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Discovery of Potent Positive Allosteric Modulators of the α3β2 Nicotinic Acetylcholine Receptor by a Chemical Space Walk in ChEMBL

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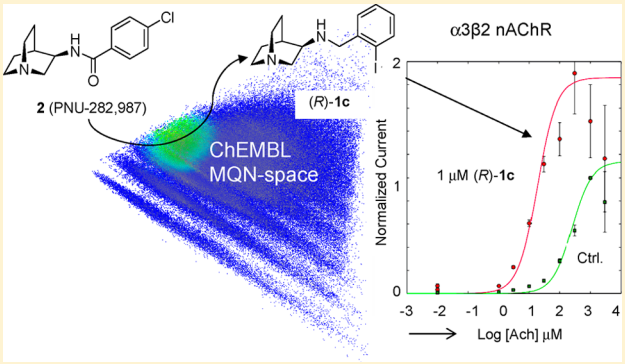
Supporting Information

ABSTRACT: While a plethora of ligands are known for the well studied α7 and α4β2 nicotinic acetylcholine receptor (nAChR), only very few ligands address the related α3β2 nAChR expressed in the central nervous system and at the neuromuscular junction. Starting with the public database ChEMBL organized in the chemical space of Molecular Quantum Numbers (MQN, a series of 42 integer value descriptors of molecular structure), a visual survey of nearest neighbors of the α7 nAChR partial agonist *N*-(3*R*)-1-azabicyclo[2.2.2]oct-3-yl-4-chlorobenzamide (PNU-282,987) pointed to *N*-(2-halobenzyl)-3-aminoquinuclidines as possible nAChR modulators. This simple "chemical space walk" was performed using a web-browser available at www.gdb.unibe.ch. Electrophysiological recordings revealed that these ligands represent a new and to date most potent class of positive allosteric modulators (PAMs) of the α3β2 nAChR, which also exert significant effects in vivo. The present discovery highlights the value of surveying chemical space neighbors of known drugs within public databases to uncover new pharmacology.



The cumulated efforts of drug discovery over the last century has produced millions of bioactive organic small molecules, and their number keeps increasing rapidly as documented in public databases such as PubChem[1] and ChEMBL.[2] For certain receptor types, thousands of small molecule modulators have been identified, suggesting a crowded chemical space where all possible opportunities have already been exploited. However, the extremely large number of possible organic molecules[3] and the existence of steep activity cliffs in many structure−activity landscapes[4] suggest that there may still be many opportunities to uncover new bioactive compounds even in such well-researched areas.

Herein we report the discovery of *N*-benzyl-3-aminoquinuclidines **1a**−**c** as positive allosteric modulators (PAMs) of the α3β2 nicotinic acetylcholine receptor (nAChR), a pharmacological profile rarely documented among thousands of known nAChR modulators[5−8] despite its therapeutic potential in the treatment of sarcopenia, the muscular atrophy that develops with aging. These PAMs were identified by a simple "chemical space walk" in ChEMBL consisting in surveying a few tens of compounds listed in the immediate chemical space vicinity of the prototypical 3-aminoquinuclidine based α7 nAChR agonist PNU-282,987 (**2**).[9] Two small yet previously overlooked structural changes were sufficient to trigger the appearance of the α3β2 nAChR PAM effect, namely, removal of the carboxamide oxygen atom of **2** and moving its *para*-chloro substituent to the *ortho* position (Figure 1).

The chemical space walking approach presented here follows the concept of chemical space vicinity as a guide to find bioactive analogues. Chemical space describes the ensemble of all possible organic small molecules in the context of drug discovery,[10−14] as well as property spaces in which dimensions correspond to numerical descriptors of the molecules and their properties.[15,16] We showed recently that proximity searches in MQN-space, a property space composed of 42 integer value descriptors of molecular structure called Molecular Quantum Numbers (MQN),[17] efficiently retrieve bioactive analogues in both retrospective studies and prospective ligand discovery applications.[18−23] Remarkably, most bioactive analogues of a drug are found in its immediate MQN-space vicinity within the city-block distance boundary $CBD_{MQN} \leq 12$. This distance

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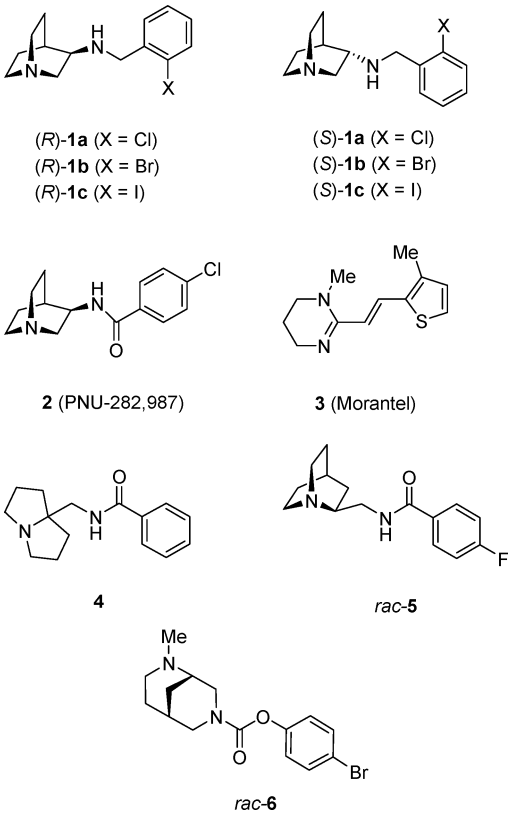

Figure 1. Structures of nAChR ligands.

constraint corresponds to thousands or even millions of analogues when searching very large databases such as the GDB. Here we used the fact that this distance constraint reduces the search to at most a few tens of compounds when applied to smaller databases such as ChEMBL or DrugBank because these databases only sparsely populate chemical space.[24] In addition, using an annotated database focused our search on compounds with already documented bioactivities, a valuable addition for addressing possible polypharmacology issues. The discovery of new pharmacology within ChEMBL in one of the arguably most crowded regions of chemical space with regards to nAChR modulators illustrates the value of chemical space walking, and clearly shows that chemical space is by far not exhausted.

RESULTS AND DISCUSSION

Virtual Screening. The cyclic amidine Morantel (**3**) was for several years the only documented $\alpha3\beta2$ nAChR PAM.[25−28] We recently surveyed analogues of the $\alpha7$ nAChR partial agonist **2** and identified three additional $\alpha3\beta2$ nAChR PAMs **4**−**6**.[29,30] However, the activities of our $\alpha3\beta2$ nAChR PAMs **4**−**6** were relatively modest and could not be improved by further structure−activity relationship studies, suggesting that a fresh approach should be tried to identify more potent analogues. Considering that **3** also acts as an $\alpha7$ nAChR agonist,[25] and that our own $\alpha3\beta2$ nAChR PAMs **4**−**6** were designed as analogues of the 3-aminoquinuclidine **2**, we specifically asked the question if 3-aminoquinuclidine type analogues of **2** might be identified displaying $\alpha3\beta2$ nAChR PAM activity.

Walking in the MQN-space vicinity ($CBD_{MQN} \leq 12$) of the $\alpha7$ nAChR partial agonist **2** within the database ChEMBL identified 115 compounds, 49 of which were 3-substituted quinuclidines (Figure 2). These MQN-nearest neighbors showed a varying degree of substructure similarity to **2** as measured by the Tanimoto similarity coefficient of a 1024 Daylight-type substructure fingerprint (T_{SF}, Figure 3). We were particularly intrigued by *N*-benzyl-3-amino-quinuclidines **1a** and **7** at rank 32 and 33 (CBD_{MQN} = 9 from **2**) because they featured a cationic secondary amine rather than an acyl type linker at position 3. Interestingly, diamines **1a** and **7** were also encountered at rank 40 and 68, respectively, of a different set of analogues of compound **2** retrieved using ChEMBL similarity searches (>0.70 threshold). The switch from a neutral carboxamido linker as encountered in essentially all quinuclidine based nAChR modulators to a cationic secondary amine linker only caused a small change in the MQN but represented a chemically significant difference which might strongly impact on the pharmacology. The 3-aminoquinuclidines **1a** and **7** were not annotated with any activity on nAChR or related ion channels, suggesting that it might be worth investigating them closer for this purpose.

Chemistry. Enantiomerically pure derivatives of **1** and **7** were prepared by reductive alkylation of (*R*)- and (*S*)-3-amino-quinuclidines with the corresponding aromatic aldehydes using sodium triacetoxyborohydride. The same approach was used to prepare further (*R*)- and (*S*)-3-aminoquinuclidine enantiomeric pairs with the following *N*-alkyl substituents: 2,4-dichlorobenzyl (**8**), 2,6-dichlorobenzyl (**9**), benzyl (**10**), 2-pyridyl-methyl (**11**), 4-pyridyl-methyl (**12**), methylene-dioxy-phenylmethyl (**13**), napthtylmethyl (**14**), dibenzyl (**15**), dihydroisoindole (**16**), and α-methylbenzyl (**17**−**19**) (Scheme 1). Additional *ortho*-substituted 3-benzylamino-quinuclidines were also prepared following the identification of **1a** as the most promising compound comprising the 2-bromo (**1b**), 2-iodo (**1c**), 2-methyl (**1d**), and 2-methoxy (**1e**) analogues.

Electrophysiology. Functional activity was measured using two electrode voltage clamp in *Xenopus* oocytes expressing recombinant human nAChR subtypes.[31] An initial screen was performed with **1a** and **7**−**9** (10 μM, *R* or *S* enantiomers) on both the $\alpha3\beta2$ nAChR and the $\alpha7$ nAChR because the $\alpha7$ subunit is closely related to the $\alpha3$ subunit and **2** and **3** are $\alpha7$ nAChR agonists. Both enantiomers of **1a** showed a strong $\alpha3\beta2$ nAChR PAM effect on the acetylcholine (ACh) signal, while the other six compounds **7**−**9** showed weak antagonistic effects at that receptor (Figure 4A/B). The ACh signal intensity increased up to 2-fold in the presence of **1a**, comparable to the best effect observed previously with **4**.[30] Both enantiomers of compound **1a** furthermore showed an agonist signal at the $\alpha7$ nAChR similarly to **2** and **3**.

Encouraged by these early results, we expanded the search to further *ortho*-substituted 3-benzylaminoquinuclidines **1b**−**e** on the one hand, and to a broader family of 3-benzylated aminoquiniclidines **10**−**19** on the other hand. This broader screening showed that the $\alpha3\beta2$ nAChR PAM effect at 10 μM occurred with other 2-halo derivatives (series **1a**−**c**), the 2-methyl (*S*)-**1d** and the 2-pyridyl analogues (*R*)- and (*S*)-**11** (Figure 4B). For **11**, the PAM effect was quite strong but also occurred on the $\alpha7$ nAChR. The (*R*)-enantiomers of the 2-halo compounds **1a**−**c** furthermore displayed $\alpha7$ nAChR agonist activities as well as a small but significant $\alpha3\beta2$ nAChR agonist activity which is absent in the parent compound **2**, and which to the best of our knowledge is unprecedented.

