Repository Drugs. IV. 4′,4‴-Sulfonylbisacetanilide (Acedapsone, DADDS)[1] and Related Sulfanilylanilides with Prolonged Antimalarial and Antileprotic Action[2]

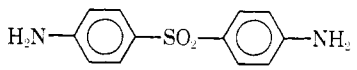
EDWARD F. ELSLAGER, ZOE B. GAVRILIS, ANNETTE A. PHILLIPS, AND DONALD F. WORTH

Research Laboratories, Parke, Davis and Company, Ann Arbor, Michigan 48106

Received January 6, 1969

Thirty-six sulfanilylanilides and related compounds were investigated as potential repository antimalarial and antileprotic agents. Seven compounds protected mice against challenge with *Plasmodium berghei* for 4 to >10 weeks following a single, subcutaneous 400-mg/kg dose. 4′,4‴-Sulfonylbisacetanilide (acedapsone, DADDS) (XIII) showed the longest duration of action and protected mice for 6–14 weeks against challenges with *P. berghei* and monkeys for 2–8 months against challenges with *Plasmodium cynomolgi*. Repository antimalarial effects were abolished or drastically reduced when DADDS was modified by: (1) replacement of the acetamide groups with a formamide function, (2) replacement of both acetamide groups with amide functions containing more than two carbon atoms, (3) N-alkylation of one acetamide function, (4) introduction of a chlorine atom at positions 2′ or 3′, or (5) replacement of the sulfone moiety by a thio, sulfinyl, oxalyl, or 2,2,2-trichloroethylidene linkage. Representative 4′,4‴-bis(N-acetylsulfanilyl)alkylenebisanilides (VIIIa–c), 4′-[N-(substituted vinyl)sulfanilyl]-acetanilides (IXa–d), 4′-[(2-acetamido-5-thiazolyl)sulfonyl]acetanilide (X), 3′,3‴-sulfonylbisacetanilide (XI), and 3,3′-[sulfonylbis(*p*-phenyleneiminomethylene)]di-2-thiazolidinethione (XII) also lacked appreciable repository action. A combination of cycloguanil pamoate and DADDS showed better activity than either component alone against drug-resistant plasmodia in experimental animals and in man. DADDS exhibited strong repository antileprotic action against *Mycobacterium leprae* in mice and in man.

4,4′-Sulfonyldianiline (diaphenylsulfone, DDS)[3] (I) has occupied a preeminent position in the treatment of human leprosy since 1941.[4] For several decades it has



I

also been known that sulfones and sulfonamides exhibit noteworthy antimalarial properties, although until recently these substances have been little used in the treatment of human malarias.[5–7] This stemmed from the knowledge that other more promising antimalarial drugs were available, from apprehension concerning the toxicity of certain compounds, and from recognition that the need for frequent dosing limited their usefulness in mass treatment.

Interest in sulfones as antimalarial agents was revived in 1960 by the report of Archibald and Ross.[8] Their observation of lower prevalence of malaria in leprosy patients under treatment with DDS led them to compare the relative antimalarial effects of 200 mg of DDS and 300 mg of chloroquine administered in single oral doses. They observed that falciparum malaria was cleared by either drug, although somewhat more slowly by DDS. Most cases of quartan malaria also responded to DDS treatment. These results, together with results of earlier clinical studies with DDS in leprosy,[4] suggested to us that the sulfones were potent enough to be considered as one class of antimalarial and antileprotic agent that might be amenable to the development of repository drugs. Therefore, we initiated a search for long-acting sulfone and sulfonamide derivatives that might provide a slow and sustained release of an active moiety from a depot site and thereby (1) enable their use in large-scale malaria eradication programs or for mass leprosy treatment, and (2) provide antimalarial substances, that, in combination with cycloguanil pamoate[2c,5,9] or related compounds,[2b,5] might effect a sequential block in the metabolic synthesis of purines and pyrimidines within the parasite and afford broader repository action against drug-resistant plasmodia than either drug alone.

During the course of this repository work, it became apparent that certain lines of *Plasmodium berghei*,[10] *Plasmodium cynomolgi*,[11] and *Plasmodium gallinaceum*,[12] made resistant to DDS or to cycloguanil or pyrimethamine, were still susceptible to the heterologous drug, with only a low order of cross-resistance. Further, a 1:1 mixture of cycloguanil hydrochloride and DDS proved highly effective against the parent, cycloguanil-resistant and DDS-resistant lines of *P. berghei*, and the rate of emergence of resistance in the parent strain was significantly less with the mixture than with either drug alone.[10] These observations reinforced interest in developing a combination of the two drugs in repository form. In a recent preliminary communication,[2a] we reported briefly on the development of 4′,4‴-sulfonylbisacetanilide (acedapsone, DADDS)[1] and related sulfanilylanilides that showed remarkable repository

(1) Acedapsone is the nonproprietary name for 4′,4‴-sulfonylbisacetanilide. In the biological literature acedapsone has also been referred to as sulfadiamine, 4,4′-diacetyldiaminodiphenyl sulfone, 4,4′-diacetylaminodiphenyl sulfone, N,N′-diacetyl-4,4′-diaminodiphenyl sulfone, and DADDS. Hansolar® is a proprietary name for acedapsone. The proprietary name for the acedapsone–cycloguanil pamoate combination is Dapolar®.

(2) For previous papers on repository drugs, see (a) E. F. Elslager and D. F. Worth, *Nature*, **206**, 630 (1965); (b) E. F. Elslager and P. E. Thompson, Abstracts, 9th National Medicinal Chemistry Symposium of the American Chemical Society, Minneapolis, Minn., June 1964, p 6A; (c) P. E. Thompson, B. J. Olszewski, E. F. Elslager, and D. F. Worth, *Am. J. Trop. Med. Hyg.*, **12**, 481 (1963).

(3) Diaphenylsulfone is the international nonproprietary name for 4,4′-sulfonyldianiline. In the biological literature diaphenylsulfone is also referred to as dapsone, 4,4′-diaminodiphenyl sulfone, bis(4-aminophenyl) sulfone, and DDS.

(4) For a review, see L. Doub in "Medicinal Chemistry," Vol. V, W. H. Hartung, Ed., John Wiley and Sons, Inc., New York, N. Y., 1961, pp 350–425.

(5) For recent reviews, see: (a) E. F. Elslager in "Annual Reports in Medicinal Chemistry, 1965," C. K. Cain, Ed., Academic Press, New York, N. Y., 1966, p 136; (b) E. F. Elslager in "Annual Reports in Medicinal Chemistry, 1966," C. K. Cain, Ed., Academic Press, New York, N. Y., 1967, p 131.

(6) J. Hill in "Experimental Chemotherapy," Vol. I, R. J. Schnitzer and F. Hawking, Ed., Academic Press, New York, N. Y., 1963, p 547.

