of nonradioactive reference compounds and injected directly for HPLC analysis. The reference compounds were those that are possible products arising from simple metabolic or degradative modification of the test compounds **4a** and **4b**. The reference mixture contained, in addition to **4a** and **4b**, **2**, uracil, uridine, 2′-deoxyuridine, the 5-halouracil, and the 5-halouridine. The reference mixture containing 5-chloro compounds was resolved with 2% CH_3OH in H_2O at a flow rate of 1 mL/min (column B) and the 5-fluoro compounds similarly with 4% CH_3OH in H_2O. The radioactive components of the urine were identified by superimposing the activity histogram, obtained by liquid scintillation counting of the column eluate, with the UV trace of the spiked urine. Assignments were confirmed by exact coincidence of UV and radioactivity chromatograms under varying HPLC conditions.

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Registry No. 1, 3736-77-4; [2-^{14}C]-1, 99285-47-9; **2**, 784-71-4; [2-^{14}C]-**2**, 99277-84-6; **4a**, 55612-15-2; [2-^{14}C]-**4a**, 106711-36-8; **4b**, 72-84-4; [2-^{14}C]-**4b**, 106711-37-9.

Folate Analogues as Inhibitors of Thymidylate Synthase

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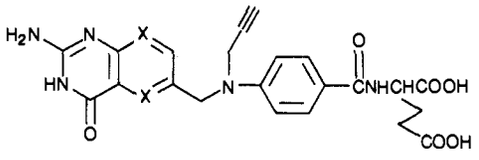
Recent demonstrations that deazafolate analogues may act as potent inhibitors of thymidylate synthase (TS) provided a firm rationale for the synthesis of N^{10}-propargyl derivatives of 8-deazafolate (**3**) and 8-deazaaminopterin (**4**). A complete assignment of the ^{1}H NMR spectra of these compounds was made possible through application of 2D (COSY) techniques at 200 MHz. Data describing the inhibition of TS derived from human leukemia (K562) cells are presented. IC_{50} values of 2.25 and 1.26 μM were determined for 8-deaza-10-propargylfolate and 8-deaza-10-propargylaminopterin, respectively. Comparison of the data for various folate analogues reveals a striking dependence of TS inhibitory potency upon the number of nitrogens in the folate pyrazine ring.

A number of folate analogues have been studied for their ability to inhibit thymidylate synthase (TS), an enzyme that catalyzes the C-methylation reaction of 2′-deoxyuridylate (dUMP) to provide 2′-deoxythymidylate (dTMP).[1-6] This one-carbon transfer reaction is critical to cell division, since it provides the sole de novo source of dTMP, an essential building block for DNA synthesis.[7] Hence, TS has long been considered a key target enzyme in the design and synthesis of antitumor agents.[7-9]

The most impressive activity demonstrated to data has been associated with N^{10}-propargyl-5,8-dideazafolate (PDDF, **1**) with K_i values reported as about 1 nM for the murine leukemia L1210 enzyme[3] and 20 nM for the human enzyme derived from either HeLa or KB cells.[10] Surprisingly, N^{10}-propargylfolate (**2**) itself was a poor inhibitor



