Center for Drug Discovery[1], University of Florida, Gainesville, Florida, USA and IVAX Laboratories[2], Miami, Florida, USA

Studies on a soft glycopyrrolate analog, SG-1

F. Ji[1], W.-M. Wu[1] and N. Bodor[1,2]

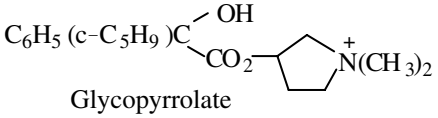
A short-acting soft drug analog (SG-1) of glycopyrrolate (G) was developed by retrometabolic design in order to minimize systemic side effects and optimize the therapeutic index. SG-1,3-(1′-hydroxycyclopentyl)phenylacetoxy-1-methyl-1-methoxycarbonylpyrrolidinium bromide, was synthesized by: (*a*) esterification of phenylacetic acid with *N*-methyl-3-pyrrolidinol by DCC to obtain *N*-methyl-3-pyrrolidinyl phenylacetate; (*b*) reaction of lithium salt of above phenylacetates with cyclopentanone to obtain *N*-methyl-3-pyrrolidinyl 3-(1′-hydroxycyclopentyl)phenylacetate; and (*c*) quarternization with methyl bromoacetate in acetonitrile to give the designed product. To evaluate the pharmacological effect of SG-1, its mydriatic activity in rabbit eyes was compared to that of glycopyrrolate. At the pharmacodynamically equivalent doses (the lowest dose that induces the maximum response) of SG-1 (1%) and glycopyrrolate (0.1%), the mydriatic activities lasted for 5 and 100 h, respectively. Compared to glycopyrrolate, the intrinsic pupil dilation potency of SG-1 was lower (~$1/10^{th}$) but the duration of action was much shorter (<$1/20^{th}$) as SG-1 is susceptible to facile enzymatic hydrolysis/deactivation in the rabbit eyes. *In vitro* metabolism and stability investigations further supported this finding. *In vitro* half lives of SG-1 in rat plasma, blood, and 20% liver and lung tissue homogenates were 15.62, 53.86, 263.43, and 318.35 min, respectively. In human plasma and blood, half-lives were 19.93 and 88.32 min, respectively. SG-1 was relatively stable under acidic conditions (pH 5 and lower). SG-1 is a promising, clinically useful short acting anticholinergic.

1. Introduction

Glycopyrrolate (G) is one of the frequently used potent anticholinergics [1]. The use of anticholinergics is often limited by their systemic side effects, most of which are the results of their pharmacological actions. These side effects include dry mouth, urinary hesitancy, retention, blurred vision, increased ocular tension, tachycardia, palpitation, decreased sweating, loss of taste, headache, nervousness, drowsiness, weakness, dizziness, insomnia, nausea, vomiting, impotence, suppression of lactation, constipation, bloated feeling, severe allergic reaction of drug idiosyncrasies including anaphylaxis, hallucinations, urticaria, and other dermal manifestations [2, 3]. Glycopyrrolate is a quaternary ammonium compound, which prevents its passage across lipid membranes and reduces its CNS-related side effects compared to atropine or scopolamine. Nevertheless, since it is eliminated mostly in unchanged form or as its active metabolites [1, 4–6], it is clinically difficult to apply to patients both systemically and locally, especially on young children and elderly or uraemic patients.

To optimize the therapeutic index, the "inactive metabolite" concept of soft drug design was applied [7–10] in this study. As illustrated in Scheme 1, a hypothetical, inactive, acid metabolite of a glycopyrrolate analog was chosen as the starting point of the present design. This was reactivated by isosteric structural modifications, and a soft analog of glycopyrrolate, SG-1, was developed. SG-1 is expected to undergo a one-step metabolism in the body into this inactive metabolite after achieving its pharmacological activity at the site of application; thus, unwanted side effects can be minimized. The synthesis and investigation of two other soft analogs of glycopyrrolate, SG and SGA, were reported previously [11]. However, the pharmacological activities of SG and SGA were relatively low. Here, the synthesis and *in vitro/in vivo* investigations of the soft nature and the anticholinergic effect of SG-1 are reported. Following synthesis, a pH profile was developed to evaluate the physicochemical stability of the compound; then, metabolism and stability were investigated *in vitro* using human and rat blood and tissue homogenates. Sub-

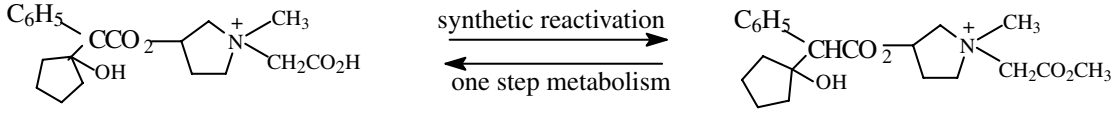
Scheme 1



Glycopyrrolate
(Lead)

Soft drug design

inactive metabolite approch



(Inactive metabolite) SG-1

sequently, pharmacological activity (mydriatic effect in rabbit eyes) was evaluated and compared to that of glycopyrrolate.

