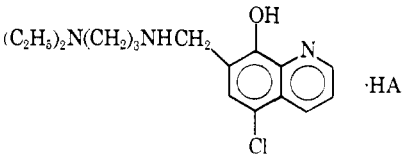
TABLE I

CLAMOXYQUIN SALTS



Compd no.	HA	Mp, °C	Yield, purified, %	Purification solvent[a]	Formula $C_{11}H_{24}ClN_3O$	Carbon, % Calcd	Carbon, % Found	Hydrogen, % Calcd	Hydrogen, % Found	Nitrogen, % Calcd	Nitrogen, % Found	Water, %[b] Calcd	Water, %[b] Found
1	Salicylic	108–110	72	A	$\cdot 2C_7H_6O_3$	62.25	61.91	6.07	6.33	7.03	6.70		
2	8-Hydroxy-7-iodoquinoline-5-sulfonic	145 dec	77	B	$\cdot 2C_9H_6INO_4S \cdot 1.5H_2O$	40.00	40.34	3.74	3.69	6.66	6.54	2.57	2.25
3	Naphthalene-1,5-disulfonic	238–240 dec	67	B	$\cdot C_{10}H_8S_2O_6 \cdot 0.25H_2O$	52.76	52.66	5.33	5.59	6.83	6.41	0.73	0.40
4	1-Hydroxy-2-naphthoic	95–97	63	C	$\cdot 2C_{11}H_8O_3 \cdot H_2O$	65.40	65.37	5.91	5.95	5.87	5.85	2.51	2.28
5	3-Hydroxy-2-naphthoic	142–143	60	D	$\cdot 2C_{11}H_8O_3$	67.09	67.02	5.77	5.96	6.02	5.76		
6	2,2′-Thiobis(4,6-dichlorophenol)	108–110	92	B	$\cdot 2C_{12}H_6Cl_4O_2S$	47.62	47.33	3.51	3.54	4.06	3.89		
7	2,2′-Methylenebis(3,4,6-trichlorophenol)	125 dec	77	E	$\cdot 2C_{13}H_6Cl_6O_2$	45.47	45.14	3.20	3.26	3.70	3.54		
8	4,4′-Methylenebis(3-hydroxy-2-naphthoic)	150 indef	95	F	$\cdot C_{23}H_{16}O_6 \cdot 0.5H_2O$	66.79	66.39	5.75	5.74	5.84	5.88	1.25	1.08

[a] A, acetone–ether; B, methanol–water; C, ethanol–water; D, ethanol; E, acetonitrile–ethanol; F, not recrystallized. [b] Water determinations are by the Karl-Fischer method.

Experimental Section[12]

General Procedure.—Aqueous or methanol solutions of 0.01–0.1 mole of 5-chloro-7-({[3-(diethylamino)propyl]amino}methyl)-8-quinolinol dihydrochloride[3] (clamoxyquin hydrochloride) were added at 25° to aqueous or methanol solutions of stoichiometric amounts of the sodium salts of the requisite acids or phenols (Table I). If the salt precipitated, it was collected and recrystallized from the solvent indicated. If not, the reaction mixture was refrigerated until crystallization occurred.

Clamoxyquin Pamoate.—A solution of 39.5 g (0.1 mole) of 5-chloro-7-({[3-(diethylamino)propyl]amino}methyl)-8-quinolinol dihydrochloride[3] (clamoxyquin hydrochloride) in 500 ml of distilled H_2O was poured slowly with vigorous stirring at 25° into a solution of 45.0 g (0.1 mole) of 4,4′-methylenebis(3-hydroxy-2-naphthoic acid) disodium salt monohydrate (sodium pamoate) in 800 ml of distilled H_2O. The resulting thick slurry was diluted to 3 l. with distilled H_2O and stirred briefly. The precipitate was collected by filtration and slurried twice with 3-l. portions of distilled H_2O. The off-white solid was collected and dried *in vacuo* at 45° for 48 hr; yield 66.0 g (93%), melting point indefinite beginning at approximately 150°.

The clamoxyquin pamoate thus obtained ranged from 1–5 μ in particle size with aggregates up to 300 μ. The X-ray diffraction pattern indicates that this material is amorphous. The compound exhibits the ultraviolet absorption maxima shown in Table II.

