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Original article

The multiobjective based design, synthesis and evaluation of the arylsulfonamide/amide derivatives of aryloxyethyl- and arylthioethyl- piperidines and pyrrolidines as a novel class of potent 5-HT$_7$ receptor antagonists

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ABSTRACT

An analysis of the virtual combinatorial library was used for refining a pilot set of 34 derivatives and designing a targeted 38-member library of the arylamide and arylsulfonamide derivatives of aryloxyethyl- and arylthioethyl- piperidines and pyrrolidines. All compounds **24**–**95** were synthesized according to an elaborated parallel solid-phase method and were biologically evaluated for their affinity for 5-HT$_7$R. Additionally, the targeted library members were tested for 5-HT$_{1A}$, 5-HT$_6$, and D$_2$ receptors. Selected compounds of particular interest were examined for their intrinsic activity at 5-HT$_7$R in vitro employing a cAMP assay. The study allowed us to identify compound **68** (4-fluoro-*N*-(1-{2-[(propan-2-yl) phenoxy]ethyl}piperidin-4-yl) benzenesulfonamide) as a potent 5-HT$_7$R ligand ($K_i = 0.3$ nM) with strong antagonistic properties ($K_b = 1$ nM) and a 1450-fold selectivity over 5-HT$_{1A}$Rs.

1. Introduction

Over the recent decades high-throughput chemistry methods have been extensively used in pharmaceutical industry and academic research for identifying and optimizing potential lead compounds. The initial ideology behind the solid-phase synthesis (SPS) underwent transformation from the synthesis of vast combinatorial libraries, counting even 10 000 members and showing a low structural diversity and a high attrition rate, to generation of designed libraries with improved physicochemical attributes. Nowadays, solid-phase chemistry often extends the fragment-based drug discovery approach, and aims at combining building blocks or fragments found in drugs or clinical candidates. Several examples show that the solid-phase chemistry has been efficient at exploring the chemical space and rapidly acquiring meaningful structure–activity relationship data for GPCR-oriented drug discovery projects [1–3].

A wide variety of different computational approaches are used to support the design of combinatorial libraries, their analysis, and identification of the most attractive subsets. Virtual combinatorial libraries (VCL) are usually created using two main strategies; a reactant-based and a product-based design [4–6]. As regards to the former, optimization is focused solely on reagent (building blocks) selection, achieved for example by clustering or similarity searching methods. In the case of product-based approaches, all possible compounds are virtually synthesized from available reactants, and the selection of a final combinatorial library is often supported by virtual screening (VS) methods for scoring and ranking of compounds. The obtained product ranking list is next analyzed to select reactants for the real (often solid-supported) synthesis. The product-based approach is computationally more expensive but seems more promising due to the possibility of

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optimizing properties of the targeted library. Recently, dedicated software for a focused library design have been developed: COLIBREE (Combinatorial Library Breeding) [7], LoFT (Library optimizer using Feature Trees) [8], GLARE (Global Library Assessment of Reagents) [9] and PICCOLO (PICking by COmbinatorial Library Optimization) [10], but the entire process can also be controlled semi-automatically using different tools.

A growing body of preclinical and clinical data supports the hypothesis that ligands affectingthe 5-HT_7 receptors (5-HT_7Rs) may help develop new therapies for the treatment of the affective disorders [11–13]. Identified in 1993, the 5-HT_7R is the latest addition to the 5-HT subtypes, and belongs to the family of G protein-coupled receptors. Its distribution in the hypothalamus (particularly in the suprachiasmatic nucleus), the thalamic and cortical regions has been associated with the control of circadian rhythms and involvement in mood regulation; the presence of 5-HT_7Rs in the hippocampus highlights their potential involvement in learning and memory as well as in emotional processes [14]. More recently, Abbas *et al.* and Sarkisyan *et al.*, have gathered strong evidence that the antidepressant-like effects of well-known atypical antipsychotics amisulpride and aripiprazole are mediated by 5-HT_7R antagonism [15,16]. 5-HT_7R has also shown some potential for pain control, prophylaxis or treatment of migraine and seizures [17].

Several excellent reviews thoroughly have dealt with the progress in the development of 5-HT_7R ligands and have also adequately described the proposed pharmacophore models for 5-HT_7R antagonism and agonism [17–19]. Arylsulfonamides, which are analogs of SB-269970 and SB-656104, hold a pre-eminent position in the group of 5-HT_7R ligands. Another distinguished class is represented by long-chain arylpiperazines (LCAP) with tetrahydrobenzindole, arylketone and amide terminal fragments.

However, these compounds are often devoid of selectivity over 5-HT_{1A}, 5-HT_{2A} or D_2 sites [20–22]. Such obstacles were recently overcome by Leopoldo et al. [23], who described a successful application of 2-substituted phenylpiperazine, leading to generation of the highly potent and selective 5-HT_7R agonists LP-44 and LP-211 (Fig. 1).

For several years our research group has been developing solid-phase methodologies for the synthesis of new arylpiperazine-based selective or multireceptor ligands of 5-HT_{1A}, 5-HT_{2A}, and 5-HT_7 receptors with potential antidepressant, anxiolytic, and antipsychotic properties [24–26]. In the present study we expanded our SPS technology platform for the synthesis of the arylamide and arylsulfonamide derivatives of aryloxyethyl- and arylthioethyl-piperidines and pyrrolidines as novel 5-HT_7R ligands. In comparison with the classic LCAP, we replaced an arylpiperazine fragment with a flexible aryloxy-/arylthio-ethyl, substituted tertiary amine core and simultaneously employed partial rigidification in the part corresponding to the alkylene spacer of LCAP. At the same time, this allowed us to explore the second structural homology by diversifying the length between an amide/sulfonamide bond and a basic nitrogen atom via introduction of three different cores: 3-aminopyrrolidine, 4-aminopiperidine and 4-aminomethylpiperidine (Fig. 2). Further modification consisted of the bioisosteric replacement of an amide bond with a sulfonamide bond. Last but not least, to additionally understand the configurational requirements for compounds binding to 5-HT_7R, we investigated enantiomers of the selected representatives of the 3-aminopyrrolidine core.

The number of compounds that can be synthesized using an elaborated protocol is proportional to the number of accessible building blocks in the commercial chemical space. Therefore, we developed an integrated VCL-VS protocol to support the solid-phase methodology and enable the selection of the most promising building blocks (which are simultaneously diverse and representative) to prioritize the synthesis and to increase the probability of identification of 5-HT_7 receptor ligands. Our project involved affinity determination of the synthesized compounds for the predefined 5-HT_7R, as well as for 5-HT_{1A}, 5-HT_6, and dopamine D_2 receptors, and functional evaluation of the selected library representatives for 5-HT_7Rs.

2. Chemistry – library synthesis

The synthesis of the designed arylamide and arylsulfonamide derivatives of aryloxyethyl- and arylthioethyl- piperidines and pyrrolidines was carried out in two stages. First, starting with the selected commercial phenols and thiols, we synthesized building blocks BB2 – aryloxylethyl bromides and arylthioethyl bromides for a solid-phase approach (for details see Supporting Information). Treatment of the respective phenol derivatives (**1**–**11**) with 1,2-dibromoethane in refluxing acetone in the presence of potassium carbonate yielded aryloxylethyl bromides **22**{*1–11*} (Scheme 1, Pathway A). Alternatively, the respective arylthiols (**12**, **13**) were treated with an excess of 2-chloroethanol in a 10% solution of sodium hydroxide to give arylthioethanol derivatives (**14**, **15**). The latter were readily converted into arylthioethyl bromide analogs (**22**{*12–13*}) upon treatment with phosphorus tribromide (Scheme 1, Pathway B).

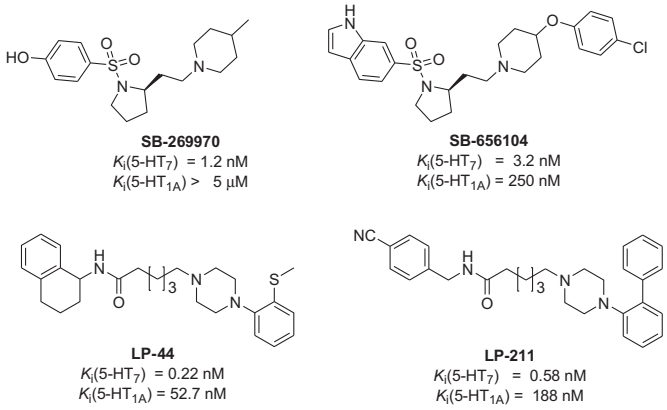


Fig. 1. The structure of model 5-HT_7R ligands.

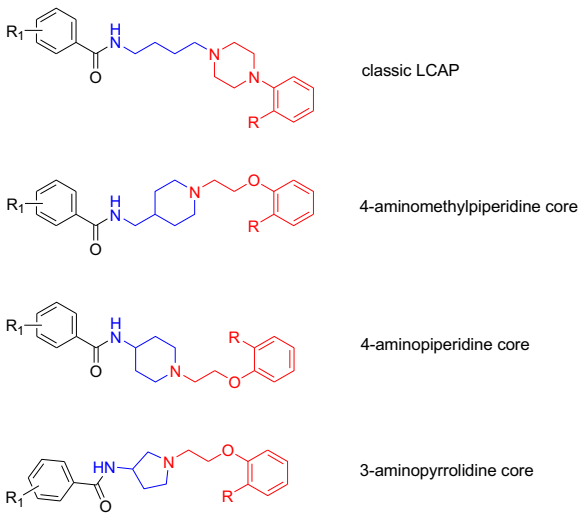
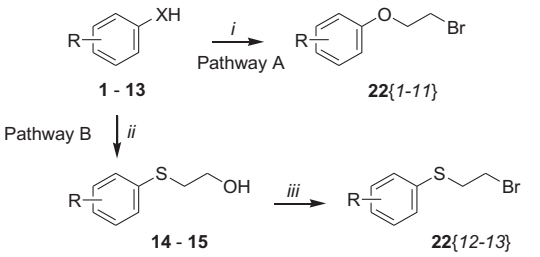


Fig. 2. General structures of the classic long-chain arylpiperazine vs the designed flexible aryloxy-/arylthio-ethyl analogs.



