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ORIGINAL PAPER

Complexation of Biologically Active Aromatic Compounds with DNA in the Presence of Theophylline

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Abstract ^{1}H NMR measurements (500 MHz) have been used to determine the equilibrium hetero-association constants of theophylline (THP) with various biologically active aromatic compounds (daunomycin, novantrone, ethidium bromide, proflavine, norfloxacin) and the complexation constants of THP with both single- and double-stranded oligonucleotides in solution. The results provide a quantitative estimation of the effect of THP on the binding of aromatic ligands with DNA, and a determination of the fraction of aromatic ligand removed from DNA on addition of THP.

Keywords Theophylline · Caffeine · Competitive binding · Hetero-association · Interceptor mechanism · Protector mechanism

1 Introduction

Methylxanthines are one of the most widely consumed types of biologically active aromatic compounds (BAC), particularly caffeine (CAF, Fig. 1), which can be found in significant amounts in coffee, tea, chocolate, etc. [1, 2]. The high content of methylxanthines in food and, consequently, their relatively high daily consumption by humans (approximately 2.5 mg kg^{-1} day^{-1} [3]) has stimulated intensive study of the question of toxicity and side effects of methylxanthines [4, 5]. For example, it was found that CAF inhibits DNA synthesis, increases chromatin condensation and causes other cellular effects [2]. On the other hand,

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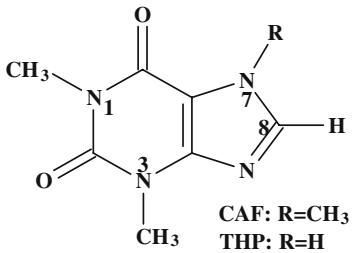

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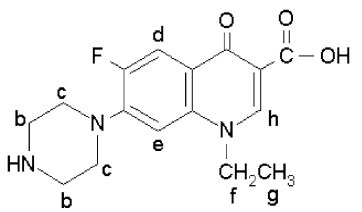

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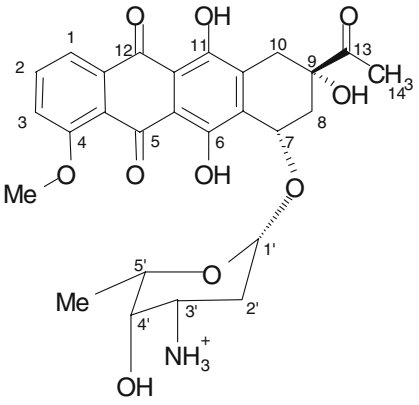

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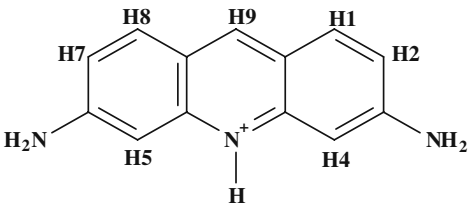

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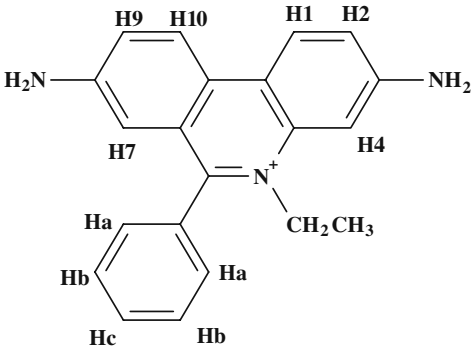

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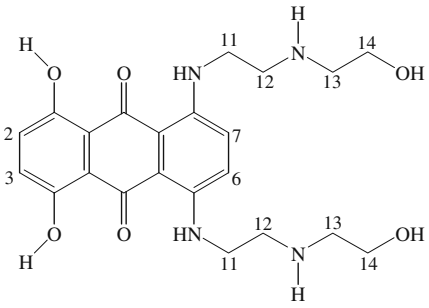

(f)

