PUBLICATIONS
Synthesis of some 2-substituted-6-phenyl- and 7-phenyl-thieno[3,2-d]pyrimidin-4(3H)-ones

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Received 13 April 1993; revised and accepted 28 November 1993

The title compounds, 7a-j and 8a-c, have been prepared through cyclocondensation of the corresponding thiophene o-aminocarbonyls (5a and 5b) with a variety of nitriles in the presence of dry hydrogen chloride gas.

Antihyperlipaemic activity has been reported in a few thieno[2,3-d]pyrimidine-2-propionic acids (1) and some 2-mercaptothieno[2,3-d]pyrimidin-4(3H)-ones (2). This laboratory has been engaged in the synthesis of thieno[2,3-d]primidines for biological evaluation. Earlier, we have reported the synthesis and biological evaluation of a series of 2-substitutedthieno[2,3-d]pyrimidin-4(3H)-ones (3) for antihyperlipaemic activity. One of the compounds, LM/1554 (R1, R2 = -(CH2)4-, X = Cl) has been the subject of extensive study as a potential antihyperlipaemic agent. In order to complete the structure activity relationship (SAR) studies of the antihyperlipaemic thienopyrimidin-4-ones, it was thought of interest to investigate into the synthesis of the hitherto unexplored thieno[3,2-c]pyrimidin-4(3H)-ones (4), isomeric with thieno[2,3-d]pyrimidin-4(3H)-ones, for their pharmacological screening.

Our approach to the synthesis of the target compounds, 2-substituted-6-phenylthieno[3, 2-d]pyrimidin-4(3H)-ones (7) and 2-substituted-7-phenylthieno[3,2-d]pyrimidin-4(3H)-ones (8) essentially involved the dry HCl gas catalysed condensation of a variety of nitriles with the corresponding o-amino carbonyl-thiophenes, 3-amino-2-carbothoxy-5-phenylthiophene (5a) and 3-amino-2-carbothoxy-4-phenylthiophene (5b) (Scheme I).

3-Amino-2-carbothoxy-5-phenylthiophene (5a) was prepared by Dickmann condensation of β-chlorocinnamonic acid (11) with equimolar quantities of thioglycolic ester (12) in sodium ethoxide (Scheme II). β-Chlorocinnamonic acid was prepared by a slight modification of the reported method involving the Vilsmeier-Haack chloroformylation of acetophenone (9) to the 3-phenyl-3-chloro-2-propenium salt (10). The salt 10 was heated with a solution of hydroxylamine hydrochloride in DMF instead of using solid hydroxylamine hydrochloride. This modification increased the yield of the β-chlorocinnamonic acid (11) by 30-35% than the reported yields (Scheme II).

The other thieno o-aminocarbonyl, 3-amino-2-carbothoxy-4-phenylthiophene (5b) was prepared by the base catalysed condensation of α-(hydroxy-methylene)phenylacrylonitrile (15) with ethyl thioglycolate. The nitrile 15 was in turn synthesised through the base catalysed reaction of phenylacetonitrile (13) with ethyl formate (Scheme III).

Thiophene o-aminocarbonyl (5a) was condensed with various alkyl- and aryl-nitriles (6a-j) in the presence of...
dry HCl to afford a series of 2-substituted-6-phenyl-thieno[3,2-d]pyrimidin-4(3H)-ones (7a-j) (Table I). The reaction consists of passing a stream of dry hydrogen chloride gas through an equimolar mixture of the reactants in dry dioxane for 12 hr at ambient temperature (Scheme IV).

When dry HCl gas was bubbled through the reaction mixture containing equimolar quantities of 5a and ethyl cyanoacetate (6d) in dioxane over a period of 12 hr at

![Scheme II](image)

![Scheme III](image)

**Table I.—Physical, analytical and spectral data of 2-substituted-6-phenylthieno[3,2-d]pyrimidin-4(3H)-ones (7a-j)**

<table>
<thead>
<tr>
<th>Compd</th>
<th>R</th>
<th>m.p.(°C) (Solvent)*</th>
<th>Yield (%)</th>
<th>Mol. formula</th>
<th>Found (%) (Calc.)</th>
<th>¹H NMR* (δ, ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>CH₃</td>
<td>308-10 (E-C)</td>
<td>45b</td>
<td>C₁₃H₁₆N₂O₅S</td>
<td>64.50 3.97 11.67</td>
<td>2.4(s,3H,2-CH₃), 7.5(m,</td>
</tr>
<tr>
<td>7b</td>
<td>CH₂Cl</td>
<td>268-70 (E-C)</td>
<td>40</td>
<td>C₁₃H₁₄ClN₂O₅</td>
<td>56.27 3.26 10.60</td>
<td>1.47-1.54(t,3H,COOH₂CH₂), 7.3-7.75(m,6H,Ar-H and H-7)</td>
</tr>
<tr>
<td>7c</td>
<td>CO₂C₂H₅</td>
<td>228-30 (B-C)</td>
<td>25</td>
<td>C₁₃H₁₄N₂O₅S</td>
<td>59.60 3.97 9.10</td>
<td>2.74-2.94(2H,COOH₂CH₂), 7.2-7.8(m,6H,Ar-H and H-7)</td>
</tr>
<tr>
<td>7d</td>
<td>CH₂CO₂C₂H₅</td>
<td>208-10 (C-PE)</td>
<td>38</td>
<td>C₁₃H₁₄N₂O₅S</td>
<td>61.42 4.63 9.20</td>
<td>1.35-1.55(t,3H,CH₂COOH₂CH₂)</td>
</tr>
<tr>
<td>7e</td>
<td>CH₃CH₂Cl</td>
<td>250-52 (E-C)</td>
<td>41</td>
<td>C₁₅H₁₆N₂OS</td>
<td>58.01 4.08 9.40</td>
<td>7.3-7.75(m,6H,Ar-H and H-7)</td>
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<tr>
<td>7f</td>
<td>C₆H₅N</td>
<td>290-92 (E-C)</td>
<td>35</td>
<td>C₁₅H₁₆N₂OS</td>
<td>70.95 4.26 9.45</td>
<td>7.3-7.75(m,6H,Ar-H and H-7)</td>
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<tr>
<td>7g</td>
<td>CH₃CH₂H</td>
<td>242-44 (E-C)</td>
<td>32</td>
<td>C₁₅H₁₆N₂OS</td>
<td>71.63 4.55 8.49</td>
<td>7.1-7.9(m,1H,Ar-H and H-7), 12.6(s,1H,NH, exchangeable with D₂O)</td>
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<tr>
<td>7h</td>
<td>CH₂C₆H₄Cl-4</td>
<td>274-75 (B-PE)</td>
<td>38</td>
<td>C₁₅H₁₆ClN₂OS</td>
<td>68.55 3.70 8.04</td>
<td>3.98(s,2H,CH₂C₆H₄Cl-4), 7.94 7.35-7.85(m,10H,Ar-H and H-7), 12.66(s,1H,NH, exchangeable with D₂O)</td>
</tr>
<tr>
<td>7i</td>
<td>CH₃CH₆H₄NO₂-4</td>
<td>263-65 (C-PE)</td>
<td>52</td>
<td>C₁₅H₁₆N₂O₅S</td>
<td>62.59 3.82 11.40</td>
<td>7.5-7.75(m,6H,Ar-H and H-7)</td>
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<tr>
<td>7j</td>
<td>CH = CHC₆H₅</td>
<td>302-4 (B-C)</td>
<td>55</td>
<td>C₂₀H₂₁N₂O₅S</td>
<td>73.17 4.47 8.00</td>
<td>7.3-7.85(m,10H,Ar-H and H-7), 12.66(s,1H,NH, exchangeable with D₂O)</td>
</tr>
</tbody>
</table>

