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Discovery of benzamide analogs as negative allosteric modulators of human neuronal nicotinic receptors: Pharmacophore modeling and structure–activity relationship studies

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ABSTRACT

The present study describes our ongoing efforts toward the discovery of drugs that selectively target nAChR subtypes. We exploited knowledge on nAChR ligands and their binding site that were previously identified by our laboratory through virtual screenings and identified benzamide analogs as a novel chemical class of neuronal nicotinic receptor (nAChR) ligands. The lead molecule, compound **1** (4-(allyloxy)-*N*-(6-methylpyridin-2-yl)benzamide) inhibits nAChR activity with an IC_{50} value of 6.0 (3.4–10.6) μM on human α4β2 nAChRs with a ~5-fold preference against human α3β4 nAChRs. Twenty-six analogs of compound **1** were also either synthesized or purchased for structure–activity relationship (SAR) studies and provided information relating the chemical/structural properties of the molecules to their ability to inhibit nAChR activity. The discovery of subtype-selective ligands of nAChRs described here should contribute significantly to our understanding of the involvement of specific nAChR subtypes in normal and pathophysiological states.

1. Introduction

Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels that mediate the physiological effects elicited by the endogenous neurotransmitter, acetylcholine (ACh). nAChR signaling influences and regulates a number of physiologically important processes (e.g., cognition, arousal, anxiety, pain processing, food intake, and reward).[1,2] Furthermore, disruption or alteration of nAChR activity has been implicated in many diseases and disorders (Alzheimer's disease, Parkinson's disease, depression, schizophrenia, and addiction).[3] Therefore, nAChRs hold considerable promise as therapeutic targets.

A major challenge in the study of the nAChRs is the diversity and complexity of these pentameric receptors. There are eleven human genes that encode nAChR subunits (α2–α10 excluding α8 and β2–β4). These subunits assemble to form multiple subtypes that are characterized by distinct biochemical properties (e.g., ion selectivity, conductance, mean channel open time, permeability to Ca^{2+}, and desensitization rate).[4–6] Importantly, each subunit is expressed in unique spatial and developmental patterns, suggesting the possibility that individual subtypes may play distinct roles in certain physiological processes.[6,7] Knowledge of these roles will pave the way toward the identification of novel therapeutic targets and allow the development of more targeted pharmacotherapy with reduced side effects. Therefore, drugs that specifically target nAChR subtypes can represent valuable research tools by providing insight into the roles that different nAChR subtypes play in physiological and pathological states.

Orthosteric binding sites of nAChRs (sites of ACh binding) contain a high level of sequence homology. At the interface between α and β subunits, ACh interacts with the 'aromatic nest', which is a group of five aromatic amino acid residues (i.e., Trp56, Trp61, Trp87, Trp150, and Tyr199). (The numbers used in this manuscript are based on the first transcribed protein). These residues are completely conserved in all nAChR subunits.[18] In addition, amino acid residues surrounding the orthosteric site (Cys 193, Cys 194, and Tyr 94) are also completely conserved or highly conserved (75–100% amino acid identity across nAChR subunits). We believe

Abbreviations: NAM, negative allosteric modulator; nAChRs, neuronal nicotinic acetylcholine receptors; HBK, HEPES-buffered Krebs; LBVS, ligand-based virtual screening; SBVS, structure-based virtual screening; SAR, structure–activity relationship; n_h, Hill coefficient.

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that this extensive level of sequence homology makes it difficult to discover and develop drugs that interact with the orthosteric binding site and pharmacologically discriminate one subtype from others. Our laboratory is addressing the issue of subtype-selectivity by targeting 'non-orthosteric' sites of nAChRs previously identified by our laboratory (e.g., allosteric sites).[16] Our hypotheses are that (1) structural variations within allosteric binding sites can provide a foundation to develop subtype-selective ligands of nAChRs and (2) a multidisciplinary approach can direct the design and synthesis of new molecules with optimized properties (i.e., selectivity and potency). The present study is a proof-of-concept study to document the utilization of allosteric binding sites to develop drugs with specificity for nAChR subtypes using a combination of multiple approaches. In particular, we focused on the subtype-selectivity for human α4β2 (hα4β2) nAChRs over human α3β4 (hα3β4) nAChRs. As one of the main subtypes expressed in the central nervous system (CNS), hα4β2 nAChRs have been implicated in various brain diseases and disorders (e.g., nicotine addiction, anxiety, and depression).[8–12] On the other hand, hα3β4 nAChR is largely expressed in the peripheral autonomic ganglia where it modulates the release of multiple neurotransmitters. Due to the roles of hα3β4 nAChR in the autonomic ganglia, activity of nicotinic drugs on this subtype has been postulated to mediate a wide range of autonomic side effects, as exemplified by those reported from usage of the classical autonomic blocker, mecamylamine (e.g., constipation, urinary retention, dilation of the pupils, and postural hypotension).[1–3] Therefore, with interest in developing safer drugs for CNS application, subtype-selectivity was pursued for the hα4β2 nAChRs over the hα3β4 nAChRs.

