Design, synthesis, and evaluation of novel, selective γ-butyrolactones sigma-2 ligands

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Abstract

Nearly 40 years after the first disclosure of sigma receptors, the sigma-2 (σ2) receptor was recently identified as the Transmembrane Protein 97 (TMEM97, also known as MAC30 (Meningioma-associated protein). This macromolecule has been associated with a number of disease states such as schizophrenia, Alzheimer’s disease, neuropathic pain, traumatic brain injury, and cancer. We have recently identified a series of novel, functionalized γ-butyrolactones that are potent σ2 receptor ligands that are drug-like and identified a potential candidate (9z) for future in vivo study.

Graphical Abstract

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

Introduction

In 1976, W. R. Martin et. al. described their efforts to classify opioids based on their impact on chronic spinal dogs. They observed that exposure to morphine (1), ketocyclazocine, (2), and (rac)-SKF-100047 (3) (Fig. 1) produced different responses in this animal model. They hypothesized that these compounds were engaging three different receptors that they labeled as the µ-opioid receptor (morphine type, MOR), the κ-opioid receptor (ketocyclazocine type, KOR), and the σ-opioid receptor (SKF-100047 like) [1]. These studies were conducted with racemic material, and follow-up studies with the individual enantiomers of SKF-100047 revealed that (−)-SKF-100047 elicited opioid mediated physiological responses through MOR and KOR, while (+)-SKF-100047’s biological activity was produced by interaction with a previously unknown, non-opioid receptor that was designated the sigma receptor (σR) [2, 3]. Nearly 17 years later, W. D. Bowen et. al. successfully demonstrated that there were two sub-types of this receptor, sigma-1 (σ1) and sigma-2 (σ2) [4], and in 1996 mammalian σ1 receptor was cloned and expressed in yeast cells [5]. A crystal structure of the human σ1 receptor was reported in 2016 [6], but to date, there is no known natural ligand for this receptor.

The true nature of the σ2 receptor, on the other hand, proved more elusive. In 2017, over 40 years after the original description of the σR, Transmembrane Protein 97 (TMEM97, also known as MAC30 (Meningioma-associated protein) was identified as the σ2 receptor [7]. The pharmacological role of σ2 remains unclear, as no natural ligand has been identified. It is known, however, that this receptor protein is present in the endoplasmic reticulum (ER) and lysosomes. There is also some evidence supporting binding of σ2 to cholesterol in these regions of the...
with potent \( \sigma_2 \) chemical space associated with a series of functionalized therapeutically useful molecules, we have been exploring the \( \gamma \)-butyrolactones. In an early publication, we described the tones as potential selective characterization and preliminary evaluation of these lactones as potential selective \( \sigma_2 \) ligands will be presented.

As part of our on-going effort to develop novel, therapeutically useful molecules, we have been exploring the chemical space associated with a series of functionalized \( \gamma \)-butyrolactones. In an early publication, we described the tones as potential selective characterization and preliminary evaluation of these lactones as potential selective \( \sigma_2 \) ligands will be presented.

### Results and discussion

Synthesis of substituted \( \gamma \)-butyrolactones was conducted as shown in Scheme 1 utilizing novel methods developed in our laboratory. The synthesis of these compounds begins with substituted ester (4). Allylation of (4) under basic conditions (LDA, HMPA) provides (5) which is then converted to (7) using a modified Prins reaction [19]. Specifically, reaction of (5) with paraformaldehyde in a hot mixture of acetic and sulfuric acid provided oxepan-2-one (6), which was converted to \( \gamma \)-butyrolactone (7) by sequential treatment with refluxing aqueous NaOH and cold sulfuric acid. Compound 7 was identified as a critical precursor in the preparation of the target series. Conversion of the primary alcohol to the corresponding tosylate (8) followed by displacement with various amines provided the final target molecules (9a, 9b and 9d-w). Alternatively, oxidation of (7) using Jones reagent followed by treatment with thionyl chloride provided the corresponding acid chloride (10), which could be reacted with an appropriate amide provide the final target molecule (9c).

Table 1 includes the in vitro binding (K\(_i\) at \( \sigma_1 \) and \( \sigma_2 \)), physicochemical properties (MW, TPSA, LogP, solubility), and preliminary data regarding metabolism (human and mouse liver microsomal stability (HLM, MLM), CYP3A4 inhibition). All compounds evaluated herein have acceptable water solubility, and properties that are consistent with Lipinski rule of 5 (MW, cLogP). The compounds prepared and tested have TPSA and cLogP values that indicate they will cross the BBB following oral administration. While many of the compounds were stable in HLMs, only three compounds had T\(_{1/2}\) values >10 mins in MLMs. Since future in vivo studies will be performed in rodents, this limits the compounds eligible for these studies.

The structure-activity relationship studies involving this series of compounds began with the unsubstituted phenyl piperazine (9a). As indicated in Table 1, this compound binds to the \( \sigma_2 \) receptor (K\(_i\) = 82 nM) and has a low level of selectivity for this receptor over \( \sigma_1 \) (K\(_i\) = 138 nM). Replacing the 1,1-diethyl lactone with a 1,1-dimethyl lactone (9b) leads to >9-fold decrease in \( \sigma_2 \) binding (K\(_i\) = 753 nM), but \( \sigma_1 \) binding decreases by ~2 fold (K\(_i\) = 279 nM). Insertion of a carbonyl into the ethyl linker chain (9c) lead to a substantial loss of binding affinity at both \( \sigma_1 \) and \( \sigma_2 \) (both K\(_i\) = 10,000 nM), as did conversion of the piperazine to the corresponding piperazin-2-one (9d). Conversion to the corresponding piperidine (9e), on the other hand, lead to a >10 fold increase in both \( \sigma_2 \) (K\(_i\) = 6.4 nM) and \( \sigma_1 \) (K\(_i\) = 2.7 nM) binding. Replacing the benzene ring of (9a) with a cyclohexyl moiety (9f) produced a similar increase in affinity at \( \sigma_2 \) (K\(_i\) = 5.4 nM), but the increase in affinity at \( \sigma_1 \) (K\(_i\) = 26 nM) was not as large as that observed in (9e). Incorporating the electron withdrawing substituents CN (9g), CF\(_3\) (9h), and Cl (9i) in the 4-position of the phenyl ring produced compounds with improved \( \sigma_2 \) affinity (K\(_i\) = 34 nM, 7.3 nM, and 12 nM), but \( \sigma_1 \) selectivity was low (2.9x, 1.9x, and 1.4x respectively). In addition, MLM

### Scheme 1 Synthesis of (9a-z)

![Scheme 1 Synthesis of (9a-z)](image_url)
stability was poor (>5 min.) and HLM stability was moderate (23, 11, and 12 min respectively). Replacing these groups with the electron donating substituents 4-OMe (9j) and 4-Me (9k) also lead to potent σ₂ binders (Ki = 53 nM, and 14 nM respectively). Interestingly, while (9j) demonstrated limited selectivity for σ₂ over σ₁, (9k) was highly selective (σ₁ Ki = 10,000 nM). In addition, both of these compounds were highly stable in HLM assays (T₁/₂ = 60 min, 40.6 min respectively), but MLM stability was low (T₁/₂ > 5 min.).

Relocating these same substituents to the 3-position (9l to 9p) and the 2-position (9q to 9u) produced highly potent σ₂ binders (Ki = 9.9 nM to 62 nM). Most of these analogs demonstrated low to moderate selectivity (2–8.5 fold) with the exception of the 2-OMe analog (9u), which exhibited a >26 fold drop in binding affinity at σ₁ (Ki = 1168 nM) over σ₂ (Ki = 44 nM). Increasing the steric bulk in the 2-position by replacing the 2-OMe with an isopropyl group (9v) improved σ₂ selectivity (σ₂ Ki = 5.9 nM vs σ₁ Ki = 195 nM), but the 2,4-di-Me analog (9w) displayed high affinity at both

Table 1 In vitro screening and physicochemical properties data for (9a)–(9z).

