Synthesis and Biological Evaluation of 1-(4-Hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)pyridinium Hydroxide Inner Salt Derivatives

NATHANIEL GRIER [x] and ROBERT A. STRELITZ

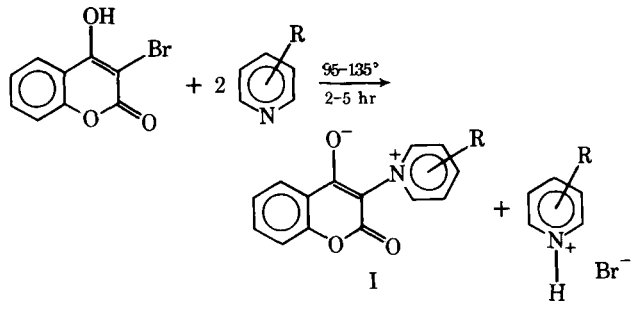
Abstract □ Eight 1-(4-hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)pyridinium hydroxide inner salts were synthesized, and the antibacterial, antifungal, anticoccidial, and anthelmintic activities were determined against different microorganisms, the protozoan *Eimeria tenella*, and trichostrongyle nematodes. All were noninhibitory to Gram-negative bacteria and the parasites. The pyridine, 4-benzylpyridine, and 2-isoquinoline inner salt derivatives controlled only *Rhizoctonia solani* of the four genera of fungi challenged.

Keyphrases □ Benzopyranylpyridinium salts, substituted—synthesized, antibacterial, antifungal, anticoccidial, anthelmintic activities evaluated □ Antibacterial agents, potential—series of substituted benzopyranylpyridinium salts synthesized, evaluated □ Antifungal agents, potential—series of substituted benzopyranylpyridinium salts synthesized, evaluated □ Anticoccidial agents, potential—series of substituted benzopyranylpyridinium salts synthesized, evaluated □ Anthelmintic agents, potential—series of substituted benzopyranylpyridinium salts synthesized, evaluated

4-Hydroxycoumarin and various 3-substituted derivatives have antimicrobial (1–3), anthelmintic (4), and insecticidal (4) properties of interest. An effort was directed to the synthesis of related compounds with possibly enhanced utility for the growth control of bacteria, fungi, protozoa, and helminths. Eight novel 1-(4-hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)pyridinium hydroxide inner salt derivatives (I) were prepared by treatment of 3-bromo-4-hydroxycoumarin with pyridines (Scheme I).

2-Alkylpyridines and quinoline failed to yield I-type compounds, apparently because of steric hindrance; isoquinoline was converted to 2-(4-hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)isoquinolinium hydroxide inner salt. A similar selectivity in betaine formation with dichloromaleimides was observed previously (5).

All of the compounds prepared are listed in Table I. When tested in preliminary screens for antimicrobial and antiparasitic properties, none exhibited sufficient inhibition and potency to encourage further study. The pyridine, 4-benzylpyridine, and 2-isoquinoline I inner salt derivatives suppressed growth of one fungus, but the eight compounds failed to suppress the growth of the Gram-negative bacteria, protozoan, and helminth at the maximum concentrations tested.



Scheme I

The pyridines were obtained from commercial sources, and 4-hydroxycoumarin was readily brominated using a known procedure (6).

DISCUSSION[1]

All of the compounds prepared were assayed for antimicrobial potency using the method of Conkey and Carlson (7). The microorganisms employed are associated with industrial and agricultural economic losses and include *Staphylococcus aureus* (ATCC 6538), *Aerobacter aerogenes*[2] (IPC 500), *Aureobasidium pullulans* (ATCC 9348), *Aspergillus niger* (ATCC 6275), *Rhizoctonia solani* (ATCC 16115), and *Verticillium albo-atrum*[3] (V3H). The compounds were dissolved or suspended in 25% methanol at concentrations of 1 mg/ml and diluted with hot sterile culture medium[4] to the desired strength. Concentrations of 1, 10, 50, and 100 μg/ml were used.

A culture medium containing the maximum concentration of 2.5% methanol failed to inhibit any of the microorganisms. All assays were run in duplicate. The control consisted of test medium medicated with 10 μg/ml of phenylmercuric acetate, which was totally effective.