Scheme 1. The synthesis of aryloxylethyl bromides (**22**{*1–11*}) and arylthioethyl bromides (**22**{*12–13*}). Reagents and conditions: *(i)* 1,2-dibromoethane, K_2CO_3, $(CH_3)_2CO$ KI, 60 °C, 48–72 h; *(ii)* 2-chloroethanol, 10% NaOH, 0 °C → 105 °C; *(iii)* PBr_3, CH_2Cl_2 anh., 30 min, argon.

The solid-phase synthesis was performed according to a five-step protocol using a BAL-linker functionalized polystyrene resin (Scheme 2). In the initial step of the synthesis, Boc-protected amines (**17**{*1–5*}, Fig. 3) were anchored to a solid support by one-pot reductive amination.

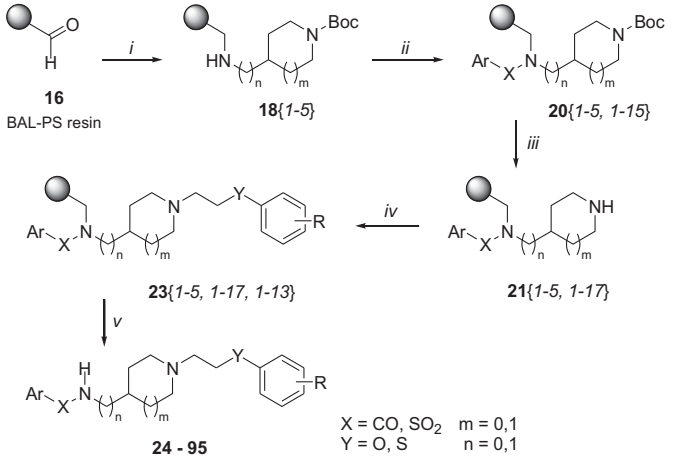
The obtained secondary amines **18**{*1–5*} were subsequently coupled with acyl **19**{*1–7*} and sulfonyl **19**{*8–15*} chlorides (Fig. 4), yielding resin-bound amides and sulfonamides of the general structure **20**. Treatment with acyl chlorides was conducted in DMF and the Hunig base, while with sulfonyl chlorides was carried out in CH_2Cl_2 in the presence of TEA [27]. Then, selective BOC removal was accomplished using Burgess's methodology involving treatment of the resin with a mixture of trimethylsilyltrifluoromethane sulphonate (TMSOTf) and 2,6-lutidine [28].

The obtained secondary amines **21** were further alkylated with aryloxylethyl bromides and arylthioethyl bromides (**22**{*1–13*}, Fig. 5) in the presence of 1,8-diazabicyclo [5,4,0]udec-7-ene (DBU) in DMF. The reaction was conducted at 60 °C for 24 h. Treatment of the resin-bound compounds of the general structure **23** with a mixture of trifluoroacetic acid/CH_2Cl_2 (90/10; v/v) yielded the final products **24**–**95**.

3. Virtual combinatorial library design and analysis

3.1. Queries definition

Based on the proposed chemical reaction pathway, two sets of reagent queries were defined (Fig. 6). The first described pattern for



Scheme 2. The solid-phase synthesis of arylamides and arylsulfonamides of the aryloxyethyl- and arylthioethyl- derivatives of 3-aminopyrrolidine, 4-aminopiperidine and 4-aminomethylpiperidine. Reagents and conditions: *(i)* amine diversity reagent **17**{*1–5*}, $NaBH_3CN$, 1% AcOH/DMF, 60 °C, 24 h; *(ii)* ArCOCl **19**{*1–7*}, DIEA, DMF, RT, 2 × 2 h or $ArSO_2Cl$ **19**{*8–15*}, TEA, CH_2Cl_2, RT, 2 × 2 h; *(iii)* TMSOTf, 2,6-lutidine, CH_2Cl_2, 2 × 30 min; *(iv)* alkylating agent **22**{*1–13*}, DBU, KI, DMF, 60 °C, 24 h; *(v)* TFA/CH_2Cl_2 (90/10; v/v), 2 h.

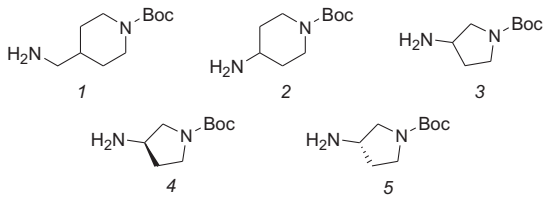


Fig. 3. The diversity of core amines **17**{*1–5*}.

different substituted aryl and heteroaryl sulfonyl and acyl chlorides (BB1 queries), and the second contained general formula for aryloxyalkyl- and arylthioalkyl halides, as well as for unfunctionalized phenols and thiols (BB2 queries). The latter two were transformed to their alkylated halide derivatives by means of Pathway A (Scheme 1), by simple combination of a given building block with a modified linker using CombiGlide [29] application from Schrödinger. Possible structural variations, such as aromatic ring size changes, migration of heteroatoms in ring systems and substitution pattern of any R-groups, were encoded by a symbolic chemical terms language applied in Instant JChem database manager [30].

3.2. Building blocks database searching

The in-house 5.5 × 10^6 building blocks (and intermediates) member library based on stock-available resources from 26 commercial vendors, was build. It was screened using substructure searching algorithm and defined automatic queries with tools applied in Instant JChem. The detailed results are shown in Table SI-2.

3.3. Building blocks filtering

In order to reduce and refine the obtained sets of building blocks, three filters were applied. The first limited BBs to those which simultaneously satisfied simple structural rules, i.e.: molecular weight ≤250 Da, the number of rotatable bonds ≤4, and the number of rings ≤2. Then, based on medicinal/organic chemistry know-how, libraries of unwanted and reactive functional R-groups were defined (e.g. amines, alcohols, carboxylic acids, esters) and were successively used to reduce the BB datasets. The resultant building block sets were hierarchically grouped using Molprint2D fingerprint and Tanimoto metric (Canvas) [31], yielding 76 and 259 clusters of BB1 and BB2, respectively. Next, centroids and a few of the structurally most diverse cluster representatives (1–5 BBs, proportionally to cluster size) were selected. Additionally, the database was supplemented with building blocks used in the synthesis of a pilot series of compounds giving 257 representatives of BB1 set and 555 agents of BB2 set (Fig. 6).

3.4. Virtual combinatorial library (VCL) generation

The selected building block databases BB1 and BB2 were iteratively combined with each core using a set of algorithms applied in CombiGlide [29]. In this way, all possible combinations (i.e. about 428 000 virtual compounds) were produced and used as an input database for the Virtual Screening Protocol.

3.5. Virtual screening (VS) protocol

Basically, the applied protocol followed the hierarchical scheme reported previously [32], and consisted of four filtering phases: physicochemical, ADME, 3D pharmacophore and docking. At first, the Lipinski rules of 5, the Veber rules and the strongest basic pK_a > 5 were applied (Calculator Plugins, JChem) [33]. Since

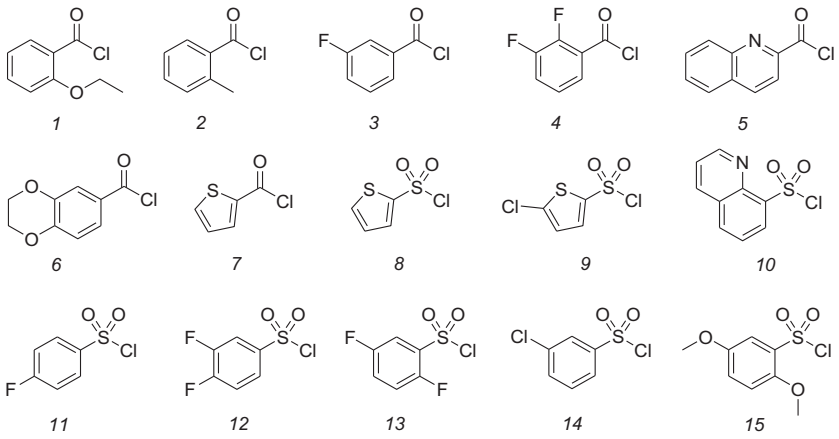


Fig. 4. The diversity of building blocks BB1 – acyl chlorides **19**{*1–7*} and sulfonyl chlorides **19**{*8–15*}.

successive stages were based on 3D structures, all possible stereoisomers were generated using LigPrep [34] from Schrödinger. The calculated ADME descriptors (QikProp) [35], i.e. the number of reactive functional groups (0–2), aqueous solubility (−6.5–0.5 mole/liter), gut-blood barrier (>500 nm/s) and blood-brain-barrier penetration coefficient (−3.0–1.2), were used to filter off compounds with unfavorable profiles. In the next phase, linear combination of six different pharmacophore models, developed on previously classified diverse groups of 5-HT_7 antagonists [36], were applied. A compound was accepted if it matched at least one of the pharmacophore models used. The remaining set of molecules was docked (Glide at SP accuracy level) to six different conformations of the previously published rhodopsin-based 5-HT_7 homology models [36], with a spatial constrain on ionic interaction between a ligand and Asp3.32 side-chain.

