was dried (Na_2SO_4) and concentrated in vacuo to yield a light yellow solid which upon recrystallization (*n*-hexane) gave 21.6 g (93%) of **2b** as a white powder: mp 67–69°. The HCl salt of **2b** was prepared and recrystallized (acetone): mp 166–167°. Anal. ($C_{14}H_{20}ClNO_2$) C, H, N.

A solution of **2b** (5.0 g, 0.021 mol) was prepared in 100 ml of 1.13 *N* H_2SO_4, 1.0 g of 10% Pd/C was added, and the mixture was hydrogenated at 55–60° and 48 psi for 48 hr. The solution was filtered, basified (15% NaOH), and extracted with $CHCl_3$. The extract was then dried ($MgSO_4$) and concentrated in vacuo to provide 4.0 g (80%) of a viscous, yellow oil which was identified by spectral methods as the diol **3** which had been previously prepared from the reaction of 2-piperidinemethanol and styrene oxide. Treatment of **3** with 48% HBr provided **2b** having identical physical and chemical properties with the sample obtained by the previous synthetic method.

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Synthesis of *N*-Hydroxythiourea

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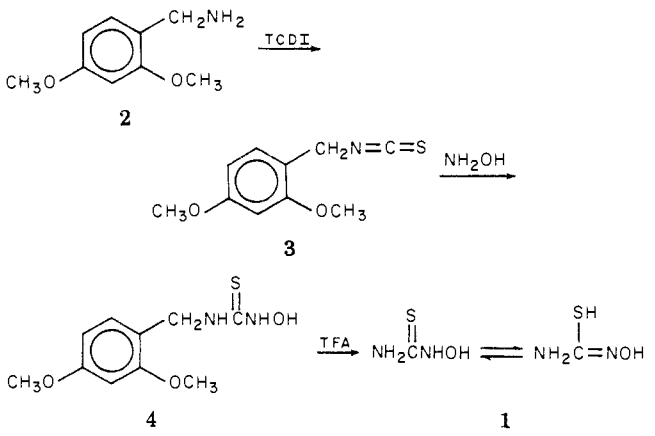
The synthesis of the title compound (1) was accomplished by the conversion of 2,4-dimethoxybenzylamine (2) into an isothiocyanate (3) using thiocarbonyl diimidazole. Treatment of 3 with hydroxylamine and removal of the DMB group with trifluoroacetic acid gave 1. *N*-Hydroxythiourea (1) showed no activity in the L1210 mouse tumor.

N-Hydroxyurea is a well-known and useful chemotherapeutic agent in the treatment of cancer.[1] It was of interest, therefore, to prepare the sulfur analog of this compound, *N*-hydroxythiourea (1), in order to examine its chemotherapeutic properties. The standard method for conversion of an amine to a thiourea, i.e., treatment with thiocyanic acid under rather vigorous conditions, would not suffice for the synthesis of 1 since neither hydroxylamine nor the product might be expected to withstand the conditions required for the reaction. In light of reports by Weygand,[2] who used the bis(2,4-dimethoxybenzyl), and Pietta,[3] who used the 2,4-dimethoxybenzyl (Dmb) protecting group for amide functions, we designed a synthesis of 1 in which the Dmb-protecting group was used (Scheme I).

Scheme I



The conversion of 2,4-dimethoxybenzylamine (2) into the isothiocyanate 3 turned out to be the most difficult step in the synthesis. Although Bach and Kjaer[4] reported the conversion of 2 into 3 using thiophosgene, these authors indicated that it decomposed on short-path distillation and were unable to report an elemental analysis for their product. In our hands also, thiophosgene was a poor reagent for the preparation of the isothiocyanate 3. However, thiocarbonyldiimidazole (TCDI) smoothly converted the amine into 3, which crystallized from hexane: mp 24–25°. Treatment of 3 with excess hydroxylamine in aqueous methanol afforded the thiourea 4 in excellent yield. The Dmb group was removed from 4 with trifluoroacetic acid giving 1 in almost 70% yield.

