Synthesis and evaluation of novel, selective, functionalized γ-butyrolactones as sigma-2 ligands

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Abstract
The sigma-2 (σ2) receptor was discovered nearly 40 years ago and was recently identified as the Transmembrane Protein 97 (TMEM97, also known as MAC30 (Meningioma-associated protein)). Aberrant σ2 activity has been linked to diseases and conditions such as schizophrenia, Alzheimer’s disease, neuropathic pain, traumatic brain injury, and cancer. The utility of σ2 as a therapeutic target is currently under investigation in numerous laboratories. Herein, we report on the synthesis and evaluation of a series of novel, functionalized γ-butyrolactones that are potent σ2 receptor ligands.

Graphical Abstract

Keywords Sigma-2 · Sigma-1 · γ-butyrolactone · Sigma receptor

Introduction
The discovery and characterization of the sigma receptors began in 1976 with W. R. Martin et. al.’s exploration of the impact of opioids on chronic spinal dogs. In these studies, they observed that the opioids morphine (1), ketocyclazocine, (2), and (rac)-SKF-100047 (3) (Fig. 1) produced different responses and hypothesized that each compound was interacting with a different receptor. They designated these receptors the μ-opioid receptor (morphine type, MOR), the κ-opioid receptor (ketocyclazocine type, KOR), and the σ-opioid receptor (SKF-100047 like) [1]. Follow-up studies conducted in the early 1980s using the individual enantiomers of SKF-100047 (3) demonstrated that the two enantiomers elicited physiological responses through different biochemical pathways. The opioid-mediated physiological response observed with (-)-SKF-100047 was determined to be the result of interactions with MOR and KOR. In addition, these studies revealed that (+)-SKF-100047 interacts with a previously unknown, non-opioid receptor that was designated the sigma receptor (σR) [2, 3]. In 1993, W. D. Bowen et. al. determined that there were two sub-types of this receptor, which were designated sigma-1 (σ1) and sigma-2 (σ2) [4]. Three years later, Glossman H, et.al. cloned and expressed the mammalian σ1 receptor in yeast cells [5], and in 2016 a crystal structure of the human σ1 receptor was reported [6]. To date, there is no known natural ligand for this receptor.

The nature and function of the σ2 receptor, on the other hand, remains the subject of intense research, but some
Results and discussion

Synthesis of substituted γ-butyrolactones was conducted as shown in Scheme 1 utilizing novel methods developed in our laboratory. The synthesis of these compounds begins with the known alkenyl alcohols (5a, 5b), which were protected as the benzyl ethers using benzyl bromide, NaH, and tetrabutylammonium iodide in THF. The alkenes were then converted to the corresponding epoxide (5c, 5d) with mCPBA. Ring opening of epoxide (6) with the enolate of 2-ethyl-N,N-dimethylbutanamide via deprotonation with LDA provided the intermediate alcohol, which cyclized to form the γ-butyrolactone ring (7) in the presence of trifluoroacetic acid. Removal of the benzyl protecting group via hydrogenation in the presence of palladium on carbon provided the corresponding alcohol, which was then reacted with tosyl chloride in the presence triethylamine to provide α-3. Reaction of α-3 with amines in refluxing THF provided the final target molecules (9). Alternatively, the previously reported γ-butyrolactone alcohol (10) was reacted with tosyl chloride in the presence triethylamine, followed by reaction with amines in refluxing THF to provide the final target molecules (9).

Tables 1 and 2 describe the in vitro binding (Ki at σ1 and σ2), physicochemical properties (MW, TPSA, LogP, solubility), and mouse liver microsomal (MLM) stability. All of the compounds are consistent with Lipinski’s rule of 5 (MW, cLogP, TPSA) and have acceptable water solubility. In addition, TPSA and cLogP of the compounds are in a range that is indicative of BBB penetration. While the majority of compounds have low MLM stability, we were able to identify 3 compounds with MLM T1/2 values > 10 min. Stability in MLM is an important factor, as future in vivo studies will be performed in rodents.

The structure activity relationship analysis of this series of compounds begins with an examination of the impact of length of the linker chain between the two rings (9a–9c). As indicated in Table 1, compounds with chain lengths of 2 (9a), 3 (9b), and 4 (9c) methylene units bind to σ2 with moderate to high potency (σ1 Ki = 82, 7.7, and 12 nM), but selectivity versus σ1 was low (σ1 Ki = 138.31, and 5.5 nM). Decreasing the size of the dialkyl side chains of the γ-butyrolactone (9d) lead to a nearly 10-fold decrease in σ2 potency (Ki = 753 nM) relative to (9a).

We next examined the impact of changes to the aryl piperazine region. Replacing the phenyl piperazine of (9a) with the corresponding 1-naphthyl piperazine (9e) led to a moderate increase in σ2 potency (Ki = 32 nM), as well as increase in selectivity over (σ1 Ki = 2167 nM) in comparison to (9a). Notably, this compound is the least soluble analog (sol = 47 μM), which is almost certainly the result of increased aromatic character of the aryl piperazine region. Employing heteroaromatic replacements for the aryl piperazine produced mixed results. While the 4-pyridine analog (4) is a moderate affinity σ2 ligand (Ki = 142 nM), with a high degree selectivity for this target over σ1 (Ki = 10,000 nM), the corresponding...
4-pyrimidine analog (9f) had limited capacity to bind to \(\sigma_2\) (K\(_I\) = 10,000 nM) and low affinity for \(\sigma_1\) (K\(_I\) = 1017 nM).

Incorporation of potential piperazine bioisosteres produced compounds with high \(\sigma_2\) potency and moderate to low selectivity versus \(\sigma_1\). Specifically, the...
homopiperazine analog (9g), 2,6-diazaspiro[3.3]heptane analog (9h), and octahydropyrrolo[3,4-c]pyrrole analog (9i) are all potent $\sigma_2$ binders ($K_i = 6.8$, 53, and 3.5 nM) with low to moderate selectivity over $\sigma_1$ ($K_i = 17$, 12, 31 nM). Interestingly, the combination of the octahydropyrrolo[3,4-c]pyrrole bioisostere and 4-pyridine substituent (9j) led to improved $\sigma_2$ potency ($K_i = 29$ nM) versus the piperazine analog (4), but decreased $\sigma_1$ selectivity ($K_i = 142$ nM). In addition, the high level of MLM stability observed with (4) was maintained with (9j) (MLM $T_{1/2} = 60$ min).

