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Sulfonium as a surrogate for Ammonium: A new $\alpha 7$ nicotinic acetylcholine receptor partial agonist with desensitizing activity

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Abstract

Weak partial agonists that promote a desensitized state of the $\alpha 7$ nicotinic acetylcholine receptor (nAChR) have been associated with anti-inflammatory effects. Exemplar compounds feature a tertiary or quaternary ammonium group. We report the synthesis, structure, and electrophysiological evaluation of 1-ethyl-4-phenylthiomorpholin-1-ium triflate, a weak partial agonist with a sulfonium isostere of the ammonium pharmacophore. These results offer new insights in understanding nAChR-ligand interactions and provide a new chemical space to target the $\alpha 7$ nAChR.

TOC Graphic

INTRODUCTION

The $\alpha 7$ nicotinic acetylcholine receptor (nAChR) is a homopentameric ligand-gated ion channel¹ with unique pharmacological and physiological properties.^{1–3} These include rapid activation and inactivation kinetics, high calcium permeability, low channel opening

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Supporting Information.

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Author Contributions

MQ designed compounds and experiments, conducted synthetic chemistry, and contributed to writing the paper. CS designed and ran voltage-clamp experiments and contributed to writing the paper. AG conducted molecular electrostatic potential calculations, modeling and docking experiments. KA and ACJF conducted X-Ray crystallographic analysis. RLP designed experiments, analyzed data, and contributed to writing the paper. NAH designed compounds and experiments, helped develop syntheses, and contributed to writing the paper.

probability, and a distinct form of concentration-dependent rapid and reversible desensitization. Besides being widely present in the central nervous system, it is also found in peripheral non-neuronal cells such as macrophages, lymphocytes, and microglial cells.^{4–8} In both cultured immune cells and mice, $\alpha 7$ is involved in the modulation of cholinergic anti-inflammatory and immune responses^{8,9} independent of ligand-related ion channel fluxes,¹⁰ suggesting a metabotropic-like function of the receptor.^{1,11–13} The possibility of $\alpha 7$ nAChRs mediating metabotropic responses through direct coupling of the nicotinic receptor intracellular domain to G-proteins has been suggested based on mutational analysis of the intracellular domain M3M4 loop.^{14–15}

The $\alpha 7$ metabotropic-like signal transduction can be selectively induced by molecular tools we define as “silent agonists”. These are nAChR ligands capable of selectively stabilizing a non-conducting state of the receptor and thereby promoting a signal transduction pathway independent of classical ion channel activation.^{13,16–19} It is this lack of classical behavior that led to the use of the term “silent”, the agonist part of the descriptor refers to the potential ability to act metabotropically. In order to screen and pharmacologically characterize such compounds, the ligand needs to satisfy two main requirements: first, upon binding to the receptor at a site overlapping the site for orthosteric agonists, it should not evoke significant activation of the receptor; we therefore set the agonist response threshold to 0.1-fold of the maximal response given by acetylcholine (ACh) at 60 μ M. Silent agonists are therefore weak partial agonists. Second, the compound must induce a non-conducting state corresponding to one of the desensitized states of the receptor, which for $\alpha 7$ is detectable by co-application of the silent agonist with a type II positive allosteric modulator (PAM) such as **1**, PNU-120596.²⁰ The PAM converts the receptor from this desensitized state to an ion-conductive state that can be readily detected and quantified by two-electrode voltage-clamping techniques. We hypothesize that desensitized states of the receptor (in the ionotropic sense) can be pharmacologically active in the metabotropic sense. Molecules acting as $\alpha 7$ nAChR silent agonists show promising anti-inflammatory and anti-nociceptive activities in both in vitro and in vivo tests,^{8,10} thus representing a new approach in which $\alpha 7$ is targeted to treat inflammation-related diseases.

