Novel DNA Bis-intercalation by MLN944, a Potent Clinical Bisphenazine Anticancer Drug*

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The new bisphenazine anticancer drug MLN944 is a novel cytotoxic agent with exceptional anti-tumor activity against a range of human and murine tumor models both in vitro and in vivo. MLN944 has recently entered Phase I clinical trials. Despite the structural similarity with its parent monophenazine carboxamide and acridine carboxamide anticancer compounds, MLN944 appears to work by a distinct mechanism of inhibiting DNA transcription rather than the expected mechanism of topoisomerase I and II inhibition. Here we present the first NMR structure of MLN944 complexed with d(ATG-CAT), DNA duplex, demonstrating a novel binding mode in which the two phenazine rings bis-intercalate at the 5'-TpG site, with the carboxamide amino linker lying in the major groove of DNA. The MLN944 molecule adopts a significantly unexpected conformation and side chain orientation in the DNA complex, with the N10 on the phenazine ring protonated at pH 7. The phenazine chromophore of MLN944 is very well stacked with the flanking DNA base pairs using the parallel base-stacking intercalation binding mode. The DNA sequence specificity and the groove recognition of MLN944 binding is determined by several site-specific hydrogen bond interactions with the central G:C base pair as well as the favorable stacking interactions with the 5'-flanking thymine. The specific binding site of MLN944 is known to be recognized by a number of important transcription factors. Our electrophoretic gel mobility shift assay results demonstrated that the c-Jun DNA binding to the AP-1 site is significantly inhibited by MLN944 in a dose-dependent manner. Thus, the exceptional biological activity of MLN944 may be due to its novel DNA binding mode leading to a unique mechanism of action.

The systemic therapies currently available for the treatment of various solid tumors of adult life, including those of lung, colon, breast, prostate, and ovary, remain primarily palliative, and there is an urgent need for more effective therapies. Although recently there has been a significant effort in developing molecular targeted therapies, cytotoxic agents remain the major form of therapy for the majority of cancers. Moreover, there is reason to believe that a therapeutic advantage remains to be gained from cytotoxic drugs with novel mechanisms of action, with improved safety profiles and different spectra of anti-tumor activity.

The bisphenazine MLN944 (XR5944) (Fig. 1a) is a novel cytotoxic agent with exceptional anti-tumor activity against a range of human and murine tumor models both in vitro and in vivo (1). It has recently entered Phase I clinical trials. Although initial reports showed that MLN944 can bind strongly to DNA (2) and that it may interfere with the normal function of topoisomerase I and II in vitro (1), recent studies have indicated that topoisomerase I and II may not be its primary cellular target and that it works by a novel mechanism of action. The mechanistic novelty of MLN944 is suggested by the fact that in the yeast Saccharomyces cerevisiae, modulation of topoisomerase I, II, and III levels did not alter the efficacy of the compound. Moreover, the functional genomics profiles of MLN944-treated yeast were unlike the profiles from yeast treated with known inhibitors of topoisomerase I and II (3). Studies in mammalian cells have also indicated a novel mechanism of action of MLN944. For example, exposure of human tumor cells to MLN944 causes arrest in both G1 and G2 phases of the cell cycle, whereas compounds that inhibit topoisomerase I or II characteristically lead to cell cycle arrest in S/G2 phase even when cells are exposed to these agents simultaneously (3). MLN944 also exhibits activity against both quiescent and proliferating tumor cells and has the ability to kill cells following only a brief exposure period (4) with a corresponding lack of schedule dependence in its in vivo anti-tumor efficacy, as is characteristic for topoisomerase inhibitors (1). The novel mechanism of action of MLN944 has recently been shown to be related to the inhibition of DNA transcription (5, 6).

