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Synthesis and Pharmacological Properties of 5-Alkyl Substituted Nicotine Analogs†

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This paper describes a concise and practical route to enantiomerically enriched 5-alkyl substituted nicotine analogs. The Vilsmeier reaction was used to construct the nicotinaldehydes ring followed by the introduction of the chiral homoallylic alcohol by organic boron reagent and the cyclization of the pyrrolidine ring through the reduction of a chiral azide. 17 analogs have been synthesized and their corresponding biological activities were tested, in which compounds **10d** and **10g** exhibit excellent IC_{50} values against RD and SY-SY5Y.

Keywords nicotine analog, asymmetric synthesis, Vilsmeier reaction, biological activities

Introduction

Nicotine attracted considerable attention from medicinal chemists because of its benefit on patients suffering from Pakinson's disease, anxiety, Alzheimer's disease, schizophrenia, ulcerative colitis and other CNS disorders.[1] However, the detrimental effects, such as actions on the cardiovascular and gastrointestinal systems, sleep disturbance and addiction, limited the use of nicotine as a therapeutic reagent.[2] As a consequence, researchers focused on synthesizing nicotine derivatives which exhibit the same effects with lower toxicity.

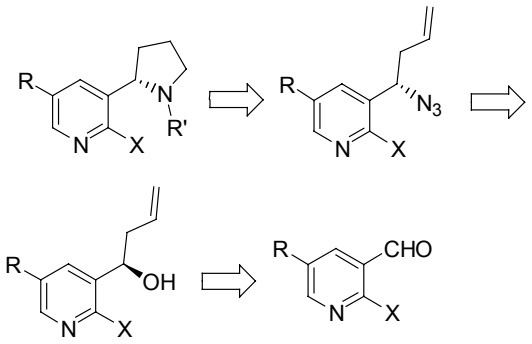
Synthesis of enantiomerically pure substituted nicotine derivatives especially with the variations at C-5 position is current research interests. SIB-1508Y, which progressed as far as Phase II clinical trials for the treatment of Parkinson's disease, confirmed this issue.[3] Nevertheless, regioselective substitution of the pyridine ring of nicotine especially at C-5 is difficult to achieve using known pyridine substitution chemistry.[4] Thus, we select an alternative route to obtain a series of nicotine derivatives from non-pyridine ring.

From the retrosynthesis (Scheme 1), it is easy to see that the difficulties rely on three aspects: (1) the construct of the C-5 substituted nicotinaldehydes; (2) the introduction of the chiral homoallylic alcohol; (3) building the pyrrolidine ring from the chiral azide.

Results and Discussion

First, the C-5 substituted nicotinaldehydes need to be constructed. Considering the lack of reactivity of pyridines toward electrophilic substitution reactions, the introduction of a formyl group onto the pyridine ring is difficult to achieve. This problem was resolved by Vilsmeier reaction that could construct the pyridine ring as well as introduce the C-5 substituent and the formyl group.[5] Therefore, various enmides **3** were prepared by condensing corresponding aldehydes with benzylamine to form initial Schiff bases and then reacted with acetic anhydride. The following Vilsmeier reaction was conducted to construct the desired nicotinaldehydes **4** using DMF and triphosgene in toluene at 95 ℃ (Scheme 2).

Scheme 1 Retrosynthesis of (*S*)-nicotine analogs



With the desired nicotinaldehydes in hand, Roush reagent was firstly used to introduce the chiral homoallylic alcohol.[6] However, this reaction was found difficult to handle together with low yield. As a consequence, (＋)-B-allyldiisopinocampheyborane was applied to get

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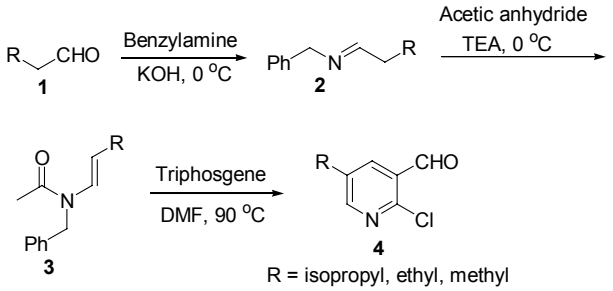
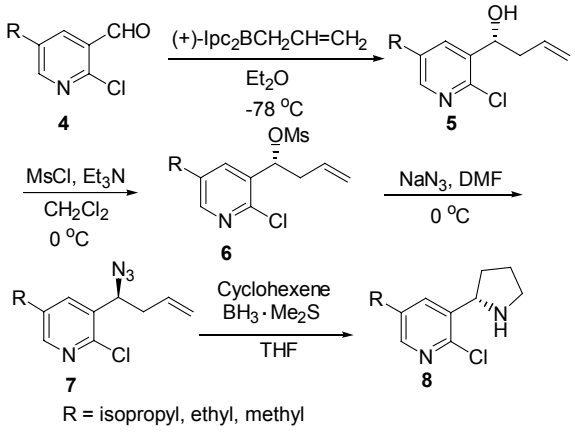
Scheme 2 Construction of the pyridine ring



compound **5** with moderate yield (49%).[7]

