The synthesis and structure–activity-relationship (SAR) for a series of N-substituted pipеразинyl carbamoyl acetyl 18—26 derivatives of tetrahydropapaverine have been carried out. The general synthetic methods of carbamoyl tetrahydropapaverine analogues involve N-substituted pipеразинyl and carbamoyl imidazole tetrahydropapaverine as starting materials. Another route for synthesizing these compounds, involving the formation of carbamoyl imidazole pipеразинyl has also been explored. Acylation of tetrahydropapaverine followed by substitution with various pipеразинyl moieties afforded the acetyl tetrahydropapaverine derivatives. Variously substituted pipеразинyls have been used to monitor the effect of electron releasing and electron withdrawing substituents upon the antispasmodic activity of the molecules. Effect of varying electron densities on the antispasmodic activity, by altering the position of these groups on the benzene ring has also been monitored. Pharmacological methods involve the in vitro antispasmodic activity studies on a freshly removed guinea pig ileum using a force displacement transducer amplifier connected to a physiograph. Among the analogues synthesized in the present study, a promising compound 7, a potent muscle relaxant as compared to papaverine has been obtained.

Key words isоquinoline; antispasmodic; pipеразинyl; papaverine; urea

An abnormal spasm in gastrointestinal, uretral and uterine smooth muscles is the most frequent cause of abdominal discomfort. The causative factor for the spasm is not clearly understood. Generally, drugs like anticholinergics and nonspecific smooth muscle relaxants have been used clinically to relieve the symptoms. Atropine-like antispasmodics do not show selectivity towards a particular muscarinic receptor subtype and thereby they also show several side effects. Their ready penetration of the central nervous system (CNS) can give rise to undesirable CNS effects. Synthesis of quaternary ammonium salts of the free bases serves as a useful technique to avoid or minimise these side effects. However, absorption after oral administration is greatly reduced in spasmyotics containing the quaternary ammonium group.

Few drugs, chemically unrelated to atropine show a direct relaxant effect on various smooth muscle types, e.g. papaverine, a benzyllsoquinoline alkaloid, is a well known smooth muscle relaxing agent with multiple activities such as cyclic nucleotide phosphodiesterase (PDE) inhibitor and Ca2+ channel blocker via specific binding to the benzothiazepine receptor site in the Ca2+ channel. Because of these multiple mechanisms it is useful as a non-specific spasmyotic agent but cannot be put to more specific use in cardiovascular and abdominal disorders. However, if we take the chemical structure of papaverine as a model, we may find that small structural differences could lead to more useful compounds.

Tetrandrine, a bis-benzyltetrahydroisoquinoline alkaloid, blocks inward calcium current most likely by acting at the benzothiazepine site of L-channel. It inhibits [3H]-diltiazem binding competitively, partly inhibits [3H]-verapamil binding and stimulates [3H]-nitrendipine binding to cardiac sarcolemmal membranes. It is reported that substituted tetrahydroisoquinolines which are active in inhibiting specific [3H]-nitrendipine binding to rat cerebral cortical membranes, are also able to inhibit the KCl-induced contraction of rat aorta. Also increase in the size of N-alkyl substituents from acetyl to propionyl, nicotinyl and finally 2-(1-piperidine)-acetyl and presence of carbonyl functionality increases the antispasmodic activity. It is reported that 4-aminopiperidine ureas act as potent selective agonists of the human \( \beta_1 \)-adrenergic receptor. Substituted ureas have also been of recent interest due to appearance of this functionality in drug candidates such as human immunodeficiency virus (HIV) protease inhibitors, FKBP12 inhibitors, CCKB-receptor antagonists and endothelin antagonists. Recently, Okuyama et al. have reported a novel Ca2+ channel blocker T-477 [(R)-(+) 2-(4-chlorophenyl)-2,3-dihydro-4-diethylaminoacetyl-4H-1,4-benzothiazine hydrochloride] which prevents brain edema in rats. We envisaged that the two spasmyotic groups i.e. the tetrahydroisoquinoline moiety and the urea/amide functionality, if brought together in a single molecule can produce very good antispasmodic agents. Considering these factors and in continuation to our earlier efforts to find out potent smooth muscle relaxants, we synthesized the N-substituted pipеразинyl carbamoyl 7—15 and N-substituted pipеразинyl acetyl 18—26 derivatives of tetrahydropapaverine and evaluated them for their antispasmodic activity.

