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Design, synthesis, and pharmacological evaluation of soft glycopyrrolate and its analog

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Glycopyrrolate is a quaternary anticholinergic drug. Like for other anticholinergics, the usefulness of this agent is limited by its side effects. In this study, based on the structure of glycopyrrolate, we designed a soft drug, methoxycarbonylphenylcyclopentylacetoxy-*N,N*-dimethyl-3-pyrrolidinium methyl sulfate (SG), and its analog, methoxycarbonylphenylcyclopentylacetoxyethyl-*N,N,N*-trimethylammonium methyl sulfate (SGA). These soft drugs are expected to be locally active, but systemically inactive in order to increase therapeutic index. SG and SGA were synthesized by (i) carboxylation of methyl phenylcyclopentylacetate, (ii) esterification with *N*-methyl-3-pyrrolidinol (for SG) or 2-chloro-*N,N*-dimethylaminoethane (for SGA), and (iii) quarternization with dimethyl sulfate. Receptor binding studies demonstrate that SG has muscarinic subtype selectivity (m_3/m_2). Guinea pig ileum pA_2 assay indicates that activity of SG is moderate, and SG is about ten times more potent than SGA. The *in vivo* characterization of SG and SGA, both in mydriasis tests and in prevention of carbachol induced bradycardia, supported its soft nature. Applying SG or SGA into rabbit eyes, the dilation of the contralateral (water-treated) pupils was not observed. Glycopyrrolate application, however, caused dilation of the contralateral pupil, indicating a systemic effect of this drug. Cardiac studies were carried out by evaluating the protective effect of soft anticholinergics against carbachol induced bradycardia. The results indicate that SG and SGA were as potent as atropine-MeBr in preventing carbachol induced bradycardia in the rat; however, their durations of action were significantly shorter. In conclusion, the newly synthesized SG and SGA showed soft nature in the body. They are anticholinergics with subtype selectivity and moderate potency, and can be used as topical antiperspirants.

1. Introduction

Anticholinergics are frequently used in a wide variety of clinical conditions mainly for inhibiting the effects of parasympathetic nervous system activity. The major limitation in the clinical use of the traditional antimuscarinic drugs is their side effects, such as dry mouth, photophobia, difficulty in urination, restlessness, irritability, disorientation, hallucinations, delirium, tachycardia, and cardiac arrhythmias. Even the topical application of anticholinergics can lead to unwanted systemic side effects because of their slow *in vivo* metabolism [1–2].

Based on the "inactive metabolite" approach of the soft drug design concept proposed by Bodor [3–4], many representatives of soft anticholinergics derived from methatropine, methscopolamine, and propantheline were synthesized and characterized in the past decade in our laboratory [5–8]. These soft drugs are expected to elicit the intended pharmacological effect at the site of application (e.g., mydriasis, antiperspirant effect), but are deactivated in a single predictable step upon entering the systemic circulation. Thus, they lack the systemic side effects and are safer to use.

Glycopyrrolate is a quarternary anticholinergic agent. Like for other anticholinergics, the usefulness of this agent is limited by its side effects. In this paper, the synthesis and evaluation of two new soft anticholinergics, SG and SGA, based on glycopyrrolate are presented. The retrometabolic design of SG based on the "inactive metabolite" approach, and the predicted metabolism of SG are displayed in Scheme 1.

