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The role of aryl-topology in balancing between selective and dual 5-HT₇R/5-HT_{1A} actions of 3,5-substituted hydantoins†

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In order to search for active and selective serotonin 5-HT₇R antagonists among 3,5-disubstituted arylpiperazine-imidazolidine-2,4-diones, the role of the introduction/deletion and the mutual orientation of aromatic rings was analyzed. Chemical modifications of 2nd generation lead structure of 3-(3-(4-(diphenylmethyl)piperazin-1-yl)-2-hydroxypropyl)-5-(4-fluorophenyl)-5-methylimidazolidine-2,4-dione (2, KKB16) were performed. New derivatives (4–18) were designed and synthesized. X-ray crystallographic analysis of the representative compound 5-(4-fluorophenyl)-3-[2-hydroxy-3-(4-phenylpiperazin-1-yl)propyl]-5-methylimidazolidine-2,4-dione (3) was performed to support molecular modeling and SAR studies. The affinity for 5-HT₇R, D₂R and 5-HT_{1A}R in radioligand binding assays for the entire series and ADME-Tox parameters *in vitro* for selected compounds (7, 10, and 13) were evaluated. Molecular docking and pharmacophore model assessment were performed. According to the obtained results, 5-methyl-5-naphthylhydantoin derivatives were found to be the new highly active 5-HT₇R agents ($K_i \leq 5$ nM) with significant selectivity over 5-HT_{1A}R and D₂R. On the contrary, the (1-naphthyl)piperazine moiety was gained with the potent dual 5-HT₇R/5-HT_{1A}R action (K_i : 11 nM/19 nM).

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Introduction

Targeting receptor 5-HT₇, the youngest member of the serotonergic system,^{1–3} seems to be a promising approach in terms of treatment of CNS disorders, such as depression⁴ and schizophrenia-like cognitive impairments.⁵ However, when considering the design of 5-HT₇R ligands, the same dilemma still remains, *viz.*, whether selective^{6,7} or multitargeted therapy^{8,9} is the better solution for CNS patients. The selective mechanism of action targeting only the desired protein gives hope for decreased probability of side-effects manifestation caused by interactions with off-targets. By going deeper according to the functional selectivity approach, it is possible

to activate not only the chosen biological target, but also the specific signal transduction pathway.⁶ It is also worth mentioning that only one selective 5-HT₇R ligand, compound JNJ-18038683, has been studied in clinical trials; however, unfortunately, its antidepressant action has not been proved due to the lack of sensitivity of performed studies.⁷ Thus, searching a potent and selective 5-HT₇R ligand seems to be a significant direction in order to verify potential advantages and disadvantages of selective therapy.

On the contrary, polypharmacology is suggested to be an appropriate solution to achieve high efficacy complex therapy, *e.g.*, for mood disorders and schizophrenia^{8,9} or in both cancer and CNS diseases.¹⁰ Some trends in search for so called “magic shotguns” against most common central nervous system disorders¹¹ indicate the importance of serotonin and dopamine targets apart from 5-HT₇, 5-HT_{1A}⁸ and D₂⁹ receptors. However, lines of evidence demonstrate various dual- and multitarget acting compounds that are useful against CNS-disorders, which involve serotonergic receptors and other GPCRs, *e.g.*, muscarinic M₄ receptors against schizophrenia¹² or “non-monoaminergic” mechanisms, *e.g.*, neurokinin NK₁ antagonists against depression.¹³ To the date, 5-HT₇R antagonists acting in multidirect ways are available in the pharmaceutical market, *e.g.*, vortioxetine, approved in 2013 by FDA for the treatment of major depressive

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disorders^{14,15} and lurasidon, the second generation antipsychotic drug.¹⁶

Our previous studies led to the synthesis of the 30-membered group of hydantoin-derived 5-HT₇ ligands.^{17–19} Nineteen of them are highly active ($K_i < 100$ nM) and selective over other GPCRs (5-HT_{1A}, D₂, 5-HT₇, α_{1A} , α_{2B} , β_1 -adrenoceptors). Behavioral studies (Porsolt's test) performed for selected compounds confirmed their antidepressant activity in mice. It is worth noting that it is not the 1st generation lead (MF-8, 1, Fig. 1) that had the strongest affinity towards 5-HT₇,^{18,20} but the lead derivative (KKB16, 2, Fig. 1) with the highest metabolic stability that turned up to cause the most significant antidepressant effect.²¹ Moreover, compound 2 also showed the highest selectivity over 5-HT_{1A}R (71-fold) within the entire series. According to the SAR analysis which included compound 2, in comparison to the majority of derivatives with (un)substituted phenyl rings,^{18,19} the observed selectivity seemed to be a consequence of the presence of diphenylmethyl group linked to piperazine.

Hence, the aforementioned issues are worth to be further studied in order to verify (i) how the changes in the number and the (mutual) spatial orientation of aromatic rings influence the activity and selectivity for 5-HT₇R among hydantoin-derived ligands and (ii) the importance of the diphenylmethyl group for beneficial metabolic stability. For this purpose, the diphenylmethyl derivative (2) was selected as the 2nd generation lead structure to be modified within “antipodal” aromatic-containing areas (blue, Fig. 1). Leads of both generations 1 and 2 and the previously investigated 5-phenyl derivative 3 (ref. 18) (Table 1) were used as reference compounds for these studies. Fifteen novel derivatives of lead 2 were designed and synthesized (4–18, Table 1). These new compounds were tested in the radioligand binding assay in order to assess the affinity towards 5-HT₇R, 5-HT_{1A} and D₂. X-ray crystallographic analysis of 3 was also performed. To elucidate the differences in 5-HT₇R activity and selectivity, molecu-

lar modeling, docking- and pharmacophore-based studies were performed. Selected compounds (7, 10 and 13) were examined for their metabolic stability and toxicity *in vitro* in comparison to both leads (1 and 2).

Results and discussion

Synthesis

The designed new derivatives (4–18) were obtained based on the previously optimized three-step pathway¹⁹ starting from Bucherer–Bergs reaction, which led to the hydantoin system formation, followed by *N*-alkylation with epichlorohydrin and solvent-free microwave-assisted condensation with simultaneous epoxide opening (Scheme 1). Compounds 15–17 were synthesized in two steps using commercially available 5,5-dimethylhydantoin as the starting material. Pure products were isolated during the pH-dependent extraction. This method allowed to eliminate the time- and cost-consuming purification by column chromatography used previously.^{18,19} All the compounds were obtained as racemic mixtures due to the non-stereoselectivity of Bucher–Bergs reaction and the application of ($\pm$)-2-(chloromethyl)oxirane as an alkylating agent.

X-ray crystallographic studies

It was hard to obtain any crystals suitable for the X-ray analysis from piperazine-hydantoin derivatives obtained as modifications of lead 1. In particular, the basic form rarely precipitated during the synthesis work up. Earlier, 5-phenyl-3-(2-hydroxy-3-(4-(2-ethoxyphenyl)piperazin-1-yl)propyl)-5-methylimidazolidine-2,4-dione hydrochloride was the only compound that gave sufficient crystals for the X-ray analysis, but in the salt form.¹⁹ The present study successfully provided the first representative crystal of the basic form (compound 3), which was appropriate for X-ray crystallographic analysis that allowed us to extend the knowledge about the 3D-

Fig. 1 Lead structures (MF-8, KKB-16) and areas of their modifications explored within this study.

