CA-10914 from the National Institutes of Health, and 780014 from the Alabama affiliate of the American Heart Association.

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2-Acetylpyridine Thiosemicarbazones. 1. A New Class of Potential Antimalarial Agents[1]

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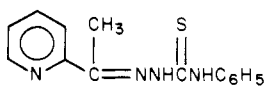
Based on the antimalarial properties observed for 2-acetylpyridine 4-phenyl-3-thiosemicarbazone (1), an extensive series of related thiosemicarbazones was prepared and tested against *Plasmodium berghei* in mice. Screening results indicated that the presence of the 2-pyridylethylidene group was critical and that certain phenyl, benzyl, phenethyl, or cycloalkyl groups at N^4 of the thiosemicarbazone moiety also contribute to antimalarial activity.

Thiosemicarbazones, a class of compounds possessing a wide spectrum of medicinal properties, have been studied for activity against tuberculosis,[2] leprosy,[3] bacterial[4] and viral[5] infections, psoriasis,[6] rheumatism,[7] trypanosomiasis,[8] and coccidiosis.[9] In the past few years, thiosemicarbazones derived from 2-formylpyridine and related aldehydes have been of great interest because of their reported antineoplastic action.[10]

Among the thousands of compounds submitted for antimalarial screening by numerous contributors to the Division of Experimental Therapeutics have been several hundred thiosemicarbazides and thiosemicarbazones. Virtually all were devoid of activity, including the well-known tuberculostat, *p*-acetamidobenzaldehyde 3-thiosemicarbazone (Thiacetazone, Tibione). One thiosemicarbazone, however, namely, 2-acetylpyridine 4-phenyl-3-thiosemicarbazone (1),[11] attracted our attention because



1

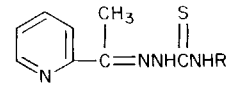
it showed activity in our primary screen. It was decided to exploit this interesting lead by ascertaining the molecular features essential for activity and utilizing them to develop a new class of antimalarial agents.

The influence on biological action was observed when the structure of **1** was modified as follows: (1) the thiocarbonyl group was replaced by a carbonyl group; (2) the pyridine moiety was replaced by another heterocyclic, aromatic, or cycloaliphatic ring system; (3) the point of attachment of the ethylidene group to the pyridine ring was changed to the 3 and 4 positions; (4) the methyl of the ethylidene group was replaced by other alkyls or hydrogen; (5) the phenyl ring at the terminal (N^4) position of the thiosemicarbazone was replaced by various substituted phenyls, other cyclic structures, and various so-called antimalarial aliphatic side chains.

This paper is one of the first to report on thiosemicarbazones possessing antimalarial activity.[12] In it, we limit our discussion to those compounds which are monosubstituted at N^4 of the thiosemicarbazone moiety.

Additional reports are in preparation which are devoted to related 2-acetylpyridine thiosemicarbazones that are disubstituted at N^4 and also to the antibacterial properties of this general class of compounds.

Table I. Antimalarial Activity of Thiosemicarbazones Derived from 2-Acetylpyridine against *Plasmodium berghei* in Mice

