Electronic supplementary Information

Design, Synthesis and Biological Evaluation of Imidazo[1,5-\(a\)]pyridine-PBD Conjugates as Potential DNA-directed Alkylating Agents

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General

(A) Chemistry

All the chemicals and reagents were obtained from Aldrich (Sigma-Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthew Company, Ward Hill, MA, USA) and were used without purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254, and visualization on TLC was achieved by UV light or iodine indicator. Column chromatography was performed with Merck 60-120 mesh silicagel. $^1$H NMR spectra were recorded on Gemini Varian VXR-unity (400 and 500 MHz) or Bruker UXNMR/XWIN-NMR (300 MHz) instruments. Chemical shifts ($\delta$) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI$^+$ software with capillary voltage 3.98 KV and ESI mode positive ion trap detector. High-resolution mass spectra (HRMS) were recorded on QSTAR XL Hybrid MS/MS mass spectrometer. Melting points were determined with an Electrothermal melting point apparatus, and are uncorrected. Optical rotations were measured on Horiba, high sensitive polarimeter, SEPA-300. High-performance liquid chromatography analyses for checking purity of synthesized compounds were recorded on a shimazu SPD-10A (uv-vis detector)LC-10AT instrument: column, Luna 5u C18(2) 250*4.60 mm Mightysil RP-18 GP 250–4.6 (5 lm); mobile phase, 70% A and 30% B in 25 min (mobile phase A = acetonitrile, B = water); flow rate, 1 mL/min; injected sample, 10 µL; column temp, 27 °C; wavelength, 289 nm. The purity of all the compounds was $\geq$90% based on analytical HPLC.

(B) Biology

(a) Cell culture

MCF-7 (breast carcinoma cells) were incubated by using DMEM media, supplemented with 10% fetal calf serum, 100 µg/mL pencillin-G and 100 µg/mL streptomycin sulfate. The cell line was maintained at 37 °C in a humidified atmosphere containing 5% CO$_2$ in the incubator.

(b) MTT cell viability assay

Cell viability was assessed by the MTT based assay, a mitochondrial function assay. It is based on the ability of viable cells to reduce the MTT to insoluble formazan crystals by mitochondrial dehydrogenase. In this assay MCF-7 cells were seeded in a 96-well plate at a density of 10,000 cells/well. After overnight incubation cells were treated with 1, 13a-13l at 4 µM concentration and incubated for 24 h. Then the medium was discarded and replaced with
10 μL MTT dye. Plates were incubated at 37°C for 2 h. The resulting formazan crystals were solubilized in 100 μL extraction buffer. The optical density (O.D) was recorded at 570 nm with micro plate reader.

(e) Thermal denaturation studies

The compounds 13a–l were subjected to DNA thermal melting (denaturation) studies using duplex form calf thymus DNA (CT-DNA) using modification reported procedure. Working solutions were produced by appropriate dilution in aqueous buffer (10 mM NaH₂PO₄/NaH₂PO₄, 1 mM Na₂EDTA, pH 7.00±0.01) containing CT-DNA, (100 μM in phosphate) and the PBD (20 μM) were prepared by addition of concentrated PBD solutions in methanol to obtain a fixed [PBD]/[DNA] molar ratio of 1:5. The DNA-PBD solutions were incubated at 37 °C for 0 h and 18 h prior to analysis. Samples were monitored at 260 nm using a Beckman DU-7400 spectrophotometer fitted with high performance temperature controller. Heating was applied at a rate of 1 °C min⁻¹ in the 40–90 °C range. DNA helix-coil transition temperatures (T_m) were determined from the maxima in the d(A260)/dT derivative plots. Results for each compound are shown as mean ± standard derivation from the least three determinations and are corrected for the effects of methanol cosolvent using a linear correction term. Ligand-induced alteration in DNA melting behavior are given by \( \Delta T_m = T_m(DNA+PBD) - T_m(DNA alone) \), where the \( T_m \) value for the PBD free CT-DNA is 69.1 ± 0.001 the fixed [PBD]/[DNA] ratio used did not result in binding saturation of the host DNA duplex for any compound examined.

(d) Cell cycle analysis

5 X 10⁵ MCF-7 cells were seeded in 60 mm dish and allowed to grow for 24 h, 2 and 4 μM concentration of 1, 13 a-13l compounds were added to the culture media, and the cells were incubated for an additional 24 h. Cells were harvested with Trypsin-EDTA, fixed with ice-cold 70% ethanol at 4 °C for 30 min, washed with PBS and incubated with 1mg/mL RNAase solution (Sigma) at 37 °C for 30 min. Cells were collected by centrifugation at 2000 rpm for 5 min and further stained with 250 μL of DNA staining solution [10 mg of Propidium Iodide (PI), 0.1 mg of trisodium citrate, and 0.03 mL of Triton X-100 were dissolved in 100 mL of sterile MilliQ water at room temperature for 30 min in the dark]. The DNA contents of 20,000 events were measured by flow cytometer (DAKO CYTOMATION, Beckman Coulter, Brea, CA). Histograms were analyzed by using Summit Software.
(e) **Immunofluorescence microscopy studies for γH2AX staining**

MCF-7 cells were seeded on glass cover slips, incubated for 24 h in the presence or absence of test compounds 1 and 13g at 4 µM concentration. Following the termination of incubation, cells were fixed with 4% paraformaldehyde, 0.02% glutaraldehyde in PBS and permeabilized by dipping the cells in 100% methanol (-20 °C). Later, cover slips were blocked with 1% BSA in phosphate buffered saline for 1 h followed by incubation with a primary antibody (γH2AX) followed by secondary antibody. At the end, cells were washed and fixed. Photographs were taken by using the confocal microscope (Olympus).

(f) **Protein extraction and Western blot analysis**

5 X 10^5 MCF-7 cells were seeded in 60 mm dish and allowed to grow for 24 h, 4 µM concentration of 1, 13f and 13g compounds were added to the culture media, and the cells were incubated for an additional 24 h. Total cell lysates from cultured MCF-7 cells were obtained by lysing the cells in ice-cold RIPA buffer (1X PBS, 1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS) and containing 100 µg/mL PMSF, 5 µg/mL Aprotinin, 5 µg/mL leupeptin, 5 µg/mL pepstatin and 100 µg/mL NaF. After centrifugation at 12,000 rpm for 10 min, the protein in supernatant was quantified by Bradford method (BIO-RAD) using Multimode variaskan instrument (Thermo-Fischer Scientifics). Fifty micrograms of protein per lane was applied in 12% SDS-polyacrylamide gel. After electrophoresis, the protein was transferred to polyvinylidine difluoride (PVDF) membrane (Amersham Biosciences). The membrane was blocked at room temperature for 2 h in 1X TBS + 0.1% Tween20 (TBST) containing 5% blocking powder (Santacruz). The membrane was washed with TBST for 5 min, and primary antibody was added and incubated at 4 °C overnight. P53, p21 antibodies were purchased from Millipore Company. β-actin was purchased from Imgenex Company. Membranes were washed with TBST three times for 15 min and the blots were visualized with chemiluminescence reagent (Thermo Fischer Scientifics Ltd.). The X-ray films were developed with developer and fixed with fixer solution purchased from Kodak Company.

(g) **p53 ELISA**

Enzyme-linked immunorsorbent assays (ELISA) for p53 were conducted by the p53 ELISA kit from Alexis Biochemical. MCF-7 cells were treated with compounds DC-81 (1), 13f and 13g at 4 µM concentration for 24 h. Cell lysates were isolated and added to microplate wells containing p53 antibody. Biotin-conjugated anti-human p53 monoclonal antibody (100 µL) was added. After the incubation period and washing steps, bound p53 was
detected by using streptavidin–HRP secondary antibody (150 µL). The coloured product obtained was detected by measuring OD at 450 nm. OD is directly proportional to the amount of p53 protein present in the sample.
**Supplementary Table 1:** IC$_{50}$ values of compounds 13a-l in MCF7 cell line.

<table>
<thead>
<tr>
<th>Compd</th>
<th>IC$_{50}$ in µM</th>
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<tbody>
<tr>
<td>DC-81</td>
<td>5 ± 0.012</td>
</tr>
<tr>
<td>13a</td>
<td>7.21 ± 0.09</td>
</tr>
<tr>
<td>13b</td>
<td>7.18 ± 0.07</td>
</tr>
<tr>
<td>13c</td>
<td>4.16 ± 0.021</td>
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<tr>
<td>13d</td>
<td>3.74 ± 0.03</td>
</tr>
<tr>
<td>13e</td>
<td>5.74 ± 0.04</td>
</tr>
<tr>
<td>13f</td>
<td>3.26 ± 0.07</td>
</tr>
<tr>
<td>13g</td>
<td>3.16 ± 0.1</td>
</tr>
<tr>
<td>13h</td>
<td>4.28 ± 0.04</td>
</tr>
<tr>
<td>13i</td>
<td>6 ± 0.09</td>
</tr>
<tr>
<td>13j</td>
<td>4.17 ± 0.08</td>
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<tr>
<td>13k</td>
<td>5.67 ± 0.10</td>
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<tr>
<td>13l</td>
<td>3.68 ± 0.011</td>
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**Supplementary Table 2:**

<table>
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<th>Complex</th>
<th>Binding energy</th>
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<td>DNA-13a</td>
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<tr>
<td>DNA-13b</td>
<td>-10.35</td>
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<tr>
<td>DNA-13c</td>
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<tr>
<td>DNA-13l</td>
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</tbody>
</table>
Spectral data and procedures for compound preparations

**Ethyl 2-hydroxyimino-2-(2-pyridyl)acetate (2)**

A solution of 495 mg (3 mmol) of ethyl (2-pyridyl) acetate (1) in 10 mL of glacial acetic acid was cooled in an ice bath and 0.207 mg (3.2 mmol) of sodium nitrite in 5 mL of water was added over a period of 20 minutes, keeping the temperature between 15 to 25 °C. The mixture was stirred for an additional 30 minutes, 50 mL of water was added, and the stirring continued for two more hours. The crystals were washed with water, washed three times with 5% sodium bicarbonate solution and again with water. Recrystallization from methanol yielded 0.488 mg (84%) of white needles. mp 147−152 °C; $^1$H NMR (300 MHz, CDCl$_3$): δ 7.65 (s, 1H), 6.95-6.89 (m, 2H), 6.75 (s, 1H), 3.98 (q, 2H, $J = 6.7$ Hz), 1.54 (t, 3H, $J = 6.7$ Hz); MS (ESI): m/z 195 (M$^+$+1).

