Synthesis and Antimicrobial Activity of 3*H*-1,2,4-Thiadiazolo Fused Heterocycles

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Received May 19, 1978, from *Syntex Research, Palo Alto, CA 94304.* Accepted for publication July 12, 1978.

Abstract □ Various fused 3*H*-1,2,4-thiadiazoles were prepared. Significant *in vitro* Gram-positive antibacterial and antifungal activities were observed for certain members of the series.

Keyphrases □ 3*H*-1,2,4-Thiadiazoles, various—synthesized, evaluated for antibacterial and antifungal activities *in vitro* □ Antibacterial activity—various 3*H*-1,2,4-thiadiazoles evaluated *in vitro* □ Antifungal activity—various 3*H*-1,2,4-thiadiazoles evaluated *in vitro* □ Structure-activity relationships—various 3*H*-1,2,4-thiadiazoles evaluated for antibacterial and antifungal activities *in vitro*

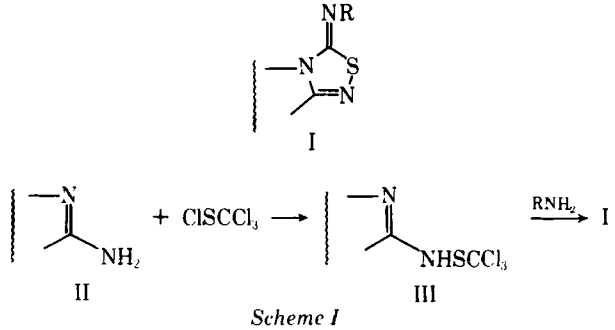
Fused ring systems represented by the general formula I are an interesting class of little-studied heterocyclic compounds for which there have been no reports of biological activity. The present report describes the synthesis and antimicrobial activity of a limited number of compounds of this type (Table I).

DISCUSSION

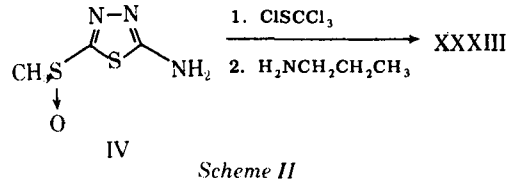
The synthetic sequences are outlined in Scheme I and are essentially those described by Potts and Kane (1). An α-amino *N*-heterocyclic compound (II) was first reacted with perchloromethylsulfur chloride to give the corresponding trichloromethyl sulfenamide (III). Treatment of III with an appropriate amine gave bicyclic compounds (I). While it was possible to isolate and characterize III, this step generally was not performed.

Oxidation of sulfides to the sulfoxides was usually done as a final step on I, using 40% peracetic acid. However, when I corresponded to fusion with a 1,3,4-thiadiazole and R was an aliphatic group, oxidation gave only complex mixtures. Thus, XXXIII was obtained by subjecting IV to the sequence of II to III to I (Scheme II). When I corresponded to fusion with a thiazole, oxidation with an aliphatic R group proceeded smoothly. Initially, the sulfides corresponding to the sulfoxides in Table I were isolated and purified. However, when the sulfides proved to lack biological activity, only the final products were thoroughly characterized.

While no attempt was made to improve yields, steric hindrance and electron-withdrawing groups on the amine adversely affected the III to I reaction. Treatment of III with 2,6-disubstituted anilines gave no reaction.



Scheme I



Scheme II

With the exception of V, VI, X, XIII, and XXXIV–XXXVI, the starting materials leading to the compounds in Table I were obtained by alkylation of commercially available 2-amino-5-mercapto-1,3,4-thiadiazole. Reaction of 2-amino-5-bromo-1,3,4-thiadiazole (2) with thiophenol gave 2-amino-5-phenylthio-1,3,4-thiadiazole, which was converted to X.

