Registry No. **5**, 53-86-1; **6**, 109908-76-1; **7**, 109908-77-2; **10** (n = 2), 109908-78-3; **10** (n = 4), 109908-83-0; **10** (acid, n = 2), 109908-92-1; **11** (n = 2), 109908-79-4; **11** (n = 4), 109908-85-2; **12** (n = 2), 109908-80-7; **12** (n = 4), 109908-86-3; **13a**, 109908-82-9; **13a** methyl ester, 109908-81-8; **13b**, 109908-88-5; **13b** methyl ester, 109908-87-4; **14**, 6260-86-2; **15**, 109908-89-6; **16**, 109908-90-9; **17**, 109908-91-0; TsCl, 98-59-9; 5-methoxy-2-methylindole, 1076-74-0; succinic anhydride, 108-30-5; p-chlorobenzoyl chloride, 122-01-0; 5-(chloroformyl)pentanoate, 102939-46-8; 3-(5'-carbomethoxypentanoyl)-*N*-(5'-carbomethoxypentanoyl)-5-methoxy-2-methylindole, 109908-84-1; (5-carboxypentyl)triphenylphosphonium bromide, 50889-29-7.

Synthesis and Physicochemical Properties of Thiadiazolo[3,2-*a*]pyrimidinesulfonamides and Thiadiazolo[3,2-*a*]triazinesulfonamides as Candidates for Topically Effective Carbonic Anhydrase Inhibitors

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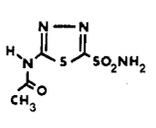
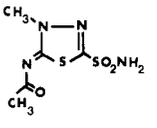
A series of bicyclic 1,3,4-thiadiazolo[3,2-a]pyrimidine- and 1,3,4-thiadiazolo[3,2-a]triazine-7-sulfonamides were synthesized from 5-amino-1,3,4-thiadiazole-2-sulfonamide and evaluated for topical efficacy as ocular hypotensive agents. The compounds were tested for the physicochemical properties of sulfonamide pK_a, free acid water solubility, $CHCl_3$/buffer partition, and transcorneal penetration (k_{in}), as well as for activity against carbonic anhydrase (I_{50}). A number of these compounds exhibited lower sulfonamide pK_a and higher water solubility than those of acetazolamide (**1**) and methazolamide (**2**), and one, **12**, brought about a small reduction in IOP in the normal rabbit eye.

An important treatment of the abnormally high intraocular pressure (IOP) associated with glaucoma is by the suppression of aqueous humor formation via inhibition of ciliary process carbonic anhydrase (CA). 1,3,4-Thiadiazole-2-sulfonamides are among the most potent CA inhibitors with enzyme–inhibitor binding constants (I_{50}) in the 10^{-8}–10^{-9} M range. However, sulfonamides in current clinical use by the oral route, such as acetazolamide (5-(acetylamino)-1,3,4-thiadiazole-2-sulfonamide, **1**) and methazolamide (5-(acetylimino)-4-methyl-Δ^2-1,3,4-thiadiazoline-2-sulfonamide, **2**), do not penetrate the cornea very well.[1] It has been shown that some 10–20 μM of free sulfonamide (of I_{50} = 10^{-8} M) at the ciliary process is necessary for complete inhibition of the enzyme and lowering of pressure.[2] However, parenteral administration of these agents causes side effects such as paresthesias, numbness and tingling, fatigue, and depression,[3] so their usefulness as ocular hypotensive agents is somewhat limited.



1



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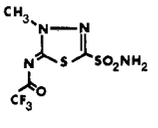

3

The suppression of aqueous humor formation by topical administration of carbonic anhydrase inhibitors was previously demonstrated by Maren et al.[1] for several 5-haloalkyl derivatives of **1** and **2**. One of these, 5-[(trifluoroacetyl)imino]-4-methyl-Δ^2-1,3,4-thiadiazoline-2-sulfonamide (**3**), possessed the requisite physicochemical properties to be topically effective in lowering IOP. A single drop of a 2% suspension lowered intraocular pressure in rabbit eye by 3.1 mmHg.[4] Spontaneous hydrolysis of the trifluoroacetyl group, however, precluded further development of this compound. Higher 5-alkyl homologues of **2** have recently been shown by Maren et al.[4] to produce similar reductions in IOP in the rabbit eye for several hours after administration of 1 drop (2% suspension).

Recently, a number of bicyclic sulfonamides related to benzothiazole-2-sulfonamide have shown promise as topically effective hypotensive agents.[5] In addition, Sugrue et al.[6] and Bar-Ilan et al.[7] have demonstrated IOP lowering in the rabbit after topical administration of 2-sulfamoyl-6-benzothiazolyl 2,2-dimethylpropionate, a prodrug that is enzymatically cleaved to 6-hydroxybenzothiazole-2-sulfonamide inside the eye.

