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In Vitro Studies with Methylproamine: A Potent New Radioprotector

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ABSTRACT

New analogues of the minor groove binding ligand Hoechst 33342 have been investigated in an attempt to improve radioprotective activity. The synthesis, DNA binding, and *in vitro* radioprotective properties of methylproamine, the most potent derivative, are reported. Experiments with V79 cells have shown that methylproamine is ~100-fold more potent than the classical aminothiol radioprotector WR1065. The crystal structures of methylproamine and proamine complexes with the dodecamer d(CGCGAATTCGCG)$_2$ confirm that the new analogues also are minor groove binders. It is proposed that the DNA-bound methylproamine ligand acts as a reducing agent by an electron transfer mechanism, repairing transient radiation-induced oxidizing species on DNA.

INTRODUCTION

In the early 1970s, the Hoechst company synthesized a wide range of bibenzimidazoles in a program aimed at the development of improved antihelmintics (1). As outlined in a recent review (2), it soon was established that these compounds also could be used as fluorescent DNA stains in a variety of cytologic techniques, with principal attention focused on the two analogues Hoechst 33342 and Hoechst 33258 (Fig. 1). X-ray crystallography (3, 4) studies of complexes with synthetic oligonucleotides confirmed early evidence from DNA binding studies that the ligands are minor groove binders with strong AT selectivity (5). The unexpected radioprotective activity of Hoechst 33342 in cultured cells was first reported in 1984 (6), subsequently confirmed (7, 8), and extended with the demonstration of *in vivo* radioprotection of mouse lung after i.v. administration (9). The potential use of radioprotectors in cancer radiotherapy prompted us to investigate the mechanism of the radioprotective activity of Hoechst 33342 and led to the hypothesis that electron donation from the ligand to damaged DNA contributed to the radioprotective effect (2). Accordingly, we synthesized the bibenzimidazole analogue in which the ethoxy substituent of Hoechst 33342 was replaced with the more powerful electron-donating *N*,*N*-dimethylamino group. This compound (proamine) provided greater radioprotection to cultured cells than Hoechst 33342 (9). We now report the synthesis and evaluation of methylproamine (Fig. 1), a new derivative that displays additional improvement in radioprotection.

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Notes: M. E. Reum is deceased. C. J. Squire is currently at the School of Biological Sciences, University of Auckland, Auckland, New Zealand. C. Clark is now at Alchemica Pty Ltd, Brisbane Technology Park, Queensland, Australia. Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org).

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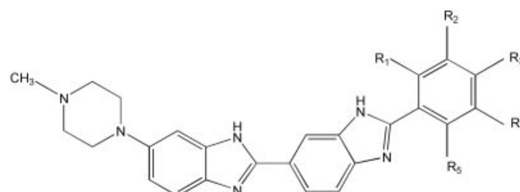

Fig. 1. Molecular structures of bibenzimidazoles. The substituents for Hoechst 33258 are R_3 = OH; Hoechst 33342, R_3 = OCH_2CH_3; and proamine, R_3 = $N(CH_3)_2$; and for methylproamine, R_1 = CH_3 and R_3 = $N(CH_3)_2$.

As well as carrying out DNA drug-binding studies and *in vitro* radioprotection experiments, we have determined the crystal structures of proamine and methylproamine complexed to the self-complementary dodecamer d(CGCGAATTCGCG)$_2$.

MATERIALS AND METHODS

Synthesis and DNA Binding. Methylproamine was synthesized according to the general procedures described previously (10, 11). The details are available as supplementary data.

DNA binding parameters (K_d, dissociation constant; *N*, number of binding sites) of the ligands were determined by exploiting the difference in fluorescence intensity between the free and DNA-bound forms, and nonlinear regression analysis of the measured fluorescence intensities as a function of DNA and ligand concentrations. The DNA used was a 20-mer oligodeoxynucleotide denoted HBC1: 5′-CAGCACGGAATTCGCAGGAC-3′ and 3′-GTCGTGCCTTAAGCGTCCTG-5′.

