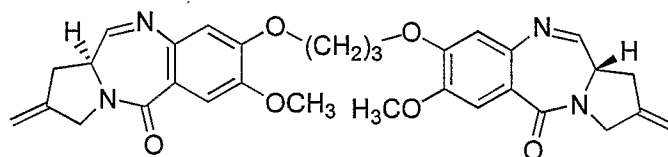
S.; Howard, P. W.; Leoni, A.; Croker, S. J.; Jenkins, T. C.; Neidle, S. and Hurley, L. H. *J. Org. Chem.* **1996**, 61, 8141).



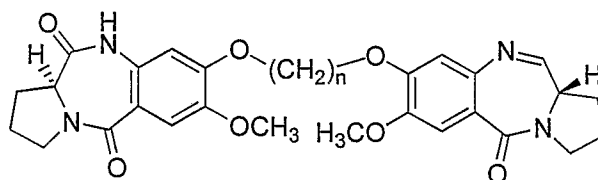
anthramycin



DC-81

DC-81 dimers ($n = 3-5$); DSB-120 ($n = 3$)

SJG-136

imine-amide PBD dimers; $n = 3-5$

- 5 Recently, PBD dimers have been developed that comprise of two C2-exo-methylene substituted DC-81 subunits tethered through their C-8 position *via* an inert propanedioxy linker (Gregson, S. J.; Howard, P. W.; Hartely, J. A.; Brooks, N. A.; Adams, L. J.; Jenkins, T. C.; Kelland, L. R. and Thurston, D. E. *J. Med. Chem.* **2001**, 44, 737). A non-cross-linking mixed imine-amide PBD dimers have been synthesized that have significant DNA binding
- 10 ability and potent antitumour activity (Kamal, A.; Ramesh, G. Laxman, N.; Ramulu, P.; Srinivas, O.; Neelima, K.; Kondapi, A. K.; Srinu, V. B.; Nagarajaram, H. M. *J. Med. Chem.* **2002**, 45, 4679). Recently, some new pyrrolobenzodiazepine (PBD) hybrids have been synthesized that have significant DNA binding ability and potent antitumour activity. (Kamal,

A.; Srinivas, O.; Ramulu, P.; Ramesh, G.; Kumar, P. P. *Bioorg. Med. Chem. Lett.* **2003**, 13, 3577).

Naturally occurring pyrrolo[2,1-c][1,4]benzodiazepines belong to a group of antitumour antibiotics derived from *Streptomyces* species. Recently, there is much impetus for the PBD systems as they can recognize and bind to specific sequence of DNA. Examples of naturally occurring PBDs include anthramycin, DC-81, tomaymycin, sibiromycin and neothramycin.

However, the clinical efficacy for these antibiotics is hindered by several limitations, such as poor water solubility, cardio toxicity, development of drug resistance and metabolic inactivation.

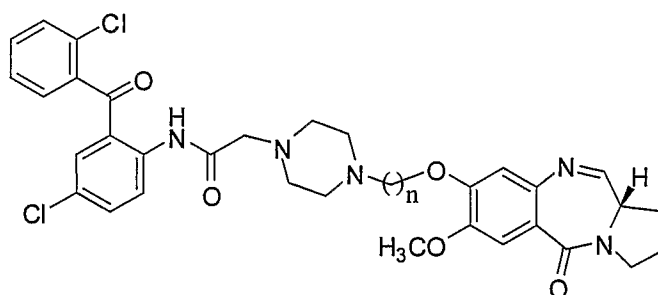
OBJECTIVES OF THE INVENTION

The main objective of the present invention is to provide novel benzophenone - piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrids, useful as antitumour agents.

Yet another object of this invention is to provide a process for the preparation of novel benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrids.

SUMMARY OF THE INVENTION

Accordingly the present invention provides a novel benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid of general formula 5



Formula 5a-e

Where in, $n = 3, 4, 5, 6, 8$

In an embodiment of the present invention the novel benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid according to claim 1 is represented by the group of the following compounds:

7-Methoxy-8-[3-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}propyloxy]- (11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (**5a**);

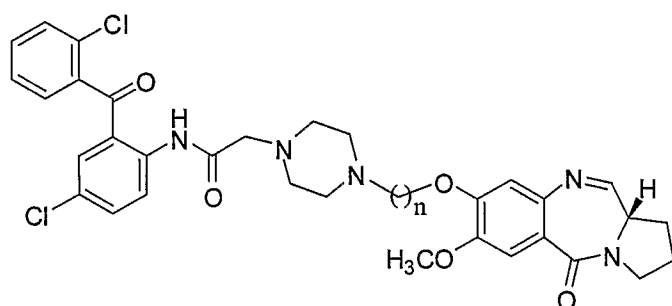
7-Methoxy-8-[4-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]butyloxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one(5b);

7-Methoxy-8-[5-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide] pentyloxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one(5c);

5 7-Methoxy-8-[6-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]hexyloxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (5d);

7-Methoxy-8-[8-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]octyloxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (5e);

10 In yet another embodiment the structural formula of the representative compounds of benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid are:



Formula 5a-e n = 3, 4, 5, 6, 8

15 In yet another embodiment the novel benzophenone-piperazine linked pyrrolo [2,1-c][1,4]benzodiazepine hybrid exhibits an *in vitro* anticancer/antitumour activity against human cancer cell lines selected from the group consisting of lung (Hop-62), cervix (SiHa), breast (MCF7, Zr-75-1), colon (Colo205), prostate (DU145, PC3) and oral (DWD, HT1080) cell lines.

20 In yet another embodiment the concentration of benzophenone linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid used for *in vitro* activity against Colo205 for IC50 is in the range of 13 to 80µm, at an exposure period of at least 48 hrs.

In yet another embodiment the concentration of benzophenone linked pyrrolo[2,1-c][1,4]benzodiazepine hybrids used for *in vitro* activity against DU145 for IC50 is in the range of 12 to 80µm, at an exposure period of at least 48 hrs.

25 In yet another embodiment the concentration of benzophenone linked pyrrolo[2,1-c][1,4]benzodiazepine hybrids used for *in vitro* activity against DWD for IC50 is in the range of 8 to 80µm, at an exposure period of at least 48 hrs.

In yet another embodiment the concentration of benzophenone linked pyrrolo[2,1-c][1,4]benzodiazepine hybrids used for *in vitro* activity against HoP62 for IC₅₀ is in the range of 11 to 48 μm , at an exposure period of at least 48 hrs.

In yet another embodiment the concentration of benzophenone linked pyrrolo[2,1-c][1,4]benzodiazepine hybrids used for *in vitro* activity against HT1080 for IC₅₀ is in the range of 14 to 36 μm , at an exposure period of at least 48 hrs.

In yet another embodiment the concentration of benzophenone linked pyrrolo[2,1-c][1,4]benzodiazepine hybrids used for *in vitro* activity against MCF7 for IC₅₀ is in the range of 22 to about 80 μm , at an exposure period of at least 48 hrs.

