Antimalarial Activities of Some 3,5-Diamino-*as*-triazine Derivatives

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In 1952 one of us (P. B. R.), together with Hitchings and others,[1] demonstrated, as part of a study relating antimalarial activity to chemical constituion, that 3,5-diamino-6-(3,4-dichlorophenyl)-*as*-triazine (**5**) was potent against *Plasmodium berghei* infections in mice, having about 230 times the activity of chlorguanide. In *P. gallinaceum* infections in chicks, however, it was less active than this standard compound. Compounds of this general type were also shown to be competitive antagonists of folic and folinic acids in the growth of *Lactobacillus casei* and of folinic acid in the growth of *Leuconostoc citrovorum*; in this, the 3,5-diamino-*as*-triazines resemble the pyrimethamine type of pyrimidine antimalarial. With the emergence of strains of *P. falciparum* resistant to antimalarial drugs, which had hitherto been used successfully in the treatment of malignant tertian malaria, attention was redirected to these unexploited *as*-triazines, their preparation being much aided by the synthesis of Settepani and Borkovec[2] which had been published in the interim.

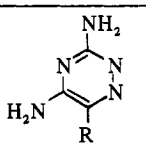
Using this synthetic pathway, a number of chlorinated, flourinated, methoxylated, and trifluoromethylated 6-aryl-*as*-triazines (Table I, **1–17**) were prepared.

The reaction of the arylglyoxylonitriles with aminoguanidine was carried out in strongly acidic solution, and the intermediate amidinohydrazone salts were isolated and identified spectroscopically; their free bases were examined for purity by thin-layer chromatography. They were used in the next step without further purification, no difficulties being encountered in the ring closure to the corresponding *as*-triazines. Only one report of a 3,5-diamino-6-benzyl-*as*-triazine was found in the literature[3] and, accordingly, an attempt was made to apply the synthesis to this type of compound. However, no reaction appeared to take place between aminoguanidine and phenylpyruvonitrile or 3,4,5-trimethoxyphenylpyruvonitrile; only the starting pyruvonitriles and the corresponding phenylacetic acids were recovered. This failure to react was thought to be a result of enolization, and to prevent this effect, the methylene group in phenylpyruvonitrile and 3,4-dichlorophenylpyruvonitrile was methylated. The resulting compounds reacted smoothly with aminoguanidine, and the resulting *as*-triazines (**13** and **14**) were obtained by treatment of the intermediates with base. 3,4-Dichlorophenylethylglyoxylonitrile reacted normally with aminoguanidine, but the amidinohydrazone of (α,α,α-trifluoro-*o*-tolyl)glyoxylonitrile could not be formed under the usual conditions.

Antimalarial Activity. All compounds were tested for antimalarial activity against *P. berghei* (Strain KBG 13) in mice and some were also tested against *P. gallinaceum* in chicks by methods previously described.[4] The results of the *P. berghei* tests are given in Table II. Compounds **1**, **2**, **3**, **6**, **9**, **16**, and **17** were tested against *P. gallinaceum* at between 100 and 160 mg/kg. Only compounds **2** and **3** showed any indication of activity in that test. (At 120 mg/kg, 2.3 days and 3.3 days increase in mean survival time was observed, respectively.)

In tests against *P. berghei*, it is apparent that compounds bearing a halogen (Cl, F) or halogen-like substituent (CF_3) in the 4 position of the aromatic group attached to the 6 position of the *as*-triazine (**3**, **7**, **9**) were always more active than compounds with the same substituent in the 2 or 3 positions (**1**, **2**, **6**, **8**). When a 4-chloro group was present, introduction of a second chloro group at either the 2 or 3 position increased activity (**4** and **5**), the 3 substituent more than the 2. The 3,4-dichloro compound (**5**) was the most active of all the compounds examined, giving cures at 5 mg/kg and still showing a considerable increase in survival