**Ethyl 2-amino-2-(2-pyridyl)acetate (3)**

To a solution of ethyl 2-hydroxyimino-2-(2-pyridyl)acetate (2) (0.194 mg, 1 mmol) in 20 mL of methanol was added catalytic amount of 10% palladium on activated carbon. The mixture was stirred overnight under hydrogen, the reaction mixture was filtered, and the filtrate was evaporated. The crude product 3 was used directly in next step without further purification.

**Ethyl 2-(benzoylamino)-2-(2-pyridyl)acetate (5a)**

To a solution containing ethyl 2-amino-2-(2-pyridyl)acetate (3) (0.180 mg, 1 mmol), triethylamine (3 mmol) in 20 mL of dichloromethane, benzoyl chloride (4a) (1 mmol) in 10 mL of dichloromethane was added under nitrogen and stirred at room temperature for about 5 h till the completion of the reaction as monitored by TLC. The reaction mixture was washed with water, extracted with dichloromethane, dried over anhydrous sodium sulphate and subjected to column chromatography using ethyl acetate/hexane (7:3) as an eluent to give compound (5a). $^1$H NMR (300 MHz, CDCl$_3$): δ 8.30 (d, 1H, $J = 7.0$ Hz), 8.22 (d, 1H, $J = 8.7$ Hz), 7.90 (d, 2H, $J = 6.7$ Hz), 7.22-7.34 (m, 3H), 6.98 (s, 1H), 6.73 (t, 1H, $J = 6.7$ Hz), 5.66 (d, 1H, $J = 6.5$ Hz), 4.39 (q, 2H, $J = 6.8$ Hz), 1.52 (t, 3H, $J = 6.8$ Hz); MS (EI): m/z 285 (M$^+$+1).

**Ethyl 2-(2-pyridyl)-2-[4-(trifluoromethyl)benzoyl]aminoacetate (5b)**

The compound 5b was prepared according to the method described for the compound 5a by employing compound 4b. $^1$H NMR (300 MHz, CDCl$_3$): δ 8.56 (d, 1H, $J = 6.7$ Hz), 8.11 (d, 1H, $J = 7.5$ Hz), 8.01 (d, 2H, $J = 8.3$ Hz), 7.70 (d, 2H, $J = 8.3$ Hz), 7.58 (d, 1H, $J = 7.5$ Hz), 7.31-7.25 (m, 1H), 5.77 (d, 1H, $J = 6.7$ Hz), 4.21 (q, 2H, $J = 7.5$ Hz), 3.92 (s, 3H), 1.25 (t, 3H, $J = 7.5$ Hz); MS (ESI): m/z 343 (M$^+$+1).
Ethyl 2-[(4-methoxybenzoyl)amino]-2-(2-pyridyl)acetate (5c)
The compound 5c was prepared according to the method described for the compound 5a by employing compound 4c (1 mmol). \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta \) 8.33 (m, 2H), 7.82 (d, 2H, \(J = 6.7 \) Hz), 7.16 (dd, 1H, \(J = 9.8, 6.8 \) Hz), 6.82 (d, 2H, \(J = 6.7 \) Hz), 6.67 (d, 1H, \(J = 6.2 \) Hz), 5.47 (d, 1H, \(J = 6.5 \) Hz), 4.32 (q, 2H, \(J = 6.9 \) Hz), 3.92 (s, 3H), 1.52 (t, 3H, \(J = 6.9 \) Hz); MS (EI): \(m/z \) 315 (M\(^{+}\)+1).

Ethyl 3-phenylimidazo[1,5-a]pyridine-1-carboxylate (6a)
To ethyl 2-(benzoylamino)-2-(2-pyridyl)acetate 5a (0.284 mg, 1 mmol), add 4 ml of POCl\(_3\) and reflux for three hours. This was poured into cold water and neutralized with NaHCO\(_3\) solution. This water layer was extracted three times with ethylacetate. The combined organic phases were dried over anhydrous Na\(_2\)SO\(_4\) and evaporated under vacuum. The residue, thus obtained was purified by column chromatography using ethylacetate and hexane as eluant to afford compound 6a (212 mg, 80%). mp 136−140 °C ; \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta \) 8.29 (d, 1H, \(J = 7.1 \) Hz), 8.26 (d, 1H, \(J = 9.0 \) Hz), 7.77 (d, 2H, \(J = 6.7 \) Hz), 7.42-7.54 (m, 3H), 7.09 (dd, 1H, \(J = 6.6, 6.4 \) Hz), 6.73 (t, 1H, \(J = 6.6 \) Hz), 4.46 (q, 2H, \(J = 6.9 \) Hz), 1.47 (t, 3H, \(J = 6.9 \) Hz); MS (ESI): \(m/z \) 267 (M\(^{+}\)+1).

Ethyl 3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridine-1-carboxylate (6b)
The compound 6b was prepared according to the method described for the compound 6a by employing compound 5b (352 mg, 1 mmol). Yield: 250 mg, 75%; \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta \) 8.30 (dd, 2H, \(J = 9.6, 6.7 \) Hz), 7.90 (d, 2H, \(J = 7.7 \) Hz), 7.70 (d, 2H, \(J = 8.6 \) Hz), 7.13 (dd, 1H, \(J = 5.7, 6.7 \) Hz), 6.80 (t, 1H, \(J = 6.7 \) Hz), 4.47 (q, 2H, \(J = 6.7 \) Hz), 1.47 (t, 3H, \(J = 6.7 \) Hz); MS (ESI): \(m/z \) 335 (M\(^{+}\)+1).

Ethyl 3-(4-methoxyphenyl)imidazo[1,5-a]pyridine-1-carboxylate (6c)
The compound 6c was prepared according to the method described for the compound 6a by employing compound 5c (314 mg, 1 mmol). Yield: 216 mg, 73%; \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta \) 8.21-8.24 (m, 2H), 7.69 (d, 2H, \(J = 9.0 \) Hz), 7.04 (dd, 1H, \(J = 9.8, 6.7 \) Hz), 6.98 (d, 2H, \(J = 8.3 \) Hz), 6.70 (t, 1H, \(J = 7.5 \) Hz), 4.45 (q, 2H, \(J = 6.7 \) Hz), 3.87 (s, 3H), 1.46 (t, 3H, \(J = 6.7 \) Hz); MS (ESI): \(m/z \) 297 (M\(^{+}\)+1).

3-Phenylimidazo[1,5-a]pyridine-1-carboxylic acid (7a)
2N sodium hydroxide (1.22 mL) was added to a solution of 6a (266 mg, 1 mmol) in THF-H\(_2\)O-MeOH (4:1:1) and the mixture stirred at room temperature for 12 h. After most of the THF and methanol were evaporated, the aqueous phase was acidified with 12N HCl to pH 7
and re-extracted with CH₂Cl₂ to give compound 7a in quantitative yield. This compound was directly used in next step.

3-[4-(Trifluoromethyl)phenyl]imidazo[1,5-a]pyridine-1-carboxylic acid (7b)
This compound was prepared according to the method described for the compound 7a by employing compound 6b (334 mg, 1 mmol) and it was directly used for the next step.

