Prodrugs of nalidixic acid and norfloxacin

M S Y Khan* & Poonam Raghuvanshi
Department of Pharmaceutical Chemistry, Hamdard University, Hamdard Nagar, New Delhi 110 062

Received 30 September 1999; accepted (revised) 1 November 2000

Prodrugs of nalidixic acid and norfloxacin using three different amino alcohols namely (i) monoethanolamine, (ii) 1-amino-2-propanol and (iii) 3-amino-1-propanol have been prepared and tested against four different organisms.

Nalidixic acid and norfloxacin are two very important drugs used very widely for the treatment of bacterial infections but it has been shown that the blood levels and the urinary recoveries after oral administration of these drugs were not sufficient. In order to achieve sustained release of these two drugs and for better clinical results several approaches have been made to synthesize the prodrugs of nalidixic acid and norfloxacin. We also considered it worthwhile to prepare various oxazolines and oxazines from these drugs using appropriate amino alcohols with the aim that these derivatives could be converted by hydrolytic mechanism to the corresponding hydroxamides or amino esters and finally to the parent drugs.

The amino alcohols (i) monoethanolamine, (ii) 1-amino-2-propanol and (iii) 3-amino-1-propanol were reacted with nalidixic acid and norfloxacin in benzene and toluene under azeotropic conditions; however, no reaction could take place and hence, the reactants were directly heated to refluxing temperature, using excess of amino alcohols. The resulting products were separated; NMR and mass spectral data of these products showed that they were not the expected oxazolines and oxazines, but the corresponding hydroxamides (1-6). Further studies are in progress for the successful conversion of the hydroxamides to cyclic products.

Meanwhile, we report the characterization and antimicrobial activity of these hydroxamides. The physical properties, IR absorption, NMR shifts and mass spectral data are recorded in Table I. The IR and NMR data indicated these compounds to be hydroxamides rather than cyclized derivatives. However, a distinct differentiation between these structures could be made on the basis of mass spectra in which, in addition to molecular ion peaks, the two most prominent peaks were due to $RC=O^+$ and $RCO-N^+\text{CH}_2$ from both the series arising out of $\alpha$-cleavage of the amide and $\beta$-cleavage of the amine moiety. Considerably abundant peaks were also present for $RCONH\text{CH}_3$ (where $R$ stands for the cyclic part of the structures of nalidixic acid and norfloxacin).

The fragmentation pattern for products (2) and (5) are shown in the Charts 1 and 2 as two examples, one each from nalidixic acid and norfloxacin respectively. All the compounds were tested against Staphylococcus aureus, Streptococcus pyogenes,
**Table 1** — Physical and spectral data of compounds 1-6

<table>
<thead>
<tr>
<th>Compd</th>
<th>Mp(°C)</th>
<th>IR (cm⁻¹)</th>
<th>NMR</th>
<th>Mass (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>218-20</td>
<td>1620 (pyridine carbonyl), 1670(CONH), 3450 (b.OH)</td>
<td>DMSO-d₆, 1.4 (t, 3H, NCH₃CH₂), 3.3-3.8 (m, 4H, 2×CH₂), 4.5 (q, 2H, NCH₂CH₂), 7.4 (d, J=8Hz, 1H, H-5), 7.3 (d, J=8Hz, 1H, H-6), 8.45 (d, J=8Hz, 1H, H-5) 8.35 (s, 1H, H-2), 8.9 (s, 1H, H-3), 9.8 (b, 1H, NH)</td>
<td>275 (M⁺ missing)</td>
</tr>
<tr>
<td>2</td>
<td>182-84</td>
<td>1615 (pyridine carbonyl), 1640 (CONH), 3400 (b.OH)</td>
<td>CDCl₃, 1.2 (d, 3H, CH- CH₃), 1.5 (t, 3H, NCH₂CH₂), 2.66 (s, 3H, CH₃), 3.46 (m, 2H, CH₂), 4.0 (m, 1H, CH- CH₃), 4.5 (q, 2H, NCH₂CH₂), 7.25 (d, J=8Hz, 1H, H-6), 8.6 (d, J=8Hz, 1H, H-5), 8.8 (s, 1H, H-2), 9.6 (b, 1H, NH)</td>
<td>289 (M⁺), 271, 245, 244, 215</td>
</tr>
<tr>
<td>3</td>
<td>180-82</td>
<td>1620 (pyridine carbonyl), 1680(CONH), 3450 (b.OH)</td>
<td>CDCl₃, 1.5 (t, 3H, NCH₂CH₂), 1.7 (m, 2H, CH₂), 2.7 (s, 3H, CH₃), 3.6 (m, 4H, 2×CH₂), 4.5 (q, 2H, NCH₂CH₂), 7.3 (d, J=7Hz, 1H, H-6), 8.6 (d, J=7Hz, 1H, H-5), 8.9 (s, 1H, H-2), 9.4 (b, 1H, NH)</td>
<td>289 (M⁺), 245, 244, 215</td>
</tr>
<tr>
<td>4</td>
<td>242</td>
<td>1620 (pyridine carbonyl), 1650, 1670 (CONH), 3400 (b.OH)</td>
<td>DMSO-d₆, 1.3 (t, 3H, CH₂CH₃), 2.9-3.1 (m, 8H, 4×CH₂), 3.65 (m, 4H, 2×CH₂), 4.35 (q, 2H, NCH₂CH₂), 6.9 (d, J=14Hz, 1H, H-8), 7.9 (J=13Hz, 1H, H-5), 8.5 (b, 1H, NH), 8.8 (s, 1H, H-2)</td>
<td>362 (M⁺), 344, 332, 331, 302</td>
</tr>
<tr>
<td>5</td>
<td>242</td>
<td>1610 (pyridine carbonyl), 1630, 1660 (CONH), 3400 (b.OH)</td>
<td>CDCl₃, 1.24 (d, 3H, CHCH₂), 1.5 (t, 3H, NCH₂CH₂), 2.45, 3.16 (unresolved signal, 10H, 5xCH₂), 3.48 (m, 1H, CHCH₂), 4.24 (q, 2H, NCH₂CH₂), 6.76 (d, J=7Hz, 1H, H-8), 8.0 (d, J=13Hz, 1H, H-5), 8.8 (s, 1H, H-2)</td>
<td>376 (M⁺), 358, 332, 331, 302</td>
</tr>
<tr>
<td>6</td>
<td>246</td>
<td>1620 (pyridine carbonyl), 1660 (CONH), 3300 (-NH), 3450 (b.OH)</td>
<td>CDCl₃, 1 drop TFA, 1.5 (t, 3H, NCH₂CH₂), 1.9 (b, 2H, CH₂), 3.6 (b, 12H, 6CH₂), 4.6 (q, 2H, NCH₂CH₂), 7.2 (d, J=7Hz, 1H, H-8), 8.2 (d, J=14Hz, 1H, H-5), 9.1 (s, 1H, H-2)</td>
<td>376 (M⁺), 331, 302, 275</td>
</tr>
</tbody>
</table>

