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Graphical Abstract
1,3-Dioxane as a scaffold for potent and selective 5-HT\textsubscript{1A}R agonist with \textit{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 \( \alpha_1 \) 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 \textit{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 $\alpha_1$-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-HT₁AR. A detailed SAR study was carried out, and a potent and selective 5-HT₁AR agonist with *in-vivo* anxiolytic, antidepressant and anti-nociceptive activity was discovered.

**Figure 2.** A structural modification approach used to design new 5-HT₁AR 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 SN2 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 SN2 conditions, from the chloro-derivative 38 [15] and the appropriate phenoxyethylanine. 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-propanediol (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-butanetriol in refluxing toluene, using p-toluenesulfonic 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-butatriol 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-butanetriol (1.7 eq.), pTSA (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$_2$O, 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.), pTSA (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 Et₂O, 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.), CoCl₂ (0.6 eq.), TMSCl (1 eq.), ACN, r.t., 17 h, quantitative yield; f) thionyl chloride (1.3 eq.), pyridine (2 eq.), dry toluene, N₂, 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.), pTSA (cat.), dry Toluene, Dean-Stark trap, N₂, reflux 65 h, 5% yield; b) thionyl chloride (1.3 eq.), pyridine (2 eq.), dry toluene, N₂, 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 Et₂O, 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.), CoCl₂ (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$_2$O, 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$_a$) and activity (pK$_b$) at human $\alpha_1$ 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 $\alpha_1$ and 5-HT$_{1A}$R receptors [12]. Binding studies in human cloned receptors have shown that at all the three $\alpha_1$ subtypes ($\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$), 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 $\alpha_1$ adrenoceptors and partial agonists at 5-HT$_{1A}$R. Compound 1 showed a selective profile towards $\alpha_{1D}$ (more than 100-fold), with respect to the $\alpha_{1A}$ and $\alpha_{1B}$ 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$_a$
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 \( \alpha_1 \) adrenoceptors, the methoxy group increased potency but decreased selectivity towards the \( \alpha_{1D} \) subtype, while leaving the affinity at the \( \alpha_1 \) subtypes in the binding studies almost unchanged.

Table 1. Affinity constants (pK\(_b^*\) or pK\(_i^\dagger\)) for \( \alpha_1 \)-adrenoceptors in isolated rat prostatic vas deferens (\( \alpha_{1A} \)), spleen (\( \alpha_{1B} \)), and thoracic aorta (\( \alpha_{1D} \)) and for human cloned \( \alpha_{1a} \), \( \alpha_{1b} \), \( \alpha_{1d} \) and 5-HT\(_1A\)R. Agonist potency (pD\(_2\)) and relative effectiveness (% E\(_{max}\)) in the agonist-induced [\(^{35}\)S]GTP\(_S\)-binding assay at 5-HT\(_1A\). All the compounds were assayed as oxalate salt.

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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 α₁ and 5-HT₁AR. 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 (pKᵢ) at all α₁ subtypes, while leaving unchanged the affinity at 5-HT₁A.R. During the functional studies, the antagonist potency of 4 at α₁ subtypes was slightly decreased, while the selectivity towards the α₁D subtype was almost the same (about 10-fold). At 5-HT₁A.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-HT₁A.R. During the functional studies at α₁, the most significant variation was a complete loss of α₁D selectivity, due to a more than 10-fold decrease in potency at this subtype and a concomitant, although small, increase at the α₁A and α₁B 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 diastereomers were obtained (t and c). The most important change was in the selectivity 5-HT₁A/α₁, which was greatly reduced, as a result of a significant decrease in 5-HT₁A.R affinity. Combining the two variations, as in 8, was generally negative for both affinity and potency for α₁ and 5-HT₁A receptor systems. The exception was the increase in efficacy at 5-HT₁A.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 α₁b and α₁d receptors, with an α₁d/α₁a selectivity ratio of about 12-fold higher than that of compound 2. The affinity at 5-HT₁A.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$_2$O$_2$), 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.

<table>
<thead>
<tr>
<th></th>
<th>12</th>
<th>H$_2$O$_2$$^b$</th>
<th>oligomycin A</th>
<th>rotenone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>31.2 ± 0.6</td>
<td>195 ± 1.7$^*$</td>
<td>29 ± 3.4$^*$</td>
<td>74.1 ± 4.5$^*$</td>
</tr>
</tbody>
</table>

$^a$ According to[18]

$^b$ H$_2$O$_2$ 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$_2$O$_2$, 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$_2$O$_2$ 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

<table>
<thead>
<tr>
<th>Compd [µM]</th>
<th>H$_2$O$_2$ (195 µM)</th>
<th>Oligomycin A (30 µM)</th>
<th>Rotenone (75 µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 (1 µM)</td>
<td>79 ± 5</td>
<td>83 ± 2</td>
<td>61 ± 4</td>
</tr>
<tr>
<td>12 (0.1 µM)</td>
<td>89 ± 3</td>
<td>69 ± 2</td>
<td>63 ± 9</td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th>Compd</th>
<th>$P_{app}$ AP(cm/sec)</th>
<th>$P_{app}$ BL(cm/sec)</th>
<th>ER $P_{app}$BL/$P_{app}$AP</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>2.6*10^{-5}</td>
<td>1.26*10^{-5}</td>
<td>0.49</td>
</tr>
<tr>
<td>diazepam</td>
<td>1.46*10^{-5}§</td>
<td>1.23*10^{-5}§</td>
<td>0.84§</td>
</tr>
<tr>
<td>FD-4</td>
<td>1.03*10^{-6}§</td>
<td>2.08*10^{-7}§</td>
<td>0.20§</td>
</tr>
</tbody>
</table>

* 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]