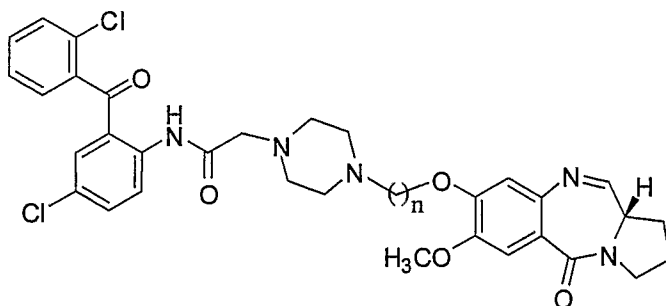
In yet another embodiment the concentration of benzophenone linked pyrrolo[2,1-c][1,4]benzodiazepine hybrids used for *in vitro* activity against PC3 for IC₅₀ is in the range of 28 to about 80 μm , at an exposure period of at least 48 hrs.

In yet another embodiment the concentration of benzophenone linked pyrrolo[2,1-c][1,4]benzodiazepine hybrids used for *in vitro* activity against SiHa for IC₅₀ is in the range of 36 to about 80 μm , at an exposure period of at least 48 hrs.

In yet another embodiment the concentration of benzophenone linked pyrrolo[2,1-c][1,4]benzodiazepine hybrids used for *in vitro* activity against Zr-75-1 for IC₅₀ is in the range of 29 to about 80 μm , at an exposure period of at least 48 hrs.

The present invention further provides a pharmaceutical composition comprising benzophenone linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid, its derivatives, analogues, salts or mixture thereof optionally with pharmaceutically acceptable carriers, adjuvants and additives.

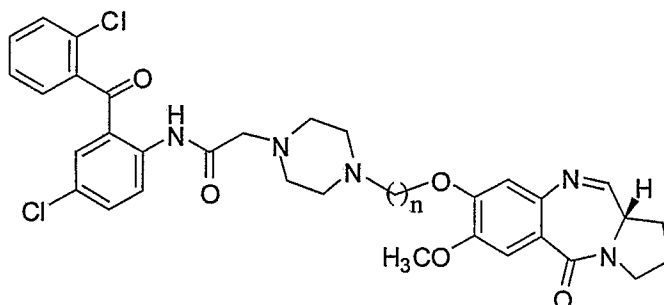
In yet another embodiment the benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid used is represented by a general formula 5,



Formula 5

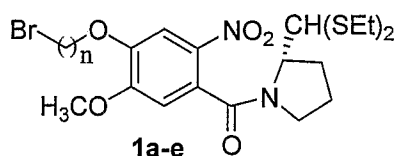
wherein $n = 3, 4, 5, 6, \text{ or } 8$.

The present invention further provides a process for the preparation of benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid of formula 5

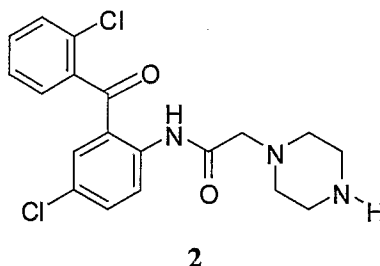


wherein $n = 3, 4, 5, 6, 8$ and the said process comprising the steps of:

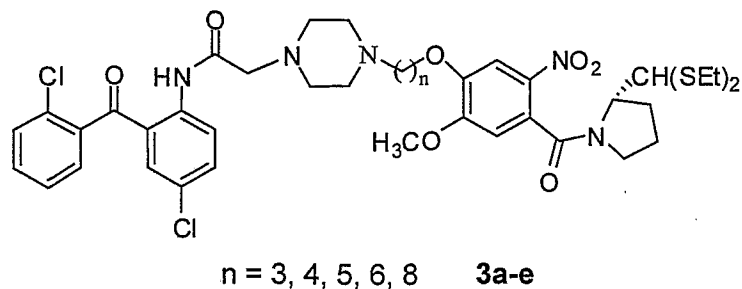
- 5 a) reacting (2*S*)-*N*-[(*n*-bromoalkoxy)-3-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethylthioacetal of formula 1



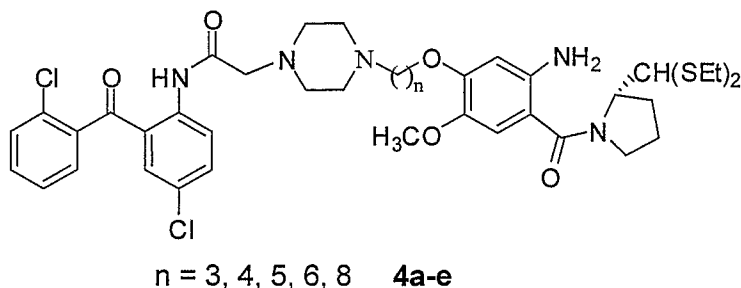
with *N*1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide of below formula 2



in an aprotic water miscible organic solvent, in the presence of anhydrous mild inorganic base, under refluxing temperature in an oil bath, for a period of about 24 to 48 hrs, followed by the removal of inorganic base by filtration and evaporating the organic solvent to obtain the resultant crude product and purifying it by column chromatography to obtain the desired product of 2*S*-*N*-[4-{*n*-(*N*1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide)alkyl}oxy-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal of formula 3a-e,



- b) reducing (2S)-N-[4-{n-(N-1-[4-chloro-2-(2-chlorobenzoyl) phenyl]-2-piperazinoacetamide]alkyl)oxy-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl-thioacetal of formula **3a-e** with $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, in an alcohol, under reflux, followed by the evaporation of alcohol and adjusting the pH of the resultant product layer to about 8 to 9 by using a base like Sodium bicarbonate and potassium bicarbonate, followed by extraction with ethyl acetate and washing the combined organic phase with brine solution and evaporating the solvent to obtain the desired product of 2S-N-[4-{n-(N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamid-e]alkyl)oxy-5-methoxy-2-amino benzoyl]pyrrolidine-2-carboxaldehyde diethylthioacetal of formula **4a-e**,



- c) reacting the above said amino compound of formula **4** obtained in step (b) with a deprotecting agent of the kind of mercuric chloride by known method to obtain the desired compound of formula **5**

In yet another embodiment the mild inorganic base used in steps (a) is potassium carbonate.

In yet another embodiment the aprotic organic solvent used in step (a) is acetone and acetonitrile

In yet another embodiment the organic solvent used in step (c) is acetonitrile and acetone

In yet another embodiment the alcohol used in step (b) is selected from methanol and ethanol.

In yet another embodiment the compounds of formula **5a-e** obtained are represented by a group of the following compounds:

7-Methoxy-8-[3-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}propyloxy]-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one(**5a**);

5 7-Methoxy-8-[4-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide} butyloxy]-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one(**5b**);

7-Methoxy-8-[5-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}pentyloxy]-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one(**5c**);

10 7-Methoxy-8-[6-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}hexyloxy]-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (**5d**);

7-Methoxy-8-[8-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}octyloxy]-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (**5e**);

15 In still another embodiment the benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid of formula **5a-e** exhibits an *in vitro* anticancer/antitumour activity against human cancer cell lines selected from the group consisting of lung, cervix, breast, colon, prostate and oral cell lines.