The concentration of stock solutions of the ligands was determined spectrophotometrically in the solvent comprising 0.1% trifluoroacetic acid in 45% methanol in water (v/v). The extinction coefficients ($M^{-1}cm^{-1}$) for methylproamine, proamine, and Hoechst 33342 were 39,300 (389 nm), 54,000 (400 nm), and 40,300 (349 nm), respectively.

X-ray Crystallography. The two crystal structures were determined using standard macromolecular crystallographic techniques. Important crystal data are given in Table 1. Full details of the crystallography are available as supplementary data, including the crystallization conditions (Supplementary Table SD1), a complete summary of data collection and refinement (Supplementary Table SD2), and the inter-ring torsion angles (Supplementary Table SD3). Atomic coordinates and structure factors have been deposited in the Nucleic Acid Database[5] with identity codes DD0060 (proamine) and DD0059 (methylproamine).

In Vitro Radioprotection Experiments

Drug Solutions. The purity of proamine and methylproamine was confirmed by high-performance liquid chromatography before use. Hoechst 33258 and 33342 were obtained pure from Sigma-Aldrich (St. Louis, MO). Stock solutions were prepared in 10 mM acetic acid in 50% methanol (v/v), and concentrations were determined spectroscopically using the extinction coefficients (determined in-house) given in the DNA-binding section. Aliquots of each stock solution were dispensed into 1.5-ml Eppendorf tubes, lyophilized, and stored as dried pellets.

Cell Cultures. V79 hamster fibroblasts were passaged as monolayers in α-MEM with 10% FCS. Cells for survival experiments were set up in 25-ml

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Table 1 *Crystal data for proamine and methylproamine complexes with d(CGCGAATTCGCG)$_2$*

	Proamine	Methylproamine
Cell parameters (Å)		
a	25.31	25.44
b	40.40	40.64
c	65.92	66.35
Space group	$P2_12_12_1$	$P2_12_12_1$
Final R factor	0.214	0.232

flasks so that they were in log-phase growth with a target density of 2×10^6 cells per flask at the time of drug treatment and irradiation. The lyophilized drug pellets were dissolved in 10 mM acetic acid in 50% methanol (v/v), diluted into medium, and added to the cultures. Because drug uptake depends on cell density as well as nominal drug concentration, the volume of the medium was adjusted to 5 ml/2×10^6 cells based on cell counts from replicate flasks. The concentration of the drug stocks was such that the final concentration of methanol did not exceed 0.5%. No-drug controls treated with the appropriate amounts of the vehicle were included in some experiments, but separate studies demonstrated that such inclusion had no effect on viability or radiosensitivity.

Irradiation and Assay of Clonogenic Survival. Drug-treated cultures and the appropriate controls were maintained at 37°C for 2 h and transferred to polystyrene boxes just before irradiation (^{137}Cs-GammaCell 40 irradiator; Nordion International, Inc., Kanata, Canada; dose rate of 0.68 Gy/min). During the longest irradiation time (~20 min), the temperature decreased to 33°C. Immediately after irradiation, the cultures were returned to the 37°C incubator for an additional period; therefore, the total duration of drug exposure was 3 h. They then were plated out for clonogenic survival by standard methods. Clonogenic survival was calculated relative to that for nonirradiated drug-treated cultures. The drug-only survival was >85%; the detailed results for the effect of drug concentration on survival are available as supplementary data (Supplementary Fig. SD3).

RESULTS AND DISCUSSION

DNA Binding. The bibenzimidazole ligands (exemplified by Hoechst 33258) have been shown by affinity cleavage (12), foot printing (13) and X-ray crystallographic studies (8) to bind at discrete sites in the minor groove characterized by 3–4 consecutive AT bp. Equilibrium binding studies have established that the dissociation constants (K_d) for the high affinity sites generally range from 1–100 nM, depending on conditions. For example, ionic strength has a significant effect on the binding affinity of substituted Hoechst derivatives because the ligands are positively charged (14). Accordingly, the results of the present study (Table 2) are compared with data from previous studies that involved the use of buffers and NaCl salt solutions at ~0.1 M.

