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

Benjamin E Blass (tud12939@temple.edu)
Temple University School of Pharmacy https://orcid.org/0000-0003-2449-4503
Rong Gao
Temple University School of Pharmacy
Kevin M. Blattner
Temple University School of Pharmacy
John C. Gordon
Temple University School of Pharmacy
Douglas A. Pippin
Praeventix Inc
Daniel J. Canney
Temple University School of Pharmacy

Research Article

Keywords: Sigma-2, Sigma-1, γ-butyrolactone, Sigma receptor

Posted Date: June 30th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-661542/v1

Version of Record: A version of this preprint was published at Medicinal Chemistry Research on July 28th, 2021. See the published version at https://doi.org/10.1007/s00044-021-02771-0.

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.

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**) (Figure 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 elicits opioid mediated physiological responses through MOR and KOR, while (+)-SKF-100047's biological activity is produced by interaction with a previously unknown, non-opioid receptor that was designated the sigma receptor (σR).[2] Nearly 17 years later, W. D. Bowen et. al. successfully demonstrated that there are two sub-types of this receptor, sigma-1 (σ_1) and sigma-2 (σ_2),[3] and in 1996 mammalian σ_1 receptor was cloned and expressed in yeast cells.[4] A crystal structure of the human σ_1 receptor was reported in 2016,[5] but to date, there is no known natural ligand for this receptor.

The true nature of the σ_2 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 σ_2 receptor.[6] The pharmacological role of σ_2 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 σ_2 to cholesterol in these regions of the cell[7] and that it may play a role the regulation of the Niemann-Pick protein NPC1.[8] Despite the lack of a clear understanding of the pharmacological role of σ_2, this macromolecule has been the focus of efforts to develop therapies in a number of disease states such as schizophrenia,[9] Alzheimer's disease,[10] neuropathic pain,[11] traumatic brain injury,[12] and cancer.[13]

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 γ-butyrolactones. In an early publication, we described the identification of muscarinic receptor ligands from this class.[14] Further assessment of this family of compounds by the Psychoactive Drug Screening Program demonstrated that members of this family that have limited binding potency (>10,000 nM) to the family of muscarinic family of receptors, but they are potent σR binders with varying levels of σ_1/σ_2 selectivity. Given the therapeutic potential of this receptor, a drug discovery program focused on the identification of novel, drug-like functionalized γ-butyrolactones with potent σ_2 binding was established. The synthesis, characterization and preliminary evaluation of these lactones as potential selective σ_2 ligands will be presented.

Results And Discussion

Synthesis of substituted γ-butyrolactones was conducted as shown in Scheme 1 utilizing novel methods developed in our laboratory. The synthesis of these compounds begins with disubstituted ester (**4**). Allylation of (**4**) under basic conditions (LDA, HMPA) provides (**5**) which is then converted to (**7**) using a modified Prins reaction,[15] Specifically, reaction of (**5**) with paraformaldehyde in a hot mixture of acetic and sulfuric acid provided oxepan-2-one (**6**), which was converted to γ-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 σ_1 and σ_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 3 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 σ_2 receptor (K_i = 82 nM) and has a low level of selectivity for this receptor over σ_1 (K_i = 138 nM). Replacing the 1,1-diethyl lactone with a 1,1-dimethyl lactone (**9b**) leads to >9-fold decrease in σ_2 binding (K_i = 753 nM), but σ_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 σ_1 and σ_2 (both K_i = 10,000 nM), as did conversion of the piperazine to the corresponding piperazin-2-one (**9d**). Conversion to the corresponding piperadine (**9e**), on the other hand, lead to a >10 fold increase in both σ_2 (K_i = 6.4 nM) and σ_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 σ_2 (K_i = 5.4 nM), but the increase in affinity at σ_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 σ_2 affinity (K_i = 34 nM, 7.3 nM, and 12 nM), but σ_1 selectivity was low (2.9x, 1.9x, and 1.4x respectively). In addition, MLM stability was poor (> 5 min.) and HLM stability was moderate (23, 11, and 12 minutes respectively). Replacing these groups with the electron donating substituents 4-OMe (**9j**) and 4-Me (**9k**) also lead to potent σ_2 binders (K_i = 53 nM, and 14 nM respectively). Interestingly, while (**9j**) demonstrated limited selectively for σ_2 over σ_1, (**9k**) was highly selective (σ_1 K_i = 10,000 nM). In addition, both of these compounds were highly stable in HLM assays ($T_{1/2}$ = 60 min, 40.6 min respectively), but MLM stability was low ($T_{1/2}$ > 5 min.).

Relocating these same substituents to the 3-position (**9l** to **9p**) and the 2-position (**9q** to **9u**) produced highly potent σ_2 binders (K_i = 9.9 nM to 62 nM), Most of these analogs demonstrated low to moderate selectivity (2 to 8.5 fold) with the exception of the 2-OMe analog (**9u**), which exhibited a >26 fold drop in binding affinity at σ_1 (K_i = 1168 nM) over σ_2 (K_i = 44 nM). Increasing the steric bulk in the 2-position by replacing the 2-OMe with an isopropyl group (**9v**) improved σ_2 selectivity (σ_2 K_i = 5.9 nM vs σ_1 K_i = 195 nM), but the 2,4-di-Me analog (**9w**) displayed high affinity at both σ_2 (K_i = 9.2 nM) and σ_1 (K_i = 10 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 to 37.8 min.), none of them were stable in MLM ($T_{1/2}$ <5 min.).

We next assessed the impact of replacing the phenyl ring with a pyridine ring. Interestingly, incorporating a 2-pyrdime (**9x**, σ_2 K_i = 268 nM) or a 4-pyridine (**9z,** σ_2 K_i = 142 nM) lead to a 3.2 x and 1.7x loss in σ_2 binding affinity when compared to the phenyl analog (**9a**, σ_2 K_i = 82 nM). However, the 3-pyridine analog (**9y,** σ_2 K_i =10,000 nM) displayed a >100-fold drop in affinity over (**9a**). σ_1 affinity was also diminished in all three pyridine analogs, but the decrease in affinity was not uniform. While (**9x**) σ_1 binding affinity (K_i = 1499 nM) was ~10x less than (**9a**) (K_i = 138 nM), both (**9y**) and (**9z**) were >120x less potent binders for this target (σ_1 Ki = 10,000 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 to 60 min.), only (**9z**) was stable in MLM ($T_{1/2}$ = 60 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 mM), Cyp 3A4 inhibition was low (IC_{50} = 7400 nM), and their physicochemical properties (MW, TPSA, cLogP) are all within the drug-like properties defined by Lipinski.[16]

Conclusion

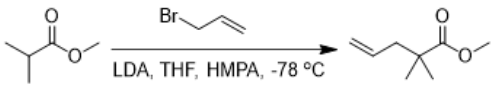
In summary, a series of substituted lactones with drug-like physicochemical properties (MW, TPSA, cLogP) have been investigated as potential selective σ_2R ligands. We have determined that conversion of either of the piperidine amine units to the corresponding amide causes a significant loss in activity at both σRs (**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 σ_2 over σ_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 σ_2 ligand (K_i = 142 nM), with excellent selectivity for this target over σ_1 (K_i = 10,000 nM) and it is highly stable in both MLM and HLM ($T_{1/2}$ = 60 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 σ_2 activity[17] such as schizophrenia, Alzheimer's disease, neuropathic pain, traumatic brain injury, and cancer.

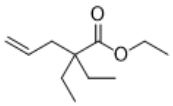
Experimental Methods And Materials

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 (δ values) were reported in ppm relative to TMS. For multiplicity, s = singlet, d = doublet, t = triplet, m = multiplet. Purity (%) and mass spectral data were determined with a Waters Agilent 1200 HPLC/MS (Zorbax SB-C18, 2.1 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

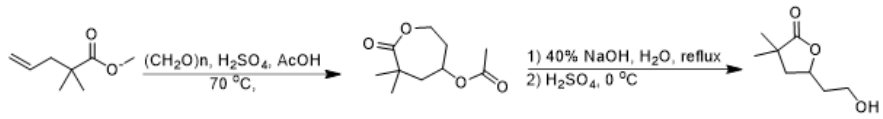
and Agilent 6130 quadrupole MS. All compounds were purified to 95% purity or greater as determined by HPLC/MS and 1H-NMR. Melting points were recorded on a capillary melting point apparatus.