Chemistry. In the present research we report the synthesis and physicochemical properties of a series of sulfonamides derived from 1,3,4-thiadiazolo[3,2-a]pyrimidine, 1,3,4-thiadiazolo[3,2-a]triazine, and related ring systems. All were prepared from the readily obtainable 5-amino-1,3,4-thiadiazole-2-sulfonamide (**4**) by annulation to the 1,3,4-thiadiazole nucleus at the 4,5-positions. The inclusion of ring heteroatoms such as N and S, as well as electron-withdrawing functionalities, i.e., carbonyl and sulfone, was anticipated to yield intermediates such as **6** and **18**, which would show high intrinsic water solubility. These thiadiazolo[3,2-a]triazinesulfonamides could then be modified via the 5-position of the triazine ring to give less water soluble, more lipophilic derivatives with low sulfonamide pK_a. Simple thiadiazolo[3,2-a]triazinesulfonamides could also be obtained from the corre-

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(1) Maren, T. H.; Jankowska, L.; Sanyal, G.; Edelhauser, H. F. *Exp. Eye Res.* **1983**, *36*, 457.
(2) Maren, T. H. *Physiol. Rev.* **1967**, *47*, 595.
(3) Epstein, D. L.; Grant, W. M. *Arch. Ophthalmol. (Chicago)* **1977**, *95*, 1378.
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(5) Schoenwald, R. D.; Eller, M. E.; Dixson, J. A.; Barfknecht, C. F. *J. Med. Chem.* **1984**, *27*, 810.
(6) Sugrue, M. F.; Gautheron, P.; Schmitt, C.; Viader, M. P.; Conquet, P.; Smith, R. L.; Shace, N. N.; Stone, C. A. *J. Pharmacol. Exp. Ther.* **1984**, *232*, 534.
(7) Bar-Ilan, A.; Pessah, N. I.; Maren, T. H. *J. Ocular Pharmacol.* **1986**, *2*, 109.

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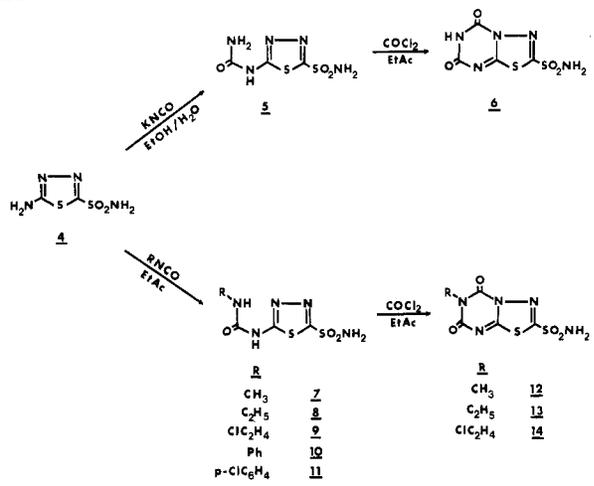
Scheme I



sponding 5-(alkylureido)-1,3,4-thiadiazole-2-sulfonamides (**7**,**8**) and 5-[(haloalkyl)ureido]-1,3,4-thiadiazole-2-sulfonamides (**9**) (obtained from **4** and alkyl isocyanates) by cyclization with $COCl_2$. Reaction of **4** with aryl isocyanates gave (*N'*-arylureido)-1,3,4-thiadiazole-2-sulfonamides **10** and **11**, which, however, could not be cyclized with phosgene.

Reaction of **4** with the appropriate alkyl or aryl isocyanate in THF at reflux gave the corresponding 5-(*N'*-alkylureido)-1,3,4-thiadiazole-2-sulfonamides (**7**–**9**) (80–95%) and 5-(*N'*-arylureido)-1,3,4-thiadiazole-2-sulfonamides (**10**–**11**) (55–82%) (Scheme I). However, reaction of **4** with several alkyl isothiocyanates or with phenyl isothiocyanate did not afford the corresponding 5-(*N'*-substituted thioureido) sulfonamides, even at higher temperatures in various solvents.

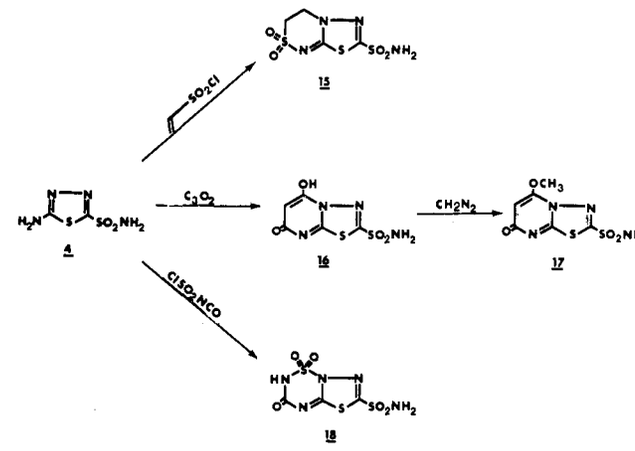
The ureas **7**–**11** were readily characterized by their ^1H NMR spectra, which typically showed NH signals at δ 5.7–9.5 as singlets or triplets, depending on the nature of R, and SO_2NH_2 signals at δ 8.4–9.1 as broad singlets. Assignment of the thiadiazole ring was aided by ^{13}C NMR, which displayed C-2 at 151–153 ppm; C-5 and the urea carbonyl both resonated at 163-164 ppm, making their assignments uncertain. The alkyl or aryl substituents showed the expected shifts.

