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2-Acetylpyridine Thiosemicarbazones XI: 2-(α-Hydroxyacetyl)pyridine Thiosemicarbazones as Antimalarial and Antibacterial Agents

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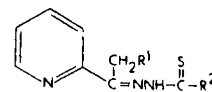
Abstract □ A series of 2-(α-hydroxyacetyl)pyridine thiosemicarbazones was synthesized as potential antimalarial and antibacterial agents. Their synthesis was achieved by the condensation of N^4-mono- or N^4,N^4-disubstituted thiosemicarbazides with 2-(α-hydroxyacetyl)pyridine. The latter was prepared by selective bromine oxidation of (2-pyridinyl)-1,2-ethanediol. The new compounds show potent inhibitory activity against penicillin-sensitive as well as penicillin-resistant *Neisseria gonorrhoeae* (MIC, 0.5-0.004 μg/mL), against *Neisseria meningitidis* (MIC, 0.5-0.032 μg/mL), and *Staphylococcus aureus* (MIC, 0.5-2 μg/mL). Good *in vitro* antimalarial effects against *Plasmodium falciparum* (Smith strain; ID_{50}, 6.7-38 ng/mL) were observed in most of these new agents, but only 3 of 12 compounds exhibit moderate *in vivo* activity against *Plasmodium berghei*. These new agents appear to be less toxic to the host and more water soluble than the corresponding 2-acetylpyridine thiosemicarbazones.

Keyphrases □ Thiosemicarbazone derivatives—2-(α-hydroxyacetyl)pyridine thiosemicarbazones, antimalarial and antibacterial activity □ Antimalarial agents—potential, 2-(α-hydroxyacetyl)pyridine thiosemicarbazones □ Antibacterial agents—potential, 2-(α-hydroxyacetyl)pyridine thiosemicarbazones

Members of a series of N^4-mono- and N^4,N^4-disubstituted 2-acetylpyridine thiosemicarbazones (I) have been reported by us to possess antimalarial (1, 2), antibacterial (3), and antiviral (4) properties. Based on these studies, the structure-activity relationship of this class of compounds has been defined. Thus, N^4,N^4-disubstitution of the thiosemicarbazone moiety appears to be essential for optimal activity against *Plasmodium berghei* in the mouse (2) and several bacterial genera (3). Replacement of the sulfur atom with oxygen leads to inactive compounds. Furthermore, biological activity is limited to those compounds in which the ethylidene group is attached to the 2-position, rather than the 3- or 4-position, of the pyridine ring. The effects resulting from placement of a functional group on the ethylidene function, however, have not been studied. N^4,N^4-Disubstituted 2-acetylpyridine thiosemicarbazones, in particular, exhibit potent antimalarial properties *in vitro* as well as *in vivo*. Against *P. berghei* in mice, the 4-(2-pyridinyl)piperazine and 3-azabicyclo[3.2.2]nonane analogues produced cures of all infected test animals at a dosage of 80 and 160 mg/kg, respectively (2). Although potent inhibitory activity against *Plasmodium falciparum* (Smith) *in vitro* (ID_{50} = 3.6 ng/mL) was observed, 2-acetylpyridine 4,4-dimethyl-3-thiosemicarbazone shows no significant *in vivo* activity against *P. berghei* in mice due to its high host toxicity.

As antibacterial agents, 2-acetylpyridine thiosemicarbazones show excellent activity against both penicillin-sensitive and -resistant strains of *Neisseria gonorrhoeae* with MIC (minimum inhibitory concentration) values in the range of 0.002-0.062 μg/mL. These agents were also found to inhibit the growth of *Neisseria meningitidis* as well as *Staphylococcus aureus*, with the MIC in the range of 0.016-0.062 μg/mL and 0.125-0.5 μg/mL, respectively. 2-Acetylpyridine 4,4-dimethyl-3-thiosemicarbazone [I, $R^2 = N(CH_3)_2$] is by far the most potent compound against *N. gonorrhoeae* among the analogues tested, with an MIC of 0.002-0.008 μg/mL. However, the substantial toxicity observed in mice (2) has limited its potential as a clinical agent.