3.6. Post-docking filter

The developed post-docking filter (see experimental) was used to reduce the final subset to unique compounds with a correct binding mode. The subset of unique compounds contained only the highly classified stereogenic form of the representatives of 3-aminopyrrolidine derivatives. The elaborated classification models were based on active and inactive 5-HT_7R ligands, and involved structural interaction fingerprints (SIFt) and support vector machine (SVM) method. Compounds whose docking pose had a negative value of the decision function were deleted from the final set.

3.7. Ranking scheme

The final results were analyzed using two different ranking approaches, i.e. BB-based and product-based. In the former, the ranking of building blocks was generated on the basis of the presence of a given BB in the final set of VCL. A substructure searching of final VCL in Instant JChem was performed for each building block used for its generation. Unique compounds containing queried structure were counted. Following the same idea, the occurrence of each building block in vendor's stocks was estimated. The product-based ranking list of compounds was made by merging various scoring functions, such as docking scoring function (Glide Score), SVM-RBF decision function and rankings of BBs occurrence. For each of those parameters, separate rankings were created and merged into one using the MIN rule of data fusion [37,38].

4. Results and discussion

Our initial studies were directed towards structural analogs of the arylamide derivatives of classic long-chain arylpiperazines in which an arylpiperazine pharmacophore was replaced with a flexible aryloxy-/arylthio-ethyl amine fragments (Fig. 2). A pilot 34-member library was evaluated for 5-HT_7Rs and those compounds displayed high to low affinity ranging from 5 to 2674 nM (Table 1). The primary focus being on the amine core fragments (4-aminomethylpiperidine, 4-aminopiperidine, and 3-aminopyrrolidine) which indicated that a larger distance between the amide/

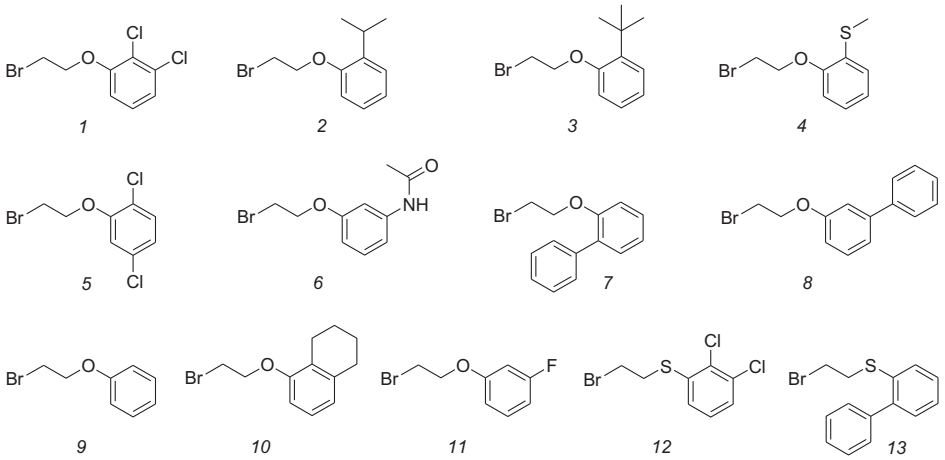


Fig. 5. The diversity of building blocks BB2 – N-alkylating agents aryloxylethyl bromides **22**{*1–11*} and arylthioethyl bromides **22**{*12–13*}.

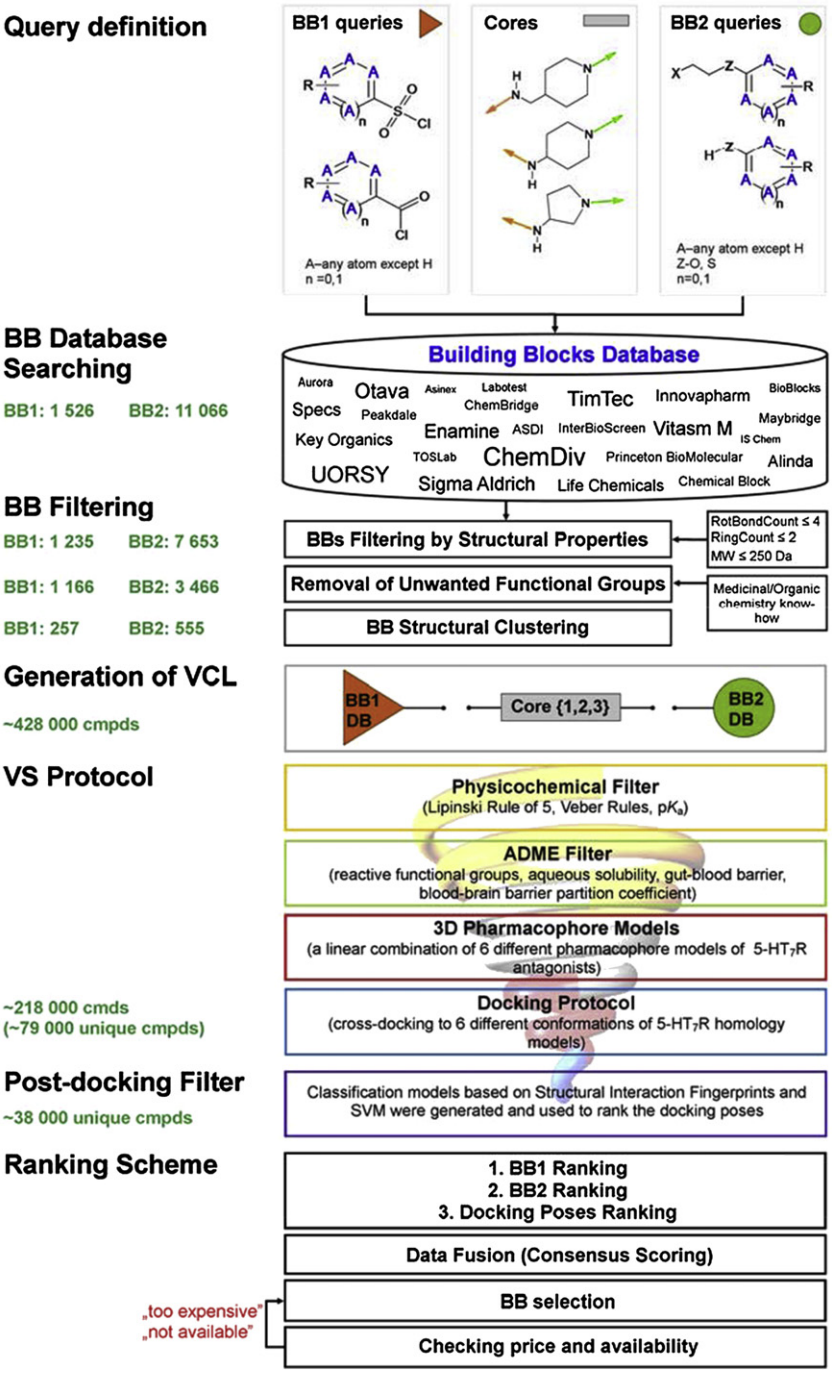


Fig. 6. A scheme of the applied VCL-VS protocol.

sulfonamide group and the basic nitrogen atom negatively influenced the binding to 5-HT$_7$R.

In general, arylamide derivatives from the **24**–**57** set displayed low to moderate affinity for the 5-HT$_7$Rs ($K_{i5\text{-}HT7} > 100$ nM). The only exceptions were pyrrolidyn-3-yl amides which contained a biphenyl-oxy fragment (**46**, **48**). Bioisosteric replacement of an amide bond with sp^2 hybridization and planar configuration with sulfonamide bond with sp^3 hybridization and tetrahedral configuration dramatically increased the affinity of compounds for 5-HT$_7$ receptors. All the arylsulfonamide derivatives displayed high affinity for the 5-HT$_7$Rs ($K_i < 100$ nM).

It was found that the introduction of substituents in the *meta* position of the phenyl ring (e.g. BB2 – **22**{*6*} and **22**{*8*}) in the aryloxy-/arylthio-ethyl fragment negatively influenced the binding of compounds **45** and **50** to 5-HT$_7$Rs. On the other hand, an isopropyl and a phenyl substituent in an *ortho* position accommodated better in the receptor pocket; of the *ortho*-substituted building blocks, **22**{*3*} containing *tert*-butyl moiety proved to be less preferred (e.g. **35** *vs* **36**). Interestingly, this data is consistent with that reported by Leopoldo *et al.* who described the enhanced 5-HT$_7$ receptor affinity of compounds containing *ortho*-phenyl- and *ortho*-isopropyl- piperazines [23]. Another interesting finding was that arylsulfonamide derivatives containing an *ortho*-methylsulfanyl substituent displayed high affinity for 5-HT$_7$Rs.

In an attempt to better understand the structural requirements for the investigated flexible analogs of arylpiperazines for the

Table 1
The binding data of the pilot 34-member library representatives (**24**–**57**) for 5-HT$_7$R.

