Syntheses and Biological Activities of Basically Substituted Isoalloxazines

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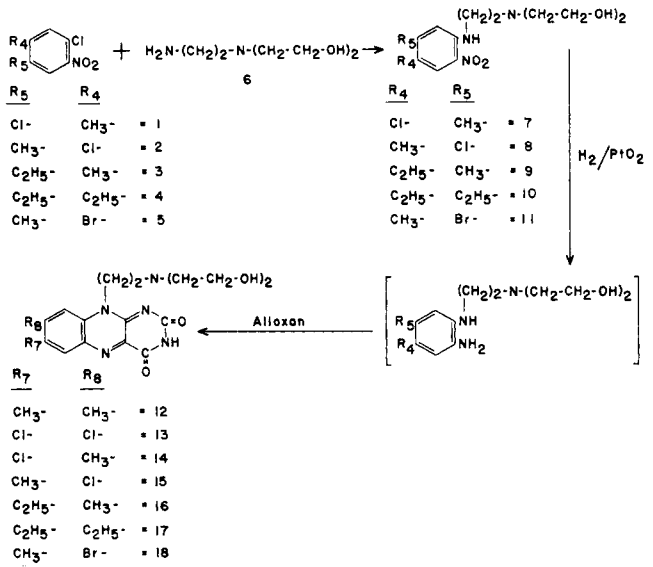
A number of flavins possessing a 2-[bis(2-hydroxyethyl)amino]ethyl side chain in place of the usual D-ribityl side chain at position 10 have been synthesized and evaluated for riboflavin antagonistic activity in the rat. Two flavins of those synthesized are potent antagonists of the vitamin.

In 1973,[1] we defined the essential structural features required in an analogue of riboflavin to ensure biological activity in a form resembling riboflavin in action or in a form antagonistic to the action of riboflavin. Much of the work in which these conclusions were based has now been reviewed.[2] We have abandoned[3] further modifications of the D-ribitylisoalloxazine structure at positions 7 and 8 and have now turned our attention to a new class of antagonists bearing modifications of the side chain at position 10 of the isoalloxazine nucleus. A logical approach was to make use of the basic isoalloxazine structures that, as the 10-D-ribityl derivatives, provided us with the most active vitamin-like or antagonistic analogues of riboflavin, namely, 7-chloro-8-methyl-,[4-7] 7-methyl-8-chloro-,[4,8] 7-ethyl-8-methyl-,[9-14] and 7,8-diethyl-10-(1′-D-ribityl)isoalloxazine.[2,15-17]

Our earlier syntheses of two-arm mustards of flavins[18] involved the use of a 2-[bis(2-hydroxyethyl)amino]ethyl side chain at position 10. This led to the synthesis of 7,8-dimethyl-10-[2-[bis(2-hydroxyethyl)amino]ethyl]isoalloxazine (**12**) which was found to be a strong, reversible antagonist of riboflavin in the rat.



This is a report of the syntheses and biological evaluation of a series of additional flavins based on the above basic flavin structures but possessing the 2-[bis(2-hydroxyethyl)amino]ethyl side chain at position 10.

The results of the evaluation of the flavins as replacements for, or antagonists of, riboflavin are given in Table I. Flavin **12** was found to be a reversible antagonist of riboflavin and this confirms our earlier finding.[18] Flavin **14** was approximately equivalent to flavin **12** as an antagonist of riboflavin in terms of growth, but it was less toxic. Flavins **16** and **17** were found to be potent reversible antagonists of riboflavin.

This new class of riboflavin analogues appears to possess none of the vitamin-like properties of many of the antagonistic analogues of riboflavin possessing the D-ribityl side chain.[1-3,5,19] However, when provided with suboptimal amounts of riboflavin, flavin **17** provided supplementary riboflavin-like growth activity and also provided riboflavin-like protection against the induction of hepatomas by 3′-methyl-4-dimethylaminobenzene.[20]

Flavins **13** and **15** were essentially inert in the rat. Flavin **15** was inert as an antagonist in *Lactobacillus casei*. When flavin **15** was autoclaved in the presence of the microbiological assay medium, it formed a red, stable semiquinone as had been observed in several flavins possessing the D-ribityl side chain (ref 2, p 354).

