Synthesis and computer-aided analysis of the role of linker for novel ligands of the 5-HT₆ serotonin receptor among substituted 1,3,5-triazinylpiperazines

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A B S T R A C T

A series of 2-amino-4-(4-methylpiperazin-1-yl)-1,3,5-triazines was designed based on previously published 2-amino-4-benzyl-(4-methylpiperazin-1-yl)-1,3,5-triazines in order to evaluate the role of a linker between the triazine moiety and an aromatic substituent for the human serotonin 5-HT₆ receptor affinity. As new linkers two carbon atoms (ethyl or ethenyl) or an oxyalkyl chain (methoxy, 2-ethoxy, 2-propoxy) were introduced. Affinities of the compounds for the 5-HT₆R as the main target, and for the 5-HT₁AR, 5-HT₇R and D₂R as competitive ones, were determined in the radioligand binding assays. Docking to the 5-HT₆R homology model was performed to support SAR analysis. Results showed that the branching of the methoxyl linker increased affinity for the human 5-HT₆R whereas an unsaturated bond within the linker dramatically reduced desirable activity. Both experimental and theoretical studies confirmed the previously postulated beneficial role of the aromatic size for interaction with the 5-HT₆R. Thus, the largest naphthyl moiety yielded the highest activity. In particular, 4-(4-methylpiperazin-1-yl)-6-(1-(naphthalen-1-yloxy)ethyl)-1,3,5-triazin-2-amine (**24**), the most potent 5-HT₆R agent found (Kᵢ = 23 nM), can be a new lead structure for further search and development.

1. Introduction

The serotonin 5-HT₆ receptors (5-HT₆Rs) are mainly distributed in the brain in the areas relevant with cognition. Potential utility of the 5-HT₆R ligands, especially antagonists, could be in the treatment of cognition deficits in diseases such as Alzheimer's disease (AD), depression/anxiety and schizophrenia [1,2]. Most of compounds advanced to clinical trials (phase III) were evaluated with therapeutic indications of cognitive impairments associated with AD, e.g. idalopirdine or intepirdine **1** [3] (Fig. 1), and have shown no higher efficacy versus placebo as adjuncts to donepezil [4]. However, new promising 5-HT₆R ligands have appeared and are now tested in phase I trials, e.g. AVN-492 (**2**; Fig. 1) [5]. Furthermore, positive effects were also observed for the 5-HT₆R antagonists in obesity and metabolic related disorders [6,7]. Recently the 5-HT₆R antagonists (e.g. **3**; Fig. 1) showed also neuropathic pain-alleviating effects in a rat spinal nerve ligation model [8].

Thus, 5-HT₆R is an attractive target in the search for biologically active substances with promising pharmacological utility. So far, intensive works done by academic and pharmaceutical industry researchers led to obtain many potent compounds with different chemical structures. In 2006 Holenz et al. [9] have suggested a classification of 5-

HT₆R ligands into the following groups: indole derivatives, indole-like derivatives, aryl-piperazines, bi- and tricyclic piperazines, and arylsulfonyl derivatives. Recently very interesting aryl-piperazines, designed as multi-target-directed ligands blocking 5-HT₆Rs, inhibiting acetyl/butyrylcholinesterase and amyloid β anti-aggregation, were described by Więckowska et al. (**4**, Fig. 1) [10].

On the other hand, in our recent studies, we discovered a totally new chemical family of potent 5-HT₆R agents among 2,4,6-trisubstituted derivatives of 1,3,5-triazine, that goes beyond the classification of Holenz, despite of a presence of methyl-piperazine fragment [11]. Benzyl derivatives of 4-piperazinyl-1,3,5-triazine were the most active members demonstrating submicromolar 5-HT₆R affinities (e.g. **5**; Fig. 1, Table 1).

The computer-aided structure-activity relationship (SAR) analysis, performed for that group, has indicated an important role of a size and properties of aromatic fragments linked by methylene spacer to the 1,3,5-triazine ring [11]. It was especially remarkable, that the potent 5-HT₆R affinity of arylmethyl compounds, containing methylene spacer (**5** and **10–12**; Table 1), significantly decreased with the spacer deletion in the case of all corresponding aryl-triazines (**6** and **7**, Table 1). This observation suggests that presence of linker between the triazine and aromatic moieties is a crucial structural feature for the desired receptor
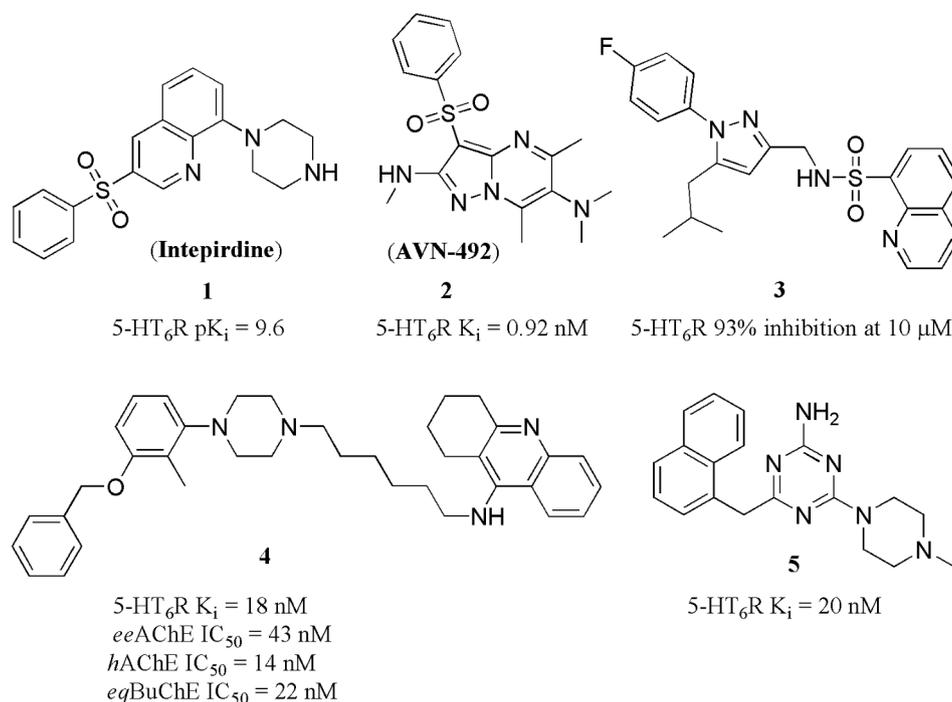