<table>
<thead>
<tr>
<th></th>
<th>C&lt;sub&gt;max&lt;/sub&gt; [a]</th>
<th>t&lt;sub&gt;max&lt;/sub&gt; (min)</th>
<th>AUC&lt;sub&gt;(0-t)&lt;/sub&gt; (nmol/g·min)</th>
<th>AUC&lt;sub&gt;(0-∞)&lt;/sub&gt; (nmol/g·min)</th>
<th>t&lt;sub&gt;½&lt;/sub&gt; (min)</th>
<th>Brain/Plasma (B/P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>0.095</td>
<td>30</td>
<td>17.4</td>
<td>18.1</td>
<td>102.6</td>
<td>≈420</td>
</tr>
<tr>
<td>Brain*</td>
<td>157.9</td>
<td>60</td>
<td>7167.6</td>
<td>7173.4</td>
<td>6.2 (15-45 min)</td>
<td>142 (45-480 min)</td>
</tr>
</tbody>
</table>

[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 (QPMDCK) were calculated with QiKProp [25]. Compound 12 showed a QPlogBB of 0.467 (for CNS penetration -3< QPlogBB <1.2) and a QPMDCK of 1250 (for CNS penetration QPMDCK >500), suggesting that is able to cross the BBB by passive diffusion. Moreover, the predicted QPMDCK 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_{1/2}) 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<0.001 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).
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\textsubscript{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\textsubscript{1A}R rather than for the \(\alpha\)-adrenergic receptor (5-HT1a/\(\alpha\)1D 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 \textit{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\textsubscript{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\textsubscript{1A}R ligands was discovered. Several compounds acted as potent 5-HT\textsubscript{1A}Rs agonists, among which 12 was the most potent, with a maximal activity of 92% compared to 8-OH-DPAT. *In 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\textsubscript{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 $\delta$ (ppm) using tetramethylsilane (TMS) as internal standard or the $^{13}$C signal of the solvent (CDCl$_3$ $\delta$ 77.04, CD$_3$OD $\delta$ 49.8, DMSO-d$_6$ $\delta$ 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$_2$O 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$_2$O 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$_{25}$H$_{28}$NO$_3$: 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$_{26}$H$_{30}$NO$_4$: 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$_{25}$H$_{28}$NO$_3$: 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), 3.30 (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$_{25}$H$_{28}$NO$_3$: 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,
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-$d_6$) $\delta$ 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) $\delta$ 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: 420.5280. 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.

4.1.1.7. 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-$d_6$) $\delta$ 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) $\delta$ 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:
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-$d_6$) $\delta$ 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) $\delta$ 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-$d_6$) $\delta$ 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) $\delta$ 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-$d_6$) $\delta$ 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) $\delta$ 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-$d_6$) $\delta$ 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) $\delta$ 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-$d_6$) $\delta$ 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) $\delta$ 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-$d_6$) $\delta$ 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) $\delta$ 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-$d_6$) $\delta$ 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) $\delta$ 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, 128.85, 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_{26}H_{31}NO_{6}: 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 (I3)

White solid (54% yield). ^1H NMR (600 MHz, DMSO-d6) 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), 7.38 – 7.50 (m, 2H). ^13C 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 (I4)

White solid (45% yield). ^1H NMR (600 MHz, DMSO-d6) 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). ^13C 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, 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 (I5)

White solid (55% yield). ^1H NMR (600 MHz, DMSO-d6) 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) $\delta$ 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$_{23}$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-$_d_6$) $\delta$ 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) $\delta$ 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$_{25}$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$_2$SO$_4$ 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-$d$) $\delta$ 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,
13C NMR (151 MHz, CDCl₃) 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. ¹H 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). ¹³C NMR (151 MHz, CDCl₃) 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. ¹H 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). ¹³C NMR (151 MHz, CDCl₃) 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-$d$) $\delta$ 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$_3$) $\delta$ 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-$d$) $\delta$ 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$_3$) $\delta$ 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-$d$) $\delta$ 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$_3$) $\delta$ 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) $\delta$ 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$_3$) $\delta$ 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) $\delta$ 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$_3$) $\delta$ 28.86, 46.82, 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) $\delta$ 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$_3$) $\delta$ 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) $\delta$ 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$_3$) $\delta$ 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) $\delta$ 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$_3$) $\delta$ 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\( _2\)SO\(_4\) 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-\(d\)) \( \delta \) 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\(_3\)) \( \delta \) 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-\(d\)) \( \delta \) 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\(_3\)) \( \delta \) 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$_2$SO$_4$ 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-$d$) $\delta$ 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-$d$) $\delta$ 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-$d$) $\delta$ 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-$d$) $\delta$ 2.00 – 2.14 (m, 2H), 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-butanetriol (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 Et₂O. The organic phase was washed with NaHCO₃ saturated solution, brine, dried over anhydrous Na₂SO₄ 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). ¹H NMR (200 MHz, DMSO-d₆) 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). ¹H NMR (200 MHz, DMSO-d₆) 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-butanetriol (2 eq., for 41) or 2-(hydroxymethyl)propane-1,3-diol (2 eq., for 43), TMSCl (1 eq.) andCoCl₂ (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 Na₂SO₄ 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). ¹H 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-\(d\)) \(\delta\) 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 \(\alpha_i\) 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 \(\alpha_{iA}\), \(\alpha_{iB}\) and \(\alpha_{iD}\) adrenoceptors subtypes, respectively, as already reported [34]. Compound potency and efficacy were measured by \([^{35}\text{S}]\text{GTP}\gamma\text{S}\) 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 \([^{35}\text{S}]\text{GTP}\gamma\text{S}\) 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$_2$O$_2$ 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$_2$O$_2$ (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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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 $\alpha_1$ 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.