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(22) The signal for C-10 was hidden in the background noise because it was split into a quintet, so that the peak heights became as low as those of noise signals.

3-[(1,2,3-Thiadiazol-5-ylthio)methyl]cephalosporins[1]

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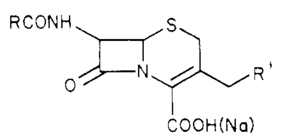
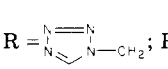
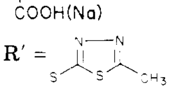
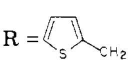
The syntheses of ten 3-[(1,2,3-thiadiazol-5-ylthio)methyl]cephalosporins, made by displacement of the 3′-acetoxy group by the novel thiol derivatives, potassium 1,2,3-thiadiazole-5-thiolate and dipotassium 1,2,3-thiadiazole-4-carboxylate-5-thiolate, are described. Several of the compounds showed good in vitro antibacterial activity against both Gram-positive and Gram-negative organisms. The subcutaneous in vivo activities against *Staphylococcus aureus* were generally less than that of cefazolin. Four of the compounds were administered orally and all were active; the 7β-(thiophen-2-acetamido) and 7β-(D-2-amino-2-phenylacetamido)-3-[(1,2,3-thiadiazol-5-ylthio)methyl] compounds were equally active by either route, with a PD_{50} of ca. 1 mg/kg.

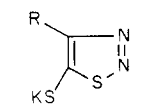
One of the more important reactions in the preparation of cephalosporin antibiotics is the displacement, by nucleophiles, of the allylic acetoxy group at C_3. A wide range of heteroaromatic thiols have been used in the reaction, and some of the products have shown enhanced activity against Gram-negative bacteria,[2] depending on the nature of the heterocycle and the substituent at C_7. Among those compounds which have reached advanced testing or clinical use are compounds derived from displacement of the acetoxy group with 5-methyl-1,3,4-thiadiazole-2-thiol [e.g., cefazolin (**1a**)[3]], 1-methyl-1,2,3,4-tetrazole-5-thiol,[4] and 1,2,3-triazole-5-thiol.[5] Recent work in these laboratories[6] has led to the synthesis of the novel 1,2,3-thiadiazole-5-thiol derivatives **2a** and **2b**. It was of interest to de-



1a, R = ; R′ = 

b, R = ; R′ = $OCOCH_3$

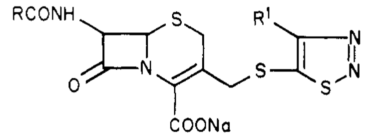
c, R = $C_6H_5CH(NH_2)$; R′ = $OCOCH_3$

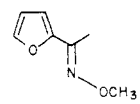
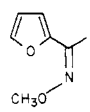
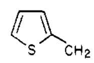
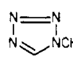
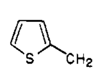


2a, R = H
b, R = COOK

termine the antibacterial activities of cephalosporins incorporating these previously inaccessible groups at the 3 position.

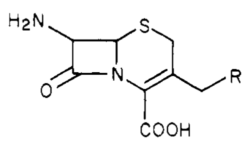
Table I. In Vitro Antibacterial Activities of 3-[(1,2,3-Thiadiazol-5-ylthio)methyl]cephalosporins



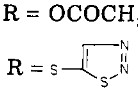
			min inhib concn, μg/mL[a]				
compd	R	R'	S.a.[b] (S)	S.a.[c] (R)	S.p.[d]	E.c.[e]	K.p.[f]
5	$C_6H_5OCH_2$	H	<0.0008	0.025	<0.0008	200	0.8
6	$N{\equiv}CCH_2$	H	0.025	3.1	0.013	25	25
7		H	0.3	0.3	nt[g]	10	0.8
8		H	0.6	10	nt	160	5
9	$C_6H_5CH(OH)$	H	0.5	3.1	<0.0008	25	0.1
10	$C_6H_5CH(NH_2)$	H	1.6	3.1	0.003	25	0.1
11		H	0.006	0.05	<0.0008	100	0.2
12		H	0.05	1.6	0.025	50	6.3
13		COONa	0.4	1.6	0.05	200	25
14	$C_6H_5CH(OH)$	COONa	0.8	25	0.2	25	25
1a (cefazolin)			3.12	0.2	0.0125	3.1	0.4
1b (cephalothin)			0.2	0.2	0.025	50	1.6
1c (cephaloglycin)			6.25	6.25	0.1	25	1.6