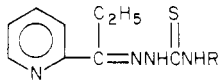


no.	R	mp, °C	formula	synth meth[b]	yield, %	recryst solvent	increase in mean surv time and no. of cures at dosage[a] 40	80	160	320	640
1	C_6H_5	182–183[c]	$C_{14}H_{14}N_4S$	A[d]	88	EtOH	3.1	4.7	11.1A	T(1/5), C(1/5)	T(2/5), C(2/5)
2	2-FC_6H_4	152–153	$C_{14}H_{13}FN_4S$	B	11	MeOH	0.0	2.6	6.8A	C(3/5)	
3	3-FC_6H_4	159–160	$C_{14}H_{13}FN_4S$	A	25	CH_3CN	4.4	5.8	6.6A	C(3/5)	C(4/5)
4	4-FC_6H_4	168–169	$C_{14}H_{13}FN_4S$	B	47	EtOH	1.3	3.7	3.9	7.7 A	T(1/5)
5	2-ClC_6H_4	154–156	$C_{14}H_{13}ClN_4S$	B	28	EtOH	0.5	0.5	2.3	6.1 A	8.1 A
6	3-ClC_6H_4	138–139	$C_{14}H_{13}ClN_4S$	A[e]	64	EtOH	0.3	0.3	2.5	5.1	7.1 A
7	4-ClC_6H_4	158–160	$C_{14}H_{13}ClN_4S$	B	64	EtOH	0.5	1.5	1.7	4.3	8.3 A
8	2-BrC_6H_4	152–154	$C_{14}H_{13}BrN_4S$	B	61	EtOH	0.1		0.5		0.9
9	3-BrC_6H_4	144–148	$C_{14}H_{13}BrN_4S$	B	49	EtOH	0.1		0.3		0.9
10	4-BrC_6H_4	189–190	$C_{14}H_{13}BrN_4S$	A[f]	80	CH_3CN	0.3		0.3		0.5
11	2,3-$Cl_2C_6H_3$	186–189[q]	$C_{14}H_{12}Cl_2N_4S$	B	25	CH_3CN-$CHCl_3$	-0.2		0.2		0.6
12	2,4-$Cl_2C_6H_3$	180–181	$C_{14}H_{12}Cl_2N_4S$	A[g]	57	EtOH	0.1		0.3		0.7
13	2,5-$Cl_2C_6H_3$	143–144	$C_{14}H_{12}Cl_2N_4S$	B	32	EtOH	-0.1		0.1		0.1
14	2,6-$Cl_2C_6H_3$	214–218[q]	$C_{14}H_{12}Cl_2N_4S$	B	50	CH_3CN	0.1		0.9		0.1
15	3,4-$Cl_2C_6H_3$	158–160	$C_{14}H_{12}Cl_2N_4S$	A	25	EtOH	0.5		0.5		0.5
16	3,5-$Cl_2C_6H_3$	164–166	$C_{14}H_{12}Cl_2N_4S$	B	25	EtOH	-0.2		-0.2		0.0
17	2,3,4-$Cl_3C_6H_2$	204–205[q]	$C_{14}H_{11}Cl_3N_4S$	A	30	$CHCl_3$	0.1		0.1		-0.3
18	2,4,5-$Cl_3C_6H_2$	168–169	$C_{14}H_{11}Cl_3N_4S$	B	19	EtOH	-0.3		0.1		-0.1
19	2-$O_2NC_6H_4$	146–149	$C_{14}H_{13}N_5O_2S$	B	14	EtOH	0.1	0.1	0.9	5.7	4.5
20	3-$O_2NC_6H_4$	179–181	$C_{14}H_{13}N_5O_2S$	B	19	CH_3CN	0.1		-0.3		-0.1
21	4-$O_2NC_6H_4$	193–195	$C_{14}H_{13}N_5O_2S$	B	50	EtOH	0.1		0.1		0.5
22	2-$CH_3C_6H_4$	164–166	$C_{15}H_{16}N_4S$	B	53	EtOH	0.1	0.5	0.9	5.1	9.9 A
23	3-$CH_3C_6H_4$	149–150	$C_{15}H_{16}N_4S$	B	39	EtOH	0.4	3.0	7.4	10.3, C(2/5)	C(4/5)
24	4-$CH_3C_6H_4$	160–161	$C_{15}H_{16}N_4S$	B	38	EtOH	0.3	2.1	4.5	C(3/5)	C(4/5)
25	2,6-$Me_2C_6H_3$	205–208	$C_{16}H_{18}N_4S$	B	66	EtOH	0.4	0.4	2.0	5.8	11.4 A
26	2-EtC_6H_4	157–159	$C_{16}H_{18}N_4S$	B	55	EtOH	0.3	0.3	0.7	3.1	5.1
27	4-EtC_6H_4	182–184	$C_{16}H_{18}N_4S$	B	79	EtOH	0.3	1.5	2.1	3.1	5.7
28	4-$(CH_3)_2CHC_6H_4$	168–171	$C_{17}H_{20}N_4S$	A[h]	81	CH_3CN	0.5	1.9	2.1	5.9	5.5
29	4-BuC_6H_4	148–149	$C_{18}H_{22}N_4S$	B	61	EtOH	0.3		3.9		9.9 A, T(3/5)
30	2-$CH_3OC_6H_4$	173–175	$C_{15}H_{16}N_4OS$	B	54	EtOH	0.1	0.4	2.0	4.6	6.6 A
31	3-$CH_3OC_6H_4$	138–140	$C_{15}H_{16}N_4OS$	B	16	EtOH	0.3	0.4	8.1 A	4.3, C(2/5)	9.6, C(2/5)
32	4-$CH_3OC_6H_4$	175–176	$C_{15}H_{16}N_4OS$	B	70	EtOH	2.1		9.7 A		1.7
33	4-HOC_6H_4	210–211[q]	$C_{14}H_{14}N_4OS$	B	30	EtOH-$CHCl_3$	0.1		0.3		0.6, T(2/5)
34	4-$C_2H_5OCOC_6H_4$	159–160	$C_{17}H_{18}N_4O_2S$	A	25	EtOH	0.3		0.3		0.6, T(2/5)
35	*p*-$C_6H_4SO_2C_6H_4$-*p*	228–231	$C_{28}H_{26}N_6O_2S_3$	B	80	*r*	0.1		0.1		0.3
36	3-[$(C_2H_5)_2$-NHCH$_2$]-4-OHC_6H_3·2HCl	200[q]	$C_{19}H_{27}Cl_2N_5OS$·H_2O	B	45	MeOH-Et_2O	2.0	5.6	8.8 A	T(5/5)	
37	$C_6H_5CH_2$	141–143	$C_{15}H_{16}N_4S$	B	38	EtOH	0.5		3.7		10.6 A, T(2/5)
38	3-$FC_6H_4CH_2$	157–159	$C_{15}H_{15}FN_4S$	B	72	CH_3CN	0.4	1.4	3.4	5.8	9.4 A
39	2-$ClC_6H_4CH_2$	172–174	$C_{15}H_{15}ClN_4S$	B	50	EtOH	0.3		0.3		1.9