The parent compounds of MLN944, phenazine carboxamides (such as MLN576 and MLN612) as well as the closely related acridine carboxamides (such as N-(2-(dimethylamino)ethyl)acridine-4-carboxamide (DACA),1) are both DNA intercalators and dual DNA topoisomerase I/II inhibitors (7–9). Crystal structures of 9-amino-DACA-DNA complexes showed that acridine rings intercalate duplex DNA at a CpG site (10, 11). MLN944 has also been shown to be an efficient DNA intercalator by its induction of dose-dependent supercoiling of plasmid DNA. Moreover, MLN944 has been shown to bind double-stranded DNA with high affinity and to have a strong G-C sequence preference (2). Therefore, it is surprising that MLN944 works by a distinct mechanism of action other than that of topoisomerase I/II inhibition. It is possible that the

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1 The abbreviations used are: DACA, N-(2-(dimethylamino)ethyl)acridine-4-carboxamide; NOE, nuclear Overhauser effect; NOESY, NOE spectroscopy; EMSA, electrophoretic mobility assay.
exceptional biological activity of MLN944 may be related to a distinct DNA binding mode and hence its novel mechanism of action. In this study, we determined the structure of a complex between MLN944 and DNA duplex d(ATGCAb), using a combination of NMR spectroscopy and molecular dynamics calculation. The result shows that MLN944 does indeed have a novel binding mode with a duplex DNA. The drug bis-intercalates at the 5′-(TpG):(CpA) site, with the aminoalkyl linker lying in the major groove of the DNA. This not only is the first structure of a phenazine derivative binding with DNA but is also the first solution structure of a DNA complex among all of the closely related acridine carboxamide compounds. A number of novel features of drug binding with DNA in the solution state that have not been reported before are also shown in our study.

EXPERIMENTAL PROCEDURES

Sample Preparation—The DNA oligonucleotides were synthesized using β-cyanoethylphosphoramidite solid-phase chemistry on an Expe- dite 90 nucleic acid synthesis system (Applied Biosystems, Inc.) with the DNA oligonucleotides were purified by HPLC reverse-phase high-pressure liquid chromatography. MLN576 (XR11576) and MLN944 (XR5944) were provided by Millennium Pharmaceuticals, Inc. (Cambridge, MA) and Xenova Ltd. (Slough, UK). The NMR samples were prepared by dissolving DNA oligonucleotide powder into 50 mM sodium phosphate buffer at pH 7 in either pure D2O (98%) or D2O/H2O (10%90%). The D2O samples were lyophilized and resuspended in 99.98% D2O two more times. The DNA-drug complexes were prepared by adding an appropriate amount of drug stock solution to the DNA sample, followed by lyophilization and redissolution in D2O. The final concentrations of DNA oligonucleotides were 1–4 mM.

NMR Experiments—Both one- and two-dimensional NMR experi- ments were carried out on a Bruker Avance 600-MHz spectrometer. Standard homonuclear two-dimensional NMR experiments were used to assign exchangeable and nonexchangeable proton chemical shifts of the complex, including DQF-COSY, TOCSY, and NOESY. The mixing times were set at 50, 100, 150, and 200 ms for NOESY and at 30 and 60 ms for TOCSY. The NMR experiments for samples in water solution were performed with WATERGATE or jump-return (NOE11) water suppression techniques. The relaxation delay was set to 2 s. The acquisition data points were set to 4096 × 512. The 60° shifted sine bell functions were applied to both dimensions of NOESY and TOCSY spectra. The 5 order poly- nominal functions were employed for the base-line corrections. The final data points were 4096 × 1024. Peak assignment and integration were achieved using Sparky (University of California, San Francisco). Distances between nonexchangeable protons were assigned based on the NOE cross-peaks integrated at 50–200-ms mixing times. The peak volumes were referenced using the distance H5–H6 of cytosine (2.45 Å). Unassigned protons were replaced by pseudoatoms, and the appropriate volumes were referenced using the distance H5–H6 of cytosine (2.45 Å).

