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Bioorganic & Medicinal Chemistry Letters 17 (2007) 5872–5875
Bioorganic & Medicinal Chemistry Letters

Synthesis of poison-frog alkaloids 233A, 235U, and 251AA and their inhibitory effects on neuronal nicotinic acetylcholine receptors

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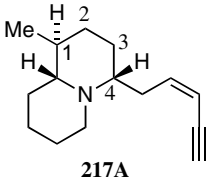
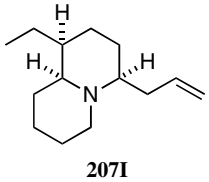
Received 12 July 2007; revised 29 July 2007; accepted 2 August 2007
Available online 26 August 2007

Abstract—We previously reported that the synthetic quinolizidine 1-epi-**207I** is a relatively selective blocker of α7 nicotinic acetylcholine receptors. We now synthesized the analogous poison frog alkaloids **233A**, **235U**, and **251AA**, and investigated the biological activities at two major types of neuronal nicotinic receptors. Electrophysiological study showed that the alkaloid **233A** blocked α7 and α4β2 currents with similar potencies. Alkaloids **235U** and **251AA** also showed similar potencies for blockade of α7 and α4β2 currents. Thus, based on these studies, it would appear that C4 substituents greater in length than the allyl of 1-epi-**207I** reduce α7-potency without affecting α4β2-potency.

Indolizidine and quinolizidine ring systems have been found in microbial, plant, and animal sources,[1] and many alkaloids containing these nuclei show intriguing biological activities.[2] Among them, the 1,4-disubstituted quinolizidine alkaloids have been detected in amphibian skin, and now about 20 alkaloids are assigned to this class.[3] The absolute stereochemistry of **217A**[4] and **207I**[5] is known, but the structures of other alkaloids belonging to this class are tentative.

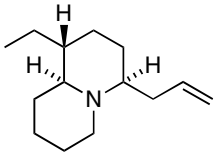


217A

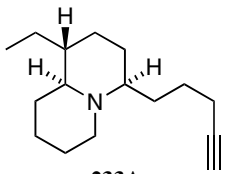


207I

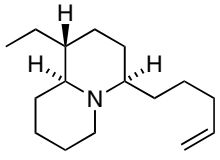
Neuronal nicotinic acetylcholine (ACh) receptors are pentameric oligomers that function as ligand-gated ion channels, and 12 subunits (α2–α10 and β2–β4) have been identified.[6] In particular, α7 and α4β2 nicotinic receptors are the most abundant subtypes in mammalian brain.[7] We have synthesized several poison-frog alkaloids and investigated their pharmacological effects on nicotinic receptors.[8] Recently, we found that the quinolizidine 1-epi-**207I** selectively blocks α7 nicotinic receptors (IC_{50} = 0.6 μM).[9] The blocking effect on α7 nicotinic receptors is ninefold more potent than that at α4β2 nicotinic receptors, and 15-fold more potent than that at α3β4 nicotinic receptors.[9]



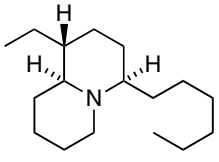
1-epi-**207I**



233A



235U



251AA

Keywords: Poison-frog alkaloids; Neuronal nicotinic acetylcholine receptors; 1-epi-**207I**; **233A**; **235U**; **251AA**.
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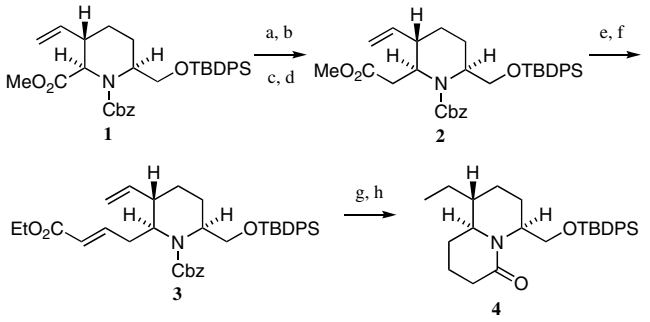
0960-894X/$ - see front matter
doi:10.1016/j.bmcl.2007.08.045

