Title: NON-CROSS-LINKING PYRROLO[2,1-C][1,4]BENZODIAZEPINES AS POTENTIAL ANTITUMOUR AGENTS AND PROCESS THEREOF

Abstract: The present invention relates to novel pyrrolo[2,1-c][1,4]benzodiazepines compounds of general formula V as shown below, which are useful as potential antitumour agents and a process of preparing these compounds; particularly the present invention provides a process for the preparation of 7-methoxy-8-n-[7-methoxy-(11aS)-2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxyalkyl]yloxyl-(11aS)-2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one, with varying aliphatic chain length and its 2-hydroxy derivatives. (F) wherein R and R₁ is H and/or OH; and n is 3 to 5.
NON-CROSS-LINKING PYRROLO (2,1-C) (1,4) BENZODIAZEPINES AS POTENTIAL ANTITUMOUR AGENTS AND PROCESS THEREOF

Filed of the Invention

The present invention provides novel pyrrolo[2,1-c] [1,4]benzodiazepines which are useful as potential antitumour agents. This invention relates to a process for the preparation of new pyrrolo[2,1-c][1,4]benzodiazepines compounds useful as antitumour agents. More particularly, it provides a process for the preparation of 7-methoxy-8-[n-{7-methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy}alkyloxy]-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4] benzodiazepin-5-one, with aliphatic chain length variation compounds and its 2-hydroxy derivatives having anticancer (antitumour) activity. The structural formula of novel pyrrolo[2,1-c][1,4]benzodiazepine is as follows.

\[ \begin{array}{c}
\text{H} \\
\text{N} \\
\text{O} \\
\text{OCH₃} \\
\text{H₃C}-
\end{array} \]

where R and R₁ is H and/or OH; and n is 3 to 5

Background and Prior art references

mixed imine-amide PBD dimers have been synthesized that have significant DNA binding
ability and potent anti tumour activity (Kamal, A.; Laxman, N.; Ramesh, G.; Ramulu, P
and Srinivas, O. US Pat. No. 636233. dt 26-03-2002.; Kamal, A.; Ramesh, G.; Laxman,
N.; Ramulu, P.; Srinivas, O.; Neelima, K.; Kondapi, A. K.; Srinu, V. B.; Nagarajaram, H.

Naturally occurring pyrrolo[2,1-c][1,4]benzodiazepines belongs to a group of
antitumour antibiotics derived from *Streptomyces* species. Recently, there is much

![Anthramycin](image1.png) ![DC-81](image2.png)

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

impetus for the PBD systems as they can recognize and bind to specific sequence of DNA.
Examples of naturally occurring PBD's 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, cardiotoxicity and metabolic inactivation.

The compounds of present invention has a difference with C-8 linked secondary
amine congeners by the replacement of phenolic hydrogen of (11aS)-8-Hydroxy-7-
methoxy-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (DC-81).

Pyrrolo[2,1-c][1,4]benzodiazepine-5-ones are a class of compounds that bind to
DNA by non-covalent interactions such as hydrophobic, Vanderwaal's interactions and
hydrogen bonding between ring substituents and DNA, are also responsible for the
influence on the sequence selectivity.

**Objects of the invention**

The main object of the present invention is to provide novel compound and its
derivatives having anti-tumor activity i.e. Anti-cancer activities
Another object of the present invention is to provide new pyrrolo[2,1-c][1,4]benzodiazepines useful as antitumour agents.

Another objective of the present invention is to provide a process for the preparation of novel pyrrolo[2,1-c][1,4] benzodiazepines useful as antitumour agents.

Another object of the invention is to provide process for the preparation of novel compounds.

Another object of the invention is to provide a pharmaceutical composition for the treatment of cancer and other tumors.

One more object of the invention is to provide a method of treating subjects suffering from cancer and related diseases.

Summary of the Invention

Accordingly the present invention provides novel pyrrolo[2,1-c][1,4]benzodiazepine of formula V wherein R = H, OH, R₁ = H, OH and n is 3 to 5 and a process thereof.

![Formula V](attachment:image)

Detailed Description of the Invention

Accordingly, the present invention provides pyrrolo[2,1-c][1,4]benzodiazepine compounds of general formula V,

![Formula](attachment:image)

where R and R₁ is H and/or OH; and n is 3 to 5

One embodiment of the invention provides pyrrolobenzodiazepine having structural formula shown below where R = R₁ = H and n = 3.

![Structural Formula](attachment:image)
One embodiment of the invention provides pyrrolobenzodiazepine having structural formula shown below, where $R = R_1 = H$ and $n = 4$.

One more embodiment of the invention provides pyrrolobenzodiazepine having structural formula shown below, where $R = R_1 = H$ and $n = 5$.

Still another embodiment of the invention provides pyrrolobenzodiazepine having structural formula shown below where $R = OH, R_1 = H$ and $n = 3$.

Another embodiment of the invention provides pyrrolobenzodiazepine having structural formula shown below, where $R = OH, R_1 = H$ and $n = 4$.

Still another embodiment of the invention provides pyrrolobenzodiazepine having structural formula shown below, where $R = OH, R_1 = H$ and $n = 5$. 
In another embodiment, the invention provides a pyrrolobenzodiazepine compound having structural formula shown below, where $R = H$, $R_1 = OH$ and $n = 3$.

![Structural formula 1](image1)

In another embodiment, the invention provides a pyrrolobenzodiazepine compound having structural formula shown below, where $R = H$, $R_1 = OH$ and $n = 4$.

![Structural formula 2](image2)

In another embodiment, the invention provides a pyrrolobenzodiazepine compound having structural formula shown below, where $R = H$, $R_1 = OH$ and $n = 5$.

![Structural formula 3](image3)

Another embodiment of the invention relates to the activity of the pyrrolo[2,1-c][1,4]benzodiazepine of formula V against human tumor cell lines.

Still another embodiment of the invention provides growth inhibition activity of these compounds against various types of cancer cells like leukemia, non-small-cell lung, colon, CNS, melanoma, ovarian, prostate and breast cancer.