* B = Benzene; C = chloroform; E = ethanol; PE = pet. ether (40-60°C)
* Ethyl cyanoacetate was used as the nitrile component in normal reaction conditions
* Determined by mass spectra
* Acrylonitrile was used as the nitrile component
* ¹H NMR were taken in CDCl₃
room temperature, the workup of the reaction mixture yielded a product that was found to be devoid of the ester group in the 2-position as indicated by its $^1$H NMR and IR spectra. It was, however, found to be identical with 2-methylthieno[3,2-$d$]-pyrimidin-4($3H$)-one (7a) obtained by the condensation of 5a with acetonitrile (6a). The formation of 7a in the reaction of 5a with 6d may be rationalised through the side-chain hydrolysis and decarboxylation of the expected product 2-carbethoxymethyl-6-phenylthieno[3,2-$d$]-pyrimidin-4($3H$)-one (7d). The desired 2-carbethoxy methyl compound 7d could, however, be obtained under carefully controlled conditions (vide Experimental Section) (Scheme V).

The unsaturated nitrile, cinnamoni trile (6j), on reaction with 5a yielded the expected product, 2-styrylthieno[3,2-$d$]-pyrimidin-4($3H$)-one (7j). Acrylonitrile (6e), on the other hand, yielded the unexpected 2-(2'-chloroethyl)-6-phenylthieno[3,2-$d$]-pyrimidin-4($3H$)-one (7e) when reacted with 5a under similar conditions (Scheme VI).

Similarly, 3-amino-2-carbethoxy-4-phenylthiophene (5b) was reacted with the nitriles, acetonitrile (6a), chloroacetonitrile (6b) and acrylonitrile (6e) under the catalysis of dry HCl gas to afford the corresponding 2-substituted-7-phenylthieno[3,2-$d$]-pyrimidin-4($3H$)-ones (8a-c) in excellent yields (76-82%; Table II). Thiophene 5b was also cyclo-condensed with formamide at 160-180°C to obtain 7-phenylthieno[3,2-$d$]-pyrimidin-4($3H$)-one (8d) in good yield (Scheme VII; Table II).

All the 2-substituted thieno[3,2-$d$]-pyrimidin-4($3H$)-ones (7 and 8) were colorless crystalline solids melting $>200°C$. While the 6-phenylthieno[3,2-$d$]-pyrimidin-4($3H$)-ones (7) were freely soluble in chloroform and insoluble in ethanol, the 7-phenylthienopyrimidin-4-ones (8) was sparingly soluble in both the solvents.

The mass spectra of these compounds (7 and 8) exhibited intense molecular ion peaks (M$^+$). Their IR spectra exhibited strong vC=O around 1660-1640 and vN-H around 3500-3400 cm$^{-1}$ due to the corresponding 4-oxo function and the cyclic amide function. The compounds 7c and 7d possessing an ethoxycarbonyl substituent at their 2-position exhibited an additional strong vC=O band around 1740 cm$^{-1}$.

A D$_2$O exchangeable singlet corresponding to the NH proton at 3-position appeared at $\delta$12.6-12.8 in the PMR spectra of the 6-phenylthieno[3,2-$d$]-pyrimidin-4($3H$)-ones (7f-h). This NH singlet was also observed in the PMR spectra of the 7-phenylthieno[3,2-$d$]-pyrimidin-4($3H$)-ones around $\delta$11.5 (compounds 8a and 8d) or 14.1 (compounds 8b and 8c).
Table II—Physical, analytical and spectral data for 2-(un)-substituted-7-phenylthieno[3,2-d]pyrimidin-4(3H)-ones (8a-d)

<table>
<thead>
<tr>
<th>Compd</th>
<th>R</th>
<th>m.p.°C (Solvent)*</th>
<th>Yield (%)</th>
<th>Mol. formula (M+)*</th>
<th>Found (%) (Calc.)</th>
<th>'H NMRd (δ, ppm)</th>
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<tr>
<td>8a</td>
<td>CH3</td>
<td>223-253 (C)</td>
<td>80</td>
<td>C8H10N2O3 (242)</td>
<td>64.20</td>
<td>4.01</td>
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<td></td>
<td>(64.38)</td>
<td>1.46</td>
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<tr>
<td>8b</td>
<td>C1H3Cl</td>
<td>255-266 (E)</td>
<td>82</td>
<td>C8H10ClN2O3 (276)</td>
<td>56.67</td>
<td>3.47</td>
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<td>(56.83)</td>
<td>2.86</td>
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<tr>
<td>8c</td>
<td>C1H3CH3</td>
<td>190-192 (C)</td>
<td>76</td>
<td>C9H14N2O3 (280)</td>
<td>57.45</td>
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<td>(57.72)</td>
<td>3.78</td>
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<tr>
<td>8d</td>
<td>H</td>
<td>227-258 (E)</td>
<td>86</td>
<td>C8H10N2O3 (242)</td>
<td>62.98</td>
<td>3.62</td>
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<td></td>
<td></td>
<td>(63.12)</td>
<td>3.53</td>
</tr>
</tbody>
</table>

* C = Chloroform; E = Ethanol

** Determined by mass spectra

' Acrylonitrile was used as the nitrile component

d 'H NMR spectra were taken in CDCl3.

