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Assessment of atypical DNA intercalating agents in biological and *in silico* systems

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Abstract

Non-covalent genotoxic interaction between DNA and classical planar fused-ring intercalating agents, has been well understood for some time especially in the context of frameshift mutagenesis in bacterial systems. Recent evidence, however, suggests that a rather wide structural range of small non-fused ring molecules may also be capable of partial or complete DNA intercalation in mammalian cells. The present paper will review recent studies on the identification and characterization of such atypically-structured molecules utilizing both cell-based and three-dimensional computational analyses focusing principally on prediction and detection of these atypical molecules. Mechanistic aspects of genotoxicity of such non-covalent binding molecules, with emphasis on marketed pharmaceuticals, will also be discussed. A review and presentation of new data using catalytic DNA topo II inhibitors, confirms the notion that topoisomerase II poisoning arising via intercalation is the major mechanism of genotoxicity of these drugs.

Keywords: DNA intercalation; *In silico*; Chinese hamster V79 cells; DNA topoisomerase II

1. Entrance into the field

In the course of reviewing information regarding the genotoxicity of antihistamines in the Physician's Desk Reference (PDR), it became evident that (1) many of these antihistamines were clastogenic when tested in the standard battery of regulatory genetic toxicology assays even though they did not possess specific structural elements (i.e. Ashby alerts) that would have been expected to confer genotoxicity, but that (2) they did share a generic base structure comprised of two or three unfused ring [1]. In addition, the clastogenic antihistamines also possessed a dialkyl amino group, which seemed to be important for genotoxicity, since antihistamines lacking this moiety were uniformly non-genotoxic. Of great interest was the structural similarity of these molecules to unfused multi-ring molecules (Fig. 1) which were identified to be active in a series of naked DNA studies aimed at the discovery of molecules that would enhance the clinical antitumor activity of the DNA groove-binding antibiotic bleomycin ([2–5] and references therein). These studies had shown that this enhancement was due to facilitation of bleomycin DNA binding and nicking arising from intercalation-induced topological modifications to the DNA.

The relative simplicity of the amplification assays using naked DNA prompted the question of whether this bleomycin enhancement assay could be applied to cultured mammalian cells. The standard Chinese hamster V79-based micronucleus (MN) assay was modified

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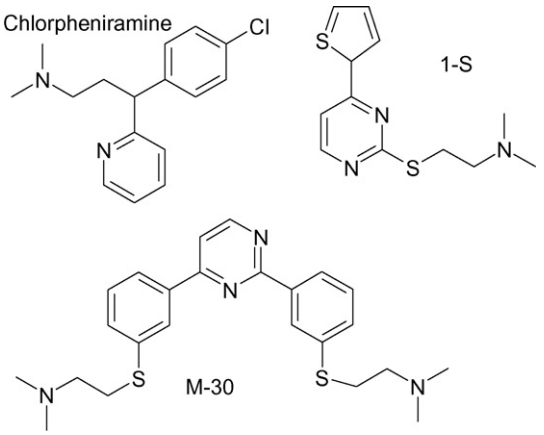

Fig. 1. Structures of atypical DNA intercalating agents (1-S and M-30) in comparison to the structure of the antihistamine, chlorpheniramine.

using a sub-clastogenic dose (usually 3 μg/mL) of bleomycin, in the presence and absence of a test compound to be evaluated for DNA intercalation activity. This assay has been described in detail elsewhere previously but briefly, V79 cells are treated with a non-clastogenic concentration of bleomycin (bleomycin induced MN frequency is linear with dose over a rather large range [6]) for about 5 min to allow interaction with DNA, test article is added, cells are incubated for 3 h, and media is replaced with fresh non-drug containing media with cytochalasin B. After approximately 16 h, cells are harvested, affixed to slides, and stained for determination of MN frequency in binucleated cells. Using this system, it was possible to reproducibly demonstrate amplification of bleomycin-induced MN formation by classical intercalating agents such as imipramine, acridines, etc. [6]. Fig. 2 illustrates the dose-responsive

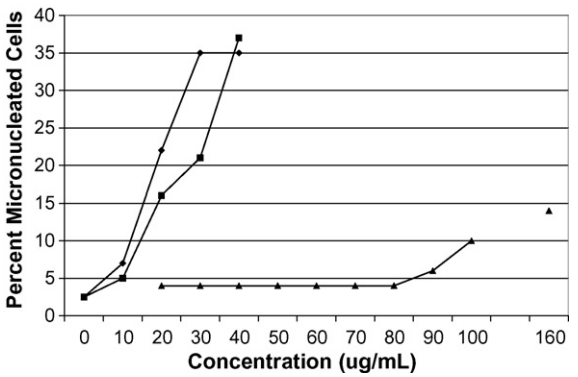

Fig. 2. Typical bleomycin amplification results with known DNA intercalators. V79 cells were treated with a non-clastogenic concentration of bleomycin in the presence of increasing concentrations of intercalating agent and the formation of micronuclei was measured as percent binucleated cells containing MN. Bleomycin alone was not clastogenic (not shown).

increase in bleomycin-dependent MN formation by imipramine, ethidium bromide and promethazine. It is seen that, even among classical intercalators, there is a wide range of "potencies" for bleomycin amplification with ethidium bromide requiring roughly 10 times the concentration of either imipramine or promethazine to observe the same degree of enhancement. Aliphatics and single-ring compounds were uniformly unable to enhance bleomycin-induced damage, whereas molecules with two or three fused or unfused rings often were quite active [6]. Some molecules such as proflavine, doxorubicin, and ellipticine, were, themselves, clastogenic in the V79 MN assay and were, thus, not readily testable as bleomycin amplifiers.

It should be stated that, as with any biological system, there may be alternative explanations for the above observations. In the present situation, enhancement of bleomycin DNA nicking could arise due to mechanisms other than intercalation dependent topological DNA changes such as increased cellular bleomycin uptake, enhanced cellular redox potential or alterations in cellular DNA repair (discussed in [6]). To date, however, none of these alternative amplification processes have been demonstrated in our studies.

In the initial study [6] approximately 50 compounds were evaluated for intercalative activity and relative intercalation potency values were made based on maximum fold-increases in MN formation and on the test article concentration required to produce that level of enhancement. Subsequently, a more accurate characterization of an intercalation potency factor (IPF) was applied utilizing the slope of the plot of test article concentrations and fold increase. Using this approach, clearly different potencies for intercalation were seen in comparing typical and atypical intercalators. Thus, molecules like imipramine yielded an approximate nine-fold increase in bleomycin-dependent MN formation at 40 μg/mL whereas an atypical molecule such as chlorpheniramine yielded a four-fold increase in MN formation only at 250 μg/mL. Perhaps surprisingly, tamoxifen was among the most potent atypicals amplifying approximately seven-fold at 10 μg/mL. The relative intercalative potencies, while certainly governed by structural features of each molecule, may also be affected greatly by, for example, cellular uptake, transport or metabolism, and thus, the absence of a positive amplification response does not necessarily mean the absence of intercalation ability.

We have also evaluated a proprietary series of five closely structurally-related drug candidates [6] some of which were positive and some negative in chromosome aberration assays. Visual inspection did not