Fig. 1. Structures of described 5-HT$_6$R ligands. *ee*AChE – acetylcholinesterase from the electric eel; *h*AChE – recombinant human acetylcholinesterase; *eq*BuChE – butyrylcholinesterase from equine serum.

Table 1

Structures of tested compounds (**5–26**).

| X	R				
	phenyl	Cl-phenyl	indole	naphthyl (1)	naphthyl (2)
–	**6**	**7**	–	**8**	**9**
−CH$_2$−	**10**	**11**	**12**	**5**	–
−CH$_2$CH$_2$−	**13**	–	–	–	–
−CH=CH−	**14**	**15**	**16**	**17**	**18**
−O−CH$_2$−	**19**	**20**	–	**21**	**22**
−O−CH(CH$_3$)−	–	**23**	–	**24**	–
−O−C(CH$_3$)$_2$−	–	**25**	–	**26**	–

action. However, there were not enough data to fully analyze that hypothesis within our previous studies due to only two linker-variants in account (Table 1). In this context, our present study reports on deeper insight into the role of the linker between the triazine moiety and the aromatic substituent. Apart from two linkers described earlier, two carbon atoms (ethyl or ethenyl) or an oxyalkyl chain (methoxy, 2-ethoxy, 2-propoxy) were under consideration. Herein, we describe synthesis of new compounds, molecular modeling docking studies and receptors radioligand binding assays. Computer-aided SAR analysis has been performed on the basis of a series of 22 compounds (**5–26**) that contains 5 corresponding aromatic moieties with different linkers (Table 1).
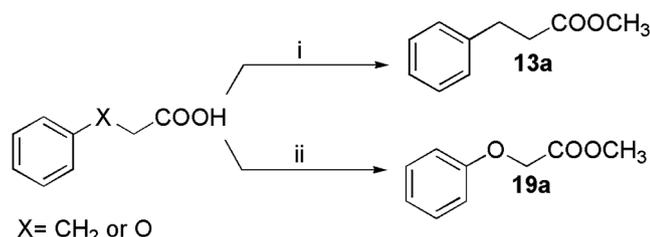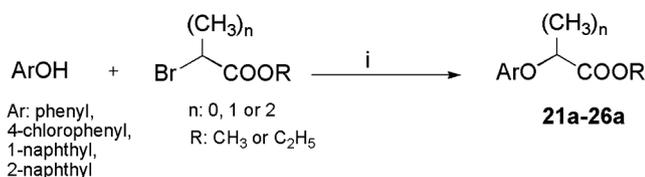
2. Synthesis

Synthesis of compounds **5–7**, **10–12**, **14–18** and **20** was described earlier, as follows: **5**, **10** and **20** were reported by Latacz et al. [12],

compounds **6** and **7** by Łażewska et al. [13], compounds **11** and **12** by Łażewska et al. [11], whereas compounds with vinyl linker (**14–18**) by Kamińska et al. [14]. Compounds **8**, **9**, **13**, **19**, and **21–26** were prepared from the proper esters (**8a, 9a, 13a, 19a, 21a–26a**) and 4-methylpiperazin-1-yl biguanide dihydrochloride in the presence of sodium methoxide (Scheme 1).

The reaction was carried out at reflux temperature as described previously [13] (for **9, 13, 19, 23** and **25**) or at room temperature (for **8, 21, 22, 24** and **26**). Commercially unavailable esters (**13a, 19a,** and **21a–26a**) were obtained by two methods (Schemes 2a and 2b). The first method comprised esterification of commercially available aromatic acids with methanol in the presence of sulfuric acid or with iodomethane and DBU (Scheme 2a). In the second synthetic procedure, proper phenols or naphthols were alkylated with suitable bromo-esters in the presence of potassium carbonate in acetone (Scheme 2b). The structures and purity of the final compounds **8, 9, 13, 19,** and **21–26** were confirmed by means of spectral (^{1}H NMR, ^{13}C NMR, MS, IR) and
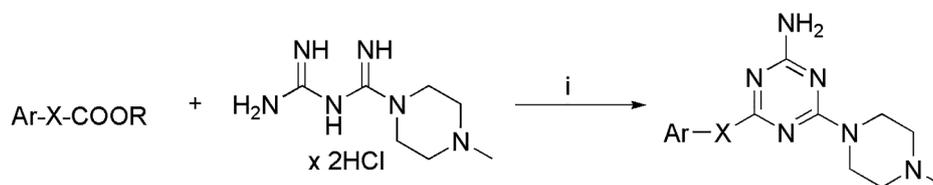
Scheme 1. Synthesis of compounds **8, 9, 13, 19, 21–26**. (i) Reagents and conditions: CH$_3$ONa, rt or reflux: 24–48 h; 5–65%.

