Accepted Manuscript

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PII: S0223-5234(16)30069-1

DOI: 10.1016/j.ejmech.2016.01.059

Reference: EJMECH 8349

To appear in: *European Journal of Medicinal Chemistry*

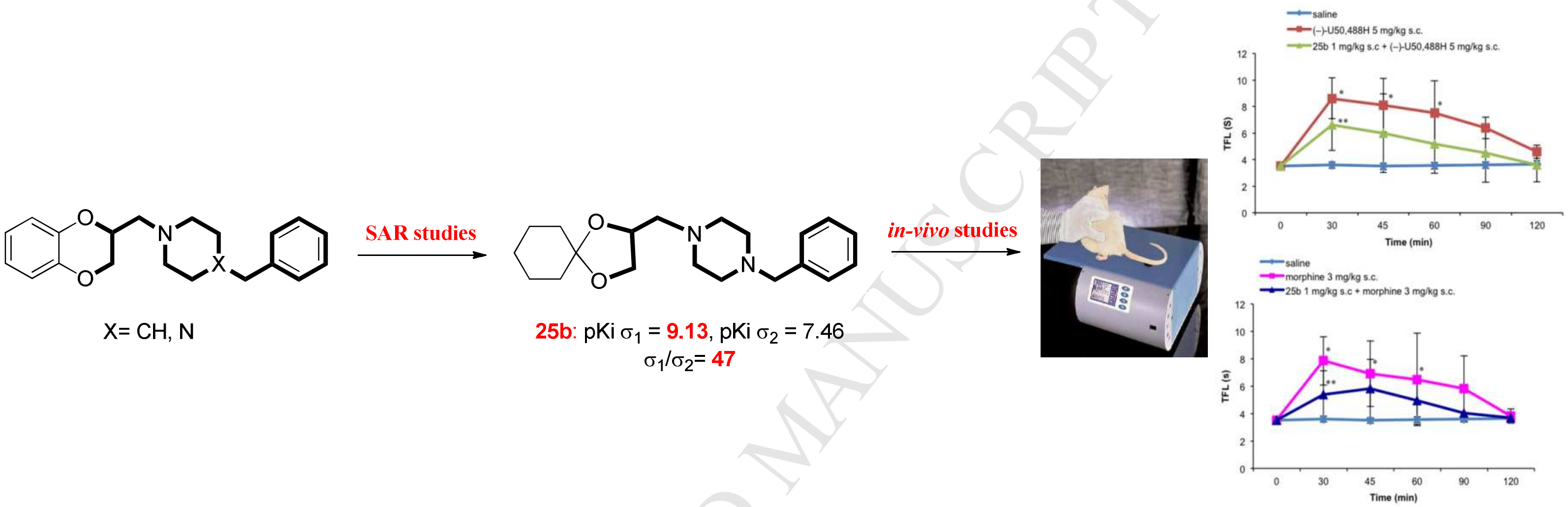
Received Date: 1 October 2015

Revised Date: 13 January 2016

Accepted Date: 30 January 2016

Please cite this article as: 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, *European Journal of Medicinal Chemistry* (2016), doi: 10.1016/j.ejmech.2016.01.059.

Graphical Abstract



Scouting New Sigma Receptor Ligands: Synthesis, Pharmacological Evaluation and Molecular Modeling of 1,3-Dioxolane-Based Structures and Derivatives.

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Abstract

Herein we report the synthesis and biological activity of new sigma receptor (σR) ligands obtained by combining different substituted five-membered heterocyclic rings with appropriate σR pharmacophoric amines. Radioligand binding assay, performed on guinea pig brain membranes, identified **25b** (1-(1,4-dioxaspiro[4.5]decan-2-ylmethyl)-4-benzylpiperazine) as the most interesting compound of the series, displaying high affinity and selectivity for σ_1R ($pKi\sigma_1 = 9.13$; $\sigma_1/\sigma_2 = 47$). The ability of **25b** to modulate the analgesic effect of the κ agonist (−)-U-50,488H and μ agonist morphine was evaluated *in vivo* by radiant heat tail-flick test. It exhibited anti-opioid effects on both κ and μ receptor-mediated analgesia, suggesting an agonistic behavior at σ_1R. Docking studies were performed on the theoretical σ_1R homology model. The present work represents a new starting point for the design of more potent and selective σ_1R ligands.

Keywords: Sigma receptors ligands; sigma-1; sigma-2; piperidine; piperazine; 1,3-dioxolane; receptor-mediated analgesia.

1. Introduction

Sigma receptors (σRs) were discovered in 1976 and initially classified as an additional class of opioid receptorse [1]. Subsequently, σRs were mischaracterized as PCP/NMDA glutamate receptor complexes, due to the poor selectivity of the ligands employed [2,3]. However, these hypotheses were disproved [4,5]. Today, the σR is recognized as a unique entity with no homology to opioid receptors or other mammalian proteins[6]. Further radioligand binding studies and biochemical analysis suggested that sigma receptors exist as two different and distinct subtypes, named sigma-1 receptor (σ_1R) and sigma-2 receptor (σ_2R) [7,8]. The σ_1R has been recently characterized and cloned from guinea pig [9], human [10], mouse [11,12], and rat tissues [13]. It is present mainly in the endoplasmic reticulum membrane (ER), the mitochondria associated ER membrane (MAM) and the plasma membrane [14]. σ_1R consists of two transmembrane domains with both the amino and carboxy termini on the cytoplasmic side, whereas the loop between the transmembrane domains is located within the endoplasmic reticulum [15]. σ_1R has been shown to act as a unique ligand-regulated molecular chaperone that modulates the activity of several proteins, such as the N-methyl-D-aspartate (NMDA) receptor[16] and several ion channels [17]. Neurosteroids such as progesterone and dehydroepiandrosterone have been postulated to be the endogenous σ_1R ligands[18-20]. Moreover, it has been shown that several exogenous compounds can interact with the σ_1R. Among them, the dextrorotatory benzomorphans SKF10047 and pentazocine [21-25], haloperidol and NE-100 represent relevant σ_1R ligands [22, 25-27].

High affinity σ_1R ligands have been considered to play an important role in the treatment of various neurological disorders, including depression, schizophrenia, neuropathic pain, and Alzheimer's disease [28-34]. Unlike σ_1Rs, σ_2Rs have not yet been cloned. This subtype is mainly located in lipid rafts where it modulates calcium signalling through sphingolipid products. Very recently it has been proposed that the progesterone receptor membrane component 1, which binds directly to the heme group and regulates lipid and drug metabolism and hormone signalling, represents the σ_2R binding site [35]. Activation of σ_2R appears to be involved in the regulation of cellular proliferation and cell death [36]. For these reasons, the antagonism or inhibition of σ_2R function could mitigate cell death [37]. Furthermore, it has been reported that σ_2R ligands can be used as biomarkers for tumour cell proliferation and thus they could be exploited for tumor imaging [37,38]. Therefore, due to the broad diagnostic and therapeutic potential, the development of potent and selective σ_1R or σ_2R ligands is a primary challenge in medicinal chemistry.

In a previously published paper we reported a series of 1,4-benzodioxane-based piperazines and piperidines as novel σR ligands with a good affinity for both receptor subtypes but lacking in adequate selectivity among sigma subtypes and sigma /5-HT1A receptors (Chart 1, **1a**,**b**) [39]. Parallel SAR studies conducted by our research group on α_1-adrenoreceptor demonstrated the bioequivalence of the 1,3-dioxolane moiety with the 1,4-benzodioxane nucleus [40]. This approach has successfully led to the discovery of a novel class of α_1-adrenoreceptors antagonists and, more recently, the identification of potent and selective 5-HT_{1A} receptor agonists and NOP receptor ligands [41, 42].

Thus, in this work we have applied the same strategy to explore a series of 1,3-dioxolane-based compounds, obtained by replacing the 1,4-benzodioxane moiety, in order to verify whether the above mentioned approach could be advantageous also for the class of σR ligands (Chart 1, **8a**,**b**). In addition, focusing our attention on the 1,3-dioxolane scaffold, we applied the classical medicinal chemistry approach described in Chart 2, such as annular oxygen bio-isosteric substitutions (Group II and III) and externalization of the annular oxygen (Group IV) to investigate the effect on activity of a series of five-membered heterocyclic rings or opened analogues (Group V). Moreover, on the basis of previously obtained results showing that the phenyl groups at position 2 on the 1,3-dioxolane scaffold are not essential for the binding to 5-HT_{1A}R and NOP receptors, we planned the synthesis of the conformationally restricted spiro-dioxolanes (Group VI) [41, 43]. All the compounds were tested for affinity and selectivity at σ_1 and σ_2R subtypes and detailed SAR studies were drawn up. In addition nociceptive effect was evaluated *in vivo*. In order to rationalize the pharmacological results and support and guide the chemical exploration, *in-silico* docking studies were performed on the theoretical σ_1 three-dimensional model.

2. Results and discussion

2.1. Chemistry

All the compounds (**8-25a,b)** were prepared by alkylation of the commercially available 4-benzylpiperidine or 1-benzylpiperidine with the suitable intermediate.

For Group I and II compounds acetalization of the selected ketone with the proper glycerol derivative provided the corresponding 1,3-dioxolane, oxathiolane and dithiolane-intermediates from which either the chloro or the tosyl derivatives **3**-**7** were obtained (Scheme 1). In case of **4**, the diastereomeric mixture was separated by silica gel flash chromatography yielding *cis*-**4** and *trans*-**4**. The separated isomers were characterized by means of NOESY experiments and ^{1}H-NMR studies (Figure 1S, panel a, Supporting Informations). The furane derivatives **14a,b** (Group III) (Scheme 2) were prepared starting from the key intermediate **13**, obtained in three steps, as recently described (see experimental section).

The cyclopentanone derivatives **16a,b** were obtained in good yields by Mannich reaction between diphenylcyclopentanone (**15**) in the presence of aqueous paraformaldehyde and 4-benzylpiperidine or 1-benzylpiperazine as hydrochloride salt to ensure the acidic reaction conditions [44]. Reduction of **16a,b** by $NaBH_4$ provided the corresponding cyclopentanol derivatives **17a,b** (Group IV) (Scheme 2). The *cis*/*trans* diastereomeric pairs were separated by using flash column chromatography and their relative stereochemistry was elucidated by NOESY experiments and H^1-NMR studies (Figure 1S, panel b, Supporting Informations).

For the 1,3-dioxolane opened analogues **22a,b** and **23a,b** (Group V), the 3-chloropropane-1,2-diol, previously protected as *tert*-butyldiphenylsilyl ether **18**, or the 2-chloroethanol was reacted with the bromodiphenylmethane to yield the alkyl halides **19** and **20** (Scheme 3).

The spiro-dioxolane derivatives **25a,b** (Group VI) were readily prepared starting from the key intermediate **24** (Scheme 2).

2.2. Biological activity

2.2.1. Binding affinity

The compounds in Groups I-VI were evaluated for their affinity at both σ_1R and σ_2R (Table 1-3). Since most of the molecules share the same chemical features with previously published 5-HT_{1A}R ligands [41, 43, 44] we also evaluated the binding affinities at 5-HT_{1A}R. Furthermore, the affinity at α_1 adrenoceptors was determined (values not shown) and the compounds showed practically no activity at these receptors.

Compounds **1a** and **1b** were our starting points. In a previously published paper we reported that they display good affinity for both receptor subtypes but lacking in adequate selectivity [39]. Replacing the 1,4-benzodioxane group with the 2,2-diphenyl-dioxolane moiety (**8a** and **8b**) the affinity of both derivatives was increased by about 5/10-fold while the selectivity remained absent, as in the case of the parent compounds. However, **8a** and **8b** show lower affinities for 5-HT_{1A}R with respect to **1a** and **1b** with good selectivity ratios (σ/5HT_{1A}) of 251 and >589, respectively. Replacement of one of the two phenyl rings with a cyclohexyl group at position 2 of the 1,3-dioxolane ring led to a different effect for the two series (piperidine and piperazine), both in terms of affinity and stereoselectivity. Compounds *cis*-**9a** and *trans*-**9a** showed a marked decrease in affinity at both receptor subtypes. A decrease in σ/5HT_{1A} selectivity was also observed. In this case the stereochemistry seems not to play a significant role. On the contrary, for the piperazine derivatives a certain degree of stereoselectivity at σ_1R site was observed. Compound *trans*-**9b** maintained the affinity at σ_1 subtype while, at σ_2 receptor subtype, the value is slightly decreased (7.79 vs 8.38). On the other hand, the *cis* isomer **9b** is more than 10-fold less active at σ_1R while having the same affinity at σ_2 subtype with respect to the *trans* isomer **9b**. Moreover, for both isomers *cis*-**9b** and *trans*-**9b** the σ/5HT$_{1A}$ selectivity is conserved (>56 and >933 respectively).

With the substitution of the second phenyl ring, to give the 2,2-dicyclohexyl derivatives (**10a** and **10b**), the affinities of the piperidine series are further decreased, while, in the case of the piperazine series, they are maintained. However, the very small decrease in affinity at σ_1R together with the small increase at σ_2R drastically reduced the selectivity observed with *trans*-**9b**. The Piperazine series is confirmed to be more selective towards σ receptors with respect to the 5-HT$_{1A}$R.

Isosteric substitutions oxygen/sulphur/methylene of compound **8a** and **8b** were also evaluated. Replacement of oxygen with sulphur, to give the 1,3-oxathiolane **11a** and **11b**, reduced the affinity at both σR subtypes of different extent: 4- 5-fold in the case of piperidine **11a** and a large reduction of 40-50-fold in the case of piperazine **11b**. The same trend, although to a lesser extent, is also observed with the introduction of a second sulphur atom to give the 1,3-dithiolane derivatives **12a** and **12b.** As a result, the piperidine couple (**11a** and **12a**) is more potent than the piperazine one, at both σR subtypes.

Isosteric substitution of one annular oxygen atom of the 1,3-dioxolane with a methylene unit gave the tetrahydrofurane derivatives **14a** and **14b** endowed with lower affinity values. It is a quite large decrease of about 40 to 70-fold either for piperazine and piperidine, at both σR subtypes.

All isosters of **8a** and **8b** retain good σ/5HT$_{1A}$ selectivity displaying low affinity values for 5HT$_{1A}$R.

Replacing the oxygen atom in the tetrahydrofuran ring with a carbonyl group, to give the cyclopentanones derivative **16a** and **16b**, a further reduction of affinity is observed, although an increase of selectivity (20-fold) for the piperazine derivative **16b** is observed.

The reduction of the carbonyl group gives two couples of diastereoisomeric cyclopentanols ***cis*-17a**, *trans*-**17a**, and *cis*-**17b** and *trans*-**17b**. For both piparazine and piperidine couples, a recovery of affinity is observed with respect to the parent cyclopentanones, with a clear lack of diastereoselectivity since each diasteromeric couple shows similar affinity values at both receptor subtypes. It is worth noting that the least active of the four cyclopentanols, *trans*-**17a**, is the only one to show a certain degree of selectivity (39-fold). Interestingly, all the pentanol derivatives, except *trans*-**17a**, show a marked increase in affinity towards 5HT$_{1A}$R, with reversed σ/5HT$_{1A}$ selectivity.

Compounds **22a** and **22b** are open analogues of **8a** and **8b** while **23a** and **23b**, obtained by removal of the hydroxymethylene group, could be considered their molecular simplification. Opening of the 1,3-dioxolane ring causes a drop in affinity at both σR subtypes: of about 4- 7-fold in the case of piperazine derivatives and, a more pronounced decrease, about 44- 90-fold, in the case of piperidine derivatives. The removal of the hydroxymethylene group does not cause a significant variation in affinites. Once more, piperazines **22b** and **23b** highlight good $\sigma/5HT_{1A}$ selectivity values while the corresponding piperidines displayed poor or no selectivity.

Considering that the best results, in terms of affinity, were obtained with the 1,3-dioxolane scaffold and that the phenyl rings in position 2 do not seem to be essential for affinity (see compounds *trans*-**9b** and **10b**) we planned the synthesis of the conformationally restricted spiro-dioxolanes **25a** and **25b**.

The piperazine derivative **25b** showed the highest affinity at σ_1R with a pKi value of 9.13 and a selectivity ratio (σ_1/σ_2) of 47 fold. The same profile was observed for the piperidine derivative **25a**, although the affinity for σ_1 subtype and the selectivity ratio was of a lesser extent. Compound **25b** also displays the highest $\sigma/5HT_{1A}$ selectivity value (1349) in the whole series.

As far as the differences in affinity between σ_1R and σ_2R subtypes are concerned, in most cases higher values are obtained for the former, although the selectivity is quite low. Only compound **25b** is outstanding in this respect (pK_i σ_1 = 9.13, σ_1/σ_2 = 47). Furthermore, it is worth noting that, excluding the 1,3-oxathiolane and the 1,3-dithiolane derivatives **11** and **12**, it clearly appears that at the σ_2 binding site the piperazine derivatives are more active than the corresponding piperidines, with the exception of cyclopentanones (**16**) and spiro derivatives (**25**). In the case of the sulphur-containing derivatives (1,3-oxathiolanes and 1,3-dithiolanes), the piperidines show affinity values higher than those seen with the piperazines, with a reversed trend of activity. Therefore, it seems that the introduction of one or two sulphur atoms is responsible for this effect. However, as the number of compounds is too limited, more compounds are needed in order to generalize this observation.

2.2.2. In vivo analgesic activity

Given the implication of σ_1Rs in opioid-mediated analgesia [45] we analysed the ability of compound **25b**, on the bases of its affinity (pKi = 9.13) and selectivity (47-fold), to modulate the analgesic effect of the systemically injected KOP agonist, *trans*-(1*S*,2*S*)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide [(−)-U50,488H] [46]. Our results demonstrate that the systemic administration of **25b** (1 mg/kg sc) did not affect tail withdrawal latencies during the entire observation time (data not shown). Injection of the KOP agonist (−)-U-50,488H, at a dose of 5 mg/kg s.c., significantly increased the nociceptive latency by following thermal stimulation, which demonstrated a clear analgesic effect (* $P< 0.05$ vs saline treated rats). Pre-treatment with **25b** at 1 mg/kg s.c., followed by (−)-U-50,488H (5 mg/kg s.c.) caused a reduction in the opioid analgesic effect which was significant only at 30 minutes after the last administration (*$P<0.05$ vs (−)-U-50,488H treated rats) (Figure 1). In the next experimental protocol, the injection of morphine at the dose of 2 mg/kg s.c. (chosen in a dose range of 1 to 10 mg/kg) determined a significant analgesic effect ($^{*}P<0.05$ vs saline treated rats). The double treatment with **25b** 1 mg/kg s.c. plus morphine 2 mg/kg s.c., diminished MOP-induced analgesia (Figure 2); values were significant only at 30 minutes of observation ($^{**}P<0.05$ vs morphine treated rats). These results are consistent with an agonistic behavior at σ_1R of compound **25b**.

