Soft Drugs. 21. Design and Evaluation of Soft Analogs of Propantheline

G. BROUILLETTE, M. KAWAMURA, G. N. KUMAR, AND N. BODOR†

Abstract □ The soft drug approach was applied to synthesize seven soft analogs of propantheline, which by design display predictable and controllable decomposition to inactive metabolites. Their synthesis involved the quaternization of several different amine groups with the chloromethyl ester of 9-methylxanthen-9-carboxylic acid. The rates of disappearance were measured for all of the compounds and were found to be more rapid than that of propantheline bromide in a variety of chemical and biological media under in vitro conditions. One of the soft analogs was found to be equipotent with propantheline in an in vitro assay. This soft analog was found to be equipotent with propantheline, in vivo, in protecting the rats against indomethacin-induced gastric ulceration and in inducing mydriasis in rabbits on intravenous administration. The pupil sizes returned faster to predrug levels with the soft analog than with propantheline, indicating increased metabolic liability of the soft analog. The equipotency of this soft analog coupled with increased metabolic liability proves the rationality of the soft drug approach for the design of safer therapeutic agents with higher therapeutic indices.

Introduction

Soft drugs are defined as “biologically active, therapeutically useful chemical compounds (drugs) characterized by a predictable and controllable in vivo destruction (metabolism) to nontoxic moieties, after achieving their therapeutic role”.1 The soft drug approach combines structure–activity relationships with structure–metabolism relationships to design compounds with higher therapeutic indexes, the single most important characteristic of a drug. The soft analog approach is one of the methods used to design soft drugs.2 Soft analogs are compounds that have a dose similarity to known active drugs or bioactive compounds. However, they have a specific metabolically sensitive spot built into their structure to provide a one-step detoxification to metabolites which are inactive and nontoxic.

Bodor et al.2 applied the soft analog approach to design a new class of anticholinergic compounds. These compounds differ from the traditional anticholinergics in that only one carbon atom separates the quaternary nitrogen from the carbonyl oxygen, thus breaking the 7 Å rule. Shortening the alkyl bridge increased the hydrolytic rate of the new compounds. These anticholinergic agents were found to be as effective as or even more effective than atropine in both in vitro and in vivo studies with significantly shorter durations of anticholinergic action. This decreased duration of action is potentially important for attenuating systemic toxicity, since the amount of circulating active drug is minimized.

Propantheline bromide (Pro-Banthine) is a quaternary ammonium anticholinergic agent which has been widely used in clinical practice to inhibit gastric acid secretion in patients with peptic ulcer disease.1 Suppression of hyperhidrosis by the topical application of propantheline has also been reported.3 Allergic eczema, erythema, and severe contact dermatitis have been reported with the application of propantheline preparations.5,6 The primary metabolic products of propantheline have been reported to be xanthoic acid and its glucuronide.7 As with other anticholinergic drugs, the frequency and severity of side effects of propantheline are dose related. Infants, geriatric patients, and narrow-angle glaucoma patients are especially susceptible to adverse events associated with the compounds.8 Anticholinergics are known to inhibit eccrine sweating on topical application.9 Since their action could not be localized, the topical application of the traditional anticholinergics resulted in unwanted systemic anticholinergic activity, such as dry mouth, mydriasis, etc. In this study, soft analog design was applied to propantheline with the intention of developing soft drugs which are equipotent to the parent drug and display facile hydrolysis to an acid, an aldehyde, and an amine (all inactive compounds).

This paper describes the synthesis, chemical stability, hydrolytic stability in biological media, and in vitro anticholinergic activity of seven soft drugs based on propantheline and the in vivo activity of the most promising candidate.

Materials and Methods

All chemicals used were reagent grade. Acetylcholinesterase was obtained from Sigma Chemical Company. Other chemicals were obtained from Aldrich Chemical Company and solvents from Fisher Scientific. All melting points were recorded using a Fisher-J ohns melting point apparatus and are uncorrected. NMR data were recorded with a Varian T-90 NMR spectrometer and are reported in parts per million (δ) relative to tetramethylsilane. All quaternary compounds were dissolved in DMSO-d₆ and other compounds were dissolved in CDCl₃ for NMR analysis. The elemental analyses were carried out at Atlantic Microlab, Atlanta, GA, and are within ±0.4% of the calculated values. Thin layer chromatography was carried out using EM Science DC-plastic foil plates coated to a thickness of 0.2 mm with silica gel 60 containing a fluorescent (254 nm) indicator.