Scheme 2a. Synthesis of esters **13a** and **19a**. (i) Reagents and conditions: CH$_3$OH, H$_2$SO$_4$, rt: 144 h, 77% (ii) CH$_3$I, DBU, toluene, rt 25 h, 80%.

Scheme 2b. Synthesis of esters **21a–26a**. (i) Reagents and conditions: acetone:H$_2$O (4:1), K$_2$CO$_3$, reflux: 4–15 h, 38–83%.

chromatographic methods (TLC).

3. *In vitro* pharmacological studies

3.1. Affinity for human serotonin 5-HT$_6$ receptor

All compounds were screened for human 5-HT$_6$R affinity in the radioligand binding assay with [³H]-LSD as a specific radioligand. Results from these studies are collected in Table 2. For comparison, affinity data for our previous triazine compounds (**5–7** and **10–12**) were included [11]. Nine (**13, 18, 20–26**) out of all new 5-HT$_6$R ligands investigated (**8, 9, 13–26**) had significant affinity for the 5-HT$_6$R with K$_i$ values below 250 nM, and compounds **24** and **26** displayed particularly potent affinities (K$_i$: 23 nM and 33 nM, respectively) in the range of the most active compounds found previously (**5, 11** and **12**). In contrary, two compounds described previously (**6, 7**) and three new compounds (**14–16**) showed very low affinities for the target 5-HT$_6$R.

3.2. Intrinsic activity for human serotonin 5-HT$_6$ receptor of compounds 5, 12, 24 and 26

Selected compounds (**5, 12, 24** and **26**) were additionally determined on their intrinsic activity in functional tests. The cAMP accumulation assay in HEK293 cells expressing the human recombinant 5-HT$_6$R was used. Results, expressed as K$_b$ values, are shown in Table 3. Addition of considered compounds resulted in a blockade of 5-carboxyamidotryptamine (5-HT$_3$R agonist) activity, leading to dose-dependent

Table 2
Affinity of investigated compounds for serotonin (5-HT$_6$, 5-HT$_{1A}$, 5-HT$_7$) and dopamine D$_2$ receptors.

Cpds	K$_i$ (nM)			
	5-HT$_6$[a]	D$_2$[b]	5-HT$_{1A}$[c]	5-HT$_7$[d]
5	20	895	5060	5372
6	4189	10,680	2937	10,840
7	2708	–[e]	–[e]	–[e]
8	3103	15,590	2221	68,110
9	5188	–[e]	–[e]	–[e]
10	95	965	17,560	9263
11	52	1359	2189	6665
12	22	1946	9453	16,790
13	218	606	2447	2114
14	7077	11,190	10,470	319,700
15	11,190	2524	1858	27,690
16	10,470	7562	4337	13,400
17	894	460	574	8015
18	206	2336	1782	3465
19	507	1348	8726	9531
20	226	2729	3020	5701
21	150	1262	225	4943
22	191	1548	4136	5533
23	84	1900	1395	5880
24	23	319	2357	9574
25	88	554	158	2411
26	33	1036	3349	2305

[a] [³H]-LSD binding assay in HEK293 cells stably expressing the human serotonin 5-HT$_6$R.
[b] [³H]-Raclopride binding assay in HEK293 cells stably expressing the human dopamine D$_2$ receptor.
[c] [³H]-Raclopride binding assay in HEK293 cells stably expressing the human serotonin 5-HT$_{1A}$ receptor.
[d] [³H]-5-Carboxyamidotryptamine binding assay in HEK293 cells stably expressing the human serotonin 5-HT$_7$ receptor.
[e] Not tested.

Table 3
Functional activity of investigated compounds (**5, 12, 24** and **26**) for serotonin 5-HT$_6$ receptor.

Cpd	5	12	24	26
5-HT$_6$R functional antagonism K$_b$ [nM]	26	18	14	150

increase of cAMP level in cells co-treated with 5-CT and forskolin. On the basis of the obtained results, all the compounds can be classified as potent 5-HT$_6$R antagonists with K$_b$ values corresponding to K$_i$ ones in the radioligand binding assays (**5, 12, 24,** Table 2) or a bit higher (**26**).

3.3. Selectivity in respect to other GPCRs (D₂, 5HT₁ₐ, 5HT₇)

Most of the compounds (excluding **7** and **9**), tested for affinities towards serotonin receptors 5-HT₁ₐ and 5-HT₇ as well as for dopamine D₂R, predominantly demonstrated low affinities (Kᵢ > 1.2 μM; Table 2). It is notable that all active 5-HT₆R agents displayed significantly lower affinities for the competitive serotonin and dopamine receptors tested. However, some compounds (**5**, **10**, **13**, **17**, **21**, **24** and **25**) had a moderate affinity for dopamine D₂ and 5-HT₁ₐ receptors with Kᵢ values < 1 μM. Compound **24** was the most active at dopamine D₂ receptor, with Kᵢ of 319 nM. The highest affinity for 5-HT₁ₐ showed compounds **21** and **25** (Kᵢ: 225 nM and 158 nM, respectively). None of the tested compounds showed significant affinity for the 5-HT₇ receptor (Kᵢ > 2.1 μM). Compounds **11**, **12**, **23** and **26** were the most selective among the potent 5-HT₆R ligands with Kᵢ < 100 nM (Table 2).