Flavins **12**, **13**, **15**, and **16** were found to be devoid of antimalarial activity by the Walter Reed Army Institute of Research.

Because of the inertness of flavin **15**, flavin **18** was not tested in the rat.

Experimental Section

Biological Procedures. Female weanling Wistar rats (CFN strain from Carworth Inc.; this strain is no longer produced) were fed a riboflavin-deficient diet[7,21] until they were deficient.[17] The evaluation of the new flavins as replacements for, or as antagonists of, riboflavin was carried out essentially as we have described before.[3,5,8] The results are shown in Table I.

The *L. casei* 7469 used for the assay of flavin **15** was obtained from the American Type Culture Collection, Rockville, Md.; the procedure used was like that described before.[3,5]

Chemical Syntheses. Melting points were determined in open Pyrex capillary tubes in an electrically heated, modified Drechsel-type bath and are corrected (thermometers calibrated against U.S.P. melting point reference standards). Boiling points and decomposition points are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. GLC were determined on a Varian Aerograph, Model 600D; the column was packed with 3% OV1 on WHP80-100.

4,5-Disubstituted 2-Chloronitrobenzenes. 2,5-Dichloro-4-nitrotoluene[4] (**1**) (GLC pure), 2,4-dichloro-5-nitrotoluene[4] (**2**), and 2-bromo-4-chloro-5-nitrotoluene[19] (**5**) were prepared as described in the literature.

2-Chloro-4-methyl-5-ethylnitrobenzene (3). 2-Nitro-4-ethyl-5-methylaniline[9] (15.1 g, 0.084 mol) was converted to **3**, as described for the preparation of **1**, to produce yellow needles. Recrystallization from EtOH yielded 10.2–11.0 g (62–66%): mp 33–34 °C. Anal. ($C_9H_{10}ClNO_2$) C, H, Cl, N.

2-Chloro-4,5-diethylnitrobenzene (4). 2-Nitro-4,5-diethylaniline[15] (22.0 g, 0.113 mol) was converted to **4**, as described for the preparation of **1**, to yield a yellow liquid: 12.3 g (50%); bp 185–187 °C (24 mmHg); 101–104 °C (0.3 mmHg). Anal. ($C_{10}H_{12}ClNO_2$) C, H, Cl, N.

4,5-Disubstituted 2-Nitro-*N*-2-[bis(2-hydroxyethyl)amino]ethylanilines. 4-Chloro-5-methyl-2-nitro-*N*-2-[bis(2-hydroxyethyl)amino]ethylaniline (7). 2-[Bis(2-hydroxyethyl)amino]ethylamine dihydrochloride[22,23] (**6**) (11.0 g, 0.05 mol) was suspended in 50 mL of EtOH, 2% phenolphthalein solution added, and the mixture stirred while enough of a solution of 7.5 g of $NaOCH_3$ dissolved in 50 mL of EtOH was added to impart a pale pink color to the suspension. The EtOH solution was filtered from NaCl, evaporated to dryness, and extracted from a residue of NaCl into 25 mL of pyridine. The pyridine solution of the free base plus 5.15 g (0.025 mol) of **1** were heated in a sealed tube at 150–155 °C for 15 h and then processed as described for the preparation of 4,5-dimethyl-2-nitro-*N*-2-[bis(2-hydroxy-

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Table I. Growth of Rats Administered No Flavin, One of the Aminoflavins, or Mixtures of an Aminoflavin and Riboflavin