Synthesis (Scheme 1) 9-Methylxanthen-9-carboxylic acid (1)—Xanthene-9-carboxylic acid (9.94 g, 44 mmol) was dissolved in tetrahydrofuran (25 mL) and was added dropwise at −20 °C to a solution of lithium diisopropylamide [prepared from diisopropylamine (13.7 mL, 98 mmol), tetrahydrofuran (80 mL), and butyllithium (58 mL, 1.7 M solution in n-hexane)]. Hexamethyldiphosphoramide (9 mL, 50 mmol) was then added, and the mixture was stirred for 30 min. Methyl iodide (2.72 mL, 44 mmol) was then added. The reaction mixture was stirred for another 2 h and was allowed to equilibrate to room temperature. The solvent was evaporated, dilute hydrochloric acid (100 mL, 1 M) was added, and the mixture was twice extracted with ether. The organic phase was washed with water followed by saturated sodium chloride, then dried over anhydrous magnesium sulfate, filtered, and evaporated to yield a tan solid. Recrystallization from methanol yielded 7.27 g (68.9%) of pure compound (3).

Chloromethyl 9-Methylxanthen-9-carboxylate (2)—A mixture of 1 (2.4 g, 9.9 mmol), NaHCO₃ (3.14 g, 38 mmol), H₂O (10 mL), methylene chloride (30 mL) and the phase-transfer catalyst tetrabutylammonium hydrogen sulfate (TBA) (0.34 g, 1 mmol) was stirred vigorously at...
room temperature. A methylene chloride solution (2.5 mL) of chloromethyl sulfate (1.89 g, 11.5 mmol) previously prepared by the method of Binderup and Hansen\textsuperscript{10} was added dropwise over a 15 min period. The mixture was stirred for 1 h. The organic layer was separated, dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The crude ester was purified on a silica gel column (n-hexane:chloroform, 2:1), and the solvent was removed in vacuo. The resulting acetic acid was removed under reduced pressure. A compound of the amine. 1H NMR (CDCl\textsubscript{3}): δ 2.3–2.0 (m, 5H, 2CH\textsubscript{2} and CH). N-Methyldiisopropylamine\textsuperscript{11}—Diisopropylamine (65 mL) was added dropwise, with stirring, to a flask containing formic acid (100 mL). The mixture was stirred and placed at 70 °C until carbon dioxide gas no longer evolved. The solution was stirred at approximately 70 °C for 2 h. The mixture was filtered and concentrated under reduced pressure. The crude mixture was purified on a silica gel column (n-hexane:chloroform, 2:1), and the solvent was removed in vacuo. The resulting acetic acid was removed under reduced pressure. A saturated aqueous potassium hydroxide solution (20 mL) was added to the stock residue, and the mixture was then extracted with chloroform. The organic phase was separated, dried over anhydrous magnesium sulfate, and the solvent was removed in vacuo. The residue was vacuum distilled at 111 °C to yield 4.15 g of N-acetoxyquinuclidinide (69.4%). \textsuperscript{1}H NMR (CDCl\textsubscript{3}): δ 4.7–5.0 (m, 1H, CHO), 2.5–3.5 (m, 6H, 3CH\textsubscript{2}N), 2.1 (s, 3H, CH\textsubscript{3}), 1.2–2.0 (m, 5H, 2CH\textsubscript{2} and CH). N-Methylmorpholine\textsuperscript{11}—Diisopropylamine (65 mL) was added dropwise, with stirring, to a flask containing formic acid (100 mL). The mixture was stirred and placed at 70 °C until carbon dioxide gas no longer evolved. The solution was stirred at approximately 70 °C for 2 h. The mixture was filtered and concentrated under reduced pressure. The crude product was acidified with concentrated HCl (40 mL) and concentrated under reduced pressure. Potassium hydroxide pellets were added to neutralize the product and to cause it to separate from the aqueous solution. The compound was distilled at 111–112 °C, yielding 40 g (75%) of N-methylmorpholine. \textsuperscript{1}H NMR (CDCl\textsubscript{3}): δ 2.7–3.2 (septet, 2H, 2CH\textsubscript{2}), 2.2 (s, 3H, CH\textsubscript{3}), 0.9–1.3 (d, 12H, 4CH\textsubscript{3}). (Hydroxymethyl)-3-acetoxyquinuclidinium Chloride 9-Methylxan-thene-9-carboxylate (3)—A mixture of compound 2 (2 g, 7.4 mmol) and 3-acetoxyquinuclidine (0.86 mL, 7.4 mmol) in 15 mL of chloroform was gently refluxed overnight under a stream of dry nitrogen. The reaction mixture was washed with 1 N HCl (30 mL, four times) to remove any unreacted amine. The organic solvent was removed under vacuum, and the resulting oil was washed with ether to remove unreacted chloromethyl ester. Final purification was accomplished by using a Sephadex LH20 column with ethyl acetate/ethanol, 4:1, as the eluent to yield 1.9 g of white solid (30%). UV (methanol): 224, 252, and 284 nm. The synthesis of compounds 4–9 was accomplished by following the same method as described above with appropriate substitution of the amine.