| Entry | R¹ | R² | X | Y | Z | R¹ | MW | TPSA | cLogP | σ₂* | σ₁** | σ₂/σ₁ ratio | HLM | MLM | Sol | CYP3A4 |
|-------|----|----|---|---|---|----|----|------|------|-----|-----|-------------|-----|------|-----|--------|
| 9a    | Et | Et | CH₂ | N | CH₂ | Ph | 330 | 33 | 3.5 | 82 | 138 | 1.7 | 48 | 2 | 200 | 10000 |
| 9b    | Me | Me | CH₂ | N | CH₂ | Ph | 302 | 33 | 2.6 | 753 | 279 | 0.4 | 60 | 14 | 192 | 10000 |
| 9c    | Et | Et | C(O) | N | CH₂ | Ph | 344 | 50 | 3.3 | 10000 | 10000 | 1.0 | 39 | 3 | 189 | 10000 |
| 9d    | Et | Et | CH₂ | N | CH₂ | Ph | 344 | 50 | 3.3 | 5289 | 10000 | 1.9 | 60 | 9.6 | 140 | 10000 |
| 9e    | Et | Et | CH₂ | CH₂ | CH₂ | Ph | 329 | 30 | 4.7 | 6.4 | 2.7 | 0.4 | 46 | 2 | 200 | 10000 |
| 9f    | Et | Et | CH₂ | N | CH₂ | Cyc-hex | 337 | 33 | 3.8 | 5.4 | 26 | 4.8 | 60 | 4.3 | 147 | 10000 |
| 9g    | Et | Et | CH₂ | N | CH₂ | 4-CN-Ph | 355 | 57 | 3.2 | 34 | 98 | 2.9 | 24 | 3.5 | 200 | 8570 |
| 9h    | Et | Et | CH₂ | N | CH₂ | 4-CF₃-Ph | 398 | 33 | 4.5 | 7.3 | 14 | 1.9 | 11 | 3 | 25 | 10000 |
| 9i    | Et | Et | CH₂ | N | CH₂ | 4-Cl-Ph | 365 | 33 | 4.1 | 12 | 17 | 1.4 | 12 | 2 | 88 | 10000 |
| 9j    | Et | Et | CH₂ | N | CH₂ | 4-OMe-Ph | 361 | 42 | 3.4 | 53 | 79 | 1.5 | 60 | 5.4 | 185 | 10000 |
| 9k    | Et | Et | CH₂ | N | CH₂ | 4-Me-Ph | 344 | 33 | 3.8 | 14 | 10000 | 714 | 41 | 2 | 200 | 10000 |
| 9l    | Et | Et | CH₂ | N | CH₂ | 3-CN-Ph | 355 | 57 | 3.2 | 46 | 159 | 3.5 | 9.9 | 3.2 | 200 | 10000 |
| 9m    | Et | Et | CH₂ | N | CH₂ | 3-CF₃-Ph | 398 | 33 | 4.5 | 12 | 65 | 5.4 | 9.8 | 2.9 | 109 | 10000 |
| 9n    | Et | Et | CH₂ | N | CH₂ | 3-Cl-Ph | 365 | 33 | 4.1 | 9.9 | 84 | 8.5 | 6.7 | 2.1 | 107 | 10000 |
| 9o    | Et | Et | CH₂ | N | CH₂ | 3-Me-Ph | 344 | 33 | 3.8 | 30 | 59 | 2.0 | 15.2 | 2 | 200 | 10000 |
| 9p    | Et | Et | CH₂ | N | CH₂ | 3-OMe-Ph | 360 | 42 | 3.4 | 62 | 169 | 2.7 | 37.8 | 2 | 200 | 10000 |
| 9q    | Et | Et | CH₂ | N | CH₂ | 2-CN-Ph | 355 | 57 | 3.2 | 61 | 351 | 5.8 | 3.6 | 2 | 200 | 10000 |
| 9r    | Et | Et | CH₂ | N | CH₂ | 2-CF₃-Ph | 398 | 33 | 4.5 | 17 | 67 | 3.9 | 8.9 | 2 | 72 | 10000 |
| 9s    | Et | Et | CH₂ | N | CH₂ | 2-Cl-Ph | 365 | 33 | 4.1 | 7.0 | 35 | 5.0 | 11 | 2 | 200 | 10000 |
| 9t    | Et | Et | CH₂ | N | CH₂ | 2-Me-Ph | 344 | 33 | 3.8 | 9.3 | 36 | 3.9 | 22 | 2 | 194 | 7400 |
| 9u    | Et | Et | CH₂ | N | CH₂ | 2-OMe-Ph | 360 | 42 | 3.4 | 44 | 1168 | 26.5 | 20 | 2.3 | 200 | 10000 |
| 9v    | Et | Et | CH₂ | N | CH₂ | 2-iPr-Ph | 373 | 33 | 4.6 | 5.9 | 195 | 33.1 | 7.5 | 2.4 | 37 | 10000 |
| 9w    | Et | Et | CH₂ | N | CH₂ | 2,4-di-Me-Ph | 359 | 33 | 4.0 | 9.2 | 10 | 1.0 | 27 | 2 | 126 | 10000 |
| 9x    | Et | Et | CH₂ | N | CH₂ | 2-Py | 331 | 46 | 2.6 | 268 | 1499 | 5.6 | 46 | 2 | 200 | 10000 |
| 9y    | Et | Et | CH₂ | N | CH₂ | 3-Py | 331 | 46 | 2.2 | 10000 | 10000 | 1.0 | 52 | 5.4 | 193 | 10000 |
| 9z    | Et | Et | CH₂ | N | CH₂ | 4-Py | 331 | 46 | 2.2 | 142 | 10000 | 70.4 | 60 | 60 | 199 | 10000 |

*Sigma-2 assays: Conducted with PC12 membrane preparations. Radioligand: [3H]-DTG, Kd = 9.9 nM. Reference standard: Haloperidol, Ki = 13.9 nM **Sigma-1 assays: Conducted with HEK293 membrane preparations. Radioligand: [3H]-Pentazocine, Kd = 6.5 nM. Reference standard: Haloperidol, Ki = 3.54 nM.
\( \sigma_2 (K_i = 9.2 \text{ nM}) \) and \( \sigma_1 (K_i = 10 \text{ nM}) \). In addition, while some of these compounds demonstrated moderate stability in HLM assays (9o, 9p, 9s, 9t, and 9u \( T_{1/2} = 11.4-37.8 \text{ min} \)), none of them were stable in MLM (\( T_{1/2} < 5 \text{ min} \)).

We next assessed the impact of replacing the phenyl ring with a pyridine ring. Interestingly, incorporating a 2-pyridine (9x, \( \sigma_2 K_i = 268 \text{ nM} \)) or a 4-pyridine (9z, \( \sigma_2 K_i = 142 \text{ nM} \)) lead to a 3.2x and 1.7x loss in \( \sigma_2 \) binding affinity when compared to the phenyl analog (9a, \( \sigma_2 K_i = 82 \text{ nM} \)). However, the 3-pyridine analog (9y, \( \sigma_2 K_i = 10,000 \text{ nM} \)) displayed a >100-fold drop in affinity over (9a). \( \sigma_1 \) affinity was also diminished in all three pyridine analogs, but the decrease in affinity was not uniform. While (9x) \( \sigma_1 \) binding affinity (\( K_i = 1499 \text{ nM} \)) was ~10x less than (9a) (\( K_i = 138 \text{ nM} \)), both (9y) and (9z) were >120x less potent binders for this target (\( \sigma_1 K_i = 10,000 \text{ nM} \) for both). Evaluation of these compounds in MLM and HLM assays demonstrated that while all three were highly stable in HLM (\( T_{1/2} = 46.3 \text{ to } 60 \text{ min} \)), only (9z) was stable in MLM (\( T_{1/2} = 60 \text{ min} \)). We also determined the aqueous solubility and Cyp3A4 inhibitory capacity of all of the aforementioned compounds. As noted in Table 1 and discussed above, the majority of compounds had high solubility (>100 \( \mu \text{M} \)), Cyp 3A4 inhibition was low (\( IC_{50} = 7400 \text{ nM} \)), and their physicochemical properties (MW, TPSA, cLogP) are all within the drug-like properties defined by Lipinski [20].

### Conclusion

In summary, a series of substituted lactones with drug-like physicochemical properties (MW, TPSA, cLogP) have been investigated as potential selective \( \sigma_2 \)R ligands. We have determined that conversion of either of the piperidine amine units to the corresponding amide causes a significant loss in activity at both \( \sigma_2 \)Rs (9c, 9d), while replacement of the aliphatic amine of the piperazine is well tolerated (9e). In addition, we have shown that the electronic, steric, and lipophilic character of the ring appended to the piperazine moiety is critical to identifying compounds that are both 1) highly selective for \( \sigma_2 \) over \( \sigma_1 \), and 2) highly stable in HLM and MLM (9f-9z). Importantly, all of the compounds examined are soluble in aqueous media and none appear to have significant impact on Cyp3A4 activity.

Based on our finding, we have identified (9z) as our preliminary lead compound for future studies and will be advancing this compound into mouse in vivo PK studies. Unlike the other compounds described above, (9z) is a moderate affinity \( \sigma_2 \) ligand (\( K_i = 142 \text{ nM} \)), with excellent selectivity for this target over \( \sigma_1 \) (\( K_i = 10,000 \text{ nM} \)) and it is highly stable in both MLM and HLM (\( T_{1/2} = 60 \text{ min} \)). 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 \( \sigma_2 \) activity [21–23] such as schizophrenia, Alzheimer’s disease, neuropathic pain, traumatic brain injury, and cancer.

### 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 (\( \delta \) 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 \( \mu \text{m} \), 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 6130 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.

