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ORIGINAL PAPER

Quantification of the interceptor action of caffeine on the in vitro biological effect of the anti-tumour agent topotecan

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Abstract Using published in vitro data on the dependence of the percentage of apoptosis induced by the anti-cancer drug topotecan in a leukaemia cell line on the concentration of added caffeine, and a general model of competitive binding in a system containing two aromatic drugs and DNA, it has been shown to be possible to quantify the relative change in the biological effect just using a set of component concentrations and equilibrium constants of the complexation of the drugs. It is also proposed that a general model of competitive binding and parameterization of that model may potentially be applied to any system of DNA-targeting aromatic drugs under in vitro conditions. The main reasons underpinning the proposal are the general feature of the complexation of aromatic drugs with DNA and their interaction in physiological media via hetero-association.

Keywords Hetero-association · Topotecan · Interceptor–protector mechanism · Competitive binding

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Introduction

Biologically active aromatic compounds belonging to the camptothecin family are currently one of the most promising types of drugs for treatment of various cancers. The cytotoxic activity of these compounds is thought to be due to their ability to induce single-strand breaks in nuclear DNA in the presence of topoisomerase I (Liu et al. 2000). Topotecan (TPT) is a camptothecin derivative used in the clinic for treating various tumours (Pizzolato and Saltz 2003), either as a single agent or as a component of combinational chemotherapy when administered together with other drugs (Bernacki et al. 2000). The mechanism of synergistic action of TPT–drug systems is normally not known, which is a barrier to rational design of more effective protocols of disease treatment. However, when both drugs in combination are aromatic molecules, the observed synergistic biological effects may be understood in terms of their direct interaction in physiological media via π–π stacking (to be referred to as hetero-association when one of the interacting drugs acts as an interceptor of the other drug) [for a review see Evstigneev (2010)]. Such an interpretation has been given to explain the synergistic effects in xanthine–drug (Traganos et al. 1991; Davies et al. 2001; Piosik et al. 2003; Hernandez Santiago et al. 2009; Woziwodska et al. 2011), vitamin B_2–drug (Munoz et al. 1995; Ramu et al. 2000; Evstigneev et al. 2005) and chlorophyllin–drug (Pietrzak et al. 2008; Osowski et al. 2010) combinations. It is thought that, by variation of the concentration of the interceptor molecule (xanthine, vitamin B_2 or chlorophyllin) in such combinations it might be possible to modulate the biological activity of the main drug. The latter may be used to regulate the toxicity level of anti-tumour agents or carcinogens (Piosik et al. 2002; Evstigneev et al. 2006a), regulate the rate of drug

degradation (Evstigneev et al. 2006b), optimize the solubility of the drug (Evstigneev et al. 2006c) or solve specific biochemical tasks (Bedner et al. 2001). Recently we proposed that relative biological effect in a combination of aromatic drugs may be quantified (Evstigneev et al. 2008), which may make it possible to regulate the activity of a drug by a predictable change in the synergistic effect.

The anti-tumour agent topotecan (TPT) has long been known to interact synergistically with caffeine (CAF), a xanthine-based aromatic molecule, which was interpreted in terms of TPT–CAF hetero-association and interference with the binding of TPT to the DNA–enzyme complex (Traganos et al. 1993). Based on published in vitro cellular data for the TPT–CAF system, in the present work we provide the first demonstration that the relative biological effect in combinations of aromatic drugs may be described quantitatively just using a set of equilibrium complexation constants specific to the given system and measured in independent physico-chemical experiments.

Experimental

Materials

Topotecan from Molekula and caffeine from Sigma (for molecular structures see Fig. 1) were used without further purification. The samples were lyophilized from D_2O solutions and re-dissolved in 0.1 M phosphate buffer in 99.95% D_2O at pD 7.1, containing 10^{-4} M ethylenediamine tetra-acetic acid (EDTA).

NMR measurements

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian 11.74-T Unity-Plus spectrometer operating at 500 MHz for protons, with attenuation of the residual water peak by pre-saturation during the relaxation delay. Under conditions of the experiment (pD 7.1) topotecan exists as a mixture of lactone (ring E closed, see Fig. 1a) and carboxylate (ring E opened, see Fig. 1b) forms, which is manifested by the existence of two sets of peaks in one-dimensional (1D) NMR spectra of TPT–CAF solution corresponding to non-exchangeable TPT protons. Signal assignments of the non-exchangeable protons of TPT in both forms and of CAF were obtained using two-dimensional homonuclear total correlated spectroscopy (TOCSY) and rotational nuclear Overhauser effect spectroscopy (ROESY) experiments, and were in agreement with previous assignments (Bocian et al. 2004). Chemical shift measurements of the non-exchangeable protons of the aromatic molecules were made as a function of concentration of CAF at $T = 298$ K maintaining the concentration

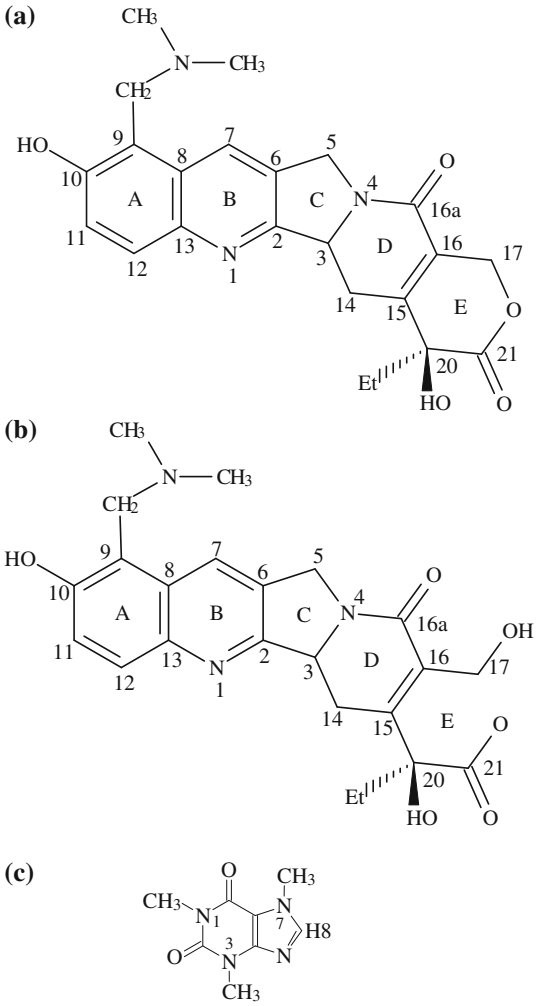

Fig. 1 Structures of **a** the lactone form of topotecan, **b** the carboxylate form of topotecan and **c** caffeine

of TPT constant (see titration curves, Fig. 2). Chemical shifts were measured relative to an internal reference tetramethylammonium bromide (TMA) and recalculated with respect to sodium 2,2-dimethyl-2-silapentane-5-sulphonate (DSS), i.e. $\delta_{DSS} = \delta_{TMA} + 3.178$ (ppm). The sample temperature was regulated using a Varian VT unit. The method of sample preparation and NMR experimental details have been described elsewhere (Davies et al. 2001).

Analysis of TPT–CAF hetero-association

The dynamic equilibrium in solution containing two types of interacting aromatic molecules X (topotecan) and Y (caffeine) in the NMR concentration range (millimolar) in Scheme 1 must also take into account the self-association reactions of X and Y and the formation of aggregates of higher dimensions than dimers for self-association or 1:1 hetero-association complexes of X and Y (Davies et al.

