Supplementary Material (ESI)

Design and synthesis of DNA-intercalative naphthalimide-benzothiazole/cinnamide derivatives: Cytotoxicity evaluation and topoisomerase IIα inhibition

N. Sankara Rao, a,b Narayana Nagesh,*c V. Lakshma Nayak, a Satish Sunkari, a,b Ramya Tokala, d Gaddam Kiranmai, c Phanindranath Regur, c Nagula Shankaraiah,*d and Ahmed Kamal,*a,b,e

a Medicinal Chemistry and Pharmacology, b Academy of Scientific and Innovative Research (AcSIR), CSIR-Indian Institute of Chemical Technology, Hyderabad 500 007, India.
c CSIR-Centre for Cellular and Molecular Biology, Hyderabad 500007, India.
d Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER), Hyderabad 500 037, India.
e School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi, 110062, India.

Corresponding authors: Dr Ahmed Kamal, E-mail.: ahmedkamal@iict.res.in; and Dr. Narayana Nagesh, E-mail.: nagesh@ccmb.res.in; Dr Nagula Shankaraiah, E-mail.: shankar@niperhyd.ac.in

Table of contents

1. Experimental Section (Chemistry & Biology) 2-14

2. Copies of 1H and 13C NMR spectra of products 15-34

3. Table 1. Cytotoxic activity……………………………………………………………………………………35-36
Experimental section
Chemistry

All the chemicals and reagents used in this study 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. The reactions were monitored by TLC performed on silica gel glass plates containing 60 GF-254, and visualized was done by using a UV light or iodine indicator. Column chromatography was performed using Merck 60-120 mesh silica gel. $^1$H NMR spectra were recorded on Bruker UXNMR/XWIN-NMR (300 MHz) or Inova Varian-VXRunity (400, 500 MHz) instruments. $^{13}$C NMR spectra were recorded on a Bruker UXNMR/XWIN-NMR (75 MHz) instrument. Chemical shifts ($\delta$) were reported in ppm downfield from an internal standard TMS and coupling constants are expressed in Hz. Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), quartet (q) ds (double singlet), dd (double doublet), m (multiplet) and br s (broad singlet). ESI spectra were recorded on a Micro mass Quattro LC using ESI+ software with a capillary voltage of 3.98 kV and an ESI mode positive ion trap detector. High-resolution mass spectra (HRMS) were recorded on a QSTAR XL hybrid MS-MS mass spectrometer. Melting points were determined with an electrothermal melting point apparatus, and are uncorrected.

2-(2-(Piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (8)

To 1,8,-naphthalic anhydride (6)(198 mg, 1 mmol),2-(piperazin-1-yl)ethanamine (7) (137 mg, 1.1 mmol) in ethanol (10 ml) was added and stirred for 8 h at 60 °C under reflux condition. After completion of the reaction as checked by TLC, the solvent was evaporated, and extracted with ethyl acetate. The combined organic fractions were washed with water followed by brine solution, dried over Na$_2$SO$_4$ and purified by column chromatography using pure ethyl acetate to obtain the pure product 8 as white solid. (250 mg, 81% yield); mp: 106 °C; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.59 (d, J = 7.5 Hz, 2H), 8.21 (d, J = 8.3 Hz, 2H), 7.75 (t, J = 7.5 Hz, 2H), 4.32 (t, J = 6.7 Hz, 2H), 2.90 (m, 4H), 2.67 (t, J = 6.7 Hz, 2H), 2.57 (bs, 4H); MS (ESI): m/z 310 [M+H]$^+$. Ethyl 2-(4-(2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl)piperazin-1-yl)acetate (9)

To a solution of compound 8(309 mg, 1 mmol) in dry DMF (15 ml) was added, anhydrous K$_2$CO$_3$ (276 mg, 2 mmol), $\alpha$-bromoethylacetate (250 mg, 1.5 mmol) and the mixture was stirred at room temperature for 24 h. The reaction was monitored by TLC using ethyl acetate–hexane (8:2). After completion of the reaction as indicated by the TLC, K$_2$CO$_3$ was removed by filtration,
diluted with water and extracted with dichloromethane (2x20 ml). The combined organic phases were washed with water followed by brine solution, dried over Na₂SO₄ and evaporated under vacuum. The residue, thus obtained was purified by column chromatography using pure ethyl acetate and hexane to afford pure compound 9 as yellow solid. (335 mg, 85% yield); mp: 144 °C; ¹H NMR (300 MHz, CDCl₃) δ 8.56 (d, J = 8.3 Hz, 2H), 8.17 (d, J = 7.5 Hz, 2H), 7.75 (t, J = 8.3 Hz, 2H), 4.30 (t, J = 6.7 Hz, 2H), 4.16 (q, J = 6.7 Hz, 2H), 3.13 (s, 2H), 2.73 – 2.63 (m, 6H), 2.60 – 2.53 (m, 4H), 1.27 (t, J = 6.7 Hz, 3H); MS (ESI): m/z 396 [M+H]^+.

2-(4-(2-(1,3-Dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl)piperazin-1-yl)acetic acid (10)

To a solution of compound 9 (395 mg, 1 mmol) in THF (15 ml) and water (2 ml), LiOH.H₂O (84 mg, 2 mmol) was added and the mixture was stirred at room temperature for 12 h. The reaction was monitored by TLC using ethyl acetate. After completion of the reaction as indicated by the TLC, the solvent was removed under vacuum and neutralized with dilute HCl up to pH 7. After neutralization the reaction mixture was extracted with dichloromethane (2x20 ml). The combined organic phases were washed with water followed by brine solution, dried over Na₂SO₄ and evaporated under vacuum to obtain compound 10. This crude compound was purified by recrystallization by using ethyl acetate as solvent to obtain the pure product 10 as white solid. (293 mg, 80% yield); mp: 187 °C; m/z 368 [M+H]^+.

N-(Benzo[d]thiazol-2-yl)-2-(4-(2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl)piperazin-1-yl)acetamide (3a)

To a solution of 2-aminobenzothiazole (150 mg, 1 mmol) in dichloromethane (20 ml) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC hydrochloride) (210 mg, 1.1 mmol) and 1-hydroxy-1,2,3-benzotriazole (HOBt) (13.5 mg, 0.1 mmol). Then compound 10 (1 mmol) was added and the reaction mixture was stirred at room temperature for 14 h and the reaction was monitored by TLC. After completion of reaction, water was added to reaction mixture and extracted with dichloromethane (2x30 ml). The organic layer was dried with Na₂SO₄ and evaporated under vacuum to afford the crude product. This was further purified by column chromatography using pure ethyl acetate as solvent system to obtain the pure product 3a as yellow solid; (167 mg, yield 82%); mp: 221°C; ¹H NMR (300 MHz, CDCl₃) δ 10.37 (bs, 1H), 8.58 (d, J = 7.1 Hz, 2H), 8.19 (d, J = 8.1 Hz, 2H), 7.80 – 7.72 (m, 4H), 7.40 (t, J = 7.1 Hz, 1H), 7.28 (t, J = 7.1 Hz, 1H), 4.32 (t, J = 6.6 Hz, 2H), 3.23 (s, 2H), 2.99 – 2.70 (m, 6H), 2.65 (bs, 4H); ¹³C NMR (75 MHz, CDCl₃) δ 169.24, 164.14, 157.06, 148.41, 133.91, 132.17, 131.53, 131.17, 128.12, 127.52,
126.89, 126.20, 123.94, 122.56, 121.37, 120.97, 61.01, 55.48, 53.66, 53.04, 37.33; IR (KBr) 
(v_max/cm^{-1}): 3328, 2925, 2821, 1694, 1655, 1621, 1584, 1531, 1448, 1342; MS (ESI): m/z 500 [M+H]^+.

