Studies in Enteric Anthelmintics: Activity Profile of Prodrug of a Bisbenzimidazole [1]

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Prodrug, Anthelmintic Activity, Bisbenzimidazole, Comparative Efficacy

The comparative anthelmintic activity of a possible prodrug, 2,2′-dicarbomethoxyamino-5,5′-dibenzimidazolyl methanol (**2**) with its parent compound 2,2′-dicarbomethoxyamino-5,5′-dibenzimidazolyl ketone (**1**) and the reference drug mebendazole (**3a**) is reported. At a dose of 25 mg/kg, compound **2** was 100% effective against *Ancylostoma ceylanicum* in hamsters. Compound **2** also exhibited a similar order of activity against *Syphacia obvelata, Hymenolepis nana* and *H. diminuta* at a dose of 100 mg/kg. The drug exhibited lethal effects against metamorphic forms of *A. ceylanicum* at a dose of 100 mg/kg. However the trichostrongylids, *Nippostrongylus brasiliensis* remained unaffected up to a dose of 250 mg/kg of **2.** Both **1** and **3a** exhibited inferior activity than **2** except against adult *A. ceylanicum*. The activity of **1** and **2** has been explained on the basis of their ability to resist systemic hydrolysis resulting in higher concentration of the active drug in biophase.

Introduction

Despite extensive work carried out towards design and synthesis of ideal benzimidazole-2-carbamate anthelmintics, it has not been possible to eliminate some of the basic limitations associated with this class of drugs [2–4]. The major limitations with benzimidazole drugs are their poor absorption through the gastrointestinal tract and irratic pharmacophoric behaviour in biophase [5]. Further, benzimidazole-2-carbamate molecules are extremely sensitive to even minor structural changes and, therefore, skeletal modifications play a detrimental role in successful drug design [6]. In an effort to obtain a benzimidazole anthelmintic which is devoid of the inherent limitations as discussed above, a series of methyl 5(6)-substituted benzimidazole-2-carbamates were synthesized in this laboratory [7–11] of which methyl 5(6)-[α-hydroxy-α-phenyl]methylbenzimidazole-2-carbamate (**3b**), an active metabolite of mebendazole (**3a**), was found to display excellent anthelmintic activity against a variety of intestinal and tissue-dwelling helminths [12].

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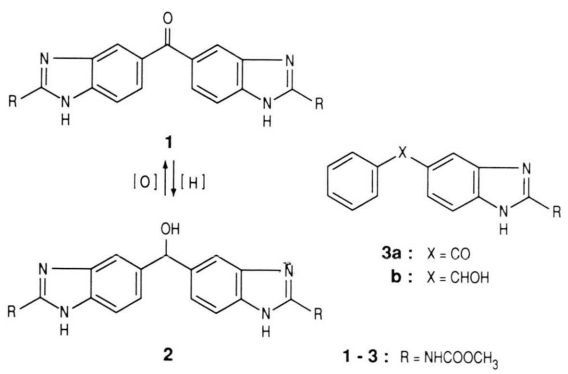
In a further probe in this direction, 2,2′-dicarbomethoxyamino-5,5′-dibenzimidazolyl methanol (**2**) was synthesized as a possible prodrug of 2,2′-dicarbomethoxyamino-5,5′-dibenzimidazolyl ketone (**1**), a compound synthesized in this laboratory and found to possess high order of activity against different luminal and tissue-dwelling helminths [13]. The basis for synthesizing **2** also lies in the fact that this molecule is expected to be highly sensitive to biological oxidoredox reactions by virtue of two electron-deficient rings attached to secondary alcohol.

The present communication is, therefore, concerned with the synthesis and anthelmintic activity of **2** against different helminth parasites in experimental animals.



Materials and Methods

Synthesis

The synthesis of 2,2′-dicarbomethoxyamino-5,5′-dibenzimidazolyl methanol (**2**) was achieved by cyclization of bis-(3,4-diaminophenyl) methanol with 1,3-dicarbomethoxy-S-methylisothiourea (yield 40%, m.p. >300 °C).

