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Original article

Towards the development of 5-HT$_7$ ligands combining serotonin-like and arylpiperazine moieties

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ABSTRACT

Many known 5-HT$_7$ ligands contain either a serotonin-like or an arylpiperazine structure that, in published SAR studies, are generally supposed to bind the same receptor pocket. Conversely, we explored the hypothesis that two such moieties can co-exist in the same ligand, binding to different pockets. We thus designed and synthesized a set of compounds including both a 5-hydroxyindol-3-ylethyl and a 1-arylpiperazine moieties connected by a short linker. The compounds were tested for their affinity for human 5-HT$_7$ serotonin receptor. We further prepared a novel series of 5-HT$_7$ ligands, where the 5-hydroxyindol-3-ylethyl moiety was bioisosterically replaced by a 3-hydroxyanilinoalkyl one. Among the newly synthesized compounds, potent ligands at the 5-HT$_7$ receptor, behaving as antagonists in functional tests, were identified, even if they showed limited subtype selectivity. Docking studies within a model of the 5-HT$_7$ receptor showed that the binding site can actually accommodate both moieties, with the serotonin-like one in the putative orthosteric site and the arylpiperazine one occupying an accessory pocket. The present results demonstrate that it is possible to devise and develop new 5-HT$_7$ ligands merging two privileged structures in the same molecule.

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT, Fig. 1) is an important neurotransmitter that mediates a wide range of physiological functions by interacting with seven serotoninergic receptor families (5-HT$_{1-7}$) subdivided into at least 14 subpopulations [1]. Drugs which interact specifically with serotonin receptors are clinically approved for the treatment of migraine (sumatriptan: 5-HT$_{1D/1F}$ agonist), psychosis (risperidone: 5-HT$_2$/D$_2$ antagonist), anxiety disorders (buspirone: 5-HT$_{1A}$ agonist) and emesis (ondansetron: 5-HT$_3$ antagonist) [2–4]. The most recently discovered and cloned member of the 5-HT receptor family is 5-HT$_7$, which belongs to the G protein-coupled receptor (GPCR) superfamily and is positively coupled to adenylyl cyclase. The 5-HT$_7$ receptor was cloned independently by three laboratories in 1993 [5–7]. Since its discovery, the 5-HT$_7$ receptor has been cloned from different species, including rat, man, mouse, pig, guinea pig, honeybee, *Xenopus laevis*, *Aedes aegypti* and *Caenorhabditis elegans* [8]. It is expressed in the central and peripheral nervous system and also in the periphery. In the latter, the 5-HT$_7$ receptor has been detected predominantly in smooth muscle cells of the cardiovascular, gastrointestinal, and reproductive system, where it was consistently shown to induce smooth muscle relaxation [9]. In the central nervous system, the 5-HT$_7$ receptor is most abundant in hippocampus, thalamus, hypothalamus and cerebral cortex. The presence in the hypothalamus correlates with its involvement in circadian rhythm, thermoregulation, and endocrine regulation. Thalamic and cortical 5-HT$_7$ receptors may be of importance for sleep, mood regulation and epilepsy. The distribution of 5-HT$_7$ receptors in the hippocampus is of relevance for its role in learning and memory [10]. Finally, its localization in the spinal cord is in agreement with putative functions in nociception and locomotion. A consistent body of evidence, using both 5-HT$_7$ receptor knockout mice and selective ligands, supports the involvement of this receptor in various pathophysiological

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processes. Therefore, this receptor has become an attractive target for drug discovery and in the past years intense efforts have led to the identification of different structural classes of 5-HT_7 receptor agents [for a recent review see Ref. [11]].

Ligands characterized by a 1-arylpiperazine moiety are well known for their affinity to several members of the family of serotoninergic receptors, and recently different research groups have modified this template in order to identify selective 5-HT_7 receptor ligands [12]. On the other hand, it is known that appropriate molecular modifications of tryptamine can lead to derivatives that display an enhanced selectivity for different 5-HT receptor subtypes [13], even if the features conferring a subtype selective profile are not fully established.

A number of studies defining 5-HT_7 pharmacophore and homology (using rhodopsin crystal structures as templates) models have been published [12c,14], and they are characterized by at least four pharmacophoric features: a positive ionizable atom, a H-bonding acceptor group, and two hydrophobic regions. In these models, it is generally assumed that the 1-arylpiperazine and the indolylethylamine fragments are reciprocal bioisosteres, with their aromatic portions occupying the same hydrophobic region of the 5-HT_7 receptor.

In some structures of shorter 5-HT_7 ligands (**a**–**c** in Fig. 1), the basic amine group is bound to a serotonin-like fragment, i.e. a tryptamine or a 2-aminotetralin one, where the 5-hydroxyl and the 8-hydroxyl groups can be strictly superposed, and conserve a significant role for structure–activity relationships. On the other hand, in other ligands, such as aminochromanes (**d**, **e**), 5-aryl-2-aminotetralines (**f**) and mercaptopyridines (**g**) the similarity with the tryptamine scaffold of 5-HT is less evident, with the lack of a hydroxyl group or its bioisostere mimicking the 5-hydroxyl group of 5-HT. 1-Arylpiperazines (**h**–**j**), an important class of 5-HT_7 ligands, resemble more this second chemotype. In fact, the 7'-methoxy group of **h** can be replaced by a hydrogen atom with no loss of receptor affinity [14e]. Moreover, the 1-arylpiperazine moiety can be connected to a lipophilic tail with good 5-HT_7 affinity, as in compounds **i** and **j**, while no "longer" 5-HT_7 ligands with a tryptamine or 8-hydroxy-2-aminotetralin scaffold have been described.

These observations led us to hypothesize that a serotonin-like moiety could be combined with an arylpiperazine one, as they may occupy two distinct hydrophobic cavities. Although such distinct binding pockets have been proposed both in ligand-based and receptor-based models [14], compounds including both moieties have not been reported so far, nor has this hypothesis been investigated through structure–activity relationships.

We thus synthesized a first series of putative 5-HT_7 ligands, characterized by the presence of a serotonin-like scaffold, where the basic nitrogen is enclosed in a piperazine nucleus, linked to different lipophilic portions through its second nitrogen. As this lipophilic region showed SARs that resembled those of known 1-arylpiperazine ligands, we thus explored the possibility to bioisosterically replace the tryptamine moiety. In a previous work on melatonin receptor ligands, we had observed that a 2-(3-methoxyanilino)ethyl and a 3-(3-methoxyanilino)propyl fragment could replace the 5-methoxyindol-3-ylethyl portion of melatonin [15]. We therefore synthesized a novel series of putative 5-HT_7 ligands, composed by a 2-(3-hydroxyanilino)ethyl or a 3-(3-hydroxyanilino)propyl moiety, potentially mimicking the indole scaffold of 5-HT, and by an arylpiperazine moiety. Furthermore, we explored the SARs for this novel class of compounds and analyzed their possible binding modes at the 5-HT_7 receptor by docking simulations within a homology-based model of the receptor. Selected compounds, endowed with good receptor affinity, were further examined for their intrinsic activity and selectivity against different 5-HT receptor subtypes.

2. Chemistry

A previously reported method [16] was used to prepare the [(4-substituted-piperazin-1-yl)ethyl]-indole derivatives **5i**–**p** (Scheme 1); the procedure involves acylation of the suitable indole **1a**–**d** with oxalyl chloride, followed by reaction with the appropriate *N*-substituted-piperazine to give the (4-substituted-piperazin-1-yl) ethane-1,2-dione derivatives **3a**–**h**; these latter are then converted to the corresponding 5-hydroxyindole derivatives **5i**–**p** by reduction with $LiAlH_4$ and subsequent *O*-deprotection by hydrogenolysis or by treatment with boron tribromide.

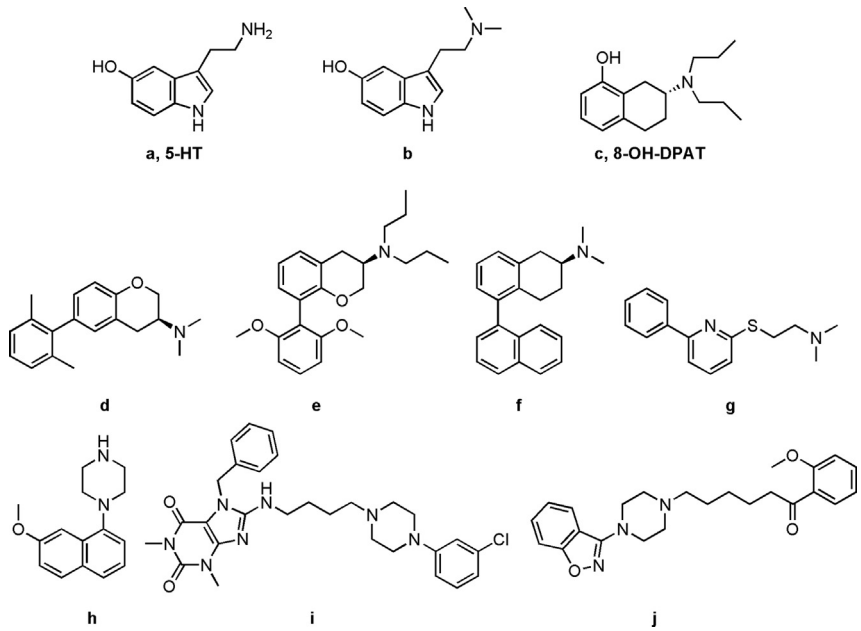
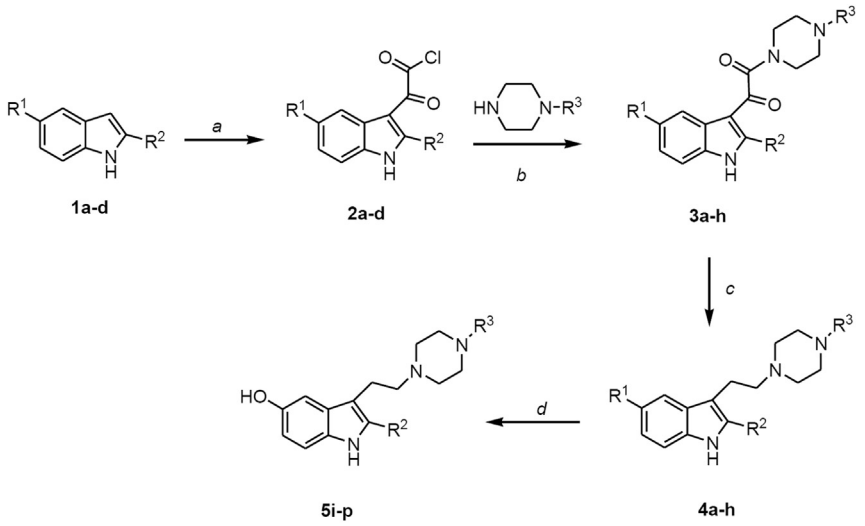


Fig. 1. Representative 5-HT_7 ligands [12d,14b,55–58].



	R^1	R^2	R^3		R^2	R^3
a	OCH_3	H	α-naphthyl	**5i**	H	α-naphthyl
b	OCH_3	Et	α-naphthyl	**5j**	Et	α-naphthyl
c	OCH_3	Me	α-naphthyl	**5k**	Me	α-naphthyl
d	OCH_2Ph	H	biphenyl-3-yl	**5l**	H	biphenyl-3-yl
e	OCH_2Ph	H	biphenyl-4-yl	**5m**	H	biphenyl-4-yl
f	OCH_2Ph	H	Ph	**5n**	H	Ph
g	OCH_3	H	n-Bu	**5o**	H	n-Bu
h	OCH_3	H	cyclopentyl	**5p**	H	cyclopentyl

Scheme 1. *Reagents and conditions*: a) $(COCl)_2$, THF, RT, 1 h; b) TEA, THF, RT, 1.5 h; c) $LiAlH_4$, THF, reflux, 1 h; d) BBr_3, CH_2Cl_2, RT, 2 h (for **5i**–**k** and **5o**–**p**) or H_2, 10% Pd/C, MeOH, RT, 6 h (for **5l**–**n**).

The (anilinoethyl)-piperazino derivatives **10h**–**l** were prepared as depicted in Scheme 2. Briefly, the (4-substituted-piperazine) acetamido derivatives **8d**–**g** were synthesized by *N*-acylation of anilines (**6a**–**c**) with bromoacetyl chloride in the presence of $NaHCO_3$ followed by substitution of the intermediate bromoacetamides **7a**–**c** with the suitable *N*-substituted-piperazine. The target compounds **10h**–**j** were then obtained by $LiAlH_4$ reduction of the amides **8d**–**f**, followed by *O*-deprotection of the intermediates **9d**–**f** by treatment with BBr_3 (for **10h**–**i**) or by hydrogenolysis catalyzed by palladium on carbon (for **10j**). The nitro-compound **9g,** obtained by BH_3 amide reduction of **8g**, was hydrogenated over 10% Pd/C to give the corresponding anilino derivative **10k** that could be converted into the *N*-methanesulfonamido target compound **10l**, by treatment with methanesulfonyl chloride.

Following a similar stepwise synthetic route (after changing the *N*-acylating reagent to 3-bromopropanoyl chloride), the (anilinopropyl)-piperazino derivatives **14b**–**o** were obtained from the corresponding anilines **6a**, **11b**–**i** as shown in Scheme 3. In a similar manner, the higher homologs **18a**–**d**, **18f**–**h** and the piperidine analog **18e** were synthesized starting from 3-benzyloxyaniline (**6b**) or 3-benzyloxy-*N*-methylaniline (**11i**) [17] and the suitable ω-alkanoylchloride (Scheme 4). The phenol-hexaneamido derivative **16i** was obtained by *O*-debenzylation with BCl_3 of the corresponding benzyloxy derivative **16f** (Scheme 4).

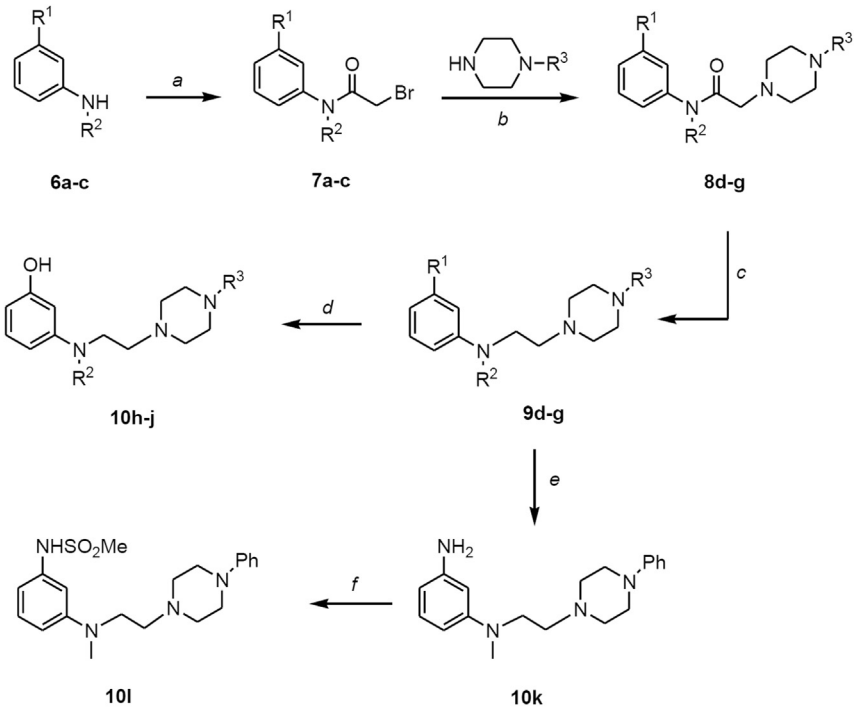
The *N*-acetamido- (**14p**) and the *N*-methanesulfonamido- (**14q**) target compounds were obtained by catalytic hydrogenation (10% Pd/C) of the nitro-compound **14f** (Scheme 3) and subsequent treatment of the crude aniline intermediate with acetic anhydride or methanesulfonyl chloride, respectively.

Reductive amination of the aniline derivative **17d** with acetaldehyde or benzaldehyde in the presence of $NaBH_3CN$ and subsequent *O*-debenzylation yielded, respectively, the *N*-ethyl- (**18j**) or *N*-benzyl- (**18k**) final product (Scheme 4).

Compounds **14r**–**u** were prepared from the ethyl ester derivative **14g** following the step sequence shown in Scheme 5. In particular **14g** was reduced by $LiAlH_4$ to the hydroxymethyl derivative **14t**, that was oxidized by MnO_2 to the carboxaldehyde derivative **14u**. By treatment of **14g** with magnesium nitride in methanol we obtained a mixture of the primary amide **14r** and of the methyl ester derivative **14s**, that was separated by chromatography.

N-substituted-[3-(4-phenylpiperazin-1-yl)propyl]anilines **24**–**27** were prepared from the aniline derivative **14h** (Scheme 6) that can be converted to the *N*-propyl-anilino derivative **24** by *N*-acylation with propionic anhydride, followed by BH_3 amide reduction and subsequent *O*-demethylation with boron tribromide. Alternatively, **14h** is converted to the *N*-benzyl- (**25**) or to the *N*-benzenesulfonyl- (**27**) target compound by reaction with benzyl bromide or benzenesulfonyl chloride, respectively, and subsequent *O*-demethylation with BBr_3. The *N*-phenyl derivative **26** was achieved by palladium-catalyzed amination [$Pd(OAc)_2$/BINAP/KO*t*Bu] of **14h** with iodobenzene and subsequent *O*-demethylation with boron tribromide.

Preparation of the 4-substituted piperidine derivatives **30b**–**c** started with the reaction of **12a** with the suitable piperidine giving the corresponding piperidine-propanamido derivatives **28b**–**c**, that were then reduced with BH_3, and finally *O*-deprotected by treatment with BBr_3 (Scheme 7).



	R^1	R^2	R^3		R^2	R^3
a	OCH_3	Me				
b	OCH_2Ph	H		**10h**	Me	Ph
c	NO_2	Me		**10i**	Me	Bn
d	OCH_3	Me	Ph	**10j**	H	Ph
e	OCH_3	Me	Bn			
f	OCH_2Ph	H	Ph			
g	NO_2	Me	Ph			

Scheme 2. *Reagents and conditions*: a) 2-bromoacetyl chloride, $NaHCO_3$, EtOAc, H_2O, RT, 20 min; b) TEA, DMF, RT, 1–16 h; c) $LiAlH_4$, THF, reflux, 2 h (for **9d**–**f**) or BH_3, THF, RT, 16 h (for **9g**); d) BBr_3, CH_2Cl_2, RT, 2 h (for **10h**–**i**) or H_2, 10% Pd/C, MeOH, 60 °C, 3 h (for **10j**); e) H_2, 10% Pd/C, EtOH, EtOAc, RT, 5 h; f) methanesulfonyl chloride, TEA, THF, RT, 1 h.

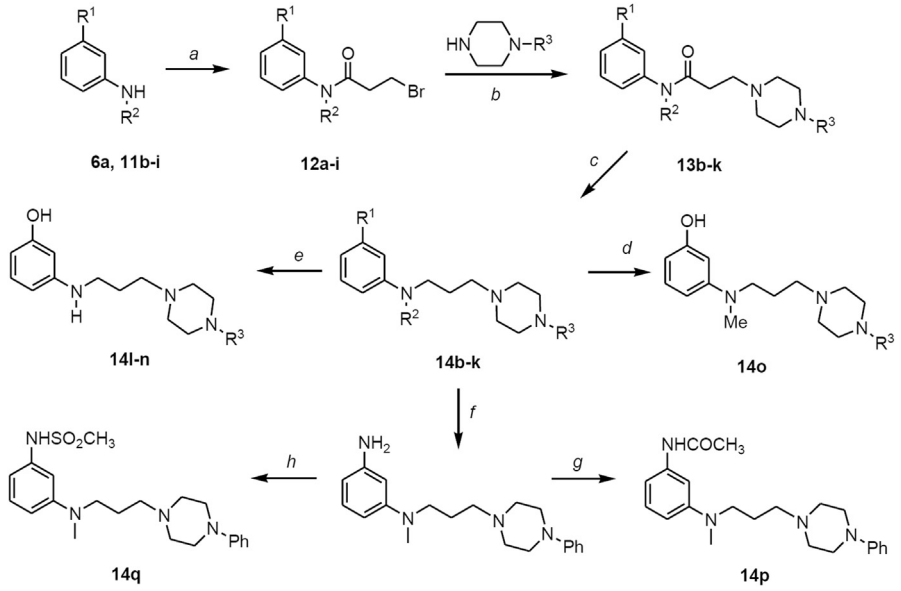
Phenolalkyl-piperazino derivative **32a**–**c** were synthesized by condensation of the appropriate carboxylic acid with *N*-phenyl piperazine in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), followed by amide reduction with 1 M B_2H_6 in THF and *O*-deprotection (for **32c**) (Scheme 8).

Compound **35** was prepared from 3-(benzyloxy)phenol following the three-step sequence (*O*-alkylation, amide reduction, *O*-debenzylation) illustrated in Scheme 9.

The tetralin derivative **37** was synthesized by reductive amination of 8-methoxy-2-tetralone with *N*-phenyl-piperazine and subsequent *O*-demethylation with BBr_3 (Scheme 10).

3. Results and discussion

Binding affinities at the human 5-HT_7 receptor for the newly synthesized compounds that combine a serotonin-like (5-hydroxyindol-3-ylethylamine) with an arylpiperazine moiety are reported in Table 1. Compared with 5-HT, the phenylpiperazinyl derivative **5n** showed moderate binding affinity (K_i = 63 nM), suggesting that the presence of the phenyl-piperazine moiety did not prevent accommodation of the 5-hydroxyindole portion within the 5-HT_7 binding site. Bulkier aromatic substituents, in the *m*-biphenyl and *p*-biphenyl derivatives **5l** and **5m**, abolished affinity for the receptor. On the other hand, an α-naphthyl group (**5i**) had a favorable effect and led to a sixfold increase of binding affinity compared to the phenyl derivative **5n**. When small alkyl groups were inserted at position 2 of the indole ring in the α-naphthyl derivative, a methyl group (**5k**) was tolerated and an ethyl one (**5j**) doubled 5-HT_7 binding affinity. This behavior differs from what observed for other tryptamine derivatives. In fact, insertion of a methyl or an ethyl group in position 2 of *N*,*N*-dimethyl-5-methoxytryptamine led to a decrease of binding affinity, with an increase from tenfold to fiftyfold in K_i values [14b,18]. Replacement of the aromatic substituent on the piperazine nitrogen with an alkyl one, such as a *n*-butyl chain (**5o**) or a cyclopentyl ring (**5p**), led to derivatives devoid of any significant 5-HT_7 binding affinity. This observation is not in line with what reported for a series of 6-bromo-1-(homo-piperazinyl)ethylindoles, in which the presence of alkyl substituents on the homo-piperazine nitrogen afforded compounds with binding affinities in the nanomolar range [19]. Thus, both a phenyl- and an α-naphthyl-piperazine moiety could be combined with a serotonin-like moiety with limited loss of receptor affinity. This preliminary result was confirmed preparing compound **37**, where the 5-hydroxyindol-3-ylethyl substructure

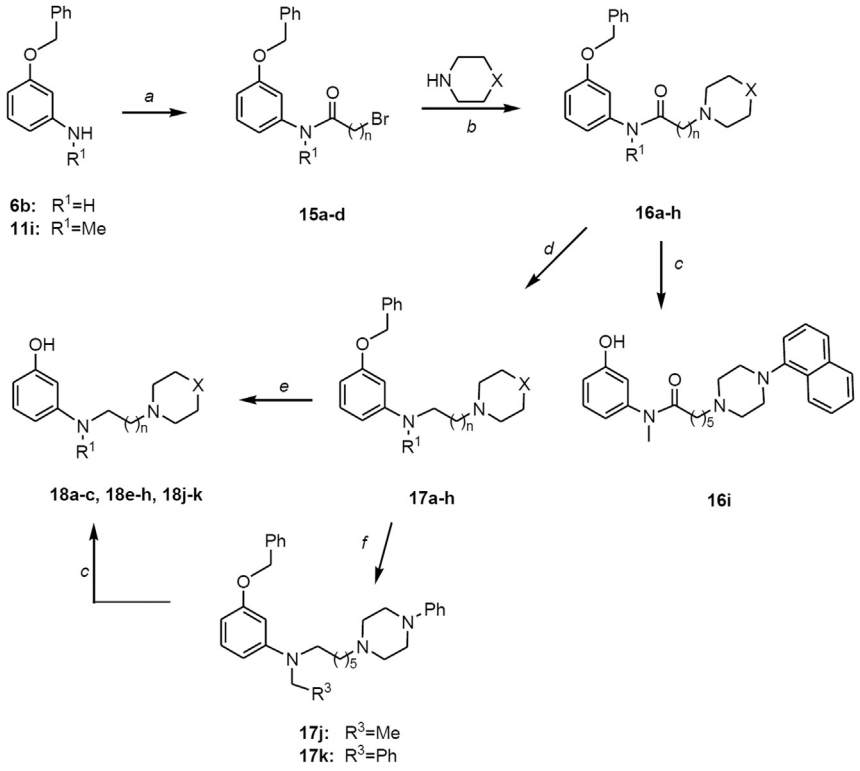


	R^1	R^2	R^3		R^1	R^2	R^3
a	OCH_3	Me	Ph	**i**	OCH_2Ph	Me	Ph
b	Me	Me	Ph	**j**	OCH_3	Me	α-naphthyl
c	Br	Me	Ph	**k**	OCH_3	Me	β-naphthyl
d	Cl	Me	Ph	**14l**		H	Ph
e	F	Me	Ph	**14m**		Me	α-naphthyl
f	NO_2	Me	Ph	**14n**		Me	β-naphthyl
g	CO_2Et	H	Ph	**14o**			Ph
h	OCH_3	H	Ph				

Scheme 3. *Reagents and conditions*: a) 3-bromopropanoyl chloride, $NaHCO_3$, EtOAc, H_2O, RT, 20 min; b) TEA, DMF, RT, 1–16 h; c) BH_3, THF, RT, 16 h; d) H_2, 10% Pd/C, MeOH, 60 °C, 3 h; e) BBr_3, CH_2Cl_2, RT, 2 h; f) H_2, 10% Pd/C, EtOH, EtOAc, RT, 5 h; g) Ac_2O, TEA, THF, RT, 1 h; h) methanesulfonyl chloride, TEA, THF, RT, 1 h.

was replaced by a 8-hydroxytetralin one, which had been shown to be a bioisostere on several 5-HT receptors.

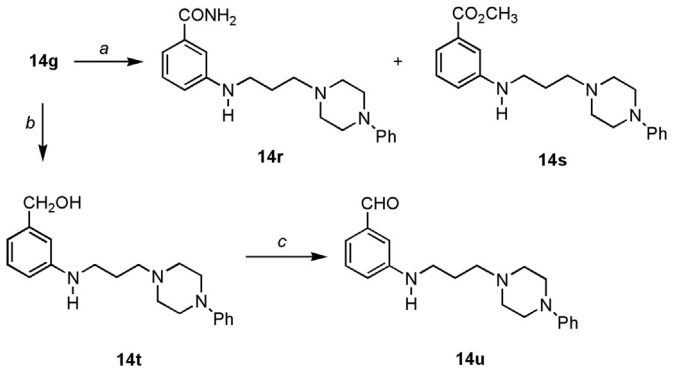
To investigate the binding mode of our indole derivatives, we built a homology model of the 5-HT_7 receptor based on the three-dimensional coordinates of the 5-HT_{1B} serotonin receptor co-crystallized with the agonist dihydroergotamine. Crystal structures of different GPCRs have shown that agonist and antagonist compounds can be accommodated within binding sites having very similar location and size, with only subtle differences [20]. Therefore, given the level of accuracy of GPCR homology models, we considered the 5-HT_{1B} crystal structure as a suitable template and we used the 5-HT_7 model for docking studies on our compounds, for which a functional characterization was not systematically carried out. As already observed by Kolaczkowsky et al. [14e] for a rhodopsin-based 5-HT_7 receptor model, the putative binding region is characterized by the presence of Asp162 (3.32 according to Ballesteros–Weinstein's nomenclature [21]) on transmembrane (TM) helix 3 which is supposed to be the counterpart for basic groups of ligands and which divides the binding site into two portions, one located within the helix bundle and delimited by TM3, 5 and 6 and one which lays towards the extracellular side of TM7. Accordingly, in the best induced-fit docking solution of compound **5i** the protonated piperazine nitrogen interacts with Asp162$^{3.32}$ through a salt bridge and the ligand molecule spans the helix bundle from TM5 to TM7 (Fig. 2a). The indole ring of **5i** is located in a cavity delimited by TM3, TM5 and TM6 and is engaged in several hydrophobic contacts with Val163$^{3.33}$, Ala247$^{5.46}$, Phe343$^{6.51}$ and Phe344$^{6.52}$; the α-naphthyl-piperazine moiety is accommodated towards the extracellular portion of the helix bundle, close to TM7, and undertakes a number of favorable interactions with Phe158$^{3.28}$, Ile159$^{3.29}$, Leu232 (extracellular loop 2) and Leu370$^{7.39}$. Interestingly, the region occupied by the α-naphthyl substituent of compound **5i** is partially superposed to that occupied by the cyclic tripeptide of dihydroergotamine in the crystallized 5-HT_{1B} receptor (Fig. 2a). A 100 ns long molecular dynamics (MD) simulation of the 5-HT_7–**5i** complex was conducted in an explicit solvated lipid bilayer. The stability of the receptor structure was confirmed by the time evolution of the root mean squared deviation (RMSD) calculated for TM alpha carbons, which showed a plateau after 30 ns of simulation (Fig. S1). Compound **5i** retained its initial conformation during the whole MD simulation as well as the key ionic interaction with Asp162$^{3.32}$, and subtle rearrangements involving the indole and the α-naphthyl rings were detected (Fig. 2b). In particular, an outward shift of the extracellular half of



	n	R^1	X		n	R^1	X
a	3	Me	N-Ph	**f**	5	Me	N-α-naphthyl
b	4	Me	N-Ph	**g**	5	Me	N-*m*-CF_3-Ph
c	5	Me	N-Ph	**h**	5	Me	N-(7-Cl-quinolin-4-yl)
d	5	H	N-Ph	**j**	5	Et	N-Ph
e	5	Me	C-Ph	**k**	5	Bn	N-Ph

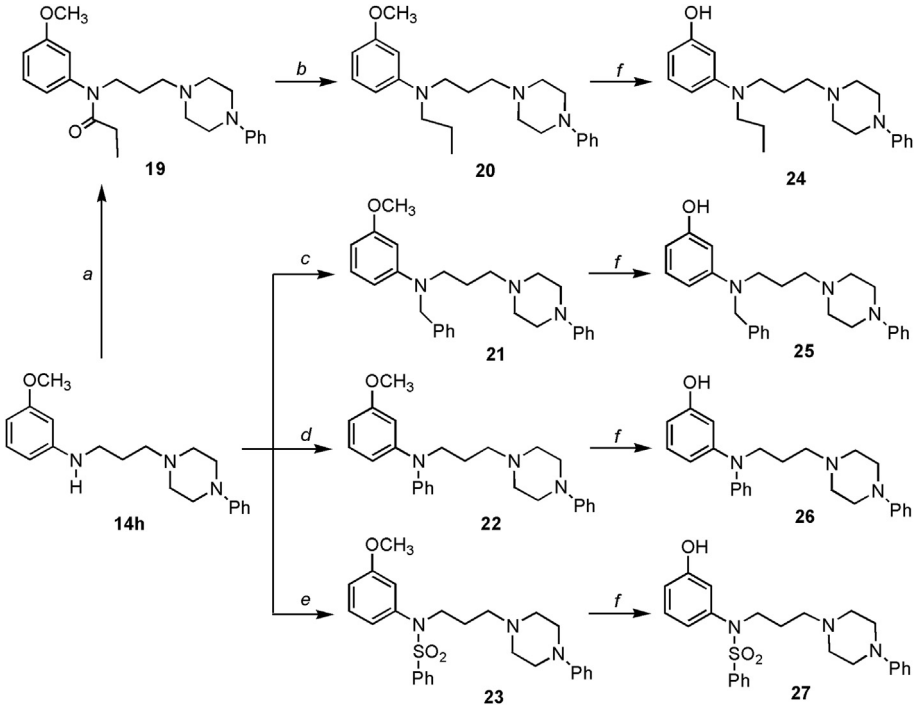
Scheme 4. *Reagents and conditions*: a) ω-bromoalkanoyl chloride, $NaHCO_3$, EtOAc, H_2O, RT, 20 min; b) TEA, DMF, RT, 1–16 h; c) BCl_3, CH_2Cl_2, RT, 1 h; d) $LiAlH_4$, THF, reflux, 2 h; e) BCl_3, CH_2Cl_2, RT, 2 h (for **18e**–**h**) or H_2, 10% Pd/C, MeOH, 60 °C, 3 h (for **18a**–**c**); f) CH_3CHO (for **17j**) or benzaldehyde (for **17k**), MeOH, AcOH, $NaBH_3CN$, RT, 16 h.

