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Bioorganic & Medicinal Chemistry Letters 18 (2008) 3440–3445
Bioorganic & Medicinal Chemistry Letters

Bis-tetrahydroisoquinoline derivatives: AG525E1, a new step in the search for non-quaternary non-peptidic small conductance Ca^{2+}-activated K^+ channel blockers

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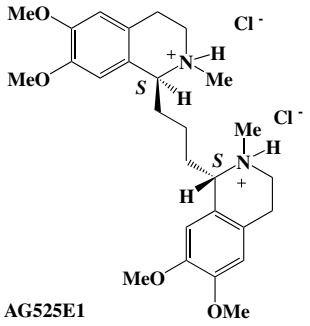
Received 18 February 2008; revised 21 March 2008; accepted 25 March 2008
Available online 29 March 2008

Abstract—So far, small conductance Ca^{2+}-activated K^+ channel (SK) blockers mostly consist of quaternary ammonium derivatives or peptides. Due to their physicochemical properties, these blockers are not suitable to study the physiological roles of SK channels in the central nervous system in vivo. Herein, we report the discovery of a chiral bis-tertiary amine with SK blocking properties from chemical modulation of laudanosine. AG525E1 has an affinity for SK channels (K_i = 293 nM) approximately 100-fold higher than the tertiary compound laudanosine ($K_i \sim 30$ μM) and similar to the charged compound dequalinium (K_i = 221 nM). AG525E1 equipotently blocks SK1, SK2 and SK3 currents in transfected cell lines. Because of its basic and lipophilic properties, it can reach central SK targets.

Small conductance Ca^{2+}-activated K^+ (SK) channels are found in several types of neurons as well as in other cell types. The SK channels are selective for K^+ ions and their opening depends upon an increase of the intracellular Ca^{2+} concentration. These channels underlie the medium postspike afterhyperpolarization (mAHP), which plays an important role in modulating the firing rate and the firing pattern of neurons.[1,2] Three SK channel subtypes have been cloned[1] and exhibit a differential location in the brain. The distribution of the SK channel subtypes was investigated in the rat by using *in situ* hybridization and immunohistochemistry and revealed that SK1 and SK2 subtypes are mostly expressed in the cortex and hippocampus[3] whilst SK3 subtype expression is higher in the monoaminergic cell regions. These features attract great attention to SK channels as putative targets for the treatment of various CNS diseases such as cognitive dysfunction,[4–8] neuronal hyperexcitability,[9] dopamine-related disorders,[10–12] and depression.[7]



AG525E1

Keywords: Apamin; Tetrahydroisoquinoline; Patch-clamp; Neurons; Motor activity; Stereoisomer.
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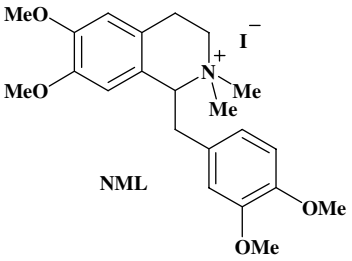
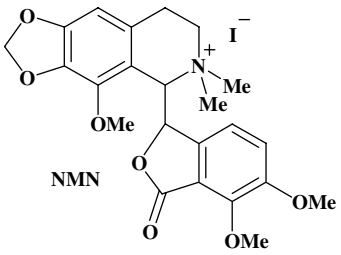
doi:10.1016/j.bmcl.2008.03.069



NML



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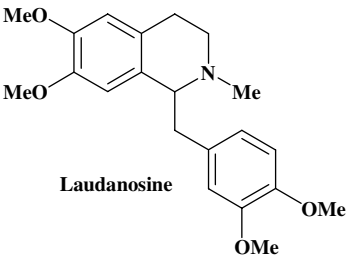
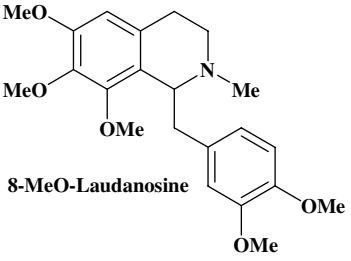


