Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supplementary Information

Carbazole-pyrrolo[2,1-*c*][1,4]benzodiazepine conjugates: Design, synthesis, and biological evaluation

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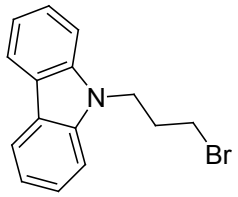
Material and Methods

All chemicals and reagents were obtained from Aldrich (Sigma–Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) or Spectrochem Pvt. Ltd (Mumbai, India) and were used without further purification. ^{1}H NMR spectra were recorded on a Bruker UXNMR/XWIN-NMR (300 MHz) or Varian VXR-Unity (200 MHz). Chemical shifts have been expressed in (ppm) down field from TMS. Coupling constants are reported in Hertz (Hz). EI mass spectra were recorded on a VG-7070H Micromass mass spectrometer at 200 ^{o}C, 70 eV, with a trap current of 200 μA and 4 kV of acceleration voltage. FAB mass spectra were recorded on a LSIMS-VG-AUTOSPEC Micromass spectrometer. LC mass spectra and ESI mass spectra were recorded on LC-MSD-Trap-SL spectrometer and Q-STAR-XL Hybrid spectrometer respectively. Elemental analysis was within ±0.4% of the theoretical values. All reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) with UV light, iodine as probing agents. Column chromatography was performed using Acme silica gel (100-200 mesh). Yields were not optimized. All solvents and reagents were used without further purification unless otherwise specified.

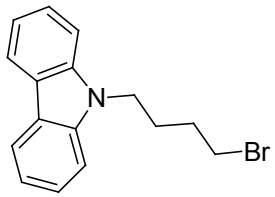
Experimental Section

9H-(3-Bromopropyl)-carbazole (7a)



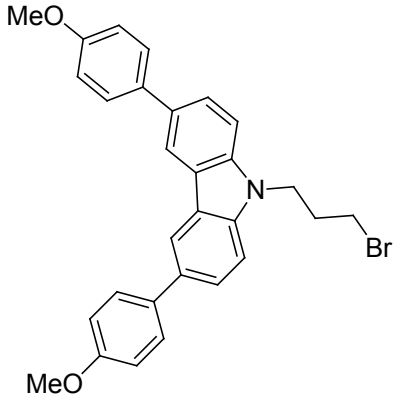
To a solution of carbazole (**6a**) (167 mg, 1 mmol) in dry acetonitrile (15 mL) was added, anhydrous K_2CO_3 (552 mg, 4 mmol), 1,3-dibromopropane (0.30 mL, 3 mmol) and the mixture was stirred at reflux temperature for 24 hr. The reaction was monitored by TLC using ethyl acetate-hexane (1:9). After completion of the reaction as indicated by the TLC, K_2CO_3 was removed by filtration and the solvent evaporated under reduced pressure, diluted with water and extracted with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and evaporated under vacuum. The residue, thus obtained was purified by column chromatography using ethyl acetate and hexane (1:9) to afford compound **1a** as white solid. Yield (259 mg, 90%); ^{1}H NMR ($CDCl_3$, 300 MHz); δ 8.05 (d, 2H, *J* = 7.7 Hz, carbazole-**H**), 7.40 (t, 2H, *J* = 8.3 Hz, carbazole-**H**), 7.35 (d, 2H, *J* = 7.7 Hz, carbazole-**H**), 7.18 (t, 2H, *J* = 7.7 Hz, carbazole-**H**), 4.50 (t, 2H, *J* = 6.9 Hz, -N-C**H$_2$**-), 3.34 (t, 2H, *J* = 6.9 Hz, -C**H$_2$**-Br), 2.24-2.45 (m, 2H, -C**H$_2$**-); ESIMS: *m/z* 289 $(M+H)^+$.

9*H*-(4-bromobutyl)-carbazole (7b)



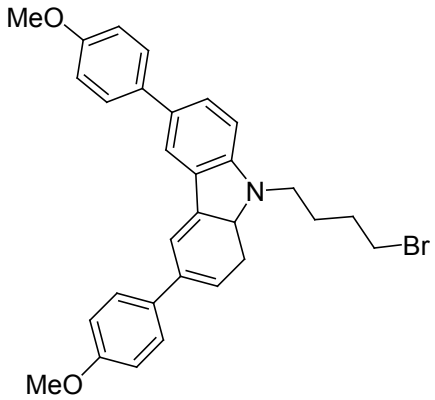
The compound **7b** was prepared according to the method described for compound **7a** by employing carbazole (167 mg, 1 mmol), and 1,4-dibromobutane (0.35 mL, 3 mmol). Yield (278 mg, 92%); ^{1}H NMR ($CDCl_3$, 300 MHz); δ 8.05 (d, 2H, *J* = 7.7 Hz, carbazole-**H**), 7.40 (t, 2H, *J* = 8.3 Hz, carbazole-**H**), 7.35 (d, 2H, *J* = 7.7 Hz, carbazole-**H**), 7.18 (t, 2H, *J* = 7.7 Hz, carbazole-**H**), 4.34 (t, 2H, *J* = 6.9 Hz, -N-C**H$_2$**-), 3.34 (t, 2H, *J* = 6.9 Hz, -C**H$_2$**-Br), 2.09-2.19 (m, 2H, -C**H$_2$**-), 1.82-1.95 (m, 2H, *J* = 6.6 Hz, -C**H$_2$**-); ESIMS: *m/z* 303 $(M+H)^+$.

9-(3-bromopropyl)-3,6-bis(4-methoxyphenyl)-9*H*-carbazole (7c)



The compound **7c** was prepared according to the method described for compound **7a** by employing 3,6-bis(4-methoxyphenyl)-9*H*-carbazole (**6b**) (379 mg, 1mmol), and 1,3-dibromopropane (0.30 mL, 3 mmol). Yield (455 mg, 91%); ^{1}H NMR ($CDCl_3$, 300 MHz); δ 8.29 (s, 2H, carbazole-**H**), 7.68-7.71 (m, 6H, carbazole-**H,** carbazole-Ar**H**), 7.53 (d, 2H, *J* = 8.3 Hz, carbazole-**H**), 7.02 (d, 4H, *J* = 8.3 Hz, carbazole-Ar**H**), 3.56 (t, 2H, *J* = 6.0 Hz, -N-C$\mathbf{H_2}$-), 3.87 (s, 6H, -OC$\mathbf{H_3}$), 3.41 (t, 2H, *J* = 6.0 Hz, -C$\mathbf{H_2}$-Br), 2.24-2.48 (q, 2H, *J* = 6.0 Hz, -C$\mathbf{H_2}$-); ESIMS: *m/z* 500 $(M)^+$.

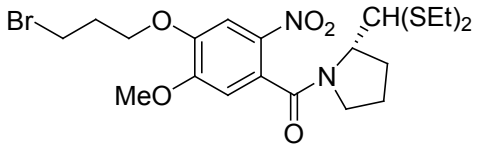
9-(4-bromobutyl)-3,6-bis(4-methoxyphenyl)-9*H*-carbazole (7d)



The compound **7d** was prepared according to the method described for compound **7a** by employing 3,6-bis(4-methoxyphenyl)-9*H*-carbazole (**6b**) (379 mg, 1mmol), and 1,4 dibromobutane (0.36 mL, 3 mmol). Yield (469 mg, 91%); ^{1}H NMR ($CDCl_3$, 300 MHz); δ 8.30 (s, 2H, carbazole-**H**), 7.69-7.70 (m, 6H, carbazole-**H,** carbazole-Ar**H**), 7.55 (d, 2H, *J* = 8.3 Hz, carbazole-**H**), 7.05 (d, 4H, *J* = 8.3 Hz, carbazole-Ar**H**), 4.35 (t, 2H, *J* = 6.9 Hz,

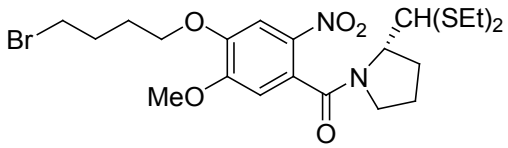
-N-C**H₂**-), 3.88 (s, 6H, -OC**H₃**), 3.34 (t, 2H, *J* = 6.9 Hz, -C**H₂**-Br), 2.05-2.13 (m, 2H, -C**H₂**-), 1.85-1.95 (m, 2H, -C**H₂**-); ESIMS: *m/z* 517 $(M+H)^+$.

(2*S*)-*N*-[4-(3-Bromopropyloxy)-5-methoxy–2-nitrobenzoyl]pyrrolidine-2-carboxaledhyde diethylthioacetal (8a)



To a solution of compound **1** (400 mg, 1 mmol) in dry acetone (15 mL) was added, anhydrous K_2CO_3 (414 mg, 3 mmol), 1,3-dibromopropane (0.12 mL, 1.2 mmol) and the mixture was stirred at reflux temperature for 12 h. The reaction was monitored by TLC using ethyl acetate-hexane (1:1). After completion of the reaction as indicated by the TLC, K_2CO_3 was removed by filtration and the solvent evaporated under reduced pressure, diluted with water and extracted with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and evaporated under vacuum. The residue, thus obtained was purified by column chromatography using ethyl acetate and hexane (2:3) to afford compound **8a** as yellow liquid (Yield 484 mg, 93%); ^{1}H NMR ($CDCl_3$, 300 MHz); δ 7.67 (s, 1H, PBD-Ar**H**), 6.83 (s, 1H, PBD-Ar**H**), 4.88 (d, 1H, *J* = 3.7 Hz, -C**H**$(SEt)_2$), 4.74-4.76 (m, 1H, -N-C**H**-), 4.11 (t, 2H, *J* = 6.0 Hz, -O-C**H₂**-), 3.95 (s, 3H, -OC**H₃**), 3.45 (t, 2H, -C**H₂**-Br), 3.20-3.32 (m, 2H, -N-C**H₂**-), 2.68-2.88 (m, 4H, *J* = 7.5, Hz, -S(C**H₂**$CH_3)_2$), 1.75-2.35 (m, 6H, 3 X –C**H₂**-), 1.25-1.40 (q, 6H, *J* = 7.5, 5.2 Hz, -S(CH_2C**H₃**$)_2$); ESIMS: *m/z* 521 $(M)^+$.

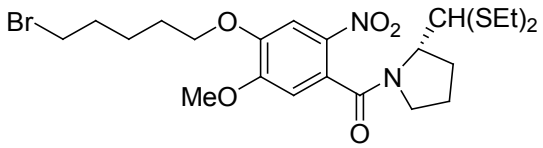
(2*S*)-*N*-[4-(4-bromobutyloxy)-5-methoxy–2-nitrobenzoyl]pyrrolidine-2-carboxaledhyde diethylthioacetal (8b)



The compound **8b** was prepared according to the method described for compound **8a** and 1,4-dibromobutane (0.14 mL, 1.2 mmol). Yield (492 mg, 92%); ^{1}H NMR ($CDCl_3$, 300 MHz); δ 7.65 (s, 1H, PBD-Ar**H**), 6.74 (s, 1H, PBD-Ar**H**), 4.83 (d, 1H, *J* = 3.7 Hz, -C**H**$(SEt)_2$), 4.71-4.60 (m, 1H, -N-C**H**-), 4.11 (t, 2H, *J* = 6.8 Hz, -OC**H₂**-), 3.96 (s, 3H, -OC**H₃**), 3.50 (t, 2H, *J* = 6.0 Hz, -C**H₂**-Br), 3.14-3.28 (m, 2H, -N-C**H₂**-), 2.64-2.81 (m,

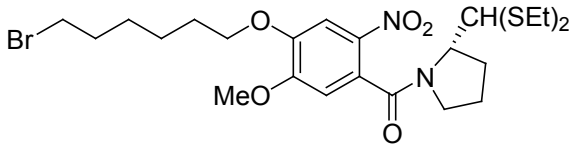
4H, -S(C**H₂**CH$_3$)$_2$), 1.65-2.34 (m, 8H, 4 X –C**H₂**-), 1.27-1.39 (q, 6H, *J* = 7.5, 5.2 Hz, -S(CH$_2$C**H₃**)$_2$); ESIMS: *m/z* 536 (M+H)$^+$.

(2*S*)-*N*-[4-(5-bromopentyloxy)-5-methoxy–2-nitrobenzoyl]pyrrolidine-2-carboxaledhyde diethylthioacetal (8c)



The compound **8c** was prepared according to the method described for compound **8a** and 1,5-dibromopentane (0.16 mL, 1.2 mmol). Yield (505 mg, 92%); ^{1}H NMR (CDCl$_3$, 300 MHz); δ 7.62 (s, 1H, PBD-Ar**H**), 6.76 (s, 1H, PBD-Ar**H**), 4.82-4.87 (d, 1H, *J* = 3.8 Hz, -C**H**(SEt)$_2$), 4.72-4.62 (m, 1H, -N-C**H**-), 4.13-4.05 (t, 2H, *J* = 6.0 Hz, -OC**H₂**-), 3.94 (s, 3H, -OC**H₃**), 3.49-3.43 (t, 2H, *J* = 6.0 Hz, -C**H₂**-Br), 3.16-3.30 (m, 2H, -N-C**H₂**-), 2.63-2.83 (m, 4H, -S(C**H₂**CH$_3$)$_2$), 1.61-2.36 (m, 10H, 5 X –C**H₂**-), 1.28-1.40 (q, 6H, *J* = 7.5, 5.2 Hz, -S(CH$_2$C**H₃**)$_2$); ESIMS: *m/z* 550 (M+H)$^+$.

(2*S*)-*N*-[4-(4-bromohexyloxy)-5-methoxy–2-nitrobenzoyl]pyrrolidine-2-carboxaledhyde diethylthioacetal (8d)

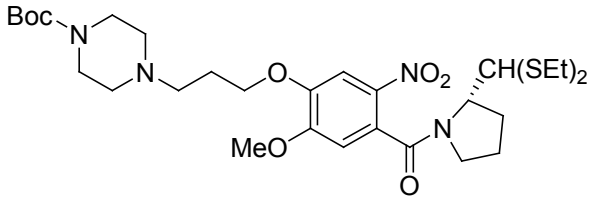


The compound **8d** was prepared according to the method described for compound **8a** and 1,6-dibromohexane (0.18 mL, 1.2 mmol). Yield (507 mg, 90%); ^{1}H NMR (CDCl$_3$, 300 MHz); δ 7.67 (s, 1H, PBD-Ar**H**), 6.83 (s, 1H, PBD-Ar**H**), 4.82-4.86 (d, 1H, *J* = 3.8 Hz, -C**H**(SEt)$_2$), 4.75-4.68 (m, 1H, -N-C**H**-), 4.14-4.08 (t, 2H, *J* = 6.0 Hz, -OC**H₂**-), 3.96 (s, 3H, -OC**H₃**), 3.49-3.44 (t, 2H, *J* = 6.0 Hz, -C**H₂**-Br), 3.20-3.34 (m, 2H, -N-C**H₂**-), 2.69-2.85 (m, 4H, -S(C**H₂**CH$_3$)$_2$), 1.61-2.33 (m, 12H, 6 X –C**H₂**-), 1.31-1.39 (q, 6H, *J* = 7.5, 5.2 Hz, -S(CH$_2$C**H₃**)$_2$); ESIMS: *m/z* 564 (M+H)$^+$.