Table I. 6-R-3,5-Diamino-*as*-triazines



Compd	R	Mp, °C	Recrystn[a] solvent	Yield, %	Formula	Analysis
1	Ph-2-Cl	189–191	A	27	$C_9H_8ClN_5$	C,[b] H, Cl, N
2	Ph-3-Cl	190–197	B, C	43	$C_9H_8ClN_5$	
3	Ph-4-Cl[c,d]	218–221	A, D	37	$C_9H_8ClN_5$	C, H, Cl, N
4	Ph-2-4-Cl_2[c]	220–222	A	8	$C_9H_7Cl_2N_5$	C,[e] H, Cl, N
5	Ph-3,4-Cl_2[c]	222–224	B, C	23	$C_9H_7Cl_2N_5$	
6	Ph-2-F	231–232	B, E	61	$C_9H_8FN_5$	C, H, N
7	Ph-4-F	257–260	B, E	35	$C_9H_8FN_5$	C, H, N
8	Ph-3-CF_3	199–200	B, E	34	$C_{10}H_8F_3N_5$	C, H, N
9	Ph-4-CF_3	232–233	B, E	36	$C_{10}H_8F_3N_5$	C, H, N
10	Ph-3,5-$(CH_3)_2$	232–245	E	8	$C_{11}H_7F_6N_5$	C, H, N
11	Ph-4-OCH_3[d]	215–217	A, B	50	$C_{10}H_{11}N_5O$	
12	Ph-3,4,5-$(OCH_3)_3$[d]	300–303	B, C	19	$C_{12}H_{15}N_5O_3$	C, H, N
13	$C(CH_3)_2$-Ph	272–274	A, B	17	$C_{12}H_{15}N_5$	C, H, N
14	$C(CH_3)_2$-Ph-3,4-Cl_2	251–253	A, B	7	$C_{12}H_{13}Cl_2N_5 \cdot 0.5H_2O$	C, H, Cl, N
15	$(CH_2)_2$-Ph-3,4-Cl_2	206–208	A, F	24	$C_{11}H_{11}Cl_2N_5$	C, H, Cl, N
16	Ph-4-OCF_3[f]	210–212	E	70	$C_{10}H_8F_3N_5O$	C, H, N
17	Ph-4-CF_3[g]	341–350	B, E[h]	60	$C_{10}H_7F_3N_4O$	C, H, N

[a] A = MeOH; B = H_2O; C = DMSO; D = $CHCl_3$; E = EtOH; F = EtOAc; G = Me_2CO. [b] Calcd, 48.8; found, 47.7. [c] British Patent 759,014[1b] reported **3**, **4**, and **5** with mp of 218–220, 220–222, and 219–220°, respectively. [d] Settepani and Bŏrkevec[2] reported **3**, **11**, and **12** with mp of 215–222, 219–219.5, and 295–297°, respectively. [e] Calcd C, 42.3; found, 41.8. [f] We thank Mr. Sie Yearl Chai for the preparation of this compound. [g] The triazine moiety is 3-amino-1,2,4-triazin-5-ol. [h] Basified with concd NH_4OH.

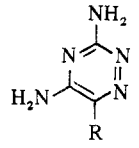
Table II. Activity of Compounds from Table I against *P. berghei* in Mice

Compd[a]	Increase in mean survival time at highest dose tested,[b] days (cured/treated)	Minimum dose giving cure, mg/kg (cured/treated)[c]	Minimum dose showing activity, mg/kg	Increase in mean survival time at minimum dose, days
1	6.7		160	3.5
2	4.9		320	3.5
3	9.3[d]		10	6.3
4	(5/5)	80 (3/5)	40	6.9
5	(3/5)[d]	5 (3/5)	0.63	14.0
6	8.4		160	4.0
7	(5/5)	80 (1/5)	20	4.1
8	5.8		640	5.8
9	(5/5)	10 (2/5)	2.5	3.1
10	2.9		640	2.9
11	3.4[e]		80	2.8
12	0.0[e]			
13	0.9			
14	1.7			
15	0.9			
16	(2/5)	640 (2/5)	160	8.9
17	1.5			
18[f]	(3/5)[g]	40 (8/25)	10	8.0
19[h]	Cures[e]	80 (2/15)	20	9.6

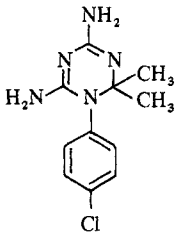
[a]Numbers refer to those in Table I. [b]Highest dose tested is 640 mg/kg, unless otherwise specified (no toxic deaths were encountered at that dose). [c]Cure is defined as a survival time of 60 days of the treated mouse over its control. [d,e]Highest dose tested with no toxic deaths occurring was *d*, 40 mg/kg, *e*, 320 mg/kg. [f]Pyrimethamine. [g]Highest dose tested with no toxic deaths occurring was 80 mg/kg. [h]Cycloguanil pamoate.

time at doses as low as 0.63 mg/kg.