Group no.	Flavin used[a]	Wt gained, g[b]	P[c]	Survivors,[d] %
1	Flavin deficient	3 ± 1		100
2	7,8-Dimethylaminoflavin (12)	−10 ± 2	"0"	40
3	7,8-Dimethylaminoflavin (12) plus Rb (20 μg/day)	35 ± 3	"0"	100
4	7,8-Dichloroaminoflavin (13)	6 ± 3	0.302	100
5	7-Chloro-8-methylaminoflavin (14)	−8 ± 2	0.0003	100
6	7-Methyl-8-chloroaminoflavin (15)	0 ± 2	0.270	100
7	7-Ethyl-8-methylaminoflavin (16)	−25 ± 3	"0"	60
8	7-Ethyl-8-methylaminoflavin (16) plus Rb (20 μg/day)	−2 ± 2	0.084 "0"[e]	100
9	7-Ethyl-8-methylaminoflavin (16) plus Rb (40 μg/day)	21 ± 3	"0"	100
10	7,8-Diethylaminoflavin (17)	−26 ± 3[f]	"0"	25
11	7,8-Diethylaminoflavin (17) plus Rb (20 μg/day)	−9 ± 3	0.0002 "0"[g]	90
12	7,8-Diethylaminoflavin (17) plus Rb (40 μg/day)	7 ± 3	0.192 0.001[h]	100
13	7,8-Diethylaminoflavin (17) plus Rb (60 μg/day)	33 ± 3	"0"	100

[a] All aminoflavins were administered at 2 mg/rat/day in 0.5 mL of H_2O. When riboflavin (Rb) was added to the analogues, the mixed flavins were administered in 0.5 mL of H_2O. [b] Group 1 consisted of 40 rats (four groups of ten each); group 2 consisted of 20 rats (two groups of ten each); all other groups consisted of ten rats each. Average starting weights ± SEM for all groups were from 61 ± 2 g to 67 ± 3 g. Weight gained is the average weight gained ± SEM for the 28-day test period. [c] Probability (Student's t test); the numbers represent the P values for the differences between the group in that line and the flavin-deficient group (group 1). The symbol "0" means the P value is less than 0.0001. [d] The percentage of the test animals that survived the 28-day test period. [e] P value for the differences between groups 7 and 8. [f] Average weight gained for animals living 14 days or longer. [g] P value for the difference between groups 10 and 11. [h] P value for the difference between groups 11 and 12.

ethyl)amino]ethylaniline[18] to yield 6.3–6.4 g (79–81%) of product as orange needles: mp 118–120 °C (EtOH). Anal. ($C_{13}H_{20}ClN_3O_4$) C, H, Cl, N.

4-Ethyl-5-methyl-2-nitro-*N*-2-[bis(2-hydroxyethyl)amino]ethylaniline (9). As described for the preparation of **7**, **3** (5.00 g, 0.25 mol) was converted to **9** except that the heating period was increased to 24 h. The product was dissolved in a mixture of 1250 mL of H_2O and 30 mL of concentrated HCl, freed of unreacted **3** by Et_2O extraction, and, following evaporation to 200 mL under reduced pressure, neutralized with NH_4OH to produce 5.5 g of product. Recrystallization from 50% EtOH yielded 4.4 g (57%) of orange needles: mp 73–74 °C. Anal. ($C_{15}H_{25}N_3O_4$) C, H, N.

4,5-Diethyl-2-nitro-*N*-2-[bis(2-hydroxyethyl)amino]ethylaniline Hydrochloride (10). As described for the preparation of **9**, **4** (5.3 g, 0.025 mol) was converted to **10** except that, following the addition of NH_4OH, the product was extracted into Et_2O. Removal of the solvent produced the product. To avoid excessive losses occurring during recrystallization, the combined product resulting from three batches was converted to the hydrochloride salt by passing dry HCl gas into an absolute Et_2O solution of the product. The salt was recrystallized from 6 N HCl to yield 15.8 g (61%) of yellow needles: mp 150–152 °C. Anal. ($C_{16}H_{28}ClN_3O_4$) C, H, Cl, N.

