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Soft Drugs–XIV. Synthesis and Anticholinergic Activity of Soft Phenylsuccinic Analogs of Methatropine

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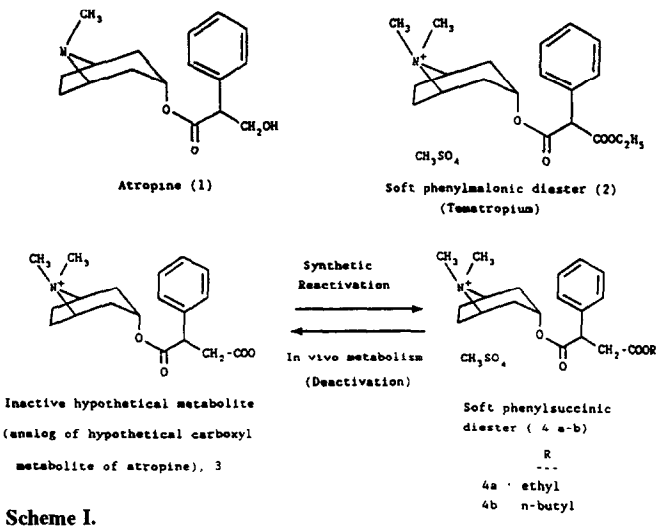
Abstract—Three soft drug analogs and a metabolite of methatropine based on phenylsuccinic structural moiety were synthesized and tested for activity. In an *in vivo* assay, the soft drugs were found to be two orders of magnitude less potent than methatropine while the carboxylate metabolite was found to be one order of magnitude less potent than the soft drugs. A structural isomer of compound **4a** was found to be less potent. All the soft drugs tested elicited shorter durations of mydriatic action in rabbit eyes compared to atropine. The untreated eye was dilated in the atropine treated animals while no dilation occurred in the soft drug treated animals indicating facile systemic metabolism of the soft drugs to inactive moieties, possibly the carboxylate metabolite. In *in vitro* stability studies, the soft drugs have been found to be more hydrolytically labile than atropine. The shorter duration of mydriatic action of compound **4a** coupled with increased hydrolytic lability make this a candidate for further study.

Introduction

Anticholinergic drugs such as atropine (**1**, Scheme I) and scopolamine are well known for their antispasmodic and mydriatic activity as well as their toxic side effects. The soft drug design approach of Bodor,[1] has been used to design less toxic anticholinergic agents,[2] and other biologically active drugs that have improved pharmacokinetic and pharmacological properties.[3,4] Recently, several soft quaternary diester analogs of atropine with a phenylmalonic acid basic structure (**2**) (tematropium) have been synthesized[5] and shown to have very short duration's of mydriatic activity compared to atropine.[6] Other studies from our laboratories on soft phenylmalonic and phenylsuccinic analogs of methscopolamine have recently been reported.[7,8] The short duration of action of tematropium has also been demonstrated *in vivo* in rats by blocking acetylcholine or electrical vagal stimulation induced bradycardia for 3 min versus 2 h for atropine or atropine methyl nitrate.[9] The short duration of mydriatic and cardiac activity of the atropine soft analogs should be related to their facile *in vivo* esterase hydrolysis to the relatively inactive monoester carboxyl metabolite based on the inactive metabolite soft drug design concept.

In this study, we extended this concept to include a methylene group in the side chain ester part to get compounds based on phenylsuccinic structural moiety (**4 a–b**). The compounds designed are expected to degrade *in vivo* to an inactive carboxylate metabolite (**3**). In this paper we wish to report the synthesis, mydriatic and antispasmodic (pA_2 values) activity, and *in vitro* stability studies on selected phenylsuccinic soft drug analogs of atropine.

Keywords: Anticholinergics, atropine, soft drugs, mydriatics.



Scheme I.

Materials and Methods

All melting points are uncorrected and were recorded on a Fisher–Johns melting point apparatus. NMR spectra were recorded with a Varian T-90 NMR spectrometer and are reported in parts per million relative to tetramethylsilane. Elemental analysis were carried out for C, H and N at Atlantic Microlab, Inc., Atlanta, GA, and were within ± 0.4% of the calculated values.

