Novel indole-based sigma-2 receptor ligands: synthesis, structure-affinity relationship and antiproliferative activity†

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We report the synthesis and biological evaluation of a series of indole-based σ2 receptor ligands derived from siramesine. In vitro competition binding assays showed that these analogues possessed high to moderate affinity and selectivity for σ2 receptors. Structure-affinity relationship analyses of these indole-based σ2 receptor ligands were performed. In the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, 1a and 1b displayed significant and comparable antiproliferative activity in DU145, MCF7 and C6 cells to siramesine. In cell cycle analyses, compounds 1a, 1b and siramesine were found to induce a G1 phase cell cycle arrest in DU145 cells using flow cytometry. The combination of 5,6-dimethoxyisoindoline scaffold and N-(4-fluorophenyl)indole moiety was identified as a new σ2 receptor ligand deserving further investigation as an antitumor agent.

1. Introduction

Two subtypes of sigma (σ) receptors, termed σ1 and σ2, have been identified.5 Both subtypes display different distributions in the central nervous system and peripheral organs. The σ1 receptor contains 223 amino acids with two transmembrane regions.2,3 It functions as “ligand-operated receptor chaperone” and regulates various ion channels, G protein-coupled receptors, lipids, and other signaling proteins.4,5 In contrast, the σ2 receptor has not been cloned so far, and its molecular weight was estimated to be 21.5 kD. Recently, progesterone receptor membrane component 1 (PGRMC1) was reported as the putative σ2 receptor binding site.6

It is interesting that both subtypes are expressed in a variety of human and rodent tumor cell lines.7,8 However, the expression of the σ2 receptor was found to be higher than that of the σ1 receptor. In proliferating tumor cells, the density of the σ2 receptor was about 8-10-fold higher than that in quiescent tumor cells.9–11 Moreover, σ2 receptor ligands can rapidly internalize into tumor cells and activate apoptosis via multiple pathways.12–14 Thus, the σ2 receptor may both serve as a receptor-based biomarker to distinguish different proliferative states of solid tumors and as a promising target for the treatment of cancer.16

In the past decades, morphans, indoles (siramesine analogues), granatanes, flexible benzamides and N-cyclohexylpiperazines have been reported to serve as selective σ2 receptor ligands.17 Among these ligands, siramesine (also known as Lu-28-179) and its analogues, conformationally flexible amines such as RHM-1, and PB28 analogues were more extensively investigated.17 Their structures are presented in Fig. 1. Although clinical trials of siramesine for the treatment of depression and anxiety were paused in 2002, it proved to be non-toxic and well tolerated in humans. Most importantly, siramesine was demonstrated to induce cell death in many tumorigenic and immortalized cells via different apoptotic pathways.12–14,18 To obtain selective σ2 receptor ligands with antiproliferative activity, we used siramesine as the lead compound to design a series of novel indole-based compounds. It was reported that the indole residue and the butyl chain between the indole and the spirocyclic piperidine moieties were important to maintain the σ2 receptor selectivity for siramesine derivatives.17 We introduced different functional groups to develop new σ2 receptor ligands. Moreover, we also introduced the substituents with fluorine atom to find PET radiotracers for σ2 receptor tumor imaging. The design concept is shown in Fig. 2. First, by keeping the 4-fluorophenyl ring at the indole N-atom and the butyl chain constant,
The 3H-spiro(2-benzofuran-1,4'-piperidinyl) moiety was replaced by different pharmacophores including $\sigma_1$ preferred group C and $\sigma_2$ preferred group A, B, or D (1). Secondly, the 4-fluorophenyl ring at the indole N-atom was replaced by a 2-fluoroalkyl group (2). As a third approach, both the 4-fluorophenyl ring at the indole N-atom and the 3H-spiro(2-benzofuran-1,4'-piperidinyl) moiety were modified (3). Fourth, 4-fluorophenyl was replaced by the 4-iodophenyl group, while the 3H-spiro(2-benzofuran-1,4'-piperidinyl) moiety was replaced by C (4). Finally, the indole core was replaced by 4-fluoro-benzophenone (5). Moreover, the structure–affinity relationships (SAR) of these analogues for $\sigma_2$ receptors were analyzed. The 3-(4,5-dimethyiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to investigate the antiproliferative activity of the most potent ligands. In order to further support this, cell cycle analysis was carried out to examine the effects of these potent compounds on the cell cycle progression using flow cytometry in DU145 cells.

2. Results and discussion

2.1 Chemistry

The synthetic routes of fluorophenylindole derivatives 1a–1g are depicted in Scheme 1. All compounds in this series were prepared from the key bromobutyl derivative 6. Compounds 6,
were synthesized according to the method reported in the literature. Compound 7 or 8 reacted with intermediates 9–11 under basic conditions, followed by deprotection to obtain compounds 12–15 with yields of 56–95%.

N-Alkylation of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (16), 5,6-dimethoxyisoindoline (17), 4-phenylpiperidine-4-carbonitrile (18) or 12–15 with compound 6 provided compounds 1a, 1b, 1c, or 1d–1g, respectively, with yields of 29–84%.

The synthetic route of compound 2 is presented in Scheme 2. Protection of compound 19 with TBDMS chloride, followed by N-alkylation of compound 20 with 2-bromoethanol and tosylation of compound 21 with p-TsCl provided compound 22. Deprotection of TBDMS and fluorination of compound 22 with TBAF by a one-pot reaction afforded compound 23 with yield of 56%. Tosylation of compound 23 led to compound 24, which reacted with 3H-spiro[2-benzofuran-1,4′-piperidine] (25) to obtain target compound 2.

