Published in final edited form as:
J Med Chem. 2013 November 14; 56(21): 8943–8947. doi:10.1021/jm401267t.

Expeditious Synthesis, Enantiomeric Resolution and Enantiomer Functional Characterization of (4-(4-bromophenyl)-3a, 4, 5, 9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide (4BP-TQS): an Allosteric agonist-Positive Allosteric Modulator of α7 Nicotinic Acetylcholine Receptors

Ganesh A. Thakur[‡,‖,*], **Abhijit R. Kulkarni**[‡], **Jeffrey R. Deschamps**[†], and **Roger L. Papke**[§,*]

[‡]Department of Pharmaceutical Sciences, Bouvé College of Pharmacy, Northeastern University, Boston, MA, 02115, USA

[‖]Center for Drug Discovery, Northeastern University, Boston, MA, 02115, USA

[†]Naval Research Laboratory, Code 6930, 4555 Overlook Avenue, Washington, DC 20375

[§]Department of Pharmacology and Therapeutics, University of Florida, P.O. Box 100267, Gainesville, FL 32610-0267

Abstract

An expeditious microwave-assisted synthesis of 4BP-TQS, its enantiomeric separation and their functional evaluations is reported. Electrophysiological characterization in *Xenopus* oocytes revealed that activity exclusively resided in the (+)-enantiomer **1b** (GAT107) and (−)-enantiomer **1a** did not affect its activity, when co-applied. X-ray crystallography studies revealed the absolute stereochemistry of **1b** to be 3a*R*, 4*S*, 9b*S*. Compound **1b** represents the most potent ago-PAM of α7 nAChRs available to date and is considered for further *in vivo* evaluation.

INTRODUCTION

Nicotinic acetylcholine receptors (nAChRs), members of Cys-loop superfamily of cationic ligand-gated ion channels are involved in the physiological responses to the neurotransmitter acetylcholine (ACh) and are distributed throughout the central and peripheral nervous systems.[1–2] Several distinct nAChR subtypes have been identified based on subunit composition and stoichiometry.[3] The homopentameric α7 nAChR subtype is distinguished from the other nAChRs by its relatively high permeability to Ca^{+2}, rapid activation and desensitization (<100 ms) following exposure to agonists and sensitivity to antagonists such as α-bungarotoxin and methyllycaconitine.[4,5] It has been considered a promising target for improving cognitive impairments in diseases such as Alzheimer's (AD) and schizophrenia[6] as well as for treatment of inflammation and neuropathic pain.[7] In recent years, a variety of

[*]**Corresponding Authors** G.A.T.: Phone: +1-617-373-8163; Fax, +1-617-373-8886; g.thakur@neu.edu. [*]R.L.P.: Phone: +1-352 392-4712; rlpake@ufl.edu.

ASSOCIATED CONTENT

Supporting Information

Crystal data and structure refinement, atomic coordinates and equivalent isotropic displacement parameters, bond lengths and angles, anisotropic displacement parameters, hydrogen coordinates and isotropic displacement parameters, circular dichroism and torsion angles for compound **1b** are provided. Chiral HPLC separation details as well as ^{1}H NMR, ^{13}C NMR, DQ-COSY, HSQC, HMBC and ROESY spectra of compound **1b** in DMSO-d_6 solutions and methods for its electrophysiological studies including the raw data of Fig.2A are provided. The material is available free of charge at http://pubs.acs.org

structurally distinct, subtype-selective and potent α7 nAChR agonists have been developed and profiled.[8] However, rapid desensitization of α7 nAChRs in response to high agonist concentration *in vitro* [9] together with the possibility of endogenous tone disruption[10] has led to concerns regarding utility of such agonists as clinical candidates.[2]

An alternative therapeutic approach has been the development of positive allosteric modulators (PAMs) which can synergize and augment orthosteric-site-mediated signaling of endogenous neurotransmitter ACh without, in most cases, directly activating or desensitizing the receptor.[11] Selective PAMs of α7 nAChR have been reported and classified as type I and type II (Figure 1).[11,12] Type I PAMs increase peak agonist-evoked responses but have little or no effect on the decay rate of macroscopic currents or the equilibrium desensitization of α7 nAChR,[13] whereas type II PAMs increase peak currents as well as slow down the apparent desensitization profile of the agonist response.[12,14,15] Both types of PAMs have been reported to show *in vivo* efficacy in animal model of cognition, however, only type II PAMs are efficacious in neuropathic pain models.[16] Additional PAMs displaying the properties intermediate to the type I and type II have also been identified.[17,18]

Recently, 4-(4-bromophenyl)-3*a*,4,5,9*b*-tetrahydro-3*H* cyclopenta[c]quinoline-8-sulfonamide (**1**, 4BP-TQS, Figure 1), a substituent analog of TQS, was shown to possess allosteric agonism in addition to type II PAM activity (ago-PAM).[19,20] Compound **1** was shown to cause agonism through a site topographically distinct from the ACh site and was a more potent and efficacious agonist of α7 nA-ChR than ACh (8-fold lower EC_{50} and 45-fold larger maximal response).[19] Acting as an allosteric agonist, **1** produced less equilibrium desensitization than would be seen with orthosteric agonists. A relatively slow-desensitizing allosteric agonist might be particularly beneficial compared to a ligand with only PAM activity, under conditions of severe loss of endogenous acetylcholine, such as in advanced Alzheimer's disease. Although, several examples of ago-PAMs have been reported for G-protein coupled receptors, availability of such modulators for ion-channel receptors is limited.