Synthesis

Syntheses of **4a**, **4b** and the carboxylic metabolite **3** were accomplished by a previously described method with substitution of tropine for scopine[8] (Scheme III).

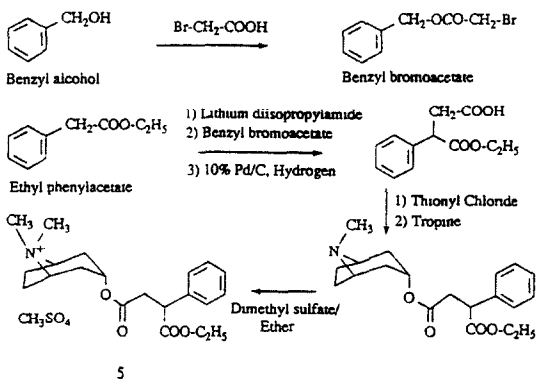
(2R,2S) 4-Alkyl 1-[3α-8-methyl-8-azabicyclo [3.2.1] oct-3-yl] 2-phenylbutanedioic acid and dimethyl sulfate salt (4a). White crystals, 44%, m.p. 134–136 °C, m^+, 360. NMR (DMSO-d_6) 1.2 (t, 3H, CH_3), 1.3–2.2 (m, 8H bicyclic), 2.4–2.7 (3s, 9H) $N^+(CH_3)_2$, $CH_3SO_4^-$, 2.7–3.7 (m, 4H, $N^+(CH)_2$, CH_2–COO), 4.1 (m, 3H, COO–CH_2, Ar–CH–COO), 5 (t, 1H, bicyclic CH–OCO), 7.3 (m, 5H, aromatic). Anal. $C_{22}H_{33}SNO_8$, $^1/_2H_2O$.

*(2R,2S) (±)4-*n*-Butyl 1-[3α-(8,8-dimethyl-8-azabicyclo [3.2.1] oct-3-yl)] 2-phenylbutanedioic acid methyl sulfate (4b).* Hygroscopic oil, 75%. NMR (DMSO-d_6): 7.3 (5H, s, aromatic); 4.9 (1H, t, O–CH); 4.0 (2H, q, O–CH_2); 3.9 (1H, t, CH–Ar); 3.7 (2H, m, $OOCCH_2$); 3.5 (2H, m, $N(CH)_2$); 3.3 (3H, s, CH_3SO_4); 3.1–2.9 (6H, 2s, 2N–CH_3); 2.1–1.5 (12H, m, bicyclic & CH_2–CH_2); 0.9 (3H, t, CH_3). Anal. for $C_{24}H_{37}NO_8S$, 1 H_2O.

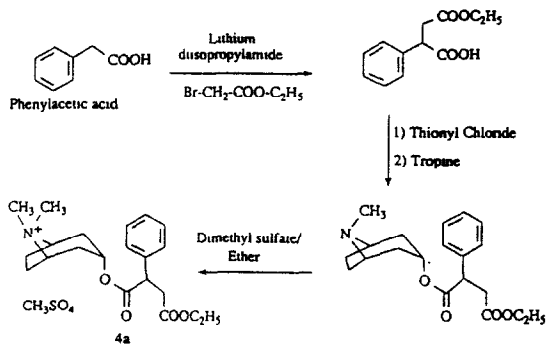
(2R,2S) (±) 1-[3α-(8,8-Dimethyl-8-azabicyclo [3.2.1] oct-3-yl)] 2-phenylbutanedioic acid methyl sulfate (3). White crystals, 83%, m.p. 171–173 °C, m^+: 332. NMR (DMSO-d_6) 1.3–2.2 (m, 4H) bicyclic, 2.4–2.7 (3s, 9H) $N^+(CH_3)_2$ and $CH_3SO_4^-$, 2.7–3.7 (m, 4H) $N^+(CH)_2$, CH_2–COO, 4.1 (m, 1H) Ar–CH–COO, 4.9 (t, 1H) bicyclic CH–OCO, 7.3 (m, 5H) aromatic and 10.5 (s, 1H) COOH.