The 2-halo series **1a**−**c** also evoked an agonist current at the $\alpha3\beta4$ nAChR at 10 μM, again with a stronger effect with the (*R*)-enantiomers, but no activity at the $\alpha4\beta2$ nAChR,

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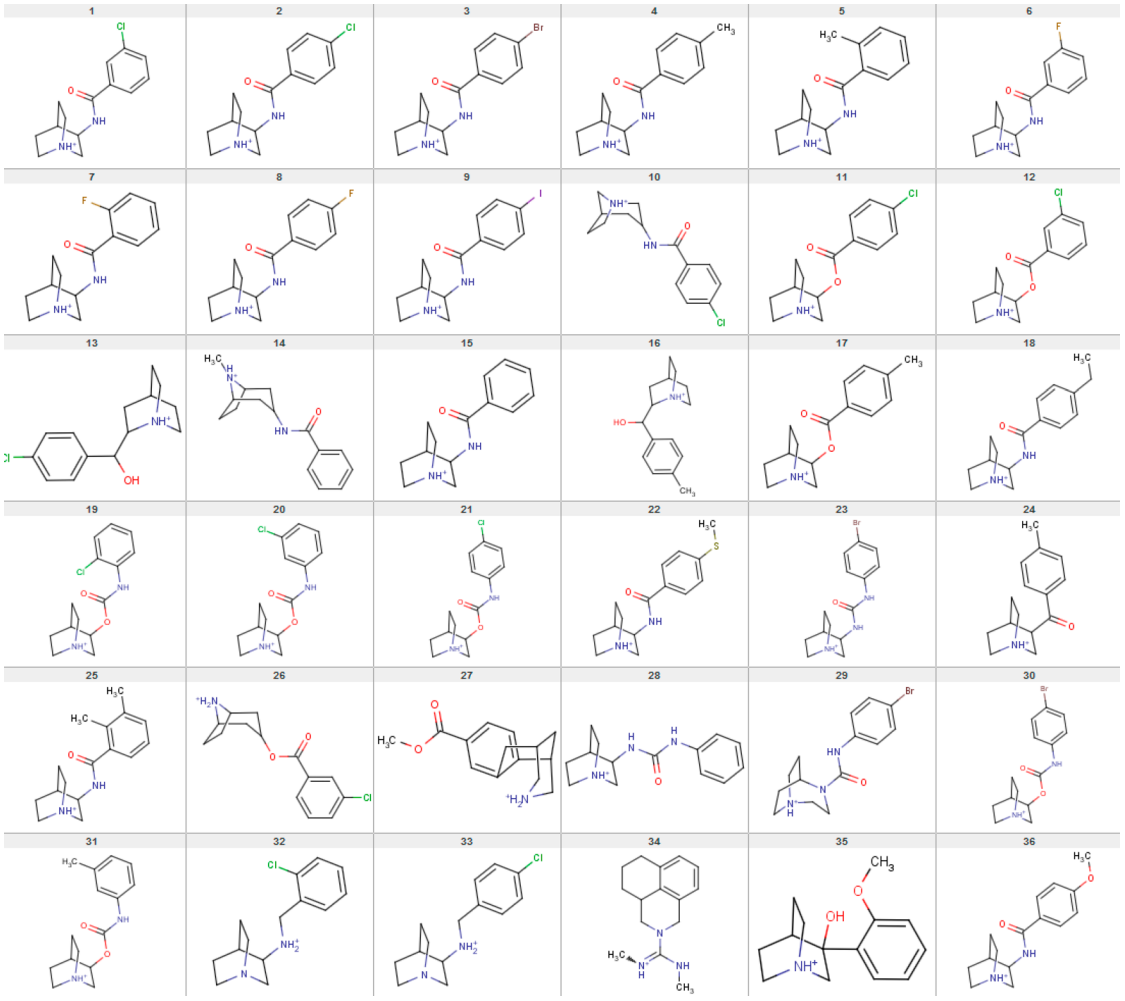

Figure 2. Structure of the first 36 MQN-neighbors of **2** in ChEMBL.

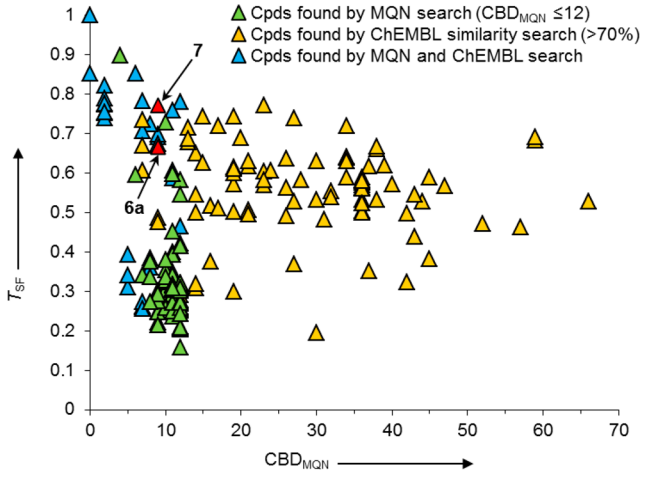

Figure 3. Nearest neighbors of **2** retrieved from ChEMBL using the online MQN search ($CBD_{MQN} \leq 12$, from www.gdb.unibe.ch) and using ChEMBL online similarity search (>70% similarity, www.ebi.ac.uk/chembl/). Compounds from both searches were compared in terms of CBD_{MQN} (x-axis) and substructure fingerprint similarity T_{SF} (y-axis). The compounds found only with MQN search are marked in green, the ones only found by ChEMBL similarity search are shown in yellow, and the compounds found with both searches are marked in blue. Compounds **1a** and **7** are highlighted in red. See Figure 2 for the structures of the first 36 MQN-neighbors of **2** in ChEMBL.

suggesting that the $\alpha3\beta2$ nAChR PAM effect is triggered by interaction with the $\alpha3$ rather than the $\beta2$ subunit (Figure 4C). This assignment was also supported by the observed agonistic effect of (*R*)-**1c** at 1 μM on the $\alpha7$ and $\alpha3\beta4$ nAChRs in addition to its $\alpha3\beta2$ nAChR PAM effect, and is consistent with the high degree of similarity between the $\alpha7$ and the $\alpha3$ subunits (Figure 4D).

Further investigations focused on (*R*)-**1c**, since this ligand showed the strongest $\alpha3\beta2$ nAChR PAM effect at 1 μM, and on (*S*)-**1a** as a slightly weaker but more selective $\alpha3\beta2$ nAChR PAM ligand. The full concentration activity profile of (*R*)-**1c** was recorded against the $\alpha3\beta2$, $\alpha7$ and the $\alpha3\beta4$ nAChR (Figure 5, Table 1). The IC_{50} curve at the $\alpha3\beta2$ nAChR showed up to 4-fold potentiation peaking at 1 μM, before fully desensitizing the receptor, while no potentiation was observed at the $\alpha7$ or the $\alpha3\beta4$ nAChR. (*R*)-**1c** exhibited partial agonist activity at all three nAChRs with EC_{50}'s found in the single digit micromolar range and relatively low evoked current amplitudes (10−14%). Most remarkably, the $\alpha3\beta2$ nAChR PAM effect of (*R*)-**1c** at 1 μM was sustained over a broad ACh concentration range. This sustained PAM effect was much stronger than that previously observed with compound **4**.[30] A similar sustained PAM effect was observed with (*S*)-**1a** at 3 μM (Figure 6). Overall (*S*)-**1a** was the most selective $\alpha3\beta2$ nAChR PAM ligand across the different measurements, and seemed the best suited for *in vivo* evaluation.

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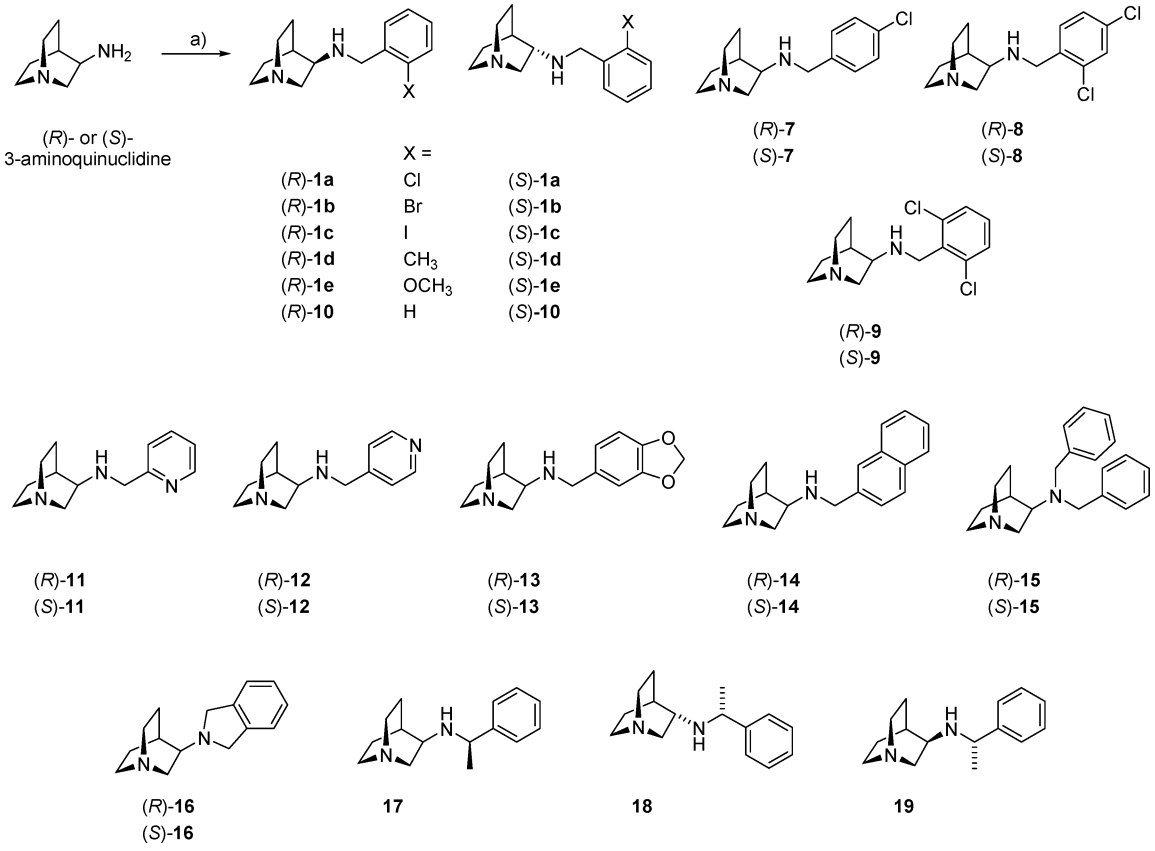
Scheme 1. Reductive Alkylation of Optically Pure 3-Aminoquinuclidines with Various Aldehydes[a]



[a]Conditions: (a) (i) Corresponding aldehyde, MeCN. (ii) $NaHB(OAc)_3$, MeCN, Ar, RT, 12 h, then preparative RP-HPLC.

As $\alpha7$ quinuclidine ligands were shown to cross-react with the $5HT_{3A}$ receptors by causing competitive inhibition with 5HT, it is important to assess if molecules acting as allosteric modulators at the $\alpha3\beta2$ interact with the orthosteric site of the $5HT_{3A}$ receptors. To probe the putative activity of compounds at the human $5HT_{3A}$ receptors, these proteins were expressed in *Xenopus* oocytes and a protocol of competition with pre- and coapplication was designed. These experiments revealed that (*R*)-**1c** and (*S*)-**1a** acted as $5HT_{3A}$ antagonists with IC_{50} = 4.5 ± 0.6 and 9.9 ± 1.6 μM, respectively, which is slightly weaker than the antagonist activity reported for **2** (IC_{50} = 4.5 μM).[9]

In Vivo Effects. Because the reference compound **2** is primarily reported to act on the CNS, a preliminary investigation of compound (*S*)-**1a** was carried out for CNS effects by recording body temperature and behavioral effects in BALB/c male mice upon intraperitoneal injection (i.p.) of the compound at 2.5 and 5 mg/kg in comparison with the parent compound **2** or vehicle (DMSO) (Figure 7). Compounds **2** and (*S*)-**1a** dose-dependently reduced body temperature, indicative of a rapid central mechanism (Figure 7A). The differential dose and time-dependence on body temperature by **2** and (*S*)-**1a** may reflect differences in pharmacokinetics. Exploration behavior in the open field was increased time-dependently by both **2** and (*S*)-**1a** (Figure 7B). The elevated plus maze test revealed that mice treated with (*S*)-**1a** after 1h at 2.5 mg/kg spent more time in the open arms, but to a lesser extent mice treated with **2** (Figure 7C). Since the PAM effects of (*S*)-**1a** are expected at the lower dose, data from open arms may reflect the distinct pharmacology between **2** and (*S*)-**1a**. Since this effect was absent after 2h we interpret it as pro-stress behavior rather than a general anxiolytic effect. A recent study has shown differential effects of **2** on stress and anxiety in an open field depending on genetic variability.[32]

Only mice treated with 5 mg/kg of (*S*)-**1a** presented significant impairment of motor control in the rotarod performance test (Figure 7D). A pronounced anxiety-like behavior was observed in the light-dark preference test with both doses of (*S*)-**1a** (Figure 7E). This is consistent with the overall reduction of exploration behavior observed with 5 mg/kg (*S*)-**1a** but not with **2** (Figure 7B), and in agreement with studies showing the positive involvement of nAChRs in anxiety.[33] For example, varenicline, a partial agonist at nAChRs, was found to produce anxiolytic effects in rodent models of anxiety.[34]

Interestingly, the anxiogenic effect of (*S*)-**1a** observed in the light-dark preference test was not reproduced in the hole-board test. BALBc male mice receiving 2.5 mg/kg of (*S*)-**1a** showed increased head dipping activity (Figure 7F), indicative of an anxiolytic effect, thus reflecting an overall more complex modulation of anxiety behavior. How $\alpha3\beta2$ nAChRs modulate anxiety in BALB/c mice, as suggested by our preliminary findings with the $\alpha3\beta2$ nAChR PAM (*S*)-**1a**, needs to be tested in independent experiments. Additional studies will be necessary to determine whether the hole-board or elevated plus maze tests are suitable to detect specific pharmacological effects of $\alpha3\beta2$ nAChR modulators.

