Journal of Medicinal Chemistry

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Synthesis and Nicotinic Receptor Activity of Chemical Space Analogues of *N*-(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl-4-chlorobenzamide (PNU-282,987) and 1,4-Diazabicyclo[3.2.2]nonane-4-carboxylic Acid 4-Bromophenyl Ester (SSR180711)

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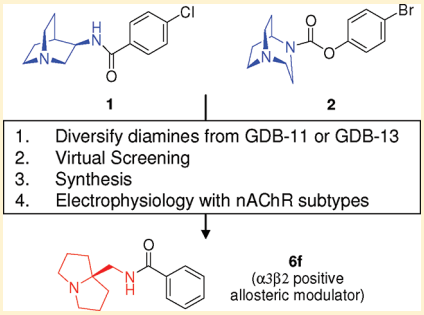
Supporting Information

ABSTRACT: The Chemical Universe Generated Databases up to 11 atoms of CNOF (GDB-11) and up to 13 atoms of CNOClS (GDB-13) were used to enumerate analogues of the diamine part of two known $\alpha7$ nicotinic receptor agonists and construct libraries of virtual analogues of these drugs. The libraries were scored using structure-based (docking to the nicotine binding site of the acetylcholine binding protein 1uw6.pdb) or ligand-based (similarity to the parent drugs) methods, and the top-scoring virtual ligands were inspected for easily accessible synthetic targets. In total, 21 diamines were prepared and acylated with aromatic carboxylic or oxycarbonic acids to produce 85 analogues of the parent drugs. The compounds were profiled by electrophysiology in *Xenopus* oocytes expressing human nicotinic acetylcholine receptor (nAChR) subtypes $\alpha7$, $\alpha3\beta2$, $\alpha4\beta2$, $\alpha3\beta4$, or $\alpha4\beta4$. Characterization of selected compounds revealed eight inhibitors of the $\alpha7$ nicotinic receptor and three positive allosteric modulators of the $\alpha3\beta2$ nAChR.



INTRODUCTION

Nicotinic acetylcholine receptors (nAChRs) are widely expressed in the central and peripheral nervous system as well as on non-neuronal cells.[1] nAChRs are composed by the assembly of five subunits around an axis of pseudo symmetry, and binding of the ligand occurs in the extracellular domain at the interface between two subunits. Most nAChR are heteromeric and composed by the assembly of two or more distinct protomers. To date, 17 genes encoding for nAChR have been identified in mammals, yielding a large repertoire of receptor subtypes. Many of these are implicated in fundamental biological and physiological processes and their associated pathologies, leading to intense pharmacology research to discover nAChR modulators. Thousands of ligands have been reported that act on various nAChR as partial or full agonists, antagonists, or positive or negative allosteric modulators. Annotated lists of nAChR ligands are available in public databases such as ChEMBL[2] or in the online compound catalog of Tocris.[3] Nicotinic ligands are structurally highly diverse and comprise small molecule natural products (e.g., nicotine, epibatidine) and synthetic ligands (e.g., varenicline, morantel), most often acting as subtype selective agonists, as well as larger natural products (e.g., ivermectin, methyllycaconitine, tubocurarine) and peptide toxins (e.g., α-bungarotoxin, α-conotoxins) typically acting as antagonists or allosteric modulators (Figure 1).

Despite the large number and structural diversity of already known nAChR ligands, there is a continued demand for new nAChR ligands, in particular with new subtype selectivity and activity profiles. Although nAChR ligands span a very broad area of chemical space, we asked the question whether new ligands and activities might be uncovered using a highly focused, in depth exploration of chemical space around selected known ligands. This approach would be somewhat similar to the drug design concept of systematic analoging, whereby new drugs can be derived from known drugs by optimization of side activities through structural optimization.[4] Herein, we report the discovery of new nAChR modulators with original activity profiles among analogues of the structurally closely related ligands *N*-(3*R*)-1-azabicyclo[2.2.2]oct-3-yl-4-chlorobenzamide (PNU-282,987) and 1,4-diazabicyclo[3.2.2]nonane-4-carboxylic acid 4-bromophenyl ester (SSR180711) (Figure 2).[5−7] The ligands were identified by enumeration of over 500000 virtual analogues by de novo drug design,[8,9] virtual screening using either docking to the nicotinic binding site of the acetylcholine binding protein (AChBP) or ligand-based similarity to the parent drugs, chemical synthesis of 85 selected analogues, and profiling by electrophysiology in *Xenopus* oocytes expressing various human nAChR subtypes ($\alpha7$, $\alpha3\beta2$, $\alpha4\beta2$, $\alpha3\beta4$, or $\alpha4\beta4$).

The enumeration of virtual analogues of **1** (PNU-282,987) and **2** (SSR180711) was based on our recently reported

Received: January 10, 2012
Published: May 16, 2012

dx.doi.org/10.1021/jm300030r | *J. Med. Chem.* 2012, 55, 4605−4618

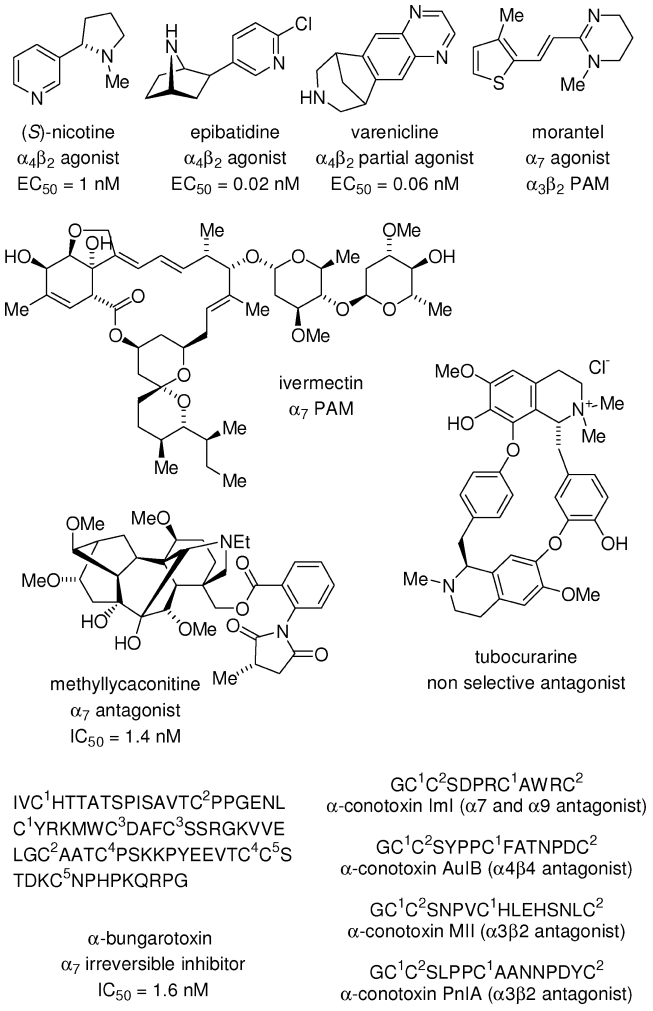


Figure 1. Structures of selected synthetic and natural nAChR modulators. PAM = positive allosteric modulator. Peptides are written in one-letter code aminoacids with uppercase numbers at cysteines indicating disulfide bridge connectivities using the SMILES formalism for cycles.[10,11] Data from ref 3.

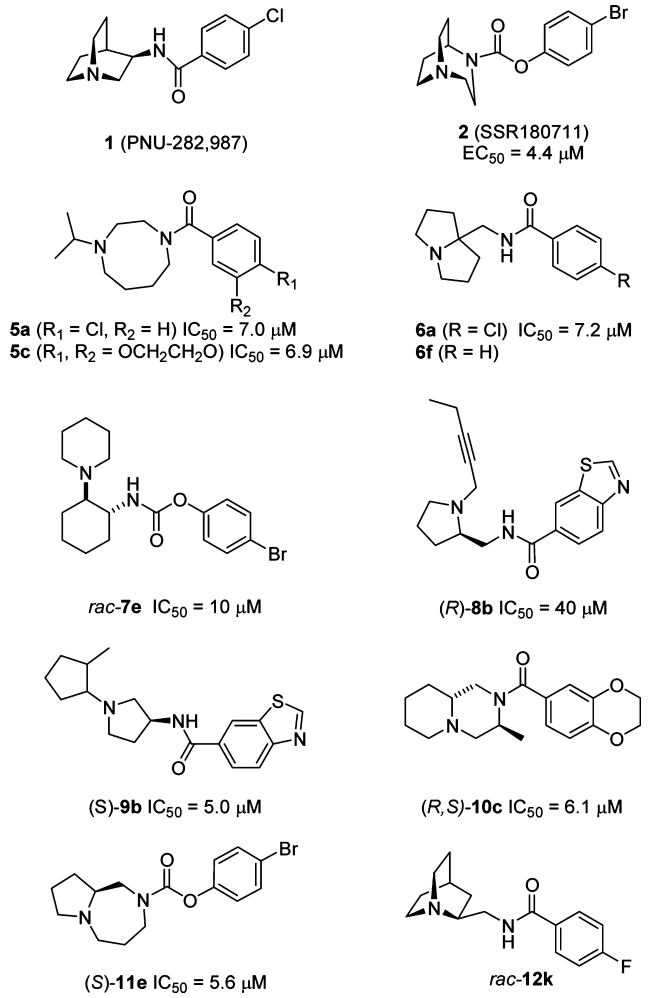


Figure 2. Structures of **1** and **2** and related $\alpha7$ and $\alpha3\beta2$ nAChR modulators identified by virtual screening, synthesis, and electrophysiology assay in *Xenopus* oocytes. The IC_{50} values given are for inhibition of human $\alpha7$ nAChR measured by electrophysiology. Compounds **6a**, **6f**, and *rac*-**12k** are positive modulators of the $\alpha3\beta2$ nAChR.

Chemical Universe Generated Databases up to 11 atoms (GDB-11) and up to 13 atoms (GDB-13).[12−14] These publicly available databases list 26.4 million molecules up to 11 atoms of C, N, O, and F, respectively, and 977 million molecules up to 13 atoms of C, N, O, S, and Cl that are virtually possible following selected criteria for chemical stability and synthetic feasibility and have been used previously to guide the synthesis of new inhibitors for the *N*-methyl D-aspartate (NMDA) receptor glycine site and the glutamate transporter GLT-1.[15−17] While the starting ligands were partial agonists of the $\alpha7$ nAChR, our study uncovered eight antagonists of the $\alpha7$ nAChR and three positive allosteric modulators of the $\alpha3\beta2$ nAChR. Thus, the relatively narrow chemical space defined by the monoacylated bicyclic diamine chemotype of the $\alpha7$ nAChR agonists **1** and **2** provided sufficient structural diversity to significantly vary the activity profiles, illustrating the value of an in depth exploration of a local chemical space for drug discovery. $\alpha7$ nAChR modulators might find application in treating neurological disorders and cancer.[18,19] The positive allosteric modulators of the $\alpha3\beta2$ nAChR reported here seem to be significantly more potent than the effect reported for the $\alpha7$ nAChR agonist Morantel.[20−23] Positive allosteric modulators of the $\alpha3\beta2$ nAChR might find applications in the treatment of sarcopenia, the muscular atrophy that develops with aging.