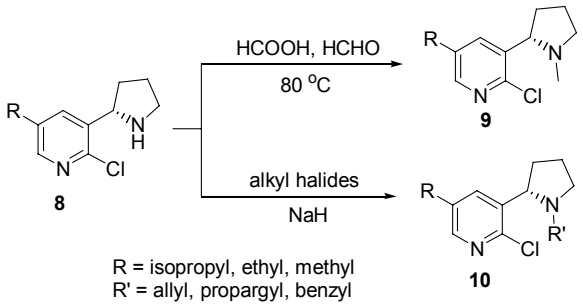
Compound **5** was mesylated with MsCl in the presence of triethylamine to afford compound **6**. The unstable compound **6** was directly treated with sodium azide in DMF at 60 ℃ to access compound **7** in 85% yield over two steps. Finally, borane dimethyl sulfide complex was used to reduce the azide group followed by cyclization of pyrrolidine ring to provide chiral compound **8**. At the same time, (—)-B-allyldiisopinocampheyborane was used to obtain the corresponding (*R*)-nicotine analogs (Scheme 3).

Scheme 3 Construction of the pyrrolidine



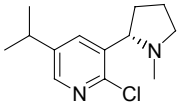
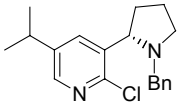
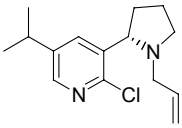
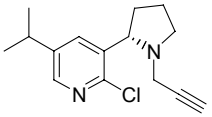
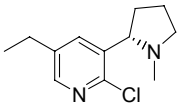
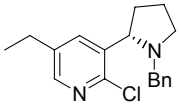
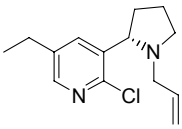
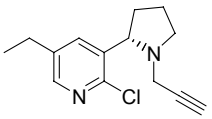
Selective *N*-alkylation was a challenge as nicotine's two nitrogen atoms are nucleophilic and can compete for electrophiles.[8] Finally, the methylation of the nicotine ring was carried out using Mannich reaction[9] and other alkyl groups were introduced by sodium hydride (Scheme 4).

Scheme 4 *N*-Alkylation of nicotine pyrrolidine ring



Overall, 17 nicotine analogs were successfully synthesized through the route we developed and ready for the biological test. Up to date, 17 nicotinic acetylcholine receptors subunits have been identified and nicotine receptors are pentamers of these subunits. In our experiment, we were interested in two types of nicotinic acetylcholine receptors, human neuromuscular $\alpha1\beta1\gamma\delta$ receptors and ganglionic $(\alpha7)_5$ receptors, to test the effects of the compounds. As references reported, RD rhabdomyosarcoma cells expressed human neuromuscular $\alpha1\beta1\gamma\delta$ receptors,[10] and SH-SY5Y neuroblastoma cells expressed human ganglionic $(\alpha7)_5$ receptors.[11] Therefore, the corresponding biological activities of those nicotine analogs against RD and SY-SY5Y were tested and the results were summarized in Table 1.

Table 1 Structures and biological activities of the nicotine analogs

No.	Structure	SY-SY5Y IC_{50}[a]/ ($\mu mol \cdot L^{-1}$)	RD IC_{50}[b]/ ($\mu mol \cdot L^{-1}$)
9a		17.25	62.49
10a		15.75	29.04
10b		62.08	85.70
10c		19.94	77.96
9b		35.65	43.58
10d		4.88	22.26
10e		14.71	27.07
10f		21.12	62.98

