Synthesis and Properties of the Sulfonyl Analogues of 4(5)-Aminoimidazole-5(4)-carboxamide, 4(5)-(Formylamino)imidazole-5(4)-carboxamide, Guanine, and Xanthine[1]

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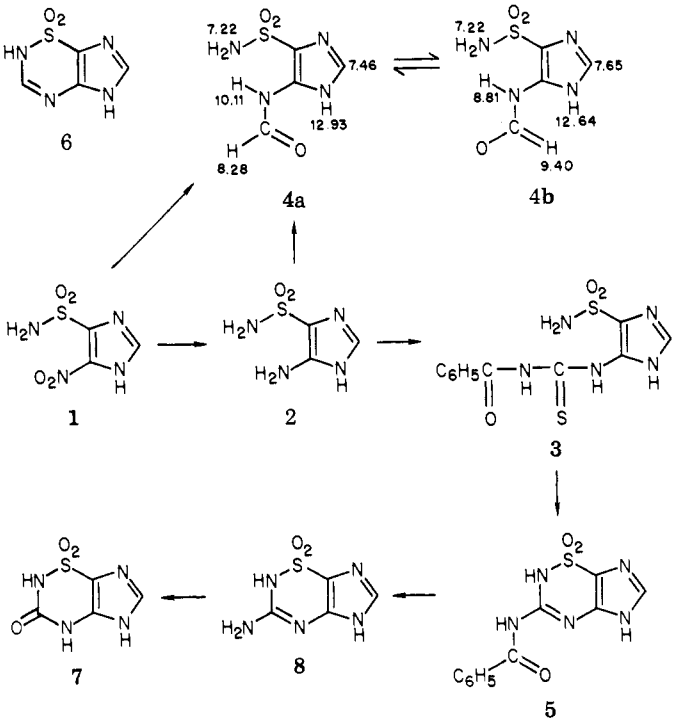
Reduction of 4(5)-nitroimidazole-5(4)-sulfonamide afforded the sulfonamide analogue of 4(5)-aminoimidazole-5-(4)-carboxamide (AICA). This was formylated to afford the sulfonamide analogue of formyl-AICA and was ring closed to the unsubstituted 6-sulfonyl analogue of guanine, 3-aminoimidazo[4,5-*e*]-1,2,4-thiadiazine 1,1-dioxide. Diazotization of the latter afforded the corresponding 6-sulfonyl analogue of xanthine. None of the imidazole-sulfonamides or the purine 6-sulfonyl analogues inhibited the growth of L1210 cells in culture nor were they substrates for or significant inhibitors of human hypoxanthine–guanine phosphoribosyltransferase or milk xanthine oxidase.

The substitution of a sulfonamide for a carboxamide moiety either in compounds that are essential for cellular metabolism or in their biogenic precursors has been one approach to the development of agents of potential chemotherapeutic value.[2] Our initial rationale for the exchange of these two functional groups in the purine series was that the sulfonyl analogues might sufficiently resemble the transition state for amination or deamination so that they would behave as transition-state analogue inhibitors of enzymes affecting purine metabolism. The synthesis of the sulfonyl analogue of hypoxanthine, **6** (Scheme I), was recently accomplished by rapid closure of the unstable 4(5)-aminoimidazole-5(4)-sulfonamide, **2**, with $HC(OEt)_3$.[3] Because of the structural resemblance of **2** to the imidazole intermediate in purine biosynthesis, 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), it appeared appropriate to isolate and characterize **2**, if possible, and to examine its biological activity. We now report studies on the properties of **2** and its formyl derivative **4**, as well as the synthesis and physical and biological properties of the 6-sulfonyl analogues of guanine, **8**, and xanthine, **7**, obtained by ring closure of **2**.

