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Radioligand and computational insight in structure – Activity relationship of saccharin derivatives being ipsapirone and revospirone analogues

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ABSTRACT

Schizophrenia and depression are diseases that significantly impede human functioning in society. Current antidepressant drugs are not fully effective. According to literature data, the effect on D_2R or 5-$HT_{1A}R$ can effectively reduce the symptoms of depression or schizophrenia. Recent research hypothetized that the synergism of both of these receptors can improve the effectiveness of therapy. Ipsapirone, a representative of long-chain arylpiperazines, is a known 5-$HT_{1A}R$ ligand that has antidepressant effect. This compound has no affinity for the D_2R. Bearing in mind, we decided to design ligands with improved affinity to D_2R and confirmed that in some cases elongation of the carbon linker or arylpiperazine exchange may have beneficial influence on the binding to D2R and 5-HT1AR. Four groups of ligands being ipsapirone analogues with butyl, pentyl, hexyl and stiffened xylene chains were designed. All compounds were obtained in *solvent-free* reactions supported by a microwave irradiation with an efficiency mainly above 60%. All ligands containing 1-(2-pyrimidinyl)piperazine exhibited high affinity to 5-$HT_{1A}R$. In this case, chemical modifications within the chain did not affect the affinity to D_2R. In the case of ligands containing 1-phenylpiperazine, 1-(3-trifluoromethylphenyl)piperazine, 1-(1-naphthyl) piperazine, and 1-(4-chlorophenyl)piperazine, elongation of carbon linker increases of affinity to D_2R. For ligands containing 1- (2-pyridyl) piperazine, and 1-(2,3-dichlorophenyl)piperazine, we observed an opposite effect. For ligands containing 1-phenylpiperazine, 1-(2-methoxyphenyl)piperazine and 1-(2-pyridyl)piperazine, chain elongation had no effect on 5-$HT_{1A}R$ binding. In turn of ligands containing 1-(3-trifluoromethylphenyl) piperazine and 1- (2,3-dichlorophenyl)piperazine, we observed that elongation of carbon linker has a positive influence to 5-$HT_{1A}R$. Molecular modelling was used to support the SAR study.

Central nervous system (CNS) disorders such as depression or schizophrenia in the near future may become one of the main dysfunctions that impede human functioning in society. Despite the advances made in pharmacy in the context of CNS disorders, the currently used drugs still have numerous side effects.[1] Treatment of depression or schizophrenia is related with affecting on D_2R, 5-$HT_{1A}R$ as well as 5-HT_6R or 5-HT_7R.[2–6] 5-$HT_{1A}R$ and D_2R are involved in depression or schizophrenia disorders.[7–9] In major depressive disorders (MDD) patients, 5-$HT_{1A}R$ agonists (such as buspirone or gepirone) are thought to act synergistically to increase 5-HT neurotransmission in postsynaptic structures (in the cortex and the limbic areas) and thus may exhibit antidepressant properties.[4,10] Nevertheless, improvement in therapy can be obtained by acting additionally on D_2R. Dual D_2R/5-$HT_{1A}R$ action seems to be more effective than single one. In a rat study, stimulation of the 5-$HT_{1A}R$ was shown to increase dopamine secretion in prefrontal cortex resulting in a reduction of schizophrenia-specific symptoms. Sumiyoshi et al.[10] showed that buspirone or tandospirone in combination with haloperidol significantly improved the cognitive abilities of patients suffering from schizophrenia. Thus dual D2R/5-HT1AR ligands seem to be much more promising, with a broader spectrum of activity than single ones, however this hypothesis must be confirmed in further research.[4]

Ipsapirone belongs to a well-characterized group of azapirones. As it was mentioned above an anti-depressant effect is related to the release of serotonin in synaptic cleft in corticolimbic structures due to the substance's high affinity to postsynaptic 5-$HT_{1A}R$. Affecting these receptors may have additional modulatory effect on other neurotransmitter systems such as the glutamatergic system in various parts of brain.[11]

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Ipsapirone exhibits anxiolytic, anti-depressant, and anti-aggressive properties in animal models.[12] as it acts as a postsynaptic partial agonist to 5-HT_{1A}R with $K_i = 7.9$ nM and no activity to D_2R.[13–15] In the literature[16–18] there is described only one analogue of ipsapirone with ethyl linker (**A**) that does not bind to 5-HT_{1A}R, two analogues of ipsapirone with propyl linker that bind to 5-HT_{1A}R with $K_i = 400$ nM for **B** and $K_i = 2$ nM for **C** (revospirone) and four four-carbon analogues of ipsapirone with known affinity to D_2R (four analogues) as well as 5-HT_{1A}R/5-HT_{2A}R (one analogue) (Table 1). In our previous research[19] with saccharin being a terminal moiety of LCAP, we demonstrated that carbon linker extended up to six carbons may have a beneficial influence on affinity mostly to D_2R (Table 1). Taking into account that acting on both D_2R and 5-HT_{1A}R may have a beneficial influence in treatment of depression or schizophrenia, the aim of our work is to perform the full structure activity relationship analysis for ipsapirone analogues to confirm our hypothesis that elongation of carbon linker has a beneficial influence on affinity to D_2R/5-HT_{1A}R.

