Phenoxypropoxybiguanides, Prodrugs of DHFR−Inhibiting Diaminotriazine Antimalarials

Norman P. Jensen,*,† Arba L. Ager,‡ Robert A. Bliss,† Craig J. Canfield,§ Barbara M. Kotecka,|| Karl H. Rieckmann,|| Jacek Terpinski,† and David P. Jacobus†

Jacobus Pharmaceutical, Box 5290, Princeton, New Jersey 08540, Department of Microbiology & Immunology, University of Miami School of Medicine, Miami, Florida 33136, Pharmaceutical Systems, Inc., Talent, Oregon 97540, and Australian Army Malaria Institute, Enoggera QLD 4052, Australia

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A total of 34 analogues of the biguanide PS-15 (**5s**), a prodrug of the diaminotriazine WR-99210 (**8s**), have been prepared. Several of them, such as **5b** (PS-33) and **5m** (PS-26), maintain or exceed the in vivo activity of PS-15 while not requiring the use of highly regulated starting materials. The putative diaminotriazine metabolites of these new analogues (compounds **8**) have also been prepared and shown to maintain the activity against resistant *P. falciparum* strains. The structure−activity relationships of biguanides **5** and putative metabolites **8** are discussed.

Introduction

Worldwide there is no doubt that malaria is a disease deserving the continual deployment of resources to find effective protective and curative agents. It is estimated that there are 300 million new cases with the result of 2 million deaths a year.[1,2] Furthermore, the burden of this disease is borne disproportionately by the third world and by its young children.[3] In Africa alone about $^1/_2$ million children up to the age of 4 die of malaria every year.[4] Compounding the problem is the widespread resistance to older antimalarial agents.[5−7] This paper describes a series of dihydrofolate reductase inhibitors that are both potent and not cross-resistant with older antimalarials.

We have previously reported on the prototype antimalarial of the present series, PS-15[8,9] (**5s** in Chart 1). This member of the series has been demonstrated to have in vivo antimalarial activity in a mouse and two monkey models.[10,11] It has also shown in vitro activity against *Mycobacterium avium*[12] and in vivo activity against *Pneumocystis carinii*.[13]

As depicted in Chart 1, PS-15 and its congeners are actually prodrugs that require metabolic oxidative cyclization to triazine structures. In the case of PS-15 the antimalarial activity of the triazine metabolite **8s** (WR-99210) was reported as early as 1973,[14,15] but animal studies indicated gastrointestinal intolerance and poor bioavailability.[16,17] These drawbacks precluded human development, but WR-99210 has consistently been demonstrated to be active against field strains that are resistant to other antimalarials that work as antifolates or by other mechanisms.[18−21] As reported previously,[8] PS-15 is 3 times as potent as its metabolite WR-99210 in an in vivo model. This prodrug success is portended by analogy with the widely used antimalarial proguanil (see Chart 1), which has similar structural elements and metabolic activation. It has been over 50 years since it was first predicted[22] that this drug had a much more active metabolite that was later identified as cycloguanil.[23]

Although PS-15 is a very attractive candidate for further development on the basis of its biological activities, it has a significant practical drawback. The logical starting material, 2,4,5-trichlorophenol, has severe governmental restrictions on its manufacture and on the disposal of any wastes associated with its manufacture or the manufacture of pesticides derived from it.[24] These regulations were promulgated because base-catalyzed reactions can give the highly toxic tetrachlorodioxin known as TCDD or simply dioxin. TCDD can be formed in the manufacture of 2,4,5-trichlorophenol or, as a contaminant, in the manufacture of the herbicide 2,4,5-T, a component of "Agent Orange".[25] Levels of dioxin must be shown to be less than the very low limit of 0.1 ppb. Such stringent regulations of the manufacture of the starting material for PS-15 make other analogues in the series of similar activity (but without such a safety or regulatory liability) highly desirable.

Chemistry

Because the new biguanides **5** of this report are prodrugs of the triazines **8**, in most cases the corresponding triazines **8** were also prepared in order to follow the in vitro as well as in vivo SAR. Scheme 1 depicts the general synthetic pathways used to prepare both **5** and **8**. The target biguanides **5** were prepared starting with a suitably substituted phenol or thiophenol **1**, which was converted to a bromide **2**. Bromides **2** could then be used to prepare intermediate aminooxy compounds **3** or, utilizing the method of Mamalis,[26] converted directly to desired triazine products **8** by alkylation of an *N*-hydroxytriazine. The aminooxy intermediates **3** were converted to the desired biguanides **5** by reaction with suitably substituted dicyandiamides **4**. Table 1 tabulates the reaction conditions used to convert intermediates **3** to the target biguanides **5** and provides

* To whom correspondence should be addressed. Phone: 609-921-7447. Fax: 609-799-1176. E-mail: romich@aol.com.
† Jacobus Pharmaceutical.
‡ University of Miami School of Medicine.
§ Pharmaceutical Systems, Inc.
|| Australian Army Malaria Institute.