Table 1 Structures and radioligand binding results for compounds 1–18

				K_i [nM]		
				D_2R^1	5-HT _{1A} R ²	5-HT ₇ R ³

Cpd	Group	R ¹	R ²	D_2R^1	5-HT _{1A} R ²	5-HT ₇ R ³
1 ^a	A	4-Fluorophenyl	2-MeO-phenyl	715	121	3
2 ^a	A	4-Fluorophenyl	Diphenylmethyl	261	5570	79
3 ^a	A	4-Fluorophenyl	Phenyl	2906	2733	223
4	A	4-Fluorophenyl	Benzyl	20 030	377 500	2085
5	A	4-Fluorophenyl	Benzoyl	10 080	6216	3609
6	A	4-Fluorophenyl	(Naphthalene-1-yl)methyl	5233	5577	2172
7	A	4-Fluorophenyl	1-Naphthyl	295	19	11
8	B	4-Fluorophenyl	Phenoxy	4353	5211	165
9	B	4-Fluorophenyl	4-Cl-Phenoxy	4116	21 470	172
10	A	1-Naphthyl	2-MeO-phenyl	256	326	5
11	A	1-Naphthyl	2-CN-phenyl	416	1225	19
12	A	1-Naphthyl	Diphenylmethyl	273	413 200	224
13	A	2-Naphthyl	2-MeO-phenyl	153	128	3
14	A	2-Naphthyl	2-CN-phenyl	264	129	3
15	A	Methyl	2-MeO-phenyl	2130	489	125
16	A	Methyl	2-CN-phenyl	3429	1155	209
17	A	Methyl	Diphenylmethyl	1152	15 150	824
18	C	4-Fluorophenyl	—	5848	1551	888
Ref ^{a-c}				9 ^a	20 ^b	18 ^c

^a Compounds from the previously published and pharmacologically described series. ^{1–3} Radioligands used: [³H]-raclopride (D_2R), [³H]-8-OH-DPAT (5-HT_{1A}R), [³H]-5-CT (5-HT₇R). ^{a–c} Reference ligands for GPCRs investigated: ^aolanzapine, ^bbupirone, ^cclozapine, nt - not tested.

properties of this chemical group and also supported molecular modeling.

The molecular geometry of the crystal structure of compound 3 with the atom numbering scheme is shown in Fig. 2.

Scheme 1 Synthesis pathway for compounds 4–18: (i) KCN, (NH₄)₂CO₃, 50% EtOH, 50 °C, 24 h; (ii) 2-(chloromethyl)oxirane, NaOH, H₂O, rt, 10 h; (iii) R₂-commercially available derivative of piperazine/piperidine or isohexahydroquinoline, solvent-free reaction, microwave irradiation 300–450 W, 2 min.

The flexible aliphatic linker, which binds hydantoin and piperazine rings, shows an extended conformation, with the torsion angles being N3–C6–C7–C8 = 179.4(2)° and C6–C7–C8–N2 = 147.8(2)°. The larger deviation of one value from 180° was due to the intramolecular contact C9–H9A...O1, for which the following parameters were observed: H9A...O1 = 2.59 Å, C9...O1 = 3.128(3) Å and the angle C9–H9A...O1 = 114°. The mutual orientation of the 4-fluorophenyl substituent at C5 and hydantoin ring differed in comparison to the geometry of the aforementioned (2-ethoxyphenyl)piperazine compound with the phenyl ring at C5 atom, as determined earlier.¹⁹ The angle between the planes of the aromatic ring and the hydantoin ring was 62.2(1)°, while it was 86.73(5)° in the compared compound.

In the presented structure, the piperazine ring adopts chair conformation, where the substituents at N2 and N4

Fig. 2 The molecular structure of compound 3 showing the atom numbering scheme. Displacement ellipsoids are drawn at the 30% probability level.

were equatorial. The torsion angles of C14–C13–N4–C10 and C18–C13–N4–C10 were 25.7(3)° and –157.3(3)°, respectively, which indicated that the phenyl ring at N4 atom was almost coplanar with the piperazine moiety. The angle between the planes of aromatic and piperazine (C9, C10, C11, and C12) rings was 10.3(2)°. We observed a higher value of the corresponding angle of 26.8(2)° for another hydantoin derivative with a *N*-phenylpiperazine moiety.²²

The main intermolecular interactions were based on O–H···O and N–H···N hydrogen bonds (Fig. 3). One oxygen atom (O2) of hydantoin was involved in the hydrogen bond with hydroxyl group, whereas the second oxygen atom (O4) was involved in C–H···O intermolecular interactions. The nitrogen atom (N1) of hydantoin was engaged in the hydrogen bond with the nitrogen atom (N2) of piperazine ring. Furthermore, the fluorine atom made C–H···F contacts. The parameters of these interactions are listed in Table 2.

Radioligand binding assays

Radioligand competition binding assays were applied to determine affinity and selectivity profiles of the newly synthesized compounds (4–18) for human serotonin 5-HT₇R, 5-HT_{1A}R, and dopaminergic D_{2L}R, which stably expressed in HEK-293 cells (Table 1). Five compounds from the series (7, 10, 11, 13, and 14) were highly active toward 5-HT₇R (3 nM ≤ *K_i* ≤ 19 nM), whereas the other five (8, 9, 12, 15, and 16) demonstrated rather moderate activity (125 nM ≤ *K_i* ≤ 224 nM). Moreover, almost all the most active 5-HT₇R agents (8–16), excluding compound 7, showed selectivity over 5-HT_{1A}R and D₂R. The active compound 7 also had potent affinity toward 5-HT_{1A}R (*K_i* = 19 nM).

ADME-Tox studies *in vitro*

Preliminary *in vitro* studies on ADME-Tox parameters were performed for the selected most active 5-HT₇R ligands (7, 10 and 13) in comparison to the results for both leads (1 and 2)

Fig. 3 Packing of molecules in the unit cell projected along [010] direction. Dashed lines indicate hydrogen bonds.

Table 2 The parameters of intermolecular interactions in structure 3

D–H···A	H···A (Å)	D···A (Å)	D–H–A (°)	Symmetry code
N1–H1N···N2	2.08(4)	2.981(3)	170(3)	–x + 1, y – 1/2, –z – 1/2
O1–H1···O2	1.90(4)	2.756(3)	167(4)	–x + 1, y – 1/2, –z – 1/2
C8–H8B···F1	2.61	3.319(3)	128.6	–x + 1, –y + 1, –z
C11–H11A···F1	2.77	3.464(3)	127.6	–x + 1, –y + 1, –z
C21–H21B···O4	2.36	3.334(3)	169.9	–x + 1, –y, –z
C26–H26···O1	2.81	3.498(4)	130.3	–x + 1, –y, –z
C27–H27···O4	2.50	3.368(4)	152.4	–x + 1, –y, –z

and the metabolically stable drug, aripiprazole. For this purpose, mouse liver microsomes (MLMs) and human embryonic kidney (HEK-293) or *hepatoma* (HepG2) cell lines were used according to previously described protocols.^{20,21}

Metabolic stability

The incubation of 5-HT₇R ligands (2, 7, 10 and 13) in the presence of MLMs, followed by UPLC-MS analyses, involving ion fragmentation and supported by *in silico* simulation with MetaSite software, allowed to determine the metabolic stability, metabolic pathways and the most probable structures of 5-HT₇R ligands' metabolites (details in ESI† Table S3, Fig. S1–S12).