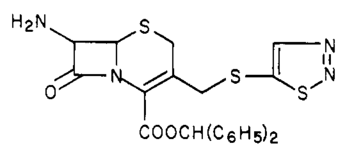
[a] See Experimental Section for procedure used. [b] *Staphylococcus aureus* ATCC 6538 P (penicillin G sensitive). [c] *Staphylococcus aureus* ATCC 14154 (penicillin G resistant). [d] *Streptococcus pyogenes* ATCC 8668. [e] *Escherichia coli* ATCC 25922. [f] *Klebsiella pneumoniae* ATCC 10031. [g] nt, not tested. [h] This compound was tested as the free acid rather than the sodium salt.

Chemistry. Reactions between 7-aminocephalosporanic acid (**3a**) and the thiolate **2a** gave the displacement



3a, R = $OCOCH_3$

b, R = 



4

product **3b**, which, after treatment with diphenyldiazomethane and chromatographic purification, gave the ester **4** in an overall yield of 49%. Acylation of **4**, under various conditions, gave the appropriate 7-(acylamino) products. A number of compounds were made by displacement of the acetoxyl group from cephalosporanic acids which already incorporated an acylamino group at C_7. In these cases, the crude products were esterified by treatment with diphenyldiazomethane prior to purification. For bioassay, the diphenylmethyl-protecting group was removed by treatment with trifluoroacetic acid/anisole, and the resultant acids were converted to sodium salts.

Antibacterial Activity. The compounds were first tested in vitro against seven bacterial strains; the results against five of these are shown in Table I; none of the compounds was active against the remaining two, *Pseudomonas aeruginosa* and *Proteus vulgaris*. Some of the compounds were also tested in vivo, by subcutaneous or oral administration, against *Staphylococcus aureus* (Smith), and the results are shown in Table II.

Table II. In Vivo[a] Antibacterial Activities

	PD_{50}, mg/kg: *S. aureus* (Smith)	
compd	subcutaneous	oral
5	0.54 (0.24)[b]	1.93 (1.03)[c]
10	0.93 (0.24)[b]	<1.25 (1.03)[c] 1.48 (NR)[d]
11	0.86 (0.61)[b]	<1.25 (1.03)[c] 1.53 (NR)[d]
12	0.41 (0.18)[b]	2.27 (1.03)[c]
13	1.8 (1.0)[b]	

[a] See Experimental Section for procedure used. [b] PD_{50} for cefazolin, run in the same assay. [c] PD_{50} for cephaloglycin, run in the same assay. [d] No standard agent was run in this assay.

Discussion

The results will be examined to determine, if possible, the most promising compound(s) in the series, and, by means of specific comparisons, to determine the effect of the 1,2,3-thiadiazole system relative to that of other 3 substituents, namely, the 2-methyl-1,3,4-thiadiazole of cefazolin (**1a**) and the acetoxymethyl of cephalothin (**1b**) and cephaloglycin (**1c**).

With the exception of the 7-(phenylglycyl) compound **10**, all the compounds showed good activity against penicillin-sensitive *S. aureus*; the phenoxyacetyl compound **5** was particularly active. All the compounds tested showed high activity against *S. pyogenes*, especially **5** and the thienyl derivative **11**. Against resistant *S. aureus*, however, the activity of the 1,2,3-thiadiazoles decreased markedly, with the exception of **11**, which, while not very potent, showed equal activity against both resistant and sensitive strains. Cephalosporins bearing the 7-(*syn*-2-methoximinofur-2-yl) side chain have been noted previously to be resistant to β-lactamase inactivation,[7] and it appears that the effect is carried over to the 1,2,3-thiadiazole series. The anti isomer **8** was less active overall and was also susceptible to β-lactamase. The compounds showed a wide variation of in vitro activities against Gram-negative organisms. Compounds **7**, **9** and **10** have activities comparable to that of cefazolin, the most active of the standard agents. The results of in vivo testing are shown in Table II. For each result, the PD_{50} of the standard, run in the same assay, is shown in parentheses, since the values vary according to the severity of the challenge. All compounds showed activity by both subcutaneous and oral routes. The subcutaneous PD_{50} values are somewhat higher than those of the standard cefazolin. The oral activities of **10** and **11** were comparable to that of the standard cefaloglycin. Furthermore, the compounds showed approximately equal PD_{50} values by either route; this property has been used as a criterion for selection of potential oral cephalosporins.[8] With the exception of **10** (see below), the existence of oral activity in the 1,2,3-thiadiazole series is unexpected. Despite considerable efforts, it has not been found possible to predict the occurrence of oral activity in cephalosporins, apart from the fact that most 7-(phenylglycyl) compounds show this property.[9] Indeed, the oral activities of a number of 3-(heterocyclic substituted thiomethyl) compounds were found in many cases to be lower than those of the corresponding 3-(acetoxymethyl) or 3-methyl compounds.[10] The presence of the 4′-carboxylate in **13** and **14** resulted in a decrease in activity, notwithstanding the fact that a number of cephalosporins bearing an acidic substituent (e.g., CH_2COOH, CH_2SO_3H) on the 3-heterocycle have been reported to have good antibacterial activities.[11]

