Synthesis of Congeners and Prodrugs of the Benzene Maleimide Photoadduct Mitindomide as Potential Antitumor Agents. 2[1]

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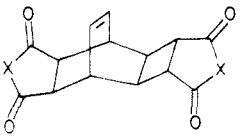
Potential prodrugs of the highly insoluble, diimide antitumor agent mitindomide (**1b**) were synthesized by several different methods. The condensation reaction between mitindomide and formaldehyde cleanly gave the stable bis(hydroxymethyl) compound **7a**, which was partially soluble in water (ca. 0.8%) and showed improved activity in the P-388 screen. When this compound was treated with secondary amines, good yields of Mannich bases could be isolated. The compound from *N*-methylpiperazine (**7b**) had excellent properties and is a candidate for clinical trials. Condensation with other aldehydes gave either no reaction or compounds with poor activity. A water-soluble ester was prepared from **7a** and succinic anhydride, but had reduced potency and activity. Oxidation of the double bond of **1a** with ozone gave an inactive diacid, whereas the dihydro compound was as active as the olefin. When other aromatics (anisole, *p*-xylene, mesitylene) were photolyzed with maleimide, the resulting photoproducts were found to be inactive. Diimides from other ring system were synthesized from the corresponding anhydrides and found to be inactive. However, the bis(hydroxymethyl) derivative of one of these (**12a**) was active in the P-388 screen.

In 1959 the photochemical addition of maleic anhydride to benzene to form a stable 2:1 adduct was first reported.[2a] Primarily by chemical means, the structure of this high melting, insoluble material was established[2b,3] as **1a**. Later,[2c,4,5] it was shown that maleimide reacted with benzene in a similar manner, to produce an adduct whose structure was presumed to be **1b**. Interconversion of



1a: X = O
b: X = NH

compounds (such as **1a** → **1b**) was attempted on the adducts of maleic anhydride and maleimide, respectively, and toluene.[4] However, conclusive results showing that the compounds had the same carbon ring system were not obtained. Diimides such as **1b** are generally very stable, insoluble, high melting compounds and such properties led to attempts to use these compounds in polymer systems.[6-8] In addition, compound **1b** is a metal ion complexing agent and can selectively remove zinc and copper from aqueous solutions.[9,10] A number of aromatic compounds[4,11] react with maleimide under the appropriate conditions.

Our interest in this area was stimulated when a random screening program revealed that **1b** had strong and reproducible in vivo antitumor activity in a number of test systems.[12] However, **1b** is insoluble in all common solvents except aqueous solutions above pH 10, and formulation has been virtually impossible.[13] The structure of **1b** has now been firmly established by X-ray crystallographic studies on two derivatives,[14,15] providing a firm basis for further work in this area. The synthesis of prodrugs with improved solubility and selectivity and the synthesis of congeners with higher potency, greater activity, and less toxicity has recently been an area of intense activity. There are, to the best of our knowledge, no published reports on the mechanism of antitumor activity of **1b**. Its rigid, nonplanar structure makes it an unlikely candidate for intercalation. Furthermore, a negative[13] (*p*-nitrobenzyl)pyridine test reduces the possibility that acylation/alkylation is a possible mode of action.

On the basis, in part, of a theory that many antitumor drugs are chelating agents,[16] Creighton et al.[17a] synthesized a series of bis(diketopiperazines), such as **2a** and **2b**. These nonpolar derivatives of EDTA might be able to cross cell membranes, and, in fact, both of these diimides showed strong antitumor activity in several test systems. Despite extensive clinical trials and other studies, the mechanism of action of these drugs is not clear.[18] They are difficult to formulate due to insolubility. Apparently, in an effort

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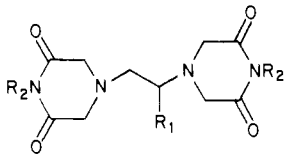
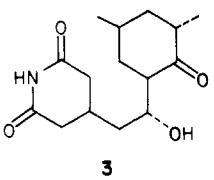
to overcome this problem, compound **2c** was synthesized and shown to have good antitumor activity.[19] More recently, other diimides related to **2a** have been shown to have antimetastatic activity.[17b] Perusal of the literature for cyclic imides with antitumor activity revealed the antibiotic cycloheximide (**3**),[20] aminoglutethimide (**4**),[21] the *N*-phthaloylglutamides (**5**),[22] and sesbanimide (**6**).[23]



2a: $R_1 = R_2 = H$
b: $R_1 = CH_3$, $R_2 = H$
c: $R_1 = H$, $R_2 = CH_2N\langle O \rangle$



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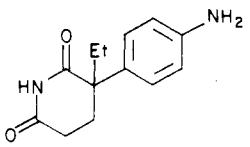

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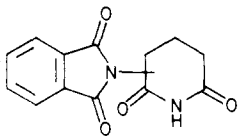

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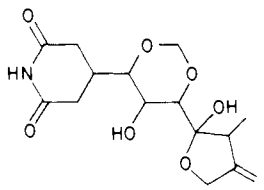

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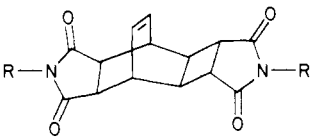
Although imides have been known to function as active hydrogen compounds in Mannich type reactions,[24,25] only recently has this area attracted the attention of medicinal chemists.[26-28] Such derivatives, formed by reaction of formaldehyde or other aldehydes and secondary amines, would seem to offer opportunities for increased water solubility. In addition, congeners with various substituents or other diimides with new ring systems would give insight into structure–activity relationships in this class of compounds. The synthesis and screening of the above-mentioned compounds is the subject of this work.

Chemistry. The photochemical addition of maleimide to benzene was carried out essentialy as described in the literature.[5] The higher yield obtained (85% vs. 65%) may reflect a somewhat higher concentration of acetophenone photosensitizer (0.22 M vs. 0.15 M) and/or the fact that the quartz probe was cleaned at regular intervals. Similar photochemical reactions with *p*-xylene, mesitylene, and anisole gave much lower yields. Whereas *p*-xylene gave, almost exclusively, the expected product **10d** and only traces of **10e**, anisole gave about a 1:1:1 mixture of **10a**, **10b**, and **10c**, contrary to a report[11b] that **10a** was the exclusive product.