RESULTS AND DISCUSSION

Virtual Screening. The drug candidates **1** and **2** are representative of a family of $\alpha7$ nAChR agonists consisting of a bicyclic scaffold comprising a bridgehead tertiary amine and a primary or secondary amine acylated with various aromatic carboxylic acids or carbamates, providing a favorable situation for structural diversification via enumeration of analogues of the diamine part. We set out to explore the chemical space around these ligands by enumerating analogues of the diamine parts with the help of the databases GDB-11 or GDB-13 and combining the resulting diamines with various aromatic acyl groups to generate a large library of virtual analogs.

Enumeration from GDB-11 and Selection by Docking (Autodock and Glide). A first library DiamA was extracted from GDB-11 by selecting the 72740 nonaziridine diamines with heavy atom formula $C_{4-9}N_2$ containing a tertiary aliphatic amine and a primary or secondary amine separated by a two-carbon spacer. The library was expanded by also selecting the

dx.doi.org/10.1021/jm300030r | J. Med. Chem. 2012, 55, 4605−4618

103528 tertiary amines with heavy atom formula $C_{10}N$ in GDB-11 and attaching a primary amino group at all β-carbons relative to the tertiary amine or by inserting a secondary amino group in all $\beta-\gamma$ carbon−carbon bonds relative to the tertiary amine, which provided an additional 160832 diamines with 12 heavy atoms again excluding aziridines. The combined set of 233572 diamines was then derivatized by attaching the five different aromatic acyl groups a−e (Figure 3) occurring frequently in

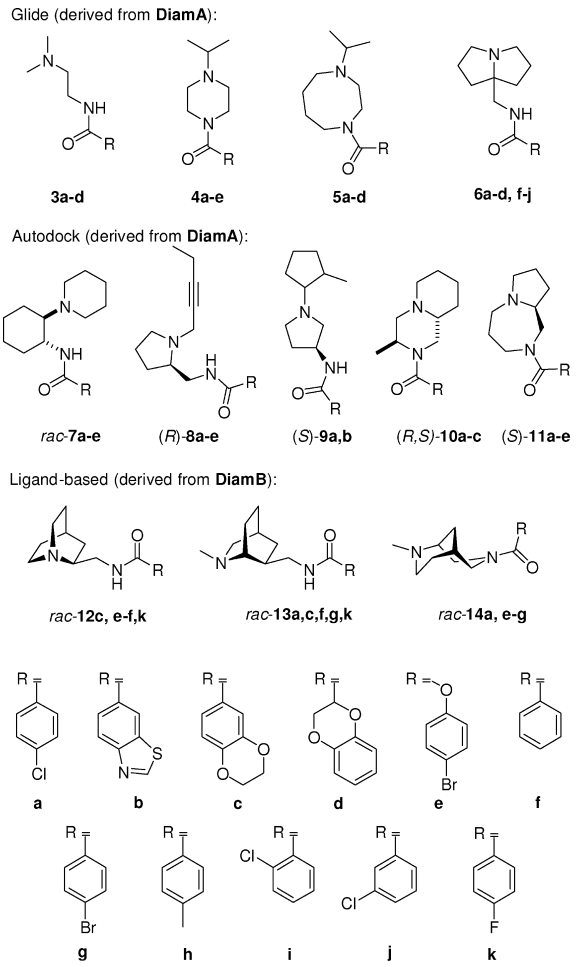


Figure 3. Synthesized analogues of **1** and **2** derived from virtual screening.

bioactive analogues of the parent drugs[24] to the free amino group to generate 1167860 analogues of **1** and **2**.

We initially chose docking to the nicotinic binding site of the AChBP 1UW6.pdb as a model because this approach has been shown to provide useful criteria for selecting nicotinic ligands.[25,26] The library of virtual acylated diamines was ranked by docking using Autodock 3.0.5[27] and Glide.[28] Both programs correctly positioned nicotine in its crystallographic position. Because of the limited throughput of the method, docking was performed with a randomly selected subset of 72745 structures taken from DiamA (6.2% of the entire library). These structures produced 507030 stereoisomers using CORINA[29] (LigPrep was used for Glide) to be considered for docking. The docking score showed a typical Gaussian curve distribution (Autodock, −12.9 to −4.6 kcal/mol estimated binding energy; Glide, −9.1 to −1.5 GScore; see Figure S1 in the Supporting Information). The docking poses of high-scoring ligands were generally very similar to the poses obtained for the reference ligands (Figure S2 in the Supporting Information). Although the docking scores of **1** and **2** were only average, the docking scores were considered to guide the selection of analogues fitting into the nicotinic binding site but with scaffolds significantly different from those of the parent molecules. 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 not previously described in the α7 nAChR literature. Five diamines were taken from the Autodock series and four scaffolds from the Glide series, featuring both chiral and achiral diamines (Figure 3). For the chiral diamines, the higher scoring enantiomer was considered as the synthetic target and is shown in the figure.

Enumeration from GDB-13 and Selection by Ligand Similarity. A second round of enumeration was undertaken aiming at ligand-based virtual screening to select analogues of **1** and **2**. In this case, a library of virtual diamines was assembled from the Chemical Universe Database GDB-13, which was at that point the newest database available. We followed simple criteria relating to the structural composition of the diamines using restrictions in values of the MQN descriptors, because this method produces high-scoring compounds for ligand-based virtual screening of very large databases such as PubChem, the fragment subset of Pubchem, and GDB-13.[30−34] Capitalizing on the results of the first round of enumeration and selection from GDB-11 discussed above, we limited the size of the diamine to a maximum of nine carbon atoms and two nitrogen atoms, suppressed all unsaturations, and focused on bicyclic diamines since these seemed the most relevant as analogues of the parent diamines. Thus, all molecules with up to nine carbon atoms and exactly two nitrogen atoms, two cycles of size 4−8, and containing only single bonds, were selected from GDB-13, which produced library DiamB with 35666 diamines. A further selection was performed by allowing only *N*-methyl groups as terminal carbon atoms, thus avoiding consideration of multiple analogues of the parent drugs containing the same diamine scaffold appended with methyl groups, yet allowing *N*-methyl amines related to the tropane scaffold to be retained. The selection was further limited by considering only 5-, 6-, or 7-membered rings, one tertiary amine, a maximum of two acyclic carbon atoms, and imposing at least two bonds shared by the two rings. These additional criteria focused the selection on "non-zero bridged" bicyclic systems having a globular shape similar to those of the parent drugs. This tighter selection left only 344 diamines for further consideration.

To estimate if the diamines in library DiamB led to analogues that were similar to the parent drugs, the shape similarity of their acylated derivatives was quantified using the ROCS Tanimoto-Combo score. The ROCS (rapid overlay of chemical structures) TanimotoCombo score measures the similarity between 3D shapes of molecules by maximizing an overlap function between molecular shapes, considering these shapes as continuous functions constructed from atom-centered electrostatic and volume Gaussians.[35] The score is maximized by comparing various conformers of both query and reference molecule. This 3D shape-based approach is well-validated for ligand-based virtual screening.[36] To perform the shape similarity scoring, the 35666 diamines in DiamB were virtually monoacylated on available primary or secondary amines either with the 4-chlorobenzoyl group to obtain virtual analogues of **1** or with the 4-bromophenoxycarbonyl group to obtain virtual analogues of **2**, providing two sets of 56788 structures. These were

dx.doi.org/10.1021/jm300030r | J. Med. Chem. 2012, 55, 4605−4618

converted to 374728 different 3D stereoisomers, which were individually scored. The ROCS score of the highest scoring stereoisomer was retained for each 2D structure.

The shape similarity data showed that most of the virtual ligands generated had a score higher than the threshold of 1.4 considered to be sufficient to predict bioactivity (Figure S3 in the Supporting Information). Indeed, from the 56294 successfully generated diamines, 74.9% had a ROCS score higher than 1.4 to one of the two reference drugs, and 19.2% had a score higher than 1.4 to both reference drugs (in each case with the corresponding acyl group). The percentages were even higher with the more restricted subset of 344 analogues, with 93.3% having a ROCS score above 1.4 for one reference compound and 54.1% for both reference compounds, highlighting the focused design of the virtual library. The subset of 344 bicyclic diamines was inspected to identify targets for synthesis and testing. Most of the diamines giving high-scoring compounds comprised ring size analogues of the diamines cores of **1** and **2** and as such were already described in the patent literature. Nevertheless, the bicyclic diamines **12**−**14** were identified as potentially interesting structures, which had not been previously described in the nicotinic receptor ligand literature (Figure 3). In addition to the ligands derived from virtual screening, analogues **15**−**28** were also prepared in the course of the investigations, in part during activity optimization attempts of selected active compounds (see below) and in part due to synthetic opportunities that arised in the course of the various syntheses (Figure 4).

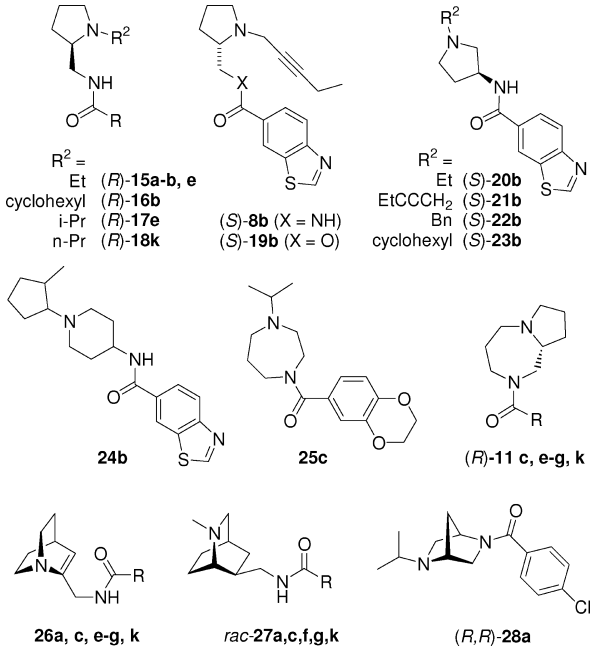


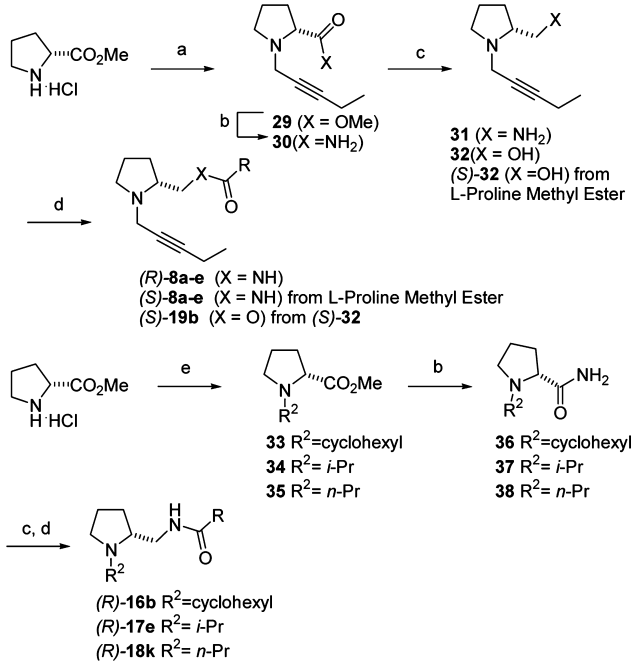
Figure 4. Further analogues prepared in this study.

