References

(1) G. L. Grunewald, A. M. Warner, S. J. Hays, R. H. Bussell, and M. K. Seals, *J. Med. Chem.*, **15**, 747 (1972) (paper 1).
(2) E. Vogel, W. Schröck, and W. A. Böll, *Angew. Chem.*, 78, 753 '1966).
(3) E. Vogel, M. Biskup, W. Pretzer, and W. Böll, *Angew. Chem., Int. Ed. Engl.*, 3, 642 (1964); E. Vogel, W. Pretzer, and W. A. Böll, *Tetrahedron Lett.*, 3613 (1965); E. Vogel, *Chimia*, **22**, 21 (1968).
(4) N. M. Yoon and H. C. Brown, *J. Amer. Chem. Soc.*, **90**, 2927 (1968).
(5) R. A. Turner, "Screening Methods in Pharmacology," Academic Press, New York, N. Y., 1965, p 47.
(6) Department of Pharmacology, University of Edinburgh, "Pharmacological Experiments on Isolated Preparations," Livingstone Ltd., Edinburgh and London, 1968, p 60.
(7) J. R. Sowa and C. C. Price, *J. Org. Chem.*, 34, 474 (1969); M. H. Benn, P. Kazmaier, and C. Watanatada, *Chem. Commun.*, 1685 (1970).
(8) B. Belleau, *Ann. N. Y. Acad. Sci.*, 139, 580 (1967).

New Compounds

Hydroxylamine Derivatives as Potential Antimalarial Agents. 2. Hydroxamates and Amidoximes†

John B. Hynes* and Linda G. Hack

Department of Pharmaceutical Chemistry, College of Pharmacy, Medical University of South Carolina, Charleston, South Carolina 29401.

Received March 6, 1972

In the preceding paper it was reported that terephthalohydroxamic acid (**1a**), as well as its dibenzoylated derivative **1b**, displayed significant antimalarial activity.[1] In the same year, the antimalarial properties of bis(trihalomethyl)arenes such as **2** were also reported.[2] A total of 24 compounds related to one or both of these structures was prepared and evaluated for possible activity enhancement.

1,4-$RONHCOC_6H_4CONHOR$

1a, R = H
1b, R = C_6H_5CO

1,4-$CCl_3C_6H_4CCl_3$

2

These included trihalomethylbenzohydroxamates (**3–7**), amidoximes (**8–12**), *N,O*-bis(trihalomethylbenzoyl)hydroxylamines (**13–19**), and derivatives of **1b** (**20–26**) (*cf.* Tables I, II, and III). None of these displayed significant activity against *Plasmodium berghei* in mice.[3,‡] In view of these results as well as those obtained earlier,[1] it appears doubtful that significant activity enhancement can be obtained by structural modification of **1a**.

It should be noted that the amidoximes **8**, **9**, **10**, and **12** showed significant toxicity.§ This was particularly disappointing in the case of **8**, which can be considered to be isosteric with one of the tautomeric forms of **1a**. However, several of the amidoximes were subsequently used to prepare a series of bis(trihalomethyl-1,2,4-oxadiazoles), which will be described in a forthcoming communication.

Experimental Section

Each of the raw materials employed in this research was obtained commercially with the exception of 4-trichloromethylbenzoyl chloride. The latter compound was prepared according to the literature method involving the $FeCl_3$-catalyzed hydrolysis of $\alpha,\alpha,\alpha,\alpha',\alpha',\alpha'$-hexachloro-*p*-xylene.[4] Compounds **3** and **4** were prepared from this acid chloride by reaction with the appropriate hydroxylamine hydrochloride in the presence of imidazole according to the method of Koenig and Deinzer.[1,5] Compound **5** was prepared from methyl 3-trifluoromethylbenzoate by reaction with hydroxylamine in the presence of excess NaOH in MeOH.[1]

4-Trifluoromethylbenzohydroxamic Acid (6). To a solution of 21 g (0.1 mole) of 4-trifluoromethylbenzoyl chloride in 300 ml of Et_2O was added 7.0 g (0.1 mole) of $NH_2OH \cdot HCl$ and 11.0 g of Na_2CO_3. Next, 20 ml of H_2O was added dropwise over 2 hr and the stirring was continued for 3 hr longer. The solid which formed was separated by filtration and then combined with the residue resulting from evaporation of the Et_2O layer. After washing with H_2O the solid was recrystallized from H_2O (*cf.* Table I).

