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New *N*- and *O*-arylpiperazinylalkyl pyrimidines and 2-methylquinazolines derivatives as 5-HT_7 and 5-HT_{1A} receptor ligands: Synthesis, structure-activity relationships, and molecular modeling studies

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ABSTRACT

Based on our earlier studies of structure activity relationships on 4-substituted piperazine derivatives, in this work we synthesized a novel set of long-chain arylpiperazines with the purpose of elucidating if some structural modifications in the terminal fragment could affect the binding affinity for the 5-HT_7 and 5-HT_{1A} receptors. In this new series, the quinazolinone system of the previous derivatives was replaced by a 6-phenylpyrimidine or a 2-methylquinazoline, which were used as versatile building blocks for the preparation of new compounds. A 4-arylpiperazine moiety through a five methylene chain was anchored at the nitrogen or oxygen atom of the heterocyclic scaffolds. The substituents borne by the piperazine nucleus were phenyl, phenylmethyl, 3- or 4-chlorophenyl, and 2-ethoxyphenyl. Binding tests, performed on human cloned 5-HT_7 and 5-HT_{1A} receptors, showed that, among the newly synthesized derivatives, 4-[5-[4-(2-ethoxyphenyl)-1-piperazinyl]pentoxy]-6-phenyl-pyrimidine (**13**) and 3-[5-[4-(2-ethoxyphenyl)-1-piperazinyl]pentyl]-2-methyl-4(3*H*)-quinazolinone (**20**) displayed the best affinity values, K_i = 23.5 and 8.42 nM for 5-HT_7 and 6.96 and 2.99 nM for 5-HT_{1A} receptors, respectively. Moreover, the functional properties for both compounds were further evaluated using the cAMP assay. Finally, a molecular modeling study has been performed for 5-HT_7 and 5-HT_{1A} receptor homology models to investigate the binding mode of *N*- and *O*-alkylated pyrimidinones/pyrimidines **4**–**13**, 2-methylquinazolinones/quinazolines **17**–**22**, and previously reported 2- and 3-substituted quinazolinones **23**–**30**.

1. Introduction

The heterocyclic quinazoline and quinazolinone are frequently encountered as scaffolds in medicinal chemistry. They possess wide pharmacological applications such as antibacterial, antidiabetic, anti-inflammatory, and many others.[1] Besides, the quinazoline system has been found in natural products, such as alkaloids,[2,3] which showed medicinal applications.[4–6] Accordingly, a lot of comprehensive studies regarding the quinazolines/quinazolinones functionalization to synthesize new effective drugs have been done.[7–9] Furthermore, quinazoline derivatives were reported as CNS depressants[10] and anticonvulsants,[11] and this system is also present in an extensively studied class of serotoninergic ligands, called long-chain arylpiperazines (LCAPs).[12–16] The serotonin neurotransmitter interacts with a large number of receptors which, following the IUPHAR classification[17] are classified into seven families (5-HT_{1-7}). Except 5-HT_3 receptor, they all belong to a G protein-coupled receptor (GPCR) family, the largest and most versatile group of cell membrane signaling proteins. The 5-HT_{1A} receptor (5-HT_{1A}R) was the first of the 5-HTRs to be cloned. It is coupled to adenylyl cyclase via $G_{i/0}$ and is widely distributed in CNS and in peripheral tissues. Probably, it was one of the most studied of the serotonin receptor family and is still a current one. It is generally believed to be involved in anxiety, depression, epilepsy, and disorders of mood, learning, and memory.[18] The 5-HT_7 receptor (5-HT_7R) discovered in 1993 is positively coupled to adenylyl cyclase via a G_s protein.[19] This receptor is located in the brain and peripheral tissues. In particular, it is largely distributed

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in hippocampus and thalamus suggesting its involvement in CNS disorders as well.[12,19,20] In light of the fact that both receptors are co-expressed in the same brain structures and modulate the same second messenger but exerting opposite roles, it is little wonder that exist a cross-talk between them. From the functional point of view, agents that act as partial agonist at 5-HT_{1A}R and antagonist at 5-HT_7R might hold antidepressant-like properties.[21] So, the study of interaction between these receptors is going to be of increasing importance as clinical interest for the treatment of depression and anxiety as well as in emotional learning and memory.[22,23]

Recently, we have reported new LCAPs bearing a quinazolinone system as a terminal fragment, which displayed high-to-low affinity for 5-HT_7R (K_i = 6.88–1082 nM, compounds **23**–**30**, Table 2), and high-to-moderate affinity for 5-HT_{1A}R (K_i = 1.04–268 nM, compounds **23**–**30**, Table 2).[24,25] Here we present the synthesis of a novel series of LCAPs. This work deals with the modification on the quinazolinone scaffold as terminal fragment, in order to elucidate how these changes influence both the affinity for 5-HT_7R and 5-HT_{1A}R, and the binding modes in receptor homology models. In particular, the quinazolinone system was replaced by 6-phenyl-4(3*H*)-pyrimidinone as a result of splitting bicyclic quinazolinone system (benzo cracking).[26] To the best of our knowledge, this is the first time that a 6-phenylpyrimidine is used as building block scaffold for the preparation of 5-HT_7 and 5-HT_{1A} receptor ligands. In confirmation of this, no hit was found within sets of 4166 5-HT_{1A}R and 720 5-HT_7R ligands with K_i < 100 nM stored in ChEMBL v. 11 database[27] for 4-oxy-pyrimidine/pyrimidinone substractural query (using Instant JChem).[28] Furthermore, the pyrimidines, simple or fused with other heterocycles, represent versatile synthetic intermediates and constitute an important class of compounds for new drug development.[29–31]

Further changes to the quinazolinone system regarding, the methylation in the 2-position which gave the 2-methyl-4(3*H*)-quinazolinone and the shift of the anchoring point of the arylpiperazinylalkyl moiety to the nitrogen or oxygen atom. Following the results from the previous investigation only derivatives with five methylene unit spacer were prepared and the same arylpiperazine fragments (i.e. phenyl, phenylmethyl, 3-chloro-, 4-chloro-, and 2-ethoxyphenyl) were used (Fig. 1).

Herein we present the synthesis and the binding properties of new *N*- and *O*-arylpiperazinylalkyl pyrimidine and 2-methylquinazoline derivatives towards 5-HT_7 and 5-HT_{1A} receptors, followed with in vitro evaluation of antagonistic properties of selected compounds **13** and **20**. Furthermore, a molecular modeling study has been performed for 5-HT_7 and 5-HT_{1A} receptor homology models to investigate the binding mode of *N*- and *O*-alkylated pyrimidinones/pyrimidines **4**–**13**, 2-methylquinazolinones/quinazolines **17**–**22**, and previously reported 2- and 3-substituted quinazolinones **23**–**30**.

2. Results and discussion

2.1. Chemistry

The synthetic procedure adopted for the preparation of the new pyrimidine **4**–**13** and quinazoline **17**–**22** derivatives is outlined in Scheme 1. The 6-phenyl-4(3*H*)-pyrimidinone (**1**) and the 2-methyl-4(3*H*)-quinazolinone (**14**) reacted with an excess of 1,5-dibromopentane or 1-chloro-5-bromopentane in the presence of potassium carbonate and a catalytic amount of potassium iodide to obtain derivatives **2**, **3**, **15**, and **16**. Halo derivatives **2** and **3** were prepared by using traditional heating method, **15** and **16** by using microwave irradiation. From the reaction mixture, derivatives **2** and **15** (alkylated at the nitrogen atom) were isolated in fair yields (53% and 47%, respectively), and in addition, it was also possible to isolate with a low yield derivatives **3** and **16** (10% and 23%, respectively), alkylated at the oxygen atom as confirmed by ^{1}H NMR and IR spectral data (Table 1). ^{1}H NMR spectra of compounds **2** and **15** show a signal of two protons of a methylene unit at δ 3.92 and 4.03, respectively, attributable to a *N*-alkylation ($CONCH_2$). The shift of these signals for compounds **3** and **16** at δ 4.53 and 4.39, respectively, is due to an *O*-alkylation (OCH_2). IR spectra further confirmed such findings; compounds **2** and **15** display peaks at 1671 and 1673 cm^{-1}, respectively, characteristic for the C=O stretching, which were absent in compounds **3** and **16**.

