Segregation of Activity Profile in Benzimidazoles: Effect of Spacers at 5(6)-Position of Methyl Benzimidazole-2-carbamates [1]

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Anthelmintic Activity, Benzimidazole-2-carbamates, Spacers, Segregation of Efficacy

The design and synthesis of a series of methyl 5(6)-substituted benzimidazole-2-carbamates as possible anthelmintics have been described. A rational analysis of the structural parameters which segregate the activity of resulting benzimidazole-2-carbamates against enteric and tissue dwelling helminths is presented. The influence of single and multiple spacers, which link the pharmacophores at 5(6)-position of benzimidazole-2-carbamate, on the activity against *Ancylostoma ceylanicum* (hookworm), *Syphacia obvelata* (pinworm), *Hymenolepis nana* (tapeworm), *Litomosoides carinii* and *Acanthocheilonema viteae* (filarial worm) has been presented. This analysis indicates that for activity against intestinal helminth the presence of one spacer holding the pharmacophore approximately 3 Å apart from the parent nucleus is usually preferred. While for activity against tissue dwelling parasite, the repetition of the benzimidazole-2-carbamate nucleus joined together through the 5,5′-position with one spacer kept apart by distance of 3 Å unit is usually desired.

Introduction

Among a large number of prototype structures explored to generate new drugs for helminth diseases, the benzimidazoles have emerged as one of the most versatile classes of heterocycles which has undergone extensive molecular modification since the discovery of thiabendazole in 1960 [2, 3]. Efforts to optimize the biological profile of benzimidazole has resulted in the discovery of a number of novel anthelmintics [4] such as mebendazole (**27**), flubendazole (**28**), fenbendazole (**15**), ciclobendazole (**26**), oxibendazole (**9**) and the recently introduced luxabendazole, all being the derivative of carbendazim (**1**) which is an established fungicide rather than an anthelmintic.

A careful analysis of the disposition of various pharmacophores on different positions of benzimidazoles indicates that the anthelmintic profile of benzimidazole is governed by the nature of substituents present at its 2- and 5(6)-positions. More detail studies carried out in this direction have unequivocally established the need of a methoxycarbonylamino function at 2-position except for triclabendazole (**VIII**) which has methylthio function at 2-position. In addition, it has been observed that the biological potency of the molecule can be modulated by introducing a proper pharmacophore at 5-position. In majority of the cases the groups linked to the 5-position through a "spacer" like O, S, SO, SO_2, CO, CHOH, CONH, NHCO etc. have a marked effect on the overall biological profile of the molecule. It appears that these spacers, play a major role in inducing biological response against both tissue and intestinal helminths. Accordingly, a large variety of 2,5-disubstituted benzimidazoles were prepared with or without a spacer at their 5(6)-position in this laboratory. Evaluation of these compounds for their anthelmintic activity resulted in the development of a number of compounds, some being specific to intestinal roundworms and tapeworms like *Ancylostoma ceylanicum, Syphacia obvelata, Hymenolepis nana* etc., while other showed better activity against the tissue-dwelling helminths like *Litomosoides carinii* and *Acanthocheilonema viteae*. However, no attempt has been made to study the effect of above spacers in segregating the biological activity against intestinal and tissue dwelling helminths. The present paper attempts to rationalize the effect of spacers in segregating the activity pro-

