Chemotherapy of Filariasis – On the Search of New Agents Effective on the Reproductive System of Female Adult Worms [1]


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Benzimidazole-2-carbamates, 4-Substituted Quinolines, Substituted Pyrroles, Macrofilaricides, Female Adult Filarial Worms, Chemosterilization

The design and synthesis of a series of alkyl 5(6)-substituted benzimidazole-2-carbamates (1–13), 7-chloro-4-(4-substituted phenyl)aminoquinolines (14–16), 1,2-dimethyl-3-methoxy-carbonyl-4,5-disubstituted pyrroles (17–19) and some compounds belonging to the class piperonitrile (20), dihydroquinoline (21), pyridine (22), pyrroloquinoline (23) and tetrahydropyrimidine (24) have been carried out as possible antifilarial agents. All these compounds have been evaluated for their activity against male and female adult worms of *Litomosoides carinii* in cotton rats. The effect of these compounds was also observed on the reproductive system (condition of developing microfilariae and their release from uterus) of adult female worms. In this study, three types of compounds were discovered: (a) those which showed activity on both the male and female adult worms and also had sterilizing effects on surviving adult females (1–3, 6–9, 13, 19), (b) those which only sterilized the adult females (14–16, 21, 24), and (c) those which had no effect on female reproduction but killed only adult worms (4, 5, 11, 12, 17, 18, 20, 22, 23). This tends to open up a new avenue in the chemotherapy of filariasis and the future scope of work on chemosterilization of adult females has been discussed.

Introduction

Filariasis, a major communicable disease of the tropics, is responsible for a wide range of disabling effects such as elephantiasis of legs, arms and genitals, the River blindness and Calabar swellings [2]. It is estimated that nearly 400 million people carry different forms of filariasis caused by infestations due to *Wuchereria bancrofti*, *Brugia malayi*, *Onchocerca volvulus*, *Loa loa*, *Acanthocheilonema pectans*, and *Mansonella ozzardi* [3]. In addition several hundred million people are exposed to the risk of acquiring these infections [4].

The successful control and eradication of filariasis may depend on several factors, the most obvious being the elimination of the vectors of the disease (mosquitoes, black gnat, tabanid flies and culicoides) and application of a clinically acceptable drug to remove microfilariae and adult worms from the infected hosts while the third factor relates to immunoprotections in endemic areas. The first approach suffers from several climatic, economic and administrative bottle-necks and is, thus, not very practical in the tropical countries. In such a situation, till the advent of an effective immunotherapeutic measure, chemotherapy leading to radical cure of the disease remains as the only viable tool to combat filariasis in man. Radical cure of filariasis involves killing of the microfilariae and adult worms, however sudden death of micro- and macrofilariae may lead to anaphylactic reactions because of significant titre of liberated proteins from the dead worms. This situation is likely to be grave especially in patients with heavy worm burden. In order to circumvent this problem, interference with the reproductive system of the adult female filarids was considered as an useful approach. The sterilization of the adult female filarids will not only bring down blood microfilaraemia and interrupt the transmission cycle, but will also help the host to assimilate the decaying worms died at different intervals with no anaphylactic reaction. Based on this rationale, studies directed towards evaluation of nitrogen heterocycles for their ability to induce sterility in female filarial worms were undertaken. The results presented in this communication are, therefore, concerned with the design, syntheses and evaluation of antifilarial activities of a variety of heterocycles (1–24).

Material and Methods

1. Synthesis of compounds

The syntheses of some of the 2,5-disubstituted benzimidazoles (4, 5, 8–12) were carried out as re-
ported earlier [5–10] while the other benzimidazo- 
ezoles (1–3, 6, 7) were prepared either by cyclizing 
the corresponding 4-substituted o-phenylene-
diamines with 1,3-dicarbalkoxy-S-methylisothio-
ureas or by acetylation of the required 2-amino-
benzimidazoles with acetic anhydride and pyridine. 
All the benzimidazole-2-carbamates were charac-
terized by their IR spectra which showed character-
istic carbamate bands at 1700–1730 cm⁻¹.

