Quasi-atomistic Receptor Surrogates for the 5-HT_{2A} Receptor: A 3D-QSAR Study on Hallucinogenic Substances

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Abstract

The 5-HT_{2A} receptor is known to act as the biological target for a series of hallucinogenic substances including substituted phenylalkylamines, tryptamines and LSD. A prerequisite for a hallucinogenic effect is an agonistic binding mode to the high-affinity state of the receptor. Attempts to establish a quantitative structure-activity relationship for such compounds are typically based on homology models or 3D-QSAR.

In this paper, we describe a surrogate for the 5-HT_{2A} receptor derived by means of *quasi-atomistic receptor modeling* (software *Quasar*), a more recently developed 3D-QSAR technique. This approach allows for the simulation of local induced fit, H-bond flip-flop, and solvation phenomena. The QSARs are established based on a family of receptor-surface models, generated by a genetic algorithm combined with cross-validation. The surrogate for the 5-HT_{2A} receptor yielded a cross-validated q^2 of 0.954 for the 23 compounds defining the training set. A series of 7 test compounds was then used to validate the model, resulting in a RMS deviation of 0.40 kcal/mol between $\Delta G^0_{prd.}$ and $\Delta G^0_{exp.}$. The largest individual deviation was 0.61 kcal/mol, corresponding to an uncertainty of a factor 2.7 in the binding affinity. A scramble test with negative outcome ($q^2 = 0.144$, slope $= -0.019$) demonstrates the sensitivity of the model with respect to the biological data. Subsequently, the surrogate was used to estimate the activity of a series of 53 hypothetical congeneric compounds, some of which are predicted to be close in activity to LSD.

1 Introduction

Hallucinogens are substances which provoke far-reaching and thorough mental and psychic changes including disorientation, derealization, and depersonalization, whereas consciousness and memory seem to remain unaltered. According to their chemical structure, hallucinogens—in the strictest sense—can be classified as phenylalkylamines (phenethylamines and amphetamines), indolealkylamines such as tryptamines and ergolines such as LSD. Large numbers of derivatives of such compounds are synthesized by chemists in underground laboratories and subsequently misused as *designer drugs*. The driving force behind such illicit activity is to produce substances with stronger psychological effects by minor—often arbitrary—variations of the molecular structures. Aiming at a more effective criminal prosecution and a faster registration of substances with a dependency and abuse potential, the prediction of the effects and binding affinities of new hallucinogens or designer drugs is highly desirable.

The 5-HT_{2A} receptor is known to act as the biological target for a series of hallucinogenic substances including substituted phenylalkylamines, tryptamines, and LSD [1]. Prerequisites for a hallucinogenic effect include an agonistic binding behaviour for the high-affinity state of the receptor. In addition, a protonated N atom (aminoethyl side chain of the phenylalkylamines and tryptamines or ring D of LSD, respectively; cf. Figure 1, Table 1) is also mandatory, whereby a primary amine functionality yields optimal activity. Hydroxylation and methoxylation of position 2 of the phenylalkylamines or position 5 of the tryptamines and substitution with hydrophobic groups (e.g. halide atoms) in position 4 of the phenylalkylamines or position 7 of the tryptamines leads to a greatly increased affinity towards the 5-HT_{2A} receptor. The receptor also exhibits stereoselectivity for the *R*-enantiomer of the phenylalkylamines and the *S*-enantiomer of the tryptamines. For an overview of pharmacology and structure-activity relationships, see, for example, refs. 2–8.

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Key words: Hallucinogens, 5-HT_{2A} receptor agonists, designer drugs, 3D-QSAR, quasi-atomistic receptor modeling, software *Quasar*

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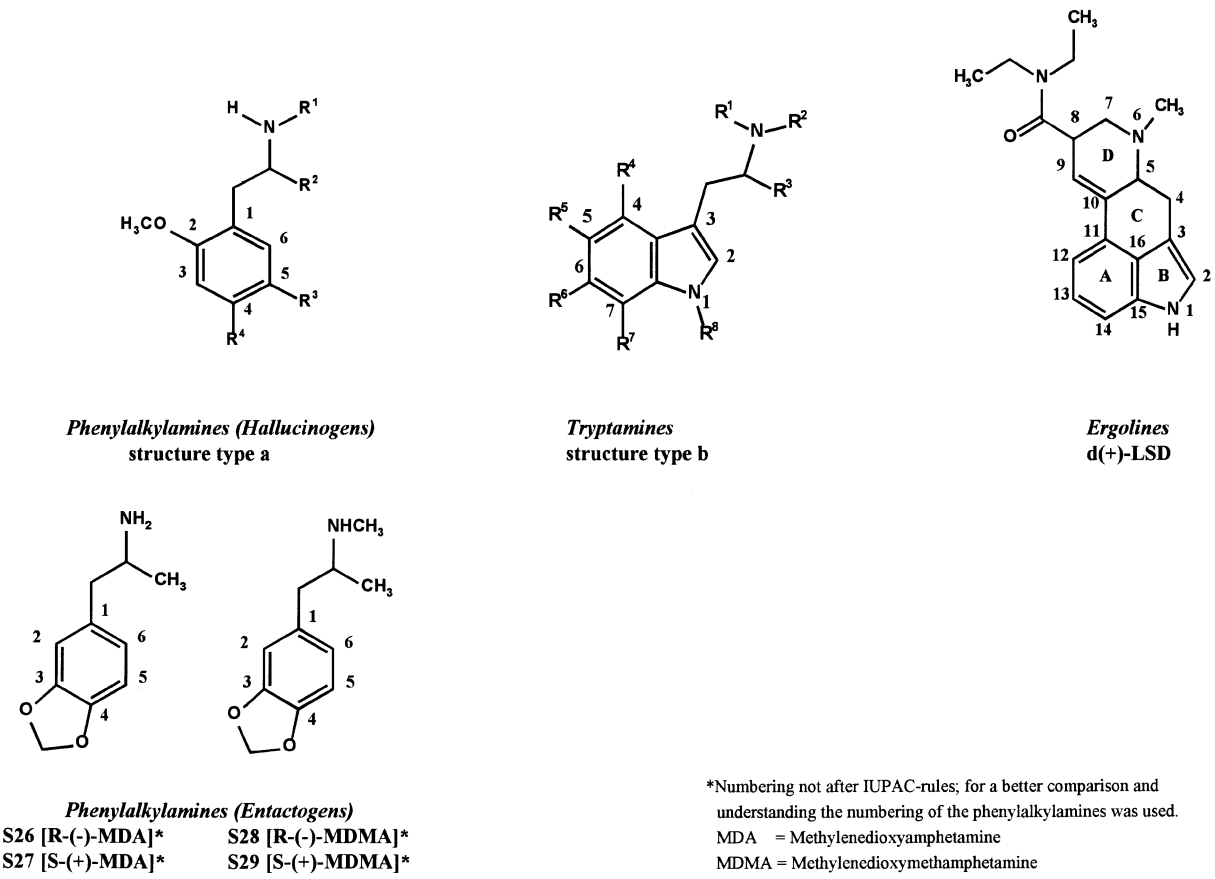

Figure 1. Molecular structures of the 5-HT_{2A} agonists defining the training and test set used for the *Quasar* study: phenylalkylamines, tryptamines, LSD (an ergoline), and entactogens. Cf. also Table 1.

