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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Homologation as a lead modification approach *en* route to a series of lactone-based muscarinic ligands

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Abstract Previous work identified the lactone ring as a useful scaffold for the design of muscarinic ligands and reported a lactone-based ligand with an IC_{50} of 340 nM. Using homologation as a lead modification approach, a new series of lactone-based compounds have been designed, synthesized, and screened in muscarinic binding assays. The approach provided a series of compounds with improved % inhibition values and identified the highest affinity lactone-based ligand reported to date. The results of these efforts and the structure–activity relationship for this series of lactones-based ligands are discussed.

Keywords Homologation · Lactone · Muscarinic receptors

Introduction

The muscarinic acetylcholine receptor system is classified into five different subtypes (M_1–M_5). In general, M_1, M_3, and M_5 receptor subtypes are coupled via Gq-like proteins; while M_2 and M_4 subtypes are coupled to Gi-proteins (Burstein *et al.*, 1998; Koch *et al.*, 2005). The receptors have been targeted in drug discovery efforts for the treatment of various disorders including overactive bladder, Alzheimer's disease, pain, cognitive impairment, drug addiction, schizophrenia, and Parkinson's disease (Abrams *et al.*, 2006; Wess *et al.*, 2007). To date, efforts to develop subtype selective ligands for muscarinic acetylcholine receptors (mAChRs) have been hampered by a lack of X-ray crystal structures of the proteins and the high degree of homology among the receptor subtypes (Felder *et al.*, 2000). However, the recent availability of the X-ray crystal structures for the M_2 and M_3 subtypes should be useful in the design of future ligands (Haga *et al.*, 2012; Kruse *et al.*, 2012). Our interest in the development of subtype-selective muscarinic ligands led to previous reports detailing the identification of substituted lactones as lead muscarinic compounds (Ahungena and Canney, 1996; Ahungena *et al.*, 2003). The general pharmacophoric elements of the lactone-based ligands are illustrated in the Fig. 1 where the lactone oxygens serve as a H-bond acceptor moieties while different nitrogen-containing heterocycles provide the requisite cationic group. These groups may be separated by linkers of varying sizes. Later work involved molecular modifications of those leads that included the addition of aromatic groups with a variety of substitution patterns. These efforts led to an increase in receptor affinity (Bhandare and Canney, 2011) and produced a lactone-based muscarinic ligand with an IC_{50} of 340 nM.

We herein report a continuation of that work with the goal of further improving affinity for muscarinic receptors and preliminary evaluation of specific ligands for subtype selectivity. The design, synthesis, and evaluation of a homologous series of lactone-based compounds as muscarinic ligands are included in the approach. The results of these efforts and the structure–activity relationship for this series of lactones-based ligands are discussed.

Electronic supplementary material The online version of this article (doi:10.1007/s00044-013-0692-3) contains supplementary material, which is available to authorized users.

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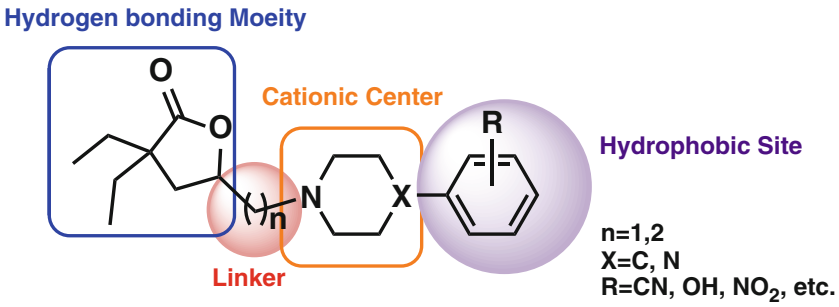


Fig. 1 General structural features of the newly designed ligands

Chemistry

The synthesis and testing of lactone-based compounds **1a**–**9a**, **11a**–**15a** (Table 1) has been published previously (Bhandare and Canney, 2011). Scheme 1 shows the synthesis of a homologous series of lactone-based muscarinic ligands beginning with the olefinic ester as starting material. A previously published modified Prins reaction was used to prepare precursor **2** from the olefinic ester in good yield (Gao and Canney, 2009). Compound **2** was treated with triethylamine and tosyl chloride in dichloromethane (DCM) to afford intermediate tosylate **3** in 69 % yield. Displacement reactions involving **3** and the appropriate secondary amines under refluxing conditions (72 h) afforded the target ligands **1b**–**15b** in 50–75 % yield. Precursor **4** was synthesized using a previously described method (Ahungena and Canney, 1996). Displacement of the iodine of lactone **4** by 1-(4-fluorobenzyl)piperazine under refluxing condition (48 h) afforded **10a** in 12 % yield (Ahungena *et al.*, 2003; Ahungena and Canney, 1996).

