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Effect of the rigidification of propranolol, a mixed β-adrenoceptor and 5-HT$_{1A}$R antagonist

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Propranolol is a popular β adrenergic antagonists that, together with pindolol, binds also to serotoninergic receptors, namely 5-HT$_{1A/B}$. In this work the rigidification of the propranolol structure by locking its hydroxyl group within a 1,3-dioxolane ring was investigated. Constrained derivatives of propranolol were synthesized, fully characterized and tested for their affinity at β-adrenoreceptors and 5-HT$_{1A/B/C}$ receptors using radioligand binding assay. The constrained derivatives were inactive, as expected, at β$_{1/2/3}$ adrenergic receptors. Although less expected, these derivatives failed to bind also to 5-HT$_{1A/B/C}$ receptors. The rigidification of propranolol is detrimental for 5-HT$_{1A}$R activity.

1. Introduction

Among the fourteen serotonin (5-hydroxytryptamine, 5-HT) receptor subtypes, grouped into seven major families (5-HT$_1$-5-HT$_7$), the 5-HT$_{1A}$ receptor (5-HT$_{1A}$R) still represents an attractive therapeutic target for drug discovery (Fiorino et al. 2014). 5-HT$_{1A}$R is involved in many physiological and pathological processes including anxiety (Jastrzebska-Wiesek et al. 2018), depression (Pytka et al. 2016), schizophrenia (Cuisiat et al. 2007), neuroprotection (Marco et al. 2011; Coyner et al. 2016), learning and memory (Stiedl et al. 2015), pain perception (Di Cesare Mannelli et al. 2017), gastrointestinal (Javid et al. 2018) urogenital (Sakai et al. 2013) cardiovascular response (Pham-Le et al. 2011) and sexual behavior (Motofei et al. 2008). More recently, it was found that 5-HT plays a role in oncogenesis, contributing to cell proliferation in different tumors (Chilmonczyk et al. 2017; Shinka et al. 2011).

Although a number of 5-HT$_{1A}$R agonists have been identified and approved for therapeutic use (i.e. buspirone, trazodone, vilazodone, nemonapride), clinical candidates with an antagonist profile are still lacking.

Preclinical data have shown that several 5-HT$_{1A}$ antagonists are able to inhibit the growth of tumor cell lines, opening a new scenario in cancer therapy (Chilmonczyk et al. 2017; Siddiqui et al. 2006). 5-HT$_{1A}$ antagonists have also been explored as potential adjunctive therapies for selective serotonin reuptake inhibitors (SSRI) (Childers et al. 2010). This class of drugs is extensively used in the treatment of the major depressive disorder (MDD) although it suffers from a slow onset of clinical action and limited efficacy, due to the induction of a negative feed-back mechanisms mediated by auto-receptors (5-HT$_{1A}$, 5-HT$_{1B}$, α$_2$-adrenoceptors) and postsynaptic receptors (e.g., 5-HT$_3$) (Albert et al. 2014). Pindolol, a mixed β-adrenoceptor/5-HT$_{1A}$R antagonist, was proven to accelerate and even increase, the clinical action of SSRI, preferentially blocking inhibitory 5-HT$_{1A}$ autoreceptors (Artigas et al. 2018).

Similarly to pindolol, propranolol, the first clinically successful β-blocker, binds also to 5-HT$_{1A}$R, behaving as antagonist (Fig. 1). Thus, with the aim to identify novel and clean 5HT$_{1A}$ antagonists, propranolol was chosen as a starting point for structural modifications. To separate the β-adrenergic from the serotoninergic activity and to improve the 5HT$_{1A}$R/β selectivity, we applied the conformational restriction of the ligand as a well-established strategy in drug design. We had already successfully used this approach in the case of naftopidil (Sorbi et al. 2009). More recently, a series of

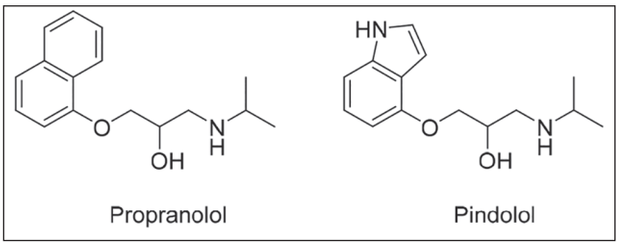


Fig. 1: Chemical structures of two mixed β-adrenoceptor / 5-HT$_{1A}$R antagonists.

compounds obtained by ring closure were reported as potent and selective protein kinase inhibitors (Assadieskandar et al. 2018).

In the present work, the effect of the rigidification of the propranolol structure, by locking its hydroxyl group within a 1,3-dioxolane ring (Fig. 2), was investigated. We knew that the hydroxyl group plays a key role for the β-adrenergic activity, as its contribution to the binding process, through the hydrogen bond, is of paramount importance. Then, if the interaction with the β-adrenoceptors, as it seems, involves the alcoholic function, its incorporation into a ring most probably would decrease the affinity for the β-adrenoceptors, maintaining, hopefully, the 5-HT1A activity and thus enhancing 5-HT1A/β selectivity.