Regarding the metabolic stability, the new compounds 7, 10 and 13 were less stable than aripiprazole (Table S3, Fig. S1–S9 vs. Fig. S10, ESI†). It was seen that ~65–70% of compounds 7 and 13 were metabolized into four or six metabolites, respectively, whereas ~40% of compound 10 was metabolized into four metabolites. However, the metabolic stability of 10 was the closest to the most stable lead 2 (~20% remaining, Table 3) that metabolized into one metabolite (Table S3, Fig. S11 and S12, ESI†).

Furthermore, the metabolic stability of the lead 2 and compounds 7, 10 and 13 were determined by pharmacokinetic *in vitro* studies performed using MLMs in order to calculate the half-life period (*t*_{1/2}) and hepatic metabolic clearance CL_{int} and compared to the previously obtained data for the reference aripiprazole and the 1st generation lead 1 (ref. 20) (Table 3).

The comparison of pharmacokinetic values (*t*_{1/2}, CL_{int}) for both leads (1 (ref. 20) and 2) showed their similar metabolic stabilities in MLMs. In contrast, the newly synthesized derivatives (7, 10 and 13) were less stable than both leads (1 and 2) and the reference aripiprazole, but they demonstrated rather satisfying values of both *t*_{1/2} and CL_{int}. The CL_{int} value of the most stable compound 10 was only ~1.7-fold higher than that of the leads (1 and 2) and ~2.3-fold higher than that of metabolically stable aripiprazole, whereas the CL_{int} of the most labile compound 7 was ~3.5-fold higher than that of both leads and almost 5-fold higher in comparison to the value of aripiprazole (Table 3).

Toxicity

Compounds 7, 10 and 13 were tested for their safety in HEK-293 and HepG2 cell lines at four concentration

Table 3 Pharmacokinetic properties of 5-HT₇R ligands and the reference drug aripiprazole, estimated *in vitro* in MLMs

	Aripiprazole	1 ^a	2	7	10	13
<i>t</i> _{1/2} (min)	217.0 ^a	157.5 ^a	157.5	45.0	94.0	62.5
Mouse CL _{int} (ml min ⁻¹ kg ⁻¹)	12.5 ^a	17.2 ^a	17.2	60.3	29.0	43.4
% of remaining substrate after 120 min of incubation	ND ^b	ND ^b	~20	~65	~40	~70

^a Values in MLMs estimated previously.²⁰ ^b ND = not determined.

intervals (0.1–100 μM) for 72 h. The antiproliferative drug doxorubicin (DX) was used as a positive control at 1 μM. Additionally, the mitochondrial toxin carbonyl cyanide 3-chlorophenylhydrazone (CCCP) was used at 10 μM for assays with HepG2 (Fig. 4).

All the examined compounds significantly influenced the viability of both cell lines, but only at the highest used dose (100 μM). However, the comparison of the obtained results with the effect of DX at 1 μM or CCCP at 10 μM indicated rather weak cytotoxic and hepatotoxic effects of the examined 5-HT₇R ligands (Fig. 4). Regarding the previously described effects of leads 1 and 2 on HEK-293 cell viability,^{20,21} compounds 7, 10 and 13 were in the range of toxicity of lead 2.

Molecular modeling and SAR analysis

To support the discussion about aromatic ring localization and its influence on affinity towards 5-HT₇R, appropriate interfeature distances were measured and compared with known pharmacophore model requirements.²³ Moreover, all the compounds (1–18) were docked to homology models of 5-HT₇R in order to define key protein–ligand interactions. Additionally, due to the occurred 5-HT₇/5-HT_{1A} dual action for compound 7, docking studies with 5-HT_{1A}R homology models were also applied.

Previously, we confirmed¹⁹ that analogs of lead structure MF-8 (1) fitted very well to the pharmacophore model of 5-HT₇R antagonists reported by López-Rodríguez *et al.*²³ For this model, the optimal distance between the basic center (N2) and the hydrophobic (aromatic) area (ring centroid) was 5.4–6.4 Å; thus, the arylpiperazine moiety perfectly met this requirement (Fig. 5A). Radioligand binding results showed a significant relationship between N2-ring centroid distances and 5-HT₇R affinities of compounds 2–8 and 18. Both an increase and a decrease of this distance beyond the optimum range resulted in a significant reduction of 5-HT₇R affinity. For example, compounds 4 (Fig. 5B) and 6 (Fig. 5C) with N2-ring centroid distances >6.4 Å showed *K*_i = 2085 nM and 2172 nM, respectively, while compound 18 (Fig. 5D) with N2-ring centroid distance <5.4 Å was also less active (*K*_i = 888 nM). Intriguing results were observed for the diphenylmethyl-

Fig. 4 The effect of compounds 7, 10, 13 and doxorubicin (DX) on HEK-293 viability after 72 h of incubation (A). The effect of compounds 7, 10, 13, and doxorubicin (DX) and carbonyl cyanide 3-chlorophenylhydrazone (CCCP) on HepG2 viability after 72 h of incubation (B). The statistical significance was evaluated by a one-way ANOVA, followed by Bonferroni's comparison test (***) *p* < 0.001 compared with negative control).

Fig. 5 Comparison of distances between the basic center (N2) and aromatic centroids (presented as green lines and expressed in [Å]) for compounds: 3 (A), 4 (B), 6 (C), 18 (D), 2 (E) and 7 (F).

containing lead 2 ($K_i = 79$ nM, Fig. 5E) in comparison to the inactive benzyl compound (4, Fig. 5B). The presence of an additional aromatic ring changed the geometry, such that one of the phenyl groups was closer to the basic center (6.30 Å), resulting in a significant 5-HT₇R activity improvement. Moreover, not only the presence of the additional phenyl ring, but also the distance between these two phenyl rings was beneficial (*e.g.* compound 2 *vs.* 6). Fused rings, as is observed in naphthalene moiety, linked to piperazine *via* a methylene group (6, Fig. 5C), resulted in a strong decrease in the 5-HT₇R affinity, whereas the introduction of diphenylmethyl group (2, 12, and 17) improved the 5-HT₇R affinity.

Moreover, the naphthyl group linked directly to piperazine (7, Fig. 5F) provided very high affinity ($K_i = 11$ nM) and additionally, this structure turned out to be the first dual 5-HT₇/5-HT_{1A} ligand coming from the present and previously described studies.^{18,19}

The analysis of binding modes for compounds 4, 6, 7 and 18 (Fig. 6A) showed the influence of the above-discussed distances on receptor–ligand interactions. Although all compounds (4, 6, 7 and 18) interacted with the aromatic cluster formed by Phe6.51 and Phe6.52, the significant decrease in activity for compounds 4, 6, and 18 seemed to be a consequence of the loss of the salt bridge with Asp3.32, which is the key interaction linked with high 5-HT₇R affinity that is maintained only for the most active compound (7).

