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Chemotherapeutic Evaluation of Some N^1- and N^4-Heterocyclic Derivatives of Sulfanilamide.

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The clinical success which has attended the use of sulfapyridine, and more recently, sulfathiazole, has stimulated an active interest in the heterocyclicsulfanilamide derivatives as chemotherapeutic agents. A review of the compounds of this class which have been tested against experimental infections lists the following groups of nuclear-coupled N^1-heterocyclicsulfanilamides other than those of pyridine and thiazole: those of tetrazole,[1] 1,3,4-thiadiazole,[1] pyrrolene,[2] pyrrolidine,[2] 5-pyrazolone,[1] pyrimidine,[1] benzothiazole[1] and quinoline.[3] Among the N^1-acylheterocyclics were the furoyl- and nicotinyl-derivatives.[4]

The substitution of halogen in the 5-position of 2-sulfanilamidopyridine (sulfapyridine) destroyed its activity, whereas substitution of nitro or amino groups in this position was claimed to enhance the antistreptococcic activity, but slightly depress the antipneumococcic activity.[5] The substitution of an amino group in the 6-position left the activities practically unaltered.[3,6] 3-Sulfanilamidopyridine[5] and it 6-chlor[5] and 6-brom[5] derivatives were as active as sulfapyridine, the 6-ethoxy derivative was slightly active,[5] the 6-amino[5] and 6-hydroxy[5] derivatives, inactive. The substitution of halogen in the 2-position of 5-sulfanilamidopyridine resulted in an active compound,[5] whereas the corresponding amino and nitro compounds were inactive.[5]

The therapeutic activity of 2-sulfanilamidothiazole (sulfathiazole) has, with one exception, been more sensitive to the effects of substitution than that of sulfapyridine. Substitution of a methyl group in the 4-position (sulfamethylthiazole) did not affect its antistreptococcic,[6,7] antipneumococcic,[6,7] or antistaphylococcic ac-

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tivity[8, 9] appreciably, although the substitution of a phenyl group in the 4-position diminished these activities.[7, 8]

The other groups of N^1-heterocyclic derivatives possess little or no therapeutic action, except 2-sulfanilamidopyrimidine (sulfadiazine). This compound is claimed to be as active as sulfapyridine and sulfathiazole against streptococcic,[1, 10] pneumococcic[1, 10] and staphylococcic[1, 10] infections, and considerably more active than either drug against experimental infections with Friedländer's bacillus.[10] The 4-methyl derivative, 2-(sulfanilamido)-4-methylpyrimidine, is reported to be as active as the parent sulfadiazine, whereas 4-sulfanilamidopyrimidine and 5-sulfanilamidouracil are inactive.[1]

N^4-2-pyridylsulfanilamide and N^4-2-quinylsulfanilamide were inactive against streptococcic and pneumococcic infections,[11] whereas N^4-bromotetronylsulfanilamide was claimed to be as active as sulfanilamide against streptococcic infections.[12]

Of the N^4-heterocyclic-acyl-sulfanilamides, the 2-furoyl[13] and 2-thenoyl derivatives[13] showed decreased activity; N^4-quinolinylsulfanilamide, activity equal to that of sulfanilamide[14] and N^4-(5-pyrrolidone-2-carbonyl)-sulfanilamide, even greater activity.[15] N^4-nicotinylsulfanilamide was claimed to be both more active[16] and less active than sulfanilamide.[13]

In the present study a number of previously unreported heterocyclic derivatives are compared to sulfapyridine, sulfathiazole, and sulfadiazine against experimental infections of mice. All mice dying within the 21-day period were autopsied and those not dying of the given infection were discarded from the series.