The 7-chloro-4-(4-substituted phenyl)amino-
quinolines (14–16) were obtained from 4,7-di-
chloroquinoline as described by us earlier [11, 12]. 
All the compounds showed molecular ion peaks at 
their respective m/z values as given in Table II. The 
syntheses of 1,2-dimethyl-3-methoxycarbonylpyr-
roles (17–19) were carried out [13] by reacting 
methyl-N-methylaminocrotonates with substitut-
ed nitrostyrenes. All the pyrrole esters exhibited 
IR absorption at 1720–1750 cm⁻¹ showing the 
presence of an ester function. Other characteriza-
tion data are recorded in Table III. 1,3,5-Tri-
cyano-3-phenylpentane (20) was prepared by 
method reported by Kaddah and coworkers [14, 
15] while the representative candidates (21–24) 
from other heterocycles designed to explore newer 
avenues in filarial chemotherapy, were prepared 
by the methods reported by us earlier [16–19]. The 
physical constants of compounds 20–24 are 
recorded in Table IV.

All the compounds recorded in Tables I–IV 
were analyzed for their C, H and N analyses and 
the results were within ±0.5% of the calculated 
values.

2. Evaluation of antifilarial activity of compounds 
with their effect on reproductive system of adult 
female worms

The micro- and macrofilaricidal activity of the 
compounds was evaluated against Litomosoides 
carinii infection in cotton rats (Sigmodon hispi-
dus). L. carinii was transmitted to cotton rats 
through the vector Liponyssus bacoti by the meth-
and Hawking and Sewell [20]. At the end of pre-
patent period, animals showing 250 or more 
microfilariae per 5 μl of the blood were chosen for 
screening. Five animals formed an experimental 
group. Blood samples of experimental and control 
animals were examined before starting the treat-
ment. The compounds were suspended in water in 
the presence of Tween 80 and were administered 
intraperitoneally and/or orally for 5 consecutive 
days. Blood smears of animals were examined for 
microfilariae at weekly intervals for up to 6 weeks 
from the start of treatment. On day 42, all the 
treated and control animals were sacrificed and the 
condition of male and female worms was ob-
erved. The micro- and macrofilaricidal action of 
the compounds were assessed as described by 
Laemmmler et al. [21]. The uteri of the adult female 
worms were also examined to ascertain any de-
formity of developing microfilariae or ova and the 
release of microfilariae from uterus.

Results and Discussion

The antifilarial activity of compounds 1–24 
against the microfilariae, adult male and female 
worms and their action on the reproductive system 
of the female worms both by intraperitoneal and 
oral routes are recorded in Tables I–IV. Based on 
the broad-spectrum of anthelmintic activity asso-
ciated with various benzimidazole drugs [22] a to-
total of thirteen benzimidazoles (1–13) carrying dif-
ferent pharmacophores at their 2 and 5 positions 
were valuated against L. carinii. Of these, com-
 pounds 1–3, 6–8 and 9 showed marked effect on 
the reproductive system of the females. The com-
 pounds also caused 75–100% death of male and 
female adult worms. Compounds 4, 5 and 10–12, 
though killed 50–100% of the adult male and 
female filariids, could not check the release of micro-
filariae from the uterus of the female worms. Thus 
all the benzimidazoles, having action on the re-
production of microfilariae, also had lethal effects 
on the male and female adult worms. Nevertheless, 
compounds with lethal action on adult worms may 
not inhibit the reproductive function of adult fe-
dmale worms (cf. 4, 5, 10–12). It was also observed 
that the death of adult worms and/inhibition of 
normal reproduction of female worms were 
achieved only by those benzimidazoles which were 
absorbed by gastrointestinal tract when adminis-
tered by oral route.

Amongst 7-chloro-4-substituted quinolines [11, 
12], compounds 14–16 were found to cause com-
plete sterilization of adult female worms at an in-
traperitoneal dose of 30 mg/kg given for 5 days 
(Table II). This indicates that 7-chloro-4-(4-substi-
tuted phenyl)aminoquinolines have high potential-
ity of interfering with the reproductive system of 
adult female filariids.
Table I: Characterization data and antifilarial activity of 2,5-disubstituted benzimidazoles