3-(4-Methoxyphenyl)imidazo[1,5-a]pyridine-1-carboxylic acid (7c)
This compound was prepared according to the method described for the compound 7a by employing compound 6c (296 mg, 1 mmol) and it was directly used for the next step.

tert-Butyl 4-[(3-phenylimidazo[1,5-a]pyridin-1-yl)carbonyl]-1-piperazinecarboxylate (8a)
Dichlormethane (10 mL) solution of compound 7a (1 mmol) was charged with EDC (1 mmol), HOBt (1mmol) at 0 °C. After twenty minutes Boc protected piperazine dissolved in dichlomethne was added. The solution was stirred at room temperature for 16 h under an atmosphere of nitrogen and then diluted with dichloromethane (20 mL), washed with saturated aqueous sodium bicarbonate solution (50 mL), brine (50 mL), dried over anhydrous sodium sulphate, filtered, and concentrated in vacuo. The crude product was purified by silica gel chromatography using ethylacetate and hexane as eluant to afford compound 8a (345 mg, 85%). mp 166−170 °C ; ¹H NMR (300 MHz, CDCl₃): δ 8.32 (d, 1H, J = 9.0 Hz), 8.29 (d, 1H, J = 7.1 Hz), 7.75 (d, 2H, J = 7.3 Hz), 7.42-7.54 (m, 3H), 6.99 (t, 1H, J = 6.9 Hz), 6.70 (t, 1H, J = 6.9 Hz), 4.49 (brs, 2H), 3.78 (brs, 2H), 3.53 (s, 4H), 1.47 (s, 9H); MS (ESI): m/z 407 (M⁺+1).

tert-Butyl 4-[(3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-ylcarbonyl)-1-piperazinecarboxylate (8b)
The compound 8b was prepared according to the method described for the compound 8a by employing compound 7b (306 mg, 1 mmol).
Yield: 388 mg, 82%; ¹H NMR (300 MHz, CDCl₃): δ 8.37 (d, 1H, J = 9.0 Hz), 8.29 (d, 1H, J = 7.5 Hz), 7.90 (d, 2H, J = 8.3 Hz), 7.70 (d, 2H, J = 9.0 Hz), 7.05 (dd, 1H, J = 6.7, 5.2 Hz), 6.79 (t, 1H, J = 7.5 Hz), 4.31-4.59 (brs, 2H), 3.70-3.88 (brs, 2H), 3.60-3.49 (m, 4H), 1.47 (s, 9H); MS (ESI): m/z 475 (M⁺+1).

tert-Butyl 4-[3-(4-methoxyphenyl)imidazo[1,5-a]pyridin-1-yl]carbonyl-1-piperazinecarboxylate (8c)
The compound 8c was prepared according to the method described for the compound 8a by employing compound 7c (268 mg, 1 mmol).
Yield: 305 mg, 70%; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.31 (d, 1H, $J = 8.6$ Hz), 8.20 (d, 1H, $J = 7.6$ Hz), 7.64 (d, 2H, $J = 8.6$ Hz), 7.01 (d, 2H, $J = 8.6$ Hz), 6.96 (dd, 1H, $J = 6.7, 8.6$ Hz), 6.68 (t, 1H, $J = 6.7$ Hz), 4.33-4.64 (brs, 2H), 3.88 (s, 3H), 3.65-3.84 (brs, 2H), 3.53 (s, 4H), 1.47 (s, 9H); MS (ESI): m/z 437 (M$^+$+1).

(3-Phenylimidazo[1,5-a]pyridin-1-yl)(piperazino)methanone (9a)

To a solution of Boc-protected compound 8a (406 mg, 1 mmol) in dry dichloromethane was added trifluoroacetic acid (1.2 mL, 15.8 mmol) at 0 °C and stirred under nitrogen for 8 h, the reaction mixture was then concentrated in vacuum to afford the compound 9a and then it was used directly in the next step.

Piperazino3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-ylmethanone (9b)

This compound was prepared according to the method described for the compound 9a by employing compound 8b (474 mg, 1 mmol) and it was directly used for the next step.

[3-(4-Methoxyphenyl)imidazo[1,5-a]pyridin-1-yl](piperazino)methanone (9c)

This compound was prepared according to the method described for the compound 9a by employing compound 8c (436 mg, 1 mmol) and it was directly used for the next step.

(2S)-N-[4-(3-Bromopropoxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal (11a)

To a solution of compound 10 (400 mg, 1 mmol) in dry acetone (15 ml) was added, anhydrous K$_2$CO$_3$ (553 mg, 4 mmol), 1,3-dibromopropane (256 mg, 1.2 mmol) and the mixture was stirred at reflux temperature for 48 h. The reaction was monitored by TLC using EtOAc-hexane (1:1). After completion of the reaction as indicated by TLC, K$_2$CO$_3$ was removed by filtration and the solvent was evaporated under reduced pressure, diluted with water and extracted with ethyl acetate. The combined organic phases were dried over Na$_2$SO$_4$ and evaporated under vacuum. The residue, thus obtained was purified by column chromatography using ethyl acetate and hexane (2:3) to afford compound 11a as yellow liquid (500 mg, 96%). $^1$H NMR (CDCl$_3$, 200 MHz): $\delta$ 7.65 (s, 1H), 6.80 (s, 1H), 4.86 (d, 1H, $J = 4.3$ Hz), 4.72-4.61 (m, 1H), 4.25 (t, 2H, $J = 6.0$ Hz), 3.95 (s, 3H), 3.65-3.55 (m, 2H), 3.31-3.15 (m, 2H), 2.90-2.60 (m, 4H), 2.40-1.70 (m, 6H), 1.45-1.21 (m, 6H); MS (ESI): m/z 521 (M$^+$+1)$^+$.

(2S)-N-[4-(4-Bromobutoxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal (11b)

The compound 11b was prepared according to the method described for compound 11a by employing 10 (400 mg, 1 mmol) and 1,4-dibromobutane (268 mg, 1.2 mmol) to afford the
compound 11b (492 mg, 92%). \(^1\)H NMR (CDCl\(_3\), 200 MHz): \(\delta \) 7.65 (s, 1\( \text{H} \)), 6.80 (s, 1\( \text{H} \)), 4.87 (d, 1\( \text{H} \), \(J = 4.3 \) Hz), 4.71-4.60 (m, 1\( \text{H} \)), 4.15 (t, 2\( \text{H} \), \(J = 6.0 \) Hz), 3.95 (s, 3\( \text{H} \)), 3.59-3.42 (m, 2\( \text{H} \)), 3.30-3.15 (m, 2\( \text{H} \)), 2.85-2.65 (m, 4\( \text{H} \)), 2.40-1.60 (m, 8\( \text{H} \)), 1.40-1.21 (m, 6\( \text{H} \)); MS (ESI): \(m/z\) 536 (M+1\(^+\)).

**(2S)-N-[4-(5-Bromopentyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal (11c)**

The compound 11c was prepared according to the method described for compound 11a by employing 10 (400 mg, 1 mmol) and 1,5-dibromopentane (270 mg, 1.2 mmol) to afford the compound 11c (522 mg, 94%). \(^1\)H NMR (CDCl\(_3\), 200 MHz): \(\delta \) 7.64 (s, 1\( \text{H} \)), 6.80 (s, 1\( \text{H} \)), 4.83 (d, 1\( \text{H} \), \(J = 4.3 \) Hz), 4.72-4.62 (m, 1\( \text{H} \)), 4.15-4.05 (t, 2\( \text{H} \), \(J = 6.0 \) Hz), 3.95 (s, 3\( \text{H} \)), 3.51-3.43 (m, 2\( \text{H} \)), 3.30-3.16 (m, 2\( \text{H} \)), 2.85-2.65 (m, 4\( \text{H} \)), 2.40-1.61 (m, 10\( \text{H} \)), 1.41-1.23 (m, 6\( \text{H} \)); MS (ESI): \(m/z\) 550 (M+1\(^+\)).

4-\[(6-Bromohexyl)oxy]-5-methoxy-2-nitrophenyl2-[di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylmethanone (11d)

The compound 11d was prepared according to the method described for compound 11a by employing 10 (400 mg, 1 mmol) and 1,6-dibromopentane (292 mg, 1.2 mmol) to afford the compound 11d (507 mg, 90%). \(^1\)H NMR (CDCl\(_3\), 200 MHz): \(\delta \) 7.63 (s, 1\( \text{H} \)), 6.80 (s, 1\( \text{H} \)), 4.83 (d, 1\( \text{H} \), \(J = 4.3 \) Hz), 4.74-4.62 (m, 1\( \text{H} \)), 4.14-4.05 (t, 2\( \text{H} \), \(J = 6.0 \) Hz), 3.95 (s, 3\( \text{H} \)), 3.51-3.43 (m, 2\( \text{H} \)), 3.30-3.16 (m, 2\( \text{H} \)), 2.85-2.65 (m, 4\( \text{H} \)), 2.40-1.61 (m, 12\( \text{H} \)), 1.41-1.23 (m, 6\( \text{H} \)); MS (ESI): \(m/z\) 564 (M+1\(^+\)).

**(4-3-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxypropyl)piperazino)(3-phenylimidazo[1,5-a]pyridin-1-yl)methanone (12a)**

To a solution of 2S-N-[4-(3-bromopropoxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal (11a) (521 mg, 1.0 mmol) in dry DMF (20 mL) was added anhydrous K\(_2\)CO\(_3\) (5.0 mmol) and (3-Phenylimidazo[1,5-a]pyridin-1-yl)(piperazino)methanone of formula (9a) (306 mg, 1.0 mmol). The reaction mixture was stirred at room temperature for 24 h and the reaction was monitored by TLC using ethyl acetate-hexane (60%) as a solvent system. After the completion of reaction ice was added to the reaction mixture followed by extraction with ethyl acetate (3×20 mL) and finally washed with brine solution. Then the solvent was evaporated under vacuum to afford the crude product. This was further purified by column chromatography using ethyl acetate-hexane (80%) as a solvent system to obtain the pure product 12a as a yellow solid. Yield 560 mg, 75%; mp 71-76 \(^\circ\)C; \(^1\)H NMR (CDCl\(_3\), 300 MHz): \(\delta \) 1.35 (q, 6\( \text{H} \), \(J = 6.4 \) Hz), 1.48-2.35 (m,
11H), 2.51-2.94 (m, 9H), 3.15-3.35 (m, 2H), 3.92 (s, 3H), 4.20 (t, 2H, J = 6.0 Hz), 4.65-4.75 (m, 1H) 4.68 (d, 1H, J = 3.7 Hz), 6.75 (t, 1H, J = 6.4 Hz), 6.81 (s, 1H), 7.04 (t, 1H, J = 6.9 Hz), 7.44-7.60 (m, 3H), 7.69 (s, 1H), 7.77 (d, 2H, J = 6.9 Hz), 8.28-8.34 (m, 2H). ESIMS: m/z 747 (M++1).

