Synthesis of Trimethoprim Variations. Replacement of CH_2 by Polar Groupings†

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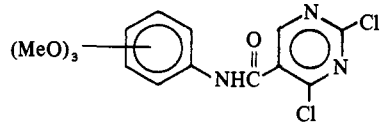
The antimalarial efficacy of 5-aryl-2,4-diaminopyrimidines, as a function of the electronic demands of aryl substituents, was defined by the extensive studies of Hitchings[1] and coworkers. Although their work also indicated an activity dependence on the nature of the functionality uniting the aryl and pyrimidine rings, comparatively few studies have dealt with this structural parameter.

An examination of this parameter in trimethoprim [2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine] congeners appeared particularly pertinent in light of its recently reported activity against drug-resistant strains of malaria.[2] We wish to describe structural variations of trimethoprim in which the CH_2 group that connects the aryl and pyrimidine rings has been replaced by polar functionality.

The target compounds listed in Table I were prepared by the synthetic techniques detailed in the Experimental Section. Several of these syntheses were atypical and merit comment. Although aminolysis of chloropyrimidines[3] are generally high temp processes, the known promotional influence of carbonyl functionality in nucleophilic displacements presented the possibility of obtaining **4** by treating



4a

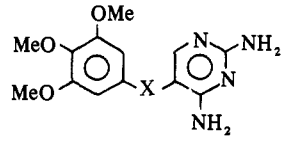
4a with NH_3-EtOH at atm pressure. Reaction of **4a** with refluxing NH_3-EtOH over extended time periods failed to effect complete amination of **4a**. Spectral data of the reaction product suggested that under these conditions, EtOH was a competing nucleophile. Displacement of both Cl required high temp and pressure amination techniques.

The direct condensation of 3,4,5-trimethoxybenzaldehyde with 2,4,5-triaminopyrimidine to yield **7** was intolerably slow and isolation of product difficult. These synthetic deficiencies were alleviated by an initial preparation of the Schiff's base from 3,4,5-trimethoxybenzaldehyde and *i*-$PrNH_2$. An amine exchange between this Schiff's base and 2,4,5-triaminopyrimidine occurred readily, giving good yields of **7**.

Compds **9** and **10**, obtained virtually quantitatively, appear to undergo facile thermal alteration.‡ Purification by sublimation or recrystallization failed. Attempts to sublime either **9** or **10** resulted in the isolation of 3,4,5-trimethoxyaniline, with destruction of **9** and **10**.

Biological Tests.§ The target structures did not affect the mean survival time of *Plasmodium berghei* infected mice sufficiently, at the maximum dose levels (640 mg/kg), to be classified as active. Compds **2**, **3**, and **6–9** in the avian activity screen (*P. gallinaceum*) failed to improve the survival time of the test animals beyond day 1, at a dose level of 320 mg/kg. At a drug concn of 0.1%, **1** produced 100% abnormal oocyst and 100% suppression of oocyst in the mosquito (*Aedes aegypti*) screen. However, the next lower dose level (0.01%) failed to produce a response.

Table I.

No.	X	Mp, °C	Recrystn solvent	Formula	Analysis[a]	Antimalarial act,[b,c] Δ ST, days
						
1	O	204–205	THF	$C_{13}H_{16}N_4O_4$	H, N; C[d]	*e*
2	N=N[f]	270–272	H_2O	$C_{13}H_{18}N_6O_7S$	N, O, S	0.2
3	CONH[g]	140–142	H_2O	$C_{14}H_{19}N_5O_5$	C, H; N[h]	0.1
4	NHCO[g]	226–228	H_2O	$C_{14}H_{19}N_5O_5$	C, N	3.3
5	CO_2–	222–224	EtOH	$C_{14}H_{16}N_4O_5$	C, H, N	0.2
6	CH=N	185–185.5	PhH	$C_{14}H_{17}N_5O_3$	C, H, N	2.5
7	CH_2NH[g,i]	169–172		$C_{18}H_{27}N_5O_7$	C, H, N	0.7
8	CH_2NH	193.5–195	PhH	$C_{14}H_{19}N_5O_3$	C, H, N	0
9	NHCONH[g]	*j*		$C_{14}H_{20}N_6O_5$	C, H, N	0.5
10	NHCSNH	*j*		$C_{14}H_{18}N_6O_3S$	C, H, N, S	0.6

[a] Where analyses are indicated by symbols of elements analytical results were obtained for those elements within ±0.4% of the theoretical value. [b] Mice were treated 3 days postinfection sc with a single dose of the compd being screened. The change in survival time (ΔST) is an indication of activity against *P. berghei* (see ref 6). [c] ΔST at 640 mg/kg. [d] C, calcd, 53.42; found 53.85. [e] Not evaluated in the rodent screen. [f] H_2SO_4 salt. [g] Monohydrate. [h] N, calcd, 20.77; found 21.33. [i] HOAc salt. [j] Decompd on heating.