Compound **1** is by far the most potent ago-PAM of α7 nAChR available for investigating the effect of such modulation in biological system.[20] However, it has been studied exclusively *in vitro* as a racemate.[19,20] It has three chiral centers with the cyclopentene and 4-bromophenyl rings oriented *cis*- to each other. Neither the individual contributions of each enantiomer towards racemate's dual activity (ago-PAM) at α7 nAChR nor the effect of presence of one enantiomer on the activity of the other is known.

In this work, we have developed an expeditious, microwave-assisted synthesis of **1**, performed separation of its enantiomers followed by their functional evaluations and identified the (+)-enantiomer **1b** (GAT107) as the bioactive enantiomer having 3a*R*, 4*S*, 9b*S* absolute stereochemistry.

RESULT AND DISCUSSION

Chemistry

The tetrahydro-3*H*-cyclopenta[c]quinoline scaffold of **1** is synthetically accessible using the three-component Povarov cyclization (aza-Diels-Alder reaction).[21,22] The reaction between 4-aminosulfonamide, 4-bromobenzaldehyde and cyclopentadiene in the presence of 20 mole % $InCl_3$ at room temperature, over 24 hour was reported to give the desired product in only 37% yield and the *cis*-diasteromer **1** was reported to be the major product.[20] We recently developed a microwave-accelerated and high yielding synthesis of cyclopentene ring-fused tetrahydroquinolines.[23] In this work, we extend the application of this methodology to the synthesis of **1**. Our optimized procedure employed $InCl_3$ (20 mole%) in acetonitrile, for 15

minutes at 100°C under microwave irradiation, which gave **1** in 70% yield and *cis*-diasteromer was obtained exclusively (Scheme 1). The reaction conditions were highly reproducible and **1** could easily be scaled-up to multi-gram quantities. Compound **1** contains three chiral centers and the *cis*- stereochemical orientation of cyclopentene and 4-bromophenyl rings was confirmed based on the coupling constant between protons H-3a and H-4 ($J_{3a,4}$ = 3.5Hz; see Experimental) in ^{1}H NMR.

Enantiomeric resolution of racemate **1** was undertaken by chiral HPLC. Several analytical conditions were tried, and a hexane/ethanol (60:40) mixture as a mobile phase with Chiralpak IA was found to provide baseline separation of two enantiomers (see SI Fig. 1). These optimized conditions were used for enantiomeric excess determination and were further extended to the preparative method for separation of enantiomer pair **1a** and **1b**. The preparative chiral HPLC method was scaled up for the separation of gram quantities of racemic **1** to provide enantiomer **1a** and **1b** in > 99% enantiomeric excess (ee).

The CD spectra of the enantiomers of **1** were measured using their solutions in chloroform (2.45 mM for **1a** and 2.55 mM for **1b**) and were mirror images of each other, thus, confirming their enantiomeric nature (See SI, Fig. 2). Enantiomers exhibited a strong Cotton effect at shorter wavelength (272 nm). The structure of **1b** was established using 1D NMR spectra as well as ^{13}C NMR, DQ-COSY, HSQC, HMBC and ROESY correlations (see SI).

In-vitro Biological Evaluation

Functional characterization of enantiomers in Xenopus oocytes—Oocytes expressing human α7 nAChR were studied with two-electrode voltage clamp (see Methods in SI).[24] Cells were given control applications of 60 μM ACh and then treated with 10 μM of **1a** or **1b**. As shown in Figure 2A, 1a neither evoked a response nor significantly enhanced the size of responses evoked by subsequent applications of ACh at the control concentration. Likewise, co-application of **1a** with ACh did not potentiate ACh evoked responses. In contrast, **1b** was very effective both as an allosteric agonist when applied alone and as a PAM when co-applied with ACh. Note also that the potentiating effects of **1b** were long lasting, suggesting that it may remain bound to receptors after bath applications, such that there was very good potentiation of responses to the application of ACh alone four minutes after the application of **1b** alone. During this wash period, currents returned to baseline but receptors nonetheless remained primed for potentiated ACh responses.

Concentration dependence of 1b: allosteric activation and potentiation—Using our standard 20-second application protocol, we evaluated the concentration-response relationship for **1b**-evoked net-charge response.[24] With this protocol we estimated an EC_{50} of 28 ± 3 μM (Figure 2B). The potentiation of subsequent ACh-evoked responses showed similar concentration dependence. It should be noted that these data are based on relatively brief applications of **1b**, and since the potentiating effects are only slowly reversible, it is likely that the potency for allosteric activation and potentiation might be increased with longer incubations.

Enantiomer competition and potentiation time course—In order to determine whether the inactive enantiomer might function as a competitive antagonist of **1b**, oocytes were treated with either 10 μM **1b** alone or in combination with 10 μM **1a**. As shown in Figure 3A, neither the allosteric activation (t = 0), nor the potentiation of subsequent ACh-evoked responses by **1b** were reduced when **1a** was co-applied with the active isomer. In this experiment two pre-incubation times were tested, 30 seconds and 5 minutes. As expected, the longer incubations produced greater allosteric activation and larger

potentiation of the subsequent ACh-evoked responses. The decay rate of the **1b** potentiation was too slow to measure over the course of 20 minutes.