The antimicrobial activities of the compounds at the 100-μg/ml concentration are reported in Table II. All but 1-(4-hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)-4-*tert*-butylpyridinium hydroxide inner salt inhibited *S. aureus*. The 1-pyridinium, 1-(4-benzyl)pyridinium, and 2-isoquinolinium derivatives also prevented growth of *R. solani*. No activity was evident for any compound at the three lower concentrations.

An *in vitro* chemotherapy screen was run with Gram-negative bacteria such as *Escherichia coli*[5] (MSD 2017 and 3293), *Pseudomonas aeruginosa* (MSD 3210 and 3301), and *Proteus vulgaris* (MSD 1810). Stock solutions of the eight compounds containing 2 mg/ml of dimethyl sulfoxide were diluted with hot sterile culture medium[6] to provide concentrations of 200, 100, 80, 60, 40, 20, 10, 5, and 1 μg/ml.

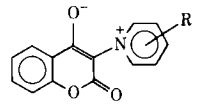
A culture medium with the maximum concentration of 1% dimethyl sulfoxide as the solvent control was noninhibitory. All assays were run in duplicate. Tetracycline was used as a control and prevented growth completely at a concentration of 20 μg/ml. No test compounds were antibacterial at the indicated concentrations.

The betaines were evaluated in *in vitro* and *in vivo* parasitology assays (8, 9). Ova and larva of trichostrongyle nematodes, which cause helminthiasis, were subjected to a maximum concentration of 0.1 mg/ml of the test compounds and were unaffected. Thiabendazole, at a concentration of 1 μg/ml, suppressed both forms of the parasite.

The coccidiosis screen employed 2-week-old chicks fed a basal ration containing 0.05% (w/w) betaine. On the 2nd day of the experiment, an inoculum was administered orally containing 50,000 sporulated oocysts of the protozoan *Eimeria tenella*. After an additional 6 days, a comparative evaluation was made between the

[1] The broad spectrum antimicrobial test was run by the Product Development Laboratory, Chemical Division; all other screens were run in the Merck Sharp & Dohme Research Labs., Rahway, N.J.
[2] Institute of Paper Chemistry, Appleton, Wis.
[3] Collection n, University of California, Riverside, Calif.
[4] Difco tryptone glucose extract agar for bacteria and Difco Sabouraud maltose agar for the fungi.
[5] Field isolates.
[6] Difco brain heart infusion agar.

Table I—1-(4-Hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)pyridinium Hydroxide Inner Salt Derivatives



Compound	R	Melting Point	Yield, %	Recrystallization Solvent	Empirical Formula	Analysis, % Calc.	Found
I*a*	H	244–245°	38	Methanol	$C_{14}H_9NO_3$	C 70.29	70.00
						H 3.79	3.78
						N 5.86	5.81
I*b*	4-Methyl	>300°	34	Ethanol	$C_{15}H_{11}NO_3$	C 71.13	71.05
						H 4.38	4.41
						N 5.53	5.47
I*c*	4-Ethyl	253–256°	60	Tetrahydrofuran–petroleum ether (bp 30–60°)	$C_{16}H_{13}NO_3$	C 71.90	71.73
						H 4.90	4.91
						N 5.24	5.30
I*d*	4-*tert*-Butyl	288–290°	22	Isopropanol	$C_{18}H_{17}NO_3$	C 73.20	73.09
						H 5.80	5.75
						N 4.74	4.64
I*e*	4-Benzyl	195–198°	65	Isopropanol	$C_{21}H_{15}NO_3$	C 76.58	76.27
						H 4.59	4.79
						N 4.25	4.46
I*f*	3-Chloro	281–283°	20	Dimethylformamide	$C_{14}H_8ClNO_3$	C 61.44	60.92
						H 2.95	2.98
						N 5.12	4.96
						Cl 12.96	13.06
I*g*	3-Hydroxy	>350°	38	Ethanol	$C_{14}H_9NO_4$	C 65.88	65.40
						H 3.55	3.59
						N 5.49	5.76
I*h*	3,4-Benzo	299–300°	32	Isopropanol	$C_{18}H_{11}NO_3$	C 74.73	74.36
						H 3.83	3.57
						N 4.84	4.77