4-Methyl-5-bromo-2-nitro-*N*-2-[bis(2-hydroxyethyl)amino]ethylaniline (11). As described for the preparation of **7**, **5** (6.26 g, 0.025 mol) was converted to **11** to produce 5.1–5.8 g (56–64%) of orange needles: mp 117–119 °C. Anal. ($C_{13}H_{20}BrN_3O_4$) C, H, Br, N.

4-Methyl-5-chloro-2-nitro-*N*-2-[bis(2-hydroxyethyl)amino]ethylaniline (8). A mixture of **6** (119 g, 0.54 mol) and **2** (55.1 g, 0.268 mol) in 300 mL of dry pyridine was refluxed for 24 h. The product was worked up as described for **7** to produce 23.8 g (28%) of orange needles: mp 113–114 °C (EtOH). Anal. ($C_{12}H_{20}ClN_3O_4$) C, H, Cl, N.

7,8-Disubstituted 10-[2-[Bis(2-hydroxyethyl)amino]ethyl]isoalloxazines. 7-Chloro-8-methyl-10-[2-[bis(2-hydroxyethyl)amino]ethyl]isoalloxazine Hydrochloride (14). A solution of **7** (9.70 g, 0.03 mol) in 300 mL of absolute EtOH was hydrogenated over 500 mg of PtO_2 for 4 h at 4.6 kg/cm^2. Dry HCl gas was passed into the catalyst-free filtrate, and then the solution was evaporated to dryness. To a hot solution of the residue in 100 mL of absolute EtOH was added a hot solution of 8.0 g of alloxan in 200 mL of absolute EtOH; the mixture was refluxed on the steam bath for 10 min and then stored in the dark at room temperature for 3 days. The collected crude flavin was extracted into 250 mL of 50% EtOH, filtered, and refrigerated. The product (4.23 g) was recrystallized from 180 mL of 60% EtOH to yield 3.92 g (30%) of greenish yellow crystals: mp 255–256 °C dec. Anal. ($C_{17}H_{21}Cl_2N_5O_4$) C, H, Cl, N.

7-Methyl-8-chloro-10-[2-[bis(2-hydroxyethyl)amino]ethyl]isoalloxazine Hydrochloride (15). As described for **14**, **8** (15.9 g, 0.050 mol) was treated with 12 g of alloxan to produce 6.90 g (32%) of yellow crystals: mp 269–270 °C dec. Anal. ($C_{17}H_{21}Cl_2N_5O_4$) C, H, Cl, N.

7-Ethyl-8-methyl-10-[2-[bis(2-hydroxyethyl)amino]ethyl]isoalloxazine Hydrochloride (16). Except that hydrogenation was continued for 24 h, **9** (5.00 g, 0.016 mol) was converted to a crystalline flavin which, when recrystallized from 75% EtOH, produced 3.54 g (52%) of yellow needles: mp 261–262 °C dec. Anal. ($C_{19}H_{26}ClN_5O_4$) C, H, Cl, N.

7,8-Diethyl-10-[2-[bis(2-hydroxyethyl)amino]ethyl]isoalloxazine Hydrochloride (17). As described for **16**, except that the hydrogenation solvent was EtOH, **10** (5.00 g, 0.014 mol) produced a crystalline flavin which, when recrystallized from 60% EtOH, yielded 2.68–2.74 g (44–45%) of yellow crystals: mp 254–256 °C dec. Anal. ($C_{20}H_{28}ClN_5O_4$) C, H, Cl, N.

7-Methyl-8-bromo-10-[2-[bis(2-hydroxyethyl)amino]ethyl]isoalloxazine Hydrochloride (18). As described for **14**, **11** (5.00 g, 0.014 mol) yielded 1.54 g (23%) of yellow needles: mp 266–267 °C dec. Anal. ($C_{17}H_{21}BrClN_5O_4$) H, N; C: calcd, 43.0; found, 42.2.