(4-4-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxyl]butylpiperazino)(3-phenylimidazo[1,5-a]pyridin-1-yl)methanone (12b)

This compound was prepared according to the method described for compound 12a, employing compound 11b (535 mg, 1 mmol) and compound 9a (1 mmol) to obtain the pure product 12a as a yellow solid. Yield 570 mg, 75%; mp 75-80 °C; ¹H NMR (CDCl₃, 300MHz): δ 1.33 (q, 6H, J = 7.5 Hz), 1.70-2.33 (m, 11H), 2.46-2.86 (m, 10H), 3.16-3.31 (m, 2H), 3.93 (s, 3H), 4.12 (t, 2H, J = 6.0 Hz), 4.61-4.70 (m, 1H), 4.81 (d, 1H, J = 3.7 Hz), 6.70 (t, 1H, J = 6.7 Hz), 6.76 (s, 1H), 6.99 (dd, 1H, J = 7.5, 6.7 Hz), 7.42-7.55 (m, 3H), 7.62 (s, 1H), 7.72 (d, 2H, J = 7.5 Hz), 8.27 (d, 1H, J = 7.5 Hz), 8.33 (d, 1H, J = 9.0 Hz). ESIMS: m/z 761 (M+).

(4-5-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxyl]pentylpiperazino)(3-phenylimidazo[1,5-a]pyridin-1-yl)methanone (12c)

This compound was prepared according to the method described for compound 12a, employing compound 11c (549 mg, 1 mmol) and compound 9a (1 mmol) to obtain the pure product 12c as a yellow solid. Yield 581 mg, 75%; mp 64-68 °C; ¹H NMR (CDCl₃, 300MHz): δ 1.34 (q, 6H, J = 7.7 Hz), 1.44-2.36 (m, 13H), 2.46-2.86 (m, 11H), 3.15-3.32 (m, 2H), 3.93 (s, 3H), 4.08 (t, 2H, J = 6.3 Hz), 4.61-4.72 (m, 1H), 4.81 (d, 1H, J = 3.5 Hz), 6.70 (t, 1H, J = 6.3 Hz), 6.76 (s, 1H), 6.99 (dd, 1H, J = 7.5, 6.2 Hz), 7.42-7.55 (m, 3H), 7.61 (s, 1H), 7.72 (d, 2H, J = 6.9 Hz), 8.27 (d, 1H, J = 7.5 Hz), 8.33 (d, 1H, J = 9.0 Hz). ESIMS: m/z 775 (M++1).

(4-6-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxyl]hexylpiperazino)(3-phenylimidazo[1,5-a]pyridin-1-yl)methanone (12d)

This compound was prepared according to the method described for compound 12a, employing compound 11d (563 mg, 1 mmol) and compound 9a (1 mmol) to obtain the pure product 12d as a yellow solid. Yield 552 mg, 70%; mp 65-70 °C; ¹H NMR (CDCl₃, 300MHz): δ 1.20-1.28 (m, 2H), 1.36 (q, 6H, J = 7.1 Hz), 1.40-1.68 (m, 3H), 1.73-2.39 (m, 9H), 2.40-2.88 (m, 12H), 3.15-3.31 (m, 2H), 3.93 (s, 3H), 4.01-4.13 (m, 2H), 4.57-4.71 (m, 1H), 4.81 (d, 1H, J = 3.7 Hz), 6.70 (t, 1H, J = 6.8 Hz), 6.75 (s, 1H), 7.00 (dd, 1H, J = 6.7, 6.0 Hz), 7.42-7.55 (m, 3H), 7.61 (s, 1H), 7.72 (d, 2H, J = 6.9 Hz), 8.27 (d, 1H, J = 7.5 Hz), 8.33 (d, 1H, J = 9.0 Hz). ESIMS: m/z 775 (M++1).
7.40-7.56 (m, 3H), 7.61 (s, 1H), 7.70-7.75 (m, 2H), 8.27 (d, 1H, J = 6.7 Hz), 8.34 (d, 1H, J = 9.0 Hz). ESIMS: m/z 789 (M+).

(4-3-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxo]propylpiperazino)3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-ylmethanone (12e)

This compound was prepared according to the method described for compound 12a, employing compound 11a (521 mg, 1 mmol) and compound 9b (1 mmol) to obtain the pure product 12e as a yellow solid. Yield 611 mg, 75%; mp 83-88 °C; 1H NMR (CDCl 3, 300 MHz): δ 1.10-1.29 (m, 1H), 1.35 (q, 6H, J = 7.4 Hz), 1.73-2.35 (m, 10H), 2.62-2.90 (m, 9H), 3.15-3.31 (m, 2H), 3.93 (s, 3H), 4.20 (t, 2H, J = 6.8 Hz), 4.60-4.71 (m, 1H), 4.82 (d, 1H, J = 3.7 Hz), 6.76 (s, 1H), 6.80 (dd, 1H, J = 7.3, 6.9 Hz), 7.06 (dd, 1H, J = 6.4, 5.4 Hz), 7.65 (s, 1H), 7.80 (d, 2H, J = 8.3 Hz), 7.91 (d, 2H, J = 8.1 Hz), 8.30 (d, 1H, J = 7.3 Hz), 8.38 (d, 1H, J = 9.2 Hz). ESIMS: m/z 815 (M+).

(4-4-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxo]butylpiperazino)3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-ylmethanone (12f)

This compound was prepared according to the method described for compound 12a, employing compound 11b (535 mg, 1 mmol) and compound 9b (1 mmol) to obtain the pure product 12f as a yellow solid. Yield 622 mg, 75%; mp 75-80 °C; 1H NMR (CDCl 3, 300 MHz): δ 1.33 (q, 6H, J = 7.3 Hz), 1.68-2.36 (m, 12H), 2.48-2.88 (m, 10H), 3.12-3.31 (m, 2H), 3.93 (s, 3H), 4.06-4.19 (m, 2H), 4.61-4.71 (m, 1H), 4.81 (d, 1H, J = 3.2 Hz), 6.72-6.82 (m, 2H), 7.04 (t, 1H, J = 7.8 Hz), 7.62 (s, 1H), 7.81 (d, 2H, J = 8.1 Hz), 7.92 (d, 2H, J = 7.9 Hz), 8.30 (d, 1H, J = 7.1 Hz), 8.35 (d, 1H, J = 8.8 Hz). ESIMS: m/z 844 (M++1).

(4-5-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxo]pentylpiperazino)3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-ylmethanone (12g)

This compound was prepared according to the method described for compound 12a, employing compound 11c (549 mg, 1 mmol) and compound 9b (1 mmol) to obtain the pure product 12g as a yellow solid. Yield 590 mg, 70%; mp 84-89 °C; 1H NMR (CDCl 3, 300 MHz): δ 1.33 (q, 6H, J = 7.5 Hz), 1.46-2.36 (m, 13H), 2.46-2.89 (m, 10H), 3.14-3.34 (m, 2H), 3.93 (s, 3H), 4.08 (t, 2H, J = 6.2 Hz), 4.60-4.72 (m, 1H), 4.82 (d, 1H, J = 3.5 Hz), 6.72-6.82 (m, 2H), 7.06 (dd, 1H, J = 6.7, 6.4 Hz), 7.61 (s, 1H), 7.81 (d, 2H, J = 8.3 Hz), 7.89 (d, 2H, J = 8.1 Hz), 8.31 (d, 1H, J = 6.9 Hz), 8.36 (d, 1H, J = 9.2 Hz). ESIMS: m/z 829 (M+).
(4-6-[4-([Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxy]hexylpiperazino)3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-ylmethanone (12h)

This compound was prepared according to the method described for compound 12a, employing compound 11d (563 mg, 1 mmol) and compound 9b (1 mmol) to obtain the pure product 12h as a yellow solid. Yield 685 mg, 80%; mp 85-90 °C; 1H NMR (CDCl 3, 300 MHz): δ 1.17-1.70 (m, 14H), 1.72-2.17 (m, 6H), 2.39-2.89 (m, 1H), 3.13-3.33 (m, 2H), 3.94 (s, 3H), 4.07 (t, 2H, J = 6.1 Hz), 4.60-4.72 (m, 1H), 4.81 (d, 1H, J = 3.7 Hz), 6.72-6.83 (m, 2H), 7.05 (dd, 1H, J = 9.0, 6.4 Hz), 7.61 (s, 1H), 7.81 (d, 2H, J = 8.3 Hz), 7.92 (d, 2H, J = 8.1 Hz), 8.29 (d, 1H, J = 7.1 Hz), 8.37 (d, 1H, J = 9.0 Hz). ESIMS: m/z 858 (M++1).

