CNS Depressant Effects of Schiff Bases Derived from 4,6-Dichloro-5-pyrimidinecarboxaldehydes and Substituted Anilines

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Abstract □ Several Schiff bases were prepared by the reaction of 4,6-dichloro-5-pyrimidinecarboxaldehydes with substituted anilines. When the aniline was substituted at the *ortho*-position by a mercapto group, cyclization to the corresponding benzothiazoline resulted. A representative number of the new compounds showed CNS depressant activity in mice at doses ranging from 12.7 to 400 mg/kg ip.

Keyphrases □ Schiff bases—prepared from 4,6-dichloro-5-pyrimidinecarboxaldehydes and substituted anilines, CNS depressant activity □ 4,6-Dichloro-5-pyrimidinecarboxaldehydes—reacted with substituted anilines to form Schiff bases, CNS depressant activity □ Anilines, substituted—reacted with 4,6-dichloro-5-pyrimidinecarboxaldehydes to form Schiff bases, CNS depressant activity □ CNS depressant activity—Schiff bases formed from 4,6-dichloro-5-pyrimidinecarboxaldehydes and substituted anilines

Anils and related isosteres such as stilbenes and diazo-type compounds are known to have varying chemotherapeutic effects (1). Certain Schiff bases derived from pyridoxal have been implicated in biological mechanisms involving transamination and oxidation (2). Relatively little has been reported, however, about the biological effects of Schiff bases derived from halogenated pyrimidinecarboxaldehydes (3). During an investigation of the synthesis of fused pyrimidine systems, Schiff bases were prepared from 4,6-dichloro-5-pyrimidinecarboxaldehyde intermediates (4) and substituted anilines. A representative number of these compounds were found to be central nervous system (CNS) depressants. In view of the paucity of information available for these types of derivatives, their preparation and observed biological responses are now reported.

CHEMISTRY

The Schiff bases II*a*–II*k* (Table I) were prepared by treating various substituted anilines in acetic acid solution with one equivalent of the pyrimidinecarboxaldehydes I*a* and I*b* at room temperature (Scheme I). In certain instances, chloroform was added to the acetic acid solution of the amine to improve solubility. Yields varied from 30 to 85%.

In one experiment, heating *p*-chloroaniline in acetic acid for a few minutes on a steam bath with one equivalent of I*a* resulted not only in Schiff-base formation but also in displacement of the 6-chloro group by the amine. The product thus afforded was 4-chloro-6-(*p*-chloroanilino)-5-[*N*-(*p*-chlorophenyl)formimidoyl]-2-(methylthio)pyrimidine (III*a*). Similarly, heating *p*-chloroaniline with I*b* gave 4-chloro-6-(*p*-chloroanilino)-5-[*N*-(*p*-chlorophenyl)-formimidoyl]-2-phenylpyrimidine (III*b*). The preparation of similar types of Schiff bases was recently reported (5); in that report the reaction of 2,4-diamino-6-chloro-5-pyrimidinecarboxaldehyde with two equivalents of various substituted anilines, in boiling ethanol containing hydrochloric acid, resulted in Schiff-base formation with concomitant displacement of the chloro group by the amine.

When *o*-mercaptoaniline was allowed to react with I*a* in acetic acid at room temperature, the product obtained was 2-(4,6-dichloro-2-methylthio-5-pyrimidinyl)benzothiazoline (IV*a*). The Schiff base initially formed presumably must then undergo a cyclo-addition process initiated by attack of the mercapto group on the azomethine linkage. In identical fashion, reaction of I*b* with *o*-mercaptoaniline afforded 2-(4,6-dichloro-2-phenyl-5-pyrimidinyl)benzothiazoline (IV*b*). Hull (6) also observed thiazoline formation in the reaction of 4-hydroxy-6-methyl-2-piperidinopyrimidine-5-carboxaldehyde with *o*-mercaptoaniline.