4. Molecular docking studies

Molecular docking studies were performed to take an insight into the binding mode of the tested compounds to the 5-HT₆R model. For this purpose, the recently developed 5-HT₆R homology models [11] based on the β₂ adrenergic receptor template [15–17] and trained on 1,3,5-tiazine derivatives were used. For each compound, five top-scored complexes were considered, of which the best with a binding mode coherent to the previously described [11] was analyzed. The molecular docking results indicated that all newly investigated compounds (**13–26**), generally, showed very consistent binding mode to the previously reported 1,3,5-triazine derivatives [11]. However, some influence of linker properties on both, strength and manner of binding has been observed (Fig. 2), and was in good concordance with results of the radioligand binding assay.
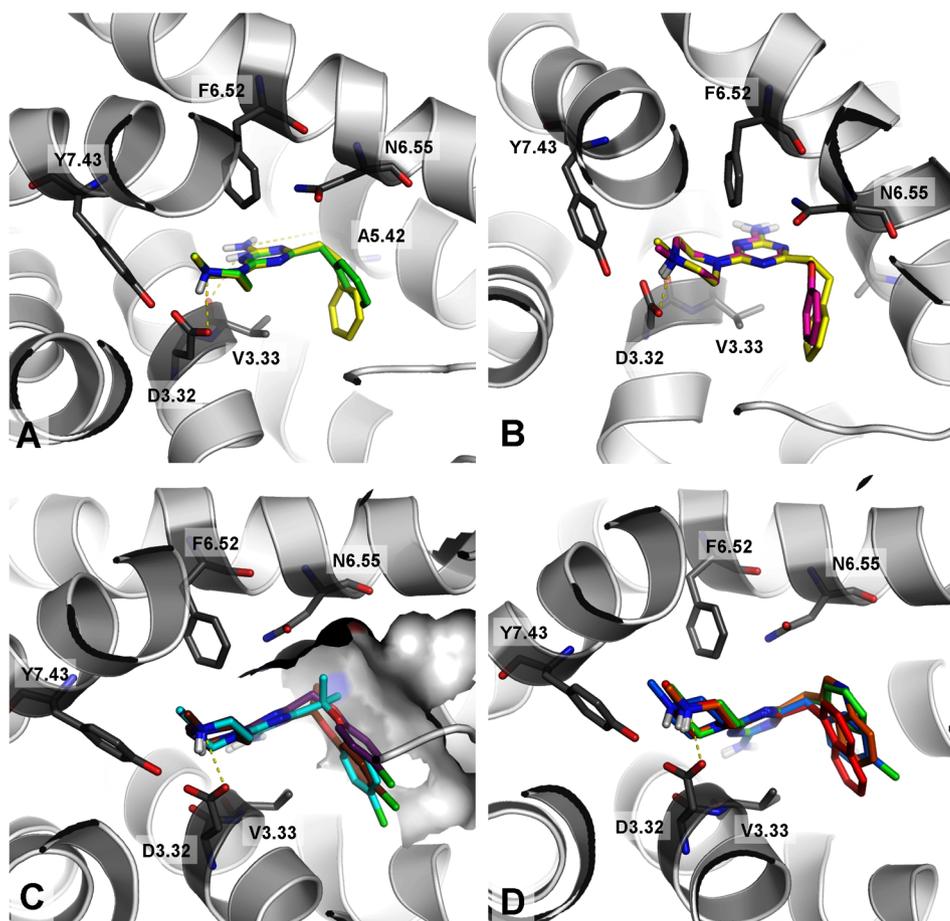
5. Molecular modeling-aided SAR discussion

Results of both, radioligand binding assay and docking analysis, have provided an interesting insight on SAR within this chemical group, accentuating a role of the linkers between aromatic and triazine moieties.

The most potent compounds for 5-HT₆R (Kᵢ < 35 nM) found in this (**24** and **26**) and previous (**5** and **12**) studies belong to the flexible linker-containing derivatives of 1,3,5-triazines with fused (hetero)aromatic rings of the 1-naphthyl (**5**, **24**, **26**) or 3-indole (**12**). In contrast, linker-free aryl derivatives of the 1,3,5-triazine (**6–9**; Kᵢ > 2 μM) and the compounds with vinylene linker (**14–16**, Kᵢ > 7 μM) were very weak 5-HT₆R agents, almost regardless the kind of their aromatic substituent. However, significant impact of the aromatic substituent is distinctly seen in the case of more active compounds (**5**, **10–13**, **18–26**). Generally, the phenyl derivatives (**6**, **10**, **14**, **19**) were less active than their corresponding naphthyl analogues (**8**, **5**, **17–18**, **21–22**). As impact of the linker, the following relationships can be observed: (i) an elongation of the methylene chain to the ethylene one (**13**) slightly decreased the 5-HT₆R affinity, whereas the inflexible vinylene linker (**14–17**) caused much stronger activity decrease; (ii) an introduction of oxygen instead of carbon within the ethylene linker resulted in decrease of the affinity, e.g. **13** vs **19**, while (iii) branching in the methoxyl linker (−OCH(CH₃)− or −OC(CH₃)₂−) increased the 5-HT₆R affinity in comparison to the straight-chain (e.g. **20** vs **23** vs **25** or **21** vs **24** vs **26**), and let obtain compounds with comparable affinity to the most active methylene-linked analogues (e.g. **11** vs **23** *vs* **25** or **5** vs **24** *vs* **26**).

Results of the docking studies (Fig. 2) indicated that, in addition to the crucial salt bridge interaction with D3.32 found previously [11], all of the docked compounds formed at least one specific aromatic interaction (CH–π or π–π stacking) with F6.52 and partially with F6.51. The

Fig. 2. The interactions of 1,3,5-triazinylpiperazine derivatives with different modification of linker with 5-HT₆R. (A) A comparison of the binding modes of analogs with a different carbon linker length between the 1,3,5-triazine and the aromatic ring (**10** – green, **13** – yellow). (B) A comparison of the binding modes of compound **13** (yellow) and its analog **19** (magenta) with oxygen atom in a linker. (C) Illustration of orientation of analogs with branching in the methoxyl linker (**20** – violet, **23** – brown, and **25** – cyan). (D) A superposition of the poses with different aromatic ring, i.e.: phenyl (**10** – green), 4-chlorophenyl (**11** – blue), 3-indolyl (**12** – orange), and 1-naphtyl (**5** – red).