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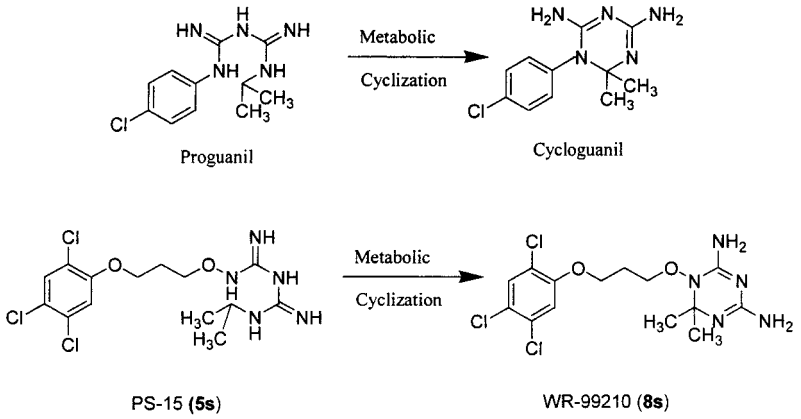
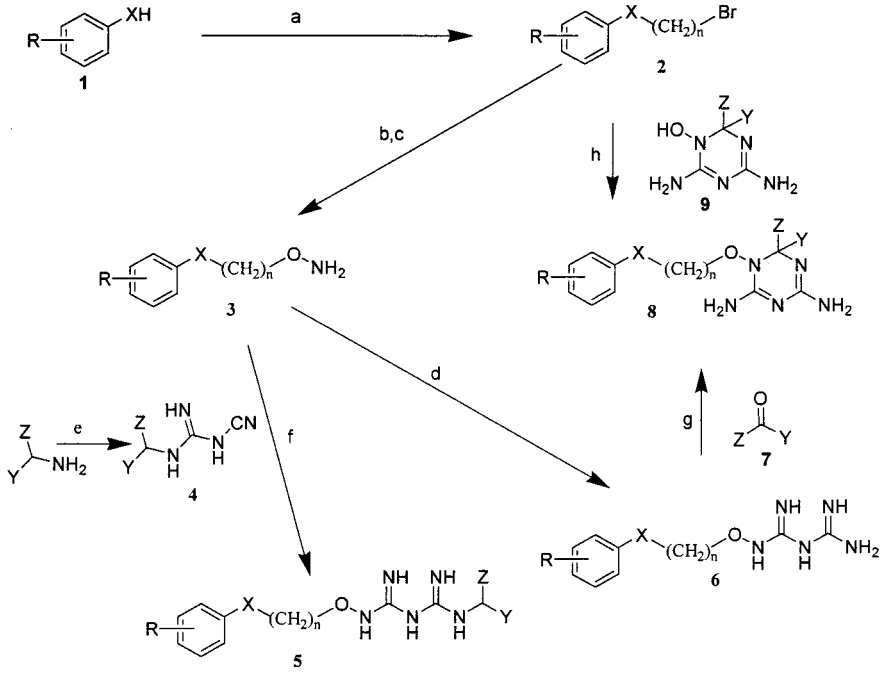
Chart 1



Scheme 1[a]



[a] Reagents: (a) 1,3-dibromopropane (for $n = 3$), NaOH, tetrabutylammonium hydrogen sulfate; (b) AcNHOH, NaOH, or KOH; alcoholic solvent; (c) concentrated HCl, MeOH; (d) dicyandiamide, aqueous EtOH, heat, and then aqueous NaOH to neutralize; (e) sodium dicyanamide, HCl, alcoholic solvent, heat; (f) EtOAc, heat; (g) HCl, MeOH; (h) room temp, DMF.

a structure key to both **5** and their metabolite triazines **8**. The aminooxy intermediates **3** were also used in an alternative path to the triazines **8**. In this pathway compounds **3** were reacted with dicyandiamide to give the monosubstituted biguanides, which then were converted to the final triazines **8** by reaction with ketones **7**.

In Vitro Activity

Table 2 tabulates data on the in vitro activity of the putative triazine metabolites **8**. While the biguanide prodrugs **5** are the target drugs of work described in this paper, these corresponding triazine metabolites **8** were prepared in the majority of cases in order to determine the potential antimalarial activity of the analogues free of ADME (absorption, distribution, metabolism, excretion) effects. It has already been reported[8] that WR-99210 (**8s**) is some 8000 times more potent in vitro than its prodrug PS-15 (**5s**). It was therefore necessary to make and test the putative triazine metabolites in vitro in order to determine what structural features of the metabolites are necessary for activity. When a general SAR of these features was in hand, further SAR work could be done to determine what features are necessary for metabolic cyclization to the active triazines (**8**) and to achieve the other ADME properties necessary for the therapeutic blood levels necessary for in vivo activity.