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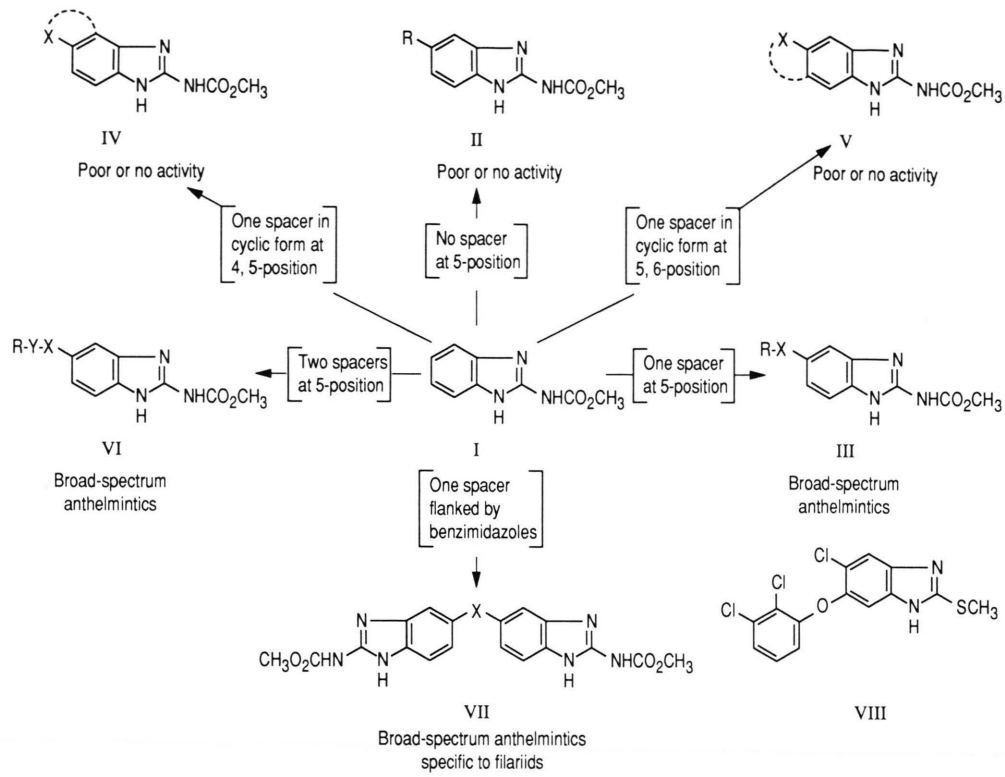


file of benzimidazoles against the intestinal and tissue-dwelling helminths.

Materials and Methods

Synthesis

The synthesis of various methyl 5(6)-substituted benzimidazole-2-carbamates (Table I–V) was achieved by cyclization of the corresponding *o*-phenylenediamines with 1,3-dicarbomethoxy-S-methylisothioureas according to literature procedures [2].

Parasites and hosts

All the benzimidazoles were evaluated against *Ancylostoma ceylanicum* (hookworm) in hamsters, *Syphacia obvelata* (oxyurid) in mice, *Hymenolepis nana* (cestode) in rats, *Litomosoides carinii* in cotton rats and *Acanthocheilonema viteae* in *Mastomys natalensis (coucha)*. Compounds being insoluble in water were made to fine suspensions with little amount of Tween 80 [5]. Two to three animals were used for each dose level and two to five replicates were done. The assessment of efficacy was done on autopsy under deep ether anaesthesia.

1. Ancylostoma ceylanicum (Hookworm)

The drug testing was carried out using golden hamsters of either sex (50–60 g) infected orally with 50 ± 5 L_3 (3rd stage larvae) of *A. ceylanicum* [6]. On day 17 post-inoculation, infection was checked by ovoscopic examination. Hamsters found positive were administered orally or intraperitoneally with compounds under test. The efficacy was expressed in terms of absolute clearance of parasite from the host and percent worm reduction.

2. Syphacia obvelata (Oxyurid)

The compounds were evaluated in either sex of Swiss albino mice (20–25 g) carrying naturally acquired infection of *S. obvelata*. The criterion of efficacy was the absolute clearance of the parasites from the treated mice [5, 6].

3. Hymenolepis nana (Cestode)

The efficacy assessment was made against egg (200) induced infection of *H. nana* in albino male rats (35–40 g). The basis of drug efficacy was the complete elimination of worms along with scolices from the treated animals [7, 8].

4. Litomosoides carinii (Filarial worm)

The micro- and macrofilaricidal activities of compounds were evaluated against *L. carinii* infection in cotton rats *(Sigmodon hispidus)*. The infection was transmitted to cotton rats through the vector *Liponyssus bacoti* by the method of Hawking and Sewell [9]. At the end of the prepatent period, animals showing 250 or more microfilariae per 5 ml of blood were chosen for screening. Five animals formed an experimental group. Blood samples for experimental and control animals were examined before starting the treatment. All the compounds were given intraperitoneally (except compound **65** which was also administered orally) for 5 consecutive days. Blood smears of animals examined for microfilariae at weekly intervals up to 6 weeks from the start of the treatment. On day 42, all the treated and control animals were sacrified and the condition of adult male and female worms observed. The micro- and macro filaricidal action was assessed as described by Laemmler *et al.* [10]. For monitoring the macrofilaricidal activity, blood samples of the rodents infected with *L. carinii* were examined at weekly intervals up to day 91.