It has been well-established that regioselectivity varies from one scaffold to another and within the same scaffold. Different synthetic outcomes can be obtained depending on solvent, nature of electrophiles, and other conditions such as temperature, base used for deprotonation, and nature of the substituent at the 2-position of the heterocyclic compound.[32–35] For these reasons, it is very difficult establishing predictable and robust protocols for *N*- versus *O*-alkylation reactions.[36,37] The different percentage in the alkylated product mixtures depends on the competing pathways when these lead to different products. Prevalence of thermodynamic or kinetic control determines the final composition of the product.[38] Due to the poor regioselectivity of the ambident nucleophiles in the alkylation reaction, 4-(5-chloropenthoxy)-6-phenyl-pyrimidine (**3**) and 4-(5-bromopenthoxy)-2-methylquinazoline (**16**) were more difficult to be obtained. Nevertheless, they were isolated from the reaction mixture and used in preparation of the final compounds.

Compounds **4**–**13** and **17**–**22** were prepared from halo derivatives **2**, **3**, **15**, and **16** by reaction with an excess of the substituted piperazine, in the presence of potassium carbonate and a catalytic amount of potassium iodide using traditional heating or by microwave irradiation (Scheme 1).

2.2. Structure–affinity relationship studies

New derivatives **4**–**13** and **17**–**22** were tested on human cloned 5-HT_7 and 5-HT_{1A} serotonin receptors expressed in CHO-K1 cells following a previously reported procedure.[39] Binding assays on 5-HT_7 and 5-HT_{1A} receptors were carried out by using [^{3}H]-5-HT and [^{3}H]-8-OH-DPAT as radioligands, respectively. Results, expressed as K_i (nM), are summarized in Table 2.

The structure-affinity relationship (SAR) analysis in regard to the aromatic part of the arylpiperazine moiety showed that like in our previous study, 3-ClC_6H_4 (**6**, **11**, and **18**) and 2-$EtOC_6H_4$ (**8**, **13**, and **20**) were the most active derivatives at both receptors, whereas analogous with 4-ClC_6H_4 (**7**, **12**, and **19**) and $CH_2C_6H_5$ (**5** and **10**) displayed the lowest affinity. Unsubstituted arylpiperazines (**4**, **9**, **17**, and **21**) showed moderate affinity for 5-HT_7 (K_i = 53.7–613 nM) and higher for 5-HT_{1A} (K_i = 18.6–111 nM).

The influence of the terminal fragment on 5-HT_7 and 5-HT_{1A} receptor affinity can be directly traced in *N*-alkylated derivatives, comparing appropriately substituted arylpiperazines from quinazolinone series **26**–**30** with 2-methylquinazolinones **17**–**20** and 6-phenylpyrimidinones **4**–**8** (Table 2). The introduction of the methyl substituent at the 2-position of the quinazolinone system did not significantly change affinities for both receptors, as can be seen from a comparison of the affinities of 2-$EtOC_6H_4$ derivatives **20** and **30** which are similar and the highest for both series. An exception was observed for the phenyl derivative **17**, which showed a 6-fold increase of affinity for the 5-HT_7R compared to **26** (K_i = 53.7 *vs* 307 nM); and the 4-chlorophenyl derivatives **19** *vs* **29**, that conversely, demonstrated a 25-fold decrease of affinity for this receptor (K_i = 327 and 12.9 nM, respectively). On the other hand, the benzo cracking strategy (compounds **4**–**8**) caused a decrease in affinity for both receptors.

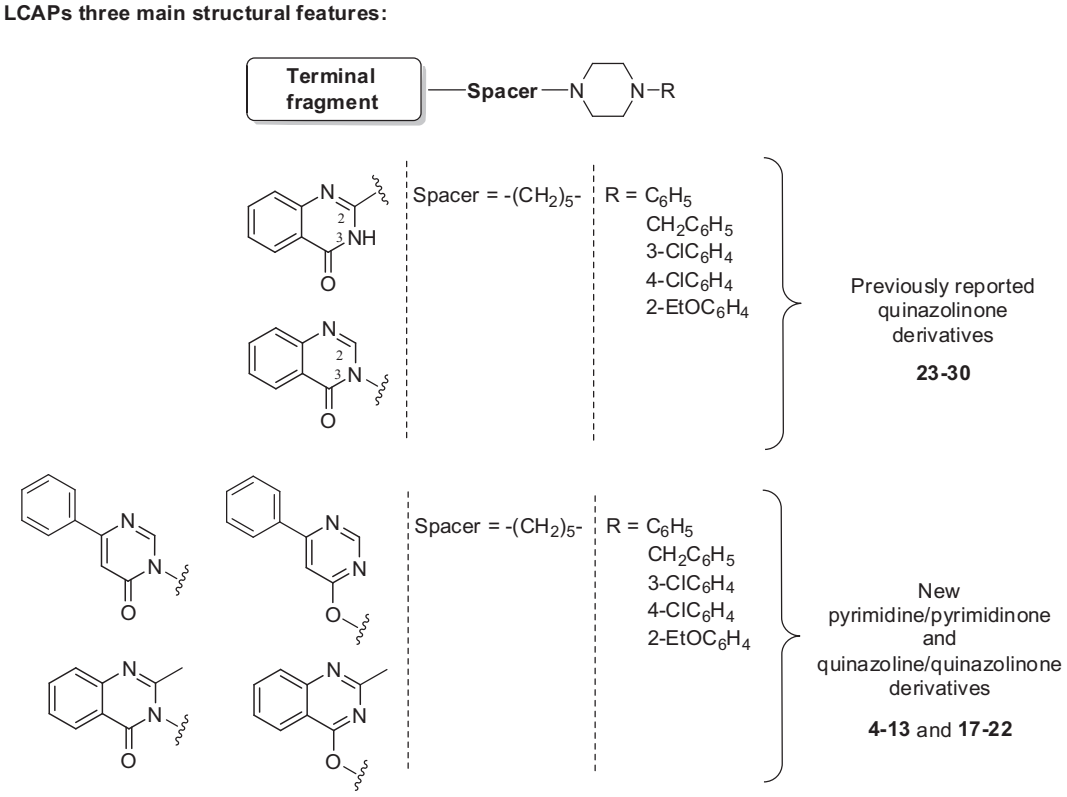


Fig. 1. Structural features of previously and newly synthesized LCAPs.

Shifting the anchoring point of the arylpiperazinylalkyl moiety from the nitrogen (**4**–**8** and **17**–**20**) to the oxygen atom (**9**–**13** and **21**, **22**) determined a general slight increase of affinity in the pyrimidine derivatives (**4**–**8** *vs* **9**–**13**), whereas no homogenous results was obtained for 2-methylquinazolinone derivatives (**17**, **19** *vs* **21**, **22**).

2.3. In vitro functional evaluation

Compounds **13** and **20** which possess the best 5-HT_7/5-HT_{1A} binding properties, were selected in order to investigate the functional activity. The cAMP functional assays were performed in HEK293 cell lines with stable expression of human 5-HT_7R and 5-HT_{1A}R. The results were reported as% inhibition of control agonist response at 10^{-6} M and using as reference the antagonist SB-269970 (97% at 10^{-6} M). The results of the functional assays are shown in Table 3. Compounds **13** and **20** produced a weak inhibition of cAMP on 5-HT_7R (5.5% and 19.5%, respectively), suggesting a functional behavior as weak antagonists. On the other hand compound **30** showed stronger antagonistic properties producing at the same concentration a reduction of cAMP accumulation to 42% (Table 3).[24]