As described in the Experimental Section, two different assays were utilized in determining the in vitro antimalarial activity. Both are whole-cell systems utilizing multidrug-resistant parasites. The two strains, W-2 and K1, are resistant to both chloroquine and DHFR inhibitors such as pyrimethamine. Several analogues had subnanomolar activity against both strains, which indicates that the outstanding activity against resistant strains reported in the literature for WR-99210 (**8s**) is shared by a wide variety of analogue metabolites in this

Table 1. Structure Key to Analogues **5** and **8** and Reaction Conditions for Formation of, Melting Points of, and Salts of Biguanides **5**

					final reaction converting **3** to **5**				
compd	R	X	*n*	Y, Z	reaction solvent	reaction temp (°C)/time (h)	recryst solvent	mp (°C)	salt
5a	H	O	3	methyl	2-ME[a]	80/5	MeCN/EtOAc	146−151	2HCl
5b	4-Cl	O	3	methyl	DME	70/15	MeCN	160−162	2HCl
5c	4-F	O	3	methyl	MeCN	70/15	MeCN	145−150	2HCl
5d	4-Br	O	3	methyl	DME	70/15	not recryst	162−167	2HCl
5e	4-NO_2	O	3	methyl	DME	50/4	EtOH/EtOAc	162−165	1.5HCl
5f	4-CH_3	O	3	methyl	DME	70/15	MeCN	155−161	2HCl
5g	4-CH_2CH_3	O	3	methyl	DME	70/15	MeCN	145−150	2HCl
5h	4-*tert*-butyl	O	3	methyl	EtOAc	50/24	MeCN	151−153	2HCl
5i	4-OCH_3	O	3	methyl	DME	reflux/6	MeCN	139−140	2HCl
5j	3-CH_3	O	3	methyl	DME	reflux/6	MeCN	159−161	2HCl
5k	3-CF_3	O	3	methyl	DME	reflux/6	not recryst	135−137	2HCl
5l	2,4-Cl	O	3	methyl	EtOAc	50/5	water	65−70	HCl·H_20
5m	3,4-Cl	O	3	methyl	EtOAc	50/5	ETOH/AcOEt	160−162	2HCl
5n	2,4-F	O	3	methyl	DME	reflux/6	MeCN	135−147	2HCl
5o	3,4-F	O	3	methyl	DME	reflux/6	not recryst	140−142	2HCl
5p	2,4-CH_3	O	3	methyl	DME	50/15	MeCN/EtOAc	143−147	2HCl
5q	3,4-CH_3	O	3	methyl	DME	reflux/2.5	MeCN	144−145	2HCl
5r	3-CH_3,4-$CH(CH_3)_2$	O	3	methyl	DME	reflux/6	MeCN	137−139	2HCl
5s	2,4,5-Cl	O	3	methyl	DME	reflux/3.5	water	102−103	HCl H_20
5t	2,4,5-F	O	3	methyl	DME	reflux/5	MeCN	145−150	2HCl
5u	2,3,5-F	O	3	methyl	DME	reflux 6	not recryst	146−145	2HCl
5v	2,3,5-CH_3	O	3	methyl	DME	70/15	MeCN	151−159	2HCl
5w	3,4,5-OCH_3	O	3	methyl	DME	reflux/6	EtOH/EtOAc	127−129	2HCl·H_2O
5x	4-Cl	S	3	methyl	DME	reflux/5	not recryst	144−150	2HCl
5y	4-F	S	3	methyl	DME	reflux/6	EtOH/EtOAc	144−146	1.5HCl
5z	4-CH_3	S	3	methyl	DME	reflux/3	MeCN/EtOAc	131−136	2HCl
5aa	2,5-Cl	S	3	methyl	DME	reflux/4	not recryst	135−140	2HCl
5bb	2,4,5-Cl	O	2	methyl	DME	reflux/5	not recryst	155−161	2HCl
5cc	3-CF_3	none	1	methyl	DME	reflux/5	MeCN	154−155	2HCl·0.5H_2O
5dd	3,4-Cl	none	1	methyl	DME	reflux/5	not recryst	155−157	1.5HCl
5ee	2,4,5-Cl	O	3	*n*-nonyl,H	DME	reflux/5	not recryst	141−143	2HCl
5ff	2,4,5-Cl R attached directly to X	O	3	$(CH_2)_5$	EtOAc	75/3	MeCN	139−140	HCl
5gg	1-naphthyl	O	3	methyl	DME	50/2	no reaction	162−165	2HCl
5hh	2-naphthyl	O	3	methyl	DME: 2-ME1:1	reflux/5	MeCN	149−151	2HCl·0.5H_2O

[a] 2-ME = 2-methoxyethanol.

Table 2. In Vitro Antimalarial Activity, Recrystallization Solvents, Melting Points, and Salts of Triazines **8**