In the search for agents with lower toxicity, increased water-solubility, and improved structure-activity relationship, the synthesis of a new series of 2-(α-hydroxyacetyl)pyridine thiosemicarbazones (II) was undertaken. These compounds were screened for their antimalarial properties as well as their antibacterial activity.



I, R^1 = H
II, R^1 = OH

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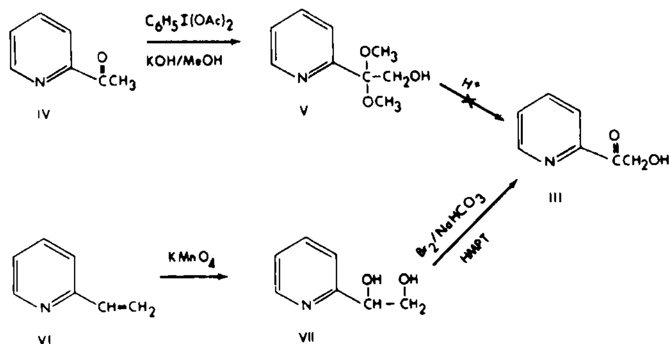
BACKGROUND

The preparation of the target compounds involved the synthesis of the unknown key intermediate, 2-(α-hydroxyacetyl)pyridine (III), followed by its condensation with an appropriately substituted thiosemicarbazide. The method used to convert 2,6-diacetylpyridine to 2,6-bis(α-hydroxyacetyl)-pyridine was applied to 2-acetylpyridine (IV) (Scheme I). In the procedure reported by Moriarty *et al.* (5), 2,6-diacetylpyridine was treated with iodosobenzene diacetate under KOH–MeOH catalysis to give a moderate yield of the corresponding dimethyl ketal. The latter was hydrolyzed to 2,6-bis(α-hydroxyacetyl)pyridine by the action of p-toluenesulfonic acid in acetone.



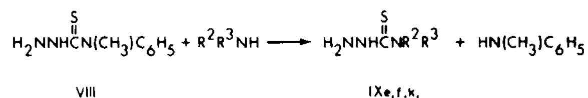
Scheme I

In our case, IV was readily converted to the corresponding ketal (V); however, the recommended technique for hydrolysis to III gave unchanged material. The use of acetic, hydrochloric, or hydriodic acids under a variety of conditions also gave either starting material or decomposition products.

An alternate avenue which was investigated involved the oxidation of 2-vinylpyridine (VI) to (2-pyridinyl)-1,2-ethanediol (VII) by potassium permanganate according to a procedure of Furuyama *et al.* (6). Further oxidation of VII to III was achieved in 35% yield using Br_2–$NaHCO_3$ with hexamethylphosphoramide serving as the coreactant. This method had been reported (7) to selectively oxidize secondary alcohols to their corresponding ketones in the presence of a primary hydroxy group. The product (III), a low-melting solid, was found to be best purified on a silica gel flash column.

Condensation of III with the appropriate thiosemicarbazides gave the desired thiosemicarbazones (IIa,d–k) in moderate yield (*cf.* Table I). Three of the thiosemicarbazides, precursors of IIc, f, and k, had not been reported previously and were prepared by a new procedure in which 4-methyl-4-phenyl-3-thiosemicarbazide [VIII (8)] is heated under reflux with a secondary amine (Scheme II). This transamination reaction gave better yields of N^4,N^4-disubstituted thiosemicarbazides (IX) than the older method involving an amine displacement reaction of methyl hydrazinecarbodithioate (9).