Methyl 2,2-dimethylpent-4-enoate (**4**, R^{1a}, R^{1b} = Me): This reaction was performed in oven-dried glassware under a nitrogen atmosphere. To a well-stirred solution of freshly prepared lithium diisopropylammide (1M, 1.10 equiv) in dry 35 ml tetrahydrofuran, isobutyric acid methyl ester (3.32 g, 32.6 mmol, 1.0 equiv) was added dropwise during 0.5 hours at -78 ^{o}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 x 100mL). The extract was washed with 10% $NaHCO_3$ (200mL) and brine (200mL). The solution was then dried over $MgSO_4$, concentrated in vacuo and distilled (bp. 85.5~86.5 ^{o}C/3.5mm Hg) to provide 3.47g (75% yield) of the product as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73 (1H, dd, J = 9.4, 17.7, H-4), 5.04 (2H, dd, J = 1.9, 13.5, H-5), 4.12 (3H, s, OCH_3), 2.28 (2H, d, J = 7.4, H-3), 1.17 (6H, s, H-2'); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.42 (C=O), 134.42 (CH, C-4), 117.88 (CH_2, C-5), 60.35 ($CO_2\underline{C}H_3$) 44.91 (C, C-2), 42.25 (CH_2, C-3), 24.92 ($CH(\underline{C}H_3)_2$, C-2)

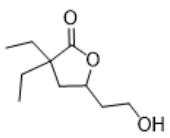


Ethyl 2,2-diethylpent-4-enoate (**4**, R^{1a}, R^{1b} = Et): The title compound was prepared according to the procedure for methyl 2,2-dimethylpent-4-enoate, except 2-ethyl-butyric acid ethyl ester was substituted for isobutyric acid methyl ester. The product was isolated as a colorless oil. (66% yield) ^{1}H NMR (400 MHz, $CDCl_3$) δ 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, $O\underline{CH_2}CH_3$), 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, $OCH_2\underline{CH_3}$), 0.80 (6H, t, J = 7.5, H-4'). ^{13}C NMR (101 MHz, $CDCl_3$) δ 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 (CH2, C-3), 29.8 (CH2, C-3'), 14.3 (CH3, $OCH_2\underline{CH_3}$), 10.5 (CH3, C-4').

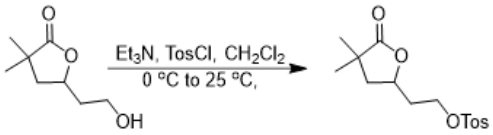


5-(2-hydroxy-ethyl)-3,3-dimethyl-dihydro-furan-2-one: A mixture of glacial acetic acid (28.6 g, 477 mmol, 53.6 equiv), paraformaldehyde (0.80 g, 26.7 mmol, 3.0 equiv) and H_2SO_4 (0.5 g, 4.45 mmol, 0.57equiv) was stirred for 30min at 70 ^{o}C before methyl 2,2-dimethylpent-4-enoate (1.26 g, 8.9mmol, 1.0 equiv) was added dropwise during 10 min. The reaction mixture was then maintained at 70~80 ^{o}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 x 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.

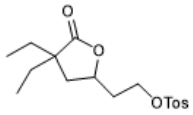
A mixture of the crude oil and 30% NaOH (7.1 g NaOH, 177 mmol, 20 equiv) aqueous solution was refluxed for 2 hours. The mixture was cooled in an ice bath and excess 30% H_2SO_4 was added until acidic (pH < 2). The resulting mixture was extracted with ethyl acetate (3 x 200 mL), the combined organic phase was washed with 10% $NaHCO_3$, (400 mL), brine (400mL), 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 (1.05g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.70-4.60 (1H, m, CH), 3.90-3.78 (2H, m, $-CH_2\underline{CH_2}OH$), 2.22 (1H, dd, J = 5.9, 12.7, CH_2), 1.98 – 1.87 (2H, m, $-\underline{CH_2}CH_2OH$), 1.80 (1H, dd, J = 5.9, 12.7, CH_2), 1.29 (3H, s, CH_3), 1.28 (3H, s, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.26 (CO), 75.01 (CH), 59.58 ($-CH_2\underline{CH_2}OH$), 43.93 (CH_2), 40.62 ($\underline{C}(CH_3)_2$), 38.69 ($-\underline{CH_2}CH_2OH$), 25.31 (CH_3), 24.61 (CH_3); Rf, 0.34 (Hexane: Ethyl Acetate 1:1); Anal. Calcd for $C_8H_{14}O_3$: C, 60.74; H, 8.92; Found: C, 60.47; H, 8.86.



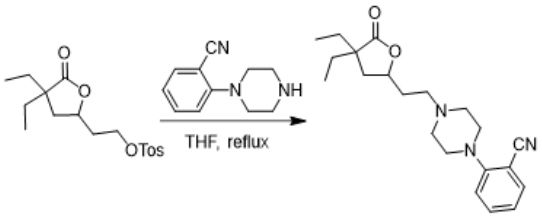
Synthesis of 3,3-diethyl-5-(2-hydroxyethyl)dihydrofuran-2(3H)-one: The title compound was prepared according to the procedure for 5-(2-Hydroxy-ethyl)-3,3-dimethyl-dihydro-furan-2-one, except ethyl 2,2-diethylpent-4-enoate was substituted for methyl 2,2-dimethylpent-4-enoate. The product was isolated as a colorless oil. (76% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.62 (dtd, J = 5.3, 7.3, 9.5, 1H), 3.78 (t, J = 6.1, 2H), 3.20 (s, 1H), 2.19 (dd, J = 6.8, 13.1, 1H), 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, $CDCl_3$) δ 181.46 (CO), 75.10 (CH), 58.91 ($CH_2\underline{CH_2}OH$), 48.77 (C), 39.13 (CH_2), 37.76 ($\underline{CH_2}CH_2OH$), 29.21 ($\underline{CH_2}CH_3$), 28.30 ($\underline{CH_2}CH_3$), 8.83 ($CH_2\underline{CH_3}$), 8.73 ($CH_2\underline{CH_3}$); Rf, 0.36 (Hexane: Ethyl Acetate 5:2); Anal. Calcd for $C_{10}H_{18}O_3$: C, 64.49; H, 9.74; Found: C, 64.20; H, 9.57.



2-(4,4-dimethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate: To a stirred solution of 5-(2-Hydroxy-ethyl)-3,3-dimethyl-dihydro-furan-2-one (0.316 g, 2 mmol, 1.0 equiv) and Et_3N (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 ^{o}C. The resulting mixture was stirred at 0 ^{o}C for 1 hour 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 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), 7.29 (2H, m, CH), 4.39 (1H, m, CH), 4.10 (2H, m, $CH_2\underline{CH_2}$OTos), 2.38 (3H, s, CH_3), 2.09 (1H, m, CH_2), 1.93 (2H, m, $\underline{CH_2}CH_2$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, CDCl3) δ 181.26 (CO), 145.16 (C), 132.53 (C), 130.03 (CH), 127.84 (CH), 72.93 (CH), 66.83 ($CH_2\underline{CH_2}O$-SO_2), 42.99 (CH_2), 40.23 (C), 34.97 ($\underline{CH_2}CH_2O$-SO_2), 24.82 (CH_3), 24.12 (CH_3), 21.57 (CH_3); HRMS (CI): [M+H] 313.1; Anal. Calcd for $C_{15}H_{20}O_5S$: C, 57.67; H, 6.45; Found: C, 57.85; H, 6.63.

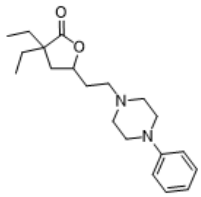


Synthesis of 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate: The title compound was prepared according to the procedure for 2-(4,4-dimethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate, except 3,3-diethyl-5-(2-hydroxyethyl)dihydrofuran-2(3H)-one was substituted for 5-(2-Hydroxy-ethyl)-3,3-dimethyl-dihydro-furan-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, $CH_2\underline{CH_2}$OTos), 2.46 (3H, s, CH_3), 2.21 – 1.84 (3H, m, $\underline{CH_2}CH_2$OTos and CH_2), 1.83 – 1.68 (1H, m, CH_2), 1.58 (4H, t, J = 7.4 Hz, $\underline{CH_2}CH_3$), 0.89 (6H, dt, J = 7.5, 18.0 Hz, $CH_2\underline{CH_3}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.33 (CO), 145.30 (SO_2-C), 132.72 ($\underline{C}CH_3$), 130.15 (CH), 128.03 (CH), 73.18 (CH), 66.95 ($CH_2\underline{CH_2}O$-SO_2), 48.67 (C), 37.53 (CH_2), 35.82 ($\underline{CH_2}CH_2O$-SO_2), 29.14 ($\underline{CH_2}CH_3$), 28.23 ($\underline{CH_2}CH_3$), 21.76 (CH_3), 8.81 ($CH_2\underline{CH_3}$), 8.74 ($CH_2\underline{CH_3}$). Anal. Calcd for $C_{17}H_{24}O_5S$: C, 59.98; H, 7.11; Found: C, 60.27; H, 7.25.

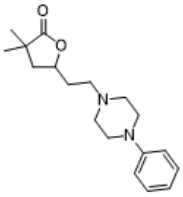


2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (**9q**): 2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl 4-methylbenzenesulfonate (0.102 g, 0.3mmol, 1.0 equiv) was treated with 2-piperazin-1-yl-benzonitrile (168.3 mg, 0.9 mmol, 3.0 equiv) in dry tetrahydrofuran and refluxed for 72 hours. The tetrahydrofuran was evaporated under reduced pressure, the residue dissolved in dichloromethane, washed with H_2O, 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, $N\underline{CH_2}CH_2N$), 2.81 – 2.51 (6H, m, $NCH_2\underline{CH_2}N$ and $CH_2\underline{CH_2}N$), 2.14 (1H, dd, J = 13.1, 6.8 Hz, CH_2), 1.86 (3H, m, $\underline{CH_2}CH_2N$ and CH_2), 1.67 – 1.53 (4H, m, $\underline{CH_2}CH_3$), 0.92 (6H, dt, J = 20.1, 7.5 Hz, $CH_2\underline{CH_3}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.82 (CO), 155.57 (C), 134.43 (CH), 133.95 (CH), 122.03 (C-$\underline{C}$N), 118.81 (CH), 118.50

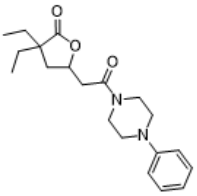
(CH), 106.13 (C-CN), 75.50 (CH), 54.44 (C), 53.22 ($NCH_2\underline{CH_2}N$), 51.34 ($N\underline{CH_2}CH_2N$), 48.71 ($N\underline{CH_2}CH_2$), 37.75 (CH_2), 33.60 ($NCH_2\underline{CH_2}$), 29.35 ($\underline{CH_2}CH_3$), 28.39 ($\underline{CH_2}CH_3$), 8.89 ($CH_2\underline{CH_3}$), 8.81 ($CH_2\underline{CH_3}$); MS (LC/MS, $M+H^+$): 356.2.



