5, 111478-66-1; 6 (isomer 1), 123835-99-4; 6 (isomer 2), 123836-00-0; 7 (isomer 1), 123836-01-1; 7 (isomer 2), 123836-02-2; 8, 123836-03-3; 9, 123836-04-4; 10, 123836-05-5; 11, 123836-06-6; 12, 106647-58-9; 12 4,6-diene derivative, 123836-07-7; 12 5,7-diene derivative, 106647-60-3; 13, 123836-08-8; 14, 123930-01-8; 15, 123836-09-9; 16, 106647-61-4; 17, 123836-10-2; 18, 123836-11-3; 19, 123836-12-4; 20, 123836-13-5; 20a, 123836-14-6; 21, 106647-71-6; 21a, 124018-42-4; PTAD, 4233-33-4; $ClCH_2OCH_3$, 107-30-2; Ca, 7440-70-2; ethyl bromodifluoroacetate, 667-27-6; 1,1'-thiocarbonyldiimidazole, 6160-65-2.

Antitumor Properties of Tetrahydrobenz[*a*]anthraquinone Derivatives[1]

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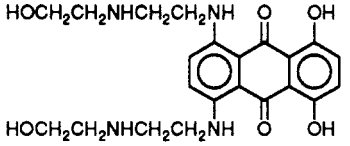
The compound 8,11-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-6-methoxy-1,2,3,4-tetrahydro-7,12-benz[*a*]-anthraquinone (7) was synthesized from 3,6-dimethoxyphthalic anhydride and 6-methoxy-1,2,3,4-tetrahydronaphthalene by a Friedel–Crafts reaction, cyclization to form a dihydroxyanthraquinone, and conversion into the amino-substituted derivative by reaction with 2-[(2-hydroxyethyl)amino]ethylamine. The new compound, a ring D analogue of mitoxantrone, showed growth inhibition, at micromolar concentrations, of murine leukemia 1210, human lung H125, human breast MCF7, human ovary 121, and human colon WiDr and increased the life span of leukemic mice by 38%.

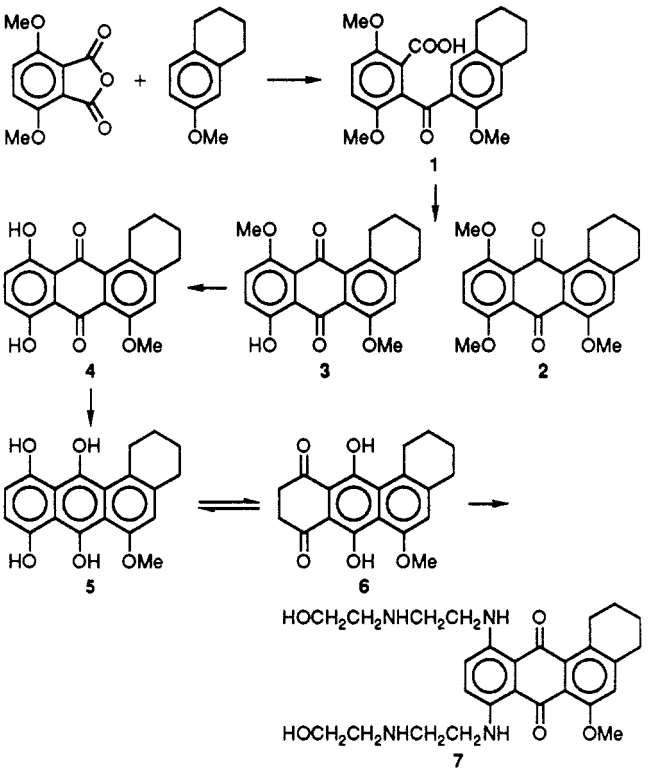
The compound 5,8-bis[[2-[(2-hydroxyethyl)amino]-ethyl]amino]-1,4-dihydroxyanthraquinone (DHAQ, mitoxantrone) is used clinically to treat a variety of human cancers, particularly lung carcinoma,[2,3] leukemia,[4-6] melanoma and lymphoma,[7-9] Hodgkins disease,[7,10] and breast cancer.[7,8,11,12]



DHAQ

Drugs in this class are loosely related to the antineoplastic anthracyclines such as doxorubucin and daunorubicin, which are also anthracenediones; these compounds, as well as DHAQ and its many derivatives, have been shown to bind strongly to DNA[13-16] and are reputed to exercise their antitumor activity by this route.