Distance-restrained Molecular Dynamics Simulation—Structure Calculations were performed using NOE-restrained molecular dynamics simulation in the program X-PLOR (version 3.85b1) (12). The starting model of the DNA d(ATGCAb):MLN944 complex was constructed in Insight II 2000.1 (Accelrys), with the intercalation site conformations deduced from the NOE data. The partial charges were obtained from X-PLOR or from the representative fragments in Insight II. The CHARMM force field was used for the calculation. The skewed bichromonic energy function was used for distance constraints from NOE data. A total of 161 distances were set to 4096 × 1024. Peak assignment and integration were achieved using Sparky (University of California, San Francisco). Distances between nonexchangeable protons were assigned based on the NOE cross-peaks integrated at 50–200-ms mixing times. The peak volumes were referenced using the distance H5–H6 of cytosine (2.45 Å). Unassigned protons were replaced by pseudoatoms, and the appropriate correction was applied to the measured distance.

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strong NOE cross-peak with the drug C9 methyl group. This led to the assignment of its vicinal proton H7 and then of H6. The drug H3 proton was clearly identified by having two COSY cross-peaks with both H2 and H4, whereas H2 was assigned by its NOE cross-peak with the carboxamide linker and was further confirmed by a number of NOE cross-peaks with its neighboring DNA bases. The exchangeable protons of both DNA and drug were assigned by using two-dimensional NOESY. The chemical shifts of all resonances are tabulated in Table I.

Free DNA hexamer d(ATGCAT)2 forms a regular B-type double helix in solution, as indicated by standard sequential connectivities and intrasugar interactions in NOESY and COSY spectra. The exchangeable proton NMR spectrum in H2O revealed two clear imino proton resonances at 12.6 ppm (G3HN1) and 13.65 (T2HN3) ppm, suggesting Watson-Crick configuration in the d(ATGCAT)2-MLN944 complex, as indicated by the intraresidue H6/H8 protons to the H1 proton of any DNA nucleotide. It has a much slower exchange rate with water than that of the DNA imino protons of both T2 and G3. This proton has NOE interactions with several other protons of MLN944, including 9-methyl protons (strong), linker protons H1 (medium to strong), and H8 (weak), and was thus assigned as HN10 on the N10 of ring B of MLN944. The protonation of MLN944 phenazine ring—The exchangeable proton NMR spectrum in H2O of the DNA-MLN944 complex at pH 7 also reveals a very interesting proton resonance in the imino region at 11.22 ppm. Inspection of the two-dimensional NOESY spectra indicates that this proton is not an imino proton of any DNA nucleotide. It has a much slower exchange rate with water than that of the DNA imino protons of both T2 and G3. This proton has NOE interactions with several other protons of MLN944, including 9-methyl protons (strong), linker proton Ha1 (medium to strong), and H8 (weak), and was thus assigned as HN10 on the N10 of ring B of MLN944. The presence of the HN10 at pH 7 is quite remarkable, since the pKa

### Table I

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<th>H6/H8</th>
<th>H2/H5/Me</th>
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<th>H4′</th>
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<td>4.96</td>
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<td>5.74</td>
<td>5.27</td>
<td>5.49</td>
<td>4.22</td>
<td>4.27</td>
</tr>
<tr>
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<td>8.01</td>
<td>6.18</td>
<td>6.37</td>
<td>2.30</td>
<td>2.08</td>
<td>4.96</td>
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### FIG. 2.

The expanded regions of the nonexchangeable two-dimensional NOESY spectra of the DNA-MLN944 complex. Bottom, aromatic H1′ region. The sequential assignment pathway is shown. Top, aromatic H2/H2′/methyl region. The intermolecular NOE cross-peaks between MLN944 and DNA are labeled with asterisks and arrows (for C4H6 only).
The value of the N10 is only 1.0–1.3 (13). This unexpected drug phenazine conformation, which could not possibly exist in the bulk solution, is clearly induced and stabilized by the microenvironment of DNA binding pocket. MLN944 is formulated as the dimesylate salt, with the amino nitrogen protonated at pH 7 and positively charged (Fig. 1a). The amide proton HN is also observable and assigned in the exchangeable proton NOESY spectra (Table I).