Cyclization of 5-*N'*-alkylureas **7**–**9** with phosgene in EtOAc gave the corresponding 3-alkyl-derived 2,3-dihydro-2,4-dioxo-4*H*-[1,3,4]thiadiazolo[3,2-*a*][1,3,5]triazine-7-sulfonamides **12**–**14** in good yields. However, analogous cyclization did not occur with phenyl derivatives **10** and **11**. Cyclization of the alkyl derivatives **7**–**9** did not occur with $CSCl_2$.

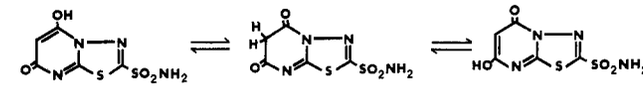
Bicyclic thiadiazolo[2,3-*c*]thiadiazine- and thiadiazolo[3,2-*a*]pyrimidine-7-sulfonamides were obtained directly from **4** by reaction with ethenesulfonyl chloride and carbon suboxide to give **15** and **16** (Scheme II). The regiochemistry of the addition reactions may be rationalized by considering the mechanism of the reaction and the NMR spectra of the products. Because of the mild conditions used during the formation of **15**, nucleophilic attack at sulfur should occur first, followed by Michael addition of the heterocyclic nitrogen to the α,β-unsaturated sulfonamide. ^1H and ^{13}C NMR analysis is in agreement with the assigned structure, **15**. Similar arguments may be advanced in determining the regiochemistry of addition in **18**. However, in this case, nucleophilic addition occurs first with the more electrophilic isocyanate followed by cyclization at the sulfonyl chloride.

Compound **4** reacts with carbon suboxide to give the thiadiazolopyrimidine **16**. It can exist as three distinct

Scheme II



tautomers (**16a**–**c**). Our assignment of structure **16a** was obtained by analysis of its NMR data. Structure **16b** was



16a 16b 16c

easily eliminated from consideration by the presence of a 1 H singlet at δ 5.5 in the ^1H NMR and a signal at 86.2 ppm, characteristic of β-enone carbons, in the ^{13}C NMR. Tautomer **16a** is preferred over **16c**, as a favored intramolecular five-membered-ring hydrogen bond may form with N-3 in **16a**, whereas an unfavorable four-membered ring would need to form between the N-7 and the OH in **16c**. ^{13}C data provide evidence for this, since hydrogen bonding would cause deshielding at C-2 by inductive withdrawal by the more electropositive N-3. Indeed, C-2 (157.6 ppm) is deshielded relative to the other compounds prepared (151–155 ppm). Unfortunately, the OH absorption normally associated with intramolecular hydrogen bonding is unobservable in its IR spectrum due to broad NH_2 absorption.

Further functionalization of **16** was performed by alkylation with diazomethane to give **17** in good yield (Scheme II). This product showed the expected NMR, IR, and analytical data.

Pharmacological Evaluation. The transcorneal penetration of bicyclic thiadiazolo[3,2-*a*]pyrimidinesulfonamides and thiadiazolo[3,2-*a*]triazinesulfonamides was studied in anesthetized rabbits by application of saturated aqueous solutions (pH 7.5) for 10 min, followed by assay of the anterior aqueous humor for concentration of drug. The first-order rate constants (k_{in}) for transcorneal penetration are shown in Table I along with the physicochemical parameters of sulfonamide pK_a, free acid water solubility, lipid solubility, as measured by $CHCl_3/H_2O$ distribution coefficient, and activity against carbonic anhydrase (I_{50}). The data may be compared to other compounds studied.[1,4]

All compounds tested showed approximately the same activity against the enzyme, with I_{50} values ranging from 1.4 to 5×10^{-8} M. It is evident that the nature of the ring substituents in compounds **6** and **12**–**17** has a small effect on the I_{50} value. The similarity of this value to 2×10^{-8} for **1** and **2** may demonstrate a certain insensitivity of the I_{50} value to the nature of the ring components attached to the sulfonamide.

