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

Jujhar Singh, M. Seth, Nigar Fatma, Satyavan Sharma, A. P. Bhaduri, and R. K. Chatterjee

Central Drug Research Institute, Lucknow 226001, India

Z. Naturforsch. **45c**, 1210–1214 (1990); received April 27/August 20, 1990

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-methoxycarbonyl-4,5-disubstituted pyrroles (**17–19**) and some compounds belonging to the class pimelonitrile (**20**), dihydroquinoline (**21**), pyridine (**22**), pyridoquinoline (**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 disabilitating 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 pestans,* 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 filariids was considered as an useful approach. The sterilization of the adult female filariids 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**).

Reprint requests to Dr. Satyavan Sharma, Medicinal Chemistry Division, C.D.R.I., Lucknow 226001, India.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/90/1100–1210 $ 01.30/0

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 benzimidazoles (**1–3, 6, 7**) were prepared either by cyclizing the corresponding 4-substituted *o*-phenylenediamines with 1,3-dicarbalkoxy-S-methylisothioureas or by acetylation of the required 2-aminobenzimidazoles with acetic anhydride and pyridine. All the benzimidazole-2-carbamates were characterized by their IR spectra which showed characteristic carbamate bands at 1700–1730 cm^{-1}.

The 7-chloro-4-(4-substituted phenyl)aminoquinolines (**14–16**) were obtained from 4,7-dichloroquinoline 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-methoxycarbonylpyrroles (**17–19**) were carried out [13] by reacting methyl-N-methylaminocrotonates with substituted nitrostyrenes. All the pyrrole esters exhibited IR absorption at 1720–1750 cm^{-1} showing the presence of an ester function. Other characterization data are recorded in Table III. 1,3,5-Tricyano-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

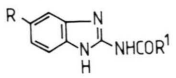
The micro- and macrofilaricidal activity of the compounds was evaluated against *Litomosoides carinii* infection in cotton rats (*Sigmodon hispidus*). *L. carinii* was transmitted to cotton rats through the vector *Liponyssus bacoti* by the method of Hawking and Sewell [20]. At the end of prepatent period, 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 treatment. The compounds were suspended in water in the presence of Tween 80 and were administered intraperitoneally and/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 observed. The micro- and macrofilaricidal action of the compounds were assessed as described by Laemmler *et al.* [21]. The uteri of the adult female worms were also examined to ascertain any deformity 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 associated with various benzimidazole drugs [22] a total of thirteen benzimidazoles (**1–13**) carrying different pharmacophores at their 2 and 5 positions were valuated against *L. carinii*. Of these, compounds **1–3, 6–8** and **9** showed marked effect on the reproductive system of the females. The compounds 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 microfilariae from the uterus of the female worms. Thus all the benzimidazoles, having action on the reproduction 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 female 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 administered by oral route.

Amongst 7-chloro-4-substituted quinolines [11, 12], compounds **14–16** were found to cause complete sterilization of adult female worms at an intraperitoneal dose of 30 mg/kg given for 5 days (Table II). This indicates that 7-chloro-4-(4-substituted phenyl)aminoquinolines have high potentiality of interfering with the reproductive system of adult female filariids.

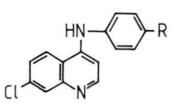
Table I: Characterization data and antifilarial activity of 2,5-disubstituted benzimidazoles