Mitindomide reacted smoothly with aqueous formaldehyde with use of DMF as a solvent to give the bis(hydroxymethyl) compound **7a**, which was easily purified by recrystallization from hot water and was quite stable under these conditions. Mannich base compounds **7b–e** were made directly from mitinomide, formaldehyde, and the appropriate secondary amine or via the bis(hydroxymethyl) compound **7a** and the corresponding amine. Attempts to use other aldehydes such as acetaldehyde or benzaldehyde were not successful, but glyoxylic acid reacted rapidly with **1b** to give a good yield of **7i**. Since **7i** was unstable in solution, it was converted to the more stable acetate **7j**, using acetic anhydride in pyridine. Diacetate **7f** was made in a similar manner from **7a**, and since it was highly crystalline, its structure was determined by X-ray crystallography.[14] The bis(hemisuccinate) ester **7h** was synthesized with use of succinic anhydride and pyridine.



7a: $R = CH_2OH$
b: $R = CH_2N\langle \rangle NCH_3$
c: $R = CH_2N\langle \rangle NCH_3 \cdot HCl$
d: $R = CH_2N\langle S$
e: $R = CH_2N\langle O$
f: $R = CH_2OAc$
g: $R = CH_2N(Et)_2$
h: $R = CH_2OCOCH_2CH_2CO_2H$
i: $R = CH(OH)CO_2H$
j: $R = CH(OAc)CO_2H$
k: $R = CH_3$
l: $R = CO_2Et$
m: $R = CO\text{-}t\text{-}Bu$
n: $R = CH_2CH_2N(Et)_2 \cdot HCl$

Mitindomide was readily alkylated by reaction of the preformed imide sodium salt (NaH in DMF) with an excess of methyl iodide to yield *N,N'*-dimethylmitindomide (**7k**). Acylation of the sodium salt with ethyl chloroformate gave the dicarboethoxy compound **7l**. The dicarbo-*tert*-butoxy compound **7m** was conveniently synthesized by the reaction of **1b** and di-*tert*-butyl carbonate in DMF with use of DMAP as a catalyst. Both **7l** and **7m** could be hydrogenated to dihydro derivatives **8c** and **8d**, respectively, with use of large amounts of Pd/C, acetic acid as solvent, and extended reaction times (4–7 days). Direct hydrogenation of **1b** proved impossible, whereas hydrogenation of **7a** gave complex mixtures, from which no pure products could be isolated. Dihydro compound **8c** could not be converted to dihydromitindomide (**8a**) by any of the conditions attempted using acid- or base-catalyzed cleavage; the imide rings appeared to open under the reaction conditions. However, compound **8d** was readily deprotected with trifluoroacetic acid in methylene chloride to give good yields of **8a**, which was easily converted into the bis(hydroxymethyl) compound **8b**.

The double bond in mitindomide and its various derivatives is rather unreactive as judged by the difficulty in hydrogenation, as mentioned above, and the complete lack of reactivity of **7l** to *m*-chloroperbenzoic acid, osmium tetraoxide, and lead tetracetate. However, both **1b** and **7a** react with ozone in acetic acid. Vigorous oxidative workup using hydrogen peroxide and a catalytic amount

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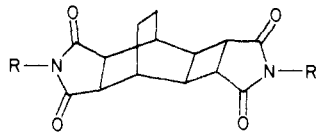
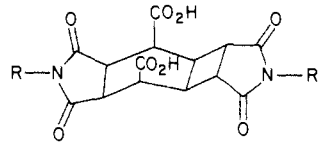


8a: R = H
b: R = CH_2OH
c: R = CO_2Et
d: R = CO_2-*t*-Bu

of selenium dioxide gave reasonable yields of diacids **9a** and **9b**, respectively.



9a: R = H
b: R = CH_2OH

Other diimides (**11b–d**) were synthesized form the corresponding dianhydrides by the following procedure: the dianhydride was mixed with concentrated aqueous ammonia and the solution evaporated to dryness to form a mixture of amides and ammonium salts; the dry material was pyrolyzed and sublimed at 250–300 °C in vacuo. Diimide **11a** was synthesized by photochemical dimerization of maleimide.

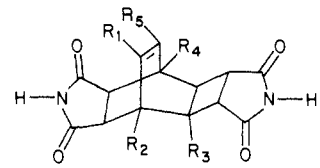
Results and Discussion

Aqueous solubility and in vivo antitumor screening (P-388) for most of the compounds synthesized in this study are summarized in Table I. Since mitindomide is essentially insoluble in all common solvents, our initial goal was to synthesize potential prodrugs with improved aqueous solubility. This was readily accomplished via the bis(hydroxymethyl) compound **7a**, some of the Mannich base compounds, and certain other derivatives. Thus, compounds **7a**, **7b**, **7c**, **7h**, and **7j** have considerable aqueous solubility and retain antitumor activity. Normally, N-alkylation is not a useful approach to prodrugs, since even the methyl group is difficult to remove under physiological conditions and often the alkylated derivative is inactive.[27] For example, *N,N'*-dimethyl derivative **7k** shows only weak activity at the highest dose level tested and the water-soluble (diethylamino)ethyl derivative **7n**[13] showed no activity. However, the addition of aldehydes to imides is known to be a reversible reaction[29] and in some cases *N*-hydroxymethyl derivatives have been shown to be prodrugs under physiological conditions.[26]

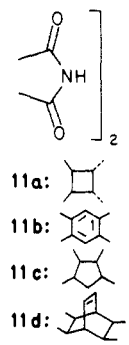
Interestingly, *N,N'*-dicarboethoxymitindomide (**7l**) is active in the P-388 screen, even though chemical hydrolysis would not be expected to yield **1b**.[30] It has been shown, in some cases, that carbamidases will cleave carbamates in vivo and release the active form of the drug.[31] Dihydro derivative **8c** is at least as active as **7l**, and dihydro compound **8b** is about as active as **7a**; this indicates the lack of importance of the double bond in the antitumor activity of mitindomide. However, when the double bond is cleaved to give diacids **9a** and **9b**, little or no activity is seen in these compounds. This may be due to the ionic, highly polar nature of these groups.