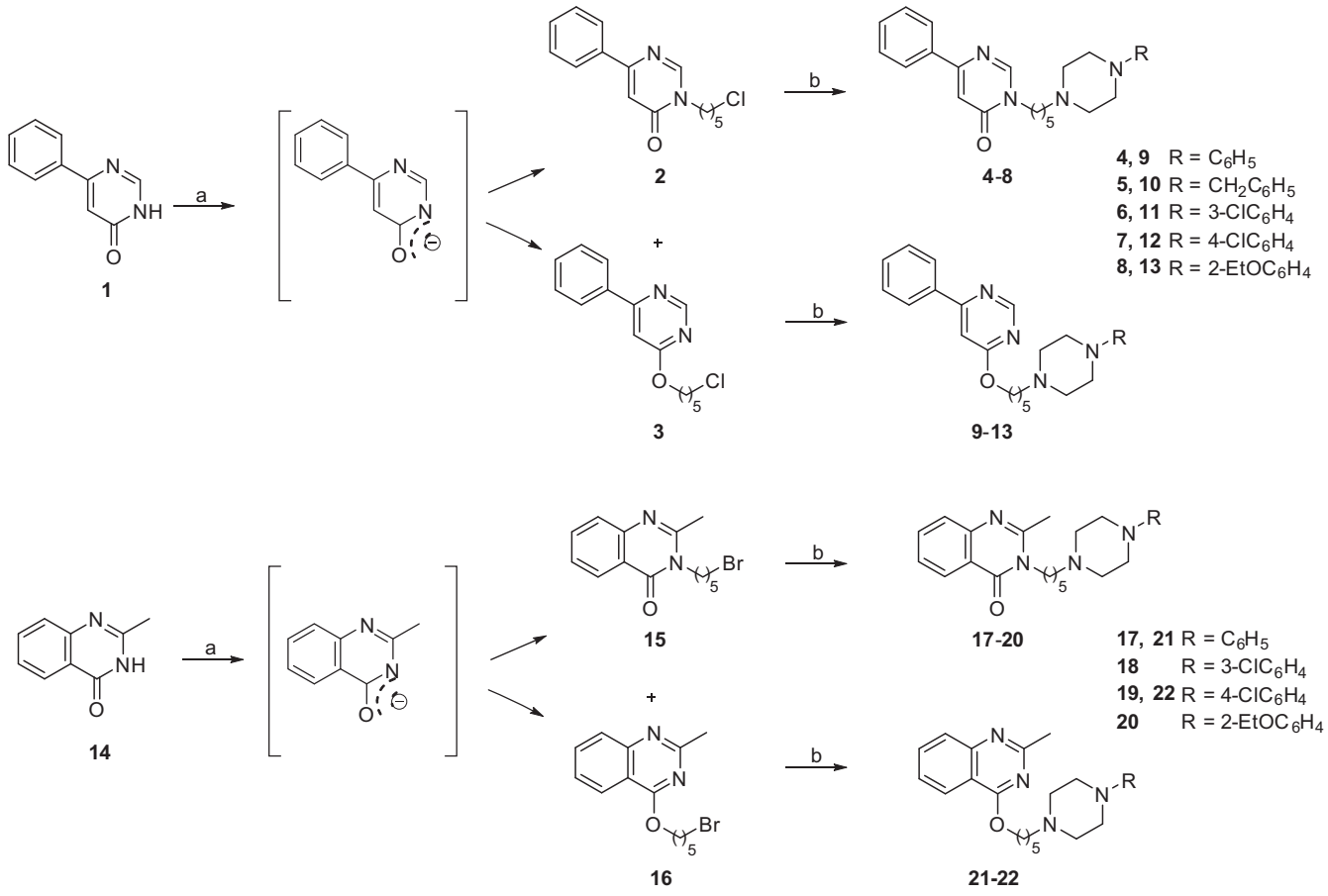
Interestingly, the *O*-arylpiperazinylalkyl 6-phenyl-pyrimidine derivative (**13**) showed a significant decrease of inhibition of cAMP levels on 5-HT_7R compared to *N*-arylpiperazinylalkyl 4(3*H*)-quinazolinone derivative (**30**). Additionally, the antagonistic properties of compounds **13** and **20** were also evaluated against the 5-HT_{1A}R (6% and 18%, respectively).

2.4. Molecular modeling studies

Molecular docking of *N*- and *O*-alkylated pyrimidinones/pyrimidines **4**–**13**, 2-methyl quinazolinones/quinazolines **17**–**22**, and previously reported 2- and 3-substituted quinazolinones **23**–**30**[24] (Table 2) was performed with the use of recently developed 5-HT_7 and 5-HT_{1A}Rs homology models based on dopaminergic D3 receptor template (PDB ID: 3PBL) and trained on long-chain arylpiperazine derivatives.[39] For each compound, five top-scored complexes were considered, of which the best with a binding mode coherent to the previously described[39] was analyzed.

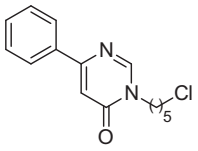
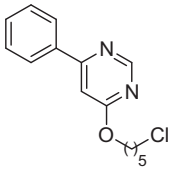
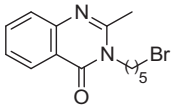
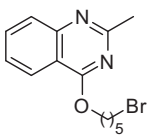
Binding modes and interacting residues of the analyzed compounds were similar, despite the adopted ligand conformations for 5-HT_7R and 5-HT_{1A}R were slightly different. Generally, the compounds preferred an L-shape conformation when bound to 5-HT_7R and an extended conformation if complexed with 5-HT_{1A}R. According to our previous study,[39] this result is attributed to the different size of the cavity within transmembrane helices (TMHs) 2, 3, 7, and the first extracellular loop 1 (EL1), which was smaller in 5-HT_7R than in 5-HT_{1A}R. Comparison of the docking poses of ligand **20**, which had the highest affinity for the 5-HT_7R and 5-HT_{1A}R in this new series, shows different orientations for the terminal 2-methyl-4(3*H*)-quinazolinone fragment (Fig. 2A). In the case of 5-HT_7R this moiety points towards the extracellular loops and interacts with Thr2.64, Arg7.36, and Cys146 of the EL2 (Fig. 2A, left), whereas in the 5-HT_{1A}R it interacts with Tyr2.64, Phe3.28, Asn7.39, and Trp7.40 (Fig. 2A, right). The 2-ethoxyphenylpiperazine moiety of **20** is hosted in a small cavity between TMHs 5 and 6, in which the aromatic ring formed a CH-π or π-π interaction with the Phe6.51 in both receptors (Fig. 2B). The orientation of the 2-ethoxygroup is, however, different; in the 5-HT_7R the *ortho* substituent seems to interact with Cys3.36 (Fig. 2B, left), whereas in the 5-HT_{1A}R with Ser5.42 and Thr5.43 (Fig. 2B, right).

From the SAR studies it was found that the introduction of a chloro atom at the *meta* position of the phenylpiperazine ring increases the affinity for both receptors with respect to the *para*-chloro analogous. The increased affinity could be justified by the binding pose of the 3-chlorophenylpiperazine derivative (see **6** *vs* **7**), which shows a favourable orientation towards the Cys3.36, by



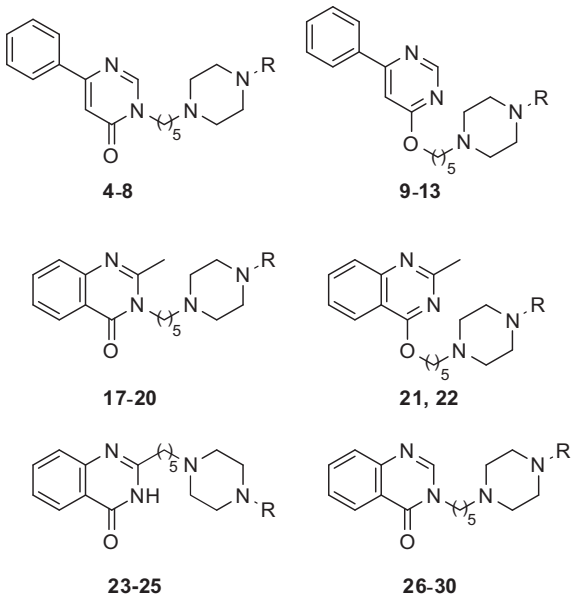
Scheme 1. Reagents and conditions: (a) $X(CH_2)_5Br$, K_2CO_3, KI catalytic amount, CH_3CN or $CO(CH_3)_2$, microwave or reflux; (b) substituted piperazines, K_2CO_3, KI catalytic amount, CH_3CN, microwave or reflux.

Table 1
Spectral data of halo derivatives **2**, **3**, **15**, and **16**.

	Compound	^{1}H NMR, δ (ppm)		IR, (cm^{-1}) C=O
		$CONCH_2$	OCH_2	
2		3.92	–	1673
3		–	4.53	–
15		4.03	–	1671
16		–	4.39	–

establishing an additional halogen bond interaction (Fig. 2C).[40] As a general trend, a similar behavior was found for the chlorophenylpiperazine derivatives (**11** *vs* **12** and **18** *vs* **19**) along the series (Table 2).

Table 2
Binding properties of derivatives **4**–**13**, **17**–**22**, **23**–**30**, reference compounds SB-269970 and 8-OH-DPAT.