Compd. No.	R	R^1	m.p. [°C]	Molecular formula	Dose (mg/kg) × 5	Antifilaria activity % death of adult worms Males	Females	Total	Effect on adult female worms
1	$-CO-C_6H_4-N=C(NHCOOMe)_2$	OMe	>290	$C_{21}H_{20}N_6O_7$	30 (ip)	100	75	83	All sterilized
					100 (oral)	88	86	86	
2	$-CH(OH)-C_6H_4-N=C(NHCOOMe)_2$	OMe	>290	$C_{21}H_{22}N_6O_7$	30 (ip)	100	83	90	All sterilized
					100 (oral)	96	83	89	
3	$-CH(OH)-C_6H_4-N=C(NHCOOEt)_2$	OEt	250	$C_{24}H_{28}N_6O_7$	30 (ip)	75	29	48	All sterilized
					100 (oral)	25	55	42	
4	$-CH(OH)-C_6H_5$	OMe	>290	$C_{16}H_{15}N_3O_3$	30 (ip)	100	100	100	No sterilization
5	$-CH(OH)-C_6H_4-NH_2(m)$	OMe	>290	$C_{16}H_{16}N_4O_3$	30 (ip)	50	50	50	No sterilization
6	$-CO-C_6H_5$	Et	238	$C_{17}H_{15}N_3O_2$	30 (ip)	75	100	91	All sterilized
					100 (oral)	100	86	90	
7	$-CH(OH)-R^2$	OMe	>300	$C_{19}H_{18}N_6O_5$	30 (ip)	100	100	100	All sterilized
8	$-CO-R^3$	Me	>300	$C_{19}H_{16}N_6O_3$	30 (ip)	100	87	93	All sterilized
9	$-CO-C_6H_5$	A	>300	$C_{29}H_{20}N_6O_3$	30 (ip)	100	100	100	No sterilization
					100 (oral)	83	70	75	
10	B	OMe	126–128	$C_{24}H_{33}N_5O_4$	30 (ip)	0	0	0	No sterilization
11	$-CO-C_6H_5$	Me	280	$C_{16}H_{13}N_3O_2$	30 (ip)	100	100	100	No sterilization
12	$-S-C_6H_5$	Me	270	$C_{15}H_{13}N_3OS$	30 (ip)	100	100	100	No sterilization
13	C	OMe	178–179	$C_{21}H_{25}N_5O_4$	30 (ip)	48	0	100	All sterilized

R^2 = 2-Methoxycarbonylaminobenzimidazol-5-yl.
R^3 = 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.



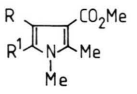
Compd. No.	R	m.p. [°C]	Molecular formula	Mass M^+ at m/z	Dose (mg/kg × 5)	Antifilarial activity % death of adult worms Males	Females	Total	Effect on adult female worms
14	$-SO_2-C_6H_4-NCS(p)$	250	$C_{22}H_{14}ClN_3O_2S_2$	451	30 (ip)	0	0	0	All sterilized
15*	$-NH-CS-R^1$	220	$C_{25}H_{24}ClN_5S$	461	30 (ip)	0	0	0	All sterilized
16*	$-CO-R^2$	190	$C_{21}H_{21}ClN_4O$	380	30 (ip)	0	0	0	All sterilized

* R^1 = 1-phenylpiperazin-4-yl
R^2 = 1-methylpiperazin-4-yl.

Compounds reported in Tables III and IV are 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.

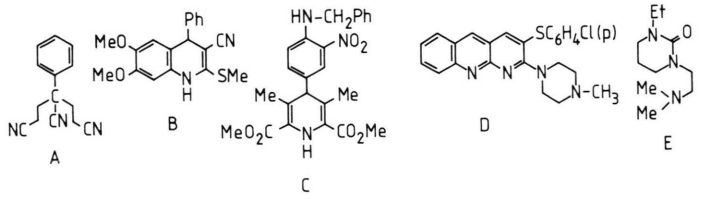


Compd. No.	R	R^1	m.p. [°C]	Molecular formula	Mass M^+ at m/z	Antifilarial activity Dose mg/kg × 5	% death of adult worms Male	Female	Total	Effect on adult female worms
17	3,4-Methylene dioxyphenyl	CH = NNHCSEt	170 (d)	$C_{19}H_{21}N_3O_4S_2$	418*	30 (ip)	50	50	50	No sterilization
18	3,4-Dimethoxyphenyl	Me	203–204	$C_{20}H_{25}N_3O_4S_2$	303	30 (ip) 100 (oral)	100 25	100 63	100 50	No sterilization
19	C_6H_5	$-CH_2OCH_2-R^2$	236	$C_{30}H_{32}N_2O_5$	500	30 (ip) 100 (oral)	81 94	50 92	65 93	All sterilized

R^2 = 1,3-Dimethyl-2-methoxycarbonyl-4-phenylpyrrol-5-yl.
* Indicates ($M^+ - 1$).

