Synthesis and Evaluation of Novel, Selective, Functionalized γ-butyrolactones as Sigma-2 Ligands

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

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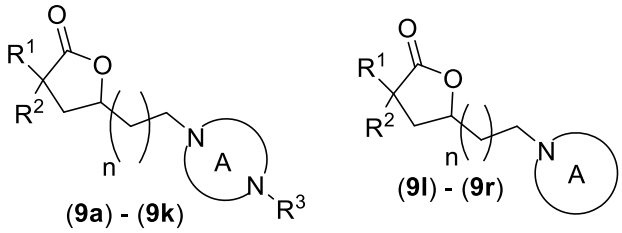
Abstract: The sigma-2 (σ2) receptor was discovered nearly 40 years ago and was recently identified as the Transmembrane Protein 97 (TMEM97, also known as MAC30 (Meningioma-associated protein). Aberrant σ2 activity has been linked to disease and conditions such as schizophrenia, Alzheimer's disease, neuropathic 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 the opioids on chronic spinal dogs. In these studies, they observed that the opioids morphine (**1**), ketocyclazocine, (**2**), and (rac)-SKF-100047 (**3**) 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) 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 designate the sigma receptor (σR).[2] In 1993, W. D. Bowen et. al. determined that

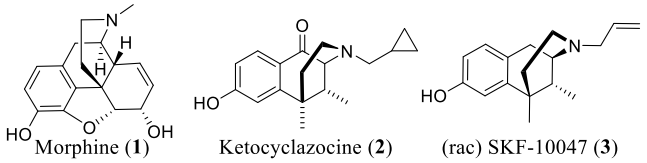
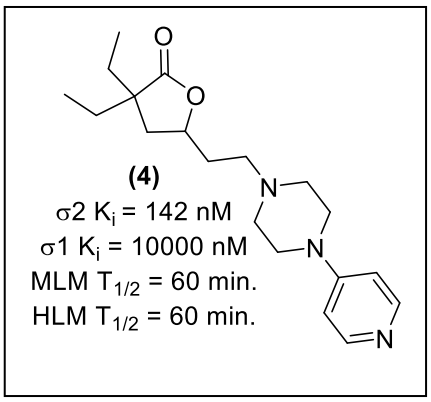


Figure 1: Structures of morphine (**1**), ketocyclazocine, (**2**), and (rac)-SKF-100047 (**3**)

there were two sub-types of this receptor, which were designated sigma-1 (σ_1) and sigma-2 (σ_2).[3] Three years later, Glossman H, et.al. cloned and expressed the mammalian σ_1 receptor in yeast cells,[4] and in 2016 a crystal structure of the human σ_1 receptor was reported.[5] To date, there is no known natural ligand for this receptor.

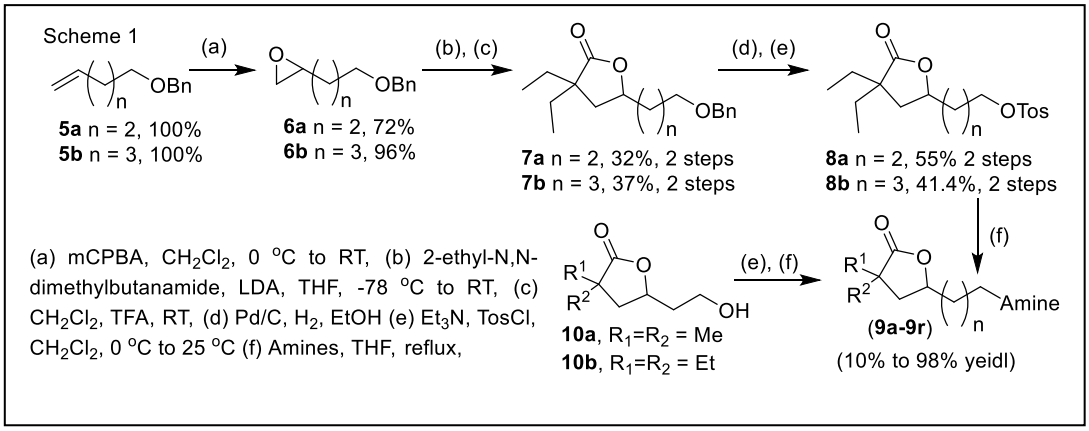
The nature and function of the σ_2 receptor, on the other hand, remains the subject of intense research, but some progress has been made. The natural ligand of this receptor remains a mystery, but A.C. Krusea et. al. have demonstrated that the protein originally described as the σ_2 receptor is identical to Transmembrane Protein 97 (TMEM97, also known as MAC30 (Meningioma-associated protein).[6] The σ_2 receptor is present in lysosomes and the endoplasmic reticulum (ER) and there is evidence that cholesterol binds this receptor.[7] Regulation of the Niemann-Pick protein NPC1 has also been suggested by H. Runz et. al.[8[Although the pharmacological role of σ_2 remains unclear, substantial effort has been devoted to the development of σ_2 binders based on the premise that aberrant σ_2 pharmacology contributes to the progression of diseases and conditions such as Alzheimer's disease,[9] traumatic brain injury,[10] neuropathic pain,[11] schizophrenia,[12] and cancer.[13]



(4)
$\sigma 2$ K_i = 142 nM
$\sigma 1$ K_i = 10000 nM
MLM $T_{1/2}$ = 60 min.
HLM $T_{1/2}$ = 60 min.