2001; Hernandez Santiago et al. 2009; Evstigneev et al. 2005, 2006a, c):

$$X_1 + X_i \overset{K_X}{\leftrightarrow} X_{i+1} \quad \text{(a)}, \quad Y_1 + Y_j \overset{K_Y}{\leftrightarrow} Y_{j+1} \quad \text{(b)},$$
$$X_i + Y_j \overset{K_h}{\leftrightarrow} X_i Y_j \quad \text{(c)}, \quad Y_j X_i + Y_l \overset{K_h}{\leftrightarrow} Y_j X_i Y_l \quad \text{(d)}, \qquad (1)$$
$$X_k + Y_j X_i \overset{K_h}{\leftrightarrow} X_i Y_j X_k \quad \text{(e)}$$

where X_1 and Y_1 correspond to the monomers of the anti-tumour agent and CAF, and X_i, X_k, Y_j and Y_l are the aggregates containing i, k monomers of the anti-tumour agent and j, l monomers of CAF, respectively; K_X and K_Y are the equilibrium self-association constants for X and Y, and K_h is the hetero-association constant. The analytical expressions for the observed dependence of proton chemical shifts of both X and Y components for reactions (1) take the form (Davies et al. 2001; Hernandez Santiago et al. 2009; Evstigneev et al. 2005, 2006a, c)

It follows that Eq. (2) is a function of two unknown quantities, δ_h and K_h, which may be determined from the concentration dependences of δ (see Fig. 2) separately for each proton. The numerical procedure of finding δ_h and K_h accomplishes the fitting of the concentration dataset by computational variation of these two variables in order to reach the minimum of the discrepancy function $\Delta = \sum_i (\delta_i - \delta_{ei})^2$, where δ_e is the experimentally observed proton chemical shift measured at the given concentration point i and δ_i is calculated from Eq. (2). To verify that the fitting procedure has reached global minimum of Δ, the minimization procedure was run numerous times with random initial values of the search parameters.

Molecular modelling

Calculations of the spatial structures of 1:1 TPT–CAF complex were carried out by the methods of molecular

$$\begin{cases} \delta_X = \frac{x_1}{x_0}\left[\begin{array}{l}\delta_{mX}\left(2(1+K_X x_1) - \frac{1}{(1-K_X x_1)^2}\right) + 2\delta_{dX}\left(\frac{1}{(1-K_X x_1)^2} - 1 - K_X x_1\right) \\ \quad +\delta_{hX}\frac{K_h y_1}{(1-K_X x_1)^2(1-K_Y y_1)}\left(1 + \frac{K_h y_1}{2(1-K_Y y_1)}\right)\end{array}\right] \\ \delta_Y = \frac{y_1}{y_0}\left[\begin{array}{l}\delta_{mY}\left(2(1+K_Y y_1) - \frac{1}{(1-K_Y y_1)^2}\right) + 2\delta_{dY}\left(\frac{1}{(1-K_Y y_1)^2} - 1 - K_Y y_1\right) \\ \quad +\delta_{hY}\frac{K_h x_1}{(1-K_Y y_1)^2(1-K_X x_1)}\left(1 + \frac{K_h y_1}{1-K_Y y_1}\right)\end{array}\right] \end{cases} \qquad (2)$$

where δ_{hX} and δ_{hY} are the chemical shifts of X or Y protons in the hetero-complex, respectively, and x_0 and y_0 are the total concentrations. The values of δ_m and δ_d and the equilibrium self-association constants are known from self-association studies of TPT (Bocian et al. 2004) and CAF (Davies et al. 2001). The monomer concentrations, x_1 and y_1, may be derived from the solution of the mass conservation law for Scheme 1:

mechanics using X-PLOR software [version 3.851 (Brunger 1992) with the Charmm22 force field]. The initial structure of the 1:1 complex was built up using the values of magnetic shielding, $\Delta\delta = \delta_m - \delta_h$, calculated from the titration curves. Modelling of the water–salt environment of the intercalated complexes was carried out using 1,100 TIP3P water molecules (Jorgensen et al. 1983) placed in a rectangular box (1,100 molecules). The

$$\begin{cases} x_0 = \frac{x_1}{(1-K_X x_1)^2}\left[1 + K_h\frac{y_1}{1-K_Y y_1} + \frac{K_h^2}{2}\frac{y_1^2}{(1-K_Y y_1)^2} + K_h^2\frac{x_1 y_1}{(1-K_Y y_1)(1-K_X x_1)}\right] \\ y_0 = \frac{y_1}{(1-K_Y y_1)^2}\left[1 + K_h\frac{x_1}{1-K_X x_1} + \frac{K_h^2}{2}\frac{x_1^2}{(1-K_X x_1)^2} + K_h^2\frac{x_1 y_1}{(1-K_X x_1)(1-K_Y y_1)}\right] \end{cases} \qquad (3)$$

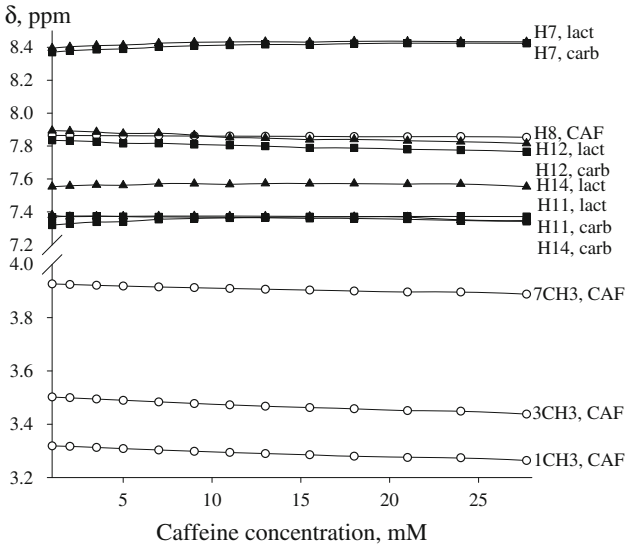

Fig. 2 Dependence of ^{1}H NMR chemical shifts of the non-exchangeable protons of topotecan and caffeine on the concentration of caffeine in Na-phosphate aqueous buffer solution, pD 7.1, $T = 298$ K. Protons of topotecan in the lactone (lact) and carboxylate (carb) forms are differentiated

topology of the TPT and CAF molecules and parameterization of their atomic interactions were created by the XPLO2D programme (Kleywegt 1998) using crystal structures from the PDB data bank. Parameters of non-valent interactions corresponded to the MM3 force field (Allinger 1977).

Results and discussion

As a result of investigation of the dependence on the concentration of added CAF of the percentage of apoptotic cells observed in HL-60 cell cultures treated with TPT [see Fig. 3a in Traganos et al. (1993)], it was found that caffeine exerts a concentration-dependent protector effect against the toxic action of TPT on the cell culture. These observations were explained in terms of the consequence of hetero-association between TPT and CAF, which lowers the fraction of 'topoisomerase I—DNA'-bound topotecan and thereby decreases its toxicity. Although it was thought for quite a long time that the TPT molecule does not bind with DNA alone but requires the presence of the enzyme, it has been clearly demonstrated (Yao et al. 1998) that TPT binds with DNA specifically in the lactone form in the absence of the enzyme. This stimulated a series of investigations of TPT-DNA systems [see for instance Mazzini et al. (2004); Streltsov et al. (2001) and Bocian et al. (2008)] eventually leading to general recognition that TPT binding with DNA has its own significance. Hence, the principal aim of the present work is to explore the possibility of describing the experimental apoptosis–concentration curve from Traganos et al. (1993) using a model (Evstigneev et al. 2008; Evstigneev 2010) based on the assumption that non-covalent complexations between TPT, CAF and DNA are the main determinants of the observed change in biological effect of the antibiotic on addition of CAF.