2-(4-(2-(1,3-Dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl)piperazin-1-yl)-N-(6-
methoxybenzo[d]thiazol-2-yl)acetamide (3b)

This compound was prepared according to the method described for compound 3a by 
employing compound 10 (367 mg, 1 mmol), EDCI (210 mg, 1.1 mmol), HOBt (13.5 mg, 0.1 
mmol) and 2-amino-6-methoxybenzothiazole (180 mg, 1 mmol) to obtain the pure product 3b as 
yellow solid; (184 mg, yield 85%); mp: 215°C; 1H NMR (300 MHz, CDCl_3) δ 10.28 (bs, 1H), 8.59 
(d, J = 7.5 Hz, 2H), 8.20 (d, J = 7.5 Hz, 2H), 7.77 (t, J = 7.5 Hz, 2H), 7.63 (d, J = 8.3 Hz, 1H), 
7.24 (d, J = 2.5 Hz, 1H), 6.99 (dd, J = 9.0, 2.5 Hz, 1H), 4.33 (t, J = 6.7 Hz, 2H), 3.87 (s, 3H), 3.23 
(s, 2H), 2.99 – 2.71 (m, 6H), 2.66 (s, 4H); 13C NMR (125 MHz, CDCl_3) δ 168.99, 164.14, 156.82, 
155.08, 142.63, 133.91, 133.43, 131.56, 131.18, 128.15, 126.90, 122.60, 121.55, 115.19, 104.25, 
61.00, 55.78, 55.50, 53.63, 53.06, 37.33; IR (KBr) (v_max/cm^{-1}): 3313, 2929, 2819, 1699, 1656, 
1618, 1576, 1513, 1438, 1375; MS (ESI): m/z 530 [M+H]^+; HRMS calcd for C_{28}H_{28}N_{5}O_{4}S 
[M+H]^+: 530.18565, found 530.18478.

2-(4-(2-(1,3-Dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl)piperazin-1-yl)-N-(6-
ethoxybenzo[d]thiazol-2-yl)acetamide (3c)

This compound was prepared according to the method described for compound 3a by 
employing compound 10 (367 mg, 1 mmol), EDCI (210 mg, 1.1 mmol), HOBt (13.5 mg, 0.1 
mmol) and 2-amino-6-ethoxybenzothiazole (194 mg, 1 mmol) to obtain the pure product 3c as 
yellow solid. (191 mg, yield 86%); mp: 173°C; 1H NMR (300 MHz, CDCl_3) δ 10.31 (bs, 1H), 8.19 
(d, J = 7.3 Hz, 2H), 8.19 (d, J = 7.5 Hz, 2H), 7.76 (t, J = 7.7 Hz, 2H), 7.61 (d, J = 8.8 Hz, 1H), 
7.21 (d, J = 2.2 Hz, 1H), 6.96 (dd, J = 8.8, 2.2 Hz, 1H), 4.32 (t, J = 6.6 Hz, 2H), 4.06 (q, J = 13.9, 
6.9 Hz, 2H), 3.21 (s, 2H), 2.79 – 2.71 (m, 6H), 2.65 (bs, 4H), 1.45 (t, J = 6.9 Hz, 3H); 13C NMR 
(75 MHz, CDCl_3) δ 168.97, 164.09, 156.08, 155.00, 142.43, 133.88, 133.31, 131.47, 131.13, 
128.06, 126.85, 122.49, 121.46, 115.58, 104.88, 64.00, 60.94, 55.44, 53.55, 53.00, 37.24, 14.79; 
IR (KBr) (v_max/cm^{-1}): 3304, 2944, 2818, 1694, 1656, 1623, 1591, 1565, 1463, 1387; MS (ESI): 
m/z 544 [M+H]^+.

2-(4-(2-(1,3-Dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl)piperazin-1-yl)-N-(6-
methylbenzo[d]thiazol-2-yl)acetamide (3d)
This compound was prepared according to the method described for compound \textbf{3a} by employing compound \textbf{10} (367 mg, 1 mmol), EDCI (210 mg, 1.1 mmol), HOBt (13.5 mg, 0.1 mmol) and 2-amino-6-methylbenzothiazole (164 mg, 1 mmol) to obtain the pure product \textbf{3f} as yellow solid. (170mg, yield 81%); mp: 194°C; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 10.34 (bs, 1H), 8.59 (d, $J = 8.3$ Hz, 2H), 8.19 (d, $J = 8.3$ Hz, 2H), 7.77 (t, $J = 8.3$ Hz, 2H), 7.66 – 7.57 (m, 2H), 7.23 – 7.17 (m, 1H), 4.34 (t, $J = 6.7$ Hz, 2H), 3.26 (s, 2H), 2.84 – 2.74 (m, 6H), 2.70 (bs, 4H), 2.50 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 169.10, 164.13, 156.28, 146.32, 133.91, 132.29, 131.53, 131.17, 128.11, 126.89, 125.55, 121.15, 120.50, 61.01, 55.48, 53.59, 53.04, 37.29, 21.42; IR (KBr) ($v_{\text{max}}$/cm$^{-1}$): 3327, 2923, 2818, 1696, 1659, 1591, 1537, 1460, 1384; MS (ESI): m/z 514 [M+H]$^+$; HRMS calcd for C$_{28}$H$_{28}$N$_5$O$_3$S [M+H]$^+$ 514.19074, found 514.18998.

$N$-(5,6-Dimethylbenzo[d]thiazol-2-yl)-2-(4-(2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl)piperazin-1-yl)acetamide (3e)

This compound was prepared according to the method described for compound \textbf{3a} by employing compound \textbf{10} (367 mg, 1 mmol), EDCI (210 mg, 1.1 mmol), HOBt (13.5 mg, 0.1 mmol) and 2-amino-5,6-dimethylbenzothiazole (178 mg, 1 mmol) to obtain the pure product \textbf{3g} as yellow solid. (176mg, yield 82%); mp: 201°C; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 10.37 (bs, 1H), 8.59 (d, $J = 7.5$ Hz, 2H), 8.19 (d, $J = 8.3$ Hz, 2H), 7.76 (t, $J = 8.3$ Hz, 2H), 7.52 (s, 1H), 7.50 (s, 1H), 4.33 (t, $J = 6.7$ Hz, 2H), 3.22 (s, 2H), 2.80 – 2.73 (m, 6H), 2.65 (bs, 4H), 2.38 (s, 3H), 2.37 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 169.03, 164.12, 156.28, 146.91, 135.30, 133.90, 133.24, 131.51, 131.16, 129.51, 126.88, 122.54, 121.33, 121.30, 61.03, 55.48, 53.59, 53.03, 37.29, 20.21, 20.01; IR (KBr) ($v_{\text{max}}$/cm$^{-1}$): 3331, 2945, 2820, 1699, 1659, 1645, 1591, 1537, 1460, 1384; MS (ESI): m/z 528 [M+H]$^+$; HRMS calcd for C$_{28}$H$_{27}$N$_6$O$_3$S [M+H]$^+$ 514.19074, found 514.18998.