TM5 was observed at the beginning of the MD simulation, allowing a slight rotation of the indole ring of **5i** and the consequent formation of a hydrogen bond between its 5-hydroxyl group and Ser243$^{5.42}$, which was maintained throughout the MD simulation. Although no mutagenesis data are available for this amino acid, replacement of the corresponding serine of the 5-HT_{1A} receptor with an alanine decreased 5-HT binding affinity [22]. Moreover, the serine residue in position 5.42 of the β_1 receptor is hydrogen bonded to the hydroxyl groups of isoprenaline [23], suggesting that the amino acid in this position may interact with polar groups of other aminergic neurotransmitters. The α-naphthyl substituent of **5i** slightly rearranged during MD simulation. In particular, it moved slightly away from Phe158$^{3.28}$ on TM3 and was accommodated in a crevice located at the tips of TM6 and TM7, delimited by Phe343$^{6.51}$, Leu346$^{6.54}$ and Leu370$^{7.39}$. The shape and the location of this pocket could also explain the loss of binding affinity brought by the introduction of *meta*- or *para*-biphenyl substituents (compounds **5l** and **5m**). Indeed, these bulkier groups could not be accommodated, due to unfavorable steric clashes. This hydrophobic region, extending beyond the anionic site of Asp162$^{3.32}$, can thus represent the binding pocket for the arylpiperazine moieties of our hybrid ligands.



Scheme 5. *Reagents and conditions*: a) Mg_3N_2, MeOH, 80 °C, 24 h; b) $LiAlH_4$, THF, RT, 1 h; c) MnO_2, CH_2Cl_2, RT, 18 h.

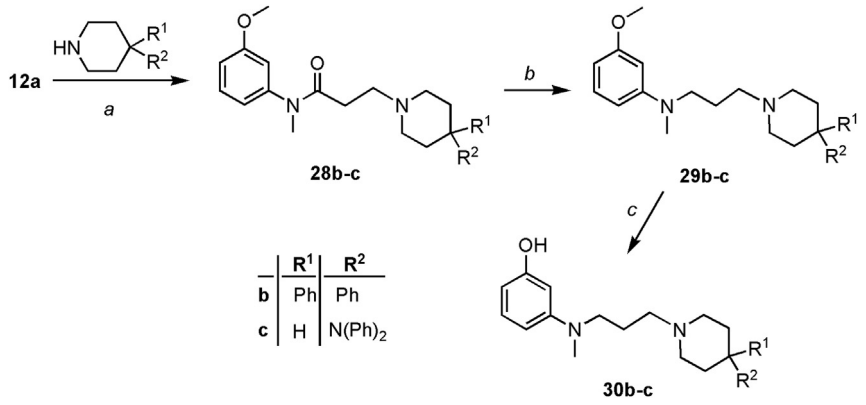
The binding model depicted in Fig. 2b is also consistent with the possibility to replace the serotonin-like portion with bioisosteric substructures. In fact, compound **37**, having a 8-hydroxytetralin scaffold, showed binding affinity similar to that of the serotonin-like derivative **5n** (K_i = 81.4 nM and K_i = 63.1 nM, respectively). To further test this hypothesis, we replaced the indole scaffold with an aniline one and we prepared a small series of anilino-alkyl-piperazine derivatives, exemplified by compound **10h** (Table 2). In



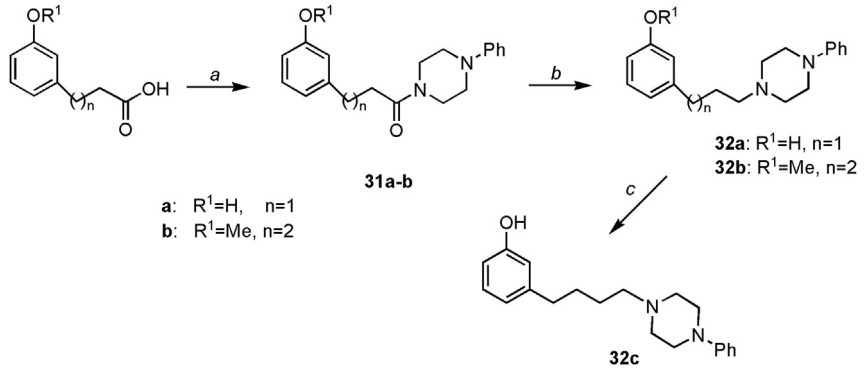
Scheme 6. *Reagents and conditions*: a) $(EtCO)_2O$, TEA, THF, RT, 24 h; b) BH_3, THF, RT, 16 h; c) benzyl bromide, TEA, toluene, 80 °C, 16 h; d) $Pd(OAc)_2$, (±)-BINAP, *tert*-BuO^-K^+, iodobenzene, toluene, reflux, 24 h; e) benzenesulfonyl chloride, TEA, THF, RT, 48 h; f) BBr_3, CH_2Cl_2, RT, 2 h.

fact, successful bioisosteric replacement of the 5-methoxyindol-3-ylethyl fragment with a *meta*-methoxyanilinoethyl or a *meta*-methoxyanilinopropyl one had already been described for melatonin (*N*-acetyl-5-methoxytryptamine) derivatives [15], and the anilino scaffold had resulted more versatile and synthetically feasible. Fig. 3 depicts *N*,*N*-dimethyl-5-hydroxytryptamine (**b**, Fig. 1) in the bioactive conformation proposed by Vermeulen et al. [14b], superposed to the tetralin derivative 8-OH-DPAT (**c**, Fig. 1) and to 3-{[2-(dimethylamino)ethyl](methyl)amino}phenol. The close superposition of this last compound highlights the possibility for a *meta*-hydroxyanilino-ethylamine fragment to bioisosterically replace the other two scaffolds.

Compound **10h** carries a hydroxyl group in *meta* position, topologically corresponding to position 5 of the indole ring of 5-HT, and a spacer with two methylene units connects the aniline nitrogen to the arylpiperazine portion. Compound **10h** showed a limited loss of binding affinity (Table 2), being about three times less potent than the corresponding indole derivative **5n**. An investigation on the importance of the hydroxyl group was carried out inserting in *meta* position substituents with different size and ability to form hydrogen bonds. While amino (**10k**) and nitro (**9g**) substituents led to a decrease of binding affinity, a methylsulfonylamino group had a positive effect, leading to a K_i value of 57.0 nM for compound **10l**. Replacement of the phenyl ring on the piperazine nitrogen with a benzyl group markedly reduced binding affinity (**10i**), which stresses the role of the arylpiperazine moiety. Removal of the methyl substituent from the aniline nitrogen slightly reduced binding affinity (**10j**), while replacement of N-methyl with an oxygen atom (**35**) almost abolished binding affinity. On the other hand, replacement with a methylene, as in the phenylpropyl derivative **32a,** led to a recovery in potency ($K_i = 40.0$ nM). SARs related to the lengthening of the alkyl spacer were also investigated, progressively increasing the number of methylene groups from two to six. Addition of one methylene group had a positive effect on binding affinity, since the phenylpiperazine derivative **14o** had $K_i = 20.0$ nM, compared to $K_i = 161.4$ nM for the shorter analog **10h**. As already observed in the indole series, replacement of the phenyl substituent with an α-naphthyl one significantly increased binding affinity (**14m**, $K_i = 2.9$ nM), while a β-naphthyl group (**14n**) was hardly tolerated. When docked into the 5-HT_7 receptor binding site, compound **14m** showed an accommodation and a pattern of interactions similar to those described for the indole derivative **5i**. The *meta*-hydroxyl group is hydrogen-bonded to Ser243$^{5.42}$, the protonated piperazine nitrogen interacts with Asp162$^{3.32}$ and the naphthyl substituent occupies the region defined by Phe343$^{6.51}$, Leu346$^{6.54}$ and Leu370$^{7.39}$ (Fig. 2c). Within the anilinopropyl series, a slight increase in binding affinity was registered when the aniline nitrogen was demethylated (**14l**) or replaced by a methylene group, with $K_i = 3.1$ nM for compound **32c**. The *meta*-hydroxyl group was fundamental to achieve high potency. In fact, its replacement with other substituents reduced binding affinity, as observed for a methyl group (**14b**), for halogens, such as fluoro (**14e**), chloro (**14d**) and bromo (**14c**), and for acetylamino (**14p**) and methylsulfonylamino (**14q**) groups. A similar decrease of binding affinity was obtained, on the anilino-NH scaffold, with hydroxymethyl (**14t**), methoxycarbonyl (**14s**) and carboxamido (**14r**) groups, with only the formyl substituent (**14u**) maintaining the same binding affinity as the *meta*-hydroxyl derivative **14l**. Four different substituents were introduced on the aniline nitrogen to look for additional interactions with the binding site. Our receptor model suggested that the 5-HT_7 receptor binding site has room enough to accommodate substituents at nitrogen slightly bulkier than the methyl group. Indeed, a *n*-propyl chain (**24**) did not affect binding affinity, while the phenyl (**26**) and benzyl (**25**) groups reduced K_i to about 90 nM. Interestingly, the phenylsulfonyl derivative **27** was the best one, with higher binding affinity than the reference **14o**. Docking of **27** into the receptor model gave two families of solutions. One of them was consistent with our hypothesis (Fig. 2d), while the other one showed inverted orientation, i.e. with the



Scheme 7. *Reagents and conditions*: a) TEA, DMF, RT, 16 h; b) BH_3, THF, RT, 16 h; c) BBr_3, CH_2Cl_2, RT, 2 h.

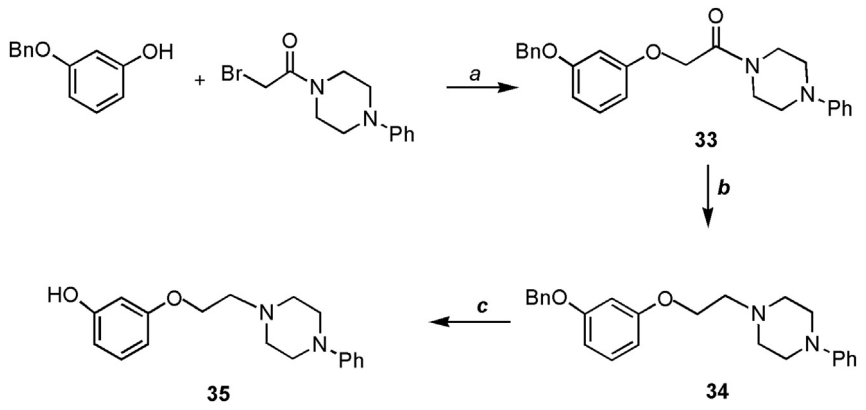


Scheme 8. *Reagents and conditions*: a) *N*-phenylpiperazine, EDC, HOBT, Et_3N, CH_2Cl_2, 2 h at 0 °C and 16 h at RT; b) B_2H_6, THF, RT, 4 h; c) BBr_3,CH_2Cl_2, RT, 2 h.

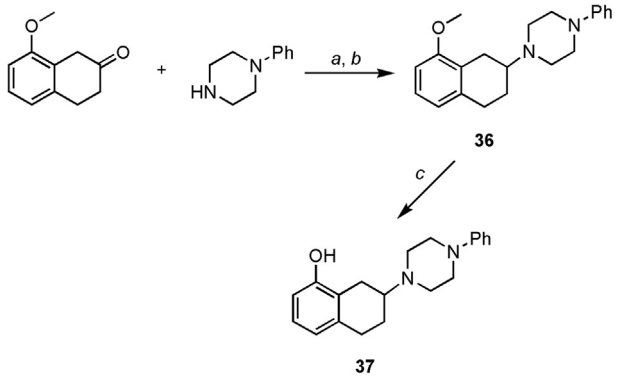
phenyl-piperazine moiety in the serotonin-like binding cavity and vice versa. These alternative solutions can be the result of model errors, due to its homology-based nature, or simply a consequence of the choice to select a model adapted, by MD refinement, to a smaller ligand (**14m**), lacking the N-phenylsulfonyl group.

Attempts to probe the size of the binding site portion accommodating the arylpiperazine moiety with bulkier substituents were not successful, as derivatives carrying 4,4′-diphenylpiperidinyl (**30b**) and 4-(*N*,*N*-diphenylamino)piperidinyl (**30c**) groups markedly reduced binding affinity. Lengthening of the alkyl spacer to four (**18a**), five (**18b**) and six (**18c**) methylene units did not significantly affect binding affinity, ending up with the most potent compound **18c** having $K_i = 12.0$ nM.

Selected anilino-alkyl-piperazines with two to six methylene spacers were tested in functional assays, to evaluate their agonist or antagonist behavior. Compound **18a** produced no increase of cAMP level when tested up to the concentration of 10^{-5} M. On the other hand it decreased the accumulation of cAMP stimulated by 5-HT, with a $K_B = 57$ nM. In the same test, compounds **10l**, **14o**, **18b** and **18c** at the concentration of 1 μM produced a reduction of cAMP accumulation induced by 5-HT to 48%, 49%, 69% and 56%, respectively (Table 2), suggesting antagonist behavior.



Scheme 9. *Reagents and conditions*: a) K_2CO_3, DMF, 40 °C, 16 h; b) B_2H_6, THF, RT, 4 h; c) BCl_3, CH_2Cl_2, RT, 1 h.



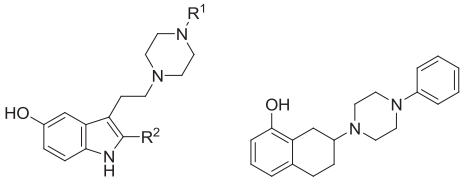
Scheme 10. *Reagents and conditions*: a) PTSA, toluene, reflux, 24 h; b) H_2, EtOH/THF, PtO_2, RT, 24 h; c) BBr_3,CH_2Cl_2, RT, 2 h.

The antagonists **18a** and **18c**, having a four-methylene and six-methylene spacers, respectively, were docked into the 5-HT_7 receptor binding site. Both were accommodated similarly to the shorter **14m,** with the same polar interactions formed by the piperazine nitrogen and hydroxyl substituent (Fig. 4). The hydroxylanilino moiety is accommodated within the serotonin-like binding pocket where agonists are supposed to bind, while the phenyl ring on the piperazine lies in the region of the binding site delimited by TM2, 3 and 7. This binding pose could be consistent with their antagonist behavior. Indeed, for the 5-HT_7 receptor [24] and, in general, for biogenic amine receptors [25] it has been proposed that antagonist and agonist compounds occupy the same binding region, with antagonists undertaking interactions with an additional portion of the receptor.

Other structural modifications were evaluated in the presence of the six-methylene spacer. Replacement of the phenyl-piperazine with a 4-phenyl-piperidine (**18e**) remarkably decreased binding affinity, and insertion of an α-naphthyl ring on the piperazine had a moderate negative effect (**18f**), opposite to what observed for shorter derivatives. Recovery of binding affinity could be attained by the anilide derivative **16i** having $K_i = 8.1$ nM. The 5-HT_7 receptor was poorly tolerant to substituents on the piperidine nitrogen of compounds with a six-methylene linker. Indeed, insertion of a trifluoromethyl group in *meta* position on the phenyl ring (**18g**) reduced binding affinity, and a bulkier 7-chloro-quinolin-4-yl fragment replacing the phenyl ring led to compound **18h** devoid of any binding affinity. Substituents on the aniline nitrogen were tolerated also in this longer series, even if an ethyl chain (**18j**) and a benzyl group (**18k**) slightly decreased binding affinity compared to the methyl derivative **18c**.

Four compounds from both the indole (**5n**, **5i**) and the aniline series (**14o**, **16i**) were also tested on other 5-HT receptors (Table S1) [26]. These compounds interacted with different receptor subtypes with high affinity, in particular with the 5-HT_{1A} receptor, which is known to bind different classes of 5-HT_7 ligands This behavior highlights the necessity to optimize the structure of these compounds to gain receptor subtype selectivity while maintaining their good 5-HT_7 binding affinity.

Table 1
Binding affinity of the newly synthesized indole and tetralin derivatives for human 5-HT_7 receptors.



5i-p **37**

Compd.	R^1	R^2	K_i (nM)[a] (95% confidence interval) or % displacement at fixed conc.
5-HT			1.4 (0.8–2.2)
5n	Ph	H	63.1 (35.8–111.1)
5l	*m*-Biphenyl	H	88%@10^{-5} 42%@10^{-6}
5m	*p*-Biphenyl	H	32%@10^{-5} 10%@10^{-7}
5i	α-Naphthyl	H	11.6 (6.8–19.7)
5k	α-Naphthyl	Me	12.8 (8.7–18.8)
5j	α-Naphthyl	Et	6.6 (3.5–12.6)
5o	*n*-Butyl	H	−9%@10^{-7}
5p	*c*-Pentyl	H	1%@10^{-7}
37[b]			81.4 (44.3–149.4)

[a] K_i values were calculated from IC_{50} values, obtained from competition curves by the method of Cheng and Prusoff [33], and are the mean of four determinations performed in duplicate.
[b] Tested as racemate.

4. Conclusions

Our data and models are consistent with the existence of two separate binding pockets in the 5-HT_7 receptor. The first one can interact with the serotonin-like 5-hydroxyindole substructure or its bioisosteres in the putative orthosteric site, and the second one can accommodate an arylpiperazine moiety. For this reason the two substructures can be combined to yield new ligands endowed with good receptor affinity and antagonist potency, while subtype selectivity still needs further investigations.

5. Experimental section

5.1. Chemistry

Melting points were determined on a Buchi B-540 capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra and ^{13}C NMR were recorded on a Bruker AVANCE 200 spectrometer (^{1}H: 200 MHz; ^{13}C: 50 MHz) using $CDCl_3$ as solvent unless stated otherwise. Chemical shifts (δ scale) are reported in parts per million (ppm) relative to the central peak of the solvent. Coupling constants (J values) are given in Hz. EI-MS spectra (70 eV) were taken on a Fisons Trio 1000 instrument. Only molecular ions (M^+) and base peaks are given. ESI-MS spectra were taken on a Waters Micromass Zq instrument. Only molecular ions $(M + H)^+$ are given. Infrared spectra were obtained on a Nicolet Avatar 360 FT-IR spectrometer; absorbances are reported in ν (cm^{-1}). Elemental analyses for C, H and N were performed on a Carlo Erba analyzer, and the results are within 0.4% of the calculated values. Column chromatography purifications were performed under "flash" conditions using Merck 230–400 mesh silica gel. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} plates. The starting indoles **1a**–**d**, anilines **6a**–**c** and **11b**–**h**, 3-(3-hydroxyphenyl)propionic acid, 3-(benzyloxy)phenol, 8-methoxy-3,4-dihydronaphthalen-2(1*H*)-one, 4-phenylpiperidine and all the 4-substituted-piperazines were commercially available.

5.1.1. General procedure for 1-(5-substituted-indol-3-yl)-2-(4-substituted-piperazin-1-yl)ethane-1,2-dione derivatives ***3a–h***

A solution of oxalyl chloride (0.14 mL, 1.6 mmol) in dry THF (2.5 mL) was added dropwise to an ice-cooled stirred solution of the suitable indole **1a**–**c** (1.3 mmol) in dry THF (6.5 mL), under N_2. Upon completion of the addition, the reaction mixture was allowed

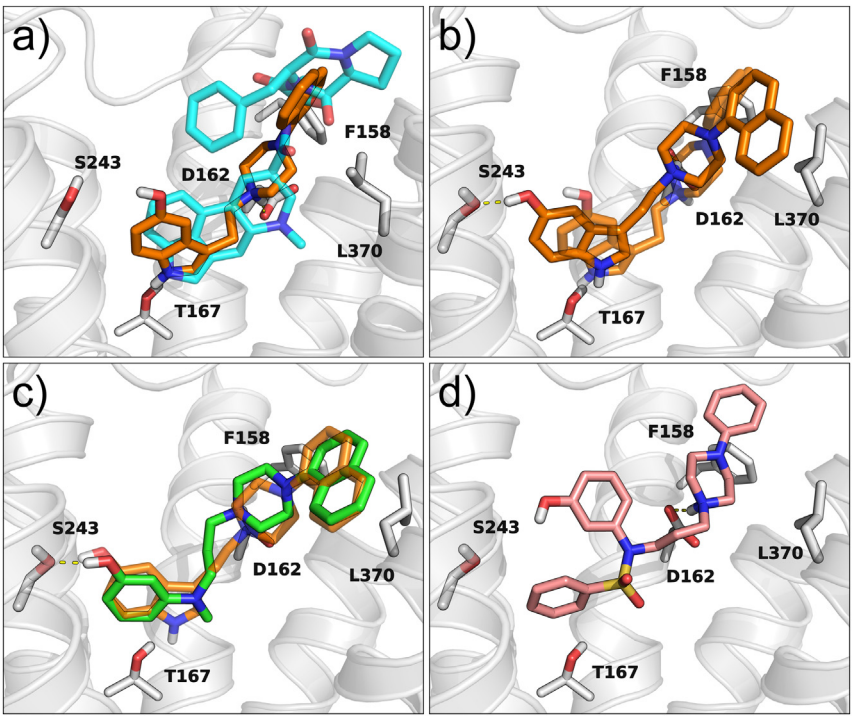


Fig. 2. a) Induced-fit docking of compound **5i** (orange) within the 5-HT$_7$ receptor model (light gray). Dihydroergotamine is represented as transparent cyan carbons. b) MD simulation of the 5-HT$_7$–**5i** complex: superposition of the starting (transparent sticks) and the final energy-minimized structures (opaque sticks and cartoons). c) Best docking pose of **14m** (green carbons) within the 5-HT$_7$ binding site. **5i** (transparent orange carbons) is depicted for comparison. d) Best docking pose of **27** (pink carbons) within the 5-HT$_7$ binding site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to warm to room temperature and stirred at this temperature for 1 h. The solvent and excess oxalyl chloride were removed under reduced pressure and the residue dissolved in dry THF. After cooling to 0 °C, a solution of TEA (0.42 mL) and of the suitable *N*-substituted-piperazine (1.95 mmol) in dry THF (6.5 mL) was added, and the resulting mixture was stirred at room temperature for 1.5 h. The solvent was removed by distillation *in vacuo*, the residue partitioned between EtOAc and water, and the aqueous phase extracted with EtOAc. The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure, to give a crude residue that was purified by flash-chromatography on silica gel: cyclohexane/EtOAc 1:1 as eluant (EtOAc/MeOH, 95:5 for **3g** and **3h**).

*5.1.1.1. 1-(5-Methoxy-1H-indol-3-yl)-2-[4-(naphthalen-1-yl)piperazin-1-yl]ethane-1,2-dione (**3a**).* White amorphous solid (0.33 g, 62%); ^{1}H NMR (acetone-d_6):δ = 3.15 (m, 4H, piperazine), 3.80 (m, 2H, piperazine), 3.87 (s, 3H, $\underline{CH_3}$), 4.01 (m, 2H, piperazine), 6.93 (dd, 1H, J = 2.0 and 8.0, Ar-$\underline{H}$), 7.18 (m, 1H, Ar-$\underline{H}$), 7.40–7.65 (m, 5H, Ar-$\underline{H}$), 7.81 (d, 1H, J = 2.0, Ar-$\underline{H}$), 7.89 (m, 1H, Ar-$\underline{H}$), 8.16 (m, 1H, Ar-$\underline{H}$), 8.29 (m, 1H, Ar-$\underline{H}$), 11.23 (brs, 1H, $\underline{NH}$); ESI-MS (*m/z*): 414 (M + H)$^+$.

*5.1.1.2. 1-(2-Ethyl-5-methoxy-1H-indol-3-yl)-2-[4-(naphthalen-1-yl) piperazin-1-yl]ethane-1,2-dione (**3b**).* Yellowish amorphous solid (0.30 g, 52%); ^{1}H NMR ($CDCl_3$): δ = 1.41 (t, 3H, J = 7.5, $CH_2\underline{CH_3}$), 3.18 (q, 2H, J = 7.5, $\underline{CH_2}CH_3$), 3.22 (m, 4H, piperazine), 3.41 (m, 2H, piperazine), 3.75 (m, 2H, piperazine), 3.90 (s, 3H, O$\underline{CH_3}$), 6.90 (dd, 1H, J = 2.0 and 8.0, Ar-H), 7.08 (d, 1H J = 8.0, Ar-H), 7.26 (m, 1H, Ar-H), 7.38–7.63 (m, 5H, Ar-H), 7.85 (m, 1H, Ar-H), 8.20 (m, 1H, Ar-H), 8.75 (brs, 1H, $\underline{NH}$); ESI-MS (*m/z*): 442 (M + H)$^+$.

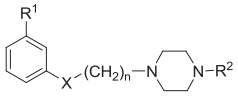
*5.1.1.3. 1-(5-Methoxy-2-methyl-1H-indol-3-yl)-2-[4-(naphthalen-1-yl)piperazin-1-yl]ethane-1,2-dione (**3c**).* Yellowish amorphous solid (0.24 g, 43%); ^{1}H NMR (DMSO-d_6): δ = 2.62 (s, 3H, $\underline{CH_3}$), 3.00 (m, 2H, piperazine), 3.13 (m, 2H, piperazine), 3.61 (m, 2H, piperazine), 3.79 (s, 3H, O$\underline{CH_3}$), 3.94 (m, 2H, piperazine), 6.84 (dd, 1H, J = 2.0 and 8.0, Ar-$\underline{H}$), 7.14 (m, 1H, Ar-$\underline{H}$), 7.33 (d, 1H, J = 8.0, Ar-$\underline{H}$), 7.39–7.65 (m, 5H, Ar-$\underline{H}$), 7.90 (m, 1H, Ar-$\underline{H}$), 8.19 (m, 1H, Ar-$\underline{H}$), 12.01 (brs, 1H, $\underline{NH}$); ESI-MS (*m/z*): 428 (M + H)$^+$.

*5.1.1.4. 1-[5-(Benzyloxy)-1H-indol-3-yl]-2-[4-(biphenyl-3-yl)piperazin-1-yl]ethane-1,2-dione (**3d**).* White solid (0.45 g, 67%); mp: 221–222 °C (EtOAc); ^{1}H NMR ($CDCl_3$): δ = 3.25 (m, 2H, piperazine), 3.36 (m, 2H, piperazine), 3.75 (m, 2H, piperazine), 3.96 (m, 2H, piperazine), 5.17 (s, 2H, $\underline{CH_2}$Ph), 6.95 (m, 1H, Ar-$\underline{H}$), 7.06 (dd, 1H, J = 2.5 and 8.5, Ar-$\underline{H}$), 7.13–7.17 (m, 2H, Ar-$\underline{H}$), 7.31–7.60 (m, 12H, Ar-$\underline{H}$), 7.98 (m, 2H, Ar-$\underline{H}$), 8.80 (brs, 1H, $\underline{NH}$); MS (EI, 70 eV): *m/z* 515 (M^+), 175 (100).