2. Investigations, results and discussion

2.1. Synthesis

3-(1′-Hydroxycyclopentyl)phenylacetyloxy-1-methyl-1-methoxycarbonylmethyl-pyrrolidinium bromide (SG-1) was synthesized as shown in Scheme 2. Esterification [12] of phenylacetic acid (**1**) with 1-methyl-3-pyrrolidinol (**2**) by DCC (1,3-dicyclohexylcarbodiimide) in methylene chloride with 4-pyrrolidinopyridine as a catalyst at room temperature gave 1-methyl-3-pyrrolidinyl phenylacetate (**3**) with a yield of 91%. Treatment of **3** in THF (tetrahydrofuran) with LDA (lithium diisopropylamide) at −78 °C and then with cyclopentanone at the same temperature for 4 h [12–14] gave 1-methyl-3-pyrrolidinyl (1′-hydroxycyclopentyl)phenylacetate (**4**) in 90% yield. Quaternarization of **4** with methyl bromoacetate in acetonitrile produced the desired 3-(1′-hydroxycyclopentyl)phenylacetyloxy-1-methyl-1-methoxycarbonylmethylpyrrolidinium bromide (SG-1) with a yield of 35%.

2.2. pH profile

In the pH range of 4.60–8.60 at 37 °C, the rate of hydrolysis of SG-1 (0.2 mM) was strongly dependent on the pH of buffer solution. As shown in Table 1, the compound was more stable under acidic conditions. At pH 4.60, the half-life was 86.22 days; however, at pH 8.6, it decreased to 14 min. At biological pH (7.4), the compound showed a half-life of 2 h. Fig. 1 presents the pH profile as log k (h^{-1}) vs. pH. Results indicate a base-catalyzed hydrolysis of SG-1 with a correlation coefficient of 0.996.

Table 1: Effect of pH on the stability of SG-1 at 37 °C

pH	$k \times 10^{-4}$ (min^{-1})	$t_{1/2}$, (hr)	r^2
4.60	0.06	2069.2	0.903
5.18	0.36	323.73	0.997
5.72	1.12	103.25	0.997
6.60	6.81	16.95	0.999
7.40	57.7	2.00	0.999
7.75	114.5	1.00	0.999
8.70	520.6	0.23	0.981

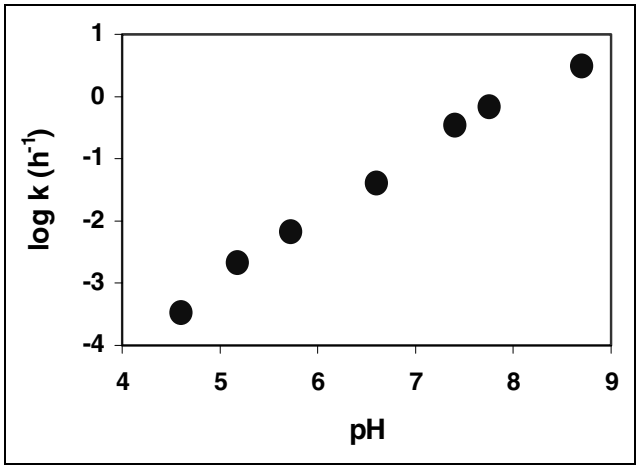


Fig. 1: pH profile of SG-1 at 37 °C

The disappearance of SG-1 and the appearance of its hydrolytic product in the buffer solution were studied in details at pH 7.4, and they are presented in Fig 2. Disappearance of SG-1 concurred with formation of the hydrolytic product, the corresponding inactive acid metabolite. This confirms that degradation of SG-1 was in agreement with the principles of soft drug design.