Methoxy-(2*S*)-[*N*-{3-[4-(*N*-*ter*-butoxycaronyl)piperazino]propyloxy}-5-methoxy–2-nitrobenzoyl]pyrrolidine-2-carboxaledhyde diethylthioacetal (10a)

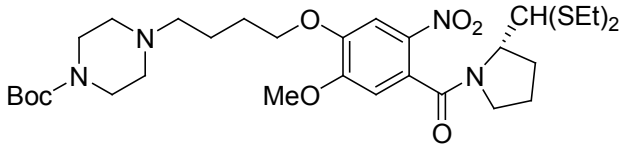
To a solution of compound **9a** (521 mg, 1 mmol) in dry acetonitrile (15 mL) was added, *N*-boc piperazine (186 mg 1.0 mmol) and anhydrous K$_2$CO$_3$ (414 mg, 3 mmol), and the mixture was stirred at reflux temperature for 12 h. The reaction was monitored by

TLC using ethyl acetate-hexane (1:1). After completion of the reaction as indicated by the TLC, K_2CO_3 was removed by filtration and the solvent evaporated under reduced pressure, diluted with water and extracted with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and evaporated under vacuum. The residue, thus obtained was purified by column chromatography using ethyl acetate and hexane (4:6) to afford compound **10a** as yellow liquid (Yield 564 mg, 90%).



^{1}H NMR ($CDCl_3$, 300 MHz); δ 7.66 (s, 1H, PBD-Ar**H**), 6.77 (s, 1H, PBD-Ar**H**), 4.88 (d, 1H, *J* = 3.7 Hz, -C**H**(SEt)$_2$), 4.70-4.60 (m, 1H, -N-C**H**-), 4.18-4.13 (t, 2H, *J* = 6.5 Hz, -OC**H$_2$**-), 3.95 (s, 3H, -OC**H$_3$**), 3.48-3.42 (m, 4H, 2 X piperzinyl-C**H$_2$**-), 3.29-3.21 (m, 2H, -NC**H$_2$**-), 2.88-2.70 (m, 4H, -S(C**H$_2$**CH$_3$)$_2$), 2.58 (t, 2H, *J* = 6.0 Hz, -NC**H$_2$**-), 2.52-2.45 (m, 4H, 2 X piperzinyl-C**H$_2$**-), 2.40-2.32 (m, 4H, 2 X -C**H$_2$**-), 2.02-2.06 (m, 2H, -C**H$_2$**-), 1.48 (s, 9H, 3 X Boc-C**H$_3$**), 1.42-1.32 (q, 6H, *J* = 7.5, 5.2 Hz, -S(CH$_2$C**H$_3$**)$_2$); ESIMS: *m/z* 627 (M+H)$^+$.

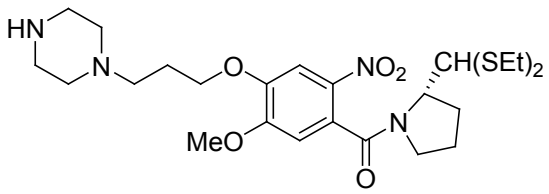
Methoxy-(2*S*)-[N-{3-[4-(*N-ter*-butoxycaronyl)piperazino]butyloxy}-5-methoxy–2-nitrobenzoyl]pyrrolidine-2-carboxaledhyde diethylthioacetal (10b)



The compound **10b** was prepared according to the method described for compound **10a** by employing compound **9b** (535 mg, 1mmol), to afford **10b**. (Yield 576 mg, 90%); ^{1}H NMR ($CDCl_3$, 300 MHz); δ 7.68 (s, 1H, PBD-Ar**H**), 6.79 (s, 1H, PBD-Ar**H**), 4.89 (d, 1H, *J* = 3.7 Hz, -C**H**(SEt)$_2$), 4.78-4.65 (m, 1H, -N-C**H**-), 4.18-4.13 (t, 2H, *J* = 6.5 Hz, -OC**H$_2$**-), 3.95 (s, 3H, -OC**H$_3$**), 3.51-3.40 (m, 4H, 2 X piperzinyl-C**H$_2$**-), 3.28-3.20 (m, 2H, -NC**H$_2$**), 2.88-2.70 (m, 4H, -S(C**H$_2$**CH$_3$)$_2$), 2.55 (t, 2H, *J* = 6.0 Hz, -C**H$_2$**), 2.51-2.45 (m, 4H, 2 x piperzinyl-C**H$_2$**-), 2.37-2.32 (m, 4H, 2 X -C**H$_2$**-), 2.20-1.67 (m, 4H, 2 X -C**H$_2$**-) 1.50 (s, 9H, 3 X Boc-C**H$_3$**), 1.41-1.33 (m, 6H, *J* = 7.5 Hz, -S(CH$_2$C**H$_3$**)$_2$); ESIMS: *m/z* 641 (M+H)$^+$.

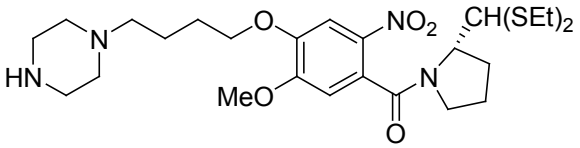
Methoxy-(2*S*)-[*N*-{3-[4-piperazino]propyloxy}-5-methoxy–2-nitrobenzoyl]pyrrolidine-2-carboxaledhyde diethylthioacetal (11a)

To a DCM solution (anhydrous, 15 mL) of compound **10a** (625 mg, 1 mmol) in a round-bottomed flask was added TFA (0.765 mL, 10 mmol) at room temperature. After that, the reaction mixture was stirred at room temperature for 12 h and TLC analysis indicated that compound **10a** was consumed. Then the reaction was diluted with CH_2Cl_2 (20 mL) and washed with saturated Na_2CO_3 solution until the pH value of the aqueous layer was 9. Then the aqueous layer was extracted with DCM (3 x 10 mL) and the combined organic solvent was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford compound **11a** as a light yellow glass (Yield 484 mg, 92%).



^{1}H NMR ($CDCl_3$, 200 MHz); δ 7.67 (s, 1H, PBD-Ar**H**), 6.73 (s, 1H, PBD-Ar**H**), 4.87 (d, 1H, *J* = 3.8 Hz, -C**H**(SEt)$_2$), 6.21 (bs, 1H, -N**H**), 4.75-4.68 (m, 1H, -N-C**H**-), 4.17-4.14 (t, 2H, *J* = 6.5 Hz, -OC**H$_2$**-), 3.95 (s, 3H, -OC**H$_3$**), 3.28-3.20 (m, 2H, -NC**H$_2$**), 2.88-2.70 (m, 4H, -S(C**H$_2$**CH$_3$)$_2$), 2.62-2.71 (m, 4H, 2 X piperzinyl-C**H$_2$**-), 2.58 (t, 2H, *J* = 6.0 Hz, -C**H$_2$**-), 2.47-2.40 (m, 4H, 2 X piperzinyl-C**H$_2$**-), 2.38-2.30 (m, 4H, 2 X -C**H$_2$**-), 2.02-2.06 (m, 2H, -C**H$_2$**-), 1.46-1.32 (m, 6H, *J* = 7.5 Hz, -S(CH$_2$C**H$_3$**)$_2$); ESIMS: *m/z* 527 $(M+H)^+$.

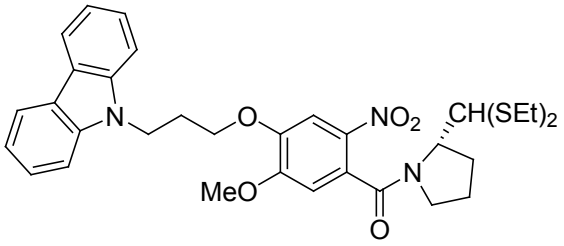
Methoxy-(2*S*)-[N-{3-[4-piperazino]butyloxy}-5-methoxy–2-nitrobenzoyl]pyrrolidine-2-carboxaledhyde diethylthioacetal (11b)



The compound **11b** was prepared according to the method described for compound **11a** by employing compound **10b** (641 mg, 1mmol), to afford **11b**. (Yield 486 mg, 90%); ^{1}H NMR ($CDCl_3$, 300 MHz); δ 7.64 (s, 1H, PBD-Ar**H**), 6.75 (s, 1H, PBD-Ar**H**), 6.11 (bs, 1H, -N**H**), 4.88 (d, 1H, *J* = 3.7 Hz, -C**H**(SEt)$_2$), 4.72-4.65 (m, 1H, -NC**H**-), 4.14-4.08 (t, 2H, *J* = 6.5 Hz, -OC**H$_2$**-), 3.97 (s, 3H, -OC**H$_3$**), 3.28-3.21 (m, 2H, -

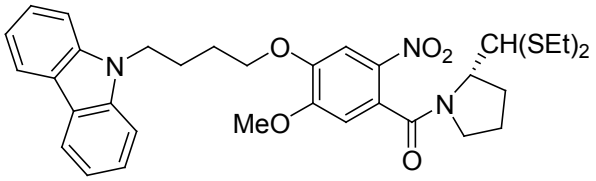
NC**H₂**), 2.87-2.71 (m, 4H, -S(C**H₂**CH₃)₂), 2.62-2.71 (m, 4H, 2 X piperzinyl-C**H₂**-), 2.58 (t, 2H, *J* = 6.0 Hz, -C**H₂**-), 2.47-2.40 (m, 4H, 2 X piperzinyl-C**H₂**-), 2.38-2.30 (m, 4H, 2 X -C**H₂**-), 2.20-1.67 (m, 4H, 2 X -C**H₂**-), 1.46-1.32 (m, 6H, *J* = 7.5 Hz, -S(CH₂C**H₃**)₂); ESIMS: *m/z* 541 $(M+H)^+$.

(2*S*)-*N*-{4-(3-[4-(9*H*-9-Carbazoly)-2-methoxyphenoxy]propyl)oxy-5-methoxy-2-nitrobenzoyl}-pyrrolidine-2-carboxaldehyde diethyl thioacetal (9a)



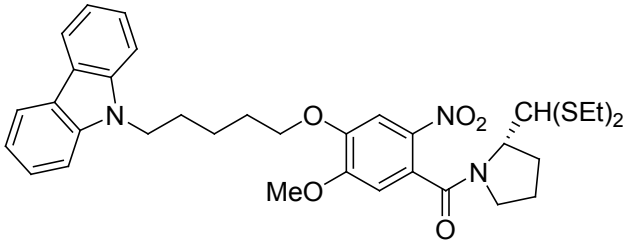
To a solution of (2*S*)-[*N*-{3-bromopropyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehydediethylthioacetal (**8a**) (521 mg, 1mmol), and in dry acetone (15 mL) was added, anhydrous K_2CO_3 (414 mg, 3 mmol), and carbazole (**6a**) (200 mg, 1.2 mmol) the mixture was stirred at reflux temperature for 24 hours. The reaction was monitored by TLC using ethyl acetate-hexane (1:1). After completion of the reaction as indicated by the TLC, K_2CO_3 was removed by filtration and the solvent evaporated under reduced pressure, diluted with water and extracted with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and evaporated under vacuum. The residue, thus obtained was purified by column chromatography using ethyl acetate and hexane (1:1) to afford compound **9a** as yellow solid. Yield (437 mg, 72%); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.02 (d, 2H, *J* = 7.5 Hz, carbazole-**H**), 7.55 (s, 1H, PBD-Ar**H**), 7.40 (t, 2H, *J* = 8.3 Hz, carbazole-**H**), 7.35 (d, 2H, *J* = 7.7 Hz, carbazole-**H**), 7.18 (t, 2H, *J* = 7.5 Hz, carbazole-**H**), 6.86 (s, 1H, PBD-Ar**H**), 4.87 (d, 1H, *J* = 3.7, Hz, -C**H**(SEt)₂), 4.68-4.75 (m, 1H, -N-C**H**-), 4.61 (t, 2H, *J* = 6.8 Hz, -OC**H₂**-), 4.05 (s, 3H, -OC**H₃**), 3.91-4.0 (m, 2H, -NC**H₂**-), 3.18-3.20 (m, 2H, -NC**H₂**-), 2.69-2.87 (m, 4H, -S(C**H₂**CH₃)₂), 2.45 (t, 2H, *J* = 6.8 Hz, -C**H₂**-), 2.25-2.21 (m, 2H, -C**H₂**-), 1.97-1.72 (m, 2H, -C**H₂**-), 1.39-1.36 (m, 6H, -S(CH₂C**H₃**)₂); FABMS: *m/z* 608 $(M+H)^+$.

(2*S*)-*N*-{4-(4-[4-(9*H*-9-Carbazoly)-2-methoxyphenoxy]butyl)oxy-5-methoxy-2-nitrobenzoyl}-pyrrolidine-2-carboxaldehyde diethyl thioacetal (9b)



The compound **9b** was prepared according to the method described for compound **9a** by employing (2*S*)-[*N*-{4-bromobutyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehydediethylthioacetal (**8b**) (535 mg, 1mmol), and carbazole (**6a**) (200 mg, 1.2 mmol). Yield (459 mg, 74%); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.04 (d, 2H, *J* = 7.5 Hz, carbazole-**H**), 7.55 (s, 1H, PBD-Ar**H**), 7.42 (t, 2H, *J*= 8.3 Hz, carbazole-**H**), 7.30 (d, 2H, *J* = 7.7 Hz, carbazole-**H**), 7.18 (t, 2H, *J* = 7.7, carbazole-**H**), 6.78 (s, 1H, PBD-Ar**H**), 4.82 (d, 1H, *J* = 3.7 Hz, -C**H**$(SEt)_2$), 4.65-4.71 (m, 1H, -NC**H**-), 4.60 (t, 2H, *J* = 6.9 Hz, -OC$\mathbf{H_2}$-), 4.05 (t, 2H, *J* = 6.4 Hz, -NC$\mathbf{H_2}$-), 3.92 (s, 3H, -OC$\mathbf{H_3}$), 3.22-3.27 (m, 2H, -NC$\mathbf{H_2}$-), 2.65-2.82 (m, 4H, -S(C$\mathbf{H_2}$$CH_3)_2$), 2.20-2.01 (m, 4H, -C$\mathbf{H_2}$-), 1.92-1.85 (m, 2H, -C$\mathbf{H_2}$-), 1.75-1.82 (m, 2H, -C$\mathbf{H_2}$-), 1.39-1.37 (m, 6H, -S(CH_2C$\mathbf{H_3}$)$_2$); FABMS: *m/z* 622 $(M+H)^+$.