N-Hydroxythiourea (1) crystallized in two polymorphic forms when allowed to crystallize slowly but could be obtained in a single form when crystallization was rapid. The compound was unstable at ambient temperatures in the laboratory and was stored in a refrigerator freezer compartment. Its NMR spectrum indicated that it exists as a mixture of enthiol and thione tautomers and the ir spectrum indicated an azomethine function (C=N, 1610 cm^{-1}) but showed no strong band readily assignable to a thiocarbonyl group.

The use of the Dmb-protecting group in the synthesis of thiocarbonyl derivatives of very sensitive molecules is exemplified in this work. The very mild conditions used in this procedure should allow the synthesis of such de-

rivatives of many other sensitive amino compounds.

Dissappointingly, 1 showed no activity against the L1210 mouse tumor at 400 mg/kg by intraperitoneal injection as determined by the National Cancer Institute.

Experimental Section

All melting points were measured on a Nagle-Kopler micro hot stage. All infrared spectra were recorded on a Perkin-Elmer Model 257. All NMR spectra were recorded on a Hitachi HA-100.

2,4-Dimethoxybenzyl Isothiocyanate (3). To a stirred solution of *N,N'*-thiocarbonyldiimidazole[5] (14.95 g, 84 mmol) in dry $CHCl_3$ (100 ml) was added a solution of 2,4-dimethoxybenzylamine[3] (11.69 g, 70 mmol) in $CHCl_3$ (30 ml) over 30 min under ice–water cooling. The reaction mixture was stirred for 3 hr at room temperature and evaporated in vacuo. The residue was extracted with warm hexane repeatedly (8 × 100 ml) and the combined hexane extracts were evaporated to ca. 200 ml. The solution was passed through a short column of silica gel (10 g) and eluted with 300 ml of hexane. The combined eluate was evaporated to ca. 50 ml and cooled to −10° overnight to give 7.0 g (60%) of colorless prisms: mp 24–25°; ir ($CHCl_3$) 2080, 2175 cm^{-1} (−N=C=S); NMR ($CDCl_3$) δ 3.79 (s, 3 H, OCH_3) 3.82 (s, 3 H, OCH_3), 4.58 (s, 2 H, CH_2N), 6.38–6.50 (m, 2 H, Ar), 7.15 ppm (d, 1 H, Ar). Anal. Calcd for $C_{10}H_{11}NO_2S$: C, 57.41; H, 5.30; N, 6.70; S, 15.30. Found: C, 57.27; H, 5.31; N, 6.64; S, 15.26.

***N*-(2,4-Dimethoxybenzyl)-*N'*-hydroxythiourea (4).** A mixture of **3** (4.18 g, 20 mmol), $NH_2OH{\cdot}HCl$ (2.78 g, 40 mmol), and $NaHCO_3$ (3.36 g, 40 mmol) in 20 ml of 75% aqueous methanol was stirred at room temperature for 1 hr. The mixture was diluted with 50 ml of water and cooled in ice–water. The precipitate was collected, washed with water, and dried to give 4.4 g of crystals: mp 105–106°. Recrystallization from ethyl acetate gave 4.0 g (83%) of prisms: mp 108–109°; ir (Nujol) 3350, 3160, 3040 cm^{-1} (OH, NH); NMR (Me_2SO-d_6) δ 3.80 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), (d, 2 H, CH_2N, J = 6 Hz), 6.44–6.60 (m, 2 H, Ar), 7.10 (d, 1 H, Ar), 7.94 (t, 1 H, J = 6 Hz, CH_2NH), 9.4 (1 H, broad, SH), 10.1 ppm (1 H, broad, −NOH). Anal. Calcd for $C_{10}H_{14}N_2O_3S$: C, 49.59; H, 5.83; N, 11.56; S, 13.21. Found: C, 49.66; H, 5.81; N, 11.54; S, 13.14.