Recently, we also proved that the introduction of an additional phenyl ring into position 5 of hydantoin caused a significant decrease in 5-HT₇R affinity.¹⁹ Additionally, the exchange of phenyl to methyl group (resulting in 5,5-dimethylhydantoin moiety) led to the decrease in 5-HT₇R activity ($K_i = 125$ nM for 15 *vs.* $K_i = 3$ nM for the 1st generation lead 1). However, this decrease is not as severe as in the case of the presence of two phenyl rings (5,5-diphenylhydantoin,

Fig. 6 (A) Influence of the basic center–aromatic centroid distance on the binding modes for compounds 4 (brown), 6 (pink), 7 (red) and 18 (olive); (B) the comparison of binding modes of lead structure, 1 (yellow) and its analogs with 2-naphthyl hydantoin moiety – 10 (cyan) and dimethylhydantoin – 15 (violet); (C) the comparison of binding modes of 1-naphthyl and 2-naphthyl hydantoin derivatives with (2-cyanophenyl)piperazine moiety – 11 (orange) and 14 (green).

Fig. 7 Binding mode of compound 7 in 5-HT₇R (A) and 5-HT_{1A}R (B) homology models.

189-fold decrease with respect to 1).¹⁹ Interestingly, the exchange of the phenyl ring at position 5 of hydantoin to 1- or 2-naphthyl was beneficial and did not disrupt the high 5-HT₇R affinity, making compounds 10, 11, 13 and 14 the most active in the entire series (4–18). For the naphthyl derivatives with the (2-methoxyphenyl)piperazine fragment (10 and 13), the 5-HT₇R affinity was almost identical as that for the 1st generation lead 1. No visible difference in 5-HT₇R affinity between compounds 10 and 13 seemed to be due to the very well fitting of both aromatic moieties (1-naphthyl and 2-naphthyl) into the hydrophobic pocket formed by ECL2 and TM2, 3, and 7 (Fig. 6B). In comparison, the absence of the aromatic group in the case of compound 15 (with methyl group) lacked this additional stabilization, which resulted in decreased activity. For compounds with the (2-cyanophenyl)piperazine moiety (11 and 14), a slight difference was visible, indicating that 2-naphthyl (14) was more preferable relative to 1-naphthyl (11) group. Molecular docking of this pair of compounds (11 and 14) resulted in a coherent binding mode to 5-HT₇R. However, there was a slight difference in hydantoin orientation, which might have caused the weaker stabilization of this moiety by Arg7.36 (reported previously by our group¹⁹) for compound 11, resulting in ~4-fold lower 5-HT₇R affinity than that for compound 14 (Fig. 6C).

It is worth mentioning that the introduction of 1- and 2-naphthyl groups instead of the 4-fluorophenyl ring allowed the retention of 5-HT₇R selectivity over 5-HT_{1A}R and D₂R. However, the selectivity was slightly less than that in the cases of both 1st generation lead 1 (the highest 5-HT₇/D₂ selectivity ratio) and 2nd generation lead 2 (the highest 5-HT₇/5-HT_{1A} selectivity ratio).

As the naphthylpiperazine derivative (7), molecular docking to 5-HT_{1A}R and 5-HT₇R homology models confirmed that structure 7 fitted very well into both binding pockets and formed the following interactions: (i) a salt bridge with Asp3.32 and (ii) CH- π/π - π interaction between naphthyl group linked with piperazine and Phe6.52 side chain (Fig. 7). In comparison to the binding mode of dual ligands to 5-HT_{1A}R from different chemical classes (2-benzoxazolones and 2-benzothiazolones) published previously,²⁴ compound 7 also

interacted with Tyr7.43, while the hydrogen bond formation with Trp7.40 and Pro144 from EL2 was not observed. Regarding the docking to 5-HT₇R, the hydantoin moiety was hydrogen bonded by Arg7.43, which was similar to that observed for the terminal heterocycle moiety of above-mentioned 2-benzoxazolone and 2-benzothiazolone derivatives.

The size, type and spatial orientation of the aromatic rings influenced not only the activity and selectivity, but also the metabolic stability of the investigated series (1–18). The lead 2, with two unfused phenyl rings linked *via* a methylene group to the piperazine and with mono-aromatic substituents at position 5 of hydantoin, has shown the highest metabolic stability, predominantly in humans,²¹ but also in mouse liver microsomes. On the contrary, the presence of fused naphthyl rings either on piperazine (7) or hydantoin (10 and 13) sides resulted in some decrease in metabolic stabilities, particularly in the case of the β -naphthyl derivative (13). On the contrary, the introduction of the naphthyl moiety, regardless of the topology, did not significantly affect the safety, which was in the range of that for the lead 2 for all the new derivatives (7, 10 and 13).

Conclusions

Based on the 2nd generation lead (KKB16, 2) and SAR analysis of the previous series, we have enriched the library of hydantoin-derived 5-HT₇R ligands by introducing 15 new members with diverse receptor affinities, particularly potent in the case of 5-naphthylhydantoin derivatives. The performed X-ray analysis, molecular modeling and consequently, the comprehensive SAR-studies enabled to validate the role of changes in the number and (mutual) spatial orientation of aromatic rings for the 5-HT₇R activity and selectivity. We proved that the 1- and 2-naphthyl moieties in position 5 of hydantoin were crucial features for the potent action on 5-HT₇R while maintaining distinct selectivity towards 5-HT_{1A}R and D₂R. In turn, the introduction of 1-naphthyl group onto the piperazine side resulted in significant 5-HT₇R and 5-HT_{1A}R dual action. The ADMET studies performed *in vitro* for selected compounds (7, 10, and 13) confirmed their low

cytotoxicity. The precise pharmacokinetic studies *in vitro* revealed a generally lower metabolic stability of naphthyl derivatives (7, 10, and 13) in comparison to that of leads 1 and 2 and aripiprazole. However, the result was more satisfying from “druglikeness” point of view for 1-naphthyl derivatives, particularly in the case of the naphthylpiperazine derivative (7). Bearing these results in mind, it would be worthy to further investigate whether both the dual receptor action and lower (compared to the leads) metabolic stability *in vitro* are reflected in the pharmacological action *in vivo*, with special respect to antidepressant properties.

Material and methods

Synthesis

^1H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury VX 300 MHz PFG instrument (Varian Inc., Palo Alto, CA, USA) in DMSO- d_6 at ambient temperature using the solvent signal as an internal standard. Data are reported using following abbreviations: s, singlet; bs, broad singlet; d, doublet; t, triplet; m, multiplet; Ph, phenyl; Pp, piperazine; and Ar, aromatic. Thin-layer chromatography was performed on pre-coated Merck silica gel 60 F $_{254}$ aluminum sheets, and the solvent system used was methylene chloride/methanol 95:5. The mass of compounds 4–24 were recorded on a Waters ACQUITYTM UPLC (Waters, Milford, MA, USA) coupled to a Waters TQD mass spectrometer (electrospray ionization mode, ESI-tandem quadrupole). Retention times (t_R) are given in minutes. The UPLC/MS purities of all final compounds were determined (%). Syntheses under microwave irradiation were performed in a Samsung MW71B household microwave oven. Procedures for the preparation of 5-methyl-5-naphthylhydantoins (20, 21) and 5-(4-fluorophenyl)-5-methylhydantoin (19) and their spectral data have already been published.^{17,25,26} Procedure for the *N*-alkylation has been described before.^{19,27} Physicochemical data for oxirane 22 is available in the literature²⁷ and that for oxiranes 23 and 24 can be found in Supplementary material.

