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A desirability function-based scoring scheme for selecting fragment-like class A aminergic GPCR ligands

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ABSTRACT A physicochemical property-based desirability scoring scheme for fragment-based drug discovery was developed for class A aminergic GPCR targeted fragment libraries.

Physicochemical property distributions of known aminergic GPCR-active fragments from the ChEMBL database were examined and used for a desirability function-based score. Property-distributions such as logD (at pH = 7.4), PSA, pK_a (strongest basic center), number of nitrogen atoms, number of oxygen atoms, and the number of rotatable bonds were combined into a desirability score (FrAGS). The validation of the scoring scheme was carried out using both public and proprietary experimental screening data. The scoring scheme is suitable for the design of aminergic GPCR targeted fragment libraries and might be useful for preprocessing fragments before structure based virtual or wet screening.

KEYWORDS G-protein coupled receptors, fragment-based drug discovery, desirability function, aminergic fragment library

INTRODUCTION Fragment based drug discovery (FBDD) implies the screening of small-sized, simple, polar molecules in high-concentrations. Fragment screening is an alternative lead-discovery approach to high-throughput screening campaigns with remarkable advantages.

Fragment based lead discovery samples the notably smaller fragment space with respect to the lead-like or drug-like space. An illustration of this point is that the number of compounds with up to 17 heavy atoms¹ is about 10^{11} while the estimated number of drug like molecules up to 30 heavy atoms² is 10^{60} . Another inevitable aspect is the documented increase of molecular weight and lipophilic character during lead-optimization^{3,4}, and fragments as typically soluble, low molecular weight and polar compounds provide more operational freedom in optimizing them to compounds with favorable pharmacokinetic properties.

G-Protein Coupled Receptors (GPCRs) are key modulators of cell-signaling and constitute the largest cell-membrane receptor superfamily. Ligands of these receptors are neurotransmitters, peptides, hormones, etc. GPCRs are either named as seven-transmembrane receptors (7TM) for having a common structure of seven α helices ending in an extracellular N terminus and an intracellular C terminus. The superfamily is classified into A, B, C, and Frizzled-type receptors, hereby class A constitutes the largest family, including aminergic, chemokine, glycoprotein hormone receptors and neuropeptide receptors. G-protein coupled receptors are appearing prevalently in drug discovery and representing the therapeutic target for a wide range of small-molecule drugs of central-nervous system, cardiovascular and inflammation related diseases⁵. Aminergic receptors, the largest group of class A, are muscarinic achetylcholine, adrenoceptors, dopamine-, histamine-, serotonin-, octopamine-, and trace amine-receptors. In the present study

we collected fragment-like aminergic GPCR ligands from public databases and analyzed their characteristic physicochemical features to derive a scoring scheme suitable to screen compound libraries for aminergic class A GPCR ligands.

COMPILING LIBRARIES Active set: GPCR-SARfari⁶ was downloaded from the ChEMBL database of the European Bioinformatics Institute (as part of European Molecular Biology Laboratory, EMBL), and was processed using Knime.com AG's Konstanz Information Miner (Knime)⁷. GPCR SARfari (version 3.00, June 2012) contains 947914 entries, including molecular structures, and in vitro activity data for GPCR-targets. Collected data were processed in several steps. Only binding data were kept, while data of functional assays and those related to ADME properties were discarded. Activities given as IC₅₀, logIC₅₀, K_i and logK_i were converted to pActivity and were treated on an equal footing with pK_i values. When ligand activities for several species were available only the highest activity was kept. Counter ions of salts were stripped¹⁴, and only one entry was kept with the highest pK_i value. This process resulted in 166699 entries, containing structurally unique compounds with activity data on several GPCR targets. Size-independent ligand efficiency⁸ (SILE) metrics were used to classify compounds as active or inactive on a target. SILE is an empirically derived measure suitable for the comparison of compounds with different sizes:

$$SILE = pK_i / N_{heavy}^{0.3}$$

The cut-off for sorting of GPCR-active fragments was determined by evaluating the relationship between pK_i and SILE in the $8 \leq N_{heavy} \leq 22$ range (see Table 1 in the Supporting Information). $SILE \geq 1.95$ was accepted as the activity threshold that corresponds to K_i values suitable for fragment-screening activity on GPCR targets (100μM-10μM). After removing

activities under SILE <1.95, the resulting data-set of 144759 entries was used for identifying GPCR-active fragments. Unlike several prevalent methodologies using molecular weight as size-determining definition, our measure was the number of heavy atoms⁹. Molecular-weight based filters penalize halogen and sulphur atoms including sulphonamide-type molecules, due to their proportionately higher atom-weight. Since one heavy atom stands for 13.286¹⁰ Da molecular weight, the commonly used RO3¹¹ cut-off (300 Da) may be converted to $N_{\text{heavy}} \leq 22$. Removing molecules with less than 8 or more than 22 heavy atoms resulted in 10477 unique fragments with listed binding activity data on several GPCRs. Compounds considered to be GPCR-like fragments (2370), were defined having SILE of at least 1.95 on at least four different GPCR-targets. The active set contains activity data related to 139 different receptors (and subtypes), out of which 87 receptors are aminergic GPCRs¹² (serotonine, β -adrenerg, dopamine, histamine, trace-amine, octopamine, muscarinic-acetylcholine receptors) and 52 other not-aminergic class-A GPCRs (opioid-, adenosine-, sphingosine-1-phosphate-, thromboxane-, melatonine-, orphan-, and angiotensin-receptors). It was found that the majority of the fragments (2183 out of 2370, 92%) had exclusively aminergic GPCR activity data, serving as training set.

