granules with a standard deviation of 5.14. The relatively high standard deviation indicates intergranule variability in strength. To meet the requirement of statistical significance, the data were evaluated by Duncan's multiple-range test (7) which classifies the means into subsets. A statistical difference exists between different subsets but not between members of the same subset. The first subset is aspirin granules; the second, sugar–starch pellets; the third, lactose with 10% methylcellulose, lactose with 20% polyvinylpyrrolidone and dicalcium phosphate with 10% methylcellulose; and the fourth, lactose granulated with water and dicalcium phosphate granulated with water. The use of 20% polyvinylpyrrolidone or 10% methylcellulose in preparing lactose granules increased granule strength over those made by aqueous granulation. Also, 10% methylcellulose increased the granule strength of dicalcium phosphate over the straight aqueous granulation. The increase in strength of 20% polyvinylpyrrolidone over 10% methylcellulose for lactose granules does not have statistical significance.

Measurements were also made on 10–12-, 16–18-, and 20–25-mesh aspirin granules; the effect of granule size on the strength of these granules is shown in Fig. 6. Each value represents the average of 20 measurements, and the vertical bracketed lines are the 95% confidence limits. A linear relationship was found to exist between the strength of these granules and granule size.

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NOTES

Schiff Base Derivatives of Anti-Inflammatory *O*-Substituted Hydroxylamines

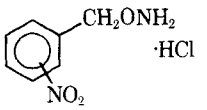
GLENN H. HAMOR and LESLIE D. WATSON*

Abstract □ The synthesis of 12 azomethine (Schiff base) derivatives by the reaction of various nitrobenzaldehydes and salicylaldehydes with the anti-inflammatory compounds, *m*-nitrobenzyloxyamine hydrochloride and 4-thiazolylmethoxyamine dihydrochloride, and with congeneric *O*-substituted hydroxylamines is described. These Schiff bases, which are structurally related to the naturally occurring pyridoxal derivatives, showed no activity in the carrageenin-induced rat paw edema test. These compounds possessed no antimalarial activity and no activity against the L-1210 mouse lymphoid leukemia test system.

Keyphrases □ Hydroxylamines, *O*-substituted—synthesis of Schiff base derivatives, pharmacological screening □ Azomethine (Schiff base) derivatives—synthesis, pharmacological screening □ Anti-inflammatory hydroxylamines—synthesis, pharmacological screening of Schiff base derivatives

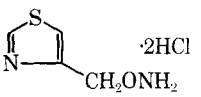
Potent anti-inflammatory activity in the carrageenin-induced rat paw edema test is shown by *m*-nitrobenzyloxyamine hydrochloride (I), *p*-nitrobenzyloxyamine hydrochloride (II), and 4-thiazolylmethoxyamine dihydrochloride (III) (1). Moreover, I, II, and III also possess powerful inhibitory activity *in vitro* against the histamine-forming enzyme, specific histidine decarboxylase (1–3).

The present work describes the synthesis and biological activity of Schiff base derivatives of these anti-inflammatory *O*-substituted hydroxylamines (I and III) and congeneric compounds. These Schiff bases are



I: *meta*

II: *para*



III

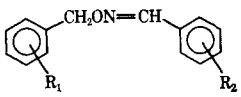
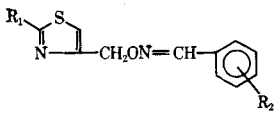
structurally related to naturally occurring pyridoxal derivatives.

Pyridoxal phosphate, the coenzyme of mammalian histidine decarboxylases, is attached to the apoenzyme by an azomethine linkage (Schiff base) with the ϵ-amino group of a lysine unit in the enzyme molecule (4). Schiff bases of pyridoxal phosphate are highly reactive and can react with an amino acid more rapidly than can the free aldehyde (5).

It is hoped that the synthetic Schiff bases described in this report might compete with pyridoxal phosphate for the amino group of apoenzyme-bound histidine.

The 12 Schiff bases synthesized are listed and their analytical data and melting points are given in Table I. The syntheses of the starting benzyloxyamines (1) (with the exception of 2-chloro-4,5-methylenedioxybenzyloxyamine hydrochloride which is described in the *Experimental* section) and 4-thiazolylmethoxyamine dihydrochloride (6) are reported in other publications. The Schiff bases were prepared by standard methods (7, 8)

Table I—Schiff Bases

Compound	R_1	R_2	Formula	Melting Point	Recrystallization Solvent	Yield, %	Anal. Calcd.	Anal. Found
								
