(2,2,2-trifluoroethoxy)benzamide (8.3 g, 0.02 mol), and excess 88% formic acid (5.5 g, 0.12 mol) were combined and stirred under reflux. After 12 h the mixture was cooled and 2.8 mL of concentrated HCl was added. Heating under reflux was continued for another 5 h. The solution was then cooled, made strongly basic with 10% NaOH, diluted with H_2O, and extracted with CH_2Cl_2. Evaporation of solvent yielded 6 g of crude product which was purified by recrystallization from cyclohexane: mp 99–102 °C; yield 5.2 g (61%). Compounds of this type were also conveniently prepared by the Borch reductive amination procedure.[9]

Method D. 2-[2,5-Bis(2,2,2-trifluoroethoxy)benzamidomethyl]-1,1-dimethylpiperidinium Iodide (53). Compound 48 (2.7 g, 0.0063 mol) was heated with 10 mL of CH_3I in a sealed tube at 55 °C. After 1.5 h the tube was opened and the contents rinsed out with CH_3OH. Solvents were removed under vacuum and the residue was recrystallized from EtOAc–*i*-PrOH to give 53 as a white powder: mp 170–172.5 °C; yield 2.4 g (71%).

Method E. *N*-(1-Formyl-2-piperidylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)benzamide (54). Trichloroacetaldehyde (2.82 g, 0.019 mol) was added dropwise at 0 °C to a stirred solution of compond **33** (7.24 g, 0.0175 mol) in 70 mL of $CHCl_3$. After the addition was complete, the solution was allowed to warm to room temperature, stirred for 1 h, and then heated under reflux for 3 h. The solution was cooled, washed with 5% HCl, dried, and concentrated. The residual gummy solid was dissolved in hot benzene. Slow addition of hexane to the cloud point induced crystallization of **54** as a finely divided ivory powder: mp 90–94 °C; yield 4.6 g (58%).

2-(*tert*-Butylaminomethyl)pyridine. The general procedure used to prepare various 2-(alkylaminomethyl)pyridines is illustrated by the following example. A solution of 21.9 g (0.3 mol) of *tert*-butylamine in 30 mL of absolute EtOH was added dropwise over 1.5 h to a stirred solution of 32.1 g (0.3 mol) of 2-pyridinecarboxaldehyde in 40 mL of absolute EtOH maintained at 10–20 °C. After the addition was complete, the mixture was stirred 1 h at 25 °C, heated to reflux for 6 h and cooled. GLC analysis showed that conversion to the Schiff base was essentially quantitative. The mixture was diluted with 150 mL of absolute EtOH and hydrogenated over 1 g of 10% Pd/C in a Parr apparatus. After removal of catalyst and solvent, the crude product was purified by distillation: bp 75–80 °C (1.2 mm); yield 40.4 g (82%). Purity was established by NMR and comparative GLC traces.

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Notes

Synthesis of 5-Chloro-3′-nitro-4′-substituted Salicylanilides, a New Series of Anthelmintic and Antimicrobial Agents[1]

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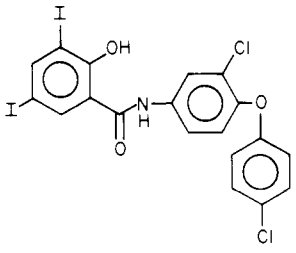
A number of 5-chloro-3′-nitro-4′-substituted salicylanilides (**6–23**) have been synthesized by treating 4′,5-dichloro-3′-nitrosalicylanilide (**5**) with various sodium aryl oxides, alkoxides, or amines. These compounds have been tested against *Hymenolepis nana* infection in rats and have also been evaluated for their in vitro antimicrobial activity against various strains of bacteria and fungi. In the former test **17** was the most active cestodicidal agent showing activity at 30 mg/kg. In the antimicrobial screening, **22** inhibited the growth of all the bacteria and fungi used while **6** was active against the pencillin resistant *Staphylococcus aureus* at a minimum inhibitory concentration of 0.00609 μg/mL.