PHARMACOLOGY

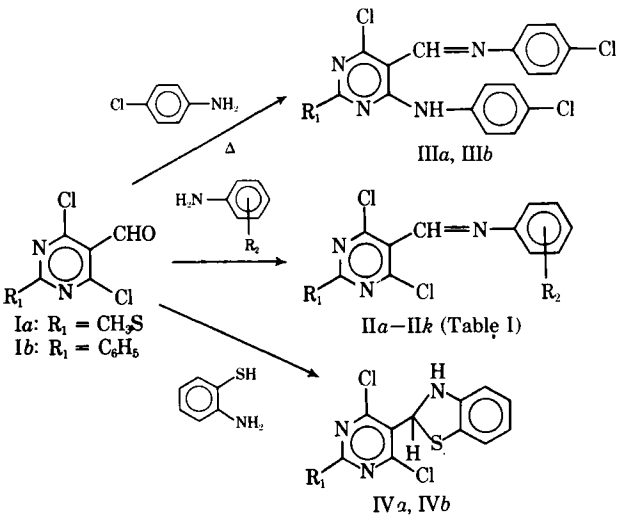
The title compounds were administered orally or intraperitoneally to each of three mice at four separate doses: 400, 127, 40, and 12.7 mg/kg. The animals were observed for a minimum of 2 hr during which time signs of stimulation (*i.e.*, increased spontaneous motor activity, hyperactivity on tactile stimulation, and twitching) or depression (*i.e.*, decreased spontaneous motor activity and decreased respiration) were noted.

Compounds II*a*, II*c*, II*d*, II*f*, II*g*, II*h*, II*i*, and II*k* as well as IV*a* and IV*b* were found to have CNS depressant activity when administered in doses ranging from 40 to 400 mg/kg ip, as manifested by decreased motor activity and respiration. In addition to depressing the CNS, IV*a* moderately lowered blood pressure in hypertensive rats when administered orally at a dose of 100 mg/kg.

Compounds III*a* and III*b* decreased the motor activity and respiration of mice when they were administered at a dose of 12.7 mg/kg ip.

EXPERIMENTAL[1]

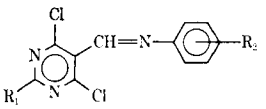
4,6-Dichloro-5-[*N*-(*o*-chlorophenyl)formimidoyl]-2-methylthiopyrimidine (IIc)—This example typifies the method used to



Scheme I

[1] Melting points were determined with a Thomas-Hoover capillary melting-point apparatus and are uncorrected. The IR spectra were determined in potassium bromide disks using a Perkin-Elmer model 21 spectrophotometer. NMR spectra were determined on a Varian A-60 spectrometer in deuterochloroform, using tetramethylsilane as the internal reference. The intermediate pyrimidinecarboxaldehydes (I*a* and I*b*) were previously described (Ref. 4). The various substituted anilines were all commercially available. The nomenclature used is recommended by *Chemical Abstracts*.

Table I—Properties of Schiff Bases of 4,6-Dichloropyrimidine-5-carboxaldehydes



Compound	R_1	R_2	Yield, %	Melting Point	Crystallization Solvent	Formula	Analysis, % Calc.	Found
II*a*	CH_3S	4-Cl	30	103–105°	Pentane	$C_{12}H_8Cl_3N_3S$	C 43.33 H 2.42 Cl 31.98 N 12.64	43.70 2.34 31.78 12.40
II*b*	CH_3S	3-Cl; 4-OH; 6-Cl	67	139–141°	Cyclohexane	$C_{12}H_7Cl_4N_3OS$	C 37.62 H 1.84 Cl 37.02 N 10.97	37.65 2.20 37.07 10.91
II*c*	CH_3S	2-Cl	45	112–115°	Heptane	$C_{12}H_8Cl_3N_3S$	C 43.33 H 2.42 Cl 31.98 N 12.64	43.61 2.57 31.91 12.62
II*d*	CH_3S	2-CH_3; 5-Cl	85	145–147°	Ethanol	$C_{13}H_{10}Cl_3N_3S$	C 45.04 H 2.91 Cl 30.68 N 12.12	45.17 2.65 30.57 12.05
II*e*	CH_3S	4-$OCH_2C_6H_5$	62	105–108°	Heptane	$C_{19}H_{15}Cl_2N_3OS$	C 56.44 H 3.74 Cl 17.54 N 10.39	56.66 3.87 17.26 10.52
II*f*	C_6H_5	4-Cl	70	190–193°	Chloroform	$C_{17}H_{10}Cl_3N_3$	C 56.30 H 2.78 Cl 29.33 N 11.59	56.00 2.50 29.38 11.40
II*g*	C_6H_5	2-Cl	70	157–159°	Ethyl acetate	$C_{17}H_{10}Cl_3N_3$	C 56.30 H 2.78 Cl 29.33 N 11.59	56.00 2.81 29.20 11.52
II*h*	C_6H_5	4-OCH_3	39	142–144°	Cyclohexane	$C_{18}H_{13}Cl_2N_3O$	C 60.35 H 3.66 Cl 19.79 N 11.73	60.31 3.54 19.53 11.72
II*i*	C_6H_5	4-$OCH_2C_6H_5$	69	168–171°	Cyclohexane	$C_{24}H_{17}Cl_2N_3O$	C 66.37 H 3.94 Cl 16.33 N 9.67	66.54 3.64 16.01 9.90
II*j*	C_6H_5	2-$CONH_2$	65	159–161°	Ethanol	$C_{18}H_{12}Cl_2N_4O$	C 58.24 H 3.26 Cl 19.10 N 15.09	58.24 3.52 19.15 15.05
II*k*	C_6H_5	4-$NHCOCH_3$	39	215–218°	Ethanol	$C_{19}H_{14}Cl_2N_4O$	C 59.24 H 3.66 Cl 18.40 N 14.54	59.56 3.78 18.17 14.44

