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Potential Radioprotective Agents—V. Melatonin Analogs. Oral Activity of *p*-Aminopropiophenone and its Ethylene Ketal

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Abstract—Seven new amides of 5-methoxytryptamine were synthesized and tested for radioprotective activity in mice. One of them, the heptafluorobutyramide **4**, is moderately active (57 % survivors), the rest demonstrate little or no activity. Of twelve compounds that had been found to exhibit high radioprotective activity by ip injection, only two [*p*-aminopropiophenone (**9**) and its ethylene ketal **8**] retain that high activity (92–95 % survivors) when administered orally. Three are moderately active: *p*-aminobenzonitrile (**10**, 55 %), 5-methoxytryptamine octanoic amide (**11**, 50 %), and *p*-aminobenzophenone (**12**, 48 %).

Introduction

Protection from radiation damage by a variety of compounds has been the subject of excellent reviews.[1,2] An extensive program sponsored by the U.S. Army Medical Research and Development Command produced a number of protective amino-thiol derivatives, most notably *S*-2-(3-aminopropylamino)ethyl phosphorothioic acid [$H_2N(CH_2)_3NH(CH_2)_2SPO_3H_2$, WR2721].[3] *In vivo*, WR2721 undergoes hydrolysis (dephosphorylation) to the free mercaptan, which is taken up by normal tissues more efficiently than by solid tumors.[4] It is currently undergoing clinical trials for use in both radiation therapy and chemotherapy.[5] Minor side effects that are somewhat limiting include nausea, vomiting, sneezing, allergic reactions and hypotension.[6] Thus, the search continues for effective radioprotectors for both civilian and military needs.[7] Recent developments include significant radioprotection by *N*-acyldehydroalanines,[8] zinc aspartate,[9] neo-mercazole,[10] leukotrienes,[7] dimethylaminodithiazines,[11] a mixture of five active agents,[12] ammonium trichloro(dioxyethylene-*O*-*O*'-)tellurate,[13] nitroxides,[14] (16*RS*)-15-deoxy-16-hydroxy-16-methylprostaglandin E_1, methyl ester,[15] and hyperthermia.[16]

In the study of a series of homologs of melatonin, we observed that the simple expedient of extending the chain length of the side-chain amide group from two carbons to six or eight carbons, more than doubled the radioprotective activity in mice irradiated with 6 mV photons produced by a linear accelerator.[17] It seemed possible that other structural modifications of the side-chain might also produce active compounds. Furthermore, there is a continuing search for oral activity among those compounds that demonstrate radioprotective activity by ip administration.

Results and Discussion

The new amides of 5-methoxytryptamine were easily prepared by treating a pyridine solution of the amine with the requisite acid chloride. They were assayed for radioprotective activity by ip injection into male, Swiss Webster mice, which were then irradiated with 950 cGy of 6 mV photons produced by a linear accelerator.[18] Only the heptafluorobutyramide **4** gave moderate protection, and the rest are unimpressive (see Table 1). These results may be compared with a similar experiment in which WR2721 gave 100 % protection when injected in aqueous solution, and 50 % protection when suspended in soybean oil.[19]

In addition to the hexanoic and octanoic amides of 5-methoxytryptamine mentioned earlier,[17] excellent radioprotective activity had been observed with substituted anilines,[20] and with an ethylene ketal.[21] The activity by oral administration of some of these compounds was determined, providing the results in Table 2. *p*-Aminopropiophenone (**9**) and its ethylene ketal **8** gave excellent protection (92–95 %); *p*-aminobenzonitrile (**10**), 5-methoxytryptamine octanoic amide (**11**), and *p*-aminobenzophenone (**12**) exhibited moderate activity (48–55 %), and the rest (**13–19**) showed little or no activity. The ethylene ketal **8** may be functioning as a pro-drug, as it would be expected to hydrolyze to *p*-aminopropiophenone under acidic conditions. The latter did not evidence the degree of toxicity here that is found when it is administered intraperitoneally at this high dosage (117 mg/kg).[22] It was tested for radioprotective activity by Fitzgerald *et al.* by ip injection at a much lower dosage, namely 30 mg/kg, where it gave 42 % survival.[23] Storer and Coon tested it also by ip injection at 20, 30, 40, and 50 mg/kg, and observed 45, 50, 60 and 72.5 % survivals, respectively.[24] However, neither group tested it by oral administration.

