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Thiolesters of Orotic Acid†

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The chemical reactivity associated with thiolesters[1] has been utilized to produce noncompetitive metabolic inhibitors.[2-4] Since orotic acid is an essential intermediate in the biogenesis of pyrimidines, a series of potential metabolite analogs was prepared for biological study containing thiolester moieties. These compounds were synthesized by a direct condensation between orotoyl chloride[5] and the appropriate thiol. The poor solubility of these derivatives precluded testing them effectively in liquid cultures, and they were accordingly examined for toxicity to microbial growth using a disk assay technique.[6]

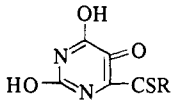
Experimental Section

All of the thiols were purchased from commercial sources. Orotoyl chloride was prepared by a previously reported procedure[5] and was used immediately for the condensation reaction.

All melting points were determined in open glass capillaries using a Thomas-Hoover Uni-Melt apparatus and are uncor. Microanalyses were carried out by Mrs. Delaney Blocker of the Analytical Laboratories of North Texas State University using an F & M Model 185 GPC carbon-nitrogen-hydrogen analyzer.

S-(Substituted)thiolesters of Orotic Acid (Table I). All of these deriv were prepd in a comparable fashion. A sample of 0.01 mole of freshly prepd orotoyl chloride was placed in a reaction flask and treated with 5–10 ml (excess) of the appropriate thiol. The react mixt was magnetically stirred and heated at the indicated temp for about 1 hr and then allowed to come to room temp with continued stirring for an additional 5 hr. The resulting ppt was taken up in warm AcOH, treated with Darco G-60, and filtered through a Celite pad. Upon cooling to room temp, a small amount of orotic acid pptd which was removed. The resulting clear filtrate was treated with H_2O to ppt the thiolesters which were recrystd and dried *in vacuo* overnight at 50–60° prior to elemental analysis.

Table I. *S*-(Substituted)thiolesters of Orotic Acid[a]



R	Mp, °C	Reaction temp, °C	Recrystn solvent	Empirical formula[b]
n-Pr	208–209	Reflux	H_2O	$C_8H_{10}N_2O_3S$
n-Bu	187–188	Reflux	AcOH-H_2O	$C_9H_{12}N_2O_3S$
n-Hept	150–151	Reflux	AcOH	$C_{12}H_{18}N_2O_3S$
n-Dec	148–149	125–150	AcOH	$C_{15}H_{24}N_2O_3S$
n-Tetradec	131–132	125–150	AcOH	$C_{19}H_{32}N_2O_3S$
Cyclohex	248–249	Reflux	AcOH	$C_{11}H_{14}N_2O_3S$
Benzyl	228–230	125–150	AcOH	$C_{12}H_{10}N_2O_3S$

[a] Replicate syntheses gave varying yields of analytically pure products ranging between 10 and 30%. [b] All compds were analyzed for C, H, N and were within ±0.3% of the theoretical value.

†This work was supported in part by a grant from the Robert A. Welch Foundation (B-342) and the North Texas State University Faculty Research Fund.

Biological Assays. Of the eight microorganisms studied, the *n*-propyl, *n*-butyl, and benzyl thiolesters were inhibitory to growth of *Lactobacillus plantarum* and *Pediococcus cerevisiae* at about 60 μg/disk but were ineffective toward growth of *Escherichia coli*, *L. bulgaricus*, *Leuconostoc dextranicum*, *Streptococcus faecalis*, *L. casei*, and *Strep. lactis* at 100 μg/disk. The other thiolesters herein reported were nontoxic at 100 μg/disk to growth of these bacteria.

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Potential Anticancer Agents. 4. Schiff Bases from Benzaldehyde Nitrogen Mustards

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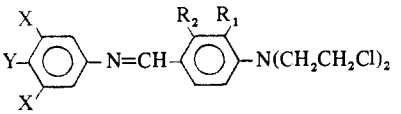
We have reported in an earlier communication the synthesis and study of Schiff bases from substituted benzaldehyde N mustards and various arylamines.[1] A number of compounds from this series displayed significant activity against Dunning leukemia (solid), lymphoid leukemia (L 1210), and Walker carcinosarcoma 256 (intramuscular). Compounds derived from 4-[*N*,*N*-bis(2-chloroethyl)amino]-*m*-anisaldehyde were in general more active against L 1210 lymphoid leukemia. A significant observation in our earlier work was that the presence of a halogen in the meta position of the arylamines induced activity of a high order. Further, the introduction of an additional halogen group in another available meta position of the aniline moiety considerably enhances the antileukemic activity with reduction in toxicity. The work has now been extended and Schiff bases of structure I from various 3,5-dihalo-substituted anilines have been prepared and studied for biological activity.