(2R,2S) (±) 1-Ethyl 4-[3α-(8,8-dimethyl-8-azabicyclo [3.2.1] oct-3-yl)] 2-phenylbutanedioic acid methyl sulfate (5) (Scheme II). Benzyl bromoacetate and butyl bromoacetate were synthesized as previously described.[8] Ethyl phenylacetate (3.25 g, 0.02 moles) dissolved in 50 ml of anhydrous THF taken in a three-neck flask was cooled in a salt ice bath under a brisk stream of dry nitrogen. Lithium diisopropyl amide solution (16 ml of 1.5 M solution, 0.022 moles) was injected slowly through the stopper. The mixture was stirred for 1 h and benzyl bromoacetate was added (4.6 g, 0.02 moles). It was then dissolved in 10 ml of dry THF. The mixture was stirred for 2 h and the reaction was stopped by addition of 1 ml of water. The solvent was evaporated and the residue was suspended in 50 ml of diethyl ether. The ether layer was washed with dilute HCl, followed by washing with saturated bicarbonate solution. The ether solution was dried and evaporated to dryness. The crude compound in methylene chloride was subjected to deprotection without further purification by subjecting it to hydrogenation at 30 lbs/sq.in. for 4 h in a Paar apparatus using 2 g of 10% palladium on carbon as the catalyst. The mixture was filtered and the deprotected acid was extracted into a saturated bicarbonate solution. Neutralization, extraction with diethyl ether and evaporation yielded 3.3 g of thick liquid. The end product was obtained in pure form by silica gel column chromatography using *n*-hexane/acetone = 2/1 (3.65 g, 55%). m.p. 97 °C. NMR ($CDCl_3$): 8.3 (hump, 1H, COOH); 7.3 (s, 5H, aromatic); 4.1 (m, 3H, Ar–CH, COO–CH_2); 2.8 (m, 2H, CH–$\underline{CH_2}$); 1.2 (t, 3H, CH_3).

1-Ethyl 2-phenylbutanedioic acid (1.11, 0.005 m) dissolved in 25 ml of diethyl ether was reacted with 1 ml of thionyl chloride and the mixture was refluxed at 50 °C for 2 h. The solvent was removed under reduced pressure. The reddish



Scheme II. Synthetic pathway to **5**.



Scheme III. Synthetic pathway to **4a/4b** (from Ref. 8).

brown oily acid chloride was redissolved in 20 ml of dry benzene and to it was added dropwise a solution 0.71 g of tropine (0.005 m) in 5 ml of dry benzene and this was stirred for 4 h. The tropine ester was extracted into 1N HCl, the acid layer was neutralized and the diester was extracted into chloroform. The solvent was removed under vacuum to yield a viscous brownish liquid (0.77 g, 45%). TLC with toluene/acetone (3:1) showed a single spot. The diester (0.6 g, 0.0016 m) dissolved in ether was reacted with 0.5 g of dimethyl sulfate under stirring, overnight. The white precipitate formed was collected on a filter and crystallized from ethanol/diethyl ether. Extremely hygroscopic white solid, 62%. NMR (DMSO-d_6) 7.3 (s, 5H, aromatic); 4.9 (t, 1H, COO–CH, bicyclic); 4.1 (m, 3H, Ar–CH, O–$\underline{CH_2}$); 3.3–2.7 (m, 4H, CH–$\underline{CH_2}$, N–$(CH)_2$); 2.4–2.6 (3s, 9H, N–$(CH_3)_2$, CH_3–SO_4); 2.2 (m, 8H, bicyclic); 1.2 (t, 3H, CH_3).

Mydriatic activity

Mydriatic studies were carried out according to a previously published study.[6] Twelve male New Zealand rabbits weighing 2.5 to 3 kg were used in the study. The studies were performed in a temperature and noise controlled room. A dose of 50 µl of the drug at the different concentrations (% w/v) given on the figures was administered unilaterally into one eye. The untreated eye served as an indicator of the systemic absorption of the drug and the potential systemic side effects. The control experiment was performed by the administration of normal saline to the rabbits on a separate occasion. At appropriate time intervals, the pupil diameters of both eyes were measured and the differences in pupil

diameter between each time point and the zero time point were calculated for both treated and control eyes and reported as mydriatic response.

Antispasmodic activity (pA_2 values)

The guinea pig ileum assay method[5,10] was employed to determine the pA_2 values of the soft atropine analogs compared to atropine. Dose response curves were plotted and pA_2 values were calculated using a Schild plot.

