Synthesis and Anthelmintic Activity of 5(6)-(Benzimidazol-2-ylcarbamoyl) and (4-Substituted piperazin-1-yl)benzimidazoles[1]

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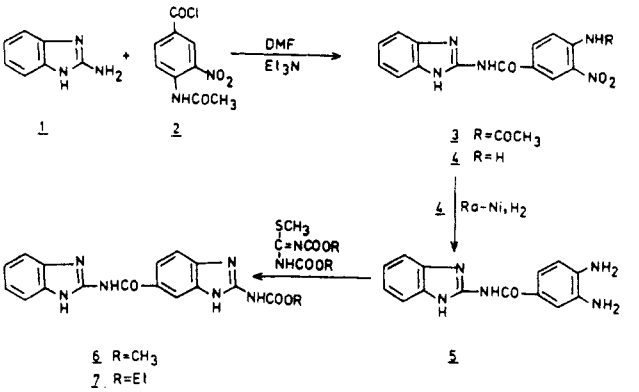
The synthesis of alkyl 5(6)-(benzimidazol-2-ylcarbamoyl)benzimidazole-2-carbamates (**6**, **7**), and alkyl 5(6)-(4-substituted piperazin-1-yl)benzimidazole-2-carbamates (**31–40**) has been carried out. When the compounds were tested for their anthelmintic activity against *Ancylostoma ceylanicum* in hamsters, *Hymenolepis nana* in rats, *Litomosoides carinii* in cotton rats, and *Dipetalonema viteae* in *Mastomys natalensis*, methyl 5(6)-(4-benzoylpiperazin-1-yl)benzimidazole-2-carbamate (**31**), methyl 5(6)-[4-(2-furoyl)piperazin-1-yl]benzimidazole-2-carbamate and methyl 5(6)-[4-[(diethylamino)carbonyl]piperazin-1-yl]benzimidazole-2-carbamate (**36**) showed 100% elimination of tapeworms *H. nana* at three oral doses of 100–250 mg/kg. Compounds **34** and **36** also killed the microfilariae and adult worms of *L. carinii* in cotton rats at an intraperitoneal dose of 30 mg/kg given for 5 days.

Despite numerous attempts to develop new structural prototypes in the search for more effective anthelmintics, the benzimidazoles still remain as one of the most versatile class of compounds possessing high activity against both enteric and tissue-dwelling helminths[2,3] and, therefore, are useful substructures for further molecular exploration. The chemistry and biological profile of various pharmacophores at the 5(6)-position of alkyl benzimidazole-2-carbamates has been worked out in detail;[2] however, the synthesis and anthelmintic evaluation of benzimidazoles carrying a pharmacophore derived from diethylcarbamazine[4] has received only limited attention. Thus, the synthesis of a series of alkyl 5(6)-(benzimidazol-2-ylcarbamoyl)benzimidazole-2-carbamates (**6**, **7**) and alkyl 5(6)-(4-substituted piperazin-1-yl)benzimidazole-2-carbamates (**31–40**) was initiated. Each of the benzimidazole analogues prepared has been tested for their antihookworm, antitapeworm, and antifilarial activities in experimental animals, and the results are reported in this paper.

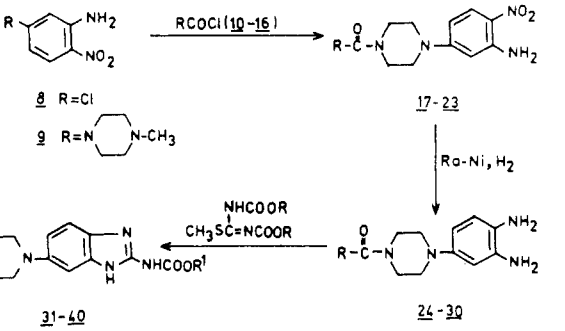
Chemistry. Reaction of 2-aminobenzimidazole (**1**)[6] with 4-acetamido-3-nitrobenzoyl chloride (**2**) in dry DMF and triethylamine gave 2-[*N*-(4-acetamido-3-nitrobenzoyl)-amino]benzimidazole (**3**), which was selectively hydrolyzed with KOH to yield 2-[*N*-(4-amino-3-nitrobenzoyl)-amino]benzimidazole (**4**). Reduction of **4** afforded the corresponding diamino compound 2-[*N*-(3,4-diaminobenzoyl)amino]benzimidazole (**5**), which on cyclocondensation with 1,3-dicarbalkoxy-*S*-methylisothioureas in refluxing ethanol yielded alkyl 5(6)-(benzimidazol-2-ylcarbamoyl)benzimidazole-2-carbamates (**6**, **7**) (Scheme I).