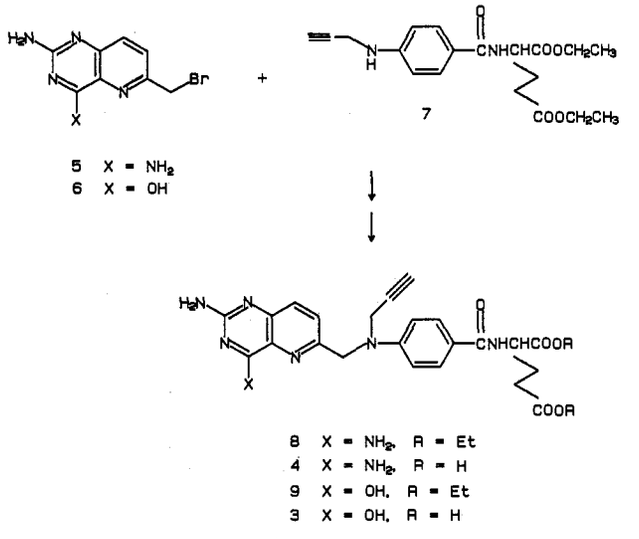
1, X = C
2, X = N

of the bacterial enzyme, having an IC_{50} of 3.9 μM against the *L. casei* enzyme,[6] very similar to the value of 5.5 μM against TS from K562 cells found in this study. N^{10}-Propargylaminopterin has also been prepared and was a very poor inhibitor of the enzyme (IC_{50} = 20 μM).[5] The preparation and evaluation of the 8-deaza analogue **3** became, therefore, of considerable interest; the 4-amino congener **4** was prepared for comparison purposes.

Chemistry

Previously described procedures[11,12] were used to prepare 6-(bromomethyl)-2,4-diamino- (**5**) and 2-amino-6-(bromomethyl)-4-oxopyrido[3,2-*d*]pyrimidine (**6**). Diethyl *N*-[4-(propargylamino)benzoyl]glutamate (**7**) was prepared

Scheme I



5 X = NH_2
6 X = OH

8 X = NH_2, R = Et
4 X = NH_2, R = H
9 X = OH, R = Et
3 X = OH, R = H

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in a significantly improved yield of 52% by a modification of the procedure of Piper et al.[5] Alkylation of **7** with either **5** or **6** afforded a reasonable yield of ester **8** and a low yield of **9**. Mild saponification[11,12] gave the target N^{10}-propargyl-8-deazaaminopterin **4** and the corresponding folate analogue **3** (Scheme I).

An alternative approach to **3**, involving direct alkylation of 8-deazafolate[11-13] by propargyl bromide, was evaluated. Usual conditions of alkylation in a polar, aprotic solvent such as dimethylformamide, dimethylacetamide or dimethyl sulfoxide, with or without catalytic amounts of potassium iodide, gave rise to complex mixtures. This unpleasant phenomenon presumably arose from inability of the alkylating agent to discriminate among the several nucleophilic centers in 8-deazafolate in an aprotic solvent. Ethanol, a protic solvent in which both reactants were soluble, was evaluated in the hope that selective solvation would enhance the regiospecificity of the reaction. This approach was successful and gave rise to **3** in 74% yield. The product was identical in all respects with **9** prepared by the alternative route. Direct alkylation with propargyl bromide was then tried on 8-deazaaminopterin, under the same conditions, as an alternative route to **4**. Again, a greatly improved yield (77%) was achieved. Final confirmation of N^{10} as the alkylation site was provided by FAB mass spectrometry. In addition to a clearly discernible molecular ion at m/e 535 (FAB, MH^+), a major cleavage occurred between C^9 and N^{10}, as expected on the basis of "benzylic" cleavage. The pyrido[3,2-*d*]pyrimidine fragment (m/e 176) did not contain the propargyl function. Cleavage of the amide, however, gave rise to the protonated deazapteroyl function containing the propargyl moiety (m/e 332), establishing N^{10} as the alkylation site.

A complete analysis of the 1D 200-MHz ^1H NMR spectrum of the N^{10}-propargyl derivatives could not be accomplished because of near equivalence of the propargyl methylene signal with those of other methylene groups in the molecule. The application of 2D COSY techniques, however, permitted immediate and unambiguous assignment of all proton resonances in the molecule. The cross peaks readily permit tracking through the glutamate portion of the molecule, beginning with the downfield glutamate NH to Glu α-CH to Glu β-CH_2 to Glu γ-CH_2. The only other nonobvious coupling corresponds to a weak coupling typical of the system $HC{\equiv}CCH_2N$.[15] These data enable an unambiguous assignment of the propargyl methylene and acetylene protons to signals at δ 4.45 and 3.27, respectively.

Biological Data

Data describing TS inhibition by a number of folate analogues are presented in Table I. The quinazoline analogue **1** is at least 2 orders of magnitude more potent than the pyridopyrimidine **3**, which in turn may bind a little more tightly than 10-propargylfolate **2**. Aminopterin derivative **4** was much like **3**, with an IC_{50} of 1.26 μM.