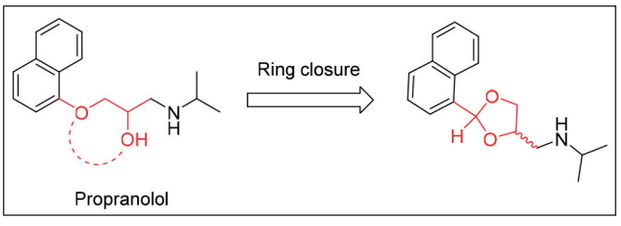


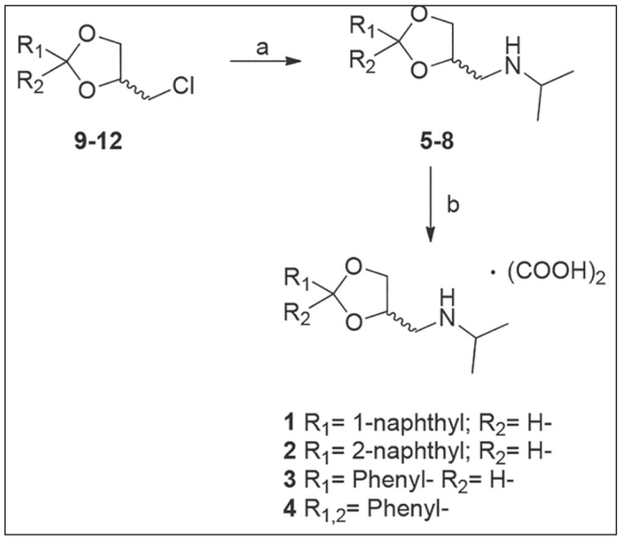
Fig. 2: Cyclization of the propandiol chain of propranolol yields 1,3-dioxolane-based compounds.

Consequently we set out to prepare and test for β adrenergic and 5-HT1A receptor activity some 1,3-dioxolane-based analogues of propranolol, in particular the two positional isomers of naphthalene and the 2,2-diphenyl and monophenyl derivatives. This would allow us to study also the contribution of each diastereomer (*Z*/*E*) to the binding affinity.

2. Investigations, results and discussion

2.1. Synthesis

The tested compounds **1**-**4** were prepared as reported in the Scheme.



Scheme: Reagents and conditions: a) isopropyl amine (2 eq.), KI (cat.), neat, 110°C. b) oxalic acid (1.1. eq.) dry Et_2O, r.t., N_2, 48h.

The appropriate aliphatic chlorides **9**-**12**, prepared as previously reported (Brasili et al. 2003), were reacted with isopropyl amine to afford the amines **5-8**. The free amines were converted into the corresponding oxalate salts **1-4** by treatment with oxalic acid in dry diethyl ether. The isopropyl amine derivatives **5-7** were initially obtained as mixtures of *Z/E* isomers, in the ratio of about 2:1 in favour of the *E* form. This ratio is probably due to the steric hindrance created by the aromatic substituent in the *Z* isomers, making the nucleophilic attach by the amine group more difficult with respect to the *E* form. The *Z/E* isomers were easily separated by flash chromatography, the *E* form being the first to be eluted.

The assignment of the *Z/E* isomerism was performed on the free amines **5-7** by {^{1}H-^{1}H} NOESY (Jeneer et al. 1979; Wagner et al. 1982). In Fig. 3, the NOESY spectra for the exemplary compound **5-***Z* is shown (for **6** and **7**-*Z* see SI).

Focusing on the protons in positions 2 and 4, a cross-peak is observed for the *Z* isomers whereas no correlation was found for the *E* isomers. Based on the above considerations, the configuration of the substituents at the carbon atom in position 2 and 4 of the [1,3]-dioxolane ring was assigned for compounds **5-7**, in accordance to previous studies (Mucci et al. 1995; Malmusi et al. 1996).

2.2. Pharmacology

Compounds **1-4** were submitted to the PDSP (Psychoactive Drug Screening Program, at National Institute of Mental Health) to evaluate their profile against $\beta_{1/2/3}$ adrenergic and 5-$HT_{1A/B/D}$ receptors

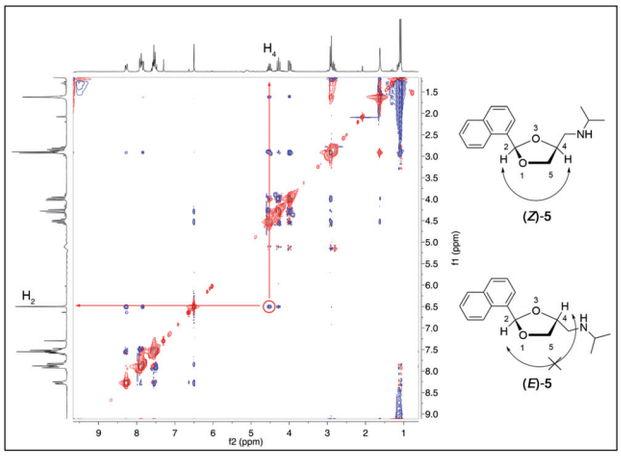
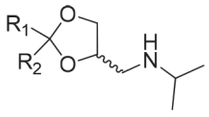
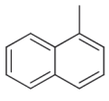
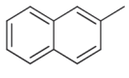


Fig. 3: NOESY spectra of the representative compound (***Z***)**-5**. The red circle highlights the correlation between the protons in positions 2 and 4 indicating a *Z* type conformation.