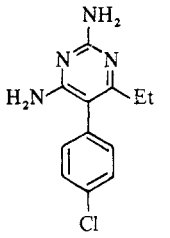
In the original description of compound **5**, Hitchings, *et al.*,[1] stated that it had 230 times the activity of chlorguanide against *P. berghei* in mice; this may be compared with a figure of 200 for pyrimethamine,[5] both determinations being conducted in the same manner. However, while pyrimethamine (**19**) was some 60 times as active as chlorguanide



as-triazine
R, see Table I



cycloguanil (**18**)



pyrimethamine (**19**)

against *P. gallinaceum* in chicks,[5] **5** was described as being less active than that standard in this test.[1]

As already mentioned, the *as*-triazines, if active at all against the avian parasite, show a low order of activity. The only bis(trifluoromethyl) compound available was the 3,5-disubstituted derivative (**10**). This compound was not particularly active.

When the aromatic group at position 6 was substituted in the 4 position by a methoxyl group (**11**), activity against *P. berghei* was reduced as compared to the corresponding 4-chloro compound; the 3,4,5-trimethoxy compound (**12**) was inactive. However, when the 4 substituent was trifluoromethoxy, a "pseudohalogen,"[6] as in **16**, some curative activity returned. Insertion of alkyl groups between the aromatic and *as*-triazine rings (**13**, **14**, **15**) resulted in complete† loss of antimalarial activity. Replacement of the 5-amino group in the triazine ring of **9** by a hydroxyl group eliminated antimalarial activity† (**17**).

As mentioned above, the 3,4-dichloro compound (**5**) was found to be the most active member of the series, however, it, together with the 4-chloro compound (**3**), was also the most toxic; the maximum tolerated dose (MTD) for both these compounds was 40 mg/kg. The 4-trifluoromethyl compound [3,5-diamino-6-(α,α,α-trifluoro-*p*-tolyl)-*as*-triazine (**9**)] combined excellent activity against *P. berghei* (about 0.5–0.25 as active as **5** or, say, 0.33 as active as pyrimethamine) with relatively low toxicity [the MTD was >640 mg/kg for **9**, 40 mg/kg for **5**, and about 80 mg/kg for pyrimethamine (**19**)]. This excellent profile prompted the testing of **9** against "triazine-resistant *P. berghei*" [*i.e.*, *P. berghei* KGB-173 strains made resistant to cycloguanil [4,6-diamino-1-(*p*-chlorophenyl)-1,2-dihydro-2,2-dimethyl-*as*-triazine (**18**)], the active metabolite of chlorguanide; in this experiment the strain was about 100-fold resistant to cycloguanil].[7] Doses of 46 and 114 mg/kg per day of **9** in the diet suppressed the resistant strain to the extent of 94.9 and 99.8%, respectively; at these levels the parent (sensitive) strain was totally suppressed. In comparison, the ED_{90} for the parent strain was found to be 4 mg/kg per day and that for the resistant strain 38 mg/kg per day. Pyrimethamine (**19**) has an ED_{70} of 1.1 mg/kg per day against the sensitive and 126 mg/kg per day against the resistant strain.‡

Infections caused by *P. cynomolgi* in the rhesus monkey were cured by doses of **9** of 3.1 and 10.0 mg/kg per day, the infection not recrudescing on splenectomy after 32–34 days. Pyrimethamine (**19**) was curative at 0.3 mg/kg per day.‡ The activity of **9** was also assessed against two strains of *P. falciparum* in the owl monkey (*Aotus trivirgatus*). Against the Malayan Camp-CH/Q strain, which is resistant to pyrimethamine (**19**) but susceptible to chlorquine, **9** was suppressive at about 25 mg/kg, though no accurate assessment could be made because of the toxicity of the compound in this species. Against the Vietnam Oak Knoll strain, which is resistant to chlorquine but susceptible to pyrimethamine (**19**), **9** was suppressive at 6.25 mg/kg. The owl monkey appeared to be rather sensitive to the toxic effects of **9**, the maximum tolerated dose in this species being less than 25 mg/kg per day; in contrast the rhesus monkey tolerated doses of up to 316 mg/kg per day.

