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Identification of novel serotonin 2C receptor ligands by sequential virtual screening

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ABSTRACT

Pharmacophore modelling, docking and virtual screening have become important tool in drug discovery process. Serotonin 2C (5-HT_{2C}) receptor ligands have got major attention for their therapeutic uses as antidepressant and anorectic agents. Two step pharmacophore and docking based virtual screening was done using 5-HT_{2C} agonists. Two common feature pharmacophore directed virtual hits had submicromolar activity. Refined pharmacophore with excluded volumes was constructed and combined with homology model based docking. Best hit from this virtual screening showed IC_{50} of 20.1 nM. Similarity search of this hit compound resulted more active ligand with 7.8 nM activity.

1. Introduction

Serotonin or 5-hydroxytryptamine (5-HT) is a major neurotransmitter in animals, both vertebrates and invertebrates, which regulates many important physiological processes.[1] 5-HT receptors are member of the seven transmembrane (7-TM) receptor superfamily, known as G-protein coupled receptors (GPCRs). GPCRs comprise the largest integral membrane protein family in the human genome and have over 1000 members.[2,3] Ligands for the GPCRs also vary a lot; ions, organic odorants, amines, peptides, proteins, lipids, nucleotides and even photons are able to mediate their message through these proteins. 5-HT receptors belong to amine subfamily of class A GPCR and comprise seven members; 5-HT_1 to 5-HT_7, where only 5-HT_3 is an ion channel.[4] More than 14 5-HT receptor subtypes have been identified, with splice variants and isoforms created by mRNA editing.[5]

The 5-HT_2 receptor family has three subtypes, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}, which exhibit closely related sequence and signal transduction pathways. The 5-HT_{2C} receptors (5-HT_{2C}Rs) couple to multiple cellular signalling system and regulate a variety of physiological functions and behaviours. 5-HT_{2C}Rs are therapeutic targets for conditions such as schizophrenia, anxiety, depression, Parkinson's disease, drug addiction and obesity.[6] This receptor is highly expressed and was first identified in the choroid plexus, where it may serve to regulate ion exchange between the brain and the cerebrospinal fluid. Receptor mRNA and protein are also found widely distributed throughout the brain, including the cortex, amygdala, basal ganglia, hippocampus and thalamus.[7,8] It couples Gαq which lead to the hydrolysis of membrane phosphoinositides, resulting in the formation of diacylglycerol (DAG) and inositol phosphates, which then act as signalling molecules to activate, for example, protein kinase C (PKC) and elevate intracellular calcium, respectively.[9]

Although there are some reports of 5-HT_{2C} selective agonists, it is proved difficult to get highly specific 5-HT_{2C} agonists because of its close sequence homology with the other two members, that is, 5-HT_{2A} and 5-HT_{2B}.[10] 5-HT_{2C} receptor agonists have shown good promise against obesity. Obesity is a chronic health condition, in US it is a common problem and in past few decades it became a worldwide threat.[11] In addition 5-HT_{2C} receptor knockout mouse is severely obese and defective in food intake.[12,13]

In the present study two step virtual screening was done to get novel 5-HT_{2C}R ligands. First, 3D pharmacophore was made using highly active 5-HT_{2C}R agonists with antiobesity properties. Then using this pharmacophore virtual screening of commercial database has been done and biological results show some moderately active hits with submicromolar activity. In the second phase some inactive ligands were included in the previously used training set and pharmacophore with excluded volumes (HipHopRefine) were made. This modified pharmacophore directed virtual screening hits

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were docked in homology model of 5-HT_{2C}R. By the fit values, F-scoring and binding mode pattern inside active site of the model, a set of compounds were tested further for biological activity. This time nanomolar active hit was found and similarity search of the hit revealed more active leads. The overall work flow of the molecular modelling and biological assay is schematically shown in Figure 1.

2. Results and discussion

2.1. Common feature pharmacophore and validation

HipHop module does not use any activity data of training set compounds, instead it tries to generate hypothesis taking each training set compound as reference, in such a way that it satisfies features of all other compounds also. As it is assumed that all compounds are equally important and contain important features putting 2 in principal number which ensures all of the chemical features in the compound will be considered in building hypothesis space. In maximum omitting features column, value of 0 forces mapping of all features of training set compounds.

In HipHop pharmacophore generation, hypothesis run returns 10 hypotheses with ranking scores. All our 10 hypotheses have four features; the first four have one ring aromatic, one positive ionizable and two hydrophobic regions and the last six have one ring aromatic, two hydrophobic and one hydrogen bond donor features. The ranking score of the hypotheses ranges from 60.94 to 54.22 (Table 1). The hypothesis 1 (hypo-1) was selected as best hypothesis because of its top score of 60.94 and good fit values with all training set compounds (Table 2). Hypo-1 consists of four features, one ring aromatic, one positive ionizable and two hydrophobic features. The 3D orientation of the features and their inter feature distances are shown in Figure 2. Compound **7** fit hypo-1 perfectly with maximum fit value of 4 (Fig. 3) The N atom of piperazine map with positive ionizable feature, benzofuran part with ring aromatic and one hydrophobic and trifluoromethane part with another hydrophobic feature of the pharmacophore.