We recently reported a series of novel, selective γ-butyrolactones sigma-2 ligands that included the identification of (**4**). This compound was found to have moderate affinity σ_2 ligand (K_i = 142 nM), excellent selectivity for this target over σ_1 (K_i = 10,000 nM) and high stability in the presence of both mouse and human liver microsomes (MLM, HLM $T_{1/2}$ = 60 min.). Herein we report follow up studies that describe the synthesis and characterization of a related series of novel γ-butyrolactones in which we explore (1) the impact of altering the length of the linker between the two ring systems, and (2) replacements of the aryl piperazine moiety with alternative ring systems.

Results and discussion: Synthesis of substituted γ-butyrolactones was conducted as shown in Scheme 1 (missing compound #s) utilizing novel methods developed in our laboratory. The synthesis of these compounds begins with the known benzyl protected alkenyl alcohols (**5**), which were converted to the corresponding epoxide (**6**) with mCPBA. Ring opening of epoxide (**6**) with the enolate of 2-ethyl-N,N-dimethylbutanamide via deprotonation with LDA provided an 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 triethyl amine to provide (**8**). Reaction of (**8**) 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 triethyl amine, followed by amines in refluxing THF to provided the final target molecules (**9**).



Tables 1 and 2 describe the *in vitro* binding (K_i at σ_1 and σ_2), 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) 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 had low MLM stability, we were able to identify 3 compounds with MLM $T_{1/2}$ values > 10 minutes. 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 systems (**9a**-**9c**). As indicated in table 1, compounds with chain lengths of 2 (**9a**), 3 (**9b**), and 4 (**9c**) methylene units bind to σ_2 with moderate to high potency (σ_2 K_i = 82, 7.7, and 12 nM), but selectivity versus σ_1 was low (σ_1 K_I = 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 σ_2 potency (K_i = 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 σ_2 potency (K_i = 32 nM), as well as increase in selectivity over (σ_1 K_I = 2167 nM) in comparison to (**9a**). Notably, this compound is the least soluble analog (sol = 47 mM), which is almost certainly the increase aromatic character of the aryl piperazine region. Employing heteroaromatic replacements for the aryl piperazine produced mixed results. While the 4-pyridine analog (**9f**) is a moderate affinity σ_2 ligand (K_i = 142 nM), with a high degree selectivity for this target over σ_1 (K_i = 10,000 nM), the corresponding 4-pyrimidine analog (**9g**) had limited capacity to bind to σ_2 (KI = 10000 nM) and low affinity for σ_1 (K_i = 1017 nM).

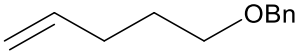
Incorporation of potential piperazine bioisosteres produced compounds with high σ_2 potency and moderate to low selectivity versus σ_1. Specifically, the homopiperazine analog (**9h**), 2,6-diazaspiro[3.3]heptane analog (**9i**), and octahydropyrrolo[3,4-c]pyrrole analog (**9j**) are all potent σ_2 binders (K_i = 6.8, 53, and 3.5 nM) with low to moderate selectivity over σ_1 (K_i = 17, 12, 31 nM). Interestingly, the combination of the octahydropyrrolo[3,4-c]pyrrole bioisostere and 4-pyrdine substituent (**9k**) led to improved σ_2 potency (K_I = 29 nM) versus the piperazine analog (**9f**), but decreased σ_1 selectivity (K_I = 142 nM). In addition, the high level of MLM stability observed with (**9f**) was maintained in (**9k**) (MLM $T_{1/2}$ = 60 min).

We next turned our attention to replacing the aryl piperazine moiety with tetrahydroisoquinolines. The unsubstituted tetrahydroisoquinoline analog (**9l**) is a potent σ_2 binder (K_i = 6.1 nM), with moderate σ_1 selectivity (K_I = 125 nM). Incorporating halogens in the 7-position of the tetrahydroisoquinoline nucleus produced compounds (**9m**-**9o**) with potency similar to that observed with the unsubstituted analog (7-F (**9m**) σ_2 K_i =7.4 nM, 7-Cl (**9n**) σ_2 K_i =2.8 nM, 7-Br (**9o**) σ_2 K_i =7.4 nM). Interestingly, while the 7-F (**9m**) and 7-Cl (**9n**) analogs had moderate selectivity for σ_2 over σ_1, the 7-Br analog (**9o**) was nearly equipotent at these two receptors (σ_1 K_i = 4.7 nM). Insertion of a pyridine nitrogen into the tetrahydroisoquinoline framework substantially diminished σ_2 binding. The 2,6-naphthyridine (**9p**) and 2,7-naphthyridine (**9q**) analogs had limited capacity to bind to σ_2 (K_i = 10,000 nM), while the 1,7-naphthyridine (**9r**) demonstrated moderate σ_2 binding (K_i = 277 nM). As noted in tables 1 and 2, the majority of compounds are highly soluble in aqueous media (> 100 μM) and their physicochemical properties (MW, TPSA, cLogP) are consistent with drug-like properties as defined by Lipinski. [14]