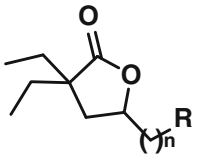
Results and discussion

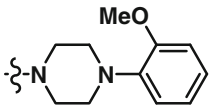
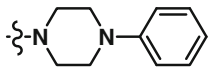
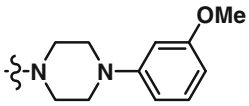
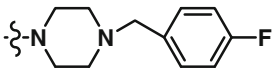
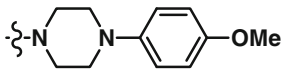
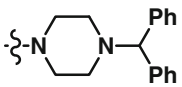
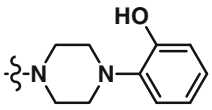
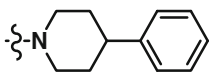
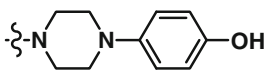
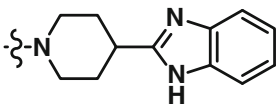
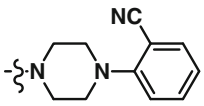
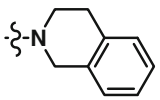
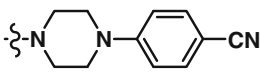
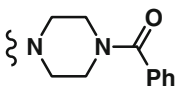
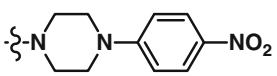
Previously reported lactone-based muscarinic ligands were used in the design of a homologous series of compounds (**1b**–**15b** Table 1). Novel homologs were prepared in modest to good yield using a newly developed route involving a modified Prins reaction followed by the displacement of the corresponding tosylated intermediate by secondary amines (Scheme 1). Binding studies were performed at CEREP using rat cerebral cortex membranes as per a previously published method (Richards, 1990).

Preliminary data presented in Table 1 and Fig. 2 represent the percent inhibition of specific binding of radioligand at a single concentration (10 μM) of test compound. The inclusion of the methylene spacer between the H-bonding lactone ring and the amine-containing heterocyclic rings (see Fig. 1) resulted in an increase in affinity for each of the homologs tested. Based on the limited number of compounds evaluated here, the substitution pattern on the aromatic rings of the test compounds was found to have a profound effect on affinity but the electronic nature of the substituents were not as relevant. For example, the unsubstituted compounds **9a** and **9b** inhibited specific binding of radioligand by 16 and 74 %, respectively. The para-substituted compounds **3a**, **3b**, **5a**, **5b**, **7a**, **7b**, **8a**, and **8b** contain substituents with electron donating (**3a**, **3b**; **5a**, **5b**) and electron withdrawing (**7a**, **7b**; **8a**, **8b**). In each case, the homologous compound in the pair [**3b** (56 %), **5b** (61 %), **7b** (57 %), and **8b** (70 %)] exhibits higher % inhibition regardless of the electronic nature of the substituent (Fig. 2). The para-substituted compounds were similar to or less than the unsubstituted **9b** in their ability to inhibit specific binding. The ortho-substituted compounds, **1a**, **1b**, **4a**, **4b**, **6a**, and **6b**, show a similar trend with the homologs **1b** (82 %), **4b** (81 %), and **6b** (83 %) exhibiting higher % inhibition than the parent lactones regardless of the nature of the substituent. However, the ortho-substituted compounds were found to have slightly higher % inhibition values than the corresponding unsubstituted **9b**, suggesting that ortho substitution may be preferred to para. The substitution on the ortho position may influence the orientation of the aromatic ring with respect to the piperazine and this may have effected in improvement in the % inhibition values over the other positions on the ring.

Several additional piperazine (**10a**, **10b**, **11a**, **11b**, and **15b**) derivatives were also prepared and tested. In our previous work, **11a** was found to have a high % inhibition value and was chosen for further evaluation (IC_{50} = 340 nM). Among this new series of lactones, **11b** showed the highest percent inhibition and was chosen for further evaluation. The IC_{50} value (non-selective) for **11b** was determined to be 17 nM, the highest affinity of any of the lactone-based muscarinic ligands reported to date. Due to its high affinity in the general muscarinic assay, **11b** underwent further screening to evaluate possible subtype selectivity (see Fig. 3). Compound **11b** was tested for the ability to inhibit the specific binding of radioligand to muscarinic receptor subtypes hM_1–hM_5 at a concentration of 10 nM. The % inhibition values for the five subtypes were found to be 22, 56, 34, 62, and 14 % for hM_1–hM_5, respectively, demonstrating that no receptor selectivity was exhibited for the compound.