2.3. Molecular Modeling

In order to better understand the affinities of the compounds disclosed here, docking studies on the σ_1R homology model, previously built by us and presented here (see experimental section), were performed. According to our results, the putative σ_1 binding site was delimited by: (*i*) one hydrophobic region located inside the protein including F58, A86, V104, L105, L106, L124, Y147, (ii) one hydrophilic core placed around the polar residues D126, E150, T151, (*iii*) a region much more exposed outside the protein showing the F83, V84, F107, I128, S130, T132, F133, H134 residues. The model refinement was performed exploring the docking mode of known σ_1 ligands and then comparing the results with the literature data. In particular, compound **I** (1-benzyl-6',7'-dihydrospiro[piperidine-4,4'-thieno[3,4-c]pyran], Figure 3) [47] was chosen for its high binding affinity at σ_1R and for a considerable structural similarity with leading members of our series. The docking results highlight the importance of a salt-bridge with the D126 side-chain and of one H-bond between the spirocyclic oxygen atom and T151 (Figure 4); the data, being in agreement with the literature, supported the reliability of the obtained σ_1R homology model [47]. The relevance of the interactions with D126 and T151 was also confirmed by mutagenesis studies [48], validating, once again, the computational protocol. In addition, the MOE dock scoring funtions revealed the docking protocol ability to efficiently rank any selected conformer in accordance with the affinity trend (Table S1, Supporting Information). Among the compounds investigated here, a number of them displayed a salt-bridge interaction with D126 and some hydrophobic contacts with V84, A86, V104, L105, L106, L124, I128, while the compounds with the highest affinity also showed additional H-bonds with T151 and/or S130. In more detail, the dioxolane derivatives **8a** and **8b** shared the same docking mode, displaying the key salt-bridge interaction between the piperidine or piperazine protonated nitrogen atom and D126 and the H-bond interaction between the dioxolane core and T151, while the diphenyl portion and the benzylic ring were properly engaged in π–π stacking with F83, F107, F133, and Y147, respectively (Figure 5, the *S* enantiomers were revealed by calculations to be the most stable). These results are in agreement with the affinity data showing that piperidine **8a** and piperazine **8b** bind equally both σR subtypes. Conversely, the replacement of one or two phenyl rings with the cyclohexyl group led to a different effect, the piperazines *cis*/*trans*-**9b** and **10b** showing higher pK_i values than the corresponding piperidines *cis*/*trans*-**9a** and **10a** at both σR subtypes. In particular, it was observed that the most active piperazines *trans*-**9b** and **10b** displayed a docking mode comparable with that described for **8a** and **8b**, maintaining the two driving interactions with the key residues D126 and T151, although, in this case, both of them are exerted by the two piperazine nitrogen atoms, while the oxygen ring is not engaged in any H-bond. On the contrary, the piperidine derivatives **9a** and **10a**, were characterized by a switched binding mode, orienting the cyclohexyl or dicyclohexyl portion towards Y147 and therefore lacking the key salt-bridge interaction with D126 (Figure 6, the **10a** and **10b** *S* and *R* enantiomers were revealed by calculations to be the most stable). In this case, only the H-bond with T151 was maintained. These results could be an indication of the different affinities observed in the binding experiments for these two series. All the structural modifications applied to the dioxolane scaffold, leading to the ring-opened derivatives (**22a**,**b**; **23a**,**b**: pK_i= 6.7-7.9) oxathiolane- (**11a**,**b**: pK_i= 7.9, 7.2), dithiolane- (**12a**,**b**: pK_i= 7.8, 6.5), tetrahydrofuran- (**14a**,**b**: pK_i= 6.7, 7.0), cyclopentanone- (**16a**,**b**: pK_i= 5.9, 6.2) or cyclopentanol- (*cis/trans*-**17a**, **b**: pK_i = 6.5-7.4) analogues, proved to be detrimental for binding to σR . With the exception of oxathiolanes or dithiolanes, the above-mentioned compounds properly located the diphenyl and the benzyl substituents towards F83, F107, F133, and Y147, respectively, thus resulting in only the salt-bridge interaction with D126 or in the H-bond with T151. Conversely, both interactions were maintained in compound **22b** through the piperazine nitrogen atom and the hydroxyl group, respectively.

Interestingly, when the 1,3-dioxolane portion was replaced with a 1,3-oxathiolane or 1,3-dithiolane, compounds in the piperidine series (**11a**, **12a**) performed better (p$K_i\sigma_1$ = 7.82, 7.92) than the corresponding piperazine derivatives (**11b**, **12b**; p$K_i\sigma_1$ = 6.52, 7.20), at both σR subtypes. According to our calculation, **11a** and **12a** oriented the diphenyl and the benzyl substituent inside and quite outside the protein, respectively, displaying a salt-bridge between the protonated nitrogen atom and D126. On the contrary, the corresponding piperazine **11b** and **12b** showed an inversed docking mode which prevented any contact with D126, exhibiting only a weak H-bond with T151 (data not shown).

Lastly, **25a** and **25b**, the most interesting members of this series, outstanding for their affinity (pK_i = 8.70, 9.13) and selectivity (σ_1 / σ_2 = 10, 47) at σ_1R, shared the by following interactions: (*i*) a salt-bridge between the piperidine or piperazine nitrogen atom and D126; (*ii*) an H-bond between the dioxolane oxygen atom and S130: (*iii*) π–π stacking and hydrophobic contacts with Y147 and with V84, I128, F133. Moreover, compound **25b** displayed one additional H-bond between the piperazine nitrogen atom and T151 (Figure 7; the **25a** and **25b** *S* enantiomers were revealed by calculations to be the most stable). Significantly, the docking pose of **25b** was comparable with that of the previously described agonist **I**

(pK_i=9.36), displaying the same hydrophilic contacts with D126 and T151. Notably, the bond distances measured for the two protein-ligand complexes were slightly lower for **I**, giving an indication for the slightly greater affinity of **I** with respect to **25b**. On the basis of these results, it could be hypothesized that the replacement of the piperazine ring with a shorter basic linker between the benzyl group and the dioxaspiro-core on **25b** could efficiently guarantee the proper pattern of an H-bond acceptor and basic features to interact with D126, T151 and also with S130. Moreover, additional aromatic moieties linked to the spiro-decane scaffold could be introduced, in order to further stabilize the protein-ligand complex by means of π–π stacking interactions with both the two aforementioned hydrophobic pockets, including F58, Y147 and F83, F107, F133. These results could represent a new starting point for the design of structural analogues of **25b**.

3. Conclusions

Starting from **1a** and **1b** and replacing the 1,4-benzodioxane moiety with a variety of five-membered heterocyclic rings, a new class of σR ligands was obtained. Structure-affinity studies were performed leading to these conclusions:

a) all the compounds exhibited a preference for σ_1R subtype respect to σ_2R, although the selectivity, in most of the cases, is quite low;
b) the best results in terms of affinity and selectivity were obtained with the 1,3-dioxolane scaffold;
c) isosteric substitutions of the dioxolane atoms or molecular simplification led to a general decrease in affinity;
d) aromatic substituents at position 2 on the 1,3-dioxolane ring do not seem to be essential for σR affinity;
e) with few exceptions, piperazine-based compounds were more potent than the corresponding piperidines;
f) the computational results, in agreement with the biological data, proved the reliability of the σ_1R model.

In particular, compound **25b** was outstanding for its high affinity (pK_i=9.13) and selectivity (σ_1/σ_2 = 47) at σ_1R subtypes. *In-vivo* studies suggested that **25b** acts as a σ_1R agonist since it is able to reduce both (−)-U50,488H- and morphine-mediated analgesia. Therefore, **25b** could represent a new starting point for the development of more active and selective ligands. Further research along this line is in progress and will be disclosed in due course.

4. Experimental Part

4.1. Chemistry

All the reagents, solvents and other chemicals were used as purchased from Sigma-Aldrich without further purification unless otherwise specified. Air- or moisture-sensitive reactants and solvents were employed in reactions carried out under nitrogen atmosphere unless otherwise noted. Flash column chromatography purifications (medium pressure liquid chromatography) were carried out using Merck silica gel 60 (230-400 mesh, ASTM). The structures of all isolated compounds were ensured by Nuclear magnetic resonance (NMR) and Mass spectrometry. ^{1}H and ^{13}C-NMR (1D and 2D experiments) spectra were recorded on a DPX-200 Avance (Bruker) spectrometer operating at 200.13 MHz and on a DPX-400 Avance (Bruker) spectrometer operating at 400.13 MHz. Chemical shifts are expressed in δ (ppm). ^{1}H NMR chemical shifts are relative to tetramethylsilane (TMS) as internal standard. ^{13}C-NMR chemical shifts are relative to TMS at δ 0.0 or to the ^{13}C signal of the solvent: $CDCl_3$ δ 77.04, CD_3OD δ 49.8, DMSO-d_6 δ 39.5. NMR data are reported as follows: chemical shift, number of protons/carbons, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broadened), coupling constants (Hz) and assignment (Diox = 1,3-Dioxolane; Ar = Phenyl; Cyc = Cyclohexyl; Ts = Tosyl; Piper = Piperidine or Piperazine; Ph = Phenyl ; Bz = Benzyl, Oxath = 1,3-Oxathiolane; Dithio = 1,3-Dithiolane; Fur = Tetrahydrofurane; Cyclopent = Cyclopentanone or Cyclopentanol; Dosd = 1,4-Dioxaspiro[4.5]decane). ^{1}H-^{1}H Correlation spectroscopy (COSY), ^{1}H-^{13}C heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) experiments were recorded for determination of ^{1}H-^{1}H and ^{1}H-^{13}C correlations respectively. NOESY experiments have been performed to assign the correct stereochemistry. HR-MS experiments were carried out using a LC-MS mass spectrometer (6520 Accurate-Mass Q-TOF LC/MS - Agilent Technologies) equipped with an ion spray ionization source (ESI). MS (+) spectra were acquired by direct infusion (5 ml/min) of a solution containing the appropriate sample as oxalate salt (10 nmol/ml), dissolved in a 0.1% acetic acid solution, with mobile phase methanol/water 50:50, at the optimum ion voltage of 4800 V. The yields reported are based on a single experiment and are not optimized. The final compounds were converted into hydrogen oxalate. Melting points were determined with a Stuart SMP3 and they are uncorrected. The purity of the salts was confirmed by elemental analysis on a Carlo Erba 1106 Analyzer and the values obtained are within ±0.4% of the calculated ones. The purity was higher than 97%. The oxalate salts were tested for the biological activity.

The compounds **3** [40], **6** [43], **7** [43], **13**[44], **15** [44], **24** [43] were obtained as previously reported.

4.1.1. (2-Cyclohexyl-2-phenyl-[1,3]-dioxolan-4-yl)methanol (2)

An excess of glycerol (26.56 mmol) and a catalytic amount of p-toluenesulfonic acid (0.53 mmol) were added to a solution of cylohexylphenyl ketones (13.28 mmol) in toluene (250 mL). The mixture was refluxed and water was removed in a Dean-Stark trap until the formation of water stopped. After completion of the reaction a mixture of CH_2Cl_2/H_2O was added. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The organic layers were combined, washed with a saturated solution of $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The unassigned diastereoisomeric mixture of the title compound was obtained as a yellow oil (3.31 g, 12.61 mmol, 95% yield) and used without further purification.

^{1}H NMR (DMSO, 200 MHz): δ = 0.91-1.22 (5H, m, Cyc), 1.49-1.72 (7H, m, Cyc, OH), 3.58 (1H, dd, J = 7.2, 8.9 Hz, CHa-5 Diox), 3.76 (1H, dd, J = 7.2, 8.2 Hz, CHb-5 Diox), 4.00 (2H, m, CH_2OH), 4.21-4.35 (1H, m, CH-4 Diox), 7.21-7.40 (5H, m, Ar). ESI-HRMS calcd for $C_{16}H_{23}O_3$ $[M+H]^+$ 263.1642, found 263.1645.

4.1.2. (2-Cyclohexyl-2-phenyl-1,3-dioxolan-4-yl)methyl 4-methylbenzenesulfonate (4)

p-Toluenesulfonyl chloride (8.40 mmol) was added at 0°C to a solution of **2** (7.63 mmol) and triethylamine (1.0 mmol) in dry CH_2Cl_2 (10 mL). The mixture was stirred at room temperature for 12 h. Ice water was added and the mixture was extracted with CH_2Cl_2. The organic extracts were collected, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to yield the title compound. Single pure *cis/trans* diastereomer was obtained by using flash column chromatography (cyclohexane/ethyl acetate 97.5/2.5) as an oil.

Cis-**4** (1.81 g, 4.35 mmol, 57% yield)

^{1}H NMR ($CDCl_3$, 200 MHz): δ = 0.91-1.12 (5H, m, Cyc), 1.49-1.78 (6H, m, Cyc), 2.48 (3H, s, CH_3), 3.38 (1H, dd, J = 7.4, 8.1 Hz, CHa-5 Diox), 3.71 (1H, dd, J = 2.4, 4.9 Hz, CHaTs), 3.90 (1H, dd, J= 4.7, 4.9 Hz CHbTs), 4.01 (1H, dd, J = 6.3, 7.4 Hz, CHb-5 Diox), 4.11 (1H, m, CH-4 Diox), 7.18-7.41 (7H, m, Ar, CH-3, CH-5 Ts), 7.71 (2H, d, J= 8.2 Hz, CH-2, CH-6 Ts). ESI-HRMS calcd for $C_{23}H_{29}O_5S$ $[M+H]^+$ 417.1730, found 417.1729.

Trans-**4** (0.48 g, 1.14 mmol, 15% yield)

^{1}H NMR ($CDCl_3$, 200 MHz): δ = 0.98-1.15 (5H, m, Cyc), 1.43-1.77 (6H, m, Cyc), 2.47 (3H, s, CH_3), 3.61-3.78 (2H, m, CH_2-5 Diox), 3.84-4.07 (3H, m, CH_2Ts, CH-4 Diox), 7.19-7.36 (5H, m, Ar), 7.37 (2H, d, J= 8.3 Hz, CH-3, CH-5 Ts), 7.82 (2H, d, J= 8.3 Hz, CH-2, CH-6 Ts). ESI-HRMS calcd for $C_{23}H_{29}O_5S$ $[M+H]^+$ 417.1730, found 417.1729.

4.1.3. 4-(Chloromethyl)-2,2-dicyclohexyl-1,3-dioxolane (5)

The title compound was obtained as an oil (1.09 g, 3.80 mmol, 74%) starting from dicyclohexyl ketone (5.14 mmol) and 3-chloro-1,2-propanediol (7.71 mmol), by following the same procedure described for **2**.

^{1}H NMR ($CDCl_3$, 200 MHz): δ = 1.04–1.41 (10H, m, Cyc), 1.54–1.87 (12H, m, 2x Cyc.), 3.44 (1H, dd, J = 7.9, 10.6 Hz, CHa-5 Diox), 3.57–3.71 (2H, m, CH_2Cl), 4.08 (1H, dd, J = 5.3, 10.6 Hz, CHb-5 Diox), 4.30–4.43 (1H, m, CH-4 Diox). ESI-HRMS calcd for $C_{16}H_{28}O_2{}^{35}Cl$ $[M+H]^+$ 287.1772, found 287.1773. Calcd for $C_{16}H_{28}O_2{}^{37}Cl$ $[M+H]^+$ 289.1743, found 289.1744.

4.1.4. General procedure for the synthesis of the amines 8-12a,b; 14a,b; 21a,b; 23a,b

A large excess of 4-benzylpiperidine or 1-benzylpiperazine (5-10 equiv.) and a catalytic amount of KI were added to a solution of chloromethyl (**3**, **5-7**) or tosyl derivative (**4, 13, 24**) (0.34-2.25 mmol) in 2-methoxyethanol. The resulting mixture was stirred and was refluxed for 20 h. The solvent was evaporated under vacuum, $CHCl_3$ was added, and the residue was washed with a solution of 5% NaOH (2x) and with brine. The organic layer was dried over Na_2SO_4 and the solvent was evaporated under vacuum to give the desired amine as an oil. The residue was purified by using flash column chromatography.

4.1.5. 4-Benzyl-1-[(2,2-diphenyl-1,3-dioxolan-4-yl)methyl]piperidine (8a)

The title compound was obtained from **3** [40] and 4-benzylpiperidine and was purified by using flash column chromatography (cyclohexane/ethyl acetate 70/30) to give **8a** (0.44 g, 1.06 mmol, 78% yield) as an oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.41-1.63 (5H, m, CH_2-3, CH-4, CH_2-5), 1.89-2.16 (2H, m, CHa-2, CHa-6 Piper), 2.43-2.59 (3H, m, CHaN, CH_2Ph), 2.61 (1H, dd, J = 6.2, 12.1, CHbN), 2.77-2.94 (1H, m, CHb-2/CHb-6 Piper), 2.99-3.19 (1H, m, CHb-2/CHb-6 Piper), 3.77 (1H, dd, J = 7.2, 7.6 Hz, CHa-5 Diox), 4.15 (1H, dd, J = 7.6, 7.8 Hz, CHb-5 Diox), 4.27-4.50 (1H, m, CH-4 Diox), 7.01-7.37 (11H, m, Ar_2, Ph), 7.42-7.69 (4H, m, Ar_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 31.8 (CH_2, C-3/C-5 Piper), 32.2 (CH_2, C-3/C-5 Piper), 37.0 (CH, C-4 Piper), 42.8 (CH_2, CH_2Ph), 53.9 (CH_2, C-2/C-4 Piper), 54.4 (CH_2, C-2/C-4 Piper), 60.7 (CH_2, CH_2N), 68.6 (CH_2, C-5 Diox), 75.4 (CH, C-4 Diox), 110.1 (C, C-2 Diox), 125.7 (CH, C-4 Ph), 126.3 (4CH, C-2, C-6 Ar_2), 128.0 (2CH, C-3, C-5 Ph), 128.2 (4CH, C-3, C-5 Ar_2), 128.3 (2CH, C-4 Ar_2), 128.9 (2CH, C-2, C-6 Ph), 138.3 (C, C-1 Ph), 142.7 (2C, C-1 Ar_2). ESI-HRMS calcd for $C_{28}H_{32}NO_2$ $[M+H]^+$ 414.2428, found 414.2430.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.03 g, 0.07 mmol, yield 44%).

mp: 200-202 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 1.32-1.53 (5H, m, CH_2-3, CH-4, CH_2-5), 2.41-2.52 (2H, m, CH_2Ph), 2.71-2.91 (2H, m, CH_2N), 3.01-3.27 (2H, m, CHa-2,CHa-6 Piper), 3.29-3.39 (1H, m, CHb-2/CHb-6 Piper), 3.42-3.53 (1H, m, CHb-2/CHb-6 Piper), 3.74 (1H, dd, J = 7.2, 7.9 Hz, CHa-5 Diox), 4.12 (1H, dd, J = 7.2, 7.5 Hz, CHb-5 Diox), 4.40-4.64 (1H, m, CH-4 Diox), 7.11-7.51 (15H, m, Ar_2, Ph).

ESI-HRMS calcd for $C_{28}H_{32}NO_2$ $[M+H]^+$ 414.2428, found 414.2430. Anal. Calcd. for $C_{30}H_{33}NO_6$: C, 71.55; H, 6.61; N, 2.78; Found C, 71.51; H, 6.42; N, 2.63.

4.1.6. 1-Benzyl-4-[(2,2-diphenyl-1,3-dioxolan-4-yl)methyl]piperazine (8b)

The title compound was obtained from **3** [40] and 1-benzylpiperazine and was purified by using flash column chromatography (cyclohexane/ethyl acetate 95/5) to give **8b** (0.75 g, 1.8 mmol, 80% yield) as an oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.52-2.83 (10H, m, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper, CH_2N), 3.52 (2H, s, CH_2Ph), 3.79 (1H, dd, J = 7.1, 7.6 Hz, CHa-5 Diox), 4.16 (1H, dd, J = 7.1, 7.9 Hz, CHb-5 Diox), 4.35-4.48 (1H, m, CH-4 Diox), 7.20-7.41 (11H, m, Ph, Ar_2), 7.48-7.69 (4H, m, Ar_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 52.7 (2CH_2, C-2, C-6 Piper) 53.4 (2CH_2, C-3, C-5 Piper), 61.0 (CH_2N), 62.8 (CH_2, CH_2Ph) 69.0 (CH_2, C-5 Diox), 74.9 (CH, C-4 Diox), 60.7 (CH_2, CH_2N), 110.1 (C, C-2 Diox), 126.2 (4CH, C-3, C-5 Ar_2), 127.5 (CH, C-4 Ph), 120.1 (2CH, C-2, C6 Ar), 128.2 (2CH, C-2, C-6 Ar’), 128.3 (2CH, C-3, C-5 Ph), 129.5 (2CH, C-2, C-6 Ph), 137.2 (C, C-1 Ph). 142.4 (C, C-4 Ar), 142.5 (C, C-4 Ar’). 142.7, (2C, C-1 Ar, Ar’). ESI-HRMS calcd for $C_{27}H_{31}N_2O_2$ $[M+H]^+$ 415.2380, found 415.2382.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.24 g, 0.47 mmol, yield 51%).

mp: 228-229 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 2.61-3.12 (10H, m, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper, CH_2N), 3.71 (1H, dd, J = 7.1, 8.0 Hz, CHa-5 Diox), 3.91-4.21 (3H, m, CHb-5 Diox, CH_2Ph), 4.25-4.46 (1H, m, CH-4 Diox), 7.19-7.36 (15H, m, Ar_2, Ph).