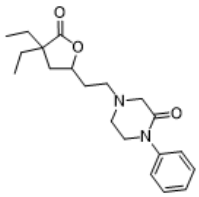
3,3-diethyl-5-(2-(4-phenylpiperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (**9a**): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (**9q**), except 2-piperazin-1-yl-benzonitrile was substituted for 2-piperazin-1-yl-benzonitrile (50.6 mg, 51% yield)**:** ^{1}H NMR (400 MHz, D_2O) 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_2CH_2N and $CH_2\underline{CH_2}N$), 2.39 – 2.07 (3H, m, $\underline{CH_2}CH_2N$ and CH_2), 1.98 (1H, dd, J = 13.4, 9.4 Hz, CH_2), 1.61 (4H, m, $\underline{CH_2}CH_3$), 0.87 (6H, dt, J = 12.1, 7.5 Hz, $CH_2\underline{CH_3}$); ^{13}C NMR (101 MHz, D_2O) δ 187.92 (CO), 150.20 (C), 132.89 (CH), 127.03 (CH), 121.14 (CH), 79.53 (CH), 56.52 (C) , 54.13 ($NCH_2\underline{CH_2}N$), 52.41 ($N\underline{CH_2}CH_2N$), 50.87 ($N\underline{CH_2}CH_2$), 39.37 (CH_2), 32.81 ($NCH_2\underline{CH_2}$), 31.91 ($\underline{CH_2}CH_3$), 30.68 ($\underline{CH_2}CH_3$), 11.00 ($CH_2\underline{CH_3}$), 10.87 ($CH_2\underline{CH_3}$); 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. Melting point of di HCl salt: 239 ºC



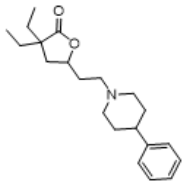
Synthesis of 3,3-dimethyl-5-(2-(4-phenylpiperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (**9b**): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (**9q**), except 2-(4,4-dimethyl-5-oxotetrahydrofuran-2-yl)ethyl 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, $CDCl_3$) δ 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, $N\underline{CH_2}CH_2N$), 2.66 (4H, m, $NCH_2\underline{CH_2}N$), 2.61 (2H, m, $CH_2\underline{CH_2}N$), 2.26 (1H, m, CH_2), 1.90 (3H. m, $\underline{CH_2}CH_2N$ and CH_2), 1.34 (3H, s, CH_3), 1.33 (3H, s, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 184.35 (CO), 152.68 (C), 130.07 (CH), 121.21 (CH), 117.49 (CH), 77.42 (CH), 55.67 ($NCH_2\underline{CH_2}N$), 54.31 (CH_2), 50.29 ($N\underline{CH_2}CH_2N$), 44.18 ($CH_2\underline{CH_2}N$), 41.71 ($\underline{C}(CH_3)_2$), 33.74 ($\underline{CH_2}CH_2N$), 25.27 (CH_3), 24.59 (CH_3). LC/MS [M+H]= m/z 303.2.



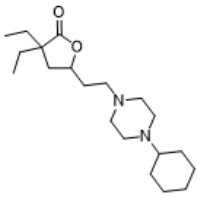
Synthesis of 3,3-diethyl-5-(2-oxo-2-(4-phenylpiperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (**9c**): The title compounds was prepared according to the procedures described by R. Goa.[18] ^{1}H NMR (400 MHz, MeOD) δ 7.80-7.68 (2H, m, CH), 7.67-7.49 (3H, m, CH), 4.09 (4H, broad, $N\underline{CH_2}CH_2N$), 3.86-3.62 (4H, m, $NCH_2\underline{CH_2}N$), 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, $\underline{CH_2}CH_3$), 0.94 (6H, dt, J = 18.7, 7.5 Hz, $CH_2\underline{CH_3}$). ^{13}C NMR (101 MHz, MeOD) δ 182.79 (CO), 170.27 (CO), 143.18 (C), 131.74 (CH), 131.40 (CH), 122.20 (CH), 75.73 (CH), 56.13 ($\underline{C}(CH_2CH_3)_2$), 49.93 ($CH_2$), 44.34 ($NCH_2\underline{CH_2}N$), 40.21 ($NCH_2\underline{CH_2}N$), 39.68 ($N\underline{CH_2}CH_2N$), 38.30 ($\underline{CH_2}C(O)$-N), 30.06 ($\underline{CH_2}CH_3$), 29.14 ($\underline{CH_2}CH_3$), 9.04 ($CH_2\underline{CH_3}$), 8.93 ($CH_2\underline{CH_3}$). MS (LC/MS, $M+H^+$): 345.2



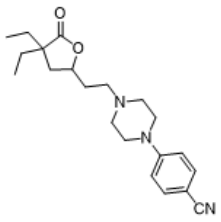
Synthesis of 4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)-1-phenylpiperazin-2-one (**9d**): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (**9q**), except 1-phenylpiperazin-2-one was substituted for 2-piperazin-1-yl-benzonitrile (50.4 mg, yield 56%). The product was isolated as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1H NMR (400 MHz, $CDCl_3$) δ 7.42-7.34 (2H, m, CH), 7.31-7.21 (3H, m, CH), 4.51 (1H, m, CH), 3.75-3.61 (2H, m, $N\underline{CH_2}CH_2N$), 3.29 (2H, dd, J= 13.3, 16.3 Hz, $N\underline{CH_0}CO$), 2.82 (2H, t, J= 5.7 Hz, $CH_2\underline{CH_2}N$), 2.62 (2H, t, J= 7.0 Hz, $NCH_2\underline{CH_2}N$), 2.14 (1H, dd, J= 6.8, 13.1 Hz, CH_2), 1.89-1.78 (3H, m, $\underline{CH_2}CH_2N$, CH_2), 1.68-1.55 (4H, m, $\underline{CH_2}CH_3$), 0.92 (6H, dt, J= 7.5, 21.0 Hz, $CH_2\underline{CH_3}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.62 (CO), 141.54 (CO), 129.54 (C), 127.27 (CH), 125.91 (CH), 124.53 (CH), 74.86 (CH), 53.88 ($\underline{C}(CH_2CH_2)_2$), 50.80 ($N\underline{CH_2}CH_2N$), 50.21 ($NCH_2\underline{CH_2}N$), 49.43 ($CH_2$), 48.62 ($CH_2\underline{CH_2}N$), 37.6.2 ($N\underline{CH_2}CO$), 33.28 ($\underline{CH_2}CH_2N$), 29.20 ($\underline{CH_2}CH_3$), 28.25 ($\underline{CH_2}CH_3$), 8.77 ($CH_2\underline{CH_3}$), 8.70 ($CH_2\underline{CH_3}$), LC/MS [M+H]= m/z 345.2



Synthesis of 3,3-diethyl-5-(2-(4-phenylpiperidin-1-yl)ethyl)dihydrofuran-2(3H)-one (**9e**): The title compound was prepared according to the procedure for 22-(4-(2-(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)**:** ^{1}H NMR (400 MHz, D_2O) δ 7.39 (5H, tt, 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, $\underline{CH_2}CH_3$), 0.92 (6H, dt, J = 7.5, 12.7, $CH_2\underline{CH_3}$); ^{13}C NMR (101 MHz, D_2O) δ 187.89, 146.67, 131.85, 130.03, 129.64, 79.59, 52.33, 41.74, 39.25, 32.90, 31.85, 30.60, 10.89, 10.76; MS (LC/MS, M+H$^+$): 330.2; Anal. Calcd for $C_{21}H_{32}ClNO_2$: C, 68.93; H, 8.81; N, 3.83; Found: C, 68.87; H, 8.93; N,3.79. Melting point of HCl salt: 239.5 ^{o}C

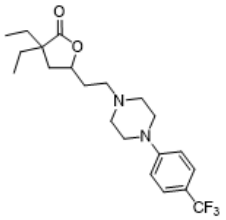


Synthesis of 5-(2-(4-cyclohexylpiperazin-1-yl)ethyl)-3,3-diethyldihydrofuran-2(3H)-one (**9f**): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (**9q**), except 1-cyclohexyl-piperazine was substituted for 2-piperazin-1-yl-benzonitrile (56.6 mg, 56%). The product was isolated as a clear oil. ^{1}H 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); ^{13}C 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

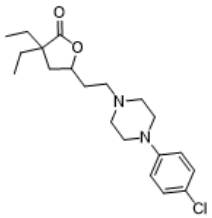


Synthesis of 4-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (**9g**): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (**9q**), except 4-piperazin-1-yl-benzonitrile was substituted for 2-piperazin-1-yl-benzonitrile (48.mg, 45% yield)**:** ^{1}H NMR (400 MHz, MeOD) δ 7.69 – 7.54 (2H, m, CH), 7.23 – 7.02 (2H, m, CH),