General model of competitive binding in the *X*–*Y*–DNA system

Let the *X* ligand be the main drug, i.e. topotecan, exerting its biological effect by complexation with DNA, and the *Y* ligand be the 'interceptor/protector' molecule, i.e. caffeine, which is able to bind non-covalently with both DNA and the *X* ligand. The hetero-association *X*–*Y* lowers the concentration of free molecules of *X* in solution, and so the biological response of drug *X* changes on addition of *Y*. This hetero-association process is termed the 'interceptor' mechanism. In addition, *Y* may complex with DNA, block the potential binding sites available for *X* molecules resulting in lowering of the fraction of *X*–DNA complexes in the overall dynamic equilibrium in solution and cause a change in the biological response of drug *X*; this process is termed the 'protector' mechanism. The strengths of the hetero-association, and *X*- and *Y*-binding with DNA, are characterized by equilibrium binding constants K_h, K_{XN} and K_{YN}, which can be measured independently in physico-chemical experiments.

An important point of the model is the choice of DNA receptor in the analysis. The distribution and the concentration of free-from-histones regions of nuclear DNA, N_0, available for *X* and *Y* binding, are typically unknown, and so it was suggested to represent N_0 in terms of short DNA fragments, which allows for non-cooperative binding of *X* and/or *Y* ligands to any set of such fragments neighbouring within the free-from-histones DNA sequence. For aromatic intercalators, typically three adjacent nucleotide sites are blocked and the cooperativity of overall binding is not pronounced, which enables a tetramer sequence to be the smallest binding fragment of DNA, and so N_0 represents the concentration of tetramers.

It was also suggested that the 'protector' and 'interceptor' mechanisms could be quantified using the criterion R_D, the relative decrease in proportion of *X*-DNA complexes on addition of the ligand *Y* as summarized in Eq. (4). R_D is calculated for two limiting situations:

– The condition of 'switched-off' X–Y hetero-association and 'switched-on' complexation of Y with DNA ($K_h = 0$, $K_{YN} \neq 0$), i.e. $f^X_{C(C)}$, and
– The condition of 'switched-on' X–Y hetero-association and 'switched-off' complexation of Y with DNA ($K_h \neq 0$, $K_{YN} = 0$), i.e. $f^X_{C(h)}$:

$$R_{\mathrm{D}} = \frac{f^X_{C(0)} - f^X_{C(C)}}{f^X_{C(0)} - f^X_{C(h)}}, \tag{4}$$

where $f^X_{C(0)}$ is the mole fraction of X–DNA complexes with 'switched-off' hetero-association and Y–DNA complexation. The range $R_{\mathrm{D}} > 1$ corresponds to predominance of Y–DNA complexation over X–Y hetero-association (i.e. the 'protector' action of ligand Y), and $R_{\mathrm{D}} < 1$ corresponds to hetero-association being the major contribution to the displacement of drug molecules from DNA (i.e. the 'interceptor' action of ligand Y).

An estimate of the amount of drug X displaced from DNA due to the presence of Y ligand can be made using the quantity A_{D}, which corresponds to the relative amount of X molecules removed from DNA on addition of Y,

$$A_{\mathrm{D}} = \frac{f^X_{C(0)} - f^X_C}{f^X_{C(0)}}, \tag{5}$$

where f^X_C is the relative amount of drug X bound to DNA in the presence of Y.

As long as the drug X exerts its biological action mainly via complexation with DNA, and so, within the current model, the quantity A_{D} may be used as an indication of the biological effect of the drug X in the three-component system, X–Y–DNA.

The final key point of the model is the choice of concentrations of the components in three-component mixtures. It was suggested to use a set of quasi-physiological concentrations for x_0 and N_0, and y_0 varied over a vast range of concentrations in order to represent the concentration-dependent protector effect:

$$x_0 = 10\ \mu\mathrm{M}, \quad y_0\text{—var}, \quad N_0 = 10\ \mu\mathrm{M}. \tag{6}$$

The concentrations in (6) were defined against in vitro data on detoxification of cultured leukaemia cell lines, containing the antibiotics mitoxantrone (NOV) and doxorubicin (DOX), by caffeine added at a particular concentration: $y_0 \approx 5$ mM (Evstigneev et al. 2006a). It is important to note that mitoxantrone and doxorubicin, as well as topotecan, are currently considered as topoisomerase inhibitors (Traganos et al. 1991; Liu et al. 2000; Dezhenkova et al. 2008), which indirectly supports the appropriateness of using a similar estimated value of N_0 for TPT, DOX and NOV. Nevertheless, the availability of the concentration-dependent dataset for the TPT–CAF system (Traganos et al. 1993) provides an opportunity to test the appropriateness of the concentrations (6) not only for topotecan but also for a wide concentration range of the interceptor molecule. For now, it is assumed that the quasi-physiological concentrations (6) are appropriate for the TPT–CAF–DNA system. The sensitivity of the model to the choice of concentrations will be tested below.

To estimate the key quantities (R_{D}, A_{D}) of the model, it is also necessary to determine the equilibrium binding constants, K_h, K_{XN} and K_{YN}, for this system.

Determination of the equilibrium binding constants in the TPT–CAF–DNA system

Determination of the binding constants requires knowledge of the DNA binding site of topotecan. Unfortunately, the DNA binding properties of topotecan are currently a matter of debate, and therefore, the TPT-DNA complexation constant, K_{XN}, is difficult to determine precisely; for example, it has been suggested that the binding of TPT with oligonucleotides occurs by external stacking to the flanking base pairs (Bocian et al. 2004; Mazzini et al. 2004), whereas the binding to polymeric DNA occurs by non-intercalative external mode (Streltsov et al. 2001) or classical intercalation (Lipfert et al. 2010). Recently, binding of TPT to nicked DNA was reported (Bocian et al. 2008), which is thought to be a useful model of the cleavable topoisomerase I—DNA complex and, in our view, more closely represents the native-like conditions of TPT action in vitro. It is therefore reasonable to take $K_{XN} = 3{,}800\ \mathrm{M}^{-1}$ for 'TPT-nicked DNA' complexation (Bocian et al. 2008). Importantly, this constant reflects the binding of only the lactone form of TPT, as it has been recognized that the carboxylate form of TPT has much weaker affinity to DNA and little, if any, biological effect (Yao et al. 1998). Once the nicked DNA is selected as a potential target for the TPT binding, it follows that the quantity N_0 in Eq. (6) represents now the concentration of nicked sites.

The caffeine complexation constant to nicked sites of DNA, K_{YN}, is not known from literature; however, it was previously measured by NMR with respect to DNA tetranucleotide, i.e. $K_{YN} = 246\ \mathrm{M}^{-1}$ (Davies et al. 2001). Tetranucleotide is less stiff in terms of formation of the binding site as compared with regular polymeric DNA, which is manifested by typically lower magnitudes of drug–tetranucleotide binding constants with respect to polymeric DNA binding constants for the same drugs (Davies et al. 2001). For this reason, the order of the CAF affinity to the nicked DNA site and to the DNA tetranucleotide may be assumed equal. Hence, as an initial approximation, for the CAF–DNA complexation constant, K_{YN}, it is reasonable to take the value $K_{YN} = 246\ \mathrm{M}^{-1}$

derived from drug–tetranucleotide studies and also used previously in analysis of CAF–drug–DNA systems (Davies et al. 2001; Evstigneev et al. 2006a, 2008). The appropriateness of such an approximation is also supported by the fact that the hetero-association constants between CAF and aromatic drugs, mimicking the unstacked DNA bases in the nicked site, on average, are close in value to $K_{YN} = 246\ M^{-1}$ (Evstigneev 2010).