2-Amino-5-trifluoromethyl-1,3,4-thiadiazole (3), 3-mercapto-5-amino-1,2,4-thiadiazole (4), 2-amino-5-mercaptothiazole (5), and 3-amino-6-mercaptopyridazine (6) were prepared by literature methods and converted into XIII, XXXIV, XXXV, and XXXVI, respectively. Commercially available 2-amino-1,3,4-thiadiazole and 2-amino-5-methyl-1,3,4-thiadiazole were used to prepare V and VI. The amines were either commercially available or were prepared by standard synthetic transformations.

RESULTS

In vitro bacteriostatic and fungistatic activities are presented in Table II along with values for cephalothin sodium[1] and micronazole nitrate[2]. In addition, fungicidal activities are given for XIX, XXII, and XXVII (Table III).

Several generalities emerge. Significant activity within the series was realized only when the 1,2,4-thiadiazole was fused to a 1,3,4-thiadiazole. Replacement of one nitrogen (XXXV) or the sulfur by a vinyl group (XXXVI) of the 1,3,4-thiadiazole resulted in loss of activity. Slight activity was retained in the isomeric 1,2,4-thiadiazole derivative (XXXIV). Furthermore, of all Y groups tested, only the sulfoxides (and to a lesser extent the sulfone IX) were active.

The phenyl sulfoxide seemed to be the most effective Y group but was difficult to make, and the remaining comparisons of R groups were done with methyl sulfoxides. Significant activity was found only when R was an aryl group. Both antifungal and antibacterial activities appeared, at least in part, to be a function of lipophilicity. With the exception of sulfonamide derivatives, no Gram-negative activity was observed.

The most impressive results were antifungal, with *in vitro* fungistatic and fungicidal activities of some compounds at least as high as those of micronazole nitrate.

EXPERIMENTAL[3]

All compounds were prepared in a manner analogous to the synthesis of VII and VIII.

6-Methylthio-3-phenylimino-3*H* - 1,3,4-thiadiazolo[2,3-*c*][1,2,-4]thiadiazole (VII)—To a solution of 8.82 g (60 mmoles) of 2-amino-5-methylthio-1,3,4-thiadiazole in 500 ml of tetrahydrofuran with 6 g of sodium bicarbonate was added 7.2 ml (65 mmoles) of perchloromethylsulfur chloride. After stirring at room temperature for 1 hr, a solution of 6 g (65 mmoles) of aniline and 30 ml (200 mmoles) of triethylamine in 500 ml of tetrahydrofuran was added.

The mixture was stirred for another hour at room temperature and then filtered, and the filtrate was evaporated under reduced pressure. The residue was passed through a 100-g pad of silica gel with methylene chloride. The eluent was evaporated, and the residue was crystallized from methylene chloride–hexane to give 9 g of VII.

6-Methylsulfinyl-3-phenylimino-3*H* - 1,3,4-thiadiazolo[2,3-*c*][1,2,4]thiadiazole (VIII)—To a solution of 9 g (32 mmoles) of VII in 100 ml of chloroform was added 10 ml (~50 mmoles) of 40% peracetic

[1] Keflin, Eli Lilly and Co.
[2] Micatin, Johnson & Johnson.
[3] Melting points were determined in a Thomas-Hoover capillary apparatus and are uncorrected. NMR spectra were obtained in chloroform-d_1 with Varian A-60 and HA-100 instruments, and mass spectra were determined with a Varian-MAT CH4 spectrometer. Elemental analyses were performed by the analytical department of Syntex Research, Institute of Organic Chemistry. Chromatography was done on Merck silica gel 60.