**HPLC Method**—The mobile phase consisted of acetonitrile:water, 45:55, with final concentrations of 0.1% heptanesulfonic acid, 0.2% acetic acid, and 0.1% THF. The system consisted of a Hamilton reversed-phase C-18 (5 µm) column, a rhodamine detector, and a UV detector set at 254 nm. The flow rate was 1.5 mL/min. The soft quaternary compounds (3–9) had retention times between 2.4 and 4.4 min whereas the metabolite (1) had a retention time of 8.5 min.

**Chemical Stability**—The chemical stability of propantheline bromide and soft quaternary compounds (3–9) was tested in phosphate buffers (0.05 M; pH 1.75, 7.40, and 9.50). A buffer solution (2 mL) was equilibrated at 37 °C. At time 0, 50 µL of a 0.1 M stock solution of test compound in DMSO was added and mixed well. The solution was kept at 37 °C while being shaken. Samples (50 µL) were withdrawn at appropriate time intervals and immediately diluted with 450 µL of ice-cold acetonitrile and vortexed. Samples were kept refrigerated until analysis. The mixture was analyzed by HPLC for both the original compound and degradation products. The kinetics were followed for 4 half-lives.

**Stability in Biological Media**—The following biological media were used in the study: fresh human blood (withdrawn into a heparinized vial and stored on ice until needed); 80% human plasma (obtained from Civitan Regional Blood Center, Gainesville, FL); whole rat blood (obtained from male Sprague-Dawley rats by cardiac puncture and collected into heparinized syringe); 20% rat liver homogenate (supernatant obtained by homogenizing liver from Sprague-Dawley rats in pH 7.4, 0.1 M phosphate buffer and centrifugation); 20% rat intestinal homogenate (supernatant obtained by homogenizing rat intestine in pH 7.4, 0.1 M phosphate buffer and centrifugation); and acetylcholinesterase from bovine erythrocytes (10.8 units (20 mg) added to 20 mL of 0.1 M phosphate buffer of pH 7.4)

To the biological medium (2 mL) was added 10 µL of the stock solution of the compound, and the sample was mixed. The mixture was kept at 37 °C while being shaken. Samples (50 µL) were withdrawn at appropriate time intervals and immediately diluted with 450 µL of ice-cold acetonitrile to stop enzymatic reaction and vortexed. The supernatant after centrifugation was analyzed by HPLC for both the original compound and degradation products. The kinetics were followed for four half-lives.

