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Quantification of the interrelationships of receptor pharmacologies within a tricyclic privileged structural scaffold through application of modified forward selection☆

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ABSTRACT

Many neuropsychiatric drugs interact with more than one molecular target, and therapeutic indices might be improved by prospectively designing compounds with profiles optimized against a combination of targets. The dibenzo-epine scaffold is considered a privileged structure, and this scaffold has been explored rigorously in the search for potential novel neuropharmacologic treatments. Members of this chemical class are known to interact with many receptors and transporters, particularly those of the biogenic amine class. In this study, four points of diversity within a dibenzo-epine scaffold were varied systematically and the pharmacologic profiles of the compounds were assessed across 14 receptors and transporters thought to be important to clinical profiles of efficacy and safety. The resulting data were analyzed using a modified forward selection linear regression procedure, thus revealing potential pharmacophoric relationships of the assessed targets within this chemical class. The results highlight a strong covariance across numerous targets. Moreover, the outcome quantifies the innately problematic issue of prospectively designing compounds with defined affinities across multiple targets. Finally, an exploration of the correspondence of binding affinities to *in vitro* functional activity reveals an additional layer of complexity central to prospectively designing compounds to engage multiple targets. The apparent relatedness of the 5-HT_{2a} and D_2 activities suggests that the structural pharmacophores of these receptors overlap more closely with each other than with members of their respective families.

1. Introduction

The initial discoveries of imipramine and clozapine, the foundational neuropsychiatric drugs with tricyclic structures, spawned decades of research aimed at designing novel therapies for psychiatric disorders (Hippius, 1989). Consequently, dibenzo-epine scaffolds were rigorously explored in search of novel neuropharmacologic drugs. Indeed, many new chemotherapeutics were brought to the marketplace based on the dibenzo-epine scaffold and hence, it is considered to be a privileged structure. The advent of receptor pharmacology revealed that members of this privileged structure can interact with a wide variety of targets, and that they can inhibit biogenic amine receptors and transporters, in particular (Coward, 1992). Recently, there has been renewed appreciation for neuropsychiatric agents that engage multiple mechanisms. In fact, it has been hypothesized that complex neuropsychiatric disorders may only be optimally treated by drugs that engage multiple nodes within networked systems (Roth et al., 2004).

A fundamental challenge in designing individual small molecules that selectively engage multiple targets in a network is the concurrent need to avoid engaging undesired targets. The single agent, multi-target approach presupposes that the pharmacophoric requirements of the desired targets are distinct enough from those of undesired targets that adequate separation of the corresponding affinities can be achieved (Xie et al., 2012). The vast and varied clinical success realized with members of the dibenzo-epine scaffold implies that this class of compounds is well suited to exploring the pharmacophoric interdependencies of a subset of key receptors and transporters. A collection of twenty-four compounds from this class, systematically varied across four points of differentiation, were evaluated at fourteen receptors and transporters. The set

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contained five approved neuropsychiatric drugs and several active drug metabolites. The attendant pharmacophoric relationships of the test set were evaluated using a straightforward statistical method. The results quantify the covariate nature of the receptor affinities within the compound subset, producing models that explain considerable variance and reveal unexpected relationships.

2. Materials and methods

2.1. Compounds

Compounds 1 (CAS#5747-48-8), 2 (1977-09-9), 3 (5747-63-7), 4 (2058-52-8), 5 (858670-47-0), 6 (5747-55-7), 7 (21636-40-8), 8 (2058-53-9), 9 (14028-44-5), 10 (27833-64-3), 11 (179418-95-2), 12 (3527-47-7), 13 (56296-18-5), 14 (1977-07-7), 15 (858669-84-8), 16 (1977-08-8), 17 (6104-71-8), 18 (5786-21-0), 19 (5001-00-3), 20 (1977-11-3), 21 (138246-83-0), 22 (1977-12-4), 23 (858670-48-1), and 24 (5542-88-1) were synthesized at AstraZeneca Pharmaceuticals, LP (Wilmington, DE) or Adesis, Inc. (New Castle, DE). All chemical structures were verified, and each has a purity of >95%.

2.2. Radioligand binding

Radioligand binding was performed on membranes prepared from stably transfected cells expressing human recombinant receptors or transporters. Radioligand binding at dopamine (catalog #220320, DAT), norepineprine (#204410, NET), and serotonin (#274030, SET) transporters was evaluated according to standard validated protocols under conditions defined by the contractor (Ricerca Biosciences, Concord, OH, USA; http://www.ricerca.com). Compounds were evaluated in duplicate across eight, half-log concentrations (0.3–300 μM). Radioligand binding at adrenergic α_{1a} (catalog #FAST-005B), serotonin 5-HT_{1a} (#FAST-0500B), serotonin 5-HT_{2a} (#FAST-0505B), serotonin 5-HT_{2b} (#FAST-0506B), serotonin 5-HT_{2c} (#FAST-507B), dopamine D_1 (#FAST-0100B), dopamine D_2 (#FAST-0101B), dopamine D_3 (#ES-0173B), histamine H_1 (#FAST-0170B), muscarinic M_1 (#FAST-0260B) and M_3 (#ES-212B) receptors was evaluated according to standard validated protocols under conditions defined by the contractor (Euroscreen, Gosselies, Belgium; http://www.euroscreen.com). Compounds were first evaluated at 0.1 nM, 10 nM, and 1 μM to establish approximate IC_{50} values. Compounds were then evaluated in duplicate across ten concentrations bracketing the approximate IC_{50}. Reference standards were run as an integral part of all assays to verify results.