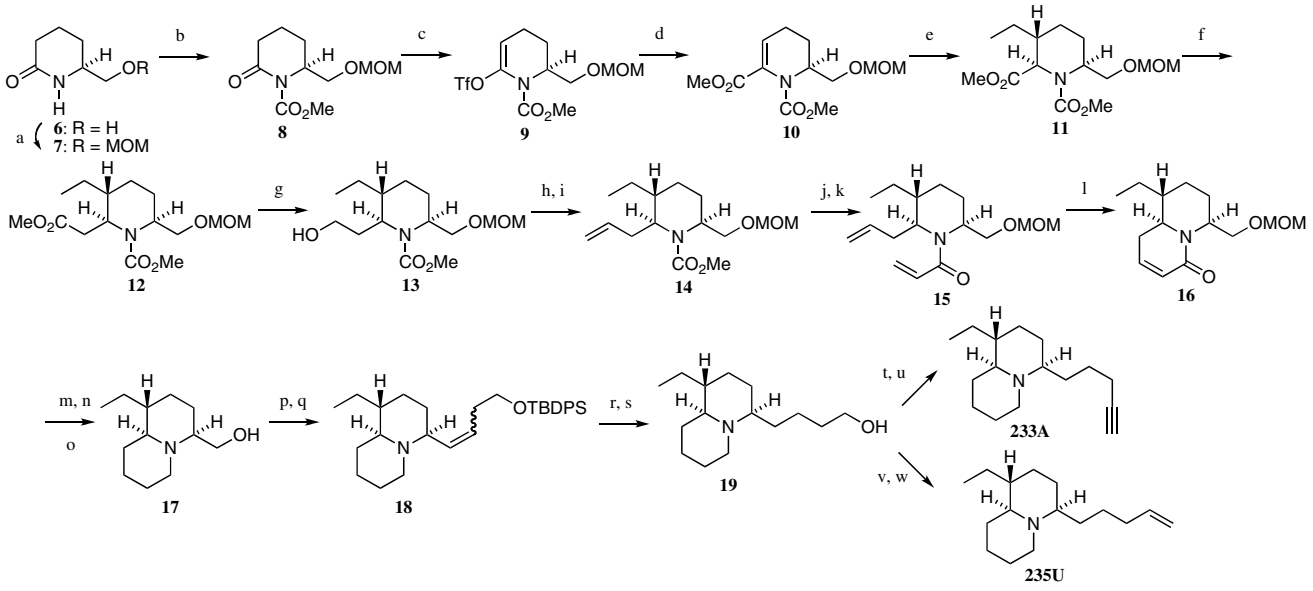


Scheme 1. Reagents and conditions: (a) LiOH, MeOH–H_2O, reflux; (b) $ClCO_2Et$, Et_3N, THF, 0 °C; (c) CH_2N_2, Et_2O; (d) $PhCO_2Ag$, Et_3N, MeOH, rt (69% for four steps); (e) DIBAL, CH_2Cl_2, −78 °C; (f) $(EtO)_2P(O)CH_2CO_2Et$, NaH (78% for two steps); (g) H_2, 20% $Pd(OH)_2$, MeOH, 1 atm; (h) Me_3Al, CH_2Cl_2, (14% for two steps).

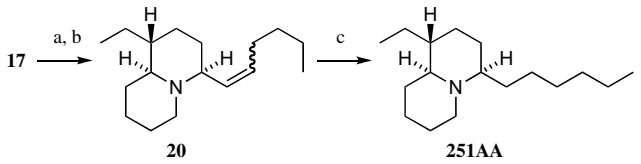
Here, we report the synthesis and determination of the relative stereochemistry of the 1,4-disubstituted quinolizidine poison-frog alkaloids **233A**, **235U**, and **251AA**. Furthermore, we compared their effects on two major types of nicotinic receptors to clarify the essential moiety in 1,4-disubstituted quinolizidines required for selective blockade of the α7 subtype.

The synthesis began with the trisubstituted piperidine **1**,[10] which was converted to the homologated ester **2** using the Arndt-Eistert sequence. Reduction of **2** with DIBAL at −78 °C provided the corresponding aldehyde, which was directly used for a Horner–Emmons olefination to afford the α,β-unsaturated ester **3**. Hydrogenation of both double bonds and hydrogenolysis of the Cbz-protecting group were performed by treatment of **3** with 20% $Pd(OH)_2$ in MeOH under hydrogen atmosphere at 1 atm to give rise to the aminoester, which was subjected to lactam formation using Weinreb's reaction.[11] Unfortunately, we could isolate the desired lactam **4** in only 14% yield[12] (Scheme 1).