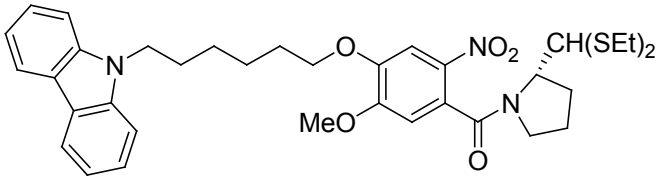
(2*S*)-*N*-{4-(5-[4-(9*H*-9-Carbazoly)-2-methoxyphenoxy]pentyl)oxy-5-methoxy-2-nitrobenzoyl}-pyrrolidine-2-carboxaldehyde diethyl thioacetal (9c)



The compound **9c** was prepared according to the method described for compound **9a** by employing(2*S*)-[*N*-{5-bromopentyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehydediethylthioacetal (**8c**) (549 mg, 1mmol), and carbazole (**6a**) (200 mg, 1.2 mmol). Yield (445 mg, 70%); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.05 (d, 2H, *J* = 7.5 Hz, carbazole-**H**), 7.55 (s, 1H, PBD-Ar**H**), 7.34-7.43 (m, 4H, *J* = 8.3, 6.9 Hz, carbazole-**H**), 7.20 (d, 2H, *J* = 7.7 Hz, carbazole-**H**), 6.78 (s, 1H, PBD-Ar**H**), 4.82 (d, 1H, *J* = 3.7 Hz, -C**H**$(SEt)_2$), 4.65-4.69 (m, 1H, -NC**H**-), 4.35 (t, 2H, *J* = 6.6 Hz, -OC$\mathbf{H_2}$-), 4.01 (m, 2H, -

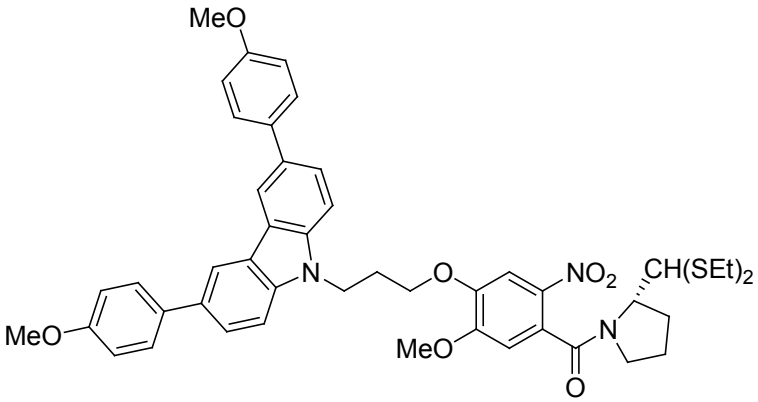
NC**H₂**-), 3.92 (s, 3H, -OC**H₃**), 3.20-3.26 (m, 2H, -NC**H₂**-), 2.65-2.82 (m, 4H, -S(C**H₂**CH$_3$)$_2$), 1.56-2.28 (m, 10H, 5 X -C**H₂**-), 1.37-1.39 (m, 6H, -S(CH$_2$C**H₃**)$_2$); FABMS: *m/z* 636 (M+H)$^+$.

(2*S*)-*N*-{4-(6-[4-(9*H*-9-Carbazoly)-2-methoxyphenoxy]hexyl)oxy-5-methoxy-2-nitrobenzoyl}-pyrrolidine-2-carboxaldehyde diethyl thioacetal (9d)



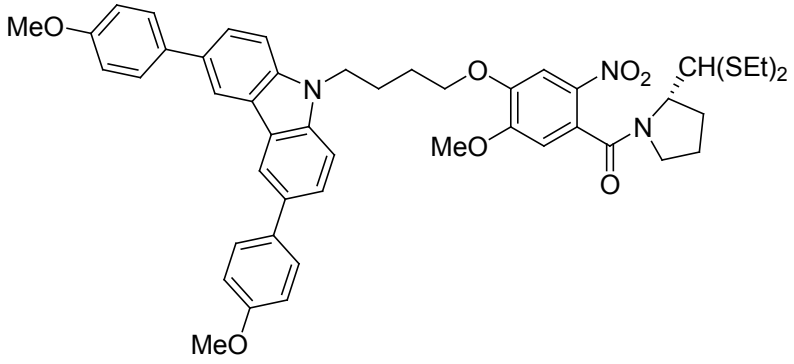
The compound **9d** was prepared according to the method described for compound **9a** by employing (2*S*)-[*N*-{6-bromohexyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehydediethylthioacetal (**8d**) (563 mg, 1mmol), and carbazole (**6a**) (200 mg, 1.2 mmol). Yield (493 mg, 76%); ^{1}H NMR (CDCl$_3$, 300 MHz): δ 8.04 (d, 2H, *J* = 7.5 Hz, carbazole-**H**), 7.55 (s, 1H, PBD-Ar**H**), 7.32-7.41 (m, 4H, *J* = 6.7, 7.5 Hz, carbazole-**H**), 7.19 (d, 2H, *J* = 7.7 Hz, carbazole-**H**), 6.74 (s, 1H, PBD-Ar**H**), 4.82 (d, 1H, *J* = 3.7 Hz, -C**H**(SEt)$_2$), 4.63-4.72 (m, 1H, -NC**H**-), 4.32 (t, 2H, *J* = 6.8 Hz, -OC**H₂**-), 3.99 (t, 2H, *J* = 6.8 Hz, -NC**H₂**-), 3.89 (s, 3H, -OC**H₃**), 3.18-3.27 (m, 2H, -NC**H₂**-), 2.65-2.84 (m, 4H, *J* = 7.5 Hz, -S(C**H₂**CH$_3$)$_2$), 2.32-2.05 (m, 2H, -C**H₂**-), 1.76-1.97 (m, 6H, *J* = 6.8 Hz, 3 X -C**H₂**-), 1.45-1.52 (m, 4H, 2 X -C**H₂**-), 1.31-1.39 (q, 6H, *J* = 7.5 Hz, -S(CH$_2$C**H₃**)$_2$); FABMS: *m/z* 650 (M+H)$^+$.

(2*S*)-*N*-{4-(3-[4-(3,6-di(4-methoxyphenyl)-9*H*-9-carbazoly)-2-methoxyphenoxy]propyl)oxy-5-methoxy-2-nitrobenzoyl}-pyrrolidine-2-carboxaldehyde diethyl thioacetal (9e)



The compound **9e** was prepared according to the method described for compound **9a** by employing (2*S*)-[*N*-{3-bromopropyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehydediethylthioacetal (**8a**) (521 mg, 1mmol), and 3,6-bis(4-methoxyphenyl)-9*H*-carbazole (**6b**) (455 mg, 1.2 mmol). Yield (582 mg, 69%); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.21 (s, 2H, carbazole-**H**), 7.56-7.61 (m, 6H, *J* = 8.3 Hz, 2 X carbazole-**H,** 4 X carbazole-Ar**H**), 7.51 (s, 1H, PBD-Ar**H**), 7.43 (d, 2H, *J* = 8.3 Hz, carbazole-**H**), 6.94 (d, 4H, *J* = 9.0 Hz, carbazole-Ar**H**), 6.82 (s, 1H, PBD-Ar**H**), 4.82 (d, 1H, *J* = 3.7 Hz, -S(C**H$_2$**CH$_3$)$_2$), 4.62-4.71 (m, 1H, -NC**H**-), 4.32 (t, 2H, *J* = 6.8 Hz, -OC**H$_2$**-), 4.07-4.11 (t, 2H, *J* = 6.8 Hz, -NC**H$_2$**-), 4.05 (s, 3H, -OC**H$_3$**), 3.85 (s, 6H, 2 X -OC**H$_3$**), 3.04-3.21 (m, 2H, -NC**H$_2$**-), 2.65-2.88 (m, 4H, -S(C**H$_2$**CH$_3$)$_2$), 2.44-2.46 (m, 2H, *J* = 6.0 Hz, -C**H$_2$**-), 2.23-2.04 (m, 2H, -C**H$_2$**-), 1.64-1.88 (m, 2H, -C**H$_2$**-), 1.38-1.35 (m, 6H, *J* = 7.5 Hz -S(CH$_2$C**H$_3$**)$_2$); FABMS: *m/z* 821(M+H)$^+$.

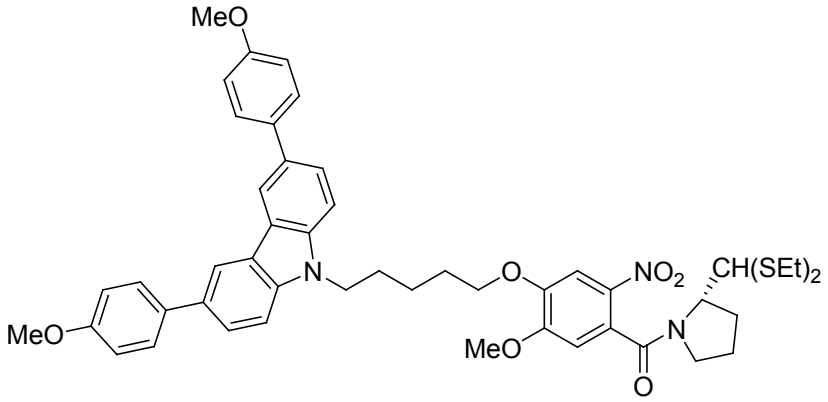
(2*S*)-*N*-{4-(4-[4-(3,6-di(4-methoxyphenyl)-9*H*-9-carbazoly)-2-methoxyphenoxy]butyl)oxy-5-methoxy-2-nitrobenzoyl}-pyrrolidine-2-carboxaldehyde diethyl thioacetal (9f)



The compound **9f** was prepared according to the method described for compound **9a** by employing (2*S*)-[*N*-{4-bromobutyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehydediethylthioacetal (**8b**) (535 mg, 1 mmol), and 3,6-bis(4-methoxyphenyl)-9*H*-carbazole (**6b**) (455 mg, 1.2 mmol). Yeild (583 mg, 70%); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.21 (s, 2H, carbazole-**H**), 7.56-7.61 (m, 6H, *J* = 8.3 Hz, 2 X carbazole-**H,** 4 X carbazole-Ar**H**), 7.51 (s, 1H, PBD-Ar**H**), 7.44 (d, 2H, *J* = 8.3 Hz, carbazole-**H**), 6.94 (d, 4H, *J* = 9.0 Hz, carbazole-Ar**H**), 6.82 (s, 1H, PBD-Ar**H**), 4.85 (d, 1H, *J* = 3.7 Hz, -C**H**(SEt)$_2$), 4.68-4.72 (m, 1H, -NC**H**-), 4.44 (t, 2H, *J* = 6.7 Hz, -OC**H$_2$**-), 4.02 (t, 2H, , *J* = 6.4 Hz, -NC**H$_2$**-), 3.92 (s, 3H, -OC**H$_3$**), 3.85 (s, 6H, 2 X -OC**H$_3$**), 3.21-3.27 (m, 2H, -

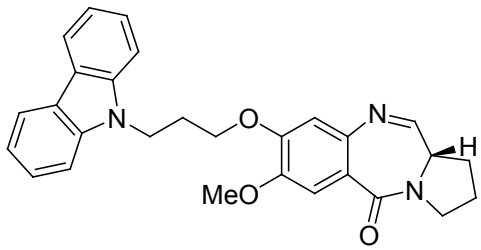
NC**H₂**-), 2.65-2.88 (m, 4H, -S(C**H₂**CH$_3$)$_2$), 2.39-2.34 (m, 2H, -C**H₂**-), 2.02-2.23 (m, 4H, -C**H₂**-), 1.78-1.94 (m, 2H, -C**H₂**-), 1.42-1.37 (m, 6H, -S(CH$_2$C**H₃**)$_2$); FABMS: *m/z* 835 $(M+H)^+$.

(2*S*)-*N*-{4-(5-[4-(3,6-di(4-methoxyphenyl)-9*H*-9-carbazoly)-2-methoxyphenoxy]pentyl)oxy-5-methoxy-2-nitrobenzoyl}-pyrrolidine-2-carboxaldehyde diethyl thioacetal (9g)



The compound **9g** was prepared according to the method described for compound **9a** by employing (2*S*)-[*N*-{5-bromopentyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehydediethylthioacetal (**8c**) (549 mg, 1mmol), and 3,6-bis(4-methoxyphenyl)-9*H*-carbazole (**6b**) (455 mg, 1.2 mmol). Yeild (610 mg, 72%); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.2 (s, 2H, carbazole-**H**), 7.54-7.62 (m, 6H, *J* = 8.3 Hz, 2 X carbazole-**H,** 4 X carbazole-Ar**H**), 7.52 (s, 1H, PBD-Ar**H**), 7.45 (d, 2H, *J* = 8.3 Hz, carbazole-**H**), 6.96 (d, 4H, *J* = 9.0 Hz, carbazole-Ar**H**), 6.81 (s, 1H, PBD-Ar**H**), 4.82 (d, 1H, *J* = 3.7 Hz, -C**H**(SEt)$_2$), 4.63-4.67 (m, 1H, -NC**H**-), 4.45 (t, 2H, *J* = 6.6 Hz, -OC**H₂**-), 4.05 (t, 2H, *J* = 6.0 Hz, -NC**H₂**-), 3.92 (s, 3H, -OC**H₃**), 3.85 (s, 6H, 2 X -OC**H₃**), 3.22-3.25 (m, 2H, -NC**H₂**-), 2.65-2.82 (m, 4H, -S(C**H₂**CH$_3$)$_2$), 1.56-2.28 (m, 10H, 5 X –C**H₂**-), 1.36-1.39 (m, 6H, -S(CH$_2$C**H₃**)$_2$); FABMS: *m/z* 849 $(M+H)^+$.

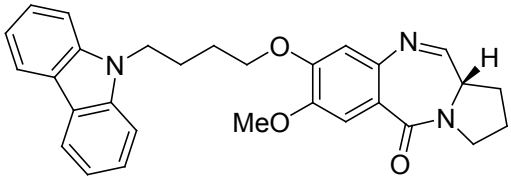
7-Methoxy-8-[3-(9*H*-9-carbazoly)propoxy]-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*] [1,4]benzodiazepin-5-one (4a)



To the compound **9a** (607 mg, 1 mmol) in methanol (20 mL) was added $SnCl_2.2H_2O$ (1.12 mg, 5 mmol) and reflux for 5 hrs until the TLC indicated the reaction was completed. The methanol was evaporated under vacuum, the aqueous phase was then carefully adjusted to pH 8 with 10% $NaHCO_3$ solution and the extracted with ethyl acetate and chloroform (2x30 mL and 2x30 mL). The combined organic phases was dried over Na_2SO_4 and evaporated under vacuum to afford the crude amino diethylthioacetal (520 mg, 90%), which was used directly in the next step due to its potential stability problem.

