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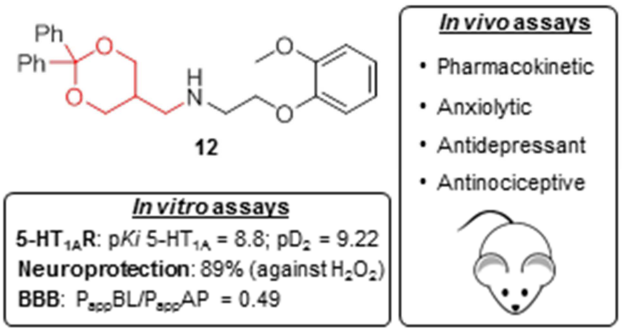
Graphical Abstract



1,3-Dioxane as a scaffold for potent and selective 5-HT_{1A}R agonist with *in-vivo* anxiolytic, anti-depressant and anti-nociceptive activity

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Abstract

A series of compounds generated by ring expansion / opening and molecular elongation / simplification of the 1,3-dioxolane scaffold were prepared and tested for binding affinity at 5-HT_{1A}R and α_1 adrenoceptors. The compounds with greater affinity were selected for further functional studies. N-((2,2-diphenyl-1,3-dioxan-5-yl)methyl)-2-(2-methoxyphenoxy)ethan-1-ammonium hydrogen oxalate (**12**) emerged as highly potent full agonist at the 5-HT_{1A}R (pKi 5-HT_{1A} = 8.8; pD_2 = 9.22, $\%E_{max}$ = 92). The pharmacokinetic data in rats showed that the orally administered **12** has a high biodistribution in the brain compartment. Thus, **12** was further investigated *in-vivo*, showing an anxiolytic and antidepressant effect. Moreover, in the formalin test, **12** was able to decrease the late response to the noxious stimulus, indicating a potential use in the treatment of chronic pain.

Keywords

1,3-dioxane, 5-HT_{1A} receptor agonist, anxiolytic, anti-depressant, antinociceptive activity

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Note: The authors declare no competing financial interest.

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter within the central and peripheral nervous systems, which exerts its actions through its interaction with seven distinct receptors (5-HT_{1-7}R). In the 5-HT_1 subfamily, five subtypes have been identified (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-ht_{1E} and 5-HT_{1F}), all of which belong to the G-protein-coupled receptors (GPCRs). The 5-HT_{1A}R is widely recognized as a relevant therapeutic target for several psychiatric disorders, such as anxiety, depression and schizophrenia [1–3] but also for other pathological conditions, including cognitive deficits [4], neurodegenerative disorders, like Parkinson's and Alzheimer's diseases [5–7], ischemic stroke [8], neuropathic pain [9] and cancer [10].

In the last three decades, a number of 5-HT_{1A}R ligands have been developed. They range from full agonists (8-OH-DPAT, S-14506 and Xaliproden), partial agonists (vilazodone, buspirone, tandospirone, BMY 7378), inverse agonists (spiperone [11]), biased agonists (F15599 also known as NLX-101,[2]) to neutral antagonists (WAY 100635).

However, it should be noted that some of these also share affinity for other receptor types (alpha adrenoceptors, dopamine receptors) and/or 5-HT_1 subtypes or subfamilies (e.g. 8-OH-DPAT for 5-HT_7). Therefore, there is still a need to develop potential clinical candidates with a high degree of selectivity and full agonist potency.

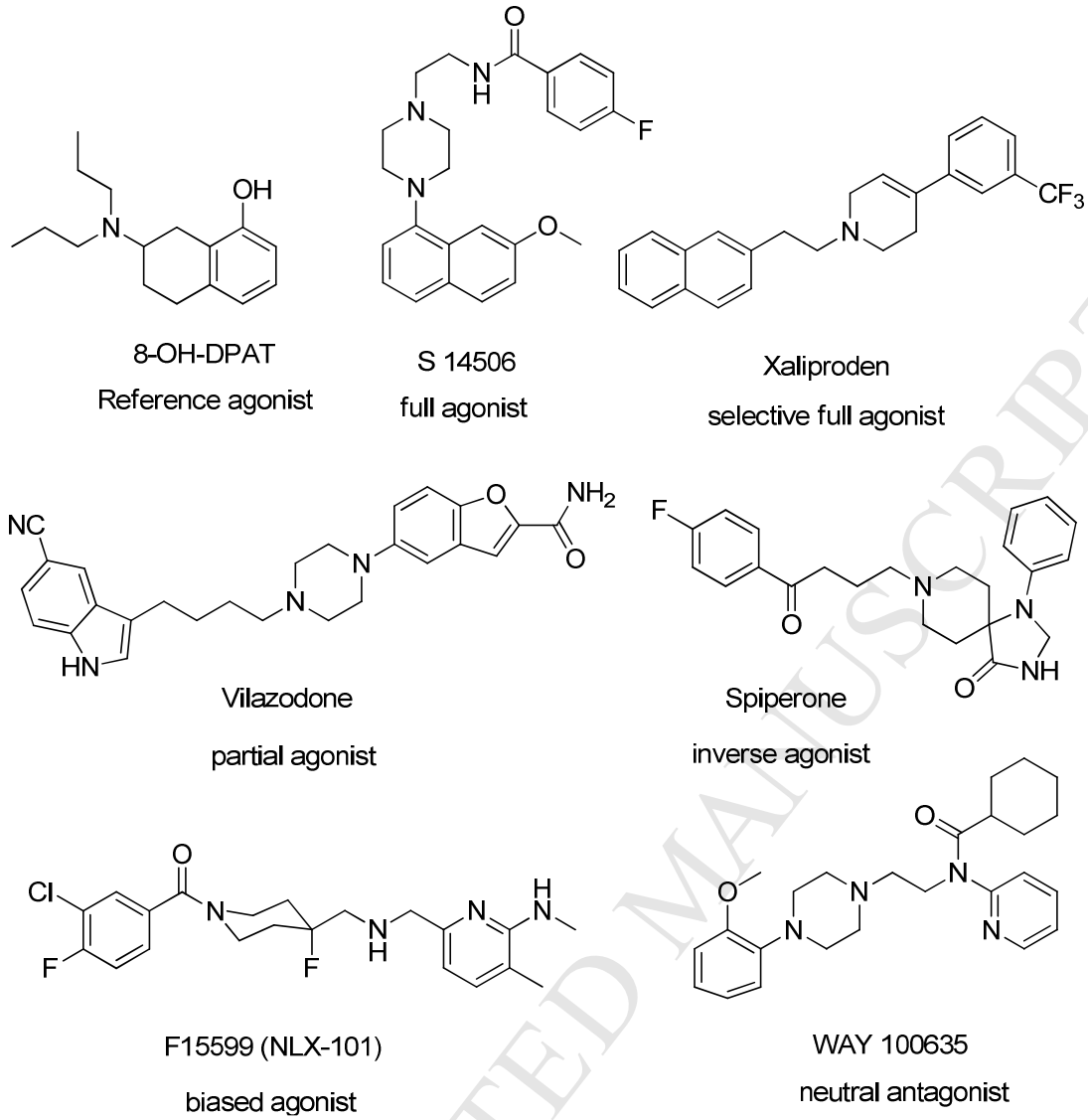


Figure 1: Representative 5-HT_{1A}R ligands

Starting from compounds acting at α_1-adrenoceptors, a series of 5-HT_{1A}R ligands built on the 1,3-dioxolane scaffold was identified [12]. The 1,3-dioxolane has proved to be a versatile and useful scaffold for different classes of drugs. We had successfully employed this moiety to improve potency and selectivity of ligands acting at $alpha_1$adrenergic, 5-HT_{1A} serotoninergic, sigma and TAAR5 receptors.[13][14][15][16]

In this work, we explored the distance between the basic centre and the 2,2-diphenyl portion (Figure 2a, b), the expansion (Figure 2c, d), the opening and the simplification of the 1,3-dioxolane ring (Figure 2e, f), as a way to increase the intrinsic activity and selectivity for 5-HT_{1A}R. A detailed SAR study was carried out, and a potent and selective 5-HT_{1A}R agonist with *in-vivo* anxiolytic, antidepressant and anti-nociceptive activity was discovered.

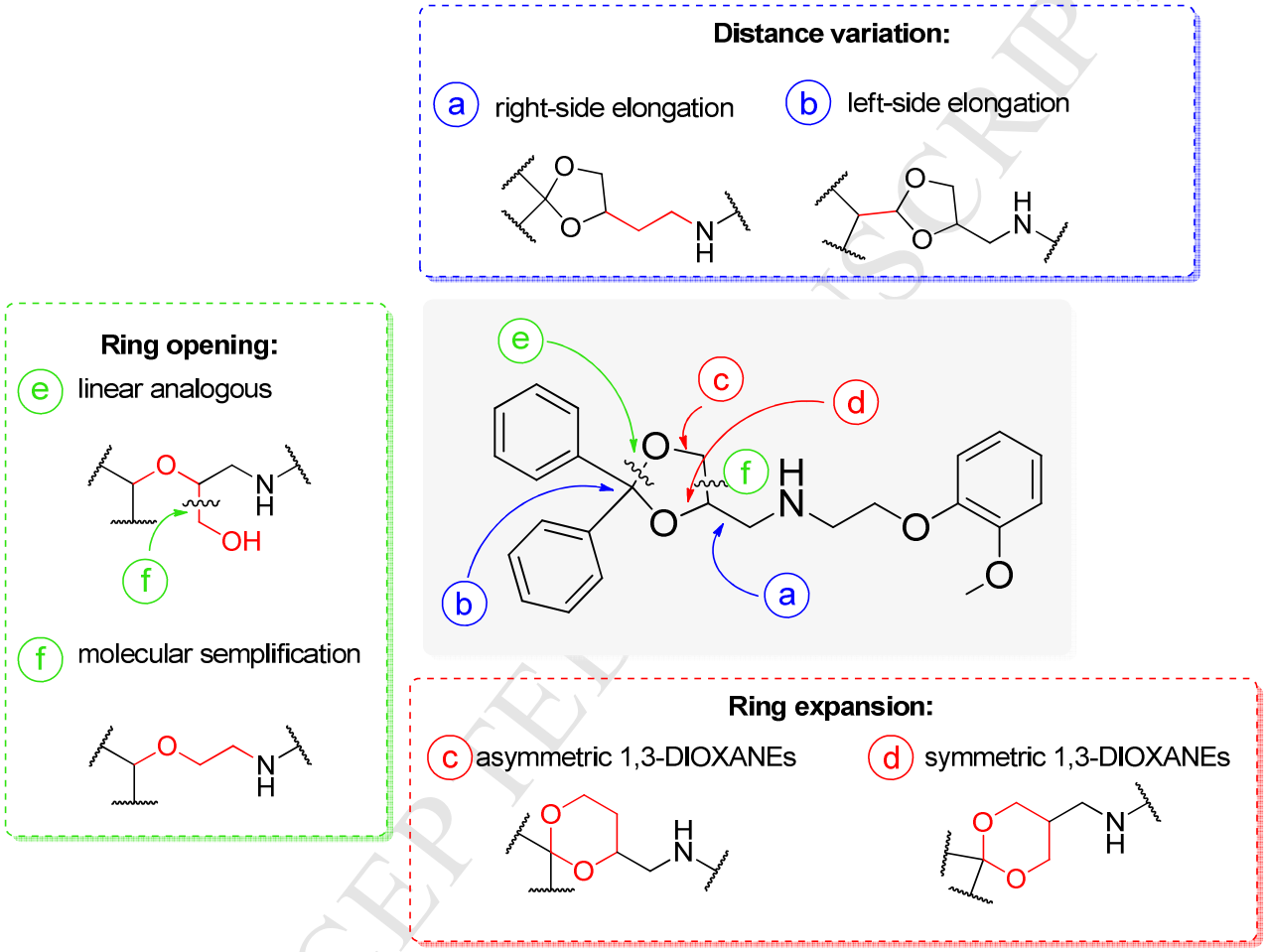


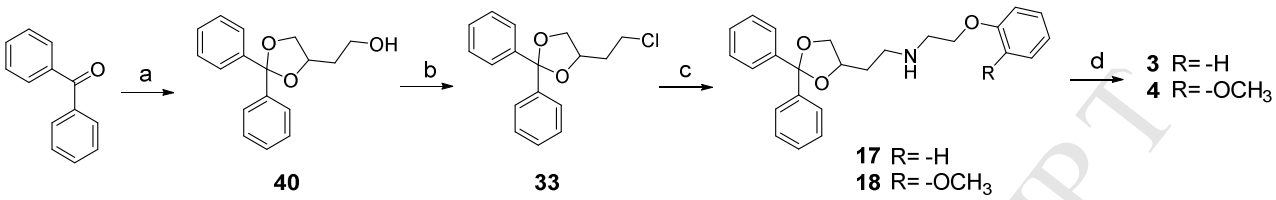
Figure 2. A structural modification approach used to design new 5-HT_{1A}R agonists: a,b) study of the distance between the basic center and the diphenyl portion; c,d) ring expansion; e,f) ring opening and molecular simplification.

2. Results and Discussion

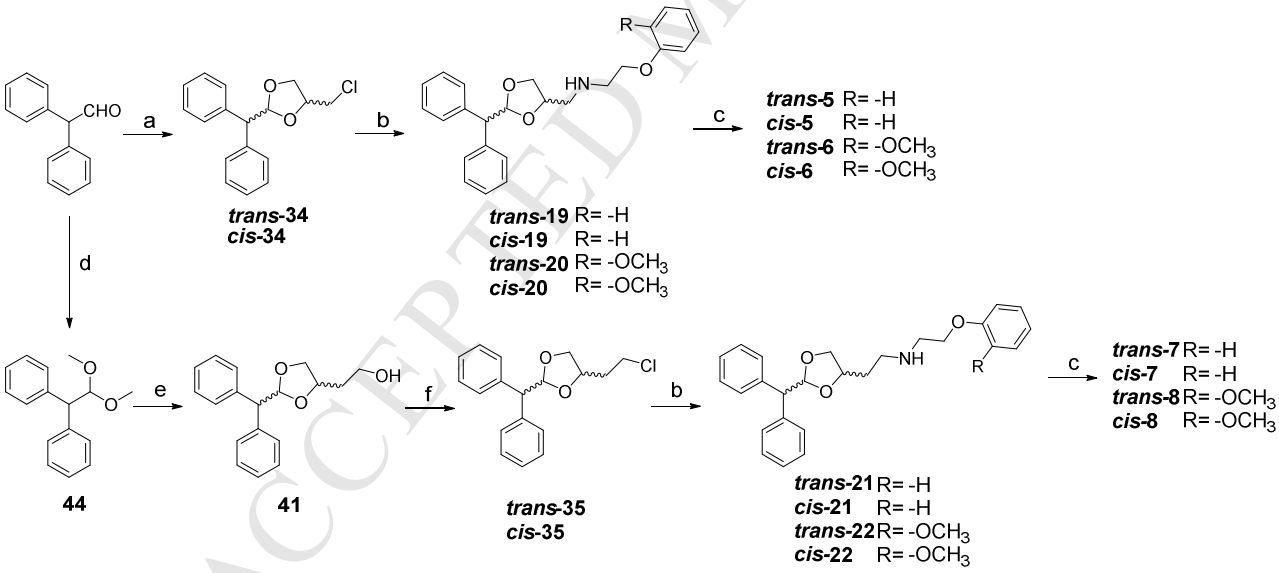
2.1 Chemistry

All the final compounds **3-16** were tested as oxalate salts, prepared through reaction of the respective free amines **17-30** with anhydrous oxalic acid, followed by crystallization from dry diethyl ether (Scheme 1-4). The free amines **17-30** were directly obtained by standard S_N2 reaction between the appropriate aliphatic chloride **33-39** and the 2-phenoxyethan-1-amine or 2-(2-methoxyphenoxy)ethan-1-amine, prepared as previously reported [13]. The reaction was performed in 2-methoxyethanol at reflux temperature for 18-24 hours, using potassium iodide as a catalyst (Scheme 1-4). The two amines **27-28** were synthesized, under the same S_N2 conditions, from the chloro-derivative **38** [15] and the appropriate phenoxyethylamine. The de-protection of the hydroxyl group by treatment of **31-32** with tetra-n-butylammonium fluoride (TBAF) in THF at room temperature for 24 hours led to amines **27-28** in high yield (Scheme 4). With the sole exception of **34** [13], which was obtained directly by condensation of benzophenone with 3-chloro-1,2-propandiol (Scheme 2), the aliphatic chlorides **33** [17]**,** and the brand-new **35-37** were easily prepared by treatment of the respective alcohols **40-43** with thionyl chloride in dry toluene, under nitrogen atmosphere, using pyridine as a base (Scheme 1-3). The two diastereomers of the intermediates **34** and **35** were separated by flash chromatography (Scheme 2). Aliphatic chloride **39** was synthesized as previously reported [15]. Finally, the synthesis of the alcohols **40-43** followed two different procedures. **40** and **42** were jointly prepared (in stoichiometric ratio 13:1) through the condensation of benzophenone and 1,2,4-butantriol in refluxing toluene, using p-toluensulfonic acid (pTSA) as a catalyst and a Dean-Stark trap to remove the water formed, and easily separated by flash chromatography (Scheme 1 and 3). On the contrary, the same condensation procedure did not lead to the preparation of alcohols **41** and **43**. Therefore, for the synthesis of **41** and **43,** the respective carbonyl starting compounds (benzophenone for **41** and 2,2-diphenylacetaldehyde for **43**) were firstly converted into the dimethyl-acetals **44**, **45**, by refluxing in methanol using pTSA as a catalyst and trimethyl orthoformate as a water scavenger. The condensation of **44** with 1,2,4-butantriol and **45** with 2-(hydroxymethyl)propane-1,3-diol in acetonitrile at room temperature, in

the presence of cobalt chloride ($CoCl_2$) and trimethylsilyl chloride (TMSCl), led to the quantitative preparation of alcohols **41** and **43**, respectively (Scheme 2 and 3).