The equilibrium constant of TPT–CAF hetero-association, K_h, is not available in the literature and was determined in the present work by means of ^{1}H NMR spectroscopic measurements (see "Materials and methods"). The unambiguous global minimum of the discrepancy between experimental and calculated chemical shifts and reliable values of the search parameters, δ_h and K_h, were obtained only for the CAF protons. A similar numerical analysis of the TPT protons requires determination of the concentration of lactone and carboxylate forms at each concentration point. However, there is partial overlap of their signals in the 1D ^{1}H NMR spectra, and any derived results would be far less reliable than those for CAF protons. The results of calculations are presented in Table 1.

It can be seen in Table 1 that the hetero-association parameters for the TPT–CAF system follow the same pattern as previously reported for other CAF–drug systems (Davies et al. 2001; Evstigneev 2010), i.e. the magnitude of the hetero-association constant, K_h, is in between the values for the self-association constants of TPT (K_X) and CAF (K_Y) molecules. The shielding of CAF protons, $\Delta\delta_{hY} = \delta_{mY} - \delta_{hY}$, is positive, which is an indication of the ring-current effect, commonly observed in π-stacked complexes (Evstigneev 2010). Additional support for this conclusion comes from analysis of the titration curves for TPT aromatic protons (see Fig. 2) which follow the same trend (upfield shift on CAF concentration increase) as do the CAF protons. Molecular modelling calculations of the 1:1 TPT–CAF complex, performed in water, suggest that CAF molecule is located over the A and B rings of the TPT chromophore (Fig. 3) and is nearly parallel to it. Such an orientation of the interacting molecules in the hetero-complex appears to be favourable in terms of both hydrophobic and van der Waals interactions.

Table 1 Parameters of self- and hetero-association of topotecan (X) with caffeine (Y) at $T = 298$ K

CAF proton	δ_{hY}, ppm	δ_{mY}, ppm[a]	δ_{dY}, ppm[a]	Equilibrium constant, M^{-1}
H8	7.72	7.89	7.83	$K_X = 3{,}800 \pm 1{,}000$[a]
7Me	3.82	3.95	3.82	$K_Y = 11.8 \pm 0.3$[b]
3Me	3.30	3.54	3.33	$K_h = 320 \pm 30$
1Me	3.15	3.35	3.16	

[a] Estimated from the equilibrium constant at $T = 308$ K reported for the lactone form (Bocian et al. 2004). Independent ^{1}H NMR measurements of the self-association of TPT carboxylate form have shown that K_X for lactone and carboxylate forms are similar within the error of their determination (unpublished result)

[b] Taken from Davies et al. (2001)

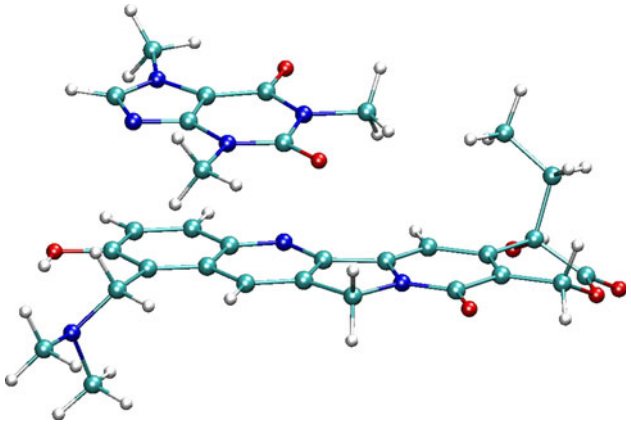

Fig. 3 Calculated spatial structure of the topotecan–caffeine 1:1 hetero-complex

Description of competitive binding in the TPT–CAF–DNA system

The general model of the X–Y–DNA equilibrium, outlined above, as well as other models available in literature (Pietrzak et al. 2006), are based on the assumption that only three main components (drug, interceptor and DNA) form the dynamic equilibrium in solution. Under physiological conditions topotecan (X) exists as a mixture of the lactone (L) and carboxylate (C) forms, which makes the system four-component. Although the C-form of TPT is known to exert no biological effect in vitro and does not bind with DNA (Yao et al. 1998; Bocian et al. 2008), it can still interact with CAF (Y) and lower the effective concentration of the interceptor itself. Let us further consider the application of the general three-component competitive binding model to the four-component system TPT–CAF–DNA (L-C-Y-N).

The key assumptions of the L-C-Y-N model are the following:

(i) The C-form does not bind with DNA; the L-form binds with DNA with an equilibrium complexation constant $K_{LN} = 3{,}800\ M^{-1}$ and exerts a biological effect proportional to the fraction of LN complexes;

(ii) The DNA receptor is taken to be a mixture of short DNA fragments (at least tetramer or higher), allowing for non-cooperative binding of the *L*-form in 1:1 stoichiometry;

(iii) The total concentrations of *L*- and *C*-forms, $L_0 + C_0 = 10$ μM, and the DNA receptor, $N_0 = 10$ μM, correspond to quasi-physiological conditions (6). Let us initially take the interrelation between *L*- and *C*-forms as 1:1, which corresponds to conditions close to physiological (Yao et al. 1998);

(iv) The concentration of the interceptor molecule *Y* is varied over a wide range from μM to an upper limit, mM, at which the protector effect is observed in vitro (Traganos et al. 1993). Concentrations in the millimolar range require that indefinite self-association of the interceptor molecule, Y_i, needs to be considered, whereas micromolar concentrations of TPT rule out any self-association of the *L*- and *C*-forms or interactions between them, *LC*. Such conditions restrain the set of the most probable compositions of hetero-complexes between *L*, *C* and *Y* molecules to the four main ones: LY_i, CY_i, Y_jLY_i and Y_jCY_i.

These assumptions enable the basic scheme of dynamic equilibrium in *L*-*C*-*Y*-*N* solution to be written

self-association

$$Y + Y_i \overset{K_Y}{\leftrightarrow} Y_{i+1}$$

hetero-association

$$L + Y_i \overset{K_{LY}}{\leftrightarrow} LY_i, \quad LY_i + Y_j \overset{K_{LY}}{\leftrightarrow} Y_jLY_i \qquad (7)$$

$$C + Y_i \overset{K_{CY}}{\leftrightarrow} CY_i, \quad CY_i + Y_j \overset{K_{CY}}{\leftrightarrow} Y_jCY_i$$

DNA complexation

$$L + N \overset{K_{LN}}{\leftrightarrow} LN, \quad Y + N \overset{K_{YN}}{\leftrightarrow} YN,$$

where *i* and *j* are the numbers of interceptor molecules in the aggregates.

The hetero-association constants, K_{LY} and K_{CY}, between caffeine and the lactone/carboxylate forms are unlikely to be significantly different, and it is reasonable to make them equal to the hetero-association constant K_h determined from the ^{1}H NMR data in solution, i.e. $K_{LY} = K_{CY} = K_h = 320\ \mathrm{M}^{-1}$. The appropriateness of such an assumption is based on the fact that the *L*- and *C*-forms self-associate with similar equilibrium constants (see footnote in Table 1), and the CAF binding occurs to the A/B rings of the TPT molecule (see Fig. 3), which are minimally affected by the ring-opening process during lactone–carboxylate interconversion.