(7) L. T. Coggeshall, J. Maier, and C. A. Best, *J. Am. Med. Assoc.*, **117**, 1077 (1941).

(8) H. M. Archibald and C. M. Ross, *J. Trop. Med. Hyg.*, **63**, 25 (1960).

(9) Camolar®.

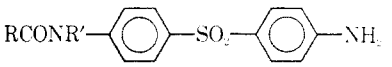
(10) P. E. Thompson, A. Bayles, B. Olszewski, and J. A. Waitz, *Am. J. Trop. Med. Hyg.*, **14**, 198 (1965).

(11) S. P. Ramakrishnan, P. C. Basu, H. Singh, and N. Singh, *Bull. World Health Organ.*, **27**, 213 (1962).

(12) A. Bishop, *Parasitology*, **53**, 10P (1963).

TABLE I

4′-SULFANILYLANILIDES

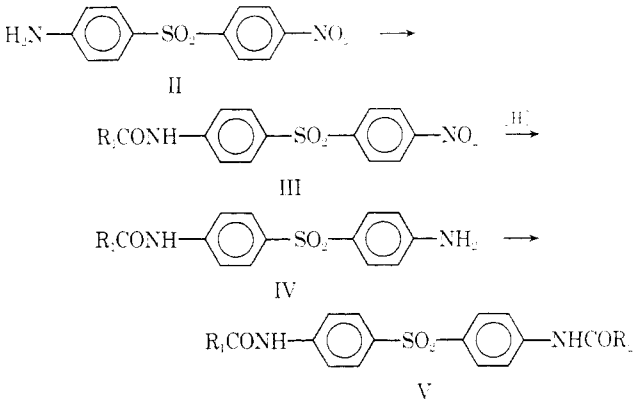


No.	RCONR	Mp, °C	Yield purifd, %	Purifcn solvent	Formula	Analyses[25]	Solubility in pH 7 PB, mg/ml	Repository act., PMW[a]
1	HCONH	168–170	72	MeCN	$C_{13}H_{12}N_2O_3S$	C, H, N, S	0.27	–
2	CH_3CONH	239–241[b]	94	DMF–H_2O	$C_{14}H_{14}N_2O_3S$	C, H, N	0.04	+
3	CH_3CH_2CONH	214–215[c]	62	MeOH	$C_{15}H_{16}N_2O_3S$	C, H, N	0.027	+
4	$CH_3CONCH_2CH_2CH_3$	126–128	75	C_6H_6	$C_{17}H_{20}N_2O_3S$	C, H, N	0.12	–
5	$CH_3(CH_2)_5CONH$	163–164	70	*i*-PrOH	$C_{19}H_{24}N_2O_3S$	C, H, N	0.001	– +
6	$CH_3(CH_2)_{10}CONH$	156–158[d]	69	*i*-PrOH	$C_{24}H_{34}N_2O_3S$	C, H, N	<0.0005	+ + +

[a] Drugs were suspended in 5 ml/kg of benzyl benzoate–castor oil (40:60) and administered subcutaneously to groups of 15–25 female albino mice in a single 400-mg/kg dose.[2b,c] Subgroups of five mice were challenged by the intraperitoneal injection of 15 million *Plasmodium berghei* at various intervals, usually at 1, 3, 5, 7, and 9 weeks, for susceptibility to malaria.[2b,c] Activity is based on the number of weeks 50% of the mice were protected (PMW), and activity ratings were assigned within the following ranges (weeks protected): + + + +, >10; + + +, 7–10; + +, 4–7; +, 1–4; –, <1. [b] Lit.[16] mp 242–243°. [c] Lit.[17] mp 201–202°; K. Miura [*J. Pharm. Soc. Japan*, **62**, 230 (1942)] reports mp 210–211°. [d] K. Miura and Y. Bando [*ibid.*, **63**, 75 (1943)] reports mp 160°.

action against *P. berghei*[13] and *Mycobacterium leprae*[14a] in mice. The present paper describes the synthesis and properties of such substances in detail.

The 4′-sulfanilylanilides (IV) (**1–6**, Table I), including 4′-sulfanilylformanilide (MFDDS) (**1**) and 4′-sulfanilylacetanilide (MADDS) (**2**), were synthesized by acylation of *p*-[(*p*-nitrophenyl)sulfonyl]aniline (II) with formic acid or the appropriate acid chloride,[15,16] followed by catalytic hydrogenation of the intermediate 4′-[(*p*-nitrophenyl)sulfonyl]anilides (III) in THF or DMF over Raney nickel. Earlier attempts[15] to prepare MFDDS (**1**) by reduction of the corresponding nitro compound failed because the amide linkage was attacked, but no difficulties were encountered in the present work. N-Propyl-4′-sulfanilylacetanilide (**4**) was



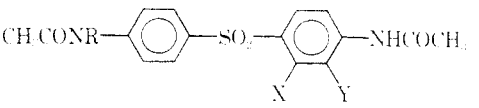
II

III

IV

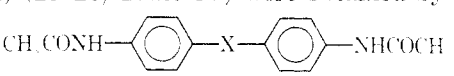
V

obtained by the catalytic hydrogenation of N-allyl-4′-[(*p*-nitrophenyl)sulfonyl]acetanilide. Acylation of the 4′-sulfanilylanilides (IV) with the appropriate acid chloride, anhydride, or ester afforded the unsymmetrical 4′-(N-acylsulfanilyl)anilides[15,17] V ($R_1 \neq R_2$) (**8**, **10**, **12**, **13**, **16**, Table II). The symmetrical 4′-(N-acylsulfanilyl)anilides[16,18,19] V ($R_1 = R_2$) (**7**, **9**, **11**, **14**, **15**, **17**, **18**, Table II), including 4′,4‴-sulfonylbisformanilide (DFDDS) (**7**) and 4′,4‴-sulfonylbisacetanilide (DADDS) (**9**), were prepared by acylation of 4,4′-sulfonyldianiline (DDS) (I) with excess formic acid, acetic anhydride, or the appropriate acid chloride. The 2′,3′-substituted 4′,4‴-sulfonylbisacetanilides VI



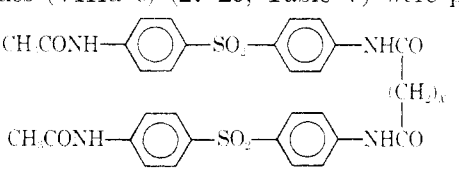
VI

(**19–22**, Table III) and other 4′,4‴-bisacetanilides interrupted with S, SO, COCO, or $CHCCl_3$ functions (VIIa–d) (**23–26**, Table IV) were obtained by treating



VIIa, X = S
b, X = SO
c, X = COCO
d, X = $CHCCl_3$

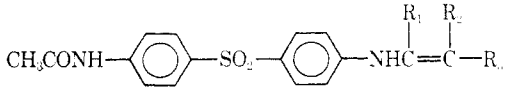
the corresponding diamines with excess Ac_2O in AcOH. Representative 4′,4‴-bis(N-acetylsulfanilyl)alkylenebisanilides (VIIIa–c) (**27–29**, Table V) were prepared



VIIIa, $x = 2$
b, $x = 4$
c, $x = 8$

by the acylation of 2 moles of 4′-sulfanilylacetanilide (MADDS) (**2**) with 1 mole of succinyl chloride, adipyl chloride, and sebacyl chloride in a pyridine–acetone mixture.