Experimental Section

Melting points were taken on a Kofler block under microscopic magnification. Pmr spectra were measured with a Varian Associates A-60 spectrometer on 10–15% solution in DMSO-d_6 and confirmed the suggested structures. The molecular composition of the compounds was routinely determined on an AEI MS-902 mass spectrometer at low resolution. High-resolution spectra, whenever necessary, were recorded on the same instrument, and the data evaluated with a Digital Equipment PDP-8 computer. Thin-layer chromatography was performed on silica gel plates irrigated with $CHCl_3$–MeOH (8:2) and saturated with NH_4OH.

Glyoxylonitriles and Pyruvonitriles. The following glyoxylonitriles and pyruvonitriles were prepared by the action of CuCN on the corresponding aroyl halide.[8] The conditions commonly employed involved heating a stirred mixture of the aroyl halide and

†Increases in survival time of less than 2.5 days are not statistically significant.

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CuCN to 130–200° and following the course of the reaction spectroscopically from samples taken from the reaction mixture. After completion of the reaction, the mixture was cooled and the slurry was taken up in $CHCl_3$ or C_6H_6, filtered, and distilled *in vacuo*, unless stated otherwise: *o*-chlorophenylglyoxylonitrile,[9] mp 35–37°, lit.[9] bp 101–105° (1.5–1.6 mm); *m*-chlorophenylglyoxylonitrile,[9] mp 36–40°, lit.[9] mp 35–37°; *p*-chlorophenylglyoxylonitrile,[10,11] mp 35–37°, lit.[10] mp 41–42.5°, lit.[11] mp 37–39°; 2,4-dichlorophenylglyoxylonitrile, mp 58–70°; *o*-fluorophenylglyoxylonitrile, bp 68–70° (0.1 mm); *p*-fluorophenylglyoxylonitrile,[11] bp 99–104° (aspirator vacuum), lit.[11] mp 20–22°; (α,α,α-trifluoro-*o*-tolyl)glyoxylonitrile, bp 53–56° (0.75 mm); (α,α,α-trifluoro-*m*-tolyl)glyoxylonitrile), bp 53–56° (0.75 mm); (α,α,α-trifluoro-*p*-tolyl)glyoxylonitrile, bp 39–40° (0.25 mm); (α,α,α,α′,α′,α′-hexafluoro-3,5-xylyl)glyoxylonitrile, bp 44–46.5° (0.25 mm); *p*-methoxyphenylglyoxylonitrile,[11,12] mp 52–55°, lit.[11] mp 56–57°, lit.[12] mp 57–58°; 3,4,5-trimethoxyphenylglyoxylonitrile, mp 134–135° (recrystd from petroleum ether), lit.[13] mp 133–134°; 3,3-dimethyl-3-phenylpyruvonitrile, bp 75–77° (0.5 mm); 3-(3,4-dichlorophenyl)-3,3-dimethylpyruvonitrile, bp 91–93° (0.15 mm); *p*-trifluoromethoxyphenylglyoxylonitrile, bp 172–173° (760 mm). Under the described conditions we were unable to prepare 2,6-dichlorophenylglyoxylonitrile.

The following compounds were prepared according to a procedure of Mauthner[14] (Et_2O, pyridine, HCN): phenylpyruvonitrile, mp 54–64°; 3,4,5-trimethoxyphenylpyruvonitrile, mp 125–135°.

3,5-Diamino-6-(α,α,α-trifluoro-*p*-tolyl)-*as*-triazine (9). (α,α,α-Trifluoro-*p*-tolyl)glyoxylonitrile (226.0 g, 1.21 moles) in 250 ml of DMSO was added dropwise to a stirred solution of aminoguanidine HCO_3 (169.8 g, 1.26 moles) in 1880 ml of 8 *N* HNO_3 at 0–5°. The suspension [which at the start of the reaction consisted mostly of (α,α,α-trifluoro-*p*-tolyl)glyoxylonitrile] was stirred overnight at room temperature, and the ppt was filtered the next morning. The amidinohydrazone HNO_3 (358.7 g) was used in the next step without further purification.