The effect of the 1,2,3-thiadiazole can also be assessed by comparing **12** and cefazolin, which differ only in that cefazolin (**1a**) has a 5-methyl-1,3,4-thiadiazole as the 3′-heterocycle. Gram-positive activities against *Streptococcus pyogenes* in vitro are similar, but cefazolin is more active against Gram-negative organisms in vitro and against *S. aureus* in vivo. Similar comparisons can be made between **11** and cephalothin (**1b**) and between **10** and cephaloglycin (**1c**) to determine the effect of the 1,2,3-thiadiazol-5-ylthio group relative to that of the acetoxy group present in both standards. The compounds bearing the acetoxy groups are, in almost every case, somewhat less active than the thiadiazole compounds. The differences are particularly pronounced against *S. pyogenes* and *Klebsiella pneumoniae*. The 1,2,3-thiadiazole moiety is thus an interesting and potentially promising heterocycle for introduction into the cephalosporin antibiotics. The occurrence of oral activity in all the compounds tested is particularly noteworthy.

Experimental Section

Melting points are uncorrected; infrared spectra were recorded with a Perkin-Elmer 237B spectrometer and ultraviolet spectra with a Cary 14 instrument. NMR spectra were obtained with Varian A-60 and HA-100 and Bruker WH 90 instruments. Chemical shifts are in parts per million and coupling constants (J) in hertz. $MgSO_4$ was used as drying agent. The purities of the bioassay samples were determined to be not less than ca. 95% by the absence of extraneous peaks in the NMR spectra. In addition, a small quantity of each sodium salt was converted back to the free acid and compared by TLC (silica gel, 3:1 CH_2Cl_2–CH_3COOH) to a sample of the acid before conversion to the sodium salt. In all cases, the two samples of acid were found to be identical and homogeneous.

7β-Amino-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4-carboxylic Acid (3b). A solution of 7-aminocephalosporanic acid (2.62 g, 0.96 mM), potassium 1,2,3-thiadiazole-5-thiolate[6] (1.72 g, 1.1 mM), and $NaHCO_3$ (0.88 g, 1.03 mM) in water (40 mL) and acetone (30 mL) was refluxed for 2 h. The solution was cooled and acidified to pH 3.5 with 2 N hydrochloric acid; the precipitated solid was filtered, washed with acetone, and dried, to give **3b** (2.16 g, 68%) as an off-white solid: UV (MeOH) 273 nm, 305 (ϵ 6800, 4500); NMR (Me_2SO-d_6) 3.45, 3.71 (two d, J = 18 Hz, 2-H), 4.23 (s, CH_2S thiadiazole), 4.77 (d, J = 4 Hz, 8-H), 5.52 (d, J = 4 Hz, 7-H), 8.86 ppm (s, 4′-H).