Compd[a]	{*Core*,*BB1*,*BB2*}	K_i 5-HT$_7$ [nM]	Compd[a]	{Core,*BB1*,*BB2*}	K_i 5-HT$_7$ [nM]
24	{***1,1,4***}	1188 ± 132	**41**	{***2,9,4***}	17 ± 2
25	{***1,2,3***}	1500 ± 116	**42**	{***2,9,7***}	12 ± 1
26	{***1,2,8***}	2674 ± 178	**43**	{***2,12,13***}	93 ± 6
27	{***1,2,10***}	409 ± 36	**44**	{***3,1,2***}	204 ± 17
28	{***1,3,5***}	415 ± 22	**45**	{***3,1,6***}	3372 ± 410
29	{***1,5,7***}	334 ± 17	**46**	{***3,1,7***}	85 ± 5
30	{***1,7,2***}	407 ± 28	**47**	{***3,1,8***}	1104 ± 75
31	{***1,15,2***}	27 ± 2	**48**	{***3,2,7***}	59 ± 4
32	{***2,1,2***}	311 ± 34	**49**	{***3,2,12***}	427 ± 18
33	{***2,1,13***}	329 ± 21	**50**	{***3,3,8***}	709 ± 86
34	{***2,2,12***}	815 ± 64	**51**	{***3,3,10***}	2224 ± 252
35	{***2,3,3***}	1672 ± 145	**52**	{***3,5,12***}	1108 ± 87
36	{***2,3,7***}	391 ± 14	**53**	{***3,6,1***}	161 ± 6
37	{***2,5,5***}	873 ± 97	**54**	{***3,9,2***}	7 ± 1
38	{***2,5,13***}	865 ± 66	**55**	{***3,9,4***}	5 ± 0.5
39	{***2,7,4***}	1144 ± 120	**56**	{***3,11,7***}	5 ± 0.4
40	{***2,7,7***}	276 ± 19	**57**	{***3,12,5***}	97 ± 11

[a] Physicochemical data for compounds with $K_{i(5\text{-HT7})} > 90$ nM is given in Table SI-1.

interaction with 5-HT$_7$ receptors, we designed and synthesized a targeted library of compounds **58**–**95**. To achieve this, we made use of the biological results obtained for the pilot set (Table 1), and analysis of the commercially available BB chemical space. The search of building block libraries of 26 vendors with defined queries (Fig. 6) resulted in 1526 BB1 and 11066 BB2. Their combination with 3 cores would give a virtual library of about 51×10^6 compounds (without stereoisomers), hence, a BB filtering was applied prior to VCL generation. The remaining blocks were clustered, and only several representatives (including centroids) were used to obtain the VCL of 428 000 compounds (0.84% of the initial chemical space). In the successive stage, the previously elaborated multistep virtual screening protocol [32] (physicochemical filter, ADME filter, 3D pharmacophore models, docking protocol) allowed a 5-fold reduction in the number of compounds.

Since only one constraint, i.e. an ionic interaction of a ligand with the crucial Asp3.32 side chain, was implied in docking, an automated procedure for selecting poses matching the common binding mode was developed and used. This was based on SIFt, the machine learning method (support vector machine – SVM) and the tool was trained on a set of active 5-HT$_7$R ligands ($K_{i(5\text{-HT7R})} < 300$ nM) and inactive compounds ($K_{i(5\text{-HT7R})} > 5$ μM) with diverse structures. In the final set of 38000 compounds, the frequency of BBs was analyzed (Table 2) and used as a clue for selecting reagents for the targeted library.

Since the experimental data is of primary importance, a number of building blocks not preferable to the pilot series were excluded. From the previously applied set of acyl chlorides, we chose only **19**{*5*} and additionally, the well-scored benzoyl chlorides **19**{*4*} and **19**{*1*}. We extended the set of sulfonyl chlorides by applying several highly (**19**{*8*}, **19**{*13*}) and moderately (**19**{*10*}, **19**{*14*}) positioned representatives (Table 2). Regarding to the choice of alkylating agents (**22**{*1–13*}), we excluded a few non-tolerated fragments from the pilot series (e.g. **22**{*3*}, **22**{*6*}, **22**{*8*}), as well as arylthioethyl bromides. On the other hand, we introduced two representatives of the most highly scored aryloxyethyl building blocks (**22**{*9*} and **22**{*11*}). Several of the top-ranked BBs were unaffordable or caused unpredicted synthetic problems, as in the case of 8-(2-chloroethoxy) quinoline, the second-scored representative of the BB2 set (Table 2). The latter compound was readily cyclized to the corresponding 2,3-dihydropyrido [1,2,3-de]-1,4-benzoxazinium salt via the intramolecular quaternization of a quinoline heteroatom [39].

All the members of the target library were biologically evaluated for their affinity for 5-HT$_7$Rs and due to the structural homology and involvement in the pathology of psychiatric disorders for the 5-HT$_{1A}$R, 5-HT$_6$R and D$_2$ dopamine receptors (Table 3). Compounds **58**–**95** displayed diversified affinity for 5-HT$_7$, their K_i value ranging from 0.3 nM to 2375 nM, high to low affinity for 5-HT$_{1A}$ (9–903 nM) and comparable affinity for D$_2$ receptors (10–1059 nM). The compounds tested displayed the lowest affinity for 5-HT$_6$ sites, ranging from 91 to 2759 nM. Within the targeted library, some compounds were found as highly potent 5-HT$_7$R ligands with acceptable selectivity over other receptors (Table 3).

Structure–activity relationship studies within the target library confirmed the results of virtual screening by showing that the sulfonamide bond with sp^3 hybridization was more beneficial than the amide in the interaction with 5-HT$_7$Rs. It is noteworthy that, the highly ranked *p*-F-benzenesulfonamide fragment (**19**{*11*}) turned out to be advantageous to 5-HT$_7$R affinity (e.g. **61**, **68**, **81**, **82**, **83**, **92**). Furthermore, introduction of building blocks with an additional fluoro atom (**19**{*12*} or **19**{*13*}) either only slightly decreased the affinity for 5-HT$_7$Rs (**56** *vs* **84** and **86**) or did not influence it at all (**83** *vs* **87**). This was also in accordance with the frequency scoring presented in Table 2.

Furthermore, it was difficult to determine precisely the most favorable amine core for the binding to 5-HT$_7$Rs by defining the optimal distance between the H-bond donor group and the positively charged nitrogen atom. Nevertheless, the 4-aminomethylpiperidine core with a low conformational restriction was less preferential. That finding was in agreement with VS results where compounds with 4-aminomethylpiperidine moiety (**17**{*1*}) amounted to 18% of the final set.

As already demonstrated in the pilot series, the affinity of compounds **58**–**95** for 5-HT$_7$Rs also depended crucially upon the kind of substitution in the aryloxyethyl fragment. Being highly ranked, the unsubstituted phenoxy building block (**22**{*9*}) yielded compounds with either high (**82**) or moderate (e.g. **67** and **79**) 5-HT$_7$R affinity. Surprisingly, the most highly scored building block **22**{*11*} (Table 2) containing a fluoro atom in the *meta* position turned out to be very unfavorable for the interaction with 5-HT$_7$R (and other receptors, as well). That was much below expectations compared to the data presented by Volk et al. who described the *meta*-fluorophenylpiperazine derivative as a 5-HT$_7$R ligand with $K_i = 11$ nM [21]. On the other hand, the considerably less widely populated *ortho*-isopropylphenoxy fragment (**22**{*2*}) was found to be highly beneficial for 5-HT$_7$R affinity; all the arylsulfonamides containing that substituent displayed good affinity for 5-HT$_7$ sites ($K_i < 60$ nM), of which **68** showed outstanding affinity ($K_i = 0.3$ nM). The importance of the substituent in the *ortho* position of the aryloxyethyl fragment was additionally evidenced by the high affinity of compounds with methylsulfanyl- and phenyl-substituted building blocks, **22**{*4*} and **22**{*7*}, respectively (Tables 2 and 3). The above analysis showed that computational results were generally in good agreement with the biological data reinforcing the relevance of its application in building blocks filtering.

In regards to the data on the affinity of compounds **58**–**95** for 5-HT$_{1A}$Rs, it was demonstrated that of the secondary amine cores **17**{*1–3*}, 3-aminopyrrolidine moiety (**17**{*3*}) was preferential for those sites. Importantly enough, compounds **64**, **81**, **89** containing a methylsulfanyl substituent in the *ortho* position (**22**{*4*}) were always classified as potent 5-HT$_{1A}$ ligands, regardless of other building blocks used. This finding shows that flexible aryloxyethyl amine fragment, substituted with electron-donating moiety in the *ortho* position, preserves, e.g., *ortho*-methoxyphenylpiperazine (a well-known 5-HT$_{1A}$ privileged structure) high affinity for 5-HT$_{1A}$ sites. Irrespective of piperazine opening, the influence of the substitution pattern, similar to that observed in the classic LCAP, supports the concept that aryloxyalkyl piperidines and pyrrolidines may be regarded as arylpiperazine biomimetics. This hypothesis may be further confirmed by compound **77** with a 2,3-dichlorophenoxy fragment (**22**{*1*}), which, like 2,3-diCl-phenylpiperazines showed high affinity for 5-HT$_{1A}$Rs

Table 2
The frequency and ranking of building blocks BB1 and BB2 in a set of 38 000 compounds. BB in shades were used for generation of the targeted library.