5. Acanthocheilonema viteae (Filarial worm)

The micro- and macrofilaricidal activities of the compounds were assessed against *A. viteae* in *Mastomys natalensis* as described for *Litomosoides carinii*. The infection was transmitted to *Mastomys* through the vector *Ornithodoros mobata* by the method of Worms *et al.* [11].

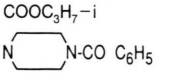
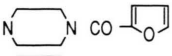
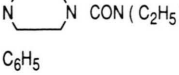
Results

1. A. ceylanicum

In general all the 5(6)-substituted benzimidazole-2-carbamates exhibited a wide-spectrum of activity against the hookworm, *A. ceylanicum* in hamsters. Incorporation of a pharmacophore with no spacer at 5(6)-position of the benzimidazole-2-carbamate (Table I) gave biological activity though not of very high order. Most of the compounds (**1–8**) caused 47–100% removal of the worms at the dose of 250 mg/kg. The most effective compounds of this class are **3** and **4** showing activity at 1.25 mg/kg and 6.25 mg/kg respectively. Parbendazole (**1**) was found to remove 100% *A. ceylanicum* worm at a dose of 25 mg/kg.

Incorporation of a pharmacophore at 5(6)-position of benzimidazole-2-carbamate through a spacer was found to have pronounced effect in evoking biological response against *A. ceylanicum*. It was observed that introduction of a spacer like O, S, SO, SO_2 between the pharmacophore and benzimidazole nucleus at 5-position had only marginal effect in eliciting anthelmintic activity against

Table I. Anthelmintic profile of benzimidazole-2-carbamate (type II) with no "spacer" at 5(6)-position.

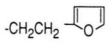
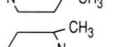
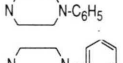
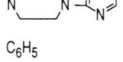
Compd. No.	R	Activity against *A. ceylanicum** Dose [mg/kg]	% Clearance	*S. obvelata** Dose [mg/kg]	% Clearance	*H. nana** Dose [mg/kg]	% Clearance	Reference
1	$-(CH_2)_3CH_3$ (parbendazole)	25	100	10–12	90	250	92	[13–15]
2	$COOCH_3$	250	100	250	100	250	0	[16]
3	$COOC_3H_{7}$-n	1.25	88	250	0	250	0	[16]
4	$COOC_3H_7$-i	6.25	99	250	100	250	0	[16]
5		250	0	–		250 × 3	100	[17]
6		250 × 3	58.3	–		250	100	[17]
7		250 × 3	47.2	–		250	100	[17]
8	C_6H_5	25 × 3	100	–		250	0	[18]

* The drugs were administered orally.
0 Inactive.
– Not done.

the hookworm. The compounds (**9–24**) in general, exhibited no activity at a single oral dose of 250 mg/kg except for compound **17,** which caused 100% elimination of the worms at a dose of 12.5 mg/kg.

The most potent activity was obtained when a carbonyl group was introduced at the 5(6)-position of benzimidazole-2-carbamate. In majority of the cases compounds (**27, 29, 31–35**) exhibited appreciable activity against *A. ceylanicum*. The best being mebendazole (**27**) showing 100% clearance of the parasite at a dose of 1 mg/kg. Attempts to attach an electron donating group to the carbonyl group thereby reducing its electrophilic character retained the anthelmintic profile against *A. ceylanicum*. However, none of the compounds (**31–35**) thus generated exceeded the potency of mebendazole (Table II).

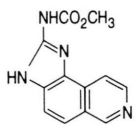
Table II. Anthelmitic profile of benzimidazole-2-carbamates (type III) with one "spacer" at 5(6)-position.