Comparison of 1b to racemic 1—Although our data indicate that the inactive isomer is not a competitive antagonist of **1b**, a racemic preparation of **1** would be expected to have less activity than the purified preparation of **1b** at the same concentration. As shown in Figure 3B, the potentiating activity of 30 μM of **1** co-applied with 300 μM of the α7-selective agonist choline was significantly less than that of 30 μM **1b**.

X-ray crystal structure—To define the absolute configuration of C3a, C4 and C9b, we explored different crystallization conditions for bioactive enantiomer **1b**. Crystals suitable for X-ray investigation were obtained when compound **1b** was crystallized from CH_2Cl_2. As shown in Figure 4, the stereochemistry of the active enantiomer was confirmed to be *cis* and the absolute configuration was revealed to be 3a*R*, 4*S*, 9b*S*.

CONCLUSION

In conclusion, we have developed a novel, microwave-accelerated synthesis of 4BP-TQS and performed separation of its enantiomer using chiral HPLC. The second eluted (+)-enantiomer **1b** was shown to possess almost all α7 ago-PAM activity, whereas, the (−)-enantiomer **1a** had negligible activity as an allosteric agonist or as a PAM and it did not affect activity of enantiomer **1b** when applied together. X-ray crystallography studies revealed the absolute stereochemistry of the active enantiomer **1b** to be 3a*R*, 4*S*, 9b*S*. This critical information will be valuable for further lead optimization efforts. Compound **1b** represents the most potent ago-PAM of α7 nAChR available to date, and is a valuable tool to investigate the biological functions controlled by α7 nAChR both *in vitro* and *in vivo*.

EXPERIMENTAL SECTION

Chemistry

All commercial chemicals and solvents were purchased from Sigma-Aldrich Inc. and Alfa Aesar and unless otherwise specified they were used without further purification. Biotage Initiator microwave system was used for the synthesis. The progress of the reaction was monitored by thin layer chromatography (TLC) using commercially prepared silica gel 60 F_{254} glass-backed plates. All compounds were visualized under ultraviolet (UV) light. NMR spectra and other 2D spectra were recorded in DMSO-d_6, unless otherwise stated, on a Varian 500MHz. Chemical shifts are recorded in parts per million (δ) relative to internal tetramethylsilane (TMS). Multiplicities reported in Hertz (Hz). LCMS analysis was performed using a Waters Alliance reverse-phase HPLC, (electrospray ionization). The CD spectra of enantiomers (dissolved in $CHCl_3$) were measured on a Jasco J-815 CD spectropolarimeter. Specific rotations of enantiomers were measured at 589 nm with a Jasco polarimeter model P-2000 equipped with a Na lamp. The volume of the cell was 100 mm and were specific rotations were recorded in methanol (c = 1.0).

4-(4-Bromophenyl)-3*a*,4,5,9*b*-tetrahydro-3*H*-cyclopenta[c]quinoline-8-sulfonamide (1)

In a microwave vial, cyclopentadiene (2.14 g, 32.43 mmol, 3 equiv.) was added to a suspension of 4–bromobenzaldehyde (2 g, 10.81 mmol), 4-aminosulfonamide (1.86 g, 10.81 mmol) and indium trichloride (476 mg, 2.15 mmol, 0.2 equiv.) in acetonitrile (15 mL). The reaction vial was placed in a microwave synthesizer and heated to 100°C for 15 min. The contents were added to aqueous Na_2CO_3 solution (0.1 M; 30 mL) and extracted with chloroform (3×50 mL). The combined organic layer was washed with water (20 mL), brine (30 mL), and (Na_2SO_4) and concentrated under reduced pressure. The residue was purified

by chromatography (EtOAc/Hexane = 20/80 → 50/50) to yield compound 1 as a white solid (3.07 g; yield 70%). See SI for the details of enantiomer separation and characterization.

(3a*R*,4*S*,9b*S*)-4-(4-Bromophenyl)-3a,4,5,9btetrahydro-3*H*-cyclopenta[c]quinoline-8-sulfonamide (1b)