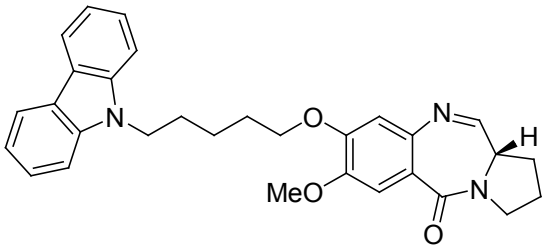
A solution of amino diethylthioacetal (577 mg, 1.0 mmol), $HgCl_2$ (613 mg, 2.26 mmol) and $CaCO_3$ (246 mg, 2.46 mmol) in acetonitrile-water (4:1) was stirred slowly at room temperature overnight until complete consumption of starting material as indicated by the TLC. The clear organic supernatant liquid was extracted with ethyl acetate and washed with saturated 5% $NaHCO_3$ (20 mL), brine (20 mL) and the combined organic phase was dried over Na_2SO_4. The organic layer was evaporated in vacuum to afford a white solid, which was first eluted on a column chromatography with ethyl acetate to remove mercuric salts, and then with MeOH-$CHCl_3$ (1:24) to obtain the pure product **4a.** Yield (258 mg, 57%). Mp 79-80 ^oC; $[\alpha]_D^{27}$ +115.6 (c = 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.05 (d, 2H, , J = 7.5 Hz, carbazole-**H**), 7.61 (d, 1H J = 4.5 Hz, -N=C**H**-), 7.56 (s, 1H, PBD-Ar**H**), 7.45 (t, 2H, J = 8.3 Hz, carbazole-**H**), 7.35 (d, 2H, J = 7.7 Hz, carbazole-**H**), 7.18 (t, 2H, J = 7.74 Hz, carbazole-**H**), 6.85 (s, 1H, PBD-Ar**H**), 4.12-4.23 (t, 2H, J = 6.8 Hz, -OC$\mathbf{H_2}$-), 3.97-4.0 (m, 2H, -NC$\mathbf{H_2}$-), 3.95 (s, 3H, -OC$\mathbf{H_3}$), 3.52-3.73 (m, 3H, -NC$\mathbf{H_2}$-, -NC**H**-), 2.46-1.85 (m, 6H, 3 X -C$\mathbf{H_2}$-); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.7, 162.4, 150.8, 147.8, 140.6, 140.3, 125.5, 122.7, 120.3, 120.1, 118.7, 111.5, 110.4, 108.5, 68.8, 56.1, 53.7, 48.3, 46.7, 29.7, 28.8, 24.2; FABMS: *m/z* 454 $(M+H)^+$, 485 $(M+MeOH)^+$. Elemental Analysis $C_{28}H_{27}N_3O_3$: calcd C, 74.15, H, 6.00, N, 9.27; found C, 74.10, H, 5.97, N, 9.25.

7-Methoxy-8-[4-(9*H*-9-carbazoly)butoxy]-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*] [1,4]benzodiazepin-5-one (4b)



This compound was prepared according to the method described for the compound **4a**, reduction of compound **9b** (621 mg, 1.0 mmol) gives amino diethylthioacetal (532 mg, 90%) then this aminodiethylthioacetal intermediate (592 mg, 1 mmol) on deprotection $HgCl_2$ (613 mg, 2.26 mmol), $CaCO_3$ (246 mg, 2.46 mmol) in acetonitrile-water (4:1) affords the pure product **4b.** Yield (271 mg, 58%). Mp 83-82 ^oC; $[\alpha]_D^{27}$ +109.5 (*c* = 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.05 (d, 2H, *J* = 7.3 Hz, carbazole-**H**), 7.63 (d, 1H, *J* = 4.6 Hz, -N=C**H**-), 7.55 (s, 1H, PBD-Ar**H**), 7.45 (t, 2H, *J* = 8.3 Hz, carbazole-**H**), 7.32-7.18 (m, 4H, *J* = 8.0 Hz, carbazole-**H**), 6.64 (s, 1H, PBD-Ar**H**), 4.35 (t, 2H, *J* = 6.5 Hz, -OC$\mathbf{H_2}$-), 3.92-4.2 (m, 2H, -NC$\mathbf{H_2}$-), 3.90 (s, 3H, -OC$\mathbf{H_3}$), 3.42-3.74 (m, 3H, -NC$\mathbf{H_2}$-, -NC**H**-), 2.29-2.34 (t, 2H, -C$\mathbf{H_2}$-), 2.12-1.72 (m, 6H, 3 X –C$\mathbf{H_2}$-); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.7, 162.4, 150.8, 147.8, 140.6, 140.3, 125.5, 122.7, 120.3, 120.1, 118.7, 111.5, 110.4, 108.5, 68.8, 56.1, 53.7, 46.7, 44.3, 29.7, 26.6, 25.8, 24.2; FABMS: *m/z* 468 $(M+H)^+$, 490 $(M+Na)^+$. Elemental Analysis $C_{29}H_{29}N_3O_3$: calcd C, 74.50, H, 6.25, N, 8.99; found C, 74.48, H, 6.20, N, 8.92.

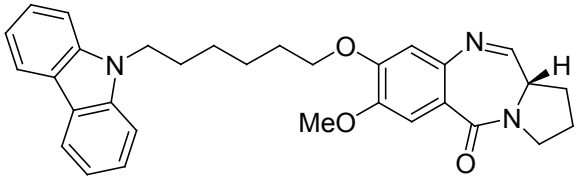
7-Methoxy-8-[5-(9*H*-9-carbazoly)pentyloxy]-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*] [1,4]benzodiazepin-5-one (4c)



This compound was prepared according to the method described for the compound **4a,** reduction of compound **9c** (635 mg, 1.0 mmol) gives amino diethylthioacetal (533 mg, 88%) then this aminodiethylthioacetal intermediate (605 mg, 1 mmol) on deprotection $HgCl_2$ (613 mg, 2.26 mmol), $CaCO_3$ (246 mg, 2.46

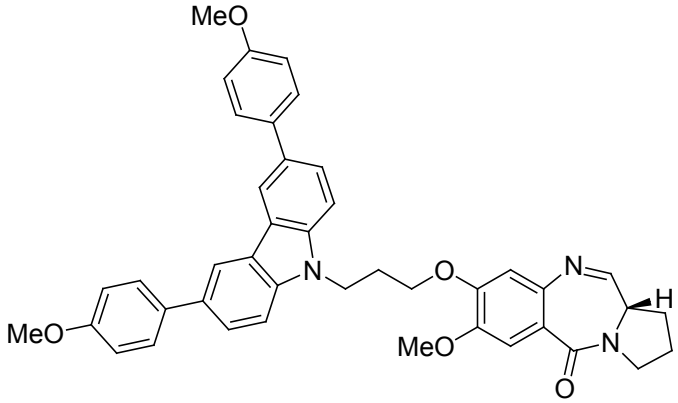
mmol) in acetonitrile-water (4:1) affords the pure product **4c**. Yield (279 mg, 58%). Mp 85-84 °C; $[\alpha]_D^{27}$ +110.8 (*c* = 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.05 (d, 2H, *J* = 7.3 Hz, carbazole-**H**), 7.64 (d, 1H, *J* = 4.6 Hz, -N=C**H**-), 7.56 (s, 1H, PBD-Ar**H**), 7.32-7.42 (m, 4H, *J* = 8.0 Hz, carbazole-**H**), 7.17-7.23 (m, 2H, carbazole-**H**), 6.78 (s, 1H, PBD-Ar**H**) 4.37 (t, 2H, *J* = 6.5 Hz, -OC**H₂**-), 3.95-4.01 (m, 2H, -NC**H₂**-), 3.90 (s, 3H, -OC**H₃**), 3.42-3.74 (m, 3H, -NC**H₂**-, -NC**H**-), 2.29-1.64 (m, 10H, 5 X -C**H₂**-); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.7, 162.4, 150.8, 147.8, 140.6, 140.3, 125.5, 122.7, 120.3, 120.1, 118.7, 111.5, 110.4, 108.5, 68.8, 56.1, 53.7, 46.7, 43.3, 29.7, 29.1, 28.5, 24.2, 23.3; FABMS: *m/z* 482 $(M+H)^+$, 504 $(M+Na)^+$. Elemental Analysis $C_{30}H_{31}N_3O_3$: calcd C, 74.82, H, 6.49, N, 8.73; found C, 74.78, H, 6.42, N, 8.69.

7-Methoxy-8-[6-(9*H*-9-carbazoly)hexyloxy]-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*] [1,4]benzodiazepin-5-one (4d)



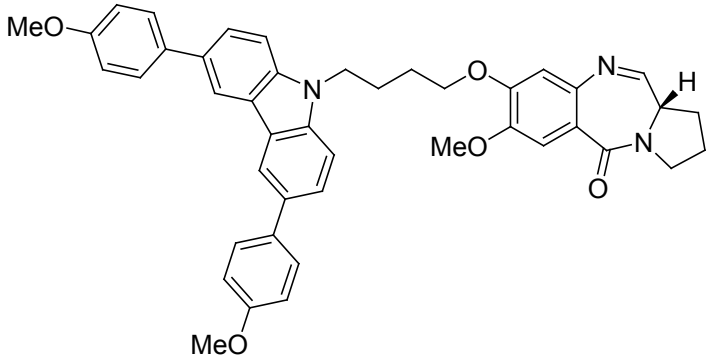
This compound was prepared according to the method described for the compound **4a,** reduction of compound **9d** (649 mg, 1.0 mmol) gives amino diethylthioacetal (551 mg, 89%) then this aminodiethylthioacetal intermediate (619 mg, 1 mmol) on deprotection $HgCl_2$ (613 mg, 2.26 mmol), $CaCO_3$ (246 mg, 2.46 mmol) in acetonitrile-water (4:1) affords the pure product **4d**. Yield (277 mg, 56%). Mp 87-86 °C; $[\alpha]_D^{27}$ +112.8 (*c* = 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.05 (d, 2H, *J* = 8.1 Hz, carbazole-**H**), 7.66 (d, 1H, *J* = 4.4 Hz, -N=C**H**-), 7.55 (s, 1H, PBD-Ar**H**), 7.38-7.45 (m, 4H, carbazole-**H**), 7.18-7.26 (t, 2H, *J* = 7.7 Hz, carbazole-**H**), 6.79 (s, 1H, PBD-Ar**H**), 4.38 (t, 2H, *J* = 6.6 Hz, -OC**H₂**-), 4.01-4.18 (m, 2H, -NC**H₂**-), 3.92 (s, 3H, -OC**H₃**), 3.54-3.88 (m, 3H, -NC**H₂**-, -NC**H**-), 2.48-1.71 (m, 12H, 6 X -C**H₂**-); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.7, 162.4, 150.8, 147.8, 140.6, 140.3, 125.5, 122.7, 120.3, 120.1, 118.7, 111.5, 110.4, 108.5, 68.8, 56.1, 53.7, 46.7, 43.3, 29.7, 29.5, 28.5, 26.4, 25.8, 24.3; FABMS: *m/z* 496 $(M+H)^+$, 528 $(M+MeOH)^+$. Elemental Analysis $C_{31}H_{33}N_3O_3$: calcd C, 75.13, H, 6.71, N, 8.48; found C, 75.15, H, 6.70, N, 8.42.

7-Methoxy-8-{3-[3,6-di(4-methoxyphenyl)-9*H*-9-carbazoly]propoxy}-(11a*S*)-1,2,3,11a- tetra hydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (4e)



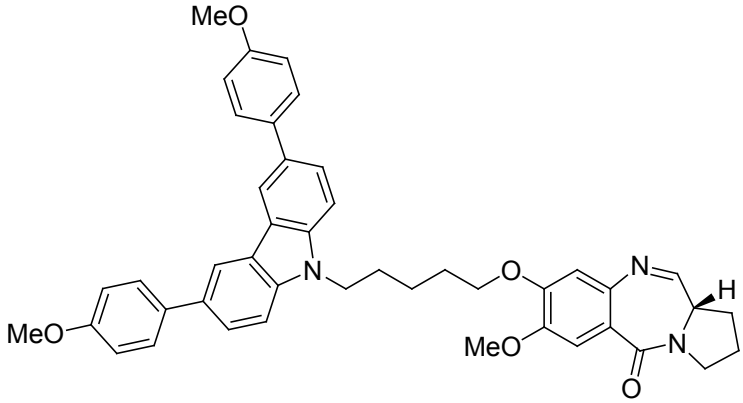
This compound was prepared according to the method described for the compound **4a** reduction of compound **9e** (820 mg, 1.0 mmol) gives amino diethylthioacetal (711 mg, 90%) then this aminodiethylthioacetal intermediate (790 mg, 1 mmol) on deprotection $HgCl_2$ (613 mg, 2.26 mmol), $CaCO_3$ (246 mg, 2.46 mmol) in acetonitrile-water (4:1) affords the pure product **4e.** Yield (332 mg, 50%). Mp 79-78 ^{o}C; $[\alpha]_D^{27}$ +113.6 (*c* = 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.25 (s, 2H, carbazole-**H**), 7.59-7.68 (m, 7H, *J* = 8.3, *J* = 4.5 Hz, carbazole-**H,** carbazole-Ar**H,** -N=C**H**-), 7.54 (s, 1H, PBD-Ar**H**), 7.49 (d, 2H, *J* = 8.3 Hz, carbazole-**H**), 7.07 (d, 4H, *J* = 8.3 Hz, carbazole-Ar**H**), 6.58 (s, 1H, PBD-Ar**H**), 4.26 (t, 2H, *J* = 6.8 Hz, -OC$\mathbf{H_2}$-), 4.06-4.15 (m, 2H, -NC$\mathbf{H_2}$-), 4.01 (s, 3H, -OC$\mathbf{H_3}$), 3.95 (s, 6H, 2 X -OC$\mathbf{H_3}$), 3.81-3.71 (m, 3H, -NC$\mathbf{H_2}$-, -NC**H**-), 2.48-1.72 (m, 6H, 3 X –C$\mathbf{H_2}$-); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.7, 162.4, 158.6, 150.8, 147.8, 140.6, 139.5, 134.4, 132.8, 128.2, 125.3, 123.7, 120.3, 118.7, 114.2, 111.5, 110.4, 108.7, 68.9, 56.2, 55.3, 53.4, 48.3, 46.7, 29.7, 28.7, 24.3; FABMS: *m/z* 666 $(M+H)^+$, 688 $(M+Na)^+$. Elemental Analysis $C_{42}H_{39}N_3O_5$: calcd C, 75.77, H, 5.90, N, 6.31; found C, 75.75, H, 5.85, N, 6.30.