Diphenylmethyl 7β-Amino-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4-carboxylate (4). Diphenyldiazomethane (1.94 g, 10 mM) and **3b** (2.13 g, 0.65 mM) were stirred in the dark in MeCN (50 mL) for 3.5 days. The solution was filtered, the filtrate was evaporated, and the residue therefrom was chromatographed on four 100 × 20 cm × 0.75 mm thick preparative silica gel plates (CH_2Cl_2–MeOH, 98:2) to give 2.13 g (70%) of **4** as a foam: UV (MeOH) 275 nm (ϵ 8400); NMR ($CDCl_3$) 3.30, 3.56 (two d, J = 18 Hz, 2-H), 3.90, 4.08 (two d, J = 13 Hz, CH_2S thiadiazole), 4.71 (d, J = 4 Hz, 8-H), 4.88 (d, J = 4 Hz, 7-H), 6.89 (s, $CHPh_2$), 8.30 ppm (s, 4′-H).

Sodium 7β-(Phenoxyacetamido)-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4-carboxylate (5). A solution of phenoxyacetyl chloride (200 mg, 1.17 mM) in CH_2Cl_2 (1 mL) was added with stirring to a solution of **4** (300 mg, 0.63 mM) and pyridine (2 mL) in CH_2Cl_2 (30 mL). After 2 h, water was added and the organic layer was washed with 2 N hydrochloric acid, aqueous $NaHCO_3$, and brine, then dried, and evaporated. The residue was chromatographed on one 100-cm silica gel plate (Et_2O), to afford 224 mg (58%) of **diphenylmethyl 7β-(phenoxyacetamido)-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4-carboxylate** as a gum: NMR ($CDCl_3$) 3.33, 3.55 (two d, J = 17 Hz, 2-H), 3.97, 4.11 (two d, J = 13 Hz, CH_2S thiadiazole), 4.57 (s, $PhOCH_2$), 4.98 (d, J = 5 Hz, 8-H), 5.88 (dd, J = 9 and 5 Hz, 7-H), 8.34 ppm (s, 4′-H). This material was dissolved in anisole (2 mL) at 0 °C, and CF_3COOH (8 mL) was added with ice cooling. The mixture was stirred at 0 °C for 5 min and then quickly evaporated to dryness. The residue was partitioned between EtOAc and 0.2 N $NaHCO_3$. The aqueous solution was washed with Et_2O, then acidified with 2 N hydrochloric acid, and extracted with EtOAc (50 mL). The extract was washed with brine, dried, filtered, and reduced to 10 mL. A solution of sodium 2-ethylhexanoate (200 mg, 1.27 mM) in EtOAc (2 mL) was then added, and the mixture was stirred for 30 min. The solid was filtered and washed with EtOAc and then hexane, to afford 95 mg (56%) of **5** as a buff powder: mp ~180 °C; UV (H_2O) 268 nm (ϵ 11600); NMR (Me_2SO-d_6) 3.35, 3.55 (two d, J = 18 Hz, 2-H), 4.38, 4.54 (two d, J = 13 Hz, CH_2S thiadiazole), 4.60 (s, $PhOCH_2$), 5.00 (d, J = 4 Hz, 8-H), 5.53 (dd, J = 8 and 4 Hz, 7-H), 9.00 (d, J = 8 Hz, NH), 9.02 ppm (s, 4′-H).

Sodium 7β-(Cyanoacetamido)-3-[(1,2,3,-thiadiazol-5-ylthio)methyl]ceph-3-em-4-carboxylate (6). Cyanoacetic acid (40 mg, 0.47 mM), dicyclohexylcarbodiimide (DCC; 80 mg, 0.30 mM), and **4** (120 mg, 0.25 mM) were stirred in CH_2Cl_2 (10 mL) for 2 h. Water and EtOAc were added, and the organic layer was dried and evaporated. The residue was chromatographed on silica gel (CH_2Cl_2–acetone, 10:1) to afford 128 mg (93%) of **diphenylmethyl 7β-(cyanoacetamido)-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4-carboxylate**: mp 82–84 °C; UV (MeOH) 257 nm (ϵ 8600); NMR ($CDCl_3$) 3.55, 3.77 (two d, J = 18 Hz, 2-H), 3.73 (s, CH_2S thiadiazole), 4.15 (s, CH_2CN), 5.20 (d, J = 4 Hz, 8-H), 5.74 (dd, J = 8 and 4 Hz, 7-H), 6.96 (s, $CHPh_2$), 8.71 ppm (s, 4′-H). This compound was converted, in 68% yield, as described above, to the sodium salt **6**: mp ~150 °C dec; UV (H_2O) 259 nm (ϵ 6800); NMR (Me_2SO-d_6) 3.40 (brs, 2-H), 3.72 (s, CH_2S thiadiazole), 4.43 (s, CH_2CN), 4.94 (d, J = 4 Hz, 8-H), 5.44 (dd, J = 8 and 4 Hz, 7-H), 8.98 ppm (s, 4′-H).

