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OVICIDAL ACTIVITY OF 6-SUBSTITUTED-2- AND -4-AMINOQUINOLINES AND THEIR MONO AND BIS (THIOUREA) DERIVATIVES AGAINST HUMAN HOOKWORM

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Abstract.

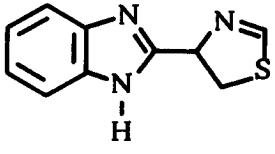
A series of 6-methoxy- and 6-methyl-2- and -4- quinolyl mono and bis thioureas was synthesized as potential anthelmintic agents against human hookworm. Tests for ovicidal activity against Necator americanus *isolated from human patients showed the 6-methoxy-2- and -4-quinolyl mono thioureas to cause better than 90 % ovicidal effects, and the 6-substituted-2- and 4-aminoquinolines to give 100 % inhibition of egg survival. This publication therefore reports a new type of agents with activity against human hookworm.*

Introduction

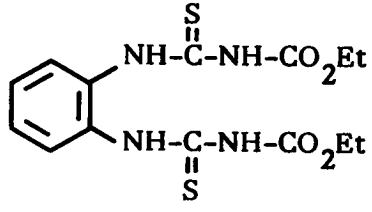
Hookworm infestation is one of the most prevalent helminic diseases of man, particularly in the tropical and subtropical regions.[1] In Thailand, the prevalence of hookworm infestation is as high as 80 % in the southern region and 75 % in the northeast.[2] Although these infestations are generally not fatal in well nourished people, they steadily weaken vitality, and if left untreated, can lead to death. Despite the fact that effective agents for treatment of hookworm and other helminthiases have been found in mebendazole and pyrantel, more than one course of therapy is often necessary to eliminate all parasites in a given case, and different drugs are frequently employed. Development of resistance to some drugs has also been experienced.[3] So a need for new agents exists.

Many of the compounds found to have some effect against the helminthiases have been heterocyclic compounds containing an N-C-S function, e.g., Thiabendazole (I) and some of its derivatives, Thiophanate (a bisthiourea) (II), tetramisole, some pyrantel

analogs and cyanine derivatives.[4] Of interest also are several mono and bis isothiocyanates, including amoscanate (III)[5] and bitoscanate (1, 4-phenylenediisothiocyanate).[6] These arylisothiocyanates are effective antihookworm agents but have serious toxic effects. Since the isothiocyanate function is highly reactive and would not be expected to remain unchanged *in vivo,* derivatives such as thioureas, or ureas, would appear to be promising and more stable compounds for anthelminic testing. A series of arylureas has already been found to have appreciable activity against human hookworm infestation.[7] To examine the effects of arylthioureas, a series of 2- and 4-quinolyl thioureas (IV) and 2- and 4-quinolyl-bis (thioureas) (V) were synthesized and tested for their ovicidal effects against human hookworm. The parent 2- and 4-aminoquinolines, as possible degradation products of isothiocyanates, were also screened.



I



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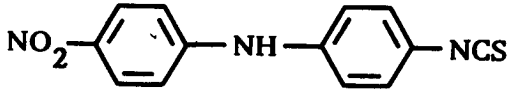
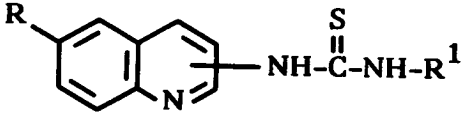
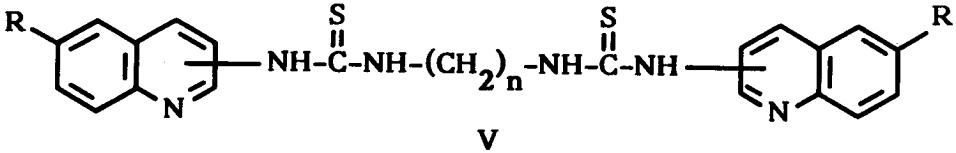


III

Little is known of the mechanisms by which the existing antihookworm agents act against the parasites. The benzimidazoles, such as mebendazole, have an inhibitory effect on glucose uptake, but the basis for it is unknown.[8] The benzimidazoles also inhibit fumarate-stimulated oxidation of NADH in susceptible sheep nematodes,[9] but the inhibitory action of the isothiocyanates is unexplained.



IV



V

Results and Discussion

The desired mono thioureas (IV) were obtained from the reaction of 6-substituted-2-aminoquinolines and -4-aminoquinolines with alkane isothiocyanates. Condensation with diisothiocyanates gave the corresponding bis thioureas (V). The substituted 2-aminoquinolines and 4-aminoquinolines were prepared from the corresponding chloroquinolines. These were obtained by the reaction of the quinoline-N-oxides with phosphorus oxychloride which gave a mixture of the 2- and 4-chloro derivatives.[10] The two isomers were separated by successive partial neutralization with concentrated aqueous ammonia. By this procedure, 6-methoxyquinoline-N-oxide gave the 2-chloro and 4-chloro derivatives in a 1.0:0.6 ratio. From 6-methylquinoline-N-oxide, the 2-chloro isomer was the major product (65 % yield).