The psychoactive properties of hallucinogens are mediated by the 5-HT_{2A} receptor subtype for which two affinity states exists: a high- and a low-affinity state [9–11]. In radioaffinity-labelling studies, radiolabelled 5-HT_{2A} agonists such as [^{125}I] 2,5-dimethoxy-4-iodoamphetamine ([^{125}I] DOI) and [^{3}H] 2,5-dimethoxy-4-bromoamphetamine ([^{3}H] DOB) have been shown to bind preferably to the high-affinity state. Compared to the low-affinity state they display a 50-fold higher affinity. In contrast, 5-HT_{2A} antagonists such as [^{3}H] ketanserin show comparable affinities for both states of the receptor [9, 12, 13].

To understand the activity of hallucinogenic substances at the molecular level, several receptor models for the 5-HT_{2A} receptor have been constructed, and numerous 3D-QSAR studies were carried out [14–23]. Since the three-dimensional structure of the 5-HT_{2A} receptor is not presently available, various molecular models have been generated in homology to the structure of bacteriorhodopsin, which has been determined by means of high-resolution electron cryomicroscopy [24]. Bacteriorhodopsin acts as a proton pump and has a different signal-transduction pathway. Not surprisingly, an apparent lack of primary sequence homology is observed between the two proteins. Consequently, models for the 5-HT_{2A} receptor based on the structure of bacteriorhodopsin should be interpreted cautiously. To establish a 3D-QSAR on the other hand, no direct knowledge of the receptor topology is necessary—unfortunately, however, most of the studies were based on data sets consisting only of hallucinogen subgroups such as the phenylalkylamines or tryptamines [20–22, 25].

Here we present a study combining a more diverse data set including phenylalkylamines, tryptamines and LSD (an ergoline) as hallucinogens and also entactogenic substances. The term entactogen (lat. *tactus* = to touch, griech. *en* = within, griech. *gen* = to produce) was first introduced by Nichols [26] and stands for "*to produce a touching within*". It describes the unique effects of this substance class which acts by enhancing the readiness to communicate rather than being hallucinogenic. A new 3D-QSAR approach named *quasi-atomistic receptor modeling* (software *Quasar*) was also used. This technique bridges 3D-QSAR and receptor fitting by combining receptor-surface models populated with atomistic properties (hydrogen bonds, salt bridges, aromatic and aliphatic regions, solvent) and individually adapted ligand-surrogate envelopes, simulating a flexible receptor cavity [27].

Table 1. Phenylalkylamine and tryptamine derivatives used for the QSAR data set. Cf. also Figure 1.

Phenylalkylamines

	structure type	R^1	R^2	R^3	R^4
S1	a	H	H	H	CH_3
S2	a	H	H	H	Br
S3 [R-(−)-DOM]	a	H	CH_3	OCH_3	CH_3
S4 [R-(−)-DOB]	a	H	CH_3	OCH_3	Br
S5 [S-(+)-DOB]	a	H	CH_3	OCH_3	Br
S6 [α-desMeDOB]	a	H	H	OCH_3	Br
S7 [R-(−)-DOI]	a	H	CH_3	OCH_3	I
S8 [S-(+)-DOI]	a	H	CH_3	OCH_3	I
S9 [R-(−)-DON]	a	H	CH_3	OCH_3	NO_2
S10 [R-(−)-N-MeDOM]	a	CH_3	CH_3	OCH_3	CH_3
S11 [α-Demethyl-DOM]	a	H	H	OCH_3	CH_3

Tryptamines

	structure type	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8
S20 [5-OH-DMT]	b	CH_3	CH_3	H	H	OH	H	H	H
S30 [5-HT]	b	H	H	H	H	OH	H	H	H
S17 [DMT]	b	CH_3	CH_3	H	H	H	H	H	H
S21 [6-TMT]	b	CH_3	CH_3	H	H	H	CH_3	H	H
S22 [7-Br-DMT]	b	CH_3	CH_3	H	H	H	H	Br	H
S23 [5-OMe-7-Me-DMT]	b	CH_3	CH_3	H	H	OCH_3	H	CH_3	H
S14 [5-OMeT]	b	H	H	H	H	OCH_3	H	H	H
S24 [5-OMe-DET]	b	C_2H_5	C_2H_5	H	H	OCH_3	H	H	H
S19 [4-OMeDMT]	b	CH_3	CH_3	H	OCH_3	H	H	H	H
S31 [6-OMe-DMT]	b	CH_3	CH_3	H	H	H	OCH_3	H	H
S18 [1-TMT]	b	CH_3	CH_3	H	H	H	H	H	CH_3
S16 [S-(+)-5-OMe-α-MeT]	b	H	H	CH_3	H	OCH_3	H	H	H
S13 [R-(−)-α-MeT]	b	H	H	CH_3	H	H	H	H	H
S12 [S-(+)-α-MeT]	b	H	H	CH_3	H	H	H	H	H

Phenylalkylamines:
DOM = 2,5-Dimethoxy-4-methylamphetamine
DOB = 2,5-Dimethoxy-4-bromoamphetamine
α-desMeDOB = α-Demethyl-2,5-dimethoxy-4-bromoamphetamine
DOI = 2,5-Dimethoxy-4-iodoamphetamine
DON = 2,5-Dimethoxy-4-nitroamphetamine
N-MeDOM = N-Methyl-2,5-dimethoxy-4-methylamphetamine
α-Demethyl-DOM = α-Demethyl-2,5-dimethoxy-4-methylamphetamine

Tryptamines:
5-OH-DMT = 5-Hydroxy-N,N-dimethyltryptamine
5-HT = 5-Hydroxytryptamine
DMT = N,N-Dimethyltryptamine
6-TMT = 6,N,N-Trimethyltryptamine
7-Br-DMT = 7-Bromo-N,N-dimethyltryptamine
5-OMe-7-Me-DMT = 5-Methoxy-7,N,N-trimethyltryptamine
5-OMeT = 5-Methoxytryptamine
5-OMe-DET = 5-Methoxy-N,N-diethyltryptamine
4-OMeDMT = 4-Methoxy-N,N-dimethyltryptamine
6-OMe-DMT = 6-Methoxy-N,N-dimethyltryptamine
1-TMT = 1,N,N-Trimethyltryptamine
S-(+)-5-OMe-α-MeT = S-(+)-5-Methoxy-α-methyltryptamine
α-MeT = α-Methyltryptamine

In contrast to other 3D-QSAR approaches, problems associated with the various ligand molecules binding to an "averaged receptor model" are avoided, as the adaption of the receptor-envelope to the individual ligand topologies is simulated (local induced-fit mechanism). Moreover, H-bond flip-flop and simulation of solvation effects are included in the algorithm. H-bond flip-flop particles would seem to be particularly important as they are capable of mimicking amino-acid residues at the true biological receptor (Ser, Thr, Tyr, Cys, His, Asn and Gln) capable to engage in differently directed hydrogen bonds with different ligand molecules. This is of utmost importance in a virtual

experiment where a series of ligand molecules binds "simultaneously" to the receptor surrogate. In addition, a solvent-accessible binding pocket or different degrees of binding-site tightness may be simulated. Ligand-receptor interactions are evaluated based on a directional force field for hydrogen bonds and salt bridges which allows for the simulation of ligand selectivity, including the discrimination of stereoisomers [27].