The presence of electron-withdrawing ring functionalities in **12**–**15** and **17** leads to an expected decrease in sulfonamide pK_a relative to **1** and **2**. That the corresponding free acid water solubilities are not greater but

Table I. Physicochemical Parameters and Physiological Evaluation of Thiadiazolo[3,2-*a*]pyrimidinesulfonamides and Thiadiazolo[3,2-*a*]triazinesulfonamides

compd	pK_a (sulfonamide)	I_{50} vs. CA $\times 10^8$ M	water solubility, mM	partition coefficient at pH 7.4 ($CHCl_3$/buffer)	k_{in}, $h^{-1} \times 10^{-3}$	effect on IOP after 1 h, mmHg
1	7.4	2	3.2	0.001	2.0	0
2	7.4	2	5	0.06	6.0	−0.6 ± 0.2
3	6.6	2	8	0.3	14	−3.1 ± 0.3
6	8.3	2	4.0	0.005		0
12	7.3	2	5.6	0.02	1.5	−1.0
13	7.3	2	4.8	0.01	1.8	0
14	7.1	1.8	3.5	0.02	3	0
15	6.7	1.4	2.0	0.006	1.5	0
16	7.8	3.7	17	0.003		
17	7.1	5	5.0	0.010	0.8	0
18	7.2	5	58	0.006		0

generally equal to those of **1** and **2** may be attributed to the greater molecular size of the bicyclic sulfonamides.

The failure of these new compounds (**6**, **12–18**) to reduce IOP to any large extent in the normal rabbit eye probably relates to inadequate lipid solubility as reflected by very low $CHCl_3$/buffer partition. Compounds **3** and **15**, for example, are similar with respect to sulfonamide pK_a, inhibitory potency, and water solubility, yet the $CHCl_3$/buffer partition coefficient of **3** is 50 times greater than that of **15**. With an approximately 10-fold greater k_{in}, **3** is topically effective in lowering IOP[1] whereas **15** produces no measurable effect. Compound **12**, with one of the highest partition coefficients of the series, did have a small effect on IOP after 1 h.

Experimental Section

Chemistry. Melting points were measured with a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were obtained with a Perkin-Elmer 283 IR spectrometer. ^1H NMR and ^{13}C NMR spectra were taken on a Varian EM 360 L and a JEOL FX 100 spectrometer and mass spectra with an AEI MS 30. Chemical shifts are reported as parts per million relative to Me_4Si as an internal standard. Precoated (silica gel 60 F_{254}) TLC sheets were used to examine the purity of compounds and obtain R_f values. All compounds were analyzed (C, H, N), and values obtained were within ±0.4% of the theoretical values. Ethenesulfonyl chloride,[8] carbon suboxide,[9] and phosgene[10] were obtained by literature procedures. Chlorosulfonyl isocyanate and alkyl and aryl isocyantes were obtained from Aldrich and used without further purification.

5-Ureido-1,3,4-thiadiazole-2-sulfonamide (5). To a solution of **4** (5.4 g, 30 mmol) in 150 mL of absolute ethanol heated to 50 °C was slowly added dropwise KNCO (4.9 g, 60 mmol) dissolved in 3 mL of H_2O and 60 mmol of HCl in an equivalent volume of ethanol. The solution was cooled and filtered. Evaporation of the solvent gave 6.0 g (90%) of **5**: mp 194–196 °C (from acetone/water, 60:40); IR ($CHBr_3$) 3350, 3270, 1700, 1510, 1420, 1350, 1220, 1120, 1100, 930, 810 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.1 (s, 1 H, N*H*), 5.7 (s, 2 H, NH_2), 8.6 (s, 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6) 152.4 (C-2), 158.2 (C-5), 172.0 ppm (C=O). Anal. ($C_3H_5N_5O_3S_2$) C, H.

2,3-Dihydro-2,4-dioxo-4*H*-[1,3,4]thiadiazolo[3,2-*a*][1,3,5]triazine-7-sulfonamide (6). To a suspension of **5** (2.24 g, 10 mmol) in 100 mL of ethyl acetate was added $COCl_2$ (2 g, 20 mmol), and the solution was refluxed for 1 h. The solution was cooled, and neutralized with NH_4OH, and then filtered. Recrystallization from water gave 2.5 g of **6** (99%): mp 238 °C; IR ($CHBr_3$) 3360, 3280, 1760, 1690, 1600, 1420, 1350, 1160, 1130, 980, 880 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.5 (s, 1 H, N*H*), 8.7 (s, 2 H, SO_2NH_2). Anal. ($C_4H_3N_5O_4S_2$) C, H, N.