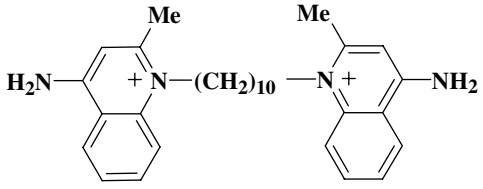
Laudanosine



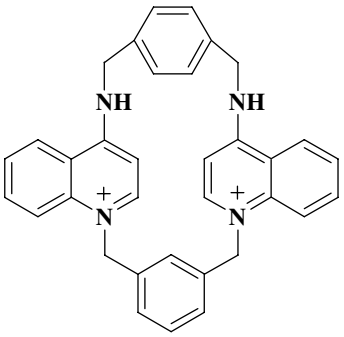
8-MeO-Laudanosine

SK channel blockers consist of peptidic toxins such as apamin,[13] leiurotoxin I,[14] PO5,[15] tityus κ,[16] tamapin,[17] BmSKTx1,[18] and non-peptidic compounds such as tubocurarine,[19] atracurium,[20] dequalinium[21] and UCL derivatives.[22,23] We reported that *N*-methyl-laudanosine (NML)[24–26] and *N*-methyl-noscapine (NMN)[26] also block SK channels. Unlike apamin, these two molecules are medium affinity blockers with a high reversibility as shown in patch-clamp experiments.[26] Besides their low affinities, the quaternary ammonium function is probably the major drawback of these pharmacological tools for investigating their CNS effects after systemic injection. Indeed, this permanent positive charge imposes to inject the drugs by an invasive route, such as the intracerebroventricular one.[27] Some tertiary derivatives have also been reported to block SK channels[28–30] and in the first part of our program 8-MeO-laudanosine was found to be almost equipotent to NML at interacting with apamin-sensitive sites with a K_i of 5.8 μM versus 1.6 μM for the latter.[31] Previous studies had shown that the presence of two positive charges or two basic centres improves the affinity of the blockers.[32–34] In order to find new compounds with significant affinity and appropriate physicochemical properties, bis-tertiary amines based on the scaffold of laudanosine were prepared and tested for their affinity on SK channels.[35] We report herein the results obtained with the most potent basic compound in this series.

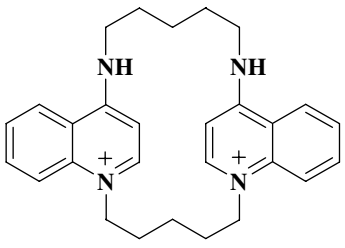
The bis-tertiary amine was obtained from 6,7-dimethoxy-isoquinoline which was classically prepared using a modification of the Pomeranz–Fritsch synthesis (Scheme 1).[36] The bis-alkylation was subsequently performed by using the Reissert compound pathway