Scheme I



The DHAQ series of compounds was developed by mimicking the type and stereochemistry of the pertinent functional groups in the hydroxyquinone chromophore of the anthracyclines. Thus, as a structural unit, the unique features of DHAQ eloquently represent an effective arrangement of functional features, i.e. a quinone with a side

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0022-2623/90/1833-0490$02.50/0

chain amino component. The development of the DHAQ type of compound was also inspired by the need for antitumor agents without the cardiotoxicity displayed by the anthracyclines. In fact, mitoxantrone shows a very low incidence of cardiac failure when used clinically.[17-20]

Introduction of a D ring into this system was designed to introduce a nonpolar zone into the molecule and thus provide a substituted benz[a]anthracene ring system. Compounds containing this type of structural arrangement have been shown to be competitive with steroid hormones. By resembling steroids, the new compounds described here would be expected to be attracted to endocrine systems.[21,22] To explain antitumor activity in the anthraquinones, DNA interaction has been proposed as a step in the stabilization (primarily by intercalation) of the planar chromophore of the compounds through an interaction with the grooves of DNA as a route toward a modification of its conformation and function. The presence of a lipophilic D ring into this system would be expected to influence this interaction.

Reaction of phthalic anhydride with 6-methoxy-1,2,3,4-tetrahydronaphthalene gave only one keto acid, compound 1 (Scheme I), which would be the expected isomer due to the anticipated steric hindrance of the alternate ortho position. Cyclization of this keto acid with sulfuric acid was expected to give the trimethoxy compound 2,[23] but analysis indicated that in the major product one methoxy group had been cleaved. Attack of the methoxy group in the tetrahydronaphthalene moiety was ruled out by the fact that subsequent reactions showed the presence of a hydroquinone.

Thus, HBr gave compound 4. These reactions indicate that the intermediate monohydroxy compound could be structure 3 or the alternate isomer where position 8 is present as a methyl ether and position 11 is cleaved to the free hydroxy form. Assignment of structure 3 is based primarily on the preferred sensitivity to acids of the methoxy group which is positioned ortho to a ketone as opposed to a carboxylic acid group in aroylbenzoic acids.[24]

Mild reduction of compound 4 in hydrosulfite or zinc in dilute acid gave tetrol 5 which is tautomeric with the reactive species 6. Compound 6 reacted easily with 2-[(2-hydroxyethyl)amino]ethyl amine to give, after air oxidation, the final product 7.

Compound 7 displayed anticancer activity against both murine and human tumor cell lines, causing 50% inhibition of growth of the following at micromolar concentrations: murine leukemia L1210, 2.0×10^{-6} M; human nonsmall cell lung carcinoma, 2.2×10^{-7} M; human breast carcinoma MCF7, 1.0×10^{-6} M; human ovarian carcinoma A121, 1.0×10^{-7} M; and human colon carcinoma WiDr, 9.6×10^{-7} M.

In DBA/2J mice implanted ip with L1210 leukemia cells life span was increased by 38%, demonstrating statistically significant antileukemic activity.

Experimental Section

Chemical Studies. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Thin-layer chromatography was performed on Kodak silica gel plates, and the R_f values were determined by UV absorbance or exposure to iodine vapors. Microanalyses were performed by Atlantic Microlab, Norcross, GA.

3,6-Dimethoxy-2-[3-(2-methoxy-5,6,7,8-tetrahydronaphthoyl)]benzoic Acid (1). To a stirred suspension of 3.0 g (14 mmol) of 3,6-dimethoxyphthalic anhydride and 5.8 g (36 mmol) of 6-methoxy-1,2,3,4-tetrahydronaphthalene in 150 mL of CH_2Cl_2 was slowly added over a period of 10 min 3.0 g (22 mmol) of $AlCl_3$, and the mixture was left at 25 °C for 18 h. Evaporation of the CH_2Cl_2 at 40 °C and addition to ice and HCl gave a thick oil, which was extracted with EtOAc from which the free acid was extracted with 8% $NaHCO_3$. Neutralization with HCl gave a solid mass, which was recrystallized from EtOH to give 0.65 g (12.2% yield) of benzoic acid derivative 1, mp 217–218 °C. Anal. ($C_{21}H_{22}O_6$) C, H.

6,11-Dimethoxy-8-hydroxy-1,2,3,4-tetrahydrobenz[a]anthracene-7,12-dione (3). A mixture of 100 mg (27.0 mmol) of keto acid 1, 10 mg of H_3BO_3, and 5 mL of concentrated H_2SO_4 was heated on the steam bath for 50 min and then poured onto ice. The orange-brown solid was dissolved in EtOAc and washed with water. Evaporation of the solvent gave an orange red mass, which was crystallized from MeOH to give 30 mg (32.9% yield) of quinone 3, mp 195–197 °C, as the major product. Anal. ($C_{20}H_{18}O_5$) C, H.