^{1}H NMR (500 MHz, DMSO) δ 7.59 (d, *J* = 8.5 Hz, 2H, H-12, H-14), 7.43 (d, *J* = 2.5 Hz, 1H, H-9), 7.41 (d, *J* = 8.5 Hz, 2H, H-11, H-15), 7.34 (dd, *J* = 8.5, 2.0 Hz, 1H, H-7), 6.97 (s, 2H, NH_2), 6.80 (d, *J* = 8.5 Hz, 1H, H-6), 6.39 (br s, 1H, NH), 5.92 – 5.86 (m, 1H, H-1), 5.64 – 5.59 (m, 1H, H-2), 4.62 (d, *J* = 3.5 Hz, 1H, H-4), 4.06 (br d, *J* = 9.0 Hz, 1H, H-9b), 2.98 – 2.88 (m, 1H, H-3a), 2.33 (ddd, *J* = 16.0, 10.0, 2.5 Hz, 1H, H-3eq), 1.64 (ddd, *J* = 15.5, 10.0, 1.0 Hz, 2H, H-3ax). ^{13}C NMR (126 MHz, DMSO) δ 149.87 (ArC-5), 142.32 (ArC-10), 134.92 (C-1), 133.09 (ArC-8), 131.79 (ArC-12 and ArC-14), 130.68 (C-2), 129.52 (ArC-11, ArC-15), 127.42 (ArC-9), 124.73 (ArC-7), 124.67 (ArC-9a), 120.64 (ArC-13), 115.70 (ArC-6), 56.10 (C-4), 45.70 (C-9b), 45.68 (C-3a), 31.85 (C-3). Chemical purity = 99.7 % by HPLC and ee = 99.88%. m/z = 405.1 $[M+H]^+$; $[\alpha]^{25}_{D}$ = +4.3° (c = 1, MeOH).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grants from National Institute on Drug Abuse (DA027113 to GAT), National Institute of General Medical Sciences (GM057481 to RLP). X-ray crystallographic studies were supported by the National Institute on Drug Abuse (NIDA) under contract Y1-DA1101. We are thankful to Myrick Dennis for help with recording CD spectra, Roger Kautz for help with recording 2D NMR spectra, Avinash Chaudhary for his help in chiral separation and ee determination and Ralph Loring for helpful comments on the manuscript.

ABBREVIATIONS

PAM	positive allosteric modulator
AD	Alzheimer's disease
ee	enantiomeric excess
TMS	tetramethylsilane
ACh	acetyl choline
nAChR	nicotinic acetylcholine receptors
4BP-TQS	4-(4-bromophenyl)-3*a*,4,5,9*b* tetrahydro-3*H*-cyclopenta[c]quinoline-8-sulfonamide
CRC	concentration response curve.

REFERENCES

1. Taly A, Corringer PJ, Guedin D, Lestage P, Changeux JP. Nicotinic receptors: allosteric transitions and therapeutic targets in the nervous system. Nat. Rev. Drug. Discov. 2009; 8:733–750. [PubMed: 19721446]
2. Hurst R, Rollema H, Bertrand D. Nicotinic acetylcholine receptors: From basic science to therapeutics. Pharmacol. Ther. 2013; 137:22–54. [PubMed: 22925690]
3. Gotti C, Clementi F, Fornari A, Gaimarri A, Guiducci S, Manfredi I, Moretti M, Pedrazzi P, Pucci L, Zoli M. Structural and functional diversity of native brain neuronal nicotinic receptors. Biochem. Pharmacol. 2009; 78:703–711. [PubMed: 19481063]