**NMR Structure Determination**—Many intermolecular NOE cross-peaks between MLN944 and DNA are observed in two-dimensional NOESY, as summarized in Fig. 3. The strong NOE interaction between C4H5 and MLN944-M9 places the methyl group of the drug phenazine ring position 9 (Fig. 1a) in the major groove of the DNA double helix. In the meantime, the NOE interactions between the DNA T2 methyl group and MLN944-H1 and H2 place the carboxamide aminoalkyl linker in the major groove as well. The aromatic protons H2, H3, and H4 on the drug display multiple NOE cross-peaks with the DNA T2 and G3 aromatic protons H8/H6/Me and sugar protons H1/H2/H2', whereas the drug aromatic protons H6, H7, and H8 show similar NOE interactions with the C4 and A5 residues of DNA, indicating that the MLN944 phenazine ring intercalates between the (T2:A5) and (G3:C4) base pairs. The ring A of MLN944 stacks between the T2pG3 and C4pA5 step of the complementary strand. These intermolecular NOEs suggest a parallel base-stacking intercalation of both phenazine rings of MLN944 at the two symmetric (T2pG3):(C4pA5) and (C4pA5):(T2pG3) steps, with the long axis of each phenazine parallel to the long axes of the flanking DNA base pairs and the aminoalkyl linker in the major groove of the DNA duplex. The stacking position of the phenazine rings is further defined by the intermolecular NOE interactions between drug aromatic ring protons and DNA residues. The MLN944 H2 and H3 are close to DNA T2H1 and to a lesser extent G3H1, which are both located in the DNA minor groove, indicating that the phenazine ring A is inserted deeply into the DNA base pair stack. H6/H7/H8 of MLN944 ring C show similar, but weaker, NOE interactions with DNA C4 and A5 residues, indicating that the drug phenazine ring is closer to the T2pG3 side than to the C4pA5 side of the complementary strand. Furthermore, the G3H1 in the minor groove is one of the most upfield-shifted DNA proton resonances upon drug binding (Table I), as discussed before, indicating the close proximity of the MLN944 A ring with the T2pG3 sugar backbone. The same phenomenon was not observed between the drug C ring and C4pA5 backbone. In addition, MLN944-H4 has a strong NOE interaction with DNA A5H2, whereas MLN944-H6 does not have much NOE interaction with A5H2. These observations suggest that the MLN944 phenazine A ring end inserts more deeply toward the DNA minor groove and is closer to the DNA sugar backbone, whereas the C ring end is positioned farther from the DNA sugar backbone and more toward the DNA major groove.

A starting model of the d(ATGCAT)2-MLN944 complex con-

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2 W. Denny, personal communication.
constructed using the above mentioned information was subjected to NOE-restrained molecular dynamics calculation in X-PLOR (12). A total of 161 distance restraints, of which 51 are from intermolecular NOE interactions between MLN944 and DNA, were incorporated into the restrained molecular dynamics calculation. The restrained molecular dynamics calculation produced a very stable, converged family of structures (Fig. 4 and Table II, Protein Data Bank accession number 1X95), indicating a well defined binding conformation. The pairwise root mean square deviation of the refined structures is 0.38 Å.

**Global Complex Structure and DNA Conformation**—A representative model of refined complex structures with MLN944 and DNA hexamer d(ATGCAT)$_2$ is shown in stereo view in Fig. 5a. The two phenazine chromophores of MLN944 bis-intercalate at the TpG and CpA steps of the DNA, wrapping the two central G:C base pairs, with the long axes of chromophores parallel to the long axes of the flanking DNA base pairs. The carboxamide aminoalkyl linker lies in the major groove of the DNA duplex. The 2-fold symmetry of both DNA and drug are retained. The linker runs diagonally across the major groove such that the drug has the appearance of a backward “Z” when viewed from the major groove. The drug exhibits a left-handed twist counter to the right-handed twist of the DNA helix.

