Synthesis and Antimalarial Effects of 2-(3,4-Dichloroanilino)-7-[[(dialkylamino)alkyl]amino]-5-methyl-*s*-triazolo[1,5-*a*]pyrimidines (1,2)

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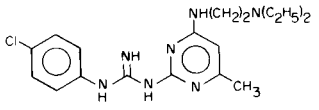
3,4-Dichlorophenylisothiocyanate (**10**) was allowed to react with 2-methyl-2-thiopseudourea to give methyl 4-(3,4-dichlorophenyl)dithioallophanimidate (**11**) (41%), which upon treatment with hydrazine afforded 3-amino-5-(3,4-dichloroanilino)-*s*-triazole (**12**) (54-91%). Ring-closure with ethyl acetoacetate in acetic acid afforded 2-(3,4-dichloroanilino)-5-methyl-*s*-triazolo[1,5-*a*]-pyrimidin-7-ol (**13**) (81%). Chlorination with phosphorus oxychloride gave 7-chloro-2-(3,4-dichloroanilino)-5-methyl-*s*-triazolo[1,5-*a*]pyrimidine (**14**) (98%), which was condensed with various amines to yield the desired 2-(3,4-dichloroanilino)-7-[[(dialkylamino)alkyl]amino]-5-methyl-*s*-triazolo[1,5-*a*]pyrimidines (**6**a-d). The structures of the *s*-triazolo[1,5-*a*]pyrimidines were based on nmr spectroscopy and ring stability considerations. Several of the amino-*s*-triazolo[1,5-*a*]pyrimidines possessed antimalarial activity against *P. berghei* in mice.

During the British war-time antimalarial program, it was discovered that various 1-phenyl-3-(4-amino-2-pyrimidinyl)guanidines (3,4), exemplified by 1-(*p*-chlorophenyl)-3-(4-[[2-(diethylamino)ethyl]amino]-6-methyl-2-pyrimidinyl)guanidine (**1**) (3), exhibited strong antimalarial effects against *Plasmodium gallinaceum* in chicks (3,5).



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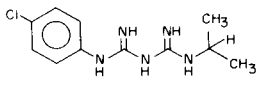
The drug also displayed promising effects against *P. cathemerium* and *P. relictum* in canaries (6), *P. knowlesi* in rhesus monkeys (7), and *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale* in man (6,8-11). Although it might be tacitly assumed that strains of malarial parasites that are resistant to related compounds such as chlorguanide (**2**) and its active metabolite cycloguanil (**3**)



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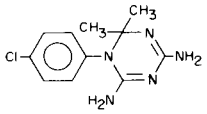

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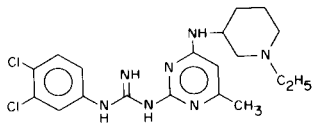
would also be cross-resistant to the guanidinopyrimidine **1**, subsequent studies demonstrated conclusively that this was not the case. Thus, no cross-resistance was observed when **1** was tested against a strain of *P. gallinaceum* that was resistant (20-40-fold) to chlorguanide (12,13), a strain of *P. berghei* that was resistant (100-fold) to sulfadiazine and cross-resistant with chlorguanide (14), and strains of *P. knowlesi* that were resistant to chlorguanide (2,400-fold) (7) and pyrimethamine (>2 x 10^6-fold) (15). Furthermore, when a normal drug-sensitive strain of *P. gallinaceum* was subjected for nearly two and one-half years to intensive treatment with **1**, no drug resistance was acquired (12,13).

Recent confirmation that there is no apparent cross-resistance between **1** and folate antagonists such as chlorguanide, cycloguanil, and pyrimethamine against *P. berghei* in mice (16) sparked a rebirth of interest in the guanidinopyrimidines, and led to the discovery of an array of new congeners that were considerably more active and less toxic than **1** (16). Among them, 1-(3,4-dichlorophenyl)-3-[[4-[(1-ethyl-3-piperidyl)amino]-6-methyl-2-pyrimidinyl]]guanidine (**4**) was designated for preclinical toxicological studies and clinical trial (16-18).