The mass conservation law, associated with Scheme 7, can be written as

$$\begin{cases} C_0 = C_1 + \sum_{i=1}^{\infty}[CY_i] + \sum_{i=1}^{\infty}\sum_{j=1}^{\infty}[Y_jCY_i] \\ L_0 = L_1 + \sum_{i=1}^{\infty}[LY_i] + \sum_{i=1}^{\infty}\sum_{j=1}^{\infty}[Y_jLY_i] + [LN] \\ Y_0 = \sum_{i=1}^{\infty} i[Y_i] + \sum_{i=1}^{\infty} i[CY_i] + \sum_{i=1}^{\infty} i[LY_i] \\ \qquad + \sum_{i=1}^{\infty}\sum_{j=1}^{\infty}(i+j)[Y_jCY_i] + \sum_{i=1}^{\infty}\sum_{j=1}^{\infty}(i+j)[Y_jLY_i] + [YN] \\ N_0 = N_1 + [LN] + [YN] \end{cases} \qquad (8)$$

where square brackets […] denote concentrations of particular types of complexes; the subscripts '0' or '1' denote total or monomeric concentrations, respectively.

Sums appearing in Eqs. (8) and corresponding to the complexes with one hetero-stack can be reduced to indefinite geometrical progressions, which allows them to be deconvoluted analytically:

$$\sum_{i=1}^{\infty}[Y_i] = \sum_{i=1}^{\infty}K_Y^{i-1}Y_1^i = \frac{Y_1}{1-K_Y\cdot Y_1}, \qquad \sum_{i=1}^{\infty} i[Y_i] = \sum_{i=1}^{\infty} iK_Y^{i-1}Y_1^i = \frac{Y_1}{(1-K_Y\cdot Y_1)^2}$$

$$\sum_{i=1}^{\infty}[CY_i] = K_h\cdot C_1\cdot\sum_{i=1}^{\infty}[Y_i] = \frac{K_h\cdot C_1\cdot Y_1}{1-K_Y\cdot Y_1}, \quad \text{by analogy: } [LY_i] = \frac{K_h\cdot L_1\cdot Y_1}{1-K_Y\cdot Y_1}$$

$$\sum_{i=1}^{\infty} i[CY_i] = K_h\cdot C_1\cdot\sum_{i=1}^{\infty} i[Y_i] = \frac{K_h\cdot C_1\cdot Y_1}{(1-K_Y\cdot Y_1)^2}, \quad \text{by analogy: } \sum_{i=1}^{\infty} i[LY_i] = \frac{K_h\cdot L_1\cdot Y_1}{(1-K_Y\cdot Y_1)^2} \qquad (9)$$

Deconvolution of the complexes with two hetero-stacks (Y_j LY_i, Y_j CY_i) in Eqs. (8) is less straightforward. It has been shown (Veselkov et al. 2001) that the double summation over such types of complexes results in overestimation of their contribution to the dynamic equilibrium in solution due to double-counting of physically equivalent 'reflected' complexes (for example, the Y_j *L* Y_i complex and its 'reflected' analogue, Y_i *L* Y_j, are equivalent). The suggested method of correction for the double summation of the 'reflected' complexes (Veselkov et al. 2001) was used in the present work to deconvolute the double summation in Eqs. (8) (subscripts '*non-corr*' and '*corr*' stand for non-corrected and corrected sums, respectively):

$$\sum_{i=1}^{\infty}\sum_{j=1}^{\infty}[Y_jCY_i]_{non-corr} = \sum_{i=1}^{\infty}[Y_i]\cdot C_1\cdot\sum_{j=1}^{\infty}[Y_j] = \frac{K_h^2\cdot C_1\cdot Y_1^2}{(1-K_Y\cdot Y_1)^2}$$

$$\sum_{i=1}^{\infty}\sum_{j=1}^{\infty}[Y_jCY_i]_{corr} = \frac{1}{2}\cdot\left(\sum_{i=1}^{\infty}\sum_{j=1}^{\infty}[Y_jCY_i]_{non-corr} + \sum_{i=1}^{\infty}[Y_iCY_i]\right)$$

$$\sum_{i=1}^{\infty}[Y_iCY_i] = C_1\cdot K_h^2\cdot\left(Y_1^2 + K_Y^2\cdot Y_1^4 + K_Y^4\cdot Y_1^6 + \cdots\right) = \frac{C_1\cdot K_h^2\cdot Y_1^2}{1-K_Y^2\cdot Y_1^2}$$

The final equations for the corrected sums of the $\sum_{i=1}^{\infty}\sum_{j=1}^{\infty}[Y_jCY_i]$- and $\sum_{i=1}^{\infty}\sum_{j=1}^{\infty}[Y_jLY_i]$-type take the form

$$\sum_{i=1}^{\infty}\sum_{j=1}^{\infty}\left[Y_jCY_i\right]_{corr} = \frac{1}{2}\cdot\left[\frac{K_h^2\cdot C_1\cdot Y_1^2}{(1-K_Y\cdot Y_1)^2}+\frac{K_h^2\cdot C_1\cdot Y_1^2}{1-K_Y^2\cdot Y_1^2}\right] = \frac{K_h^2\cdot C_1\cdot Y_1^2}{1-K_Y\cdot Y_1-K_Y^2\cdot Y_1^2+K_Y^3\cdot Y_1^3}$$
$$\sum_{i=1}^{\infty}\sum_{j=1}^{\infty}\left[Y_jLY_i\right]_{corr} = \frac{K_h^2\cdot L_1\cdot Y_1^2}{1-K_Y\cdot Y_1-K_Y^2\cdot Y_1^2+K_Y^3\cdot Y_1^3} \quad (10)$$

For the final equations of the remaining summations in Eq. (8) of the $\sum_{i=1}^{\infty}\sum_{j=1}^{\infty}(i+j)\left[Y_jCY_i\right]$- and $\sum_{i=1}^{\infty}\sum_{j=1}^{\infty}(i+j)\left[Y_jLY_i\right]$-types it is more convenient to use a partition function formalism:

$$\sum_{i=1}^{\infty}\sum_{j=1}^{\infty}(i+j)\left[Y_jCY_i\right]_{corr} = \frac{\partial\sum_{i=1}^{\infty}\sum_{j=1}^{\infty}\left[Y_jCY_i\right]_{corr}}{\partial\ln Y_1} = \frac{K_h^2\cdot C_1\cdot Y_1^2\cdot\left(K_Y^2\cdot Y_1^2+K_Y\cdot Y_1+2\right)}{(1-K_Y\cdot Y_1)^3(1+K_Y\cdot Y_1)^2}$$
$$\sum_{i=1}^{\infty}\sum_{j=1}^{\infty}(i+j)\left[Y_jLY_i\right]_{corr} = \frac{\partial\sum_{i=1}^{\infty}\sum_{j=1}^{\infty}\left[Y_jLY_i\right]_{corr}}{\partial\ln Y_1} = \frac{K_h^2\cdot L_1\cdot Y_1^2\cdot\left(K_Y^2\cdot Y_1^2+K_Y\cdot Y_1+2\right)}{(1-K_Y\cdot Y_1)^3(1+K_Y\cdot Y_1)^2} \quad (11)$$

Equations (9–11) enable the mass conservation law (8) to be written as

$$\begin{cases} C_0 = C_1+\dfrac{K_hC_1Y_1}{1-K_YY_1}+\dfrac{K_h^2C_1Y_1^2}{1-K_YY_1-K_Y^2Y_1^2+K_Y^3Y_1^3} \\ L_0 = L_1+\dfrac{K_hL_1Y_1}{1-K_YY_1}+\dfrac{K_h^2L_1Y_1^2}{1-K_YY_1-K_Y^2Y_1^2+K_Y^3Y_1^3} \\ \quad +K_{LN}L_1N_1 \\ Y_0 = \dfrac{Y_1}{(1-K_YY_1)^2}+\dfrac{K_hY_1}{(1-K_YY_1)^2}(C_1+L_1) \\ +\dfrac{K_h^2Y_1^2\left(K_Y^2Y_1^2+K_YY_1+2\right)}{(1-K_YY_1)^3(1+K_YY_1)^2}(C_1+L_1)+K_{YN}Y_1N_1 \\ N_0 = N_1+K_{LN}L_1N_1+K_{YN}Y_1N_1 \end{cases} \quad (12)$$

The system of equations (12) provides the basis of the method of quantification of competitive binding of topotecan and caffeine with DNA. Its solution against the monomer concentrations L_1, C_1, Y_1 and N_1 enables the mole fractions $f_C^X = \frac{K_{LN}L_1N_1}{L_0}$ of the DNA-bound lactone form of TPT to be computed under different circumstances defined in Eqs. (4, 5), and finally to compute the key quantities R_D and A_D.