The amidinohydrazone HNO_3 (233.75 g) was added to 2.5 l. of 10% KOH in EtOH and refluxed under N_2 for 1 hr. Then H_2O (1.054 l.) was added, and the whole was vacuum evaporated until a cryst ppt separated. A total of 67.9 g of cryst compound was obtained.

3-Amino-6-(α,α,α-trifluoro-*p*-tolyl)-*as*-triazin-5-ol (17). The aqueous mother liquor from the preceding reaction was acidified with concd HCl to pH 1, when a solid began to separate; 105.5 g of **17** was obtained by filtration.

The high-resolution mass spectrum showed the molecular ion at 256.0582 (calcd 256.0570) and a fragment peak at 186.0314 for the elemental composition $C_9H_5OF_3$ (calcd 186.0292), indicating the hydroxyl function to be attached at position 5 of the *as*-triazine nucleus rather than at position 3.

3,5-Diamino-6-(α,α,α-trifluoro-*p*-tolyl)-*as*-triazine Pamoate. 3,5-Diamino-6-(α,α,α-trifluoro-*p*-tolyl)-*as*-triazine hydrochloride (2.8 g) was dissolved in boiling MeOH (200 ml), 4.51 g of the disodium salt of pamoic acid dissolved in MeOH (25 ml) was added, and the whole was left at room temperature. After 24 hr, H_2O was added and the solution was concd *in vacuo*. The resulting crystals were filtered, yielding 3.1 g of the pamoate salt, mp 193–198°. *Anal.* ($C_{43}H_{32}O_6N_{10}F_6$) C, H, N.

Acknowledgments. Tests with *P. berghei* and *P. gallinaceum* were conducted by Dr. Leo Rane, those with drug-resistant *P. berghei* by Dr. Paul Thompson, those with *P. cynomolgi* by Dr. E. Ross Hart, and those with *P. falciparum* in *Aotus trivirgatus* by Dr. Leon H. Schmidt. To these gentlemen the authors extend their thanks.

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Synthesis of Halogenated Anthraldehydes and Their Conversion to Antimalarial Amino Alcohols†

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As part of the current U. S. Army Research Program on malaria, we undertook the syntheses of substituted anthracene amino alcohols. The goal was increased activity against the drug-resistant strain of *Plasmodium falciparum*. The intensive antimalaria testing program during World War II did not include anthracene compounds,[1] but shortly thereafter, several anthracenes with C_9-$CH_2NHCH_2CH(OH)R$ groups[2] were found to be inactive against *P. gallinaceum* in chickens, while tetrahydroanthracene aminomethanols with unspecified side-chain position and 9,10-dihydroanthracene 9-aminomethanols were slightly active and inactive, respectively.[3]

An earlier unsuccessful attempt to prepare anthracene 9-aminomethanols was noted in 1948.[4] However, in 1968, the synthesis of 9-(2-di-*n*-heptylamino-1-hydroxyethyl)-anthracene was reported by Duncan, *et al.*,[5] and this compound was curative[6] against *P. berghei* in mice (Table II) at 320 mg/kg. Our work reports the preparation of 10-haloanthracene 9-amino alcohols, some of which are shown to be curative at considerably lower dose levels (*e.g.*, 80 mg/kg) in the same test.

We prepared the intermediate substituted 9-anthraldehydes in Table I by Vilsmeier[7] or reductive[8] formylation of the corresponding anthrone or 2-chloroanthraquinone, respectively. Anthrone intermediates to aldehydes D and E were prepared by the method of Bergmann and Loewenthal[9] from the corresponding 3-arylphthalides.

Aldehydes A and B gave corresponding 9-anthrylethylene oxides in 67 and 96% yields, respectively, by methylene transfer with dimethylsulfonium methylide.[5,18] A marked substituent effect was noted with aldehydes C, D, and E: none of the corresponding epoxides could be obtained from these compounds using several modifications of the dimethylsulfonium methylide procedure. That ylid attack on the aldehyde carbonyl of E had occurred was indicated by the disappearance of the aldehyde proton peak at 10.88 ppm in the nmr spectrum of the crude reaction mixture.

Readily available 10-chloro-9-anthraldehyde (B of Table I) was used as a model compound in a search for alternate routes to amino alcohols. Attempts at entry into the more

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