compd	recryst solvent	mp (°C)	salt	W-2[a] stain	K-1[b] stain
8b	acetone	215−217	HCl		0.63 ± 0 (n = 3)
8c	water	197−198	HCl		0.63 ± 0 (n = 3)
8d	60% AcOH	220−221	HCl		0.40 ± 0.14 (n = 3)
8e	acetone	204−206	HCl	0.0299	0.32 ± 0 (n = 3)
8i	acetone	180−182	1.75HCl[c]	0.0342	0.32 ± 0 (n = 3)
8j	water	190−191	HCl·H_2O	0.0416	0.37 ± 0.24 (n = 3)
8k	water	182−183	1.5HCl[c]	0.0299	2.50 ± 0 (n = 3)
8l	acetone	219.5−221	HCl	0.0379	1.67 ± 0.72 (n = 3)
8n	water	180−182	1.5HCl[c]	0.0285	0.42 ± 0.18 (n = 3)
8o	acetone	196−198	2HCl	0.0332	0.38 ± 0.14 (n = 4)
8r	EtOH/EtOAc/ether	185−187	HCl	0.0719	11.7 ± 7.6 (n = 3)
8s	acetone	195−197.5	HCl	0.0470	1.43 ± 0.75 (n = 14)
8t	water	184−185	1.5HCl[c]	0.0320	0.94 ± 0.34 (n = 6)
8u	acetone	180−182	2HCl	0.0301	0.63 ± 0 (n = 3)
8w	ether	175−177	HCl·0.5H_2O	0.211	1.25 ± 0 (n = 3)
8x	water	195−197	HCl	0.0288	2.08 ± 0 (n = 3)
8y	water	202−203	HCl	0.0222	1.25 ± 0 (n = 4)
8aa	water	205−206	HCl·H_2O	0.0254	2.08 ± 0.72 (n = 3)
8bb	EtOH	244−245	HCl	0.278	40.0 ± 0 (n = 3)
8cc	acetone	206−208	2HCl	0.184	40.0± 0 (n = 3)
8dd[d]	80% EtOH	229−230	HCl	0.0719	10.0 ± 0 (n = 3)
8ee	90% EtOH	182−184	HCl·H_2O	>0.5	>640 (n = 3)
8ff	90% EtOH	231−232.5	HCl	0.113	66.7 ± 23.1 (n = 3)
8hh	acetone	199−200	HCl	0.0333	1.56 ± 0.63 (n = 4)
cycloguanil				>0.5	152 ± 24 (n = 3)
chloroquine				70	
pyrimethamine					1745 ± 482 (n = 4)

[a] IC_{50} in ng/mL reported. See Experimental Section for assay details. [b] IC_{90} reported in ng/mL. See Experimental Section for assay details. [c] Based on halide analysis. [d] This triazine is known as clociguanil or BRL-50216. See the following: Knight, D. J.; Peters, W. The Antimalarial Activity of *N*-Benzyloxydihydrotriazines. *Ann. Trop. Med. Parasit.* **1986**, *74*, 393−404.

series. On the phenyl ring, both very electron-withdrawing substituents such as nitro (**8e**) or electron-donating substituents such as methoxy (**8i** and **8w**) are compatible with high activity. The bulk introduced by replacing the phenyl group with a naphthyl group (**8gg** and **8hh**) is also very well tolerated. Furthermore, the linker oxygen next to phenyl may be changed to sulfur (**8x**, **8y**, and **8aa**) without appreciable loss of activity. When the comparative in vivo data reported in the literature[27] for the WR-99210 related triazines were corroborated,

Table 3. In Vivo Antimalarial Activity of Biguanides **5** in the *P. berghei* Antimalarial Mouse Model

compd	mg/kg ED_{90} 6-day parasitemia[a]	mg/kg ED + 100% survival[b]	mg/kg complete survival dose[c]
5a	124	165	540
5b	<16	26	79
5c	<16	30	90
5d	<32	64	135
5e	*d*	*d*	*d*
5f	113	265	445
5g	96	158	505
5h	>128	460	1480
5i	>128	>128[e]	
5j	>128	>128[e]	
5k	27	73	140
5l	17	28	64
5m	<16	16	33
5n	>128	>128[e]	
5o	<16	27	77
5p	120	170	235
5q	>128	>128[e]	
5r	<128	128	
5s	<16	30	76
5t	18	71	128
5u	74	107	180
5v	<128	>128[e]	
5w	*d*	*d*	*d*
5x	73	112	182
5y	70	109	183
5z	92	117	265
5aa	<128	>128[e]	
5bb	*d*	*d*	*d*
5cc	*d*	*d*	*d*
5dd	< 8	22	32
5ee	*d*	*d*	*d*
5ff	> 256	275	470
R attached directly to X			
5gg	> 256	185	495
5hh	<128	<128[e]	
chloroquine	3	4	256
pyrimethamine	1.2	6	400

[a] On day 6, a sample of blood was examined to determine the percentage of cells infected. ED_{90} is the dose necessary to reduce the percentage of infected cells to 10% of the level found in the infected untreated controls. [b] An ED + 100% dose is that dose needed to increase the survival of the treated animals to twice the number of days. These values are calculated using linear regression analysis and Microsoft Excel software. [c] The experiment is run for 31 days. Therefore, a complete survival dose means that all animals survived for the 31 days. [d] Not tested. These analogues were prepared but not tested in vivo because the triazine metabolites were not very active or because they were deemed likely to have undesirable metabolic characteristics. [e] These analogues were only tested at 128 mg/kg and had increased survival time at that level, but not as much as 100%.

it was found that shortening the linker between the phenyl ring and the biguanide group (**8bb**, **8cc**, and **8dd**) degrades activity.