*5.1.1.5. 1-[5-(Benzyloxy)-1H-indol-3-yl]-2-[4-(biphenyl-4-yl)piperazin-1-yl]ethane-1,2-dione (**3e**).* Yellowish solid (0.50 g, 75%); mp: 216–217 °C (EtOAc); ^{1}H NMR (DMSO-d_6): δ = 3.16 (m, 2H, piperazine), 3.32 (m, 2H, piperazine), 3.49 (m, 2H, piperazine), 3.77 (m, 2H, piperazine), 5.14 (s, 2H, $\underline{CH_2}$Ph), 6.98 (dd, 1H, J = 2.5 and 8.5, Ar-$\underline{H}$), 7.03 (d, 1H, J = 8.5, Ar-$\underline{H}$), 7.23–7.70 (m, 15H, Ar-$\underline{H}$), 7.74 (d, 1H, J = 2.5, Ar-$\underline{H}$), 12.23 (brs, 1H, $\underline{NH}$); ESI-MS (*m/z*): 516 (M + H)$^+$.

*5.1.1.6. 1-[5-(Benzyloxy)-1H-indol-3-yl]-2-(4-phenylpiperazin-1-yl) ethane-1,2-dione (**3f**).* White solid (0.43 g, 75%); mp: 136–137 °C (EtOAc); ^{1}H NMR ($CDCl_3$): δ = 3.16 (m, 2H, piperazine), 3.27 (m, 2H, piperazine), 3.68 (m, 2H, piperazine), 3.91 (m, 2H, piperazine), 5.14 (s, 2H, $\underline{CH_2}$Ph), 6.91–7.02 (m, 4H, Ar-$\underline{H}$), 7.23–7.51 (m, 8H, Ar-$\underline{H}$), 7.78 (d, 1H, J = 3.0, Ar-$\underline{H}$), 7.95 (d, 1H, J = 2.0, Ar-$\underline{H}$), 9.73 (brs, 1H, $\underline{NH}$); ^{13}C NMR ($CDCl_3$): δ = 185.5, 166.3, 156.1, 150.8, 137.1, 135.7, 131.5, 129.3, 128.6, 127.9, 127.7, 126.1, 120.8, 116.9, 115.4, 114.5, 112.8, 104.6, 70.6, 50.0, 49.6, 46.1,41.5; MS (EI, 70 eV): *m/z* 439 (M^+), 330 (100).

Table 2
Binding affinity of the newly synthesized anilino-alkyl-piperazine derivatives for human 5-HT_7 receptors (K_i (nM) or % displacement at fixed concentration) and functional evaluation at the cAMP test.



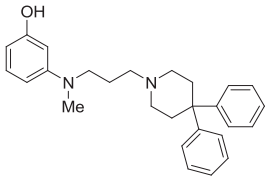
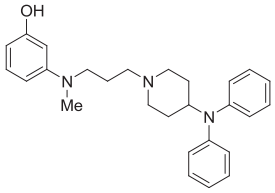
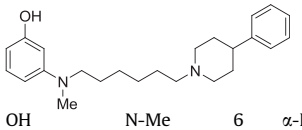
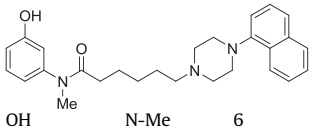
Compd.	R^1	X	n	R^2	K_i (nM)[a] (95% confidence interval) or % displacement at fixed conc.	% Serotonin response on cAMP accumulation at 1 μM
10h	OH	N-Me	2	Ph	161.4 (78.3–332.9)	
10k	NH_2	N-Me	2	Ph	85%@10^{-5} 40%@10^{-6}	
9g	NO_2	N-Me	2	Ph	332.0 (96.3–1177.0)	
10l	$NHSO_2Me$	N-Me	2	Ph	57.0 (38.9–83.2)	48%
10i	OH	N-Me	2	Bn	82%@10^{-5} 42%@10^{-6}	
10j	OH	NH	2	Ph	252.1 (144.4–555.5)	
35	OH	O	2	Ph	42%@10^{-5} 21%@10^{-6}	
32a	OH	CH_2	2	Ph	40.0 (22.9–69.9)	
14o	OH	N-Me	3	Ph	20.0 (12.0–33.3)	49%
14m	OH	N-Me	3	α-Naphthyl	2.9 (1.0–8.3)	
14n	OH	N-Me	3	β-Naphthyl	434.2 (117.6–1602.0)	
32c	OH	CH_2	3	Ph	3.1 (1.3–7.6)	
14l	OH	NH	3	Ph	10.1 (5.2–19.6)	
14b	Me	N-Me	3	Ph	84.8 (26.1–275.2)	
14e	F	N-Me	3	Ph	111.7 (55.2–226.3)	
14d	Cl	N-Me	3	Ph	179.2 (72.9–440.5)	
14c	Br	N-Me	3	Ph	161.2 (49.5–525.3)	
14p	NHCOMe	N-Me	3	Ph	161.1 (73.8–351.5)	
14q	$NHSO_2Me$	N-Me	3	Ph	39.6 (14.9–104.7)	
14t	CH_2OH	NH	3	Ph	108.8 (8.9–1325.0)	
14s	COOMe	NH	3	Ph	77.9 (40.8–148.6)	
14r	$CONH_2$	NH	3	Ph	63.3 (32.7–122.4)	
14u	CHO	NH	3	Ph	13.2 (7.2–24.1)	
24	OH	N-*n*Pr	3	Ph	20.8 (11.8–36.4)	
26	OH	N-Ph	3	Ph	90.1 (28.3–287.0)	
25	OH	N-Bn	3	Ph	92.5 (47.0–181.8)	
27	OH	N-SO_2Ph	3	Ph	12.1 (6.2–23.8)	
30b					242.1 (150.8–388.6)	
30c					310.0 (170.1–564.9)	
18a	OH	N-Me	4	Ph	30.0 (14.0–59.3)	39% IC_{50} = 540 nM K_B = 57 nM
18b	OH	N-Me	5	Ph	16.7 (7.7–35.9)	69%
18c	OH	N-Me	6	Ph	12.0 (8.3–17.2)	56%
18e					226.0 (141.9–360.0)	
18f	OH	N-Me	6	α-Naphthyl	39.4 (18.2–85.6)	
16i					8.1 (4.6–14.2)	
18g	OH	N-Me	6	*m*-CF_3-Ph	95.6 (45.5–201.3)	

Table 2 (*continued*)

Compd.	R^1	*X*	*n*	R^2	K_i (nM)[a] (95% confidence interval) or % displacement at fixed conc.	% Serotonin response on cAMP accumulation at 1 μM
18h	OH	N-Me	6	7-Cl-quinolin-4-yl	74%@10^{-5} 28%@10^{-6}	
18j	OH	N-Et	6	Ph	191.0 (51.8–703.7)	
18k	OH	N-Bn	6	Ph	137.5 (90.0–210.3)	

[a] K_i values were calculated from IC_{50} values, obtained from competition curves by the method of Cheng and Prusoff [33], and are the mean of four determinations performed in duplicate.

*5.1.1.7. 1-(4-Butylpiperazin-1-yl)-2-(5-methoxy-1H-indol-3-yl) ethane-1,2-dione (**3g**).* Yellowish solid (0.36 g, 82%); ^{1}H NMR ($CDCl_3$): δ = 0.92 (t, 3H, *J* = 7.0, $CH_2CH_2CH_2\underline{CH_3}$), 1.39 (m, 4H, $CH_2\underline{CH_2}\underline{CH_2}CH_3$), 2.43 (m, 6H, piperazine and $\underline{CH_2}CH_2CH_2CH_3$), 3.52 (m, 2H, piperazine), 3.76 (m, 2H, piperazine), 3.88 (s, 3H, $O\underline{CH_3}$), 6.89 (dd, 1H, *J* = 2.0 and 8.0, Ar-H), 7.24 (d, 1H, *J* = 8.0, Ar-H), 7.71 (brs, 1H, Ar-H), 7.79 (d, 1H, *J* = 2.0, Ar-H), 9.87 (brs, 1H, NH); ESI-MS (*m/z*): 344 $(M + H)^+$.

*5.1.1.8. 1-(4-Cyclopentylpiperazin-1-yl)-2-(5-methoxy-1H-indol-3-yl)ethane-1,2-dione (**3h**).* Yellowish solid (0.28 g, 62%); ^{1}H NMR ($CDCl_3$): δ = 1.41–1.86 (m, 8H, *c*-pentyl), 2.55 (m, 5H, piperazine and CH), 3.55 (m, 2H, piperazine), 3.79 (m, 2H, piperazine), 3.90 (s, 3H, $O\underline{CH_3}$), 6.92 (dd, 1H, *J* = 2.0 and 10.0, Ar-H), 7.27 (d, 1H, *J* = 10, Ar-H), 7.77 (brs, 1H, Ar-H), 7.80 (d, 1H, *J* = 2.0, Ar-H), 9.65 (brs, 1H, NH); MS (EI, 70 eV) *m/z*: 355 (M^+), 174 (100).

*5.1.2. General procedure for 3-[2-(4-substituted-piperazin-1-yl) ethyl]-1H-indoles **4a**–**h***

$LiAlH_4$ (0.2 g, 5.4 mmol) was added portionwise to an ice-cooled suspension of the suitable ethane-1,2-dione derivative **3a**–**h** (0.9 mmol) in dry THF (9 mL) under a N_2 atmosphere. Once the addition was completed, the reaction mixture was refluxed for 1 h. After cooling to 0 °C, water was added dropwise to destroy the excess hydride and the resulting mixture was filtered on Celite. The filtrate was concentrated *in vacuo*, and partitioned between water and EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure to afford a crude residue which was purified by flash chromatography on silica gel (cyclohexane/EtOAc 1:1 or EtOAc/MeOH 9:1 for **4g**–**h** as eluant).

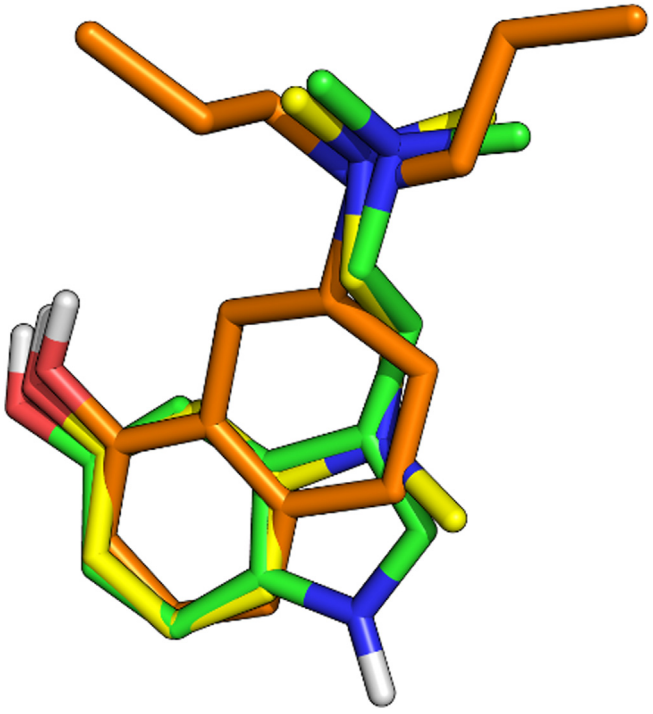


Fig. 3. Superposition of *N,N*-dimethyl-5-hydroxytryptamine (**b** in Fig. 1, green carbons), 8-OH-DPAT (**c** in Fig. 1, orange carbons) and 3-{[2-(dimethylamino)ethyl](methyl)amino}phenol (yellow carbons). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

*5.1.2.1. 5-Methoxy-3-{2-[4-(naphthalen-1-yl)piperazin-1-yl]ethyl}-1H-indole (**4a**).* Amorphous solid (0.23 g, 68%); ^{1}H NMR ($CDCl_3$): δ = 2.06 (m, 2H, $\underline{CH_2}$Ar), 2.82–3.09 (m, 6H, piperazine and $\underline{CH_2}$-piperazine), 3.24 (m, 4H, piperazine), 3.90 (s, 3H, $O\underline{CH_3}$), 6.88 (dd, 1H, *J* = 2.0 and 8.0, Ar-H), 7.07–7.30 (m, 4H, Ar-H), 7.39–7.60 (m, 4H, Ar-H), 7.84 (m, 1H, Ar-H), 7.92 (brs, 1H, NH), 8.23 (m, 1H, Ar-H); ESI-MS (*m/z*): 386 $(M + H)^+$.

*5.1.2.2. 2-Ethyl-5-methoxy-3-{2-[4-(naphthalen-1-yl)piperazin-1-yl] ethyl}-1H-indole (**4b**).* Amorphous solid (0.32 g, 85%); ^{1}H NMR ($CDCl_3$): δ = 1.32 (t, 3H, *J* = 7.5, $CH_2\underline{CH_3}$), 2.86 (m, 10H, $\underline{CH_2}CH_3$, piperazine and $Ar\underline{CH_2}\underline{CH_2}N$), 3.26 (m, 4H, piperazine), 3.90 (s, 3H $O\underline{CH_3}$), 6.81 (dd, 1H, *J* = 2.0 and 8.0, Ar-H), 7.06 (d, 1H, *J* = 2.0, Ar-H), 7.19 (m, 2H, Ar-H), 7.50 (m, 4H, Ar-H), 7.73 (brs, 1H, NH), 7.85 (m, 1H, Ar-H), 8.24 (m, 1H, Ar-H); ESI-MS (*m/z*): 414 $(M + H)^+$.

*5.1.2.3. 5-Methoxy-2-methyl-3-{2-[4-(naphthalen-1-yl)piperazin-1-yl]ethyl}-1H-indole (**4c**).* Amorphous solid (0.26 g, 74%); ^{1}H NMR ($CDCl_3$): δ = 2.40 (s, 3H, $\underline{CH_3}$), 2.70 (m, 2H, $\underline{CH_2}$Ar), 2.93 (m, 6H, piperazine and $\underline{CH_2}$-piperazine), 3.23 (m, 4H, piperazine), 3.88 (s,

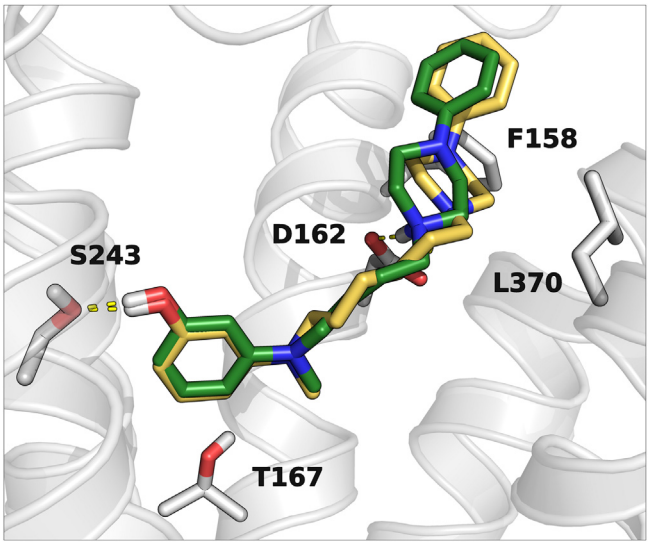


Fig. 4. Superposition of the best docking poses of **18a** (green carbons) and **18c** (yellow carbons) within the 5-HT_7 binding site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3H, O$\underline{CH_3}$), 6.78 (dd, 1H, J = 2.0 and 8.0, Ar-$\underline{H}$), 7.03 (d, 1H, J = 2.0, Ar-$\underline{H}$), 7.15 (m, 2H, Ar-$\underline{H}$), 7.38–7.59 (m, 4H, Ar-$\underline{H}$), 7.69 (brs, 1H, $\underline{NH}$), 7.83 (m, 1H, Ar-$\underline{H}$), 8.23 (m, 1H, Ar-$\underline{H}$); ESI–MS (m/z): 400 $(M + H)^+$.

*5.1.2.4. 5-(Benzyloxy)-3-{2-[4-(biphenyl-3-yl)piperazin-1-yl]ethyl}-1H-indole (**4d**).* White solid (0.33 g, 75%); mp: 99–100 °C (EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ = 2.78 (m, 6H, piperazine and $\underline{CH_2}$Ar), 3.00 (m, 2H, $\underline{CH_2}$-piperazine), 3.35 (m, 4H, piperazine), 5.14 (s, 2H, $\underline{CH_2}$Ph), 6.95–7.62 (m, 18H, Ar-$\underline{H}$), 7.93 (brs, 1H, $\underline{NH}$); MS (EI, 70 eV): m/z 487 (M^+), 251 (100).

*5.1.2.5. 5-(Benzyloxy)-3-{2-[4-(biphenyl-4-yl)piperazin-1-yl]ethyl}-1H-indole (**4e**).* White solid (0.28 g, 64%); mp: 92–93 °C (EtOAc/*n*-hexane); ^{1}H NMR (acetone-d_6): δ = 2.70 (m, 6H, piperazine and $\underline{CH_2}$Ar), 2.97 (m, 2H, $\underline{CH_2}$-piperazine), 3.32 (m, 4H, piperazine), 5.19 (s, 2H, $\underline{CH_2}$Ph), 6.89 (dd, 1H, J = 2.0 and 8.0, Ar-$\underline{H}$), 7.09 (m, 2H, Ar-$\underline{H}$), 7.24 (m, 2H, Ar-$\underline{H}$), 7.29–7.48 (m, 7H, Ar-$\underline{H}$), 7.53–7.68 (m, 6H, Ar-$\underline{H}$), 9.92 (brs, 1H, $\underline{NH}$); ESI–MS (m/z): 488 $(M + H)^+$.

*5.1.2.6. 5-(Benzyloxy)-3-[2-(4-phenylpiperazin-1-yl)ethyl]-1H-indole (**4f**).* White solid (0.26 g, 71%); mp: 133–134 °C (EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$): δ = 2.77 (m, 6H, piperazine and $\underline{CH_2}$Ar), 2.99 (m, 2H, $\underline{CH_2}$-piperazine), 3.29 (m, 4H, piperazine), 5.14 (s, 2H, $\underline{CH_2}$Ph), 6.85–7.00 (m, 4H, Ar-$\underline{H}$), 7.05 (d, 1H, J = 2.0, Ar-$\underline{H}$), 7.17 (d, 1H, J = 2.0, Ar-$\underline{H}$), 7.25–7.53 (m, 8H, Ar-$\underline{H}$), 7.92 (brs, 1H, $\underline{NH}$); ^{13}C NMR ($CDCl_3$): δ = 153.1, 151.3, 137.7, 131.6, 129.1, 128.5, 127.8, 127.6, 122.4, 119.7, 116.1, 114.1, 112.9, 111.8, 102.5, 71.0, 59.2, 53.3, 49.2, 23.0; MS (EI, 70 eV): m/z 411 (M^+), 175 (100).

*5.1.2.7. 3-[2-(4-Butylpiperazin-1-yl)ethyl]-5-methoxy-1H-indole (**4g**).* Oil (0.18 g, 63%); ^{1}H NMR ($CDCl_3$): δ = 0.93 (t, 3H, J = 7.0, $CH_2CH_2CH_2\underline{CH_3}$), 1.20–1.60 (m, 4H, $CH_2\underline{CH_2}\underline{CH_2}CH_3$), 2.39–3.00 (m, 14H, piperazine, $\underline{CH_2}$Ar and 2 × $\underline{CH_2}$N), 3.87 (s, 3H, O$\underline{CH_3}$), 6.85 (dd, 1H, J = 2.0 and 8.0, Ar-$\underline{H}$), 7.02 (d, 1H, J = 2.0, Ar-$\underline{H}$), 7.03 (d, 1H, J = 2.0, Ar-$\underline{H}$), 7.23 (d, 1H, J = 8.0, Ar-$\underline{H}$), 8.04 (brs, 1H, $\underline{NH}$); ESI–MS (m/z): 316 $(M + H)^+$.

*5.1.2.8. 3-[2-(4-Cyclopentylpiperazin-1-yl)ethyl]-5-methoxy-1H-indole (**4h**).* Amorphous solid (0.21 g, 71%); ^{1}H NMR ($CDCl_3$): δ = 1.26–2.00 (m, 8H, *c*-pentyl), 2.45–3.00 (m, 13H, piperazine, $\underline{CH_2}$Ar, $\underline{CH_2}$N and $\underline{CH}$), 3.86 (s, 3H, O$\underline{CH_3}$), 6.86 (dd, 1H, J = 2.5 and 8.0, Ar-$\underline{H}$), 7.02 (d, 1H, J = 2.5, Ar-$\underline{H}$), 7.05 (d, 1H, J = 2.5, Ar-$\underline{H}$), 7.23 (d, 1H, J = 8.0, Ar-$\underline{H}$), 7.93 (brs, 1H, $\underline{NH}$); MS (EI, 70 eV): m/z 327 (M^+), 167 (100).

*5.1.3. O-Demethylation with BBr_3. General procedure for 5-hydroxyindole derivatives **5i**–**k** and **5o**–**p***

A solution of BBr_3 (1 M in CH_2Cl_2, 3 mL, 3 mmol) diluted with dry CH_2Cl_2 (6 mL) was added dropwise to a solution of the suitable methoxy derivative **4a**–**c** or **4g**–**h** (1 mmol) in dry CH_2Cl_2 (4 mL) at 0 °C, and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was neutralized with a 2 N aqueous solution of Na_2CO_3 and extracted with EtOAc. The organic phases were combined, dried (Na_2SO_4) and concentrated under reduced pressure, to give a crude product that was purified by flash chromatography (silica gel; EtOAc for **5i**–**k** or EtOAc/MeOH 9:1 for **5o**–**p** as eluant) and crystallization.

*5.1.3.1. 3-{2-[4-(Naphthalen-1-yl)piperazin-1-yl]ethyl}-1H-indol-5-ol (**5i**).* Pink Solid (0.16 g, 44%); ^{1}H NMR ($CDCl_3$): δ = 2.96 (m, 8H, piperazine and $\underline{CH_2}\underline{CH_2}$Ar), 3.24 (m, 4H, piperazine), 6.80 (dd, 1H, J = 2.0 and 8.0, Ar-$\underline{H}$), 6.99 (d, 1H, J = 2.0, Ar-$\underline{H}$), 7.06 (d, 1H, J = 2.0, Ar-$\underline{H}$), 7.10–7.27 (m, 2H, Ar-$\underline{H}$), 7.48 (m, 4H, Ar-$\underline{H}$ and $\underline{NH}$), 7.85 (m, 2H, Ar-$\underline{H}$), 8.21 (m, 1H, Ar-$\underline{H}$); ^{13}C NMR (acetone-d_6): δ = 150.5, 149.9, 134.9, 128.9, 128.5, 128.3, 125.9, 125.7, 125.1, 123.6, 123.1, 122.9, 114.5, 112.7, 112.6, 111.5, 111.2, 102.5, 59.2, 53.6, 53.1, 22.9; MS (EI, 70 eV) m/z: 371 (M^+), 146 (100); Anal. calcd for $C_{24}H_{25}N_3O$: C 77.60, H 6.78, N 11.31, found C 77.81, H 6.90, N 11.03.

*5.1.3.2. 2-Ethyl-3-{2-[4-(naphthalen-1-yl)piperazin-1-yl]ethyl}-1H-indol-5-ol (**5j**).* White solid (0.28 g, 70%); mp: 200–201 °C (CH_2Cl_2/petroleum ether); ^{1}H NMR ($CDCl_3$): δ = 1.29 (t, 3H, J = 7.5, $\underline{CH_3}CH_2$), 2.76 (q, 2H, J = 7.5, $CH_3\underline{CH_2}$), 2.77 (m, 2H, $\underline{CH_2}$Ar) 2.90 (m, 6H, piperazine and $\underline{CH_2}CH_2$Ar), 3.25 (m, 4H, piperazine), 6.73 (dd, 1H, J = 2.0 and 8.0, Ar-$\underline{H}$), 6.89 (d, 1H, J = 2.0, Ar-$\underline{H}$), 7.14 (m, 2H, Ar-$\underline{H}$), 7.37–7.59 (m, 4H, Ar-$\underline{H}$), 7.69 (brs, 1H, $\underline{NH}$), 7.85 (m, 1H, Ar-$\underline{H}$), 8.21 (m, 1H, Ar-$\underline{H}$); ^{13}C NMR ($CDCl_3$): δ = 149.7, 149.5, 138.3, 134.7, 130.3, 129.3, 128.9, 128.4, 125.9, 125.8, 125.3, 123.5, 123.5, 114.8, 111.1, 111.0, 107.9, 103.1, 59.4, 53.7, 52.6, 21.6, 19.5, 14.4; IR (nujol): ν = 3308 cm^{-1}; ESI–MS (m/z): 400 $(M + H)^+$; Anal. calcd for $C_{26}H_{29}N_3O$: C 78.16, H 7.32, N 10.52, found C 78.01, H 7.21, N 10.29.

*5.1.3.3. 2-Methyl-3-{2-[4-(naphthalen-1-yl)piperazin-1-yl]ethyl}-1H-indol-5-ol (**5k**).* White solid (0.30 g, 79%); mp: 192–193 °C (CH_2Cl_2/petroleum ether); ^{1}H NMR ($CDCl_3$): δ = 2.39 (s, 3H, $\underline{CH_3}$), 2.70 (m, 2H, $\underline{CH_2}$Ar), 2.90 (m, 6H, piperazine and $\underline{CH_2}CH_2$Ar), 3.23 (m, 4H, piperazine), 6.68 (dd, 1H, J = 2.0 and 8.0, Ar-$\underline{H}$), 6.91 (d, 1H, J = 2.0, Ar-$\underline{H}$), 7.13 (m, 2H, Ar-$\underline{H}$), 7.37–7.58 (m, 4H, Ar-$\underline{H}$), 7.64 (brs, 1H, $\underline{NH}$), 7.83 (m, 1H, Ar-$\underline{H}$), 8.21 (m, 1H, Ar-$\underline{H}$); ^{13}C NMR (DMSO-d_6): δ = 150.6, 149.9, 134.8, 132.6, 130.1, 129.5, 128.7, 128.6, 126.5, 126.3, 125.8, 123.8, 123.4, 115.0, 111.1, 110.2, 107.9, 102.2, 59.4, 53.7, 53.2, 22.1, 11.8; IR (nujol): ν = 3238 cm^{-1}; ESI–MS (m/z): 386 $(M + H)^+$; Anal. calcd for $C_{25}H_{27}N_3O$: C 77.89, H 7.06, N 10.90, found C 77.92, H 7.16, N 10.85.

*5.1.3.4. 3-[2-(4-Butylpiperazin-1-yl)ethyl]-1H-indol-5-ol (**5o**).* Amorphous solid (0.12 g, 42%); ^{1}H NMR (acetone-d_6): δ = 0.90 (t, 3H, J = 7.0, $CH_2CH_2CH_2\underline{CH_3}$), 1.16–1.54 (m, 4H, $CH_2\underline{CH_2}\underline{CH_2}CH_3$), 2.28–2.88 (m, 14H, piperazine, $\underline{CH_2}CH_2CH_2CH_3$ and $\underline{CH_2}\underline{CH_2}$Ar), 6.68 (dd, 1H, J = 2.0 and 8.0, Ar-$\underline{H}$), 6.96 (d, 1H, J = 2.0, Ar-$\underline{H}$), 7.09 (d, 1H, J = 2.0, Ar-$\underline{H}$), 7.18 (d, 1H, J = 8.0, Ar-$\underline{H}$), 7.61 (brs, 1H, $\underline{NH}$), 9.70 (brs, 1H, $\underline{OH}$); ^{13}C NMR ($CDCl_3$): δ = 150.0, 131.2, 128.0, 122.6, 112.8, 112.7, 111.9, 103.7, 58.9, 58.4, 52.6, 29.7, 28.6, 22.4, 20.7, 14.0; IR (nujol): ν = 3452, 3142 cm^{-1}; ESI–MS (m/z): 302 $(M + H)^+$; Anal. calcd for $C_{18}H_{27}N_3O$: C 71.72, H 9.03, N 13.94, found C 71.63, H 9.21, N 14.02.

*5.1.3.5. 3-[2-(4-Cyclopentylpiperazin-1-yl)ethyl]-1H-indol-5-ol (**5p**).* Amorphous solid (0.15 g, 47%); ^{1}H NMR (acetone-d_6): δ = 1.32–1.89 (m, 8H, *c*-pentyl), 2.35–3.31 (m, 13H, piperazine, $\underline{CH_2}\underline{CH_2}$Ar and $\underline{CH}$), 6.68 (dd, 1H, J = 2.0 and 8.0, Ar-$\underline{H}$), 6.95 (d, 1H, J = 2.0, Ar-$\underline{H}$), 7.09 (d, 1H, J = 2.0, Ar-$\underline{H}$), 7.18 (d, 1H, J = 8.0, Ar-$\underline{H}$), 7.63 (brs, 1H, $\underline{NH}$), 9.70 (brs, 1H, $\underline{OH}$); ^{13}C NMR ($CDCl_3$): δ = 149.9, 131.2, 128.0, 122.6, 113.0, 112.8, 111.9, 103.7, 67.6, 59.0, 52.7, 51.8, 30.2, 24.1, 22.4; IR (nujol): ν = 3165, cm^{-1}; ESI–MS (m/z): 314 $(M + H)^+$; Anal. calcd for $C_{19}H_{27}N_3O$: C 72.81, H 8.68, N 13.41, found C 72.85, H 8.74, N 13.58.

*5.1.4. O-Debenzylation by hydrogenolysis. General procedure for 5-hydroxyindole derivatives **5l**–**n***

A solution of the benzyloxy derivative **4d**–**f** (0.73 mmol) was dissolved in anhydrous MeOH (12 mL) and then hydrogenated (4 atm) at room temperature in the presence of 10% Pd/C (0.053 g) for 6 h. The reaction mixture was filtered on Celite, the filtrate was evaporated under reduced pressure to yield a crude residue that was purified by crystallization.