ESI-HRMS calcd for $C_{27}H_{31}N_2O_2$ $[M+H]^+$ 415.2380, found 415.2382. Anal. Calcd. for $C_{31}H_{34}N_2O_{10}$: C, 62.62; H, 5.76; N, 4.71; Found C, 62.65; H, 5.82; N, 4.82.

4.1.7. *Cis*-4-benzyl-1-[(2-cyclohexyl-2-phenyl-1,3-dioxolan-4-yl)methyl]piperidine (*cis*-9a)

The title compound was obtained from *cis*-**4** and 4-benzylpiperidine and was purified by using flash column chromatography (cyclohexane/ethyl acetate 95/5) to give *cis*-**9a** (0.27 g, 0.65 mmol, 94% yield) as a yellow oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.81-1.26 (5H, m, Cyc), 1.31-1.86 (11H, m, Cyc, CH_2-3, CH-4, CH_2-5 Piper), 1.87-2.13 (2H, m, CHa-2, CHa-6 Piper), 2.22-2.39 (1H, m, CHb-2/CHb-6 Piper), 2.41-2.52 (2H, m, CH_2N), 2.55 (2H, d, J = 5.6 Hz, CH_2Ph), 3.01-3.13 (1H, m, CHb-2/CHb-6 Piper), 3.61-3.78 (2H, m, CH_2-5 Diox), 4.23-4.38 (1H, m, CH-4 Diox), 7.11 (2H, d, J = 7.4 Hz, CH-2, CH-6 Ph), 7.23 (1H, t, J = 7.2 Hz, CH-4 Ph), 7.32-7.49 (7H, m, CH-3, CH-4 Ph, CH-2, XH-3, CH-4, CH-5, CH-6 Ar); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 26.1 (2CH_2, Cyc) 26.2 (CH_2, Cyc), 26.3 (CH_2, Cyc), 26.8 (CH_2, Cyc), 31.3 (CH_2, C-3/C-5 Piper), 31.9 (CH_2, C-3/C-5 Piper), 37.1 (CH, C-4 Piper), 42.7 (CH_2, CH_2Ph), 47.1 (CH, C-1 Cyc), 52.9 (CH_2, C-2/C-4 Piper), 54.2 (CH_2, C-2/C-4 Piper), 60.2 (CH_2, CH_2N), 68.6 (CH_2, C-5 Diox), 74.6 (CH, C-4 Diox), 112.5 (C, C-2 Diox), 125.8 (CH, C-4 Ph), 126.4 (2CH, C-3, C-5 Ar), 127.7 (2CH, C-2, C-5 Ar), 128.3 (2CH, C-3, C-5 Ph), 128.9 (CH, C-4 Ar), 129.0 (2CH, C-2, C-6 Ph), 139.3 (C, C-1 Ar), 140.6 (C, C-1 Ph). ESI-HRMS calcd for $C_{28}H_{38}NO_2$ $[M+H]^+$ 420.2897, found 420.2895.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.18 g, 0.36 mmol, yield 55%).

mp: 205-207 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 0.71-1.31 (5H, m, Cyc), 1.41-1.81 (11H, m, Cyc, CH_2-3, CH-4, CH_2-5 Piper), 2.44-2.51 (2H, m, CH_2Ph), 2.52-2.73 (2H, m, CH_2N), 2.71-3.32 (4H, m, CH_2- 2/CH_2-6 Piper), 3.38-3.43 (1H, m, CHa-5 Diox), 4.05-4.13 (1H, m, CHb-5 Diox), 4.23-4.49 (1H, m, CH-4 Diox), 7.11-7.42 (10H, m, Ar, Ph).

ESI-HRMS calcd for $C_{28}H_{38}NO_2$ $[M+H]^+$ 420.2897, found 420.2895. Anal. Calcd. for $C_{30}H_{39}NO_6$: C, 70.70; H, 7.71; N, 2.75; Found C, 70.83; H, 7.77; N, 2.96.

4.1.8. *Cis*-1-benzyl-4-[(2-cyclohexyl-2-phenyl-1,3-dioxolan-4-yl)methyl]piperazine (*cis*-9b)

The title compound was obtained from *cis*-**4** and 1-benzylpiperazine and was purified by using flash column chromatography (cyclohexane/ethyl acetate 85/15) to give *cis*-**9b** (0.37 g, 0.88 mmol, 99% yield) as an oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.91-1.24 (5H, m, Cyc), 1.32-1.62 (6H, m, Cyc), 2.47 (2H, d, J = 4.8 Hz, CH_2N), 2.52-2.98 (8H, m, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper), 3.31 (1H, dd, J = 8.3, 8.6 Hz, CHa-5 Diox), 3.61 (2H, s, CH_2Ph), 4.19 (1H, dd, J = 6.1, 8.23 Hz, CHb-5 Diox), 4.27-4.41 (1H, m, CH-4 Diox), 7.21-7.45 (10H, m, Ar, Ph).

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 25.8 (2CH_2, Cyc), 25.9 (CH_2, Cyc), 26.5 (CH_2, Cyc), 26.7 (CH_2, Cyc), 47.1 (CH, C-1 Cyc), 51.7 (2CH_2, C-3,C-5 Piper), 52.5 (2CH_2, C-2,C-6 Piper), 60.5 (CH_2, CH_2N), 62.1 (CH_2, CH_2Ph), 68.2 (CH_2, C-5 Diox), 75.1 (CH, C-4 Diox), 112.7 (C, C-2 Diox), 125.9 (CH, C-4 Ph), 126.6 (2CH, C-3, C-5 Ar), 127.2 (CH, C-4 Ar), 127.3 (2CH, C-2, C-5 Ar), 127.4 (C, C-1 Ar), 128.2 (2CH, C-3, C-5 Ph), 129.4 (2CH, C-2, C-6 Ph), 142.3 (C, C-1 Ph). ESI-HRMS calcd for $C_{27}H_{37}N_2O_2$ $[M+H]^+$ 421.2850, found 421.2853.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.19 g, 0.32 mmol, yield 40%).

mp: 225-226 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 0.79-1.32 (5H, m, Cyc), 1.45-1.77 (6H, m, Cyc), 2.47-2.71 (10H, m, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper, CH_2N), 3.21(1H, dd, J= 7.3, 8.2 Hz, CHa-5 diox), 3.95 (2H, s, CH_2Ph), 4.20-4.41 (2H, m, CHb-5, CH-4 Diox), 7.20-7.48 (10H, m, Ar, Ph).
ESI-HRMS calcd for $C_{27}H_{37}N_2O_2$ $[M+H]^+$ 421.2850, found 421.2853. Anal. Calcd. for $C_{31}H_{40}N_2O_{10}$: C, 61.99; H, 6.71; N, 4.66; Found C, 61.86; H, 6.56; N, 4.47.

4.1.9. *Trans*-4-benzyl-1-[(2-cyclohexyl-2-phenyl-1,3-dioxolan-4-yl)methyl]piperidine (*trans*-9a)

The title compound was obtained from *trans*-**4** and 4-benzylpiperidine and was purified by using flash column chromatography (cyclohexane/ethyl acetate 90/10) to give *trans*-**9a** (0.31 g, 0.74 mmol, 82% yield) as a yellow oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.93-1.23 (5H, m, Cyc), 1.35-1.84 (11H, m, Cyc, CH_2-3, CH-4, CH_2-5 Piper), 1.91-2.17 (2H, m, CHa-2, CHa-6 Piper), 2.43-2.72 (4H, m, CH_2N, CH_2Ph), 2.72-2.90 (1H, m, CHb-2/CHb-6 Piper), 2.97-3.15 (1H, m, CHb-2/CHb-6 Piper), 3.58 (1H, dd, J = 6.5, 7.3 Hz, CHa-5 Diox), 3.83 (1H, dd, J = 7.0, 7.3 Hz, CHb-5 Diox), 4.02-4.21 (1H, m, CH-4 Diox), 7.12-7.46 (10H, m, Ar, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 25.8 (2CH_2, Cyc) 25.9 (CH_2, Cyc), 26.5 (CH_2, Cyc), 26.7 (CH_2, Cyc), 31.4 (CH_2, C-3/C-5 Piper), 32.0 (CH_2, C-3/C-5 Piper), 37.3 (CH, C-4 Piper), 42.9 (CH_2, CH_2Ph), 46.5 (CH, C-1 Cyc), 53.4 (CH_2, C-2/C-4 Piper), 54.0 (CH_2, C-2/C-4 Piper), 60.3 (CH_2, CH_2N), 68.1 (CH_2, C-5 Diox), 73.9 (CH, C-4 Diox), 112.1 (C, C-2 Diox), 125.8 (CH, C-4 Ph), 126.2 (2CH, C-3, C-5 Ar), 127.5 (CH, C-4 Ar), 127.7 (2CH, C-2, C-5 Ar), 127.8 (C, C-1 Ar), 128.5 (2CH, C-3, C-5 Ph), 129.6 (2CH, C-2, C-6 Ph), 141.4 (C, C-1 Ph). ESI-HRMS calcd for $C_{28}H_{38}NO_2$ $[M+H]^+$ 420.2897, found 420.2895.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.10 g, 0.20 mmol, yield 45%).

mp: 198-200 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 0.73-1.26 (5H, m, Cyc), 1.35-1.82 (11H, m, Cyc, CH_2-3, CH-4, CH_2-5 Piper), 2.45-2.59 (2H, m, CH_2Ph), 2.61-2.92 (2H, m, CH_2N), 2.71-3.32 (4H, m,

CH_2- 2/CH_2-6 Piper), 3.62 (1H, dd, J = 6.1, 7.8 Hz, CHa-5 Diox), 3.76 (1H, dd, J = 7.3, 7.8 Hz, CHb-5 Diox), 4.11-4.26 (1H, m, CH-4 Diox), 7.10-7.48 (10H, m, Ar, Ph).

ESI-HRMS calcd for $C_{28}H_{38}NO_2$ $[M+H]^+$ 420.2897, found 420.2895. Anal. Calcd. for $C_{30}H_{39}NO_6$: C, 70.70; H, 7.71; N, 2.75; Found C, 70.65; H, 7.58; N, 2.59.

4.1.10. *Trans*-1-benzyl-4-[(2-cyclohexyl-2-phenyl-1,3-dioxolan-4-yl)methyl]piperazine (*trans*-9b)

The title compound was obtained from *trans*-**4** and 1-benzylpiperazine and was purified by using flash column chromatography (cyclohexane/ethyl acetate 90/10) to give *trans*-**9b** (0.37 g, 0.88 mmol, 99% yield) as an oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.91-1.27 (5H, m, Cyc), 1.49-1.79 (6H, m, Cyc), 2.62 (2H, br s, CH_2N), 2.41-2.70 (8H, m, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper), 3.59 (1H, dd, J = 6.4, 7.1 Hz, CHa-5 Diox), 3.72-3.91 (3H, m, CHb-5 Diox, CH_2Ph), 4.12-4.32 (1H, m, CH-4 Diox), 7.21-7.48 (10H, m, Ar, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 25.8 (2CH_2, Cyc), 25.9 (CH_2, Cyc), 26.5 (CH_2, Cyc), 26.7 (CH_2, Cyc), 46.2 (CH, C-1 Cyc), 51.1 (2CH_2, C-3,C-5 Piper), 51.9 (2CH_2, C-2,C-6 Piper), 60.2 (CH_2, CH_2N), 61.6 (CH_2, CH_2Ph), 67.8 (CH_2, C-5 Diox), 73.5 (CH, C-4 Diox), 113.2 (C, C-2 Diox), 125.9 (CH, C-4 Ph), 126.1 (2CH, C-3, C-5 Ar), 127.4 (CH, C-4 Ar), 127.5 (2CH, C-2, C-5 Ar), 127.7 (C, C-1 Ar), 128.6 (2CH, C-3, C-5 Ph), 129.4 (2CH, C-2, C-6 Ph), 142.4 (C, C-1 Ph). ESI-HRMS calcd for $C_{27}H_{37}N_2O_2$ $[M+H]^+$ 421.2850, found 421.2851.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.16 g, 0.26 mmol, yield 35%).

mp: 230-232 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 0.83-1.22 (5H, m, Cyc), 1.49-1.76 (6H, m, Cyc), 2.69-3.05 (10H, m, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper, CH_2N), 3.56 (1H, dd, J= 6.6, 8.1 Hz, CHa-5 Diox), 3.73 (2H, dd, J = 6.6, 7.7 Hz, CHb-5 Diox), 3.91-4.22 (3H, m, CH_2Ph, CH-4 Diox), 7.22-7.48 (10H, m, Ar, Ph).

ESI-HRMS calcd for $C_{27}H_{37}N_2O_2$ $[M+H]^+$ 421.2850, found 421.2851. Anal. Calcd. for $C_{31}H_{40}N_2O_{10}$: C, 61.99; H, 6.71; N, 4.66; Found C, 61.92; H, 6.72; N, 4.73.

4.1.11. 4-Benzyl-1-[(2,2-dicyclohexyl-1,3-dioxolan-4-yl)methyl]piperidine (10a)

The title compound was obtained from **5** and 4-benzylpiperidine and was purified by using flash column chromatography (cyclohexane/ethyl acetate 90/10) to give **10a** (0.37 g, 0.87 mmol, 71% yield) as a yellow oil.

^{1}H NMR ($CDCl_3$, 200 MHz): δ = 0.92-1.39 (10H, m, Cyc_2), 1.51-1.86 (17H, m, Cyc_2, CH_2-3, CH-4, CH_2-5 Piper), 2.31-2.49 (2H, m, CHa-2, CHa-6 Piper), 2.59 (2H, J= 6.1 Hz, CH_2Ph), 2.63-2.83 (2H, m, CH_2N), 2.99-3.12 (1H, m, CHb-2/CHb-6 Piper), 3.13-3.39 (1H, m, CHb-2/CHb-6 Piper), 3.43 (1H, dd, J = 7.6, 9.0 Hz, CHa-5 Diox), 4.17 (1H, dd, J = 6.3, 7.4 Hz, CHb-5 Diox), 4.38-4.56 (1H, m, CH-4 Diox), 7.17 (2H, dd, J = 1.5, 7.4 Hz, CH-2, CH-6 Ph), 7.23 (1H, t, J = 7.1 Hz, CH-4 Ph), 7.32 (2H, dd, J = 7.1, 7.4 Hz, CH-3, CH-5 Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 26.0 (4CH_2, Cyc_2) 26.1 (2CH_2, Cyc_2) 26.8 (2CH_2, Cyc_2), 26.9 (2CH_2, Cyc_2), 31.6 (CH_2, C-3/C-5 Piper), 32.0 (CH_2, C-3/C-5 Piper), 36.9 (CH, C-4 Piper), 42.9 (CH_2, CH_2Ph), 44.2 (2CH, C-1 Cyc), 53.8 (CH_2, C-2/C-4 Piper), 54.6 (CH_2, C-2/C-4 Piper), 60.7 (CH_2, CH_2N), 70.6 (CH_2, C-5 Diox), 75.9 (CH, C-4 Diox), 116.9 (C, C-2 Diox), 125.8 (CH, C-4 Ph), 128.2 (2CH, C-3, C-5 Ph), 128.9 (2CH, C-2, C-6 Ph), 140.6 (C, C-1 Ph). ESI-HRMS calcd for $C_{28}H_{44}NO_2$ $[M+H]^+$ 426.3367, found 426.3368.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.06 g, 0.12 mmol, yield 40%).

mp: 210-212 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 0.88-1.23 (10H, m, Cyc_2), 1.43-1.81 (17H, m, Cyc_2, CH_2-3, CH-4, CH_2-5 Piper), 2.41-2.52 (2H, m, CH_2Ar), 2.52-2.73 (2H, m, CH_2N), 2.71-3.32 (4H, m, CH_2- 2/CH_2-6 Piper), 3.40 (1H, dd, J = 7.4, 8.1 Hz, CHa-5 Diox), 4.11 (1H, dd, J = 7.1, 7.4 Hz, CHb-5 Diox), 4.20-4.49 (1H, m, CH-4 Diox), 7.11-7.39 (5H, m, Ar).

ESI-HRMS calcd for $C_{28}H_{44}NO_2$ $[M+H]^+$ 426.3367, found 426.3368. Anal. Calcd. for $C_{30}H_{45}NO_6$: C, 69.87; H, 8.80; N, 2.72; Found C, 69.91; H, 8.93; N, 2.82.

4.1.12. 1-Benzyl-4-[(2,2-dicyclohexyl-1,3-dioxolan-4-yl)methyl]piperazine (10b)

The title compound was obtained from **5** and 1-benzylpiperazine and was purified by using flash column chromatography (cyclohexane/ethyl acetate 90/10) to give **10b** (0.47 g, 1,10 mmol, 90% yield) as a yellow oil.

^{1}H NMR ($CDCl_3$, 200 MHz): δ = 0.91-1.44 (10H, m, Cyc_2), 1.50-1.81 (12H, m, Cyc_2), 2.49-2.72 (10H, m, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper, CH_2N), 3.43 (1H, dd, J = 7.5, 9.0 Hz, CHa-5 Diox), 3.60 (2H, s, CH_2Ar), 4.12 (1H, dd, J = 6.0, 7.5 Hz, CHb-5 Diox), 4.22-4.41 (1H, m, CH-4 Diox), 7.17-7.41 (5H, m, CH-2, CH-3, CH-4, CH-5, CH-6 Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 26.1 (4CH_2, Cyc_2), 26.3 (2CH_2, Cyc_2), 26.8 (2CH_2, Cyc_2), 26.9 (2CH_2, Cyc_2), 43.0 (CH, C-1 Cyc), 44.0 (CH, C-1 Cyc'), 52.2 (2CH_2, C-3,C-5 Piper), 53.1 (2CH_2, C-2,C-6 Piper), 60.5 (CH_2, CH_2N), 62.5 (CH_2, CH_2Ph), 70.7 (CH_2, C-5 Diox), 75.6 (CH, C-4 Diox), 116.1 (C, C-2 Diox), 127.1(CH, C-4 Ph), 128.0 (2CH, C-3, C-5 Ph), 129.1 (2CH, C-2, C-6 Ph), 136.4 (C, C-1 Ph). ESI-HRMS calcd for $C_{27}H_{43}N_2O_2$ $[M+H]^+$ 427.3319, found 427.3322.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.28 g, 0.47 mmol, yield 50%).

mp: 226-228 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 0.89-1.27 (10H, m, Cyc_2), 1.45-1.78 (12H, m, Cyc_2), 2.67-3.04 (10H, m, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper, CH_2N), 3.33-3.40 (1H, m, CHa-5 Diox), 3.80-3.93 (2H, m, CH_2Ar), 4.01-4.12 (1H, m, CHb-5 Diox), 4.18-4.37 (1H, m, CH-4 Diox), 7.26-7.44 (5H, m, Ar).

ESI-HRMS calcd for $C_{27}H_{43}N_2O_2$ $[M+H]^+$ 427.3319, found 427.3322. Anal. Calcd. for $C_{31}H_{46}N_2O_{10}$: C, 61.37; H, 7.64; N, 4.62; Found C, 61.45; H, 7.71; N, 4.44.