The hydrazone of III, *i.e.*, X, reacted with methyl and phenyl isothiocy-

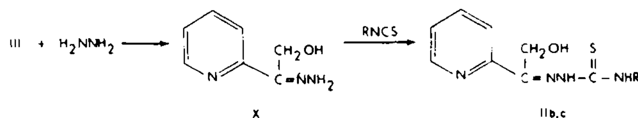


Scheme II

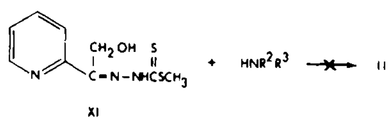
Table I—2-(α-Hydroxyacetyl)pyridine Thiosemicarbazones

Compound	R	mp, °C	Method[a,b]	Yield, %	Formula
IIa	NH_2	81–83[c]	A	55	$C_8H_{10}N_4OS$
IIb	$NHCH_3$	165–166[d]	B	30	$C_9H_{12}N_4OS$
IIc	NHC_6H_5	140–141[c]	B	30	$C_{14}H_{14}N_4OS$
IId	$N(CH_3)_2$	132–134[e]	A	90	$C_{10}H_{14}N_4OS$
IIe	N N– N	147–149[c]	C	43	$C_{17}H_{20}N_6OS$
IIf	N	133–135[c]	A	35	$C_{16}H_{22}N_4OS$
IIg	N	185–186[f]	A	60	$C_{12}H_{16}N_4OS$
IIh	N	128–129[g]	C	50	$C_{14}H_{20}N_4OS$
IIi	N O	163–165[h]	A	33	$C_{12}H_{16}N_4O_2S$
IIj	N	134–136[g]	A	50	$C_{13}H_{18}N_4OS$
IIk	N $NCO_2C_2H_5$	122–124[c]	C	50	$C_{15}H_{21}N_5O_3S$

[a] Key: (A) reaction of III with a thiosemicarbazide prepared by the reaction of methyl hydrazinecarbodithioate with an amine; (B) reaction of 2-(α-hydroxyacetyl)pyridine hydrazone with an isothiocyanate; (C) reaction of III with thiosemicarbazides IXe, h, k. [b] ^1H-NMR ($CDCl_3$): IIa–c, N^2–H (~14.5 ppm), $-CH_2-$ (d, ~4.6 ppm); IId–k, N^2–H (~15.3 ppm), $-CH_2-$ (s, ~4.7 ppm). [c] EtOAc. [d] Dimethylformamide–$CHCl_3$. [e] EtOAc–MeOH. [f] Dimethylformamide–MeOH. [g] CH_3CN. [h] $CHCl_3$–EtOH.



Scheme III



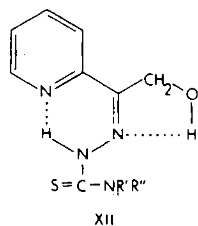
Scheme IV

anates to give the corresponding N^4-monosubstituted thiosemicarbazones (IIb,c) (Scheme III).

Another route for the preparation of II which was considered was the displacement of the $-SCH_3$ function of XI with either primary or secondary amines. Although this method (1, 2) proved to be useful for the synthesis of 2-acetylpyridine thiosemicarbazones, in the case of the α-hydroxy analogue, the reaction of carbodithioate (XI) with amines gave little or no product (Scheme IV).

A determination and comparison of the water solubility at 37°C of α-hydroxylated 2-acetylpyridine thiosemicarbazones with analogues lacking the hydroxy group revealed that appreciable hydrophilic enhancement occurred. For example, IId and f increased water solubility 1.3-fold and 7.5-fold, respectively, by having the α-hydroxy group present in the molecule.

It is of interest to note that while the N^4,N^4-disubstituted 2-acetylpyridine thiosemicarbazones exist in three tautomeric forms in $CDCl_3$, as indicated by NMR spectral data (10), only a single tautomer was observed for both N^4-mono- and N^4,N^4-disubstituted 2-(α-hydroxyacetyl)pyridine thiosemicarbazones. The C-methyl protons of 2-acetylpyridine N^4,N^4-dimethylthiosemicarbazone [I, $R^2 = N(CH_3)_2$] are seen as an unequal triplet centered at δ 2.5 ppm ($CDCl_3$) and the thiosemicarbazone NH proton appears as three peaks, *i.e.*, at δ 15, 14.5, and 8 ppm. These data suggest that the N^4, N^4-disubstituted 2-acetylpyridine thiosemicarbazones form three tautomers in $CDCl_3$. However,the N^4-monosubstituted 2-acetylpyridine thiosemicarbazones exist solely as a single *E*-isomer, with N^2 proton resonance at $\delta \sim 8$ ppm. The methylene protons of the N^4,N^4-disubstituted 2-(α-hydroxyacetyl)pyridine thiosemicarbazones and those of the unsubstituted (IIa) and monosubstituted (IIb,c) analogues resonate as a singlet and as doublets, respectively, at $\delta \sim 4.7$ ppm. The NH proton on N^2 appears in all analogues as a singlet at $\delta \sim 15$ ppm. The NMR data suggest that the 2-(α-hydroxyacetyl)pyridine thiosemicarbazones exist primarily as the *Z*-isomer with NH forming an internal hydrogen bond with the pyridine ring nitrogen, as shown in XII. Furthermore, the lack of coupling between the methylene protons and