Table I. Thymidylate Synthase Inhibitory Activity of Various Folate Analogues[a]

compound	thymidylate synthase		
	IC_{50}, μM	K_i, μM	inhibition
folate	290	40[b]	competitive[b]
10-methylfolate		1.0[b]	competitive[b]
10-propargylfolate (**2**)	5.5		
8-deazafolate	55		
8-deaza-10-methylfolate	2.5		
8-deaza-10-propargylfolate (**3**)	2.4		
5,8-dideazafolate[18]	2.7		
5,8-dideaza-10-methylfolate[18]	0.29		
5,8-dideaza-10-propargylfolate (**1**)[10]	0.037	0.007[c]	competitive[c]

[a] The enzyme assays were performed as described in the Experimental Section with TS from K562 cells unless otherwise indicated. K_m of this enzyme for 5,10-methylenetetrahydrofolate was 28.5 μM. [b] Reference 16 (TS from human AML cells). [c] Reference 17 (TS from human AML cells).

Jones et al. originally reported[3] competitive inhibition by **1** of TS derived from murine leukemia L1210 cells. Cheng and co-workers described[10] noncompetitive inhibition of HeLa- and KB-derived TS but competitive inhibition of AML TS.[17] Very recently, Santi conducted more detailed studies using the *Lactobacillus casei* enzyme[19] and demonstrated that the initial kinetics, determined by initiating the reaction by addition of enzyme, were competitive, changing over a few minutes to noncompetitive as the covalent dUMP–enzyme interaction was stabilized by association with **1** to form a very slowly dissociating ternary complex. These workers also made the important observation that **1** could be released unchanged from the enzyme, establishing that the noncompetitive kinetics observed did not arise from covalent modification of the enzyme by the inhibitor.

This information raises an intriguing question; namely, why does replacement of the pyrazine ring nitrogens with CH lead to so dramatic an increase in enzyme affinity? Steric arguments cannot be made, since a pyridine-type nitrogen with its lone pair can hardly be regarded as more sterically demanding than C–H. Simple hydrophobicity arguments also seem untenable, since face-to-face stacking of aromatic heterocycles based upon hydrophobicity or water exclusion from the nonpolar faces is commonplace.[20]

Certainly, however, the "edges" of the molecule represent its hydrophilic regions and pyridine-type nitrogens readily undergo hydrogen-bonding interactions with water.[20] It is conceivable that hydration of nitrogen at position 5 sterically hinders the approach of a folate or 8-deazafolate to the tetrahydrofolate binding site of TS.

Alternatively, although it is not easy to see why this should be so, there may be conformational differences separated by significant energy differences among **1**, **2**, and **3**. Since such differences might arise from differential solvation, it might not be possible to differentiate between the last two possibilities. Nevertheless, a computer graphic aided energy-minimization study has been initiated to

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attempt to deal with this problem, the results of which will be reported elsewhere.

A striking parallel to these observations, conceivably amenable to the same kind of interpretation, was reported by Benkovic in his studies with glycinamido ribonucleotide transformylase (GAR TFase).[21] Benkovic found that 10-formylfolate and a series of deaza analogues were, in general, substrates for the enzyme. Since the normal cofactor is a reduced folate (5,10-methenyltetrahydrofolate), it was remarkable that the fully aromatic 5,8-dideaza-10-formylfolate was an even better substrate based on the ratio V_{rel}/K_m^{app}. The 5-deaza analogue was less effective, the folate still less (by 2 and 3 orders of magnitude, respectively), and the 8-deaza was an inhibitor of the enzyme. These data are also generally consistent with the hypothesis outlined above.

Experimental Section

The ^{1}H NMR spectra were recorded on an IBM FT-200 NMR spectrometer in $(CD_3)_2SO$ with Me_4Si as an internal standard. Melting points were determined on a Thomas-Hoover melting point apparatus and were not corrected. Mass spectra were taken on a Varian 112S or LKB-GCMS 9000S spectrometer. UV spectra were taken on a Beckman DU-8 spectrometer. Elemental analysis was performed by MicAnal Laboratories, Tucson, AZ. All thin-layer chromatography was done on precoated TLC sheets, silica gel 60 F254, layer thickness 0.2 mm.