DETAILED DESCRIPTION OF THE INVENTION

Accordingly, the present invention provides a process for preparation of pyrrolo[2,1-c][1,4]benzodiazepine hybrids of formula **5a-e** of the drawing accompanying the specification where n = 3-6, and 8 which comprises reacting {N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide of formula **2** with (2S)-N-[(n-bromoalkyloxy)-3-methoxy-2-nitrobenzoyl]}pyrrolidine-2-carboxaldehyde diethyl thioacetal of formula **1** in presence of CH₃COCH₃/K₂CO₃ for a period of 48 h with isolating (2S)-{N1-[n-{4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}alkyl]oxy-5-methoxy-2-nitrobenzoyl} pyrrolidine-2-carboxaldehyde diethylthioacetal of formula **3a-e** by conventional methods. Reducing the above nitro compound of formula **3a-e** with SnCl₂.2H₂O in presence of organic solvent with reflux temperature, resulting with the formation of (2S)-N-1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide[alkyloxy]-5-methoxy-2-aminobenzoyl}

30 pyrrolidine-2-carboxaldehyde diethylthioacetal **4a-e**. Reacting the above said amino compound of formula **4a-e** with known deprotecting agents in a conventional manner to give novel pyrrolo[2,1-c][1,4]benzodiazepine hybrids of formula **5a-e** where 'n' is as stated above.

The precursors, {N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide of formula **2** (Kentaro Hirai, Teruyuki Ishiba, Hirohiko Sugimoto, Toshio Fujishita, Yuji sukinoki,

and Katsumi Hirose; *J. Med. Chem.* 1981, 24, 20–27). and (2S)-N-(4-hydroxy-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxaldehyde diethylthioacetal of formula 1 (Thurston, D. E.; Morris, S. J.; Hartley, J. A. *Chem. Commun.* 1996, 563-565) have been prepared by literature methods.

5

Some representative compounds of formula 5a-e for the present inventions are given below

7-Methoxy-8-[3-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]propyloxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one(5a);

10 7-Methoxy-8-[4-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]butyloxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one(5b);

7-Methoxy-8-[5-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]pentyloxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one(5c);

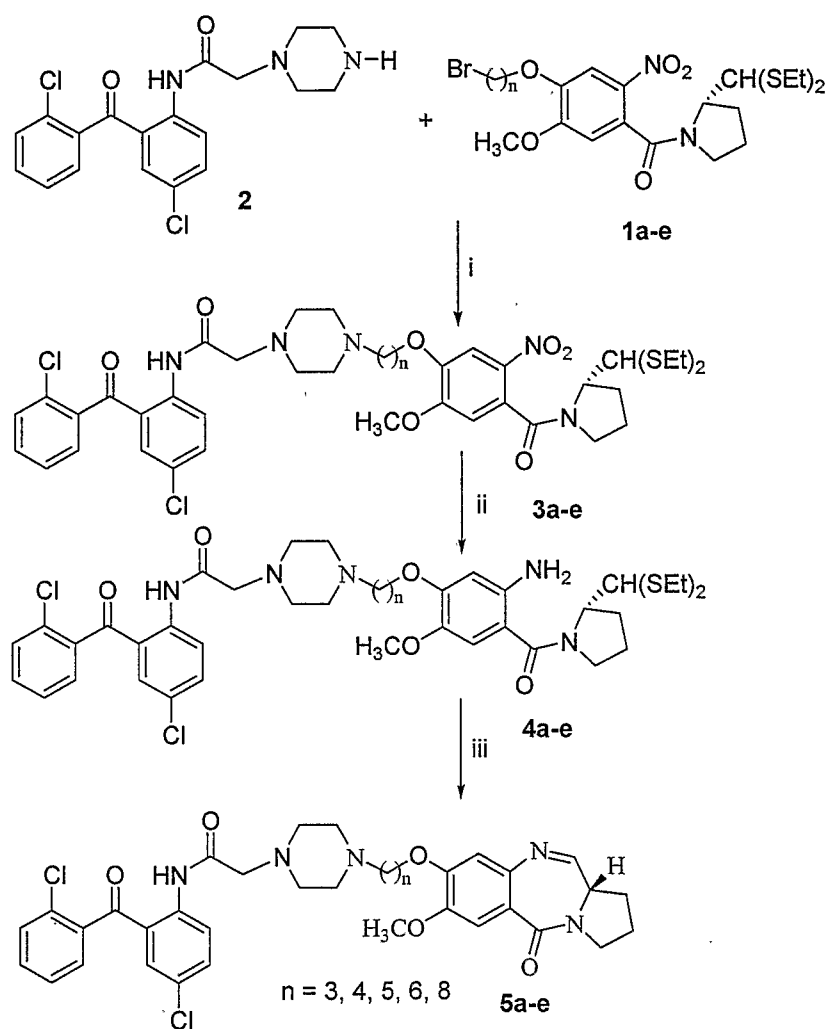
15 7-Methoxy-8-[6-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]hexyloxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (5d);

7-Methoxy-8-[8-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]octyloxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (5e);

20 These new analogues of pyrrolo[2,1-c][1,4]benzodiazepine hybrids linked at C-8 position have shown promising DNA binding activity and efficient anticancer activity in various cell lines. The molecules synthesized are of immense biological significance with potential sequence selective DNA-binding property. This resulted in design and synthesis of new congeners as illustrated in Scheme-1, which comprise:

1. The ether linkage at C-8 position of DC-81 intermediates with {N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide moiety.
2. Refluxing the reaction mixtures for 48 h.
3. Synthesis of C-8 linked PBD antitumour antibiotic hybrid imines.
4. Purification by column chromatography using different solvents like ethyl acetate, hexane, dichloromethane and methanol.

30



Reagents and conditions: (i) K_2CO_3 , acetone, 18 h, reflux, 90-92%; (ii) $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, MeOH, 2 h, reflux, 81-87%; (iii) $\text{HgCl}_2 \cdot \text{CaCO}_3$, $\text{CH}_3\text{CN-H}_2\text{O}$ (4:1), 12 h, rt, 68-71%.

Scheme: 1

The following examples are given by way of illustration and therefore should not be construed to the present limit of the scope of invention.

Example-1

2*S*-*N*-[4-(3-{*N*1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]propyl)oxy-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal (**3a**)

To a solution of 2*S*-*N*-[4-(3-bromopropoxy)-5-methoxy-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethylthioacetal (**1a**) (521 mg, 1.0 mmol) in dry acetone (20 mL) was added anhydrous potassium carbonate (690 mg, 5.0 mmol) and *N*1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide (**2**) (392 mg, 1.0 mmol). The reaction mixture was refluxed in an oil bath for 24 h and the reaction was monitored by TLC using ethyl acetate-hexane (6:4) as a solvent system. The potassium carbonate was then removed by suction filtration and the solvent was evaporated under vacuum to afford the crude product. This was further purified by column chromatography using ethyl acetate:hexane (6:4) as a solvent system to obtain the pure product (**3a**) (670 mg, 80% yield).