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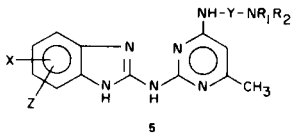
The overall promise of **4** and related guanidinopyrimidines stimulated an investigation of hypothetical metabolites that might result from *in vivo* dehydrogenation involving the unsubstituted nitrogen of the guanidine linkage and the *ortho* position of the adjacent phenyl or pyrimidine rings. The former cyclization would afford 2-[(4-amino-6-methyl-2-pyrimidinyl)amino]benzimidazoles (**5**), which were shown in the preceding communication



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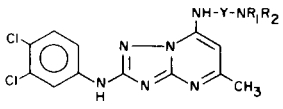
to have remarkable antimalarial effects (1). The latter ring closure, which would lead to *s*-triazolo[1,5-*a*]pyrimidines of types **6** or **7**, is the subject of the present communication. In this regard, it is noteworthy that



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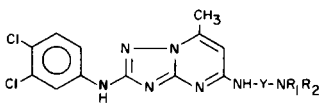

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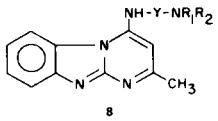
various 4-[[(dialkylamino)alkyl]amino]-2-methylpyrimido-[1,2-*a*]benzimidazoles (**8**), which could be considered formally to result from cyclization of the *ortho* position of the aniline ring of the 2-(arylamino)-4-[[(dialkylamino)-



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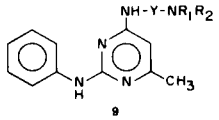

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alkyl]amino]-6-methylpyrimidine antimalarials **9** (19) with the pyrimidine ring nitrogen, lacked appreciable antimalarial activity (20).

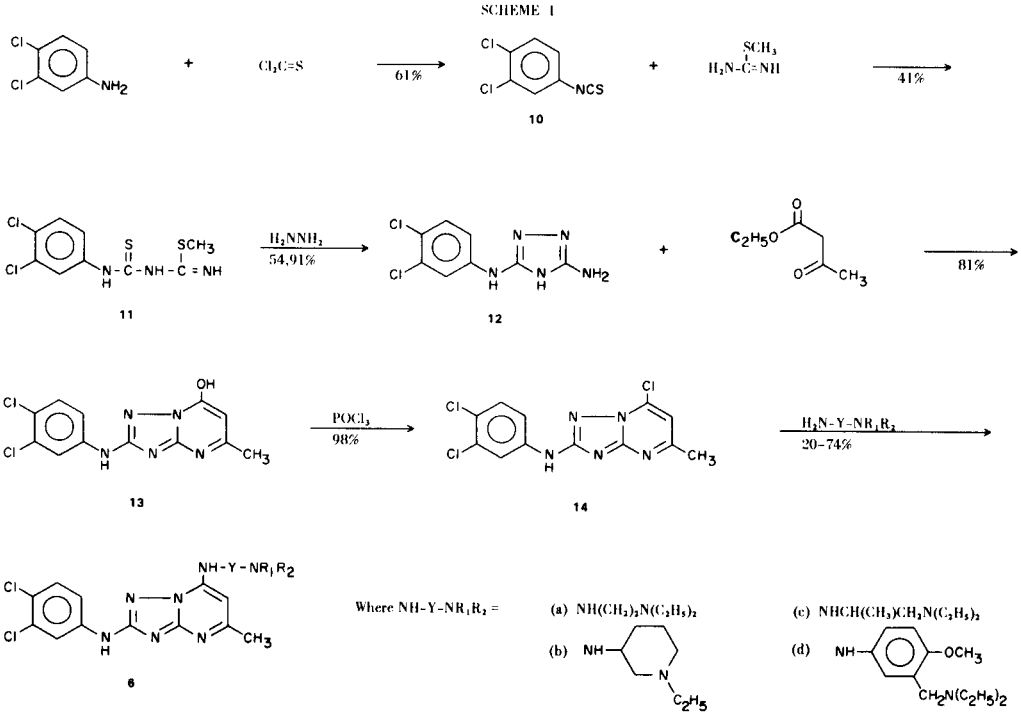
Chemistry.

The 2-(3,4-dichloroanilino)-7-[[(dialkylamino)alkyl]-amino]-5-methyl-*s*-triazolo[1,5-*a*]pyrimidines described in the present communication were synthesized according to the route outlined in Scheme 1. Thiophosgene in concentrated hydrochloric acid was treated with 3,4-dichloroaniline in toluene to give 3,4-dichlorophenyl-isothiocyanate (**10**) (21) in 61% yield. Utilizing the triazole synthesis developed by Davidson (22), the isothiocyanate (**10**) was allowed to react with 2-methyl-2-thiopseudourea to give methyl 4-(3,4-dichlorophenyl)-dithioallophanimidate (**11**) (41%), which upon treatment with hydrazine afforded the key intermediate 3-amino-5-(3,4-dichloroanilino)-*s*-triazole (**12**) in 54-91% yield.