Treatment of 5-chloro-2-nitroaniline (**8**) with anhydrous piperazine in presence of K_2CO_3 in a steel bomb gave 1-(3-amino-4-nitrophenyl)piperazine (**9**)[7] which was condensed with different acid chlorides (RCOCl: **10**, R = Ph; **11**, R = cyclohexyl; **12**, R = 2-furyl; **13**, R = diethylamino; **14**, R = 1-hydroxy-2-naphthyl; **15**, R = 4-nitrophenyl; **16**, R = 2-pyrazinyl) in the presence of triethylamine in THF to yield 1-(3-amino-4-nitrophenyl)-4-substituted-piperazines (**17–23**). Hydrogenation of **17–23** afforded the corresponding 1-(3,4-diaminophenyl)-4-substituted-piperazines (**24–30**), which were treated in situ with 1,3-dicarbalkoxy-*S*-methylisothioureas in refluxing ethanol to form alkyl 5(6)-(4-substituted piperazin-1-yl)benzimidazole-2-carbamates (**31–40**) (Scheme II).

Scheme I



Scheme II



Results and Discussion

For the preliminary antihookworm[8,9] and antitapeworm[10,11] screening, the compounds were given initially at three oral doses of 250 mg/kg to hamsters infected with

Table I. Antitapeworm Activity of Compounds **31**, **34**, and **36** against *Hymenolepis nana* in Rats

compd	dose (oral), mg/kg × 3	% redn worms with scolices	% animals freed of worms
31	250	100	100
	100	100	100
	50	100	85
34	250	100	100
36	250	100	100
mebendazole	400	0	0

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Table II. Antifilarial Activity of Compounds **34** and **36** against *Litomosoides carinii* in Cotton Rats

compd	dose (ip), mg/kg × 5	% removal of microfilariae	persistence of microfilaricidal effect	% death of adult worms on day 42
34	30	>90	up to 42 days	90
36	30	>95	up to 42 days	100
DEC	6 (base)	>95	up to 7 days	nil

Ancylostoma ceylanicum and rats infected with *Hymenolepis nana*. Only compounds **31**, **34**, and **36** exhibited anthelmintic activity. A dose of 250 mg/kg of **34** and **36** given for 3 days removed 58.3 and 47.2% of hookworms from hamsters. In the antitapeworm evaluation compounds **31**, **34**, and **36** eliminated 100% of *H. nana* worms along with their scolices when treated with three oral doses of 250 mg/kg. At a lower dose (100 mg/kg × 3), compounds **34** and **36** showed no activity while **31** caused 100% removal of the tapeworms. However, when the dose of **31** was reduced further (50 mg/kg), it cleared 100% of the parasites from only 85% of the treated animals (Table I). In the simultaneous controlled trials, mebendazole removed 100% of the *A. ceylanicum* worms from hamsters at an oral dose of 1 mg/kg but was ineffective against *H. nana* in rats up to a dose of 400 mg/kg × 3.

The antifilarial testing[12,13] was carried out with an initial dose of 30 mg/kg given intraperitoneally for 5 days to cotton rats infected with *Litomosoides carinii*. In this study compounds **34** and **36** showed marked micro- and macrofilaricidal activities., Compound **34** decreased the microfilarial count gradually,. and on day 21 all microfilariae were removed from the blood. This effect persisted for 42 days. On autopsy, 90% of the adult worms were found to be dead.

Like **34**, compound **36** also exhibited slow action on the microfilariae. However, the onset of its microfilaricidal action was faster than **34** as it eliminated >95% of the microfilariae from the blood on day 14. It also caused 100% suppression of microfilaraemia up to 42 days. When the animals were sacrificed, all the adult male and female worms were found degenerated. Diethylcarbamazine (DEC) was used as the standard drug that showed activity only against microfilariae of *L. carinii* (Table II). At lower doses these compounds did not exhibit appreciable filaricidal efficacy.

Compounds **34** and **36** were also tested against *Dipetalonema viteae* infection in *Mastomys natalensis* at an intraperitoneal dose of 30 mg/kg given for 5 days, but neither compound showed any noteworthy effect. At a higher dose (50 mg/kg × 5) both of the compounds were still ineffective.