In the validation test with a set of 832 compounds, where number of true actives are 344, the *E* value of 1.972 indicates the model is good for virtual screening. The database mining was done using BEST flexible search and 81.57% of actives were retrieved. Besides, GH was also calculated, which is 0.71 where value from 0.7 to 0.8 indicates good model (Table 3).

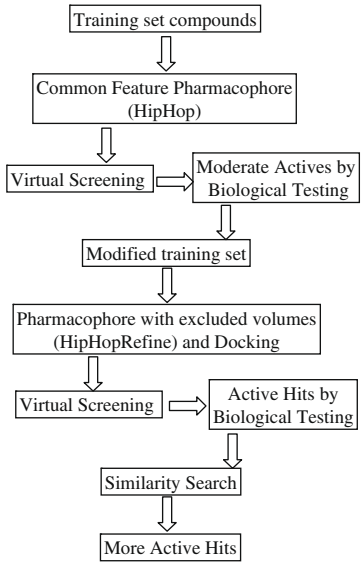


Figure 1. Flow chart of two step virtual screening using HipHop, HipHopRefine and FlexX docking.

Table 1
Score of 10 common feature hypotheses (HipHop)

No	Features	Score	Direct hit	Partial hit	Fit
1	RPHH	60.94	1111111	0000000	4
2	RPHH	60.54	1111111	0000000	4
3	RPHH	59.92	1111111	0000000	4
4	RPHH	59.24	1111111	0000000	4
5	RHHD	55.93	1111111	0000000	4
6	RHHD	55.93	1111111	0000000	4
7	RHHD	55.34	1111111	0000000	4
8	RHHD	55.05	1111111	0000000	4
9	RHHD	54.94	1111111	0000000	4
10	RHHD	54.22	1111111	0000000	4

R, ring aromatic; P, positive ionizable; H, hydrophobic; D, hydrogen bond donor. Direct hit mask indicates whether (1) or not (0) a training set molecule mapped every feature. Partial hit mask indicates whether (1) or not (0) a molecule mapped all but one feature.

Table 2
Fit values of HipHop training set compounds with best hypothesis (hypo-1)

Compound	Fit value
1	2.579
2	2.789
3	2.812
4	3.459
5	3.050
6	3.430
7	4.000

Fit values were calculated using Best Flexible search option.

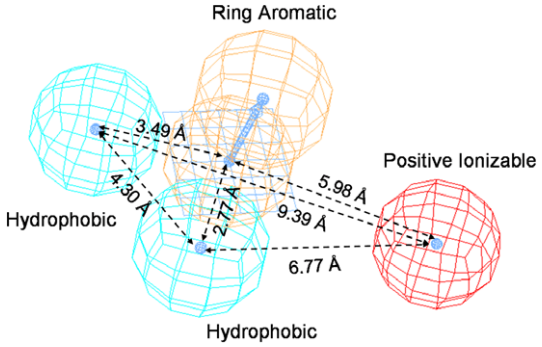


Figure 2. Top scored four features HipHop pharmacophore with inter feature distances.

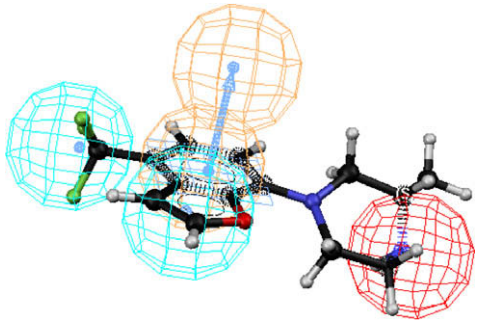


Figure 3. Best HipHop pharmacophore (hypo-1) mapped with training set compound **7**.

Table 3
Statistical parameters and scores of virtual screening (validation) using hypo-1

Parameters	
1. Total molecules in database (D)	832
2. Total no. of actives in database (A)	344
3. Total hits (Ht)	396
4. Active hits (Ha)	323
5. % Yield of actives [(Ha/Ht) × 100]	81.57
6. % Ratio of actives [(Ha/A) × 100]	93.89
7. Enrichment factor (E) [(Ha × D)/(Ht × A)]	1.972
8. False negatives [A − Ha]	21
9. False positives [Ht − Ha]	63
10. Goodness of hit (GH)[a]	0.71

[a] [(Ha/4HtA)(3A + Ht) × (1 − (Ht − Ha)/(D − A))]; GH score of 0.7–0.8 indicates a very good model.