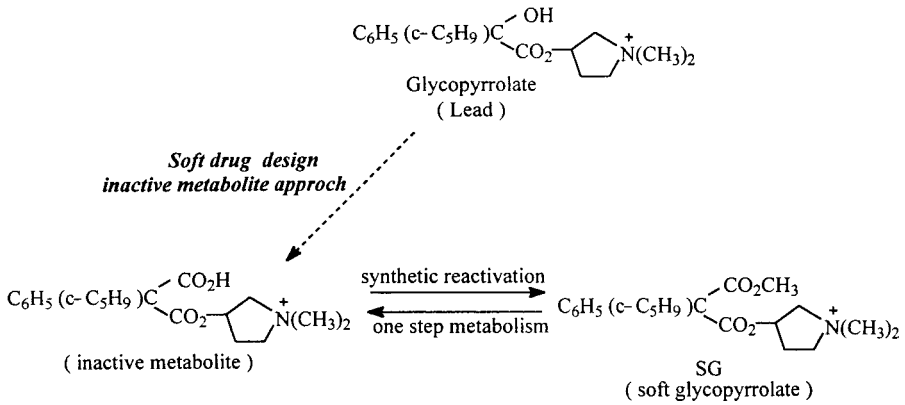
The anticholinergic activity of these compounds, *in vitro*, was characterized by receptor binding affinity studies and a guinea pig ileum assay method. The *in vivo* anticholinergic effects and the soft drug nature were demonstrated by the mydriatic responses of rabbit eyes upon topical administration and by the duration of protective action against carbachol induced bradycardia in the rat after i.v. administration.

2. Investigations, results and discussion

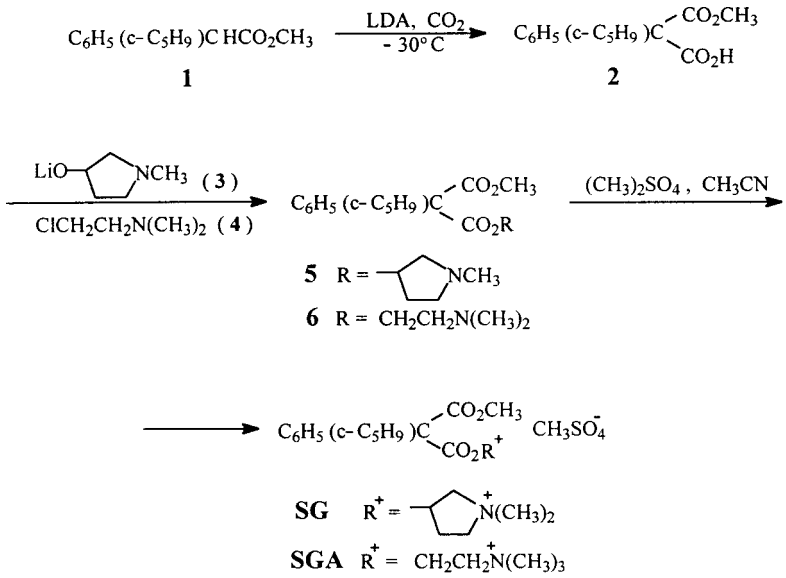
2.1. Synthesis

Methoxycarbonylphenylcyclopentylacetoxy-*N,N*-dimethyl-3-pyrrolidinium methyl sulfate (SG) and methoxycarbonyl-

Scheme 1



Scheme 2



phenylcyclopentylacetoxyethyl-*N,N,N*-trimethylammonium methyl sulfate (SGA) were synthesized as shown in Scheme 2.

Methyl phenylcyclopentylacetate (**1**) was carboxylated [9] with lithium diisopropylamide (LDA) and carbon dioxide at low temperature to give monomethyl phenylcyclopentylmalonate (**2**) in the yield of 85%. Esterificaton [10] of **2** with lithium *N*-methyl-3-pyrrolidoxide (**3**) or 2-chloro-*N,N*-dimethylaminoethane (**4**) gave methyl *N*-methyl-3-pyrrolidinyl phenylcyclopentylmalonate (**5**) or methyl *N,N*-dimethylaminoethyl phenylcyclopentylmalonate (**6**). Quaternization of **5** and **6** with dimethyl sulfate in acetonitrile at room temperature gave the desired SG and SGA in 88–89% yield.

2.2. pA_2 Values and receptor binding

To evaluate the relative potency of the newly synthesized soft drugs, guinea pig ileum assay (pA_2) and receptor binding affinity studies were performed (Table). The results demonstrate that pA_2 values of SG and SGA (7.37 ± 0.11 and 6.72 ± 0.14) are lower than that of glycopyrrolate and atropineMeBr (8.71 ± 0.10 and 8.44 ± 0.09), but fell into a range similar to that of the soft anticholinergics synthesized earlier in our laboratory [11]. In the receptor binding affinity studies, radioligand binding assays were performed on m_1, m_2, m_3, and m_4 human cloned muscarinic receptor subtypes. As displayed in the Table, the receptor binding affinities of SG and SGA are less than those of the parent compound glycopyrrolate and the reference compound atropineMeBr; however, SG shows some subtype selectivity, particularly on m_3, which was not observed in the case of atropine and glycopyrrolate. The results suggest that SG has a moderate *in vitro* anticholinergic activity, and that SG is about ten times more potent than SGA.