One more embodiment of the invention relates to a process for the preparation of compounds, the said process comprising steps of:

a) reacting a compound of formula (I) with formula (II) in presence of mild base in water miscible aprotic organic solvent at reflux temperature for a period of 16h to 48h,

b) pouring the reaction mixture of step (a) into water, extracting with ethyl acetate separating organic layer and discarding aqueous layer,

c) evaporating the organic layer of step (b) to obtain a residue which is purified to get compound of formula (III),
d) reducing the nitro compound of formula (III) in halogenated solvent, adding stannous chloride dihydrate, refluxing for a period of 0.5h to 2h,
e) adjusting the pH of the reaction mixture of step (d) to 8.0 using alkali bicarbonate solution,
f) extracting the step(e) solution with ethylacetate, evaporating the ethylacetate extract under reduced pressure to obtain crude compound of formula (IV),
g) providing a solution of compound of formula (IV) in a mixture of acetonitrile/H₂O adding mercuric chloride, calcium carbonate, stirring at room temperature for 6h to 12h,
h) filtering and evaporating the organic layer under reduced pressure to obtain a residue which is diluted with ethyl acetate,
i) adding saturated solution of sodium bicarbonate to ethylacetate solution of step (h) at room temperature,
j) filtering the solution of step (I) through celite bed, evaporating the filtrate to obtain a residue containing crude compound of formula V, and
k) purifying crude compound of step (j) using silica gel as an adsorbent to obtain pure compound of formula (V) wherein R and R₁ are as defined earlier.

Still another embodiment, wherein the aprotic organic solvent used in step (a) is selected from a group consisting of tetrahydrofuran, acetone or dimethyl formamide.

Yet another embodiment, the mild base used in step (a) is selected from a group of sodium carbonate, potassium carbonate, lithium carbonate, barium carbonate and cesium carbonate.

Yet another embodiment, in step (d) the halogenated solvent used is selected from a group consisting of carbon tetra chloride, chloroform and dichloromethane, preferably dichloromethane.

Still another embodiment of the invention relates to the process, wherein in step (c) the alkali carbonate solution used is selected from a solution of sodium bicarbonate potassium bicarbonate or lithium bicarbonate.

One more embodiment of the invention provide pharmaceutical composition effective against human cancer cell lines, said composition comprising effective amount of compound pyrrolo[2,1-c][1,4]benzodiazepine of general formula V, where R and R₁ is H and/or OH; and n is 3 to 5, along with pharmaceutically acceptable additives.
The composition is administered to mammals including human beings. The composition is administered orally, systemically or by any other conventional methods. The pharmaceutically acceptable additives are selected from a group consisting of carriers, diluents, solvents, filler, lubricants, excipients, binders and stabilizers.

Another embodiment of the invention, the said composition inhibits the growth of cancer cells.

Still another embodiment, the said composition inhibits the growth of the cancer cells such as leukemia, non-small-cell lung, colon, CNS, melanoma, ovarian, prostate and breast cancer.

The present process provides a process for preparation of pyrrolo[2,1-c][1,4]benzodiazepines of formula V of the drawing accompanying the specification wherein R is H, OH, R₁ is H, OH and n is 3 to 5 which comprises: (2S)-N-[4-(n-bromoalkoxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal wherein R₁ is H, OH and n is 3-5 compounds of formula I with secondary amine of formula II wherein R is H and OH in presence of an inorganic mild bases like K₂CO₃, CsCO₃ and BaCO₃ in presence of aprotic water miscible organic solvents like acetone, THF, and DMF upto refluxing temperature for a period upto 12-48 hours isolating (2S)-N-{4-[n-(7-methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-loxy)alkyloxy]-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethyl thioacetal and III wherein R is H, OH, R₁ is H, OH and n is 3 to 5 by conventional methods, reducing the above nitro compounds of formula III with SnCl₂ .2H₂O in presence of organic solvent up to a reflux temperature, isolating the (2S)-N-{4-[n-(7-methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodi-azepine-5-one-8-loxy)alkoxy]-5-methoxy-2-aminobenzoyl}pyrrolidine-2-carboxaldehyde diethyl thioacetal of formula IV wherein R is H, OH, R₁ is H, OH and n is 3 to 5 by known methods, reacting the above said amino compound of formula IV with known deprotecting agents in a conventional manner to give novel pyrrolo[2,1-c][1,4]benzodiazepines of formula V wherein R is H, OH, R₁ is H, OH and n is 3 to 5.


Some representative compounds of formula V present invention are given below

1) 7-Methoxy-8-{3-[7-methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4] benzodiazepine-5-one-8-yloxy]proproxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

2) 7-Methoxy-8-{4-[7-methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4] benzodiazepine-5-one-8-yloxy]butoxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

3) 7-Methoxy-8-{5-[7-methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4] benzodiazepine-5-one-8-yloxy]pentyloxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

4) 7-Methoxy-8-{3-[7-methoxy-(2R)-hydroxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4] benzodiazepine-5-one-8-yloxy]proproxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

5) 7-Methoxy-8-{4-[7-methoxy-(2R)-hydroxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4] benzodiazepine-5-one-8-yloxy]butoxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

6) 7-Methoxy-8-{5-[7-methoxy-(2R)-hydroxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4] benzodiazepine-5-one-8-yloxy]pentyloxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

7) 7-Methoxy-8-{3-[7-methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4] benzodiazepine-5-one-8-yloxy]proproxy}-(2R)-hydroxy-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

8) 7-Methoxy-8-{4-[7-methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4] benzodiazepine-5-one-8-yloxy]butoxy}-(2R)-hydroxy-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

9) 7-Methoxy-8-{5-[7-methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4] benzodiazepine-5-one-8-yloxy]pentyloxy}-(2R)-hydroxy-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one

The process of preparation of new pyrrolo[2,1-c][1,4]benzodiazepines is disclosed and claimed in applicants co pending application no........
These new analogues of pyrrolo[2,1-c][1,4]benzodiazepines linked at C-8 position have shown promising 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 secondary amine.
2. Refluxing the reaction mixture for 24-48 h.
3. Synthesis of C-8 linked PBD antitumour antibiotic imines.
4. Purification by column chromatography using different solvents like ethylacetate, hexane and methanol.

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

**Brief description of accompanying drawing**

Figure 1 represents schematic diagram of preparing compound of general formula V.