We next turned our attention to replacing the aryl piperazine moiety with tetrahydroisoquinolines. The unsubstituted tetrahydroisoquinoline analog (9k) is a potent $\sigma_2$ binder ($K_i = 6.1$ nM), with moderate $\sigma_1$ selectivity ($K_i = 125$ nM). Incorporating halogens in the 7-position of the tetrahydroisoquinoline nucleus produced compounds (9l-9n) with potency similar to that observed with the

### Table 2 In vitro screening and physicochemical properties data for (9k)–(9q)

| Entry | R<sup>1</sup> | R<sup>2</sup> | N | A | MW | TPSA | cLogP | $\sigma_2$ | $\sigma_1$ | $\sigma_2/\sigma_1$ ratio | MLM | Sol (µM) |
|-------|-------------|-------------|---|---|----|------|-------|----------|----------|---------------------|-----|----------|
| 9k    | Et          | Et          | 1 |   | 301 | 30   | 4     | 6.1      | 125      | 20.5                | 2.0 | 194      |
| 9l    | Et          | Et          | 1 |   | 319 | 30   | 4     | 7.4      | 68       | 9.2                 | 2.0 | 151      |
| 9m    | Et          | Et          | 1 |   | 336 | 30   | 5     | 2.8      | 59       | 21.1                | 2.0 | 91       |
| 9n    | Et          | Et          | 1 |   | 380 | 30   | 5     | 8.9      | 4.7      | 0.5                 | 2.0 | 111      |
| 9o    | Et          | Et          | 1 |   | 302 | 42   | 3     | 10000    | 10000    | 1.0                | 4.7 | 191      |
| 9p    | Et          | Et          | 1 |   | 302 | 42   | 3     | 10000    | 1156     | 0.1                | 2.9 | 192      |
| 9q    | Et          | Et          | 1 |   | 302 | 42   | 3     | 277      | 10000    | 36.1               | 3.3 | 194      |

*Sigma-2 assays: Conducted with PC12 membrane preparations. Radioligand: $[^3]$H-DTG, $K_d = 9.9$ nM, Reference standard: Haloperidol, $K_i = 13.9$ nM

*Sigma-1 assays: Conducted with HEK293 membrane preparations. Radioligand: $[^3]$H-Pentazocine, $K_d = 6.5$ nM, Reference standard: Haloperidol, $K_i = 3.54$ nM
Conclusions

In summary, a series of substituted lactones with drug-like physicochemical properties (MW, TPSA, cLogP) have been investigated as potential selective σ₂ ligands. We have determined that increasing the length of the linker chain from two (9a) to four carbons (9c) leads to increase σ₂ potency, but selectivity over σ₁ decreases. In addition, we have demonstrated that σ₂ potency and selectivity for σ₂ over σ₁ is maintained when the phenyl ring of the aryl piperazine is replaced with 1-naphthylene (9e) or 4-pyridine (4), but replacement with a 4-pyrimidine (9f) leads to a significant lose of σ₂ potency. Replacement of the piperazine ring with bioisosteres such as homopiperazine (9g) 2,6-diazaspiro[3.3]heptane (9h), and octahydropyrrolo[3,4-c]pyrrole (9i) was tolerated with respect to σ₂ potency, but σ₁ selectivity was substantially decreased. Incorporation of tetrahydroisoquinolines (9k–9n) in place of the aryl piperazine also produced high potency σ₂ binders, but naphthyridine analogs examined to date had limited σ₂ binding capacity. We anticipate these studies will help us further evaluate the potential value of this series for the identification of novel therapeutic agents for the treatment of diseases associated with abnormal σ₂ activity. Future studies will be focused on the identification of highly potent, selective, novel σ₂ binders that have improved MLM stability.

Experimental methods and materials

Reagents were purchased from Fisher Scientific, VWR International, Sigma Aldrich, and Combi-Blocks, Inc. Chromatographic purification of compounds (normal phase and reverse phase) were carried out on a Teledyne Isco CombiFlash RF system. H-NMR spectra were obtained on a Bruker 400-MHz NMR. Chemical shift values (δ values) were reported in ppm relative to TMS. For multiplicity, s = singlet, d = doublet, t = triplet, m = multiplet. Purity (%) and mass spectral data were determined with a Waters Agilent 1200 HPLC/MS (Zorbax SB-C18, 2.1 × 30 mm, 3.5 μm, 100% water/0.1% formic acid to 100% acetonitrile/0.1% formic acid over 4.0 min, 1.0 mL/min.) with a diode array detector from 210–400 nm and Agilent 6100 quadrupole MS. All compounds were purified to 95% purity or greater as determined by HPLC/MS and 1H-NMR. Melting points were recorded on a capillary melting point apparatus.

Preparation of ((pent-4-en-1-yloxy)methyl)benzene (5c)

To a dry round bottom flask under nitrogen was added 1.4 g of 60% NaH dispersion (0.834 g NaH, 34.5 mmol, 2eq), followed by ~200 mg of tetrabutylammonium iodide. 18 mL of dry THF was added and the reaction was cooled to 0 °C using an ice bath. Pent-4-en-1-ol (1.5 g, 17.4 mmol, 1 eq.) was added dropwise. The reaction was stirred at 0 °C for 5 min and then benzyl bromide (3.57 g, 21 mmol, 1.2 eq.) was added. The reaction was warmed to room temperature and stirred overnight. The reaction was quenched with sat. NH₄Cl (15 mL) and then extracted 3×10 mL diethyl ether. The combined organic layers were dried over Na₂SO₄ filtered and concentrated onto Celite under reduced pressure. The crude material was purified by flash chromatography (silica; ethyl acetate/hexanes, 0–5%). Percent yield: 100%. ¹H NMR (400 MHz, CDCl₃) δ 7.45–7.26 (m, 5H), 5.87 (m, 1H), 5.01 (m, 1H), 4.55 (s, 2H), 4.38 (s, 2H), 3.35 (t, J = 6.5 Hz, 2H), 2.20 (q, J = 17.2 Hz, 1H), 1.77 (m, 2H), 1.95 (m, 1H).