In vitro *studies in rat and human plasma*

The plasma was obtained by centrifugation of freshly heparinized blood. The stability studies were carried out by adding aliquot of drug stock solution to plasma to obtain a final concentration of 0.25 mM. The plasma was kept at 37 °C while shaking. Samples of 0.1 ml were withdrawn at appropriate time intervals and mixed with 0.4 ml of ice-cold acetonitrile to stop the enzymatic reaction. The sample was then centrifuged and the supernatant was analyzed for the drug and its product(s) by HPLC. The kinetics were followed for four half-lives.

In vitro *studies in rat liver homogenate*

A suspension of freshly obtained rat liver was made in isotonic p*H* 7.4 phosphate buffer. The protein content was adjusted to 50 mg per ml. The liver suspension was equilibrated at 37 °C. An aliquot of soft drug stock solution was added to obtain a final concentration of 0.25 mM. Sampling, deproteination and analysis were done as described below.

Analytical method for in vitro *stability studies*

A high pressure liquid chromatography (HPLC) method was developed to assay soft drugs and their products in the buffer media. The system consisted of SP 8810 precision isocratic pump, SP 4290 autosampler with 20 µl loop, reverse phase Waters Nova-Pak CN 5 mm x 10 cm radial-pak column, SP 8450 UV/Vis detector and SP 4290 integrator. The mobile phase consisted of 60 % of 2.5 mM potassium dihydrogen phosphate buffer containing 5 mM of 1-octane sulfonic acid sodium (p*H* 6.0) and 40% acetonitrile. The flow rate was 1 ml/min. The detection was made at 254 nm. Area under peak was used as a measure of the concentration. The concentration vs area under the peak showed linearity ($r = 0.998$) for the range of 1–10 µg of the injected compounds with a detection limit of 0.2 µg of the injected sample.

The authors have conducted the animal studies in accordance with the guidelines set forth in the Declaration of Helsinki and The Guiding Principles in the Care and Use of Animals (DHEW Publication, NIH 80-23).

Results and Discussion

Synthesis of the soft ethyl (**4a**) and butyl (**4b**) phenyl-succinic analogs of atropine and a structural isomer of **4a** (**5**) was accomplished by following suitable synthetic routes to selectively obtain the appropriate isomers. The *in vitro* and *in vivo* activities (Table 1) and *in vitro* stability (Table 2) of the compounds is tabulated below. The mydriatic data is presented in Figures 1 and 2 and Table 1.

Table 1. *In vivo* and *in vitro* anticholinergic activity

Compound	pA_2*	Mydriatic activity in rabbits		
		AUC (6h) (treated)	AUC (6h) (untreated)	MRT (h)
1	8.95	12.4	7 40	40.0
2	7.85@	7.0	0.20	3.8
3	5.20	--	--	--
4a	6.50	6.5	0.15	4.1
5	6.10	5.7	0.40	3.3
4b	5.80	--#	--	--

* Guinea pig ileum assay (Ref. 10).
@ From Ref. 5.
# Insignificant mydriatic activity; MRT: mydriatic recovery time (time taken for the pupil to reach within 0.75 mm of the predrug size).

Table 2. *In vitro* stability studies

Compound	Half life (min)		
	Rat plasma	Rat liver (20%)	Human plasma
1	1315	324	1391
2	3	10	268
4a	26	72	630
4b	17	313	629

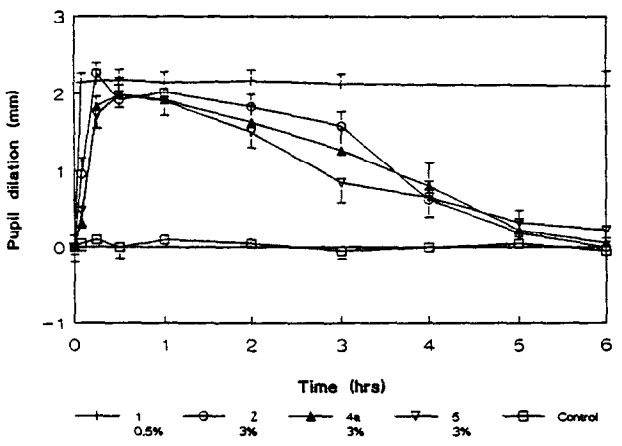


Figure 1. Mydriatic activity in **treated** rabbit eye. Each value is the mean of twelve readings. Error bars represent S.E.M.