Parasites and hosts

Compounds **1, 2** and reference drug, mebendazole (**3a**) were evaluated against *Ancylostoma ceylanicum* (hookworm) in hamsters, *Nippostrongylus brasiliensis* (trichostrongylid) in rats, *Syphacia obvelata* (oxyurid) in mice, *Hymenolepis nana* (cestode) in rats and *H. diminuta* (cestode) in rats. Compounds being insoluble in water were made to fine suspensions with little amount of Tween 80 [14]. 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 60 ± 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.

For larvicidal action, the test compounds were either fed or injected intraperitoneally on day 1, 3 and 6 post-infection for evaluating effects on L_3, L_4 and L_5 stages, respectively [15]. Treated and untreated hamsters were sacrificed on day 17 post-infection when L_3 reached patency in the intestine. The efficacy assessment was similar to that described above.

2. Nippostrongylus brasiliensis (trichostrongylid)

Male rats of UF strain (30–40 g) infected subcutaneously with 500 L_3 served as experimental host. The therapeutic trials were initiated on day 9 post-infection (p.i.). The treated and untreated animals were autopsied on the third day of the last dose and efficacy assessment was made on percent worm reduction as described for *A. ceylanicum* [15].

3. 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 [6, 14].

4. 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 [16].

5. Hymenolepis diminuta (cestode)

The parasite was maintained through its intermediate host, grain beetles (*Tribolium confusum*), in laboratory bred albino rats (35–40 g). Rats found positive by ovoscopic examination on day 16 were used in the drug evaluation. The criterion of efficacy was the same as for *H. nana*.

Results

1. Ancylostoma ceylanicum

(a) Adulticidal action

Both the compounds **1** and **2** were found efficacious. Compound **2** however showed better activity than **1** as 100% efficacy was achieved at a dose of 25 mg/kg as against 50 mg/kg for compound **1** (Table I). At a lower dose of 12.5 mg/kg, compound **1** exhibited poor activity (40.4% worm reduction) whereas compound **2** reduced the worm-load to the tune of 97.1%. Mebendazole (**3a**) exerted excellent activity at a single oral dose of 1 mg/kg.

(b) Larvicidal action

Since compound **2** had remarkable efficacy, only this along with reference drug (**3a**) were investigated for larvicidal action. Promising action of compound **2** was recorded at a single oral dose of 100 mg/kg against L_3, L_4 and L_5 stages of

Table I. Effect of compounds **1, 2** and **3a** against adult helminth parasites.

Parasite (host)	Compounds	Dose mg/kg *per os*	Animal cured Treated (replicates)	Percent host clearance	Worm recovery mean with (range)	Percent worm reduction
A. ceylanicum (hamster)	**1**	50 × 1	9/9 (3)	100	0	100
		25 × 1	4/8 (3)	50	9.15 (6–20)	60.6
		12.5 × 1	0/5 (2)	0	15.65 (9–22)	40.4
		6.25 × 1	0/5 (2)	0	24.67 (21–35)	0
	2	25 × 1	6/6 (2)	100	0	100
		12.5 × 1	6/15 (5)	40	0.86 (4–8)	97.1
		6.25 × 1	0/9 (3)	0	4.44 (1–7)	83.2
		3.12 × 1	0/9 (3)	0	8.87 (5–15)	66.2
		1.56 × 1	0/9 (3)	0	19.00 (13–29)	24.6
		0.78 × 1	0/6 (2)	0	27.75 (20–35)	0
	3a	1.00 × 1	6/6 (2)	100	0	100
		0.5 × 1	1/6 (2)	16.66	6.75 (0–11)	74.6
N. brasiliensis (rat)	**1**	250 × 1	0/6 (2)	0	88.62 (61–111)	0
	2	250 × 1	0/4 (2)	0	84.60 (65–108)	0
	3a	250 × 1	6/6 (2)	100	0	100
		100 × 1	0/6 (2)	0	56.50 (45–75)	38.7
S. obelata (mouse)	**1**	100 × 1	6/6 (2)	100	–	–
		50 × 1	4/6 (2)	66.6	–	–
		25 × 1	0/6 (2)	0	–	–
	2	100 × 1	6/6 (2)	100	–	–
		50 × 1	8/9 (3)	88.8	–	–
		25 × 1	7/12 (4)	58.3	–	–
		12.5 × 1	0/6 (2)	0	–	–
	3a	250 × 1	6/6 (2)	100	–	–
		100 × 1	6/9 (3)	66.6	–	–
		50 × 1	0/4 (2)	0	–	–
H. nana (rat)	**1**	250 × 1	6/6 (2)	100	–	–
		100 × 1	4/8 (2)	50	–	–
		50 × 1	2/12 (3)	16.6	–	–
	2	100 × 1	5/5 (2)	100	–	–
		50 × 1	6/12 (4)	50.0	–	–
		25 × 1	0/6 (2)	0	–	–
	3a	400 × 3	0/6 (2)	0	–	–
H. diminuta (rat)	**1**	100 × 3	0/4 (2)	0	–	–
	2	100 × 3	4/4 (2)	100	–	–
	3a	100 × 3	0/6 (2)	0	–	–

