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Potential Radioprotective Agents — IV. Schiff Bases

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Abstract—Twelve Schiff bases were prepared using salicylaldehyde, one with 5-chlorosalicylaldehyde, one with benzaldehyde, and a series of anilines substituted in the *m*- or *p*-positions. They were assayed for radioprotective activity in male, Swiss mice irradiated with a nearly lethal dose (950 cGy) of 6 mV photons produced by a linear accelerator, and were compared with the parent amines. Schiff base formation reduced toxicity of the parent amines; its effect on radioprotective activity was erratic, increasing activity in some cases, decreasing activity in others, and having no effect in still others. Radioprotective activity appears to be unrelated to a number of molecular descriptors. The highest radioprotection (100 %) was observed for mixtures of *p*-aminopropiophenone with its Schiff base, or with the Schiff base of 1-(*p*-aminophenyl)-1-propanol (95 %).

Introduction

The revelation of wide-spread exposure to radiation from uranium mining and processing,[1] as well as the possibility of nuclear accidents such as those at Three Mile Island, Chernobyl, and Goiania[2] continue to emphasize the need for compounds that afford protection in response to emergency as well as to military needs.[3] A further potential application of radioprotective agents is in the radiation therapy of cancer where the agent selectively protects healthy cells vs. cancer cells.

Compounds demonstrating radioprotective activity in mice when injected intraperitoneally include substituted anilines, amides of 5-methoxytryptamine, and aminoketones such as *p*-aminopropiophenone.[4-6] In our hands, the acute toxicity of *p*-aminopropiophenone was ameliorated by converting it to the corresponding Schiff base with salicylaldehyde.[4] In the present study other amines, some of them very toxic, were converted to Schiff bases, which were then assayed for radioprotective activity in mice.

Results and Discussion

Salicylaldehyde Schiff bases were chosen for this study because of their relative stability, brought about by intramolecular hydrogen bonding,[7] as shown in Figure 1.

Apparent toxicity of the Schiff bases was *estimated* from survival data collected in the radiation protective assay.

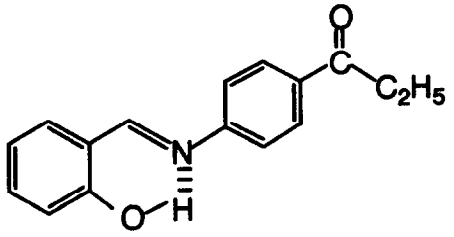


Figure 1. Stabilization of Schiff base **2** by H-bonding.

As illustrated in Figure 2, mice in the control group die in the period 5–14 days post radiation. Consequently, it is assumed that those mice treated with a test compound, and which die in the first four days, die (at least primarily) from the toxicity of the compound, and not from radiation. For example, only 60 % of the mice treated with 4-aminobenzotrifluoride (the precursor to Schiff base **8**) were alive on day 5. Also, 67 % of mice treated with 2-(*p*-nitrophenyl)ethyl amine (precursor to Schiff base **13**) survived to day 5. Mice which died on

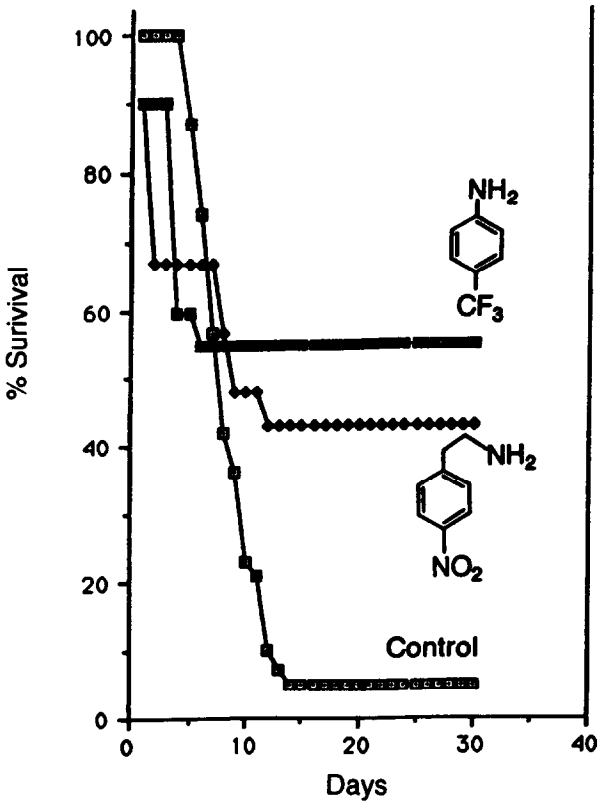


Figure 2. Survival of mice following irradiation..