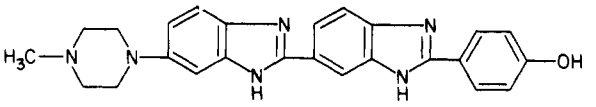
Conclusion

With the demonstration of antifilarial activity in some benzimidazole anthelmintics[5,14] the search for an ideal pharmacophore responsible for optimizing the activity against different filarial parasites within this class of compounds seems appropriate. The present study suggests that the presence of a piperazine at position 5(6) of alkyl benzimidazole-2-carbamates improves the chances of finding an anthelmintic with activity against intestinal and tissue nematodes. This is evident by the filaricidal efficacy associated with benzoyl, furoyl, and diethylcarbamoyl substituents at 4-position of piperazine. This observation is supported by the high antifilarial activity exhibited by HOE-33258, which contains one *N*-methylpiperazine residue at position 5(6) of 2-[(4-hydroxyphenyl)benzimidazol-5-yl]benzimidazole.[14] Thus, a proper choice of the group attached to the nitrogen of the piperazine in alkyl 5(6)-piperazin-1-ylbenzimidazole-2-carbamates may yield better benzimidazole antifilarials due to additive/synergistic effect and warrent a detailed structure–activity analysis.

Table III. Physical Data of the Compounds

compd	R	R^1	mol formula[a]	mp, °C	yield, %
7	ethyl		$C_{18}H_{16}N_6O_3$	265	45
17	phenyl		$C_{17}H_{18}N_4O_3$	185	80
18	cyclohexyl		$C_{17}H_{24}N_4O_3$	186	78
20	diethylamino		$C_{15}H_{23}N_5O_3$	126	68
21	1-hydroxy-2-naphthyl		$C_{21}H_{20}N_4O_4$	182	70
22	4-nitrophenyl		$C_{17}H_{17}N_5O_5$	195	82
23	2-pyrazinyl		$C_{15}H_{16}N_6O_3$	152	65
25	cyclohexyl		$C_{17}H_{26}N_4O$[b]		75
26	2-furyl		$C_{15}H_{18}N_4O_2$[b]		68
27	diethylamino		$C_{15}H_{25}N_5O$[b]		72
28	1-hydroxy-2-naphthyl		$C_{21}H_{22}N_4O_2$[b]		45
29	4-aminophenyl		$C_{17}H_{21}N_5O$[b]		58
30	2-pyrazinyl		$C_{15}H_{18}N_6O$[b]		52
32	phenyl	ethyl	$C_{22}H_{23}N_5O_3$	225	62
33	cyclohexyl	methyl	$C_{20}H_{27}N_5O_3$	>250	40
34	2-furyl	methyl	$C_{18}H_{19}N_5O_4$	230	45
35	2-furyl	ethyl	$C_{19}H_{21}N_5O_4$	218	40
36	diethylamino	methyl	$C_{18}H_{26}N_6O_3$	205	50
37	1-hydroxy-2-naphthyl	methyl	$C_{24}H_{23}N_5O_4$	130	35
38	4-(*N*,*N*'-dicarbomethoxyguanidino)phenyl	methyl	$C_{25}H_{28}N_8O_7$	>260	50
39	4-(*N*,*N*'-dicarbethoxyguanidino)phenyl	ethyl	$C_{28}H_{34}N_8O_7$	>260	42
40	2-pyrazinyl	methyl	$C_{18}H_{19}N_7O_3$	102	50

[a] The compounds gave satisfactory C, H, and N analyses. [b] Denotes products used in situ.



HOE-33258

Experimental Section

Structures of all compounds were checked routinely by IR spectra on Perkin-Elmer 157 and 177 infracord photometers. The NMR spectra were recorded on Varian 360L (60-MHz) and Perkin-Elmer R 32 (90-MHz) spectrometers using Me_4Si as internal reference. Mass spectra were taken on a Jeol JMS D-300 instrument. The purity of compounds was checked on silica gel G plates, and spots were located by iodine vapors, $KMnO_4$ spray, or Dragondorff reagent spray. Melting points were taken in sulfuric acid bath and are uncorrected. The physical data of the compounds are recorded in Table III.

2-[*N*-(4-Acetamido-3-nitrobenzoyl)amino]benzimidazole (3). To a stirred solution of **2** (5.9 g, 24 mmol) in 20 mL of dry

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DMF and Et_3N (2.46 g, 24 mmol) was added a solution of 2-aminobenzimidazole (1; 1.6 g, 12 mmol) in 20 mL of dry DMF dropwise at room temperature. Stirring was continued for 2 h; the reaction mixture was then refluxed for 5 h, cooled, and poured into 250 mL of H_2O. The precipitate obtained was filtered, washed with 3 × 20 mL of H_2O, dried, and recrystallized from EtOH: yield 1.5 g (59%); mp 146 °C; IR (KBr) 3310 (NH), 1680, 1620 (CO), 1510, 1335 (NO_2) cm^{-1}; EI MS, m/e 339 (M^+). Anal. ($C_{16}H_{13}N_5O_4$) C, H, N.