In Vivo Activity

The in vivo activity of the biguanides **5** (Table 3) did not completely mirror the in vitro activity of the corresponding triazine metabolites **8**. Some analogues with substituents on the phenyl ring, such as **5i**, **5j**, and **5r**, whose corresponding triazines had excellent in vitro activity, showed only marginal in vivo activity. Even the unsubstituted phenyl analogue **5a** was inactive. While comparative metabolic data on many of the analogues are needed to establish the relative importance of several ADME factors, it is suggested that the primary reason for the lack of in vivo activity of these analogues is that the degradative metabolism of the prodrug biguanides and their metabolite triazines is occurring just as quickly or more quickly than the desired metabolic cyclization to the triazines depicted in Chart 1. The lack of in vivo activity of unsubstituted analogue **5a** suggests that even P450 oxidative attack on a phenyl ring is occurring as quickly as the desired oxidative cyclization. The lack of in vivo activity for the substituted naphthyl isomers **5gg** and **5hh** suggests the same thing for oxidative metabolic attack on a naphthalene ring. When these considerations are used as a working hypothesis, later analogue synthesis concentrated on analogues where the phenyl is substituted with electron-withdrawing groups such as halide to deactivate the phenyl ring toward metabolic oxidation and without easily metabolized groups such as methyls, methoxys, or nitros. Several analogues that fit this description, such as **5b**−**d, 5l**, **5m**, **5o**, and **5dd**, all have in vivo activity equal to that of PS-15 (**5s**). The 3,4-dichloro analogue **5m** was consistently found to be twice as active as PS-15. Several more analogues of this type, such as **5k**, **5t**, and **5u**, have only slightly less activity than PS-15. The analogues **5x**, **5y**, and **5aa** also fit this profile with regard to the phenyl group substituents, and they also have significant activity. However, they also have a sulfur as a linker group and are not as active as corresponding analogues (**5b**, **5c**) with the oxygen linker.

Toxicology

In work already reported[28] on the toxicology of this series in mice, it was found that with survival as an end point PS-15(**5s**) and PS-22 (**5c**) were slightly less toxic than Proguanil in a 90-day test in which the drugs were administered in the feed. However, it was also found in this work that PS-22 caused testicular damage at levels of 400 and 600 ppm in the feed. Preliminary experiments have indicated that other analogues in the series with equivalent efficacy are free of this effect. Experiments to confirm this are now underway.

Conclusions and Summary

By use of the working hypothesis that analogues of PS-15 should not have easily metabolized substituents on the phenyl group and that electron-withdrawing groups on the phenyl are necessary to deactivate the phenyl itself to metabolic degradation, analogues can be prepared that can maintain or exceed the in vivo antimalarial activity of PS-15. In addition, the analogues reported here do not require the use of the heavily regulated starting material 2,4,5-trichlorophenol and they also maintain activity against malarial strains resistant to widely used antimalarials. Several of these new compounds such as **5b** (PS-33) and **5m** (PS-26) are presently undergoing further toxicity studies as part of a commercial development program.

Experimental Section

Chemistry. Melting points were determined on a Laboratory Devices' Mel-Temp II heating block type melting point apparatus and are uncorrected. Infrared spectra were determined on a Mattson Instrument Galaxy series 5000 FTIR spectrometer. NMR spectra were determined on a Varian 60T 60 MHz spectrometer using TMS as an internal standard. TLC was performed on Baker $Si250F_{254}$ silica gel plates using UV/

fluorescence visualization. Visualization was, where noted, also aided by the use of a diazonium salt spray prepared by dissolving 1.0 g of 4-nitroaniline in 200 mL of 2 N HCl. An aliquot of this solution was then titrated to colorlessness with 2 N sodium nitrite just prior to use as a spray. After the spraying with the diazonium spray, the plate was sprayed with a 5% sodium carbonate solution to develop the TLC spots. A TLC eluent found to be useful for the biguanides and triazines was a 12:6:3:2 mixture of $CHCl_3$/acetone/*n*-butanol/formic acid. Aldrich Chemical Co. 200−400 or 70−230 mesh 60A^0 silica gel was used for column chromatography. Elemental analyses were done by Robertson Microlit of Madison, NJ.

3-(4-Fluorophenoxy)propyl Bromide (2c). A mixture of 4-fluorophenol (500 g, 4.46 mol), 1,3-dibromopropane (1250 mL, 12.32 mol), 24% sodium hydroxide (750 mL, 5.7 mol), and 1 g of tetrabutylammmonium hydrogen sulfate was stirred and heated at 80−85 °C for 15 h. The pH was adjusted to 14 by the addition of more sodium hydroxide, and heating was continued for 16 h more, at which time a TLC of the reaction mixture (eluent of hexane/ethyl acetate 5:1 with visualization by spraying with *p*-nitrophenyldiazonium chloride spray) indicated that the starting phenol was almost entirely consumed. After the mixture was cooled, the lower phase was separated and excess dibromopropane was remove on a rotary evaporator. The residue was distilled through a short Vigreux column at 1 Torr. A total of 873 g (84%) of product was collected at 95−99 °C. ^{1}H NMR ($CDCl_3$): δ 2.23 (m, 2H), 3.50 (t, 2H), 4.02 (t, 2H), 6.7−7.2 (m, 4H).