4.1.13. 4-Benzyl-1-[(2,2-diphenyl-1,3-oxathiolan-5-yl)methyl]piperidine (11a)

The title compound was obtained from **6** [43] and 4-benzylpiperidine and was purified by using flash column chromatography (cyclohexane/ethyl acetate 90/10) to give **11a** (0.12 g, 0.28 mmol, 82% yield) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.31-1.53 (5H, m, CH_2-3, CH-4, CH_2-5 Piper), 1.91-2.27 (2H, m, CHa-2, CHa-6 Piper), 2.54 (2H, d, J = 6.5 Hz, CH_2Ph), 2.62-3.22 (6H, m, CH_2N, CHb-2, CHb-6 Piper, CH_2-4 Oxath), 4.22-4.44 (1H, m, CH-5 Oxath), 7.04-7.42 (13H, m, Ar_2, Ph), 7.62 (2H, d, J = 7.1 Hz, Ar_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 32.1 (CH_2, C-3/C-5 Piper), 32.2 (CH_2, C-3/C-5 Piper), 34.7 (CH_2, C-4 Oxath), 37.5 (CH, C-4 Piper), 43.1 (CH_2, CH_2Ph), 54.1 (CH_2, C-2/C-4 Piper), 55.0 (CH_2, C-2/C-4 Piper), 62.4 (CH_2, CH_2N), 70.3 (CH, C-5 Oxath), 89.3 (C, C-2 Oxath), 125.7 (CH, C-4 Ph), 127.0 (4CH, C-2, C-6 Ar_2), 127.6 (2CH, C-4 Ar_2), 128.1 (2CH, C-3, C-5 Ph), 128.3 (4CH, C-3, C-5 Ar_2), 129.1 (2CH, C-2, C-6 Ph), 140.5 (C, C-1 Ph), 143.4 (2C, C-1 Ar_2). ESI-HRMS calcd for $C_{28}H_{32}NOS$ $[M+H]^+$ 430.2199, found 430.2201.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.04 g, 0.08 mmol, yield 48%).

mp: 182-184 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 1.31-1.97 (5H, m, CH_2-3, CH-4, CH_2-5 Piper), 2.39-2.64 (2H, m, CH_2Ph), 2.67-2.99 (2H, m, CHa-2, CHa-6 Piper), 3.02-3.19 (2H, m, CH_2-4 Oxath), 3.32-3.64 (4H, m, CHb-2, CHb-6 Piper, CH_2N),4.31-4.50 (1H, m, CH-5 Oxath), 7.10-7.43 (13H, m, Ar_2, Ph), 7.60 (2H, d, J = 7.0 Hz , Ar_2).

ESI-HRMS calcd for $C_{28}H_{32}NOS$ $[M+H]^+$ 430.2199, found 430.2201. Anal. Calcd. for $C_{30}H_{33}NO_5S$: C, 69.34; H, 6.40; N, 2.70; Found C, 69.51; H, 6.61; N, 2.83.

4.1.14. 1-Benzyl-4-[(2,2-diphenyl-1,3-oxathiolan-5-yl)methyl]piperazine (11b)

The title compound was obtained from **6** [43] and 1-benzylpiperazine and was purified by using flash column chromatography (cyclohexane/ethyl acetate 65/35) to give **11b** (0.22 g, 0.51 mmol, 88% yield) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.48-2.89 (10H, m, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper, CH_2-4 Oxath), 3.12-3.28 (2H, m, CH_2N), 3.54 (2H, s, CH_2Ph), 4.20-4.45 (1H, m, CH-5 Oxath), 7.16-7.44 (13H, m, Ar_2, Ph), 7.63 (2H, d, J = 7.1 Hz, Ar_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 34.8 (CH_2, C-4 Oxath), 52.1 (2CH_2, C-3,C-5 Piper), 52.9 (2CH_2, C-2,C-6 Piper), 60.6 (CH_2, CH_2N), 62.6 (CH_2, CH_2Ph), 70.5 (CH, C-5 Oxath), 89.8 (C, C-2 Oxath), 126.2 (CH, C-4 Ph), 127.1 (4CH, C-2, C-6 Ar_2), 127.5 (2CH, C-4 Ar_2), 128.0 (2CH, C-3, C-5 Ph), 128.3 (4CH, C-3, C-5 Ar_2), 129.0 (2CH, C-2, C-6 Ph), 139.8 (C, C-1 Ph), 143.2 (2C, C-1 Ar_2). ESI-HRMS calcd for $C_{27}H_{31}N_2OS$ $[M+H]^+$ 431.2152, found 431.2154.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.10 g, 0.16 mmol, yield 50%).

mp: 210-212 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 2.71-3.12 (11H, m, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper, CH_2-4 Oxath, CHaN), 3.28 (1H, dd, J = 2.2, 4.5 Hz, CHbN), 4.03 (2H, s, CH_2Ph), 4.17-4.34 (1H, m, CH-5 Oxath), 7.16-7.44 (13H, m, Ar_2, Ph), 7.59 (2H, d, J = 7.0 Hz, Ar_2).

ESI-HRMS calcd for $C_{27}H_{31}N_2OS$ $[M+H]^+$ 431.2152, found 431.2154. Anal. Calcd. for $C_{31}H_{34}N_2O_9S$: C, 60.97; H, 5.61; N, 4.59; Found C, 61.12; H, 5.76; N, 4.31.

4.1.15. 4-Benzyl-1-[(2,2-diphenyl-1,3-dithiolan-4-yl)methyl]piperidine (12a)

The title compound was obtained from **7** [43] and 4-benzylpiperidine and was purified by using flash column chromatography (cyclohexane/ethyl acetate 98/2) to give **12a** (0.24 g, 0.55 mmol, 68% yield) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.30-1.54 (5H, m, CH_2-3, CH-4, CH_2-5 Piper), 1.91-2.21 (2H, m, CHa-2, CHa-6 Piper), 2.53 (2H, d, J = 6.1 Hz, CH_2Ph), 2.70-3.12 (4H, m, CH_2-5 Dithio, CHb-2, CHb-6 Piper), 3.16-3.40 (2H, m, CH_2N), 3.99-4.17 (1H, m, CH-4 Dithio), 7.04-7.42 (11H, m, Ar_2, Ph), 7.58 (2H, d, J = 7.1 Hz, Ar_2), 7.62 (2H, d, J = 7.1 Hz, Ar_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 31.9 (CH_2, C-3/C-5 Piper), 32.0 (CH_2, C-3/C-5 Piper), 37.7 (CH, C-4 Piper), 44.6 (CH_2, C-5 Dithio), 43.0 (CH_2, CH_2Ph), 54.0 (CH_2, C-2/C-4 Piper), 54.8 (CH_2, C-2/C-4 Piper), 56.5 (CH, C-4 Dithio), 62.6 (CH_2, CH_2N), 68.5 (C, C-2 Dithio), 125.7 (CH, C-4 Ph), 127.2 (4CH, C-2, C-6 Ar_2), 127.9 (2CH, C-4 Ar_2), 128.2 (2CH, C-3, C-5 Ph), 128.4 (4CH, C-3, C-5 Ar_2), 129.2 (2CH, C-2, C-6 Ph), 140.6 (C, C-1 Ph), 143.8 (2C, C-1 Ar_2). ESI-HRMS calcd for $C_{28}H_{32}NS_2$ $[M+H]^+$ 446.1971, found 446.1972.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.12 g, 0.22 mmol, yield 40%).

mp: 164-166 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 1.26-1.41 (2H, m, CHa-3, CHa-5 Piper), 1.52-1.76 (3H, m, CHb-3, CH-4, CHb-5 Piper), 2.39-2.64 (2H, m, CH_2Ph), 2.81-4.04 (1H, m, CHa-2/CHa-6 Piper), 3.32-3.64 (7H, m, CHa-2/CHa-6 Piper, CHb-2, CHb-6 Piper, CH_2N, CH-5 Dithio), 4.27-4.50 (1H, m, CH-3 Oxath), .704-7.38 (11H, m, Ar_2, Ph), 7.52 (2H, d, J = 7.0 Hz, Ar_2), 7.63 (2H, d, J = 7.0 Hz, Ar_2).

ESI-HRMS calcd for $C_{28}H_{32}NS_2$ $[M+H]^+$ 446.1971, found 446.1972. Anal. Calcd. for $C_{30}H_{33}NO_4S_2$: C, 67.26; H, 6.21; N, 2.61; Found C, 67.33; H, 6.28; N, 2.52.

4.1.16. 1-Benzyl-4-[(2,2-diphenyl-1,3-dithiolan-4-yl)methyl]piperazine (12b)

The title compound was obtained from **7** [43] and 1-benzylpiperazine and was purified by using flash column chromatography (cyclohexane/ethyl acetate 98/2) to give **12b** (0.16 g, 0.36 mmol, 42% yield) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.34-2.84 (10H, m, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper, CH_2-5 Dithio), 3.12-3.34 (2H, m, CH_2N), 3.55 (2H, s, CH_2Ph), 3.91-4.17 (1H, m, CH-4 Dithio), 7.13-7.41 (11H, m, Ar_2, Ph), 7.56 (2H, d, J = 7.2 Hz, Ar_2) 7.66 (2H, d, J = 7.2 Hz, Ar_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 44.5 (CH_2, C-5 Dithio), 52.1 (2CH_2, C-3, C-5 Piper), 53.0 (2CH_2, C-2,C-6 Piper), 56.7 (CH, C-4 Dithio), 61.3 (CH_2, CH_2N), 62.7 (CH_2, CH_2Ph), 68.6 (C, C-2 Dithio), 125.5 (CH, C-4 Ph), 127.3 (4CH, C-2, C-6 Ar_2), 127.8 (2CH, C-4 Ar_2), 128.1 (2CH, C-3, C-5 Ph), 128.5 (4CH, C-3, C-5 Ar_2), 129.3 (2CH, C-2, C-6 Ph), 140.7 (C, C-1 Ph), 143.6 (2C, C-1 Ar_2). ESI-HRMS calcd for $C_{27}H_{31}N_2S_2$ $[M+H]^+$ 447.1923, found 447.1925.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.1 g, 0.16 mmol, yield 50%).

mp: 211-213 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 2.59-3.12 (10H, m, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper, CH_2-5 Dithio), 3.11-3.35 (2H, m, CH_2N), 3.96-4.27 (3H, m, CH_2Ph, CH-4 Dithio), 7.16-7.52 (13H, m, Ar_2, Ph), 7.63 (2H, d, J = 7.1 Hz, Ar_2).

ESI-HRMS calcd for $C_{27}H_{31}N_2S_2$ $[M+H]^+$ 447.1923, found 447.1925. Anal. Calcd. for $C_{31}H_{34}N_2O_8S_2$: C, 59.41; H, 5.47; N, 4.47; Found C, 59.77; H, 5.69; N, 4.45.

4.1.17. 4-Benzyl-1-[(5,5-diphenyltetrahydrofuran-2-yl)methyl]piperidine (14a)

The title compound was obtained from **13** [44] and 4-benzylpiperidine and was purified by using flash column chromatography (cyclohexane/ethyl acetate 70/30) to give **14a** (0.21 g, 0.52 mmol, 90% yield) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.30-1.54 (5H, m, CH_2-3, CH-4, CH_2-5 Piper), 1.73-1.89 (1H, m, CHa-3 Fur), 1.99-2.24 (3H, m, CHb-3 Fur, CHa-2, CHa-6 Piper), 2.53 (2H, d, J = 6.1 Hz, CH_2Ph), 2.61-3.19 (3H, m, CHa-4 Fur, CHb-2, CHb-6 Piper), 3.21-3.42 (3H, m, CHa-4 Fur, CH_2N), 4.31-4.49 (1H, m, CH-2 Fur), 7.09-7.49 (15H, m, Ar_2, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 25.6 (CH_2, C-3 Fur), 31.3 (CH_2, C-3/C-5 Piper), 32.1 (CH_2, C-3/C-5 Piper), 36.8 (CH, C-4 Piper), 38.8 (CH_2, C-4 Fur), 42.8 (CH_2, CH_2Ph), 53.7 (CH_2, C-2/C-4 Piper), 54.8 (CH_2, C-2/C-4 Piper), 60.6 (CH_2, CH_2N), 67.6 (CH, C-2 Fur), 88.2 (C, C-5 Fur), 125.7 (CH, C-4 Ph), 125.9 (4CH, C-2, C-6 Ar_2), 126.85(2CH, C-4 Ar_2), 128.2 (2CH, C-3, C-5 Ph), 128.4 (4CH, C-3, C-5 Ar_2), 128.8 (2CH, C-2, C-6 Ph), 140.7 (C, C-1 Ph), 146.6 (2C, C-1 Ar_2). ESI-HRMS calcd for $C_{29}H_{34}NO$ $[M+H]^+$ 412.2635, found 412.2636.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.04 g, 0.08 mmol, yield 42%).

mp: 206-208 °C; ^{1}H NMR (DMSO, 200 MHz): δ = ^{1}H NMR (DMSO, 200 MHz): δ = 1.28-1.81 (5H, m, CH_2-3, CH-4, CH_2-5 Piper), 1.92-2.05 (1H, m, CHa-3 Fur), 2.62 (2H, d, J = 6.2 Hz, CH_2Ph), 2.63-2.75 (1H, m, CHb-3 Fur), 2.78-3.21 (4H, m, CH_2-2, CH_2-6 Piper), 3.38-4.02 (4H, m, CH_2N, CH_2-4 Fur), 4.32-4.47 (1H, m, CH-2 Fur), 7.09-7.49 (15H, m, Ar_2, Ph).

ESI-HRMS calcd for $C_{29}H_{34}NO$ $[M+H]^+$ 412.2635, found 412.2636. Anal. Calcd. for $C_{31}H_{35}NO_5$: C, 74.23; H, 7.03; N, 2.79; Found C, 74.41; H, 7.33, N, 2.89.

4.1.18. 1-Benzyl-4-[(5,5-diphenyltetrahydrofuran-2-yl)methyl]piperazine (14b)

The title compound was obtained from **13** [44] and 1-benzylpiperazine and was purified by using flash column chromatography (cyclohexane/ethyl acetate 70/30) to give **14b** (0.03 g, 0. 07 mmol, 20% yield) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.01-1.22 (1H, m, CHa-3 Fur), 1.28-1.41 (1H, m, CHb-3 Fur), 1.61-1.83 (1H, m, CHa-4 Fur), 1.96-2.13 (1H, m, CHb-4 Fur), 2.41-2.87 (10H, m, CH_2N, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper), 3.51 (2H, s, CH_2Ph), 4.33-4.49 (1H, m, CH-2 Fur), 7.09-7.49 (15H, m, Ar_2, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 25.6 (CH_2, C-3 Fur), 38.8 (CH_2, C-4 Fur), 52.1 ($2CH_2$, C-3,C-5 Piper), 53.0 ($2CH_2$, C-2,C-6 Piper), 60.5 (CH_2, CH_2N), 62.1 (CH_2, CH_2Ph), 65.2 (CH, C-2 Fur), 88.5 (C, C-5 Fur), 125.8 (CH, C-4 Ph), 125.8 (4CH, C-2, C-6 Ar_2), 127.0 (2CH, C-4 Ar_2), 128.1 (2CH, C-3, C-5

Ph), 128.5 (4CH, C-3, C-5 Ar_2), 128.9 (2CH, C-2, C-6 Ph), 140.6 (C, C-1 Ph), 145.1 (2C, C-1 Ar_2). ESI-HRMS calcd for $C_{28}H_{33}N_2O$ $[M+H]^+$ 413.2587, found 413.2586.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.03 g, 0.05 mmol, yield 51%).

mp: 222-224 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 1.49-1.71 (1H, m, CHa-3 Fur), 1.83-2.01 (1H, m, CHb-3 Fur), 2.32-2.51 (1H, m, CHa-4 Fur), 2.55-2.71 (1H, m, CHb-4 Fur), 2.74-3.31 (10H, m, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper, CH_2N), 3.82 (2H, s, CH_2Ph), 4.38-4.45 (1H, m, CH-2 Fur), 7.03-7.58 (15H, m, Ar_2, Ph).

ESI-HRMS calcd for $C_{28}H_{33}N_2O$ $[M+H]^+$ 413.2587, found 413.2586. Anal. Calcd. for $C_{32}H_{36}N_2O_9$: C, 64.85; H, 6.12; N, 4.73; Found C, 64.98; H, 6.35; N, 4.64.

4.1.19. 5-[(4-benzylpiperidin-1-yl)methyl]-2,2-diphenylcyclopenta-1-one (16a)

4-Benzylpiperidin-1-ium chloride (1.85 g, 8.75 mmol) and aqueous paraformaldehyde (0.08 g, 2.7 mol) were added to a solution of **15** [44] (0.51 g, 2.16 mmol) in 5 mL of anhydrous ethanol. The reaction mixture was refluxed for 1 h and then an additional amount of paraformaldehyde (0.06g, 2.0 mol) was added. The mixture was refluxed for further 12 h. After cooling to room temperature, the solvent was removed under reduced pressure. The crude, dissolved in CH_2Cl_2, was washed with a solution of 5% NaOH and brine and dried over anhydrous Na_2SO_4. The crude extract was purified by using flash column chromatography on silica gel (cyclohexane/ethyl acetate 70/30) to give the title compound (0.64 g, 1.51 mmol, 70% yield) as an oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.21-1.49 (2H, m, CHa-3, CHa-5 Piper), 1.52-2.05 (5H, m, CH-4, CHb-3, CHb-5 Piper, CH_2-4 Cyclopent), 2.15-2.41 (4H, m, CHa-2, CHa-6 Piper, CH_2-3 Cyclopent), 2.51-2.99 (7H, m, CHb-2, CHb-6 Piper, CH_2N, CH_2Ph, CH-5 Cyclopent), 7.04-7.44 (15H, m, Ar_2, Ph);^{13}C NMR ($CDCl_3$, 100 MHz): δ = 19.2 (CH_2, C-4 Cyclopent), 31.9 (CH_2, C-3/C-5 Piper), 32.2 (CH_2, C-3/C-5 Piper), 37.7 (CH, C-4 Piper), 39.5 (CH, C-5 Cyclopent), 40.1 (CH_2, C-3 Cyclopent.) 42.6 (CH_2, CH_2Ph), 53.7 (CH_2, C-2/C-4 Piper), 55.1 (CH_2, C-2/C-4 Piper), 61.3 (CH_2, CH_2N), 62.9 (C, C-2 Cyclopent), 125.5 (CH, C-4 Ph), 126.8 (2CH, C-4 Ar_2), 128.0 (4CH, C-3, C-5 Ar_2), 128.2 (2CH, C-3, C-5 Ph), 128.5 (4CH, C-2, C-6 Ar_2), 129.1 (2CH, C-2, C-6 Ph), 140.6 (C, C-1 Ph) 142.3 (2C, C-1 Ar_2), 214.2 (C, C-1 Cyclopent). ESI-HRMS calcd for $C_{30}H_{34}NO$ $[M+H]^+$ 424.2635, found 424.2637.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.08 g, 0.15 mmol, yield 85%).

mp: 163-165 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 1.28-1.81 (6H, m, CH_2-3, CH-4, CH_2-5 Piper, CHa-4 Cyclopent), 2.12-2.37 (1H, m, CHb-4 Cyclopent), 2.61 (2H, d, J = 6.1 Hz, CH_2Ph), 2.65-3.39 (9H, m, CH_2-2, CH_2-6 Piper, CH_2N, CH_2-3, CH-5 Cyclopent), 7.08-7.41 (15H, m, Ar_2, Ph).
ESI-HRMS calcd for $C_{30}H_{34}NO$ $[M+H]^+$ 424.2635, found 424.2637. Anal. Calcd. for $C_{32}H_{35}NO_5$: C, 74.83; H, 6.87; N, 2.73; Found C, 74.94; H, 7.03; N, 2.72.