¹H NMR (CDCl₃): δ 1.22-1.39 (m, 6H), 1.69-2.32 (m, 8H), 2.51-2.56 (m, 4H), 2.66-2.86 (m, 8H), 3.19-3.26 (m, 4H), 3.92 (s, 3H), 4.06-4.13 (t, 2H, *J*=6.79 Hz), 4.63-4.70 (m, 1H), 4.81-4.83 (d, 1H, *J* = 3.77 Hz), 6.75 (s, 1H), 7.23-7.60 (m, 7H), 8.87-8.90 (d, 1H, *J* = 9.06 Hz), 12.30 (s, 1H); FABMS: 832 (M+H)⁺.

2*S*-*N*-[4-{*N*1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]propyl)oxy-5-methoxy-2-aminobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal (**4a**)

2*S*-*N*-[4-{*N*1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide] propyl)oxy-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethylthioacetal of formula **3a** (832 mg, 1.0 mmol) was dissolved in methanol (10 mL), SnCl₂·2H₂O (1.12 g, 5.0 mmol) was added and refluxed until the TLC indicated the completion of the reaction. The methanol was then evaporated in vacuum and the aqueous layer was then adjusted to pH 8 with 10% NaHCO₃ solution and extracted with ethyl acetate (60 mL). The combined organic phase was dried over Na₂SO₄ and evaporated under vacuum to afford the crude amino diethylthioacetal **4a** (790 mg, 97% yield), which was directly used in the next step.

7-Methoxy-8-[3-{*N*1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]propyloxy)-(11a*S*)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (**5a**)

A solution of 2*S*-*N*-[3-{*N*1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide
[propyl]oxy-5-methoxy-2-aminobenzoyl}pyrrolidine-2-carboxaldehyde diethyl thioacetal of
formula **4a** (802 mg, 1.0 mmol), HgCl₂ (576 mg, 2.26 mmol) and CaCO₃ (225 mg, 2.46 mmol)
in acetonitrile-water (4:1) was stirred slowly at room temperature overnight until complete
5 consumption of starting material as indicated by the TLC. The clear organic supernatant
liquid was extracted with ethyl acetate and washed with saturated 5% NaHCO₃ (20 mL),
brine (20 mL) and the combined organic phase was dried over Na₂SO₄. The organic layer
was evaporated in vacuum to afford a white solid, which was first eluted on a column
chromatography with ethyl acetate to remove mercuric salts, and then with ethyl acetate to
10 obtain the pure product **5a** (330 mg, 58% yield).

¹H NMR (CDCl₃): δ 1.24-1.54 (m, 4H), 1.66-1.94 (m, 4H), 2.00-2.38 (m, 4H), 2.94-3.05
(m, 2H), 3.29-3.41 (m, 6H), 3.56-4.23 (m, 4H), 6.80 (s, 1H), 7.25-7.70 (m, 8H), 8.87-8.89 (d,
1H, *J* = 4.28 Hz), 12.44 (s, 1H);
FABMS: 678 (M+H)⁺.

Example-2

2*S*-*N*-[4-(4-{*N*1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]butyl)oxy-5-
methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethyl thioacetal (**3b**)

This compound was prepared according to the method described for the compound
3a by employing 2*S*-*N*-[4-(4-bromobutoxy)-5-methoxy-2-nitrobenzoyl] pyrrolidine-2-
20 carboxaldehyde (**1b**) diethylthioacetal (535 mg, 1.0 mmol) was added anhydrous potassium
carbonate (690 mg, 5.0 mmol) and *N*1-4-chloro-2-(2-chlorobenzoyl)phenyl]-2-
piperazinoacetamide **2** (392 mg, 1.0 mmol) to obtain the pure product **3b** (710 mg, 84%
yield).

¹H NMR (CDCl₃): δ 1.22-1.39 (m, 8H), 1.70-2.32 (m, 8H), 2.52-2.57 (m, 4H), 2.68-2.85 (m,
25 8H), 3.19-3.27 (m, 4H), 3.92 (s, 3H), 4.06-4.12 (t, 2H, *J* = 6.55 Hz), 4.64-4.70 (m, 1H), 4.82-4.83
(d, 1H, *J* = 3.77 Hz), 6.75 (s, 1H), 7.22-7.60 (m, 7H), 8.87-8.90 (d, 1H, *J* = 9.06 Hz), 12.30 (s,
1H); FABMS: 846 (M+H)⁺.

2*S*-*N*-{4-[4-{*N*1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]butyl)oxy-5-
30 methoxy-2-aminobenzoyl}pyrrolidine-2-carboxaldehyde diethyl thioacetal (**4b**)

This compound was prepared according to the method described for the compound
4a by reducing 2*S*-*N*-{4-[4-{*N*1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]
butyl)oxy-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal **3b** (846

mg, 1.0 mmol) using $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (1.12 g, 5.0 mmol). The amino compound **4b** obtained was (795 mg, 97% yield).

7-Methoxy-8-[4-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}butyloxy]-
 5 (11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (**5b**)

This compound was prepared according to the method described for the compound **5a** employing 2S-N-4-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}butyl oxy-5-methoxy-2-aminobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal **4b** (816 mg, 1.0 mmol) and HgCl_2 (582 mg, 2.26 mmol), CaCO_3 (230 mg, 2.46 mmol) in acetonitrile-water
 10 (4:1) to obtain the pure product **5b** (330 mg, 58% yield).

^1H NMR (CDCl_3): δ 1.23-1.43 (m, 4H), 1.53-2.18 (m, 6H), 2.27-2.38 (m, 2H), 2.96-3.11 (m, 4H), 3.31-3.43 (m, 4H), 3.56-3.94 (m, 4H), 4.00-4.12(m, 2H), 6.78 (s, 1H) 7.25-7.75 (m, 8H), 8.86-8.90 (d, 1H, $J = 4.36$ Hz), 12.45(s, 1H);

FABMS: 692 ($\text{M}+\text{H}$)⁺.

Example-3

2S-N-[4-{5-(N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}pentyl)oxy-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehydediethyl thioacetal (**3c**)

This compound was prepared according to the method described for the compound **3a** by employing 2S-N-[4-(5-bromopenyloxy)-5-methoxy-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethylthioacetal (**1c**) (549 mg, 1.0 mmol) was added anhydrous potassium carbonate (690 mg, 5.0 mmol) and N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide **2** (392 mg, 1.0 mmol) to obtain the pure product **3c** (740 mg, 87% yield).

^1H NMR (CDCl_3): δ 1.20-1.39 (m, 8H), 1.72-2.33 (m, 10H), 2.50-2.56 (m, 8H) 2.60-2.85 (m, 4H), 3.19-3.26 (m, 4H), 3.91 (s, 3H), 4.05-4.13 (m, 2H), 4.62-4.69 (m, 1H), 4.80-4.83(d, 1H, $J = 3.77\text{Hz}$), 6.74 (s, 1H), 7.22-7.60(m, 7H), 8.86-8.90 (d, 1H, $J = 9.06\text{Hz}$), 12.30(s, 1H);
 25 FABMS: 860 ($\text{M}+\text{H}$)⁺.