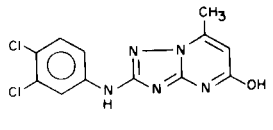
The reaction of the aminotriazole (**12**) with ethyl acetoacetate in acetic acid gave 2-(3,4-dichloroanilino)-5-methyl-*s*-triazolo[1,5-*a*]pyrimidin-7-ol (**13**) (81%), which upon chlorination with phosphorus oxychloride gave 7-chloro-2-(3,4-dichloroanilino)-5-methyl-*s*-triazolo[1,5-*a*]-pyrimidine (**14**) in 98% yield. The condensation of **14** with *N*,*N*-diethylethylenediamine, 3-amino-*N*-ethylpiperidine, N^1,N^1-diethyl-1,2-propanediamine, and N^{α},N^{α}-diethyl-6-methoxy-α,3-toluenediamine (1) neat at 105-145° afforded the desired 2-(3,4-dichloroanilino)-7-[[(dialkylamino)alkyl]amino]-5-methyl-*s*-triazolo[1,5-*a*]pyrimidines (**6**a-d) in 20-74% yield.

The reaction of the aminotriazole **12** with ethyl acetoacetate could conceivably lead to four probable isomers

SCHEME I



involving condensation on the primary amine and the ring NH, namely **13** and **15-17**. Previous work with the pyrimido[1,2-*a*]benzimidazoles (**8**) indicated that in the



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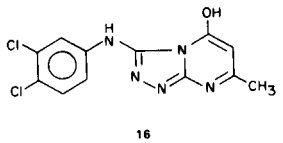

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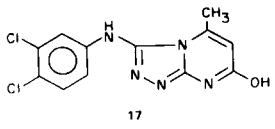

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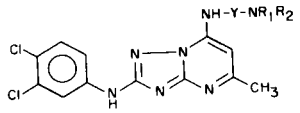
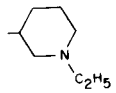
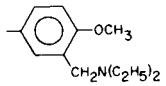
chloro compounds and amines derived from **15** and **17**, the ring methyl absorption in the nmr would be expected to be split by coupling with the adjacent ring proton, while in the isomeric structures **13** and **16** the methyl signal would appear as a singlet (20). In the nmr spectrum of the chloro compound that was obtained, the methyl signal appeared as a clean singlet at 2.85 δ (in trifluoroacetic acid relative to sodium-2,2-dimethyl-2-silapentane-5-sulfonate), thus eliminating structures **15** and **17** for the hydroxy compound. Moreover, it is also known that the triazolo[1,5-*a*]pyrimidine ring system **13** is more stable than the triazolo[4,3-*a*]pyrimidine ring system **16** (23-25). Since the cyclization with ethyl acetoacetate was effected in boiling acetic acid, compound **16**, even if formed, would be expected to rearrange to **13**. Therefore, it can

TABLE I

Effects of 2-(3,4-Dichloroanilino)-7-[[(dialkylamino)alkyl]amino]-5-methyl-*s*-triazolo[1,5-*a*]pyrimidines Against *Plasmodium berghei* in Mice



		Drug diet, 6 days SD90 (a),			Single s.c. dose ΔMST; T or C (c) after mg./kg.					
No.	-Y-NR1R2	No. of mice	mg./kg. per day	Q (b)	640	320	160	80	40	20
6a	$(CH_2)_2N(C_2H_5)_2$	14	135	0.6	6.6	5.2 5.4	4.8 5.0	0.8 0.6	0.4 0.4	0.2 0.4
6b					5.8	3.2 3.2	0.4 0.6	0.2 0.4	0.2 0.2	0.0 0.2
6c	$CH(CH_3)CH_2N(C_2H_5)_2$					0.7	0.5	0.5	0.3	0.3
6d		14	>30	<2.4	4.1	3.7 3.9	0.5 0.7	0.3 0.3	0.3 0.3	0.1 0.1
1		21	68	1.1	T5		T5 7.9; T3	7.9; T2	6.3 6.9	2.7
4		28	10	7.5	C5 C5 C3, T2 C4, T1	C5 C5	C5 C5 C5 C5	16.3; C3 16.9; C3	9.3; Cl 11.7; Cl 13.4; C3 12.4; C3	6.6 4.5

(a) SD_{90} represents the daily dose (mg./kg.) required for 90% suppression of the parasitemia in treated mice relative to control mice. The SD_{90} was estimated graphically using semi-log paper. (b) The quinine equivalent Q is the ratio of the SD_{90} of quinine·HCl (74.5 mg. base/kg./day) to the SD_{90} of the test substance under comparable experimental conditions. (c) ΔMST is the mean survival time (days) of treated mice (MSTT) minus the mean survival time (days) of control mice (MSTC). In the present study the MSTC ranged from 6.1 to 6.2 days. T signifies the number of toxic deaths occurring on days 2-5 after infection which are attributed to drug action. C indicates the number of mice surviving at 60 days post infection and termed "cured"; data to establish parasitological cure based on sub-inoculation are unavailable. Each entry at each dose level represents results with a 5-animal group.

be deduced with reasonable certainty that the triazolopyrimidines that were obtained according to Scheme I correspond to structures **6**, **13**, and **14** as depicted.