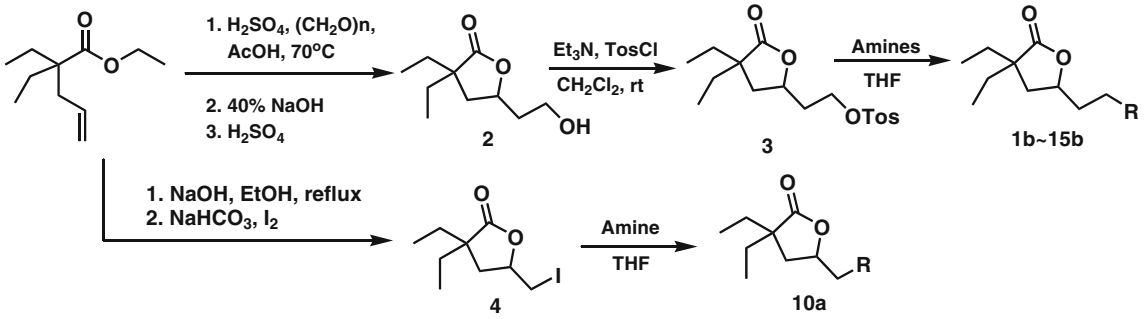
Table 1 Preliminary binding data of compounds **1a–15a** and **1b–15b**



R	#	*n*	% Inhib[a, b]	R	#	*n*	% Inhib[a, b]
	**1a**	1	32		**9a**	1	16
	1b	2	82		**9b**	2	74
	**2a**	1	9		**10a**	1	28
	2b	2	75		**10b**	2	63
	**3a**	1	26		**11a**	1	97
	3b	2	56		**11b**	2	99
	**4a**	1	46		**12a**	1	68
	4b	2	81		**12b**	2	86
	**5a**	1	7		**13a**	1	46
	5b	2	61		**13b**	2	66
	**6a**	1	31		**14a**	1	44
	6b	2	83		**14b**	2	57
	**7a**	1	18		**15a**	1	5
	7b	2	57		**15b**	2	33
	**8a**	1	18				
	8b	2	70				

[a] For details regarding the evaluation of results, see "Experimental" section

[b] % Inhibition at 10 μM



Scheme 1 Synthesis of a homologous series of lactone-based muscarinic ligands **10a**, **1b**–**15b**

