Synthesis of Potential Antimalarial Agents. VI.[1] Preparation of 3-(*p*-Chlorophenyl)-8-{[4-(diethylamino)-1-methylbutyl]amino}pyrido[2,3-*b*]pyrazine

CARROLL TEMPLE, JR.,* JERRY D. ROSE, AND JOHN A. MONTGOMERY

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205

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Previously we reported that a series of 6-amino-3-(*p*-substituted-phenyl)-8-{[4-(diethylamino)-1-methylbutyl]amino}pyrido[2,3-*b*]pyrazines (*e.g.*, VI) showed activity against mice infected with the sensitive strain of *Plasmodium berghei*.[2] To determine the effect of the 6-amino group on activity, the synthesis of a compound without this group was undertaken. Reaction of 2,4-dichloro-3-nitropyridine (I)[3] with 2-amino-5-diethylaminopentane gave a complex mixture containing I, II, III, and IV, which was separated by column chromatography. A pure sample of IV was obtained in 45% yield. Amination of IV and reduction of the NO_2 of the resulting compound VII with Ra Ni gave the corresponding diaminopyridine, which was condensed *in situ* with *p*-chlorophenylglyoxal to give V. The assignment of the position of the chlorophenyl group is based on analogy with the major product obtained in the condensation reactions of other 2,3-diaminopyridines.[4]

The test results showed that V is more active and less toxic than the corresponding 6-amino compound VI (see Table I). These data suggest that the 6-amino group of VI inhibits, either sterically or electronically, the function of the pyridine ring N, which is probably necessary for antimalarial activity.[5]

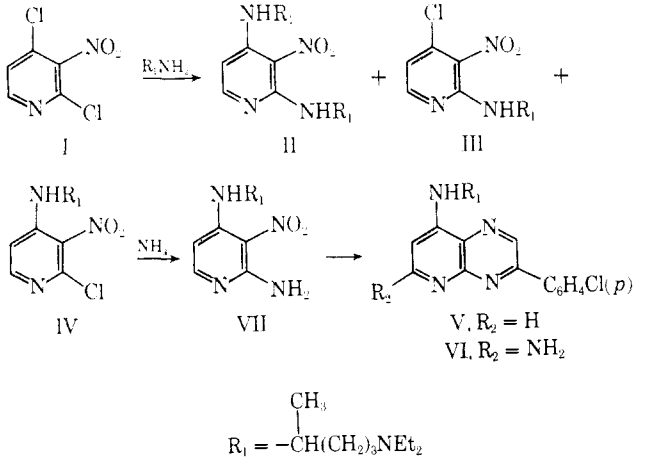

TABLE I

ANTIMALARIAL ACTIVITY[a]

Increase in MST, Days[b]

	Dosage, mg/kg		
Compd	160	320	640
V	11.9 A	5 C	5 C
VI	6.0	16.1 A (2C)	3 C (2T)
Chloroquine	10.0 A		(5T)

[a] Tests were carried out in 5 mice infected with a lethal dose of *P. berghei* by L. Rane and coworkers, Malaria Screening Laboratory, University of Miami, Miami, Fla. [For a description of the test procedure, see T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967)]. Results were supplied by the Walter Reed Army Institute of Research, Washington, D. C. [b] MST, mean survival time over controls (6.2 ± 0.49 days); A, active (MST of treated animals is greater than twice the MST of control group); C, number of cures (mice surviving to 60 days); T, number of toxic deaths (mice dying before 6.2 ± 0.49 days).

Experimental Section[6]

Reaction of I with 2-Amino-5-diethylaminopentane.—A soln of 2-amino-5-diethylaminopentane (17.0 g, 107 mmoles) and I (20.7 g, 107 mmoles) in MeOH (250 ml) was stirred at room temperature for 24 hr, then refluxed under N_2 for 15 hr. Evaporation of the solvent *in vacuo* gave a fluorescent orange oil which was dissolved in $CHCl_3$ and poured onto a silica gel H column (300 g) [prewashed with $CHCl_3$–MeOH (9:1)]. Elution with $CHCl_3$–MeOH (9:1) gave 4 fractions, the first containing crude I (5.4 g, ~26% recovery). The residue from each of the remaining fractions was converted into the corresponding HCl salt by treatment with excess 1 *N* ethanolic HCl, and evaporation of the whole *in vacuo* to give a brittle hygroscopic glass. The first component isolated in this manner was identified as III; yield 0.4 g (1%). Amination of this material with saturated ethanolic NH_3 in a stainless steel bomb at 60° for 1 hr gave a yellow oil which was identified as 4-amino-2-{[4-(diethylamino)-1-methylbutyl]amino}-3-nitropyridine by comparison of its chromatographic behavior and spectral characteristics with those of an authentic sample;[7] λ_{max} nm ($\epsilon \times 10^{-3}$), pH 7, 255 (sh), 348 (8.2).