7-Methoxy-8-{4-[3,6-di(4-methoxyphenyl)-9*H*-9-carbazoly]butoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (4f)



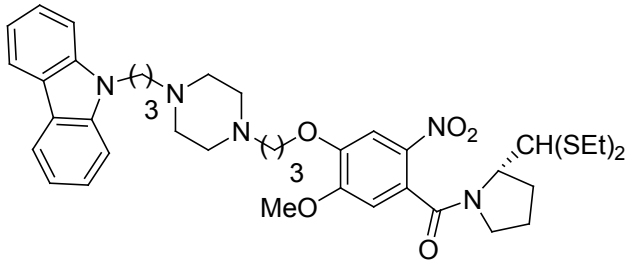
The compound **4f** was prepared according to the method described for the compound **4a,** reduction of compound **9f** (834 mg, 1.0 mmol) gives amino diethylthioacetal (723 mg, 90%) then this aminodiethylthioacetal intermediate (804 mg, 1 mmol) on deprotection $HgCl_2$ (613 mg, 2.26 mmol), $CaCO_3$ (246 mg, 2.46 mmol) in acetonitrile-water (4:1) affords the pure product **4f.** Yield (394 mg, 58%). Mp 90-89 ^{o}C; $[\alpha]_D^{27}$ +114.6 (*c* = 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.21 (s, 2H, carbazole-**H**), 7.54-7.69 (m, 8H, *J* = 8.3, *J* = 4.5 Hz, carbazole-**H,** carbazole-Ar**H,** -N=C**H**-, PBD-Ar-**H**), 7.50 (d, 2H, *J* = 8.3 Hz, carbazole-**H**), 7.03 (d, 4H, *J* = 8.30 Hz, carbazole-Ar**H**), 6.56 (s, 1H, PBD-Ar-**H**), 4.35 (t, 2H, *J* = 6.5 Hz, -OC$\mathbf{H_2}$-), 3.92-4.2 (m, 2H, -NC$\mathbf{H_2}$-), 4.02 (s, 3H, -OC$\mathbf{H_3}$), 3.85 (s, 6H, 2 X -OC$\mathbf{H_3}$), 3.81-3.71 (m, 3H, -NC$\mathbf{H_2}$-, -NC**H**-), 2.48-1.62 (m, 8H, 4 X –C$\mathbf{H_2}$-); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.7, 162.4, 158.6, 150.8, 147.8, 140.6, 139.5, 134.4, 132.8, 128.2, 125.3, 123.7, 120.3, 118.7, 114.2, 111.5, 110.4, 108.7, 68.8, 56.1, 55.3, 53.7, 46.7, 44.3, 29.7, 26.6, 25.8, 24.2; FABMS: *m/z* 680 $(M+H)^+$, 702 $(M+Na)^+$. Elemental Analysis $C_{43}H_{41}N_3O_5$: calcd C, 75.97, H, 6.08, N, 6.18; found C, 75.95, H, 6.04, N, 6.20.

7-Methoxy-8-{4-[3,6-di(4-methoxyphenyl)-9*H*-9-carbazoly]pentyloxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (4g)



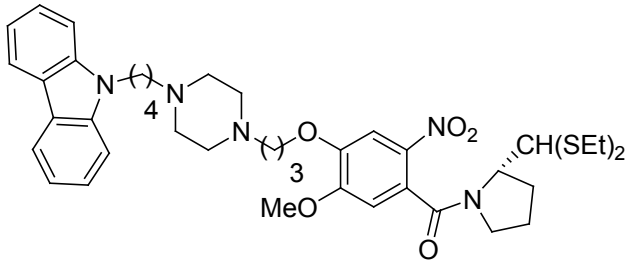
The compound **4g** was prepared according to the method described for the compound **4a**, reduction of compound **9g** (848 mg, 1.0 mmol) gives amino diethylthioacetal (728 mg, 89%) then this aminodiethylthioacetal intermediate (818 mg, 1 mmol) on deprotection $HgCl_2$ (613 mg, 2.26 mmol), $CaCO_3$ (246 mg, 2.46 mmol) in acetonitrile-water (4:1) affords the pure product **4g.** Yield (374 mg, 54%). Mp 95-94 ^{o}C; $[\alpha]_D^{27}$ +116.2 (*c* = 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.22 (s, 2H, carbazole-**H**), 7.54-7.68 (m, 8H, *J* = 8.3, *J* = 4.5 Hz, carbazole-**H,** carbazole-Ar**H,** -N=C**H**-, PBD-Ar-**H**), 7.52 (d, 2H, *J* = 8.3 Hz, carbazole-**H**), 7.0 (d, 4H, *J* = 8.30 Hz, carbazole-Ar**H**), 6.54 (s, 1H, PBD-Ar-**H**), 4.35 (t, 2H, *J* = 6.5 Hz, -OC$\mathbf{H_2}$-), 3.92-4.2 (m, 2H, -NC$\mathbf{H_2}$-), 3.95 (s, 3H, -OC$\mathbf{H_3}$), 3.85 (s, 6H, 2 X -OC$\mathbf{H_3}$), 3.42-3.74 (m, 3H, -NC$\mathbf{H_2}$-, -NC**H**-), 2.49-1.64 (m, 10H, 5 X –C$\mathbf{H_2}$-); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.7, 162.4, 158.6, 150.8, 147.8, 140.6, 139.5, 134.4, 132.8, 128.2, 125.3, 123.7, 120.3, 118.7, 114.2, 111.5, 110.4, 108.7, 68.8, 56.1, 55.3, 53.7, 46.7, 43.3, 29.7, 29.1, 28.5, 24.2, 23.3; FABMS: *m/z* 694 $(M+H)^+$, 716 $(M+Na)^+$. Elemental Analysis $C_{44}H_{43}N_3O_5$: calcd C, 76.17, H, 6.25, N, 6.06; found C, 76.14, H, 6.20, N, 6.00.

(2*S*)-*N*-{4-(3-{4-[3-(9*H*-9-Carbazoly)propyloxy]piperazino}propyl)oxy-5-methoxy-2-nitrobenzoyl}-pyrrolidine-2-carboxaldehyde diethylthioacetal (12a)



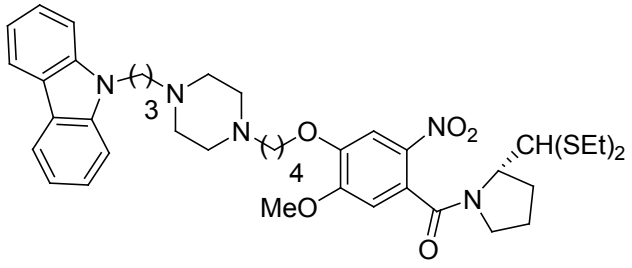
The compound **12a** was prepared according to the method described for compound **4a** by employing (2*S*)-[*N*-{4-(3-(piperazin-1-yl)propyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehydediethylthioacetal (**11a**) (526 mg, 1mmol), and 9-(3-bromopropyl)-9*H*-carbazole (**7a**) (345 mg, 1.2 mmol). Yeild (535 mg, 73%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.04 (d, 2H, *J* = 7.5 Hz, carbazole-**H**), 7.65 (s, 1H, PBD-Ar**H**), 7.33-7.43 (m, 4H, *J* = 8.3 Hz, carbazole-**H**), 7.18 (t, 2H, *J* = 7.7 Hz, carbazole-**H**), 6.78 (s, 1H, PBD-Ar**H**), 4.85 (d, 1H, *J* = 3.7 Hz, -C**H**$(SEt)_2$), 4.63-4.70 (m, 1H, -NC**H**-), 4.61-4.63 (t, 2H, *J* = 6.8 Hz, -OC$\mathbf{H_2}$-), 4.16-4.10 (m, 2H, -NC$\mathbf{H_2}$-), 3.92 (s, 3H, -OC$\mathbf{H_3}$), 3.19-3.27 (m, 2H, -NC$\mathbf{H_2}$-), 2.63-2.85 (m, 4H, -S(C$\mathbf{H_2}CH_3)_2$), 2.64-2.42 (m, 12H, 6 X –NC$\mathbf{H_2}$-(piperazinyl)), 2.26-1.52 (m, 8H, 4 X –C$\mathbf{H_2}$-), 1.31-1.39 (m, 6H, *J* = 6.79 Hz, -S(CH_2C$\mathbf{H_3})_2$); FABMS: *m/z* 735 $(M+H)^+$.

(2*S*)-*N*-{4-(3-{4-[4-(9*H*-9-Carbazoly)butyloxy]piperazino}propyl)oxy-5-methoxy-2-nitrobenzoyl}-pyrrolidine-2-carboxaldehyde diethylthioacetal (12b)



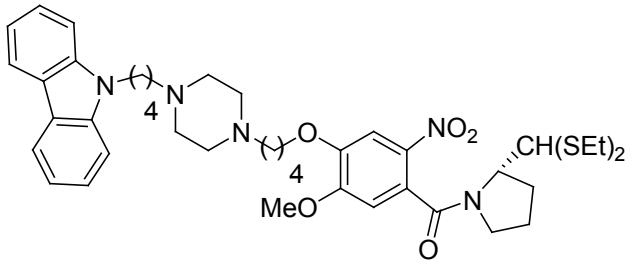
The compound **12b** was prepared according to the method described for compound **4a** by employing (2*S*)-[*N*-{4-(3-(piperazin-1-yl)propyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehydediethylthioacetal (**11a**) (526 mg, 1mmol), and 9-(4-bromobutyl)-9*H*-carbazole (**7b**) (362 mg, 1.2 mmol). Yeild (531 mg, 71%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.04 (d, 2H, *J* = 7.5 Hz, carbazole-**H**), 7.61 (s, 1H, PBD-Ar**H**), 7.32-7.40 (m, 4H, *J* = 7.5 Hz, carbazole-**H**), 7.18 (t, 2H, *J* = 7.5 Hz, carbazole-**H**), 6.78 (s, 1H, PBD-Ar**H**), 4.84 (d, 1H, *J* = 3.7 Hz, -C**H**$(SEt)_2$), 4.64-4.73 (m, 1H, -NC**H**-), 4.38-4.41 (t, 2H, *J* = 6.7 Hz, -OC$\mathbf{H_2}$-), 4.12-4.16 (m, 2H, -NC$\mathbf{H_2}$-), 3.94 (s, 3H, -OC$\mathbf{H_3}$), 3.20-3.24 (m, 2H, *J* = 6.7 Hz, -NC$\mathbf{H_2}$-), 2.68-2.84 (m, 4H, *J* = 6.7 Hz, -S(C$\mathbf{H_2}CH_3)_2$), 2.48-2.63 (m, 12H, *J* = 7.5 Hz, 6 X -NC$\mathbf{H_2}$-(piperazinyl)), 2.24-1.53 (m, 10H, 5 X –C$\mathbf{H_2}$-), 1.32-1.38 (m, 6H, *J* = 6.7 Hz, -S(CH_2C$\mathbf{H_3})_2$); FABMS: *m/z* 749 $(M+H)^+$.

(2*S*)-*N*-{4-(4-{4-[3-(9*H*-9-Carbazoly)propyloxy]piperazino}butyl)oxy-5-methoxy-2-nitrobenzoyl}-pyrrolidine-2-carboxaldehyde diethylthioacetal (12c)



The compound **12c** was prepared according to the method described for compound **4a** by employing (2*S*)-[*N*-{4-(4-(piperazin-1-yl)butyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehydediethylthioacetal (**11b**) (540 mg, 1mmol), and 9-(3-bromopropyl)-9*H*-carbazole (**7a**) (345 mg, 1.2 mmol). Yeild (495 mg, 69%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.04 (d, 2H, *J* = 7.5 Hz, carbazole-**H**), 7.65 (s, 1H, PBD-Ar**H**), 7.33-7.40 (m, 4H, *J* = 8.3 Hz, carbazole-**H**), 7.18 (t, 2H, *J* = 7.5 Hz, carbazole-**H**), 6.78 (s, 1H, PBD-Ar**H**), 4.84 (d, 1H, *J* = 3.7 Hz, -C**H**$(SEt)_2$), 4.66-4.72 (m, 1H, -NC**H**-), 4.47 (t, 2H, *J* = 6.0 Hz, -OC$\mathbf{H_2}$-), 4.15-4.17 (m, 2H, *J* = 6.0 Hz, -NC$\mathbf{H_2}$-), 3.94 (s, 3H, -OC$\mathbf{H_3}$), 3.19-3.23 (m, 2H, *J* = 6.7 Hz, -NC$\mathbf{H_2}$-), 2.68-2.89 (m, 4H, *J* = 7.9 Hz, -S(C$\mathbf{H_2}$$CH_3$)$_2$), 2.35-2.63 (m, 12H, 6 X -NC$\mathbf{H_2}$-(piperazinyl)), 2.33-1.74 (m, 10H, 5 X –C$\mathbf{H_2}$-),1.38 (m, 6H, *J* = 7.5 Hz, -S(CH_2C$\mathbf{H_3}$)$_2$); FABMS: *m/z* 749 $(M+H)^+$.

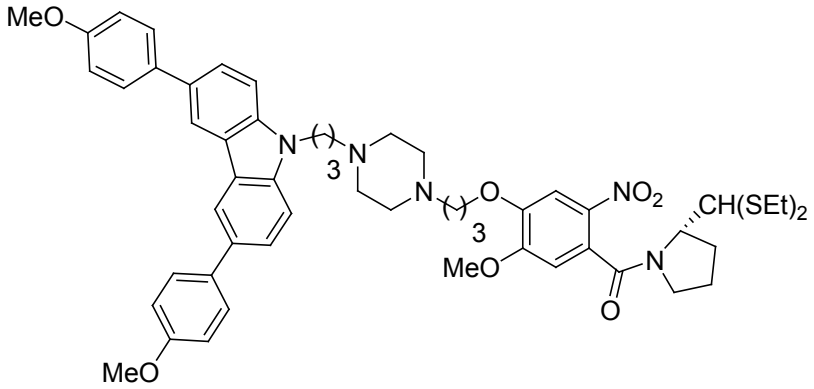
(2*S*)-*N*-{4-(4-{4-[4-(9*H*-9-Carbazoly)butyloxy]piperazino}butyl)oxy-5-methoxy-2-nitrobenzoyl}-pyrrolidine-2-carboxaldehyde diethylthioacetal (12d)



The compound **12d** was prepared according to the method described for compound **4a** by employing (2*S*)-[*N*-{4-(4-(piperazin-1-yl)butyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehydediethylthioacetal (**11b**) (540 mg, 1 mmol), and 9-(4-bromobutyl)-9*H*-carbazole (**7b**) (362 mg, 1.2 mmol). Yeild (563 mg, 74%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.04 (d, 2H, *J* = 7.5 Hz, carbazole-**H**), 7.61 (s, 1H, PBD-

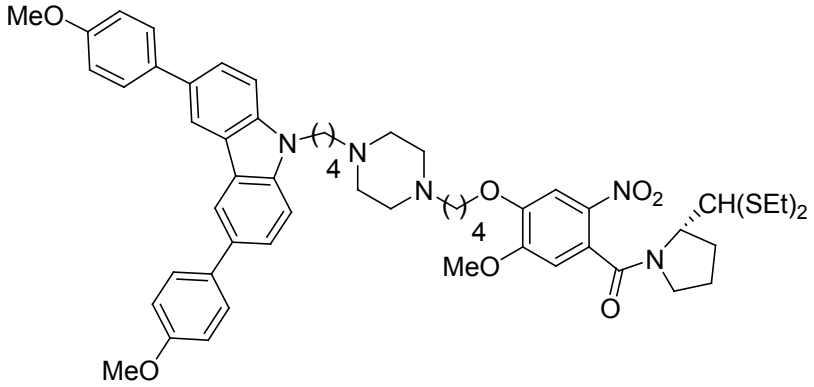
Ar**H**), 7.34-7.44 (m, 4H, *J* = 8.3 Hz, carbazole-**H**), 7.18 (t, 2H, *J* = 7.5 Hz, carbazole-**H**), 6.78 (s, 1H, PBD-Ar**H**), 4.83 (d, 1H, *J* = 3.7 Hz, -C**H**$(SEt)_2$), 4.63-4.72 (m, 1H, -NC**H**-), 4.32-4.44 (t, 2H, *J* = 6.0 Hz, -OC$\mathbf{H_2}$-), 4.09-4.15 (m, 2H, -NC$\mathbf{H_2}$-), 3.94 (s, 3H, -OC$\mathbf{H_3}$), 3.20-3.26 (m, 2H, -NC$\mathbf{H_2}$-), 2.63-2.88 (m, 4H, -S(C$\mathbf{H_2}$$CH_3)_2$), 2.58-2.04 (m, 12H, 6 X –NC$\mathbf{H_2}$-), 2.29-1.52 (m, 12H, 6 X –C$\mathbf{H_2}$-), 1.30-1.38 (m, 6H, -S(CH_2C$\mathbf{H_3}$)$_2$); FABMS: *m/z* 763 $(M+H)^+$.