Based on a series of ligand molecules with individually adapted receptor envelopes (training set), *Quasar* allows to generate a family of receptor models using a genetic algorithm combined with cross-validation. Model building involves the following basic steps:

(1) *Construction of a receptor envelope and its individual adaption to the topology of the very ligands.*
(2) *Generation of an initial population of parent models by random distribution of atomistic properties (positively charged salt bridges, negatively charged salt bridges, H-bond donors, H-bond acceptors, H-bond flip-flop particles, electrically neutral hydrophobic particles, positively charged hydrophobic particles, negatively charged hydrophobic particles, solvent particles, and void elements) on the envelope surface.*
(3) *Simulation of the evolution (cross-over and mutation events).*
(4) *Validation of the model family (external test set, scramble test).*

Finally, the obtained model family must be validated through an external set of ligand molecules not used for model construction (test set). The sensitivity towards the biological data (i.e. the binding affinities) may be established by means of a scramble test. Here, the ΔG^0 data of the training set are randomly scrambled with respect to the true biological values and the simulation is repeated under otherwise identical conditions. If a solution for the ligands of the training set is nonetheless found and if the ligands of the test set are predicted similarly well—when compared with the true simulation using unscrambled ΔG^0 data—the model is worthless as it is not sensitive to the biological data it should establish a QSAR for. If the genetic algorithm even fails to identify a reasonable model for the scrambled training set, force-field and the model surrogate are thought to adequately represent the interaction at the true biological receptor.

In the present investigation the validated family of quasi-atomistic receptor models for the 5-HT_{2A} receptor is subsequently used for predictions of the binding affinities of 53 congeneric compounds, most of them not yet synthesized and tested for affinity towards the 5-HT_{2A} receptor. Due to the structural resemblance of these molecules to hallucinogenic templates one can assume that the new substances which possess a high binding affinity for our system might possibly also act as potent hallucinogens.

2 Methods

2.1 Selection of the Data Set

For our study, radioligand-binding data determined for the rat-brain cortical 5-HT_{2A} receptor labelled with the antagonist [^{3}H] ketanserin were used (cf. Table 2 and refs. 28–35). As agonists and antagonists show a different binding behaviour for the two affinity states of the 5-HT_{2A} receptor, a data set consisting of pure agonists also determined against a receptor labelled with an agonistic acting radioligand e.g. [^{3}H] DOB or [^{125}I] DOI would seem to be preferable. Unfortunately, only few data determined against agonistic-binding substances are presently available. This holds also for (±)-2,3-dimethoxyphenyl-1-[2-(4-piperidine)-methanol] ([^{3}H] MDL 100.907) [36], a more specific antagonist for the 5-HT_{2A} receptor than ketanserin for which only insufficient data exists. As for the safe validation of a receptor surrogate at least 20–30 substances are recommended [37], we had to select the mixed-mechanism approach with the biological data determined against [^{3}H] ketanserin. The scramble test (cf. below) did, however, demonstrate a sufficiently high sensitivity towards the biological data, thus, supporting the assumption that the systematic error possibly introduced by the mixed-mechanism approach is comparable in magnitude for all substances tested.

The various phenylalkylamine and α-methylated tryptamine ligands used in our study exist in two stereoisomeric forms. As frequently observed with chiral bioligands, the different isomers display a different biological activity: for the phenylalkylamine ligands the *R*-enantiomer is the more active one while for the tryptamine ligands the *S*-enantiomer is more potent. For a statistical significant validation of the receptor surrogate, the binding affinity of the ligands of the training set should preferably span 3 to 4 orders of magnitude in their affinity towards the true biological receptor [37, 38], a requirement which is met by our ligand data set (cf. Table 2).

2.2 Building of the Molecules/Geometry Optimization/Conformational Search

The three-dimensional structures of the 5-HT_{2A} agonists (cf. Table 1) were constructed using the X-ray crystal structures of 4-ethyl-2,5-dimethoxyamphetamine, 5-hydroxy-N,N-dimethyltryptamine and LSD [39–41] as templates and the model building tools of the *SYBYL 6.3* software [42]. All molecules were generated in the N-protonated form, as this tautomer prevails at physiological pH (pH 7.4) [2, 43] at which the affinity was determined [28–35].

The initial geometry optimization was performed *in vacuo* using the Tripos standard force field [44]. Subsequently, we explored the conformational space of each of the ligand

molecules by scanning all rotatable bonds in 5-degree increments and retained all conformers refined to within 20 kcal/mol of the global energy minimum. The ensemble of structures was then sampled and analyzed by means of the software *Starmaker* and *Starcompare* [45] in order to identify a common spatial orientation of the protonated N atom as a pharmacophorically important group among the phenylalkylamines and the tryptamines.

A first pharmacophore model was based on the superposition of the atoms defining the aromatic six-membered ring (henceforth referred to as the primordial alignment). The second hypothesis—used for the *Quasar* study—included the energy minimization of the ligands of the primordial alignment and the superimposition of the N-atom, its proton and the aromatic ring (for details of this technique, cf. ref. 38). All ligand molecules were subsequently refined in aqueous solution using the MM2 force-field as implemented in *MacroModel 6.0* [46]. Electrostatic partial charges and solvation energies were then calculated using the *AMSOL 6.4* software package [47]. Thereafter, the alignment of the ligand molecules was adjusted a final time.

2.3 *Quasar: Construction of Quasi-Atomistic Receptor Models and Estimation of Relative Free Energies of Ligand Binding*

Quasi-atomistic receptor modeling (software *Quasar* [27]) allows the construction of a receptor-surface model—a three-dimensional envelope, populated with atomistic properties—about any molecular framework of interest, e.g. a pharmacophore. Based on our pharmacophore hypothesis (cf. above)—prior to the analysis—23 substances of the data set were defined as a training set, 7 substances (**S2, S4, S6, S11, S14, S17, S26**) as a test set. The selection of the ligands for the training and the test set followed the regulation of each functional group of the test set being implemented in the training set to avoid extrapolation of the afterwards obtained results. For the training set of ligand molecules an "averaged receptor envelope" using the default-values was generated. The envelope was optimized by means of energy minimization and adapted to each ligand molecule of both training and test set as a (1 : 1) ligand-receptor complex, thus allowing for individual adaption of the receptor envelope to the respective ligand topology.