General procedure for the synthesis of final compounds (4–18)

5-(4-Fluorophenyl)-5-methyl-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione (17, 3.5 mmol, 1.0 eq.) with the appropriate piperazine derivative (3.0 mmol, 0.9 eq.) in a 50 mL flat-bottom flask were irradiated in a household microwave (450 MW, 2–3 min). The progress of the reaction was monitored by TLC (DCM/MeOH 95:5). The crude product was dissolved in methylene chloride. The resultant organic phase was washed with 2% aqueous HCl solution, followed by water, and then dried over Na₂SO₄, filtered through cotton and concentrated under vacuum to obtain the desired product. Majority of the final products were transformed into hydrochloride salts by dissolving in 3–4 mL of 1.25 M HCl solution in ethanol. After 30 minutes of stirring at room temperature, the resultant precipitates were filtered, washed with 2-propanol and dried.

3-[3-(4-Benzylpiperazin-1-yl)-2-hydroxypropyl]-5-(4-fluorophenyl)-5-methylimidazolidine-2,4-dione hydrochloride (4). White solid. Yield 32%. LC/MS $\pm$: purity 98.05% t_R = 3.69, (ESI) m/z [M + H] 441.29. 10.44 (s, 1H, NH⁺), 9.01 (s, 1H, N¹H), 7.56–7.52 (m, 2H, Ar), 7.51–7.43 (m, 5H, Ar), 7.31–7.19 (m, 2H, Ar), 5.94 (s, 1H, CHOH), 4.23 (s, 1H, CHOH), 3.65–3.28 (m, 10H, N₃–CH₂, Ph–CH₂, Pp–2,6-H), 3.21–3.04 (s, 4H, Pp–CH₂, Pp–3,5-H), 1.72 (s, 3H, CH₃). ^{13}C NMR δ (ppm): 175.89, 169.58, 163.42, 160.99, 155.95, 155.86, 136.35, 136.32, 136.28, 135.19, 130.47, 128.98, 128.34, 128.27, 127.58, 115.81, 115.60, 62.95, 62.46, 59.35, 42.47, 25.95.

3-[3-(4-Benzoylpiperazin-1-yl)-2-hydroxypropyl]-5-(4-fluorophenyl)-5-methylimidazolidine-2,4-dione hydrochloride (5). White solid. Yield 29%. LC/MS $\pm$: purity 96.84% t_R = 3.59, (ESI) m/z [M + H] 455.31. ^1H NMR δ (ppm): 10.54 (s, 1H, NH⁺), 9.02 (s, 1H, N¹H), 7.56–7.53 (m, 2H, Ar), 7.37–7.12 (m, 7H, Ar), 5.98 (s, 1H, CHOH), 4.53 (m, 1H, CHOH), 4.35 (s, 2H, N₃–CH₂), 3.80–3.32 (m, 6H, Pp–3,5-H, Pp–CH₂), 3.30–2.92 (m, 4H, Pp–2,6-H), 1.72 (s, 3H, CH₃). ^{13}C NMR δ (ppm): 175.91, 175.87, 163.42, 160.99, 155.97, 155.89, 136.35, 128.94, 128.35, 128.27, 128.03, 127.03, 115.81, 115.59, 65.37, 63.42, 62.98, 62.95, 58.84, 58.73, 25.51, 15.63.

5-(4-Fluorophenyl)-3-(2-hydroxy-3-[4-[(naphthalen-1-yl)-methyl]piperazin-1-yl]propyl)-5-methylimidazolidine-2,4-dione (6). White solid. Yield 43%. LC/MS $\pm$: purity 95.24% t_R = 4.77, (ESI) m/z [M + H] 491.34. ^1H NMR δ (ppm): 9.02 (s, 1H, N¹H), 8.43 (s, 1H, Ar), 8.11–7.99 (m, 2H, Ar), 7.95 (s, 1H, Ar), 7.73–7.48 (m, 5H, Ar), 7.22 (t, J = 8.9, 2H, Ar), 4.88 (s, 2H, CH₂-naphthyl), 4.26 (s, 1H, CHOH), 4.00–3.01 (m, 12H, Pp–2,3,5,6-H, Pp–CH₂, N₃–CH₂), 1.69 (s, 3H, CH₃). ^{13}C NMR δ (ppm): 175.90, 175.85, 163.40, 160.97, 155.97, 155.88, 136.38, 136.35, 136.33, 136.30, 133.90, 132.55, 130.80, 129.18, 128.36, 128.34, 128.28, 128.26, 127.47, 126.73, 125.82, 124.57, 115.80, 115.59, 62.96, 62.93, 42.43, 25.44.

5-(4-Fluorophenyl)-3-[2-hydroxy-3-[4-(naphthalen-1-yl)-piperazin-1-yl]propyl]-5-methylimidazolidine-2,4-dione hydrochloride (7). White solid. Yield 36%. LC/MS $\pm$: purity 97.74% t_R = 4.77, (ESI) m/z [M + H] 477.31. ^1H NMR δ (ppm): 10.82 (bs, 1H, NH⁺), 9.07 (s, 1H, N¹H), 8.14–8.12 (d, J = 8.7 Hz, 1H, Ar), 7.96–7.87 (m, 1H, Ar), 7.67–7.65 (d, J = 8.2 Hz, 1H, Ar), 7.63–7.49 (m, 4H, Ar), 7.46–7.43 (t, J = 7.8 Hz, 1H, Ar), 7.24 (t, J = 7.6 Hz, 2H, Ar), 7.17–7.16 (d, J = 7.2 Hz, 1H, Ar), 6.00 (s, 1H, CHOH), 4.38 (s, 1H, CHOH), 3.82–3.13 (m, 12H, Pp–2,3,5,6-H, Pp–CH₂, N₃–CH₂), 1.75 (s, 3H, CH₃). ^{13}C NMR δ (ppm): 175.95, 175.90, 163.44, 161.01, 155.99, 155.91, 148.16, 136.40, 136.37, 136.33, 134.75, 128.86, 128.37, 128.31, 126.55, 126.40, 126.23, 124.46, 123.62, 115.83, 115.61, 115.52, 63.28, 62.99, 53.26, 42.62, 25.53.

5-(4-Fluorophenyl)-3-[2-hydroxy-3-(4-phenoxypiperidin-1-yl)-propyl]-5-methylimidazolidine-2,4-dione hydrochloride (8). LC/MS $\pm$: purity 100.00% t_R = 4.55, (ESI) m/z [M + H] 442.28. ^1H NMR δ (ppm): δ 10.56 (s, 1H, NH⁺), 9.05 (s, 1H, N¹H), 7.62–7.50 (m, 2H, Ar), 7.30 (dd, J = 14.3, 7.4 Hz, 8H), 7.27–7.19 (m, 2H, Ar), 7.07–6.90 (m, 3H, Ar), 5.92 (s, 1H, CHOH), 4.74 (m, 1H, CH₂OH), 4.55 (m, CH₂OH, 1H), 4.29 (s, 1H, CH–O), 3.55–3.25 (m, 4H, N₃–CH₂, Pp–CH₂), 3.25–2.88 (m, 4H, Pp–2,6-H), 2.29–2.09 (m, 2H,

Pp-3-H), 2.01–1.98 (m, 2H, Pp-5-H), 1.72 (s, 3H, CH₃). ¹³C NMR δ (ppm): 175.86, 163.42, 160.99, 157.10, 156.79, 155.96, 136.36, 130.09, 128.35, 128.26, 121.68, 121.47, 116.73, 116.13, 115.81, 115.59, 70.53, 66.87, 63.43, 63.18, 63.09, 62.95, 59.54, 59.21, 51.61, 50.86, 48.75, 47.75, 47.63, 42.52, 26.56, 25.48.