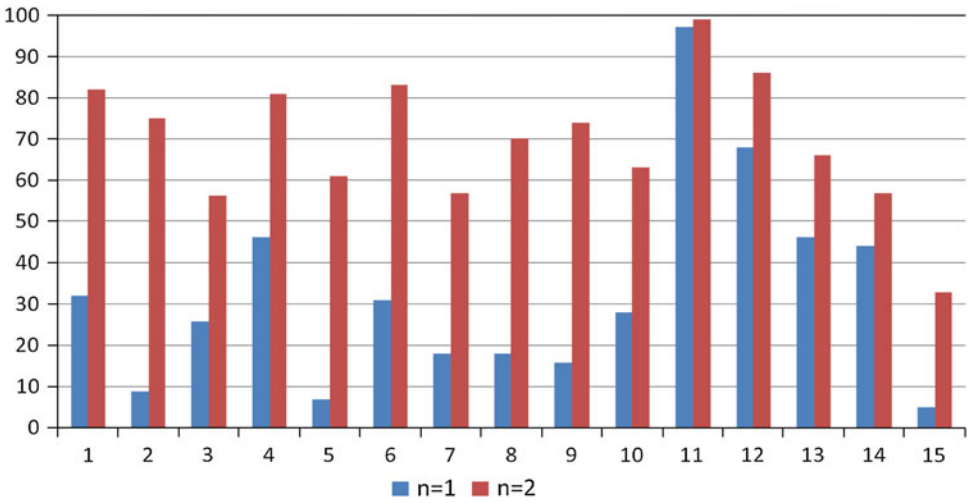


Fig. 2 Comparison of % inhibition values of homologs to the parent lactones

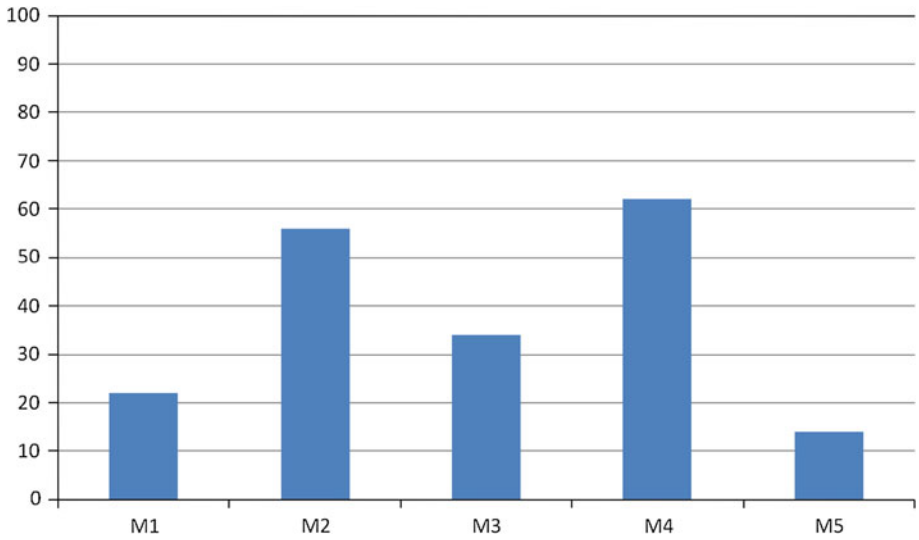


Fig. 3 Subtype selectivity data for hM_1–hM_5 receptor subtypes for compound **11b**

Conclusion

In summary, a homologous series of lactone-based muscarinic ligands was synthesized using a facile-modified Prins reaction followed by displacement of the protected alcohol with the appropriate amine. Based on the limited number of compounds screened here, it appears that the position of aromatic substitution may affect receptor affinity but the electronic nature of the substitution does not have a strong effect. Compound **11b** was identified as the highest affinity lactone-based ligand reported to date (IC_{50} = 17 nM). Compound **11b** was evaluated for

selectivity for muscarinic subtypes and found to show no selectivity. While these data confirm that the lactone nucleus is a useful scaffold for the design of muscarinic ligands and that changes in aromatic substitution patterns may be useful in improving affinity, additional lead modification techniques will be required to improve selectivity for the target subtypes. Compounds **6b**, **11b**, and **12b** will be utilized in future lead modification efforts to design subtype-selective muscarinic ligands.

Experimental

All the commercial chemicals were purchased from Sigma-Aldrich or Fisher scientific. Dry solvents were purchased from Fisher Scientific. Column Chromatography was run using Silicycle SiliaFlash P60 Silica Gel. Analytical TLC was performed using Silicycle Precoated 60-f-254 TLC. Melting points were recorded on a Thomas-Hoover Unimelt Capillary melting point apparatus. The ^{1}H NMR (300, 400 MHz) spectra were recorded on UnityINOVA 300 or AVANCE 400 spectrometer. The ^{13}C NMR (101 MHz) spectra were recorded on AVANCE 400 spectrometer. The data are presented as follows: chemical shift in parts per million (ppm) on the δ scale relative to internal tetramethylsilane (TMS) (coupling constant(s) in hertz). The following abbreviations are used to denote signal patterns: s = singlet, d = doublet, t = triplet, and m = multiplet. The purity was analyzed using two mobile phases in 10–90 % acetonitrile in water solvent gradient containing 0.1 % formic acid (method A) and 10–90 % methanol in water solvent gradient containing 0.1 % trifluroacetic acid (method B). Elemental analysis was carried out by Atlantic Microlab Inc, Atlanta, GA. LC–MS (ESI) analyses were done on an Agilent technologies 1200 series instrument on target compounds using 5–95 % acetonitrile in water (containing 0.1 % formic acid) gradient solvent system and was found to be >95 %.

Binding studies

The target compounds **1a–b** to **15a–b** were evaluated in radioligand binding assays performed by CEREP (86600 CELLE L'EVESCAULT, France) using rat cerebral cortex membranes expressing muscarinic receptor subtypes M_1–M_5. The competitive binding assays were performed according to the previously reported method (Richards, 1990). The assays were done in duplicate and are presented as supplied by CEREP. Briefly, [^{3}H]-QNB (0.05 nM) and the test compound (10 μM) were incubated with rat cerebral cortex membranes for 90 min at room temperature. Following incubation, the reaction was terminated by filtration. Atropine (1 μM) was used to determine non-specific binding. The bound radioactivity was measured with scintillation counter. For interpretation of this type of preliminary data, CEREP suggests the following guidelines: 50 % inhibition or higher represent significant effects (i.e., 50 % is a common cut-off value for further investigation; determination of IC_{50} or EC_{50} values from concentration response curves). Results showing an inhibition between 20 and 50 % indicate weak to moderate effects; inhibition less than 20 % are considered inactive.

Chemistry

*Procedure for intermediate **3***

Over a mixture of the corresponding 5-membered lactone (**2**, 1.0 Eq), Et_3N (1.5 Eq) in dry DCM, a solution of *p*-TosCl (1.25 Eq) in DCM was added dropwise at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and allowed to stir overnight at room temperature. Then, the reaction mixture was diluted with DCM (100 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; 10–20 % EtOAc/Hexane) to afford desired tosylate (69 % yield).