**Example 1**

A solution of (2S)-N-[4-(3-bromopropoxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal of formula I (521 mg, 1 mmol), 8-hydroxy-7-methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (248 mg, 1 mmol) of the formula II and K₂CO₃ (414 mg, 3 mmol) in dry acetone (20 mL) was refluxed for 48 h. After the completion of reaction as indicated by TLC, EtOAc, the reaction mixture was poured on to the water and then extracted with ethylacetate. Evaporation of the organic layer gave the crude product, which was further purified by column chromatography on silica gel eluting with EtOAc:hexane (8:2) gave the pure (2S)-N-[4-[3-(7-Methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)propoxy]-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal III.

1H NMR (CDCl₃) δ 1.35-1.45 (m, 6H), 1.70-2.45 (m, 10H), 2.72-2.90 (m, 4H), 3.20-3.28 (m, 3H), 3.50-3.58 (m, 1H), 3.62-3.75 (m, 1H) 3.80-3.90 (m, 4H), 4.20 (t, 2H), 4.35 (t, 2H), 4.65-4.75 (m, 1H), 4.85 (d, 1H, J = 4.28 Hz), 6.25 (s, 1H), 6.82 (s, 1H), 7.48 (s, 1H), 7.72 (s, 1H); MS (FAB) 689 [M + H]+.

The (2S)-N-[4-[3-(7-Methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)propoxy]-5-methoxy-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal III.
xaldehyde diethyl thioacetal III (688 mg, 1.0 mmol) was dissolved in dichloromethane (5 mL), methanol (10 mL) and added SnCl₂2H₂O (1.124 g, 5.0 mmol) was refluxed for 1.5 h. The reaction mixture was then carefully adjusted to pH 8 with saturated NaHCO₃ solution and then extracted with ethyl acetate (3x20 mL). The combined organic phase was dried over Na₂SO₄ and evaporated under vacuum to afford the crude (2S)-N-[4-[3-(7-Methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-ylotoxypropoxy]-5-methoxy-2-aminobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal IV

A solution of the (2S)-N-[4-[3-(7-Methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-ylotoxypropoxy]-5-methoxy-2-aminobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal IV (658 mg, 1 mmol), HgCl₂ (794 mg, 2.93 mmol) and CaCO₃ (300 mg, 3 mmol) in CH₃CN/H₂O (4:1, 15 mL) was stirred at room temperature for 12 h until TLC (EtOAc), indicates complete loss of starting material. Then organic layer is evaporated in vacuum and the residue is diluted with EtOAc. To this saturated NaHCO₃ was added slowly at room temperature and the mixture is filtered through celite and washed with ethylacetate. The filterate is evaporated in vacuum to get crude 7-Methoxy-8-{[3-[7-methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-ylotoxypropoxy]-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one of formula V, which was further purified by column chromatography on silica gel eluting with methanol:EtOAc (2:8).

¹H NMR (CDCl₃) δ 1.65-2.45 (m, 10H), 3.15-3.25 (m, 2H), 3.48-3.75 (m, 4H), 3.78 – 3.88 (m, 4H), 3.90 (s, 3H), 4.25-4.35 (m, 5H), 6.18 (s, 1H), 6.82 (s, 1H), 7.48 (s, 1H), 7.52 (s, 1H), 7.65 (d, 1H, J = 4.8 Hz); MS (FAB) 535 [M + H]+.

**Example 2**

A solution of (2S)-N-[4-(4-bromobutoxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal of formula I (535 mg, 1 mmol), 8-hydroxy-7-methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrol[2,1-c][1,4]benzodiazepine-5-one (248 mg, 1 mmol) of the formula II and K₂CO₃ (414 mg, 3 mmol) in dry acetone (20 mL) was refluxed for 48 h. After the completion of reaction as indicated by TLC, EtOAc, the reaction mixture was poured on to the water and then extracted with ethylacetate. Evaporation of the organic layer gave the crude product, which was further purified by column chromatography on silica gel eluting with EtOAc:hexane (8:2) gave the pure (2S)-N-[4-(4-(7-Methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]ben-
zodiazepine-5-one-8-yloxy)butoxy]-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thiacetal III.

1H NMR (CDCl₃) δ 1.35-1.45 (m, 6H), 1.70-2.45 (m, 12H), 2.72-2.90 (m, 4H), 3.20-3.28 (m, 3H), 3.50-3.58 (m, 1H), 3.62-3.75 (m, 1H) 3.80-3.90 (m, 4H), 4.20 (t, 2H), 4.35 (t, 2H), 4.65-4.75 (m, 1H), 4.85 (d, 1H, J = 4.28 Hz), 6.25 (s, 1H), 6.82 (s, 1H), 7.48 (s, 1H), 7.72 (s, 1H); MS (FAB) 703 [M + H]+.

The (2S)-N-{4-[4-(7-Methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)butoxy]-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal (702 mg, 1.0 mmol) of the formula III was dissolved in dichloromethane (5 mL), methanol (10 mL) and added SnCl₂.2H₂O (1.124 g, 5.0 mmol) was refluxed for 1.5 h. The reaction mixture was then carefully adjusted to pH 8 with saturated NaHCO₃ solution and then extracted with ethylacetate (3x20 mL). The combined organic phase was dried over Na₂SO₄ and evaporated under vacuum to afford the crude (2S)-N-{4-[4-(7-Methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrrolo[2,1-c]

[1,4]benzodiazepine-5-one-8-yloxy)butoxy]-5-methoxy-2-aminobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal IV.

A solution of the (2S)-N- {4-[4-(7-Methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)butoxy]-5-methoxy-2-aminobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal of formula IV. (672 mg, 1 mmol), HgCl₂ (794 mg, 2.93 mmol) and CaCO₃ (300 mg, 3 mmol) in CH₃CN/H₂O (4:1, 15 mL) was stirred at room temperature for 12 h until TLC (EtOAc), indicates complete loss of starting material. Then organic layer is evaporated in vacuum and the residue is diluted with EtOAc. To this saturated NaHCO₃ was added slowly at room temperature and the mixture is filtered through celite and washed with ethylacetate. The filtrate is evaporated in vacuum to get crude 7-Methoxy-8-{4-[7-methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)butoxy]-11aS)-1,2,3,11a-tetrahy-dro-5H-pyrrrolo[2,1-c][1,4]benzodiazepine-5-one of formula V, which was further purified by column chromatography on silica gel eluting with methanol:EtOAc (2:8).