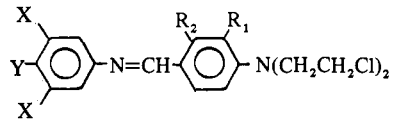
I

X = Cl, Br
Y = Cl, CN, OH, COOH, OMe, OEt, H
R_1 = H, OMe
R_2 = H, Me

Chemistry. The Schiff bases (Table I) were obtained as monohydrochlorides by heating the requisite pure amine hydrochlorides with mustard aldehydes in EtOH[1] and were found to be of analytical purity with yields varying between 60 and 75%.

Biological Results. Fourteen representative compounds were screened for antitumor activity by C.C.N.S.C. The re-

Table I



No.	X	Y	R_1	R_2	Mp, °C	Formula[a]
1	Cl	Cl	H	CH_3	218-220	$C_{18}H_{17}Cl_5N_2 \cdot HCl$
2	Cl	Cl	OCH_3	H	203-205	$C_{18}H_{17}Cl_5N_2O \cdot HCl$
3	Cl	Cl	OC_2H_5	H	195-197	$C_{19}H_{19}Cl_5N_2O \cdot HCl$
4	Cl	COOH	H	CH_3	247	$C_{19}H_{18}Cl_4N_2O_2 \cdot HCl$
5	Cl	COOH	OCH_3	H	140-141	$C_{19}H_{18}Cl_4N_2O_3 \cdot HCl$
6	Cl	COOH	OC_2H_5	H	167-169	$C_{20}H_{20}Cl_4N_2O_3 \cdot HCl$
7	Cl	OH	H	H	150-152	$C_{17}H_{16}Cl_4N_2O \cdot HCl$
8	Cl	OH	H	CH_3	240-242	$C_{18}H_{18}Cl_4N_2O \cdot HCl$
9	Cl	OH	OCH_3	H	255-256	$C_{18}H_{18}Cl_4N_2O_2 \cdot HCl$
10	Cl	OH	OC_2H_5	H	235-238	$C_{19}H_{20}Cl_4N_2O_2$
11	Cl	OCH_3	H	CH_2	221-222	$C_{19}H_{20}Cl_4N_2O \cdot HCl$
12	Cl	OCH_3	OCH_3	H	168-170	$C_{19}H_{20}Cl_4N_2O_2 \cdot HCl$
13	Cl	OCH_3	OC_2H_5	H	135-138	$C_{20}H_{22}Cl_4N_2O_2 \cdot HCl$
14	Cl	OC_2H_5	H	H	232-235	$C_{19}H_{20}Cl_4N_2O \cdot HCl$
15	Cl	OC_2H_5	H	CH_3	227-229	$C_{20}H_{22}Cl_4N_2O \cdot HCl$
16	Cl	OC_2H_5	OCH_3	H	145-148	$C_{20}H_{22}Cl_4N_2O_2 \cdot HCl$
17	Br	H	H	CH_3	230-232	$C_{18}H_{18}Br_2Cl_2N_2 \cdot HCl$
18	Br	H	OCH_3	H	152-154	$C_{18}H_{18}Br_2Cl_2N_2O \cdot HCl$
19	Br	H	OC_2H_5	H	187-190	$C_{19}H_{20}Br_2Cl_2N_2O \cdot HCl$
20	Br	COOH	H	CH_3	170-172	$C_{19}H_{18}Br_2Cl_2N_2O_2 \cdot HCl$
21	Br	COOH	OC_2H_5	H	180-182	$C_{20}H_{20}Br_2Cl_2N_2O_3 \cdot HCl$
22	Br	OH	H	H	215-218	$C_{17}H_{16}Br_2Cl_2N_2O \cdot HCl$
23	Br	OH	H	CH_3	235-236	$C_{18}H_{18}Br_2Cl_2N_2O \cdot HCl$
24	Br	OH	OCH_3	H	230	$C_{18}H_{18}Br_2Cl_2N_2O_2 \cdot HCl$
25	Br	OH	OC_2H_5	H	230-232	$C_{19}H_{20}Br_2Cl_2N_2O_2 \cdot HCl$
26	Br	OCH_3	H	CH_3	222-224	$C_{19}H_{20}Br_2Cl_2N_2O \cdot HCl$
27	Br	OCH_3	OCH_3	H	175-177	$C_{19}H_{20}Br_2Cl_2N_2O_2 \cdot HCl$
28	Br	OCH_3	OC_2H_5	H	142-144	$C_{20}H_{22}Br_2Cl_2N_2O_2 \cdot HCl$
29	Br	OC_2H_5	H	CH_3	230-233	$C_{20}H_{22}Br_2Cl_2N_2O \cdot HCl$
30	Br	OC_2H_5	OCH_3	H	170-172	$C_{20}H_{22}Br_2Cl_2N_2O_2 \cdot HCl$