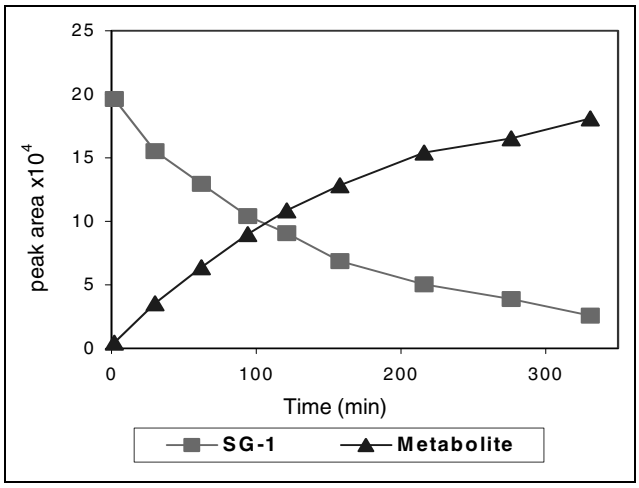


Fig. 2: Degradation of SG-1 in pH 7.4 buffer at 37 °C

Scheme 2

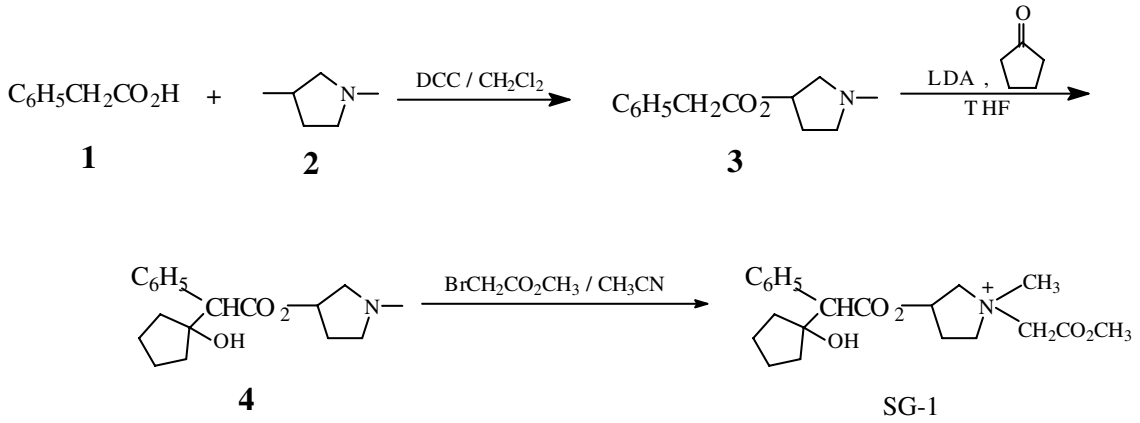


Table 2: Pseudo-first-order rate constant (k, min^{-1}) and half-life ($t_{1/2}$, min) for the disappearance of SG-1 in biological media at 37 °C

Medium	$k \times 10^{-2}$ (min^{-1})	$t_{1/2}$ (min)	r^2
Rat Plasma	4.44 ± 0.1	15.62 ± 0.44	0.997
Blood	1.29 ± 0.1	53.86 ± 4.58	0.996
20% Liver	0.26 ± 0.01	263.43 ± 8.73	0.992
20% Lung	0.22 ± 0.01	318.35 ± 18.95	0.998
Human Plasma	3.48 ± 0.21	19.93 ± 1.17	0.993
Human Blood	0.79 ± 0.04	88.32 ± 5.01	0.995

2.3. In vitro studies

To demonstrate the soft drug nature of SG-1, a series of *in vitro* studies were performed. Freshly collected human and rat plasma and blood and rat tissue homogenates were used. All experiments were carried out in triplicate using media collected from three different individuals. Stability was determined by measuring the pseudo-first-order rate constant (k, min^{-1}) and half-life ($t_{1/2}$, min) of the disappearance of SG-1 from the corresponding biological media.

As shown in Table 2, the *in vitro* hydrolysis of SG-1 in rat plasma and blood was relatively fast with half-lives of 15.62 and 53.86 min, respectively. In 20% liver and 20% lung homogenates, the half-lives were 263.43 min and 318.35 min, respectively. In human plasma and blood, the hydrolysis rate of SG-1 was also relatively fast with half-lives of 19.93 and 88.32 min, respectively. The longer half-lives in both human and rat blood compared to plasma, indicate that binding of the compound to blood cells was relatively significant. In all biological media, metabolism of SG-1 resulted in the corresponding predicted and inactive acid metabolite. These *in vitro* kinetic results suggest that SG-1 is susceptible toward enzymatic hydrolysis in the body; therefore, shorter duration of action and markedly reduced side effects are expected after topical administration.