Synthesis. The synthesis of the various ligands is described below starting with the acylation of known diamines, followed by the synthesis and acylation of further diamines in the order of structural complexity. The selection of carboxylic acids and carbamates used for acylation was dictated by the availability of the reagents and synthetic yields when preparing the five acyl groups a−e known to lead to nAChR activity (Figure 3) and in selected cases by efforts to optimize activities as detailed in the electrophysiology section.

Derivatives of Known Diamines. The amide derivatives **3a**−**d**, **4a**−**e**, **15a**,**b**,**e**, **22b**, and **28a** were prepared in one step by acylation of the corresponding commercially available free diamines. Compounds **6**,[37−39] **7**,[40] **13**, and **27**[41] were obtained by derivatization of the corresponding diamines, which were synthesized according to the literature. Although the 4- and 5-member ring diamine analogues of **7** were also high-scoring in virtual screening, their synthesis failed in our hands, although these diamines have been described in the literature.[42]

2-Aminomethyl-pyrrolidine Derivatives (Scheme 1). Diamine **31** was prepared starting with alkylation of D-proline

Scheme 1. Synthesis of 2-(Aminomethyl)pyrrolidine 8a−e, 16b, 17e, and 18k[a]



[a]Conditions: (a) 1-Bromo-2-pentyne, LiI, $NaHCO_3$, acetonitrile, 60 °C, 46 h (65%, **29**). (b) THF/NH_3 aqueous, 60 °C, 14 h (20−59%). (c) $LiAlH_4$, Et_2O, reflux, 12 h (13−31%) [32% (*S*)-**31**, 6% (*S*)-**32**]. (d) $ArCO_2H$, EDC, HOBt, *N*-methyl morpholine, CH_2Cl_2, 25 °C, 14 h, (10−61%) or ArOCOCl, NEt_3, THF, 2 h (3−32%). (e) Cyclohexanone, NEt_3, $NaBH_3CN$ (quant., **33**) or acetone, $NaHCO_3$, $NaBH(OAc)_3$, MeOH, 25 °C, 48 h (**34**) or 1-bromopropane, $NaHCO_3$, CH_3CN, 60 °C, 48h (78%, **35**).

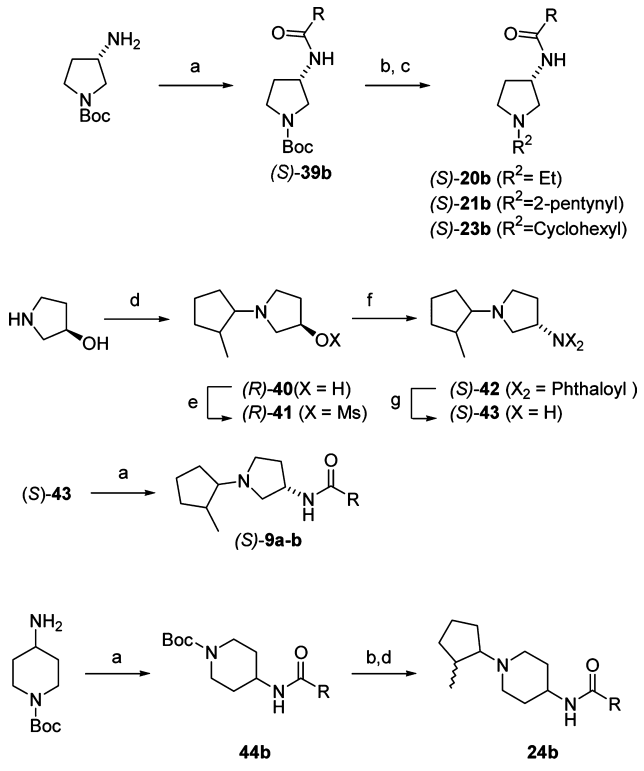
methyl ester hydrochloride with 1-bromo-2-pentyne[43] in the presence of $NaHCO_3$ and LiI in ACN at 60 °C to give amino ester **29** in 65% yield. Amide **30** was obtained in 54% yield by dissolving intermediate **29** in THF/NH_3 25% (1:9) and heating up to 60 °C. Reduction with $LiAlH_4$ in Et_2O, quenched with water and NaOH,[44] and purification by column chromatography finally afforded diamine **31** in 32% yield together with a small amount of amino alcohol **32**. Diamine **31** was then acylated to afford ligands (*R*)-**8a**−**e**. Using the same procedure, the enantiomer (*S*)-**8b** was prepared starting with L-proline methyl ester hydrochloride. The corresponding ester (*S*)-**19b** was obtained by acylation of alcohol (*S*)-**32** obtained as a byproduct of the reduction step. Amides **16b**, **17e**, and **18k** were synthesized starting from D-proline methyl ester hydrochloride by reductive amination with cyclohexanone (**33**) or acetone (**34**) or alkylation with 1-bromopropane (78% **35**), followed by treatment with an aqueous solution of ammonia

dx.doi.org/10.1021/jm300030r | J. Med. Chem. 2012, 55, 4605−4618

(51% **36**, 20% over two steps **37**, and 59% **38**), reduction of the amide by $LiAlH_4$ and acylation.

3-Aminopyrrolidine and 4-Aminopiperidine Derivatives (Scheme 2). (*S*)-(−)-1-Boc-3-aminopyrrolidine was acylated

Scheme 2. Synthesis of 3-Aminopyrrolidine and 4-Aminopiperidine Derivatives (*S*)-20b, 21b, and 23b and (*S*)-9a,b[a]

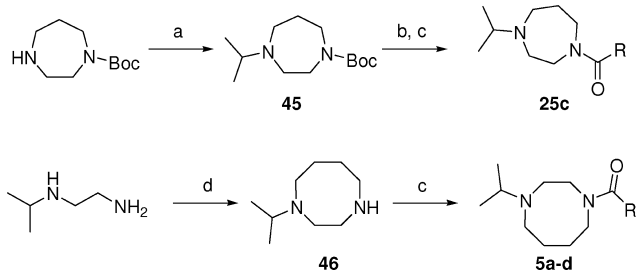


[a]Conditions: (a) $ArCO_2H$, EDC, HOBt, *N*-methyl morpholine, CH_2Cl_2, 25 °C, 14 h, or ArOCOCl, NaOH, 3 h (9−89%). (b) TFA, CH_2Cl_2. (c) Acetaldehyde, $NaBH_3CN$, MeOH, 30 min [(*S*)-**20b**, 10%] or cyclohexanone, $NaBH_3CN$, MeOH [(*S*)-**21b**: 50%] or 1-bromo-2-pentyne, K_2CO_3, DMF, 25 °C, 7 h [(*S*)-**23b**, 75%]. (d) 2-Methyl-cyclopentanone, $NaBH_3CN$, MeOH/AcOH, 25 °C, 14 h [(*R*)-**40**, 54%; **24b**, 27%]. (e) MsCl, Et_3N, THF, −10 to 25 °C, 14 h (90%). (f) Potassium phthalimide, DMF, 90 °C, 38 h (96%). (g) $NH_2NH_2·H_2O$, Et_2O, reflux, 3 h (94%).

with 1,3-benzothiazole-6-carboxylic acid providing compound (*S*)-**39b**, which was deprotected by TFA before being submitted to reductive amination or alkylation to give the amides **20b**, **21b**, and **23b**. Diamine (*S*)-**43** was prepared starting with reductive alkylation of (*R*)-3-pyrrolidinol with 2-methylcyclopentanone using $NaBH_3CN$ to give amino alcohol (*R*)-**40** in 54% yield as a 1:1 diastereomeric mixture. The alcohol was mesylated to **41**, substituted with potassium phthalimide to (*S*)-**42**, and deprotected with hydrazine in Et_2O to provide (*S*)-**43**, which was then used to prepare the corresponding amides (*S*)-**9a,b**. The 4-aminopiperidineamide **24b** was obtained from 4-amino-1-Boc-piperidine, which was acylated to give **44b** before being deprotected and submitted to a reductive amination with 2-methylcyclopentanone.

Diazepine and Diazocine Derivatives (Scheme 3). Diazepine **45** was prepared by reductive amination of 1-Boc-hexahydro-1,4-diazepine with acetone. The corresponding amide **25c** was then obtained after Boc deprotection with

Scheme 3. Synthesis of Diazepine 25c and Diazocines 5a−d[a]



[a](a) Acetone, $NaBH_3CN$, HCl cat., MeOH (93%). (b) TFA, CH_2Cl_2. (c) $ArCO_2H$, EDC, HOBt, *N*-methyl morpholine, CH_2Cl_2, 25 °C, 14 h (21−59%), or ArOCOCl, NaOH (10%). (d) 1,4-Dibromobutane, Na_2CO_3, *o*-xylene, 130 °C, 18 h (42%).

TFA and acylation of the free secondary amine. Diazocines **5a−d** were synthesized starting from a dialkylation reaction of *N*-isopropylethylenediamine with 1,4-dibromobutane using Na_2CO_3 in *o*-xylene, as previously described in a methodology study of synthesis of cyclic diamines.[45]

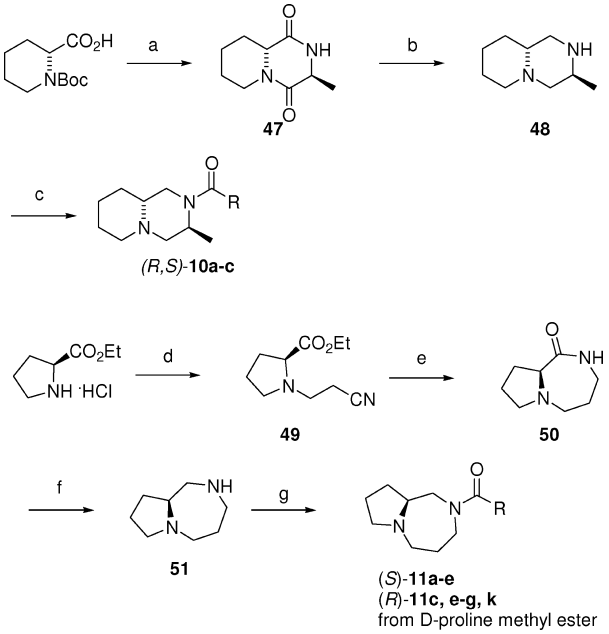
Bicyclic Amine Derivatives (R,S)-10, (S)-11, and (R)-11 (Scheme 4). Bicyclic diamine **48**, which has been reported in a study of the reactivity of 2-azaquinolizinium oxides,[46] was prepared starting with peptide coupling between (*R*)-(+)-*N*-Boc-2-piperidinecarboxylic acid and L-alanine ethyl ester. The resulting dipeptide was deprotected and cyclized in a one-pot reaction to give diketopiperazine **47** in 34% yield. Finally, reduction with $LiAlH_4$ and distillation gave diamine **48** in 22% yield, which was converted to amides (*R,S*)-**10a−c**. Diamine **51** was prepared as described in a methodology study of reductive cyclization with nitriles.[7,47] Thus, alkylation of L-proline ethylester with acrylonitrile gave **49** in 58% yield. The reduction of the nitrile to the corresponding amine with Nickel Raney gave the lactam **50** directly in 57% yield. Reduction of the lactame with $LiAlH_4$ in dry THF and purification by distillation finally gave the diamine in 36% yield. Diamine **51** was then acylated to the corresponding amides (*S*)-**11a−e**, which were purified by preparative RP-HPLC. Following the same procedure, the enantiomer (*R*)-**11c,e−g,k** were obtained starting from D-proline methyl ester hydrochloride.