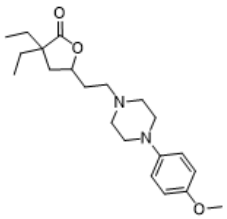
4.59 (1H, ddd, J = 15.8, 9.3, 3.7 Hz, CH), 4.31 – 3.30 (10H, m, N$\underline{CH_2}CH_2N$, $NCH_2\underline{CH_2}N$, and $CH_2\underline{CH_2}N$), 2.36 – 2.21 (2H, m, CH_2), 2.21 – 2.06 (1H, m, CH_2), 1.96 (1H, dd, J = 13.3, 9.4 Hz, CH_2), 1.65 (4H, ddd, J = 17.4, 8.7, 6.2 Hz, $\underline{CH_2}CH_3$), 0.95 (6H, dt, J = 13.3, 7.5 Hz, $CH_2\underline{CH_3}$); ^{13}C 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 ($\underline{C}(CH_2CH_2)_2$), 52.76 (N$\underline{CH_2}CH_2N$), 49.91 ($NCH_2\underline{CH_2}N$), 45.91 ($CH_2\underline{CH_2}N$), 38.33 ($CH_2$), 31.73 ($\underline{CH_2}CH_2N$), 30.04 ($\underline{CH_2}CH_3$), 29.17 ($\underline{CH_2}CH_3$), 9.00 ($CH_2\underline{CH_3}$), 8.91 ($CH_2\underline{CH_3}$); MS (LC/MS, M+H$^+$): 356.2; Anal. Calcd for $C_{21}H_{30}ClN_3O_2$: C, 64.35; H, 7.72; N, 10.72; Found: C, 64.46; H, 7.65; N, 10.65. Melting point of di-HCl salt: 213~214^{o}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-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (**9q**) except 1-(4-(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, $CDCl_3$) 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, N$\underline{CH_2}CH_2N$) 2.69-2.48 (6H, m, $NCH_2\underline{CH_2}N$, and $CH_2\underline{CH_2}N$), 2.15 (1H, dd, J= 6.8, 13.1 Hz, CH_2), 1.98-1.78 (3H, m, CH_2), 1.69-1.58 (4H, m, $\underline{CH_2}CH_3$), 0.94 (6H, dt, J= 7.5, 19.0 Hz, $CH_2\underline{CH_3}$) ^{13}C NMR (101 MHz, MeOD) 183.18 (CO), 154.91 (C), 127.30 (CH), 125.01 (CF_3), 115.75 (CH), 77.59 (CH), 55.95 ($\underline{C}(CH_2CH_2)_2$), 54.07 (N$\underline{CH_2}CH_2N$), 50.13 ($NCH_2\underline{CH_2}N$), 48.67 ($CH_2\underline{CH_2}N$), 38.45 ($CH_2$), 34.36 ($\underline{CH_2}CH_2N$), 30.26 ($\underline{CH_2}CH_3$), 29.32 ($\underline{CH_2}CH_3$), 9.04 ($CH_2\underline{CH_3}$), 8.95 ($CH_2\underline{CH_3}$), LC/MS [M+H]= m/z 399.2

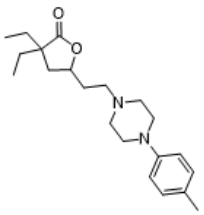


Synthesis of 5-(2-(4-(4-chlorophenyl)piperazin-1-yl)ethyl)-3,3-diethyldihydrofuran-2(3H)-one (**9i**): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (**9q**), except 1-(4-chlorophenyl)piperazine was substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (Yield: 83%). ^{1}H NMR (400 MHz, $CDCl_3$) 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, N$\underline{CH_2}CH_2N$) 2.68-2.48 (6H, m, $NCH_2\underline{CH_2}N$, and $CH_2\underline{CH_2}N$), 2.15 (1H, dd, J= 6.9, 12.8 Hz, CH_2), 1.96-1.77 (3H, m, CH_2), 1.70-1.56 (4H, m, $\underline{CH_2}CH_3$), 0.94 (6H, dt, J= 7.5, 19.2 Hz, $CH_2\underline{CH_3}$) ^{13}C NMR (101 MHz, MeOD) δ 183.16 (CO), 151.40 (C), 129.89 (CH), 125.55 (C), 118.53 (CH), 77.61 (CH), 55.51 ($\underline{C}(CH_2CH_2)_2$), 54.20 (N$\underline{CH_2}CH_2N$), 50.12 ($NCH_2\underline{CH_2}N$), 49.94 ($CH_2\underline{CH_2}N$), 38.46 ($CH_2$), 34.37 ($\underline{CH_2}CH_2N$), 30.26 ($\underline{CH_2}CH_3$), 29.33 ($\underline{CH_2}CH_3$), 9.07 ($CH_2\underline{CH_3}$), 8.98 ($CH_2\underline{CH_3}$), LC/MS [M+H]= m/z 365.2.

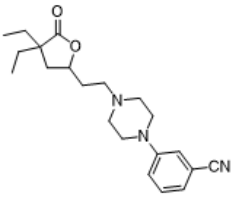


Synthesis of 3,3-diethyl-5-(2-(4-(4-methoxyphenyl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (**9j**): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (**9q**), except 1-(4-methoxy-phenyl)-piperazinewas substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (67.1 mg, 62% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 – 6.75 (4H, m, CH), 4.48 (1H, ddd, J = 19.8, 8.4, 6.4 Hz, CH), 3.76 (3H, s, CH_3), 3.14 – 2.99 (4H, m, N$\underline{CH_2}CH_2N$), 2.67 – 2.46 (6H, m, $NCH_2\underline{CH_2}N$, and $CH_2\underline{CH_2}N$), 2.15 – 2.07 (1H, m, CH_2), 1.92 – 1.79 (3H, m, CH_2), 1.62 (4H, qd, J = 7.4, 4.7 Hz, $\underline{CH_2}CH_3$), 0.97 – 0.88 (6H, m, $CH_2\underline{CH_3}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.90 (CO), 153.93 (C), 145.74 (C), 118.29 (CH), 114.53 (CH), 75.71 (CH), 55.67 ($O\underline{CH_3}$), 54.59 ($\underline{C}(CH_2CH_2)_2$), 53.51 (N$\underline{CH_2}CH_2N$),

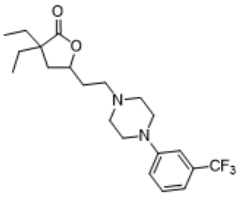
50.69 ($NCH_2\underline{CH_2}N$), 48.72 ($CH_2\underline{CH_2}N$), 37.81 (CH_2), 33.91 ($\underline{CH_2}CH_2N$), 29.35 ($\underline{CH_2}CH_3$), 28.41 ($\underline{CH_2}CH_3$), 8.90 ($CH_2\underline{CH_3}$), 8.82 ($CH_2\underline{CH_3}$). MS (LC/MS, $M+H^+$): 361.2.



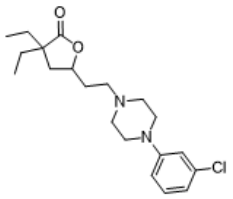
Synthesis of 3,3-diethyl-5-(2-(4-(p-tolyl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (**9k**): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (**9q**), except 1-p-tolyl-piperazine was substituted for 2-piperazin-1-yl-benzonitrile (52.8 mg, 51% yield)**:** ^{1}H 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, $N\underline{CH_2}\underline{CH_2}N$, and $CH_2\underline{CH_2}N$), 2.30 – 2.01 (6H, m, CH_3 and CH_2), 1.88 (1H, dd, J = 13.3, 9.4 Hz, CH_2), 1.58 (4H, m, $\underline{CH_2}CH_3$), 0.87 (6H, dt, J = 13.7, 7.5 Hz, $CH_2\underline{CH_3}$); ^{13}C 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 ($\underline{C}(CH_2CH_2)_2$), 52.04 ($N\underline{CH_2}CH_2N$), 50.53 ($NCH_2\underline{CH_2}N$), 49.93 ($CH_2\underline{CH_2}N$), 38.31 ($CH_2$), 31.68 ($\underline{CH_2}CH_2N$), 30.03 ($\underline{CH_2}CH_3$), 29.17 ($\underline{CH_2}CH_3$), 20.76 ($CH_3$), 9.01 ($CH_2\underline{CH_3}$), 8.92 ($CH_2\underline{CH_3}$); MS (LC/MS, $M+H^+$): 345.2; Anal. Calcd for $C_{21}H_{34}Cl_2N_2O_2$: 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^{o}C.