Elemental analyses (% C, H and N) of all the compounds were found to be satisfactory (+ 0.4%). All the thieno[3,2-d]pyrimidin-4(3H)-ones synthesised and characterised are being screened for their antihyperlipaemic activity in various animal models.

Experimental Section

Melting points were determined in open capillaries and are uncorrected. IR spectra were recorded on a Perkin-Elmer 337 Grating spectrophotometer (νmax in cm⁻¹), mass spectra on a Varian Atlas CH-7 Electron Impact mass spectrometer at 70 eV ionising beam and using direct insertion probe, and 'H NMR spectra on a Varian A-60 or EM-360 spectrometer at 60 MHz (chemical shifts in δ, ppm) using TMS as internal standard.

Ethyl thioglycolate, acetonitrile, phenylacetonitrile, benzonitrile, ethyl cyanoacetate, cinnamonic acid, acrylonitrile and 4-chlorophenylacetonitrile were available commercially. Other nitriles namely, ethyl cyanoformate, chloroacetonitrile and 4-nitrophenoxyacetonitrile were prepared by literature methods.

β-Chlorocinnamonic acid (11)

Phosphorous oxychloride (33 g, 0.2 mole) was added dropwise with cooling (0-6°C) and stirring to the ice-cold dimethylformamide (30 g, 0.4 mole). To this cold mixture, acetoephone (9 g, 0.1 mole) was added dropwise maintaining the temperature of the reaction mixture between 45-55°C. After the addition was complete, the reaction mixture was allowed to stand for 30 min at room temperature. To the above reaction mixture, containing 3-chloro-3-phenyl-2-propenium salt (10), was added 5 ml of the total solution of hydroxylamine hydrochloride (28 g, 0.4 mole) in dry DMF (40 ml) and the reaction mixture heated at 70-80°C. The remaining 35 ml solution of hydroxylamine hydrochloride in DMF was added thereafter at such a rate that the temperature of the reaction mixture rise above 160°C. After completion of the addition, the reaction mixture was allowed to cool to room temperature and then diluted with cold water (200-250 ml) when an oil separated out that was extracted with solvent ether. The organic layer was dried (Na2SO4) and concentrated. The crude oil was distilled under vacuum and pure β-chlorocinnamonic acid was collected 132-135°C/10 mm Hg, yield 13.6 g (85%), δ20 1.1955, n20 1.6040 (ref.9); IR(CHCl3): 2200 (C=O) cm⁻¹.

3-Amino-2-carbethoxy-phenylthiophene (5a)

To a well stirred solution of β-chlorocinnamonic acid (11; 16.4 g, 0.1 mole) was added a solution of ethyl thioglycolate (12.0 g, 0.1 mole) in sodium ethoxide (sodium 2.3 g, 0.1 g atom in 100 ml ethanol) at room temperature. After the addition was complete, the reaction mixture was refluxed on a boiling water-bath for 10 min, cooled and poured on to crushed ice. The separated solid was filtered, dried and crystallized from ethanol-chloroform to afford colorless crystals of 5a, yield 15.6 g (63%), m.p. 100-102°C (lit.6 m.p. 101-104°C).

3-Amino-2-carbethoxy-4-phenylthiophene (5b)

A mixture of 3-hydroxy-2-phenylacrylonitrile11
ethyl thioglycolate (12; 12.0 g, 0.1 mole) and 5 drops of conc. HCl was heated on a water-bath at 80°C for 15 min. The reaction mixture was allowed to cool to room temperature and sodium ethoxide (0.15 mole) in alcohol (30 ml) added to it. The reaction mixture was heated thereafter on a water-bath for 30 min. The ethanol removed (under vacuum) and residue quenched with ice-water mixture (150 ml) and acidified (gl. acetic acid). The solid that separated out was filtered, washed with aq. NaHCO₃ (satu.) and water, dried and recrystallized from ethanol to give 2(11) m.p. 73°C, yield 35%.

2-Substituted-6-phenyl- and 7-phenylthieno[3,2-d]pyrimidin-4(3H)-ones (7a-j and 8a-c): General procedure

A stream of dry hydrogen chloride gas was passed through a mixture of the appropriate thiophene-o-aminocarboxylic acid 5 (0.02 mole) and an appropriate nitrile (0.022 mole) in dioxane (30 ml) for about 12 hr. The reaction mixture was diluted with ice-water and basified with 10% aq. ammonium hydroxide. The solid obtained was washed with water, dried and crystallized from a suitable solvent (Tables I and II). With aromatic nitriles the yields of the product were improved by heating the reaction mixture on a water-bath for 2 hr after passing hydrogen chloride gas. In case of the reaction with acetonitrile (6a) excess of it was used as a solvent. In case of the reaction with ethyl cyanoacetate (6d), the temperature was maintained below 10°C throughout the course of the reaction and solid NaHCO₃ was used instead of aq. NH₄OH for neutralization during the workup procedure.

7-Phenylthieno[3,2-d]pyrimidin-4(3H)-one (8d)

A mixture of 3-amino-2-carbethoxy-4-phenyl-thiophene (5b) (2.47 g, 0.01 mole) and formamide (25 ml) was refluxed at 160-180°C for 6 hr and then allowed to cool to room temperature. The solid separated was filtered, washed with water, dried and crystallized from ethanol (m.p. 227°C, yield 86%).