3-{3-[4-(4-Chlorophenoxy)piperidin-1-yl]-2-hydroxypropyl}-5-(4-fluorophenyl)-5-methylimidazolidine-2,4-dione hydrochloride (9). White solid. Yield 58%. LC/MS $\pm$: purity 100.00% t_R = 5.10, (ESI) m/z [M + H] 476.25 ¹H NMR δ (ppm): 10.55 (bs, 1H, N¹H), 9.04 (s, 1H, N¹H), 7.60–7.48 (m, 2H, Ar), 7.40–7.28 (m, 2H, Ar), 7.26–7.21 (m, 2H, Ar), 7.11–6.97 (m, 2H, Ar), 5.92 (s, 1H, CHOH), 4.74 (m, 1H, CH₂OH), 4.55 (m, CH₂OH, 1H), 4.28 (s, 1H, CH–O), 3.55–3.25 (m, 4H, N₃–CH₂, Pp–CH₂), 3.27–2.97 (m, 4H, Pp-2,6-H), 2.26–2.09 (m, 2H, Pp-3-H), 2.01–1.98 (m, 2H, Pp-5-H), 1.72 (s, 3H, CH₃). ¹³C NMR δ (ppm): 175.86, 163.42, 160.99, 155.99, 155.96, 155.88, 155.67, 136.35, 129.86, 128.34, 128.26, 125.36, 125.13, 118.54, 117.90, 115.81, 115.59, 71.04, 67.44, 63.41, 63.17, 62.95, 59.49, 59.15, 51.57, 50.79, 48.67, 47.62, 42.52, 28.28, 28.20, 26.44, 25.95, 25.48.

3-{2-Hydroxy-3-[4-(2-methoxyphenyl)piperazin-1-yl]propyl}-5-methyl-5-(naphthalen-1-yl)imidazolidine-2,4-dione (10). White solid. Yield 48%. LC/MS $\pm$: purity 99.04% t_R = 4.69, (ESI) m/z [M + H] 489.57. ¹H NMR δ (ppm): 8.79 (s, 1H, N¹H), 7.98–7.94 (m, 2H, Ar), 7.92–7.79 (m, 1H, Ar), 7.75–7.72 (d, J = 8.1 Hz, 1H, Ar), 7.60–7.44 (m, 3H, Ar), 6.98–6.78 (m, 4H, Ar), 4.92 (t, J = 5.2 Hz, 1H, CHOH), 4.16–3.99 (m, 1H, CHOH), 3.74 (s, 3H, OCH₃), 3.67–3.47 (m, 2H, N₃–CH₂), 2.94 (m, Pp-2,6-H, 4H), 2.57–2.49 (m, 4H, Pp-3,5-H), 2.40–2.39 (d, J = 5.9 Hz, 2H, Pp–CH₂), 1.92 (s, 3H, CH₃). ¹³C NMR δ (ppm): 177.07, 156.58, 156.54, 152.39, 141.68, 134.46, 133.98, 133.93, 131.02, 130.93, 130.22, 129.63, 127.07, 126.10, 125.37, 124.50, 122.77, 121.26, 118.32, 112.28, 65.41, 65.21, 63.46, 63.39, 55.72, 54.07, 50.48, 26.72.

3-{2-Hydroxy-3-[4-(2-cyanophenyl)piperazin-1-yl]propyl}-5-methyl-5-(naphthalen-1-yl)imidazolidine-2,4-dione hydrochloride (11). White solid. Yield 44%. LC/MS $\pm$: purity 97.53% t_R = 4.65, (ESI) m/z [M + H] 484.52. ¹H NMR δ (ppm) 10.93 (s, 1H, NH⁺), 8.95 (s, 1H, N¹H), 8.95 (s, 1H, Ar), 7.99–7.95 (m, 2H, Ar), 7.88–7.70 (m, 3H, Ar), 7.68–7.45 (m, 4H, Ar), 7.27–7.07 (m, 2H, Ar), 6.05 (s, 1H, CHOH), 4.47 (s, 1H, CHOH), 3.82–3.49 (m, 6H, Pp-3,5-H; Pp–CH₂), 3.49–3.11 (m, 6H, N₃–CH₂, Pp-2,6-H), 1.98 (s, 3H, CH₃). ¹³C NMR δ (ppm): 177.10, 177.06, 156.16, 154.20, 134.99, 134.74, 134.47, 133.77, 130.95, 130.90, 130.33, 129.73, 129.66, 127.25, 127.17, 126.94, 126.85, 126.14, 125.43, 124.55, 124.36, 123.48, 119.83, 118.37, 105.58, 63.69, 48.40, 26.62.

3-{3-[4-(Diphenylmethyl)piperazin-1-yl]-2-hydroxypropyl}-5-methyl-5-(naphthalen-1-yl)imidazolidine-2,4-dione hydrochloride (12). White solid. Yield 33%. LC/MS $\pm$: purity 98.69% t_R = 5.86, (ESI) m/z [M + H] 549.60 ¹H NMR δ (ppm): 10.57 (bs, 1H, NH⁺), 8.94 (s, 1H, N¹H), 7.97 (m, 2H, Ar), 7.87–7.70 (m, 2H, Ar), 7.62–7.47 (m, 3H, Ar), 7.44–7.42 (d, J = 7.5 Hz, 4H, Ar), 7.33–7.23 (t, J = 7.4 Hz, 4H, Ar), 7.22–7.17 (m, 2H, Ar), 6.00 (s, 1H, CHOH), 4.43 (m, 2H, CHOH, Ph–CH–Ph), 3.63–3.40 (m, 4H, Pp-3,5-H), 3.20–3.13 (m, 4H, Pp-2,6-H), 2.79–2.77 (m, 2H, Pp–

CH₂), 1.96 (s, 3H, CH₃). ¹³C NMR δ (ppm): 177.07, 177.03, 156.15, 142.41, 134.47, 133.75, 130.95, 130.89, 130.33, 129.72, 129.65, 129.16, 129.02, 127.63, 127.18, 126.90, 126.12, 125.42, 124.46, 124.32, 74.17, 63.68, 52.63, 48.26, 42.33, 26.27.

3-{2-Hydroxy-3-[4-(2-methoxyphenyl)piperazin-1-yl]propyl}-5-methyl-5-(naphthalen-2-yl)imidazolidine-2,4-dione hydrochloride (13). White solid 42%. LC/MS $\pm$: purity 97.39% t_R = 4.84, (ESI) m/z [M + H] 489.57. ¹H NMR δ (ppm): 10.63 (bs, 1H, NH⁺), 9.11 (s, 1H, s, 1H, N¹H), 8.03 (s, 1H, Ar), 7.99–7.88 (m, 3H, Ar), 7.66–7.62 (m, 1H, Ar), 7.53–7.50 (m, 2H, Ar), 6.99–6.93 (m, 2H, Ar), 6.88–6.87 (m, 2H, Ar), 5.97 (s, 1H, CHOH), 4.33 (s, 1H, CHOH), 3.76 (s, 3H, OCH₃), 3.61 (s, 1H, CHOH), 3.55–3.39 (m, 6H, Pp-3,5-H; Pp–CH₂), 3.21 (s, 2H, N₃–CH₂), 3.14–2.91 (m, 4H, Pp-2,6-H), 1.83 (s, 3H, CH₃). ¹³C NMR δ (ppm): 175.97, 175.97, 156.08, 156.01, 156.01, 152.20, 139.84, 137.54, 137.49, 132.95, 132.76, 128.58, 127.84, 126.94, 124.83, 124.33, 123.82, 121.25, 118.58, 112.30, 63.51, 63.09, 55.78, 52.95, 47.17, 42.52, 24.98.