Various 4′-[N-(substituted vinyl)sulfanilyl]acetanilides (IXa–d) (**30–33**, Table VI) were obtained by the



	R_1	R_2	R_3
IXa,	H	CN	CN
b,	CN	CN	CN
c,	H	CN	$CO_2C_2H_5$
d,	H	$CO_2C_2H_5$	$CO_2C_2H_5$

(13) (a) P. E. Thompson, B. Olszewski, and J. A. Waitz, *Am. J. Trop. Med. Hyg.*, **14**, 343 (1965); (b) P. E. Thompson, *Intern. J. Leprosy*, **35**, 605 (1967); (c) P. E. Thompson and A. Bayles, unpublished results.

(14) (a) C. C. Shepard, *Proc. Soc. Exp. Biol. Med.*, **124**, 430 (1967); (b) C. C. Shepard, D. H. McRae, and J. A. Habas, *ibid.*, **122**, 893 (1966).

(15) H. Heymann and C. Heidelberger, *J. Am. Chem. Soc.*, **67**, 1986 (1945).

(16) G. W. Raiziss, L. W. Clemence, M. Severac, and J. C. Moetsch, *ibid.*, **61**, 2763 (1939).

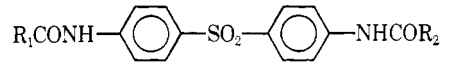
(17) H. A. Shonle and A. M. VanArendonk, *ibid.*, **65**, 2375 (1943).

(18) H. Heymann and L. F. Fieser, *ibid.*, **67**, 1979 (1945).

(19) V. A. Zasosov, *J. Gen. Chem. USSR*, **17**, 471 (1947).

TABLE II

4′-(N-ACYLSULFANILYL)ANILIDES

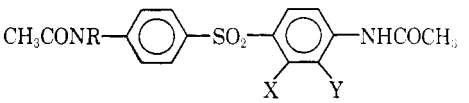


No.	R_1	R_2	Mp, °C	Yield purifd, %	Purifcn solvent	Formula	Analyses[25]	Solubility in pH 7 PB, mg/ml	Repository act., PMW[a]
7	H	H	240, 267–269[b]	75	DMF–H_2O	$C_{14}H_{12}N_2O_4S$	C, H, N, S	0.013	++
8	H	CH_3	287–289[c]	48	DMF–H_2O	$C_{15}H_{14}N_2O_4S$	C, H, N, S	0.012	++
9	CH_3	CH_3	287–288[d]	75	DMF	$C_{16}H_{16}N_2O_4S$	C, H, N	0.0030	++++
10	CH_3	C_2H_5	231–233[e]	79	EtOH	$C_{17}H_{18}N_2O_4S$	C, H, N	0.0090	+++
11	C_2H_5	C_2H_5	225–227[f]	67	EtOH	$C_{18}H_{20}N_2O_4S \cdot 0.2H_2O$	C, H, N, H_2O	0.0035	+
12	CH_3	$(CH_2)_4CH_3$	204–208[g]	75	EtOH–H_2O	$C_{20}H_{24}N_2O_4S$	C, H, N	<0.0001	+++
13	CH_3	C_6H_4OH-*o*	265–271	76	DMF–MeOH	$C_{21}H_{18}N_2O_5S$	C, H, N	<0.001	—
14	$(CH_2)_4CH_3$	$(CH_2)_4CH_3$	189–191[h]	79	EtOH	$C_{24}H_{32}N_2O_4S$	C, H, N	<0.0001	+
15	C_6H_5	C_6H_5	287–289[i]	66	DMF–EtOH	$C_{26}H_{20}N_2O_4S$	C, H, N	<0.0001	—
16	CH_3	$(CH_2)_{10}CH_3$	172–173[j]	72	EtOH	$C_{26}H_{36}N_2O_4S$	C, H, N	<0.0001	++
17	$(CH_2)_{10}CH_3$	$(CH_2)_{10}CH_3$	148–149[k]	81	EtOH	$C_{36}H_{56}N_2O_4S$	C, H, N	<0.0002	—
18	$(CH_2)_{14}CH_3$	$(CH_2)_{14}CH_3$	144–146[l]	88	EtOH	$C_{54}H_{72}N_2O_4S$	C, H, N	<0.0002	—

[a] See footnote *a*, Table I. [b] Lit.[18] mp 268–270°. [c] Lit.[15] mp 289–290°. [d] Lit.[16] mp 285°. [e] Lit.[17] mp 227–228°. [f] Lit.[19] mp 222°. [g] Lit.[17] mp 197–198°. [h] Lit.[19] mp 186°. [i] Lit.[19] mp 287°. [j] Lit.[17] mp 168–170°. [k] G. A. H. Buttle, T. Dewey, G. H. Foster, W. H. Grey, S. Smith, and D. Stephenson [*Biochem. J.*, **32**, 1101 (1938)] report mp 148°. [l] Lit.[19] mp 145°.

TABLE III

2′,3′-SUBSTITUTED 4′,4‴-SULFONYLBISACETANILIDES

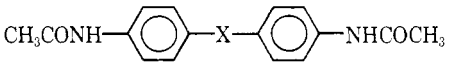


No.	R	X	Y	Mp, °C	Yield purifd, %	Purifcn solvent	Formula	Analyses[25]	Solubility in pH 7 PB, mg/ml	Repository act., PMW[a]
19	H	H	Cl	269–270	78	EtOH–H_2O	$C_{16}H_{15}ClN_2O_4S$	C, H, N	0.002	—
20	H	Cl	H	211–213	73	EtOH–H_2O	$C_{16}H_{15}ClN_2O_4S \cdot 0.3H_2O$	C, H, N, H_2O	0.004	+
21	H	H	CH_3	256–258	96	EtOH–Et_2O[b]	$C_{17}H_{18}N_2O_4S$	C, H, N	0.02	+++
22	$(CH_2)_2CH_3$	H	H	143–145	63	EtOH	$C_{19}H_{22}N_2O_4S$	C, H, N	0.09	+

[a] See footnote *a*, Table I. [b] Product was triturated with EtOH and Et_2O.