4.1.20. 5-[(4-benzylpiperazin-1-yl)methyl]-2,2-diphenylcyclopenta-1-one (16b)

The title compound was obtained from **15** [44] and 4-benzylpiperazinium chloride (1.85 g, 8.75 mmol) by following the same procedure described for **16a**. The crude extract was purified by using flash column chromatography on silica gel (cyclohexane/ethyl acetate 70/30) to give **16b** (0.59 g, 1.39 mmol, 66% yield) as a yellow oil.
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.75-1.91 (1H, m, CHa-4 Cyclopent), 2.21-2.35 (1H, m, CHb-4 Cyclopent), 2.42-2.91 (13H, m, CH_2N, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper, CH_2-3, CH-5 Cyclopent), 3.53 (2H, s, CH_2Ph), 7.05-7.40 (15H, m, Ar_2, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 19.9 (CH_2, C-4 Cyclopent), 39.7 (CH, C-5 Cyclopent), 40.3 (CH_2, C-3 Cyclopent), 52.2 (2CH_2, C-3,C-5 Piper), 53.2 (2CH_2, C-2,C-6 Piper), 60.5 (CH_2, CH_2N), 61.9 (CH_2, CH_2N), 62.9 (C, C-2 Cyclopent), 125.8 (CH, C-4 Ph), 126.8 (2CH, C-4 Ar_2), 128.1 (4CH, C-3, C-5 Ar_2), 128.2 (2CH, C-3, C-5 Ph), 128.6 (4CH, C-2, C-6 Ar_2), 129.1 (2CH, C-2, C-6 Ph), 140.7 (C, C-1 Ph), 142.4 (2C, C-1 Ar_2), 216.1 (C, C-1 Cyclopent). ESI-HRMS calcd for $C_{29}H_{33}N_2O$ $[M+H]^+$ 425.2587, found 425.2589.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.13 g, 0.22 mmol, yield 74%).
mp: 220-222 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 1.53-1.72 (1H, m, CHa-4 Cyclopent), 2.12-2.39 (1H, m, CHb-4 Cyclopent), 2.54-3.11 (13H, m, CH_2N, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper, CH_2-3, CH-5 Cyclopent), 3.52 (2H, s, CH_2Ph), 7.05-7.40 (15H, m, Ar_2, Ph).
ESI-HRMS calcd for $C_{29}H_{33}N_2O$ $[M+H]^+$ 425.2587, found 425.2589. Anal. Calcd. for $C_{33}H_{36}N_2O_9$: C, 65.55; H, 6.00; N, 4.63; Found C, 65.87; H, 6.31; N, 4.71.

4.1.21. 5-[(4-Benzylpiperidin-1-yl)methyl]-2,2-diphenylcyclopenta-1-ol (17a)

The title compound was obtained as diastereomeric mixture from **16a** (1.18 mmol) using an excess of $NaBH_4$ (1.8 mmol) at 0 °C in ethanol. The resulting mixture was stirred for 30 minutes at room temperature, then concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 and water. The organic layer was separated and the aqueous one was extracted with CH_2Cl_2. The organic layers were combined, washed with water, dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The *cis/trans* diastereomeric mixture was separated by using flash column chromatography (cyclohexane/ethyl acetate 90/10).

***Cis*-17a** (0.04 g, 0.10 mmol, 9 % yield) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.20-1.97 (11H, m, CH_2-3 Cyclopent, CHa-2, CHa-6 Piper, CH_2-3, CH-4, CH_2-5 Piper, CH_2-4 Cyclopent), 2.15-2.31 (3H, m, OH, CHb-2, CHb-6 Piper), 2.37-2.72 (2H, m, CH_2Ph), 2.74-2.89 (2H, m, CH_2N), 3.12-3.24 (1H, m, CH-5 Cyclopent), 4.99 (1H, d, J = 5.9 Hz, CH-1 Cyclopent), 7.01-7.46 (15H, m, Ar_2, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 20.0 (CH_2, C-4 Cyclopent), 31.8 (CH_2, C-3/C-5 Piper), 32.4 (CH_2, C-3/C-5 Piper), 37.6 (CH, C-4 Piper), 29.8 (CH_2, C-2 Cyclopent), 35.1 (CH, C-3 Cyclopent), 42.6 (CH_2, CH_2Ph), 53.7 (CH_2, C-2/C-4 Piper), 55.1 (CH_2, C-2/C-4 Piper), 59.0 (C, C-1 Cyclopent), 61.6 (CH_2, CH_2N), 63.5 (C, C-2 Cyclopent), 125.5 (CH, C-4 Ph), 126.9 (2CH, C-4 Ar_2), 128.2 (4CH, C-3, C-5 Ar_2), 128.4 (2CH, C-3, C-5 Ph), 128.6 (4CH, C-2, C-6 Ar_2), 129.2 (2CH, C-2, C-6 Ph), 140.4 (C, C-1 Ph) 142.6 (2C, C-1 Ar_2). ESI-HRMS calcd for $C_{30}H_{36}NO$ $[M+H]^+$ 426.2791, found 426.2793.

The free amine was then converted into the corresponding hydrogen oxalate from diethyl ether (0.02 g, 0.04 mmol, yield 65%).

mp: 187-189 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 1.31-2.01 (7H, m, CH_2-3 Cyclopent, CH_2-3, CH-4, CH_2-5 Piper), 2.11-2.44 (2H, m, CH_2-4 Cyclopent, CHa-2/CHa-6 Piper), 2.41-2.58 (3H, m, CHa-2/CHa-6 Piper CH_2Ph), 2.63-2.92 (3H, m, CHa-2/CHa-6, CHb-2, CHb-6 Piper), 3.10-3.45 (3H, m, CH_2N, CH-5 Cyclopent), 4.31 (1H, m, OH), 4.90 (1H, d, J = 3.3 Hz, CH-1 Cyclopent), 7.03-7.48 (15H, m, Ar_2, Ph). ESI-HRMS calcd for $C_{30}H_{36}NO$ $[M+H]^+$ 426.2791, found 426.2793. Anal. Calcd. for $C_{32}H_{37}NO_5$: C, 74.54; H, 7.23; N, 2.72; Found C, 74.60; H, 7.31; N, 2.88.

***Trans*-17a** (0.22 g, 0.53 mmol, 45 % yield) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.14-1.71 (7H, m, CH_2-3 Cyclopent, CH_2-3, CH-4, CH_2-5 Piper), 1.83-2.18 (5H, m, OH, CHa-2, CHa-6 Piper, CH_2-4 Cyclopent), 2.40-2.88 (7H, m, CHb-2, CHb-6 Piper, CH_2Ph, CH_2N, CH-5 Cyclopent), 4.36 (1H, d, J = 9.3 Hz, CH-1 Cyclopent), 7.01-7.49 (15H, m, Ar_2, Ph). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 20.1 (CH_2, C-4 Cyclopent), 31.7 (CH_2, C-3/C-5 Piper), 32.4 (CH_2, C-3/C-5 Piper), 37.7 (CH, C-4 Piper), 29.9 (CH_2, C-2 Cyclopent), 35.2 (CH, C-3 Cyclopent), 42.6 (CH_2, CH_2Ph), 53.8 (CH_2, C-2/C-4 Piper), 55.2 (CH_2, C-2/C-4 Piper), 59.1 (C, C-1 Cyclopent), 61.4 (CH_2, CH_2N), 63.3 (C, C-2 Cyclopent), 125.4 (CH, C-4 Ph), 126.8 (2CH, C-4 Ar_2), 128.1 (4CH, C-3, C-

5 Ar_2), 128.4 (2CH, C-3, C-5 Ph), 128.5 (4CH, C-2, C-6 Ar_2), 129.1 (2CH, C-2, C-6 Ph), 140.3 (C, C-1 Ph) 142.5 (2C, C-1 Ar_2). ESI-HRMS calcd for $C_{30}H_{36}NO$ $[M+H]^+$ 426.2791, found 426.2792.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.13 g, 0.20 mmol, yield 49%).

mp: 219-221 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 1.24-2.01 (7H, m, CH_2-3 Cyclopent, CH_2-3, CH-4, CH_2-5 Piper), 2.53-3.38 (11H, m, CH_2-2, CH_2-6 Piper, CH_2Ph, CH_2N, CH_2-4, CH-5 Cyclopent), 4.23 (1H, d, J = 9.3 Hz, CH-1 Cyclopent), 4.33 (1H, m, OH), 7.10-7.44 (15H, m, Ar_2, Ph).

ESI-HRMS calcd for $C_{30}H_{36}NO$ $[M+H]^+$ 426.2791, found 426.2792. Anal. Calcd. for $C_{32}H_{37}NO_5$: C, 74.54; H, 7.23; N, 2.72; Found C, 74.67; H, 7.44; N, 2.93.

4.1.22. 5-[(4-Benzylpiperazin-1-yl)methyl]-2,2-diphenylcyclopenta-1-ol (17b)

The title compound was obtained as diastereomeric mixture from **16b** (12.3 mmol) by following the same procedure described for **17a**.

***Cis*-17b** (0.16 g, 0.37 mmol, 3 % yield) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.41-1.53 (1H, m, CHa-4 Cyclopent), 1.68-1.89 (1H, m, CHb-4 Cyclopent), 2.23-2.88 (14H, m, OH,CH_2N, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper, CH_2-3, CH-5 Cyclopent), 3.51 (2H, s, CH_2Ph), 4.99 (1H, d, J = 4.8 Hz, CH-1 Cyclopent), 7.01-7.47 (15H, m, Ar_2, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 20.0 (CH_2, C-4 Cyclopent), 29.7 (CH_2, C-2 Cyclopent), 35.1 (CH, C-3 Cyclopent), 52.2 (2CH_2, C-3,C-5 Piper), 53.4 (2CH_2, C-2,C-6 Piper), 59.1 (C, C-1 Cyclopent), 60.3 (CH_2, CH_2N), 61.6 (CH_2, CH_2N), 63.5 (C, C-2 Cyclopent), 125.5 (CH, C-4 Ph), 126.9 (2CH, C-4 Ar_2), 128.2 (4CH, C-3, C-5 Ar_2), 128.4 (2CH, C-3, C-5 Ph), 128.6 (4CH, C-2, C-6 Ar_2), 129.2 (2CH, C-2, C-6 Ph), 140.4 (C, C-1 Ph) 142.6 (2C, C-1 Ar_2). ESI-HRMS calcd for $C_{29}H_{35}N_2O$ $[M+H]^+$ 427.2744, found 427.2743.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.04 g, 0.06 mmol, yield 62%).

mp: 210-212 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 1.35-1.61 (1H, m, CHa-4 Cyclopent), 1.69-1.87 (1H, m, CHb-4 Cyclopent), 2.08-2.20 (1H, m, CHa-3, Cyclopent), 2.17-3.02 (12H, m, CH_2N, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper, CHb-3, CH-5 Cyclopent), 3.62 (2H, s, CH_2Ph), 4.28 (1H, m, OH), 4.99 (1H, d, J = 3.3 Hz, CH-1 Cyclopent), 7.01-7.47 (15H, m, Ar_2, Ph).

ESI-HRMS calcd for $C_{29}H_{35}N_2O$ $[M+H]^+$ 427.2744, found 427.2743. Anal. Calcd. for $C_{33}H_{38}N_2O_9$: C, 65.33; H, 6.31; N, 4.62; Found C, 65.56; H, 6.59; N, 4.65.

***Trans*-17b** (0.31 g, 0.74 mmol, 6 % yield) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.23-1.42 (2H, m, CH_2-4 Cyclopent), 1.83-2.16 (3H, m, OH, CH_2-3 Cyclopent), 2.25-2.90 (11H, m, CH_2N, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper, CH-5 Cyclopent), 3.54 (2H, s, CH_2Ph), 4.38 (1H, d, J = 6.7 Hz, CH-1 Cyclopent), 7.05-7.51 (15H, m, Ar_2, Ph).). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 20.2 (CH_2, C-4 Cyclopent), 29.8 (CH_2, C-2 Cyclopent), 35.1 (CH, C-3 Cyclopent), 42.6 (CH_2, CH_2Ph), 52.1 (2CH_2, C-3, C-5 Piper), 53.3 (2CH_2, C-2, C-6 Piper), 59.1 (C, C-1 Cyclopent), 61.3 (CH_2, CH_2N), 63.3 (C, C-2 Cyclopent), 125.4 (CH, C-4 Ph), 126.8 (2CH, C-4 Ar_2), 128.1 (4CH, C-3, C-5 Ar_2), 128.4 (2CH, C-3, C-5 Ph), 128.5 (4CH, C-2, C-6 Ar_2), 129.1 (2CH, C-2, C-6 Ph), 140.2 (C, C-1 Ph) 142.6 (2C, C-1 Ar_2). ESI-HRMS calcd for $C_{29}H_{35}N_2O$ $[M+H]^+$ 427.2744, found 427.2745.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.07 g, 0.12 mmol, yield 56%).

mp: 224-226 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 1.37-1.54 (1H, m, CHa-4 Cyclopent), 1.78-2.05 (1H, m, CHb-4 Cyclopent), 2.05-2.22 (1H, m, CHa-3, Cyclopent), 2.41-3.12 (12H, m, CH_2N, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper, CHb-3, CH-5 Cyclopent), 3.66 (2H, s, CH_2Ph), 4.11 (1H, m, OH), 4.22 (1H, d, J = 9.2 Hz, CH-1 Cyclopent), 7.01-7.47 (15H, m, Ar_2, Ph).

ESI-HRMS calcd for $C_{29}H_{35}N_2O$ $[M+H]^+$ 427.2744, found 427.2745. Anal. Calcd. for $C_{33}H_{38}N_2O_9$: C, 65.33; H, 6.31; N, 4.62; Found C, 65.41; H, 6.42; N, 4.70.

4.1.23. 1-[(Tert-butyldiphenylsilyl)oxy]-3-chloropropan-2-ol (18)

Tert-butyldiphenylsilyl chloride (3.50 ml, 13.56 mmol) and imidazole (1.20 g, 17.18 mmol) were added to a solution of 3-chloropropane-1,2-diol (1.00 g, 9.04 mmol) in dry DMF (15 mL). The mixture was stirred at room temperature for 5 h. The reaction was quenched with saturated aqueous NH_4Cl and extracted with EtOAc (3 ×30 mL). The combined organic layer was washed with 1.0 M aqueous HCl for three times and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography to give the corresponding silyl ether as a colorless oil (2.21 g, 6.33 mmol, 70% yield).

^{1}H-NMR (200MHz, $CDCl_3$): δ 1.09 (9H, s, *t*-Bu), 2.54 (1H, d, J = 6.2; OH), 3.64 (1H, dd, J = 5.7, 11.0 Hz, CHa-Cl), 3.71 (1H, dd, J = 5.3, 11.0 Hz, CHb-Cl), 3.74 (1H, dd, J= 5.4, 10.2 Hz, CHa-O), 3.81 (1H, dd, J = 4.6, 10.2 Hz, CHb-O), 3.92 (1H, m, C$\underline{H}$OH), 7.32-7.51 (6H, m, CH-3, CH-4, CH-5 Si-Ar_2), 7.56-7.76 (4H, m, CH-2, CH-6 Si-Ar_2). ESI-HRMS calcd for $C_{19}H_{26}{}^{35}ClO_2Si$ $[M+H]^+$ 349.1386, found 349.1388. Calcd for $C_{19}H_{26}{}^{37}ClO_2Si$ $[M+H]^+$ 351.1356, found 351.1358.

4.1.24. (2-(Benzhydryloxy)-3-chloropropoxy)(tert-butyl)diphenylsilane (19)

Bromodiphenylmethane (0.51 g, 2.05 mmol) was added to a solution of **18** (2.1 g, 6.2 mmol) in toluene (10 mL). The mixture was stirred under reflux for 24 h. The solvent was evaporated under reduced pressure and the solid residue obtained was taken up with EtOAc (30 mL). The organic layer was washed with brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography to give the title compound as a dark oil (0.65 g, 1.27 mmol, 62% yield).

^{1}H-NMR (200MHz, $CDCl_3$): δ 1.05 (9H, s, *t*-Bu), 3.69-3.85 (5H, m, Cl-CH_2, CH_2-O, C<u>H</u>OH), 5.58 (1H, s, C<u>H</u>Ar_2), 7.25-7.48 (16H, m, Ar_2, CH-3, CH-4, CH-5 Si-Ar_2), 7.61-7.69 (4H, m, CH-2, CH-6 Si-Ar_2). ESI-HRMS calcd for $C_{32}H_{36}ClO_2Si$ $[M+H]^+$ 515.2168, found 515.2169.

4.1.25. [(2-Chloroethoxy)methylene]dibenzene (20)

The title compound was obtained from bromodiphenylmethane (0.47 g, 1.92 mmol) and 2-chloroethanol (0.4 g, 5.0 mmol) as an oil (0.1 g, 0.5 mmol, 26% yield) by following the same procedure described for **19**.

^{1}H NMR ($CDCl_3$, 200 MHz): δ = 3.74 (2H, t, J = 6.2 Hz, OCH_2), 3.80 (2H, t, J=6.2 Hz, CH_2Cl), 5.5 (1H, s, $CHAr_2$), 7.21-7.49 (10H, m, Ar_2). ESI-HRMS calcd for $C_{15}H_{16}{}^{35}ClO$ $[M+H]^+$ 247.0885, found 247.0883. ESI-HRMS calcd for $C_{15}H_{16}{}^{37}ClO$ $[M+H]^+$ 249.0855, found 249.0853.

4.1.26. 1-{2-(Benzhydryloxy)-3-[(tert-butyldiphenylsilyl)oxy]propyl}-4-benzylpiperidine (21a)

The title compound was obtained from **19** and 4-benzylpiperidine as a yellow oil (0.28 g, 0.43 mmol, 74% yield) by following the general procedure described in the **4.1.4.** section.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.02-1.38 (14H, m, *t*-Bu, CH_2-3, CH-4, CH_2-5 Piper), 1.81-2.11 (2H, m, CHa-2, CHa-6 Piper), 2.56-2.94 (6H, m, CH_2N, CH_2Ph, CHa-2, CHb-6 Piper), 3.61-3.83 (3H, m, CH_2-O, C<u>H</u>OH), 5.58 (1H, s, $CHAr_2$), 7.23-7.72 (25H, m, Si-Ar_2, Ar_2, Ph). ESI-HRMS calcd for $C_{44}H_{52}NO_2Si$ $[M+H]^+$ 654.3762, found 654.3764.

4.1.27. 1-{2-(Benzhydryloxy)-3-[(tert-butyldiphenylsilyl)oxy]propyl}-4-benzylpiperazine (21b)

The title compound was obtained from **19** and 1-benzylpiperazine as a colorless oil (0.32 g, 0.49 mmol, 90% yield) by following the general procedure described in the **4.1.4.** section.

^1H NMR ($CDCl_3$, 400 MHz): δ = 1.03 (9H, s, *t*-Bu), 2.39-2.79 (10H, m, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper, CH_2N), 3.51 (2H, s, CH_2Ph), 3.60-3.84 (3H, m, CH_2-O, C$\underline{H}$OH), 5.81 (1H, s, $CHAr_2$), 7.21-7.74 (25H, m, Si-Ar_2, Ar_2, Ph). ESI-HRMS calcd for $C_{43}H_{51}N_2O_2Si$ $[M+H]^+$ 655.3714, found 655.3716.

4.1.28. 2-(Benzhydryloxy)-3-(4-benzylpiperidin-1-yl)propan-1-ol (22a)

TBAF (0.46 ml, 0.49 mmol) was added to a solution of **21a** (0.28 g, 0.43 mmol) in THF (10 mL). The mixture was stirred at room temperature for 24 h, diluted with water, and extracted with EtOAc. The combined organic layers were washed with brine and dried over $MgSO_4$. Solvent was removed under vacuum. The residue was purified by flash column chromatography to give the title compound as a colorless oil (0.12 g, 0.29 mmol, 68% yield).