**In Vitro Anticholinergic Activity**—An adult male Duncan-Hartley guinea pig was fasted for 24 h with free access to water and then sacrificed by exsanguination. A section of terminal ileum, approximately 20 cm long, was obtained and trimmed to remove connective tissue. The intestine was maintained in oxygenated Tyrode solution. A section of ileum (2 cm) was suspended in a jacketed organ bath containing 10 mL of Tyrode solution preequilibrated at 37 °C and oxygenated with 95% oxygen and 5% carbon dioxide. The tissue was attached via silk thread to a myograph under 0.5 g tension. The longitudinal contractions were monitored isometrically by a force displacement transducer, and signals were recorded on a desk-top physiograph.

The cumulative dose–response curves were then generated according to the method of Van Rossum,\textsuperscript{12} Response profiles to the agonist (carbachol) both in the absence and in the presence of varying concentrations of an antagonist (atropine or propantheline or soft quaternary compounds 3–9) were compared. The pA\textsubscript{2} value, an empirical parameter which defines the negative logarithm of the molar concentration of the antagonist which produces a 2-fold shift to the right of a concentration–response curve, was used as a measure of comparison between the affinities of atropine, propantheline, and soft quaternary analogs 3–9.

**In Vivo Anticholinergic Activity on Chemically-Induced Gastric Ulceration in the Rat (Propantheline vs 4)**—Male Sprague-Dawley rats (100–160 g) were fasted, with free access to water, for 18 h. The rats were randomly divided into three groups, with eight animals in each group. Three rats were used as fasting controls and received no drugs. A suspension of indomethacin (3.5 mg/mL), as well as solutions of propantheline (10 mg/mL) and compound 4 (10 mg/mL), was prepared using sterile water. Groups I, II, and III received indomethacin (30 mg/kg, 4 mL/kg) by intraperitoneal injection. Group I then immediately received sterile water (1 mL), group II received an aqueous solution of propantheline bromide (40 mg/kg), and group III received an equimolar aqueous solution of compound 4 (36 mg/kg) by gastric intubation. Five hours after drug administration, the rats were sacrificed by an ether overdose. The stomachs were removed immediately, opened along the greater curvature, and rinsed with distilled water. The total areas of ulcerative lesions were measured using a 10× dissecting microscope.

**In Vivo Anticholinergic Activity (Mydriasis) on Parenteral Administration into Rabbits (Propantheline vs 4)**—An aqueous solution of compound 4 (0.43 mg/kg) or propantheline bromide (0.5 mg/kg) was injected via the ear vein into three male New Zealand
The spectral, analytical, and physicochemical data of the compounds synthesized is presented in Table 1. Initial attempts to make chloromethyl esters of xanthene-9-carboxylic acid were unsuccessful due to decarboxylation. Hence a methyl group was substituted for hydrogen at C-9, which prevented decarboxylation. Thus compound 5, which has the same quaternary head as that of propantheline, is in fact a structural isomer of the lead compound due to deletion of a methylene group in the bridge component and the introduction of a methyl group at C-9. All the soft drugs were found to be >97% pure, as determined by HPLC.

All the compounds were extremely stable in pH 1.75 buffer (Table 2), with half-lives ranging from 28 to 1700 h. In contrast, the hydrolytic rates were significantly higher in both pH 7.40 (58–750 min) and 8.50 (25–170 min) buffers. Propantheline was found to be more stable than all the soft analogs at each pH tested. Compounds 3, 6, and 8 were the most hydrolytically labile compounds. These compounds, probably due to the presence of electron-withdrawing groups (or lack of electron-donating substituents), make the ester more susceptible to nucleophilic attack. On the other hand, compounds with alliphatic straight/branched-chain amines (5 and 9) as well as compounds with electron-donating substituents (4 and 7) have been found to have slower hydrolytic rates. Acetylcholinesterase is the enzyme responsible for the hydrolysis of acetylcholine. Interestingly, all soft drugs, which have only one carbon bridge, are better substrates for this enzyme than propantheline, which has a two-carbon bridge. The results of the chemical stability studies clearly established that the “soft” acyloxyalkyl linkage significantly influences the rate of hydrolysis. The major decomposition product of all the soft analogs was 9-methylxanthene-9-carboxylic acid. The decomposition product of propantheline was xanthene-9-carboxylic acid. The hydrolysis kinetics followed pseudo-first-order profiles.