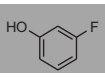
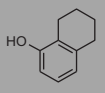
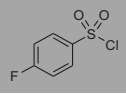
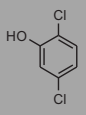
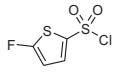
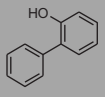
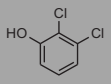
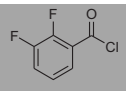
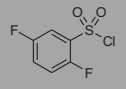
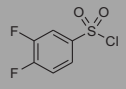
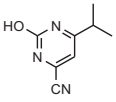
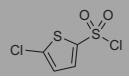
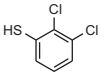
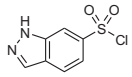
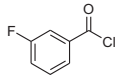
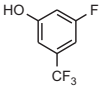
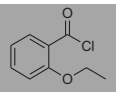
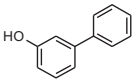
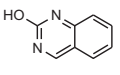
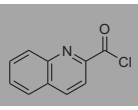
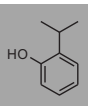
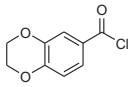
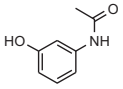
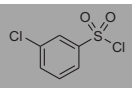
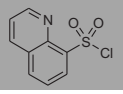
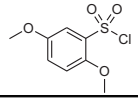
BB1					BB2				
	Structure	Rank	Freq.	BB avail.		Structure	Rank	Freq.	BB avail.
19[a]		1	430	4	22{*11*}		1	248	4
19[a]		2	428	2	22[b]		2	228	7
19[a]		4	419	4	22{*9*}		5	206	7
19{*8*}		5	402	11	22{*10*}		75	140	6
19{*11*}		7	392	9	22{*5*}		82	134	7
19[a]		13	366	2	22{*7*}		89	131	7
19[a]		23	336	3	22{*1*}		109	117	5
19{*4*}		27	315	3	22{*4*}		158	82	2
19{*13*}		34	308	8	22{*13*}		161	80	1
19{*12*}		35	307	9	22[a]		166	75	1
19{*9*}		43	280	13	22{*12*}		174	70	3
19[a]		55	266	1	22[a]		185	64	1
19{*3*}		102	167	7	22[a]		194	57	1
19{*1*}		115	142	1	22{*8*}		217	42	4
19{*7*}		130	122	7	22[a]		225	41	2

Table 2 (*continued*)

BB1					BB2				
	Structure	Rank	Freq.	BB avail.		Structure	Rank	Freq.	BB avail.
19{*5*}		135	116	4	22{*2*}		229	39	4
19{*6*}		160	84	7	22{*6*}		253	32	6
19{*2*}		164	81	7	22{*3*}		502	4	5
19{*14*}		175	67	8					
19{*10*}		188	44	9					
19{*15*}		236	8	12					

Freq. – frequency of a given BB in the final set of 38 000 compounds.
BB avail. – BB availability in vendors' stocks.
[a] Building blocks recognized as unaffordable.
[b] Building block with unpredicted chemistry.

[40]. On the contrary, the 2,5-dichlorophenoxy fragment (**22**{*5*}), which did not fit the substitution pattern of LCAP for the 5-HT_{1A}R, displayed low affinity for 5-HT_{1A} sites.

As regards to the structural similarity between 5-HT_7 and 5-HT_{1A} receptors, designing selective 5-HT_7R ligands is often problematic. On the basis of the above data we traced structural requirements for 5-HT_7/5-HT_{1A} selectivity. Regarding to the arylsulfonamide fragment, introduction of a chlorine atom into position 5 of the thiophenesulfonamide core, regardless additional chiral switch from racemic mixture to respective R/S enantiomers, increased the 5-HT_7/5-HT_{1A}Rs selectivity (compound **73** vs compounds **90**, **93** and **74** vs **91**, **94**, respectively). The important element determining 5-HT_7/5-HT_{1A}R selectivity was the kind of a substituent at the phenyl ring in the aryloxy fragment. As proof, compounds containing an unsubstituted phenyl ring (**82** and **79**) displayed low 5-HT_7/5-HT_{1A} selectivity. The same relationship was observed in the case of compounds containing tetrahydronaphthalenyloxy moiety (**75**, **83**, **87**). Although ligands with an *ortho*-methylsulfanyl substituent showed high affinity for 5-HT_7R (**64**, **81**, **89**), their closer examination revealed that, in contrast to the data concerning arylpiperazines [23], the modification did not enhance the selectivity over 5-HT_{1A}Rs within the evaluated series. Compounds containing electron-donating substituents in the *ortho* position either displayed no 5-HT_7/5-HT_{1A} selectivity (**81**), or even bound preferentially to 5-HT_{1A} sites (**89**, **64**). In contrast, library members with isopropyl and phenyl substituents in the *ortho* position displayed the highest 5-HT_7 preference with outstanding 5-HT_7/5-HT_{1A} selectivity ratio for compound **68** equaling 1453-fold. Thus, like in the case of LCAP [23], hydrophobic and $\pi-\pi$ interaction properties in the *ortho* position accounted better selectivity for the 5-HT_7/5-HT_{1A}R.

In the series of pyrrolidyn-3-yl arylsulfonamides we observed a slight preference for the *R* enantiomers **90**–**92** over their *S* counterparts **93**–**95** in respect to their affinity for 5-HT_7Rs. Moreover, an increase of selectivity of the *R* enantiomer over 5-HT_{1A} sites was also observed. Of the evaluated enantiomers, compound **91** displayed the highest 5-HT_7/5-HT_{1A} selectivity ratio ($S_{1A/7} = 19$). The results obtained for the (*R*) enantiomers **90** and **92** were in line with the virtual screening data, more interestingly, they were consistent with the stereospecificity of SB-269970 for 5-HT_7Rs [41].

Since, like aripiprazole and amisulpride, some D_2Rs ligands display high affinity for 5-HT_7 sites, we also tested the targeted library for D_2 receptors. We found that majority of the compounds were classified as potent D_2R ligands. The influence of amine cores and the kind of arylamide and arylsulfonamide moieties were not very clear. However, the properties of a substituent at the phenyl ring in the aryloxyethyl fragment affected differentially the interaction with D_2Rs. Interestingly, a fluoro atom in the *meta* position was the least preferential substituent for D_2R affinity. It was later found that the selected (*R*) enantiomers of pyrrolidyn-3-yl arylsulfonamides (**90**–**92**) displayed a higher 5-HT_7/D_2 selectivity, that observation was consistent with the results obtained for 5-HT_7/5-HT_{1A} selectivity. On the other hand, their (*S*)-counterparts may become templates for designing multireceptor ligands in this class of derivatives.

4.1. The functional effect of selected ligands on 5-HT_7 receptors

Five selected library representatives were evaluated in a functional model in CHO cells, which stably expressed the human 5-HT_7 receptor. All the compounds behaved like potent antagonists at h5-HT_7R (Table 4), and methiothepin was used as a reference compound. Interestingly, the most potent compound in the 5-HT_7 affinity binding assay **68** displayed the K_B value equaling 1 nM, which indicated superior antagonistic activity compared to the other congeners tested. Furthermore, the potency depended on stereochemistry. Examination of the antagonistic activity of a pair of enantiomers (**91** and **94**) provided additional support for the preference of 5-HT_7Rs for *R* enantiomers.

Table 3
The binding data of the second set of library members (**58**–**95**) for 5-HT$_7$Rs and 5-HT$_{1A}$, 5-HT$_6$, and D$_2$ receptors.

Compd	{*Core*,*BB1*,*BB2*}	$K_i \pm$ SEM [nM]				$S_{1A/7}$[b]
		5-HT$_7$	5-HT$_{1A}$	5-HT$_6$	D$_2$	
58	{***1,9,7***}	20 ± 2	101 ± 9	410 ± 26	32 ± 3	5
59	{***1,8,11***}	2222 ± 180	841 ± 58	2759 ± 180	1059 ± 99	<1
60	{***1,11,2***}	60 ± 5	141 ± 15	313 ± 26	50 ± 3	2
61	{***1,11,7***}	13 ± 1	290 ± 22	315 ± 33	46 ± 4	22
62	{***1,11,11***}	1362 ± 118	903 ± 48	2124 ± 194	839 ± 75	<1
63	{***1,14,2***}	46 ± 5	104 ± 7	177 ± 10	66 ± 5	2
64	{***1,14,4***}	78 ± 6	18 ± 2	147 ± 8	50 ± 1	<1
65	{***1,14,11***}	2195 ± 257	283 ± 33	831 ± 39	560 ± 62	<1
66	{***2,1,11***}	2375 ± 194	456 ± 35	3785 ± 415	76 ± 9	<1
67	{***2,8,9***}	155 ± 11	237 ± 7	862 ± 62	84 ± 7	2
68	{***2,11,2***}	0.3 ± 0.1	436 ± 21	240 ± 29	51 ± 5	1453
69	{***2,12,7***}	7 ± 1	313 ± 28	199 ± 17	63 ± 4	45
70	{***3,4,7***}	21 ± 3	48 ± 4	1799 ± 128	15 ± 2	2
71	{***3,5,2***}	178 ± 14	30 ± 3	1761 ± 186	79 ± 7	<1
72	{***3,5,7***}	41 ± 3	45 ± 6	1335 ± 94	24 ± 1	1
73	{***3,8,2***}	29 ± 4	21 ± 2	258 ± 20	16 ± 1	<1
74	{***3,8,7***}	10 ± 1	45 ± 3	267 ± 17	16 ± 2	5
75	{***3,8,10***}	27 ± 2	13 ± 1	102 ± 8	72 ± 5	<1
76	{***3,8,11***}	1438 ± 79	270 ± 29	1779 ± 140	729 ± 41	<1
77	{***3,9,1***}	14 ± 2	28 ± 4	91 ± 8	67 ± 8	2
78	{***3,9,5***}	58 ± 5	717 ± 58	225 ± 31	78 ± 6	12
79	{***3,9,9***}	121 ± 9	65 ± 7	957 ± 74	318 ± 24	<1
80	{***3,10,2***}	10 ± 1	23 ± 1	504 ± 44	10 ± 1	2
81	{***3,11,4***}	11 ± 1	10 ± 1	357 ± 26	41 ± 3	1
82	{***3,11,9***}	9 ± 1	9 ± 1	248 ± 21	11 ± 1	1
83	{***3,11,10***}	16 ± 2	22 ± 1	96 ± 8	72 ± 5	1
84	{***3,12,7***}	7 ± 0.5	92 ± 5	327 ± 23	24 ± 3	13
85	{***3,12,11***}	293 ± 17	230 ± 9	951 ± 88	523 ± 19	<1
86	{***3,13,7***}	9 ± 1	81 ± 4	515 ± 54	18 ± 2	9
87	{***3,13,10***}	16 ± 1	10 ± 1	156 ± 17	60 ± 4	<1
88	{***3,14,2***}	4 ± 0,6	31 ± 2	245 ± 15	25 ± 3	8
89	{***3,14,4***}	15 ± 1	5 ± 0.6	185 ± 7	36 ± 2	<1
90	{***4,9,2***}	6 ± 0.3	49 ± 6	200 ± 17	20 ± 1	8
91	{***4,9,7***}	4 ± 0.2	75 ± 6	202 ± 23	39 ± 1	19
92	{***4,11,7***}	2 ± 0.2	22 ± 3	102 ± 4	16 ± 2	11
93	{***5,9,2***}	28 ± 2	89 ± 7	537 ± 49	25 ± 1	3
94	{***5,9,7***}	12 ± 1	147 ± 12	551 ± 34	8 ± 1	12
95	{***5,11,7***}	10 ± 1.1	32 ± 2	271 ± 17	10 ± 1	3
Clozapine[a]		18 ± 2	143 ± 11	4 ± 0.3	72 ± 5	–
Olanzapine[a]		185 ± 21	3442 ± 408	5 ± 0.7	7 ± 1	–