^1H NMR ($CDCl_3$, 400 MHz): δ = 1.21-1.43 (5H, m, CH_2-3, CH-4, CH_2-5 Piper), 1.82-2.12 (2H, m, CHa-2, CHa-6 Piper), 2.19 (1H, br s, OH), 2.31-2.54 (2H, m, CH_2Ph), 2.59-2.71 (2H, m, CH_2N), 2.79-2.91 (1H, m, CHa-2/CHa-6 Piper), 2.93-3.03 (1H, m, CHa-2/CHa-6 Piper), 3.58-3.86 (3H, m, CH_2-OH, CHO), 5.52 (1H, s, $CHAr_2$), 7.12-7.41 (15H, m, Ar_2, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 31.6 (CH_2, C-3/C-5 Piper), 32.0 (CH_2, C-3/C-5 Piper), 36.9 (CH, C-4 Piper), 42.9 (CH_2, CH_2Ph), 53.8 (CH_2, C-2/C-4 Piper), 54.6 (CH_2, C-2/C-4 Piper), 62.0 (CH_2, CH_2N), 66.0 (CH_2, CH_2OH), 72.2 (CH, CHO), 82.0 (CH, $CHAr_2$), 125.8 (CH, C-4 Ph), 127.1 (4CH, C-2, C-6 Ar_2), 127.6 (CH, C-4 Ar), 127.7 (CH, C-4 Ar’), 128.2 (2CH, C-3, C-5 Ph), 128.4 (2CH, C-3, C-5 Ar), 128.5 (2CH, C-3, C-5 Ar’), 128.9 (2CH, C-2, C-6 Ph), 140.6 (C, C-1 Ph), 142.1 (C, C-1 Ar), 142.2 (C, C-1 Ar’). ESI-HRMS calcd for $C_{28}H_{34}NO_2$ $[M+H]^+$ 416.2584, found 416.2586.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.08 g, 0.16 mmol, yield 61%).

mp: 174-176 °C; ^1H NMR (DMSO, 200 MHz): δ = 1.07-1.71 (5H, m, CH_2-3, CH-4, CH_2-5 Piper), 2.41-2.71 (4H, m, CH_2Ph, CHa-2, CHa-6 Piper), 2.85-3.27 (4H, m, CH_2N, CHa-2, CHa-6 Piper), 3.42-3.61 (2H, m, CH_2-O), 3.67-3.81 (1H, m, CHOH), 3.91 (1H, m, OH) 5.77 (1H, s, $CHAr_2$), 7.11-7.78 (15H, m, Ar_2, Ph).

ESI-HRMS calcd for $C_{28}H_{34}NO_2$ $[M+H]^+$ 416.2584, found 416.2586. Anal. Calcd. for $C_{30}H_{35}NO_6$: C, 71.27; H, 6.98; N, 2.77; Found C, 71.55; H, 7.13; N, 2.94.

4.1.29. 2-(Benzhydryloxy)-3-(4-benzylpiperazin-1-yl)propan-1-ol (22b)

The title compound was obtained from **21b** (0.30 g, 0.46 mmol) as a colorless oil (0.12 g, 0.29 mmol, 63% yield) by following the procedure described for **22a**.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.21 (1H, br s, OH), 2.31-2.61 (8H, m, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper), 2.63-2.71 (2H, m, CH_2N), 3.49 (2H, s, CH_2Ph), 3.53-3.61 (1H, m, CHO), 3.74-3.88 (2H, m, CH_2OH), 5.57 (1H, s, $CHAr_2$), 7.19-7.44 (15H, m, Ar_2, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 52.0 ($2CH_2$, C-3,C-5 Piper), 53.2 ($2CH_2$, C-2,C-6 Piper), 61.5 (CH_2, CH_2N), 62.5 (CH_2, CH_2Ph), 66.0 (CH_2, CH_2OH), 72.2 (CH, CHO), 82.0 (CH, $CHAr_2$), 125.6 (CH, C-4 Ph), 127.0 (4CH, C-2, C-6 Ar_2), 127.5 (CH, C-4 Ar), 127.8 (CH, C-4 Ar'), 128.3 (2CH, C-3, C-5 Ph), 128.5 (2CH, C-3, C-5 Ar), 128.6 (2CH, C-3, C-5 Ar'), 128.9 (2CH, C-2, C-6 Ph), 141.0 (C, C-1 Ph), 142.2 (C, C-1 Ar), 142.3 (C, C-1 Ar'). ESI-HRMS calcd for $C_{27}H_{33}N_2O_2$ $[M+H]^+$ 417.2537, found 417.2536.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.08 g, 0.14 mmol, yield 54%).

mp: 201-203 °C; ^{1}H NMR (DMSO, 400 MHz): δ = 2.58-2.96 (10H, m, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper, CH_2N), 3.44-3.73 (3H, m, C<u>H</u>OH, CH_2O CH_2Ar), 3.76 (2H, s, CH_2Ph), 3.89 (1H, m, OH) 5.76 (1H, s, $CHAr_2$), 7.11-7.49 (15H, m, Ar_2, Ph).

ESI-HRMS calcd for $C_{27}H_{33}N_2O_2$ $[M+H]^+$ 417.2537, found 417.2536. Anal. Calcd. for $C_{31}H_{36}N_2O_{10}$: C, 62.41; H, 6.08; N, 4.70; Found C, 62.52; H, 6.11; N, 4.68.

4.1.30. 1-[2-(Benzhydryloxy)ethyl]-4-benzylpiperidine (23a)

The title compound was obtained from **20** (0.64 g, 1.66 mmol) and 4-benzylpiperidine as a yellow oil (0.27 g, 0.70 mmol, 42% yield) by following the general procedure described in the **4.1.4.** section.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.32-1.51 (5H, m, CH_2-3, CH-4, CH_2-5 Piper), 1.87-2.09 (2H, m, CHa-2, CHa-6 Piper), 2.52 (2H, d, J = 6.5 Hz, CH_2Ph), 2.72 (2H, t, J = 6.0 Hz, CH_2N), 2.90-3.10 (2H, m, CHb-2, CHb-6 Piper), 3.62 (2H, t, J = 6.0 Hz, CH_2O), 5.34 (1H, s, $CHAr_2$), 7.04-7.42 (15H, m, Ar_2, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 31.7 (CH_2, C-3/C-5 Piper), 32.1 (CH_2, C-3/C-5 Piper), 37.0 (CH, C-4 Piper), 42.8 (CH_2, CH_2Ph), 53.9 (CH_2, C-2/C-4 Piper), 54.5 (CH_2, C-2/C-4 Piper), 56.8 (CH_2, CH_2O), 62.0 (CH_2, CH_2N), 85.5 (CH, $CHAr_2$), 125.7 (CH, C-4 Ph), 126.8 (4CH, C-2, C-6 Ar_2), 127.5 (2CH, C-4 Ar_2), 128.2 (2CH, C-3, C-5 Ph), 128.4 (4CH, C-3, C-5 Ar_2), 128.9 (2CH, C-2, C-6 Ph), 140.6 (C, C-1 Ph), 142.0 (2C, C-1 Ar_2). ESI-HRMS calcd for $C_{27}H_{32}NO$ $[M+H]^+$ 386.2478, found 386.2476.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.07 g, 0.15 mmol, yield 33%).

mp: 165-167 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 1.27-1.58 (2H, m, CHa-3, CHa-5 Piper), 1.59-1.87 (3H, m, CHb-3, CH-4, CHb-5 Piper), 2.39-2.64 (2H, m, CH_2Ph), 2.67-2.99 (2H, m, CHa-2, CHa-6

Piper), 3.20 (2H, t, J= 5.2 Hz, CH_2N), 3.31-3.43 (2H, m, CHb-2, CHb-6 Piper), 3.66 (2H, t, J= 5.2 Hz, CH_2O), 5.52 (1H, s, $CHAr_2$), 7.12-7.50 (15H, m, Ar_2, Ph).

ESI-HRMS calcd for $C_{27}H_{32}NO$ $[M+H]^+$ 386.2478, found 386.2476. Anal. Calcd. for $C_{29}H_{33}NO_5$: C, 73.24; H, 6.99; N, 2.95; Found C, 73.56; H, 7.10; N, 3.12.

4.1.31. 1-[2-(Benzhydryloxy)ethyl]-4-benzylpiperazine (23b)

The title compound was obtained from **20** (0.38 g, 1.54 mmol) and 1-benzylpiperazine as a colorless oil (0.15 g, 0.40 mmol, 26% yield) by following the general procedure described in the **4.1.4.** section.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.40-2.64 (8H, m, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper), 2.69 (2H, t, J = 6.6 Hz, CH_2N), 3.49 (2H, s, CH_2Ph), 3.60 (2H, t, J = 6.6 Hz, CH_2-O), 5.38 (1H, s, $CHAr_2$), 7.12-7.44 (15H, m, Ar_2, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 51.7 (2CH_2, C-3,C-5 Piper), 52.8 (2CH_2, C-2,C-6 Piper), 55.8 (CH_2, CH_2O), 61.9 (CH_2, CH_2N), 62.8 (CH_2, CH_2Ph), 82.4 (CH, $CHAr_2$), 125.6 (CH, C-4 Ph), 126.6 (4CH, C-2, C-6 Ar_2), 127.3 (2CH, C-4 Ar_2), 128.1 (2CH, C-3, C-5 Ph), 128.3 (4CH, C-3, C-5 Ar_2), 128.8 (2CH, C-2, C-6 Ph), 140.5 (C, C-1 Ph), 142.3 (2C, C-1 Ar_2). ESI-HRMS calcd for $C_{26}H_{31}N_2O$ $[M+H]^+$ 387.2431, found 387.2434.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.10 g, 0.18 mmol, yield 49%).

mp: 218-220 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 2.62-2.89 (4H, m, CH_2-3, CH_2-5 Piper), 2.91-3.18 (6H, m, CH_2-2, CH_2-6 Piper, CH_2N), 3.62 (2H, t, J = 6.3 Hz, CH_2-O), 3.76 (2H, s, CH_2Ph), 5.50 (1H, s, $CHAr_2$), 7.14-7.52 (15H, m, Ar_2, Ph).

ESI-HRMS calcd for $C_{26}H_{31}N_2O$ $[M+H]^+$ 387.2431, found 387.2434. Anal. Calcd. for $C_{30}H_{34}N_2O_9$: C, 63.59; H, 6.05; N, 4.94; Found C, 63.31; H, 6.11; N, 4.76.

4.1.32. 1-(1,4-dioxaspiro[4.5]decan-2-ylmethyl)-4-benzylpiperidine (25a)

The title compound was obtained from **24** [43] and 4-benzylpiperidine by following the general procedure described in the **4.1.4.** section. The crude extract was purified by using flash column chromatography (cyclohexane/ethyl acetate 60/40) to give **25a** as a colorless oil (0.42 g, 1.28 mmol, 96% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.13-178 (15H, m, CH_2-6, CH_2-7, CH_2-8, CH_2-9, CH_2-10 Dosd, CH_2-3, CH-4, CH_2-5 Piper), 1.88-2.21 (2H, m, CHa-2, CHa-6 Piper), 2.61 (2H, d, J = 6.5 Hz, CH_2Ph), 2.66-2.78 (1H, m, CHaN), 2.80-2.92 (1H, m, CHb-N), 3.05-3.28 (1H, m, CHb-2/CHb-6 Piper), 3.31-3.54 (1H, m, CHb-2/CHb-6 Piper), 3.61 (1H, dd, J = 7.1, 8.0 Hz, CHa-3 Dosd), 4.16 (1H, dd, J = 7.1, 7.7 Hz, CHb-3 Dosd), 4.40-4.71 (1H, m, CH-2 Dosd), 7.17 (2H, d, J = 7.1 Hz, CH-2, CH-6 Ph), 7.23 (1H, t, J = 7.3 Hz, CH-4 Ph), 7.32 (2H, dd, J = 7.1 7.3 Hz , CH-3, CH-5 Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 23.8 (CH_2, C-8 Dosd), 24.0 (CH_2, C-7/C-9 Dosd), 25.0 (CH_2, C-7/C-9 Dosd), 31.8 (CH_2, C-3/C-5 Piper), 32.1 (CH_2, C-3/C-5 Piper), 34.9 (CH_2, C-6/C-10 Dosd), 36.5 (CH_2, C-6/C-10 Dosd), 37.7 (CH, C-4 Piper), 42.4 (CH_2, CH_2Ph), 53.5 (CH_2, C-2/C-4 Piper), 55.1 (CH_2, C-2/C-4 Piper), 61.2 (CH_2, CH_2N), 67.9 (CH_2, C-3 Dosd), 72.3 (CH, C-2 Dosd), 110.5 (C, C-5 Dosd), 125.8 (CH, C-4 Ph), 128.2 (2CH, C-3, C-5 Ph), 129.1 (2CH, C-2, C-6 Ph), 140.6 (C, C-1 Ph). ESI-HRMS calcd for $C_{21}H_{32}NO_2$ $[M+H]^+$ 330.2428, found 330.2429.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.18 g, 0.43 mmol, yield 38%).

mp: 155-157 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 1.16-1.89 (15H, m, CH_2-6, CH_2-7, CH_2-8, CH_2-9, CH_2-10 Dosd, CH_2-3, CH-4, CH_2-5 Piper), 2.40-2.61 (2H, m, CH_2Ph), 2.69-3.24 (4H, m, CH_2-2, CH_2-6 Piper), 3.26-3.49 (2H, m, CH_2N), 3.61 (1H, dd, J = 7.1, 7.8 Hz, CHa-3 Dosd), 4.05 (1H, dd, J = 7.1, 8.0 Hz, CHb-3 Dosd), 4.28-4.51 (1H, m, CH-2 Dosd), 7.04-7.42 (5H, m, Ph).

ESI-HRMS calcd for $C_{21}H_{32}NO_2$ $[M+H]^+$ 330.2428, found 330.2429. Anal. Calcd. for $C_{23}H_{33}NO_6$: C, 65.85; H, 7.93; N, 3.34; Found C, 65.97; H, 8.06, N, 3.57.

4.1.33. 1-(1,4-dioxaspiro[4.5]decan-2-ylmethyl)-4-benzylpiperazine (25b)

The title compound was obtained from **24** [43] and 1-benzylpiperazine by following the general procedure described for the synthesis of the amines. The crude extract was purified by using flash column chromatography (cyclohexane/ethyl acetate 30/70) to give **25b** as colorless oil (0.15 g, 0.47 mmol, 45% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.11-1.80 (10H, m, CH_2-6, CH_2-7, CH_2-8, CH_2-9, CH_2-10 Dosd), 2.29-2.76 (10H, m, CH_2N, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper), 3.53 (2H, s, CH_2Ph), 3.58 (1H, dd, J = 5.1, 7.3 Hz, CHa-3 Dosd), 4.07 (1H, dd, J = 6.2, 7.3 Hz, CHb-3 Dosd), 4.17-4.36 (1H, m, CH-2 Dosd), 7.05-7.40 (5H, m, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 23.8 (CH_2, C-8 Dosd), 24.0 (CH_2, C-7/C-9 Dosd), 25.0 (CH_2, C-7/C-9 Dosd), 34.9 (CH_2, C-6/C-10 Dosd), 36.5 (CH_2, C-6/C-10 Dosd), 53.0 (2CH_2, C-3, C-5 Piper), 53.9 (2CH_2, C-2, C-6 Piper), 61.4 (CH_2, CH_2N), 63.2 (CH_2Ph), 68.0 (CH_2, C-3 Dosd), 72.2 (CH, C-2 Dosd), 110.7 (C, C-5 Dosd), 125.9 (CH, C-4 Ph), 128.4 (2CH, C-3, C-5 Ph), 129.3 (2CH, C-2, C-6 Ph), 140.8 (C, C-1 Ph). ESI-HRMS calcd for $C_{20}H_{31}N_2O_2$ $[M+H]^+$ 331.2380, found 331.2382.

The free amine was converted into the corresponding hydrogen oxalate from diethyl ether (0.10 g, 0.19 mmol, yield 40%).

mp: 215-217 °C; ^{1}H NMR (DMSO, 200 MHz): δ = 1.18-1.71 (10H, m, CH_2-6, CH_2-7, CH_2-8, CH_2-9, CH_2-10 Dosd), 2.61-3.11 (10H, m, CH_2N, CH_2-2, CH_2-3, CH_2-5, CH_2-6 Piper), 3.55 (1H, dd, J = 6.9, 7.6 Hz, CHa-3 Dosd), 3.92 (2H, s, CH_2Ph), 4.00 (1H, dd, J = 6.3, 7.6 Hz, CHb-3 Dosd), 4.18-4.39 (1H, m, CH-2 Dosd), 7.24-7.49 (5H, m, Ph).

ESI-HRMS calcd for $C_{20}H_{31}N_2O_2$ $[M+H]^+$ 331.2380, found 331.2382. Anal. Calcd. for $C_{24}H_{34}N_2O_{10}$: C, 56.46; H, 6.71; N, 5.49; Found C, 56.72; H, 6.97; N, 5.53.

4.2. Biological activity

4.2.1. Radioligand binding assay at σ_1 receptors

In vitro σ-binding experiments were carried out as previously reported [49]. σ_1 Binding assays were performed on guinea pig brain membranes according to experimental protocol described by DeHaven et al [50]. Briefly, 500 μg of membrane protein was incubated with 3 nM [^{3}H]-(+)-pentazocine (29 Ci/mM; the value of the apparent dissociation constant (Kd) was 14 ± 0.3 nM, n = 3) in 50 mM Tris-HCl (pH 7.4). Test compounds were added in concentrations ranging from 10−5 to 10−11 M. Nonspecific binding was assessed in the presence of 10 μM of unlabeled haloperidol. The reaction was performed for 150 min at 37 °C and terminated by filtering the solution through Whatman GF/B glass fiber filters which were presoaked for 1 h in a 0.5% poly(ethylenimine) solution. Filters were washed with ice cold buffer (2 × 4 mL). Regarding σ_2-binding assays [51], the membranes were incubated with 3 nM [^{3}H]DTG (53.3 Ci/mM; Kd = 11 ± 0.8 nM; n = 3) in the presence of 400 nM (+)-SKF10,047 in order to mask σ_1 sites. Nonspecific binding was evaluated with DTG (5 μM). Incubation was carried out in 50 mM Tris-HCl (pH 8.0) for 120 min at room temperature, and assays were terminated by the addition of ice-cold 10 mM Tris-HCl (pH 8.0).

Each sample was filtered through Whatman GF/B glass fibers filters, which were presoaked for 1 h in a 0.5% poly(ethylenimine) solution, using a Millipore filter apparatus. The filters were washed twice with 4 mL of ice-cold buffer. Radioactivity was counted in 4 mL of "Ultima Gold MV" in a 1414 Winspectral PerkinElmer Wallac liquid scintillation counter. Inhibition constants (Ki values) were calculated using the EBDA/LIGAND program purchased from Elsevier/Biosoft. Each concentration was tested in duplicate and each experiment was repeated three times. The Ki values agreed to ± 20%.

4.2.2. Radioligand Binding Assay at Human Recombinant 5-HT$_{1A}$R

A human cell line (HeLa) stably transfected with genomic clone G-21 coding for the human 5-HT$_{1A}$ serotoninergic receptor was used. The cells were grown as monolayers in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and gentamycin (100 μg/mL) under 5% CO_2 at 37 °C. The cells were detached from the growth flask at 95% confluence by a cell scraper and were lysed in ice-cold Tris (5 mM) and EDTA buffer (5 mM, pH 7.4). The homogenates were centrifuged for 20 min at 40000g, and the pellets were re-suspended in a small volume of ice-cold Tris/EDTA buffer (above) and immediately frozen and stored at 70 °C until use. On the day of experiment, cell membranes (80-90 μg of protein) were re-suspended in binding buffer (50 mM Tris, 2.5 mM $MgCl_2$, and 10 mM pargiline, pH 7.4). The membranes were incubated in a final volume of 0.32 mL for 30 min at 30 °C with 1 nM [^{3}H]8-OH-DPAT, in the absence or presence of various concentrations of the competing drugs (1pM to 1 μM); each experimental condition was performed in triplicate. Non specific binding was determined in the presence of 10 μM 5-HT [52]. Ki values agreed to ± 10%.

4.2.3. *In vivo biological assay*

4.2.3.1. Animals

Male Sprague-Dawley rats (Harlan, Italy), weighing 180–200 g, were used.