TABLE II

Abs methanol λ, mμ	Abs methanol $E^{1\%}_{1cm}$	0.1 *N* NaOH λ, mμ	0.1 *N* NaOH $E^{1\%}_{1cm}$
362	96	364	135
300	125	300	96
289	164	288	153
278	140	236	1526
238	1685		

The solubility of clamoxyquin pamoate in pH 7 0.1 *M* phosphate buffer is 0.012%. Solutions of this drug (0.01%) in methanol or pH 7 phosphate buffer are stable for more than 2 weeks.

Acknowledgment.—The authors wish to express their appreciation to Dr. Loren M. Long for encouragement in this investigation and to Dr. Paul E. Thompson, Dr. D. H. Kaump, and co-workers for the biological evaluation of these compounds. We are also indebted to Mr. Charles E. Childs and associates for the microanalyses and to Dr. J. M. Vandenbelt and associates for the physical-chemical data.

(12) Melting points (corrected) were taken in open capillary tubes in a Thomas-Hoover capillary melting point apparatus.

Synthesis and Antiviral and Antibacterial Activity of Certain N-Dialkylaminomethylisatin β-Thiosemicarbazones

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Thiosemicarbazones of different carbonyl compounds have shown antiviral[2–8] and tuberculostatic[9–15] activity, including the activity of isatin 3-thiosemicarbazone (I) against the pox group of viruses in human beings and type 2 polio in ERK cells.[16] Bauer and Sadler[7] in-

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(1) Author to whom inquiries should be addressed.

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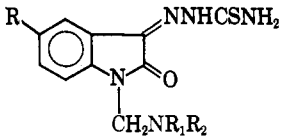
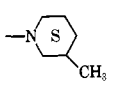
TABLE I

N-DIALKYLAMINOMETHYLISATIN β-THIOSEMICARBAZONES

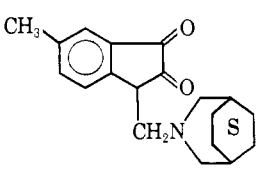


No.	NR_1R_2	R	Yield, %	Mp, °C[a]	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N	Infrared, cm⁻¹ NH	Infrared, cm⁻¹ C=O
1		H	70	170	$C_{15}H_{19}N_5OS$[b]	56.71	6.03	22.05	56.78	6.15	22.16	3205, 3334	1692
2		H	77	185	$C_{18}H_{23}N_5OS$[c]	60.48	6.48	19.59	60.54	6.56	19.44	3067, 3165, 3333	1675
3	$N(CH_3)_2$	H	36	168–170	$C_{12}H_{15}N_5OS \cdot 0.5H_2O$	...	...	24.45	...	...	24.48 24.65 24.04	3096, 3333	1689
4		H	72	202–204	$C_{14}H_{17}N_5O_2S \cdot 0.5H_2O$	51.25	5.50	21.34	51.30	5.37	21.78	3106, 3205, 3333	1684
5		Br	92	198–200	$C_{18}H_{22}BrN_5OS$	49.56	5.08	16.05	49.64	5.22	15.90	3100, 3200, 3400	1675
6		CH_3	98	196–200	$C_{19}H_{25}N_5OS$	61.44	6.78	18.85	61.63	6.92	18.72	3100, 3200, 3400	1684
7		CH_3	90	206	$C_{16}H_{21}N_5OS$[d]	57.99	6.39	21.13	57.90 58.02	6.45 6.39	20.93	3096, 3175, 3334	1686
8		H	60	165–166	$C_{16}H_{21}N_5OS$	...	...	21.13	...	...	21.32	3125, 3230, 3350	1680

[a] All compounds melt with decomposition. [b] *Anal.* Calcd: S, 10.10. Found: S, 9.86. *Anal.* Calcd: S, 8.97. Found: S, 8.80. [d] *Anal.* Calcd: S, 9.67. Found: S, 9.82.

vestigated several derivatives of I and found that the N-methyl and N-ethyl derivatives were more active than the parent compound (I). In this work we have prepared a series of isatin β-thiosemicarbazones (Table I) by replacing the N-methyl group with the N-dialkylaminomethyl group.

