Antimalarial Properties of a Variety of Substituted *p*-Sulfamoylphenylazo Compounds

R. E. HARMON, B. L. GELLER, S. K. GUPTA, M. H. HERBERT, and D. CHITHARANJAN

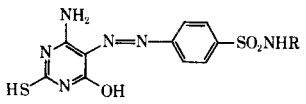
Abstract □ A wide variety of substituted *p*-sulfamoylphenylazo compounds were prepared, and their activity against *Plasmodium berghei* in mice was studied. A study of the effects of substitution in the sulfamoyl group revealed that substitution by pyrimidine, methylpyrimidine, and hexylresorcinol greatly increased the antimalarial activity, while substitution by thiazole, methylthiazole, and 2-methyl-3,4-thiadiazole did not lead to a comparable increase in activity. The least activity was seen in compounds with 2-pyridyl as the substituent. Coupling compounds derived from sulfa drugs, hexylresorcinol, and 1,3-dimethyl-6-aminouracil were either active or curative at lower doses than the remainder of the compounds evaluated. With pyrimidine as the substituent, an amino group in position 4 or 5 was necessary for antimalarial activity. It was also found that coplanarity is not an essential structural requirement for antimalarial activity.

Keyphrases □ *p*-Sulfamoylphenylazo compounds, substituted—antimalarial properties evaluated □ Antimalarials, evaluation—*p*-sulfamoylphenylazo compounds □ UV spectrophotometry—identification, structure □ IR spectrophotometry—identification, structure

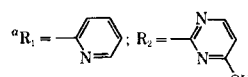
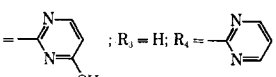
Since Lythgoe *et al.* (1) found that azo coupling occurs in the 5-position of the pyrimidine ring, various substituted 5-arylazopyrimidines have been synthesized and their mode of action in various biological systems studied (2). It was found that at least one amino group adjacent to the arylazo link is necessary for optimum activity and that the aryl group should be unsubstituted or contain electron-releasing substituents for maximum biological activity (3–5). The inhibitory actions of 5-phenylazo-2,4,6-triaminopyrimidine and 5-phenylazo-2,4-diamino-6-hydroxypyrimidine were unaffected by most bases and nucleotides involved in nucleic acid synthesis. This finding, in conjunction with the findings of Roy-Burman and Sen (5) that the inhibitory effects of arylazopyrimidines in the *Streptococcus faecalis* (ATCC 8043) system could be more efficiently reversed by 5-formyltetrahydrofolic acid than by folic acid itself, would seem to indicate that arylazopyrimidines may act as folic acid antagonists and interfere with the enzymatic conversion of folic acid to 5-formyltetrahydrofolic acid. Recent work by Hampshire *et al.* (6) on the inhibitory effects of 5-arylazo-2,4,6-triaminopyrimidines on folic acid reductase from rat liver indicates that these compounds exhibit a wide range of activities, depending on the aryl substituent. There is a strong indication that some sulfonylphenylazo compounds have a high synergistic effect when used in combination therapy (7).

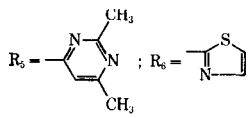
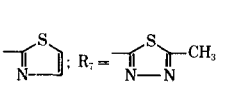
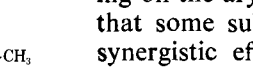
The reactions of various diazotized sulfa drugs with hexylresorcinol, 2,6-diaminopyridine, 3-phenylazo-2,6-diaminopyridine, 1-phenyl-3-methyl-5-pyrazolone, 1-phenyl-3-carbethoxy-5-pyrazolone, and several substituted pyrimidines were studied as an extension of the authors' earlier investigation (2) of the antimalarial

Table I—Activity Data of Substituted *p*-Sulfamoylphenylazo Compounds



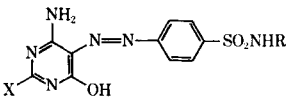
R[a]	M.p.	UV Data mμ	$\epsilon_{max.}$ (10^4)	Dose, mg./kg.	IST[b]	Activity in *P. berghei*[c]
R_1	300°	400	1.21	640	18.0	++
		286	10.1			
		240	14.1			
R_4	221–227°	392	3.56	160	15.8	++
		285	5.70	640	29.0	++++
		240	11.1			

[a] R_1 =  ; R_2 =  ; R_3 = H; R_4 =  ;

R_5 =  ; R_6 =  ; R_7 = 

[b] Increase in (mean survival time of the treated group minus mean survival time of the control group) mean survival time of control mice (M.S.T.) was 6 days. [c] + = 100% increase in survival time, 6.0 ± 0.5 days; ++ = greater than 100% increase in survival time; +++ = greater than 100% increase in survival time but not curative; ++++ = curative, less than 30-days survival. See *Reference 8* for procedures used in evaluating compounds for antimalarial activity.