TABLE IV

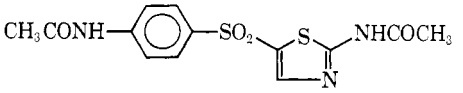
OTHER 4′4‴-BISACETANILIDES



No.	X	Mp, °C	Yield purifd, %	Purifcn solvent	Formula	Analyses[25]	Solubility in pH 7 PB, mg/ml	Repository act., PMW[a]
23	S	219–221[b]	83	EtOH	$C_{16}H_{16}N_2O_2S$	C, H, N	0.007	—
24	SO	289 dec[c]	59	DMF–H_2O	$C_{16}H_{16}N_2O_3S$	C, H, N	0.02	—
25	COCO	245–247[d]	42	Me_2CO–H_2O	$C_{18}H_{16}N_2O_4 \cdot H_2O$	C, H, N, H_2O	0.002	—
26	$CHCCl_3$	270–271[e]	59	DMF–H_2O	$C_{18}H_{17}Cl_3N_2O_2$	C, H, N	<0.0001	—

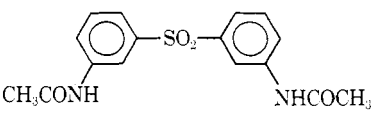
[a] See footnote *a*, Table I. [b] Lit.[16] mp 223–224°. [c] S. Sugasawa and K. Sakurai [*J. Pharm. Soc. Japan*, **60**, 22 (1940)] report mp 278°. [d] R. Kuhn, E. F. Möller, and G. Wendt [*Chem. Ber.*, **76**, 405 (1943)] report mp 251°. [e] S. Kirkwood and P. H. Phillips [*J. Am. Chem. Soc.*, **69**, 934 (1947)] report mp 275° dec.

condensation of 4′-sulfanilylacetanilide (MADDS) (**2**) with (ethoxymethylene)malononitrile, tetracyanoethylene, ethyl 2-cyano-3-ethoxyacrylate, and diethyl (ethoxymethylene)malonate, respectively. 4′-[(2-Acetamido-5-thiazolyl)sulfonyl]acetanilide (X), a thiazole congener of DADDS (**9**), was prepared by acetylation of



X

N-(5-sulfanilyl-2-thiazolyl)acetamide with Ac_2O in AcOH, while 3′,3‴-sulfonylbisacetanilide (XI), a position isomer of DADDS, was obtained from 3,3′-sulfonyldianiline and AcCl in pyridine. The condensation of

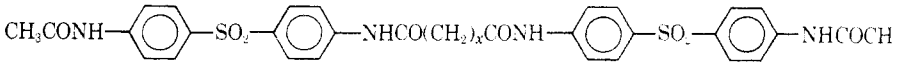


XI

4,4′-sulfonyldianiline (DDS) with formaldehyde and 2-thiazoline-2-thiol afforded 3,3′-[sulfonylbis(*p*-phenyleneiminomethylene)]di-2-thiazolidinethione (XII).

TABLE V

4′,4‴-BIS(N-ACETYLSULFANILYL)ALKYLENEBISANILIDES

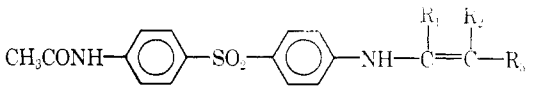


No.	x	Mp, °C	Yield purifd, %	Purifen solvent	Formula	Analyses[25]	Solubility in pH 7 PB, mg/ml	Repository act., PMW[a]
27	2	359 dec	74	DMF-H_2O	$C_{22}H_{30}N_4O_8S_2 \cdot 0.25H_2O$	C, H, N, H_2O	<0.0001	—
28	4	308–310	69	DMF-EtOH-H_2O	$C_{24}H_{34}N_4O_8S_2 \cdot 0.5H_2O$	C, H, N, H_2O	0.0006	—
29	8	256–259	70	DMF-EtOH	$C_{28}H_{42}N_4O_8S_2 \cdot H_2O$	C, H, N, H_2O	<0.0001	—

[a] See footnote *a*, Table I.

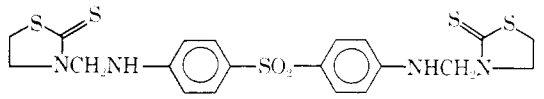
TABLE VI

4′-[N-(SUBSTITUTED VINYL)SULFANILYL]ACETANILIDES



No.	R_1	R_2	R_3	Mp, °C	Yield purifd, %	Purifen solvent	Procedure	Formula	Analyses[25]	Repository act., PMW[a]
30	H	CN	CN	310–311 dec	16	DMF-H_2O	I	$C_{18}H_{14}N_4O_3S$	C, H, N	—
31	CN	CN	CN	238–241 dec	68	MeCN	II	$C_{19}H_{13}N_5O_3S$	C, H, N	—
32	H	CN	$CO_2C_2H_5$	236–239	43	*i*-PrOH	II	$C_{20}H_{19}N_3O_5S$	C, H, N	—
33	H	$CO_2C_2H_5$	$CO_2C_2H_5$	188–190	33	*i*-PrOH	II	$C_{22}H_{24}N_2O_7S$	C, H, N	—

[a] See footnote *a*, Table I.



XII

4′,4‴-Sulfonylbisacetanilide (acedapsone, DADDS) (**9**) and the related sulfanilylanilides described in the present communication were supplied to Dr. P. E. Thompson and coworkers of these laboratories for evaluation as potential repository antimalarial agents utilizing *Plasmodium berghei* in the mouse. As in previous work,[2b,2c] drugs were suspended in 5 ml/kg of benzyl benzoate–castor oil (40:60) and given to groups of 15–25 albino mice in a single 400-mg/kg sc dose. Subgroups of treated mice were then challenged by the intraperitoneal injection of 15 million *P. berghei* at weekly or biweekly intervals. Assessment of repository action was based on the period of protection against patent infections afforded by a single dose of the drug. Activity is expressed as the number of weeks 50% of the mice were protected (PMW), and activity ratings (Tables I–VI) are assigned within the following ranges (weeks protected): ++++, >10; +++, 7–10; ++, 4–7; +, 1–4; —, <1.