$N$-(Benzo[d]thiazol-6-yl)-2-(4-(2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl)piperazin-1-yl)acetamide (4a)

This compound was prepared according to the method described for compound \textbf{3a} by employing compound \textbf{10} (367 mg, 1 mmol), EDCI (210 mg, 1.1 mmol), HOBt (13.5 mg, 0.1 mmol) and 6-aminobenzothiazole (150 mg, 1 mmol) to obtain the pure product \textbf{4a} as yellow solid. (165mg, yield 81%); mp: 103°C; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.41 (bs, 1H), 8.91 (s, 1H), 8.61 (d, $J = 7.1$ Hz, 3H), 8.23 (d, $J = 8.1$ Hz, 2H), 8.06 (d, $J = 8.6$ Hz, 1H), 7.78 (t, $J = 8.1$ Hz, 2H), 7.42 – 7.37 (m, 1H), 4.38 (t, $J = 6.9$ Hz, 2H), 3.16 (s, 2H), 2.82 – 2.71 (m, 6H), 2.67 (bs, 4H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 168.54, 164.18, 153.22, 149.86, 135.37, 134.80, 133.98, 131.58,
131.21, 128.16, 126.93, 123.55, 122.58, 118.62, 111.93, 61.80, 55.53, 53.46, 53.40, 37.36; IR (KBr) \( v_{\text{max}}/\text{cm}^{-1} \): 3286, 2935, 2817, 1698, 1659, 1625, 1589, 1566, 1474, 1380; MS (ESI): m/z 500 [M+H]⁺; HRMS calcd for C\(_{27}\)H\(_{25}\)N\(_5\)O\(_3\)S [M+H]⁺ 500.1751, found 500.1777.

\( \text{2-(4-(2-(1,3-Dioxo-1H-benzo}[de]\text{isoquinolin-2(3H)-yl})ethyl)piperazin-1-yl)-N-(2-methylbenzo}[d]\text{thiazol-6-yl})acetamide (4b) } \\
\)

This compound was prepared according to the method described for compound 3a by employing compound 10 (367 mg, 1 mmol), EDCI (210 mg, 1.1 mmol), HOBt (13.5 mg, 0.1 mmol) and 6-amino-2-methylbenzothiazole (164 mg, 1 mmol) to obtain the pure product 4b as yellow solid. (158mg, yield 76%); mp: 98 °C; \( ^1\text{H NMR (300 MHz, CDCl}_3 \) \( \delta \) 9.25 (bs, 1H), 8.59 (d, \( J = 7.1 \text{ Hz, 2H} \)), 8.04 (s, 1H), 7.77 (t, \( J = 7.9 \text{ Hz, 2H} \)), 7.70 (d, \( J = 4.3 \text{ Hz, 2H} \)), 4.34 (t, \( J = 6.4 \text{ Hz, 2H} \)), 2.83 (s, 3H), 2.79 – 2.68 (m, 6H), 2.65 (bs, 4H); \( ^{13}\text{C NMR (126 MHz, CDCl}_3 \) \( \delta \) 168.36, 168.20, 164.15, 153.86, 136.06, 133.94, 131.53, 131.18, 130.95, 128.11, 126.89, 122.52, 121.44, 117.33, 112.86, 61.73, 55.50, 53.33, 37.27, 20.13; IR (KBr) \( v_{\text{max}}/\text{cm}^{-1} \): 3302, 2934, 2818, 1697, 1658, 1625, 1589, 1568, 1463, 1379; MS (ESI): m/z 514 [M+H]⁺; HRMS calcd for C\(_{28}\)H\(_{28}\)N\(_5\)O\(_3\)S [M+H]⁺ 514.1907, found 514.1903.

\( (E)-2-(4-Cinnamoylpiperazin-1-yl) \text{ethyl-1H-benzo}[de]\text{isoquinoline-1, 3(2H)-Dione (5a) } \\
\)

To a solution of compound 8 (1mmol) in dichloromethane (20 ml) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) (210 mg, 1.1 mmol) and 1-hydroxy-1,2,3-benzotriazole (HOBt) (13.5 mg, 0.1 mmol). Then cinnamic acid (148 mg, 1 mmol) was added and the reaction mixture was stirred at room temperature for 14 h and the reaction was monitored by TLC. After completion of reaction, water was added to reaction mixture and extracted with dichloromethane (2x30 ml). The organic layer was dried with Na\(_2\)SO\(_4\) and evaporated under vacuum to afford the crude product. This was further purified by column chromatography using pure ethyl acetate as solvent system to obtain the pure product 5a as yellow solid. (179mg, yield 84%); mp: 143°C; \( ^1\text{H NMR (300 MHz, CDCl}_3 \) \( \delta \) 8.58 (d, \( J = 7.5 \text{ Hz, 2H} \)), 8.20 (d, \( J = 8.3 \text{ Hz, 2H} \)), 7.77 (t, \( J = 7.5 \text{ Hz, 2H} \)), 7.65 (d, \( J = 15.4 \text{ Hz, 1H} \)), 7.55 – 7.47 (m, 2H), 7.41 – 7.32 (m, 3H), 6.85 (d, \( J = 15.4 \text{ Hz, 1H} \)), 4.40 (t, \( J = 6.6 \text{ Hz, 2H} \)), 3.79 – 3.63 (m, 4H), 2.84 (t, \( J = 6.6 \text{ Hz, 2H} \)), 2.73 (bs, 4H); \( ^{13}\text{C NMR (75 MHz, CDCl}_3 \) \( \delta \) 165.38, 164.15, 142.83, 135.07, 133.95, 131.44, 131.16, 129.55, 128.69, 127.67, 126.84, 122.34, 118.18, 116.78, 55.38, 53.20, 52.82, 45.36, 41.71, 36.82; IR (KBr) \( v_{\text{max}}/\text{cm}^{-1} \): 1699, 1654, 1613, 1590, 1436, 1381, 1346, 1333; MS (ESI): m/z 440 [M+H]⁺; HRMS calcd for C\(_{28}\)H\(_{26}\)N\(_3\)O\(_3\) [M+H]⁺ 440.19687, found 440.19427.
(E)-2-(2-(4-(3-(3-Methoxyphenyl)acryloyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (5b)

This compound was prepared according to the method described for compound 5a by employing compound 8 (367 mg, 1 mmol), EDCI (210 mg, 1.1 mmol), HOBt (13.5 mg, 0.1 mmol) and 3-methoxy cinnamic acid (178 mg, 1 mmol) to obtain the pure product 5b as yellow solid.

(180mg, yield 79%); mp: 120°C; 1H NMR (300 MHz, CDCl3) δ 8.59 (d, J = 8.1 Hz, 2H), 8.20 (d, J = 8.3 Hz, 2H), 7.75 (t, J = 7.9 Hz, 2H), 7.61 (d, J = 15.4 Hz, 1H), 7.28 (dd, J = 10.3, 7.7 Hz, 1H), 7.10 (d, J = 7.7 Hz, 1H), 7.02 (s, 1H), 6.93 – 6.87 (m, 1H), 6.84 (d, J = 15.4 Hz, 1H), 4.39 (t, J = 6.6 Hz, 2H), 3.83 (s, 3H), 3.78 – 3.65 (m, 4H), 2.83 (t, J = 6.6 Hz, 2H), 2.73 (bs, 4H); 13C NMR (75 MHz, CDCl3) δ 165.24, 164.07, 159.65, 142.61, 136.43, 133.91, 131.38, 131.09, 129.64, 127.95, 126.79, 122.30, 120.19, 117.12, 115.12, 112.81, 55.34, 55.17, 53.19, 52.80, 45.38, 41.73, 36.84; IR (KBr) (v max/cm⁻¹): 1698, 1659, 1603, 1590, 1512, 1488, 1437, 1373, 1367; MS (ESI): m/z 470 [M+H]+; HRMS calcd for C28H28N3O4 [M+H]+ 470.20743, found 470.20496.