*5.1.4.1. 3-{2-[4-(Biphenyl-3-yl)piperazin-1-yl]ethyl}-1H-indol-5-ol (**5l**).* White solid (148 mg, 51%); mp: 194–195 °C dec. (EtOAc/petroleum ether); ^{1}H NMR (DMSO-d_6): δ = 2.64 (m, 6H, piperazine

and CH_2Ar), 2.80 (m, 2H, CH_2CH_2Ar), 3.25 (m, 4H, piperazine), 6.60 (dd, 1H, J = 2.0 and 8.0, Ar-H), 6.85 (d, 1H, J = 2.0, Ar-H), 6.97–7.49 (m, 9H, Ar-H), 7.65 (m, 2H, Ar-H), 8.55 (brs, 1H, NH), 10.48 (brs, 1H, OH); ^{13}C NMR (DMSO-d_6): δ = 152.1, 150.6, 141.6, 141.4, 131.3, 129.9, 129.2, 128.4, 127.7, 127.3, 123.4, 117.8, 115.0, 114.1, 112.2, 112.1, 111.6, 102.7, 59.3, 53.3, 48.8, 23.1; IR (nujol): ν = 3198 cm^{-1}; MS (EI, 70 eV) m/z: 397 (M^+), 251 (100); Anal. calcd for $C_{26}H_{27}N_3O$: C 78.56, H 6.85, N 10.57, found C 78.77, H 6.97, N 10.75.

*5.1.4.2. 3-{2-[4-(Biphenyl-4-yl)piperazin-1-yl]ethyl}-1H-indol-5-ol (**5m**).* Gray solid (122 mg, 42%); mp: 216–217 °C dec. (MeOH); ^{1}H NMR (acetone-d_6): δ = 2.67 (m, 6H, piperazine and CH_2Ar), 2.88 (m, 2H, CH_2CH_2Ar), 3.27 (m, 4H, piperazine), 6.68 (dd, 1H, J = 2.5 and 8.0, Ar-H), 6.97 (d, 1H, J = 2.5, Ar-H), 6.97–7.61 (m, 12H, Ar-H and NH), 9.73 (brs, 1H, OH); ^{13}C NMR ($CDCl_3$): δ = 150.8, 150.6, 140.5, 131.3, 130.8, 129.2, 128.4, 127.6, 126.7, 126.3, 123.5, 116.0, 112.1, 112.0, 111.7, 102.7, 59.0, 53.0, 48.3, 26.8; IR (nujol): ν = 3162 cm^{-1}; ESI-MS (m/z): 398 (M + H)$^+$; Anal. calcd for $C_{26}H_{27}N_3O$: C 78.56, H 6.85, N 10.57, found C 7 8.69, H 6.90, N 10.43.

*5.1.4.3. 3-[2-(4-Phenylpiperazin-1-yl)ethyl]-1H-indol-5-ol (**5n**).* White solid (145 mg, 62%); mp: 202–203 °C (MeOH/Et_2O); ^{1}H NMR (acetone-d_6): δ = 2.69 (m, 6H, piperazine and CH_2Ar), 2.90 (m, 2H, CH_2CH_2Ar), 3.22 (m, 4H, piperazine), 6.70 (ddd, 1H, J = 0.5, 2.0 and 9.0, Ar-H), 6.78 (m, 1H, Ar-H) 6.94–6.99 (m, 3H, Ar-H), 7.13 (d, 1H, J = 2.0, Ar-H), 7.17–7.26 (m, 3H, Ar-H), 7.53 (brs, 1H, NH), 9.67 (brs, 1H, OH); ^{13}C NMR (DMSO-d_6): δ = 151.6, 150.6, 131.3, 129.3, 128.4, 123.4, 119.1, 115.7, 112.1, 112.1, 111.6, 102.7, 59.3, 53.2, 48.7, 23.1; IR (nujol): ν = 3205 cm^{-1}; MS (EI, 70 eV): m/z 321 (M^+), 175 (100); Anal. calcd for $C_{20}H_{23}N_3O$: C 74.74, H 7.21, N 13.07, found C 74.96, H 7.19, N 13.32.

*5.1.5. N-Acylation of anilines with ω-bromoalkanoyl chlorides. General procedure for ω-bromoalkanamido derivatives **7a–c**, **12a–i** and **15a–d***

The suitable ω-bromoalkanoyl chloride (0.7 mmol) was added dropwise to a stirred ice-cooled solution of the suitable aniline (0.33 mmol) and $NaHCO_3$ (0.10 g, 1.22 mmol) in EtOAc (0.4 mL) and water (0.4 mL), and the resulting mixture was stirred at room temperature for 20 min. Water was added and the aqueous phase was extracted three times with EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure to afford a crude residue that was used for the next step, without further purification.

*5.1.5.1. 2-Bromo-N-(3-methoxyphenyl)-N-methylacetamide (**7a**).* The product was prepared starting from 3-methoxy-*N*-methylaniline and 2-bromoethanoyl chloride (Scheme 2). Oil; MS (EI, 70 eV) m/z: 257–259 (M^+), 136 (100) [27].

*5.1.5.2. N-[3-(Benzyloxy)phenyl]-2-bromo-N-methylacetamide (**7b**) [28].* The product was prepared starting from 3-benzyloxyaniline and 2-bromoethanoyl chloride (Scheme 2). Oil; ESI-MS (m/z): 334–336 (M + H)$^+$.

*5.1.5.3. 2-Bromo-N-methyl-N-(3-nitrophenyl)acetamide (**7c**) [27].* The product was prepared starting from 3-nitro-*N*-methylaniline and 2-bromoethanoyl chloride (Scheme 2). Oil; ESI-MS (m/z): 272–274 (M + H)$^+$.

*5.1.5.4. 3-Bromo-N-(3-methoxyphenyl)-N-methylpropanamide (**12a**).* The product was prepared starting from 3-methoxy-*N*-methylaniline and 3-bromopropanoyl chloride (Scheme 3). Oil; ESI-MS (m/z): 272–274 (M + H)$^+$.

*5.1.5.5. 3-Bromo-N-methyl-N-m-tolylpropanamide (**12b**).* The product was prepared starting from 3-methyl-*N*-methylaniline and 3-bromopropanoyl chloride (Scheme 3). Oil; ESI-MS (m/z): 256–258 (M + H)$^+$.

*5.1.5.6. 3-Bromo-N-(3-bromophenyl)-N-methylpropanamide (**12c**).* The product was prepared starting from 3-bromo-*N*-methylaniline and 3-bromopropanoyl chloride (Scheme 3). Oil; ESI-MS (m/z): 320–322–324 (M + H)$^+$.

*5.1.5.7. 3-Bromo-N-(3-chlorophenyl)-N-methylpropanamide (**12d**).* The product was prepared starting from 3-chloro-*N*-methylaniline and 3-bromopropanoyl chloride (Scheme 3). Oil; ESI-MS (m/z): 276–278–280 (M + H)$^+$.

*5.1.5.8. 3-Bromo-N-(3-fluorophenyl)-N-methylpropanamide (**12e**).* The product was prepared starting from 3-fluoro-*N*-methylaniline and 3-bromopropanoyl chloride (Scheme 3). Oil; ESI-MS (m/z): 260–262 (M + H)$^+$.

*5.1.5.9. 3-Bromo-N-methyl-N-(3-nitrophenyl)propanamide (**12f**).* The product was prepared starting from 3-nitro-*N*-methylaniline and 3-bromopropanoyl chloride (Scheme 3). Oil; ESI-MS (m/z): 300–302 (M + H)$^+$.

*5.1.5.10. Ethyl 3-(3-bromo-N-methylpropanamido)benzoate (**12g**).* The product was prepared starting from ethyl 3-aminobenzoate and 3-bromopropanoyl chloride (Scheme 3). Oil; ESI-MS (m/z): 287–289 (M + H)$^+$.

*5.1.5.11. 3-Bromo-N-(3-methoxyphenyl)propanamide (**12h**).* The product was prepared starting from 3-methoxyaniline and 3-bromopropanoyl chloride (Scheme 3). Oil; ESI-MS (m/z): 258–260 (M + H)$^+$.

*5.1.5.12. N-[3-(Benzyloxy)phenyl]-3-bromo-N-methylpropanamide (**12i**).* The product was prepared starting from 3-benzyloxy-*N*-methylaniline [17] and 3-bromopropanoyl chloride (Scheme 3). Oil; MS (EI, 70 eV) m/z: 347–349 (M^+), 91 (100).

*5.1.5.13. N-[3-(Benzyloxy)phenyl]-4-bromo-N-methylbutanamide (**15a**).* The product was prepared starting from 3-benzyloxy-*N*-methylaniline [17] and 4-bromobutanoyl chloride (Scheme 4). Oil; MS (EI, 70 eV) m/z: 361–363 (M^+), 91 (100).

*5.1.5.14. N-[3-(Benzyloxy)phenyl]-5-bromo-N-methylpentanamide (**15b**).* The product was prepared starting from 3-benzyloxy-*N*-methylaniline [17] and 5-bromopentanoyl chloride (Scheme 4). Oil; ^{1}H NMR ($CDCl_3$): δ = 1.67–1.87 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.09 (t, 2H, J = 7.0, CH_2CO), 3.25 (s, 3H, CH_3), 3.31 (t, 2H, J = 6.5, CH_2Br), 5.09 (s, 2H, CH_2Ph), 6.76–6.79 (m, 2H, Ar-H), 7.00 (dd, 1H, J = 2.0 and 8.0, Ar-H), 7.27–7.46 (m, 6H, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 172.6, 159.6, 145.0, 136.3, 130.6, 128.7, 128.2, 127.5, 119.6, 114.3, 114.0, 70.2, 37.3, 33.3, 33.0, 32.3, 24.1; MS (EI, 70 eV) m/z: 375–377 (M^+), 91 (100).

*5.1.5.15. N-[3-(Benzyloxy)phenyl]-6-bromo-N-methylhexanamide (**15c**).* The product was prepared starting from 3-benzyloxy-*N*-methylaniline [17] and 3-bromohexanoyl chloride (Scheme 4). Oil; MS (EI, 70 eV) m/z: 389–391 (M^+), 91 (100).

*5.1.5.16. N-[3-(Benzyloxy)phenyl]-6-bromohexanamide (**15d**).* The product was prepared starting from 3-benzyloxyaniline and 3-bromohexanoyl chloride (Scheme 4). Oil; ESI-MS (m/z): 375–377 (M + H)$^+$.

*5.1.6. General procedure for ω-(4-substituted-piperazin-1-yl) alkanamides **8d–g**, **13b–k** and **16a–h***

The suitable *N*-substituted-piperazine (0.6 mmol) and TEA (0.83 mL) were added to a solution of the appropriate crude ω-bromoalkanamide (from 0.5 mmol of substituted-aniline) in DMF (0.14 mL) and the resulting mixture was stirred at room temperature for 1–16 h, monitoring the progress of the reaction by TLC on silica gel. The reaction mixture was poured into water, and extracted with EtOAc. After drying over Na_2SO_4, the combined organic phases were concentrated *in vacuo* and the resulting crude product was purified by column flash chromatography on silica gel (EtOAc or cyclohexane/EtOAc, 1:1 for **8f** as eluant) and/or crystallization.

*5.1.6.1. N-(3-Methoxyphenyl)-N-methyl-2-(4-phenylpiperazin-1-yl) acetamide (**8d**).* The product was prepared starting from **7a** and *N*-phenylpiperazine (Scheme 2). Oil (154 mg, 91%); ^{1}H NMR ($CDCl_3$): δ = 2.67 (m, 4H, piperazine), 3.05 (s, 2H, CH_2CO), 3.21 (m, 4H, piperazine), 3.29 (s, 3H, NCH_3), 3.85 (s, 3H, OCH_3), 6.86 (m, 6H, Ar-H), 7.30 (m, 3H, Ar-H); MS (EI, 70 eV): *m/z* 339 (M^+), 175 (100).

*5.1.6.2. 2-(4-Benzylpiperazin-1-yl)-N-(3-methoxyphenyl)-N-methyl-acetamide (**8e**).* The product was prepared starting from **7a** and *N*-benzylpiperazine (Scheme 2). Oil (116 mg, 66%); ^{1}H NMR ($CDCl_3$): δ = 2.51 (m, 8H, piperazine), 2.96 (s, 2H, CH_2CO), 3.26 (s, 3H, NCH_3), 3.52 (s, 2H, CH_2Ph), 3.83 (s, 3H, OCH_3), 6.81 (m, 3H, Ar-H), 7.32 (m, 6H, Ar-H); MS (EI, 70 eV): *m/z* 353 (M^+), 91 (100).

*5.1.6.3. N-[3-(Benzyloxy)phenyl]-2-(4-phenylpiperazin-1-yl)acetamide (**8f**).* The product was prepared starting from **7b** and *N*-phenylpiperazine (Scheme 2). White solid (160 mg, 80%); mp 119–120 °C (EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$): δ = 2.82 (m, 4H, piperazine), 3.24 (s, 2H, CH_2CO), 3.29 (m, 4H, piperazine), 5.09 (s, 2H, CH_2Ph), 6.76 (dd, 1H, *J* = 2.0 and 8.0, Ar-H), 6.98 (m, 4H, Ar-H), 7.25–7.48 (m, 9H, Ar-H), 9.19 (brs, 1H, NH); ESI-MS (*m/z*): 402 $(M + H)^+$.

*5.1.6.4. N-Methyl-N-(3-nitrophenyl)-2-(4-phenylpiperazin-1-yl) acetamide (**8g**).* The product was prepared starting from **7c** and *N*-phenylpiperazine (Scheme 2). Yellow oil (136 mg, 77%); ^{1}H NMR ($CDCl_3$): δ = 2.71 (m, 4H, piperazine), 3.14 (s, 2H, CH_2CO), 3.20 (m, 4H, piperazine), 3.31 (s, 3H, NCH_3), 6.87 (m, 3H, Ar-H), 7.28 (m, 2H, Ar-H), 7.62 (m, 2H, Ar-H), 8.14 (m, 1H, Ar-H), 8.24 (m, 1H, Ar-H); ESI-MS (*m/z*): 355 $(M + H)^+$.

*5.1.6.5. N-Methyl-3-(4-phenylpiperazin-1-yl)-N-m-tolylpropanamide (**13b**).* The product was prepared starting from **12b** and *N*-phenylpiperazine (Scheme 3). Amorphous solid (106 mg, 63%); ^{1}H NMR ($CDCl_3$): δ = 2.33 (apt, 2H, *J* = 7.0, CH_2CO), 2.39 (s, 3H, Ar-CH_3), 2.51 (m, 4H, piperazine), 2.76 (apt, 2H, *J* = 7.0, $COCH_2CH_2N$), 3.15 (m, 4H, piperazine), 3.27 (s, 3H, NCH_3), 6.82–7.35 (m, 9H, Ar-H); ESI-MS (*m/z*): 338 $(M + H)^+$.

*5.1.6.6. N-(3-Bromophenyl)-N-methyl-3-(4-phenylpiperazin-1-yl) propanamide (**13c**).* The product was prepared starting from **12c** and *N*-phenylpiperazine (Scheme 3). Amorphous solid (140 mg, 70%); ^{1}H NMR ($CDCl_3$): δ = 2.34 (apt, 2H, *J* = 7.0, CH_2CO), 2.52 (m, 4H, piperazine), 2.75 (apt, 2H, *J* = 7.0, $COCH_2CH_2N$), 3.16 (m, 4H, piperazine), 3.29 (s, 3H, NCH_3), 6.93–7.38 (m, 9H, Ar-H); ESI-MS (*m/z*): 402–404 $(M + H)^+$.

*5.1.6.7. N-(3-Chlorophenyl)-N-methyl-3-(4-phenylpiperazin-1-yl) propanamide (**13d**).* The product was prepared starting from **12d** and *N*-phenylpiperazine (Scheme 3). Amorphous solid (144 mg, 81%); ^{1}H NMR ($CDCl_3$): δ = 2.34 (apt, 2H, *J* = 7.0, CH_2CO), 2.53 (m, 4H, piperazine), 2.76 (apt, 2H, *J* = 7.0, $COCH_2CH_2N$), 3.16 (m, 4H, piperazine), 3.28 (s, 3H, NCH_3), 6.90 (m, 3H, Ar-H), 7.10–7.45 (m, 6H, Ar-H); ESI-MS (*m/z*): 358 $(M + H)^+$.

*5.1.6.8. N-(3-Fluorophenyl)-N-methyl-3-(4-phenylpiperazin-1-yl) propanamide (**13e**).* The product was prepared starting from **12e** and *N*-phenylpiperazine (Scheme 3). Amorphous solid (128 mg, 75%); ^{1}H NMR ($CDCl_3$): δ = 2.36 (apt, 2H, *J* = 7.0, CH_2CO), 2.53 (m, 4H, piperazine), 2.76 (apt, 2H, *J* = 7.0, $COCH_2CH_2N$), 3.16 (m, 4H, piperazine), 3.29 (s, 3H, NCH_3), 6.82–7.47 (m, 9H, Ar-H); ESI-MS (*m/z*): 342 $(M + H)^+$.

*5.1.6.9. N-Methyl-N-(3-nitrophenyl)-3-(4-phenylpiperazin-1-yl) propanamide (**13f**).* The product was prepared starting from **12f** and *N*-phenylpiperazine (Scheme 3). Yellow amorphous solid (147 mg, 80%); ^{1}H NMR ($CDCl_3$): δ = 2.38 (apt, 2H, *J* = 7.0, CH_2CO), 2.58 (m, 4H, piperazine), 2.81 (apt, 2H, *J* = 7.0, $COCH_2CH_2N$), 3.18 (m, 4H, piperazine), 3.36 (s, 3H, NCH_3), 6.88 (m, 3H, Ar-H), 7.25 (m, 2H, Ar-H), 7.64 (m, 2H, Ar-H), 8.13 (m, 1H, Ar-H), 8.23 (m, 1H, Ar-H); ESI-MS (*m/z*): 369 $(M + H)^+$.

*5.1.6.10. Ethyl 3-[3-(4-phenylpiperazin-1-yl)propanamido]benzoate (**13g**).* The product was prepared starting from **12g** and *N*-phenylpiperazine (Scheme 3). White solid (86 mg, 45%); mp 127–128 °C (EtOAc) ^{1}H NMR ($CDCl_3$): δ = 1.30 (t, 3H, *J* = 7.0, CH_2CH_3), 2.60 (m, 2H, CH_2CO), 2.79 (m, 6H, piperazine and $COCH_2CH_2N$), 3.31 (m, 4H, piperazine), 4.31 (q, 2H, *J* = 7.0, CH_2CH_3), 6.93 (m, 3H, Ar-H), 7.25–7.41 (m, 3H, Ar-H), 7.74 (m, 1H, Ar-H), 7.86 (m, 1H, Ar-H), 8.04 (m, 1H, Ar-H), 11.15 (brs, 1H, NH); ESI-MS (*m/z*): 382 $(M + H)^+$.

*5.1.6.11. N-(3-Methoxyphenyl)-3-(4-phenylpiperazin-1-yl)propanamide (**13h**).* The product was prepared starting from **12h** and *N*-phenylpiperazine (Scheme 3). Amorphous solid (151 mg, 89%); ^{1}H NMR ($CDCl_3$): δ = 2.59 (m, 2H, CH_2CO), 2.80 (m, 6H, piperazine and $COCH_2CH_2N$), 3.33 (m, 4H, piperazine), 3.80 (s, 3H, CH_3), 6.64 (dd, 1H, *J* = 2.0 and 8.0, Ar-H), 6.95 (m, 4H, Ar-H), 7.16–7.43 (m, 4H, Ar-H), 10.95 (brs, 1H, NH); ESI-MS (*m/z*): 340 $(M + H)^+$.

*5.1.6.12. N-[3-(Benzyloxy)phenyl]-N-methyl-3-(4-phenylpiperazin-1-yl)propanamide (**13i**).* The product was prepared starting from **12i** and *N*-phenylpiperazine (Scheme 3). Oil (131 mg, 61%); ^{1}H NMR ($CDCl_3$): δ = 2.35 (apt, 2H, *J* = 7.0, CH_2CO), 2.52 (m, 4H, piperazine), 2.76 (apt, 2H, *J* = 7.0, $COCH_2CH_2N$), 3.16 (m, 4H, piperazine), 3.27 (s, 3H, NCH_3), 5.09 (s, 2H, CH_2Ph), 6.81–6.99 (m, 6H, Ar-H), 7.22–7.43 (m, 8H, Ar-H); MS (EI, 70 eV): *m/z* 429 (M^+), 91 (100).

*5.1.6.13. N-(3-Methoxyphenyl)-N-methyl-3-[4-(naphthalen-1-yl) piperazin-1-yl]propanamide (**13j**).* The product was prepared starting from **12a** and 1-(naphthalen-1-yl)piperazine (Scheme 3). Amorphous solid (103 mg, 51%); ^{1}H NMR ($CDCl_3$): δ = 2.29 (apt, 2H, *J* = 7.0, CH_2CO), 2.61 (m, 4H, piperazine), 2.70 (apt, 2H, *J* = 7.0, $COCH_2CH_2N$), 3.10 (m, 4H, piperazine), 3.24 (s, 3H, NCH_3), 3.80 (s, 3H, OCH_3), 6.82 (m, 3H, Ar-H), 7.09 (m, 1H, Ar-H), 7.30–7.61 (m, 5H, Ar-H), 7.85 (m, 1H, Ar-H), 8.21 (m, 1H, Ar-H); ESI-MS (*m/z*): 404 $(M + H)^+$.

*5.1.6.14. N-(3-Methoxyphenyl)-N-methyl-3-[4-(naphthalen-2-yl) piperazin-1-yl]propanamide (**13k**).* The product was prepared starting from **12a** and 1-(naphthalen-2-yl)piperazine (Scheme 3). Oil (95 mg, 47%); ^{1}H NMR ($CDCl_3$): δ = 2.34 (apt, 2H, *J* = 7.0, CH_2CO), 2.58 (m, 4H, piperazine), 2.79 (apt, 2H, *J* = 7.0, $COCH_2CH_2N$), 3.27 (m, 4H, piperazine), 3.28 (s, 3H, NCH_3), 3.85 (s, 3H, OCH_3), 6.75–6.93 (m, 3H, Ar-H), 7.08 (m, 1H, Ar-H), 7.22–7.44 (m, 4H, Ar-H), 7.70 (m, 3H, Ar-H); ESI-MS (*m/z*): 404 $(M + H)^+$.

*5.1.6.15. N-[3-(Benzyloxy)phenyl]-N-methyl-4-(4-phenylpiperazin-1-yl)butanamide (**16a**).* The product was prepared starting from **15a** and *N*-phenylpiperazine (Scheme 4). Oil (51 mg, 23%); ^{1}H NMR ($CDCl_3$): δ = 1.81 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.14 (apt, 2H, J = 7.0, $\underline{CH_2}CO$), 2.32 (apt, 2H, J = 7.0, $COCH_2CH_2\underline{CH_2}N$), 2.55 (m, 4H, piperazine), 3.16 (m, 4H, piperazine), 3.26 (s, 3H, $N\underline{CH_3}$), 5.08 (s, 2H, $\underline{CH_2}Ph$), 6.79–6.99 (m, 6H, Ar-H), 7.26–7.43 (m, 8H, Ar-H); MS (EI, 70 eV): *m/z* 443 (M^+), 91 (100).

*5.1.6.16. N-[3-(Benzyloxy)phenyl]-N-methyl-5-(4-phenylpiperazin-1-yl)pentanamide (**16b**).* The product was prepared starting from **15b** and *N*-phenylpiperazine (Scheme 4). Oil (94 mg, 41%); ^{1}H NMR ($CDCl_3$): δ = 1.54 (m, 4H, $CH_2\underline{CH_2}\underline{CH_2}CH_2$), 2.13 (apt, 2H, J = 7.0, $\underline{CH_2}CO$), 2.34 (t, 2H, J = 7.5, $\underline{CH_2}$-piperazine), 2.57 (m, 4H, piperazine), 3.19 (m, 4H, piperazine), 3.25 (s, 3H, $\underline{CH_3}$), 5.08 (s, 2H, $\underline{CH_2}Ph$), 6.78–6.99 (m, 6H, Ar-H), 7.23–7.46 (m, 8H, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 172.9, 159.6, 151.3, 145.3, 136.4, 130.4, 129.1, 128.7, 128.2, 127.5, 119.8, 119.6, 116.0, 114.2, 114.0, 70.2, 58.3, 53.2, 49.0, 37.2, 33.8, 26.4, 23.5; MS (EI, 70 eV): *m/z* 457 (M^+), 91 (100).

*5.1.6.17. N-[3-(Benzyloxy)phenyl]-N-methyl-6-(4-phenylpiperazin-1-yl)hexanamide (**16c**).* The product was prepared starting from **15c** and *N*-phenylpiperazine (Scheme 4). Oil (94 mg, 40%); ^{1}H NMR ($CDCl_3$): δ = 1.27 (m, 2H, $CH_2CH_2\underline{CH_2}CH_2CH_2$), 1.57 (m, 4H, $CH_2\underline{CH_2}CH_2\underline{CH_2}CH_2$), 2.09 (apt, 2H, J = 7.5, $\underline{CH_2}CO$), 2.42 (apt, 2H, J = 7.5, $\underline{CH_2}N$), 2.66 (m, 4H, piperazine), 3.24 (m, 4H, piperazine), 3.25 (s, 3H, $\underline{CH_3}$), 5.09 (s, 2H, $\underline{CH_2}Ph$), 6.77–6.99 (m, 6H, Ar-H), 7.24–7.45 (m, 8H, Ar-H); MS (EI, 70 eV): *m/z* 471 (M^+), 91 (100).

*5.1.6.18. N-[3-(Benzyloxy)phenyl]-6-(4-phenylpiperazin-1-yl)hexanamide (**16d**).* The product was prepared starting from **15d** and *N*-phenylpiperazine (Scheme 4). Oil (103 mg, 45%); ^{1}H NMR ($CDCl_3$): δ = 1.28 (m, 2H, $CH_2CH_2\underline{CH_2}CH_2CH_2$), 1.61 (m, 4H, $CH_2\underline{CH_2}CH_2\underline{CH_2}CH_2$), 2.13 (apt, 2H, J = 7.5, $\underline{CH_2}CO$), 2.43 (apt, 2H, J = 7.5, $\underline{CH_2}$-piperazine), 2.71 (m, 4H, piperazine), 3.21 (m, 4H, piperazine), 5.08 (s, 2H, $\underline{CH_2}$-Ph), 6.73–6.98 (m, 5H, Ar-H), 7.21–7.55 (m, 9H, Ar-H), 9.03 (brs, 1H, NH); ESI-MS: *m/z* 458 $(M + H)^+$.

*5.1.6.19. N-[3-(Benzyloxy)phenyl]-N-methyl-6-(4-phenylpiperidin-1-yl)hexanamide (**16e**).* The product was prepared starting from **15e** and 4-phenylpiperidine (Scheme 4). Oil (94 mg, 40%); ^{1}H NMR ($CDCl_3$): δ = 1.19 (m, 2H, $CH_2CH_2\underline{CH_2}CH_2CH_2$), 1.58 (m, 4H, $CH_2\underline{CH_2}CH_2\underline{CH_2}CH_2$), 1.81 (m, 2H, piperidine), 2.01–2.25 (m. 6H, $\underline{CH_2}N$, $\underline{CH_2}CO$ and piperidine), 2.45 (m, 1H, CH), 2.60 (m, 4H, piperidine), 3.20 (s, 3H, $\underline{CH_3}$), 5.01 (s, 2H, $\underline{CH_2}Ph$), 6.71 (m, 2H, Ar-H), 6.92 (m, 1H, Ar-H), 7.16–7.42 (m, 11H, Ar-H); ESI-MS: *m/z* 471 $(M + H)^+$.

*5.1.6.20. N-[3-(Benzyloxy)phenyl]-N-methyl-6-[4-(naphthalen-1-yl)piperazin-1-yl]hexanamide (**16f**).* The product was prepared starting from **15c** and 1-(naphthalen-1-yl)piperazine (Scheme 4). Oil (99 mg, 38%). ^{1}H NMR ($CDCl_3$): δ = 1.32 (m, 2H, $CH_2CH_2\underline{CH_2}CH_2CH_2$), 1.61 (m, 2H, $CH_2CH_2\underline{CH_2}CH_2$), 1.78 (m, 2H, $CH_2CH_2\underline{CH_2}$), 2.13 (apt, 2H, J = 7.0, $\underline{CH_2}CO$), 2.79 (m, 2H, $\underline{CH_2}$-piperazine), 2.91–3.51 (m, 8H, piperazine), 3.22 (s, 3H, $\underline{CH_3}$), 5.09 (s, 2H, $\underline{CH_2}Ph$), 6.80 (m, 2H, Ar-H), 6.99 (m, 1H, Ar-H), 6.38 (m, 1H, Ar-H), 7.28–7.55 (m, 9H, Ar-H), 7.61 (m, 1H, Ar-H), 7.84 (m, 1H, Ar-H), 8.13 (m, 1H, Ar-H); ESI-MS: *m/z* 522 $(M + H)^+$.

*5.1.6.21. N-[3-(Benzyloxy)phenyl]-N-methyl-6-{4-[3-(trifluoromethyl)phenyl]piperazin-1-yl}hexanamide (**16g**).* The product was prepared starting from **15c** and 1-[3-(trifluoromethyl)phenyl] piperazine (Scheme 4). Oil (124 mg, 46%); ^{1}H NMR ($CDCl_3$): δ = 1.19 (m, 2H, $CH_2CH_2\underline{CH_2}CH_2CH_2$), 1.56 (m, 4H, $CH_2\underline{CH_2}CH_2\underline{CH_2}CH_2$), 2.02 (apt, 2H, J = 7.0, $\underline{CH_2}CO$), 2.55 (m, 2H, $\underline{CH_2}$-piperazine), 2.68 (m, 4H, piperazine), 3.19 (s, 3H, $\underline{CH_3}$), 3.31 (m, 4H, piperazine), 5.01 (s, 2H, $\underline{CH_2}Ph$), 6.76 (m, 2H, Ar-H), 6.86–7.08 (m, 4H, Ar-H), 7.19–7.40 (m, 7H, Ar-H). ESI-MS: *m/z* 540 $(M + H)^+$.