[a] Standard drugs added as a reference.
[b] Ratio of affinity for 5-HT$_{1A}$ vs 5-HT$_7$ receptors.

5. Conclusions

Aiming to develop of novel 5-HT$_7$ receptor antagonists, a 34-member pilot library of the arylamide and arylsulfonamide derivatives of differently substituted aryloxy- and arylthio-ethyl piperidines and pyrrolidines was synthesized using a solid-phase methodology. Successively, a computational approach involving a combinatorial library design and a multistep virtual screening, followed by post-docking filtering and building block ranking within compounds satisfying the desired 5-HT$_7$R binding pattern allowed us to identify critical molecular substructures and provided rationale data for designing the targeted 38-member library of 5-HT$_7$R ligands. A few compounds of the series, i.e. **61**, **69**, **91**, displayed high affinity for 5-HT$_7$Rs and moderate selectivity over 5-HT$_{1A}$, 5-HT$_6$, and D$_2$ sites. The arylsulfonamide derivative **68** showed high affinity for 5-HT$_7$Rs (K_i = 0.3 nM) with high selectivity over 5-HT$_{1A}$R and D$_2$R, and was classified as a potent antagonist (K_b = 1 nM). Structure-activity relationship studies demonstrated that *ortho*-phenyl or *ortho*-isopropyl substitutes at the aryloxyethyl fragment were preferred for an interaction with 5-HT$_7$Rs. Furthermore, the sulfonamide bond turned out to be favorable for those sites. It seems that the replacement of arylpiperazine pharmacophore with flexible aryloxyethyl piperidine and pyrrolidine fragments opens up the possibility of exploring a new chemical space and designing new selective 5-HT$_7$R ligands or multireceptor ligands targeted on monoamine receptors. Further investigation of this series of derivatives may result in discovering of novel psychotropic agents.

Table 4
The receptor binding data and antagonistic activities for 5-HT$_7$ receptors of some selected library members.

Compd	{*Core*,*BB1*,*BB2*}	5-HT$_7$	
		K_i [nM]	K_b [nM]
61	{***1,11,7***}	13	5.1
68	{***2,11,2***}	0.3	1
69	{***2,12,7***}	7	4.6
91	{***4,9,7***}	4	7.4
94	{***5,9,7***}	12	21
Clozapine		18	–
Methiothepin		–	0.075

6. Experimental

6.1. Chemistry

Solution and solid-phase organic transformations and resin washes were carried out at ambient temperature, unless indicated otherwise. Organic solvents (from Aldrich and Chempur) were of reagent grade and were used without purification. BAL linker MBHA-

resin (loading 1.1 mmol/g) was purchased from Iris Chemicals. The reagents were from Aldrich, Alfa Aesar, Chembridge, Fluorochem.

Analytical HPLC were run on a Waters Alliance HPLC instrument, equipped with a ChromolithSpeedROD column (4.6 × 50 mm). Standard conditions were eluent system A (water/0.1% TFA), system B (acetonitrile/0.1% TFA). A flow rate of 5 mL/min and a gradient of (0–100)% B over 3 min were used. Detection was performed on a PDA detector.

^{1}H NMR and ^{13}C NMR spectra were obtained in a Varian BB 200 spectrometer using TMS (0.00 ppm) in chloroform-d_1, and were recorded at 300 and 75 MHz, respectively; *J* values are in hertz (Hz), and splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), m (multiplet).

Melting points (mp) were determined with a Büchi apparatus and are uncorrected.

Elemental analysis for C, H, and N were carried out by a micro method using the elemental Vario EI III elemental analyzer. The results for elemental analyses were found within ±0.4% of the theoretical values.

LC/MS were carried out on a system consisted of a Waters Acquity UPLC, coupled to a Waters TQD mass spectrometer. All the analyses were carried out using a Acquity UPLC BEH C18, 50 × 2.1 mm column, at 40 °C. A flow rate of 0.3 mL/min and a gradient of (5–95)% B over 5 min was used. Eluent A: water/0.1% HCO_2H; eluent B: acetonitrile/0.1% HCO_2H. Retention times (R_t) are given in minutes. The UPLC/MS purity of all the test compounds and key intermediates was determined to be >98%.

Abbreviations used: DIEA, *N*,*N*-diisopropylethylamine; EtOAc, ethyl acetate; TEA, triethylamine.

6.2. Solid-phase synthesis on BAL-MBHA-PS-resin

6.2.1. Preparation of amine-bound resin **16** via reductive amination

The dried MBHA-BAL resin was divided into five reactors containing a suspension sodium cyanoborohydride ([$NaBH_3CN$] = 5.5 mmol, 5 equiv) and the amine ([Diversity reagent **17**, **17**{*1–5*}] = 5.5 mmol, 5 equiv, Fig. 3), in a 1% acetic acid in 3 mL of DMF. The reactors were placed in the oven for 24 h at 60 °C. Then the resin was drained; washed with 10% AcOH in DMF (1 × 5 mL) then with DMF (3 × 5 mL), MeOH (1 × 5 mL), and CH_2Cl_2 (3 × 5 mL), and dried under low vacuum.

6.2.2. Preparation of support-bound carboxamide derivatives

1 mL of a solution containing acyl chloride ([Diversity reagent **19**, **19**{*1–7*}] = 0.40 mmol, 5 equiv, Fig. 4) in DMF was added to the resin followed by addition of DIEA (0.88 mmol, 11 equiv). The reactors were shaken for 2 h at room temperature. The resin was drained and washed with DMF (3 × 5 mL), MeOH (1 × 5 mL), and CH_2Cl_2 (3 × 5 mL), and dried under low vacuum. The procedure described above was repeated.

6.2.3. Preparation of support-bound sulfonamide derivatives

1 mL of a solution containing sulfonyl chloride ([Diversity reagent **19**, **19**{*8–15*}] = 0.40 mmol, 5 equiv, Fig. 4) in CH_2Cl_2 was added to the resin followed by addition of TEA (0.88 mmol, 11 eq). The reactors were shaken for 2 h at room temperature. The resin was drained and washed with DMF (3 × 5 mL), MeOH (1 × 5 mL), and CH_2Cl_2 (3 × 5 mL), and dried under low vacuum. The procedure described above was repeated.

6.2.4. Boc-deprotection protocol

The resin was treated with 1 mL of a freshly prepared solution of TMSOTf (1.5 M) and 2,6-lutidine (1 M) in dry CH_2Cl_2. The resin was shaken for 30 min and was washed with CH_2Cl_2 (3 × 1 mL). The reaction was repeated and the resin was washed with CH_2Cl_2 (3 × 3 mL), DMF (3 × 3 mL), MeOH (5 × 3 mL), and CH_2Cl_2 (3 × 3 mL), and dried under low vacuum. The efficacy of the reaction was confirmed by a positive chloranil test.

6.2.5. Alkylation with aryloxyalkylbromides and arylthioalkylbromides

The deprotected resin **21** was swelled in CH_2Cl_2 for 30 min, and to the resin was added a solution of 0.8 ml of 0.65 M solution of alkylating agent **22** ([Diversity reagent **22**, **22**{*1-13*}], 0.52 mmol, 6.5 eq, Fig. 5) in DMF, followed by addition of DBU (1.04 mmol, 13 eq) and KI (0.08 mmol, 1 eq). The reactors were placed in 60 °C for 24 h. The resulting resin **23** was drained, washed with DMF (3 × 1 mL), MeOH (1 × 1 mL), CH_2Cl_2 (3 × 1 mL) and dried under vacuum.

6.2.6. Cleavage of the final products

A 1.5 mL of a mixture of TFA/CH_2Cl_2 (9/1, v/v) was dispensed into glass vials containing the resin. The cleavage was carried out for 120 min, then the mixture was filtrated and the resin was washed with a mixture of TFA/CH_2Cl_2 (8/2; v/v), and the collected filtrates were evaporated with a stream of argon on Eva parallel evaporator.