In the antistreptococcic and antipneumococcic evaluations, treatment was withheld for 3 hours to allow infection to become established. This method of assay was considered preferable to the prophylactic method of Bieter and co-workers,[17] in which the drug is

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TABLE I.
Streptococcic Infection of Mice

	Treatment	No. of Mice	No. of deaths daily during 21 days									No. of Survivors	% Survivors	Efficacy of each drug compared to that of every other drug*								
			1	2	3	4	5	6	7	8	9-21			I	H	G	F	E	D	C	B	A
	None	45	43		1		1					None	None	0	—	0	0	—	—	—	—	—
A	Sulfapyridine	44					3	2	1		5	33	75	+	0	+	+	+	+	+	0	
B	Sulfathiazole (1)	43	1				3	2	4		7	26	61	+	0	+	+	0	+	0		
C	2-(Sulfanilamido)-5-ethyl-4-thiazolone (2)	43		1		1	7	3	6	2	2	21	49	+	0	+	+	0	+			
D	2-(Sulfanilamido)-4, 5, 6, 7-tetrahydrobenzothiazole (1)	20	1			4	4	5	1	1	1	3	15	0	+	0	+	—				
E	2-(Sulfanilamido)-5-methyl-1, 3, 4-thiadiazine (1)	30		1	1		1				10	17	57	+	0	+	+					
F	N^4-Nicotinylsulfathiazole (1)	15	11	3								1	7	0	—	0						
G	N^4-Acetylsulfanilyl-2-methyl-4, 5-dihydroimidazole (3)	20	19	1								None	None	0	—							
H	2-(4-Nitrobenzenesulfonamido) thiazole (1)	20		1		1	4	3				11	35	+								
I	2-(Sulfanilamido)-6-amino-isonicotinic acid (4)	20	20									None	None									

Infection: 0.5 cc of a 10^{-4} broth dilution of an 18-hr broth culture (Richards) intraperitoneally (1000 fatal doses).

Treatment: 10 mg of drug suspended in 0.2 cc of 15% gum acacia orally 3 hours after infection, then once daily for 3 successive days (total 40 mg). At time of first treatment, blood cultures of 6 mice, selected at random from the series, averaged more than 7 colonies per cc.

Drugs were synthesized and donated by the (1) Maltbie Chemical Company, Newark, N. J.; (2) Sharp and Dohme, Glenolden, Pa.; (3) Monsanto Chemical Company, St. Louis, Mo.; and (4) Charles Pfizer and Company, Inc., New York, N. Y.

*The drugs in the left hand column are evaluated against those designated by letters A to I along the top by the symbols: +: better than; —: worse than; and 0: not significantly different from. This table was derived by the extraction of χ^2 from the original data. P values less than 0.05 were accepted as evidence of valid difference. Where significant difference occurs, "better than" or "worse than" were determined by inspection of original data.

administered in the ration for 48 hours before infection, because it more closely approximates clinical conditions, namely, the treatment of an established infection.

Streptococcic Infection. Table I shows that the 3 compounds: 2-(sulfanilamido)-5-methyl-1,3,4-thiadiazine, 2-(4-nitrobenzenesulfonamido)-thiazole and 2-(sulfanilamido)-5-ethyl-4-thiazolone (sulfaethylthiazolone) have activities comparable to that of sulfathiazole, but not as pronounced as that of sulfapyridine.

Pneumococcic Infection. Since the activity of sulfathiazole has been shown to be practically equal to that of sulfapyridine,[6, 10, 18, 19] the latter drug was used as a standard of comparison. Table II shows that sulfaethylthiazolone is practically as active as sulfapyridine.

Staphylococcic Infection. Since sulfaethylthiazolone showed adequate antistreptococcic and antipneumococcic activity, its therapeutic action was compared to that of sulfathiazole and sulfadiazine against staphylococcic infections. Reference to Table III shows that all 3 drugs possess comparable activity.

In considering the correlation between structure and therapeutic activity, it is generally accepted that the active phenylsulfonamides either possess a free N^4-amino group or a structure which is capable of yielding this group *in vivo*. However, the converse is not necessarily true. The activity of 2-(4-nitrobenzenesulfonamido) thiazole is accounted for by the fact that blood levels of 1.9, 3.6, and 2.8 mg % of diazotizable material, calculated as sulfathiazole, were observed in mice one, 4, and 7 hours, respectively, after the oral administration of 20 mg. Traces were observed at the end of 24 hours. The inactivity of nicotinylsulfathiazole is explained by the fact that mice which received 20 mg orally failed to show during 24 hours any appreciable free or conjugated diazotizable material which could be detected by the Bratton and Marshall[20] method, or our modification, which gives significantly higher recovery of free and conjugated sulfathiazole.[21]

Toxicity studies showed sulfaethylthiazolone to be less acutely toxic than sulfapyridine or sulfathiazole. Of 8 mice which received 25 mg orally twice daily until the time of death, 6 died between the eleventh and twenty-third days and 2 survived. Blood counts made

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TABLE II. Pneumococcic Infection of Mice.