Overall, the effects observed upon i.p. administration of (*S*)-**1a** indicated that the compound is able to cross the blood-brain barrier, and exerts significant CNS related biological effects, part of which might reflect potentiation/agonistic or even

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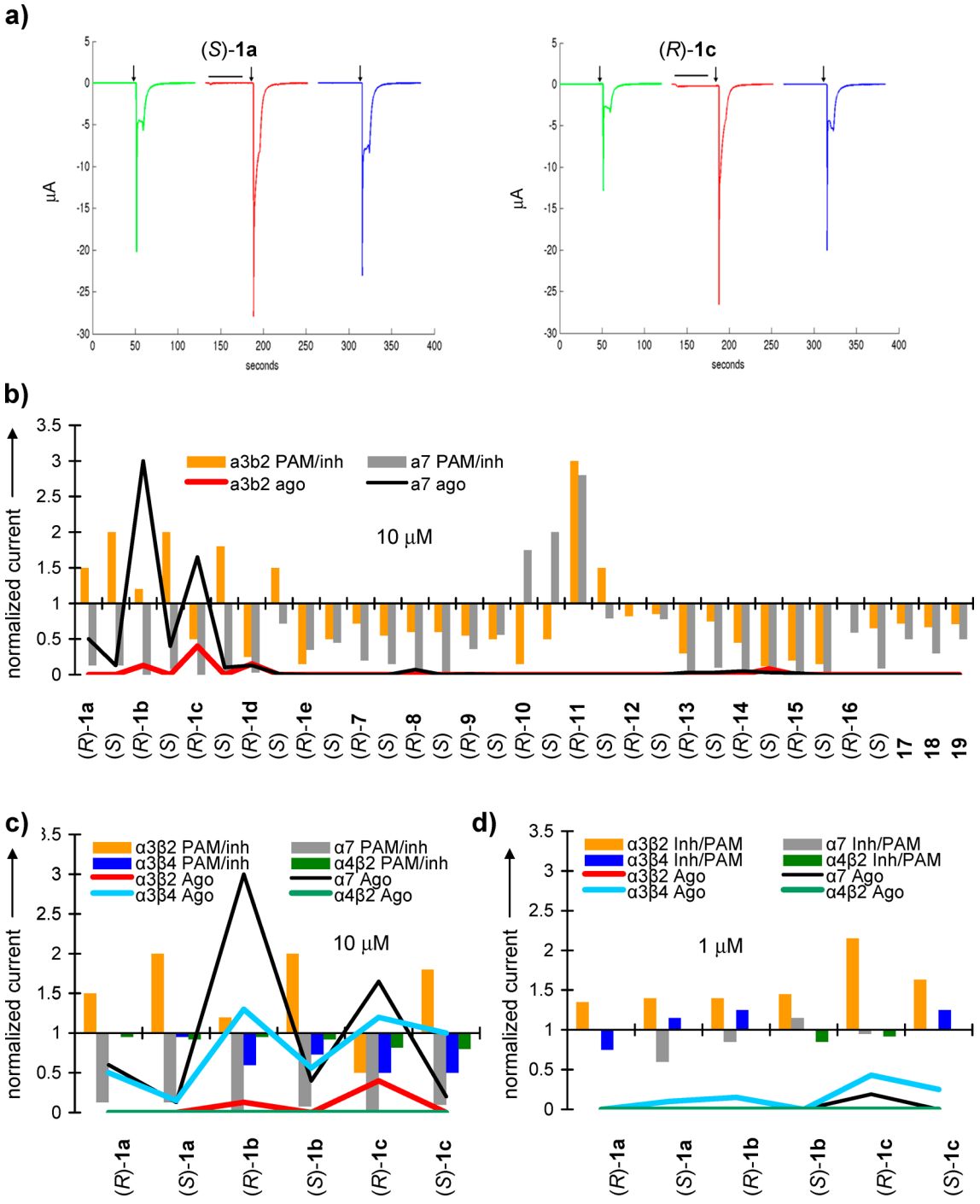

Figure 4. Electrophysiology data for 3-benzylaminoquinuclidines at various human nAChR subtypes. (A) Selected electrophysiology traces with (*S*)-**1a** (1 μM) and (*R*)-**1c** (1 μM). Cells expressing the human $\alpha3\beta2$ nAChR were challenged first with a reference ACh pulse (200 μM, 5 s, green trace, arrows indicate the timing of the onset of the ACh application), and then the response to the incubation of the test compound at 1 μM (red trace, bare above the trace indicate the 30 s incubation, agonist effect) was recorded followed by the response to the same pulse of ACh as the initial one (inhibition or PAM effect). At 100 s after the second ACh pulse, a third ACh test pulse was applied (blue trace). (B) Screening for the effects of compounds at 10 μM at the human $\alpha3\beta2$ and $\alpha7$ nAChR following the procedure described in (A). Ago: ratio of the signal recorded upon addition of compound versus the reference ACh initial signal. Inh/PAM: ratio of the 2nd ACh signal (after 30 s compound incubation) versus the reference ACh initial signal, showing either inhibition (<1), PAM ($\gg$ 1), or no effect (= 1, horizontal line). (C, D) The same experimental setup as in (A) was used to profile the 2-halobenzyl series **1a**–**c** at different nAChR subtypes at 10 μM (C) and 1 μM (D).

desensitization effects on the $\alpha3\beta2$ and/or other related nAChRs as documented above, although additional effects on other neuroactive targets cannot be excluded at this point. In the case of (*S*)-**1a**, it should be noted that the six targets documented for this compound in ChEMBL are unrelated to such behavioral effects (human DNA polymerase, histone-lysine *N*-methyltransferase, lysosomal α-glucosidase and vitamin D, a microbial fructose-1,6-bisphosphate aldolase and a viral protein).

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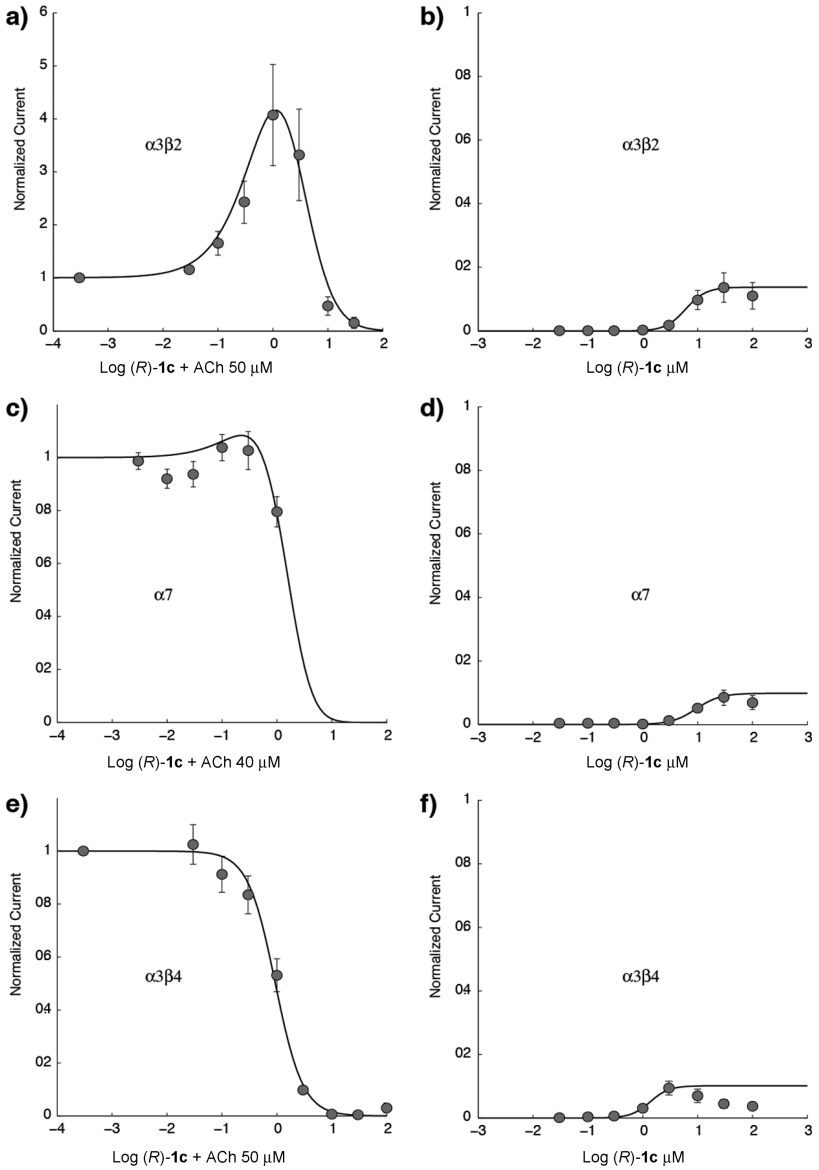

Figure 5. Concentration–inhibition and concentration–activation curves of (*R*)-**1c** on various human nAChR subtypes expressed in *Xenopus* oocytes ($\alpha3\beta2$ for A and B; $\alpha7$ for C and D; $\alpha3\beta4$ for E and F). Inhibition curves (A, C, and E) were measured by applying brief pulses of ACh (50 μM for A and E; 40 μM for C; they were used for normalization) in the presence of increasing concentrations of compound (*R*)-**1c**. The dose–response curves (B, D, and F) were obtained by measuring an initial brief reference pulse of ACh (1280 μM that was used for normalization) followed by applying a series of pulses of increasing concentration of compound (*R*)-**1c** (see Table 1). Bars indicate SEM (for A, $n = 6$; B, $n = 5$; C, $n = 4$; D, $n = 5$; E, $n = 5$; and F, $n = 5$). Note that, in most cells expressing the $\alpha3\beta4$ receptor, exposure to (*R*)-**1c** caused only inhibition such as that shown in (E); however, potentiation was also observed in a few cells (data not shown). These differences might be caused by heterogeneity in receptor expression and especially in the ratio of α- versus β-subunits expressed by the different cells.

Table 1. Electrophysiology Measurements of (*R*)-1c on *Xenopus* Oocytes Expressing Different Human nAChR Subtypes[a]

human nAChR	IC_{50} [μM]	EC_{50} [μM]
$\alpha3\beta2$	2.57 ± 0.09	6.07 ± 0.89 (14%)
$\alpha7$	2.78 ± 0.20	9.39 ± 1.65 (10%)
$\alpha3\beta4$	0.94 ± 0.21	1.36 ± 0.01 (10%)

[a] The IC_{50} was determined by applying brief pulses of ACh (50 μM for $\alpha3\beta2$ and $\alpha3\beta4$; 40 μM for $\alpha7$) in the presence of increasing concentrations of compound (*R*)-**1c**. The EC_{50} was measured by evoking an initial brief reference pulse of ACh (1280 μM) followed by applying a series of pulses of increasing concentration of compound (*R*)-**1c** (see Figure 5 for SEM).

CONCLUSION

The experiments above demonstrate the identification of *ortho*-halogenated 3-benzylamino quinuclidines **1a–c** as a new class of $\alpha3\beta2$ nAChR PAMs. The initial ligand selection was guided by a chemical space walk in the chemical space of the ChEMBL database organized by MQN for fast searching of nearest neighbors as publicly available at www.gdb.unibe.ch. In this chemical space walk, a limited number of molecules of the known $\alpha7$ nAChR partial agonist **2** within the distance boundary $CBD_{MQN} \leq 12$ were surveyed visually to select compounds for experimental evaluation. This straightforward approach should be generally useful to uncover new pharmacology within public databases.