Table: Radioligand binding assay of compounds 1-4 at human recombinant $\beta_{1/2/3}$ adrenoceptor and 5-$HT_{1A/B/D}$ receptors



Compd.	R_1	R_2	% of inhibition[a]					
			β_1[b]	β_1[b]	β_3[b]	5-HT_{1A}[c]	5-HT_{1B}[d]	5-HT_{1D}[d]
***(Z)*-1**		H-	17.7	-4.2	6.4	0.2	12.9	-3.0
***(E)*-1**			16.2	8.4	4.8	2.2	6.3	-3.0
***(Z)*-2**		H-	12.3	-3.7	9.0	-9.4	-5.9	-12.8
***(E)*-2**			15.2	4.3	9.2	0.4	3.1	11.3
***(Z)*-3**		H-	9.4	11.6	17.9	-3.0	1.0	-9.3
***(E)*-3**			11.3	10.3	0.0	-10.0	1.1	-12.2
4			9.5	11.3	3.2	-7.7	1.4	-16.9

The SD is within±10 % of the nominal value.
[a]Non-specific binding in the presence of 10 µM of the appropriate reference compound is set as 100% inhibition; total binding in the absence of test compound or reference compound is set as 0% inhibition.
[b]Alprenolol, [c]8-OH-DPAT, [d]ergotamine were used as reference compounds.

by radioligand binding assays, in human recombinant cell lines (Table). The compounds were tested at 10 µM in a primary binding assay and the results, expressed as percentage of radioligand binding inhibition, are reported in the Table. The binding results showed that the rigidification of the propranolol structure gives derivatives that are inactive at both receptor systems. The lack of activity at $\beta_{1/2/3}$ adrenoceptors was not surprising as it was demonstrated that the alkylation (Crowther and Smith 1968) or removal (Howe and Shanks 1966) of the hydroxyl group from the propranolol structure leads to inactive derivatives. More surprising was the lack of activity at 5-$HT_{1A/B/D}$ receptors as extensive structure-activity relationship studies had shown that the hydroxyl group in the lateral chain of propranolol is not essential for the binding, at least at one serotoninergic receptor subtype (Pierson et al. 1989; Ismaiel et al. 1997).

3. Experimental

All commercially available chemicals and solvents were reagent grade and were used without further purification unless otherwise specified. Reactions were monitored by thin-layer chromatography on silica gel plates (60F-254, E. Merck) and visualized with UV light or cerium ammonium sulphate. The following solvents and reagents have been abbreviated: ethyl ether (Et_2O), dimethyl sulfoxide (DMSO), ethyl acetate (EtOAc), dichloromethane (DCM), methanol (MeOH). NMR spectra were recorded on a Bruker 200 spectrometer with ^{1}H at 200 MHz and ^{13}C at 50 MHz. Proton chemical shifts were referenced to a TMS internal standard. Chemical shifts are reported in parts per million (ppm, δ units). Coupling constants are reported in Hertz (Hz). Splitting patterns are designed as s, singlet; d, doublet; t, triplet; q quartet; dd, double doublet; spt, septet; m, multiplet; b, broad. NOE spectra were acquired in $CDCl_3$, at 25°C, with a mixing time of 1200 ms on a 200 MHz Bruker spectrometer. LC-MS analysis was performed on a 6310A Ion Trap (Agilent Technologies) mass spectrometer. Melting points were recorded on a Stuart, SMP3 (Barloworld Scientific Limited Stone, Staffordshire, UK) and are uncorrected. The elemental analysis was performed on a Carlo Erba 1106 Analyzer and the values obtained are within ±0.4 % of the calculated ones. All the compounds showed a level of purity above 97 %. The yields reported are based on a single experiment and are not optimized. The oxalate salts of all tested compounds were used for the pharmacological evaluations. The chlorides **9-12** were obtained as previously reported (Brasili et al. 2003).

3.1. General procedure for the synthesis of [1,3]-dioxolan-4-ylmethyl-isopropylamines 5-8

Chlorides **9-12** (1 eq.) were placed in a Parr reactor. Isopropylamine (2 eq.) and a catalytic amount of KI were added. The mixture was reacted at 110 °C for 45-62 h. The isopropylamine was evaporated under reduced pressure and the crude was dissolved in AcOEt. The organic phase was washed with 5 % di NaOH (3 x 50 ml) and brine (1 x 50 ml), dried over anhydrous Na_2SO_4, and concentrated. The crude was chromatographed to afford the title compound.

*3.1.1. N-((-2-(Naphthalen-1-yl)-1,3-dioxolan-4-yl)methyl) propan-2-amine (**5**)*

The *Z*/*E* isomers (yield, 80 %) were separated by flash chromatography using silica gel as stationary phase and AcOEt 100% as eluting system, giving **(*Z*)-** and **(*E*)-5** pairs as oil.