Other congeners of mitindomide were synthesized by photochemical reactions using anisole, *p*-xylene, and mesitylene. The corresponding compounds **10a–c** (mixture), **10d**, and **10f** were inactive. All of these compounds showed some toxicity, in term of weight loss, at the highest dosages but no increase in life span. Because of their insoluble nature, leading to difficult formulation, screening as more soluble bis(hydroxymethyl) adducts may be warranted. On the basis of information to date, substituents in ring positions derived from the aromatic ring interfere greatly with antitumor activity.

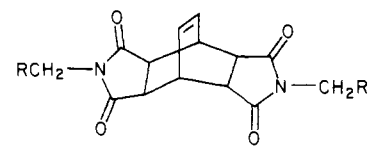
Several other types of diimides (**11a–d**) were synthesized in order to gain some insight into the minimum structural requirements for antitumor activity. All of these compounds were high melting, insoluble in water, and inactive in the P-388 test system (not all data shown). The bicyclo[2.2.2]octene diimide **11d** was also converted to the water-soluble bis(hydroxymethyl) compound **12a** and Mannich base **12b**. Interestingly, both of these compounds showed activity, although neither was as active or potent as the corresponding mitindomide derivatives. Thus, in at least one other ring system, a simple diimide derivative has shown antitumor activity. The exact implication of this observation awaits further research into structure–active relationships and the mechanism of action of these drugs.



10a: R_1 = OCH_3
b: R_2 = OCH
c: R_3 = OCH_3
d: R_2 = R_4 = CH_3
e: R_5 = R_3 = CH_3
f: R_1 = R_3 = R_4 = CH_3
all other R's equal to H



11a:
11b:
11c:
11d:



12a: R = OH
b: R = N⟨ ⟩NCH$_3$

Experimental Section

Melting points were taken with a Kofler hot stage microscope and are uncorrected. NMR spectra were determined with either a Varian T-60 or a Brucker WP-300 spectrometer, equipped with an Aspect 2000 data system. Chemical shifts are in ppm relative to either $HCCl_3$ (δ 7.24), Me_2SO (2.49), or H_2O (4.65), depending on the solvent used. IR spectra were taken on a Perkin-Elmer 299 spectrophotometer. Mass spectra were recorded on a Varian-MAT 112S spectrometer interfaced with an SS200 data system. All microanalyses were done by Atlantic Microlabs Inc., Atlanta, GA. Unless otherwise noted, all solvents and chemicals were used as received.

3a,3b,4,4a,7a,8,8a,8b-Octahydro-4,8-ethenopyrrolo[3',4':3,4]cyclobut[1,2-*f*]isoindole-1,3,5,7(2*H*,6*H*)-tetrone (1b; Mitindomide). With heating, 62.5 g (0.644 mole) of maleimide (Aldrich, mp 91–94 °C) was dissolved in 125 mL of acetophenone and the solution poured into 4.6 L of benzene in a stirred 5-L photochemical reactor. With use of a quartz probe and a 450-W Hanovia mercury vapor lamp, the solution (N_2 purge) was irradiated for periods of time varying between 12 and 24 h. At the end of each period, the suspension of photoproduct and benzene was removed and filtered. After washing of the solid with a small volume of benzene, the filtrate was returned to the reactor and the solid was vacuum dried. The quartz probe was cleaned and irradiation continued. After about 80 h, no more product formation was observed and a total of 74.3 g (84.8%) of crude **1b**

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was isolated. This material can be purified by a complex series of solvent washings and precipitations; however, best results were obtained via the preparation and purification of **7a**. Pure **1b** showed the following: mg >400 °C; IR (Nujol) 1765, 1700 cm^{-1}; ^1H NMR (300 MHz, Me_2SO-d_6) δ 11.3 (br s, NH), 6.30 (m, vinyl), 3.08 (br s, CH), 2.77 (s, CH), 2.56 (s, CH), 2.54 (s, CH); ^1H NMR (300 MHz, TFA-*d*) 9.75 (s, NH), 9.55 (s, NH), 6.61 (m, vinyl), 3.62 (br s, CH), 3.19 (s, CH), 3.10 (s, CH), 2.99 (s, CH); UV (dioxane) λ_{max} 253 (log ε 2.546), 260 nm (sh, 2.442); MS, *m/z* 272 (M^+, 5), 244 (M - 28, 2), 216 (3), 201 (4), 175 (3), 130 (7), 129 (7), and 78 (100). Anal. ($C_{14}H_{12}N_2O_4$) C, H, N.

Bis(hydroxymethyl) Derivative 7a. To a slurry of 25.0 g (0.0919 mol) of crude **1b** in 500 mL of DMF was added 20.7 mL (0.276 mol) of 37% aqueous formaldehyde, and the mixture was heated at 74 °C for 2 h. The initial turbid mixture became clear during this period. The solvent was removed in vacuo and the brown-yellow solid recrystallized from hot (steam bath) water. The white solid was dried in vacuo to yield 25.3 g (83%, overall 70% based on maleimide) of **3a**: mp >300 °C; IR (Nujol) 1765, 1688 cm^{-1}; ^1H NMR (300 MHz, Me_2SO-d_6) δ 6.37 (br t, *J* = 6 Hz, *OH*), 6.30 (*m, olefin*), 4.75 (d, *J* = 6 Hz, CH_2), 4.64 (d, *J* = 6 Hz, CH_2), 3.21 (br s, CH), 2.90 (s, CH), 2.66 (s, CH), 2.60 (s, CH); MS, *m/z* 272 ($M^+ - 2CH_2O$, 10), 78 (100). Anal. ($C_{16}H_{16}N_2O_6 \cdot 0.75H_2O$).

Mannich Base Compounds 7b–g. Typical Procedures. Bis[(*N*-methylpiperazino)methyl] Derivative 7b. Method A. A solution of 6.70 g (0.0202 mol) of **7a**, 400 mL of DMF, and 4.47 mL (0.0404 mol) of *N*-methylpiperazine was heated at 73 °C for 18 h. The solvent was removed in vacuo. The residue was dissolved in 300 mL of warm (60 °C) water and the solution was then cooled to −5 °C as quickly as possible in order to avoid decomposition. This yielded 4.5 g (45%) of a white solid: mp 250–255 °C dec; IR (KBr) 1765, 1695 cm^{-1}; ^1H NMR (300 Hz, Me_2SO-d_6) δ 6.33 (m, olefinic), 4.26 (s, CH_2), 4.16 (s, CH_2), 2.09 (s, NCH_3); MS, *m/z* 496 (M^+, 6), 384 ($M^+ - 112$, 6), 70 (100). Anal. ($C_{26}H_{36}N_6O_4$) C, H, N.