(E)-2-(2-(4-(3-(4-Ethoxyphenyl)acryloyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (5c)

This compound was prepared according to the method described for compound 5a by employing compound 8 (367 mg, 1 mmol), EDCI (210 mg, 1.1 mmol), HOBt (13.5 mg, 0.1 mmol) and 4-ethoxy cinnamic acid (192 mg, 1 mmol) to obtain the pure product 5c as yellow solid.

(190mg, yield 81%); mp: 164°C; 1H NMR (300 MHz, CDCl3) δ 8.59 (d, J = 8.3 Hz, 2H), 8.21 (d, J = 8.3 Hz, 2H), 7.75 (t, J = 7.5 Hz, 2H), 7.62 (d, J = 15.1 Hz, 1H), 7.45 (d, J = 8.3 Hz, 2H), 6.87 (d, J = 8.3 Hz, 2H), 6.71 (d, J = 15.1 Hz, 1H), 4.41 (t, J = 6.7 Hz, 2H), 4.05 (q, J = 7.0 Hz, 2H), 3.73 (bs, 4H), 2.86 (t, J = 6.7 Hz, 2H), 2.75 (bs, 4H), 1.42 (t, J = 7.0 Hz, 3H); 13C NMR (125 MHz, CDCl3) δ 165.74, 164.07, 159.65, 142.61, 136.43, 133.91, 131.38, 131.09, 129.64, 127.95, 126.79, 122.30, 120.19, 117.12, 115.12, 112.81, 55.34, 55.17, 53.19, 52.80, 45.38, 41.73, 36.84; IR (KBr) (v max/cm⁻¹): 1698, 1659, 1603, 1590, 1512, 1488, 1437, 1373, 1367; MS (ESI): m/z 484 [M+H]+; HRMS calcd for C29H30N3O4 [M+H]+ 484.22308, found 484.22032.

(E)-2-(2-(4-(3-(3-Trifluoromethyl)phenyl)acryloyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (5d)

This compound was prepared according to the method described for compound 5a by employing compound 8 (367 mg, 1 mmol), EDCI (210 mg, 1.1 mmol), HOBt (13.5 mg, 0.1 mmol) and 3-trifluoromethylcinnamic acid (216 mg, 1 mmol) to obtain the pure product 5e as yellow solid.
solid. (184 mg, 75%); mp: 189°C; ¹H NMR (300 MHz, CDCl₃) δ 8.60 (d, J = 6.9 Hz, 2H), 8.21 (d, J = 8.3 Hz, 2H), 7.80 – 7.73 (m, 3H), 7.69 – 7.56 (m, 3H), 7.50 (d, J = 7.5 Hz, 1H), 6.92 (d, J = 15.8 Hz, 1H), 4.37 (t, J = 6.7 Hz, 2H), 3.75 – 3.60 (m, 4H), 2.78 (t, J = 6.7 Hz, 2H), 2.67 (bs, 4H); ¹³C NMR (75 MHz, CDCl₃) δ 164.78, 164.19, 140.91, 136.05, 134.00, 131.55, 131.21, 131.12, 129.33, 128.12, 126.93, 125.97, 125.92, 123.99, 123.93, 123.88, 123.84, (q, J = 4.4, 8.7 Hz), 122.51, 119.06, 55.53, 53.44, 52.98, 45.88, 42.24, 37.26; IR (KBr) (v max/cm⁻¹): 1694, 1683, 1625, 1608, 1591, 1456, 1390, 1376, 1366, 1347; MS (ESI): m/z 508 [M+H]⁺; HRMS calcd for C₂⁸H₂⁵F₃N₃O₃ [M+H]⁺ 508.18425, found 508.18288.

(E)-2-((2-(4-(3-(2,4-Dimethoxyphenyl)acryloyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (5e)

This compound was prepared according to the method described for compound 5a by employing compound 8 (367 mg, 1 mmol), EDCI (210 mg, 1.1 mmol), HOBt (13.5 mg, 0.1 mmol) and 2,4-dimethoxycinnamic acid (208 mg, 1 mmol) to obtain the pure product 5f as yellow solid. (206 mg, yield 85%); mp: 199°C; ¹H NMR (300 MHz, CDCl₃) δ 8.61 (d, J = 7.5 Hz, 2H), 8.22 (d, J = 8.3 Hz, 2H), 7.86 – 7.73 (m, 3H), 7.40 (d, J = 8.3 Hz, 1H), 6.90 (d, J = 15.8 Hz, 1H), 6.52 – 6.41 (m, 2H), 4.38 (t, J = 6.7 Hz, 2H), 3.87 (s, 3H), 3.84 (s, 3H), 3.76 – 3.71 (m, 4H), 2.78 (t, J = 6.7 Hz, 2H), 2.67 (bs, 4H); ¹³C NMR (126 MHz, CDCl₃) δ 166.38, 164.13, 161.91, 159.54, 138.29, 133.92, 131.51, 131.14, 130.53, 128.08, 126.86, 122.49, 117.39, 115.36, 104.92, 98.41, 55.50, 55.41, 55.35, 53.32, 53.13, 37.13; IR (KBr) (v max/cm⁻¹): 1697, 1656, 1607, 1591, 1539, 1455, 1437, 1389, 1376; MS (ESI): m/z 500 [M+H]⁺; HRMS calcd for C₂⁹H₃₀N₅O₅ [M+H]⁺ 500.21800, found 500.21515.

(E)-2-((2-(4-(3-(4-(Dimethylamino)phenyl)acryloyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (5f)

This compound was prepared according to the method described for compound 5a by employing compound 8 (367 mg, 1 mmol), EDCI (210 mg, 1.1 mmol), HOBt (13.5 mg, 0.1 mmol) and 4-dimethylaminocinnamic acid (191 mg, 1 mmol) to obtain the pure product 5g as yellow solid. (196 mg, yield 84%); mp: 182°C; ¹H NMR (500 MHz, CDCl₃) δ 8.59 (d, J = 8.0 Hz, 2H), 8.21 (d, J = 8.20 Hz, 2H), 7.75 (t, J = 8.0 Hz, 2H), 7.61 (d, J = 15.2 Hz, 1H), 7.40 (d, J = 8.8 Hz, 2H), 6.67 – 6.63 (m, 3H), 4.37 (t, J = 6.7 Hz, 2H), 3.74 – 3.60 (m, 4H), 2.99 (s, 6H), 2.76 (t, J = 6.7 Hz, 2H), 2.64 (bs, 4H); ¹³C NMR (125 MHz) δ 166.15, 164.12, 151.23, 143.19, 133.91, 131.52, 131.14, 129.19, 128.10, 126.87, 123.15, 122.53, 111.80, 111.50, 55.52, 53.24, 45.65,
42.01, 40.12, 37.24, 29.61; IR (KBr) (v max/cm\(^{-1}\)):
1689, 1655, 1600, 1584, 1517, 1467, 1415, 1373, 1335; MS (ESI): m/z 483 [M+H] \(^+\); HRMS calcd for C\(_{29}\)H\(_{31}\)N\(_4\)O\(_3\) [M+H] \(^+\)483.23907, found 483.23609.