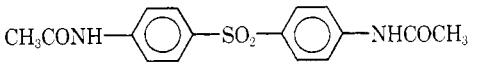
Among the 4′-sulfanilylanilides (IV) (Table I), 4′-(N-acylsulfanilyl)anilides (V) (Table II), and 2′,3′-substituted 4′,4‴-sulfonylbisacetanilides (VI) (Table III), nine compounds (**5–10**, **12**, **16**, **21**) protected mice against challenge with *P. berghei* for periods ranging from 4 to >10 weeks. 4′,4‴-Sulfonylbisacetanilide (acedapsone, DADDS) (**9**) conferred the longest protection and was designated for expanded studies (*vide infra*). Repository antimalarial effects were abolished or drastically reduced when DADDS was modified by (1) replacement of the acetamide groups with a formamide function (MFDDS, **1**), (2) replacement of both acetamide groups with amide functions containing more than two carbon atoms (**11**, **14**, **15**, **17**, **18**), (3) alkylation of one acetamide function (**4**, **22**), (4) introduction of a chlorine atom at positions 2′ or 3′ (**19**, **20**), or (5) replacement of the sulfone moiety by a thio, sulfinyl, oxalyl, or 2,2,2-trichloroethylidene linkage (**23–26**, Table IV). The 4′,4‴-bis(N-acetylsulfanilyl)alkylenebisanilides (VIIIa–e) (**27–29**, Table V), 4′-[N-(substituted vinyl)sulfanilyl]acetanilides (IXa–d) (**30–33**, Table VI), 4′-[(2-acetamido-5-thiazolyl)sulfonyl]acetanilide (X), 3′,3‴-sulfonylbisacetanilide (XI), and 3,3′-[sulfonylbis(*p*-phenyleneiminomethylene)]di-2-thiazolidinethione (XII) also lacked appreciable repository action. Solubility studies with the sulfanilylanilides in pH 7 phosphate buffer (Tables I–VI, Experimental Section) indicate that the length of repository action of a given amide cannot be reliably predicted on the basis of aqueous solubility.

4′,4‴-Sulfonylbisformanilide (DFDDS) (**7**, Table II) is currently undergoing clinical evaluation as an oral prophylactic antimalarial agent alone, and in combination with chloroquine and primaquine, under the auspices of the Walter Reed Army Institute of Research.[20a] In the present work, both the repository and therapeutic antimalarial properties of DFDDS were examined. DFDDS showed only modest repository activity (Table II), but exhibited strong therapeutic activity against *P. berghei* in the mouse when administered continuously in the diet for 6 days. The SD_{90} (daily dose required for 90% suppression of the parasitemia) for DFDDS, DDS, and quinine was <0.3, 0.5, and 74.5 mg/kg, respectively. Therefore DFDDS had an order of activity similar to DDS against the sensitive parent line and was approximately 250 times as potent as quinine. These values are consistent with the results of Aviado[20b] who reported the SD_{50} of DDS and DFDDS to be 0.5 and 0.25 mg/kg, respectively, following single subcutaneous injections daily for 3 days. By contrast, the SD_{90} of DFDDS against a line of

(20) (a) Personal communication, Dr. D. P. Jacobus, Walter Reed Army Institute of Research, Washington, D.C.; (b) D. M. Aviado, *Exp. Parasitol.*, **20**, 88 (1967); (c) personal communication, Dr. T. H. Maren, The University of Florida, Gainesville, Fla.

P. berghei made completely resistant (>600-fold) to DDS was >137 mg/kg/day.[13c] Thus, the DDS-resistant strain was >450-fold cross-resistant to DFDDS. These data, coupled with observation that the antimalarial activity of DFDDS is reversed by PABA,[20c] suggest that the principal mode of action of DFDDS and DDS is similar.

4′,4‴-Sulfonylbisacetanilide (acedapsone, DADDS) (XIII) has received extensive evaluation as a repository



XIII

antimalarial agent alone and in combination with cycloguanil pamoate.[2a,13a,b,21,22] Against trophozoite-induced *P. berghei* infections in the mouse, a single 100–400-mg/kg sc dose of DADDS almost invariably prevented or suppressed patent infections for 6–14 weeks.[2a,13a] In contrast to mice, rats were not protected against *P. berghei* challenges by a subcutaneous dose of DADDS. Metabolic studies subsequently showed that this host specificity was due to the fact that mice deacetylated DADDS efficiently while rats did not.[13b] These observations agree with other evidence which indicates that at least one free amino group is essential for microbiological activity by a sulfone.[4] It is also noteworthy that the repository action of DADDS would have been missed had rats instead of mice been routinely employed as the screening host.

In rhesus monkeys, a 50-mg/kg im dose of DADDS prevented patent *P. cynomolgi* infections for 63–268 (average 158) days, and greatly suppressed the parasitemia for many weeks longer.[2a,13a] Therapeutic tests against established patent *P. cynomolgi* infections in monkeys showed that DADDS suppressed the parasitemia slowly during the acute phase. Studies against sporozoite-induced infections of *P. cynomolgi* in rhesus monkeys indicated that DADDS lacked appreciable activity against tissue stages, but had long suppressive action against blood forms.[13b] A comparison of DADDS, cycloguanil pamoate, and a 1:1 mixture against lines of *P. berghei* highly resistant to either DDS or to cycloguanil hydrochloride demonstrated that the mixture had broader repository action against these drug-resistant lines than DADDS or cycloguanil pamoate alone.[2a,13a]

The results of clinical antimalarial studies with the cycloguanil pamoate–DADDS mixture have been encouraging.[21,22] The drug, suspended in a benzyl benzoate–castor oil (40:60) vehicle, was administered in a single, intramuscular dose at 4-month intervals in doses ranging from 3.2–10 mg/kg of each component according to the age of the subject. Approximately 1000 subjects have received one dose, 650 a second, and 600 a third. The few local reactions that have been noted were similar to or milder than those reported following the use of cycloguanil pamoate alone. The concensus of the clinical results to date is as follows. Against *Plasmodium vivax*, the minimum period of protection is 5 months in strains susceptible to chlorguanide and no protection in strains highly resistant to chlorguanide. Against *Plasmodium falciparum*, the following results were obtained: (1) strains susceptible to chlorguanide and pyrimethamine, 4 months protection; (2) strains resistant to chlorguanide and pyrimethamine, 3 months; (3) strains from southeast Asia resistant to chlorguanide, pyrimethamine, and the 4-aminoquinolines, variable protection, approximately 2 months. Some additional prolongation of effects may be obtained, and the emergence of resistant strains made less likely, when a single oral dose of a 4-aminoquinoline is given concurrently with the cycloguanil pamoate–DADDS injection.[21] It is concluded that the administration of the cycloguanil pamoate–DADDS combination at 4-month intervals is particularly suitable for use: (a) for the protection of itinerant laborers, migrants, and nomads; (b) as a causal prophylactic for nonimmunes visiting malarious areas; (c) in the consolidation phase of malaria eradication campaigns and in problem areas; and (d) in the attack phase in conjunction with spraying.