40	3-$ClC_6H_4CH_2$	160–162	$C_{15}H_{15}ClN_4S$	B	48	EtOH		0.0	1.6	0.0	−0.4
41	4-$ClC_6H_4CH_2$	158–160	$C_{15}H_{15}ClN_4S$	B	64	EtOH	0.5	0.5	2.1	4.3	6.9 A
42	2,4-$Cl_2C_6H_3CH_2$	152–155	$C_{15}H_{14}Cl_2N_4S$	B	37	EtOH	0.4		1.0		2.0
43	3,4-$Cl_2C_6H_3CH_2$	157–158	$C_{15}H_{14}Cl_2N_4S$	B	37	EtOH	0.1		0.5		0.1
44	2-$CH_3C_6H_4CH_2$	152–154	$C_{16}H_{18}N_4S$	B	48	EtOH	0.1	2.5	5.9, C(1/5)	7.7, C(1/5)	8.9, C(3/5)
45	3-$CH_3C_6H_4CH_2$	143–144	$C_{16}H_{18}N_4S$	B	22	EtOH	0.3		0.7		6.5
46	4-$CH_3C_6H_4CH_2$	149–150	$C_{16}H_{18}N_4S$	B	14	MeOH	0.1	1.7	4.3	5.7	
47	3,4-$Me_2C_6H_3CH_2$	153–154	$C_{17}H_{20}N_4S$	B	60	MeOH	0.3		0.1		2.1
48	2,4-$Me_2C_6H_3CH_2$	148–149	$C_{17}H_{20}N_4S$	B	65	MeOH	0.5	0.1	3.1	6.1 A	8.9 A
49	2-$CH_3OC_6H_4CH_2$	120–123	$C_{16}H_{18}N_4OS$	B	28	EtOH	0.1	0.3	1.3	3.5	6.9 A
50	3-$CH_3OC_6H_4CH_2$	115–117	$C_{16}H_{18}N_4OS$	B	22	EtOH	0.3	0.3	0.9	4.5	5.9
51	4-$CH_3OC_6H_4CH_2$	134–136	$C_{16}H_{18}N_4OS$	B	44	EtOH	0.3	1.7	2.9	5.9, T(1/5)	7.9 A, T(3/5)
52	$C_6H_5CH_2CH_2$	134–135	$C_{16}H_{18}N_4S$	A[i]	63	CH_3CN	−0.2	0.6	2.0	6.6 A	8.8, C(3/5)
53	4-$FC_6H_4CHCH_3$	118–120	$C_{16}H_{17}FN_4S$	A	76	EtOH	1.1	5.5	8.5 A	5.2, C(1/5)	T(4/5)
54	$(C_6H_5)_3C$	179–180	$C_{27}H_{24}N_4S$	A[j]	23	CH_3CN	0.1		0.1		0.1
55	cyclohexyl	156	$C_{14}H_{20}N_4S$	A[k]	72	EtOH	3.9	3.9	9.6, C(2/5)	10.4, C(3/5)	C(2/5), T(3/5)
56	cyclooctyl	134–135	$C_{16}H_{22}N_4S$	B	52	MeOH	1.1	1.2	3.4	4.8	10.7 A, T(1/5)
57	1-adamantyl	165.5–167	$C_{18}H_{24}N_4S$	A[l]	71	EtOH	0.7	1.1	3.7	8.5 A	9.2, C(1/5)
58	2-pyridyl	185–187	$C_{13}H_{13}N_5S$	A[m]	34	EtOH	1.5	2.7	4.5	6.5 A	8.1 A
59	3-pyridyl	174.5–176[q]	$C_{13}H_{13}N_5S$	A	72	EtOH	0.3		0.5		1.3
60	4-pyridyl	153–155	$C_{13}H_{13}N_5S$	B	31	EtOH	0.1		0.3		0.9, T(2/5)
61	2-picolyl	141–145	$C_{14}H_{15}N_5S$	B	39	EtOH	0.1		0.3		0.9, T(3/5)
62	3-picolyl	149–151	$C_{14}H_{15}N_5S$	B	60	EtOH	0.1		0.3		0.6, T(2/5)
63	4-picolyl	155–158	$C_{14}H_{15}N_5S$	B	45	EtOH	0.3		0.4, T(1/5)		0.0, T(2/5)
64	6-MeO-3-pyridazinyl	194[q]	$C_{13}H_{14}N_6OS$	B	33	EtOH	0.1		0.1		0.2, T(2/5)
65	6-MeO-4-Me-8-quinolyl	236[q]	$C_{19}H_{19}N_5OS$	B	27	EtOH	0.1		0.1		0.3
66	9-acridyl	196–200[q]	$C_{21}H_{17}N_5S$	B	30	*r*	0.3		0.3		0.5
67	$-CH_2CH_2-$	214–216	$C_{18}H_{22}N_8S_2$	A[n]	53	*r*	0.1		0.1		0.1
68	$HOCH_2CH_2$	130–133	$C_{10}H_{14}N_4OS$	B	23	EtOH	0.3		0.5		T(5/5)
69	$CH_2=CHCH_2$	107–108	$C_{11}H_{14}N_4S$	A[o]	74	MeOH	0.5	2.3	3.1	3.0	T(5/5)
70	$C_2H_5OCOCH_2$	143–144	$C_{12}H_{16}N_4O_2S$	A	87	EtOH	0.1		0.1		0.3
71	1,1,3,3-Me_4Bu	143–144	$C_{16}H_{26}N_4S$	A	49	MeOH	1.1	1.3	0.9	2.9	8.7 A
72	$(C_2H_5)_2N(CH_2)_3CH(CH_3)\cdot 2HBr$	200–201[q]	$C_{17}H_{31}Br_2N_5S$	B	30	MeOH–Et_2O	0.3		T(1/5)		T(5/5)
73	$(CH_3)_2NCH(CH_3)CH_2\cdot HBr$	161–162[q]	$C_{13}H_{22}BrN_5S$	A	44	CH_3CN–Et_2O	0.1		T(4/5)		T(5/5)
74	$(C_2H_5)_2NCH_2CH_2\cdot 2HBr$	231[q]	$C_{14}H_{25}Br_2N_5S$	A	76	MeOH–CH_3CN	0.3		0.5	T(5/5)	T(5/5)
75	H	158–160[p]	$C_8H_{10}N_4S$	A	87	EtOH	T(5/5)		T(5/5)		T(5/5)

[a] Time in days and dosage in mg/kg. Abbreviations used are: A, active; C, cure; T, toxic. These terms are defined in the Biological Method paragraph given under the Experimental Section. [b] See Experimental Section for details. Method A: the reaction of a 4-substituted 3-thiosemicarbazide with 2-acetylpyridine. Superscripts in this column refer to precursor thiosemicarbazides. An "A" lacking a superscript indicates that the thiosemicarbazide was not in the literature and is reported by us in Table IV. Method B: the reaction of II with an amine. Yields are given for the final step and have not been optimized. [c] Mp 187–189 °C, ref 13; thiosemicarbazide, mp 141 °C, ref 14. [d] Method C: the reaction of 2-acetylpyridine hydrazone with an isothiocyanate (phenyl) gave a 94% yield. [e] Mp 120 °C, ref 15. [f] Mp 189 °C, ref 16. [g] Mp 218 °C, ref 17. [h] Mp 96 °C, ref 16. [i] Mp 114–115 °C, ref 19. [j] Mp 165–166 °C dec, ref 19. [k] Mp 146–147 °C, ref 19. [l] Mp 212.5–213 °C, ref 20. [m] Mp 194 °C, ref 14. [n] Mp 225 °C, ref 21. [o] Mp 96.5–97 °C, ref 22. [p] Mp 158–160 °C, ref 23. [q] Decomposition. [r] Washed with EtOH.

Table II. Antimalarial Activity of Thiosemicarbazones Derived from 2-Propionylpyridine against *Plasmodium berghei* in Mice



no.	R	mp, °C	formula	yield, %[b]	recryst solvent	increase in mean surv time and no. of cures at dosage[a] 40	80	160	320	640
76	C_6H_5	137	$C_{15}H_{16}N_4S$	32[c]	CH_3CN	0.0	1.2	2.6	C(1/5)	C(1/5)
77	2-ClC_6H_4	163–164	$C_{15}H_{15}ClN_4S$	63[d]	CH_3CN	0.1		0.1		0.3
78	3-ClC_6H_4	140–142	$C_{15}H_{15}ClN_4S$	20[e]	EtOH	0.3		0.3		0.5
79	4-ClC_6H_4	128–129	$C_{15}H_{15}ClN_4S$	56[f]	EtOH	0.3		0.7		1.1
80	4-BrC_6H_4	115–116	$C_{15}H_{15}BrN_4S$	40[g]	CH_3CN	0.3		0.3		0.7
81	4-$O_2NC_6H_4$	166	$C_{15}H_{15}N_5O_2S$	45[h]	EtOH	0.1		0.1		0.3
82	4-$C_2H_5OCOC_6H_4$	189	$C_{18}H_{20}N_4O_2S$	82	EtOH	0.3		0.5		0.5
83	$(C_6H_5)_3C$	188–190	$C_{28}H_{26}N_4S$	60[i]	$CHCl_3$	0.1		0.1		0.3
84	1-adamantyl	152–153	$C_{19}H_{26}N_4S$	67[j]	CH_3CN		0.3	3.7	6.3 A	9.1 A
85	$C_2H_5OCOCH_2$	145–146	$C_{13}H_{18}N_4O_2S$	74	MeOH	0.3		0.3		0.5