Acknowledgement

We are thankful to CTDRI, Lucknow for microanalyses and mass spectra, and Dr. H. H. Patel, Princeton University, USA for PMR spectra.

References
PHARMACOLOGICAL EVALUATION OF LM-2616:
A BETα₂-ADRENOCEPTOR ANTAGONIST WITH
BETα₁-ADRENOCEPTOR AGONISTIC ACTIVITY

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The compound LM-2616 (2,7,9-Trimehyl-4 [N-methyl-piprazino] pyrido(3',2':4,5)thieno(3,2-)(f) pyrimidine)
was found to inhibit isoprenaline-induced positive chronotropic responses in atropinized and non-atropinized dogs.
In guinea-pig heart preparation, LM-2616 also inhibited isoprenaline-induced positive chronotropic responses.
Terbutaline, a specific BETα₂-adrenoceptor agonist produced dose dependent inotropic responses which were sig­nificantly antagonized by butoxamine. However, LM-2616 potentiated isoprenaline induced positive inotropic re­sponses. In guinea-pig tracheal chain and rat uterus, LM-2616, as well as terbutaline, produced dose dependent relaxation. This effect was blocked by butoxamine in both the preparations. On guinea-pig tracheal chain it also potentiated isoprenaline induced relaxation. LM-2616 was also found to exhibit local-anaesthetic activity. The LD₅₀ of the compound was found to be 480 mg/kg. Our data indicates that LM-2616 appears to be a specific BETα₁-adrenoceptor antagonist with BETα₂-agonistic activity.

KEY WORDS: Thienopyrimidines, BETα₁-adrenoceptor agonist, BETα₂-adrenoceptor antagonist, terbutaline, butoxamine

INTRODUCTION

Lands et al., (1967) subclassified the BETα-adrenoceptors into BETα₁- and BETα₂-adrenoceptors. A further sub-division of cardiac-BETα₂-adrenoceptors into those mediating only chronotropic and those mediating only inotropic effects was suggested by Bonelli (1977). Recently it has been reported by Kaumann and Lemoine (1987) and Bristow (1986) that not only BETα₁-adrenoceptors but also BETα₂-adrenoceptors contribute to the increased contractile force of human myocardium. BETα₂-adrenoceptors have been identified in the heart of dog (Einstein et al., 1979) and of man (Brooke, et al., 1983, Wilson, 1984). These cardiac-BETα₂-adreno­ceplors mediate positive inotropic responses (Molenaar and Summers, 1987).

Recently, some of the thienopyrimidine derivatives have been shown to possess antihypertensive (Hennig, et al., 1983), anti-arrhythmic (Troxler and Wiskott, 1976) and anti-throm­bic or platelet aggregation inhibitory activity (Amselem et al., 1983). We have developed in our laboratory a novel route of synthesis of the functionized condensed pyrimidines by re­action of nitriles with ortho-aminocarbonyl compounds (Shishoo et al., 1990). A number of
thienopyrimidine compounds were synthesized and screened. We present here a preliminary report on the autonomic effects of the compound LM-2616 which appears to possess beta,-adrenoceptor agonistic activity along with beta,-adrenoceptor antagonistic activity (Fig. 1).

MATERIAL AND METHODS

Dog blood pressure and heart rate

Mongrel dogs of either sex weighing 9–12 kg were anesthetized with 30 mg/kg pentobarbitone. Femoral vein was cannulated for the injection of various drugs and carotid artery was cannulated with arterial cannula for recording of blood pressure and heart rate. Arterial cannula was connected to a polygraph through a pressure transducer.

In the first series of experiments, the responses to adrenaline, noradrenaline, isoprenaline, acetylcholine and histamine were obtained in the absence and presence of the compound LM-2616. In the second series of experiments, the dogs were atropinised (2 mg/kg i.v.) and the responses to all these drugs were obtained as mentioned above in the absence and the presence of LM-2616.

Isolated guinea-pig heart preparations

Guinea-pigs of either sex weighing 400–500 g were injected heparin (18 mg/kg, i.p.) one hour before they were sacrificed by a sharp blow on to the head. Hearts were quickly excised from the body and mounted as per the Langendorff's technique. The hearts were perfused with Chenoweth-Koelle solution (pH = 7.4) maintained at 37°C and continuously bubbled with carbogen. The force of contraction and the rate were recorded on a polygraph using force displacement transducer.

After 10 minutes of stabilization of heart, responses to graded doses of LM-2616 (1 mcg to 300 mcg) and terbutaline (1 mcg to 100 mcg) were obtained. The hearts were then perfused with butoxamine (1.0 x 10^-6M) and the responses to terbutaline were recorded. In another set of experiments the responses to isoprenaline (0.1 mcg to 10 mcg) were taken in the presence and absence of LM-2616 (50 mcg/ml).

FIGURE 1 Structure of LM-2616.
Isolated smooth muscle preparation

Guinea pigs of either sex were sacrificed by a sharp blow on to the head and the trachea was isolated and kept in a Petri dish containing Kreb's bicarbonate solution. Spiral tracheal chain preparations made and mounted in the organ baths containing Kreb's bicarbonate solution maintained at 37°C and continuously bubbled with carbogen. The preparations were exposed to carbachol (1.37 x 10^{-6} M) and the maximum spasm was induced before administration of any drug.

For isolated rat uterine preparation female Wistar rats weighing 150–200 g were given diethylstilbestrol (100 mcg/100 g body weight) 24 hours before they were sacrificed by a sharp blow on to the head and cutting neck blood vessels. Uterine horns were quickly dissected-out and mounted in organ baths containing deJalon solution maintained at 37°C. These horns were later depolarized with 80 mM KCl.

Both the preparations were allowed to stabilize for 30 minutes and responses to LM-2616 per-se (8.25 x 10^{-8} to 2.75 x 10^{-6} M) and terbutaline (3.6 x 10^{-8} to 1.09 x 10^{-5} M) were elicited in the presence and absence of butoxamine (1.33 x 10^{-4} M). The interaction of LM-2616 was studied with isoprenaline on guinea-pig tracheal chain. The dose response curves of LM-2616 were obtained also in the presence of propranolol (3.38 x 10^{-4} M) and practolol (3.75 x 10^{-4} M).