Preparation of ((hex-5-en-1-yloxy)methyl)benzene (5d)

The title compound was prepared according to the procedure for ((pent-4-en-1-yloxy)methyl)benzene, except hex-5-en-1-ol was substituted for pent-4-en-1-ol. Percent yield: 100%. ¹H NMR (400 MHz, CDCl₃) δ 7.26–7.11 (m, 5H), 5.68 (m, 1H), 4.88 (dq, J = 1.9, 17.0 Hz, 1H), 4.82 (m, 1H), 4.38 (s, 2H), 3.35 (t, J = 6.5 Hz, 2H), 1.95 (m, 2H).
Preparation of 2-(3-(benzyloxy)propyl)oxirane (6a)

To a round bottom flask is added ((pent-4-en-1-yloxy)methyl)benzene (3.15 g, 17.9 mmol, 1 eq.) and CH₂Cl₂ (45 mL). The resulting solution is then cooled to 0 °C with an ice bath and then 3-chloroperbenzoic acid (5.25 g (6.82 g, 77% purity), 30 mmol, 1.67 eq.) was added in portions. The reaction was allowed to warm to room temperature and stir overnight. The solution was filtered through a plug of Celite and washed filter with CH₂Cl₂. The solution was then washed with 3 × 10 mL of 1 N NaOH (aq.) solution, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude oil was used in the next step without further purification. Percent yield: 72%. ¹H NMR (400 MHz, CDCl₃) δ 7.41–7.24 (m, 5H), 4.53 (s, 2H), 3.55 (m, 2H), 2.95 (m, 1H), 2.75 (dd, J = 4.2, 5.0 Hz, 1H), 2.48 (dd, J = 2.6, 5.0 Hz, 1H), 1.89–1.55 (m, 4H).

Preparation of 2-(4-(benzyloxy)butyl)oxirane (6b)
The title compound was prepared according to the procedure for 2-(3-(benzyloxy)propyl)oxirane, except ((hex-5-en-1-yloxy)methyl)benzene was substituted for ((pent-4-en-1-yloxy)methyl)benzene. Percent yield: 96%. ¹H NMR (400 MHz, CDCl₃) δ 7.40–7.25 (m, 5H), 4.52 (s, 2H), 3.51 (t, J = 6.4 Hz, 2H), 2.93 (m, 1H), 2.76 (dd, J = 4.0, 5.0 Hz, 1H), 2.48 (dd, J = 2.7, 5.1 Hz, 1H), 1.76–1.65 (m, 2H), 1.64–1.50 (m, 4H).

Preparation of 5-(3-(benzyloxy)propyl)-3,3-diethyldihydrofuran-2(3H)-one (7a)
A dry round bottom flask was placed under N₂ atmosphere and then charged with 1 M LDA solution (THF/Hexanes, 23 mL, 23 mmol, 2.3 eq.) and cooled to −78 °C. While at −78 °C, 2-ethyl-N,N-dimethylbutanamide (2.86 g, 20 mmol, 2 eq.) was added dropwise. The reaction was stirred at −78 °C for 30 min, then allowed to warm to 0 °C and stir at that temperature for 15 min. Then the reaction was warmed to room temperature and stirred for 5 min before cooling back to 0 °C with an ice bath. At 0 °C, 2-(3-(benzyloxy)propyl)oxirane (2.0 g, 10 mmol, 1 eq.) was added. The reaction was stirred at 0 °C for 15 min and then warmed to room temperature. After 48 h, the reaction was quenched with sat. NH₄Cl solution (aq.) and extracted 3 × 20 mL CH₂Cl₂. The combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure.

The crude material was then dissolved in CH₂Cl₂ (30 mL) and trifluoroacetic acid (5 mL) was added slowly. The resulting solution was allowed to stir at room temperature for 40 min before being slowly quenched with sat. NaHCO₃ solution (aq.) and extracted with 3 × 10 mL CH₂Cl₂. The combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (silica; ethyl acetate/hexanes, 0–30%). Percent yield: 32%. ¹H NMR (400 MHz, CDCl₃) δ 7.35–7.18 (m, 5H), 4.46 (s, 2H), 3.48 (m, 2H), 2.05 (dd, J = 6.7, 13.1 Hz, 1H), 1.81–1.68 (m, 5H), 1.57 (q, J = 7.5 Hz, 4H), 0.88 (dt, J = 7.4, 16.5 Hz, 6H).

Preparation of 5-(4-(benzyloxy)butyl)-3,3-diethyldihydrofuran-2(3H)-one (7b)
The title compound was prepared according to the procedure for 5-(3-(benzyloxy)propyl)-3,3-diethyldihydrofuran-2(3H)-one, except 2-(4-(benzyloxy)butyl)oxirane was substituted for 2-(3-(benzyloxy)propyl)oxirane. Percent yield: 37%. ¹H NMR (400 MHz, CDCl₃) δ 7.40–7.19 (m, 5H), 4.55 (s, 2H), 4.40 (m, 1H), 3.48 (m, 2H), 2.09 (dd, J = 6.8, 13.1 Hz, 1H), 1.77 (dd, J = 9.3, 13.3 Hz, 1H), 1.72–1.34 (m, 10H), 0.89 (dt, J = 7.4, 19.7 Hz, 6H).

Preparation of 3,3-diethyl-5-(3-hydroxypropyl)dihydrofuran-2(3H)-one
To a round bottom flask was added 10% Pd/C (182 mg, 20% wt) followed by a solution of 5-(3-(benzyloxy)
propyl)-3,3-diethylidihydrofuran-2(3H)-one (910 mg, 3.13 mmol, 1 eq.) in EtOH (18 mL). The reaction was put under H₂ (1 atm) using a balloon and stirred at room temperature under a H₂ atmosphere overnight. The reaction was then filtered through a plug of Celite and the filtrate was concentrated under reduced pressure. The crude product was used in next step without further purification. Percent yield: 100%. ¹H NMR (400 MHz, MeOD) δ 4.61 (b, 1H), 4.38 (m, 1H), 3.48 (m, 2H), 2.11 (dd, J = 6.8, 13.3 Hz, 1H), 1.73 (dd, J = 9.3, 13.1 Hz, 1H), 1.68–1.32 (m, 8H), 0.81 (dt, J = 7.6, 18.8 Hz, 6H).

Preparation of 3,3-diethyl-5-(4-hydroxybutyl)dihydrofuran-2(3H)-one

The title compound was prepared according to the procedure for 3,3-diethyl-5-(3-hydroxypropyl)dihydrofuran-2(3H)-one, except 5-(4-(benzyloxy)butyl)-3,3-diethylidihydrofuran-2(3H)-one was substituted for 5-(3-(benzyloxy)propyl)-3,3-diethylidihydrofuran-2(3H)-one. Percent yield: 100%. ¹H NMR (400 MHz, MeOD) δ 4.38–4.23 (m, 3H), 2.08 (dd, J = 6.5, 13.0 Hz, 1H), 1.77–1.63 (m, 1H), 1.63–1.20 (m, 10H), 0.76 (dt, J = 7.4, 17.7 Hz, 6H).