[a] Time in days and dosage in mg/kg. Abbreviations used are: A, active; C, cure. These terms are defined in the Biological Method paragraph given under the Experimental Section. [b] All compounds were made by method A. Superscripts in this column refer to precursor thiosemicarbazides. New thiosemicarbazides are given in Table IV. [c] Mp 141 °C, ref 14. [d] Mp 130–131 °C, ref 15. [e] Mp 120 °C, ref 15. [f] Mp 187–188 °C, ref 15. [g] Mp 189 °C, ref 16. [h] Mp 190 °C, ref 17. [i] Mp 165–166 °C dec, ref 19. [j] Mp 212.5–213 °C, ref 20.

Biological Results. Replacement of the thiocarbonyl group of **1** by a carbonyl gave compound **161** which was devoid of antimalarial activity, providing an indication of the essentiality of the sulfur atom in this class of compounds.

A number of thiosemicarbazones were prepared in which a wide variety of aromatic and heterocyclic aldehydes and ketones were used to form the alkylidene portion of the molecule. It became evident from the test data (cf. Tables I–III) that none of the aldehydes or ketones except 2-acetylpyridine (and to some extent, 2-propionylpyridine) would impart antimalarial activity. In some instances, the N^4 position of the thiosemicarbazone was substituted with a so-called antimalarial side chain (e.g., **36**, **72–74** and **145–158**). This approach failed, however, even when the thiosemicarbazones were derived from 2-acetylpyridine as in **72** and **74**.

In an attempt to confirm the optimum point of attachment of the ethylidene group to the pyridine ring, three active 2-pyridylethylidene thiosemicarbazones, R = C_6H_5 (**1**), 2-pyridyl (**58**), adamantyl (**57**), were prepared also as their 3- (**86**, **112**, and **131**, respectively) and 4-pyridylethylidene (**87**, **113**, and **132**, respectively) isomers. All 3- and 4-pyridyl compounds were found to be totally inactive.

Replacement of the ethylidene function of **1** by methylidene, to give compounds analogous to the type being studied for antileukemic[10] properties, destroyed activity (cf. **88**, **104**, and **123**). A propylidene group, on the other hand, appeared only to diminish activity in analogous compounds and in no case transformed an inactive compound into an active one (cf. Table II). Use of di-2-pyridinylmethanone as a precursor (**114** and **133**) abolished activity.

Keeping the 1-(2-pyridylethylidene) 3-thiosemicarbazone portion of **1** constant, the nature of the phenyl group at N^4 was modified by placement of one, two, or three substituents about the ring. Of the monofluorophenyl compounds, the 2 and 3 substituted (**2** and **3**) were curative at a fairly high dose of 320 mg/kg, whereas the 4-fluorophenyl (**4**) only slightly prolonged the life of the test animals at this dose level. All the monochlorophenyl derivatives (**5–7**) were active at 640 mg/kg. The three isomeric bromophenyl compounds (**8–10**) were inactive, as were the dichlorophenyl (**11–16**), trichlorophenyl (**17** and **18**), and the three isomeric nitrophenyl compounds (**19–21**).

Of the other substituted phenyls, 3- and 4-tolyl (**23** and **24**, respectively) were curative at the next to highest level, whereas only minimal activity was seen when the substituent was 2-tolyl (**22**), 2,6-dimethyl (**25**), 4-butyl (**29**), or 2- and 4-methoxy (**30** and **32**).

Of the group of benzyl derivatives tested, benzyl itself (**37**) and 4-chlorobenzyl (**41**) showed only slight activity at the highest test level of 640 mg/kg. The 2,4-dimethylbenzyl compound **48** was marginally active at the next lower dose and the best of the benzyl group, 2-methyl (**44**), gave cures at 160 mg/kg. Extension of the methylene side chain to give the phenethyl derivative **52** gave some enhanced activity over the benzyl compound. Further extension of the chain was not pursued in this study.

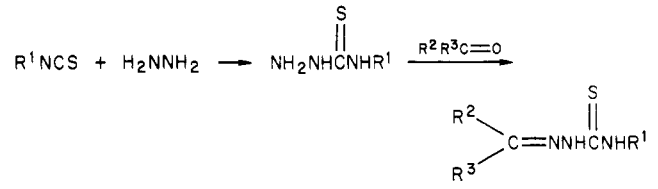
Not only was the cyclohexyl derivative **55** the most effective of the three cycloaliphatics (**55–57**) prepared and, in fact, in the entire series, but it was also one of the few compounds in the present group to be curative at the 160 mg/kg level.

Of the heterocycles (mainly pyridyl and picolyl) placed in the N^4 of the thiosemicarbazone moiety, only 2-pyridyl (**58**) imparted antimalarial activity. The latter was, however, only marginally active. The "dapsone" derivative **35** was disappointingly inactive, as were all the precursor thiosemicarbazides which were tested.