Compd. No.	R	X	Activity against *A. ceylanicum* Dose [mg/kg]	% Clearance	*S. obvelata* Dose [mg/kg]	% Clearance	*H. nana* Dose [mg/kg]	% Clearance	*L. carinii*⁺ Dose [mg/kg]	% Clearance	*A. viteae*⁺ Dose [mg/kg]	% Clearance	Reference
9	C_3H_7 (n)	O		–		–	250	99.5		–		–	[14]
10	C_6H_5	O		–		–	250	100	150	85 (maf)[a]		–	[14, 1
11	$C_6H_4NH_2$ (4)	O	250	0	250	0	250 × 3	0		–		–	[20]
12	C_6H_4NCS (4)	O	250	0	250	0	250 × 3	0		–		–	[20]
13	$C_6H_4NHCOCH_3$ (4)	O	250	0	250	0	250	0		–		–	[20]
14	C_3H_7 (n)	S		–		–	250	80.5		–		–	[14]
15	C_6H_5	S		–		–	250	100		–		–	[14]
16	$C_6H_4NH_2$ (4)	S	250	0	250	0	250	0		–		–	[21]
17	C_6H_4NCS (4)	S	12.5	100		–	30	100		–		–	[21]
18	$C_6H_4NHCOCH_3$ (4)	S	250	0	250	0	250	0		–		–	[21]
19		S	250	0	250	0	250	0		–		–	[22]
20	C_6H_5	SO		–	10 (31 ppm)	100	40 (125 ppm)	100		–		–	[23]
21	$C_6H_4NH_2$ (4)	SO_2	250	0	250	0	250	0		–		–	[21]
22	C_6H_4NCS (4)	SO_2	250	0	250	0	250	0		–		–	[21]
23	$C_6H_4NHCOCH_3$ (4)	SO_2	250	0	250	0	250	0		–		–	[21]
24		SO_2	250	0	250	0	250	0		–		–	[22]
25	CH_3	CO	250	0	40	0	250	0		–		–	[24, 2
26		CO		–	2.5	100		–		–		–	[25]
27	C_6H_5	CO	1	100	250	100	250	100	10	100 (maf)	6.25	100	[26, 2
28	C_6H_4F (4)	CO		–	0.16	100	50	0	150	99 (maf)		–	[19, 2
29		CO	100	100		–	250	100		–		–	[24]
30		CO	3.12	100	0.63	100	250 × 3	100		–		–	[25, 2
31		CO	1.56	59	12.5 × 3	100	250 × 3	100	30	0	50	0	[30]
32		CO	6.25	65	250	100	250 × 3	100	30	0	50	0	[30]
33		CO	6.25 3.12	100 83–97		–	50	100		–		–	[31]
34		CO	6.25 3.12	100 77–100		–	50	10		–		–	[31]
35		CO	6.25 3.12	100 88–100	12.5 × 3	100	25	100	30	97 (mf)[b] 100 (maf)		–	[31]
36	C_6H_5	CHOH	5	99.1	50	100	100 × 3	100		–		–	[29]
37		CHOH	3.12	100	25 × 3	100	250 × 3	100		–		–	[29]

⁺ Compounds were given to animals intraperitoneally for 5 days.
[a] Macrofilariae (adult filarial worms).
[b] Microfilariae.

Table III. Anthelmintic profile of benzimidazole-2-carbamates (type IV and V) with "one spacer" in cyclic form at 4(5)- and 5(6)-position.

d.	Structure	Activity against *A. ceylanicum* Dose [mg/kg]	% Clearance	*S. obvelata* Dose [mg/kg]	% Clearance	*H. nana* Dose [mg/kg]	% Clearance	*L. carinii* Dose [mg/kg]	% Clearance	*A. viteae* Dose [mg/kg]	% Clearance	Reference
		250	0	–		250	0	30	0	50	0	[32]
		250 × 3	50	–		250	0	30	0	50	0	[33]
		250 × 3	26.6	–		250	0	30	0	50	0	[33]
		250	65	–		250	0	30	0	50	0	[34]
		250	55	–		250	0	–		–		[35]
		250	0	–		250	0	–		–		[35]
		250	87	–		250	100	–		–		[36]
		250	0	–		250	100	–		–		[36]
		250	0	–		250	100	–		–		[36]



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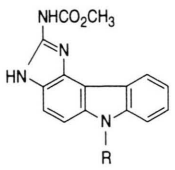
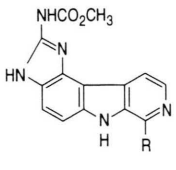
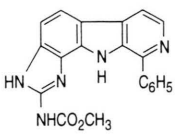


39, R=CH_3
40, R=$CH_2C_6H_5$



42, R=H
43, R=C_6H_5



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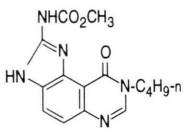

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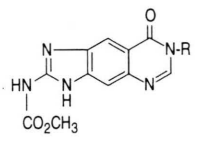


45, R=CH_3
46, R=C_4H_{9}-n

Reduction of the keto group of mebendazole (**27**) and its, thienyl congener **30** gave the corresponding hydroxy compounds **36** and **37.** Of these **36** has been found to be the active metabolite of **27** showing 99% clearance of the hookworm at a single oral dose of 5 mg/kg. On the other hand the hydroxy derivative **37** of the thienyl congener of the mebendazole was found to be equipotent to the parent molecule showing 100% activity at a dose of 3.12 mg/kg.