The synthetic routes of compounds 3a–3c are depicted in Scheme 3. Reduction of indole-3-acetic acid (26) and indole-3-propanoic acid (27) with LiAlH₄ gave the corresponding alcohols 28 and 29 in 86% and 79% yield, respectively. Alkylation with 1,4-dibromobutane yielded 30 and 31, followed by fluorination with DAST to obtain 32 and 33. Finally, compound 32 or 33 reacted with intermediate 16 to provide 3a and 3b, respectively. Compound 32 reacted with 25 to obtain 3c.

The synthetic routes of compounds 4 and 5 are depicted in Scheme 4. Synthesis of compound 4 was similar to that of compound 1c. Ullmann reaction between compound 19 and 1,4-diiodobenzene instead of the 4-fluorophenyl residue provided 34 which was subsequently treated with PBr₃ to yield 35. Finally, reaction between 35 and intermediate 18 provided target compound 4. N-Alkylation of intermediate 16 with 5-bromo-1-(4-fluorophenyl)pentan-1-one (36) gave compound 5 with yield of 62%.

In vitro radioligand competition studies and structure–affinity relationship analyses

The affinities of the indole-based analogues for the σ₁ and σ₂ receptors were determined by radioligand competition binding.
were used as radioligands for the low nanomolar affinity and non-selectivity for \( \sigma \) receptors.

More recently, Niso et al. revealed its high affinity with substitution at the meta- and para-positions but increased the subtype selectivity. Compound 1d with substitution at the para-position of the phenyl ring in group D showed comparable affinity to compound 1g with substitution at the meta-position but had a somewhat higher subtype selectivity. Substitution at the para-position with an increased length of the fluoroooligoethoxylated chain (\( n = 2, 3 \)) dramatically decreased the affinities for \( \sigma_1 \) and \( \sigma_2 \) receptors (1e and 1f). In the literature, compound 1a was reported to possess nanomolar affinity (\( K_i(\sigma_2) = 5.34 \) nM) and high subtype selectivity (\( K_i(\sigma_1)/K_i(\sigma_2) = 260 \)) for \( \sigma_2 \) receptors. However, our sample displayed only moderate affinity (\( K_i(\sigma_2) = 49.2 \) nM) and selectivity (\( K_i(\sigma_1)/K_i(\sigma_2) = 10.8 \)). The above discrepancy may result from the different experimental conditions employed by different groups. It is interesting to note that compound 1b with the 5,6-dimethoxyisoindoline moiety displayed comparable affinity and selectivity to compound

### Scheme 3

**Synthetic routes of compounds 3a-3c.** Reagents and conditions: (a) Ar atmosphere, \( 0^\circ C \), LiAlH\(_4\), anhydrous THF, 4 h, 86% for 28, 79% for 29; (b) Ar atmosphere, 110 \(^\circ\)C, 1,4-dibromobutane, NaH, DMF, overnight, 19% for 30, 34% for 31; (c) Ar atmosphere, \(-78^\circ\)C, DAST, anhydrous \( \text{CH}_2\text{Cl}_2 \), 2 h, 58% for 32, 87% for 33; (d) \( \text{K}_2\text{CO}_3 \), NaI, \( \text{CH}_3\text{CN} \), \( 80^\circ\)C, 4 h, for 3a, 32, 16, 43%; for 3b, 33, 16, 36%; for 3c, 32, 25, 41%.

### Scheme 4

**Synthetic routes of compounds 4 and 5.** Reagents and conditions: (a) 1,4-diiodobenzene, \( \text{K}_2\text{CO}_3 \), copper powder, DMF, \( 120^\circ\)C, 5 h, 23%; (b) \( \text{PBr}_3 \), anhydrous \( \text{CH}_2\text{Cl}_2 \), \( 0^\circ\)C, 2 h, 58%; (c) \( \text{K}_2\text{CO}_3 \), NaI, \( \text{CH}_3\text{CN} \), \( 80^\circ\)C, 4 h, 49%. (d) \( \text{K}_2\text{CO}_3 \), NaI, \( \text{CH}_3\text{CN} \), \( 80^\circ\)C, 4 h, 62%.
In order to find new scaffolds and new \( \sigma_2 \) receptor ligands as potent antitumor agents, antiproliferative activity of compounds 1a and 1b was evaluated in MCF7 (breast cancer), DU145 (androgen-independent human prostate cancer) and C6 (rat glioma) cells using the MTT assay. Antiproliferative activity of siramesine was also determined in these cells as comparison. The effects of these compounds on cellular viability were analyzed using different concentrations between 100 nM and 100 \( \mu \)M. The results expressed as \( EC_{50} \) values are shown in Table 2. All \( EC_{50} \) values were found to be in the micromolar range. Compound 1a and siramesine showed notable antiproliferative effects in MCF7 cells with \( EC_{50} \) values of 20.9 and 23.6 \( \mu \)M, respectively, which are consistent with that reported in the literature (with \( EC_{50} \) values of 17.8 and 12.3 \( \mu \)M, respectively).^{25} It is interesting to note that the new compound 1b exhibited the highest activity in MCF7 cells. Moreover, compound 1b displayed notable and comparable antiproliferative effects to compound 1a and siramesine in DU145 cells. However, all of the three compounds displayed a higher \( EC_{50} \) value in C6 cells than those in the human DU145 and MCF7 tumor cells. Besides compound 1a and siramesine, the indole-based compound 1b with the 5,6-dimethoxyindolino moiety seems to be promising as an anti-tumor agent and warrants further evaluation.