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>R</th>
<th>R¹</th>
<th>m.p. [°C]</th>
<th>Molecular formula</th>
<th>Dose (mg/kg x 5)</th>
<th>Antifilarial activity</th>
<th>Effect on adult female worms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% death of adult worms</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>-CO-C₆H₄-N=C(NHCOOMe)₂</td>
<td>OMe</td>
<td>&gt;290</td>
<td>C₂₈H₂₀N₆O₇</td>
<td>30 (ip)</td>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>-CH(OH)-C₆H₄-N=C(NHCOOMe)₂</td>
<td>OMe</td>
<td>&gt;290</td>
<td>C₂₈H₂₂N₆O₇</td>
<td>30 (ip)</td>
<td>100</td>
<td>88</td>
</tr>
<tr>
<td>3</td>
<td>-CH(OH)-C₆H₄-N=C(NHCOOEt)₂</td>
<td>OEt</td>
<td>250</td>
<td>C₂₈H₂₀N₆O₇</td>
<td>30 (ip)</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>4</td>
<td>-CH(OH)-C₆H₅</td>
<td>OMe</td>
<td>&gt;290</td>
<td>C₁₆H₁₅N₃O₃</td>
<td>30 (ip)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>-CH(OH)-C₆H₄-NH(m)</td>
<td>OMe</td>
<td>&gt;290</td>
<td>C₁₆H₁₅N₃O₃</td>
<td>30 (ip)</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>-CO-C₆H₅</td>
<td>Et</td>
<td>238</td>
<td>C₁₆H₁₅N₂O₂</td>
<td>30 (ip)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>-CH(OH)-R²</td>
<td>OMe</td>
<td>&gt;300</td>
<td>C₁₆H₁₅N₃O₂</td>
<td>30 (ip)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>-CO-R³</td>
<td>Me</td>
<td>&gt;300</td>
<td>C₁₆H₁₅N₃O₂</td>
<td>30 (ip)</td>
<td>100</td>
<td>87</td>
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<tr>
<td>9</td>
<td>-CO-C₆H₅</td>
<td>A</td>
<td>&gt;300</td>
<td>C₂₈H₂₀N₃O₃</td>
<td>30 (ip)</td>
<td>100</td>
<td>83</td>
</tr>
<tr>
<td>10</td>
<td>B</td>
<td>OMe</td>
<td>126-128</td>
<td>C₂₈H₂₀N₄O₄</td>
<td>30 (ip)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>-CO-C₆H₅</td>
<td>Me</td>
<td>280</td>
<td>C₁₆H₁₅N₂O₂</td>
<td>30 (ip)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>12</td>
<td>-S-C₆H₅</td>
<td>Me</td>
<td>270</td>
<td>C₁₆H₁₅N₃Os</td>
<td>30 (ip)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>13</td>
<td>C</td>
<td>OMe</td>
<td>178-179</td>
<td>C₂₈H₂₀N₄O₄</td>
<td>30 (ip)</td>
<td>48</td>
<td>0</td>
</tr>
</tbody>
</table>

R² = 2-Methoxycarbonylaminobenzimidazol-5-yl.
R³ = 2-Acetylaminobenzimidazol-5-yl.
A = 5-Benzoylbenzimidazol-2-yl.
B = 1-(Diethylaminopropyl)-2,5-dimethyl-3-methoxycarbonylpyrrol-4-yl.
C = 2,5-Dimethyl-3-(1-methylpiperazin-4-yl)carbonylfuran-4-yl.

Table II: Characterization data and antifilarial activity of 7-chloro-4-substituted quinolines.

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>R</th>
<th>m.p. [°C]</th>
<th>Molecular formula</th>
<th>Mass M⁺ at m/z</th>
<th>Dose (mg/kg x 5)</th>
<th>Antifilarial activity</th>
<th>Effect on adult female worms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>% death of adult worms</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>14</td>
<td>-SO₂-C₆H₄-NCS(p)</td>
<td>250</td>
<td>C₂₂H₂₁Cl₁N₁O₄S₂</td>
<td>451</td>
<td>30 (ip)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15*</td>
<td>-NH-CS-R¹</td>
<td>220</td>
<td>C₂₂H₂₁Cl₁N₁S</td>
<td>461</td>
<td>30 (ip)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16*</td>
<td>-CO-R²</td>
<td>190</td>
<td>C₂₁H₂₁Cl₁No</td>
<td>380</td>
<td>30 (ip)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* R¹ = 1-phenylpiperazin-4-yl
R² = 1-methylpiperazin-4-yl.
Compounds reported in Tables III and IV are a novel class of compounds hitherto unexplored in the filarial chemotherapy. Among these novel template molecules, compounds 19, 21 and 24 were found to have pronounced effect in sterilizing the female worms. Other compounds (7, 18, 20, 22 and 23) were only lethal to the adult male and female worms.

