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## Thiolesters of Orotic Acid<sup>†</sup>

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The chemical reactivity associated with thiolesters<sup>1</sup> has been utilized to produce noncompetitive metabolic inhibitors.<sup>2-4</sup> 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<sup>5</sup> 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.<sup>6</sup>

## Experimental Section

All of the thiols were purchased from commercial sources. Orotol chloride was prepared by a previously reported procedure<sup>5</sup> 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

Table I. S-(Substituted)thiolesters of Orotic Acid<sup>a</sup>

R	Mp, °C	Reaction temp, °C	Recrystn solvent	Empirical formula <sup>b</sup>
<i>n</i> -Pr	208-209	Reflux	H <sub>2</sub> O	C <sub>8</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub> S
<i>n</i> -Bu	187-188	Reflux	AcOH-H <sub>2</sub> O	C <sub>9</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub> S
<i>n</i> -Hept	150-151	Reflux	AcOH	C <sub>12</sub> H <sub>18</sub> N <sub>2</sub> O <sub>3</sub> S
<i>n</i> -Dec	148-149	125-150	AcOH	C <sub>15</sub> H <sub>24</sub> N <sub>2</sub> O <sub>3</sub> S
<i>n</i> -Tetradec	131-132	125-150	AcOH	C <sub>19</sub> H <sub>32</sub> N <sub>2</sub> O <sub>3</sub> S
Cyclohex	248-249	Reflux	AcOH	C <sub>11</sub> H <sub>14</sub> N <sub>2</sub> O <sub>3</sub> S
Benzyl	228-230	125-150	AcOH	C <sub>12</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub> S

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

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<sub>2</sub>O

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to ppt the thiolesters which were recrystd and dried *in vacuo* overnight at 50-60° prior to elemental analysis.

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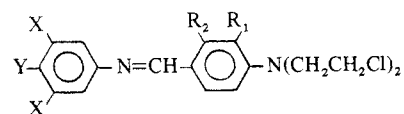
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## Potential Anticancer Agents. 4. Schiff Bases from Benzaldehyde Nitrogen Mustards

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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.<sup>1</sup> 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.



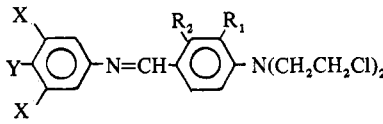
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X = Cl, Br  
Y = Cl, CN, OH, COOH, OMe, OEt, H  
R<sub>1</sub> = H, OMe  
R<sub>2</sub> = H, Me