### 2.4 Cell cycle analysis

To further examine the antitumor activity of compounds 1a and 1b, their effects on the cell cycle progression were analyzed by flow cytometry in DU145 cells. Cell cycle phase distribution in control DU145 cells and cells treated with different concentrations of 1a, 1b and siramesine at 24 h time point is presented in Fig. 3. The percentages of \( G_1 \), \( S \) and \( G_2 \) phases of the untreated DU145 cells (control) are 58.2\%, 38.6\% and 3.25\%, respectively. Treatment with compound 1a or 1b or siramesine increased the percentage of \( G_1 \) cells in a dose-dependent manner. After treatment with 40 \( \mu \)M 1a or 30 \( \mu \)M 1b, the percentages of \( G_1 \) cells increased to 84.1\% and 80.5\%, respectively. At the same time, the percentages of \( S \) cells decreased to 15.9\% and 19.3\%, respectively. The percentage of \( G_2 \) phase cells was maintained at 75.7–77.2\% after treatment with 15 to 25 \( \mu \)M siramesine. These data suggest that compounds 1a and 1b and siramesine could induce cell cycle delay and arrest the cell cycle progression predominantly at the \( G_1 \) phase in DU145 cells. It was reported that \( \sigma_2 \)

### 2.3 Antiproliferative activity

Recently, a series of compounds with the indole moiety were reported to display antiproliferative activity in MCF7 and MCF7/adr cells.\(^{25} \) In Table 1 binding affinities of indole-based analogues for \( \sigma_1 \) and \( \sigma_2 \) receptors were presented in triplicate. \(^{25} \)

### Table 1 Binding affinities of indole-based analogues for \( \sigma_1 \) and \( \sigma_2 \) receptors

<table>
<thead>
<tr>
<th>Compound</th>
<th>( K_i(\sigma_1) ) (nM)</th>
<th>( K_i(\sigma_2) ) (nM)</th>
<th>( K_i(\sigma_1)/K_i(\sigma_2) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>530.8 ± 181.1</td>
<td>49.2 ± 11.7</td>
<td>10.8</td>
</tr>
<tr>
<td>1a(^b)</td>
<td>1390 ± 20</td>
<td>5.34 ± 1.22</td>
<td>260</td>
</tr>
<tr>
<td>1b</td>
<td>255.6 ± 14.8</td>
<td>53.8 ± 2.1</td>
<td>4.8</td>
</tr>
<tr>
<td>1c</td>
<td>2.58 ± 0.82</td>
<td>3.03 ± 0.75</td>
<td>0.9</td>
</tr>
<tr>
<td>1d</td>
<td>614 ± 137</td>
<td>68.0 ± 0.04</td>
<td>9.0</td>
</tr>
<tr>
<td>1e</td>
<td>1110 ± 252</td>
<td>458 ± 51</td>
<td>2.4</td>
</tr>
<tr>
<td>1f</td>
<td>2158 ± 404</td>
<td>1879 ± 11</td>
<td>1.1</td>
</tr>
<tr>
<td>1g</td>
<td>257 ± 6.28</td>
<td>48.4 ± 2.65</td>
<td>5.3</td>
</tr>
<tr>
<td>2</td>
<td>246 ± 59.4</td>
<td>44.0 ± 28.1</td>
<td>5.6</td>
</tr>
<tr>
<td>3a</td>
<td>493.5 ± 84.1</td>
<td>27.5 ± 0.7</td>
<td>17.9</td>
</tr>
<tr>
<td>3b</td>
<td>262.5 ± 62.9</td>
<td>28.5 ± 4.9</td>
<td>9.2</td>
</tr>
<tr>
<td>3c</td>
<td>11.0 ± 0.5</td>
<td>29.8 ± 1.6</td>
<td>0.4</td>
</tr>
<tr>
<td>4</td>
<td>386 ± 94</td>
<td>18.5 ± 5.7</td>
<td>20.9</td>
</tr>
<tr>
<td>5</td>
<td>16.6 ± 1.1</td>
<td>12.4 ± 0.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Siramesine</td>
<td>4.69 ± 2.36</td>
<td>3.08 ± 0.68</td>
<td>1.5</td>
</tr>
<tr>
<td>Siramesine(^h)</td>
<td>10.5 ± 2.6</td>
<td>12.6 ± 0.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Siramesine(^i)</td>
<td>17</td>
<td>0.12</td>
<td>140</td>
</tr>
<tr>
<td>ISO-1</td>
<td>102.3 ± 15.1</td>
<td>28.2 ± 0.9</td>
<td>3.6</td>
</tr>
<tr>
<td>ISO-1(^f)</td>
<td>330 ± 25</td>
<td>6.95 ± 1.63</td>
<td>47.5</td>
</tr>
</tbody>
</table>

\(^a\) Values are means ± standard deviation (SD) of three experiments performed in triplicate. \(^b\) From ref. 25. \(^c\) IC\(50\) value, from ref. 24. \(^d\) From ref. 27.

- **C6** 76.5 ± 4.6 44.1 ± 9.9 43.1 ± 6.2

**Table 2 EC\(50\) values of compounds 1a and 1b in different tumor cells**

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>( EC_{50} ) (( \mu )M)</th>
<th>1a</th>
<th>1b</th>
<th>Siramesine</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF7</td>
<td>20.9 ± 6.3</td>
<td>17.0 ± 6.5</td>
<td>23.6 ± 7.8</td>
<td></td>
</tr>
<tr>
<td>MCF7(^b)</td>
<td>17.8 ± 0.4</td>
<td>—</td>
<td>12.3 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>DU145</td>
<td>28.8 ± 3.9</td>
<td>26.9 ± 6.9</td>
<td>13.9 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td>76.5 ± 4.6</td>
<td>44.1 ± 9.9</td>
<td>43.1 ± 6.2</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Values are means ± standard deviation (SD) of two to three experiments performed in triplicate. \(^b\) From ref. 25.
ligands can induce the tumor cell death by multiple signaling pathways.\textsuperscript{15} 10 \( \mu \)M siramesine decreased the expression levels of cyclin D1, which are responsible for progression through the G\(_1\) phase in MDA-MB-435 cells in a time-dependent manner. Thus, siramesine may block G\(_1\)-phase progression by decreasing cyclin D1 expression. In addition, siramesine also mainly decreased cyclin B1 and pRb in MDA-MB-435 cells. The investigation of the detailed mechanism in which compounds 1a and 1b impair the G\(_1\) phase of the cell cycle progression in DU145 cells is in progress.

