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Pharmacophore design and database searching for selective monoamine neurotransmitter transporter ligands

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Abstract

Neuronal monoamine transporters (MATs) are involved in the pathophysiology and treatment of mental health conditions such as depression, attention deficit hyperactivity disorder, substance abuse and neurodegenerative disorders including Alzheimer's disease and Parkinson's disease. Various structural classes of compounds have been synthesized and tested *in vitro* for activity against transporters of three monoamine signaling molecules: noradrenaline (NET); serotonin (SERT) and dopamine (DAT). We have developed and validated a number of pharmacophore models describing the interaction of two classes of compounds with each of these three MATs. These pharmacophores explain the selectivity of binding to the MATs for various compound classes and have been used to search *in silico* databases for novel, potentially selective ligands. These ligands, after confirmation of their activities, will provide tools for investigating the function of MATs as well as the potential for new therapeutic agents in mental health applications. The database searches also retrieved close analogues of known MAT ligands, further validating the approach.

Keywords: Noradrenaline transporter; Dopamine transporter; Serotonin transporter; Pharmacophores; Database searching

1. Introduction

The three closely related monoamine neurotransmitters dopamine, serotonin and noradrenaline mediate signal transduction between neurons *via* interaction with specific receptors. Attenuation of the signal is caused by the reuptake of neurotransmitters by specific transporter proteins. The balance of interaction between neurotransmitter, receptor and transporter is important for a number of disease states including depression, attention deficit hyperactivity disorder, drug dependence and neurodegenerative conditions such as Alzheimer's and Parkinson's diseases. Therefore, compounds which selectively modulate the effect of the monoamine transporter proteins (MATs) are important therapeutic and research tools.

While there are a number of approaches used for investigating these interactions, the focus of this paper is the development of computer-generated pharmacophores for ligands which interact with dopamine transporter (DAT), serotonin transporter (SERT) and noradrenaline transporter (NET). Computational approaches to investigating the ligand-transporter interaction can be either transporter-based or ligand-based. As there are no crystal structures for MATs and very few crystal structures available for similar transporters generally [1,2] and these are of low resolution (>3 Å), accurate structural data is hard to come by for most transporters. Homology models of the three MATs based on the distantly related crystal structures have been published [3,4], however the low level of relationship has rendered transporter-based investigation difficult to date.

The ligand-based approach as used thus far can be divided into 3D-QSAR modeling and pharmacophore modeling. The 3D-QSAR CoMFA technique has been used successfully for DAT and SERT [5–8], in particular with good correlation data for the DAT. However this technique is limited to structurally related compounds. The pharmacophore approach has previously been used in particular for the DAT, with excellent outcomes from *in silico* database screening [9–11]. Three distinct structural classes of DAT modulators were discovered using this technique. These pharmacophores previously used for searching consisted of atomic constituents rather than chemical features, which may result in an even greater diversity

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of retrieved hits. A pharmacophore based on derivatives of 1-aryl-3-[4-arylpiperazin-1-yl]-1-propane for interaction at the SERT has been published [12]. However, no similar attempts have been made for the NET, or to distinguish differences in binding between the three MATs.

Most of the synthetic chemistry and drug development involving MATs has been centred on DAT and SERT with a large number of selective modulators being tested, especially with the possibility of therapeutic benefit in treatment of depression [13,14]. There are a wide variety of structures that have been tested, including rigid tropane-based cocaine analogues [6,15,16], mazindol analogues [5] and lobeline analogues [17], as well as more flexible structures such as GBR-12909 piperidine derivatives [18]. Much less effort has been spent on the NET with selective modulators mainly restricted to the rigid tropane and tricyclic decane derivatives, with a few exceptions. A series of papers describes the synthesis of GBR compounds with high affinities for all three MATs [19–23] and is of particular interest to this study.