2-Trifluoromethylbenzohydroxamic Acid (7). To a mixture of 3.5 g of $NH_2OH \cdot HCl$ (0.025 mole), 5.5 g of Na_2CO_3, and 150 ml of Et_2O was added 5.5 ml of H_2O. Then 5.25 g (0.025 mole) of 2-trifluoromethylbenzoyl chloride was added dropwise with stirring at room temperature over 0.5 hr. The solid which separated was removed by filtration, and the filtrate was evaporated to dryness *in vacuo*. Extraction of the residue with 70 ml of Et_2O and evaporation yielded a white solid which was purified by recrystallization from EtOAc.

Amidoximes (8–10 and 12). Compounds **10** and **12** were prepared according to the literature methods (*cf.* Table I). Terephthal- and isophthalamidoximes, **8** and **9**, were obtained by heating the appropriate nitrile at reflux with 4 equiv of NH_2OH, obtained by neutralizing the hydrochloride with NaOH in EtOH for 24 and 48 hr, respectively.

4-(Hydroxycarbamyl)benzamidoxime (11). A solution of 3.4 g of MeONa (0.063 mole) in 50 ml of MeOH was added to 4.35 g (0.025 mole) of $NH_2OH \cdot HCl$ in 60 ml of MeOH. After filtration, 4.4 g (0.025 mole) of ethyl 4-cyanobenzoate was added followed by an additional 3.4 g of MeONa. After stirring at room temperature for 6 days, the white solid was separated by filtration and dissolved in 10 ml of H_2O. Adjusting the pH to 5.5 with 1 *N* HCl and cooling produced the crude crystalline solid.

***N,O*-Bis(4-trichloromethylbenzoyl)hydroxylamine (13).** Numerous attempts were made to prepare 4-trichloromethylbenzohydroxamic acid. However, the only compound which could be isolated from any of the procedures in pure form was **13**. The following example afforded the title compound in poor yield but high purity. A mixture of 3.1 g of imidazole, 1.0 g (0.015 mole) of $NH_2OH \cdot HCl$, and 100 ml of MeCN was stirred in a system protected from moisture for 0.5 hr. Next, 7.7 g (0.03 mole) of 4-trichloromethylbenzoyl chloride was added dropwise over 1 hr. After stirring at room temperature for 4 hr, the solution was added to 300 ml of cold H_2O, and the resulting solid was separated by filtration and washed several times with H_2O.

***N,O*-Bis(trihalomethylbenzoyl)hydroxylamines (14–19).** Each of these compounds was prepared in a similar manner. The following procedure for compound **14** is representative. To a stirred solution of 2.1 g (0.01 mole) of 4-trifluoromethylbenzohydroxamic acid (**6**) in 10 ml of pyridine was added dropwise 2.1 g (0.01 mole) of 4-trifluoromethylbenzoyl chloride over 0.5 hr. After stirring for an additional 3 hr at room temperature, the mixture was poured into 12 ml of HCl (37%) containing 12 g of ice. The crude solid which formed was separated by filtration, washed with H_2O, and vacuum dried over P_2O_5.

†This work was supported by U. S. Army Medical Research and Development Command Contract No. DADA 17-69-C-9066.

‡Testing of all compounds was carried out by Dr. L. Rane of the University of Miami.

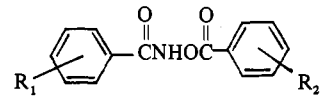
§Deaths due to toxicity occur in 3–5 days (mean survival time for controls was 6.1 days).