6-Methoxy-8,11-dihydroxy-1,2,3,4-tetrahydrobenz[a]anthracene-7,12-dione (4). A. From Monophenol 3. A mixture of 65 mL of 48% HBr, 65 mL of AcOH, and 850 mg of quinone 3 was heated on the steam bath for 18 h. The solution, which showed the presence of a mixture of compounds of which the major component was 8,11-diol 4, was poured into ice water and filtered to give a red solid, which was recrystallized from MeOH to give 800 mg (83.4% yield) of pure compound 4, mp 241–242 °C. Anal. ($C_{19}H_{16}O_5$) C, H.

B. From 3,6-Diacetoxyphthalic Anhydride. A solution of 13.6 g (0.05 mol) of 3,6-diacetoxyphthalic anhydride and 80 g (0.49 mol) of 6-methoxy-1,2,3,4-tetrahydronaphthalene in 200 mL of CH_2Cl_2 was added over a period of 15 min to a stirred slurry of 40 g of $AlCl_3$ (0.30 mol) in 400 mL of CH_2Cl_2. The dark solution was left at 25 °C for 18 h, and evaporated at 40 °C to a syrup, which was added to ice and HCl and extracted with EtOAc. The solution was extracted with saturated $NaHCO_3$ (3 × 100 mL), which was acidified with HCl to give a yellow oil which was treated with 10 mL of acetic anhydride and heated on the steam bath for 1 h. Vacuum evaporation of the acetic anhydride left an oil, which was treated with 200 mg of H_3BO_3 and 500 mL of concentrated H_2SO_4. After 10 min on the steam bath, the hot, dark blue solution was added to ice and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with $NaHCO_3$ and H_2O and dried (Na_2SO_4) to give, after evaporation and recrystallization from MeOH, 2.8 g (16.8% yield) of dihydroxyquinone 4, mp 241–242 °C.

6-Methoxy-1,2,3,4-tetrahydrobenz[a]anthracene-7,8,11,12-tetrol (5). A solution of 250 mg (0.77 mmol) of dihydroxyquinone 4 in 25 mL of Et_2O was added to a mixture of 5 g of Zn powder in 25 mL of 3 N H_2SO_4 and the mixture was vigorously stirred. Conversion to the tetrol was monitored by silica gel thin-layer chromatography in 50% EtOAc–benzene, where the tetrol appears as a faster running bright green fluorescent spot under UV light. In a typical run, 2 h are required. The appearance of a light yellow spot close to the origin indicates that the compound is being destroyed. The ether is washed with water, dried (Na_2SO_4), and crystallized from benzene to give 150 mg (59.6% yield) of tetrol 5, mp 229–230 °C. Anal. ($C_{19}H_{18}O_5$) C, H.

8,11-Bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-1,2,3,4-tetrahydro-6-methoxy-7,12-benz[a]anthraquinone (7). Preparation of the Free Amine. A mixture of 458 mg (1.4 mmol) of tetrol 5 and 1.46 g (14.0 mmol) of 2-[(2-hydroxyethyl)amino]ethylamine was heated at 55–60 °C under N_2 for 4

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h. EtOH (20 mL) was added and the solution was heated at 55–60 °C for 16 h. Dry air was then bubbled into the solution for 4 h while the volume of EtOH was maintained at approximately 15–20 mL. The volume was then reduced to 5–10 mL and chilled to give 398.4 mg (56.1%) of a violet-blue solid. Recrystallization from benzene gave 362 mg (51.0% yield) of amino derivative 7, mp 172–173 °C. Anal. ($C_{27}H_{36}N_4O_5$) C, H, N.

Preparation of the Hydrochloride Salt. To the free amine (271.5 mg) in 100 mL of isopropyl alcohol was added 3 mL of concentrated HCl and the solution was evaporated on a rotary evaporator at 40 °C. Benzene (50 mL) and EtOH (50 mL) were added, and the solution was again evaporated. Then 50 mL of isopropyl alcohol was added and the solution was evaporated. The compound was recrystallized from isopropyl alcohol to give 161.3 mg of the deep blue dihydrochloride, mp 239–240 °C. Anal. ($C_{27}H_{38}Cl_2N_4O_5 \cdot 0.5H_2O$) C, H, N, Cl.

Growth Inhibition Studies. IC_{50} Determinations. Leukemia L1210 cells were diluted to a concentration of 1×10^5 cells/mL in RPMI 1640 plus 20% HI-FCS plus 20 mmol Hepes. Cells were distributed into 13 × 100 mm sterile, borosilicate-glass culture tubes and randomized before 1-mL aliquots of test compound or control solution were added. This 1:2 dilution of cells with test solution resulted in a final inoculum of 5×10^4 cells/mL in a 2-mL total volume of RPMI 1640 plus 10% HI-FCS 20 mmol Hepes. Tubes were stoppered with silicon stoppers and incubated in an upright position in a 37 °C incubator for 48 h.