Condensation between **4** and *syn*-2-(methoxyimino)furan-2-acetic acid,[12] as described above, gave 40% of **diphenylmethyl 7β-[*syn*-2-(methoxyimino)-2-(furan-2-yl)acetamido]-3-[(1,-2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4-carboxylate** as a gum: NMR ($CDCl_3$) 3.48 (br s, 2-H), 4.02 (s, CH_2S thiadiazole), 4.02 (s, OCH_3), 5.02 (d, J = 4 Hz, 8-H), 5.89 (dd, J = 8 and 4 Hz, 7-H), 6.86 (s, $CHPh_2$), 8.31 ppm (s, 4′-H). This was converted, as described above, into **sodium 7β-[*syn*-2-(methoxyimino)-2-(furan-2-yl)acetamido]-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4-carboxylate (7)**: mp 149–152 °C; UV (H_2O) 219 nm, 278 (ε 25 200, 21 000); NMR (Me_2SO-d_6) 3.53 (br s, 2-H), 4.01 (s, CH_2S thiadiazole), 4.07 (s, OCH_3), 5.02 (d, J = 4 Hz, 8-H), 5.58 (dd, J = 8 and 4 Hz, 7-H), 9.08 (s, 4′-H), 9.50 ppm (d, J = 8 Hz, NH). Similarly, reaction between 4 and *anti*-2-(methoxyimino)furan-2-acetic acid[12] gave 52% of the *anti*-diphenylmethyl ester as a gum: UV (MeOH) 221 nm, 278 (ε 23 200, 21 000); NMR ($CDCl_3$) 3.37, 3.60 (two d, J = 18 Hz, 2-H), 4.05 (s, CH_2S thiadiazole), 4.09 (s, OCH_3), 5.03 (d, J = 4 Hz, 8-H), 5.91 (dd, J = 8 and 4 Hz, 7-H), 6.90 (s, $CHPh_2$), 8.33 ppm (s, 4′-H). This was converted in 30% yield into **sodium 7β-[*anti*-2-(methoxyimino)-2-(furan-2-yl)acetamido]-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4-carboxylate (8)**: mp ~180 °C dec; UV (H_2O) 269 nm (ε 16 200); NMR (Me_2SO-d_6) 3.50 (br s, 2 H), 4.11 (s, CH_2S thiadiazole), 4.09 (s, OCH_3), 5.08 (d, J = 4 Hz, 8-H), 5.49 (dd, J = 8 and 4 Hz, 7-H), 9.04 (s, 4′-H) 9.44 ppm (d, J = 8 Hz, NH).

Sodium 7β-(D-Mandelamido)-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4-carboxylate (9). The amine 4 (250 mg, 0.52 mM) and D-mandelic acid *O*-carboxyanhydride[13] (178 mg, 1 mM) were stirred in CH_2Cl_2 (15 mL) for 2 h. Water was added and the CH_2Cl_2 solution was washed with 0.2 N $NaHCO_3$, 2 N hydrochloric acid, and water, then dried, and evaporated to give 300 mg (88%) of **diphenylmethyl 7β-(D-mandelamido)-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4-carboxylate** as a gum: UV (MeOH) 267 nm (ε 10 800); NMR ($CDCl_3$) 3.24, 3.45 (two d, J = 18 Hz, 2-H), 3.96 (s, CH_2S thiadiazole), 4.88 (d, J = 4 Hz, 8-H), 5.08 (s, CHOH), 5.67 (dd, J = 8 and 4 Hz, 7-H), 6.95 (s, $CHPh_2$), 8.30 ppm (s, 4′-H). This compound was converted in 77% yield to the sodium salt 9: mp ~150 °C dec; UV (H_2O) 264 nm, 311 (ε 9900, 3900); NMR (Me_2SO-d_6) 3.31, 3.53 (two d, J = 18 Hz, 2-H), 4.46 (s, CH_2S thiadiazole), 4.96 (d, J = 5 Hz, 8-H), 5.11 (s, CHOH), 5.54 (dd, J = 10 and 5 Hz, 7-H), 8.55 (d, J = 10 Hz, NH), 8.95 ppm (s, 4′-H).