Preparation of 3-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)propyl 4-methylbenzenesulfonate (8a)

To a solution of triethylamine (493 mg, 4.85 mmol, 1.5 eq.) and p-toluenesulfonyl chloride (744 mg, 3.90 mmol, 1.2 eq.) in CH₂Cl₂ (25 mL) was added a solution of 3,3-diethyl-5-(3-hydroxypropyl)dihydrofuran-2(3H)-one (650 mg, 3.25 mmol, 1.0 eq.) in CH₂Cl₂ (5 mL) at 0 °C. The reaction was allowed to stir at room temperature overnight before being washed with 2 × 20 mL of sat. NaHCO₃ solution (aq.). The organic layer was dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (silica; ethyl acetate/hexanes, 0–40%). Percent yield: 55%. ¹H NMR (400 MHz, CDCl₃) δ 7.69 (d, J = 8.3 Hz, 2H), 7.28 (d, J = 8.1 Hz, 2H), 4.23 (m, 1H), 4.05–3.91 (m, 2H), 2.36 (s, 3H), 2.00 (dd, J = 6.7, 13.2 Hz, 1H), 1.84–1.40 (m, 9H), 0.80 (dt, J = 7.5, 19.5 Hz, 6H).

Preparation of 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate (8b)

The title compound was prepared according to the procedure for 3-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)propyl 4-methylbenzenesulfonate, except 3,3-diethyl-5-(4-hydroxybutyl)dihydrofuran-2(3H)-one was substituted for 3,3-diethyl-5-(3-hydroxypropyl)dihydrofuran-2(3H)-one. Percent yield: 46.5%. ¹H NMR (400 MHz, CDCl₃) δ 7.71 (d, J = 8.3 Hz, 2H), 7.28 (d, J = 8.1 Hz, 2H), 4.24 (m, 1H), 4.00–3.90 (m, 2H), 2.38 (s, 3H), 2.00 (dd, J = 6.7, 13.0 Hz, 1H), 1.76–1.29 (m, 11H), 0.84 (dt, J = 7.5, 22.5 Hz, 6H).

Preparation of 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroacetate (9c)

To a small vial was added 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate (25 mg, 0.0679 mmol, 1 eq.) and 1-phenylpiperazine (23.1 mg, 0.142 mmol, 2.1 eq.). Both were dissolved in THF (1.7 mL). The reaction mixture was allowed to reflux for 72 h and then cooled to room temperature. The mixture was filtered, the precipitate was washed with THF, and the combined organic layers were concentrated under reduced pressure. The crude product was then purified by HPLC (CH₃CN/H₂O, 0.1% Trifluoroacetic acid), 0~100%) to give desired product as a trifluoroacetic acid salt. Percent yield: 41.4%. ¹H NMR (400 MHz, CDCl₃) δ 189.1, 149.8, 131.4, 129.1, 115.9, 78.8, 57.1, 41.4.
Preparation of 3,3-diethyl-5-(2-(4-phenylpiperazin-1-yl) ethyl)dihydrofuran-2(3H)-one dihydrochloride (9a)

The title compound was prepared according to the procedure for 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 1-phenylpiperazine was substituted for 2-piperazin-1-yl-benzonitrile. In addition, the TFA salt was dissolved in 20 ml 4.0 N HCl and then stripped of solvent to provide the HCl salt. Percent yield: 58.3%. 

\[ \text{LC/MS } [\text{M} + \text{H}] = m/z \ 359.2. \]

1H NMR (400 MHz, D2O) \( \delta \) 7.43 (m, 2H), 7.27 – 7.13 (m, 3H), 4.69 (m, 1H), 4.11 – 3.09 (m, 10H), 2.39 – 2.07 (m, 3H), 1.98 (dd, \( J = 13.4, 9.4 \) Hz, 1H), 1.61 (m, 4H), 0.87 (dt, \( J = 12.1, 7.5 \) Hz, 6H); 13C NMR (101 MHz, D2O) \( \delta \) 187.9, 150.2, 132.9, 127.0, 121.1, 79.5, 56.5, 54.1, 52.4, 50.8, 39.4, 32.8, 31.9, 30.7, 11.0, 10.9; MS (LC/MS, M+H+): 331.2;

Anal. Calcd for C20H32Cl2N2O2: C, 59.55; H, 8.00; N, 6.94; Found: C, 59.62; H, 8.11; N, 6.90.

Preparation of 3,3-diethyl-dihydro-5-(3-(4-phenylpiperazin-1-yl)propyl)furan-2(3H)-one (9b)

The title compound was prepared according to the procedure for 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroacetate, except 3-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)propyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and the crude product was purified by flash chromatography (silica; MeOH: dichloromethane, 0~10%) Percent yield: 32%. 

1H NMR (400 MHz, CDCl3) \( \delta \) 7.32 (td, \( J = 1.1, 7.7, 2H \)), 7.00 (t, \( J = 7.4, 1H \)), 6.95 (d, \( J = 8.6, 1H \)), 4.40 (m, 1H), 3.70 (m, 4H), 3.35 (m, 2H), 3.16 (t, \( J = 8.1, 2H \)), 3.01 (b, 2H), 2.16 (dd, \( J = 6.7, 13.4, 1H \)), 2.01 (m, 2H), 1.81 (m, 2H), 1.64 (m, 5H), 0.94 (dt, \( J = 7.5, 22.4, 6H \)). 13C NMR (101 MHz, DMSO) \( \delta \) 178.2, 148.5, 131.1, 120.8, 117.3, 75.3, 55.1, 54.2, 50.1, 49.8, 44.4, 30.9, 23.7, 23.5. LC/MS [M + H] = m/z 345.2.

Preparation of 3,3-dimethyl-5-(2-(4-phenylpiperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (9d)

The title compound was prepared according to the procedure for 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-dimethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and the crude product was purified by flash chromatography (silica; MeOH: dichloromethane, 0~10%) Percent yield: 45.2%. 

1H NMR (400 MHz, CDCl3) \( \delta \) 7.32 (m, 2H), 6.99 (d, \( J = 7.9 \) Hz, 2H), 6.91 (t, \( J = 7.2 \) Hz, 1H) 4.58 (m, 1H), 3.26 (t, \( J = 5.0 \) Hz, 4H), 2.66 (m, 4H), 2.61 (m, 2H), 2.26 (m, 1H), 1.90 (m, 3H), 1.34 (s, 3H), 1.33 (s, 3H). 13C NMR (101 MHz, MeOH) \( \delta \) 178.2, 148.5, 131.1, 120.8, 117.3, 75.3, 55.1, 54.2, 50.1, 49.8, 44.4, 30.9, 23.7, 23.5. LC/MS [M + H] = m/z 303.2.