Fig. 1 Structures of aromatic ligands: **a** caffeine and theophylline, **b** norfloxacin, **c** daunomycin, **d** proflavine, **e** ethidium bromide, **f** novantrone

when CAF is administered together with aromatic cytotoxic drugs (such as doxorubicin, ethidium bromide, novantrone), there is a remarkable reduction in the in vitro toxicity of the drugs acting on nuclear DNA [6–9]. The effect was investigated in detail using ^{1}H NMR measurements [10–12], and the results were interpreted in terms of the competing action of two basic molecular processes, viz. the interceptor (hetero-association of CAF and drug) and protector (competition of CAF and drug for DNA binding sites) mechanisms of action of caffeine on DNA-binding aromatic ligands. This analysis led to a series of investigations

of the hetero-association of CAF with various aromatic BACs which has provided detailed structural and thermodynamic information on the complexation process [7, 10, 11, 13–17]. However, caffeine is not the only methylxanthine compound present in significant amounts in human blood plasma on consumption of caffeinated food. There are also relatively large amounts of caffeine derivatives such as paraxanthine, theophylline and theobromine [1], which, in principle, can act as effective interceptors of aromatic BACs, though there is little known about the hetero-association of these compounds with BACs and their influence on drug–DNA binding except a few reports of the complexation of theophylline with the dye Acridine Orange [14], and some nucleotide derivatives [18–21]. Theophylline (THP, Fig. 1) differs from CAF by the absence of the 7-methyl group in the xanthine chromophore and exhibits a different spectrum of pharmacological activity from CAF [22]. It has also been reported that the effect of THP on the action of aromatic BACs results in apparent synergetic biological effects [23], though the interpretation of these effects was only discussed in [24], in which it was suggested that the direct interaction between the drug and methylxanthine (hetero-association) might inhibit drug intercalation into DNA and modulate the DNA-related toxicity. The relative importance of the protector and interceptor mechanisms of the action of THP on the complexation of aromatic drugs with DNA is investigated by proton nuclear magnetic resonance (NMR) spectroscopy in the present work. This necessitates determination of the equilibrium hetero-association constants for complexation of THP with a range of aromatic drug compounds (such as daunomycin, novantrone, ethidium bromide, proflavine, norfloxacin) and with the same DNA oligomer, d(TGCA), as measured previously for the same set of drug molecules [10–12, 17]. The results are compared with the effect of CAF under similar conditions.

2 Materials and Methods

Daunomycin (DAU) from Fluka, novantrone (NOV), norfloxacin (NOR), proflavine (PF), ethidium bromide (EB) and THP from Sigma (Fig. 1), d(TGCA) (Metabion) were used without further purification. The samples were lyophilised from D_2O solutions and redissolved in 0.1 M phosphate buffer in 99.95% D_2O at pD 7.1, containing 10^{-4} M EDTA. All measurements were made at $T = 298$ K except for the study of NOV-THP, which was performed at $T = 318$ K in order to reach a sufficient concentration of the antibiotic NOV for NMR studies.

^{1}H NMR spectra (500 MHz) were recorded on a Bruker DRX spectrometer with the residual water peak saturated during relaxation. Chemical shift measurements of the non-exchangeable protons of the aromatic molecules were made as a function of concentration of the ligand (DAU/NOV/PF/EB/DNA oligomer with THP) or theophylline (THP–NOR). Chemical shifts were measured relative to TMA (tetramethylammonium bromide) as an internal reference and recalculated with respect to DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate), i.e. DSS = TMA + 3.178 (ppm).

3 Results and Discussion

3.1 Hetero-Association of THP with Aromatic Drugs

Hetero-association in the THP–drug system was analysed as previously [10–12] using concentration dependences of proton chemical shifts. An example of the experimental

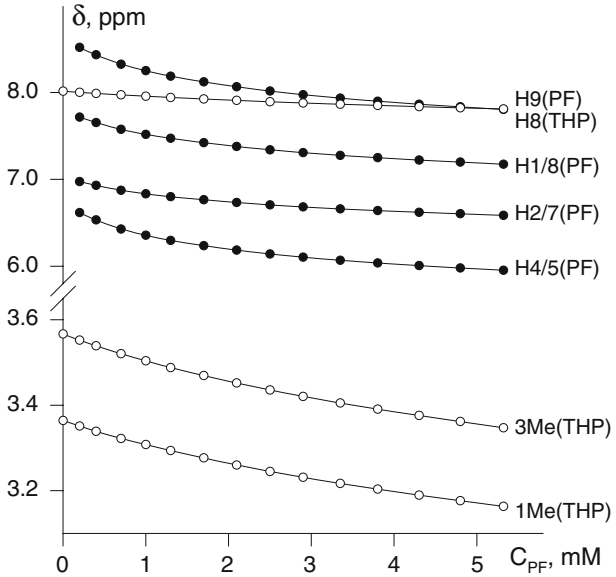

Fig. 2 Experimental dependence of the proton chemical shifts of proflavine (*PF*) and theophylline (*THP*) in mixed solutions (T = 298 K, pD 7.1, 0.1 M Na phosphate buffer) at constant concentration of THP (C_{THP} = 2 mM) and varying concentrations of PF (C_{PF})