In the medicinal chemistry, hit-to-lead is a well-known approach, where a desirable pharmacological profile for protein/receptor is expected *via* various chemical modifications.[20] In the literature there are numerous reports about SAR (***S**tructure **A**ctivity **R**elationship*) study within LCAPs (***L**ong **C**hain **A**rylpiperazines*) including carbon linker and various arylpiperazine exchange (Table 2).[7,21–24] In general, *N*-arylpiperazine itself exhibits moderate affinity and selectivity to the following receptors: serotonin[21], dopamine[25], noradrenaline[26] as well as monoamine transporters.[9–10] The functionalization of *N*-arylpiperazine by introducing a long chain core attached to the basic nitrogen of piperazine core may have beneficial influence on affinity to receptors.[21] For example, 1-(2-methoxyphenyl)piperazine binds to 5-HT_{1A}R with K_i = 68 nM. Introducing phthalimide with ethyl linker (**I**)to basic nitrogen atom resulted in loss of activity to 5-HT_{1A}R ($K_i > 1000$ nM) (Table 2). Further elongation of carbon linker resulted in gain of affinity to 5-HT_{1A}R in the following pattern: propyl linker (**J**) $K_i = 13$ nM[21], butyl linker (**K** – NAN-190) $K_i = 0.6$ nM[21], pentyl linker (**L**) $K_i = 7.2$ nM[27], hexyl linker (**M**) $K_i = 22$ nM.[27] According to the NAN-190 SAR study, optimal carbon linker length is around 4 atoms. Shorter linker decreases affinity, and similar effects are observed for longer linkers as well. For salicylamide and 1,3-benzoxazine-2,4-dione in terminal position, the same trend has been observed (Table 2). Considering the SAR study for trazodone, elongation of carbon linker up to 6 atoms resulted in a near 5-fold increase of affinity to 5-HT_{1A}R.[23] Further research revealed that using other *N*-arylpiperazines such as 1-(2-methoxyphenyl)piperazine or 1-(3-methoxyphenyl)piperazine instead of 1-(3-chlorophenyl)piperazine dramatically increased affinity to D_2R/5-HT_{1A}R (Table 3).

In light of the above consideration, to fully perform structure activity relationship study of ipsapirone, we designed four groups of compounds. Compounds with flexible butyl, pentyl, hexyl chain and more rigid xylene linker have been chosen to find out the optimal length of carbon linker. Additionally, these four groups include various *N*-arylpiperazines in order to find their influence on binding to selected receptors (Fig. 1). In this study, we focused mostly on dual D_2R/5-HT_{1A}R effect, however, to examine their selectivity, binding to 5-HT_{2A}R, 5-HT_6R and 5-HT_7R were examined as well. We used molecular modelling to support the SAR study.

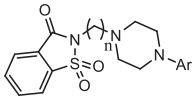
Designed compounds were obtained in solvent-free reactions with microwave irradiation. The procedure has already been described in our previous publications.[19,31] Ligands from groups I, II and IV were obtained in three independent synthetic routes using two-step reactions (Scheme 1 and Scheme 2). For SAR study, ligands from group of III were taken from our previous publication.[19]

In the first step, a saccharin **1** is *N*-alkylated with appropriate alkyl halide **2i-2k**. The first step was carried out in the presence of 3 equivalents of potassium carbonate, 0.1 equivalents of tetrabutylammonium bromide (TBAB), and 2–3 drops of *N,N*-dimethylformamide (DMF) or acetonitrile (ACN). Intermediates **3i** and **3k** were purified *via* crystallization from methanol, while **3j** was purified using column chromatography. Reaction conditions for the second step were similar to those for the first one. Final products were purified using column chromatography. Pure ligands were transformed into HCl salts and subjected to radioligand assays.

The aim of this research was to elucidate SAR of LACPs being saccharin derivatives with increased affinity to D_2R/5-HT_{1A}R. All ligands were tested in radioligand binding assays according to a known procedure.[32] The assays were performed *via* the displacement of the respective radioligands from cloned human receptors, all stably expressed in HEK293 cells (except for 5-HT_{2A}R which was expressed in CHO cells): [^{3}H]-8-OH-DPAT for 5-HT_{1A}R, [^{3}H]-ketanserin for 5-HT_{2A}R, [^{3}H]-LSD for 5-HT_6R, [^{3}H]-5-CT for 5-HT_7R and [^{3}H]-raclopride for D_2R.