3. Conclusion

We have developed a series of indole-based \( \sigma_2 \) receptor ligands derived from siramesine. Structure–affinity relationship analyses indicated the high importance of the indole moiety and \( \sigma_2 \) preferred group to improve the selectivity for \( \sigma_2 \) receptors. In the MTT experiments, compound 1b displayed notable and comparable antiproliferative effects to compound 1a and siramesine in DU145 cells and exhibited the highest activity in MCF7 cells. Cell cycle analysis by flow cytometry demonstrated that compounds 1a, 1b and siramesine impaired the cell cycle progression predominantly at the G\(_1\) phase in DU145 cells. The indole-based compound 1b with the 5,6-dimethoxyisoindoline moiety shows potential as an antitumor agent and warrants further evaluation.

4. Experiments

4.1. Chemistry

All the chemicals or reagents were purchased from chemical suppliers and used without further purification unless otherwise noted. NMR spectra were recorded on a Varian Inova-400 spectrometer or on a Bruker Avance III NMR spectrometer at 400 (\( ^1 \)H), 376 (\( ^19 \)F), and 100 MHz (\( ^13 \)C), respectively. The chemical shifts of the spectra were reported in parts per million (ppm) with tetramethylsilane (TMS) as an internal standard, and coupling constants are reported in Hertz (Hz). MS spectra were obtained using a Xevo TQ-S spectrometer (Waters) with electrospray (ESI) as the ionization method. High-resolution mass spectrometry (HRMS) was performed on a LCT Premier XE ESI-TOF mass spectrometry instrument (Waters, USA). Chromatographic separations were carried out using Merck Silica Gel 60 (63–200 \( \mu \)m). TLC detections were carried out using Merck Silica Gel 60 F\(_{254}\) sheets and TLCs were developed by visualization under UV light (\( \lambda = 254 \)nm). Microanalyses were carried out using a Hekatech CHNS elemental analyser EuroEA 3000 or an Elementar 240C device (PerkinElmer). The HPLC analyses were performed using an AGILENT 1100 HPLC (Agilent Technologies, USA) equipped with a DAD detector. Analyses were carried out using a Nucleodur C18 ISIS column (250 x 4 mm, 5 \( \mu \)m, Macherey-Nagel, Germany) with an eluent of acetonitrile/H\(_2\)O (0.1% TFA) (30:70) at a flow rate of 0.5 mL min\(^{-1}\). Cell cycle analysis was performed using a BD FACSCalibur flow cytometer (BD Biosciences, California, USA), and DNA distributions were analyzed using ModeFit LT MacIntel (Verity Software House, Topsham, ME, USA).

4.1.1. tert-Butyl 4-{2-(2-fluoroethoxy)ethoxy} phenyl)piperazine-1-carboxylate (Boc-13).

Compounds 7 (168 mg, 0.60 mmol) and 10 (190 mg, 0.72 mmol) were dissolved in CH\(_3\)CN (25 mL), followed by addition of K\(_2\)CO\(_3\) (124 mg, 0.90 mmol) and NaI (27 mg, 0.18 mmol). The mixture was heated under reflux and stirred overnight. After cooling and filtration, the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (PE (petroleum ether) : EE (ethyl acetate) = 1:1) to afford Boc-13 (203 mg, 92%). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 6.92–6.80 (m, 4H), 4.59 (dt, \( J = 47.7, 4.2 \) Hz, 2H), 4.10 (t, \( J = 5.8 \) Hz, 2H), 3.86 (t, \( J = 5.0 \) Hz, 2H), 3.62 (dt, \( J = 33.7, 4.2 \) Hz, 2H), 3.57 (t, \( J = 5.0 \) Hz, 4H), 3.00 (t, \( J = 4.8 \) Hz, 4H), 1.48 (s, 9H).

4.1.2. tert-Butyl 4-{2-[2-(2-fluoroethoxy)ethoxy]phenyl)piperazine-1-carboxylate (Boc-14). The procedure described for the synthesis of Boc-13 was applied to compounds 7 (152 mg, 0.55 mmol) and 11 (188 mg, 0.61 mmol) to afford Boc-14 (171 mg, 75%). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 6.91–6.80 (m, 4H), 4.57 (dt, \( J = 47.7, 4.2 \) Hz, 2H), 4.09 (t, \( J = 47.7, 4.2 \) Hz, 2H)}
4.8 Hz, 2H), 3.84 (t, 𝑗 = 4.8 Hz, 2H), 3.81–3.70 (m, 6H), 3.57 (t, 𝑗 = 5.0 Hz, 4H), 3.00 (t, 𝑗 = 4.8 Hz, 4H), 1.48 (s, 9H).

4.1.3. tert-Butyl 4-[3-(2-fluoroethoxy)phenyl]piperazine-1-carboxylate (Boc-15). The procedure described for the synthesis of Boc-13 was applied to compounds 8 (414 mg, 1.49 mmol) and 9 (188 mg, 2.90 mmol) to afford Boc-15 (311 mg, 64%). H NMR (400 MHz, CDCl3): δ 7.18 (t, 𝑗 = 8.2 Hz, 1H), 6.57 (d, 𝑗 = 8.2 Hz, 1H), 6.51 (s, 1H), 6.44 (d, 𝑗 = 8.1 Hz, 1H), 4.74 (dt, 𝑗 = 47.4, 4.1 Hz, 2H), 4.20 (dt, 𝑗 = 27.9, 4.1 Hz, 2H), 3.57 (t, 𝑗 = 4.7 Hz, 4H), 3.14 (t, 𝑗 = 4.4 Hz, 4H), 1.48 (s, 9H).