1	*m*-NO_2	*m*-NO_2	$C_{14}H_{11}N_3O_5$	94–96°	Me_2CO–H_2O	85	C, 55.82 H, 3.68 N, 13.95	C, 56.00 H, 4.00 N, 13.68
2	*m*-NO_2	*p*-NO_2	$C_{14}H_{11}N_3O_5$	131.5–132°	Me_2CO–H_2O	77	C, 55.82 H, 3.68 N, 13.95	C, 56.02 H, 3.69 N, 14.06
3	*m*-NO_2	*o*-OH	$C_{14}H_{12}N_2O_4$	83.5–85.5°	EtOH	75	C, 61.75 H, 4.45 N, 10.29	C, 61.97 H, 4.44 N, 10.66
4	*m*-NO_2	2-OH 5-Cl	$C_{14}H_{11}ClN_2O_4$	113–114°	Me_2CO–EtOH	88	C, 54.82 H, 3.62 N, 9.14	C, 54.87 H, 3.74 N, 9.09
5	*m*-Cl	*o*-CH	$C_{14}H_{12}ClNO_2$	36–37°	MeOH	83	C, 64.24 H, 4.63 N, 5.35	C, 64.29 H, 4.70 N, 5.22
6	*m*-Cl	2-OH 5-Cl	$C_{14}H_{11}Cl_2NO_2$	82°	MeOH	82	C, 56.77 H, 3.75 N, 4.73	C, 56.88 H, 3.75 N, 4.72
7	*p*-Cl	*o*-OH	$C_{14}H_{12}ClNO_2$	94–95°	MeOH	93	C, 64.24 H, 4.63 N, 5.35	C, 64.26 H, 4.74 N, 5.18
8	*p*-Cl	2-OH 5-Cl	$C_{14}H_{11}Cl_2NO_2$	95–98°	MeOH	90	C, 56.77 H, 3.75 N, 4.73	C, 56.83 H, 3.93 N, 4.65
9	*p*-Cl	H	$C_{14}H_{12}ClNO$	31–32°	MeOH	83	C, 68.42 H, 4.93 N, 5.70	C, 68.50 H, 5.14 N, 5.46
10	2-Cl-4,5-methylenedioxy	2-OH 5-Cl	$C_{15}H_{11}Cl_2NO_4$	130–132.5°	EtOH	60	C, 52.96 H, 3.27 N, 4.12	C, 53.06 H, 3.36 N, 3.96
								
11	H	*m*-NO_2	$C_{11}H_9N_3O_3S$	98–99°	EtOH	61	C, 50.20 H, 3.42 N, 15.96	C, 50.47 H, 3.59 N, 15.72
12	H	*p*-NO_2	$C_{11}H_9N_3O_3S$	127–128°	EtOH–H_2O	51	C, 50.20 H, 3.42 N, 15.96	C, 50.03 H, 3.54 N, 15.45

involving reaction of the appropriate aldehyde with the oxyamine hydrochloride in ethanol.

BIOLOGICAL RESULTS

Anti-Inflammatory Screening—Selected compounds were submitted for pharmacological testing; preliminary results[1] are shown in Table II. The four compounds screened, namely, *m*-nitrobenzaldehyde *O*-(*m*-nitrobenzyl)oxime (Compound 1), *p*-nitrobenzaldehyde *O*-(*m*-nitrobenzyl)oxime (Compound 2), salicylaldehyde *O*-(*m*-nitrobenzyl)oxime (Compound 3), and 5-chlorosalicylaldehyde *O*-(*m*-chlorobenzyl)oxime (Compound 6), showed no anti-inflammatory activity in the carrageenin-induced rat paw edema test. In fact, several of the compounds produced a slight increase in edema. This lack of anti-inflammatory activity is in marked contrast to the potent activity of the parent *m*-nitrobenzyloxyamine (1). These four compounds displayed no significant CNS or cardiovascular effects in animal testing. Preliminary results obtained for the related thiazole derivative, 5-chlorosalicylaldehyde *O*-(4-thiazolylmethyl)oxime, indicate that it lacks anti-inflammatory activity as well as being less toxic than the parent 4-thiazolylmethoxyamine dihydrochloride (9).

Anticancer Screening—Seven of the compounds, Compound 1 (NSC 131323), Compound 2 (NSC 131324), Compound 3 (NSC 131406), Compound 4 (NSC 132327), Compound 5 (NSC 132326), Compound 11 (NSC 132328), and Compound 12 (NSC 132329), were submitted[2] for screening against the L-1210 mouse lymphoid leukemia test system (10). None of the compounds possessed any significant antitumor activity.

Antimalarial Screening—Seven compounds (Compounds 1–5, 11, and 12) were tested for antimalarial activity against *Plasmodium bergheii* in mice[3] and were found to be inactive.

EXPERIMENTAL

The syntheses of representative compounds reported in Table I are described here. All melting points were taken on a Fisher-Johns hot stage and are uncorrected. Elemental microanalyses were performed[4]. The IR spectra of all compounds were determined on a Perkin-Elmer Infracord apparatus in mineral oil mulls and are in agreement with the assigned structures.