NH$_2$ group of the 1,3,5-triazine ring was additionally hydrogen bonded with carbonyl oxygen of V3.33 (Fig. 2A). A comparison of the binding mode of analogs with a different carbon linker length (ranged from 0 to 2) between 1,3,5-triazine and aromatic moieties (Fig. 2A), showed that the methylene is an optimal linker followed by an ethylene. The less active analog in the series, without linker (**6**) was not docked, probably due to its conformational rigidity. For the similar reason, stiffened vinylene-linked derivatives (**14–16**) were the least active 5-HT$_6$R ligands within the whole series. However, the low linker-flexibility seems to be partly compensated by presence of profitable aromatic moieties of the naphthalene in the case of **17** and **18**. Both compounds (**17** and **18**) exhibited submicromolar 5-HT$_6$R-affinities, with predominant activity of the β-naphthyl derivative (**18**).

In contrast, a decrease of the affinity for the derivative with the ethylene linker (**13**) can be attributed to its higher flexibility leading to the dispersion of the spatial position of the phenyl ring in the cavity of the binding crevice formed by helices 4–6 and extracellular loop 2. An introduction of oxygen instead of carbon into the ethylene linker led to the further decrease of the affinity (from K$_i$ = 218 nM to 507 nM). A comparison of the binding modes of compound **13** and its analog **19** with oxygen atom (Fig. 2B) indicated that the methoxyl linker induces a larger rigidity of this fragment of molecule, causing a difficult adaptation to the binding pocket compared to the more flexible analog with the ethylene linker. The branching of the methoxyl linker by one or two methyl groups led to improve the affinity significantly. Molecular docking indicated (Fig. 2C) that methyl substituents better filling the hydrophobic binding cavity formed by helices 5 and 6. A comparison of the binding mode of analogs with different aromatic substituents showed that an increase of the activity may result from an enlargement of hydrophobic surface of a substituent that interacts stronger with the hydrophobic crevice formed by helices 3–5 and extracellular loop 2. This is in line with our previous study among arylmethyl 1,3,5-triazine derivatives [11].

SAR analysis indicted that a new promising group of alkylether-linked aryl triazine derivatives was found in this study as an extension of the previous active group of arylmethyl derivatives. The influence of both, a kind of the linker and a kind of the aromatic substituent, are pivotal for the 5-HT$_6$R interaction of the 1,3,5-triazine derivatives investigated and some balance of linker-aromatic moiety can be observed. However, the population of representative compounds is still not enough to find a regular principle guiding this relationship. Due to the small number of representatives, further studies are necessary to well define an influence of the ether-linker, in "collaboration" with a variety of aromatic substituents, on the 5-HT$_6$R affinity and selectivity. In this context, the most active α-naphthyl derivative with branched ether linker (**24**) could be a lead structure for further chemical modifications.

6. Conclusions

A series of new compounds with a different linker between the triazine and aromatic moieties were designed based on our previous 5-HT$_6$R ligands found among 4-benzyl-1,3,5-triazines. The evaluated linkers showed different influence on the 5-HT$_6$R affinity. The observed positive effect decreased in order: methylene ≥ branched methoxylene > methoxylene > ethylene ≫ vinylene ~ lack of linker. Replacement of the phenyl (4-chlorophenyl) substituent with the naphthyl ring resulted in an increase of the 5-HT$_6$R affinity. Results of the studies accent two following structural factors as beneficial in the search for active ligands: the branched methoxylene linkers and the bulky aromatic system. The role of both factors is worth to be further investigated in separate expanded studies. Summing up, this study allowed to identify a new group of potent 5-HT$_6$R agents among (branched) methoxylene derivatives of 1,3,5-triazines. The most potent member, 4-(4-methylpiperazin-1-yl)-6-(1-(naphthalen-1-yloxy)ethyl)-1,3,5-triazin-2-amine (**24**), seems to be a good lead structure for further modifications.

7. Experimental

Reagents were purchased from Alfa Aesar (Karlsruhe, Germany) or Sigma Aldrich (Darmstadt, Germany). Methanol was dried over calcium oxide. Reaction progress was verified using thin layer chromatography (TLC), which was carried out on 0.2 mm Merck silica gel 60 F254 plates. Spots were visualized by UV light or treatment with Dragendorff reagent. Melting points (mp) were determined using MEL-TEMP II apparatus and are uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were obtained on a Varian Mercury-VX 300 MHz spectrometer or 400 MHz Spectrometer in DMSO-d_6. Chemical shifts in ^{1}H NMR spectra were reported in parts per million (ppm) on the δ scale using the solvent signal as an internal standard. Data are reported as follows: chemical shift, multiplicity (s, singlet; br.s, broad singlet; d, doublet; t, triplet; m, multiplet), coupling constant J in Hertz (Hz), number of protons, proton's position (Ind-indole, Naph- naphthalene, Ph-phenyl, Pp-piperazine). LC-MS were carried out on a system consisting of a Waters Acquity UPLC, coupled to a Waters TQD mass spectrometer. Retention times (t$_R$) are given in minutes. The UPLC/MS purity of all final compounds was determined (%).