4. Seguela P, Wadiche J, Dineley-Miller K, Dani JA, Patrick JW. Molecular cloning, functional properties, and distribution of rat brain alpha 7: a nicotinic cation channel highly permeable to calcium. J. Neurosci. 1993; 13:596–604. [PubMed: 7678857]
5. Dajas-Bailador F, Wonnacott S. Nicotinic acetylcholine receptors and the regulation of neuronal signalling. Trends Pharmacol. Sci. 2004; 25:317–324. [PubMed: 15165747]
6. Wallace TL, Porter RH. Targeting the nicotinic alpha7 acetylcholine receptor to enhance cognition in disease. Bio-chem. Pharmacol. 2011; 82:891–903.
7. Loram LC, Taylor FR, Strand KA, Maier SF, Speake JD, Jordan KG, James JW, Wene SP, Pritchard RC, Green H, Van Dyke K, Mazarov A, Letchworth SR, Watkins LR. Systemic administration of an alpha-7 nicotinic acetylcholine agonist reverses neuropathic pain in male sprague dawley rats. J. Pain. 2012; 13:1162–1171. [PubMed: 23182225]
8. Mazurov AA, Speake JD, Yohannes D. Discovery and development of alpha7 nicotinic acetylcholine receptor modulators. J. Med. Chem. 2011; 54:7943–7961. [PubMed: 21919481]
9. Bertrand D, Bertrand S, Ballivet M. Pharmacological properties of the homomeric alpha 7 receptor. Neurosci. Lett. 1992; 146:87–90. [PubMed: 1475054]
10. Papke RL, Kem WR, Soti F, Lopez-Hernandez GY, Horenstein NA. Activation and desensitization of nicotinic alpha7-type acetylcholine receptors by benzylidene anabaseines and nicotine. J. Pharmacol. Exp. Ther. 2009; 329:791–807. [PubMed: 19223664]
11. Williams DK, Wang J, Papke RL. Positive allosteric modulators as an approach to nicotinic acetylcholine receptor- targeted therapeutics: advantages and limitations. Bio-chem. Pharmacol. 2011; 82:915–930.
12. Gronlien JH, Hakerud M, Ween H, Thorin-Hagene K, Briggs CA, Gopalakrishnan M, Malysz J. Distinct profiles of alpha7 nAChR positive allosteric modulation revealed by structurally diverse chemotypes. Mol. Pharmacol. 2007; 72:715–724. [PubMed: 17565004]
13. Broad LM, Zwart R, Pearson KH, Lee M, Wallace L, McPhie GI, Emkey R, Hollinshead SP, Dell CP, Baker SR, Sher E. Identification and pharmacological profile of a new class of selective nicotinic acetylcholine receptor potentiators. J. Pharmacol. Exp. Ther. 2006; 318:1108–1117. [PubMed: 16738207]
14. Hurst RS, Hajos M, Raggenbass M, Wall TM, Higdon NR, Lawson JA, Rutherford-Root KL, Berkenpas MB, Hoffmann WE, Piotrowski DW, Groppi VE, Allaman G, Ogier R, Bertrand S, Bertrand D, Arneric SP. A novel positive allosteric modulator of the alpha7 neuronal nicotinic acetylcholine receptor: in vitro and in vivo characterization. J. Neurosci. 2005; 25:4396–4405. [PubMed: 15858066]
15. Williams DK, Wang J, Papke RL. Investigation of the molecular mechanism of the alpha7 nicotinic acetylcholine receptor positive allosteric modulator PNU-120596 provides evidence for two distinct desensitized states. Mol. Pharmacol. 2011; 80:1013–1032. [PubMed: 21885620]
16. Freitas K, Ghosh S, Ivy Carroll F, Lichtman AH, Imad Damaj M. Effects of alpha 7 positive allosteric modulators in murine inflammatory and chronic neuropathic pain models. Neuropharmacology. 2012; 65:156–164. [PubMed: 23079470]
17. Dunlop J, Lock T, Jow B, Sitzia F, Grauer S, Jow F, Kramer A, Bowlby MR, Randall A, Kowal D, Gilbert A, Comery TA, Larocque J, Soloveva V, Brown J, Roncarati R. Old and new pharmacology: positive allosteric modulation of the alpha7 nicotinic acetylcholine receptor by the 5-hydroxytryptamine(2B/C) receptor antagonist SB-206553 (3,5-dihydro-5-methyl-N-3-pyridinylbenzo[1,2-b-4,5-b']di pyrrole-1(2H)-carboxamide). J. Pharmacol. Exp. Ther. 2009; 328:766–776. [PubMed: 19050173]
18. Dinklo T, Shaban H, Thuring JW, Lavreysen H, Stevens KE, Zheng L, Mackie C, Grantham C, Vandenberk I, Meulders G, Peeters L, Verachtert H, De Prins E, Lesage AS. Characterization of 2-[[4-fluoro-3-(trifluoromethyl)phenyl]amino]-4-(4-pyridinyl)-5-thiazolemethanol (JNJ-1930942), a novel positive allosteric modulator of the {alpha}7 nicotinic acetylcholine receptor. J. Pharmacol. Exp. Ther. 2011; 336:560–574. [PubMed: 21084390]
19. Gill JK, Savolainen M, Young GT, Zwart R, Sher E, Millar NS. Agonist activation of alpha7 nicotinic acetylcholine receptors via an allosteric transmembrane site. Proc. Natl. Acad. Sci. U.S.A. 2011; 108:5867–5872. [PubMed: 21436053]

20. Gill JK, Dhankher P, Sheppard TD, Sher E, Millar NS. A series of alpha7 nicotinic acetylcholine receptor allosteric modulators with close chemical similarity but diverse pharmacological properties. Mol. Pharmacol. 2012; 81:710–710. [PubMed: 22328718]

21. Kouznetsov VV. Recent synthetic developments in a powerful imino Diels-Alder reaction (Povarov reaction): application to the synthesis of N-polyheterocycles and related alkaloids. Tetrahedron. 2009; 65:2721–2750.

22. Sridharan V, Suryavanshi PA, Menendez JC. Advances in the Chemistry of Tetrahydroquinolines. Chem. Rev. 2011; 111:7157–7259. [PubMed: 21830756]

23. Kulkarni AR, Thakur GA. Microwave-assisted expeditious and efficient synthesis of cyclopentene ring-fused tetrahydroquinoline derivatives using three-component Povarov reaction. Tet. Lett. 2013 (http://dx.doi.org/101016/j.tetlet.2013.09.107).

24. Papke RL, Porter Papke JK. Comparative pharmacology of rat and human alpha7 nAChR conducted with net charge analysis. Br. J. Pharmacol. 2002; 137:49–61. [PubMed: 12183330]

