(Table I) caused a significant inhibition of MAO B, either in vitro or in rat brain after oral dosing.

A number of metabolites of **4a** were isolated from rat, dog, and monkey urine. Of these, the 1-(1-hydroxyethyl) (**4b**), 1-(2-hydroxyethyl) (**4c**), and 1-(1-vinyl) (**4f**) analogues had high MAO A-I, as shown in Table I, and the 1-(1,2-dihydroxyethyl) (**4d**) and 1-(1-acetyl) (**4e**) analogues had moderate potency. 1-Acetic acid **4g**, however, had essentially no MAO inhibition. None had detectable MAO B-I.

Our working hypothesis is that the inhibitory activity of these compounds is due to noncovalent binding to the flavin prosthetic group of the enzyme. Quantum mechanical calculations for a similar interaction have been published.[10]

Chemistry

Phenoxathiins were oxidized to the sulfones with hydrogen peroxide in acetic acid at or over a temperature of 95 °C. 1-Substituted derivatives were prepared by lithiation, generally using butyl lithium in tetrahydrofuran at temperatures below −30 °C and treating the lithio compound with the appropriate electrophile (Scheme I). Compound **4a** prepared by this procedure using ethyl iodide was contaminated with 1,9-diethyl homologue as well as smaller amounts of other impurities (possibly 4- or 6-ethylated), which required extensive chromatography to yield pure product. This compound therefore was best made by a two-step process involving reaction of the lithio compound with acetaldehyde to yield **4b**, followed by removal of the hydroxy group by means of Pearlman's $Pd(OH)_2$ on carbon catalyst[11] and hydrogen.

A general synthetic method (Scheme II) for preparing phenoxathiins with various substitution patterns involved formation of the diaryl sulfide from an *o*-nitroaryl halide and an *o*-hydroxythiophenol, followed by cyclization with displacement of the nitro function by the phenoxide oxygen. Various bromophenoxathiins were made by this method and converted by way of lithium-for-bromine exchange followed by electrophilic substitution to alkyl derivatives, and via boronates to hydroxy compounds. Migration to the 4-position was not observed. The products could then be oxidized to the 10,10-dioxides (after protection of any free hydroxyl function).

Biological Methods

MAO activities were determined by a double-label assay using tritiated serotonin and [^{14}C]-2-phenethylamine.[12] Ex vivo MAO inhibition was determined after removing rat brains 3 h after oral administration of the substance to be tested. Portions of a washed mitochondrial preparation from rat brain were incubated at 37 °C with [^{3}H]serotonin (0.19 mM, 5 Ci/mol) and [^{14}C]-β-phenethylamine (10 μM, 3 Ci/mol) in a total volume of 0.30 mL of 30 mM, pH 7.4, potassium phosphate buffer for 20 min. At these substrate concentrations, which are approximately twice the K_m concentrations at MAO A and MAO B sites, the activity with each substrate was independent of the other. Therefore it was possible to evaluate MAO A-I and MAO B-I simultaneously. This procedure gives IC_{50} values that approximate twice the K_i values for competitive inhibitors and has provided a reliable way to rank inhibitory potencies.[12] The 5-hydroxytryptophan potentiation test, the Porsolt test for antidepressant activity, and a mother-reared/peer-deprived Rhesus monkey test were performed following published methods.[13-15]

In summary, 1-ethylphenoxathiin 10,10-dioxide (**4a**, 1370U87) is a potent monoamine oxidase inhibitor selective for MAO A. It is reversibly bound to and displaceable from the enzyme. Compound **4a** gives positive results in standard antidepressant tests in rats and in a personality disorder paradigm in Rhesus monkeys. A negligible rise in blood pressure is found in rats ingesting tyramine after pretreatment with this compound. A number of other phenoxathiin dioxides also inhibit MAO A selectively, including five of the six isolated metabolites of this compound. This series is unique in having potent MAO inhibitors that contain no nitrogen.

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Nitroheterocycle Reduction as a Paradigm for Intramolecular Catalysis of Drug Delivery to Hypoxic Cells

Bioreductive activation is a well-established concept for achieving selective toxicity of antitumor agents to hypoxic cells.[1-3] The reduction of nitroheterocycles such as nitroimidazoles[2] and nitroacridines[3] provides a widely ex-