Preparation of 3,3-diethyl-5-(2-(4-(naphthalen-1-yl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one trifluoroacetate (9e)

The title compound was prepared according to the procedure 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 1-(naphthalen-1-yl)piperazine for 1-phenylpiperazine and the crude product was purified by HPLC (CH3CN/H2O, 0~100%) to give desired product as a trifluoroacetic acid salt. Percent yield: 33%. 

1H NMR (400 MHz, CDCl3) \( \delta \) 8.07 (m, 1H), 7.88 (m, 1H), 7.66 (d, \( J = 8.2 \) Hz, 1H), 7.52 (m, 2H), 7.44 (t, \( J = 7.7 \) Hz, 1H), 7.16 (d, \( J = 7.4 \) Hz, 1H), 7.00 (t, \( J = 7.4 \) Hz, 2H), 6.99 (d, \( J = 7.9 \) Hz, 2H), 6.91 (t, \( J = 7.2 \) Hz, 1H) 4.58 (m, 1H), 3.26 (t, \( J = 5.0 \) Hz, 4H), 2.66 (m, 4H), 2.61 (m, 2H), 2.26 (m, 1H), 1.90 (m, 3H), 1.34 (s, 3H), 1.33 (s, 3H). 13C NMR (101 MHz, MeOH) \( \delta \) 178.2, 148.5, 131.1, 120.8, 117.3, 75.3, 55.1, 54.2, 50.1, 49.8, 44.4, 30.9, 23.7, 23.5. LC/MS [M + H] = m/z 345.2.
4.49 (m, 1H), 3.81 (t, J = 9.3 Hz, 2H), 3.54–3.05 (m, 8H), 2.36 (m, 1H), 2.25 (dd, J = 6.8, 13.3 Hz, 1H), 2.08 (m, 1H), 1.88 (dd, J = 9.3, 13.3 Hz, 1H), 1.65 (q, J = 7.5 Hz, 4H), 0.95 (dt, J = 7.4, 17.7 Hz, 6H), 13C NMR (101 MHz, DMSO) δ 179.7, 147.5, 134.3, 128.4, 127.8, 126.1, 125.9, 125.8, 124.1, 123.1, 115.20, 74.1, 52.4, 51.6, 49.6, 47.8, 36.4, 29.9, 28.3, 27.6, 8.5, 8.4. LC/MS [M + H] = m/z 381.2.

Preparation of 3,3-diethyl-5-(2-(4-(pyridin-4-yl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (4)

The title compound was prepared according to the procedure for 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 1-(pyridin-4-yl)piperazine for 1-phenylpiperazine. Percent yield: 37%. 1H NMR (400 MHz, CDCl3) δ 8.27 (d, J = 5.7 Hz, 2H), 6.67 (d, J = 5.9 Hz, 2H), 4.50 (m, 1H), 3.35 (t, J = 5.2 Hz, 4H) 2.68–2.46 (m, 6H), 2.15 (dd, J = 6.6, 13.0 Hz, 1H), 1.95–1.77 (m, 3H), 1.69–1.57 (m, 4H), 0.93 (dt, J = 7.5, 19.3 Hz, 6H). 13C NMR (101 MHz, MeOD) δ 183.2, 157.3, 148, 109.4, 77.5, 55.4, 53.7, 50.1, 46.7, 38.4, 34.3, 30.2, 29.3, 9.1, 8.9 LC/MS [M + H] = m/z 332.2.

Preparation of 3,3-diethyl-5-(2-(4-(pyrimidin-4-yl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (9f)

The title compound was prepared according to the procedure for 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 4-(piperazin-1-yl)pyrimidine for 1-phenylpiperazine. In addition the crude product was purified by flash chromatography (silica; MeOH:dichloromethane, 0~10%). Percent yield: 52%. 1H NMR (400 MHz, CDCl3) δ 8.52 (s, 1H), 8.13 (d, J = 6.2 Hz, 1H), 6.44 (dd, J = 1.0, 6.3 Hz, 1H), 4.42 (m, 1H), 3.62 (b, 4H), 2.52 (m, 6H), 2.08 (dd, J = 7.0, 13.1 Hz, 1H), 1.82 (q, J = 6.8 Hz, 2H), 1.77 (dd, J = 9.4, 13.1 Hz, 1H), 1.56 (m, 4H) 0.86 (dt, J = 7.5, 20.0 Hz, 6H). 13C NMR (101 MHz, DMSO) δ 178.9, 159.8, 156.7, 154.3, 102.4, 74.1, 52.5, 51.0, 41.8, 35.7, 31.4, 27.2, 26.5, 7.5, 7.4. LC/MS [M + H] = m/z 333.20.

Preparation of 3,3-diethyl-5-(2-(4-(phenyl-1,4-diazepan-1-yl)ethyl)dihydrofuran-2(3H)-one (9g)

The title compound was prepared according to the procedure for 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 1-phenyl-1,4-diazepane for 1-phenylpiperazine. The crude product was purified by flash chromatography (silica; MeOH:dichloromethane, 0~10%). Percent yield: 68%. 1H NMR (400 MHz, CDCl3) δ 7.22 (m, 2H), 6.73–6.64 (m, 3H), 4.45 (m, 1H), 3.56 (t, J = 6.2 Hz, 2H), 2.80 (t, J = 4.9 Hz, 2H), 2.73–2.56 (m, 4H), 2.09 (dd, J = 6.8, 13.1 Hz, 1H), 1.97 (b, 2H), 1.90–1.70 (m, 3H), 1.62 (q, J = 7.5 Hz, 4H), 0.93 (dt, J = 7.6, 18.3 Hz, 6H). 13C NMR (101 MHz, DMSO) δ 180, 148.5, 129.1, 115, 111.2, 70.5, 54.2, 53.7, 52.8, 48.3, 47.7, 47.5, 36.7, 33.6, 28.2, 27.5, 26.8, 8.5, 8.4. MS (LC/MS, M + H+): 345.2.