Local anaesthetic activity and acute toxicity study

Local anaesthetic activity of the compound was tested by infiltration method in guinea-pig (Patel et al., 1965) and surface anaesthesia method in rabbits (Chance and Lobstein, 1944) and the responses were compared with that of lignocaine. Acute toxicity of LM-2616 was determined using mice (20–30 g). The compound was administered by intraperitoneal route.

Analysis of results

The pA_2 value was determined by the following formula:

\[ pA_2 = -\log[B] + \log(x - 1) \]

Where B is concentration of the antagonist, x is the ratio between the EC_{50} of the agonist in the absence and the presence of the antagonist.

All the results were analysed using Student’s t test and the value of P less than 5% (p < 0.05) was taken as significant.

RESULTS

Dog blood pressure and heart rate

The compound LM-2616 (1 mg/kg and 3 mg/kg) produced negative chronotropic effect (5.5 ± 1.3%) and decrease in blood pressure (2.35 ± 1.33%) but these were not statistically significant. The adrenaline and isoprorenaline induced positive chronotropic effects were significantly inhibited by the compound LM-2616 in the presence and absence of atropine (Fig. 2b, 3b). However, the isoprorenaline induced hypotensive responses was found to be poten-
tiated in the unatropinized and atropinized dog (Fig. 2b). The compound LM-2616 did not significantly affect noradrenaline induced increase in heart rate and increase in blood pressure. Acetylcholine and histamine induced effects on blood pressure and heart rate were not significantly affected by LM-2616 (Fig. 3a, 3b).

![Graph showing the effect of various doses of LM-2616 on isoprenaline induced change in blood pressure (a) and heart rate (b) on unatropinized and atropinized dogs. Each bar indicates the mean ± SEM of 5 experiments.](image)
Isolated guinea-pig heart

In this preparation the compound LM-2616 inhibited the isoprenaline induced chronotropic responses and potentiated isoprenaline induced positive inotropic responses (Fig. 4a, 4b). Terbutaline, a specific beta₂-adrenoceptor agonist produced a dose dependent inotropic responses which were significantly antagonized by butoxamine (Fig. 5). The pA₂ value of butoxamine was found to be 9.18.

**Figure 3** Effect of LM-2616 on adrenaline, noradrenaline, histamine and acetylcholine induced change in blood pressure (a) and heart rate (b) on unatropinized and atropinized dogs. Each bar indicates the mean ± SEM of 5 experiments.
FIGURE 4 Effect of LM-2616 on isoprenaline induced positive chronotropic (a) and inotropic (b) responses on guinea-pig heart. Each bar indicates mean ± SEM of 6 experiments.

Estrogen primed rat uterus

The compound LM-2616 (8.25 x 10^-4 to 2.75 x 10^-3 M) and terbutaline (3.6 x 10^-3 to 1.09 x 10^-3 M) produced a dose dependent relaxation of depolarized (KCl, 80 mM) rat uterus. Butoxamine (1.33 x 10^-4 M) inhibited the responses to LM-2616 and terbutaline. There was
LM-2616: A NOVEL β, ADRENOCEPTOR ANTAGONIST

Guinea-pig heart

The compound LM-2616 and terbutaline produced dose dependent relaxation of guinea-pig heart. Each point indicates mean ± SEM experiments.

Propranolol (3.38 x 10^-6M) also inhibited the responses to LM-2616 and pA2 value was found to be 5.77. However, practolol (3.75 x 10^-6M) failed to inhibit the responses to LM-2616 (Fig. 6).

Guinea-pig tracheal chain

The compound LM-2616 and terbutaline produced dose dependent relaxation of guinea-pig tracheal chain preparation contracted with carbachol. These effects were inhibited by the butoxamine (1.89 x 10^-5M). The pA2 values of butoxamine were found to be 9.04 and 9.16 for LM-2616 and terbutaline respectively (Fig. 8, 9). Isoprenaline (4.04 x 10^-9 to 1.21 x 10^-5M) also produced dose dependent relaxation of this preparation. LM-2616 (9.17 x 10^-5M) potentiated the responses to isoprenaline (Fig. 10).

Local anaesthetic activity

The compound LM-2616 was found to produce 100% local-anaesthesia with the onset of action within 5 minutes. The duration of action with 3 mg and 6 mg dose in guinea pigs (Infiltration anaesthesia) was 61.7 ± 4.4 and 58.75 ± 7.7 minutes respectively (Table 2). In rabbits (Surface anaesthesia) the onset of action with 2 mg and 4 mg of LM-2616 was within 4 minutes and duration of action was 25 ± 5.14 and 50 ± 2.83 minutes respectively (Table 3). These results were also comparable with lignocaine.
FIGURE 6  Effect of LM-2616 and its interaction with propranolol, practolol and butoxamine on isolated rat uterus. Each point indicates mean ± SEM of 6 experiments.

FIGURE 7  Effect of terbutaline and its interaction with butoxamine on isolated rat uterus. Each point indicates mean ± SEM of 6 experiments.
FIGURE 8. Effect of LM-2616 and its interaction with butoxamine on isolated guinea pig tracheal chain. Each point indicates mean ± SEM of experiments.

FIGURE 9. Effect of terbutaline and its interaction with butoxamine on isolated guinea pig tracheal chain. Each point indicates mean ± SEM of 6 experiments.
Acute toxicity test

The LD₅₀ of the compound LM-2616 was found to be 480 mg/kg. The compound also appeared to be non-irritant.

DISCUSSION

In the present investigation the compound LM-2616, a thieno (3,2-d) pyrimidine derivative was found to specifically inhibit the adrenaline and isoprenaline induced positive chronotropic effects (Fig. 2a, 3a) whereas, isoprenaline induced hypotensive response was found to be potentiated in dog (Fig. 2b). The results of our finding also support two different populations of beta₁-adrenoceptors, one mediating the chronotropic responses and the other mediating inotropic responses. It appears that the inhibition of positive chronotropic actions of adrenaline and isoprenaline is through the sub-type of beta₁-adrenoceptors mediating chronotropic effect and the potentiation of isoprenaline induced hypotensive effect is mediated through beta₂-adrenoceptors (Fig. 2a, 2b).