1H NMR (CDCl₃) δ 1.65-2.45 (m, 10H), 3.15-3.25 (m, 2H), 3.48-3.75 (m, 4H), 3.78 – 3.88 (m, 4H), 3.90 (s, 3H), 4.25-4.35 (m, 5H), 6.18 (s, 1H), 6.82 (s, 1H), 7.48 (s, 1H), 7.52 (s, 1H), 7.65 (d, 1H, J = 4.8 Hz); MS (FAB) 549 [M + H]+.

Example 3

A solution of (2S)-N-[4-(5-bromopentnyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal of formula I. (549 mg, 1 mmol), 8-hydroxy-7-methoxy-
(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (248 mg, 1 mmol) II and K$_2$CO$_3$ (414 mg, 3 mmol) in dry acetone (20 mL) was refluxed for 48 h. After the completion of reaction as indicated by TLC, EtOAc, the reaction mixture was poured on to the water and then extracted with ethylacetate. Evaporation of the organic layer gave the crude product, which was further purified by column chromatography on silica gel eluting with EtOAc:hexane (8:2) gave the pure (2S)-N-[4-[5-(7-Methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yl oxy) pentyloxy]-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thiaocetal III. $^1$H NMR (CDCl$_3$) δ 1.30-1.40 (m, 6H), 1.65-2.35 (m, 14H), 2.65-2.75 (m, 4H), 3.18-3.32 (m, 3H), 3.45-3.75 (m, 2H), 3.80-3.85 (s 4H), 3.85-4.0 (m, 5H), 4.65-4.72 (m, 1H), 4.85 (d, 1H, $J = 4.38$ Hz), 6.0 (s, 1H), 6.78 (s, 1H), 7.52 (s, 1H), 7.65 (s, 1H); MS (FAB) 717 [M + H]$^+$.

The (2S)-N-[4-[5-(7-Methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yl oxy)pentyloxy]-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal III (716 mg, 1.0 mmol) was dissolved in dichloromethane (5 mL), methanol (10 mL) and added SnCl$_2$.2H$_2$O (1.124 g, 5.0 mmol) was refluxed for 1.5 h. The reaction mixture was then carefully adjusted to pH 8 with saturated NaHCO$_3$ solution and then extracted with ethyl acetate (3x20 mL). The combined organic phase was dried over Na$_2$SO$_4$ and evaporated under vacuum to afford the crude (2S)-N-[4-[5-(7-Methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yl oxy)pentyloxy]-5-methoxy-2-aminobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal of formula IV.

A solution of (2S)-N-[4-[5-(7-Methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yl oxy)pentyloxy]-5-methoxy-2-aminobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal IV (686 mg, 1 mmol), HgCl$_2$ (794 mg, 2.93 mmol) and CaCO$_3$ (300 mg, 3 mmol) in CH$_3$CN/H$_2$O (4:1, 15 mL) was stirred at room temperature for 12 h until TLC (EtOAc), indicates complete loss of starting material. Then organic layer is evaporated in vacuum and the residue is diluted with EtOAc. To this saturated NaHCO$_3$ was added slowly at room temperature and the mixture is filtered thorough celite and washed with ethylacetate. The filtrate is evaporated in vacuum to get crude 7-Methoxy-8-{5-[7-methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yl oxy)butoxy}-{(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one of formula V, which was further purified by column chromatography on silica gel eluting with methanol:EtOAc (2:8).
$^1$H NMR (CDCl$_3$) $\delta$ 1.20-2.45 (m, 14H), 3.10-3.20 (m, 2H), 3.20-3.28 (m, 1H), 3.40-3.78 (m, 5H), 3.80 (s, 3H), 3.98 (s, 3H), 4.10-4.22 (m, 4H), 6.0 (s, 1H), 7.02 (s, 1H); 7.50 (s, 1H), 7.55 (s, 1H), 7.65 (d, 1H, $J = 5.1$ Hz); MS (FAB) 563 [M + H]$^+$.

**Example 4**

A solution of (2S)-N-[4-(3-bromopropoxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal of formula I (521 mg, 1 mmol), 8-hydroxy-7-methoxy-(2R)-hydroxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (264 mg, 1 mmol) of the formula II and K$_2$CO$_3$ (414 mg, 3 mmol) in dry acetone (20 mL) was refluxed for 24-48 h. After the completion of reaction as indicated by TLC, EtOAc, the reaction mixture was poured on to the water and then extracted with ethylacetate. Evaporation of the organic layer gave the crude product, which was further purified by column chromatography on silica gel eluting with EtOAc gave the pure (2S)-N-{4-[3-(7-Methoxy-(2R)-hydroxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)propoxy]-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethyl thioacetal III.

The (2S)-N-{4-[3-(7-Methoxy-(2R)-hydroxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)propoxy]-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethyl thioacetal III (704 mg, 1.0 mmol) was dissolved in dichloromethane (5 mL), methanol (10 mL) and added SnCl$_2$·2H$_2$O (1.124 g, 5.0 mmol) was refluxed for 1.5 h. The reaction mixture was then carefully adjusted to pH 8 with saturated NaHCO$_3$ solution and then extracted with ethyl acetate (3×20 mL). The combined organic phase was dried over Na$_2$SO$_4$ and evaporated under vacuum to afford the crude (2S)-N-{4-[3-(7-Methoxy-(2R)-hydroxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)propoxy]-5-methoxy-2-aminobenzoyl}pyrrolidine-2-carboxaldehyde diethyl thioacetal IV.

A solution of the (2S)-N-{4-[3-(7-Methoxy-(2R)-hydroxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)propoxy]-5-methoxy-2-aminobenzoyl}pyrrolidine-2-carboxaldehyde diethyl thioacetal IV (674 mg, 1 mmol), HgCl$_2$ (794 mg, 2.93 mmol) and CaCO$_3$ (300 mg, 3 mmol) in CH$_3$CN/H$_2$O (4:1, 15 mL) was stirred at room temperature for 12 h until TLC (EtOAc), indicates complete loss of starting material. Then organic layer is evaporated in vacuum and the residue is diluted with EtOAc. To this saturated NaHCO$_3$ was added slowly at room temperature and the mixture is filtered through celite and washed with ethylacetate. The filtrate is evaporated
in vacuum to get crude 7-Methoxy-8-\{3-[7-methoxy-(11\alpha)-1,2,3,10,11,11\alpha-hexahydro-5H-pyrrolo[2,1-c]][1,4]benzodiazepine-5-one-8-yloxy)propoxy]-\{11\alpha\)-1,2,3,11\alpha-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one of formula \text{V}, which was further purified by column chromatography on silica gel eluting with methanol:EtOAc (3:7).