Method B. A mixture of 2.00 g (0.007 53 mol) of **1b**, 1.14 mL (0.0154 mol) of 37% aqueous formaldehyde, and 1.63 mL (0.0147 mol) of *N*-methylpiperazine was heated in 150 mL of DMF at 70 °C for about 6 h. The reaction was worked up exactly as described in method A to yield 1.7 g (47%) of **7b**, which was identical in all respects with that obtained under method A.

Dihydrochloride Salt 7c. To a mixture of 2.50 g (5.04 mmol) of **7b** in 200 mL of water was added 100.8 mL (10.08 mmol) of 0.100 N HCl. The clear solution was freeze-dried to yield 3.10 g of **7c**: mp >300 °C; ^1H NMR (300 MHz, Me_2SO-d_6) δ 6.50 (m, vinyl), 4.34 (s, CH_2), 4.23 (s, CH_2), 2.66 (s, NCH_3). Anal. ($C_{26}H_{38}Cl_2N_6O_4 \cdot 1.5H_2O$) C, H, Cl, N.

Bis(thiamorpholinomethyl) Derivative 7d. With use of method B, pure compound **7d** was obtained directly in 100% yield: mp 275–285 °C dec; ^1H NMR (300 MHz, TFA-*d*) δ 6.61 (m, vinyl), 5.08 (s, CH_2), 4.99 (s, CH_2); MS-CI, *m/z* 503 ($M^+ + 1$, 11), 388 (100). Anal. ($C_{24}H_{30}N_4O_4S_2 \cdot 0.5H_2O$) C, H, N, S.

Bis(morpholinomethyl) Derivative 7e. With use of method B, pure compound **7e** was obtaining directly in 100% yield: mp >300 °C; ^1H NMR (300 MHz, TFA-*d*) δ 6.61 (m, vinyl), 5.15 (s, CH_2), 5.05 (s, CH_2); MS, *m/z* 470 (M^+, 2), 100 (100). Anal. ($C_{24}H_{30}N_4O_6 \cdot 0.5H_2O$) C, H, N.

Bis[(diethylamino)methyl] Derivative 7g. With use of method B, pure compound **7g** was obtained directly in 100% yield: mp 240 °C dec; ^1H NMR (60 MHz, Me_2SO-d_6) 6.33 (m, vinyl), 4.33 (s, CH_2), 4.23 (s, CH_2), 1.03 (t, *J* = 7 Hz, CH_3); MS, *m/z* 442 (M^+, 1), 86 (100). Anal. ($C_{24}H_{34}N_4O_4 \cdot 0.33H_2O$) C, H, N.

Bis[(acetyloxy)methyl] Derivative 7f. Compound **7a** (3.98 g, 12.0 mmol) was mixed with excess acetic anhydride with pyridine as the solvent. After 19 h, the solvents were removed in vacuo, the residue was dissolved in chloroform, and the solution was extracted with dilute hydrochloric acid. After drying and evaporation, the solid was recrystallized from boiling acetic acid to yield **7f** (52%): mp 288–290 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 6.39 (m, vinyl), 5.49 (s, CH_2), 5.38 (s, CH_2), 2.05 (s, Ac), 2.02 (s, Ac); MS-CI, *m/z* 417 ($M^+ + 1$, 100), 387 ($M^+ - 59$, 56). Anal. ($C_{20}H_{20}N_2O_8$) C, H, N.

Bis[(succinyloxy)methyl] Derivative 7h. To a chilled solution (5 °C) of 4.0 g (0.012 mol) of **7a** in 200 mL of dry pyridine was added 12.0 g (0.12 mol) of succinic anhydride, and the mixture was allowed to come to room temperature. After 1 h the solvent was removed in vacuo, and 100 mL of ice water was added. With vigorous stirring (glass rod), a white solid was produced, which was collected by filtration, dried, and recrystallized from acetonitrile–benzene. This yielded 4.3 g (67%) of **7h**: mp >300 °C (gas evolution at 90–100 °C); ^1H NMR (300 MHz, Me_2SO-d_6) δ 12.2 (br s, CO_2H), 6.30 (m, vinyl), 5.36 and 5.26 (s, CH_2), 3.2–2.1 (complex). Anal. ($C_{24}H_{24}O_{12}N_2$) C, H, N. For screening, compound **7h** was converted to a sodium salt. To a slurry of 2.4 g of **7h** in 150 mL of water was added solid $NaHCO_3$ until the pH was 6.33. The clear solution was freeze-dried to yield 2.8 g of a white powder.

Bis[[(2-carboxyacetyl)oxy]methyl] Derivative 7j. To a slurry of 6.0 g (0.022 mol) of **1b** in 200 mL of DMF was added 4.3 g (0.046 mol, based on 1.1 mol/mol of water from ^1H NMR analysis) of glyoxylic acid. The mixture was heated to 70 °C for 10 min, at which time it became homogeneous. After heating for an additional 2 h, the solvent was removed on a rotary evaporator in vacuo, and the residue dissolved in 100 mL of water and freeze-dried. This yielded 9.9 g (106%) of a fluffy, white, hydroscopic solid. ^1H NMR (D_2O) analysis showed δ 6.43 (m, vinyl, 2.0 H), 5.87 (s, NCH, 1.0 H), and 5.75 (s, NCH, 1.0 H), indicating that pure compound **7i** had been formed. However, aqueous solutions of **7i** were unstable, and it was converted to the acetyl derivatives as follows. To a solution of 2.8 g (ca. 0.0067 mol) of **7i** in 150 mL of pyridine was added 6.3 mL (0.067 mole) of acetic anhydride. After 3 days, the solvent was removed in vacuo, the residue dissolved in a small volume of water, and the solution passed over a column of Dowex 50W-X8 (H^+) resin. The effuent was freeze-dried to yield 2.6 g (77%) of **7j**, which showed the following: mp 160 °C dec; IR (KBr) 3450, 2950, 1780, 1720, and 1625 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 6.70 (s, NCH), 6.58 (s, NCH), 6.38 (m, vinyl), 3.4–2.7 (complex), and 2.17 (br s, Ac). Anal. ($C_{22}H_{20}N_2O_{12} \cdot 2.5H_2O$) C, H, N.