The results obtained in guinea pig heart also support these findings. In this preparation the compound LM-2616 inhibited the isoprenaline induced positive chronotropic responses (Fig. 4a). However, the isoprenaline induced positive inotropic effect was potentiated by the compound LM-2616 (Fig. 4b). Terbutaline produced a dose dependent positive inotropic effect which was competitively blocked by butoxamine, a specific beta₂-adrenoceptor antagonist (Fig. 5). The pA₂ value of butoxamine for this preparation was found to be 9.18. The pA₂ value was not different in the rat uterus and guinea pig tracheal preparations.

![Graph showing the effect of isoprenaline and its interaction with LM-2616 on guinea pig tracheal chain. Each point indicates the mean ± SEM of 4 experiments.](image-url)
(Table 1). Thus our results also indicate the presence of beta_2 adrenoceptors in the heart which are responsible for the inotropic effect.

The presence of beta_2-adrenoceptors in rat uterus has been reported by Tothill, (1967). Further evidence for beta_2-adrenoceptor agonistic activity for the compound LM-2616 was obtained from the results of rat uterus where the compound LM-2616 per-se produced a dose dependent relaxation. This was not blocked by practolol, a specific beta_1-adrenoceptor antagonist but blocked competitively by propranolol and butoxamine, the specific beta_2-adrenoceptor antagonist (Fig. 6). Terbutaline, a specific beta_2-adrenoceptor agonist also produced a dose dependent relaxation of depolarized rat uterus and which was also blocked by butoxamine (Fig. 7). The pA2 values of butoxamine was found to be identical when tested against the compound LM-2616 or terbutaline (Table 1). Identical results were obtained on guinea-pig tracheal chain (Fig. 8, 9). Isoprenaline produced a dose dependent relaxation of guinea-pig tracheal chain. The responses to isoprenaline were potentiated by the compound LM-2616 without altering the maxima (Fig. 10).

The antagonism of adrenaline and isoprenaline induced positive chronotropic responses by the compound LM-2616 suggests its possible usefulness in cardiac arrhythmias. Propranolol, a non selective beta-adrenoceptor antagonist is very commonly used in cardiac arrhythmias. It is not uncommon that many of the beta-adrenoceptor blockers possess local anesthetic activity. This activity is attributed mainly to membrane stabilizing activity and is specifically beneficial for anti-arrhythmic activity (Black and Prichard, 1973; Shand, 1975). The compound LM-2616 was also found to possess local anesthetic activity (Table 2 and 3).

A compound with beta_2-agonistic activity could be the drug of choice in the treatment of heart failure. Further, cardiac arrhythmias are many a times associated with heart failure (Satoskar el at., 1993). For this reason, during quinidine therapy for atrial flutter, digitalization of the patient is often necessary (Bigger and Hoffman, 1980). The compound LM-2616 appears to possess beta_2-adrenoceptor antagonistic activity, alongwith the beta_2-adrenoceptor agonistic activity. This may provide additional benefit to this compound for use as an anti-arrhythmic agent in patients with a problem of heart failure. In fact a combination of beta_2-adrenoceptor antagonist and beta_1-agonist have been shown to be ideal in heart failure (Mugge, el at., 1985; Zerkowski, et al., 1986). This is because in heart failure there is increased sympathetic tone (Cohn, et al., 1984) that gives rise to useful inotropic stimulation, but may result in excessive tachycardia and arrhythmias. The beta_2-adrenoceptor antagonist will prevent this tachycardia and the beta_1-adrenoceptor agonist, will produce vasodilation, reducing afterload on the heart. Till date there is no compound with both (beta_1-adrenoceptor antagonist and beta_2-adrenoceptor agonist) activities in market.

<table>
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<th>Agonist</th>
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<tr>
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<tr>
<td></td>
<td>Rat uterus</td>
<td>9.08</td>
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<tr>
<td></td>
<td>Guineapig heart</td>
<td>9.18</td>
</tr>
<tr>
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<td>Guineapig tracheal chain</td>
<td>9.04</td>
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<tr>
<td></td>
<td>Rat uterus</td>
<td>8.92</td>
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</table>
Preliminary toxicological studies indicate that this compound is safe, as it has the median lethal doses (LD₅₀) 480 mg/kg. Our data indicates that the compound LM-2616 possesses both these activities and may have potential to be developed as anti-arrhythmic agent.

ACKNOWLEDGEMENTS

The work was supported by a research grant from Council of Scientific and Industrial Research, New Delhi. Thanks are also due to Dr. K.C. Dave, Associate Dean, N.I.I.L. Municipal Medical College for his critical evaluation of the manuscript and valuable suggestions.

References


LM-2616: A NOVEL β-ADRENOCEPTOR ANTAGONIST


Synthesis and Antihyperlipaemic Activity of Some Novel N-Cyanovinylformamidines

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Summary

Potent antihyperlipaemic activity has been observed in a series of novel N-cyanovinylformamidines when tested in hyperlipaemic rats. Two of the compounds (11 and 15) were found more potent than gemfibrozil at 50 mg/kg/d dose level in reducing serum cholesterol and triglyceride levels and also in elevating serum HDL level. A good three-dimensional structural similarity has been observed between these two compounds and clofibrate and gemfibrozil, respectively. Acute toxicity studies carried out in mice indicated compound 11 to be safe even at a dose level of 4.5 g/kg. The title compounds were synthesized by the reaction of u-cyanoketene S,N-acetals with formimidacacetate under controlled reaction conditions.
Zusammenfassung

Synthese und antihyperlipämische Wirkung neueriger N-Cyanovinylformamidine

Eine Reihe neueriger N-Cyanovinylformamidine zeigte in Untersuchungen an hyperlipämischen Ratten eine potentielle lipidenkende Wirkung. Zwei der getesteten Substanzen (II und 15) waren bei einer Dosis von 50 mg/kg Tag wirksam als Gemfibrozil am Einfluss auf die Senkung der Serumtriglyceride und Cholesterin bei den proteren Tieren. Bei diesen beiden Verbindungen wurde eine starke dreidimensionale Strukturähnlichkeit mit Clofibrate beobachtet. Untersuchungen der akuten Toxizität zeigten die Sicherheit der Verbindung II bei der Mäuse bis zu der Dosis von 4,5 g/kg. Die untersuchten Verbindungen wurden durch die Synthese von α-Cyanoketen-S,N-Acetalen mit Formamidinacetat unter kontrollierten Bedingungen synthetisiert.