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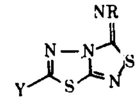
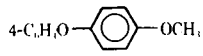
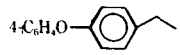
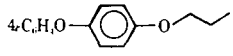
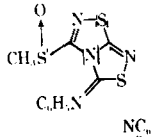
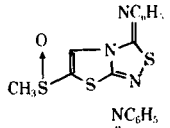
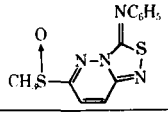


Table I—Physical Properties of Title Compounds

Compound	Y	R	Melting Point	Yield[a], %	Formula	Mass Spectrum, m/e (M+)	Analysis, % Calculated C	Calculated H	Calculated N	Found C	Found H	Found N
V	H	C_6H_5	148–151° [b]	49	$C_9H_6N_4S_2$	234	46.14	2.58	23.91	46.22	2.63	23.69
VI	CH_3	C_6H_5	139–142° [b]	30	$C_{10}H_8N_4S_2$	248	47.98	3.25	22.56	47.98	3.10	22.72
VII	CH_3S	C_6H_5	143–144° [b]	60	$C_{10}H_8N_4S_3$	280	42.84	2.88	19.99	42.50	3.01	19.90
VIII	$CH_3S(\rightarrow O)$	C_6H_5	167–168° [b]	25	$C_{10}H_8N_4OS_3$	296	40.53	2.72	18.90	40.11	2.69	18.82
IX	$CH_3S(\rightarrow O)_2$	C_6H_5	191–192° [b]	22	$C_{10}H_8N_4O_2S_3$	312	38.45	2.58	17.94	38.90	2.56	17.76
X	$C_6H_5S(\rightarrow O)$	C_6H_5	133–134° [c]	25	$C_{15}H_{10}N_4OS_3$	358	50.26	2.81	15.63	50.26	2.68	15.79
XI	$C_6H_5CH_2S(\rightarrow O)$	C_6H_5	167–168° [b]	35	$C_{16}H_{12}N_4OS_3$	372	51.59	3.24	15.04	51.47	3.15	15.22
XII	$CH_3CH_2CH_2S(\rightarrow O)$	C_6H_5	122–123° [b]	40	$C_{12}H_{12}N_4OS_3$	324	44.43	3.73	17.27	44.20	3.78	17.28
XIII	CF_3	C_6H_5	90–91° [b]	76	$C_{10}H_5F_3N_4S_2$	302	39.73	1.67	18.53	39.81	1.71	18.36
XIV	$CH_3S(\rightarrow O)$	4-C_6H_4Cl	188–189° [b]	33	$C_{10}H_7ClN_4OS_3$	330–332	36.31	2.13	16.94	36.49	1.81	16.91
XV	$CH_3S(\rightarrow O)$	3-C_6H_4Cl	157–159° [b]	36	$C_{10}H_7ClN_4OS_3$	330–332	36.31	2.13	16.94	36.12	2.06	16.77
XVI	$CH_3S(\rightarrow O)$	2-C_6H_4Cl	181–182° [b]	14	$C_{10}H_7ClN_4OS_3$	330–332	36.31	2.13	16.94	36.56	2.14	16.84
XVII	$CH_3S(\rightarrow O)$	2,4-$C_6H_3Cl_2$	198–199° [b]	4	$C_{10}H_6Cl_2N_4OS_3$	364–368	32.88	1.66	15.34	32.59	1.39	15.27
XVIII	$CH_3S(\rightarrow O)$	4-$C_6H_4OCH_3$	190–192° [b]	20	$C_{11}H_{10}N_4O_2S_3$	326	40.48	3.09	17.17	40.13	3.39	16.77
XIX	$CH_3S(\rightarrow O)$	4-C_6H_4*n*-C_4H_9	134–135° [b]	32	$C_{14}H_{16}N_4OS_3$	352	47.71	4.58	15.90	47.54	4.86	15.75
XX	$CH_3S(\rightarrow O)$	4-C_6H_4CN	186–189° [b]	15	$C_{11}H_7N_5OS_3$	321	41.11	2.20	21.79	41.16	2.16	21.43
XXI	$CH_3S(\rightarrow O)$	4-$C_6H_4CO_2C_2H_5$	195–197° [b]	18	$C_{13}H_{12}N_4O_3S_3$	368	42.38	3.29	15.21	42.51	3.27	15.01
XXII	$CH_3S(\rightarrow O)$	4-$C_6H_4CF_3$	168–169° [b]	12	$C_{11}H_7F_3N_4OS_3$	364	36.26	1.94	15.38	35.92	1.88	14.99
XXIII	$CH_3S(\rightarrow O)$	4-$C_6H_4OC(=O)C_6H_5$	186–188° [c]	31	$C_{17}H_{12}N_4O_3S_3$	416	49.03	2.90	13.45	48.68	2.95	13.20
XXIV	$CH_3S(\rightarrow O)$	4-$C_6H_4SO_2NH_2$	212–214° [c]	15	$C_{10}H_9N_5O_3S_4$	375	31.99	2.42	18.65	31.81	2.71	18.47
XXV	$CH_3S(\rightarrow O)$	4-$C_6H_4SO_2N(CH_3)_2$	218–219° [b]	12	$C_{12}H_{13}N_5O_3S_4$	403	35.72	3.25	17.36	35.52	3.31	17.00
XXVI	$CH_3S(\rightarrow O)$	4-SO_2N(morpholino)	218–219° [c]	15	$C_{14}H_{15}N_5O_4S_4$	445	37.74	3.39	15.79	37.65	3.47	15.49
XXVII	$CH_3S(\rightarrow O)$	4-$C_6H_4OC_6H_5$	152–153° [b]	38	$C_{16}H_{12}N_4O_2S_3$	388	49.47	3.11	14.42	49.48	3.05	14.41
XXVIII	$CH_3S(\rightarrow O)$	4-C_6H_4O-C_6H_4-$C(CH_3)_3$	121–122° [b]	20	$C_{20}H_{20}N_4O_2S_3$	444	54.03	4.54	12.60	54.18	4.56	12.45