The stability studies of soft analogs and propantheline were carried out in various biological media (Table 2). These studies provide useful information regarding the hydrolytic liability of the compounds synthesized. Propantheline was more stable than all the soft analogs in all the media tested. Just as in buffers, the major metabolic products of propantheline and the soft analogs were xanthene-9-carboxylic acid and 9-methylxanthene-9-carboxylic acid, respectively. Interestingly, compound 5, which has the same quaternary head (diisopropylmethylammonium) as propantheline, was the most stable of all the soft drugs. Compounds 3, 6, and 8 have shorter half-lives compared to other soft analogs. This is consistent with their stability profile in buffers. The hydrolytic rates were higher in human plasma (80%) than in whole human blood, probably indicating that erythrocyte binding of the compounds is significant. The rat liver and intestine homogenates (20%) also rapidly hydrolyzed the soft analogs. The overall results of the in vitro stability study demonstrate that the “soft” acyloxyalkyl linkage provides a metabolically sensitive spot which allows the facile decomposition of the soft analogs to inactive moieties. Hence, the soft analogs of propantheline meet the in vitro stability criterion for ideal soft drugs.

The in vitro anticholinergic activity was determined by the classical guinea pig ileum assay (Table 3). Initially, all the compounds were tested for their stability in Tyrode solution under the test conditions. All the compounds, including propantheline, were stable for 5.5 h with hydrolysis less than 10%. The results of this assay indicated that the soft

### Table 1

<table>
<thead>
<tr>
<th>No.</th>
<th>N\textsuperscript{R}\textsubscript{2}</th>
<th>Mp, °C</th>
<th>yield, %</th>
<th>NMR Data</th>
<th>Anal.</th>
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<tr>
<td>1</td>
<td>205–209</td>
<td>69</td>
<td>9.8–10.4 (bs, 1H, COOH)</td>
<td>C,H</td>
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<tr>
<td>2</td>
<td>55–57</td>
<td>91</td>
<td>5.5 (s, 2H, CH\textsubscript{2})</td>
<td>C,H,Cl</td>
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<tr>
<td>3</td>
<td>162–183</td>
<td>30</td>
<td>5.6 (s, 2H, CH\textsubscript{2}O), 4.8–5.1 (m, 1H, CHO), 2.9–4.1 (m, 6H, N(CH\textsubscript{2}O)), 2.2 (s, 3H, CH\textsubscript{3}CO), 1.6–2.5 (m, 5H, bicyclic)</td>
<td>C,H,N,Cl</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>166–168</td>
<td>85</td>
<td>5.2 (s, 2H, CH\textsubscript{2}O), 3.2–3.4 (m, 3H, CH\textsubscript{3}N and CH\textsubscript{3}), 3.1 (s, 3H, CH\textsubscript{3}N), 1.3–2.5 (m, 4H, (CH\textsubscript{2}\textsubscript{3})), 1.0–1.2 (d, 3H, CH\textsubscript{3})</td>
<td>C,H,N,Cl</td>
<td></td>
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<tr>
<td>5</td>
<td>170–180</td>
<td>5</td>
<td>5.2 (s, 2H, CH\textsubscript{2}O), 3.4–4.0 (septet, 2H, 2CH\textsubscript{3}N), 3 (s, 3H, CH\textsubscript{3}N), 1.2–1.4 (d, 6H, 2CH\textsubscript{3}O), 0.7–1.1 (d, 6H, 2CH\textsubscript{3}O)</td>
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<td>6</td>
<td>178–181</td>
<td>34</td>
<td>5.7 (s, 2H, CH\textsubscript{2}O), 3.6–4.1 (bm, 4H, 2CH\textsubscript{3}N), 3.1 (s, 3H, CH\textsubscript{3}N), 3–3.6 (bm, 4H, 2CH\textsubscript{3}O)</td>
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<td>7</td>
<td>161–163</td>
<td>25</td>
<td>10.2 (s, 1H, imidazole H-2), 7.1 (d, 1H, imidazole H-5), 6.9–7.5 (m, 9H, xanthene and imidazole H-4), 6.3 (s, 2H, CH\textsubscript{2}O), 4 (s, 3H, CH\textsubscript{3}N)</td>
<td>C,H,N,Cl</td>
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<tr>
<td>8</td>
<td>173–175</td>
<td>27</td>
<td>8.9–9.1 (d, 2H, pyridine H-2 and H-6), 8.4–8.7 (t, 1H, pyridine H-4), 7.8–8.2 (t, 2H, pyridine H-3 and H-5), 6.7 (s, 2H, CH\textsubscript{3})</td>
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<tr>
<td>9</td>
<td>173–175</td>
<td>65</td>
<td>5.4 (s, 2H, CH\textsubscript{2}O), 2.9–3.4 (g, 6H, 3CH\textsubscript{3}N), 0.9–1.3 (t, 9H, 3CH\textsubscript{3})</td>
<td>C,H,N,Cl</td>
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\textsuperscript{a} See structure for 3–9 in Scheme 1. \textsuperscript{b} Common signals: 6.9–7.4 (m, 8H, xanthene), 1.8 (s, 3H, CH\textsubscript{3}).