**Chart 2**

*Pseudomonas pyocyanea* and *Escherichia coli* by Disc-plate method. The activity is given in **Table II**.

From these data the following conclusions could be drawn:

1. Compound 1 exhibited equivalent potency against *Pseudomonas pyocyanea* but less antimicrobial activity against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli* as compared to nalidixic acid. Compounds 2 and 3 showed no anti-microbial activity.

2. Compound 5 exhibited antimicrobial activity of nearly half potency against *Staphylococcus aureus*, *Pseudomonas pyocyanea* and *Escherichia coli* as compared to norfloxacin. It showed no activity against *Streptococcus pyogenes*. Compounds 4 and 6 revealed no antimicrobial effects except a weak action against *Escherichia coli* by Compound 6.

**Experimental Section**

Melting points were recorded in open capillary tubes and are uncorrected. IR (KBr) spectra were recorded on a Perkin-Elmer 157 spectrometer, 1H NMR spectra on a Varian 60 MHz instrument using TMS as internal standard and the mass spectra on a JMSD 3000 spectrometer. Microanalyses were within ±0.4% of the theoretical values.

Nalidixic acid and norfloxacin (1.0 g) each was refluxed for 6-8 hr separately with an excess of (i) monoethanolamine (ii) 1-amino-2-propanol and (iii)
Table II — In vitro Antimicrobial activity (zone of inhibition in mm)

<table>
<thead>
<tr>
<th>Compd</th>
<th>Staphylococcus aureus</th>
<th>Streptococcus pyogenes</th>
<th>Pseudomonas pyocyanea</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>*12.66±0.66</td>
<td>*11.33±0.66</td>
<td>16.66±0.66</td>
<td>*15.33±0.66</td>
</tr>
<tr>
<td>Nalidixic acid (control)</td>
<td>16.66±0.66</td>
<td>16.66±0.66</td>
<td>16.66±0.66</td>
<td>17.33±0.66</td>
</tr>
<tr>
<td>5</td>
<td>**11.33±0.66</td>
<td>—</td>
<td>*14.00±0.00</td>
<td>*13.33±0.66</td>
</tr>
<tr>
<td>Norfloxacin (control)</td>
<td>21.33±0.66</td>
<td>17.33±0.66</td>
<td>26.33±0.80</td>
<td>28.33±0.66</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.001
Note: Figures indicate the mean value ± S.E
Comparisons: compound 1 vs nalidixic acid, compound 5 vs norfloxacin.

3-amino-1-propanol (10 mL of each amino alcohol was used). On cooling a solid mass separated out, which was filtered and crystallized from methanol-methylene chloride mixture.

Antibacterial studies
A filter paper disc method was employed for in vitro study of antibacterial effect against two Gram negative (Escherichia coli and Pseudomonas pyocyanea) and two Gram positive (Staphylococcus aureus and Streptococcus pyogenes) organisms.

Diameter of the filter paper disc was 6 mm. All drugs were used at conc. of 50μg/mL and emulsions were made in Tween-80. Nalidixic acid and Norfloxacin were used as standards for comparison.

Acknowledgement
The authors are thankful to (late) Hakim Abdur Hameed Sahib and Mr A Mueed for providing facilities to carry out this work. One of the authors (PR) is thankful to UGC, New Delhi for the award of Senior Research Fellowship.

References
5 Dumitriu S & Blascu V, J Biomater Appl, 9, 1995, 289.