

## Preparation, antiprotozoal and antibacterial evaluation and mutagenicity of some metronidazole derivatives

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Preparation of metronidazole derivatives **2-5** is described and the results of antimicrobial evaluation and mutagenicity are given. Compounds **2** and **5** at higher concentrations show better antiprotozoal activity than the parent drug. When tested against few strains of *Bacteroides fragilis*, an anaerobic microorganism, compound **2** is found to be active on four strains, compound **4** to be active against two strain whereas **3** and **5** are effective against only one strain. In mutagenicity assay **4** is found to be less mutagenic than the parent drug.

Prodrugs have been designed as reversible derivatives of a drug to eliminate a variety of undesirable properties of the drug such as poor absorption, unwanted side effects, toxicity, etc. Various linkages are employed in forming a prodrug, ester formation being the most common; presence of esterases in the biological system is the reason. Metronidazole, 2-(2-methyl-5-nitroimidazol-1-yl)ethanol **1** is an antibacterial and antiprotozoal agent used in the treatment of trichomoniasis, giardiasis, amoebiasis, acute ulcerative colitis and dental infections. For more than two decades, interest has been increasing towards the optimization of pharmacological and pharmacokinetic properties of metronidazole through chemical modifications. Aiming at the development of a water-soluble prodrug for parenteral administration, low molecular weight prodrugs, such as metronidazole phosphate<sup>1</sup>, amino acid<sup>2,3</sup>, and dicarboxylic acid monoesters<sup>4</sup> have been synthesised. Macromolecular derivatives of the drug have also been prepared as a mean of achieving greater pharmacological activity, reduced toxicity or higher cell-selective uptake<sup>5-7</sup>. Still metronidazole faces the problems relating to the low aqueous solubility, mutagenicity/toxicity and poor absorption characteristics.

The present communication comprises a study on the preparation, antimicrobial evaluation, mutagenicity testing of some metronidazole derivatives **2-5**. Effect of substituents on the lipophilicity of the esters prepared is also being reported.

### Results and Discussion

The general route for the synthesis of compound **2-4**

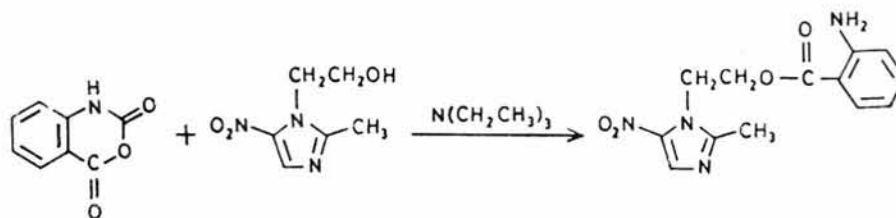
is depicted in **Scheme I**. Before converting the acid into acid chloride, the OH and/or NH<sub>2</sub> group was protected by acetylation. The acid chloride was allowed to react with metronidazole to form the ester **2-4**. The ester **5**, anthranilate ester, was prepared directly by reacting metronidazole with isatoic anhydride **6** and triethylamine in *N,N*-dimethylformamide as depicted in **Scheme II**.

The antiprotozoal activity of the synthesised compounds and the standard (metronidazole) was tested *in vitro*, in microplate assay against an axenic culture of *Entamoeba histolytica* (HM1-IMSS strain) using nitro blue tetrazolium (NBT) reduction<sup>8</sup>. NBT is metabolically reduced in viable cells to form a dark blue (formazan) product. This was measured directly by absorption at 540 nm using enzyme-linked immunosorbent assay (ELISA) microplate reader. Percentage of non-viable amoeba (percent inhibition) is calculated by the formula:

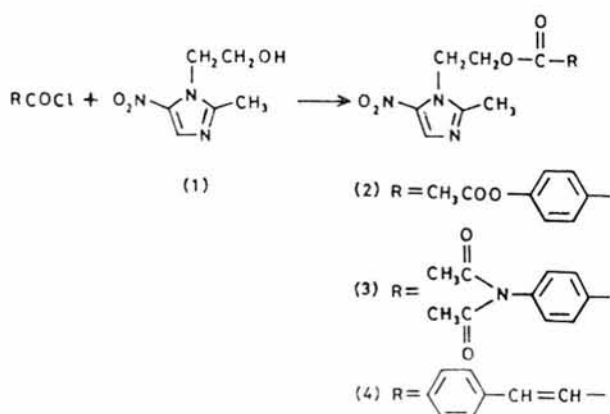
Percent inhibition

$$= 100 - \left[ \frac{(\text{Test OD}_{540} - \text{Blank OD}_{540})}{(\text{Control OD}_{540} - \text{Blank OD}_{540})} \times 100 \right]$$