2-Aminomethyl Quinuclidine Derivatives (Scheme 5). Diamine **54**, reported as an intermediate in the synthesis of a serotonin-3 receptor ligand, was prepared by adapting the literature.[48] Starting with the deprotonation and reduction of the commercial 2-methylene-3-quinuclidinone hydrochloride, followed by a SN2′ substitution by treatment with thionyl chloride (58%), a substitution of chloride by reaction with an aqueous solution of ammonia gave the desired diamine **54** in a quantitative yield. The corresponding amides **26a,c,e−g,k** were then prepared directly from **54**. The saturated analogues (*rac*-**12c,e,f,k**) were obtained by acylation of the hydrogenated diamine **55** or by reduction under hydrogen conditions of the amide compounds.

Bicyclic Amine Derivatives rac-14 (Scheme 6). Bicyclic diamine *rac*-**62** was synthesized in eight steps starting from the commercial 5-norbonen-2-yl-acetate, which was submitted to a dihydroxylation reaction under osmium tetroxide condition to give compound *rac*-**56** (quant.). A Lemieux−Johnson reaction, followed by a reductive amination with benzylamine, afforded the bicyclic compound *rac*-**57** (62%). After reduction of the acetal and a swern oxidation, keto-compound *rac*-**58** was transformed to oxime *rac*-**59**, which was submitted to a Beckmann

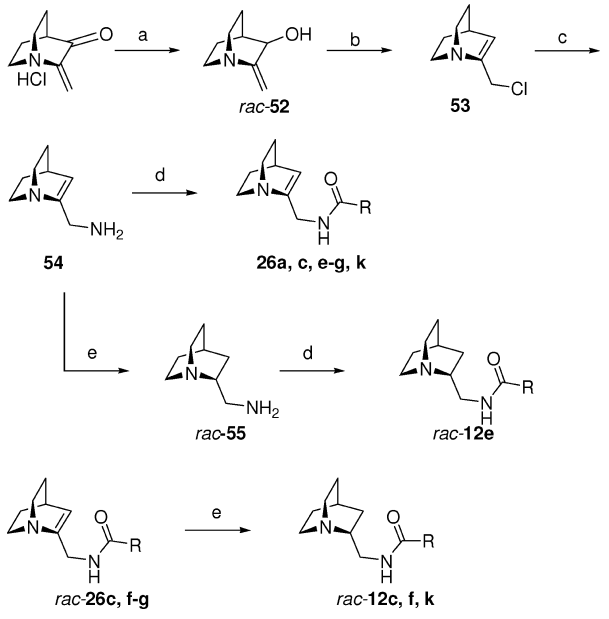
dx.doi.org/10.1021/jm300030r | *J. Med. Chem.* 2012, 55, 4605−4618

Scheme 4. Synthesis of Bicyclic Amides (*R,S*)-10, (*S*)-11, and (*R*)-11[a]



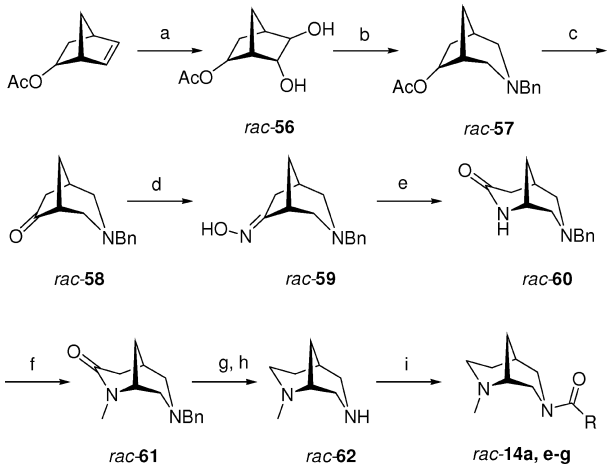
[a]Conditions: (a) Ala-OEt·HCl, EDC, HOBt, Et_3N, 25 °C, 15 h, then CF_3CO_2H, 25 °C, 1 h, then Et_3N, MeOH, reflux, 4 h (34%). (b) $LiAlH_4$, THF, reflux, 14 h (22%). (c) $ArCO_2H$, EDC, HOBt, *N*-methyl morpholine, CH_2Cl_2, 25 °C, 14 h (8−52%). (d) Acrylonitrile, aqueous KOH, 50−70°C, 2.5 h (58%). (e) H_2 (48 bar), Raney-Ni, MeOH, 80 °C, 6 h (57%). (f) $LiAlH_4$, THF, reflux, 14 h (36%). (g) $ArCO_2H$, EDC, HOBt, *N*-methyl morpholine, CH_2Cl_2, 25 °C, 14 h, or ArOCOCl, NaOH or NEt_3 3 h (8−52%).

Scheme 5. Synthesis of 2-Aminomethyl Quinuclidine Derivatives 12c,f,k and 26a,c,e−g,k[a]



[a]Conditions: (a) K_2CO_3, CH_2Cl_2, H_2O, 25 °C, 3 days then $NaBH_4$, MeOH, 0 °C, 30 min (51%). (b) Thionyl chloride, CH_2Cl_2, 16 h (58%). (c) NH_4OH, MeOH, 25 °C, 4 h (quant.). (d) $ArCO_2H$, EDC, HOBt, *N*-methyl morpholine, CH_2Cl_2, 25 °C, 14 h or ArOCOCl, NEt_3, THF, 2 h (5−48%). (e) Pd/C, H_2, MeOH, 4 h (3−76%).

Scheme 6. Synthesis of Bicyclic Amine Derivatives *rac*-14a,e−g[a]



[a]Conditions: (a) *N*-Methyl morpholine *N*-oxide, OsO_4, acetone, H_2O, 40 °C, 18 h (quant.). (b) $NaIO_4$, DCE, H_2O, 0 °C, 18 h then benzylamine, $NaBH(OAc)_3$, DCE, 18 h (62%). (c) (i) KOH, MeOH, H_2O, 25 °C, 18 h (52%); (ii) oxalyl chloride, DMSO, CH_2Cl_2, −78 °C, 15 min then NEt_3, −78 °C, 15 min (89%). (d) Hydroxylamine hydrochloride, NaOAc, MeOH, reflux, 3 h (65%). (e) H_2SO_4 concentrated, 100 °C, 4 h (46%). (f) NaH, THF, 0 °C, 30 min then MeI, 25 °C, 18 h (82%). (g) BH_3·THF, THF, reflux, 12 h then MeOH, 25 °C, 1 h (67%). (h) $Pd(OH)_2$, H_2, MeOH, reflux, 6 h (quant.). (i) $ArCO_2H$, PyBOP, DIEA, DCM, 14 h (13−22%) or ArOCOCl, NEt_3, THF, 2 h (13%).

rearrangement by treatment with concentrated sulfuric acid to give amide *rac*-**60** (46%). A methylation of the amide (82%), followed by reduction with borane (67%) and deprotection of the benzylated amine under $Pd(OH)_2/H_2$ conditions, afforded the desired bicylic diamine *rac*-**62**, which was directly used to form the bicyclic amides compounds *rac*-**14a,e−g**.

Electrophysiology. Activity was measured by electrophysiology in oocytes expressing various recombinant human nAChR subtypes.[49] Compound effects were assessed by first recording the current 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.

α7 nAChR Activity. The initial activity screening was performed against the α7 nAChR subtype, for which **1** and **2** are partial agonists (Table S1 in the Supporting Information). Compounds **2a**−(*S*)-**11e** (36 compounds, Figure 3) synthesized as examples of high-scoring analogues by docking were investigated. 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 36 ligands tested, 12 showed weak activity (less than 30% inhibition at 10 μM), 19 showed moderate inhibition (30−70% inhibition at 10 μM), and 5 showed a strong inhibition of the acetylcholine response (>70% at 10 μM, observed for **5c**, **6d**, **8a,b**, and **9b**).

The strongest hits inspired the preparation of further analogues to see if activity could be improved (Figure 4). Analogues of pyrrolidines **8b** and **9b** were prepared bearing simplified alkyl residues at the tertiary amine in form of analogues **15a−23b**, including the enantiomer (*S*)-**8b** and an ester analogue (*S*)-**19b** obtained as a side product. In addition, the ring-expanded analogue of **9b** was prepared as **24b** derived from the achiral 4-amino-piperidine. The diazepine compound

dx.doi.org/10.1021/jm300030r | J. Med. Chem. 2012, 55, 4605−4618

25c was also prepared with an intermediate ring size between the weakly active piperazine **4c** (19% inhibition) and its more active 8-membered ring analogue **5c** (75% inhibition). (*R*)-Enantiomers of (*S*)-**11** were also prepared because the bromophenyl carbamate showed a much higher shape similarity (ROCS score) to **2** than (*S*)-**11**, the enantiomer originally selected by its docking score, to test if any (*R*)-**11** derivative might act as an agonist. However, none of these derivatives showed any significant activity at the α7 nAChR, with no detectable agonist effect and less than 50% inhibition of the ACh signal at 10 μM ligand.

Inhibition of the α7 nAChR was characterized in more detail by measuring IC_{50} values for compounds **5a,c**, **6a**, **7e**, **8b**, **9b**, **10c**, and (*S*)-**11e**, thus including one compound for each of the acylated diamines investigated (except for **3** and **4**, which showed only very weak activities with all derivatives). Most values were in the 5−10 μM range, which corresponds to the affinity range for the parent ligand **2** (Table 1). Closer

Table 1. Activities on the Human α7 nAchR[a]

compd	IC_{50} or EC_{50} (μM)	activity type
5a	7.0 ± 1.1	mixed antagonist
5c	6.9 ± 1.0	ND
6a	7.2 ± 1.2	mixed antagonist
7e	10 ± 0.8	ND
(*R*)-**8b**	40 ± 6	ND
(*S*)-**8b**	45 ± 1	ND
9b	5.0 ± 0.7	ND
10c	6.1 ± 1.5	noncompetitive antagonist
(*S*)-**11e**	5.6 ± 1.7	competitive antagonist to ACh
2	4.4[b]	partial agonist[b]

[a]Electrophysiological assay in *Xenopus* oocytes. See the Supporting Information for details. [b]Data from ref 6, indicated as EC_{50} = 4.4 μM (2.5−7.8 μM), which corresponds to a binding affinity of K_i = 14 nM. ND, not determined. See Figure S4 in the Supporting Information for electrophysiology traces.

investigation of the inhibition mechanism was carried out in four cases. This showed that only (*S*)-**11e** behaved as a competitive inhibitor of acetylcholine and, therefore, could be assigned to binding to its binding pocket as anticipated from the virtual screening procedure.