Another definition for GPCR-active fragments was also used in order to check how much the property distributions depend on the definition of actives. The activity threshold of $\text{SILE} \geq 1.95$ was replaced by $\text{pK}_i = 4$. Note that while pK_i is independent from molecular size, the SILE definition requires higher affinity for larger ligands in accordance with their higher available maximal affinity⁸. Activities over $\text{pK}_i \geq 4$ were kept resulting a set of 165459 entries. Keeping molecules with the number of heavy atoms between 8 and 22 resulted in 10950 unique fragments. Compounds considered to be GPCR-like fragments, were defined having a pK_i value of at least 4 on at least four different GPCR-targets. This set of 2478 fragments was used for

checking whether the property distributions of the active set are dependent on the method for defining actives (see later).

Reference set: For the determination of the essential GPCR fragment parameters we created a reference set derived from the ChEMBL¹³. Compounds of the active GPCR set were removed from the 1292344 ChEMBL (version 16) entries. Ligand structures in salt forms were stripped¹⁴, and only one entry was kept in case of duplicates, resulting in structurally unique compounds. After filtering by $N_{\text{heavy}} = [8; 22]$, 309962 fragments remained, out of which 5000 fragments were picked out randomly to generate a set proportional in size with the active set.

SELECTION OF RELEVANT PHYSICOCHEMICAL-DESCRIPTORS AND SCORING The examination of molecular physicochemical property distributions in drug-discovery related chemical databases is a commonly used methodology for library design.^{5, 15} Fragments were characterized by widely used physicochemical descriptors such as clogP, clogD (at pH = 7.4), polar surface area (PSA) (at pH = 7.4), number of rotatable bonds, number of hydrogen bond acceptors, number of hydrogen bond donors, acidic dissociation constant pK_a (related to the strongest center), basic dissociation constant pK_a (related to the strongest center), number of nitrogen atoms and number of oxygen atoms using ChemAxon's JChem for Excel.¹⁶ All the descriptors were calculated for all the fragments of both the active and the reference sets, followed by the calculation of the distribution-functions for each property. The corresponding distribution-functions were described by medians, means and standard deviations shown in Table 1.

Table 1. Mean, median, and standard deviation values of the calculated physicochemical properties.

The distributions of the hydrogen bond donor and acceptor-count and logP do not differ markedly in their medians and standard deviations comparing the active and reference sets, however in case of the aminergic GPCR-like fragments the polar surface area (at pH = 7.4) has median lower than that of the reference set. Lower logD values at pH = 7.4 are related to the basic character of the aminergic GPCR fragments. Considering the number of rotatable bonds, aminergic fragments are typically less flexible containing generally 0 or 1 rotatable bond than molecules in the reference set. Fragments of the active and inactive sets were also classified by their acid-base character. The classification method and the results are shown in Table 2. 83% of the active fragments have basic character, 16% of the fragments were neutral, none of them were acidic, and only 1% were zwitter-ionic. In contrast, 15 % of the randomly sampled reference fragments were basic, 71% neutral, 12% acidic, and 3% zwitter-ionic. The dominantly basic character of the active set causes that although they have less O atoms, smaller PSA and slightly higher logP than the reference molecules their logD values at pH=7.4 are lower. Summarizing, these aminergic fragments constitute a set of small, rigid molecules, containing few heteroatoms, that are most often (~83%) basic nitrogens.

Table 2. Classification of the acid-base characters of active and inactive fragments

Differences in property-distributions were identified by visual inspection and by the analysis of medians, means and standard deviations, comparing aminergic-fragments, and ChEMBL inactive reference fragments (see Table 1). As a result, logD (pH = 7.4), number of nitrogen atoms, number of oxygen atoms, number of rotatable bonds, strongest basic pK_a, and PSA (at pH = 7.4) were identified as descriptors that are able to characterize aminergic-fragments. Differences between selected property distributions were checked by Mann-Whitney U-tests.¹⁷ All compared property distribution pairs are different at a significance level of $p = 0.05$. The following section

will present some general statements about aminergic fragments, providing standpoints for developing a desirability scoring function.

LogD distribution of the amine-like fragments has a mean of 0.90, with a standard deviation of ± 1.62 , correlating with their predominantly basic character, differing from the logD distribution of the random fragments (mean = 1.65, standard deviation = ± 2.24), however there is no notable difference in the corresponding logP distributions. The number of nitrogen atoms represents that aminergic GPCR-like fragments have at least 1 nitrogen-atom (~40 % contain 1, ~40 % contain 2). The number of oxygen atoms puts restraint to the number of oxygens (~ 30% do not contain any). Aminergic fragments are less flexible with a mean of 2.64 rotatable bonds, and with 10 % not having any rotatable bonds, unlike random fragments, with a mean of 3.35 rotatable bonds. Only 0.2 % of aminergic fragments have no basic-center. 83 % of them have at least one basic center, that is identified as having no acidic pK_a lower than 7, and a basic pK_a higher than 7. The distribution of basic pK_a has a mean value of 7.92 with a standard deviation of 2.84. In contrast, 50% of the reference fragments are neutral (pK_a mean = 5.96, standard deviation = 2.59). The polar surface area of a molecule is defined as the surface sum over all polar atoms. PSA is a commonly used medicinal chemistry metric for the optimization of passive membrane permeability. The widely known Rule of Three¹¹ filter for fragment-like compounds limits polar surface area to be at most 60 Å². The training set has a median value of 37.3 Å² and a mean value of 39.67 Å² for polar surface area, with a standard deviation of 21.04. The lower values of PSA of the active set are due to the smaller number of oxygen atoms.

The examined physicochemical properties were calculated for the fragments of the alternative active set selected by pActivity cut-off ($pK_i \geq 4$). The corresponding, medians, means and standard deviations are shown in Table 3.

Table 3. Mean, median, and standard deviation values of the calculated physicochemical properties of the active set defined by pActivity metrics

Comparing property distributions based on SILE and pActivity thresholds we concluded that these are highly similar and the SILE active set was used in all further studies.

