Preliminary Evaluation of Mesoionic 6-Substituted 1-Methylimidazo[2,1-*b*][1,3]thiazine-5,7-diones as Potential Novel Prodrugs of Methimazole

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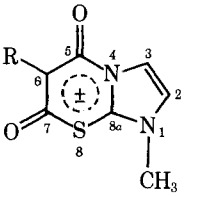
Abstract □ A series of five 6-alkyl- and 6-aryl-mesoionic 1-methylimidazo[2,1-*b*][1,3]thiazine-5,7-diones was synthesized and found to produce 1-methyl-3*H*-imidazole-2-thione (methimazole) upon alkaline hydrolysis or treatment with amine or thiol reagents. The alkaline hydrolysis followed a second-order rate expression, being dependent on both substrate and hydroxide-ion concentrations. The rate constants for the five derivatives fell within 6–15 × 10^{-2} liter/mole min at 40°. These compounds were stable in aqueous acidic solutions and in human serum or rat liver homogenate under conditions producing rapid hydrolysis of the methimazole prodrug 1-carbethoxy-3-methylimidazole-2-thione (carbimazole).

Keyphrases □ Methimazole—potential new prodrugs, evaluation of mesoionic 6-substituted 1-methylimidazo[2,1-*b*][1,3]thiazine-5,7-diones □ Prodrugs—of methimazole, evaluation of mesoionic 6-substituted 1-methylimidazo[2,1-*b*][1,3]thiazine-5,7-thiones □ Thyroid inhibitors—methimazole evaluation of mesoionic 6-substituted 1-methylimidazo[2,1-*b*][1,3]thiazine-5,7-diones

Considerable interest has been directed toward the development of prodrugs to modify the pharmacodynamic properties of known therapeutic agents, and several labile functionalities have been employed to accomplish this goal (1, 2). During previous investigations (3–8) involving the syntheses and chemistry of bicyclic mesoionic heterocycles related to the purinones, several examples were found in which the novel structures of these heterocycles rendered them susceptible to nucleophilic attack, often leading to ring-opened products (5, 6).

The present study examined the feasibility of exploiting the nucleophilic susceptibility of mesoionic 6-substituted 1-methylimidazo[2,1-*b*][1,3]thiazine-5,7-diones[1] (I) in the development of a methimazole prodrug (II) in which both lipophilicity and lability might be controlled by substituents eliminated from the released drug.



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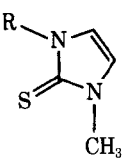

II: R = H
III: R = $CO_2C_2H_5$

EXPERIMENTAL[2]

UV spectrophotometer[3] was employed for the collection of kinetic data wherein sample temperature was maintained with a circulating constant-temperature bath[4].

Synthesis—Five derivatives of I (Table I) were synthesized using a procedure generally useful in the synthesis of various similar mesoionic heterocycles (5, 8). Appropriately substituted malonic acids, obtained commercially or by ethoxycarbonation (9) of arylacetic acid esters, were converted in good yield by the use of phosphorus oxychloride to the corresponding bis-2,4,6-trichlorophenyl esters (10). Heating of a mixture of these esters and II neat at 120–140° produced I*a*–I*e* in moderate yields[5].

Anhydro-5-hydroxy-1-methyl-7-oxo-6-phenyl-7*H*-imidazo[2,1-*b*][1,3]thiazinium Hydroxide (I*d*)—The following procedure illustrates the preparation of the compounds in Table I. A mortar-ground mixture of II (3.0 g, 0.027 mole) and bis-2,4,6-trichlorophenyl phenylmalonate (14.4 g, 0.027 mole) was heated *via* an oil bath at 120° for 25 min while a slow nitrogen stream was introduced to carry off subliming trichlorophenol. The resulting melt was cooled and triturated with cold anhydrous ether (50 ml), filtered, and washed repeatedly with cold ether–acetone (2:1). The resulting pale-orange solid was recrystallized from acetone with carbon treatment to give I*d* as fine white needles (3.0 g, 44%, mp 287–289° dec. [lit. (11)mp 271–273° dec.]; IR (KBr): 3170 (w), 3140 (w), 1599 (s), 1581 (s), and 1572 (s) cm^{-1}; NMR (dimethyl sulfoxide-d_6): δ 8.10 (d, 1H, J = 2 Hz, 3-H), 7.81 (d, 1H, J = 2 Hz, 2-H), 7.50–7.15 (m, 5H, phenyl), and 3.77 (s, 3H, CH_3); UV (water): 234 (4.2 log ε), and 299 (3.9) nm.