2,6-Dimethyl Derivative 7k. A slurry of 4.5 g (0.017 mol) of **1b**, 1.5 g (0.063 mol) of NaH, and 20 mL of DMF was stirred for 3 h. After the mixture was cooled with use of an ice bath, 4.0 mL (0.064 mol) of methyl iodide was added and stirring continued overnight at room temperature. With caution, a large excess of dilute hydrochloric acid was added, and the white precipitate was collected by suction filtration and washed with a large volume of water. After drying, the solid was recrystallized from methylene chloride/hexane to yield 2.9 g (58%) of **7k**, which showed the following: mp 300–303 °C; IR (KBr) 3060, 2960, 2930, 1770, and 1690 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.37 (m), 3.47 (br s), 3.02 and 2.91 (s, NCH_3), 2.73 (br s), and 2.67 (br s); MS, *m/z* 300 (M^+, 33), 150 (29), and 78 (100). Anal. ($C_{16}H_{16}N_2O_4 \cdot 0.25H_2O$) C, H, N.

2,6-Dicarbethoxy Derivative 7l. A slurry of 10.0 g (0.0368 mol) of **1b**, 3.53 g (0.147 mol) of NaH, and 50 mL of DMF was stirred for 5 h. After the mixture was cooled with use of an ice bath, 17 mL (0.178 mol) of ethyl chloroformate was added slowly via syringe. Stirring was continued overnight at room temperature. The reaction mixture was added to 200 mL of water and the precipitate collected by suction filtration and washed with water. After drying, the solid was recrystallized from methylene chloride/hexane to yield 7.0 g (46%) of **7l**, which showed the following mp 240 °C dec; IR (KBr) 2990, 1810, 1770, and 1725 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.44 (m), 4.40 and 4.35 (q, *J* = 7 Hz), 3.50 (br s), 2.80 (s), 2.75 (br s), 1.36 and 1.34 (t, *J* = 7 Hz); MS, *m/z* 416 (M^+, 2), 388 ($M^+ - 28$, 3), 371 ($M^+ - 45$, 6), and 78 (100). Anal. ($C_{20}H_{20}N_2O_2O_8 \cdot 0.5H_2O$) C, H, N.

9,10-Dihydro-2,6-dicarbethoxy Derivative 8c. To a solution of 7.0 g (0.017 mol) of **7l** in 100 mL of acetic acid was added 1.5 g of 5% Pd/C. This mixture was treated with H_2 gas (initial pressure 40 psi) for 7 days. After vacuum filtration through a Celite pad, the filtrate was concentrated in vacuo and the residue dissolved in methylene chloride, washed with water, dried, and concentrated. Ther solid was recrystallized from chloroform/hexane to yield 5.8 g (83%) of **8c**, which showed the following: mp 208 °C dec; IR ($CHCl_3$) 1807, 1765, and 1725 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.42 and 4.41 (q, *J* = 7.0 Hz), 3.88 (br s), 2.72 (br s), 2.49 (br s), 1.88 and 1.57 (d, *J* = 9.0 Hz), and 1.37 (t, *J* = 7.0 Hz); MS, *m/z* 418 (M^+, 1), 373 (M - 45, 10), 346 (6), 303 (11), and 171 (100). Anal. ($C_{20}H_{22}N_2O_8 \cdot 0.5H_2O$) C, H, N.

9,10-Dihydro Derivative 8a. A mixture of 5.0 g (0.018 mol) of **1b**, 0.22 g (0.0018 mol) of 4-(dimethylamino)pyridine, 16 g (0.073

Table I. Aqueous Solubility[a] and Antitumor Activity in the P-388 Lymphocytic Leukemia System[b]