Potassium Acetohydroxamate Hydrate. A solution of 350 g (5.0 mol) of hydroxylamine hydrochloride in 2.8 L of methanol was stirred and cooled to 10 °C, and a solution of 660 g (10 mol of 85%) of KOH in 1.2 L of methanol was added dropwise while maintaining the reaction mixture temperature at 10−15 °C. After half of the KOH was added, stirring was continued and 450 g (5.1 mol) of EtOAc was added followed by the remainder of the KOH. The KCl was removed by filtration, and the filtrate was concentrated at 1 Torr and 30 °C for 2 days to give 680 g of product. A 580 g portion of this material was dissolved in 0.96 kg of 2-methoxyethanol. The resultant solution was assayed by the $FeCl_3$/colorimetric method[29] and determined to contain 4.26 mol of product.

3-(4-Fluorophenoxy)propyl Acetohydroxamate. To a solution of 580 g (4.26 mol) of potassium acetohydroxamate hydrate in 0.96 kg of 2-methoxyethanol was added 873 g (3.74 mol) of 3-(4-fluororophenoxy)propyl bromide, and the reaction mixture was heated for 15 h at 60 °C. After the mixture was cooled to room temperature, the KBr was removed by filtration and washed with 100 mL of 2-methoxyethanol. The filtrate and washings were concentrated on a rotavap, diluted with 2 L of EtOAc, and washed with 300 mL of water before being concentrated at high vacuum to yield 845 g of crude product, mp 56−59 °C, which was used without further purification. A 1.2 g sample was purified for analytical purposes by chromatography on silica gel using 2:1 EtOAc/hexane to yield 0.9 g of pure material, mp 67.5−68.5 °C. IR (KBr): 3114 cm^{-1} (NH), 1668 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$): δ 1.93 (s, 3H), 2.08 (m, 2H), 4.05 (t, 2H), 4.08 (t, 2H), 6.85−7.10 (m, 4H), ∼9 (br, 1H).

3-(4-Fluorophenoxy)propyloxyamine Hydrochloride (3c). To a solution of 844 g (3.71 mol) of 3-(4-fluorophenoxy)-propyl acetohydroxamate in 2.0 L of methanol was added 330 mL of concentrated HCl. After 15 h, the starting material was consumed, as determined by TLC utilizing the diazonium spray. The mixture was filtered to remove 6.5 g of solid, and the filtrate was concentrated at oil-pump vacuum to give 810 g of crude product, mp 80−85 °C, which was recrystallized from 2.0 L of EtOAc to give material pure enough for further reactions, mp 128−130 °C. Analytically pure material was obtained by dissolving a small sample in 1:1 2-propanol/EtOAc and filtering and cooling the filtrate to precipitate the product, mp 130−132 °C. ^{1}H NMR (DMSO-d_6): δ 2.10 (m, 2H), 4.05 (t, 2H), 4.20 (t, 2H), 6.8−7.3 (M, 4H), ∼10 (br, 3H).

3,4-Dichlorobenzyloxyamine Hydrochloride (3dd). To a solution of 8.55 g (0.114 mol) of acetohydroxamic acid and 12.6 g (0.113 mol) of 36% NaOH in 50 mL of DMSO was added 19.5 g (0.10 mol) of 3,4-dichlorobenzyl chloride. The mixture was heated 15 h at 60 °C, and the solvents were removed in vacuo. The residue was extracted 4 × 50 mL with acetone, and the extracts were filtered before being concentrated in vacuo. The residue was then dissolved in 100 mL of MeOH, and 10 mL of concentrated HCl was added. After 24 h, the solvent was removed in vacuo and the residue was triturated with 100 mL of EtOAc. The precipitate was collected on a filter, resuspended in 100 mL of hot THF, and re-collected on a filter to yield 19.55 g (85%) of analytically pure product, 195 °C (dec). ^{1}H NMR (DMSO-d_6): δ 5.20 (s, 2H), 7.4−7.9 (m, 3H), 10.7 (br s, 3H).

1-[3-(4-Fluorophenoxy)propyloxy]-5-isopropylbiguanide (6c). A mixture of 617 g (2.78 mol) of 3-(4-fluorophenoxy)propyloxyamine hydrochloride, 360 g (2.85 mol) of isopropyldicyandiamide,[30] and 2.0 kg of EtOAc was stirred and heated at 70 °C for 15 h. After the mixture was cooled, an additional 1 L of EtOAc was added and a small amount of 1,5-bis(isopropyl)biguanide, which was present in the isopropyldicyandiamide starting material, was removed by filtration. The filtrate was shaken well with 735 g (3.3 mol) of 18% NaOH. The organic layer was separated and stirred with 100 g of anhydrous K_2CO_3 and decanted and dried with another 100 g of anhydrous K_2CO_3 before being concentrated to about a 2 L volume on a rotavap. This residue was cooled in an ice bath, and the resultant precipitate was collected and washed 2 × 500 mL with ice-cold EtOAc to give 693 g of material, mp 111−113 °C. Further purification was achieved by dissolving in 1.0 L of hot ethanol, filtering through an 0.45 μm filter to remove some solid particles, and cooling in an ice bath. Collection of the resultant precipitate gave 659 g of colorless crystals, mp 111.5−114.0 °C. IR: 3484, 3360, 3299, 3108, 1681, 1606, 1566 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.03 (d, 6H), 2.0 (m, 2H), 3.75−4.10 (m, 5H) 4.8 (br s, H), 5.9 (br s, 1H), 6.5 (br s, 2H), 6.8−7.2 (m, 4H).