**Example 5**

A solution of \((2\alpha)-N-\{4-(4-bromobutoxy)-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxaldehyde diethyl thioacetal of formula \text{I} (535 mg, 1 mmol), 8-hydroxy-7-methoxy-(2\alpha)-hydroxy-(11\alpha)-1,2,3,10,11,11\alpha-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (264 mg, 1 mmol) of the formula \text{II} and \(K_2CO_3\) (414 mg, 3 mmol) in dry acetone (20 mL) was refluxed for 48 h. After the completion of reaction as indicated by TLC, EtOAc, the reaction mixture was poured on to the water and then extracted with ethylacetate. Evaporation of the organic layer gave the crude product, which was further purified by column chromatography on silica gel eluting with EtOAc gave the pure \((2\alpha)-N-\{4-(7-methoxy-(2\alpha)-hydroxy-(11\alpha)-1,2,3,10,11,11\alpha-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)butoxy]-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxaldehyde diethyl thioacetal \text{III}.

The \((2\alpha)-N-\{4-(7-methoxy-(2\alpha)-hydroxy-(11\alpha)-1,2,3,10,11,11\alpha-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)butoxy]-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxaldehyde diethyl thioacetal (718 mg, 1.0 mmol) of the formula \text{III} was dissolved in dichloromethane (5 mL), methanol (10 mL) and added \(SnCl_2\cdot2H_2O\) (1.124 g, 5.0 mmol) was refluxed for 1.5 h. The reaction mixture was then carefully adjusted to pH 8 with saturated \(NaHCO_3\) solution and then extracted with ethyl acetate (3x20 mL). The combined organic phase was dried over \(Na_2SO_4\) and evaporated under vacuum to afford the crude \((2\alpha)-N-\{4-(7-Methoxy-(2\alpha)-hydroxy-(11\alpha)-1,2,3,10,11,11\alpha-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)butoxy]-5-methoxy-2-aminobenzoyl\)-pyrrolidine-2-carboxaldehyde diethyl thioacetal \text{IV}.

A solution of the \((2\alpha)-N-\{4-(7-methoxy-(2\alpha)-hydroxy-(11\alpha)-1,2,3,10,11,11\alpha-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)butoxy]-5-methoxy-2-aminobenzoyl\)pyrrolidine-2-carboxaldehyde diethyl thioacetal of formula \text{IV}. (688 mg, 1 mmol), \(HgCl_2\) (794 mg, 2.93 mmol) and \(CaCO_3\) (300 mg, 3 mmol) in \(CH_3CN/H_2O\) (4:1, 15 mL) was stirred at room temperature for 12 h until TLC (EtOAc), indicates complete loss of starting material. Then organic layer is evaporated in vacuum and the residue is diluted with EtOAc. To this saturated \(NaHCO_3\) was added slowly at room temperature and the
mixture is filtered through celite and washed with ethylacetate. The filtrate is evaporated in vacuum to get crude 7-methoxy-8-{4-[7-methoxy-(2R)-hydroxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy]butoxy} (11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one of formula V, which was further purified by column chromatography on silica gel eluting with methanol:EtOAc (3:7).

**Example 6**

A solution of (2S)-N-{4-(5-bromopentyl)oxy}-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal of formula I (549 mg, 1 mmol), 8-hydroxy-7-methoxy-(2R)-hydroxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (264 mg, 1 mmol) II and K₂CO₃ (414 mg, 3 mmol) in dry acetone (20 mL) was refluxed for 48 h. After the completion of reaction as indicated by TLC, EtOAc, the reaction mixture was poured on to the water and then extracted with ethylacetate. Evaporation of the organic layer gave the crude product, which was further purified by column chromatography on silica gel eluting with EtOAc gave the pure (2S)-N-{4-[5-(7-Methoxy-(2R)-hydroxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)pentyl]oxy}-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal III.

The (2S)-N-{4-[5-(7-Methoxy-(2R)-hydroxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)pentyl]oxy}-5-methoxy-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal III (732 mg, 1.0 mmol) was dissolved in dichloromethane (5 mL), methanol (10 mL) and added SnCl₂.2H₂O (1.124 g, 5.0 mmol) was refluxed for 1.5 h. The reaction mixture was then carefully adjusted to pH 8 with saturated NaHCO₃ solution and then extracted with ethylacetate (3x20 mL). The combined organic phase was dried over Na₂SO₄ and evaporated under vacuum to afford the crude (2S)-N-{4-[5-(7-Methoxy-(2R)-hydroxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)pentyl]oxy}-5-methoxy-2-aminobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal of formula IV.

A solution of (2S)-N-{4-[5-(7-Methoxy-(2R)-hydroxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)pentyl]oxy}-5-methoxy-2-aminobenzoic]pyrrolidine-2-carboxaldehyde diethyl thioacetal IV. (702 mg, 1 mmol), HgCl₂ (794 mg, 2.93 mmol) and CaCO₃ (300 mg, 3 mmol) in CH₃CN/H₂O (4:1, 15 mL) was stirred at room temperature for 12 h until TLC (EtOAc:methanol), indicates complete loss of starting
material. Then organic layer is evaporated in vacuum and the residue is diluted with EtOAc. To this saturated NaHCO₃ was added slowly at room temperature and the mixture is filtered through celite and washed with ethylacetate. The filtrate is evaporated in vacuum to get crude 7-Methoxy-8-{5-[7-methoxy-(2R)-hydroxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy]butoxy} (11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one of formula V, which was further purified by column chromatography on silica gel eluting with methanol:EtOAc (3:7).

Example 7.

A solution of (4R)-hydroxy-(2S)-N-[4-(3-bromopropoxy)-5-methoxy-2-nitrobenzoyl]-pyrrolidine-2-carboxaldehyde diethyl thioacetal of formula I (537 mg, 1 mmol), 8-hydroxy-7-methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (248 mg, 1 mmol) of the formula II and K₂CO₃ (414 mg, 3 mmol) in dry acetone (20 mL) was refluxed for 48 h. After the completion of reaction as indicated by TLC, EtOAc, the reaction mixture was poured on to the water and then extracted with ethylacetate. Evaporation of the organic layer gave the crude product, which was further purified by column chromatography on silica gel eluting with EtOAc gave the pure (4R)-hydroxy-(2S)-N-[4-[3-(7-Methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)propoxy]-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal III.