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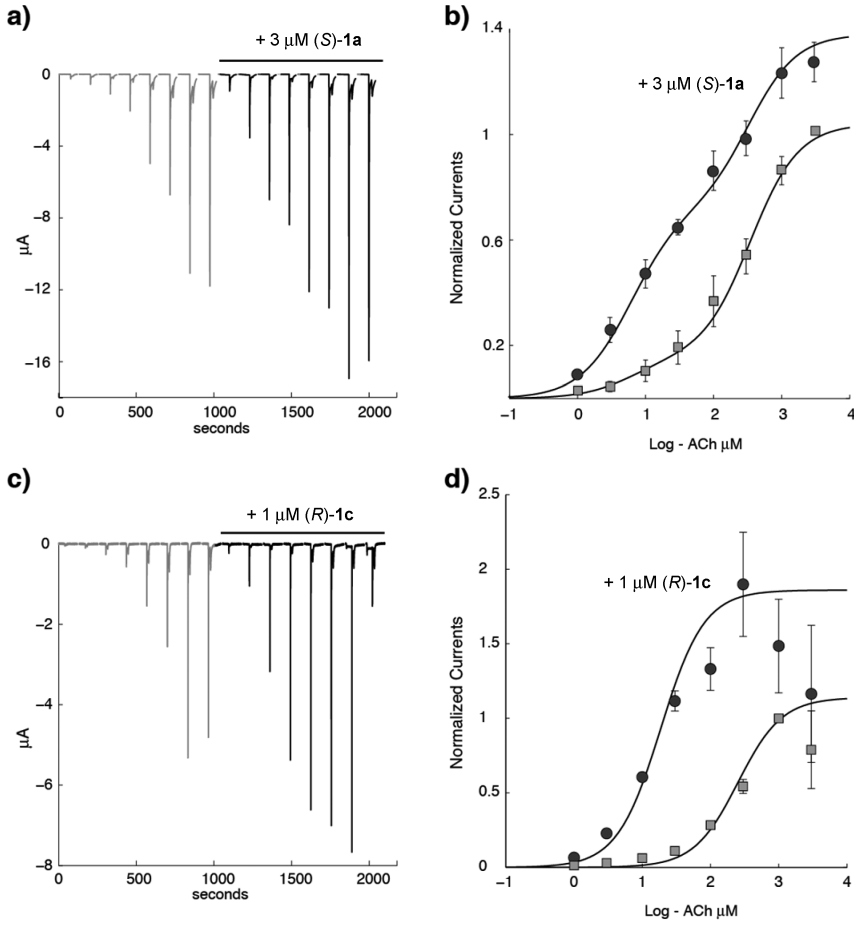

Figure 6. Positive allosteric modulation of (*S*)-**1a** (at 3 μM) and (*R*)-**1c** (at 1 μM) at the human $\alpha3\beta2$ nAChR expressed in *Xenopus* oocytes. (A, C) Currents evoked by brief ACh pulses of different concentrations were recorded in the absence (gray lines) and presence (dark lines) of compound at the human $\alpha3\beta2$ nAChR. The positive allosteric modulator was continuously applied during the second part of the experiment (indicated by the bar above the traces) at a concentration of 3 μM (*S*)-**1a** and at 1 μM for (R)-**1c**. (B) Plot of the peak inward current evoked by ACh in the absence (squares) and presence (circles) of the PAM (see A). The continuous gray line describes the best fit obtained with the Hill equation presenting an EC_{50} of 349 μM ± 72 μM (n_H = 1.23 ± 0.95) for ACh alone. The dark line is the best fit obtained with the Hill equation presenting an EC_{50} of 6.30 ± 1.09 μM (n_H = 1.16 ± 0.20) for a sustained concentration of 3 μM (*S*)-**6a** and application of increasing concentrations of ACh. Compound (*S*)-**1a** causes a shift of the concentration activation curve toward higher potency and an increase of the maximal current amplitude. Bars indicate SEM (n = 5). (D) Plot of the dose−response curves recorded in absence or presence of the compound (*R*)-**1c** (see C). In control condition the EC_{50} was 253 ± 51 μM (n_H = 1.35 ± 0.09) and in presence of 1 μM (*R*)-**1c** the EC_{50} was shifted to 18.6 ± 3.08 μM (n_H = 1.36 ± 0.01). Note the concomitant increase in maximal evoked current observed in the presence of the PAM. Bars indicate SEM (n = 6).

The aminoquinuclidine derivatives **1a−c** are closely related to the $\alpha7$ agonist **2** used as reference molecule, yet display a distinctive PAM effect on the $\alpha3\beta2$ nAChR which does not occur with **2**. The occurrence of the PAM effect in both enantiomeric series of **1a−c** is somewhat surprising, yet parallels the fact that analogues of **2** derived from the enantiomeric (*S*)-3-aminoquinuclidine such as RG4387/MEM3454 have also been reported as $\alpha7$ nAChR agonists.[35] Our observation therefore further confirms that both enantiomers of 3-aminoquinuclidine type ligands may act as nAChR modulators. The most potent compound was (*R*)-**1c**, which exhibited an agonist activity at the $\alpha3\beta2$ nAChR and a strong PAM activity with up to 10-fold potentiation of the ACh response in the concentration-activation curve, which is a much stronger effect than with the previously reported $\alpha3\beta2$ nAChR **3−6**. The somewhat less active but more selective $\alpha3\beta2$ nAChR PAM (*S*)-**1a** was investigated in different behavioral models in BALB/c male mice and induced significant behavioral effects indicating CNS activity and possibly mediated via PAM at $\alpha3\beta2$ nAChR. Further studies will be necessary to explore the pharmacological profiles of these new and potent $\alpha3\beta2$ nAChR PAMs.

METHODS

Virtual Screening. The ChEMBL "chemical space walk" in search of analogues of **2** was performed using a dedicated ChEMBL MQN-browser (freely available at www.gdb.unibe.ch) constructed as described previously for other databases.[19−21,23] The ChEMBL database was downloaded in June 2012 and processed in SMILES format. Ionization state of each molecule was adjusted to pH 7.4, and counterions were removed. Molecular Quantum Numbers were calculated for each molecule using an in-house developed Java program which is utilizing Java Chemistry library (JChem) from Chemaxon, Ltd. as a starting point. In the MQN-browser search, the ChEMBL database is preorganized for fast searching by city-block distance CBD_{MQN} as similarity measure. Substructure similarity calculations were performed using a Daylight-type 1024 bit hash fingerprint using the Tanimoto similarity (T_{SF}) coefficient.[36]

Synthesis. *General.* Reagents were purchased in the highest purity available from Fluka, Sigma Aldrich, and Alfa Aesar. Dry solvents were obtained directly from a solvent drying system. High resolution mass

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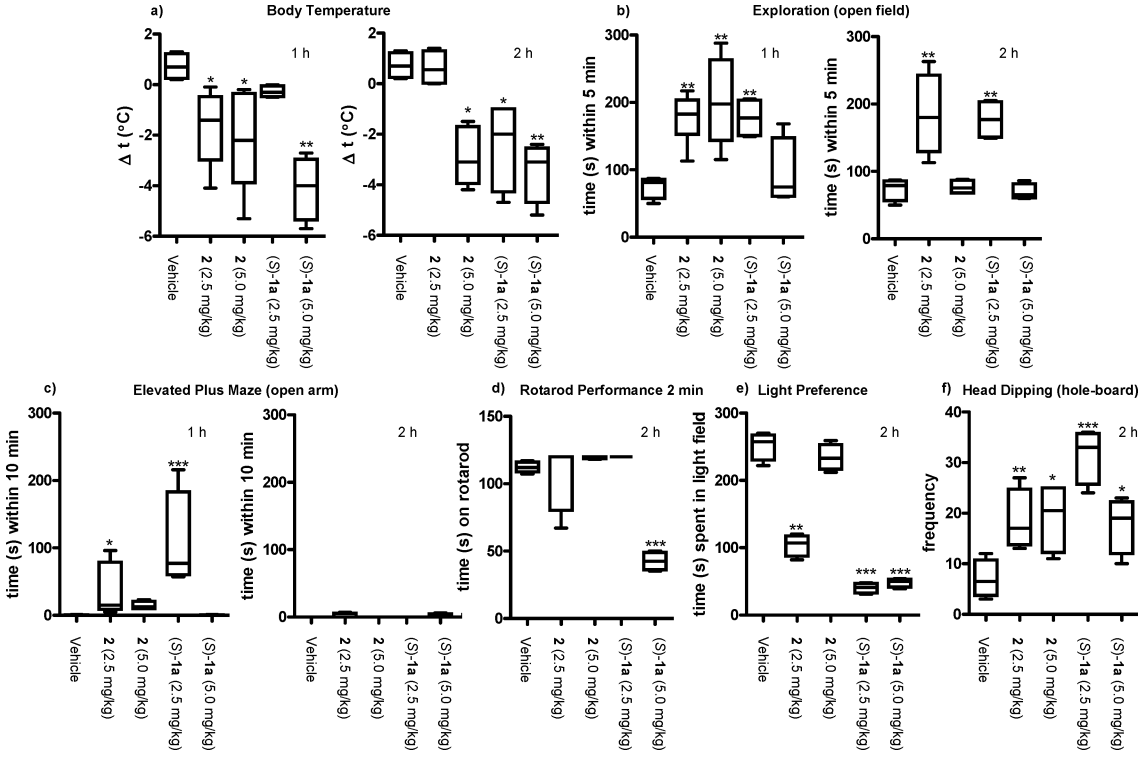

Figure 7. Box and whisker plots showing *in vivo* effects recorded with (*S*)-**1a** and **2** after i.p. in BALB/c male mice. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. $n = 3–6$.

spectra were provided by the "Service of Mass Spectrometry" at the Department of Chemistry and Biochemistry in Bern and were obtained by electron spray ionization in the positive ion mode recorded on a Thermo Scientific LTQ OrbitrapXL. ^{1}H and ^{13}C NMR spectra (MeOD or d_6-DMSO) were obtained at 300 or 75 MHz, respectively (Bruker AV300). ^{1}H and ^{13}C chemical shifts are quoted relative to solvent signals.[37] Compounds purity was determined by analytical RP-UHPLC. All compounds had purities ≥ 95%. Analytical RP-UHPLC was performed on a Dionex UltiMate 3000 RSLC system (DAD-3000 RS Photodiode Array Detector) using a Dionex Acclaim RSLC 120 column (C18, 3.0 × 50 mm, particle size 2.2 μm, 120 Å pore size) at a flow rate of 1.2 mL min^{-1} operating at 214 nm. Data recording and processing was done with Dionex Chromeleon Management System Version 6.8. Preparative RP-HPLC was performed with a Waters Delta Prep LC4000 Chromatography System using a Reprospher 100 (C18-DE, 30 × 100 mm, particle size 5 μm, 100 Å pore size) column from Dr. Maisch GmbH and a Waters 489 Tunable Absorbance Detector operating at 214 nm. Eluents for analytical RP-UHPLC were as follows: A: milliQ-deionized water with 0.1% TFA and D: HPLC-grade acetonitrile/miliQ-deionized water (9/1) with 0.1% TFA. The gradient applied was in 2.2 min from 100% A to 100% D, unless otherwise mentioned. Eluents for RP-HPLC were as follows: A: milliQ-deionized water with 0.1% TFA and D: HPLC-grade acetonitrile/miliQ-deionized water (2/3) with 0.1%TFA.

General Procedure for Reductive Amination.[38] (*R* or *S*)-3-Aminoquinuclidine dihydrochloride (1 equiv.) and the corresponding benzaldehyde (1 equiv.) were mixed in dry acetonitrile and stirred at RT under Ar atmosphere for up to 1 h. The reaction mixture was treated with sodium triacetoxyborohydride (3 equiv.) and stirred overnight. The mixture was concentrated, and the residue was quenched by a combination of RP-HPLC eluents A and D and directly applied on RP-HPLC.

Aldehydes used were 2-chlorobenzaldehyde, 2-bromobenzaldehyde, 2-iodobenzaldehyde, 2-methylbenzaldehyde, 2-methoxybenzaldehyde, 4-chlorobenzaldehyde, 2,4-dichlorobenzaldehyde, 2,6-dichlorobenzaldehyde, benzaldehyde, 2-pyridinecarboxaldehyde, 4-pyridinecarboxaldehyde, 1,3-benzodioxole-5-carboxaldehyde, 2-naphthaldehyde, and *ortho*-phthalic dicarboxaldehyde.

*(R)-N-(2-Chlorobenzyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((R)-**1a**).* The residue obtained from the general procedure for reductive amination was dissolved in A:D/1:1 (30 mL) and purified by RP-HPLC (from 100% A to 65% A and 35% D in 30 min, 30 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*R*)-**1a** (83 mg, 0.17 mmol, 35%) as a white solid. ^{1}H NMR (300 MHz, MeOD): δ = 7.67–7.61 (m, 1H), 7.58–7.52 (m, 1H), 7.51–7.38 (m, 2H), 4.56–4.30 (m, 2H), 4.05–3.80 (m, 2H), 3.54–3.34 (m, 5H), 2.69–2.56 (m, 1H), 2.34–2.11 (m, 2H), 2.11–1.95 (m, 2H). ^{13}C NMR (75 MHz, MeOD): δ = 135.9, 133.2, 132.4, 131.4, 131.2, 129.0, 54.0, 51.0, 47.6, 47.2, 23.6, 22.8, 17.7. HR-MS (ESI) *m/z* calculated for $C_{14}H_{20}N_2{}^{35}Cl$ (M+H)$^+$ 251.1310, found 251.1306. RP-UHPLC: t_R = 1.2 min, RP-HPLC: t_R = 19.8 min. M_P = 144.3–144.4 °C. (*R*)-**1a** (2.4 mg) was dissolved in DMSO (500 μL) to yield a 10 mM stock solution for electrophysiology.