7.1. Chemistry

7.1.1. Synthesis of esters

Esters **8a** and **9a** were commercially available. Esters **13a**, **19a**, and **21a–26a** were obtained according the procedure described by Łażewska et al. [11] as seen in Schemes 2a and 2b, and some of them (**13a**, **19a**, **21a**, **22a**, **23a**, **25a**) are known in Chemical Abstract Database:

Methyl 3-phenylpropanoate (**13a**) CAS: 103-25-3. *Methyl 2-phenoxyacetate* (**19a**) CAS: 2065-23-8. *Ethyl (naphthalen-1-yloxy)acetate* (**21a**) CAS: 41643-81-6. *Ethyl (naphthalen-2-yloxy)acetate* (**22a**) CAS: 6036-14-2. *Methyl 2-(4-chlorophenoxy)propanoate* (**23a**) CAS: 18671-89-1. *Ethyl 2-(4-chlorophenoxy)-2-methylpropanoate* (**25a**) CAS: 55162-41-9

7.1.2. Synthesis of triazines (8, 9, 13, 19, 21–26)

Triazines (Table 1) were obtained as presented in Scheme 1 by cyclization of 4-methylpiperazin-1-yl biguanidine dihydrochloride with the appropriate carboxylic acid methyl ester in the presence of sodium methoxide according to the previously published procedure [13] (at reflux: **9**, **13**, **19**, **23**, **25**) or with modification (at room temperature; **9**, **21**, **22**, **24**, **26**). Crystallization from acetonitrile (**21**), acetonitrile/water (**8**), ethyl acetate (**22**), methanol (**9**, **13**, **19**, **23**, **25**, **26**), and methanol/water (**24**) was performed.

7.1.2.1. 4-(4-methylpiperazin-1-yl)-6-(naphthalen-1-yl)-1,3,5-triazin-2-amine (8). Obtained as white solid (Yield 5%) from commercially available methyl 1-naphthoate, mp 173-176 °C. C$_{18}$H$_{20}$N$_6$ (MW 320.39). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.72 (dd, J = 3.52, 5.86 Hz, 1H, Naph-8-*H*), 7.87–8.11 (m, 3H, Naph-2,4,5-*H*), 7.45–7.63 (m, 3H, Naph-3,6,7-*H*), 7.01 (br. s, 2H, N*H$_2$*), 3.76 (br. s, 4H, Pp-2,6-*H$_2$*), 2.33 (br. s, 4H, Pp-3,5-H$_2$), 2.19 (s, 3H, -C*H$_3$*). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 173.23, 167.42, 164.97, 135.98, 133.89, 130.63, 128.73, 128.44, 126.80, 126.55, 126.21, 125.48, 54.83, 46.27, 43.03. LC/MS$^{+/−}$: purity: 100%, t$_R$ = 3.21, (ESI) *m/z* [M + H] $^+$ 321.38.

7.1.2.2. 4-(4-Methylpiperazin-1-yl)-6-(naphthalen-2-yl)-1,3,5-triazin-2-amine (9). Obtained as white solid (Yield 13%) from commercially available methyl 2-naphthoate, mp 230–231 °C. C$_{18}$H$_{20}$N$_6$ (MW 320.39). ^{1}H NMR (300 MHz, DMSO-d_6): δ: 8.86 (s, 1H, Naph-1-*H*), 8.39–8.36 (dd, J = 1.79 Hz, J = 8.71 Hz, Naph-4-*H*), 8.06–8.03 (m, 1H, Naph-3-*H*), 7.99–7.94 (m, 2H, Naph-5,8-*H*), 7.61–7.53 (m, 2H, Naph-6,7-*H*), 6.96 (br.s, 2H, N*H$_2$*), 3.84 (br.s, 4H, Pp-*2,6-H*), 2.25 (s, 4H, Pp-*3,5-H*), 2.20 (s, 3H, Pp-C*H$_3$*). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.13, 167.68, 165.30, 134.97, 132.92, 129.46, 128.46, 128.08, 128.06, 127.82, 126.89, 125.29, 54.93, 46.29, 43.06. LC/MS$^{+/−}$: purity:

100%, t_R = 3.83, (ESI) *m/z* [M + H]$^+$ 321.19.

7.1.2.3. 4-(4-Methylpiperazin-1-yl)-6-phenethyl-1,3,5-triazin-2-amine (13).

Obtained as white solid (Yield 10%) from ester **13a**, mp 137–140 °C. $C_{16}H_{22}N_6$ (MW 298.37). ^1H NMR (300 MHz, DMSO-d_6): δ: 7.29–7.13 (m, 5H, Ph-2,3,4,5,6-*H*), 6.72 (br.s, 2H, N*H₂*), 3.69 (br.s, 4H, Pp-2,6-*H*), 2.96 (def. t, 2H, Ph-CH₂-C*H₂*), 2.67 (def. t, 2H, Ph-C*H₂*), 2.29 (t, *J* = 5.04 Hz, 4H, Pp-3,5-*H*), 2.19 (s, 3H, Pp-C*H₃*). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 176.95, 167.22, 167.22, 164.91, 142.09, 128.73, 128.70, 126.23, 54.85, 46.28, 42.83, 33.00. LC/MS$^{+/−}$: purity: 99.22%, t_R = 2.30, (ESI) *m/z* [M + H]$^+$ 299.26.

7.1.2.4. 4-(4-Methylpiperazin-1-yl)-6-(phenoxymethyl)-1,3,5-triazin-2-amine (19).

Obtained as white solid (Yield 9%) from ester **19a**, mp 128–130 °C. $C_{15}H_{20}N_6O$ (MW 300.36). ^1H NMR (400 MHz, DMSO-d_6): δ: 7.27 (t, *J* = 7.8 Hz, 2H, Ph-*3,5-H*), 7.06–6.90 (m, 5H, Ph-2,4,6-*H* + N*H₂*), 4.77 (s, 2H, OC*H₂*), 3.67 (t, *J* = 4.7 Hz, 4H, Pp-2,6-*H*), 2.28 (br.s, 4H, Pp-3,5-*H*), 2.18 (s, 3H, Pp-C*H₃*). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 173.41, 167.26, 164.77, 158.89, 129.83, 121.06, 115.08, 70.03, 54.76, 46.22, 42.87. LC/MS$^{+/−}$: purity: 100%, t_R = 2.50, (ESI) *m/z* [M + H]$^+$ 301.25.