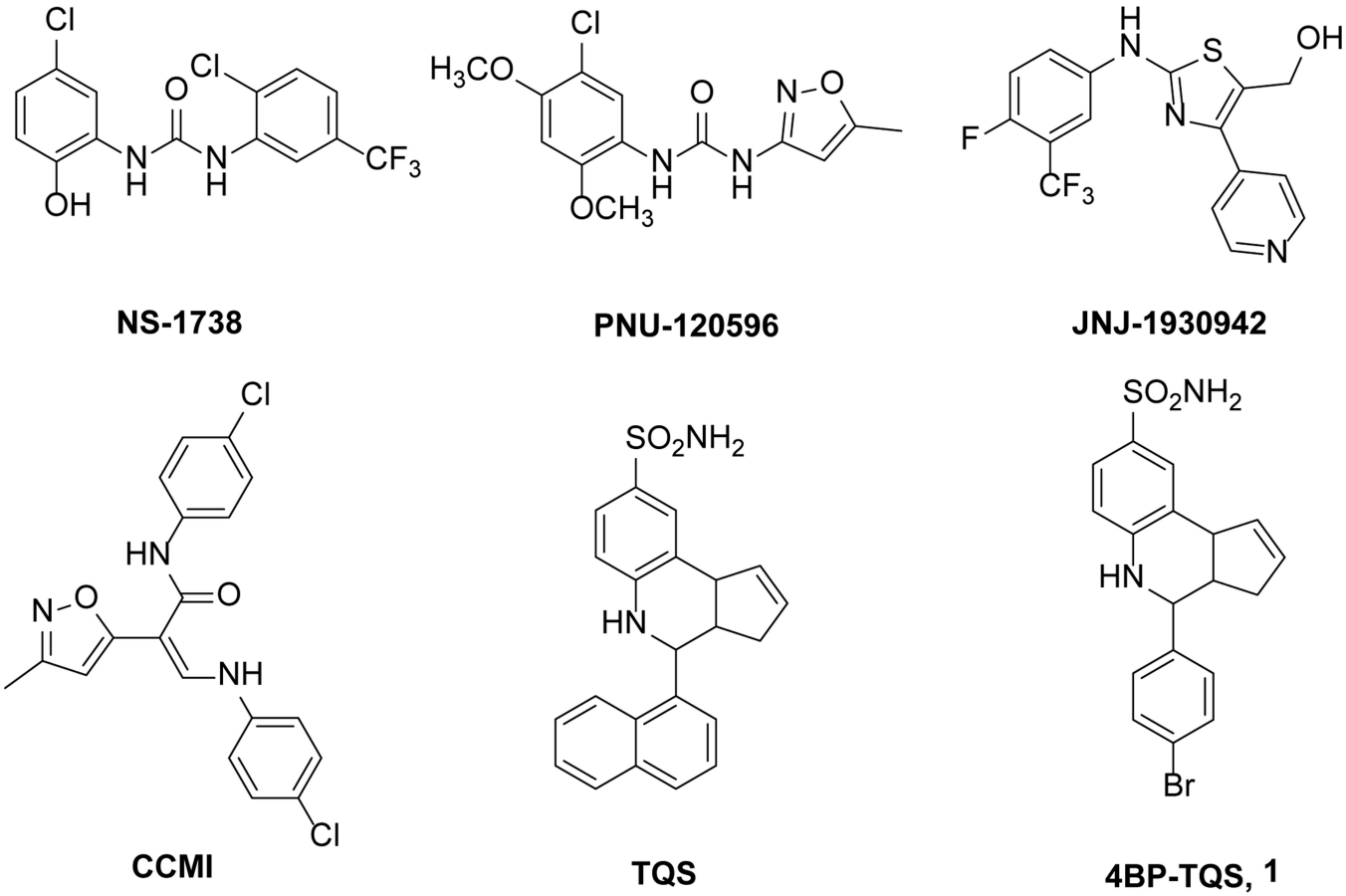


Figure 1.

Representative α7nAChR PAMs: type I PAMs [(*Z*)-*N*-(4-chlorophenyl)-3-((4-chlorophenyl)amino)-2-(3-methylisoxazol-5-yl)acrylamide (CCMI), NS-1738], type II PAMs [PNU-120596, 4-(naphthalen-1-yl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide (TQS)], intermediate acting PAM (JNJ-1930942) and an allosteric agonist-type II PAM compound (4BP-TQS, 1).

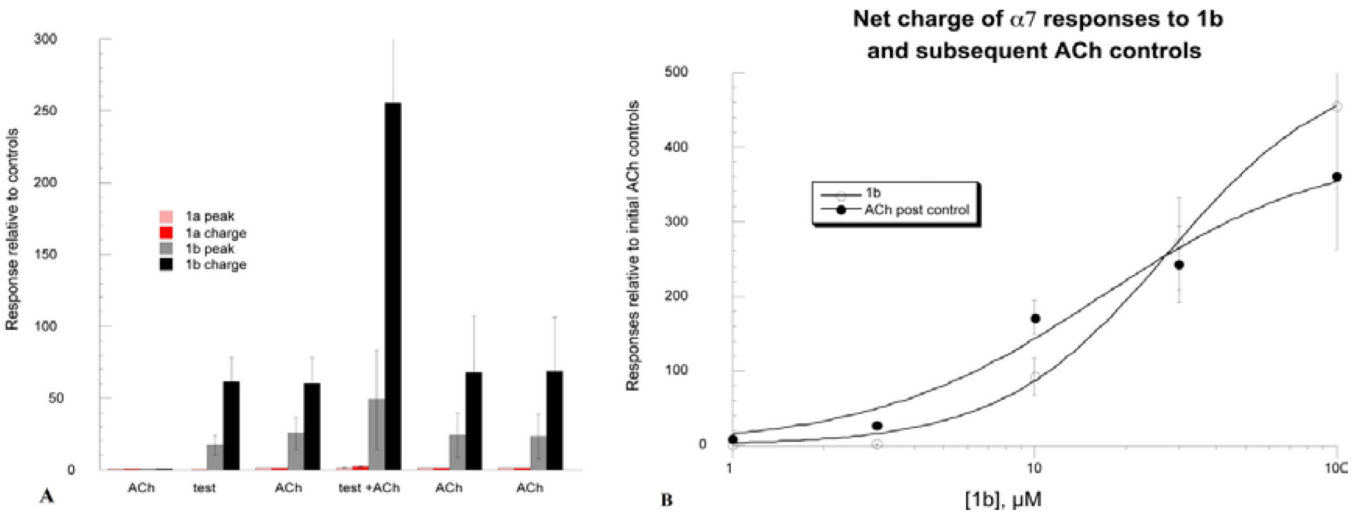


Figure 2.