compd no.	solubility	dose per inj,[c] mg/kg	survivors, day 4[d]	wt diff, g (T − C)[d]	% T/C[d]
1b	INS	200	3/6, 5/6	−7.3, −3.1	TOX, TOX
		100	5/6, 6/6	−3.4, −3.4	177, 94
		50	6/6, 6/6	−2.2, −2.0	168, 172
		25	6/6, 6/6	−0.1, −0.8	142, 149
		12.5	6/6	0.2	119
7a	0.8	200	0/6		TOX
		100	3/6, 0/6	−8.2	TOX, TOX
		50	6/6, 6/6	−5.6, −6.5	195, 208
		25	6/6, 6/6	−2.5, −3.3	140, 157
		12.5	6/6	−0.7	105
7b	1.5	200	5/6	−9.0	TOX
		166.7	3/6	−5.1	TOX
		100	6/6, 6/6	−3.7, −4.9	211, 200
		60	6/6	−3.1	165
		50	5/6	−1.3	106
		30	6/6	−0.2	116
		25	6/6	−0.6	99
7c	vsol	191.2	0/6		TOX
		114.7	2/6	−5.9	TOX
		68.8	6/6	−4.6	172
		41.3	6/6	−1.8	130
		24.8	6/6	1.2	113
7d	INS	400	6/6	−5.7	TOX
		200	6/6, 6/6	−5.1, −5.2	166, 194
		100	6/6, 6/6	−2.9, −2.1	148, 165
		50	6/6, 6/6	−1.4, −2.1	121, 116
		25	6/6	−1.3	99
7e	INS	200	3/6, 4/6	−6.2, −5.9	TOX, TOX
		100	6/6, 6/6	−4.8, −3.9	120, 174
		50	6/6, 6/6	−1.7, −1.5	141, 148
		25	6/6, 6/6	−0.9, −1.0	120, 125
7f	INS	400	5/5	−2.2	208
		200	6/6, 5/5	1.4, −1.4	180, 133
		100	6/6, 5/5	0.3, −1.4	155, 118
		50	6/6, 5/5	−0.4, −0.7	140, 115
		25	6/6	−0.1	117
7g	INS	400	5/6	−0.9	111
		200	6/6, 6/6	−0.7, −0.6	139, 125
		100	6/6, 6/6	−0.8, −0.4	117, 116
		50	6/6, 6/6	−1.0, −0.5	111, 102
		25	6/6	0.0	103
7h	vsol[e]	200	2/6, 4/6	−6.7, −6.9	TOX, TOX
		100	6/6, 5/6	−3.1, −4.4	165, TOX
		50	6/6, 6/6	0.0, −0.6	115, 116
		25	6/6, 6/6	0.3, −0.7	103, 107
7j	vsol[e]	400	3/6	−5.7	TOX
		200	6/6, 6/6	−1.2, −0.5	175, 125
		100	6/6, 6/6	−0.3, 0.1	123, 113
		50	6/6	−0.3	110
		25	6/6	0.2	103
7k	INS	400	6/6	−2.0	135
		200	6/6, 6/6	−1.4, −0.8	104, 111
		100	6/6	−0.8	111
		50	6/6	−0.9	108
7l	INS	200	6/6, 0/6	−6.5	TOX, TOX
		100	6/6, 6/6	−5.5, −4.6	145, 152
		50	6/6, 6/6	−0.8, −0.6	135, 121
		25	6/6, 6/6	−1.2, 0.2	124, 116
8b	1.0	200	5/6	−4.7	TOX
		100	6/6	−3.3	205
		50	6/6	−1.8	145
		25	6/6	0.1	102
8c	INS	200	6/6, 5/6	−4.0, −5.2	222, TOX
		100	6/6, 6/6	−2.2, −1.2	157, 142
		50	6/6, 6/6	−0.4, −0.3	127, 115
		25	6/6, 6/6	−0.7, −0.5	111, 104
9a	INS	400	6/6	−0.2	96
		200	6/6, 6/6	−0.1, 1.1	92, 91
		100	6/6	−0.7	97
		50	6/6	0.0	94
9b	vsol[e]	400	6/6, 6/6	−0.3, 0.1	129, 131
		200	6/6, 6/6	−1.3, 0.5	120, 114
		100	6/6, 6/6	−0.7, 0.5	120, 107
		50	6/6, 6/6	−0.9, −1.2	92, 105

Table I (Continued)

compd no.	solubility	dose per inj,[c] mg/kg	survivors, day 4[d]	wt diff, g (T - C)[d]	% T/C[d]
10a–c	INS	400	6/6	-2.0	98
		200	6/6, 6/6	-0.4, -0.3	101, 90
		100	6/6	-0.3	101
		50	6/6	0.0	99
10d	INS	200	6/6	-6.1	97
		100	6/6	-4.8	109
		50	6/6	-3.7	108
		25	6/6	-1.5	103
10f	INS	400	6/6	-5.3	101
		200	6/6	-1.8	97
		100	6/6	-1.5	109
		50	6/6	-0.9	108
11d	INS	200	5/6	-4.3	92
		100	5/6	-2.4	97
		50	6/6	-1.1	100
		25	6/6	-0.7	105
12a	0.5	200	6/6, 6/6	-3.4, -4.1	160, 152
		100	6/6, 6/6	-4.0, -3.9	137, 144
		50	6/6, 6/6	-2.5, -1.8	127, 140
		25	6/6, 6/6	-1.6, -0.1	114, 132
12b	2	400	3/6	-6.4	TOX
		200	6/6, 6/6	-3.6, -3.1	122, 128
		100	6/6, 6/6	-1.0, -0.5	126, 113
		50	6/6	0.0	108

[a] At room temperature in g/100 mL of water; INS = less than 0.1, vsol = greater than 5. [b] Screening was carried out under the auspices of the National Cancer Institute. For detailed explanations of procedures and data, see Instruction 14, Screening Data Summary Interpretation and Outline of Current Screen Drug Evaluation Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD 20205. [c] Q01D×09. Single dose for 9 days, given in milligrams/kilogram per injection. [d] For many compounds results are shown in two columns. These represent independent testing results done at different times. Abbreviations: survivors day 4, live animal on the fourth day of testing/total animals; wt diff, g (T - C), the difference in body weight in grams between test and control animals; % T/C, the median lifetime of test animal divided by the median lifetime of control animals, times 100. [e] As sodium salt; see Experimental Section.

mol) of di-*tert*-butyl carbonate, and 150 mL of DMF was stirred for 0.5 h, at which time it became homogeneous. About 150 mL of water was added, and the solid was collected by suction filtration and washed well with water. After drying, the solid was recrystallized from methylene chloride/hexane to yield 7.3 g (84%) of **7m**, which showed the following: mp >300 °C; IR ($CHCl_3$) 2980, 2935, 1800, 1765, and 1718 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.53 (m), 3.56 (br s), 2.93–2.75 (complex), 1.60 (s), and 1.57 (s); TLC (5% methanol in chloroform) R_f 0.62. Hydrogenation of 5 g (0.012 mol) of **7m**, dissolved in 150 mL of acetic acid, with 5 g of 5% Pd/C was conducted at room temperature with an initial H_2 pressure of 40 psi for 4 days. With use of a Celite pad, the catalyst was removed by suction filtration and the filtrate concentrated in vacuo. Note: Heating the reaction mixture to 50 °C appeared to cause considerable decomposition, and the product could not be completely purified; hovever, ^1H NMR analysis indicated that reduction was complete. A 2.1-g sample of the above reaction product (**8d**) was stirred with a mixture of 21 mL of trifluoroacetic acid and 63 mL of methylene chloride for 40 min, and the solvents were evaporated. The resulting white powder was slurried with water and collected by suction filtration and dried. This yielded 1.43 g of **8a**, which showed the following: mp >300 °C; ^1H NMR (300 MHz, Me_2SO-d_6) δ 11.3 (br s), 3.28 (s), 2.78 (s), 2.50 (s), 2.08 (s), 1.85 and 1.28 (d, J = 9.0 Hz); MS, m/z 274 (M^+, 30), 99 (100). Anal. ($C_{14}H_{14}N_2O_4$) C, H, N.