According to Structure – Linker Relationship, most of the obtained ligands exhibited affinity to the mixed D_2R/5-HT_{1A}R profile. In the group of ligands with no substituents of the *N*-arylpiperazine moiety (**5a**, **5b**, **5c**), slightly decreased activity was observed upon linker chain elongation. Ligands still exhibited high affinity to 5-HT_{1A}R with $K_i \leq 20$ nM. The opposite pattern was observed with respect to D_2R. We observed moderate or no affinity to the remainder of tested receptors. Because saccharin core is structurally similar to phthalimide, we expected that in case of 1-(2-methoxyphenyl)piperazine (ligands **5b**, **6b**

Table 1
Saccharin carbon linker comparison on affinity (K_i [nM]) to D_2R/5-HT_{1A}R/5-HT_{2A}R.[16–19]



Ligand No.	n	Ar	h-D1	h-D2	h-D3	h-D4	h-D5	h-5-HT1A	h-5-HT2A	h-5-HT2C	h-α1
A	2	3-CF3-Ph	nd	nd	nd	nd	nd	NA	nd	nd	nd
B	3	3-CF3-Ph	nd	nd	nd	nd	nd	400	700	NA	nd
C*	3	2-Pyrimidinyl	nd	nd	nd	nd	nd	2	nd	nd	nd
D	4	Ph	nd	38	66	136	1629	nd	nd	nd	nd
E		4-Cl-Ph	nd	177	287	36	230	nd	nd	nd	nd
F = 5e		3-CF3-Ph	NA	113	107	NA	1756	100	2250	NA	1500
G		2-OMe-Ph	nd	360	187	79	1132	nd	nd	nd	nd
H** =5f		2-Pyrimidinyl	nd	1600	nd	nd	nd	7.9	14,980	nd	229
7a	6	Ph	nd	48	nd	nd	nd	20	321	nd	nd
7d		4-Cl-Ph	nd	28	nd	nd	nd	84	nd	nd	nd
7e		3-CF3-Ph	na	7	nd	nd	nd	25	167	nd	nd
7b		2-OMe-Ph	na	61	nd	nd	nd	91	nd	nd	nd
7f		2-Pyrimidinyl	na	51	nd	nd	nd	24	183	nd	nd

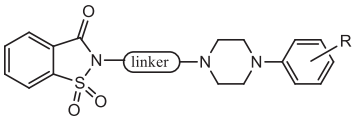
NA – not active (Ki > 5 μM) nd - not determined, * - revospirone, ** - ipsapirone, h-human receptor type

Table 2
Phthalimide, benzoxazine and salicylamide effects of carbon linker on affinity to *h*-5-HT$_{1A}$R[27–30].

Ligand No	Ar	n	K_i [nM]	Ligand No	n	K_i [nM]	Ligand No	n	K_i [nM]
I		2	>1000	**I-1**	2	na	**I-2**	2	155
J		3	13	**J-1**	3	46	**J-2**	3	21
K		4	0.6*	**K-1**	4	3.2	**K-2**	4	38
L		5	7.2	**L-1**	5	20	**L-2**	5	4
M		6	22	**M−1**	6	18	**M−2**	6	3
N		2	>1000	**N-1**	2	na	**N-2**	2	na
O		3	200	**O-1**	3	402	**O-2**	3	143
P		4	10	***P*-1**	4	2.8	**P-2**	4	na
R		5	8.5	**R-2**	5	na	**R-2**	5	na
S		6	1	**S-1**	6	na	**S-2**	6	na

*NAN-190, na – not available, h – human receptor type.

Table 3

K_i [nM] ± SEM of radioligand binding for all synthetized compounds for tested receptors. 

Ligand No.	R	linker	*h*-D$_2$R	*h*-5-HT$_{1A}$R	*h*-5-HT$_{2A}$R	*h*-5-HT$_6$R	*h*-5-HT$_7$R
5a	H	butyl ***group I***	249	3	474	4664	631
5b	2-OMe		7	8	382	161	96
5c	2,3-diCl		26	1	2580	2662	372
5d	4-Cl		571	78	318	2729	536
5e	3-CF$_3$		113**	100**	2250**	na	na
5f	2-Pyrimidyl		1224	15	14,980	16,800	1406
6a	H	pentyl ***group II***	165	16	313	1836	148
6b	2-OMe		20	3	584	1991	125
6c	2,3-diCl		17	29	126	197	149
6d	4-Cl		254	101	139	703	191
6e	3-CF$_3$		34	21	162	1354	35
6f	2-Pyrimidyl		954	6	6306	7097	2673
6 g	2-Pyridyl		92	13	1438	2626	179
6 h	1-Naphtyl		20	16	105	101	36
7a	H	hexyl* ***group III***	48*	20*	321*	971*	192*
7b	2-OMe		1	10	764	779	105
7c	2,3-diCl		132*	58*	209*	920*	119*
7d	4-Cl		28*	84*	299	72	487
7e	3-CF$_3$		7*	25*	167*	52*	58*
7f	2-Pyrimidyl		3274	56	3530	5708	2515
7 g	2-Pyridyl		239*	13*	1290*	2352*	552*
7 h	1-Naphtyl		12*	13*	44*	77*	23*
8b	2-OMe	xylene ***group IV***	2	77	4754	10,090	97

na - not available, * - Ref.[19]; ** - Ref.[33]; high affinity $K_i < 50$ nM, moderate affinity 50 nM $< K_i <$ 500 nM, low affinity 500 nM $< K_i <$ 1000 nM, not active $K_i >$ 1000 nM. Each compound was tested in triplicate at 7 concentrations (0.1 nM – 100 μM). The inhibition constants (K_i) were calculated from the Chenge-Prusoff equation[37]. Results were expressed as means of at least two separate experiments (SEM ≤ 25%; h – human receptor type.