### Table 2

<table>
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<tr>
<th>Compound</th>
<th>pH 1.75</th>
<th>pH 7.40</th>
<th>pH 8.50</th>
<th>Rat Blood</th>
<th>Human Blood</th>
<th>Human Plasma (80%)</th>
<th>Rat Liver (20%)</th>
<th>Rat Intestine (20%)</th>
<th>AChEs (Bovine)</th>
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<tr>
<td>3</td>
<td>37 h</td>
<td>86</td>
<td>30</td>
<td>19</td>
<td>28</td>
<td>17</td>
<td>18</td>
<td>23</td>
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<tr>
<td>4</td>
<td>190 h</td>
<td>270</td>
<td>68</td>
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<td>75</td>
<td>55</td>
<td>110</td>
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<td>180</td>
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<td>5</td>
<td>1700 h</td>
<td>750</td>
<td>170</td>
<td>110</td>
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<td>160</td>
<td>610</td>
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<tr>
<td>6</td>
<td>28 h</td>
<td>58</td>
<td>25</td>
<td>10</td>
<td>20</td>
<td>15</td>
<td>6</td>
<td>17</td>
<td>50</td>
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<tr>
<td>7</td>
<td>320 h</td>
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<td>94</td>
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<td>63</td>
<td>54</td>
<td>150</td>
<td>140</td>
<td>180</td>
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<tr>
<td>Propantheline</td>
<td>b</td>
<td>1200</td>
<td>330</td>
<td>1600</td>
<td>370</td>
<td>370</td>
<td>880</td>
<td>610</td>
<td>1500</td>
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</tbody>
</table>

\textsuperscript{a} Each value is the mean of five readings. \textsuperscript{b} No decomposition observed for six-day period.
significant difference was found between the propantheline-of ulcerative lesions compared to the control group. No treatedanimalsshowedsignificantdifferenceintheformation
respectively. The propantheline-treated and compound
administration of indomethacin.16 When administered im-
rabbits. Gastriculcerationinratswaschemicallyinducedby
ative activity in rats and systemic anticholinergic activity
systems. Compound
acid, was tested and found to be inactive even at very high
1,2-dimethylpyrrolidine
The order of anticholinergic potency of the soft analogs was
amine
622
/Journal of Pharmaceutical Sciences
p
quaternaryanalogsarepotentanticholinergiccompounds. The
valuesreportedinliterature. Compound 4 (1,2-dimethylpyr-
rolidinium soft analog) was the most potent anticholinergic
of all the soft analogs of this study, with a pA2 value of 8.70.
The order of anticholinergic potency of the soft analogs was
1,2-dimethylpyrrolidine > 3-acetoxyquinuclidine > triethyl-
amine > pyridine > N-methyldiisopropylamine > N-methyl-
morpholine > N-methylimidazole. This order of potency is
roughly identical to the potency of an analogous series of
anticholinergic agents based on α-cyclopentylphenylacetic
acid.2 The metabolic product, 9-methylxanthene-9-carboxylic
acid, was tested and found to be inactive even at very high
concentrations.