The inclusion of alcohol in the buffer system obviates problems with adsorption of the ligands to glassware, but it also has an impact on binding affinity to DNA, as illustrated by the data in Table 2. For example, addition of ethanol to 25% increases K_d by a factor of ~70 (Table 2, *rows 7* and *8*), and increasing it from 10% to 20% approximately doubles the K_d (*rows 3* and *4*). Although the K_d in the last two entries of Table 2 is higher than may be expected from the other data, one can reasonably conclude that the affinities of these two ligands are similar. More generally, considering the effects of alcohol, the data in Table 2 indicate that the DNA binding affinities of proamine, methylproamine, Hoechst 33258, and Hoechst 33342 are of a similar order of magnitude (*e.g.,* in the range of 40–200 nM in 10% ethanol).

One notable and unexpected feature of the binding data for proamine compared with methylproamine is the binding site size. Because the HBC1 20-mer only has a single binding site, one would expect the value of N to be 0.05 bp^{-1}. The observed value for proamine is 0.098, whereas that for methylproamine is much closer to the expected value of 0.05. One possible interpretation for this difference is that the 20-mer associates with two proamine molecules but only one methylproamine. It may be that in solution two proamine molecules can be accommodated side by side in the minor groove, as has been found recently in crystal structures of, for example, distamycin/d(GTATATAC)$_2$, but there is no other evidence to support this speculation (15). In the case of methylproamine, the *ortho*-methyl group may prevent such a side-by-side dimer conformation.

X-ray Crystallography

DNA Structure and Ligand-Binding Sites. The DNA in both structures adopts a B-type conformation and packs within the crystal in an analogous pattern to the native DNA and other dodecamer/ligand complexes that crystallize in the same $P2_12_12_1$ space group. The binding sites of proamine and methylproamine are similar and cover ~5 bp over the 5′-AATTC sequence. The methyl group projects out of the groove. The piperazine ring systems are located at the C9 end of the binding site where the minor groove widens. The structure of the methylproamine complex is shown in Fig. 2

Ligand Conformation. Proamine and methylproamine have almost identical binding sites, hydrogen bonding interactions, and van der Waals contacts. The ligand NH donors form bifurcated hydrogen bonds to acceptor atoms on the floor of the groove. Despite the similar ligand-binding sites in the proamine and methylproamine complexes, the inter-ring torsion angles of the ligands are different. In the proamine ligand, the torsion angle between the central benzimidazoles is 24° and that between the benzimidazole and phenyl ring is 17°. In the methylproamine complex, the corresponding angles are 8° and 20°. Nevertheless, whereas the individual torsion angles differ significantly among these and similar complexes, the sums of the angles in each complex are confined to a relatively narrow range because of the requirement that the whole ligand must fit the groove in an isohelical manner (3, 4). The individual proamine/methylproamine inter-ring torsion angles will be influenced to varying extents by the bulk of the NMe_2 group, partial charge delocalization between phenyl and the

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Table 2 *DNA binding parameters for methylproamine and related ligands*

The data from referenced sources were originally given as association constant $K_{ass} = 1/K_d$ (rows 5, 6, 9, and 10) and the number of bp per binding site (rows 5–8). These data have been recalculated and presented as dissociation constant and number of binding sites per bp. The sequence for the 20-mer HBC1 is given in "Materials and Methods"; the 12-mer sequences are self-complementary, namely, d(CCGGAATTCCGG)$_2$ for 12-mer-A and d(CGCGAATTCGCG)$_2$ for 12-mer-B.

Row	Ligand	DNA	Reference	NaCl, nM	EtOH, %	K_d, nM	N, bp^{-1}
1	Hoechst 33342	20-mer (HBC1)	This study	100	10	42.3	0.085
2	Proamine			100	10	114	0.098
3	Methylproamine			100	10	124	0.064
4	Methylproamine			100	20	250	0.075
5	Hoechst 33258	12-mer-A	14	100	0	3.1	0.083
6	Hoechst 33258	12-mer-B		100	0	2.5	0.083
7	Hoechst 33258	Calf thymus DNA	14	100	0	3.3	0.033
8	Hoechst 33258			100	25	230	0.029
9	Hoechst 33258	Calf thymus DNA	23	140	1.3	38	0.056
10	Hoechst 33342			140	1.3	35	0.056