\((E)-2-(2-(4-(3-(Benzo[d][1,3]dioxol-5-yl)acryloyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (5g)\)

This compound was prepared according to the method described for compound 5a by employing compound 8 (367 mg, 1 mmol), EDCI (210 mg, 1.1 mmol), HOBT (13.5 mg, 0.1 mmol) and 3,4-methylanedioxycinnamic acid (192 mg, 1 mmol) to obtain the pure product 5h as yellow solid. (202 mg, yield 86%); mp: 151 °C; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 8.59 (d, \(J = 7.5\) Hz, 2H), 8.20 (d, \(J = 7.5\) Hz, 2H), 7.75 (t, \(J = 7.5\) Hz, 2H), 7.56 (d, \(J = 15.1\) Hz, 1H), 7.03 – 6.95 (m, 2H), 6.79 (d, \(J = 7.5\) Hz, 1H), 6.68 (d, \(J = 15.1\) Hz, 1H), 5.99 (s, 2H), 4.38 (t, \(J = 6.7\) Hz, 2H), 3.75 – 3.60 (m, 4H), 2.81 (t, \(J = 6.7\) Hz, 2H), 2.69 (bs, 4H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 165.47, 164.14, 148.90, 148.09, 142.56, 133.94, 131.46, 131.15, 129.53, 128.04, 126.85, 123.71, 122.42, 114.75, 108.39, 106.24, 101.34, 55.41, 53.25, 52.88, 45.44, 41.83, 36.94; IR (KBr) (v max/cm\(^{-1}\)):
1699, 1654, 1604, 1598, 1591, 1433, 1391, 1366; MS (ESI): m/z 484 [M+H] \(^+\); HRMS calcd for C\(_{28}\)H\(_{26}\)N\(_3\)O\(_5\) [M+H] \(^+\)484.18670, found 484.18378.

\((E)-2-(2-(4-(3-(3-Hydroxy-4-methoxyphenyl)acryloyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (5i)\)

This compound was prepared according to the method described for compound 5a by employing compound 8 (367 mg, 1 mmol), EDCI (210 mg, 1.1 mmol), HOBT (13.5 mg, 0.1 mmol) and 3-hydroxy-4-methoxycinnamic acid (194 mg, 1 mmol) to obtain the pure product 5i as yellow solid. (181 mg, yield 77%); mp: 112 °C; \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 8.60 (d, \(J = 7.1\) Hz, 2H), 8.22 (d, \(J = 8.2\) Hz, 2H), 7.77 (t, \(J = 8.2\) Hz, 2H), 7.56 (d, \(J = 15.4\) Hz, 1H), 7.12 (d, \(J = 1.9\) Hz, 1H), 6.98 (dd, \(J = 8.3, 1.9\) Hz, 1H), 6.81 (d, \(J = 8.3\) Hz, 1H), 6.71 (d, \(J = 15.4\) Hz, 1H), 4.37 (t, \(J = 6.7\) Hz, 2H), 3.91 (s, 3H), 3.71 – 3.60 (m, 4H), 2.76 (t, \(J = 6.7\) Hz, 2H), 2.64 (bs, 4H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 165.62, 164.19, 148.03, 145.80, 142.51, 133.94, 131.56, 131.19, 128.85, 128.14, 126.90, 122.57, 121.35, 115.05, 112.63, 110.49, 55.91, 55.54, 53.41, 53.04, 45.72, 42.11, 37.26; IR (KBr) (v max/cm\(^{-1}\)): 1697, 1658, 1602, 1590, 1511, 1437, 1384, 1345; MS (ESI): m/z 486 [M+H] \(^+\); HRMS calcd for C\(_{28}\)H\(_{26}\)N\(_3\)O\(_5\) [M+H] \(^+\)486.20235, found 486.19960.

\((E)-2-(2-(4-(3-(2,4-Difluorophenyl)acryloyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (5i)\)

This compound was prepared according to the method described for compound 5a by employing compound 8 (367 mg, 1 mmol), EDCI (210 mg, 1.1 mmol), HOBT (13.5 mg, 0.1 mmol) and 3-hydroxy-4-methoxycinnamic acid (194 mg, 1 mmol) to obtain the pure product 5i as yellow solid. (181 mg, yield 77%); mp: 112 °C; \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 8.60 (d, \(J = 7.1\) Hz, 2H), 8.22 (d, \(J = 8.2\) Hz, 2H), 7.77 (t, \(J = 8.2\) Hz, 2H), 7.56 (d, \(J = 15.4\) Hz, 1H), 7.12 (d, \(J = 1.9\) Hz, 1H), 6.98 (dd, \(J = 8.3, 1.9\) Hz, 1H), 6.81 (d, \(J = 8.3\) Hz, 1H), 6.71 (d, \(J = 15.4\) Hz, 1H), 4.37 (t, \(J = 6.7\) Hz, 2H), 3.91 (s, 3H), 3.71 – 3.60 (m, 4H), 2.76 (t, \(J = 6.7\) Hz, 2H), 2.64 (bs, 4H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 165.62, 164.19, 148.03, 145.80, 142.51, 133.94, 131.56, 131.19, 128.85, 128.14, 126.90, 122.57, 121.35, 115.05, 112.63, 110.49, 55.91, 55.54, 53.41, 53.04, 45.72, 42.11, 37.26; IR (KBr) (v max/cm\(^{-1}\)): 1697, 1658, 1602, 1590, 1511, 1437, 1384, 1345; MS (ESI): m/z 486 [M+H] \(^+\); HRMS calcd for C\(_{28}\)H\(_{26}\)N\(_3\)O\(_5\) [M+H] \(^+\)486.20235, found 486.19960.
This compound was prepared according to the method described for compound 5a by employing compound 8 (367 mg, 1 mmol), EDCI (210 mg, 1.1 mmol), HOBr (13.5 mg, 0.1 mmol) and 2,4-difluorocinnamic acid (184 mg, 1 mmol) to obtain the pure product 5j as yellow solid. (196 mg, yield 85%); mp: 214°C; 1H NMR (500 MHz, CDCl3) δ 8.61 (d, J = 7.2 Hz, 2H), 8.23 (d, J = 8.1 Hz, 2H), 7.77 (t, J = 7.7 Hz, 2H), 7.64 (d, J = 15.7 Hz, 1H), 7.48 (dd, J = 15.0, 8.5 Hz, 1H), 6.94 (d, J = 15.7 Hz, 1H), 6.92 – 6.82 (m, 2H), 4.38 (t, J = 6.7 Hz, 2H), 3.72 – 3.58 (m, 4H), 2.77 (t, J = 6.7 Hz, 2H), 2.65 (bs, 4H); 13C NMR (75 MHz, CDCl3) δ 165.13, 164.15, 134.58, 133.93, 131.57, 131.16, 130.62, 128.14, 126.90, 122.57, 119.87, 111.92, 111.60, 104.85, 104.51, 104.17, 55.53, 53.38, 53.04, 45.85, 42.23, 37.31; IR (KBr) (vmax/cm⁻¹): 1699, 1655, 1609, 1526, 1471, 1425, 1388, 1344, 1338; MS (ESI): m/z 476 [M+H]^+; HRMS calcd for C27H24F2N3O3 [M+H]^+ 476.17802, found 476.17503.