Key words
N-Cyanovinylformamidine derivatives, antihyperlipaemic activity, in vivo studies, Lipid reducers

1. Introduction

Since hyperlipidemia has been recognised as a primary risk factor of atherosclerosis and coronary heart diseases there has been intense effort to identify the chemical entities that are capable of regulating plasma levels of lipids. Present study is an effort in the same direction. Earlier, antihyperlipaemic activity has been reported in series of thienopyrimidine derivatives from our laboratory. 2-Chloromethyl-5,6,7,8-tetrahydrobenzo(b)thieno(2,3-d)pyrimidine (I) (see Scheme 1; LM-1554) has been the subject of intensive preclinical studies [1]. Recently, ethyl-2-cyano-3,3-bis-(4-fluoro phenyl)-propionate (II) was found to be a moderate inhibitor of HMG-CoA [2].

Apart from a close structural similarity between compound II and N-cyanovinylformamidine (V, see Scheme 1), a good three-dimensional structural similarity of two compounds (II and 15) was observed with clofibrate (III) and gemfibrozil (IV), respectively, through the overlapping of the energy-minimised three-dimensional conformers (Fig. 1 and 2). Therefore, the N-cyanovinylformamidines were thought to be potential candidates for antihyperlipaemic activity. The synthesis and biological activity of a series of N-cyanovinylformamidines is reported.

2. Materials and methods

2.1. Chemistry

The primary synthesis of pyrimidines involves the condensation of a 1,3-dicarbonyl compound or its analogues with an amine or amine and is presumed to proceed through non-isolable open chain vinylamidine intermediate. There are very few reports on isolation of this hypothetical intermediate [3-7].

Earlier, the isolation of novel and otherwise difficultly isolable N-cyanovinylaminidines intermediates in the reaction of α-cyanoketen-S,N-acetal with amidines, namely acetamidine, benzamidine and morpholinecarboxamidine, under controlled reaction conditions has been reported from this laboratory [8]. Essentially under similar experimental condition, a series of N-[2-carbethoxy-2-cyano-1-(substituted amino) vinyl]-formamidines (V) was synthesised through condensation of 2-cyano-3-substituted aminoacrylate (VI) with formamidine acetate under controlled reaction conditions (Scheme 2).

2.2. Chemical synthesis

Melting points were determined in open capillaries and are uncorrected. The IR spectra were recorded in potassium bromide on a Perkin-Elmer 837 Grating spectrophotometer (Perkin-Elmer Corp., Connecticut, USA). The 1H-NMR spectra were taken on a Varian A-60 spectrometer (Varian Analytical Instruments, California, USA) using TMS as internal standard. The mass spectra were obtained on a Varian Atlas CH-7 spectrophotometer at 70 eV using beam focusing direct insertion probe. Satisfactory microanalysis (± 0.4 % of the calculated values) was obtained for all compounds.
2.2.1. Reagents
The u-cyanokcylene S.N-acclals (VI) and formamidine acetate [9] were synthesized by literature methods.

2.2.2. N-(2-Carbethoxy-2-cyano-1-(substituted amino)vinyl)
formamide synthesis - general procedure
To a solution of 0.01 mol of sodium hydroxide in 50 ml of ethanol, formamidineacetate (0.01 mol) was added under stirring. After stirring for 0.5 h, 2-cyano-3-(methylthio)-3-(substituted amino)acrylate (0.01 mol) was added slowly with constant stirring. The reaction mixture was stirred for 1 h and allowed to stand at room temperature for 12 h. The reaction mixture was poured into ice-water. Solid obtained was filtered, washed with water, dried and crystallized from a suitable solvent.

2.3. Biological methods
2.3.1. General conditions of experimental animals
The experiments were carried out using laboratory animals such as mice (Swiss strain) and albino rats (Wistar strain). Inhouse breeding of these animals was carried out at the Department of Pharmacology, Cadila Pharmaceuticals Ltd., Ahmedabad (India). The animals were housed at 24 ± 1 °C and air humidity of 50-70% with 14-h light and 10-h dark cycle. The animals were given food prepared instantly by mixing wheat 80%, gram 15%, mineral oil 2%, milk powder 2.5%, salt 0.5%, and boiled to cook, and tap water ad libitum, unless specified in any particular method. For all the studies animals were selected at random.

2.3.2. Antihypertension activity of various compounds
Albino Wistar rats (12-14 weeks old) of either sex, weighing 160-200 g, were divided into eleven groups of five each. First group was kept as a control group, second group as a cholesterol control group, third group as a standard group and rest are test groups. The treatment was given for seven days. The control group received plain water and cholesterol control group, third group as a standard group and rest are test groups. The treatment was given for seven days. The control group received plain water and cholesterol control group, third group as a standard group and rest are test groups. The treatment was given for seven days. The blood samples were collected before and after the treatment from the retinal-orbital plexuses of rat eye [10].

Blood samples were analysed for serum cholesterol, triglycerides and HDL levels by using their respective enzyme immuno assay kits (Miles India Ltd., Baroda, India). Differences between final and initial levels of cholesterol, triglycerides and HDL levels were calculated as % change in initial levels (Table 2).

2.3.3. Acute toxicity study of compound 11
Acute toxicity study of compound 11 was carried out in albino Wistar mice of either sex. Mice (6-8 weeks old, 20-25 g) were divided into eight groups of 10 mice each and different doses of compound 11 were administered orally to each animal. The animals were kept under observation for 7 days.