5-(*N'*-Methylureido)-1,3,4-thiadiazole-2-sulfonamide (7). A suspension of **4** (5.4 g, 30 mmol) in 100 mL of ethyl acetate was refluxed for 1 h with methyl isocyanate (3.4 g, 60 mmol). Evaporation of the solvent gave 6.4 g of **7** (90%): mp 208–211 °C (from H_2O); R_f 0.15 ($CHCl_3$/MeOH, 4:1); IR ($CHBr_3$) 3350, 3280, 1680, 1530, 1350, 1240, 920, 800 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.9 (d, 3 H, CH_3), 6.7 (t, 1 H, N*H*), 8.30 (br s, 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6) 26.7 (CH_3), 153.9 (CSO_2NH_2), 163.4 (C=O), 163.6 ppm (NHC=N). Anal. ($C_4H_7N_5O_3S_2$) C, H, N.

5-(*N'*-Ethylureido)-1,3,4-thiadiazole-2-sulfonamide (8). A suspension of **4** (3.6 g, 20 mmol) in 50 mL of THF was refluxed for 3 h with ethyl isocyanate (2.8 g, 40 mmol). Evaporation of the solvent gave 1.9 g of **8** (75%): mp 257–259 °C dec; R_f 0.45 ($CHCl_3$/MeOH; 4:1); ^1H NMR (DMSO-d_6) δ 1.1 (t, 3 H, CH_3), 3.33 (m, 2 H, CH_2), 6.9 (t, 1 H, N*H*), 8.4 (s, 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6) 14.8 (CH_3), 34.5 (C_2), 153.0 (C-2), 163.2 ppm (C-5, C=O). Anal. ($C_5H_9N_5O_3S_2$) C, H, N.

5-[*N'*-(Chloroethyl)ureido]-1,3,4-thiadiazole-2-sulfonamide (9). A suspension of **4** (1.8 g, 10 mmol) in 100 mL of DMF was refluxed for 1 h with chloroethyl isocyanate (1.56 g, 15 mmol). Evaporation of the solvent gave 1.7 g of **9** (60%): mp 282 °C (from H_2O); IR ($CHBr_3$) 3300 (br), 1675, 1505, 1430, 1350, 910 cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.5 (m, 4 H, C_2H_4), 7.9 (t, 1 H, N*H*), 9.1 (s, 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6) 41.2 (NCH_2), 43.3 (CH_2Cl), 153.0 (C-2), 162.7 (C=O), 163.1 ppm (C-5). Anal. ($C_5H_8ClN_5O_3S_2$) C, H, N.

5-(*N'*-Phenylureido)-1,3,4-thiadiazole-2-sulfonamide (10). To an ice-cold suspension of **4** (3.0 g, 16.6 mmol) in 100 mL of dry THF was added dropwise over 20 min a solution of freshly distilled phenyl isocyanate (2.02 g, 17 mmol) in 25 mL of THF. The solution was then refluxed under N_2 for 6 h. The clear solution was rotoevaporated to give 4.69 g of white solid. Recrystallization from EtOH/H_2O gave 4.05 g (82%) of pure **10** as white flakes: mp 255–258 °C dec; IR ($CHBr_3$) 3380, 3290, 2800 (br), 1730, (CO), 1585, 1545, 1343, 760 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.1–7.8 (m, 5 H, Ar *H*), 8.43 (s, 2 H, SO_2NH_2), 9.32 (s, 1 H, N*H*), 11.66 (br s, 1 H, ArN*H*); ^{13}C NMR (DMSO-d_6) 119.2 (Ar *C*-2), 123.5 (Ar *C*-4), 129.0 (Ar *C*-3), 138.0 (Ar *C*NH), 151.5 (C-2), 162.9 (CO), 163.8 ppm (C-5). Anal. ($C_9H_9N_5O_3S_2$) C, H, N.

5-[*N'*-(4-Chlorophenyl)ureido]-1,3,4-thiadiazole-2-sulfonamide (11). Preparation was similar to that of **10**,

(8) Rondestvedt, C. S. *J. Am. Chem. Soc.* **1954**, *76*, 1926.
(9) Diels, O.; Meyerheim, G. *Ber. Dtsch. Chem. Ges.* **1907**, *40*, 355.
(10) Illani, G. *Gazz. Chim. Ital.* **1951**, *81*, 439.

except that pure **11** precipitated from the reaction mixture on cooling. The filtrate was rotoevaporated to give a white solid, which was recrystallized from EtOH/H_2O (55%): mp 285 °C dec; IR ($CHBr_3$) 3385, 3335, 3240, 2700 (br), 1693 (CO), 1357, 1185, 1173, 825 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.50 (AB quartet, 2 H, Ar *H*), 7.76 (AB quartet, 2 H, Ar *H*), 8.51 (s, 2 H, SO_2NH_2), 9.53 (s, 1 H, N*H*), 11.80 (br s, 1 H, N*H*); ^{13}C NMR (DMSO-d_6) 120.8 (Ar *C*-2), 127.2 (Ar *C*Cl), 128.8 (Ar *C*-3), 137.0 (Ar *C*NH), 151.6 (C-2), 162.9 (*C*O), 163.8 ppm (C-5). Anal. ($C_9H_8ClN_5O_3S_2$) C, H, N.