Results and Discussion

Compounds 7—15 and 18—26 were synthesized using tetrahydropapaverine 5 as the starting material. Tetrahydropapaverine efficiently displaced one of the imidazole rings of carboxylateimidazole to give Tetrahydropapaverine carbamoyl imidazole 6 on refluxing it with carboxylateimidazole (CDI) in tetrahydrofuran (THF) for 24 h. The carbamoyl imidazole 6 obtained, did not require further purification for use in subsequent steps. Stirring of 6 with MeI (4 molar eq) in dichloromethane for 12 h at room temperature produced the imidazolium salt quantitatively. This imidazolium salt again required no additional purification for final conversion to ureas. Although the salt is hygroscopic in nature, but it could be stored for several days without detectable decomposition. Addition of 1-substituted pipеразинyls, morpholine to a solution of imidazolium salt in dichloromethane with triethyl-
amine at room temperature afforded tetrasubstituted ureas 7—15 (Chart 1). These compounds were purified by column chromatography and obtained in 75—85% yield. The activation of the leaving imidazole ring was found to be necessary for the formation of desired ureas since the carbamoyl imidazoles were unreactive towards piperazines even under refluxing conditions for prolonged periods. Addition of triethylamine (1 molar eq) resulted in improved yields of ureas.

Another route, where the imidazole ring of carbonyldimidazole is displaced by piperazine [1-(4-fluorophenyl)piperazine] instead of tetrahydropapaverine was also tried. Cations of carbamoyl imidazolium salt of piperazine carbamoyl imidazole 16 on reaction with tetrahydropapaverine gave 10 (Chart 2) in equally good yield as obtained by the method reported in Chart 1. However, this approach was not utilized for the synthesis of other derivatives in the series as it included the formation of separate cationic carbamoyl imidazolium salt for each derivative. The compounds 7—15 and 18—26 were characterized on the basis of their 1H-NMR, mass spectral data, IR absorption methods and elemental analysis. The biological data obtained for these compounds is reported in Table 1.

Amide 3 was prepared by the condensation of homoveratryl amine 1 and homoveratric acid 2 in xylene with azeotropic removal of water. Cyclization of 3 to 3,4-dihydroisoquinoline 4 was achieved by Bischler–Napierlaski fashion, in refluxing toluene with phosphorous oxychloride. Reduction of 3,4-dihydroisoquinoline with sodium borohydride in methanol resulted in the formation of tetrahydropapaverine 10 (Chart 1).

Antispasmodic activity studies of compounds 7—15 and 18—26 were performed on guinea pig ileum. Acetylcholine was used to induce sustained contraction in guinea pig ileum. When the contraction response to acetylcholine reached a steady level, papaverine was added cumulatively. Papaverine inhibited the acetylcholine-induced contraction in a concentration-dependent manner. Similarly, acetylcholine-induced contraction inhibition by tetrahydropapaverine derivatives 7—15 and 18—26 was studied. Dose–response curves were obtained for papaverine and the derivatives. The results showed that the test compounds 7—15 and 18—26 more potently inhibited the acetylcholine-induced contractions than papaverine. Table 1 summarizes the concentration of pa-
paverine and test compounds producing 50% relaxation (IC_{50}) of acetylcholine induced contraction. IC_{50} values were determined graphically from dose–response curves obtained by measuring antispasmodic activity at different concentrations of the compounds in duplicate of triplicates.

As compared to paverine the \(N\)-substituted carbamoyl \(7\–15\) and acetyl \(18\–26\) derivatives of tetrahydropapaverine exhibited better antispasmodic activities. Tetrasubstituted

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**Table 1. Structural Formula of Tetrahydropapaverine Derivatives and Their IC_{50} in Inhibiting Acetylcholine Induced Contraction in Guinea Pig Ileum**

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>IC_{50} ((\mu M))</th>
<th>Compound</th>
<th>R</th>
<th>IC_{50} ((\mu M))</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.30</td>
<td>18</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.35</td>
<td>19</td>
<td>0.81</td>
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<tr>
<td>9</td>
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<td>20</td>
<td>1.07</td>
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<td>21</td>
<td>1.13</td>
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</tr>
<tr>
<td>11</td>
<td>2.81</td>
<td>22</td>
<td>3.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
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<td>24</td>
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</tr>
<tr>
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<td>3.06</td>
<td>25</td>
<td>6.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>3.01</td>
<td>26</td>
<td>6.10</td>
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</tr>
</tbody>
</table>