4.1.4. General procedure for the syntheses of compounds 12, 13, 14, and 15. The Boc-protected group of compound Boc-12, Boc-13, Boc-14, or Boc-15 was cleaved using TFA in dichloromethane solution at 0 °C for 1 h. Compounds 12–15 were obtained in nearly quantitative yields and used for the next step without further purification.

4.2. General procedure for the syntheses of 1a–1g

3-(4-Bromobutyl)-1-(4-fluoroaryl)indole (6) and the respective amine (12–18) were dissolved in CH2CN, followed by addition of K2CO3. The mixture was heated under reflux and stirred overnight. After cooling and filtration, the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (PE: EtOAc = 1:1) to afford 1a–1g.

2.4-[1-(4-Fluorophenyl)-1H-indol-3-yl]butyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (1a). The synthesis of 1a was similar to that reported in the literature.25 3-(4-Bromobutyl)-1-(4-fluoroaryl)indole (18) (321 mg, 0.92 mmol), 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (5) (176 mg, 0.77 mmol) and K2CO3 (233 mg, 1.69 mmol) dissolved in CH2CN (25 mL) afforded 1a (185 mg, 44%) as light-yellow oil. H NMR (400 MHz, CDCl3): δ 7.88–7.66 (m, 1H), 7.50–7.40 (m, 3H), 7.28–7.13 (m, 4H), 7.10 (s, 1H), 6.60 (s, 1H), 6.52 (s, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.57 (s, 2H), 2.90–2.80 (m, 4H), 2.72 (t, 𝑗 = 5.9 Hz, 2H), 2.62–2.54 (m, 2H), 1.89–1.82 (m, 2H), 1.82–1.70 (m, 2H); 13C NMR (100 MHz, CDCl3): δ 160.7, 147.5, 147.2, 136.3, 136.0, 128.9, 126.1, 126.0, 125.9, 125.6 (2C), 122.7, 122.0, 121.9, 120.0, 120.0, 120.0, 119.4, 119.1, 116.6, 116.3 (2C), 110.4, 57.7, 50.4 (2C), 41.9, 33.5 (2C), 27.3, 24.5, 23.6; MS (ESI+); m/z = calc. for C28H29FNO3 [M + H]+ 452.3, found 452.7. HRMS (ESI): m/z calced. for C28H29FNO3 [M + H]+, 452.2502, found 452.2505. Anal. calced. for C28H29FNO3-HCl/1H2O: C 73.16, N 8.53, H 6.45; found: C 73.19, N 8.48, H 6.69.

2.4. 3-(4-[2-(2-Fluoroethoxy)ethoxy]phenyl)piperazin-1-yl]butyl-1-(4-fluorophenyl)indole (1H, 1d). Compounds 6 (180 mg, 0.52 mmol) and 12 (132 mg, 0.59 mmol) and K2CO3 (680 mg, 4.92 mmol) in CH2CN (25 mL) afforded 1d as white solid (210 mg, 83%). H NMR (400 MHz, CDCl3): δ 7.65 (d, 𝑗 = 7.6 Hz, 1H), 7.48–7.40 (m, 3H), 7.24–7.12 (m, 4H), 7.08 (s, 1H), 6.93–6.83 (m, 4H), 4.71 (dt, 𝑗 = 47.4, 4.1 Hz, 2H), 4.16 (dt, 𝑗 = 27.9, 4.2 Hz, 2H), 3.10 (t, 𝑗 = 4.8 Hz, 4H), 2.84 (t, 𝑗 = 7.4 Hz, 2H), 2.61 (t, 𝑗 = 4.7 Hz, 4H), 2.46 (t, 𝑗 = 7.6 Hz, 2H), 1.86–1.75 (m, 2H), 1.72–1.61 (m, 2H); 13C NMR (100 MHz, CDCl3): δ 160.8, 152.5, 145.6, 136.4, 136.1, 128.9, 125.9 (2C), 125.1, 122.5, 119.8, 119.4, 118.0 (2C), 117.8, 116.4 (2C), 115.5 (2C), 110.2, 82.1, 67.7, 58.6 (2C), 53.4, 50.4 (2C), 28.0, 26.9, 25.0; MS (ESI+); m/z = calcd. for C50H53F2N2O3 [M + H]+, 499.3, found 499.5; Anal. calcd. for C50H53F2N2O3 (489.60): C 73.60, H 6.79, N 5.88; found: C 73.79, H 7.12, N 8.26.

2.5 3-[4-[4-[2-(2-Fluoroethoxy)ethoxy]phenyl]piperazin-1-yl]butyl-1-(4-fluorophenyl)indole (1e). Compounds 6 (488 mg, 1.41 mmol) and 13 (138 mg, 0.51 mmol) and K2CO3 (84 mg, 0.61 mmol) in CH2CN (25 mL) afforded 1e as light-yellow oil (43 mg, 16%). H NMR (400 MHz, CDCl3): δ 7.65 (t, 𝑗 = 7.6 Hz, 1H), 7.50–7.40 (m, 3H), 7.24–7.13 (m, 4H), 7.08 (s, 1H), 6.91–6.82 (m, 4H), 4.58 (dt, 𝑗 = 47.7, 4.1 Hz, 2H), 4.09 (t, 𝑗 = 4.8 Hz, 2H), 3.89–3.75 (m, 4H), 3.10 (t, 𝑗 = 4.7 Hz, 4H), 2.84 (t, 𝑗 = 7.4 Hz, 2H), 2.62 (t, 𝑗 = 4.5 Hz, 4H), 2.46 (t, 𝑗 = 7.6 Hz, 2H), 1.85–1.74 (m, 2H), 1.72–1.62 (m, 2H); 13C NMR (100 MHz, CDCl3): δ 160.8, 152.8, 146.0, 136.3, 136.0, 128.9, 125.8, 125.8 (2C), 125.1, 122.5, 119.6 (2C), 118.0 (2C), 117.8, 116.4, 115.4 (2C), 110.2, 83.2, 70.6, 70.1, 68.0, 58.6 (2C), 53.4, 50.5 (2C), 28.0, 26.8, 25.0. HRMS (EI): m/z calcd. for C29H22F2N2O3 [M + H]+ 534.2932, found 534.296.