7.1.2.5. 4-(4-methylpiperazin-1-yl)-6-((naphthalen-1-yloxy)methyl)-1,3,5-triazin-2-amine (21).

Obtained as white solid (Yield 50%) from ester **21a**, mp 153-155 °C. $C_{19}H_{22}N_6O$ (MW 350.42). ^1H NMR (300 MHz, DMSO-d_6) δ: 8.31–8.17 (m, 1H, Naph-8-*H*), 7.91–7.78 (m, 1H, Naph-5-*H*), 7.63–7.41 (m, 3H, Naph-4,6,7-*H*), 7.36 (t, *J* = 7.90 Hz, 1H, Naph-3-*H*), 6.89 (m, 3H, Naph-2-*H* + N*H₂*), 4.98 (s, 2H, -O-C*H₂*), 3.63 (br.s, 4H, Pp-2,6-*H*), 2.32–2.10 (m, 7H, Pp-3,5-*H* + Pp-C*H₃*). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.39, 167.26, 164.77, 154.42, 134.49, 127.84, 126.86, 126.55, 125.62, 125.49, 122.30, 120.54, 106.24, 70.47, 54.70, 46.19, 42.86. LC/MS$^{+/−}$: purity: 100%, t_R = 3.85, (ESI) *m/z* [M + H]$^+$ 351.16.

7.1.2.6. 4-(4-methylpiperazin-1-yl)-6-((naphthalen-2-yloxy)methyl)-1,3,5-triazin-2-amine (22).

Obtained as white solid (Yield 32%) from ester **22a**, mp 119 °C. $C_{19}H_{22}N_6O$ (MW 350.42). ^1H NMR (300 MHz, DMSO-d_6) δ: 7.80 (d, *J* = 8.79 Hz, 2H, Naph-5,8-*H*), 7.73 (d, *J* = 8.21 Hz, 1H, Naph-4-*H*), 7.42 (t, *J* = 7.03 Hz, 1H, Naph-7-*H*), 7.37–7.24 (m, 2H, Naph-3,6-*H*), 7.19 (dd, *J* = 2.34, 8.79 Hz, 1H, Naph-1-*H*), 6.95 (br.s, 2H, N*H₂*), 4.88 (s, 2H, -O-C*H₂*), 3.66 (br.s, 4H, Pp-2,6-*H*), 2.37–2.01 (m, 7H, Pp-3,5-*H* + Pp-C*H₃*). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.21, 167.27, 164.75, 156.77, 134.57, 129.68, 128.97, 127.93, 127.12, 126.81, 124.07, 119.12, 107.71, 70.19, 54.72, 46.19. LC/MS$^{+/−}$: purity: 99.45%, t_R = 3.72, (ESI) *m/z* [M + H]$^+$ 351.16.

7.1.2.7. 4-(1-(4-Chlorophenoxy)ethyl)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-amine (23).

Obtained as white solid (Yield 65%) from **23a**, mp 132–133 °C. $C_{16}H_{21}ClN_6O$ (MW 348.83). ^1H NMR (400 MHz, DMSO-d_6): δ: 7.27 (d, *J* = 8.61 Hz, 2H, Ph-3,5-*H*), 6.96 (br.s, 2H, N*H₂*), 6.87 (d, *J* = 9.0 Hz, Hz, 2H, Ph-2,6-*H*), 4.88 (q, *J* = 6.65 Hz, 1H, OC*H*-CH₃), 3.66 (br.s, 4H, Pp-2,6-*H*), 2.27 (br.s, 4H, Pp-3,5-*H*), 2.18 (s, 3H, Pp-C*H₃*), 1.53 (d, *J* = 6.65 Hz, 3H, CH-C*H₃*). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 176.80, 167.44, 164.92, 157.30, 129.30, 124.54, 117.30, 76.45, 54.69, 46.21, 42.91, 20.73. LC/MS$^{+/−}$: purity: 100%, t_R = 3.58, (ESI) *m/z* [M + H]$^+$ 349.24.

7.1.2.8. 4-(4-methylpiperazin-1-yl)-6-(1-(naphthalen-1-yloxy)ethyl)-1,3,5-triazin-2-amine (24).

Obtained as white solid (Yield 29%) from ester **24a**, mp 134-136 °C. ^1H NMR (300 MHz, DMSO-d_6) δ: 8.32–8.18 (m, 1H, Naph-8-*H*), 7.89–7.75 (m, 1H, Naph-5-*H*), 7.56–7.43 (m, 2H, Naph-6,7-*H*), 7.43–7.36 (m, 1H, Naph-4-*H*), 7.35–7.23 (m, 1H, Naph-3-*H*), 7.08–6.72 (m, 3H, Naph-2-*H* + -N*H₂*), 5.15–5.00 (m, 1H, -C*H*(CH₃)), 3.60 (br.s, 4H. Pp-2,6-*H₂*), 2.33–2.02 (m, 7H, Pp-3,5-*H₂* + Pp-C*H₃*), 1.65 (d, *J* = 6.45 Hz, 3H, C*H₃*). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 177.23, 167.49, 164.97, 154.04, 134.55, 127.79, 126.82, 126.50, 125.68, 125.54, 122.50, 120.35, 106.98, 76.84, 54.66, 46.20, 20.94. LC/MS$^{+/−}$: purity: 100%, t_R = 4.17, (ESI) *m/z* [M + H]$^+$ 365.25.