Scheme I



Results and Discussion

Chemistry. Previous efforts by others to obtain **2** by catalytic reduction of **1** and isolation as the hydrochloride salt found the compound too unstable to be isolated[4-8] and our initial studies verified this. Catalytic reduction of **1** under neutral conditions, however, afforded **2** as the free base, which proved to be sufficiently stable to be manipulated if the temperature was kept sufficiently low. Although even mild heating caused rapid decomposition of the solid, a dilute (10^{-4} M) buffered (pH 7) aqueous solution of **2** maintained at 37 °C did not lose UV optical density over a period of 3 weeks. The solid showed only minimal discoloration when kept at −30 °C over extended periods. Thus, the amine, **2** can be prepared, manipulated, and stored for reasonable lengths of time with appropriate care.

The more stable formyl derivative, **4**, could be prepared by reaction of **2** with formic acid, or more conveniently by reduction of **1** with Pd/C and 98% formic acid,[9] and then warming the solution slightly. Attempts to ring close **4** to the sulfonyl analogue of hypoxanthine, **6**, by heating under acidic (acetic anhydride), neutral [DMF, dimethoxymethyl acetate, $HC(OEt)_3$], or basic (pyridine) conditions were not successful. No reaction occurred with mild heating, while prolonged heating at elevated temperatures caused gradual decomposition. An NMR spectrum of **4** in Me_2SO-d_6 indicated the presence of both the trans (*Z*)(**4a**) and cis (*E*)(**4b**) conformers. As observed with related N-substituted amides,[10] the *Z* conformer, **4a**, predominated (70%).

Reaction of the amine, **2**, with benzoyl isothiocyanate[11,12] afforded 4(5)-[*N'*-(benzoylthiocarbamyl)amino]-

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Table I. UV Spectral Properties and pK_a Values

compd	charge	pH	λ_{max}, nm ($\epsilon \times 10^{-3}$)	apparent pK_a[a-e]
2	+	1	220 (9.5), 243 sh (5.2)	
	0	7	225 (10.2)	9.43[a] (0.07) [22]
	-	13	227 (8.1)	
4		1	237 (11.7)	
		7	246 (10.0)	8.7[a] (0.1) [22]
		13	223 (7.6), 252 sh (6.6)	
7	0	0	230 (4.2)	
	-1	7	226 (4.5)	2.55[a,c] (0.05) [26]
	-2	10	228 (4.5)	8.99[a] (0.08)
	-3	13	229 (4.4)	11.00[a] (0.06)
8	0	1	228 (3.4)	
	-	9	242 sh (3.1), 255 (3.6)	6.84[a,d] (0.06) [24]
	-2	14	240 (2.8), 257 sh (2.7)	11.6[e]

[a] Determined potentiometrically in 0.001 M solutions with a Beckman Research Model pH meter by procedures described previously.[18] pK_a values for 7 were calculated by a least-squares analysis[28] using activity corrections with a Monroe 1665 programable calculator. [b] Standard deviation in parentheses. Temperature (°C) in brackets. [c] For comparison, the pK_a values of xanthine are 7.70 and 11.92.[29] [d] The pK_a values of guanine are 9.32 and 12.62.[30] [e] Estimated from isobestic spectra.

imidazole-5(4)-sulfonamide (3). S-Methylation of the anion of 3 was accompanied by ring closure of the thiadiazine ring to afford the benzoyl derivative, 5. This could be converted in good yield to the sulfonyl analogue of guanine, 3-aminoimidazo[4,5-*e*]-1,2,4-thiadiazine 1,1-dioxide (8). Diazotization of 8 afforded the 6-sulfonyl analogue of xanthine, 7, which was recently prepared by a different route.[8]

As observed for the sulfonyl analogue of hypoxanthine,[3] 6, the pK_a values of the imidazo[4,5-*e*]-1,2,4-thiadiazine 1,1-dioxides, 7 and 8, are considerably lower than those of the corresponding purine analogues, xanthine and guanine, respectively. This was particularly evident in the 6-sulfonyl analogue of xanthine, 7, which exhibited a first pK_a of 2.55 (Table I). The similarity in structure between 7 and saccharin was reflected in the slight sweet taste exhibited by 7.

Biological Studies. None of the 6-sulfonylpurine analogues, 6, 7, or 8, nor the imidazole sulfonamides, 2 and 4, inhibited the growth of L1210 cells in culture when tested at concentrations up to 10^{-4} M.