After determining the characteristic descriptors showing remarkable difference in their medians and standard deviations with respect to randomly sampled reference fragments we used these descriptors to derive a desirability scoring scheme.

Common approaches for multiparametric filtering¹⁸ include desirability functions¹⁹ that are used to convert preferred descriptor distributions into a scoring function. A desirability function maps the value of a property onto a score in the range of [0; 1]. Desirable and undesirable property-ranges (x) are defined by the function through series of inflection points²⁰, identifying the desirable and undesirable regions of the properties with a certain desirability score of $y(x)$. Molecules not satisfying the criteria of the examined descriptor receive a desirability score of 0. Molecules with desirable properties gain a score higher than 0 and at most 1. After calculating a desirability score for all of the properties, the scores may be combined by summation or multiplication into an overall desirability measure. Desirability functions make it possible to define smooth boundaries for a property, rather than using a rigid cut-off, thus avoiding rejection of compounds based on an uncertain property value close to a criterion boundary. The selected properties were next translated to desirability functions. The two properties describing the number of heteroatoms and the number of rotatable bonds are considered to be discrete variables, while logD (at pH = 7.4), PSA (at pH = 7.4) and basic pK_a (strongest) are continuous (see the Supporting Information).

The number of nitrogen atoms is an important feature of aminergic GPCR-fragments due to their basic character that gives a major contribution to the interaction with the binding site. However not all fragments are supposed to be sorted out that lack nitrogen atoms. The desirable property range for the number of nitrogen atoms is between 0 and 5, namely 0 and 3-5 nitrogen atoms are scored with $y(x) = 0.5$, and 1-3 nitrogen atoms are scored with $y(x) = 1$. A score value of $y(x) = 0$ was assigned to fragments containing more than 5 nitrogen atoms (Figure 1 in Supporting Information).

The active fragments of the training set contained mostly 0-3 oxygen atoms, supporting the account of oxygen atoms most probably as hydrogen-bond acceptors. Fragments having 0-3 oxygen atoms are considered to be desirable with a score of $y(x) = 1$ (see in Figure 2 in Supporting Information).

In addition to the gain in conformational entropy a recent paper¹¹ has revealed the positive influence of the restriction of molecular flexibility by the number of rotatable bonds in ADME/PK properties suggesting an upper bound of 3. The distribution of the rotatable bond count for the training set showed that ~ 8 % of the active fragments possessed rigid planar structure, ~ 21 % contained only 1 and ~ 27 % only 2 rotatable bonds. None of the active fragments contained more than 7 rotatable bonds. Consequently all planar, non-flexible fragments and molecules containing 3 or less rotatable bonds are desirable (score of 1). Less-desirable property-ranges of 4-7 rotatable bonds are mapped with a monotonic decreasing score function (see Figure 3 in Supporting Information).

The desirability function for logD (at pH = 7.4) prefers mostly fragments in the logD range of 1 and 2, and decreases monotonously towards -2 and 5, accepting more basic and more polar fragments (see Figure 4 in Supporting Information).

The desirability function of the polar surface area (at pH = 7.4) is more permissive than the upper bound of the Rule of Three ($PSA \leq 60 \text{ \AA}^2$), because fragments on the range of 0 and 100 \AA^2 are not discarded. In contrast, these fragments are favored by a score of a hump function with a maximum at 40 \AA^2 and linearly decreasing towards 0 and 100 (see Figure 5 in Supporting Information).

Though 83 % of the fragments of the training set are basic, the desirability function of the strongest basic pK_a does not totally discard an acidic fragment, in this manner the property range of the strongest basic pK_a was mapped with a constant score of 0.1 in the 0-6 pK_a range, and with a hump function covering the values of 6 and 13, penalizing fragments with basicity stronger than $pK_a > 13$, and preferring fragments with a pK_a value between 8 and 10 (see in Figure 6 in Supporting Information).

Desirability scores may be combined either by summation or by multiplication into an overall desirability measure. A disadvantage of the multiplicative approach is that it discards molecules if they receive a desirability score of zero for a single property. In contrast, the additive approach reduces the impact of a single property-score when a large number of properties are involved¹⁸. Taking these aspects into consideration we defined the “Fragment Aminergic GPCR Score” (FrAGS) as the sum of the individual property-scores, providing a score with a range between 0 and 6.

VALIDATION OF THE Fragment Aminergic GPCR Score (FrAGS) Validation was carried out by three complementary approaches. First, the active training set was mixed with random inactive ChEMBL compounds to check whether the score is able to sort out the active compounds. The second approach used an independent active set from the PubChem database²¹. The third validation used data of a HTS and a fragment screening (FS) campaign on aminergic GPCR targets provided by Gedeon Richter Plc. Compounds not satisfying our fragment criterion ($8 < N_{\text{heavy}} < 22$) were removed from the HTS data set. The effectiveness of the FrAGS was determined by the Enrichment Factor (EF), and by the comparison of False Negative Rates (FNR) and True Negative Rates (TNR) as functions of the score. Furthermore, Receiver Operating Characteristic (ROC) curves were also investigated.

ChEMBL VALIDATION The inactive set was created similarly to the reference set generated for deriving the FrAGS. The entire ChEMBL database¹³ of about 1300000 entries was filtered by the previously identified active fragments. Counter ions of salts were removed; finally 96539 unique fragments were kept. This set was merged with the 2183 active fragments to give a validating set with 2.2 % actives. Relevant properties were calculated for the compiled 98722 fragments, and FrAGS were calculated. The enrichment factor increases monotonically up to the score of about 5 and oscillates for higher scores (see Figure 7 in Supporting Information). Its highest value before the oscillatory region is about 2.5. The corresponding ROC curve is shown in Figure 1 (True negative rates and false negative rates as functions of the FrAGS are shown in Figure 8 in Supporting Information.)