2-[*N*-(4-Amino-3-nitrobenzoyl)amino]benzimidazole (4). A 10% aqueous KOH solution was added in portions to a stirred suspension of **3** (1.0 g, 29 mmol) in 20 mL of EtOH at room temperature until a clear solution was obtained. Stirring was continued at this temperature for another 2 h. The solvent was evaporated and the residue washed with 2 × 10 mL of H_2O and 2 × 10 mL of 50% aqueous EtOH, dried, and recrystallized from EtOH to give 0.6 g (70%) of **4**: mp >270 °C; IR (KBr) 3450, 3340 (NH_2), 1625 (CO), 1570, 1340 (NO_2) cm^{-1}; EI MS, m/e 297 (M^+). Anal. ($C_{14}H_{11}N_5O_3$) C, H, N.

2-[*N*-(3,4-Diaminobenzoyl)amino]benzimidazole (5). A solution of **4** (1.0 g, 3.3 mmol) and Raney nickel (~200 mg) in 30 mL of EtOH was shaken in a Paar hydrogenator for 6 h at 3.5 kg/cm^2 pressure. The catalyst was filtered off and washed with 3 × 10 mL of EtOH, and the combined filtrate and washings were evaporated in vacuo to give the crude product, which was used for next step without further purification; yield of crude **5** 0.75 g (84%).

Methyl 5(6)-(Benzimidazol-2-ylcarbamoyl)benzimidazole-2-carbamate (6). A mixture of **5** (0.8 g, 2.9 mmol) and 1,3-dicarbomethoxy-*S*-methylisothiourea (0.62 g, 3 mmol) in 25 mL of EtOH was refluxed on water bath for 6 h. The solid separated was filtered and washed with 3 × 10 mL of water and 2 × 10 mL of EtOH. Recrystallization from acetic acid–water gave **6**: yield 0.48 g (48%); mp >270 °C; NMR (TFA) δ 3.62 (s, 3, OCH_3), 7.0–8.05 (m, 7, Ar-*H*); IR (KBr) 1715, 1630 (CO) cm^{-1}. Anal. ($C_{17}H_{14}N_6O_3$) C, H, N.

Similarly, **7** was obtained from **5** and 1,3-dicarbethoxy-*S*-methylisothiourea in refluxing EtOH.

1-(3-Amino-4-nitrophenyl)-4-(2-furoyl)piperazine (19). A mixture of **9** (1.1 g, 4.9 mmol), 2-furoyl chloride (**12**; 0.65 g, 5 mmol), and Et_3N (0.5 g, 5 mmol) in 50 mL of dry DMF was refluxed for 3 h. The solvent was removed in vacuo and the residue crystallized from EtOH to give 1.2 g (77%) of **19**: mp 182 °C; NMR (TFA) δ 3.3–3.6 (br s, 4, 2 NCH_2), 3.8–4.2 (br s, 4, 2, NCH_2), 6.4–7.3 (m, 5, Ar-*H*), 7.9 (d, 1, Ar-*H*), ortho to NO_2, J = 9 Hz); IR (KBr) 3450, 3300 (NH_2), 1625 (CO), 1580, 1335 (NO_2) cm^{-1}. Anal. ($C_{15}H_{16}N_4O_4$) C, H, N.

Similarly, compounds **17**, **18**, and **20–23** were synthesized from **10**, **11**, **13–16**, and **9** in dry THF.

1-(3,4-Diaminophenyl)-4-benzoylpiperazine (24, R = Ph). **17** (1.0 g, 3 mmol) was reduced with Raney nickel (~200 mg) in EtOH in a Paar hydrogenator and worked up as described in the preparation of **5**. The product thus obtained was used in next step as such without further purification; yield of crude **24** 0.7 g (78%).

Compounds **25–30** were obtained similarly by reduction of **10–23**, respectively.

Methyl 5(6)-(4-Benzoylpiperazin-1-yl)benzimidazole-2-carbamate (31). A solution of **24** (0.8 g, 2.7 mmol) and 1,3-dicarbomethoxy-*S*-methylisothiourea (0.56 g, 2.7 mmol) in 20 mL of EtOH was refluxed for 8 h. The solid that separated was filtered off, washed with 3 × 10 mL of H_2O and 3 × 5 mL of EtOH, and dried. Recrystallization from AcOH–H_2O gave 0.6 g (59%) of **31**: mp 230 °C; NMR (TFA) δ 3.6 (s, 3, OCH_3), 3.4–4.1 (br s, 8, 4 NH_2), 7.1–7.8 (m, 8, Ar-*H*); IR (KBr) 3310 (NH), 1720, 1630 (CO) cm^{-1}. Anal. ($C_{20}H_{21}N_5O_3$) C, H, N.