2,3-Dihydro-3-methyl-2,4-dioxo-4*H*-[1,3,4]-thiadiazolo[3,2-*a*][1,3,5]triazine-7-sulfonamide (12). A suspension of **7** (2.35 g, 10 mmol) in 100 mL of ethyl acetate was refluxed for 30 min with $COCl_2$ (4 g, 40 mmol). Evaporation of the solvent gave 1.9 g of **12** (72%): mp 318 °C (from H_2O); IR ($CHBr_3$) 3360, 3280, 1760, 1680, 1560, 1420, 1380, 1240, 1130, 1080, 1040, 980, 940, 760, 730, cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.0 (s, 3 H, CH_3), 8.5 (s, 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6) 29.1 (CH_3), 145.3 (C-6), 152.1 (C-2), 159.7 (C-4), 168.4 ppm (C-7a). Anal. ($C_5H_5N_5O_4S_2$) C, H, N.

2,3-Dihydro-3-ethyl-2,4-dioxo-4*H*-[1,3,4]thiadiazolo[3,2-*a*][1,3,5]triazine-7-sulfonamide (13). A suspension of **8** (2.51 g, 10 mmol) in 100 mL of ethyl acetate was refluxed for 6 h with $COCl_2$ (4 g, 40 mmol). Evaporation of the solvent gave 2.1 g of **13** (75%): mp 220–222 °C (from H_2O). Anal. ($C_6H_7N_5O_4S_2$) C, H, N.

2,3-Dihydro-3-(chloroethyl)-2,4-dioxo-4*H*-[1,3,4]-thiadiazolo[3,2-*a*][1,3,5]triazine-7-sulfonamide (14). A suspension of **9** (1.43 g, 5 mmol) in 100 mL of ethyl acetate was refluxed for 1 h with $COCl_2$ (1 g, 10 mmol). Evaporation of the solvent gave 2.7 g of **14** (85%): mp 258 °C dec (from H_2O); ^1H NMR (DMSO-d_6) δ 3.9 (d, 2 H, CH_2), 4.2 (d, 2 H, CH_2), 9.1 (s, 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6) 39.4 (NCH_2), 43.0 (CH_2Cl), 144.7 (C-6), 151.1 (C-2), 159.7 (C-4), 168.6 ppm (C-7a). Anal. ($C_6H_6ClN_5O_4S_2$) C, H, N.

3,4-Dihydro-2,2-dioxo[1,3,4]thiadiazolo[2,3-*c*][1,2,4]thiadiazine-7-sulfonamide (15). A solution of **4** (3.6 g, 20 mmol), Et_3N (2.5 g, 25 mmol), and 300 mL of dry THF was cooled to −30 °C in dry ice–acetone. Ethenesulfonyl chloride (2.6 g, 23 mmol) was added at −30 °C, and the solution was allowed to warm to room temperature over 4 h with stirring. Rotoevaporation gave 3.8 g of **15** (60%): mp 265 °C dec; R_f 0.32 ($CHCl_3$/MeOH, 4:1), IR ($CHBr_3$) 3400, 3250, 3100, 1540, 1490, 1360, 1300, 1270, 770 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.75 (m, 2 H, CH_2), 4.70 (m, 2 H, CH_2), 8.85 (br s, 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6) 41.9 (C-4), 47.5 (C-5), 155.1 (C-2), 166.0 ppm (C-7a). Anal. ($C_4H_6N_4O_4S_3$) C, H, N.

2-Oxo-4-hydroxy-2*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-7-sulfonamide (16). A solution of **4** (1.8 g, 10 mmol) in 100 mL of THF was diluted to 1 L with Et_2O and cooled to −40 °C in dry ice/acetone. Carbon suboxide (C_3O_2) (2.1 g, 30 mmol) was added and the solution allowed to warm to room temperature over 2 h. Rotoevaporation of the solvent gave 2.2 g of **16** (80%): mp 214–216 °C (from H_2O); R_f 0.65 ($CHCl_3$/MeOH, 4:1); IR ($CHBr_3$) 3350, 1650, 1560, 1490, 1360, 1250, 1080, 940, 850 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.5 (s, 1 H, C*H*), 9.0 (br s, 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6) 86.2 (C-5), 57.6 (C-2), 160.5 (C-4), 162.4 (C-6), 168.6 ppm (C-7a). Anal. ($C_5H_4N_4O_4S_2$) C, H, N.