Figure 1. Receiver operating characteristics of the ChEMBL validation data

Second validation was carried by retrieving aminergic GPCR targeted HTS data from PubChem. PubChem is a public repository for biological properties of small molecules hosted by the National Institutes of Health (NIH, USA), containing bioassay data on more than 700,000 compounds, providing information about HTS and fragment-screening data on GPCR-targets suitable for our validation.²¹ High-throughput screenings for allosteric-binders, or targeting β -arrestin pathways, or used as counter-screening were sorted out. Only confirmed actives with at least 10 μ M activity were taken into consideration. The following aminergic GPCR targets were represented in HTS campaigns: 5HT_{1A} with 7 confirmed actives, 5HT_{1E} with 51 confirmed actives, TAAR₁ with 375 confirmed agonists and with 36 confirmed antagonists. Molecules satisfying fragment-size criterion (heavy atom count between 8 and 22) were kept providing 200 fragments with confirmed active data on aminergic-GPCRs (5HT_{1A}, 5HT_{1E}, TAAR₁). This dataset does not overlap with ChEMBL actives used as training set. 31 confirmed 5HT₁-active fragments and 169 TAAR₁-active fragments were added to a set of inactive fragments extracted from the corresponding primary screening sets by filtering all inactive compounds by the number of heavy atoms ($8 \leq N_{\text{heavy}} \leq 22$). After removing duplicates 296510 fragments remained out of which 3100 and 16900 randomly sampled inactive molecules from the 5HT₁ and TAAR₁ screens, respectively, were selected for validation. FrAGS values were calculated for the 3131 5HT₁ set of fragments and 17069 TAAR₁ set of fragments, followed by the calculation of TPR, FPR, TNR, FNR, ROC and EF (shown in Figures 2, 3 and Table 4 and in Figures 9, 10 and 11, 12 for 5HT₁ and TAAR₁ in Supporting Information). In the case of the 5HT₁ validation the highest enrichment resulted a remarkable ratio of 7.98 ($EF_{5,0} = 7.98$). The ROC curve of the 5HT₁ validation set (Figure 2 in Supporting Information) appears to be separated into two parts, one below and another above FPR=0.8. It raises the question if the compounds of the two subsets

belong to different structural classes. Therefore we examined the structural similarity of the two distinct sets. The distance matrices of fingerprints were calculated for the entire 5HT₁ validation set, and separately for the two subsets. The similarity of the three sets was characterized by the means and standard deviations calculated for their pairwise distances. The mean distance was 0.66 ± 0.09 , and 0.64 ± 0.09 for the first and the second subset, respectively. Furthermore the entire 5HT₁ set has a mean distance of 0.66 ± 0.09 , demonstrating the structural diversity of the validation set. The TAAR₁ EF curve shows a 3.41 fold enrichment at FrAGS = 5. The enrichment monotonously increases until FrAGS = 5, and oscillates over 5.

Figure 2. Receiver operating characteristics of the PubChem validation data on 5HT₁

Figure 3. Receiver operating characteristics of the PubChem validation data on TAAR₁

The third validation was carried out on experimental screening data of fragments, provided by Gedeon Richter Plc. The targets of the screening campaigns were aminergic GPCR receptors. Only fragment sized molecules were examined from the HTS campaign. 9302 fragments were screened by high-throughput screening, and 3038 fragments were evaluated with the fragment-screening approach.

In the high-throughput screening dataset 41 fragments were found to be active on the aminergic GPCR target, and 9261 fragments were inactive. The enrichment factor increases monotonically until FrAGS of about 5, and oscillates over this value. The corresponding false negative rates, true negative rates and the ROC curve is shown in Figure 4 and in Figures 13 and 14 in Supporting Information.

Figure 4. Receiver operating characteristics of the HTS validation data

Fragment-screening identified 33 active, and 3038 inactive fragments. The plot of enrichment factor versus FrAGS is shown in Figure 16 in Supporting Information. The enrichment factor increases monotonically until about FrAGS=5. The ROC curve is shown in Figure 5 and the plots of false negative rates and true negative rates are shown in Figure 15 and 16 in Supporting Information.

Figure 5. Receiver operating characteristics of the fragment screening validation data

DEFINING FrAGS CUT-OFF Ligand-based filters are typically used for selecting a set of compounds of a large library for further virtual or experimental studies. For the determination of the appropriate cut-off the enrichment curves of the validation studies were examined (Table 4 and Figures 7, 9, 11, 13 and 15 in Supporting Information). The validation test using ChEMBL compounds showed, that the enrichment is about 2-3 fold over about FrAGS = 4.5. The enrichments are much higher in the other two validation studies using PubChem and the experimental HTS and fragment screening data. Remarkable oscillation of the enrichment was observed over FrAGS > 5 that is due to the small number of compounds having high score values. It is proposed that a cut off value of 5.0 is an appropriate choice for the selection of promising aminergic fragments. Our three independent validation studies show enrichments around or above 3 using this cut off value.

Table 4. Enrichment factors at selected FrAGS values obtained in validation studies

CONCLUSIONS Comparing the distributions of several physicochemical descriptors calculated for fragment-like aminergic GPCR ligands with those of randomly selected fragments revealed that the following descriptors are markedly different for the two sets of compounds: logD (at pH = 7.4), PSA (at pH = 7.4) , pK_a (strongest basic center), number of nitrogen atoms,

number of oxygen atoms, and the number of rotatable bonds. Desirability functions based on the statistical differences in the property distributions were defined and were combined to a Fragment Aminergic GPCR Score (FrAGS). This score can take values between 0 and 6, the higher the score the more likely the fragment is an aminergic GPCR ligand. The scoring scheme was verified with independent experimental data collected in real-life screening situations and it was found that a score cut off value of 5 is appropriate to achieve an enrichment of around or over 3. The scoring scheme is suitable for screening large libraries of fragment-like compounds for aminergic GPCR ligands, and thus it is a useful tool for compiling focused fragment libraries for drug discovery projects.