In the intercalation pocket, the rise between two intercalated T2:A5 and G3:C4 base pairs is −6.32 Å, much larger than that of the perpendicular intercalation binding site, as exemplified...
Effect of MLN944 on the c-Jun DNA Binding to the AP-1 Site by Electrophoretic Gel Mobility Shift Assay (EMSA)—The AP-1 family of transcription factors are dimeric complexes that specifically bind to a consensus DNA promoter region, namely the AP-1 binding site 5′-TGAGTCA. c-Jun is a major member of the AP-1 transcription factors, whose activity has been implicated in cell proliferation/transformation and cancer development (18, 19). We carried out electrophoretic gel mobility shift assays (EMSA) of c-Jun DNA binding to the AP-1 site in the absence and presence of MLN944. The EMSA results demonstrated that the c-Jun DNA binding to the AP-1 site is significantly inhibited by MLN944 in a dose-dependent manner. Specifically, the DNA binding of c-Jun was clearly inhibited by MLN944 at the 50 nM concentration and was almost completely blocked by the drug at the 25 μM concentration (Fig. 6, a and d). In contrast, MLN944 does not block the DNA binding of transcription factor NF-xB to its consensus promoter region 5′-GGGACATTGCC at the concentration of 25 μM (Fig. 6c).

To demonstrate the specificity of the MLN944 activity, we also tested the inhibitory activity of other known DNA intercalators, including ethidium bromide and topotecan (topoisomerase I inhibitor), on the DNA binding of c-Jun to the AP-1 site. No evident inhibition of c-Jun DNA binding was shown by these two other DNA intercalating compounds at the 25 μM concentration (Fig. 6b).

DISCUSSION

Unexpected MLN944 Conformation—The MLN944 molecule adopts a significantly unexpected conformation and side chain orientation. The N10 of the phenazine ring of MLN944 is protonated (Fig. 1a) in the DNA complex at pH 7, and this HN10 proton is clearly observed in the NMR data. The carbonyl group of carboxamide forms an internal hydrogen bond to the protonated phenazine ring N10, with the N10 proton in the bulk solution, is clearly induced and stabilized by the microenvironment of DNA binding pocket. It has been observed in the crystal structure that 9-amino-6-bromo-DACA can adopt both the acid (protonated, positively charged N10) and conjugate base (nonprotonated neutral N10) forms at pH 6.5 (11); however, the acridine ring pKa of 9-amino-DACA is 8.3 (13), much higher than that of the phenazine ring of MLN944. Furthermore, the protonated phenazine ring configuration is the only drug conformation observed in our DNA complex, since there is no sign of a second conformation or an exchange process in the NMR data. Although it is well accepted that the negatively charged microenvironment of DNA could stabilize an otherwise unstable conformation, our result is very significant, since it provides for the first time direct experimental evidence, in solution state, of how dramatically DNA local environment can affect the physical property and conformation of a ligand.

MLN944 Base-stacking Interactions with DNA and Binding Site Specificity of 5′-T—Both the phenazine chromophores of MLN944 are deeply inserted into the flanking DNA base pairs (Fig. 5b). Each drug aromatic phenazine ring is very well stacked with both the central G3:C4′ (or G3′:C4) base pair and the T2 (or T2′) base of the T2:A5′ (or T2′:A5) base pair at the intercalation site (Figs. 1b and 5c). The long axis of the phenazine chromophore of MLN944 is almost completely aligned with that of the central G/C base pair (G3′:C4′ or G3:C4′). In the interaction with the central G/C base pair, the six-member ring of the guanine G3 (or G3′) base is stacked on the drug phenazine A ring, whereas its base pair partner, cytosine C4′ (or C4), is stacked on the drug C ring. In the interaction with the T-G base pair (T2:A5′ or T2′:A5) on the other side of the same phenazine chromophore, the thymine T2 (or T2′) base is completely stacked very well on the drug A ring, whereas the adenine A5′ (or A5) base is unstacked with the drug chromophore.