(E)-2-(2-(4-(3-(2,5-Difluorophenyl)acryloyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (5j)

This compound was prepared according to the method described for compound 5a by employing compound 8 (367 mg, 1 mmol), EDCI (210 mg, 1.1 mmol), HOBr (13.5 mg, 0.1 mmol) and 2,5-difluorocinnamic acid (184 mg, 1 mmol) to obtain the pure product 5k as yellow solid. (184 mg, yield 80%); mp: 165°C; 1H NMR (500 MHz, CDCl3) δ 8.59 (d, J = 7.5 Hz, 2H), 8.22 (d, J = 8.3 Hz, 2H), 7.77 (t, J = 7.5 Hz, 2H), 7.61 (d, J = 15.8 Hz, 1H), 7.21 – 7.14 (m, 1H), 7.09 – 7.00 (m, 2H), 6.97 (d, J = 15.8 Hz, 1H), 4.35 (t, J = 6.7 Hz, 2H), 3.72 – 3.57 (m, 4H), 2.75 (t, J = 6.7 Hz, 2H), 2.65 (bs, 4H); 13C NMR (75 MHz, CDCl3) δ 164.76, 164.11, 134.23, 133.92, 131.49, 131.14, 126.87, 122.47, 121.30, 121.20, 117.38, 117.19, 117.05, 116.87, 115.19, 114.86, 55.46, 53.36, 52.93, 45.84, 42.20, 37.24; IR (KBr) (vmax/cm⁻¹): 1699, 1655, 1603, 1517, 1459, 1417, 1387, 1346, 1335; MS (ESI): m/z 476 [M+H]^+; HRMS calcd for C27H24F2N3O3 [M+H]^+ 476.17802, found 476.17639.

(E)-2-(2-(4-(3-(3,4-Difluorophenyl)acryloyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (5k)

This compound was prepared according to the method described for compound 5a by employing compound 8 (367 mg, 1 mmol), EDCI (210 mg, 1.1 mmol), HOBr (13.5 mg, 0.1 mmol) and 3,4-difluorocinnamic acid (184 mg, 1 mmol) to obtain the pure product 5l as yellow solid. (182 mg, yield 79%); mp: 179°C; 1H NMR (500 MHz, CDCl3) δ 8.59 (d, J = 7. Hz, 2H), 8.20 (d, J = 8.3 Hz, 2H), 7.77 (t, J = 7.5 Hz, 2H), 7.53 (d, J = 15.4 Hz, 1H), 7.37 – 7.30 (m, 1H), 7.25
- 7.12 (m, 2H), 6.75 (d, J = 15.4 Hz, 1H), 4.35 (t, J = 6.5 Hz, 2H), 3.70 – 3.60 (m, 4H), 2.76 (t, J = 6.5 Hz, 2H), 2.65 (bs, 4H); \( ^{13} \text{C} \text{ NMR} \) (125 MHz) \( \delta \) 164.75, 164.16, 140.31, 133.95, 132.52, 131.57, 131.18, 128.14, 126.92, 124.38, 122.57, 118.26, 117.68, 117.54, 115.90, 115.76, 55.51, 53.98, 48.75, 42.24, 37.30; IR (KBr) (\( \nu_{\max} / \text{cm}^{-1} \)): 1699, 1655, 1605, 1527, 1465, 1414, 1371, 1344, 1327; MS (ESI): m/z 476 [M+H]+; HRMS calcd for C\(_{27}\)H\(_{24}\)F\(_2\)N\(_3\)O\(_3\) [M+H]+ 476.17802, found 476.17538.

\( (E) - 2 - (2 - (4 - (3,5 - \text{Difluorophenyl}) \text{acryloyl}) \text{piperazin-1-yl})\text{ethyl}) - 1H - \text{benzo[de]isoquinoline-1,3(2H)-dione (5l)} \)

This compound was prepared according to the method described for compound 5a by employing compound 8 (367 mg, 1 mmol), EDCI (210 mg, 1.1 mmol), HOBt (13.5 mg, 0.1 mmol) and 3,5-difluorocinnamic acid (184 mg, 1 mmol) to obtain the pure product 5m as yellow solid. (181 mg, 83%); mp: 206°C; \(^{1} \text{H} \text{NMR} \) (500 MHz, CDCl\(_3\)) \( \delta \) 8.60 (d, \( J = 6.9 \) Hz, 2H), 8.22 (d, \( J = 7.9 \) Hz, 2H), 7.77 (t, \( J = 7.9 \) Hz, 2H), 7.52 (d, \( J = 15.8 \) Hz, 1H), 7.03 – 6.99 (m, 2H), 6.85 (d, \( J = 15.8 \) Hz, 1H), 6.81 – 6.76 (m, 1H), 4.36 (t, \( J = 6.9 \) Hz, 2H), 3.71 – 3.58 (m, 4H), 2.77 (t, \( J = 6.9 \) Hz, 2H), 2.64 (bs, 4H); \(^{13} \text{C} \text{ NMR} \) (75 MHz, CDCl\(_3\)) \( \delta \) 164.83, 164.66 (d, \( J = 13.17 \) Hz), 164.46, 164.16, 161.54, 161.36 (d, \( J = 13.17 \) Hz), 140.08, 133.97, 131.19, 126.91, 122.51, 119.83, 110.45, 110.11, 104.94, 104.61, 55.48, 53.38, 52.92, 45.89, 42.24, 37.27; IR (KBr) (\( \nu_{\max} / \text{cm}^{-1} \)): 1698, 1652, 1604, 1541, 1498, 1418, 1369, 1324, 1311; MS (ESI): m/z 476 [M+H]+; HRMS calcd for C\(_{27}\)H\(_{24}\)F\(_2\)N\(_3\)O\(_3\) [M+H]+ 476.17802, found 476.17530.

\( (E) - 2 - (2 - (4 - (3,4,5 - \text{Trifluorophenyl}) \text{acryloyl}) \text{piperazin-1-yl})\text{ethyl}) - 1H - \text{benzo[de]isoquinoline-1,3(2H)-dione (5m)} \)

This compound was prepared according to the method described for compound 5a by employing compound 8 (367 mg, 1 mmol), EDCI (210 mg, 1.1 mmol), HOBt (13.5 mg, 0.1 mmol) and 3,4,5-trifluorocinnamic acid (202 mg, 1 mmol) to obtain the pure product 5n as yellow solid. (187 mg, yield 78%); mp: 215°C; \(^{1} \text{H} \text{NMR} \) (300 MHz, CDCl\(_3\)) \( \delta \) 8.62 (d, \( J = 6.7 \) Hz, 2H), 8.24 (d, \( J = 8.3 \) Hz, 2H), 7.78 (t, \( J = 8.3 \) Hz, 2H), 7.49 (d, \( J = 15.8 \) Hz, 1H), 7.17 – 7.08 (m, 2H), 6.78 (d, \( J = 15.8 \) Hz, 1H), 6.31 – 6.26 (m, 1H), 4.36 (t, \( J = 6.7 \) Hz, 2H), 3.71 – 3.58 (m, 4H), 2.77 (t, \( J = 6.7 \) Hz, 2H), 2.64 (bs, 4H); \(^{13} \text{C} \text{ NMR} \) (126 MHz, CDCl\(_3\)) \( \delta \) 164.32, 164.16, 139.42, 133.96, 131.55, 131.18, 128.13, 126.91, 122.53, 119.52, 111.60, 111.56, 111.47, 111.43, 55.48, 53.35, 52.90, 45.85, 42.23, 37.22; IR (KBr) (\( \nu_{\max} / \text{cm}^{-1} \)): 1698, 1687, 1630, 1537, 1451, 1389, 1363, 1348; MS (ESI): m/z 494 [M+H]+; HRMS calcd for C\(_{27}\)H\(_{23}\)F\(_3\)N\(_3\)O\(_3\) [M+H]+ 494.16860, found 494.16584.
Biology