the hydroxyl on the N^4,N^4-disubstituted thiosemicarbazones suggests that the hydroxyl function forms another internal hydrogen bond with the N^1 nitrogen atom of the thiosemicarbazone function. The fact that a coupling between methylene protons and the hydroxyl exists in IIa–c indicates that the hydroxyl function of unsubstituted and monosubstituted analogues is not involved in hydrogen bond formation as seen in the N^4,N^4-disubstituted thiosemicarbazones IId–k. A substantial downfield chemical shift of the proton on N^2 of thiosemicarbazones as a result of internal hydrogen bonding has been reported (11–13).

EXPERIMENTAL SECTION[1]

(2-Pyridinyl)-1,2-ethanediol (VII)—2-Vinylpyridine (VI, 75.6 g, 0.72 mol) was dissolved in 900 mL of acetone, and the solution was cooled in an ice bath to 0–5°C. A chilled solution of $KMnO_4$ (75.9 g, 0.48 mol) and $MgSO_4$ (28.8 g, 0.24 mol) in 1.5 L of H_2O was added slowly with agitation[2] to the 2-vinylpyridine solution at such a rate as to maintain the temperature of the mixture at ~5°C. After addition of all the $KMnO_4$ solution (1.5 h), the mixture was stirred at 5°C for 1 h and then at ambient temperature overnight.

A small amount of hydroquinone was added to the resulting thick, dark-brown slurry which was then filtered through diatomaceous earth[3]. The filtrate was concentrated to ~200 mL, and 750 mL of 95% EtOH was added. The filter cake was extracted with 500 mL of 95% EtOH, and the filtrate and extract were combined and stored overnight at 5°C. The mixture was filtered to remove K_2SO_4, and the filtrate was then concentrated under reduced pressure to a volume of 250 mL. The crude VII (37.1 g, 37% yield) was collected and recrystallized from MeOH–CH_3CN to give light-brown crystals, mp 93–96°C. Further recrystallization from EtOH–CH_3CN gave colorless crystals of (2-pyridinyl)-1,2-ethanediol, mp 94–96°C [lit. (14) mp 97°C].

2-(α-Hydroxyacetyl)pyridine (III)—(2-Pyridinyl)-1,2-ethanediol (10.8 g, 77.7 mmol) was suspended in 600 mL of CH_2Cl_2. To the suspension was added 3.8 g of hexamethylphosphoramide and 132 mL of 16% aqueous $NaHCO_3$ solution. The mixture was cooled to 0–5°C with an ice bath and to the mixture Br_2 (18 g, 112.5 mmol) in 60 mL of CH_2Cl_2 was added in a dropwise manner with vigorous stirring. The mixture was stirred at room temperature until the Br_2 was consumed (8–12 h). The organic layer was separated, and the aqueous layer was extracted twice with CH_2Cl_2. The organic layer and extracts were combined, dried over Na_2SO_4, and evaporated to dryness under reduced pressure. The oil was purified on a silica gel flash column, using CH_3CN as eluant to give 3.8 g (36% yield) of III. Recrystallization of III from benzene–cyclohexane gave colorless needles, mp 68–70°C, which gradually turned brown on standing. IR (KBr): 3280, 1710, 1585, 1438, 1222, 1005, 975, 776, and 625 cm^{-1}; ^1H-NMR ($CDCl_3$): δ 3.40 (br s, 1, OH), 5.09 (s, 2, CH_2), 7.45 and 8.12 (m, 3, ArH), and 8.68 ppm (d, 1, ArH).