Investigation of $\alpha 7$ nAChR silent agonists led to the identification of several different structural frameworks (Figure 1).^{17,18,21} In those molecules, a nitrogen center was present either in the form of a quaternary ammonium nitrogen or a tertiary nitrogen capable of being protonated at physiologic pH. We have demonstrated that while preferable for silent activity, a permanent charge is not strictly necessary if other suitable features are present.²¹ The minimal pharmacophore for silent agonism was identified as the tetraethyl ammonium cation, and that in multiple series of alkylammonium compounds, the criteria of silent agonism appears correlated with the molecular volume surrounding the ammonium nitrogen center.¹⁸ Uniquely different scaffolds show silent activity (Figure 1), and the pharmacophore(s) for silent agonism are the subject of current studies in our laboratories. NS-6740 is an archetype $\alpha 7$ silent agonist with demonstrated $\alpha 7$ -associated anti-inflammatory activity.^{8,10}

Recently, diEPPs (*N,N*-diethyl-*N'*-phenylpiperazines) emerged as promising $\alpha 7$ silent agonists.^{18,21} In our previous work we examined a number of substituents on the phenyl ring

which revealed a SAR with a strong preference for a para trifluoromethyl or carboxamide substituent, or a carboxamide substituent in the meta position.²¹

To further elucidate the pharmacophore for $\alpha 7$ silent agonists, in the present work we focused on the ammonium nitrogen center of diEPP and investigated its replacement with a sulfonium group. The idea behind this was that we could maintain a charge in the same place as an ammonium, but tinker with the charge distribution, and the size and shape of the ring. Whereas sulfur is certainly part of some well-known scaffolds such as sulfonamides, sulfones, and sulfides,²² the sulfonium group is far less common in medicinal chemistry. There is an interesting history associated with use of sulfonium as an ammonium isostere. According to the definition of isosterism given by Erlenmeyer in 1932,²³ a sulfonium cation is identified as an isosteric replacement for quaternary nitrogen. Indeed, in Erlenmeyer's classification, atoms, ions, or molecules can be considered isosteric if they have identical peripheral layers of electrons. Focusing on the nAChR literature with sulfonium derivatives, Hunt and Renshaw²⁴ reported the ability of trimethylsulfonium iodide (TMS-I) to increase blood pressure, accelerate heart rate, and induce pupil dilatation via a nicotinic action, similarly to tetramethylammonium cation. These data suggested that TMS-I was an active nAChR ligand, albeit less active than the corresponding ammonium derivative. In the same study, triethylsulfonium bromide, besides not having detectable muscarinic action, showed virtually no nicotinic activity. More recently, TMS-I was reported to show agonist activity on frog muscle nAChR.²⁵ Of course because TMS-I is an active methylating agent, these older data must be considered with caution. One rare example of a sulfonium compound that is active at a nAChR appears in the pharmacopeia, trimetaphan camsilate, which is a tricyclic sulfonium thienoimidazolidine salt that has been used to target ganglionic nAChR as a competitive antagonist.²⁶ There are a few other examples of biologically active sulfonium compounds including the well-known cofactor S-adenosylmethionine and sulfonium isosteres of dopamine targeting the dopamine receptor D2.²⁷

We considered that the relatively unexploited sulfonium group might well serve as an alternate ligand for π -cation recognition in the $\alpha 7$ receptor.^{28,29} We hypothesized that the new molecule 1-ethyl-4-phenylthiomorpholin-1-ium, **6**, (EPTM) in which the ammonium nitrogen of **4** is replaced with a sulfonium group, would retain silent agonism activity at the $\alpha 7$ receptor.

RESULTS AND DISCUSSION

Chemistry

The title compound 1-ethyl-4-phenylthiomorpholin-1-ium trifluoromethane sulfonate, **7**, was synthesized in two steps (Scheme 1). Commercially available thiomorpholine was coupled with iodobenzene in the presence of *L*-proline and copper iodide, using the synthetic protocol followed for the synthesis of our previous diEPP analogues library.²¹ The copper-catalyzed Ullmann-type aryl amination provided the coupled 4-phenylthiomorpholine intermediate **6** in 61 % yield. Initial attempts to ethylate the sulfur center with bromo- or iodo-ethane in tetrahydrofuran (THF) at 90 °C were unsuccessful, necessitating use of the more electrophilic reagent, ethyl trifluoromethane sulfonate. Ethylation in anhydrous

dichloromethane at room temperature satisfactorily alkylated the sulfur center to produce the target compound.