![Synthesis of methyl 2,2-dimethylpent-4-enoate (5a, \( R_1 \), \( R_2 = \text{Me} \)): This reaction was performed in oven-dried glassware under a nitrogen atmosphere. To a well-stirred solution of freshly prepared lithium diisopropylammmide (1 M, 1.10 equiv) in dry 35 ml tetrahydrofuran, isobutyrac acid methyl ester (3.32 g, 32.6 mmol, 1.0 equiv) was added dropwise during 0.5 h at −78 °C. The mixture was allowed to stir at this temperature for 30 min followed by the addition of allyl bromide (5.35 g, 44.0 mmol) and Hexamethylphosphoramide (HMPA) (2.91 g, 16.3 mmol) dropwise over 0.5 h. The reaction mixture was stirred overnight at room temperature, quenched with 10% HCl (while cooling in ice bath) until acidic (pH = 2). The organic layer was separated and the aqueous layer was extracted with hexanes (3 × 100 mL). The extract was washed with 10% NaHCO3 (200 mL) and brine (200 mL). The solution was then dried over MgSO4, concentrated in vacuo and distilled (bp. 85.5–86.5 °C/3.5 mm Hg) to provide 3.47 g (75% yield) of the product as a colorless oil.](image_url)
Synthesis of ethyl 2,2-diethylpent-4-enoate (5b, R1, R2 = Et): The title compound was prepared according to the procedure for methyl 2,2-dimethylpent-4-enoate, except 2-ethyl-butrylic acid ethyl ester was substituted for isobutyric acid methyl ester. The product was isolated as a colorless oil. (66% yield) \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 5.68 (1H, dd, \(J = 9.9, 17.2, H-4\)), 5.16–4.97 (2H, m, H-5), 4.14 (2H, q, \(J = 7.1\), OCH\(_2\)CH\(_3\)), 2.33 (2H, d, \(J = 7.4, H-3\)), 1.59 (6H, dt, \(J = 6.5, 7.5, H-3^{'}) \), 1.26 (3H, t, \(J = 7.1\), OCH\(_2\)CH\(_3\)), 0.80 (6H, t, \(J = 7.5, H-4^{'}) \). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 175.6 (CO), 135.5 (CH, C-4), 115.2 (CH\(_2\), C-5), 61.8 (CH\(_2\)), 46.3 (C, C-2), 32.2 (CH\(_2\), C-3), 29.8 (CH\(_2\), C-3'), 14.3 (CH\(_3\), OCH\(_2\)CH\(_3\)), 10.5 (CH\(_3\), C-4').

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\begin{align*}
\text{Synthesis of 5-(2-hydroxy-ethyl)-3,3-dimethyl-dihydro-furan-2-one (7a, R1, R2 = Me): A mixture of glacial acetic acid (28.6 g, 477 mmol, 53.6 equiv), paraformaldehyde (0.80 g, 26.7 mmol, 3.0 equiv) and H\(_2\)SO\(_4\) (0.5 g, 4.45 mmol, 0.57equiv) was stirred for 30 min at 70 °C before methyl 2,2-dimethylpent-4-enoate (5a) (1.26 g, 8.9 mmol, 1.0 equiv) was added dropwise during 10 min. The reaction mixture was then maintained at 70–80 °C and allowed to stir overnight. Acetic acid was removed under reduced pressure and the reaction was quenched with 10% NaHCO\(_3\) solution. The mixture was then extracted with ethyl acetate (3 × 50 mL) and the combined organic phase was concentrated in vacuo to give a crude oil. The crude oil was used for next step without further purification.}
\end{align*}
\]

A mixture of the crude oil and 30% NaOH (7.1 g NaOH, 177 mmol, 20 equiv) aqueous solution was refluxed for 2 h. The mixture was cooled in an ice bath and excess 30% H\(_2\)SO\(_4\) was added until acidic (pH< 2). The resulting mixture was extracted with ethyl acetate (3 × 200 mL), the combined organic phase was washed with 10% NaHCO\(_3\), (400 mL), brine (400 mL), dried over MgSO\(_4\) and concentrated in vacuo to give a crude product which was further purified by column chromatography (Ethyl acetate/Hexanes, 10–60%) to provide the product as a clear oil (1.05 g, 73%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 4.70–4.60 (1H, m, CH), 3.90–3.78 (2H, m, –CH\(_2\)CH\(_3\)OH), 2.22 (1H, dd, \(J = 5.9, 12.7, H_2\)), 1.98–1.87 (2H, m, –CH\(_2\)CH\(_2\)OH), 1.80 (1H, dd, \(J = 5.9, 12.7, H_2\)), 1.29 (3H, s, CH\(_3\)), 1.28 (3H, s, CH\(_3\)). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 182.26 (CO), 75.01 (CH), 59.58 (~CH\(_2\)CH\(_3\)OH), 43.93 (CH\(_2\)), 40.62 (~C(CH\(_3\))), 38.69 (~CH\(_2\)CH\(_3\)OH), 25.31 (CH\(_3\)), 24.61 (CH\(_3\)); Rf, 0.34 (Hexane: Ethyl Acetate 1:1); Anal. Calcd for C\(_9\)H\(_{13}\)O\(_3\): C, 60.74; H, 8.92; Found: C, 60.47; H, 8.86.

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\begin{align*}
\text{Synthesis of 3,3-diethyl-5-(2-hydroxyethyl)dihydrofuran-2(3H)-one (7b): The title compound was prepared according to the procedure for 5-(2-Hydroxy-ethyl)-3,3-dimethyl-dihydro-furan-2-one, except ethyl 2,2-diethylpent-4-enoate was substituted for methyl 2,2-dimethylpent-4-enoate. The product was isolated as a colorless oil. (76% yield): \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 4.62 (dd, \(J = 5.3, 7.3, 9.5, 1.1\)), 3.78 (dt, \(J = 6.1, 2.2\)), 3.20 (s, 1H), 2.19 (dd, \(J = 6.8, 13.1, 1.1\)), 1.97–1.81 (m, 3H), 1.70–1.56 (m, 4H), 0.93 (dt, \(J = 7.5, 20.7, 6H\)); \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 181.46 (CO), 75.10 (CH), 58.91 (~CH\(_2\)CH\(_3\)OH), 48.77 (C), 39.13 (CH\(_2\)), 37.76 (~CH\(_2\)CH\(_2\)OH), 29.21 (CH\(_2\)CH\(_3\)), 28.30 (~CH\(_2\)CH\(_2\)), 8.83 (~CH\(_2\)CH\(_3\)), 8.73 (~CH\(_2\)CH\(_2\)); Rf, 0.36 (Hexane: Ethyl Acetate 5:2); Anal. Calcd for C\(_{10}\)H\(_{15}\)O\(_3\): C, 64.49; H, 9.74; Found: C, 64.20; H, 9.57.
\end{align*}
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\begin{align*}
\text{2-(4,4-dimethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulphonate (8a): To a stirred solution of 5-(2-Hydroxy-ethyl)-3,3-dimethyl-dihydro-furan-2-one (0.316 g, 2.5 mmol, 1.0 equiv) and Et\(_3\)N (0.152 g, 1.5 mmol, 1.5 equiv) in dry dichloromethane, a solution of p-TosCl (0.475 g, 2.5 mmol, 1.25 equiv) in dichloromethane was added drop wise at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and allowed to stir overnight at room temperature. Then, the reaction mixture was diluted with dichloromethane (50 mL), washed with 10 % HCl, brine, dried over MgSO\(_4\) and concentrated in}
\end{align*}
\]

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\begin{align*}
\text{OCH}_3\text{, 2.28 (2H, d, \(J = 7.4\), H-3), 1.17 (6H, s, H-2')); \(^{13}\)C}\text{ NMR (101 MHz, CDCl}_3\text{) \(\delta\) 177.42 (C=O), 134.42 (CH, C-4), 117.88 (CH\(_2\), C-5), 60.35 (~CO\(_2\)CH\(_3\)), 44.91 (C, C-2), 42.25 (CH\(_2\), C-3), 24.92 (CH(CH\(_3\))\(_2\)), 2.33 (2H, d, \(J = 7.4, H-3\)), 1.59 (6H, dt, \(J = 6.5, 7.5, H-3^{'}) \), 1.26 (3H, t, \(J = 7.1\), OCH\(_2\)CH\(_3\)), 0.80 (6H, t, \(J = 7.5, H-4^{'}) \). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 175.6 (CO), 135.5 (CH, C-4), 116.2 (CH\(_2\), C-5), 61.8 (CH\(_2\)), 46.3 (C, C-2), 32.2 (CH\(_2\), C-3), 29.8 (CH\(_2\), C-3'), 14.3 (CH\(_3\), OCH\(_2\)CH\(_3\)), 10.5 (CH\(_3\), C-4').}
\end{align*}
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vacuo to afford yellowish oil. This crude product was then purified by flash chromatography (silica gel; Ethyl acetate/Hexanes, 0–40%) to afford desired tosylate as a clear oil (424 mg, 67% yield). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.72 (2H, m, CH$_2$), 7.29 (2H, m, CH$_2$), 4.39 (1H, m, CH), 4.10 (2H, m, CH$_2$CH$_2$OTos), 2.38 (3H, s, CH$_3$), 2.09 (1H, m, CH$_2$), 1.93 (2H, m, CH$_2$CH$_2$OTos), 1.65 (1H, m, CH$_2$), 1.16 (6H, s, CH$_3$), 1.15 (6H, s, CH$_3$); $^{13}$C NMR (101 MHz, CDCl$_3$) $^{13}$C NMR (101 MHz, CDCl$_3$) δ 181.26 (CO), 145.16 (C), 132.53 (C), 130.03 (CH), 127.84 (CH), 72.93 (CH), 66.83 (CH$_2$CH$_2$O-SO$_2$), 42.99 (CH$_2$), 40.23 (C), 34.97 (CH$_2$CH$_2$O-SO$_2$), 24.82 (CH$_3$), 24.12 (CH$_3$), 21.57 (CH$_3$); HRMS (Cl): [M + H] $^+$ 313.1; Anal. Calcd for C$_{15}$H$_{20}$O$_5$S: C, 57.67; H, 6.45; Found: C, 57.85; H, 6.63.