Table 1. Radioprotective activity of mixtures

Test Mixture	Survivors[a]
Mixture of *p*-Aminopropiophenone and **2**, 56-44[b]	19/21, 90%
Mixture of *p*-Aminopropiophenone and **2**, 70-30[c]	21/21, 100%
Mixture of *p*-Aminopropiophenone and **6**, 70-30[c]	21/21, 95%

[a]Male, Swiss mice were injected with warm solutions of the mixtures in soybean oil at a dose level of 198 mg/kg 30 min before irradiation with 950 cGy of 6 mV photons.
None of the control mice (soybean oil only) survived.
[b]Dose of *p*-amino-propiophenone, 86 mg/kg.
[c]Dose of *p*-aminopropiophenone, 60 mg/kg.

day 5 or later were assumed to have died from radiation, though in some instances, toxicity could have been a contributing factor. All of the Schiff bases are relatively non-toxic in that 95–100 % of the mice survive to day 5. Ten of the compounds (**2–5, 7, 8, 11–14**) appear to be less toxic than their corresponding amines; (the other amines are themselves non-toxic).

With respect to radioprotective activity, only Schiff base **11** (prepared from benzaldehyde and 4-aminochalcone) performed well (71 % survival), five others (**1, 3, 6, 9,** and **14**) exhibited moderate activity (40–55 %), while the rest demonstrated little or no activity. Only two of the Schiff bases, **9** and **14**, performed better than their amine precursors. Amines that are precursors to bases **1-4** and **8** have been assayed for radioprotective activity at dosages different from those employed here.[8]

To understand these results, an attempt was made to regress the biological response onto the following molecular descriptors, all of which are calculated: ionization potential, energy of the lowest unoccupied molecular orbital (LUMO), the energy gap between the highest occupied molecular orbital and the LUMO, heat of formation, the difference in heats of formation between the neutral and radical ion, dipole moment, nonpolar saturated surface area, nonpolar unsaturated surface area, polar surface area, total surface area, and percentage polar surface area.[6] We found no statistically significant relationship between radioprotective activity of the Schiff bases and any of the molecular descriptors.

While the Schiff bases *per se* were not highly radioprotective, mixtures of *p*-aminopropiophenone with Schiff base **2** or with Schiff base **6** were excellent protectors (Table 1). The 70:30 mixtures contain *p*-aminopropiophenone at a dosage of 60 mg/kg; alone at that level, a protection of 86 % was obtained for *p*-aminopropiophenone,[9] while the mixtures gave 95–100 %. Additional assays are required to establish whether or not that difference is significant.

Experimental

General

Chemicals were purchased from Aldrich Chemical Co., Milwaukee. 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.

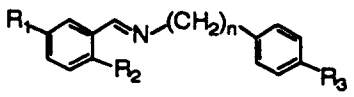
Synthesis of Schiff bases. The following representative procedure was followed for all the Schiff bases, with appropriate minor variations. Salicylaldehyde (0.70 mL, 6.57 mmol) was added dropwise to a stirred solution of 4-aminobenzotrifluoride (0.80 mL, 6.37 mmol) in 5 mL of ethanol and 0.1 mL of a solution made by dissolving 3.08 g of KOH in 20 mL of ethanol and 12 mL of H_2O. The solution was stirred for 10 min, then evaporated overnight in an open dish. The residue was recrystallized in ethanol to give the product (**8**) in 79 % yield.

Most of the Schiff bases (listed in Table 2) were recrystallized from ethanol or ethanol–water. The bis-compound (**9**), made from 4,4'-diaminobenzophenone and a 2:1 mole ratio of salicylaldehyde, was recrystallized from DMSO–ethanol–H_2O.

Synthesis of 1-(p-aminophenyl)-1-propanol. Sodium borohydride (2.00 g) was added gradually to a warm, stirred solution of *p*-aminopropiophenone (2.00 g) in 40 mL of ethanol. The mixture was stirred at reflux for 2 h, then evaporated under vacuum. The residue was extracted three times with methylene chloride (50 mL); the combined extracts were diluted with petroleum ether and refrigerated to give the crude product in two crops: 1.34 g. Two recrystallizations from CH_2Cl_2–petroleum ether gave the analytical sample of 1-(*p*-aminophenyl)-1-propanol (precursor of Schiff base **6**), mp 62–63°. Anal. Calcd for $C_9H_{13}NO$: 71.49 % C; 8.66 % H; 9.27 % N. Found: 71.58 % C; 8.69 % H; 9.04 % N.

