manner[6,9,10]. Shibamoto *et al.*[8] observed in different tumours that, results from *in vivo* and *in vitro* experiments did not differ significantly. A synergistic increase in the BNC with many micronuclei and also a decreased cell progression are evident in the cells treated with HT immediately after 4.0 Gy. This indicates the presence of multiple lesions and unrepaired residual lesions remaining after cell division. Thus hyperthermia immediately after irradiation appears to enhance the cytogenetic damage by inhibiting repair of radiation-induced lesions, which may also convert some of the potentially lethal damage to lethal damage. This could explain the high increase in the cells with many micronuclei. *In vivo* studies on mouse bone marrow after whole body irradiation and local hyperthermia have shown that moderate post irradiation hyperthermia significantly increased the micronuclei frequency and also produced more cells with >1 micronucleus than in the animals receiving only radiation[11].

Even though the micronuclei frequency may not be directly correlated with the number of the chromosome aberrations, it can possibly be used as an index of the response of the mitotically active cells like the proliferating cancer cells and the normal cell renewal systems to combined modality treatment in cancer therapy. This is very important in that many cells which have received lesions will not be able to undergo more than one division after which they will be eliminated from the division pool and hence will not be available for studies at a later stage. Such an elimination has been observed in mouse bone marrow where there was a drastic reduction in cells bearing chromosome aberrations at 48 h after irradiation from the peak value of chromosome lesions observed at 24 h (ref 12).

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# Photoinduced DNA strand scission by 1,8-naphthalimide-derived intercalators

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**DNA cleavage by non-enzymatic chemical reagents has both basic and applied value. We report here photo-induced strand scission properties of novel DNA intercalators, 4-nitro(*N*-hexylamine)1,8-naphthalimide and 4-amino(*N*-hexylamine)1,8-naphthalimide. The results indicate that the cleavage takes place via a free-radical mechanism and the efficiency of cleavage is unaltered over a long range of pH and ionic strength. These intercalators, therefore, provide a probe for studying DNA conformation, conformational micro-heterogeneity, DNA–ligand interactions and have potential use in genome targeting.**

CHEMICAL modification of DNA strand has facilitated DNA sequencing[1], conformational studies[2] and understanding of interaction of small molecules to DNA[3]. Novel mechanistic approaches have been used for DNA cleavage[4], which are also important for their application in chemotherapy[5], gene regulation[6] and genome targeting[7]. A variety of synthetic[8] and natural products[9] have been used in these studies. 1,8-Naphthalimide-derived synthetic DNA intercalators, 4-nitro-(*N*-hexylamine)1,8-naphthalimide **a** and 4-amino(*N*-hexylamine)1,8-naphthalimide **b** (Figure 1) have strong fluorescent properties and have been shown to intercalate with the double helical DNA (unpublished results). Also, a substantial increase in the life-time of the excited state is observed upon intercalation. This increased life-time of the excited state and binding of **a** and **b** to DNA, prompted us to investigate if these intercalators have DNA-cleavage property. We report here that compounds **a** and **b** indeed cause DNA cleavage upon irradiation with visible light.

The DNA-cleavage analysis was carried out by comparing the reduction in intensity of the supercoiled DNA and reciprocal increase in relaxed DNA (and also aggregated DNA under stronger cleavage conditions). At a fixed DNA/intercalator ratio, irradiation time was varied and increased cleavage was observed with

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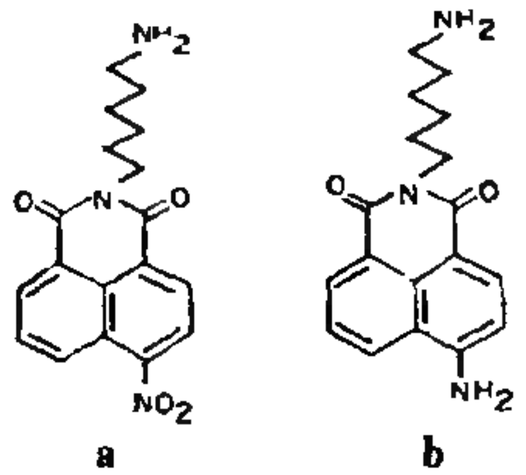


