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Potential Central Nervous System Antitumor Agents. Hydantoin Derivatives

Geoffrey W. Peng, Victor E. Marquez, and John S. Driscoll*

Drug Development Branch, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014. *Received January 10, 1975*

Hydantoin derivatives of varying lipophilic character were prepared as nitrogen mustard carriers for CNS antitumor evaluation. Activity was studied in the murine ependymoblastoma brain tumor system. Multiple cures were observed for three of the four analogs examined. The compounds were also active in the intraperitoneal leukemia L1210 and P388 systems as well as in B16 melanoma and Lewis lung carcinoma.

The design of drugs which may be useful in the chemotherapy of tumors of the central nervous system contains numerous challenges. In addition to possessing antitumor activity, a compound should have structural features which allow it to circumvent natural defense mechanisms, such as the blood-brain barrier (BBB). There appear to be significant differences in the anatomical structure of brain tumors and their surrounding areas compared to normal brain.[1] While the neoplasm appears to alter the BBB in such a way that drug penetration is sometimes enhanced,[2-4] the changes are not constant among different types of brain tumors. This indicates that the permeability properties are not altered to the same extent[5] and the BBB is a factor which must be considered. The situation is complex and in seeking new antitumor drugs, one should be concerned with the type of structure which (a) penetrates the BBB,[6] (b) does so in significant concentrations, and (c) has antitumor activity.

The principle of using a carrier for an antitumor active functional group, e.g., a nitrogen mustard, is not new, and phenylalanine mustard (sarcolysin) is an example of this application. In a recent review of CNS antitumor agents,[7] Broder and Rall concluded that new drug emphasis should be placed on alkylating agents which are able to cross the BBB. The reports that 5,5-diphenylhydantoin (DPH) penetrated the BBB in significant concentrations[8] and localized in brain tumors relative to surrounding normal brain tissue,[9] but had no antitumor activity, prompted a study of hydantoins as carriers for nitrogen mustard groups in an attempt to prepare agents which might have utility as drugs for CNS and brain tumors. Mauger and Ross have previously used the hydantoin ring as a carrier in a series of active bis(2-chloroethyl)aminoarylhydantoins which were tested against the Walker tumor system.[10]

The importance of the partition coefficient as a parameter in CNS drug activity has been shown and quantitated.[11] DPH has a log P value of 2.47 in the octanol–water system.[12] This value approximates an optimum value (log P_0) of about 2.0 found to be characteristic of many CNS agents.[11] The addition of an alkylating group to DPH to yield the DPH mustard **16** should significantly alter the partition coefficient of the compound relative to DPH. An approximation of the log P of **16** as the neutral molecule can be made as seen in eq 1. The modification made to con-

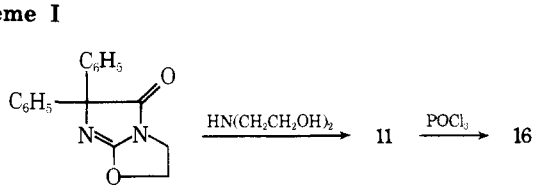
$$\log P(\text{DPH}) + \log P(\text{Et}_3\text{N}) + 2\pi(\text{Cl}) = \log P(\mathbf{16}) \quad (1)$$

$$2.47 + 1.44 + 2(0.39) = 4.69$$

fer antitumor activity on DPH might, therefore, alter its log P value in such a way as to minimize its CNS properties. Because of the large increase in lipophilic character caused by the addition of the $CH_2CH_2N(CH_2CH_2Cl)_2$ group, the carrier hydantoin was modified in order to compensate. An additional four derivatives (**17–20**) were chosen for synthesis based on their estimated log P values (1.5–2.7). After **16–20** were synthesized, attempts were made to measure the partition coefficients of several of these molecules by the method of Hansch.[12,13] These experiments, however, were not successful because of solution decomposition problems. This is often the case with bis(2-chloroethyl) derivatives.

Chemistry. The initial member of the series, **16**, was prepared by two procedures. An earlier hydantoin study[14] had produced the bicyclic compound **21**. During an investigation of the alkylating properties of **21**, it was allowed to react with diethanolamine to produce **11** which was subsequently converted to **16** (Scheme I).