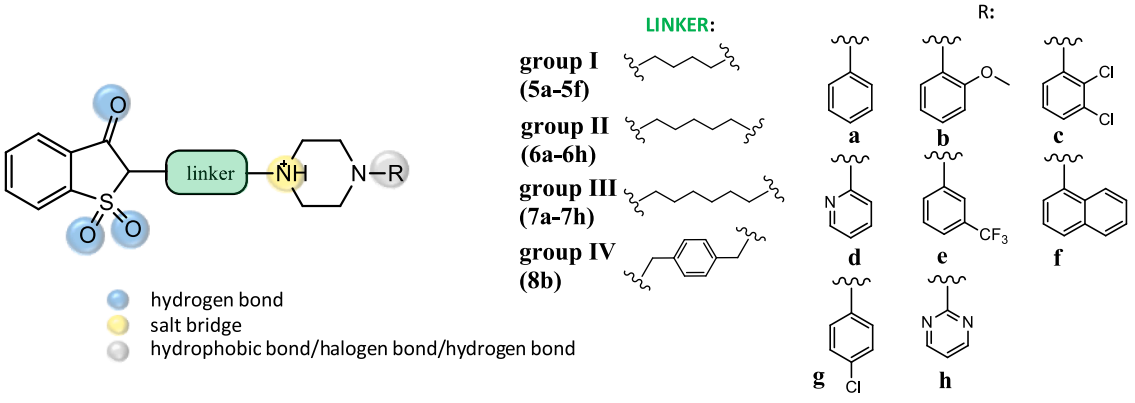
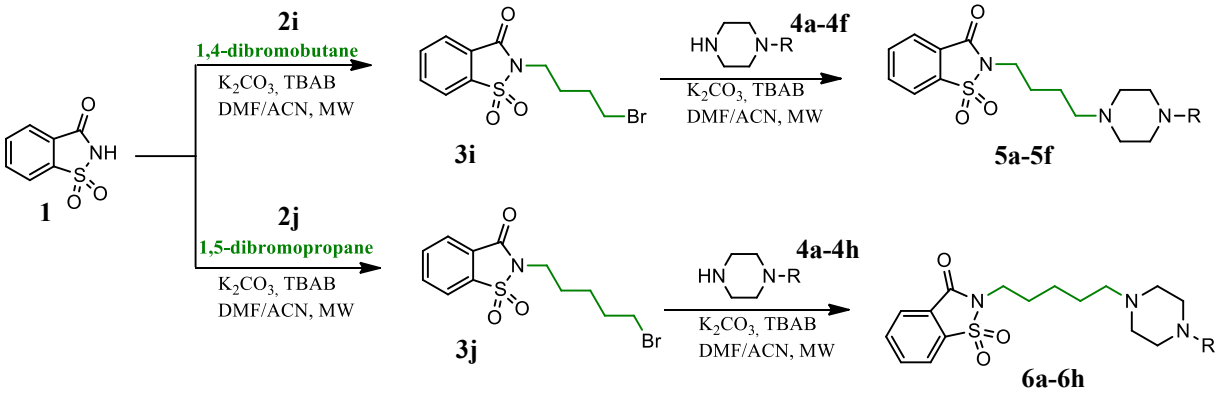
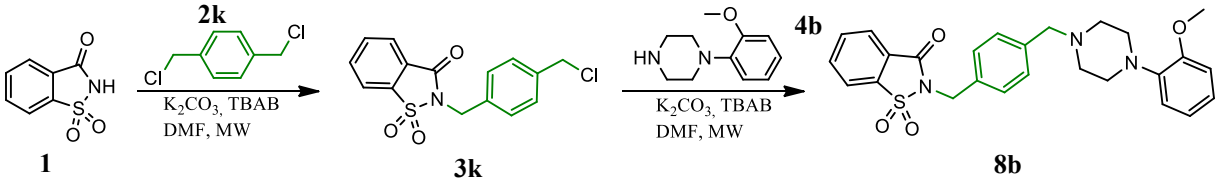


Fig. 1. Structure – Activity Relationship analysis plan leading to dual D$_2$R/5-HT$_{1A}$R ligands.



Scheme 1. Synthetic pathway leading to new compounds with flexible linker.