Table II—Antimicrobial Activity[a] of 1-(4-Hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)pyridinium Hydroxide Inner Salts

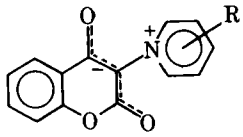
Compound	*S. aureus*	*A. aerogenes*	*A. pullulans*	*A. niger*	*R. solani*	*V. albo-atrum*
I*a*	+	—	—	—	+	—
I*b*	+	—	—	—	—	—
I*c*	+	—	—	—	—	—
I*d*	—	—	—	—	—	—
I*e*	+	—	—	—	+	—
I*f*	+	—	—	—	—	—
I*g*	+	—	—	—	—	—
I*h*	+	—	—	—	+	—
Phenylmercuric acetate	+	+	+	+	+	+

[a] + = complete inhibition; — = no inhibition.

test group and control chicks fed the unmedicated diet. No activity was found for any compounds. Amprolium, at a concentration of 0.003% (w/w), controlled the infection.

EXPERIMENTAL[7]

The compounds (Table I), except I*g*, were prepared in the manner described for I*a*. No attempt was made to improve yields. Upon crystallization, all of the compounds were yellow crystalline solids; their IR spectra in mineral oil[8] mulls exhibited intensive absorption in the 1680–1600-cm^{-1} region. These bands probably involve strong mixing of two C⋯O and two C⋯C valence bond motions[9]:



[7] Melting points were taken in open capillary tubes with a Thomas-Hoover apparatus and are uncorrected. IR spectra were obtained with a Perkin-Elmer model 621 grating spectrophotometer; TLC was performed with Analtech Inc. precoated plates.

[8] Nujol.

[9] The clarification of IR spectral band assignments in this region for coumarin and chromone structures was detailed by Büchi *et al.* (10).

1-(4-Hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)pyridinium Hydroxide Inner Salt (I*a*)—Five grams (0.021 mole) of powdered 3-bromo-4-hydroxycoumarin was suspended in 25 ml of anhydrous pyridine, and the mixture was heated at 95° for 4 hr. The solution, which resulted shortly on warming, gradually deposited solids. After cooling to 20°, the mixture was suction filtered and the product was washed with cold methanol. The yield was 4.3 g (85.7%). Recrystallization from methanol (40 ml) provided 1.9 g (37.8%); single spot TLC on silica gel, R_f 0.45 [chloroform–methanol (9:1 v/v)].

3-Hydroxy-1-(4-hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)pyridinium Hydroxide Inner Salt (I*g*)—A finely ground mixture of 1.2 g (0.005 mole) of 3-bromo-4-hydroxycoumarin and 0.95 g (0.01 mole) of 3-hydroxypyridine was heated in an oil bath at 135° for 2 hr. A clear melt was obtained at 95°. The liquid solidified on cooling and was then washed with cold ethanol; 0.8 g (63%) of betaine was isolated. Recrystallization of 0.5 g twice from 30–35 ml of dimethylformamide provided 0.3 g; single spot TLC on silica gel, R_f 0.44 [chloroform–methanol (9:1 v/v)].

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Antimalarials V: Aminobenzothiazoles

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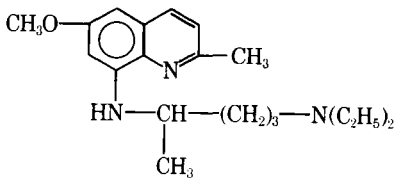
Abstract □ Four mono- and dialkylated 4-aminobenzothiazoles (VII–X) were prepared as analogs of potent causal prophylactic drugs in the 8-aminoquinoline series. Compounds VII and VIII were toxic at 80 mg/kg in the chick; IX was inactive at 640 mg/kg. In a sporozoite-induced mouse test system, X was inactive at 30 mg/kg and toxic at 480 mg/kg. None of the compounds was active as a suppressive drug.