2.3. Mydriatic activity

To evaluate the *in vivo* topical pharmacological activities and soft nature of SG and SGA, mydriatic activities were investigated in a group of three rabbits. To each animal, 100 µl of pharmacodynamic equivalent doses of glycopyrrolate (0.2% w/v), SG (2%), or SGA (3%) were applied to one eye, while in the other eye only normal saline was instilled as control. In Fig. 1, the results indicate that in the drug-treated eye, at the dose used, mydriatic activities of SG and SGA were a little lower than those of glycopyrrolate; however, the duration of action was much shorter. The control eye dilations were also recorded at different time intervals and used as an index of systemic absorption of drugs. As shown in Fig. 2, there was absolutely no mydriatic response in the control untreated eye after SG or SGA treatment, while a measurable pupil dilation (>1 mm

Table: pA_2 Values and receptor binding affinities

	Subtypes of cloned muscarinic receptors [a]				pA_2 [b]
	m_1	m_2	m_3	m_4	
Glycopyrrolate	N/A	9.65 ± 0.33 (1.03 ± 0.03)	10.01 ± 0.34 (0.98 ± 0.05)	N/A	8.71 ± 0.11
SG	7.54 ± 0.05 (0.98 ± 0.11)	6.95 ± 0.02 (1.02 ± 0.11)	7.81 ± 0.01 (1.03 ± 0.04)	8.02 ± 0.02 (0.87 ± 0.06)	7.37 ± 0.11
SGA	6.62 ± 0.12 (0.93 ± 0.03)	6.54 ± 0.20 (1.04 ± 0.08)	6.46 ± 0.29 (0.90 ± 0.03)	6.84 ± 0.21 (0.88 ± 0.14)	6.72 ± 0.14
Atropine MeBr	9.35 ± 0.2 (0.94 ± 0.04)	8.84 ± 0.43 (1.03 ± 0.03)	9.10 ± 0.32 (1.17 ± 0.05)	8.83 ± 0.74 (0.94 ± 0.02)	8.44 ± 0.09

[a] Data of the receptor binding experiments represent the mean ±S.D. of 3–5 experiments. The numbers in parentheses denote Hill slopes
[b] pA_2 values were determined on 4–6 ileum strips obtained from different animals. Data represent the mean ±S.D

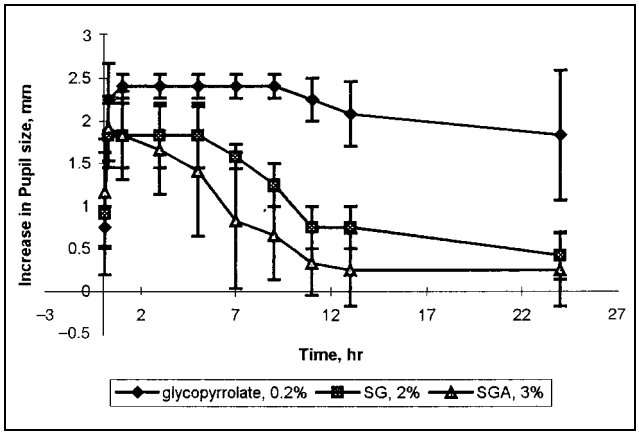


Fig. 1: Mydriatic activities of glycopyrrolate, SG, and SGA (drug-treated eye)

increase in pupil size) of the untreated eye was observed after glycopyrrolate treatment. The short duration of action and the lack of mydriatic activity in the untreated eye indicate a lower systemic activity and suggest the soft, safer nature of SG and SGA.