curves for each system is given in Fig. 2, which shows that the chemical shifts of both ligand and THP move to low frequency on increasing the concentration of PF. A qualitatively similar dependence was observed for all other systems studied in this work and for all CAF–ligand systems measured in previous work [10, 11, 15, 17]. Hence, the NMR observations can be interpreted by analogy with the CAF–ligand systems as a consequence of sandwich-type aggregation of molecules in solution, characterised by significant overlapping of the aromatic chromophores and a corresponding increase in magnetic shielding within the complex. A similar conclusion was also made previously [14] for the hetero-association of THP with Acridine Orange based on absorption spectrophotometry.

The quantitative analysis of the hetero-association of THP with aromatic BACs was carried out using the methodology developed previously for caffeine systems [10, 11, 15–17]. The latter is based on a statistical–thermodynamical model of molecular hetero-association [25], which takes into account the interaction of two types of aromatic compounds: X (ligand) and Y (THP) in solution and which are able to form indefinite self-association and hetero-complexes of different types. The corresponding equations describing the NMR experimental proton chemical shift of the ligand and theophylline take the form [10, 11, 25]:

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

Table 1 Calculated magnitudes of equilibrium constants of the hetero-association of THP and CAF with biologically active aromatic compounds

Ligand	K_h(l/mol)[a]	
	THP	CAF [ref.]
DAU	190 ± 30	72 ± 4 [10]
PF	180 ± 20	160 ± 17 [10]
EB	102 ± 6	62 ± 4 [10]
NOV	100 ± 10	256 ± 30 [15]
NOR	49 ± 2	30 ± 10 [17]

[a]Values are given at $T = 298$ K except that for NOV–THP system, which is measured at $T = 318$ K

where δ_h are the chemical shifts of the protons of X and Y molecules within the hetero-complex and K_h is the equilibrium hetero-association constant. The quantities δ_m, δ_d and the equilibrium self-association constants K_X, K_Y are known from the literature [11, 15, 17, 26, 27]. The monomer concentrations x_1 and y_1 can be found from the solution of the systems of equations written from the mass conservation law [10, 11, 25]:

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

Equations (1) and (2) contain two unknown parameters, δ_h and K_h, which can be obtained from the concentration dependences of the proton chemical shifts (see Fig. 2) by means of minimisation of the discrepancy function $\Delta = \sum_i (\delta_i - \delta_{ei})^2$. The discrepancy function reflects the deviation of the calculated δ_i using formula (1) and experimental δ_{ei} chemical shifts. The results of the calculations of the equilibrium hetero-association constants of THP with different aromatic drug molecules are given in Table 1.

It is found in Table 1 that the magnitudes of the equilibrium constants of THP–ligand hetero-association are in general similar to those for the hetero-association of CAF with the same ligands, although being somewhat higher (except for the NOV–THP system). In previous work [19, 20], it was shown that the absence of the 7-methyl group in the structure of THP molecules should lead to more compact stacking of the aromatic chromophores than in CAF. In the case of the sandwich-type hetero-association discussed in this work, compact stacking will always result in effective dispersive interactions of aromatic chromophore currents and, as a consequence, may lead to the increase in the hetero-association constant of THP compared to CAF with the same aromatic drugs. On the other hand, the hetero-association of NOV–THP appears to be energetically less favourable than NOV-CAF (see Table 1). It was concluded previously [15] that, in the stabilisation of the NOV–CAF hetero-complex, a major role is played by hydrophobic interactions of the side chains of NOV and CAF molecules. It seems that a similar situation is also found in the NOV–THP system, although the absence of the hydrophobic 7-methyl group in THP must inevitably lower the hydrophobic contribution to complexation, which is reflected in the reduction of the equilibrium constant.