The ethylation reaction with ethyl trifluoromethane sulfonate proceeded slowly, and after complete consumption of starting compound **6**, provided a mixture of products **7** and **8**. NMR analysis of the crude product before column chromatography purification suggested the presence of two main components in a 1 to 2.2 ratio corresponding to the preferred alkylation at sulfur and alkylation at nitrogen (Figure S1). No evidence for an S,N di-ethylated derivative was found. The product mixture resulted in a challenging chromatographic separation problem, which compromised the isolated yield. In an attempt to increase the chemoselectivity of the ethylating step to target the desired sulfur center, 4-phenylthiomorpholine was first converted to the correspondent anilinium hydrochloride salt, which was subsequently treated with ethyl trifluoromethane sulfonate. This protocol failed to give improvement in obtaining the desired compound at room temperature. Although the reaction mixture appeared to be less complex, the reaction proceeded very slowly and the desired compound was barely detectable by TLC analysis after weeks. Heating the reaction at 30 °C resulted in a more complex reaction mixture, with no improvement in the rate of conversion toward **7** versus **8**. Presumably the charged nitrogen electrostatically lowered the modest nucleophilicity of the sulfide sulfur atom. We tentatively identified the desired product by ¹H-NMR after chromatography and confirmed its structure by single crystal X-ray diffraction (Figure 2). C₁₂H₁₈N₁S1·CF₃SO₃, Monoclinic, P21/n, a = 21.3292(12) Å, b = 7.9492(4) Å, c = 21.6158(12) Å, V = 3196.1(3) Å³, Z = 8, 83919 reflections measured (7332 independent; R_{int} = 2.61%), T = 173 K, R₁ = 2.72, wR₂ = 7.25. (see supplemental information).

Compound Stability in Ringer's solution and water

Although we considered an S-ethyl sulfonium compound to be less reactive than an S-methyl compound towards nucleophiles, we still wished to demonstrate that **7** was stable under the aqueous conditions in which we would characterize its electrophysiology, at room temperature (~ 25 °C). We used ¹H-NMR to assess the hydrolytic stability of **7** in both water and the Ringer's buffer solution used in the electrophysiological tests on oocytes (pH 7.2) (Figure S2). In particular, for Ringer's buffer stability analysis, we focused on the possibility of ethyl group transfer to HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)), which was present in our Ringer's solutions. Spectra were collected on freshly dissolved **7**, and then after 1 h, 3 h, 19 h and up to 60 days later. Comparison between NMR spectra at time zero and at the different time points revealed no differences in the spectra, thus confirming the stability of **7** in aqueous media and in the Ringer's solution used in electrophysiology experiments.

Electrophysiology

The title compound, 1-ethyl-4-phenylthiomorpholin-1-ium trifluoromethane sulfonate **7** was assayed using *Xenopus laevis* oocytes expressing human nAChRs and two-electrode voltage clamping. It was tested on α7 nAChRs at 10, 30, 100, 300 μM, and 1 mM to evaluate its partial agonism activity, **7** showed virtually no activation of the α7 nAChR when applied

alone, as evidenced by the CRC analysis: $I_{\max} = 0.07 \pm 0.01 \mu\text{M}$ relative to the ACh maximum response at $100 \mu\text{M}$, with an EC_{50} of $40 \pm 17 \mu\text{M}$. (Figure 3, top)

With respect to the permanent positive charge, and their structural and electronic similarities, both **4** and N-phenyl N'-ethyl piperazine, **5**, represent relevant derivatives to be compared to **7**. Clearly, **7** is less efficacious than the ammonium compound **4** ($I_{\max} = 0.07 \pm 0.01 \mu\text{M}$ vs $0.47 \pm 0.02 \mu\text{M}$) (Figure 3, top) thus showing a notably decrease in agonism at the $\alpha 7$ nAChR. Interestingly, the potency evaluation revealed **7** was 5 times more potent than **4** as a partial agonist ($\text{EC}_{50} = 40 \pm 17 \mu\text{M}$ vs $221 \pm 16 \mu\text{M}$), suggesting that the reduced efficacy was not associated with reduced affinity. Compound **5**, the nitrogen isostere of **7**, showed no activation of the $\alpha 7$ receptor subtype at the tested concentrations (10, 30, 100, and $300 \mu\text{M}$), with an $I_{\max}$ effectively zero.