2.2. Virtual screening and biological testing

Catalyst pharmacophore can be used for virtual screening to get hits. Pharmacophore features are associated with position constraints which consist of the ideal location of a particular feature in 3D-space surrounded by a spherical tolerance.[14] To be retrieved as hit, a candidate ligand must possess appropriate functional groups which can reside within the tolerance spheres of the pharmacophoric features. Database searching was performed with BEST flexible search method which manipulates the conformers so as to minimize the distances between pharmacophore features and mapped atom in the molecule.

A total of 16,560 Chemdiv GPCR compounds were screened using hypo-1. Total 5686 hits were found using BEST flexible search. To reduce the number of returned hits, drug like properties were observed and molecular weight cutoff <350 was set. After this the number of hits reduced to 872, then considering the fit values, different scaffolds and visual observation total 21 compounds were available for biological testing. In the in vitro assay, two compounds (VH1 and VH2) showed submicromolar activity of 424 nM and 589 nM with 81% and 75% inhibition at 10 μM concentration respectively. Seven compounds (VH3–VH9) had activity in micro molar range (Table 4).

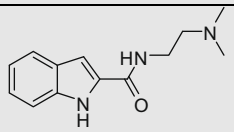
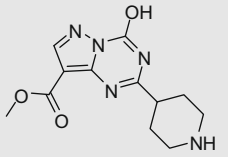
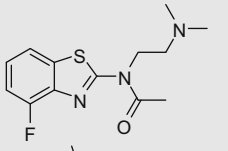
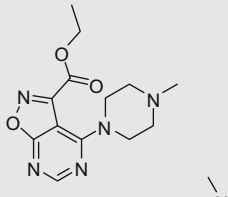
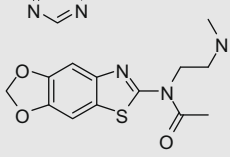
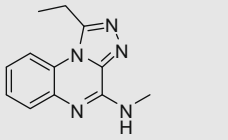
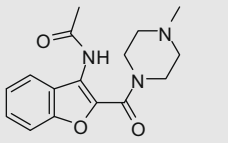
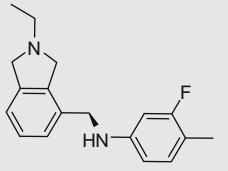
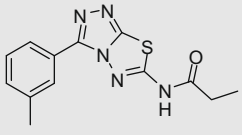
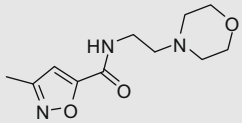
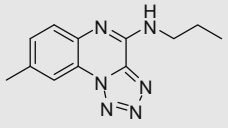
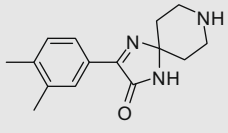
2.3. Pharmacophore with excluded volume (HipHopRefine)

Biological testing of HipHop hits (VH1 and VH2) showed submicromolar activity towards the receptor. 3D-database searching with typical pharmacophore queries containing a minimal number of features usually generates large number of hits. Typical pharmacophore has another limitation in case of steric properties. For example if inactive compounds that contain the same pharmacophore feature as active molecules, and are inactive due to incompatible steric clashes with the target, might come as false positive.

HipHopRefine module of Catalyst has new algorithm to overcome this difficulties. Using the information from inactive compounds of training set, it places excluded volume in the hypothesis and approximate steric interactions. It has been reported that use of excluded volumes in the pharmacophore can reduce number of hits, as well as number of false positives.[15]

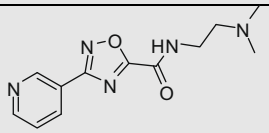
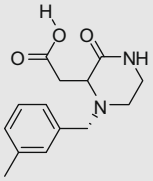
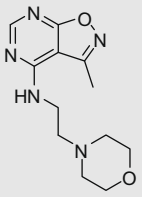
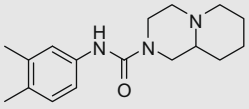
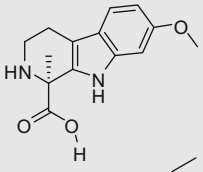
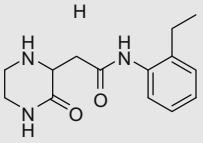
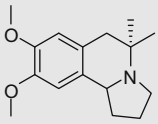
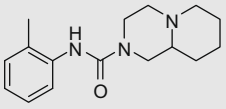
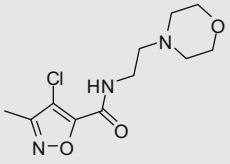
The HipHopRefine pharmacophore contains same features like HipHop with the extra excluded volumes. The HipHopRefine pharmacophore with excluded volumes are shown in Figure 4. Total 29 excluded volumes were formed in the pharmacophore. Normally the fit values with HipHopRefine are less than that of HipHop with any compound. In case of negative compounds it can reduce dramatically and helps to remove false positives. For example, here compound **8** is inactive and it shows fit value of 3.369 with HipHop, but 1.127 with the HipHopRefine. In the same way VH13 has fit value of 1.99 and 1.22 with HipHop and HipHopRefine,