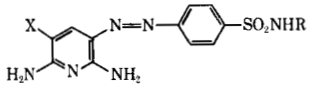
Table II—Activity Data of Substituted *p*-Sulfamoylphenylazo Compounds



R[a]	X	M.p.	UV Data mμ	$\epsilon_{max.}$ (10^4)	Dose, mg./kg.	IST[b]	Activity in *P. berghei*[c]
R_1	NH_2	268–270°	392	7.1	320	20.6	++
			288	1.54	640	21.8	++
			235	7.4			
R_2	NH_2	248–250°	394	2.9	160	13.0	+
			264	7.5	640	22.0	+++
R_5	NH_2	250°	395	7.8	80	13.2	+
			250	7.8	160	12.8	+
					640	21.0	++
R_6	OH	>300°	398	10.93	320	13.8	+
			255	7.01	640	16.8	++
R_3	SCH_3	283–285°	398	11.52	640	15.4	++
			255	6.21			
R_1	SCH_3	200°	397	7.52	80	12.8	+
			247	8.52	160	11.2	+
					640	22.0	+++
R_7	SCH_3	225°	399	10.89	640	16.2	++
			253	7.38			

[a,b,c] See Table I.

Table III—Activity Data of Substituted *p*-Sulfamoylphenylazo Compounds



R[a]	X	M.p.	UV Data mμ	UV Data $\epsilon_{max.}$ (10^4)	Dose, mg./kg.	IST[b]	Activity in *P. berghei*[c]
R_1	H	237–239°	449	9.96	40	13.2	+
			284	5.53	160	15.8	++
			220	6.42	640	19.0	++
R_2	H	225–235°	451	8.06	40	18.8	++
			272	4.30	160	27.2	+++
			240	6.22			
R_5	H	251–257°	499	7.80	160	14.6	++
			273	5.61	640	17.6	++
			247	4.52			
R_6	H	150°	451	9.75	160	15.2	++
			272	6.00	640	33.0	+++
R_7	H	263–266°	453	8.19	640	16.0	++
			270	4.68			
R_2	N_2Ph	221–223°	472	17.57	160	12.4	+
			310	4.29	320	14.0	++
			263	8.00	640	20.8	++
R_4	N_2Ph	215–219°	428	6.92	160	16.4	++
			240	6.63	320	22.7	+++
					640	34.0	++++
R_6	N_2Ph	222–227°	428	7.09	640	17.2	++
			276	4.02			

[a,b,c] See Table I.

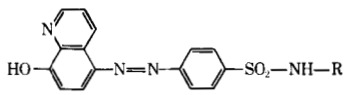
and anticancer properties of arylazopyrimidines. The structures and activities of the compounds are shown in Tables I–VIII.

EXPERIMENTAL

All melting points were determined using a Thomas-Hoover Unimelt apparatus. All the compounds melted with decomposition at or around the temperatures indicated in the tables. The UV spectra were determined by dissolving 10 mg. of the compound in 500 ml. of 1% sodium hydroxide solution. Spectrophotometers (Beckman model DB and Cary model 14) were used to determine the UV spectra. The IR spectra were obtained from mineral oil (Nujol) mulls on a Beckman IR-8 spectrophotometer.

The general procedure for the preparation of substituted *p*-sulfamoylphenylazo compounds was as follows. All the compounds were synthesized using procedures analogous to those previously reported in the literature (2). In a typical preparatory method, 0.05 mole of the sulfonamide drug was dissolved in 100 ml. of 3 *N* HCl. On cooling to −5°, the amine hydrochloride was precipitated. It was diazotized by adding 0.05 mole of $NaNO_2$ in 25 ml. of H_2O. The temperature was maintained at 0°. The pyrimidine (0.05 mole) was dissolved in 3 *N* HCl and cooled to −5°; the solution of the diazonium salt was added to it slowly and with stirring. The addition took about 20 min. The temperature was maintained at 10° for 1 hr. and then at room temperature for 12 hr. A thick slurry containing the *p*-sulfamoylphenylazo compound as a bright-yellow or orange solid formed. The azo compound was filtered, washed with 95% EtOH, and recrystallized from boiling 2-ethoxyethanol. The compounds were analyzed for C, H, and N and were within the normal limits.