	Treatment	No. of Mice	No. of deaths daily during 21 days									No. of Survivors	% Survivors	Efficacy of each drug compared to that of every other drug*					
			1	2	3	4	5	6	7	8	9-21			I	H	F	E	C	A
	None	75	14	49	12							None	None	0	—	0	—	—	—
A	Sulfapyridine	73		2	6	1	4	2	19	4	8	27	37	+	0	+	0	0	
C	2-(Sulfanilamido)-5-ethyl-4-thiazolone (2)	73		1	5	6	12	2	7	4	17	19	26	+	0	+	0		
E	2-(Sulfanilamido)-5-methyl-1, 3, 4-thiadiazine (1)	15		2	4	3	4					2	13	0	0	0			
F	N^4-Nicotinylsulfathiazole (1)	15		7	8							None	None	0	0				
H	2-(4-Nitrobenzenesulfonamido)thiazole (1)	15			1		1	2	5	1	3	2	13	0					
I	2-(Sulfanilamido)-6-amino-isonicotinic acid (4)	15	1	9	2	2	1					None	None						

Infection: 0.5 cc of a 10^{-5} broth dilution of an 18-hr broth culture (Binda, Type II) subcutaneously (650 fatal doses).

Treatment: 20 mg of drug suspended in 0.2 cc of 15% gum acacia orally 3 hours after infection, then once daily for 5 successive days (total 120 mg). (1) (2) (4)* See Legend of Table I.

TABLE III. Staphylococcic Infection of Mice.

	Treatment	No. of Mice	No. of deaths daily during 21 days									No. of Survivors	% Survivors	Efficacy of each drug compared to that of every other drug*		
			1	2	3	4	5	6	7	8	9-21			J	C	B
	None	71	6	12	12	12	3	4	2	2	3	15	21	—	—	—
B	Sulfathiazole (1)	64	1				1	1	1		12	48	75	0	0	
C	2-(Sulfanilamido)-5-ethyl-4-thiazolone (2)	64	1	2	1	2	2	1		3	14	38	59	0		
J	Sulfadiazine	65		1	2		3	5	2		8	44	68			

Infection: 0.2 cc of a broth suspension from an 18-hr blood agar culture (F.D.A. 209) intravenously (160 million cocci).

Treatment: 10 mg of drug suspended in 0.2 cc of 15% gum acacia orally at time of infection and 6 hours later, then twice daily for 4 days, followed by single daily treatment for 5 days (total 150 mg). (1) (2)* See legend of Table I.

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every second day showed a mean fall in erythrocytes from 9,200,000 to 5,821,000 during 16 days. In some of the fatalities, the erythrocyte count fell to 3,000,000 and less. No evidence of urolithiasis was observed in 30 rats (weighing approximately 200 g) which received 100 mg orally twice daily for 12 days, although 11 animals died within this time.

Sulfadiazine, on the contrary, produced renal failure in a number of infected mice. Since it was impossible to determine whether this was secondary to the damage caused by staphylococci, a series of normal mice and a group of normal rats were treated orally with similar and smaller doses of sulfadiazine. Gross inspection and microscopic examination (frozen sections of fresh tissue) of kidneys showed crystalline deposits, predominantly in the urinary papilla and often sufficiently massive to be capable of completely obstructing the urinary outflow. A more detailed study of this toxic manifestation will be reported elsewhere.[22]

Conclusions. 2-(Sulfanilamido)-5-ethyl-4-thiazolone (sulfaethylthiazolone) has less antistreptococcic activity and approximately the same antipneumococcic activity as sulfapyridine. Its antistaphylococcic activity is of the same order as that of sulfathiazole and sulfadiazine.

Sulfadiazine produces urolithiasis medicamentosa capable of causing death by acute suppression of urine in mice and rats, whereas sulfaethylthiazolone is free of this defect, but may cause fatal anemia.

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Erythrocyte Phosphatase Activity in Hemolysed Sera and Estimation of Serum "Acid" Phosphatases.

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Erythrocytes contain an apparently specific phosphatase[1,2,3] classified by Folley and Kay[4] as phosphomonoesterase A_4. This enzyme

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