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Exploring α7-Nicotinic Receptor Ligand Diversity by Scaffold Enumeration from the Chemical Universe Database GDB

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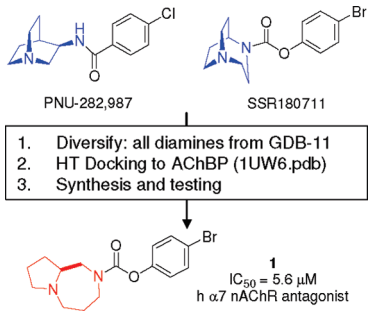
ABSTRACT Virtual analogues (1167860 compounds) of the nicotinic α7-receptor (α7 nAChR) ligands PNU-282,987 and SSR180711 were generated from the chemical universe database GDB-11 by extracting all aliphatic diamine analogues of the aminoquinuclidine and 1,4-diazabicyclo[3.2.2]nonane scaffolds of these ligands and converting them to the corresponding aryl amides using five different aromatic acyl groups. The library was ranked by docking to the nicotinic binding site of the acetylcholine binding protein (AChBP, 1UW6.pdb) using Autodock and Glide. Thirty-eight ligands derived from the best docking hits were synthesized and tested for modulation of the acetylcholine signal at the human α7 nAChR receptor expressed in *Xenopus* oocytes, leading to competitive and noncompetitive antagonists with IC_{50} = 5−7 μM. These experiments demonstrate the first example of using GDB in a fragment-based approach by diversifying the scaffold of known drugs.

KEYWORDS Virtual libraries, virtual screening, nicotinic receptor, docking, electrophysiology



The development of new drugs critically depends on new chemical entities.[1] At the level of small molecule drugs, discovering new structures is difficult because many small molecules are already in the public domain or under patent protection. This problem might be circumvented by de novo drug design approaches that consider the chemical space of yet unknown molecules as a source for innovation.[2,3] Recently, we reported the chemical universe database GDB enumerating all organic molecules up to a size of 11 and 13 atoms possible under simple chemical stability and synthetic feasibility rules.[4−6] GDB contains at least 1000-fold more compounds than other data sets that contain similarly sized molecules. New ligands for the NMDA receptor glycine site were readily identified in GDB-11 through a combination of ligand-based and structure-based virtual screening, synthesis, and testing.[7,8] However, with MW < 200 Da, GDB molecules lie in the size range of fragments[9] and cannot serve in a general sense as a source for drug molecules that are typically much larger (MW ∼ 340 Da, 20−30 nonhydrogen atoms).[10] Inspired by the idea of using known drugs as starting points for new drugs,[11] we herein report the first example of using GDB in a fragment-based approach by diversifying the diamine scaffold of the known nicotinic α7 acetylcholine receptor (α7 nAChR) ligands PNU-282,987 (**5**)[12] and SSR180711 (**6**),[13] leading to the identification of the new analogues **1**−**4** (Figure 1).

The α7 nAChR is an important drug target whose modulation may find application in treating neurological disorders and cancer.[14,15] Most α7 nAChR ligands described to date are amines or diamines, including natural products and synthetic alkaloid analogues.[16] These ligands bind either at the orthosteric binding site located at the interface between two α7-subunits[17] or directly to the channel entrance to block the ion current.[18] While these ligands were identified mostly by serendipity and activity screening, the report of the crystal structure of the *Lymnaea signalis* acetylcholine binding protein with bound nicotine (AChBP, 1UW6.pdb), which shares a high degree of structural homology with the nicotinic receptors, marked a turning point in our comprehension of the receptor. In particular, it was shown recently that docking to this structure could be used for structure-based discovery of new ligands.[17] Similarly, we chose docking to the nicotinic site in 1UW6.pdb as a virtual screening tool to guide the selection of new α7 nAChR ligands from a GDB-derived ligand library.

GDB was used to diversify the structure of PNU-282,987 (**5**) and SSR180711 (**6**), which are both competitive ligands to acetylcholine binding at the nicotinic site. These ligands and related analogues such as JN403[19] published in the course of this study feature a bicyclic diamine scaffold including a tertiary amine and a primary or secondary amine separated by a two-carbon spacer, the latter amine being acylated as an aromatic amide or carbamate. Diversification of the diamine scaffold up to 12 atoms was realized starting from GDB-11,