7β-(D-2-Amino-2-phenylacetamido)-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4-carboxylic Acid (10). The amine 4 (249 mg, 0.5 mM), DCC (103 mg, 0.5 mM), and *N*-(*tert*-butoxycarbonyl)-D-α-phenylglycine (125 mg, 0.5 mM) were stirred in THF (10 mL) for 3 h. The solvent was removed under vacuum and the residue was chromatographed on one preparative silica gel plate (hexane–acetone, 20:1) to afford 265 mg (82%) of **diphenylmethyl 7β-[D-2-(*tert*-butoxyformamido)-2-phenylacetamido]-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4-carboxylate** as a gum: UV (MeOH) 260 nm, 268, 280 (ε 8400, 8800, 8500); NMR ($CDCl_3$) 1.41 (s, *t*-Bu), 3.22, 3.45 (two d, J = 18 Hz, 2-H), 3.97 (s, CH_2S thiadiazole), 4.89 (d, J = 4 Hz, 8-H), 5.76 (dd, J = 8 and 4 Hz, 7-H), 6.85 (s, $CHPh_2$), 8.30 ppm (s, 4′-H). This compound was dissolved in anisole (0.5 mL) and cooled to 0 °C; TFA (2 mL) was added, and the solution was stirred at 0 °C for 30 min. The solvents were removed under vacuum, and the residue was triturated with Et_2O–hexane (1:1), to afford 135 mg (79%) of **7β-(D-2-amino-2-phenylacetamido)-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4-carboxylic acid trifluoroacetate** as a pale brown solid: NMR (CF_3COOH) 3.70 (br s, 2-H), 3.90, 4.60 (two d, J = 13 Hz, CH_2S thiadiazole), 5.26 (d, J = 4 Hz, 8-H), 5.81 (dd, J = 8 and 4 Hz, 7-H), 8.92 ppm (s, 4′-H).

Sodium 7β-(Thiophen-2-acetamido)-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4-carboxylate (11). A solution of Keflin (645 mg, 1.54 mM), the thiolate **2a** (253 mg, 1.63 mM), and $NaHCO_3$ (100 mg, 1.19 mM) in water (6 mL) was heated to 50 °C for 24 h. The cooled solution was acidified with 2 N hydrochloric acid and extracted with EtOAc. Diphenyldiazomethane (1 g, 5.22 mM) was added to the dried extract; after 1 h, the solution was evaporated and the residue was chromatographed on two 100 × 20 cm × 0.5 nm thick silica gel plates ($CHCl_3$–acetone, 15:1) to give 151 mg (15%) of **diphenylmethyl 7β-(thiophen-2-acetamido)-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4-carboxylate** as a gum: UV (MeOH) 260 nm, 269 (ε 9000, 8900); NMR ($CDCl_3$) 3.37 (br s, 2-H), 3.79 (s, CH_2CONH), 4.00 (s, CH_2S thiadiazole), 4.92 (d, J = 4 Hz, 8-H), 5.78 (dd, J = 9 and 4 Hz, 7-H), 6.70 (d, J = 9 Hz, NH), 7.25 (s, $CHPh_2$), 8.31 ppm (s, 4′-H). Anal. ($C_{29}H_{24}N_4O_4S_4$) C, H. This compound was converted, in 65% yield, to the sodium salt 11: mp ~175 °C dec; UV (H_2O) 232 nm, 260 (ε 14 200, 9800); NMR (Me_2SO-d_6) 3.41 (br s, 2-H), 3.72 (s, CH_2CONH), 4.43 (br s, CH_2S thiadiazole), 4.96 (d, J = 4 Hz, 8-H), 5.47 (dd, J = 8 and 4 Hz, 7 H), 9.01 ppm (s, 4′-H).