Scheme I



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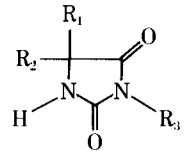
A more general method employed a 3-(β-chloroethyl)hydantoin (Scheme II). Physical and chemical data are shown in Table I. Several of the intermediate products were oils. In those instances, yields were computed based on the first crystalline material isolated.

Table I. Physical and Chemical Data



Compd no.	R_1	R_2	R_3	Yield, %	Mp, °C	Mol formula	Analyses
2	$CH_2CH_2CH_2CH_2CH_2$		H	88	215–215.5[a]	$C_8H_{12}N_2O_2$	C, H, N
4	C_2H_5	C_2H_5	H	55	164–165.5[b]	$C_7H_{12}N_2O_2$	C, H, N
6	C_6H_5	C_6H_5	CH_2CH_2Cl	47	150–152	$C_{17}H_{15}N_2O_2Cl$	C, H, N
7	$CH_2CH_2CH_2CH_2CH_2$		CH_2CH_2Cl	73	156–158	$C_{10}H_{15}N_2O_2Cl$	C, H, N
8	CH_3	CH_3	CH_2CH_2Cl	77	94.5–96	$C_7H_{11}N_2O_2Cl$	C, H, N
11	C_6H_5	C_6H_5	$CH_2CH_2N(CH_2CH_2OH)_2$	87	145	$C_{21}H_{25}N_3O_4$	C, H, N
12	$CH_2CH_2CH_2CH_2CH_2$		$CH_2CH_2N(CH_2CH_2OH)_2$	63	100–102	$C_{14}H_{25}N_3O_4$	C, H, N
15	H	C_6H_5	$CH_2CH_2N(CH_2CH_2OH)_2$	28[c]	108–110	$C_{15}H_{21}N_3O_4$	C, H, N
16	C_6H_5	C_6H_5	$CH_2CH_2N(CH_2CH_2Cl)_2$	68	146–147.5	$C_{21}H_{23}N_3O_2Cl_2$	C, H, N
17	$CH_2CH_2CH_2CH_2CH_2$		$CH_2CH_2N(CH_2CH_2Cl)_2$	51	125–126	$C_{14}H_{23}N_3O_2Cl_2$	C, H, N
18	CH_3	CH_3	$CH_2CH_2N(CH_2CH_2Cl)_2$	24[d]	70–72	$C_{11}H_{19}N_3O_2Cl_2$	C, H, N
19	C_2H_5	C_2H_5	$CH_2CH_2N(CH_2CH_2Cl)_2$	18[e]	157–158	$C_{13}H_{24}N_3O_2Cl_3$[f]	C, H, N
20	H	C_6H_5	$CH_2CH_2N(CH_2CH_2Cl)_2$	41	74–75	$C_{15}H_{19}N_3O_2Cl_2$	C, H, N

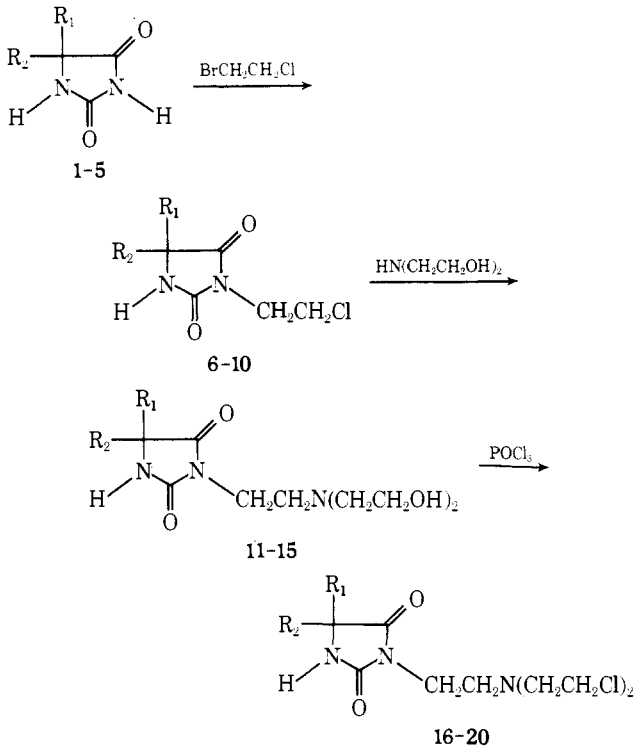
[a]Lit.[16] mp 215–215.5°. [b]Lit.[16] mp 165°. [c]From 5-phenylhydantoin (compound **5**). [d]From 5,5-dimethyl-3-(2-chloroethyl)hydantoin (compound **8**). [e]From 5,5-diethylhydantoin (compound **4**). [f]HCl salt.