As an alternative strategy for construction of the quinolizidine ring system, we examined the RCM reaction of diene **15** to form the quinolizidinone **16** (Scheme 2). The known amide **6**[13] was transformed into the MOM ether **7**, which was converted to the urethane **8**. Enoltriflation of **8** with Comins' reaction[14] provided the enoltriflate **9**, which was converted to the enaminoester **10** according to Cacchi's conditions.[15] A Michael-type conjugate addition[16] to **10** with diethylcuprate afforded the ethyl adduct **11**, as a single stereoisomer. The ester **11** was homologated to give rise to the ester **12**, which was converted to the alcohol **13**. Swern oxidation of **13** followed by Wittig reaction provided the terminal olefin **14**. Hydrolysis of the methyl urethane moiety and then treatment of the resulting piperidine with acryloyl chloride afforded **15**. The RCM reaction of **15** with Grubbs' second-generation catalysis[17] proceeded smoothly to give the quinolizidinone derivative **16** in good yield. Hydrogenation of **16**, reduction of the lactam moiety with $LiAlH_4$, followed by deprotection of the MOM protective group with acid, afforded the quinolizidinol **17**, which was used directly in the next reaction. Swern oxidation of **17**, followed by Wittig reaction of the resulting aldehyde, provided the olefin



Scheme 2. Reagents and conditions: (a) MOMCl, $(i\text{-}Pr)_2NEt$, CH_2Cl_2, rt (76%); (b) *n*-BuLi, $ClCO_2Me$, THF, −78 to 0 °C (95%); (c) LiHMDS, 2-[*N*,*N*-bis(trifluoromethylsulfonyl)amino]-5-chloropyridine (Comins' reagent), THF, −78 to −35 °C (92%); (d) $Pd(Ph_3P)_4$, CO, MeOH, Et_3N, DMF, 75 °C (76%); (e) $Et_2CuMgBr$, THF, −78 to −10 °C (92%); (f) 1—LiOH, MeOH–H_2O, reflux; 2—$ClCO_2Et$, Et_3N, THF, 0 °C; 3—CH_2N_2, Et_2O; 4—$PhCO_2Ag$, Et_3N, MeOH, rt (76% for four steps); (g) super-hydride, THF, 0 °C (97%); (h) Swern oxidn; (i) *n*-BuLi, $CH_3P^+Ph_3I^-$, THF, 0 °C–rt (73% for two steps); (j) 2 M KOH/*i*-PrOH, 120 °C in sealed tube; (k) acryloyl chloride, Et_3N, CH_2Cl_2, 0 °C–rt (71% for two steps); (l) Grubbs' second-generation catalyst, CH_2Cl_2, rt (85%); (m) 10% Pd/C, H_2, EtOAc, 1 atm; (n) $LiAlH_4$, THF, reflux; (o) concd HCl, MeOH, reflux; (p) Swern oxidn; (q) *n*-BuLi, $TBDPSO(CH_2)_3P^+Ph_3Br^-$, THF, 0 °C–rt (72% for five steps); (r) 10% Pd/C, H_2, EtOAc, 1 atm; (s) TBAF, THF rt (64% for two steps); (t) Swern oxidn; (u) *t*-BuOK, $(MeO)_2P(O)CHN_2$, THF, −78 °C–rt (47% for two steps); (v) Swern oxidn; (w) *n*-BuLi, $CH_3P^+Ph_3I^-$, THF, 0 °C–rt (55% for two step).



Scheme 3. Reagents and conditions: (a) Swern oxidn; (b) *n*-BuLi, *n*- $C_5H_{11}P^+Ph_3Br^-$, THF, 0 °C–rt (61% for two steps); (c) 10% Pd/C, H_2, EtOAc (90%).

18 as a mixture of *E*- and *Z*-forms. Hydrogenation of **18**, followed by treatment of the resulting silyl ether with TBAF, gave the alcohol **19**. Swern oxidation of **19** and Seyferth-Gilbert reaction[18] of the resulting aldehyde furnished quinolizidine **233A**[19] (Scheme 2). The alcohol **19** was converted to **235U**[20] by Swern oxidation and Wittig reaction of the resulting aldehyde (Scheme 2).

The crude alcohol **17** was subjected to Swern oxidation and Wittig olefination of the resulting aldehyde to afford the olefin **20**, which was converted to the alkaloid **251AA**[21] by hydrogenation over Pd/C (Scheme 3).

The synthetic **233A** co-chromatographed on a capillary GC column with the natural alkaloid from a Madagascan mantellid frog. The GC–MS for **233A**, **235U**, and **251AA** and the GC-FTIR for **233A** and **235U**were virtually identical with those of the natural alkaloids. Alkaloids **235U** and **251AA**(originally detected in certain mantellid frogs only in trace amounts) could no longer be detected in extracts.