The results obtained are given in **Table I**. From the percentage inhibition, IC<sub>50</sub> (inhibitory concentration) values were calculated graphically. The IC<sub>50</sub> values are given in **Table II**. The IC<sub>50</sub> values suggest that compounds **2** and **5** are almost equipotent to the parent compound metronidazole. At higher concentrations (18 and 27 µg/mL, respectively) compounds **2** and **5** showed a higher percentage inhibition (**Table I**). Rest



Scheme II



Scheme I

of the derivatives had  $IC_{50}$  values greater than that of metronidazole (standard), compound **4** being least active.

The compounds synthesised and the standard (metronidazole) were tested for their antimicrobial activity using disc diffusion test, against five clinical isolates of anaerobic bacteria *Bacteroides fragilis*. The results obtained in terms of diameter of the zone of inhibition are shown in Table III. The antimicrobial agent showing a zone of inhibition of 10-12 mm diameter and above is classified as sensitive. Thus, metronidazole was shown to be active against all strains; compound **2** active against all but strain 5; compound **4** active against strains 3 and 4 whereas compound **3** and **5** were effective only against strain 3 of *B. fragilis*.

Mutagenicity of the synthesised compounds was tested by Ames Salmonella Mutagenicity Assay<sup>9</sup> using

TA-100 strain of *Salmonella typhimurium* supplied by Professor B N Ames (Berkley, CA, USA). The results are shown in Table IV. The results of mutagenicity expressed as Rev/n mole indicate that the relative mutagenic activity of the compound **2-5** and the standard metronidazole **1** are in the order :  $4 < 1 < 2 < 3 < 5$ . In the assay **4** was found to be less mutagenic than the parent drug.

The lipophilic character of the compounds synthesised and the standard (metronidazole) was estimated by calculating log *P* (logarithm of partition coefficient) using 'Shake Flask' method<sup>10</sup> followed by UV spectrophotometric analysis. The results are shown in Table V. The esters prepared showed an increased lipophilicity.

### Experimental Section

**General.** <sup>1</sup>H-NMR (300 MHz) and <sup>13</sup>C NMR spectra were obtained on Varian EM360L NMR spectrometer and Bruker AC 300 F (300 MHz) spectrophotometer, respectively. Mass spectra were obtained on a VG Micromass 7070F mass spectrometer. Infrared spectra were recorded on a Perkin-Elmer 882 Infrared spectrophotometer using KBr disc. UV absorption was recorded on a Perkin-Elmer Lambda 15 UV/VIS spectrophotometer. Elemental analyses were performed on a Perkin-Elmer 2400 CHN elemental analyser.

**Synthesis of metronidazole esters 2, 3 and 4.**  
**General procedure.** Firstly the corresponding acids of **2** and **3** having OH and NH<sub>2</sub> functions, respectively were acetylated to protect these groups by treatment

Table I—Antiprotozoal activity against *Entamoeba histolytica* of the compounds synthesised and comparison with metronidazole

| Conc.<br>(µg/mL) | <b>1</b> |              | <b>2</b> |              | <b>3</b> |              | <b>4</b> |              | <b>5</b> |              |
|------------------|----------|--------------|----------|--------------|----------|--------------|----------|--------------|----------|--------------|
|                  | OD       | % inhibition | OD       | % inhibition | OD       | % inhibition | OD       | % inhibition | OD       | % inhibition |
| 2.25             | 0.161    | 36.29        | 0.139    | 20.30        | 0.124    | 18.80        | 0.140    | 20.74        | 0.149    | 27.40        |
| 4.50             | 0.170    | 42.90        | 0.153    | 30.70        | 0.147    | 26.29        | 0.149    | 27.40        | 0.151    | 28.80        |
| 9.00             | 0.188    | 48.80        | 0.164    | 38.50        | 0.148    | 27.03        | 0.168    | 41.48        | 0.159    | 35.18        |
| 18.0             | 0.192    | 59.25        | 0.211    | 73.70        | 0.177    | 48.50        | 0.177    | 48.40        | 0.161    | 66.66        |
| 27.0             | 0.194    | 60.70        | 0.227    | 79.00        | 0.202    | 67.03        | 0.188    | 56.29        | 0.164    | 68.88        |