Scheme 2. Synthetic pathway leading to new compounds with rigid linker.

and **7b**) the binding pattern to 5-HT_{1A}R would be similar to **I-M**. Ligands with four (**5b**) or five (**6b**) carbon atoms in the linker showed the highest affinity with K_i = 8 nM and K_i = 3 nM, respectively. Adding an extra carbon to the linker (6 carbons in total) resulted in slight loss of affinity (**7b**, K_i = 10 nM). Increased rigidity of the carbon linker by introducing xylene moiety resulted in further loss of activity to 5-HT_{1A}R (**8b**, K_i = 77 nM). In this case increasing rigidity did not have any effect on affinity to D_2R. Ligands **5b**, **6b**, **7b** and **8b** did not exhibit affinity to 5-HT_{2A}R and 5-HT_6R, however, exhibited moderate affinity to 5-HT_7R. A different binding pattern was observed when 1-(2,3-dichlorophenyl)piperazine was present in the ligands. Elongation of the carbon linker resulted in a decrease of affinity to 5-HT_{1A}R in the following pattern: **5c** K_i = 1 nM, **6c** K_i = 29 nM and **7c** K_i = 58 nM. According to D_2R, ligand with propyl linker was the most active in this group (**6c**, K_i = 17 nM). Shortening (**5c**) as well as elongation (**7c**) of the carbon linker resulted in a decrease of activity when compared to **6c** with K_i = 26 nM and K_i = 132 nM, respectively. Ligands with *para*-Cl substituent of arylpiperazine moiety were less active than those with 2,3-dichloro substituents. Ligand with four carbons (**5d**) as well as six carbons (**7d**) exhibits similar affinity to 5-HT_{1A}R, while ligand with five carbons (**6d**) exhibits slight worse affinity with K_i = 101 nM. Elongation of the carbon linker resulted in increased of affinity only in term to D_2R. The same ligands exhibited moderate affinity to 5-HT_{2A}R, 5-HT_6R and 5-HT_7R or were not active towards these receptors. With respect to 1-(3-trifluoromethylphenyl) piperazine ligands, elongation of carbon linker resulted in increased affinity to

5-HT_{1A}R. Ligand with short ethyl chain (**A**) linker was inactive.[16–18] Elongation of ethyl chain to 3 atoms (i.e., propyl) (**B**) resulted in increased affinity with K_i = 400 nM.[16–18] Further elongation caused increase of affinity in the following pattern: **5e** K_i = 100 nM, **6e** K_i = 25 nM and **7e** K_i = 21 nM. The same pattern was observed in term of D_2R. Ligands exhibited high affinity to 5-HT_7R as well. With respect to 5-HT_{1A}R, the binding pattern of ligands with 1-(2-Pyrimidinyl)piperazine was similar to those with 1-(2-methoxyphenyl)piperazine. Ligands with short linkers: propyl (**C**), butyl (**5f**), and pentyl (**6f**), exhibited high affinity to 5-HT_{1A}R with the K_i = 2 nM, K_i = 15 nM and K_i = 6 nM, respectively. So far, we reported that investigated ligands exhibit affinity to D_2R. Ligands with 1-(2-pyrimidinyl)piperazine do not exhibit the mentioned affinity, making them highly selective towards tested receptors. For 1-(2-pyridinyl)piperazine ligands (**6g** and **7g**, K_i = 13 nM), we did not observe differences in the binding to

5-HT_{1A}R. During conducted research, we found that ligands **6h** and **7h** showed multifunctional binding, where the structure-carbon length relationship may be difficult to analyse. According to Structure – Arylpiperazine Relationship, in the first group of ligands (**5a-5c**, **5f**) where four-carbon linker was present, high affinity to 5-HT_{1A}R was observed in the range of K_i between 1 and 15 nM. Introduction of *para*-Cl substituent to *N*-arylpiperazine core (**5d**, K_i = 78 nM) as well as *meta*-CF_3 substituent (**5e**, K_i = 100 nM) resulted in considerable decrease of affinity. Generally, we observed less affinity of the ligands **5a-5f** to D_2R than to 5-HT_{1A}R. Ligands with *ortho*-OCH_3 (**5b**) and 2,3-dichloro (**5c**) substituent only exhibited high affinity with K_i = 7 nM and K_i = 26 nM, respectively. In most cases, these ligands were not active toward 5-HT_{2A}R, 5-HT_6R and 5-HT_7R. In the second group of ligands where five-carbon linker was present, the affinity to 5-HT_{1A}R was also very high – $K_i \leq$ 29 nM. One exception was **6d**, where introducing of *para*-Cl substituent resulted in the decrease of activity with K_i = 101 nM. For D_2R, an interesting relationship was observed with respect to ligands with only *ortho* and/or *meta* positions occupied (**6b**, **6c**, **6e** and **6i**). In that case, ligands exhibited high affinity with $K_i \leq$ 34 nM. We indicated that most of ligands with pentyl linker (**6a-6i**) did not exhibit high affinity to 5-HT_{2A}R, 5-HT_6R and 5-HT_7R except of ligand **6i** which behaved as a multifunctional ligand. Ligands with six-carbon linker seem to be less selective than those with five or four carbons. Ligand **7e** exhibits high affinity to four types of receptors: D_2, 5-HT_{1A}, 5-HT_6 and 5-HT_7. Ligands **6h** and **7h** exhibit affinity to three type of receptors:D_2, 5-HT_{1A} and 5-HT_7.