Condensation of III with N^4,N^4-Disubstituted Thiosemicarbazides—2-(α-Hydroxyacetyl)pyridine (III) (2.2 g, 16 mmol) and 16 mmol of an N^4, N^4-disubstituted thiosemicarbazide were heated under reflux in 60 mL of MeOH, with two drops of glacial HOAc as catalyst, for 1.5 h. The solvent was evaporated to dryness under reduced pressure, and the crude product was recrystallized.

2-(α-Hydroxyacetyl)pyridine Hydrazone (X)—2-(α-Hydroxyacetyl)pyridine (2 g, 14.6 mmol) and 85% hydrazine hydrate (2 mL, 53.8 mmol) in 30 mL of MeOH were heated under reflux for 3.5 h. The solvent was evaporated to dryness under reduced pressure, and the resulting oil was extracted several times with hot C_6H_6. The C_6H_6 extracts were combined, dried over Na_2SO_4, and evaporated to a small volume. The crystals of X which formed were collected (1.2 g, 54% yield) and recrystallized from C_6H_6 to give light-straw needles, mp 113–115°C; ^1H-NMR ($CDCl_3$): δ 3.60 (br s, 1, OH), 4.57 (s, 2, CH_2O), 7.18–7.87 (m, 3, pyridinyl-H), 8.41 (br s, 2, NH_2), and 8.65 ppm (d, 1, pyridinyl H).

2-(α-Hydroxyacetyl)pyridine 4-Methylthiosemicarbazone (IIb)—2-(α-Hydroxyacetyl)pyridine hydrazone (X) (1.5 g, 10 mmol) and 0.75 g (10 mmol) of methyl isothiocyanate were heated in CH_3CN under reflux for 2 h. The solvent was evaporated to dryness under reduced pressure, and the residue was recrystallized. 2-(α-Hydroxyacetyl)pyridine 4-phenylthiosemicarbazone (IIc) was made in a similar manner using phenyl isothiocyanate.

3-Azabicyclo[3.2.2]nonane-3-thiocarboxylic Acid Hydrazide (IXf)—A solution of 5 g (27.6 mmol) of 4-methyl-4-phenylthiosemicarbazide (8) in 10 mL of EtOH was combined with 3.43 g (27.6 mmol) of 3-azabicyclo[3.2.2]-nonane, and the solution was heated under reflux for 5 h. The mixture was chilled; the crystals which separated were collected and recrystallized from EtOH. This afforded 3.36 g (61% yield) of colorless rods of 3-azabicyclo[3.2.2]nonane-3-thiocarboxylic acid hydrazide, mp 164–165°C dec.; IR (KBr): 3244, 2930, 2860, 1604, 1515, 1343, 1216, 1009, and 975 cm^{-1}.

1-(2-Pyridinyl)piperazine-4-thiocarboxylic acid hydrazide (IXc) was prepared by the method described above in 47% yield, mp 184–185°C (from EtOH). 1-Carbethoxypiperazine-4-thiocarboxylic acid hydrazide (IXk) was prepared by the method described above in 63% yield, mp 158–159°C (from EtOH).

Methyl 3-[1-(2-pyridinyl)-2-hydroxyethylidene]hydrazinecarbodithioate (XI)—Compound III (0.25 g, 1.8 mmol) and methyl hydrazinecarbodithioate (1) (0.23 g, 1.8 mmol) in 25 mL of MeOH containing one drop of HOAc was heated on a steam bath for 1.5 h. The solvent was evaporated under reduced pressure and the yellow solid obtained was recrystallized from C_6H_6 to give 0.36 g (85% yield) of XI as yellow needles, mp 122–124°C.

Solubility Determination—A saturated solution of the thiosemicarbazone,

[1] Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Microanalyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, Mich. Analytical results were ±0.3% of the calculated values. IR spectra of solid samples were obtained in KBr disks on a Perkin-Elmer Model 283 spectrophotometer. ^1H-NMR spectra were run on a JEOL-FX90Q spectrometer using Me_4Si as an internal standard.