*(S)-N-(2-Chlorobenzyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((S)-**1a**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A:D/1:1 (30 mL) and purified by RP-HPLC (from 100% A to 65% A and 35% D in 30 min, 30 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*S*)-**1a** (71 mg, 0.14 mmol, 30%) as a white solid. ^{1}H NMR (300 MHz, MeOD): δ = 7.67–7.61 (m, 1H), 7.58–7.52 (m, 1H), 7.51–7.38 (m, 2H), 4.52–4.28 (m, 2H), 4.05–3.80 (m, 2H), 3.54–3.34 (m, 5H), 2.69–2.56 (m, 1H), 2.34–2.11 (m, 2H), 2.11–1.95 (m, 2H). ^{13}C NMR (75 MHz, MeOD): δ = 135.9, 133.2, 132.4, 131.4, 131.2, 129.0, 54.0, 51.0, 47.6, 47.2, 23.6, 22.8, 17.7. HR-MS (+ESI) *m/z* calculated for $C_{14}H_{20}N_2{}^{35}Cl$ (M+H)$^+$ 251.1310, found 251.1307. RP-UHPLC: t_R = 1.2 min, RP-HPLC: t_R = 19.8 min. M_P = 143.3–144.4 °C. (*S*)-**1a** (2.4 mg) was dissolved in DMSO (500 μL) to yield a 10 mM stock solution for electrophysiology.

*(R)-N-(2-Bromobenzyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((R)-**1b**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A:D/1:1 (8 mL) and purified by RP-HPLC (from 100% A to 40% A and 60% D in 25 min, 40 mL·min^{-1}). The purest fractions

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(>95%) were collected and lyophilized, yielding (*R*)-**1b** (196 mg, 0.37 mmol, 37%) as a colorless solid. ^{1}H NMR (300 MHz, d_6-DMSO) δ = 7.77−7.60 (m, 2H), 7.50−7.29 (m, 2H), 4.34 (dd, *J* = 12 Hz, 9 Hz, 2H), 3.98−3.67 (m, 2H), 3.51−3.11 (m, 5H), 2.68−2.55 (m, 1H), 2.33−2.15 (m, 1H), 2.10−1.76 (m, 3H). ^{13}C NMR (75 MHz, d_6-DMSO) δ = 132.8, 131.8, 131.6, 131.0, 128.0, 124.3, 52.3, 48.7, 48.6, 45.4, 45.0, 21.6, 21.5, 16.3. HR-MS calculated for $[M + H]^+$ $C_{14}H_{20}N_2{}^{79}Br$ 295.0799, found 295.0799. RP-UHPLC: t_R = 1.2 min, RP-HPLC: t_R = 7.5 min. M_P = 155.3−157.8 °C. (*R*)-**1b** (3.72 mg) was dissolved in DMSO (711 μL) to yield a 10 mM stock solution for electrophysiology.

*(S)-N-(2-Bromobenzyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((S)-**1b**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A:D/1:1 (8 mL) and purified by RP-HPLC (from 100% A to 40% A and 60% D in 25 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*S*)-**1b** (202 mg, 0.38 mmol, 37%) as a colorless solid. ^{1}H NMR (300 MHz, d_6-DMSO) δ = 7.76−7.66 (m, 2H), 7.51−7.41 (m, 1H), 7.41−7.32 (m, 1H), 4.34 (dd, *J* = 12 Hz, 9 Hz, 2H), 3.93−3.71 (m, 2H), 3.44−3.14 (m, 5H), 2.64−2.55 (m, 1H), 2.32−2.13 (s, 1H), 2.10−1.74 (m, 3H). ^{13}C NMR (75 MHz, d_6-DMSO) δ = 132.9, 131.8, 131.6, 131.1, 128.0, 124.4, 52.3, 48.6, 48.6, 45.4, 45.0, 21.6, 21.5, 16.3. HR-MS calculated for $[M + H]^+$ $C_{14}H_{20}N_2{}^{79}Br$ 295.0799, found 295.0799. RP-UHPLC: t_R = 1.2 min, RP-HPLC: t_R = 7.2 min. M_P = 155.3−157.1 °C. (*S*)-**1b** (3.36 mg) was dissolved in DMSO (642 μL) to yield a 10 mM stock solution for electrophysiology.

*(R)-N-(2-Iodobenzyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((R)-**1c**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A:D/7:4 (11 mL) and purified by RP-HPLC (from 100% A to 40% A and 60% D in 25 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*R*)-**1c** (101 mg, 0.35 mmol, 35%) as a transparent, sticky solid. ^{1}H NMR (300 MHz, MeOD) δ = 8.07−7.94 (m, 1H), 7.69−7.60 (m, 1H), 7.57−7.44 (m, 1H), 7.29−7.13 (m, 1H), 5.22−4.75 (s, 1H), 4.46 (dd, *J* = 15 Hz, 9 Hz, 2H), 4.12−3.82 (m, 2H), 3.58−3.32 (m, 5H), 2.81−2.65 (m, 1H), 2.39−1.93 (m, 4H). ^{13}C NMR (75 MHz, MeOD) δ = 141.5, 135.5, 132.6, 132.4, 130.4, 101.8, 55.7, 54.3, 50.4, 47.4, 47.1, 23.4, 22.8, 17.8. HR-MS calculated for $[M + H]^+$ $C_{14}H_{20}N_2I$ 343.0666, found 343.0668. RP-UHPLC: t_R = 1.3 min, RP-HPLC: t_R = 6.6 min. (*R*)-**1c** (3.68 mg) was dissolved in DMSO (645 μL) to yield a 10 mM stock solution for electrophysiology.

*(S)-N-(2-Iodobenzyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((S)-**1c**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A:D/3:5 (9 mL) and purified by RP-HPLC (from 100% A to 40% A and 60% D in 25 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*S*)-**1c** (107 mg, 0.37 mmol, 37%) as a transparent, sticky solid. ^{1}H NMR (300 MHz, MeOD) δ = 8.06−7.94 (m, 1H), 7.72−7.62 (m, 1H), 7.55−7.43 (m, 1H), 7.27−7.11 (m, 1H), 5.18−4.77 (s, 1H), 4.45 (dd, *J* = 12 Hz, 9 Hz, 2H), 4.09−3.81 (m, 2H), 3.60−3.33 (m, 5H), 2.80−2.64 (m, 1H), 2.41−1.95 (m, 4H). ^{13}C NMR (75 MHz, MeOD) δ = 141.4, 135.7, 132.6, 132.4, 130.4, 101.8, 55.7, 54.2, 50.5, 47.4, 47.1, 23.4, 22.8, 17.8. HR-MS calculated for $[M + H]^+$ $C_{14}H_{20}N_2I$ 343.0666, found 343.0666. RP-UHPLC: t_R = 1.3 min, RP-HPLC: t_R = 6.7 min. (*S*)-**1c** (3.02 mg) was dissolved in DMSO (529 μL) to yield a 10 mM stock solution for electrophysiology.

*(R)-N-(2-Methylbenzyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((R)-**1d**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A (7 mL) and purified by RP-HPLC (from 100% A to 40% A and 60% D in 25 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*R*)-**1d** (139 mg, 0.30 mmol, 30%) as a colorless solid. ^{1}H NMR (300 MHz, MeOD) δ = 7.57−7.42 (m, 1H), 7.39−7.15 (m, 3H), 5.13 (s, 1H), 4.36 (dd, *J* = 12.0, 6.0 Hz, 2H), 4.12−3.99 (m, 1H), 3.96−3.81 (m, 1H), 3.58−3.28 (m, 5H), 2.75−2.64 (dd, *J* = 6.0, 3.0 Hz, 1H), 2.52−2.41 (s, 3H), 2.36−1.95 (m, 4H). ^{13}C NMR (75 MHz, MeOD) δ = 139.0, 132.3, 131.7, 131.0, 130.5, 127.9, 54.3, 50.2, 50.0, 49.7, 49.4, 49.1, 48.8, 48.5, 48.3, 47.5, 47.2, 23.4, 23.0, 19.4, 17.8. HR-MS calculated for $[M + H]^+$ $C_{15}H_{23}N_2$ 231.1856, found 231.1851. RP-UHPLC: t_R = 1.2 min, RP-HPLC: t_R = 6.1 min. M_P = 152.8−155.4 °C. (*R*)-**1d** (2.90 mg) was dissolved in DMSO (633 μL) to yield a 10 mM stock solution for electrophysiology.

*(S)-N-(2-Methylbenzyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((S)-**1d**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A (7 mL) and purified by RP-HPLC (from 100% A to 40% A and 60% D in 25 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*S*)-**1d** (49 mg, 0.11 mmol, 11%) as a colorless solid. ^{1}H NMR (300 MHz, MeOD) δ = 7.52−7.46 (m, 1H), 7.38−7.24 (m, 3H), 5.08−4.80 (s, 5H), 4.34 (dd, *J* = 14 Hz, 7.4 Hz, 2H), 4.07−3.96 (m, 1H), 3.94−3.81 (m, 1H), 3.54−3.33 (m, 5H), 2.71−2.64 (m, 1H), 2.45 (s, 3H), 2.31−2.12 (m, 2H), 2.12−1.97 (m, 2H). ^{13}C NMR (75 MHz, MeOD) δ = 138.9, 132.2, 131.5, 131.0, 130.6, 127.8, 54.2, 50.4, 48.6, 47.5, 47.2, 23.4, 22.9, 19.3, 17.7. HR-MS calculated for $[M + H]^+$ $C_{15}H_{23}N_2$ 231.1856, found 231.1855. RP-UHPLC: t_R = 1.2 min, RP-HPLC: t_R = 7.1 min. M_P = 152.3−154.4 °C. (*S*)-**1d** (1.99 mg) was dissolved in DMSO (434 μL) to yield a 10 mM stock solution for electrophysiology.

*(R)-N-(2-Methoxybenzyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((R)-**1e**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A (7 mL) and purified by RP-HPLC (from 100% A to 40% A and 60% D in 25 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*R*)-**1e** (196 mg, 0.82 mmol, 82%) as a transparent, hard solid. ^{1}H NMR (300 MHz, MeOD) δ = 7.52−7.40 (m, 2H), 7.15−7.06 (m, 1H), 7.06−6.97 (m, 1H), 5.14−4.82 (s, 1H), 4.38 (d, *J* = 12 Hz, 1H), 4.25 (d, *J* = 12 Hz, 1H), 3.96−3.76 (s, 5H), 3.53−3.31 (m, 5H), 2.71−2.64 (m, 1H), 2.30−1.96 (m, 4H). ^{13}C NMR (75 MHz, MeOD) δ = 159.4, 133.0, 132.9, 122.1, 119.7, 112.1, 56.2, 53.4, 50.1, 47.3, 47.0, 46.4, 23.1, 22.7, 17.6. HR-MS calculated for $[M + H]^+$ $C_{15}H_{23}ON_2$ 247.1805, found 247.1801. RP-UHPLC: t_R = 1.2 min, RP-HPLC: t_R = 4.9 min. (*R*)-**1e** (2.19 mg) was dissolved in DMSO (462 μL) to yield a 10 mM stock solution for electrophysiology.

*(S)-N-(2-Methoxybenzyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((S)-**1e**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A (7 mL) and purified by RP-HPLC (from 100% A to 50% A and 50% D in 20 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*S*)-**1e** (163 mg, 0.68 mmol, 68%) as a transparent, hard solid. ^{1}H NMR (300 MHz, MeOD) δ = 7.51−7.41 (m, 2H), 7.14−7.06 (m, 1H), 7.07−6.98 (m, 1H), 5.27−4.76 (s, 1H), 4.37 (d, *J* = 12 Hz, 1H), 4.26 (d, *J* = 12 Hz, 1H), 3.97−3.76 (m, 5H), 3.55−3.32 (m, 5H), 2.71−2.65 (m, 1H), 2.30−1.97 (m, 4H). ^{13}C NMR (75 MHz, MeOD) δ = 159.4, 133.1, 133.0, 122.1, 119.7, 112.1, 56.1, 53.3, 50.1, 47.3, 47.0, 46.4, 23.1, 22.7, 17.6. HR-MS calculated for $[M + H]^+$ $C_{15}H_{23}ON_2$ 247.1805, found 247.1801. RP-UHPLC: t_R = 1.2 min, RP-HPLC: t_R = 5.1 min. (*S*)-**1e** (1.97 mg) was dissolved in DMSO (415 μL) to yield a 10 mM stock solution for electrophysiology.