Chemicals. [5-^{3}H]dUMP (22 Ci/mmol) and dUMP were obtained from Moravek Biochemicals, Inc., Brea, CA, and Sigma Chemical Co., St. Louis, MO, respectively. Tetrahydrofolate was prepared by the chemical synthesis as described.[22] The purity and the concentration of the folate compounds used were determined spectrophotometrically. All other chemicals used were of reagent grade or higher.

Enzyme Purification and Assays. TS was purified approximately 2480-fold to a specific activity of 89.4 units/mg of protein by procedures described previously[23] from K562 cells. The enzyme assay was performed by the tritium release procedure of Roberts[24] as reported by Dolnick and Cheng[23] with 28 μM [5-^{3}H]dUMP (1.07 mCi/μmol) and 300 μM 5,10-methylenetetrahydrofolate. One unit of enzyme activity was defined as the conversion of 1 nmol of the substrate (dUMP) per min and the concentration of the enzyme was 0.12 unit/mL. The reaction was started by the addition of the enzyme and the incubation was for 50 min at 37 °C. All assays were conducted in duplicate and repeated at least once with similar results.

Diethyl *N*-[4-(Propargylamino)benzoyl]-L-glutamate (7). A mixture of diethyl *N*-(4-aminobenzoyl)-L-glutamate (8.06 g, 25.0 mmol), propargyl bromide (2.8 mL as 80% solution in toluene, 25.5 mmol), $NaHCO_3$ (2.66 g, 31.7 mmol), and anhydrous EtOH (50 mL) was refluxed with stirring for 16 h. Examination by TLC (EtOAc–hexane, 2:3) showed base-line impurities and unchanged amine, desired product, and a coproduct presumed to be the dipropargyl compound with R_f values of 0.20, 0.50, and 0.65, respectively. The mixture was filtered and ethanol was evaporated. EtOAc (100 mL) was added to the residue and NaBr precipitated. This mixture was washed with H_2O (3 × 100 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to leave an orange oil (9.84 gm).

A 3.28-g sample of the crude material, corresponding to 2.69 g of starting ester, was applied to a silica gel (230–400 mesh) flash column. The mixture was separated with EtOAc–hexane (2:3). Twenty-five-milliliter fractions were collected. Fractions 12–23 contained monoalkylated product contaminated with dialkylated compound. Fractions 24–38 contained only desired product. Evaporation of the pure fractions yielded 752 mg of *N*-[4-(propargylamino)benzoyl]glutamate. Fractions containing di- and monoalkylation products were combined and evaporated. The oil was taken up in EtOAc and hexane was added until the solution became cloudy. After refrigeration the precipitate was filtered, yielding an additional 806 mg of desired product: yield 1.56 g (52%); mp 98 °C; mass spectrum, *m/e* 360 (M^+); UV λ_{max} (ϵ_{max}) pH 1, 285 nm (6900) and 230 (8000); pH 7, 287 (19000); pH 12, 282 (19400) and 230 (4300); ^{1}H NMR ($(CD_3)_2SO$) δ 8.3 (d, 1, CON*H*), 7.7 and 6.6 (d, 2 ea, C_6H_4), 4.4 (s, 2, $CH_2C{\equiv}C$) 3.2 (s, 1, $HC{\equiv}C$). Anal. ($C_{19}H_{24}N_2O_5$) C, H, N.