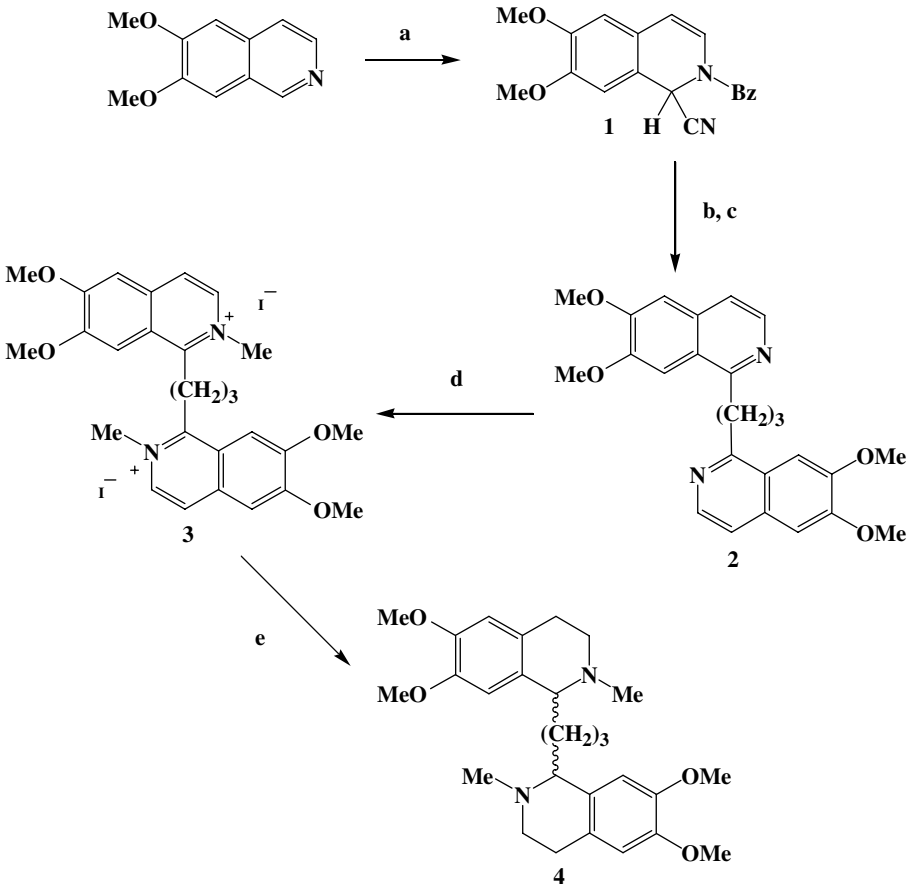
Dequalinium



UCL1684



UCL1848



Scheme 1. Reagents and conditions: (a) Me_3SiCN, BzCl, $AlCl_3$, CH_2Cl_2, rt, 70%; (b) I-$(CH_2)_3$-I, NaH, DMF, −10 °C; (c) NaOH, EtOH/H_2O, reflux, 31%; (d) MeI, DMF, Δt until dissolution, 97%; (e) $NaBH_4$, MeOH, rt, 67%.

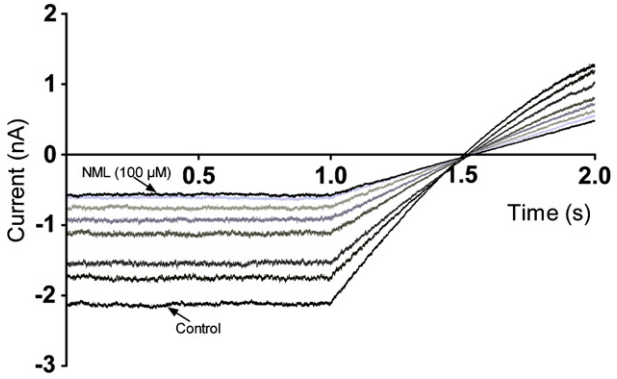


Figure 1. SK3 current traces recorded at a holding potential of −50 mV during 1 s followed by a 1 s ramp from −50 mV to +50 mV. The current was blocked by increasing concentration of AG525E1. Their effect is represented by different grey levels. From the bottom to the top trace, the concentrations used were 0.3, 1, 3, 5, 10, and 30 µM. NML was used at a supra-maximal concentration as a reference.