It has been observed that introduction of a phenoxy group in a biologically active molecule may lead to compounds with enhanced activity.[2,3] One example is the discovery of 3′-chloro-4′-(*p*-chlorophenoxy)-3,5-diiodosalicylanilide (rafoxanide, **1**).[4] Based on this observation, the synthesis of various 5-chloro-3′-nitro-4′-aryloxysalicylanilides (**6–9**), as the structural analogues of the well-known cestodicide, 2,5′-dichloro-4′-nitrosalicylanilide (**2**),[5] has been carried out. Unlike **2**, in which ring B is substituted by two electron-withdrawing groups (Cl and NO_2) at the 2 and 4 positions, respectively, the compounds reported in this communication carry one electron-withdrawing NO_2 group at the 3 position and one electron-donating aryloxy group in the 4 position of ring B. Compounds **10–23** with other electron-donating groups like ethoxy, dialkylamino, and cyclic imino have also been prepared for structure–activity relationship studies. These compounds have been tested for their in vivo cestodicidal activity. In addition, they have also been subjected to in vitro antimicrobial screening and the results are reported



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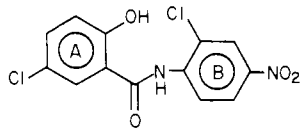

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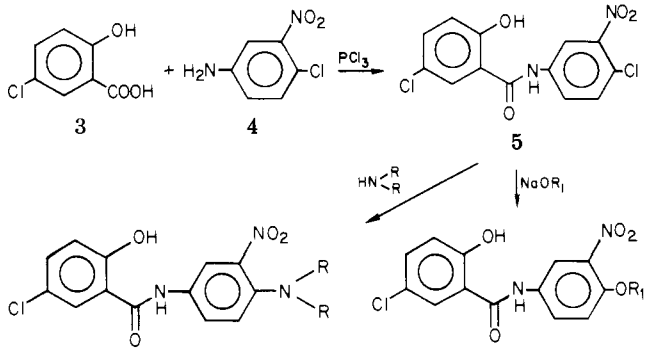
Chemistry. Condensation of 5-chlorosalicyclic acid (**3**) with 4-chloro-3-nitroaniline (**4**) in the presence of phosphorus trichloride in refluxing toluene or xylene gave 4′,5-dichloro-3′-nitrosalicylanilide (**5**)[6] which when treated with sodium aryloxide or sodium ethoxide yielded the corresponding 4′-aryloxy- (**6–9**) and 4′-ethoxy- (**10**) salicylanilides, respectively. **5** was also allowed to react with various primary and secondary amines in pyridine to yield 5-chloro-3′-nitro-4′-aminosalicylanilides (**11–23**).



3 4 5

11–23 6–10

Cestodicidal Activity. All the compounds were evaluated for their cestodicidal activity against *Hymenolepis nan* infection in rats by the technique of Steward[7] in the Parasitology Division of this Institute and the results are summarized in Table I. The compounds were given orally at dosages of 250, 100, 50, and 30 mg/kg using three animals per experimental group. The most potent compound of this series, **17**, is active at a single oral dose of 30 mg/kg while **13**, **14**, and **21** were as active as **2**. Compounds **18** and **22** were one-half as active as **2** while **6**, **12**, **15**, **16**, **19**, and **20** showed activity only at a dosage of 250 mg/kg. The other compounds were inactive at 250 mg/kg.

The cestodicidal activity results show that (a) introduction of a aryloxy group at the 4′ position in **2** causes either lowering (**6**) or loss (**7–9**) of activity; (b) compounds with 4′-ethoxy (**10**) or dibutylamino (**23**) also do not possess cestodicidal activity; (c) all the compounds carrying 4′-cyclic imino or amino (**11–12**) were active at a dose of 250 mg/kg or lower. These studies indicate that, in addition to the higher electron density in ring B of **2**, the geometry and the associated steric interactions between groups at 3 and 4 positions of ring B are important for determining cestodicidal activity.