In the absence of a PAM, silent agonists are functional antagonists of ACh, so inhibition experiments provide an alternative measure of drug potency. The antagonism of $\alpha 7$ ACh-evoked responses by **7** was evaluated in an inhibition CRC (iCRC) by co-application at 0.3, 1, 3, 10, 30, 100, and $300 \mu\text{M}$ with $60 \mu\text{M}$ ACh (Figure 3, bottom). The corresponding IC_{50} values for **7** and **4** were $73 \pm 8 \mu\text{M}$ and $55 \pm 19 \mu\text{M}$, respectively. Both compounds showed a similar inhibitory effect on ACh responses of the $\alpha 7$ receptor, thus confirming their competitive binding to its orthosteric site. Conversely, **5** lacked the ability to inhibit $\alpha 7$ ACh responses in the range of 0.3– $100 \mu\text{M}$, with less than 20 % ACh response inhibition at the highest concentration tested ($300 \mu\text{M}$). The iCRC results for **5** suggest that if it binds at all to the $\alpha 7$ nAChR, it does so poorly.

The first step in characterizing sulfonium **7** was successfully met; it is a very weak partial agonist of $\alpha 7$ but does competitively bind to the orthosteric site. To distinguish it as a special type of weak partial agonist, i.e. be a “silent agonist”, it should promote a desensitized state of the receptor, which we detected via co-applications with the positive allosteric modulator **1** (10, 30, and $100 \mu\text{M}$ of tested compound + $10 \mu\text{M}$ **1**). The responses (Figure 4) were normalized to the responses evoked by control $60 \mu\text{M}$ ACh in the same cells. Compound **7** induced responses 3-fold greater than the ACh control at $10 \mu\text{M}$, 5-fold at $30 \mu\text{M}$, and 25-fold at $100 \mu\text{M}$.

In order to provide a specific demonstration of the conversion of receptors desensitized by a prolonged application of compound **7**, to conducting states by a type II PAM, we applied $10 \mu\text{M}$ PNU120596 to receptors previously exposed to $100 \mu\text{M}$ **7** for 30s. As shown in Figure 5 (See inset), the small currents have largely desensitized at the point of the PAM application which generates renewed current that only decays upon washout of the PAM and partial agonist. As is usually the case with $\alpha 7$ nAChR, desensitization promoted by **7** was readily reversible, so that a subsequent ACh evoked response was essentially identical to the initial ACh control.

Further, **7** was tested on the human heteromeric nicotinic receptor $\alpha 4\beta 2$ (Figure S3) and failed to show agonism, but it did show antagonism when co-applied with $30 \mu\text{M}$ ACh, with an IC_{50} of $51 \pm 3.6 \mu\text{M}$. Whereas **4** did show modest potentiation by **1** (Figure 4), **5** failed to show significant potentiated responses. The data argue that the new sulfonium **7** is an

exceptional silent agonist. A silent agonist must evoke minimal channel opening response (<0.1 ACh response, by the criteria we have previously published^{18,21}), and, as noted above, in the bound state, the receptor must promote some receptors to be in a PAM-sensitive desensitized state. Compound **7** resulted in extremely weak $\alpha 7$ activation, with an $I^{\max}$ at $100 \mu\text{M}$ equal to 0.07 ± 0.01 relative to the ACh maximum response. Coapplication of **7** with $10 \mu\text{M}$ **1** resulted in 3 to 25-fold larger responses compared to the control ACh response, and 2–3 orders of magnitude larger than the response to **7** applied alone. These potentiated responses were greater than the ones evoked by **4**, or **5** (which was fully inactive). Indeed, at 10, 30, and $100 \mu\text{M}$, the potentiated responses of **4** were equal to half or less than the magnitude of the corresponding ones for **7**. With regard to receptor subtype specificity, $30 \mu\text{M}$ **7** failed to produce measureable activation of either muscle type or ganglionic $\alpha 3\beta 4$ human nAChR; competition experiments with co-application of **7** with ACh ($30 \mu\text{M}$ for muscle, $100 \mu\text{M}$ for ganglionic) revealed weak antagonism by **7** at these receptors.