Synthesis of 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate (8b): The title compound was prepared according to the procedure for 2-(4,4-dimethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate, except 3,3-diethyl-5-(2-hydroxyethyl)dihydrofuran-2(3H)-one was substituted for 5-(2-Hydroxyethyl)-3,3-dimethyl-dihydrofuran-2-one, 69% yield. The product was isolated as a colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.79 (2H, d, J = 8.3 Hz, CH), 7.36 (2H, d, J = 8.0 Hz, CH), 4.55–4.33 (1H, m, CH), 4.14 (2H, dd, J = 6.5, 13.3 Hz, CH$_2$CH$_2$OTos), 2.46 (3H, s, CH$_3$), 2.21–1.84 (3H, m, CH$_2$CH$_2$OTos and CH$_2$), 1.83–1.68 (1H, m, CH$_2$), 1.58 (4H, t, J = 7.4 Hz, CH$_2$CH$_2$), 0.89 (6H, dt, J = 7.5, 18.0 Hz, CH$_3$CH$_2$); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 180.33 (CO), 145.30 (SO$_2$-C), 132.72 (CCH$_3$), 130.15 (CH), 128.03 (CH), 73.18 (CH), 66.95 (CH$_2$CH$_2$O-SO$_2$), 48.67 (C), 37.53 (CH$_2$), 35.82 (CH$_2$CH$_2$O-SO$_2$), 29.14 (CH$_2$CH$_2$), 28.23 (CH$_2$CH$_2$), 21.76 (CH$_3$), 8.81 (CH$_2$CH$_3$), 8.74 (CH$_2$CH$_3$). Anal. Calcd for C$_{17}$H$_{20}$O$_5$S: C, 59.98; H, 7.11; Found: C, 60.27; H, 7.25.

Synthesis of 2-(4-(4-hydroxy-4-dimethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9q): 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate (0.102 g, 0.3 mmol, 1.0 equiv) was treated with 2-piperazin-1-yl-benzonitrile (168.3 mg, 0.9 mmol, 3.0 equiv) in dry tetrahydrofuran and refluxed for 72 h. The tetrahydrofuran was evaporated under reduced pressure, the residue dissolved in dichloromethane, washed with H$_2$O, and brine, then dried over MgSO$_4$ and concentrated in vacuo to give a crude product which was purified by flash chromatography (silica gel; 2–8% MeOH in dichloromethane) to afford pure product as a yellow oil. (53.4 mg, 50% yield). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.62–7.42 (2H, m, CH), 7.01 (2H, dd, J = 7.8, 5.0 Hz, CH), 4.48 (1H, dq, J = 9.2, 6.7 Hz, CH), 3.35–3.17 (4H, m, NCH$_2$CH$_2$N), 2.81–2.51 (6H, m, NCH$_2$CH$_2$N and CH$_2$CH$_2$N), 2.14 (1H, dd, J = 13.1, 6.8 Hz, CH$_2$), 1.86 (3H, m, CH$_2$CH$_2$N and CH$_2$), 1.67–1.53 (4H, m, CH$_2$CH$_3$), 0.92 (6H, dt, J = 20.1, 7.5 Hz, CH$_2$CH$_3$); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 180.82 (CO), 155.57 (C), 134.43 (CH), 133.95 (CH), 122.03 (C-CN), 118.81 (CH), 118.50 (CH), 106.13 (C-CN), 75.50 (CH), 54.44 (C), 53.22 (NCH$_2$CH$_2$N), 51.34 (NCH$_2$CH$_2$N), 48.71 (NCH$_2$CH$_2$), 37.75 (CH$_2$), 33.60 (NCH$_2$CH$_2$), 29.35 (CH$_2$CH$_3$), 28.39 (CH$_2$CH$_3$), 8.89 (CH$_2$CH$_3$), 8.81 (CH$_2$CH$_3$); MS (LC/MS, M + H$^+$): 356.2.

3,3-diethyl-5-(2-(4-phenylpiperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (9a): The title compound was prepared according to the procedure for 2-(4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9q), except 2-piperazin-1-yl-benzonitrile was substituted for 2-piperazin-1-yl-benzonitrile (50.6 mg, 51% yield): $^1$H NMR (400 MHz, D$_2$O) δ 7.43 (2H, m, CH), 7.27–7.13 (3H, m, CH), 4.69 (1H, m, CH), 4.11–3.09 (10H, m, NCH$_2$CH$_2$N and CH$_2$CH$_2$N), 2.39–2.07 (3H, m, CH$_2$CH$_2$N and CH$_2$), 1.98 (1H, dd, J = 13.4, 9.4 Hz, CH$_2$), 1.61 (4H, m, CH$_2$CH$_2$), 0.87 (6H, dt, J = 12.1, 7.5 Hz, CH$_3$CH$_2$); $^{13}$C NMR (101 MHz, D$_2$O) δ 187.92 (CO), 150.20 (C), 132.89 (CH), 127.03 (CH), 121.14 (CH), 79.53 (CH), 56.52 (C), 54.13 (NCH$_2$CH$_2$N), 52.41 (NCH$_2$CH$_2$N), 50.87 (NCH$_2$CH$_2$), 39.37 (CH$_2$), 32.81 (NCH$_2$CH$_2$), 31.91 (CH$_2$CH$_3$), 30.68 (CH$_2$CH$_3$), 11.00 (CH$_2$CH$_3$), 10.87 (CH$_2$CH$_3$); MS (LC/MS, M + H$^+$): 331.2; Anal. Calcd for C$_{20}$H$_{32}$Cl$_2$N$_2$O$_2$: C, 59.55; H, 8.00; N, 6.94; Found: C, 59.62; H, 8.11; N, 6.90. Melting point of di HCl salt: 239 °C.

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Synthesis of 3,3-dimethyl-5-(2-(4-phenylpiperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (9b): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9q), 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 2-piperazin-1-yl-benzonitrile was substituted for 2-piperazin-1-yl-benzonitrile. The crude product was purified by flash chromatography (silica; MeOH:dichloromethane, 0% ~ 10%) to provide the product as an oil (50.9 mg, yield 56%).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.32 (2H, m, CH), 6.99 (2H, d, $J = 7.9$ Hz, CH), 6.91 (1H, t, $J = 7.2$ Hz, CH) 4.58 (1H, m, CH), 3.26 (4H, t, $J = 5.0$ Hz, NCH$_2$CH$_2$N), 2.66 (4H, m, NCH$_2$CH$_2$N), 2.61 (2H, m, CH$_2$CH$_2$N), 2.26 (1H, m, CH$_3$), 1.90 (3H, m, CH$_2$CH$_2$N and CH$_2$), 1.34 (3H, s, CH$_3$), 1.33 (3H, s, CH$_3$). $^{13}$C NMR (101 MHz, MeOD) $\delta$ 184.35 (CO), 152.68 (C), 130.07 (CH), 121.21 (CH), 117.49 (CH), 77.42 (CH), 55.67 (NCH$_2$CH$_2$N), 54.31 (CH$_2$), 50.29 (NCH$_2$CH$_2$N), 44.18 (CH$_2$CH$_2$N), 41.71 (C(CH$_3$)$_2$), 33.74 (CH$_2$CH$_2$N), 25.27 (CH$_3$), 24.59 (CH$_3$).

LC/MS $[M + H] \equiv m/z$ 303.2.