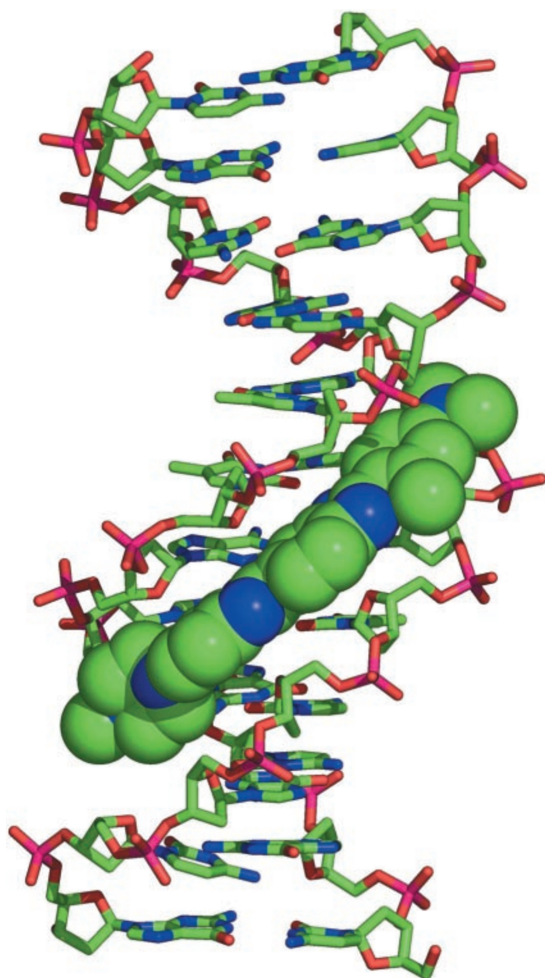

Fig. 2. Crystal structure of methylproamine/d(CGCGAATTCGCG)$_2$. Molecular model of the complex generated from the crystal structure coordinates. For the sake of clarity, hydrogen atoms are not shown in either the DNA or the ligand.

NMe_2 substituent, and the binding site location imposed by the NMe_2 substituent. The presence of the *ortho*-methyl group of methylproamine increases the phenyl-benzimidazole ring torsion angle to minimize any phenyl-benzimidazole steric clashes with the walls of the minor groove, which influences the relative orientation of the two benzimidazoles. The significantly smaller torsion value for the methylproamine benzimidazole-benzimidazole linkage also may indicate an increase in electron density within the benzimidazole moieties caused by electron donating from the *ortho*-methyl group.

The impact of the *ortho*-methyl group of methylproamine on the phenyl-benzimidazole torsion angle was evaluated by a conformational search using the SYBYL molecular modeling program (Tripos Associates, St. Louis, MO) and the AMBER force field (UCSF Kollman Group, San Francisco, CA). The results are available as supplementary data (Supplementary Fig. SD1). The analysis shows that the *ortho*-methyl group severely restricts rotation of the phenyl ring because of intramolecular and intermolecular effects but interestingly indicates that there is a narrow window of torsion angles at ~205°, in which the methyl group of methylproamine could be accommodated inside the minor groove.

Radioprotection by Methylproamine *in Vitro*. The well-established radiosensitization by electron-affinic nitroimidazoles, as well as the fact that one of the early mechanisms proposed for sensitization by nitroimidazoles was suppression of recombination of electrons and holes in irradiated DNA, provided the initial basis for the hypothesis that electron donation might contribute to radioprotection by Hoechst 33342 (16). This lead us to synthesize the bibenzimidazole analogue, in which the ethoxy group of Hoechst 33342 was replaced with the more strongly electron-donating *N*,*N*-dimethylamino group. This compound (proamine) proved to be a more active radioprotector of cultured cells than Hoechst 33342 (9). The same assay system was used to investigate methylproamine, and the survival curves for V79 cells show that there is an additional marked improvement in radioprotective activity (Fig. 3). The drug is present before (for 2 h), during, and after irradiation. Separate experiments established that there is no radioprotective effect from drug exposure only after irradiation (data not shown). From the data in Fig. 3, methylproamine at a concentration of only 30 μM confers a dose-modifying factor of 2.1 at the 10% survival level. (The dose-modifying factor is defined as the ratio of radiation doses in the protected and control situations that yield the same cell kill.)