(2*S*)-*N*-{4-(3-{4-[3-(3,6-di(4-methoxyphenyl)propyloxy]piperazino}propyl)oxy-5-methoxy-2-nitrobenzoyl}-pyrrolidine-2-carboxaldehyde diethyl thioacetal (12e)



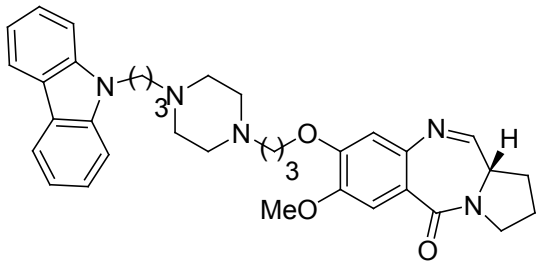
The compound **12e** was prepared according to the method described for compound **9a** by employing (2*S*)-[*N*-{4-(3-(piperazin-1-yl)propyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehydediethylthioacetal (**11a**) (526 mg, 1 mmol), and 9-(3-bromopropyl)-3,6-bis(4-methoxyphenyl)-9*H*-carbazole (**7c**) (600 mg, 1.2 mmol). Yeild (662 mg, 70%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.25 (s, 2H, carbazole-**H**), 7.69 (s, 1H, PBD-Ar**H**), 7.54-7.60 (m, 6H, *J* = 8.30 Hz, 2 X carbazole-**H,** 4 X carbazole-Ar**H**), 7.42-7.48 (d, 2H, *J* = 8.30 Hz, carbazole-**H**), 6.98 (d, 4H, *J* = 9.06 Hz, carbazole-Ar**H**), 6.78 (s, 1H, PBD-Ar**H**), 4.84 (d, 1H, *J* = 3.7 Hz, -C**H**$(SEt)_2$), 4.64-4.71 (m, 1H, -NC**H**-), 4.32-4.42 (t, 2H, *J* = 6.4 Hz, -OC$\mathbf{H_2}$-), 4.09-4.15 (m, 2H, -NC$\mathbf{H_2}$-), 3.92 (s, 3H, -OC$\mathbf{H_3}$), 3.78 (s, 6H, 2 X -OC$\mathbf{H_3}$), 3.23-3.28 (m, 2H, -NC$\mathbf{H_2}$-), 2.71-2.89 (m, 4H, S(C$\mathbf{H_2}$$CH_3)_2$), 2.64-2.31 (m, 12H, 6 X –NC$\mathbf{H_2}$-(piperazinyl)), 2.26-1.52 (m, 8H, 4 X –C$\mathbf{H_2}$-), 1.38-1.42 (m, 6H, *J* = 6.79 Hz, -S(CH_2C$\mathbf{H_3}$)$_2$); FABMS: *m/z* 947 $(M+H)^+$.

(2*S*)-*N*-{4-(4-{4-[4-(3,6-di(4-methoxyphenyl)butyloxy]piperazino}butyl)oxy-5-methoxy-2-nitrobenzoyl}-pyrrolidine-2-carboxaldehyde diethyl thioacetal (12f)



The compound **12f** was prepared according to the method described for compound **9a** by employing (2*S*)-[*N*-{4-(3-(piperazin-1-yl)butyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehydediethylthioacetal (**11b**) (540 mg, 1mmol), and 9-(4-bromobutyl)-3,6-bis(4-methoxyphenyl)-9*H*-carbazole (**7d**) (619 mg, 1.2 mmol). Yeild (701 mg, 72%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.25 (s, 2H, carbazole-**H**), 7.69 (s, 1H, PBD-Ar**H**), 7.53-7.62 (m, 6H, *J* = 8.30 Hz, 2 X carbazole-**H,** 4 X carbazole-Ar**H**), 7.41-7.46 (d, 2H, *J* = 8.30 Hz, carbazole-**H**), 6.98 (d, 4H, *J* = 9.06 Hz, carbazole-Ar**H**), 6.78 (s, 1H, PBD-Ar**H**), 4.83 (d, 1H, *J* = 3.7 Hz, -C**H**$(SEt)_2$), 4.63-4.73 (m, 1H, -NC**H**-), 4.32-4.41 (t, 2H, *J* = 6.0 Hz, -OC$\mathbf{H_2}$-), 4.09-4.17 (m, 2H, -NC$\mathbf{H_2}$-), 3.94 (s, 3H, -OC$\mathbf{H_3}$), 3.76 (s, 6H, 2 X -OC$\mathbf{H_3}$), 3.22-3.27 (m, 2H, -NC$\mathbf{H_2}$-), 2. 2.63-2.88 (m, 4H, -S(C$\mathbf{H_2}$$CH_3$)$_2$), 2.48-2.04 (m, 12H, 6 X –NC$\mathbf{H_2}$-), 2.28-1.52 (m, 12H, 6 X –C$\mathbf{H_2}$-), 1.31-1.38 (m, 6H, -S(CH_2C$\mathbf{H_3}$)$_2$); FABMS: *m/z* 975 $(M+H)^+$.

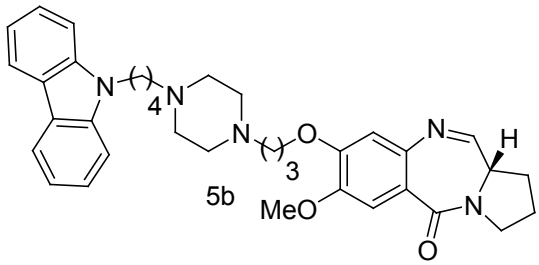
7-Methoxy-8-(3-{4-[3-(9*H*-9-carbazoly)propyloxy]piperazino}propyloxy)-(11a*S*)-1,2,3,11a-tetra hydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (5a)



This compound was prepared according to the method described for the compound **4a,** reduction of compound **12a** (734 mg, 1.0 mmol) gives amino diethylthioacetal (591 mg, 84%) then this aminodiethylthioacetal intermediate (704

mg, 1 mmol) on deprotection $HgCl_2$ (613 mg, 2.26 mmol), $CaCO_3$ (246 mg, 2.46 mmol) in acetonitrile-water (4:1) affords the pure product **5a**. Yield (324 mg, 56%). Mp 106 °C; $[\alpha]_D^{27}$ +145.2 (*c* = 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.05 (d, 2H, *J* = 7.3 Hz, carbazole-**H**), 7.62 (d, 1H, *J* = 4.5 Hz, -N=C**H**-), 7.56 (s, 1H, PBD-Ar**H**), 7.35-7.55 (m, 4H, carbazole-**H**), 7.20 (t, 2H, *J* = 7.1 Hz, carbazole-**H**), 6.85 (s, 1H, PBD-Ar**H**), 4.28-4.34 (m, 2H, -OC**H₂**-), 4.08-4.18 (m, 2H, -NC**H₂**-), 3.90 (s, 3H, -OC**H₃**), 3.53-3.84 (m, 3H, -NC**H₂**-, -NC**H**-), 2.52-2.76 (m, 12H, -NC**H₂**-), 1.52-2.52 (m, 8H, 4 X -C**H₂**-); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.7, 162.4, 150.8, 147.8, 140.6, 140.3, 125.5, 122.7, 120.3, 120.1, 118.7, 111.5, 110.4, 108.5, 67.8, 56.1, 55.8, 54.7, 52.4, 51.8, 46.6, 40.5, 32.1, 29.4, 25.2, 24.3; FABMS: *m/z* 580 $(M+H)^+$ 612 $(M+MeOH)^+$; Elemental Analysis $C_{35}H_{41}N_5O_5$: calcd C, 72.51, H, 7.13, N, 12.08; found C, 72.48, H, 7.10, N, 11.94.

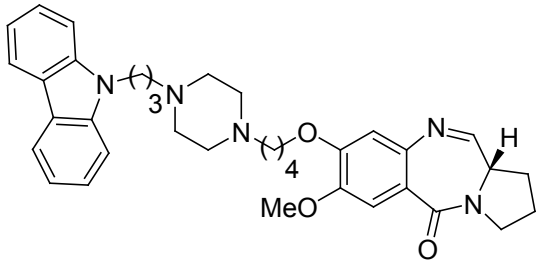
7-Methoxy-8-(3-{4-[4-(9*H*-9-carbazoly)butyloxy]piperazino}propyloxy)-(11a*S*)-1,2,3,11a-tetra hydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (5b)



This compound was prepared according to the method described for the compound **4a**, reduction of compound **12b** (748 mg, 1.0 mmol) gives amino diethylthioacetal (639 mg, 89%) then this aminodiethylthioacetal intermediate (718 mg, 1 mmol) on deprotection $HgCl_2$ (613 mg, 2.26 mmol), $CaCO_3$ (246 mg, 2.46 mmol) in acetonitrile-water (4:1) affords the pure product **5b**. Yield (344 mg, 58%). Mp 106-105 °C; $[\alpha]_D^{27}$ +151.5 (*c* = 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.10 (d, 2H, *J* = 7.7 Hz, carbazole-**H**), 7.61 (d, 1H, *J* = 4.5 Hz, -N=C**H**-), 7.56 (s, 1H, PBD-Ar**H**), 7.35-7.48 (m, 4H, carbazole-**H**), 7.19 (t, 2H, *J* = 7.7 Hz, carbazole-**H**), 6.78 (s, 1H, PBD-Ar**H**), 4.41 (t, 2H, *J* = 6.61 Hz, -OC**H₂**-), 4.08-4.16 (m, 2H, -NC**H₂**-), 3.94 (s, 3H, -OC**H₃**), 3.52-3.80 (m, 3H, -NC**H₂**-, -NC**H**-), 2.52 (m, 12H, -NC**H₂**-), 2.52-1.68 (m, 10H, 5 X -C**H₂**-); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.7, 162.4, 150.8, 147.8, 140.6, 140.3, 125.5, 122.7, 120.3, 120.1, 118.7, 111.5, 110.4, 108.5, 67.8, 56.2, 55.8,

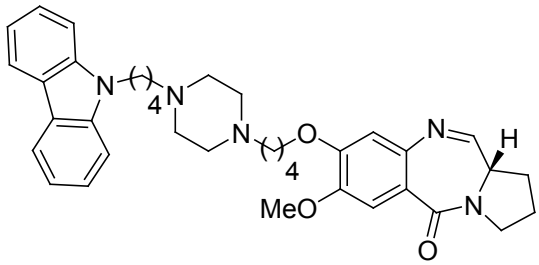
54.7, 53.6, 52.5, 51.8, 46.8, 29.6, 27.5, 27.1, 26.3, 25.4, 24.13 ; FABMS: *m/z* 594 $(M+H)^+$, 616 $(M+Na)^+$. Elemental Analysis $C_{36}H_{43}N_5O_5$: calcd C, 72.82, H, 7.30, N, 11.79; found C, 72.78, H, 7.31, N, 11.72.

7-Methoxy-8-(4-{4-[3-(9*H*-9-carbazoly)propyloxy]piperazino}butyloxy)-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (5c)



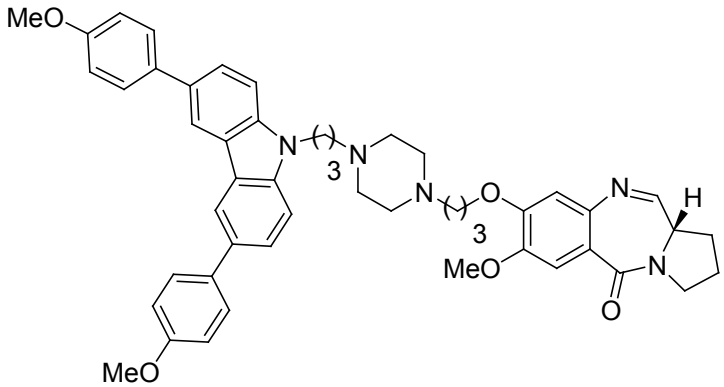
This compound was prepared according to the method described for the compound **4a**, reduction of compound **12c** (748 mg, 1.0 mmol) gives amino diethylthioacetal (631 mg, 88%) then this aminodiethylthioacetal intermediate (718 mg, 1 mmol) on deprotection $HgCl_2$ (613 mg, 2.26 mmol), $CaCO_3$ (246 mg, 2.46 mmol) in acetonitrile-water (4:1) affords the pure product **5c**. Yield (296 mg, 50%). Mp 107-106 ^oC; $[\alpha]_D^{27}$ +150.5 (*c* = 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.10 (d, 2H, *J* = 7.7 Hz, carbazole-**H**), 7.61 (d, 1H, *J* = 4.5 Hz, -N=C**H**-), 7.65 (s, 1H, PBD-Ar**H**), 7.35-7.48 (m, 4H, carbazole-**H**), 7.20 (t, 2H, *J* = 7.7 Hz, carbazole-**H**), 6.78 (s, 1H, PBD-Ar**H**), 4.35-4.41 (t, 2H, *J* = 6.6 Hz, -OC$\mathbf{H_2}$-), 4.08-4.16 (m, 2H, -NC$\mathbf{H_2}$-), 3.94 (s, 3H, -OC$\mathbf{H_3}$), 3.52-3.81 (m, 3H, -NC$\mathbf{H_2}$-, -NC**H**-), 2.52-2.71 (m, 12H, -NC$\mathbf{H_2}$-), 1.62-2.51 (m, 10H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.7, 162.4, 150.8, 147.8, 140.6, 140.3, 125.5, 122.7, 120.3, 120.1, 118.7, 111.5, 110.4, 108.5, 67.8, 56.2, 55.4, 54.2, 53.6, 52.5, 51.8, 46.8, 30.4, 29.6, 26.4, 25.4, 24.2; FABMS: *m/z* 594 $(M+H)^+$, 616 $(M+Na)^+$; Elemental Analysis $C_{36}H_{43}N_5O_5$: calcd C, 72.82, H, 7.30, N, 11.79; found C, 72.74, H, 7.25, N, 11.74.