FIGURES

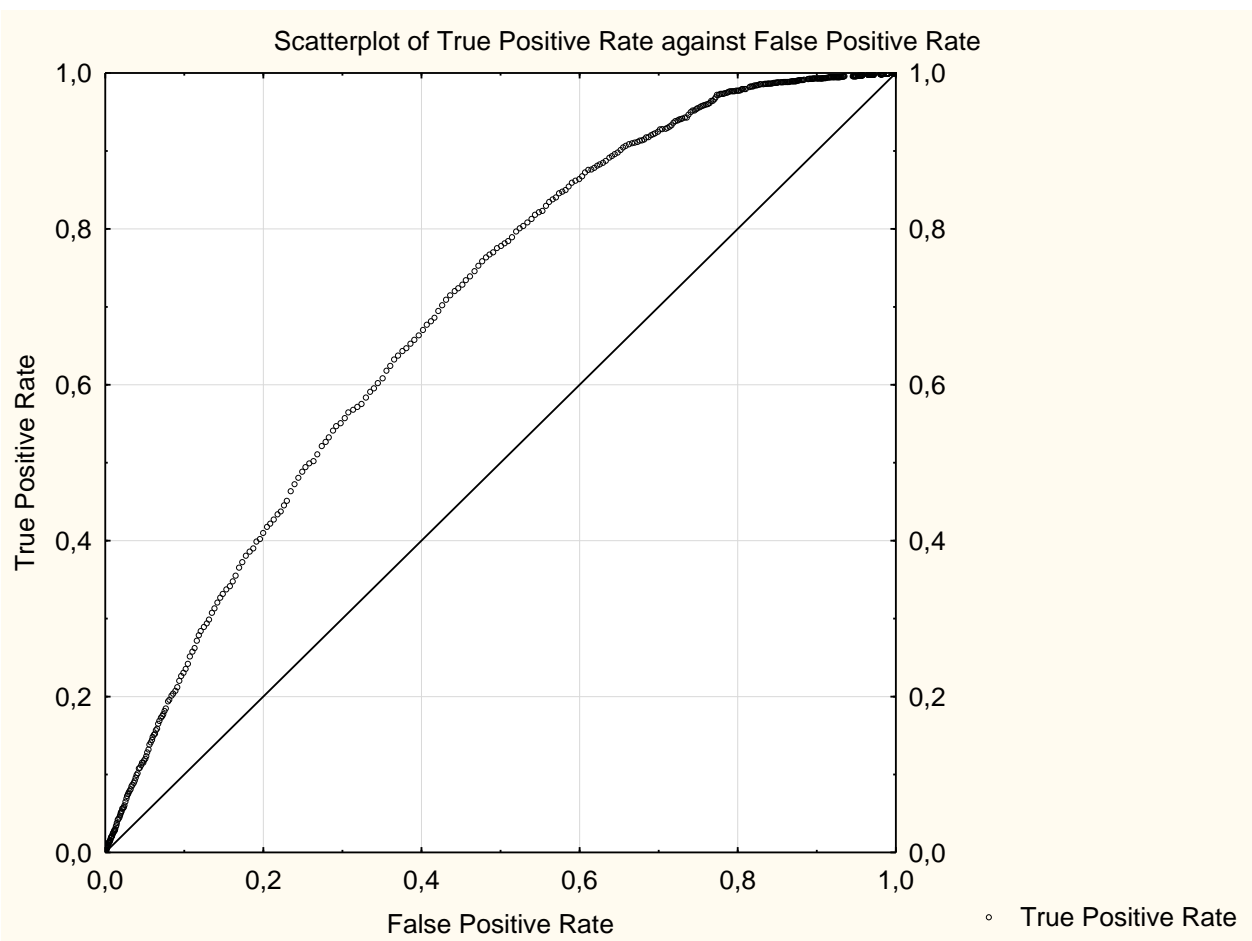


Figure 1. Receiver operating characteristics of the ChEMBL validation data

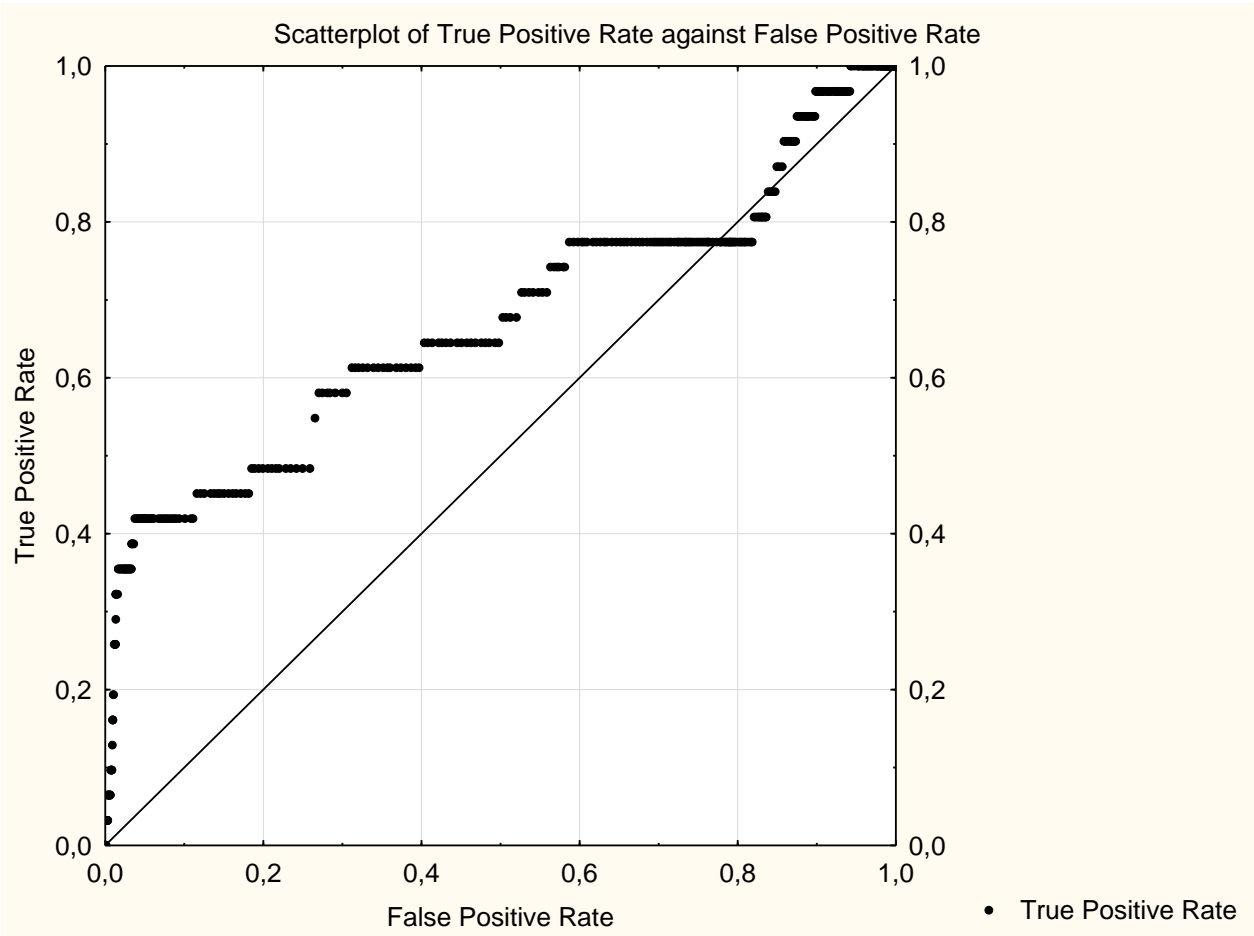