Microbiological Studies. The in vitro growth inhibitory activity of these compounds against *Bacillus subtilis*, *Staphylococcus aureus* (gram-positive, resistant to 2500 units of penicillin/mL), *Escherichia coli*, *Agrobacterium tumefacience*, and *Salmonella typhi* (gram-negative), and the fungi *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes*, *Microsporum canis*, and *Aspergillus niger* was determined. The most promising compound of this series was 5-chloro-3′-nitro-4′-phenoxysalicylanilide (**6**) which inhibits the growth of pencillin-resistant *S. aureus* at a minimum inhibitory concentration (MIC) of 0.00609 μg/mL, while **11–14**, **16**, and **17** were as active as 3,4′,5-tribromosalicylanilide (MIC, 0.0975 μg/mL) and **8**, **12**, and **22** inhibit growth at 0.195 μg/mL. These compounds as also 3,4′,5-tribromosalicylanilide showed lower activity against *A. tumefacience* (MIC, 12.5–25 μg/mL) in comparison to tetracycline (MIC, 0.78 μg/mL). They were also inactive against the other three bacteria used.

All the compounds except the 4′-ethoxy (**10**) and 4′-dibutylamino (**23**) show marked growth inhibitory property against *S. aureus*. This indicates that introduction of electron-donating groups like phenoxy and amino increases the antibacterial activity in **2**.[8]

Consistent with their antibacterial activity, an enhanced, though selective, antifungal activity was also observed in the 4′-aryloxysalicylanilides (**6–9**); these and 3,4′,5-tribromosalicylanilide inhibited the growth of *M. canis* at one-half the inhibitory concentration of amphotericin B (MIC, 1.56 μg/mL) but were equipotent to amphotericin B against *T. mentagrophytes*. On the other hand, the corresponding 4′-amino compounds (**11–21**) were found to be either less active or inactive.

Thus, in general, these compounds were found to possess better activity against the gram-positive bacteria than the fungi used. Although **6**, which shows powerful growth inhibitory property against *S. aureus*, *T. mentagrophytes*, and *M. canis*, is less active or inactive against the other strains of bacteria and fungi, **22** shows an all-round activity against all the microorganisms tested.

On the basis of the above discussion it can be concluded that whereas the cestodicidal activity of these compounds was only marginally improved by 2′,5-dichloro-4′-nitrosalicylanilide, the antibacterial activity, particularly that of compound **6** against *S. aureus*, is significantly enhanced as compared to 3,4′,5-tribromosalicylanilide and tetracycline.

Experimental Section

The structures of all the compounds were checked routinely by IR spectra recorded on Perkin-Elmer 137 and 337 Infracord spectrophotometers. Melting points were taken in sulfuric acid bath and are uncorrected.

4′,5-Dichloro-3′-nitrosalicylanilide (5). PCl_3 (3 mL) was added dropwise to a refluxing solution (140 °C) of 5-chlorosalicylic acid (17.25 g, 0.1 mol) and 4-chloro-3-nitroaniline (17.25 g, 0.1 mol) in dry xylene (200 mL) and the reaction mixture refluxed for 4 h. Solvent was removed by steam distillation, and the residue was filtered, washed with hot water, dried, and crystallized from acetone: yield 22.3 g (68%); mp 246 °C (lit.[6] mp 246 °C).

5-Chloro-3′-nitro-4′-phenoxysalicylanilide (6). A solution of sodium phenoxide (made according to Brewster et al.[9] by heating of NaOH and 25 mL of phenol) and **5** (3.27 g, 0.01 mol) in phenol (30 mL) was refluxed for 36 h. Excess phenol was removed by steam distillation, and the residual solid was filtered, dried, and crystallized from acetone–water: yield 2.9 g (76%).

Compounds **7–9** were made in a similar manner by treating **5** with appropriate sodium aryloxide.

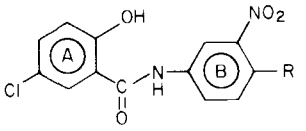
5-Chloro-3′-nitro-4′-ethoxysalicylanilide (10). A mixture of NaOEt (obtained from 0.3 g of sodium and 20 mL of absolute EtOH) and **5** (3.27 g, 0.01 mol) in absolute EtOH (30 mL) was refluxed for 12 h. Solvent was removed from the reaction mixture, and the residue was washed with water, dried, and crystallized from EtOH: yield 2.5 g (75%).