4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-[3′-(4-fluorophenoxy)propoxy]-1,3,5-triazine Hydrochloride (8c). A mixture of 53 g (0.24 mol) of 3-(4-fluorophenoxy)propyloxyamine hydrochloride, 29 g (0.34 mol) of dicyandiamide, 600 mL of ethanol, and 25 mL of water was stirred and refluxed for 7 h. The solvent was removed on a rotary evaporator, and 20 mL of 24% NaOH and 200 mL of water were added to the residue. The mixture was cooled in an ice bath. The resultant precipitate was collected on a filter and washed with 50 mL of water before extraction with 300, 50, and 50 mL portions of EtOAc. The EtOAc extracts were dried with K_2CO_3, filtered, and concentrated in vacuo to give about 48 g of crude intermediate as an oil that crystallized on standing but was used immediately as an oil. It was dissolved in a mixture of 250 mL of methanol, 400 mL of acetone, and 30 mL of concentrated HCl. After 3 days at room temperature, the mixture was concentrated in vacuo at oil-pump vacuum to an oil, which was dissolved in 200 mL of acetone and cooled in a refrigerator to yield 32 g of dihydrochloride. A 15 g portion of the dihydrochloride was dissolved in 50 mL of hot water and cooled in a refrigerator to yield 12.2 g of product, mp 197−198 °C. ^{1}H NMR (DMSO-d_6): δ 1.40 (s, 6H), 2.1 (m, 2H), 4.0 (m, 4H), 6.9−7.5 (m, 4H), 7.5 (br s, 2H), 8.1 (br s, 1H), 8.75 (br s, 1H), 9.6 (s, 1H).

4,6-Diamino-1,2-dihydro-2,2-(pentylene-1,5)-1-[3′-(2,4,5-trichlorophenoxy)propyloxy]-1,3,5-triazine Hydrochloride (8ff). To a solution of 1.00 g of 3-(2,4,5-trichlorophenoxy)-propyloxybiguanide[17,26] (2.82 mmol) and 1.2 g (12.2 mmol) of cyclohexanone in 10 mL of MeOH was added 0.7 g of concentrated HCl. After the mixture was left standing for 4 days, the resultant precipitate was collected on a filter and washed with 2 mL of MeOH to give 1.2 g of product, mp 229−230 °C. Recrystallization from 25 parts of 90% EtOH gave analytically pure material, mp 231−232.5 °C. ^{1}H NMR (DMSO-d_6): δ 1.2−1.9 (m, 6H), 2.2 (m, 2H), 3.15 (t, 4H), 4.1 (m, 4H), 7.4 (s, 1H), 7.6 (br s, 2H), 7.7 (s, 1H), 8.0 (br s, 1H), 8.4 (br s, 1H), 9.4 (br s, 1H).

4,6-Diamino-1,2-dihydro-2-nonyl-1-[3′-(2,4,5-trichlorophenoxy)propyloxy]-1,3,5-triazine Hydrochloride (8ee). A mixture of 3.54 g (10 mmol) of 3-(2,4,5-trichlorophenoxy)-propyloxybiguanide[17,26] and 1.0 mL of concentrated HCl in 10 mL of EtOH was concentrated to dryness in vacuo and triturated with 5 mL of ethyl ether and 5 mL of EtOH. The resultant precipitate was collected on a filter to give 1.7 g (4.3 mmol) of hydrochloride, mp 105−108 °C, which was stirred for 5 days with 1.0 g (6.4 mM) of decylaldehyde and 15 mL of DME. The resultant 1.2 g of precipitate (mp 170−173 °C) was collected on a filter and recrystallized from 8 mL of 90% EtOH to give 0.8 g of analytically pure product, mp 182−184 °C. ^{1}H NMR (DMSO-d_6): δ 0.8 (t, 3H), 1.25 (m, 16H), 2.15 (m, 2H), 4.1 (t, 2H), 4.9 (m, 1H), 7.4 (s, 1H), 7.5 (br s, 2H), 7.7 (s, 1H), 8.0 (br s, 1H), 9.0 (br s, 1H), 9.8 (br s, 1H).