Table 4
Biological test results of HipHop virtual hits for 5-HT_{2C}R

ID	Structure	% Inhibition @ 10 μM	IC_{50} (nM)
VH1		81	424
VH2		75	589
VH3		74	1999
VH4		76	2052
VH5		66	2732
VH6		57	5110
VH7		53	5294
VH8		57	5627
VH9		50	9223
VH10		21	>10,000
VH11		22	>10,000
VH12		40	>10,000

(*continued on next page*)

Table 4 (*continued*)

ID	Structure	% Inhibition @ 10 μM	IC_{50} (nM)
VH13		11	>10,000
VH14		28	>10,000
VH15		24	>10,000
VH16		0	>10,000
VH17		29	>10,000
VH18		16	>10,000
VH19		22	>10,000
VH20		14	>10,000
VH21		7	>10,000

respectively. So using the same fit value cutoff used in HipHop, many false positives can be eliminated from HipHopRefine hits.

2.4. Virtual screening, docking and biological testing

The HipHopRefine pharmacophore was used to screen the same Chemdiv GPCR database. This time the number of hits reduced to 1592 using BEST flexible search option. Molecular weight cut off <350 was used to get 530 hits. Then all these 530 compounds were docked inside the active site of 5-HT_{2C}R homology model. Finally 39 compounds were selected for biological test based on fit values, F-score of docking, interaction with important residues inside active site and structural diversity. Any hits which were ordered before for biological testing were excluded from the list.

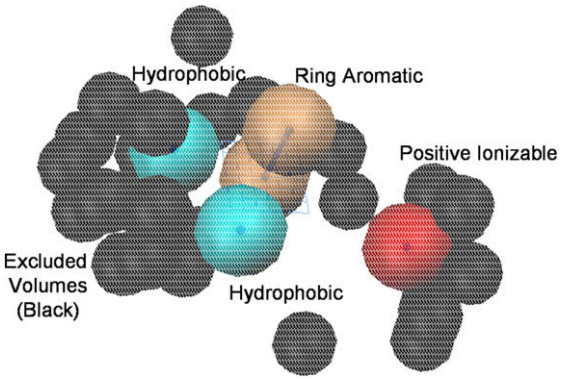


Figure 4. Pharmacophore with excluded volumes (HipHopRefine).

Out of 39 compounds, the best hit VH22 showed 20.1 nM activity towards the 5-HT_{2C}R. Compound VH23 had submicromolar activity and another three compounds (VH24–VH26) had micro molar activity (Table 5). Percent inhibition and IC_{50} values of 15 selected hits are presented in Table 5. The predicted binding mode of VH22 in docking simulation shows the H-bonding of the compound with D134, S110 and Y358 of the receptor (Fig. 5).

2.5. Similarity search of HipHopRefine best hit

Thirty three compounds were tested for biological activity. Out of 33, the best hit S1 was 7.8 nM, two compounds (S2–S3) showed two digit nM activity. Six compounds with submicromolar (S4–S9), 15 compounds with micro molar (S10–S24) activity and the rest were found inactives (Table 6). Structure–activity relationship (SAR) of the hits (Table 6) show that, the presence of piperazine ring with small hydrophobic groups and halogen and/or oxygen containing side chain (electronegative groups) in the phenyl part are necessary for the activity (e.g., S1, S2). Absence of piperazine ring or substitution of with other aliphatic or cyclic groups can reduce the activity (e.g., S15, S27). Addition of aromatic groups with piperazine ring also reduces the activity (e.g., S14).

3. Conclusion

In this study, we have used seven active 5-HT_{2C} agonists from literature to generate common feature hypothesis by HipHop module of Catalyst. This four feature pharmacophore was used to screen Chemdiv GPCR library. Based on fit values and drug like properties, 21 hits were tested for biological activity. Two submicromolar hits (424 nM and 589 nM) were found by in vitro assay. To improve the quality of the pharmacophore model and to get more active hits, pharmacophore with excluded volumes (HipHopRefine) was made. Inactive compounds in the training set give information about steric interactions by excluded volumes and supposed to reduce false positives. Besides, homology modelling of 5-HT_{2C}R was done using the X-ray crystal structure of beta 2 adrenergic receptor. Hits from HipHopRefine were further docked inside the model and finally a set of 37 compounds were biologically tested. This time the most active hit with 20.1 nM activity was returned. Similarity search of this hit using the same database was done and after biological testing the best hit with 7.8 nM activity was found.