Conclusions: In summary, a series of substituted lactones with drug-like physicochemical properties (MW, TPSA, cLogP) have been investigated as potential selective σ_2R ligands. We have determined that increasing the length of the linker chain from two (**9a**) to four carbons (**9c**) leads to increase σ_2 potency, but selectivity over σ_1 decreases. In addition, we have demonstrated that σ_2 potency and selectivity for σ_2 over σ_1 Is maintained when the phenyl ring of the aryl piperazine is replaced with 1-napthylene (**9e**) or 4-pyridine (**9f**), but replacement with a 4-pyrimidine (**9g**) leads to a significant lose of σ_2 potency. Replacement of the piperazine ring with bioisosteres such as homopiperazine (**9h**) 2,6-diazaspiro[3.3]heptane (**9i**), and octahydropyrrolo[3,4-c]pyrrole (**9j**) was tolerated with respect to σ_2 potency, but σ_1 selectivity was substantially decreased. Incorporation of tetrahydroisoquinolines (**9l**-**9o**) in place of the aryl piperazine also

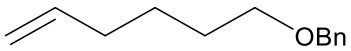
produced high potency σ_2 binders, but naphthyridine analogs examined to date had limited σ_2 binding capacity. We anticipate these studies will help us further evaluate the potential value of this series for the identification of novel therapeutic agents for the treatment of diseases associated with abnormal σ_2 activity. Future studies will be focused on the identification of highly potent, selective, novel σ_2 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 (□ 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 x 30 mm, 3.5 µm, 100% water/0.1% formic acid to 100% acetonitrile/0.1% formic acid over 4.0 minutes, 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.

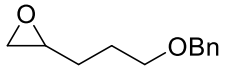


Preparation of ((pent-4-en-1-yloxy)methyl)benzene: To a dry round bottom flask under nitrogen was added 1.4 g of 60% NaH dispersion (0.834 g NaH, 34.5 mmol, 2eq.), followed by ~200 mg of tetrabutylammonium iodide. 18 mL of dry THF was added and the reaction was cooled to 0°C using an ice bath. Pent-4-en-1-ol (1.5 g, 17.4 mmol, 1 eq.) was added dropwise. The reaction was stirred at 0°C for 5 minutes 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. NH_4Cl (15 mL) and then extracted 3x10 mL diethyl ether. The combined organic layers were dried over Na_2SO_4, filtered and concentrated onto Celite under reduced pressure. The crude material

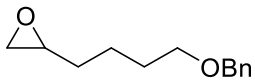
was purified by flash chromatography (silica; ethyl acetate/hexanes, 0% ~ 5%). Percent yield: 100%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45-7.26 (m, 5H), 5.87 (m, 1H), 5.08 (dq, J= 1.8, 17.2 Hz, 1H), 5.01 (m, 1H), 4.55 (s, 2H), 3.54 (t, J= 6.5 Hz, 2H), 2.20 (q, J= 7.6 Hz, 2H), 1.77 (quin, J= 7.6 Hz, 2H).



Preparation of ((hex-5-en-1-yloxy)methyl)benzene: 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%. ^{1}H NMR (400 MHz, $CDCl_3$) 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), 1.57-1.46 (m, 2H), 1.41-1.29 (m, 2H).

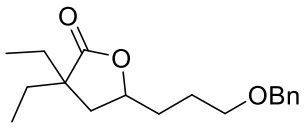


Preparation of 2-(3-(benzyloxy)propyl)oxirane (**6a**): To a round botton flask is added ((pent-4-en-1-yloxy)methyl)benzene (3.15 g, 17.9 mmol, 1 eq.) and CH_2Cl_2 (45 mL). Resulting solution is then cooled to 0ºC with an ice bath and then 3-chloroperbenzoic acid (5.25 g (6.82 g, 77% purity), 30 mmol, 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 CH_2Cl. The solution was then washed with 3x10 mL of 1N NaOH (aq.) solution, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude oil was used in the next step without further purification. Percent yield: 72%. ^{1}H NMR (400 MHz, $CDCl_3$) 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).



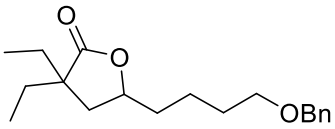
Preparation of 2-(4-(benzyloxy)butyl)oxirane (**6b**): The title compound was prepared according to the procedure for 2-(3-(benzyloxy)propyl)oxirane, except ((hex-5-en-1-yloxy)methyl)benzene was substituted for ((pent-4-en-1-yloxy)methyl)benzene. Percent yield: 96%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.25 (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).



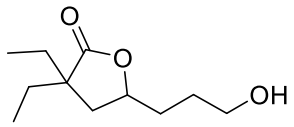
Preparation of 5-(3-(benzyloxy)propyl)-3,3-diethyldihydrofuran-2(3H)-one (**7a**): A dry round bottom flask was placed under N_2 atmosphere and then charged with 1M LDA solution (THF/Hexanes, 23 mL, 23 mmol, 2.3 eq.) and cooled to -78^{O}C. While at -78^{O}C, 2-ethyl-N,N-dimethylbutanamide (2.86 g, 20 mmol, 2 eq.) was added dropwise. The reaction was stirred at -78^{O}C for 30 minutes, then allowed to warm to 0^{O}C and stir at that temperature for 15 minutes. Then the reaction was warmed to RT and stirred for 5 minutes before cooling back to 0^{O}C with an ice bath. At 0^{O}C, 2-(3-(benzyloxy)propyl)oxirane (2.0 g, 10 mmol, 1 eq.) was added. The reaction was stirred at 0^{O}C for 15 minutes and then warmed to RT. After 48 hour, the reaction was quenched with sat. NH_4Cl solution (aq.) and extracted 3x20 mL CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure.