(4-3-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxy]propylpiperazino)[3-(4-methoxyphenyl)imidazo[1,5-a]pyridin-1-yl]methanone (12i)

This compound was prepared according to the method described for compound 12a, employing compound 11a (521 mg, 1 mmol) and compound 9c (1 mmol) to obtain the pure product 12i as a yellow solid. Yield 621 mg, 80%; mp 85-90 °C; 1H NMR (CDCl 3, 300 MHz): δ 1.19-1.29 (m, 2H), 1.34 (q, 6H, J = 7.5 Hz), 1.73-2.35 (m, 8H), 2.62-2.87 (m, 10H), 3.14-3.32 (m, 2H), 3.88 (s, 3H), 3.93 (s, 3H), 4.18 (t, 2H, J = 5.8 Hz), 4.61-4.69 (m, 1H), 4.82 (d, 1H, J = 3.7 Hz), 6.68 (t, 1H, J = 6.6 Hz), 6.76 (s, 1H), 6.97 (dd, 1H, J = 6.6 Hz), 7.00 (d, 2H, J = 8.6 Hz), 7.25 (s, 1H), 7.63 (d, 2H, J = 6.9 Hz), 8.19 (d, 1H, J = 7.1 Hz), 8.31 (d, 1H, J = 9.0 Hz). ESIMS: m/z 777 (M++1).

(4-4-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxy]butylpiperazino)[3-(4-methoxyphenyl)imidazo[1,5-a]pyridin-1-yl]methanone (12j)

This compound was prepared according to the method described for compound 12a, employing compound 11b (535 mg, 1 mmol) and compound 9c (1 mmol) to obtain the pure product 12j as a yellow solid. Yield 593 mg, 75%; mp 79-84 °C; 1H NMR (CDCl 3, 300 MHz): δ 1.18-1.29 (m, 2H), 1.36 (q, 6H, J = 7.5 Hz), 1.69-2.34 (m, 10H), 2.49-2.88 (m, 10H), 3.13-3.31 (m, 2H), 3.88 (s, 3H), 3.93 (s, 3H), 4.11 (t, 2H, J = 6.0 Hz), 4.61-4.70 (m, 1H), 4.82 (d, 1H, J = 3.7 Hz), 6.68 (t, 1H, J = 7.0 Hz), 6.76 (s, 1H), 6.97 (dd, 1H, J = 9.8, 6.7 Hz), 7.01 (d, 2H, J = 9.0 Hz), 7.61 (s, 1H), 7.63 (d, 2H, J = 9.0 Hz), 8.19 (d, 1H, J = 6.7 Hz), 8.32 (d, 1H, J = 9.0 Hz). ESIMS: m/z 791 (M++1).

(4-5-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxy]pentylpiperazino)[3-(4-methoxyphenyl)imidazo[1,5-a]pyridin-1-yl]methanone (12k)
This compound was prepared according to the method described for compound 12a, employing compound 11c (549 mg, 1 mmol) and compound 9c (1 mmol) to obtain the pure product 12k as a yellow solid. Yield 563 mg, 70%; mp 80-85 °C; \(^1\)H NMR (CDCl\(_3\), 300 MHz): \(\delta\) 1.33 (q, 6H, J = 7.6 Hz), 1.48-2.35 (m, 12H), 2.47-2.87 (m, 10H), 3.14-3.31 (m, 2H), 3.88 (s, 3H), 3.93 (s, 3H), 4.08 (t, 2H, J = 6.0 Hz), 4.61-4.71 (m, 1H), 4.81 (d, 1H, J = 3.7 Hz), 6.68 (t, 1H, J = 7.1 Hz), 6.76 (s, 1H), 6.97 (dd, 1H, J = 6.7, 6.4 Hz), 7.01 (d, 2H, J = 8.6 Hz), 7.61-7.65 (m, 3H), 8.22 (d, 1H, J = 7.1 Hz), 8.32 (d, 1H, J = 9.2 Hz). ESIMS: m/z 805 (M\(^+\)+1).

\((4-6-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxy]hexylpiperazino)[3-(4-methoxyphenyl)imidazo[1,5-a]pyridin-1-yl] Methanone (12l)

This compound was prepared according to the method described for compound 12a, employing compound 11d (563 mg, 1 mmol) and compound 9c (1 mmol) to obtain the pure product 12l as a yellow solid. Yield 614 mg, 75%; mp 87-92 ºC; \(^1\)H NMR (CDCl\(_3\), 300 MHz): \(\delta\) 1.18-1.67 (m, 18H), 1.71-2.36 (m, 8H), 2.63-3.07 (m, 6H), 3.15-3.33 (m, 2H), 3.89 (s, 3H), 3.94 (s, 3H), 4.02-4.13 (m, 2H), 4.59-4.70 (m, 1H), 4.81 (d, 1H, J = 3.7 Hz), 6.69-6.79 (m, 2H), 7.02 (d, 3H, J = 8.3 Hz), 7.58-7.64 (m, 3H), 8.21 (d, 1H, J = 6.7 Hz), 8.28 (d, 1H, J = 9.0 Hz). ESIMS: m/z 819 (M\(^+\)+1).

\((11aS)-7-Methoxy-8-(3-4-[(3-phenylimidazo[1,5-a]pyridin-1-yl)carbonyl]piperazino propoxy)-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13a)

A solution of the nitro thioacetal 12a (746 mg, 1mmol) and SnCl\(_2\),2H\(_2\)O (1.12 g, 5 mmol) in MeOH (20 mL) was refluxed for 1.5 h or until the TLC (EtOAc/Hexane, 1:1) indicated that reaction was completed. The reaction mixture was cooled and adjusted to pH 8 (sat. aq. NaHCO\(_3\)) and then extracted with EtOAc (3 x 30 mL). The combined organic phase was washed with brine (1 x 15 mL), dried (Na\(_2\)SO\(_4\)) and the solvent removed by rotary evaporation in vacuo to afford the amino diethyl thioacetal and due to stability problems directly used in the next step. A solution of amino thioacetal (1 mmol), HgCl\(_2\) (1.19 mg, 4.4 mmol), and CaCO\(_3\) (480 mg, 4.8 mmol) in acetonitrile–water (4:1) was stirred slowly at r.t. for 24 hours until TLC (EtOAc) indicates complete loss of starting material. The reaction mixture was diluted with ethyl acetate (30 mL) and filtered through celite. The clear yellow organic supernatant was extracted with ethyl acetate (2 X 20 mL). The organic layer was washed with sat. aq. NaHCO\(_3\) (2 x 20 mL), brine (2 x 20 mL) and the combined organic phase was dried (Na\(_2\)SO\(_4\)). The organic layer was evaporated under vacuum and the crude product was purified by column chromatography (2% MeOH–CHCl\(_3\)) to afford the
compound 13a as a white solid; (355 mg, 60% yield). mp 110–115 °C; [α]D27 +326.6° (c = 0.5, CHCl3); 1H NMR (CDCl3, 300 MHz): δ 1.54-1.70 (m, 6H), 2.00-2.12 (m, 2H), 2.26-2.34 (m, 2H), 2.53-2.63 (m, 6H), 3.51-3.61 (m, 1H), 3.66-3.73 (m, 1H), 3.76-3.85 (m, 1H), 3.94 (s, 3H), 4.08-4.21 (m, 2H), 6.69 (t, 1H, J = 7.5 Hz), 6.79 (s, 1H), 6.97 (dd, 1H, J = 6.7, 6.5 Hz), 7.40-7.55 (m, 4H), 7.60 (d, 1H, J = 6.7 Hz). 13C NMR (CDCl3, 150 MHz): δ 24.1, 26.1, 29.5, 46.5, 53.4, 53.6, 54.8, 56.0, 67.1, 110.4, 114.2, 120.9, 121.6, 122.5, 128.3, 129.0, 129.1, 140.5, 147.6, 162.4, 164.5; IR (KBr) (Umax/cm⁻¹): ν 3382 (br), 2927, 1599, 1508, 1433, 1378, 1238, 1126, 1075, 1006, 774, 693; ESIMS: m/z 593 (M+1); HRMS (ESI m/z) for C34H37N6O4 calcd 593.2876, found 593.2887 (M+1); purity 97%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

(11aS)-7-Methoxy-8-(4-4-[[(3-phenylimidazo[1,5-a]pyridin-1-yl)carbonyl]piperazino]butoxy)-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13b)
The compound 13b was prepared according to the method described for the compound 13a, employing the compound 12b (761 mg, 1.0 mmol). The crude  product was purified by column chromatography (MeOH/CHCl3, 2%) to afford the compound 13b as a white solid (363 mg, 60% yield). mp 65–70 °C; [α]D27 +270.4° (c = 0.5, CHCl3); 1H NMR (CDCl3, 500 MHz): δ 1.68-1.78 (m, 2H), 1.85-1.96 (m, 2H), 1.99-2.10 (m, 4H), 2.25-2.36 (m, 2H), 2.52 (t, 2H, J = 7.8 Hz), 2.64 (t, 4H, J = 4.9 Hz), 2.72-3.00 (brs, 4H), 3.54-3.62 (m, 1H), 3.7.-3.74 (m, 1H), 3.78-3.84 (m, 1H), 3.93 (s, 3H), 4.03-4.16 (m, 2H), 6.71 (t, 1H, J = 7.8 Hz), 6.79 (s, 1H), 6.97-7.01 (m, 1H), 7.44-7.54 (m, 4H), 7.64 (d, 1H, J = 3.9 Hz), 7.74 (d, 2H, J = 7.8 Hz), 8.28 (t, 2H, J = 7.8 Hz); 13C NMR (CDCl3, 150 MHz): δ 21.4, 22.5, 24.1, 26.7, 29.5, 46.5, 53.0, 53.6, 56.0, 57.6, 68.5, 110.3, 111.4, 114.3, 120.0, 120.9, 121.7, 122.7, 128.3, 129.0, 129.2, 135.4, 140.3, 147.7, 150.6, 162.4, 163.3; IR (KBr) (Umax/cm⁻¹): ν 3419 (br), 2923, 1631, 1433, 1378, 1165, 1116, 1061, 609; ESIMS: m/z 607 (M+1); HRMS (ESI m/z) for C35H39N6O4 calcd 607.3032, found 607.3011 (M+1); purity 96%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