Preparation of 3,3-diethyl-5-(2-(6-phenyl-2,6-diazaspiro[3.3]heptan-2-yl)ethyl)dihydrofuran-2(3H)-one (9h)

The title compound was prepared according to the procedure for 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 2-phenyl-2,6-diazaspiro[3.3]heptane was substituted for 1-phenylpiperazine. Percent yield:
Preparation of 3,3-diethyl-5-(2-(5-phenylhexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)ethyl)dihydrofuran-2(3H)-one (9i)

The title compound was prepared according to the procedure for 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroac except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 2-(pyridin-4-yl)octahydropyrrolo[3,4-c]pyrrole was substituted for 1-phenylpiperazine. Percent yield: 68%. 1H NMR (400 MHz, CDCl3) δ 6.83 (m, 2H, J = 7.1 Hz, 1H), 6.78 (s, 2H, J = 8.0 Hz, 2H), 2.85 (b, 2H, J = 2.9, 3.9 Hz, 2H), 2.72 (m, 2H, J = 6.8 Hz, 2H), 2.02 (dd, J = 6.7, 12.9 Hz, 1H, J = 7.5, 14.0 Hz, 6H). 13C NMR (101 MHz, DMSO) δ 179.9, 163.2, 153.2, 141.9, 111.7, 75.6, 58.2, 54.1, 50.9, 47.2, 37.9, 36.2, 34.1, 28.7, 27.4, 8.7, 8.6. MS (LC/MS, M + H⁺): m/z 358.2.

Preparation of 3,3-diethyl-5-(2-(5-(pyridin-4-yl)hexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)ethyl)dihydrofuran-2(3H)-one (9j)

The title compound was prepared according to the procedure for 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroac except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 1,2,3,4-tetrahydro-isoquinoline was substituted for 1-phenylpiperazine. In addition the crude product was purified by flash chromatography (silica; MeOH:dichloromethane, 0~10%) and converted to the HCl salt using HCl in ether. Percent yield: 36.4%. 1H NMR (400 MHz, MeOH) δ 7.17–7.39 (m, 4H), 4.63–4.84 (m, 1H), 4.49 (s, 2H, J = 4.3 Hz, 4.49 Hz), 3.75–3.63 (m, 2H, J = 5.0 Hz, 5.1 Hz), 3.54–3.37 (m, 2H, J = 2.2 Hz, 2.3 Hz), 2.36–2.24 (m, 2H), 1.95–1.81 (m, 2H, J = 9.4, 13.3, 13.3 Hz, 1.95 Hz), 1.75–1.53 (m, 4H, 1.53 Hz), 0.94–0.82 (m, 6H, J = 7.5, 12.2 Hz, 6H). 13C NMR (101 MHz, MeOH) δ 183.24, 132.92, 130.75, 130.38, 129.74, 129.17, 128.70, 77.07, 55.67, 55.33, 55.28, 52.24, 39.25, 32.87, 30.89, 30.02, 27.35, 9.85, 9.77. MS (LC/MS, M + H⁺): m/z 358.2; Anal. Calcd for C19H28ClNO2: C, 67.54; H, 8.35; N, 4.15; Found: C, 67.60; H, 8.36; N, 4.14.
Preparation of 3,3-diethyl-5-(2-(7-fluoro-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)dihydrofuran-2(3H)-one (9l)

The title compound was prepared according to the procedure for 3,3-diethyl-5-(2-(4-(naphthalen-1-yl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 7-fluoro-1,2,3,4-tetrahydroisoquinoline was substituted for 1-phenylpiperazine.

Percent yield: 37%. 1H NMR (400 MHz, CDCl3) δ 7.06 (dd, J = 5.8, 8.3, 1H), 6.84 (td, J = 2.7, 8.5 1H), 6.73 (dd, J = 2.5, 9.5, 1H), 4.54 (m, 1H), 3.62 (s, 2H), 2.86 (m, 2H), 2.75 (m, 2H), 2.68 (m, 2H), 2.16 (dd, J = 6.8, 13.0, 4H), 1.90 (m, 3H), 1.64 (qt, J = 1.7, 7.6, 4H), 0.94 (dt, J = 7.5, 15.8, 6H). 13C NMR (101 MHz, DMSO) δ 178.7, 165.1, 138.5, 133.6, 131.5, 118.5, 115.3, 75.8, 48.3, 46.1, 43.1, 37.5, 35.9, 32.9, 28.7, 28.1, 27.2, 8.7, 8.5. LC/MS [M + H] = m/z 320.1.

Preparation of 5-(2-(7-chloro-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-3,3-diethyldihydrofuran-2(3H)-one (9m)

The title compound was prepared according to the procedure for 3,3-diethyl-5-(2-(4-(naphthalen-1-yl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 7-chloro-1,2,3,4-tetrahydroisoquinoline was substituted for 1-phenylpiperazine.

Percent yield: 41%. 1H NMR (400 MHz, CDCl3) δ 7.02 (dd, J = 2.2, 8.2, 1H), 6.95 (m, 2H), 4.45 (m, 1H), 3.52 (s, 2H), 2.77 (m, 2H), 2.63 (m, 4H), 2.07 (dd, J = 6.7, 13.0, 1H), 1.83 (m, 3H), 1.55 (qd, J = 1.2, 7.3, 4H), 0.85 (dt, J = 7.5, 15.3, 6H). 13C NMR (101 MHz, DMSO) δ 179.9, 162.1, 137.6, 131.2, 129.5, 126.6, 117.3, 75.1, 47.8, 45.4, 43.6, 36.8, 35.4, 33.6, 28.2, 27.5, 26.6, 8.5, 8.4. LC/MS [M + H] = m/z 336.1.

Preparation of 5-(2-(3,4-dihydro-2,6-naphthyridin-2(1H)-yl)ethyl)-3,3-diethyldihydrofuran-2(3H)-one (9o)

The title compound was prepared according to the procedure for 3,3-diethyl-5-(2-(4-(naphthalen-1-yl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 1,2,3,4-tetrahydro-2,6-naphthyridine dihydrochloride was substituted for 1-phenylpiperazine.

Percent yield: 45%. 1H NMR (400 MHz, CDCl3) δ 8.29 (s, 1H), 8.24 (d, J = 4.7 Hz, 1H), 6.87 (d, J = 5.0 Hz, 1H), 4.50 (m, 1H), 3.55 (s, 2H), 2.82 (t, J = 5.7 Hz, 2H), 2.76–2.66 (m, 2H), 2.66–2.56 (m, 2H), 2.07 (dd, J = 6.7, 13.0 Hz, 1H), 1.92–1.72 (m, 3H), 1.55 (qd, J = 2.1, 7.5 Hz, 4H), 0.85 (dt, J = 7.7, 15.8 Hz, 6H). 13C NMR (101 MHz, DMSO) δ 179.9, 161.6, 148.8, 148.5, 135.6, 132.8, 120.1, 120.1, 75.0, 47.8, 45.4, 43.7, 36.7, 33.5, 28.1, 27.5, 24.2, 8.5, 8.4. LC/MS [M + H] = m/z 303.2.