Crude material was then dissolved in CH_2Cl_2 (30 mL) and trifluoroacetic acid (5 mL) was added slowly. The resulting solution was allowed to stir at room temperature for 40 minutes before being slowly quenched with sat. $NaHCO_3$ solution (aq.) and extracted with 3x10 mL CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (silica; ethyl acetate/hexanes, 0% ~ 30%). Percent yield: 32%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.18 (m, 5H), 4.46 (s, 2H), 4.35 (m, 1H), 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).

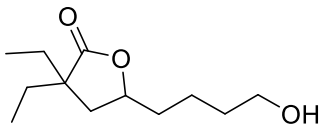


Preparation of 5-(4-(benzyloxy)butyl)-3,3-diethyldihydrofuran-2(3H)-one (**7b**): The title compound was prepared according to the procedure for 5-(3-(benzyloxy)propyl)-3,3-diethyldihydrofuran-2(3H)-one, except 2-

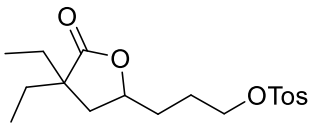
(4-(benzyloxy)butyl)oxirane was substituted for 2-(3-(benzyloxy)propyl)oxirane. Percent yield: 37%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.19 (m, 5H), 4.55 (s, 2H), 4.40 (m, 1H), 3.53 (t, J= 6.4 Hz, 2H), 2.09 (dd, J= 6.8, 13.1 Hz, 1H), 1.77 (dd, J= 9.3, 13.3 Hz, 1H), 1.72-1.34 (m, 10H), 0.89 (dt, J= 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-diethyldihydrofuran-2(3H)-one (910 mg, 3.13 mmol, 1 eq.) in EtOH (18 mL). The reaction was put under H_2 (1 atm) using a balloon and stirred at room temperature under H_2 atm 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%. ^{1}H 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).

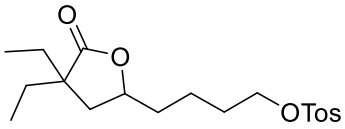


Preparation of 3,3-diethyl-5-(4-hydroxybutyl)dihydrofuran-2(3H)-one: The title compound was prepared according to the procedure for 3,3-diethyl-5-(3-hydroxypropyl)dihydrofuran-2(3H)-one, except 5-(4-(benzyloxy)butyl)-3,3-diethyldihydrofuran-2(3H)-one was substituted for 5-(3-(benzyloxy)propyl)-3,3-diethyldihydrofuran-2(3H)-one. Percent yield: 100%. ^{1}H 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).

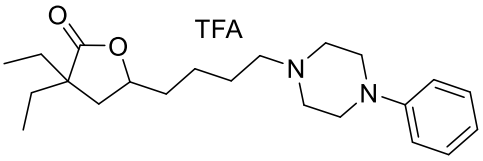


Preparation of 3-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)propyl 4-methylbenzenesulfonate (**8a**): To a solution of triethylamine (493 mg, 4.85 mmol, 1.5 eq.) and p-toluenesulfonyl chloride (744 mg, 3.90 mmol, 1.2 eq.) in CH_2Cl_2 (25 mL) was added a solution of 3,3-diethyl-5-(3-hydroxypropyl)dihydrofuran-2(3H)-one (650 mg, 3.25

mmol, 1.0 eq.) in CH_2Cl_2 (5 mL) at $0^{O}C$. The reaction was allowed to stir at RT overnight before being washed with 2x20 mL of sat. $NaHCO_3$ solution (aq.). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (silica; ethyl acetate/hexanes, 0% ~ 40%). Percent yield: 55%. ^{1}H NMR (400 MHz, $CDCl_3$) 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).

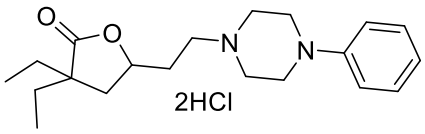


Preparation of 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate (**8b**): The title compound was prepared according to the procedure for 3-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)propyl 4-methylbenzenesulfonate, except 3,3-diethyl-5-(4-hydroxybutyl)dihydrofuran-2(3H)-one was substituted for 3,3-diethyl-5-(3-hydroxypropyl)dihydrofuran-2(3H)-one. Percent yield: 4.65%. ^{1}H NMR (400 MHz, $CDCl_3$) 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).