(*Z*)-5 ^{1}H NMR (200 MHz, Chloroform-*d*) δ 1.08 (d, *J* = 6.3 Hz, 6H, CH_3), 2.76 – 2.95 (m, 3H, CH_2N, CH *i*Pr), 3.99 (dd, *J* = 6.3, 7.8 Hz, 1H, H-5 diox), 4.28 (dd, *J* = 6.9, 7.8 Hz, 1H, H-5' diox), 4.46-4.58 (m, 1H, H-4 diox), 6.50 (s, 1H, H-2 diox), 7.44 – 7.63 (m, 3H, H-3,6,7 Napht), 7.80 – 7.95 (m, 3H, H-2,4,8 Napht), 8.20 – 8.33 (m, 1H, H-5 Napht). ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.34 (CH_3); 21.66 (CH_3); 49.05 (CH *i*Pr); 49.62 (CH_2N); 68.54 (diox C-5); 75.19 (C-4 diox); 102.62 (C-2 diox); 123.63 (C-7 Napht); 123.91 (C-6 Napht); 125.09 (C-8 Napht); 125.83 (C5 Napht); 126.42 (C-3 Napht); 128.59 (C-4 Napht); 129.80 (C-2 Napht); 130.89 (C8 Napht); 132.50 (C-4a Napht); 133.71 (C-1 Napht). MS (ESI): *m*/*z* [M + H]$^+$: 271.1.

(*E*)-5 ^{1}H NMR (200 MHz, Chloroform-*d*) δ 1.15 (d, *J* = 6.3 Hz, 6H, CH_3), 2.74 – 3.11 (m, 3H, CH_2N, CH *i*Pr), 3.91 (dd, *J* = 6.6, 8.0 Hz, 1H, H-5 diox), 4.35 (dd, *J* = 6.4, 8.0 Hz, 1H, H-5' diox), 4.42 – 4.59 (m, 1H, H-4 diox), 6.63 (s, 1H, H-2 diox), 7.42 – 7.64 (m, 3H, H-3,6,7 Napht), 7.74 – 7.83 (m, 1H, H-2 Napht), 7.84 – 7.96 (m, 2H, H-4,8 Napht), 8.19 – 8.31 (m, 1H, H-5 Napht). ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.52 (CH_3); 20.91 (CH_3); 47.68 (CH *i*Pr); 50.14 (CH_2N); 68.37 (diox C-5); 73.72 (C-4 diox); 102.46 (C-2 diox); 123.98 (C-7 Napht); 124.16 (C-6 Napht); 124.99 (C-8 Napht); 125.83 (C5 Napht); 126.45 (C-3 Napht); 128.56 (C-4 Napht); 129.77 (C-2 Napht); 130.79 (C8 Napht); 132.58 (C-4a Napht); 133.79 (C-1 Napht). MS (ESI): *m*/*z* [M + H]$^+$: 271.2

*3.1.2. N-((-2-(Naphthalen-2-yl)-1,3-dioxolan-4-yl)methyl) propan-2-amine (**6**)*

The *Z*/*E* isomers (yield, 48 %) were separated by flash chromatography using silica gel as stationary phase and a gradient eluting system from AcOEt 100% to AcOEt 99%/MeOH 1%, giving **(*Z*)-** and **(*E*)-6** pairs as oil.

(*Z*)-6 ^{1}H NMR (200 MHz, Chloroform-*d*) δ 1.11 (d, *J* = 6.3 Hz, 6H, CH_3), 2.77 – 3.01 (m, 3H, CH_2N, CH *i*Pr), 3.96 (dd, *J* = 6.1, 7.8 Hz, 1H, H-5 diox), 4.22 (dd, *J* = 6.9, 7.8 Hz, 1H, H-5' diox), 4.39-4.51 (m, 1H, H-4 diox), 6.03 (s, 1H, H-2 diox), 7.46 – 7.59 (m, 2H, H-6,7 Napht), 7.64 (dd, *J* = 1.7, 8.6 Hz, 1H, H-5 Napht), 7.84 – 7.95 (m, 3H, H-3,4,8 Napht), 7.97 – 8.01 (m, 1H, H-1 Napht). ^{13}C NMR (50 MHz, $CDCl_3$) d 21.99 (CH_3); 22.20 (CH_3); 49.24 (CH *i*Pr); 49.67 (CH_2N); 68.79 (C-5 diox); 75.81 (C-4 diox); 104.57 (C-2 diox); 123.81 (C-6 Napht); 126.29 (C-7 Napht); 126.44 (C-1 Napht); 126.57 (C-4 Napht); 127.76 (C-8 Napht); 128.30 (C-5 Napht), 128.39 (C-3 Napht); 132.93 (C-4a Napht); 133.96 (C-8a Napht); 134.68 (C-2 Napht). MS (ESI): *m*/*z* [M + H]$^+$: 271.1