Average overall yields of the crude products were between 38 and 57% and were calculated on the basis of the initial loading of the resin. The LC/MS of the identified compounds **24**–**95** revealed an average purity exceeding 82%. The crude residue was redissolved in CH_2Cl_2 or CH_2Cl_2/MeOH mixture, and purified using silica gel columns and mixture CH_2Cl_2/MeOH, to elute the pure products **24**–**95**.

6.3. Spectroscopic data for selected library members

The compound characterization data for the key final compounds from Table 3 are summarized below. The synthesis and characterization data for the remaining final compounds are in the Supporting Information.

6.3.1. 4-Fluoro-N-({1-[2-(biphenyl-2-yloxy)ethyl]piperidin-4-yl} methyl) benzenesulfonamide (**61**)

Yellow oil, 38 mg (54% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.5); initial LC/MS purity 88%, t_R = 3.05. MW 468.58. Monoisotopic Mass 468.2, $[M + H]^+$ 469.5. Anal. Calcd for $C_{26}H_{29}FN_2O_3S$: C, 65.43; H, 5.72; N, 6.36; found: C, 63.18; H, 5.48; N, 6.32%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08–1.20 (m, 2H), 1.34–1.42 (m, 1H), 1.56–1.60 (m, 2H), 1.92–1.99 (d, 2H), 2.68–2.71 (t, 2H), 2.79–2.88 (m, 4H), 4.04–4.08 (t, 2H), 4.47 (b s, 1H), 6.94–6.97 (d, 1H), 7.00–7.05 (td, 1H), 7.15–7.23 (m, 2H), 7.29–7.40 (m, 5H), 7.49–7.54 (m, 2H), 7.83–7.90 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.62, 33.64, 46.76, 53.03, 55.87, 63.18, 112.52, 115.95, 116.44, 122.01, 126.97, 127.79, 128.71, 129.19, 129.24, 129.31, 130.59, 136.00, 136.04, 138.18, 154.10, 163.07, 166.44.

6.3.2. 3-Chloro-N-({1-[2-(2-methylsulfanylphenoxy)ethyl] piperidin-4-yl}methyl) benzenesulfonamide (**64**)

Yellow oil, 39 mg (57% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.5); initial LC/MS purity 90%, t_R = 2.75. $C_{21}H_{27}ClN_2O_3S_2$, MW 455.03, Monoisotopic Mass 454.1, $[M + H]^+$ 455.4. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16–1.28 (m, 2H), 1.43–1.48 (m, 1H), 1.65–1.69 (m, 2H), 2.08–2.17 (td, 2H), 2.41 (s, 3H), 2.83–2.87 (t, 4H), 3.00–3.04 (m, 2H), 4.12–4.16 (t, 2H), 4.54–4.56 (m, 1H), 6.79–6.82 (dd, 1H), 6.92–6.98 (td, 1H), 7.08–7.14 (m, 2H), 7.43–7.48 (t, 1H), 7.53–7.57 (d, 1H), 7.71–7.75 (m, 1H), 7.84–7.85 (t, 1H).

6.3.3. 4-Fluoro-N-(1-{2-[(propan-2-yl)phenoxy]ethyl}piperidin-4-yl) benzenesulfonamide (**68**)

Yellow oil, 28 mg (44% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.7); initial LC/MS purity

87%, t_R = 2.82. MW 420.54. Monoisotopic Mass 420.2, $[M+H]^+$ 421.1. Anal. Calcd for $C_{22}H_{29}FN_2O_3S$: C, 62.83; H, 6.95; N, 6.66; found: C, 62.71; H, 6.92; N, 6.61%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16–1.21 (m, 6H), 1.45–1.55 (m, 2H), 1.75–1.81 (m, 2H), 2.17–2.25 (m, 2H), 2.76–2.80 (t, 2H), 2.83–2.88 (m, 2H), 3.16–3.29 (m, 2H), 4.03–4.06 (t, 2H), 4.63–4.66 (d, 1H), 6.77–6.80 (dd, 1H), 6.89–6.94 (td, 1H), 7.09–7.21 (m, 4H), 7.88–7.92 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.59, 26.34, 29.61, 52.50, 56.06. 62.57, 111.27, 116.10, 116.40, 121.96, 126.17, 126.75, 129.30, 129.71, 136.48, 154.15, 163.17, 166.55.

6.3.4. 3,4-Difluoro-N-{1-[2-(biphenyl-2-yloxy)ethyl]piperidin-4-yl} benzenesulfonamide (**69**)

Yellow oil, 32 mg (45% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.7); initial LC/MS purity 82%, t_R = 2.84. MW 472.54, Monoisotopic Mass 472.2, $[M+H]^+$ 473.1. Anal. Calcd for $C_{25}H_{26}F_2N_2O_3S$: C, 63.54; H, 5.55; N, 5.93; found: C, 63.58; H, 5.51; N, 5.90%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33–1.46 (m, 2H), 1.66–1.71 (m, 2H), 2.01–2.09 (td, 2H), 2.65–2.72 (m, 4H), 3.10–3.12 (m, 1H), 4.00–4.04 (t, 2H), 4.57–4.59 (d, 1H), 6.93–6.95 (m, 1H), 7.00–7.05 (td, 1H), 7.26–7.38 (m, 6H), 7.47–7.51 (m, 2H), 7.63–7.69 (m, 1H), 7.71–7.75 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 33.05, 50.84, 52.22, 56.77, 66.95, 112.71, 116.72, 116.97, 118.06, 118.30, 121.18, 123.91, 126.78, 127.75, 128.57, 129.60, 130.86, 131.18, 138.52, 148.32, 148.50, 151.15, 151.32, 151.87, 155.65.

6.3.5. 4-Fluoro-N-(1-{2-[2-(methylsulfanyl)phenoxy]ethyl} pyrrolidin-3-yl)benzenesulfonamide (**81**)

Yellow oil, 26 mg (42% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.5); initial LC/MS purity 85%, t_R = 2.38. $C_{19}H_{23}FN_2O_3S_2$, MW 410.52, Monoisotopic Mass 410.1, $[M+H]^+$ 411.0. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.62–1.64 (m, 1H), 2.06–2.14 (m, 1H), 2.27–2.34 (m, 1H), 2.40–2.42 (m, 1H), 2.43 (s, 3H), 2.57–2.60 (m, 1H), 2.77–3.03 (m, 3H), 3.87 (b s, 1H), 4.03–4.07 (m, 2H), 5.38 (b s, 1H), 6.77–6.79 (d, 1H), 6.96–7.02 (m, 1H), 7.04–7.14 (m, 4H), 7.79–7.84 (m, 2H).

6.3.6. 3-Chloro-N-(1-{2-[(propan-2-yl)-phenyloxy]ethyl} pyrrolidin-3-yl)benzenesulfonamide (**88**)

Yellow oil, 25 mg (39% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.7); initial LC/MS purity 80%, t_R = 2.93. $C_{21}H_{27}ClN_2O_3S$, MW 422.96 Monoisotopic Mass 422.1, $[M+H]^+$ 423.1. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15–1.22 (m, 7H), 1.55–1.61 (m, 1H), 2.09–2.28 (m, 1H), 2.48–2.53 (q, 1H), 2.60–2.64 (dd, 2H), 2.83–2.87 (m, 1H), 2.90–2.97 (m, 1H), 3.21–3.31 (m, 1H), 3.89 (b s, 1H), 4.00–4.03 (t, 2H), 5.06 (b s, 1H), 6.77–6.80 (dd, 1H), 6.91–6.96 (td, 1H), 7.11–7.17 (td, 1H), 7.20–7.23 (dd, 1H), 7.37–7.42 (t, 1H), 7.47–7.52 (ddd, 1H), 7.71–7.74 (ddd, 1H), 7.85–7.86 (t, 1H).

6.3.7. 5-Chloro-(R)-N-(1-{2-[(propan-2-yl)-phenyloxy]ethyl} pyrrolidin-3-yl)thiophene-2-sulfonamide (**90**)

Yellow oil, 32 mg (49% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.5); initial LC/MS purity 72%, t_R = 2.19. $C_{19}H_{25}ClN_2O_3S_2$, Monoisotopic Mass 428.1, $[M+H]^+$ 429.0. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19–1.21 (dd, 6H), 1.64–1.74 (m, 1H), 2.14–2.26 (m, 1H), 2.35–2.44 (m, 1H), 2.58–2.63 (dd, 1H), 2.73–2.77 (dd, 1H), 2.89–2.93 (t, 2H), 2.97–3.05 (m, 1H), 3.27–3.33 (m, 1H), 3.92–3.99 (m, 1H), 4.04–4.08 (t, 2H), 6.79–6.82 (dd, 1H), 6.86–6.87 (d, 1H), 6.91–6.97 (td, 1H), 7.12–7.17 (td, 1H), 7.20–7.23 (dd, 1H), 7.36–7.38 (d, 1H).

6.3.8. 5-Chloro-(R)-N-{1-[2-(biphenyl-2-yloxy)ethyl]pyrrolidin-3-yl}thiophene-2-sulfonamide (**91**)

Yellow oil, 39 mg (56% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.5); initial LC/MS purity 82%, t_R = 2.83. MW 462.02, Monoisotopic Mass 462.1 $[M+H]^+$463.3. Anal. Calcd for $C_{22}H_{23}ClN_2O_3S_2$: C, 57.07; H, 5.01; N, 6.05; found: C, 57.16; H, 4.99; N, 6.06%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.49–1.60 (m, 1H), 2.05–2.21 (m, 2H), 2.35–2.40 (dd, 1H), 2.46–2.50 (dd, 1H), 2.73–2.78 (m, 3H), 3.78 (b s, 1H), 3.99–4.05 (t, 2H), 5.05 (b s, 1H), 6.86–6.87 (d, 1H), 6.94–6.96 (d, 1H), 7.02–7.07 (td, 1H), 7.28–7.41 (m, 6H), 7.47–7.51 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.65, 51.10, 53.47, 53.82, 58.99, 63.92, 112.71, 122.32, 127.08, 127.23, 128.08, 129.00, 129.55, 130.89, 131.37, 137.25, 138.32, 139.52, 154.19.