*IC_{50} of paverine=7.31 \(\mu M\). IC_{50} were calculated using a mean of at least 3 measurements (all duplicates) for 6 concentrations in the range 0.1–20 \(\mu M\).*
ures 7—15 were found to be more potent as compared to amides 18—26. Among the tetrasubstituted ureas, the presence of electron withdrawing groups chloro and fluoro at meta or para position of their respective phenyl rings in compounds 7—10 lead to more potent compounds as compared to 14 and 15 having electron releasing methyl group at meta and para position of phenyl ring. The compounds not having aromatic ring on N-substituent 11 and 12 decreased the antispasmodic activity in comparison to compounds possessing aromatic ring like 7—10. Among the compounds having electron withdrawing substituents at the meta and para positions, the meta isomers 7 and 9 exhibited better activity in comparison to the corresponding para isomers 8 and 10. However, compound 14 having electron releasing methyl group at meta position was found to be less active than 15 having the methyl group at para position.

Considering the structure of T-477 and pirenzipine (1) (a cholinomimetic, selective to M1 receptors), we envisaged that compounds 7—15, these compounds exhibited a decline in the antispasmodic activity. Removal of solvent under vacuum gave a viscous oil which was dissolved in CH2Cl2 (100 ml), and washed twice with 100 ml portions of water. The organic layer was dried with anhydrous Na2SO4, filtered and concentrated under vacuum to yield the required tetra-substituted urea as an oil which was column chromatographed (ethylacetate–hexane) to obtain pure 7 (3.14 g, 84%) mp: 125—127 °C. 1H-NMR (CDCl3, 300 MHz): δ: 2.75 (8H, m), 2.97 (2H, m), 3.21 (4H, m), 3.60 (3H, s), 3.81 (6H, s), 3.83 (3H, s), 5.10 (1H, t, J=6.0 Hz), 6.29 (1H, s), 6.51 (1H, s), 6.73 (3H, s), 6.88 (4H, m). IR (KBr) cm−1: 1680. MS (m/z): 550 (M+H)1. Anal. Calcd for C24H27N3O5: C, 65.88; H, 6.22; N, 9.60. Found: C, 65.74; H, 6.60; N, 7.64. 1H-NMR (CDCl3, 300 MHz): δ: 2.98 (2H, m), 3.24 (4H, m), 3.70 (3H, s), 3.83 (6H, s), 3.85 (3H, s), 5.11 (1H, t, J=6.0 Hz), 6.29 (1H, s), 6.59 (1H, s), 6.72 (3H, m), 6.91 (4H, m). IR (KBr) cm−1: 1634. MS (m/z): 550 (M+H)1. Anal. Calcd for C24H27N3O5Cl: C, 67.74; H, 6.60; N, 7.64. Found: C, 67.71; H, 6.63; N, 7.76.

Conclusion

N-Substituted tetrahydropapaverine derivatives showed better antispasmodic activity in comparison with papaverine in guinea pigs in preliminary screenings. Tetrasubstituted ureas were found to be more active than trisubstituted urea derivatives. Presence of electron withdrawing groups and aromatic ring in the N-substituents increased the activity. Structure activity relationship studies revealed the importance of chloro and fluoro group at the 3 or 4 positions of phenyl ring. Compound 7 was found to be the most potent antispasmodic agent.

Experimental

Melting points are recorded in open capillary tubes on Büchi melting point B-540 instrument and are uncorrected. Solvent system used throughout the experimental work for running TLC plates was ethylacetate–hexane. The 1H-NMR spectra were recorded in CDCl3, as solvent (using TMS as internal standard) on a Bruker Avance Spectrospin 300 instrument at 300 MHz. Mass spectra were run on a MALDI Kratos Analytical Compact SEQ mass spectrometer using α-cyano-4-hydroxy cinnamic acid (4-HCCA) as matrix under positive linear reflectance mode and JEOL JMS-DX 303 mass spectrometer. IR spectra were recorded using KBr discs on a Shimadzu FTIR-8300 spectrophotometer. Elemental analysis was carried out on Heraeus Rapid CHN analysers.