(Scheme 1).[37] The Reissert compound was obtained by the reaction of the corresponding isoquinoline with benzoyl chloride in the presence of trimethylsilyl cyanide in a good yield.[38] This derivative was deprotonated by sodium hydride in DMF and the resulting Reissert anion was alkylated by using a half equivalent of the appropriate bis-electrophilic reagent.[39] The alkylated Reissert compound was hydrolysed to give the bis-isoquinoline derivative (**2**). The bis-isoquinoline dissolved in DMF with a mild heating was methylated by methyl iodide to obtain the corresponding bis-isoquinolinium derivative (**3**).[34] Finally, the mixture of bis-tetrahydroisoquinoline stereoisomers (**4**) was obtained by the reduction of the bis-isoquinolinium analogue with an excess of sodium borohydride (Scheme 1).[40] After biological screening at 10 µM, the three stereoisomers were separated by semi-preparative chiral HPLC.[41] AG525E1 was the first eluted enantiomer and was subsequently isolated as a dihydrochloride. In our binding conditions as previously reported (see [25,34,42]), NML and dequalinium (DQ+) had an affinity (K_i) for the apamin-sensitive sites of ~1600 nM and ~220 nM, respectively.[30] The affinities of the three stereoisomers were 293 ± 22 nM, 1422 ± 116 nM and 1885 ± 105 nM for the first eluted (AG525E1), the meso form and the second eluted isomer, respectively. The difference in affinity between the two enantiomers (AG525E1: K_i = 293 ± 22 nM; second enantiomer: K_i = 1885 ± 105 nM) indicates that the stereochemistry is an important feature for interacting with SK channels, while enantiomers of NML were previously found to be equipotent on apamin-sensitive sites.[25] The absolute configuration of AG525E1 was determined by X-ray crystallography after the crystallisation of the dihydrochloride from MeCN.[43] This experiment showed

that it possesses a *S*,*S* configuration associated with a dextrorotatory activity. The affinity of this enantiomer (K_i = 293 nM) is similar to that of the reference charged compound dequalinium (K_i = 221 nM) but clearly superior to that of laudanosine ($K_i \sim 30$ μM.[24] As is the case for quaternary compounds,[34] the development of non-quaternary bis-derivatives is highly favourable for the interaction with SK channels. From laudanosine, an approximately 100-fold increase in affinity is observed. An extensive binding profile performed in another laboratory on 65 receptors or channels and 7 enzymes at 10 μM confirms that the compound has a high affinity for SK channels (61% inhibition of specific binding) versus other targets. AG525E1 has weaker affinity (% inhibition of specific binding) for serotonin 5-HT_{1A} (39%), sigma2 (39%), histamine H_3 (37%) and dopamine D_2 receptors (31%) and the verapamil site of the L type calcium channel (36%).

Whole-cell patch-clamp experiments in transfected cell lines were used to confirm that the compound effectively blocks SK currents (Fig. 1).[44] AG525E1 completely blocked SK1, SK2 and SK3 currents with IC_{50}'s of 3.8 ± 0.8 μM (n = 13), 2.6 ± 0.3 μM (n = 13) and 3.0 ± 0.4 μM (n = 14), respectively.

The affinity for the apamin-sensitive binding sites of SK channels depends on the presence of positive charges in the structure,[32] but, in order to develop compounds with potential effect on CNS, the basic character of the molecule is important in terms of bioavailability. Unlike NML, dequalinium and UCL1684, the bis-tertiary amine AG525E1 does not bear one or two permanent positive charge(s). Therefore, physicochemical parameters such as pK_a and log P were determined using potentiometric titration procedures on a Sirius® PCA200 apparatus.[45] AG525E1 possesses two values of pK_a, namely 9.3 and 8.1. Therefore, at physiological pH, this stereoisomer has mostly (~83%) a positive charge on both nitrogens, and a small fraction (~17%) is ionised just once whilst the free base represents a minor proportion (<1%). The lipophilicity of the compound was also measured by potentiometric titration using the same equipment.[45] The log P value is 2.90. More generally from a theoretical point of view, the structure of AG525E1 is in accordance with the classical Lipinski's Rule of Five. Indeed, this stereoisomer possesses six H bond acceptors and has a log P which is smaller than 5. It has a molecular weight of ~527 daltons and does not possess H bond donors.[46] All these parameters support the ability of the compound to reach CNS targets after crossing the blood–brain barrier. Preliminary experiments in rats showed that, following ip injection, the compound tends to increase motor activity (data not shown) as previously reported for apamin.[10] Of course, this effect is quite unspecific and the compound will be better characterized in further pharmacological and psychopharmacological experiments since the non-selectivity for SK channel subtypes probably leads to a global behaviour resulting from combined SK channel subtype blockade.