Using the set of concentrations defined above, the results of computation of R_D and A_D are given in Fig. 4. The A_D curve demonstrates a saturation profile when approaching ca. 10 mM concentration of CAF, which agrees with the level of detoxification of cell culture under the same concentrations of the interceptor molecule (Traganos et al. 1993). The R_D curve changes insignificantly in the whole range of y_0 and lies in the region $R_D < 1$. This result means that the interceptor mechanism (the hetero-association TPT–CAF) dominates in the net removal of the TPT drug from DNA on addition of CAF, which is in general agreement with the same qualitative conclusion made previously (Traganos et al. 1993). However, the previous work totally neglected the protector mechanism (competition of CAF and TPT for DNA binding sites), assuming that the binding of TPT with the enzyme–DNA complex is not affected by competition with CAF. As discussed above,

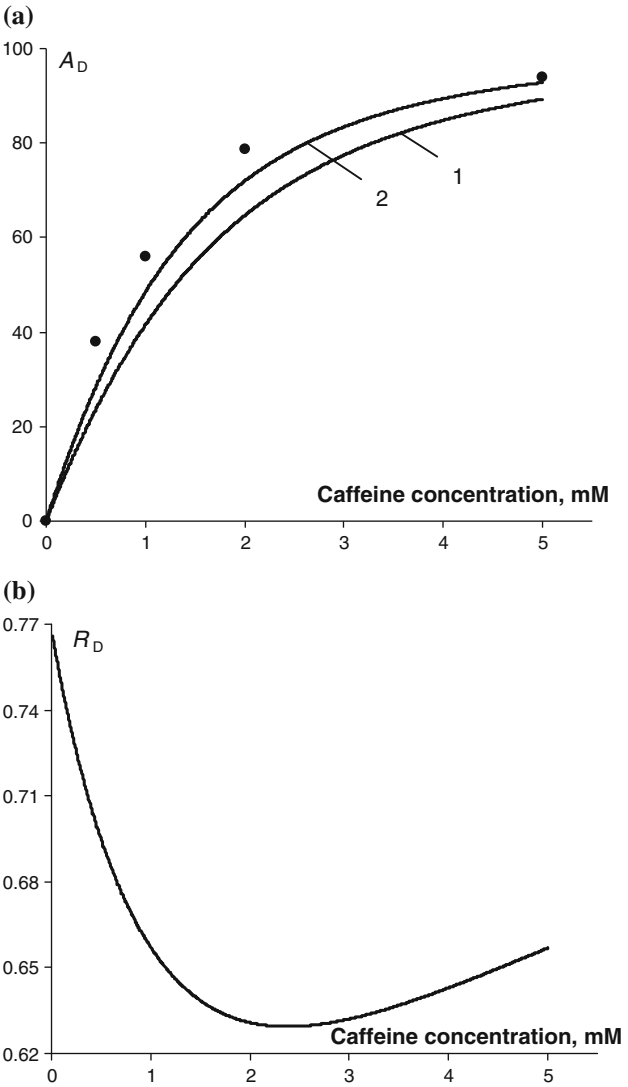

Fig. 4 Dependence on caffeine concentration of **a** the A_D factor [*solid points* represent experimental A_D factor, A_D^{exp}, recalculated using Eq. (13); curve 1 is a theoretical A_D factor calculated using Eq. (5) with $K_h = 320\ M^{-1}$, $K_{YN} = 246\ M^{-1}$; curve 2 is a theoretical A_D factor calculated using Eq. (5) with $K_h = 400\ M^{-1}$, $K_{YN} = 300\ M^{-1}$], and **b** R_D factor

the biological effect of TPT may be exerted at the DNA level, which is also being targeted by CAF, and, hence, competition for DNA binding sites may occur. It is shown in Fig. 4b that in the millimolar range $R_D \approx 0.6$, which approximately corresponds to a 40:60 ratio of the contributions of the protector and interceptor mechanisms to the net removal of TPT from DNA, and so consideration of the competition with CAF is important for correct evaluation of the mutual binding of aromatic drugs to DNA.

It is interesting that, for the majority of aromatic drugs studied before (Evstigneev et al. 2006a, 2008; Evstigneev 2010), the protector mechanism dominates [excluding chlorophyllin, which does not bind with DNA and/or does not enter through cell membrane, thereby exerting purely interceptor mechanism (Pietrzak et al. 2008)] and the predominance of the interceptor mechanism was only observed for the antibiotic mitoxantrone (NOV) (Evstigneev et al. 2006a, 2008; Evstigneev 2010). For both systems it is found that K_h for the TPT–CAF and NOV–CAF systems are the highest compared with other aromatic drugs investigated (Evstigneev et al. 2008; Evstigneev 2010).

Quantification of the change in biological effect of topotecan on addition of caffeine

To ascertain whether the quantity A_D may be used to predict the change in the biological effect of topotecan on addition of caffeine in vitro, the corresponding experimental apoptosis–concentration curve, $A(y_0)$, measured in human leukaemia cell line HL-60 was taken from Table 3 (Fig. 3a) in Traganos et al. (1993) (see also Table 2 in the present work). However, these data cannot be used straightforwardly for comparison with A_D. $A(y_0)$ is a measure of the 'absolute' biological effect in the sense that it always contains the intrinsic biological activity of the drug itself, I_{drug}, in the absence of CAF ($I_{drug} = I_{TPT} = 33\%$ in Table 2), whereas A_D is a net removal of the drug on addition of the interceptor and does not contain any intrinsic biological activity of the drug, thereby representing the biological effect 'relative' to I_{drug}.

Table 2 Apoptosis–concentration curve, $A(y_0)$, and the A_D factor

y_0, mM	$A(y_0)$, %*	A_D^{exp}, %
0	33	0
0.5	20.5	37.9
1	14.5	56.1
2	7	78.8
5	2	93.9

* Taken from Traganos et al. (1993)

To normalize the $A(y_0)$ curve against I_{TPT} we follow the previously formulated procedure (Evstigneev et al. 2008):

- Denoting $A(y_0) = I_{TPT-CAF}$ it is possible to compute the percentage of detoxification of the cell culture on addition of CAF as $I_{TPT} - I_{TPT-CAF}$;
- The detoxification must then be normalized against I_{TPT}, giving the experimental A_D factor:

$$A_D^{exp} = \frac{100\%}{I_{TPT}}(I_{TPT} - I_{TPT-CAF}). \quad (13)$$

The A_D^{exp} factor calculated using Eq. (13) (Table 2) can now be compared with the theoretical A_D calculated from Eq. (5) (see also Fig. 4a).