Comp.	R	K_i[a] (nM)	
		5-HT_7	5-HT_{1A}
4	C_6H_5	613 ± 68	111 ± 21
5	$CH_2C_6H_5$	1328 ± 192	772 ± 156
6	3-ClC_6H_4	94.7 ± 10	17.8 ± 2.8
7	4-ClC_6H_4	1310 ± 179	167 ± 20
8	2-$EtOC_6H_4$	45.1 ± 4.6	25.9 ± 3.7
9	C_6H_5	143 ± 17	33.5 ± 4.1
10	$CH_2C_6H_5$	990 ± 177	1032 ± 114
11	3-ClC_6H_4	44.8 ± 7.8	17.4 ± 2.0
12	4-ClC_6H_4	1377 ± 252	90.3 ± 6.5
13	2-$EtOC_6H_4$	23.5 ± 2.9	6.96 ± 0.76
17	C_6H_5	53.7 ± 12	18.6 ± 2.1
18	3-ClC_6H_4	35.1 ± 6.9	8.21 ± 1.1
19	4-ClC_6H_4	327 ± 47	100 ± 14
20	2-$EtOC_6H_4$	8.42 ± 0.78	2.99 ± 0.26
21	C_6H_5	276 ± 27	24.7 ± 2.0
22	4-ClC_6H_4	181 ± 20	52.1 ± 4.4
23[b]	C_6H_5	228 ± 12	43.5 ± 5.4
24[b]	3-ClC_6H_4	11.9 ± 3.2	7.33 ± 0.77
25[b]	4-ClC_6H_4	101 ± 26	116 ± 20
26[b]	C_6H_5	307 ± 57	28.9 ± 4.6
27[b]	$CH_2C_6H_5$	1082 ± 100	268 ± 25
28[b]	3-ClC_6H_4	35.8 ± 7.0	6.28 ± 0.86
29[b]	4-ClC_6H_4	12.9 ± 0.85	51.5 ± 11
30[b]	2-$EtOC_6H_4$	6.88 ± 0.66	1.04 ± 0.13
SB-269970		0.71 ± 0.06	9024 ± 181
8-OH-DPAT		388 ± 58	2.65 ± 0.10

[a] Each value is the mean ± SD of the data from three separate experiments.
[b] Data from Ref. 24.

Relative positions of terminal fragments can be compared in Fig. 2D which shows the binding modes of unsubstituted phenylpiperazines (**4**, **9**, **17**, **21**, **23**, and **26**; Table 2). Generally, the subset of analogs is docked in similar conformations (L-shape for the 5-HT_7R and extended for 5-HT_{1A}, Fig. 2D) making explanation of 12- and 6-fold differences between the most and the least actives, for 5-HT_7R and 5-HT_{1A}R, respectively. Interestingly, in case of 5-HT_7R, terminal fragments of phenylpyrimidines (**4**, **9**), 2-methylquinazolines (**17**, **21**) and quinazolines (**23**, **26**), despite different linker anchoring points, occupied similar positions. As regards 5-HT_{1A}R, due to limited space between TMH2 and 7, the more distant aromatic rings of terminal fragments are well aligned at the expense of differences in linker conformations.

Table 3
Antagonist activity of selected compounds for 5-HT_7R and 5-HT_{1A}R.

Comp.	% inh[a] 5-HT_7	% inh[b] 5-HT_{1A}
13	5.5	6
20	19.5	18
30[c]	42	NT[d]
SB-269970	97	–

[a] % inhibition of control agonist response (5-CT 10 nM) at 10^{-6} M.
[b] % inhibition of control agonist response (8-OH-DPAT 1 μM) at 10^{-6} M.
[c] Data from Ref. 24.
[d] NT = not tested.

3. Conclusions

In conclusion, we described the synthesis of new LCAPs with structural modifications in the terminal fragment and in the anchoring position of the arylpiperazinylalkyl moiety. We reported the simultaneous preparation and isolation of *N*- and *O*-alkylated pyrimidine and 2-methylquinazoline derivatives. The newly synthesized piperazine derivatives have been evaluated for binding affinities at the human cloned 5-HT_7R and 5-HT_{1A}R and the main structure-affinity relationships were outlined.

Preliminary data on functional activity showed partial inhibition of cAMP levels for 4(3*H*)-quinazolinone derivative (**30**), while derivatives 6-phenyl-pyrimidine (**13**) and 2-methyl-4(3*H*)quinazoline (**20**) showed only weak inhibition values. These results suggest a possible correlation between scaffold types and intrinsic activity of the new ligands.

Docking studies revealed coherent binding mode for all compounds in both receptors. This observation is well matched with our previous model and confirmed that L-shape is more suitable than extended conformation for 5-HT_7R. In addition, it was outlined that planar bicyclic system is preferable with respect to a single heterocyclic ring with a bulky substituent in the interaction with both receptors.

4. Experimental

4.1. Chemistry

Melting points were determined in an Electrothermal IA9200 apparatus using glass capillary tubes and are uncorrected. Infrared spectra were recorded on a Perkin Elmer series FTIR 1600 spectrometer in KBr disks. Elemental analyses for C, H, and N were within ±0.4% of theoretical values and were obtained with a Carlo Erba Elemental Analyzer Mod. 1108 apparatus. ^{1}H NMR spectra were performed on a Varian Inova Unity 200 spectrometer (200 MHz) in DMSO-d_6 or $CDCl_3$ solution. Tetramethylsilane was used as the internal standard; chemical shifts and coupling constants (*J*) are given in δ values (ppm) and in Hertz (Hz), respectively. Signal multiplicities are abbreviated as follow: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Microwave irradiation experiments were carried out with a CEM Discover instrument using closed Pyrex glass tubes (ca. 10 mL) with Teflon-coated septa. Thin-layer chromatography was utilized to monitor the progress of reactions and to test the purity of all the synthesized compounds, using Merck aluminium sheet coated with silica gel 60 F_{254} and detection with ultraviolet light at 254 and 366 nm of wavelength. Purification of synthesized compounds was performed by flash column chromatography using Merck silica