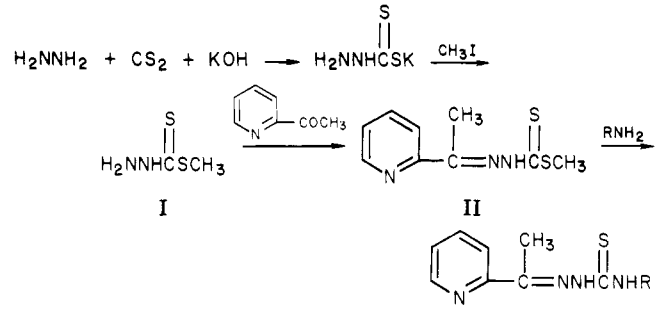
It was concluded, therefore, that the critical structural feature for a thiosemicarbazone exhibiting antimalarial activity is the 2-pyridylethylidene moiety. At N^4, the presence of an unsubstituted phenyl ring yields a more effective compound than when the phenyl ring is substituted. Some N^4-benzyl and -phenethyl compounds are also active, as are some cycloaliphatics such as adamantyl and, especially, cyclohexyl. N^4-Substitution by linear aliphatics or heterocyclics, on the other hand, contributes little or nothing to the antimalarial activity of the 2-acetylpyridine thiosemicarbazones. Because our experience with 2-propionylpyridine derivatives is still limited, no conclusion can be reached as yet regarding their therapeutic utility. Preliminary work indicates that substitution of a methyl group on N^2 serves to diminish antimalarial activity.

Expansion of the 2-acetylpyridine thiosemicarbazone series to include compounds in which N^4 is disubstituted

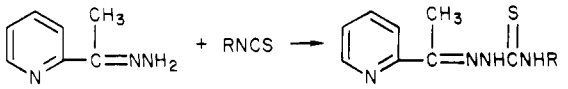
Scheme I



Scheme II



Scheme III



is now in progress. The early results from this study suggest that this type of structural modification serves to improve antimalarial activity.

Chemistry. The thiosemicarbazones reported herein were made by one of three routes.

Method A consisted of condensation of a thiosemicarbazide, prepared from an aryl, aralkyl, or alkyl isothiocyanate and hydrazine, with an aldehyde or ketone (Scheme I). Table IV presents the properties of previously unreported thiosemicarbazides made in the course of applying this method.

Method B, employed exclusively for the preparation of 2-acetylpyridine thiosemicarbazones, involved the condensation of 2-acetylpyridine with methyl hydrazinecarbodithioate (I) to form methyl 3-[1-(2-pyridyl)ethylidene]hydrazinecarbodithioate (II). The *S*-methyl group of the latter compound, upon displacement by an amine, formed the desired thiosemicarbazone (Scheme II). Through the use of the common intermediate II and readily available amines it was possible to form most of the compounds given in Table I in essentially a one-step reaction. As might be expected, the rate of the displacement reaction roughly paralleled the basicity of the amine, the weaker ones sometimes requiring ca. a 24-h reflux time.

Method C, an alternative preparative technique studied during the latter part of this study, involved the condensation of an isothiocyanate with the hydrazone of 2-acetylpyridine (III) (Scheme III).

The semicarbazone required for this investigation was made by the reaction of phenyl isocyanate with 2-acetylpyridine hydrazone.

Experimental Section

Melting points were taken on a Fisher-Johns hot stage interfaced with a Bailey Instruments BAT-8 digital thermometer. Infrared spectra were run as KBr pellets on a Perkin-Elmer 283 or a Beckman IR-5 spectrometer. NMR spectra were run on a Varian T60-A spectrometer using Me_4Si as an internal standard. Microanalyses were performed by the Baron Consulting Co. and Spang Microanalytical Laboratory. Satisfactory elemental analyses (±0.4% of calculated values) were obtained for all compounds, except where noted otherwise.

Thiosemicarbazones. Method A. Equimolar quantities of a 4-substituted 3-thiosemicarbazide and an aldehyde or a ketone in MeOH were heated on a steam bath for 1–3 h and, in some instances, up to 16 h. The reaction mixture was cooled and the thiosemicarbazone which separated from solution was collected and recrystallized.

Method B. Methyl Hydrazinecarbodithioate (I).[25] To a cooled solution of 198 g (3.0 mol) of KOH (quantity adjusted for 85% purity) in 240 mL of water and 200 mL of 2-propanol was added 171 mL (3.0 mol) of 85% hydrazine hydrate. Ice-cooled carbon disulfide (182 mL, 229 g, 3.0 mol) was added dropwise to the stirred solution, which was maintained at <10 °C over about 100 min. The bright-yellow mixture was stirred for an additional 1 h, after which ice-cooled iodomethane[26] (187 mL, 426 g, 3.0 mol) was added dropwise over a 2-h period. As the MeI was added the color of the mixture diminished in intensity and gradually became white. Stirring was continued for an additional 90 min, and the white precipitate was collected with the aid of a filter dam, washed with ice-cold water, and again collected. The crude product was recrystallized from CH_2Cl_2 to give 185 g (50%) of colorless prisms of methyl hydrazinecarbodithioate: mp 81–83 °C (lit. mp 82 °C,[25] 80–82 °C[27]); IR 3275, 3200 (br), 1510, 1155, 1010, 945 cm^{-1}; NMR ($CDCl_3$) δ 2.65 (s, 3 H, SCH_3).

Methyl 3-[1-(2-pyridyl)ethylidene]hydrazinecarbodithioate (II). Methyl hydrazinecarbodithioate (I; 213.6 g, 1.74 mol) and 212.0 g (1.75 mol) of 2-acetylpyridine in 500 mL of 2-PrOH were mechanically stirred. The reaction mixture turned yellow as the I dissolved and then the yellow product began to precipitate. The reaction mixture was stirred for an additional 2 h and cooled overnight. The crystals were collected, washed with cold 2-PrOH, and air-dried to yield 370 g (94%) of II, mp 126–129 °C (lit.[27] mp 131–132.5 °C). The compound was used without further purification: IR 3170, 1490, 1470, 1440, 1280, 1070, 780 cm^{-1}; NMR ($CDCl_3$) δ 2.42 (s, 3 H), 2.43 (s, 3 H), 2.65 (s, 3 H, SCH_3), 2.67 (s, 3 H, SCH_3), 7.10–8.77 (m, 4 H); TLC R_f 0.67–0.70 (silica gel, CH_3OH).

2-Acetylpyridine Thiosemicarbazones. To 2.4 g (0.02 mol) of II dissolved in 50 mL of either warm MeOH or EtOH[28] was added 0.02 mol of amine. The solution was heated under reflux until the evolution of methyl mercaptan almost completely ceased. Methyl mercaptan was detected by the yellow color it imparts to moistened $Pb(OAc)_2$ paper placed at the mouth of the reflux condenser. Reaction times were about 8 h; however, weakly basic amines required up to 24 h. The resultant thiosemicarbazones frequently crystallized from the hot solution as the reaction progressed. The more soluble thiosemicarbazones, however, separated from solution only after cooling.