1-(3,4-Dimethoxybenzyl)-2-carbamoylimidazol-1-yl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (12) Synthesized in a manner similar to that described for 7. The product was obtained as a white solid (80%) mp: 90—91 °C. 1H-NMR (CDCl3, 300 MHz): δ: 2.77 (8H, m), 2.97 (2H, m), 3.21 (4H, m), 3.60 (3H, s), 3.81 (6H, s), 3.83 (3H, s), 5.10 (1H, t, J=6.0 Hz), 6.29 (1H, s), 6.51 (1H, s), 6.73 (3H, s), 6.88 (4H, m). IR (KBr) cm−1: 1680. MS (m/z): 550 (M+H)1. Anal. Calcd for C24H27N3O5Cl: C, 67.74; H, 6.60; N, 7.64. Found: C, 67.71; H, 6.63; N, 7.76.

1-(3,4-Dimethoxybenzyl)-2-carbamoyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (14) Synthesized in a manner similar to that described for 7. The product was obtained as pale yellow solid (80%) mp 90—91 °C. 1H-NMR (CDCl3, 300 MHz): δ: 2.38 (3H, s), 2.59 (8H, s), 2.90 (2H, m), 3.08 (4H, m), 3.82 (3H, s), 3.85 (6H, s), 3.86
(3H, s), 5.65 (1H, t, J = 6.0 Hz), 6.28 (1H, s), 6.50 (1H, s), 6.62 (3H, m), 6.70 (4H, m). IR (KBr) cm⁻¹: 1679. MS (m/z): 650 (M⁺H⁺). Anal. Calc'd for C₂₇H₄₁N₃O₅Cl: C, 66.25; H, 6.60; N, 7.24. Found: C, 66.31; H, 6.51; N, 7.31.

1-(3,4-Dimethoxybenzyl)-2-[4-(3-fluorophenyl)piperazine-1-yl]carbamoyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (18) A solution of 1-(3-chlorophenyl)piperazine (0.97 g, 5.5 mmol) in DMF (10 ml) was added slowly to a solution of 17 (2 g, 47 mmol) and activated K₂CO₃ (2 g, 14.4 mmol) in DMF (15 ml). The mixture was stirred for 3 h at 60 °C. After the completion (TLC) of reaction, stirred the mixture reaction with water (500 ml) and extracted with ethyl acetate (3 × 50 ml). The organic layer was washed with brine and dried over Na₂SO₄. It was finally concentrated and chromatographed on silica gel using ethylacetate–hexane (1:10) as eluent. The product was obtained as a gummy material (81%). 1H-NMR (CDCl₃, 300 MHz): 2.26 (8H, m), 2.59 (2H, m), 2.90 (4H, m), 3.02 (4H, m), 3.25 (2H, s), 3.68 (3H, s), 3.85 (3H, s), 5.67 (1H, t, J = 6.0 Hz), 6.24 (1H, s), 6.43 (1H, s), 6.46 (1H, t, J = 6.0 Hz), 6.90 (4H, m), 7.22 (4H, m). IR (KBr) cm⁻¹: 1634. MS (m/z): 564 (M⁺H⁺). Anal. Calc'd for C₂₇H₃₇N₃O₅: C, 68.18; H, 6.79; N, 7.45. Found: C, 68.05; H, 6.61; N, 7.59.

1-(3,4-Dimethoxybenzyl)-2-[4-(4-fluorophenyl)piperazine-1-yl]carbamoyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (21) A solution of toluene (23 g, 32 mmol) in THF (50 ml) was added 1-(4-fluorophenyl)piperazine (20.77 g, 127.4 mmol) in THF (50 ml). The mixture was stirred at room temperature for 4 h. After the completion (TLC) of reaction, evaporated off the solvent, diluted the residue with water (150 ml) and extracted with ethyl acetate (3 × 50 ml). The organic layer was washed with brine and dried over Na₂SO₄. It was finally concentrated and chromatographed on silica gel using ethylacetate–hexane (1:10) as eluent. The product was obtained as a gummy material (83%). 1H-NMR (CDCl₃, 300 MHz): 2.22 (8H, m), 2.67 (8H, m), 2.87 (2H, m), 3.04 (4H, m), 3.25 (2H, s), 3.68 (3H, s), 4.00 (2H, t, J = 6.0 Hz), 7.13 (2H, d, J = 6.0 Hz). IR (KBr) cm⁻¹: 1695. MS (m/z): 274 (M⁺). Anal. Calc'd for C₂₇H₃₇N₃O₅: C, 61.30; H, 5.51; N, 20.42. Found: C, 61.21; H, 5.59; N, 20.53.