7.1.2.9. 4-(2-(4-Chlorophenoxy)propan-2-yl)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-amine (25).

Obtained as white solid (Yield 62%) from ester **25a**, mp 137–138 °C. $C_{17}H_{23}ClN_6O$ (MW 362.86). ^1H NMR (400 MHz, DMSO-d_6): δ: 7.22 (d, *J* = 8.99 Hz, 2H, Ph-3,5-*H*), 6.90 (br.s, 2H, N*H₂*), 6.67 (d, *J* = 9.0 Hz, Hz, 2H, Ph-2,6-*H*), 3.66 (br.s, 4H, Pp-2,6-*H*), 2.26 (br.s, 4H, Pp-3,5-*H*), 2.18 (s, 3H, Pp-C*H₃*), 1.59 (s, 6H, C-(C*H₃*)₂). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 178.66, 167.57, 165.05, 155.25, 129.14, 124.93, 120.71, 81.36, 54.70, 46.22, 42.93, 26.95. LC/MS$^{+/−}$: purity: 100%, t_R = 3.76, (ESI) *m/z* [M + H]$^+$ 363.26.

7.1.2.10. 4-(4-methylpiperazin-1-yl)-6-(2-(naphthalen-1-yloxy)propan-2-yl)-1,3,5-triazin-2-amine (26).

Obtained as creamy solid (Yield 10%) from ester **26a**, mp 210-213 °C. $C_{21}H_{26}N_6O$ (MW 378.47). ^1H NMR (300 MHz, DMSO-d_6) δ: 8.30–8.15 (m, 1H, Naph-8-*H*), 7.90–7.73 (m, 1H, Naph-5-*H*), 7.56–7.32 (m, 3H, Naph-4,6,7-*H*), 7.29–7.14 (m, 1H, Naph-3-*H*), 6.90 (br.s, 2H, -N*H₂*), 6.48 (d, *J* = 7.62 Hz, 1H, Naph-2-*H*), 3.63 (br.s, 4H, Pp-2,6-*H₂*), 2.35–2.06 (m, 7H, Pp-3,5-*H₂* + Pp-C*H₃*), 1.70 (s, 6H, 2x C*H₃*). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 179.07, 167.63, 165.13, 151.76, 134.71, 127.83, 127.36, 126.55, 126.08, 125.47, 122.86, 120.40, 111.0, 81.54, 54.70, 46.22, 42.93, 26.93. LC/MS$^{+/−}$: purity: 99.33%, t_R = 4.32, (ESI) *m/z* [M + H]$^+$ 379.21.

7.2. Radioligand binding studies

7.2.1. Radioligand binding assay for serotonin 5-HT₆ receptor

The affinity of the synthesized compounds for human serotonin 5-HT₆R stably expressed in HEK293 cells in radioligand binding assays *via* the displacement of [^{3}H]-LSD was performed as described previously [11].

7.2.2. Intrinsic activity for human serotonin 5-HT₆ receptor of compounds 5, 12, 24 and 26

The functional properties of compounds on 5-HT₆R were evaluated using their ability to inhibit cAMP production induced by 5-CT (1000 nM) – a 5-HT₆R agonist. Compounds were tested in triplicate at 8 concentrations (10^{-11}–10^{-4} M). The level of adenylyl cyclase activity was measured using frozen recombinant 1321N1 cells expressing the Human Serotonin 5-HT₆R (PerkinElmer). Total cAMP was measured using the LANCE cAMP detection kit (PerkinElmer), according to the manufacture's directions. For quantification of cAMP levels, cells (5 μL) were incubated with mixture of compounds (5 μL) for 30 min at room temperature in 384-well white opaque microtiter plate. 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 (TR-FRET) was detected by an Infinite M1000 Pro (Tecan) using instrument settings from LANCE cAMP detection kit manual. K_b values were calculated from Cheng–Prusoff equation [18] specific for the analysis of functional inhibition curves: $K_b = IC_{50}/(1 + A/EC_{50})$ where A is an agonist concentration, IC_{50} is the concentration of antagonist producing a 50% reduction in the response to agonist, and EC_{50} is the agonist concentration which causes a half of the maximal response.

7.2.3. Radioligand binding studies for serotonin receptors (5HT₁ₐ, 5HT₇) and dopamine D₂

Tests for human serotonin (5-HT₂ₐ and 5-HT₇) and human dopamine D₂ receptors were conducted in the radioligand binding assays as described by Canale et al. [19].

7.3. Molecular modeling studies

The building of homology models of 5-HT$_6$ receptor, and validation of these models on the 1,3,5-triazine derivatives were previously described [11]. Since 5-HT$_6$R homology models built on β$_2$ adrenergic template showed coherent for the whole set of compounds, and explained the main structure-activity relationships, they were used in this study.

3-Dimensional structures of the ligands were prepared using LigPrep v3.6 [20], and the appropriate ionization states at pH = 7.4 ± 1.0 were assigned using Epik v3.4 [21]. Compounds with stereogenic centres obtained as racemates (**23** and **24**) or those with unknown Z/E configurations (**14**–**18**) were docked in all possible, R and S or Z and E, configurations, respectively. One low energy ring conformation per ligand was generated. The Protein Preparation Wizard was used to assign the bond orders, appropriate amino acid ionization states and to check for steric clashes. The receptor grid was generated (OPLS3 force field [22]) by centring the grid box with a size of 12 Å on D3.32 side chain. Automated flexible docking was performed using Glide v6.9 at SP level [23].

Acknowledgements

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