For activity as antimetabolites, purine analogues usually require conversion to the corresponding ribonucleotides.[13] This is accomplished for many 6-oxopurines by hypoxanthine–guanine phosphoribosyltransferase (HGPRTase, EC 2.4.2.8), which catalyzes the transfer of the phosphoribosyl moiety of 5-phosphoribosyl 1-pyrophosphate to the 9 position of hypoxanthine and guanine.[14] The enzyme from humans will bind 6-oxo- or 6-thiopurines and some of the corresponding 8-aza analogues but will not bind 6-aminopurines.[14] In general, the structural requirements for substrate binding are rather restricted. Examination of the aminoimidazolesulfonamide, 2, and the 6-sulfonyl analogues of hypoxanthine and guanine, 6 and 8, revealed that they were neither substrates for nor inhibitors of human HGPRTase.

Milk xanthine oxidase (EC 1.2.3.2) is a rather nonspecific enzyme that will accept not only a variety of purines as substrate[15,16] but will also oxidize aldehydes[16] and a number of heterocycles related to purines, including pteridines.[16,17] It is also inhibited by a variety of heterocyclic systems, including pyrazolopyrimidines,[18,19] imidazoles,[20] triazoles[20,21] and benzo[1,2,4]thiadiazine 1,1-dioxides.[22-24] The inhibitory effect of the benzo[1,2,4]thiadiazine 1,1-dioxides suggested that the imidazo[4,5-*e*]-1,2,4-thiadiazine dioxide analogues of hypoxanthine and xanthine, 6 and 7, which are even closer in structure to the normal substrates, might be either substrates for or inhibitors of xanthine oxidase. However, neither was a substrate for nor a significant inhibitor of this enzyme.

The xanthine analogue, 7, did show a slight (25%) inhibition of the oxidation of xanthine when the analogue was present in twofold higher concentration than substrate. The 2-sulfonyl analogue of xanthine, 1,5-dihydroimidazo-[4,5-*c*][1,2,6]thiadiazin-4(3*H*)-one 2,2-dioxide, also showed no inhibition of xanthine oxidase.[25] These results suggest that tetrahedrally arranged substituents, such as the sulfonamide oxygens of 6–8 which extend above and below the plane of the thiadiazine ring, represent a steric deterrent to the binding of such analogues to the active sites of xanthine oxidase and HGPRTase. They may also interfere with binding to other enzyme systems involving purines.

Experimental Section

NMR spectra were determined in $(CD_3)_2SO$ using Me_4Si as an internal standard with a JEOL PFT-100 NMR spectrometer, UV spectra were determined with a Cary 15 recording spectrophotometer, and IR spectra were determined with a Perkin-Elmer Infracord spectrophotometer. Melting points were determined with a Mel-Temp apparatus and are uncorrected. Microanalyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, Mich.

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4(5)-Aminoimidazole-5(4)-sulfonamide (2). 4(5)-Nitroimidazole-5(4)-sulfonamide[6] (1; 5.00 g; 26.0 mmol) in 900 mL of EtOH was hydrogenated at 1 atm with 5% Pd/C (10.0 g; 1 h), the mixture was filtered, and the filtrate was evaporated to dryness under vacuum at *temperatures below 20 °C* to yield 3.39 g (80%) of **2**, as a tan solid: mp 162 °C dec; MS *m/e* (relative intensity) 162 (8) (M^+), 128 (6), 83 (11), 66 (6), 65 (8), 64 (100); NMR δ 11.52 (br, 1, exch, N-*H*), 7.16 (s, 1, C-*H*), 6.85 (br, 2, exch, SO_2NH_2), 5.15 (br, 2, exch, C-NH_2). Anal. ($C_3H_6N_4O_2S$) C, H, N, S.