Using a genetic algorithm an initial population of 200 receptor models was developed using both cross-over and mutation events. For generation of the models default values were used with exception of the following ones: ligand-receptor polarization was enabled, the weighting of solvation effect was set to 0.1, 4 cross-validation groups were used, and the site-saturation rate was set to 0.6. The optimal duration of the evolution was determined by a series of identical simulations differing solely in the maximum number of evolution steps (1000, 2000, . . . , 10 000) and was set to 8000.

In the *Quasar* concept [27], the approach of Blaney *et al*. [48] for estimating the binding energy of an individual ligand molecule bound to a macromolecular receptor, $E_{bdg.}$, is augmented by two terms accounting for the change in ligand-internal energy [see also refs. 38, 49] and local induced fit, respectively:

$$E_{bdg.} \approx E_{lig\text{-}rec} - T\Delta S_{bdg.} - \Delta G_{solv.lig.} + \Delta E_{int.lig.} + \Delta E_{env.adapt.lig.} \quad (1)$$

The ligand-receptor interaction energy, $E_{lig\text{-}rec}$, is determined by means of a directional force field [27, 38, 49, 50]. The entropy term, $T\Delta S_{bdg.}$, is calculated based on the number of freely rotatable bonds, excluding terminal methyl groups [51]. By default, the ligand desolvation energies, $\Delta G_{solv.lig.}$, are estimated based on W. Clark Still's implicit-solvent model [52]. For the 5-HT_{2A} agonists, we preferred to determine the solvation energies by means of *AMSOL 6.4* [47] as this program was also used to calculate the atomic partial charge model (cf. above). The term $\Delta E_{int.lig.}$ accounts for a potential increase of the ligand-internal energy while bound to the receptor surrogate (relative to a strain-free reference conformation in aqueous solution). This correction would seem to be necessary as the internal energy of a ligand molecule may increase while maximizing its interaction with the receptor. $\Delta E_{env.adapt.lig.}$ is associated with the energy uptake upon modifying the mean receptor envelope to an entity individually adapted to the topology of the very ligand molecule (→local induced fit; see also ref. 27).

Free energies of ligand binding, $\Delta G^0_{prd.}$, are then predicted by establishing a linear regression between $\Delta G^0_{exp.}$ and $\Delta E_{bdg.}$ using solely the ligand molecules of the training set:

$$\Delta G^0_{prd.} = |a|\Delta E_{bdg.} + b \quad (2)$$

Slope and intercept of [2] are inherent to a given receptor model within the model family and are subsequently applied to predict the relative binding energy of ligand molecules different from those in the training set. The family of receptor models is then validated based upon their ability to predict free energies of ligand binding for an external set of test ligand molecules not used during model construction. A more serious challenge to the receptor surrogate is provided by the scramble test (cf. above). Other criteria include the cross-validated q^2 value, the *lack-of-fit* (cf. ref. 27) for the ligands of the training set as well as the uniformness of the distribution of the properties mapped onto the receptor envelope, e.g. larger hydrophobic areas or solvent-accessible regions. Finally, the number and distribution of the atomistic properties might also represent valuable criteria.

2.4 Prediction of Binding Affinities of New Compounds

The model family was then used to predict the binding affinities of 53 compounds, most of which have not yet been synthesized and tested (cf. Figure 5, Table 3). For these compounds, conformational search, alignment and construction of individual ligand envelopes in *Quasar* were carried out under the same conditions as for the training and the test set.

A simultaneous consideration of all rotatable bonds for ligands such as **ko3** would lead to a too large number of possible conformers and a more difficult evaluation/interpretation of the results. So for this ligands *all* of the rotatable bonds were evaluated step by step. After every conformational search the molecules were again energy minimized and the total energy was checked. As reference points the corresponding atoms of the amide side-chain of the LSD-molecule were chosen and every conformer was compared with this (software *Starmaker/Starcompare*, cf. above).

3 Results and Discussion

The relative alignment of substituted derivatives of both tryptamines and phenylalkylamines is often discussed controversially (see, e.g., refs. 14, 25, 32, 53, 54). In our primordial pharmacophore model, the aromatic ring systems were oriented analogously to a previous study in our laboratory [25] and in agreement with other 3D-QSAR simulations [14, 32, 54]. As the ammonium functionality has been shown to engage in a strong salt bridge with an aspartate residue at the true biological receptor [55], we selected a more stringent alignment protocol (cf. above), where special emphasis was put on the spatial orientation of the $>$N-H$\rightarrow$H-bond donor. This was accomplished by means of a vector-alignment concept (H-extension and lone-pair vectors associated with ideal H-bond geometries; for details, see ref. 50). Comparison of these vectors and the electrostatic field exerted by the ligand molecules suggests that—in spite of the structural difference—phenylalkylamines, tryptamines, and ergolines might well bind to the same receptor site displaying a similar topology. Such a protocol has been referred to as *receptor mediated alignment* [38]. The ligand superposition is shown in Figure 2.

Using a population of 200 receptor models, the system—comprising 23 ligand molecules of the training set and the enclosing individual envelopes, each defined by 238 discrete positions—was allowed to evolve for 8000 cross-over cycles. The transcription-error rate (expressed by random mutations) was set to 0.02 and the minimal difference between any of the receptor models was required to be at least 0.1, i.e. 24 of the 238 properties.

This simulation resulted in a cross-validated q^2 of 0.954, averaged over all 200 models defining the receptor family. The RMS deviation (cf. Equation 3) of experimental and predicted free energies of ligand binding

$$\text{RMS deviation} = \sqrt{\frac{\sum\left(\Delta G^0_{\text{prd.}} - \Delta G^0_{\text{exp.}}\right)^2}{n_{\text{lig.}}}} \qquad (3)$$

for the ligand molecules of the training set ($n_{\text{lig.}} = 23$) was 0.23 kcal/mol. The largest individual deviation, obtained for ligand **S10**, was 0.74 kcal/mol which corresponds to an uncertainty in the binding affinity of a factor 3.3.

A test set of seven ligand molecules was then used to validate the model. Here, the RMS deviation of experimental and predicted free energies of ligand binding was calculated to be 0.40 kcal/mol. The largest individual deviation, obtained for ligand **S2**, was 0.61 kcal/mol which corresponds to an uncertainty in the binding affinity of a factor 2.7. A summary

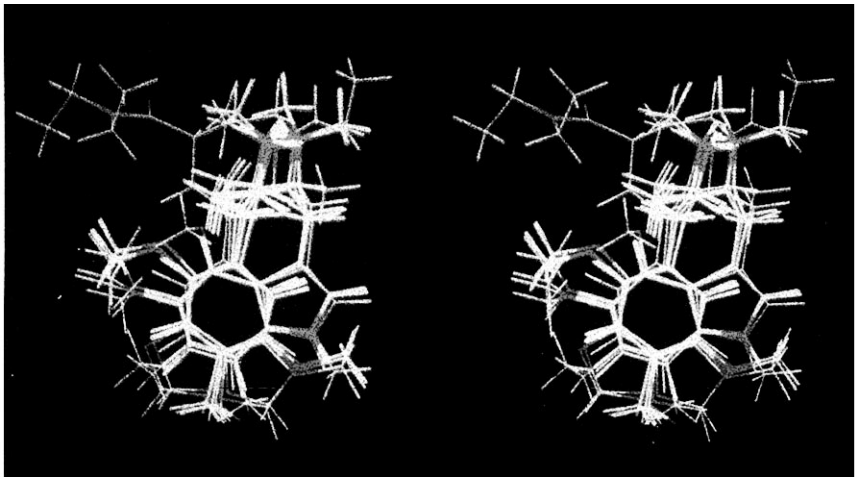

Figure 2. Stereoview of the ligand alignment used for the *Quasar* study.

of the results is given in Table 2; the receptor surrogate is shown in Figure 3; experimental and predicted binding affinities are compared in Figure 4.