It may be concluded that amongst the 24 compounds screened, only 14 compounds exhibited sterilization effect and these represented different class of organic compounds such as benzimidazoles (1–13), quinolines (14–16), pyrroles (17–19), pimelonitrile (20), dihydroquinoline (21), pyridine (22), pyridoquinoline (23) and tetrahydropyrimidine (24). Except a few cases, it was not possible to separate the adulticidal and the chemosterilization effects. Only these compounds (14–16) were found to selectively sterilize the female adult worms without killing the adult male or female worms. These observations are likely to evoke basic studies on the mechanism of interference with

Table III. Characterization data and antifilarial activity of substituted pyrroles.

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>Structure</th>
<th>m.p. [°C]</th>
<th>Molecular formula</th>
<th>Mass M+ at m/z</th>
<th>Antifilarial activity</th>
<th>Effect on adult female worms</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>3,4-Methylene dioxyphenyl</td>
<td>170(d)</td>
<td>C₁₀H₁₃N₂O₄S₂</td>
<td>418*</td>
<td>30 (ip)</td>
<td>50</td>
</tr>
<tr>
<td>18</td>
<td>3,4-Dimethoxy-phenyl</td>
<td>203–</td>
<td>C₂₀H₂₅N₃O₂S₂</td>
<td>303</td>
<td>30 (ip)</td>
<td>100</td>
</tr>
<tr>
<td>19</td>
<td>C₆H₅_–CH₂OCH₃–R²</td>
<td>236</td>
<td>C₃₀H₃₂N₃O₃</td>
<td>500</td>
<td>30 (ip)</td>
<td>81</td>
</tr>
</tbody>
</table>

R² = 1,3-Dimethyl-2-methoxycarbonyl-4-phenylpyrrol-5-yl.
* Indicates (M+ – 1).

Table IV. Characterization data and antifilarial activity of miscellaneous compounds.

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>Structure</th>
<th>m.p. [°C]</th>
<th>Molecular formula</th>
<th>Mass M+ at m/z</th>
<th>Antifilarial activity</th>
<th>Effect on adult female worms</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>A</td>
<td>59–60</td>
<td>C₁₄H₁₃N₃</td>
<td>223</td>
<td>30 (ip)</td>
<td>100</td>
</tr>
<tr>
<td>21</td>
<td>B</td>
<td>160–162</td>
<td>C₁₉H₁₅ClN₂O₂</td>
<td>326</td>
<td>30 (ip)</td>
<td>0</td>
</tr>
<tr>
<td>22</td>
<td>C</td>
<td>206–207</td>
<td>C₂₂H₂₅N₃O₆</td>
<td>451</td>
<td>30 (ip)</td>
<td>50</td>
</tr>
<tr>
<td>23</td>
<td>D</td>
<td>148</td>
<td>C₂₃H₂₁ClN₄S</td>
<td>420</td>
<td>30 (ip)</td>
<td>100</td>
</tr>
<tr>
<td>24</td>
<td>E</td>
<td>Oil</td>
<td>C₁₀H₁₁N₃O</td>
<td>199</td>
<td>30 (ip)</td>
<td>0</td>
</tr>
</tbody>
</table>
the reproductive physiology of adult filarial worms and may promote a renewed search for pharmacophores. For example, the profile of biological activity of compounds 1–13 provokes a search for the optimum pharmacophore which may be used as a substituent at position 5(6) of benzimidazole nucleus to achieve 100% sterilization effect by oral route of administration at lower doses. This appears to be feasible provided their mechanism of action of chemosterilization of adult female worms is studied in detail. One such mechanism may involve inhibition of the biosynthesis of ecdysteroids which have recently been shown to be associated with macrofilaricidal action and inhibition of microfilarial production in Brugia pahangi [23].

Acknowledgements

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