*General procedure for the synthesis of **1b–15b***

Tosylate **3** (1.0 Eq) was treated with appropriate amines (3.0 Eq) in dry THF and refluxed for 72 h. The THF was evaporated under reduced pressure, the residue was dissolved in DCM, 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 DCM) to afford pure product. For **1b**, **2b**, **4b**, **5b**, **7b–12b**, and **14b**, the purified product was then dissolved in ether and treated with HCl solution (2.0 M in diethyl ether) to afford the hydrochloride salt which was recrystallized with isopropanol or a MeOH/Ether mixture.

*Procedure for the synthesis of **10a***

Iodomethyl lactone **4** (1.0 Eq) and the appropriate secondary amine (5.0 Eq) in anhydrous tetrahydrofuran (35 mL) was stirred at reflux under a nitrogen atmosphere for 48 h. After 48 h, the mixture was concentrated under reduced pressure and the residue was dissolved in dichloromethane and purified by flash silica gel chromatography using methanol (0–3 %) in dichloromethane.

*2-(4,4-Diethyl-5-oxotetrahydrofuran-2-yl)ethyl-4-methylbenzenesulfonate (**3**)* Colorless crystal. Yield: 69 %. mp: 57–58 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (d,

$J = 8.3$ Hz, 2H), 7.36 (d, $J = 8.0$ Hz, 2H), 4.55–4.33 (m, 1H), 4.14 (dd, $J = 6.5$, 13.3 Hz, 3H), 2.46 (s, 3H), 2.21–1.84 (m, 3H), 1.83–1.68 (m, 1H), 1.58 (t, $J = 7.4$ Hz, 4H), 0.89 (dt, $J = 7.5$, 18.0 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.33, 145.30, 132.72, 130.15, 128.03, 77.68, 77.36, 77.04, 73.18, 66.95, 48.67, 37.53, 35.82, 29.14, 28.23, 21.76, 8.81, 8.74. Anal. Calcd for $C_{17}H_{24}O_5S$: C, 59.98; H, 7.11; Found: C, 60.27; H, 7.25.

Dihydrochloride salt of 3,3-diethyl-5-(2-(4-(2-methoxyphenyl)piperazin-1-l)ethyl)dihydrofuran-2(3H)-one ***(1b)*** White solid. Yield: 55 %. mp: 228–229 °C. ^{1}H NMR (400 MHz, D_2O) δ 7.21–7.10 (m, 2H), 7.04 (d, $J = 8.2$ Hz, 1H), 6.97 (t, $J = 7.6$ Hz, 1H), 4.60 (m, 1H), 3.89 (s, 3H), 3.71 (m, 4H), 3.54–3.19 (m, 6H), 2.37–2.21 (m, 2H), 2.14 (s, 1H), 1.95 (dd, $J = 9.4$, 13.3 Hz, 1H), 1.78–1.52 (m, 4H), 1.09–0.80 (m, 6H); ^{13}C NMR (101 MHz, D_2O) δ 183.21, 154.76, 139.55, 127.76, 123.21, 121.35, 114.18, 77.01, 57.07, 55.83, 54.06, 50.79, 50.50, 50.29, 50.21, 50.07, 49.86, 49.65, 49.43, 49.22, 39.21, 32.60, 30.91, 30.05, 9.86, 9.77. 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.

Dihydrochloride salt of 3,3-diethyl-5-(2-(4-(3-methoxyphenyl)piperazin-1-yl)ethyl) dihydrofuran-2(3H)-one ***(2b)*** White solid. Yield: 52 %. mp: 203–205 °C. ^{1}H NMR (400 MHz, DMSO) δ 7.20 (t, $J = 8.2$ Hz, 1H), 6.66–6.45 (m, 3H), 4.58 (m, 1H), 3.77 (s, 3H), 3.61 (s, 2H), 3.26 (s, 2H), 3.16 (d, $J = 8.9$ Hz, 4H), 2.25 (dd, $J = 6.8$, 13.3 Hz, 3H), 1.86 (dd, $J = 9.4$, 13.1 Hz, 1H), 1.69–1.45 (m, 4H), 0.89 (dt, $J = 7.5$, 10.2 Hz, 6H); ^{13}C NMR (101 MHz, MeOH) δ 183.23, 163.09, 150.73, 132.18, 77.00, 56.75, 55.73, 53.34, 50.78, 50.50, 50.29, 50.07, 49.86, 49.65, 49.43, 49.22, 39.17, 32.55, 30.89, 30.03, 9.86, 9.78. 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.

3,3-Diethyl-5-(2-(4-(4-methoxyphenyl)piperazin-1-yl)ethyl) dihydrofuran-2(3H)-one ***(3b)*** Yellowish oil. Yield: 53 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84-6.95 (m, 4H), 4.45–4.48 (m, 1H), 3.70 (s, 3H), 3.04–3.01 (m, 4H), 2.57–2.47 (m, 6H), 2.10–2.05 (m, 1H), 1.80–1.75 (m, 3H), 1.58–1.53 (m, 4H), 0.90–0.82 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 181.10, 154.13, 145.94, 118.49, 114.73, 75.91, 55.87, 54.79, 53.70, 50.89, 48.92, 38.00, 34.11, 29.55, 28.61, 9.10, 9.02. MS (ESI) (m/z) 361.1 $(M + H)^+$. HPLC analysis: >95 % purity using two different mobile phases.