2.4. Effects on carbachol induced bradycardia

The extent and duration of action of the bradycardia protective effect of SG and SGA were evaluated in comparison with atropineMeBr. With i.v. administration of carbachol at a dose of 5 μg/kg to male Sprague-Dawley rats, the temporary development of sinus bradycardia and Mobitz II A-V block can be evoked safely and reproducibly. This effect can be antagonized by previous administration of an anticholinergic agent, e.g., atropine, scopolamine, glycopyrrolate, propantheline, and tematropium. The full protection against carbachol induced bradycardia by the anticholinergics was regarded as their ability to protect against both the lengthening of PP cycle (sinus bradycardia) and the development of Mobitz II type A-V block. Bradycardia protective effects of different anticholinergics differ greatly in respect of their potency and duration of action.

In Fig. 3, AtropineMeBr showed a long duration of action against carbachol induced bradycardia in rat. The effect lasted for a long period. The potency of action of SG and SGA were about the same order of magnitude as that of atropineMeBr, as they were effective at the same dose range (0.2 and 2 μmol/kg). However, the anticholinergic action of SG and SGA lasted only for about 30–40 min

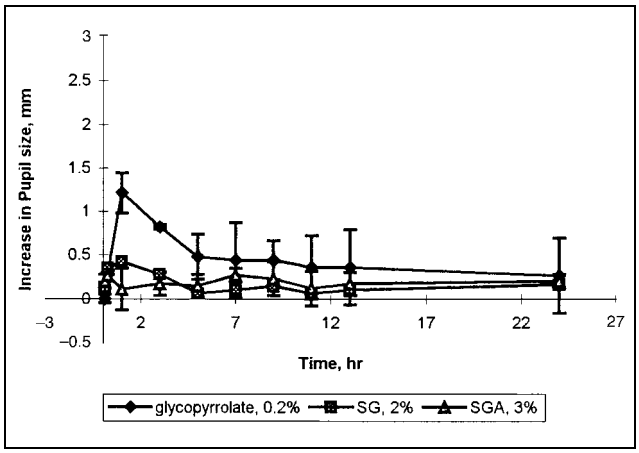


Fig. 2: Mydriatic activities of glycopyrrolate, SG, and SGA (control eye)

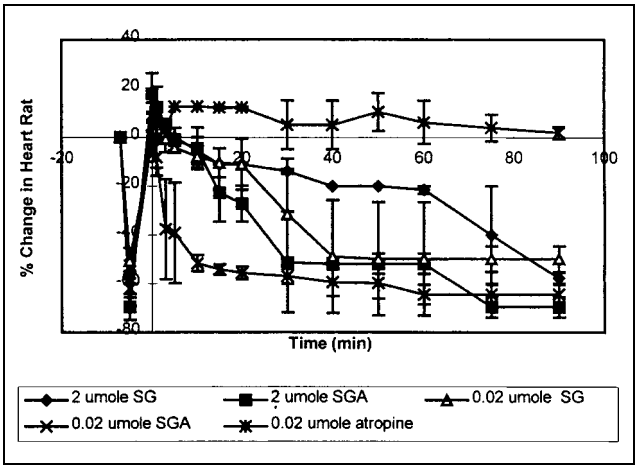


Fig. 3: Bradycardia protective effects of atropine, SG, and SGA

(except in the case of SG 2 μmol/kg, where the action lasted a little longer) indicating the "soft" nature of SG and SGA.

In conclusion, by soft drug design using the inactive metabolite approach, new anticholinergic agents, SG and SGA, were synthesized in order to reduce the systemic side effects of traditional anticholinergics. In the body, they are expected to be cleaved by esterases ubiquitous in blood and tissues to form the inactive acid metabolite and, thus, significantly shortening their duration of action. In this study, the *in vitro* determination of receptor binding affinity and pA_2 values demonstrate that SG is an anticholinergic with moderate potency. Mydriasis tests in rabbits and the studies on the effect on prevention of carbachol induced bradycardia in rats both demonstrated the *in vivo* activity and soft nature of SG and SGA. In the mydriasis studies, the duration of action of SG and SGA was much shorter than that of glycopyrrolate. The lack of pupil dilation in the water-treated eye also suggested that the soft drug was metabolized into an inactive metabolite upon entering the systemic circulation. The potency of the bradycardia protective effect of SG and SGA were proved to be the same as that of atropineMeBr, but the duration of action was significantly shorter. Based on these findings, it can be concluded that SG and SGA although showed lower *in vitro* activities than atropineMeBr and glycopyrrolate, their *in vivo* activities are fairly good and their behaviours in the body are in accordance with their soft nature.