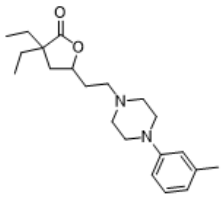
Synthesis of 3-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (**9l**): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (**9q**), except 3-(piperazin-1-yl)benzonitrile was substituted 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: 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (1H, m, CH), 7.09 (3H, m, CH), 4.49 (1H, m, CH), 3.22 (4H, t, J= 5.0 Hz, $N\underline{CH_2}CH_2N$), 2.61 (4H, t, J= 5.2 Hz, $NCH_2\underline{CH_2}N$), 2.56 (2H, m, $CH_2\underline{CH_2}N$) 2.14 (1H, dd, J= 6.7, 13.1 Hz, CH_2), 1.85 (3H, m, CH_2), 1.62 (4H, m, $\underline{CH_2}CH_3$) 0.92 (6H, dt, J= 7.7, 19.0 Hz, $CH_2\underline{CH_3}$). ^{13}C NMR (101 MHz, MeOD) δ 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 ($\underline{C}(CH_2CH_2)_2$), 52.83 ($N\underline{CH_2}CH_2N$), 48.60 ($NCH_2\underline{CH_2}N$), 48.07 ($CH_2\underline{CH_2}N$), 37.67 ($CH_2$), 33.54 ($\underline{CH_2}CH_2N$), 29.22 ($\underline{CH_2}CH_3$), 28.28 ($\underline{CH_2}CH_3$), 8.77 ($CH_2\underline{CH_3}$), 8.71 ($CH_2\underline{CH_3}$). LC/MS [M+H]= m/z 356.20.



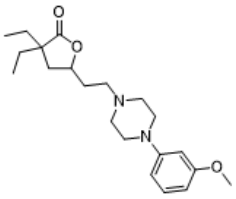
Synthesis of 3,3-diethyl-5-(2-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (**9m**): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (**9q**), except 1-(3-(trifluoromethyl)phenyl)piperazine was substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (Yield: 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 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, $N\underline{CH_2}CH_2N$), 3.33 (4H, m, $NCH_2\underline{CH_2}N$), 3.06 (2H, b, $CH_2\underline{CH_2}N$), 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, $\underline{CH_2}CH_3$) 0.92 (6H, dt, J= 7.4, 16.6 Hz, $CH_2\underline{CH_3}$) ^{13}C NMR (101 MHz, MeOD) δ 182.32 (CO), 151.54 (C), 132.87 (C), 132.55 (CH), 13126 (CF_3), 121.08 (CH), 118.35 (CH), 114.01 (CH), 76.12 (CH), 54.90 ($\underline{C}(CH_2CH_2)_2$), 53.11 ($N\underline{CH_2}CH_2N$), 49.94 ($NCH_2\underline{CH_2}N$), 47.51 ($CH_2\underline{CH_2}N$), 38.35 ($CH_2$), 31.83 ($\underline{CH_2}CH_2N$), 30.09 ($\underline{CH_2}CH_3$), 29.19 ($\underline{CH_2}CH_3$), 8.98 ($CH_2\underline{CH_3}$), 8.90 ($CH_2\underline{CH_3}$). LC/MS [M+H]= m/z 399.2.



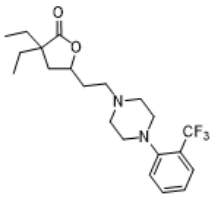
Synthesis of 5-(2-(4-(3-chlorophenyl)piperazin-1-yl)ethyl)-3,3-diethyldihydrofuran-2(3H)-one (**9n**): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (**9q**), 1-(3-chlorophenyl)piperazine was substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (Yield: 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 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_2CH_2N) 2.55-2.35 (6H, m, NCH_2CH_2N and CH_2CH_2N), 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_2CH_3), 0.81 (6H, dt, J= 7.5, 19.0 Hz, CH_2CH_3) ^{13}C NMR (101 MHz, $CDCl_3$) δ 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 ($C(CH_2CH_2)_2$), 54.51 (NCH_2CH_2N), 52.87 (NCH_2CH_2N), 48.59 (CH_2CH_2N), 48.29 (CH_2), 37.67 (CH_2CH_2N), 29.24 (CH_2CH_3), 28.29 (CH_2CH_3), 8.78 (CH_2CH_3), 8.71 (CH_2CH_3), LC/MS [M+H]= m/z 365.2



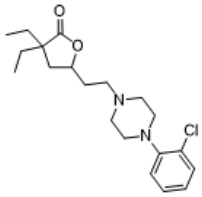
Synthesis of 3,3-diethyl-5-(2-(4-(o-tolyl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (**9o**): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (**9q**), except 1-(3-methylphenyl)piperazine was substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (Yield: 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 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_2CH_2N, NCH_2CH_2N, and CH_2CH_2N), 2.33 (3H, s, CH_3), 2.28 (1H, m, CH_2), 2.21 (1H, dd, J= 6.7, 13.1 Hz, 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_2CH_3) 0.92 (6H, dt, J= 7.4, 16.8 Hz, CH_2CH_3) ^{13}C NMR (101 MHz, MeOD) δ 182.29 (CO). 151.16 (C), 140.28 (CH), 130.22 (CCH_3), 123.33 (CH), 118.77 (CH), 115.18 (CH), 76.10 (CH), 54.89 ($C(CH_2CH_2)_2$), 53.35 (NCH_2CH_2N), 49.94 (NCH_2CH_2N),, 48.25 (CH_2CH_2N), 38.35 (CH_2), 31.79 (CH_2CH_2N), 30.08 (CH_2CH_3), 29.19 (CH_2CH_3), 21.70 (CH_3), 8.98 (CH_2CH_3), 8.90 (CH_2CH_3). LC/MS [M+H]= m/z 345.2.



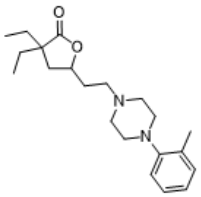
Synthesis of 3,3-diethyl-5-(2-(4-(3-methoxyphenyl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (**9p**): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (**9q**), except 1-(3-methoxy-phenyl)-piperazine was substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (61.7 mg, 57% yield)**.** ^{1}H NMR (400 MHz, DMSO) δ 7.16 (1H. t, J = 8.2 Hz, CH), 6.65 – 6.35 (3H. m, CH), 4.54 (1H, s, CH), 3.82 (2H, d, J = 8.9 Hz, CH_2), 3.57 (2H, s, CH_2), 3.16 (6H, dd, J = 27.5, 16.8 Hz, NCH_2CH_2N and CH_2CH_2N), 2.28 – 2.04 (3H, m), 1.82 (1H, dd, J = 13.1, 9.4 Hz, CH_2), 1.64 – 1.44 (4H, m, CH_2CH_3), 0.85 (6H, dt, J = 10.2, 7.5 Hz, CH_2CH_3); ^{13}C NMR (101 MHz, MeOD) δ 182.37 (CO), 162.23 ($COCH_3$), 149.87 (C), 131.32 (CH), 110.3 (CH), 106.9 (CH), 98.6 (CH), 76.14, 55.89 ($COCH_3$), 54.87 ($C(CH_2CH_2)_2$), 52.48 (NCH_2CH_2N), 49.92 (NCH_2CH_2N), 38.31 (CH_2CH_2N), 31.69 (CH_2), 30.03 (CH_2CH_2N), 29.17 (CH_2CH_3), 9.01 (CH_2CH_3), 8.92 (CH_2CH_3). MS (LC/MS, $M+H^+$): 361.2; Anal. Calcd for $C_{21}H_{34}Cl_2N_2O_3$: C, 58.20; H, 7.91; N, 6.46; Found: C, 58.24; H, 7.93; N, 6.46.



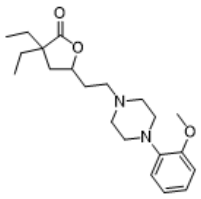
Synthesis of 3,3-diethyl-5-(2-(4-(2-(trifluoromethyl)phenyl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (**9r**): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (**9q**), except 1-(2-(trifluoromethyl)phenyl)piperazine was substituted for 2-piperazin-1-yl-benzonitrile. In addition the crude product was purified by flash chromatography (silica; MeOH:dichloromethane, 0% ~ 10%). The product was isolated as a clear oil (Yield: 83%). ^{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, N$\underline{CH_2}CH_2$N), 2.72-2.45 (6H, m, NCH$_2\underline{CH_2}$N and CH$_2\underline{CH_2}$N), 2.15 (1H, dd, J= 6.8, 13.1 Hz, CH_2), 1.88 (3H, m, CH_2), 1.64 (4H, m, $\underline{CH_2}CH_3$) 0.94 (6H, dt, J= 7.5, 21.5 Hz, $CH_2\underline{CH_3}$), ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.91 (CO), 145.40 (C), 131.22 (CH), 125.86 (CF_3), 125.58 ($\underline{C}CF_3$), 125.53 (CH), 123.67 (CH), 122.51 (CH), 73.63 (CH), 52.93 ($\underline{C}(CH_2CH_2)_2$), 51.79 (N$\underline{CH_2}CH_2$N), 49.81 (NCH$_2\underline{CH_2}$N), 46.93(CH$_2\underline{CH_2}$N), 35.99 ($CH_2$), 31.95, ($\underline{CH_2}CH_2$N) 27.61 ($\underline{CH_2}CH_3$), 26.66 ($\underline{CH_2}CH_3$), 7.12 ($CH_2\underline{CH_3}$), 7.05 ($CH_2\underline{CH_3}$), LC/MS [M+H]= m/z 399.20



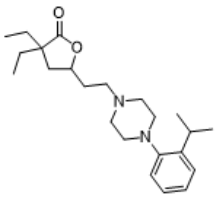
Synthesis of 5-(2-(4-(2-chlorophenyl)piperazin-1-yl)ethyl)-3,3-diethyldihydrofuran-2(3H)-one (**9s**): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile (**9q**), except 1-(2-chlorophenyl)piperazine was substituted 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, dd, 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, N$\underline{CH_2}CH_2$N), 3.45 (2H, m, N$\underline{CH_2}CH_2$N), 3.39-3.20 (4H, m, NCH$_2\underline{CH_2}$N), 3.12 (2H, m, CH$_2\underline{CH_2}$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, $\underline{CH_2}CH_3$), 0.92 (6H, dt, J= 7.5, 17.2 Hz, $CH_2\underline{CH_3}$) ^{13}C NMR (101 MHz, MeOD) δ182.32 (CO), 148.54 (C), 131.81 (CH), 130.05 ($\underline{C}Cl$), 129.26 (CH), 128.46 (CH), 122.03 (CH), 76.13 (CH), 54.99 ($\underline{C}(CH_2CH_2)_2$), 53.75 (N$\underline{CH_2}CH_2$N), 49.94 (NCH$_2\underline{CH_2}$N), 49.86 (CH$_2\underline{CH_2}$N), 38.35 ($CH_2$), 31.84 ($\underline{CH_2}CH_2$N), 30.08 ($\underline{CH_2}CH_3$), 29.21 ($\underline{CH_2}CH_3$), 8.99 ($CH_2\underline{CH_3}$), 8.91 ($CH_2\underline{CH_3}$) LC/MS [M+H]= m/z 365.20.