Pooled control data	Parasite	No. of animals	Worm recovery mean with range
	A. ceylanicum	25	27.65 (19–35)
	N. brasiliensis	08	90.25 (69–115)
	S. obvelata	15	32.5 (21–70)
	H. nana	12	22.75 (15–66)
	H. diminuta	12	8.50 (5–12)

A. ceylanicum. Comparable efficacy was found with compound **3** (Table II). Intraperitoneal route of administration was superior to *per os* route.

2. N. brasiliensis

Compounds **1** and **2** showed no activity against this infection even at the initial dose of 250 mg/kg. However at a similar dose compound **3a** exhibited 100% efficacy.

3. S. obvelata

Promising action of compounds **1** and **2** was recorded against *S. obvelata.* At the dose of 100 mg/kg both the compounds showed 100% parasite

Table II. Efficacy of compounds **2** and **3a** against developing forms of *A. ceylanicum*.

Dose mg/kg × 1	Larval stage	Compound **2** Animal cured Treated (R)*	% Host clearance	Worm recovery mean with range	% Worm reduction	Compound **3a** Animal cured Treated (R)	% Host clearance	Worm recovery mean with range	% W reduc
100 (p.o.)	L_3	0/6 (2)	0	8.5 (4–12)	69.60	0/6 (2)	0	10.25 (8–15)	66.7
	L_4	5/6 (2)	83.33	1.36 (0–3)	94.80	6/6 (2)	100	0	100.0
	L_5	6/6 (2)	100.0	0	100.0	6/6 (2)	100	0	100.0
50 (p.o.)	L_3	0/6 (2)	0	10.50 (6–13)	56.3	0/6 (2)	0	18.50 (10–29)	41.2
	L_4	3/6 (2)	50.0	2.60 (1–5)	91.1	6/6 (2)	100.0	0	100.0
	L_5	5/5 (2)	100.0	0	100.0	6/6 (2)	100.0	0	100.0
25 (p.o.)	L_3	0/6 (2)	0	18.40 (14–21)	41.7	0/6 (2)	0	22.75 (20–27)	25.6
	L_4	1/6 (2)	16.66	4.80 (3–8)	82.7	0/6 (2)	0	6.50 (4–9)	78.5
	L_5	3/6 (2)	50.0	1.12 (0–3)	97.6	2/6 (2)	33.33	1.50 (0–3)	93.8
12.5 (p.o.)	L_3	0/4 (2)	0	23.70 (18–27)	24.8	0/6 (2)	0	25.65 (19–31)	16.2
	L_4	0/5 (2)	0	8.35 (5–14)	70.2	0/6 (2)	0	10.55 (7–15)	65.3
	L_5	0/4 (2)	0	3.83 (2–5)	85.4	0/6 (2)	0	4.66 (2–7)	81.4
100 (i.p.)	L_3	1/6 (2)	16.66	3.0 (1–9)	87.1	6/6 (2)	100.0	0	100.0
	L_4	9/9 (3)	100.00	0	100.0	6/6 (2)	100.0	0	100.0
	L_5	5/5 (2)	100.00	0	100.0	6/6 (2)	100.0	0	100.0
50 (i.p.)	L_3	0/4 (2)	0	7.75 (2–12)	66.6	6/6 (2)	100.0	0	100.0
	L_4	4/10 (4)	40.00	2.18 (1–5)	91.9	3/5 (2)	60.0	1.66 (0–5)	94.9
	L_5	5/5 (2)	100.0	0	100.0	6/6 (2)	100.0	0	100.0
25 (i.p.)	L_3	0/4 (2)	0	14.5 (11–17)	45.6	0/6 (2)	0	14.66 (10–18)	48.1
	L_4	0/5 (2)	0	3.8 (1–7)	86.5	1/5 (2)	20.0	5.0 (2–10)	84.8
	L_5	4/6 (2)	66.66	1.45 (0–2)	95.7	2/5 (2)	40.0	1.33 (0–4)	97.2
12.5 (i.p.)	L_3	0/4 (2)	0	21.25 (17–25)	31.3	0/5 (2)	0	20.25 (14–23)	33.9
	L_4	0/5 (2)	0	7.60 (3–14)	72.9	0/5 (2)	0	6.20 (2–10)	79.7
	L_5	0/6 (2)	0	2.64 (1–4)	92.4	1/5 (2)	20	7.95 (0–4)	93.6
		Pooled control data		Route	No. of animals	Worm recovery mean with range			
				Oral	25	29.85 (24–38)			
				Intraperitoneal	30	30.65 (27–37)			