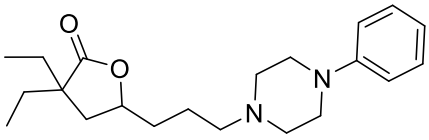


Preparation of 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroacetate (**9c**): To a small vial was added 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate (25 mg, 0.0679 mmol, 1 eq.) and 1-phenylpiperazine (23.1 mg, 0.142 mmol, 2.1 eq.) then both were dissolved in THF (1.7 mL). The reaction mixture was allowed to reflux for 72 hours 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. Crude product was then purified by HPLC (CH_3CN/H_2O, 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 7.24 (t, J= 7.9 Hz, 2H), 6.92 (t, J= 7.4 Hz, 1H), 6.87 (d, J= 8.3 Hz, 2H), 4.29 (m, 1H), 3.78-3.47 (m,

4H), 3.34-3.14 (m, 2H), 3.09-2.79 (m, 4H), 2.04 (dd, J= 6.8, 13.1 Hz, 1H), 1.88-1.64 (m, 3H), 1.64-1.34 (m, 8H), 0.85 (dt, J= 7.5, 20.9 Hz, 6H) LC/MS [M+H]= m/z 359.2

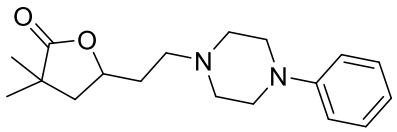


Preparation of 3,3-diethyl-5-(2-(4-phenylpiperazin-1-yl)ethyl)dihydrofuran-2(3H)-one dihydrocholoride (**9a**): The title compound was prepared according to the procedure for 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonat and 1-phenyl-piperazinewas substituted for 2-piperazin-1-yl-benzonitrile**.** Percent yield: 58.3% ^{1}H NMR (400 MHz, D_2O) δ 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); ^{13}C NMR (101 MHz, D_2O) δ 187.92, 150.20, 132.89, 127.03, 121.14, 79.53, 56.52, 54.13, 52.41, 50.87, 39.37, 32.81, 31.91, 30.68, 11.00, 10.87; MS (LC/MS, $M+H^+$): 331.2; Anal. Calcd for $C_{20}H_{32}Cl_2N_2O_2$: C, 59.55; H, 8.00; N, 6.94; Found: C, 59.62; H, 8.11; N, 6.90

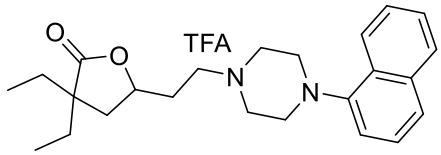


Preparation of 3,3-diethyl-dihydro-5-(3-(4-phenylpiperazin-1-yl)propyl)furan-2(3H)-one (**9b**): The title compound was prepared according to the procedure for 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroacetate, except 3-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)propyl 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) δ 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) LC/MS [M+H]= m/z 345.2

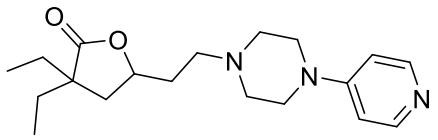


Preparation of 3,3-dimethyl-5-(2-(4-phenylpiperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (**9d**): The title compound was prepared according to the procedure for 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-dimethyl-5-oxotetrahydrofuran-2-yl)ethyl 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) δ 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). LC/MS [M+H]= m/z 303.2

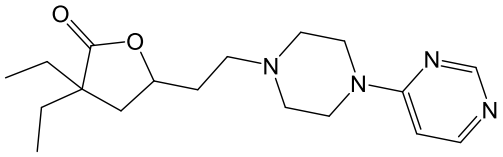


Preparation of 3,3-diethyl-5-(2-(4-(naphthalen-1-yl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one trifluoroacetate (**9e**): The title compound was prepared according to the procedure 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 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 Crude product was then purified by HPLC (CH_3CN/H_2O, 0.1% Trifluoroacetic acid), 0%~100%) to give desired product as a trifluoroacetic acid salt. Percent yield: 33%. 1H NMR (400 MHz, CDCl3) δ 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), 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

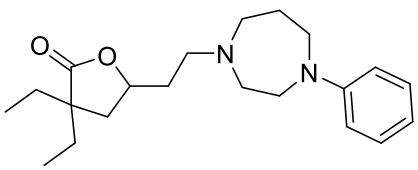
(m, 1H), 1.88 (dd, J= 9.3, 13.3 Hz, 1H), 1.65 (q, J= 7.5 Hz, 4H), 0.95 (dt, J= 7.4, 17.7 Hz, 6H) LC/MS [M+H]= m/z 381.2



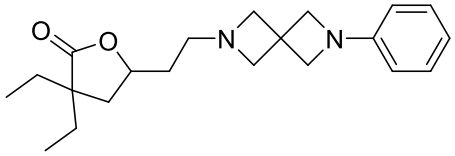
Preparation of 3,3-diethyl-5-(2-(4-(pyridin-4-yl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (**9f**): The title compound was prepared according to the procedure 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 1-(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) LC/MS [M+H]= m/z 332.2



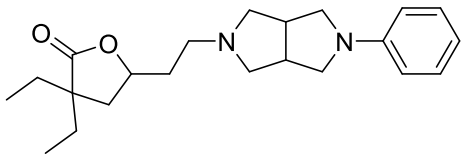
Preparation of 3,3-diethyl-5-(2-(4-(pyrimidin-4-yl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (**9g**): The title compound was prepared according to the procedure for 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 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) LC/MS [M+H]= m/z 333.20