In view of the promise of DADDS as a repository antimalarial agent, the drug was supplied to Dr. Charles C. Shepard, Communicable Disease Center, Atlanta, Ga., for evaluation as a repository antileprotic agent against *Mycobacterium leprae* in mice. A single 6-mg/kg sc dose of DADDS administered to mice at 2-month intervals afforded nearly complete suppression of *M. leprae*, while larger doses were completely suppressive.[14a] Thus, the repository effects of DADDS against *M. leprae* were even more striking than against *P. berghei*. These results are in accord with earlier observations that the minimum effective level of DDS in the diet against *M. leprae* in mice was about fivefold less than was necessary for activity against *P. berghei*.[10,14b] The results of a preliminary clinical trial with DADDS in lepromatous leprosy have also been promising.[23] In this study, a single intramuscular 225-mg dose of DADDS administered once every 77 days was as effective as oral DDS given in a dosage of 100 mg daily over a treatment period of 48 weeks.[23]

It is apparent from a variety of observations that DADDS is absorbed very slowly following intramuscular or intraperitoneal administration.[13a,b] Chemical analyses showed only trace amounts of DADDS or related sulfones in either the blood or urine of monkeys given 50 mg/kg or of rats given 400 mg/kg. Assays of the injected muscle in monkeys at various intervals after dosage confirmed that the drug is absorbed slowly. The prolonged antimalarial effects and low toxicity of DADDS concur with chemical evidence of slow release from the injection site and very low blood levels of active moiety. Studies in monkeys showed that DADDS implanted subcutaneously in dialysis sacks had protective action while the sacks were in place but not following their removal. Chemical analyses of the bag contents showed the average drug release rate during protection was only 1.0 mg/day.[13b] Thin layer chromatography of the urine of mice, rats, and monkeys given DADDS parenterally showed that in each species some of the drug was metabolized to MADDS and DDS, although the amount of DDS in rat urine was particu-

(21) D. F. Clyde, Abstracts, Eighth International Congresses on Tropical Medicine and Malaria, Teheran, Iran, Sept 7–15, 1968, p 1380.

(22) (a) R. H. Black, W. B. Hennessy, B. McMillan, B. B. Dew, and J. C. Biggs, *Med. J. Australia*, **2**, 801 (1966); (b) A. B. G. Laing, G. Pringle, and F. C. T. Lane, *Am. J. Trop. Med. Hyg.*, **15**, 838 (1966); (c) K. H. Rieckmann, *Trans. Roy. Soc. Trop. Med. Hyg.*, **61**, 189 (1967); (d) W. Chin, G. R. Coatney, and H. K. King, *Am. J. Trop. Med. Hyg.*, **16**, 13 (1967); (e) W. Chin, P. G. Contacos, G. R. Coatney, M. H. Jeter, and E. Alpert, *ibid.*, **16**, 580 (1967).

(23) C. C. Shepard, J. G. Tolentino, and D. H. McRae, *ibid.*, **17**, 192 (1968).

larly low.[18b] Presumably deacylases present in host tissues are involved in these transformations.[2a]

The above patterns of drug release favor continuous suppressive action, with less likelihood of toxicity from high drug blood levels. The over-all results with DADDS alone and in combination with cycloguanil pamoate encourage further evaluation of the cycloguanil pamoate–DADDS mixture in connection with the prevention and eradication of malaria, and of DADDS alone for the prophylaxis and treatment of leprosy.

Experimental Section[24,25]

Solubilities were estimated by suspending 0.1 g of sample in 10 ml of 0.1 *N* pH 7 phosphate buffer at 25° with mild agitation for 18–24 hr. After centrifugation, the uv absorption maxima from the clear supernatant solution were compared with those obtained from a solution of known concentration. Chromatography studies were done with Eastman Chromograph Sheet 6060 silica gel with fluorescent indicator.

4′-Sulfanilylanilides (IV) (Table I). 4′-Sulfanilylformanilide (MFDDS) (1).—A solution of 23 g (0.075 mole) of 4′-[(*p*-nitrophenyl)sulfonyl]formanilide[15] in 250 ml of THF was hydrogenated over 2 g of Ra-Ni at an initial pressure of 3.6 kg/cm². When the calculated amount of H_2 had been absorbed, the catalyst was removed by filtration and the filtrate was concentrated to dryness on a rotary evaporator. Crystallization of the residue from MeCN gave 15 g (72%), mp 168–170°.

The uv absorption curves (MeOH) showed maxima at 294 mμ (ε 26,100) and 256 mμ (ε 22,200) which are typical of 4′-sulfanilylanilides. The ir absorption curves (KBr disk) showed the expected carbonyl peak at 1690 cm⁻¹ and peaks at 3470 and 3370 cm⁻¹. The nmr curve (DMSO-d_6) shows 1 H singlets at δ 10.54 (CONH) and 8.37 (HCO), a 4 H singlet at 7.78 (sulfanilyl ring protons), a typical $A_2'B_2'$ pattern centered at 7.10 (formanilide ring protons), and a 2 H singlet at 6.10 (NH_2).

N-Propyl-4′-sulfanilylacetanilide (4).—A MeOH solution of 3.6 g (0.010 mole) of N-allyl-4′-[(*p*-nitrophenyl)sulfonyl]acetanilide[26] was hydrogenated in a Parr shaker over Ra-Ni. When the calculated amount of H_2 had been absorbed, the catalyst was removed by filtration and the residue was concentrated to dryness on a rotary evaporator. Recrystallization from C_6H_6 gave 2.7 g (75%) of colorless crystals, mp 126–128°.

4′-Sulfanilylheptananilide (5).—A solution of 78 g (0.20 mole) of 4′-[(*p*-nitrophenyl)sulfonyl]heptananilide[27] in 400 ml of DMF was hydrogenated over 3 g of Ra-Ni at an initial pressure of 3.6 kg/cm². After 40 hr the calculated amount of H_2 had been absorbed. The catalyst was removed by filtration and the filtrate was diluted with 500 ml of H_2O. The precipitate was collected and recrystallized from *i*-PrOH to give 50 g (70%) of an off-white solid, mp 163–164°.

4′-(N-Acylsulfanilyl)anilides (V) (Table II). 4′,4‴-Sulfonylbisformanilide (DFDDS) (7) was prepared by formylation of DDS (I) in essentially the manner described by Heymann and Fieser.[18] Recrystallization from DMF–H_2O proved to be more convenient than EtOH–H_2O. However, the product softened and resolidified at about 240° before melting at 267–269°. After storage for several years, it was observed that changes had taken place in the ir absorption (KBr disk) particularly at 810, 1180, and 3000–3200 cm⁻¹.

Proceeding on the assumption that a change in crystal form might have taken place, a small sample was heated at 245–260° for 15 min. The ir absorption curve obtained from this material was different from both previous curves. Next, a portion of the heated material was recrystallized from Me_2CO–H_2O to yield a sample which gave an ir curve which was not significantly different from the curve obtained originally. The uv absorption curves (MeOH) and microanalyses (C, H, N) were within the normal range for all samples. Further, all of these preparations melted at 267–269°. However, the original sample and the material obtained from acetone–H_2O softened and resolidified at about 240°. This indicates that DFDDS can exist in at least two different crystal forms, and that at least partial interconversion takes place on standing or on heating and recrystallization from Me_2CO–H_2O.

DFDDS (7), MFDDS (1), and DDS (I) are readily distinguished by tlc. For example, development on silica gel with EtOAc and examination under a uv lamp at 254 mμ shows DDS, the fastest moving component, as a deep blue spot, MFDDS following as a bright blue spot, and DFDDS moving slowest as a purple-gray spot.