Synthesis of 4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)-1-phenylpiperazin-2-one (9d): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9q), except 1-phenylpiperazin-2-one was substituted for 2-piperazin-1-yl-benzonitrile (50.4 mg, yield 56%). The product was isolated as a clear oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.42–7.34 (2H, m, CH), 7.31–7.21 (3H, m, CH), 4.51 (1H, m, CH), 3.91–3.61 (2H, m, NCH$_2$CH$_2$N), 3.30 (2H, dd, $J = 13.3$, 16.3 Hz, NCH$_2$CO), 2.82 (2H, t, $J = 5.7$ Hz, CH$_2$CH$_2$N), 2.62 (2H, d, $J = 7.0$ Hz, NCH$_2$CH$_2$N), 2.14 (1H, dd, $J = 6.8$, 13.1 Hz, CH$_2$), 1.89–1.78 (3H, m, CH$_2$CH$_2$N, CH$_2$), 1.68–1.55 (4H, m, CH$_2$CH$_2$N), 0.92 (6H, dt, $J = 7.5$, 21.0 Hz, CH$_3$CH$_2$). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 180.62 (CO), 141.54 (CO), 129.54 (C), 127.27 (CH), 125.91 (CH), 124.53 (CH), 74.86 (CH), 53.88 (C(CH$_2$CH$_2$)$_2$), 50.80 (NCH$_2$CH$_2$N), 50.21 (NCH$_2$CH$_2$N), 49.43 (CH$_2$), 48.62 (CH$_2$CH$_2$N), 37.62 (NCH$_2$CO), 33.28 (CH$_2$CH$_2$N), 29.20 (CH$_2$CH$_2$), 28.25 (CH$_2$CH$_3$), 8.77 (CH$_2$CH$_3$), 8.70 (CH$_2$CH$_3$). MS (LC/MS, M + H$^+$) 345.2.

Synthesis of 3,3-diethyl-5-(2-oxo-2-(4-phenylpiperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (9e): The title compound was prepared according to the procedures described by R. Goa [24]. $^1$H NMR (400 MHz, MeOD) $\delta$ 7.80–7.68 (2H, m, CH), 7.67–7.49 (3H, m, CH), 4.09 (4H, broad, NCH$_2$CH$_2$N), 3.86–3.62 (4H, m, NCH$_2$CH$_2$N), 3.30 (1H, dt, $J = 3.3$, 1.6 Hz, CH), 2.95 (2H, ddd, $J = 21.3$, 16.2, 6.0 Hz, CH$_2$), 2.34 (1H, dd, $J = 13.3$, 6.8 Hz, CH$_2$), 2.03 (1H, dd, $J = 13.3$, 9.5 Hz, CH$_2$), 1.77–1.49 (4H, m, CH$_2$CH$_3$), 0.94 (6H, dt, $J = 18.7$, 7.5 Hz, CH$_2$CH$_3$). $^{13}$C NMR (101 MHz, MeOD) $\delta$ 182.79 (CO), 143.18 (C), 131.74 (CH), 131.40 (CH), 122.20 (CH), 75.73 (CH), 56.13 (C(CH$_2$CH$_2$N)$_2$), 49.93 (CH$_2$), 44.34 (NCH$_2$CH$_2$N), 40.21 (NCH$_2$CH$_2$N), 39.68 (NCH$_2$CH$_2$N), 38.30 (C(CH$_2$C(O)-N)), 30.06 (CH$_2$CH$_3$), 29.14 (CH$_2$CH$_3$), 9.04 (CH$_2$CH$_3$), 8.93 (CH$_2$CH$_3$).
was prepared according to the procedure for 22-(4-(4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9q), except 4-phenyl-piperidine was substituted for 2-piperazin-1-yl-benzonitrile (48.5 mg, 49% yield): 1H NMR (400 MHz, D2O) δ 7.39 (5H, t, J = 7.3, 14.3, CH), 4.71 (s, 1H), 3.72 (s, 2H), 3.36 (s, 2H), 3.17 (s, 2H), 2.98 (s, 1H), 2.37 (dd, J = 6.9, 13.4, 1H), 2.31–2.10 (m, 4H), 2.02 (dd, J = 9.4, 13.5, 3H), 1.78–1.53 (4H, m, CH2CH2), 0.92 (6H, dt, J = 7.5, 12.7, CH2CH3); 13C NMR (101 MHz, D2O) δ 187.89, 146.67, 131.85, 130.03, 129.59, 52.33, 41.74, 39.25, 32.90, 31.85, 30.60, 10.89, 10.76; MS (LC/MS, M+): 320.2; Anal. Calcd for C21H32ClNO2: C, 68.93; H, 8.81; N, 3.79. Melting point of HCl salt: 239.5 °C

Synthesis of 5-(2-(4-(4-(triﬂuoromethyl)phenyl)piperazin-1-yl)ethyl) dihydrofuran-2(3H)-one (9h): The title compound was prepared according to the procedure for 2-(4-(2-(4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9q) except 1-cyclohexyl-piperazine was substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil. 1H NMR (400 MHz, DMSO) δ 4.60–4.49 (m, 1H), 3.93–3.45 (m, 8H), 3.23 (s, 3H), 2.25–2.01 (m, 5H), 1.89–1.72 (m, 3H), 1.68–1.02 (m, 11H), 0.91–0.76 (m, 6H); 13C NMR (101 MHz, DMSO) δ 179.73, 74.15, 64.22, 52.26, 48.34, 47.85, 44.84, 36.45, 28.27, 27.60, 25.90, 24.57, 24.36, 8.54, 8.48; MS (LC/MS, M+H+): 337.3

Synthesis of 4-(4-(2-(4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9g): The title compound was prepared according to the procedure for 2-(4-(2-(4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9q), except 4-piperazin-1-yl-benzonitrile was substituted for 2-piperazin-1-yl-benzonitrile (48.5 mg, 45% yield): 1H NMR (400 MHz, MeOD) δ 7.69–7.54 (2H, m, CH), 7.23–7.02 (2H, m, CH), 4.59 (1H, dd, J = 15.8, 9.3, 3.7 Hz, CH), 4.31–3.30 (10H, m, NCH2CH2N, NCH2CH2N, and CH2CH2N), 2.36–2.21 (2H, m, CH2), 2.21–2.06 (1H, m, CH2), 1.96 (1H, dd, J = 13.3, 9.4 Hz, CH2), 1.65 (4H, ddd, J = 17.4, 8.7, 6.2 Hz, CH2CH2), 0.95 (6H, dt, J = 13.3, 7.5 Hz, CH2CH3); 13C NMR (101 MHz, MeOD) δ 182.32 (CO), 153.74 (C), 134.73 (CH), 120.40 (CN), 116.55 (CH), 102.99 (C), 76.15 (CH), 54.93 (C CH2CH2), 52.76 (NCH2CH2N), 49.91 (NCH2CH2N), 45.91 (CH2CH2N), 38.33 (CH2), 31.73 (CH2CH2N), 30.04 (CH2CH3), 29.17 (CH2CH3), 9.00 (CH2CH3), 8.91 (CH2CH3); MS (LC/MS, M+): 356.2; Anal. Calcd for C21H32ClNO2: C, 64.46; H, 7.65; N, 10.72; Found: C, 64.46; H, 7.65; N, 10.65. Melting point of di-HCl salt: 213–214°C

Synthesis of 3,3-diethyl-5-(2-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)ethyl) dihydrofuran-2(3H)-one (9h): The title compound was prepared according to the procedure for 2-(4-(2-(4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9q) except 1-(trifluoromethyl)phenyl-piperazine was substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (Yield: 73%). 1H NMR (400 MHz, CDCl3) δ 7.48 (2H, d, J = 8.6 Hz, CH), 6.93 (2H, d, J = 8.6 Hz, CH), 4.50 (1H, m, CH), 3.29 (4H, t, J = 5.0 Hz, NCH2CH2N), 2.69–2.48 (6H, m, NCH2CH2N, and CH2CH2N), 2.15 (1H, dd, J = 6.8, 13.1 Hz, CH2), 1.98–1.78 (3H, m, CH2), 1.69–1.58 (4H, m, CH2CH3), 0.94 (6H, dt, J = 7.5, 19.0 Hz, CH2CH3); 13C NMR (101 MHz, MeOD) 183.18 (CO), 154.91 (C), 127.30 (CH), 125.01 (CF3), 115.75 (CH), 77.59 (CH), 55.95 (C CH2CH2), 54.07 (NCH2CH2N), 50.13 (CH2CH2N), 48.67 (CH2CH2N), 38.45 (CH2), 34.36 (CH2CH3), 30.26 (CH2CH3), 29.32 (CH2CH3), 9.04 (CH2CH3), 8.95 (CH2CH3); LC/MS [M + H] = m/z 399.2
Synthesis of 5-(2-(4-(4-chlorophenyl)piperazin-1-yl)ethyl)-3,3-diethylidihydrofuran-2(3H)-one (9i): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9q), except 1-(4-chlorophenyl)piperazine was substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (Yield: 83%). 1H NMR (400 MHz, CDCl₃) δ 7.20 (2H, d, J = 9.0 Hz, CH), 6.84 (2H, d, J = 9.0 Hz, CH), 4.49 (1H, m, CH), 3.17 (4H, t, J = 5.0 Hz, NCH₂CH₂N) 2.68-2.48 (6H, m, NCH₂CH₂N, and CH₂CH₂N), 2.15 (1H, dd, J = 6.9, 12.8 Hz, CH₂), 1.96–1.77 (3H, m, CH₂), 1.70–1.56 (4H, m, CH₂CH₃), 0.94 (6H, d, J = 9.0 Hz, CH₂), 1H NMR (400 MHz, MeOD) δ 183.16 (CO), 151.40 (C), 129.89 (CH), 125.55 (C), 118.53 (CH), 77.61 (CH), 55.51 (C(CH₂CH₂)₂), 54.20 (NCH₂CH₃N), 50.12 (NCH₂CH₃N), 49.94 (CH₂CH₃N), 38.46 (CH₂), 34.37 (CH₂CH₂N), 30.26 (CH₂CH₃), 29.33 (CH₂CH₃), 9.07 (CH₂CH₃), 8.98 (CH₂CH₃), LC/MS [M+H] = m/z 365.2.