*(R)-N-(4-Chlorobenzyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((R)-**7**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A:D/1:1 (15 mL) and purified by RP-HPLC (from 100% A to 85% A and 15% D in 10 min, then in 40 min to 100% D, 25 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*R*)-**7** (15 mg, 0.054 mmol, 54%) as a white solid. ^{1}H NMR (300 MHz, MeOD): δ = 7.57−7.52 (m, 2H), 7.50−7.45 (m, 2H), 4.36−4.24 (m, 2H), 3.97−3.86 (m, 1H), 3.85−3.75 (m, 1H), 3.56−3.31 (m, 5H), 2.63−2.56 (dd, *J* = 6.1, 3.1 Hz, 1H), 2.28−2.10 (m, 2H), 2.10−1.96 (m, 2H). ^{13}C NMR (75 MHz, MeOD): δ = 137.0, 133.3, 131.1, 130.4, 53.7, 50.5, 50.4, 47.5, 47.1, 23.6, 22.9, 17.7. HR-MS (+ ESI) *m*/*z* calculated for $C_{14}H_{20}N_2{}^{35}Cl$ $(M+H)^+$ 251.1310, found 251.1311. RP-UHPLC: t_R = 1.3 min, RP-HPLC: t_R = 17.1 min. M_P = 132.4−132.5 °C. (*R*)-**7** (2.39 mg) was dissolved in DMSO (500 μL) to yield a 10 mM stock solution for electrophysiology.

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*(S)-N-(4-Chlorobenzyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((S)-**7**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A:D/1:1 (15 mL) and purified by RP-HPLC (from 100% A to 85% A and 15% D in 10 min, then in 40 min to 100% D, 25 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*S*)-**7** (27 mg, 0.056 mmol, 56%) as a white solid. ^{1}H NMR (300 MHz, MeOD): δ = 7.57−7.52 (m, 2H), 7.50−7.45 (m, 2H), 4.38−4.26 (m, 2H), 3.98−3.87 (m, 1H), 3.86−3.76 (m, 1H), 3.59−3.34 (m, 5H), 2.63−2.56 (dd, *J* = 6.1, 3.1 Hz, 1H), 2.28−2.10 (m, 2H), 2.10−1.96 (m, 2H). ^{13}C NMR (75 MHz, MeOD): δ = 137.3, 133.3, 131.0, 130.7, 54.0, 50.7, 50.4, 47.7, 47.4, 23.8, 23.1, 18.0. HR-MS (+ESI) *m*/*z* calculated for $C_{14}H_{20}N_2{}^{35}Cl$ (M+H)$^+$ 251.1310, found 251.1311. RP-UHPLC: t_R = 1.3 min, RP-HPLC: t_R = 17.1 min. M_P = 134.1−134.2 °C. (*S*)-**7** (2.39 mg) was dissolved in DMSO (500 μL) to yield a 10 mM stock solution for electrophysiology.

*(R)-N-(2,4-Dichlorobenzyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((R)-**8**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A:D/1:1 (15 mL) and purified by RP-HPLC (from 100% A to 100% D in 45 min, 25 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*R*)-**8** (20 mg, 0.039 mmol, 39%) as a transparent, sticky solid. ^{1}H NMR (300 MHz, MeOD): δ = 7.66−7.62 (m, 2H), 7.47−7.42 (m, 1H), 4.46−4.28 (m, 2H), 3.93−3.78 (m, 2H), 3.51−3.25 (m, 5H), 2.66−2.58 (m, 1H), 2.33−2.09 (m, 2H), 2.09−1.94 (m, 2H). ^{13}C NMR (75 MHz, MeOD): δ = 137.3, 136.7, 134.1, 130.9, 130.8, 129.1, 53.9, 51.2, 47.9, 47.6, 47.2, 23.7, 22.8, 17.8. HR-MS (+ESI) *m*/*z* calculated for $C_{14}H_{19}N_2{}^{35}Cl_2$ (M+H)$^+$ 285.0920, found 285.0925. RP-UHPLC: t_R = 1.4 min, RP-HPLC: t_R = 14.5 min. (*R*)-**8** (2.55 mg) was dissolved in DMSO (500 μL) to yield a 10 mM stock solution for electrophysiology.

*(S)-N-(2,4-Dichlorobenzyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((S)-**8**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A:D/1:1 (15 mL) and purified by RP-HPLC (from 100% A to 100% D in 45 min, 25 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*S*)-**8** (20 mg, 0.039 mmol, 39%) as a transparent, sticky solid. ^{1}H NMR (300 MHz, MeOD): δ = 7.66−7.62 (m, 2H), 7.47−7.42 (m, 1H), 4.46−4.28 (m, 2H), 3.93−3.78 (m, 2H), 3.51−3.25 (m, 5H), 2.66−2.58 (m, 1H), 2.33−2.09 (m, 2H), 2.09−1.94 (m, 2H). ^{13}C NMR (75 MHz, MeOD): δ = 137.2, 136.7, 134.0, 131.2, 130.8, 129.1, 53.9, 51.4, 47.9, 47.6, 47.2, 23.7, 22.8, 17.8. HR-MS (+ESI) *m*/*z* calculated for $C_{14}H_{19}N_2{}^{35}Cl_2$ (M+H)$^+$ 285.0920, found 285.0926. RP-UHPLC: t_R = 1.4 min, RP-HPLC: t_R = 14.5 min. (*S*)-**8** (2.56 mg) was dissolved in DMSO (500 μL) to yield a 10 mM stock solution for electrophysiology.

*(R)-N-(2,6-Dichlorobenzyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((R)-**9**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A (6 mL) and purified by RP-HPLC (from 100% A to 30% A and 70% D in 30 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*R*)-**9** (112 mg, 0.44 mmol, 43%) as a colorless solid. ^{1}H NMR (300 MHz, MeOD) δ = 7.57−7.50 (m, 2H), 7.50−7.42 (m, 1H), 5.07−4.84 (s, 1H), 4.56 (d, *J* = 12 Hz, 2H), 4.02−3.82 (m, 2H), 3.53−3.32 (m, 5H), 2.77−2.68 (m, 1H), 2.35−1.96 (m, 4H). ^{13}C NMR (75 MHz, MeOD) δ = 137.9, 132.9, 131.0, 130.2, 54.2, 51.7, 47.6, 47.2, 46.3, 23.6, 22.9, 17.8. HR-MS calculated for [M + H]$^+$ $C_{14}H_{19}N_2{}^{35}Cl_2$ 285.0920, found 285.0923. RP-UHPLC: t_R = 1.2 min, RP-HPLC: t_R = 7.5 min. M_P = 161.1−162.3 °C. (*R*)-**9** (3.32 mg) was dissolved in DMSO (647 μL) to yield a 10 mM stock solution for electrophysiology.

*(S)-N-(2,6-Dichlorobenzyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((S)-**9**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A (6 mL) and purified by RP-HPLC (from 100% A to 30% A and 70% D in 30 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*S*)-**9** (89 mg, 0.35 mmol, 35%) as a colorless solid. ^{1}H NMR (300 MHz, MeOD) δ = 7.60−7.50 (m, 2H), 7.50−7.41 (m, 1H), 5.05−4.88 (s, 1H), 4.57 (dd, *J* = 15 Hz, 9 Hz, 2H), 4.03−3.82 (m, 2H), 3.55−3.33 (m, 5H), 2.79−2.68 (m, 1H), 2.36−2.14 (m, 2H), 2.13−1.97 (m, 2H). ^{13}C NMR (75 MHz, MeOD) δ = 137.9, 132.9, 131.0, 130.2, 54.2, 51.7, 47.6, 47.2, 46.3, 23.6, 22.9, 17.8. HR-MS calculated for [M + H]$^+$ $C_{14}H_{19}N_2{}^{35}Cl_2$ 285.0920, found 285.0923. RP-UHPLC: t_R = 1.2 min, RP-HPLC: t_R = 7.3 min. M_P = 161.6−163.5 °C. (*S*)-**9** (4.32 mg) was dissolved in DMSO (842 μL) to yield a 10 mM stock solution for electrophysiology.

*(R)-N-Benzyl-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((R)-**10**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A:D/1:1 (10 mL) and purified by RP-HPLC (from 100% A to 100% D in 45 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*R*)-**10** (87 mg, 0.2 mmol, 19%) as a colorless solid. ^{1}H NMR (300 MHz, d_6-DMSO) δ = 10.70−9.42 (m, 2H), 7.59−7.48 (m, 2H), 7.48−7.34 (m, 3H), 4.22 (dd, *J* = 12 Hz, 3 Hz, 2H), 3.84−3.59 (m, 2H), 3.38−3.12 (m, 5H), 2.23−2.05 (m, 1H), 2.04−1.88 (m, 1H), 1.88−1.72 (m, 2H). ^{13}C NMR (75 MHz, d_6-DMSO) δ = 131.8, 130.2, 129.1, 128.7, 51.8, 49.0, 48.5, 45.5, 45.0, 21.7, 21.6, 16.4. HR-MS calculated for [M + H]$^+$ $C_{14}H_{21}N_2$ 217.1699, found 217.1696. RP-UHPLC: t_R = 1.1 min, RP-HPLC: t_R = 5.8 min. M_P = 200.0−202.0 °C. (*R*)-**10** (2.22 mg) was dissolved in DMSO (500 μL) to yield a 10 mM stock solution for electrophysiology.

*(S)-N-Benzyl-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((S)-**10**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A:D/1:1 (10 mL) and purified by RP-HPLC (from 100% A to 40% A and 60% D in 25 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*S*)-**10** (188 mg, 0.42 mmol, 42%) as a colorless solid. ^{1}H NMR (300 MHz, MeOD) δ = 7.57−7.51 (m, 2H), 7.50−7.44 (m, 2H), 4.87 (s, 1H), 4.29 (dd, *J* = 12 Hz, 6 Hz, 2H), 3.95−3.70 (m, 2H), 3.46−3.35 (m, 4H), 2.63−2.54 (m, 1H), 2.25−2.09 (m, 2H), 2.09−1.95 (m, 2H). ^{13}C NMR (75 MHz, d_6-DMSO) δ = 159.1, 158.6, 131.8, 130.1, 129.1, 128.7, 118.9, 51.7, 48.9, 48.5, 45.4, 44.9, 40.3, 40.0, 39.8, 39.5, 39.2, 38.9, 38.7, 21.6, 21.5, 16.4. HR-MS calculated for [M + H]$^+$ $C_{14}H_{21}N_2$ 217.1699, found 217.1700. RP-UHPLC: t_R = 1.1 min, RP-HPLC: t_R = 5.5 min. M_P = 198.5−199.9 °C. (*S*)-**10** (2.98 mg) was dissolved in DMSO (671 μL) to yield a 10 mM stock solution for electrophysiology.

*(R)-N-(Pyridin-2-ylmethyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((R)-**11**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A (8 mL) and purified by RP-HPLC (100% A for 10 min followed by a gradient to 50% A and 50% D in 20 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*R*)-**11** (183 mg, 0.82 mmol, 82%) as a transparent, sticky solid. ^{1}H NMR (300 MHz, MeOD) δ = 8.69−8.63 (m, 1H), 8.01−7.92 (m, 1H), 7.61−7.54 (d, *J* = 7.9 Hz, 1H), 7.54−7.46 (m, 1H), 5.21−5.03 (s, 1H), 4.54−4.43 (s, 2H), 3.98−3.79 (m, 2H), 3.57−3.31 (m, 5H), 2.66−2.57 (m, 1H), 2.37−1.91 (m, 4H). ^{13}C NMR (75 MHz, MeOD) δ = 152.5, 149.6, 140.1, 125.5, 124.8, 53.9, 50.7, 50.4, 47.5, 47.2, 23.8, 22.9, 17.8. HR-MS calculated for [M + H]$^+$ $C_{13}H_{20}N_3$ 218.1652, found 218.1654. RP-UHPLC: t_R = 1.0 min, RP-HPLC: t_R = 3.5 min. (*R*)-**11** (12.28 mg) was dissolved in DMSO (2757 μL) to yield a 10 mM stock solution for electrophysiology.

*(S)-N-(Pyridin-2-ylmethyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((S)-**11**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A (8 mL) and purified by RP-HPLC (100% A for 10 min followed by a gradient to 50% A and 50% D in 10 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*S*)-**11** (148 mg, 0.66 mmol, 66%) as a transparent, sticky solid. ^{1}H NMR (300 MHz, MeOD) δ = 8.73−8.61 (m, 1H), 8.04−7.88 (m, 1H), 7.61−7.55 (d, *J* = 7.9 Hz, 1H), 7.53−7.46 (m, 1H), 5.30−5.00 (s, 1H), 4.59−4.42 (s, 2H), 4.00−3.77 (m, 2H), 3.58−3.30 (m, 5H), 2.68−2.55 (m, 1H), 2.37−1.93 (m, 4H). ^{13}C NMR (75 MHz, MeOD) δ = 152.4, 149.5, 140.0, 125.4, 124.8, 53.8, 50.6, 50.3, 47.4, 47.1, 23.7, 22.8, 17.7. HR-MS calculated for [M + H]$^+$ $C_{13}H_{20}N_3$ 218.1652, found 218.1653. RP-UHPLC: t_R = 1.0 min, RP-HPLC: t_R = 3.5 min. (*S*)-**11** (8.02 mg) was dissolved in DMSO (1800 μL) to yield a 10 mM stock solution for electrophysiology.