(11aS)-7-Methoxy-8-[(5-4-[(3-phenylimidazo[1,5-a]pyridin-1-yl)carbonyl]piperazino]pentyl)oxy]-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13c)
The compound 13c was prepared according to the method described for the compound 13a, employing the compound 12c (774 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/CHCl3, 2%) to afford the compound 13c as a white solid (372 mg, 60% yield). mp 86–90 °C; [α]D27 +276.6° (c = 0.5, CHCl3); 1H NMR (CDCl3, 500 MHz): δ 1.54-1.70 (m, 6H), 2.00-2.12 (m, 2H), 2.26-2.34 (m, 2H), 2.53-2.63 (m, 6H), 3.51-3.61 (m, 1H), 3.66-3.73 (m, 1H), 3.76-3.85 (m, 1H), 3.94 (s, 3H), 4.08-4.21 (m, 2H), 6.69 (t, 1H, J = 7.5 Hz), 6.79 (s, 1H), 6.97 (dd, 1H, J = 6.7, 6.5 Hz), 7.40-7.55 (m, 4H), 7.60 (d, 1H, J = 4.5 Hz), 7.74 (dd, 2H, J = 6.7, 6.3 Hz), 8.25-8.35 (m, 2H); 13C NMR (CDCl3, 150 MHz): δ 24.1, 26.1, 29.5, 46.5, 53.0, 53.6, 54.8, 56.0, 67.1, 110.4, 114.2, 120.9, 121.6, 122.5, 125.2, 128.3, 129.0, 129.1, 140.5, 147.6, 162.4, 164.5; IR (KBr) (Umax/cm⁻¹): ν 3419 (br), 2923, 1631, 1433, 1378, 1165, 1116, 1061, 609; ESIMS: m/z 607 (M+1); HRMS (ESI m/z) for C35H39N6O4 calcd 607.3032, found 607.3011 (M+1); purity 96%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.
(11aS)-7-Methoxy-8-[6-4-[3-phenylimidazo[1,5-a]pyridin-1-yl]carbonyl]piperazino-hexyl]oxy]-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13d)

The compound 13d was prepared according to the method described for the compound 13a, employing the compound 12d (788 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/CHCl₃, 2%) to afford the compound 13d as a white solid (348 mg, 55% yield). mp 78–83 °C; [α]D²⁷ +253.7° (c = 0.5, CHCl₃); ¹H NMR (CDCl₃, 300 MHz): δ 1.55-1.77 (m, 8H), 1.81-1.95 (m, 4H), 1.99-2.11 (m, 4H), 2.25-2.43 (t, 2H, J = 7.1 Hz), 2.49-2.53 (brs, 4H), 3.48-3.56 (m, 1H), 3.64-3.71 (m, 1H), 3.72-3.84 (m, 1H), 3.93 (s, 3H), 3.97-4.13 (m, 4H), 6.67-6.76 (m, 2H), 6.98 (dd, 1H, J = 6.6, 6.4 Hz), 7.41-7.57 (m, 4H), 7.61 (d, 1H, J = 4.1 Hz), 7.73 (d, 2H, J = 6.9 Hz), 8.32 (dd, 2H, J = 8.1, 6.8 Hz); ¹³C NMR (CDCl₃, 150 MHz): δ 24.1, 25.7, 26.4, 27.1, 28.7, 29.5, 46.5, 53.5, 53.6, 56.0, 58.5, 68.8, 110.2, 111.4, 114.2, 119.9, 120.9, 121.6, 122.5, 125.1, 128.3, 129.0, 129.1, 129.5, 135.2, 140.4, 147.7, 150.7, 162.3, 163.4; IR (KBr) (Umax/cm⁻¹): ν 3351 (br), 2931, 1599, 1507, 1466, 1429, 1372, 1312, 1235, 1126, 1075, 1005, 868, 772, 693; ESIMS: m/z 635 (M⁺+1); HRMS (ESI m/z) for C₃₇H₄₃N₆O₄ calcd 635.3345, found 635.3317 (M⁺+1); purity 95%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

(11aS)-7-Methoxy-8-3-[4-(3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-yl]carbonyl)piperazino-propoxy-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13e)

The compound 13e was prepared according to the method described for the compound 13a, employing the compound 12e (815 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/CHCl₃, 2%) to afford the compound 13e as a white solid (343 mg, 52% yield). mp 115–120 °C; [α]D²⁷ +246.9° (c = 0.5, CHCl₃); ¹H NMR (CDCl₃, 300 MHz): δ 1.50-1.71 (m, 8H), 1.86-1.94 (m, 2H), 2.00-2.10 (m, 2H), 2.27-2.35 (m, 2H), 2.41 (t, 2H, J = 7.7 Hz), 2.52-2.61 (brs, 6H), 3.53-3.59 (m, 1H), 3.67-3.72 (m, 1H), 3.76-3.83 (m, 1H), 3.93 (s, 3H), 3.99-4.11 (m, 2H), 4.13-4.24 (brs, 2H), 6.69 (t, 1H, J = 6.7 Hz), 6.73 (s, 1H), 6.97 (t, 1H, J = 7.7 Hz), 7.43-7.46 (m, 2H), 7.52 (t, 2H, J = 7.7 Hz), 7.60 (d, 1H, J = 3.8 Hz), 7.74 (d, 2H, J = 7.7 Hz), 8.27 (d, 1H, J = 7.2 Hz), 8.32 (d, 1H, J = 7.4 Hz); ¹³C NMR (CDCl₃, 150 MHz): δ 23.9, 24.2, 26.3, 28.7, 29.6, 35.3, 53.5, 53.7, 56.1, 56.2, 58.5, 68.8, 110.3, 111.5, 114.3, 120.1, 120.9, 121.7, 122.6, 125.2, 125.8, 128.4, 129.1, 129.2, 129.6, 132.7, 140.5, 140.6, 147.7, 150.8, 162.5, 163.4; IR (KBr) (Umax/cm⁻¹): ν 3331 (br), 2931, 1599, 1507, 1467, 1429, 1367, 1315, 1237, 1126, 1074, 1007, 868, 774, 748, 692; ESIMS: m/z 621 (M⁺+1); HRMS (ESI m/z) for C₃₆H₄₁N₆O₄ calcd 621.3189, found 621.3187 (M⁺+1); purity 99%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.
MHz): \(\delta\) 1.47-1.63 (m, 6H), 2.02-2.11 (m, 4H), 2.26-2.34 (m, 2H), 2.59 (t, 4H, 4.7 Hz), 3.52-3.63 (m, 1H), 3.67-3.83 (m, 1H), 3.93 (s, 3H), 4.09-4.20 (m, 2H), 6.74-6.82 (m, 2H), 7.02 (dd, 1H, \(J = 6.7, 6.4\) Hz), 7.43 (s, 1H), 7.5 (d, 2H, \(J = 8.3\) Hz), 7.92 (d, 2H, \(J = 7.5\) Hz), 8.26 (d, 1H, \(J = 7.5\) Hz), 8.33 (d, 1H), \(J = 8.0\) Hz);

\(^{13}\)C NMR (CDCl\(_3\), 150 MHz): \(\delta\) 24.1, 26.2, 29.5, 46.6, 53.4, 53.6, 54.8, 56.0, 67.2, 110.5, 111.4, 114.9, 120.1, 121.1, 121.4, 123.0, 125.9, 126.0, 126.1, 128.4, 135.6, 147.7, 150.6, 162.4, 163.1; IR (KBr) (U\(_{\text{max}}/\text{cm}^{-1}\)): \(\nu\) 3384 (br), 2952, 1601, 1534, 1510, 1462, 1432, 1381, 1324, 1240, 1167, 1125, 1066, 1010, 775, 743, 690, 608; ESIMS: \(m/z\) 661 (M\(^++\)1); HRMS (ESI \(m/z\)) for C\(_{35}\)H\(_{36}\)F\(_3\)N\(_6\)O\(_4\) calcd 661.2750, found 661.2751 (M\(^+\)1); purity 98%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

(11aS)-7-Methoxy-8-4-[4-(3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-yl carbonyl)piperazino]butoxy-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13f)