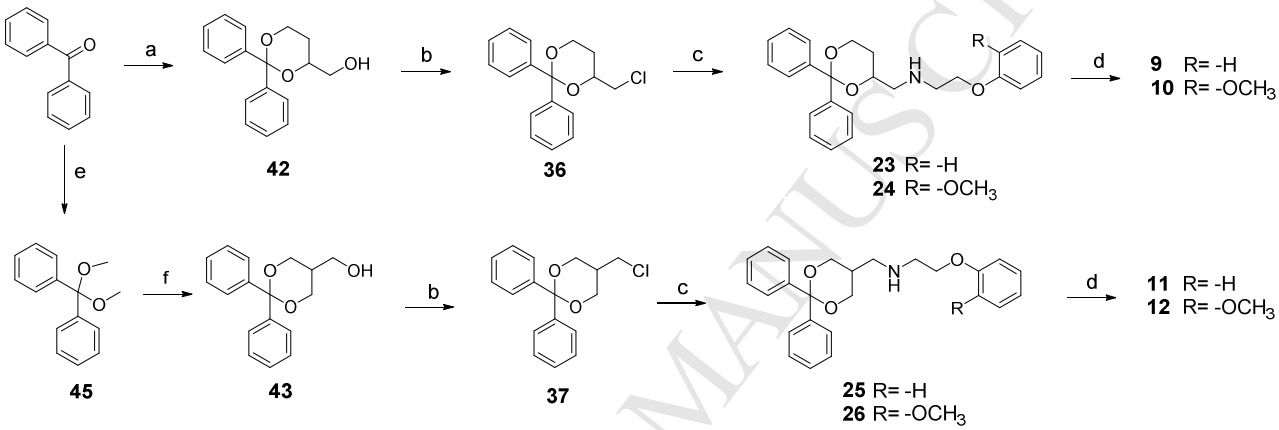


Scheme 1. Reagents and conditions: **a)** 1,2,4-butantriol (1.7 eq.), *p*TSA (cat.), dry Toluene, Dean-Stark trap, N_2, reflux 65 h, 65% yield; **b)** thionyl chloride (1.3 eq.), pyridine (2 eq.), dry toluene, N_2, 0°C to reflux, 45 min, 64% yield; **c)** 2-phenoxyethan-1-amine or 2-(2-methoxyphenoxy)ethan-1-amine (6.4 eq.), KI (cat.), 2-methoxyethanol, reflux, 24 h, 74% yield (for **17**) and 55% yield (for **18**); **d)** oxalic acid (1.2 eq.), dry Et_2O, r.t., 24 h, 63% yield (for **3**) and 60% yield (for **4**).

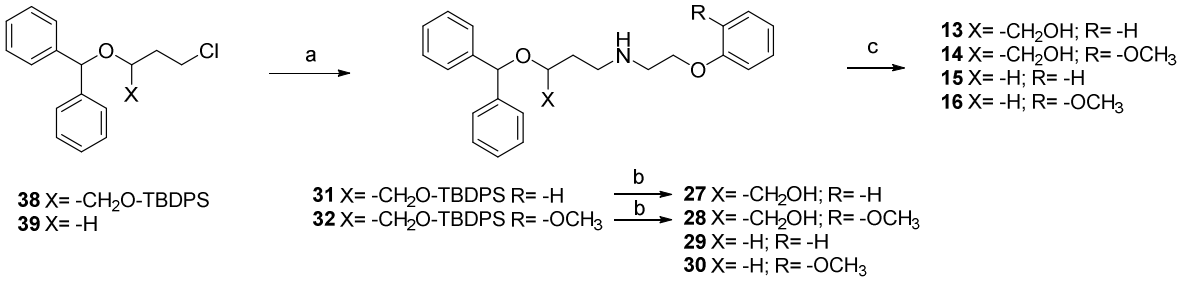


Scheme 2. Reagents and conditions**: a)** 1-Chloro-2,3-propandiol (2 eq.), *p*TSA (cat.), dry Toluene, Dean-Stark trap, N_2, reflux 18 h, 5% yield (***cis*-34**) and 42% yield (***trans*-34**); **b)** 2-phenoxyethan-1-amine or 2-(2-methoxyphenoxy)ethan-1-amine (6.4 eq.), KI (cat.), 2-methoxyethanol, reflux, 18-24 h, 66% yield (for **trans-19**), 54% yield (for **cis-19**), 20% yield (for **trans-20**), 39% yield (for

cis-20); **c)** oxalic acid (1.2 eq.), dry Et_2O, r.t., 24 h, 37% yield (for **trans-5**), 57% yield (for **cis-5**), 41% yield (for **trans-6**), 40% yield (for **cis-6**), 59% yield (for **trans-7**), 30% yield (for **cis-7**), 45% yield (for **trans-8**), 62% yield (for **cis-8**); **d)** trimethyl orthoformate (10 eq.), pTSA (cat.), MeOH, reflux, 5 h, 75% yield; **e)** 1,2,4-butantriol (2 eq.), $CoCl_2$ (0.6 eq.), TMSCl (1 eq.), ACN, r.t., 17 h, quantitative yield; **f)** thionyl chloride (1.3 eq.), pyridine (2 eq.), dry toluene, N_2, 0°C to reflux, 45 min, 39% yield (***cis*-35**) and 45% yield (***trans*-35**).



Scheme 3. Reagents and conditions: **a)** 1,2,4-butantriol (1.7 eq.), *p*TSA (cat.), dry Toluene, Dean-Stark trap, N_2, reflux 65 h, 5% yield; **b)** thionyl chloride (1.3 eq.), pyridine (2 eq.), dry toluene, N_2, 0°C to reflux, 45 min, 88.5% yield (**36**) and 77% yield (**37**); **c)** 2-phenoxyethan-1-amine or 2-(2-methoxyphenoxy)ethan-1-amine (6.4 eq.), KI (cat.), 2-methoxyethanol, reflux, 24 h, 20% yield (for **23**), 31% yield (for **24**), 20% yield (for **25**), 36% yield (for **26**); **d)** oxalic acid (1.2 eq.), dry Et_2O, r.t., 24 h, 26% yield (for **9**), 30% yield (for **10**), 41% yield (for **11**), 57% yield (for **12**); **e)** trimethyl orthoformate (10 eq.), pTSA (cat.), MeOH, reflux, 5 h, 82% yield; **f)** 2-(hydroxymethyl)propane-1,3-diol (2 eq.), $CoCl_2$ (0.6 eq.), TMSCl (1 eq.), ACN, r.t., 17 h, quantitative yield.



Scheme 4. Reagents and conditions: **a)** 2-phenoxyethan-1-amine or 2-(2-methoxyphenoxy)ethan-1-amine (6.4 eq.), KI (cat.), 2-methoxyethanol, reflux, 24 h, 77% yield (for **31**), 40% yield (for **32**), 41% yield (for **29**), 20% yield (for **30**); b) TBAF (1.2. eq.), THF, r.t., 24 h, 57% yield (for **27**) and 87% yield (for **28**); c) oxalic acid (1.2 eq.), dry Et_2O, r.t., 24 h, 54% yield (for **13**), 45% yield (for **14**), 55% yield (for **15**), 30% yield (for **16**).

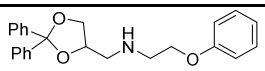
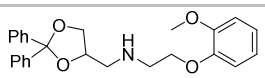
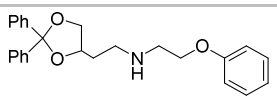
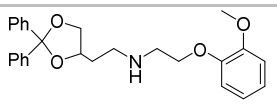
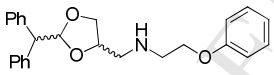
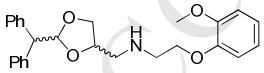
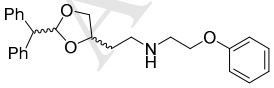
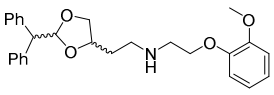
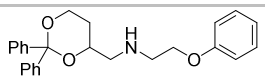
2.2 Structure–affinity and structure-activity relationship studies

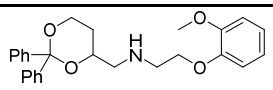
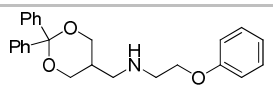
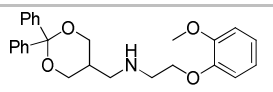
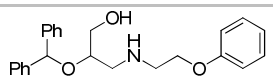
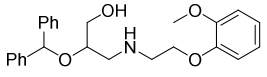
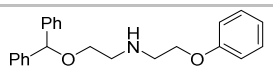
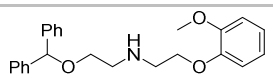
Compounds **3-16** were tested for binding affinity (pK_i) and activity (pK_b) at human α_1 and 5-HT_{1A} receptors. The most active and selective compounds were chosen for their functional characterization (pD_2 and $\%E_{max}$).

In a previous paper, we showed that compounds **1** and **2** bind to both α_1 and 5-HT_{1A}R receptors [12]. Binding studies in human cloned receptors have shown that at all the three α_1 subtypes ($\alpha_{1a,}$ α_{1b} and α_{1d}), the affinities of both compounds are quite similar, with compound **2** showing a small, but scarcely significant, preference with respect to **1**. In the case of 5-HT_{1A}R, compound **2** has a higher affinity than **1**, of about one order of magnitude (9.22 vs 8.45). During the functional studies, both behaved as antagonists at α_1 adrenoceptors and partial agonists at 5-HT_{1A}R. Compound **1** showed a selective profile towards α_{1D} (more than 100-fold), with respect to the α_{1A} and α_{1B} subtypes. The functional data for the 5-HT_{1A}R indicated that the agonist potency decreased by about 28-fold, going from **1** to **2** (pD_2 of 8.8 and 7.36 respectively). This is contrary to the trend observed in the binding experiment, where the affinity of **1** was 6-fold lower than that of compound **2** (pK_i

8.45 and 9.22 respectively). Therefore, the presence of the methoxy group in the *ortho* position had a positive effect on binding and a negative one on agonist potency. Furthermore, during the functional studies at α_1 adrenoceptors, the methoxy group increased potency but decreased selectivity towards the α_{1D} subtype, while leaving the affinity at the α_1 subtypes in the binding studies almost unchanged.

Table 1. Affinity constants (pK_b* or p*Ki*†) for α_1-adrenoceptors in isolated rat prostatic vas deferens (α_{1A}), spleen (α_{1B}), and thoracic aorta (α_{1D}) and for human cloned α_{1a}, α_{1b}, α_{1d} and 5-HT_{1A}R. Agonist potency (pD_2) and relative effectiveness (% Emax)‡ in the agonist-induced [^{35}S]GTPγS-binding assay at 5-HT_{1A}. All the compounds were assayed as oxalate salt.

Cmp	Structure	pK_b*			pK_i†				5-HT_{1A}	%Emax
		α_{1A}	α_{1B}	α_{1D}	α_{1a}	α_{1b}	α_{1d}	5-HT_{1A}	pD_2	5-HT_{1A}
1		6.16	5.86	8.37	7.43	7.20	7.94	8.45	8.8	24.4
2		7.53	7.36	8.65	7.71	7.33	8.03	9.22	7.36	31.6
3		6.34	6.49	7.04	7.23	7.02	7.52	6.14		
4		6.74	6.93	8.17	8.14	7.83	8.24	9.30	6.16	22.1
Trans-5		6.29	6.26	6.70	6.92	6.99	7.06	8.28		
Cis-5		6.22	6.83	6.72	6.37	6.99	7.53	7.94		
Trans-6		6.78	6.82	7.64	7.51	7.56	8.15	8.70		
Cis-6		6.16	6.75	7.52	6.01	7.43	8.36	8.50		
Trans-7		5.71	6.66	7.22	7.05	6.78	7.02	7.24		
Cis-7		6.14	6.43	7.15	6.77	6.97	7.12	7.22		
Trans-8		6.23	7.13	7.25	7.22	7.51	7.80	7.81	6.24	53
Cis-8		6.58	6.91	7.67	7.30	7.19	7.78	7.59	5.86	82.1
9		6.12	6.97	7.55	7.41	7.25	8.30	8.73	7.85	79.5

Cmp	Structure	pK_b*			pK_i†				5-HT_{1A}	%Emax
		α_{1A}	α_{1B}	α_{1D}	α_{1a}	α_{1b}	α_{1d}	5-HT_{1A}	pD_2	5-HT_{1A}
10		6.99	7.19	8.68	7.61	7.8	8.99	9.11	8.49	65.9
11		5.52	6.76	6.76	6.54	6.55	6.75	7.63	5.87	88.7
12		5.98	6.80	7.06	7.47	7.5	7.6	8.79	9.22	91.6
13		<5	6.98	7.07	6.85	5.64	7.24	8.34	7.51	69.5
14		6.51	7.02	7.46	7.08	7.04	8.23	8.81	7.53	90.2
15		7.65	7.01	7.73	7.77	7.60	8.92	8.87	7.85	78.9
16		6.18	7.11	7.75	7.35	5.52	8.00	8.14	7.38	77.3
BMY-7378					6.41§	6.15§	8.89§	8.90§	9.27§	26§
8-OH DPAT					6.82§	<6§	<6§	8.43§	7.83§	100§
WAY 100635					7.73¶	7.18¶	8.34¶	9.48¶	n.a.	-

*Each experiment was performed in triplicate.

†The data are expressed as means of 2–3 separate experiments performed in duplicate;

*†Standard deviation is within ±10% of the value.

‡According to [18]

§See [12];

¶See [19].

In the present study, we wanted to further investigate the importance of other structural elements on activity, potency and selectivity, such as the distance between the basic center and the lipophilic diphenyl portion, and the enlargement and opening of the 1,3-dioxolane ring (Figure 2 a-f). The newly synthesized compounds are reported in Table 1, together with the binding affinities and activities at both α_1 and 5-HT_{1A}R. As can be seen, compound **4**, having the nitrogen atom moved

away by the insertion of a methylene in the lateral chain, with respect to **2,** showed a small increase in binding affinity (pK_i) at all α_1 subtypes, while leaving unchanged the affinity at 5-HT_{1A}R. During the functional studies, the antagonist potency of **4** at α_1 subtypes was slightly decreased, while the selectivity towards the α_{1D} subtype was almost the same (about 10-fold). At 5-HT_{1A}R, the agonist potency was also decreased by about 17-fold. The same modification made on the desmethoxy derivative **1** to give **3** produced different results. In fact, the binding affinities were decreased and the decrease was much larger for 5-HT_{1A}R. During the functional studies at $\alpha_{1,}$ the most significant variation was a complete loss of α_{1D} selectivity, due to a more than 10-fold decrease in potency at this subtype and a concomitant, although small, increase at the α_{1A} and α_{1B} subtypes. In compound **6** the diphenyldioxolane was replaced with a benzhydryldioxolane to increase the distance between the diphenyl moiety and the central amine and two disatereomers were obtained (**t** and **c**). The most important change was in the selectivity 5-$HT_{1A}/\alpha_{1,}$ which was greatly reduced, as a result of a significant decrease in 5-HT_{1A}R affinity. Combining the two variations, as in **8,** was generally negative for both affinity and potency for α_1 and 5-HT_{1A} receptor systems. The exception was the increase in efficacy at 5-HT_{1A}R for both diastereomers, which was accompanied by a decrease in potency. A similar trend was observed with the desmethoxy derivatives **5c,t** and **7c,t**. As far as the stereochemistry is concerned, no clear difference emerged for the two pairs of diastereomers.

The study of the expansion of the dioxolane ring was achieved in two ways: (i) by the insertion of a methylene unit adjacent to the oxygen atom in position 1 of the 1,3-dioxolane to give the asymmetric 1,3-dioxanes **9**,**10** (Figure 2 c); (ii) by the insertion of the same methylene unit adjacent to the oxygen atom in position 3 to give the symmetric 1,3-dioxanes **11, 12** (Figure 2 d).

Compound **10** showed a significant enhancement of the affinity at α_{1b} and α_{1d} receptors, with an α_{1d}/α_{1a} selectivity ratio of about 12-fold higher than that of compound **2**. The affinity at 5-HT_{1A}R

was practically unchanged, with a consequential loss of 5-HT_{1A}/α_1 selectivity. During the functional studies, compound **10** showed an increase in α_{1D} selectivity (31-fold) and, at 5-HT_{1A}R, a significant increase in agonist potency (about 10-fold) and efficacy (doubled).

With respect to **2,** compound **12** showed a decrease, although limited, in affinity at both receptor systems, the exception being the affinity at the α_{1b} receptor subtype. Also the antagonist potencies at the three α_1 receptor subtypes were decreased. At 5-HT_{1A}R the agonist potency was enhanced by about 72-fold, while the efficacy was three times the one observed with compound **2**.