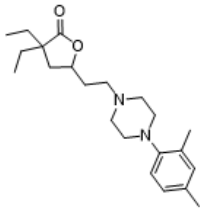
Synthesis of 3,3-diethyl-5-(2-(4-(o-tolyl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (**9t**): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile, except 1-o-Tolyl-piperazine was substituted for 2-piperazin-1-yl-benzonitrile (46.6 mg, 45% yield)**:** ^{1}H 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, N$\underline{CH_2}CH_2$N), 3.43 – 3.26 (4H, m, NCH$_2\underline{CH_2}$N), 3.24 – 3.07 (4H, m, NCH$_2\underline{CH_2}$N and CH$_2\underline{CH_2}$N), 2.34 – 2.02 (6H, m, CH_3 and CH_2), 1.90 (1H, dd, J = 13.3, 9.4 Hz, CH_2), 1.60 (4H, ddd, J = 17.2, 8.6, 6.4 Hz, $\underline{CH_2}CH_3$), 0.89 (6H, dt, J = 14.0, 7.5 Hz, $CH_2\underline{CH_3}$); ^{13}C NMR (101 MHz, MeOD) δ 182.40 (CO), 150.67 (C), 134.07 ($\underline{C}CH_3$), 132.33 (CH), 127.96 (CH), 125.84 (CH), 120.39 (CH), 76.21 (CH), 54.96 ($\underline{C}(CH_2CH_2)_2$), 54.81 (N$\underline{CH_2}CH_2$N), 53.99 (NCH$_2\underline{CH_2}$N), 53.80 (CH$_2\underline{CH_2}$N), 50.26 ($CH_2$), 49.93 ($\underline{CH_2}CH_2$N), 38.33 ($\underline{CH_2}CH_3$), 31.77 ($\underline{CH_2}CH_3$), 30.05 ($\underline{CH_2}CH_3$), 29.18 ($\underline{CH_2}CH_3$), 17.84 ($CH_3$), 9.01 ($CH_2\underline{CH_3}$), 8.92 ($CH_2\underline{CH_3}$); MS (LC/MS, M+H$^+$): 345.3. melting point of di-HCl salt 233^{o}C



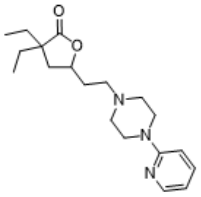
Synthesis of 3,3-diethyl-5-(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (**9u**): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile, except 1-(2-methoxy-phenyl)-piperazine was substituted for 2-piperazin-1-yl-benzonitrile (61.7 mg, 57% yield)**:** ^{1}H NMR (400 MHz, D_2O) δ 7.06 (2H, ddd, J = 7.8, 7.2, 1.5 Hz, CH), 6.96 (1H, dd, J = 8.1, 1.3 Hz, CH), 6.93 – 6.82 (1H, m, CH), 4.50 (1H, dt, J = 9.2, 7.5 Hz, CH), 3.80 (3H, s, OCH_3), 3.72 – 3.22 (10H, m, NCH_2CH_2N , NCH_2CH_2N and CH_2CH_2N), 2.28 – 2.10 (2H, m, CH_2CH_2N), 2.10 – 1.96 (1H, m, CH_2), 1.86 (1H, dd, J = 13.3, 9.4 Hz, CH_2), 1.68 – 1.42 (4H, m, CH_2CH_3), 1.00 – 0.75 (6H, m, CH_2CH_3); ^{13}C NMR (101 MHz, D_2O) δ 182.35 (CO), 153.90 (C), 138.69 ($COCH_3$), 126.90 (CH), 122.35 (CH), 120.49 (CH), 113.32 (CH), 76.15 (CH), 56.21 ($C(CH_2CH_2)_2$), 54.97 (NCH_2CH_2N), 53.20 ($COCH_3$), 49.93 (NCH_2CH_2N), 49.35 (CH_2CH_2N), 38.35 (CH_2), 31.74 (CH_2CH_2N), 30.05 (CH_2CH_3), 29.19 (CH_2CH_3), 9.00 (CH_2CH_3), 8.91 (CH_2CH_3); MS (LC/MS, M+H$^+$): 361.2; Anal. Calcd for $C_{21}H_{34}Cl_2N_2O_3$: C, 58.20; H, 7.91; N, 6.46; Found: C, 58.05; H, 7.95; N, 6.39. melting point of di-HCl salt: 228~229 ^{o}C



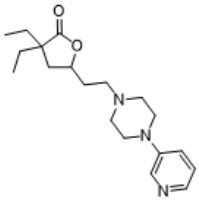
Synthesis of 3,3-diethyl-5-(2-(4-(2-isopropylphenyl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (**9v**): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile, except 1-(2-Isopropyl-phenyl)-piperazinewas substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (44.8 mg, 40% yield)**.** ^{1}H 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, NCH_2CH_2N, 3.41 (1H, dd, J = 13.7, 6.8 Hz, $CH(CH_3)_2$), 3.33 – 3.12 (6H, m, NCH_2CH_2N and NCH_2CH_2N), 3.02 (2H, d, J = 10.7 Hz, CH_2CH_2N), 2.31 – 2.03 (3H, m, CH_2), 1.83 (1H, dd, J = 13.2, 9.3 Hz, CH), 1.69 – 1.34 (4H, m, CH_2CH_3), 1.16 (6H, d, J = 6.9 Hz, $CH(CH_3)_2$), 0.85 (6H, dt, J = 10.6, 7.5 Hz, CH_2CH_3); ^{13}C NMR (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 ($C(CH_2CH_2)_2$), 51.57 (NCH_2CH_2N), 51.43 (NCH_2CH_2N), 49.55 (CH_2CH_2N), 47.87 (CH_2), 36.43 (CH_2CH_2N), 29.72 (CH_2CH_3), 28.36 (CH_2CH_3), 27.66 ($CH(CH_3)_2$), 26.24 ($CH(CH_3)_2$), 23.99 ($CH(CH_3)_2$), 8.55 (CH_2CH_3), 8.51 (CH_2CH_3); MS (LC/MS, M+H$^+$): 373.3.