**Table II**— $IC_{50}$  of the metronidazole **1** and compounds synthesised

| Compounds | $IC_{50}$ ( $\mu$ g/mL) |
|-----------|-------------------------|
| <b>1</b>  | 12.60                   |
| <b>2</b>  | 12.78                   |
| <b>3</b>  | 18.37                   |
| <b>4</b>  | 20.39                   |
| <b>5</b>  | 14.54                   |

**Table III**—Antimicrobial activity of the synthesised compounds and metronidazole against *Bacteroides fragilis*

| Compound | Diameter (in mm) of zone of inhibition against <i>Bacteroides fragilis</i> |          |          |          |          |
|----------|----------------------------------------------------------------------------|----------|----------|----------|----------|
|          | Strain 1                                                                   | Strain 2 | Strain 3 | Strain 4 | Strain 5 |
| <b>1</b> | 30                                                                         | 16       | 20       | 20       | 12       |
| <b>2</b> | 20                                                                         | 10       | 20       | 16       | 0        |
| <b>3</b> | 0                                                                          | 0        | 16       | 0        | 0        |
| <b>4</b> | 0                                                                          | 0        | 24       | 10       | 0        |
| <b>5</b> | 0                                                                          | 0        | 16       | 8        | 0        |

**Table IV**—Ames Salmonella mutagenicity assay of the compounds **2-5** and metronidazole

| Dose ( $\mu$ moles) | Revertants/Plate Compound |          |          |          |          |
|---------------------|---------------------------|----------|----------|----------|----------|
|                     | <b>1</b>                  | <b>2</b> | <b>3</b> | <b>4</b> | <b>5</b> |
| 0.05                | 176                       | 376      | 445      | 504      | 294      |
|                     | 160                       | 430      | 450      | 554      | 304      |
|                     | 818                       | 2852     | 3224     | 1070     | 3316     |
| 1.00                | 1204                      | 2700     | 3200     | 1054     | 3644     |
|                     | 3300                      | 54       | 13       | 690      | 462      |
| 5.00                | 3344                      |          | 2        | 715      | 507      |
| Rev/n mole          | 0.89                      | 2.5      | 2.91     | 0.56     | 3.35     |

with acetic anhydride in the presence of pyridine. These acids were then converted to their corresponding acid chlorides by reaction with thionyl chloride in tetrahydrofuran. The esters **2-4** of metronidazole were prepared by condensation of appropriate acid chloride with metronidazole in the presence of pyridine under anhydrous condition and stirring for 24 hr. The solvent was then removed *in vacuo* and the ester was precipitated by addition of sodium carbonate solution (1M). The prepared ester was extracted with chloroform. Removal of the solvent yielded the product which was recrystallised from acetone.

**2-(2-Methyl-5-nitroimidazol-1-yl)ethyl 4-acetoxybenzoate 2.** Yield 62%; m.p. 126–128°C;  $\lambda_{\text{max}}^{\text{MeOH}}$  310 nm; IR: 1759 (C=O), 1720 (C=O) 1530 and 1363  $\text{cm}^{-1}$  (C-NO<sub>2</sub> stretch); <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  2.32 [3H, s, C(=O)-CH<sub>3</sub>], 2.49 (3H, s, CH<sub>3</sub> at position-2' of imidazole ring), 4.66–4.72 (4H, m, CH<sub>2</sub>CH<sub>2</sub>), 7.17–7.19 (2H, d, H-

**Table V**—Log *P* determination of metronidazole and the compounds synthesised

| Compound | $\lambda_{\text{max}}$<br>H <sub>2</sub> O<br>(nm) | $\lambda_{\text{max}}$<br>Octanol<br>(nm) | $E_{1\%}^{1\text{cm}}$<br>water | $E_{1\%}^{1\text{cm}}$<br>octanol | log <i>P</i> |
|----------|----------------------------------------------------|-------------------------------------------|---------------------------------|-----------------------------------|--------------|
| <b>1</b> | 319.4                                              | 310.2                                     | 579.8                           | 658.7                             | −0.21        |
| <b>2</b> | 229.8                                              | 246                                       | 501.2                           | 516.4                             | 1.391        |
| <b>3</b> | 333.4                                              | 248.8                                     | 485.3                           | 295.5                             | 1.389        |
| <b>4</b> | 279                                                | 287.6                                     | 564                             | 582.7                             | 1.875        |
| <b>5</b> | 322.8                                              | 311.8                                     | 343                             | 373.8                             | 1.427        |

