New derivatives of isoniazide, pyrazinamide and 2-aminobutanol and their anti-tubercular activity

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Some new s-triazine derivatives having isoniazide, pyrazinamide and 2-aminobutanol moeity have been synthesized by condensation of substituted s-triazene (2a, b, c) with isoniazide, pyrazinamide and 2-aminobutanol. The products have been characterized by spectral data and evaluated for antitubercular activity. Some of them have shown moderate antitubercular activity values in terms of \( \mu g/mL \) determined by broth dilution technique.

Tuberculosis is as old as mankind. It is a systemic infectious disease which is caused in man by organisms such as Mycobacterium tuberculosis and Mycobacterium bovis. The present active drugs are relatively toxic and tubercular bacteria are developing resistance fairly rapidly to this generation of antitubercular drugs. It is therefore necessary to look for new series of anti-tubercular drugs. Literature survey for pyrazinamides reveals that N-acyl derivative of pyrazinamide, pyrazine 2-carboxylic acid ester as intermediates for pyrazinamide and for isonicotinic acid hydrazide and substituted 4-(isonicotinoyl hydrazine) quinolones, substituted 1,6-naphthyridines have been reported. As part of our ongoing search for better antibacterials, we report the preparation of substituted s-triazene derivatives of isoniazide, pyrazinamide and 2-aminobutanol (6-11), in good yields (Scheme I). Structures of these compounds have been established by spectral data and elemental analysis.

Experimental Section

Preparation of 2a, 2b and 2c. Cyanuric chloride (24 mmoles) was dissolved in dry acetone and cooled to \(-50^\circ C\). To this was added TEA (24 mmoles) and the isonicotinic acid hydrazide (42 mmoles) in small portions at a time and the reaction mixture stirred for over 2 hr at \(0^\circ C\). Acetone was evaporated and the product was washed with suitable solvents, filtered in vacuo and dried to get pure 2a in 70% yield, m.p. 218°C. Compounds 2b and 2c were prepared in similar manner. 2b: yield 80%, m.p. 245°C, 2c: yield 76%, m.p. 220°C.

Preparation of 6-11. Accurately weighed 2a (5.26 mmoles) was dissolved in dry methylene chloride and to this was added TEA (5.26 mmoles) and the temperature raised to 38 to 40°C. To this isonicotinic acid hydrazide (5.26 mmoles) was added and the reaction mixture stirred for 2 hr, methylene chloride was removed and the product was filtered in vacuo and dried to get 6. The compounds 7-11 were prepared in similar manner. The physical data are given in Table I.

6: \(^1\)H NMR (DMSO-d_6): 7.74 (d, 2H, Ha), 8.70 (d, 2H, Hb), 4.68 (bs, 1H, Hc), 10.13 (bs, 1H, Hd).
7: \(^1\)H NMR (DMSO-d_6): 9.19 (d, 1H, H-3), 8.72 (q, 1H, H-5), 8.86 (d, 1H, H-6), 8.26 (bs, 1H, H-7), 7.86 (bs, 1H, H-8).
8: \(^1\)H NMR (DMSO-d_6): 0.83-0.88 (t, 6H, Ha a'), 1.04-1.18 (quintate, 2H, Hb), 1.29-1.38 (quintate, 2H, Hb'), 2.45-2.56 (m, 2H, Hc c'), 3.08-3.13 (quintate, 2H, He) 3.25-3.30 (quintate, 2H, He'), 1.80-2.90 (b, '2 H, NH1OH).

Antitubercular activity. The compounds synthesized in the present investigation have been subjected to in vitro screening by tube dilution technique employing the human virulent H37 Rv strain of M. tuberculosis. In this method Kirchner’s medium containing Tween-80 as described by Rake et al. was used. The compounds were dissolved in absolute ethanol to get concentration of 200, 100, 50, 25, 12.5 \(\mu g/mL\). The sterile normal bovine serum (0.5ml) was added to each test tube. The inoculum of standard suspension (0.1 mL) of M. tuberculosis (H37 Rv strain) was added at the end. The medium was inoculated according to the recommendation of WHO. The tubes were incubated at 37°C for ten days and then examined for the presence or absence of growth of the test organisms. The lowest concentration which showed no visible growth was taken as an end point i.e. minimum inhibitory concentration (MIC).

A drug free control was set up with each test and was kept for comparison. Control tube with Ethambutol was used as standard drug which inhibited the growth of M. tuberculosis at a concentra-
Table I — Physical and spectral data of compounds 6-11

| Product | Yield | m.p. (°C) | IR(KBr) (v<sub>max</sub> in cm<sup>-1</sup>) | Electronic spectra | Antitubercular activity
<table>
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<tbody>
<tr>
<td></td>
<td>(%)</td>
<td></td>
<td>Bonded NH &amp; CH</td>
<td>C=O amide I</td>
<td>C=O amide II</td>
</tr>
<tr>
<td>6</td>
<td>49.26</td>
<td>136-38</td>
<td>3300-3000</td>
<td>1669</td>
<td>1559</td>
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<tr>
<td>7</td>
<td>58.88</td>
<td>161-64</td>
<td>3427</td>
<td>1715</td>
<td>1614</td>
</tr>
<tr>
<td>8</td>
<td>43.71</td>
<td>178-79</td>
<td>3400-3100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3100-2500&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1500-780&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>9</td>
<td>41.00</td>
<td>148-50</td>
<td>3427</td>
<td>1715, 1669</td>
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<td>10</td>
<td>55.80</td>
<td>150-53</td>
<td>3121</td>
<td>1715</td>
<td>1612</td>
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<tr>
<td>11</td>
<td>31.60</td>
<td>141-43</td>
<td>3300-3000</td>
<td>1669</td>
<td>1559.6</td>
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<sup>a</sup>Intramolecular hydrogen bonding.  
<sup>b</sup>—HC2—CH—stretching-OH in chelate form.  
<sup>c</sup>Finger print region.
tion of 0.4µg/mL. All the compounds have shown anti-tubercular activity in the range of 10-20 µg/mL concentration levels compared with standard drug Ethambutol 0.4 µg/mL tested against human virulent the strain of *M. tuberculosis*.

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**References**