The compound 13f was prepared according to the method described for the compound 13a, employing the compound 12f (829 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/CHCl\(_3\), 2%) to afford the compound 13f as a white solid (377 mg, 56% yield). mp 119–124 °C; \([\alpha]_{D}^{27}\) +253.3° (\(c = 0.5, \text{CHCl}_3\)); \(\text{H NMR (CDCl}_3, 300 MHz): \(\delta\) 1.66-1.79 (m, 2H), 1.84-2.18 (brs, 2H), 1.88-1.96 (m, 2H), 2.00-2.10 (m, 2H), 2.25-2.34 (m, 2H), 2.46 (t, 2H, \(J = 7.5\) Hz), 2.56 (t, 4H, \(J = 4.5\) Hz), 3.50-3.62 (m, 1H), 3.65-3.72 (m, 1H), 3.74-3.83 (m, 1H), 3.93 (s, 3H), 4.03-4.16 (m, 2H), 4.24-4.54 (brs, 2H), 6.74-6.81 (m, 2H), 7.03 (dd, 1H, \(J = 6.7, 6.5\) Hz), 7.44 (s, 1H), 7.61 (d, 1H, \(J = 4.5\) Hz), 7.77 (d, 2H, \(J = 8.3\) Hz), 7.90 (d, 2H, \(J = 7.5\) Hz), 8.28 (d, 1H, \(J = 7.5\) Hz), 8.35 (d, 1H, \(J = 8.0\) Hz); \(^{13}\)C NMR (CDCl\(_3\), 150 MHz): \(\delta\) 23.1, 24.1, 26.7, 29.5, 46.6, 53.4, 53.6, 56.0, 57.9, 68.6, 110.3, 111.4, 114.9, 120.0, 121.2, 121.4, 122.9, 125.9, 126.0, 128.3, 134.8, 135.7, 140.5, 147.6, 150.7, 162.3, 164.6; IR (KBr) (U\(_{\text{max}}/\text{cm}^{-1}\)): \(\nu\) 3378 (br), 2932, 1602, 1509, 1433, 1375, 1323, 1239, 1168, 1123, 1067, 1009, 851, 775, 741, 689, 607; ESIMS: \(m/z\) 675 (M\(^++\)1); HRMS (ESI \(m/z\)) for C\(_{36}\)H\(_{38}\)F\(_3\)N\(_6\)O\(_4\) calcd 675.2906, found 675.2901 (M\(^+\)1); purity 97%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

(11aS)-7-Methoxy-8-(5-[4-(3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-yl carbonyl)piperazino]pentyloxy)-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13g)

The compound 13g was prepared according to the method described for the compound 13a, employing the compound 12g (843 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/CHCl\(_3\), 2%) to afford the compound 13g as a white solid.
(378 mg, 55% yield). mp 120–125 °C; [α]D 27 +194.0° (c = 0.5, CHCl3); 1H NMR (CDCl3, 500 MHz): δ 1.48-1.64 (m, 6H), 1.85-1.95 (m, 2H), 1.98-2.10 (m, 2H), 2.24-2.34 (m, 2H), 2.40 (t, 2H, J = 7.4 Hz), 2.47-2.60 (brs, 4H), 3.50-3.59 (m, 1H), 3.65-3.71 (m, 1H), 3.73-3.86 (m, 1H), 3.91 (s, 3H), 3.97-4.11 (m, 2H), 4.36-4.59 (brs, 2H), 6.65-6.83 (m, 2H), 7.01 (s, 1H), 7.43 (s, 1H), 7.59 (s, 1H), 7.77 (d, 2H, J = 7.4 Hz), 7.90 (d, 2H, J = 7.4 Hz), 8.28 (d, 1H, J = 6.5 Hz), 8.34 (d, 1H, J = 9.3 Hz); 13C NMR (CDCl3, 150 MHz): δ 23.8, 24.1, 26.4, 28.7, 29.5, 46.6, 53.6, 56.1, 58.3, 68.7, 110.3, 111.4, 114.9, 120.0, 121.2, 121.3, 122.9, 125.9, 126.0, 128.3, 133.1, 135.7, 140.5, 147.7, 150.8, 162.4, 164.6; IR (KBr) (Umax/cm⁻¹): ν 3384 (br), 2928, 1601, 1534, 1510, 1462, 1432, 1366, 1324, 1261, 1239, 1166, 1125, 1066, 1011, 850, 774, 742, 691, 605; ESIMS: m/z 690 (M++1); HRMS (ESI m/z) for C37H40F3N6O4 calcd 689.3063, found 689.3076 (M+)+; purity 99%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

(11aS)-7-Methoxy-8-(6-[4-(3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-yl carbonyl)piperazino]hexyloxy)-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4] diazepin-5-one (13h)

The compound 13h was prepared according to the method described for the compound 13a, employing the compound 12h (857 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/CHCl3, 2%) to afford the compound 13h as a white solid (386 mg, 55% yield). mp 75–80 °C; [α]D 27 +212.5° (c = 0.5, CHCl3); 1H NMR (CDCl3, 300 MHz): δ 1.45-1.62 (m, 10H), 1.83-1.93 (m, 2H), 2.01-2.10 (m, 2H), 2.26-2.34 (m, 2H), 2.39 (t, 2H, J = 6.9 Hz), 2.55 (t, 4H, J = 4.7 Hz), 3.51-3.61 (m, 1H), 3.67-3.72 (m, 1H), 3.75-3.84 (m, 1H), 3.93 (s, 3H), 3.98-4.12 (m, 2H), 4.13-4.28 (brs, 2H), 6.71-6.80 (m, 2H), 7.03 (dd, 1H, J = 6.6, 6.4 Hz), 7.43 (s, 1H), 7.60 (d, 1H, J = 4.3 Hz), 7.79 (d, 2H, J = 8.3 Hz), 7.91 (d, 2H, J = 8.1 Hz), 8.29 (d, 1H, J = 7.1 Hz), 8.37 (d, 1H, J = 9.2 Hz); 13C NMR (CDCl3, 150 MHz): δ 24.2, 25.8, 26.6, 27.2, 28.8, 29.6, 46.6, 53.5, 53.7, 56.1, 58.6, 68.9, 110.4, 111.5, 115.0, 120.0, 121.3, 121.4, 123.0, 126.0, 126.1, 128.4, 133.1, 134.9, 135.7, 140.5, 147.8, 150.8, 162.4, 164.6; IR (KBr) (Umax/cm⁻¹): ν 3343 (br), 2933, 1601, 1534, 1509, 1454, 1431, 1379, 1323, 1237, 1167, 1125, 1066, 1008, 849, 774, 738, 689, 607; ESIMS: m/z 704 (M⁺+1); HRMS (ESI m/z) for C38H42F3N6O4 calcd 703.3219, found 703.3218 (M+1⁺); purity 96%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

(11aS)-7-Methoxy-8-[3-[4-[3-(4-methoxyphenyl)imidazo[1,5-a]pyridin-1-yl]carbonyl]piperazino]propoxy]-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13i)
The compound 13i was prepared according to the method described for the compound 13a, employing the compound 12i (776 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/CHCl₃, 2%) to afford the compound 13i as a white solid (311 mg, 50% yield). mp 73–78 °C; [α]D²⁷ +208.6° (c = 0.5, CHCl₃); ¹H NMR (CDCl₃, 300 MHz): δ 1.64-1.88 (m, 4H), 1.99-2.13 (m, 4H), 2.26-2.36 (m, 2H), 2.52-2.64 (m, 6H), 3.52-3.62 (m, 1H), 3.66-3.73 (m, 1H), 3.75-3.84 (m, 1H), 3.89 (s, 3H), 3.94 (s, 3H), 4.06-4.28 (m, 4H), 6.66 (t, 1H, J = 6.6 Hz), 6.80 (s, 1H), 6.95 (dd, 1H, J = 6.9, 6.7 Hz), 7.00 (d, 2H, J = 8.4 Hz), 7.44 (s, 1H), 7.62 (d, 1H, J = 4.3 Hz), 7.67 (d, 2H, J = 8.4 Hz), 8.19 (d, 1H, J = 6.9 Hz), 8.28 (d, 1H, J = 9.2 Hz); ¹³C NMR (CDCl₃, 150 MHz): δ 24.1, 26.1, 29.5, 46.5, 53.4, 53.6, 54.8, 55.3, 56.0, 67.1, 110.4, 111.5, 114.0, 114.4, 120.0, 120.8, 121.6, 121.8, 122.3, 129.8, 135.0, 140.5, 147.6, 162.3, 163.5; IR (KBr) (Umax/cm⁻¹): υ 3393 (br), 2928, 1604, 1508, 1460, 1380, 1250, 1178, 1122, 1008, 837, 749, 689; ESIMS: m/z 623 (M⁺+1); HRMS (ESI m/z) for C₃₅H₃₉N₆O₅ calcd 623.2981, found 623.2979 (M⁺+1); purity 91%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

(11aS)-7-Methoxy-8-[4-[4-[3-(4-methoxyphenyl)imidazo[1,5-a]pyridin-1-yl]carbonylpiperazino]butoxy]-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13j)