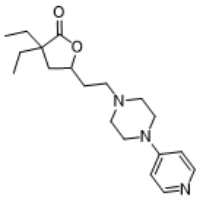
Synthesis of 5-(2-(4-(2,4-dimethylphenyl)piperazin-1-yl)ethyl)-3,3-diethyldihydrofuran-2(3H)-one (**9w**): The title compound was prepared according to the procedure for 2-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile, except 1-(2,4-Dimethyl-phenyl)-piperazinewas substituted for 2-piperazin-1-yl-benzonitrile. The product was isolated as a clear oil (52.8 mg, 49% yield)**.** ^{1}H 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, CH_2CH_2N), 3.33 – 3.02 (8H, m, NCH_2CH_2N and NCH_2CH_2N), 2.31 – 2.07 (9H, m, CH_2CH_2N, CH_3, CH_3, and CH_2), 1.83 (1H, dd, J = 13.2, 9.3 Hz, CH2), 1.67 – 1.39 (4H, m, CH_2CH_3), 0.85 (6H, dt, J = 10.6, 7.5 Hz, CH_2CH_3); ^{13}C NMR (101 MHz, DMSO) δ 179.75 (CO), 147.31 (C), 132.64 (CCH_3), 131.80 (CCH_3), 131.62 (CH), 127.05 (CH), 118.82 (CH), 74.31 (CH), 52.11 ($C(CH_2CH_2)_2$), 51.55 (NCH_2CH_2N), 51.38 (NCH_2CH_2N), 48.24 (CH_2CH_2N), 47.85 (CH_2), 36.44 (CH_2CH_2N), 28.33 (CH_2CH_3), 27.64 (CH_2CH_3), 20.32 (CH_3), 17.27 (CH_3), 8.54 (CH_2CH_3), 8.49 (CH_2CH_3); 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). ^{1}H NMR (400 MHz, D_2O) 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, N$\underline{CH_2}CH_2$N, NCH$_2\underline{CH_2}$N, and CH$_2\underline{CH_2}$N), 2.26 (3H, m, CH_2), 2.00 (1H, dd, J = 13.5, 9.4 Hz, CH_2), 1.76 – 1.46 (4H. m, $\underline{CH_2}CH_3$), 0.88 (6H, dt, J = 11.8, 7.5 Hz, CH$_2\underline{CH_3}$); ^{13}C NMR (101 MHz, D_2O) δ 187.89 (CO), 155.57 (C), 147.93 (CH), 140.42 (CH), 117.97 (CH), 115.85 (CH), 79.49 (CH), 56.71 ($\underline{C}(CH_2CH_2)_2$), 53.74 (N$\underline{CH_2}CH_2$N), 52.39 (NCH$_2\underline{CH_2}$N), 46.15 (CH$_2\underline{CH_2}$N), 39.38 ($CH_2$), 31.88 ($\underline{CH_2}CH_2$N), 30.66 ($\underline{CH_2}CH_3$), 26.67 ($\underline{CH_2}CH_3$), 10.99 (CH$_2\underline{CH_3}$), 10.86 (CH$_2\underline{CH_3}$); MS (LC/MS, M+H$^+$): 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%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (1H, b, CH), 8.10 (1H, b, CH), 7.17 (2H, m, CH), 4.49 (1H, m, CH), 3.23 (4H, t, J = 5.3 Hz, N$\underline{CH_2}CH_2$N) 2.68-2.48 (6H, m, NCH$_2\underline{CH_2}$N, and CH$_2\underline{CH_2}$N), 2.14 (1H, dd, J= 6.7, 13.0 Hz, CH_2), 1.95-1.78 (3H, m, $\underline{CH_2}CH_2$N and CH_2), 1.69-1.57 (4H, m, $\underline{CH_2}CH_3$), 0.93 (6H, dt, J= 7.5, 19.1 Hz, CH$_2\underline{CH_3}$).^{13}C NMR (101 MHz, $CDCl_3$) δ 180.55 (CO), 146.48 (C), 140.96 (CH), 138.56 (CH), 123.58 (CH), 122.98 (CH), 75.12 (CH), 54.55 ($\underline{C}(CH_2CH_2)_2$), 52.68 (N$\underline{CH_2}CH_2$N), 48.59 (NCH$_2\underline{CH_2}$N), 47.72 (CH$_2\underline{CH_2}$N), 37.64 ($CH_2$), 32.89 ($\underline{CH_2}CH_2$N), 29.23 ($\underline{CH_2}CH_3$), 28.28 ($\underline{CH_2}CH_3$), 8.77 (CH$_2\underline{CH_3}$), 8.71 (CH$_2\underline{CH_3}$). 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-4-yl)piperazine for 1-phenylpiperazine. The product was isolated as a clear oil (Yield: 37%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (2H, d, J= 5.7 Hz, CH), 6.67 (2H, d, J= 5.9 Hz, CH), 4.50 (1H, m, CH), 3.35 (4H, t, J = 5.2 Hz, N$\underline{CH_2}CH_2$N) 2.68-2.46 (6H, m, NCH$_2\underline{CH_2}$N, and CH$_2\underline{CH_2}$N), 2.15 (1H, dd, J= 6.6, 13.0 Hz, CH_2), 1.95-1.77 (3H, m, CH_2), 1.69-1.57 (4H, m, $\underline{CH_2}CH_3$), 0.93 (6H, dt, J= 7.5, 19.3 Hz, CH$_2\underline{CH_3}$) ^{13}C NMR (101 MHz, MeOD) δ 183.19 (CO), 157.31 (C), 148.03 (CH), 109.36, (CH) 77.54 (CH), 55.46 ($\underline{C}(CH_2CH_2)_2$), 53.71 (N$\underline{CH_2}CH_2$N), 50.13 (NCH$_2\underline{CH_2}$N), 46.71 (CH$_2\underline{CH_2}$N), 38.43 ($CH_2$), 34.38 ($\underline{CH_2}CH_2$N), 30.25 ($\underline{CH_2}CH_3$), 29.31 ($\underline{CH_2}CH_3$), 9.05 (CH$_2\underline{CH_3}$), 8.96 (CH$_2\underline{CH_3}$) LC/MS [M+H]= m/z 332.2

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).

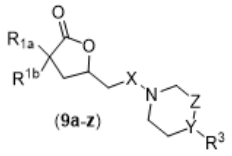
Sigma-1 and sigma-2 competitive radioligand-binding studies: Competitive binding assays were conducted by the Psychoactive Drug Screening Program (PDSP) at The University of North Carolina, Chapel Hill under the direction of Professor Bryan Roth. Assay conditions can be found in the PDSP assay protocol book at https://pdsp.unc.edu/pdspweb/content/UNC-CH%20Protocol%20Book.pdf.

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 (McMasters et al., 2007).

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..[19] 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**) – (**9z**)



Entry	R^{1a}	R^{1b}	X	Y	Z	R^3	MW	TPSA	cLogP	σ_2	σ_1	σ2/σ1 ratio	HLM	MLM	Sol	CYP3A4
										K_i (nM)			$T_{½}$ (min.)		mM	(IC_{50} nM)
9a	Et	Et	CH_2	N	CH_2	Ph	330	33	3.5	82	138	1.7	48	2	200	10000
9b	Me	Me	CH_2	N	CH_2	Ph	302	33	2.6	753	279	0.4	60	14	192	10000
9c	Et	Et	C(O)	N	CH_2	Ph	344	50	3.3	10000	10000	1.0	39	3	189	10000
9d	Et	Et	CH_2	N	C(O)	Ph	344	50	3.3	5289	10000	1.9	60	9.6	140	10000
9e	Et	Et	CH_2	CH_2	CH_2	Ph	329	30	4.7	6.4	2.7	0.4	46	2	200	10000
9f	Et	Et	CH_2	N	CH_2	Cyc-hex	337	33	3.8	5.4	26	4.8	60	4.3	147	10000
9g	Et	Et	CH_2	N	CH_2	4-CN-Ph	355	57	3.2	34	98	2.9	24	3.5	200	8570
9h	Et	Et	CH_2	N	CH_2	4-CF_3-Ph	398	33	4.5	7.3	14	1.9	11	3	25	10000
9i	Et	Et	CH_2	N	CH_2	4-Cl-Ph	365	33	4.1	12	17	1.4	12	2	88	10000
9j	Et	Et	CH_2	N	CH_2	4-OMe-Ph	361	42	3.4	53	79	1.5	60	5.4	185	10000
9k	Et	Et	CH_2	N	CH_2	4-Me-Ph	344	33	3.8	14	10000	714	41	2	200	10000
9l	Et	Et	CH_2	N	CH_2	3-CN-Ph	355	57	3.2	46	159	3.5	9.9	3.2	200	10000
9m	Et	Et	CH_2	N	CH_2	3-CF_3-Ph	398	33	4.5	12	65	5.4	9.8	2.9	109	10000
9n	Et	Et	CH_2	N	CH_2	3-Cl-Ph	365	33	4.1	9.9	84	8.5	6.7	2.1	107	10000
9o	Et	Et	CH_2	N	CH_2	3-Me-Ph	344	33	3.8	30	59	2.0	15.2	2	200	10000
9p	Et	Et	CH_2	N	CH_2	3-OMe-Ph	360	42	3.4	62	169	2.7	37.8	2	200	10000
9q	Et	Et	CH_2	N	CH_2	2-CN-Ph	355	57	3.2	61	351	5.8	3.6	2	200	10000
9r	Et	Et	CH_2	N	CH_2	2-CF3-Ph	398	33	4.5	17	67	3.9	8.9	2	72	10000
9s	Et	Et	CH_2	N	CH_2	2-Cl-Ph	365	33	4.1	7.0	35	5.0	11	2	200	10000
9t	Et	Et	CH_2	N	CH_2	2-Me-Ph	344	33	3.8	9.3	36	3.9	22	2	194	7400
9u	Et	Et	CH_2	N	CH_2	2-OMe-Ph	360	42	3.4	44	1168	26.5	20	2.3	200	10000
9v	Et	Et	CH_2	N	CH_2	2-iPr-Ph	373	33	4.6	5.9	195	33.1	7.5	2.4	37	10000
9w	Et	Et	CH_2	N	CH_2	2,4-	359	33	4.0	9.2	10	1.0	27	2	126	10000

						di-Me-Ph										
9x	Et	Et	CH_2	N	CH_2	2-Py	331	46	2.6	268	1499	5.6	46	2	200	10000
9y	Et	Et	CH_2	N	CH_2	3-Py	331	46	2.2	10000	10000	1.0	52	5.4	193	10000
9z	Et	Et	CH_2	N	CH_2	4-Py	331	46	2.2	142	10000	70.4	60	60	199	10000

Declarations

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.