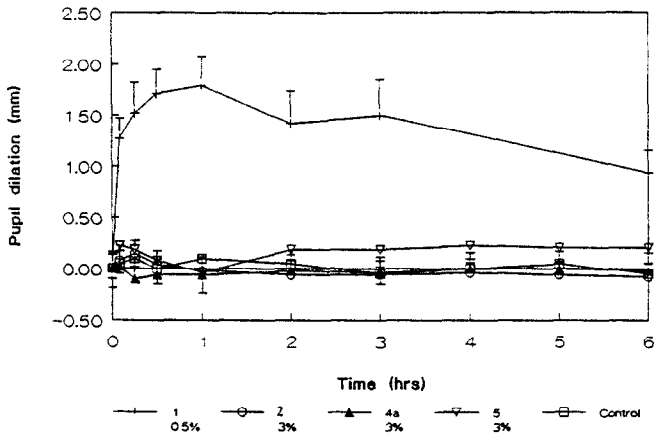


Figure 2. Mydriatic activity in **untreated** eye after unilateral administration. Each value is the mean of twelve readings. Error bars represent S.E.M.

The guinea pig ileum assay (Table 1) indicates that the phenylsuccinic analogs are at least a log unit less potent than the corresponding phenylmalonic analogs (**5**, **7**, **8**). Compound **5**, which has a longer bridge, is about 5 times less potent than its structural isomer, compound **4a**. Compound **4b**, which has a bulkier side chain than compound **4a**, is also less potent. This is consistent with the earlier reported observations (**7**). The carboxylate metabolite **3**, was one log unit less potent than the most active compound **4a**.

The mydriatic activity of **4a** was found to be comparable to atropine and **2** but the isomer, **5**, was slightly less active. Soft drug **4b** was inactive at concentrations up to 5%. Atropine elicited a very long mydriatic response compared to very short response with all the three soft drugs tested. This is consistent with the higher *in vitro* hydrolytic rates in biological media observed with the soft drugs (Table 2). The soft drugs on hydrolysis would yield the carboxylate metabolite **3** which is much less potent and thus effectively diminishing the action. Figure 2 shows the mydriatic response in the untreated eye after unilateral treatment. The untreated eyes of atropine treated animals showed dilation while the soft drug treated animals did not. These results indicate rapid systemic deactivation of the soft drugs, as the soft drug design predicted, which leads to the inactive carboxyl metabolite (**3**) and therefore no control eye dilation. Atropine which is not deactivated systemically dilates the untreated eye.

In vitro stability of atropine and soft drugs is presented in Table 2. In all the media tested, atropine has been found to be more stable compared to soft drugs. The increased hydrolytic lability of soft drugs would minimize their accumulation in the systemic circulation with a consequent avoidance of side effects. Both soft drugs **4a** and **4b**, on hydrolysis, produced carboxylate metabolite **3** quantitatively.

Compound **5** differs from compound **4a** in having an additional methylene group in the bridge (between tropine and aromatic moiety). The α-carbon in compound **4a** bears an ethyl acetate moiety compared to ethyl formate moiety in compound **5**. Thus these two compounds (**4a** and **5**) are in fact structural isomers. The original intention was to extend the concept of soft drug design by inclusion of a methylene group into the ester part of the phenylmalonic soft drugs (e.g. tematropium). This extension was thought to decrease the steric hinderance and thus promote hydrolytic rate possibly reducing the duration of mydriatic activity. Compound **5** was included in the study to examine the effect of lengthening the bridge part on the anticholinergic activity of the molecule. Compound **5** has been found to be less active than **4a** which reflects the significance of the length of the bridge or the interatomic distance between the basic tropane moiety and the bulky aromatic substituent. Similarly, compound **4b** which has a bulkier substituent has been found to be less active than **4a**. In turn, **4a** is less active than **2**, even though the mydriatic activity of **4a** and **2** are comparable. These results further confirm the observation that the hydrophobic pocket of the cholinergic receptor is of limited size.[11]

The short duration of mydriatic activity coupled with high hydrolytic lability make compound **4a** a candidate for further study.

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