Figure 2. Receiver operating characteristics of the PubChem validation data on 5HT₁

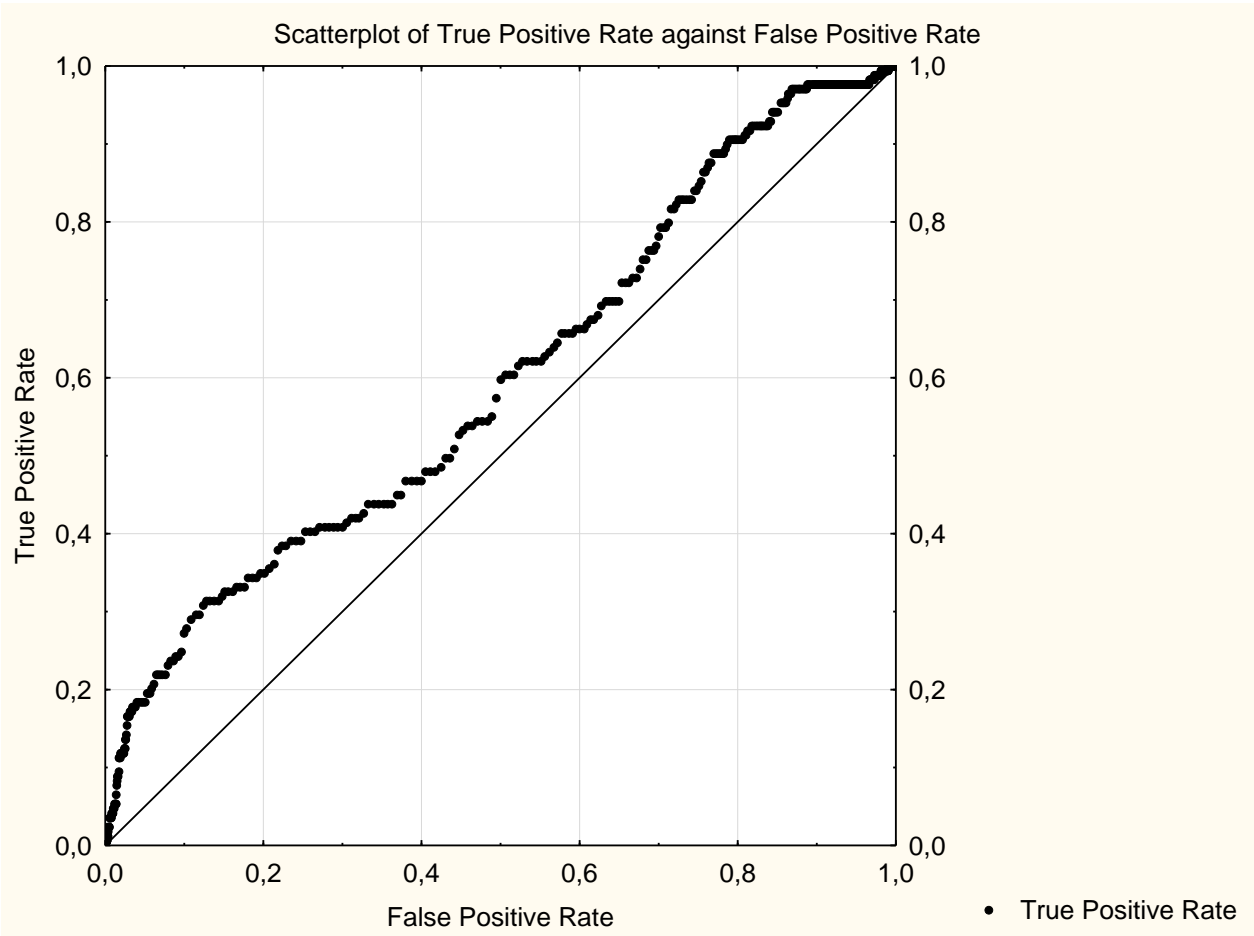


Figure 3. Receiver operating characteristics of the PubChem validation data on TAAR₁