3" and 5" of phenyl ring), 7.94 (1H, s, H-4' of imidazole ring) and 7.96–7.98 (2H, d, H-2" and 6" of phenyl ring), <sup>13</sup>C NMR:  $\delta_c$  14.39 (−CH<sub>3</sub> at 2'-position of imidazole ring), 21.19 [O−C(=O)CH<sub>3</sub>], 45.30 (−CH<sub>2</sub>−CH<sub>2</sub>−O), 63.00 (−CH<sub>2</sub>−CH<sub>2</sub>−O), 121.99 (C-3" and C-5" of phenyl ring), 126.53 (C-1" of phenyl ring), 131.21 (C-2" and C-6" of phenyl ring), 131.95 (C-2' of imidazole ring), 133.29 (C-4' of imidazole ring), 150.84 (C-5' of imidazole ring), 154.82 (C-4" of phenyl ring), 165.28 [CH<sub>2</sub>−O−C(=O) Ar] and 168.18 ppm [−O−C(=O) CH<sub>3</sub>]; MS: m/z 291 (M<sup>+</sup>−2CH<sub>3</sub>), 213 (25.4%), 198, 180 (18, 7), 171 (40.0), 154 (12.6), 138 (100), 121 (67.5), 101 (71.1) and 87 (93.0). Anal. Calcd for C<sub>15</sub>H<sub>15</sub>O<sub>6</sub>N<sub>3</sub>: C, 54.05; H, 4.53; N, 12.61%. Found: C, 55.45; H, 3.16; N, 11.79%.

**2-(2-Methyl-5-nitroimidazol-1-yl)ethyl 4-(N,N-diacetyl)aminobenzoate 3.** Yield 68.5%, m.p. 184–186°C,  $\lambda_{\text{max}}^{\text{MeOH}}$  236.6 nm; IR: 1719 (C=O), 1526 and 1365  $\text{cm}^{-1}$  (C-NO<sub>2</sub> stretch); <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  2.28 (6H, s, COCH<sub>3</sub>), 2.51 (3H, s, 2'-methyl of imidazole ring), 4.67–4.76 (4H, m, CH<sub>2</sub>CH<sub>2</sub>), 7.24–7.26 (2H, d, H-3" and 5" of phenyl ring), 7.97 (1H, s, H-4' of imidazole ring) and 8.01–8.03 (2H, d, H-2" and 6" of phenyl ring); <sup>13</sup>C NMR:  $\delta_c$  14.42 (2'-methyl of imidazole ring), 26.92 (COCH<sub>3</sub>), 45.08 (−CH<sub>2</sub>−CH<sub>2</sub>−O), 63.21 (−CH<sub>2</sub>−CH<sub>2</sub>−O), 129.31 (C-3" and C-5" of phenyl ring), 131.11 (C-2" and C-6" of phenyl ring), 133.30 (C-4' of imidazole ring), 138.58 (C-2' of imidazole ring), 142.26 (C-1" of phenyl ring), 144.10 (C-5" of imidazole ring), 150.75 (C-4" of phenyl ring), 165.01 (−CH<sub>2</sub>−CH<sub>2</sub>−O−CO−Ar) and 172.39 ppm (N−CO−CH<sub>3</sub>); MS: m/z 374, [M<sup>+</sup>], 332 (41.8%), 317 (23.7), 286 (3.1), 244 (3.1), 204 (3.3), 190 (100), 162 (22.2), 154 (3.7), 146 (36.0), 131 (28.0), 120 (50.4), 103 (15.3), 95 (5.8), 81 (7.1) and 77 (13.8). Anal. Calcd for C<sub>17</sub>H<sub>18</sub>O<sub>6</sub>N<sub>4</sub>: C, 55.54; H, 4.84; N, 14.96%. Found C, 55.42; H, 3.47; N, 14.85%.