7-Methoxy-8-(4-{4-[4-(9*H*-9-carbazoly)butyloxy]piperazino}butyloxy)-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (5d)



This compound was prepared according to the method described for the compound **4a,** reduction of compound **12d** (762 mg, 1.0 mmol) gives amino diethylthioacetal (658 mg, 90%) then this aminodiethylthioacetal intermediate (732 mg, 1 mmol) on deprotection $HgCl_2$ (613 mg, 2.26 mmol), $CaCO_3$ (246 mg, 2.46 mmol) in acetonitrile-water (4:1) affords the pure product **5d**. Yield (334 mg, 55%). Mp 109-108 ^oC; $[\alpha]_D^{27}$ +152.9 (*c* = 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.11 (d, 2H, *J* = 7.5 Hz, carbazole-**H**), 7.68 (d, 1H, *J* = 4.3 Hz, -N=C**H**-), 7.62 (s, 1H, PBD-Ar**H**), 7.35-7.48 (m, 4H, carbazole-**H**), 7.20 (t, 2H, *J* = 6.8 Hz, carbazole-**H**), 6.78 (s, 1H, PBD-Ar**H**), 4.34-4.45 (t, 2H, *J* = 6.6 Hz, -OC$\mathbf{H_2}$-), 4.17-4.02 (m, 2H, -NC$\mathbf{H_2}$-,), 3.96 (s, 3H, -OC$\mathbf{H_3}$), 3.52-3.81 (m, 3H, -NC$\mathbf{H_2}$-, -NC**H**-), 2.59-2.78 (m, 12H, -NC$\mathbf{H_2}$-), 1.61-2.54 (m, 12H, -C$\mathbf{H_2}$-); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.7, 162.4, 150.8, 147.8, 140.6, 140.3, 125.5, 122.7, 120.3, 120.1, 118.7, 111.5, 110.4, 108.5, 67.8, 56.2, 55.8, 54.7, 53.6, 52.5, 51.8, 46.8, 29.6, 27.5, 27.1, 26.3, 25.4, 24.13 ; FABMS: *m/z* 608 $(M+H)^+$, 630 $(M+Na)^+$; Elemental Analysis $C_{37}H_{45}N_5O_5$: calcd C, 73.12, H, 7.46, N, 11.52; found C, 73.08, H, 7.40, N, 11.48.

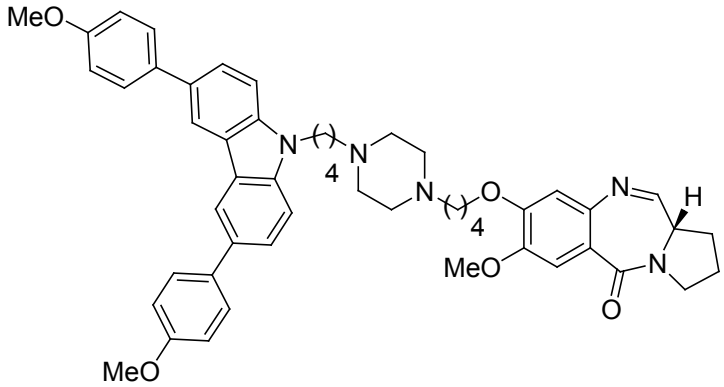
7-methoxy-8-[3-(4-{3-[3,6-di(4-methoxyphenyl)-9H-9-carbazoly]propyloxy}piperazino)proyloxy]-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (5e)



This compound was prepared according to the method described for the compound **4a,** reduction of compound **12e** (946 mg, 1.0 mmol) gives amino diethylthioacetal (778 mg, 85%) then this aminodiethylthioacetal intermediate (916 mg, 1 mmol) on deprotection $HgCl_2$ (613 mg, 2.26 mmol), $CaCO_3$ (246 mg, 2.46 mmol) in acetonitrile-water (4:1) affords the pure product **5e**. Yield (443 mg, 56%). Mp 110-109 ^oC; $[\alpha]_D^{27}$ +162.9 (*c* = 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.23

(s, 2H, carbazole-**H**), 7.51-7.66 (m, 8H, *J* = 8.3, *J* = 4.5 Hz, carbazole-**H,** carbazole-Ar**H**, PBD-Ar**H**, -N=C**H**-), 7.51 (d, 2H, *J* = 8.3 Hz, carbazole-**H**), 7.02 (d, 4H, *J* = 8.30 Hz, carbazole-Ar**H**), 6.56 (s, 1H, PBD-Ar**H**), 4.25-4.38 (t, 2H, *J* = 6.0 Hz, -OC**H₂**-), 4.06-4.17 (m, 2H, -NC**H₂**-), 3.90 (s, 3H, -OC**H₃**), 3.85 (s, 6H, 2 X -OC**H₃**), 3.53-3.84 (m, 3H, -NC**H₂**-, -NC**H**-), 2.42-2.84 (m, 12H, -NC**H₂**-), 2.41-1.65 (m, 8H, 4 X -C**H₂**-); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.7, 162.4, 158.6, 150.8, 147.8, 140.6, 139.5, 134.4, 132.8, 128.2, 125.3, 123.7, 120.3, 118.7, 114.2, 111.5, 110.4, 108.7, 67.8, 56.1, 55.8, 55.3, 54.7, 52.4, 51.8, 46.6, 40.5, 32.1, 29.4, 25.2, 24.2; FABMS: *m/z* 793 $(M+H)^+$, 815 $(M+Na)^+$, 824 $(M+MeOH)^+$; Elemental Analysis $C_{49}H_{53}N_5O_5$: calcd C, 74.31, H, 6.75, N, 8.84; found C, 74.28, H, 6.70, N, 8.80.

7-methoxy-8-[3-(4-{3-[3,6-di(4-methoxyphenyl)-9H-9-carbazoly]butyloxy}piperazino)butyloxy]-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (5f)



This compound was prepared according to the method described for the compound **4a,** reduction of compound **12f** (974 mg, 1.0 mmol) gives amino diethylthioacetal (802 mg, 90%) then this aminodiethylthioacetal intermediate (944 mg, 1 mmol) on deprotection $HgCl_2$ (613 mg, 2.26 mmol), $CaCO_3$ (246 mg, 2.46 mmol) in acetonitrile-water (4:1) affords the pure product **5f**. Yield (475 mg, 58%). Mp 112-113 °C; $[\alpha]_D^{27}$ +172.2 (*c* = 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.22 (s, 2H, carbazole-**H**), 7.54-7.68 (m, 8H, *J* = 8.3, *J* = 4.5 Hz, carbazole-**H,** carbazole-Ar**H**, PBD-Ar**H**, -N=C**H**-), 7.52 (d, 2H, *J* = 8.3 Hz, carbazole-**H**), 7.0 (d, 4H, *J* = 8.30 Hz, carbazole-Ar**H**), 6.54 (s, 1H, PBD-Ar**H**), 4.24-4.42 (t, 2H, *J* = 6.6 Hz, -OC**H₂**-), 4.06-4.17 (m, 2H, -NC**H₂**-), 3.96 (s, 3H, -OC**H₃**), 3.85 (s, 6H, 2 X -OC**H₃**), 3.53-3.80 (m, 3H, -NC**H₂**-, -NC**H**-), 2.48-2.79 (m, 12H, , -NC**H₂**-), 1.61-2.39 (m, 12H, 6 X -C**H₂**); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.7, 162.4, 158.6, 150.8, 147.8, 140.6, 139.5, 134.4,

132.8, 128.2, 125.3, 123.7, 120.3, 118.7, 114.2, 111.5, 110.4, 108.7, 67.8, 56.2, 55.8, 55.3, 54.7, 53.6, 52.5, 51.8, 46.8, 29.6, 27.5, 27.1, 26.3, 25.4, 24.13 ; FABMS: *m/z* 821 $(M+H)^+$, 844 $(M+Na)^+$, 852 $(M+MeOH)^+$; Elemental Analysis $C_{51}H_{57}N_5O_5$: calcd C, 74.70, H, 7.01, N, 8.54; found C, 74.62, H, 6.95, N, 7.94.

Methods:

Model Building and Parametrization.

All small molecules were prepared using SYBYL6.9. Tripos force field and Gasteiger–Huckel partial atomic charges were applied with distance dependent dielectric constant and Powell's conjugate gradient energy minimization method was used until a convergence criterion of 0.001 kcal/mol was reached.

The self-complementary 14mer d(5'-CGCA**G**AATTCTGCG-3')2 DNA was built in its canonical B form and minimized using nucgen and sander program of AMBER, respectively [1]. To get the initial coordinates of the ligand in the minor groove of DNA for the formation of covalent bond between DNA and ligand, we performed non-covalent docking procedure (GOLD 3.2) [2]. The entire conformational space of the DNA molecule was scanned during docking. Based on the scores and intermolecular interactions such as Coulomb and van der Waals, this method was used to investigate the probable sites of heterodimers interaction or where the ligand would prefer to bind energetically with the DNA, that is, whether major or minor grooves or specific sequences of nucleobases. The parameter set for docking was as follows: number of islands 5, population size of 100, number of operations was 100,000, a niche size of 2 and a selection pressure of 1.1 and the van der Waals and hydrogen bonding were set to 4.0 and 2.5 respectively.

Binding of the ligands in the minor groove does not have a large impact on the conformational change in the DNA, as enunciated by the minimization of the complex. The duplex from the average twist angle is slightly underwound in the presence of the ligand when compared to input canonical structure of B-DNA which leads to narrowing of the minor groove along the ligand binding site by the non-covalent interaction between linker and AT region of the DNA. AT region of the DNA is usually associated with narrowing of minor groove, responsible for the widening of major groove of DNA and

opening of other base pairs from the major groove side. Conspicuous structural distortions due to ligand binding, as also indicated by the molecular minimization, are largely confined to some helicoidal parameters around the covalent binding site (Fig. 1).

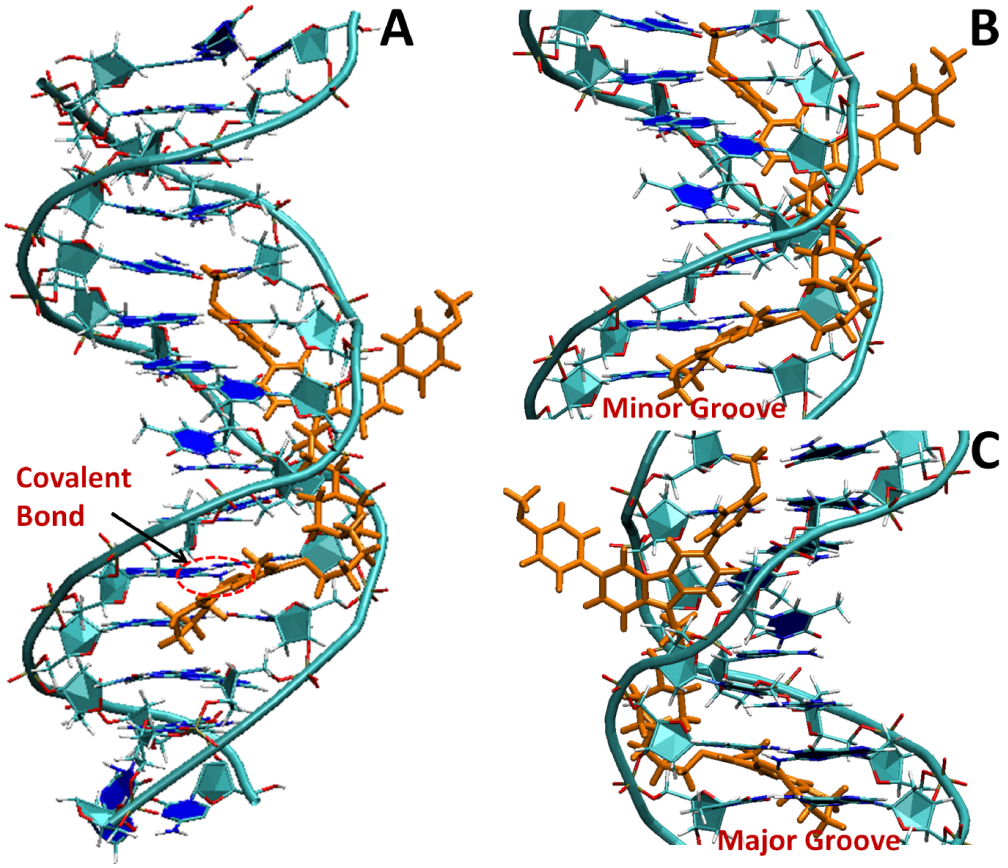


Figure 1: (A) The energy minimized structure of DNA-**5f** complex showing the minor groove binding mode of the molecule **5f,** red dotted circle shows the covalent bonding of the C11 with the Guanine (A**G**A) nitrogen. (B) View from the minor groove (C) View from the major groove shows the structural change in the confined area of covalent binding site (for better picture).

1. Pearlman, D.A.; Case, D.A.; Caldwell, J.W.; Ross, W.S.; Cheatham, III, T.E.; DeBolt, S.; Ferguson, D.; Seibel, G.; Kollman, P. *Comp. Phys. Commun.* **1995**, *91*, 1-41.
2. Kamal, A.; Rajender, D.; Reddy, R.; Reddy, M. K.; Balakishan, G.; Shaik, T. B.; Chourasia, M.; Sastry, G. N. *Bioorg. Med. Chem.* **2009**, *17*, 1557–1572.

Biological Assays

Cell culture

The MCF-7 (human breast cancer cells) were incubated by using Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal calf serum, 100 µg/mL pencillin-G and 100 µg/mL streptomycin sulfate. The cell line was maintained at 37 ^{o}C in a humidified atmosphere containing 5% CO_2 in the incubator.

MTT cell viability assay

Cell viability was assessed by the MTT 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 over night incubation cells were treated with **1**, **6b**, **4c**, **4f**, **5a** and **5f** at 2, 4, 8 and 16 µM concentration (Fig 2a) and incubated for 6, 12, 24 and 48 h (Fig 2b) respectively. Then the medium was discarded and replaced with 10 µL MTT dye. Plates were incubated at 37℃ for 2 h. The resulting formazan crystals were solubilized in 100 µL extraction buffer. The optical density (O.D) was read at 570 nm with micro plate reader.

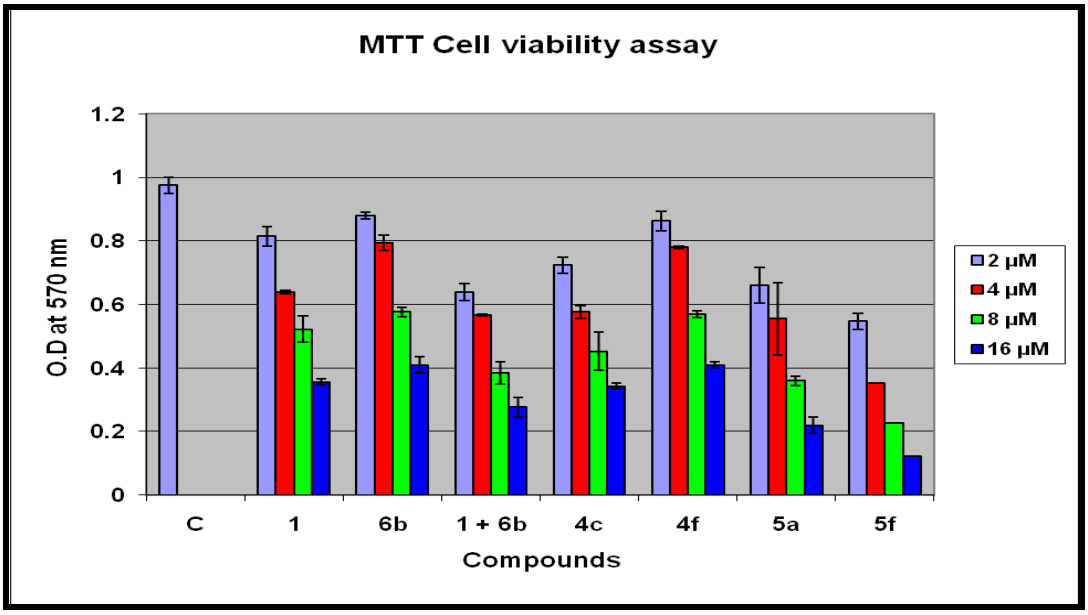


Figure 2a. The MTT assay was conducted at increasing concentration ranging from 2-16 µM in MCF-7 breast cancer cell line with the compounds such as **1**, **6b**, **1+6b**, **4c**, **4f**, **5a** and **5f**. Here **1** and **6b** are the starting materials used. **1+6b** is the treatment where in we

have used both starting materials. We have conducted each experiment thrice and are considered to be statistically significant.