There is a very strong π-stacking interaction between the T2 base and ring A of the drug phenazine. The thymine T2 is completely stacked over the phenazine ring A, with the electron-negative O4 group of thymine T2 positioned right above the
protonated, electropositive N10 imide edge of the MLN944 phenazine chromophore (Fig. 5c). It would not be possible to have such favorable interactions if cytosine were in place of the thymine T2, since the electropositive N4 amino group of cytosine would have an unfavorable interaction with the electropositive N10 imide group and would therefore destabilize the stacking interactions. Furthermore, the 5-methyl group of thymine T2, which is known to increase hydrophobic interactions and stabilize ligand binding, is well stacked over the carboxamide group of MLN944. These favorable electrostatic and hydrophobic interactions may be the reason for the site-specific requirement of 5'-T, as in the present TpG binding site, instead of the predicted CpG site (2). The strong stacking interaction of the MLN944 phenazine ring with the intercalated base pairs may provide an energetic basis for the tight binding (stronger than the binding with poly(GC), whose $K_b = 1.6 \times 10^9 \text{ M}^{-1}$ (2)).

The drug aromatic chromophore resembles a B-DNA base pair when viewing into the minor groove, with the N5 of the diazine of MLN944 located right above the N2 of the central guanine G3 in the minor groove. Moreover, the electronegative sugar O4' atom of G3 at the binding site is in close contact (2.7 Å) with the π-phenezine aromatic ring, probably due to the relatively low electronegativity of O4'. Such a phenomenon has also been observed in a number of nucleic acid structures (see Ref. 20 and the references therein), where the interactions between the sugar O4' atom and the π-electron system often stabilize the nucleic acid structures.

**MLN944 Linker-DNA Interactions and Sequence Specificity**—The carboxamide aminooalkyl linker of MLN944 plays a major role in DNA groove recognition as well as in sequence specificity of drug binding. The two γ-amino groups of carboxamide aminooalkyl linker of MLN944 are protonated at pH 7 and therefore positively charged as γ-NH$_2$(+) (Fig. 1b), which facilitates the binding of drug linker in the normally very electronegative DNA major groove. The strong electronegative potential of the DNA major groove always facilitates the binding of drugs with positively charged side chains (21–23). MLN944 binding dramatically changes the electrostatic distribution of the DNA major groove to a more electropositive potential (Fig. 5d).

When the ring A end of MLN944 is deeply inserted into the intercalating pocket and is in close proximity to the DNA sugar backbone, the site-specific interactions of the drug carboxamide aminooalkyl linker with the two central guanines, G3 and G3', are facilitated. The two γ-amino groups of the linker are located very close to the O6 and N7 of the two symmetrically related guanine residues for strong hydrogen-bonding interactions. Specifically, one hydrogen of the γ-amino group is 1.7 Å from the O6 atom of one guanine and forms a strong hydrogen bond and the second hydrogen of the same γ-amino group is 2.3 Å from the N7 atom of the same guanine to form a second hydrogen bond (Fig. 5e).

The parallel base-stacking intercalation binding mode has been observed in a number of drugs (10, 11, 21, 24). Although the chromophore structure of MLN944 is similar to that of the acridine carboxamides such as 9-amino-DACA, the base-stacking interactions of the two drugs with DNA base pairs is somewhat different (10, 11). The phenazine ring of MLN944 is shifted more toward the major groove side of DNA compared with the acridine ring of DACA. The long axis of the phenazine ring is almost completely aligned with that of the central G:C base pair, whereas the acridine ring in 9-amino-DACA bisects the angle between the long axes of the intercalated base pairs. In addition, the protonated conformation of the drug phenazine ring, which occurs unexpectedly in the microenvironment of the DNA, indirectly induces a favorable side chain orientation to form a site-specific hydrogen bond interaction with the two central guanines (Fig. 5, c and e). In contrast to the carboxamide plane of 9-amino-DACA being co-planar with the acridine chromophore, the plane of the MLN944 carboxamide group is about 15° with the phenazine plane, so that the amide proton is 2.8 Å to the N7 of guanine G3 (or G3'), to form a possible hydrogen bond. Furthermore, the N-H–N angle is −160°, favorable for hydrogen bond formation (25).

The existence of two hydrogen bond acceptors at the major groove side of guanine, the above mentioned site-specific interactions between drug and DNA, and the favorable charge-charge interactions between the drug linker and the DNA major groove are important determinants in the sequence specificity and the binding groove selectivity of MLN944.