4′,4‴-Sulfonylbisacetanilide (Acedapsone, DADDS) (9).—A mixture of 24.8 g (0.10 mole) of DDS (I), 75 ml of HOAc, and 25.5 g (0.25 mole) of Ac_2O was heated under reflux for 1 hr. After cooling to 25°, the precipitate was collected by filtration and washed well with H_2O, EtOH, and then Et_2O. Recrystallization from DMF gave 25 g (75%), mp 287–288°.

4′-(N-Acetylsulfanilyl)salicylanilide (13).—An intimate mixture of 14.5 g (0.050 mole) of MADDS (2) and 15 g (0.070 mole) of phenyl salicylate was heated to 170° when all of the MADDS had dissolved. After heating and stirring the solution at 200–215° for 1.8 hr, the mixture was cooled, and the solid was removed from the flask and broken up in a blender under cyclohexane. The insolubles were collected by filtration, washed with cyclohexane, and recrystallized twice from DMF–MeOH to give 15.5 g (76%), mp 265–271°.

2′,3′-Substituted 4′,4‴-Sulfonylbisacetanilides (VI) (Table III) and Other 4′,4‴-Bisacetanilides (VII) (Table IV). 3′-Chloro-4′,4‴-sulfonylbisacetanilide (20).—A mixture of 10 g (0.035 mole) of 3-chloro-4,4′-sulfonyldianiline[28,29] and 7.2 g (0.070 mole) of Ac_2O in 25 ml of AcOH was heated under reflux for 2 hr. Upon cooling, a precipitate formed, which was collected by filtration and washed with H_2O, EtOH, and Et_2O. Three recrystallizations from EtOH–H_2O gave 9.5 g, mp 211–213°.

Compounds **19**, **21**, and **22** (Table III) were prepared in the same manner from Ac_2O and 2-chloro-4,4′-sulfonyldianiline,[30] 4-sulfanilyl-*o*-toluidine,[30] and N-propyl-4,4′sulfonyldianiline,[29,31] respectively. When **22** did not precipitate from the AcOH, the solution was poured into cold H_2O and extracted with $CHCl_3$. Concentration and dilution with Et_2O gave a solid which was recrystallized from EtOH.

4′,4‴-Bis(N-acetylsulfanilyl)alkylenebisanilides (VIII) (Table V). 4′,4‴-Bis(N-acetylsulfanilyl)succinanilide (27).—To a suspension of 14.5 g (0.05 mole) of 4′-sulfanilylacetanilide (MADDS) (2) in 180 ml of Me_2CO and 8 ml of pyridine was slowly added with stirring 3.9 g (0.025 mole) of succinyl chloride while maintaining the temperature below 40°. The reaction mixture was stirred at room temperature for 20 hr, then poured into 250 ml of cold 5% HCl. The precipitate was collected by filtration, washed with H_2O, and dried *in vacuo* at 45° for 20 hr. Crystallization from DMF–H_2O gave 12.4 g (74%) of colorless crystals, mp 359° dec.

4′-[N-(Substituted Vinyl)sulfanilyl]acetanilides (IX) (Table VI). 4′-[N-(2,2-Dicyanovinyl)sulfanilyl]acetanilide (30). Procedure I.—A solution of 5.8 g (0.02 mole) of 4′-sulfanilylacetanilide (MADDS) (2) and 2.5 g (0.02 mole) of (ethoxymethylene)-malononitrile in 25 ml of DMF was heated at 100° for 3.5 hr. After cooling, the solution was poured into H_2O and the precipitate was collected. Two recrystallizations from DMF–H_2O gave 1.2 g (16%) of gold crystals, mp 310–311° dec.

4′-[N-(1,2,2-Tricyanovinyl)sulfanilyl]acetanilide (31). Procedure II.—To a solution of 5.8 g (0.02 mole) of 4′-sulfanilylacetanilide (MADDS) (2) in 25 ml of DMF was added portionwise 2.6 g (0.02 mole) of tetracyanoethylene. A slightly exothermic reaction occurred and the mixture initially turned dark blue in color, then became colorless. It was stirred for 15 min at room temperature, the temperature was raised to 50–60° for 15 min, and the product was poured into a mixture of ice and H_2O. A yellow gum precipitated which gradually solidified on standing. The product was

(24) Melting points (corrected) were taken on a Thomas–Hoover capillary melting point apparatus.

(25) Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values. Water determinations by the Karl Fischer method.

(26) K. Maruyama and K. Kawanabe, *Meiji Yakka Daigaku Kenkyu Kiyo*, **2**, 69 (1963); *Chem. Abstr.*, **60**, 14417*d* (1964).

(27) V. A. Zasosov, *J. Gen. Chem. USSR*, **17**, 477 (1947).

(28) S. S. Berg, *J. Chem. Soc.*, 1991 (1949).

(29) B. R. Baker, M. V. Querry, and A. F. Kadish, *J. Org. Chem.*, **15**, 402 (1950).

(30) G. P. Youmans and L. Doub, *Am. Rev. Tuberc.*, **54**, 287 (1946).

(31) A. L. Rawlins, U. S. Patent 2,454,835 (1948).

collected by filtration and crystallized three times from MeCN to give 5.3 g (68%) of a bright yellow solid, mp 238–241° dec.

4′[(2-Acetamido-5-thiazolyl)sulfonyl]acetanilide (X).—A mixture of 29.7 g (0.10 mole) of N-(5-sulfanilyl-2-thiazolyl)acetamide,[32] 75 ml of HOAc, and 13 g (0.13 mole) of Ac_2O was heated to boiling when a thick slurry formed. DMF (50 ml) was added and the mixture was heated under reflux for 1.5 hr. After cooling, the precipitate was collected by filtration and washed (H_2O). Recrystallization from DMF–EtOH gave 28.5 g (84%), mp 313–314° dec. *Anal.* ($C_{13}H_{13}N_3O_4S_2$) C, H, N.

3′,3‴-Sulfonylbisacetanilide (XI).—To a solution of 9.3 g (0.038 mole) of 3,3′-sulfonyldianiline[33] in 100 ml of Me_2CO and 7.5 ml of pyridine was added dropwise 8.8 g (0.11 mole) of AcCl, keeping the temperature below 40° by adjusting the rate of addition. After standing 24 hr at room temperature, the mixture was poured into 5% HCl, and the resulting precipitate collected. Recrystallization from EtOH gave 6.4 g (51%), mp 215–218°. *Anal.* ($C_{16}H_{16}N_2O_4S$) C, H, N.

3,3′-[Sulfonylbis(*p*-phenyleneiminomethylene)]di-2-thiazolidinethione (XII).—A mixture of 24.8 g (0.10 mole) of DDS, 23.8 g (0.20 mole) of 2-thiazoline-2-thiol, and 18 ml (0.20 mole) of formalin in 500 ml of *i*-PrOH was stirred and heated under reflux for 24 hr. The product was collected by filtration of the hot reaction mixture and recrystallized from DMF–H_2O to give 18 g (35%), mp 225–226°. *Anal.* ($C_{20}H_{22}N_4O_2S_3$) C, H, N, S.