3.2 Complexation of THP with the DNA Oligonucleotide, d(TGCA)

In previous work [11, 12, 17], it was suggested that a physiologically relevant model of cellular histone-free DNA can be formulated in terms of short double-stranded DNA oligonucleotides having a length which allows just one ligand to be accommodated at a time; the tetrameric DNA sequence d(TGCA) was successfully used in such an approach. The short length of the DNA sequence enables all the molecules in the NMR titration experiment to be in fast exchange conditions so that the additive model applies in the determination of the equilibrium constant for complexation of aromatic molecule with DNA. Hence, analysis of the NMR titration experiments for the complexation of THP with d(TGCA) in aqueous solution was made in an analogous manner to the THP–ligand hetero-association discussed above.

The experimental dependences of the chemical shifts of THP protons as a function of oligonucleotide concentration are shown in Fig. 3, and the fact that there are changes in chemical shifts reflects the existence of interactions between THP and d(TGCA) in solution. However, short oligonucleotides can exist in solution as an equilibrium between single-stranded (N) and duplex (N_2) forms [28], and that equilibrium also needs to be taken into account. The model of the dynamic equilibrium must include the complexation of THP (D) with both forms of DNA, viz. the formation of 1:1 (DN) and 1:2 (DN_2) complexes:

$$\begin{array}{llll} D + D \overset{K_D}{\longleftrightarrow} D & \text{(a)} & N + N \overset{K_N}{\longleftrightarrow} N_2 & \text{(b)} \\ D + N \overset{K_{11}}{\longleftrightarrow} DN & \text{(c)} & D + N_2 \overset{K_{12}}{\longleftrightarrow} DN_2 & \text{(d)} \end{array}, \tag{3}$$

where K_D, K_N are the self-association constants of THP and oligonucleotide, respectively, and K_{11}, K_{12} are the equilibrium constants for the formation of 1:1 and 1:2 complexes, respectively. Taking into account the concentration range used in the NMR experiment (millimolars), the reactions of THP (3a) and d(TGCA) (3b) self-association must also be included.

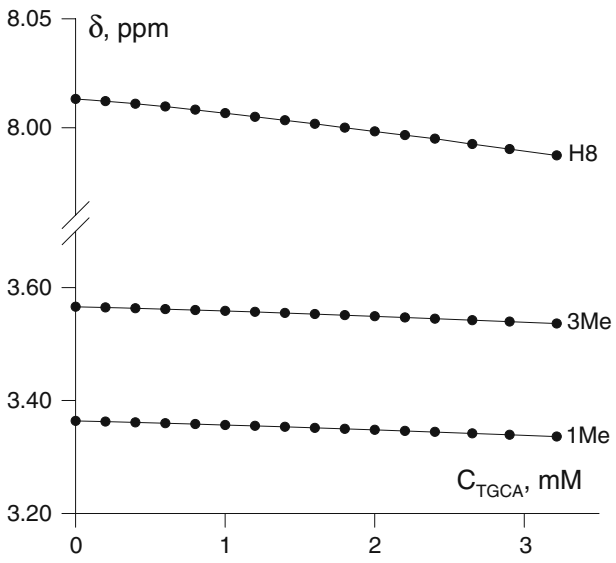

Fig. 3 Experimental dependence of the chemical shifts of theophylline protons in the mixed solution with d(TGCA) (T = 298 K, pD 7.1, 0.1 M Na phosphate buffer) at constant concentration of THP (C_{THP} = 2 mM) and varying concentrations of tetranucleotide (C_{TGCA})

Table 2 Calculated magnitudes of equilibrium constants of the complexation of THP and CAF with the deoxytetranucleotide 5′-d(TGCA) at $T = 298$ K