We then conducted electrophysiological studies to examine the effects of **233A**, **235U**, and **251AA** on α7 and α4β2 nicotinic receptors expressed in *Xenopus laevis* oocytes. Oocytes were prepared as described previously.[9,22] In brief, stage V–VI oocytes were selected and nanoinjected with 23 ng of cDNAs encoding nicotinic receptor subunits into the nucleus. The α7 subunit cDNA was injected alone, whereas the mixture of α4 and β2 subunit cDNAs was injected in a ratio of 1:1. Oocytes were incubated at 19 °C in standard oocyte saline solution for 5–7 days. An oocyte was placed in a 300-μl tube-like chamber in which Ringer's solution (82.5 mM NaCl, 2.5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM Hepes, pH 7.4), containing 1 μM atropine to block endogenous muscarinic receptors, was perfused by gravity (15 ml/min). Current responses were recorded under the two-electrode voltage–clamp method. To apply a pulse of ACh to the oocyte, the perfusion fluid was switched to one containing ACh, using a three-way Teflon solenoid valve (Parker Hannifin Corp., General Valve Division, Fairfield, NJ) controlled by a PC computer with pClamp7 software (Axon Instruments, Union City, CA). Animal experiments were performed in accordance with the guidelines approved by the University of Toyama Animal Research Committee.

Concentration–inhibition curves for alkaloids were fitted by the equation $I = I_{max} - I_{max}/[1 + (IC_{50}/A)^{nH}]$, using Prism software (GraphPad Software, Inc., San Diego, CA), in which I is the percent response, I_{max} is the maximal response normalized to the current evoked by ACh (in the absence of alkaloids), A is the alkaloid concentration, IC_{50} is the alkaloid concentration eliciting the half-maximal inhibition, and nH is the Hill coefficient. The statistical data were represented as means ± standard error of the mean (SEM).

After preincubation with the synthetic alkaloid for 3 min, current was elicited by 5-s application of ACh (100 μM for α7 or 1 μM for α4β2) in combination with the alkaloid to oocytes expressing either α7 or α4β2 nicotinic receptors. The alkaloid **233A** (3 μM) effectively reduced the peak amplitude of both α7 and α4β2 nicotinic receptor-mediated currents (Fig. 1a). The alkaloid **235U** (3 μM) or **251AA** (3 μM) also reduced the peak amplitude of both currents (Fig. 1a). The concentration–inhibition curves of **233A** (1–10 μM) for α7 and α4β2 nicotinic receptors were similar with IC_{50} values of 7.4 μM and 5.1 μM, respectively (Fig. 1b). Similarly, **235U** inhibited both α7 and α4β2 currents in a concentration-dependent manner with IC_{50} values of 5.2 μM and 3.5 μM, respectively. In