In summary, a new scaffold of tertiary SK channel blockers has been obtained from the structure of laudanosine. We demonstrate the feasibility of targeting central SK channels after the systemic administration of non-quaternized compounds. Further efforts will be directed towards the discovery of molecules with a higher selectivity for SK channels versus other targets, on the one hand, and subtype-specific blockers, on the other hand. Although the search for high affinity ligands is frequently a challenge in medicinal chemistry, medium affinity blockers with a high reversibility, as shown in patch-clamp experiments for NML,[26] will be valuable tools for electrophysiological exploration of SK channel physiology.

Acknowledgments

The technical assistance of Y. Abrassart, S. Counerotte, and J.-C. Van Heugen is gratefully acknowledged. A.G. was supported by a Research Fellow grant of the «Fonds pour la formation à la Recherche Industrielle et Agricole (F.R.I.A.)» and J.-F.L. is a Senior Research Associate of the 'Fonds National de la Recherche Scientifique' (F.N.R.S.) of Belgium. C.L. is the recipient of a 'First DEI' Ph.D. Studentship from the Walloon Region of Belgium (Contract No. 516131). This work was financially supported in part by F.N.R.S. Grant Nos. 3.4525.98 and 9.4560.03 and by grants from Fonds Spéciaux pour la Recherche 2002 and 2003 of the University of Liège and the Fondation Léon Frédéricq of the Faculty of Medicine of the University of Liège.