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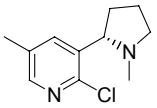
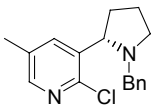
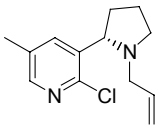
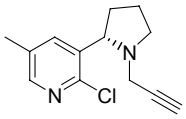
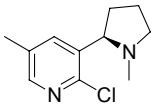
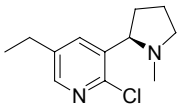
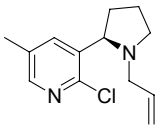
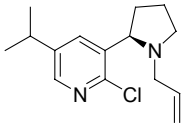
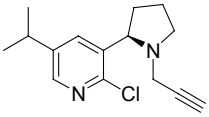
Continued

No.	Structure	SY-SY5Y IC_{50}[a]/ (μmol•L^{-1})	RD IC_{50}[b]/ (μmol•L^{-1})
9c		63.23	91.42
10g		5.97	22.81
10h		26.16	69.17
10i		34.95	45.72
9d		94.28	112.35
9e		62.41	37.74
10j		69.05	88.22
10k		14.71	32.40
10l		12.74	60.63
	Mecamylamine	1.25	26.50
	DMSO	0	0

[a] All compounds showed negative agonist activities against RD. [b] All compounds showed negative agonist activities against SY-SY5Y.

The data showed that all of the compounds had negative agonist activities against RD and SY-SY5Y. Among these compounds, **10d** and **10g** showed exciting IC_{50} values toward these two cell lines which were close to that of Mecamylamine. We also found that the molecules bearing methyl or ethyl group at the Py-5 position generally had lower IC_{50} value than those of isopropyl at the same position. All the compounds with *S* configuration had better IC_{50} values than those with *R* configuration. Based on the SAR analysis, our next generation targets were concentrated on the synthesis of various *N*-substituted aromatic or hetero-aromatic drugs.

Conclusions

In summary, an eight-step synthetic route was developed to a series of enantiomerically enriched 5-alkyl substituted nicotine analogs. The key steps involved the construction of the nicotinaldehydes ring through Vilsmeier reaction, the introduction of the chiral homoallylic alcohol by an organic boron reagent and the cyclization of the pyrrolidine ring through the reduction of a chiral azide. According to this method, 17 nicotine analogs were synthesized, in which compounds **10d** and **10g** exhibited good activities against RD and SY-SY5Y cell.

Experimental

Chemistry

Unless stated otherwise, all reagents and solvents were obtained from commercial sources without further purification. Column chromatography was carried out on silica gel (300—400 μm). ^{1}H NMR and ^{13}C NMR spectra were recorded on a spectrometer (400 and 100 MHz, respectively) using TMS as internal standard. HRMS data were determined by EI ionization.

(*E*)-*N*-Benzyl-*N*-(3-methylbut-1-enyl) acetamide (3)[5] Isovaleraldehyde (14.3 mL, 0.13 mol) was added to benzyl amine (10.9 mL, 0.10 mol) dropwise at 0 ℃ under nitrogen. Then, solid potassium hydroxide (2.8 g, 0.05 mol) was added. The resulting mixture was stirred for 2 h and the additional potassium hydroxide (1.0 g) was added. The mixture was cooled to 0 ℃ for 12 h and the supernatant liquid (compound **2**) was taken out for the next step.

Compound **2** was cooled to 0 ℃ before the addition of triethylamine (18.1 mL, 0.13 mol) and acetic anhydride. Then, the mixture was warmed to r.t. and kept stirring for 3 h. Compound **3** was gained by distillation (142 ℃/10 mmHg) as colorless oil (16.2 g, 75% for 2 steps).

2-Chloro-5-isopropylnicotinaldehyde (4)[5] To a solution of triphosgene (154.9 g, 521.8 mmol) in toluene (300 mL) was added DMF (40.6 mL, 521.8 mmol) at 0 ℃. The mixture was stirred for 30 min before the solution of compound **3** (16.2 g, 74.5 mmol) in toluene (30 mL) was added. The mixture was stirred for 2 h before heated to 95 ℃ and kept stirring for another 3 h. Upon cooling, the organic layer was discarded. The aqueous layer was extracted with DCM (60 mL×3) and washed with water. The organic layer was dried with anhydrous sodium sulfate and concentrate under vacuum. The crude product was further purified by column chromatography to get compound **4** as yellow oil (7.9 g, 58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.40 (s, 1H), 8.44 (d, J=2.4 Hz, 1H), 8.05 (d, J=2.4 Hz, 1H), 3.03—2.96 (m, 1H), 1.28 (d, J=7.2 Hz, 6H).