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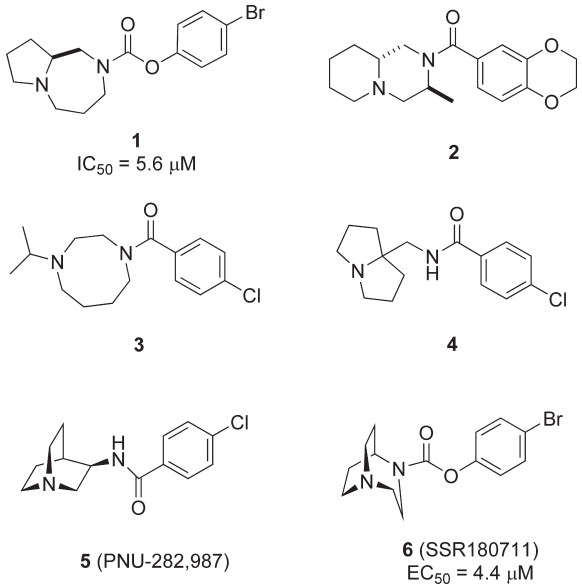

Figure 1. Structures of α-7AChR ligands.

which contains 26.4 million organic structures of up to 11 atoms of C, N, O, and F, as outlined in Figure 2. First, all diamines with heavy atom formula $C_{1-9}N_2$ were extracted, and the nonaziridine subset containing one tertiary aliphatic amine and one primary or secondary amine separated by a two-carbon spacer was retained, providing 72 740 structures. Second, all tertiary amines with heavy atom formula $C_{1-10}N$ were extracted (103 528 compounds), and diamines with the heavy atom formula $C_{1-10}N_2$ were generated by attaching either a primary amino group at all β-carbons relative to the tertiary amine (153 359 compounds) or by inserting a secondary amino group in all $\beta-\gamma$ carbon−carbon bonds relative to the tertiary amine (31138 compounds), again excluding aziridines. The combined set provided 233 572 unique diamines with variable numbers of cycles and rotatable bonds, covering a broad range of scaffold analogues (Table S1 in the Supporting Information). These scaffolds were then derivatized by attaching five different aromatic acyl groups (Figure 2A−E)[20] to the secondary or primary amino group to generate 1167 860 acylated virtual ligands.

Docking was performed using the programs Autodock 3.0.5[21] and Glide,[22] both correctly positioning nicotine in its crystallographic position. A random selection of 72 745 analogues of the library (6.2% of the 1167 860 acylated virtual ligands) was expanded to 507 030 stereoisomers using CORINA[23] (for Autodock, LigPrep was used for Glide), and these were then docked to the nicotinic binding site of 1UW6.pdb, resulting in typical Gaussian curve distribution of docking scores (Autodock: −12.9 to −4.6 kcal/mol estimated binding energy; glide: −9.1 to −1.5 GScore; see Figure S1A,B in the Supporting Information). Although the docking scores of the reference ligands **5** and **6** were only average, this ranking method was considered valid for selecting new ligands fitting into the nicotinic binding site but with significantly different structures. Indeed, shape-based selection of the same library relative to **5** or **6** using

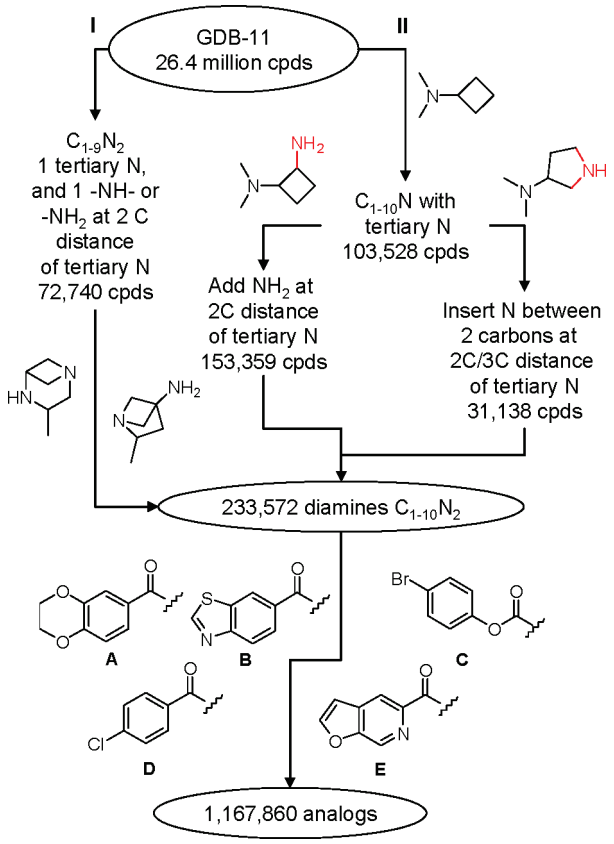

Figure 2. Assembly of the diamine scaffold library from GDB-11 via two different routes. Route I, direct extraction of diamines from GDB-11; route II, extraction of monoamines from GDB followed by addition or insertion of a primary or second amine at a distance of two carbon atoms. The newly formed bond and atom are indicated in red on the illustrated examples. The selected acyl groups are attached to the primary or secondary amino group to form the analogues of **5** and **6**. See Table S1 in the Supporting Information for details on database composition.