Antimalarial Effects.

The 2-(3,4-dichloroanilino)-7-[[(dialkylamino)alkyl]-amino]-5-methyl-*s*-triazolo[1,5-*a*]pyrimidines (**6**a-d) described in the present communication were evaluated for antimalarial effects utilizing *P. berghei* infections in mice. Initially compounds **6**a-d were administered in single subcutaneous doses ranging from 20 to 640 mg./kg. to mice infected with a normal drug-sensitive strain of *P. berghei* (26,27) (Table I). Compound **6**a increased the mean survival time of mice by >100% at a dose of 640 mg./kg. and is thus considered to be active. Compounds **6**b and d increased the mean survival time of mice 5.8 and 4.1 days, respectively, at the same dosage, but did not prolong the survival time sufficiently to satisfy the criterion for activity (27). None was curative at any dose level, and these substances were all significantly less active than the reference drug 1-(3,4-dichlorophenyl)-3-[[4-[(1-ethyl-3-piperidyl)amino]-6-methyl-2-pyrimidinyl]]guanidine (**4**) (16).

Two of the *s*-triazolo[1,5-*a*]pyrimidines (**6**a and d) were also administered continuously for 6 days in the diet of mice infected with another drug-sensitive strain of *P. berghei* (28,29). Compound **6**a caused a 90% suppression of the parasitemia relative to control animals at a dose of 135 mg./kg. per day, but was only 0.6 times as potent as quinine hydrochloride and was much less active than **4** (SD_{90} = 10 mg./kg. per day, Q = 7.5).

EXPERIMENTAL (30)

Methyl 4-(3,4-Dichlorophenyl)dithioallophanimidate (**11**).

To a solution of 7.0 g. (0.025 mole) of 2-methyl-2-thiopseudourea sulfate in 50 ml. of water was added 50 ml. of ethanol and 26 ml. of 10% sodium carbonate solution. This solution was warmed almost to boiling, 10.2 g. (0.05 mole) of 3,4-dichlorophenylisothiocyanate (21) was added, and the suspension was shaken for a few minutes and allowed to stir overnight at room temperature. The solid was collected, washed successively with 1:1 ethanol-water and water, and recrystallized from ethanol-water to give 6.0 g. (41%) of product, m.p. 141-142.5°. An additional recrystallization of 1.0 g. of the material from ethanol-water gave 0.6 g. of analytically pure product, m.p. 145-147°.

Anal. Calcd. for $C_9H_9Cl_2N_3S_2$: C, 36.73; H, 3.08; N, 14.28; Cl, 24.10; S, 21.80. Found: C, 36.77; H, 3.20; N, 14.57; Cl, 23.78; S, 21.67.

3-Amino-5-(3,4-dichloroanilino)-*s*-triazole (**12**).

A solution of 5.0 g. (0.017 mole) of methyl 4-(3,4-dichlorophenyl)dithioallophanimidate (**11**) in 175 ml. of ethanol and 1.28 g. (0.034 mole) of 85% hydrazine hydrate was heated under reflux for 2 hours. The mixture was concentrated to dryness, and the residue was slurried in 250 ml. of warm water and recrystallized first from aqueous ethanol and then from acetonitrile to give 1.6 g. (39%) of the product, m.p. 210-213°. Concentration of the filtrate gave an additional 0.6 g. of product, m.p. 211-213°. Total yield, 54%.

Anal. Calcd. for $C_8H_7Cl_2N_5$: C, 39.37; H, 2.89; N, 28.69. Found: C, 39.41; H, 2.99; N, 28.67.

In a subsequent 0.045 mole run, the reaction mixture was concentrated to a small volume, poured into water, filtered, and dried to give a 91% yield of the product, m.p. 213-215°, of adequate purity for use in the next step.

2-(3,4-Dichloroanilino)-5-methyl-*s*-triazolo[1,5-*a*]pyrimidin-7-ol (**13**).

A mixture of 2.4 g. of **12** (0.01 mole) and 1.3 g. of ethyl acetoacetate in 35 ml. of acetic acid was heated under reflux for 7.5 hours. The suspension was filtered, and the solid was rinsed with methanol, collected, and dried to give 2.5 g. (81%) of the product, m.p. >380°.