In this study, we report the discovery of a novel chemical class of nAChR antagonists that allosterically modulate receptor activity using multiple approaches. As a rational drug design strategy, we utilized knowledge gained from our previous studies[13–18] by incorporating ligand-based modeling, structure-based modeling, pharmacology, and medicinal chemistry. Previously, our laboratory has identified several classes of novel negative allosteric modulators (NAMs) of nAChRs.[13–16] Effects of those NAMs were insurmountable with increasing concentrations of the orthosteric agonist, epibatidine, suggesting their non-competitive mechanisms of action. Importantly, some of these NAMs showed selectivity for hα4β2 nAChRs over hα3β4 nAChRs. Utilizing a combination of homology modeling, blind docking, and site-directed mutagenesis, an allosteric binding site where these NAMs bind was also identified.[16–18] This site is located approximately 10 Å from the orthosteric site at the interface between α and β subunits. Since major amino acid residues comprising this site are located at the β subunit, we named this site the 'β subunit site'. The 'β subunit site' is sequentially and structurally diverse among subtypes, which provides an explanation for the relative subtype-selectivity shown with some of the NAMs[17]: among residues positioned within the 'β subunit site', Thr58, Ser133, Ser138, Ser142, Phe118, and Ser137 showed reduced sequence conservation (less than 26% amino acid identity across nAChR subunits). Other amino acids (Glu60 and Ser97) that contribute to interactions between ligands and receptors occurring through the 'β subunit site' also share a limited degree of sequence conservation among subunits (amino acid identity of 63% and 39%, respectively).[17] The diversity within the 'β subunit site' and the identification of subtype-selective NAMs acting on this site provide support for our approach of targeting allosteric binding sites to develop selective nAChR antagonists. In this study, the physicochemical properties of NAMs that selectively target hα4β2 nAChRs as well as the biochemical characteristics of their binding sites (i.e., the 'β subunit site') mentioned above were exploited. Utilization of ligand-based and structure-based approaches using the previously obtained knowledge led to the discovery of a lead compound of this study (i.e., Compound **1**, (4-(allyloxy)-*N*-(6-methylpyridin-2-yl)benzamide) that acts as a NAM of nAChRs. Furthermore, 26 analogs of compound **1** were either synthesized or purchased to determine the structural features that are relevant for potency and relative selectivity toward nAChRs.

2. Chemistry

Target compounds **8**–**15** were obtained through reaction between commercially available 6-substituted-2-aminopyridines **6a**–**e** with 4-propoxybenzoyl chloride (**7a**) or 4-allyloxybenzoyl chloride (**7b**) as shown in Scheme 1. Benzoyl chlorides **7a**,**b** were readily prepared by heating 4-propoxybenzoic acid or 4-allyloxybenzoic acid to reflux in thionyl chloride.[19] 4-Allyloxybenzoic acid (**18**) was made through the reaction between 4-hydroxybenzoic acid (**16**) and allyl bromide. Since ester **17** was also formed as an undesired side product of the reaction above, **17** was converted to the desired acid **18** by treatment with NaOH in ethanol/water (Scheme 2).

Target compounds **19**–**30** were synthesized in high yield by reacting a variety of aliphatic and aromatic amines with bromopyridine **12** through Buchwald-Hartwig amination[20] (Scheme 3). In contrast, Buchwald–Hartwig reactions using chloropyridine **10** failed to give the desired products. Also, Buchwald–Hartwig coupling using compound **9** as a precursor in an attempt to produce compounds **34** and **35** was unsuccessful, likely due to the presence of the allyl group in **9**. An alternative route was thus required to synthesize target compounds **34** and **35**. 2-Amino-6-bromopyridine (**6b**) was first protected by acylation to provide **31**, followed by Buchwald-Hartwig amination with either propylamine or butylamine to yield protected alkylaminopyridines **32** and **33**, respectively. The acetyl group was removed by treatment with sodium hydroxide, followed by acylation of the resulting primary aromatic amines with **7b** to afford target compounds **34** and **35** (Scheme 4).

3. Results

3.1. Pharmacophore and ligand-based virtual screening

In order to identify novel chemical entities that exhibit subtype-selectivity, information obtained from our previous studies was utilized.[13–18] Four NAMs (i.e., KAB-18, DDR-5, DDR-13, and DDR-18) previously identified by our laboratory[16] were selected based on their preference for hα4β2 nAChRs against hα3β4 nAChRs and used here to generate an initial pharmacophore (Fig. 1). In parallel, structure-based virtual screening (SBVS) using the allosteric binding sites for these four NAMs (i.e., β subunit site) was performed and led to the identification of four novel scaffolds that inhibit the activity of nAChRs with relative selectivity for hα4β2 nAChRs.[14] Among top hits from this SBVS, Hit 2 ((5-amino-*N*-(6-methylpyridin-2-yl)-2-(piperidin-1-yl)benzamide) shares structural similarities with the four NAMs used to generate the initial pharmacophore (Fig. 1). In addition, Hit 2 has lower molecular weight by lacking the substitutions linked to the piperidine ring and thereby possesses potential to exhibit improved bioavailability (Fig. 1). As Hit 2 shows a preference for hα4β2 nAChRs, shares structural similarity with the four NAMs used for initial pharmacophore development, and possesses more desirable drug properties with regard to in vivo bioavailability, the initial pharmacophore was then refined here using Hit 2 from the SBVS. The refined pharmacophore reported here features three hydrophobic regions (HYD1, HYD2, and HYD3) and one hydrogen bond acceptor (HBA) (Fig. 2). This refined pharmacophore model was utilized for ligand-based virtual screening (LBVS) as described below.