2.6. 3-[4-[4-[2-(2-Fluoroethoxy)ethoxy]phenyl]piperazin-1-yl]butyl-1-(4-fluorophenyl)indole (1f). Compounds 6 (142 mg, 0.41 mmol) and 14 (129 mg, 0.41...
mmol) and K₂CO₃ (70 mg, 0.51 mmol) in CH₂CN (25 mL) afforded 1f (68 mg, 29%). ¹H NMR (400 MHz, CDCl₃): δ 7.65 (d, J = 7.6 Hz, 1H), 7.47–7.40 (m, 3H), 7.24–7.14 (m, 4H), 7.08 (s, 1H), 6.91–6.82 (m, 4H), 4.56 (dt, J = 47.7, 4.1 Hz, 2H), 4.08 (t, J = 4.8 Hz, 2H), 3.83 (t, J = 4.8 Hz, 2H), 3.80–3.68 (m, 6H), 3.10 (t, J = 4.7 Hz, 4H), 2.84 (t, J = 7.4 Hz, 2H), 2.61 (t, J = 4.8 Hz, 4H), 2.46 (t, J = 7.6 Hz, 2H), 1.85–1.75 (m, 2H), 1.74–1.63 (m, 2H); ¹³C NMR (100 MHz, CDCl₃): δ 160.7, 152.9, 145.9, 136.3, 136.0, 128.9, 125.8, 125.7, 125.1, 122.4, 119.5 (2C), 118.0 (2C), 117.7, 116.3 (2C), 115.3 (2C), 110.1, 83.1, 70.8, 70.5, 70.3, 69.9, 67.8, 58.6, 53.4 (2C), 50.5 (2C), 27.9, 26.8, 24.9; MS (ESI⁺): m/z = calc. for C₁₉H₁₃F₂N₂O₂ [M + H⁺] 297.3, found 297.2. Anal. calcd. for C₁₉H₁₃F₂N₂O₂: C 69.1, H 8.5, N 8.0, F 9.4.

4.2.7. 3-(4-[(2-Fluoroethoxy)phenyl]piperazin-1-yl)butyl-1-(4-fluorophenyl)-1H-indole (1g). Compounds 6 (259 mg, 0.75 mmol) and 15 (168 mg, 0.75 mmol) and K₂CO₃ (132 mg, 0.97 mmol) in CH₂CN (25 mL) afforded 1g as light-yellow oil (130 mg, 35%). ¹H NMR (400 MHz, CDCl₃): δ 7.68–7.61 (m, 1H), 7.47–7.39 (m, 3H), 7.22–7.11 (m, 5H), 7.07 (s, 1H), 6.59–6.53 (m, 1H), 6.49 (t, J = 2.3 Hz, 1H), 6.44–6.36 (m, 1H), 4.71 (dt, J = 47.1, 4.2 Hz, 2H), 4.17 (dt, J = 27.8, 4.2 Hz, 2H), 3.18 (t, J = 5.2 Hz, 4H), 2.83 (t, J = 7.4 Hz, 2H), 2.57 (t, J = 5.0 Hz, 4H), 2.43 (t, J = 7.6 Hz, 2H), 1.87–1.71 (m, 2H), 1.73–1.60 (m, 2H); ¹³C NMR (100 MHz, CDCl₃): δ 160.7, 159.4, 152.7, 136.3, 136.0, 129.7, 128.8, 128.5 (2C), 125.1, 122.4, 119.8, 119.3, 117.7, 116.3 (2C), 110.1, 109.3, 104.6, 103.2, 82.0, 67.0, 58.7, 53.2 (2C), 48.9 (2C), 27.9, 26.8, 24.9; ¹⁹F NMR (376 MHz, CDCl₃): δ −120.7, −228.8; MS (ESI⁺): m/z = calc. for C₁₉H₁₃F₂N₂O₂ [M + H⁺] 390.3, found 390.5; HRMS (EI): m/z calc. for C₁₉H₁₃F₂N₂O₂ [M + H⁺] 390.2670, found 390.2673. Anal. calcd. for C₁₉H₁₃F₂N₂O₂H₂O: C 57.98, H 6.95, N 8.28; found: C 71.18, H 6.87, N 8.01.

4.2.8. 3-(4-[(2-Fluoroethyl)silyl]oxy)butyl-1H-indole (20). To a solution of 19 (2.10 g, 11.1 mmol) in CH₂Cl₂ (40 mL), TBDMSCl (2.06 g, 13.7 mmol) and imidazole (1.43 g, 21.0 mmol) were added. The mixture was stirred at room temperature for 2 h. After filtration, the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (PE:EE = 1:5) to afford 20 (2.80 g, 84%). ¹H NMR (400 MHz, CDCl₃): δ 7.87 (s, 1H), 7.58 (d, J = 7.9 Hz, 1H), 7.32 (d, J = 8.1 Hz, 1H), 7.20–7.13 (m, 1H), 7.12–7.04 (m, 1H), 6.95 (s, 1H), 3.63 (t, J = 6.5 Hz, 2H), 2.75 (t, J = 7.5 Hz, 2H), 1.80–1.68 (m, 2H), 1.65–1.58 (m, 2H), 0.87 (s, 9H).