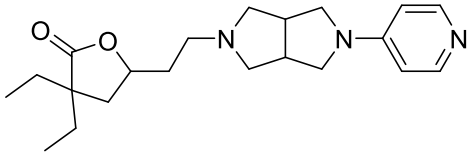
Preparation of 3,3-diethyl-5-(2-(4-phenyl-1,4-diazepan-1-yl)ethyl)dihydrofuran-2(3H)-one (**9h**): The title compound was prepared according to the procedure for 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 1-phenyl-1,4-diazepane for 1-phenylpiperazine. The crude product was purified by flash chromatography (silica; MeOH:dichloromethane, 0% ~ 10%). Percent yield: 68%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (m, 2H), 6.73-6.64 (m, 3H), 4.45 (m, 1H), 3.56 (t, J= 4.8 Hz, 2H), 3.50 (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); MS (LC/MS, M+H$^+$): 345.2



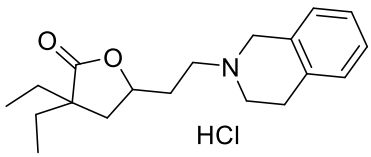
Preparation of 3,3-diethyl-5-(2-(6-phenyl-2,6-diazaspiro[3.3]heptan-2-yl)ethyl)dihydrofuran-2(3H)-one (**9i**): The title compound was prepared according to the procedure for 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroacetate except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 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: 10%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (m, 2H), 6.67 (t, J= 7.4 Hz, 1H), 6.37 (d, J= 8.2 Hz, 2H), 4.36 (m, 1H), 3.85 (s, 4H), 3.29 (s, 4H), 2.48 (t, J= 7.1 Hz, 2H), 2.04 (dd, J= 6.7, 13.0 Hz, 1H), 1.71 (dd, J= 9.4, 13.1 Hz, 1H), 1.67-1.43 (m, 6H), 1.83-1.61 (m, 6H), 0.85 (dt, J= 7.5, 21.9 Hz, 6H); MS (LC/MS, M+H$^+$): 343.2



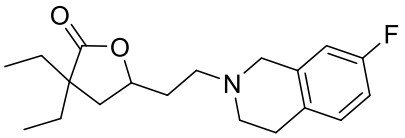
Preparation of 3,3-diethyl-5-(2-(5-phenylhexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)ethyl)dihydrofuran-2(3H)-one (**9j**): The title compound was prepared according to the procedure for 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroac except 2-(4,4-dieethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 2-phenyloctahydropyrrolo[3,4-c]pyrrole dihydrochloride was substituted for 1-phenylpiperazine.. Percent yield: 98%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (m, 2H), 6.64 (t, J= 7.1 Hz, 1H), 6.57 (d, J= 8.1 Hz, 2H), 4.37 (m, 1H), 3.29(t, J= 8.0 Hz, 2H), 3.08 (dt, J= 2.9, 9.3 Hz, 2H), 2.85 (b, 2H), 2.72 (m, 2H), 2.47 (t, J= 6.8 Hz, 2H), 2.32 (dd, J= 3.9, 8.9 Hz, 2H), 2.02 (dd, J= 6.7, 12.9 Hz, 1H), 1.86-1.61 (m, 3H), 1.51 (q, J= 7.5 Hz, 4H), 0.81 (dt, J= 7.5, 14.0 Hz, 6H); MS (LC/MS, M+H$^+$): m/z 357.2



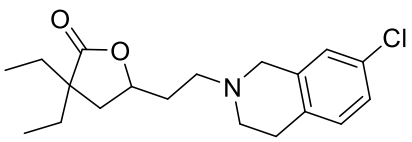
Preparation of 3,3-diethyl-5-(2-(5-(pyridin-4-yl)hexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)ethyl)dihydrofuran-2(3H)-one (**9k**): The title compound was prepared according to the procedure for 3,3-diethyl-5-(4-(4-phenylpiperazin-1-yl)butyl)dihydrofuran-2(3H)-one trifluoroac except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 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 for1-phenylpiperazine. Percent yield: 68% ^{1}H NMR (400 MHz, $CDCl_3$) δ8.13 (dd, J= 1.4, 3.5 Hz, 2H), 6.32 (dd, J= 1.5, 3.5 Hz, 2H), 4.37 (m, 1H), 3.45 (dd, J= 8.3, 9.2 Hz, 2H), 3.12 (dt, J= 3.4, 9.9 Hz, 2H), 2.90 (m, 2H), 2.62 (m, 2H), 2.50 (t, J= 7.4 Hz, 2H), 2.46 (m, 2H), 2.02 (dd, J= 6.8, 13.0 Hz, 1H), 1.85-1.61 (m, 3H), 1.52 (q, J= 7.5 Hz, 4H), 0.82 (dt, J= 5.7, 13.2 Hz, 6H); MS (LC/MS, M+H$^+$): m/z 358.2