Incorporation of one spacer in a cyclic structure, fused to the 4,5-position (**38–44**) or 5,6-position (**45–46**) led to the benzimidazole-2-carbamates with poor or no activity against *A. ceylanicum* (Table III).

Enlargement of the inter-pharmacophoric distances by introducing two space (CONH or NHCO) in benzimidazole-2-carbamates (Table IV) had detrimental effect on the biological activity. The compounds with a CONH spacer (**47–55**) exhibited significant activity, though not of very high order against experimental hookworm infection. The noteworthy compounds of this class are **53** and **54,** which caused 100% elimination of the parasite at single oral doses of 25 mg/kg and 50 mg/kg respectively. Inter change of the spacer CONH into NHCO compounds (**56–59**) led to loss of activity in most of the cases. Reduction of the CO group of NHCO into $NHCH_2$ was found to give compound (**60**) with no anthelmintic activity.

In general, the bisbenzimidazole-2-carbamates joined together through one spacer (**62–67**) O, S, CO and CH_2 exhibited almost 100% clearance of the hookworm at the dose ranging 12.5–50 mg/kg. Reduction of **65** into its corresponding hydroxy derivative **67** exhibited better activity against *A. ceylanicum* than its parent drug. However, conversion of **63** into its sulphone **64** was associated with lowering of anthelmintic activity. It was also observed that increase in the size of spacer led to the compounds (**68–73**) which were associated with either poor activity or complete loss of activity against *A. ceylanicum.*

2. S. obvelata

The benzimidazole-2-carbamates having no spacer at 5(6)-position exhibited almost a similar pattern of activity against *S. obvelata* as reported for *A. ceylanicum.* Perbendazole (**1**) caused 90% of the clearance of the parasite at an oral dose of 10–12 mg/kg, while 100% reduction of worm load achieved by compounds **2** and **4** at a higher dose of 250 mg/kg (Table I).

Introduction of one spacer like O, S, SO or SO_2 has been found to marginally improve the biologi-

Table IV. Anthelmintic profile of benzimidazole-2-carbamates (type VI) with "two spacer" at 5(6)-position.

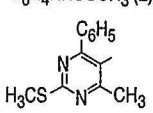
Compd. No.	R	X	Y	Activity against *A. ceylanicum* Dose [mg/kg]	% Clearance	*S. obvelata* Dose [mg/kg]	% Clearance	*H. nana* Dose [mg/kg]	% Clearance	*L. carinii* Dose [mg/kg]	% Clearance	*A. viteae* Dose [mg/kg]	% Clearance	Reference
47	CH_3	CO	NH	250	95	–		250	100	–		–		[31]
48	C_6H_5	CO	NH	250	87	–		250	0	–		–		[31]
49		CO	NH	250	0	–		250	0	–		–		[31]
50		CO	NH	250 50	100 55.5	–		250	100	–		–		[31]
51		CO	NH	250	95	–		250	0	–		–		[31]
52		CO	NH	250	75	–		250	0	–		–		[31]
53		CO	NH	25 10	100 57–82	–		25	100	–		–		[31]
54	C_2H_5	CO	NC_2H_5	50 25	100 90	–		3.12	100	–		–		[31]
55		CO	NH	250	0	–		250	0	–		–		[17]
56	C_6H_5	NH	CO	250	0	–		250	0	–		–		[37]
57		NH	CO	250	50	–		250	50	–		–		[37]
58		NH	CO	250	100	–		250	100	–		–		[37]
59	$C_6H_4NHCOCH_3$ (2)	NH	CO	250	0	–		250 × 3	100	30 × 5	0	–		[38]
60		NH	CH_2	250	0	–		250	0	30 × 5	0	–		[39]

Table V. Anthelmintic profile of benzimidazole-2-carbamates (type VII) with "one spacer" at 5(6)-position in bis benzimidazole.