*5.1.6.22. N-[3-(Benzyloxy)phenyl]-6-[4-(7-chloroquinolin-4-yl) piperazin-1-yl]-N-methylhexanamide (**16h**).* The product was prepared starting from **15c** and 7-chloro-4-(piperazin-1-yl)quinoline (Scheme 4). Oil (81 mg, 29%); ^{1}H NMR ($CDCl_3$): δ = 1.27 (m, 2H, $CH_2CH_2\underline{CH_2}CH_2CH_2$), 1.58 (m, 2H, $\underline{CH_2}CH_2CH_2$), 1.97 (m, 2H, $CH_2CH_2\underline{CH_2}$), 2.14 (m, 2H, $\underline{CH_2}CO$), 3.01–3.68 (m, 6H), 4.18 (m, 2H), 4.42 (m, 2H), 3.18 (s, 3H, $\underline{CH_3}$), 5.01 (s, 2H, $\underline{CH_2}Ph$), 6.75 (m, 2H. Ar-H), 6.92 (m, 1H, Ar-H), 7.16 (m, 1H, Ar-H), 7.18–7.41 (m, 6H, Ar-H), 7.48 (m, 1H, Ar-H), 7.88 (m, 1H, Ar-H), 8.22 (m, 1H, Ar-H), 8.32 (m, 1H Ar-H). ESI-MS: *m/z* 557 $(M + H)^+$.

*5.1.7. Reduction of the alkanamido derivatives with $LiAlH_4$. General procedure for compounds **9d–f** and **17a–h***

$LiAlH_4$ (0.59 g, 15.5 mmol) was added portionwise to an ice-cooled solution of the suitable alkanamido derivative **8d–f**, **16a–h** (2.5 mmol) in dry THF (30 mL) under a N_2 atmosphere. Once the addition was completed, the reaction mixture was refluxed for 2 h. After cooling to 0 °C, water was added dropwise to destroy the excess hydride and the resulting mixture was filtered on Celite. The filtrate was concentrated *in vacuo* and partitioned between water and EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure to afford a crude residue which was purified by flash chromatography on silica gel.

*5.1.7.1. 3-Methoxy-N-methyl-N-[2-(4-phenylpiperazin-1-yl)ethyl]aniline (**9d**).* The product was prepared starting from **8d** (Scheme 2). Purification by flash chromatography on silica gel (EtOAc as eluant). Oil (292 mg, 36%); ^{1}H NMR ($CDCl_3$): δ = 2.63 (apt, 2H, J = 7.5, $\underline{CH_2}CH_2NCH_3$), 2.70 (m, 4H, piperazine), 2.98 (s, 3H, $N\underline{CH_3}$), 3.23 (m, 4H, piperazine), 3.54 (apt, 2H, J = 7.5, $\underline{CH_2}NCH_3$), 3.82 (s, 3H, $O\underline{CH_3}$), 6.35 (m, 3H, Ar-H), 6.91 (m, 3H, Ar-H), 7.23 (m, 3H, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 160.9, 151.3, 150.6, 130.0, 129.1, 119.7, 116.0, 105.3, 101.0, 98.7, 55.1, 54.9, 53.7, 50.5, 49.1, 38.7; MS (EI, 70 eV): *m/z* 325 (M^+), 150 (100).

*5.1.7.2. N-[2-(4-Benzylpiperazin-1-yl)ethyl]-3-methoxy-N-methylaniline (**9e**).* The product was prepared starting from **8e** (Scheme 2). Purification by flash chromatography on silica gel (EtOAc/cyclohexane 8:2 as eluant). Oil (246 mg, 29%); ^{1}H NMR ($CDCl_3$): δ = 2.53 (m, 10H, piperazine and $\underline{CH_2}CH_2NCH_3$), 2.93 (s, 3H, $N\underline{CH_3}$), 3.46 (m, 2H, $\underline{CH_2}NCH_3$), 3.51 (s, 2H, $\underline{CH_2}Ph$), 3.78 (s, 3H, $O\underline{CH_3}$), 6.29 (m, 3H, Ar-H), 7.08–7.33 (m, 6H, Ar-H); MS (EI, 70 eV): *m/z* 339 (M^+), 91 (100).

*5.1.7.3. 3-(Benzyloxy)-N-[2-(4-phenylpiperazin-1-yl)ethyl]aniline (**9f**).* The product was prepared starting from **8f** (Scheme 2). Purification by flash chromatography on silica gel (EtOAc/cyclohexane 4:6 as eluant). Oil (822 mg, 85%); ^{1}H NMR ($CDCl_3$): δ = 2.69 (m, 6H, piperazine and $\underline{CH_2}CH_2NH$), 3.22 (m, 6H, piperazine and $\underline{CH_2}NH$), 4.38 (brs, 1H, NH), 5.06 (s, 2H, $\underline{CH_2}Ph$), 6.35 (m, 3H, Ar-H), 6.93 (m, 3H, Ar-H), 7.07–7.45 (m, 8H, Ar-H); MS (EI, 70 eV): *m/z* 387 (M^+), 91 (100).

*5.1.7.4. 3-(Benzyloxy)-N-methyl-N-[4-(4-phenylpiperazin-1-yl) butyl]aniline (**17a**).* The product was prepared starting from **16a** (Scheme 4). Purification by flash chromatography on silica gel (EtOAc/cyclohexane 7:3 as eluant). Amorphous solid (257 mg, 24%); ^{1}H NMR ($CDCl_3$): δ = 1.63 (m, 4H, $CH_2\underline{CH_2}\underline{CH_2}CH_2$), 2.46 (m, 2H, $\underline{CH_2}$-piperazine), 2.65 (m, 4H, piperazine), 2.93 (s, 3H, $\underline{CH_3}$), 3.26 (m, 4H, piperazine), 3.34 (m, 2H, $\underline{CH_2}NCH_3$), 5.06 (s, 2H, $\underline{CH_2}Ar$), 6.35

(m, 3H, Ar-H), 6.92 (m, 3H, Ar-H), 7.10–7.48 (m, 8H, Ar-H); MS (EI, 70 eV): *m/z* 429 (M^+), 91 (100).

*5.1.7.5. 3-(Benzyloxy)-N-methyl-N-[5-(4-phenylpiperazin-1-yl)pentyl]aniline (**17b**).* The product was prepared starting from **16b** (Scheme 4). Purification by flash chromatography on silica gel (EtOAc/cyclohexane 7:3 as eluant). Oil (764 mg, 69%); ^{1}H NMR ($CDCl_3$): δ = 1.36 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.61 (m, 4H, $CH_2CH_2CH_2CH_2CH_2$), 2.44 (apt, 2H, J = 8.0, CH_2-piperazine), 2.65 (m, 4H, piperazine), 2.92 (s, 3H, CH_3), 3.25 (m, 4H, piperazine), 3.31 (t, 2H, J = 7.5, CH_2NCH_3), 5.07 (s, 2H, CH_2Ar), 6.35 (m, 3H, Ar-H), 6.91 (m, 3H, Ar-H), 7.11–7.49 (m, 8H, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 160.1, 151.2, 150.7, 137.4, 129.8, 129.1, 128.6, 127.9, 127.6, 119.8, 116.1, 105.5, 101.4, 99.6, 69.9, 58.6, 53.2, 52.7, 49.0, 38.4, 26.7, 26.6, 25.1; MS (EI, 70 eV): *m/z* 443 (M^+), 91 (100).

*5.1.7.6. 3-(Benzyloxy)-N-methyl-N-[6-(4-phenylpiperazin-1-yl)hexyl]aniline (**17c**).* The product was prepared starting from **16c** (Scheme 4). Purification by flash chromatography on silica gel (EtOAc/cyclohexane 7:3 as eluant). Oil (320 mg, 28%); ^{1}H NMR ($CDCl_3$): δ = 1.35 (m, 4H, $CH_2CH_2CH_2CH_2CH_2CH_2$), 1.58 (m, 4H, $CH_2CH_2CH_2CH_2CH_2CH_2$), 2.45 (m, 2H, CH_2-piperazine), 2.66 (m, 4H, piperazine), 2.92 (s, 3H, CH_3), 3.29 (m, 6H, piperazine and CH_2NCH_3), 5.06 (s, 2H, CH_2Ph), 6.34 (m, 3H, Ar-H), 6.86 (m, 3H, Ar-H), 7.10–7.48 (m, 8H, Ar-H); MS (EI, 70 eV): *m/z* 457 (M^+), 91 (100).

*5.1.7.7. 3-(Benzyloxy)-N-[6-(4-phenylpiperazin-1-yl)hexyl]aniline (**17d**).* The product was prepared starting from **16d** (Scheme 4). Purification by flash chromatography on silica gel (EtOAc as eluant). Oil (443 mg, 40%); ^{1}H NMR ($CDCl_3$): δ = 1.38 (m, 4H, $CH_2CH_2CH_2CH_2CH_2CH_2$), 1.59 (m, 4H, $CH_2CH_2CH_2CH_2CH_2CH_2$), 2.50 (m, 2H, CH_2-piperazine), 2.71 (m, 4H, piperazine), 3.11 (m, 2H, CH_2NH), 3.30 (m, 4H, piperazine), 3.65 (brs, 1H, NH), 5.05 (s, 2H, CH_2Ph), 6.21 (m, 3H, Ar-H), 6.83–7.61 (m, 11H, Ar-H); ESI-MS: *m/z* 444 $(M + H)^+$.

*5.1.7.8. 3-(Benzyloxy)-N-methyl-N-[6-(4-phenylpiperidin-1-yl)hexyl]aniline (**17e**).* The product was prepared starting from **16e** (Scheme 4). Purification by flash chromatography on silica gel (EtOAc/cyclohexane 8:2 as eluant). Oil (513 mg, 45%); ^{1}H NMR ($CDCl_3$): δ = 1.22 (m, 4H, $CH_2CH_2CH_2CH_2CH_2CH_2$), 1.57 (m, 4H, $CH_2CH_2CH_2CH_2CH_2CH_2$), 1.83 (m, 2H, piperidine), 1.88–2.57 (m, 9H, piperidine, and CH_2N), 2.95 (s, 3H, CH_3), 3.24 (m, 2H, CH_2NCH_3), 5.02 (s, 2H, CH_2Ph), 6.70 (m, 2H, Ar-H), 6.95 (m, 1H, Ar-H), 7.13–7.45 (m, 11H, Ar-H); ESI-MS: *m/z* 457 $(M + H)^+$.

*5.1.7.9. 3-(Benzyloxy)-N-methyl-N-{6-[4-(naphthalen-1-yl)piperazin-1-yl]hexyl}aniline (**17f**).* The product was prepared starting from **16f** (Scheme 4). Purification by flash chromatography on silica gel (EtOAc/cyclohexane 7:3 as eluant). Oil (532 mg, 42%); ^{1}H NMR ($CDCl_3$): δ = 1.36 (m, 4H, $CH_2CH_2CH_2CH_2CH_2CH_2$), 1.60 (m, 4H, $CH_2CH_2CH_2CH_2CH_2CH_2$), 2.65 (m, 2H, CH_2-piperazine), 2.71 (m, 4H, piperazine), 2.90 (s, 3H, CH_3), 3.29 (m, 6H, piperazine and CH_2NCH_3), 5.08 (s, 2H, CH_2Ph), 6.78 (m, 2H, Ar-H), 6.99 (m, 1H, Ar-H), 7.13 (m, 1H, Ar-H), 7.25–7.59 (m, 9H, Ar-H), 7.63 (m, 1H, Ar-H), 7.82 (m, 1H, Ar-H), 8.15 (m, 1H, Ar-H); ESI-MS: *m/z* 508 $(M + H)^+$.

*5.1.7.10. 3-(Benzyloxy)-N-methyl-N-(6-{4-[3-(trifluoromethyl)phenyl]piperazin-1-yl}hexyl)aniline (**17g**).* The product was prepared starting from **16g** (Scheme 4). Purification by flash chromatography on silica gel (EtOAc/cyclohexane 7:3 as eluant). Oil (670 mg, 51%); ^{1}H NMR ($CDCl_3$): δ=1.37 (m, 4H, $CH_2CH_2CH_2CH_2CH_2CH_2$), 1.59 (m, 4H, $CH_2CH_2CH_2CH_2CH_2CH_2$), 2.43 (m, 2H, CH_2-piperazine), 2.69 (m, 4H, piperazine), 2.90 (s, 3H, CH_3), 3.31 (m, 6H, piperazine and CH_2NCH_3), 5.02 (s, 2H, CH_2Ph), 6.76 (m, 2H, Ar-H), 6.83–7.09 (m, 4H, Ar-H), 7.15–7.42 (m, 7H, Ar-H). ESI-MS: *m/z* 526 $(M + H)^+$.

*5.1.7.11. 3-(Benzyloxy)-N-{6-[4-(7-chloroquinolin-4-yl)piperazin-1-yl]hexyl}-N-methylaniline (**17h**).* The product was prepared starting from **16h** (Scheme 4). Purification by flash chromatography on silica gel (EtOAc as eluant). Oil (528 mg, 39%); ^{1}H NMR (CD_3OD): δ = 1.42 (m, 4H, $CH_2CH_2CH_2CH_2CH_2CH_2$), 1.58 (m, 2H, $CH_2CH_2CH_2$), 1.81 (m, 2H, $CH_2CH_2CH_2$), 3.20 (s, 3H, CH_3), 3.23 (m, 2H, CH_2NCH_3), 3.49 (m, 4H, piperazine), 3.79 (m, 2H), 3.92 (m, 2H), 4.38 (m, 2H), 5.18 (s, 2H, CH_2Ph), 7.10 (m, 3H, Ar-H), 7.30–7.51 (m, 7H, Ar-H), 7.78 (m, 1H, Ar-H), 8.05 (m, 1H, Ar-H), 8.24 (m, 1H, Ar-H), 8.78 (m, 1H, Ar-H). ESI-MS: *m/z* 543 $(M + H)^+$.

*5.1.8. Reduction of the alkanamido derivatives with BH_3. General procedure for compounds **9g**, **14b–k***

A 1 M solution of BH_3 in THF (3 equiv) was added dropwise to an ice-cooled solution of the suitable alkanamido derivative **8g**, **13b–k** (1 mmol) in dry THF (7 mL) under a N_2 atmosphere. Once the addition was completed, the reaction mixture was stirred at room temperature for 16 h; 6 N HCl (0.7 mL) [a 1.25 N solution of HCl in EtOH for **14g**] was added and the resulting mixture was heated to reflux for 1 h. After cooling to room temperature the reaction mixture was made alkaline with 1 N NaOH (2.7 mL) and then extracted with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated to afford a crude residue which was purified by flash chromatography on silica gel and/or crystallization.

*5.1.8.1. N-Methyl-3-nitro-N-[2-(4-phenylpiperazin-1-yl)ethyl]aniline·HCl (**9g**).* The product was prepared starting from **8g** (Scheme 2). Purification by flash chromatography on silica gel (EtOAc/cyclohexane 1:1 as eluant); the product was then dissolved in a solution of HCl in MeOH and the solvent was removed by distillation under vacuum obtaining the final product as white solid (207 mg, 55%); ^{1}H NMR (DMSO-d_6): δ = 3.01 (s, 3H, CH_3), 3.20 (m, 4H, piperazine), 3.31 (m, 2H, CH_2-piperazine), 3.61 (m, 2H, piperazine), 3.82 (m, 2H, piperazine), 3.96 (m, 2H, CH_2NCH_3) 6.86 (m, 1H, Ar-H), 7.02 (m, 2H, Ar-H), 7.25–7.34 (m, 3H, Ar-H), 7.43–7.52 (m, 3H, Ar-H), 11.50 (brs, 1H, NH^+); ESI-MS (*m/z*): 341 $(M + H)^+$. Anal. calcd for $C_{19}H_{25}ClN_4O_2 \cdot 0.15\ H_2O$: C 60.12, H 6.72, N 14.76, found C 60.28, H 6.93, N 14.55.

*5.1.8.2. N,3-Dimethyl-N-[3-(4-phenylpiperazin-1-yl)propyl]aniline (**14b**).* The product was prepared starting from **13b** (Scheme 3). Purification by flash chromatography on silica gel (EtOAc/cyclohexane 7:3 as eluant). Oil (145 mg, 45%); ^{1}H NMR ($CDCl_3$): δ = 1.82 (m, 2H, $CH_2CH_2CH_2$), 2.34 (s, 3H, Ar-CH_3), 2.46 (t, 2H, J = 7.5, CH_2-piperazine), 2.63 (m, 4H, piperazine), 2.95 (s, 3H, NCH_3), 3.25 (m, 4H, piperazine), 3.41 (t, 2H, J = 7.5, CH_2NCH_3), 6.57 (m, 3H, Ar-H), 6.90 (m, 3H, Ar-H), 7.22 (m, 1H, Ar-H), 7.23–7.34 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 151.3, 149.5, 138.8, 129.1, 129.0, 119.7, 118.6, 117.0, 116.0, 113.0, 109.5, 55.8, 53.3, 50.7, 49.2, 38.2, 24.3, 22.0; ESI-MS (*m/z*): 324 $(M + H)^+$. Anal. calcd for $C_{21}H_{29}N_3$: C 77.97, H 9.04, N 12.99, found C 77.90, H 9.18, N 13.07.

*5.1.8.3. 3-Bromo-N-methyl-N-[3-(4-phenylpiperazin-1-yl)propyl]aniline (**14c**).* The product was prepared starting from **13c** (Scheme 3). Purification by flash chromatography on silica gel (EtOAc/cyclohexane 3:7 as eluant). Oil (314 mg, 81%); ^{1}H NMR ($CDCl_3$): δ = 1.80 (m, 2H, $CH_2CH_2CH_2$), 2.43 (t, 2H, J = 7.0, CH_2-piperazine), 2.62 (m, 4H, piperazine), 2.94 (s, 3H, CH_3), 3.25 (m, 4H, piperazine), 3.41 (t, 2H, J = 7.0, CH_2NCH_3), 6.65 (dd, 1H, J = 2.0 and 8.0, Ar-H), 6.78–7.00 (m, 5H, Ar-H), 7.07 (dd, 1H, $J_1 = J_2$ = 8.0, Ar-H), 7.27 (d, 1H, J = 7.0, Ar-H), 7.31 (d, 1H, J = 8.0, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 151.3, 150.6,

130.3, 129.1, 123.5, 119.7, 118.6, 116.1, 114.8, 110.5, 55.4, 53.3, 50.3, 49.2, 38.1, 24.1. ESI-MS (*m/z*): 388–390 $(M + H)^+$. Anal. calcd for $C_{20}H_{26}BrN_3$: C 61.86, H 6.75, N 10.82, found C 61.69, H 6.69, N 12.63.

*5.1.8.4. 3-Chloro-N-methyl-N-[3-(4-phenylpiperazin-1-yl)propyl]aniline (**14d**).* The product was prepared starting from **13d** (Scheme 3). Purification by flash chromatography on silica gel (EtOAc/cyclohexane 3:7 as eluant). Oil (250 mg, 73%); ^{1}H NMR ($CDCl_3$): δ = 1.82 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.45 (t, 2H, J = 7.0, $\underline{CH_2}$-piperazine), 2.64 (m, 4H, piperazine), 2.96 (s, 3H, $\underline{CH_3}$), 3.28 (m, 4H, piperazine), 3.44 (t, 2H, J = 7.0, $\underline{CH_2}NCH_3$), 6.70 (m, 3H, Ar-H), 6.94 (m, 3H, Ar-H), 7.16 (t, 1H, Ar-H), 7.28–7.37 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 151.4, 150.5, 135.1, 130.1, 129.2, 119.7, 116.1, 115.7, 111.9, 110.1, 55.5, 53.3, 50.3, 49.2, 38.2, 24.2. ESI-MS (*m/z*): 344 $(M + H)^+$. Anal. calcd for $C_{20}H_{26}ClN_3$: C 69.85, H 7.62, N 12.22, found C 70.01, H 7.78, N 12.35.

*5.1.8.5. 3-Fluoro-N-methyl-N-[3-(4-phenylpiperazin-1-yl)propyl]aniline (**14e**).* The product was prepared starting from **13e** (Scheme 3). Purification by flash chromatography on silica gel (EtOAc/cyclohexane 3:7 as eluant). White solid (245 mg, 75%); mp: 64–65 °C (Et_2O/petroleum ether); ^{1}H NMR ($CDCl_3$): δ = 1.79 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.42 (t, 2H, J = 7.0, $\underline{CH_2}$-piperazine), 2.61 (m, 4H, piperazine), 2.94 (s, 3H, $\underline{CH_3}$), 3.23 (m, 4H, piperazine), 3.40 (t, 2H, J = 7.0, $\underline{CH_2}NCH_3$), 6.31–6.53 (m, 3H, Ar-H), 6.84–7.32 (m, 6H, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 164.2 (d, J = 239.5), 151.3, 151.1 (d, J = 10.5), 130.1 (d, J = 10.5), 129.1, 119.7, 116.0, 107.5 (d, J = 2), 102.3 (d, J = 21.5), 99.0 (d, J = 26), 55.5, 53.3, 50.4, 49.2, 38.3, 24.2; ESI-MS (*m/z*): 328 $(M + H)^+$. Anal. calcd for $C_{20}H_{26}FN_3$: C 73.36, H 8.00, N 12.83, found C 73.21, H 7.91, N 12.85.

*5.1.8.6. N-Methyl-3-nitro-N-[3-(4-phenylpiperazin-1-yl)propyl]aniline (**14f**).* The product was prepared starting from **13f** (Scheme 3). Purification by flash chromatography on silica gel (EtOAc as eluant). Yellow oil (241 mg, 68%); ^{1}H NMR ($CDCl_3$): δ = 1.86 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.47 (t, 2H, J = 7.5, $\underline{CH_2}$-piperazine), 2.65 (m, 4H, piperazine), 3.03 (s, 3H, $\underline{CH_3}$), 3.27 (m, 4H, piperazine), 3.51 (t, 2H, J = 7.5, $\underline{CH_2}NCH_3$), 6.95 (m, 4H, Ar-H), 7.31 (m, 3H, Ar-H), 7.51 (m, 2H, Ar-H); ESI-MS (*m/z*): 355 $(M + H)^+$.

*5.1.8.7. Ethyl 3-[3-(4-phenylpiperazin-1-yl)propylamino]benzoate (**14g**).* The product was prepared starting from **13g** (Scheme 3). Purification by flash chromatography on silica gel (EtOAc/cyclohexane 1:1 as eluant). Amorphous solid (253 mg, 69%); ^{1}H NMR ($CDCl_3$): δ = 1.37 (t, 3H, J = 7.0, $CH_2\underline{CH_3}$), 1.87 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.58 (t, 2H, J = 7.0, $\underline{CH_2}$-piperazine), 2.66 (m, 4H, piperazine), 3.26 (m, 6H, piperazine and $\underline{CH_2}NH$), 4.35 (q, 2H, J = 7.0, $\underline{CH_2}CH_3$), 4.89 (brs, 1H, NH), 6.75–6.99 (m, 4H, Ar-H), 7.17–7.43 (m, 5H, Ar-H); ESI-MS (*m/z*): 368 $(M + H)^+$.

*5.1.8.8. 3-Methoxy-N-[3-(4-phenylpiperazin-1-yl)propyl]aniline (**14h**).* The product was prepared starting from **13h** (Scheme 3). Purification by flash chromatography on silica gel (EtOAc as eluant). Amorphous solid (292 mg, 90%); ^{1}H NMR ($CDCl_3$): δ = 1.86 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.57 (t, 2H, J = 7.0, $\underline{CH_2}$-piperazine), 2.66 (m, 4H, piperazine), 3.24 (m, 6H, piperazine and $\underline{CH_2}NH$), 3.79 (s, 3H, $\underline{CH_3}$), 4.78 (brs, 1H, NH), 6.16–6.30 (m, 3H, Ar-H), 6.92 (m, 3H, Ar-H), 7.09 (dd, 1H, $J_1 = J_2$ = 8.0, Ar-H), 7.30 (m, 2H, Ar-H); ESI-MS (*m/z*): 326 $(M + H)^+$.

*5.1.8.9. 3-(Benzyloxy)-N-methyl-N-[3-(4-phenylpiperazin-1-yl)propyl]aniline (**14i**).* **The product** was prepared starting from **13i** (Scheme 4). Purification by flash chromatography on silica gel (EtOAc/cyclohexane, 7:3 as eluant). Oil (286 mg, 69%); ^{1}H NMR ($CDCl_3$): δ = 1.81 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.43 (apt, 2H, J = 7.0, $\underline{CH_2}$-piperazine), 2.62 (m, 4H, piperazine), 2.93 (s, 3H, $\underline{CH_3}$), 3.23 (m, 4H, piperazine), 3.39 (apt, 2H, J = 7.0, $\underline{CH_2}NCH_3$), 5.06 (s, 2H, $\underline{CH_2}Ph$), 6.37 (m, 3H, Ar-H), 6.90 (m, 3H, Ar-H), 7.11–7.49 (m, 8H, Ar-H); MS (EI, 70 eV): *m/z* 415 (M^+), 91 (100).

*5.1.8.10. 3-Methoxy-N-methyl-N-{3-[4-(naphthalen-1-yl)piperazin-1-yl]propyl}aniline (**14j**).* The product was prepared starting from **13j** (Scheme 3). Purification by flash chromatography on silica gel (EtOAc/cyclohexane 1:1 as eluant). Oil (323 mg, 83%);^{1}H NMR ($CDCl_3$): δ = 1.86 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.52 (apt, 2H, J = 7.0, $\underline{CH_2}$-piperazine), 2.77 (m, 4H, piperazine), 2.97 (s, 3H, $N\underline{CH_3}$), 3.19 (m, 4H, piperazine), 3.44 (apt, 2H, J = 7.0, $\underline{CH_2}NCH_3$), 3.82 (s, 3H, $O\underline{CH_3}$), 6.28 (m, 2H, Ar-H), 6.41 (m, 1H, Ar-H), 7.15 (m, 2H, Ar-H), 7.38–7.59 (m, 4H, Ar-H), 7.84 (m, 1H, Ar-H), 8.22 (m, 1H, Ar-H); ESI-MS (*m/z*): 390 $(M + H)^+$.

*5.1.8.11. 3-Methoxy-N-methyl-N-{3-[4-(naphthalen-2-yl)piperazin-1-yl]propyl}aniline (**14k**).* The product was prepared starting from **13k** (Scheme 3). Purification by flash chromatography on silica gel (EtOAc/cyclohexane 1:1 as eluant). Oil (222 mg, 57%); ^{1}H NMR ($CDCl_3$): δ = 1.84 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.46 (apt, 2H, J = 7.0, $\underline{CH_2}$-piperazine), 2.67 (m, 4H, piperazine), 2.96 (s, 3H, $N\underline{CH_3}$), 3.34 (m, 4H, piperazine), 3.42 (apt, 2H, J = 7.0, $\underline{CH_2}NCH_3$), 3.82 (s, 3H, $O\underline{CH_3}$), 6.29 (m, 2H, Ar-H), 6.40 (m, 1H, Ar-H), 7.16 (m, 2H, Ar-H), 7.25–7.45 (m, 3H, Ar-H), 7.73 (m, 3H, Ar-H); ESI-MS (*m/z*): 390 $(M + H)^+$.

*5.1.9. O-Demethylation of methoxyanilino-derivatives with BBr_3. General procedure for compounds **10h–i**, **14l–n***

A solution of BBr_3 (1 M in CH_2Cl_2, 3 mL, 3 mmol) diluted with dry CH_2Cl_2 (6 mL) was added dropwise to a solution of the suitable methoxy derivative **9d–e**, **14h**, **14j–k** (1 mmol) in dry CH_2Cl_2 (4 mL) at 0 °C, and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was neutralized with a 2 N aqueous solution of Na_2CO_3 and extracted with EtOAc. The organic phases were combined, dried (Na_2SO_4) and concentrated under reduced pressure, to give a crude product that was purified by flash chromatography on silica gel and/or crystallization.

*5.1.9.1. 3-{Methyl[2-(4-phenylpiperazin-1-yl)ethyl]amino}phenol (**10h**).* The product was prepared starting from **9d**. Purification by flash chromatography on silica gel (cyclohexane/EtOAc 1:1) and subsequent crystallization. White solid (106 mg, 34%); mp: 155–156 °C (Et_2O); ^{1}H NMR ($CDCl_3$): δ = 2.63 (t, 2H, J = 8.0, $\underline{CH_2}CH_2NCH_3$), 2.66 (m, 4H, piperazine), 2.95 (s, 3H, $N\underline{CH_3}$), 3.24 (m, 4H, piperazine), 3.53 (t, 2H, J = 8.0, $\underline{CH_2}NCH_3$), 5.51 (brs, 1H, OH), 6.24 (m, 3H, Ar-H), 6.90 (m, 3H, Ar-H), 7.08 (t, 1H, Ar-H), 7.28 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 157.0, 151.1, 150.6, 130.2, 129.1, 119.9, 116.1, 104.7, 103.4, 99.2, 54.7, 53.6, 50.2, 49.0, 38.7; MS (EI, 70 eV): *m/z* 311 (M^+), 136 (100); Anal. calcd for $C_{19}H_{25}N_3O$: C 73.28, H 8.09, N 13.49, found C 73.52, H 8.33, N 13.20.