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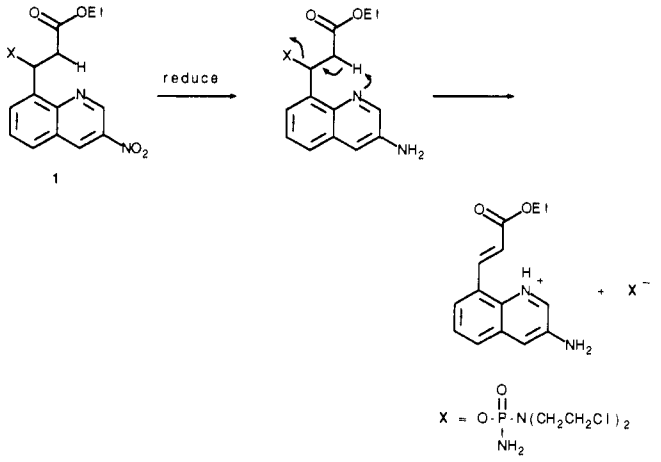
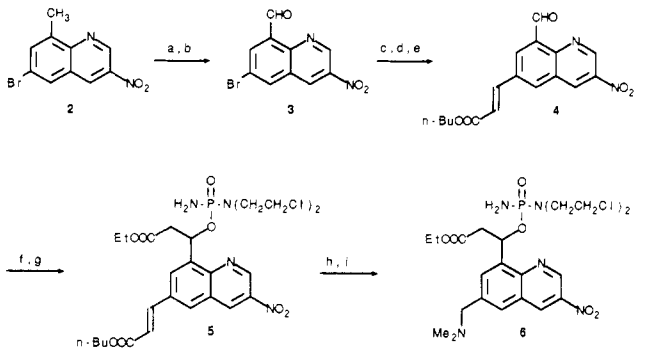
Scheme I



Scheme II[a]



[a] (a) *N*-Bromosuccinimide (66%); (b) *N*-methylpiperazine, H_2O, CH_3CN (75%); (c) $HOCH_2CH_2OH$, H^+ (87%); (d) $H_2C{=}CHCOO$-*n*-Bu, $Pd(OAc)_2$, NaOAc (70%); (e) AcOH, H_2O (95%); (f) $H_2C{=}C(OEt)OSiMe_2$-t-Bu, $MgBr_2$, then aqueous AcOH (79%); (g) TlO-*t*-Am, then $Cl_2P(O)N(CH_2CH_2Cl)_2$, then NH_3 (68%); (h) OsO_4, $Me_2NCH_2CH_2OAc$, HIO_4, then $NaIO_4$ (46%); (i) Me_2NH, Bu_4NBH_3CN (22%).

ploited pathway for the generation of toxic metabolites under hypoxic conditions. Another approach to the selective delivery of a cytotoxic species might utilize the reductive process to enhance catalysis of a specific activation reaction. On the basis of our interest in the activation mechanisms of cyclophosphamide and the mechanistic precedent involving drug activation by a β-elimination reactions,[4] we sought to test the hypothesis outlined in Scheme I, where X is phosphoramide mustard (PDA). Although β-elimination of PDA occurs readily from substrates in which the carbonyl group is an aldehyde or ketone, this reaction does not occur when the activating group is an ester (e.g., 1). If the nitro group in 1 were to undergo reduction, however, the increase in basicity of the quinoline nitrogen resulting from reduction of the nitro group would provide a mechanism for the intramolecular base-catalyzed elimination of PDA as shown. We report here the synthesis and evaluation of a nitroquinoline phosphoramidate as a novel type of mechanism-based hypoxia-selective alkylating agent.

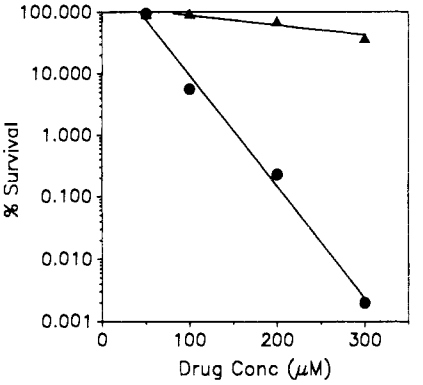


Figure 1. Survival of HT-29 cells following treatment with phosphoramidate **6**. Cells were treated under aerobic or hypoxic conditions for 4 h. Survival was measured by using a clonogenic assay: (▲) aerobic, (●) hypoxic. The data points represent the mean of quadruplicate determinations for each of two separate experiments, range ± 20%. The solid lines represent the linear regression of the data points.

Chemistry. Synthesis of a nitroquinoline phosphoramidate was accomplished as outlined in Scheme II. 3-Nitro-6-bromo-8-methylquinoline (2) was converted to the α,α-dibromo derivative which was hydrolyzed to aldehyde 3. Following protection of the aldehyde group, the solubilizing (dimethylamino)methyl group was appended in masked form via a palladium-catalyzed coupling reaction with butyl acrylate.[5] Subsequent hydrolysis of the acetal afforded aldehyde 4. Unfortunately, the nitroquinolines decomposed rapidly in the presence of strong base, so an alternate to the obvious anionic routes was sought for the conversion of 4 to 5. This was ultimately accomplished via the magnesium bromide promoted reaction of 4 with *tert*-butyldimethylsilyl ketene acetal to give the β-hydroxy ester.[6] Attempts to generate a variety of alkali metal alkoxides of the β-hydroxy ester by reaction with base led to rapid decomposition. Deprotonation of the β-hydroxy ester was finally achieved by reaction with thallous *tert*-amyl oxide;[7] the resulting thallous alkoxide was phosphorylated and the product reacted with ammonia in situ to afford phosphoramidate 5. The synthesis of **6** was completed by hydroxylation of the acrylate double bond to the diol,[8] periodate cleavage to the aldehyde, and reductive amination with dimethylamine/cyanoborohydride.[9] Surprisingly, acrylate 5 was resistant to oxidation by osmium tetroxide/periodate in the absence of (dimethylamino)ethyl acetate.[8]