(A) Raw data showing the effectiveness of **1b**, but not **1a**, as an allosteric agonist and a PAM (10μM) is included as Fig. 3 in SI. Note the lack of response to applications of 1a alone and the ineffectiveness of 1a to enhance ACh evoked responses. In contrast, **1b** functioning as an allosteric agonist evoked large responses when applied alone and greatly enhanced ACh evoked responses. Averaged values for the experiment shown. Data were normalized to the average of two ACh control responses obtained prior to first application of the compounds **1a** and **1b**. Each bar represents the average (±SEM of at least 4 oocytes). (B)Concentration response curves (CRCs) for **1b** allosteric activation and potentiation of subsequent ACh-evoked responses. Cells expressing α7 were tested for their responses to the application of 60 μM ACh alone and then tested with **1b** at the indicated concentrations. The data represent the responses of at least four oocytes for each condition, normalized to the average of the two initial ACh control responses.

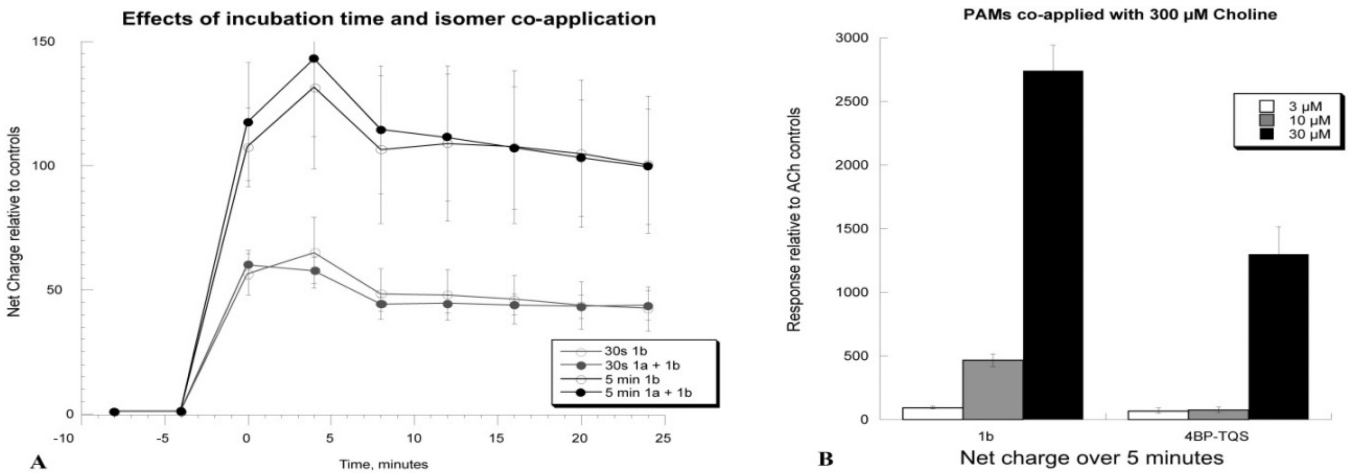


Figure 3.

(**A**) Extended pre-incubations with **1b** produced greater allosteric activation and increased the potentiation of ACh-evoked responses by **1b**. These effects were not significantly affected when **1a** was co-applied, suggesting that **1a** is not a competitive antagonist of the active isomer. Data were normalized to the average of two responses evoked by ACh alone prior to the application of compounds **1a** and **1b**. The points represent the average responses of at least four oocytes to either the GAT compounds alone (at t = 0) or the application of 60 µM ACh (all other points). Note that although the responses to 5 minute incubations had both transient and sustained components, the data plotted are for the standard 120-second interval beginning with the start of drug application. (**B**) The potentiating activity of **1b** compared to **1**. Oocytes expressing α7 were evaluated for responses to control applications of 60 µM ACh and then incubated with 300 µM choline and either **1b** or **1** at the indicated concentrations. Net-charge responses were calculated for the entire 5-minute incubation periods. Data were normalized to the average of two responses evoked by ACh alone prior to the application of the choline and PAMS. The bars represent the average responses of at least 4 oocytes