*5.1.9.2. 3-{[2-(4-Benzylpiperazin-1-yl)ethyl]methylamino}phenol (**10i**).* The product was prepared starting from **9e**. Purification by flash chromatography on silica gel (cyclohexane/EtOAc 2:8) and subsequent crystallization. White solid (159 mg, 49%); mp: 99–100 °C (Et_2O/petroleum ether); ^{1}H NMR ($CDCl_3$): δ = 2.56 (m, 10H, piperazine and $\underline{CH_2}CH_2NCH_3$), 2.92 (s, 3H, $N\underline{CH_3}$), 3.47 (apt, 2H, J = 7.5, $\underline{CH_2}NCH_3$), 3.54 (s, 2H, $\underline{CH_2}$-Ar), 6.15 (m, 2H, Ar-H), 6.27 (m, 1H, Ar-H), 7.06 (m, 1H, Ar-H), 7.22–7.34 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 157.3, 150.5, 137.5, 130.1, 129.4, 128.3, 127.2, 104.3, 103.7, 99.5, 63.0, 54.5, 53.3, 52.7, 50.0, 38.6; ESI-MS (*m/z*): 326 $(M + H)^+$; Anal. calcd for $C_{20}H_{27}N_3O$: C 73.81, H 8.36, N 12.91, found C 73.98, H 8.44, N 12.95.

*5.1.9.3. 3-[3-(4-Phenylpiperazin-1-yl)propylamino]phenol (**14l**).* The product was prepared starting from **14h**. Purification by flash chromatography on silica gel (cyclohexane/EtOAc 3:7) and subsequent crystallization. White solid (208 mg, 67%); mp: 140–141 °C (EtOAc); ^{1}H NMR ($CDCl_3$): δ = 1.87 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.56 (t, 2H, J = 7.0, $\underline{CH_2}$-piperazine), 2.66 (m, 4H, piperazine), 3.17 (t, 2H J = 6.5, $\underline{CH_2}NH$), 3.23 (m, 4H, piperazine), 6.05 (m, 1H, Ar-H), 6.12–6.21 (m, 2H, Ar-H), 6.85–7.05 (m, 4H, Ar-H), 7.25–7.33 (m, 2H, Ar-H); IR (nujol): ν = 3390, 3340 cm^{-1}; ^{13}C NMR ($CDCl_3$): δ = 157.1, 151.1, 150.2, 130.2, 129.1, 119.9, 116.1, 105.7, 104.5, 99.7, 57.1, 53.2, 49.1, 43.4, 25.7. ESI-MS (m/z): 312 $(M + H)^+$; Anal. calcd for $C_{19}H_{25}N_3O$: C 73.28, H 8.09, N 13.49, found C 73.09, H 7.78, N 13.55.

*5.1.9.4. 3-(Methyl{3-[4-(naphthalen-1-yl)piperazin-1-yl]propyl}amino)phenol (**14m**).* The product was prepared starting from **14j**. Purification by flash chromatography on silica gel (EtOAc as eluant) and subsequent crystallization. Beige solid (255 mg, 68%); mp: 124–125 °C (Et_2O/petroleum ether); ^{1}H NMR ($CDCl_3$): δ = 1.85 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.56 (apt, 2H, J = 7.0, $\underline{CH_2}$-piperazine), 2.83 (m, 4H, piperazine), 2.89 (s, 3H, $\underline{CH_3}$), 3.21 (m, 4H, piperazine), 3.38 (apt, 2H, J = 7.0, $\underline{CH_2}NCH_3$), 6.18 (m, 2H, Ar-H), 6.31 (m, 1H, Ar-H), 7.09 (m, 2H, Ar-H), 7.37–7.59 (m, 4H, Ar-H), 7.85 (m, 1H, Ar-H), 8.21 (m, 1H, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 157.2, 150.9, 149.4, 134.7, 130.1, 128.8, 128.4, 125.9, 125.8, 125.3, 123.6, 123.5, 114.8, 104.7, 103.4, 99.5, 56.0, 53.8, 52.6, 50.6, 38.3, 24.2. ESI-MS (m/z): 376 $(M + H)^+$; Anal. calcd for $C_{24}H_{29}N_3O$: C 76.76, H 7.78, N 11.19, found C 76.99, H 7.83, N 11.32.

*5.1.9.5. 3-(Methyl{3-[4-(naphthalen-2-yl)piperazin-1-yl]propyl}amino)phenol (**14n**).* The product was prepared starting from **14k.** Purification by flash chromatography on silica gel (gradient cyclohexane/EtOAc 1:1 to EtOAc as eluant) and subsequent crystallization. White solid (198 mg, 53%); mp: 161–162 °C (acetone); ^{1}H NMR ($CDCl_3$): δ = 1.84 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.47 (apt, 2H, J = 7.5, $\underline{CH_2}$-piperazine), 2.69 m (4H, piperazine), 2.92 (s, 3H, $\underline{CH_3}$), 3.37 (m, 6H, piperazine and $\underline{CH_2}NCH_3$), 6.18 (m, 2H, Ar-H), 6.33 (m, 1H, Ar-H), 7.04–7.14 (m, 2H, Ar-H), 7.25–7.45 (m, 3H, Ar-H), 7.72 (m, 3H, Ar-H); MS (EI, 70 eV): m/z 325 (M^+), 91 (100); ^{13}C NMR ($CDCl_3$): δ = 156.8, 150.9, 149.0, 134.5, 130.0, 128.7, 128.5, 126.7, 126.2, 125.2, 123.4, 119.4, 110.3, 104.9, 103.1, 99.2, 55.8, 53.2, 50.5, 49.4, 38.3, 24.2. ESI-MS (m/z): 376 $(M + H)^+$; Anal. calcd for $C_{24}H_{29}N_3O$: C 76.76, H 7.78, N 11.19, found C 76.59, H 7.71, N 11.27.

*5.1.10. N^1-Methyl-N^1-[2-(4-phenylpiperazin-1-yl)ethyl]benzene-1,3-diamine·HCl (**10k**)*

A solution of the nitro compound **9g** (1.0 mmol) in EtOH (10 mL) and EtOAc (10 mL) was hydrogenated over 10% Pd/C (0.075 g) at 1 atm of H_2 for 5 h at room temperature. The catalyst was filtered on Celite, the filtrate was concentrated under reduced pressure to give a crude oily amine that was dissolved in a solution of HCl in EtOH and stirred for 1 h. After removing the solvent by distillation under vacuum, the resulting solid was purified by crystallization in EtOH/Et_2O. White solid (280 mg, 81%). ^{1}H NMR (DMSO-d_6): δ = 2.98 (s, 3H, $\underline{CH_3}$), 3.20 (m, 4H, piperazine), 3.30 (apt, 2H, J = 7.0, $\underline{CH_2}$-piperazine), 3.63 (m, 2H, piperazine), 3.82–3.95 (m, 4H, piperazine and $\underline{CH_2}NCH_3$), 6.66 (m, 1H, Ar-H), 6.82 (m, 1H, Ar-H), 6.87 (m, 1H, Ar-H), 6.93 (m, 1H, Ar-H), 7.10 (m, 2H, Ar-H), 7.27 (m, 3H, Ar-H), 10.25 (brs, 2H, NH_2), 11.50 (brs, 1H, NH^+); ESI-MS (m/z): 311 $(M + H)^+$. Anal. calcd for $C_{19}H_{27}ClN_4$·0.3 EtOH: C 65.26, H 8.05, N 15.53, found C 65.12, H 8.24, N 15.31.

*5.1.11. N-(3-{Methyl[2-(4-phenylpiperazin-1-yl)ethyl]amino}phenyl)methanesulfonamide·HCl (**10l**)*

Methanesulfonyl chloride (0.05 mL, 0.65 mmol) was added to a solution of **10k** (0.25 g, 0.63 mmol) and TEA (0.18 mL) in dry THF (5 mL), and the resulting mixture was stirred at room temperature for 1 h. After removing the solvent by distillation at reduced pressure, the residue was taken up in EtOAc, and washed with 1 N NaOH and with brine. After drying over Na_2SO_4, the organic layer was concentrated under reduced pressure and the resulting crude product was purified by column flash chromatography on silica gel (EtOAc/MeOH, 95:5 as eluant) to give a crude oily amine that was dissolved in a solution of HCl in EtOH and stirred for 1 h. After removing the solvent by distillation under vacuum, the resulting solid was purified by crystallization in EtOH/Et_2O White solid (216 mg, 81%). ^{1}H NMR (DMSO-d_6): δ = 2.91 (s, 3H, $CH_2N\underline{CH_3}$), 2.97 (s, 3H, $SO_2\underline{CH_3}$), 3.10 (m, 4H, piperazine), 3.25 (apt, 2H, J = 7.0, $\underline{CH_2}$-piperazine), 3.58 (m, 2H, piperazine), 3.80 (m, 2H, piperazine), 3.84 (apt, 2H, J = 7.0, $\underline{CH_2}NCH_3$), 6.57–6.69 (m, 3H, Ar-H), 6.87 (t, 1H, Ar-H), 7.01 (m, 2H, Ar-H), 7.14 (t, 1H, Ar-H), 7.24–7.29 (m, 2H, Ar-H), 9.51 (s, 1H, NH), 11.60 (brs, 1H, NH^+). ESI-MS (m/z): 389 $(M + H)^+$. Anal. calcd for $C_{20}H_{29}ClN_4O_2S$·0.5 H_2O: C 55.35, H 6.97, N 12.91, found C 55.49, H 7.13, N 12.79.

*5.1.12. O-Debenzylation of benzyloxyanilino-derivatives by hydrogenolysis. General procedure for compounds **10j**, **14o**, **18a–c***

A solution of the suitable benzyloxy-anilino derivative **9f**, **14i**, **17a–c** (1 mmol) in dry MeOH (18 mL) was hydrogenated over 10% Pd/C (0.075 g) at 4 atm of H_2 for 3 h at 60 °C. The catalyst was filtered on Celite, the filtrate was concentrated under reduced pressure to give a crude residue that was purified by flash chromatography on silica gel and/or crystallization.

*5.1.12.1. 3-[2-(4-Phenylpiperazin-1-yl)ethylamino]phenol (**10j**).* The product was prepared starting from **9f**. Purification by flash chromatography on silica gel (EtOAc/cyclohexane, 6:4 as eluant) and subsequent crystallization. Beige solid (119 mg, 40%); mp: 159–160 °C (EtOAc); ^{1}H NMR ($CDCl_3$): δ = 2.69 (m, 6H, piperazine and $\underline{CH_2}CH_2NH$), 3.21 (m, 6H, piperazine and $\underline{CH_2}NH$), 4.35 (brs, 1H, $\underline{NH}$), 6.10 (dd, 1H, $J_1 = J_2$ = 2.0, Ar-H), 6.21 (m, 2H, Ar-H), 6.84–6.98 (m, 3H, Ar-H), 7.03 (dd, 1H, $J_1 = J_2$ = 8.0, Ar-H), 7.29 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 156.9, 151.2, 149.9, 147.9, 130.2, 129.1, 119.9, 116.2, 106.0, 99.8, 56.6, 52.9, 49.1, 40.2; IR (nujol): ν = 3423 cm^{-1}; MS (EI, 70 eV): m/z 297 (M^+), 122 (100). Anal. calcd for $C_{18}H_{23}N_3O$: C 72.70, H 7.80, N 14.13, found C 72.39, H 8.03, N 14.00.

*5.1.12.2. 3-{Methyl[3-(1-phenylpiperazin-4-yl)propyl]amino}phenol (**14o**).* The product was prepared starting from **14i** (Scheme 4). Purification by flash chromatography on silica gel (EtOAc as eluant) and subsequent crystallization. Gray solid (172 mg, 53%); mp: 177–178 °C (EtOAc); ^{1}H NMR ($CDCl_3$): δ = 1.82 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.44 (apt, 2H, J = 7.0, $\underline{CH_2}$-piperazine), 2.63 (m, 4H, piperazine), 2.90 (s, 3H, $\underline{CH_3}$), 3.24 (m, 4H, piperazine), 3.37 (t, 2H, J = 7.0, $\underline{CH_2}NCH_3$), 6.16 (m, 2H, Ar-H), 6.31 (m, 1H, Ar-H), 6.83–7.32 (m, 6H, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 156.7, 151.3, 150.9, 130.0, 129.1, 119.7, 116.0, 105.0, 103.0, 99.1, 55.7, 53.3, 50.5, 49.1, 38.3, 24.2; ESI-MS (m/z): 326 $(M + H)^+$; Anal. calcd for $C_{20}H_{27}N_3O$: C 73.81, H 8.36, N 12.91, found C 74.10, H 8.65, N 12.89.

*5.1.12.3. 3-{Methyl[4-(1-phenylpiperazin-4-yl)butyl]amino}phenol (**18a**).* The product was prepared starting from **17a** (Scheme 4). Purification by flash chromatography on silica gel (EtOAc, as eluant) and subsequent crystallization. White solid (217 mg, 64%); mp: 169–170 °C (EtOAc); ^{1}H NMR ($CDCl_3$): δ = 1.59 (m, 4H, $CH_2\underline{CH_2}\underline{CH_2}CH_2$), 2.45 (t, 2H, J = 7.0, $\underline{CH_2}$-piperazine), 2.65 (m, 4H, piperazine), 2.90 (s, 3H, $\underline{CH_3}$), 3.26 (m, 6H, piperazine and $\underline{CH_2}NCH_3$), 6.13 (m, 2H, Ar-H), 6.32 (m, 1H, Ar-H), 6.83–7.32 (m, 6H, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 156.9, 151.2, 150.8, 130.0, 129.1, 119.8, 116.1, 104.8, 103.1, 99.3, 58.4, 53.2, 52.6, 49.0, 38.4, 24.7, 24.2; ESI-

MS (*m/z*): 340 $(M + H)^+$; Anal. calcd for $C_{21}H_{29}N_3O$: C 74.30, H 8.61, N 12.38, found C 74.15, H 8.44, N 12.27.

*5.1.12.4. 3-{Methyl[5-(1-phenylpiperazin-4-yl)pentyl]amino}phenol (**18b**).* The product was prepared starting from **17b** (Scheme 4). Purification by flash chromatography on silica gel (EtOAc, as eluant) and subsequent crystallization. White solid (141 mg, 40%); mp: 144–145 °C (EtOAc); ^{1}H NMR ($CDCl_3$): δ = 1.33 (m, 2H, $CH_2CH_2\underline{CH_2}CH_2CH_2$), 1.61 (m, 4H, $CH_2\underline{CH_2}CH_2\underline{CH_2}CH_2$), 2.45 (apt, 2H, J = 8.0, $\underline{CH_2}$-piperazine), 2.69 (m, 4H, piperazine), 2.88 (s, 3H, $\underline{CH_3}$), 3.25 (m, 6H, $\underline{CH_2}NCH_3$ and piperazine), 6.14 (m, 2H, Ar-$\underline{H}$), 6.25 (m, 1H, Ar-$\underline{H}$), 6.84–7.10 (m, 4H, Ar-$\underline{H}$), 7.24–7.32 (m, 2H, Ar-$\underline{H}$); ^{13}C NMR ($CDCl_3$): δ = 157.2, 151.1, 150.8, 130.0, 129.1, 119.9, 116.2, 104.5, 103.3, 99.4, 58.7, 53.3, 52.5, 48.9, 38.4, 26.4, 26.3, 25.0; ESI-MS (*m/z*): 354 $(M + H)^+$; Anal. calcd for $C_{22}H_{31}N_3O$: C 74.75, H 8.84, N 11.89, found C 74.55, H 8.71, N 11.96.

*5.1.12.5. 3-{Methyl[6-(4-phenylpiperazin-1-yl)hexyl]amino}phenol (**18c**).* The product was prepared starting from **17c** (Scheme 4). Purification by flash chromatography on silica gel (EtOAc as eluant) and subsequent crystallization. White solid (183 mg, 50%); mp: 125–126 °C (EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$): δ = 1.35 (m, 4H, $CH_2CH_2\underline{CH_2}\underline{CH_2}CH_2CH_2$), 1.57 (m, 4H, $CH_2\underline{CH_2}CH_2CH_2\underline{CH_2}CH_2$), 2.41 (apt, 2H, J = 7.5, $\underline{CH_2}$-piperazine), 2.62 (m, 4H, piperazine), 2.89 (s, 3H, $\underline{CH_3}$), 3.25 (m, 4H, piperazine), 3.28 (apt, 2H J = 7.0, $\underline{CH_2}NCH_3$), 6.13 (m, 2H, Ar-$\underline{H}$), 6.26 (m, 1H, Ar-$\underline{H}$), 6.82–7.10 (m, 4H, Ar-$\underline{H}$), 7.22–7.30 (m, 2H, Ar-$\underline{H}$); ^{13}C NMR ($CDCl_3$): δ = 157.1, 151.2, 150.8, 130.0, 129.1, 119.8, 116.1, 104.6, 103.2, 99.4, 58.7, 53.2, 52.6, 48.8, 38.4, 27.3, 26.8, 26.4, 26.3; ESI-MS (*m/z*): 368 $(M + H)^+$; Anal. calcd for $C_{23}H_{33}N_3O$: C 75.16, H 9.05, N 11.43, found C 74.91, H 8.86, N 11.56.

*5.1.13. O-Debenzylation of benzyloxyanilino-derivatives with BCl_3. General procedure for compounds **16i**, **18e–h**, **18j–k***

A solution of BCl_3 (1 M in CH_2Cl_2, 3 mL, 3 mmol) was added dropwise to a solution of the suitable benzyloxy-anilino derivative **16f, 17e–h**, or **17j–k** (1 mmol) in dry CH_2Cl_2 (6 mL) at 0 °C, and the resulting mixture was stirred at room temperature for 1 h. The reaction mixture was neutralized with 2 N NaOH, the organic layer was washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure, to give a crude product that was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 8: 2 as eluant). The product was dissolved in a solution of HCl in MeOH and the solvent was removed by distillation under vacuum to give the desired final product as hydrochloride.

*5.1.13.1. N-(3-Hydroxyphenyl)-N-methyl-6-[4-(naphthalen-1-yl) piperazin-1-yl]hexanamide·HCl (**16i**).* The product was prepared starting from **16f** (Scheme 4). White solid (398 mg, 85%); ^{1}H NMR (CD_3OD): δ = 1.35 (m, 2H, $CH_2CH_2\underline{CH_2}CH_2CH_2$), 1.70 (m, 4H, $CH_2\underline{CH_2}CH_2\underline{CH_2}CH_2$), 2.20 (apt, 2H, $\underline{CH_2}CO$), 3.20 (m, 4H, piperazine), 3.22 (s, 3H, $\underline{CH_3}$), 3.50 (m, 4H, piperazine), 3.70 (m, 2H, $\underline{CH_2}$-piperazine), 6.67 (m, 2H, Ar-$\underline{H}$), 6.82 (m, 1H, Ar-$\underline{H}$), 7.21–7.58 (m, 5H, Ar-$\underline{H}$), 7.62 (m, 1H, Ar-$\underline{H}$), 7.90 (m, 1H, Ar-$\underline{H}$), 8.22 (m, 1H, Ar-$\underline{H}$). ESI-MS (*m/z*): 432 $(M + H)^+$. Anal. calcd for $C_{27}H_{34}ClN_2O_2 \cdot 0.15\ H_2O$ C 69.29, H 7.32, N 8.98, found C 69.49, H 7.51, N 8.69.

*5.1.13.2. 3-{Methyl[6-(4-phenylpiperidin-1-yl)hexyl]amino}phenol·HCl (**18e**).* The product was prepared starting from **17e** (Scheme 4). White solid (322 mg, 80%); ^{1}H NMR (CD_3OD): δ = 1.58 (m, 4H, $CH_2CH_2\underline{CH_2}\underline{CH_2}CH_2CH_2$), 1.70 (m, 2H, $CH_2\underline{CH_2}CH_2CH_2CH_2CH_2$), 1.90 (m, 2H, $CH_2\underline{CH_2}CH_2CH_2CH_2CH_2$), 2.10–2.30 (m, 4H, piperidine), 3.10 (m, 1H, $\underline{CH}$), 3.26 (m, 4H, $\underline{CH_2}N$), 3.38 (s, 3H, $\underline{CH_3}$), 3.83 (m, 4H, $\underline{CH_2}N$), 7.19 (m 3H, Ar-$\underline{H}$), 7.44–7.58 (m, 6H, Ar-$\underline{H}$). ESI-MS (*m/z*): 367 $(M + H)^+$. Anal. calcd for $C_{24}H_{35}ClN_2O \cdot 0.5\ H_2O$: C 71.05, H 8.77, N 6.90, found C 70.88, H 8.61, N 6.69.

*5.1.13.3. 3-(Methyl{6-[4-(naphthalen-1-yl)piperazin-1-yl]hexyl} amino)phenol·HCl (**18f**).* The product was prepared starting from **17f** (Scheme 4). White solid (385 mg, 85%); ^{1}H NMR (CD_3OD): δ = 1.60 (m, 4H, $CH_2CH_2\underline{CH_2}\underline{CH_2}CH_2CH_2$), 1.72 (m, 2H, $CH_2\underline{CH_2}CH_2CH_2CH_2CH_2$), 2.00 (m, 2H, $CH_2\underline{CH_2}CH_2CH_2CH_2CH_2$), 3.36 (m, 4H, piperazine), 3.40 (s, 3H, $\underline{CH_3}$) 3.62–3.95 (m, 8H, piperazine, $\underline{CH_2}N$ and $\underline{CH_2}NCH_3$), 7.18 (dd, 1H, Ar-$\underline{H}$), 7.23 (m, 2H, Ar-$\underline{H}$), 7.38 (m, 1H, Ar-$\underline{H}$), 7.52–7.75 (m, 4H, Ar-$\underline{H}$), 7.81 (m, 1H, Ar-$\underline{H}$), 8.06 (m, 1H, Ar-$\underline{H}$), 8.40 (m, 1H, Ar-$\underline{H}$); ESI-MS (*m/z*): 418 $(M + H)^+$. Anal. calcd for $C_{27}H_{36}ClN_3O \cdot 0.2$ MeOH: C 70.95, H 8.06, N 9.13, found C 71.21, H 8.28, N 9.33.

*5.1.13.4. 3-[Methyl(6-{4-[3-(trifluoromethyl)phenyl]piperazin-1-yl} hexyl)amino]phenol·HCl (**18g**).* The product was prepared starting from **17g** (Scheme 4). White solid (368 mg, 78%); ^{1}H NMR (CD_3OD): δ = 1.42 (m, 4H, $CH_2CH_2\underline{CH_2}\underline{CH_2}CH_2CH_2$), 1.57 (m, 2H, $CH_2\underline{CH_2}CH_2CH_2CH_2CH_2$), 1.81 (m, 2H, $CH_2\underline{CH_2}CH_2CH_2CH_2CH_2$), 3.19 (m, 6H, piperazine and $\underline{CH_2}N$), 3.22 (s, 3H, $\underline{CH_3}$), 3.62 (m, 4H, piperazine), 3.91 (m, 2H, $\underline{CH_2}NCH_3$), 6.94 (m, 1H, Ar-$\underline{H}$), 7.08 (m, 2H, Ar-$\underline{H}$), 7.21 (m, 1H, Ar-$\underline{H}$), 7.32 (m, 2H, Ar-$\underline{H}$), 7.42 (m, 2H, Ar-$\underline{H}$). ESI-MS (*m/z*): 436 $(M + H)^+$. Anal. calcd for $C_{24}H_{33}ClF_3N_3O \cdot 0.3$ MeOH: C 60.60, H 7.16, N 8.72, found C 60.65, H 7.01, N 8.63.

*5.1.13.5. 3-({6-[4-(7-Chloroquinolin-4-yl)piperazin-1-yl]hexyl}methylamino)phenol (**18h**)·HCl.* The product was prepared starting from **17h** (Scheme 4). White solid (381 mg, 78%); ^{1}H NMR (CD_3OD): δ = 1.41 (m, 4H, $CH_2CH_2\underline{CH_2}\underline{CH_2}CH_2CH_2$), 1.58 (m, 2H, $CH_2\underline{CH_2}CH_2CH_2CH_2CH_2$), 1.82 (m, 2H, $CH_2\underline{CH_2}CH_2CH_2CH_2CH_2$), 3.22 (s, 3H, $\underline{CH_3}$), 3.25 (m, 2H), 3.51 (m, 4H, piperazine), 3.79 (m, 2H), 3.92 (m, 2H), 4.38 (m, 2H), 6.98 (m, 1H, Ar-$\underline{H}$), 7.08 (m, 2H, Ar-$\underline{H}$), 7.42 (m, 2H, Ar-$\underline{H}$), 7.80 (m, 1H, Ar-$\underline{H}$), 8.08 (m, 1H, Ar-$\underline{H}$), 8.22 (d, 1H, Ar-$\underline{H}$), 8.78 (d, 1H, Ar-$\underline{H}$). ESI-MS (*m/z*): 453.5 $(M + H)^+$. Anal. calcd for $C_{26}H_{34}Cl_2N_4O \cdot 0.1\ H_2O \cdot 0.15$ MeOH: C 63.31, H 7.07, N 11.29, found C 63.60, H 7.24, N 11.19.

*5.1.13.6. 3-{Ethyl[6-(4-phenylpiperazin-1-yl)hexyl]amino}phenol (**18j**)·HCl.* The product was prepared starting from **17j** (Scheme 4). White solid (342 mg, 82%); ^{1}H NMR (CD_3OD): δ = 1.19 (t, 3H, $CH_2\underline{CH_3}$), 1.40 (m, 4H, $CH_2CH_2\underline{CH_2}\underline{CH_2}CH_2CH_2$), 1.50 (m, 2H, $CH_2\underline{CH_2}CH_2CH_2CH_2CH_2$), 1.76 (m, 2H, $CH_2\underline{CH_2}CH_2CH_2CH_2CH_2$), 3.19 (m, 6H), 3.60 (m, 6H), 3.85 (m, 2H), 6.90–7.10 (m, 6H, Ar-$\underline{H}$), 7.30 (m, 2H, Ar-$\underline{H}$), 7.43 (m, 1H, Ar-$\underline{H}$); ESI-MS (*m/z*): 382 $(M + H)^+$. Anal. calcd for $C_{24}H_{36}ClN_3O \cdot 0.3\ H_2O$: C 68.08, H 8.71, N 9.92, found C 67.91, H 8.52, N 9.78.

*5.1.13.7. 3-{Benzyl[6-(4-phenylpiperazin-1-yl)hexyl]amino}phenol (**18k**)·HCl.* The product was prepared starting from **17k** (Scheme 4). White solid (379 mg, 79%); ^{1}H NMR (CD_3OD): δ = 1.42 (m, 6H, $CH_2CH_2\underline{CH_2}\underline{CH_2}CH_2CH_2$), 1.75 (m, 2H, $CH_2\underline{CH_2}CH_2CH_2CH_2CH_2$), 3.20 (m, 6H, piperazine and $\underline{CH_2}N$), 3.78 (m, 6H, piperazine and $\underline{CH_2}N$), 4.77 (s, 2H, $\underline{CH_2}Ph$), 6.81–7.08 (m, 6H, Ar-$\underline{H}$), 7.10–7.23 (m, 8H, Ar-$\underline{H}$); ESI-MS (*m/z*): 444 $(M + H)^+$. Anal. calcd for $C_{29}H_{38}ClN_3O$: C 72.55, H 7.98, N 8.75, found C 72.81, H 8.15, N 8.90.

*5.1.14. 3-(Benzyloxy)-N-ethyl-N-[6-(4-phenylpiperazin-1-yl)hexyl] aniline (**17j**)*

A solution of acetaldehyde (132 mg, 3.0 mmol) in MeOH (16 mL) was added dropwise to a stirred cooled (0 °C) solution of **17d** (1.33 g, 3.0 mmol), AcOH (0.47 mL) and sodium cyanoborohydride (0.35 g, 5.55 mmol) and the resulting mixture was stirred at room temperature for 16 h. MeOH was removed by distillation *in vacuo*, then a 1N NaOH aqueous solution (5 mL) was added, and the

aqueous phase was extracted with CH_2Cl_2; after drying over Na_2SO_4, the combined organic layers were concentrated under reduced pressure to give a crude residue which was purified by flash chromatography on silica gel (EtOAc/cyclohexane 8:2 as eluant). Oil (848 mg, 60%). ^{1}H NMR ($CDCl_3$): δ = 1.15 (t, 3H, J = 7.0, $CH_2\underline{CH_3}$), 1.38 (m, 4H, $CH_2CH_2\underline{CH_2}\underline{CH_2}CH_2CH_2$), 1.56 (m, 2H, $CH_2CH_2CH_2CH_2\underline{CH_2}CH_2$), 1.83 (m, 2H, $CH_2\underline{CH_2}CH_2CH_2CH_2CH_2$), 2.62–3.24 (m, 8H, piperazine and 2 × $\underline{CH_2}$N), 3.30 (q, 2H, J = 7.0, $\underline{CH_2}CH_3$), 3.56 (m, 4H, piperazine), 5.02 (s, 2H, $\underline{CH_2}$Ph), 6.22 (m, 3H, Ar-H), 6.82–7.52 (m, 11H, Ar-H). ESI-MS: m/z 472 $(M + H)^+$.

5.1.15. N-Benzyl-3-(benzyloxy)-N-[6-(4-phenylpiperazin-1-yl) hexyl]aniline (**17k**)

The product was prepared according to the above described procedure for **17j**, using benzaldehyde instead of acetaldehyde. Purification by flash chromatography on silica gel (EtOAc/cyclohexane 7:3 as eluant). Oil (1.04 g, 65%) ^{1}H NMR ($CDCl_3$): δ = 1.31 (m, 4H, $CH_2CH_2\underline{CH_2}\underline{CH_2}CH_2CH_2$), 1.58 (m, 2H, $CH_2CH_2CH_2CH_2\underline{CH_2}CH_2$), 1.92 (m, 2H, $CH_2\underline{CH_2}CH_2CH_2CH_2CH_2$), 2.91 (m, 4H, piperazine), 3.38 (m, 2H, $\underline{CH_2}$-piperazine), 3.60 (m, 6H, piperazine and $\underline{CH_2}$NAr), 4.78 (d, 2H, J = 9.0, N$\underline{CH_2}$Ph), 4.98 (s, 2H, O$\underline{CH_2}$Ph), 6.30 (m, 2H, Ar-H), 6.90–7.41 (m, 17H, Ar-H). ESI-MS: m/z 534 $(M + H)^+$.