In conclusion, it has been shown that, modification of typical pharmacophore and combination of docking with pharmacophore based virtual screening can improve the activity of hits. These hits

Table 5
Biological test results of selected HipHopRefine virtual hits for 5-HT_{2C}R

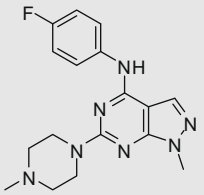
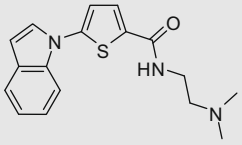
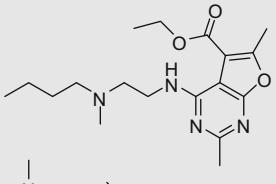
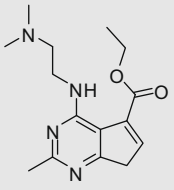
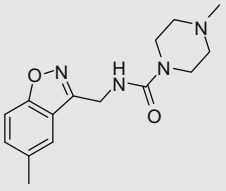
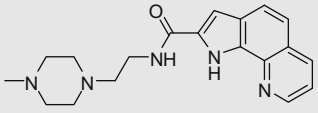
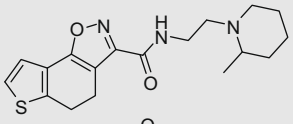
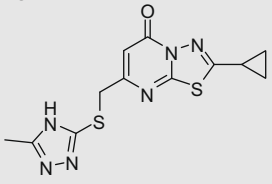
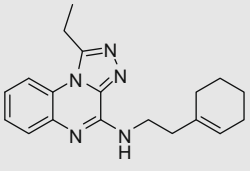
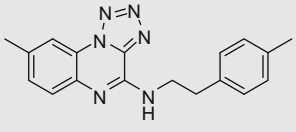
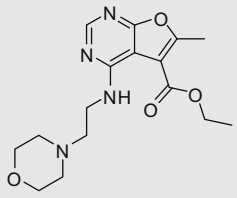
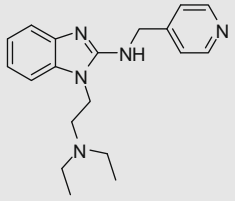
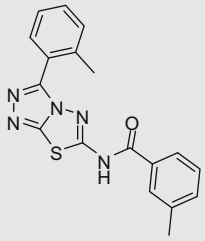
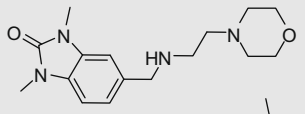
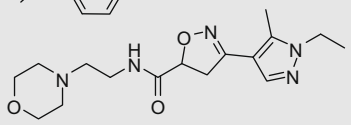
ID	Structure	% Inhibition @ 10 μM	IC_{50} (nM)
VH22		99	20.1
VH23		96	222
VH24		71	4169
VH25		65	4482
VH26		56	8928
VH27		43	>10,000
VH28		40	>10,000
VH29		38	>10,000
VH30		35	>10,000
VH31		30	>10,000

Table 5 (*continued*)

ID	Structure	% Inhibition @ 10 μM	IC_{50} (nM)
VH32		26	>10,000
VH33		24	>10,000
VH34		0	>10,000
VH35		0	>10,000
VH36		0	>10,000

are under optimization for further drug development to get more druggable lead. Different pharmacophoric approach along with docking can be a good starting point in the drug discovery process to get bioactive 5-HT_{2C} agonists.

4. Methods

4.1. Generation of common feature pharmacophore (HipHop) and validation

The common feature hypothesis or HipHop pharmacophore model is the automated tool within Catalyst that is based on alignment of common features present in highly potent compounds. For HipHop pharmacophore analysis of 5-HT_{2C} agonists, seven highly active agonists (Fig. 6) from Prous Science Integrity[16] were carefully selected considering their structural diversity. The compounds were drawn in 2D/3D sketcher module inside Catalyst, energetically minimized and saved. The conformational models of them were built using the BEST conformer generation method having up to 250 conformers with a 20 kcal/mol energy cutoff.[17]

During spreadsheet generation Principal column and maximum omitted feature (MaxOmitFeat) were set 2 and 0, respectively, for all the training set compounds. Spacing was set to 200 pm and all other parameters were kept as default. HipHop pharmacophore models are derived by comparing a set of conformational models and a number of three-dimensional configurations of chemical fea-

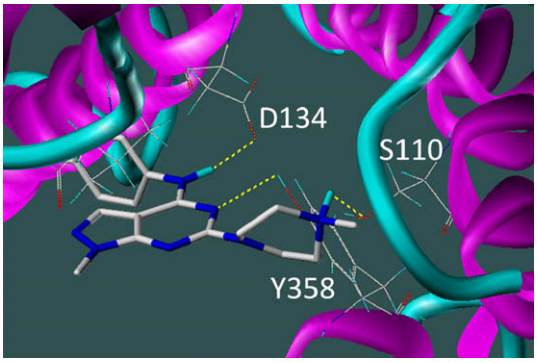


Figure 5. Docking simulation of VH22 in 5-HT_{2C}R homology model showing H-bonding with D134, S110 and Y358.

tures shared among the training set molecules. Hydrophobic, Hydrogen Bond Acceptor, Hydrogen Bond Donor, Positive Ionizable and Ring Aromatic features were used to generate the pharmacophore, based on the atom properties of training set compounds.