(*E*)-6 ^{1}H NMR (200 MHz, Chloroform-*d*) δ 1.14 (d, *J* = 6.3 Hz, 6H, CH_3), 2.69 – 3.09 (m, 3H, CH_2N, CH *i*Pr), 3.84 (dd, *J* = 6.6, 8.0 Hz, 1H, H-5 diox), 4.33 (dd, *J* = 6.4, 8.0 Hz, 1H, H-5' diox), 4.43-4.55 (m, 1H, H-4 diox), 6.15 (s, 1H, H-2 diox), 7.48-7.57 (m, 2H, H-6,7 Napht), 7.62 (dd, *J* = 1.7, 8.5 Hz, 1H, H-5 Napht), 7.84 – 7.95 (m, 3H, H-3,4,8 Napht), 7.98-7.99 (m, 1H, H-1 Napht). ^{13}C NMR (50 MHz, $CDCl_3$) d 22.39 (CH_3); 22.67 (CH_3); 49.03 (CH *i*Pr); 49.42 (CH_2N); 68.81 (C-5 diox); 75.87 (C-4 diox); 103.57 (C-2 diox); 123.80 (C-6 Napht); 126.05 (C-7 Napht); 126.20 (C-1 Napht); 126.43 (C-4 Napht); 127.74 (C-8 Napht); 128.28 (C-5 Napht), 128.36 (C-3 Napht); 132.93 (C-4a Napht); 133.83 (C-8a Napht); 135.36 (C-2 Napht). MS (ESI): *m*/*z* [M + H]$^+$: 271.1.

*3.1.3. N-((-2-Phenyl-1,3-dioxolan-4-yl)methyl)propan-2-amine (**7**)*

The *Z*/*E* isomers (yield, 58 %). were separated by flash chromatography using silica gel as the stationary phase and a gradient eluting system from AcOEt 100% to AcOEt 95%/MeOH 5%, giving **(*Z*)-** and **(*E*)-7** pairs as oil

(*Z*)-7 ^{1}H NMR (200 MHz, Chloroform-*d*) δ 1.13 (d, *J* = 6.3 Hz, 6H, CH_3), 2.05 (bs, 1H, NH), 2.73 – 3.06 (m, 3H, CH_2N, CH *i*Pr), 3.78 (dd, *J* = 6.6, 8.1 Hz, 1H, H-5 diox), 4.28 (dd, *J* = 6.4, 8.0 Hz, 1H, H-5' diox), 4.38-4.51 (m, 1H, H-4 diox), 5.98 (s, 1H, H-2 diox), 7.33 – 7.58 (m, 5H, Ph). ^{13}C-NMR (50 MHz, DMSO-d_6) d 20.31 (CH_3); 20.81 (CH_3); 48.35 (CH *i*Pr); 49.82 (CH_2N); 68.67 (C-5 diox); 74.06 (C-4 diox); 104.55 (C-2 diox); 126.52 (C-2,6 Ph); 128.44 (C-3,5 Ph); 129.52 (C-4 Ph); 136.92 (C-1 Ph). MS (ESI): *m*/*z* [M + H]$^+$: 221.2.

(*E*)-7 ^{1}H NMR (200 MHz, Chloroform-*d*) δ 1.10 (d, *J* = 6.2 Hz, 6H, CH_3), 1.83 (bs, 1H, NH), 2.68 – 3.04 (m, 3H, CH_2N, CH *i*Pr), 3.90 (dd, *J* = 7.0, 7.8 Hz, 1H, H-5 diox), 4.16 (dd, *J* = 6.0, 7.8 Hz, 1H, H-5' diox), 4.30 – 4.52 (m, 1H, H-4 diox), 5.85 (s, 1H, H-2 diox), 7.36 – 7.63 (m, 5H, Ph). ^{13}C-NMR (50 MHz, DMSO-d_6) d 19.86(CH_3); 20.38 (CH_3); 46.91 (CH *i*Pr); 50.11 (CH_2N); 68.35 (C-5 diox); 73.29 (C-4 diox); 103.67 (C-2 diox); 126.54 (C-2,6 Ph); 128.39 (C-3,5 Ph); 129.36 (C-4 Ph); 137.41 (C-1 Ph). MS (ESI): *m*/*z* [M + H]$^+$: 221.1.

*3.1.4. N-((2,2-Diphenyl-1,3-dioxolan-4-yl)methyl)propan-2-amine (**8**)*

The title compound was purified by flash chromatography using silica gel as the stationary phase and a gradient eluting system from AcOEt 99 % - MeOH 1 % to AcOEt 80 % - MeOH 20 %, giving **8** as oil (77 % yield). ^{1}H NMR (200 MHz, Chloroform-*d*) δ 1.06 (d, *J* = 6.2 Hz, 3H, CH_3), 1.09 (d, *J* = 6.2 Hz, 3H, CH_3), 2.65 – 2.97 (m, 3H, CH_2N, CH *i*Pr), 3.87 (dd, *J* = 6.3, 7.8 Hz, 1H, H-5 diox), 4.12 (dd, *J* = 6.8, 7.8 Hz, 1H, H-5' diox), 4.30-4.43 (m, 1H, H-4 diox), 7.18 – 7.44 (m, 6H, H-3,4,5,3',4',5' Ph), 7.44 – 7.65 (m, 4H, H-2,6,2',6' Ph). ^{13}C-NMR (50 MHz, DMSO-d_6) d 22.42 (CH_3); 22.66 (CH_3); 48.99 (CH *i*Pr); 50.03 (CH_2N); 68.26 (C-5 diox); 76.07 (C-4 diox); 109.95 (C-2 diox); 126.15 (C-2,6 Ph); 126.28 (C-2',6' Ph); 128.00 (C-4 Ph); 128.03 (C-4' Ph); 128.06 (C-3,5 Ph); 128.14 (C-3',5' Ph); 142.19 (C-1 Ph); 142.46 (C-1' Ph). MS (ESI): *m*/*z* [M + H]$^+$: 297.2.