We confirmed a fully conducted SAR study based on *in vitro* assays *via* molecular modelling approach using docking for selected ligands (Fig.2, Fig.3) Due to lack of crystalline structure of 5-HT_{1A}R, a homology model based on 5-HT_{1B}R (PDB 4IAR) template with ergotamine was created using Swiss-Model server.[34] The 5-HT_{1A}R homology model was validated using also Swiss-Model server as well as QMEAN by parameter determination. The amino acids sequence identity was>41% and the coverage was 0.89 – such values correspond with literature.[35–36] The percent of residues in favoured regions of the Ramanchadron plot was also determined (Supplementary Material). For D_2R, we used the crystal

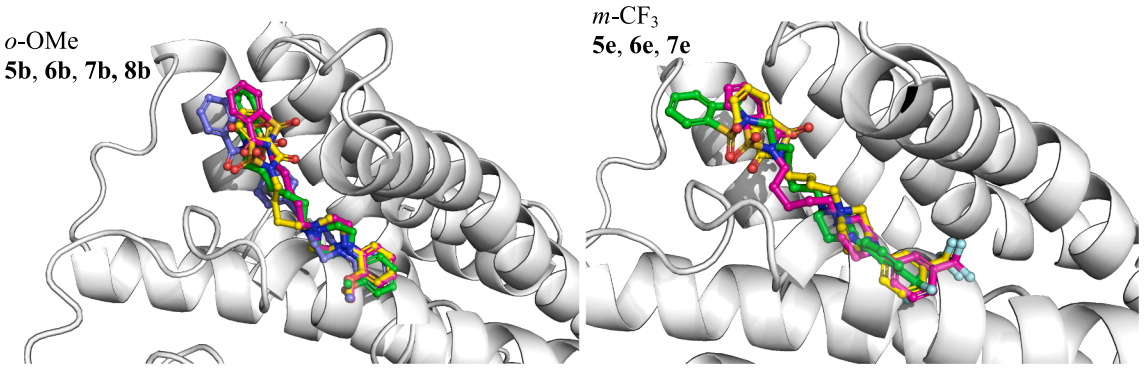


Fig. 2. Aligned poses of selected ligands to D_2R structure crystal (PDB: 6LUQ) obtained in the docking. Green ligands represent hexyl linker, magenta ligands represent pentyl linker, yellow ligands represent butyl linker, silver ligand represents propyl linker, purple ligand represents xylene linker.

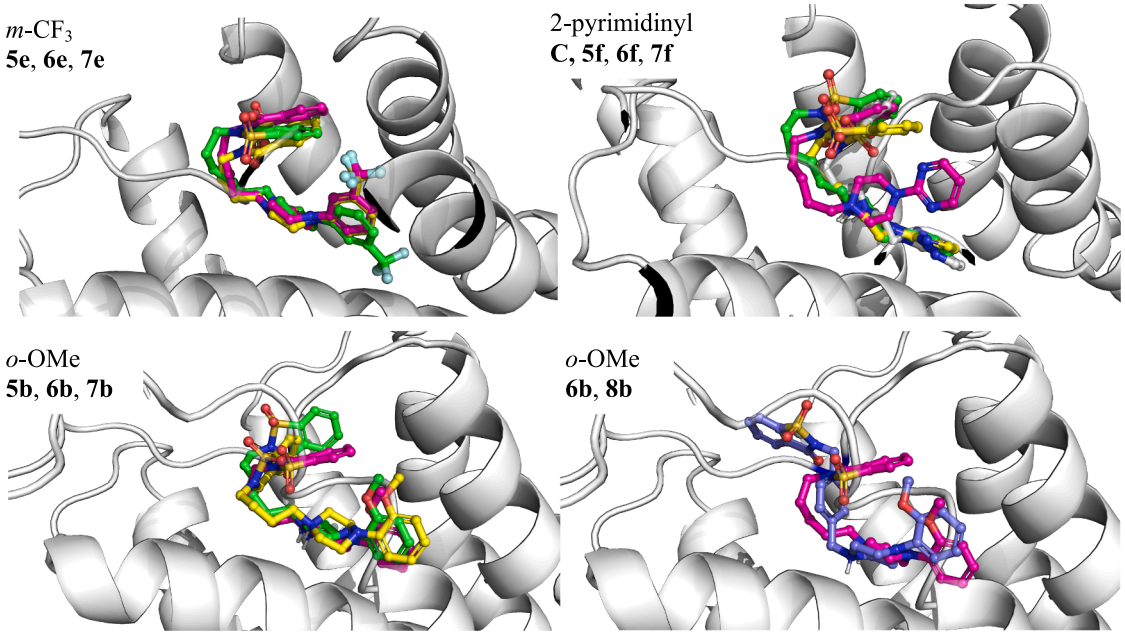


Fig. 3. Aligned poses of selected ligands to the homology model of 5-$HT_{1A}R$ obtained in the docking. Green ligands represent hexyl linker, magenta ligands represent pentyl linker, yellow ligands represent butyl linker, silver ligand represents propyl linker, purple ligand represents xylene linker.