*R = Replicate.

clearance in 100% of treated animals. At a dose of 50 mg/kg compound **2** showed superior activity (88.6% host clearance) than compound **1** (66.6% efficacy). Compound **3a** was found much inferior (Table I).

4. H. nana

Compound **2** at the dose of 100 mg/kg was fully effective whereas the same amount of compound **1** cured only 50% of the treated mice. At lower dose (50 mg/kg), compound **1** cleared only 16.6% of the treated mice whereas compound **2** cured 50%. The reference drug (**3a**) had no effect on this parasite (Table I).

5. H. diminuta

Compound **2** eliminated all the parasites from the treated rats at three oral doses of 100 mg/kg. Compounds **1** and **3a** in parallel trials, failed to exert any action at this dose level (Table I).

Discussion

As discussed earlier, the main objective of synthesizing 2,2′-dicarbomethoxyamino-5,5′-dibenzimidazolyl methanol (**2**) as the possible prodrug of 2,2′-dicarbomethoxyamino-5,5′-dibenzimidazolyl ketone (**1**) was to develop an anthelmintic agent which has better profile of activity against the major intestinal helminths. This was based on our earlier experience wherein one of the metabolites of mebendazole, methyl 5(6)-[α-hydroxy-α-phenyl]methyl benzimidazole-2-carbamate, obtained by reduction of the keto function, was found to possess better activity than the parent drug both against the intestinal and tissue-dwelling helminths. In the present study also, somewhat similar results were obtained. Compound **2** exhibits anthelmintic activity better than its parent drug (**1**) against intestinal helminths (*A. ceylanicum, S. obvelata, H. nana* and *H. diminuta*). This would indicate that compound **2** remains unabsorbed

through the gastrointestinal tract most of the time and only a small portion of it reaches the extra-intestinal organs leading to poor activity against tissue-dwelling worms. This is probably due to the fact that compound **2** is sensitive to oxidoredox reaction in biophase and a major amount to the drug gets converted into **1.** However, it is not clear if the net anthelmintic activity is the resultant of the additive or synergistic effects of both the bio-transformations.

The biological profile of compound **2** is very close to mebendazole (**3a**). Against *A. ceylanicum,* compound **2** is inferior to mebendazole but against *N. brasiliensis, S. obvelata* and *Hymenolepis* spp., it is superior to that of both mebendazole and parent drug (**1**). Thus, compound **2** may provide an useful lead to carry out further molecular modifications in benzimidazole anthelmintics to generate better drugs to combat both individual and mixed helminthiases.

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