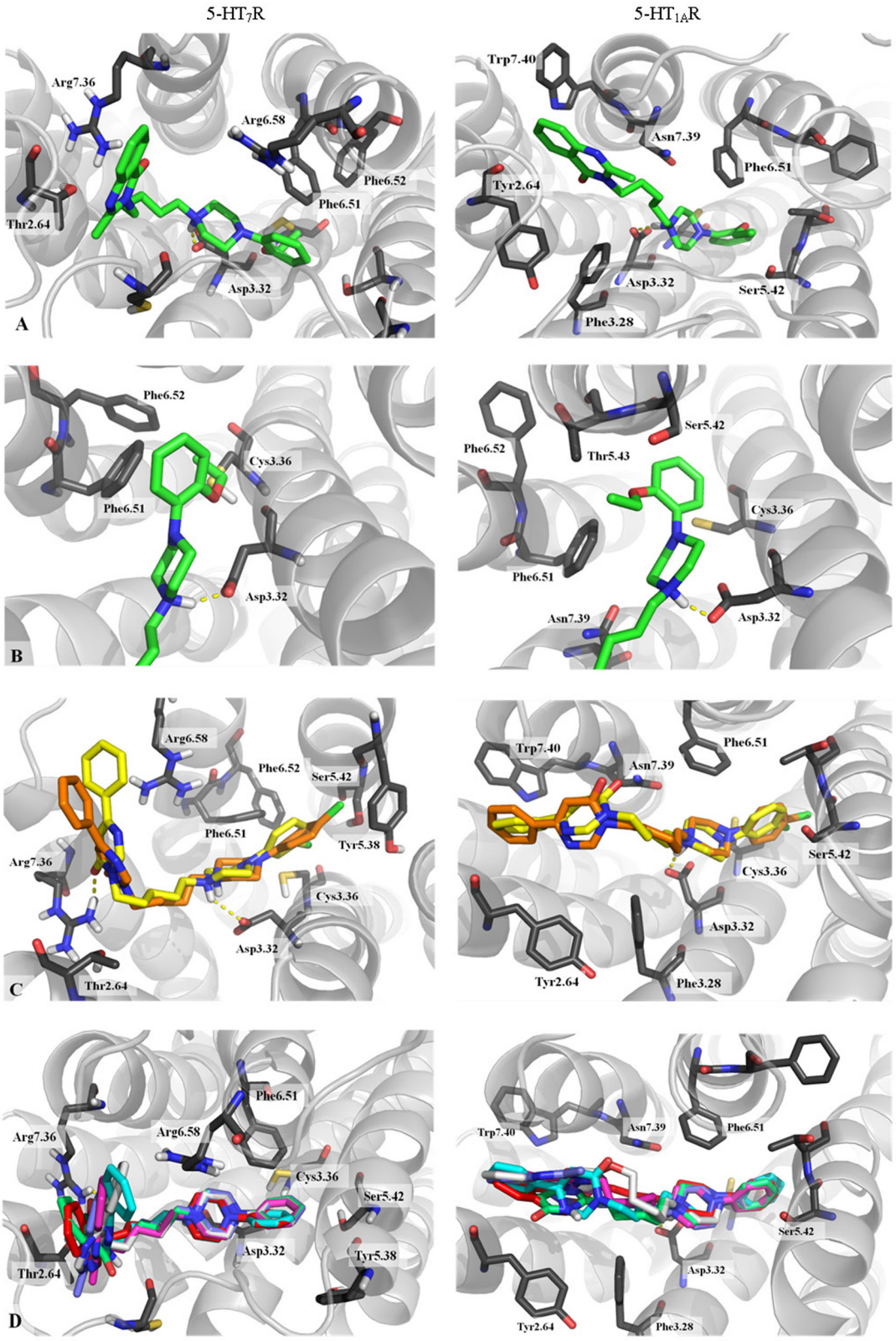


Fig. 2. Panel illustrating molecular modeling results of the new *N*-/*O*-alkyl derivatives and of the previously reported analogs for 5-HT_7 (left) and 5-HT_{1A} receptors (right). (A) Docking pose of the high-affinity ligand **20** (green) in 5-HT_7 and 5-HT_{1A} binding sites. (B) Details of the 2-ethoxyphenyl moiety orientation within the binding pocket (compound **20**). (C) Orientation of the 3-chloro **6** (yellow) and 4-chlorophenylpiperazine **7** derivatives (orange). (D) Comparison of the binding modes of phenylpiperazine derivatives **4** (cyan), **9** (grey), **17** (magenta), **21** (purple blue), **23** (limegreen), and **26** (red).

gel (0.040–0.063 mm). All chemicals and solvents were reagent grade and were purchased from commercial source.

4.1.1. General procedure for the preparation 3-(5-chloropentyl)-6-phenyl-4(3H)-pyrimidinone (**2**) and 4-(5-chloropenthoxy)-6-phenyl-pyrimidine (**3**)

Acetone was added (30 mL) to a mixture of pyrimidinone **1** (2.90 mmol), 1-bromo-5-chloro-pentane (5.80 mmol), potassium carbonate (4.34 mmol), and of a catalytic amount of potassium iodide and the mixture was refluxed under stirring for 9 h. After being cooled, the solid was removed by filtration and the solution was concentrated to dryness. Recrystallization with cyclohexane gave pure compound **2**. From the solution compound **3** was isolated by flash chromatography using a mixture of cyclohexane/ethyl acetate (9:1, v/v).

Compound **2**: yield 53%, mp 103.9–104.5 °C. IR (KBr, selected lines) cm^{-1} 2944, 2361, 1673, 1593, 1450, 691. ^1H NMR (DMSO-d_6) δ 1.30–1.50 (m, 2H, CH_2), 1.60–1.85 (m, 2H + 2H, CH_2CH_2), 3.65 (t, J = 6.6 Hz, 2H, CH_2Cl), 3.92 (t, J = 7.2 Hz, 2H, $CONCH_2$), 6.97 (s, 1H, NCH), 7.40–7.58 (m, 3H, aromatic), 7.98–8.15 (m, 2H, aromatic), 8.59 (s, 1H, CCH). Anal. ($C_{15}H_{17}ClN_2O$) C, H, N.

Compound **3**: yield 10%, mp 41.0–44.0 °C. IR (KBr, selected lines) cm^{-1} 2956, 1592, 1541, 1466, 1219, 868, 696. ^1H NMR (DMSO-d_6) δ 1.44–1.65 (m, 2H, CH_2), 1.70–1.90 (m, 2H + 2H, CH_2CH_2), 3.67 (t, J = 6.6 Hz, 2H, CH_2Cl), 4.39 (t, J = 6.4 Hz, 2H, OCH_2), 7.45–7.60 (m, 3H + 1H, aromatic + NCH), 8.15–8.25 (m, 2H, aromatic), 8.84 (d, J = 1.2 Hz, 1H, CCH). Anal. ($C_{15}H_{17}ClN_2O$) C, H, N.

4.1.2. General procedure for the synthesis of 3-[5-(4-substituted-1-piperazinyl)pentyl]-4(3H)-pyrimidinones (**4**–**8**) and 4-[5-(4-substituted-1-piperazinyl)pentoxy]-pyrimidines (**9**–**13**)

Acetonitrile (4 mL) was added to a mixture of chloroderivative **2** or **3** (0.90 mmol), properly substituted piperazine (1.08 mmol), sodium carbonate (1.08 mmol), and a catalytic amount of potassium iodide. The mixture was refluxed under stirring for 9 h. After being cooled, the solid was removed by filtration and the solution was concentrated to dryness. The following new compounds were obtained using this procedure.

4.1.3. 3-[5-(4-Phenyl-1-piperazinyl)pentyl]-6-phenyl-4(3H)-pyrimidinone (**4**)

The title compound was obtained by recrystallization from acetonitrile (51%), mp 139.6–140.4 °C. IR (KBr, selected lines) cm^{-1} 2929, 2823, 1665, 1592, 1451, 1239, 750, 695. ^1H NMR ($CDCl_3$) δ 1.30–1.58 (m, 2H, CH_2), 1.60–1.95 (m, 2H + 2H, CH_2CH_2), 2.60 (t, J = 7.2 Hz, 2H, CH_2N), 2.70–2.95 (m, 4H, piperazine), 3.20–3.45 (m, 4H, piperazine), 3.98 (t, J = 7.4 Hz, 2H, $CONCH_2$), 6.82–6.98 (m, 3H + 1H, aromatic + NCH), 7.20–7.34 (m, 2H, aromatic), 7.42–7.52 (m, 3H, aromatic), 7.88–8.10 (m, 2H, aromatic), 8.19 (s, 1H, CCH). Anal. ($C_{25}H_{30}N_4O$) C, H, N.

4.1.4. 3-[5-[4-(Phenylmethyl)-1-piperazinyl]pentyl]-6-phenyl-4(3H)-pyrimidinone (**5**)

The title compound was purified by flash chromatography using a mixture of ethyl acetate/methanol (8:2, v/v) as eluent (26%), mp 106.2–106.5 °C. IR (KBr, selected lines) cm^{-1} 2947, 2802, 1673, 1596, 1451, 688. ^1H NMR (DMSO-d_6) δ 1.19–1.55 (m, 2H + 2H, CH_2CH_2), 1.60–1.79 (m, 2H, CH_2), 2.22 (t, J = 7.4 Hz, 2H, CH_2N), 2.30–2.42 (m, 8H, piperazine), 3.39 (s, 2H, $CH_2C_6H_5$), 3.90 (t, J = 7.0 Hz, 2H, $CONCH_2$), 6.96 (s, 1H, NCH), 7.18–7.38 (m, 5H, aromatic), 7.42–7.55 (m, 3H, aromatic), 8.00–8.15 (m, 2H, aromatic), 8.58 (s, 1H, CCH). Anal. ($C_{26}H_{32}N_4O$) C, H, N.

4.1.5. 3-[5-[4-(3-Chlorophenyl)-1-piperazinyl]pentyl]-6-phenyl-4(3H)-pyrimidinone (**6**)

The title compound was obtained by recrystallization from acetonitrile (14%), mp 112.2–112.7 °C. IR (KBr, selected lines) cm^{-1} 2938, 1666, 1596, 1489, 1449, 1245, 692. ^1H NMR (DMSO-d_6) δ 1.22–1.42 (m, 2H, CH_2), 1.42–1.62 (m, 2H, CH_2), 1.62–1.82 (m, 2H, CH_2), 2.31 (t, J = 6.8 Hz, 2H, CH_2N), 2.38–2.49 (m, 4H, piperazine), 3.18–3.22 (m, 4H, piperazine), 3.93 (t, J = 7.0 Hz, 2H, $CONCH_2$), 6.75–6.80 (m, 1H, aromatic), 6.80–6.97 (m, 2H, aromatic), 6.98 (s, 1H, NCH), 7.18–7.25 (m, 1H, aromatic), 7.42–7.78 (m, 3H, aromatic), 8.00–8.15 (m, 2H, aromatic), 8.61 (s, 1H, CCH). Anal. ($C_{25}H_{29}ClN_4O$) C, H, N.