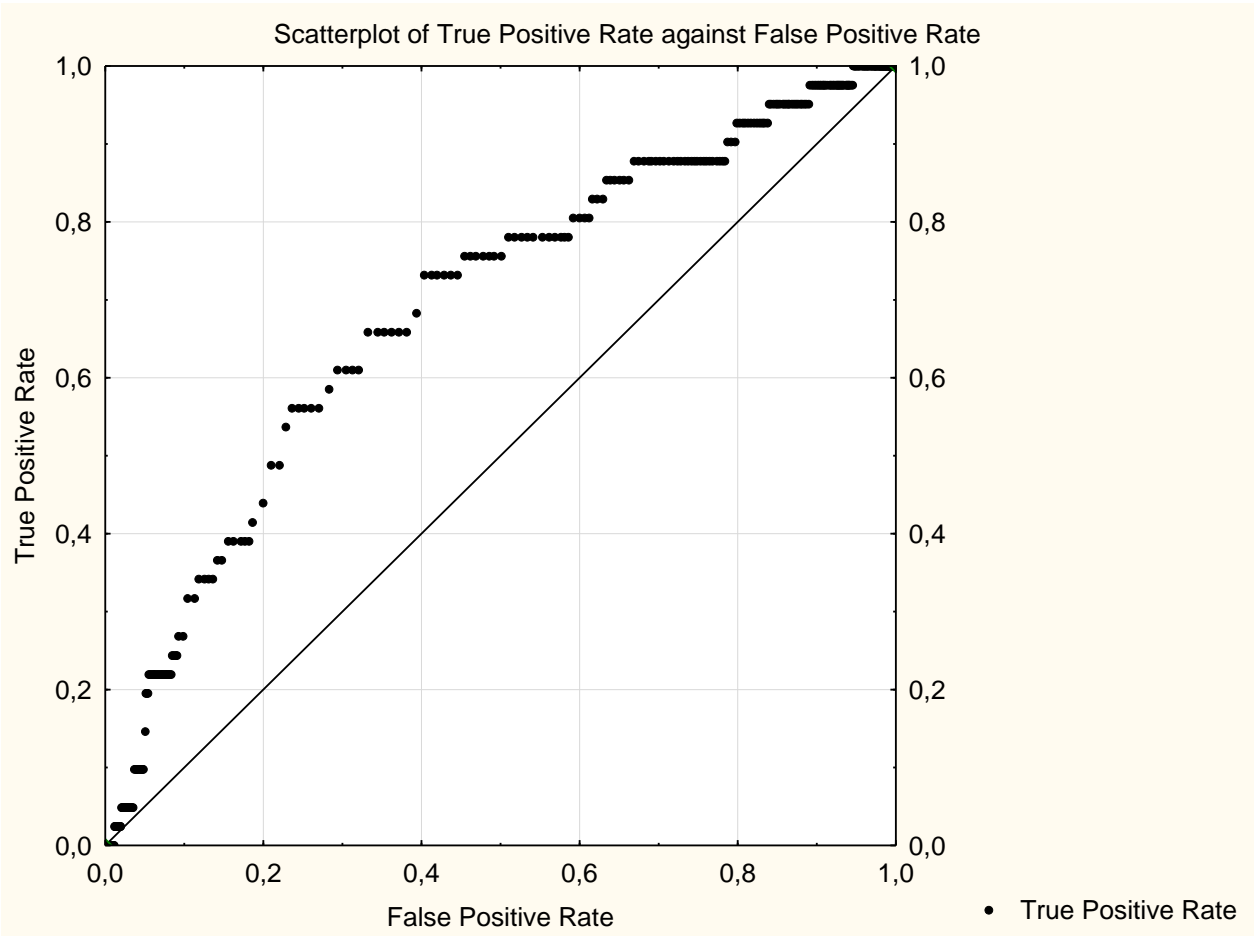


Figure 4. Receiver operating characteristics of the GPCR HTS validation data

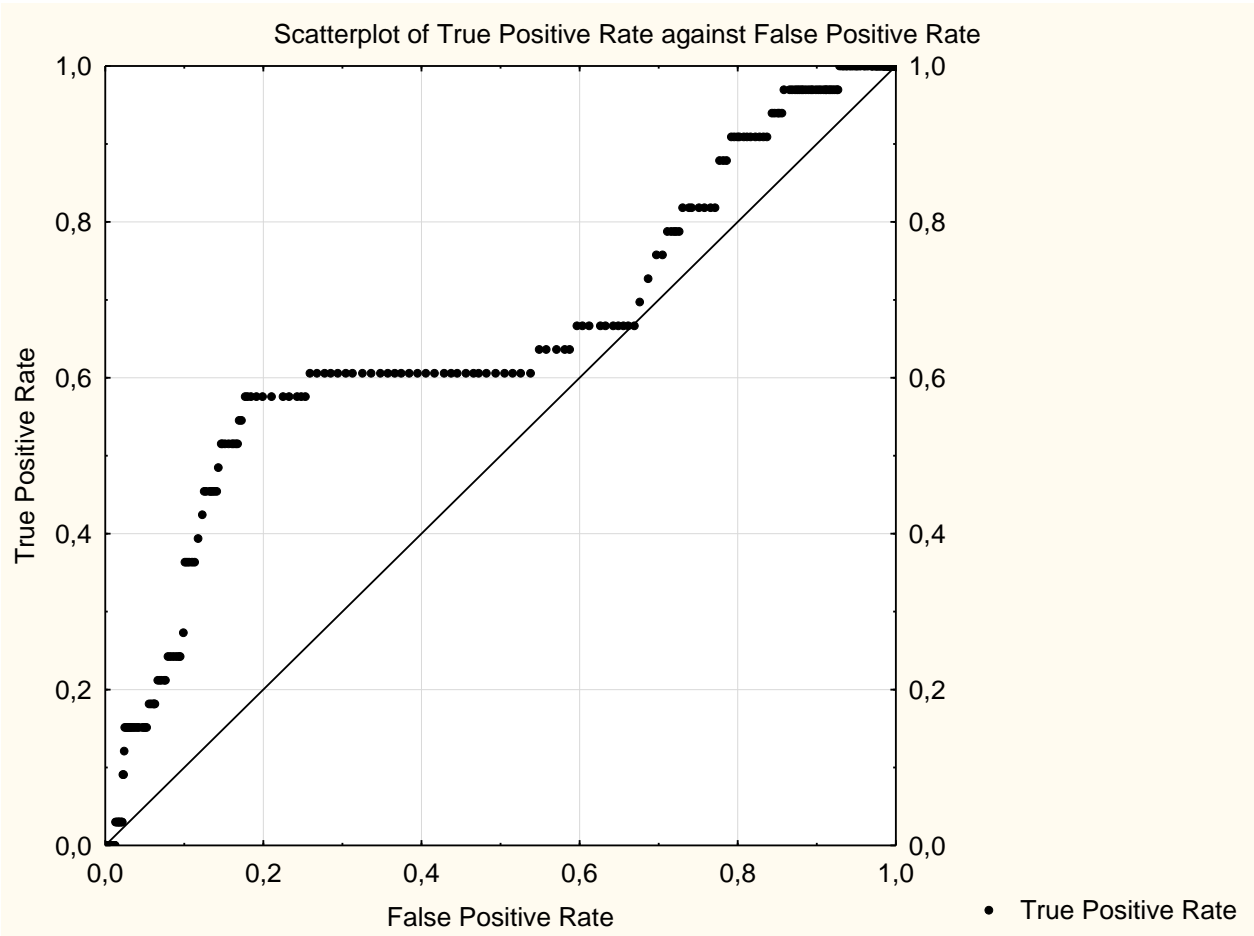


Figure 5. Receiver operating characteristics of the GPCR fragment screening validation data

TABLES

ACTIVE SET (SILE \geq 1.95)	median	mean	standard deviation
PSA (at pH = 7.4)	37.30	39.67	21.04
logD (at pH = 7.4)	0.97	0.90	1.62
number of nitrogen atoms	2.00	2.28	1.26
number of oxygen atoms	1.00	1.08	0.95
number of rotatable bonds	2.00	2.64	1.89
pK _a (strongest basic)	8.55	7.92	2.85
logP	2.18	2.04	1.53
hydrogen bond donor count	1.00	0.89	0.96
hydrogen bond acceptor count	3.00	2.70	1.13
REFERENCE SET	median	mean	standard deviation
PSA (at pH = 7.4)	56.49	60.49	29.28
logD (at pH = 7.4)	2.03	1.65	2.24
number of nitrogen atoms	2.00	2.17	1.48
number of oxygen atoms	2.00	2.14	1.52
number of rotatable bonds	3.00	3.36	2.10
pK _a (strongest basic)	7.00	5.97	2.60
logP	2.41	2.20	1.80
hydrogen bond donor count	1.00	1.27	1.12
hydrogen bond acceptor count	3.00	3.23	1.50

Table 1. Mean, median, and standard deviation values of the calculated physicochemical properties

	pKa (acidic)	pKa (basic)	actives	inactives
acid	< 7	> 7 or absent	0	12
base	> 7 or absent	> 7	83	15
neutral	> 7 or absent	< 7 or absent	16	71
zwitterion	< 7	> 7	1	3

Table 2. Classification of the acid-base characters of active and inactive fragments

ACTIVE SET ($pK_i \geq 4$)	median	mean	standard deviation
PSA (at pH = 7.4)	37.97	40.22	21.38