Diethyl *N*-[4-[[(2,4-Diaminopyrido[3,2-*d*]pyrimidin-6-yl)methyl]propargylamino]benzoyl]-L-glutamate (8). Method A. To a solution of 2,4-diamino-6-(bromomethyl)pyridopyrimidine (**5**)[11] from 2,4-diamino-6-(hydroxymethyl)pyridopyrimidine (0.5 g, 2.62 mM) in 50 mL of dimethylacetamide (distilled from CaH_2) was added 1.9 g (2 equiv) of **7**. The reaction mixture was heated at 100 °C for 2 h. TLC in $CHCl_3$–MeOH (8:2) showed absence of starting material. The solvent was removed in vacuo, and the residue was suspended in water, stirred with 20 mL of 5% $NaHCO_3$ solution, and extracted with three 30-mL portions of $CHCl_3$. The organic extract was dried over anhydrous sodium sulfate and concentrated. The oil was applied to a silica gel column (60–200 mesh). Elution with $CHCl_3$–CH_3OH (96:4) gave unreacted **7**. Elution with $CHCl_3$–CH_3OH (90:10) gave 400 mg (28.6%) of the desired compound: mp 100 °C; mass spectrum, *m/e* 533 (M^+); UV λ_{max} (ϵ_{max}) pH 1, 300 nm (17400) and 224 (23500); pH 7, 300 (17400) and 223 (25000); pH 12, 297 (19400); ^{1}H NMR ($(CD_3)_2SO$) δ 8.4 (d, 1, N*H*C=O), 7.8 and 6.8 (d, 2 each, C_6H_4), 7.5 (d of d, 2, C^7*H*, C^8*H*), 4.8 (s, 2, CH_2N^{10}), 4.4 (s, 2, $CH_2C{\equiv}C$), 3.2 (s, 1, $HC{\equiv}C$). A small amount was recrystallized from EtOAc and hexanes for an analytical sample. Anal. ($C_{27}H_{31}N_7O_5{\cdot}0.5$EtOAc) C, H, N. The presence of 0.5EtOAc was verified by NMR.

Method B. Diethyl 8-deazaaminopterin[12] (100 mg, 0.2 mM) was added to 2 mL of EtOH. Propargyl bromide (1.25 mL, 80% in toluene) was added and the reaction mixture was refluxed for 16 h. TLC in $CHCl_3$–MeOH (8:2) showed the absence of starting material and a single spot at R_f 0.7. Solvent was removed in vacuo, and the residue was taken up in H_2O and brought to pH 7 with 5% $NaHCO_3$. The mixture was extracted three times with 30-mL portions of $CHCl_3$, dried over Na_2SO_4, filtered, and evaporated to yield 83.6 mg (77%) of the desired compound, identical in all respects with the compound formed by method A.

Diethyl *N*-[4-[[(2-Amino-3,4-dihydro-4-oxopyrido[3,2-*d*]-pyrimidin-6-yl)methyl]propargylamino]benzoyl]-L-glutamate (9). Method A. To a solution of 2-amino-4-oxo-6-(bromomethyl)pyridopyrimidine (**6**)[11] from 2-amino-4-oxo-6-(hydroxymethyl)pyridopyrimidine (0.5 g, 2.62 mM) in 50 mL of dry DMAC was added 1.9 g (2 equiv) of **7**. The reaction mixture was heated at 100 °C for 2 h. TLC in $CHCl_3$–MeOH (8:2) showed absence of starting material. Workup as above yielded an orange oil. The oil was applied to a silica gel column (60–200 mesh). The silica gel (52 g) had been deactivated by addition of 8 mL of deionized H_2O. The deactivated silica gel was then packed as a $CHCl_3$ slurry. Elution with $CHCl_3$–CH_3OH (96:4) yielded unreacted **7**. Elution with $CHCl_3$–MeOH (90:10) gave 84 mg (6.5%) of the desired compound: mp 132 °C; mass spectrum, *m/e* 534 (M^+); UV λ_{max} (ϵ_{max}) pH 1, 299 nm (28000) and 249 (14300); pH 7, 300 (26500) and 220 (35600); pH 12, 294 (25400) and 227 (27600); ^{1}H NMR ($(CD_3)_2SO$) δ 7.8 and 6.8 (d, 2 each, C_6H_4), 7.3 and 7.5 (d, 1 ea, C^7*H*, C^8*H*), 4.8 (s, 2, CH_2N^{10}), 3.2 (s, 1, $HC{\equiv}C$). A small amount was recrystallized from EtOAc and hexanes for an analytical sample. Anal. ($C_{27}H_{30}N_6O_6{\cdot}0.5$EtOAc) C, H, N.