Table I. Amidoximes and Hydroxamic Acids

Name	No.	Mp, °C	Formula	Analyses[a]	Yield, %	Recrystallization medium and no.
O-Methyl-4-trichloromethylbenzohydroxamate	3	143–144	$C_9H_8Cl_3NO_2$	C, H, N, Cl	49	CCl_4 (1)
O-Ethyl-4-trichloromethylbenzohydroxamate	4	163–165	$C_{10}H_{10}Cl_3NO_2$	C, H, N, Cl	69	CCl_4 (1)
3-Trifluoromethylbenzohydroxamic acid	5	134–135	$C_8H_6F_3NO_2$	C, H, N, F	69	H_2O (2)
4-Trifluoromethylbenzohydroxamic acid	6	178–180	$C_8H_6F_3NO_2$	C, H, N, F	54	H_2O (2)
2-Trifluoromethylbenzohydroxamic acid	7	163–164	$C_8H_6F_3NO_2$	C, H, N, F	21	EtOAc (1)
Terephthalamidoxime	8	244–246[b] dec	$C_8H_{10}N_4O_2$	C, H, N	62	H_2O (1)
Isophthalamidoxime	9	200–203[c]	$C_8H_{10}N_4O_2$	C, H, N	57	EtOH–H_2O (1) (95:5)
4-Carbethoxybenzamidoxime	10	143–145[d]	$C_{10}H_{12}N_2O_3$	C, H, N	73	H_2O (2)
4-(Hydroxycarbamyl)benzamidoxime	11	185 dec	$C_8H_9N_3O_3$	C, H, N	20	H_2O (2)
Trichloroacetamidoxime	12	130–131[e]			64	C_6H_6–hexane (1) (1:1)

[a] Where analyses are indicated only by symbols of the elements, the results were within ±0.4% of the theoretical values. The analyses were performed by Galbraith Laboratories, Knoxville, Tenn. [b] Lennaers and Eloy[6] reported mp 180° dec. [c] Goldberg[7] reported mp 193°. [d] Müller[8] reported mp 135°. [e] Narayanan and Bernstein.[9]

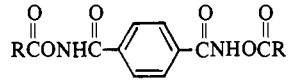
Table II. N,O-Diaroylated Hydroxylamines



R_1	R_2	No.	Mp, °C	Formula	Analyses[a]	Yield, %	Recrystallization medium and no.
4-CCl_3	4-CCl_3	13	178–180	$C_{16}H_9Cl_6NO_3$	C, H, N, Cl	12	MeOH (3)
4-CF_3	4-CF_3	14	193–194	$C_{16}H_9F_6NO_3$	C, H, N, F	59	CCl_4 (1)
3-CF_3	4-CF_3	15	120–121	$C_{16}H_9F_6NO_3$	C, H, N, F	72	CCl_4 (1)
3-CF_3	3-CF_3	16	93.5–95	$C_{16}H_9F_6NO_3$	C, H, N, F	60	CCl_4 (1)
4-CF_3	3-CF_3	17	124–125.5	$C_{16}H_9F_6NO_3$	C, H, N, F	65	CCl_4 (1)
4-CF_3	4-CCl_3	18	174–175.5	$C_{16}H_9Cl_3F_3NO_3$	C, H, N, Cl, F	55	CCl_4 (2)
3-CF_3	4-CCl_3	19	134–135	$C_{16}H_9Cl_3F_3NO_3$	C, H, N, Cl, F	30	CCl_4 (2)

[a] *Cf.* footnote *a*, Table I.

Table III. Symmetrical O,O′-Disubstituted Terephthalohydroxamates



R	No.	Mp, °C	Formula	Analyses[a]	Yield, %	Recrystallization medium and no.
4-$CCl_3C_6H_4$	20	207.5–210	$C_{24}H_{14}Cl_6N_2O_6$	C, H, N, Cl	28	Dioxane (2)–acetone (1)
4-$CF_3C_6H_4$	21	245 dec	$C_{24}H_{14}F_6N_2O_6$	C, H, N, F	33	MeOH (1)
3-$CF_3C_6H_4$	22	214–215.5	$C_{24}H_{14}F_6N_2O_6$	C, H, N, F	26	EtOH (1)–MeOH (1)
3,4,5-$(CH_3O)_3C_6H_2$	23	203–204	$C_{28}H_{28}N_2O_{12}$	C, H, N	46	MeOH (1)
C_6F_5	24	210 dec	$C_{22}H_6F_{10}N_2O_6$	C, H, N, F	28	EtOH (1)
C_6H_5NH	25	215 dec	$C_{22}H_{18}N_4O_6$	C, H, N	6	EtOH (1)[b]
$(C_6H_5)_2N$	26	205–207	$C_{34}H_{26}N_4O_6$	C, H, N	43	EtOH (1)

[a] *Cf.* footnote *a*, Table I. [b] By extraction from a large quantity of intractable white solid.