Dihydrochloride salt of 3,3-diethyl-5-(2-(4-(2-hydroxyphenyl)piperazin-1-l)ethyl)dihydrofuran-2(3H)-one ***(4b)*** White solid. Yield: 49 %. mp: 252 °C. ^{1}H NMR (400 MHz, D_2O) δ 7.33 (d, $J = 8.3$ Hz, 1H), 7.25 (t, $J = 7.8$ Hz, 1H), 7.03 (t, $J = 8.0$ Hz, 2H), 4.72 (m, 1H), 3.95–3.36 (m, 10H), 2.37 (dd, $J = 7.0$, 13.5 Hz, 1H), 2.24 (dd, $J = 10.0$, 19.4 Hz, 2H), 2.02 (dd, $J = 9.4$, 13.5 Hz, 1H), 1.82–1.47 (m, 4H), 1.01–0.75 (m, 6H); ^{13}C NMR (101 MHz, D_2O) δ 187.89, 152.26, 136.50, 131.01, 123.97, 123.39, 119.52, 79.51, 56.59, 54.12, 52.40, 51.58, 39.37, 32.82, 31.89, 30.67, 11.00, 10.87. Anal. Calcd for $C_{20}H_{32}Cl_2N_2O_3$: C, 57.28; H, 7.69; N, 6.68; Found: C, 57.37; H, 7.64; N, 6.59.

Dihydrochloride salt of 3,3-diethyl-5-(2-(4-(4-hydroxyphenyl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one ***(5b)*** White solid. Yield: 58 %. mp: 235 °C. ^{1}H NMR (400 MHz, D_2O) δ 7.16 (d, $J = 9.0$ Hz, 2H), 6.94 (d, $J = 9.0$ Hz, 2H), 4.71 (m, 1H), 3.46 (m, 10H), 2.36 (dd, $J = 6.9$, 13.5 Hz, 1H), 2.23 (dd, $J = 9.2$, 19.4 Hz, 2H), 2.01 (dd, $J = 9.4$, 13.5 Hz, 1H), 1.77–1.50 (m, 4H), 0.90 (dt, $J = 7.5$, 12.5 Hz, 6H); ^{13}C NMR (101 MHz, D_2O) δ 187.92, 155.62, 143.21, 123.52, 119.36, 79.53, 56.53, 54.17, 52.42, 52.07, 39.38, 32.83, 31.92, 30.68, 11.00, 10.87. Anal. Calcd for $C_{20}H_{32}Cl_2N_2O_3$: C, 57.28; H, 7.69; N, 6.68; Found: C, 57.53; H, 7.74; N, 6.62.

2-(4-(2-(4,4-Diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile ***(6b)*** Yellowish oil. Yield: 50 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50–7.40(m, 2H), 6.97–6.93 (m, 2H), 4.44–4.40 (m, 1H), 3.21–3.19 (m, 4H), 2.67–2.55 (m, 6H), 2.11–2.06, (m, 1H), 1.85–1.75 (m, 3H), 1.58–1.55, (m, 4H), 0.90–0.82, (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 181.02, 155.77, 134.63, 134.15, 122.23, 119.01, 118.70, 106.22, 75.70, 54.63, 53.41, 51.54, 48.90, 37.95, 33.80, 29.55, 28.59, 9.08, 9.01. MS (ESI) (m/z) 356.1 $(M + H)^+$. HPLC analysis: >95 % purity using two different mobile phases.

Hydrochloride salt of 4-(4-(2-(4,4-diethyl-5-oxotetrahydrofuran-2-yl)ethyl)piperazin-1-yl)benzonitrile ***(7b)*** White solid. Yield: 56 %. mp: 213–214 °C; ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.68–7.52 (m, 2H), 7.12 (d, $J = 9.1$ Hz, 2H), 4.58 (m, 1H), 4.33–2.97 (m, 10H), 2.35–2.20 (m, 2H), 2.20–2.07 (m, 1H), 1.95 (dd, $J = 9.4$, 13.3 Hz, 1H), 1.74–1.53 (m, 4H), 0.94 (dt, $J = 7.5$, 13.3 Hz, 6H). ^{13}C NMR (101 MHz, MeOH-d_4) δ 183.18, 154.60, 135.59, 121.26, 117.41, 103.85, 77.01, 55.79, 53.62, 50.77, 50.50, 50.29, 50.07, 49.86, 49.65, 49.44, 49.22, 46.77, 39.19, 32.59, 30.90, 30.03, 9.85, 9.77. 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.