Preparation of 5-(2-(7-bromo-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-3,3-diethyldihydrofuran-2(3H)-one (9n)

The title compound was prepared according to the procedure for 3,3-diethyl-5-(2-(4-(naphthalen-1-yl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 7-bromo-1,2,3,4-tetrahydroisoquinoline was substituted for 1-phenylpiperazine.

Percent yield: 37%. 1H NMR (400 MHz, CDCl3) δ 7.13 (dd, J = 1.8, 8.0 Hz, 1H), 7.06 (d, J = 1.4 Hz, 1H), 6.86 (d, J = 8.6 Hz, 1H), 4.43 (m, 1H), 3.49 (s, 2H), 2.73 (t, J = 5.4 Hz, 2H), 2.62 (m, 2H), 2.56 (m, 2H), 2.06 (dd, J = 6.8, 13.0 Hz, 1H), 1.91–1.69 (m, 3H), 1.52 (q, J = 7.6 Hz, 4H), 0.83 (dt, J = 5.6, 12.8 Hz, 6H). 13C NMR (101 MHz, MeOH) δ 184.9, 134.6, 131.5, 130.9, 128.6, 121.3, 126.6, 76.5, 55.2, 55.1, 54.9, 51.8, 38.9, 32.6, 31.2, 30.6, 27.9, 9.6, 9.4. LC/MS [M + H] = m/z 380.10.
Preparation of 5-(2-(3,4-dihydro-2,7-naphthyridin-2(1H)-yl)ethyl)-3,3-diethylidihydrofuran-2(3H)-one (9p)

The title compound was prepared according to the procedure for 3,3-diethyl-5-(2-(4-naphthalen-1-yl)piperazin-1-yl)ethyl dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 1,2,3,4-tetrahydro-2,6-naphthyridine dihydrochloride was substituted for 1-phenylpiperazine. Percent yield: 90%. ¹H NMR (400 MHz, CDCl₃) δ 8.36–8.23 (m, 2H), 7.02 (d, J = 5.0 Hz, 1H), 4.52 (m, 1H), 3.64 (m, 2H), 2.92–2.8 (m, 2H), 2.80–2.63 (m, 4H), 2.15 (dd, J = 6.8, 13.1 Hz, 1H), 1.99–1.79 (m, 3H), 1.63 (qd, J = 1.8, 7.4 Hz, 4H), 0.92 (dt, J = 7.5, 15.6 Hz, 6H). ¹³C NMR (101 MHz, DMSO) δ 182.7, 150.1, 148.3, 144.6, 131.4, 129.3, 77.5, 55.9, 55.1, 54.6, 53.1, 38.9, 33.2, 30.8, 27.9, 9.4, 9.2. LC/MS [M + H] = m/z 303.2.

Preparation of 5-(2-(5,8-dihydro-1,7-naphthyridin-7(6H)-yl)ethyl)-3,3-diethylidihydrofuran-2(3H)-one (9q)

The title compound was prepared according to the procedure for 3,3-diethyl-5-(2-(4-naphthalen-1-yl)piperazin-1-yl)ethyl dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 5,6,7,8-tetrahydro-2,6-naphthyridine dihydrochloride was substituted for 1-phenylpiperazine. Percent yield: 90%. ¹H NMR (400 MHz, CDCl₃) δ 8.34 (d, J = 4.9 Hz, 1H), 7.40 (d, J = 7.7 Hz, 1H), 7.06 (dd, J = 4.8, 7.6 Hz, 1H), 4.52 (m, 1H), 3.71 (m, 2H), 2.96–2.81 (m, 2H), 2.80–2.64 (m, 4H), 2.13 (dd, J = 6.8, 13.0 Hz, 1H), 2.01–1.77 (m, 3H), 1.61 (qd, J = 1.7, 7.5 Hz, 4H), 0.91 (dt, J = 7.4, 15.5 Hz, 6H). ¹³C NMR (101 MHz, DMSO) δ 180.1, 148.2, 146.6, 135.8, 134.9, 125.8, 75.2, 47.8, 45.1, 43.7, 36.8, 35.5, 33.7, 28.2, 27.5, 8.5, 8.4. LC/MS [M + H] = m/z 303.2.

Computational values

TPSA and cLogP values were calculated using the Dotmatics software suite (Dotmatics LLC The Old Monastery, Windhill Bishops, Stortford Herts, CW23 2ND UK).

Sigma-1 and sigma-2 competitive radioligand-binding studies

Competitive binding assays were conducted by the Psychoactive Drug Screening Program (PDS) at The University of North Carolina, Chapel Hill under the direction of Professor Bryan Roth. Assay conditions can be found in the PDS assay protocol book at https://pdsn.unc.edu/pdsweb/content/UNC-CH%20Protocol%20Book.pdf. A brief description of the assays is provided.

Sigma-2 receptor binding assay

Kᵢ values for test compounds for the sigma-2 receptor were determined using a filtration assay in a 96 well polypropylene plate using membranes prepared from HEK293T cells stably transfected with the sigma-1 receptor or PC12 cells. The membranes were prepared from cultured cells rinsed with PBS, lysed in cold 50 mM Tris-HCL (pH 7.4), centrifuged at 20000 x g, pellets resuspended in buffer and then stored at −80 C until used. In a final volume of 250 uL of assay buffer (50 mM Tris-HCL, 10 mM MgCl₂, 1 mM EDTA, pH 7.4) the membranes were incubated with 5–7 nM [³H]-1,3-di-(2-tolyl)guanidine ([³H]-DTG, Kᵢ = 9.9 nM) and test compound (11 concentrations) at room temperature for 90 min. Nonspecific binding was defined with 10 uM haloperidol. Membranes were then collected by rapid filtration on to filter mats pretreated with 0.3% polyethylenimine, washed 4x with cold assay buffer, dried, microscintillant added and then counted in a Microbeta scintillation counter. IC₅₀ values were determined using a three-parameter non-linear curve fitting program in Prism 4.0 (GraphPad Software). Kᵢ values were calculated from the IC₅₀ values using the Cheng-Prusoff equation [17]. The reference standard haloperidol had a Kᵢ = 13.9 nM.

Sigma-1 receptor binding assay

Kᵢ values for test compounds for the sigma-1 receptor were determined using the sigma-2 method except that membrane from HEK-293 cells stably transfected with the sigma-1 receptor or PC12 cells were used and 2-10 nM [³H]-Pentazocine (Kᵢ = 6.5 nM) was the radioligand. Nonspecific binding was defined with 10 uM haloperidol. The reference standard haloperidol had a Kᵢ = 3.54 nM.