Table II. Antitumor Activity[a,b]

Compd no.	L1210 lymphoid leukemia[c]		P388 lymphocytic leukemia[d]		B16 melano-carcinoma[d,e]		Lewis lung carcinoma[d,f]		Ependymo-blastoma[g]	
	OD[h]	T/C[i]	OD	T/C	OD	T/C	OD	T/C	OD	T/C
16	100	121	50	200[c]	6	161			50	117
17	35	137	12.5	324	6	161			12.5	271
18	6	138	3	243	2	138	3	157	3	227
19	25	141	6	292	6	183	3	162	3	267
20	25	115	12.5	250						

[a]Protocols and tumor systems described in ref 15. [b]In a minimum of one additional experiment, these compounds were determined to have a T/C value not more than 15% lower than the value shown in this table. All compounds with active T/C values (see text for definition of activity) are confirmed active (at least one additional active test). [c]Day 1, 5, 9 treatment schedule. [d]QD 1-9 treatment schedule unless noted otherwise. [e]Intraperitoneal tumor implantation. [f]Subcutaneous tumor implantation. [g]QD 1-5 treatment schedule. [h]Optimum dose (mg/kg/dose). [i]T/C = (treated survival/control survival) × 100%.

Scheme II



1, 6, 11, 16, $R_1 = R_2 = C_6H_5$
2, 7, 12, 17, $R_1 + R_2 = (CH_2)_5$
3, 8, 13, 18, $R_1 = R_2 = CH_3$
4, 9, 14, 19, $R_1 = R_2 = C_2H_5$
5, 10, 15, 20, $R_1 = H$; $R_2 = C_6H_5$

Antitumor Activity. All compounds described (1–21) were tested in the lymphoid leukemia L1210 system by standard NCI protocols.[15] Only compounds **16–20** showed enough activity to be further tested in additional tumor systems. In addition to leukemia L1210, these compounds were tested against lymphocytic leukemia P388, B16 melanocarcinoma, Lewis lung carcinoma, and the ependymoblastoma mouse brain tumor.[15] All T/C results reported in Table II have been reproduced in separate experiments to give values not more than 15% lower than the value in the table.

Discussion

Compounds **16–20** show substantial, reproducible activity in most of the test systems employed (Table II). Among these compounds, the 5-phenyl derivatives **16** and **20** were inactive in the L1210 system. (Compounds described here are considered active if they give reproducible T/C activity[15] values equal to or greater than the following: L1210 leukemia, 125%; P388 leukemia, 125%; B16 melanoma, 140%; Lewis lung carcinoma, 125%; ependymoblastoma, 140%.) L1210 activity would have to be considered only moderate for compounds **17–19**. It can be noted that there is a significant difference between the optimal doses of the diphenyl analog **16** and the other derivatives. Compounds **17–19** exhibited no intracerebral L1210 activity. This might be expected, however, based on the marginal L1210 intraperitoneal activity.

Substantial activity is noted in the leukemia P388 tests with the pentamethylene (**17**) and diethyl (**19**) derivatives having the greatest activity. Multiple cures (30-day survivors) were observed with both compounds.

Compounds **16**, **17**, and **19** had approximately the same level of activity in the B16 melanocarcinoma tumor model. Compound **18** almost reached the level of reproducible activity.