The (4R)-hydroxy-(2S)-N-[4-[3-(7-Methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)propoxy]-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal III (704 mg, 1.0 mmol) was dissolved in dichloromethane (5 mL), methanol (10 mL) and added SnCl₂·2H₂O (1.124 g, 5.0 mmol) was refluxed for 1.5 h. The reaction mixture was then carefully adjusted to pH 8 with saturated NaHCO₃ solution and then extracted with ethyl acetate (3x20 mL). The combined organic phase was dried over Na₂SO₄ and evaporated under vacuum to afford the crude (2S)-N-[4-[3-(7-Methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)propoxy]-5-methoxy-2-aminobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal IV

A solution of the (4R)-hydroxy-(2S)-N-[4-[3-(7-Methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)propoxy]-5-methoxy-2-aminobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal IV (674 mg, 1 mmol),
HgCl₂ (794 mg, 2.93 mmol) and CaCO₃ (300 mg, 3 mmol) in CH₂CN/H₂O (4:1, 15 mL) was stirred at room temperature for 12 h until TLC (EtOAc), indicates complete loss of starting material. Then organic layer is evaporated in vacuum and the residue is diluted with EtOAc. To this saturated NaHCO₃ was added slowly at room temperature and the mixture is filtered through celite and washed with ethylacetate. The filtrate is evaporated in vacuum to get crude 7-Methoxy-8-\{3-[7-methoxy-(11αS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy]propoxy\}-(4R)-hydroxy-(11αS)-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one of formula V, which was further purified by column chromatography on silica gel eluting with methanol:EtOAc (3:7).

**Example 8**

A solution of (4R)-hydroxy-(2S)-N-\{4-(4-bromobutoxy)-5-methoxy-2-nitrobenzoyl\}pyrrolidine-2-carboxaldehyde diethyl thioacetal of formula I (551 mg, 1 mmol), 8-hydroxy-7-methoxy-(11αS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (248 mg, 1 mmol) of the formula II and K₂CO₃ (414 mg, 3 mmol) in dry acetone (20 mL) was refluxed for 48 h. After the completion of reaction as indicated by TLC, EtOAc, the reaction mixture was poured on to the water and then extracted with ethylacetate. Evaporation of the organic layer gave the crude product, which was further purified by column chromatography on silica gel eluting with EtOAc gave the pure (4R)-hydroxy-(2S)-N-\{4-(4-(7-Methoxy-(11αS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)butoxy\}-5-methoxy-2-nitrobenzoyl\}pyrrolidine-2-carboxaldehyde diethyl thioacetal III.

The (4R)-hydroxy-(2S)-N-\{4-(4-(7-Methoxy-(11αS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)butoxy\}-5-methoxy-2-nitrobenzoyl\}pyrrolidine-2-carboxaldehyde diethyl thioacetal (718 mg, 1.0 mmol) of the formula III was dissolved in dichloromethane (5 mL), methanol (10 mL) and added SnCl₂·2H₂O (1.124 g, 5.0 mmol) was refluxed for 1.5 h. The reaction mixture was then carefully adjusted to pH 8 with saturated NaHCO₃ solution and then extracted with ethylacetate (3x20 mL). The combined organic phase was dried over Na₂SO₄ and evaporated under vacuum to afford the crude (2S)-N-\{4-(4-(7-Methoxy-(11αS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)butoxy\}-5-methoxy-2-aminobenzoyl\}pyrrolidine-2-carboxaldehyde diethyl thioacetal IV.
A solution of the (4R)-hydroxy-(2S)-N-[4-(7-Methoxy-(11aS))-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy]butoxy]-5-methoxy-2-aminobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal of formula IV. (688 mg, 1 mmol), HgCl2 (794 mg, 2.93 mmol) and CaCO3 (300 mg, 3 mmol) in CH3CN/H2O (4:1, 15 mL) was stirred at room temperature for 12 h until TLC (EtOAc), indicates complete loss of starting material. Then organic layer is evaporated in vacuum and the residue is diluted with EtOAc. To this saturated NaHCO3 was added slowly at room temperature and the mixture is filtered through celite and washed with ethylacetate. The filtrate is evaporated in vacuum to get crude 7-Methoxy-8-(4-[7-methoxy-(11aS))-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy]butoxy)-(4R)-hydroxy-(11aS)-1,2,3,11a-tetrahy-dro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one of formula V, which was further purified by column chromatography on silica gel eluting with methanol:EtOAc (2:8).

Example 9

A solution of (4R)-hydroxy-(2S)-N-[4-(5-bromopentloxy)-5-methoxy-2-nitrobenzoyl]-pyrrolidine-2-carboxaldehyde diethyl thioacetal of formula I. (565 mg, 1 mmol), 8-hydroxy-7-methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one (248 mg, 1 mmol) II and K2CO3 (414 mg, 3 mmol) in dry acetone (20 mL) was refluxed for 48 h. After the completion of reaction as indicated by TLC, EtOAc, the reaction mixture was poured on to the water and then extracted with ethylacetate. Evaporation of the organic layer gave the crude product, which was further purified by column chromatography on silica gel eluting with EtOAc gave the pure (4R)-hydroxy-(2S)-N-[4-(5-(7-Methoxy-(11aS))-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)pentloxy]-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal III.

The (4R)-hydroxy-(2S)-N-[4-(5-(7-Methoxy-(11aS))-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)pentloxy]-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal III (732 mg, 1.0 mmol) was dissolved in dichloromethane (5 mL), methanol (10 mL) and added SnCl2.2H2O (1.124 g, 5.0 mmol) was refluxed for 1.5 h. The reaction mixture was then carefully adjusted to pH 8 with saturated NaHCO3 solution and then extracted with ethyl acetate (3x20 mL). The combined organic phase was dried over Na2SO4 and evaporated under vacuum to afford the crude
(4R)-hydroxy-(2S)-N-{4-[5-(7-Methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-
pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)pentyloxy]-5-methoxy-2-aminobenzo-
yl}pyrrolidine-2-carboxaldehyde diethyl thioacetal of formula IV.