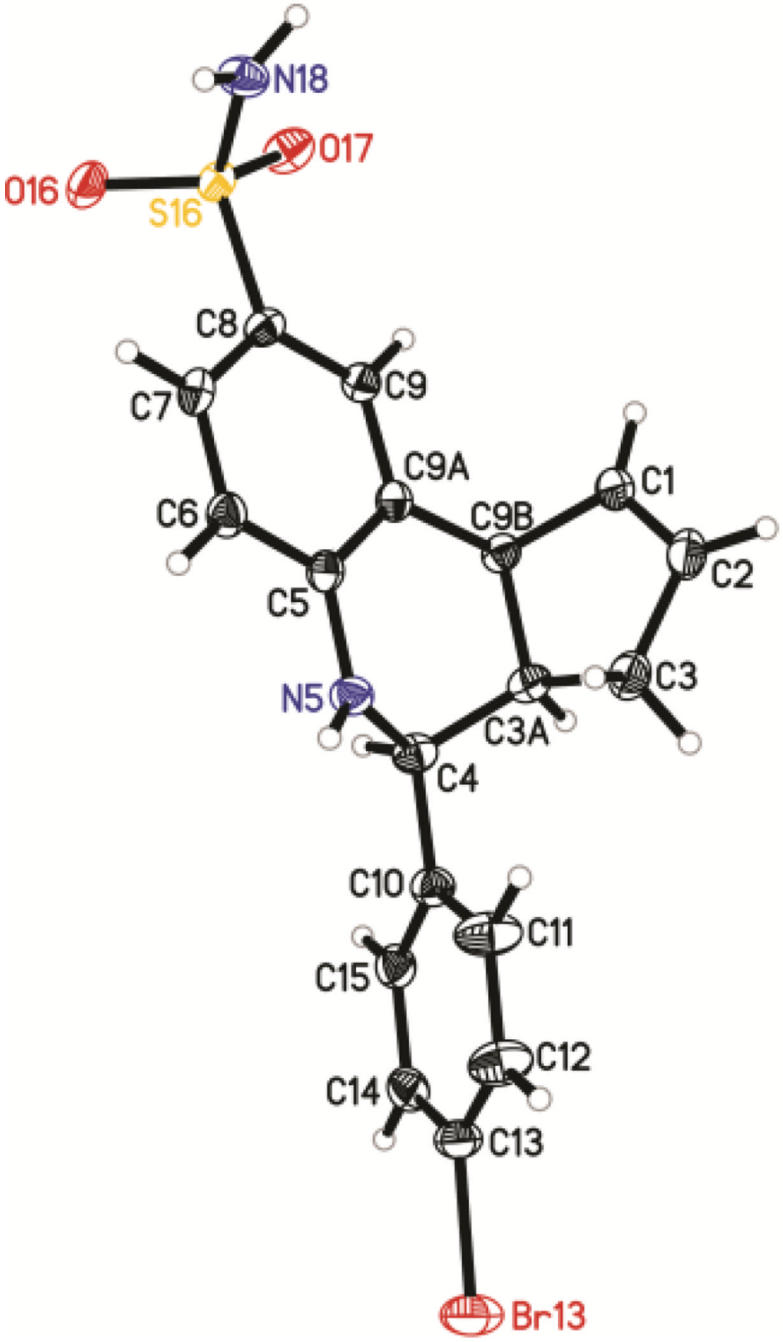
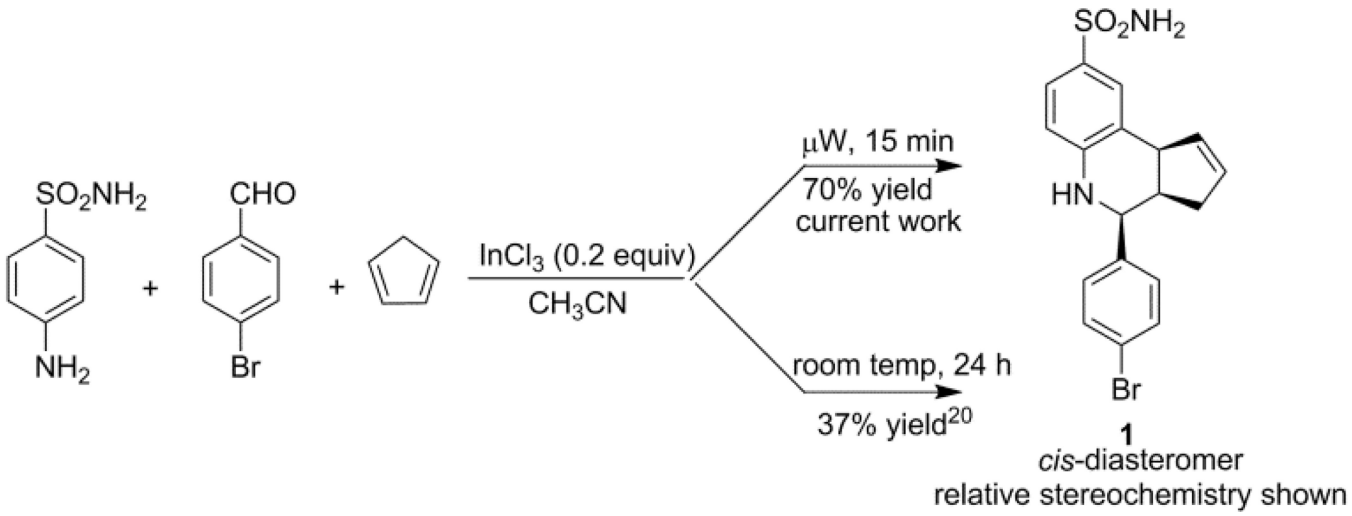


Figure 4.
The molecular structure of **1b** as determined by x-ray diffraction. For clarity only one of the two molecules in the asymmetric unit is shown; displacement ellipsoids are at the 50% level.



scheme 1.
Improved synthesis of compound 1