2.4. Mydriatic activity

The *in vivo* pharmacological activity and the soft nature of SG-1 were evaluated in rabbits. The mydriatic effects of SG-1 and glycopyrrolate (G) were compared following topical administration. Figs. 3 and 4 display the dose-mydriatic response relationships of SG-1 and G, respectively. Increasing doses of the corresponding compounds were administered until maximum pupil dilations were achieved. Both compounds produced similar maximum pupil dilations, but the intrinsic potency of SG-1 on pupil dilation was about 1/10th of G. In agreement with soft drug design principles, SG-1 showed much shorter duration of action than G and also produced no observable control eye dilation in all cases.

To compare the relative duration of mydriatic activities of SG-1 and G, the lowest concentrations that produced the maximum mydriatic response were used and were considered as pharmacologically equipotent doses. Fig. 5 compares the mydriatic activity of the pharmacologically equipotent doses of SG-1 (1%) and G (0.1%). The mydriatic activities of SG-1 and G lasted for 5 h and 5 days, respectively, indicating that SG-1 was rapidly hydrolyzed in the eye after achieving its pharmacological effect.

To ensure that the acid metabolite of SG-1 was inactive, its pharmacological activity was also determined. As shown in Fig. 5, after topical administration of 1% acid metabolite there was no sign of mydriatic response in the rabbit eye indicating that SG-1 is indeed a safe compound for clinical application.

In conclusion, SG-1, a new anticholinergic compound, was developed based on the inactive metabolite approach of soft drug design to minimize the systemic side effects of traditional anticholinergics. SG-1 was chemically relatively stable under acidic conditions (pH 4–5 buffer solutions). In the body, SG-1 was easily hydrolyzed by ubiqui-