Because $\Delta G^0_{prd.}$ is averaged over the *n* members (here $n = 200$) of the receptor family in the *Quasar* concept, each value is associated with a standard deviation S_{Ki}, describing the variation over the various individual models. This value may be interpreted in terms of a safe or less safe prediction of an individual compound. For ligand molecules defining the training set, this range is typically rather narrow; of greater interest, however, are the corresponding values of the test set. For the 5-HT_{2A} receptor, the largest variation of $\Delta G^0_{prd.}$ within the training set was observed for ligand **S9** ($\sigma = 0.17$ kcal/mol); for the test set, ligand **S26** displayed the largest variation ($\sigma = 0.18$ kcal/mol). These comparable values suggest that the training-set selection is representative for the ligand molecules defining the test set. The individual ΔG^0-values, converted into K_i-values, are given in Table 2. Next, we performed a *scramble test* using a randomly scrambled $\Delta G^0_{exp.}$ data for the ligands of the training set but otherwise identical settings for the simulated evolution. The simulation yielded a cross-validated q^2 of 0.144 (normal simulation: 0.954) with individual deviations as large as 2.8 kcal/mol, corresponding to an uncertainty in the binding affinity of a factor 96. The low q^2 and the negative slope of the regression ($a = -0.019$) indicate that the receptor-surrogate is indeed sensitive to the biological data it should establish a QSAR for (cf. Figure 4). A final simulation explicitly allowing for a solvent-accessible binding pocket led to slightly inferior results, suggesting that solvent might not be involved in ligand binding at the true biological receptor.

Finally, the quasi-atomistic receptor surrogate was used to predict the activity of a series of 53 congeneric ligands (cf. Figure 5, Table 3). Based on their structural resemblance to hallucinogenic templates they are believed to display a potential hallucinogenic activity in man. True predictions should always be interpreted with care as the possibility of an extrapolation with respect to the topologies and the functionalities within the training set can seldom be fully excluded. When using a genetic evolved family of receptor models, the variation of the predicted activities within the individual models may provide a hint whether or not extrapolation might be present. The ultimate assessment is given by the subsequent (or independent) synthesis and determination of the biological activity. With respect to our candidate molecules, this is indeed the case for some of the compounds which shall, therefore, be discussed in more detail.

Compound **pm8**, a rigid congener of DOB with a bromo substituent in position 4 was predicted with a high binding affinity of $K_i = 19$ nM (cf. Table 4). Independently to this study, the compound **pm8** was synthesized as a racemate and tested by Parker *et al.* [56]. It was shown to be a very potent derivative which even slightly surpassed LSD in potency and is so far the most potent ligand known for the 5-HT_{2A} receptor. As can be seen from experimental data (cf. Table 2), DOI possesses a higher binding affinity for the [^{3}H] ketanserin labeled 5-HT_{2A} receptor of the rat than DOB. Thus, one could expect to improve the activity of **pm8** by

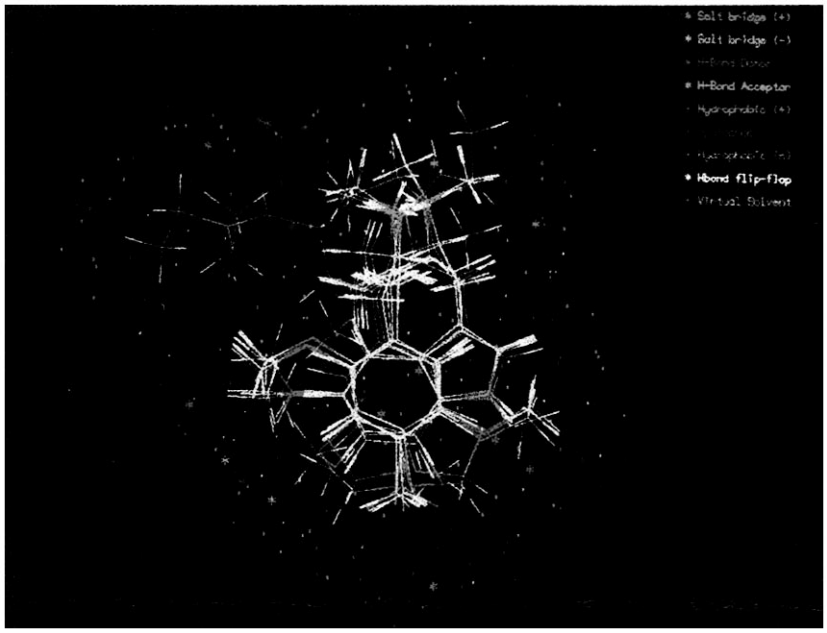

Figure 3. Quasi-atomistic receptor model for the 5-HT_{2A} receptor system as generated by *Quasar*. The individual properties mapped onto the envelope are colorcoded; the most frequent particle distribution among the 200 models is shown (cf. legend).

Table 2. K_i-values of the QSAR data set, descending order.