Sodium 7β-(1*H*-Tetrazol-1-ylacetamido)-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4-carboxylate (12). Sodium 7β-(1*H*-tetrazol-1-ylacetamido)-3-(acetoxymethyl)ceph-3-em-4-carboxylate[14] and the thiolate **2a** were reacted together, and the product was esterified and purified, as described above, to give 22% of **diphenylmethyl 7β-(1*H*-tetrazol-1-ylacetamido)-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4-carboxylate** as a gum: NMR ($CDCl_3$) 3.47 (br s, 2-H), 3.84, 4.12 (two d, J = 13 Hz, CH_2S thiadiazole), 4.99 (d, J = 5 Hz, 8-H), 5.19 (s, CH_2CONH), 5.78 (dd, J = 8 and 5 Hz, 7-H), 6.85 (s, $CHPh_2$), 8.29 (d, J = 8 Hz, NH), 8.37 (s, tetrazole H), 8.84 ppm (s, 4′-H). This compound was deblocked and converted to the sodium salt as described previously, to afford 62% of 12: mp 165–175 °C dec; UV 262 nm, 309 (ε 7800, 4000); NMR (Me_2SO-d_6) 3.3–3.4 (br, 2-H and CH_2S thiadiazole), 4.98 (d, J = 5 Hz, 8-H), 5.34 (s, CH_2CONH), 5.50 (dd, J = 8 and 5 Hz, 7-H), 9.02 (s, tetrazole H), 9.32 (s, 4′-H), 9.44 ppm (d, J = 8 Hz, NH).

Disodium 7β-(Thiophen-2-acetamido)-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4,4′-dicarboxylate (13). Reaction between Keflin and dipotassium 1,2,3-thiadiazole-4-carboxylate-5-thiolate (**2b**), as described above, gave after esterification and chromatography 33% of **bis(diphenylmethyl) 7β-(thiophen-2-acetamido)-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4,4′-dicarboxylate**: NMR ($CDCl_3$) 3.14, 3.39 (two d, J = 18 Hz, 2-H), 3.79 (s, CH_2CONH), 3.82, 4.01 (two d, J = 13 Hz, CH_2S thiadiazole), 4.79 (d, J = 5 Hz, 8-H), 5.69 (dd, J = 9 and 5 Hz, 7-H), 6.92 ppm (s, $CHPh_2$). This compound was deblocked and converted, in 42% yield, to the disodium salt 13: mp 196–200 °C dec; UV (H_2O) 264 nm, 301 (ε 2900, 1500); NMR (Me_2SO-d_6) 3.3 (br s, 2-H), 3.74 (s, CH_2CONH), 4.02, 4.40 (two d, J = 13 Hz, CH_2S thiadiazole), 4.96 (d, J = 5 Hz, 8-H), 5.50 (dd, J = 9 and 5 Hz, 7-H), 9.00 ppm (d, J = 9 Hz, NH).

Disodium 7β-(D-Mandelamido)-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4,4′-dicarboxylate (14). Reaction between sodium 7β-(D-mandelamido)-3-(acetoxymethyl)ceph-3-em-4-carboxylate[15] and the thiolate **2b**, as described above, gave, after esterification and chromatography, 60% of **bis(diphenylmethyl) 7β-(D-mandelamido)-3-[(1,2,3-thiadiazol-5-ylthio)methyl]ceph-3-em-4,4′-dicarboxylate**: mp 85–90 °C; UV (MeOH) 265 nm, 301 (ε 11 000, 13 000); NMR ($CDCl_3$) 3.22, 3.50 (two d, J = 18 Hz, 2-H), 3.89, 4.08 (two d, J = 13 Hz, CH_2S thiadiazole), 4.90 (d, J = 4 Hz, 8-H), 5.10 (s, CHOH), 5.75 ppm (dd, J = 9 and 4 Hz, 7-H), 6.97 (s, $CHPh_2$). Anal. ($C_{45}H_{34}N_4O_7S_3$) C, H. This compound was deblocked and converted, in 70% yield, into the disodium salt 14 as a pale yellow solid: mp ~200 °C dec; UV 265 nm, 302 (ε 7100, 6500); NMR (Me_2SO-d_6) 3.4 (br s, 2-H and CH_2S thiadiazole), 4.94 (d, J = 5 Hz, 8-H), 5.11 (s, CHOH), 5.51 ppm (s, CHOH), 5.51 (dd, J = 8 and 5 Hz, 7-H).