Compound **14** is the open analogue of **2,** obtained by breaking the C2-O1 bond of the 1,3-dioxolane ring. This molecular variation caused a decrease in affinity at both receptor systems, with the exception of the affinity at α_{1d} subtype, showing a significant decrease in 5-HT1A/α_1 selectivity. These results are in agreement with the antagonist potency trend at the α_1 subtypes. The agonist potency at 5-HT_{1A}R was retained, while the efficacy was increased by about 3-fold.

Molecular simplification of **14**, by removing the hydroxymethyl moiety, to give **16**, interestingly gave an increase in binding affinity and antagonist potency at the α_1 receptor subtype, the exception being the potency at the α_{1b} subtype. At 5-HT_{1A}R, the affinity and potency remained unchanged, with a small variation in efficacy.

Compounds **9**, **11**, **13** and **15** were synthesized in order to confirm the effects on the activity of the previously described *ortho*-methoxy group (compound **2** vs **1**). The methoxy group improved, with some exceptions, the pharmacological parameters at both receptor systems. In particular, the most significant variation is the potency of compound **12,** which shows a pD2 of 9.22, 2240-fold higher than the desmethoxy derivative **11,** showing a pD2 of 5.87.

Overall, the above described structural modifications allowed the identification of compound **12,** which is the most interesting in the series, due to its high potency at 5-HT_{1A}R. In direct comparison with the starting point **2,** compound **12** clearly showed enhancement of the pharmacological profile

at 5-HT_{1A}: the selectivity ratio 5-HT_{1A}/α_1 was maintained and, despite a limited reduction in affinity, an increase of about two orders of magnitude in agonist potency and three in efficacy was observed. These results allowed us to consider **12** as one of the most potent 5-HT_{1A}R full agonists. Therefore, compound **12** was chosen for further pharmacological studies.

2.3. *In vitro studies*

2.3.1. Citotoxicity

Firstly, the cytotoxicity (IC_{50}) of **12** was determined by MTT assay, on SH-SY5Y human neuroblastoma cell line, across a wide range of concentrations (0.1-100 μM, see Experimental Section). Cell viability assay was also performed for hydrogen peroxide (H_2O_2), oligomycin A and rotenone, to determine their corresponding IC_{50}. The results, reported in Table 2, showed a dose-dependent cytotoxicity for the above-mentioned compounds at the tested concentrations.

Table 2. Cytotoxicity (IC_{50} μM)[a] of the tested compounds.

12	**H_2O_2**[b]	**oligomycin A**	**rotenone**
31.2 ± 0.6	195 ± 1.7*	29 ± 3.4*	74.1 ± 4.5*

*According to[18]

[a] The IC_{50} values were determined after 24 h incubation of the cells SH-SY5Y with the compounds, by varying concentrations in the range 0.1-100 μM. [b] H_2O_2 was tested in the range 1-500 μM.

2.3.2. Neuroprotective capacity

An assessment of the ability of **12** to prevent the death of human neuroblastoma SH-SY5Y cell lines caused by H_2O_2, oligomycin A and rotenone was carried out *in vitro* [20]. These neurotoxins were used at a concentration equal to their IC_{50}. As reported in Table 3, compound **12,** at 1 μM, showed neuroprotective activity against H_20_2 and oligomycin A damage, while a minimal effect was observed against rotenone.

Table 3. *Neuroprotective effect of **12** on human neuroblastoma cell lines after the addition of three toxic insults

Compd [μM]	**H_2O_2 (195 μM)**	**Oligomycin A(30 μM)**	**Rotenone (75 μM)**
12 (1 μM)	79 ± 5	83 ± 2	61 ± 4
12 (0.1 μM)	89 ± 3	69 ± 2	63 ± 9

*According to[18]

The data are expressed as percentages of neuroprotection ±SD of three independent experiments.

2.3.3. Bi-directional transport studies

An evaluation of the ability of **12** to permeate the MDCK-MDR1 monolayers was carried out *in vitro.* It is well-known that these cell lines mimic the BBB and express the P-glycoprotein (P-gp), which is involved in the drug efflux transport [21]. Transport studies were conducted in both Apical-to-Basolateral and Basolateral-to-Apical directions and the results are shown in Table 4. Compound **12** was found to have non-significant differences in P_{app} values between the AP-to-BL and BL-to-AP directions. The efflux ratio (ER), which was calculated using the equation ER = P_{app}, BL-AP / P_{app}, AP-BL, was found to be less than 2. This result indicates that **12** is not likely to be considered a suitable substrate for P-gp transport. Therefore, **12** was able to permeate the monolayer, by passive diffusion, with permeability that was comparable to that of diazepam. The

permeability for the control (Fluorescein isothiocyanate–dextran, FD-4) was within the expected values.

Table 4. [*]Bi-directional Transport Across MDCKII-MDR1 cells of tested compound **12** and reference compounds.

Compd	P_{app} AP(cm/sec)	P_{app} BL(cm/sec)	ER[a] P_{app}BL/P_{app}AP
12	$2.6*10^{-5}$	$1.26*10^{-5}$	0.49
diazepam	$1.46*10^{-5}$[§]	$1.23*10^{-5}$[§]	0.84[§]
FD-4	$1.03*10^{-6}$[§]	$2.08*10^{-7}$[§]	0.20[§]

[*]According to[22]

[§]See [18]

2.4. *Pharmacokinetic studies*

Before proceeding to the *in vivo* studies, preliminary pharmacokinetic analysis was performed in rats. Compound **12** was administered *per os* at a dose of 10 mg/Kg and brain and plasma concentrations were quantified following the previously reported and validated bioanalytical method [23]. The concentration vs. time curves are reported in Figure 3 and the pharmacokinetic parameters for brain and plasma are summarized in Table 5.

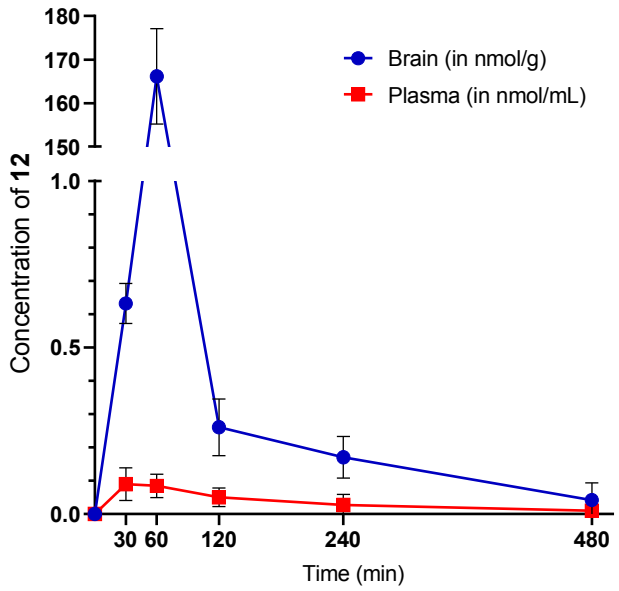


Figure 3. Cerebral (blue circles) and plasmatic (red squares) concentrations (nmol/g and nmol/mL, respectively) of **12** after an oral dose of 10 mg/kg.

Table 5. Pharmacokinetic parameters in rat brain and plasma after an oral dose of **12** (10mg/kg), calculated using *"PK Solutions"* software.[24]

8	**C_{max}**[a]	**t_{max} (min)**	**$AUC_{(0-t)}$ (nmol/g·min)**	**$AUC_{(0-\infty)}$ (nmol/g·min)**	**$t_{½}$ (min)**	**Brain/Plasma (B/P)**
Plasma*	0.095	30	17.4	18.1	102.6	
Brain#	157.9	60	7167.6	7173.4	6.2 (15-45 min) 142 (45-480 min)	≈420

[a] concentrations are expressed as nmol/mL in the plasma and in nmol/g in the brain.

Comparing brain and plasma curves, it is possible to observe a different profile of **12** in the two compartments. The areas under the brain or plasma concentration vs. time (AUC0-t) were 7167 and 17 nmol/g·min, respectively. This difference is reflected in the high brain/plasma ratio (B/P), which was calculated as the ratio between the brain and the plasma AUC(0-t). Compound **12** showed a B/P

ratio value of 420, demonstrating its elevate capability to permeate the blood-brain barrier (BBB). These data were supported by in silico BBB-passage prediction for non-active transport. Predicted brain/blood partition coefficient (QPlogBB) and predicted apparent MDCK cell permeability (QPPMDCK) were calculated with QiKProp [25]. Compound **12** showed a QPlogBB of 0.467 (for CNS penetration -3< QPlogBB <1.2) and a QPPMDCK of 1250 (for CNS penetration QPPMDCK >500), suggesting that is able to cross the BBB by passive diffusion. Moreover, the predicted QPPMDCK is in accordance with the bi-directional transport studies on MDCK-MDR1 monolayers.

As shown in Table 5, the concentration of **12** in the rat brain increased rapidly, reaching a maximum of 157.9 ± 8.7 nmol/g (C_{max}) in the first 60 minutes (t_{max}). The effect of **12** in the behavioral studies was evaluated at this time point. The initial increased concentrations of **12** in the brain occurred during stable plasma levels, as observed for (*R*)-8-OH-DPAT, dipropylaminotetraline (DPAT) derivatives and (*S*)-UH-301. The concentration then decreased, following a biphasic trend: rapidly during the first phase (60-120 minutes) and more slowly during the second phase (120-480 min) with two half-lives ($t_{½}$) of 6.16 and 142 min, respectively. This trend follows the one reported for (*R*)-8-OH-DPAT [26]. The rapid decrease of **12** in the brain does not seems to be linked to a drug efflux transport by P-gp, as suggested by the *in vitro* transport studies.

In the plasma, the concentration of **12** was lower than in the brain, with a C_{max} of 0.095 nmol/mL during the first 30 minutes, but with a monophasic and slow elimination rate, resulting in a $t_{1/2}$ of 102.6 min, 3-times higher than the reference drug 8-OH-DPAT ($t_{1/2}$ = 27 min) [26].

According to the high bio-distribution of **12** in brain, this compound was tested *in vivo* for its activity on the central nervous system.

2.5. In vivo behavioural studies

Compound **12** was assessed in adult male Sprague-Dawley rats for anxiolytic, locomotor and anti-depressant activity.

2.5.1. Anxiolytic effect

The anxiolytic effect of **12** was evaluated in rats using the Elevated Plus Maze test (EPM) [27]. Compound **12** was administered *per os* at three different concentrations (5, 10 and 20 mg/Kg). 8-OH-DPAT (0.5 mg/kg, i.p.) was used as a positive control. The percentage of time spent by the rat between the open arms of the maze (Figure 4A) and the number of entries (Figure 4B) were used as a measure of the anxiolytic effect of the compound. The administration of **12** at the dosages of 10 and 20 mg/kg significantly increased the percentage of the time spent by the rat in the open-arm section (20% and 18% with a $P<0.001$ and $P<0.01$ respectively) with an effect that is comparable to 8-OH-DPAT (19% at 0.5 mg/kg). At the same time, rats administered with **12** spent less time in the closed arms with respect to the rat administered with the vehicle. In addition, **12**, at 10 and 20 mg/kg, was able to increase ($P<0.05$) the number of the open arm entries, whereas no differences were observed between 8-OH-DPAT and the control group. Overall, these data show that **12** exhibits anxiolytic-like activity in the EPM test.

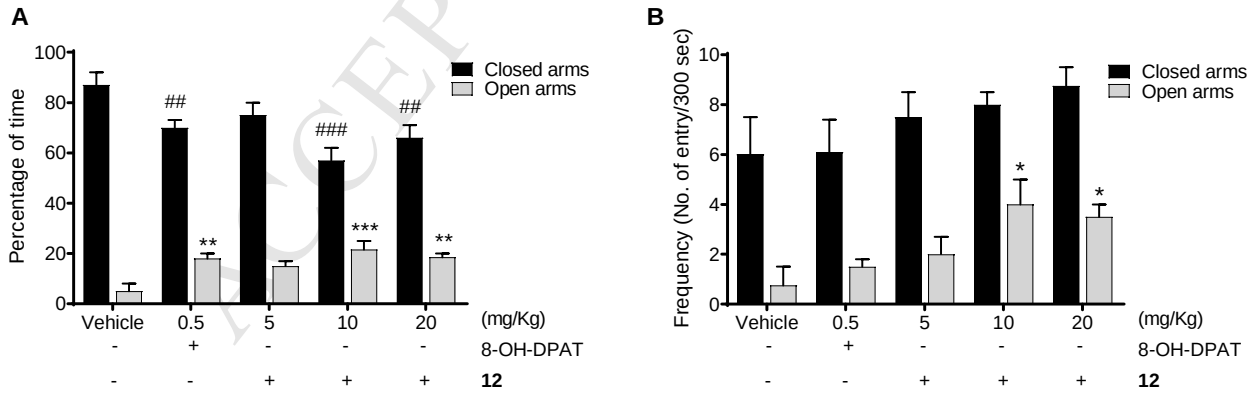


Figure 4. Elevated plus maze test for the evaluation of the anxiolytic effect of **12** in rats administered per os with 5, 10 and 20 mg/Kg. (**A**) Percentage of time spent by the rat in the open

and closed arms of the maze. (**B**) Number of entries in the open and closed arms of the maze, in the 300 sec test session. 8-OH-DPAT, (0.5 mg/Kg, i.p.) was used as a positive control. **P*<0.05, ***P*<0.01, ****P*<0.001, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001 vs. vehicle treated rats (Anova followed by Dunnett's test).

2.5.2. Locomotor activity and anxiolytic effect

The potentiality of compound **12** to possess excitatory activity was evaluated using the Open Field test [27]. The test measures the total distance tracked by the rats as an index of the locomotory activity following CNS excitation. Compound **12** was administered *per os* at 10 mg/Kg. 8-OH-DPAT (at 0.5 mg/Kg) was used as a reference. As reported in Figure 5A, the rats administered both **12** and 8-OH-DPAT ran a comparable total distance, with the respect to the untreated group. This lack of significant variation in the locomotor activity revealed an absence of excitatory effect for compound **12**. In addition, the Open Field test confirmed the anxiolytic effect of **12** observed in the Elevated Pluz Maze test, by measuring the attitude of the rats to explore the open field area of the maze (time spent and number of entries). Due to their nature, anxious rats avoid bright and open spaces, preferring to stay close to the walls of the field (thigmotaxis). On the contrary, a decreased level of anxiety in the animals leads to increased exploratory behavior. The administration of compound **12** at the dose of 10 mg/kg induced an anti-thigmotactic effect, as indicated by a significant increase in the percentage of the time spent and number of entries into the central area of the open field (Figure 5B-C), with respect to the group of untreated and control animals.

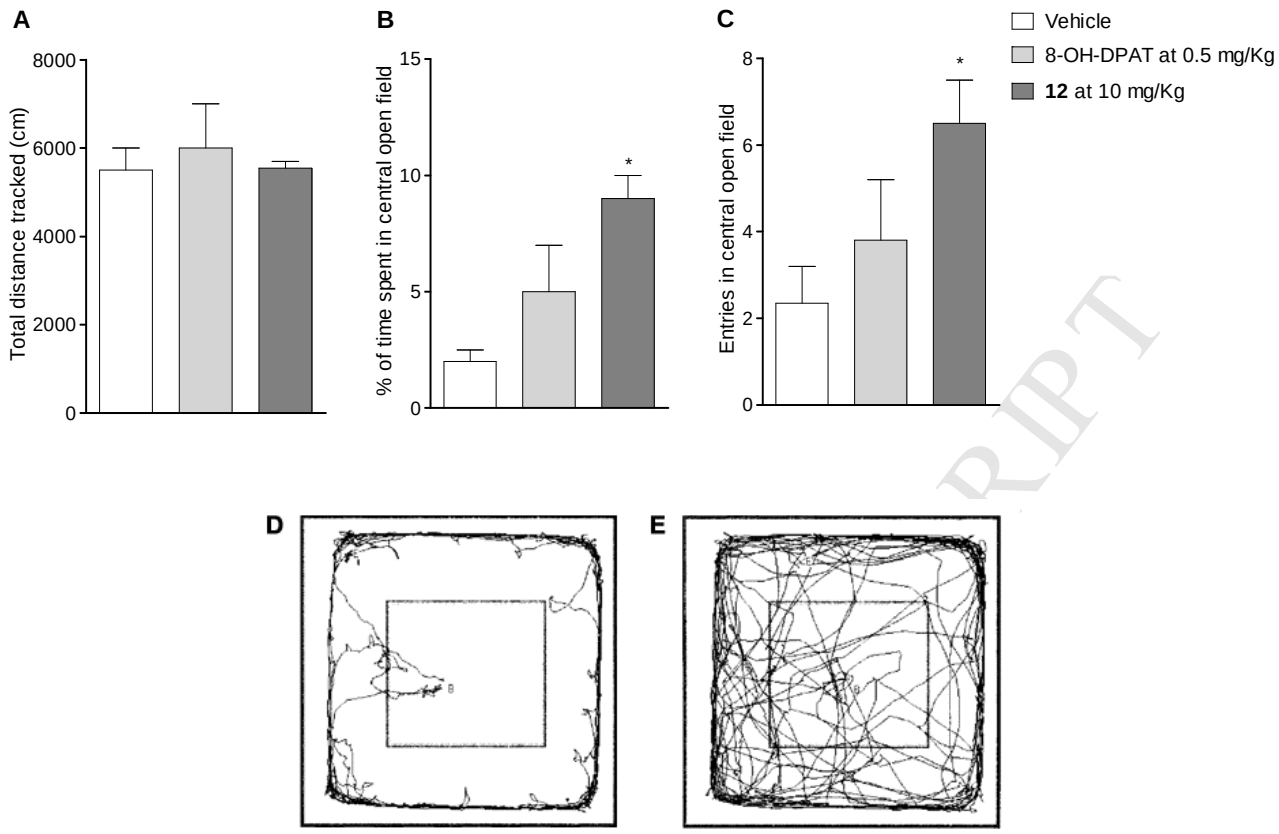


Figure 5. Open Field Test for the evaluation of the locomotor activity and anxiolytic effect of **12** (10 mg/Kg per os) in rats. 8-OH-DPAT (0.5 mg/Kg, i.p.) was used as a positive control. (**A**) Total distance tracked (in cm) by the treated and untreated rats. (**B**) Percentage of time spent in the central open field. (**C**) Number of entries in the central open field. **P*<0.05 vs. vehicle treated rats (Anova followed by Dunnett's test). Moving traces of untreated (**D**) and treated (**E**) mice in the open field test.