prepare II*a*–II*k* (Table I). To 2.6 g of *o*-chloroaniline in 50 ml of acetic acid was added 4.5 g of 4,6-dichloro-2-methylthio-5-pyrimidinecarboxaldehyde (I*a*). The reaction mixture was allowed to stand at room temperature for a few minutes, during which time a crystalline product was deposited. This material was collected on a filter (6.5 g) and recrystallized from heptane, giving 3 g of product, mp 112–115°; IR (KBr): 6.17 (C=N) μm.

2-(4,6-Dichloro-2-methylthio-5-pyrimidinyl)benzothiazoline (IV*a*)—This compound was prepared from 2.5 g of *o*-mercaptoaniline and 4.6 g of I*a* in 50 ml of acetic acid. In a few minutes, a yellow precipitate was formed. After 0.5 hr at room temperature, the reaction mixture was filtered and the collected solid was recrystallized from cyclohexane, giving 1.1 g of product, mp 116–118°; IR: 3.05 (NH) μm; NMR: δ 4.04 (s, 1H, NH, disappears on deuteration) and 7.11 (s, 1H, H—C(—S)—N) ppm.

Anal.—Calc. for $C_{12}H_9Cl_2N_3S_2$: C, 43.64; H, 2.75; Cl, 21.47; N, 12.72. Found: C, 43.75; H, 2.77; Cl, 21.29; N, 12.59.

2-(4,6-Dichloro-2-phenyl-5-pyrimidinyl)benzothiazoline (IV*b*)—This compound was prepared in similar fashion from 2.5 g of *o*-mercaptoaniline and 5.0 g of I*b*. Recrystallization from cyclohexane gave 1.5 g of product, mp 156–158°; IR: 3.02 (NH) μm; NMR: δ 3.95 (s, 1H, NH, disappears on deuteration) and 7.23 (s, 1H, H—C(—S)—N) ppm.

Anal.—Calc. for $C_{17}H_{11}Cl_2N_3S$: C, 56.68; H, 3.08; Cl, 19.68; N, 11.66. Found: C, 56.94; H, 2.90; Cl, 19.39; N, 11.71.

4-Chloro-6-(*p*-chloroanilino)-5-[*N*-(*p*-chlorophenyl)formimidoyl]-2-(methylthio)pyrimidine (III*a*)—To a solution of 2.5 g of *p*-chloroaniline in 50 ml of acetic acid was added 4.46 g of I*a*. The reaction mixture was heated on a steam bath for approximately 10 min and then cooled to room temperature, and 25 ml of water was added. The precipitate which formed was collected on a filter. Recrystallization from benzene–pentane gave 1.1 g of product, mp 204–206°; IR: 6.08 (C=N) μm.

Anal.—Calc. for $C_{18}H_{13}Cl_3N_4S$: C, 51.02, H, 3.09; Cl, 25.10; N, 13.20. Found: C, 51.25; H, 3.09; Cl, 25.30; N, 13.20.

4-Chloro-6-(*p*-chloroanilino)-5-[*N*-(*p*-chlorophenyl)formimidoyl]-2-phenylpyrimidine (III*b*)—Compound III*b* was prepared in similar fashion from 3.8 g of *p*-chloroaniline and 6.6 g of I*b* in 100 ml of chloroform containing 40 ml of acetic acid. After heating on a steam bath for 0.5 hr, the reaction mixture was chilled in ice. The yellow precipitate which was formed amounted to 3.6 g after recrystallization from chloroform, mp 235–237°; IR: 6.12 (C=N) μm.

Anal.—Calc. for $C_{23}H_{15}Cl_3N_4$: C, 60.88; H, 3.33; Cl, 23.44; N, 12.35. Found: C, 61.03; H, 3.35; Cl, 23.40; N, 12.47.