(continued)

Table I—*Continued*

Compound	Y	R	Melting Point	Yield[a], %	Formula	Mass Spectrum, *m/e* (M^+)	Analysis, % Calculated C	H	N	Found C	H	N
XXIX			164–165°[b]	21	$C_{17}H_{14}N_4O_3S_3$	418	48.79	3.27	13.39	48.54	3.28	13.03
XXX			145–147°[b]	39	$C_{18}H_{16}N_4O_2S_3$	416	51.90	3.87	13.45	52.03	3.77	13.18
XXXI			147–149°[b]	36	$C_{20}H_{20}N_4O_3S_3$	460	52.16	4.38	12.16	52.40	4.37	12.13
XXXII		4-$C_6H_4OC_6H_5$	157–158°[b]	50	$C_{17}H_{14}N_4O_2S_3$	402	50.73	3.51	13.92	50.68	3.39	13.81
XXXIII		$CH_2CH_2CH_3$	130–131°[c]	85	$C_7H_{10}N_4OS_3$	262	32.05	3.84	21.36	31.90	3.95	21.30
XXXIV			152–154°[b]	40	$C_{10}H_8N_4OS_3$	296	40.53	2.72	18.90	40.25	2.68	18.90
XXXV			198–200°[b]	33	$C_{11}H_9N_3OS_3$	295	44.73	3.07	14.23	44.51	2.81	13.89
XXXVI			195–196°[b]	24	$C_{12}H_{10}N_4OS_2$	290	49.64	3.47	19.30	49.43	3.66	19.26

[a] Yields are overall values for II → III → I and the oxidation step except where there was no oxidation. [b] Recrystallized from methylene chloride–hexane. [c] Recrystallized from acetone–hexane.

Table II—Antimicrobial Activities[a]