The in vivo activity studies were conducted in two test
systems. Compound 4, the most potent compound of the
series, and propantheline were evaluated for their antulcer-
active activity in rats and systemic anticholinergic activity
(mydriatic response as the indicator of systemic activity) in
rabbits. Gastric ulceration in rats was chemically induced by
administration of indomethacin.16 When administered im-
mediately after the ulcerative agent, the anticholinergic
compounds were shown to inhibit the formation of lesions. The
ulcerative lesions in rats of control, propantheline-treated and
compound 4 treated groups were 8.69 ± 1.58, 2.31 ± 0.65 and
2.38 ± 0.76 mm2 (mean ± standard error of the mean),
respectively. The propantheline-treated and compound 4
treated animals showed significant difference in the formation
of ulcerative lesions compared to the control group. No
significant difference was found between the propantheline-
treated animals and compound 4 treated animals when
analyzed by Student's t-test. This proved that the soft
quaternary analog 4 is as effective as propantheline as an
anticholinergic under in vivo conditions.

Another in vivo activity experiment was performed by
intravenous injection of equimolar doses of propantheline and
compound 4 to rabbits. Then the mydriatic activity (an
anticholinergic response) was followed as a measure of the
systemic persistence of the administered drugs (Figure 1). The
mydriatic activity persisted for up to 4 h in the propantheline-
treated animals compared to less than 2 h in the soft analog
4 treated animals. At 2 h, a significant difference in dilation
of the pupil was observed between propantheline-treated and
compound 4 treated animals (P < 0.05). The results of this
experiment demonstrated that the soft quaternary analog 4
elicit a mydriatic response to the same degree as the parent
compound, i.e., propantheline, but with a shorter duration.
The reduction in duration is due to the increased systemic
hydrolytic liability of the compound, thus fulfilling the impor-
tant criterion intended in the soft drug design. The 1.5–2 h
mydriatic activity of 4 suggests that its enzymatic hydrolysis
in the eye is somewhat slower than in the general circulatory
system.

Table 3—In Vitro Anticholinergic Activity Determined by Guinea Pig Ileum
Assay

<table>
<thead>
<tr>
<th>Compound</th>
<th>pA2</th>
<th>r</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>8.25</td>
<td>0.976</td>
<td>-0.83</td>
</tr>
<tr>
<td>4</td>
<td>8.70</td>
<td>0.998</td>
<td>-1.01</td>
</tr>
<tr>
<td>5</td>
<td>7.86</td>
<td>0.988</td>
<td>-1.23</td>
</tr>
<tr>
<td>6</td>
<td>7.66</td>
<td>0.993</td>
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</tr>
<tr>
<td>7</td>
<td>7.60</td>
<td>0.980</td>
<td>-0.90</td>
</tr>
<tr>
<td>8</td>
<td>7.88</td>
<td>0.939</td>
<td>-0.68</td>
</tr>
<tr>
<td>9</td>
<td>8.12</td>
<td>0.998</td>
<td>-1.11</td>
</tr>
<tr>
<td>Propantheline</td>
<td>8.93</td>
<td>0.989</td>
<td>-1.24</td>
</tr>
<tr>
<td>Atropine</td>
<td>9.20</td>
<td>0.984</td>
<td>-0.80</td>
</tr>
</tbody>
</table>

Values represent average of nine experiments.

Conclusions

Compound 4 fulfills the requirements of an ideal soft drug
candidate, because it retains the potency of the lead compound
while being more metabolically labile. 4 is equipotent with
propantheline in both in vitro and in vivo anticholinergic
assays. It is also less stable compared to propantheline in
both the buffers and biological homogenates. All the soft
anals synthesized and examined in this study were rapidly
hydrolyzed to yield inactive metabolites. These soft analogs
are likely to exert their pharmacological action only near the
site of administration (e.g., topically on the skin), where they
are likely to undergo facile metabolism to inactive metabolites.
The equipotency of the soft analog 4 coupled with increased
metabolic liability proves that the soft drug approach is a
rational method to design safer therapeutic agents with higher
therapeutic indices.

References and Notes

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