**2-(2-Methyl-5-nitroimidazol-1-yl)ethyl cinnamate**

**4.** Yield 68.2%, m p 100-102°C,  $\lambda_{\max}^{\text{MeOH}}$  282.6 nm; IR: 1711 (C=O stretch), 1526 and 1361  $\text{cm}^{-1}$  (C-NO<sub>2</sub> stretch); <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  2.52 (3H, s, CH<sub>3</sub> at 2' position of imidazole ring), 4.43-4.56 (2H, t, -CH<sub>2</sub>-CH<sub>2</sub>-O-CO), 4.63-4.66 (2H, t, -CH<sub>2</sub>-CH<sub>2</sub>-O-CO), 6.32-6.37 (1H, d, CO-CH=CH-Ph), 7.37-7.41 (3H, m, H-3", 4" and 5" of phenyl ring), 7.49-7.52 (2H, m, H-2" and 6" of phenyl ring), 7.62-7.67 (1H, d, CO-CH=CH-Ph) and 7.97 (1H, s, H-4' of imidazole ring); <sup>13</sup>C NMR:  $\delta_c$  14.30 (methyl at C-2' of imidazole ring), 45.17 (-CH<sub>2</sub>-CH<sub>2</sub>-O), 62.43 (-CH<sub>2</sub>-CH<sub>2</sub>-O), 116.40 (CO-CH=CH-Ph), 128.18 (C-2" and C-6" of phenyl ring), 128.90 (C-3" and C-5" of phenyl ring), 130.73 (C-4" of phenyl ring), 133.14 (C-4' of imidazole ring), 133.78 (C-1" of phenyl ring), 146.28 (-CH=CH-Ph), 150.86 (C-5' of imidazole ring) and 166.15 ppm (O-CO-CH); MS: m/z 301 (4.7) [M<sup>+</sup>], 255 (33.9%), 175 (14.2), 147 (11.5), 131 (100), 103 (49.2) and 77 (30.0). Anal. Calcd. for C<sub>15</sub>H<sub>15</sub>O<sub>4</sub>N<sub>3</sub>: C, 59.79; H, 5.01; N, 13.94%. Found C, 60.32; H, 3.42, N, 14.28%.

**Synthesis of anthranilate ester 5.** A mixture of metronidazole, isatoic anhydride and triethylamine in *N,N*-dimethylformamide was heated at 80°C for 4h to give 5.

**2-(2-Methyl-5-nitroimidazol-1-yl)ethyl anthranilate 5.** Yield 70.85%, m p 118-120°C;  $\lambda_{\max}^{\text{MeOH}}$  313.2 nm; IR: 3499 and 3390 (NH<sub>2</sub> stretch), 1695 (C=O stretch), 1522 and 1358  $\text{cm}^{-1}$  (C-NO<sub>2</sub> stretch); <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  2.48 (3H, s, CH<sub>3</sub>), 4.60-4.69 (4H, m, CH<sub>2</sub>-CH<sub>2</sub>), 5.73 (2H, s, NH<sub>2</sub>), 6.57-6.65 (2H, q, H-4" and 5" of phenyl ring), 7.23-7.28 (1H, t, H-3" of phenyl ring), 7.61-7.63 (1H, d, H-6" of phenyl ring) and 7.97 (1H, s, H-4' of imidazole ring); <sup>13</sup>C NMR:  $\delta_c$  14.29

(CH<sub>3</sub>), 45.40 (CH<sub>2</sub>-CH<sub>2</sub>-O), 62.05 (CH<sub>2</sub>-CH<sub>2</sub>-O), 109.22 (C-1" of phenyl ring), 116.32 (C-3" of phenyl ring), 116.80 (C-5" of phenyl ring), 130.59 (C-6" of phenyl ring), 133.28 (C-4' of imidazole ring), 134.68 (C-4" of phenyl ring), 138.51 (C-2" of imidazole ring), 150.91 (C-2" of phenyl ring, C-5' of imidazole ring) and 167.28 ppm (C=O); MS: m/z 290 (37.0%) [M<sup>+</sup>], 273 (29.0), 244 (5.3), 164 (19.0), 131 (17.1), 120 (100), 101 (21.2), 92 (33.2) and 86 (26.4); Anal. Calcd. for C<sub>13</sub>H<sub>14</sub>O<sub>4</sub>N<sub>4</sub>: C, 53.79; H, 4.86; N, 19.30%. Found C, 54.72; H, 3.59; N, 19.15%.

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