ROCS[24] primarily led to very close analogues of **5** and **6** already described in the patent literature.

Analysis of the docking results showed that the docking poses of high-scoring ligands were generally very similar to the poses obtained for the reference ligands **5** and **6**. The diamine portion of the ligands occupied the same space as the pyrrolidine ring of nicotine and the aromatic group partly overlaid with the pyridine ring of nicotine (Figure S1C in the Supporting Information). The 1000 top scoring compounds from each docking series were visually inspected to select structures for synthesis and testing. Easily accessible acyclic, monocyclic, or bicyclic diamines were chosen, focusing on compounds that were unknown or at least not previously described in the α7 nAChR literature. Five scaffolds were taken from the Autodock series, and four scaffolds were taken from the Glide series. The diamines were prepared by chemical synthesis, and each diamine was then acylated with 2−5 acyl groups A−E (Figure 2), providing a total of 38 ligands for testing.

The activity was measured by electrophysiology in oocytes expressing the recombinant human α7 nAChR.[25] Compound effects were assessed by first recording the current

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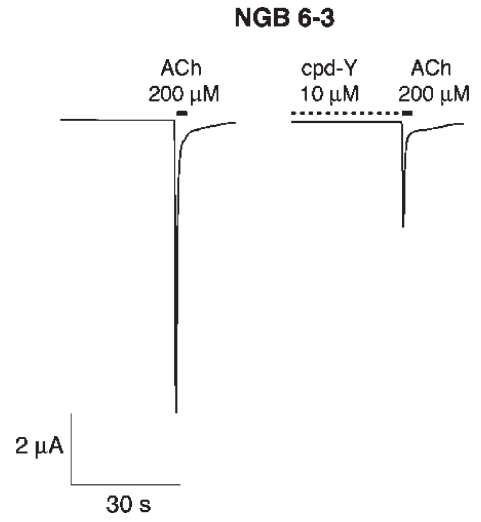

Figure 3. Typical ACh-evoked current recorded in an oocyte expressing the human α7 nAChR in control and following compound exposure. The dashes line indicates the timing of compound application. Note that exposure to this compound caused no detectable inward current and therefore failed to activate the receptors.

evoked by a brief ACh test pulse (200 μM, 5 s) and then by recording the response to the same ACh test pulse following a 30 s incubation in presence of the compound. Typical responses recorded with this protocol are illustrated in Figure 3.

To obtain an overview of the receptor sensitivity effects of the compounds, these were characterized at 1, 10, and 100 μM. All ligands behaved as antagonists of acetylcholine, evoking no current during exposure to the compound alone but causing a reduction of the subsequent ACh-evoked current at the three concentrations tested. Out of the 38 ligands tested, 13 showed weak activity (less than 30% inhibition at 10 μM), 19 showed moderate inhibition (30−70% inhibition at 10 μM), and 6 showed a strong inhibition of the Ach response (>70% at 10 μM). Compounds **1** and **2** derived from the Autodock scoring and **3** and **4** (Figure 1) derived from the Glide scoring, which although derived from known diamines[26−31] were unprecedented ligands, were characterized in more detail.

Ligands **1**−**4** inhibited the ACh-evoked current with IC_{50} values in the range 5−7 μM, which is comparable to the EC_{50} value of 4.4 μM reported for SSR180711 (**6**) under the same conditions (Figure 3 and Table 1).[13] While both **5** and **6** are partial agonists of the receptor and evoked current when applied in isolation, ligands **1**−**4** were all antagonists causing only inhibition of the receptor (Figure 4). The EC_{50} value of acetylcholine was shifted from 87 ± 10 to 169 ± 24 μM in the presence of 6 μM ligand **1** without affecting the maximum response, indicating a competitive inhibition by **1** at the acetylcholine binding site (Figure 5). Ligand **2** by contrast showed noncompetitive inhibition by lowering the maximum response of acetylcholine without affecting its EC_{50}, indicative of a noncompetitive inhibition (Figure S5 in the Supporting Information). A possible interpretation is that the molecule enters the ionic pore and may cause open channel blockade. Ligands **3** and **4** affected both the EC_{50} and the maximum response of acetylcholine, suggesting mixed binding at both the nicotinic side and

Table 1. Activity of Ligands **1**−**4** and Reference **6** on the Human α7 nAchR[a]

compound	IC_{50} or EC_{50} (μM)	activity type
1	5.6 ± 1.7	competitive antagonist to ACh
2	6.1 ± 1.5	noncompetitive antagonist
3	7.0 ± 1.1	mixed antagonist
4	7.2 ± 1.2	mixed antagonist
6	4.4[b]	partial agonist[b]

[a] Electrophyiological assay in *Xenopus* oocytes. See Figure 4 and the Supporting Information for details. [b] Data from ref 13 are indicated as EC_{50} = 4.4 μM (2.5−7.8 μM), which corresponds to a binding affinity of K_i = 14 nM.