See Table V for a listing of the important peaks found in the IR spectra and Table VI for a correlation of NMR spectra of representative members of this group of compounds.[29]

Method C. Typical Procedure. To a solution of 1.35 g (0.01 mol) of 2-acetylpyridine hydrazone[30] in 4 mL of CH_3CN was added 1.35 g of phenyl isothiocyanate, resulting in a mildly exothermic reaction. The solution was heated for 0.5 h at ~60 °C and cooled, causing crystallization of 1. The IR spectrum was identical with that obtained from 1 made by methods A and B.

2-Acetylpyridine 4-Phenylsemicarbazone (161). To a solution of 1.35 g (0.01 mol) of 2-acetylpyridine hydrazone in 5 mL of CH_3CN was added dropwise 1.2 g (0.01 mol) of phenyl isocyanate. An exothermic reaction began immediately and crystals separated. The white product was collected from the cooled reaction mixture, affording 2.3 g (92%) of 2-acetylpyridine 4-phenylsemicarbazone, mp 170–173 °C. An analytical sample, mp 171–173 °C, was prepared by recrystallization from CH_3CN. Anal. ($C_{14}H_{14}N_4O$) C, H, N.

Biological Method. The compounds described herein were tested at the Leo Rane Laboratory, University of Miami, Miami, FL, against a drug-sensitive strain of *Plasmodium berghei* (strain KBG 173) in mice. Young ICR/HA Swiss mice, ranging in weight from 18 to 22 g, are administered intraperitoneally a standard inoculum of plasmodia. The latter consists of 0.5 mL of a 1:100 dilution of heparinized heart's blood containing 4×10^7 cells, a minimum of 90% of which are parasitized. The cells are drawn from donor mice which had been infected 1 week earlier with

Table III. Thiosemicarbazones Inactive against *Plasmodium berghei* in Mice (Excluding Derivatives of 2-Acetylpyridine and 2-Propionylpyridine)

$R^3R^2C{=}NNHCNHR^1$ (S)

no.	R^1	R^2	R^3	mp, °C	formula	synth meth[a]	yield,[b] %	recryst solvent
86	C_6H_5	CH_3	3-pyridyl	177–178	$C_{14}H_{14}N_4S$	A	35	EtOH
87	C_6H_5	CH_3	4-pyridyl	193.5–195	$C_{14}H_{14}N_4S$	A	63	MeOH
88	C_6H_5	H	2-pyridyl	196–199[c]	$C_{13}H_{12}N_4S$			
89	4-ClC_6H_4	H	4-FC_6H_4	174–175	$C_{14}H_{11}ClFN_3S$	A	56	CH_3CN
90	4-ClC_6H_4	H	2,6-$Cl_2C_6H_3$	210–211	$C_{14}H_{10}Cl_3N_3S$	A	78	CH_3CN
91	4-ClC_6H_4	H	4-$CH_3OC_6H_4$	192–193	$C_{15}H_{14}ClN_3OS$	A	74	CH_3CN
92	4-ClC_6H_4	H	3,4-$(MeO)_2C_6H_3$	203–204.5	$C_{16}H_{16}ClN_3O_2S$	A	77	CH_3CN
93	4-ClC_6H_4	H	3,4-$OCH_2OC_6H_3$	210–211	$C_{15}H_{12}ClN_3O_2S$	A	70	$CHCl_3$
94	4-ClC_6H_4	H	4-$(CH_3)_2NC_6H_4$	204–206	$C_{16}H_{17}ClN_4S$	A	91	CH_3CN
95	4-ClC_6H_4	H	5-O_2N-2-furyl	203–204	$C_{11}H_9N_5O_3S$	A	90	CH_3CN
96	4-ClC_6H_4	H	$C_6H_5CH{=}CH$ (trans)	199–200	$C_{16}H_{14}ClN_3S$	A	90	CH_3CN
97	4-ClC_6H_4	CH_3	3,4-$Cl_2C_6H_3$	186–188	$C_{15}H_{12}Cl_3N_3S$	A	65	CH_3CN
98	4-ClC_6H_4	CH_3	4-BrC_6H_4	194–195	$C_{15}H_{13}BrClN_3S$	A	30	CH_3CN
99	2-pyridyl	H	C_6H_5	148–150	$C_{13}H_{12}N_4S$	A	46[d]	CH_3CN
100	2-pyridyl	H	4-FC_6H_4	160–161	$C_{13}H_{11}FN_4S$	A	33	CH_3CN
101	2-pyridyl	H	2,6-$Cl_2C_6H_3$	185–186	$C_{13}H_{10}Cl_2N_4S$	A	27	CH_3OH
102	2-pyridyl	H	3,4-$Me_2OC_6H_3$	205–206	$C_{15}H_{16}N_4O_2S$	A	67	CH_3CN
103	2-pyridyl	H	3,4-$OCH_2OC_6H_3$	195–197	$C_{14}H_{12}N_4O_2S$	A	49	CH_3CN
104	2-pyridyl	H	2-pyridyl	189–191	$C_{12}H_{11}N_5S$	A	58	CH_3CN
105	2-pyridyl	H	4-pyridyl	193–194	$C_{12}H_{11}N_5S$	A	77	CH_3CN
106	2-pyridyl	H	2-thienyl	170–171	$C_{11}H_{10}N_4S_2$	A	38	CH_3CN
107	2-pyridyl	H	3-indolyl	179–181	$C_{15}H_{13}N_5S$	A	44	CH_3CN
108	2-pyridyl	CH_3	4-FC_6H_4	203–204	$C_{14}H_{13}FN_4S$	A	62	CH_3CN
109	2-pyridyl	CH_3	4-ClC_6H_4	192–193	$C_{14}H_{13}ClN_4S$	A	50	EtOH
110	2-pyridyl	CH_3	4-BrC_6H_4	213–214	$C_{14}H_{13}BrN_4S$	A	70	CH_3OH
111	2-pyridyl	CH_3	1-adamantyl	192–193	$C_{18}H_{24}N_4S$	A	37	CH_3CN
112	2-pyridyl	CH_3	3-pyridyl	207–209	$C_{13}H_{13}N_5S$	A	48	EtOH
113	2-pyridyl	CH_3	4-pyridyl	209–211	$C_{13}H_{13}N_5S$	A	66	CH_3CN
114	2-pyridyl	2-pyridyl	2-pyridyl	150–153	$C_{17}H_{14}N_6S$	A	53	$CHCl_3$
115	3-pyridyl	H	C_6H_5	182–183	$C_{13}H_{12}N_4S$	A	64[e]	EtOH
116	3-pyridyl	H	4-FC_6H_4	191–192[h]	$C_{13}H_{11}FN_4S$	A	80	EtOH
117	3-pyridyl	H	3,4-$OCH_2OC_6H_3$	206–207	$C_{14}H_{12}N_4O_2S$	A	81	MeOH
118	4-pyridyl	CH_3	C_6H_5	153–155	$C_{14}H_{14}N_4S$	B	27	EtOH
119	1-adamantyl	H	4-FC_6H_4	207–208[h]	$C_{18}H_{22}FN_3S$	A	85[f]	*i*
120	1-adamantyl	H	2,6-$Cl_2C_6H_3$	233–234	$C_{18}H_{21}Cl_2N_3S$	A	35	EtOH
121	1-adamantyl	H	4-$CH_3OC_6H_4$	215	$C_{19}H_{25}N_3OS$	A	89	EtOH
122	1-adamantyl	H	3,4-$(MeO)_2C_6H_3$	193–194	$C_{20}H_{27}N_3O_2S$	A	49	EtOH
123	1-adamantyl	H	2-pyridyl	196–198[h]	$C_{17}H_{22}N_4S$	A	79	EtOH
124	1-adamantyl	H	4-pyridyl	215–216	$C_{17}H_{22}N_4S$	A	72	EtOH
125	1-adamantyl	CH_3	C_6H_5	195–198[h]	$C_{19}H_{25}N_3S$	A	61	*i*
126	1-adamantyl	CH_3	4-FC_6H_4	216	$C_{19}H_{24}FN_3S$	A	65	EtOH
127	1-adamantyl	CH_3	4-ClC_6H_4	212–215[h]	$C_{19}H_{24}ClN_3S$	A	57	EtOH
128	1-adamantyl	CH_3	3,4-$Cl_2C_6H_3$	218–220	$C_{19}H_{23}Cl_2N_3S$	A	19	EtOH
129	1-adamantyl	CH_3	4-BrC_6H_4	228–230	$C_{19}H_{24}BrN_3S$	A	54	MeOH
130	1-adamantyl	CH_3	1-adamantyl	208.5–210.5[h]	$C_{23}H_{35}N_3S$	A	52	EtOH
131	1-adamantyl	CH_3	3-pyridyl	192–195[h]	$C_{18}H_{23}N_4S$	A	45	*i*
132	1-adamantyl	CH_3	4-pyridyl	215–216[h]	$C_{18}H_{24}N_4S$	A	63	EtOH