$\alpha3\beta2$ nAChR Activity. Additional subtypes of the nAChR were tested with the different compounds to assess the functional diversity of the prepared chemical space analogues. Activity profiling was performed in oocytes expressing the $\alpha3\beta2$, $\alpha4\beta2$, $\alpha3\beta4$, and $\alpha4\beta4$ nAChR subtypes (Table S1 in the Supporting Information). These data showed that a positive deflection was specifically observed at $\alpha3\beta2$, suggesting a possible positive allosteric modulation of this receptor subtype in compounds **6a**, **6f**, *rac*-**12k**, and *rac*-**14e** (Figure 5A). To probe the effect of the aromatic acyl group on the $\alpha3\beta2$ nAChR modulatory effect, we focused our attention on analoging **6a** due to its simple synthesis and its elegant achiral diamine scaffold, yielding analogues **6b−k**. Activity profiling showed that the positive modulatory effect on $\alpha3\beta2$ was preserved in several derivatives of **6a**, in particular the unsubstituted benzamide **6f**.

Closer investigation of **6a**, **6f**, *rac*-**12k**, and *rac*-**14e** at the $\alpha3\beta2$ nAChR by testing the effect at 1, 10, and 100 μM ligand on a 50 μM acetylcholine pulse showed that only **6f** and *rac*-**12k** preserved the positive modulatory effect at a high ligand concentration (Figure 5B). Ligands **6f**, *rac*-**12k**, and *rac*-**14e** were further characterized by measuring the concentration−activation relationship for acetylcholine in the absence or presence of a prepulse of the putative allosteric modulator (10 μM, Figure 5C). This showed that only **6f** induced a sustained potentiation characterized by an increase of the amplitude of the ACh-evoked currents over the entire range of the concentration−activation curve and caused a shift of the agonist response toward a higher sensitivity. These features are typical of an allosteric effect and cannot be attributed to potentiation such as that observed with a coagonist activity.

Allosteric modulators are compounds that, by definition, bind on the receptor at a site that is distinct from the natural ligand binding site, also called orthosteric binding site. The physical interaction of the allosteric modulator with the receptor complex yields either an increase of the receptor function or a reduction of the overall activity.[50] While most allosteric modulators have a molecular structure quite different from the natural ligand, molecules sharing some analogies with the ligand have also been shown to influence receptor functions. For example, tubocurarine that is known to inhibit the nAChRs was found to potentiate the ACh-evoked currents at the $\alpha3\beta4$ receptors by a mechanism that is probably distinct from the true allosteric modulation.[51] Another example of receptor modulation, including the $\alpha3\beta2$ receptors, was provided by studies of the anthelminthic compound morantel. This molecule, which acts as an agonist at the invertebrate nAChRs, was shown to enhance, albeit to a limited extend, the ACh-evoked current at the $\alpha3\beta2$. Moreover, it was recently proposed that morantel binds at the interface between the β- and the α-subunit.[20−23] Altogether, the data presented above in Figure 5 indicate that the mode of action of compound **6f** is distinct from that previously reported for morantel.

As it is known that $\alpha3\beta2$ nAChRs are expressed at the presynaptic ending of the neuromuscular junction, it can be postulated that a positive allosteric modulator should increase the release of neurotransmitter from the motor nerve. This effect could find immediate applications in diseases, or conditions, in which neuromuscular transmission is impaired, for example, in the treatment of sarcopenia, a progressive neuromuscular atrophy that develops with aging.

Similarity Analysis. Most of the 85 compounds tested in this study showed measurable levels of activity in terms of an inhibitory effect on the α7 nAChR or a positive modulation of the $\alpha3\beta2$ nAChR. However, none of the compounds showed agonistic effects on the α7 nAChR, which was the main characteristic of the two reference compounds **1** and **2**. A similarity analysis was performed to test if the differences in observed activities could be traced back to inherent structural features of the diamines. The diamine libraries DiamA and DiamB and the synthesized examples were compared to **1** and **2** in terms of shape and substructure similarity. A random set of small molecules of similar size from the public access database of commercially available druglike ligands zinc is not commercial (ZINC) was included in each case as a control.[52]

A shape similarity analysis based on the ROCS Tanimoto Combosscore was performed on both diamine series using the 4-bromophenyl carbamates for comparison to **2** and the 4-chlorobenzamides for comparison with **1**, as used above for ligand-based similarity selection. Both acylated diamine series scored significantly higher than a control set of 39853 compounds from ZINC (with 16 ≤ hac ≤ 22 corresponding to the range covered by the carbamates and amides) and

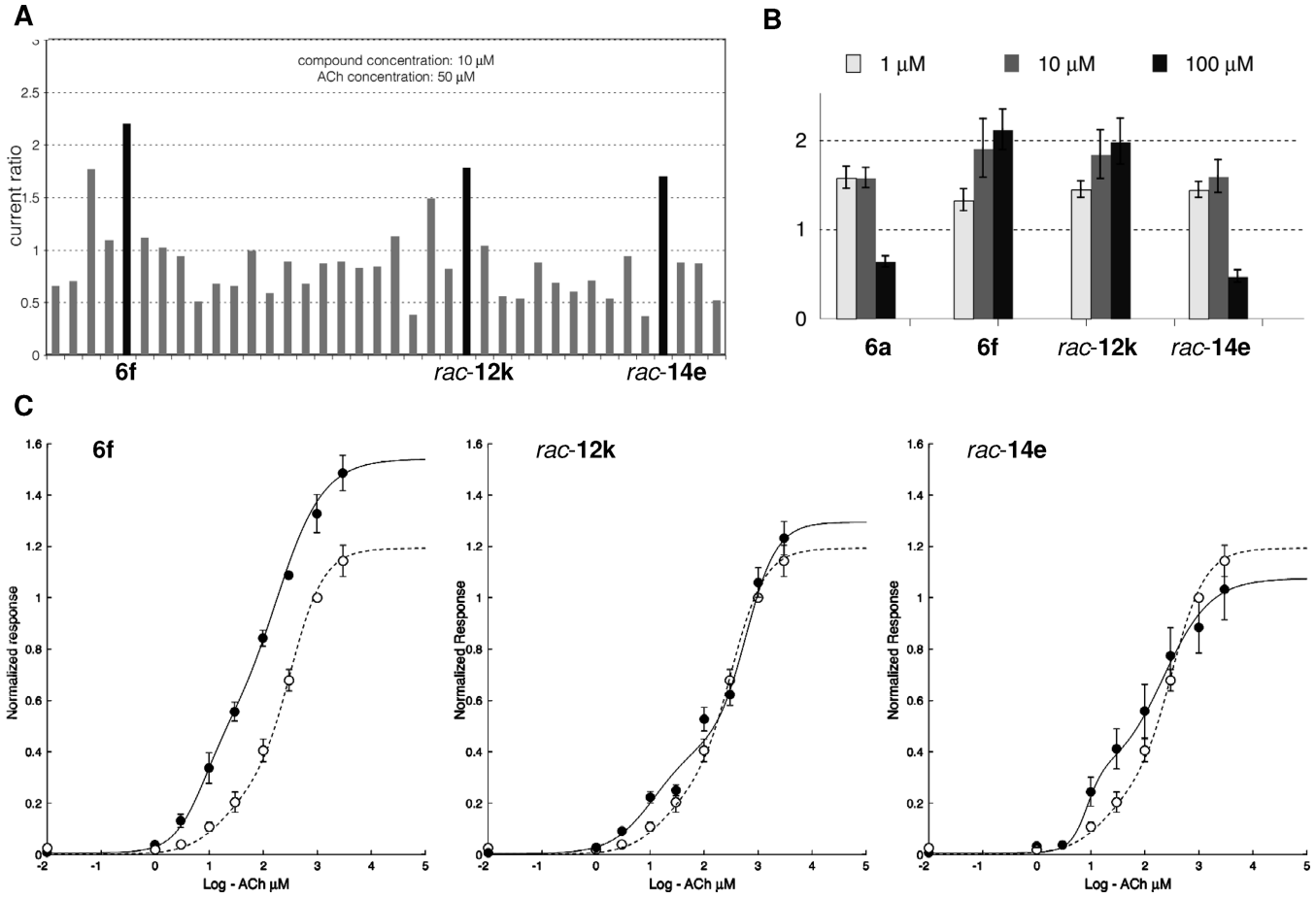


Figure 5. Positive modulation at the human $\alpha3\beta2$ nAChR. (A) Screening for the effects of compounds at the human $\alpha3\beta2$. Cells expressing the human $\alpha3\beta2$ nAChR were challenged first with a reference ACh test pulse (50 μM, 5 s), and the response to the same test pulse was repeated after 30 s of exposure to a fixed concentration of compound. Plot of the ratio of the peak currents, recorded after compound exposure over peak current recorded in control, yields a ratio where unity corresponds to the absence of effect and values superior to unity indicate a potentation of the ACh response. See Table S1 in the Supporting Information for complete data set. (B) Effects of four positive allosteric modulators at three concentrations. Data were collected and analyzed as in panel A. Note that exposure to a concentration of 100 μM inhibits the ACh-evoked current. (C) Effects of a fixed concentration of the positive allosteric modulator (10 μM) on the ACh concentration activation curve. Note that compound **6f** causes a shift of the concentration activation curve toward a higher sensitivity and an increase of the maximal current amplitude.

Table 2. Similarity Values for Virtual Libraries

data set	no. of compds	ROCS to **1** (% > 1.4)[a]	ROCS to **2** (% > 1.4)[b]	% > 1.4 for **1** and **2** (**1** or **2**)[c]	T_{SF} to **1** (% > 0.7)[a]	T_{SF} to **2** (% > 0.7)[b]	% > 0.7 for **1** and **2** (**1** or **2**)[c]
ZINC[d]	39853	1.12 ± 0.17 (6.0)	1.11 ± 0.13 (2.2)	1.4 (6.7)	0.28 ± 0.08 (0.1)	0.25 ± 0.04 (0)	0.0 (0.1)
DiamA	44410	1.43 ± 0.19 (57.0)	1.42 ± 0.15 (53.0)	34.1 (75.9)	0.77 ± 0.10 (73.6)	0.76 ± 0.08 (73.1)	68.4 (78.3)
DiamB	56294	1.34 ± 0.19 (41.7)	1.42 ± 0.12 (52.4)	19.2 (74.9)	0.82 ± 0.06 (99.9)	0.79 ± 0.05 (94.3)	94.3 (100.0)
subs344	344	1.50 ± 0.19 (77.0)	1.49 ± 0.15 (70.4)	54.1 (93.3)	0.82 ± 0.06 (100.0)	0.81 ± 0.05 (99.7)	99.7 (100.0)

[a]ROCS Tanimoto Comboscore and T_{SF} for the 4-chlorobenzamide. [b]ROCS Tanimoto Comboscore and T_{SF} for the 4-bromophenylcarbamate of the diamines from DiamA, DiamB. or subs344 or for the compounds from ZINC. The 1024 bits Daylight type substructure fingerprint from ChemAxon was used. [c]Refers to the scores of the corresponding acylated diamines or of the ZINC cpds. [d]Random selection from ZINC with $16 \leq$ hac ≤ 22 covering the molecular sizes of compounds derived from acylation of the diamines libraries DiamA and DiamB.

contained a much more significant fraction of compounds scoring above 1.4 than the control set (Table 2). The diamine libraries also showed a high degree of similarity to the reference drugs when compared by substructure similarity using the Tanimoto similarity coefficient T_{SF} for a 1024 bit daylight type substructure fingerprint. Both the average T_{SF} and the fraction of high similarity compounds ($T_{SF} > 0.7$) were much higher in these libraries as compared to the control set extracted from ZINC.