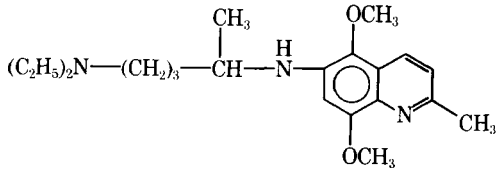
Keyphrases □ 4-Aminobenzothiazoles, mono- and dialkylated—synthesis, antimalarial activity screened □ Antimalarial agents, potential—mono- and dialkylated 4-aminobenzothiazoles screened □ Structure–activity relationships—mono- and dialkylated 4-aminobenzothiazoles, antimalarial activity

The history of the development of causal prophylactic antimalarials in the 6- and 8-aminoquinoline series, such as I and II, is well known; convenient summaries are available (1, 2). More recently developed additions to the series (III) are among its more potent members (3, 4).

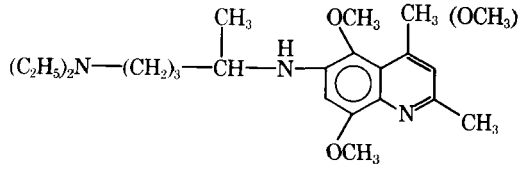
The pharmacological analogy between quinolines and benzothiazoles was drawn first by Bogert and Abrahamson (5), who prepared 2-phenylbenzothiazole-6-carboxylic acid as an analog of cinchophen. Benzothiazole amino alcohols, having the side chain at the 6-position, were later found not to be curative



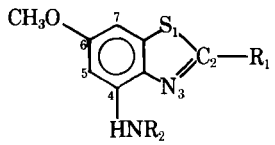
I



II



III



IV: $R_1 = CH_3$, $R_2 = (CH_2)_3N(C_2H_5)_2$

V: $R_1 = H$, $R_2 = NH(CH_2)_2N(C_2H_5)_2$

VI: $R_1 = CH_3$, $R_2 = NH(CH_2)_6N(C_2H_5)_2$

and to be highly toxic in mice at 160–640 mg/kg (6). A few other inactive benzothiazoles derived from 2-amino- or 2-mercaptobenzothiazole were reported (7). Compound IV was reported to be inactive (test system unreported) (8) and V was synthesized but not evaluated (9). Compound VI was found to be inactive (10) in the test system then in vogue (7).

Benzothiazoloquinones, as analogs of quinolinequinones, have been found to have effective prophylactic activity against *Plasmodium gallinaceum* in the chick (11).

The purposes of the present work were to prepare additional members of the 4-aminobenzothiazole series and to determine their prophylactic activity in a standard animal test system.

DISCUSSION

A general literature procedure (9) was used for the sequence 4-methoxy-2-nitroaniline → 1-chloro-6-methoxy-4-nitrobenzodithiazole (55%) → 1-hydroxy-6-methoxy-4-nitrobenzodithiazole (21%, but not isolated in most runs) → 6-methoxy-2-methyl-4-nitrobenzothiazole (43% overall from 1-chloro precursor) → 4-amino-6-methoxy-2-methylbenzothiazole (57%). The amine was alkylated by 3-dimethylaminopropyl chloride to give 4-(3-dimethylaminopropyl)amino-6-methoxy-2-methylbenzothiazole (VII·2HCl·H_2O, 24%).

The alkylation of 4-amino-6-methoxy-2-methylbenzothiazole by 2-bromo-5-diethylaminopentane (12) to VIII gave an unacceptable poor yield. An alternative procedure, recently used for the analogous alkylation of 1,4-dimethoxy-2-naphthylamine (13), involved the condensation of the amine with 5-diethylamino-2,2-dimethoxypentane followed by reduction of the intermediate anil by sodium borohydride. 4-(4-Diethylamino-2-methylbutyl)amino-6-methoxy-2-methylbenzothiazole (VIII·HCl·H_2O) was obtained in 21% yield.

The precursor for both IX and X was prepared from 1-hydroxy-6-methoxy-4-nitrobenzodithiazole (an intermediate in the 2-methyl series described previously) by the sequence 2-mercapto-4-methoxy-6-nitroaniline (9) (65%) → 2-(4-chlorophenyl)-6-methoxy-4-nitrobenzothiazole (85%) → 4-amino-2-(4-chlorophenyl)-6-methoxybenzothiazole (71%). Alkylation of the amine by 3-dimethylaminopropyl chloride gave 2-(4-chlorophenyl)-4-(3-dimethyl-