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, 140 The Fenway, 360 Huntington Avenue, Boston, Massachusetts, 02115, United States
‡Center for Drug Discovery, Northeastern University, 360 Huntington Avenue, Boston, Massachusetts, 02115, United States
§Naval Research Laboratory, Code 6930, 4555 Overlook Avenue, Washington, DC 20375, United States
∥Department of Pharmacology and Therapeutics, University of Florida, P.O. Box 100267, Gainesville, Florida 32610-0267, United States

Supporting Information

ABSTRACT: An expeditious microwave-assisted synthesis of 4BP-TQS, its enantiomeric separation, and their functional evaluation 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 coapplied. X-ray crystallography studies revealed the absolute stereochemistry of 1b to be 3aR,4S,9bS. 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++, rapid activation, and desensitization (<100 ms) following exposure to agonists and sensitivity to antagonists such as α-bungarotoxin and methylycaconitine.4,5 It has been considered a promising target for improving cognitive impairments in diseases such as Alzheimer’s disease (AD) and schizophrenia6 as well as for treatment of inflammation and neuropathic pain.7 In recent years, a variety of 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 vitro9 together with the possibility of endogenous tone disruption10 has led to concerns regarding their utility 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 types I and 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 nAChRs 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,

Received: April 29, 2013
Published: October 3, 2013
however, only type II PAMs are efficacious in neuropathic pain models. Additional PAMs displaying the properties intermediate to the types I and II have also been identified. Recently, 4-(4-bromophenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide (1, 4BP-TQS, Figure 1), a substituent analogue of TQS, was shown to possess allosteric agonism in addition to type II PAM activity (ago-PAM). I was shown to cause agonism through a site topographically distinct from the ACh site and was a more potent and efficacious agonist of α7 nAChR than ACh (8-fold lower EC50 and 45-fold larger maximal response). Acting as an allosteric agonist, I 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 ACh such as in advanced AD. Although, several examples of ago-PAMs have been reported for G-protein coupled receptors, availability of such modulators for ion-channel receptors is limited.

I is by far the most potent ago-PAM of α7 nAChR available for investigating the effect of such modulation in biological systems. However, it has been studied exclusively in vitro as a racemate. It has three chiral centers, with the cyclopentene and 4-bromophenyl rings oriented cis to each other. Neither the individual contributions of each enantiomer toward 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 I, performed separation of its enantiomers followed by their functional evaluations, and identified the (+)-enantiomer 1b (GAT107) as the bioactive enantiomer having 3aR,4S,9bS absolute stereochemistry.

**RESULTS AND DISCUSSION**

**Chemistry.** The tetrahydro-3H-cyclopenta[c]quinoline scaffold of I is synthetically accessible using the three-component Povarov cyclization (aza-Diels–Alder reaction). The reaction between 4-aminosulfonamide, 4-bromobenzaldehyde, and cyclopentadiene in the presence of 20 mol % InCl3 at room temperature over 24 h was reported to give the desired product in only 37% yield and the cis-diasteromer 1 was reported to be the major product. We recently developed a microwave-accelerated and high yielding synthesis of cyclopentene ring-fused tetrahydroquinolines. In this work, we extend the applicability of this methodology to the synthesis of I. Our optimized procedure employed InCl3 (20 mol %) in acetonitrile for 15 min at 100 °C under microwave irradiation, which gave I in 70% yield and cis-diasteromer was obtained exclusively (Scheme 1). The reaction conditions were highly reproducible, and I could easily be scaled up to multigram quantities. I 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 (J3a,4 = 3.5 Hz, see Experimental Section) in 1H NMR.

Enantiomeric resolution of racemic I 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 Supporting Information (SI) Figure 1). These optimized conditions were used for enantiomeric excess (ee) determination and were further extended to the preparative method for separation of the (+)-enantiomer 1a and the (-)-enantiomer 1b (Scheme 1). The preparative chiral HPLC method was scaled up for the separation of gram quantities of racemic I to provide enantiomer 1a and 1b in >99% ee.