Anal. Calcd. for $C_{12}H_9Cl_2N_5O$: C, 46.47; H, 2.92; N, 22.58; Cl, 22.86. Found: C, 46.44; H, 2.94; N, 22.40; Cl, 22.74.

7-Chloro-2-(3,4-dichloroanilino)-5-methyl-*s*-triazolo[1,5-*a*]pyrimidine (**14**).

A suspension of 40 ml. of phosphorus oxychloride and 2.0 g. (0.0065 mole) of **13** was heated under reflux for 5 hours. The mixture was cooled and poured into iced water with vigorous stirring. The solid that formed was collected and dried to give 2.1 g. (98%) of the product, m.p. 301-304.5°.

Anal. Calcd. for $C_{12}H_8Cl_3N_5$: C, 43.86; H, 2.45; N, 21.31. Found: C, 43.24; H, 2.57; N, 20.90.

2-(3,4-Dichloroanilino)-7-[[2-(diethylamino)ethyl]amino]-5-methyl-*s*-triazolo[1,5-*a*]pyrimidine (**6**a).

A suspension of 1.97 g. (0.006 mole) of the chloro compound **14** in 50 ml. of *N,N*-diethylethylenediamine was heated at 135-145° for 4 hours. A solution was first obtained, then a solid appeared. The reaction mixture was filtered hot, and the solid obtained was washed with water and slurried with boiling ethanol to give 1.8 g. (74%) of the product, m.p. 275.5-276.5°.

Anal. Calcd. for $C_{18}H_{23}Cl_2N_7$: C, 52.95; H, 5.68; N, 24.01. Found: C, 53.06; H, 5.69; N, 24.03.

2-(3,4-Dichloroanilino)-7-[(1-ethyl-3-piperidyl)amino]-5-methyl-*s*-triazolo[1,5-*a*]pyrimidine (**6b**).

A suspension of 2.0 g. (0.006 mole) of the chloro compound **14** in 55 ml. (0.38 mole) of 3-amino-*N*-ethylpiperidine was heated to 130° to obtain solution and then at 95-105° for 1.5 hours. A small amount of insoluble material was removed from the hot mixture by filtration and discarded. The filtrate was cooled to 50° and the solid that formed was collected and slurried in refluxing ethanol to give 0.7 g. (28%) of the product, m.p. 267-270°.

Anal. Calcd. for $C_{19}H_{23}Cl_2N_7$: C, 54.29; H, 5.51; N, 23.33; Cl, 16.87. Found: C, 54.00; H, 5.53; N, 23.17; Cl, 17.12.

2-(3,4-Dichloroanilino)-7-[[2-(diethylamino)-1-methylethyl]-amino]-5-methyl-*s*-triazolo[1,5-*a*]pyrimidine (**6c**).

A suspension of 2.0 g. (0.006 mole) of **14** in 20 ml. of N^1,N^1-diethyl-1,2-propanediamine was heated at 105-115° for 2.5 hours. The mixture was allowed to cool to room temperature and filtered. The solid was washed with water and recrystallized from ethanol to give 1.0 g. of solid. The original reaction mixture filtrate was poured into 150 ml. of water and the solid that formed was collected and recrystallized from ethanol to give 0.5 g. of solid. The combined 1.5 g. of material was slurried in boiling

ethanol and then recrystallized from ethyl acetate to give 0.5 g. (20%) of the product, m.p. 224-228°.

Anal. Calcd. for $C_{19}H_{25}Cl_2N_7$: C, 54.03; H, 5.97; N, 23.21. Found: C, 54.14; H, 5.86; N, 23.53.

2-(3,4-Dichloroanilino)-7-[[3-[(diethylamino)methyl]-*p*-anisidino]-5-methyl-*s*-triazolo[1,5-*a*]pyrimidine (**6d**).

A suspension of 2.0 g. (0.006 mole) of **14** in 20 ml. of N^{α},N^{α}-diethyl-6-methoxy-α,3-toluenediamine (1,31) was heated at 115-125° for 5 hours, allowed to remain at room temperature for 16 hours, and then heated for an additional 3 hours at 115-125°. The reaction mixture was slurried in cold ethanol to provide 2.0 g. of crude solid. Trituration with boiling acetonitrile gave 1.3 g. (43%) of the product, m.p. 209.5-211.5°.

Anal. Calcd. for $C_{24}H_{27}Cl_2N_7O$: C, 57.60; H, 5.44; N, 19.59. Found: C, 57.28; H, 5.49; N, 19.53.

Acknowledgments.

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