References

1. Martin W. R.; Eades, C. E.; Thompson, J. A; Huppler, R. E. The effects of morphine and nalorphine-like drugs in the non-dependent and morphine-dependent chronic spinal dog. Journal of Pharmacology and Experimental Therapeutics, 1976, 197:517-532.
2. (a) Su, T. P. Evidence for sigma opioid receptor: binding of [3H]SKF-10047 to etorphine inaccessible sites in guinea-pig brain. Journal of Pharmacology and Experimental Therapeutics, 1982, 223, 2, 284–290. (b) Khazan, N.; Young, G. A.; El-Fakany, E. E.; Hong, O.; Caliigaro, D. Sigma receptors mediated the psychotomimetic effects of N-allylnormetazocine (SKF-10,047), but not its opioid agonistic-antagonistic properties. Neuropharmacology, 1984, 23:983-987.
3. Bowen, W. D.; de Costa, B. R.; Hellewell, S. B.; Walker, J. M.; Rice, K. C. [3H]-(+)-Pentazocine: a potent and highly selective benzomorphan-based probe for sigma-1 receptors. Molecular Neuropharmacology, 1993, 3:117-126.
4. Hanner, M.; Moebius, F. F.; Flandorfer, A.; Knaus, H. G.; Striessnig, J.; Kepner, E.; Glossman, H. Purification, molecular cloning, and expression of the mammalian sigma-1 binding site, Proceedings of the National Academy of Sciences of the United States of America, 1996, 93, 8072-8077.
5. Schmidt, H. R.; Zheng, S.; Guripinar, E.; Koehl, A.; Manglik, A.; Kruse, A. C. Crystal structure of the human σ1 receptor, Nature, 2016, 532, 527-530.
6. Alon, A.; Schmidt, H. R.; Wood, M. D.; Sahn, J. J.; Martin, S. F.; Krusea, A. C.; Identification of the gene that codes for the σ2 receptor, Proceedings of the National Academy of Science, 2017, 114, 7160-7165.
7. Bartz, F.; Kern, L.; Erz, D.; Zhu, M.; Gilbert, D.; Meinhof, T.; Wirkner, U.; Erfle, H.; Muckenthaler, M.; Pepperkok, R.; Runz, H. Identification of cholesterol-regulating genes by targeted RNAi screening. Cell Metabolism, 2009, 10, 1, 63-75.
8. Ebrahimi-Fakhar, D.; Wahlster, L.; Bartz, F.; Werenbeck-Ueding, J.; Praggastis, M.; Zhang, J.; Joggerst-Thomalla, B.; Theiss, S.; Grimm, D.; Ory, D. S.; Runz, H. Reduction of TMEM97 increases NPC1 protein levels and restores cholesterol trafficking in Niemann-pick type C1 disease cells. Human Molecular Genetics, 2016, 25, 16, 3588-3599.
9. Guo, L.; Zhen, X. Sigma-2 receptor ligands: neurobiological effects. Current Medicinal Chemistry, 2015 22, 989–1003.
10. (a) Yi, B.; Sahn, J. J.; Ardestani, P. M.; Evans, A. K.; Scott, L. L.; Chan, J. Z.; Iyer, S.; Crisp, A.; Zuniga, G.; Pierce, J. T.; Martin, S. F.; Shamloo, M. Small molecule modulator of sigma 2 0zreceptor is neuroprotective and reduces cognitive deficits and neuroinflammation in experimental models of Alzheimer's disease. Journal of Neurochemistry, 2017, 140, 561-575. (b) Izzo, N. J.; Staniszewski, A.; To, L.; Fa, M.; Teich, A. F.; Saeed, F.; Wostein, H.; Walko, T.; Vaswani, A.; Wardius, M.; Syed, Z.; Ravenscroft, J.; Mozzoni, K.; Silky, C.; Rehak, C.; Yurko, R.; Finn, P.; Look, G.; Rishton, G.; Safferstein, H.; Miller, M.; Johanson, C.; Stopa, E.; Windisch, M.,;Hutter-Paier, B.; Shamloo, M.; Arancio, O.; LeVine, H.; Catalano, S. M. Alzheimer's therapeutics targeting amyloid beta 1–42 oligomers I: Abeta 42 oligomer binding to specific neuronal receptors is displaced by drug candidates that improve cognitive deficits. PLoS One, 2014, 9, e111898. (c) Izzo, N. J.; Xu, J.; Zeng, C.; Kirk, M. J.; Mozzoni, K.; Silky, C.; Rehak, C.; Yurko, R.; Look, G.; Rishton, G.; Safferstein, H.; Cruchaga, C.; Goate, A.; Cahill, M. A.; Arancio, O.; Mach, R. H.; Craven, R.; Head, E.; LeVine, H.; Spires-Jones, T. L.; Catalano, S. M. Alzheimer's therapeutics targeting amyloid beta 1–42 oligomers II: Sigma-2/PGRMC1 receptors mediate Abeta 42 oligomer binding and synaptotoxicity. PLoS One, 2014, 9, No. e111899.

11. Sahn, J. J.; Mejia, G. L.; Ray, P. R.; Martin, S. F.; Price, T. J. Sigma 2 receptor/Tmem97 agonists produce long lasting antineuropathic pain effects in mice. ACS Chemical Neuroscience, 2017, 8, 1801-1811.
12. Vazquez-Rosa, E.; Watson, M. R.; Sahn, J. J.; Hodges, T. R.; Schroeder, R. E.; Cintron-Perez, C. J.; Shin, M. K.; Yin, T. C.; Emery, J. L.; Martin, S. F.; Liebl, D. J.; Pieper, A. A. Neuroprotective Efficacy of a Sigma 2 Receptor/TMEM97 Modulator (DKR-1677) after Traumatic Brain Injury, ACS Chemical Neuroscience, 2019, 10, 3, 1595-1602.
13. (a) Vilner, B. J.; John, C. S.; Bowen, W. D. Sigma-1 and Sigma-2 receptors are expressed in a wide variety of human and rodent tumor cell lines, Cancer Research, 1995, 408-413. (b) Asong, Gladys; Zhu, Xue Y.; Bricker, Barbara; Andey, Terrick; Amissah, Felix; Lamango, Nazarius; Ablordeppey, Seth Y. New analogs of SYA013 as sigma-2 ligands with anticancer activity, Bioorganic and Medicinal Chemistry, 2019, 27, 12, 2629-2636.
14. Gao, R.; Bhandare, R. R.; Canney, D. J. Homologation as a lead modification approach en route to a series of lactone-based muscarinic ligands, Medicinal Chemistry Research, 2014, 23, 1023-1030.
15. Gao, R.; Canney, D. J. A modified Prins reaction for the facile synthesis of structurally diverse substituted 5-(2-hydroxyethyl)-3, 3-dihydrofurane-2(3H)-ones. Tetrahedron Letters, 2009, 50, 43, 5914–5916.
16. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings". Advanced Drug Delivery Review, 2001, 46, 1–3, 3–26.
17. (a) Abate, C.; Niso, M.; Berardi, F. “Sigma-2 receptor: past, present and perspectives on multiple therapeutic exploitations.” Future Medicinal Chemistry, 2018, 10, 16, 1997-2018. (b) Blass, B. E.; Rogers, J. P. “The sigma-2 (σ-2) receptor: a review of recent patent applications: 2013-2018,” Expert Opinion on Therapeutic Patents, 2018, 28, 9, 655-663. (c) Zeng, C.; Mach, R. H. “The Evolution of the Sigma-2 (σ2) Receptor from Obscure Binding Site to Bona Fide Therapeutic Target” Advances in experimental medicine and biology, 2017, 964, 49-61.
18. Gao, R. Design, synthesis and evaluation of novel muscarinic ligands, Temple University School of Pharmacy Ph.D. Thesis, 2013, 1-233.
19. Yang, J; Jamei, M; Yeo, KR; Rostami-Hodjegan, A and Tucker, GT. “Misuse of the well-stirred model of hepatic drug clearance.” Drug Metabolism and Disposition, 2007, 35, 501-502.

Figures

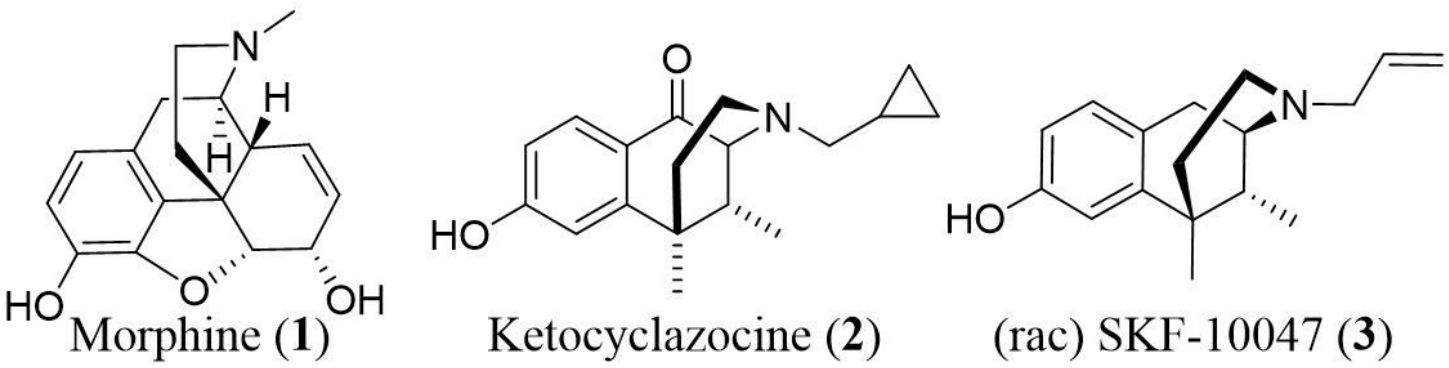


Figure 1

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

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- scheme1.jpg