5.1.16. N^1-Methyl-N^1-[3-(4-phenylpiperazin-1-yl)propyl]benzene-1,3-diamine

A solution of the nitro compound **14f** (0.38 g, 1.07 mmol) in EtOH (10 mL) and EtOAc (10 mL) was hydrogenated over 10% Pd/C (0.075 g) at 1 atm of H_2 for 5 h at room temperature. The catalyst was filtered on celite, the filtrate was concentrated under reduced pressure to give a crude oily amine that was used for the next step, without further purification (342 mg, near quantitative yield). ESI-MS (m/z): 325 $(M + H)^+$.

5.1.17. N-(3-{Methyl[3-(4-phenylpiperazin-1-yl)propyl]amino} pheny)acetamide (**14p**)

Ac_2O (0.045 mL, 0.48 mmol) was added to a solution of N^1-methyl-N^1-[3-(4-phenylpiperazin-1-yl)propyl]benzene-1,3-diamine (0.15 g, 0.46 mmol) and TEA (0.07 mL) in dry THF (4 mL), and the resulting mixture was stirred at room temperature for 1 h. After removing the solvent by distillation at reduced pressure, the residue was taken up in EtOAc, and washed with 1 N NaOH and with brine. After drying over Na_2SO_4, the organic layer was concentrated under reduced pressure and the resulting crude product was purified by column flash chromatography on silica gel (EtOAc/MeOH, 95:5 as eluant). Amorphous solid (145 mg, 86%); ^{1}H NMR ($CDCl_3$): δ = 1.80 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.15 (s, 3H, CO$\underline{CH_3}$), 2.42 (apt, 2H, J = 7.0, $\underline{CH_2}$-piperazine), 2.59 (m, 4H, piperazine), 2.93 (s, 3H, N$\underline{CH_3}$), 3.21 (m, 4H, piperazine), 3.39 (t, 2H, J = 7.0, $\underline{CH_2}$NCH$_3$), 6.49 (dd, 1H, J = 2.0 and 8.0, Ar-H), 6.66 (dd, 1H, J = 1.5 and 8.0, Ar-H), 6.81–6.97 (m, 3H, Ar-H), 7.04–7.08 (m, 2H, Ar-H), 7.13 (dd, 1H, $J_1 = J_2$ = 8.0, Ar-H), 7.21–7.32 (m, 1H, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 168.2, 151.3, 150.0, 138.9, 129.5, 129.1, 119.6, 116.0, 108.3, 107.5, 103.7, 55.7, 53.3, 50.6, 49.1, 38.3, 24.8, 24.2; IR (nujol): ν = 3311, 1657 cm^{-1}; ESI-MS (m/z): 367 $(M + H)^+$; Anal. calcd for $C_{22}H_{30}N_4O$: C 72.10, H 8.25, N 15.29, found C 72.28, H 8.19, N 15.41.

5.1.18. N-(3-{Methyl[3-(4-phenylpiperazin-1-yl)propyl]amino} phenyl)methanesulfonamide (**14q**)

The product was prepared according the above described procedure for **14p**, using methanesulfonyl chloride (0.05 mL, 0.65 mmol) instead of Ac_2O. Purification by column flash chromatography on silica gel (EtOAc as eluant). Oil (157 mg, 85%); ^{1}H NMR ($CDCl_3$): δ = 1.79 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.42 (apt, 2H, J = 7.0, $\underline{CH_2}$-piperazine), 2.60 (m, 4H, piperazine), 2.94 (s, 3H, N$\underline{CH_3}$), 2.99 (s, 3H, NHSO$_2\underline{CH_3}$), 3.21 (m, 4H, piperazine), 3.39 (apt, 2H, J = 7.0, $\underline{CH_2}$NCH$_3$), 6.47–6.58 (m, 3H, Ar-H), 6.83–6.97 (m, 3H, Ar-H), 7.13–7.32 (m, 3H, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 151.2, 150.3, 138.0, 130.2, 129.1, 119.8, 116.1, 109.1, 108.0, 104.4, 55.7, 53.2, 50.5, 49.1, 38.9, 38.4, 24.1; IR (nujol): ν = 3248, 1148 cm^{-1}; ESI-MS (m/z): 403 $(M + H)^+$; Anal. calcd for $C_{21}H_{30}N_4O_2S$: C 62.66, H 7.51, N 13.92, found C 62.54, H 7.38, N 13.78.

5.1.19. 3-[3-(4-Phenylpiperazin-1-yl)propylamino]benzamide (**14r**)

To a stirred ice-cooled solution of the ester **14g** (0.12 g, 0.33 mmol) in MeOH (3 mL) in a 5 mL microwave vial, was added magnesium nitride (0.165 g, 1.64 mmol). The vial was sealed immediately and allowed to warm to room temperature in a water bath. After approximately 1 h, the reaction was heated at 80 °C for 24 h. After cooling to room temperature the reaction mixture was diluted with dichloromethane (12 mL) and water (10 mL), the aqueous phase was neutralized with 1 N HCl and then extracted (2 × 10 mL) with dichloromethane. After drying over Na_2SO_4, the combined organic layers were concentrated under reduced pressure and the resulting crude product was purified by column flash chromatography on silica gel (EtOAc/MeOH, 9:1 as eluant) and subsequent crystallization. White solid (14 mg, 13%); mp: 147–148 °C (EtOAc); ^{1}H NMR ($CDCl_3$): δ = 1.87 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.57 (t, 2H, J = 6.5, $\underline{CH_2}$-piperazine), 2.65 (m, 4H, piperazine), 3.23–3.28 (m, 6H, piperazine and $\underline{CH_2}$NH), 4.95 (brs, 1H, $\underline{NH}$), 5.71 (brs, 1H, $\underline{NH}$), 6.07 (brs, 1H, $\underline{NH}$), 6.75 (dd, 1H, J = 2.0 and 8.0, Ar-H), 6.85–7.34 (m, 8H, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 170.0, 151.2, 149.0, 134.5, 129.3, 129.1, 119.8, 116.4, 116.0, 115.1, 111.1, 57.3, 53.3, 49.3, 43.4, 25.5; IR (nujol): ν = 3321, 3151, 1661 cm^{-1}; ESI-MS (m/z): 339 $(M + H)^+$; Anal. calcd for $C_{20}H_{26}N_4O$: C 70.98, H 7.74, N 16.55, found C 71.11, H 7.86, N 16.37.

From chromatographic purification we also isolated the methyl ester derivative **14s** (87 mg, 75%) as a result of **14g** transesterification.

5.1.20. Methyl 3-[3-(4-phenylpiperazin-1-yl)propylamino]benzoate (**14s**)

White solid; mp: 102–103 °C (EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$): δ = 1.88 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.59 (t, 2H, J = 6.5, $\underline{CH_2}$-piperazine), 2.67 (m, 4H, piperazine), 3.24–3.29 (m, 6H, piperazine and $\underline{CH_2}$NH), 3.90 (s, 3H, $\underline{CH_3}$), 4.93 (brs, 1H, $\underline{NH}$), 6.79 (ddd, 1H, J = 1.0, 2.5, 8.0, Ar-H), 6.89 (m, 1H, Ar-H), 6.95 (m, 1H, Ar-H), 6.99 (m, 1H, Ar-H), 7.18–7.38 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 167.6, 151.2, 148.7, 131.0, 129.2, 129.1, 119.8, 118.2, 117.5, 116.1, 112.8, 57.3, 53.3, 52.0, 49.2, 43.4, 25.5; IR (nujol): ν = 3384, 1707 cm^{-1}; ESI-MS (m/z): 354 $(M + H)^+$; Anal. calcd for $C_{21}H_{27}N_3O_2$: C 71.36, H 7.70, N 11.89, found C 71.02, H 7.58, N 12.05.

5.1.21. {3-[3-(4-Phenylpiperazin-1-yl)propylamino]phenyl} methanol (**14t**)

$LiAlH_4$ (0.085 g, 2.24 mmol) was added portionwise to an ice-cooled stirred solution of **14g** (1.13 mmol) in dry THF (10 mL) under nitrogen atmosphere, and the resulting mixture was stirred for 1 h at room temperature. Water was added dropwise to destroy the excess of hydride, the resulting mixture was filtered on Celite, and the filtrate was concentrated *in vacuo* and partitioned between EtOAc and water. The combined organic phases were washed with brine, dried (Na_2SO_4) and concentrated to afford a crude residue which was purified by crystallization. White solid (334 mg, 91%); mp: 142–143 °C (EtOAc); ^{1}H NMR ($CDCl_3$): δ = 1.87 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.57 (t, 2H, J = 6.5, $\underline{CH_2}$-piperazine) 2.65 (m, 4H, piperazine), 3.23–3.28 (m, 6H, piperazine and $\underline{CH_2}$NH), 4.62 (s, 2H, $\underline{CH_2}$OH), 4.74 (brs, 1H, $\underline{NH}$), 6.55 (dd, 1H, J = 2.0 and 8.0, Ar-H), 6.61 (m, 2H, Ar-H), 6.92 (m, 3H, Ar-H), 7.17 (dd, 1H, $J_1 = J_2$ = 8.0, Ar-H), 7.31 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 151.2, 149.0, 142.3, 129.4, 129.1, 119.8, 116.0, 115.6, 112.1, 111.0, 65.5, 57.2, 53.3, 49.2, 43.4, 25.8;

IR (nujol): $\nu = 3332, 3121\ cm^{-1}$; ESI–MS (*m/z*): 326 $(M + H)^+$; Anal. calcd for $C_{20}H_{27}N_3O$: C 73.81, H 8.36, N 12.91, found C 73.59, H 8.18, N 12.77.

5.1.22. 3-[3-(4-Phenylpiperazin-1-yl)propylamino]benzaldehyde (**14u**)

Activated manganese dioxide (1.28 g) was added to a solution of the alcohol derivative **14t** (0.35 g, 1.08 mmol) in dry dichloromethane (15 mL), and the resulting mixture was stirred for 18 h at room temperature. The mixture was filtered, the filter cake was washed with hot acetone and the combined filtrates were concentrated under reduced pressure to yield a crude residue that was purified by flash chromatography (EtOAc as eluant) and crystallization. Yellow solid (261 mg, 75%); mp: 87–88 °C (EtOAc); ^1H NMR ($CDCl_3$): $\delta = 1.88$ (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.58 (t, 2H, $J = 6.5$, $\underline{CH_2}$-piperazine) 2.66 (m, 4H, piperazine), 3.23–3.32 (m, 6H, piperazine and $\underline{CH_2}NH$), 5.13 (brs, 1H, $\underline{NH}$), 6.84–7.35 (m, 9H, Ar-$\underline{H}$), 9.93 (s, 1H, $\underline{CHO}$); ^{13}C NMR ($CDCl_3$): $\delta =$ 193.1, 151.2, 149.2, 137.5, 129.7, 129.2, 120.0, 119.9, 119.5, 116.1, 110.8, 57.3, 53.3, 49.3, 43.4, 25.4; IR (nujol): $\nu = 3346, 1677\ cm^{-1}$; ESI–MS (*m/z*): 324 $(M + H)^+$; Anal. calcd for $C_{20}H_{25}N_3O$: C 74.27, H 7.79, N 12.99, found C 74.45, H 7.92, N 12.63.

5.1.23. N-(3-Methoxyphenyl)-N-[3-(4-phenylpiperazin-1-yl) propyl]propionamide (**19**)

TEA (0.186 mL, 1.34 mmol) and propionic anhydride (0.171 mL, 1.34 mmol) were added to a solution of **14h** (0.29 g, 0.89 mmol) in dry THF (12 mL) and the resulting mixture was stirred at room temperature for 24 h. The solvent was removed by distillation *in vacuo*, water was added and the aqueous phase was extracted (3×) with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure to give a crude residue that was purified by flash chromatography on silica gel (EtOA as eluant). Oil (308 mg, 91%); ^1H NMR ($CDCl_3$): $\delta = 1.06$ (t, 3H, $J = 7.0$, $CH_2\underline{CH_3}$), 1.82 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.09 (m, 2H, $\underline{CH_2}$-piperazine), 2.41 (q, 2H, $J = 7.0$, $\underline{CH_2}CH_3$), 2.57 (m, 4H, piperazine), 3.17 (m, 4H, piperazine), 3.77 (t, 2H, $J = 7.5$, $\underline{CH_2}NAr$), 3.82 (s, 3H, $O\underline{CH_3}$), 6.71–6.93 (m, 6H, Ar-$\underline{H}$), 7.21–7.32 (m, 3H, Ar-$\underline{H}$); ESI–MS (*m/z*): 382 $(M + H)^+$.

5.1.24. 3-Methoxy-N-[3-(4-phenylpiperazin-1-yl)propyl]-N-propylaniline (**20**)

A 1 M solution of BH_3 in THF (2.43 mL) was added dropwise to an ice-cooled solution of **19** (0.31 g, 0.81 mmol) in dry THF (6 mL) under a N_2 atmosphere. Once the addition was completed, the reaction mixture was stirred at room temperature for 16 h; 6 N HCl (0.7 mL) was cautiously added and the resulting mixture was heated to reflux for 1 h. After cooling to room temperature the reaction mixture was made alkaline with 1 N NaOH (2.7 mL) and then extracted with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated to afford a crude residue which was purified by flash chromatography on silica gel (cyclohexane/EtOAc, 7:3 as eluant). Oil (193 mg, 65%); ^1H NMR ($CDCl_3$): $\delta = 0.92$ (t, 3H, $J = 7.0$, $CH_2CH_2\underline{CH_3}$), 1.63 (m, 2H, $CH_2\underline{CH_2}CH_3$), 1.80 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.43 (t, 2H, $J = 7.0$, $\underline{CH_2}$-piperazine), 2.62 (m, 4H, piperazine), 3.23 (m, 6H, piperazine and $\underline{CH_2}N$), 3.35 (t, 2H, $J = 7.0$, $\underline{CH_2}NAr$), 3.80 (s, 3H, $O\underline{CH_3}$), 6.22 (m, 2H, Ar-$\underline{H}$), 6.33 (dd, 1H, $J = 2.0$ and 8.5, Ar-$\underline{H}$), 6.83–6.98 (m, 3H, Ar-$\underline{H}$), 7.12 (dd, 1H, $J_1 = J_2 = 8.5$, Ar-$\underline{H}$), 7.23–7.32 (m, 2H, Ar-$\underline{H}$); ESI–MS (*m/z*): 368 $(M + H)^+$.

5.1.25. N-Benzyl-3-methoxy-N-[3-(4-phenylpiperazin-1-yl)propyl] aniline (**21**)

TEA (0.5 mL, 3.6 mmol) and benzyl bromide (0.37 mL, 3.11 mmol) were added to a solution of **14h** (0.20 g, 0.61 mmol) in toluene (4 mL), and the resulting mixture was heated at 80 °C for 16 h. After cooling to room temperature, water was added and the aqueous phase was extracted (3×) with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure to give a crude residue that was purified by flash chromatography on silica gel (EtOAc/cyclohexane, 3:7 as eluant). Oil (207 mg, 82%); ^1H NMR ($CDCl_3$): $\delta = 1.90$ (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.45 (t, 2H, $J = 7.0$, $\underline{CH_2}$-piperazine), 2.61 (m, 4H, piperazine), 3.23 (m, 4H, piperazine), 3.49 (t, 2H, $J = 7.0$, $\underline{CH_2}NCH_2Ph$), 3.75 (s, 3H, $O\underline{CH_3}$), 4.57 (s, 2H, $\underline{CH_2}Ph$), 6.27 (m, 2H, Ar-$\underline{H}$), 6.38 (dd, 1H, $J = 2.0$ and 8.0, Ar-$\underline{H}$), 6.92 (m, 3H Ar-$\underline{H}$), 7.11 (dd, 1H, $J_1 = J_2 = 8.0$, Ar-$\underline{H}$), 7.22–7.35 (m, 7H, Ar-$\underline{H}$); ESI–MS (*m/z*): 416 $(M + H)^+$.

5.1.26. 3-Methoxy-N-phenyl-N-[3-(4-phenylpiperazin-1-yl)propyl] aniline (**22**)

A Schlenk flask was charged with palladium acetate (0.01 g, 0.044 mmol), (±)-BINAP (0.031 g, 0.05 mmol), potassium *tert*-butoxide (0.147 g, 1.3 mmol) and toluene (2 mL) under a nitrogen atmosphere. To this mixture a solution of **14h** (0.3 g, 0.92 mmol) and iodobenzene (0.1 mL, 0.92 mmol) in dry toluene (2 mL) was added dropwise via syringe. After the addition was completed, the mixture was heated at 110 °C for 24 h. After cooling to room temperature EtOAc was added, the mixture was filtered and the filtrate was washed with brine. After drying over Na_2SO_4, the organic layer was concentrated under reduced pressure and the resulting crude product was purified by column flash chromatography on silica gel (cyclohexane/EtOAc 7:3 as eluant). Oil (162 mg, 44%); ^1H NMR ($CDCl_3$): $\delta = 1.91$ (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.47 (t, 2H, $J = 7.0$, $\underline{CH_2}$-piperazine), 2.61 (m, 4H, piperazine), 3.23 (m, 4H, piperazine), 3.76 (s, 3H, $O\underline{CH_3}$), 3.80 (t, 2H, $J = 7.0$, $\underline{CH_2}NPh$), 6.54 (m, 3H, Ar-$\underline{H}$), 6.84–7.32 (m, 11H, Ar-$\underline{H}$); MS (EI, 70 eV): *m/z* 401 (M^+), 226 (100).

5.1.27. N-(3-Methoxyphenyl)-N-[3-(4-phenylpiperazin-1-yl) propyl]benzenesulfonamide (**23**)

TEA (0.23 mL, 1.8 mmol) and benzenesulfonyl chloride (0.18 mL, 1.41 mmol) were added to a solution of **14h** (0.25 g, 0.77 mmol) in dry THF (7.5 mL), and the resulting mixture was stirred at room temperature under nitrogen for 48 h. After removing the solvent by distillation *in vacuo*, the residue was partitioned between water and EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure to afford a crude residue which was purified by flash chromatography on silica gel (EtOAc/cyclohexane, 7:3 as eluant). White solid (250 mg, 70%); ^1H NMR ($CDCl_3$): $\delta = 1.60$ (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.48 (t, 2H, $J = 7.0$, $\underline{CH_2}$-piperazine), 2.56 (m, 4H, piperazine), 3.18 (m, 4H, piperazine), 3.62 (t, 2H, $J = 7.0$, $\underline{CH_2}NSO_2$), 3.76 (s, 3H, $O\underline{CH_3}$), 6.62 (m, 2H, Ar-$\underline{H}$), 6.88 (m, 4H, Ar-$\underline{H}$), 7.24 (m, 3H, Ar-$\underline{H}$), 7.54 (m, 5H, Ar-$\underline{H}$); ESI–MS (*m/z*): 466 $(M + H)^+$.

5.1.28. 3-{[3-(4-Phenylpiperazin-1-yl)propyl]propylamino}phenol (**24**)

The product was prepared starting from **20** using the above general *O*-demethylation procedure with boron tribromide. Purification by flash chromatography on silica gel (cyclohexane/EtOAc 1:1 as eluant) and subsequent crystallization. White solid (339 mg, 96%); mp: 132–133 °C (Et_2O/petroleum ether); ^1H NMR ($CDCl_3$): $\delta = 0.91$ (t, 3H, $J = 7.5$, $CH_2CH_2\underline{CH_3}$), 1.67 (q, 2H, $J = 7.5$, $CH_2\underline{CH_2}CH_3$), 1.84 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.45 (t, 2H, $J = 7.0$, $\underline{CH_2}$-piperazine), 2.66 (m, 4H, piperazine), 3.16–3.36 (m, 8H, piperazine, 2 × $\underline{CH_2}NAr$), 5.86 (brs, 1H, $\underline{OH}$), 6.10 (m, 2H, Ar-$\underline{H}$), 6.25 (dd, 1H, $J = 2.0$ and 8.0, Ar-$\underline{H}$), 6.84–7.07 (m, 4H, Ar-$\underline{H}$), 7.26–7.33 (m, 2H, Ar-$\underline{H}$); ^{13}C NMR ($CDCl_3$): $\delta = 157.1, 151.2, 149.7, 130.0, 129.1, 119.9, 116.1, 104.5, 102.6, 99.0, 55.9, 53.3, 52.8, 49.0, 48.9, 24.6, 20.3, 11.5$; ESI–MS (*m/z*): 354 $(M + H)^+$; Anal. calcd for $C_{22}H_{31}N_3O$: C 74.75, H 8.84, N 11.89, found C 74.98, H 8.59, N 11.66.

*5.1.29. 3-{Benzyl[3-(4-phenylpiperazin-1-yl)propyl]amino}phenol (**25**)*

The product was prepared starting from **21** using the above general *O*-demethylation procedure with boron tribromide. Purification by flash chromatography on silica gel (cyclohexane/EtOAc 3:7 as eluant) and subsequent crystallization. White solid (228 mg, 57%); mp: 166–167 °C (EtOAc); ^{1}H NMR ($CDCl_3$): δ = 1.89 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.44 (t, 2H, J = 7.0, $\underline{CH_2}$-piperazine), 2.54 (m, 4H, piperazine), 3.16 (m, 4H, piperazine), 3.45 (t, 2H, J = 7.0, $\underline{CH_2}NCH_2Ph$), 4.44 (s, 2H, $\underline{CH_2}Ph$), 6.08 (m, 1H, Ar-$\underline{H}$), 6.16 (dd, 1H, J = 2.0 and 8.0, Ar-$\underline{H}$), 6.31 (dd, 1H, J = 2.0 and 8.0, Ar-$\underline{H}$), 6.83–6.94 (m, 3H, Ar-$\underline{H}$), 7.06 (dd, 1H, $J_1 = J_2$ = 8.0, Ar-$\underline{H}$) 7.13–7.35 (m, 7H, Ar-$\underline{H}$); ^{13}C NMR ($CDCl_3$): δ = 157.3, 151.1, 150.3, 138.5, 130.2, 129.1, 128.6, 126.7, 126.4, 119.9, 116.1, 104.4, 103.6, 99.5, 55.6, 53.5, 53.1, 48.8, 48.5, 24.7; ESI-MS (*m/z*): 402 $(M + H)^+$; Anal. calcd for $C_{26}H_{31}N_3O$: C 77.77, H 7.78, N 10.46, found C 77.39, H 7.52, N 10.30.

*5.1.30. 3-{Phenyl[3-(4-phenylpiperazin-1-yl)propyl]amino}phenol (**26**)*

The product was prepared starting from **22** using the above general *O*-demethylation procedure with boron tribromide. Purification by flash chromatography on silica gel (cyclohexane/EtOAc 1:1 as eluant) and subsequent crystallization. White solid (248 mg, 64%); mp: 172–173 °C (Et_2O); ^{1}H NMR ($CDCl_3$): δ = 1.87 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.44 (t, 2H, J = 7.0, $\underline{CH_2}$-piperazine), 2.57 (m, 4H, piperazine), 3.19 (m, 4H, piperazine), 3.74 (t, 2H, J = 7.0, $\underline{CH_2}$NPh), 6.33 (m, 2H, Ar-$\underline{H}$), 6.51 (m, 1H, Ar-$\underline{H}$), 6.83–7.13 (m, 7H, Ar-$\underline{H}$), 7.21–7.32 (m, 4H, Ar-$\underline{H}$); ^{13}C NMR ($CDCl_3$): δ = 156.8, 151.1, 149.5, 147.6, 130.1, 129.4, 129.1, 123.4, 122.7, 119.9, 116.1, 110.8, 107.1, 105.9, 55.6, 53.1, 50.0, 48.9, 24.8; ESI-MS (*m/z*): 387 $(M + H)^+$; Anal. calcd for $C_{25}H_{29}N_3O$: C 77.48, H 7.54, N 10.84, found C 77.13, H 7.30, N 10.96.

*5.1.31. N-(3-Hydroxyphenyl)-N-[3-(4-phenylpiperazin-1-yl)propyl] benzenesulfonamide (**27**)*

The product was prepared starting from **23** using the above general *O*-demethylation procedure with boron tribromide. Purification by flash chromatography on silica gel (cyclohexane/EtOAc 3:7 as eluant). Amorphous solid (333 mg, 74%); ^{1}H NMR ($CDCl_3$): δ = 1.69 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.55 (t, 2H, J = 7.0, $\underline{CH_2}$-piperazine), 2.68 (m, 4H, piperazine), 3.19 (m, 4H, piperazine), 3.55 (t, 2H, J = 7.0, $\underline{CH_2}NSO_2$), 6.42 (m, 2H, Ar-$\underline{H}$), 6.55 (dd, 1H, J = 2.0 and 8.0, Ar-$\underline{H}$), 6.83–6.93 (m, 3H, Ar-$\underline{H}$), 7.06 (dd, 1H, $J_1 = J_2$ = 8.0, Ar-$\underline{H}$), 7.22–7.30 (m, 2H, Ar-$\underline{H}$), 7.36–7.58 (m, 5H, Ar-$\underline{H}$); ^{13}C NMR ($CDCl_3$): δ = 156.9, 151.0, 139.3, 137.7, 132.7, 129.8, 129.1, 128.8, 127.6, 120.0, 119.1, 116.1, 115.5, 55.5, 53.1, 48.6, 48.4, 24.9; IR (nujol): ν = 1164 cm^{-1}; ESI-MS (*m/z*): 452 $(M + H)^+$; Anal. calcd for $C_{25}H_{29}N_3O_3S$: C 66.49, H 6.47, N 9.31, found C 66.20, H 6.55, N 9.66.

*5.1.32. 3-(4,4-Diphenylpiperidin-1-yl)-N-(3-methoxyphenyl)-N-methylpropanamide (**28b**)*

4,4-diphenylpiperidine [29] (0.6 mmol) and TEA (0.83 mL) were added to a solution of 3-bromo-*N*-(3-methoxyphenyl)-*N*-methylpropanamide (**12a**) (0.5 mmol) in DMF (0.14 mL) and the resulting mixture was stirred at room temperature for 16 h, monitoring the progress of the reaction by TLC on silica gel. The reaction mixture was poured into water, and extracted with EtOAc. After drying over Na_2SO_4, the combined organic phases were concentrated *in vacuo* and the resulting crude product was purified by column flash chromatography on silica gel (EtOAc as eluant). Amorphous solid (103 mg, 48%); ^{1}H NMR ($CDCl_3$): δ = 2.28 (t, 2H, J = 7.5, $\underline{CH_2}CO$), 2.41 (m, 8H, piperidine), 2.60 (t, 2H J = 7.5, $COCH_2\underline{CH_2}N$), 3.24 (s, 3H, $N\underline{CH_3}$), 3.82 (s, 3H, $O\underline{CH_3}$), 6.68–6.76 (m, 2H, Ar-$\underline{H}$), 6.87 (ddd, 1H, J = 1.0, 3.0 and 8.0, Ar-$\underline{H}$), 7.09–7.34 (m, 11H, Ar-$\underline{H}$); ESI-MS (*m/z*): 429 $(M + H)^+$.

*5.1.33. 3-[4-(Diphenylamino)piperidin-1-yl]-N-(3-methoxyphenyl)-N-methylpropanamide (**28c**)*

This compound was prepared using the above described procedure for **28b**, using diphenylpiperidin-4-ylamine [30] instead of 4,4-diphenylpiperidine Purification by flash chromatography on silica gel (EtOAc as eluant). Oil (113 mg, 51%); ^{1}H NMR ($CDCl_3$): δ = 1.39 (m, 2H, piperidine), 1.99 (m, 4H, piperidine), 2.27 (t, 2H, J = 7.5, $\underline{CH_2}CO$), 2.66 (t, 2H, J = 7.5, $COCH_2\underline{CH_2}N$), 2.82 (m, 2H, piperidine), 3.23 (s, 3H, $N\underline{CH_3}$), 3.80 (s, 3H, $O\underline{CH_3}$), 3.81 (m, 1H, $\underline{CH}$), 6.67–7.01 (m, 9H, Ar-$\underline{H}$), 7.19–7.33 (m, 5H, Ar-$\underline{H}$); ESI-MS (*m/z*): 444 $(M + H)^+$.

*5.1.34. N-[3-(4,4-Diphenylpiperidin-1-yl)propyl]-3-methoxy-N-methylaniline (**29b**)*

A 1 M solution of BH_3 in THF (3 mL) was added dropwise to an ice-cooled solution of **28b** (0.43 g, 1 mmol) in dry THF (7 mL) under a N_2 atmosphere. Once the addition was completed, the reaction mixture was stirred at room temperature for 16 h; 6 N HCl (0.9 mL) was cautiously added and the resulting mixture was heated to reflux for 1 h. After cooling to room temperature the reaction mixture was made alkaline with 1 N NaOH (3 mL) and then extracted with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated to afford a crude residue which was purified by flash chromatography on silica gel (EtOAc as eluant). Oil (270 mg, 65%); ^{1}H NMR ($CDCl_3$): δ = 1.78 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.34 (t, 2H, J = 7.5, $\underline{CH_2}$-piperidine), 2.54 (m, 8H, piperidine), 2.79 (s, 3H, $N\underline{CH_3}$), 3.28 (t, 2H, J = 7.5, $\underline{CH_2}NCH_3$), 3.83 (s, 3H, $O\underline{CH_3}$), 6.16 (m, 3H, Ar-$\underline{H}$), 7.00–7.32 (m, 11H, Ar-$\underline{H}$); ESI-MS (*m/z*): 415 $(M + H)^+$.