Compound	Bacteriostatic A	B	C	D	E	F	Fungistatic G	H	I	J	K
V	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	—[c]	—[c]	—[c]	—[d]	—[d]
VI	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	—[c]	—[c]	—[c]	—[d]	—[d]
VII	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	—[c]	—[c]	—[c]	—[d]	—[d]
VIII	6.25	25	200	12.5	200	50	3	1	3	100	30
IX	12.5	25	200	200	—[b]	—[b]	3	1	3	30	30
X	0.05	0.8	—[b]	200	200	—[b]	1	1	3	30	10
XI	50	100	—[b]	—[b]	—[b]	—[b]	—[c]	1	—[c]	—[d]	—[d]
XII	6.25	50	—[b]	—[b]	—[b]	—[b]	3	1	3	—[d]	30
XIII	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	—[c]	—[c]	—[c]	—[d]	—[d]
XIV	3.12	3.12	—[b]	50	—[b]	—[b]	1	0.1	1	10	30
XV	50	50	—[b]	—[b]	—[b]	—[b]	1	1	1	30	10
XVI	6.25	12.5	—[b]	—[b]	—[b]	—[b]	3	1	3	30	30
XVII	1.6	3.12	—[b]	—[b]	—[b]	—[b]	1	0.1	1	10	10
XVIII	6.25	25	—[b]	200	—[b]	—[b]	—	—	—	—	—
XIX	1.6	1.6	—[b]	—[b]	—[b]	—[b]	1	0.1	1	3	3
XX	100	12.5	200	50	—[b]	200	3	3	3	300	30
XXI	25	50	—[b]	—[b]	—[b]	—[b]	1	1	3	—[d]	—[d]
XXII	25	12.5	—[b]	—[b]	—[b]	—[b]	1	0.1	1	10	10
XXIII	50	1.6	—[b]	—[b]	—[b]	—[b]	—[c]	1	—[c]	—[d]	—[d]
XXIV	12.5	12.5	100	25	200	100	—[c]	—[c]	—[c]	—[d]	—[d]
XXV	25	12.5	200	100	—[b]	200	100	10	30	—[d]	—[d]
XXVI	50	12.5	—[b]	—[b]	—[b]	—[b]	100	30	100	—[d]	—[d]
XXVII	0.1	0.8	200	200	—[b]	—[b]	1	0.1	1	3	10
XXVIII	3.12	3.12	—[b]	—[b]	—[b]	—[b]	—[d]	1	—[d]	—[d]	—[d]
XXIX	3.12	3.12	—[b]	—[b]	—[b]	—[b]	1	0.1	1	—[d]	—[d]
XXX	12.5	3.12	—[b]	—[b]	—[b]	—[b]	3	0.1	1	—[d]	—[d]
XXXI	3.12	200	—[b]	200	—[b]	—[b]	100	1	100	—[d]	—[d]
XXXII	1.6	1.6	200	200	200	—[b]	1	0.1	—[d]	—[d]	—[d]
XXXIII	12.5	25	100	25	—[b]	100	30	10	30	300	100
XXXIV	100	25	—[b]	—[b]	—[b]	—[b]	10	3	10	100	30
XXXV	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	—[c]	—[c]	—[c]	—[d]	—[d]
XXXVI	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	—[c]	30	—[c]	—[d]	—[d]
Cephalothin sodium	0.05	<0.008	50	0.05	—[b]	—[b]	—	—	—	—	—
Micronazole nitrate	—	—	—	—	—	—	3	0.1	3	30	0.2

[a] Values are minimum inhibitory concentrations in micrograms per milliliter. Assay procedures are described under *Experimental*. A = *Staphylococcus aureus* (ATCC 6538P), B = *Streptococcus pyogenes* (ATCC 8668), C = *Escherichia coli* (ATCC 25922), D = *Klebsiella pneumoniae* (ATCC 10031), E = *Pseudomonas aeruginosa* (ATCC 10145), F = *Proteus vulgaris* (ATCC 9484), G = *Microsporum gypseum* (ATCC 14683), H = *Epidermophyton floccosum* (ATCC 15643), I = *Trichophyton mentagrophytes* (ATCC 11481), J = *Candida albicans* (ATCC 10231), and K = *Cryptococcus neoformans* (ATCC 13690). [b] >200. [c] >100. [d] >300.