Constants (l/mol)	THP	CAF [10]
K_{11}	1000 ± 200	34 ± 4
K_{12}	230 ± 50	246 ± 18

According to scheme (3), the system of equations for the mass conservation law and the observed chemical shifts of THP protons takes the form:

$$\begin{cases} N_0 = N + 2K_N N^2 + K_{11} DN + 2K_{12} K_N DN^2 \\ D_0 = D + 2K_D D^2 + K_{11} DN + K_{12} K_N DN^2 \\ \delta_D = \dfrac{D}{D_0} \left(\delta_m + 2K_D D\delta_d + K_{11} N\delta_{11} + K_N K_{12} N^2 \delta_{12} \right), \end{cases} \tag{4}$$

where D_0, N_0 are the total concentrations of THP and oligonucleotide in solution and δ_{11}, δ_{12} are the chemical shifts of THP protons within the various complexes. After the solution of the first two equations in (4) with respect to monomer concentrations D and N, the remaining unknown quantities are the equilibrium complexation constants and chemical shifts within the complexes. All these parameters can be determined from experimental concentration dependences of THP proton chemical shifts in solution (Fig. 3) using the numerical procedure applied above for the hetero-association analysis. Results of these calculations are presented in Table 2.

From a comparison of the calculated parameters of THP/CAF-d(TGCA) in Table 2, it is seen that complexation of the methylxanthines with the duplex form of DNA is similar within experimental error, whereas the affinity of THP to the single-stranded DNA is much higher than that of CAF. We were not able to find any study in literature dealing with the interaction of xanthines with single-stranded DNA; however, an analogous effect with respect to the complexation of THP and CAF with single-stranded RNA was reported in [29, 30]. It was shown [30] that the absence of the 7-methyl group in the structure of THP (see Fig. 1a) not only creates the necessary steric conditions for insertion of the molecule into RNA but also provides an additional site for hydrogen bonding, which considerably increases the affinity to RNA of THP compared to CAF. Although the comparison of RNA- and DNA-binding properties is ambiguous in the general case, we suggest that similar reasons may explain the increased affinity to single-stranded DNA of THP compared to CAF reported in Table 2 in the present work.

3.3 Quantitation of the Protector and Interceptor Mechanisms of Action on the Binding of Aromatic BACs with DNA in the Presence of THP

The computational scheme for analysis of the competitive complexation of two different aromatic BACs with DNA takes into account the dynamic equilibrium of all possible types of interaction in solution [11, 12, 17], viz. for aromatic components X (Ligand) and Y (interceptor), there is the self-association of both X and Y, hetero-association between X and Y and the complexation of both X and Y with the DNA fragment. A quantitative estimate of the influence of the interceptor molecule on ligand binding with DNA is given by two factors: R_D and A_D.

R_D is considered for two limiting circumstances [11, 12]:

(a) 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., $f^X_{C2(C)}$, and
(b) 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., $f^X_{C2(h)}$,

and is defined as

$$R_D = \frac{f^X_{C2(0)} - f^X_{C2(C)}}{f^X_{C2(0)} - f^X_{C2(h)}}, \tag{5}$$

where $f^X_{C2(0)}$ is the mole fraction of X–DNA complexes with 'switched off' hetero-association and Y-DNA complexation. The range of $R_D > 1$ corresponds to the predominance of Y–DNA complexation over X–Y hetero-association (i.e. the 'protector action' of ligand Y) and $R_D < 1$ 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 ligand Y can be made using the quantity A_D [11, 12], which corresponds to the relative amount of X molecules removed from DNA on addition of Y,

$$A_D = \frac{f^X_{C2(0)} - f^X_{C2}}{f^X_{C2(0)}}, \tag{6}$$

where f^X_{C2} is the relative amount of drug X bound to DNA in the presence of Y.

If the fraction of X–DNA complexes correlates with the biological activity of X, then the fraction of ligand X removed from DNA, A_D, can act as a quantitative measure of the change of biological effect in a mixture of DNA-acting aromatic drugs. The details of computation of the f-quantities used in (5) and (6) are given in [11, 12]. The following input data were used in the calculations; the hetero-association constants of THP–BAC (see Table 1), the THP-d(TGCA) complexation constant (see Table 2) and the BAC-d(TGCA) complexation constants [12]. The results are summarised in Fig. 4.