2S-N-{4-[5-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}pentyl)oxy-5-methoxy-2-aminobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (**4c**)

This compound was prepared according to the method described for the compound **4a** by reducing A solution of 2S-N-[4-[5-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}pentyl)oxy-5-methoxy-2-aminobenzoyl}pyrrolidine-2-carboxaldehyde diethyl thioacetal **3c** (860 mg, 1.0 mmol) using $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (1.12 g, 5.0 mmol). The amino compound **4c** obtained was (810 mg, 97% yield).

7-Methoxy-8-[5-{*N*1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]pentyloxy}-(11*a*S)-1,2,3,11*a*-tetrahydro-5H-pyrrolo[2,1-*c*][1,4] benzodiazepine -5-one (**5c**)

This compound was prepared according to the method described for the compound **5a** employing 2*S*-*N*-[5-{*N*1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]pentyloxy-5-methoxy-2-aminobenzoyl]pyrrolidine-2-carboxaldehyde diethylthioacetal **4c** (830 mg, 1.0 mmol) and HgCl₂ (590 mg, 2.26 mmol), CaCO₃ (244 mg, 2.46 mmol) in acetonitrile-water (4:1) to obtain the pure product **5c** (330 mg, 58% yield).

¹H NMR (CDCl₃): δ 1.22-1.39 (m, 4H), 1.72-2.33 (m, 8H), 2.60-2.85 (m, 4H), 2.66-2.86 (m, 3H), 3.19-3.26 (m, 4H), 3.92 (s, 3H), 4.06-4.13 (t, 2H, *J*=6.79 Hz), 6.75 (s, 1H), 7.23-7.60 (m, 8H), and 8.87-8.90 (d, 1H, *J* = 4.41 Hz), 12.30 (s, 1H);

FABMS: 706 (M+H)⁺.

Example-4

2*S*-*N*-[4-{6-(*N*1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]hexyloxy-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethylthioacetal (**3d**)

This compound was prepared according to the method described for the compound **3a** by employing 2*S*-*N*-[4-(6-bromohexyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethylthioacetal **1d** (563 mg, 1.0 mmol) was added anhydrous potassium carbonate (690 mg, 5.0 mmol) and *N*1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide **2** (392 mg, 1.0 mmol) to obtain the pure product (**3d**) (775 mg, 88% yield).

¹H NMR (CDCl₃): δ 1.21-1.48 (m, 8H), 1.69-1.86 (m, 10H), 1.98-2.15 (m, 4H), 2.23-2.39 (m, 4H), 2.59-2.98 (m, 6H), 3.18-3.30 (m, 4H), 3.93 (s, 3H), 4.02-4.12 (m, 2H), 4.63-4.69 (m, 1H), 4.81-4.83 (d, 1H, *J* = 3.77 Hz), 6.78 (s, 1H), 7.25-7.61 (m, 7H), 8.86-8.90 (d, 1H, *J* = 9.06 Hz), 12.33 (s, 1H); FABMS: 874 [M+H]⁺.

2*S*-*N*-[4-{6-{*N*1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]hexyloxy-5-methoxy-2-aminobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal (**4d**)

This compound was prepared according to the method described for the compound **4a** by reducing 2*S*-*N*-[4-{6-{*N*1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]hexyloxy-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal **3d** (874 mg, 1.0 mmol) using SnCl₂·2H₂O (1.12 g, 5.0 mol). The amino compound **4d** obtained was (842 mg, 97% yield).

7-Methoxy-8-[6-{*N*1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide]hexyloxy}-(11*a*S)-1,2,3,11*a*-tetrahydro-5H-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (**5d**)

This compound was prepared according to the method described for the compound **5a** employing 2S-N-[6-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}hexyl)oxy-5-methoxy-2-aminobenzoyl]pyrrolidine-2-carboxaldehyde diethylthioacetal **4d** (844 mg, 1.0 mmol) and HgCl₂ (597 mg, 2.26 mmol), CaCO₃ (250 mg, 2.46 mmol) in acetonitrile-water (4:1) to obtain the pure product **5d** (485 mg, 58% yield).

¹H NMR (CDCl₃): δ 1.22-1.39 (m, 6H), 1.69-2.32 (m, 8H), 2.51-2.56 (t, 4H, *J*=6.64 Hz), 2.66-2.86 (m, 3H), 3.19-3.26 (m, 4H), 3.92 (s, 3H), 4.06-4.13 (t, 2H, *J*=6.79 Hz), 6.75 (s, 1H), 7.23-7.60 (m, 8H), 8.87-8.90 (d, 1H, *J* = 5.06 Hz), 12.30 (s, 1H); MS(FAB): 720 (M+H)⁺.

Biological Activity: some of *in vitro* biological activity studies were carried out at the National Cancer Institute, Maryland, USA.

Cytotoxicity:

The compounds 7-Methoxy-8-[3-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}propyloxy]-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (**5a**);

7-Methoxy-8-[4-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}butyloxy]-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (**5b**);

7-Methoxy-8-[5-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}pentyloxy]-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (**5c**);

7-Methoxy-8-[6-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}hexyloxy]-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (**5d**);

7-Methoxy-8-[8-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}octyloxy]-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (**5e**);

BIOLOGICAL ACTIVITY OF C8-LINKED BENZOPHENONE-PIPERAZINE-PBD HYBRIDS
***In vitro* Cytotoxicity**

The C8-linked benzophenone-piperazine-PBD hybrids have been tested against sixty human tumour cell lines derived from nine cancer types (leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer) as per NCI protocol. For each compound, dose response curves for individual cell lines have been measured at a minimum of five concentrations at 10 fold dilutions. A protocol of 48 h continuous drug exposure has been used, and a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth. The concentration for

50% cell growth inhibition (GI_{50}), total cell growth inhibition (TGI, 0% growth) and 50% cell death (LC_{50} , 50% growth) compared with the control has been calculated (Figure-2).

Compounds **5b** and **5d** have been evaluated for their *in vitro* cytotoxicity in eleven cell lines from nine human cancer cell lines of colon (Colo205), lung (Hop-62), cervix (SiHa), prostate (DU145, PC3), oral (DWD, HT1080), and breast (MCF7, Zr-75-1) origin. The results are expressed as percent of cell growth determined relative to that of untreated control cells (Table 1). The representative compounds 5b and 5d have shown significant cytotoxicity against some cancer cell lines.

Table-1. Log GI_{50} (concentration in mol/L causing 50% growth inhibition) values for benzophenone-piperazine-PBD hybrids

CANCER CELL LINES	Compound 5b	Compound 5d
Leukemia	-6.02	-6.39
Non-small-cell-lung	-6.86	-6.74
Colon	-5.87	-5.41
CNS	-5.11	-5.43
Melanoma	-4.34	-5.31
Ovarian	-5.40	-5.18
Renal	-5.39	-5.18
Prostate	-5.50	-5.82
Breast	-6.14	-6.64

Each cancer type represents the average of six to eight different cancer cell lines.