The second component was identified as IV: yield 18.6 g (45%); λ_{max} nm ($\epsilon \times 10^{-3}$), pH 7, 248 (16.4), 370 (2.0). *Anal.* ($C_{11}H_{25}ClN_4O_2 \cdot HCl \cdot 1.5H_2O$) C, H, Cl, N.

The third component (12.0 g) was dissolved in aq NaOH (pH >11), and the soln was extd with $CHCl_3$ (4 × 200 ml). The combined exts were washed with H_2O, dried (Na_2SO_4), and evapd to dryness *in vacuo* to give II; yield 7.6 g (16%); λ_{max} nm ($\epsilon \times 10^{-3}$), pH 7, 265 (sh), 367 (9.6). *Anal.* ($C_{22}H_{34}N_6O_2$) C, H, N.

2-Amino-4{[4-(diethylamino)-1-methylbutyl]amino}-3-nitropyridine (VII).—A soln of IV (15.0 g, 39.6 mmoles) in ethanolic NH_3 (600 ml, saturated at 0°) was heated in a glass-lined stainless steel bomb at 80° for 3 hr, then at 65° for 18 hr. The bomb was chilled and opened and its contents evapd to dryness. The gum was dissolved in $CHCl_3$ (100 ml); the resulting soln was filtered to remove inorg salt and evapd to dryness *in vacuo*. A soln of the residue in EtOH (200 ml) was charcoaled, filtered, acidified with 1 *N* ethanolic HCl (100 ml), and evapd to dryness. An aq (200 ml) soln of the glassy residue was charcoaled, filtered, and adjusted to pH 10 with 50% NaOH soln. The mixture was extd with $CHCl_3$ (3 × 100 ml), and the combined exts were washed with H_2O, dried (Na_2SO_4), and evapd to dryness *in vacuo*. The residual amber oil was dried by prolonged evacuation on the oil pump; yield 7.2 g (62%); λ_{max} nm ($\epsilon \times 10^{-3}$), pH 7, 350 (9.4). *Anal.* ($C_{14}H_{25}N_5O_2$) C, H, N.

3-(*p*-Chlorophenyl)-8-{[4-(diethylamino)-1-methylbutyl]-amino}pyrido[2,3-*b*]pyrazine·2HCl·H_2O (V).—A soln of VII (5.00 g, 16.9 mmoles) in EtOH (200 ml) was hydrogenated over Ra Ni catalyst (~15 g) at an initial H_2 pressure of 3.5 kg/cm². The catalyst was removed by filtration under N_2, and the colorless filtrate was treated with solid *p*-chlorophenylglyoxal monohy-

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* To whom correspondence should be addressed.

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(6) Silica gel H was obtained from Brinkmann Instruments, Inc., and Raney Active Catalyst No. 28 from W. R. Grace & Co. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

(7) C. Temple, Jr., A. G. Laseter, J. D. Rose, and J. A. Montgomery, unpublished results.

drate (3.73 g, 20.0 mmoles). The resulting red soln was stirred under N_2 at room temperature for 72 hr, then refluxed for 1 hr. After treatment with charcoal the soln was evapd *in vacuo* to give a dark gummy residue that was dissd in H_2O (300 ml) containing concd HCl (5 ml). The resulting soln was warmed, treated with charcoal, and filtered through Celite. The orange filtrate was adjusted to pH 11 with 50% NaOH and extracted with $CHCl_3$ (3 × 150 ml). The combined extract was washed with H_2O, dried (Na_2SO_4), and evapd to dryness. An EtOH soln (100 ml) of the residue was acidified with 3 *N* ethanolic HCl (10 ml) and dild with Et_2O (300 ml). After vigorous stirring for several hours, an orange powder deposited slowly. The mixture was dild to 1 l. with Et_2O and filtered under N_2. The orange powder was recrystd under the same conditions to give a hygroscopic orange solid that was collected by filtration and dried *in vacuo* over P_2O_5 at 78°: yield 3.85 g (46%): mp sintering and gradual decpn 220–230° (Mel-Temp); λ_{max} nm ($\epsilon \times 10^{-3}$), pH 7, 243 (27.4), 293 (21.4), 338 (12.6), 385 (10.0); pmr (8% DMSO-d_6 w/v), δ 1.28 (m, 9, CH_3), 1.82 (m, 4, CH_2), 3.05 (m, 6, CH_2N), 4.24 (m, 1, CHN), 7.22 (d, 1, 7-CH), 8.04 (q, C_6H_4), 8.55 (6-CH), 9.57 (NH), 9.63 (2-CH). *Anal.* ($C_{22}H_{28}ClN_5 \cdot 2HCl \cdot H_2O$) C, H, Cl, N.