Table 1. Radioprotective activity of amides of 5-methoxytryptamine

No.	Name	Dosage, mg/kg	Survivors
4	Heptafluorobutyramide	416	12/21, 57%
2	2,5-Dichlorobenzenesulfonamide	412	8/21, 38%
7	p-Toluamide	332	8/21, 38%
1	p-Trifluoromethylbenzamide	390	4/18, 29%
3	3,4-Dichlorobenzamide	392	4/20, 20%
5	3,4-Methylenedioxybenzamide	365	3/19, 14%
6	p-Cyanobenzamide	344	1/21, 5%
	Control, soybean oil only		6/82, 7%

Male, Swiss Webster mice were injected ip with solutions of suspensions of 1.076 mequiv/kg of the test compounds in soybean oil 30 minutes before irradiation with 950 cGy of 6 mV photons. Thirty-day survivals are shown.

Table 2. Oral activity of radioprotectants

No.	Name	Dosage, mg/kg	Survivors
8	p-Aminopropiophenone ethylene ketal[20,21]	152	19/20, 95%
9	p-Aminopropiophenone[20,21]	117	36/39, 92%
10	p-Aminobenzonitrile[20]	92.7	11/20, 55%
11	5-Methoxytryptamine octanoic amide[17]	341	10/20, 50%
12	p-Aminobenzophenone[20,21]	154	19/40, 48%
13	p-Bromoaniline[20]	135	7/20, 35%
14	p-Nitroaniline[20]	109	5/20, 25%
15	1-(p-Aminophenyl)-1-propanol[22]	118.6	5/22, 23%
16	p-Aminoacetophenone[20]	106	1/21, 5%
17	2-Amino-5-chloropyridine[20]	101	0/20
18	5-Amino-2-chloropyridine[20]	101	0/20
19	5-Methoxytryptamine hexanoic amide[17]	311	0/20
	WR2721[19]	167	0/20
	Control, soybean oil only		2/80, 2.5%

Male, Swiss Webster mice were injected by gavage with solutions or suspensions of the test compounds in soybean oil (except for WR2721, which was dissolved in water) 30 minutes before irradiation with 950 cGy of 6 mV photons. Thirty-day survivals are shown.

Experimental

General

Chemicals were purchased from Aldrich Chemical Co., Milwaukee. ^{1}H NMR spectra were obtained on a Varian EM 390 Spectrophotometer. IR spectra were obtained on a Perkin–Elmer 237 Spectrophotometer. Melting points were taken on a Thomas–Hoover capillary melting point apparatus and are uncorrected. Chemical analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, and agreed to within 0.4 % of theoretical for the elements listed.

Synthesis

Method A. 5-Methoxy-N -(4'-trifluoromethylbenzoyl)-tryptamine (1). 4-Trifluoromethylbenzoyl chloride (1.00 g, 4.79 mmol) was added dropwise to a stirred solution of 829 mg (4.36 mmol) of 5-methoxytryptamine in 21 mL of

pyridine at room temperature. The solution was stirred for 6 h, diluted with ice water to turbidity, and refrigerated overnight. Filtering gave the crude product in 78 % yield. Recrystallization in ethanol–water gave the analytical sample, mp 140–142 °C; IR 3410, 3240, 1630 cm^{-1}; anal. $C_{19}H_{17}N_2F_3O_2$ (C, H, N).