It can be seen in Fig. 4a that the mean discrepancy between the theoretical (curve 1) and experimental (solid points) A_D averaged over four experimental points is about 12% (in A_D units), and that the theoretical curve is systematically lower than the experimental. Taking into account the complexity of the systems studied (the search for a quantitative link between a biological effect and physico-chemical parameters of molecular interaction), this result is good enough to state that the model and the parameters used in the analysis represent in vitro conditions of TPT–CAF mutual binding with DNA. The question is: what might be the source of the systematic discrepancy? The model used is purely physico-chemical; i.e. it utilizes only physico-chemical parameters of interaction in order to describe the relative biological effect. The key 'biological' assumption is that the fraction of the drug removed from DNA by CAF is proportional to the change in biological effect. It follows that the ability of the model to describe the apoptosis–concentration curve even in relative units must depend on (i) the equilibrium complexation constants (K_h, K_{LN} and K_{YN}), (ii) the concentrations of components (L_0, C_0 and N_0) and (iii) the type of aromatic and/or interceptor molecule used. It would be quite natural to always question the correspondence between the quantities measured in separate physico-chemical experiment and those in real cells. These three issues are discussed in more detail below:

- The dependence on L_0, C_0 and N_0

Previously, for various CAF–drug (Evstigneev et al. 2006a, d, 2008) and vitamin–drug (Evstigneev et al. 2005, 2008) systems, we showed that an order of magnitude variation of the drug and DNA concentrations, x_0 and N_0, in incremental or decremental directions, affects the A_D curve by less than 10%. This result was also confirmed for the TPT–CAF system in the present work, viz. the variation of x_0 or N_0 at $y_0 = 2$ mM within the range 0.001–0.1 mM resulted in less than 7% change in A_D and

even less for R_D, which is insignificant. This result is considered to be very important because, as discussed above, the concentration of nicked DNA sites, N_0, as the targets for TPT binding, and the antibiotic concentration, x_0, are difficult to estimate. Such a property of the proposed analytical scheme, however, is not unexpected because the computation of A_D with respect to the intrinsic biological activity of the drug (which strongly depends on x_0) as in Eq. (13), makes the dependence on x_0 for A_D itself naturally weak. This means that the error in estimation of the total concentration $L_0 + C_0$ and the interrelation between *L*- and *C*-forms does not strongly affect the quality of the A_D^{exp} description [note that A_D^{exp} was computed from the published data (Traganos et al. 1993) measured for TPT concentration of 0.15 μM, whereas the quasi-physiological concentration of the drug used in the present work is $L_0 + C_0 = 10$ μM. The concentration 0.15 μM cannot be used directly in this analysis because it may underestimate the real in-cell concentration of the drug (Evstigneev et al. 2005, 2006a; Evstigneev 2010). As for the N_0 parameter, it is worth pointing out the model study (Evans et al. 2004) of TPT in vitro kinetics, which resulted in concentration of DNA sites available for TPT binding, $B_T = 29$ μM, close to that used in the present work. It is also important to note that the same set of quasi-physiological parameters (Eq. 6) was also previously utilized in the analysis of doxorubicin–caffeine and mitoxantrone–caffeine systems (Evstigneev et al. 2006a, 2008), which led to results agreeing with experiment. It follows that the set of quasi-physiological concentrations appears to be virtually independent of the system studied, hence the approach used in the present work may have general application to the class of DNA-targeting aromatic drugs;

– The dependence on K_h, K_{LN} and K_{YN}

The difference in the K_{LN} constant from 3,800 M^{-1} (used in the present work) up to 20,000 M^{-1} measured for TPT binding with oligonucleotides (Mazzini et al. 2004) leads to less than a 5% shift in A_D and even less for R_D, and therefore is considered insignificant, whereas the dependence on K_h and K_{YN} is the most pronounced. The latter is expected, as both constants directly characterize the interceptor and protector mechanisms. If K_h and K_{YN} are changed within the typical 20% error of their determination, viz. $K_h = 400$ M^{-1} and $K_{YN} = 300$ M^{-1}, there is an average discrepancy of 6% between the experimental and theoretical A_D curves (see Fig. 4a, curve 2), which is a very good result. The fact that these constants, when used in the competitive binding model, give good correspondence with biological experiment suggests that the complexation parameters measured in independent physico-chemical experiments may be used to quantify the in vitro biological data. However, as shown above, it is very important that these constants are measured with high accuracy. The estimation, $K_{YN} = 246$ M^{-1}, used by us is very approximate and requires further experimental adjustment;

– The assumption of proportionality between the relative biological effect and the mole fraction A_D of drug removed from DNA on addition of interceptor

We can point to the results of work (Dashwood and Guo 1993) reporting a correlation between the antimutagenic potency of chlorophyllin (CHL) against the action of various DNA-targeting aromatic carcinogens (IQ), and the hetero-association constant between them. K_h is a measure of the fraction of IQ molecules intercepted by CHL and thereby inaccessible for DNA binding and exerting the mutagenic activity (as already discussed above, CHL does not exert the protector mechanism). Indirectly this confirms the assumption of proportionality between the quantity of drug–DNA complexes and relative biological effect.

In summary, it may be concluded that the good correspondence between the experimental and theoretical estimation of the relative change in biological effect in the TPT–CAF system, found in the present work, is not due to a lucky set of numbers used as input, but reflects the pattern noted before for other aromatic ligands targeting DNA (Dashwood and Guo 1993; Traganos et al. 1991, 1993; Munoz et al. 1995; Ramu et al. 2000; Bedner et al. 2001; Davies et al. 2001; Piosik et al. 2002, 2003; Pietrzak et al. 2006, 2008; Evstigneev et al. 2005, 2006a, b, c, d, 2008; Hernandez Santiago et al. 2009; Osowski et al. 2010; Evstigneev 2010; Woziwodska et al. 2011). Hence, the general model and its parameterization used in the present work may potentially have general application to the description of relative change in biological effect in other drug–interceptor–DNA systems.

Conclusions

There are two principal outcomes of the present work:

(i) Using published in vitro data on the dependence of the percentage of apoptosis induced by the drug topotecan in a leukaemia cell line on the concentration of caffeine, and the general model of competitive binding in a system containing two aromatic drugs and DNA (Evstigneev et al. 2008; Evstigneev 2010), it has been possible to quantify the relative change in biological effect just using a set of component concentrations unspecific to the drug–interceptor combination, and equilibrium constants of drug–interceptor complexation. This is probably the first

example of the establishment of a quantitative interrelation between parameters measured in independent physico-chemical experiments and in vitro biological experiments for the class of DNA-binding drugs;

(ii) It is proposed that the general model of competitive binding and the parameterization of that model may potentially be applied to any system of DNA-targeting aromatic drugs under in vitro conditions. The main reason is the general feature of aromatic drug binding to DNA and complexation in physiological media via hetero-association. It is, however, important to note that the model of competitive binding will work only if the protector and interceptor mechanisms are significant in the net biological effect of the given drug.