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Hydroxylamine Derivatives as Potential Antimalarial Agents. 1. Hydroxamic Acids[1]

JOHN B. HYNES

Department of Pharmaceutical Chemistry, College of Pharmacy, Medical University of South Carolina, Charleston, South Carolina 29401

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Hydroxamic acids have been found to exert a number of diverse pharmacologic actions including antituberculous, antifungal, and antileukemic activities.[2] In addition, certain arylhydroxamic acids inhibit nucleic acid biosynthesis *in vitro*.[3-5] Since the quinoline type antimalarials such as chloroquine, quinacrine, and quinine have been reported to function at least in part by blocking enzymatic synthesis of DNA and RNA,[6] a series of hydroxamic acids was synthesized and evaluated as a potential new class of antimalarial drugs.

A total of 33 mono- and dihydroxamic acids and related compounds was prepared and tested for *in vivo* antimalarial activity against *Plasmodium berghei* in mice.[7,8] Pertinent physical and chemical data for new compounds or those for which the melting points were significantly different from the literature values are summarized in Table I. The preparation and properties of other compounds have been described earlier.

Of the compounds tested, two showed an increase in mean survival time of infected mice of greater than 100%. They were terephthalohydroxamic acid (**2**) and dibenzoylterephthalohydroxamate (**15**) and the pertinent testing data for them are summarized in Table II. The most probable structure of **15** is based upon ir data.

p-HONHCOC$_6$H$_4$CONHOH
2

p-C$_6$H$_5$COONHCOC$_6$H$_4$CONHOCOC$_6$H$_5$
15

The similar level of activity of these two compounds suggests that **15** is enzymatically converted into **2**. However, the lack of activity of the diacetyl derivative **14** is difficult to explain on this basis.

All of the derivatives of **2** involving ring substitution, **4**, **5**, **6**, and **7**, were inactive with the exception of the tetrafluoro analog **3** which was slightly active (Δ = 2 days at 640 mg/kg). Similarly, alkylation of the hydroxamic acid portion of the molecule (**11**, **12**, and **13**) resulted in inactive compounds.

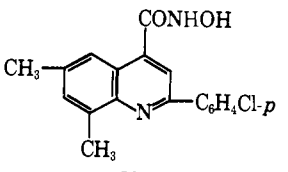
Compounds **8**, **10**, and **20** as well as adipohydroxamic acid[9] were prepared in order to evaluate the effect of altering the distance separating the two hydroxamic acid functions. Since none of these showed appreciable activity, one may conclude that this value is extremely critical.

The pyridine analog of **2**, 2,5-pyridinedicarbohydroxamic acid (**16**), as well as its 3-position isomers, **17**, **18**, and **19**, were also prepared and evaluated for antimalarial activity. As would be expected from the inactivity of **8**, only **16** showed appreciable activity but was also toxic.[10]

In addition to 4-carboxybenzohydroxamic acid (**1**) two related compounds, terephthaldihydrazide[11] and terephthaldioxime,[12] were also prepared and found to be devoid of activity.

A series of 4-substituted benzohydroxamic acids [p-XC$_6$H$_4$CONHOH (X = SO_2NH_2, NO_2, Br, Cl, I)] was prepared as described in the literature.[5,13] Only the 4-Br derivative showed appreciable activity (Δ = 3.1 days at 640 mg/kg). In addition, 3,4,5-trimethoxy-, 2-bromo-3,4,5-trimethoxy-, 2-hydroxy-3,4,5-trimethoxy-, and 3,5-dichlorobenzohydroxamic acids as well as 3,4,5-trimethoxyphenylacetohydroxamic acid were prepared as described earlier[5] and found to be inactive.

Compound **21** was synthesized as an analog of the highly potent antimalarials, the quinoline methanols.[14] It was also inactive.



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