Compd. No.	X	Activity against *A. ceylanicum* Dose [mg/kg]	% Clearance	*S. obvelata* Dose [mg/kg]	% Clearance	*H. nana* Dose [mg/kg]	% Clearance	*L. carinii* Dose [mg/kg]	% Clearance	*A. viteae* Dose [mg/kg]	% Clearance	Reference
62	O	25 125	100 94–100	100 × 3	100	100	60	–		–		[20]
63	S	50 25	100 64–100	–		100	70	–		–		[40]
64	SO_2	250 × 3	100	–		250 × 3	100	–		–		[40]
65	CO	50 25	100 50	100 50	100 66.6	250 100	100 50	10(ip)(maf) 100(oral)(maf)	100 100	25(ip)(maf) 150(oral)(maf)	100 100	[26]
66	CH_2	50	93.5	250	0	250	100	30 × 5	0	50 × 5	0	[26]
67	CHOH	25 12.5	100 97.1	100 50	100 88.8	100 50	100 50	–		–		[27]
68	$-S(CH_2)_2S-$	250	0	–		250	0	30 × 5	0	–		[24]
69	$-SO_2(CH_2)_2SO_2-$	250	0	–		250	0	30 × 5	0	–		[24]
70	$-CONH(CH_2)_2NHCO-$	250	50	–		250	0	–		–		[31]
71	$-CONH\,C_6H_4SO_2C_6H_4NHCO-$	250	48.2	–		250	0	–		–		[31]
72	$-CONH\,C_6H_4CH_2CH_2NHCO-$	250	50	–		250	0	–		–		[31]
73	$-NHCO\,C_6H_4CONH-$	250 50	100 66.6	–		250	0	–		–		[31]

cal profile of the compounds. All the compounds (**9–24**) of this class except **20** were found to be inactive against *S. obvelata* at a single oral dose 250 mg/kg. Ricobendazole (**20**) caused 100% removal of the worms at a dose of 10 mg/kg (31 ppm) in experimental animals. Replacement of the above spacer by a carbonyl function was found to have marked effect on the overall biological profile in general and activity against *S. obvelata* in particular. Most of the compounds (**25–35**) of this class exhibit 100% elimination of the helminth at a dose ranging from 0.16–250 mg/kg. The most effective compounds flubendazole (**28**) and **30** showing activity a dose of 0.16 mg/kg and 0.63 mg/kg, respectively (Table II). Partial reduction of **27** into **36** increases the biological profile (100% clearance of worm at 50 mg/kg), while in the case of **37** a mark decrease in anthelmintic activity (100% activity at 3 doses of 25 mg/kg) was observed.

Among the bisbenzimidazole-2-carbamates having one spacer flanked by two benzimidazole-2-carbamates, the optimal activity was achieved with compound (**65**) having carbonyl function, which showed 100% activity at a dose of 100 mg/kg. When the dose was lowered to 50 mg/kg, it only eliminated 66.6% of the parasite. The metabolite (**67**) of compound (**65**) exhibited a better spectrum of activity against this parasite. It eliminated 88.8% and 100% of the worms at a dose of 50 mg/kg and 100 mg/kg respectively.

3. H. nana

The activity of various benzimidazole-2-carbamates, having different substitution pattern at 5(6)-position is given in Table I–V. The compounds having no spacer showed poor activity against the dwarf tapeworm, *H. nana* in mice. Compounds (**1, 5–7**) were found to eliminate the 100% tapeworm at a dose of 250 mg/kg, while other compounds (**2–4, 8**) were found to be inactive at this dose level (Table I).

Unlike the activity against *S. obveleta* the introduction of one spacer like O, S, SO, SO_2 had marked influence in evoking biological activity. Compounds (**9, 10, 14–17** and **20**) removed 85-100% of *H. nana* at the single oral dose, ranging from 30–250 mg/kg. The best compound was found to be **17** and **20** exhibited 100% activity at a single oral dose of 30 mg/kg and 40 mg/kg (125 ppm) respectively. An interesting profile of activity was also observed, when carbonyl was introduced at the 5(6)-position of the benzimidazole-2-carbamate. In general, the compounds killed 100% of the tapeworm at a dose ranging from 25–250 mg/kg. Compounds having a morpholino or piperazinyl residue liked to carbonyl function (**33–35**) exhibited 100% elimination of the worm at a dose of 25–50 mg/kg (Table II). Conversion of **27** and **30** into the corresponding hydroxy compounds **36** and **37,** respectively, did not alter the biological profile significantly. The resulting compounds showed almost equipotent activity against *H. nana* to their parent drug.