The potency of methylproamine compared with other radioprotectors also is evident from the data summarized in Table 3.

In a cell culture experiment with Chinese hamster ovary cells, comparable with the methylproamine experiments described here, a dose-modifying factor of 1.9 was reported for 4 mM WR1065 (17). A more recent article reported a dose-modifying factor of 1.5 for radioprotection of V79 cells by 4 mM WR1065 (18). Thus, methylproamine is at least 100 times more potent than WR1065 (the active metabolite of WR2721) for the V79 cell line.

The data in Table 3 also demonstrate the improved radioprotective potency of methylproamine compared with other bibenzimidazoles,

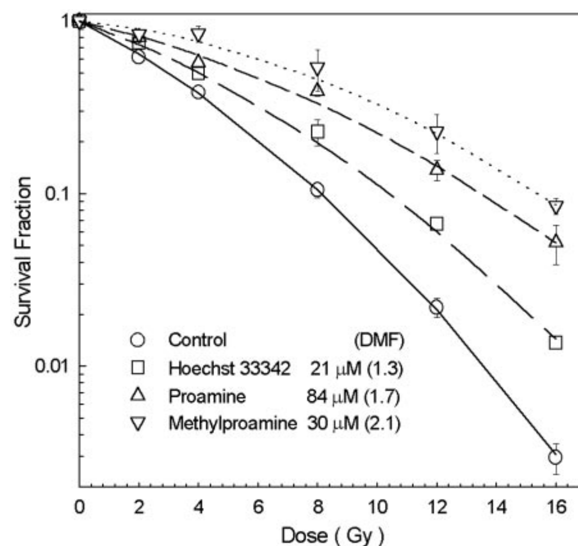

Fig. 3. Survival curves for cultured V79 cells. The cells were treated with the indicated concentrations of the radioprotectors for 2 h before and during irradiation, for a total of 3 h. Clonogenic survival was calculated relative to the corresponding drug-only control. The dose-modification factors (*DMF*) were calculated from the radiation doses corresponding to 10% survival.

Table 3 *Radioprotection of cultured cells*

Cell line	Radioprotector	Concentration	Dose-modification factor	Reference
HT29	Hoechst 33342	8.7 μM	1.5	6
KHT	Hoechst 33342	10 μM	1.8	7
V79	Hoechst 33342	21 μM	1.3	2
V79	Proamine	84 μM	1.7	2
V79	Methylproamine	30 μM	2.1	Fig. 3
CHO	WR1065	4 mM	1.9	17
V79	WR1065	4 mM	1.5	18
Human kidney	Cysteamine	2 mM	1.4	24
V79	Dithiothreitol	10 mM	1.4	25

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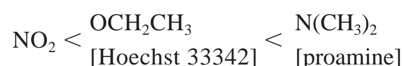
and this difference additionally is emphasized in the results of studies of the effect of drug concentration on the clonogenic survival of cells exposed to 12 Gy irradiation and unirradiated controls (Supplementary Fig. SD3). However, those results also reveal a relatively low concentration window between radioprotective activity and cytotoxicity for methylproamine and Hoechst 33342, although proamine would appear to be less cytotoxic, at least for V79 cells.

The underlying hypothesis connecting radioprotection with electron donation was investigated by synthesis and evaluation of the *para*-nitro analogue (Fig. 1; $R_3 = NO_2$) in the cell culture system. Inclusion of the nitro compound at a concentration of 20 μM in cultures irradiated at 12 Gy reduced survival twofold compared with the radiation-only control (data not shown). In other words, this nitro-bibenzimidazole is in fact a radiosensitizer. Thus, the structure-activity relationship for *in vitro* radioprotection in the series of ligands with different *para*-substituents is



$$NO_2 < \underset{[\text{Hoechst 33342}]}{OCH_2CH_3} < \underset{[\text{proamine}]}{N(CH_3)_2}$$