The chloroquinoline-N-oxides were converted to the amines by reaction with gaseous ammonia.[11] The quinoline N-oxides were prepared by reaction of the 6-substituted quinolines with H_2O_2 in acetic acid.[12] Physical properties of the prepared mono and bis thioureas are listed in Table 1.

The ^{1}H-NMR spectra of the 2-quinolyl mono thioureas all showed a peak at ~ 12.45 ppm as broad singlets, exchangeable with D_2O, due to the N-H adjacent to the alkyl chain. This can be attributed to H-bonding with the quinoline nitrogen, forming a 6-membered chelate ring. This peak was not present in the 4-quinolyl thioureas, the only NH peak observed for the 4-quinolyl compounds being at 6.60-6.80 ppm. The appearance of a broad singlet, exchangeable with D_2O, at 9.25-9.45 ppm in the 2-quinolyl thioureas was due to the N-H adjacent to the ring. The 2-quinolyl bis (thioureas) showed broad singlets for NH at 10.65-10.75 and 12.35-12.70 ppm. IR spectra showed characteristic C=S absorptions for the mono and bis thioureas at 1235-1260 cm^{-1}.

Ovicidal effects on hookworm eggs. Determination of the ovicidal effects of the test compounds was carried out by the test tube cultivation method of Sasa *et al.*[13] This required isolation of hookworm eggs from faecal specimens of human patients, which in the Bangkok region involves infestations by *Necator americanus*. The isolated eggs are cultivated in the presence of test compounds, and the degree of inhibition of egg survival is calculated from the eggs surviving a seven day cultivation period. Bitoscanate, used clinically, gives 72-100 % reduction in egg counts and 25-96 % cures vs. *N. americanus* in humans.[9]

Ovicidal effects are tabulated in Table 2. Isolated egg samples were first incubated in the presence of 50 mg of test compound, using mebendazole as control substance. The 6-methoxy-2-quinoline thioureas and bis(thioureas) all slowed better than 90 % inhibition of egg survival, but the best activity was shown by 6-methoxy-2-aminoquinoline. The 6-methyl-2-quinoline mono thioureas were much less inhibitory, while the bis(thioureas)

showed no inhibitory activity. Again, the underivatized 6-methyl-2-aminoquinoline showed complete inhibition. For the 4-quinolyl thioureas, only the 6-methoxy compounds were tested. Similar activity was shown, with the two longer chain mono thioureas giving nearly complete inhibition. The only bis(thiourea) of the 4-quinolyl series, however, did give 78.5 % inhibition. Again, 4-amino-6-methoxyquinoline gave 100 % inhibition.

The compounds showing the greatest inhibitory activity were retested at a dose of 25 mg. The activity of the 6-methoxy-2-quinolyl derivatives was substantially less at this level, although the three aminoquinolines still gave 100 % inhibition. The solubilities of the test compounds probably limit their activities, since the bis(thioureas) were the least soluble, and the underivatized aminoquinolines were the most soluble in the test medium. It is apparent that the aminoquinoline moiety is of more importance to the ovicidal effects on hookworm eggs than the N-C-S function.

Materials and Methods

Melting points were taken on an Electrothermal Melting Point apparatus and are uncorrected. ^{1}H-NMR data were obtained with a Varian EM 360L spectrometer with Me_4Si as internal standard. IR spectra were recorded on a Jasco A 302 spectrophotometer either with KBr pellets or Nujol mulls. UV spectra were measured with a Unichem SP 800 instrument, and mass spectra were determined with a Dupont GC-MS 409 spectrometer. TLC was carried out on 1.5 mm Merck silica gel PF 254 strips, and the purified compounds each showed a single spot. Elemental analyses were done by the Scientific and Technological Research Equipment Center of Chulalongkorn University, Bangkok.

2-Chloro-6-methoxyquinoline and 4-chloro-6-methoxyquinoline. These compounds were prepared from 6-methoxyquinoline-1-oxide[10] by the method of Bachman.[10] The 2-chloro compound was obtained in 59 % yield, mp 105-106° (lit[10] 106-107°), and the 4-chloro derivative was obtained in 38 % yield, mp 72-74° (lit[14] 76-77°).

2-chloro-6-methylquinoline. The same procedure gave a 65 % yield of yellow needles, mp 111-113° (lit[15] 111-112°).

2-Amino-6-methoxyquinoline. This was obtained from the procedure of Bachman[11] in 95 % yield as white needles, mp 178-180° (lit[11] 179.5-181°).