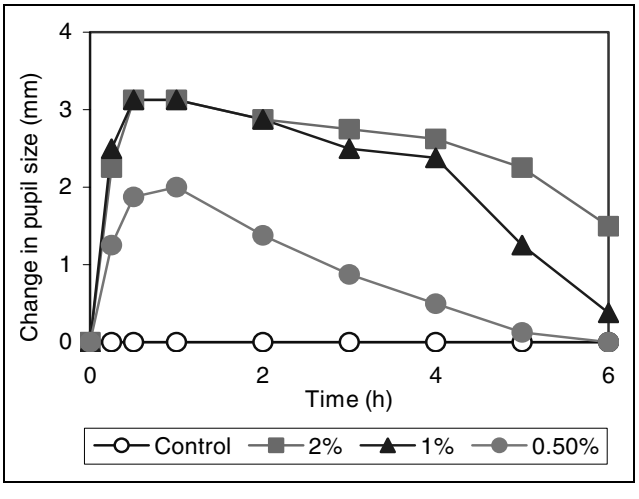


Fig. 3: Mydriatic activity of SG-1 in rabbit eyes

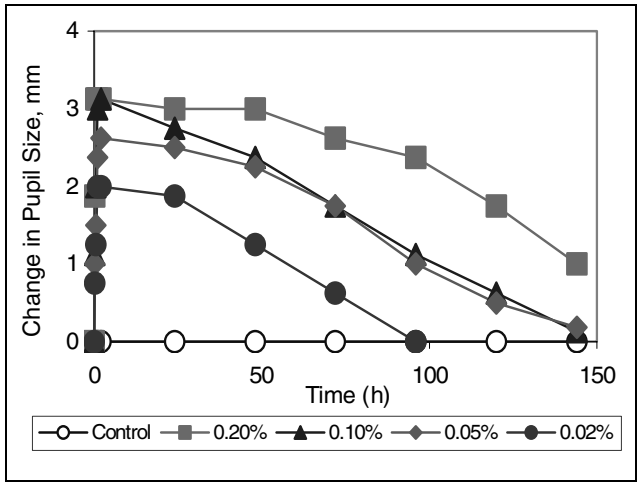


Fig. 4: Mydriatic activity of glycopyrrolate (G) in rabbit eyes

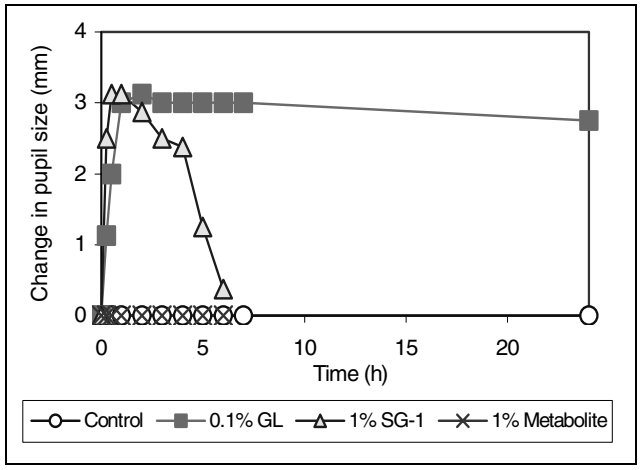


Fig. 5: Mydriatic activity of glycopyrrolate (G) and SG-1 at pharmacologically equipotent doses. The inactive metabolite of SG-1 is also included; it completely lacked activity.

tous esterases in blood and tissues to form the predicted inactive acid metabolite. The *in vivo* pharmacological activity of SG-1 on the mydriatic response in rabbit eyes was less than that of glycopyrrolate. However, the duration of action was much shorter, indicating that SG-1 is a good candidate for clinical conditions that require only a short duration of cholinergic effects. Therefore, the safeness of potential clinical applications of the new soft anticholinergic SG-1 was demonstrated.

3. Experimental

3.1. Synthesis

3.1.1. 1-Methyl-3-pyrrolidinyl phenylacetate **(3)**

To a mixture of 1-methyl-3-pyrrolidinol (**2**, 1.07 g, 10.58 mmol, 1 eq), phenylacetic acid (**1**, 1.73 g, 12.7 mmol, 1.2 eq), and 4-pyrrolidinopyridine (0.14 g, 0.95 mmol, 0.09 eq) in methylene chloride (30 ml) 1 M DCC methylene chloride (13 ml, 13 mmol) was added at 0 °C by syringe. The mixture was stirred at 0 °C for 1 h and at room temperature for 23 h. The mixture was filtered. The filtrate was extracted with hydrochloric acid (30 ml, 1 N). The acidic solution was washed with ethyl ether three times and then made basic with solid potassium carbonate. The aqueous solution was extracted with ethyl ether twice. After drying with anhydrous sodium sulfate, removal of the ether under reduced pressure gave the oily product (**3**, 2.11 g, 91%). ^{1}H NMR($CDCl_3$): 1.83–1.88, 2.23–2.33, 2.58–2.63, 2.705–2.712, 2.74–2.75, 2.80–2.8 (6H, 5m, $CH_2CH_2NHCH_2$), 2.36 (3H, s, NCH_3), 3.62(2H, s, CH_2CO_2), 5.20(1H, m, CO_2CH), 7.24–7.35 (5H, m, Ph) ppm.
$C_{13}H_{17}N$

3.1.2. 1- Methyl-3-pyrrolidinyl (1′-hydroxycyclopentyl)phenylacetate **(4)**

To 2 M LDA tetrahydrofuran solution (16 ml, 32 mmol, 4 eq) in 30 ml of anhydrous THF, 1-methyl-3-pyrrolidinyl phenylacetate (**3**, 1.75 g, 7.99 mmol, 1 eq) in 15 ml of anhydrous THF was added at −78 °C. The mixture was stirred for 2 h at −78 °C. Then, to the above solution cyclopentanone (6.72 g, 80 mmol, 10 eq) was added at −78 °C. The mixture was stirred for 3.5 h and stayed for 18 h at −78 °C. To the cold mixture, 40 ml of water was added. The mixture was extracted with ethyl ether three times. The combined organic solution was concentrated to give an oil. The product was mixed with 20 ml of 1N HCl and extracted with ethyl ether twice. The aqueous solution was made basic with solid potassium carbonate and extracted with ethyl ether twice. After drying with anhydrous sodium sulfate, removal of ether under reduced pressure gave a crude product (2.42 g) that consisted of the designed product **4** and starting material **3** (**4/3**=10/1 by NMR, yield of **4** was 90%). The crude product could be used for the next step.