This establishes the trend of increasing radioprotection with increasing electron richness in the phenyl ring of the ligand, which is consistent with the hypothesis that at least a component of the radioprotection is caused by electron donation (reduction) from the DNA-bound ligand to the transient radiation-induced oxidizing species on DNA. The results of pulse radiolysis experiments with methylproamine and Hoechst 33342 add additional support to this hypothesis, with evidence for intramolecular oxidation of DNA-bound ligands (19). The addition of the *ortho*-methyl group to proamine to form methylproamine may simply be an extension of the structure-activity series indicated previously, or there could be different reasons for the improved radioprotective activity of methylproamine over proamine, perhaps stemming from the restriction of the rotation around the benzimidazole-phenyl linkage.

The potency of methylproamine and proamine radioprotection is remarkable. It has been established clearly from studies with amino thiols that radioprotective efficacy is better for positively charged analogues (20), and this can be attributed to enhanced DNA association, particularly to the regions of high electronegativity (21). However, it is not clear whether the potency of methylproamine (and bibenzimidazoles generally) is simply because of overt targeting to DNA or because of more subtle differences in the radioprotective mechanism.

Regardless of mechanistic considerations, it is clear that methylproamine has a potential use as a radioprotector in cancer radiotherapy. There are a number of situations in which normal tissues in the vicinity of a tumor are either dose limiting or subject to troublesome acute reactions. In many of these cases, the normal tissue at risk (*e.g.,* rectal mucosa, oral mucosa, or skin) would be accessible to topical application of a radioprotector, with the prospect of limiting systemic delivery by formulation design. However, previous attempts to protect skin (*e.g.,* by topical application of WR2721) have been unsuccessful, the failure attributed to poor penetration through the stratum corneum (22). In this context, the high potency of methylproamine could confer considerable advantages over existing radioprotectors because much lower concentrations are required for it to achieve the desired level of radioprotection.