Incorporation of the spacer in a cyclic form, which, in turn, forms the hetero atom of pyridine, indole or azaindole skeleton (**33–43**) led to complete loss of activity against *H. nana*. However, formation of a quinazolone skeleton utilizing the spacer present at 5(6)-position of benzimidazole-2-carbamates (**44–46**) gave 100% response against *H. nana* a dose of 250 mg/kg (Table III).

Among the benzimidazole-2-carbamates having two spacers at 5(6)-position, the presence of a carbonyl (CO) and NH was found to elicit wide spectrum of activity against *H. nana*. It was observed that compounds having CONH spacer where CO is directly linked to benzimidazole-2-carbamate exhibited better activity than the benzimidazole-2-carbamate having CONH, where NH group is directly linked to benzimidazole. The effective compounds of the former class were compounds (**47, 50, 53** and **54**) of which optimal activity exhibited by **54** at a dose of 3.12 mg/kg. The active compound belonging to a latter class are **57–59** showing 50–100% elimination of the tapeworm at a dose of 250 mg/kg (Table IV).

In the bisbenzimidazole-2-carbamate, where the two heterocycles are separated by different spacers. The compounds (**62–67**) having spacers like O, S, SO_2, CO, CH_2 and CHOH were found to kill the parasite 70–100% at a dose of 100–250 mg/kg. Substitution of any of these spacers with other spacers having more functionality (**68–73**) led to complete loss of activity, indicating to the fact that internuclear distance in such compounds may be essential for activity (Table V).

4. L. carinii and A. viteae

It is difficult to draw the effect of the spacer at 5(6)-position of benzimidazole-2-carbamate on the

antifilarial activity against *L. carinii* and *A. viteae*. However, available data show that compounds having one spacer are associated with marked antifilarial activity for example, the oxygen congener (**10**) of mebendazole (**27**) showed 85% clearance of the adultworm in cotton rats infected with *L. carinii*. Mebendazole (**27**) and flubendazole (**28**) themselves show 100% activity against the macrofilaria of *L. carinii* and *A. viteae* at the dose ranging from 6.25–150 mg/kg given for 5 days. No activity was observed by substitution of phenyl group by 4-methyl piperidine (**31**) or 2-methylpiperidine (**32**) except **35** which have 2-pyridylpiperazine function, killed 97% of the microfilariae and 100% adultworm of *L. carinii* at a dose of 30 × 5 mg/kg.

Incorporation of one spacer in a cyclic structures **38–41** was associated with no activity against both the above filarial parasites. Similarly, no improvement in the antifilarial activity profile could be observed in benzimidazole-2-carbamates (**59–60**) having two spacers at 5(6)-position.

Among the bisbenzimidazole-2-carbamates joined at their 5(6)-position through one spacer like O, S, SO_2, CO, CH_2, CHOH etc. was found to have no activity against *L. carinii* and *A. viteae* except **65,** which caused 100% death of the microfilariae and adultworm of the above parasite, both by intraperitoneal (10–25 mg/kg) and oral (100–150 mg/kg) routes of administration.

Discussion

The examination of the anthelmintic profile of different methyl 5-substituted benzimidazole-2-carbamates against the intestinal nematodes *(A. ceylanicum, S. obvelata)*, tapeworm *(H. nana)* and filarids *(L. carinii, A. viteae)* summarized in Tables I–V, point out to the structure activity correlate which may be summarized as follows.

The benzimidazole-2-carbamates having no spacer (Table I), in general show poor or no activity against most of the helminths tested. This is probably due to the fact that compounds of type II though resist hydroxylation at 5-position to form inactive metabolites, do not exhibit a characteristic geometry of the pharmacophore thereby indicating to the fact that presence of a spacer is essential for anthelmintic activity. This observation may be best supported by compound **8,** which represents all the structural features of mebendazole except for the fact that a carbonyl spacer at 5(6)-position has been removed. The net result is that compounds with no spacer have either poor or no activity.