3. Experimental

3.1. Materials

Hexamethonium bromide, carbamylcholine bromide (carbachol), and atropine methylbromide (atropineMeBr) were obtained from Sigma. Chemicals used for synthesis were reagent or HPLC grade, and were obtained from Aldrich and Fisher Scientific. Melting points were taken on a Fisher-Johns apparatus and are uncorrected. NMR spectra were recorded on a Varian 300 NMR spectrometer and are reported in ppm relative to TMS. Elemental analyses were performed by Atlantic Microlab., Inc., all the results were in an acceptable range. Animal studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the National Institute of Health.

3.2. Synthesis

3.2.1. Monomethyl phenylcyclopentylmalonate (**2**)

To 9.5 ml (19 mmol) of 2 M LDA (lithium diisopropylamide) solution in 20 ml of anh. tetrahydrofuran, methyl α-phenylcyclopentylacetate (**1**, 3.42 g, 15.7 mmol) in 15 ml of anh. tetrahydrofuran was added slowly by syringe at $-40 \sim -50$ °C. Fast CO_2 stream was passed through the above solution at $-25 \sim -35$ °C for 20 min. Then tetrahydrofuran was removed under reduced pressure. The residue was treated with 25 ml of 1 N hydrochloric acid solution at 0 °C. The solution was extracted with ethyl ether

(3 × 25 ml). The ether solution concentrated to around 30 ml was shaken with 30 ml of 10% potassium carbonate solution. The aqueous solution was extracted with ethyl ether (2 × 25 ml, ether solution discarded), acidified with concentrated HCl solution, and extracted with ethyl ether. After drying with sodium sulfate, removal of the solvent gave pure monomethyl phenylcyclopentylmalonate (**2**, 3.49 g, 85%). ^{1}H NMR($CDCl_3$): 1.38–1.55, 1.74–1.88 [8 H, m, $CH(C\underline{H}_2)_4$], 3.08 [1 H, quintet, $C\underline{H}(CH_2)_4$], 3.77 (3 H, s, CH_3), 7.25–7.40 (5 H, m, Ph) ppm.
$C_{15}H_{18}O_4$

3.2.2. Methyl N-methyl-3-pyrrolidinyl phenylcyclopentylmalonate (**5**)

A mixture of monomethyl phenylcyclopentylmalonate (**2**, 1.31 g, 5 mmol), thionyl chloride (0.66 g, 6 mmol) and *N,N*-dimethylformamide (2 drops) in 25 ml of anh. ethyl ether was refluxed for 2 h. The ether was removed under reduced pressure to give oily monomethyl phenylcyclopentylmalonic chloride, which was dissolved in anh. tetrahydrofuran (ready for use). To 1-methyl-3-pyrrolidinol (1.03 g, 10.2 mmol) in 15 ml of anh. tetrahydrofuran, 7.5 ml (12 mmol) of 1.6 M n-LiBu solution in heptane was added by syringe at 0 °C. The reaction mixture was stirred at 0 °C and room temperature for 30 min, separately. To this mixture, the above prepared monomethyl phenylcyclopentylmalonic chloride was added by syringe at 0 °C. Then the mixture was stirred at room temperature for 19 h. Tetrahydrofuran was removed under reduced pressure. The residue was treated with 20 ml of saturated sodium bicarbonate solution. The solution was extracted with ethyl ether (3 × 25 ml). After drying with sodium sulfate, removal of the solvent gave crude oily product, which was purified by flash chromatography on silica gel with EtOAc, then 1:100 NH_4OH/MeOH to give pure methyl *N*-methyl-3-pyrrolidinyl phenylcyclopentylmalonate (**5**, 0,27 g, 16%). ^{1}H NMR($CDCl_3$): 1.28–1.50, 1.72–1.88 [8 H, m, $CH(C\underline{H}_2)_4$], 2.15–2.55, 2.85–2.92(6 H, m, $CH_2NCH_2CH_2$), 2.27, 2.32 (3 H, s, NCH_3), 2.96 [1 H, quintet, $C\underline{H}(CH_2)_4$], 3.75 (3 H, s, CO_2CH_3), 5.21–5.30 (1 H, br, CO_2CH), 7.23–7.39 (5 H, m, Ph) ppm.
$C_{20}H_{27}NO_4$