structure of D_2R (PDB 6LUQ) with endogenously bound haloperidol. Selected forms for all docked ligands as well as interactions are presented in Supplementary data. Endogenously bound haloperidol adopts a linear conformation and forms a conservative hydrophobic π-π stacking with W407 and F411 which are located in a hydrophobic binding pocket deep in the receptor. In general, the ligands considered (Fig. 2) adopt a coherent haloperidol binding mode. In the opposition to 5-$HT_{1A}R$ ligands, D_2R ligands adopt more linear conformation. All ligands form salt bridge with D3.32. In case of **5e**, **6e** and **7e**, 1-(3-trifluoromethylphenyl)piperazine reaches out hydrophobic binding pocket deep in the receptor which is located between transmembrane helix (TMh) 3 and TMh6 (CH-π stacking with: F411 – **7e**; F410 – **7e**, **6e**; W407 – **6e**, **5e**). Terminal moiety of the ligands is orientated in the outer part of receptor in the direction to TMh2 and TMh7. Carbonyl group forms hydrogen bond interaction with W100 for **7e** and **5e** as well as hydrogen bond with T433 for **6e**. Very high affinity to D_2R of **7e** may be explained through additional occurrence of hydrophobic CH-π stacking with W100 and aromatic ring of saccharin. On the other hand, lowered affinity of **5e** may be caused through lack of CH-π stacking with F411 or F410. In this case we observed worse parameters of salt bridge (Table 4, Supplementary Material). According to ligands with 1-(2-methoxyphenyl) piperazine **5b** – **8b** very high affinity with $K_i \leq 20$ nM may be explained through coherent binding mode and formation of conserve salt bridges with D3.32 as well as hydrogen/hydrophobic bonds with appropriate amino acids. The *N*-phenylpiperazine binding pocket is hydrophobic and located deeper in the receptor, where *o*-methoxyphenyl rings bind preferentially in the cavity between TMh3, TMh6 helices forming CH-π type interactions with W407 and F411. Saccharin which is placed in outer part of the receptor interacts with hydrogen bond with T433 (for **7b** and **6b**), and W100 (for **8b**, **6b** and **5b**). Stiffened carbon linker additionally interacts with F189 *via* CH– π interaction.

Because groups of ligands with *meta*-CF_3 (**A**, **B**, **5e**, **6e**, **7e**) are well characterised in *in vitro* assays, we chose them as the first group to perform docking in the range of 2–6 carbons to 5-$HT_{1A}R$. All ligands are characterized by a bent form and key salt bridge interaction with D3.32. Ligands with high affinity (**6e**, $K_i = 21$ nM and **7e**, $K_i = 25$ nM) to 5-$HT_{1A}R$ exhibit consistent binding mode for azapirone[37] as well as for saccharin derivatives.[19] 1-(3-trifluoromethylphenyl)piperazine in the ligands reaches out to a hydrophobic binding pocket placed deep in the receptor, which is located near TMh3 and TMh6. Aromatic ring of *N*-arylpiperazine interacts with F6.51 and F6.52 *via* CH-π stacking. Terminal moiety of the ligands is oriented in the outer part of receptor in the