4(5)-(Formylamino)imidazole-5(4)-sulfonamide (4). To a mixture of 2.26 g (11.8 mmol) of 4(5)-nitroimidazole-5(4)-sulfonamide and 6.5 g of 10% Pd/C was added 100 mL of 97–100% HCO_2H. The reaction mixture was stirred for 1 h; TLC (silica gel; EtOAc/CH_3OH, 4:1) indicated complete conversion to **2**. The solution was filtered, and the filtrate was heated at 50–60 °C for 6 h and then evaporated to dryness in vacuo. The residue was washed with MeOH (50 mL) and collected to give 1.43 g (64%) of **4**: mp 213 °C dec; NMR δ values shown in Scheme I, $J_{H,H}$ for NH and CH ~2 Hz for **4a** and ~10 Hz for **4b**; MS *m/e* (relative intensity) 190 (55) (M^+), 163 (100), 111 (50), 83 (70), 81 (48), 80 (14), 64 (74). Anal. ($C_4H_6N_4O_3S$) C, H, N, S.

4(5)-[*N'*-(Benzoylthiocarbamyl)amino]imidazole-5(4)-sulfonamide (3). A solution of 4(5)-aminoimidazole-5(4)-sulfonamide (**2**; 3.35 g, 20.7 mmol) and benzoyl isothiocyanate[26] (4.0 g, 24.5 mmol) in dimethylacetamide (DMAC; 20 mL) was sitrred at 20 °C for 2 h. The solution was added dropwise to CH_2Cl_2 (600 mL) to precipitate **3**. The product was collected and dried at 60 °C in vacuo for 1 day to yield 6.17 g (92%) of colorless flakes: mp 217 °C dec; NMR δ 13.13 (br, 1, exch, N-*H*), 13.05 (br, 1, exch, N-*H*), 11.95 (br, 1, exch, N-*H*), 8.03 to ~7.47 (m, 6, C_6H_5 and C_2-H), 7.35 (br, 2, exch, SO_2NH_2). Anal. ($C_{11}H_{11}N_5O_3S$) C, H, N, S.

3-(Benzoylamino)imidazo[4,5-*e*]-1,2,4-thiadiazine 1,1-Dioxide (5). A solution of 4(5)-*N'*-[(benzoylthiocarbamyl)-amino]imidazole-5(4)-sulfonamide (**3**; 5.88 g, 18.1 mmol) and CH_3I (4.0 g, 28.2 mmol) in 0.1 N NaOH (600 mL) was stirred for 7 h. The reaction mixture was acidified with HOAc to precipitate **5**, yield 2.60 g (49%). This was recrystallized from EtOH to give colorless flakes (dried over $CaCl_2$ in vacuo at 60 °C for 2 days): mp >320 °C; NMR δ 13.77 (br, 1, exch, N-*H*), 12.46 (br, 1, N-*H*), 11.84 (br, 1, N-*H*), 8.09–7.99 (m, 3), 7.69–7.46 (m, 3, aromatic C-*H*). Anal. ($C_{11}H_9N_5O_3S$) C, H, N, S.

3-Aminoimidazo[4,5-*e*]-1,2,4-thiadiazine 1,1-Dioxide (8). 3-(Benzoylamino)imidazo[4,5-*e*]-1,2,4-thiadiazine 1,1-dioxide (**5**; 1.50 g, 5.2 mmol) was added to a solution of Na (0.50 g, 21.7 mmol) in CH_3OH (150 mL). The resulting solution was heated at reflux for 3 h, cooled, and then acidified with 1.0 N HCl to pH 3.5 to precipitate **8**, yield 0.90 g (94%). The product was recrystallized from H_2O and dried over $CaCl_2$ at 80 °C in vacuo for 2 days to obtain colorless plates: mp >320 °C; NMR δ 13.12 (br, 1, exch, N-*H*), 10.94 (br, 1, exch, N-*H*), 7.75 (s, 1, C-*H*), 6.60 (br, 2, exch, NH_2); IR (KBr) ν 3500 and 3400 (NH_2), 1330 and 1165 cm^{-1} (SO_2); MS *m/e* (relative intensity) 187 (14) (M^+), 64 (69), 54 (6), 52 (15), 48 (34). Anal. ($C_4H_5N_5O_2S$) C, H, N, S.