*5.1.35. 1-{3-[(3-Methoxyphenyl)methylamino]propyl}-N,N-diphenylpiperidin-4-amine (**29c**)*

The product was prepared using the above described procedure starting from **28c**. Purification by flash chromatography on silica gel (EtOAc/cyclohexane 1:1 as eluant). Oil (202 mg, 47%); ^{1}H NMR ($CDCl_3$): δ = 1.50 (m, 2H, piperidine), 1.72 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.03 (m, 4H, piperidine), 2.34 (t, 2H, J = 7.0, $\underline{CH_2}$-piperidine), 2.88 (s, 3H, $N\underline{CH_3}$), 2.97 (m, 2H, piperidine), 3.30 (t, 2H, J = 7.0, $\underline{CH_2}NCH_3$), 3.77 (s, 3H, $O\underline{CH_3}$), 3.85 (m, 1H, $\underline{CH}$), 6.29 (m, 3H, Ar-$\underline{H}$), 6.84 (m, 4H, Ar-$\underline{H}$), 7.05 (m, 3H, Ar-$\underline{H}$), 7.22–7.32 (m, 4H, Ar-$\underline{H}$); ESI-MS (*m/z*): 430 $(M + H)^+$.

*5.1.36. 3-{3-[(4,4-Diphenylpiperidin-1-yl)propyl]methylamino} phenol (**30b**)*

The product was prepared starting from **29b** using the above general *O*-demethylation procedure with boron tribromide. Purification by flash chromatography on silica gel (EtOAc as eluant). Amorphous solid (116 mg, 29%); ^{1}H NMR ($CDCl_3$): δ = 1.76 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.31 (t, 2H, J = 7.5, $\underline{CH_2}$-piperidine), 2.52 (m, 8H, piperidine), 2.82 (s, 3H, $N\underline{CH_3}$), 3.29 (t, 2H, J = 7.0, $\underline{CH_2}NCH_3$), 6.13 (m, 2H, Ar-$\underline{H}$), 6.25 (ddd, 1H, J = 1.5, 3.0 and 8.0, Ar-$\underline{H}$), 7.04 (dd, 1H, J_1=J_2 = 8.0, Ar-$\underline{H}$), 7.11–7.18 (m, 2H, Ar-$\underline{H}$), 7.21–7.30 (m, 8H, Ar-$\underline{H}$); ^{13}C NMR ($CDCl_3$): δ = 157.6, 150.8, 130.0, 128.4, 127.1, 125.8, 104.3, 103.7, 99.5, 56.1, 50.6, 50.5, 44.6, 38.1, 35.6, 29.7, 24.2; ESI-MS (*m/z*): 401 $(M + H)^+$; Anal. calcd for $C_{27}H_{32}N_2O$: C 80.96, H 8.05, N 6.99, found C 81.24, H 8.22, N 6.68.

*5.1.37. 3-({3-[4-(Diphenylamino)piperidin-1-yl]propyl} methylamino)phenol (**30c**)*

The product was prepared starting from **29c** using the above general *O*-demethylation procedure with boron tribromide. Purification by flash chromatography on silica gel (CH_2Cl_2/MeOH, 95:5). Amorphous solid (166 mg, 40%); ^{1}H NMR ($CDCl_3$): δ = 1.45–1.81 (m, 4H, piperidine and $CH_2\underline{CH_2}CH_2$), 1.94–2.16 (m, 4H, piperidine), 2.36 (t, 2H, J = 7.0, $\underline{CH_2}$-piperidine), 2.86 (s, 3H, $N\underline{CH_3}$), 3.00 (m, 2H,

piperidine), 3.29 (t, 2H, $J = 7.0$, $\underline{CH_2}NCH_3$), 3.87 (m, 1H, $\underline{CH}$), 6.12 (m, 2H, Ar-$\underline{H}$), 6.25 (ddd, 1H, $J = 1.5$, 3.0 and 8.0, Ar-$\underline{H}$), 6.80–6.86 (m, 4H, Ar-$\underline{H}$), 6.95–7.07 (m, 3H, Ar-$\underline{H}$), 7.23–7.31 (m, 4H, Ar-$\underline{H}$); ^{13}C NMR ($CDCl_3$): $\delta = 157.1, 150.8, 146.0, 130.0, 129.3, 122.7, 121.8, 104.7, 103.5, 99.5, 55.7, 55.0, 53.4, 50.7, 38.2, 30.2, 24.2$; ESI-MS ($m/z$): 416 $(M + H)^+$; Anal. calcd for $C_{27}H_{33}N_3O$: C 78.03, H 8.00, N 10.11, found C 78.36, H 8.35, N 10.01.

*5.1.38. 3-(3-Hydroxyphenyl)-1-(4-phenylpiperazin-1-yl)propan-1-one (**31a**)*

Phenyl piperazine (500 mg, 3.08 mmol), 3-(3-hydroxyphenyl) propionic acid (570 mg, 3.4 mmol) and Et_3N (1.3 mL, 9.24 mmol) were added to an ice-cooled solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide [EDC] (885 mg, 4.62 mmol) and *N*-hydroxybenzotriazole [HOBT] (582 mg, 4.3 mmol) in CH_2Cl_2 (16.6 mL). The resulting mixture was stirred for 2 h at 0 °C and at room temperature overnight. The organic solution was washed with water, dried (Na_2SO_4), and concentrated under vacuum, obtaining a crude residue that was used for the next step without further purification. Oil (827 mg, 86%). ^{1}H NMR ($CDCl_3$): $\delta = 2.41$ (t, 2H, $J = 7.5$, $\underline{CH_2}CO$), 2.67 (t, 2H, $J = 7.5$, $\underline{CH_2}Ar$), 3.20 (m, 4H, piperazine), 3.61 (m, 2H, piperazine), 3.85 (m, 2H, piperazine), 6.68–6.99 (m, 6H, Ar-$\underline{H}$), 7.17–7.35 (m, 3H, Ar-$\underline{H}$); ESI-MS (m/z): 333 $(M + Na)^+$.

*5.1.39. 4-(3-Methoxyphenyl)-1-(4-phenylpiperazin-1-yl)butan-1-one (**31b**)*

This product was obtained according to the above described procedure for **31a** starting from 4-(3-methoxyphenyl)butanoic acid [31] (0.7 g, 3.6 mmol) instead of 3-(3-hydroxyphenyl)propionic acid. Purification by flash chromatography on silica gel (cyclohexane/EtOAc, 6:4 as eluant). Oil (770 mg, 74%); ^{1}H NMR ($CDCl_3$): $\delta = 2.03$ (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.37 (apt, 2H, $J = 7.5$, $\underline{CH_2}CO$), 2.69 (apt, 2H, $J = 7.5$, $\underline{CH_2}Ar$), 3.15 (m, 4H, piperazine), 3.57 (m, 2H, piperazine), 3.79 (m, 2H, piperazine), 3.80 (s, 3H, $O\underline{CH_3}$), 6.72–6.85 (m, 3H, Ar-$\underline{H}$), 6.86–6.95 (m, 3H, Ar-$\underline{H}$), 7.18–7.34 (m, 3H, Ar-$\underline{H}$); ESI-MS (m/z): 339 $(M + H)^+$.

*5.1.40. 3-[3-(4-Phenylpiperazin-1-yl)propyl]phenol·HCl (**32a**)*

A 1 M solution of B_2H_6 in THF (20 mL, 16 mmol) was added dropwise to an ice-cooled solution of **31a** (827 mg, 2.5 mmol) in THF (18 mL) and the reaction mixture underwent stirring for 4 h at room temperature. 6 M HCl (7 mL) was added to the solution at 0 °C and the resulting mixture was evaporated under vacuum. The solid was dissolved in EtOAc and washed with an 7% aqueous solution of $NaHCO_3$. The organic layer was washed with water, dried (Na_2SO_4), and evaporated under vacuum obtaining a crude product (1.02 g, 3.6 mmol). The oily product was dissolved in a solution of EtOH (10 mL) and conc. HCl (300 mL, 3.6 mmol) and the mixture was stirred for 1 h. After removing the solvent by distillation under vacuum, the resulting solid was purified by crystallization in EtOH. White solid (196 mg, 59%); ^{1}H NMR (DMSO-d_6): $\delta = 2.01$ (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.57 (m, 2H, $\underline{CH_2}$-piperazine), 3.01 (m, 6H, piperazine and $\underline{CH_2}Ar$), 3.58 (m, 2H, piperazine), 3.78 (m, 2H, piperazine), 6.62 (m, 2H, Ar-$\underline{H}$), 6.82 (ddd, 1H, $J = 1.5$, 3.0 and 8.0, Ar-$\underline{H}$), 7.00 (m, 3H, Ar-$\underline{H}$), 7.15 (dd, 1H, $J_1 = J_2 = 8.0$, Ar-$\underline{H}$), 7.30 (m, 2H, Ar-$\underline{H}$); 9.38 (s, 1H, $\underline{OH}$), 11.00 (brs, 1H, $\underline{NH}^+$); ESI-MS (m/z): 297 $(M + H)^+$. Anal. calcd for $C_{19}H_{24}N_2O$: C 76.99, H 8.16, N 9.45, found C 76.79, H 8.01, N 9.39.

*5.1.41. 1-[4-(3-Methoxyphenyl)butyl]-4-phenylpiperazine (**32b**)*

This compound was obtained by reduction of 4-(3-methoxyphenyl)-1-(4-phenylpiperazin-1-yl)butan-1-one (0.2 g, 0.6 mmol) with 1 M B_2H_6 in THF according to the above described procedure for compound **32a**. Purification by flash chromatography on silica gel (cyclohexane/EtOAc, 7:3 as eluant). Oil (130 mg, 67%); ^{1}H NMR ($CDCl_3$): $\delta = 1.68$ (m, 4H, $CH_2\underline{CH_2}\underline{CH_2}CH_2$), 2.42 (t, 2H, $J = 7.0$, $\underline{CH_2}Ar$), 2.63 (m, 6H, piperazine and $\underline{CH_2}N$), 3.22 (m, 4H, piperazine), 3.81 (s, 3H, $O\underline{CH_3}$), 6.73–6.98 (m, 6H, Ar-$\underline{H}$), 7.17–7.32 (m, 3H, Ar-$\underline{H}$); ESI-MS (m/z): 325 $(M + H)^+$.

*5.1.42. 3-[4-(4-Phenylpiperazin-1-yl)butyl]phenol (**32c**)*

The product was prepared starting from **32b** using the above described general *O*-demethylation procedure with boron tribromide. Purification by flash chromatography on silica gel (cyclohexane/EtOAc 1:1 as eluant) and subsequent crystallization. White solid (226 mg, 73%); mp: 143–144 °C (Et_2O/petroleum ether); ^{1}H NMR ($CDCl_3$): $\delta = 1.63$ (m, 4H, $CH_2\underline{CH_2}\underline{CH_2}CH_2$), 2.48 (t, 2H, $J = 7.0$, $\underline{CH_2}Ar$), 2.58 (t, 2H, $J = 7.0$, $\underline{CH_2}N$), 2.69 (m, 4H, piperazine), 3.26 (m, 4H, piperazine), 6.59 (m, 2H, Ar-$\underline{H}$), 6.70 (d, 1H, $J = 7.5$, Ar-$\underline{H}$), 6.82–6.98 (m, 3H, Ar-$\underline{H}$), 7.12 (dd, 1H, $J_1 = J_2 = 7.5$, Ar-$\underline{H}$), 7.24–7.32 (m, 2H, Ar-$\underline{H}$); ^{13}C NMR ($CDCl_3$): $\delta = 156.2, 151.1, 144.0, 129.4, 129.1, 120.2, 119.9, 116.2, 115.5, 113.0, 58.6, 53.2, 48.8, 35.6, 29.2, 26.0$; ESI-MS ($m/z$): 311 $(M + H)^+$; Anal. calcd for $C_{20}H_{26}N_2O$: C 77.38, H 8.44, N 9.02, found C 77.02, H 8.12, N 9.33.

*5.1.43. 2-[3-(Benzyloxy)phenoxy]-1-(4-phenylpiperazin-1-yl) ethanone (**33**)*

2-Bromo-1-(4-phenylpiperazin-1-yl)ethanone [32] (1.5 g, 6.28 mmol) was added to a solution of 3-benzyloxyphenol (1.9 g, 9.42 mmol) and K_2CO_3 (1.35 g, 9.42 mmol) in DMF (5 mL), and the resulting mixture was stirred at 40 °C overnight. After removing the solvent by distillation under vacuum, EtOAc was added and the organic solution was washed with 1 M NaOH and then with brine. After drying over Na_2SO_4, the organic layer was concentrated under reduced pressure and the resulting crude product was purified by column flash chromatography on silica gel (EtOAc/hexane, 1:1 as eluant). White solid (0.88 g, 35%). ^{1}H NMR ($CDCl_3$): $\delta = 3.10$ (m, 4H, piperazine), 3.71 (m, 4H, piperazine), 4.62 (s, 2H, $O\underline{CH_2}CO$), 4.98 (s, 2H, $\underline{CH_2}Ph$), 6.52 (m, 3H, Ar-$\underline{H}$), 6.85 (m, 3H, Ar-$\underline{H}$), 7.05–7.40 (m, 8H, Ar-$\underline{H}$). ESI-MS (m/z): 425 $(M + Na)^+$.

*5.1.44. 1-{2-[3-(Benzyloxy)phenoxy]ethyl}-4-phenylpiperazine·HCl (**34**)*

This compound was obtained by reduction of **33** (0.63 g, 1.56 mmol) with 1 M B_2H_6 in THF according to the above described procedure for compound **32a**. The crude product was dissolved in a saturated solution of HCl in MeOH and then crystallized by MeOH/Et_2O. White solid (0.58 g, 87%); ^{1}H NMR (DMSO-d_6): $\delta = 3.02$–3.37 (m, 4H, piperazine), 3.57 (m, 4H, piperazine), 3.83 (m, 2H, $\underline{CH_2}$-piperazine), 4.40 (m, 2H, $O\underline{CH_2}$), 5.10 (s, 2H, $\underline{CH_2}Ph$), 6.64 (m, 3H, Ar-$\underline{H}$), 6.85 (m, 1H, Ar-$\underline{H}$), 7.01 (m, 2H, Ar-$\underline{H}$), 7.20–7.50 (m, 8H, Ar-$\underline{H}$), 10.81 (brs, 1H). ESI-MS (m/z): 389 $(M + H)^+$.

*5.1.45. 3-[2-(4-Phenylpiperazin-1-yl)ethoxy]phenol·HCl (**35**)*

This compound was obtained starting from compound **34** and using the above described general *O*-debenzylation procedure with BCl_3. White solid (237 mg, 71%); ^{1}H NMR (DMSO-d_6): δ=3.15 (m, 4H, piperazine), 3.60 (m, 4H, piperazine), 3.81 (m, 2H, $\underline{CH_2}$-piperazine), 4.37 (m, 2H, $O\underline{CH_2}$), 6.42 (m, 3H, Ar-$\underline{H}$), 6.85 (m, 1H, Ar-$\underline{H}$), 6.98 (m, 2H, Ar-$\underline{H}$), 7.08 (m, 1H, Ar-$\underline{H}$), 7.25 (m, 2H, Ar-$\underline{H}$); ESI-MS (m/z): 299 $(M + H)^+$. Anal. calcd for $C_{18}H_{23}ClN_2O_2 \cdot 0.5$ MeOH: C 63.33, H 7.18, N 7.98, found C 63.51, H 7.40, N 7.83.

*5.1.46. 1-(8-Methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)-4-phenylpiperazine (**36**)*

N-Phenylpiperazine (1.4 g, 8.7 mmol) and *p*-toluenesulfonic acid (0.053 g, 0.29 mmol) were added to a solution of 8-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (0.5 g, 2.85 mmol) in dry toluene (25 mL). This solution was heated to reflux in a Dean Stark

apparatus for 24 h, removing the water of reaction. The solvent was removed by distillation under vacuum, the resulting crude residue was dissolved in EtOH/THF (20 mL + 20 mL) and then hydrogenated (4 atm) at room temperature in the presence of PtO_2 (0.05 g) for 24 h. The reaction mixture was filtered on celite, the filtrate was evaporated under reduced pressure to yield a crude residue that was purified by flash-chromatography on silica gel (cyclohexane/EtOAc 8:2 as eluant). White solid (688 mg, 75%); mp: 119–120 °C (Et_2O/petroleum ether); ^1H NMR ($CDCl_3$): δ = 1.65 (dddd, 1H, J = 5.0, 6.0, 11.0, 16.5, H_{3a}), 2.17 (m, 1H, H_2), 2.53 (dd, 1H, J = 11.0, 16.5, H_{4a}), 2.73 (m, 1H, H_{3b}), 2.90 (m, 6H, NCH_2CH_2N, H_{1a} and H_{1b}), 3.10 (ddd, 1H, J = 1.5, 5.0, 16.5, H_{4b}), 3.28 (m, 4H, NCH_2CH_2N), 3.84 (s, 3H, CH_3), 6.69 (d, 2H, J = 8.0, Ar-H), 6.74 (d, 1H, J = 8.0, Ar-H), 6.88 (m, 1H, Ar-H), 6.97 (m, 2H, Ar-H), 7.11 (dd, 1H, $J_1 = J_2$ = 8.0, Ar-H), 7.25–7.33 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$): δ = 157.6, 151.4, 137.7, 129.1, 126.1, 124.6, 120.7, 119.6, 116.1, 106.7, 60.4, 55.2, 49.6, 49.2, 29.7, 25.9, 25.8; MS (EI, 70 eV): m/z 322 (M^+, 100).

5.1.47. 7-(4-Phenylpiperazin-1-yl)-5,6,7,8-tetrahydronaphthalen-1-ol (**37**)

The product was prepared according to the above described general *O*-demethylation procedure with BBr_3 starting from **36**. Purification by flash-chromatography on silica gel (cyclohexane/EtOAc 1:1 as eluant) and crystallization. White solid (194 mg, 63%); mp: 211 °C dec. (EtOAc); ^1H NMR (DMSO-d_6): δ = 1.50 (dddd, 1H, J = 4.5, 6.0, 11.0, 16.5, H_{6a}), 2.02 (m, 1H, H_7), 2.39 (dd, 1H, J = 11.0, 16.0, H_{5a}), 2.60–2.85 (m, 7H, NCH_2CH_2N + H_{8a} + H_{8b} + H_{6b}), 2.86 (dd, 1H, J = 4.5, 16.0, H_{5b}), 3.15 (m, 4H, NCH_2CH_2N), 6.52 (d, 1H, J = 7.5, Ar-H), 6.58 (d, 1H, J = 8.0), 6.77 (dd, 1H, $J_1 = J_2$ = 7.0, Ar-H), 6.87 (dd, 1H, J = 7.5, 8.0, Ar-H), 6.93 (m, 2H, Ar-H), 7.21 (m, 2H, Ar-H), 9.17 (brs, 1H, NH); ^{13}C NMR (CD_3OD): δ = 154.9, 151.3, 137.2, 128.6, 125.8, 121.9, 119.7, 119.1, 116.0, 111.1, 60.8, 49.1, 47.8, 29.0, 25.6, 25.4; MS (EI, 70 eV): m/z 308 (M^+), 120 (100); Anal. calcd for $C_{20}H_{24}N_2O$: C 77.89, H 7.84; N 9.08 found C 77.95, H 7.99, N 8.97.

5.2. Pharmacology

5.2.1. HEK293 cell culture and membrane preparation

HEK293 cells, stably expressing the human serotonin 5-HT_7 receptor gene, were grown in Falcon flasks in DMEM (Cambrex, Verviers, Belgium) supplemented with 10% FBS (Cambrex), and 2 μg/mL puromincine (Sigma–Aldrich, St Louis, MO) at 37 °C in a 5% CO_2 atmosphere. For radioligand binding experiments, the cells, at 80% of confluence, were collected in PBS/EDTA (Lonza, Basel, Switzerland, cat.# BE02-017F) and centrifuged at 1000 × g for 10 min. The pellet obtained was resuspended in the same buffer used in binding experiments and homogenized with Ultra Turrax. The homogenate was centrifuged at 48,000 × g for 90 min. The final pellet was stored at −80 °C until the day of experiment. The protein concentration of membrane suspension was determined using the Bradford method (Pierce, Rockford, IL, USA) with bovine albumin as standard.

5.2.2. Competition binding assay

Competition binding experiments were performed incubating membranes (5–10 μg of protein/sample) with a single concentration of [^{3}H]5-HT (5 nmol/L) (Biotrend, Cologne, Germany), in the presence of test compounds, in 96-well filter plates (MultiScreen system, cat # MAFBN0B10, Millipore, Billerica, MA, USA) for 30 min at 25 °C in a total volume of 200 μL/well of Tris–HCl 50 mM, pH 7.4, containing 10 μmol/L pargyline, 4 mmol/L $CaCl_2$ and 0.05% ascorbic acid. Non-specific binding was determined in the presence of 100 μM cold 5-HT (Sigma–Aldrich). At the end of incubation, bound and free radioligand were separated by filtering the 96-well filter plates using a Millipore filtration apparatus (Multiscreen$_{HTS}$ vacuum manifold). Filter plates were then washed several times with ice-cold buffer (50 mM Tris–HCl, pH 7.4) and filter-bound radioactivity measured using a MicroBeta counter (PerkinElmer) after addition of 30 μL/well of OptiPhase SuperMix scintillation cocktail (PerkinElmer). Compounds were first tested at fixed (10–1 and/or 0.1 μmol/L) concentrations and those active at these concentrations (50% inhibition vs control) were repeated at scalar concentrations (ranging from 10^{-5} to 10^{-10} mol/L).

5.2.3. Data evaluation

Data were analyzed using nonlinear regression with GraphPad PRISM commercial software. Test compound concentrations causing a half maximal inhibition of control values (IC_{50} calculated by GraphPad Prism software) were calculated. Inhibitory binding constant (K_i) values were calculated from IC_{50} values, according to the Cheng and Prusoff equation [33], $K_i = IC_{50}/(1 + [C]/K_d)$, where $[C]$ is the concentration of the radioligand and K_d its dissociation constant.

5.2.4. Human 5-HT_7 functional assays

Agonist and antagonist behavior were evaluated by Cerep [26] in cAMP functional assays performed in CHO cells, according to the method of Adham et al. [34]. Agonist behavior was determined by evaluating the concentration-dependent elevation of intracellular cAMP levels produced by test compounds. Serotonin showed EC_{50} = 29 nM. Antagonist behavior of test compounds was determined evaluating the effect on cAMP accumulation stimulated by 300 nM 5-HT.

IC_{50} values (concentration causing a half-maximal inhibition of the control specific agonist response) were determined by nonlinear regression analysis of the concentration–response curves generated with mean replicate values using Hill equation curve fitting ($Y = D + [(A - D)/(1 + (C/IC_{50})nH)]$, where Y = specific response, D = minimum specific response, A = maximum specific response, C = compound concentration, and nH = slope factor). This analysis was performed using a software developed at Cerep (Hill software). The apparent dissociation constants (K_B) were calculated using the modified Cheng and Prusoff equation ($K_B = IC_{50}/(1 + (A/EC_{50}A))$, where A = concentration of reference agonist in the assay, and $EC_{50}A$ = EC_{50} value of the reference agonist). The reference compound mesulergine had K_B = 16 nM.

5.2.5. Binding assays on other serotonin receptors

Affinity of compounds **5n**, **5i**, **14o** and **16i** for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_{4e}, 5-HT_{5a} and 5-HT_6 receptors was evaluated by Cerep [26]. % Displacement of reference ligands at the concentration of 1 μM of **5n**, **5i**, **14o** and **16i** are reported in Supplementary Material Table S1.

5.3. Molecular modeling

The X-ray structure of the 5-HT_{1B} serotonin receptor in complex with the agonist dihydroergotamine (PDB code: 4IAQ) [35] was used as the template for 5-HT_7 receptor modeling. The thermostabilized apocytochrome b_{562} RIL segment and all non-protein atoms were removed and hydrogen atoms were added using the Protein Preparation Wizard [36] implemented in Maestro 9.2 [37]. The amino acid sequence of the human 5-HT_7 receptor was retrieved from the Universal Protein Resource (UniProt ID: P34969) [38,39]. Initial alignment was carried out with Prime 3.0 [40] and subsequently optimized considering conserved sequence motifs of class A GPCRs [21,41] (Fig. S2). Since the intracellular loop (ICL) 3 of the 5-HT_{1B} receptor was replaced with the thermostabilized apocytochrome b_{562} RIL, the ICL3 sequence of the 5-HT_7 receptor was excluded from homology modeling and Ala270, at the N-

terminus of ICL3, was directly linked to Ser317 belonging to the C-terminus of ICL3. This should not influence docking results, since the putative binding pocket of the 5-HT$_7$ receptor is located in the extracellular portion of the helix bundle. The all-hydrogen 5-HT$_7$ model structure obtained with Prime 3.0 was subjected to a restrained minimization using Impact 5.7 [42] and the OPLS2005 force field [43] to an RMSD value of 0.3 Å calculated on the protein heavy atoms. The stereochemical quality of the minimized structure was evaluated using Procheck [44]. The Ramachandran plot of the final refined structure showed only Val230 in extracellular loop (ECL) 3 and Ala270 at the N terminus of IL3 in disallowed regions (Fig. S3).

Compound **5i** was built with Maestro 9.2 and subjected to an energy minimization procedure using the OPLS2005 force field to a convergence threshold of 0.05 kJ mol^{-1} Å^{-1}. Induced-Fit Docking (IFD) [45,46] was then applied to account for receptor flexibility during docking calculations. During the initial softened-potential docking, a Van der Waals (VdW) radii scaling of 0.7 and 0.5 were applied on protein and ligand non-polar atoms, respectively. Moreover, Asp142$^{2.65}$, Arg367$^{7.36}$ and Leu370$^{7.39}$ were temporarily mutated to alanines, to favor initial ligand placement. Asp162$^{3.32}$ was used to define the location of the binding site and the dimensions of bounding and enclosing boxes were set to 10 Å and 30 Å, respectively. Initial docking runs were performed in standard precision (SP) mode, applying a hydrogen-bond constraint between the protonated piperazine nitrogen of **5i** and Asp162$^{3.32}$. A core constraint was also applied on compound **5i**, to induce the heavy atoms of the indole ring to superpose to the corresponding atoms of dihydroergotamine (co-crystallized with the 5-HT$_{1B}$ receptor), with a tolerance of 1 Å. Twenty ligand poses were retained for subsequent refinement. Each ligand–receptor complex obtained at the end of the softened-potential docking run was then submitted to side chain and backbone refinements with Prime 3.0. During this phase, the previously removed side chains were re-introduced and all residues located within 5.0 Å from the ligand molecule were refined by a side chain conformational search, followed by energy minimization of the residues and the ligand molecule. To prevent Glide from searching for alternative accommodations of the ligand during the final docking stage of the IFD workflow, each ligand molecule obtained at the end of the Prime stage was not re-docked into its induced-fit receptor structure, but it was only optimized in the field of the receptor and subsequently scored using default Glide settings (VdW radii scaling of 1.0 and 0.8 for receptor and ligand non-polar atoms, respectively). The resulting ligand–protein complexes were ranked according to their IFD score and the 5-HT$_7$ receptor–**5i** complex showing the best IFD score was selected. To prevent ECL3 from interpenetrating into the lipid bilayer, a loop optimization procedure was conducted on the segment Pro351$^{6.59}$–Pro362$^{7.31}$ using Prime 3.0. To this end, the selected 5-HT$_7$ receptor–**5i** complex was aligned to the 4IAQ three-dimensional structure deposited in the Orientations of Protein in Membranes (OPM) database [47], which includes the coordinates of the putative membrane boundary planes. The orientation and position of the membrane boundaries were used to generate a low-dielectric slab-shaped region in Prime 3.0, which simulates the lipid bilayer environment during loop refinement. The resulting 5-HT$_7$ receptor–**5i** complex bearing the optimized ECL3 portion was energy-minimized to a convergence threshold of 0.5 kJ mol^{-1} Å^{-1}, applying the OPLS2005 force field and the GB/SA water solvation treatment. The resulting complex is represented in Fig. 2a.

The energy-minimized 5-HT$_7$ receptor–**5i** complex was embedded in a pre-equilibrated POPC lipid bilayer using Desmond version 2.2 [48,49] and subsequently solvated with TIP3P water molecules. Chlorine atoms were added to neutralize the net charge of the system. The simulation box measured 64 × 76 × 93 Å^3 and contained 87 lipid molecules, 12 chlorine atoms and 8284 water molecules. The system was initially relaxed using a customized version of a membrane relaxation protocol implemented in the Desmond package and subsequently simulated for 100 ns in the NPT ensemble. Temperature and pressure were set to 310 K and 1 atm, respectively, applying the Langevin coupling scheme [50], and the M-SHAKE algorithm [51] was used to constrain all bond lengths to hydrogen atoms. VdW and short-range electrostatic interactions were cut off at 9 Å and long-range electrostatic interactions were computed using the smooth Particle Mesh Ewald method [52]. A multistep RESPA scheme [53] was used to integrate the equations of motion, applying a timestep of 2 fs for the bonded and the short-range nonbonded interactions and of 6 fs for the long-range nonbonded interactions. The OPLS2005 force field was used for both the relaxation and the production phase of the MD simulation.

The last snapshot of the MD simulation was submitted to energy minimization with Desmond 2.2, applying the OPLS2005 force field to a convergence threshold of 0.5 kcal mol^{-1} Å^{-1}. The energy-minimized 5-HT$_7$ receptor–**5i** complex was then extracted from the solvated lipid bilayer and used for docking studies of compounds **14m**, **27**, **18a** and **18c**. Docking grids were generated with Glide 5.7 [54] and were centered on the **5i** molecule, setting bounding and enclosing boxes to 10 Å and 30 Å on each side, respectively. To retain the key ionic interaction with Asp162$^{3.32}$, a hydrogen-bond constraint was applied between the protonated piperazine nitrogen of ligands and Asp162$^{3.32}$ during docking runs. Twenty docking poses were collected for each ligand and the top-ranked pose according to the Emodel value was merged into the 5-HT$_7$ model structure. The resulting complexes were subjected to an energy minimization process using the OPLS2005 force field and the GB/SA water solvation treatment to a convergence threshold of 0.5 kJ mol^{-1} Å^{-1}. The energy-minimized complexes are depicted in Fig. 2c, d and 4.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.04.034.

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