Hydrochloride salt of 3,3-diethyl-5-(2-(4-(4-nitrophenyl)piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one ***(8b)*** Yellowish

solid. Yield: 47 %. mp: 184 ~ 185 °C. ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.15 (d, J = 9.3 Hz, 2H), 7.11 (d, J = 9.4 Hz, 2H), 4.59 (m, 1H), 4.37–3.08 (m, 10H), 2.36–2.21 (m, 2H), 2.21–2.08 (m, 1H), 1.95 (dd, J = 9.4, 13.2 Hz, 1H), 1.75–1.52 (m, 4H), 0.94 (dt, J = 7.5, 13.2 Hz, 6H); ^{13}C NMR (101 MHz, MeOH-d_4) δ 183.19, 156.05, 141.97, 127.53, 116.10, 77.01, 55.81, 53.54, 50.77, 50.50, 50.29, 50.07, 49.86, 49.65, 49.43, 49.22, 46.59, 39.19, 32.57, 30.89, 30.03, 9.85, 9.77. Anal. Calcd for $C_{20}H_{30}Cl_1N_3O_4{\cdot}0.5H_2O$: C, 57.07; H, 7.42; N, 9.98; Found: C, 57.02; H, 7.26; N, 9.91.

*Dihydrochloride salt of 3,3-diethyl-5-(2-(4-phenylpiperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (**9b**)* White solid. Yield: 49 %. mp: decomposed over 239 °C. ^{1}H NMR (400 MHz, D_2O) δ 7.46 (t, J = 7.8 Hz, 2H), 7.31–7.11 (m, 3H), 4.70 (m, 1H), 3.85–3.44 (m, 10H), 2.34 (d, J = 7.0 Hz, 1H), 2.23 (d, J = 29.4 Hz, 2H), 2.06–1.92 (m, 1H), 1.63 (dd, J = 10.1, 17.0 Hz, 4H), 0.90 (dt, J = 7.5, 12.1 Hz, 6H); ^{13}C NMR (101 MHz, D_2O) δ 187.92, 150.20, 132.89, 127.03, 121.14, 79.53, 56.53, 54.13, 52.41, 50.87, 39.37, 32.81, 31.91, 30.68, 11.00, 10.87. 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.

*3,3-Diethyl-5-[4-(4-fluorobenzyl)-piperazin-1-ylmethyl]-dihydrofuran-2-one (**10a**)* Pale yellow liquid. Yield: 12.32 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85–0.96 (m, 6H), 1.58–1.64 (m, 4H), 1.81–1.90 (m, 1H), 2.02–2.07 (m, 1H), 2.45–2.63 (m, 8H), 3.45 (d, 2H), 4.51–4.58 (m, 1H), 5.30 (s, 2H), 6.96–7.01 (m, 2H), 7.21–7.31 (m, 2H). LC–MS (ESI) (m/z) 349.2 $(M + H)^+$. HPLC analysis: >95 % purity using two different mobile phases.

*Dihydrochloride salt of 3,3-diethyl-5-(2-(4-(4-fluorobenzyl) piperazin-1-yl)ethyl)dihydrofuran-2(3H)-one (**10b**)* White solid. Yield: 52 %. mp: decompose over 213.5 °C. ^{1}H NMR (400 MHz, D_2O) δ 7.55 (dd, J = 5.3, 8.6 Hz, 2H), 7.26 (t, J = 8.7 Hz, 2H), 4.70 (m, 1H), 4.45 (d, J = 7.1 Hz, 2H), 3.70–3.35 (m, 10H), 2.34 (dd, J = 6.9, 13.4 Hz, 1H), 2.30–2.08 (m, 2H), 1.98 (dd, J = 9.5, 13.4 Hz, 1H), 1.63 (dddt, J = 7.1, 14.0, 21.4, 28.4 Hz, 4H), 0.88 (dt, J = 7.4, 14.7 Hz, 6H); ^{13}C NMR (101 MHz, D_2O) δ 187.84, 167.92, 165.46, 136.47, 136.38, 126.74, 119.43, 119.21, 79.41, 62.68, 56.69, 52.36, 51.85, 51.11, 39.37, 32.84, 31.85, 30.63, 26.67, 10.97, 10.85. Anal. Calcd for $C_{21}H_{33}Cl_2FN_2O_2$: C, 57.93; H, 7.64; N, 6.43; Found: C, 57.71; H, 7.69; N, 6.32.