3.2.3. Methoxycarbonylphenylcyclopentylacetoxy-N,N-dimethyl-3-pyrrolidinium methyl sulfate (SG)

To compound **5** (166 mg, 0.48 mmol) was added dimethyl sulfate (128 mg, 1.01 mmol) in 5 ml of acetonitrile at room temperature. The mixture was stirred at room temperature for 4 h. Acetonitrile was removed under reduced pressure. The residue was precipitated twice with methylene chloride/ethyl ether to get pure ethoxycarbonylphenylcyclopentylacetoxy-*N,N*-dimethyl-3-pyrrolidinium methyl sulfate (**SG**, 200 mg, 88%). ^{1}H NMR ($CDCl_3$): 1.23–1.30, 1.53, 1.74, 1.97 [8 H, m, $CH(C\underline{H}_2)_4$], 2.20–2.25, 3.47, 3.57–3.64, 3.88–3.94, 4.08–4.12 (6 H, m, $CH_2NCH_2CH_2$), 2.85, 2.99, 3.38, 3.41 [6 H, 4s, $N(CH_3)_2$], 2.92, 2.95 [1 H, m, $C\underline{H}(CH_2)_4$], 3.70 (3 H, s, CH_3SO_4), 3.81 (3 H, s, CO_2CH_3), 5.58 (1 H, br, CO_2CH), 7.26–7.33 (5 H, m, Ph) ppm.
$C_{22}H_{33}NO_8S \cdot 0.4\ H_2O$

3.2.4. Methyl N,N-dimethylaminoethyl phenylcyclopentylmalonate (**6**)

To compound **2** (2.24 g, 8.54 mmol) in 70 ml of anh. acetonitrile and 5 ml of dry DMF, 2-chloro-*N,N*-dimethylaminoethane (**4**, 2.20 g, 20.4 mmol) and Cs_2CO_3 (8.15 g, 25 mmol) were added at room temperature. The mixture was stirred at room temperature for 17 h. The solvent was removed under reduced pressure. The residue was treated with water and extracted with methylene chloride twice. After drying with sodium sulfate, removal of the solvent gave crude oily product, which was purified by flash chromatography on silica gel with EtOAc to give pure methyl *N,N*-dimethylaminoethyl phenylcyclopentylmalonate (**6**, 0.92 g, 32%). ^{1}H NMR ($CDCl_3$): 1.30–1.40, 1.46–1.51, 1.75–1.82 [8 H, m, $CH(C\underline{H}_2)_4$], 2.20 [6 H, s, $N(CH_3)_2$], 2.52 (2 H, t, $CH_2C\underline{H}_2N$), 2.97 [1 H, m, $C\underline{H}(CH_2)_4$], 3.74 (3 H, s, CO_2CH_3), 4.26 (2 H, t, $C\underline{H}_2CH_2N$), 7.26–7.40 (5 H, m, Ph) ppm.