Table III—Fungicidal Activities [a]

Compound	Fungicidal G	H	I	J	K
XIX	1	1	1	30	10
XXII	1	1	3	30	10
XXVII	1	0.1	1	—[b]	10
Micronazole nitrate	30	1	30	30	10

[a] See footnotes to Table II. [b] >300.

acid. After stirring at room temperature for 3 hr, a 10% aqueous solution of sodium metabisulfite was added until starch–iodide paper gave a negative result.

Excess saturated potassium bicarbonate solution was then added, and the layers separated. The organic phase was dried (magnesium sulfate) and evaporated under reduced pressure. The residue was chromatographed, using 1% methanol–methylene chloride to elute material that crystallized from methylene chloride–hexane; 4.2 g of VIII was obtained.

Antimicrobial Assays[4]—The bioassays were done by serial broth dilutions in chemically defined media according to the procedure described by Long *et al.* (7).

[4] The bioassays were conducted under the direction of Dr. A. Braemer and Ms. S. Hitt, Institute of Agrisciences, Syntex Research.

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ACKNOWLEDGMENTS

This paper is Contribution No. 504 from the Syntex Institute of Organic Chemistry.

Potential CNS Antitumor Agents VI: Aziridinylbenzoquinones III

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Received February 21, 1978, from the *Drug Design and Chemistry Section, Laboratory of Medicinal Chemistry and Biology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20014.* Accepted for publication June 29, 1978.

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Abstract □ Thirty-one aziridinylbenzoquinones were compared against five murine tumor models *in vivo*. Two intracerebral (ependymoblastoma and L-1210 leukemia) and three intraperitoneal (P-388 and L-1210 leukemia and B16 melanoma) systems were utilized. Excellent activity was observed for many compounds. Multiple long-term survivors were produced in the ependymoblastoma, P-388, and intraperitoneal L-1210 systems. Diethyl 2,5-bis(1-aziridinyl)-3,6-dioxo-1,4-cyclohexadiene-1,4-dicarbamate demonstrated superior activity in all five test systems. This compound also was reproducibly active against two colon tumors, a mammary tumor, and the intracerebrally implanted P-388 leukemia model.

Keyphrases □ Aziridinylbenzoquinones—evaluated as potential CNS antitumor agents *in vivo*, various test systems □ CNS antitumor activity—aziridinylbenzoquinones evaluated *in vivo*, various test systems □ Antitumor agents, CNS—aziridinylbenzoquinones evaluated *in vivo*, various test systems □ Structure-activity relationships—aziridinylbenzoquinones evaluated as potential CNS antitumor agents *in vivo*, various test systems

The antitumor activity of aziridinylbenzoquinones in murine model tumor systems has been recognized for almost 25 years (1–5). Recent reports described the activity of two members of this family, trenimon (6–8) and carbazilquinone (8–11), which have had clinical trials.

As part of a program to develop agents that might be effective against neoplasms of the central nervous system (CNS), several series of aziridinylbenzoquinones were prepared and evaluated in murine brain tumor systems (12, 13). Some of these compounds produced long-term survivors in the intracerebral ependymoblastoma tumor model. To determine which aziridinylbenzoquinones might have the greatest potential for clinical trial with emphasis on CNS neoplasms, the 31 analogs available to the National Cancer Institute (NCI) were compared in two intracerebral and three intraperitoneal tumor systems.

EXPERIMENTAL

Materials—Compounds XIV–XXXI (Table I) were synthesized as described previously (12, 13), and I–XIII were obtained from other sources[1].

Tumor Test Systems—The standard NCI protocols for the intracerebral and intraperitoneal tumors are described in Table II and Ref. 14.

Treatment—Treatment was intraperitoneal in all cases. Saline (0.1%) or hydroxypropylcellulose was used as the vehicle. Treatment in all systems other than ependymoblastoma began on Day 1 and continued once daily for 9 days. In a few instances, previous data were available with

[1] Ciba-Geigy, Bayer A. G., Sankyo Co., the U.S. Department of Agriculture, and Dr. H. S. Verter of the Inter American University of Puerto Rico.

0022-3549/79/0200-0185$01.00/0