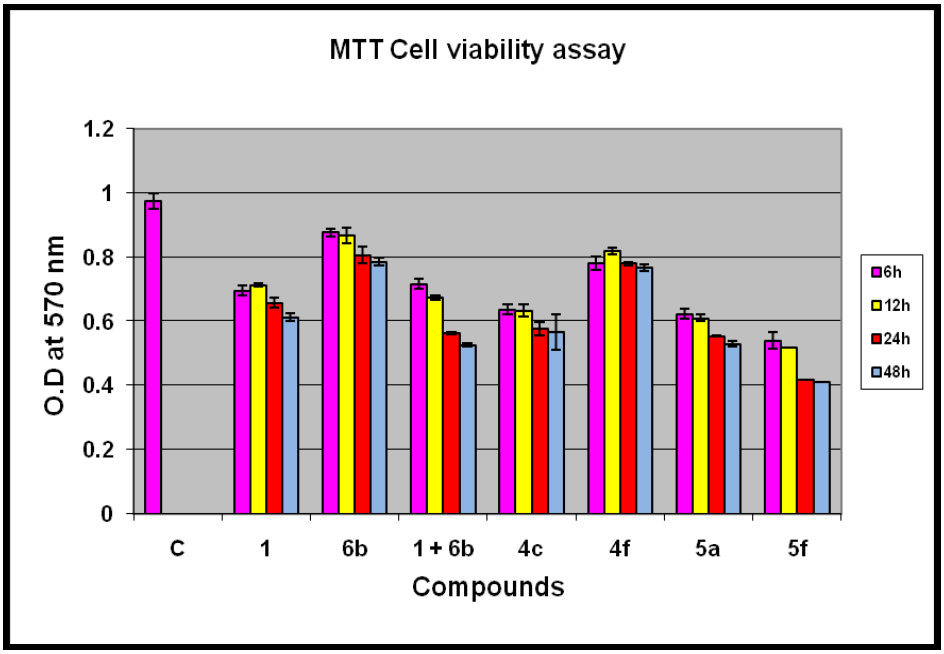


Figure 2b. The MTT assay was conducted at gradual increase of time period ranging from 6- 48h in MCF-7 breast cancer cell line with the compounds such as **1**, **6b**, **1+6b**, **4c**, **4f**, **5a** and **5f**. Here **1** and **6b** are the starting materials used. **1+6b** is the treatment where in we have used both starting materials. We have conducted each experiment thrice and are considered to be statistically significant.

BrdU cell proliferation assay

Cell proliferation analysis by the BrdU incorporation method by used to assess the effect of compounds (**1**, **6b**, **5a** and **5f**) on MCF-7 cell proliferation. MCF-7 cells were seeded at a density of 15,000 per well of 96 well plate. After 24 h time period cells were incubated with BrdU for 5h. Then the culture was treated with compounds (**1**, **6b**, **5a** and **5f**) at 4 μM concentration and incubation carried for 24 h. Fixation of cells was done for 30 min at room temperature followed by three washings. Anti BrdU antibody was added to the cell suspension and incubated for 1 h allowing it to bind to incorporated BrdU. After 1 h incubation 100 μL of anti BrdU goat anti mouse HRP secondary antibody, (1:2000) was added and allowed for 30 min, washing procedure was repeated for 3 times with PBS. TMB substrate (100 μL) was added and incubated for another 30 min at room temperature. Finally the O.D reading was taken at 450 nm. A higher O.D reading

indicates higher BrdU incorporation in the sample (which indirectly depicts the higher proliferation rate).

Cell cycle analysis

5 X 10^5 MCF-7 cells were seeded in 60 mm dish and were allowed to grow for 24 h, 4 μM concentration of **1, 6b, 4c, 4f, 5a** and **5f** 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 using Summit Software (Fig 3a and 3b).

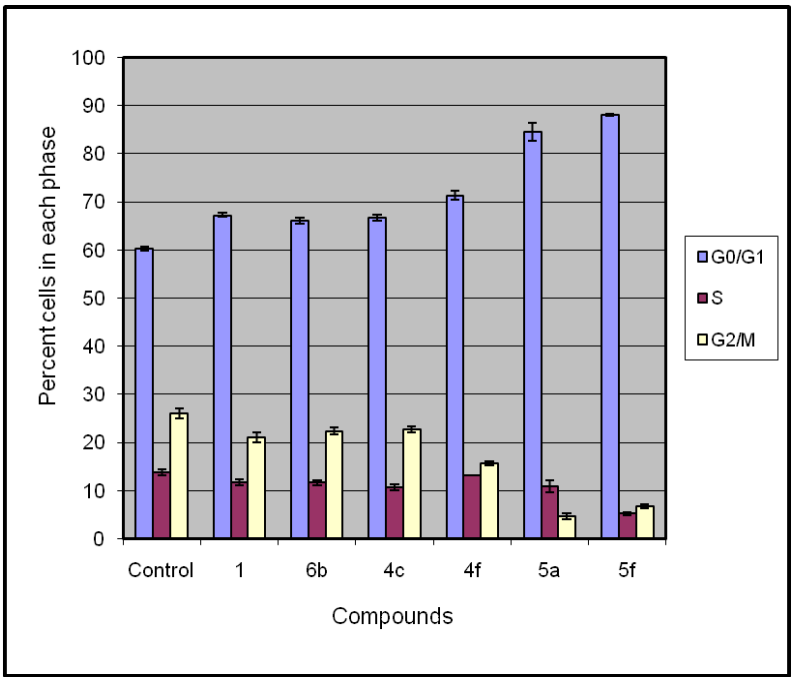


Figure 3a. Histograms of cell cycle distribution on MCF-7 cells after exposure to conjugates (**1**, **6b**, **4c**, **4f**, **5a** and **5f**) at 4 μM concentration for 24 h before cell cycle analysis.

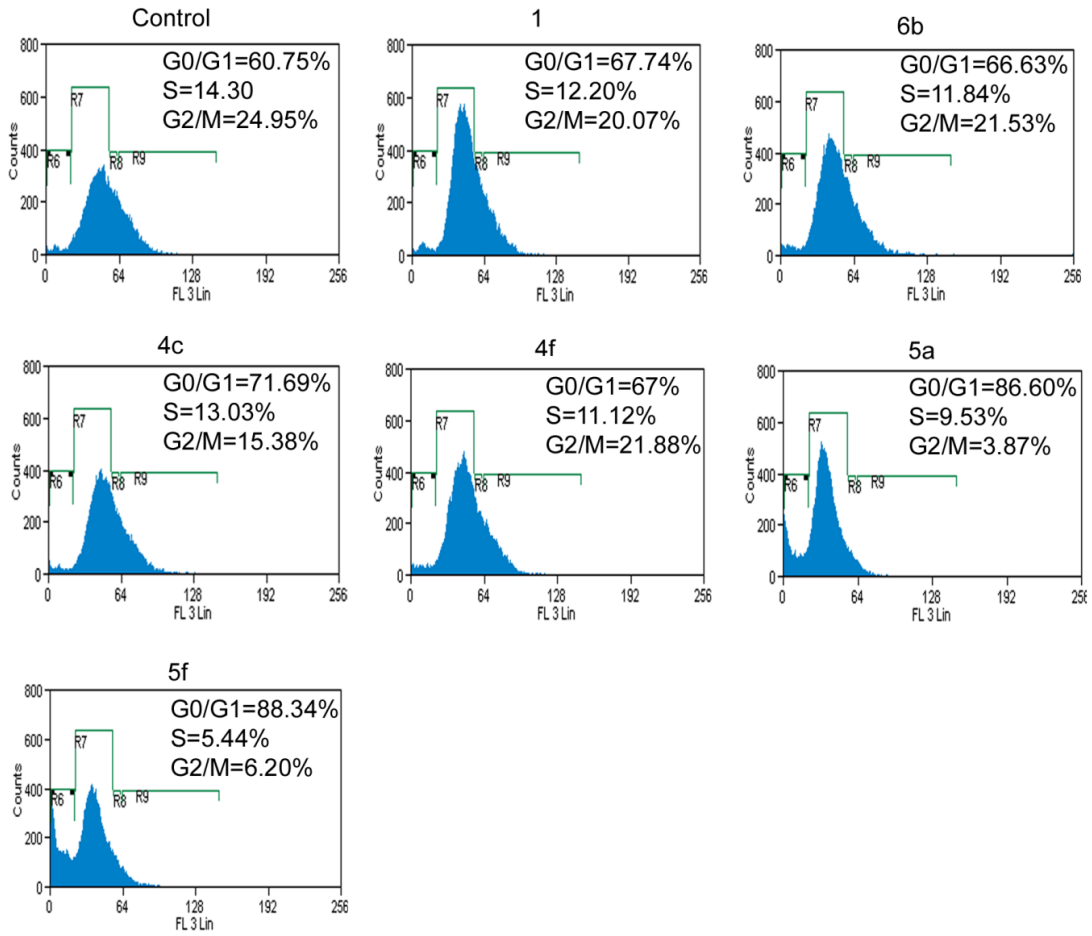


Figure **3b**. Flow cytometric analysis of cell cycle distribution on MCF-7 after exposure to PBD conjugates (**1**, **6b**, **4c**, **4f**, **5a** and **5f**) at 4 µM concentration for 24 h before cell cycle analysis (where R6 = G0, R7 = G1, R8 = S and R9 = G2/M phases).

TUNEL assay:

TUNEL assay (Terminal Transferase dUTP Nick End Labeling) was conducted by using the Apoalert DNA fragmentation Assay kit (Clone tech). Apoptosis induced nuclear DNA fragmentation was determined using this assay. This assay was conducted according to the manufacturer's recopmmendations and is based on the principle of terminal deoxy nucleotidyl transferase (TdT)-mediated dUTP nick-end-labeling. TdT catalyzes incorporation of fluorescein-dUTP at the free 3'-hydroxyl ends of fragmented DNA. Flourescein-labeled DNA can be detected via confocal microscope.

Protein extraction and Western blot analysis

Total cell lysates from cultured MCF-7 cells were obtained by lysing the cells in ice-cold RIPA buffer (1XPBS, 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 varioskan instrument (Thermo-Fischer Scientifics). Thirty 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 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 ^{o}C overnight (O/N). Cyclin D1, CDK4, c-Jun, Jun B, CREB, p53, JNK1/2, pro-caspase-7, cleaved PARP, full length pRb, *BAX* and β-actin (1:500) antibodies were purchased from Imgenex, USA. The membrane was incubated with corresponding horseradish peroxidase-labeled secondary antibody (1:2000) (Santa Cruz) at room temperature for 1 h. 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.

Study : Efficacy Study, Compound 5f

Cell Line:PC3, Human prostate Cancer Xenograft
Study Duration: 31 Days
Treatment protocols used: 20 mg/Kg, iv, d1, 5, 9
Positive control; Adriamycin 5mg/kg, iv, d1, 5, 9

Percentage of survival

Days	Control	ADR-5mg/kg I.V. Injection on day 1,5,9	5f - 20mg/kg I.V. Injection on day 1,5,9
1	100	100	100
5	100	100	100
8	100	83.3	100
12	100	83.3	100
15	100	83.3	100
19	100	83.3	100
23	100	83.3	100
27	100	83.3	100
31	100	83.3	100

Tumors weight

Days	Control	ADR-5mg/kg I.V. Injection on day 1,5,9	5f-20mg/kg I.V. Injection on day 1,5,9
1	25.0	24.5	25.0
5	25.1	22.4	24.9
8	24.9	17.2	24.3
12	25.6	16.3	24.4
15	25.3	16.1	24.3
19	25.9	16.5	24.1
23	26.1	18.2	24.6
27	26.4	18.7	25.0
31	26.5	19.3	25.2

Tumor Volume Group A: Control

Days	Mouse-1	Mouse-2	Mouse-3	Mouse-4	Mouse-5	Mouse-6
1	0.08	0.07	0.10	0.10	0.11	0.08
5	0.12	0.18	0.22	0.23	0.19	0.15
8	0.48	0.53	0.37	0.28	0.39	0.35
12	0.56	1.03	0.68	0.47	0.60	0.73
15	0.57	1.70	0.73	0.51	0.96	0.82
19	0.87	2.43	0.83	0.40	2.19	1.58
23	2.10	3.02	1.28	0.59	2.67	1.98
27	2.10	3.45	1.30	1.18	2.93	2.36
31	2.43	4.27	1.37	1.26	4.52	3.12

Tumor Volume Group B: ADR-5mg/kg I.V. Injection on day 1,5,9

Days	Mouse-1	Mouse-2	Mouse-3	Mouse-4	Mouse-5	Mouse-6
1	0.03	0.02	0.04	0.05	0.09	0.02
5	0.03	0.01	0.38	0.11	0.18	0.02
8	0.01	0.01	0.22	0.10	0.16	
12	0.15	0.03	0.82	0.05	0.32	
15	0.07	0.04	1.05	0.20	0.62	
19	0.07	0.05	1.48	0.22	0.74	
23	0.10	0.06	2.32	0.30	1.23	
27	0.05	0.05	1.18	0.24	0.92	
31	0.13	0.07	2.70	0.34	1.12	

Tumor Volume Group C: 5f-20mg/kg I.V. Injection on day 1,5,9

Days	Mouse-1	Mouse-2	Mouse-3	Mouse-4	Mouse-5	Mouse-6
1	0.07	0.10	0.00	0.07	0.13	0.07
5	0.13	0.19	0.01	0.11	0.12	0.13
8	0.13	0.21	0.01	0.20	0.43	0.09
12	0.53	0.42	0.02	0.26	0.50	0.49
15	0.73	0.55	0.06	0.47	0.59	0.73
19	0.61	0.65	0.13	0.59	0.87	1.01
23	1.13	2.35	0.22	1.25	1.24	1.78
27	1.13	1.23	0.42	0.83	1.26	1.44
31	1.32	1.75	0.67	2.33	2.02	2.25