Antibacterial Assays. (a) In Vitro. Minimum inhibitory concentrations were determined using an Ames Handititer 2 to make serial twofold dilutions from 200 to 0.0008 μg/mL, or from 160 to 0.0006 μg/mL. Media were dispensed in 0.05 mL volume into cups in the dilution tray and eight 0.05-mL diluting loops transferred fluid for serial dilution. Each cup was inoculated with 1 μL of inoculum produced by a 1:10 dilution in brain–heart infusion broth of the bacterial culture, which had been grown for 18 h at 37 °C in brain–heart infusion broth. Following inoculation, trays were incubated for 16 h at 37 °C. End points were determined by use of tetrazolium chloride dye (0.33 mL of a 2% solution for 100 mL of medium). Following incubation, trays were examined for a red precipitate and end points determined. Triplicate trays were used for each test compound against each bacteria.

(b) In Vivo. Female Swiss-Webster mice (19–21 g) were housed in groups of ten with food and water available ad libitum. *Staphylococcus aureus*, Smith strain, obtained from Sterling-Winthrop Research Institute, Rensselaer, N.Y., was inoculated into brain–heart infusion broth and grown at 37 °C for 18 h, shaking at 220 rpm. The cultures were then diluted 1 × 10^4 in 5% sterile gastric mucin for injection into mice. Each mouse was injected intraperitoneally with 0.5 mL of the 10^{-4} dilution. A solution of the test compound or standard in 0.2 mL of sterile water was injected subcutaneously in the interscapular region of the mice at 0.5 and 4 h past challenge, using four different dose levels, with ten mice per dose level. Oral doses, prepared as described above were administered by gavage. Otherwise the procedures were the same as for the subcutaneous doses. The nonmedicated control animals and the test animals were observed twice daily, and deaths were recorded per observation period up to 72 h past challenge. PD_{50} for 72 h survival was calculated using probit analysis.

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Note Added in Proof. Subsequent in vivo testing has shown that the bacterial challenge used to obtain the oral ED_{50} data in Table II was approximately 40 times the LD_{50} for untreated mice. When ca. 1600 LD_{50} doses were administered, and the initial dose of drug was given for 1 h, rather than 0.5 h, past challenge, ED_{50} values increased ca. tenfold.

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Comparative Cytotoxic and Biochemical Effects of Ligands and Metal Complexes of α-*N*-Heterocyclic Carboxaldehyde Thiosemicarbazones

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Several α-*N*-heterocyclic carboxaldehyde thiosemicarbazones and their iron and copper complexes have been tested for their cytotoxicity and inhibiting activity against DNA synthesis under controlled metal conditions. No ligands show cytotoxicity against Ehrlich cells at the concentrations tested, while some iron and copper complexes are active. In contrast, the ligands inhibit DNA synthesis at much lower concentrations than used above. Similarly, the metal complexes are effective inhibitors at concentrations much below those necessary to demonstrate cytotoxicity. In addition, the iron complexes of 1-formylisoquinoline thiosemicarbazone, 2-formylpyridine thiosemicarbazone, and 4-methyl-5-amino-1-formylisoquinoline thiosemicarbazone were shown to be three- to sixfold more active than their free ligands as inhibitors of partially purified ribonucleotide reductase to which no iron has been added. The copper complex of 2-formylpyridine thiosemicarbazone was slightly more active than the free ligand against the reductase.

Bis(thiosemicarbazones) and α-*N*-heterocyclic carboxaldehyde thiosemicarbazones comprise two interesting classes of experimental cancer chemotherapeutic agents in that both are strong metal chelating agents.[1-4] The thiosemicarbazones are potent inhibitors of ribonucleotide reductase in vitro.[5] This enzyme catalyzes a critical and possibly rate-limiting step in DNA synthesis and cell division. There is substantial evidence in the case of 3-ethoxy-2-oxobutyraldehyde bis(thiosemicarbazone) and related compounds that their copper complexes are the active in vivo forms of these agents.[6-8] Although studies have demonstrated in vivo and in vitro cytotoxic activity for iron and copper complexes of 1-formylisoquinoline thiosemicarbazone and certain 5-substituted 2-formylpyridine thiosemicarbazones, as well as in vivo activity for a number of the free ligands, there has been no concerted attempt to sort out the relationship of ligands and metal complexes which underlies these observations.[9,10] Thus, the present investigation compares the cytotoxic properties of a number of thiosemicarbazone ligands and their copper and iron complexes in a metal-free assay system involving Ehrlich ascites tumor cells. The comparison is extended to examine the inhibition of DNA synthesis and, more specifically, of ribonucleotide reductase, thought to be the

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