(*R*)-1-(2-Chloro-5-isopropylpyridin-3-yl)but-3-en-1-ol (5) The allyl magnesium bromide (6.4 g, 12.0 mmol) was added to the solution of (+)-Ipc_2BCl in diethyl ether at −78 ℃. After stirring for 15 h at −78 ℃, the mixture was warmed to 25 ℃ and stirred for another 1 h. Then, it was cooled to −78 ℃ again and the solution of **4** (1.0 g, 5.4 mmol) in diethyl ether (20 mL) was added. After stirring for 1 h, the mixture was warmed to r.t. and kept stirring for another 1 h. The mixture was then added aqueous NaOH (8.8 mL, 3 mol/L) with H_2O_2 (30% in water, 3.6 mL) and heated to reflux for 1 h. After the solvent was removed under vacuum, the residue was dissolved in ethyl acetate (50 mL). It was then washed with brine and dried over anhydrous sodium sulfate. After concentration under vacuum, the crude product was further purified by column chromatography to get compound **5** as yellow oil (540 mg, 45%). $[\alpha]_D^{25}$ +18.0 (*c* 1.0, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.16 (d, J=2.4 Hz, 1H), 7.79 (d, J=2.4 Hz, 1H), 5.91—5.85 (m, 1H), 5.23 (s, 1H), 5.20—5.08 (m, 2H), 2.99—2.93 (m, 1H), 2.71—2.68 (m, 1H), 2.38—2.31 (m, 1H), 1.28 (d, J=6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 146.7, 145.9, 143.3, 137.2, 134.2, 133.7, 119.2, 69.0, 41.8, 31.3, 23.6, 23.5; ESI-MS: 225.8 [M+1]. HRMS (EI) calcd for $C_{12}H_{16}ClNO$: 225.0920, found 225.0924.

(*S*)-3-(1-Azidobut-3-enyl)-2-chloro-5-isopropylpyridine (7) To a solution of **5** (0.50 g, 2.21 mmol) in DCM (30 mL) was added triethylamine (0.61 mL, 4.42 mmol) and methylsulfonyl chloride (0.26 mL, 3.32 mmol) successively at 0 ℃. Then, the mixture was warmed to r.t. and kept stirring for 2 h. It was quenched with water and extracted with DCM (10 mL×3). The organic layer was washed with brine and dried over anhydrous sodium sulfate. After concentration under vacuum, the crude product was used for the next step directly.

To the solution of pervious crude product in DMF (10 mL) was added sodium azide (220 mg, 3.32 mmol) at r.t. Then, the mixture was heated to 60 ℃ and stirred for 2 h. The mixture was cooled to r.t. and quenched with water. Extracted with ethyl acetate (10 mL×3), the organic layer was washed with brine and dried with anhydrous sodium sulfate. After concentration under vacuum, the crude product was further purified by column chromatography to get compound **7** as yellow oil (470 mg, 85% over two steps). $[\alpha]_D^{25}$ +1.74 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.22 (d, J=2.0 Hz, 1H), 7.61 (d, J=2.4 Hz, 1H), 5.86—5.76 (m, 1H), 5.18 (s, 1H), 5.15—5.02 (m, 2H), 3.03—2.92 (m, 1H), 2.66—2.47 (m, 2H), 1.30 (d, J=6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 147.5, 146.8, 143.5, 134.7, 133.3, 132.5, 119.2, 61.5, 39.4, 31.2, 23.5; ESI-MS: 250.8 [M+1]. HRMS (EI) calcd for $C_{12}H_{15}ClN_4$: 250.0985, found 250.0993.

(*S*)-2-Chloro-5-isopropyl-3-(pyrrolidin-2-yl)pyridine (8) To the solution of cyclohexene (2.14 mL, 21.89 mmol) in THF (3 mL) was added the solution of BH_3•Me_2S in THF (2 mol/L, 5.47 mL, 10.95 mmol) at 0 ℃ under nitrogen. The mixture was stirred at 0 ℃ for 1 h to get a white suspension. The mixture was cooled to −15 ℃ and the solution of compound **7** (860 mg, 3.65 mmol) in THF (10 mL) was added into the reaction mixture. Then, it was slowly warmed to r.t. and continued to stir for another 12 h before quenched with methanol. After the solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (30 mL) and washed with hydrochloric acid (1 mol/L, 20 mL). The organic layer was discarded, the aqueous phase was adjusted to pH=13—14 with the solution of sodium hydroxide (30%). Extracted with DCM (20 mL×3), the organic layer was washed with brine and dried over anhydrous sodium sulfate. After concentration under vacuum, the crude product was further purified by column chromatography to get compound **8** as yellow oil (720 mg, 88%). $[\alpha]_D^{25}$ +1.9 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.11 (d, J=2.4 Hz, 1H), 7.86 (d, J=2.4 Hz, 1H), 4.48 (t, J=7.6 Hz, 1H), 3.22—3.09 (m, 2H), 2.97—2.90 (m, 1H), 2.42—2.37 (m, 1H), 1.91—1.86 (m, 2H), 1.59—1.54 (m, 2H), 1.28 (d, J=6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 147.4, 145.9, 143.1, 138.5, 134.3, 58.2, 46.9, 32.8, 31.3, 25.2, 23.6; ESI-MS: 224.8 [M+1]. HRMS (EI) calcd for $C_{12}H_{17}ClN_2$: 224.1080, found 224.1078.