3-{2-Hydroxy-3-[4-(2-cyanophenyl)piperazin-1-yl]propyl}-5-methyl-5-(naphthalen-2-yl)imidazolidine-2,4-dione hydrochloride (14). White solid. Yield 38%. LC/MS $\pm$: purity 99.09% t_R = 4.77, (ESI) m/z [M + H] 484.52.

¹H NMR δ (ppm): 10.82 (bs, 1H, NH⁺), 9.10 (s, 1H, N¹H), 8.01 (s, 1H, Ar), 8.00–7.83 (m, 3H, Ar), 7.75–7.72 (t, J = 11.5 Hz, 1H), 7.65–7.60 (m, 2H, Ar), 7.53–7.50 (m, 2H, Ar), 7.22–7.12 (m, 2H, Ar), 5.98 (s, 1H, CHOH), 4.34 (s, 1H, CHOH), 3.51 (m, 6H, Pp-3,5-H; Pp–CH₂), 3.23 (m, 6H, N₃–CH₂; Pp-2,6-H), 1.81 (s, 3H, CH₃). ¹³C NMR δ (ppm): 176.02, 156.09, 156.03, 154.18, 137.53, 137.48, 134.97, 134.73, 132.95, 132.76, 131.25, 128.67, 128.58, 127.84, 126.95, 124.87, 124.34, 123.45, 119.81, 118.36, 105.54, 63.51, 63.16, 59.05, 52.71, 48.37, 42.63, 24.97.

3-{2-Hydroxy-3-[4-(2-methoxyphenyl)piperazin-1-yl]propyl}-5,5-dimethylimidazolidine-2,4-dione hydrochloride (15). White solid. Yield 39%. LC/MS $\pm$: purity 98.25% t_R = 3.04, (ESI) m/z [M + H] 377.28. ¹H NMR δ (ppm): 10.59 (s, 1H, NH⁺), 8.37 (s, 1H, N¹H), 7.08–6.88 (m, 4H, Ar), 4.28–4.27 (m, 1H, CHOH), 3.79 (s, 3H, OCH₃), 3.66–3.63 (d, J = 11.6 Hz, 1H, CHOH), 3.52–3.40 (m, 4H, Pp-3,5-H), 3.42–3.05 (m, 8H, Pp–CH₂, N₃–CH₂, Pp-2,6-H), 1.30 (s, 6H, diCH₃). ¹³C NMR δ (ppm): 178.03, 155.70, 152.29, 139.66, 124.04, 121.30, 118.75, 112.45, 63.18, 59.30, 58.31, 55.85, 52.92, 51.64, 47.27, 47.15, 42.31, 25.04.

3-{2-Hydroxy-3-[4-(2-cyanophenyl)piperazin-1-yl]propyl}-5,5-dimethylimidazolidine-2,4-dione hydrochloride (16). White solid. Yield 51%. LC/MS $\pm$: purity 100.00% t_R = 3.02, (ESI) m/z [M + H] 372.30. ¹H NMR δ (ppm): 10.93 (s, 1H, NH⁺), 8.38 (s, 1H, N¹H), 7.76 (dd, J = 7.7, 1.5 Hz, 1H, Ar), 7.68–7.58 (m, 1H, Ar), 7.24 (d, J = 8.3 Hz, 1H, Ar), 7.18 (t, J = 7.5 Hz, 1H, Ar), 5.97 (s, 1H, CHOH), 4.30 (d, J = 7.0 Hz, 1H, CHOH), 3.75–3.59 (m, 4H, Pp-3,5-H), 3.45–3.10 (m, 8H, Pp–CH₂, N₃–CH₂, Pp-2,6-H), 1.32 (s, 6H, 2xCH₃). ¹³C NMR δ (ppm): 178.02, 155.68, 154.21, 134.98, 134.74, 123.45, 119.83, 118.36, 105.57, 63.26, 59.32, 58.31, 52.68, 51.58, 48.42, 42.32, 25.04.

3-{3-[4-(Diphenylmethyl)piperazin-1-yl]-2-hydroxypropyl}-5,5-dimethylimidazolidine-2,4-dione hydrochloride (17).

White solid. Yield 54%. LC/MS±: purity 100.00% t_R = 4.64, (ESI) m/z [M + H] 437.30. ^1H NMR δ (ppm): 8.33 (s, 2H, N^1H), 7.40–7.32 (m, 10H), 4.21–4.18 (m, 2H, CHOH , Ph-CH-Ph), 3.49–2.96 (m, 12H, Pp-CH_2 , $\text{N}_3\text{-CH}_2$, Pp-2,3,5,6-H), 1.31 (s, 6H, $2\times\text{CH}_3$). ^{13}C NMR δ (ppm): 178.01, 155.69, 129.69, 128.66, 65.37, 63.47, 58.31, 42.23, 25.05, 25.03, 15.64.

5-(4-Fluorophenyl)-3-[2-hydroxy-3-(1,2,3,4-tetrahydroisoquinolin-2-yl)propyl]-5-methylimidazolidine-2,4-dione (18). White solid. Yield 42%. LC/MS±: purity 96.84% t_R = 3.88, (ESI) m/z [M + H] 398.22. ^1H NMR δ (ppm): 9.03 (bs, 1H, N^1H), 7.67 (s, 2H, Ar), 7.55–7.52 (m, 2H, Ar), 7.48–7.41 (m, 2H, Ar), 7.23 (td, J = 8.9, 1.0 Hz, 2H, Ar), 4.41–4.39 (m, 2H, $\text{N}_3\text{-CH}_2$), 4.26 (s, 1H, CHOH), 3.78–3.07 (m, 10H, Pp-CH_2 , Pp-2,3,5,6-H), 1.70 (s, 3H, CH_3). ^{13}C NMR δ (ppm): 175.90, 175.85, 163.40, 160.97, 155.97, 155.88, 136.38, 136.35, 136.33, 136.30, 131.98, 130.04, 129.26, 128.36, 128.27, 115.81, 115.59, 63.39, 62.96, 62.93, 25.57, 25.44.