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*(R)-N-(Pyridin-4-ylmethyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((R)-**12**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A (7 mL) and purified by RP-HPLC (from 100% A to 40% A and 60% D in 25 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*R*)-**12** (176 mg, 0.79 mmol, 79%) as a colorless solid. ^{1}H NMR (300 MHz, MeOD) δ = 8.94−8.79 (m, 2H), 8.19−8.09 (m, 2H), 5.72−5.17 (s, 1H), 4.58−4.40 (m, 2H), 3.92−3.76 (m, 2H), 3.61−3.33 (m, 5H), 2.62−2.52 (m, 1H), 2.42−2.24 (s, 1H), 2.22−1.91 (m, 3H). ^{13}C NMR (75 MHz, MeOD) δ = 154.6, 144.8, 144.6, 127.6, 127.2, 54.0, 51.6, 49.8, 47.5, 47.1, 24.1, 22.8, 17.8. HR-MS calculated for $[M + H]^+$ $C_{13}H_{20}N_3$ 218.1652, found 218.1655. RP-UHPLC: t_R = 0.6 min, RP-HPLC: t_R = 2.5 min. M_P = 126.4−128.4 °C. (*R*)-**12** (1.66 mg) was dissolved in DMSO (373 μL) to yield a 10 mM stock solution for electrophysiology.

*(S)-N-(Pyridin-4-ylmethyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((S)-**12**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A (7 mL) and purified by RP-HPLC (100% A for 10 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*S*)-**12** (175 mg, 0.78 mmol, 78%) as a colorless solid. ^{1}H NMR (300 MHz, MeOD) δ = 8.90−8.81 (m, 2H), 8.17−8.10 (d, *J* = 5.9 Hz, 2H), 5.30−5.06 (s, 1H), 4.54−4.40 (m, 2H), 3.88−3.75 (m, 2H), 3.55−3.33 (m, 5H), 2.59−2.51 (m, 1H), 2.39−2.25 (m, 1H), 2.22−1.90 (m, 3H). ^{13}C NMR (75 MHz, MeOD) δ = 155.4, 144.5, 144.5, 127.6, 127.2, 54.0, 51.8, 49.8, 47.6, 47.1, 24.2, 22.8, 17.8. HR-MS calculated for $[M + H]^+$ $C_{13}H_{20}N_3$ 218.1652, found 218.1649. RP-UHPLC: t_R = 0.6 min, RP-HPLC: t_R = 2.1 min. (*S*)-**12** (2.02 mg) was dissolved in DMSO (454 μL) to yield a 10 mM stock solution for electrophysiology.

*(R)-N-(1,3-Benzodioxol-5-ylmethyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((R)-**13**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A:D/2:1 (7.5 mL) and purified by RP-HPLC (from 100% A to 40% A and 60% D in 25 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*R*)-**13** (131 mg, 0.53 mmol, 53%) as a colorless, sticky solid. ^{1}H NMR (300 MHz, MeOD) δ = 7.08−6.99 (m, 2H), 6.91−6.83 (d, *J* = 7.8 Hz, 1H), 6.02−5.96 (s, 2H), 5.11−4.82 (s, 1H), 4.23 (dd, *J* = 12 Hz, 6 Hz, 2H), 3.96−3.85 (m, 1H), 3.84−3.71 (m, 1H), 3.53−3.31 (m, 5H), 2.64−2.55 (m, 1H), 2.29−1.93 (m, 4H). ^{13}C NMR (75 MHz, MeOD) δ = 150.4, 149.8, 125.6, 125.1, 111.2, 109.7, 103.0, 53.3, 51.1, 50.0, 47.4, 47.0, 23.6, 22.8, 17.7. HR-MS calculated for $[M + H]^+$ $C_{15}H_{21}O_2N_2$ 261.1598, found 261.1597. RP-UHPLC: t_R = 1.2 min, RP-HPLC: t_R = 4.2 min. (*R*)-**13** (1.84 mg) was dissolved in DMSO (377 μL) to yield a 10 mM stock solution for electrophysiology.

*(S)-N-(1,3-Benzodioxol-5-ylmethyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((S)-**13**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A:D/2:1 (7 mL) and purified by RP-HPLC (from 100% A to 70% A and 30% D in 15 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*S*)-**13** (139 mg, 0.57 mmol, 57%) as a colorless, sticky solid. ^{1}H NMR (300 MHz, MeOD) δ = 7.07−6.98 (m, 2H), 6.91−6.85 (d, *J* = 18 Hz, 1H), 6.03−5.95 (s, 2H), 5.09−4.83 (s, 1H), 4.29−4.17 (dd, *J* = 12 Hz, 6 Hz, 2H), 3.96−3.84 (m, 1H), 3.84−3.72 (m, 1H), 3.52−3.31 (m, 5H), 2.63−2.55 (m, 1H), 2.29−1.95 (m, 4H). ^{13}C NMR (75 MHz, MeOD) δ = 150.4, 149.8, 125.6, 125.1, 111.2, 109.7, 103.0, 53.3, 51.1, 50.0, 47.4, 47.0, 23.6, 22.8, 17.7. HR-MS calculated for $[M + H]^+$ $C_{15}H_{21}O_2N_2$ 261.1598, found 261.1596. RP-UHPLC: t_R = 1.2 min, RP-HPLC: t_R = 4.5 min. (*S*)-**13** (2.46 mg) was dissolved in DMSO (504 μL) to yield a 10 mM stock solution for electrophysiology.

*(R)-N-(Naphthalene-2-ylmethyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((R)-**14**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A:D/2:1 (7.5 mL) and purified by RP-HPLC (from 100% A to 40% A and 60% D in 25 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*R*)-**14** (114 mg, 0.46 mmol, 46%) as a colorless, sticky solid. ^{1}H NMR (300 MHz, MeOD) δ = 8.23−8.17 (m, 1H), 8.04−7.95 (m, 2H), 7.80−7.73 (m, 1H), 7.71−7.52 (m, 3H), 5.17−4.90 (s, 1H), 4.88−4.74 (dd, *J* = 13.6 Hz, 8.5 Hz, 2H), 4.19−4.08 (m, 1H), 3.94−3.80 (m, 1H), 3.57−3.32 (m, 5H), 2.78−2.70 (m, 1H), 2.34−1.97 (m, 4H). ^{13}C NMR (75 MHz, MeOD) δ = 135.4, 132.6, 130.7, 128.5, 127.9, 127.6, 126.5, 123.8, 120.0, 116.1, 54.2, 50.2, 48.0, 47.4, 47.1, 22.9, 17.7. HR-MS calculated for $[M + H]^+$ $C_{18}H_{23}N_2$ 267.1856, found 267.1853. RP-UHPLC: t_R = 1.4 min, RP-HPLC: t_R = 7.7 min. (*R*)-**14** (1.24 mg) was dissolved in DMSO (251 μL) to yield a 10 mM stock solution for electrophysiology.

*(S)-N-(Naphthalene-2-ylmethyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((S)-**14**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A:D/2:1 (7.5 mL) and purified by RP-HPLC (from 100% A to 40% A and 60% D in 25 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*S*)-**14** (110 mg, 0.45 mmol, 45%) as a colorless, sticky solid. ^{1}H NMR (300 MHz, MeOD) δ = 8.24−8.15 (m, 1H), 8.06−7.95 (m, 2H), 7.82−7.73 (m, 1H), 7.72−7.47 (m, 3H), 5.16−4.90 (s, 1H), 4.89−4.75 (dd, *J* = 12 Hz, 9 Hz, 2H), 4.18−4.08 (m, 1H), 3.93−3.80 (m, 1H), 3.56−3.32 (m, 5H), 2.79−2.69 (m, 1H), 2.35−1.97 (m, 4H). ^{13}C NMR (75 MHz, MeOD) δ = 135.4, 132.7, 130.7, 128.5, 127.9, 127.6, 126.5, 123.9, 120.0, 116.1, 54.2, 50.2, 48.0, 47.4, 47.1, 23.4, 22.9, 17.7. HR-MS calculated for $[M + H]^+$ $C_{18}H_{23}N_2$ 267.1856, found 267.1852. RP-UHPLC: t_R = 1.4 min, RP-HPLC: t_R = 7.7 min. (*S*)-**14** (0.51 mg) was dissolved in DMSO (103 μL) to yield a 10 mM stock solution for electrophysiology.

*(R)-N,N-Dibenzyl-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((R)-**15**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A:D/1:1 (10 mL) and purified by RP-HPLC (from 100% A to 100% D in 45 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*R*)-**15** (9 mg, 0.02 mmol, 2%) as a transparent, sticky solid. Note that (*R*)-**15** is a side-product of the alkylation reaction yielding (*R*)-**10**. ^{1}H NMR (300 MHz, MeOD) δ = 7.40−7.12 (m, 10H), 3.84−3.65 (dd, *J* = 15 Hz, 3 Hz, 4H), 3.54−3.43 (m, 1H), 3.39−3.30 (m, 1H), 3.23−3.04 (m, 4H), 2.54−2.46 (m, 1H), 2.22−2.08 (s, 1H), 2.05−1.91 (m, 1H), 1.91−1.75 (s, 1H), 1.70−1.54 (m, 1H). ^{13}C NMR (75 MHz, MeOD) δ = 139.5, 130.1, 129.5, 128.5, 58.8, 56.6, 53.4, 49.9, 49.6, 49.3, 49.0, 48.7, 48.4, 48.2, 47.9, 46.9, 24.0, 23.3, 18.5. HR-MS calculated for $[M + H]^+$ $C_{21}H_{27}N_2$ 307.2169, found 307.2159. RP-UHPLC: t_R = 1.6 min, RP-HPLC: t_R = 19.7 min. (*R*)-**15** (3.19 mg) was dissolved in DMSO (597 μL) to yield a 10 mM stock solution for electrophysiology.

*(S)-N,N-Dibenzyl-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((S)-**15**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A:D/1:1 (10 mL) and purified by RP-HPLC (from 100% A to 100% D in 45 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*S*)-**15** (13 mg, 0.03 mmol, 3%) as a transparent, sticky solid. Note that (*S*)-**15** is a side-product of the alkylation reaction yielding (*S*)-**10**. ^{1}H NMR (300 MHz, MeOD) δ = 7.49−7.17 (m, 10H), 4.06−3.79 (m, 4H), 3.59−3.33 (m, 4H), 3.27−3.10 (m, 2H), 2.72−2.54 (s, 1H), 2.29−2.11 (m, 1H), 2.08−1.96 (m, 1H), 1.95−1.81 (m, 1H), 1.79−1.62 (m, 1H). ^{13}C NMR (75 MHz, MeOD) δ = 138.3, 130.4, 129.7, 128.9, 59.2, 56.8, 52.9, 49.9, 49.6, 49.3, 49.0, 48.7, 48.5, 48.2, 47.8, 46.9, 23.9, 23.3, 18.5. HR-MS calculated for $[M + H]^+$ $C_{21}H_{27}N_2$ 307.2169, found 307.2162. RP-UHPLC: t_R = 1.6 min, RP-HPLC: t_R = 19.3 min. (*S*)-**15** (2.52 mg) was dissolved in DMSO (472 μL) to yield a 10 mM stock solution for electrophysiology.

*(R)-(2,3-Dihydro-1H-isoindole-2-yl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((R)-**16**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A (7 mL) and purified by RP-HPLC (100% A for 10 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*R*)-**16** (67 mg, 0.29 mmol, 29%) as a colorless, sticky solid. ^{1}H NMR (300 MHz, MeOD) δ = 7.62−7.40 (m, 4H), 5.27−4.91 (m, 1H), 4.91−4.75 (m, 3H), 4.33−4.19 (m, 1H), 4.17−4.02 (m, 1H), 3.88−3.76 (m, 1H), 3.65−3.43 (m, 4H), 2.89−2.78 (m,

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1H), 2.49−2.09 (m, 4H). ^{13}C NMR (75 MHz, MeOD) δ = 134.9, 130.1, 123.9, 61.1, 59.1, 47.6, 47.1, 24.1, 22.9, 17.8. HR-MS calculated for $[M + H]^+$ $C_{15}H_{21}N_2$ 229.1699, found 229.1697. RP-UHPLC: t_R = 1.1 min, RP-HPLC: t_R = 4.3 min. (*R*)-**16** (1.09 mg) was dissolved in DMSO (239 μL) to yield a 10 mM stock solution for electrophysiology.