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

Compd. No.	Structure	m.p. [°C]	Molecular formula	Mass M^+ at m/z	Dose mg/kg × 5	Antifilarial activity % death of adult worms Males	Females	Total	Effect on adult female worms
20	A	59–60	$C_{14}H_{13}N_3$	223	30 (ip) 100 (oral)	100 0	100 0	100 0	No sterilization No sterilization
21	B	160–162	$C_{18}H_{15}ClN_2O_2$	326	30 (ip)	0	0	0	All sterilized
22	C	206–207	$C_{24}H_{25}N_3O_6$	451	30 (ip)	50	50	50	No sterilization
23	D	148	$C_{23}H_{21}ClN_4S$	420	30 (ip)	100	100	100	No sterilization
24	E	Oil	$C_{10}H_{21}N_3O$	199	30 (ip)	0	0	0	All sterilized



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

The authors thank their respective research and technical staff for valuable help in carrying out the work reported in this paper.

[1] Communication No. 4537 from Central Drug Research Institute, Lucknow (India).
[2] G. W. Hunter, J. C. Swartzwelder, and D. F. Clyde (eds.), Tropical Medicine, 5th ed., W. B. Saunders Company, Philadelphia 1976.
[3] P. A. J. Janssen, Prog. Drug Res. **18,** 191 (1974).
[4] S. Sharma, Prog. Drug Res. **30,** 473 (1986).
[5] S. Gupta, J. K. Srivastava, J. C. Katiyar, J. Singh, and D. S. Bhakuni, Indian J. Expt. Biol. **25,** 871 (1987).
[6] R. Sharma, V. Ojha, J. Singh, D. S. Bhakuni, J. K. Srivastava, S. Gupta, and J. C. Katiyar, Indian J. Chem. **28B,** 702 (1989).
[7] S. S. Naim, S. K. Singh, S. Sharma, S. Gupta, A. M. Khan, M. K. Jain, S. N. Singh, R. K. Chatterjee, and J. C. Katiyar, Indian J. Chem. **29B,** 464 (1990).
[8] S. K. Singh, S. S. Naim, and S. Sharma, Indian J. Chem. **27B,** 1015 (1988).
[9] S. K. Singh, S. S. Naim, S. Sharma, S. Gupta, and J. C. Katiyar, Indian J. Chem. **28B,** 397 (1989).
[10] M. Shamim, Studies on the Synthesis of Possible Antifertility and Anthelmintic Agents, Ph.D. dissertation, Aligarh Muslim University, Aligarh 1987.
[11] P. M. S. Chauhan, S. Sharma, and D. S. Bhakuni, Indian J. Chem. **25B,** 828 (1986).
[12] P. M. S. Chauhan, S. Sharma, and D. S. Bhakuni, Indian J. Chem. **25B,** 1142 (1986).
[13] A. Mukherjee, V. L. Sharma, M. Seth, and A. P. Bhaduri, Indian J. Chem. **28B,** 537 (1989).
[14] A. M. Kaddah, A. M. Khalil, and N. I. Tawfik, Indian J. Chem. **19B,** 122 (1980).
[15] A. K. Fateen, S. M. A. Rahman, and A. M. Kaddah, Indian J. Chem. **11,** 225 (1973).
[16] A. Mukherjee, M. Seth, and A. P. Bhaduri, Indian J. Chem. **28B,** 391 (1989).
[17] A. Mukherjee, M. S. Akhtar, V. L. Sharma, M. Seth, A. P. Bhaduri, A. Agnihotri, P. K. Mehrotra, and V. P. Kamboj, J. Med. Chem. **32,** 2297 (1989).
[18] R. P. Srivastava, Neelima, and A. P. Bhaduri, Synthesis **1987,** 512.
[19] H. Singh, S. Sharma, R. N. Iyer, and N. Anand, Indian J. Chem. **14B,** 528 (1976).
[20] F. Hawking and P. Sewell, Brit. J. Pharmacol. **3,** 285 (1948).
[21] G. Laemmler, H. Herzog, and H. R. Schuetze, Bull. WHO **44,** 765 (1971).
[22] S. Sharma and S. Abuzar, Prog. Drug Res. **27,** 85 (1983).
[23] G. B. Barker, J. G. Mercet, J. A. Svoboda, M. J. Thompson, H. H. Ress, and R. E. Howells, Parasitology **99,** 409 (1989).