Abbrev.	Compound	$K_{i\ exp.} \pm S_{Ki}$ [nM]	$K_{i\ pred.} \pm S_{Ki}$ [nM]
LSD	d-(+)-LSD	$2.5 \pm 4^{a,f}$	2.8 ± 0.4
S7	R-(−)-DOI	$9.9 \pm 1^{c,f,g,h}$	11 ± 2
S8	S-(+)-DOI	$35 \pm 3^{d,g}$	38 ± 6
S3	R-(−)-DOM	$60 \pm 5^{c,f,g,h}$	96 ± 13
S5	S-(+)-DOB	$145 \pm 9^{a,b,g}$	170 ± 20
S22	7-Br-DMT	170 ± 15^{e}	160 ± 40
S9	R-(−)-DON	$210 \pm 35^{c,f,g,h}$	190 ± 50
S10	R-(−)-N-MeDOM	$260 \pm 20^{c,f,g,h}$	78 ± 10
S16	S-(+)-5-OMe-α-MeT	310 ± 20^{e}	390 ± 90
S23	5-OMe,7-Me-DMT	360 ± 60^{e}	400 ± 70
S18	1-TMT	400 ± 90^{e}	780 ± 120
S20	5-OH-DMT	480 ± 20^{e}	450 ± 90
S30	5-HT	$560 \pm 20^{e,g}$	640 ± 160
S1		825 ± 100^{a}	1300 ± 200
S24	5-OMe-DET	1120 ± 135^{e}	970 ± 190
S19	4-OMeDMT	1300 ± 50^{e}	1900 ± 300
S21	6-TMT	2500 ± 300^{e}	1400 ± 200
S12	S-(+)-α-MeT	3100 ± 500^{e}	2100 ± 300
S28	R-(−)-MDMA	3310^{h}	3800 ± 800
S13	R-(−)-α-MeT	5000 ± 300^{e}	4400 ± 700
S31	6-OMe-DMT	5400 ± 100^{e}	4000 ± 500
S27	S-(+)-MDA	13000^{h}	13900 ± 3000
S29	S-(+)-MDMA	15800^{h}	14000 ± 2000
$S4^{Testset}$	R-(−)-DOB	$24 \pm 3^{a,b,g}$	30 ± 4
$S6^{Testset}$	α-desMeDOB	34 ± 4^{a}	54 ± 12
$S11^{Testset}$	α-Demethyl-DOM	$110 \pm 8^{c,h}$	180 ± 30
$S14^{Testset}$	5-OMeT	300 ± 20^{e}	660 ± 14
$S2^{Testset}$		1030 ± 90^{a}	380 ± 100
$S17^{Testset}$	DMT	1200 ± 40^{e}	2900 ± 300
$S26^{Testset}$	R-(−)-MDA	$3420 \pm 5^{g,h}$	4300 ± 1200

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Table 3. New phenylalkylamine and tryptamine congeners. Cf. also Figure 5.

Phenylalkylamine congeners

Ligand	structure type	R^a	R^2	R^3	R^4	R^5	R^6
pn1[1]	a′	CH_3	OCH_3	H	CH_2Br	OCH_3	H
pn2[1]	a′	CH_3	OCH_3	H	CH_2Cl	OCH_3	H
pn3[1]	a′	CH_3	OCH_3	H	C_2H_4Cl	OCH_3	H
pn4[1]	a′	CH_3	OCH_3	H	CH_2F	OCH_3	H
pn5[2]	a′	CH_3	OCH_3	H	CF_3	OCH_3	H
pn6[1]	a′	CH_3	OCH_3	H	CH_2CF_3	OCH_3	H
pn7[1]	a′	CH_3	OCH_3	H	$C_2H_4CF_3$	OCH_3	H
pn8[1]	a′	CH_3	OCH_3	H	CH_2SH	OCH_3	H
pn9[1]	a′	CH_3	OCH_3	H	CH_2SCH_3	OCH_3	H
pn10[1]	a′	CH_3	OCH_3	H	CH_2OCH_3	OCH_3	H
pn11[1]	a′	CH_3	OCH_3	H	CN	OCH_3	H
pn12[1]	a′	CH_3	OCH_3	H	CH_2CN	OCH_3	H
pn13[1]	a′	CH_3	OCH_3	H	NHCOH	OCH_3	H
pn14[1]	a′	CH_3	OCH_3	H	$NHCOCH_3$	OCH_3	H
pn15[1]	a′	CH_3	OCH_3	H	$NHCOC_2H_5$	OCH_3	H
pn16[1]	a′	CH_3	OCH_3	H	$NHCOCF_3$	OCH_3	H
pn17[1]	a′	CH_3	OCH_3	H	Br	CN	H
pn18[1]	a′	CH_3	OCH_3	H	Br	NHCOH	H
pn19[1]	a′	CH_3	OCH_3	H	Br	H	OCH_3
pn20[1]	a′	CH_3	H	OCH_3	C_3H_7	OCH_3	H
pn21[1]	a′	Cl	OCH_3	H	C_2H_5	OCH_3	H

Tryptamine congeners

Ligand	structure type	X	R^a	R^4	R^5	R^6	R^7
pn22[1]	b′		CH_3	H	OCH_3	H	Br
pn23[1]	c	NH	CH_3		OCH_3	H	Br
pn24[1]	c	O	CH_3		OCH_3	H	Br
pn25[3]	d	O	CH_3		OCH_3	H	Br
pn26[1]	d	S	CH_3		OCH_3	H	Br
pn27[3]	d	O	CH_3		OCH_3	H	H
pn28[1]	b′		C_2H_5	H	H	H	H
pn29	b′		CH_3	Br	OCH_3	H	H
pn30	b′		C_2H_5	Br	OCH_3	H	H
pn31	b′		CH_3	H	OCH_3	Br	H
pn32	b′		C_2H_5	H	OCH_3	Br	H
pn33	b′		C_2H_5	H	OCH_3	H	Br

structures (not synthesized and tested):
[1]Beuerle, G.H., 1995.
structures (synthesized and tested):
[2]Nichols et al., 1994.
[3]Nichols et al., 1991.

exchange of the bromo substituent by a iodo substituent. As is shown by our study the corresponding iodo congener **pm9** (not synthesized and tested yet) has a predicted binding affinity of $K_i = 6.6$ nM, higher than **pm8**. Congeneric ligands with CH_3-, CF_3-groups or H-atoms in position 4 are predicted with a lower affinity (56 to 325 nM) which can be concluded from previous SAR-studies [4] and experimental data.

Compound **pm6**, a rigid analog of DOI, tethering the oxygen in position 2 into a ring system, was synthesized and tested independently by Monte [57]. The tethering of the oxygen in position 2 and 5 improves the activity of similar rigidified difuranyl compounds as was shown by Monte *et al*. [58]. Unfortunately the data of the pharmacological evaluations and the radioligand binding assays of **pm6** are not yet available. However, our study suggests the predicted binding affinity of **pm6** ($K_i = 8.1$ nM) is higher than the experimental binding affinity of DOI ($K_i = 9.9$ nM).

For the rigid naphthofurans **pm1**–**pm4** only moderate activities ($K_i = 74$ nM through $K_i = 6300$ nM) are calculated. Surprisingly, **pm1** shows a lower affinity ($K_i = 1019$ nM) than **pm3** ($K_i = 319$ nM). Typically, a ligand bearing a primary amine functionality displays the highest receptor