2.5.3. Anti-depressant activity

To assay the anti-depressant activity of compound **12**, the Forced Swim test (Porsolt) was used in rats [28–30]. The administration of **12** at the doses of 10 and 20 mg/kg was able to significantly reduce the time that the rats spent immobile and to increase the time spent swimming, with an effect comparable to that of 8-OH-DPAT (Figures 6A and B, respectively).

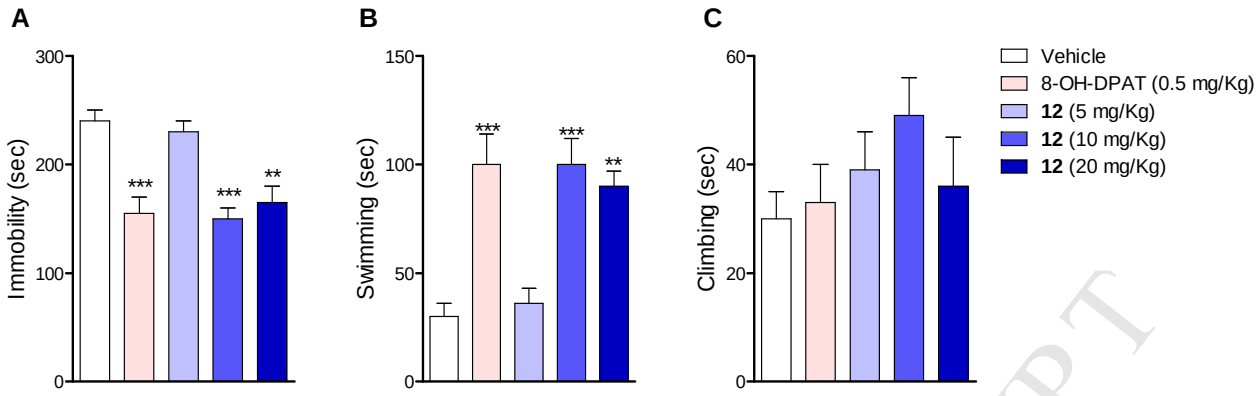


Figure 6. Porsolt's Test for the evaluation of the antidepressant activity of **12** (5, 10 and 20 mg/Kg *per os*) in rats. (**A**) time spent immobile (in sec), (**B**) time spent swimming (sec) and (**C**) time spent climbing (sec) of treated and untreated rats. 8-OH-DPAT (0.5 mg/Kg, i.p.) was used as a positive control. ***P<0.001 and **P<0.01 vs. vehicle treated rats (Anova followed by Dunnett's test)

In contrast, the administration of **12** at all doses did not significantly influence the time spent climbing (Figure 6C). The same results were obtained using the reference drug 8-OH-DPAT. Taken together, these data are in accordance with the effect of 5-HT_{1A}R agonists, whereas catecholaminergic agents cause a decrease in the time spent immobile, together with an increase in the time spent climbing [31,32]. This behaviour indicates that the anti-depressant action of **12** is strictly due to its interaction with the serotonergic system. These data are in agreement with the higher affinity of **12** for the 5-HT_{1A}R rather than for the α-adrenergic receptor (5-HT1a/α1D selectivity = 16), as shown by the binding data.

2.5.4. Anti-nociceptive activity

The formalin test was used to assess the potential analgesic activity of compound **12** *in vivo*.[33] Indeed, at the dose of 10mg/kg, i.p., **12** was able to significantly decrease perception of the II phase

of the noxious stimulus. This effect was reverted by WAY-100635 at 3mg/kg i.p. This is a confirmation that the nociceptive effect of **12** is mediated by the stimulation of 5-HT_{1A}R (Figure 3).

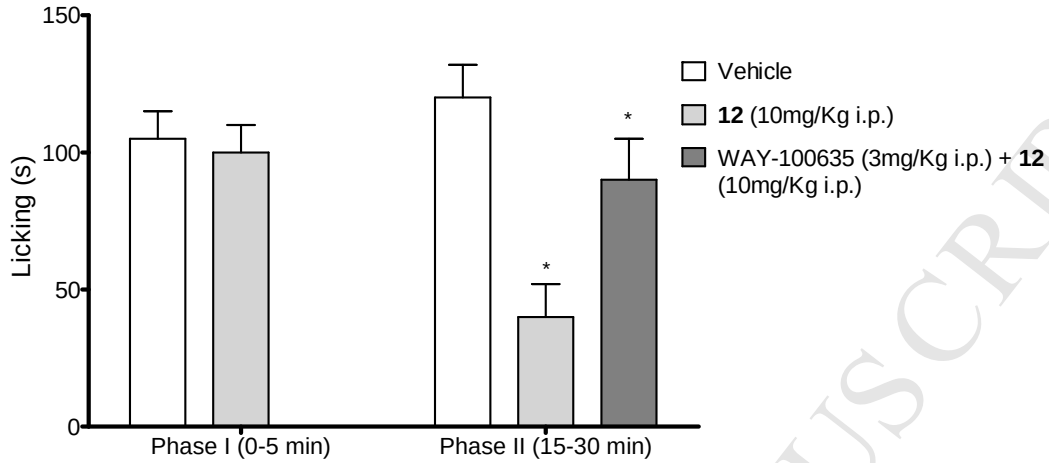


Figure 7: Formalin test to study the effect of intraperitoneal (i.p.) injection of **12** (10 mg/kg) or vehicle on the early (0-5 min) and late (15-30 min) phase of the noxious stimulus. Data are means ± S.E.M. of 8 and 10 mice per group. *$p < 0.05$ vs. mice treated with vehicle.

3. Conclusions

Starting from **Leads 1** and **2,** a new class of 1,3-dioxane-based 5-HT_{1A}R ligands was discovered. Several compounds acted as potent 5-HT_{1A}Rs agonists, among which **12** was the most potent, with a maximal activity of 92% compared to 8-OH-DPAT. I*n vitro*, compound **12** proved to penetrate the BBB. This result was confirmed by the pharmacokinetic analysis of **12** in rat brain and plasma, which showed a preferential distribution in the brain compartment. The behavioral tests in rats treated orally with **12** at a dose of 10 mg/Kg demonstrated an anxiolytic and anti-depressant effect. Compound **12** showed also a good anti-nociceptive activity that was reverted by the co-administration of the 5-HT_{1A}R antagonist WAY-100635

4. Experimental Section

4.1. Chemistry

All the reagents and solvents were commercially available from Sigma-Aldrich. The moisture-sensitive reactions were performed under an inert atmosphere of argon. Each reaction was monitored by TLC on Merck 60G F254 plates and detected at 254 nm. All the compounds were purified by flash column chromatography using silica gel 60 (230-400 mesh, ASTM) supplied by Merck, unless otherwise specified. The purity of the final compounds was assessed by elemental analysis (C,H,N) on a Carlo Erba 1106 analyzer and the results obtained are within ±0.4% of the theoretical values. The melting points were determined with Stuart SMP3 apparatus and are uncorrected. The structure elucidation was confirmed by ^{1}H and ^{13}C NMR (1D and 2D) on a DPX-200 Avance (Bruker) spectrometer at 200 MHz or on a DPX-600 Avance (Bruker) spectrometer at 600 MHz. The chemical shifts are expressed in δ (ppm) using tetramethylsilane (TMS) as internal standard or the ^{13}C signal of the solvent ($CDCl_3$ δ 77.04, CD_3OD δ 49.8, DMSO-d_6 δ 39.5). ^{1}H NMR peak patterns are as follows: s (singlet), d (doublet), t (triplet), dd (double doublet), ddd (double dd), m (multiplet), br (broad singlet). The assignment of cis-trans configuration was done by NOESY experiments. Low resolution MS analysis was performed on a 6310A Ion Trap (Agilent Technologies) whereas high resolution mass spectra were recorded on a hybrid QTOF mass spectrometer (PE SCIEX-QSTAR), both equipped with an electrospray ionization source (ESI).

*4.1.1. General procedure for the synthesis of the oxalate salts (**3-16**)*

To a solution of the appropriate amine **17-30** (1 eq.) in 5 mL of dry Et_2O at room temperature and under nitrogen atmosphere, anhydrous oxalic acid (1.2 eq.) was added. The suspension was stirred for 30 min and left to settle down for 24 h. The precipitate was collected by filtration, washed with dry Et_2O and dried to afford the title compound.

*4.1.1.1. 2-(2,2-diphenyl-1,3-dioxolan-4-yl)-N-(2-phenoxyethyl)ethan-1-ammonium hydrogen oxalate (**3**)*

White solid (60% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.92-2.00 (m, 2H), 3.08 -3.12 (m, 1H), 3.16 – 3.26 (m, 1H), 3.35 (td, *J* = 2.7, 5.1 Hz, 2H), 3.72 (dd, *J* = 6.5, 8.2 Hz, 1H), 4.08 (dd, *J* = 6.6, 8.2 Hz, 1H), 4.23 (m, 3H), 6.91 – 7.06 (m, 3H), 7.29 – 7.39 (m, 8H), 7.41 – 7.47 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 30.12, 44.68, 46.45, 63.89, 69.35, 74.26, 109.48, 115.06, 121.70, 126.15, 126.18, 128.47, 128.49, 128.54, 128.70, 130.05, 142.85, 142.91, 158.21, 164.23. M.p. [202-204°C]. HRMS m/z $[M+H]^+$ Calcd. for $C_{25}H_{28}NO_3^+$: 390.2064. Found: 390.2065. El. Anal. Calcd. for $C_{27}H_{29}NO_7$: C 67.63, H 6.10, N 2.92. Found: C 67.60, H 6.10, N 2.90.

*4.1.1.2. 2-(2,2-diphenyl-1,3-dioxolan-4-yl)-N-(2-(2-methoxyphenoxy)ethyl)ethan-1-ammonium hydrogen oxalate (**4**)*

White solid (59% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.94-1.97 (m, 2H), 3.13-3.14 (m, 1H), 3.23-3.24 (m, 1H), 3.33-3.35 (m, 2H), 3.63 – 3.83 (m, 4H), 4.09 (dd, *J* = 6.6, 8.2 Hz, 1H), 4.15 – 4.29 (m, 3H), 6.91 (td, *J* = 1.7, 7.6 Hz, 1H), 6.94 – 7.10 (m, 3H), 7.22 – 7.40 (m, 6H), 7.44 (ddd, *J* = 1.4, 8.3, 11.2 Hz, 4H). ^{13}C NMR (151 MHz, DMSO) δ 30.27, 44.93, 46.59, 55.90, 65.67, 69.39, 74.33, 109.49, 112.81, 115.36, 121.19, 122.74, 126.16, 126.18, 128.48, 128.49, 128.53, 128.70, 142.85, 142.92, 147.57, 149.90, 164.66. M.p. [178-180°C]. HRMS m/z $[M+H]^+$ Calcd. for $C_{26}H_{30}NO_4^+$: 420.2169. Found: 420.2170. El. Anal. Calcd. for $C_{28}H_{31}NO_8$: C 66.00, H 6.13, N 2.75. Found: C 66.05, H 6.10, N 2.75.

*4.1.1.3. Trans-N-((-2-benzhydryl-1,3-dioxolan-4-yl)methyl)-2-phenoxyethan-1-ammonium hydrogen oxalate (**trans-5**)*

White solid (51% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.10-3.18 (m, 2H), 3.29-3.31 (m, 2H), 3.61 (dd, *J* = 5.8, 8.6 Hz, 1H), 4.01 (dd, *J* = 6.4, 8.6 Hz, 1H), 4.14 – 4.25 (m, 3H), 4.31-4.34 (m,

1H), 5.77 (d, *J* = 5.9 Hz, 1H), 6.96-7.00 (m, 3H), 7.20 (t, *J* = 7.3 Hz, 2H), 7.24 – 7.43 (m, 10H). ^{13}C NMR (151 MHz, DMSO) δ 47.11, 49.34, 54.89, 64.12, 67.85, 72.73, 105.45, 115.04, 121.61, 126.88, 126.90, 128.62, 128.68, 129.28, 129.32, 130.03, 141.15, 158.28, 164.42. M.p. [187-192°C]. HRMS m/z $[M+H]^+$ Calcd. for $C_{25}H_{28}NO_3^+$: 390.2064. Found: 390.2065. El. Anal. Calcd. for $C_{27}H_{29}NO_7$: C 67.63, H 6.10, N 2.92. Found: C 67.65, H 6.10, N 2.90.

*4.1.1.4. Cis-N-((-2-benzhydryl-1,3-dioxolan-4-yl)methyl)-2-phenoxyethan-1-ammonium hydrogen oxalate (**cis-5**)*

White solid (57% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.74 (dd, *J* = 4.8, 13.2 Hz, 1H), 3.01 (dd, *J* = 4.3, 13.2 Hz, 1H), 3.18-3.23 (m, 2H), 3.70 (dd, *J* = 4.8, 8.6 Hz, 1H), 3.94 (dd, *J* = 6.8, 8.6 Hz, 1H), 4.11 – 4.18 (m, 2H), 4.24 (d, *J* = 5.8 Hz, 1H), 4.31 – 4.42 (m, 1H), 5.62 (d, *J* = 5.8 Hz, 1H), 6.99-7.00 (m, 3H), 7.20 (td, *J* = 1.6, 7.3 Hz, 2H), 7.24 – 7.46 (m, 10H). ^{13}C NMR (151 MHz, DMSO) δ 47.23, 50.33, 55.06, 64.23, 67.95, 72.89, 106.16, 115.03, 121.61, 126.94, 128.60, 128.62, 129.35, 129.37, 130.04, 140.99, 141.00, 158.28, 164.31. M.p. [201-203°C]. HRMS m/z $[M+H]^+$ Calcd. for $C_{25}H_{28}NO_3^+$: 390.2064. Found: 390.2060. El. Anal. Calcd. for $C_{27}H_{29}NO_7$: C 67.63, H 6.10, N 2.92. Found: C 67.60, H 6.15, N 2.91.

*4.1.1.5. Trans-N-((-2-benzhydryl-1,3-dioxolan-4-yl)methyl)-2-(2-methoxyphenoxy)ethan-1-ammonium hydrogen oxalate (**trans-6**)*

White solid (49% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.15-3.16 (m, 1H), 3.19-3.20 (m, 1H), 3.27-3.29 (m, 2H), 3.61 (dd, *J* = 5.8, 8.6 Hz, 1H), 3.75 (s, 3H), 4.02 (dd, *J* = 6.4, 8.5 Hz, 1H), 4.17 (t, *J* = 5.4 Hz, 2H), 4.21 (d, *J* = 6.0 Hz, 1H), 4.32-4.34 (m, 1H), 5.78 (d, *J* = 6.0 Hz, 1H), 6.91 (dd, *J* = 1.7, 7.7 Hz, 1H), 6.94 – 7.05 (m, 3H), 7.20 (td, *J* = 1.4, 7.2 Hz, 2H), 7.27-7.30 (m, 4H), 7.33 – 7.41 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 47.02, 49.00, 54.83, 55.74, 64.39, 67.38, 71.96, 105.49, 112.43, 113.94, 115.57, 121.73, 122.87, 127.22, 127.24, 128.78, 128.88, 130.09, 140.59,

140.64, 146.69, 148.56, 168.61. M.p. [201-203°C]. HRMS m/z $[M+H]^+$ Calcd. for $C_{26}H_{30}NO_4^+$: 420.5285. Found: 420.5280. El. Anal. Calcd. for $C_{28}H_{31}NO_8$: C 66.00, H 6.13, N 2.75. Found: C 66.02, H 6.15, N 2.70.