Despite the structural and electronic similarities of **5** and **7**, they exhibited dramatically different activity. The permanent charge of the sulfonium derivative is not essential for $\alpha 7$ silent agonism within this series, as evidenced by the observation that the m-bromo derivative of **5**²¹ is a silent agonist, suggesting that other factors may be at play. Two factors we consider as influential are that the molecular volume¹⁸ of **7** ($194 \text{ \AA}^3$) is greater than that of **5** ($188 \text{ \AA}^3$). Secondly, the molecular electrostatic potential (MEP) of **7** is revealing (Figure 6). The charge distribution for **7** is more similar to that of **4** than for **5**. Unlike **5**, the positive charge is distributed amongst the methylenes attached to the central sulfur atom, making **7** (middle) more similar to **4**, right. Compound **5** has a much more focused positive charge distribution, much of it residing on the ammonium proton. We speculate that the π -cation box of the receptor, rich in electron-donating aromatic side chains, is better able to interact with the large positively charged sulfonium group of **7** than it is with **5**. The similarity between **7** and **4** begs the question as to why **7** is both less active on its own and more greatly potentiated by the PAM than **4**, with a similar charge distribution but a smaller molecular volume. One possibility is that the thiomorpholine ring is larger and somewhat distorted relative to the piperazine ring found in **4**. (Figure 2 and supplemental information) Molecular docking studies of **4** and **7** into an $\alpha 7$ homology model failed to show distinctly different binding modes for these two compounds suggesting that the differences we observe in the activity of these compounds may arise from similar bound poses. Molecular mechanics Poisson Boltzman surface area (MMPBSA)-based estimates of binding energies suggests that the sulfonium **7** has a larger electrostatic stabilization relative to **4** by approximately -4 kcal/mol when bound in the orthosteric site.

CONCLUSION

A new sulfonium silent agonist has been synthesized and its structure confirmed by X-ray crystallography. The results confirmed the effectiveness of isoelectronic replacement of a quaternary ammonium nitrogen with a sulfonium ion to create $\alpha 7$ orthosteric ligands that are very weak partial agonists that promote desensitized states of the receptor. Part of the effectiveness of the sulfonium group may be due to the larger ring size imposed by the

presence of sulfur compared to nitrogen. The CRC data for **7** and $\alpha 7$ prove that the sulfonium functional group can bind to the orthosteric agonist site of nAChRs, and in the case of $\alpha 7$ promotes non-conducting conformational states. Specifically, this compound lacked the ability to induce significant direct ion channel activation of $\alpha 7$ and behaves as a silent agonist as evidenced by showing $\alpha 7$ -potentiated response in co-application with **1**. By introducing a sulfonium cation to replace the ammonium group in **4**, we achieved a 4-fold PAM response enhancement compared to the parent compound. These results highlight an alternative pharmacophore for nAChR silent activation compared to the known “silent” scaffolds, offering a new chemical space to target nAChRs with sulfonium-based compounds.

EXPERIMENTAL SECTION

General procedures

Reagents for chemical synthesis were purchased from Fisher Scientific (Pittsburgh, PA) or Sigma-Aldrich (St. Louis, MO). All reagents were of reagent quality or were purified before use. Organic solvents were of analytical grade or were purified by standard procedures. The progress of all reactions was monitored on EMD Millipore 0.25 mm silica gel TLC plates (with fluorescence UV indicator F₂₅₄) using the solvent system specified in the corresponding experimental protocol. Column chromatography was performed with silica gel 60 (230–400 mesh). Spots were visualized with ultraviolet light (254 nm). Melting points were obtained on an MFB-595010M Gallenkamp apparatus equipped with a digital thermometer and are uncorrected. Proton (¹H) and carbon (¹³C) NMR spectra were recorded on a Varian Mercury-300 spectrometer (300 MHz for ¹H and 75.0 MHz for ¹³C) using CDCl₃ or D₂O as solvent. Chemical shifts (δ scale) are given in parts per million (ppm) relative to the peak of the internal standard TMS (δ = 0.00 ppm) for CDCl₃ or relative to the central peak of residual solvent (δ = 4.79 for D₂O) for ¹H and relative to the central peak of residual solvent (δ = 77.16 ppm for CDCl₃) for ¹³C. Processing of the spectra was performed with MestReNova 6.0.2. Reactions were carried out in flame-dried glassware and under argon atmosphere when anhydrous conditions were required. In those cases, anhydrous solvents were used in the reactions. Compound purity was more than 95% as determined by ¹H NMR analyses.