133	1-adamantyl	2-pyridyl	2-pyridyl	244[h]	$C_{22}H_{25}N_5S$	A	79	EtOH
134	$(C_2H_5)_2N(CH_2)_3CH(CH_3)$	H	2,6-$Cl_2C_6H_3$	83–84	$C_{17}H_{26}Cl_2N_4S$	A	26[e]	pet. ether
135	$(C_2H_5)_2N(CH_2)_3CH(CH_3)$	H	4-$CH_3OC_6H_4$	123–124	$C_{18}H_{30}N_4OS$	B	38	Et_2O
136	$(C_2H_5)_2N(CH_2)_3CH(CH_3)$·HBr	H	4-pyridyl	201–203	$C_{16}H_{28}BrN_5S$	B	44	CH_3CN-MeOH
137	$(C_2H_5)_2N(CH_2)_3CH(CH_3)$·HBr	H	5-O_2N-2-furyl	177–178[h]	$C_{15}H_{26}BrN_5O_3S$	A	69	MeOH-Et_2O
138	$(C_2H_5)_2N(CH_2)_3CH(CH_3)$	CH_3	C_6H_5	64–66	$C_{18}H_{30}N_4S$	B	21	pet. ether
139	$(C_2H_5)_2N(CH_2)_3CH(CH_3)$	CH_3	4-FC_6H_4	74	$C_{18}H_{29}FN_4S$	B	82	pet. ether
140	$(C_2H_5)_2N(CH_2)_3CH(CH_3)$·2HBr	CH_3	3-pyridyl	169–170[h]	$C_{17}H_{31}Br_2N_5S$	A	50	CH_3CN
141	$(C_2H_5)_2N(CH_2)_3CH(CH_3)$·2HBr	CH_3	4-pyridyl	173–176[h]	$C_{17}H_{31}Br_2N_5S$	A	40	CH_3CN-Et_2O
142	$(C_2H_5)_2N(CH_2)_3CH(CH_3)$·HBr	CH_3	1-adamantyl	212–213	$C_{22}H_{41}BrN_4S$	A	80	EtOH
143	$(C_2H_5)_2N(CH_2)_3CH(CH_3)$·HBr	9-fluorenylidene		179–180[h]	$C_{23}H_{31}BrN_4S$	A	77	Me_2CO-Et_2O
144	$(C_2H_5)_2N(CH_2)_3CH(CH_3)$·HBr	2-adamantylidene		171–172[h]	$C_{20}H_{37}BrN_4S$	A	32	CH_3CN-Et_2O
145	$(CH_3)_2NCH(CH_3)CH_2$	H	2,6-$Cl_2C_6H_3$	178–179	$C_{13}H_{18}Cl_2N_4S$	A	80[e]	CH_3CN
146	$(CH_3)_2NCH(CH_3)CH_2$·HBr	H	4-pyridyl	219–221[h]	$C_{12}H_{20}BrN_5S$	A	76	MeOH-Et_2O
147	$(CH_3)_2NCH(CH_3)CH_2$·HBr	H	5-O_2N-2-furyl	212[h]	$C_{11}H_{18}BrN_5O_3S$	A	83	MeOH
148	$(C_2H_5)_2NCH_2CH_2$·HBr	H	C_6H_5	158	$C_{14}H_{23}BrN_4S$	B	22[e]	CH_3CN
149	$(C_2H_5)_2NCH_2CH_2$·HBr	H	4-FC_6H_4	194–195	$C_{14}H_{22}BrFN_4S$	A	98	2-PrOH-Et_2O
150	$(C_2H_5)_2NCH_2CH_2$	H	2,6-$Cl_2C_6H_4$	150–151	$C_{14}H_{20}Cl_2N_4S$	A	71	CH_3CN
151	$(C_2H_5)_2NCH_2CH_2$·HBr	H	4-$CH_3OC_6H_4$	183–184[h]	$C_{15}H_{25}BrN_4OS$	A	42	CH_3CN-C_6H_6
152	$(C_2H_5)_2NCH_2CH_2$·HBr	H	3,4-$OCH_2OC_6H_3$	218–219[h]	$C_{15}H_{23}BrN_4O_2S$	A	46	EtOH
153	$(C_2H_5)_2NCH_2CH_2$	H	4-pyridyl	125–126	$C_{13}H_{21}N_5S$	A	56	CH_3CN
154	$(C_2H_5)_2NCH_2CH_2$	H	6-CH_3O-4-quinolyl	129–130	$C_{18}H_{25}N_5OS$	A	80	CH_3CN-Et_2O
155	$(C_2H_5)_2NCH_2CH_2$·2HBr	CH_3	3-pyridyl	215–216[h]	$C_{14}H_{25}Br_2N_5S$	A	26	2-PrOH-MeOH
156	$(C_2H_5)_2NCH_2CH_2$·2HBr	CH_3	4-pyridyl	191–192[h]	$C_{14}H_{25}Br_2N_5S$	A	78	2-PrOH-Et_2O
157	$(C_2H_5)_2NCH_2CH_2$·HBr	C_6H_5	C_6H_5	173–174	$C_{20}H_{27}BrN_4S$	B	53	MeOH-Et_2O
158	$(C_2H_5)_2NCH_2CH_2$·HBr	9-fluorenylidene		145–146	$C_{20}H_{25}BrN_4S$	A	68	Me_2CO-Et_2O
159	bis(2-pyridyl)	2,6-pyridinediethylidene		224	$C_{21}H_{21}N_9S_2$	A	37	CH_3CN
160	bis(1-adamantyl)	2,6-pyridinediethylidene		255–260[h]	$C_{31}H_{43}N_7S_2$	A	24	$CHCl_3$
161	C_6H_5	CH_3	2-pyridyl	171–173	$C_{14}H_{14}N_4O$	C	55[g]	EtOH