Similarly, **32–40** were prepared by reaction of 1,3-dicarbalkoxy-*S*-methylisothioureas with **24–30**.

Anthelmintic Test Methods. Antihookworm Screening. Hamsters of either sex (40–60 g) were infected orally with 60 infective larvae (third stage) of *A. ceylanicum*. After 17–20 days of inoculation, the animals were checked for infection by ovoscopic examination and those found with positive infections were used for treatment with the test compound and standard drugs. In each experiment three to five animals were used per dose schedule. The animals were starved overnight prior to administration of the compound. The compounds, insoluble in water, were given orally as a suspension in Tween 80 at the initial dose of 250 mg/kg for 3 days. All the experimental and controlled animals were starved overnight and sacrificed on day three post-treatment. The total number of worms present in the intestine of hamsters was counted on autopsy, and the percent deparasitization was calculated by the formula $[(N - n)/N]100$, where N and n are the number of worms in control and treated animals, respectively.

Antitapeworm Screening. Newly weaned University of Freiburg strain of male albino rats (25–30 g) were infected orally by 200 mature viable ova of *H. nana*.

On the 17th day of infection, the feces of animals were examined and those showing *H. nana* eggs were used in the group of three to five animals per experimental group. The animals were starved for 5-6 h and then treated with the compounds in an initial dose of 250 mg/kg. On the third day of treatment, the treated animals were again starved for 5–6 h and then sacrificed. The intestine of each animal was individually examined for worms and scolices under a dissecting microscope. Because of the large variation in the number of adult worms produced by intubating 200 viable eggs, the absolute clearance of worms along with their scolices in a particular dose was taken up as the criterion for assessing the activity in each animal.

Antifilarial Screening. The micro- and macrofilaricidal activity of compounds was evaluated against *L. carinii* infection in cotton rats (*Sigmodon hispidus*). The filarial infection was transmitted to cotton rats through the vector *Liponyssus bacoti* by the method of Hawking and Sewell.[12] At the end of the prepatent period, animals showing 250 or more microfilariae per 5 μL of blood were chosen for screening. Blood samples of experimental and control animals were examined before starting the treatment, and three to four animals were used in each experiment. The compounds, dissolved in water or suspended in Tween 80, were given intraperitoneally for 5 days at a dose level equivalent to one-fifth of maximum tolerated dose obtained after a preliminary acute toxicity study in albino mice. Blood smears of animals were examined for microfilariae at weekly intervals up to 6 weeks since the onset of treatment. On day 42, all the treated and control group animals were sacrificed and the condition of adult male and female worms was observed. The micro- and macrofilaricidal action of the compounds was finally assessed by the method of Laemmler and co-workers.[13]

A similar method was adopted for screening the compounds against *D. viteae* in *M. natalensis*.

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Registry No. **1**, 934-32-7; **2**, 24170-23-8; **3**, 98526-39-7; **4**, 98526-40-0; **5**, 98526-41-1; **6**, 98526-42-2; **7**, 98526-43-3; **8**, 1635-61-6; **9**, 96103-52-5; **10**, 98-88-4; **11**, 2719-27-9; **12**, 1300-32-9; **13**, 88-10-8; **14**, 38077-69-9; **15**, 122-04-3; **16**, 19847-10-0; **17**, 98526-44-4; **18**, 98526-45-5; **19**, 98526-46-6; **20**, 54997-96-5; **21**, 98526-47-7; **22**, 98526-48-8; **23**, 98526-49-9; **24**, 98526-50-2; **25**, 98526-51-3; **26**, 98526-52-4; **27**, 98526-53-5; **28**, 98526-54-6; **29**, 98526-55-7; **30**, 98526-56-8; **31**, 98526-57-9; **32**, 98526-58-0; **33**, 98526-59-1; **34**, 98526-60-4; **35**, 98526-61-5; **36**, 98526-62-6; **37**, 98526-63-7; **38**, 98526-64-8; **39**, 98526-65-9; **40**, 98526-66-0; 1,3-dicarbomethoxy-*S*-methylisothiourea, 34840-23-8; 1,3-dicarboethoxy-*S*-methylisothiourea, 34840-26-1.