*(S)-(2,3-Dihydro-1H-isoindole-2-yl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt ((S)-**16**).* RP-HPLC: The residue obtained from the general procedure for reductive amination was dissolved in A (7 mL) and purified by RP-HPLC (100% A for 10 min, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding (*S*)-**16** (65 mg, 0.28 mmol, 28%) as a colorless, sticky solid. ^{1}H NMR (300 MHz, MeOD) δ = 7.64−7.41 (m, 4H), 5.31−4.95 (m, 1H), 4.96−4.80 (m, 3H), 4.37−4.23 (m, 1H), 4.16−4.01 (m, 1H), 3.93−3.74 (m, 1H), 3.67−3.45 (m, 4H), 2.91−2.79 (m, 1H), 2.47−2.10 (m, 4H). ^{13}C NMR (75 MHz, MeOD) δ = 134.8, 130.1, 123.9, 61.1, 59.0, 49.9, 49.6, 49.3, 49.0, 48.7, 48.4, 48.2, 47.6, 47.0, 24.1, 22.9, 17.8. HR-MS calculated for $[M + H]^+$ $C_{15}H_{21}N_2$ 229.1699, found 229.1696. RP-UHPLC: t_R = 1.1 min, RP-HPLC: t_R = 4.1 min. (*S*)-**16** (2.37 mg) was dissolved in DMSO (598 μL) to yield a 10 mM stock solution for electrophysiology.

*(S/R or S)-N-((R)-1-Phenylethyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt (**17** and **18**).* Into an oven-dried flask was added 3-quinuclidone hydrochloride (81.0 mg, 0.5 mmol) and (*R*)-(+)-1-phenylethylamine (60.6 mg, 0.5 mmol), and the flask was flushed with argon. To the mixture was added dry acetonitrile (2 mL), and the reaction was stirred for 30 min under argon atmosphere. Next, $NaBH(OAc)_3$ (320 mg, 1.5 mmol) was added as a solid, and the mixture was stirred at RT overnight. Then the solvent was removed under reduced pressure and the residue obtained was dissolved in A (8 mL) and purified by RP-HPLC (100% A for 15 min, then in 15 min to 60% A and 40% D, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding **17** (29 mg, 0.06 mmol, 15%) as a colorless solid, in an isomer ratio of 1:1. ^{1}H NMR (300 MHz, MeOD) δ = 7.59−7.43 (m, 5H), 4.50 (q, *J* = 6.8 Hz, 1H), 3.75−3.34 (m, 4H), 3.26−2.97 (m, 3H), 2.56 (q, *J* = 3.0 Hz, 1H of the *S*, *R*-isomer), 2.34 (q, *J* = 3.0 Hz, 1H of the *R*, *R*-isomer), 2.29−2.12 (m, 1H), 2.12−1.96 (m, 2H), 1.96−1.81 (m, 1H), 1.73 (d, *J* = 6.8 Hz, 3H of the *S*,*R*-isomer), 1.70 (d, *J* = 6.8 Hz, 3H of the *R*,*R*-isomer). ^{13}C NMR (75 MHz, MeOD) δ = 137.1, 136.7, 131.1, 131.0, 130.7, 129.2, 128.8, 60.0, 58.9, 52.7, 51.7, 50.3, 49.9, 47.3, 47.3, 47.0, 46.8, 23.9, 23.8, 22.8, 22.7, 19.9, 19.7, 17.8, 17.7. HR-MS calculated for $[M + H]^+$ $C_{15}H_{23}N_2$ 231.1856, found 231.1850. RP-UHPLC: t_R = 1.7 min (7.5 min gradient from 100% A to 100% D), RP-HPLC: t_R = 10.0 min. M_P = 172.4−173.6 °C. **17** (5.60 mg) was dissolved in DMSO (1222 μL) to yield a 10 mM stock solution for electrophysiology.

The isomers were separated by four times recrystallization by overlaying a solution in methanol with diethyl ether to yield diastereopure **18** (19 mg, 0.04 mmol, 50%). The obtained crystals were suitable for X-ray analysis. CCDC989722 contains the supplementary crystallographic data for this structure. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. ^{1}H NMR (300 MHz, MeOD) δ = 7.62−7.42 (m, 5H), 4.53 (q, *J* = 6.8 Hz, 1H), 3.73−3.63 (m, 1H), 3.55−3.33 (m, 2H), 3.28−2.97 (m, 4H), 2.66−2.49 (m, 1H), 2.28−2.16 (m, 1H), 2.12−1.95 (m, 2H), 1.91−1.80 (m, 1H), 1.72 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, MeOD) δ = 137.2, 131.1, 130.8, 129.1, 59.8, 52.5, 50.7, 47.4, 46.9, 23.8, 22.7, 20.2, 17.7. HR-MS calculated for $[M + H]^+$ $C_{15}H_{23}N_2$ 231.1856, found 231.1854. RP-UHPLC: t_R = 1.7 min (7.5 min gradient from 100%A to 100% D), RP-HPLC: t_R = 10.0 min. M_P = 195.0−198.0 °C. **18** (1.57 mg) was dissolved in DMSO (342 μL) to yield a 10 mM stock solution for electrophysiology.

*(R)-N-((S)-1-Phenylethyl)-1-azabicyclo[2.2.2]octan-3-amine, Double Trifluoroacetic Acid Salt (**19**).* RP-HPLC: The residue obtained from the same procedure used above for **19**, using (*S*)-(−)-1-phenylethylamine instead, was dissolved in A (8 mL) and purified by RP-HPLC (100% A for 1 min, then in 30 min to 100% D, 40 mL·min^{-1}). The purest fractions (>95%) were collected and lyophilized, yielding **19** (80 mg, 0.17 mmol, 35%) as a colorless solid, in an isomer ratio of 1:1. The isomers were separated by four times recrystallization by overlaying a solution in methanol with diethyl ether to yield diastereopure **19** (13 mg, 0.03 mmol, 46%). ^{1}H NMR (300 MHz, MeOD) δ = 7.62−7.45 (m, 5H), 4.52 (q, *J* = 6.8 Hz, 1H), 3.71−3.61 (m, 1H), 3.51−3.34 (m, 2H), 3.25−3.13 (m, 3H), 3.08−2.97 (m, 1H), 2.56 (q, *J* = 3.1 Hz, 1H), 2.27−2.15 (m, 1H), 2.13−1.96 (m, 2H), 1.92−1.81 (m, 1H), 1.74 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, MeOD) δ = 137.5, 131.0, 130.8, 129.1, 59.7, 52.4, 50.8, 47.4, 46.9, 23.8, 22.7, 20.3, 17.7. HR-MS calculated for $[M + H]^+$ $C_{15}H_{23}N_2$ 231.1856, found 231.1850. RP-UHPLC: t_R = 1.1 min, RP-HPLC: t_R = 20.1 min. M_P = 194.1−195.3 °C. **19** (1.35 mg) was dissolved in DMSO (294 μL) to yield a 10 mM stock solution for electrophysiology.

Electrophysiology. Oocytes were prepared using standard methods by mechanical and enzymatical dissociation. Stage 5−6 oocytes were selected manually under the microscope and injected with 10 nL of water containing 0.2 μg/μL of plasmid of interest which contained the cDNA encoding for the human nAChRs or $5HT_{3A}$ receptors. Expression of heteromeric $\alpha3\beta2$, $\alpha4\beta2$, and $\alpha3\beta4$ 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 20 °C and cells superfused with OR2 medium containing in mM: NaCl 82.5, KCl 2.5, HEPES 5, $CaCl_2·2H_2O$ 1.8, $MgCl_2·6H_2O$ 1, pH 7.4.

Functional activity was measured using two electrode voltage clamp in *Xenopus* oocytes expressing recombinant human nAChR subtypes.[31] Compound effects were evaluated by first recording the current evoked by a brief acetylcholine (ACh) test pulse (200 μM, 5 s) and then by recording the effect of the compound alone (1 or 10 μM, agonist effect) during 30 s and immediately afterward, without wash, the response to the ACh test pulse (200 μM, 5 s). Measurement of the effects of the compound alone versus the ACh response recorded in control yielded the fraction of agonistic activity of the compound. Measurement of the ACh-response recorded after compound exposure versus the control response provided the characteristics of inhibition or PAM effect.

Behavioral Assessment. *Reagents.* The different compounds were dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, D8418). All compounds were administered intraperitoneally (i.p.) at doses of 2.5 or 5.0 mg/kg in 20 μL of DMSO 60 min before behavioral assessment.

Mice. The study was conducted on 8 week old male BALB/c mice (20−25 g). Animals were manipulated according to the guide on the use and care of experimental animals (Mexican Official Norm NOM-062-ZOO-1999 published by SAGARPA in the Diario Oficial del Gobierno Mexicano paper on June 28, 2001). The animals were bred and maintained under standard environmental conditions (n = 5 per group and cage) at an ambient temperature of 22−24 °C under a 12:12 h light−dark cycle, and supplied with food and water ad libitum.

Behavioral Assessment. Drugs were tested in different behavioral tests of motility and anxiety-like behavior in four different models (Open Field Test, Elevated Plus Maze, Light-Dark Box Test and Hole Board Test). Rectal temperature was determined using a Physitemp (Physitemp Instruments Inc., Clifton, NJ) with a thermocouple probe 1−5 mm into the rectum. The temperature was measured 10 min before compound administration and 1 or 2 h postinjection. Changes in core temperature were expressed as the difference between basal and postinjection temperatures. Hypomotility was determined using the rotarod performance test based on a rotating rod with forced motor activity being applied to mice. The maximal duration of this test was 120 s at 4 rpm. Mice were trained three times on the rotarod prior to experiments. The rotarod device was manufactured in house at CUCEI. The Open Field Test was performed 1 or 2 h before

compounds or vehicle were injected. Each animal was placed in a corner of the open field apparatus (50 × 50 × 22 cm^3) and allowed free movement. We analyzed the elapsed time in motion or activity in the open field and the time that the animal remained stationary or paused at the corners. The test time was 5 min. The entire room was kept illuminated with white light during the experiment. The animals were first habituated to the open field for 5 min to avoid bias in the novelty. After each test session, the equipment was cleaned with 70% ethanol to remove animal odors. The elevated Plus-Maze Test consisted of two open arms and two enclosed horizontal perpendicular arms 50 cm above the floor (standard measurements). The junction of four arms formed a central squared platform (5 × 5 cm^2). Each mouse was placed in the central apparatus of the Plus-Maze, facing one of the open arms, and was allowed free movement. We analyzed the total number of entries into the open and closed arms and the time spent in them. An entry in an arm means the animal placed all four feet inside. The test time was 5 min. The whole room was kept illuminated with white light during the experiment. Evaluation times were 1 and 2 h, respectively, after treatment with the vehicle or test compound. The animals were first habituated to the Plus-Maze for 5 min to avoid bias in the novelty. After each test session, the equipment was cleaned with 70% ethanol to remove animal odors. The Light-Dark Box Test uses the natural aversion of rodents to bright areas compared with darker ones. In a two-compartment box, rodents will prefer dark areas, whereas anxiolytic drugs should increase the time spent in the light compartment. The apparatus consisted of a rectangular plastic box (20 × 20 × 15 cm^3) divided in two equal size compartments, one white and one black with a tunnel (4 cm) for communication between both. Mice were placed in the center near the door. The transitions between the compartments and time spent in each compartment were quantified for a period of 5 min for each mouse. After each trial, the floor of the apparatus was cleaned with 70% ethanol to remove traces and animal odor. The Hole Board Test was used for evaluated exploratory behavior; the apparatus used was made in house at CUCEI and consists of a 40 cm square plane with 16 flush-mounted cylindrical holes (diameter 3 cm) distributed 4 by 4 in an equidistant, gridlike manner. Mice were placed in the center of the board one by one and to move about freely for a period of 5 min each. The movement of the animals on the surface of the plane and the number of head dips were quantified. After each trial, the floor of the apparatus was cleaned with ethanol to remove traces of previous mouse. Differences between vehicle and drug treated animal groups were analyzed with a two-way ANOVA, followed, when appropriate, by Tukey's posthoc test. Box and whisker plots showing median and quartiles were made in GraphPad Prism 5.0.

■ ASSOCIATED CONTENT

Supporting Information

NMR spectra, UHPLC purity data, and X-ray analysis data. 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.

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■ ABBREVIATIONS

ACh, acetylcholine; CBD, city-block distance; ChEMBL, chemical database of bioactive molecules; CNS, central nervous system; i.p., intraperitoneal injection; MQN, Molecular Quantum Numbers; nAChR, nicotinic acetylcholine receptor; PAM, positive allosteric modulator; SEM, standard error of the mean; T_{SF}, Tanimoto similarity coefficient of substructure fingerprint

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