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Thiazine Dye Antagonism of Opioid Lethality in Mice

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Abstract □ The thiazine dyes, methylene blue and tolonium chloride, were administered subcutaneously alone and in combination to adult male mice in low (0.1 mg) and high (1.0 mg) doses 5 min prior to lethal intraperitoneal doses ($LD_{99.5}$) of morphine, codeine, meperidine, levorphanol, methadone, levopropoxyphene, and propoxyphene. Survivors were recorded at 2- and 24-hr intervals after each opioid challenge. Tolonium pretreatment (0.1 mg) significantly increased the number of survivors that had received lethal doses of morphine and propoxyphene in the 2-hr phase in contrast to methylene blue pretreatment at either dosage level which failed to protect animals against opioid lethality in either the 2- or 24-hr phase. At both the 2- and 24-hr intervals, the low-dosage dye combination (0.1 mg of each dye) significantly protected the animals from lethal doses of morphine, levorphanol, and methadone. The low-dosage dye combination shortened the duration of sleep induced by hexobarbital (100 mg/kg), presumably through inhibition of CNS depression.

Keyphrases □ Thiazine dyes—antagonism of opioid lethality, mice □ Methylene blue—antagonism of opioid lethality, mice □ Tolonium chloride—antagonism of opioid lethality, mice □ Opioids, lethal doses—protection by thiazine dyes

Earlier observations by Harpel and Mann (1, 2) revealed that certain thiazine dyes, methylene blue or tolonium chloride, when administered prophylactically in conjunction with either nalorphine or levallorphan, enhanced the antidotal capabilities of these specific inhibitors against toxic doses of propoxyphene hydrochloride in mice. Similarly, in a pilot study employing methadone hydrochloride in mice, the preadministration of these thiazine dyes alone reduced opioid lethality. Accordingly, the purpose of this investigation was to ascertain the antidotal effectiveness of low (0.1 mg) and high (1.0 mg) doses of methylene blue and tolonium chloride, when administered singly or in an equal dosage combination, prior to lethal doses of several opioid[1] agents in mice. It was also anticipated that insight into the mechanism and extent of protection might be realized by testing the influence of these thiazine dyes upon hexobarbital sleeping time.

EXPERIMENTAL

Adult male mice[2], weighing between 20 and 25 g, were used. Before treatment, the animals were caged in groups of 50 for several days with free access to laboratory chow[3] and water. Immediately prior to experimentation, the animals were placed singly in stainless steel, wire-mesh cages without food and water. In the toxicity studies, however, food and water were provided for mice that were alive 2 hr after opioid injections.

Immediately before each day's testing, drug solutions[4] were prepared with water distilled in this laboratory, with the exception of methadone hydrochloride, which was procured from the manufacturer in vials (10 mg/ml, calculated as the salt).

The opioid drugs and their concentrations were: morphine sulfate, 6.0%; codeine sulfate, 2.0%; meperidine hydrochloride, 2.0%; levorphanol tartrate, 1.6%; methadone hydrochloride, 1.0%; levopropoxyphene hydrochloride, 2.0%; and propoxyphene hydrochloride, 2.0%. The thiazine dyes, methylene blue and tolonium chloride, each were prepared as 0.1 and 1.0% solutions. Sodium hexobarbital was prepared as a 1.0% solution.

A precision timer was used to determine survival and sleeping times, with all measurements being recorded to the nearest minute.

To determine the effects of thiazine dyes, alone and in combination, on opioid lethality and hexobarbital sleeping time, the following pretreatment regimen was employed: (*a*) single-dye pretreatment—methylene blue (0.1 mg), methylene blue (1.0 mg), tolonium chloride (0.1 mg), and tolonium chloride (1.0 mg); and (*b*) multiple-dye pretreatment—methylene blue (0.1 mg) + tolonium chloride (0.1 mg), and methylene blue (1.0 mg) + tolonium chloride (1.0 mg).

Toxicity Studies—The intraperitoneal LD_{50}'s were determined for each opioid drug (Table I) according to the method of Litchfield and Wilcoxon (3). Based upon the data obtained, doses corresponding to 99.5% lethality were selected for use in experimentation.

Each test animal was weighed[5] to the nearest gram, and the proposed pretreatment regimen[6] was performed subcutaneously in the upper left abdomen. When two dyes were administered, the upper right abdomen was used as the second site of injection.

[1] For the purpose of this study, the following agents are designated as opioid drugs: morphine sulfate, codeine sulfate, meperidine hydrochloride, methadone hydrochloride, levorphanol tartrate, propoxyphene hydrochloride, and levopropoxyphene hydrochloride.

[2] Huntingdon Farms, HTF strain.
[3] Purina.
[4] In each case, the salt form was used for the calculation of drug concentration and dose.
[5] Triple-beam Ohaus balance, model 730.
[6] Pretreatment injections were each administered in a volume of 0.1 ml as fixed doses.