The hypothesis generation process in Catalyst was returned 10 possible pharmacophore hypotheses having different arrangement of constituent features or ranking score. After deleting the redundant hypotheses that have the same chemical characteristics and nearly the same distances between these functions, diverse configurations of hypotheses were selected according to ranking scores and fitting scores.

The best hypothesis was determined by the potential of discriminating between active and inactive compounds. To further validate the pharmacophore an external database was prepared with active 5-HT_{2C} agonists and kinase specific compounds as inactives. The 5-HT_{2C} agonists and kinase database were downloaded as SD file format from Integrity database of Prous[18] and were converted as the Catalyst database. The two databases were mixed together with a total of 832 compounds, with 344 5-HT_{2C} agonists and 488 with activity towards kinase. Then using hypo-1 the database was screened and a set of statistical parameters were observed[19] like Ht, % yield of actives, *E*, false positives, false negatives and GH.

4.2. Virtual screening of commercial database

Virtual screening of commercial database with the best pharmacophore has been carried out by Chemdiv GPCR focused library with 16,560 compounds.[20] The database was converted into Catalyst format from SD file and then imported in the working stockroom. Best Flexible search option was used for the screening purpose. To reduce the number of returned hits, molecular weight cutoff was used 350, as most of the reported agonists are small compounds. Then the compounds were selected based on fit values, structural diversities and drug likeliness for biological evaluation.

4.3. Pharmacophore with excluded volumes (HipHopRefine)

After getting the biological results from HipHop virtual hits, one inactive compound from literature (compound **8**)[21] and two inactives from the HipHop hits (VH13 and VH15) were added to the previous training set to generate HipHopRefine training set (Fig. 7). Compound **8** has K_i value of >20,000 nM and other two compounds have IC_{50} of >10,000 nM. These three inactives contribute to the generation of pharmacophore with excluded volumes in HipHopRefine. By the strategic placement of excluded volumes in the hypothesis, steric interactions can be approximated. Principal

Table 6
Biological test results of similarity search hits of VH23 for 5-HT_{2C}R

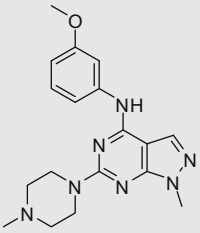
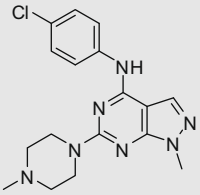
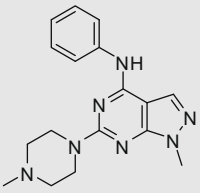
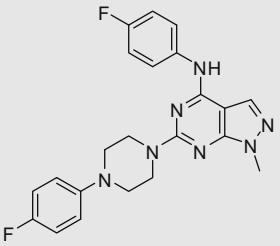
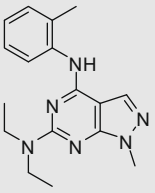
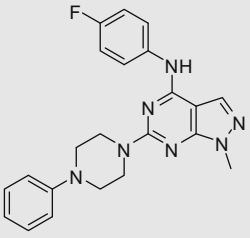
Compound ID	Structure	% Inhibition @ 10 μM	IC_{50} (nM)	Similarity index
S1		100	7.8	87.71
S2		100	29.4	89.95
S3		100	86.9	93.78
S4		96	148	95.43
S5		88	242	85.27
S6		88	416	96.31

Table 6 (*continued*)