Crystallographic studies

The crystals of compound 3 that were suitable for X-ray structure analysis were obtained from a mixture of propan-2-ol and water by the slow evaporation of the solvent at room temperature. The intensity data for the single crystal were collected using an Oxford Diffraction SuperNova four circle diffractometer, equipped with Mo ($0.71069\text{ \AA}$) $\text{K}\alpha$ radiation source and a graphite monochromator. The phase problem was solved by direct methods using SIR-2014²⁸ and all non-hydrogen atoms were refined anisotropically using weighted full-matrix least-squares on F^2 . Refinement and further calculations were carried out using SHELXL.²⁹ The hydrogen atoms bonded to carbons were included in the structure at idealized positions and were refined using a riding model with $U_{\text{iso}}(\text{H})$ fixed at $1.2 U_{\text{eq}}$ of C with the exception of hydrogen atoms in methyl group, for which $U_{\text{iso}}(\text{H})$ was fixed at $1.5 U_{\text{eq}}$. Hydrogen atoms attached to nitrogen and oxygen atoms were found from the difference Fourier map and refined without any restraints. For molecular graphics, ORTEP³⁰ and MERCURY³¹ programs were used.

$\text{C}_{23}\text{H}_{27}\text{FN}_4\text{O}_3$, M_r = 426.48, crystal size = $0.39 \times 0.13 \times 0.02\text{ mm}^3$, monoclinic, space group $P2_1/c$, a = $17.4230(7)\text{ \AA}$, b = $6.0728(2)\text{ \AA}$, c = $20.2071(7)\text{ \AA}$, V = $2077.9(1)\text{ \AA}^3$, Z = 4, T = $130(2)\text{ K}$, 26816 reflections collected, 4984 unique reflections (R_{int} = 0.0787), R_1 = 0.0738, wR_2 = 0.1793 [$I > 2\sigma(I)$].

CCDC 1831620 contains supplementary crystallographic data.

Molecular modeling studies

Pharmacophore modeling. The 3-dimensional pharmacophore model was reconstructed using the 3D QSAR Pharmacophore Generation protocol implemented in Discovery Studio 3.5. The structures of training compounds as well as the parameters were fetched from ref. 23. The conformation space of both training and the as-synthesized compounds was generated using the BEST algorithm within a relative energy threshold of 20 kcal mol^{-1} above the global

energy minimum and with the maximum number of generated conformations per ligand set to 255. The minimum distance between features was fixed at $2.5\text{ \AA}$, and the top 10 pharmacophore hypotheses were returned by the generation process, which were further used in the evaluation with the reference pharmacophore model²³ with respect to the inter-feature distances and angles.

Molecular docking

The 3-dimensional structures of the ligands were prepared using LigPrep v3.6,³² and the appropriate ionization states at $\text{pH} = 7.4 \pm 1.0$ were assigned using Epik v3.4.³³ Compounds with unknown absolute configurations were docked in R and S configurations. One low energy ring conformation per ligand was generated. The Protein Preparation Wizard was used to assign the bond orders and appropriate amino acid ionization states and to check for steric clashes. The receptor grid was generated (OPLS3 force field³⁴) by centering the grid box with a size of $12\text{ \AA}$ on the Asp3.32 side chain. Automated flexible docking was performed using Glide v6.9 at SP level.³⁵

Radioligand binding assays

Affinities for human 5-HT_{1A}, 5-HT_{7b} and D_{2L} receptors. HEK-293 cells with the stable expression of human 5-HT_{1A}, 5-HT_{7b} and D_{2L} receptors (prepared with the use of Lipofectamine 2000) were maintained at $37\text{ }^\circ\text{C}$ in a humidified atmosphere with 5% CO_2 and grown in Dulbecco's modified Eagle medium containing 10% dialyzed fetal bovine serum and $500\text{ }\mu\text{g mL}^{-1}$ G418 sulfate. For membrane preparation, cells were subcultured in 150 cm^2 flasks, grown to 90% confluence, washed twice with phosphate buffered saline (PBS), prewarmed to $37\text{ }^\circ\text{C}$ and pelleted by centrifugation (200g) in PBS containing 0.1 mM EDTA and 1 mM dithiothreitol. Prior to membrane preparation, pellets were stored at $-80\text{ }^\circ\text{C}$.

Cell pellets were thawed and homogenized in 10 volumes of assay buffer using an Ultra Turrax tissue homogenizer and centrifuged twice at $35\,000 \times g$ for 15 min at $4\text{ }^\circ\text{C}$, with incubation for 15 min at $37\text{ }^\circ\text{C}$ in between. The compositions of assay buffers were as follows: for 5-HT_{1A}R: 50 mM Tris HCl, 0.1 mM EDTA, 4 mM MgCl_2 , 10 μM pargyline and 0.1% ascorbate; for 5-HT_{7b}R: 50 mM Tris HCl, 4 mM MgCl_2 , 10 μM pargyline and 0.1% ascorbate; for dopamine D_{2L}R: 50 mM Tris HCl, 1 mM EDTA, 4 mM MgCl_2 , 120 mM NaCl, 5 mM KCl, 1.5 mM CaCl_2 and 0.1% ascorbate. All assays were incubated in a total volume of $200\text{ }\mu\text{L}$ in 96-well microtiter plates for 1 h at $37\text{ }^\circ\text{C}$, except for those for 5-HT_{1A}R, which were incubated at room temperature. The process of equilibration was terminated by rapid filtration through Unifilter plates with a FilterMate Unifilter 96 Harvester (PerkinElmer). The radioactivity bound to filters was quantified on a Microbeta TopCount instrument (PerkinElmer, USA). For competitive inhibition studies, the assay samples contained the following as radioligands (PerkinElmer, USA): 2.5 nM [^3H]-8-OH-DPAT (135.2 Ci/mmol) for 5-HT_{1A}R; 0.8 nM [^3H]-5-CT (39.2 Ci/

mmol) for 5-HT₇R or 2.5 nM [³H]-raclopride (76.0 Ci/mmol) for D_{2L}R. Non-specific binding was defined with 10 μM of 5-HT in 5-HT_{1A}R and 5-HT₇R binding experiments, whereas 10 μM of haloperidol was used in D_{2L} assays. Each compound was tested in triplicate at 7 concentrations (10⁻¹⁰–10⁻⁴ M). The inhibition constants (K_i) were calculated from the Cheng–Prusoff equation.³⁶ For all binding assays, the results were expressed as means of at least two separate experiments (SD ≤ 19%).

Study of ADME-Tox properties

The reference compound aripiprazole was synthesized and provided by Adamed Ltd. (Pieńków, Poland). The reference cytostatic drug doxorubicin (DX) and mitochondrial toxin carbonyl cyanide 3-chlorophenylhydrazone (CCCP) were provided by Sigma-Aldrich (St. Louis, MO, USA).

Mouse liver microsomes (MLMs): microsomes from liver, pooled; biological source: from mouse *mus musculus* (CD-1), male; gene information: mouse MGST-1 (56615) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

The NADPH regeneration system was purchased from Promega (Madison, WI, USA). All experiments were performed as described previously.^{20,21}

Human embryonic kidney HEK-293 cell line ATCC CRL-1573 was kindly donated by Prof. Dr. Christa Müller (Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn). HepG2 (ATCC HB-8065) cell line was kindly donated by the Department of Pharmacological Screening, Jagiellonian University Medical College. The cell cultures' growth conditions were applied as described before.^{20,21} The CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay (MTS) used for the cells' viability determination was purchased from Promega (Madison, WI, USA). The assays were performed as described previously.^{20,21} The absorbance of samples was measured using a microplate reader EnSpire (PerkinElmer, Waltham, MA USA) at 490 nm. GraphPad Prism™ software (version 5.01, San Diego, CA, USA) was used to calculate the statistical significance.

Conflicts of interest

The authors declare no competing interest.

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