Cytotoxicity. The activity of **6** was assessed against HT-29 human colon carcinoma cells in vitro as previously described.[10] Cells were exposed to drug for 4 h under

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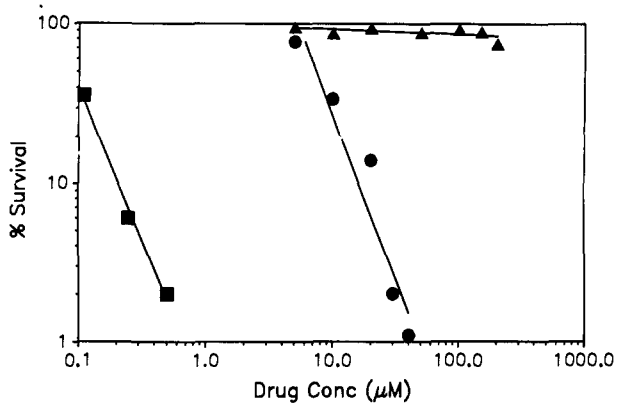

Figure 2. Survival of granulocyte/macrophage progenitors following treatment of bone marrow cells with drug for 2 h in vitro: (■) melphalan, (●) 4-hydroperoxycyclophosphamide, (▲) phosphoramidate 6. The data points represent the mean of quadruplicate determinations for each of two separate experiments, range ± 20%. The solid lines represent the linear regression of the data points.

aerobic or hypoxic conditions, and survival was measured in a clonogenic assay. The results are shown in Figure 1. Phosphoramidate **6** is 11-fold more toxic to HT-29 cells under hypoxic compared to aerobic conditions, on the basis of the slopes of the survival curves. The toxicity of **6** to bone marrow granulocyte/macrophage (GM) progenitors was compared to that of 4-hydroperoxycyclophosphamide and melphalan. The murine ex vivo GM-CFC assay[11] was used, in which fresh bone marrow cells were collected from mice and treated with drug for 2 h, and the surviving GM progenitors measured in a colony-forming assay. The well-established marrow-sparing effects of activated cyclophosphamide compared to melphalan are evident (Figure 2); phosphoramidate **6**, however, exhibits negligible toxicity to GM precursors even when compared to activated cyclophosphamide. Evidence in support of DNA cross-linking by **6** in HT-29 cells was obtained from alkaline elution experiments (Figure 3). In the absence of radiation, the elution profiles of cells treated with **6** (250 μM, 4 h) were not significantly different from control cells under either aerobic or hypoxic conditions, confirming that **6** is not acting via induction of strand breaks. DNA interstrand cross-links were produced by treatment with **6** in the presence and absence of oxygen, and the extent of cross-linking was greater under hypoxic conditions. Comparison of **6** in the absence of oxygen and an equitoxic dose of melphalan (15 μM, 1 h) showed that the extent of interstrand cross-linking was essentially identical, confirming a correlation between cross-links and cytotoxicity for this phosphoramidate under hypoxic conditions.

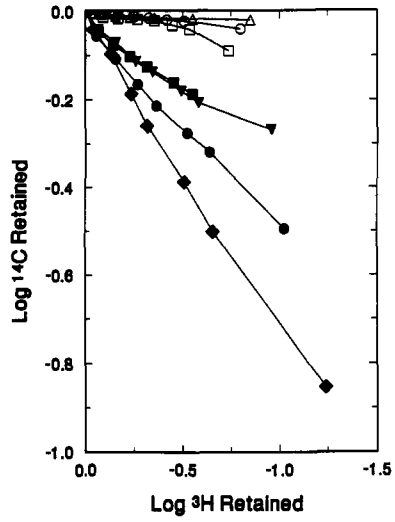

Figure 3. Alkaline elution of HT-29 cells. Cells were treated with drug (250 μM phosphoramidate **6**, 4 h, or 15 μM melphalan, 1 h), allowed to recover (16 h), and then given 400 R (filled symbols) or 0 R (open symbols) before elution: (△) control, (○) **6** aerobic, (□) **6** hypoxic, (■) **6** hypoxic + 400 R, (▼) melphalan aerobic + 400 R, (●) **6** aerobic + 400 R, (◆) 400 R.

In conclusion, we have demonstrated the feasibility of combining nitroheterocycle reduction and intramolecular catalysis to provide selective drug delivery to hypoxic cells. We are currently exploring the application of this methodology to the delivery of more potent cytotoxic agents.

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