Limited testing in a second solid tumor model, Lewis lung carcinoma, showed that **18** and **19** were active.

The ependymoblastoma intracerebral tumor system was used as the main test system for CNS antitumor activity. The advantages of this solid fragment implant system have been discussed.[1,17] The tumor is implanted in the mouse's brain and the drug is administered intraperitoneally. The DPH mustard analog, **16**, was inactive in this test system. Compounds **17–19**, however, have very good activities. Cures (60-day survivors) of at least 50% were obtained with each of these compounds. In two separate tests of the spiropentamethylene derivative **17**, 6 of 6 and 5 of 6 ependymoblastoma cures were obtained at the optimum dose (12.5 mg/kg).

At four times the optimum dose, the compounds in Table II were almost always toxic. A therapeutic index (TI) (highest active dose divided by lowest active dose) of at least two or four was generally encountered. In the very active tests, e.g., **17**, **18**, and **19** in the P388 and ependymoblastoma systems, TI values of 16–32 were not unusual.

Several derivatives (**17–19**) are active in most of the tumor systems studied. The diphenyl analog **16**, the most lipophilic of the group, was active in two of the intraperitoneal tumor systems but inactive in the brain tumor model. With the few compounds evaluated, it is impossible to make a final conclusion concerning the relative importance of the physical parameters involved. The inactivity of the 5-phenyl derivatives in the L1210 and ependymoblastoma systems might be due to high lipophilicity, the steric effects of the groups in the 5 position, or metabolic differences between aromatic and aliphatic substituted compounds. The high activity of the more moderately lipophilic derivatives in the brain tumor system is, however, consistent with the previously noted importance of drug transport properties in CNS systems.

Experimental Section

All melting points are uncorrected and recorded on a Thomas-Hoover capillary melting point apparatus. Elemental analyses were performed by NIAMD, NIH, Bethesda, Md. 5,5-Diphenylhydantoin was a gift from Ben Venue Laboratories. 5-Phenylhydantoin and 5,5-dimethylhydantoin were obtained commercially. Compounds **2** and **4** were prepared from the corresponding ketones by the method of Upham and Dermer.[16] When several compounds were prepared by comparable procedures, only one representative example is included in this section. Reference should be made to Table II for supplementary information for each compound. New compounds were identified by NMR and ir spectroscopy. Satisfactory elemental analyses (±0.4% of calculated values) are indicated by elemental symbols in Table II.

5,5-Pentamethylene-3-(2′-chlorethyl)hydantoin (**7**). 5,5-Pentamethylenehydantoin, 4.2 g (0.025 mol), was dissolved in a solution of 1.5 g (0.025 mol) of KOH in 100 ml of ethanol. To this solution was added 7.2 g (0.05 mol) of 1-bromo-2-chloroethane in one portion. The resulting solution was refluxed and stirred for 8 hr. Evaporation of the reaction mixture gave a white solid which was shaken with ethyl acetate and water in a separatory funnel. The organic layer was separated, washed (10% aqueous $NaHCO_3$), dried (Na_2SO_4), and evaporated to give 4.2 g (73%) of **7**. Recrystallization from benzene–2-propanol gave white crystals, mp 156–158°.

3-[2-[Bis(2′-hydroxyethyl)amino]ethyl]-5,5-pentamethylenehydantoin (**12**). Compound **7**, 3.0 g (0.013 mol), sodium iodide, 2.8 g (0.019 mol), and diethanolamine, 4.0 g (0.037 mol), were dissolved in 50 ml of freshly distilled *N,N*-dimethylformamide and the resulting solution was heated at 90–100° for 12 hr. After removing DMF at reduced pressure, the residue was treated with saturated aqueous $NaHCO_3$ solution and this solution was extracted with ethyl acetate. The ethyl acetate solution was washed (H_2O), dried (Na_2SO_4), and evaporated to give an oil. Compound **12** [2.4 g (63%)] was isolated by chromatographic purification on silica gel with acetone–benzene (1:1 v/v) as eluent. The analytical

sample, mp 100–102°, was prepared by recrystallization from benzene–2-propanol.