REFERENCES

1. Loewe, H., and Urbanietz, J. [Basic-substituted 2,6-bisbenzimidazole derivates, a novel class of substances with chemotherapeutic activity]. Arzneimittelforschung, *24:* 1927–1933, 1974.
2. Martin, R. F. DNA-binding bisbenzimidazoles as radioprotectors. *In:* E. A. Bump and K. Malaker (eds.), Radioprotectors. Chemical, Biological and Clinical Perspectives, pp. 151–166. New York: CRC, 1998.
3. Pjura, P. E., Grzeskowiak, K., and Dickerson, R. E. Binding of Hoechst 33258 to the minor groove of B-DNA. J. Mol. Biol., *197:* 257–271, 1987.
4. Teng, M. K., Usman, N., Frederick, C. A., and Wang, A. H. The molecular structure of the complex of Hoechst 33258 and the DNA dodecamer d(CGCGAATTCGCG). Nucleic Acids Res., *16:* 2671–2690, 1988.
5. Zimmer, C., and Wahnert, U. Nonintercalating DNA-binding ligands: specificity of the interaction and their use as tools in biophysical, biochemical and biological investigations of the genetic material. Prog. Biophys. Mol. Biol., *47:* 31–112, 1986.
6. Smith, P. J., and Anderson, C. O. Modification of the radiation sensitivity of human tumour cells by a bis-benzimidazole derivative. Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med., *46:* 331–344, 1984.
7. Young, S. D., and Hill, R. P. Radiation sensitivity of tumour cells stained *in vitro* or *in vivo* with the bisbenzimide fluorochrome Hoechst 33342. Br. J. Cancer, *60:* 715–721, 1989.
8. Denison, L., Haigh, A., D'Cunha, G., and Martin, R. F. DNA ligands as radioprotectors: molecular studies with Hoechst 33342 and Hoechst 33258. Int. J. Radiat. Biol., *61:* 561, 1992.
9. Martin, R. F., Broadhurst, S., D'Abrew, S., Budd, R., Sephton, R., Reum, M., and Kelly, D. P. Radioprotection by DNA ligands. Br. J. Cancer Suppl., *27:* S99–101, 1996.
10. Kelly, D. P., Bateman, S. A., Martin, R. F., Reum, M. E., Rose, M., and Whittaker, A. R. D. Synthesis and characterization of boron-containing bibenzimidazoles related to the minor groove binder, Hoechst 33258. Aust. J. Chem., *47:* 247–262, 1994.
11. Kelly, D. P., Bateman, S. A., Hook, R. J., Martin, R. F., Reum, M. E., Rose, M., and Whittaker, A. R. D. DNA binding compounds VI. Synthesis and characterization of 2, 5′-disubstituted bibenzimidazoles related to the DNA minor groove binder, Hoechst 33258. Aust. J. Chem., *47:* 1751–1769, 1994.
12. Martin, R. F., and Holmes, N. Use of an 125I-labelled DNA ligand to probe DNA structure. Nature (Lond.), *302:* 452–454, 1983.
13. Harshman, K. D., and Dervan, P. B. Molecular recognition of B-DNA by Hoechst 33258. Nucleic Acids Res., *13:* 4825–4835, 1985.
14. Loontiens, F. G., Regenfuss, P., Zechel, A., Dumortier, L., and Clegg, R. M. Binding characteristics of Hoechst 33258 with calf thymus DNA, poly[d(A-T)], and d(CCGGAATTCCGG): multiple stoichiometries and determination of tight binding with a wide spectrum of site affinities. Biochemistry, *29:* 9029–9039, 1990.
15. Mitra, S. N., Wahl, M. C., and Sundaralingam, M. Structure of the side-by-side binding of distamycin to d(GTATATAC)2. Acta Crystallogr. D. Biol. Crystallogr., *55* (Pt 3)*:* 602–609, 1999.
16. Adams, G. E., and Cooke, M. S. Electron-affinic sensitization. I. A structural basis for chemical radiosensitizers in bacteria. Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med., *15:* 457–471, 1969.
17. Murray, D., Prager, A., and Milas, L. Radioprotection of cultured mammalian cells by the aminothiols WR-1065 and WR-255591: correlation between protection against DNA double-strand breaks and cell killing after gamma radiation. Radiat Res, *120:* 154–163, 1989.
18. Grdina, D. J., Murley, J. S., and Kataoka, Y. Radioprotectants: current status and new directions. Oncology, *63* (Suppl 2)*:* 2–10, 2002.
19. Martin, R. F., and Anderson, R. F. Pulse radiolysis studies indicate that electron transfer is involved in radioprotection by Hoechst 33342 and methylproamine. Int. J. Radiat. Oncol. Biol. Phys., *42:* 827–831, 1998.
20. Zheng, S., Newton, G. L., Gonick, G., Fahey, R. C., and Ward, J. F. Radioprotection of DNA by thiols: relationship between the net charge on a thiol and its ability to protect DNA. Radiat. Res., *114:* 11–27, 1988.
21. Sy, D., Hugot, S., Savoye, C., Ruiz, S., Charlier, M., and Spotheim-Maurizot, M. Radioprotection of DNA by spermine: a molecular modelling approach. Int. J. Radiat. Biol., *75:* 953–961, 1999.
22. Lamperti, A., Ziskin, M. C., Bergey, E., Gorlowski, J., and Sodicoff, M. Transdermal absorption of radioprotectors in the rat using permeation-enhancing vehicles. Radiat. Res., *124:* 194–200, 1990.
23. Harapanhalli, R. S., McLaughlin, L. W., Howell, R. W., Rao, D. V., Adelstein, S. J., and Kassis, A. I. [125I/127I]iodoHoechst 33342: synthesis, DNA binding, and biodistribution. J. Med. Chem., *39:* 4804–4809, 1996.
24. Vos, O., Budke, L., and Vergroesen, A. J. Protection of tissue culture cells against ionising radiation. I. The effect of biological amines, disulphide compounds and thiols. Int. J. Radiat. Biol., *5:* 543, 1962.
25. Held, K. D. Interactions of radioprotectors and oxygen in cultured mammalian cells. I. Dithiothreitol effects on radiation-induced cell killing. Radiat. Res., *101:* 424–433, 1985.

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