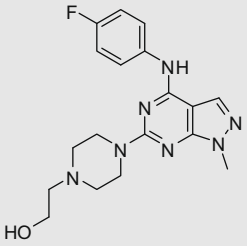
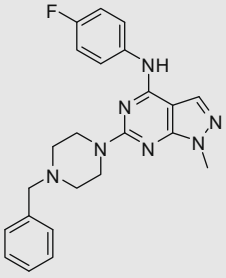
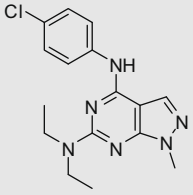
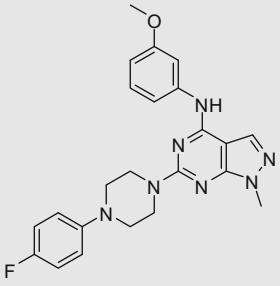
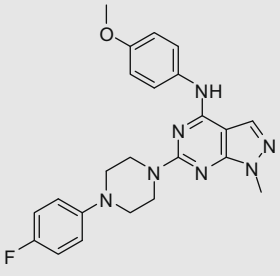
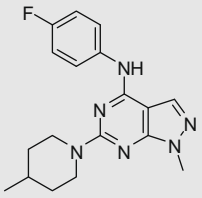
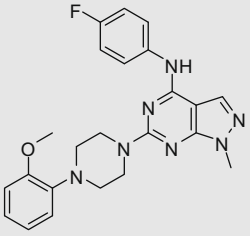
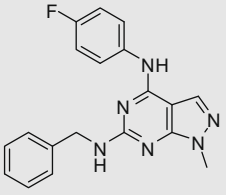
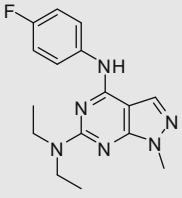
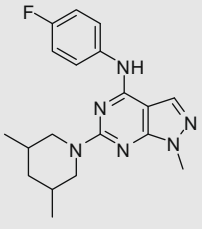
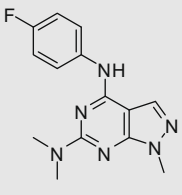
Compound ID	Structure	% Inhibition @ 10 μM	IC_{50} (nM)	Similarity index
S7		97	494	94.57
S8		96	836	92.48
S9		80	991	87.67
S10		85	1085	89.32
S11		86	1179	89.32
S12		89	1300	92.34

Table 6 (*continued*)

Compound ID	Structure	% Inhibition @ 10 μM	IC_{50} (nM)	Similarity index
S13		86	1500	91.11
S14		78	1725	85.65
S15		81	2071	91.96
S16		82	2588	86.78
S17		76	3306	97.61
S18		67	3817	91.11
S19		66	4027	93.3

(*continued on next page*)

Table 6 (*continued*)

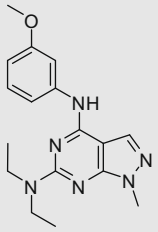
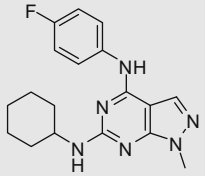
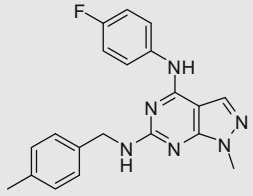
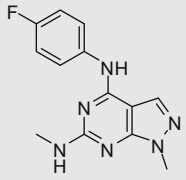
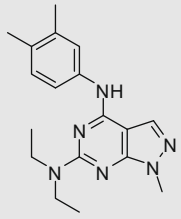
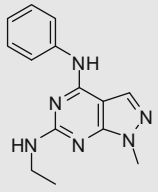
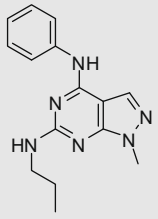
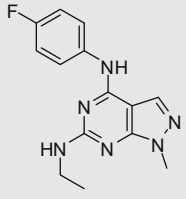
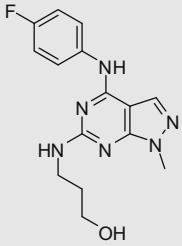
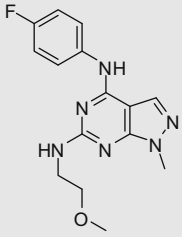
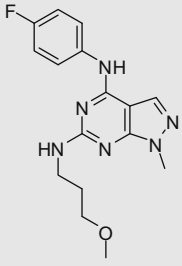
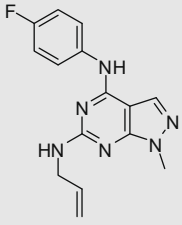
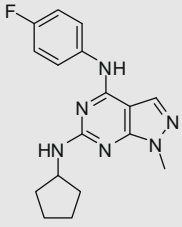
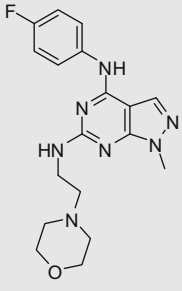
Compound ID	Structure	% Inhibition @ 10 μM	IC_{50} (nM)	Similarity index
S20		61	5422	85.78
S21		62	5713	86.09
S22		66	5833	86.4
S23		51	7787	90.48
S24		52	8491	85.65
S25		15	>10,000	87.2
S26		29	>10,000	85.19
S27		23	>10,000	93.36

Table 6 (*continued*)

Compound ID	Structure	% Inhibition @ 10 μM	IC_{50} (nM)	Similarity index
S28		5	>10,000	88.39
S29		18	>10,000	88.39
S30		28	>10,000	86.84
S31		41	>10,000	90.78
S32		35	>10,000	85.65
S33		35	>10,000	91.59

column and Maximum Omitted Features were set to 0 and 1, respectively, for these three inactives in spreadsheet. All the parameters in HipHop were kept identical during HipHopRefine generation.