The ROCS and T_{SF} data above showed that the diamine library designs enforced a substantial degree of shape and substructure similarity to the reference compounds when compared to random compounds of similar size (Figure 6). The ligands derived from DiamA showed substantially lower substructure similarity to the reference ligands (lower T_{SF}) than the ligands derived from DiamB. This dissimilarity was caused by the presence of multiple CC double bonds and triple bonds and small rings present only in the former. Some ligands derived from DiamA combined low T_{SF} similarity with high ROCS score to the reference compounds. These compounds were derived from polyunsaturated diamines often containing 3- and 4-membered rings.

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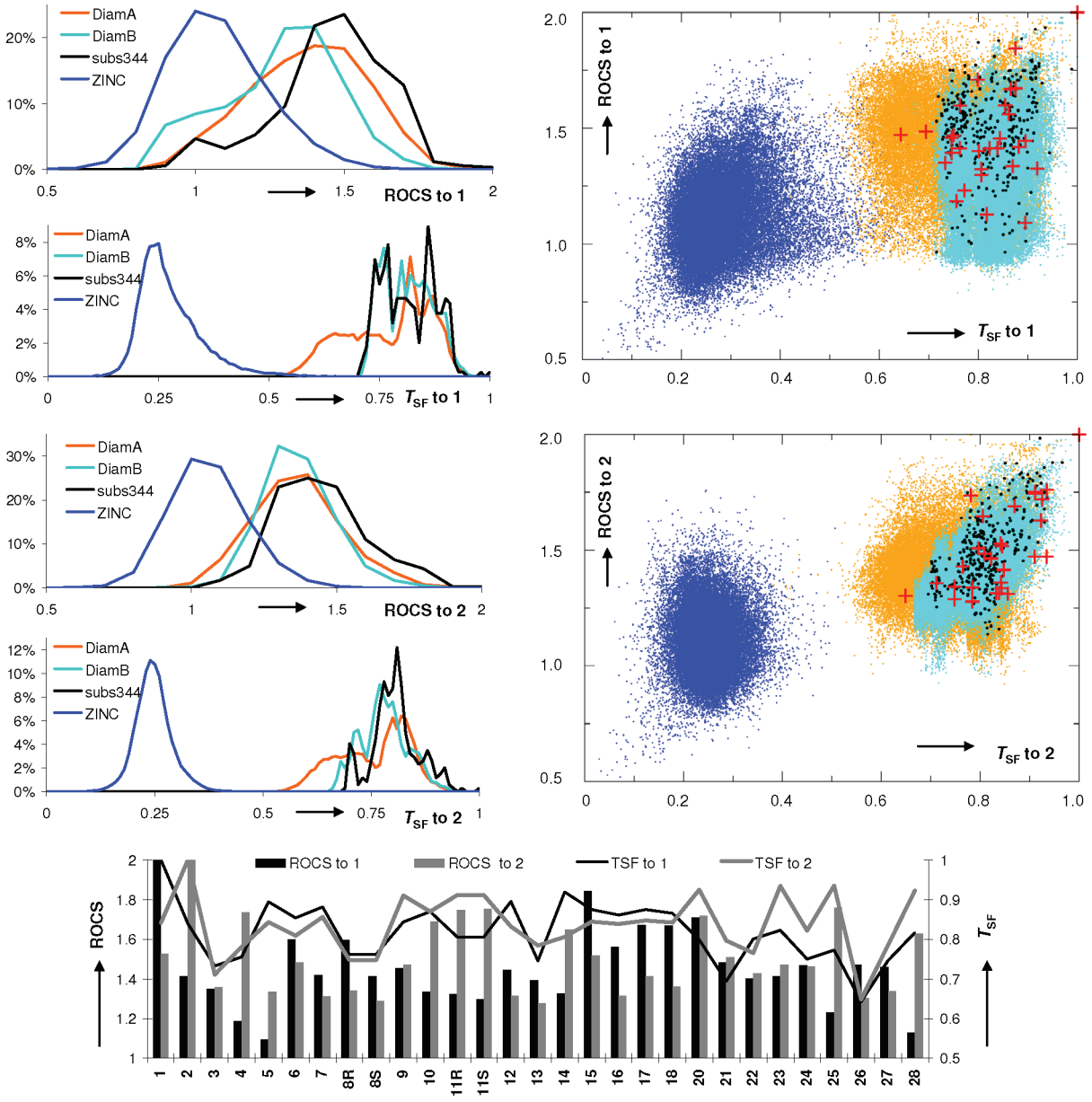

Figure 6. Shape and substructure similarities. For each diamine, the corresponding 4-chlorobenzamide derivative is compared to **1**, and the 4-bromophenyl-carbamate is compared to **2**. Compound sets: orange dots, DiamA, 44410 compounds; cyan dots, DiamB, 56294 compounds; black dots, restricted subset of 344 diamines from DiamB; and blue dots, ZINC random subset 16 ≤ hac ≤ 22, 39853 compounds. Red crosses and combined bar/line plot: synthesized compounds **3**–**28** and the two reference ligands **1** and **2**.

The diamines selected for synthesis were quite representative of the overall library design, with substantial but not extremely high similarity scores to either reference drug (in form of the derivatives with common acyl groups as discused above, Figure 6). The correlation between similarity scores and the observed activity was weak within the compound series. For example, while the acyclic *N*,*N*-dimethyl-ethylenediamine used for **3a**–**d** had a much lower shape and substructure similarity to either drugs caused by its smaller size and lacked activity, the 8-membered ring derived **5a** and **5c** showed good inhibition of the α7 nAChR despite a low shape similarity to either reference compounds. On the other hand, the *N*-ethyl-3-aminopyrrolidine **20** returned high scores toward both reference drugs, but compound **20b** did not exhibit any particularly high activity. As to the lack of α7 nAChR agonist effect among the compounds tested, it should be noted that an agonist effect requires the stabilization of the open channel state of the receptor in transition to its desensitized state and that this effect is only rarely observed when screening compound series. Antagonistic effects as those observed here are generally more common.

The similarity analysis showed that the exploration of chemical space suggested by the GDB approach guided the search somewhat further away from the parent drugs than a classical analoging series might have achieved, for example, by preparing ring size and methylated analogues of the parent diamines. While this diversification was apparently too significant to retain the agonistic effects of the parent drugs, the compounds prepared clearly covered a useful diversity range, as exemplified by the discovery of several competitive inhibitors of the α7 nAChR and of positive modulators of the $\alpha3\beta2$ nAChR. In particular, **6f** was derived from a relatively simple diamine quite substantially different from the parent

dx.doi.org/10.1021/jm300030r | *J. Med. Chem.* 2012, 55, 4605–4618

diamine that might not have been considered by classical analoging strategies.

CONCLUSION

The experiments above demonstrate the potential of the chemical universe databases GDB-11 and GDB-13 to inspire the synthesis of new analogues of **1** and **2**. Starting from these α7 nAChR partial agonists, exploration of chemical space was performed by enumeration of virtual analogues with the help of the databases, selection based on docking or ligand-based virtual screening, chemical synthesis of selected virtual hits and analogues, and testing by electrophysiology. This workflow led to the discovery of eight new α7 antagonists and three new $\alpha3\beta2$ nAChR positive modulators. Such $\alpha3\beta2$ nAChR positive modulators might have therapeutic potential for the treatment of sarcopenia. Although the identified ligands possess clear similarities to **1** and **2** when considering the overall structure of the ligands, their diamine parts are still significantly different and might not have been chosen by a pure intuition-driven design exercise. These data illustrate the innovative contribution of systematic, in depth exploration of local chemical space using the GDB databases as a support to ligand discovery.

EXPERIMENTAL SECTION

Virtual Screening. *Ligand Enumeration.* The diamine libraries were enumerated from GDB-11 or GDB-13 by in-house Java scripts using ChemAxon's academic JChem API package. In the case of GDB-11, all diamines with one nonaziridine tertiary amine and a primary or secondary amine separated by a two-carbon spacer were selected from GDB-11. A second set of nonaziridine tertiary monoamines of general formula $C_{10}N$ was extracted from GDB-11, and a primary amine was added to all a β-carbon relative to the tertiary amine or inserted in a $\beta-\gamma$ single bond relative to the tertiary amine in all possible combinations. All of the diamines from direct selection and addition/insertion were pooled, yielding 233572 unique diamines. These diamines were attached with five different aromatic moieties via virtual amide bond using ChemAxon's Reactor to form 1167860 complete molecules, which were later used for docking.

In the case of GDB-13, the subset containing only carbon and nitrogen atoms up to 11 heavy atoms was extracted (3′182′540 SMILES). From this subset, structures containing exactly two nitrogen atoms, exactly two rings, only single bonds, and only 4−8-membered rings were retained, resulting in the diamine subset (35′666 SMILES). All structures were then monoacylated at every primary or secondary amine, with either 4-chlorobenzoyl or 4-bromophenyloxycarbonyl, in analogy to the two reference structures **1** and **2**, resulting in two sets of 56′788 structures each. The two sets were submitted to ROCS calculation with **1** and **2** as references, respectively. The number of structures in both sets was reduced further by applying more restrictive filters as follows: Structures were only allowed if they contain only 5−7-membered rings, at most two acyclic carbon atoms, and at least two bonds in fused rings. Additionally, structures containing either a primary or a secondary nonacylated amine and structures containing a terminal carbon atom bound to another carbon atom were removed, resulting in the final set of 344 structures.

Docking with AutoDock. The protonation state of 72495 randomly selected compounds was set at pH 7.4. The set was then converted to 507030 stereoisomers using Corina. AutoDock 3.0.5 was applied to dock the virtual molecules into binding cavity of Ach receptor 1UW6.pdb. The receptor was trimmed to retain only chain C and chain D and was prepared using AutoDock Tools ADT3 with all default settings.

Docking with Glide. The same set of 72495 compounds was also docked using Glide (Schrodinger) docking software. A total of 72495 SMILES were converted to 3D structures using LigPrep. The resulting .mae structures were then docked into the binding site composed of only chain C and D of 1UW6.pdb. The receptor was prepared by Maestro program using default settings.

Shape Similarity Scoring with ROCS. Prior to the ROCS calculation, all molecules of the input database were submitted to "FLIPPER", which identifies all unspecified stereocenters in the molecule and enumerates both stereochemistry states of each unspecified stereocenter, resulting in all possible stereoisomers. "OMEGA" was applied on the stereoisomers to create a maximum of 200 lowest energy 3D conformers. The conformers were used as input for the ROCS calculation, in which the best "TanimotoCombo" overlap score was retrieved per stereoisomer. Lastly, the 3D structures were converted back to SMILES, and the best score per SMILES was kept as final ROCS score. The example structures **3**−**28** and the two reference structures **1** and **2** were prepared similarly prior to ROCS calculation.