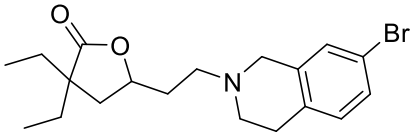
Preparation of 5-(2-(3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-3,3-diethyldihydrofuran-2(3H)-one hydrocholoride (**9l**): The title compound was prepared according to the procedure for 3,3-diethyl-5-(2-(4-(naphthalen-1-yl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 1,2,3,4-tetrahydro-isoquinolinewas 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% ^{1}H NMR (400 MHz, MeOH) δ 7.39 – 7.17 (m, 4H), 4.63 – 4.54 (m, 1H), 4.49 (s, 2H), 3.75 –3.63 (m, 2H), 3.54 – 3.37 (m, 2H), 3.22 (m, 2H), 2.36 – 2.24 (m, 2H), 2.23 – 2.08 (m, 1H), 1.95 (dd, J = 9.4, 13.3, 1H), 1.75 – 1.53 (m, 4H), 0.94 (dt, J = 7.5, 12.2, 6H); ^{13}C 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+): 302.2; Anal. Calcd for $C_{19}H_{28}ClNO_2$: C, 67.54; H, 8.35; N, 4.15; Found: C, 67.60; H, 8.36; N, 4.14



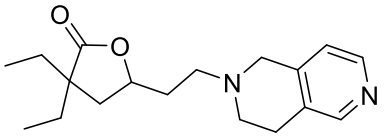
Preparation of 3,3-diethyl-5-(2-(7-fluoro-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)dihydrofuran-2(3H)-one (**9m**): The title compound was prepared according to the procedure for 3,3-diethyl-5-(2-(4-(naphthalen-1-yl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 7-fluoro-1,2,3,4-tetrahydroisoquinoline was 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) 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 (**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-chloro-1,2,3,4-tetrahydroisoquinoline was for 1 phenylpiperazine. Percent yield: 41%. 1H NMR (400 MHz, CDCl3) δ 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) LC/MS [M+H]= m/z 336.1

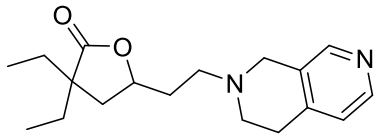


Preparation of 5-(2-(7-bromo-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-3,3-diethyldihydrofuran-2(3H)-one (**9o**): The title compound was prepared according to the procedure for 3,3-diethyl-5-(2-(4-(naphthalen-1-yl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate was substituted for 4-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)butyl 4-methylbenzenesulfonate and 7-bromo-1,2,3,4-tetrahydroisoquinoline was for 1 phenylpiperazine. Percent yield: 37%. 1H NMR (400 MHz, CDCb) 8 7.13 (dd, J= 1.8, 8.0 Hz, lH), 7.06 (d, J= 1.4 Hz, lH), 6.86 (d, J= 8.6 Hz, lH), 4.43 (m, lH), 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, lH), 1.91-1.69 (m, 3H), 1.52 (q, J= 7.6 Hz, 4H), 0.83 (dt, J= 5.6, 12.8 Hz, 6H) LC/MS [M+H]= m/z 380.10.

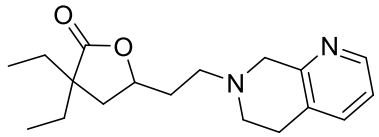


Preparation of 5-(2-(3,4-dihydro-2,6-naphthyridin-2(1H)-yl)ethyl)-3,3-diethyldihydrofuran-2(3H)-one (**9p**): The title compound was prepared according to the procedure for 3,3-diethyl-5-(2-(4-(naphthalen-1-yl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one trifluoroacetate, except 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 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 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) LC/MS [M+H]= m/z 303.2



Preparation of 5-(2-(3,4-dihydro-2,7-naphthyridin-2(1H)-yl)ethyl)-3,3-diethyldihydrofuran-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 1,2,3,4-tetrahydro-2,6-naphthyridine dihydrochloride was for 1-phenylpiperazine Percent yield: 63%. 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), 2.92-2.82 (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) LC/MS [M+H]= m/z 303.2



Preparation of 5-(2-(5,8-dihydro-1,7-naphthyridin-7(6H)-yl)ethyl)-3,3-diethyldihydrofuran-2(3H)-one (**9r**): 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-1,7-naphthyridine dihydrochloride was 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) 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 (PDSP) at The University of North Carolina, Chapel Hill under the direction of Professor Bryan Roth. Assay conditions can be found in the PDSP assay protocol book at https://pdsp.unc.edu/pdspweb/content/UNC-CH%20Protocol%20Book.pdf. A brief description of the assays is provided.

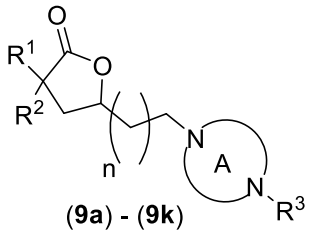
Sigma-2 receptor binding assay: K_i values for test compounds for the sigma-2 receptor were determined using a filtration assay in a 96 well polypropylene plate using membranes prepared from HEK293T cells stably transfected with the sigma-1 receptor or PC12 cells. The membranes were prepared from cultured cells rinsed with PBS, lysed in cold 50 mM Tris-HCL (pH 7.4), centrifuged at 20000 xg, 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 $MgCl_2$, 1 mM EDTA, pH 7.4) the membranes were incubated with 5-7 nM [^{3}H]- 1,3-di-(2-tolyl)guanidine ([^{3}H]-DTG, K_d = 9.9 nM) and test compound (11 concentrations) at room temperature for 90 minutes. Nonspecific binding was defined with 10 uM haloperidol. Membranes were then collected by rapid filtration on to filter mats pretreated with 0.3 % polyethyleneimine, washed 4x with cold assay buffer, dried, microscintillant added and then counted in a Microbeta scintillation counter. IC_{50} values were determined using a three-parameter non-linear curve fitting program in Prism 4.0 (GraphPad Software). K_i values were calculated from the IC_{50} values using the Cheng-Prusoff equation.[19] The reference standard haloperidol had a K_i = 13.9 nM.