2.3. In vitro functional assessment

In vitro functional assessment was performed on preparations of stably transfected cells expressing human recombinant receptors or transporters. Uptake inhibition at dopamine (catalog #316000), norepineprine (#302000), and serotonin (#364000) transporters was evaluated according to standard validated protocols under conditions defined by the contractor (Ricerca Biosciences, Concord, OH, USA; http://www.ricerca.com). Compounds were evaluated in duplicate across five concentrations (3, 30, & 300 nM, and 3 & 30 μM). Compounds were tested at adrenergic α_{1a} (catalog #FAST-005A), serotonin 5-HT_{1a} (#FAST-0500A), serotonin 5-HT_{2a} (#FAST-0505A), serotonin 5-HT_{2b} (#FAST-0506A), serotonin 5-HT_{2c} (#FAST-507A), dopamine D_2 (#FAST-0101A), histamine H_1 (#FAST-0170A), muscarinic M_1 (#FAST-0260A) and M_3 (#ES-212A) receptors in an aequorin assay format and at dopamine D_3 (ES-0173G) in a GTPγS assay format according to standard validated protocols under conditions defined by the contractor (Euroscreen, Gosselies, Belgium; http://www.euroscreen.com). Compounds were first evaluated in duplicate at 0.1 nM, 10 nM, and 1 μM for agonist activity and 0.05 nM, 5 nM, and 500 nM for antagonist activity to establish approximate EC_{50} or IC_{50} values. Compounds were tested at dopamine D_1 (#FAST-0100C) in a cAMP format. Compounds were first evaluated in duplicate at 0.1 nM, 10 nM, and 1 μM for agonist activity and antagonist activity to establish approximate EC_{50} or IC_{50} values. Compounds were then evaluated in duplicate across ten concentrations bracketing the approximate IC_{50}. Measurable agonist activity was detected in very few instances. Compounds 1, 2, 3, 5, 7, 9, 11, 17, & 18 were also evaluated for agonist activity at 5-HT_{1a} in a GTPγS format, and compounds 1, 2, 5, 7, 11, 17, & 18 were also evaluated for agonist activity at M_1 in a FLIPR assay format (n = 2 to 17 for each value, see supplemental methods). Reference standards were run as an integral part of all assays to verify results.

3. Calculation

3.1. For radioligand binding

For radioligand binding, all data were normalized to the control responses and non-linear regression curves fitted to them using logistic fits in GraphPad Prism 4.0 software (LaJolla, CA). The best curve fit of three parameter (i.e., nH = 1) or four parameter models (i.e., variable nH) was determined by comparison using the *F*-test. For fits that did not converge, a two parameter model was attempted (i.e., bottom constrained to 0); 13 of 336 (or 3.8%) radioligand binding pK_i values were determined with two parameter fits. Fits with pK_i SEMs in excess 0.5 were rejected. The upper limit value (i.e., pK_i < 5.0) is reported for data not meeting the described criteria (4 of 336). An estimated pK_i of 3.5 was used as a value for the four unfitted sets (i.e., compound 13 at D_1, D_2, M_1, & M_3) in subsequent correlation analyses.

3.2. For functional inhibition data

For functional inhibition data (i.e., antagonism), the best curve fit of three parameter (i.e., nH = 1) or four parameter models (i.e., variable nH) was determined by comparison using the *F*-test. For fits that did not converge, fits with pIC_{50} SEMs in excess of 0.5, or fits with less than 80% inhibition, the maximal inhibition at a specified concentration is reported. Only calculated pIC_{50}s were used in correlation analysis. For functional activation data (i.e., agonism), a three parameter fit was attempted. For fits that did not converge, fits with pEC_{50} SEMs greater than 0.5, maximal activation at a specified concentration is reported. Upper limit pEC_{50}s are reported for M_1 FLIPR results.

3.3. Forward selection analyses

Forward selection analyses were developed using SigmaPlot for Windows v11 (Systat Software, Inc., San Jose, CA). A global ANOVA was performed with all sets of receptor pK_is to establish statistical validity of subsequent multiple comparisons. Next, each dependent variable (i.e., pK_i at Receptor Y) was regressed across all independent variables (i.e., pK_is at Receptors X_1, X_2, … X_k, X_j,… X_n). Following univariate analysis, a modified Forward Selection procedure similar to that described by Blanchet et al., was then used to build models of explained variance (Blanchet et al., 2008). The independent variable with the highest significant correlation in univariate regression (e.g., Receptor Y vs. Receptor X_k) was assigned as the primary variable. The data were regressed again using the identified independent variable (i.e., Receptor X_k) as the primary variable and all remaining variables as secondary independent variables (i.e., pK_is at Receptors X_1, X_2, … X_j, X_l, … X_n). The second independent variable with highest significant correlation (e.g., Receptor Y vs. Receptor X_k and Receptor X_j) was identified and the procedure was repeated until no additional significant model improvement was realized. As described by Blanchet et al., stopping criteria for model improvement includes both alpha significance and an improvement in the adjusted coefficient for multiple determination (R^2_{adj}). Each final model was then evaluated using leave-one-out cross-validation (LOOCV), a cross-validation method reported to exhibit minimal bias with small data sets (Molinaro et al., 2005). Mean-squared errors (MSE) were all below 0.01. All *p*-values were below 0.005 with the singular exception of the H_1 model missing compound 13 ($p > 0.3$), implying that the H_1 model may not be robust.

4. Results

The collection of 24 compounds contains five registered drugs, including three antipsychotics [i.e., clothiapine (4), clozapine (18), and loxapine (10)], an antidepressant (amoxapine, 9), and a hypnotic (perlapine, 20). The subset also contains three known drug metabolites [i.e., norclozapine (17), norperlapine (19), and norquetiapine (1)]. All of the compounds have been described in patent