Cytotoxic activity

The cytotoxic activity of the compounds was determined using MTT assay. Cells were seeded in 200 µL DMEM, supplemented with 10% FBS in each well of 96-well microculture plates and incubated for 24 h at 37 °C in a CO₂ incubator. After 24 h of incubation cells were treated with test compounds 48 h. After 48 h of incubation, 10 µl MTT (3-(4,5-dimethylthiazol-2-y1)- 2,5-diphenyl tetrazolium bromide) (5 mg/ml) was added to each well and the plates were further incubated for 4 h. Then the supernatant from each well was carefully removed, formazone crystals were dissolved in 200 µL of DMSO and absorbance at 570 nm wavelength was recorded.

In vitro growth inhibition

The screening of anticancer activity is evaluated by the NCI, USA, according to standard procedures (http://dtp.nci.nih.gov/branches/btb/ivclsp.html).

4.2.3. CD spectroscopic studies

Circular dichroism experiments were carried out using JASCO 815 CD spectropolarimeter (Jasco, Tokyo, Japan). CD spectrum was recorded from 225 to 325 nm to find out the conformational changes in the CT DNA after complex interaction. For each CD experiment, about 15x10⁻⁶ M of CT DNA was used initially. Further, to evaluate the effect of compounds on DNA conformation, CD spectra were recorded in 1:0 and 1:1 molar ratios. CD titrations were performed in 100 mM TE (pH 7.0) at 25 °C. Each spectrum was recorded three times and the average of three scans was taken.

UV-visible spectroscopy titrations

UV-visible spectroscopy titrations were performed using ABI Lambda 40 UV-Vis spectrophotometer (Foster City, USA) at 25 °C using 1 cm path length quartz cuvette. Stock solutions of 25 µM of NBT3 and NBT6 solution and 25 µM CT DNA were prepared in 100 mM TE (pH 7.0). The compound stock solution was prepared in DMSO and diluted to the required concentration in suitable buffer solutions. The quartz cells were thoroughly cleaned with distilled water and followed by nitric acid (~ 0.1 N) after each experiment. UV-visible absorption titrations were done by adding CT DNA to the quartz cuvette containing approximately 25 µM hybrid solution. Preparation of CT DNA and the complexes were done on the same day of performing the experiment. Titrations were carried out until the hybrid absorption band remains at a fixed wavelength upon successive additions of CT DNA.
Fluorescence spectroscopy titrations

Fluorescence emission spectra were measured at 25°C using a Hitachi F4500 spectrofluorimeter (Maryland, USA) using a 1 cm path length quartz cuvette. Quartz cuvettes was thoroughly washed with distilled water and dilute nitric acid (approximately 0.1 N) to minimize non-specific binding of the molecules to the surface of the cuvette. Throughout the fluorescence experiment, the concentration of the 4b and 4a compounds were kept constant (10 µM) and titrated with increasing concentrations of CT DNA (multiples of 0.5 µM). Fluorescence spectra were recorded after each addition of CT DNA to the fluorescent cuvette. Both the compounds were excited at 350 nm and emission spectra for each titration were recorded from 355 to 500 nm. Each spectrum was recorded three times and the average of three scans was taken.

kDNA Decatenation Assay or Topo II inhibition assay

In order to test the role of synthetic hybrids 4a/4b in topo II inhibition, decatenation of kDNA was carried out using the protocol mentioned in Topo II Drug Screening Kit (TG 1009, Topogen, USA). Topoisomerase II inhibition was assayed using the ATP dependent decatenation of kDNA. Reactions were carried out in 20 µl and contained 120 mM KCl, 50 mM Tris–HCl, (pH 8), 10 mM MgCl₂, 0.5 mM dithiothreitol, 0.5 mM ATP, 30 mg/ml bovine serum albumin, 200–300 ng of kDNA, and topoisomerase II (5 units). The amount of topoisomerase II (5 units) was adjusted in preliminary experiments to decatenate approximately 100% of the kDNA under our assay conditions. Authentic decatenated DNA was used as controls to identify decatenated kDNA. kDNA in the presence of topo II enzyme was incubated with 100 µM of amonafide, 4a and 4b. The sample in which 100 µM of amonafide was added will serve as control. The reactions were incubated at 37°C for 30 min and terminated by the addition of 2 µl of a stop buffer containing 10% (w/v) SDS and 2µl of 0.5 mg/ml proteinase-K and incubated for 10 min at 37°C. After completion of the reaction, the products in the reaction mixture were separated by 1% agarose gel. The products in the agarose gel were visualized after staining with ethidium bromide (0.2 mg/ml). The gels were run at 100 V for about 40 min and visualized under UV transillumination (BIO RAD gel doc XR⁺, USA).

Molecular modelling

The crystal co-ordinates of topoisomerase-IIα subunit were retrieved from the protein data bank (PDB ID: 1ZXM). As topoisomerase-II is a homodimer and co-crystal and metal atom are present in each chain, hence the chain α was considered for molecular modelling studies. The protein
preparation tool was used for the preparation of receptor model (Schrödinger 2017-1). The tool adds missing side chains and loops and also removes water molecules with a distance of more than 5Å away from the active pocket. The ATP binding site with 20Å equally in each direction of X, Y, and Z was used for receptor grid generation. The potent ligands 4a and 4b were sketched by using 2D sketcher and different conformers were generated using Ligprep module of Schrödinger suite. The ligands were docked into the active site of topoisomerase-IIα using GLIDE-XP 7.4 (Extra Precision) mode. The docking for DNA intercalation has been performed in the same manner as mentioned above using duplex DNA obtained from protein data bank (PDB ID: 1NAB).

\[ \text{H NMR Spectrum of Compound 3a} \]
$^{13}$C NMR Spectrum of Compound 3a

$^1$H NMR Spectrum of Compound 3b
$^{13}$C NMR Spectrum of Compound 3b

$^1$H NMR Spectrum of Compound 3c
$^{13}$C NMR Spectrum of Compound 3c

$^1$H NMR Spectrum of Compound 3d
**13C NMR Spectrum of Compound 3d**

**1H NMR Spectrum of Compound 3e**
C NMR Spectrum of Compound 3e

1H NMR Spectrum of Compound 4a
\[ \text{\^{13}C NMR Spectrum of Compound 4b} \]

\[ \text{\textsuperscript{1}H NMR Spectrum of Compound 5a} \]
$^{13}$C NMR Spectrum of Compound 5a

$^{13}$C NMR Spectrum of Compound 5b
13C NMR Spectrum of Compound 5c

13C NMR Spectrum of Compound 5d
$^1$H NMR Spectrum of Compound 5d

$^{13}$C NMR Spectrum of Compound 5d

$^1$H NMR Spectrum of Compound 5e
C NMR Spectrum of Compound 5f

$^{13}$C NMR Spectrum of Compound 5f

$^1$H NMR Spectrum of Compound 5g

$^1$H NMR Spectrum of Compound 5g
C NMR Spectrum of Compound 5g

$^{13}$C NMR Spectrum of Compound 5g

$^{1}$H NMR Spectrum of Compound 5h
C NMR Spectrum of Compound 5h

$\text{F NMR Spectrum of Compound 5i}$
$^1$H NMR Spectrum of Compound 5i

$^{13}$C NMR Spectrum of Compound 5i

$^1$H NMR Spectrum of Compound 5j
$^{13}$C NMR Spectrum of Compound 5j

$^1$H NMR Spectrum of Compound 5k
$^{13}$C NMR Spectrum of Compound 5k

$^1$H NMR Spectrum of Compound 5l
Table 1: Cytotoxic activity (GI$_{50}$ µM)$^a$ of derivatives 4a and 4b on 60 human cancer cell lines.