4.4. Homology modelling of 5-HT$_{2C}$R

In the absence of X-ray crystal structure, homology modelling predicts the three-dimensional structure of a given protein sequence or target, based primarily on its alignment to one or more related proteins of known structure or templates.[22] The primary sequence of human 5-HT$_{2C}$R was collected from swissprot database (accession P28335). The homologous search of 5-HT$_{2C}$R was carried out by standard tool of sequence database searches, for example, blastp (protein–protein BLAST). The X-ray crystal structure of beta 2 adrenergic receptor (pdb code 2RH1) was obtained from the Protein Data Bank (http://www.rcsb.org/pdb/home/home.do).

Homology modelling part was done using inbuilt MODELER module of Discovery Studio (DS) version 2.0 (http://accelrys.com/) in windows operating system. The protein is then energetically minimized, checked for the structural accuracy by PROCHECK. Enrichment study was done with known compounds in FlexX module[23] to choose best scoring function. The enrichment study shows F-score can retrieve more true hits than ideal one (detail of homology modelling, energy minimization and enrichment has been submitted elsewhere).

4.5. Virtual screening combined with docking

Chemdiv GPCR database was screened using top ranked HipHopRefine pharmacophore with BEST flexible search option. Then the hits were pruned by molecular weight cut off <350. All the hits from HipHopRefine were exported to Sybyl and docked in the homology model of 5-HT$_{2C}$R. FlexX algorithm was used for docking. According to published literatures Asp134 of the receptor is vital for ligand interactions and activity. Active site was set 6.5 Å surrounding this amino acid. Then compounds were selected for biological testing, based on fit values, F-score of docking, interaction with important residues inside active site and structural diversity.

4.6. Similarity search

Similarity searching of the best active HipHopRefine hit VH22 was performed using the Chemdiv GPCR focused library database. UNITY[24] module of Sybyl 8.0 (Tripos Inc.) was used and similarity threshold was set at 85%. Among the returned hits, carefully considering the similarity and side chain atoms, total 33 compounds were again sent for biological evaluation.

4.7. Biological evaluation

[^{3}H]Mesulergine binding to serotonin 5-HT$_{2C}$ receptor was done according to the following procedure. Frozen membranes from stable CHO-K1 cell line expressing the human recombinant 5-HT$_{2C}$ receptor were used. For the binding assay, [^{3}H]Mesulergine (1 nM), receptor membrane (4 μg/well) and test compounds were added into 50 mM Tris–HCl (pH 7.7) buffer containing 0.1% ascorbic acid and 10 μM pargyline. Nonspecific binding was determined using 0.5 μM mianserin. The incubations were performed for 30 min at 37 °C, and these were terminated by rapid filtration through Whatman GF/C glass fibre filters presoaked in 1% BSA.[25] [^{3}H]Mersulergin was obtained from Amersham Biosciences (Buckinghamshire, UK). Cloned human recombinant serotonin 5-HT$_{2C}$ receptors were obtained from Euroscreen (Brussels, Belgium).

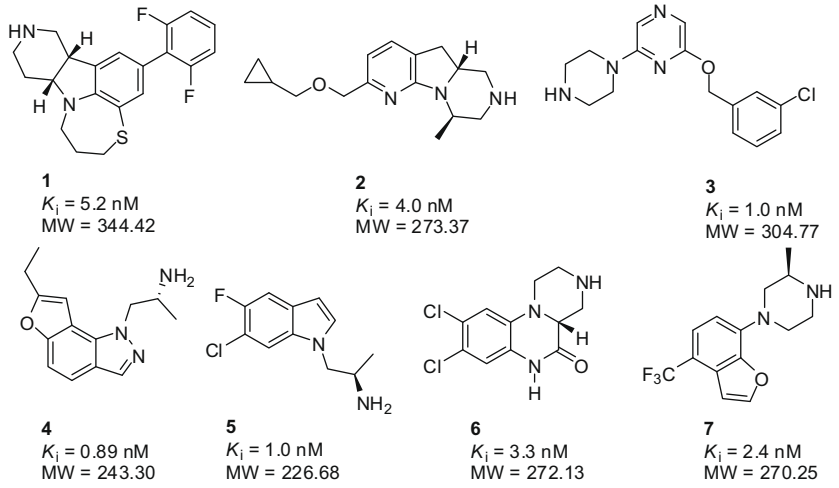


Figure 6. Training set compound for common feature hypothesis (HipHop).

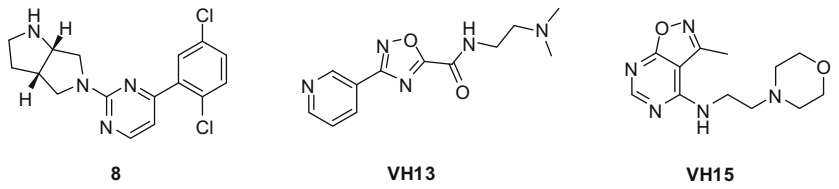


Figure 7. Three inactive ligands additionally used in the training set of common feature hypothesis with excluded volumes (HipHopRefine).

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