4-Amino-6-methoxyquinoline. The previous procedure was used to give a 90 % yield of white crystals, mp 117-118.5° (lit[16] 118-120°).

2-Amino-6-methylquinoline. The same procedure was used, and a 93 % yield of white needles was obtained, mp 147-149° (lit[17] 145.7-146.7°).

The following procedure is representative of the synthesis of the 2-aminoquinoline thioureas and bis(thioureas).

1-n-Propyl-3- [*2'-(6'-methoxy) quinolyl*] *thiourea.* A mixture of 2-amino-6-methoxyquinoline (1.0 g, 5.7 mmol) and *n*-propyl isothiocyanate (0.58 g, 5.7 mmol) was heated at 150° until it solidified. The solid was collected and crystallized from ethanol to give 1.12 g (71.4 % yield) of yellow crystals; mp 167.5-170°, UV: $^{\lambda}$max (95 % C_2H_5OH) 273 nm (36,450). IR(KBr): $^{\nu}$1230 (C=S) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.17 (t, 3H, CH_3), 1.92 (m, 2H, CH_2), 3.60-3.80 (t, 2H, NCH_2), 4.00 (s, 3H, OCH_3), 7.03-8.00 (m, 5H, quinoline H), 9.43 (bs, 1H, ArNH exchangeable with D_2O), 12.45 (bs, 1H, CH_2NH, exchangeable with D_2O). MS: m/z 275 (35 %, M^+).

The following procedure is representative of the synthesis of the 4-amino-quinoline thioureas.

1-n-Propyl-3- [*4'-(6'-methoxy) quinolyl*] *thiourea* - A mixture of 4-amino-6-methoxyquinoline (0.5 g, 2.9 mmol) and *n*-propyl isothiocynate (0.29 g, 2.9 mmol) was heated at 80° for 2 h. The crude product was collected and purified by preparative TLC (ethyl acetate:hexane 8:2, developed three times, Rf = 0.52), and crystallized from chloroform-hexane to give 0.32 g (40.3 % yield) of white crystals; mp 168-170°. UV; $^{\lambda}$max (95 % C_2H_5OH) 343 nm (9,170). IR (KBr): $^{\nu}$1220 (C=S) cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.93 (t, 3H, CH_3), 1.64 (m, 2H, CH_2), 3.43-3.83 (m, 2H, NCH_2), 3.95 (S, 3H, OCH_3), 6.67 (bs, 1H, CH_2NH, exchangeable with D_2O), 7.13-8.53 (m, 5H, quinoline H). MS: m/z 275 (2%, M^+).

Test for hookworm ovicidal activity. The method used was the test tube cultivation method[13] for determining survival of filariform larvae. Fresh faecal specimens from hookworm patients, of which the direct egg smear counts were over 10, were collected at the Tropical Medicine Hospital, Bangkok, Thailand. The cultivation of the eggs was performed by placing 3 ml of dechlorinated water in a test tube, 2.5 × 15 cm. A filter paper strip 3 × 14 cm was folded along the long axis, and 500 mg of faecal matter was smeared as thin as possible on 2/3 of the inner surface of the folded filter paper. The smeared paper was inserted into the test tube with the clean end toward the bottom and in the water. The tube was covered with polyethylene sheet (9 × 9 cm) and tied with a rubber band. The tube was stored in the dark at room temperature. The presence of larvae at the bottom of the tube was detected under a stereomicroscope. The total number of larvae was counted on the 7th day of cultivation.

Inhibitory effects of the test compounds were determined as follows: 500 mg of well-mixed faecal specimen and either 50 mg or 25 mg of test compound were mixed thoroughly and kept in a glass container with lid at room temperature. After 2 h., the specimen was cultured as described above. An equal weight of mebendazole was used as control substance, and 500 mg of untreated faecal matter served as blank. All test compounds were run in duplicate. On the 7th day of cultivation, the total number of larvae in each tube was counted as before.

Acknowledgments

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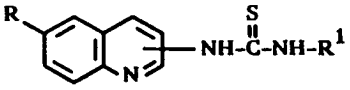
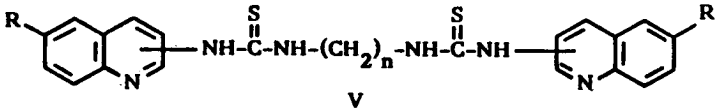
TABLE 1. PHYSICAL PROPERTIES OF THE QUINOLINE THIOUREAS