[2] A "Vibro-mixer" was found to be the most efficient.

[3] Celite.

prepared by suspending an excess quantity of the compound in distilled H_2O, was shaken in a constant-temperature bath at 37°C for 24 h, and then a 5-mL portion of the supernatant was lyophilized to constant weight. Solubilities were as follows: 2-acetylpyridine 4,4-dimethylthiosemicarbazone, 0.96 mg/mL; *N*,*N*-hexamethylene-2-[1-(2-pyridinyl)ethylidene]hydrazinecarbothioamide, 0.04 mg/mL; IId, 1.28 mg/mL; IIh, 0.30 mg/mL.

Antimalarial Studies—*In Vitro*—Antimalarial activity of the new compounds was evaluated *in vitro* against a chloroquine-resistant *P. falciparum* (Smith) by assessing the inhibition of uptake of [G-^{3}H]hypoxanthine by the parasites. A detailed procedure was given in a previous report from this laboratory (15).

In Vivo—The compounds described herein were tested[4] against a drug-sensitive strain of *P. berghei* (strain KBG 173) in mice. Five mice per dose level were infected by the intraperitoneal administration of parasitized erythrocytes. Untreated infected animals, which served as controls, died (on the average) after 6.2 d. A candidate drug was given 72 h after the mice were infected and was judged to be toxic if the infected mice died before day 6, inactive if they died between day 6 and day 12, active if the mean survival time of 6.2 d was at least doubled, and curative if the mice survived 60 d postinfection. Compounds which were active or curative at a dose of 40 mg/kg were retested at several lower dose levels, but results are not reported unless extension of mouse survival time was observed. Details of the test procedure were given in the first paper in this series and by Osdene *et al.* (16).

Antibacterial Studies—*Organisms*—Five clinical isolates of *S. aureus* were obtained from the Clinical Microbiology Laboratory, Walter Reed Army Medical Center. Five *N. meningitidis* and 25 *N. gonorrhoeae* isolates were obtained from the collections of the Department of Bacterial Diseases, Walter Reed Army Institute of Research, and the Centers for Disease Control, Atlanta, Georgia.

Testing Procedure—The standard agar plate dilution method using Mueller Hinton agar, as described by Washington and Sutter (17), was utilized to determine the MIC values of the test compounds against the *S. aureus* and *N. meningitidis* isolates tested. The *N. gonorrhoeae* isolates were similarly tested, but with GC agar plus 1% of a modified defined supplement (18). This supplement consisted of 81 mL of aqueous solution A (containing 40.5 g of dextrose and 0.002 g of cocarboxylase) which was mixed with 19 mL of aqueous solution B [83 mg of $Fe(NO_3)_3 \cdot 9H_2O$ and 1 g of L-glutamine]. It was then filter-sterilized and kept frozen until used.

The *S. aureus* inoculum was standardized after a 1-2-h incubation in Mueller Hinton broth by adjusting the turbidity to that of a 0.5 MacFarland standard (17) representing 10^7-10^8 colony-forming units (CFU)/mL, and then further diluted 20-fold in Mueller Hinton broth. The inocula of the two *Neisseria* species were standardized by suspending agar plate colonies of overnight growth in Mueller Hinton broth for *N. meningitidis* and GC broth plus 1% modified defined supplement for *N. gonorrhoeae* in order to approximate the turbidity of an overnight broth culture. The inocula were then further diluted 200-fold in the appropriate broth.

Because of the poor water solubility of the test compounds, it was necessary to dissolve them initially in dimethyl sulfoxide. The control plates also contained the highest concentration of Me_2SO present in each particular dilution series. Further dilutions were done with Mueller Hinton broth or, in the case of *N. gonorrhoeae* testing, with GC broth plus modified defined supplement (19). The concentrations of the test compounds were twofold dilutions from 32 to 0.25 μg/mL for *S. aureus* and from 1 to 0.002 μg/mL for the two *Neisseria* species.