**Figure 1.** a, 4-Nitro(*N*-hexylamine)1,8-naphthalimide b, 4-amino(*N*-hexylamine)1,8-naphthalimide.

increasing irradiation dosage (Figure 2). Cleavage efficiency of **a** at 0, 5, 10, 20, 30, 40 and 60-minute irradiation is 0, 9, 15, 20, 28, 41 and 48%, respectively (lanes 1–7 of Figure 2,*a*). Under identical conditions **b** shows 0, 16, 30, 41, 50, 65 and 90% cleavage (lanes 1–7 of Figure 2,*b*). Therefore, **b** is almost twice as efficient in DNA cleavage compared to **a** at all irradiation dosages. At higher irradiation dosage cross-linking of DNA molecules resulting into high-molecular weight aggregates is observed. Such photonicking by both **a** and **b** is increased with the increasing intercalator concentration or irradiation duration, independently. That **a** and **b** indeed interact with DNA by intercalation is shown in Figure 3 using the resolution of topoisomers of supercoiled plasmid.

Since thiourea acts as an inhibitor of the cleavage reaction and the cleavage is not affected by a wide range of salt, pH, sodium azide, etc., we are tempted to suggest that the reaction takes place via a free-radical mechanism. Hydroxyperoxide group on naphthalimides are photoinduced hydroxy free radical generators and have been used for DNA cleavage recently[10,11]. We do not detect presence of hydroxyl-free radical under the cleavage conditions. The cleavage is, therefore, effected by the intercalator directly. Photoinduced DNA cleavage has been proposed to involve abstraction of hydrogen from the deoxyribose of the backbone by the excited triplet state of the fluorophore[12]. The triplet state can arise from the $n \rightarrow \pi^*$ transition of the amide carbonyl. This leads to the generation of deoxyribose radicals. Also the formation of high-molecular weight aggregates under conditions that favour increased cleavage (i.e. high intercalator concentration or high irradiation dosage) is suggestive of transient generation of highly reactive sites on DNA, during the course of cleavage. This reactive site, probably a form of deoxyribose radical, leads to strand cleavage, cross-linking of the double helix or of two double helices depending on relative molar activity of the active species. After effecting the covalent modifications on DNA, intercalators remain chemically unaltered even on prolonged irradiation. Higher cleavage efficiency of **b** could result from its longer life-time in the bound form or due to appropriate disposition of the intercalator with respect to the target DNA site, leading to higher yield of the effector radical.