3.2. General procedure for the synthesis of [1,3]-diossolan-4-ylmethyl-isopropylammonium hydrogenoxalates 1-4

Oxalic acid (1.1 eq.) was added to a solution of the appropriate amine **5-8** (1 eq.) in 30 mL of dry Et_2O. The suspension was mixed for 30 min and the precipitate was settled down for 48 h. The titled compound was purified as following.

3.2.1. N-((-2-(Naphthalen-1-yl)-1,3-dioxolan-4-yl)methyl)propan-2-ammonium hydrogenoxalate

Z (C2-C4) pair: **(*Z*)-1**

The reaction mixture was filtered and the solid was dissolved in hot MeOH and filtered still warm and left at rt. After 48 h the crystals were filtered again and washed several times with anhydrous Et_2O. The white solid was dried under vacuum affording 214 mg of a white solid (31 % yield). M.p. 167-169 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.23 (d, *J* = 6.5 Hz, 3H, CH_3), 1.24 (d, *J* = 6.5 Hz, 3H, CH_3), 2.95 – 3.48 (m, 3H, CH_2N, CH *i*Pr), 4.02 (dd, *J* = 5.4, 8.4 Hz, 1H, H-5 diox), 4.28 (dd, *J* = 7.1, 8.5 Hz, 1H, H-5' diox), 4.69 (m, 1H, H-4 diox), 6.49 (s, 1H, H-2 diox), 7.51 – 7.65 (m, 3H, H-3,6,7 Napht), 7.87 (dd, *J* = 1.3, 7.3 Hz, 1H, H-2 Napht), 7.97-8.03 (m, 2H, H-4,8 Napht), 8.22 (d, *J* = 9.8 Hz, 1H, H-5 Napht). ^{13}C-NMR (50 MHz, DMSO-d_6) d 19.35 (CH_3); 19.45 (CH_3); 47.49 (CH *i*Pr); 50.78 (CH_2N); 68.68 (diox C-5); 73.39 (C-4 diox); 102.87(C-2 diox); 124.82 (C-7 Napht); 125.00 (C-6 Napht); 125.98 (C-8 Napht); 126.77 (C5 Napht); 127.23 (C-3 Napht); 129.28 (C-4 Napht); 130.53 (C-2 Napht); 131.38 (C8 Napht); 132.94 (C-4a Napht); 134.04 (C-1 Napht); 165.46 (ox). Anal. calcd for $C_{19}H_{23}NO_6$: C 63.15, H 6.41, N 3.88; found: C 63.79, H 6.46, N 4.27.

E (C2-C4) pair: **(*E*)-1**

The reaction mixture was filtered and washed with hot MeOH. The filtrate was washed with hot Et_2O and dried under vacuum to afford 520 mg of a white solid (90% yield). M.p. 181-183 °C.

^{1}H NMR (200 MHz, DMSO-d_6) δ 1.30 (d, *J* = 6.0 Hz, 6H, CH_3), 3.13 – 3.49 (m, 3H, CH_2N, CH *i*Pr), 3.89 (dd, *J* = 6.1, 8.6 Hz, 1H, H-5 diox), 4.37 (dd, *J* = 6.5, 8.6 Hz, 1H, H-5' diox), 4.56 – 4.78 (m, 1H, H-4 diox), 6.62 (s, 1H, H-2 diox), 7.49 – 7.63 (m, 3H,

H-3,6,7 Napht), 7.74 (dd, *J* = 1.3, 7.2 Hz, 1H, H-2 Napht), 7.97-8.01 (m, 2H, H-4,8 Napht), 8.16 (bs, 2H, NH_2), 8.22 – 8.30 (m, 1H, H-5 Napht). ^{13}C-NMR (50 MHz, DMSO-d_6) d 19.43 (CH_3); 19.49 (CH_3); 46.63 (CH *i*Pr); 50.75 (CH_2N); 68.73 (C-5 diox); 73.14 (C-4 diox); 102.30 (C-2 diox); 125.04 (C-7 Napht); 125.11 (C-6 Napht); 125.89 (C-8 Napht); 126.76 (C5 Napht); 127.12 (C5 Napht); 129.28 (C-4 Napht); 130.51 (C-2 Napht); 131.32 (C8 Napht); 133.29 (C-4a Napht); 134.20 (C-1 Napht); 165.50 (ox). Anal. calcd for $C_{19}H_{23}NO_6$: C 63.15, H 6.41, N 3.88; found: C 63.75, H 6.48, N 4.30.