Reaction of I*d* with Benzylamine—Benzylamine (170 mg, 1.6 mmoles) was added to a suspension of 20 mg (0.77 mmole) of I*d* in absolute ethanol (15 ml). The reaction mixture was stirred for 48 hr at room temperature, during which time the starting material dissolved. Reduction of the solution volume to ~5 ml by evaporation at reduced pressure and cooling gave 190 mg (72%) of *N,N'*-dibenzyl-2-phenylpropane-1,3-diamide as white crystals, mp 165–165.5°; IR (KBr): 3290 (m) and 1670 (s) cm^{-1}; NMR (dimethyl sulfoxide-d_6) δ 8.58 (t, 2H, J = 6 Hz, NH, exchangeable with deuterium oxide), 7.37 (s, 5H, phenyl), 7.25 (s, 10H, phenyl), 4.58 (s, 1H, methine), and 4.35 (d, 4H, J = 6 Hz, CH_2).

Anal.—Calc. for $C_{23}H_{22}N_2O_2$: C, 77.07; H, 6.19; N, 7.82. Found: C, 77.11; H, 6.22; N, 7.78.

The ethanolic filtrate obtained upon collection of this product was evaporated under reduced pressure, and the resulting solid residue was extracted with 10 ml of warm chloroform. The extract was filtered, and petroleum ether (10 ml) was added to give a precipitate. The precipitate was collected and recrystallized from chloroform–petroleum ether, yielding 70 mg of II (80%). This material was identical with an authentic sample of II as judged by TLC (silica gel, ethyl acetate, R_f 0.38), mixed melting point (144–146°), and IR spectra.

Reaction of I*d* with Thiobenzyl Alcohol—A solution of thiobenzyl alcohol (140 mg, 1.2 mmoles) and I*d* (150 mg, 0.58 mmole) in 10 ml of absolute ethanol was refluxed for 1.25 hr. The oily residue, obtained upon evaporation of solvent under reduced pressure, was dissolved in ethyl acetate (2 ml) and placed on a silica gel column (30 g). Elution with ethyl acetate gave 52 mg of II (79%), mp 144–146°.

1-Methyl-2(3*H*)-imidazolone (IV)—The procedure employed was a modification of a reported method (12). Aminoacetaldehyde dimethyl acetal (3.65 g, 0.035 mole) was added dropwise over 10 min to a stirred solution of methyl isocyanate (1.85 g, 0.035 mole) in 15 ml of absolute

[1] Alternative nomenclature: 6-substituted anhydro-5-hydroxy-1-methyl-7-oxo-7*H*-imidazo[2,1-*b*][1,3]thiazinium hydroxides.

[2] Melting points were determined with a Fisher–Johns hot-stage apparatus and are uncorrected. Microanalyses were performed by Atlantic Microlab Inc., Atlanta, Ga. ^{1}H-NMR spectra were recorded on a Varian T-60A spectrometer with tetramethylsilane as an internal standard. IR specrtra were recorded with a Perkin-Elmer 727B spectrophotometer and a Nicolet 7199 FT-IR interferometer. UV spectra were obtained with a Beckman model 25 spectrophotometer.

[3] Gilford model 2400.

[4] Tamson model TE-3.

[5] While this work was in progress, an alternative synthesis of I*d* was reported (11) using II and (chlorocarbonyl)phenylketene.

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Table I—Mesoionic 6-Substituted 1-Methylimidazo[2,1-*b*][1,3]thiazine-5,7-diones

Compound	R	Melting Point[a]	Yield, %	Formula	Analysis, % Calc.	Found
Ia	CH_3	150–151° (A–E)	31	$C_8H_8N_2O_2S$	C 48.97	48.54
					H 4.11	4.07
					N 14.28	13.96
					S 16.34	16.15
Ib	C_2H_5	194–195° (EA)	29	$C_9H_{10}N_2O_2S$	C 48.47	48.40
					H 5.09	4.95
					N 14.13	14.19
					S 16.17	16.15
Ic	$CH_2C_6H_5$	185–186° (M)	45	$C_{14}H_{12}N_2O_2S$	C 61.75	61.81
					H 4.44	4.47
					N 10.29	10.28
Id	C_6H_5	287–289° dec. (A)	44	$C_{13}H_{10}N_2O_2S$	C 60.45	60.18
					H 3.90	3.93
					N 10.85	10.79
					S 12.41	12.34
Ie	$4CH_3OC_6H_4$	294–296° dec. (A–M)	25	$C_{14}H_{12}N_2O_3S$	C 58.32	58.37
					H 4.20	4.24
					N 9.72	9.69
					S 11.12	11.14

[a] Crystallization solvents: A, acetone; E, diethyl ether; EA, ethyl acetate; and M, methanol.