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References

Allinger NL (1977) MM2. A hydrocarbon force field utilizing V1 and V2 torsional terms. J Am Chem Soc 99:8127–8134

Bedner E, Du L, Traganos F, Darzynkiewicz Z (2001) Caffeine dissociates complexes between DNA and intercalating dyes: application for bleaching fluorochrome-stained cells for their subsequent restraining and analysis by laser scanning cytometry. Cytometry 43:38–45

Bernacki RJ, Pera P, Gambacorta P, Brun Y, Greco WR (2000) In vitro antitumour activity of 9-nitro-camptothecin as a single agent and in combination with other antitumour drugs. Ann NY Acad Sci 922:293–297

Bocian W, Kawęcki R, Bednarek E, Sitkowski J, Pietrzyk A, Williamson MP, Hansen PE, Kozerski L (2004) Multiple binding modes of the camptothecin family to DNA oligomers. Chem Eur J 10:5776–5787

Bocian W, Kawęcki R, Bednarek E, Sitkowski J, Williamson MP, Hansen PE, Kozerski L (2008) Binding of topotecan to a nicked DNA oligomer in solution. Chem Eur J 14:2788–2794

Brunger AT (1992) X-PLOR, a system for X-PLOR crystallography and NMR. Yale University, Press

Dashwood R, Guo D (1993) Antimutagenic potency of chlorophyllin in the Salmonella assay and its correlation with binding constants of mutagen-inhibitor complexes. Env Mol Mutagen 22:164–171

Davies DB, Veselkov DA, Djimant LN, Veselkov AN (2001) Hetero-association of caffeine and aromatic drugs and their competitive binding with a DNA oligomer. Eur Biophys J 30:354–366

Dezhenkova LG, Teviashova AN, Olsuf'eva EN, Treshchalin ID, Shtil' AA, Preobrazhenskaia MN (2008) Anthracycline antibiotics and their derivatives—inhibitors of topoisomerase I. Bioorg Khim 34:430–432

Evans ND, Errington RJ, Shelley M, Feeney GP, Chapman MJ, Godfrey KR, Smith PJ, Chappell MJ (2004) A mathematical model for the in vitro kinetics of the anti-cancer agent topotecan. Math Biosci 189:185–217

Evstigneev MP (2010) DNA-binding aromatic drug molecules: physico-chemical interactions and their biological roles. Lambert Academic, Germany, p 96

Evstigneev MP, Mykhina YV, Davies DB (2005) Complexation of daunomycin with a DNA oligomer in the presence of an aromatic vitamin (B_2) determined by NMR spectroscopy. Biophys Chem 118:118–127

Evstigneev MP, Khomich VV, Davies DB (2006a) Complexation of anthracycline drugs with DNA in the presence of caffeine. Eur Biophys J 36:1–11

Evstigneev MP, Evstigneev VP, Davies DB (2006b) NMR investigation of the effect of caffeine on the hetero-association of an anticancer drug with a vitamin. Chem Phys Lett 432:248–251

Evstigneev MP, Evstigneev VP, Santiago AA, Davies DB (2006c) Effect of a mixture of caffeine and nicotinamide on the solubility of vitamin (B_2) in aqueous solution. Eur J Pharm Sci 28:59–66

Evstigneev MP, Rybakova KA, Davies DB (2006d) Complexation of norfloxacin with DNA in the presence of caffeine. Biophys Chem 121:84–95

Evstigneev MP, Lantushenko AO, Evstigneev VP, Mykhina YV, Davies DB (2008) Quantitation of the molecular mechanisms of biological synergism in a mixture of DNA-acting aromatic drugs. Biophys Chem 132:148–158

Hernandez Santiago AA, Andrejuk DD, Cervantes Tavera AM, Davies DB, Evstigneev MP (2009) Complexation of biologically active aromatic compounds with DNA in the presence of theophylline. J Biol Phys 35:115–126

Jorgensen W, Chaindrasekhar J, Madura J, Imprey R, Klein M (1983) Comparison of simple potential functions for simulating liquid water. J Chem Phys 79:926–935

Kleywegt GL (1998) Dictionaries for heteros. News Uppsala Softw Fact 5:4

Lipfert J, Klijnhout S, Dekker NH (2010) Torsional sensing of small-molecule binding using magnetic tweezers. Nucleic Acids Res 38:7122–7132

Liu LF, Desai SD, Li TK, Mao Y, Sun M, Sim SP (2000) Mechanism of action of camptothecin. Ann NY Acad Sci 922:1–10

Mazzini S, Bellucci MC, Dallavalle S, Fraternali F, Mondelli R (2004) Mode of binding of camptothecins to double helix oligonucleotides. Org Biomol Chem 2:505–513

Munoz MA, Carmona C, Hidalgo J, Guardado P, Balon M (1995) Molecular associations of flavins with betacarbolines and related indoles. Bioorg Med Chem 3:41–47

Osowski A, Pietrzak M, Wieczorek Z, Wieczorek J (2010) Natural compounds in the human diet and their ability to bind mutagens prevents DNA-mutagen intercalation. J Toxicol Environ Health Part A 73:1141–1149

Pietrzak M, Wieczorek Z, Wieczorek J, Darzynkiewicz Z (2006) The 'interceptor' properties of chlorophylline measured within the three-component system: intercalator-DNA-chlorophyllin. Biophys Chem 123:11–19

Pietrzak M, Halicka HD, Wieczorek Z, Wieczorek J, Darzynkiewicz Z (2008) Attenuation of acridine mutagen ICR-191-DNA interactions and DNA damage by the mutagen interceptor chlorophyllin. Biophys Chem 135:69–75

Piosik J, Zdunek M, Kapuscinski J (2002) The modulation by xanthines of the DNA-damaging effect of polycyclic aromatic agents. Part II. The stacking complexes of caffeine with doxorubicin and mitoxantrone. Biochem Pharmacol 63:635–646

Piosik J, Ulanowska K, Gwizdek-Wisniewska A, Czyz A, Kapuscinski J, Wegrzyn G (2003) Alleviation of mutagenic effects of polycyclic aromatic agents (quinacrine mustard, ICR-191 and ICR-170) by caffeine and pentoxifylline. Mutat Res 530:47–57

Pizzolato JF, Saltz LB (2003) The camptothecins. Lancet 361:2235–2242

Ramu A, Mehta MM, Liu J, Turyan I, Aleksic A (2000) The riboflavin-mediated photooxidation of doxorubicin. Cancer Chemother Pharmacol 46:449–458

Streltsov S, Sukhanova A, Mikheikin A, Grokhovsky S, Zhuze A, Kudelina I, Mochalov K, Oleinikov V, Jardillier JD, Nabiev I (2001) Structural basis of topotecan-DNA recognition probed by flow linear dichroism, circular dichroism, and Raman spectroscopy. J Phys Chem B 105:9643–9652

Traganos F, Kapuscinski J, Darzynkiewicz Z (1991) Caffeine modulates the effects of DNA-intercalating drugs in vitro: a flow cytometric and spectrophotometric analysis of caffeine interaction with novatrone, doxorubicin, ellipticine, and the doxorubicin analogue AD198. Cancer Res 51:3682–3689

Traganos F, Kapuscinski J, Gong J, Ardelt B, Darzynkiewicz RJ, Darzynkiewicz Z (1993) Caffeine prevents apoptosis and cell-cycle effects induced by camptothecin or topotecan in HL-60 cells. Cancer Res 53:4613–4618

Veselkov AN, Evstigneev MP, Veselkov DA, Davies DB (2001) A generalized NMR-derived statistical-thermodynamical model of hetero-association of aromatic molecules in aqueous solution. J Chem Phys 115:2252–2266

Woziwodska A, Gwizdek-Wisniewska A, Piosik J (2011) Caffeine, pentoxifylline and theophylline form stacking complexes with IQ-type heterocyclic aromatic amines. Bioorg Chem 39:10–17

Yao S, Murali D, Seetharamulu P, Haridas K, Petluru PN, Reddy DG, Hausheer FH (1998) Topotecan lactone selectively binds to double- and single-stranded DNA in the absence of topoisomerase I. Cancer Res 58:3782–3786