4.2.9. 2-(3-(4-[(2-Fluoroethyl)dimethylsilyl]oxy)butyl)-1H-indol-1-yl)ethanol (21). To a solution of compound 20 (1.88 g, 6.19 mmol) in DMF, 2-bromoethanol (1.23 g, 9.92 mmol) and NaH (240 mg, 10.0 mmol) were added. The mixture was stirred at 110 °C overnight. After cooling, the crude product was extracted with ethyl acetate, dried with anhydrous MgSO₄, and purified by silica gel column chromatography (PE:EE = 5:1) to afford 21 (805 mg, 37%). ¹H NMR (400 MHz, CDCl₃): δ 7.57 (d, J = 7.9 Hz, 1H), 7.30 (d, J = 8.2 Hz, 1H), 7.20–7.15 (m, 1H), 7.10–7.05 (m, 1H), 6.91 (s, 1H), 4.21 (t, J = 5.3 Hz, 2H), 3.92 (t, J = 5.3 Hz, 2H), 3.62 (t, J = 6.4 Hz, 2H), 2.73 (t, J = 7.5 Hz, 2H), 1.79–1.65 (m, 2H), 1.65–1.53 (m, 2H), 0.87 (s, 9H).

4.2.10. 2-(3-{4-[[[(2-Fluoroethyl)dime-}

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The procedure described for the synthesis of 32 was applied to DAST (205 mg, 1.28 mmol) and 31 (360 mg, 1.16 mmol) to afford 33 (315 mg, 87%). 1H NMR (400 MHz, CDCl3); δ 7.63 (d, J = 7.9 Hz, 1H), 7.33 (d, J = 8.2 Hz, 1H), 7.28–7.22 (m, 1H), 7.17–7.11 (m, 1H), 6.92 (s, 1H), 4.63 (dt, J = 47.3, 5.9 Hz, 2H), 4.12 (t, J = 6.8 Hz, 2H), 3.38 (t, J = 6.5 Hz, 2H), 2.92 (t, J = 7.5 Hz, 2H), 2.19–2.05 (m, 2H), 2.04–1.96 (m, 2H), 1.90–1.81 (m, 2H).

2.1.2. 3-(1-Halogen-3-yl)propan-1-ol (29). The procedure described for the synthesis of 28 was applied to 27 (3.00 g, 15.8 mmol) and LiAlH4 (2.93 g, 77.2 mmol) to afford alcohol 29 (2.18 g, 79%). 1H NMR (400 MHz, CDCl3); δ 7.93 (s, 1H), 6.75–7.55 (m, 1H), 7.37–7.30 (m, 1H), 7.23 (s, 1H), 7.19–7.14 (m, 1H), 7.12–7.07 (m, J = 8.0, 1H), 6.97 (s, 1H), 3.71 (t, J = 6.4 Hz, 2H), 2.90–2.78 (m, 2H), 2.04–1.93 (m, 2H).

2.1.3. 1-(4-Bromobutyl)-1H-indol-3-yl)ethanol (30). Under ice bath and argon atmosphere, a solution of 26 (1.00 g, 5.71 mmol) in THF (60 mL) was added to a solution of LiAlH4 (642 mg, 17.1 mmol) in THF (40 mL). The mixture was stirred for 4 h at room temperature, followed by addition of ethanolic alcohol until no H2 was formed. Then 4 M hydrochloric acid was added to adjust the pH to 5. After filtration, the solution was concentrated under reduced pressure. The crude product was extracted with ethyl acetate, dried with anhydrous MgSO4, and purified by silica gel column chromatography (PE:EE = 25:1) to afford alcohol 30 (728 mg, 1.16 mmol). 1H NMR (400 MHz, CDCl3); δ 7.60 (d, J = 7.9 Hz, 1H), 7.29 (d, J = 8.2 Hz, 1H), 7.25–7.14 (m, 1H), 7.12–7.07 (m, 1H), 6.87 (s, 1H), 4.08 (t, J = 6.8 Hz, 2H), 3.70 (t, J = 6.4 Hz, 2H), 3.35 (t, J = 6.5 Hz, 2H), 2.84 (t, J = 7.5 Hz, 2H), 2.03–1.90 (m, 4H), 1.86–1.77 (m, 2H).