A further support to the above analysis may be received from the biological profile of the compounds summarized in Table II. All the compounds **9–37** have a pharmacophore linked to the 5-position of benzimidazole-2-carbamate through a spacer like O, S, SO_2, CO and CHOH. Careful analysis of the anthelmintic activity of this class compounds would indicate that the activity broadly follows the sequence O < S < SO < CO. This is probably due to the fact the benzimidazole-2-carbamates with oxygen spacer are resistant to various metabolic reactions at 5(6)-position, thereby are less bioavailable. Contrary to this the benzimidazole-2-carbamates having a sulphur spacer at 5(6)-position may under go successive cytochrome P450 dependent oxidations in the biophase to form sulphoxide and sulphone. Consequently compounds of this class are expected to attain higher peak plasma concentration with better anthelmintic activity. This may be further supported by the high anthelmintic activity exhibited by albendazole (**14**), fenbendazole (**15**), oxfendazole (**20**), of which **20** is the active metabolite of **15.** The development of two injectable sulphoxide anthelmintics by squibb laboratories may be taken as additional support to the above hypothesis. Thus compounds having carbonyl spacers exhibit maximal anthelmintic activity. The most effective member of this class are ciclobendazole (**26**), mebendazole (**27**), flubendazole (**28**) nocodazole (**30**) and CDRI 81-470 (**35**). All these compounds are highly prone to oxido-redox enzyme systems, leading to the formation of corresponding carbinols as one of the major metabolites. These carbinols may show additive or synergistic action with other metabolites arising due to metabolic reaction at 2-position. The resultant effect is better bioavailability and higher activity against both intestinal and tissue helminths. Recently McCracken and Lipkowitz [12] have analyzed various pharmacophoric requirement in benzimidazole-2-carbamate having one spacer at 5(6)-position and have concluded by molecular modelling that the 5-substituents twisted out of plane were more active than those anthelmintics with 5-substituents in plane.

Incorporation of one spacer in cyclic forms leading to the formation of heterocycles like **38–46** generally gives rise to poor activity (Table III). This indicates to the fact that the groups attached to 5-position through a spacer should have free rotation in order to attain a preferred L-shaped cleft confirmation while approaching to the receptor site.

The benzimidazole-2-carbamates having groups at 5-position linked by two spacers like CONH (**47–54**) and NHCO (**55–60**) also exhibit moderate to poor activity. Compounds of this class though prone to various metabolic degradations and also free rotation across C-C bond and C-N bond do not exhibit activity comparable to the corresponding benzimidazoles having one spacer. This points out to the fact that in addition to the groups susceptible to enzymatic reaction and free rotation of the groups attached to such spacers, the distance between benzimidazole nucleus and the pharmacophore attach to 5(6)-position is also determinantal in evoking biological activity.

Foregoing discussion would indicate that the presence of one spacer at 5(6)-position of benzimidazole-2-carbamate plays a major role in eliciting anthelmintic response. This may be supported by the analysis of the biological activity exhibited by the bisbenzimidazole-2-carbamate (**62–73,** Table V). As observed in compounds with one spacer (Table II), the profile of activity in bisbenzimidazole attach to one spacer like O, S, SO_2, CO, CH_2 and CHOH was complimentary $O < S < SO_2 < CO$. The results summarized in Table V. The weak anthelmintic activity exhibited by bisbenzimidazole-2-carbamates (**68–73**) would further indicate that the interpharmacophoric distances linked to at 5(6)-position of the benzimidazole-2-carbamate is also detrimental in giving rise to high anthelmintic activity.

The above discussion points out that the minimal structural requirement of the substituent present at 5(6)-position of benzimidazole-2-carbamate for the optimal anthelmintic activity are (i) the presence of only one spacer like O, S and CO which are prone to various metabolic reactions in biophase resulting in either bioavailability or conversion of the drug into an active metabolite having either synergistic or additive action with the parent molecule (ii) the pharmacophore attached with the above spacers must have a free rotation across its various single bonds which probably helps in attaining a preferred L-shaped orientation while approaching to the receptor site (iii) the distance between the benzimidazole nucleus and the pharmacophore linked at 5(6)-position through one spacer is also detrimental. Usually, 3 Å + of the spacer Van der Waals-radii. Simulation of these structural requirement is expected to lead compounds with activity against the intestinal helminths. For obtaining compounds having activity against the tissue dwelling parasites the compounds must have a spacer like CHOH or SO which imparts better water solubility or repetition of the benzimidazole-2-carbamate nucleus linked through only one spacer.

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