3.2.5 Methoxycarbonylphenylcyclopentylacetoxyethyl-N,N,N-trimethylammonium methyl sulfate (SGA)

A mixture of compound **6** (0.1836 g, 0.55 mmol) and dimethyl sulfate (0.1410 g, 1.18 mmol) in 5 ml of anh. acetonitrile was stirred at room temperature for 4 h. Acetonitrile was removed under reduced pressure. The residue was precipitated by methylene chloride/ethyl ether to give pure powder precipitate (**SGA**, 0.2240 g, 89%). Mp: 137–139 °C. ^{1}H NMR ($CDCl_3$): 1.21–1.64, 1.92–2.05 [8 H, m, $CH(C\underline{H}_2)_4$], 3.00 [1 H, m, $C\underline{H}(CH_2)_4$], 3.04 [9 H, s, $N(CH_3)_3$], 3.67 (3 H, s, CH_3SO_4), 3.74 (2H br, $CH_2C\underline{H}_2N$), 3.80 (3 H, s, CO_2CH_3), 4.52 (2 H, br, $C\underline{H}_2CH_2N$), 7.27–7.32 (5 H, m, Ph) ppm.
$C_{21}H_{33}NO_8S$

3.3. Receptor binding assay

Cloned muscarinic receptor subtypes of m_1, m_2, m_3, and m_4 were obtained from RBI Co. (Boston, MA). Dissociation constants (K_d) for N-[^{3}H]-methyl-scopolamine ([^{3}H]NMS) were provided by RBI Co. ($m_1 = 0.166$, $m_2 = 0.24$, $m_3 = 0.11$, $m_4 = 0.06$). Radioligand binding studies were performed with [^{3}H]NMS following the protocol from RBI Co. Binding buffer (Phosphate Buffered Saline-PBS, at pH 7.4) consisted of 0.15 M NaCl, 1.5 mM KH_2PO_4, and 2.7 mM Na_2HPO_4. 10 mM NaF was added into the buffer as esterase inhibitor. Assay mixture (1 ml) contained 100 µl of diluted membranes (receptor protein final concentrations: $m_1 = 25$ µg/ml, $m_2 = 42$ µg/ml, $m_3 = 15.9$ µg/ml, and $m_4 = 20$ µg/ml). The final concentration of [^{3}H]NMS for m_2-m_4 binding studies was 0.5 nM, and for m_1 it was 1 nM. Specific binding was defined as the difference between the [^{3}H]NMS binding in the absence and presence of 1 µM atropine. Incubation was carried out at room temperature for 60 min. The assay was terminated by filtration through a Whatman GF/B filter. The filter was washed three times with ice-cold binding buffer, then transferred to vials, and added with 10 ml of scintiverse liquid. The detection of radioactivity was performed in a Packard 31800 liquid scintillation analyzer (Packard Instrument Inc., Downer Grove, IL). Data obtained from the binding experiments were fitted in the following equation: %[^{3}H]NMS bound = 100 − [$100x^n/k/(1 + x^n)/k$] to obtain Hill's slope *n*; then into: %[^{3}H]NMS bound = 100 − [$100x^n/IC_{50}/(1 + x^n)/IC_{50}$] to obtain IC_{50}; where x = concentration of the tested compound (in a series of concentrations). By the method of Cheng and Prusoff [12], K_i was derived from the equation: $K_i = IC_{50}/(1 + L/K_d)$, where L is the concentration of the radioligand, IC_{50} is the concentration of the drug causing 50% inhibition of specific radioligand binding, and K_d is the dissociation constant of the radioligand receptor complex. Data were analyzed by a non-linear least square curve fitting procedure using Scientist software (MicroMath Inc., Salt Lake City, UT).

3.4. Guinea pig ileum assay (pA_2 value)

The cumulative dose-response curves of the ileum longitudinal contractions to the addition of carbachol in the absence and presence of antagonists were established according to the method of Van Rossum et al. [13]. pA_2 is the negative logarithm of the molar concentration of the antagonist that produces a two-fold shift to the right of an agonist concentration-response curve. The pA_2 values unambiguously quantify the potency of an antagonist for muscarinic receptors. Male guinea pigs (Harlan Sprague Dawley Inc., Indianapolis, IN) weighting about 400 g were used after overnight fasting. Animals were sacrificed by decapitation, and the ileum (the region of 5 cm upward of the cecum) was isolated and removed. The ileum was cut into 2.5 cm pieces and suspended in an organ bath containing 30 ml of mixture of Tyrode's solution and 0.1 mM hexamethonium bromide. The organ bath was constantly aerated with oxygen and kept at 37 °C. One end of the ileum strip was attached to a fixed support at the bottom of the organ bath and the other end to an isometric force transducer (Model TRN001, Kent Scientific Corp., Conn.) operated at 2–10 g range. The ileum strip was kept at 2-gram tension, and carbachol was used as antagonist. The ileum contracted cumulatively upon the addition of consecutive doses of carbachol (10–20 µl of 2×10^{-4} M to 2×10^{-3} M in water solution). Contractions were recorded on a physiograph (Desk Model DMP-4B, Narco Biosystems Inc., Houston). After the maximum response was achieved, the ileum was washed three times, and a fresh Tyrode's solution containing appropriate concentration of the antagonist, SG, SGA, glycopyrrolate, or atropine, was replaced. An equilibration time of 10 min was allowed for antagonists before the addition of carbachol. The pA_2 values presented in the table are the mean ± S.D. of 4–6 experiments.