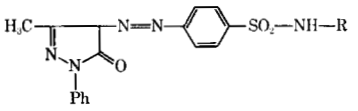
Table IV—Activity Data of Substituted *p*-Sulfamoylphenylazo Compounds



R[a]	M.p.	UV Data mμ	UV Data $\epsilon_{max.}$ (10^4)	Dose, mg./kg.	IST[b]	Activity in *P. berghei*[c]
R_1	220–224°	505	9.88	160	17.4	++
		248	10.94	640	27.3	+++
R_4	239–246°	504	10.15	160	22.3	+++
		243	11.53	640	27.0	++
R_2	200°	504	9.16	160	17.8	++
		249	8.48	640	27.3	+++
R_5	223–225°	508	11.98	160	22.0	+++
		253	10.42	640	28.0	+++
R_6	150°	508	4.52	160	17.2	++
		257	4.85	640	20.7	++
R_7	265–269°	503	5.20	640	24.0	+++
		439	4.77			
		236	7.58			

[a,b,c] See Table I.

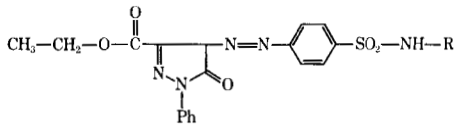
Table V—Activity Data of Substituted *p*-Sulfamoylphenylazo Compounds



R[a]	M.p.	UV Data mμ	UV Data $\epsilon_{max.}$ (10^4)	Dose, mg./kg.	IST[b]	Activity in *P. berghei*[c]
R_1	125–129°	400	7.29	80	15.6	++
		244	11.46	160	21.3	++
				320	23.0	+++
R_4	233–237°	405	6.18	160	26.3	+++
		241	10.70	640	22.0	+++
R_2	144–150°	409	7.09	40	12.6	+
		243	11.49	640	23.7	+++
R_5	273–278°	407	10.28	80	13.8	+
		246	12.41	160	15.8	++
				640	22.0	+++
R_6	180°	405	7.74	320	12.8	+
		249	9.24	640	18.5	++
R_7	147–150°	405	7.73	320	13.0	+
		250	7.55	640	22.0	+++

[a,b,c] See Table I.

Table VI—Activity Data of Substituted *p*-Sulfamoylphenylazo Compounds



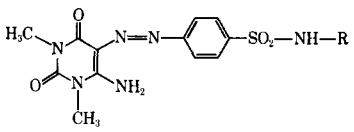
R[a]	M.p.	UV Data mμ	UV Data $\epsilon_{max.}$ (10^4)	Dose, mg./kg.	IST[b]	Activity in *P. berghei*[c]
R_3	200–208°	398	5.39	640	18.4	++
		248	8.63			
R_1	170–174°	408	2.66	640	21.8	++
		240	10.73			
R_4	218–222°	405	3.06	160	16.4	++
		241	6.61	640	27.0	+++
R_2	141–149°	410	3.55	160	12.4	+
		240	11.56	640	21.8	++
R_5	199–203°	410	7.61	160	16.0	++
		248	9.59	640	24.0	+++
R_6	170°	408	3.39	640	21.2	++
		254	8.17			

[a,b,c] See Table I.

RESULTS AND DISCUSSION

IR Spectra—The IR spectra were not very useful in characterizing the azo linkage in arylazopyrimidines, since its absorption was ob-

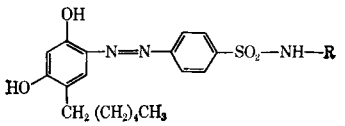
Table VII—Activity Data of Substituted *p*-Sulfamoylphenylazo Compounds



R[a]	M.p.	UV Data mμ	$\epsilon_{max.}$ (10^4)	Dose, mg./kg.	IST[b]	Activity in *P. berghei*[c]
R_1	235°	404	6.91	160	14.6	++
		243	9.93	640	24.0	+++
R_4	263–266°	404	5.91	20	13.2	+
		241	10.35	160	32.3	++++
R_2				40	13.2	+
	287–289°	409	5.56	160	18.0	++
		243	8.38	640	30.0	++++
R_5	266–269°	399	7.59	40	13.2	+
		248	9.09	160	22.0	+++
				320	35.0	++++
				640	36.0	++++

[a,b,c] See Table I.