3.2.2. N-((-2-(Naphthalen-2-yl)-1,3-dioxolan-4-yl)methyl) propan-2-ammonium hydrogenoxalate

Z (C2-C4) pair: **(*Z*)-2**

The purification was performed as described for **(*E*)-1**. The titled compound was obtained as a white solid (80 % yield). M.p. 192-194 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.26 (d, *J* = 6.5 Hz, 6H, CH_3), 2.94 – 3.50 (m, 3H, CH_2N, CH *i*Pr), 4.01 (dd, *J* = 5.2, 8.5 Hz, 1H, H-5 diox), 4.20 (dd, *J* = 7.0, 8.5 Hz, 1H, H-5' diox), 4.54-4.66 (m, 1H, H-4 diox), 6.00 (s, 1H, H-2 diox), 7.07 (bs, 2H, NH_2), 7.52 – 7.61 (m, 2H, H-6,7 Napht), 7.66 (dd, *J* = 1.7, 8.6 Hz, 1H, H-5 Napht), 7.88 – 8.04 (m, 3H, H-3,4,8 Napht), 8.05 – 8.11 (m, 1H, H-1 Napht). ^{13}C-NMR (50 MHz, DMSO-d_6) d 19.37 (CH_3); 19.49 (CH_3); 47.64 (CH *i*Pr); 50.79 (CH_2N); 68.88 (C-5 diox); 73.47 (C-4 diox); 105.01 (C-2 diox); 125.12 (C-6 Napht); 127.30 (C-7 Napht); 127.52 (C-1 Napht); 127.62 (C-4 Napht); 128.52 (C-8 Napht); 128.90 (C-5 Napht), 129.00 (C-3 Napht); 133.22 (C-4a Napht); 134.37 (C-8a Napht); 135.23 (C-2 Napht); 165.42 (ox). Anal. calcd for $C_{19}H_{23}NO_6$: C 63.15, H 6.6.41, N 3.88; found: C 63.57, H 6.34, N 4.26.

E (C2-C4) pair: **(*E*)-2**

The reaction mixture was filtered and washed with hot MeOH. The filtrate was collected, washed with hot Et_2O and dried under vacuum to afford 90 mg of a white solid (34 % yield). M.p 193-195 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.28 (d, *J* = 6.5 Hz, 6H, CH_3), 3.10 – 3.49 (m, 3H, CH_2N, CH *i*Pr), 3.82 (dd, *J* = 6.2, 8.6 Hz, 1H, H-5 diox), 4.36 (dd, *J* = 6.5, 8.6 Hz, 1H, H-5' diox), 4.56 – 4.76 (m, 1H, H-4 diox), 6.13 (s, 1H, H-2 diox), 7.43 (bs, 2H, NH_2), 7.52 – 7.64 (m, 3H, H-5,6,7 Napht), 7.94-8.03 (m, 4H, H-1,3,4,8 Napht). ^{13}C-NMR (50 MHz, DMSO-d_6) d 19.38(CH_3); 19.52(CH_3); 46.56 (CH *i*Pr); 50.73 (CH_2N); 68.83 (diox C-5); 73.30 (diox C-4); 103.67 (diox C-2); 124.92 (C-6 Napht); 127.03 (C-7 Napht); 127.30 (C-1 Napht); 127.56 (C-4 Napht); 128.51 (C-8 Napht); 128.94 (C-5 Napht); 129.02 (C-3 Napht); 133.24 (C-4a Napht); 134.25 (C-8a Napht); 135.76 (C-2 Napht); 165.48 (ox). Anal. calcd for $C_{19}H_{23}NO_6$: C 63.15, H 6.41, N 3.88; found: C 63.12, H 6.30, N 4.20.

3.2.3. N-((2-Phenyl-1,3-dioxolan-4-yl)methyl)propan-2-ammonium hydrogenoxalate

Z (C2-C4) pair: **(*Z*)-3**

The purification was performed as described for **(*Z*)-1**. The titled compound was obtained as a white solid (92 mg, 37 % yield). M. p. 171-173 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.24 (d, *J* = 6.5 Hz, 6H, CH_3), 3.09 (dd, *J* = 8.2, 12.9 Hz, 1H, CHN), 3.21 (d, *J* = 4.1 Hz, 1H, CH'N), 3.37 (spt, *J* = 6.5 Hz, 1H, CH *i*Pr), 3.94 (dd, *J* = 5.2, 8.5 Hz, 1H, H-5 diox), 4.14 (dd, *J* = 7.1, 8.5 Hz, 1H, H-5' diox), 4.45 – 4.61 (m, 1H, H-4 diox), 5.82 (s, 1H, H-2 diox), 7.39 – 7.58 (m, 5H, Ph), 8.87 (bs, 2H, NH_2). ^{13}C-NMR (50 MHz, DMSO-d_6) d 19.35(CH_3); 19.49 (CH_3); 47.64 (CH *i*Pr); 50.78 (CH_2N); 68.75 (C-5 diox); 73.29 (C-4 diox); 104.84 (C-2 diox); 127.88 (C-2,6 Ph); 129.11 (C-3,5 Ph); 130.40 (C-4 Ph); 137.71 (C-1 Ph); 165.35 (ox). Anal. calcd for $C_{15}H_{21}NO_6$: C 57.87, H 6.80, N 4.50; found: C 58.33, H 6.73, N 4.88.