*4.1.1.6. Cis-N-((-2-benzhydryl-1,3-dioxolan-4-yl)methyl)-2-(2-methoxyphenoxy)ethan-1-ammonium hydrogen oxalate (**cis-6**)*

White solid (32% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.74-2.76 (m, 1H), 3.03-3.09 (m, 1H), 3.16-3.21 (m, 2H), 3.69 (dd, *J* = 4.8, 8.6 Hz, 1H), 3.75 (s, 3H), 3.94 (dd, *J* = 6.8, 8.6 Hz, 1H), 4.10-4.15 (m, 2H), 4.23 (d, *J* = 5.8 Hz, 1H), 4.34-4.39 (m, 1H), 5.62 (d, *J* = 5.8 Hz, 1H), 6.87 – 6.94 (m, 1H), 6.95 – 7.05 (m, 3H), 7.13 – 7.23 (m, 2H), 7.26-7.30 (m, 4H), 7.33 – 7.42 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 47.11, 50.12, 54.89, 55.73, 64.29, 67.52, 71.83, 106.38, 112.45, 114.09, 115.57, 120.30, 121.71, 122.93, 127.27, 128.82, 128.92, 129.00, 130.10, 140.40, 146.68, 148.66, 168.61. M.p. [203-205°C]. HRMS m/z $[M+H]^+$ Calcd. for $C_{26}H_{30}NO_4^+$: 420.5285. Found: 420.5283. El. Anal. Calcd. for $C_{28}H_{31}NO_8$: C 66.00, H 6.13, N 2.75. Found: C 66.00, H 6.10, N 2.73.

*4.1.1.7. Trans-2-(2-benzhydryl-1,3-dioxolan-4-yl)-N-(2-phenoxyethyl)ethan-1-ammonium hydrogen oxalate (**trans-7**)*

White solid (56% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.70 – 1.87 (m, 2H), 2.90-2.95 (m, 2H), 3.22-3.24 (m, 2H), 3.40 (dd, *J* = 6.2, 8.1 Hz, 1H), 3.91 (dd, *J* = 6.0, 8.1 Hz, 1H), 3.95 – 4.00 (m, 1H), 4.02 – 4.15 (m, 3H), 5.61 (d, *J* = 6.0 Hz, 1H), 6.86 – 6.94 (m, 3H), 7.10 (td, *J* = 1.5, 7.2 Hz, 2H), 7.14 – 7.32 (m, 10H). ^{13}C NMR (151 MHz, DMSO) δ 29.67, 44.75, 46.53, 55.18, 64.05, 69.56, 73.63, 105.08, 115.05, 121.67, 126.82, 128.60, 128.63, 129.23, 129.28, 130.05, 141.34, 158.23, 164.81. M.p. [172-174°C]. HRMS m/z $[M+H]^+$ Calcd. for $C_{26}H_{30}NO_3^+$: 404.2220. Found:

404.2220. El. Anal. Calcd. for $C_{28}H_{31}NO_7$: C 68.14, H 6.33, N 2.84. Found: C 68.14, H 6.30, N 2.85.

*4.1.1.8. Cis-2-(2-benzhydryl-1,3-dioxolan-4-yl)-N-(2-phenoxyethyl)ethan-1- ammonium hydrogen oxalate (**cis-7**)*

White solid (30% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.68-1.74 (m, 1H), 1.80-1.84 (m, 1H), 2.85 – 2.96 (m, 2H), 3.24 – 3.30 (m, 2H), 3.46 (dd, *J* = 5.8, 8.1 Hz, 1H), 3.91 (dd, *J* = 6.6, 8.0 Hz, 1H), 3.90-4.20 (m, 4H), 5.56 (d, *J* = 5.9 Hz, 1H), 6.97 – 7.02 (m, 3H), 7.18 – 7.22 (m, 2H), 7.28 - 7.35 (m, 10H). ^{13}C NMR (151 MHz, DMSO) δ 29.99, 44.48, 46.58, 55.37, 64.05, 69.00, 73.79, 105.58, 115.05, 121.70, 126.87, 128.58, 128.60, 129.28, 129.32, 130.06, 141.18, 141.21, 158.23, 164.73. M.p. [172-174°C]. HRMS m/z $[M+H]^+$ Calcd. for $C_{26}H_{30}NO_3^+$: 404.2220. Found: 404.2223. El. Anal. Calcd. for $C_{28}H_{31}NO_7$: C 68.14, H 6.33, N 2.84. Found: C 68.15, H 6.35, N 2.80.

*4.1.1.9. Trans-2-(2-benzhydryl-1,3-dioxolan-4-yl)-N-(2-(2-methoxyphenoxy)ethyl)ethan-1-ammonium hydrogen oxalate (**trans-8**)*

White solid (45% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.88-1.92 (m, 2H), 3.07-3.10 (m, 2H), 3.30-3.32 (m, 2H), 3.50 (dd, *J* = 6.2, 8.1 Hz, 1H), 3.74 (s, 3H), 4.00 (dd, *J* = 6.0, 8.1 Hz, 1H), 4.03 – 4.09 (m, 1H), 4.17-4.20 (m, 3H), 5.71 (d, *J* = 6.0 Hz, 1H), 6.91 (dd, *J* = 1.7, 7.6 Hz, 1H), 6.95 – 7.10 (m, 3H), 7.17 – 7.24 (m, 2H), 7.26-7.29 (m, 4H), 7.35-7.37 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 29.65, 44.80, 46.51, 55.16, 55.91, 65.65, 69.54, 73.65, 105.10, 112.82, 115.40, 121.19, 122.74, 126.82, 128.57, 128.60, 128.63, 129.24, 129.29, 129.33, 141.33, 147.57, 149.91, 164.81. M.p. [185-187°C]. HRMS m/z $[M+H]^+$ Calcd. for $C_{27}H_{32}NO_4^+$: 434.2326. Found: 434.2325. El. Anal. Calcd. for $C_{29}H_{33}NO_8$: C 66.53, H 6.35, N 2.68. Found: C 66.50, H 6.35, N 2.65.

*4.1.1.10. Cis-2-(2-benzhydryl-1,3-dioxolan-4-yl)-N-(2-(2-methoxyphenoxy)ethyl)ethan-1-ammonium hydrogen oxalate (**cis-8**)*

White solid (62% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.73-1.75 (m, 1H), 1.79 – 1.88 (m, 1H), 2.90 – 3.03 (m, 1H), 3.26 (t, *J* = 5.3 Hz, 2H), 3.45 (dd, *J* = 5.8, 8.1 Hz, 1H), 3.76 (s, 3H), 3.91 (dd, *J* = 6.6, 8.1 Hz, 1H), 4.06 – 4.26 (m, 4H), 5.57 (d, *J* = 5.9 Hz, 1H), 6.90-6.92 (m, 1H), 6.96 – 7.06 (m, 3H), 7.20 (dd, *J* = 1.8, 8.0 Hz, 2H), 7.26-7.30 (m, 4H), 7.33 – 7.42 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 29.90, 44.51, 46.49, 55.36, 55.93, 65.62, 68.99, 73.82, 105.59, 112.84, 115.42, 121.19, 122.74, 126.86, 128.57, 128.60, 129.29, 129.33, 141.18, 141.22, 147.59, 149.93, 164.87. M.p. [159-162°C]. HRMS m/z $[M+H]^+$ Calcd. for $C_{27}H_{32}NO_4^+$: 434.2326. Found: 434.2330. El. Anal. Calcd. for $C_{29}H_{33}NO_8$: C 66.53, H 6.35, N 2.68. Found: C 66.55, H 6.36, N 2.70.

*4.1.1.11. N-((2,2-diphenyl-1,3-dioxan-4-yl)methyl)-2-phenoxyethan-1- ammonium hydrogen oxalate (**9**)*

White solid (26% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.53 (d, *J* = 12.7 Hz, 1H), 1.75 (qd, *J* = 5.0, 12.4 Hz, 1H), 2.39 (t, *J* = 1.9 Hz, 1H), 3.17-3.21 (m, 3H), 3.89 (td, *J* = 2.6, 12.1 Hz, 1H), 4.07 (dd, *J* = 4.9, 11.7 Hz, 1H), 4.16-4.18 (m, 1H), 4.30-4.32 (m, 2H), 6.98-7.01 (m, 3H), 7.22-7.24 (m, 1H), 7.27-7.29 (m, 2H), 7.34-7.36 (m, 3H), 7.43-7.45 (m, 2H), 7.54-7.57 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 27.79, 47.00, 51.47, 60.76, 62.97, 66.96, 101.32, 114.95, 122.03, 125.26, 127.26, 128.24, 128.33, 128.54, 129.60, 130.16, 139.76, 144.72, 157.85, 165.84. M.p. [192-194°C]. HRMS m/z $[M+H]^+$ Calcd. for $C_{25}H_{28}NO_3^+$: 390.2064. Found: 390.2062. El. Anal. Calcd. for $C_{27}H_{29}NO_7$: C 67.63, H 6.10, N 2.92. Found: C 67.62, H 6.11, N 2.92.

*4.1.1.12. N-((2,2-diphenyl-1,3-dioxan-4-yl)methyl)-2-(2-methoxyphenoxy)ethan-1-ammonium hydrogen oxalate (**10**)*

White solid (30% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.65 (d, *J* = 12.7 Hz, 1H), 1.87 (qd, *J* = 5.0, 12.4 Hz, 1H), 3.31-3.34 (m, 4H), 3.76 (s, 3H), 3.89 (td, *J* = 2.6, 12.1 Hz, 1H), 4.02 (dd, *J* = 4.9, 11.7 Hz, 1H), 4.18-4.20 (m, 1H), 4.43 (s, 2H), 6.85 (td, *J* = 1.7, 7.6 Hz, 1H), 7.04 (m, 3H), 7.30 – 7.32 (m, 2H), 7.37-7.43 (m, 4H), 7.48 – 7.51 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 27.76, 47.16, 51.58, 55.86, 60.78, 64.28, 67.01, 101.35, 112.52, 114.31, 115.61, 121.63, 122.94, 125.20, 127.20, 128.33, 129.60, 130.05, 139.67, 144.62, 146.88, 148.95, 165.96. M.p. [125-128°C]. HRMS m/z $[M+H]^+$ Calcd. for $C_{26}H_{30}NO_4^+$: 420.2169. Found: 420.2165. El. Anal. Calcd. for $C_{28}H_{31}NO_8$: C 66.00, H 6.13, N 2.75. Found: C 66.02, H 6.11, N 2.76.

4.1.1.13. N-((2,2-diphenyl-1,3-dioxan-5-yl)methyl)-2-phenoxyethan-1- ammonium hydrogen oxalate ***(11)***

White solid (41% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.03-2.05 (m, 1H), 2.85-2.87 (m, 2H), 3.07-3.11 (m, 2H), 3.75-3.79 (m, 2H), 4.01 – 4.22 (m, 4H), 6.92-6.96 (m, 3H), 7.19 – 7.67 (m, 12H). ^{13}C NMR (151 MHz, DMSO) δ 33.25, 40.54, 47.99, 64.04, 65.87, 100.77, 114.99, 121.32, 126.41, 126.49, 128.15, 128.76, 128.86, 128.88, 129.98, 142.40, 142.74, 158.60, 164.69. M.p. [218-220°C]. HRMS m/z $[M+H]^+$ Calcd. for $C_{25}H_{28}NO_3^+$: 390.2064. Found: 390.2066. El. Anal. Calcd. for $C_{27}H_{29}NO_7$: C 67.63, H 6.10, N 2.92. Found: C 67.65, H 6.12, N 2.90.

*4.1.1.14. N-((2,2-diphenyl-1,3-dioxan-5-yl)methyl)-2-(2-methoxyphenoxy)ethan-1-ammonium hydrogen oxalate (****12****)*

White solid (57% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.16-2.19 (m, 1H), 3.16 (d, *J* = 6.6 Hz, 2H), 3.33 (m, 2H), 3.72 (s, 3H), 3.83 (dd, *J* = 5.6, 11.8 Hz, 2H), 4.09 (dd, *J* = 3.5, 11.8 Hz, 2H), 4.20 (t, *J* = 5.1 Hz, 2H), 6.89 (td, *J* = 1.7, 7.6 Hz, 1H), 6.97 (d, *J* = 1.4 Hz, 1H), 6.99 – 7.04 (m, 2H), 7.28 (dd, *J* = 7.3, 14.3 Hz, 2H), 7.37 (dt, *J* = 7.7, 21.9 Hz, 4H), 7.43 – 7.51 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 32.10, 47.12, 47.36, 55.84, 63.65, 65.61, 100.85, 112.75, 115.16, 121.16, 122.61,

126.30, 126.57, 128.22, 128.25, 128.85, 128.98, 141.95, 142.76, 147.59, 149.83, 164.59. M.p. [196-200°C]. HRMS m/z $[M+H]^+$ Calcd. for $C_{26}H_{30}NO_4^+$: 420.2169. Found: 420.2170. El. Anal. Calcd. for $C_{28}H_{31}NO_8$: C 66.00, H 6.13, N 2.75. Found: C 66.00, H 6.15, N 2.77.

*4.1.1.15. 2-(benzhydryloxy)-3-hydroxy-N-(2-phenoxyethyl)propan-1- ammonium oxalate (**13**)*

White solid (54% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.10 – 3.29 (m, 4H), 3.58 (t, J = 4.9 Hz, 2H), 3.64-3.66 (m, 1H), 4.17 (t, J = 5.3 Hz, 2H), 5.78 (s, 1H), 6.89 – 7.04 (m, 3H), 7.20 – 7.28 (m, 2H), 7.31-7.34 (m, 6H), 7.38 – 7.50 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 47.26, 49.37, 61.00, 64.51, 73.78, 80.74, 115.02, 121.55, 127.17, 127.56, 127.68, 127.95, 128.61, 128.86, 130.03, 142.59, 143.20, 158.37, 164.77. M.p. [190-193°C]. HRMS m/z $[M+H]^+$ Calcd. for $C_{24}H_{28}NO_3^+$: 378.2064. Found: 378.2062. El. Anal. Calcd. for $C_{26}H_{29}NO_7$: C 66.80, H 6.25, N 3.00. Found: C 66.80, H 6.28, N 3.05.

*4.1.1.16. 1-(benzhydryloxy)-2-hydroxy-N-(2-(2-methoxyphenoxy)ethyl)ethan-1-ammonium hydrogen oxalate (**14**)*

White solid (45% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.25-3.28 (m, 4H), 3.60 (dd, J = 4.6, 13.5 Hz, 2H), 3.68 (t, J = 5.1 Hz, 1H), 3.74 (s, 3H), 4.17-4.19 (m, 2H), 5.79 (s, 1H), 6.91– 7.06 (m, 4H), 7.25-7.27 (m, 2H), 7.29 – 7.38 (m, 4H), 7.44-7.47 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 46.99, 48.79, 55.73, 60.55, 64.07, 72.48, 81.47, 112.40, 114.12, 121.65, 122.98, 126.62, 127.30, 127.97, 128.38, 128.78, 129.05, 141.68, 142.37, 146.66, 148.75, 166.08. M.p. [151-153°C]. HRMS m/z $[M+H]^+$ Calcd. for $C_{24}H_{28}NO_4^+$: 394.2013. Found: 394.2015. El. Anal. Calcd. for $C_{26}H_{29}NO_8$: C 64.59, H 6.05, N 2.90. Found: C 64.60, H 6.04, N 2.94.

*4.1.1.17. 2-(benzhydryloxy)-N-(2-phenoxyethyl)ethan-1- ammonium hydrogen oxalate (**15**)*

White solid (55% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.27 (t, J = 5.4 Hz, 2H), 3.37 (t, J = 5.2 Hz, 2H), 3.66 (t, J = 5.3 Hz, 2H), 4.25 (t, J = 5.2 Hz, 2H), 5.54 (s, 1H), 6.95 – 7.04 (m, 3H), 7.20 –

7.30 (m, 2H), 7.30 – 7.39 (m, 6H), 7.40 – 7.45 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 46.68, 47.26, 63.93, 64.53, 83.35, 115.06, 121.65, 127.10, 127.90, 128.84, 130.05, 142.45, 158.24, 164.71. M.p.[205-207°C]. HRMS m/z $[M+H]^+$ Calcd. for $C_{23}H_{26}NO_2^+$: 348.1958. Found: 348.1960. El. Anal. Calcd. for $C_{25}H_{27}NO_6$: C 68.64, H 6.22, N 3.20. Found: C 68.65, H 6.22, N 3.25.

4.1.1.18. 2-(benzhydryloxy)-N-(2-(2-methoxyphenoxy)ethyl)ethan-1- ammonium hydrogen oxalate ***(16)***

White solid (30% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.30-3.35 (m, 4H), 3.65 (t, *J* = 5.2 Hz, 2H), 3.75 (s, 3H), 4.20-4.23 (m, 2H), 5.54 (s, 1H), 6.91 (d, *J* = 1.7 Hz, 1H), 6.96 – 7.10 (m, 3H), 7.21 – 7.32 (m, 2H), 7.33-7.36 (m, 4H), 7.39 – 7.46 (m, 4H). ^{13}C NMR (151 MHz, DMSO) δ 46.78, 47.27, 55.84, 64.01, 64.56, 83.64, 115.61, 121.61, 122.90, 126.80, 128.09, 128.95, 130.04, 142.06, 146.90, 148.93, 166.67. M.p. [194-196°C]. HRMS m/z $[M+H]^+$ Calcd. for $C_{24}H_{28}NO_3^+$: 378.2064. Found: 378.2065. El. Anal. Calcd. for $C_{26}H_{29}NO_7$: C 66.80, H 6.25, N 3.00. Found: C 66.83, H 6.22, N 3.01.

4*.1.2. General procedure for the synthesis of amines* ***17-30***

To a solution of 2-phenoxy-ethylamine (5 eq.) or 2-(2-methoxyphenoxy-)ethylamine (5 eq.) in 2-methoxyethanol (25 mL per mmol of amine) the appropriate aliphatic chloride **33-39** (1 eq.) and KI (cat.) was added. The mixture was refluxed for 18-48 h and concentrated. The residue was suspended in $CHCl_3$ and washed with 1M NaOH, brine, dried over anhydrous Na_2SO_4 and concentrated. The crude was purified by flash chromatography to give the titled compound.