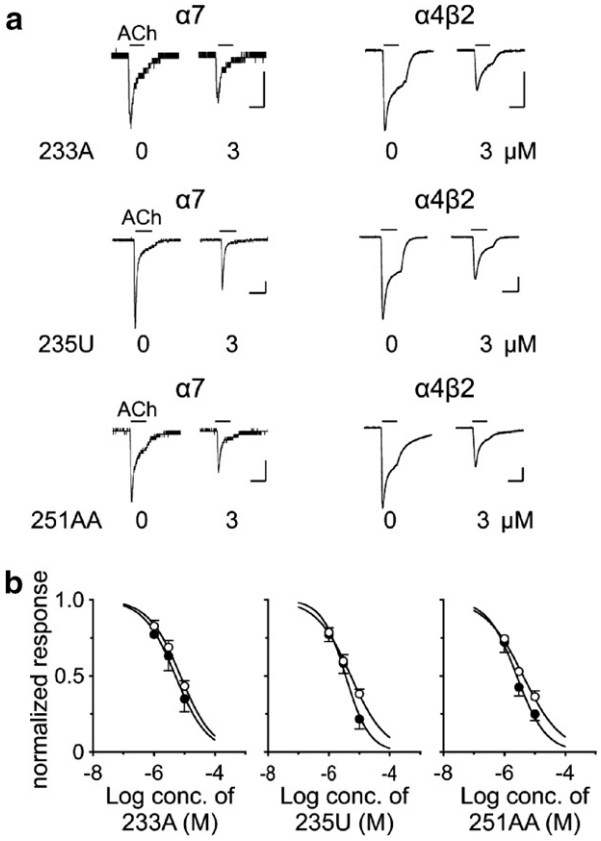


Figure 1. Inhibitory effects of quinolizidines on ACh-induced current in *Xenopus* oocytes expressing α7 or α4β2 nicotinic receptors. Current was recorded in voltage–clamp mode at −60 mV. (a) Typical traces showing inhibition by **233A**, **235U** or **251AA** at 3 μM of α7 current elicited by 100 μM ACh and α4β2 current elicited by 1 μM ACh. Horizontal bars indicate the period of perfusion with ACh for 5 s. Vertical scale bars represent 100 nA for α7 current and 500 nA for α4β2 current. (b) Concentration–inhibition curves for **233A**, **235U**, and **251AA** on α7 (○) and α4β2 (•) nicotinic receptors. Current responses to ACh in the presence of the alkaloid in each oocyte were normalized to the ACh responses (control responses) recorded in the same oocytes. Values represent means ± SEM of five to nine separate experiments.

addition, **251AA** inhibited both α7 and α4β2 currents with IC_{50} values of 4.1 μM and 2.5 μM, respectively. The blocking effects of **233A**, **235U**, and **251AA** at 10 μM on α7 and α4β2 nicotinic receptors were reversible within 10 min (data not shown).

Structures of the alkaloids **233A**, **235U**, **251AA**, and 1-epi-quinolizidine **207I** are different only in the C4 side chain, that is, 4-pentynyl- for **233A**, 4-pentenyl- for **235U**, *n*-hexyl- for **251AA**, and allyl for 1-epi-**207I**. The alkaloids **233A**, **235U**, and **251AA** blocked the responses mediated by α7 and α4β2 nicotinic receptors; however, no selectivity between these two receptor subtypes was observed. In contrast, 1-epi-**207I** acts as a selective blocker of α7 nicotinic receptors.[9] These results suggest that the α7 subtype selectivity of 1,4-disubstituted quinolizidines is remarkably dependent on the structure of the C4 side chain. Increasing the length of the 4-moiety beyond three carbons appears to markedly reduce potency at the α7 receptor, while having little effect on potency at the α4β2 receptor.

Acknowledgments

We are grateful to Dr. Jerry A. Stitzel (University of Colorado) for providing us with plasmid DNA. We also thank Dr. John A. Dani (Baylor College of Medicine, Houston, TX) for support on electrophysiological data acquisition. This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (to N.T. and H.T.). Work at NIH was supported by intramural funds of NIDDK.

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19. The spectral data of **233A** are as follows: IR (neat) 3312, 2930, 2857, 2786 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.82 (3H, t, J = 7.3 Hz), 0.95–1.03 (1H, m), 1.04–1.14 (1H, m), 1.17–1.23 (4H, m), 1.37–1.76 (13H, br m), 1.84–1.93 (4H, br m), 2.15 (2H, m), 3.24 (1H, br); ^{13}C NMR (75 MHz) δ 10.52, 18.87, 24.59, 24.72, 25.02, 26.13, 29.45, 29.74, 31.20, 33.11, 41.66, 51.44, 62.79, 67.311, 68.31, 84.38; MS *m*/*z*: 233(M^+, 100), 166, 83.
20. The spectral data of **235U** are as follows: IR (neat) 3087, 2930, 2848, 2773 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.84 (3H, t, J = 7.3 Hz), 1.03–1.94 (20H, br m), 2.03 (2H, br d-like, J = 6.0 Hz), 3.25 (1H, br), 4.94 (1H, d, J = 10.3 Hz), 5.05 (1H, d, J = 17.1 Hz), 5.79 (1H, m); ^{13}C NMR (75 MHz) δ 10.53, 25.05, 25.27, 26.00, 26.16, 29.51, 29.76, 31.24, 33.53, 34.25, 41.75, 51.55, 63.31, 67.39, 114.33, 138.71; MS *m*/*z*: 235(M^+), 166, 83 (100).
21. The spectral data of **251AA** are as follows: IR (neat) 2929, 2856, 2784 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.83 (3H, t, J = 7.3 Hz), 0.87 (3H, t, J = 7.3 Hz), 1.01 (1H, br), 1.08 (1H, quint-like, J = 6.8 Hz), 1.12–1.77 (22H, br m), 1.83 (1H, br), 1.94 (1H, br), 3.26 (1H, br); ^{13}C NMR (75 MHz) δ 10.52, 14.16, 22.70, 24.66, 25.05, 25.92, 26.20, 29.56, 29.77, 29.90, 31.31, 34.14, 41.75, 51.47, 63.43, 67.34; MS *m*/*z*: 251(M^+), 166 (100), 83.
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