Method B. N-*(2',5'-Dichlorobenzenesulfonyl)-5-methoxytryptamine (2).* 2,5-Dichlorobenzenesulfonyl chloride (270 mg, 1.10 mmol) was added gradually to a stirred solution of 190 mg (1.00 mmol) of 5-methoxytryptamine in 50 mL of pyridine at room temperature. The solution stood for 5 h and was then diluted with ice water, causing an oil to separate. The mixture was extracted 3 times with ether, and the ethereal solution was washed with 10 % HCl, and with aq. $NaHCO_3$, then dried over Na_2SO_4. Solvent evaporation left a liquid residue that was dissolved in CH_2Cl_2 and chromatographed on 18 g of Al_2O_3. Elution with 1–3 % ethanol in CH_2Cl_2 gave 257 mg (67 %) of crystalline product and recrystallization from acetone–water, and from ethanol–water gave the analytical sample, mp 107–108 °C; IR 3410, 3330, 1620 (w), 1580 (w), 1165 (st) cm^{-1}; anal. $C_{17}H_{15}N_2SCl_2O_2 \cdot H_2O$ (C, H, N, Cl).

N-*(3',4'-Dichlorobenzoyl)-5-methoxytryptamine (3).* Compound **3** was prepared by method A, 72 % and the analytical sample crystallized from $CH_3OH \cdot H_2O$, mp 136–137 °C; IR 3300–3200, 1630 cm^{-1}; anal. $C_{18}H_{16}N_2Cl_2O_2$ (C, H, N, Cl).

N-*(Heptafluorobutanoyl)-5-methoxytryptamine (4).* Compound **4** was prepared by method A, 57 % and the analytical sample crystallized from ethanol–water, mp 85–86 °C; IR 3350–3400, 1700 cm^{-1}; anal. $C_{15}H_{13}N_2O_2F_7$ (C, H, N).

5-Methoxy-N-*(3',4'-methylenedioxybenzoyl)tryptamine (5).* Compound **5** was prepared by method B, 43 %, eluted from alumina by 2–3 % ethanol in CH_2Cl_2 and the analytical sample crystallized from acetone–water, mp 104–106 °C; ^1H NMR (d-CCl_3) 3.00 (t, J = 6 Hz, 2H, $=CCH_2CH_2N$), 3.68 (t, J = 6 Hz, 2H, CH_2CH_2N), 3.75 (s, 3H, CH_3O), 5.90 (s, 2H, OCH_2O), 6.20 (br s, 1H, $CH_2NHC{=}O$), 6.6–7.3 (m, 7H, aromatic plus C=C*H*NH), 8.28 (s, 1H, C=CHN*H*); anal. $C_{19}H_{18}N_2O_4$ (N).

N-*(4'-Cyanobenzoyl)-5-methoxytryptamine (6).* Compound **6** was prepared by method A, 79 % and the analytical sample crystallized from ethanol–water, mp 176–178 °C; ^1H NMR (DMSO-d_6) 2.78 (t, 2H, $=CCH_2CH_2N$), 3.37 (t, 2H, CH_2CH_2N), 3.58 (s, 3H, CH_3O), 6.52 (broad s, 1H, CH_2NHCO), 6.9–7.9 (m, 8H, aromatic plus C=C*H*NH), 8.71 (broad s, 1H, C=CHN*H*); IR 3270, 3180, 2230, 1650 cm^{-1}; anal. $C_{19}H_{17}N_3O_2$ (H, N).

5-Methoxy-N-*(4'-methylbenzoyl)tryptamine (7).* Compound **7** was prepared by method A, 90 % and the analytical sample crystallized from ethanol–water, mp 100–101 °C; IR 3200, 1630 cm^{-1}; anal. $C_{19}H_{20}N_2O_2 \cdot 1/4H_2O$ (H, N).

Radiation-protective evaluation

Male, Swiss Webster ND4 mice were obtained from Harlan Industries, Indianapolis, weighing 21–24 g, and housed five to a cage. Compounds that were soluble in soybean oil were dissolved; those that did not dissolve were suspended by rapid stirring. The mice were injected ip or by gavage, and 30 min later they were placed in a cloth holder (in groups of 19–22 mice) which was taped to the treatment table of the instrument so that they were confined in a 20 cm^2 area 100 cm from the source. They were then irradiated over a period of approximately 5 min with 950 cGy of 6 mV photons produced by a Seimens Mevatron KD linear accelerator. Mice in the control group were injected with 0.2 mL of soybean oil.

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18. This is the most commonly used equipment for therapeutic radiation, and one of our future research projects is to use a selective radioprotective agent in a clinical setting.

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