1-(3,4-Dimethoxybenzyl)-2-[4-(4-fluorophenyl)piperazine-1-yl]carbamoyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (10) Using 16 to a solution of 16 (1.86 g, 6.8 mmol) in dichloromethane (15 ml) was added 1-(4-fluorophenyl)piperazine-1-carbamoylimidazole (12.2 g, 68 mmol) and triethylamine (0.95 ml, 6.8 mmol). The mixture was stirred at room temperature for 24 h, then washed with 1.0 ml HCl, the organic layer dried over anhydrous Na₂SO₄, filtered and concentrated under vacuum to yield the required product as an oil which was column chromatographed on silica gel (ethylacetate–hexane) to obtain pure 10 (3.05 g, 82%).

1-(3,4-Dimethoxybenzyl)-2-chloroacetetyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (17) To a solution of tetrahydrodropavine 5 (18.86 g, 55 mmol) and triethylamine (11.5 ml, 82 mmol) in dry dichloromethane (50 ml), chloroacetyl chloride (7.24 g, 64 mmol) was added slowly. During addition the reaction mixture was kept in ice. After addition the mixture was stirred at room temperature for 4 h. After the completion (TLC) of reaction, evaporated off the solvent, diluted the residue with water (150 ml) and extracted with ethyl acetate (3 × 50 ml). The collective organic portions were washed with brine and dried over Na₂SO₄. It was finally concentrated and chromatographed on silica gel using ethylacetate–hexane (1:10) as eluent. A final recrystallization from ethylacetate–hexane (1:10) afforded the product as a white solid (18.74 g, 81%) mp 98—100 °C. 1H-NMR (CDCl₃, 300 MHz): 2.76 (2H, m), 3.19 (4H, m), 3.74 (2H, s), 3.87 (6H, s), 3.91 (3H, s), 5.56 (1H, t, J = 6.0 Hz), 6.24 (1H, s), 6.53 (1H, s), 6.75 (1H, s), 7.67 (3H, m) (IR (KBr) cm⁻¹: 1642.9. MS (m/z): 420 (M⁺H⁺). Anal. Calc'd for C₂₇H₃₇N₃O₅: C, 66.25; H, 6.60; N, 7.24. Found: C, 66.17; H, 6.54; N, 7.29.

1-(3,4-Dimethoxybenzyl)-2-[4-(4-methylphenyl)piperazine-1-yl]carbamoyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (19) A solution of 1-(3-chlorophenyl)piperazine (0.97 g, 5.5 mmol) in DMF (10 ml) was added slowly to a solution of 17 (2 g, 47 mmol) and activated K₂CO₃ (2 g, 14.4 mmol) in DMF (15 ml). The mixture was stirred for 3 h at 60 °C. After the completion (TLC) of reaction, diluted the reaction mixture with water (500 ml) and extracted with ethyl acetate (3 × 50 ml). The collective organic portion was washed with brine and dried over Na₂SO₄. It was finally concentrated and chromatographed on silica gel using ethylacetate–hexane as eluent. A final recrystallization from ethylacetate–hexane (20:70) afforded the product as a white solid (18.74 g, 81%) mp 98—100 °C. 1H-NMR (CDCl₃, 300 MHz): 2.60 (8H, m), 2.87 (2H, m), 3.02 (4H, m), 3.25 (2H, s), 3.65 (3H, s), 3.86 (6H, s), 3.87 (6H, s), 5.66 (1H, t, J = 6.0 Hz), 6.22 (1H, s), 6.46 (1H, s), 6.61 (3H, m), 6.81 (4H, m). IR (KBr) cm⁻¹: 1685. MS (m/z): 580 (M⁺H⁺). Anal. Calc'd for C₂₇H₄₁N₃O₅Cl: C, 66.25; H, 6.60; N, 7.24. Found: C, 66.17; H, 6.54; N, 7.29.
tration–response were repeated in the presence of increasing concentrations of the standard antagonist papaverine (0.1—20 μM). After a further control experiment (concentration-response curve of ACh) the test compounds 7—15 and 18—26 were measured. All substances were incubated 3 min prior to the cumulative addition of ACh; after each experiment all substances were carefully washed out. For each test substance a new ileum preparation was used. Test compounds were dissolved in 46% EtOH to yield solutions (0.1—20 μM). The antispasmodic activity was assessed as a percent reduction over the initial value. The IC50 values were obtained from individual experiments with 3 to 5 different concentrations of test compounds. As EtOH in the concentration used also inhibits ACh-induced contractions the antispasmodic activity of EtOH was measured and subtracted from the values obtained with test compounds.

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References