Method B. A 200-mg sample (0.4 mM) of diethyl 8-deazafolate[11] was added to 3 mL of EtOH. Propargyl bromide (2.5 mL 80% in toluene (large excess) was added and the reaction mixture was refluxed overnight. TLC in CH_3CN–H_2O (9:1) showed the reaction to be complete. Solvent was removed in vacuo, and the residue was taken up in $CHCl_3$, washed three times with H_2O, dried over Na_2SO_4, and evaporated to yield 160 mg (74%) of the desired compound, identical in all respects with the compound formed by method A.

***N*-[4-[[(2,4-Diaminopyrido[3,2-*d*]pyrimidin-6-yl)-methyl]propargylamino]benzoyl]-L-glutamic Acid (4).** To a suspension of **8** (150 mg, 0.28 mM) in 3.75 mL of EtOH was added 1.6 mL of 1 N NaOH. The reaction mixture was stirred at room temperature for 48 h. TLC showed absence of starting

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material and a single spot (R_f 0.7) in $(CH_3)_2CHOH-NH_4OH-H_2O$ (7:1:2). The solution was acidified with 1 N HCl to pH 3-4. The precipitated solid was filtered, washed with water, and dried. **4**: yield 104 mg (78%); mp 187 °C; mass spectrum, *m/e* 765 (M^+, $4Me_3Si$ derivative); UV λ_{max} (ϵ_{max}) pH 1, 293 (23 900) and 277 (24 600); pH 7, 277 (32 400) and 223 (38 200); pH 12, 277 (32 900); ^1H NMR ($(CD_3)_2SO$) δ 7.7 and 6.8 (d of d, 4, C_6H_4), 7.4 (q, 2, C^7H, C^8H), 4.4 (s, 2, $CH_2C{\equiv}C$), 3.2 (s, 1, $HC{\equiv}C$). Anal. ($C_{23}H_{23}N_7O_5{\cdot}2H_2O$) C, H, N.

***N*-[4-[[(2-Amino-3,4-dihydro-4-oxopyrido[3,2-*d*]pyrimidin-6-yl)methyl]propargylamino]benzoyl]-L-glutamic Acid or 8-Deaza-N^{10}-propargylfolic Acid (3).** To a suspension of **9** (84 mg, 0.157 mM) in 2 mL of EtOH was added 0.8 mL of 1 N NaOH. The mixture was stirred for 48 h, at which time reaction appeared to be complete by TLC [$(CH_3)_2CHOH-NH_4OH-H_2O$ (7:1:2)]. The solution was acidified with 1 N HCl to pH 3-4. The precipitated solid was filtered washed with water and dried. **3**: yield 55 mg (73%); mp 202 °C; mass spectrum, *m/e* 766 (M + $4Me_3Si$ derivative); UV λ_{max} (ϵ_{max}) pH 1, 298 nm (18 200) and 250 (11 500); pH 7, 285 (27 400) and 220 (38 500); pH 12, 286 (11 300) and 223 (16 300); ^1H NMR δ 7.6 and 6.8 (d, 2 each, C_6H_4), 7.3 and 7.42 (d, 1 ea, C^7H and C^8H), 6.5 (s, 2-NH_2), 4.8 (s, 2, CH_2N^{10}), 3.2 (s, 1, $HC{\equiv}C$). Anal. ($C_{23}H_{22}N_6O_6{\cdot}H_2O$) C, H, N.

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Registry No. **2**, 101760-45-6; **3**, 106500-88-3; **4**, 106500-89-4; **5**, 76807-56-2; **6**, 76832-41-2; **7**, 76858-72-5; **8**, 106500-90-7; **9**, 106500-91-8; diethyl *N*-(4-aminobenzoyl)-L-glutamate, 13726-52-8; propargyl bromide, 106-96-7; diethyl 8-deazaaminopterin, 76807-59-5; diethyl 8-deazafolate, 76807-65-3; thymidylate synthase, 9031-61-2; folic acid, 59-30-3; 10-methylfolic acid, 2410-93-7; 8-deazafolic acid, 51989-25-4; 8-deaza-10-methylfolic acid, 76807-68-6.