Synthesis of 3,3-diethyl-5-(2-(4-(4-methoxyphenyl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (9j): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9q), except 1-(4-methoxyphenyl)-piperazine was substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (67.1 mg, 62% yield). 1H NMR (400 MHz, CDCl₃) δ 6.95–6.75 (4H, m, CH₂), 4.62–4.45 (1H, m, CH), 4.05–3.28 (10H, m, NCH₂CH₃N, and CH₂CH₃N), 3.76 (3H, s, CH₃), 3.14–2.99 (4H, m, NCH₂CH₃N, and CH₂CH₃N), 2.67–2.46 (6H, m, NCH₂CH₃N, and CH₂CH₃N), 2.15–2.07 (1H, m, CH₂), 1.92–1.79 (3H, m, CH₂), 1.62 (4H, qd, J = 7.4, 4.7 Hz, CH₂CH₃), 0.97–0.88 (6H, m, CH₃CH₃); 13C NMR (101 MHz, CDCl₃) δ 180.90 (CO), 153.93 (C), 145.74 (C), 118.29 (CH), 114.53 (CH), 75.71 (CH), 55.67 (OCH₃), 54.59 (C(CH₂CH₃)₂), 53.51 (NCH₂CH₃N), 50.69 (NCH₂CH₃N), 48.72 (CH₂CH₃N), 37.81 (CH₂), 33.91 (CH₂CH₃N), 29.35 (CH₂CH₃), 28.41 (CH₂CH₃), 8.90 (CH₂CH₃), 8.82 (CH₂CH₃). MS (LC/MS, M+H): 361.2.

Synthesis of 3-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9l): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9q), except 1-p-tolyl-piperazine was substituted for 2-piperazin-1-yl-benzonitrile (52.8 mg, 51% yield): 1H NMR (400 MHz, MeOD) δ 7.25–7.13 (4H, m, CH), 4.62–4.45 (1H, m, CH), 4.05–3.28 (10H, m, NCH₂CH₃N, and CH₂CH₃N), 2.30–2.01 (6H, m, CH₃ and CH₂), 1.88 (1H, dd, J = 13.3, 9.4 Hz, CH₂), 1.58 (4H, m, CH₂CH₃), 0.87 (6H, d, J = 13.7, 7.5 Hz, CH₂CH₃); 13C NMR (101 MHz, MeOD) δ 182.36 (CO), 144.75 (C), 136.86 (C), 131.47 (CH), 119.95 (CH), 76.10 (CH), 54.89 (C(CH₂CH₃)₂), 52.04 (NCH₂CH₃N), 50.53 (NCH₂CH₃N), 49.93 (CH₂CH₃N), 38.31 (CH₂), 31.68 (CH₂CH₃N), 30.03 (CH₂CH₃N), 29.17 (CH₂CH₃), 20.76 (CH₃), 9.01 (CH₂CH₃), 8.92 (CH₂CH₃); MS (LC/MS, M+H⁺): 345.2; Anal. Calcd for C₂₁H₃₄Cl₂N₂O₂: C, 60.43; H, 8.21; N, 6.71; Found: C, 60.33; H, 8.20; N, 6.61. Melting point of di-HCl salt: 213–217°C.

Synthesis of 3,3-diethyl-5-(2-(4-(4-methoxyphenyl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (9k): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9q), except 1-(4-methoxyphenyl)piperazine was substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (67.1 mg, 62% yield). 1H NMR (400 MHz, CDCl₃) δ 6.95–6.75 (4H, m, CH₂), 4.48 (1H, ddd, J = 19.8, 8.4, 6.4 Hz, CH), 3.76 (3H, s, CH₃), 3.14–2.99 (4H, m, NCH₂CH₃N, and CH₂CH₃N), 2.67–2.46 (6H, m, NCH₂CH₃N, and CH₂CH₃N), 2.15–2.07 (1H, m, CH₂), 1.92–1.79 (3H, m, CH₂), 1.62 (4H, qd, J = 7.4, 4.7 Hz, CH₂CH₃), 0.97–0.88 (6H, m, CH₃CH₃); 13C NMR (101 MHz, CDCl₃) δ 180.90 (CO), 153.93 (C), 145.74 (C), 118.29 (CH), 114.53 (CH), 75.71 (CH), 55.67 (OCH₃), 54.59 (C(CH₂CH₃)₂), 53.51 (NCH₂CH₃N), 50.69 (NCH₂CH₃N), 48.72 (CH₂CH₃N), 37.81 (CH₂), 33.91 (CH₂CH₃N), 29.35 (CH₂CH₃), 28.41 (CH₂CH₃), 8.90 (CH₂CH₃), 8.82 (CH₂CH₃). MS (LC/MS, M+H): 361.2.
was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9q), except 3-(piperazin-1-yl)benzonitrile was substituted for 2-piperazin-1-yl-benzonitrile. In addition the crude product was purified by flash chromatography (silica: MeOH:dichloromethane, 0–10%). The product was isolated as a clear oil (Yield: 73%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.30 (1H, m, CH), 7.09 (3H, m, CH), 4.49 (1H, m, CH), 3.22 (4H, t, $J = 5.0$ Hz, NCH$_2$CH$_2$N), 2.61 (4H, t, $J = 5.2$ Hz, NCH$_2$CH$_2$N), 2.56 (2H, m, CH, CH$_2$N), 1.85 (3H, m, CH$_2$), 1.62 (4H, m, CH$_2$CH$_3$), 0.92 (6H, dt, $J = 7.7$, 19.0 Hz, CH$_2$CH$_3$). $^{13}$C NMR (101 MHz, MeOD) $\delta$ 180.71 (CO), 151.13 (C), 129.92 (CH), 122.62 (CH), 119.94 (CH), 119.49 (CH), 118.47 (CN), 113.09 (CH), 75.36 (CH), 54.41 (C(CH$_2$CH$_2$)$_2$), 52.83 (NCH$_2$CH$_2$N), 48.60 (NCH$_2$CH$_2$N), 48.07 (CH$_2$CH$_2$N), 37.67 (CH$_3$), 33.54 (CH$_2$CH$_2$N), 29.22 (CH$_2$CH$_3$), 28.28 (CH$_2$CH$_3$), 8.77 (CH$_2$CH$_3$), 8.71 (CH$_2$CH$_3$). LC/MS [M + H] = m/z 356.2.

![Synthesis of 3,3-diethyl-5-(2-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (9m): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9q), except 1-(3-(trifluoromethyl)phenyl)piperazine was substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (Yield: 73%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.42 (1H, t, $J = 8.4$ Hz, CH), 7.23 (1H, d, $J = 8.1$ Hz, CH), 7.10 (1H, s, CH), 7.09 (1H, dd, $J = 2.2$, 8.1 Hz, CH), 4.46 (1H, m, CH), 3.76 (4H, b, NCH$_2$CH$_2$N), 3.33 (4H, m, NCH$_2$CH$_2$N), 3.06 (2H, b, CH$_2$CH$_3$N), 2.28 (1H, m, CH$_2$), 2.22 (1H, dd, $J = 6.7$, 12.6 Hz, CH$_2$), 2.05 (1H, m, CH$_2$), 1.86 (1H, dd, $J = 9.4$, 13.1 Hz, CH$_2$), 1.63 (4H, m, CH$_2$CH$_2$N) 0.92 (6H, dt, $J = 7.4$, 16.6 Hz, CH$_2$CH$_3$) $^{13}$C NMR (101 MHz, MeOD) $\delta$ 182.32 (CO), 152.01 (C), 135.01 (CCl), 130.11 (CH), 119.60 (CH), 116.04 (CH), 114.09 (CH), 77.24 (CH), 75.29 (C(CH$_2$CH$_2$)$_2$), 54.51 (NCH$_2$CH$_2$N), 52.87 (NCH$_2$CH$_2$N), 48.59 (CH$_2$CH$_2$N), 48.29 (CH$_2$), 37.67 (CH$_2$CH$_2$N), 29.24 (CH$_2$CH$_3$), 28.29 (CH$_2$CH$_3$), 8.78 (CH$_2$CH$_3$), 8.71 (CH$_2$CH$_3$). LC/MS [M + H] = m/z 365.2.](9m)