***N*-Hydroxythiourea (1).** A solution of 4 (2.90 g, 12 mmol) and resorcinol dimethyl ether (3.31 g) in 20 ml of CF_3COOH was stirred at room temperature for 1.5 hr. After evaporation of the acid in vacuo, 20 ml of methanol was added to the residue and the mixture was evaporated in vacuo. Dry benzene (20 ml) was added to the residue and the precipitate was collected, washed with benzene, and dried. The crystals were dissolved in tetrahydrofuran (ca. 10 ml), filtered to remove insolubles, and diluted with $CHCl_3$ to afford 0.94 g (68%) of colorless prisms: mp 97–101° dec. An analytical sample was recrystallized from ether: mp 100–101° dec; ir (Nujol) 3420, 3290, 3150 (NH, OH), 1610 cm^{-1} (C=N); NMR (Me_2SO-d_6) δ 7.35 (1 H, broad, NH_2), 7.68 (1 H, broad, NH_2), 9.40 (s, 1 H, SH), 10.12 (s, 1 H, =NOH). Anal. Calcd for CH_4N_2OS: C, 13.05; H, 4.38; N, 30.43; S, 34.76. Found: C, 13.02; H, 4.37; N, 30.30; S, 34.78.

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Some Substituted Naphthazarins as Potential Anticancer Agents

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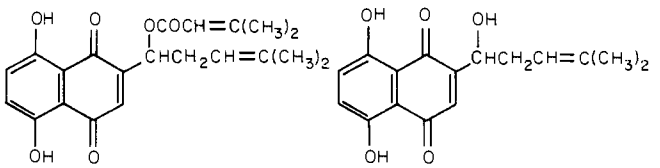
Some 2,3-bis(substituted methyl)naphthazarins and related compounds were synthesized by the Diels–Alder reaction of benzoquinone and 2,3-dimethylbutadiene followed by oxidation and substitution reactions. These compounds were prepared as potential biological alkylating agents. Screening results indicated that 1,4-diacetyl-6,7-dimethyl-4a,5,8,8a-tetrahydronaphthalene and 5,8-bis(benzoyloxy)-2,3-dimethyl-1,4-naphthoquinone possessed borderline activity against leukemia P388 and that naphthazarin diacetate possessed confirmed cytotoxicity against the cell culture of human epidermoid carcinoma of the nasopharynx.

Arnebin I (1), isolated from the roots of *Arnebia nobilis*, inhibits rat Walker carcinosarcoma 256.[1] This compound bears a close structural resemblance to shikonin (2, present in the roots of *Lithospermum erythrorhizon* Sieb et Zucc[2]) and both compounds may be biogenetically related. A crude extract prepared in this laboratory from *L. erythrorhizon* (NSC B626370) was found to possess inhibitory activity against the cell culture of human epidermoid carcinoma of the nasopharynx (9KB).



1 2

The activity of these naphthazarins, together with the fact that some antineoplastic property was noted by a number of benzoquinones and naphthoquinones substituted with one or two side chains potentially capable of biological alkylation after bioreduction,[3-7] prompted the preparation of some substituted naphthazarins for antitumor screening. This study is particularly intriguing since the naphthazarin unit is also a portion of the tetracyclic antitumor antibiotics daunomycin, adriamycin, nogalomycin,[8,9] and carminomycin.[10,11]

Chemistry. 2,3-Dimethylnaphthazarin diacetate (**5a**), prepared by Contreras[12] by the Diels–Alder adduct 4a,5,8,8a-tetrahydro-6,7-dimethyl-1,4-naphthoquinone[13-16] (**3**), was originally to be used for the preparation of 2,3-bis(substituted methyl)naphthazarins. Since the yield of **5a** was extremely low, the corresponding dibenzoate **5b** was prepared. Bromination of **5b** with *N*-bromosuccinimide in the presence of light yielded 5,8-bis(benzoyloxy)-2,3-bis(bromomethyl)-1,4-naphthoquinone (**6a**) which, upon hydrolysis, gave an almost quantitative yield of 2,3-bis(bromomethyl)naphthazarin (**6b**). The desired 2,3-bis-