Synthesis. *General.* All reagents used were purchased from commercial sources without further purification. All solvents used in reactions were distilled from technical quality. Dry solvents were obtained directly from a drying solvent system. Chromatographic purifications (flash) were performed with Silica Gel 60 from Fluka (0.04−0.063 nm; 230−400 mesh ASTM). Preparative RP-HPLC was performed with a Waters Delta Prep 4000 system with a Waters Prepak Cartridge (500 g) as column and Waters 486 Tunable Absorbance Detector or with a Waters Prep LC Controller sytem with a Dr. Maisch GmbH Reprospher C18-DE, 100 mm × 30 mm, particle size 5 μm, 100 Å pore size column, and a Waters 2489 UV/Visible Controller. The compound purity was determined by analytical RP-HPLC. All compounds whose IC_{50} values were determined had purities ≥95%. Overall, 77 compounds had purities ≥95%, and 7 compounds had 85−95% purity; see the Supporting Information, pp S138−S139, for details. Analytical RP-HPLC was performed on Waters 600E systems with a Waters Atlantis (4.6 mm × 100 mm, dC_{18}, 5 lm) column, UV detection with Waters 996 photodiode array detector. Analytical RP-UHPLC was also performed on a Dionex ULTIMATE 3000 RS chromatography system (ULTIMATE 3000 RS Photo diode array detector) using a Dionex Acclaim RSLC 120 C18, 3.0 mm × 50 mm, particle size 2.2 μm, 120 Å pore size, flow rate 1.2 mL min^{-1} column. Compounds were detected by UV absorption at 214 nm. Eluents for Waters Delta Prep 4000 system and Analytical RP-HPLC were as follows: A, water with 0.1% TFA; C, acetonitrile/water (90/10) with 0.1%TFA; and D, acetonitrile/water (40/60) with 0.1%TFA. Eluents for Waters Prep LC Controller sytem and Analytical RP-UHPLC were as follows: A, water with 0.1% TFA; and D, acetonitrile/water (90/10) with 0.1% TFA. NMR spectra were acquired on a Bruker 300 MHz or a Bruker 400 MHz instrument. ^{1}H and ^{13}C chemical shifts are quoted relative to solvent signals. Carbons multiplicities have been assigned either by attached proton test (APT), distorsionless enhancement polarization transfer (DEPT), heteronuclear multiple bond correlation spectroscopy (HMBC), or heteronuclear single quantum correlation (HSQC) spectroscopy experiments. Mass spectra were obtained by electron spray ionization (ES-MS) on a Micromass Autospec Q (Waters/Micromass) instrument in the positive mode.

Amide Formation. Procedure A. The acid (1 equiv), EDC (1.25 equiv), HOBt (1.25 equiv), *N*-methyl morpholine (2.5 equiv, 4.5 equiv when the amine was a salt), and the amine (1 equiv) were dissolved in this order in DCM (1 mL per 1 mmol of acid). The reaction was stirred for 14 h at room temperature under argon. Once the reaction was completed, it was evaporated under reduced pressure. Only for final compounds, the residue was purified using a preparative RP-HPLC, and only fractions with a purity >95% were lyophilized (unless otherwise indicated).

Procedure B. The amine (1 equiv) was mixed with the acid (3 equiv) and PyBOP (3 equiv) in dry DCM (10 mL per 1 mmol of amine) under an argon atmopshere. Then, diisopropylethylamine (5 equiv) was added, and the reaction was stirred for 14 h at room temperature under argon. Once the reaction was completed, it was evaporated under reduced pressure, and the residue was purified using a preparative RP-HPLC, and only fractions with a purity >95% were lyophilized (unless otherwise indicated).

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Carbamate Formation. Procedure C. The diamine (1 equiv) was dissolved in water (5 mL per 1 mmol), followed by the addition to the solution of NaOH in pellets (1 equiv). The solution was cooled down to 0 °C. 4-Bromophenyl chloroformate (1.2 equiv) was then added dropwise, and after some minutes, a white precipitate started appearing. After 3 h, the reaction was extracted with CH_2Cl_2 (3×), and the organics were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Unless otherwise indicated, the residue was purified using a preparative RP-HPLC, and only fractions with a purity >95% were lyophilized.

Procedure D. To a solution of 4-bromophenylchloroformate (1.2 equiv) in dry THF (10 mL per 1 mmol) cooled to 0 °C under argon were added dropwise Et_3N(2 equiv) and the diamine (1 equiv) diluted in THF. The reaction mixture was then stirred for 2 h at room temperature. The reaction was quenched with brine and extracted with EtOAc (3×), and the organics layers were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Unless otherwise indicated, the residue was purified using a preparative RP-HPLC, and only fractions with a purity >95% were lyophilized.

*(4-Chlorophenyl)(4-isopropyl-1,4-diazocan-1-yl)methanone (**5a**).* Using procedure A, this product was obtained from amine **46**. The final compound slowly hydrolyzed in 0.1% TFA (HPLC conditions). For that reason, purification was done by column chromatography (neutral alumina, CH_2Cl_2/MeOH 0.5%) to give **5a** as a yellow oil (20 mg, 0.068 mmol, 53% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.33−7.26 (m, 2H), 7.25−7.18 (m, 2H), 3.86 (br s, 1H), 3.40 (br s, 2H), 2.83−2.00 (br m, 6H), 1.70 (br s, 4H), 1.10 (br s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.5 (C), 135.8 (C), 135.0 (C), 128.6 (CH), 127.6 (CH), 54.8 (br CH_2), 54.1 (CH), 50.1 (br CH_2), 39.8 (br CH_2), 23.4 (CH_2), 20.9 (CH_3). MS (ESI$^+$) m/z 295.4 ([M + 1, Cl^{35}]$^+$), 297.2 ([M + 1, Cl^{37}]$^+$). HRMS (ESI$^+$) calcd for $C_{16}H_{24}Cl_{35}N_2O$, 295.1572 ([M + 1]$^+$); found, 295.1566.

*(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)(4-isopropyl-1,4-diazocan-1-yl)methanone (**5c**).* Using procedure A, this product was obtained from 1-isopropyl-1,4-diazocane **46**, prepared by a literature procedure.[45] The final compound slowly hydrolyzed in 0.1% TFA (HPLC conditions) For that reason, purification was done by column chromatography (neutral alumina, CH_2Cl_2/MeOH 0.5%) to give **5c** as a yellow oil (15 mg, 0.047 mmol, 37% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.98−6.79 (m, 3H), 4.25 (s, 4H), 4.11 (br s, 1H), 3.44 (br t, 2H), 2.91−2.27 (br m, 6H), 1.75 (br s, 4H), 1.15 (br d, J = 6 Hz, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.0 (C), 144.4 (C), 143.3 (C), 130.6 (C), 130.5 (C), 119.7 (CH), 117.2 (CH), 115.7 (CH), 64.4 (CH_2), 64.3 (CH_2), 54.9 (br CH_2), 54.1 (CH), 50.0 (br CH_2), 39.9 (br CH_2), 23.4 (CH_2), 21.0 (CH_3). MS (ESI$^+$) m/z 319.0 ([M + 1]$^+$). HRMS (ESI$^+$) calcd for $C_{18}H_{27}N_2O_3$, 319.2016 ([M + 1]$^+$); found, 319.2012.

*4-Chloro-N-((hexahydro-1H-pyrrolizin-7a-yl)methyl)benzamide (**6a**).* Using procedure A, this product was obtained from amine **63**. Purification by column chromatography (neutral alumina, CH_2Cl_2/MeOH 2%) gave **6a** as a yellow solid (40 mg, 0.143 mmol, 40% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.12 (br s, 1H), 7.91 (d, J = 8 Hz, 2H), 7.40 (d, J = 8 Hz, 2H), 3.63 (d, J = 6 Hz, 2H), 3.44−3.27 (m, 2H), 2.89−2.73 (m, 2H), 2.11−1.70 (m, 8H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 166.6, 137.7, 132.0, 128.8, 128.7, 78.0, 55.4, 45.6, 35.7, 24.7. MS (ESI$^+$) m/z 279.2 ([M + 1, Cl^{35}]$^+$), 2281.2 ([M + 1, Cl^{37}]$^+$). HRMS (ESI$^+$) calcd for $C_{15}H_{20}Cl^{35}N_2O$, 279.1264 ([M + 1]$^+$); found, 279.1260; mp (°C) 158−163.

*N-((Hexahydro-1H-pyrrolizin-7a-yl)methyl)benzamide, TFA Salt (**6f**).* Using procedure A, the compound was obtained from amine **63** as a colorless oil (22.7 mg, 0.06 mmol, 30% yield). ^{1}H NMR (MeOD, 300 MHz): δ 7.90 (dd, J_1 = 3 Hz, J_2 = 9 Hz, 2H), 763−7.58 (m, 1H), 7.54−7.48 (m, 2H), 3.74 (s, 2H), 3.69−3.61 (m, 2H), 3.28−3.22 (m, 2H), 2.27−2.24 (m, 8H). ^{13}C NMR (MeOD, 75 MHz): δ 173.4, 133.9, 133.8, 130.0, 128.8, 84.5, 56.7, 47.6, 35.9, 25.5. MS (ESI$^+$) m/z 245.1 ([M + 1]$^+$). HRMS (ESI$^+$) calcd for $C_{15}H_{21}N_2O$, 245.1648 ([M + 1]$^+$); found, 245.1654.

*4-bromophenyl-2-(piperidin-1-yl)cyclohexylcarbamate, TFA salt (**7e**).* Using procedure C, this product was obtained from the corresponding amine which was prepared according ref 40 as a white solid (39 mg, 0.081 mmol, 15% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.81 (br s, 1H), 7.50 (br d, J = 10 Hz, 1H), 7.47−7.40 (m, 2H), 7.09−7.01 (m, 2H), 3.85 (qd, J_1 = 10 Hz, J_2 = 4 Hz, 1H), 3.65 (br d, J = 12 Hz, 1H), 3.48−3.32 (m, 1H), 3.28−2.85 (m, 3H), 2.69−2.52 (m, 1H), 2.42−2.22 (m, 1H), 2.21−1.74 (m, 8H), 1.60−1.22 (m, 5H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 154.6, 150.2, 132.1, 123.6, 118.2, 67.2, 52.5, 50.2, 46.4, 33.6, 24.3, 23.6, 22.8, 22.3, 22.5. MS (ESI$^+$) m/z 381.4 ([M + 1, Br^{79}]$^+$), 383.4 ([M + 1, Br^{81}]$^+$). HRMS (ESI$^+$) calcd for $C_{18}H_{26}N_2O_2Br^{79}$, 381.1177 ([M + 1]$^+$]) found 381.1190; mp (°C) 145−146.

*N-(((R)-1-(Pent-2-ynyl)pyrrolidin-2-yl)methyl)benzo[d]thiazole-6-carboxamide, TFA Salt (**8b**).* Using procedure A, this product was obtained from amine **31** as a colorless oil (103 mg, 0.241 mmol, 27% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.22 (br s, 1H), 9.14 (s, 1H), 8.90 (t, J = 6 Hz, 1H), 8.59 (s, 1H), 8.14 (d, J = 8 Hz, 1H), 8.07 (d, J = 8 Hz, 1H), 4.07−3.64 (m, 6H), 3.36−3.21 (m, 1H), 2.34−1.87 (m, 6H), 1.06 (t, J = 7 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 167.7 (C), 157.3 (CH), 154.8 (C), 133.8 (C), 130.2 (C), 125.4 (CH), 123.2 (CH), 121.8 (CH), 93.2 (C), 66.6 (C), 65.5 (CH), 53.5 (CH_2), 43.9 (CH_2), 39.7 (CH_2), 27.8 (CH_2), 23.2 (CH_2), 12.9 (CH_3), 12.1 (CH_2). MS (ESI$^+$) m/z 328.1 ([M + 1]$^+$). HRMS (ESI$^+$) calcd for $C_{18}H_{22}N_3OS$, 328.1483 ([M + 1]$^+$); found, 328.1485.