Analogues and Derivatives of Tenoxicam.[1] 1. Synthesis and Antiinflammatory Activity of Analogues with Different Residues on the Ring Nitrogen and the Amide Nitrogen

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The synthesis of tenoxicam, 4-hydroxy-2-methyl-*N*-2-pyridyl-2*H*-thieno[2,3-*e*]-1,2-thiazine-3-carboxamide 1,1-dioxide (**1e**), and of the analogues with various residues on the ring nitrogen and the amide nitrogen is described. This new class of "oxicams" has pronounced antiinflammatory and analgesic properties. The very specific structure–activity relationship of isomeric and isosteric groups at the amide nitrogen has been evaluated. The substituent in position 2 also has a great influence on the pharmacological properties. Tenoxicam is presently undergoing clinical trials.

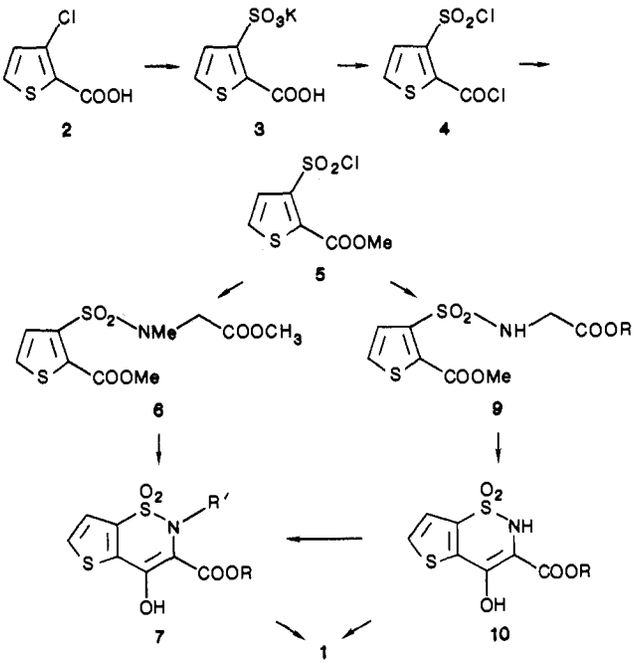
Around 1968 Lombardino and Wiseman discovered the benzo-1,2-thiazine enolamide 1,1-dioxides as a new class of potent nonsteroidal antiinflammatory agents.[2] By variation of the residues at the ring and at the amide nitrogen atoms, piroxicam has been identified as the most active derivative (Figure 1). The favorable pharmacological and pharmacokinetic properties of this compound make a "once a day dose" of 20 mg optimal to relieve pain and other symptoms of arthritic patients.[3] The potency is about 5 and 10 times higher, respectively, than that of indomethacin and phenylbutazone, the two best known drugs of the 1960s for this indication.

Recognizing the outstanding antiinflammatory potency of some of these enolamides in rats, we decided in 1973 to study the synthesis and the pharmacological activity of analogues in which the annulated benzene ring is replaced by a heterocyclic unit (Figure 1, A). It appeared to be plausible that this variation of the molecule could exert a pronounced influence on activity, similar to that found previously for the residues at the two nitrogen atoms. This paper deals with the synthesis of 4-hydroxy-2*H*-thieno-[2,3-*e*]-1,2-thiazine-3-carboxamide 1,1-dioxides **1**.[4]

Chemistry

Synthesis of the title compounds **1a–q** (Table I) started from the acid **2**, which was obtained by a "Fiesselmann" thiophene synthesis, followed by substitution of the hydroxy group by chlorine.[5] A sulfite-exchange reaction of **2** yielded the potassium sulfonate **3**, which was transformed into the bis(acid chloride) **4**, with phosphorus pentachloride in phosphorus oxychloride. Selective methanolysis

Scheme I



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(1) Trademark: Tilcotil (F. Hoffmann-La Roche & Co. Ltd.).
(2) Lombardino, J. G.; Wiseman, E. H. *Med. Res. Rev.* **1982**, *2*, 127.

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