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40. Under an inert atmosphere, $NaBH_4$ (1.76 g; 46.5 mmol) was added to a solution of the 1,3-bis[1-(6,7-dimethoxy-2-methylisoquinolylium)]-propane diiodide[34] (1.85 g; 3.1 mmol) in MeOH (100 mL) at room temperature. After 15 min, MeOH was removed under reduced pressure and the crude residue was dissolved in a 1 N aqueous HCl (100 mL). The acidic layer was washed with Et_2O (3× 20 mL), and then basified with NH_4OH. The suspension was extracted with CH_2Cl_2 (3× 30 mL). The organic layers were collected, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to afford a colourless oil, which was purified by flash chromatography on Kieselgel with a mixture of Me_2CO/MeOH (9:1, v/v) as mobile phase. The purified oil was isolated as a hydrochloride salt and further recrystallized from MeCN/Et_2O; *yield*, 67%; *m/z* 455 (100) [MH^+], 146 (11) [$THIQH^+$]; Anal. ($C_{27}H_{38}N_2O_4{\cdot}2HCl{\cdot}3/4H_2O$) C, H, N.
41. The semi-preparative resolution was performed by HPLC using a liquid chromatograph consisting of a pump Armen Instrument AP100 with an injection valve Knauer® (1–10 mL). The stationary phase was a Chiralcel® OD-H column (20 × 250 mm) preceded by a Chiralcel® OD-H precolumn (10 × 20 mm), while the mobile phase was a mixture of *n*-hexane/2-propanol (8:2, v/v) +0.05% DEA. Samples were collected by using a Büchi Fraction-Collector C-660 every 45 s. The fractions containing AG525E1 (rt: 7.3 min) were pooled and evaporated. The residue was kept in a desiccation unit under vacuum during 48 h. The purity (97.4%) and the diastereoisomeric excess (99.7%) were determined by analytical chiral HPLC using a Chiralcel® OD-H column (4.6 × 250 mm) with the same mobile phase. After this control, the solid residue was dissolved in Et_2O (5–10 mL) and the dihydrochloride was precipitated by the addition of saturated etherous HCl solution. The white precipitate was collected and kept under a dry atmosphere until biological testing. A sample was recrystallized from a mixture of MeOH and Et_2O, and analyzed as a dihydrochloride except for ^1H NMR. mp: 257–258 °C, dec.; $[\alpha]_D^{20}$: +35° (MeOH); IR (KBr, cm^{-1}): 2936, 2542, 1614, 1522, 1230, 1114, 1000. ^1H *NMR* ($CDCl_3$) δ 1.50–1.55 (m, 2H), 1.65–1.70 (m, 2H), 1.72–1.78 (m, 2H), 2.43 (s, 6H), 2.59 (dt, 2H, *J* = 5.2 and 16.0 Hz), 2.69 (dt, 2H, *J* = 5.2 and 12.4 Hz), 2.74–2.80 (m, 2H), 3.12 (dq, 2H, *J* = 5.2 and 12.4 Hz), 3.36 (t, 2H, *J* = 5.6 Hz), 3.81 (s, 6H), 3.84 (s, 6H), 6.54 (s, 4H).
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44. Whole-cell patch-clamp experiments were performed in a voltage clamp mode using an EPC-10 amplifier (Heka) and Patchmaster software (Heka). Borosilicate pipettes were pulled with a P87 puller (Sutter) and fire-polished using a Microforge (Narishige) to achieve a tip resistance between 2 and 3 MΩ. All the experiments with series resistances above 8 MΩ were discarded. An eight channel perfusion system (Ala science) was used for drug applications. The composition of the external solution was (in mM): KCl 144, $CaCl_2$ 2, $MgCl_2$ 1, Hepes 10, glucose 10. The pH was adjusted to 7.4 using KOH and the osmolarity ranged from 290 to 305 mOsm. The internal solution consisted of (in mM): KCl 130, EGTA 10, $CaCl_2$ 8.751, $MgCl_2$1.08, Hepes 10 leading to 1 mM free calcium. The pH was adjusted to 7.2 using KOH and the osmolarity ranged from 275 to 290 mOsm. Experiments were achieved with stably transfected CHO FlipIn cells (SK1, 2 or 3) which were kept under standard culture conditions (medium composition was Ham's F-12 with 10% calf serum supplemented with streptomycin/penicillin and hygromycin-B). Potassium currents were measured at −50 mV. A supra-maximal concentration of *N*-methyllaudanosine (100 μM) was used to achieve a complete inhibition of the SK current and allowed to calculate the percentage of inhibition observed with the increasing concentrations of AG525E1.
45. pK_a and log *P* values were determined using the Sirius PCA200™ analyser based on a potentiometric titration method according to the previously described methods.[47,48] Potentiometric pK_a values were determined by titrating a minimum of three aqueous solutions of the

samples containing 32–56% (weight%) methanol. In each experiment, 10 mL of a 0.5–1 mM solution of the sample was acidified to pH 1.8–2.0 with 0.5 M aqueous HCl, and then titrated with 0.5 M aqueous KOH to pH 12. The titrations were carried out at constant ionic strength ($I = 0.15$ M KCl) and temperature ($T = 25.0 \pm 0.5$ °C). Then, these p_sK_a values (the apparent ionisation constants in aqueous methanolic solution) were used to estimate the aqueous pK_a values. There after, potentiometric $\log P$ determinations were carried out. Typically, 7.5–17.5 mL of 0.5–1 mM solutions of samples was titrated under the same conditions as above but in the presence of various proportions of the partitioning solvent namely water-saturated octanol. Depending on the expected $\log P$, the phase volume ratio varied from 15–0.5 to 7.5–10 for the proportions of water–octanol, respectively. From the octanol-containing titrations, the p_oK_a and then $\log P$ values were estimated and refined by the RefinementPro™ software, where the aqueous pK_a values previously determined were used as unrefined contributions. A minimum of three titrations at different phase volume ratios were made, and the mean $\log P$ was calculated. The relevant relationships between $\log P$, pK_a, and p_oK_a are previously described.[47].

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