4.1.2.1. 2-(2,2-diphenyl-1,3-dioxolan-4-yl)-N-(2-phenoxyethyl)ethan-1-amine ***(17)***

Pale yellow liquid (74% yield). TLC (cicloexane/EtOAc 3:7): Rf = 0.38. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 1.71-1.76 (m, 1H), 1.83-1.89 (m, 1H), 1.98 (bs, 1H), 2.73-2.77 (m, 1H), 2.80-2.84 (m, 1H), 2.92 (t, *J* = 5.1 Hz, 2H), 3.63 (t, *J* = 7.5 Hz, 1H), 3.97 (t, *J* = 5.1, 2H), 4.05 (t, *J* = 7.2 Hz,

1H), 4.16-4.20 (m, 1H), 6.77 – 6.94 (m, 3H), 7.13 – 7.28 (m, 8H), 7.36 – 7.49 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 33.65, 46.64, 48.85, 67.06, 70.02, 75.60, 114.54, 120.89, 126.20, 127.96, 128.05, 128.16, 129.48, 142.64, 158.78. MS (ESI): *m*/*z* $[M + H]^+$: 389.2.

*4.1.2.2. 2-(2,2-diphenyl-1,3-dioxolan-4-yl)-N-(2-(2-methoxyphenoxy)ethyl)ethan-1-amine (**18**)*

Pale yellow liquid (55% yield). TLC (EtOAc/MeOH 95:5): Rf = 0.27. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 1.90-1.95 (m, 1H), 2.01-2.03 (m, 1H), 2.87 – 3.03 (m, 2H), 3.10 (dd, *J* = 4.5, 6.0 Hz, 2H), 3.76 (dd, *J* = 4.9, 7.5 Hz, 1H), 3.81 (s, 3H), 4.10 – 4.20 (m, 3H), 4.30 (m, 1H), 6.88 - 6.98 (m, 4H), 7.25 – 7.35 (m, 5H), 7.48 – 7.55 (m, 5H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 33.09, 46.48, 48.62, 55.78, 68.23, 69.90, 75.45, 109.71, 111.88, 114.83, 120.95, 122.01, 126.10, 126.17, 127.98, 128.07, 128.15, 130.07, 132.42, 142.47, 147.98, 149.81. MS (ESI): *m*/*z* $[M + H]^+$: 419.2.

*4.1.2.3. Trans-N-((2-benzhydryl-1,3-dioxolan-4-yl)methyl)-2-phenoxyethan-1-amine (**trans-19**)*

Pale yellow liquid (66% yield). TLC (cicloexane/EtOAc 3:7): Rf = 0.58. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 2.72 (dd, *J* = 4.3, 12.4 Hz, 1H), 2.78 (dd, *J* = 7.3, 12.4 Hz, 1H), 2.98 (t, *J* = 5.1 Hz, 2H), 3.51 (dd, *J* = 6.6, 8.1 Hz, 1H), 3.84 (dd, *J* = 6.2, 8.1 Hz, 1H), 4.00-4.02 (m, 3H), 4.14 (d, *J* = 4.5 Hz, 1H), 5.61 (d, *J* = 4.6 Hz, 1H), 6.79 – 6.84 (m, 2H), 6.88 (t, *J* = 7.4 Hz, 1H), 7.14 (t, *J* = 7.3 Hz, 2H), 7.17 – 7.30 (m, 10H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 48.63, 51.15, 55.54, 66.56, 68.54, 75.27, 105.65, 114.55, 121.04, 126.64, 126.68, 128.26, 128.28, 129.25, 129.29, 129.50, 140.10, 140.16, 158.57. MS (ESI): *m*/*z* $[M + H]^+$: 389.2.

*4.1.2.4. Cis-N-((2-benzhydryl-1,3-dioxolan-4-yl)methyl)-2-phenoxyethan-1-amine (**cis-19**)*

Pale yellow liquid (54% yield). TLC (cicloexane/EtOAc 1:9): Rf = 0.40. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 2.30 (dd, *J* = 7.7, 12.0 Hz, 1H), 2.63 (dd, *J* = 4.0, 12.0 Hz, 1H), 2.87-2.94 (m, 2H), 3.62 (dd, *J* = 4.8, 8.2 Hz, 1H), 3.95 (dd, *J* = 6.7, 8.1 Hz, 1H), 4.00 – 4.12 (m, 2H), 4.29 – 4.37 (m, 2H), 5.58 (d, *J* = 3.7 Hz, 1H), 6.93 (d, *J* = 12.0 Hz 2H), 6.99 (t, *J* = 12.0 Hz, 1H), 7.19 – 7.42 (m,

12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 48.56, 51.86, 55.01, 68.04, 68.17, 75.11, 105.81, 114.50, 121.02, 126.67, 126.72, 128.18, 128.23, 129.46, 129.52, 129.58, 139.80, 139.93, 158.59. MS (ESI): *m*/*z* $[M + H]^+$: 389.2.

*4.1.2.5. Trans-N-((2-benzhydryl-1,3-dioxolan-4-yl)methyl)-2-(2-methoxyphenoxy)ethan-1-amine (**trans-20**)*

Pale yellow liquid (20% yield). TLC (cicloexane/EtOAc 4:6): Rf = 0.16. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 2.78 (dd, *J* = 4.6, 12.3 Hz, 1H), 2.89 (dd, *J* = 7.0, 12.3 Hz, 1H), 3.06 (t, *J* = 5.4 Hz, 2H), 3.63 (dd, *J* = 6.7, 8.0 Hz, 1H), 3.83 (s, 3H), 4.07 (dd, *J* = 4.6, 6.6 Hz, 1H), 4.13-4.15 (m, 1H), 4.21 (t, *J* = 6.1 Hz, 2H), 4.24 (d, *J* = 4.5 Hz, 1H), 5.71 (d, *J* = 4.4 Hz, 1H), 6.89 – 6.98 (m, 4H), 7.20 – 7.26 (m, 2H), 7.27 – 7.33 (m, 4H), 7.36 (d, *J* = 4.2 Hz, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 48.86, 51.49, 55.60, 55.85, 68.66, 68.84, 75.78, 105.61, 111.98, 114.46, 120.92, 121.71, 126.59, 126.62, 128.22, 128.25, 129.27, 129.32, 140.21, 140.24, 148.26, 149.82. MS (ESI): *m*/*z* $[M + H]^+$: 419.2.

*4.1.2.6. Cis-N-((2-benzhydryl-1,3-dioxolan-4-yl)methyl)-2-(2-methoxyphenoxy)ethan-1-amine (**cis-20**)*

Pale yellow liquid (39% yield). TLC (cicloexane/EtOAc 1:9): Rf = 0.15. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 2.36 (ddd, *J* = 1.7, 7.3, 12.1 Hz, 1H), 2.56 (ddd, *J* = 1.7, 4.3, 12.0 Hz, 1H), 2.88 – 2.95 (m, 2H), 3.58 (ddd, *J* = 1.7, 5.2, 8.2 Hz, 1H), 3.82 (s, 3H), 3.94 (ddd, *J* = 1.7, 6.6, 8.3 Hz, 1H), 4.07 (td, *J* = 1.6, 5.5 Hz, 2H), 4.22 – 4.30 (m, 1H), 4.32 (d, *J* = 2.4 Hz, 1H), 5.59 (d, *J* = 2.4 Hz, 1H), 6.87 – 7.00 (m, 4H), 7.20-7.23 (m, 2H), 7.27 – 7.34 (m, 4H), 7.38 (d, *J* = 1.7 Hz, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 48.76, 52.02, 55.13, 55.76, 68.11, 68.54, 75.82, 105.69, 111.85, 113.93, 120.85, 121.49, 126.60, 126.63, 128.14, 128.17, 129.53, 129.55, 139.98, 140.03, 148.33, 149.72. MS (ESI): *m*/*z* $[M + H]^+$: 419.2.

*4.1.2.7. Trans-2-(2-benzhydryl-1,3-dioxolan-4-yl)-N-(2-phenoxyethyl)ethan-1-amine (**trans-21**)*

Pale yellow liquid (45% yield). TLC (cicloexane/EtOAc 1:9): Rf = 0.33. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 1.58-1.63 (m, 1H), 1.74 – 1.83 (m, 1H), 2.66 – 2.78 (m, 2H), 2.93 (t, *J* = 5.2 Hz, 2H), 3.38 (t, *J* = 7.5 Hz, 1H), 3.76 – 3.82 (m, 1H), 3.87 (dd, *J* = 5.9, 8.0 Hz, 1H), 3.98 (t, *J* = 5.1 Hz, 2H), 4.09 – 4.14 (m, 1H), 5.59 (d, *J* = 4.5 Hz, 1H), 6.81 (d, *J* = 8.0 Hz, 2H), 6.88 (t, *J* = 7.3 Hz, 1H), 7.10 – 7.15 (m, 2H), 7.16 – 7.27 (m, 10H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 38.74, 46.67, 48.71, 55.60, 68.17, 70.48, 75.26, 105.18, 114.53, 120.96, 126.58, 126.64, 128.20, 128.25, 129.27, 129.29, 129.48, 140.22, 140.25, 158.68. MS (ESI): *m*/*z* $[M + H]^+$: 403.2.

*4.1.2.8. Cis-2-(2-benzhydryl-1,3-dioxolan-4-yl)-N-(2-phenoxyethyl)ethan-1-amine (**cis-21**)*

Pale yellow liquid (34% yield). TLC (cicloexane/EtOAc 1:9): Rf = 0.22. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 1.48-1.52 (m, 1H), 1.59-1.63 (m, 1H), 2.64-2.66 (m, 2H), 2.89-2.91 (m, 2H), 3.26 (dd, *J* = 6.4, 7.7 Hz, 1H), 3.85 (dd, *J* = 6.5, 7.7 Hz, 1H), 3.96-3.99 (m, 3H), 4.19 (d, *J* = 4.5 Hz, 1H), 5.48 (d, *J* = 4.3 Hz, 1H), 6.82 (d, *J* = 7.9 Hz, 2H), 6.89 (t, *J* = 7.3 Hz, 1H), 7.09 – 7.15 (m, 2H), 7.17 – 7.30 (m, 10H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 38.74, 46.25, 48.51, 55.39, 68.17, 69.78, 75.33, 105.74, 114.54, 121.05, 126.63, 126.69, 128.17, 128.20, 129.38, 129.43, 129.51, 139.99, 140.18, 158.58. MS (ESI): *m*/*z* $[M + H]^+$: 403.2.

*4.1.2.10. Trans-2-(2-benzhydryl-1,3-dioxolan-4-yl)-N-(2-(2-methoxyphenoxy)ethyl)ethan-1-amine (**trans-22**)*

Pale yellow liquid (64% yield). TLC (EtOAc/MeOH 9:1): Rf = 0.32. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 2.01 (dt, *J* = 6.8, 14.3 Hz, 1H), 2.11 (dt, *J* = 7.1, 14.3 Hz, 1H), 3.04 – 3.08 (m, 2H), 3.19 (dd, *J* = 4.5, 6.0 Hz, 2H), 3.85 (t, *J* = 7.5 Hz, 1H), 3.91 (s, 3H), 4.24 – 4.26 (m, 3H), 4.27 (d, *J* = 4.5 Hz, 1H), 4.28-4.33 (m, 1H), 5.78 (d, *J* = 4.4 Hz, 1H), 6.98 – 7.01 (m, 4H), 7.31 – 7.33 (m, 2H), 7.36 – 7.40 (m, 4H), 7.45 (d, *J* = 4.2 Hz, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 33.45, 46.83,

48.97 56.13, 68.58, 70.25, 75.80, 105.10, 111.48, 113.95, 120.41, 121.21, 126.12, 127.70, 127.74, 128.76, 128.82, 139.70, 139.74, 147.75, 149.31. MS (ESI): *m*/*z* $[M + H]^+$: 433.2.

*4.1.2.11. Cis-2-(2-benzhydryl-1,3-dioxolan-4-yl)-N-(2-(2-methoxyphenoxy)ethyl)ethan-1-amine (**cis-22**)*

Pale yellow liquid (72% yield). TLC (cicloexane/EtOAc 5:95): Rf = 0.23. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 1.57 – 1.62 (m, 2H), 2.69-2.74 (m, 2H), 3.01 (dd, *J* = 4.8, 5.9 Hz, 2H), 3.33 (dd, *J* = 6.6, 7.7 Hz, 1H), 3.87 (s, 3H), 3.94 (dd, *J* = 6.4, 7.7 Hz, 1H), 4.12 (dd, *J* = 4.8, 5.9 Hz, 2H), 4.14 – 4.19 (m, 1H), 4.28 (d, *J* = 4.3 Hz, 1H), 5.58 (d, *J* = 4.3 Hz, 1H), 6.87 – 7.02 (m, 4H), 7.19 – 7.26 (m, 2H), 7.27 – 7.34 (m, 4H), 7.34 – 7.39 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 33.09, 46.23, 48.68, 55.45, 55.82, 68.52, 69.83, 75.33, 105.64, 111.87, 114.26, 120.92, 121.67, 126.59, 126.64, 128.13, 128.15, 129.40, 129.46, 140.06, 140.24, 148.22, 149.71. MS (ESI): *m*/*z* $[M + H]^+$: 433.2.

*4.1.2.12. N-((2,2-diphenyl-1,3-dioxan-4-yl)methyl)-2-phenoxyethan-1-amine (**23**)*

Pale yellow liquid (20% yield). TLC (EtOAc/MeOH 9:1): Rf = 0.17. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 1.43 (dd, *J* = 2.1, 13.1 Hz, 1H), 1.94 (qd, *J* = 5.3, 12.3 Hz, 1H), 2.89 (dd, *J* = 3.4, 12.5 Hz, 1H), 3.02 (dd, *J* = 8.2, 12.4 Hz, 1H), 3.13 (dt, *J* = 5.1, 12.5 Hz, 1H), 3.22 (dt, *J* = 5.1, 12.6 Hz, 1H), 3.99 – 4.13 (m, 2H), 4.18 – 4.27 (m, 3H), 6.91 – 6.95 (m, 2H), 6.96 – 6.99 (m, 1H), 7.18 – 7.23 (m, 1H), 7.25 – 7.31 (m, 5H), 7.39 (t, *J* = 7.8 Hz, 2H), 7.49 – 7.53 (m, 2H), 7.55 – 7.58 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 28.86, 48.62, 54.29, 61.15, 66.46, 68.98, 101.40, 114.54, 121.13, 125.22, 127.48, 127.78, 127.85, 128.03, 129.01, 129.56, 139.77, 144.77, 158.59. MS (ESI): *m*/*z* $[M + H]^+$: 389.2.

*4.1.2.13. N-((2,2-diphenyl-1,3-dioxan-4-yl)methyl)-2-(2-methoxyphenoxy)ethan-1-amine (**24**)*

Pale yellow liquid (31% yield). TLC (EtOAc): Rf = 0.18. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 1.34 (dd, *J* = 2.2, 12.8 Hz, 1H), 1.85-1.88 (m, 1H), 2.74 (dd, *J* = 3.5, 12.4 Hz, 1H), 2.94 (dd, *J* =

8.1, 12.4 Hz, 1H), 2.99 – 3.06 (m, 1H), 3.06 – 3.14 (m, 1H), 3.67 (s, 3H), 3.92 – 4.07 (m, 2H), 4.13-4.15 (m, 3H), 6.82-6.85 (m, 3H), 6.88-6.89 (m, 2H), 7.08 – 7.12 (m, 1H), 7.13 – 7.21 (m, 2H), 7.30 (s, 2H), 7.41 – 7.46 (m, 2H), 7.47 – 7.52 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 28.96, 48.81, 54.70, 55.81, 61.31, 68.74, 69.48, 101.29, 111.97, 114.40, 120.89, 121.71, 125.27, 127.55, 127.65, 127.72, 127.95, 128.92, 140.02, 144.96, 148.32, 149.90. MS (ESI): *m*/*z* $[M + H]^+$: 419.2.

*4.1.2.14. N-((2,2-diphenyl-1,3-dioxan-5-yl)methyl)-2-phenoxyethan-1-amine (**25**)*

Pale yellow liquid (31% yield). TLC (cicloexane/EtOAc 1:9): Rf = 0.23. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 1.96 (dt, *J* = 3.4, 7.0 Hz, 1H), 2.67 (d, *J* = 7.1 Hz, 2H), 2.91 (t, *J* = 5.1 Hz, 2H), 3.74 (dd, *J* = 7.0, 11.4 Hz, 2H), 3.98 (t, *J* = 5.1 Hz, 2H), 4.06 (dd, *J* = 4.0, 11.5 Hz, 2H), 6.80 – 6.84 (m, 2H), 6.87 (d, *J* = 1.0 Hz, 1H), 7.16 – 7.22 (m, 4H), 7.24 – 7.28 (m, 4H), 7.41 – 7.47 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 34.82, 48.80, 48.97, 64.64, 67.07, 101.22, 114.52, 120.89, 126.38, 126.65, 127.80, 128.35, 128.47, 129.60, 129.64, 141.77, 142.35, 158.76. MS (ESI): *m*/*z* $[M + H]^+$: 389.2.