4.1.6. 3-[5-[4-(4-Chlorophenyl)-1-piperazinyl]pentyl]-6-phenyl-4(3H)-pyrimidinone (**7**)

The title compound was obtained by recrystallization from acetonitrile (10%), mp 153.6–154.0 °C. IR (KBr, selected lines) cm^{-1} 2936, 1664, 1594, 1495, 1450, 1237, 812, 696. ^1H NMR (DMSO-d_6) δ 1.20–1.40 (m, 2H, CH_2), 1.40–1.60 (m, 2H, CH_2), 1.60–1.80 (m, 2H, CH_2), 2.29 (t, J = 6.6 Hz, 2H, CH_2N), 2.40–2.49 (m, 4H, piperazine), 3.02–3.15 (m, 4H, piperazine), 3.91 (t, J = 7.4 Hz, 2H, $CONCH_2$), 6.84–6.95 (m, 2H, aromatic), 6.96 (s, 1H, NCH), 7.15–7.25 (m, 2H, aromatic), 7.43–7.53 (m, 3H, aromatic), 7.99–8.11 (m, 2H, aromatic), 8.59 (s, 1H, CCH). Anal. ($C_{25}H_{29}ClN_4O$) C, H, N.

4.1.7. 3-[5-[4-(2-Ethoxyphenyl)-1-piperazinyl]pentyl]-6-phenyl-4(3H)-pyrimidinone (**8**)

The title compound was obtained after 16 h of reflux and was purified by flash chromatography using a mixture of ethyl acetate/methanol (9:1, v/v) as eluent (32%), mp 103.5–104.1 °C. IR (KBr, selected lines) cm^{-1} 2927, 2804, 1664, 1499, 1455, 1239, 699. ^1H NMR (DMSO-d_6) δ 1.20–1.40 (m, 2H + 3H, $CH_2 + CH_2CH_3$), 1.40–1.60 (m, 2H, CH_2), 1.60–1.81 (m, 2H, CH_2), 2.30 (t, J = 6.8 Hz, 2H, CH_2N), 2.38–2.58 (m, 4H, piperazine), 2.85–3.04 (m, 4H, piperazine), 3.88–4.10 (m, 4H, $CH_2CH_3 + CONCH_2$), 6.78–6.95 (m, 4H, aromatic), 6.97 (s, 1H, NCH), 7.40–7.55 (m, 3H, aromatic), 8.00–8.15 (m, 2H, aromatic), 8.60 (s, 1H, CCH). Anal. ($C_{27}H_{34}N_4O_2$) C, H, N.

4.1.8. 4-[5-(4-Phenyl-1-piperazinyl)pentoxy]-6-phenyl-pyrimidine (**9**)

The title compound was purified by flash chromatography ethyl acetate as eluent (53%), mp 70.0–70.4 °C. IR (KBr, selected lines) cm^{-1} 2946, 2817, 1592, 1360, 1227, 1006, 756, 689. ^1H NMR (DMSO-d_6) δ 1.35–1.65 (m, 2H + 2H, CH_2CH_2), 1.70–1.90 (m, 2H, CH_2), 2.28–240 (m, 2H, CH_2N), 2.40–2.60 (m, 4H, piperazine), 3.02–3.18 (m, 4H, piperazine), 4.41 (t, J = 6.4 Hz, 2H, OCH_2), 6.70–6.81 (m, 1H, aromatic), 6.83–6.98 (m, 2H, aromatic), 7.18–7.28 (m, 2H, aromatic), 7.48–7.60 (m, 1H + 3H, NCH + aromatic), 8.15–8.25 (m, 2H, aromatic), 8.85 (d, J = 1.8 Hz, 1H, CCH). Anal. ($C_{25}H_{30}N_4O$) C, H, N.

4.1.9. 4-[5-[4-(Phenylmethyl)-1-piperazinyl]pentoxy]-6-phenyl-pyrimidine (**10**)

The title compound was purified by flash chromatography using ethyl acetate and then a mixture of ethyl acetate/methanol (9.5:0.5, v/v)) as eluents (46%), mp 63.8–64.3 °C. IR (KBr, selected lines) cm^{-1} 2941, 2810, 1578, 1541, 1345, 1214, 1005, 696. ^1H NMR (DMSO-d_6) δ 1.30–1.58 (m, 2H + 2H, CH_2CH_2), 1.68–1.82 (m, 2H, CH_2), 2.25 (t, J = 6.6 Hz, 2H, CH_2N), 2.30–2.42 (m, 8H, piperazine), 3.41 (s, 2H, $CH_2C_6H_5$), 4.38 (t, J = 6.6 Hz, 2H, OCH_2), 7.18–7.38 (m, 5H, aromatic), 7.49 (s, 1H, NCH), 7.50–7.60 (m, 3H, aromatic), 8.18–8.22 (m, 2H, aromatic), 8.83 (d, J = 0.8 Hz, 1H, CCH). Anal. ($C_{26}H_{32}N_4O$) C, H, N.

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*4.1.10. 4-[5-[4-(3-Chlorophenyl)-1-piperazinyl]pentoxy]-6-phenylpyrimidine (**11**)*

The title compound was purified by flash chromatography using ethyl acetate as eluent to obtain an oil (64%). IR (neat, selected lines) cm^{-1} 2940, 2818, 1591, 1461, 1352, 1237, 987, 758, 694. ^1H NMR ($CDCl_3$) δ 1.42–1.61 (m, 2H, CH_2), 1.63–1.98 (m, 2H + 2H, CH_2CH_2), 2.58 (t, J = 7.6 Hz, 2H, CH_2N), 2.70–2.90 (m, 4H, piperazine), 3.25–3.43 (m, 4H, piperazine), 4.42 (t, J = 6.6 Hz, 2H, $CONCH_2$), 6.72–6.90 (m, 3H, aromatic), 7.08–7.24 (m, 1H + 1H, NCH + aromatic), 7.42–7.53 (m, 3H, aromatic), 7.95–8.08 (m, 2H, aromatic), 8.82 (d, J = 1.0 Hz, 1H, CCH). Anal. ($C_{25}H_{29}ClN_4O$) C, H, N.

*4.1.11. 4-[5-[4-(4-Chlorophenyl)-1-piperazinyl]pentoxy]-6-phenylpyrimidine (**12**)*

The title compound was obtained by recrystallization from acetonitrile (19%), mp 104.2–104.5 °C. IR (KBr, selected lines) cm^{-1} 2943, 1590, 1496, 1354, 1236, 1003, 811. ^1H NMR (DMSO-d_6) δ 1.38–1.61 (m, 2H + 2H, CH_2CH_2), 1.70–1.85 (m, 2H, CH_2), 2.32 (t, J = 6.8 Hz, 2H, CH_2N), 2.41–2.49 (m, 4H, piperazine), 3.02–3.15 (m, 4H, piperazine), 4.40 (t, J = 6.6 Hz, 2H, OCH_2), 6.85–6.95 (m, 2H, aromatic), 7.18–7.25 (m, 2H, aromatic), 7.44–7.58 (m, 1H + 3H, NCH + aromatic), 8.16–8.23 (m, 2H, aromatic), 8.84 (d, J = 1.0 Hz, 1H, CCH). Anal. ($C_{25}H_{29}ClN_4O$) C, H, N.

*4.1.12. 4-[5-[4-(2-Ethoxyphenyl)-1-piperazinyl]pentoxy]-6-phenylpyrimidine (**13**)*

The title compound was purified by flash chromatography using ethyl acetate as eluent to obtain an oil (27%). IR (neat, selected lines) cm^{-1} 2939, 2814, 1590, 1499, 1455, 1239, 737. ^1H NMR ($CDCl_3$) δ 1.40–1.63 (m, 2H + 3H, $CH_2 + CH_2CH_3$), 1.63–1.98 (m, 2H + 2H, CH_2CH_2), 2.65 (t, J = 6.2 Hz, 2H, CH_2N), 2.78–3.08 (m, 4H, piperazine), 3.15–3.42 (m, 4H, piperazine), 4.06 (q, J = 6.8 Hz, 2H, CH_2CH_3), 4.42 (t, J = 6.6 Hz, 2H, OCH_2), 6.80–7.08 (m, 4H, aromatic), 7.11 (s, 1H, NCH), 7.42–7.60 (m, 3H, aromatic), 7.98–8.15 (m, 2H, aromatic), 8.82 (d, J = 1.0 Hz, 1H, 1H, CCH). Anal. ($C_{27}H_{34}N_4O_2$) C, H, N.