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Synthesis and Antitumor Properties of Some Isoindolylalkylphosphonium Salts

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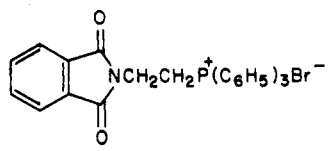
Antitumor evaluation of 2-(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)ethyltriphenylphosphonium bromide (1) revealed significant activity in P-388 lymphocytic leukemia (T/C = 160%). As a follow-up to this chemical lead, a series of closely related phosphonium salts was prepared in which the 1,3-dihydro-1,3-dioxo-2*H*-isoindole ring system was maintained or in which it was replaced by other moieties such as maleimido, bromo, methoxy, and isoindoline. Syntheses generally involved treatment of the appropriate *N*-(bromoalkyl)phthalimide with the required phosphine or condensation of the K salt of the substituted imide with β-(bromoethyl)triphenylphosphonium bromide (**12**). From the biological data obtained for these compounds, several requirements can be defined for substantial antileukemic activity. Of utmost importance is the presence of a triarylphosphonium halide moiety, coupled to an alkyl chain of two or three carbon atoms. The preferred terminus of the alkyl chain is the 1,3-dihydro-1,3-dioxo-2*H*-isoindole ring system, although the observed activity of β-(bromoethyl)triphenylphosphonium bromide (**12**) (T/C = 127%) would suggest that a superior carrier molecule could be developed.

In the course of our antitumor agents synthesis program, it became necessary to prepare 2-(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)ethyltriphenylphosphonium bromide (**1**)



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as a synthetic intermediate. This material was routinely submitted for biological evaluation and unexpectedly displayed substantial activity in the P-388 lymphocytic leukemia screen. As a follow-up to this chemical lead, a series of closely related phosphonium salts was prepared and biologically screened in an attempt to elucidate the minimum structural requirements necessary for antitumor activity, information which could then be utilized in the development of a more active second generation drug. These compounds are listed in Tables I–III. Their methods of preparation and antileukemic activity are the subjects of this report.

Chemistry. Compounds 1–4 which represent variations in the length of the carbon side chain were prepared by treatment of the appropriate *N*-(bromoalkyl)phthalimide with triphenylphosphine in refluxing mesitylene (benzene in the case of **2**). In a similar manner, reaction of *N*-(2-bromoethyl)phthalimide with the required phosphine in DMF at 50–90 °C generated analogues **5**–**11** with altered substituents at the phosphorus atom. Under the conditions of refluxing DMF, *n*-butoxydiphenylphosphine reacted with *N*-(2-bromoethyl)phthalimide to yield phosphine oxide **24**, via an Arbusov rearrangement of the intermediate phosphonium salt.

Due to the commercial unavailability of the appropriately substituted *N*-(2-bromoethyl)imides, compounds **13**–**18** and **23** were synthesized by an alternate procedure which involved condensation of **12** with the K salt of the substituted imide. Intermediate **12** was isolated from the reaction of phosphorus tribromide with β-(hydroxyethyl)triphenylphosphonium bromide, itself obtained from treatment of 2-bromoethanol with triphenylphosphine.[1]

Attempted reaction of **12** with the K salt of 1,8-naphthalimide did not result in the preparation of the desired naphthalimido phosphonium salt but unexpectedly generated the methoxy derivative **22**, a product originating from the methanol solvent.

Synthesis of phosphonium chloride **19** was achieved by treatment of **1** with Amberlite IRA-400 ion-exchange resin (Cl^- form).

Reduction of phthalimide with diborane resulted in the preparation of isoindoline which was converted to **20** by reaction with phosphonium salt **12**. The free base **21** was obtained from **20** by neutralization with $NaHCO_3$.

Biological Results and Discussion. The antimitotic activity of all compounds (see Tables I–III) was measured in lymphocytic leukemia P-388 by standard protocols of the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health.[2] Compounds are considered significantly active if they give reproducible T/C activity[2] values in the P-388 leukemia system equal to or greater than 125% where T/C represents the ratio of the mean or median survival times of the treated an-

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