*Dihydrochloride salt of 5-(2-(4-benzhydrylpiperazin-1-yl)ethyl)-3,3-diethyldihydrofuran-2(3H)-one (**11b**)* White solid. Yield: 50 %. mp: 213–215 °C. ^{1}H NMR (400 MHz, D_2O) δ 7.67 (d, J = 7.2 Hz, 4H), 7.50 (dq, J = 7.1, 14.4 Hz, 6H), 4.67 (m, 1H), 3.82–3.35 (m, 10H), 2.33 (dd, J = 6.9, 13.5 Hz, 1H), 2.18 (d, J = 33.6 Hz, 2H), 1.98 (dd, J = 9.5, 13.5 Hz, 1H), 1.62 (ddd, J = 6.8, 14.3, 28.4 Hz, 4H), 0.98–0.79 (m, 6H); ^{13}C NMR (101 MHz, D_2O) δ 187.81, 137.25, 132.85, 132.78, 130.96, 79.35, 78.44, 56.55, 52.34, 52.09, 51.50, 39.35, 32.76, 31.83, 30.61, 26.67, 10.97, 10.84. Anal. Calcd for $C_{21}H_{34}Cl_2N_2O_3{\cdot}0.5\ H_2O$: C, 64.53; H, 7.82; N, 5.57; Found: C, 64.49; H, 7.90; N, 5.57.

*Hydrochloride salt of 3,3-diethyl-5-(2-(4-phenylpiperidin-1-yl)ethyl)dihydrofuran-2(3H)-one (**12b**)* White solid. Yield: 51 %. mp: 239.5 °C. ^{1}H NMR (400 MHz, D_2O) δ 7.39 (tt, J = 7.3, 14.3 Hz, 5H), 4.75–4.67 (m, 1H), 3.75–3.68 (m, 2H), 3.39–3.31 (m, 2H), 3.22–3.12 (m, 2H), 3.03–2.95 (m, 1H), 2.37 (dd, J = 6.9, 13.4 Hz, 1H), 2.31–2.10 (m, 4H), 2.02 (dd, J = 9.4, 13.5 Hz, 3H), 1.78–1.53 (m, 4H), 0.92 (dt, J = 7.5, 12.7 Hz, 6H); ^{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. 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.

*5-(2-(4-(1H-benzo[d]imidazol-2-yl)piperidin-1-yl)ethyl)-3,3-diethyldihydrofuran-2(3H)-one (**13b**)* Yellowish semi-solid. Yield: 43 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.46 (m, 2H), 7.13–7.11 (m, 2H), 4.40–4.37 (m, 1H), 3.43 (broad s, 1H), 2.92–2.90 (m, 3H), 2.44–2.42 (m, 2H), 1.98–1.90 (m, 7H), 1.77–1.74 (m, 3H), 1.56–1.52 (m, 4H), 0.89–0.80 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 181.52, 157.99, 122.47, 76.18, 54.93. 53.98, 53.61, 48.99, 37.90, 36.93, 34.14, 31.11, 31.06, 29.52, 28.57, 9.09, 9.01. MS (ESI) (m/z) 370.1 $(M + H)^+$. HPLC analysis: >95 % purity using two different mobile phases.

*Hydrochloride salt of 5-(2-(3,4-dihydroisoquinolin-2(1H)-yl) ethyl)-3,3-diethyldihydrofuran-2(3H)-one (**14b**)* White solid. Yield: 51 %. mp: 227.5 ~ 228.5 °C. ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.39–7.17 (m, 4H), 4.63–4.54 (m, 1H), 4.49 (s, 2H), 3.75–3.54 (m, 2H), 3.51–3.40 (m, 2H), 3.22 (t, J = 6.3 Hz, 2H), 2.36–2.24 (m, 2H), 2.23–2.08 (m, 1H), 1.95 (dd, J = 9.4, 13.3 Hz, 1H), 1.75–1.53 (m, 4H), 0.94 (dt, J = 7.5, 12.2 Hz, 6H); ^{13}C NMR (101 MHz, MeOH-d_4) 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, 50.78, 50.50, 50.29, 50.07, 49.86, 49.65, 49.43, 49.22, 39.25, 32.87, 30.89, 30.02, 27.35, 9.85, 9.77. 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.

*5-(2-(4-Benzoylpiperazin-1-yl)ethyl)-3,3-diethyldihydrofuran-2(3H)-one (**15b**)* Yellowish oil. Yield: 52 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.30 (m, 4H), 4.44–4.38

(m, 1H), 3.70 (broad s, 2H); 3.37 (broad s, 2H); 2.32–2.48 (m, 6H); 2.08–2.04 (m 1H), 1.78–1.72 (m, 3H), 1.58–1.51 (m, 4H), 0.89–0.80 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) 180.92, 170.47, 135.94, 129.90, 128.68, 127.22, 75.53, 54.53, 48.78, 37.83, 33.88, 29.40, 28.45, 8.98, 8.90. MS (ESI) (m/z) 359.1 $(M + H)^+$. HPLC analysis: >95 % purity using two different mobile phases.

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