3. Molecular modelling
Superimposition of the compounds 11 and 15 with clofibrate and gemfibrozil, respectively, were carried out on HP 735 workstation using CHIRUS*2 (Molecular Simulation Inc., California, USA; ver 1.6) software. All the four structures were generated and energy-minimised using open force he'd Only the minimised conformation whose relative energy with respect to the global minimum was not exceeded 3 kcal/mol were selected in the studies. The phenyl ring and carboxyl carbon were subjected to least-square fit.

4. Results
4.1. Antihyperlipaemic activity of various compounds in hypertensive rats
The Root Mean Square Distance (RMSD) observed of superimposed the compound 11 with clofibrate is 1.21 and that of the compound 15 with gemfibrozil is 0.021. Treatment of rats with cholesterol suspension for seven days produced a significant elevation in total blood cholesterol from 74.3 ± 6.7 (SD) to 82.6 ± 8.1, total triglycerides (from 32.5 ± 7.5 to 37.8 ± 6.2) and reduction in HDL level (from 67.2 ± 6.8 to 45.8 ± 29.8). Gemfibrozil, the antihyperlipaemic drug used in therapy, decreased the cholesterol level by 10.8% and triglycerides level by 12.2% and increased the HDL level by 22.5%. Of all the compounds tested compounds 11 and 15 appeared to be the most promising antihyperlipaemic agents (Table 2).

Table 1: N-[2-carbethoxy-2-cyano-1-(substituted amino)vinyl]formamidines (1-15).

<table>
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Table 2: Effect of N-[2-carbethoxy-2-cyano-1-(substituted amino)vinyl]formamidines on hyperlipaemic rats.

<table>
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<th>Compound No.</th>
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<th>HDL</th>
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* Number of animals used: n = 5. * Significantly different from control (p < 0.05).

Change

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</table>

* Number of animals used: n = 5. * Significantly different from control (p < 0.05).
Compound 11 decreased the cholesterol level by 14.5% and triglycerides level by 34.6% and increased the HDL level by 19.5%. Compound 15 decreased the cholesterol level by 15.4% and triglycerides level by 15.1% and increased the HDL level by 19.5%. Compound II decreased the triglycerides level three-fold and increased the HDL by two times more than the standard drug gemfibrozil. Interestingly, after one week treatment of hyperlipaemic rats with compound 11, the decrease in cholesterol and triglyceride levels were found even lower than basal levels (Table I).

4.2. Acute toxicity study of compound 11
No mortality or behavioral changes have been observed in any group up to 4500 mg/kg indicating thereby the safety of compound 11.

5. Discussion
The low RMSD values 1.21 and 0.021 suggest good superimposition of the compounds II and 15 with clofibrate and gemfibrozil. The compounds also occupy the same space as occupied by known drugs (Fig. 1 and 2). The results of the biological investigation suggest that the series of N-[2-carbethoxy-2-cyano-1-(substituted amino)formamidines possess significant antihyperlipaemic activity, N-[2-carbethoxy-2-cyano-1-(4-chloroaniline)vinyl]formamide (11) being the most potent (Table 2). Treatment with compound 11 decreased cholesterol and triglyceride levels and increased HDL levels significantly (Table 2), and this antihyperlipaemic effect was found superior to that of gemfibrozil.

The acute toxicity studies indicate that compound 11 is safe as it has shown no mortality or behavioral changes at a high dose of 4.5 g/kg. Present study indicates that compound 11 has potentials of being developed as a potent hypolipidemic agent.

6. Literature

Acknowledgements
We are thankful to Mr. Y. Kamakar and Mr. S. K. Vyas, Drug design Department, Torrent Pharmaceuticals Ltd., Ahmedabad (India) for providing molecular modelling facilities.

Correspondence: Prof. Dr. Chamanlal J. Shihoo, Department of Pharmaceutical Chemistry, L. M. College of Pharmacy, Ahmedabad 380 009 (India)
SYNTHESIS AND ANTIHYPERLIPAEMIC ACTIVITY OF NOVEL N-CYANOVINYL FORMAMIDINES

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1. L.M. College of Pharmacy, Ahmedabad 380 009; 2. Cadila Laboratories, Ahmedabad 380 008

A series of novel, otherwise unsolvable N-cyanovinyl formamidine intermediates have been isolated in the reaction of cyanoketene S, N-acetals with formamidine, under carefully controlled reaction conditions.

These reactive intermediates have been cyclised to the corresponding 4-chloro, 4-amino and 4-oxopyrimidines by subtly manipulating their cyclisation conditions.

The title compounds exhibited significant antihyperlipaemic activity, when evaluated in animal models like rabbits and rats.
SYNTHESIS OF SOME NOVEL 4-AMINO-5, 7-DIMETHYL-2-SUBSTITUTED AMINOPYRIDO [2,3-d] PYRIMIDINES AS \( \alpha_1 \)-ADRENO RECEPTOR ANTAGONISTS

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Prazocin, a 4-amino-2-substituted quinazoline derivative is a specific \( \alpha_1 \)-adrenoreceptor antagonist and reported as a potent antihypertensive agent. Pyridopyrimidines are known biosteres of quinazolines. A series of 4-Amino-5, 7-dimethyl-2-substitutedaminopyrido [2,3-d] pyrimidines were synthesised as biosteres of prazocin.

The title compounds were synthesised by two step process. First, 4-amino-2-mercapto-5, 7-dimethyl-pyrido-pyrimidine was synthesised by cycloaddition of acetyl acetone with 4, 6-diamino-2-mercapto pyrimidine, followed by S-alkylation with methylidide. Final compounds were obtained by nucleophilic substituted of methylmercapto group of 4-amino-2-mercapto pyrido-pyrimidine by various amines.

All compounds of this series were evaluated for the \( \alpha_1 \)-adreno receptor antagonistic activity on rat anacoccygeus muscle using phenylephrine (5 \( \mu \)g/ml) as a agonist and prazocin (75 \( \mu \)g/ml) as standard drug. Out of all the compounds tests two compounds (I & II) have shown significant antagonistic activity. Compounds II was found equipotent to prazocin at a concentration of 75 \( \mu \)g/ml.