The animals were kept at a constant room temperature (25 ± 1 °C) under a 12:12 h light and dark cycle with free access to food and water. Each rat was used for only one experiment. 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).

4.2.3.2. Nociceptive test

Nociception was evaluated by the radiant heat tail-flick test that consisted of the irradiation of the lower third of the tail with an I.R. source [46]. The experiments were performed at room temperature (25 ± 1 °C). The basal pre-drug latency was established between 3 and 4 s, which was calculated as the average of the first three measurements performed at 5 min intervals. A cut-off latency of 10 s was established to minimize damage to the tail. Post-treatment tail flick latencies (TFLs) were determined at 30, 45, 60, 90 and 120 minutes after subcutaneous (s.c.) injection. For the double treatments **25b** was administered (1 mg/kg s.c.) followed after 45 minutes by (–)-U50,488H (5 mg/kg s.c.) or morphine (2 mg/kg s.c.); tail flick latencies were measured after 30, 45, 60, 90 and 120 minutes from the opioid administration. The behavioral tests were conducted by researchers blinded to the treatment group.

The rats were divided into the by following 6 groups (each consisting of 8-10 animals):

Group 1: saline s.c.

Group 2: **25b** 1 mg/kg s.c.

Group 3: (–)-U50,488H (Tocris, Bristol, UK) 5 mg/kg s.c.

Group 4: **25b** 1 mg/kg s.c. + (after 45 minutes) (–)-U50,488H 5 mg/kg s.c.

Group 5: morphine 2 mg/kg s.c.

Group 6: **25b** 1 mg/kg s.c. + (after 45 minutes) morphine (S.A.L.A.R.S., Como, Italy) 2 mg/kg s.c.

4.2.3.3. Statistical analysis

The data are expressed as mean ± SE. The inter-group comparisons were assessed using an initial two-way analysis of variance (ANOVA) followed by the Students' t test. Any differences were considered significant at $P<0.05$.

4.3. Molecular modeling

4.3.1. Ligand preparation

All the compounds were built, parameterised (Gasteiger-Huckel method) and energy minimised within MOE using MMFF94 forcefield [53]. For all the molecules, the (alternately) piperidine and piperazine mono-protonated forms wereconsidered for the *in silico* analyses.

4.3.2. Sigma-1 homology modeling

A σ_1 theoretical model was built using a multi-template homology modeling strategy, which was already applied by Pricl [54]. Briefly, the amino acid sequence of sigma 1 receptor (Q99720) was retrieved from the SWISSPROT database [55] while the selected templates were obtained from the Protein Data Bank [56]. In particular, the three-dimensional structure co-ordinates file of recombinant oxalate oxidase (pdb code = 2ETE; R =1.75Å) [57] and of homogentisate 1,2-dioxygenase (pdb code = 3ZDS; R =1.70Å) [58] were chosen, gaining a considerable overall similarity (>30%) with respect to the sigma-1 primary sequence.

The final model connecting loops were constructed by the loop search method implemented in MOE. The MOE output file included a series of ten models which were independently built on the basis of a Boltzmann-weighted randomized procedure [59], combined with specialized logic for the handling of sequence insertions and deletions [60]. Among the derived models, there were no significant main chain deviations. The model with the best packing quality function was selected for full energy minimization. The retained structure was minimized with MOE using the AMBER94 force field [61]. The energy minimization was carried out by the 1000 steps of the steepest descent followed by conjugate gradient minimization until the rms gradient of the potential energy was less than 0.1 kcal mol^{-1} $Å^{-1}$. The assessment of the final model was performed using Ramachandran plots, generated within MOE, showing the absence of outliers. Successively, the final model reliability was also assessed by docking analyses performed on sigma-1 ligands already discussed in the literature, and therefore by comparing the obtained results with those previously published. Concerning this issue, a series of spiro-derivatives was taken into account, focusing our attention on a careful analysis of the putative binding mode of the 1-benzyl-6',7'-dihydrospiro[piperidine-4,4'-thieno[3,4-c]pyran (compound **I**) derivative [62]. Molecular docking studies performed on unsubstituted and poorly flexible molecules are very useful and highly desirable when you want to investigate and optimize the binding site of a protein homology model. Therefore, the obtained results were also evaluated bearing in mind the information coming from mutagenesis analyses, which underlined the importance of a salt-bridge between a protonated center of the ligand and the protein D126 and also of H-bond contacts with T151, and allowed us to validate the derived sigma-1 model. Finally, the protein-agonist **I** complex stability was successfully assessed using a short ~1 ps run of molecular dynamics (MD) at constant temperature, followed by an all-atom energy minimization (LowModeMD mplemented in MOE software).

4.3.3. Docking studies

The docking studies were performed according to the by following protocol. The putative sigma-1 binding site was carefully determined and analysed on the basis of the MOE software Site Finder module [54]. Then, the most probable receptor binding site we identified was validated by a comparison with the information coming from the mutagenesis data, by following a procedure already fruitfully used [63, 64]. For all the compounds, each isomer was docked into the putative ligand binding site by means of the Surflex docking module implemented in Sybyl-X1.0 [65]. Then, for all the compounds, the best docking geometries (selected on the basis of the SurFlex scoring functions) were refined by ligand/protein complex energy minimization (CHARMM27) by means of the MOE software. To verify the reliability of the derived docking poses, the obtained ligand/protein complexes were further investigated by docking calculations (10 run), using MOE-Dock (Genetic algorithm; applied on the poses already located into the putative sigma-1 binding site). The ligand molecules were ranked with the London dG scoring function (related to the first conformer refinement process). The 10 best poses (default is 30) were retained and further refined by energy minimization in the protein binding site, followed by rescoring with the GBVI/WSA dG scoring function (calculated on the latest conformer refinement process) as reported in the Supplementary Information. The conformers showing lower energy scoring functions and rmsd values (with respect to the starting poses) were selected as the most stable and allowed us to identify the most probable conformers interacting with sigma-1.

Acknowledgments

The authors thank Ms. Rossella Gallesi for performing the elemental analysis. This work was supported by “Fondazione Cassa di Risparmio di Modena”.

Appendix A. Supplementary data

Supplementary data related to this article can be found at *ht*tp://...

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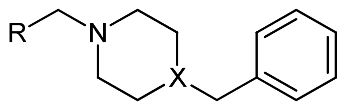
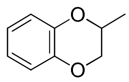
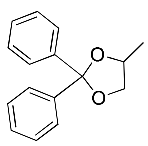
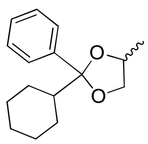
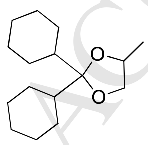
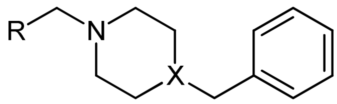
Table 1. Binding affinities (pK_i) and selectivities of Group I compounds.

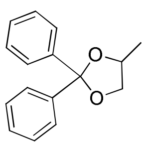
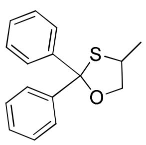
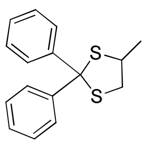
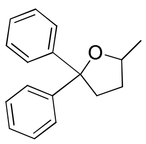
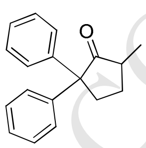
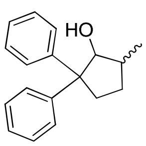


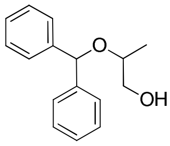
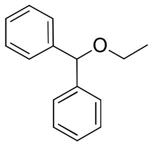
Comp.	R	X	pK_i σ_1[a,b]	pK_i σ_2[a,c]	σ_1/σ_2[d]	pK_i 5-HT_{1A}[e]	σ/5-HT_{1A}[f]
1a		CH	7.78	7.60	2	7.75	1
1b		N	7.80	7.55	2	7.16	4
8a		CH	8.66	8.20	3	6.26	251
8b		N	8.77	8.38	3	<6	>589
cis-**9a**		CH	7.28	6.84	3	<6	>19
cis-**9b**		N	7.51	7.75	1	<6	>56
trans-**9a**		CH	7.25	6.43	7	<6	>18
trans-**9b**		N	8.97	7.79	15	<6	>933
10a		CH	6.68	<6	>5	<6	>5
10b		N	8.47	8.3	2	<6	>29

[a] Each concentration was tested in duplicate and each experiment was repeated three times. The Ki values agreed to ± 20%. [b] Binding assays were performed using 3.0 nM [^{3}H]pentazocine. [c] Binding assays were performed using 3.0 nM [^{3}H]ditolylguanidine. [d] Antilog of the difference between the pKi values for σ_1 and σ_2 receptors. [e] Ki values were derived from the Cheng–Prusoff equation at one or two concentrations. Each experimental condition was performed in triplicate and agreed within 10%. [f] Antilog of the difference between the pKi values for σ receptors (higher value) and the 5-HT_{1A}R.

Table 2. Binding affinities (pK_i) and selectivities of Group II-V compounds.



Comp.	R	X	pK_i σ_1 [a,b]	pK_i σ_2 [a,c]	σ_1/σ_2 [d]	pK_i 5-HT_{1A} [e]	σ/5-HT_{1A} [f]
8a		CH	8.66	8.20	3	6.26	251
8b		N	8.77	8.38	3	<6	>589
11a		CH	7.92	7.55	2	<6	>83
11b		N	7.20	6.67	3	<6	>16
12a		CH	7.82	7.11	5	<6	>66
12b		N	6.52	5.64	8	<6	>3
14a		CH	6.76	6.69	1	<6	>6
14b		N	7.06	6.65	3	<6	>11
16a		CH	5.95	5.66	2	<6	1
16b		N	6.19	4.9	20	<6	>1.5
cis-**17a**		CH	6.55	5.66	8	8.12	0.02
cis-**17b**		N	7.45	6.62	6.8	8.14	0.2
trans-**17a**		CH	6.64	5.05	39	7.30	0.21
trans-**17b**		N	7.42	7.00	3	6.90	3

22a		CH	6.70	6.56	1	6.38	2.5
22b		N	7.95	7.75	2	<6	>89
23a		CH	7.25	6.77	3	<6	>18
23b		N	7.69	7.95	1	<6	>89

[a] Each concentration was tested in duplicate and each experiment was repeated three times. The Ki values agreed to ± 20%. [b] Binding assays were performed using 3.0 nM [^{3}H]pentazocine. [c] Binding assays were performed using 3.0 nM [^{3}H]ditolylguanidine. [d] Antilog of the difference between the pKi values for σ_1 and σ_2 receptors. [e] Ki values were derived from the Cheng–Prusoff equation at one or two concentrations. Each experimental condition was performed in triplicate and agreed within 10%. [f] Antilog of the difference between the pKi values for σ receptors (higher value) and the 5-HT_{1A}R.

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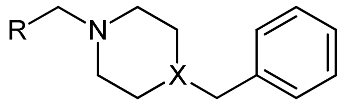
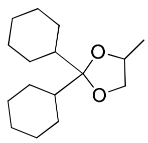
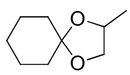
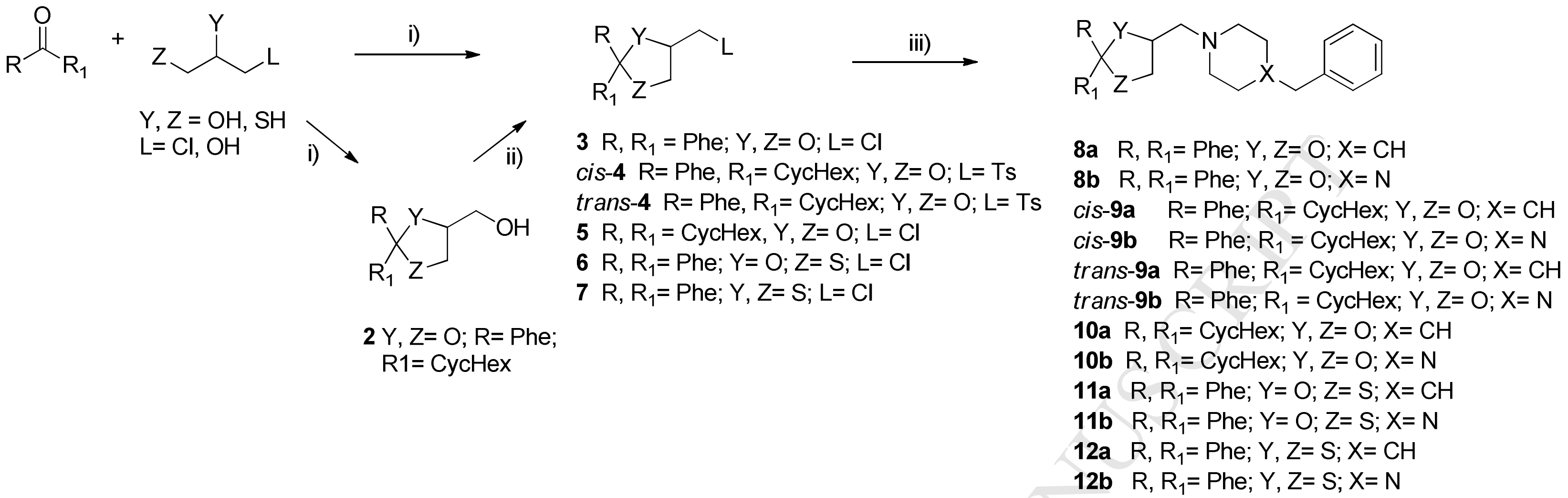
Table 3. Binding affinities (pK_i) and selectivities of Group VI compounds.

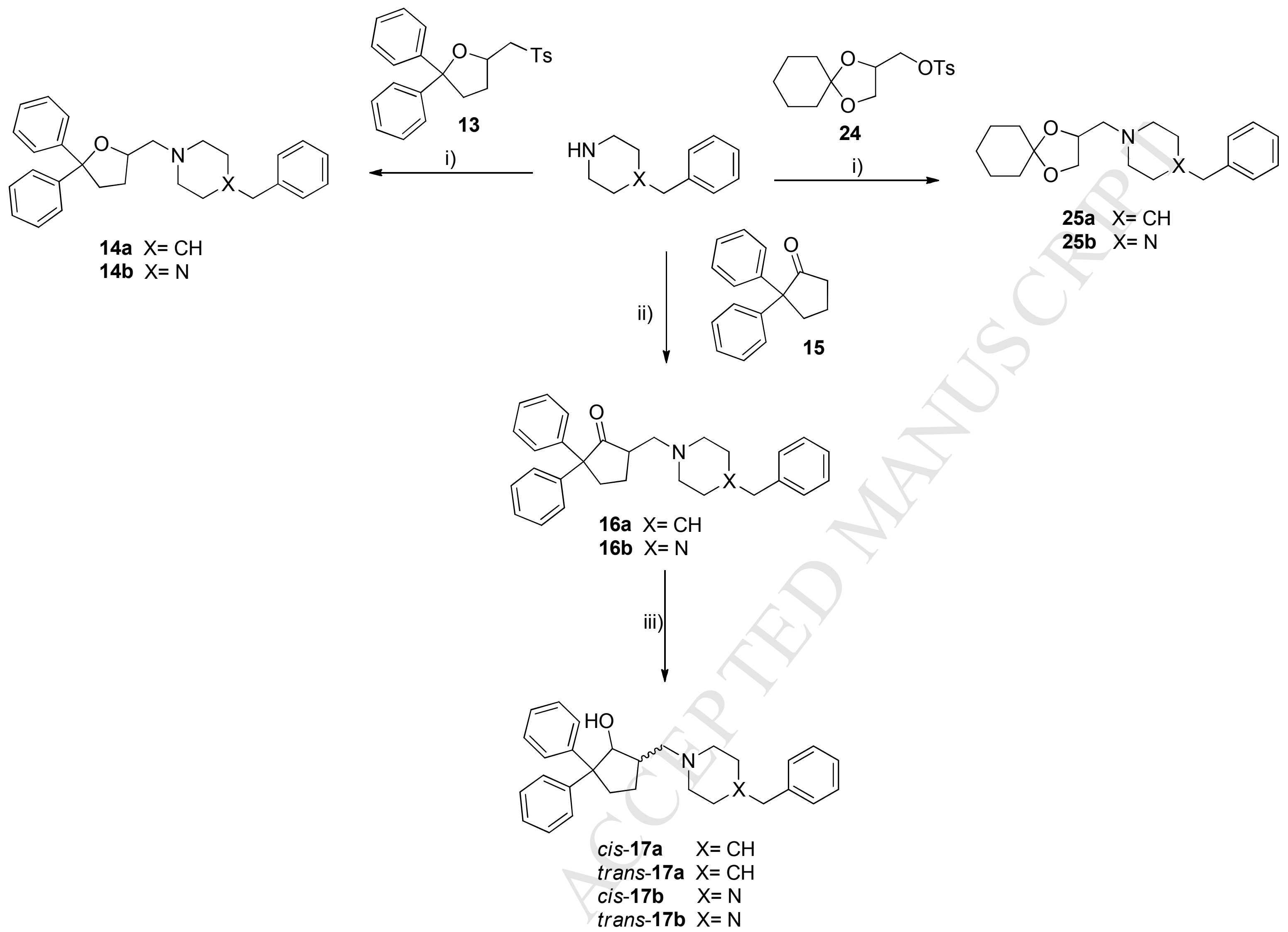


Comp.	R	X	pK_i σ_1[a,b]	pK_i σ_2[a,c]	σ_1/σ_2[d]	pK_i 5-HT_{1A}[e]	σ/5-HT_{1A}[f]
10a		CH	6.68	<6	>5	<6	>5
10b		N	8.47	8.30	2	<6	>29
25a		CH	8.70	7.72	10	6.79	81
25b		N	9.13	7.46	47	<6	>1349

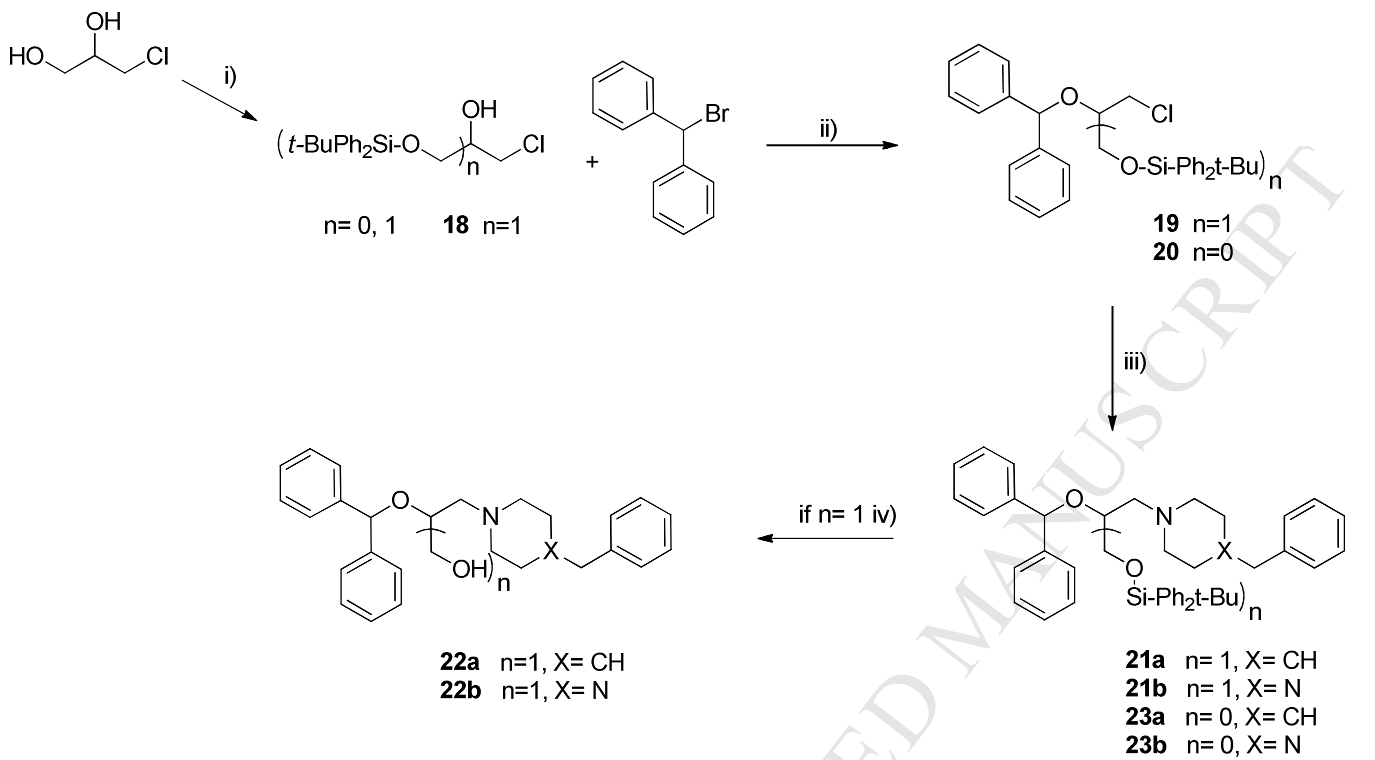
[a] Each concentration was tested in duplicate and each experiment was repeated three times. The Ki values agreed to ± 20%. [b] Binding assays were performed using 3.0 nM [^{3}H]pentazocine. [c] Binding assays were performed using 3.0 nM [^{3}H]ditolylguanidine. [d] Antilog of the difference between the pKi values for σ_1 and σ_2 receptors. [e] Ki values were derived from the Cheng–Prusoff equation at one or two concentrations Each experimental condition was performed in triplicate and agreed within 10%. [f] Antilog of the difference between the pKi values for σ receptors (higher value) and the 5-HT_{1A}R.