2.1.4. 1-(4-Bromobutyl)-3-(2-fluoroethyl)-1H-indole (32). Under argon atmosphere (–78 °C), a solution of DAST (352 mg, 2.18 mmol) in CH2Cl2 (20 mL) was added into a solution of 30 (538 mg, 1.82 mmol) in CH2Cl2 (20 mL). The mixture was stirred for 2 h, followed by addition of saturated sodium hyposulfite to quench the reaction. The crude product was extracted with ethyl acetate, dried with anhydrous MgSO4, and purified by silica gel column chromatography (PE:EE = 10:1) to afford 32 (315 mg, 58%). 1H NMR (400 MHz, CDCl3); δ 7.58 (d, J = 7.9 Hz, 1H), 7.30 (d, J = 8.2 Hz, 1H), 7.23–7.18 (m, 1H), 7.14–7.08 (m, 1H), 6.96 (s, 1H), 4.67 (dt, J = 47.2, 6.7 Hz, 2H), 4.11 (t, J = 6.9 Hz, 2H), 3.36 (t, J = 6.5 Hz, 2H), 3.15 (dt, J = 22.2, 6.5 Hz, 2H), 2.03–1.95 (m, 2H), 1.90–1.79 (m, 2H).
was removed under reduced pressure. The residue was purified by silica gel column chromatography (PE: EE = 1:5) to afford 3c (40 mg, 41%). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.58 (d, \(J = 7.9\) Hz, 1H), 7.33 (d, \(J = 8.2\) Hz, 1H), 7.29–7.16 (m, 4H), 7.15–7.07 (m, 2H), 6.99 (s, 1H), 5.06 (s, 2H), 4.66 (dd, \(J = 47.2, 6.7\) Hz, 2H), 4.10 (t, \(J = 7.1\) Hz, 2H), 3.16 (dt, \(J = 21.9, 6.7\) Hz, 2H), 2.81 (d, \(J = 11.0\) Hz, 2H), 2.47–2.29 (m, 4H), 2.03–1.81 (m, 4H), 1.75 (d, \(J = 12.5\) Hz, 2H), 1.62–1.52 (m, 2H); \(^13\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 181.0, 139.1, 136.4, 128.1, 127.8, 126.3, 121.8, 121.3, 121.0, 119.1, 109.8, 109.8, 109.7, 84.9, 83.3, 73.1, 50.4, 38.2, 26.6, 26.8, 24.8; \(^19\)F NMR (376 MHz, CDCl\(_3\)): \(\delta\) –106.7; MS (ESI+): \(m/z\) calculated for \(\text{C}_{18}\text{H}_{17}\text{BrIN} [\text{M + H}]^+\) 463.1, found 463.1.

4.2.25. 1-[4-(4-Iodophenyl)-1H-indol-3-yl]butyl-1-ol (34). Compound 19 (595 mg, 3.14 mmol), 1,4-diiodobenzene (780 mg, 2.36 mmol), \(\text{K}_2\text{CO}_3\) (3.12 g, 23.6 mmol), a catalytic amount of copper powder, and 18-crown-6 were added into 25 mL of DMF. The mixture was stirred at 120 °C for 5 h. After cooling and filtration, the crude product was extracted with ethyl acetate and washed with 1 M HCl and saturated NaCl solution. The organic layer was dried over \(\text{MgSO}_4\), and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (PE : EE = 4 : 1) to afford 34 (208 mg, 23%). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.76–7.72 (m, 2H), 7.57 (t, \(J = 7.6\) Hz, 1H), 7.46 (t, \(J = 8.2\) Hz, 1H), 7.18–7.08 (m, 4H), 7.03 (s, 1H), 3.64 (t, \(J = 6.5\) Hz, 2H), 2.76 (t, \(J = 7.4\) Hz, 2H), 1.80–1.73 (m, 2H), 1.67–1.60 (m, 2H); \(^13\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 139.6, 138.6 (2C), 135.8, 129.2, 125.7, 124.6, 122.7 (2C), 120.1, 119.4, 118.3, 110.3, 90.0, 62.9, 32.6, 26.1, 24.8; MS (ESI+): \(m/z\) calculated for \(\text{C}_{18}\text{H}_{19}\text{NO} [\text{M + H}]^+\) 329.2, found 329.2.

4.2.26. 2-[4-[1-(4-Fluorophenyl)-1H-indol-3-yl]butyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (5). Compound 36 (279 mg, 1.08 mmol), 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (153 mg, 0.67 mmol), and \(\text{K}_2\text{CO}_3\) (220 mg, 1.59 mmol) were added into 25 mL of \(\text{CH}_3\text{CN}\). The mixture was stirred at 80 °C for 4 h. After the solvent was removed under reduced pressure, the residue was purified by silica gel column chromatography (\(\text{CH}_3\text{Cl}_2: \text{CH}_2\text{Cl}_2\) = 4 : 1) to afford 5 (153 mg, 62%). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.98–7.90 (m, 2H), 7.09–7.02 (m, 2H), 6.54 (s, 1H), 6.47 (s, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 3.52 (s, 2H), 2.96 (t, \(J = 7.2\) Hz, 2H), 2.77 (t, \(J = 5.8\) Hz, 2H), 2.67 (t, \(J = 5.9\) Hz, 2H), 2.52 (t, \(J = 7.3\) Hz, 2H), 1.82–1.71 (m, 2H), 1.71–1.61 (m, 2H); \(^13\)C NMR (101 MHz, CDCl\(_3\)): \(\delta\) 198.6, 165.5, 147.5, 147.2, 133.3, 130.6 (2C), 126.5, 126.1, 115.6 (2C), 111.3, 109.4, 57.8, 55.9, 55.9, 50.9, 38.2, 28.6, 26.6, 22.3; \(^19\)F NMR (376 MHz, CDCl\(_3\)): \(\delta\) –106.7; MS (ESI+): \(m/z\) calculated for \(\text{C}_{22}\text{H}_{14}\text{FNO} [\text{M + H}]^+\) 372.2, found 372.197, calculated for \(\text{C}_{22}\text{H}_{14}\text{FNO} [\text{M + H}]^+\) 372.197, found 372.197.

4.3 In vitro radioligand competition studies

Competition assays of \(\sigma_1\) and \(\sigma_2\) receptors were performed as previously reported in the literature.28,29 The detailed procedures are provided in the ESI†.

4.4 Cell culture and antiproliferative assay

The cancer cell lines MCF7 (human mammary carcinoma), DU145 (human prostate carcinoma) and C6 (rat glioma) were routinely cultured in Beijing Normal University. The MTT assay was used to determine the antiproliferative activity of compounds 1a and 1b and siramesine in these cell lines as described previously.30,31 The procedures are shown in the ESI†.

4.5 Flow cytometry cell cycle analysis studies

1a, 1b and siramesine were cultured in DU145 cell line for 24 h to examine cell cycle arrest as described previously.32 The detailed procedures are shown in the ESI†.
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Notes and references