6.3.9. 5-Chloro-(S)-N-(1-{2-[(propan-2-yl)-phenyloxy]ethyl} pyrrolidin-3-yl)thiophene-2-sulfonamide (**93**)

Yellow oil, 27 mg (42% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.5); initial LC/MS purity 69%, t_R = 2.93. $C_{19}H_{25}ClN_2O_3S_2$, MW 428.99 Monoisotopic Mass 428.1, $[M+H]^+$ 429.0. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19–1.21 (dd, 6H), 1.59–1.71 (m, 1H), 2.12–2.25 (m, 1H), 2.31–2.40 (m, 1H), 2.54–2.60 (dd, 1H), 2.68–2.72 (dd, 1H), 2.86–2.90 (t, 2H), 2.92–3.00 (m, 1H), 3.24–3.33 (m, 1H), 3.91–3.96 (m, 1H), 4.02–4.06 (t, 2H), 6.78–6.81 (dd, 1H), 6.86–6.87 (d, 1H), 6.91–6.96 (td, 1H), 7.11–7.17 (td, 1H), 7.20–7.23 (dd, 1H), 7.36–7.37 (d, 1H).

6.3.10. 5-Chloro-(S)-N-{1-[2-(biphenyl-2-yloxy)ethyl]pyrrolidin-3-yl}thiophene-2-sulfonamide (**94**)

Yellow oil, 38 mg (55% yield) following chromatographic purification over silica gel with CH_2Cl_2/MeOH (9/0.5); initial LC/MS purity 82%, t_R = 2.84. MW 462.02, Monoisotopic Mass 462.1 $[M+H]^+$ 463.3. Anal. Calcd for $C_{22}H_{23}ClN_2O_3S_2$: C, 57.07; H, 5.01; N, 6.05; found: C, 57.12; H, 5.02; N, 6.06%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.50–1.61 (m, 1H), 2.02–2.23 (m, 2H), 2.36–2.41 (dd, 1H), 2.47–2.51 (dd, 1H), 2.73–2.77 (m, 3H), 3.79–3.82 (m, 1H), 4.00–4.04 (t, 2H), 5.05 (b s, 1H), 6.86–6.87 (d, 1H), 6.94–6.96 (d, 1H), 7.02–7.07 (td, 1H), 7.26–7.30 (m, 2H), 7.34–7.41 (m, 4H) 7.47–7.51 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.85, 51.28, 53.45, 53.71, 59.57, 112.69, 122.39, 127.13, 127.28, 128.09, 128.17, 129.05, 129.59, 129.67, 130.92, 131.40, 131.62, 137.19, 138.35, 139.58, 154.15.

6.4. Molecular modeling

6.4.1. Software

Instant JChem version 5.3.4 was used for structure database management, search and prediction of simple molecular properties. The command-line tools of JChem, i.e. Calculator Plugins (used for calculation of simple molecular descriptors) and Chemical Term Evaluator [42] (used for analyzing sdf and removing non-matching molecules) were combined to filter out building blocks and compounds (VCL) in batch mode. A set of Schrodinger's applications were used for generating combinatorial library (CombiGlide), preparing high quality 3D ligand structures taking into consideration protonation states and stereoisomers (LigPrep), calculating simple ADME descriptors (QikProp) and automated ligand-receptor docking (Glide). The Catalyst module from Discovery Studio 2.5 [43] was used to 3D pharmacophore models generation and screening.

6.4.2. Post-docking filter

To generate a post-docking filter, a set of 188 well-known diverse active molecules and of 258 diverse inactive ones of 5-HT_7R ligands and decoys (1589 molecules) were docked (Glide SP mode) to six homology models. The active and inactive subsets were prepared on the basis of the data extracted from version 09 of the ChEMBL database [44] while a decoy set was prepared following the DUD methodology [45]. The molecules with the inhibition K_i value below 300 nM were regarded as active, while in case of inactive molecules, the K_i threshold was higher than 5000 nM. Then, for the final poses, the structural interaction fingerprints (SIFt) [46,47] were generated

using in-house scripts. The results were stored in a 1D binary string, where nine bit pattern was used to describe the interaction type: any contact, backbone, side chain, polar, aromatic, hydrophobic interaction, hydrogen bond donor/acceptor and charged. On the basis on those ligand-receptor interaction description formats, training and testing sets were created by splitting the overall set in a ratio of 1:3, respectively. Vectors describing the interaction profile of known actives were labeled "+1", whereas those referring to known inactives and decoys were labeled "−1". Such representations were used to create input files to the SVMlight [48] software, separately for each receptor conformation. SVM classification models were built using training sets and a radial base function (RBF), also called the Gaussian function. This kernel function requires two parameters: C, i.e. a penalty parameter of the error term, and γ, i.e. a gamma parameter for the radial base function. The easy.py script of LibSVM [49] was used to automate the search of the above mentioned parameters by applying a systematic grid search algorithm. Test sets were used to evaluate the quality of models using different performance measures, i.e. recall, precision, enrichment factor and the Matthews correlation coefficient (MCC). For a given receptor, the best models were identified and used to rank VCL docking poses. The obtained values of the decision function were used to select only those molecules whose interaction pattern was very close to the known actives and distant from that of the known inactives and decoys. The results obtained for all the receptors were merged, and the modified MAX rule of data fusion was applied to further reduce the obtained matrix. Only those compounds were accepted which had at least one positive value of the decision function; in the case of at least two positive values, a pose with the higher value was chosen.

6.5. In vitro pharmacology

6.5.1. Cell culture and preparation of cell membranes

HEK293 cells with stable expression of human serotonin 5-HT_{1A}R, 5-HT_6, 5-HT_{7b}R or dopamine D_{2L}R (prepared with the use of Lipofectamine 2000) were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were grown in Dulbeco's Modifier Eagle Medium containing 10% dialysed foetal bovine serum and 500 μg/ml G418 sulphate. For membranes preparations, cells were subcultured in 10 cm diameter dishes, grown to 90% confluence, washed twice with prewarmed to 37 °C phosphate buffered saline (PBS) and were pelleted by centrifugation (200 g) in PBS containing 0.1 mM EDTA and 1 mM dithiothreitol. Prior to membrane preparations pellets were stored at −80 °C.

6.5.2. Radioligand binding assays

Cell pellets were thawed and homogenized in 20 volumes of assay buffer using an Ultra Turrax tissue homogenizer and centrifuged twice at 35 000 g for 20 min at 4 °C, with incubation for 15 min at 37 °C in between. The composition of the assay buffers was 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_6R: 50 mM Tris–HCl, 0.5 mM EDTA and 4 mM $MgCl_2$, 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 μl in 96-well microtitre plates for 1 h at 37 °C, except for 5-HT_{1A}R which were incubated at room temperature for 1 h. The process of equilibration is terminated by rapid filtration through Unifilter plates with a 96-well cell harvester and radioactivity retained on the filters was quantified on a Microbeta plate reader.

For displacement studies the assay samples contained as radioligands: 1.5 nM [^{3}H]-8–OH–DPAT (187 Ci/mmol) for 5-HT_{1A}R; 2 nM [^{3}H]-LSD (85.2 Ci/mmol for 5-HT_6R; 0.6 nM [^{3}H]-5-CT (39.2 Ci/mmol) for 5-HT_7R or [^{3}H]-Raclopride (74.4 Ci/mmol).

Non-specific binding is defined with 10 μM of 5-HT in 5-HT_{1A}R and 5-HT_7R binding experiments, whereas 10 μM methiothepine or 1 μM of (+)butaclamol were used in 5-HT_6R and D_{2L} assays, respectively.

Each compound was tested in triplicate at 7–8 concentrations (10^{-11}–10^{-4} M). The inhibition constants (K_i) were calculated from the Cheng-Prushoff equation [50]. Results were expressed as means of at least two separate experiments.

Membrane preparation and general assay procedures for cloned receptors were adjusted to 96-microwell format based on protocols described by us previously [22,51,52].

6.5.3. Effects on adenylate cyclase activity

The functional activity of the five selected compounds **61**, **68**, **69**, **91**, **94** on intracellular cAMP levels, studied in CHO cells which stably expressed the human 5-HT_7 receptor, was determined at CEREP (Le Bois l'Eveque, 86600 Celle L'Evescault, France) according to the previously published methods [53]. cAMP concentration was measured using HTRF method. Adenylate cyclase activity is expressed as the percentage of the maximal effect obtained with 300 nM serotonin. The compounds were tested in 5 concentrations at 10^{-4}–10^{-9} in the h5-HT_7 antagonist effect. For the antagonists, the apparent dissociation constants (K_B) were calculated using the modified Cheng Prusoff equation ($K_B = IC_{50}/(1 + (A/EC_{50}A))$, where A = concentration of reference agonist in the assay, and $EC_{50}A = EC_{50}$ value of the reference agonist).

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Appendix A. Supplementary material

Supplementary data available: experimental procedures, and spectral data for non-commercial building blocks, spectral data for target compounds, BB available resources database, and post-docking procedure description, associated with this article is available in on-line version at http://dx.doi.org/10.1016/j.ejmech.2012.07.043.

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