The starting materials for the preparation of these thiosemicarbazones were prepared by condensing isatin(s), formaldehyde, and a suitable secondary amine. The synthesis of these compounds is described elsewhere[17,18] except for one compound (II), the synthesis of which is described herein.



II

Biological Data.—Several of the compounds reported in this report have been subjected to preliminary antiviral, antibacterial, and antifungal screening procedures. The method with respect to antiviral screening involved the use of an agar diffusion technique. HeLa cells (a human carcinoma cell line) and polio virus type II (RNA type) and parainfluenza-3 (RNA type) viruses were used. These viruses are human pathogens and have in common the characteristic of causing extensive cell destruction (cytopathogenicity) in HeLa cells, thus making it possible to observe macroscopically and microscopically the protection of the cells by the drugs that have antiviral activity. Table II shows the results of the assays completed thus far.

TABLE II

ANTIVIRAL ACTIVITY[a]

Compd	Toxicity to cancer cells	Viruses: Polio II	Viruses: Parainfluenza 3
1	+	+	−
3	+	−	−
4	+	+	−

[a] Activity is shown as lowest concentration of the drug that inhibits virus. Toxicity is shown as lowest concentration of the drug toxic to cells. + = some antiviral activity (or toxicity to cancer cells), − = no observable activity.

For antibacterial and antifungal screening, filter paper disks (6.35 mm) saturated with two drops of a suspension of the test compounds (20 mg/ml in ethanol or water) were placed on the agar. Twelve[19] representative bacteria, yeasts, and fungi were chosen in order

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(19) The microbial spectrum consisted of *Staphylococcus aureus* K257 (+), *Escherichia coli* ATCC 4157 (−), *Pseudomonas aeruginosa* (−), *Klebsiella pneumoniae* ATCC 8052 (−), *Proteux vulgaris* LBa155 (−), *Mycobacterium smegmatis* (+), *Neisseria catarrhalis* (−), *Saccharomyces sp.*, *Candida albicans* ATCC 10231, *Trichophyton mentagrophytes* ATCC 9129, *Staphylococcus epidermidis*, and *Aspergillus niger* organisms.

to observe possible drug activity against a wide spectrum of different types of organisms. The test organisms included were gram-positive human pathogens, gram-negative human pathogens, acid-fast bacteria (representing the leprosy-tuberculosis group), common yeasts, and pathogenic fungi. The results are recorded in Table III.

TABLE III

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY[a]

Compd	Acid-fast bacteria	Gram-positive bacteria	Gram-negative bacteria	Fungi and yeasts
1	−	−	−	−
2	−	−	−	+
3	−	−	−	−
4	−	+	−	−
5	−	−	−	+

[a] + = inhibition, − = no inhibition.

Experimental Section[20]

N-3-Azabicyclo[3.2.2]nonylmethyl-5-methylisatin (II).—A solution of 3-azabicyclo[3.2.2]nonane (3.12 g, 0.25 mole) in 10 ml of ethanol was added to a slurry consisting of 5-methylisatin (4.02 g, 0.25 mole), 2.5 ml of 37% formalin, and 10 ml of ethanol with shaking. The resulting reaction mixture was stirred for 30 min at room temperature and then warmed for another 30 min on a steam bath. The contents of the flask, upon refrigeration overnight, gave a product melting at 160–162°. An analytical sample was prepared by three successive crystallizations from ethanol; mp 162–164°, yield 3.5 g (47%), ν_{max} 1724 cm^{-1} (C=O).

Anal. Calcd for $C_{18}H_{22}N_2O_2$: C, 72.46; H, 7.43; N, 9.39. Found: C, 72.33; H, 7.45; N, 9.41.

The nmr spectrum of II is consistent with the proposed structure. The spectrum showed a singlet at δ 1.66 attributed to the ten protons of the cyclohexane ring and a doublet at 2.73–2.8 which is attributed to the CH_2 protons (4 H) adjacent to N. A sharp singlet was observed at δ 4.5. This latter peak has been assigned to the two CH_2 protons between the two N atoms. A singlet at δ 2.36 was due to CH_3 at position 5 and a complex pattern at δ 6.96–7.51 to the protons (3 H) of the aromatic ring.