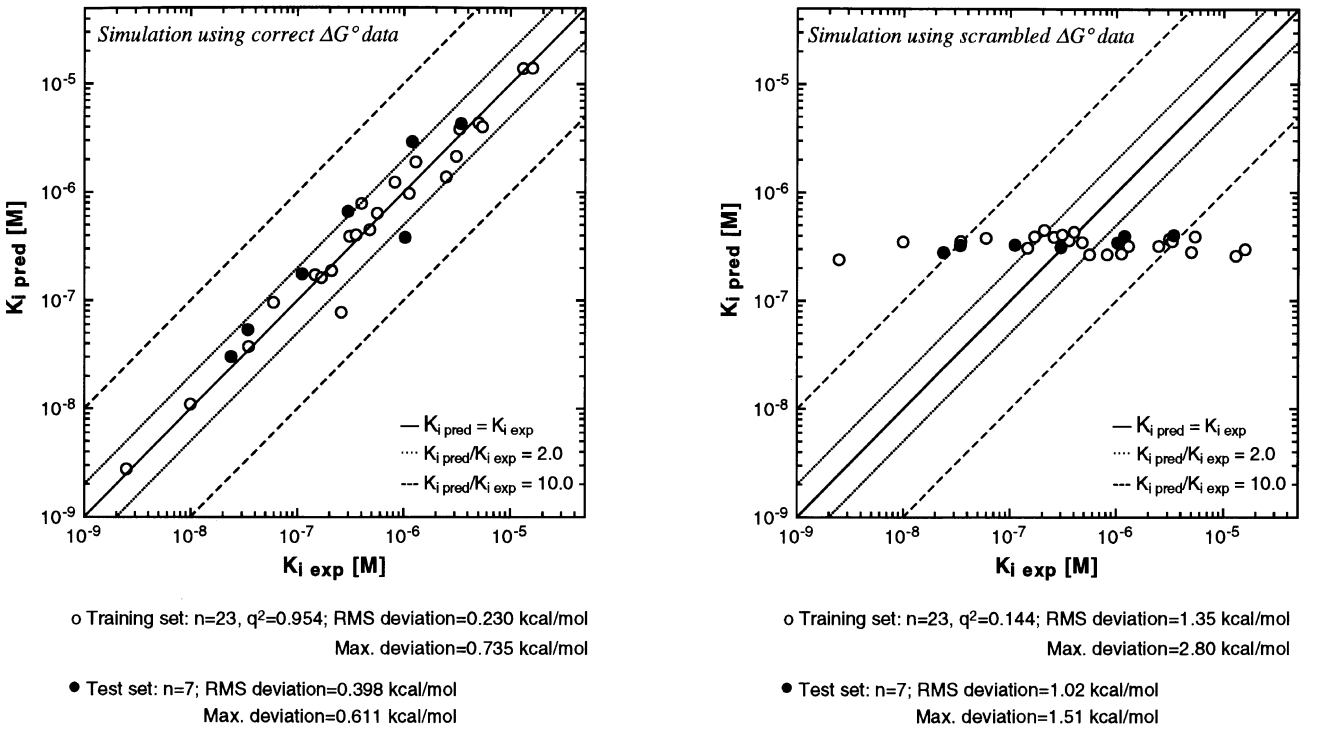

Figure 4. Graphical representation of experimental and predicted binding affinities for the 5-HT_{2A} agonists: **Left**: regular simulation; **right**: simulation using scrambled K_i-values for the training set. Open circles represent ligand molecules of the training set, filled circles those of the test set.

affinity [2, 4, 7]. On the other hand, **pm4** with its N-(propyl)$_2$ substituent is predicted to be the weakest of this series ($K_i = 6300$ nM) which is in agreement to previous SAR-studies. These results are also in accordance with studies by Monte *et al*. [59] who synthesized and tested rigid naphthofurans similar to **pm1–pm4** and concluded that such compounds do not feature an active conformation, because of the loss of LSD-like behavioural effects.

Within the new phenylalkylamine and tryptamine derivatives—apart from compound **pn5** (cf. below)—only two compounds were synthesized and tested: **pn25** and **pn27** [60]. Compound **pn25** showed a relatively low activity in animal models which is in accordance with the moderate binding affinity of $K_i = 340$ nM which is predicted by our study. The insertion of a bromo substituent leads according to SAR studies to an enhancement of the activity. In correspondence to this, the bromo derivative **pn25** showed an enhanced activity in animal models and is also predicted in our model with an higher binding affinity of $K_i = 22$ nM. These results suggest that our quasi-atomistic surrogate for the 5-HT_{2A} receptor might be useful for the estimation of the binding affinity of hypothetical compounds and synthesis planning.

Within the candidate series **pn1–pn16** compound **pn5** is the only molecule for which results from a radioligand binding study are available [61]. This substance seems to surpass DOB in potency which would not seem to be in agreement with our predicted binding affinity of $K_i = 101$ nM (DOB: $K_i = 24$ nM), because for halide substituents in position 4 a decrease in binding affinity I > Br > F is observed. This is in accordance with findings of Shulgin *et al*. [62] who examined a iodine substituent, the heaviest, was the most potent hallucinogen in this series. For a CF_3-group no increase of the binding affinity is generally calculated in our study (cf. **pm9** > **pm8** > **pm16**). But this might be a consequence of the fact that i.e. no fluoro substituent was implemented in the training set.

According to our study, the highest binding affinity of $K_i = 3.2$ nM was calculated for **ko3**. Assuming a maximal error of a factor of 10 in the prediction (cf. maximum error of the internal test set), this compound might be close in activity to **LSD** ($K_i = 2.5$ nM). This molecule represents a hybrid structure between LSD and a phenylalkylamine derivative such as DOI. It was developed expecting that the amide-oxygen functionality provides additional chances for interactions with, for instance, a receptor's Asn. Also, the diethylsubstituent at the amide-N is expected to show additional interactions with a hydrophobic region of the receptor, e.g. Ile, Phe and Trp.

In summary, two compounds (**ko3, ko2**) have calculated activities of $K_i \leq 5$ nM; additional three compounds (**pm9,**