*4.1.13. General procedure for the preparation of 3-(5-bromopentyl)-2-methyl-4(3H)-quinazolinone (**15**) and 4-(5-bromopenthoxy)-2-methylquinazoline (**16**)*

Acetonitrile (4 mL) was added to mixture of compound **14** (1.55 mmol), 1,5-dibromopentane (4.67 mmol), potassium carbonate (2.32 mmol), and of a catalytic amount of potassium iodide. The mixture and a magnetic bar were sealed in a Pyrex tube and were heated at 90 °C by microwave irradiation for 90 min (run time 3 min, microwave max power 150 W and max pressure 150 psi). After being cooled, the solid was removed by filtration and the solution was concentrated to dryness. From the obtained residue, compound **15** as a solid and compound **16** as an oil were isolated by flash chromatography using ethyl acetate/cyclohexane (5:5, v/v) as eluent.

Compound **15**: yield 47%, mp 52.0–54.6 °C. IR (KBr, selected lines) cm^{-1} 3052, 1671, 1626, 1467, 1424, 1265, 737. ^1H NMR (DMSO-d_6) δ 1.40–1.58 (m, 2H, CH_2), 1.58–1.78 (m, 2H, CH_2), 1.78–1.95 (m, 2H, CH_2), 2.61 (s, 3H, CH_3), 3.56 (t, J = 6.6 Hz, 2H, CH_2Br), 4.03 (t, J = 7.2 Hz, 2H, $CONCH_2$), 7.42–7.52 (m, 1H, aromatic), 7.52–7.60 (m, 1H, aromatic), 7.72–7.83 (m, 1H, aromatic), 8.05–8.12 (m, 1H, aromatic).

Compound **16**: yield 23%, IR (neat, selected lines) cm^{-1} 1619, 1577, 1503, 1434, 1369, 1318, 1168, 1112, 782. ^1H NMR (DMSO-d_6) δ 1.54–1.68 (m, 2H, CH_2), 1.78–1.99 (m, 2H + 2H, CH_2CH_2), 2.61 (s, 3H, CH_3), 3.58 (t, J = 6.8 Hz, 2H, CH_2Br), 4.53 (t, J = 6.4 Hz, 2H, OCH_2), 7.53–7.65 (m, 1H, aromatic), 7.76–7.95 (m, 2H, aromatic), 8.05–8.13 (m, 1H, aromatic). Anal. ($C_{14}H_{17}BrN_2O$) C, H, N.

*4.1.14. General procedure for the synthesis of 2-methyl-3-[5-(4-substituted-1-piperazinyl)pentyl]-4(3H)-quinazolinones (**17**–**20**) and 2-methyl-4-[5-(4-substituted-1-piperazinyl)pentoxy]-quinazolines (**21**, **22**)*

Acetonitrile (4 mL) was added to a mixture of bromoderivative **15** or **16** (0.73 mmol), properly substituted piperazine (0.88 mmol), sodium carbonate (0.88 mmol), and of a catalytic amount of potassium iodide. The mixture and a magnetic bar were sealed in a Pyrex tube and were heated at 90 °C by microwave irradiation for 1 h (run time 3 min, microwave max power 150 W and max pressure 150 psi). After being cooled, the solid was removed by filtration and the solution was concentrated to dryness. The following new compounds were obtained using this procedure:

*4.1.15. 2-Methyl-3-[5-(4-phenyl-1-piperazinyl)pentyl]-4(3H)-quinazolinone (**17**)*

The crude product was purified by flash chromatography using ethyl acetate, a mixture of ethyl acetate/methanol (9:1, v/v), and then ethyl acetate/methanol (8:2, v/v) as eluents (37%), mp 78.0–79.0 °C. IR (KBr, selected lines) cm^{-1} 2934, 2820, 1678, 1600, 1476, 1402, 1240, 780, 762. ^1H NMR (DMSO-d_6) δ 1.35–1.78 (m, 2H + 2H + 2H, $CH_2CH_2CH_2$), 2.32 (t, J = 6.8 Hz, 2H, NCH_2), 2.41–2.58 (m, 4H, piperazine), 2.62 (s, 3H, CH_3), 3.03–3.17 (m, 4H, piperazine), 4.04 (t, J = 7.4 Hz, 2H, $CONCH_2$), 6.71–6.82 (m, 1H, aromatic), 6.86–6.98 (m, 2H, aromatic), 7.14–7.26 (m, 2H, aromatic), 7.41–7.53 (m, 1H, aromatic), 7.53–7.62 (m, 1H, aromatic), 7.72–7.84 (m, 1H, aromatic), 8.05–8.14 (m, 1H, aromatic). Anal. ($C_{24}H_{30}N_4O$) C, H, N.

*4.1.16. 3-[5-[4-(3-Chlorophenyl)-1-piperazinyl]pentyl]-2-methyl-4(3H)-quinazolinone (**18**)*

The crude product was purified by flash chromatography using ethyl acetate, a mixture of ethyl acetate/methanol (9:1, v/v), and then ethyl acetate/methanol (8:2, v/v) as eluents (13%), mp 113.0–114.8 °C. IR (KBr, selected lines) cm^{-1} 2928, 2815, 1678, 1595, 1471, 1462, 1393, 1243, 770. ^1H NMR (DMSO-d_6) δ 1.25–1.78 (m, 2H + 2H + 2H, $CH_2CH_2CH_2$), 2.31 (t, J = 6.8 Hz, 2H, NCH_2), 2.40–2.55 (m, 4H, piperazine), 2.61 (s, 3H, CH_3), 3.05–3.20 (m, 4H, piperazine), 4.03 (t, J = 7.0 Hz, 2H, $CONCH_2$), 6.72–6.81 (m, 1H, aromatic), 6.81–6.94 (m, 2H, aromatic), 7.12–7.25 (m, 1H, aromatic), 7.41–7.60 (m, 2H, aromatic), 7.70–7.82 (m, 1H, aromatic), 8.04–8.15 (m, 1H, aromatic). Anal. ($C_{24}H_{29}ClN_4O$) C, H, N.

*4.1.17. 3-[5-[4-(4-Chlorophenyl)-1-piperazinyl]pentyl]-2-methyl-4(3H)-quinazolinone (**19**)*

The crude product was recrystallized from water (49%), mp 101.2–102.2 °C. IR (KBr, selected lines) cm^{-1} 2937, 1678, 1597, 1499, 1467, 1394, 1357, 1240, 768. ^1H NMR ($CDCl_3$) δ 1.25–1.78 (m, 2H + 2H + 2H, $CH_2CH_2CH_2$), 2.31 (t, J = 7.2 Hz, 2H, NCH_2), 2.40–2.55 (m, 4H, piperazine), 2.61 (s, 3H, CH_3), 3.02–3.15 (m, 4H, piperazine), 4.03 (t, J = 7.8 Hz, 2H, $CONCH_2$), 6.91 (d, J = 9.2 Hz, 2H, aromatic), 7.21 (d, J = 8.8 Hz, 2H, aromatic), 7.40–7.52 (m, 1H, aromatic), 7.52–7.62 (m, 1H, aromatic), 7.70–7.82 (m, 1H, aromatic), 8.04–8.15 (m, 1H, aromatic). Anal. ($C_{24}H_{29}ClN_4O$) C, H, N.