(32) L. L. Bambas, *J. Am. Chem. Soc.*, **67**, 671 (1945).
(33) Tennessee Corp., Atlanta, Ga.

Acknowledgments.—The authors wish to express their appreciation to Dr. Paul E. Thompson and coworkers of these laboratories for the antimalarial evaluation of these compounds and to Dr. Charles C. Shepard of the Communicable Disease Center, Atlanta, Ga., for the leprosy studies. We also thank Dr. David B. Capps and Dr. Leslie M. Werbel for the preparation of several of the compounds described herein, Mr. William Pearlman for the performance of the hydrogenations described, Dr. J. M. Vandenbelt and coworkers for the spectral and solubility determinations, and Mr. Charles E. Childs and associates for the microanalyses.

Repository Drugs. V. 4′,4‴-[*p*-Phenylenebis(methylidyneimino-*p*-phenylenesulfonyl)]bisacetanilide (PSBA) and Related 4′,4‴-[Bis(imino-*p*-phenylenesulfonyl)]bisanilides, a Novel Class of Long-Acting Antimalarial and Antileprotic Agents[1]

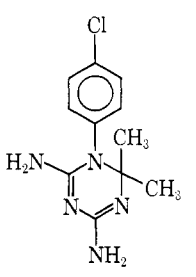
EDWARD F. ELSLAGER, ANNETTE A. PHILLIPS, AND DONALD F. WORTH

Research Laboratories, Parke, Davis and Company, Ann Arbor, Michigan 48106

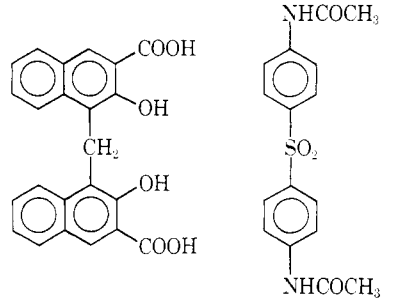
Received January 6, 1969

Various 4′,4‴-[bis(imino-*p*-phenylenesulfonyl)]bisanilides (III–VI, VIII) were synthesized as potential repository antimalarial and antileprotic agents in a search for long-acting sulfones that would be less dependent on enzymatic deacylation for activity and afford higher blood sulfone levels than 4′,4‴-sulfonylbisacetanilide (acedapsone, DADDS). The compounds were prepared by condensing the appropriate phthalaldehyde or naphthalenedicarboxaldehyde with the requisite 4′-sulfanilylanilide. Among them, 4′,4‴-[*p*-phenylenebis(methylidyneimino-*p*-phenylenesulfonyl)]bisacetanilide (PSBA) (IX) fulfilled the above requirements and showed remarkable repository effects alone against *Mycobacterium leprae* and alone or in combination with cycloguanil pamoate against *Plasmodium berghei* in mice and *Plasmodium cynomolgi* in monkeys. Structure–activity relationships are discussed.

A systematic search for various types of repository drugs in these laboratories led successively to the development of cycloguanil pamoate (I),[2–4] a long-acting antimalarial and antileishmania drug; acedapsone (DADDS) (II),[1,5–8] a repository antileprotic and antimalarial agent; and cycloguanil pamoate–acedapsone (DADDS),[1,5,6] a combination antimalarial drug with protracted action against drug-resistant strains.[9]



I



II

Among the 4′-sulfanilylanilide congeners of 4,4′-sulfonyldianiline (DDS) investigated previously,[1,5] 4′,4‴-sulfonylbisacetanilide (acedapsone, DADDS) (II) conferred the longest protection and has been studied extensively both in experimental animals and in man.[5–12]

(1) Previous paper: E. F. Elslager, Z. B. Gavrilis, A. A. Phillips, and D. F. Worth, *J. Med. Chem.*, **12**, 357 (1969).
(2) E. F. Elslager and P. E. Thompson, Abstracts, 9th National Medicinal Chemistry Symposium of the American Chemical Society, Minneapolis, Minn., June 1964, p 6A.
(3) P. E. Thompson, B. J. Olszewski, E. F. Elslager, and D. F. Worth, *Am. J. Trop. Med. Hyg.*, **12**, 481 (1963).
(4) Camolar®.
(5) E. F. Elslager and D. F. Worth, *Nature*, **206**, 630 (1965).
(6) P. E. Thompson, B. Olszewski, and J. A. Waitz, *Am. J. Trop. Med. Hyg.*, **14**, 343 (1965).
(7) (a) C. C. Shepard, *Proc. Soc. Exp. Biol Med.*, **124**, 430 (1967); (b) C. C. Shepard, D. H. McRae, and J. A. Habas, *ibid.*, **122**, 893 (1966).
(8) Acedapsone is the nonproprietary name for 4′,4‴-sulfonylbisacetanilide. In the biological literature acedapsone has also been referred to as sulfadiamine, 4,4′-diacetyldiaminodiphenyl sulfone, 4,4′-diacetylaminodiphenyl sulfone, N,N′-diacetyl-4,4′-diaminodiphenyl sulfone, and DADDS. Hansolar® is a proprietary name for acedapsone. The proprietary name for the acedapsone–cycloguanil pamoate combination is Dapolar®.
(9) For recent reviews, see (a) E. F. Elslager in "Annual Reports in Medicinal Chemistry, 1965," C. K. Cain, Ed., Academic Press, New York, N. Y., 1966, p 136; (b) E. F. Elslager in "Annual Reports in Medicinal Chemistry, 1966," C. K. Cain, Ed., Academic Press, New York, N. Y., 1967, p 131.
(10) D. F. Clyde, Abstracts, Eighth International Congresses on Tropical Medicine and Malaria, Teheran, Iran, Sept 7–15, 1968, p 1380.
(11) (a) R. H. Black, W. B. Hennessy, B. McMillan, B. B. Dew, and J. C. Biggs, *Med. J. Australia*, **2**, 801 (1966); (b) A. B. G. Laing, G. Pringle, and F. C. T. Lane, *Am. J. Trop. Med. Hyg.*, **15**, 838 (1966); (c) K. H. Rieckmann, *Trans. Roy. Soc. Trop. Med. Hyg.*, **61**, 189 (1967); (d) W. Chin, G. R. Coatney, and H. K. King, *Am. J. Trop. Med. Hyg.*, **16**, 13 (1967); (e) W. Chin, P. G. Contacos, G. R. Coatney, M. H. Jeter, and E. Alpert, *ibid.*, **16**, 580 (1967).
(12) C. C. Shepard, J. G. Tolentino, and D. H. McRae, *ibid.*, **17**, 192 (1968).