E (C2-C4) pair: **(*E*)-3**

The purification was performed as described for **(*Z*)-1**. The titled compound was obtained as a white solid (200 mg, 35% yield). M.p. 195-197°C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.27 (d, *J* = 6.5 Hz, 6H, CH_3), 3.15 – 3.24 (m, 2H, CH_2N), 3.36 (spt, *J* = 6.5, 1H, CH *i*Pr), 3.75 (dd, *J* = 6.3, 8.6 Hz, 1H, H-5 diox), 4.30 (dd, *J* = 6.5, 8.6 Hz, 1H, H-5' diox), 4.46 – 4.68 (m, 1H, H-4 diox), 5.96 (s, 1H, H-2 diox), 7.31 – 7.54 (m, 5H, Ph), 9.14 (s, 2H, NH_2). ^{13}C-NMR (50 MHz, DMSO-d_6) d 19.38 (CH_3); 19.50 (CH_3); 46.54 (CH *i*Pr); 50.70 (CH_2N); 68.72 (C-5 diox); 73.12 (C-4 diox); 103.50 (C-2 diox); 127.52 (C-2,6 Ph); 129.13 (C-3,5 Ph); 130.20 (C-4 Ph); 138.26 (C-1 Ph), 165.41 (ox). Anal. calcd for $C_{15}H_{21}NO_6$: C 57.87, H 6.80, N 4.50; found: C 58.43, H 6.78, N 4.85.

*3.2.4. N-((2,2-Diphenyl-1,3-dioxolan-4-yl)methyl)propan-2-ammonium hydrogenoxalate (**4**)*

The purification was performed as described for **(*Z*)-1**. The titled compound was obtained as a white solid (385 mg, 55% yield). M.p. 194-196 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.24 (d, *J* = 6.5 Hz, 6H, CH_3), 3.04 (dd, *J* = 8.3, 13.0 Hz, 1H, CHN), 3.20 (dd, *J* = 3.8, 13.0 Hz, 1H, CH'N), 3.37 (spt, *J* = 6.5 Hz, 1H, CH *i*Pr), 3.85 (dd, *J* = 6.4, 8.5 Hz, 1H, H-5 diox), 4.12 (dd, *J* = 6.9, 8.5 Hz, 1H, H-5' diox), 4.41-4.52 (m, 1H, H-4 diox), 6.00 (s, 2H, NH_2), 6.92 – 7.84 (m, 10H, Ph). ^{13}C-NMR (50 MHz, DMSO-d_6) d 19.26 (CH_3); 19.37 (CH_3); 47.23 (CH *i*Pr); 50.88 (CH_2N); 68.35 (C-5 diox); 73.45 (C-4 diox); 110.82 (C-2 diox); 126.69 (C-2,6 Ph); 126.83 (C-2',6' Ph); 128.96 (C-3,5 Ph); 129.08 (C-4 Ph); 129.14 (C-3,5 Ph); 142.51 (C-1 Ph); 142.68 (C-1' Ph); 164.81 (ox). Anal. calcd for $C_{21}H_{25}NO_6$: C 65.10, H 6.50, N 3.62; found: C 66.02, H 6.51, N 4.04.

3.2. Radioligand binding assay at human recombinant $\beta_{1/2/3}$ adrenoceptor subtypes and 5-$HT_{1A/B/D}$ receptors

The primary radioligand binding assays were performed as reported in literature (Besnard et al. 2012). Crude membrane fractions from stably transfected cell lines expressing human $\beta_{1/2/3}$ adrenoceptors and 5-$HT_{1A/B/D}$ receptors were suspended in an appropriate volume of buffer and dispensed into 96-well plates (50 µl per well). The compounds were tested, in quadruplicate, at a 10 µM concentration in the appropriate radioligand binding buffer (for β adrenergic receptors: 50 mM Tris HCl, 3 mM $MnCl_2$, pH 7.7; for 5-HT_1 receptors: 50 mM Tris HCl, 10 mM $MgCl_2$, 0.1 mM EDTA, pH 7.4). [^{125}I]Iodopindolol (0.1 nM), [^{3}H]8-OH-DPAT (0.5 nM) and [^{3}H]GR127543 (0.3 nM) were employed to label $\beta_{1,2,3}$, 5-HT_{1A} and 5-$HT_{1B/D}$ receptors, respectively. The radioligand was allowed to equilibrate at room temperature and in the dark for 90 minutes. Reactions were stopped by vacuum filtration onto 0.3 % polyethyleneimine soaked 96-well filter mats using a 96-well Filtermate harvester, followed by three washes with cold buffers. The filter mats were dried using microwave oven, then a scintillation cocktail was melted on top of the filters and the radioactivity retained on the filters was counted in a Microbeta scintillation counter. Raw counts per minute (cpm) data from the Microbeta counter are uploaded into the PDSP database and analyzed online using built-in analysis tools in the PDSP database. Non-specific binding in the presence of 10 µM of the appropriate reference compound was set as 100% inhibition; total binding in the absence of test compound or reference compound was set as 0% inhibition. The radioactivity in the presence of test compounds was calculated with the following equation and expressed as a percentage inhibition:

$$\% \ Inhibition = 100 - \frac{Sample\ cpm - Nonspecific\ cpm}{Total\ cpm - nonspecific\ cpm} \times 100$$

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Supplementary material: Supplementary material is available from the authors on request.

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