*4.1.2.15. N-((2,2-diphenyl-1,3-dioxan-5-yl)methyl)-2-(2-methoxyphenoxy)ethan-1-amine (**26**)*

Pale yellow liquid (36% yield). TLC (cicloexane/EtOAc 2:8): Rf = 0.28. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 1.94 – 2.02 (m, 1H), 2.62 (d, *J* = 7.1 Hz, 2H), 2.91 (t, *J* = 5.2 Hz, 2H), 3.72 (dd, *J* = 7.3, 11.5 Hz, 2H), 3.76 (s, 3H), 4.02 (t, *J* = 5.2 Hz, 2H), 4.05 (d, *J* = 4.0 Hz, 1H), 4.06 (d, *J* = 4.1 Hz, 1H), 6.78 – 6.92 (m, 4H), 7.13 – 7.20 (m, 2H), 7.22 – 7.30 (m, 4H), 7.39 – 7.48 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 34.91, 48.79, 48.95, 55.83, 64.72, 68.81, 101.19, 111.89, 114.27, 120.89, 121.60, 126.32, 126.74, 127.78, 127.79, 128.32, 128.48, 141.68, 142.59, 148.30, 149.77. MS (ESI): *m*/*z* $[M + H]^+$: 419.2.

*4.1.2.16. 2-(benzhydryloxy)-N-(2-phenoxyethyl)ethan-1-amine (**29**)*

Pale yellow liquid (40% yield). TLC (EtOAc 100%): Rf = 0.20. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 3.12 (t, *J* = 5.2 Hz, 2H), 3.23 (t, *J* = 5.1 Hz, 2H), 3.75 (t, *J* = 5.2 Hz, 2H), 4.22 (t, *J* = 5.1 Hz, 2H), 5.45 (s, 1H), 6.87 – 6.94 (m, 2H), 6.97 – 7.04 (m, 1H), 7.22 – 7.40 (m, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 47.98, 48.59, 65.36, 66.31, 84.22, 114.59, 121.35, 126.97, 127.70, 128.83, 129.56, 141.56, 158.17. MS (ESI): *m*/*z* $[M + H]^+$: 347.2.

*4.1.2.17. 2-(benzhydryloxy)-N-(2-(2-methoxyphenoxy)ethyl)ethan-1-amine (**30**)*

Pale yellow liquid (20% yield). TLC (EtOAc 100%): Rf = 0.16. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 3.22 (t, *J* = 5.2 Hz, 2H), 3.33 (t, *J* = 5.1 Hz, 2H), 3.53 (s, 3H), 3.84 (t, *J* = 5.2 Hz, 2H), 4.32 (t, *J* = 5.1 Hz, 2H), 5.55 (s, 1H), 6.83-6.86 (m, 3H), 6.92 – 6.94 (m, 1H), 7.22 – 7.31 (m, 10H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 48.74, 49.35, 56.53, 66.12, 67.08, 84.98, 112.71, 115.70, 121.70, 122.91, 127.68, 127.96, 128.32, 128.50, 129.17, 129.31, 142.65, 142.98, 148.69, 150.66. MS (ESI): *m*/*z* $[M + H]^+$: 377.2.

*4.1.2.18. 2-(benzhydryloxy)-3-((tert-butyldiphenylsilyl)oxy)-N-(2-phenoxyethyl)propan-1-amine (**31**)*

Colorless liquid (77% yield). ^{1}H NMR (200 MHz, Chloroform-*d*) δ 1.06 (s, 9H), 2.71 – 2.87 (m, 3H), 3.01 (dd, J = 7.0, 12.3 Hz, 1H), 3.31 – 3.42 (m, 1H), 3.45-3.48 (m, 1H), 3.77 (dd, J = 6.9, 12.4 Hz, 1H), 3.95 – 4.12 (m, 2H), 5.25 (s, 1H), 6.90-6.92 (m, 3H), 7.16 – 7.57 (m, 18H), 7.67-7.70 (m, 4H). MS (ESI): *m*/*z* $[M + H]^+$: 615.3.

*4.1.2.19. 2-(benzhydryloxy)-3-((tert-butyldiphenylsilyl)oxy)-N-(2-(2-methoxyphenoxy)ethyl)propan-1-amine (**32**)*

Colorless liquid (40% yield). ^{1}H NMR (200 MHz, Chloroform-*d*) δ 1.06 (s, 9H), 2.69 – 3.31 (m, 5H), 3.39 – 3.68 (m, 1H), 3.77 (dd, *J* = 6.9, 12.4 Hz, 1H), 3.88 (s, 3H), 4.03 (dd, *J* = 7.0, 12.3 Hz,

1H), 4.18 – 4.38 (m, 1H), 5.21 (s, 1H), 6.79 – 7.00 (m, 4H), 7.17 – 7.52 (m, 16H), 7.62 – 7.76 (m, 4H). MS (ESI): *m*/*z* $[M + H]^+$: 645.3.

*4.1.3. General procedure for the synthesis of amines **27** and **28***

TBAF (1.2 eq.) was added drop-wise to a solution of **31** or **32** (1 eq.) in THF (10 mL). The mixture was stirred at room temperature for 24 h, diluted with water and extracted with EtOAc. The organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude was purified by flash column chromatography to afford the title compounds.

*4.1.3.1. 2-(benzhydryloxy)-3-((2-phenoxyethyl)amino)propan-1-ol (**27**)*

Pale yellow liquid (57% yield). TLC (EtOAc/MeOH 9:1): Rf = 0.28. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 2.89 – 3.00 (m, 4H), 3.59 (dd, *J* = 3.7, 5.0 Hz, 1H), 3.70 (dd, *J* = 3.6, 11.6 Hz, 1H), 3.79 (dd, *J* = 5.1, 11.6 Hz, 1H), 3.97 (t, *J* = 5.1 Hz, 2H), 5.52 (s, 1H), 6.75 – 6.81 (m, 2H), 6.86 – 6.92 (m, 1H), 7.14 – 7.31 (m, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 48.77, 51.72, 64.34, 66.21, 74.63, 81.85, 114.52, 121.12, 126.95, 127.17, 127.62, 127.76, 128.46, 128.57, 129.53, 141.87, 142.22, 158.46. MS (ESI): *m*/*z* $[M + H]^+$: 377.2.

*4.1.3.2. 2-(benzhydryloxy)-3-((2-(2-methoxyphenoxy)ethyl)amino)propan-1-ol (**28**)*

Pale yellow liquid (57% yield). TLC (EtOAc/MeOH 98:2): Rf = 0.22. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 2.94-2.99 (m, 4H), 3.59 (t, *J* = 3.7 Hz, 1H), 3.69 (s, 3H), 3.74 (dd, *J* = 2.4, 4.0 Hz, 2H), 3.79 – 3.85 (m, 1H), 3.98 – 4.08 (m, 2H), 5.52 (s, 1H), 6.83 (s, 3H), 6.87 – 6.92 (m, 1H), 7.19 – 7.31 (m, 10H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 48.89, 51.82, 55.76, 64.29, 68.23, 74.47, 81.88, 111.95, 114.94, 120.93, 122.14, 126.92, 127.20, 127.55, 127.74, 128.41, 128.55, 141.89, 142.22, 147.93, 149.90. MS (ESI): *m*/*z* $[M + H]^+$: 407.2.

*4.1.4. General procedure for the synthesis of aliphatic chlorides **35-37***

To a solution of alcohol 41-43 (1 eq.) in dry toluene at room temperature and under nitrogen atmosphere, pyridine (2 eq.) and thionyl chloride (1.5 eq.) were added. The mixture was refluxed for 45-60 min. The solvent was removed under reduced pressure and the residue solubilized in EtOAc. The organic phase was washed with $NaHCO_3$ sat. sol., brine, dried over anhydrous Na_2SO_4 and concentrated. The crude was purified by flash column chromatography to afford the titled compound.

*4.1.4.1. Trans-2-benzhydryl-4-(2-chloroethyl)-1,3-dioxolane (**trans-35**)*

Colorless liquid. ^{1}H NMR (200 MHz, Chloroform-*d*) δ 1.75 – 2.21 (m, 2H), 3.41 – 3.55 (m, 1H), 3.63-3.66 (m, 2H), 3.87 – 4.10 (m, 2H), 4.24 (d, *J* = 4.3 Hz, 1H), 5.68 (d, *J* = 4.3 Hz, 1H), 7.15 – 7.52 (m, 10H). MS (ESI): *m*/*z* $[M + H]^+$: 302.1.

*4.1.4.2. Cis-2-benzhydryl-4-(2-chloroethyl)-1,3-dioxolane (**cis-35**)*

Colorless liquid. ^{1}H NMR (200 MHz, Chloroform-*d*) δ 1.51 – 1.79 (m, 2H), 3.32 – 3.57 (m, 3H), 3.96-4.01 (dd, *J* = 6.5, 7.9 Hz, 1H), 4.18 – 4.40 (m, 2H), 5.57 (d, *J* = 3.8 Hz, 1H), 7.18 – 7.44 (m, 10H). MS (ESI): *m*/*z* $[M + H]^+$: 302.1.

*4.1.4.3. 4-(chloromethyl)-2,2-diphenyl-1,3-dioxane (**36**)*

Colorless liquid (88% yield). ^{1}H NMR (200 MHz, Chloroform-*d*) δ 1.46 – 1.68 (m, 1H), 1.76 – 2.07 (m, 1H), 3.56 (dd, *J* = 5.0, 11.2 Hz, 1H), 3.72 (dd, *J* = 6.7, 11.2 Hz, 1H), 3.99 – 4.26 (m, 3H), 7.11 – 7.67 (m, 10H). MS (ESI): *m*/*z* $[M + H]^+$: 288.1.

*4.1.4.4. 5-(chloromethyl)-2,2-diphenyl-1,3-dioxane (**37**)*

Colorless liquid (77% yield). ^{1}H NMR (200 MHz, Chloroform-*d*) δ 2.00 – 2.14 (m, 1H), 3.73 (d, *J* = 7.3 Hz, 2H), 3.94 (dd, *J* = 5.0, 11.7 Hz, 2H), 4.18 (dd, *J* = 3.6, 11.7 Hz, 2H), 7.19 – 7.45 (m, 5H), 7.45 – 7.60 (m, 4H). MS (ESI): *m*/*z* $[M + H]^+$: 288.1.

*4.1.5. General procedure for the synthesis of alcohols **40** and **42***

To a stirring solution of benzophenone (1 eq.) in anhydrous toluene, at room temperature and under nitrogen atmosphere, 1,2,4-butantriol (2 eq.) and pTSA (cat.) were added. The mixture was refluxed for 24 h, using Dean-Stark trap to remove the forming water. The mixture was then cooled at room temperature, and diluted with Et_2O. The organic phase was washed with $NaHCO_3$ saturated solution, brine, dried over anhydrous Na_2SO_4 and concentrated. The crude was purified by column chromatography (cyclohexane:EtOAc 85:15) to give alcohols 40 and 42.

*4.1.5.1. 2-(2,2-diphenyl-1,3-dioxolan-4-yl)ethan-1-ol (**40**)*

Colorless liquid (65% yield). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.56 – 1.90 (m, 2H), 3.39 – 3.75 (m, 2H), 3.97 – 4.28 (m, 2H), 4.48 (t, *J* = 5.1 Hz, 1H), 7.16 – 7.53 (m, 10H). MS (ESI): *m*/*z* [M + H]$^+$: 270.1.

*4.1.5.2. (2,2-diphenyl-1,3-dioxan-4-yl)methanol (**42**)*

Colorless liquid (5% yield). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.60-1.65 (m, 2H), 3.38 – 3.63 (m, 2H), 3.81 – 4.12 (m, 2H), 4.70 – 4.86 (m, 1H), 7.01 – 7.63 (m, 10H). MS (ESI): *m*/*z* [M + H]$^+$: 270.1.

*4.1.6. General procedure for the synthesis of alcohols **41** and **43***

To a stirred solution of the appropriate acetals (1 eq.) in anhydrous acetonitrile (10 mL), 1,2,4-butantriol (2 eq., for **41**) or 2-(hydroxymethyl)propane-1,3-diol (2 eq., for **43**), TMSCl (1 eq.) and$CoCl_2$ (0.6 eq.) were added. The mixture was stirred at room temperature for 17-24 h and concentrated. The residue was solubilized in DCM and the organic phase was washed with Na2CO3 saturated solution, brine, dried over anhydrous Na_2SO_4 and concentrated. The crude was purified by column chromatography (cyclohexane:EtOAc 8:2) to give the desired product.

*4.1.6.1. 2-(2-benzhydryl-1,3-dioxolan-4-yl)ethan-1-ol (**41**)*

Colorless liquid (quantitative yield). ^{1}H NMR (200 MHz, Chloroform-*d*) δ 1.60 – 1.94 (m, 1H), 2.33 – 2.76 (m, 1H), 3.40 – 4.01 (m, 4H), 4.04-4.17. (m, 1H), 4.48 – 4.68 (m, 1H), 5.69 (d, *J* = 7.0 Hz, 1H), 6.88 – 7.75 (m, 10H). MS (ESI): *m*/*z* [M + H]$^+$: 284.2.

*4.1.6.2. (2,2-diphenyl-1,3-dioxan-5-yl)methanol (**43**)*

Colorless liquid (quantitative yield). ^{1}H NMR (200 MHz, Chloroform-*d*) δ 1.93 – 2.36 (m, 1H), 3.45 – 3.73 (m, 4H), 3.87 (dd, *J* = 7.0, 11.5 Hz, 2H), 7.34 (dd, *J* = 1.8, 5.1 Hz, 5H), 7.57 – 7.91 (m, 4H). MS (ESI): *m*/*z* $[M + H]^+$: 270.1.

4.2. Radioligand Binding Assay

Binding assays for recombinant human α_1 adrenoceptors and 5-HT_{1A}R were performed following published procedures [18].

4.3. Functional studies

Functional studies on isolated tissue (vas deferens prostatic portion, spleen and aorta) were used to assess antagonism toward $\alpha_{1A,}$ α_{1B} and α_{1D} adrenoceptors subtypes, respectively, as already reported [34]. Compound potency and efficacy were measured by [^{35}S]GTPγS binding in cells expressing recombinant human 5-HT_{1A}R, according to Stanton and Beer [35], with minor modifications [18].

4.4. Data Analysis

During the functional studies on isolated tissue, the concentration-response curves were analyzed as described earlier [13]. The [^{35}S]GTPγS binding data were analysed using GraphPad as reported [18].

4.5. Cytotoxicity Assays

The human neuroblastoma cell line SH-SY5Y was used for assessing the cytotoxicity of the compounds, as previously described [36]. The cells were grown in a DMEM medium (EuroClone), supplemented with 10% heat inactivated FBS, 2 mM L-glutamine, 100 U/mL penicillin and 100

µg/mL streptomycin. The cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. The results are expressed as IC_{50} values (concentrations of each drug responsible for 50% inhibition of cell growth), determined after a treatment for 24 h with compounds **12**, Oligomycin A and Rotenone in the same concentration range (0.1-100 µM), and with H_2O_2 in the range from 1-500 µM. The IC_{50} values were calculated from the dose-response curves, using the non-linear multipurpose curve fitting program GraphPad Prism 5.0.

4.6. Neuroprotective activity

The neuroprotective activity of **12** against the damage induced by H_2O_2 (195 µM) oligomycin A (30 µM) and rotenone (75 µM) was determined in the SH-SY5Y cells, using MTT assay, as described by Franchini et al. [18].

4.7. Bi-directional Transport Studies

To evaluate the ability of compound **12** to permeate the blood brain barrier (BBB), MDCKII-MDR1 cells were employed as an *in vitro* model of BBB, following the previously described protocol [37,38]. Diazepam and FD4 (fluorescein isothiocyanate-dextran) were used as internal controls for the transcellular and paracellular pathways, respectively. The apparent permeabilities (P_{app} AP and BL in cm/sec) and the efflux ratio (ER) were calculated according to the equations described in Franchini et al. [18].

4.8. In vivo study

Animals

For the pharmacokinetic and behavioral studies, the experiments were performed on male Sprague Dawley rats (Charles River Laboratories, Callo, Lecco), weighing 200–220 g on arrival, whereas

for anti-nociceptive activity male Swiss CB1 mice (Envigo, S.Pietro al Natisone (UD)) weighing 25 and 30 g were used.

The rats and mice were housed two and six per cage, respectively, in a temperature-controlled (22°C ± 1°C) colony room under a 12/12h light–dark schedule. Food and water were freely available. All animals were handled daily for a week before behavioral testing. Experimental procedures were approved by the Local Ethical Committee (IACUC) and conducted in accordance with international guidelines as well as European Communities Council Directive and National Regulations (CEE Council 86/609 and DL 116/92). All the tests were performed blind to treatment.

4.9. Pharmacokinetic studies

Twenty-five rats were treated orally with 10 mg/Kg of **12**, solubilized in 5% Tween 80 in distilled water, and administered *per os* by gastric tube. Five rats, used as control animals, received an equivalent volume of the above mentioned solvent. Five animals were sacrificed by decapitation at the following time points : 0, 30, 60, 120, 240 and 480 minutes after treatment. Compound **12** was quantified in rat brain and plasma according to Franchini et al. [23].