direction toward TMh6, TMh7 and extracellular loop (ECl) 2. Carbonyl group of **7e** forms conservative hydrogen bond interaction with N7.38. Sulfonyl group forms hydrogen bonds with K191 for **7e** and **6e** as well as with Y96, G97 and N7.38 for **6e**. Sulfonyl group of ligand **7e** forms an additional hydrogen bond with T188 and K191. Ligand **6e** forms an additional π-cation interaction between the saccharin aromatic ring and K191. Shortening the linker by one carbon atom resulted in the decrease of affinity (**5e**, $K_i = 100$ nM). It may be a result of lacking CH-π interaction with F6.51, however, ligands adopt the same conformation as **6e**. Further shortening (ligand **B**, $K_i = 400$ nM and ligand **A**, inactive) caused further decrease of affinity. It may be caused by the following changes in the binding mode: conformation of ligands **A** and **B** are not fully coherent with the rest of ligands; *N*-piperazine moiety does not fit directly in the hydrophobic binding pocket and point out more in the direction TMh6; both ligands do not form hydrophobic interactions, while ligand **A** does not form a salt bridge. The second group of ligands, with a widely known affinity to 5-HT_{1A}R, are ligands with 1-(2-pyrimidinyl)piperazine, such as revospirone (**C**), ipsapirone (**H** = **5f**) and their analogues (**6f**, **7f**). In this case, all ligands exhibit high affinity with $K_i \leq 15$ nM (**C**, **5f** and **6f**). The binding mode for these ligands is similar. Ligands form a salt bridge with D3.32 and adopt bent conformation. As typical for azapirones, aromatic part of 1-(2-pyrimidinyl)piperazine reaches hydrophobic binding pocket in deep part (parallel to TMh3 or more perpendicular toward TMh6) of the receptor forming CH-π stacking with F6.52 (**C**, **5f**, **6f**, **7f**) or F6.51 (**6f**) and W6.48 (**C**, **5f**, **7f**). All carbonyl groups interact with N7.38 and additional with Q97 (for **6f**) *via* hydrogen bonds. Sulfonyl groups interact with K191or I189 through hydrogen bonds as well. Ligand **6f**, additional interacts with T188. Slightly higher affinity of **6f** than **5f** may be explained through additional interactions such as π-cation between D3.32 and F3.28. Ligand **7f** ($K_i = 56$ nM) exhibits slightly lower affinity. It could be explained by inferior salt bridge characteristics (Table 5). Similar affinity for ligands with 1-(2-methoxyphenyl)piperazine (**5b**, **6b** and **7b**) to 5-HT_{1A}R may be explained through their coherent binding mode as well as bioconformation. Ligands form a salt bridge with D3.32 and adopt bent conformation. Interactions with amino acids are the same as previously. Increasing rigidity of the chain by introducing a xylene substituent (**8b**, $K_i = 77$ nM) resulted in the decrease of affinity. Such phenomenon may be caused by a slightly different binding mode. In this case we observed inferior salt bridge characteristics with D3.32 (Table5, Supplementary Material). *N*-arylpiperazine reaches hydrophobic binding pocket in deep part of the receptor which is consistent with previous ligands. Introducing xylene substituent instead of flexible carbon linker resulted in ligand adopting an "S-like" bioconformation (Fig. 2). Saccharin points toward TMh7 and forms hydrogen bonds with Q2.64 and Y2.63 as well as ECl2 and forms hydrogen bonds with K191.

In summary, the aim of this work was to confirm the hypothesis whether that elongation of carbon linker has beneficial influence on affinity to selected receptors by examining the influence of the carbon linker and various arylpiperazines on affinity to dopamine D_2R and serotonin 5-HT receptors. We focused mostly on mixed profile D_2R/5-HT_{1A}R. All designed ligands were obtained with a quick, eco-friendly approach using solvent-free microwave supported reactions where overall yield generally exceeded 60% (salt form). We demonstrated that positive changes in D_2R binding profile of ipsapirone may be achieved only by arylpiperazine exchange. In this case, elongation of carbon linker have no influence on the affinity to D_2R (ligands inactive) as well as to 5-HT_{1A}R (ligands active). For ligands such as 1-phenylpiperazine (**5a**, **6a**, **7a**), 1-(4-chlorophenyl)piperazine (**5d**, **6d**, **7d**), 1-(3-trifluoromethylphenyl)piperazine (**5e**, **6e**, **7e**) and 1-(1-naphthyl)piperazine (**5h**, **6h**, **7h**), derivatives, elongation of carbon linker increases of affinity to D_2R. For 1-(2-pyridyl)piperazine, and 1-(2,3-dichlorophenyl) piperazine derivatives we observed an opposite effect. We also indicated that carbon linker has no influence on affinity to 5-HT_{1A}R for ligands such as 1-phenylpiperazine (**5a**, **6a**, **7a**), 1-(2-methoxyphenyl)piperazine (**5b**, **6b**, **7b**) and 1-(2-pyridyl)piperazine derivatives (**6g**, **7g**). All of them (except of **8b**) exhibited high affinity to 5-HT_{1A}R with $K_i \leq 20$ nM. Carbon linker of ligands such as 1-(4-chlorophenyl)piperazine (**5d**, **6d**, **7d**) has also no influence on affinity to 5-HT_{1A}R, however, ligands exhibited moderate affinity. For ligand such as 1-(3-trifluoromethylphenyl)piperazine (**5e**, **6e**, **7e**), we observed that elongation of carbon linker has a positive influence to 5-HT_{1A}R. Affinity to D_2R and 5-HT_{1A}R of considered ligands was elucidated in docking tests. Ligands' binding mode is coherent with that of typical azapirones. Conserve salt bridge with D3.32 as well as appropriate hydrogen/hydrophobic bonds were identified. The conducted SAR study provides us with useful information as to which ligand should be chosen for further *in vitro/in vivo* studies as well as for the design of new series of ligands.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

^{1}H/^{13}C-NMR spectras, UPLC-MS, 5-HT_{1A}R homology model validation and selected poses for all docked ligands, K_i [nM] + SEM and whole experimental section can be found in supplementary data.

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