Table II—Rate Constants for Alkaline Hydrolysis of Ia–Ie

Compound	$k \pm SD \times 10^2$, liters/mole min[a]	Relative Rate
Ia	8.92 ± 0.06	1.00
Ib	6.25 ± 0.27	0.70
Ic	7.32 ± 0.29	0.82
Id	14.7 ± 0.12	1.65
Ie	13.3 ± 0.44	1.49

[a] Second-order rate constants in aqueous sodium hydroxide (0.05–0.40 *N*) at 40° and constant ionic strength (2.00 *M*).

ethanol maintained at 0–5°. Following the addition, the solution was stirred for 1 hr at room temperature and then refluxed for 4 hr. Concentrated hydrochloric acid (0.03 ml) in water (10 ml) was added, and the solution was stirred overnight. Addition of 5 ml of 5% $NaHCO_3$ solution transformed the color from red to pale yellow. Filtration of the solution and evaporation under reduced pressure gave a viscous oil, which crystallized on standing. Recrystallization from chloroform–petroleum ether gave 1.97 g (57%) of IV as transparent plates, mp 140–142° [lit. (12) mp 139–140.5].

Product Identification for Alkaline Hydrolysis of Ic—A suspension of Ic (0.5 g, 1.8 mmoles) in 50 ml of 1 *N* NaOH was stirred for 48 hr, during which time all material dissolved. TLC analysis (silica gel, ethyl acetate) indicated the complete disappearance of starting material (R_f 0.12) and the appearance of two new spots (R_f 0.06 and 0.38) corresponding to those of an alkaline solution of benzylmalonic acid and II. The UV absorption spectrum of the reaction solution was identical to that of a 1 *N* NaOH solution containing 1.8 mmoles each of benzylmalonic acid and II.

The extract obtained by continuous extraction of the reaction solution with ethyl acetate for 24 hr was dried (magnesium sulfate) and evaporated to give II, as evidenced by mixed melting point and comparison of IR spectra. The reaction solution was acidified (pH 1) with concentrated hydrochloric acid and continuously extracted for 6 hr with ethyl acetate. The residue of the evaporated extract was identified as benzylmalonic acid by comparison of its IR spectrum with that of an authentic sample.

Kinetics of Hydrolysis of Ia–Ie—Stock solutions were prepared by dissolving Ia–Ie (50 μmoles) in 1 ml of dimethyl sulfoxide. Sodium hydroxide solutions were prepared (0.05, 0.15, 0.30, and 0.40 *N*) by dilution of 2.00 *N* NaOH with 2.00 *M* KCl. To cells containing the alkaline solutions (2 ml), equilibrated at 40.0 ± 0.1°, was added 10 μl of the stock solution. The loss of absorbance at 300 nm was monitered with the OD_∞ value (optical density) observed to be 0 ± 2%. Pseudo-first-order rate constants were obtained from the slopes of the plots of $\ln[(OD_t - OD_\infty)/(OD_0 - OD_\infty)]$ *versus* time. Each compound was examined in triplicate. Second-order rate constants (Table II) were calculated from the linear regression of k_{obs} with the hydroxide-ion concentration.

Serum Hydrolysis of Ia, Ib, and III—Substrate Ia (20 mg), Ib (20 mg), or III (30 mg) was dissolved in 5 ml of pH 7.4 phosphate buffer solution (0.05 *M*) at 37°. The substrate solution (1 ml) was added to 3 ml of human serum[6], and the resulting solution was shaken and incubated at 37°. Aliquots (75 μl) were taken at intervals and added to cells containing 3 ml of buffer solution. Absorbance of the resulting solution was recorded at 300 (Ia and Ib) or 290 (III) nm. A blank solution lacking substrate was similarly treated.

Rat Liver Homogenate Treatment of Ia, Ib, and III—A modification of a reported procedure (13) for the hydrolysis of acetanilide was employed. A 300-g male Sprague-Dawley rat was sacrificed, and the liver was removed, rinsed in cold 0.2 *M* buffer solution[7] (pH 8.1), blotted, and weighed (11 g). The liver was minced and homogenized in 20 ml of cold buffer in a chilled tissue grinder[8] maintained in an ice bath.

Buffer solutions of Ia, Ib, or III (1 mg/ml) and homogenate were separately equilibrated (37°) immediately prior to use. Substrate solution (3 ml), buffer (1 ml), and homogenate (2 ml) were mixed and incubated at 37° in a shaker bath. At intervals, a 0.5-ml aliquot was removed and added to 2 ml of ice-cold 10% trichloroacetic acid solution, which was then centrifuged (700 rpm, 3 min), and 1 ml of the supernate was added to a cell containing 3 ml of buffer. The absorbance of this solution was recorded at 300 (Ia and Ib) or 290 (III) nm. A blank solution employing buffer instead of substrate solution was similarly treated as well as a substrate solution of III in which buffer was substituted for homogenate.