(*S*)-2-Chloro-5-isopropyl-3-(1-methylpyrrolidin-2-yl) pyridine (9a) The mixture of compound **8** (61.6 mg, 0.27 mmol), formic acid (6 mL) and formaldehyde (3 mL) was heated to 80 ℃ and kept stirring for 4 h. Then, the formic acid was removed under reduced pressure. Diluted hydrochloric acid was used to adjust the solution to pH=2 and then washed with DCM (20 mL). The aqueous layer was adjusted to pH=12 with diluted sodium hydroxide solution and extracted with DCM (10 mL×3). The organic layer was washed with brine and dried over anhydrous sodium sulfate. After concentration under vacuum, the crude product was further purified by column chromatography to get compound **9a** as yellow oil (44.6 mg, 69%). $[\alpha]_D^{25}$ −83.8 (*c* 1.80, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.12 (d, J=2.8 Hz, 1H), 7.80 (d, J=2.4 Hz, 1H), 3.52 (t, J=8.4 Hz, 1H), 3.28—3.23 (m, 1H), 2.99—2.89 (m, 1H), 2.46—2.35 (m, 2H), 2.22 (s, 1H), 1.90—1.81 (m, 2H), 1.54—1.50 (m, 1H), 1.27 (d, J=7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 148.1, 145.9, 143.4, 137.1, 134.8, 66.5, 56.8, 40.5, 33.3, 31.3, 23.7, 23.6, 22.6; ESI-MS: 238.9 [M+1]. HRMS (EI) calcd for $C_{13}H_{18}ClN_2$: 238.1237, found 238.1235.

(*S*)-3-(1-Benzylpyrrolidin-2-yl)-2-chloro-5-iso-propylpyridine (10a) To the solution of compound **8** (50 mg, 0.22 mmol) in DMF (2 mL) was added sodium hydride (60%, 35.6 mg, 0.89 mmol) at r.t. The mixture was stirred for 1 h before benzyl bromide (0.08 mL, 0.67 mmol) was added. Quenched with water until the starting material was consumed, the mixture was extracted with ethyl acetate (10 mL×3). The organic layer was washed with brine and dried over anhydrous sodium

sulfate. After concentration under vacuum, the crude product was further purified by column chromatography to get compound **10a** as yellow oil (65 mg, 94%). $[\alpha]_D^{25}$ −14.4 (*c* 1.30, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.14 (d, J=2.4 Hz, 1H), 8.04 (d, J=2.4 Hz, 1H), 7.32—7.31 (m, 4H), 7.27—7.25 (m, 1H), 3.87 (t, J=8.4 Hz, 1H), 3.80 (d, J=12.8 Hz, 1H), 3.23 (d, J=13.2 Hz, 1H), 3.19—3.14 (m, 1H), 3.02—2.92 (m, 1H), 2.47—2.42 (m, 1H), 2.38—2.32 (m, 1H), 1.87—1.84 (m, 2H), 1.59—1.54 (m, 1H), 1.30 (d, J=6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 148.1, 146.1, 143.3, 139.2, 137.8, 134.9, 128.5, 128.2, 126.9, 64.7, 58.6, 53.6, 33.1, 31.3, 23.8, 23.6, 22.7; ESI-MS: 314.9 [M+1]. HRMS (EI) calcd for $C_{19}H_{23}ClN_2$: 314.1550, found 314.1551.