Dihydro Bis(hydroxymethyl) Derivative 8b. A mixture of 1.14 g (4.14 mmol) of **8a**, 0.34 mL (12.4 mmol) of 37% aqueous formaldehyde, and 30 mL of DMF was heated at 90 °C for 12 h. The clear, colorless solution was concentrated on a rotary evaporator in vacuo and then dried overnight with moderate heat (55 °C) under high vacuum. This gave 1.68 g (121%) of **8b**, which by ^1H NMR analysis contained 0.33 mol of DMF and 0.33 mol of water. No satisfactory method of removing these solvents could be found; drying at higher temperature caused decomposition. It was used for antitumor screening without further purification and showed the following: mp >300 °C; ^1H NMR (300 MHz, Me_2SO-d_6) δ 7.94 (s, DMF), 6.36 (br s, OH), 4.79 (br s, CH_2O), 3.37 (s), 3.34 (v br s, H_2O), 2.88 (s, DMF), 2.81 (s), 2.74 (s, DMF), 2.49 (s, overlap with Me_2SO), 2.16 (s), 1.85 and 1.27 (d, J = 9.1 Hz). Anal. ($C_{16}H_{18}N_2O_6$·0.33DMF·0.33H_2O) C, H, N.

Photolysis of Anisole and Maleimide. A solution of 5.0 g (0.052 mol) of maleimide, 5 mL of acetophenone, and 170 mL of anisole was placed in a photochemical reactor equipped with a continuous nitrogen purge, which also served to stir the solution. With use of a quartz probe and a 450-W Hanovia mercury vapor lamp, the solution was irradiated for 18 h. The insoluble material was collected by suction filtration, air-dried, and then slurried with a large volume of acetone. The insoluble material was removed by filtration and oven dried to yield 1.6 g of white solid (sample A). The filtrate was evaporated and the residue oven dried to yield 5.3 g of pale yellow solid (sample B). ^1H NMR analysis of these samples indicated they had similar compositions, with sample B having a large number of minor impurities. Sample A showed the following: mp >300 °C; ^1H NMR (Me_2SO-d_6) δ 11.2 (br s, NH), 6.30 and 6.23 (complex, vinyl, isomer **10c**), 6.25 and 6.18 (complex, vinyl, isomer **10b**), 4.90 (dd, J = 7.2, 2.4 Hz, vinyl, isomer **10a**), 3.48 and 3.34 and 3.27 (s, OCH_3), 3.1–2.2 (complex); MS, m/z 302 (M^+, 10), 205 (8), and 108 (100). Anal. ($C_{15}H_{14}N_2O_5$) C, H, N.

4,8-Dimethyl Derivative 10d. With use of the procedure described for the photolysis of anisole, a mixture of 5 g of maleimide, 170 mL of *p*-xylene, and 5 mL of acetophenone was irradiated for 22 h. The acetone-insoluble portion, 2.5 g (33%), showed the following: mp >300 °C; IR (KBr) 3450, 3240, 3180, 3080, 1765, and 1704 cm^{-1}; ^1H NMR (300 MHz, Me_2SO-d_6) δ 11.3 (br s, NH), 5.97 (s, vinyl), 2.56 (s), 2.47 (t, J = 2.4 Hz), 2.31 (br s), and 1.35 (s, CH_3); MS, m/z 300 (M^+, 1), 272 (2), 203 (27), and 106 (100). Anal. ($C_{16}H_{16}N_2O_4$·0.33H_2O) C, H, N.

4,9,8a-Trimethyl Derivative 10f. With use of the procedure described for the photolysis of anisole, a mixture of 5 g of maleimide, 170 mL of mesitylene, and 5 mL of acetophenone was irradiated for 17 h. The acetone-insoluble portion, 1.7 g (21%), showed the following: mp >300 °C; IR (KBr) 3450, 3190, 3060, 1760, and 1700 cm^{-1}; ^1H NMR (300 MHz, Me_2SO-d_6) δ 11.2 (NH), 5.54 (br s, vinyl), 3.17–2.4 (complex), 1.78 (d, J = 1.2 Hz), 1.25 and 1.11 (s, CH_3); MS, m/z 314 (M^+, 11), 286 (10), 216 (9), and 120 (100). Anal. ($C_{17}H_{18}N_2O_5$·0.5H_2O) C, H, N.

3a,3b,4,4a,7a,8,8a,8b-Octahydro-4,8-dicarboxypyrrolo[3′,4′:3,4]cyclobut[1,2-*f*]isoindole-1,3,5,7(2*H*,6*H*)-tetrone (9a). A stream of ozone in oxygen was passed through a suspension of 2.0 g (7.4 mmol) of **1b** in 250 mL of acetic acid. After 1 h, the reaction mixture was light blue and most of the solid had dissolved.

Nine milliliters of acetic anhydride, 18 mL of 30% hydrogen peroxide, and ca. 20 mg selenium dioxide were added, and the mixture was heated under reflux for 4 h. Upon cooling, the product precipitated and was collected, washed with water, and dried. This gave 1.3 g (51%) of white solid, which showed the following: mg >300 °C; IR (KBr) 3420 (vb), 3220, 1780 (sh), 1760, 1720, and 1690 cm^{-1}; ^1H NMR (300 MHz, Me_2SO-d_6) δ 12.35 (br s, CO_2H), 11.50 and 11.09 (s, NH), 3.76 (m), 3.11 (br s), and 2.90 (br s). Anal. ($C_{14}H_{12}N_2O_8 \cdot 0.66H_2O$) C, H, N.

2,6-Bis(hydroxymethyl) Derivative 9b. With use of the procdure described for the synthesis of **9a**, 2.0 g (6.3 mmol) **7a** gave 1.2 g (46%) of a white solid, which showed the following: mp 294–296 °C dec; IR (KBr) 3520, 3420, 2960, 1770, and 1700 cm^{-1}; ^1H NMR (300 MHz, MeSO-d_6) δ 12.44 (br s, CO_2H), 6.42 and 6.32 (br s, OH), 4.78 and 4.74 (s, CH_2O), 3.78 and 3.21 and 2.94 and 2.85 (br s). Anal. ($C_{16}H_{16}N_2O_{10} \cdot 1.0H_2O$) C, H, N.