Experimental Section

2,4-Diamino-5-(3,4,5-trimethoxyphenoxy)pyrimidine (1). A mixt of 16.3 g of 3,4,5-trimethoxyphenol, 6.6 g of chloroacetonitrile, and 12.2 g of K_2CO_3 in 60 ml of dry Me_2CO was refluxed overnight. The mixt was added to 140 ml of 3% NaOH and extd with Et_2O. Acidification of the aq phase yielded 3 g of 3,4,5-trimethoxyphenol. Evapn of the Et_2O gave 14.3 g (88%) of 3,4,5-trimethoxyphenoxyacetonitrile, mp 65.5–66° (PhH–hexane).

To a slurry of NaH (3.7 g of 50% NaH) in 130 ml of PhH was added a soln of 16.7 g of the phenoxyacetonitrile and 11.2 g of ethyl formate. After stirring at ambient temp overnight 100 ml of H_2O was added. Neutralization of the aq phase and extn with Et_2O gave 10.5 g of a viscous oil. Treatment of this oil in 1 l. of Et_2O

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‡Intramolecular cyclization of 5-ureido and -thioureidopyrimidines with expulsion of amines, are known synthetic entries into purines. For discussion of purines see ref 3, p 339 and ref 4.

§The rodent and avian activity screens were performed by Dr. L. Rane. The mosquito activity screen was conducted by Dr. E. Gerberg. The significance of this screen is described in ref 5.

with 5.3 g of CH_2N_2 gave 8.9 g of a dark viscous oil which gradually solidified, mp 110–111° (MeOH).

A soln of guanidine (from 9.7 g of guanidine hydrochloride and 5.4 g of NaOMe) and 8.9 g of the above described methylated aldehydonitrile in 125 ml of MeOH was refluxed for 18 hr. The MeOH was evapd and the residue extd with hot THF. The residue from the THF soln was chromatogd through base-treated silica gel. The product, 1.2 g, was found in the second Me_2CO eluate.

2,4-Diamino-5-(3,4,5-trimethoxyphenylazo)pyrimidine Sulfate (2). To a soln of 20.6 g of 3,4,5-trimethoxyaniline in 250 ml of 1 *N* HCl at 0° was added 8 g of $NaNO_2$ in 40 ml of H_2O. This soln was added to 11 g of 2,4-diaminopyrimidine in 110 ml of H_2O at 0°. After 5 min sufficient $NaHCO_3$ (about 35 g) was added to raise the pH to 8. The red-brown solids, formed after standing overnight, were filtered and extd with 500 ml of boiling 1 *N* H_2SO_4. On cooling the H_2SO_4 soln pptd 3 g of **2** as a yellow solid.

2,4-Diamino-5-(3,4,5-trimethoxybenzamido)pyrimidine Monohydrate (3). To a cold soln of 2,4,5-triaminopyrimidine[7] (1 g) and 1.5 g of Et_3N in 20 ml of H_2O was added 1.95 g of 3,4,5-trimethoxybenzoyl chloride in 20 ml of THF. The mixt was stirred for 20 min and concd. The pptd solid was filtered and recrystd, yield 2 g.

2,4-Dichloro-5-pyrimidinecarbonyl Chloride. 2,4-Dihydroxy-5-pyrimidinecarboxylic acid was converted into the titled compound by the procedure Gershon[8] used to prep the analogous 6-pyrimidinecarbonyl chloride, bp 90–91° (0.3 mm), 50% yield. *Anal.* ($C_5HCl_3N_2O$) N, Cl.

2,4-Dichloro-5-pyrimidinecarbox-3,4,5-trimethoxyanilide (4a). Et_3N (1.62 g) and 2.84 g of 3,4,5-trimethoxyaniline in 100 ml of THF was added to a cold soln of 3.3 g of 2,4-dichloro-5-pyrimidinecarbonyl chloride in 50 ml of THF. After standing overnight the reaction was filtered and the THF evapd. The residue was washed with cold dil $NaHCO_3$ and recrystd from PhH–hexane, mp 196–198°. *Anal.* ($C_{14}H_{13}Cl_2N_3O_4$) N, Cl.

2,4-Diamino-5-pyrimidinecarbox-3,4,5-trimethoxyanilide Monohydrate (4). A mixt of 5 g of **4a**, 13 ml of NH_4OH, and 66 ml of liquid NH_3 was heated in an autoclave for 8 hr at 180°. The bomb was cooled, and the contents were filtered and recrystd, yield 3 g.

2,4-Diamino-5-(3,4,5-trimethoxybenzoyloxy)pyrimidine (5). To a cold soln of 1 g of 2,4-diamino-5-hydroxypyrimidine hydrochloride[9] and 3.1 g of Et_3N in 20 ml of THF–H_2O was added 1.36 g of 3,4,5-trimethoxybenzoyl chloride in 15 ml of THF. After 20 min the reaction mixt was concd, poured into a large vol of H_2O, filtered, H_2O washed, and recrystd, yield, 1.8 g.