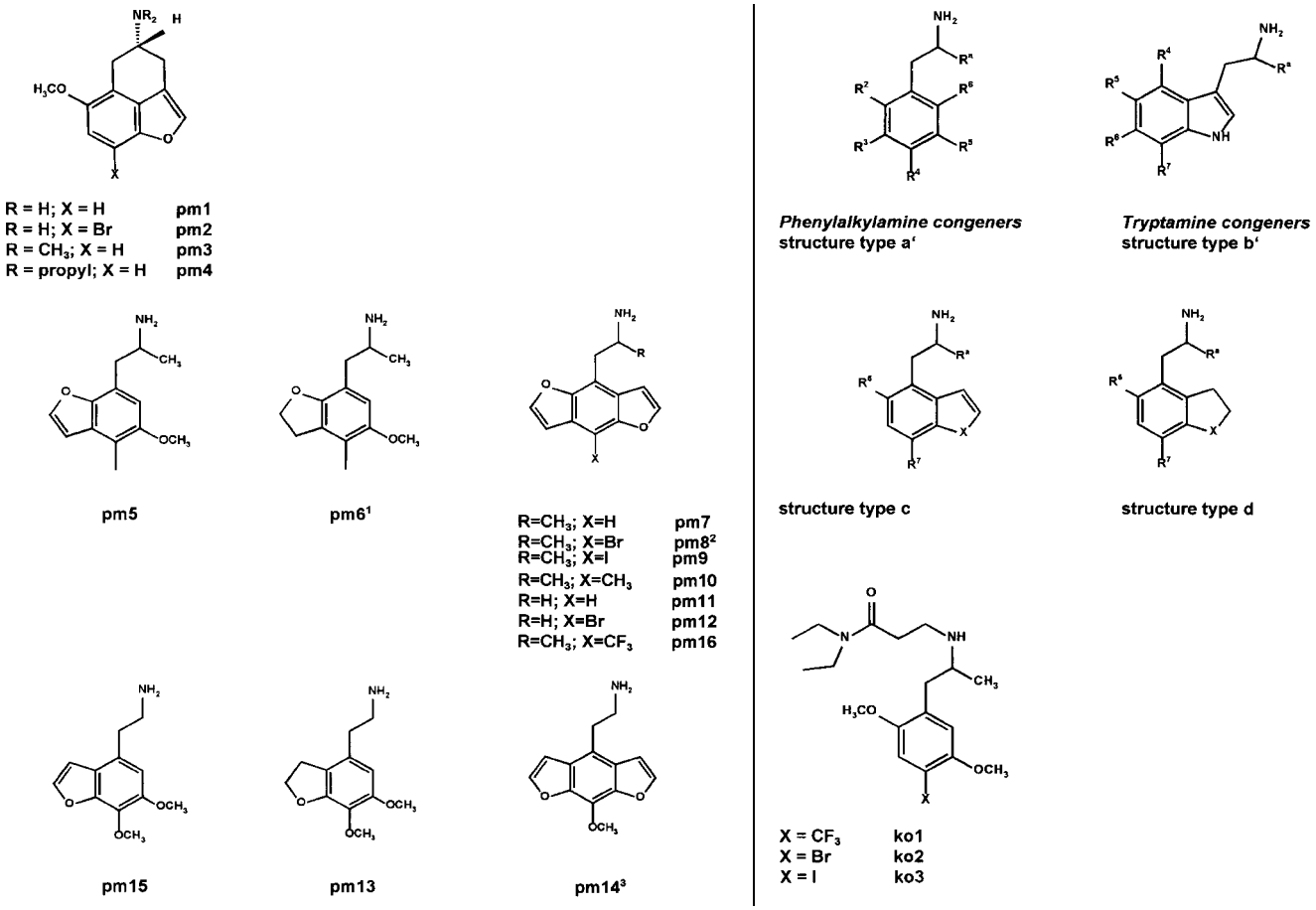

Figure 5. Molecular structures of the new 5-HT_{2A} congeneric ligands. Cf. also Table 3.

pm6, pm5) lie in the range of $5\,nM \leq K_i \leq 10\,nM$. Only for **pm8** which is predicted with a somewhat lower binding affinity of $K_i = 19\,nM$ *in vitro* and *in vivo* data of the racemate exist until now. It is the most potent ligand for the 5-HT_{2A} receptor known at the moment.

Of course, a high binding affinity is a mandatory but not sufficient criterion for hallucinogenic activity—evident for the *S*-enantiomer of DOI ($K_i = 35\,nM$), a non-hallucinogenically active substance. But as we considered the more active *R*-enantiomer for the phenylalkylamine congeners and the corresponding *S*-enantiomer for the tryptamine congeners, and as furthermore all of our new substances are lipophilic enough due to several ring systems and in some cases the α-CH_3 group to pass the blood-brain barrier one can expect that substances which show a high predicted binding affinity in our model, thus also act as hallucinogens in human.

4 Conclusions

Using *quasi-atomistic receptor-modeling* (software *Quasar*), a 3D-QSAR has been established for a series of 30 phenylalkylamine, indolealkylamine and ergoline derivatives, all known to bind to the 5-HT_{2A} receptor and most of them displaying hallucinogenic activity in man and/or behaviorally activity in animals. The *Quasar* approach allows for the simulation of local induced fit, H-bond flip-flop, and solvation phenomena. The QSARs are established based on a family of receptor-surface models, generated by a genetic algorithm combined with cross-validation. The receptor surrogate yielded a cross-validated q^2 of 0.954 for the 23 compounds defining the training set and a RMS deviation of $\Delta G^0_{prd.}$ and $\Delta G^0_{exp.}$ of 0.40 kcal/mol for the seven test compounds used to validate the model, corresponding to an uncertainty of a factor 1.9 in the binding affinity. The sensitivity of the model with respect to the biological data was demonstrated by means of a scramble test with negative outcome ($q^2 = 0.144$; slope $= -0.019$).

The surrogate was then used to estimate the activity of a series of 53 hypothetical congeneric compounds, believed to act as hallucinogens due to their topological similarity to known active substances and their putative ability to traverse the blood-brain barrier.

The most promising candidate compound is a molecule which represents a hybrid structure between LSD and phe-

Table 4. Predicted binding affinities of new compounds, index by substance classes.

substance class	Ligand	$K_{i\ pred.} \pm S_{Ki}$ [nM]
Rigid naphthofurans	pm2	74 ± 23
	pm3	320 ± 88
	pm1	1000 ± 290
	pm4	6300 ± 2600
Phenylalkylamine congeners		
	ko3	3.2 ± 1.8
	ko2	4.6 ± 3.0
	pm9	6.6 ± 1.7
	pm6*	8.1 ± 1.8
	pm5	9.5 ± 2.3
	pn1	14 ± 3.8
	pn14	16 ± 5.8
	pn15	17 ± 7.4
	pm8*	19 ± 3.9
	ko1	22 ± 14
	pn8	24 ± 4.7
	pm12	26 ± 6.9
	pn2	27 ± 6.7
	pn12	37.5 ± 13
	pn10	38.2 ± 12
	pn21	41 ± 7.4
	pm15	49 ± 14
	pn6	50 ± 14
	pm10	56 ± 11
	pm16	68 ± 16
	pn18	70 ± 18
	pn9	73 ± 17
	pn13	75 ± 22
	pn16	93 ± 32
	pn4	99 ± 21
	pn5*	101 ± 18
	pn19	105 ± 24
	pm13	130 ± 35
	pn17	170 ± 42
	pm14*	190 ± 48
	pn3	230 ± 52
	pn20	270 ± 69
	pm7	330 ± 74
	pn11	360 ± 95
	pn7	420 ± 110
	pm11	470 ± 120
Tryptamine congeners	pn25*	22 ± 3.5
	pn23	25 ± 8.5
	pn26	25.6 ± 5.8
	pn24	26.0 ± 3.8
	pn33	120 ± 45
	pn22	130 ± 48
	pn27*	340 ± 82
	pn29	530 ± 150
	pn31	700 ± 270
	pn30	750 ± 200
	pn32	760 ± 250
	pn28	3900 ± 1500

* Cf. footnotes of Figure 5 and Table 3

nylalkylamines such as 2,5-dimethoxy-4-iodoamphetamine (DOI). The binding affinity of this compound towards the 5-HT_{2A} receptor is predicted to be $K_i = 3.2$ nM, close to the experimental binding affinity of LSD ($K_i = 2.5$ nM). Some of these compounds have been synthesized in the meantime, allowing for a critical evaluation of our model.

5 Supplementary Material

The three-dimensional coordinates of all ligand molecules used in this study and the quasi-atomistic receptor surrogate for the 5-HT_{2A} receptor are freely available for distribution through "karl-artŭr.kovar@uni-tuebingen.de". Further information about recent developments of the *Quasar* software can be found at "www.biograf.ch".

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