![Synthesis of 5-(2-(4-(3-chlorophenyl)piperazin-1-yl)ethyl)-3,3-diethylidihydrofuran-2(3H)-one (9n): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9q), 1-(3-chlorophenyl)piperazine was substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (Yield: 89%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.04 (1H, t, $J = 8.1$ Hz, CH), 6.75 (1H, d, $J = 2.1$ Hz, CH), 6.67 (2H, td, $J = 1.8$, 8.2 Hz, CH), 4.37 (1H, m, CH), 3.08 (4H, t, $J = 5.0$ Hz, NCH$_2$CH$_2$N) 2.55–2.35 (6H, m, NCH$_2$CH$_2$N and CH$_2$CH$_2$N), 2.02 (1H, dd, $J = 6.8$, 13.0 Hz, CH$_2$), 1.84–1.64 (3H, m, CH$_2$), 1.56–1.46 (4H, m, CH$_2$CH$_2$N), 0.81 (6H, dt, $J = 7.5$, 19.0 Hz, CH$_2$CH$_3$) $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 180.61 (CO), 152.01 (C), 135.01 (CCl), 130.11 (CH), 119.60 (CH), 116.04 (CH), 114.09 (CH), 77.24 (CH), 75.29 (C(CH$_2$CH$_2$)$_2$), 54.51 (NCH$_2$CH$_2$N), 52.87 (NCH$_2$CH$_2$N), 48.59 (CH$_2$CH$_2$N), 48.29 (CH$_2$), 37.67 (CH$_2$CH$_2$N), 29.24 (CH$_2$CH$_3$), 28.29 (CH$_2$CH$_3$), 8.78 (CH$_2$CH$_3$), 8.71 (CH$_2$CH$_3$). LC/MS [M + H] = m/z 365.2.](9n)

![Synthesis of 3,3-diethyl-5-(2-(4-(o-tolyl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (9o): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9q), except 1-(3-methylphenyl)piperazine was substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (Yield: 77%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.20 (1H, t, $J = 7.9$ Hz, CH), 6.82 (1H, d, $J = 7.6$ Hz, CH), 6.76 (1H, s, CH), 6.75 (1H, d, $J = 7.6$ Hz, CH), 4.45 (1H, m, CH), 4.15–2.70 (10H, b, NCH$_2$CH$_2$N, NCH$_2$CH$_2$N and CH$_2$CH$_2$N), 2.33 (3H, s, CH$_3$), 2.28 (1H, m, CH$_2$), 2.21 (1H, dd, $J = 6.7$, 13.1 Hz, 9o).](9o)
CH$_2$), 2.04 (1H, m, CH$_2$), 1.85 1H, (dd, J = 9.3, 13.1 Hz, CH$_2$), 1.63 (4H, m, CH$_2$CH$_3$) 0.92 (6H, dt, J = 7.4, 16.8 Hz, CH$_2$CH$_2$); $^{13}$C NMR (101 MHz, MeOD) δ 182.29 (CO), 151.16 (C), 140.28 (CH), 130.22 (CH$_3$), 123.33 (CH), 118.77 (CH), 115.18 (CH), 76.10 (CH), 54.89 (C (CH$_2$CH$_2$)), 53.35 (NCH$_2$CH$_3$), 49.94 (NCH$_2$CH$_2$N), 48.25 (CH$_2$CH$_2$N), 38.35 (CH$_2$), 31.79 (CH$_2$CH$_2$N), 30.08 (CH$_2$CH$_3$), 29.19 (CH$_2$CH$_3$), 21.70 (CH$_3$), 8.98 (CH$_2$CH$_3$), 8.90 (CH$_2$CH$_3$). LC/MS [M + H] = m/z 345.2.

![Image](9p)

Synthesis of 3,3-diethyl-5-(2-(4-(3-methoxyphenyl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (9p): The title compound was prepared according to the procedure for 2-(4-(2-(4-(4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9q), except 1-(2-(trifluoromethyl)phenyl)piperazine was substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (Yield: 80%). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.62 (1H, d, J = 8.1 Hz, CH), 7.51 (1t, J = 7.7 Hz, CH), 7.38 (1H, d, J = 8.0 Hz, CH), 7.22 (1H, t, J = 7.7 Hz, CH), 4.50 (1H, m, CH), 2.97 (4H, t, J = 4.6 Hz, NCH$_2$CH$_2$N), 2.72-2.45 (6H, m, NCH$_2$CH$_2$N), 2.15 (1H, dd, J = 6.8, 13.1 Hz, CH$_2$), 1.88 (3H, m, CH$_2$), 1.64 (4H, m, CH$_2$CH$_2$N), 0.94 (6H, dt, J = 7.5, 21.5 Hz, CH$_2$CH$_3$), $^{13}$C NMR (101 MHz, CDCl$_3$) δ 178.91 (CO), 145.40 (C), 131.22 (CH), 125.86 (CF$_3$), 125.58 (CCF$_3$), 125.53 (CH), 123.67 (CH), 122.51 (CH), 73.63 (CH), 52.93 (C(CH$_2$CH$_2$)$_2$), 51.79 (NCH$_2$CH$_2$N), 49.81 (NCH$_2$CH$_2$N), 46.93(CH$_2$CH$_2$N), 35.99 (CH$_2$), 31.95, (CH$_2$CH$_2$N) 27.61 (CH$_2$CH$_3$), 26.66 (CH$_2$CH$_3$), 7.12 (CH$_2$CH$_3$), 7.05 (CH$_2$CH$_3$), LC/MS [M + H] = m/z 399.20.

![Image](9s)

Synthesis of 5-(2-(4-(2-chlorophenyl)piperazin-1-yl)ethyl)-3,3-diethylidihydrofuran-2(3H)-one (9s): The title compound was prepared according to the procedure for 2-(4-(2-(4-(4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (9q), except 1-(2-(chlorophenyl)piperazine was substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (Yield: 80%). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.38 (1H, d, J = 1.6, 8.2 Hz, CH), 7.25 (1H, dt, J = 1.4, 8.1 Hz, CH), 7.06 (2H, m, CH), 4.46 (1H, m, CH), 3.74 (2H, t, J = 10.3 Hz, NCH$_2$CH$_2$N), 3.45 (2H, m, NCH$_2$CH$_2$N), 3.39-3.20 (4H, m, NCH$_2$CH$_2$N), 3.12 (2H, m, CH$_2$CH$_2$N), 2.28 (1H, m, CH$_2$), 2.21 (1H, dd, J = 6.9, 12.5 Hz, CH$_2$), 2.05 (1H, m, CH$_2$), 1.85 (1H, dd, J = 9.2, 13.6 Hz, CH$_2$), 1.62 (4H, m, CH$_2$CH$_3$), 0.92 (6H, dt, J = 7.5, 17.2 Hz, CH$_2$CH$_3$); $^{13}$C NMR (101 MHz, MeOD) δ 182.32 (CO), 148.54 (C), 131.81 (CH), 130.05 (CCl), 129.26 (CH), 128.46 (CH), 122.03 (CH), 76.13 (CH), 54.99 (C(CH$_2$CH$_2$)$_2$), 53.75 (NCH$_2$CH$_2$N), 49.94 (NCH$_2$CH$_2$N), 48.86 (CH$_2$CH$_3$N), 38.35 (CH$_2$), 31.84 (CH$_2$CH$_2$N), 30.08 (CH$_2$CH$_3$), 29.21 (CH$_2$CH$_3$), 8.99 (CH$_2$CH$_3$), 8.91 (CH$_2$CH$_3$) LC/MS [M + H] = m/z 365.20.
Synthesis of 3,3-diethyl-5-(2-(4-(o-tolyl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (9t): The title compound was prepared according to the procedure for 2-(4-(2,4-diyethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonirole, except 1-o-Tolyl-piperazine was substituted for 2-piperazin-1-yl-benzonitrile (46.6 mg, 45% yield). 1H NMR (400 MHz, MeOD) δ 7.21–6.90 (4H, m, CH), 4.62–4.45 (1H, m, CH), 3.65 (2H, dd, J = 9.6, 5.4 Hz, NCH2CH3N), 3.43–3.26 (4H, m, NCH2CH3N), 3.24–3.07 (4H, m, NCH2CH3N), 2.34–2.02 (6H, m, CH3 and CH2), 1.90 (1H, dd, J = 13.3, 9.4 Hz, CH2), 1.60 (4H, ddd, J = 17.2, 8.6, 6.4 Hz, CH2CH3), 0.89 (6H, dt, J = 14.0, 7.5 Hz, CH2CH3); 13CNMR (101 MHz, MeOD) δ 182.40 (CO), 150.67 (C), 134.07 (C), 126.51 (CH), 125.20 (CH), 113.32 (CH), 76.15 (CH), 56.21 (CH2CH3), 54.97 (NCH2CH3N), 53.20 (COCH3), 49.93 (NCH2CH3N), 49.35 (CH2CH3N), 38.35 (CH2), 31.74 (CH2CH3N), 30.05 (CH3CH3), 29.19 (CH3CH3), 9.00 (CH3CH3), 8.91 (CH3CH3), MS (LC/MS, M + H+): 361.2; Anal. Calcd for C21H34Cl2N2O3: C, 58.20; H, 7.91; N, 6.39. melting point of di-HCl salt: 228–229 °C.