4-Phenylthiomorpholine (**6**)

In a flame-dried and argon flushed round-bottom flask, a mixture of iodobenzene (1.5 mL, 13.4 mmol, 1 equiv), thiomorpholine (2.02 mL, 20.1 mmol, 1.5 equiv), K₂CO₃ (3.7 g, 26.8 mmol, 2 equiv), CuI (255 mg, 1.34 mmol, 0.1 equiv), and *L*-proline (309 mg, 2.68 mmol, 0.2 equiv) in dry DMSO (32 mL, 1.6 mL/mmol thiomorpholine) was heated at 90–100 °C until completion (TLC in hexanes/ethyl acetate 95/5). To the cooled mixture was then added deionized water, the organic layer was separated, and the aqueous layer was extracted three times with ethyl acetate. The combined organic layers were washed with saturated brine, dried over MgSO₄, filtered and concentrated in vacuo. The residual oil was purified on a silica gel column eluted with a mixture of hexanes/ethyl acetate 95/5 to afford the desired product as a yellowish oil (1.47 g, 8.17 mmol, 61 % yield). *R*_f = 0.45 in hexanes/ethyl acetate 95/5. ¹H NMR (CDCl₃, 300 MHz) δ 7.26 (dd, *J* = 8.8, 7.3 Hz, 2H), 6.93 – 6.81 (m,

3H), 3.56 – 3.49 (m, 4H), 2.78 – 2.70 (m, 4H). ¹³C NMR (CDCl₃, 75 MHz) δ 151.5, 129.4, 120.0, 117.3, 52.3, 27.0.

1-Ethyl-4-phenylthiomorpholin-1-ium trifluormethane sulfonate (7)

4-phenylthiomorpholine (1.0 g, 5.58 mmol, 1 equiv) was dissolved in dry CH₂Cl₂ (50 mL) under anhydrous condition and in argon atmosphere. The solution was cooled to 0 °C and ethyl trifluoromethanesulfonate (1.1 mL, 8.37 mmol, 1.5 equiv) was added dropwise. The reaction mixture was stirred 10 min at 0 °C and then warmed up to room temperature. After complete consumption of the starting material (5 days), the reaction was quenched with 5 % aq. NH₄OH and extracted with CH₂Cl₂ (x 5). The combined organic fractions were dried over MgSO₄, filtered and evaporated under reduced pressure. A combination of silica chromatography and crystallization was used to separate **8** from **7**. The crude was first fractionated on a silica gel column eluted with 9:1 CH₂Cl₂/MeOH. In this system, **8** (TLC R_f = 0.16) elutes slightly ahead of **7** (TLC R_f = 0.09). Though there was overlap in the elution, a pure sample of **8** (80 mg, light brown oil) was obtained. Fractions containing the less mobile **7** were crystallized from CHCl₃/*i*-PrOH (stored at –20 °C and washed with ice-cold chloroform after collection by vacuum filtration) to give pure **7** as white crystals (124 mg, 0.35 mmol, 6 % yield). Mp: 75.8 – 76.7 °C. ¹H NMR (D₂O, 300 MHz) δ 7.44 (t, *J* = 7.8 Hz, 2H), 7.14 (d, *J* = 8.6 Hz, 2H), 7.08 (t, *J* = 7.4 Hz, 1H), 4.09 (dd, *J* = 14.6, 5.0 Hz, 2H), 3.83 – 3.58 (m, 4H), 3.49 (q, *J* = 7.4 Hz, 2H), 3.40 – 3.26 (m, 2H), 1.48 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (D₂O, 75 MHz) δ 147.7, 129.8, 121.5, 117.1, 44.3, 33.9, 31.5, 7.8. MS (ESI) *m/z* calculated for C₁₂H₁₈NS⁺ [M]⁺: 208.1160, found 208.1157. Compound **8**: ¹H NMR (CDCl₃, 300 MHz) δ 7.78 – 7.62 (m, 4H), 7.56 (t, *J* = 7.0 Hz, 1H), 4.95 – 4.82 (m, 2H), 4.09 – 3.95 (m, 2H), 3.87 (q, *J* = 7.2 Hz, 2H), 3.15 – 3.02 (m, 2H), 2.95 – 2.83 (m, 2H), 1.10 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃, 75 MHz) δ 138.3, 131.5, 130.8, 122.7, 68.1, 62.1, 22.4, 7.8. MS (ESI) *m/z* calculated for C₁₂H₁₈NS⁺ [M]⁺: 208.1160, found 208.1164. Note that compound **8** proved to be inactive as an α7 partial agonist and was not potentiated with **1**.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