Among them **5b-d** exhibits a wide spectrum of activity against fifty six cell lines in nine cell panels, with GI_{50} value of <20 nM. In the non-small cell lung cancer panel, the growth of HOP-62, NCI-H23 cell lines were affected by compound 5b with GI_{50} values as 20.1, 24.2 and 27.1 nM respectively. The GI_{50} values of compound 5d against colon cancer HCC-2988, HCT-116 and KM12 cell lines are 21.3, 21.8 and 23.7 nM respectively. The GI_{50} values for compound 5b against CNS SF-295, SF-539, SNB-19 and SNB-75 cell lines are in a range of 14.1-33.5 nM. Four cancer cell lines (OVCAR-4, OVCAR-5, OVCAR-8 and SK-OV-3) in the ovarian cancer cell panel were affected by compound 5d with GI_{50} values of 21.6, 23.7, 29.6 and 69.8 nM respectively. In this study compound

Compound **5b** exhibits activity against fifty-six cell lines in nine cancer cell panels with GI_{50} values of < 10 μ M. Compound 5d exhibits activity against fifty-seven cell lines in nine cancer cell panels, GI_{50} values of < 10 μ M. *In vitro* cytotoxicity of compounds **5b** and **5d** in

selected cancer cell lines has been illustrated in Table 2. The average GI₅₀ values for each cancer panel of compounds **5b** and **5d** have been illustrated in Table 3.

Table 2. *In vitro* cytotoxicity of compounds **5b** and **5d** in selected cancer cell lines

Cancer panel/cell line	GI ₅₀ (nM) 5b	GI ₅₀ (nM) 5d
Leukemia		
CCRF-CEM	13.2	15.4
HL-60(TB)	43.6	22.9
RPMI-8226	42.6	17.3
Non-small cell lung		
NCI-H226	17.6	16.7
NCI-H23	15.3	19.5
NCI-H522	65.1	17.4
Colon		
HCT-116	18.0	14.1
HCT-15	29.1	17.2
SW-620	18.6	23.1
CNS		
SF-539	31.3	32.5
U251	15.6	17.4
Melanoma		
LOX IMVI	17.9	16.5
MALME-3M	23.9	34.6
M14	16.7	16.4
SK-MEL-5	21.2	23.6
UACC-257	21.7	15.6
UACC-62	16.1	20.5
Ovarian		
OVCAR-3	21.5	22.5
Renal		
786-0	17.4	16.9
A498	21.6	22.8
ACHN	22.9	34.1
CAKI-1	23.8	22.3
TK-10	32.3	32.9
UO-31	14.1	15.2
Breast		
NCI/ADR-RES	14.9	16.6
MDA-MB-231/ATCC	17.6	16.4

The mean graph mid point values of log₁₀ TGI and log₁₀ LC₅₀ as well as log₁₀ GI₅₀ for **5b** and **5d** are listed in Table-1. As demonstrated by mean graph pattern, compounds **5b** and **5d** exhibit an interesting profile of activity and selectivity for various cell lines. The mean graph mid points of log₁₀ TGI and log₁₀ LC₅₀ have shown similar pattern to the log₁₀ GI₅₀ mean graph mid points.

Table-3. $\log_{10}$ GI₅₀, $\log_{10}$ TGI and $\log_{10}$ LC₅₀ mean graphs midpoints(MG_MID) of *in vitro* cytotoxicity data for the compounds **5b** and **5d** against human tumour cell lines.

Compound	$\log_{10}$ GI ₅₀	$\log_{10}$ TGI	$\log_{10}$ LC ₅₀
5b	-5.51	-5.94	-4.49
5d	-5.49	-5.97	-4.53

DNA-BINDING ABILITY OF NOVEL C8-LINKED BENZOPHENONE-PIPERAZINE-PBD HYBRIDS

Thermal denaturation studies

Compounds have been subjected to thermal denaturation studies with duplex-form calf thymus DNA (CT-DNA) using a modification of a reported procedure. Working solutions in aqueous buffer (10 mM NaH₂PO₄/Na₂HPO₄, 1 mM Na₂EDTA, pH 7.00 ± 0.01) containing CT-DNA (100 nm in phosphate) and the PBD (20nm) have been prepared by addition of concentrated PBD solutions in DMSO to obtain a fixed [PBD]/[DNA] molar ratio of 1:5. The DNA-PBD solutions have been incubated at 37 °C for 0 and 18 h prior to analysis. Samples have been monitored at 260 nm using a Beckman DU-800 spectrophotometer fitted with high performance temperature controller, and heated at 1 °C min⁻¹ in the 40-110 °C range. DNA helix→coil transition temperatures (T_m) have been obtained from the maxima in the $d(A_{260})/dT$ derivative plots. Drug-induced alterations in DNA melting behavior are given by: $\Delta T_m = T_m(\text{DNA} + \text{PBD}) - T_m(\text{DNA alone})$, where the T_m value for the PBD-free CT-DNA is 68.5 ± 0.01 . The fixed [PBD]/[DNA] ratio used has not resulted in binding saturation of the host DNA duplex for any compound examined.

The DNA-binding for these novel C8-linked benzophenone-piperazine-PBD hybrids has been examined by thermal denaturation studies using calf thymus (CT) DNA. Melting studies show that these compounds stabilize the thermal helix → coil or melting stabilization (ΔT_m) for the CT-DNA duplex at pH 7.0, incubated at 37 °C, where PBD/DNA molar ratio is 1:5. Interestingly, in this assay one of the benzophenone-piperazine-PBD hybrids (**5c**) elevates the helix melting temperature of CT-DNA by a margin of 9.6 °C after incubation for 18 h at 37 °C. Data for **5a-e** and DC-81 are included in Table 4 for comparison.

Table-4. Thermal denaturation data for benzophenone-piperazine-PBD hybrids with calf thymus (CT) DNA

PBD hybrids	[PBD]:[DNA] molar ratio ^b	$\Delta T_m(^{\circ}\text{C})^a$ after incubation at 37 $^{\circ}\text{C}$ for	
		0 h	18 h
5a	1:5	8.0	9.3
5b	1:5	8.5	9.5
5c	1:5	8.9	9.6
5d	1:5	8.2	9.5
5e	1:5	8.3	9.0
DC-81	1:5	0.3	0.7

^a For CT-DNA alone at pH 7.00 ± 0.01 , $T_m = 68.5^{\circ}\text{C} \pm 0.01$ (mean value from 10 separate determinations), all ΔT_m values are $\pm 0.1 - 0.2^{\circ}\text{C}$. ^b For a 1:5 molar ratio of [PBD]/[DNA], where CT-DNA concentration = $100\ \mu\text{M}$ and ligand concentration = $20\ \mu\text{M}$ in aqueous sodium phosphate buffer [10 mM sodium phosphate + 1 mM EDTA, pH 7.00 ± 0.01]. ^c The ΔT_m for PBD hybrids 5a, 5b, 5c, 5d and 5e at a [PBD]:[DNA] molar ratio of 1:5 increased to a value of 9.3°C , 9.5°C , 9.6°C , 9.5 and 9.0°C after 18 h incubation respectively.