The standardized bacterial inocula were applied to the thiosemicarbazone-containing agar plates and the controls using a Lidwell apparatus (20). Cultures were incubated under appropriate atmospheric conditions for 24 h at 37°C and read. The MIC for each compound was the lowest concentration of that compound which still inhibited all visible bacterial growth on the agar surface.

RESULTS AND DISCUSSION

Antimalarial Activity—The *in vitro* antimalarial studies (Table II) of the 2-(α-hydroxyacetyl)pyridine thiosemicarbazones and the 2-acetylpyridine thiosemicarbazones indicated that, except for IIe, f, and j, the latter compounds had greater inhibitory properties against *P. falciparum*. The lower toxicity of the 2-(α-hydroxyacetyl)pyridine thiosemicarbazones to mice was evident in the *in vivo* antimalarial screen (Table II). Similarly, these compounds, in general, were less efficacious as antimalarials than their corresponding 2-acetylpyridine thiosemicarbazones. Compound IIj showed low levels of activity, whereas the best compound of the series was IIf, which showed two of five cures and no toxicity at a dose of 320 mg/kg. However, the corresponding compound with identical N^4,N^4-substitution showed three of five mouse cures at a dose of 40 mg/kg. It appears that α-hydroxylation

[4] Tests performed at the Dr. Leo Rane Laboratory, University of Miami, Miami, Fla.

Table II—Antimalarial Activities *In Vitro* and *In Vivo* of 2-(α-Hydroxyacetyl)pyridine Thiosemicarbazones (II)

Compound	ID_{50}, ng/mL *P. falciparum* (Smith)[a]	Increase in Mean Survival Time, d[b] 40 mg/kg	80 mg/kg	160 mg/kg	320 mg/kg	640 mg/kg
IIa	>135	0.0		T(1/5)[c]		T(5/5)
IIb	54.0	0.0		0.0		0.0
IIc	38.0	0.6		0.0		0.0
IId	6.7	0.0		0.0		T(5/5)
IIe	13.0	0.2		0.8		0
IIf	15.0	1.2	0.6	4.4	C(2/5)	C(2/5)
IIg	23.0	−0.2	0.6	3.8	5.2,T(1/5)	0.4,T(2/5)
IIh	8.4	1.0	1.6	3.8	4.0,T(1/5)	2.0,T(2/5)
IIi	34.0	0.0	T(1/5)	1.6,T(1/5)	1.6,T(1/5)	0.6,T(2/5)
IIj	4.1	1.6	3.4	1.8,T(1/5)	1.4,T(2/5)	C(1/5),T(3/5)
IIk	23.0	0.0	T(3/5)	T(3/5)	T(1/5)	T(4/5)

[a] Determined in semiautomated test described in Ref. 15. [b] Test performed in Swiss mice infected with *P. berghei*. [c] (T) toxic, (C) cure; see *Experimental Section* for details.

Table III—Antibacterial Properties *In Vitro* of 2-(α-Hydroxyacetyl) (II) and 2-Acetylpyridine Thiosemicarbazones (I)

Compound	*N. gonorrhoeae* MIC, μg/mL II	I[a]	*N. meningitidis* MIC, μg/mL II	I[a]	*S. aureus* MIC, μg/mL II	I[a]
IIa	>1	0.25-0.5	>1	>1	>32	>16
IIb	0.125-1	0.5	>1	0.5-1	>32	>16
IIc	0.032-0.25	0.032-0.25	0.5-1	0.25->1	>32	8
IId	0.004-0.016	0.002-0.008	0.062-0.125	0.062-0.125	4	>16
IIe	0.016-0.062	0.004-0.031	0.125-0.25	0.062-0.25	2	2-4
IIf	0.008-0.016	0.008-0.031	0.062	0.031-0.125	0.5-1	0.25
IIg	0.004-0.016	0.008-0.062	0.062-0.125	0.016-0.031	1-4	0.5
IIh	0.004-0.016	0.016-0.062	0.032-0.062	0.031-0.125	0.5-1	2
IIi	0.032-0.125	—[b]	0.5->1	—[b]	>32	—[b]
IIj	0.004-0.016	0.016-0.062	0.032-0.062	0.031-0.062	0.5-1	0.5
IIk	0.062-0.5	0.004-0.031	>1	0.062-0.25	8-32	>16

[a] *cf.* Ref. 3. [b] Not tested in earlier study.

of the acetyl group had an overall negative effect on the antimalarial potency of this series of thiosemicarbazones.