4-Methoxy-2-oxo-4*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-7-sulfonamide (17). To an ice-cold solution of **16** (1.0 g, 4.0 mmol) in 25 mL of ether was added freshly prepared CH_2N_2 until solid began precipitating from solution. The mixture was warmed to room temperature and the solvent rotoevaporated to give a white solid. Recrystallization from H_2O gave **17**: mp 193 °C dec; IR ($CHBr_3$) 3080 (br), 1680, 1565, 1445, 1395, 1255, 1050, 945, 805 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.9 (s, 3 H, OCH_3), 5.7 (s, 1 H, C*H*), 9.0 (s, 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6), 40.3 (CH_3), 86.2 (C-5), 157.5 (C-2), 163.2 (C-4), 166.0 (C-6), 168.7 ppm (C-7a). Anal. ($C_6H_6N_4O_4S_2$) C, H, N.

2,3-Dihydro-1,1,3-trioxo[1,3,4]thiadiazolo[3,2-*b*][1,2,4,6]thiatriazine-6-sulfonamide (18). To a suspension of **4** (6.7 g, 30 mmol) in 100 mL of CH_3CN cooled to 0 °C was slowly added chlorosulfonyl isocyanate (4.94 g, 35 mmol). The solution was stirred for 1 h at room temperature before addition of pyridine (237 g, 30 mmol). The solution was then stirred overnight at room temperature. Removal of the solvent and recrystallization from acetone–water (80:20) gave 4.1 g of **18** (45%): mp 252 °C; IR ($CHBr_3$) 3450, 3280, 1780, 1550, 1430, 1350, 1140, 1000, 970, 910, 820 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.7 (s, 1 H, N*H*), 8.2 (s, 2 H, SO_2NH_2).

Pharmacological Methods. Transcorneal penetration of test compounds was studied in anesthetized white New Zealand rabbits (2.5–3.5 kg) by application for 10 min of 0.5 mL of a saturated sulfonamide solution at pH 7.5 (concentration = C_{out}) to a reservoir formed from the eyelids with forceps. C_{out} is constant since the concentration and volume are sufficiently large that drug is not depleted from the reservoir. The cornea was then thoroughly washed with a stream of water and the 200-μL sample of anterior aqueous humor immediately taken and assayed for sulfonamide content. The first-order rate constants (k_{in}) for penetration were obtained from the relationship[1]

$$k_{in} = C_a/C_{out}t$$

where C_a is the anterior chamber concentration after 10 min of exposure and t is the duration of the exposure in minutes. These may be converted into permeability constants by the following relationship:[12]

$$P = k_{in} \text{ (anterior chamber volume/corneal area)}$$

Since the volume of the anterior chamber in the rabbit eye is approximately 200 μL (0.2 mL) with a corresponding corneal area of 2.1 cm, P = 0.1 (k_{in}).

Intraocular pressure was measured in anesthetized rabbits 1 h following application of 1 drop of a 2% suspension of sulfonamdide in 1% (hydroxyethyl)cellulose.[4] A Digilab Model 30 Pneuma-Tonometer was used for all pressure measurements.

Sulfonamide Assay. Sulfonamides were assayed at 0 °C by a changing pH indicator method[11] that monitors the inhibition of carbonic anhydrase catalyzed CO_2 hydration. Aliquots of sulfonamide solution were incubated for 2 min with approxiamately 2 EU of carbonic anhydrase, 4 mL of water, and 2 mL of a phenol red indicator solution in a special CO_2 bubbler cell. One enzyme unit (EU) is defined as doubling the uncatalyzed rate. Enzyme solution was prepared from a 1/100 dilution of whole dog blood. The concentraiton of enzyme (E_0) in this system is approximately 5×10^{-9} M. The hydration reaction was initiated by addition of 1 mL of 0.5 M bicarbonate buffer (0.3 M Na_2CO_3, 0.2 M $NaHCO_3$) and the time recorded with stopwatch to obtain a color change (pH 7.2). Calibration curves using known amounts of sulfonamide were obtained for each sulfonamide under identical conditions. I_{50} values represent the molar amount of sulfonamide in the assay that reduces the EU by 50%.

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Determination of Physicochemical Parameters. Sulfonamide pK_a was determined in 50 mM phosphate buffer by monitoring changes in the UV absorption spectrum (300–210 nm) on a Beckman UV 5260 spectrophotometer. Fifteen to 25 separate absorption curves were run for each pK_a determination. Water solubilities were obtained by vortexing excess sulfonamide in 2 mL of H_2O for 2 h, filtering, and assaying for sulfonamide content. Longer vortexing times usually did not result in any additional solubilization of the sulfonamide. $CHCl_3$/buffer partition coefficients were determined by shaking 5-mL aliquots of a 0.1 mM sulfonamide solution in 50 mM phosphate buffer (pH 7.5) against 5 mL of $CHCl_3$ for 10 min and assaying both phases for sulfonamide content.