ADVANTAGES

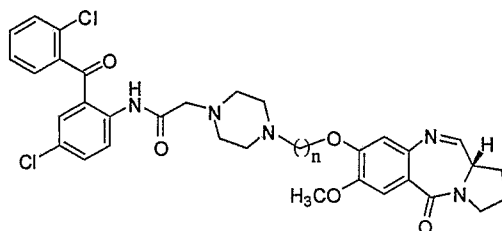
The present invention provides novel pyrrolo[2,1-c][1,4]benzodiazepine hybrids useful as antitumour agents.

The novel C8-linked benzophenone-piperazine-PBD hybrids that have been synthesized exhibited significant DNA-binding ability and showed cytotoxic activity against sixty human tumour cell lines. Some of these hybrids exhibited promising DNA-binding among these hybrids the compound **5c** show high DNA-binding ability (9.6°C).

We claim:-

1. Novel benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid of general formula 5

5



Formula 5a-e

wherein, n = 3, 4, 5, 6 or 8

2. Benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid according to claim 1 is represented by the group of the following compounds:

10

7-Methoxy-8-[3-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}propyloxy]-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (**5a**);

7-Methoxy-8-[4-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}butyloxy]-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (**5b**);

15

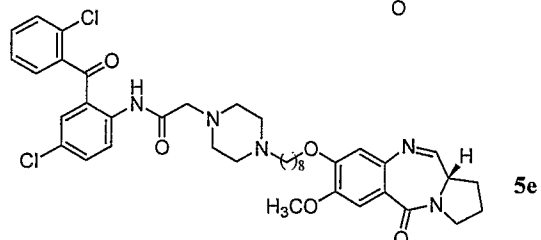
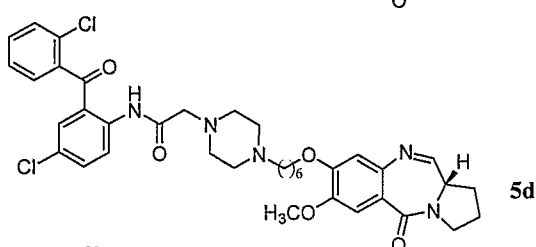
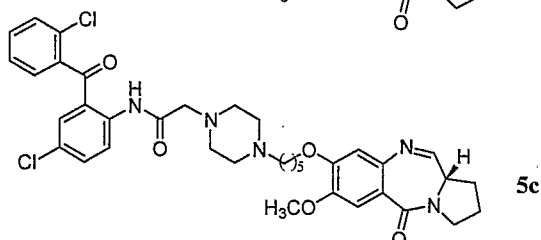
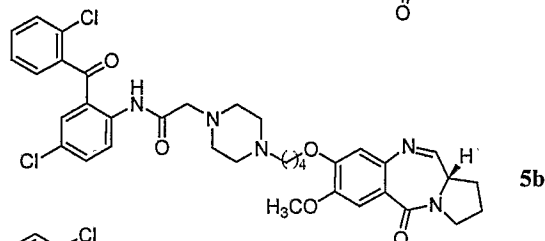
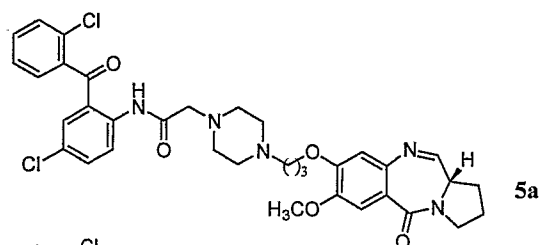
7-Methoxy-8-[5-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}pentyloxy]-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (**5c**);

7-Methoxy-8-[6-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}hexyloxy]-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (**5d**);

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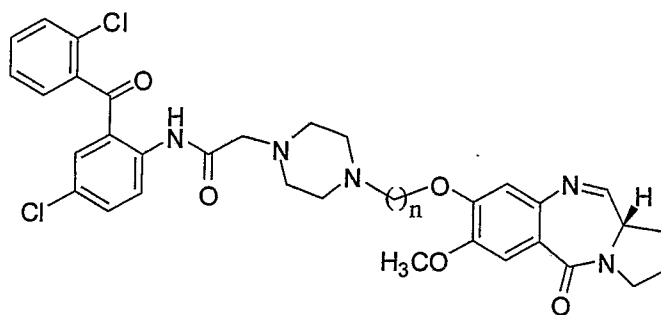
7-Methoxy-8-[8-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}octyloxy]-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (**5e**);

3. Benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid according to claim 1, wherein the structural formula of the representative compound are:



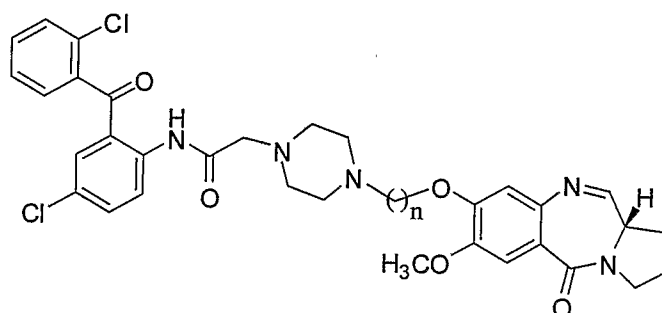
4. Benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid according to claim 1, exhibits an *in vitro* anticancer/antitumour activity against human cancer cell lines selected from the group consisting of lung (Hop-62), cervix (SiHa), breast (MCF7, Zr-75-1), colon (Colo205), prostate (DU145, PC3) and oral (DWD, HT1080) cell lines.
5. Benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid according to claim 1, wherein the concentration of the compound used for *in vitro* activity against Colo205 for IC₅₀ is in the range of 13 to 80 μm, at an exposure period of at least 48 hrs.
6. Benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrids according to claim 1, wherein the concentration of the compound used for *in vitro* activity against DU145 for IC₅₀ is in the range of 12 to 80 μm, at an exposure period of at least 48 hrs.

7. Benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrids according to claim 1, wherein the concentration of the compound used for *in vitro* activity against DWD for IC₅₀ is in the range of 8 to 80 μm, at an exposure period of at least 48 hrs.
- 5 8. Benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid according to claim 1, wherein the concentration of the compound used for *in vitro* activity against HoP62 for IC₅₀ is in the range of 11 to 40 μm, at an exposure period of at least 48 hrs.
9. Benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid according to claim 1, wherein the concentration of the compound used for *in vitro* activity against HT1080 for IC₅₀ is in the range of 14 to 30 μm, at an exposure period of at least 48 hrs.
- 10 10. Benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid according to claim 1, wherein the concentration of the compound used for *in vitro* activity against MCF7 for IC₅₀ is in the range of 22 to about 80 μm, at an exposure period of at least 48 hrs.
11. Benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid according to claim 1, wherein the concentration of the compound used for *in vitro* activity against PC3 for IC₅₀ is in the range of 28 to about 80 μm, at an exposure period of at least 48 hrs.
- 15 12. Benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid according to claim 1, wherein the concentration of the compound used for *in vitro* activity against SiHa for IC₅₀ is in the range of 36 to about 80 μm, at an exposure period of at least 48 hrs.
- 20 13. Benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid according to claim 1, wherein the concentration of the compound used for *in vitro* activity against Zr-75-1 for IC₅₀ is in the range of 29 to about 80 μm, at an exposure period of at least 48 hrs.
- 25 14.A pharmaceutical composition comprising benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid, its derivatives, analogues, salts or mixture thereof optionally with pharmaceutically acceptable carriers, adjuvants and additives.
- 15.A pharmaceutical composition according to claim 14, wherein the benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid used is represented by a general formula **5a-e**,