4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-[3′-(1-naphthyloxy)propyloxy]-1,3,5-triazine Hydrobromide (8gg). A 0.93 g (5.0 mmol) portion of 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-hydroxy-1,3,5-triazine[26] was suspended in 10 mL of DMF and stirred with 1.33 g (5.0 mmol) of 3-(1-naphthyloxy)propyl bromide (**2gg**), which was prepared in the same manner as **2c** and used crude. After 16 h of stirring, 0.10 g (0.35 mmol) of **2gg** was added, and the mixture was stirred for an additional 16 h. The solvent was removed on a rotavap, and the residue was dissolved in 20 mL of hot water. When the mixture was cooled, an oil separated, so the aqueous mixture was rewarmed to give a solution that was washed with 5 mL of EtOAc. When the mixture was cooled, crystals separated that were collected and recrystallized from acetonitrile to give 1.04 g (49%) of product as the hydrobromide salt, mp 200−201 °C. ^{1}H NMR (DMSO-d_6 + D_2O): δ 1.40 (s, 6H), 2.30 (br m, 2), 4.30 (br m, 4), 7.10 (m,1), 7.6 (br m, 4), 7.95 (m, 1), 8.10 (m, 1).

Pharmacology. In vivo[31] antimalarial activity was tested in male or female Charles River CD-1 mice that were 4−5 weeks old and weighed 20−25 g. They were housed in groups of three or four in standard plastic cages with wire tops, Bed-o-cob bedding, and 12 h/day of light and maintained at 75 °F. They were fed a standard Ralston Purina mouse chow, and the cages and water bottles were changed twice a week. Test compounds were ground in a mortar and pestle and diluted with enough vehicle to give a volume of 10 mL/kg of mouse weight. The oral doses were prepared in 0.5% hydroxyethylcellulose/0.1% Tween-80. The amount of drug was calculated on the free base weight. The mice were infected intraperitoneally on day 0 with 5×10^4 erythrocytes parasitized with *Plasmodium berghei* (KBG-173 strain) from a donor mouse having a parasitemia between 5% and 10%. On days 3, 4, and 5 the test compounds were administered bid, spaced 6 h apart, to the mice. Smears were made from tail blood on day +6 and twice a week thereafter. The smears were stained with Geimsa and examined microscopically. Parasitemias are reported as the percentage of the red blood cells that are infected. On day 6 the (suppression of) parasitemias of treated animals may be compared to the parasitemias of infected nontreated controls, but these infected nontreated controls die on days 7−12. Activity was also measured by survival. Full activity is defined as all animals living on day 31. Partial activity is defined as days of increased survival vs the infected nontreated controls.

In Vitro Activity Against K-1 Strain *P. falciparum*. Malaria parasites were maintained in continuous culture[32] using 10% O-positive human serum, erythrocytes, and RPMI-1640 LPLF medium containing 0.01 mg of folic acid per liter and 0.0005 mg/L *p*-aminobenzoic acid (GIBCO, Grand Island, NY) and supplemented with 2 g/L glucose, 50 mg/L hypoxanthine, 5.97 g/L *N*-(2-hydroxyethylpiperazine)-*N*′-2-ethanesulfonic acid, and 2.1 g/L sodium carbonate. Parasites were synchronized repeatedly with 5% sorbitol to produce experimental cultures consisting of parasites 6−12 h into the cycle.[33] Cultures were grown in flat-bottomed wells of a 96-well microculture plate. The drugs were dissolved in methanol or in DMSO and subsequently diluted with plain culture medium to obtain the desired concentration. A total of 20 μL of final drug solution was added to a well of a 96-well plate, and 2-fold serial dilutions were made with plain culture medium to cover the desired range. The parasite-infected erythrocyte suspension (80 μL) was then added to each well. The final microculture had hematocrit of 2% and 0.5% concentration of parasitized erythrocytes (>95% rings) in a culture medium containing 10% human serum. The plates were shaken gently and placed in a humidified, airtight modular incubation chamber in an atmosphere of 5% O_2, 5% CO_2, and 90% N_2. The parasites were incubated at 37.5 °C for 24−30 h to allow ring forms in the control wells to mature to schizonts. After incubation, 80 μL of supernatant was removed from the culture wells, with minimal disturbance to the red cell layer at the bottom of the wells, and 100 μL of phosphate-buffered saline was added to each well. The microculture plates were then left to stand for 1 h before removing 100 μL of supernatant from each well. After resuspension of erythrocytes in the remaining fluid, a thick blood film was made from each well, stained with Romanowsky stain, and examined microscopically. In the control wells, ring forms developed into normal schizonts with well-defined chromatin dots. In the presence of effective drug concentrations, ring forms did not mature into normal schizonts but appeared as parasites consisting of fragmented chromatin dots of unequal size scattered over a large area.[34] The minimum inhibitory concentration (MIC) for each drug was determined by assessing the lowest concentration of drug that prevented more than 90% of parasites from maturing into normal schizonts.

In Vitro Activity Against W-2 Strain *P. falciparum*. In vitro tests of activity were preformed against the Indochina W-2 clone (resistant to chloroquine, quinine, sulfadoxine, and pyrimethamine) strain of *P. falciparum* using modifications[35] of the semiautomated microdilution technique of Desjardins and others.[36] Antimalarial activity in this system was assessed by inhibition of radiolabeled hypoxanthine incorporation into parasites by graded concentrations of drugs during incubation for 66 h in a culture medium containing physiological concentrations of folic acid. All drugs studied, as well as control drugs, were tested in duplicate. In this test system the results are expressed by estimating the concentrations of drugs that produced 50% inhibitions of radiolabeled incorporation (IC_{50}).

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