**Chemistry.** The Schiff bases (Table I) were obtained as monohydrochlorides by heating the requisite pure amine hydrochlorides with mustard aldehydes in EtOH<sup>1</sup> 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 <sub>1</sub>	R <sub>2</sub>	Mp, °C	Formula <sup>a</sup>
1	Cl	Cl	H	CH <sub>3</sub>	218-220	C <sub>18</sub> H <sub>17</sub> Cl <sub>5</sub> N <sub>2</sub> ·HCl
2	Cl	Cl	OCH <sub>3</sub>	H	203-205	C <sub>18</sub> H <sub>17</sub> Cl <sub>5</sub> N <sub>2</sub> O·HCl
3	Cl	Cl	OC <sub>2</sub> H <sub>5</sub>	H	195-197	C <sub>19</sub> H <sub>19</sub> Cl <sub>5</sub> N <sub>2</sub> O·HCl
4	Cl	COOH	H	CH <sub>3</sub>	247	C <sub>19</sub> H <sub>18</sub> Cl <sub>4</sub> N <sub>2</sub> O <sub>2</sub> ·HCl
5	Cl	COOH	OCH <sub>3</sub>	H	140-141	C <sub>19</sub> H <sub>18</sub> Cl <sub>4</sub> N <sub>2</sub> O <sub>3</sub> ·HCl
6	Cl	COOH	OC <sub>2</sub> H <sub>5</sub>	H	167-169	C <sub>20</sub> H <sub>20</sub> Cl <sub>4</sub> N <sub>2</sub> O <sub>3</sub> ·HCl
7	Cl	OH	H	H	150-152	C <sub>17</sub> H <sub>16</sub> Cl <sub>4</sub> N <sub>2</sub> O·HCl
8	Cl	OH	H	CH <sub>3</sub>	240-242	C <sub>18</sub> H <sub>18</sub> Cl <sub>4</sub> N <sub>2</sub> O·HCl
9	Cl	OH	OCH <sub>3</sub>	H	255-256	C <sub>18</sub> H <sub>18</sub> Cl <sub>4</sub> N <sub>2</sub> O <sub>2</sub> ·HCl
10	Cl	OH	OC <sub>2</sub> H <sub>5</sub>	H	235-238	C <sub>19</sub> H <sub>20</sub> Cl <sub>4</sub> N <sub>2</sub> O <sub>2</sub>
11	Cl	OCH <sub>3</sub>	H	CH <sub>3</sub>	221-222	C <sub>19</sub> H <sub>20</sub> Cl <sub>4</sub> N <sub>2</sub> O·HCl
12	Cl	OCH <sub>3</sub>	OCH <sub>3</sub>	H	168-170	C <sub>19</sub> H <sub>20</sub> Cl <sub>4</sub> N <sub>2</sub> O <sub>2</sub> ·HCl
13	Cl	OCH <sub>3</sub>	OC <sub>2</sub> H <sub>5</sub>	H	135-138	C <sub>20</sub> H <sub>22</sub> Cl <sub>4</sub> N <sub>2</sub> O <sub>2</sub> ·HCl
14	Cl	OC <sub>2</sub> H <sub>5</sub>	H	H	232-235	C <sub>19</sub> H <sub>20</sub> Cl <sub>4</sub> N <sub>2</sub> O·HCl
15	Cl	OC <sub>2</sub> H <sub>5</sub>	H	CH <sub>3</sub>	227-229	C <sub>20</sub> H <sub>22</sub> Cl <sub>4</sub> N <sub>2</sub> O·HCl
16	Cl	OC <sub>2</sub> H <sub>5</sub>	OCH <sub>3</sub>	H	145-148	C <sub>20</sub> H <sub>22</sub> Cl <sub>4</sub> N <sub>2</sub> O <sub>2</sub> ·HCl
17	Br	H	H	CH <sub>3</sub>	230-232	C <sub>18</sub> H <sub>18</sub> Br <sub>2</sub> Cl <sub>2</sub> N <sub>2</sub> ·HCl
18	Br	H	OCH <sub>3</sub>	H	152-154	C <sub>18</sub> H <sub>18</sub> Br <sub>2</sub> Cl <sub>2</sub> N <sub>2</sub> O·HCl
19	Br	H	OC <sub>2</sub> H <sub>5</sub>	H	187-190	C <sub>19</sub> H <sub>20</sub> Br <sub>2</sub> Cl <sub>2</sub> N <sub>2</sub> O·HCl
20	Br	COOH	H	CH <sub>3</sub>	170-172	C <sub>19</sub> H <sub>18</sub> Br <sub>2</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>2</sub> ·HCl
21	Br	COOH	OC <sub>2</sub> H <sub>5</sub>	H	180-182	C <sub>20</sub> H <sub>20</sub> Br <sub>2</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>3</sub> ·HCl
22	Br	OH	H	H	215-218	C <sub>17</sub> H <sub>16</sub> Br <sub>2</sub> Cl <sub>2</sub> N <sub>2</sub> O·HCl
23	Br	OH	H	CH <sub>3</sub>	235-236	C <sub>18</sub> H <sub>18</sub> Br <sub>2</sub> Cl <sub>2</sub> N <sub>2</sub> O·HCl
24	Br	OH	OCH <sub>3</sub>	H	230	C <sub>18</sub> H <sub>18</sub> Br <sub>2</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>2</sub> ·HCl
25	Br	OH	OC <sub>2</sub> H <sub>5</sub>	H	230-232	C <sub>19</sub> H <sub>20</sub> Br <sub>2</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>2</sub> ·HCl
26	Br	OCH <sub>3</sub>	H	CH <sub>3</sub>	222-224	C <sub>19</sub> H <sub>20</sub> Br <sub>2</sub> Cl <sub>2</sub> N <sub>2</sub> O·HCl
27	Br	OCH <sub>3</sub>	OCH <sub>3</sub>	H	175-177	C <sub>19</sub> H <sub>20</sub> Br <sub>2</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>2</sub> ·HCl
28	Br	OCH <sub>3</sub>	OC <sub>2</sub> H <sub>5</sub>	H	142-144	C <sub>20</sub> H <sub>22</sub> Br <sub>2</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>2</sub> ·HCl
29	Br	OC <sub>2</sub> H <sub>5</sub>	H	CH <sub>3</sub>	230-233	C <sub>20</sub> H <sub>22</sub> Br <sub>2</sub> Cl <sub>2</sub> N <sub>2</sub> O·HCl
30	Br	OC <sub>2</sub> H <sub>5</sub>	OCH <sub>3</sub>	H	170-172	C <sub>20</sub> H <sub>22</sub> Br <sub>2</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>2</sub> ·HCl

<sup>a</sup>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 lymphocytic leukemia, 9, 18, 24, and 26 showed good activity.

#### Experimental Section<sup>‡,§</sup>

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

†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.

**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<sub>2</sub>O). Removal of solvent and crystn gave 1.62 g (80%) of the amine, mp 105-107°. *Anal.* (C<sub>9</sub>H<sub>9</sub>Cl<sub>2</sub>NO) 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 filt'd and washed (abs EtOH, Et<sub>2</sub>O) 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.

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