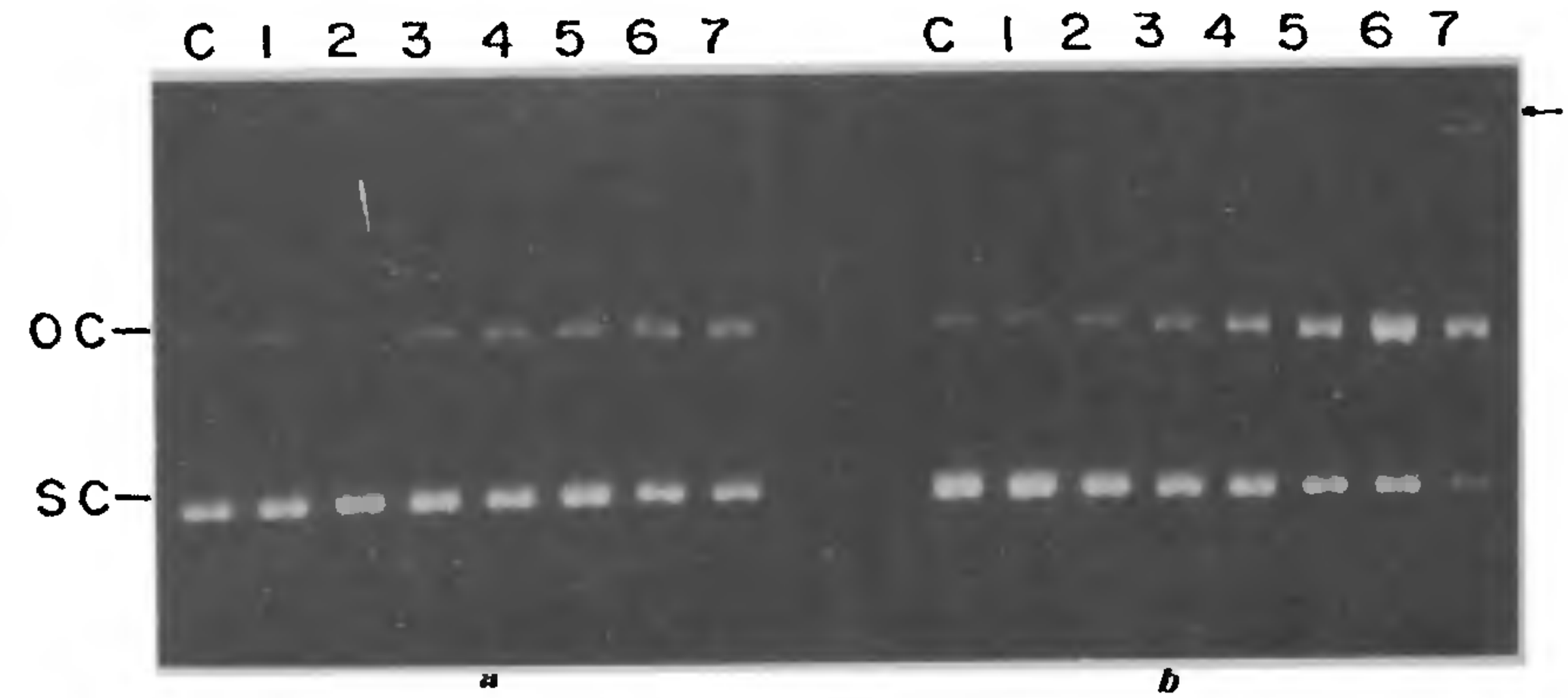


**Figure 2.** Photoinduced strand scission by intercalators **a** and **b** (panels *a* and *b* respectively). Supercoiled pBR322 plasmid DNA was treated with 1 mM intercalator in 10 mM Tris HCl (pH 8.0), 1 mM EDTA. Followed by incubation at 20 C for 15 min, the reaction mixture was irradiated using a Philips low-pressure gas discharge lamp (60 W, 600 lumen). The light source was positioned 10 cm away from the reaction mixture. Irradiation duration was 0, 5, 10, 20, 30, 40 and 60 min (lanes 1–7, respectively). Reaction mixture was extracted with phenol to remove the intercalator and electrophoresed on 1% agarose gel in TBE (89 mM Tris borate, pH 8.0, 2 mM EDTA). Gel was stained with ethidium bromide and photographed over a transilluminator. Lane C is DNA without any treatment. Arrow indicates the aggregated DNA, OC and SC denote open circular and supercoiled forms of DNA, respectively. For quantitative analysis of cleavage, the gel was scanned on a Molecular Dynamics 300A computing densitometer using Molecular Dynamics Image Quant V3.0 software.


**Figure 3.** Analysis of topoisomers on 1% agarose gel in the presence of **a** and **b**, panels ***a and b*** respectively, Supercoiled pBR322 plasmid DNA was analysed in the presence of 2 $\mu$g ml$^{-1}$ of intercalator in TBE buffer.

Amongst the DNA-binding agents, a groove binder would be expected to exhibit higher sequence specificity[13] compared to an intercalator. An intercalator at best can have specificity of two base pairs. We have observed higher degree of interaction of these intercalators with A/T base pairs compared to G/C base pairs (unpublished results). However, when intercalation occurs between A/T and G/C base pair, the cleavage can take place in the backbone sugar, irrespective of the actual preference for the intercalation. Therefore, we do not expect any remarkable base specificity and, the least, sequence specificity of the cleavage by either a or b. However, the differential affinity of these intercalators to polynucleotides of varying A/T content raises strong possibility that they might be sensitive to secondary structure heterogeneity. A cleavage agent with this property is useful in studying conformational microheterogeneity in DNA. Also, both the intercalators have nucleophilic amino function at the end of hydrocarbon chain which can be attached to DNA-recognition elements[14], e.g. triple helix potential oligonucleotide or sequence specific DNA-binding protein motifs.

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