*4.1.18. 3-[5-[4-(2-Ethoxyphenyl)-1-piperazinyl]pentyl]-2-methyl-4(3H)-quinazolinone (**20**)*

The crude product was purified by flash chromatography using ethyl acetate, a mixture of ethyl acetate/methanol (9:1, v/v), and then ethyl acetate/methanol (8:2, v/v) as eluents, obtaining compound **10** as a pure oil (12%). IR (neat, selected lines) cm^{-1} 2940, 1671, 1594, 1500, 1474, 1394, 1266, 1241, 736. ^1H NMR ($CDCl_3$) δ 1.39–1.60 (m, 2H + 3H, $CH_2 + CH_2CH_3$), 1.71–1.95 (m, 2H + 2H, CH_2CH_2), 2.65 (s, 3H, CH_3), 2.70 (t, J = 7.6 Hz, 2H, NCH_2), 2.80–3.10 (m, 4H, piperazine), 3.25–3.45 (m, 4H, piperazine),

3.98–4.20 (m, 2H + 2H, $CONCH_2 + CH_2CH_3$), 6.80–7.10 (m, 4H, aromatic), 7.38–7.50 (m, 1H, aromatic), 7.55–7.62 (m, 1H, aromatic), 7.62–7.80 (m, 1H, aromatic), 8.18–8.27 (m, 1H, aromatic). Anal. ($C_{26}H_{34}N_4O_2$) C, H, N.

4.1.19. 2-Methyl-4-[5-(4-phenyl-1-piperazinyl)pentoxy]-quinazoline (**21**)

The crude product was recrystallized from water (26%), mp 64.9–66.5 °C. IR (KBr, selected lines) cm^{-1} 2960, 2833, 1575, 1500, 1422, 1356, 1239, 1163, 1112, 777. ^1H NMR (DMSO-d_6) δ 1.42–1.70 (m, 2H + 2H, CH_2CH_2), 1.75–1.95 (m, 2H, CH_2), 2.33 (t, J = 6.4 Hz, 2H, NCH_2), 2.40–2.55 (m, 4H, piperazine), 2.61 (s, 3H, CH_3), 3.00–3.15 (m, 4H, piperazine), 4.53 (t, J = 6.4 Hz, 2H, OCH_2), 6.70–6.81 (m, 1H, aromatic), 6.83–6.96 (m, 2H, aromatic), 7.12–7.25 (m, 2H, aromatic), 7.53–7.62 (m, 1H, aromatic), 7.75–7.96 (m, 2H, aromatic), 8.02–8.18 (m, 1H, aromatic). Anal. ($C_{24}H_{30}N_4O$) C, H, N.

4.1.20. 4-[5-(4-(4-Chlorophenyl)-1-piperazinyl)pentoxy]-2-methyl-quinazoline (**22**)

The crude product was recrystallized from acetonitrile (12%), mp 109.0–111.9 °C. IR (KBr, selected lines) cm^{-1} 2941, 1578, 1497, 1424, 1351, 1163, 1109, 768. ^1H NMR ($CDCl_3$) δ 1.48–1.70 (m, 2H, CH_2), 1.70–2.00 (m, 2H + 2H, CH_2CH_2), 2.63 (t, J = 7.4 Hz, 2H, NCH_2), 2.71 (m, 3H, CH_3), 2.75–2.90 (m, 4H, piperazine), 3.25–3.39 (m, 4H, piperazine), 4.56 (t, J = 6.4 Hz, 2H, OCH_2), 6.78–6.90 (m, 2H, aromatic), 7.15–7.30 (m, 2H, aromatic), 7.42–7.58 (m, 1H, aromatic), 7.73–7.87 (m, 2H, aromatic), 8.05–8.18 (m, 1H, aromatic). Anal. ($C_{24}H_{29}ClN_4O$) C, H, N.

4.2. In vitro binding assays

Binding assays were performed using human cloned 5-$HT_{7(a)}$ and 5-HT_{1A} serotonin receptors (PerkinElmer) expressed on CHO-K1 cells. Radioligand binding assays were carried out using the condition reported on technical data sheet with some modifications. Briefly, 5-$HT_{7(a)}$ receptors[41] were resuspended in Tris HCl 50 mM pH 7.4 containing 4 mM $MgCl_2$ and incubated for 40 min at 27 °C in a final volume of 0.51 mL, consisting of 250 μL of membrane suspension (15 μg protein/sample), 250 μL of [^{3}H]-5-HT (final concentration 5 nM, s.a. 106 Ci/mmol, PerkinElmer) prepared in the same buffer used for membrane suspension and 10 μL of tested compounds. Nonspecific binding was obtained in the presence of 10 μM serotonin.

For 5-HT_{1A} binding assay,[42] receptors were resuspended in Tris HCl 50 mM pH 7.4 containing 4 mM $CaCl_2$ and incubated (10 μg protein/sample) for 1 h at 27 °C in the same volume using for 5-$HT_{7(a)}$ receptors but in presence of [^{3}H]-8-OH-DPAT (final concentration 1 nM, s.a. 137 Ci/mmol, PerkinElmer). Nonspecific binding was obtained in presence of 10 μM serotonin and, for both binding assays, a reference drug was tested. Incubations were stopped by rapid filtration under vacuum, through GF/C filters (pre-soaked with 0.3% PEI) and washed with 12 mL (4 × 3 times) of ice-cold washing buffer (Tris HCl, 50 mM, pH 7.4) using a Brandel M-48R cell harvester. The radioactivity trapped on the filters was counted in 4 mL of Ultima Gold MV (Packard) in a Tri-carb 2800 TR (PerkinElmer) liquid scintillation spectrometer with a counting efficiency of 60%. All compounds were tested in a concentration range from 10^{-5} to 10^{-10} M in triplicate and dose-inhibition curves were analyzed by the "Allfit" program to obtain the concentration of unlabeled drug that caused 50% inhibition of ligand binding.[43] The K_i values were derived from IC_{50} values according to the Cheng and Prusoff equation.[44]

4.3. Functional cAMP assay protocol

HEK293 cell lines with stable expression of human 5-HT_{1A}R and 5-HT_7R (prepared with the use of Lipofectamine 2000) were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were grown in Dulbecco's Modifier Eagle Medium containing 10% dialysed fetal bovine serum and 500 μg/mL G418 sulphate. For functional experiments, cells were subcultured in 25 cm^2 diameter dishes, grown to 90% confluence, washed twice with prewarmed to 37 °C phosphate buffered saline (PBS) and were centrifuged for 5 min (160g). The supernatant was aspirated, the cell pellet was resuspended in stimulation buffer (1 × HBSS, 5 mM HEPES, 0.5 mM IBMX, 0.1% BSA).

The functional properties of compounds **13** and **20** at 5-HT_{1A}R and 5-HT_7R were evaluated in a simplified antagonist assays, using LANCE Ultra cAMP detection kit (PerkinElmer). In a case of 5-HT_{1A}R, cells were stimulated with 1 μM of forskolin (EC_{90}) and 1 μM of agonist (*R*)-(+)-8-OH-DPAT (EC_{90}) to determine the antagonist induced increase in cellular cAMP levels; for 5-HT_7R, cells were stimulated with 10 nM of agonist 5-CT (EC_{90}) and the antagonist induced decrease in cellular production of cAMP were measured. Each compound was tested in triplicate at the one concentration 10^{-6} M.

For quantification of cAMP levels, cells (5 μL) were incubated with 5 μL mixture of compounds (tested ligand, (*R*)-(+)-8-OH-DPAT, and forskolin for 5-HT_{1A}R or tested ligand and 5-CT for 5-HT_7R) for 30 min at room temperature in 384-well white opaque microtiter plate (PerkinElmer). After incubation, the reaction was stopped and cells were lysed by the addition of 10 μL working solution (5 μL Eu-cAMP and 5 μL ULight-anti-cAMP). The assay plate was incubated for 1 h at room temperature. Time-resolved fluorescence resonance energy transfer (TRFRET) signal was detected by an Infinite M1000 Pro (Tecan) using instrument settings from LANCE Ultra cAMP detection kit manual.

4.4. Molecular modeling

The building of homology models of 5-HT_{1A} and 5-HT_7 receptors, validation of these models, and ligand-directed optimization of the binding sites were previously described.[39] The 5-HT_{1A} and 5-HT_7 receptor models selected in Induced Fit Docking (IFD) procedure were used to study the binding mode of the synthesized ligands. LigPrep was used to prepare the structures of the molecule[45] and Epik to assign the appropriate ionization states at pH = 7.4.[46] Docking was performed by using Glide at SP level.[47] The spatial constrain was imposed on the creation of an ionic interaction between the protonated amine group of the ligand and the Asp3.32 side chain. Next, ligand-receptor complexes were analyzed, and only those models were kept for which coherent, for the whole set of compounds, and closest compliance with common binding mode for monoaminergic receptor ligands was observed.[48] Final figures of the docking pose in both receptors were generated using PyMOL.[49]

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A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2016.12.039.

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