3.5. Evaluation of mydriatic activity

Using atropineMeBr as a reference, the mydriatic activity of SG and SGA were evaluated. Three healthy male New-Zealand White rabbits (Big D Co., Dade City, FL) weighting about 3.0 kg were used in the experiments. For studying mydriatic activities, to each animal, 100 µl of the pharmacodynamic equivalent dose of glycopyrrolate (0.2% w/v), SG (2%), or SGA (3%) were applied to one eye, while the other eye was instilled with only normal saline as control. The experiments were carried out in a light and temperature controlled room. At appropriate time intervals, the pupil diameters of both eyes were recorded. The difference in pupil diameter between each time point and zero time point was calculated for both treated and control eyes and reported as mydriatic response [(treated-control)/control in %]. For statistical analysis, the Student's t-test (two-sample assuming equal variances) was performed.

3.6. Antagonistic effect on carbachol induced bradycardia

Male Sprague-Dawley rats (Harlan Sprague Dawley Inc., Indianapolis, IN), weighting 300 ± 30 g, were anesthetized with 50 mg/kg sodium pentobarbital (Butler Co. Columbus, OH) via i.p. Baseline ECG recordings and all drug administrations were performed after 15 min stabilization periods. Needle electrodes were inserted s.c. into the limbs of the anesthetized rats and were joined to a GOULD 2000 recorder (GOULD Inc. Cleveland, OH). Standard leads I, II, and III were recorded at a paper speed of 25 mm/s. Recordings were taken before, during, and after the administration of any of the compounds until all basic ECG parameters returned to that of baseline. ECG recordings were evaluated for the following parameters: PP cycle length (ms), RR cycle length (ms), heart rate (1/min) by the equation of 60000/RR cycle length, and presence of Mobitz II type

A-V block (2 : 1, 3 : 1, etc.). Cholinomimetics such as carbachol have four primary effects on the cardiovascular system: vasodilation, a decrease in cardiac rate (negative chronothropic effect), a decrease in the rate of conduction in the sinoatrial (SA) and atrioventricular (AV) nodes (negative dromotropic effect), and a decrease in the force of cardiac contraction (negative inotropic effect) [14]. To evaluate the effects of carbachol, only the negative chronotropic and dromotropic effects were analyzed here. These effects of carbachol were manifested on the surface ECG as sinus bradycardia (lengthening of the PP cycle) and as development of Mobitz II type A-V block. After analyzing the ECG recordings, both heart rate and percent changes of heart rate, as compared to that of baseline, were plotted against time, and the effects of the different drugs on heart rate and on percent changes of heart rate were characterized. Each point on the figures represents the mean ± S.D. of three experiments. All drugs were dissolved in 0.9% NaCl (vehicle), and the solutions were administered by direct injections into the jugular veins on either sides of rats. Anticholinergic drugs, SG, SGA, and atropineMeBr (0.02 or 2 μmol/kg, in ~0.3 ml volume) were administered at 0 time, while carbachol (5–8 μg/kg in 0.06–1 ml volume according to the initial individual ECG response of each rat) was injected at −5, 1, 3, 5, 10, 15, 20, 30, 45, and 60 min (with some exceptions). For statistical evaluation the Student's t-test was performed.

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