The circular dichroism (CD) spectra of the enantiomers of I 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, Figure 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 13C NMR, DQ-COSY, HSCQC, 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 a two-electrode voltage clamp (see Material and Methods in SI). Cells were given control applications of 60 μM ACh and then treated with 10 μM 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, coapplication 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 coapplied with ACh. Note also that the potentiation 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 4 min 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 s application protocol, we evaluated the concentration–response relationship for 1b-evoked net-charge response. With this protocol, we...
Journal of Medicinal Chemistry

Figure 2. (A) Raw data showing the effectiveness of 1b, but not 1a, as an allosteric agonist and a PAM (10 μM) is included as Figure 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 responses evoked by ACh alone prior to the application of 60 μM ACh and then incubated with 300 μM choline and either 1a or 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 preincubations 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 coapplied, 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 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 min incubations had both transient and sustained components, the data plotted are for the standard 120 s 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 min 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 four oocytes.

estimated an EC50 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 because 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. 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 coapplied with the active isomer. In this experiment, two preincubation times were tested, 30 s and 5 min. 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 min.

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 coapplied 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 the bioactive enantiomer 1b. Crystals suitable for X-ray investigation were obtained when 1b was crystallized from CH2Cl2. As shown in Figure 4, the stereochemistry of the active enantiomer was confirmed to be...
cis and the absolute configuration was revealed to be 3aR,4S,9bS.

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.

**CONCLUSION**

In conclusion, we have developed a novel, microwave-accelerated synthesis of 4BP-TQS and performed its enantiomer separation using chiral HPLC. The second eluted (+)-enantiomer 1b was shown to possess almost all α7 nAChR 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 3aR,4S,9bS. This critical information will be valuable for further lead optimization efforts. 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 F254 glass-backed plates. All compounds were visualized under ultraviolet (UV) light. NMR spectra and other 2D spectra were recorded in DMSO-d6 unless otherwise stated, on a Varian 500 MHz. 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 CHCl3) were measured on a Jasco J-815 CD spectropolarimeter. Speciﬁc rotations were recorded in methanol ([α]D20 = +4.0° (c = 1, MeOH).

**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 1b. Chiral HPLC separation details as well as 1H NMR, 13C NMR, DQ-COSY, HSQC, ROESY spectra of 1b in DMSO-d6 solutions and methods for its electrophysiological studies including the raw data of Figure 2A. This material is available free of charge via the Internet at http://pubs.acs.org.

**AUTHOR INFORMATION**

Corresponding Authors
*G.A.T.: phone, +1-617-373-8163; fax, +1-617-373-8886; E-mail, g.thakur@neu.edu.
*R.L.P.: phone, +1-352-392-4712; E-mail, rlpake@ufl.edu.

Notes
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This work was supported by grants from National Institute on Drug Abuse (DA027113 to G.A.T.), National Institute of General Medical Sciences (GM057481 to R.L.P.). X-ray crystallographic studies were supported by the National Institute on Drug Abuse (DA027113 to G.A.T.), National Institute of General Medical Sciences (GM057481 to R.L.P.).

**ABBREVIATIONS USED**

PAM, positive allosteric modulator; AD, Alzheimer’s disease; ee, enantiomeric excess; TMS, tetramethylsiliane; ACh, acetylcholine; nAChR, nicotinic acetylcholine receptors; 4BP-TQS, (+-)4-(bromomethyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide; CRC, concentration response curve.

8946 dx.doi.org/10.1021/jm4012671 J. Med. Chem. 2013, 56, 8944—8947
REFERENCES

(2) Hurst, R.; Rollemo, H.; Bertrand, D. Nicotinic acetylcholine receptors: from basic science to therapeutics. Pharmacol. Ther. 2013, 137, 22–54.
(20) Kouznetsov, V. V. 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.