Following incubation, growth (cells/mL) was determined with a Coulter electronic cell counter. Calculations and graphing of data were performed with an Apple computer. For each concentration of compound, the program averaged the triplicates and calculated the percent control growth. The percent control growth was plotted versus compound concentration and the IC_{50} value was determined.[25]

Human nonsmall cell lung carcinoma H125, human breast carcinoma MCF7, human ovarian carcinoma A121, and human colon carcinoma WiDr cells (NCI Tumor Repository, Frederick MD) were harvested from stock cultures and added (1000–3000 cells/well) to 96-well tissue culture trays. Drug was added to each column (eight replicates) of wells in a stepwise fashion to achieve final drug concentrations ranging from 10^{-4} to 10^{-8} M. Cell growth inhibition was determined 3–5 days later with a microculture tetrazolium assay (MTT), which was based on the enzymatic reduction of colorless MTT to a purple formazan product soluble in DMSO. Absorbance at 570 nm was proportional to cell number.[26] Color formation was measured with a Biotech plate reader and data analysis was performed by an IBM software system. The drug concentration which inhibits 50% of tumor growth (IC_{50}) was determined.

Therapeutic Efficacy of 7 in Mice with L1210 Leukemia. Groups of 5 DBA/2J mice were inoculated ip with 10^6 L1210 leukemia cells and demonstrated a statistically significant ($p < 0.01$), 38% increase in life span following the daily ip administration of 5 mg/kg of 7 (day 1–5). A group of 20 control mice showed survival times ranging between 6 and 8 days with a median of 7 days and a mean of 6.6 ± 0.13. Dosages above 20 mg/kg × 5 were toxic, resulting in loss of animal weight and early death.

These results are comparable to reported work where $CD2F_1$ mice inoculated with 10^5 L1210 leukemia cells and treated with 3.1 mg/kg DHAQ on days 1, 5, and 9 showed a 43% ILS.[18]

Acknowledgment. This project was supported by the American Cancer Society with Grant No. CH391.

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Synthesis and Antihypertensive Activity of 4-(1,2-Dihydro-2-oxo-1-pyridyl)-2*H*-1-benzopyrans and Related Compounds, New Potassium Channel Activators

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Received April 10, 1989

The synthesis and antihypertensive activity of 4-(1,2-dihydro-2-oxo-1-pyridyl)-2*H*-1-benzopyran-3-ols are described. The unsubstituted pyridone adduct lead compound **7e** is highly active, with substituents on the pyridone ring leading to a decrease in activity. Strongly electron-withdrawing substituents at the C-6 position are required for optimal activity. When the 2-pyridone ring is replaced by other heterocycles such as 4-pyridone, pyrimidone, pyridazinone, pyrazinone, and 1,4-butanesultam, the activity is maintained. The removal of the 3-hydroxy function (→ **17a**) does not significantly reduce the activity. The elimination of water from the chromanols leads to the formation of the chromenes, which are among the most potent antihypertensives known. The influence of diverse substituents, in particular heterocyclic C-6 substituents, was investigated in the 4-(2-oxo-1-pyrrolidinyl)chroman-3-ol series. Chromanols esterified at the 3-hydroxy group with short-chain acids, maintain their activity. The epoxidation of the chromene double bond also produces active compounds. The rearrangement of the epoxides **22** produces the 3-keto compounds **23** and the enol derivatives **25**. The reduction of the ketone **23a** produces *cis*-chromanol **7ab** along with its trans isomer **7e**. All compounds were tested for oral antihypertensive activity in spontaneously hypertensive rats with a dose of 1 mg/kg; for selected compounds ED_{30} values as well as the duration of the antihypertensive effect were determined. 4-(1,2-Dihydro-2-oxo-1-pyridyl)-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (**18a**) is under development as a coronary vasodilator and a drug for treating angina pectoris.

Sodium channel blockers have been used for many years as local anesthetics and antiarrhythmics. Subsequently calcium channel blockers underwent a vigorous development resulting in a number of drugs that are now widely used in a range of indications. Currently there is a growing interest in the therapeutic potential of substances that modulate potassium channels.[1] There are three prototypes of this class of compounds: Pinacidil, a peripheral vasodilator; Nicorandil, an antianginal agent, and Cromakalim (**20a**), a highly potent antihypertensive drug.

Evans et al.[2] were able to show that the existence of a powerful electron-withdrawing group located at C-6 in benzopyran compounds as well as a 4-(cyclic amido) group is essential for good blood-pressure-lowering action in the

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