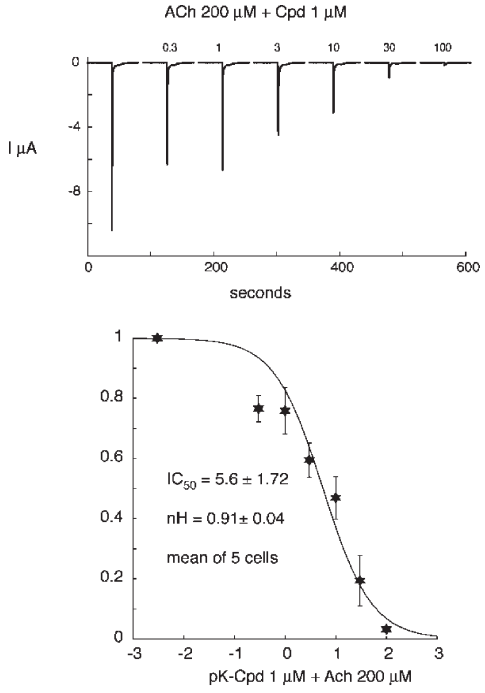

Figure 4. Electrophysiological assay of compound **1** tested for inhibition of the acetylcholine response in the human α7 nAChR expressed in oocytes. Top: μA signal recorded upon addition of a 5 s pulse of 200 μM acetylcholine after preincubation for 30 s with the indicated concentration of ligand **1**. Bottom: plot of response. See Figures S2−S4 in the Supporting Information for data on compounds **2**−**4**.

the channel or binding at an additional allosteric site (Figure S6 in the Supporting Information).

The experiments above show the first example of using GDB for diversifying the scaffold of known drugs in a fragment-based approach. Such focused scaffold diversification is particularly efficient when starting from GDB and would be difficult to realize with de novo drug design algorithms.[2,3] Although several of the diamines chosen were already known and were in fact selected as such to facilitate synthesis, none of the 38 synthesized ligands had been previously reported, including the competitive antagonist **1**. Accessing additional and exclusively novel scaffolds from the library may be readily envisioned, provided additional synthesis resources. The fact that most ligands showed significant activity (25 out of 38 ligands

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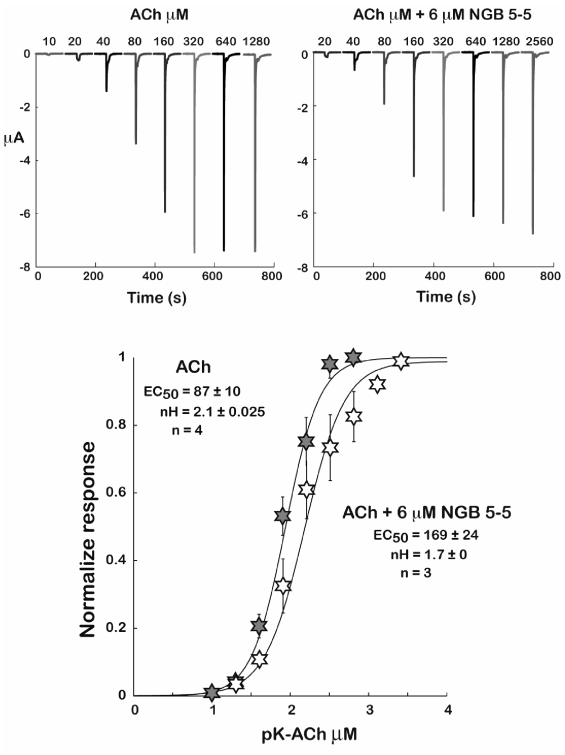

Figure 5. Competitive inhibition of the acetylcholine signal of α7 nAChR by **1** (NGB 5-5). The response was recorded for 5 s pulses of acetylcholine at the indicated concentration in the absence (upper left, gray symbols in plot) or presence of compound **1** at 6 μM (upper right, white symbols in plot).

tested, 66%) probably reflects the conservative design chosen, retaining the essential pharmacophoric features of the reference ligands. Although only a small number of compounds were investigated, the data clearly show that the docking scores did not distinguish antagonists from agonists and did not predict the cross-reactivity of some of the ligands with other binding sites as indicated by noncompetitive inhibition such as ligand **4**. This probably indicates a limitation of structure-based virtual screening in the case of the α7 nAChR. Future experiments will address virtual screening with more refined scoring functions integrating the collected structure−activity relationship data, in particular comparing agonist **6** to the new antagonist analogue **1**, and the characterization of the ligand collection with other receptor subtypes.

SUPPORTING INFORMATION AVAILABLE

Details of virtual screening, electrophysiology, and synthesis. This material is available free of charge via the Internet at http://pubs.acs.org.

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