Table VIII—Activity Data of Substituted *p*-Sulfamoylphenylazo Compounds



R[a]	M.p.	UV Data mμ	$\epsilon_{max.}$ (10^4)	Dose, mg./kg.	IST[b]	Activity in *P. berghei*[c]
R_1	235°	404	6.91	160	14.6	++
		243	9.93	640	24.0	+++
R_4	263–266°	404	5.91	20	13.2	+
		241	10.35	160	32.3	++++
R_2				40	13.2	+
	287–289°	409	5.56	160	18.0	++
		243	8.38	640	30.0	++++
R_5	266–269°	399	7.59	40	13.2	+
		248	9.09	160	22.0	+++
				320	35.0	++++
				640	36.0	++++

[a,b,c] See Table I.

scured by the strong absorption of the pyrimidine ring at 1600 $cm.^{-1}$. The absence of hydroxyl absorptions indicates that these compounds might exist in the tautomeric form (9, 10). In addition, the spectra showed medium to strong absorption bands characteristic of the pyrimidine and benzene rings at 1575 and 1625 $cm.^{-1}$, respectively; strong absorption bands at 1159 and 1320 $cm.^{-1}$ (—SO_2NH when present); medium to strong bands at 3100–3300 $cm.^{-1}$ (NH_2); and medium bands at 800–869 $cm.^{-1}$ (*p*-substituted benzene). These assignments are consistent with the assignments made by Bellamy (11) and Rao (12) for analogous compounds.

UV Spectra—Most of the substituted *p*-sulfamoylphenylazo compounds reported in Tables I–VIII were insoluble in common organic solvents such as ethanol, methanol, chloroform, and carbon tetrachloride. Therefore, they were dissolved in a 1% sodium hydroxide solution for determining the UV spectra. The UV spectral data for all the compounds are listed in Tables I–VIII. A majority of the compounds had two main UV absorption maxima around 400 and 250 mμ. In some cases the absorption maxima were shifted to about 500 and 300 mμ. But it was not possible to draw any definite conclusions about the structures and their UV absorptions.

Antimalarial Activity—All the compounds mentioned in this manuscript were tested for biological activity.[1] The effects of substitution on the antimalarial activity of a series of substituted *p*-sulfamoylphenylazo compounds are shown in Tables I–VIII. A study of the effects of substitution in the sulfamoyl group revealed an interesting pattern of activity. In general, substitution by pyrimidines and methylpyrimidines greatly increased the antimalarial activity, while substitution by thiazole, methylthiazole, and 2-methyl-3,4-thiadiazole did not lead to a comparable increase in activity. The lowest activity was observed in compounds with 2-pyridyl as the substituent.

The greatest activity was observed in the sulfamoyl compounds derived from 3-phenylazo-2,6-diaminopyrimidines (Table III). The next order of activity was seen in compounds derived from hexylresorcinol and 1-phenyl-3-methyl-5-pyrazolone. Coupling compounds derived from sulfa drugs, hexylresorcinol, and 1,3-dimethyl-6-aminouracil were either active or curative at lower doses than the other compounds evaluated. The substituent on the sulfamoyl group did not appear to exert a consistent effect on the antimalarial activity in changing from one group to the other. In the case of the pyrimidines, it has been observed that an amino group in position 4 or 5 is necessary for antimalarial activity.

It has been shown that substitution by an alkyl or aryl group in the 6-position of a 5-phenylazopyrimidine gives rise to a nonplanar configuration of the pyrimidine and benzene rings (13). Since 2,4-diamino-5-(2-chlorophenyl)pyrimidine is an antimalarial of relatively low potency (13), lack of coplanarity is not the sole requirement for antimalarial activity. This is clearly substantiated by the observation that coupling compounds derived from 1-phenyl-3-methyl-5-pyrazolone and 1-phenyl-3-carbethoxy-5-pyrazolone were found to have significant, if not curative, antimalarial activity. These compounds probably are noncoplanar since the carbon-to-nitrogen bond distance is shorter than the carbon-to-carbon bond distance. Osdene *et al.* (8) essentially brought forth the same point of view in their work on the antimalarial activity of 2,4,7-triamino-6-*ortho*-substituted arylpteridines.

[1] Tests were performed at Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC 20012

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