3.1.3. 3-(1′-Hydroxycyclopentyl)phenylacetyloxy-1-methyl-1-methoxycarbonyl-methyl-pyrrolidinium bromide (SG-1)

A mixture of 1-methyl-3-pyrrolidinyl (1′-hydroxycyclopentyl)phenylacetate (**4,** 0.82 g, 2.70 mmol) and methyl bromoacetate (0.83 g, 6.27 mmol) in 30 ml of acetonitrile was stirred at room temperature for 2.3 h. The precipitate was collected by filtration and washed with methylene chloride. Then, the precipitate was crystallized with hexane/3 : 1 CH_2Cl_2:MeOH to give pure product (SG-1, 0.43 g, 35%). ^{1}H NMR($CDCl_3$, CD_3OD): 1.49–1.84, 2.31–2.40, 2.74–2.88, 3.94–4.15[14H, 4m, (CH_2CH_2)$_2$CH and $CH_2CH_2NCH_2$], 3.51(3H, s, NCH_3), 3.76(3H, s, CO_2CH_3), 3.81(1H, s, PhC**H**), 4.26, 4.31, 4.47, 4.53(2H, dd, CH_2CO_2), 5.56(1H, t, CO_2CH), 7.29–7.42(5H, m, Ph)ppm.
$C_{21}H_{30}NBrO_5$.

3.2. pH Profile

The stability and hydrolysis of SG-1 in buffer at various pH's (4.60 to 8.70) were determined at 37 °C. The buffer solutions were prepared by mixing the following two isotonic solutions in various volume ratios to obtain different pH's. 1) 50 mM Na_2HPO_4; 2) 0.05 M KH_2PO_4 and 0.1 M NaCl. Aliquots of 20 mM SG-1 dissolved in water solution were added to these buffer solutions to give a final concentration of 0.2 mM. At appropriate time intervals, samples were taken and injected in HPLC. The disappearance of SG-1 and the formation of its hydrolysis product were determined. Stability was characterized by the pseudo-first-order rate constant (k, h^{-1}) and the half-life ($t_{1/2}$, h) of the disappearance of the compound in the buffer solutions.

3.3. In vitro studies

The metabolism and kinetics of SG-1 *in vitro* were investigated using human and rat blood and plasma, and rat 20% tissue homogenates. Stability was determined by measuring the pseudo-first-order rate constants (k, min^{-1}) and half-lives ($t_{1/2}$, min) of the disappearance of the compound from the biological media. Experiments were carried out in triplicate using biological media freshly collected from three different individuals. Rats were sacrificed by decapitation, and tissue homogenates were prepared by homogenizing the tissue with four times its weight of isotonic, pH 7.4 phosphate buffer to obtain a ~20% tissue homogenate. Aliquots of 20 mM of SG-1 solutions were added to the biological medium at 37 °C to yield final concentrations of 0.2 mM. At appropriate time intervals, samples (0.15 ml) were taken and mixed with 0.3 ml of 5% dimethyl sulfoxide in acetonitrile solution. The mixtures were centrifuged, and the supernatants were analyzed by HPLC.

3.4. HPLC methods

An HPLC method was developed for analysis of SG-1 and its hydrolysis product (acid metabolite). The HPLC system consisted of a solvent delivery system (Spectra-Physics), a variable wavelength UV detector (SP8450, operated at 230 nm) and an integrator (Spectra-Physics) was used. Supelcosil LC-18 column (25cm × 4.6mm) was used at ambient temperature, and the mobile phase consisted of acetonitrile (40%) and aqueous solution (60%) containing sodium phosphate (10 mM), acetic acid (0.1%), and triethylamine (0.1%). At a flow rate of 1 ml/min, the retention times of SG-1 and the hydrolysis product were 12.40 min and 3.85 min, respectively. A mobile phase consisting of acetonitrile (60%) and aqueous solution (40%) containing sodium phosphate (20 mM), acetic acid (0.1%), and triethylamine (0.1%) was also used. At a flow rate of 1 ml/min, the corresponding retention times of SG-1 and the hydrolysis product were 5.55 min and 2.99 min. respectively.

3.5. Evaluation of mydriatic activity

Four healthy male New-Zealand white rabbits weighting 3.0 kg were used in the experiments. To investigate the dose-mydriatic response relationships of SG-1 and glycopyrrolate, increasing doses (0.5 to 2% for SG-1 and 0.02 to 0.2% for glycopyrrolate) of the compounds (100 µl) were administered in the eyes. Drug solution was applied to one eye, while the other eye was administered with water and served 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 the zero time point was calculated for both treated and control eyes and reported as mydriatic response. Control eye dilations were recorded at different time intervals to determine whether or not systemic absorption had occurred.

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Prof. Nicholas Bodor
Center for Drug Discovery
University of Florida
Health Science Center
P.O. Box 100497
Gainesville, Florida 32610–0497
Nicholas_Bodor@ivax.com