In this work we have constructed two sets of pharmacophores for each of the MATs, the first set based on GBR-12909 derivatives and the second set based on tropane derivatives. We chose to use single training sets that incorporated ligands selective for each of the MATs, rather than individual training sets for each MAT. Individual training sets would mean six separate training sets and we believe there is sufficient data available to be incorporated into just two training sets. This also means that variability of data between different laboratories' assays as well as too much structural diversity was avoided. We constructed the two training sets based on two base structures, GBR-12909 derivatives and tropane derivatives. This approach was used, rather than covering all known MAT modulator structural classes, in order to improve the statistical validity of the pharmacophores. Database searching with the resultant pharmacophores then allows for structural diversity to be covered. By using more than one hypothetical answer (interaction pattern) for each MAT, a stepwise protocol can be used for *in silico* database searching.

2. Methods

2.1. General methodology

Pharmacophore analysis was conducted with the Catalyst® program, version 4.11 (Accelrys Inc., San Diego, CA, USA), run on a Silicon Graphics O2 workstation. Database searching was performed on the NCI2000 chemical database provided with the installation of Catalyst.

2.2. Training set selection

Training sets were compiled from published data describing the inhibition of MATs. K_i measurements were taken from the literature for the interactions with each individual MAT. K_i measurements were determined *via* the displacement of a radioactively labeled ligand for each MAT: GBR-12935 or WIN 35428 for DAT, paroxetine or citalopram for SERT and nisoxetine for NET. From the large amount of data available, a sample representative of a spread of inhibition values and structural variety was selected. Where possible the spread of inhibition values exceeded 3.5 orders of magnitude, as recommended to ensure a subtractive phase during HypoGen [24], for each MAT. This is because Catalyst defines the compound with the lowest K_i value (most active) as being the most important and penalizes against those compounds whose K_i value is more than 3.5 orders of magnitude higher than the most active compound. For NET and SERT hypothesis generation using GBR-12909 derivatives the subtractive phase was modified to consider all compounds with activities more than 2.5 orders of magnitude below the top compound. For DAT hypothesis generation using GBR-12909 derivatives, the two compounds with the lowest affinities had their value modified to artificially create a subtractive phase, as the activity spread was less than 2.5. Structural variety was ensured so that there was as little redundancy within the training set as possible. SinOne training set was constructed for the tropane-like compounds and one for the GBR-12909 derivatives, both with 32 compounds.

Conformers were generated for each compound in Catalyst using a 20 kcal/mol energy range, as recommended [25], using the ''Best'' search option. For compounds with unknown stereochemistry, conformers for both enantiomers were generated. Where stereochemistry was known, conformers were only generated for the enantiomer responsible for the highest activity. Catalyst uses the poling algorithm to sample the conformational space effectively [26–28]. For each compound the number of conformers was less than the maximum number of 255, indicating that the conformational space had been effectively sampled within the energy range.

2.3. Hypothesis generation

Pharmacophore hypotheses were generated with the HypoGen or HypoRefine algorithms within Catalyst. The HypoGen algorithm includes three phases: (1) ''Constructive Phase'', where hypotheses are generated; (2) ''Subtractive Phase'', where inactive compounds are penalized against and (3) ''Optimization Phase'', where simulated annealing is used to improve the fits of the hypotheses [24]. The HypoRefine algorithm allows the addition of excluded volume features to penalize against steric interactions causing a reduction in activity [25]. Hypotheses were generated with the possibility of two feature combinations: (1) H-bond acceptor (HBA), H-bond donor (HBD), hydrophobic (aromatic) (Har), hydrophobic (aliphatic) (Hal) and positive charge/ionisable (PC/PI) or (2) HBA, HBD, ring aromatic (RA), hydrophobic (HY) and PC/PI. These combinations of features give a good coverage of the potential interactions of the training set as well as incorporating expected interactions such as with the basic nitrogen. The features HBA, HBD and RA are vectored features, in that there are two spheres per feature, representing both sides of the interaction and the direction between them. For each feature combination the possibility of excluded volumes was explored, as were variable spatial tolerances and variable weights of