RESULTS

The mesoionic imidazothiazinediones (Ia–Ie) were white crystalline solids stable in light and air and showed no spectroscopic evidence of decomposition in 1 *N* HCl solution over 24 hr. In 1 *N* NaOH, Ic showed a gradual dissolution at room temperature as an apparent function of limited aqueous solubility and hydrolysis to benzylmalonate and II. No other products were detected by TLC or spectroscopic analyses. A potential alternative hydrolysis product, 1-methyl-2(3*H*)-imidazolone (IV), possibly resulting from hydroxide-ion attack at the bridgehead carbon position, was synthesized and found to be clearly distinguishable from II by the chromatographic and spectroscopic methods employed. Since the UV spectrum of the hydrolysis products showed no significant absorbance beyond 280 nm, the rate of alkaline hydrolysis was readily monitored by observing the loss of absorbance of the longest wavelength maxima of Ia–Ie at 300 nm.

The alkaline hydrolysis followed second-order kinetics, dependent on both substrate and hydroxide-ion concentration. The observed rate constants (Table II), showed only a twofold range of values for the five derivatives investigated. The second-order rate expression is consistent with either a rate-limiting attack by hydroxide ion or a rate-limiting decomposition of an adduct of I and hydroxide ion. No evidence was obtained concerning which pseudocarbonyl group is initially involved in the reaction.

[6] Grand Island Biological Co., Grand Island, N.Y.
[7] Trimethamine.
[8] Potter-Elvehjem, with Teflon resin pestle.

Compound II was produced in ethanolic solution from I*a* or I*d* by reaction with two equivalents of benzylamine, with complete conversion within 48 hr at room temperature or 30 min at reflux. No other products except for the corresponding malondiamide were detected by chromatographic or spectral analyses. Compound II was also produced from I*d* by reaction for 75 min with thiobenzyl alcohol in refluxing ethanol.

Alkyl derivatives I*a* and I*b* were sufficiently water soluble to allow examination of their stability at pH 7.4 in the presence of human serum. Under conditions wherein III underwent rapid hydrolysis (complete conversion within 2 hr), I*a* and I*b* showed no evidence of decomposition in the presence of human serum for 10 hr at 37°. Similarly, I*a* and I*b* showed no evidence of decomposition in the presence of rat liver homogenate over 5 hr, while III was completely hydrolyzed within 5 min under the same conditions.

DISCUSSION

Compound III, a derivative of II, is already in clinical use. Several studies in humans (14–17) have shown III to be absorbed and rapidly converted to II, an agent useful in the control of hyperthyroidism (18). Although III was designed as a potentially longer acting derivative of II (19), its conversion to II in serum is so rapid that there appears to be little therapeutic advantage over the use of II (17).

A number of bicyclic mesoionic pyrimidinedione derivatives have been reported to result from the condensation of bis-trichlorophenyl malonate esters and *sec*-amino azaheterocycles (5, 6, 20–23). These compounds, which may be viewed as 8-aza analogs of I, undergo ring opening in reaction with benzylamine by nucleophilic attack at the 5-position pseudocarbonyl group (5). However, there is some indication that anionic nucleophiles, such as hydroxide ion, attack the bridgehead carbon position of these 8-aza analogs (5). An analogous reaction of I with hydroxide ion could lead to the formation of IV. Alternatively, hydroxide-ion attack at the 5-position of I would produce a thiol ester, which would be susceptible to hydrolytic production of II. The general utility of mesoionic derivatization for prodrug modification would depend on the controllability of the hydrolytic and/or enzymic decomposition rates by use of various 6-position substituents. These substituents may also provide a means of influencing absorption and distribution by modifying the lipophilicity of the agent.

The conversion of I to II by alkaline hydrolysis and by amine and thiol reagents was demonstrated. However, the variety of substituents investigated showed only a limited influence on the rate of alkaline hydrolysis. The substituents employed exhibited a very limited range of electronic effects, being predominately electron-releasing groups. More strongly electron-withdrawing substituents would be expected to increase the electrophilicity of the pseudocarbonyl groups or to increase the concentration of the hydroxide-ion adduct, leading to an increased alkaline hydrolysis rate. Condensation of malonate esters containing *p*-nitrophenyl or acyl substituents with II was unsuccessful, probably because of the increased ease of malonate-carbanion formation. However, by analogy to the chemistry exhibited by the mesoionic pyrimidinediones (5), electrophilic substitution reactions on I should be facile at the 6-position.

In conclusion, although I*a*–I*e* produce II by alkaline hydrolysis or by reaction with amine or thiol reagents, their stability to human serum or rat liver homogenate indicate that their decomposition rate *in vivo* is likely to be insufficient. Other derivatives of I with electron-withdrawing 6-position substituents may overcome this deficiency.

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