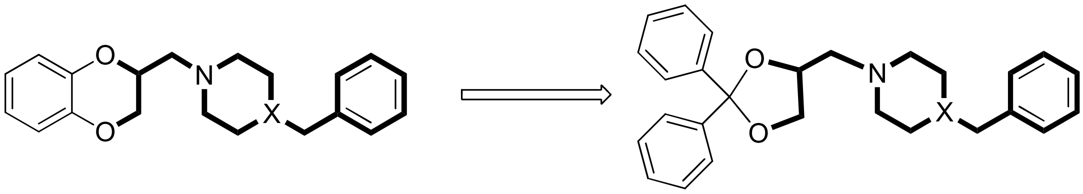
Scheme 1. Synthesis of Group I and Group II compounds. *Reagents and conditions*: i) *p*-toluensulfonic acid, toluene, reflux, 24/48 h. ii) TsCl, $N(Et)_3$, CH_2Cl_2, 0°C to 25 °C, 6 h; iii) 4-benzylpiperidine or 1-benzylpiperazine, KI, 2-methoxyethanol, reflux, 20 h.



Scheme 2. Synthesis of Group III, IV and VI compounds. *Reagents and conditions*: (i) 4-benzylpiperidine or 1-benzylpiperazine, KI, 2-methoxyethanol, reflux, 20 h.; ii) 4-benzylpiperidine or 1-benzylpiperazine as chloride salts, paraformaldehyde, C_2H_5OH, reflux, 25 h; iii) $NaBH_4$, C_2H_5OH, 0 °C to 25 °C, 24 h.



Scheme 3. Synthesis of Group V compounds. *Reagents and conditions*: i) *t*-BuPh$_2$SiCl, 1H-imidazole, DMF, 25 °C, 5 h; ii) toluene, reflux, 24 h; iii) 4-benzylpiperidine or 1-benzylpiperazine, KI, 2-methoxyethanol, reflux, 20 h.; iv) TBAF, THF, 25 °C, 24 h.



1,4-benzodioxane-based compounds

1,3-dioxolane-based compounds

1a: X= CH pKi σ1= 7.78, pKi σ2 = 7.60
1b: X= N pKi σ1 = 7.80, pKi σ2 = 7.55

Chart 1. Working hypothesis

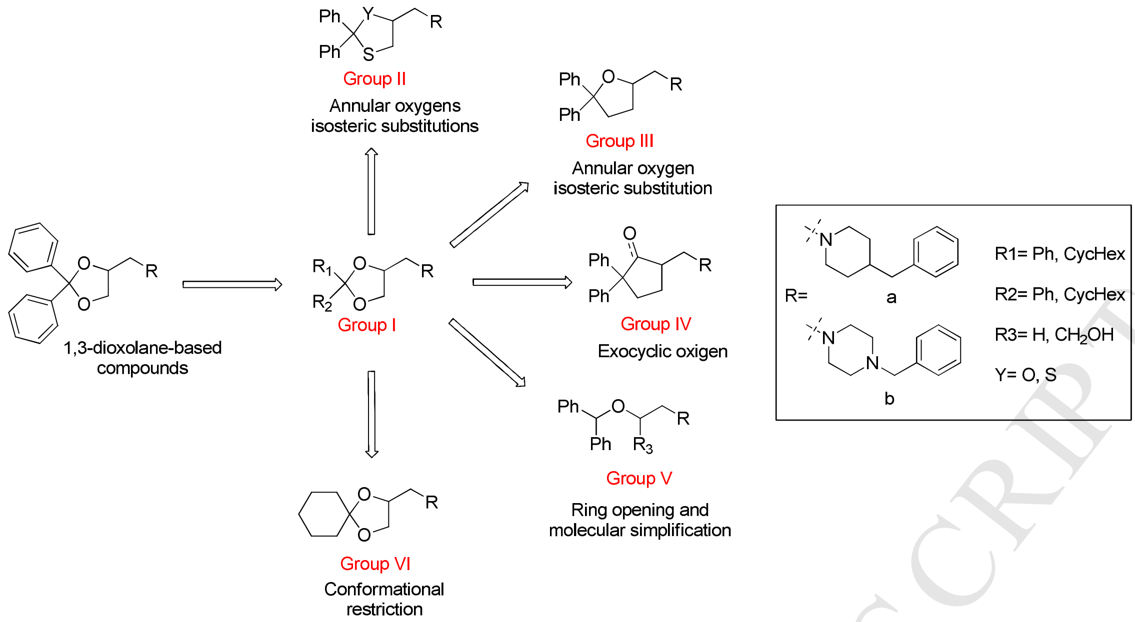


Chart 2. SAR studies

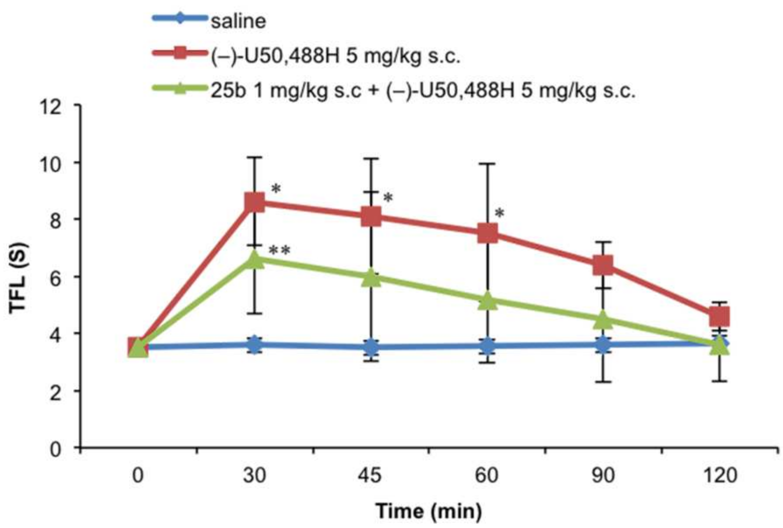


Figure 1. Effect of **25b** (1 mg/kg s.c.), on (–)-U-50,488H (5 mg/kg s.c.) analgesia. Results are expressed in seconds (s). Data are means ± SEM from 8-10 rats. *p < 0.05 vs saline-treated-rats; **p < 0.05 vs (–)-U-50,488H-treated-rats.

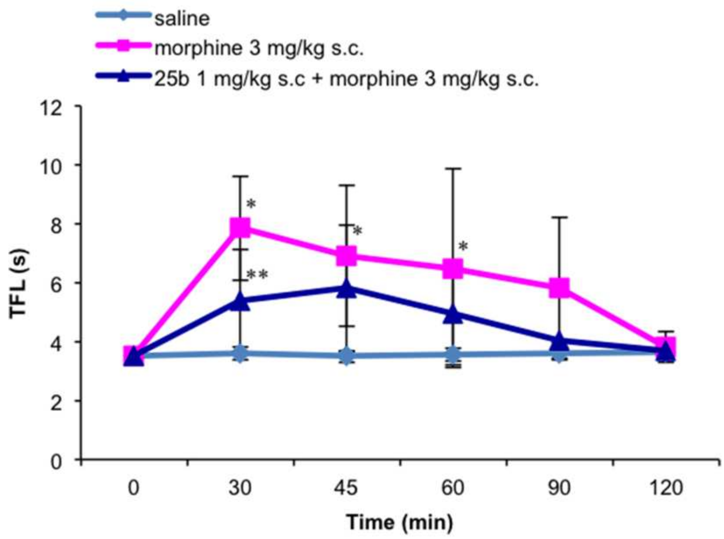
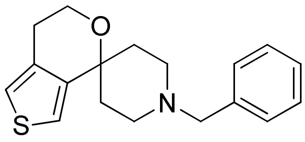


Figure 2. Effect of **25b** (1 mg/kg s.c.), on morphine (2 mg/kg s.c.) analgesia. Results are expressed in seconds (s). Data are means ± SEM from 8-10 rats. *p < 0.05 vs saline-treated-rats; **p < 0.05 vs morphine-treated-rats.



Compound I

Figure 3. Structure of the reference compound **I** ($pK_i\sigma_1$= 9.36)

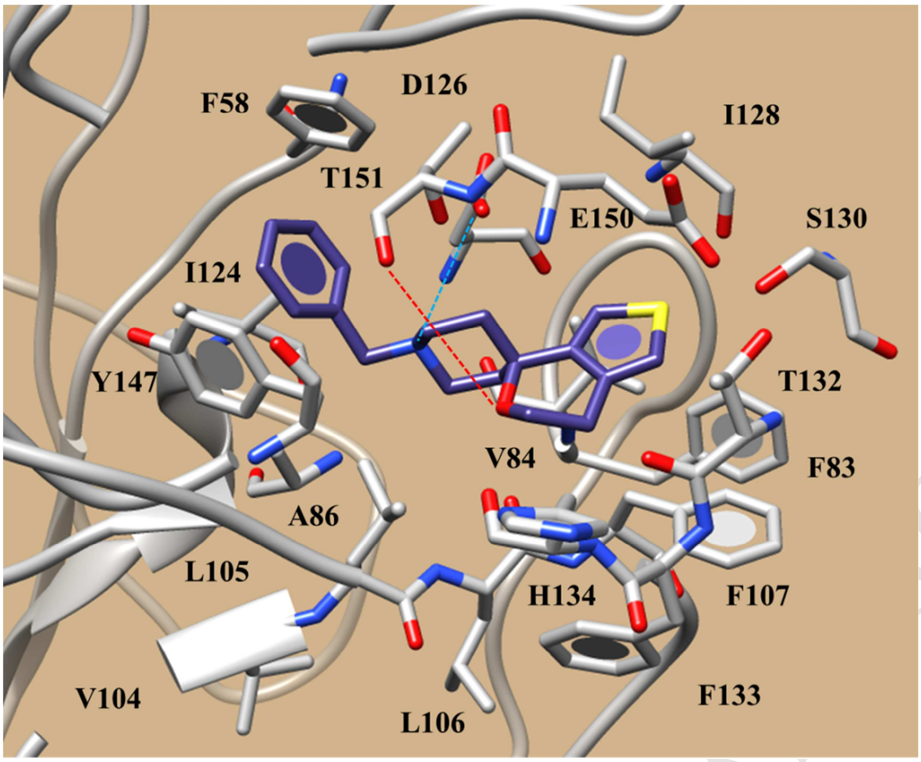


Figure 4. Ligand **I** docking pose into the putative sigma-1 binding site. Salt-bridge and H-bond contacts are displayed by a dashed line in light blue and red, respectively.

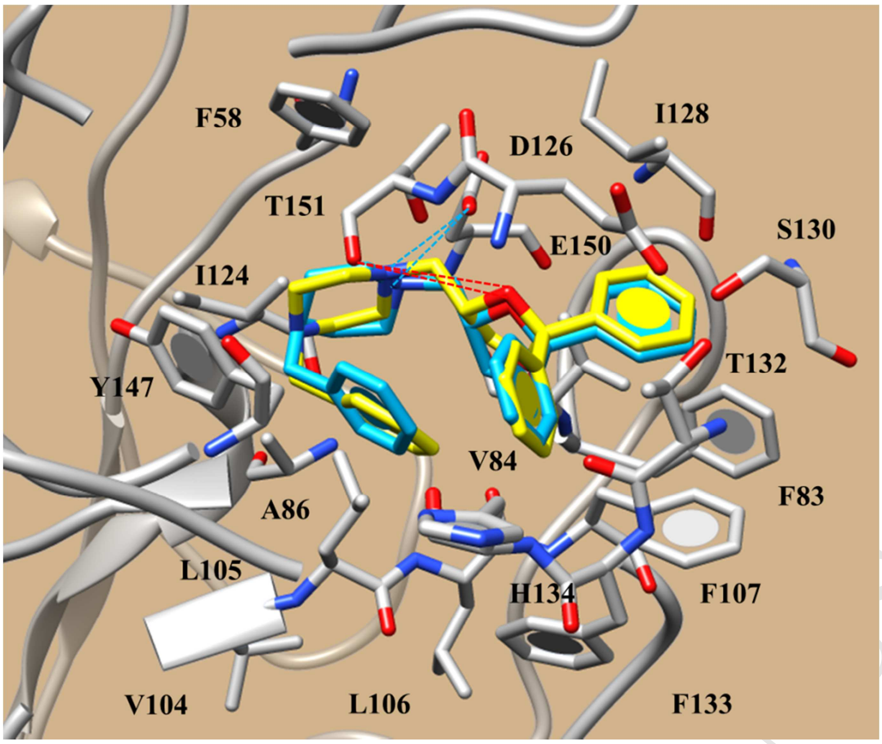


Figure 5. Compound **8a** and **8b** docking poses into the putative sigma-1 binding site. The ligands are colored by atom-type (**8a** *C* atom: cyan; **8b** *C* atom: yellow). Salt-bridge and H-bond contacts are displayed by a dashed line in light blue and red, respectively.

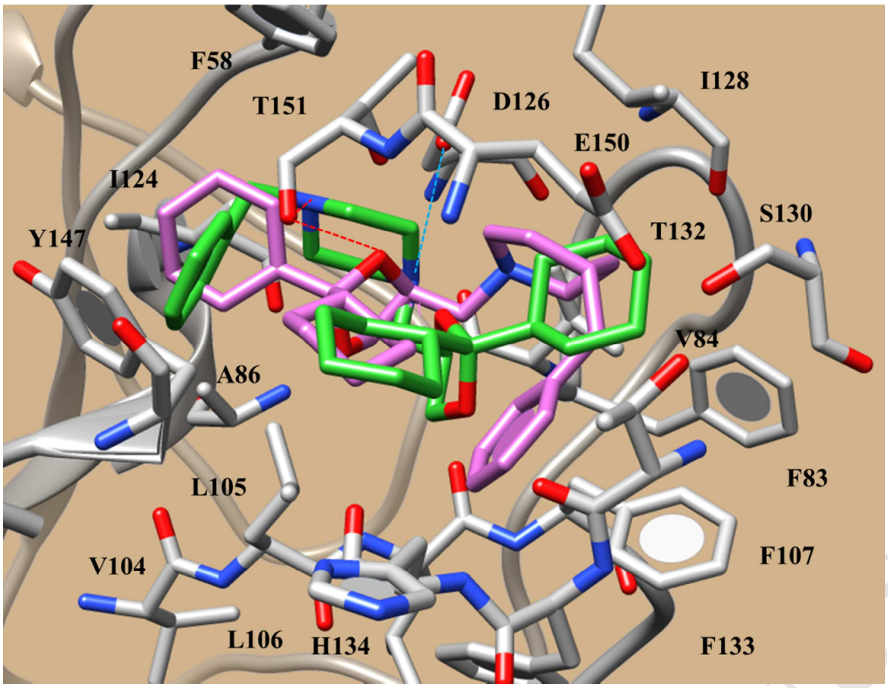


Figure 6. Compound **10a** and **10b** docking poses into the putative sigma-1 binding site. The ligands are colored by atom-type (**10a** *C* atom: light pink; **10b** *C* atom: green). Salt-bridge and H-bond contacts are displayed by a dashed line in light blue and red, respectively.

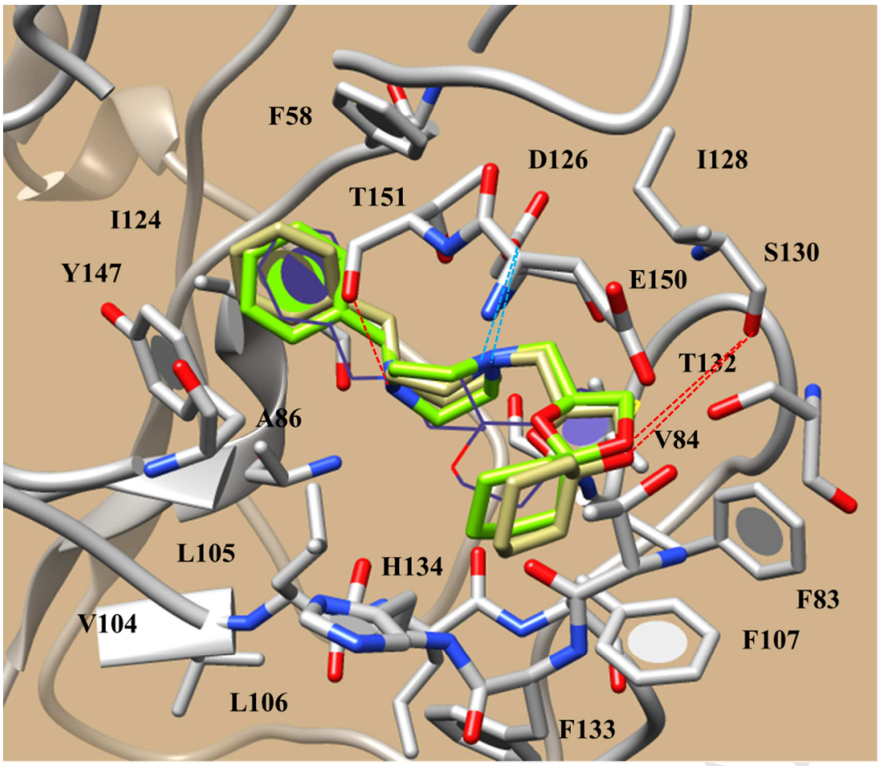


Figure 7. Compound **25a** and **25b** docking poses into the sigma-1 binding site are depicted by sticks. The ligands are colored by atom-type (**25a** *C* atom: dark khaki; **25b** *C* atom: light green). Salt-bridge and H-bond contacts are displayed by a dashed line in light blue and red, respectively. The docking mode of **I** is reported by wire (*C* atom: purple).

Highlights

- Twenty-six novel σR ligands bearing a variety of five-membered heterocyclic rings were synthesized.
- Compound **25b** exhibited the highest affinity and selectivity (pK_i σ_1 = 9.13, σ_1/σ_2 = 47).
- *In-vivo* studies showed that **25b** possesses anti-opioid effects on κ (KOP) and μ (MOP) receptor-mediated analgesia suggesting an agonistic behavior at σ_1R.
- Docking studies were performed on the theoretical σ_1R homology model