nAChR	nicotinic acetylcholine receptor
ACh	acetylcholine
PAM	positive allosteric modulator
diEPP	N,N-diethyl-N'-phenylpiperazine

EPTM	1-ethyl-4-phenylthiomorpholin-1-ium
SAR	structure-activity relationship
TMS-I	trimethyl-sulfonium iodide
THF	tetrahydrofuran
DMSO	dimethyl sulfoxide
CH₂Cl₂	methylene chloride or dichloromethane
RT	room temperature
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
CRC	concentration response curve
PEP	N-phenyl-N'-ethyl piperazine
iCRC	inhibition concentration response curve
MMPBSA	molecular mechanics Poisson Boltzman surface area

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Figure 1.
Selected $\alpha 7$ nAChR ligand structures.

Figure 2.
A drawing of the asymmetric unit of compound **7** is shown with thermal displacement ellipsoids at 40%.

Figure 3.

Concentration-response studies for **7**, **4**, and **5** with $\alpha 7$ nAChR. Top Panel: CRC for the orthosteric activation of $\alpha 7$ nAChR for **7** and **4** applied alone at 10, 30, 100, 300 μM , and 1 mM, and for **5** applied alone at 10, 30, 100, and 300 μM . The responses are measured as net charge normalized to 60 μM ACh applied alone. Each point is the average normalized response of at least four cells ($\pm$ S.E.M.). Bottom panel: iCRC for the orthosteric inhibition of $\alpha 7$ nAChR for **7**, **4**, and **5** at 0.3, 1, 3, 10, 30, 100, and 300 μM co-applied with 60 μM ACh. The responses are measured as net charge normalized to responses to 60 μM ACh

applied alone. Each point is the average normalized response of at least four cells ($\pm$ S.E.M.).

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Figure 4.

Activity of **7**, **4**, and **5** at the $\alpha 7$ nAChR. The vertical axis refers to the net charge of the compound at the given concentration co-applied with 10 μM **1**, relative to 60 μM ACh controls. Experimental values are the average normalized response of at least four cells ($\pm$ S.E.M.). In all cases these compounds showed less than 10% of the control ACh response when they were applied alone to the receptor.

Figure 5.

Desensitization by compound 7. Each trace in the figure is the averaged data from 8 cells, normalized to their control ACh responses recorded prior to the application of 7. Each trace is 210 seconds long. As shown, the response to 60 μM ACh obtained after the application of 7 was unchanged compared to the pre-application control. The inset shows the response to 100 μM 7 alone, at a 40-fold increased scale.

Figure 6. Molecular electrostatic potential surfaces for **5** (left), **7** (middle), and **4** (right). Blue represents the most positive regions on the molecular surface, and yellow the most negative. Methods were as described in the supplemental information.

Reagents and conditions: (a) K_2CO_3 (2 equiv), *L*-proline (0.2 equiv), CuI (0.1 equiv), dry DMSO, 90-100 °C, Argon, 12 h, 61%. (b) $\text{CF}_3\text{SO}_3\text{CH}_2\text{CH}_3$ (1.5 equiv), dry CH_2Cl_2 , 0 °C to RT, 5 days, 6%.

Scheme 1.

Synthesis of 1-ethyl-4-phenylthiomorpholin-1-ium trifluoromethane sulfonate, **7**.