The compound 13j was prepared according to the method described for the compound 13a, employing the compound 12j (790 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/CHCl₃, 2%) to afford the compound 13j as a white solid (318 mg, 50% yield). mp 75–80 °C; [α]D²⁷ +216.0° (c = 0.5, CHCl₃); ¹H NMR (CDCl₃, 300 MHz): δ 1.66-1.83 (6H), 1.88-1.97 (m, 2H), 2.00-2.10 (m, 2H), 2.25-2.33 (m, 2H), 2.46 (t, 2H, J = 7.1 Hz), 2.56 (t, 4H, J = 4.5 Hz), 3.50-3.61 (m, 1H), 3.66-3.72 (m, 1H), 3.75-3.84 (m, 1H), 3.88 (s, 3H), 3.93 (s, 3H), 4.02-4.23 (m, 4H), 6.66 (t, 1H, J = 6.4 Hz), 6.76 (s, 1H), 6.94 (dd, 1H, J = 6.4, 6.2 Hz), 7.03 (d, 2H, J = 8.8 Hz), 7.44 (s, 1H), 7.61 (d, 1H, J = 4.3 Hz), 7.64 (d, 2H, J = 8.6 Hz), 8.18 (d, 1H, J = 7.1 Hz), 8.28 (d, 1H, J = 9.2 Hz); ¹³C NMR (CDCl₃, 150 MHz): δ 24.1, 26.2, 29.6, 46.4, 53.4, 53.6, 54.1, 54.8, 55.2, 56.1, 67.4, 110.3, 111.5, 114.2, 114.6, 120.1, 120.8, 121.6, 121.7, 122.4, 129.8, 135.1, 136.7, 140.5, 144.6, 146.5, 149.8, 162.4, 164.6; IR (KBr) (Umax/cm⁻¹): υ 3355 (br), 2932, 1603, 1534, 1508, 1458, 1430, 1380, 1311, 1250, 1124, 1073, 1006, 868, 838, 751, 689; ESIMS: m/z 637 (M⁺+1); HRMS (ESI m/z) for C₃₆H₄₃FN₆O₅ calcd 637.3138, found 637.3113 (M⁺+1); purity 95%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.
The compound 13k was prepared according to the method described for the compound 13a, employing the compound 12k (804 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/CHCl₃, 2%) to afford the compound 13k as a white solid (377 mg, 58% yield). mp 78–83 °C; [α]D²⁷ +269.9° (c = 0.5, CHCl₃); ¹H NMR (CDCl₃, 300 MHz): δ 1.50-1.72 (m, 8 H₁), 1.84-1.95 (m, 2 H₂), 2.00-2.10 (m, 2 H₂), 2.27-2.35 (m, 2 H₂), 2.41 (t, 2 H₂, J = 6.8 Hz), 2.55 (t, 4 H₂, J = 4.5 Hz), 3.52-3.61 (m, 1 H₃), 3.66-3.73 (m, 1 H₃), 3.76-3.85 (m, 1 H₃), 3.89 (s, 3 H₃), 3.93 (s, 3 H₃), 4.00-4.12 (m, 2 H₂), 4.36-4.66 (brs, 2 H₂), 6.68 (t, 1 H₁, J = 6.2 Hz), 6.76 (s, 1 H₁), 6.97 (dd, 1 H₁, J = 6.7, 6.4 Hz), 7.01 (d, 2 H₂, J = 8.6 Hz), 7.57 (d, 1 H₁, J = 4.2 Hz), 7.61-7.65 (m, 3 H₃), 8.22 (d, 1 H₁, J = 7.1 Hz), 8.32 (d, 1 H₁, J = 9.2 Hz); ¹³C NMR (CDCl₃, 150 MHz): δ 23.8, 24.1, 26.2, 28.6, 29.5, 46.5, 53.4, 53.6, 55.3, 56.0, 58.3, 68.8, 110.2, 111.4, 114.0, 114.4, 119.9, 120.8, 121.6, 121.8, 122.3, 129.8, 135.0, 136.4, 140.4, 147.7, 150.7, 162.3, 163.4; IR (KBr) (Umax/cm⁻¹): υ 3421 (br), 2934, 1603, 1534, 1508, 1460, 1432, 1372, 1312, 1251, 1127, 1072, 1008, 869, 839, 772, 689; ESIMS: m/z 651 (M⁺+1); HRMS (ESI m/z) for C₃₇H₄₃N₆O₅ calcd 651.3294, found 651.3284 (M+1)⁺; purity 95%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

The compound 13l was prepared according to the method described for the compound 13a, employing the compound 12l (818 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/CHCl₃, 2%) to afford the compound 13l as a white solid (365 mg, 55% yield). mp 80–85 °C; [α]D²⁷ +204.8° (c = 0.5, CHCl₃); ¹H NMR (CDCl₃, 300 MHz): δ 1.40-1.59 (m, 8 H₁), 1.72-1.91 (m, 4 H₂), 2.00-2.10 (m, 2 H₂), 2.26-2.33 (m, 2 H₂), 2.37 (t, 2 H₂, J = 6.6 Hz), 2.53 (t, 4 H₂, J = 4.7 Hz), 3.51-3.63 (m, 1 H₃), 3.66-3.73 (m, 1 H₃), 3.74-3.84 (m, 1 H₃), 3.88 (s, 3 H₃), 3.93 (s, 3 H₃), 3.98-4.12 (m, 2 H₂), 4.13-4.27 (brs, 2 H₂), 6.66 (t, 1 H₁, J = 6.2 Hz), 6.73 (s, 1 H₁), 6.94 (dd, 1 H₁, J = 6.4, 5.4 Hz), 7.01 (d, 2 H₂, J = 9.0 Hz), 7.43 (s, 1 H₁), 7.61 (d, 1 H₁, J = 4.3 Hz), 7.65 (d, 2 H₂, J = 8.8 Hz), 8.20 (d, 1 H₁, J = 7.1 Hz), 8.30 (d, 1 H₁, J = 9.2 Hz); ¹³C NMR (CDCl₃, 150 MHz): δ 24.1, 25.7, 26.5, 27.1, 28.7, 29.5, 46.5, 53.4, 53.6, 55.3, 56.0, 58.5, 68.8, 110.2, 111.4, 114.0, 114.4, 120.9, 121.6, 121.9, 122.3, 124.9, 129.8, 135.0, 136.5, 140.5, 147.7, 150.7, 162.3, 163.4; IR (KBr) (Umax/cm⁻¹): υ 3414 (br), 2933, 1604, 1534, 1509, 1460, 1431, 1311, 1251, 1178, 1127, 1006, 869, 838, 773, 690; ESIMS: m/z 666.
(M$^+ + 1$); HRMS (ESI m/z) for C$_{38}$H$_{45}$N$_6$O$_5$ calcd 665.3451, found 665.3483 (M)$^+$; purity 96%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.
$^1$H NMR spectrum of compound 13a
$^{1}H$ NMR spectrum of compound 13e
Organic - 1, IICT
Hyderabad - 17

Sample ID: WS IMP 5imine
Sample: IMD5im
Raw Data: wsimp5im
Primary: wsimp5im
Project: catechol

Analyst: ramakrishna
From: Tue, 13th Jul, 2010 15:22:23
Calibration Style: (none) report

Result Table - Calculation Method Uncal

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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.740</td>
<td>72,088.00</td>
<td>5.565</td>
<td>0.200</td>
<td>0.475</td>
<td>1.745</td>
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<tr>
<td>2</td>
<td>9.380</td>
<td>15096,8995</td>
<td>313.347</td>
<td>0.760</td>
<td>99.525</td>
<td>90.255</td>
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<tr>
<td>Total</td>
<td>15168,9875</td>
<td>318.913</td>
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</tbody>
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Organic - I, IICT
Hyderabad - 17

Sample ID: WS IMP 6imine
Sample: PBD
Raw Data: wsimp6im
Primary: wsimp6im
Project: catechol

Analyst: ramakrishna
From: Tue, 13th Jul, 2010 16:00:24
Calibration: (none)
Style: report

Result Table - Calculation Method Uncal

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Retention time [min]</th>
<th>Area [mV.s]</th>
<th>Height [mV]</th>
<th>W05 [min]</th>
<th>Area [%]</th>
<th>Height [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.770</td>
<td>110.869</td>
<td>6.020</td>
<td>0.260</td>
<td>0.656</td>
<td>2.053</td>
</tr>
<tr>
<td>2</td>
<td>4.210</td>
<td>105.750</td>
<td>4.023</td>
<td>0.650</td>
<td>0.584</td>
<td>1.566</td>
</tr>
<tr>
<td>3</td>
<td>5.040</td>
<td>72.692</td>
<td>2.308</td>
<td>0.600</td>
<td>0.401</td>
<td>0.756</td>
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<tr>
<td>4</td>
<td>5.550</td>
<td>58.3057</td>
<td>2.116</td>
<td>0.500</td>
<td>0.322</td>
<td>0.666</td>
</tr>
<tr>
<td>5</td>
<td>6.220</td>
<td>95.3941</td>
<td>3.717</td>
<td>0.790</td>
<td>0.527</td>
<td>0.855</td>
</tr>
<tr>
<td>6</td>
<td>7.410</td>
<td>101.456</td>
<td>2.109</td>
<td>0.630</td>
<td>0.560</td>
<td>0.664</td>
</tr>
<tr>
<td>7</td>
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<td>0.747</td>
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<tr>
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<td>4.152</td>
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<tr>
<td>9</td>
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<td>291.330</td>
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<td>91.716</td>
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</table>

Total 18115.7705 317.649

13d

\[ \text{Structure Image} \]
Organic - 1, IICT
Hyderabad - 17

Sample ID: RIMP6imine
Sample: PBD
Primary Project: rimp6imn
catechol

Analyst: ramakrishna
From: Tue, 13th Jul, 2010 17:28:42
Calibration: (none)
Style: report

Result Table - Calculation Method Uncal

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<th>Area [mV.s]</th>
<th>Height [mV]</th>
<th>WOS [sec]</th>
<th>Area [%]</th>
<th>Height [%]</th>
</tr>
</thead>
<tbody>
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<td>4.236</td>
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<tr>
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<td>0.840</td>
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<tr>
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<td>0.810</td>
<td>96.113</td>
<td>89.798</td>
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<td>3.991</td>
<td>0.390</td>
<td>0.973</td>
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</tr>
</tbody>
</table>

13I

OMe