N-Dialkylaminomethylisatin β-Thiosemicarbazones (Table I). Method A.—To a solution of 0.01 mole of isatin N-Mannich base in 15 ml of absolute ethanol was added, in one portion, 0.91 g (0.01 mole) of thiosemicarbazide. The reaction mixture was stirred overnight at room temperature. The resulting solid was collected by filtration and washed with absolute ethanol. An analytical sample was prepared by repeated crystallizations from ethyl acetate.

Method B.—Thiosemicarbazide (0.91 g, 0.01 mole) was dissolved in 10 ml of distilled water by warming on a water bath. To this solution there was added the isatin N-Mannich base (0.01 mole) followed by 10 ml of ethanol. The reaction mixture was heated under reflux for 1.5 hr. At the end of this time the contents of the flask were cooled and the product was collected by filtration; the product was then washed with ethanol. For characterization an analytical sample was prepared by crystallization from ethyl acetate.

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(20) All melting points were taken in capillary tubes on a Thomas-Hoover apparatus and are uncorrected. The infrared spectra were determined in Nujol mull on a Perkin-Elmer Model 137 spectrophotometer. Nmr spectrum was taken on a Varian A-60 spectrometer in $CDCl_3$ using Me_4Si as an internal standard. Microanalyses were done by Dr. Alfred Bernhardt, Mülheim (Ruhr), Germany, and Galbraith Laboratories, Knoxville, Tenn., or through the courtesy of Dr. Paul Craig, Smith Kline and French Laboratories, Philadelphia, Pa.

2,4-Bis(arylamino)-5-methylpyrimidines as Antimicrobial Agents

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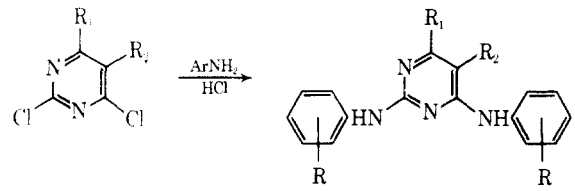
In our previous communications[1] it has been shown that 2,4-bis(arylamino)pyrimidines (A, R_1 = OH; R_2 = H and R_1 = R_2 = H) possess potent antimicrobial activities. The biological activities of these compounds have not been studied in detail so far. We have been encouraged to study 2,4-bis(arylamino)pyrimidines in some detail since some compounds of this series showed high antifungal activity.[1c] In this communication, we wish to report the synthesis and growth inhibitory activity of a number of compounds related to type A, where R_1 = H and R_2 = CH_3. These compounds have been tested against gram-positive and gram-negative bacteria and also a pathogenic strain of yeast. The growth inhibitory activity of these synthetic pyrimidines has been compared with that of neomycin (a known antifungal agent), chloramphenicol, and 6-azauracil.



A

The 2,4-bis(arylamino)-5-methylpyrimidines were synthesized by the acid-catalyzed condensation of 2,4-dichloro-5-methylpyrimidine[2] with appropriate aromatic amines.

In general, it has been observed that the antimicrobial activity of 2,4-bis(arylamino)-5-methylpyrimidines is enhanced when groups with positive σ constants are substituted in the phenyl ring, whereas groups with negative σ constants decrease the activity considerably. Thus chloro-substituted derivatives (III–V) show maximum activity, whereas minimum activity is exhibited by the hydroxy-substituted compound VI. Similar biological activities are shown by 2,4-bis(arylamino)-pyrimidines and 2,4-bis(arylamino)-6-hydroxypyrimidines which have been reported earlier.[1b,c]

Studies on the mechanism of inhibition of these active compounds are in progress and will be reported elsewhere.

Experimental Section

2,4-Bis(*p*-chloroanilino)-5-methylpyrimidine (III).—2,4-Dichloro-5-methylpyrimidine (1.63 g, 0.01 mole) was added to a warm solution of *p*-chloroaniline (3.8 g, 0.03 mole) in 2.7 ml of concentrated HCl in 20 ml of water, and refluxed on a sand bath.

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