Synthesis of 3,3-diethyl-5-(2-(4-(2-isopropylphenyl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (9v): The title compound was prepared according to the procedure for 2-(4-(2,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonirole, except 1-(2-Isopropl-phenyl)-piperazinewas substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (44.8 mg, 40% yield). 1H NMR (400 MHz, DMSO) δ 7.30 (1H, dd, J = 7.4, 1.6 Hz, CH), 7.23–7.08 (3H, m, CH), 4.66–4.43 (1H, m, CH), 3.54 (2H, t, J = 9.6 Hz, NCH2CH3N), 3.41 (1H, dd, J = 13.7, 6.8 Hz, CH (CH3)), 3.33–3.12 (6H, m, NCH2CH3N and NCH2CH3N), 3.02 (2H, d, J = 10.7 Hz, CH3CH2N), 2.31–2.03 (3H, m, CH2), 1.83 (1H, dd, J = 13.2, 9.3 Hz, CH), 1.69–1.34 (4H, m, CH2CH3), 1.16 (6H, d, J = 6.9 Hz, CH2CH3), 0.85 (6H, dt, J = 10.6, 7.5 Hz, CH3CH3); 13CNMR (101 MHz, DMSO) δ 179.77 (CO), 148.90 (C), 143.85 (C), 126.51 (CH), 125.20 (CH), 120.36 (CH), 74.31 (CH), 52.05 (CH2CH3), 51.57 (NCH2CH3N), 51.43 (NCH2CH3N), 49.55 (CH2CH3N), 47.87 (CH2), 36.43 (CH2CH3N), 29.72 (CH2CH3), 28.36 (CH2CH3), 27.66 (CH2CH3), 26.24 (CH2CH3), 23.99 (CH2CH3), 8.55 (CH2CH3), 8.51 (CH2CH3); MS (LC/MS, M + H+): 373.3.

Synthesis of 5-(2-(4-(2,4-dimethylphenyl)piperazin-1-yl)ethyl)-3,3-diethylidihydrofuran-2(3H)-one (9w): The
title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile, except 1-(2,4-Dimethyl-phenyl)-piperazinewas substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (52.8 mg, 49% yield). 1H NMR (400 MHz, DMSO) δ 7.11–6.75 (3H, m, CH), 4.55 (1H, dt, J = 11.8, 8.4 Hz, CH), 3.53 (2H, m, CH₂CH₂N), 3.33–3.02 (8H, m, NCH₂CH₂N and NCH₂CH₂N), 2.31–2.07 (9H, m, CH₂, CH₃, and CH₂), 1.83 (1H, dd, J = 13.2, 9.3 Hz, CH₂), 1.67–1.39 (4H, m, CH₂CH₃), 0.85 (6H, dt, J = 10.6, 7.5 Hz, CH₂CH₃); 13C NMR (101 MHz, DMSO) δ 179.75 (CO), 147.31 (C), 132.64 (CCH₃), 131.80 (CCH₃), 131.62 (CH), 127.05 (CH), 118.82 (CH), 74.31 (CH), 52.11 (C(CH₂CH₂)₂), 51.55 (NCH₂CH₂N), 51.38 (NCH₂CH₂N), 48.24 (CH₂CH₂N), 47.85 (CH₂), 36.44 (CH₂CH₂N), 28.33 (CH₂CH₃), 27.64 (CH₂CH₃), 20.32 (CH₃), 17.27 (CH₃), 8.54 (CH₂CH₃), 8.49 (CH₂CH₃); MS (LC/MS, M + H⁺): 359.3

Synthesis of 3,3-diethyl-5-(2-(4-(pyridin-2-yl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (9x): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile, except 1-pyridin-2-yl-piperazine was substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (41.8 mg, 42% yield).

1H NMR (400 MHz, D₂O) δ 8.10 (1H, ddd, J = 9.1, 7.2, 1.8 Hz, CH), 8.02 (1H, dd, J = 6.2, 1.7 Hz, CH), 7.34 (1H, d, J = 9.2 Hz, CH), 7.12 (1H, t, J = 6.7 Hz, CH), 4.71 (1H, ddd, J = 16.0, 9.2, 3.6 Hz, CH), 4.31–3.26 (10H, m, NCH₂CH₂N, NCH₂CH₂N, and CH₂CH₂N), 2.68–2.48 (6H, m, NCH₂CH₂N, and CH₂CH₂N), 1.95–1.78 (3H, m, CH₂CH₂N and CH₂), 1.69–1.57 (4H, m, CH₂CH₂N), 0.93 (6H, dt, J = 7.5, 19.1 Hz, CH₂CH₂N). 13C NMR (101 MHz, D₂O) δ 187.89 (CO), 155.57 (C), 147.93 (CH), 140.42 (CH), 117.97 (CH), 115.85 (CH), 79.49 (CH), 75.12 (CH), 56.71 (C(CH₂CH₂)₂), 53.74 (NCH₂CH₂N), 52.68 (NCH₂CH₂N), 48.59 (NCH₂CH₂N), 47.72 (CH₂CH₂N), 37.64 (CH₂), 32.89 (CH₂CH₂N), 29.23 (CH₂CH₂N), 28.28 (CH₂CH₂N), 8.77 (CH₂CH₂N), 8.71 (CH₂CH₂N). LC/MS [M + H⁺] = m/z 332.2

Synthesis of 3,3-diethyl-5-(2-(4-(pyridin-3-yl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (9y): The title compound was prepared according to the procedure for 7-(2-(4-phenylpiperazin-1-yl)ethyl)-6-oxaspiro[3.4]octan-5-one, except 5-(2-bromoethyl)-3,3-diethyldihydrofuran-2(3H)-one was substituted for 2-(5-oxo-6-oxaspiro[3.4]octan-7-yl)ethyl 4-methylbenzenesulfonate and 1-(pyridin-3-yl)piperazine for 1-phenylpiperazine. The product was isolated as a clear oil (Yield: 44%). 1H NMR (400 MHz, CDCl₃) δ 8.31 (1H, b, CH), 8.10 (1H, b, CH), 8.02 (1H, d, CH), 7.17 (2H, m, CH), 4.49 (1H, m, CH), 3.23 (4H, t, J = 5.3 Hz, NCH₂CH₂N), 2.68–2.48 (6H, m, NCH₂CH₂N, and CH₂CH₂N), 1.95–1.78 (3H, m, CH₂CH₂N and CH₂), 1.69–1.57 (4H, m, CH₂CH₂N), 0.93 (6H, dt, J = 7.5, 19.1 Hz, CH₂CH₂N). 13C NMR (101 MHz, CDCl₃) δ 180.55 (CO), 146.48 (C), 140.96 (CH), 138.56 (CH), 123.58 (CH), 122.98 (CH), 75.12 (CH), 54.55 (C(CH₂CH₂)₂), 52.68 (NCH₂CH₂N), 48.59 (NCH₂CH₂N), 47.72 (CH₂CH₂N), 37.64 (CH₂), 32.89 (CH₂CH₂N), 29.23 (CH₂CH₂N), 28.28 (CH₂CH₂N), 8.77 (CH₂CH₂N), 8.71 (CH₂CH₂N). LC/MS [M + H⁺] = m/z 332.2

Synthesis of 3,3-diethyl-5-(2-(4-(pyridin-4-yl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (9z): The title compound was prepared according to the procedure for 7-(2-(4-phenylpiperazin-1-yl)ethyl)-6-oxaspiro[3.4]octan-5-one, except 5-(2-bromoethyl)-3,3-diethyldihydrofuran-2(3H)-one was substituted for 2-(5-oxo-6-oxaspiro[3.4]octan-7-yl)ethyl 4-methylbenzenesulfonate and 1-(pyridin-3-yl)piperazine for 1-phenylpiperazine. The product was isolated as a clear oil (Yield: 37%). 1H NMR (400 MHz, CDCl₃) δ 8.27 (2H, d,
Sigma-1 receptor binding assay

$K_i$ 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 $[^3]$H]Pentazocine ($K_d = 6.5$ nM) was the radioligand. Nonspecific binding was defined with 10 uM haloperidol. The reference standard haloperidol had a $K_i = 3.54$ nM.

Aqueous solubility (pH 7.4) assay

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

Cytochrome P450 3A4 inhibition assay

Compounds were assessed for their ability to inhibit human cytochrome P450 3A4 using testosterone as a substrate and LC/MS/MS analysis. Expressed enzymes was used to minimize non-specific binding and membrane partitioning issues [26].

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. [27] 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 The authors declare that they have no 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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