O,O′-**Diaroylterephthalohydroxamates (20–24).** These compounds were prepared by the reaction of the disodium salt of 1a with the requisite acid chloride at reflux in dioxane as in the synthesis of 1b.[1]

O,O′-**Bis(phenylcarbamyl)terephthalohydroxamate (25).** A mixture of 3.9 g (0.02 mole) of 1a in 20 ml of pyridine was stirred in a system protected from moisture by an N_2 purge. Next, 4.8 g (0.04 mole) of PhNCO was added dropwise over 0.5 hr, and the resulting mixture was stirred at room temperature for an additional 4 hr. Addition to 25 ml of HCl (37%) containing 25 g of ice produced a white solid which was separated by filtration and washed with H_2O. After drying, most of the crude solid was found to be insoluble in boiling EtOH and other organic solvents.

O,O′-**Bis(diphenylcarbamyl)terephthalohydroxamate (26).** To a stirred solution of 4.64 g (0.02 mole) of diphenylcarbamyl chloride in 20 ml of pyridine was added portionwise 1.96 g (0.01 mole) of 1a over a 1-hr period. Next, the mixture was added to 25 ml of HCl (37%) containing 25 g of ice. The white precipitate was separated by filtration, washed with H_2O, vacuum dried without a desiccant, and then recrystallized.

Acknowledgment. The authors wish to thank Dr. Edgar A. Steck of the Walter Reed Army Institute of Research for many helpful discussions during the course of this work. This is Contribution No. 1044 to the Army Research Program on Malaria.

References

(1) J. B. Hynes, *J. Med. Chem.*, 13, 1235 (1970).
(2) E. F. Elslager, M. P. Hutt, and L. M. Werbel, *ibid.*, 13, 542 (1970).
(3) T. S. Osdene, P. B. Russell, and L. Rane, *ibid.*, 10, 431 (1967).

(4) I. G. Farbenind, A.-G., French Patent 820,696 (1937); *Chem. Abstr.*, **32**, 3422 (1938).
(5) T. Koenig and M. Deinzer, *J. Amer. Chem. Soc.*, **90**, 7014 (1968).
(6) R. Lennaers and F. Eloy, *Helv. Chim. Acta*, **46**, 1067 (1963).
(7) G. Goldberg, *Ber.*, **22**, 2976 (1889).
(8) G. Müller, *ibid.*, **18**, 2486 (1885).
(9) V. L. Narayanan and J. Bernstein, *J. Heterocycl. Chem.*, **3**, 214 (1966).

Thiolesters of Orotic Acid†

Dale R. Sargent and Charles G. Skinner*

Department of Chemistry, North Texas State University, Denton, Texas 76203. *Received May 25, 1972*

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]

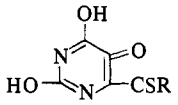
Experimental Section

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.

References

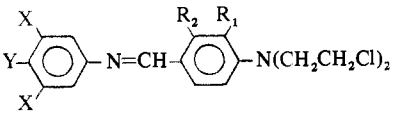
(1) A. W. Baker and G. H. Harris, *J. Amer. Chem. Soc.*, 82, 1923 (1960).
(2) J. M. Ravel, T. J. McCord, C. G. Skinner, and W. Shive, *J. Biol. Chem.*, **232**, 159 (1958).
(3) C. G. Skinner, G. F. McKenna, T. J. McCord, and W. Shive, *Tex. Rep. Biol. Med.*, **16**, 493 (1958).
(4) K. Hayashi, C. G. Skinner, and W. Shive, *ibid.*, 19, 277 (1961).
(5) B. A. Ivin and V. G. Nemets, *Zh. Obshch. Khim.*, **35**, 1294 (1965); *Chem. Abstr.*, **63**, 11557*e* (1965).
(6) E. M. Lansford, W. M. Harding, and W. Shive, *Arch. Biochem. Biophys.*, **73**, 180 (1958).

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

D. R. Shekawat, S. S. Sabnis, and C. V. Deliwala*

Department of Chemotherapy, Haffkine Institute, Bombay-12, India. *Received April 28, 1972*

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-