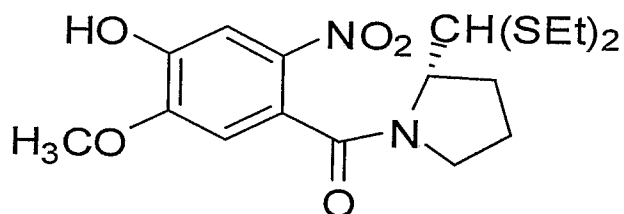
Formula 5a-e wherein n = 3, 4, 5, 6, 8

16. A process for the preparation of benzophenone linked pyrrolo[2,1-c][1,4] benzodiazepine hybrid of formula 5a-e,

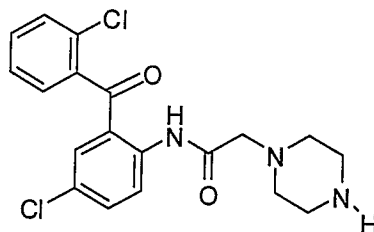


wherein n = 3, 4, 5, 6, 8 and the said process comprising the steps of:

- a) reacting (2S)-N-[4-(n-bromoalkyl)oxy-5-methoxy-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethylthioacetal of formula 1

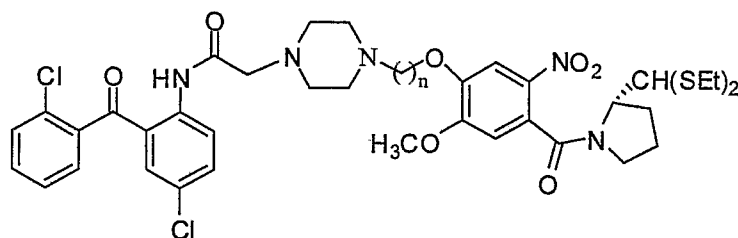


with piperazinobenzophenone derivative selected from the compound of formula 2



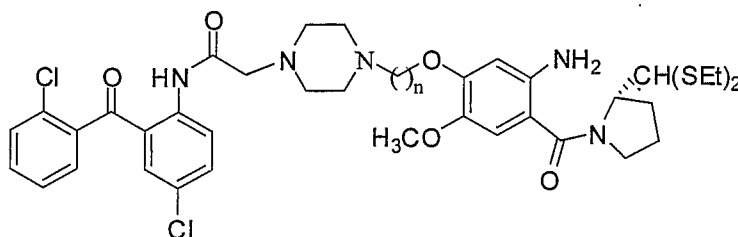
2

in the presence of K_2CO_3 , in an organic solvent, under refluxing temperature to obtain the resultant nitro compound of 2S-N-[4-{n-(N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide[alkyl]oxy-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal of formula 3a-e.



$n = 3, 4, 5, 6 \text{ or } 8$ **3a-e**

- b) reducing (2S)-N-[4-{n-(N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide)alkyl}oxy-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal of formula **3a-e** with $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, in an alcohol, under reflux, followed by the evaporation of alcohol and adjusting the pH of the resultant product layer to about 8 by using a base, followed by extraction with ethyl acetate and washing the combined organic phase with brine solution and evaporating the solvent to obtain the desired 2S-N-[4-{n-(N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide)alkyl}oxy-5-methoxy-2-aminobenzoyl]pyrrolidine-2-carboxaldehyde diethylthioacetal of formula **4a-e**,



$n = 3, 4, 5, 6 \text{ or } 8$ **4a-e**

- c) reacting (2S)-N-[4-{n-(N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazineacetamide)alkyl}oxy-5-methoxy-2-aminobenzoyl]pyrrolidine-2-carboxaldehyde diethylthioacetal of formula **4** with mercuric chloride, in a mixture of water and organic solvent, in the presence of mild inorganic, under stirring, at a temperature of about 20-30°C, for a period of 8-12 hrs, followed by the extraction of yellow organic supernatant and washing with sodium bicarbonate and brine, respectively, and evaporating the organic layer, under reduced pressure and further purified by column chromatography to obtain the desired product of benzophenone-piperazine linked pyrrolo[2,1-c][1,4] benzodiazepine hybrid of formula **5a-e**.

17. A process according to claim 16, wherein the mild inorganic base used in step (a) is potassium carbonate.

18. A process according to claim 16, wherein the aprotic organic solvent used in step (a) is acetone and acetonitrile.
19. A process according to claim 16, wherein the organic solvent used in step (c) is acetonitrile and acetone.
- 5 20. A process according to claim 16, wherein the alcohol used in step (b) is selected from methanol and ethanol.
21. A process according to claim 16, wherein the compounds of formula **5** obtained are represented by a group of the following compounds:
- 10 7-Methoxy-8-[3-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}propyloxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one(**5a**);
- 7-Methoxy-8-[4-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}butyloxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one(**5b**);
- 7-Methoxy-8-[5-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}pentyloxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one(**5c**);
- 15 7-Methoxy-8-[6-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}hexyloxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (**5d**);
- 7-Methoxy-8-[8-{N1-[4-chloro-2-(2-chlorobenzoyl)phenyl]-2-piperazinoacetamide}octyloxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (**5e**);
- 20 22. A process according to claim 16, wherein benzophenone-piperazine linked pyrrolo[2,1-c][1,4]benzodiazepine hybrid of formula **5a-e** exhibits an *in vitro* anticancer/antitumour activity against human cancer cell lines selected from the group consisting of lung, cervix, breast, colon, prostate and oral cell lines.

INTERNATIONAL SEARCH REPORT

International application No

PCT/IN2008/000714

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D487/04 A61K31/5517 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07D

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 practical, search terms used)

EPO-Internal, WPI Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 2008/120235 A (COUNCIL SCIENT IND RES [IN]; KAMAL AHMED [IN]; PRASAD BANDARI RAJENDRA) 9 October 2008 (2008-10-09) page 1, line 1 - page 20, line 23 page 10 - page 15; examples 1-10 claim 1	1-22
Y	WO 2008/020455 A (COUNCIL SCIENT IND RES [IN]) 21 February 2008 (2008-02-21) page 1, line 15 - page 3, line 12 page 5; compounds 5J-L page 11 - page 5; examples 1-5 claim 1	1-22

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

☒ See patent family annex.

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- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
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Date of the actual completion of the international search

25 February 2009

Date of mailing of the international search report

04/03/2009

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Authorized officer

Bissmire, Stewart

INTERNATIONAL SEARCH REPORT

International application No

PCT/IN2008/000714

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	WO 2007/054954 A (COUNCIL SCIENT IND RES [IN]; KAMAL AHMED [IN]; ANKATI HARI BABU [IN];) 18 May 2007 (2007-05-18) page 1, line 19 - page 3, line 9 page 8 - page 24; examples 1-12 claim 1 -----	1-22

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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