Synthesis of 4-aminochalcone and its Schiff base. Benzaldehyde (456 mg, 4.3 mmol) was added dropwise to a suspension of 580 mg (4.3 mmol) of *p*-aminoacetophenone in 3.2 mL of the KOH solution (above). The mixture was stirred 5 h, during which time a gum separated. The mixture was extracted with CH_2Cl_2, and that solution was dried over $MgSO_4$ and evaporated, leaving 943 mg of residue. Three recrystallizations in ethanol gave the analytical sample of Schiff base **11** (204 mg). Evaporation of the filtrates left a residue that

Table 2. Toxicity and radioprotective activity of Schiff cases



	R_1	R_2	n	R_3	mp	Analysis[a]	Survival (%)[b]
1	H	OH	0	$C(O)CH_3$	102-103°	CHN	11/21 (52)
2	H	OH	0	$C(O)C_2H_5$	120-122°	CHN	10/63 (16)
3	H	OH	0	$C(O)C_3H_7$	114-115°	CHN	10/21 (48)
4	H	OH	0	$C(O)C_5H_{11}$	80-82°	HN[c]	0/20 (0)
5	H	OH	0	OCF_3	69-70°	CHN[d]	5/22 (23)
6	H	OH	0	$CH(OH)C_2H_5$	77-78°	CHN	12/22 (55)
7	H	OH	0	SH	109-111°	CHN[e]	0/21 (0)
8	H	OH	0	CF_3	99-103°	CHN[f]	1/21 (5)
9	H	OH	0	$C(O)C_6H_4N{=}CHC_6H_4OH$	258-259°	CHN	8/20 (40)
10	H	OH	0	H, 3-Cl	93-95°	CHN[g]	3/42 (7)
11	H	H	0	$C(O)CH{=}CHC_6H_5$	143-145°	CHN	15/21 (71)
12	Cl	OH	0	$C(O)C_2H_5$	190-193°	CHN	5/21 (24)
13	H	OH	2	NO_2	130-131°	CHN	1/19 (5)
14	H	OH	0	H, 3-NO_2	126-127°	CHN	11/21 (52)

[a]Analyses for elements shown agree with calculated values within 0.4 %; analyses for other elements shown below.
[b]Male, Swiss mice were injected with suspensions or solutions of the test compound in soybean oil at a dose level of 0.78 mequiv/kg (except for 11), 30 minutes before irradiation with 950 cGy of 6 mV photons; 11 was tested at a dose level of 0.56 mequiv/kg. Ten out of 182 mice in the control group (soybean oil only) survived: 5 %.
[c]Calculated: 79.71 % C; found: 78.56 % C.
[d]Calcd: 20.27 % F; found: 19.43 % F.
[e]Calcd: 13.98 % S; found: 13.45 % S.
[f]Calcd: 21.49 % F; found: 20.87 % F.
[g]Calcd: 15.30 % Cl; found: 15.98 % Cl.

was chromatographed on silica gel; elution by 7 % ethanol in CH_2Cl_2 gave 515 mg of 4-aminochalcone. Two recrystallizations in ethanol–H_2O gave the analytical sample, mp 84–86°. Anal. Calcd for $C_{15}H_{13}NO$: 80.69 % C; 5.87 % H; 6.27 % N. Found: 79.86 % C; 5.95 % H; 6.20 % N.

p-*Aminobutyrophenone (precursor to Schiff base 3).* Synthesized by rearrangement of *N*-phenylbutanoic amide in polyphosphoric acid.[10] *N*-Phenylbutanoic amide (amide NH, 3240 cm^{-1}, amide C=O, 1645 cm^{-1}, mp 75–78°, 6.00 g) was stirred in 62 g of polyphosphoric acid at 190–200° for 30 min, then allowed to cool. At 100° water (25 mL) was added, and the mixture was stirred 30 min at 100–120°, then cooled to room temperature. The solution was diluted with 300 mL of water, acidified with 21 mL of conc. HCl, and gravity filtered. The filtrate was made basic (pH 9) with conc. NH_4OH, and extracted with three 150 mL portions of ether. The ethereal solution was dried over Na_2SO_4 and evaporated to give 4.04 g of crude product. The brown solid was recrystallized twice from CH_2Cl_2–hexane to give the analytical sample mp 92–93° (amine NH, 3410, 3330, 3210, ketone C=O, 1640 cm^{-1}). Anal. Calcd for $C_{10}H_{13}NO$: 73.59 % C; 8.03 % H; 8.58 % N. Found: 73.16 % C; 8.18 % H; 8.35 % N.

p-*Aminocaprophenone (precursor to Schiff base 4)*. Prepared similarly from N-phenylhexanoic amide.

Radiation-protective evaluation.

Male, Swiss ND4 mice (22–25 g) were obtained from Harlan Industries (Indianapolis) and housed five to a cage. Thirty minutes before irradiation with 950 cGy of 6 mV photons produced by a linear accelerator, they were injected intraperitoneally with a solution or suspension of the test compound in soybean oil at a dose level of 0.78 mequiv/kg. The control group was given soybean oil; most of them died 5–14 days post injection. The values reported in the last two columns of Table 2 represent 30-day survival.

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