[a] Analyzed correctly for C, H, and N.

sults of 11 active compounds are presented in Table II.† Compounds **8**, **17**, and **23** were inactive.

Present data also confirm the earlier observation that Schiff bases from 4-[*N*,*N*-bis(2-chloroethyl)amino]-*m*-anisaldehyde are in general significantly more active against L 1210 lymphoid leukemia. Compounds **24** and **27** have also shown good activity against Walker carcinosarcoma 256 (intramuscular). The introduction of an OH group between the two halogens has improved the activity of Schiff bases (**9**, **10**, **24**) but the etherification of OH resulted in the decrease of activity (**12**, **27**, **30**). The replacement of OMe by OEt in **9** increased activity and reduced the toxicity (**10**). Against 3PS lympocytic leukemia, **9**, **18**, **24**, and **26** showed good activity.

Experimental Section‡,§

Intermediates. Amines needed were prepared by reduction of the corresponding nitro compounds with Sn and HCl. 3,4,5-Trichloronitrobenzene[2] and 3,5-dibromonitrobenzene[2] were prepared by the hydrolysis of corresponding benzamides.[3] 2,6-Dihalo-4-nitroanisoles or phenetoles were prepared by heating potassium salt of appropriate phenols with alkyl sulfate.[4]

2,6-Dichloro-*p*-phenetidine. To a mixture of 2.3 g (0.01 mole) of 2,6-dichloro-4-nitrophenetole and concd HCl (20 ml) was added gradually tin metal (4 g). EtOH (2 ml) was then added, the mixt was warmed on steam bath for a few min and left overnight at room temp. The next day it was basified (10% aqueous NaOH) and extd (Et_2O). Removal of solvent and crystn gave 1.62 g (80%) of the amine, mp 105-107°. *Anal.* ($C_8H_9Cl_2NO$) C, H, N.

4-({4-[*N*,*N*-Bis(2-chloroethyl)amino]-3-ethoxybenzylidene}-amino)-2,6-dibromophenol·HCl. To a solution of 2,6-dibromo-4-aminophenol·HCl (3.0 g, 0.01 mole) in dry warm EtOH was added a concd EtOH soln of 4-[*N*,*N*-bis(2-chloroethyl)amino]-3-ethoxybenzaldehyde (2.9 g, 0.01 mole). The resulting dark red soln on standing and cooling in ice deposited a crystn solid which was filtd and washed (abs EtOH, Et_2O) to give the pure hydrochloride (3.4 g, 70%) of the Schiff base. All the other Schiff bases were similarly prepared and are recorded in Table I.

Acknowledgments. We are greatly indebted to Dr. H. B. Wood, Chief, Drug Development Branch, Cancer Chemotherapy National Service Center, for his cooperation and for making the screening data available and to Mr. M. T. Jaokar for microanalyses. Thanks are due to Dr. N. K. Dutta, Director, Haffkine Institute, Bombay, for providing facilities to carry out the present work.

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†Antitumor screening data represented by this table will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JMED-72-1196.

‡Analytical results obtained were within ±0.4% of theor values.

§Melting points are capillary melting points and are uncor.