5-Chloro-3′-nitro-4′-*N*-pyrrolidinosalicylanilide (11). A mixture of **5** (3.27 g, 0.01 mol) and pyrrolidine (0.72 g, 0.01 mol) in pyridine (40 mL) was refluxed for 24 h. Solvent was removed in vacuo and the residual solid, after washing thoroughly with hot water, was crystallized from EtOH: yield 2.7 g (75%).

Compounds **12–23** were prepared by a similar method.

Antibacterial Assay. All the bacteria were maintained on nutrient agar slants.[10] Testing was done in nutrient broth. After

Table I



No.	R	Formula[a]	Mp, °C	Yield, %	Cestodicidal act., MED,[b] mg/kg	Antibacterial act., MIC[c] in μg/mL			Antifungal act.,[d] MIC in μg/mL		
						S. aureus	*A. tumefacience*	*C. neoformans*	*T. mentagraphytes*	*M. canis*	*A. niger*
2	Yomesan				50						
6	OC_6H_5	$C_{19}H_{13}ClN_2O_5$	234	76	250	0.00609	>100	>100	1.56	0.78	25.0
7	OC_6H_4-*p*-CH_3	$C_{20}H_{15}ClN_2O_5$	166	62	*i*[e]	0.39	>100	>100	1.56	0.78	6.25
8	OC_6H_4-*m*-CH_3	$C_{20}H_{15}ClN_2O_5$	186	56	*i*	0.195	>100	>100	1.56	0.78	>100
9	OC_6H_4-*p*-Cl	$C_{19}H_{12}Cl_2N_2O_5$	167	66	*i*	0.78	25	>100	1.56	0.78	25
10	OC_2H_5	$C_{15}H_{13}ClN_2O_5$	215	75	*i*	>100	>100	>100	>100	>100	>100
11	Pyrrolidyl	$C_{17}H_{16}ClN_3O_4$	135	45	*i*	0.0975	>100	25	>100	>100	25
12	Piperidyl	$C_{18}H_{18}ClN_3O_4$	185	73	250	0.195	12.5	1.56	>100	>100	12.5
13	Homopiperidyl	$C_{19}H_{20}ClN_3O_4$	210	66	50	0.0975	>100	>100	25	25	>100
14	2-Methylpiperidyl	$C_{19}H_{20}ClN_3O_4$	235	70	50	0.0975	>100	>100	12.5	6.25	>100
15	3-Methylpiperidyl	$C_{19}H_{20}ClN_3O_4$	170	63	250	0.39	>100	25	6.25	3.125	>100
16	4-Methylpiperidyl	$C_{19}H_{20}ClN_3O_4$	193	68	250	0.0975	25	3.125	>100	>100	>100
17	Cyclohexoylamino	$C_{19}H_{20}ClN_3O_4$	220	63	30	0.0975	>100	25	3.125	3.125	25
18	2-Ethylpiperidyl	$C_{20}H_{22}ClN_3O_4$	220	65	100	0.39	>100	25	6.25	6.25	>100
19	4-Phenylpiperidyl	$C_{24}H_{22}ClN_3O_4$	155	63	250	0.39	25	3.125	6.25	6.25	>100
20	4-Hydroxy-4-phenylpiperidyl	$C_{24}H_{22}ClN_3O_5$	192	58	250	0.39	25	3.125	6.25	6.25	>100
21	4-Phenylpiperizinyl	$C_{23}H_{21}ClN_4O_4$	210	63	50	0.39	>100	12.5	3.125	1.56	25
22	4-Methylpiperizinyl	$C_{18}H_{19}ClN_4O_4$	235	66	100	0.195	25	25	0.195	0.39	25
23	Dibutylamino	$C_{21}H_{26}ClN_3O_4$	130	52	*i*	>100	>100	>100	>100	25	25
24	Tetracycline[f]						0.78				
25	Amphotericin B[g]							0.78	1.56	1.56	12.5
26	3,4',5-Tribromosalicylanilide					0.0975	12.5	6.25	1.56	0.78	12.5