IV

No	R	R^1	Position in ring	yield (%)	mp (°C)	C=S (cm^{-1})	Formula	Analyses[a]
1.	CH_3O	C_3H_7	2	71.4	167.5-170	1230	$C_{14}H_{17}N_3OS$	C,H,N
2.	CH_3O	C_7H_{15}	2	68.3	144-145	1230	$C_{18}H_{25}N_3OS$	C,H,N
3.	CH_3O	C_8H_{17}	2	53.4	138.5-140	1230	$C_{19}H_{27}N_3OS$	C,H,N
4.	CH_3	C_3H_7	2	62.7	178-180	1230	$C_{14}H_{17}N_3S$	C,H,N
5.	CH_3	C_7H_{15}	2	71.0	100-102	1230	$C_{18}H_{25}N_3S$	C,H,N
6.	CH_3	C_8H_{17}	2	77.1	104-105.5	1230	$C_{19}H_{27}N_3S$	C,H,N
7.	CH_3O	C_3H_7	4	40.3	168-170	1220	$C_{14}H_{17}N_3OS$	C,H,N
8.	CH_3O	C_7H_{15}	4	37.9	139-140	1225	$C_{18}H_{25}N_3OS \cdot \frac{1}{2} H_2O$	C,H,N
9.	CH_3O	C_8H_{17}	4	39.4	135-136	1225	$C_{19}H_{27}N_3OS$	C,H,N



V

No	R	n	Position in ring	yield (%)	mp (°C)	C=S (cm^{-1})	Formula	Analyses[a]
10.	CH_3O	3	2	81.1	243.5-245	1235	$C_{25}H_{26}N_6O_2S_2$	C,H,N
11.	CH_3O	6	2	86.0	223-225	1240	$C_{28}H_{32}N_6O_2S_2$	C,H,N
12.	CH_3O	8	2	86.2	208-210	1230	$C_{30}H_{36}N_6O_2S_2$	C,H,N
13.	CH_3O	8	4	16.9	103-104	1225	$C_{30}H_{36}N_6O_2S_2 \cdot 2H_2O$	C,H,N
14.	CH_3	3	2	56.5	242-243.5	1230	$C_{25}H_{26}N_6S_2$	C,H,N
15.	CH_3	6	2	93.9	239-240	1235	$C_{28}H_{32}N_6S_2$	C,H,N
16.	CH_3	8	2	90.2	225-226	1235	$C_{30}H_{36}N_6S_2$	C,H,N

[a]Elemental analyses within ± 0.4% were found for all compounds.

TABLE 2. OVICIDAL EFFECTS ON *N. AMERICANUS IN VITRO*[a]

Series	Compound	Inhibition (%)
A. 6-Methoxy-2-quinolyl thioureas	Control	0.0
	1	98.2
	2	92.3
	3	97.9
	10	99.1
	11	98.2
	12	98.3
	17[b]	100.0
	Mebendazole	100.0
B. 6-Methyl-2-quinolyl thioureas	Control	0.0
	4	59.1
	5	74.6
	6	60.1
	14	0.0
	15	0.0
	16	0.0
	18[c]	100.0
	Mebendazole	100.0
C. 6-Methoxy-4-quinolyl thioureas	Control	0.0
	7	36.7
	8	98.5
	9	99.3
	13	78.5
	19[d]	100.0
	Mebendazole	100.0

[a] All test compounds were run in duplicate and the amount of test compound in each tube was 50 mg. The numbers of larvae in the control of series A, B and C were 868, 193 and 275 respectively.

[b] 6-Methoxy-2-aminoquinoline

[c] 6-Methyl-2-aminoquinoline

[d] 6-Methoxy-4-aminoquinoline

TABLE 3. OVICIDAL EFFECTS ON *N. AMERICANUS IN VITRO* OF SELECTED COMPOUNDS AT A LOWER DOSE[a]

Compound	Inhibition (%)
Control	0.0
1	24.9
2	29.4
3	21.6
9	97.9
10	71.8
11	11.9
12	10.6
17[b]	100.0
18[c]	100.0
19[d]	100.0
Mebendazole	100.0

[a] All test compounds were run in duplicate and the amount of test compound in each tube was 25 mg. The number of larvae in the control was 606.

[b] 6-Methoxy-2-aminoquinoline

[c] 6-Methyl-2-aminoquinoline

[d] 6-Methoxy-4-aminoquinoline

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บทคัดย่อ

การสังเคราะห์สารประกอบชุดอนุพันธ์ของ 6-methoxy- และ 6 -methyl- ของ 2- และ 4-quinolyl thioureas (mono และ bis) เพื่อทดสอบศักยะภาพในการฆ่าพยาธิปากขอ *(Necator americanus)* พบว่าสารประกอบชุด 6-methoxy ชนิด mono thiourea มีฤทธิ์ในการยับยั้งการฟักตัวของไข่พยาธิปากขอสูงกว่า 90% ในขณะที่ 2- และ 4-aminoquinoline ก็มีฤทธิ์ยับยั้ง 100% รายงานนี้แสดงสารประกอบชนิดใหม่ที่มีฤทธิ์ต่อต้านพยาธิปากขอ