<table>
<thead>
<tr>
<th>Cancer panel/cell line</th>
<th>4a</th>
<th>4b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukaemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCRF-CEM</td>
<td>05.40 ± 0.2</td>
<td>02.94 ± 0.3</td>
</tr>
<tr>
<td>HL-60(TB)</td>
<td>10.20 ± 0.3</td>
<td>06.36 ± 0.4</td>
</tr>
<tr>
<td>MOLT-4</td>
<td>02.87 ± 0.3</td>
<td>02.52 ± 0.1</td>
</tr>
<tr>
<td>RPMI-8226</td>
<td>09.39 ± 0.3</td>
<td>02.91 ± 0.3</td>
</tr>
<tr>
<td>SR</td>
<td>01.38 ± 0.1</td>
<td>01.19 ± 0.2</td>
</tr>
<tr>
<td>Non-small lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A549/ATCC</td>
<td>03.58 ± 0.1</td>
<td>04.14 ± 0.3</td>
</tr>
<tr>
<td>EKVX</td>
<td>09.79 ± 0.2</td>
<td>07.88 ± 0.1</td>
</tr>
<tr>
<td>HOP-62</td>
<td>05.69 ± 0.3</td>
<td>03.83 ± 0.2</td>
</tr>
<tr>
<td>HOP-92</td>
<td>05.24 ± 0.2</td>
<td>02.12 ± 0.1</td>
</tr>
<tr>
<td>NCI-H226</td>
<td>07.58 ± 0.3</td>
<td>04.67 ± 0.2</td>
</tr>
<tr>
<td>NCI-H23</td>
<td>07.08 ± 0.2</td>
<td>06.60 ± 0.3</td>
</tr>
<tr>
<td>NCI-H322M</td>
<td>&gt;100 ± 0.5</td>
<td>06.88 ± 0.3</td>
</tr>
<tr>
<td>NCI-H460</td>
<td>03.58 ± 0.2</td>
<td>03.44 ± 0.3</td>
</tr>
<tr>
<td>NCI-H522</td>
<td>03.96 ± 0.1</td>
<td>03.01 ± 0.2</td>
</tr>
<tr>
<td>Colon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COLO 205</td>
<td>05.44 ± 0.3</td>
<td>04.46 ± 0.1</td>
</tr>
<tr>
<td>HCC-2998</td>
<td>05.84 ± 0.1</td>
<td>06.66 ± 0.2</td>
</tr>
<tr>
<td>HCT-116</td>
<td>03.57 ± 0.1</td>
<td>02.94 ± 0.1</td>
</tr>
<tr>
<td>HCT-15</td>
<td>05.05 ± 0.2</td>
<td>03.57 ± 0.1</td>
</tr>
<tr>
<td>HT29</td>
<td>03.61 ± 0.1</td>
<td>03.39 ± 0.2</td>
</tr>
<tr>
<td>Cell Line</td>
<td>KM12</td>
<td>SW-620</td>
</tr>
<tr>
<td>-----------</td>
<td>------</td>
<td>--------</td>
</tr>
<tr>
<td>SF-268</td>
<td>06.17± 0.1</td>
<td>04.95± 0.2</td>
</tr>
<tr>
<td>SF-295</td>
<td>04.91± 0.3</td>
<td>03.48± 0.4</td>
</tr>
<tr>
<td>SF-539</td>
<td>06.43± 0.4</td>
<td>07.59± 0.3</td>
</tr>
<tr>
<td>SNB-19</td>
<td>04.50± 0.2</td>
<td>06.23± 0.3</td>
</tr>
<tr>
<td>SNB-75</td>
<td>09.44± 0.1</td>
<td>03.52± 0.3</td>
</tr>
<tr>
<td>U251</td>
<td>04.39± 0.2</td>
<td>04.37± 0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>SF-268</th>
<th>SF-295</th>
<th>SF-539</th>
<th>SNB-19</th>
<th>SNB-75</th>
<th>U251</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS</td>
<td>06.07± 0.2</td>
<td>05.01± 0.3</td>
<td>04.91± 0.4</td>
<td>07.59± 0.3</td>
<td>06.23± 0.3</td>
<td>03.52± 0.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>C15</th>
<th>C22</th>
<th>C20</th>
<th>C10</th>
<th>C8B4</th>
<th>C24</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF-268</td>
<td>06.07± 0.2</td>
<td>05.01± 0.3</td>
<td>04.91± 0.4</td>
<td>07.59± 0.3</td>
<td>06.23± 0.3</td>
<td>03.52± 0.3</td>
</tr>
<tr>
<td>SF-295</td>
<td>04.91± 0.3</td>
<td>03.48± 0.4</td>
<td>07.59± 0.3</td>
<td>06.23± 0.3</td>
<td>03.52± 0.3</td>
<td>04.37± 0.1</td>
</tr>
<tr>
<td>SF-539</td>
<td>06.43± 0.4</td>
<td>07.59± 0.3</td>
<td>06.23± 0.3</td>
<td>03.52± 0.3</td>
<td>04.37± 0.1</td>
<td>04.37± 0.1</td>
</tr>
<tr>
<td>SNB-19</td>
<td>04.50± 0.2</td>
<td>06.23± 0.3</td>
<td>03.52± 0.3</td>
<td>04.37± 0.1</td>
<td>04.37± 0.1</td>
<td>04.37± 0.1</td>
</tr>
<tr>
<td>SNB-75</td>
<td>09.44± 0.1</td>
<td>03.52± 0.3</td>
<td>04.37± 0.1</td>
<td>04.37± 0.1</td>
<td>04.37± 0.1</td>
<td>04.37± 0.1</td>
</tr>
<tr>
<td>U251</td>
<td>04.39± 0.2</td>
<td>04.37± 0.1</td>
<td>04.37± 0.1</td>
<td>04.37± 0.1</td>
<td>04.37± 0.1</td>
<td>04.37± 0.1</td>
</tr>
</tbody>
</table>

**Note:** (a) Values are reported as GI\textsubscript{50}, the µM concentration of the compound required to cause 50% inhibition of cell growth; (b) 4a NSC : 761409 / 1); (c) 4b (NSC:761421/1).
Materials and Methods

The cell lines for the MTT assay namely, HT-29, A549 and MCF-7 cell lines were procured from the National Centre for Cell Sciences (NCCS, Pune, India). The cells were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) and an antibiotic (100 units/mL Penicillin and 100 μg/mL Streptomycin) solution (Sigma). The cells were grown in a humidified atmosphere with 5% CO₂ and 95% air at 37 °C.