(*S*)-3-(1-Allylpyrrolidin-2-yl)-2-chloro-5-isopropylpyridine (10b) The procedure was same as compound **10a**. $[\alpha]_D^{25}$ −48.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.12 (d, J=2.4 Hz, 1H), 7.87 (d, J=2.0 Hz, 1H), 5.88—5.80 (m, 1H), 5.19—5.05 (m, 2H), 3.74 (t, J=8.0 Hz, 1H), 3.35—3.30 (m, 1H), 3.20 (dd, J=5.2, 13.6 Hz, 1H), 2.98—2.91 (m, 1H), 2.80—2.75 (m, 1H), 2.43—2.31 (m, 2H), 1.88—1.83 (m, 2H), 1.54—1.50 (m, 1H), 1.27 (d, J=7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 148.0, 145.9, 143.3, 137.8, 135.7, 134.9, 116.6, 64.0, 57.1, 53.6, 33.0 31.2, 23.8, 23.5, 22.7; ESI-MS: 264.8 [M + 1]. HRMS (EI) calcd for $C_{15}H_{21}ClN_2$: 264.1393, found 264.1397.

(*S*)-2-Chloro-5-isopropyl-3-(1-(prop-2-ynyl)pyrrolidin-2-yl)pyridine (10c) The procedure was same as compound **10a**. $[\alpha]_D^{25}$ −37.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.14 (d, J=2.0 Hz, 1H), 7.79 (s, 1H), 4.01 (s, 1H), 3.47 (d, J=17.2 Hz, 1H), 3.25—3.16 (m, 2H), 2.98—2.91 (m, 1H), 2.78 (d, J=8.4 Hz, 1H), 2.48—2.39 (m, 2H), 2.22 (s, 1H), 1.90 (d, J=6.4 Hz, 2H), 1.57 (d, J=2.0 Hz, 1H), 1.28 (d, J=6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 148.2, 146.1, 143.4, 136.9, 134.7, 78.8, 72.8, 61.8, 52.4, 40.7, 33.3, 31.3, 23.7, 23.6, 22.7; ESI-MS: 262.9 [M+1]. HRMS (EI) calcd for $C_{15}H_{19}ClN_2$: 262.1237, found 262.1238.

Biology

(−)-Nicotine hydrogen tartrate, mecamylamine and probenecid were purchased from Sigma. FLIPR Membrane Potential Assay Kit and FLIPR Calcium 5 Assay Kit were purchased from Molecular Devices. Tissue culture media and fetal bovine serum (FBS) were obtained from GIBCO.

Cell lines RD rhabdomyosarcoma cells expressing endogenous human neuromuscular $\alpha1\beta1\gamma\delta$ receptors[10] and SH-SY5Y neuroblastoma cells expressing human ganglionic $(\alpha7)_5$ receptors[11] were bought from Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences, CAS, China. RD cells were grown in Dulbecco's modified Eagle's media supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin. SH-SY5Y cells were grown in a 1∶1 mixture of DMEM and Ham F12 medium (DMEM/F12) supplemented with 20% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin. The cells were cultured at 37 ℃ in a humidified atmosphere containing 5% CO_2.

Membrane potential measurements The assay was carried out according to the FLIPR Membrane Potential Assay Kit Protocol (Molecular Devices). RD cells were seed at a density of 30 000 cells/well in a black 384 well plate 24 h prior to the day of the assay. 25 μL membrane potential dye solution was added to the cells and the plate was incubated for 60 min at 37 ℃. The plate was removed from incubator, allowed to reach room temperate for 20 min. Antagonists were incubated with cells for 10 min, and the plate was transferred to FLIPR (Molecular Devices). The excitation and emission wavelengths were set to 535 nm and 560 nm, respectively, and with a cutoff of 550 nm. Fluorescence baseline was measured for 10 s followed by the addition of 100 μmol/L nicotine. Then the measurement was carried out for 120 s at 1 s intervals. The inhibitory rates of the antagonists were expressed in the percent relative to the response induced by nicotine.

Calcium fluorescence measurements The changes of calcium flux were evaluated in SH-SY5Y cells using FLIPR Calcium 5 Assay Kit (Molecular Devices). According to the manufacturer's instructions, this assay was conducted in a similar method to the membrane potential assay with modifications. Briefly, after overnight cell culture, 25 μL calcium 5 d solution, supplemented with probenecid at a final concentration of 2.5 mmol/L, was added to the cells. The plate was incubated for 60 min at 37 ℃ and 20 min at room temperate, and then the antagonists were added to the cells for 10 min incubation. After the addition of 20 μmol/L nicotine, the fluorescence measurements were taken as before, but with excitation and emission wavelengths set to 485 and 525 nm, respectively, and with a cutoff of 515 nm.

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