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Registry No. **4**, 14949-00-9; **5**, 109907-71-3; **6**, 109907-72-4; **7**, 32873-77-1; **8**, 109907-73-5; **9**, 109907-74-6; **10**, 109907-75-7; **11**, 109927-24-4; **12**, 109907-76-8; **13**, 109907-77-9; **14**, 109907-78-0; **15**, 93745-72-3; **16**, 109907-79-1; **17**, 109907-80-4; **18**, 93745-73-4; CH_2NCO, 624-83-9; C_2H_5NCO, 109-90-0; ClC_2H_4NCO, 1943-83-5; PhNCO, 103-71-9; p-ClC_6H_4NCO, 104-12-1; $H_2C{=}CHSO_2Cl$, 6608-47-5; C_3O_2, 504-64-3; $ClSO_2NCO$, 1189-71-5; carbonic anhydrase, 9001-03-0.

Design and Synthesis of Phosphonate Inhibitors of Glutamine Synthetase

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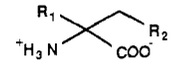
Inhibitors 1–4 have been shown previously to undergo enzymatic phosphorylation by glutamine synthetase (GS). Phosphonates 6–9 were designed as chemically stable analogues of these phosphorylated inhibitors, incorporating either a tetrahedral sulfur group (6–8) (–S–, –SO–, $–SO_2–$) or phosphinate (9) adjacent to methylphosphonic acid. Phosphonates 6–8 resemble the transiently stable phosphorylated methionine sulfone (2), whereas 9 resembles phosphorylated 2-amino-4-phosphonobutyric acid (4). When tested as inhibitors of glutamine synthetase from bacteria, mammals, and plants, analogue 9 proved to be the most potent, with a K_i value of 7.5×10^{-5} M vs. the *Escherichia coli* enzyme. Analysis of the inhibition data for 6–9 suggests that a replacement of the oxygen bridging the tetrahedral sulfur (6–8) or phosphinate (9) and the terminal phosphate with a hydrophobic methylene drastically reduces the enzyme's affinity for inhibitors. Enhanced affinity of GS for phosphonate 9 may result from interaction of the negative charge on the phosphinate with Mn^{2+} at the active site.

Glutamine synthetase (GS) catalyzes the formation of glutamine, ADP, and P_i from glutamate, ATP, and ammonia, via a tetrahedral transition state resulting from nucleophilic attack of ammonia on the activated carboxylate of glutamate (Figure 1).[1-3] Among several known transition state analogue inhibitors of GS are L-methionine (*S*)-sulfoximine (**1**),[4-6] L-methionine sulfone (**2**),[1,2] phosphinothricin (**3**),[7,8] and 2-amino-4-phosphonobutyric acid (**4**).[9] Analogues 1–4 are all phosphorylated by *Escherichia coli* GS in the presence of ATP and metal ions.[10-13] L-Methionine (*S*)-sulfoximine phosphate (**5**) is stable and binds irreversibly with ADP at the active site of GS.[14-16] Meister et al.[17] synthesized L-methionine (*SR*)-sulfoximine phosphate (**5**) and showed that it rapidly inhibited ovine brain GS in the presence of ADP and metal ions. Recent studies indicate that phosphinothricin (**3**) may also be phosphorylated by *E. coli* GS in the presence of ATP and metal ions, resulting in irreversible binding at the active site.[18] Phosphorylation of methionine sulfone (**2**) and 2-amino-4-phosphonobutyric acid (**4**) by *E. coli* GS does not result in the formation of an irreversibly bound complex.[12,18] Although the phosphorylated sulfone is unstable and cyclizes,[12] it is not clear whether the cyclization occurs before or after dissociation from the active site of GS. Phosphorylated 2-amino-4-phosphonobutyric acid (**4**) does not irreversibly inactivate *E. coli* GS, but dissociates from the active site of *E. coli* GS after being phosphorylated.[13]

This paper describes the synthesis of phosphonates 6–9, designed to resemble phosphorylated transition-state analogues 1–4, plus inhibition data for analogues 6–9 obtained with *E. coli*, ovine brain, and pea seed GS. Phosphonates 6–8 resemble methionine sulfone phosphate,[12]



#	R1	R2	#	R1	R2
1	H-	$-CH_2S(O)(NH)CH_3$	7	CH_3-	$-CH_2S(O)CH_2PO_3^{=}$
2	H-	$-CH_2S(O)_2CH_3$	8	CH_3-	$-CH_2S(O)_2CH_2PO_3^{=}$
3	H-	$-CH_2P(CH_3)O_2^-$	9	H-	$-CH_2P(O_2^-)CH_2PO_3^{=}$
4	H-	$-CH_2PO_3^{=}$	10	H-	$-CH_2C(O)CH_2PO_3^{=}$
5	H-	$-CH_2S(O)(CH_3)NPO_3^{=}$	11	H-	$-NHC(O)CH_2PO_3^{=}$
6	CH_3-	$-CH_2SCH_2PO_3^{=}$			

whereas analogue **9** most closely resembles a stable analogue of phosphorylated 2-amino-4-phosphonobutyric acid.

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