[a] The compounds were analyzed for C, H, and N and the results were within ±0.4% of the theoretical values. [b] Minimum effective dose given orally to clear 98% of *H. nana* infection in rats. [c] Minimum inhibitory concentration. [d] All the compounds synthesized were inactive against *C. albicans* except 11, 12, and 22 which show inhibition of fungal growth at a MIC of 25 μg/mL. [e] Inactive at 250 mg/kg. [f] Shows activity against *Salmonella typhi* and *Escherichia coli* at a concentration of 0.78 μg/mL. [g] Active against *Candida albicans* at a concentration of 0.39 μg/mL. [h] None of the compounds were active against *B. subtilis*, *E. coli*, and *S. typhi*.

inoculation with a loopful of culture from the slant, the seeded broths were incubated at 37 ± 1 °C for 24 h. The twofold serial dilution technique[11] was applied. A set of tubes containing only inoculated broth was kept as control. After incubation for 24 h, the last tube with no growth of the microorganism was taken to represent the minimum inhibitory concentration (MIC, expressed in μg/mL). A compound inhibiting the growth of the microbe in a concentration of 25 μg/mL was considered active.

Antifungal Assay. All the fungi were maintained on Sabouraund's agar slants.[10] Testing was done in Sabouraund's broth. Loopfuls of the fungal cultures (*C. albicans* and *C. neoformans*) from slants were inoculated into broth and the respective inoculated broths were used for testing after incubation for 24 h at 28 ± 1 °C. In the case of the other three strains, small pieces of mycelia were introduced into conical flasks containing 50 mL of broth. The flasks were then incubated with shaking for 24–48 h and the clear broths were taken out of the flasks. The compounds were tested by the serial dilution method as in case of antibacterial testing. A compound, which inhibited the growth of the fungus at 25 μg/mL concentration, was considered to be active.

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Imidazo[4,5-*f*]quinolines. 2. A Series of 9-(Substituted amino)imidazo[4,5-*f*]quinolines as Antitapeworm Agents[1,2]

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A number of 9-(substituted amino)imidazo[4,5-*f*]quinolines have been prepared and tested for anthelmintic activity in mice. All of these compounds are active in varying degrees against the tapeworm *Hymenolepis nana*.

Our success with a series of alkyl 6,7-dialkoxy-4-hydroxy-3-quinolinecarboxylates as anticoccidial agents[3] led us to consider the corresponding 6,7-diamino compounds. As a logical extension of this idea into an unexplored area, a number of imidazo[4,5-*f*]quinolin-9-ols were prepared.[1] Some of these were converted into 9-substituted amino analogues. During the biological evaluation of these latter compounds they were found to possess activity against the tapeworm *Hymenolepis nana* in mice. Since we felt there is a need for a better antitapeworm drug, a more extensive program was undertaken to study the effect of certain structural changes on the anthelmintic action of this type of imidazo[4,5-*f*]quinoline.

Chemistry. All of the compounds in Tables I and II were prepared by treating the appropriate 9-chloroimidazo[4,5-*f*]quinoline (I) with the prerequisite amine in refluxing EtOH or DMF (see Scheme I). The intermediates I were prepared by the method of Spencer et al.[1] Whenever possible, the required amine was purchased. However, in several cases it was necessary to reduce the appropriate nitro compound catalytically to the aniline which was then allowed to react directly with I. The physical properties of compounds II and III are reported in Tables I and II.

Antitapeworm Activity. The compounds prepared in this work were tested against the tapeworm *H. nana* in the mouse. The percent reduction in worm burden for each is shown in Tables I and II. Structure–activity trends are difficult to correlate but a few generalizations can be made. Of the eight most active compounds—those that show activity at a dose level of 25 mg/kg—all have substituted

Scheme I