**MLN944 Inhibits the c-Jun DNA Binding to the AP-1 Site**—The AP-1 family of transcription factors has been implicated in cell proliferation and transformation and can be induced by many stimuli, including growth factor, cytokines, and oncoproteins (see Refs. 18 and 19 and references therein). The main AP-1 components in mammalian cells are JUN and FOS proteins, where c-Jun and c-Fos are closely related with the development of various types of cancer. The AP-1 dimeric transcription factors specifically recognize a consensus DNA promoter region, the AP-1 binding site, with a palindromic base sequence of 5'-TGA(C/G)TCA, whose core subset is the TpG site that MLN944 appears specifically to bind; therefore, it is interesting to test whether the DNA binding of the AP-1 proteins can be inhibited by MLN944.

Our EMSA results demonstrated that MLN944 remarkably inhibits the c-Jun DNA binding to the AP-1 site. The DNA binding of c-Jun was clearly inhibited by MLN944 at the 50 nM concentration and was almost completely blocked by the drug at the 25 µM concentration (Fig. 6, a and d). However, MLN944 does not block the DNA binding of transcription factor NF-κB to its consensus promoter region 5'-GGGACTTTCC even at the concentration of 25 µM (Fig. 6c), which demonstrates that MLN944 does not randomly block the DNA binding of any transcription factor.

To demonstrate the specificity of the inhibitory effect of MLN944, we have also carried out the EMSA experiments on other known DNA intercalative compounds, including the known DNA intercalator ethidium bromide and the topoisomerase I inhibitor topotecan (26, 27). In contrast, although they both are DNA intercalators, these compounds do not display evident inhibition of c-Jun DNA binding to the AP-1 site at the 25 µM concentrations (Fig. 6b). These results clearly demonstrate that the effect of MLN944 is not simply due to its DNA intercalation.

The inhibitory effect of MLN944 on the AP-1 protein DNA binding can be explained by the available structure data. The AP-1 transcription factors, including Jun, Fos, and activating transcription factor proteins, are basic region leucine zipper proteins that selectively dimerize using their leucine zipper regions. The DNA binding specificities of the AP-1 proteins are imparted by the DNA major groove interactions of the AP-1 half-site (5'-T$_4$G$_3$A$_2$C$_1$G$(G$_2$T$_5$C$_5$A$_4$)) by using the conserved basic DNA-binding regions (28–31). The positioning of the MLN944 linker in the DNA major groove will clearly block the DNA binding of the AP-1 proteins. In particular, the site-specific interactions of the AP-1 proteins involved in the DNA binding include the hydrogen-bonding interactions of the invariant protein Asn side chain with the N4 atom of the AP-1 half site cytosine 3 and the O4 atom of thymine −4 as well as the van der Waals contacts between the protein Ala and Ser residues and the 5-methyl group of DNA thymine −4, specifi-
Novel DNA Binding by New Anticancer Drug MLN944

REFERENCES

Additions and Corrections


Novel DNA Bis-intercalation by MLN944, a potent clinical bisphenazine anticancer drug.

Jixun Dai, Chandanamalie Punchihewa, Prakash Mistry, Aik Teong Ooi, and Danzhou Yang

Page 46101, Fig. 6: The unit in this figure should be μM, not mM. The correct figure is shown below:

- Fig. 6:


Inhibition of MDM2-mediated p53 ubiquitination and degradation by ribosomal protein L5.

Mu-Shui Dai and Hua Lu

Page 44479: In the right column, lines 13–16, the word “not” was omitted from the sentence. The correct sentence should read: “Of note, the slight decrease of p53 in L5 siRNA-treated cells was specific to p53; it was not caused by global translational inhibition after reduction of L5 by its siRNA.”


The leucine-rich repeat protein LRIG1 is a negative regulator of ErbB family receptor tyrosine kinases.

Melanie B. Laederich, Melanie Funes-Duran, Lily Yen, Ellen Ingalla, Xiuli Wu, Kermit L. Carraway III, and Colleen Sweeney

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