*N-((S)-1-(2-Methylcyclopentyl)pyrrolidin-3-yl)benzo[d]thiazole-6-carboxamide, TFA Salt (**9b**).* Using procedure A, this product was obtained from amine (*S*)-**43** as a yellow oil (48 mg, 0.112 mmol, 25% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) diastereomeric mixture (1.5:1): δ 11.94 (br s, 0.6H), 11.80 (br s, 0.4H), 9.20 (br d, J = 8 Hz, 0.6H), 9.11 (s, 1H), 9.04 (br d, J = 8 Hz, 0.4H), 8.65 (s, 0.6H), 8.58 (d, J = 1 Hz, 0.4H), 8.19−8.04 (m, 2H), 5.28−5.08 (m, 1H), 4.06−3.81 (m, 2H), 3.32−2.87 (m, 3H), 2.70−2.51 (m, 1H), 2.49−1.50 (m, 8H), 1.12 (d, J = 7 Hz, 1.2H), 1.07 (d, J = 7 Hz, 1.8H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 166.7 (C), 166.4 (C), 156.8 (C), 155.1 (C), 133.9 (C), 130.5 (C), 125.7 (CH), 125.6 (CH), 123.3 (CH), 121.9 (CH), 71.5 (CH), 71.4 (CH), 60.6 (CH_2), 59.6 (CH_2), 54.5 (CH_2), 52.9 (CH_2), 47.9 (CH), 47.6 (CH), 35.0 (CH), 34.9 (CH), 31.3 (CH_2), 31.2 (CH_2), 30.9 (CH_2), 30.7 (CH_2), 25.5 (CH_2), 25.4 (CH_2), 19.8 (CH_2), 19.5 (CH_2), 14.0 (CH_3), 13.6 (CH_3). MS (ESI$^+$) m/z 330.2 ([M + 1]$^+$). HRMS (ESI$^+$) calcd for $C_{18}H_{24}N_3OS$, 330.1640 ([M + 1]$^+$); found, 330.1631.

*((3S,9aR)-Hexahydro-3-methyl-1H-pyrido[1,2-a]pyrazin-2(6H)-yl)(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)methanone, TFA Salt (**10c**).* Using procedure A, this product was obtained from amine **48** as a colorless oil (22 mg, 0.053 mmol, 41% yield). ^{1}H NMR (D_2O, 400 MHz at 353 K): δ 7.63−7.45 (m, 3H), 5.29 (br s, 1H), 4.89 (s, 2H), 4.88 (s, 2H), 4.63−4.38 (m, 2H), 4.29−3.80 (m, 4H), 3.69−3.55 (m, 1H), 2.59−1.93 (m, 9H). ^{13}C NMR (D_2O, 100 MHz at 353 K): δ 120.9 (CH), 118.3 (CH), 116.4 (CH), 65.4 (CH_2), 65.2 (CH_2), 57.4 (CH), 54.0 (CH_2), 47.8 (CH_2), 46.2 (br CH), 42.7 (br CH_2), 21.7 (CH_2), 21.3 (CH_2), 17.4 (CH_2), 15.3 (CH_3). MS (ESI$^+$) m/z 317.4 ([M + 1]$^+$). HRMS (ESI$^+$) calcd for $C_{18}H_{25}N_2O_3$, 317.1865 ([M + 1]$^+$); found, 317.1860.

*(S)-4-Bromophenyl Hexahydro-1H-pyrrolo[1,2-a][1,4]diazepine-2(3H)-carboxylate, TFA Salt [(S)-**11e**].* Using procedure C, this product was obtained from amine **51** as a colorless oil (26 mg, 0.059 mmol, 8% yield). ^{1}H NMR (D_2O, 400 MHz): δ 7.66 (d, J = 9 Hz, 2H), 7.14 (d, J = 7 Hz, 2H), 4.40−3.56 (m, 7H), 3.47−3.20 (m, 2H), 2.54−1.85 (m, 6H). ^{13}C NMR (D_2O, 75 MHz): mixture of four isomers δ 155.8, 155.6, 155.3, 155.0, 149.8, 149.7, 149.6, 132.6, 123.7, 118.8, 118.7, 118.7, 67.0, 66.9, 66.2, 65.5, 57.2, 57.1, 56.9, 56.8, 53.7, 53.6, 51.4, 51.3, 47.3, 47.0, 46.9, 46.8, 46.2, 43.9, 43.7, 28.1, 28.0, 27.7, 27.6, 26.2, 26.0, 25.2, 25.0, 23.3, 21.7, 21.6. MS (ESI$^+$): m/z 339.2 ([M + 1, Br^{79}]$^+$), 341.2 ([M + 1, Br^{81}]$^+$). HRMS (ESI$^+$) calcd for $C_{15}H_{20}Br^{79}N_2O_2$, 339.0708 ([M + 1]$^+$); found, 339.0698.

*N-(1-Aza-bicyclo[2.2.2]oct-2-ylmethyl)-4-fluorobenzamide, TFA Salt (rac-**12k**).* Compound **26k** (79 mg, 0.30 mmol) and Pd/C (40 mg) were diluted in dry MeOH (6 mL), and the solution was stirred for 4 h under an hydrogen atmosphere. The reaction mixture was then filtered over Celite and washed with MeOH. The filtrate was concentrated under vacuum to afford a yellow oil, which was purified by preparative HPLC to give the desired compound as a colorless oil

(3 mg, 3%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.69 (bs, 1H), 7.91 (dd, J_1 = 9 Hz, J_2 = 6 Hz, 2H), 7.06 (t, J = 9 Hz, 2H), 4.02 (ddd, J_1 = 15 Hz, J_2 =7 Hz, J_3 = 3 Hz, 1H), 3.65 (q, J = 9 Hz, 1H), 3.55−3.19 (m, 5H), 2.27−2.13 (m, 2H), 2.01−1.84 (m, 4H), 1.53−1.46 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 167.3, 165.3 (d, J = 255 Hz, 1C), 130.1 (d, J = 15 Hz, 2C), 129.5, 115.7 (d, J = 15 Hz, 2C), 59.1, 48.6, 41.2, 40.5, 28.8, 23.7, 22.8, 20.7. ^{19}F NMR (CDCl3, 376 MHz): δ −109.5, −107.9. MS (ESI$^+$): m/z 263.1 ([M + 1]$^+$). HRMS (ESI$^+$) calcd for $C_{15}H_{20}N_2OF$, 263.1554 ([M + 1]$^+$); found, 263.1554.

*N-((1-Aza-bicyclo[2.2.2]oct-2-en-2-yl)methyl)-4-fluorobenzamide (**26k**).* Using procedure A, this compound was obtained from amine **54** as yellowish solid after filtration on neutral alumina (DCM) and crystallization in hexanes (50 mg, 0.19 mmol, 38% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.85 (dd, J = 6 Hz, 2H), 7.37 (bs, 1H), 7.05 (t, J = 9 Hz, 2H), 6.43 (d, J = 6 Hz, 1H), 4.10 (d, J = 6 Hz, 2H), 3.11−3.02 (m, 2H), 2.71−2.57 (m, 3H), 1.72−1.62 (m, 2H), 1.54−1.42 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 166.2, 164.9 (d, J = 247 Hz, 1C), 146.1, 130.6 (d, J = 7.5 Hz, 1C), 129.9, 129.7 (d, J = 7.5 Hz, 2C), 115.7(d, J = 15 Hz, 2C), 49.5, 41.0, 27.4, 26.9. ^{19}F NMR ($CDCl_3$, 376 MHz): δ −108.3 MS (ESI$^+$) m/z 261.1 ([M + 1]$^+$). HRMS (ESI$^+$) calcd for $C_{15}H_{18}FN_2O$, 261.1398 ([M + 1]$^+$); found, 261.1390.

*2-Aminomethyl-1-azabicyclo[2.2.2]oct-2-ene (**54**).* 2-Chloromethyl-1-azabicyclo[2.2.2]oct-2-ene **53** (798 mg, 5.1 mmol), prepared by a literature procedure,[48] was diluted in MeOH (20 mL), and an aqueous NH_3 solution (20 mL) was added. The reaction mixture was stirred at room temperature for 4 h, and the mixture was concentrated to dryness to afford the desired compound in a quantitative yield. This material was used in the next step without purification. ^{1}H NMR (MeOD, 300 MHz): δ 6.63 (d, J = 9 Hz, 1H), 3.53 (d, J = 3 Hz, 2H), 3.08−2.99 (m, 2H), 2.73−2.69 (m, 1H), 2.66−2.56 (m, 2H), 1.78−1.69 (m, 2H), 1.53−1.43 (m, 2H). ^{13}C NMR (MeOD, 75 MHz): δ 145.5, 134.5, 51.5, 50.2, 41.8, 28.7, 28.4, 25.1. MS (ESI$^+$) m/z 139.1 [M + 1]$^+$. HRMS (ESI$^+$) calcd for $C_8H_{15}N_2$, 139.1230; found, 139.1233.

Electrophysiology. Recordings were performed as previously described.[49] Briefly, oocytes were prepared using standard methods by mechanical and enzymatical dissociation. Stage 5−6 oocytes were selected manually under the microscope and 10 nL of water containing 0.2 μg/μL of plasmid of interest, which contained the cDNA sequence for the nAChR. Expression of $\alpha3\beta2$, $\alpha4\beta2$, $\alpha3\beta4$, and $\alpha4\beta4$ was obtained by injection of 10 nL of solution containing a 1:1 ratio of the respective subtype containing plasmid at 0.2 μg/μL final concentration. Injections were performed using an automated system (roboinject, Multichannel Systems, Germany). Two or more days later, the functional properties of receptors were evaluated using an automated system equipped with two electrode voltage clamp (HiClamp, Multichannel Systems, Germany). The membrane potential of oocytes was clamped at a steady value of −80 or −100 mV, and currents evoked by ACh or compounds were recorded with a sampling frequency of 100 Hz. All recordings were performed at 18 °C, and cells were superfused with OR2 medium containing in mM: NaCl, 82.5; KCl, 2.5; HEPES, 5; $CaCl_2{\cdot}2H_2O$, 1.8; and $MgCl_2{\cdot}6H_2O$, 1; pH 7.4.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Details of the synthesis and characterization including spectra of all synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported financially by the University of Berne, the Swiss National Science Foundation. N.G.-D. is a postdoctoral fellow of the Roche Research Foundation. This work was supported by the EEC grant Neurocypres to D.B. We thank E. Neveu and M. Maver for technical assistance in some of the experiments and preparation of the figures.

■ ABBREVIATIONS USED

GDB, Chemical Universe Generated Database; nAChR, nicotinic acetylcholine receptor; AChBP, acetylcholine binding protein; NMDA, *N*-methyl D-aspartate; ROCS, rapid overlay of chemical structures; ZINC, zinc is not commercial

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