4,5-Dihydroimidazo[4,5-*e*]-1,2,4-thiadiazin-3(2*H*)-one 1,1-Dioxide (7). A solution of $NaNO_2$ (0.67 g, 9.7 mmol, in 10 mL of H_2O) was added dropwise with stirring to a chilled solution of **8** (1.50 g, 8.0 mmol) in CF_3CO_2H (50 mL). The resulting solution was allowed to warm to room temperature and stirred for 18 h. The solvent was removed under reduced pressure, and the residue was suspended in aqueous EtOH (67%, 30 mL), which was adjusted to pH 3 with NaOH (10 N), and then filtered to yield 7 (0.60 g, 40%) as a brown solid. This was chromatographed over Bio-Rad, AG-50W-X8 (H^+), eluting with water to afford **7** as colorless plates. The analytical sample was dried over P_2O_5 at 110 °C in vacuo for 3 h: mp >177 °C (gradual dec), lit.[8] mp 260 °C, for the anhydrous form; IR (KBR) ν 1680 (CO), 1380 and 1170 cm^{-1} (SO_2), lit.[8] 1682, 1375, 1167 cm^{-1}; NMR δ 13.34 (br, 2, exch, N-*H*), 11.80 (br, 1, exch, N-*H*), 7.83 (s, 1, C-*H*). Anal. ($C_4H_4N_4SO_3 \cdot H_2O$) C, H, N, S.

Biological Evaluation. Cytotoxicity Studies. Inhibition of L1210 murine leukemia cell growth in culture was the criteria by which the cytotoxicity of each analogue was determined. Cells in the logarithmic phase of growth were harvested, resuspended in RPMI 1640 medium (Grand Island Biological Co., Grand Island, N.Y.), 10% with respect to fetal bovine serum (Flow Laboratories, Rockville, Md.), and dispensed into 16 × 125 mm culture tubes (final volume 5.0 mL). A starting density of 17.5×10^4 cells/mL permitted 6–7 population doublings during the 72-h period of the experiment. A solution of each analogue was prepared (5×10^{-3} M) in phosphate-buffered saline, pH 7.4, and filter sterilized with a 0.22 μM Swinnex filter (Millipore Corp., Bedford, Mass.). Following filtration, serial dilutions were made with medium and serum before the analogue was added to the culture tubes (final concentration range: 10^{-4} to 10^{-8} M). The growth at 37 °C in control (no analogue) and drug treated cultures was monitored every 24 h with a model ZBI Coulter Counter (Coulter Electronics, Hialeah, Fla.).

HGPRTase Assay. HGPRTase activity was determined by the conversion of [8-^{14}C]hypoxanthine to radioactive inosinic acid as reported previously.[27] The reaction mixture contained 25 μmol of Tris, pH 8.0, 0.25 μmol of 5-phosphoribosyl 1-pyrophosphate, 0.5 μmol of Mg^{2+}, 0.025 μmol of [8-^{14}C]hypoxanthine (specific activity 4 Ci/mol), and 1 μg of HGPRTase (partially purified from human erythrocyte with a specific activity of 0.5 IU/mg of protein) in a total volume of 100 μL. The inhibitory effects of the analogues were tested at equimolar concentrations to that of the substrate. The reaction mixture was incubated at 37 °C in a shaker bath for 10 min; the reaction was terminated by immersion in an ethanol–solid CO_2 bath, and nucleotide formation was measured.

Xanthine Oxidase Assays. Xanthine oxidase (Worthington Biochemical Corp., XOB 4.5 units/mL) was diluted 1:30 000 and stabilized with catalase. It was incubated in 0.01 M sodium phosphate buffer at pH 7.5 and 25 °C with 5×10^{-5} M xanthine and the following concentrations of **6** and **7**: 1×10^{-4} M, equimolar, and 1×10^{-5} M. The reactions were monitored by the increase in absorbance at 290 nm with a Varian SuperScan recording spectrophotometer in the time drive mode; rates were determined from the linear portion of the plot. Only in the presence of 1×10^{-4} M **7** was there a slight (25%) inhibition of the oxidation of xanthine.

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