Aqueous solubility (pH 7.4) assay

Compounds were assessed for their solubility at pH 7.4 using the commercially available Millipore MultiScreen™Solubility filter system (Millipore, Billerica, MA). Analysis was performed by liquid chromatography tandem mass spectrometry (LC/MS/MS).
**Microsomal stability assays**

Test compounds were assessed for microsomal stability by incubating them at 37 °C in the presence of mouse or human liver microsomes and an NADPH regenerating system as described by Yang et al. [20]. Microsomal protein content was adjusted to give accurate rates of substrate consumption. Analysis was performed by Liquid Chromatography-tandem mass spectrometry (LC/MS/MS) analysis.

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**Compliance with ethical standards**

Conflict of interest Drs. Blass and Canney both have equity interests in Praeventix LLC, which have been reviewed and approved by Temple University in accordance with its conflict of interest policies. Questions regarding this interest may be directed to the Temple University Conflict of Interest Program. No other author has reported conflicts of interest to disclose at the time of publication.

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**References**

1. Martin WR, Eades CE, Thompson JA, Huppler RE. The effects of morphine and nalorphine-like drugs in the non-dependent and morphine-dependent chronic spinal dog. J Pharm Exp Ther. 1976;197:517–32

2. Su TP. Evidence for sigma opioid receptor: binding of [3H]SKF-10047 to etorphine inaccessible sites in guinea-pig brain. J Pharmacol Exp Ther. 1982;223:284–90. https://doi.org/10.1007/s00044-020-02574-9

3. Khazan N, Young GA, El-Fakany EE, Hong O, Caligari D. Sigma receptors mediated the phototinomimetic effects of N-allylnormetazocine (SKF-10,047), but not its opioid agonistic-antagonistic properties. Neuropharmacology. 1984;23:983–7. https://doi.org/10.1016/0022-3594(84)90015-7

4. Bowen WD, de Costa BR, Hellewell SB, Walker JM, Rice KC. [3H]-(+)-Pentazocine: a potent and highly selective benzomorphan-based probe for sigma-1 receptors. Mol Neuropharmacol. 1993;3:117–26.

5. Hanner M, Moebius FF, Flanderoff A, Knaus HG, Striessnig J, Kepner E, et al. Purification, molecular cloning, and expression of the mammalian sigma-1 binding site. Proc Natl Acad Sci USA. 1996;93:8072–77. https://doi.org/10.1073/pnas.93.15.8072

6. Schmidt HR, Zheng S, Guripinar E, Koehl A, Manglik A, Kruse AC. Crystal structure of the human σ1 receptor. Nature 2016;532:527–30. https://doi.org/10.1038/nature17391

7. Alon A, Schmidt HR, Wood MD, Sahj JJ, Martin SF, Krusea AC. Identification of the gene that codes for the σ2 receptor. Proc Natl Acad Sci USA 2017;114:7160–5. https://doi.org/10.1073/pnas.1705154114

8. Bartz F, Kern L, Erz D, Zhu M, Gilbert D, Meinhold T, et al. Identification of cholesterol-regulating genes by targeted RNAi screening. Cell Metab. 2009;10:63–75. https://doi.org/10.1016/j.cmet.2009.05.009

9. Ebrahimi-Fakhari D, Wahlster L, Bartz F, Werenbeck-Ueding J, Praggastis M, Zhang J, et al. Reduction of TMEM97 increases NPC1 protein levels and restores cholesterol trafficking in Niemann-Pick type C1 disease cells. Hum Mol Genet. 2016;25:3588–99. https://doi.org/10.1093/hmg/ddw204

10. Yi B, Sahj JJ, Ardestani PM, Evans AK, Scott LL, Chan IZ, et al. Small molecule modulator of sigma 2 receptor is neuroprotective and reduces cognitive deficits and neuroinflammation in experimental models of Alzheimer’s disease. J Neurochem. 2017;140:561–75. https://doi.org/10.1111/jnc.13917

11. Izzo NJ, Staniszweski A, To L, Fa M, Teich AF, Saeed F, et al. Alzheimer’s therapeutics targeting amyloid beta 1–42 oligomers I: Abeta 42 oligomer binding to specific neuronal receptors is displaced by drug candidates that improve cognitive deficits. PLoS One. 2014;9:e111898. https://doi.org/10.1371/journal.pone.0111898

12. Izzo NJ, Xu J, Zeng C, Kirk MJ, Mozzoni K, Silky C, et al. Alzheimer’s therapeutics targeting amyloid beta 1–42 oligomers II: Sigma-2/PGRMIC1 receptors mediate Abeta 42 oligomer binding and synaptotoxicity. PLoS One. 2014;9:e111899. https://doi.org/10.1371/journal.pone.0111899

13. Vazquez-Rosa E, Watson MR, Sahj JJ, Hodges TR, Schroeder RE, Cintron-Perez CJ, et al. Neuroprotective Efficacy of a Sigma 2 Receptor/TMEM97 Modulator (DKR-1677) after Traumatic Brain Injury. ACS Chem Neurosci. 2019;10:1595–602. https://doi.org/10.1021/acschemneuro.8b00543

14. Sahj JJ, Mejia GL, Ray PR, Martin SF, Price TJ. Sigma 2 receptor/Tmem97 agonists produce long lasting antineuropathic pain effects in mice. ACS Chem Neurosci. 2017;8:1801–11. https://doi.org/10.1021/acschemneuro.7b00200

15. Guo L, Zhen X. Sigma-2 receptor ligands: neurobiological effects. Curr Med Chem 2015;22:989–1003. https://doi.org/10.2174/0929867632266161014143067

16. Vilner BJ, John CS, Bowen WD. Sigma-1 and Sigma-2 receptors are expressed in a wide variety of human and rodent tumor cell lines. Cancer Res. 1995;55:408–13

17. Asong G, Zhu XY, Bricker B, Andey T, Amissah F, Lamango N, et al. New analogs of SYA013 as sigma-2 ligands with anticancer activity. Bioorg Med Chem. 2019;27:2629–36. https://doi.org/10.1016/j.bmc.2019.04.012

18. Blass BE, Gao R, Blattner, KM, Gordon JC, Pippin DA, Canney, DJ. Design, synthesis, and evaluation of novel, selective γ-butyrolactones sigma-2 ligands. Med Chem Res, 2021;1713–27. https://doi.org/10.1007/s00044-021-02771-0

19. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 2001;46:3–26. https://doi.org/10.1016/s0169-409x(00)00129-0

20. Yang J, Jamei M, Yeo KR, Rostami-Hodjegan A, Tucker GT. Misuse of the well-stirred model of hepatic drug clearance. Drug Metab Disp. 2007;35:501–2. https://doi.org/10.1124/dmd.106.013359