4.10. Behavioral studies

Twenty-five rats were divided equally into five groups and treated as follows: (1) Group 1: 0.5 mg/Kg of 8-OH-DPAT (reference drug) i.p.; (2) Group 2: 5 mg/Kg of **12** *per os*; (3) Group 3: 10 mg/Kg of **12** *per os*; (4) Group 4: 20 mg/Kg of **12** *per os*; (5) Group 5: 0.9% vehicle solution (5%Tween 80 in distilled water) *per os* (control). 8-Hydroxy-2-(di-n-propylamino) tetralin hydrobromide (8-OH-DPAT) and **12**, dissolved in 5% Tween 80 in distilled water, were administrated intraperitoneally (i.p) and *per os*, 30 and 60 minutes before the test, respectively. The experiments were performed blind.

4.10.1. Elevated Plus Maze test

The elevated plus maze test was carried out on rats.[39] The apparatus is composed of two opposite open and closed arms of the same size (50 cm long, 10 cm wide), enclosed by 40 cm high walls, elevated 50 cm above the floor and illuminated from the top. A video camera was suspended above the maze to record the trials for analysis. The rats were placed individually in the central square facing an open arm and observed for 5 min. The number of entries and the time spent in the open and closed arms were recorded. The maze was cleaned after each trial to remove any residue or odor of the animals. For the purpose of analysis, the open-arm activity was quantified as: (1) percentage of the time spent in the open and closed arms; (2) number of entries into the open and closed arms.

4.10.2. Open field test

The open field test was carried out on rats to evaluate the exploratory activity and emotional response of the animals, as previously described by Carnevale et al. [40]. Briefly, the apparatus consisted of a black-painted wooden arena (100 cm × 100 cm) with 50 cm high walls, placed in a dimly lit soundproof room. The arena was sub-divided into two areas: the central area corresponding to 25% of the total area, and the peripheral one, corresponding to the remaining area. At the beginning of the test, each rat was placed in the center of the arena and its activity was recorded for 10 min using a video tracking system (SMART 2.5 version, PanLab, Barcellona, Spain). The activities in the central zone including the percentage of time spent in the central zone and the number of entries were measured automatically.

4.10.3. Forced Swim Test (Porsolt)

The Forced Swim test was used to assess the anti-depressant activity of the compound [28]. The rats were placed into a glass cylinder (21 cm diameter) filled with water (23–25 ± 1°C; 30 cm depth) for

15 min. After the 15 min swim session, the rats were removed, dried with paper towels, and placed into a polycarbonate cage located on a heating pad for 15 min. The rats were then returned to their home cage. A 5 min swim test was conducted 24 h after the 15 min session. This test was videotaped and scored for the duration of climbing, swimming, and immobility behavior.

4.10.4. Anti-nociceptive activity

For the assessment of anti-nociceptive activity, the mice were subjected to the formalin test, in accordance with a previous publication [18].

4.11. Statistical Analysis

The data obtained from the tests which are reported in the tables and graphs in this study are the mean ± standard error (SEM) obtained from groups of 5 animals each. The statistical analysis was performed using the ANOVA test followed by the post-hoc Dunnett's test using the program GraphPad Prism version 5 for Windows (GraphPad Software, San Diego, California, USA). In all cases, $p<0.05$ was considered as a minimum level of significance.

Conflicts of interest

The authors state no conflict of interest.

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References

[1] R.L. Carhart-Harris, D.J. Nutt, Serotonin and brain function: A tale of two receptors, J.

Psychopharmacol. 31 (2017) 1091–1120. doi:10.1177/0269881117725915.

[2] M. Jastrzębska-Więsek, A. Partyka, J. Rychtyk, J. Śniecikowska, M. Kołaczkowski, A. Wesołowska, M.A. Varney, A. Newman-Tancredi, Activity of Serotonin 5-HT1AReceptor Biased Agonists in Rat: Anxiolytic and Antidepressant-like properties, ACS Chem. Neurosci. 9 (2018) 1040–1050. doi:10.1021/acschemneuro.7b00443.

[3] F. Fiorino, E. Magli, E. Kędzierska, A. Ciano, A. Corvino, B. Severino, E. Perissutti, F. Frecentese, P. Di Vaio, I. Saccone, A.A. Izzo, R. Capasso, P. Massarelli, I. Rossi, J. Orzelska-Gòrka, J.H. Kotlińska, V. Santagada, G. Caliendo, New 5-HT1A, 5HT2Aand 5HT2Creceptor ligands containing a picolinic nucleus: Synthesis, in vitro and in vivo pharmacological evaluation, Bioorganic Med. Chem. 25 (2017) 5820–5837. doi:10.1016/j.bmc.2017.09.018.

[4] O. Stiedl, E. Pappa, Ã. Konradsson-Geuken, S.O. Ã–gren, The role of the serotonin receptor subtypes 5-HT1A and 5-HT7 and its interaction in emotional learning and memory, Front. Pharmacol. 6 (2015) 1–17. doi:10.3389/fphar.2015.00162.

[5] Y. Ohno, S. Shimizu, K. Tokudome, N. Kunisawa, M. Sasa, New insight into the therapeutic role of the serotonergic system in Parkinson's disease, Prog. Neurobiol. 134 (2015) 104–121. doi:10.1016/j.pneurobio.2015.09.005.

[6] B. Vidal, S. Fieux, M. Colom, T. Billard, C. Bouillot, O. Barret, C. Constantinescu, G. Tamagnan, A. Newman-Tancredi, L. Zimmer, 18F-F13640 preclinical evaluation in rodent, cat and primate as a 5-HT1A receptor agonist for PET neuroimaging., Brain Struct. Funct. 223 (2018) 2973–2988. doi:10.1007/s00429-018-1672-7.

[7] S. Afshar, S. Shahidi, A.H. Rohani, A. Komaki, S.S. Asl, The effect of NAD-299 and TCB-2 on learning and memory, hippocampal BDNF levels and amyloid plaques in Streptozotocin-induced memory deficits in male rats., Psychopharmacol. (Heidelberg, Ger. (2018) Ahead of Print. doi:10.1007/s00213-018-4973-x.

[8] I. Marco, M. Valhondo, M. Martín-Fontecha, H. Vázquez-Villa, J. Del Río, A. Planas, O.

Sagredo, J.A. Ramos, I.R. Torrecillas, L. Pardo, D. Frechilla, B. Benhamú, M.L. López-Rodríguez, New serotonin 5-HT1Areceptor agonists with neuroprotective effect against ischemic cell damage, J. Med. Chem. 54 (2011) 7986–7999. doi:10.1021/jm2007886.

[9] L. Di Cesare Mannelli, C. Ghelardini, L. Micheli, F. Del Bello, M. Giannella, A. Piergentili, M. Pigini, W. Quaglia, Synergic stimulation of serotonin 5-HT1Areceptor and α2-adrenoceptors for neuropathic pain relief: Preclinical effects of 2-substituted imidazoline derivatives, Eur. J. Pharmacol. 810 (2017) 128–133. doi:10.1016/j.ejphar.2017.06.023.

[10] Z. Chilmonczyk, A.J. Bojarski, A. Pilc, I. Sylte, Serotonin transporter and receptor ligands with antidepressant activity as neuroprotective and proapoptotic agents, Pharmacol. Reports. 69 (2017) 469–478. doi:10.1016/j.pharep.2017.01.011.

[11] A. Newman-Tancredi, C. Conte, C. Chaput, M. Spedding, M.J. Millan, Inhibition of the constitutive activity of human 5-HT(1A) receptors by the inverse agonist, spiperone but not the neutral antagonist, WAY 100,635, Br. J. Pharmacol. 120 (1997) 737–739. doi:10.1038/sj.bjp.0701025.

[12] S. Franchini, A. Prandi, C. Sorbi, A. Tait, A. Baraldi, P. Angeli, M. Buccioni, A. Cilia, E. Poggesi, P. Fossa, L. Brasili, Discovery of a new series of 5-HT1A receptor agonists, Bioorganic Med. Chem. Lett. 20 (2010) 2017–2020. doi:10.1016/j.bmcl.2010.01.030.

[13] L. Brasili, C. Sorbi, S. Franchini, M. Manicardi, P. Angeli, G. Marucci, A. Leonardi, E. Poggesi, 1,3-Dioxolane-based ligands as a novel class of α1-adrenoceptor antagonists, J. Med. Chem. 46 (2003) 1504–1511. doi:10.1021/jm021078o.

[14] S. Guariento, S. Franchini, M. Tonelli, P. Fossa, C. Sorbi, E. Cichero, L. Brasili, Exhaustive CoMFA and CoMSIA analyses around different chemical entities: A ligand-based study exploring the affinity and selectivity profiles of 5-HT1Aligands, J. Enzyme Inhib. Med. Chem. 32 (2017) 214–230. doi:10.1080/14756366.2016.1247057.

[15] S. Franchini, U.M. Battisti, A. Prandi, A. Tait, C. Borsari, E. Cichero, P. Fossa, A. Cilia, O.

Prezzavento, S. Ronsisvalle, G. Aricò, C. Parenti, L. Brasili, Scouting new sigma receptor ligands: Synthesis, pharmacological evaluation and molecular modeling of 1,3-dioxolane-based structures and derivatives, Eur. J. Med. Chem. 112 (2016) 1–19. doi:10.1016/j.ejmech.2016.01.059.

[16] E. Cichero, S. Espinoza, M. Tonelli, S. Franchini, A.S. Gerasimov, C. Sorbi, R.R. Gainetdinov, L. Brasili, P. Fossa, A homology modelling-driven study leading to the discovery of the first mouse trace amine-associated receptor 5 (TAAR5) antagonists, MedChemComm. 7 (2016) 353–364. doi:10.1039/c5md00490j.

[17] S. Franchini, A. Tait, A. Prandi, C. Sorbi, R. Gallesi, M. Buccioni, G. Marucci, C. De Stefani, A. Cilia, L. Brasili, (2,2-Diphenyl-[1,3]oxathiolan-5-ylmethyl)-(3-phenyl-propyl)-amine: a Potent and Selective 5-HT $_{1A}$ Receptor Agonist, ChemMedChem. 4 (2009) 196–203. doi:10.1002/cmdc.200800276.

[18] S. Franchini, L.I. Manasieva, C. Sorbi, U.M. Battisti, P. Fossa, E. Cichero, N. Denora, R.M. Iacobazzi, A. Cilia, L. Pirona, S. Ronsisvalle, G. Aricò, L. Brasili, Synthesis, biological evaluation and molecular modeling of 1-oxa-4-thiaspiro- and 1,4-dithiaspiro[4.5]decane derivatives as potent and selective 5-HT1Areceptor agonists, Eur. J. Med. Chem. 125 (2017) 435–452. doi:10.1016/j.ejmech.2016.09.050.

[19] C. Sorbi, S. Franchini, A. Tait, A. Prandi, R. Gallesi, P. Angeli, G. Marucci, L. Pirona, E. Poggesi, L. Brasili, 1,3-Dioxolane-based ligands as rigid analogues of naftopidil: Structure-affinity/activity relationships at α1 and 5-HT 1A receptors, ChemMedChem. 4 (2009) 393–399. doi:10.1002/cmdc.200800277.

[20] M. Benchekroun, M. Bartolini, J. Egea, A. Romero, E. Soriano, M. Pudlo, V. Luzet, V. Andrisano, M.L. Jimeno, M.G. López, S. Wehle, T. Gharbi, B. Refouvelet, L. De Andrés, C. Herrera-Arozamena, B. Monti, M.L. Bolognesi, M.I. Rodríguez-Franco, M. Decker, J. Marco-Contelles, L. Ismaili, Novel tacrine-grafted ugi adducts as multipotent anti-alzheimer

drugs: A synthetic renewal in tacrine-ferulic acid hybrids, ChemMedChem. 10 (2015) 523–539. doi:10.1002/cmdc.201402409.

[21] A. Lopalco, H. Ali, N. Denora, E. Rytting, Oxcarbazepine-loaded polymeric nanoparticles: Development and permeability studies across in vitro models of the blood–brain barrier and human placental trophoblast, Int. J. Nanomedicine. 10 (2015) 1985–1996. doi:10.2147/IJN.S77498.

[22] N. Denora, T. Cassano, V. Laquintana, A. Lopalco, A. Trapani, C.S. Cimmino, L. Laconca, A. Giuffrida, G. Trapani, Novel codrugs with GABAergic activity for dopamine delivery in the brain, Int. J. Pharm. 437 (2012) 221–231. doi:10.1016/j.ijpharm.2012.08.023.

[23] S. Franchini, L. Taddia, D. Pinetti, G. Carnevale, L. Brasili, Development, Validation and Application of an LC-MS/MS Bioanalytical Method for the Quantification of GF449, A Novel 5-HT_{1A} Agonist, in Rat Plasma and Brain, Med. Chem. (Los. Angeles). 10 (2014) 449–459. doi:10.2174/1573406409666131128150507.

[24] PK Solutions, (2005).

[25] Schrödinger Release 2017-1: QikProp, (2017).

[26] H. Yu, T. Lewander, Pharmacokinetic and pharmacodynamic studies of (R)-8-hydroxy-2-(di-n-propylamino)tetralin in the rat, Eur Neuropsychopharmacol. 7 (1997) 165–172.

[27] H.G. Vogel, Drug Discovery and Evaluation: Pharmacological Assays, 3rd Ed., Volume 1, in: 2008: p. 2071. doi:10.1007/3-540-29837-1.

[28] R.D. Porsolt, M. Le Pichon, M. Jalfre, Depression: A new animal model sensitive to antidepressant treatments, Nature. 266 (1977) 730–732. doi:10.1038/266730a0.

[29] F. Borsini, A. Meli, Is the forced swimming test a suitable model for revealing antidepressant activity?, Psychopharmacology (Berl). 94 (1988) 147–160.

[30] R.D. Porsolt, Animal models of depression: utility for transgenic research., Rev. Neurosci. 11 (2000) 53–58.

[31] I. Lucki, The forced swimming test as a model for core and component behavioral effects of antidepressant drugs, Behav. Pharmacol. 8 (1997) 523–532. doi:10.1097/00008877-199711000-00010.

[32] J.F. Cryan, I. Lucki, Antidepressant-like behavioral effects mediated by 5-Hydroxytryptamine(2C) receptors., J. Pharmacol. Exp. Ther. 295 (2000) 1120–6. doi:2758/861806.

[33] A.W. Bannon, A.B. Malmberg, Models of Nociception: Hot-Plate, Tail-Flick, and Formalin Tests in Rodents, Wiley Online Library, 2007. doi:10.1002/0471142301.ns0809s41.

[34] S. Franchini, U.M. Battisti, A. Baraldi, A. Prandi, P. Fossa, E. Cichero, A. Tait, C. Sorbi, G. Marucci, A. Cilia, L. Pirona, L. Brasili, Structure-affinity/activity relationships of 1,4-dioxa-spiro[4.5]decane based ligands at α1 and 5-HT1Areceptors, Eur. J. Med. Chem. 87 (2014) 248–266. doi:10.1016/j.ejmech.2014.09.070.

[35] M.S.B. Josephine A. Stanton, Characterisation of a cloned human 5-HT1A receptor cell line using [35S]GTP gamma S binding, Eur. J. Pharmacol. 320 (1997) 267–275.

[36] L. Pisani, R. Farina, M. Catto, R.M. Iacobazzi, O. Nicolotti, S. Cellamare, G.F. Mangiatordi, N. Denora, R. Soto-Otero, L. Siragusa, C.D. Altomare, A. Carotti, Exploring Basic Tail Modifications of Coumarin-Based Dual Acetylcholinesterase-Monoamine Oxidase B Inhibitors: Identification of Water-Soluble, Brain-Permeant Neuroprotective Multitarget Agents, J. Med. Chem. 59 (2016) 6791–6806. doi:10.1021/acs.jmedchem.6b00562.

[37] N. Denora, V. Laquintana, A. Lopedota, M. Serra, L. Dazzi, G. Biggio, D. Pal, A.K. Mitra, A. Latrofa, G. Trapani, G. Liso, Novel L-Dopa and dopamine prodrugs containing a 2-phenyl-imidazopyridine moiety, Pharm. Res. 24 (2007) 1309–1324. doi:10.1007/s11095-007-9255-y.

[38] T. Cassano, A. Lopalco, M. De Candia, V. Laquintana, A. Lopedota, A. Cutrignelli, M. Perrone, R.M. Iacobazzi, G. Bedse, M. Franco, N. Denora, C.D. Altomare, Oxazepam-Dopamine Conjugates Increase Dopamine Delivery into Striatum of Intact Rats, Mol. Pharm.

14 (2017) 3178–3187. doi:10.1021/acs.molpharmaceut.7b00405.

[39] S. Pellow, S.E. File, Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: A novel test of anxiety in the rat, Pharmacol. Biochem. Behav. 24 (1986) 525–529. doi:10.1016/0091-3057(86)90552-6.

[40] G. Carnevale, V. Di Viesti, M. Zavatti, P. Zanoli, Anxiolytic-like effect of Griffonia simplicifolia Baill. seed extract in rats, Phytomedicine. 18 (2011) 848–851. doi:10.1016/j.phymed.2011.01.016.

Highlights

- A new series of 1,3-dioxolane analogues were prepared and tested *in vitro* for binding affinity, potency, efficacy at 5-HT_{1A}R and α_1 adrenoceptors.
- Compound **12** emerged as a potent and selective 5-HT_{1A}R agonist with an high biodistribution in the brain compartment as assessed by pharmacokinetic data in rats.
- Compound **12** exhibited anxiolytic (Elevated Plus Maze and Open Field test) and antidepressant (Forced Swim test) effect.
- Compound **12** showed anti-nociceptive activity in the formalin test.