A solution of (4R)-hydroxy-(2S)-N-{4-[5-(7-Methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-
5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one-8-yloxy)pentyloxy]-5-methoxy-2-aminobenzo-
yl}pyrrolidine-2-carboxaldehyde diethyl thioacetal IV. (702 mg, 1 mmol), HgCl2 (794
mg, 2.93 mmol) and CaCO3 (300 mg, 3 mmol) in CH3CN/H2O (4:1, 15 mL) was stirred at
room temperature for 12 h until TLC (EtOAc), indicates complete loss of starting material.
Then organic layer is evaporated in vacuum and the residue is diluted with EtOAc. To this
saturated NaHCO3 was added slowly at room temperature and the mixture is filtered
thorough celite and washed with ethylacetate. The filtrate is evaporated in vacuum to get
7-Methoxy-8-{5-[7-methoxy-(11aS)-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-
c][1,4]benzodiazepine-5-one-8-yloxy]butoxy}-(4R)-hydroxy-(11aS)-1,2,3, 11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepine-5-one of formula V, which was
further purified by column chromatography on silica gel eluting with methanol:EtOAc
(2:8).

Biological Activity. In vitro biological activity studies were carried out at National Cancer
Institute (USA).

Cytotoxicity. Compounds Va and Vc were evaluated in vitro against sixty human tumour
50
cells derived from nine cancer types (leukemia, non-small-cell lung, colon, CNS,
melanoma, ovarian, prostate, and breast cancer). For each compound, dose response curves
for each cell line were measured at a minimum of five concentrations at 10 fold dilutions.
A protocol of 48 h continuous drug exposure was used, and a sulforhodamine B (SRB)
protein assay was used to estimate cell viability or growth. The concentration causing 50 %
cell growth inhibition (GI50), total cell growth inhibition (TGI 0% growth) and 50% cell
deadth (LC50, -50% growth) compared with the control was calculated. The mean graph
midpoint values of log10 TGI and log10 LC50 as well as log10 GI50 for Va and Vc are listed
in Table 1. As demonstrated by mean graph pattern, compound Vlc exhibits an interesting
profile of activity and selectivity for various cell lines. The mean graph mid point of log10
TGI and log10 LC50 showed similar pattern to the log10 GI50 mean graph mid points.

Table 1. log10 GI50 log10 TGI and log10 LC50 mean graphs midpoints(MG_MID) of in
vitro Cytotoxicity data for the compounds Va and Vc against human tumour cell lines.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Log$_{10}$ GI50</th>
<th>Log$_{10}$ TGI</th>
<th>Log$_{10}$ LC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Va</td>
<td>-6.80</td>
<td>-5.73</td>
<td>-4.48</td>
</tr>
<tr>
<td>Vc</td>
<td>-5.29</td>
<td>-4.55</td>
<td>-4.15</td>
</tr>
</tbody>
</table>

**Table 2.** Log LC50 (concentration in mol/L causing 50% lethality) Values for Compounds Va and Vc

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Compound Va</th>
<th>Compound Vc</th>
</tr>
</thead>
<tbody>
<tr>
<td>leukemia</td>
<td>-4.08</td>
<td>-4.46</td>
</tr>
<tr>
<td>non-small-cell lung</td>
<td>-4.41</td>
<td>-4.14</td>
</tr>
<tr>
<td>colon</td>
<td>-4.59</td>
<td>-4.04</td>
</tr>
<tr>
<td>CNS</td>
<td>-4.49</td>
<td>-4.09</td>
</tr>
<tr>
<td>melanoma</td>
<td>-5.45</td>
<td>-4.42</td>
</tr>
<tr>
<td>ovarian</td>
<td>-4.15</td>
<td>-4.01</td>
</tr>
<tr>
<td>renal</td>
<td>-4.20</td>
<td>-4.02</td>
</tr>
<tr>
<td>prostate</td>
<td>-4.08</td>
<td>-4.00</td>
</tr>
<tr>
<td>Breast</td>
<td>-4.36</td>
<td>-4.05</td>
</tr>
</tbody>
</table>

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

The anticancer activity for two representative compounds has been given in Table 2. The comparison of the data of Table 2 reveals the importance of the alkane spacer. As the alkane spacer increased from 3-5 the cytotoxic activity has moderately decreased. The 3 carbon spacer of compound Va confers a suitable fit in the minor groove of double helix DNA and shows slightly higher activity in this series of compounds Va and Vc.
Claims

1. A pyrrolo[2,1-c][1,4]benzodiazepine of general formula V,

\[
\begin{array}{c}
\text{H} \\
\text{N} \\
\text{O} \\
\text{O} \\
\text{OCH}_3 \\
\text{H}_2\text{CO} \\
\text{N} \\
\text{N} \\
\text{H} \\
\text{R} \\
\text{R}_1
\end{array}
\]

where R and R₁ is H and/or OH; and n is 3 to 5.

2. A pyrrolobenzodiazepine as claimed in claim 1 has structural formula shown below where \( R = R_1 = H \) and \( n = 3 \).

\[
\begin{array}{c}
\text{H} \\
\text{N} \\
\text{O} \\
\text{(CH}_2\text{)}_3 \\
\text{OCH}_3 \\
\text{H}_2\text{CO} \\
\text{N} \\
\text{H}
\end{array}
\]

3. A pyrrolobenzodiazepine as claimed in claim 1 has structural formula shown below, where \( R = R_1 = H \) and \( n = 4 \).

\[
\begin{array}{c}
\text{H} \\
\text{N} \\
\text{O} \\
\text{(CH}_2\text{)}_4 \\
\text{OCH}_3 \\
\text{H}_2\text{CO} \\
\text{N} \\
\text{H}
\end{array}
\]

4. A pyrrolobenzodiazepine as claimed in claim 1 has structural formula shown below, where \( R = R_1 = H \) and \( n = 5 \).

\[
\begin{array}{c}
\text{H} \\
\text{N} \\
\text{O} \\
\text{(CH}_2\text{)}_5 \\
\text{OCH}_3 \\
\text{H}_2\text{CO} \\
\text{N} \\
\text{H}
\end{array}
\]

5. A pyrrolobenzodiazepine as claimed in claim 1 has structural formula shown below where \( R = \text{OH} \), \( R_1 = H \) and \( n = 3 \).

\[
\begin{array}{c}
\text{H} \\
\text{N} \\
\text{O} \\
\text{(CH}_2\text{)}_6 \\
\text{OCH}_3 \\
\text{H}_2\text{CO} \\
\text{N} \\
\text{H}
\end{array}
\]
6. A novel pyrrolobenzodiazepine as claimed in claim 1 has structural formula shown below, where $R = \text{OH}, R_1 = \text{H}$ and $n = 4$.