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).

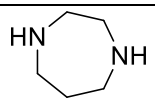
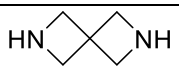
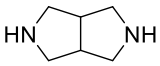
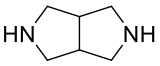
Microsomal stability assays: Test compounds were assessed for microsomal stability by incubating them at 37 ^{o}C in the presence of mouse or human liver microsomes and an NADPH regenerating system as described by Yang et. al.[21] Microsomal protein content was adjusted to give accurate rates of substrate consumption. Analysis was performed by Liquid Chromatography-tandem mass spectrometry (LC/MS/MS) analysis.

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



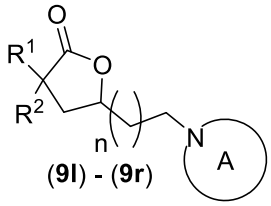
(**9a**) - (**9k**)

Entry	R^{1a}	R^{1b}	n	A	R^3	MW	TPSA	cLogP	σ_2	σ_1	σ2/σ1 ratio	MLM $T_{½}$ (min.)	Sol (mM)
									K_i (nM)				
9a	Et	Et	1	Piperazine	Phenyl	330	33	3	82	138	1.7	2.0	200
9b	Et	Et	2	Piperazine	Phenyl	344	33	4	7.7	31	4.0	2.3	182
9c	Et	Et	3	Piperazine	Phenyl	359	33	4	12	5.5	0.5	2.0	169
9d	Me	Me	1	Piperazine	Phenyl	302	33	3	753	279	0.4	14	192
9e	Et	Et	1	Piperazine	1-Naphthyl	381	33	5	32	2167	67.7	2.7	47
9f	Et	Et	1	Piperazine	4-Pyridyl	331	46	2	142	10000	38.7	60	199

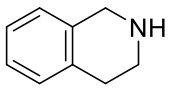
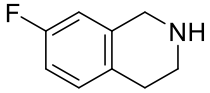
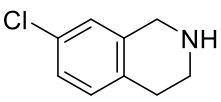
9g	Et	Et	1	Piperazine	4-Pyrimidine	332	59	2	10000	1017	0.1	8.8	198
9h	Et	Et	1		Phenyl	344	33	4	6.8	17	2.5	2.0	183
9i	Et	Et	1		Phenyl	342	33	3	53	12	0.2	5.1	197
9j	Et	Et	1		Phenyl	357	33	4	3.5	31	8.9	2.0	200
9k	Et	Et	1		4-Pyridyl	357	46	2	29	171	5.9	60	200

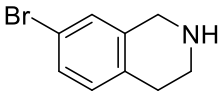
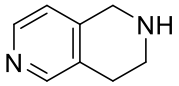
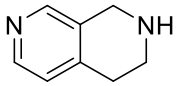
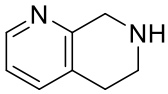
* Sigma-2 assays: Conducted with PC12 membrane preparations. Radioligand: [^{3}H]-DTG, K_d = 9.9 nM, Reference standard: Haloperidol, K_i = 13.9 nM **Sigma-1 assays: Conducted with HEK293 membrane preparations. Radioligand: [^{3}H]-Pentazocine, K_d = 6.5 nM, Reference standard: Haloperidol, K_i = 3.54 nM

Table 2. *In vitro* screening and physicochemical properties data for (**9l**) – (**9r**)



(**9l**) - (**9r**)

Entry	R^{1a}	R^{1b}	n	A	MW	TPSA	cLogP	σ_2	σ_1	$\sigma2/\sigma1$ ratio	MLM $T_{½}$ (min.)	Sol (mM)
								K_i (nM)				
9l	Et	Et	1		301	30	4	6.1	125	20.5	2.0	194
9m	Et	Et	1		319	30	4	7.4	68	9.2	2.0	151
9n	Et	Et	1		336	30	5	2.8	59	21.1	2.0	91

9o	Et	Et	1		380	30	5	8.9	4.7	0.5	2.0	111
9p	Et	Et	1		302	42	3	10000	10000	1.0	4.7	191
9q	Et	Et	1		302	42	3	10000	1156	0.1	2.9	192
9r	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

Acknowledgments: K_i determinations for compound binding to Sigma-1, and Sigma-2 were generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2013-00017-C (NIMH PDSP). The NIMH PDSP is directed by Bryan L. Roth at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscoll at NIMH, Bethesda MD, USA. For experimental details please refer to the PDSP web site https://pdsp.unc.edu/ims/investigator/web/. The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. TPSA and cLogP values were generated using the Dotmatics software suite (Dotmatics LLC The Old Monastery, Windhill Bishops, Stortford Herts, CW23 2ND UK).

Declaration of interests: 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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