3-[2-[Bis(2′-chloroethyl)amino]ethyl]-5,5-pentamethylenehydantoin (17). Compound **12**, 3.0 g (0.01 mol), was added to 25 ml of phosphorus oxychloride.[10] The mixture was heated at 70–90° for 1.5 hr. Phosphorus oxychloride was removed at reduced pressure to yield an oily residue which was treated with 10 ml of concentrated hydrochloric acid. After the initial exothermic reaction subsided, the mixture was heated on steam bath for 10 min and allowed to cool. The mixture was poured into a cold saturated NaOAc solution and the solution was extracted with ethyl acetate. After washing (H_2O), drying (Na_2SO_4), and evaporation, the ethyl acetate extract yielded an oil which solidified upon cooling. Recrystallization from 2-propanol gave 1.67 g (51%) of **17**, mp 125–126°.

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Aromatic Esters of 5-*O*-Desosaminylerythronolide A Oxime

Ronald A. LeMahieu,* Mathew Carson, Richard W. Kierstead,

Chemical Research Department

and Sidney Pestka

Roche Institute of Molecular Biology, Hoffmann-La Roche Inc., Nutley, New Jersey 07110. *Received January 27, 1975*

Several substituted aromatic esters of the C-3 hydroxyl of 5-*O*-desosaminylerythronolide A oxime were prepared. Ribosomal binding studies showed that meta substituents on the aromatic ring gave the most active analogs. The esters described were all inactive in vivo at the maximum level tested.

We recently reported the cleavage of the sugar, cladinose, from erythromycin A oxime with 1% HCl in methanol to provide 5-*O*-desosaminylerythronolide A oxime as the corresponding 2′-acetyl acetoxime **1b** after acetylation. The new C-3 hydroxyl of **1b** could be acetylated under vigorous conditions and the C-3 monoacetate was available by selective hydrolysis of the triacetate.[1]

All attempts at glycosylation of the C-3 hydroxyl were unsuccessful. In experiments using acetobromoglucose, decomposition of the reagent was apparently faster than reaction at the C-3 hydroxyl. The glycosylation of 5-*O*-desosaminyldiacetyloleandolide by treatment with methyl L-oleandroside and methanesulfonic acid to give diacetyloleandomycin has been reported.[2] When these conditions were applied to **1b** no incorporation of oleandrose was observed. Treatment of **1b** with glucose tetraacetate and boron trifluoride etherate was unsuccessful due to the instability of **1b** under the reaction conditions. A glycosylation attempt using 3,4,6-tri-*O*-acetyl-1,2-*O*-ethylorthoacetyl-α-D-glucopyranose and mercuric bromide[3] also met with failure. We decided, therefore, to introduce functionality at this hydroxyl via an ester rather than an ether linkage.

The 3″-methoxyl group of cladinose in erythromycin A is necessary for high activity since erythromycin C with a 3″-hydroxyl group is only 30% as active as erythromycin A.[4] Ribosomal binding of erythromycin A and its analogs can be correlated with antibacterial activity.[5] A hydrogen bond model for this ribosome complex has been proposed.[6] The 3″-methoxyl group is essential for binding probably by a hydrogen bond to a primary amino group of a nucleotide base in the ribosome.[6] We therefore incorporated ring substituents capable of accepting hydrogen bonds in a number of aromatic esters.

The sequence for the preparation of these aromatic esters involved heating **1b** with the appropriate acid chloride in pyridine solution followed by removal of the protecting acetyl groups by base hydrolysis. Aliphatic acid chlorides do not provide the corresponding C-3 esters probably due to competing ketene formation followed by dimerization.

That esterification took place at the C-3 hydroxyl and not at the C-11 hydroxyl was confirmed by regeneration of the 9-ketone by treatment of the *m*-chlorobenzoate **9** with nitrous acid.[1] The resultant ketone exhibited a carbonyl band at 1695 cm^{-1}, the normal position for the erythromycin ketone band. It is reported that esterification of the 11-hydroxyl of erythromycin B causes the ketone absorption to shift to 1705–1708 cm^{-1} because of a decrease in hydrogen bonding.[7]