logD (at pH = 7.4)	0.97	0.90	1.62
number of nitrogen atoms	2.00	2.28	1.26
number of oxygen atoms	1.00	1.10	0.98
number of rotatable bonds	2.00	2.76	1.96
pK_a (strongest basic)	8.54	7.83	2.99

Table 3. Mean, median, and standard deviation values of the calculated physicochemical properties of the active set defined by pActivity metrics

Fragment-GPCR-Score Cut-off	EF ChemBL	EF 5HT ₁	EF TAAR ₁	EF Richter HTS	EF Richter FS
4.5	1.79	2.00	1.39	1.33	1.09
5	2.35	7.98	3.41	2.51	2.34
5.5	2.56	22.86	4.48	1.54	2.94

Table 4. Enrichment factors at selected FrAGS values obtained in validation studies

ASSOCIATED CONTENT

Supporting Information. The desirability functions, the p-values of the Mann-Whitney U-test, Enrichment Factors and the TNR/FNR plots are available free of charge via the Internet at <http://www.springer.com/>

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ABBREVIATIONS

FrAGS, Fragment Aminergic GPCR Score; GPCR, G-Protein Coupled Receptor; PSA, polar surface area; FBDD, fragment-based drug discovery; HTS, high-throughput screening; FS, fragment-screening; 7TM, seven-transmembrane; SILE, size-independent ligand-efficiency; SMILES, simplified molecular-input line-entry system; EF, enrichment factor; TPR, true positive rate; TNR, true negative rate; FPR, false positive rate; FNR, false negative rate; ROC, receiver operating characteristic; TAAR₁, trace-amine receptor subtype 1; 5HT₁, 5-hydroxy-tryptamine receptor subtype 1

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