[a] See Experimental Section for details. [b] Yields have not been optimized. [c] Lit. mp 196–199 °C, ref 24. Submitted for testing by Dr. Frederic A. French. [d] Thiosemicarbazide, ref 14. [e] Thiosemicarbazide, see Table IV. [f] Thiosemicarbazide, ref 20. [g] Details of the preparation of this semicarbazone are given under the Experimental Section. [h] Decomposition. [i] Washed with EtOH.

Table IV. 4-Substituted 3-Thiosemicarbazides

$H_2NNHCNHR$ (S)

no.	used in synth of compd	R	mp, °C	yield, %	formula	recryst solvent
163	3	3-FC_6H_4	164–166	91	$C_7H_8FN_3S$	CH_3CN
164	15	3,4-$Cl_2C_6H_3$	174–176	91	$C_7H_7Cl_2N_3S$	CH_3CN
165	17	2,3,4-$Cl_3C_6H_2$	164–168[c]	83	$C_7H_6Cl_3N_3S$	CH_3CN
166	34, 82	4-$C_2H_5OCOC_6H_4$	137	85[a]	$C_{10}H_{13}N_3O_2S$	MeOH
167	53	4-$FC_6H_4CHCH_3$	108–109	53	$C_9H_{12}FN_3S$	CH_3CN
168	59, 115–117	3-pyridyl	162–163[c]	94	$C_6H_8N_4S$	MeOH
169	70, 85	$C_2H_5OCOCH_2$	168–169[c]	88	$C_5H_{11}N_3O_2S$	CH_3CN
170	71	1,1,3,3-Me_4Bu	98	92[b]	$C_9H_{21}N_3S$	C_6H_{12}
171	72, 134–144	$(C_2H_5)_2N(CH_2)_3CH(CH_3)$·HBr	137–139	94	$C_{10}H_{25}BrN_4S$	CH_3CN
172	73, 145–147	$(CH_3)_2NCH(CH_3)CH_2$	104–105	76	$C_6H_{16}N_4S$	C_6H_6
173	74, 148–158	$(C_2H_5)_2NCH_2CH_2$	83–83.5	63	$C_7H_{18}N_4S$	C_6H_6

[a] Anal. Calcd: S, 13.40. Found: 12.93. [b] Anal. Calcd: C, 53.16. Found: 53.63. [c] Decomposition.

Plasmodium berghei. All the untreated infected animals, which serve as controls, die after 6–8 days and with a mean survival time of 6.2 days. Every compound is tested at several dose levels. At each level, the candidate drug is given subcutaneously in a single dose as a peanut oil suspension to five mice 72 h after they are infected. The compounds are judged to be "toxic" if the infected mice die before the 6th day, i.e., before the time when the untreated mice begin to die; "active" if the mean survival time of the mice is at least doubled; and "curative" if the mice survive 60 days postinfection. Details of the test procedure were given by Osdene, Russell, and Rane.[31]

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Supplementary Material Available: Table V, infrared spectral correlation of 2-acetylpyridine 4-monosubstituted 3-thiosemicarbazones in KBr pellets, and Table VI, NMR spectral correlation of 2-acetylpyridine 4-monosubstituted 3-thiosemicarbazones and related compounds in $CDCl_3$ solution (2 pages). Ordering information is given on any current masthead page.

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Analogues of Methotrexate

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Analogues of methotrexate (MTX) were prepared by alkylation of side-chain precursors with 6-(bromomethyl)-2,4-pteridinediamine followed, where necessary, by saponification of the intermediate esters and, in two cases, by electrophilic substitution reactions in the pyridine ring portion of 3-deazamethotrexate. Effects of the various modifications on their ability to inhibit dihydrofolate reductase, cytotoxicity, and activity against L1210 leukemia in mice were examined in light of recent findings concerning active transport of MTX and related compounds and the binding features of the MTX–dihydrofolate reductase complex.

Methotrexate (MTX, 1) is perhaps the most useful *antimetabolite* presently employed in the treatment of cancer,[2] but attempts to improve the clinical activity of this agent by congener synthesis have not been successful.

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