7. A novel pyrrolobenzodiazepine as claimed in claim 1 has structural formula shown below where $R = \text{OH}, R_1 = \text{H}$ and $n = 5$.

8. A novel pyrrolobenzodiazepine as claimed in claim 1 has structural formula shown below, where $R = \text{H}, R_1 = \text{OH}$ and $n = 3$.

9. A novel pyrrolobenzodiazepine as claimed in claim 1 has structural formula shown below, where $R = \text{H}, R_1 = \text{OH}$ and $n = 4$.

10. A novel pyrrolobenzodiazepine as claimed in claim 1 has structural formula shown below, where $R = \text{H}, R_1 = \text{OH}$ and $n = 5$.

11. The compounds as claimed in claim 1 are active against human tumor cell lines.

12. The compounds as claimed in claim 11 wherein the cell lines are selected from various types of cancers like leukemia, non-small-cell lung, colon, CNS, melanoma, ovarian, prostate and breast cancer.
13. A process for the preparation of compounds as claimed in claim 1, the said process comprising steps of:
   a) reacting a compound of formula (I) with formula (II) in presence of mild base in water miscible aprotic organic solvent at reflux temperature for a period of 16h to 48h,
   b) pouring the reaction mixture of step (a) into water, extracting with ethyl acetate separating organic layer and discarding aqueous layer,
   c) evaporating the organic layer of step (b) to obtain a residue which is purified to get compound of formula (III),
   d) reducing the nitro compound of formula (III) in halogenated solvent, adding stannous chloride dihydrate, refluxing for a period of 0.5h to 2h,
   e) adjusting the pH of the reaction mixture of step (d) to 8.0 using alkali bicarbonate solution,
   f) extracting the step(e) solution with ethylacetate, evaporating the ethylacetate extract under reduced pressure to obtain crude compound of formula (IV),
   g) providing a solution of compound of formula (IV) in a mixture of acetonitrile/H₂O adding mercuric chloride, calcium carbonate, stirring at room temperature for 6h to 12h,
   h) filtering and evaporating the organic layer under reduced pressure to obtain a residue which is diluted with ethyl acetate,
   i) adding saturated solution of sodium bicarbonate to ethylacetate solution of step (h) at room temperature,
   j) filtering the solution of step (i) through celite bed, evaporating the filtrate to obtain a residue containing crude compound of formula V, and
   k) purifying crude compound of step (j) using silica gel as an adsorbent to obtain pure compound of formula (V) wherein R and R₁ are as defined earlier.

14. A process as claimed in claim 13, wherein in step (a) the aprotic organic solvent used is selected from a group consisting of tetrahydrofuran, acetone or dimethyl formamide.

15. A process as claimed in claim 13, wherein in step (a) the mild base used is selected from a group of sodium carbonate, potassium carbonate, lithium carbonate, barium carbonate and cesium carbonate.
16. A process as claimed in claim 13, wherein in step (d) the halogenated solvent used is selected from a group consisting of carbon tetra chloride, chloroform and dichloromethane.

17. The process as claimed in claim 16, wherein the solvent used is dichloromethane.

18. The process as claimed in claim 13, wherein in step (c) the alkali carbonate solution used is selected from a solution of sodium bicarbonate potassium bicarbonate or lithium bicarbonate.

19. Pharmaceutical composition effective against human cancer cell lines, said composition comprising effective amount of compound pyrrolo[2,1-c][1,4]benzodiazepine of general formula V, where R and R₁ is H and/or OH; and n is 3 to 5, along with pharmaceutically acceptable additives.

20. The composition as claimed in claim 19, wherein the composition is administered to mammals including human beings.

21. The composition as claimed in claim 19 is administered orally, systemically or by any other conventional methods.

22. The composition as claimed in claim 19, wherein pharmaceutically acceptable additives are selected from a group consisting of carriers, diluents, solvents, filler, lubricants, excipients, binders and stabilizers.

23. The composition as claimed in claim 19 wherein, the composition inhibits the growth of cancer cells.

24. The composition as claimed in claim 23, wherein cancer cells are leukemia, non-small-cell lung, colon, CNS, melanoma, ovarian, prostate and breast cancer.
Figure 1

Br(CH₂)ₙO  NO₂  (CH(SEt)₂)       HO  NO₂
H₃CO     N        H      H₃CO
R₁       R       R₁

I       II

K₂CO₃, Acetone

III

SnCl₂·2H₂O

IV

HgCl₂/CaCO₃

V

R = R₁ = H, OH
n = 3-5
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7: C07D519/00 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)

IPC 7: C07D A61K A61P

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, PAJ, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>EP 1 193 270 A (SPIROGEN LTD) 3 April 2002 (2002-04-03) Abstract; page 3, paragraph (0001); page 5, paragraph (0020), to page 7, paragraph (0036); and e.g. examples 3d (page 68), 4a-d (pages 82-86).</td>
<td>1-24</td>
</tr>
</tbody>
</table>

X Further documents are listed in the continuation of box C.

X Patent family members are listed in annex.

* Special categories of cited documents:

*A* document defining the general state of the art which is not considered to be of particular relevance

*E* earlier document but published on or after the international filing date

*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

*P* document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is combined with one or more other such documents, each combination being obvious to a person skilled in the art.

"O" document member of the same patent family

Date of the actual completion of the international search: 31 July 2003

Date of mailing of the international search report: 13/08/2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HZ Rijswijk
Tel. (+31-70) 940-5040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016

Authorized officer: Weisbrot, T
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patent document cited in search report</td>
<td>Publication date</td>
<td>Patent family member(s)</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-----------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AT 240334 T</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 757510 B2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 5635199 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2341471 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DE 69907977 D1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 0012508 A2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2002525285 T</td>
</tr>
</tbody>
</table>