Antibacterial Activity—In the assessment of the antibacterial activity of the 2-(α-hydroxyacetyl)pyridine thiosemicarbazones (Table III), the strong inhibitory effect of the 2-acetylpyridine thiosemicarbazones seen against *N. gonorrhoeae* and *N. meningitidis* (3) was also observed here. Most of the 2-(α-hydroxyacetyl)pyridine compounds had MIC values of <0.1 μg/mL against these bacterial species, and in a few instances the degree of inhibition was slightly superior to that observed with the 2-acetylpyridine thiosemicarbazones.

Against *S. aureus*, several of the 2-(α-hydroxyacetyl)pyridine thiosemicarbazones, *i.e.*, IIf, h, and j, had MIC values of 0.5–1 μg/mL. Again, there were several instances in which inhibitory activity superior to the 2-acetylpyridine thiosemicarbazones (*i.e.*, in IId, e, h, and k) was seen.

CONCLUSIONS

The biological data for the newly synthesized 2-(α-hydroxyacetyl)pyridine thiosemicarbazones indicates that N^4,N^4-disubstitution provides optimal *in vivo* and *in vitro* antimalarial and antibacterial activities. The introduction of a hydroxy function into the α-position of 2-acetylpyridine thiosemicarbazones results in compounds with increased solubility, decreased host toxicity, and in some instances, improvement of antimalarial and antibacterial activity *in vitro*. However, this is offset by the concomitant decrease in *in vivo* antimalarial effects. These results suggest that pharmacological parameters, such as tissue distribution and the rate of metabolism, play an essential role in determining the *in vivo* antimalarial activity, as well as host toxicity, of this series of compounds.

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Kinetics and Mechanism of the Alkaline Hydrolysis of Maleimide

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Abstract □ The kinetics of hydrolysis of maleimide was carried out within the $[OH^-]$ range of 2.46×10^{-6} to 2.0 M at 30°C. The observed pseudo-first-order rate constants, k_{obs}, follow the empirical equation: $k_{obs} = (A_1[OH^-] + A_2[OH^-]^2)/(1 + A_3[OH^-])$. Both ionized and un-ionized forms of maleimide have been suggested to be involved in hydrolysis. The nucleophilic attacks by hydroxide ion at the carbonyl carbon of both ionized and un-ionized maleimide and by water at the carbonyl carbon of ionized maleimide to form tetrahedral intermediates are considered to be the rate-determining steps. The observed results obtained at different 1,4-dioxane–water compositions have revealed an increase in k_{obs} with a decrease in 1,4-dioxane content which could be attributed to the higher polarity of the transition state compared with the reactant state.

Keyphrases □ Maleimide—kinetics, mechanism of hydrolysis □ Hydrolysis—maleimide, effect of temperature

Many compounds containing an imide group act as drugs (1). *N*-Ethylmaleimide is of interest because of its rapid and Michael-type reaction with the sulfhydryl group of proteins (2, 3). Usually, the alkaline hydrolysis of amides and imides have been found to occur by a stepwise mechanism involving the tetrahedral intermediate (I) (4). Biechler and Taft (5) were the first to propose an additional oxydianionic tetrahedral intermediate (II) in the alkaline hydrolysis of trifluoroacetanilide. Later, II was found to exist in many acyl transfer reactions where the acyl substrates contained electron-withdrawing substituents and hydrolytic conditions covered a reasonable range of $[OH^-]$ (6). Recently, we have observed II in the alkaline hydrolysis of methyl-*o*-methoxybenzoate (7). In the continuation of our work on mechanistic studies on aqueous cleavage of amides (8) and imides (9) in a highly alkaline medium, we initiated this study to determine if an intermediate (II) exists and to study the nature of the rate-determining step.

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