3,4,5-Trimethoxybenzylideneisopropylamine. A soln of 40 g of 3,4,5-trimethoxybenzaldehyde and 35 ml of *i*-$PrNH_2$ in 200 ml of THF was refluxed for 5 hr. Evapn of solvent left 48 g of a white solid, mp 76.5–77.5° (MeOH–H_2O). *Anal.* ($C_{13}H_{19}NO_3$) C, H, N.

2,4-Diamino-5-(3,4,5-trimethoxybenzylideneamino)pyrimidine (6). An EtOH soln, 350 ml, contg 15.4 g of 3,4,5-trimethoxybenzylideneisopropylamine and 8.1 g of 2,4,5-triaminopyrimidine was refluxed for 20 hr and then allowed to stand at room temp for 2 days. The pptd yellow solid was filtered and recrystd. Addl quants of **6** could be obtd by concn of the filtrate.

2,4-Diamino-5-(3,4,5-trimethoxybenzylamino)pyrimidine Acetate Salt (7). Low pressure hydrogenation of 2.5 g of **6** in 140 ml of HOAc over PtO_2 occurred in 15 min. The reaction mixt was filtered, poured into a large vol of Et_2O, filtered, and repeatedly washed with Et_2O, yield 2 g.

2,4-Diamino-5-(3,4,5-trimethoxybenzylamino)pyrimidine (8). An H_2O soln of 7 was made basic with NH_4OH, cooled, filtered, and recrystd.

3,4,5-Trimethoxyphenyl Isocyanate. To a slurry of NaN_3, 19.5 g in 125 ml of PhMe was added 23.1 g of 3,4,5-trimethoxybenzoyl chloride in 250 ml of PhMe. Stirring was contd until the characteristic acyl halide ir absorption was absent. The mixt was then heated on a steam bath until N_2 evoln ceased. It was cooled, filtered, and distd (14.4 g), bp 120–140° (0.1 mm). This product solidified on standing, mp 43–43.5°. *Anal.* ($C_{10}H_{11}NO_4$) C, H, N.

3,4,5-Trimethoxyphenyl Isothiocyanate. To $CSCl_2$ (25 g) suspended in 180 ml of ice H_2O was added 30.7 g of 3,4,5-trimethoxyaniline in 300 ml of $CHCl_3$, maintg the temp below 5°. After 15 min the $CHCl_3$ layer was sepd and dried over $CaCl_2$. Distn gave 24.8 g of product, bp 145° (0.5 mm). The distillate solidified on standing, mp 63° (hexane). *Anal.* ($C_{10}H_{11}NO_3S$) C, H, S.

1-[5-(2,4-Diaminopyrimidinyl)]-3-(3,4,5-trimethoxyphenyl)urea Monohydrate (9). To a soln of 2 g of 2,4,5-triaminopyrimidine in 150 ml of 50% EtOH was added 2.8 g of 3,4,5-trimethoxyphenyl isocyanate. After several hours stirring at room temp the ppt was filtered and washed with H_2O and EtOH, yield 4 g.

1-[5-(2,4-Diaminopyrimidinyl)]-3-(3,4,5-trimethoxyphenyl)-thiourea (10). Powdered 3,4,5-trimethoxyphenyl isothiocyanate (2 g) was added to a soln of 2,4,5-triaminopyrimidine in 40 ml of 75% EtOH. After 1 hr stirring the ppt was filtered and washed thoroughly with H_2O and EtOH.

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Preparation and Antitumor Activity of Derivatives of 1-Phenyl-3,3-dimethyltriazene†

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A number of 5-triazenoimidazoles are active against experimental tumors.[1] An excellent and comprehensive review of this subject has recently appeared.[2] Of the 5-triazenoimidazoles, 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DIC) in particular, is clinically useful for the induction of temporary remission in malignant melanoma.[3] The mechanism of the antitumor action of DIC remains obscure. However, on the basis of studies of the biotransformation of DIC[4,5] and the carcinogenesis of phenyltriazenes, it has been proposed that the triazenes may act as alkylating agents through the *in vivo* generation of carbonium ions.[6] Nevertheless, it is entirely possible that as a derivative of 5-aminoimidazole-4-carboxamide (AIC), the precursor of the purine base, 5-triazenoimidazole may somehow interfere with imidazole and purine metabolism.[7]

Antitumor activity has been observed in several derivatives of phenyltriazene.[8] To extend these observations and, above all, to elucidate the structural requirements for antitumor activity in the triazenes and specifically to ascertain whether an imidazole ring with a carboxamide moiety ortho to the triazeno side chain is indispensable in DIC, we have synthesized 6 derivatives of 1-phenyl-3,3-dimethyltriazene (**1a–1f**). Except for **1d**,[9] these compounds have not been

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