Cyclobutane Diimide 11a. This compound was synthesized according to the method of Boule.[32]

Diimides 11b–d. General Procedure. A 10-g portion of the corresponding commercially available dianhydride was mixed with 50 mL of concentrated aqueous ammonia until a clear solution was obtained. After evaporation of the solvent and oven drying at 100 °C, the solid was placed in sublimation apparatus and heated to about 200 °C in vacuo. After several hours the temperature was gradually raised to about 300 °C, and the product sublimed. The crude products were recrystallized from water or water/DMF mixtures. All of the compounds have been reported in the literature, but no useful physical properties were recorded. Compound **11b** showed the following: mp >300 °C; IR (KBr) 1767, 1690, cm^{-1}; ^1H NMR (60 MHz, Me_2SO-d_6) δ 11.40 (br s, NH), 7.93 (s, arom). Anal. ($C_{10}H_4N_2O_4$) C, H, N. Compound **11c** showed the following: mp 287–291 °C; MS, m/z 208 (M^+, 10), 165 (33), 137 (36), 66 (100); ^1H NMR (60 MHz, TFA-d) δ 12.07 (br s, NH), 4.27–3.67 (m), 3.00–2.63 (m). Anal. ($C_9H_8N_2O_4$) C, H, N. Compound **11d** showed the following: mp >300 °C; MS, m/z 246 (M^+, 6), 176 (15), 131 (13), 78 (100); ^1H NMR (300 MHz, Me_2SO-d_6) δ 11.14 (br s, NH), 6.11 (m, vinyl), 3.27 (m), 3.04 (br s). Anal. ($C_{14}H_{14}N_2O_6 \cdot 0.25H_2O$) C, H, N.

Bis(hydroxymethyl) Derivative 12a. This compound was synthsized by use of the same method as in the synthesis of **7a**. This gave a 50% yield (based on crude **11d**) of white needles, which showed the following: mp >300 °C; ^1H NMR (60 MHz, TFA-d) δ 6.25 (m, olefin), 5.17 (s, CH_2), 3.92 (m), 3.43 (br s). Anal. ($C_{14}H_{14}N_2O_6 \cdot 0.25H_2O$) C, H, N.

Mannich Base Derivative 12b. With use of the same procedure (method A) as in the synthesis of **7b**, compound **12b** was produced directly in 96% yield and showed the following: mp 295 °C dec; ^1H NMR (300 MHz, Me_2SO-d_6) δ 6.10 (m, olefin), 4.16 (s, CH_2), 3.3 (m), 3.18 (br s), 2.34 (br s), 2.21 (br s), 2.08 (s). Anal. ($C_{24}H_{34}N_6O_4 \cdot 0.5H_2O$) C, H, N. For screening this compound was formulated as an hydrochloride salt, by the addition of dilute hydrochloric acid to pH 7.0, followed by freeze-drying.

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Registry No. **1b**, 10403-51-7; **7a**, 96488-31-2; **7b**, 96488-32-3; **7c**, 102520-22-9; **7d**, 96488-34-5; **7e**, 96509-07-8; **7f**, 102572-81-6; **7g**, 96488-35-6; **7h**, 102520-23-0; **7i**, 102520-24-1; **7j**, 102520-25-2; **7k**, 31844-12-9; **7l**, 102520-26-3; **7m**, 102520-27-4; **8a**, 102520-28-5; **8b**, 102520-29-6; **8c**, 102520-30-9; **8d**, 102520-31-0; **9a**, 68644-36-0; **9b**, 102520-32-1; **10a**, 102572-82-7; **10b**, 102572-83-8; **10c**, 102572-84-9; **10d**, 102520-33-2; **10f**, 102535-13-7; **11b**, 2550-73-4; **11b** (dianhydride), 89-32-7; **11c**, 22031-22-7; **11c** (dianhydride), 6053-68-5; **11d**, 6787-63-9; **11d** (dianhydride), 1719-83-1; **12a**, 102520-34-3; **12b**, 102520-35-4; **12b**·HCl, 102520-36-5; maleimide, 541-59-3; benzene, 71-43-2; *N*-methylpiperazine, 109-01-3; thiamorpholine, 123-90-0; morpholine, 110-91-8; diethylamine, 109-89-7; glyoxylic acid, 298-12-4; anisole, 100-66-3; *p*-xylene, 106-42-3; mesitylene, 108-67-8.

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Comparative Antiaggregatory Activity in Human Platelets of a Benzopyranone *aci*-Reductone, Clofibric Acid, and a 2,3-Dihydrobenzofuran Analogue[1]

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A synthetic method for the preparation of *aci*-reductone 6-chloro-3,4-dihydroxy-2*H*-1-benzopyran-2-one (**3**) from 5-chlorosalicylate is presented. In human platelets, the benzopyranone derivative **3**, clofibric acid (**1**), and the 2,3-dihydrobenzofuran analogue **4** inhibited aggregation and serotonin secretory responses to adenosine diphosphate (ADP) with a rank order of potency $3 \geq 4 > 1$. Only analogues **3** and **4** consistently blocked the aggregatory responses (>50%) to arachidonic acid (AA) and U46619, a thromboxane A_2 agonist. Further, the rank order of inhibitory potency against U46619-induced serotonin secretion was $4 > 3 > 1$. Benzopyranone **3** is of interest since it was the most potent inhibitor of thrombin-induced [^3H]AA release ($3 \gg 4 = 1$) and more potent than **1** or **4** for the blockade of the ADP- or AA-mediated pathway of platelet aggregation.

Comparative antiaggregatory activities of clofibric acid (**1**) and 2-hydroxytetronic acid *aci*-reductone **2** previously have been reported.[2] Such studies are important since blood platelets are involved in the genesis of atherosclerosis.[3] Both **1**[4] and **2**[2] inhibit collagen-induced human platelet aggregation and secretion of [^{14}C]serotonin in a concentration-dependent manner at equivalent molar concentrations. Preliminary results indicated that **1** and **2** may be inhibiting platelet function by a similar mechanism—possibly involving arachidonic acid (AA) release.[2,4] However, it also might be anticipated that redox analogues such as **2** could function as antioxidants in membranes or interfere with free-radical processes involved in the biosynthetic elaboration of cyclic prostaglandin

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