[1] The authors thank Riker Laboratories, Inc., Northridge, CA 91324, for performing the pharmacological testing.

[2] To the Cancer Chemotherapy National Service Center, National Cancer Institute.

[3] Under the auspices of Walter Reed Army Institute of Research.

[4] Elek Microanalytical Laboratories, Torrance, Calif.

Table II—Pharmacology[a]

Compound	LD_{50}, mg./kg. i.p., Mice	LD_{50}, mg./kg. p.o., Rat	Carrageenin-Induced Rat Paw Edema Percent Reduction, 250 mg./kg.
1	>800	>1000	(2) Increase
2	800	>1000	4
3	>800	—	2
6	>800	>1000	(18) Increase

[a] Pharmacological testing was performed by Riker Laboratories, Inc., Northridge, Calif.

***m*-Nitrobenzaldehyde *O*-(*m*-Nitrobenzyl)oxime (Compound 1)**—To 2.4 g. (0.012 mole) of *m*-nitrobenzaldehyde dissolved in 30 ml. hot 95% EtOH was added slowly 1.8 g. (0.012 mole) of *m*-nitrobenzyloxyamine hydrochloride (1) dissolved in 40 ml. 95% EtOH. The solution was stirred for 10 min. and then filtered to give 3.0 g. (85% yield) of a white crystalline solid, m.p. 93–95°. An analytical sample, m.p. 94–96°, was obtained by two recrystallizations from acetone–H_2O.

***m*-Nitrobenzaldehyde *O*-(4-Thiazolylmethyl)oxime (Compound 11)**—To 8.1 g. (0.04 mole) of 4-thiazolylmethoxyamine dihydrochloride (6) dissolved in 600 ml. warm 95% EtOH was added, in portions, 6.0 g. (0.04 mole) of *m*-nitrobenzaldehyde dissolved in 200 ml. warm 95% EtOH. The solution was refluxed for 3 hr. and let stand overnight at room temperature. A small amount (0.5 g.) of nonsulfur-containing solid, m.p. 195.5°, was filtered off and discarded. The filtrate was diluted to 2 l. with H_2O; the resultant crystalline solid was collected and recrystallized from EtOH–H_2O to give 6.4 g. (61%) of cream-colored solid, m.p. 97–99°. One additional recrystallization yielded an analytical sample, m.p. 98–99°.

2-Chloro-4,5-methylenedioxybenzyloxyamine Hydrochloride—To a solution of 16.5 g. (0.05 mole) *N*-(2-chloro-4,5-methylenedioxybenzyloxy)phthalimide (1) in 600 ml. warm anhydrous EtOH was added 2.5 ml. (0.05 mole) hydrazine hydrate (99%), and the solution was refluxed for 4 hr. The reaction mixture was cooled, and the precipitated phthalhydrazide was removed by filtration. Addition of excess ethanolic HCl to the filtrate followed by reduction in volume by evaporation gave 10 g. (84%), m.p. 191–194°. An analytical sample was obtained by dissolving the compound in H_2O, converting to the free base with Na_2CO_3, extracting with ether, drying with Na_2SO_4, and reconverting to the hydrochloride with ethanolic HCl.

Anal.—Calcd. for $C_8H_9Cl_2NO_3$: C, 40.40; H, 3.78; N, 5.88. Found: C, 40.31; H, 4.00; N, 5.73.

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Worldwide Virtual Temperatures for Product Stability Testing

JOHN D. HAYNES

Abstract □ The market temperatures under which product stability expiration dates for products are to apply should be simulated by laboratory conditions. An actual simulation would involve a pattern of changing monthly temperatures for each location. For those products whose loss rate constant is related to temperature by the Arrhenius relationship, a single virtual temperature can be determined at which the loss rate is equivalent to that of this changing pattern of temperature. This has been calculated for each of 15 cities in the United States and 15 elsewhere. Tables I and II should serve as guides in considering the standard temperature to be incorporated into the laboratory stability test protocol for such products.

Keyphrases □ Product stability testing—simulation of worldwide virtual temperatures □ Expiration dating—guides for determining standard temperatures □ Virtual temperature—application to product stability testing

Drug products stored in pharmacies and warehouses for extended periods of time are exposed to a range of temperatures which, in the United States at least, is narrowed from the ambient range by the widespread use of air conditioning. Storage of some products is governed by restrictions printed on the labels, and these products are not the topic of this paper, although the principle employed may be applied to some of them. According to R. Blythe (1): "Twenty-seven or 68 percent of the reporting [pharmaceutical] companies conduct *field tests* on some of the products. . . in the warmer, more humid areas of the United States. . . Twenty-one