For all the ligands studied, it is seen in Fig. 4a that generally $R_D > 1$, indicating that the protector mechanism of action dominates, though its effect decreases at higher concentrations of THP. In fact, at those higher concentrations ($\geq$10 mM) $R_D \sim 1$ and the protector and interceptor mechanisms for DAU, PF, EB and NOV have similar contributions. These results are in general agreement with those previously reported for complexation with DNA of the same BACs in the presence of CAF [12], which is expected because the hetero-association and DNA complexation constants for THP and CAF are similar to each other (see Tables 1, 2). An approximate formula derived in [12] for the R_D factor

$$R_D \approx \frac{K_{12Y}}{K_h} \tag{7}$$

indicates that R_D should be constant, which is observed in Fig. 4a for theophylline at concentrations below about 0.1 mM. An important conclusion can also be deduced from analysis of Eq. (7) and the data in Table 2. It is expected that the competitive binding of the same BACs and THP with single-stranded DNA [one should substitute K_{12Y} for K_{11Y} in (7)] should be characterised by pronounced domination of the protector mechanism because the affinity of THP to single-stranded DNA is approximately five times higher than to double-stranded ($K_{11Y} > K_{12Y}$, see Table 2). In other words, if single-stranded DNA (or

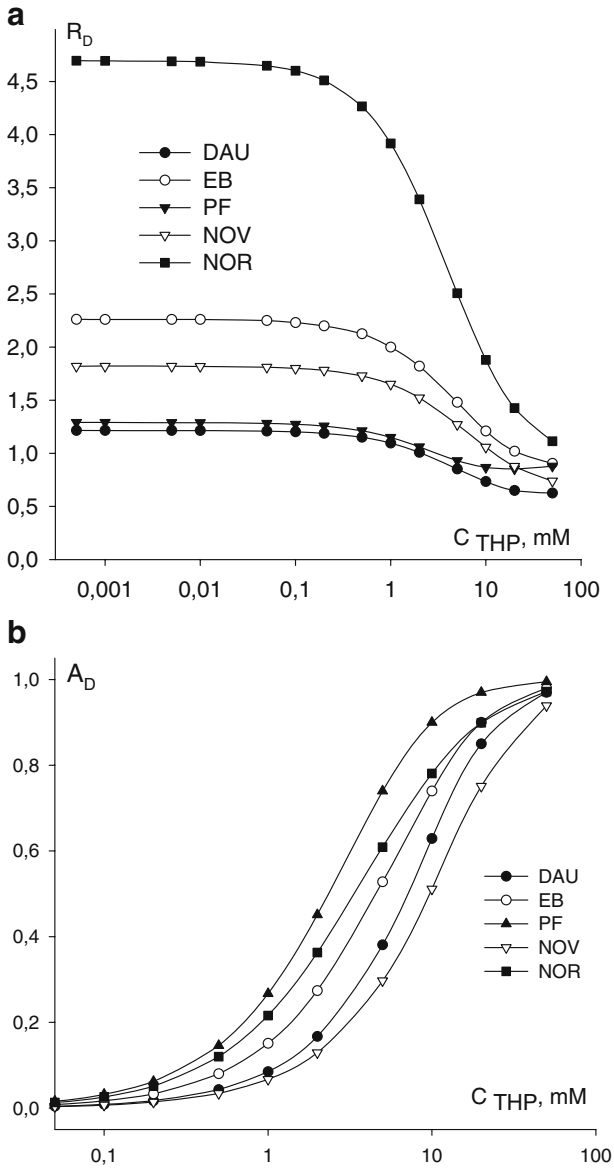

Fig. 4 Calculated dependences of the factors R_D and A_D on the concentration of theophylline (C_{THP})

probably RNA) is involved as the bioreceptor of aromatic drugs, THP is expected to be a more effective interceptor molecule than CAF.

Analysis of the calculated curves for the A_D factor in Fig. 4b suggests that the effective removal of ligand molecules from DNA ($A_D \geq 20\%$) starts at $C_{THP} > 1$ mM, which is in agreement to what was previously reported in vitro for caffeine-containing cell lines [11, 12, 17]. It also follows that the most pronounced change in biological effect should be expected on addition of THP to PF and the least pronounced to NOV. The sequence

of curves presented in Fig. 4b (from PF to NOV) can be well described by a sequence of numbers calculated by the formula:

$$A_{\mathrm{D}} \equiv \frac{K_h^2}{K_{12\mathrm{X}}} \tag{8}$$

which has previously been suggested as an approximation of A_{D} for various combinations of aromatic compounds [11, 12, 17]. Equation (8) suggests that the difference in the effect of the interceptor action of THP and CAF on the complexation of BAC ligands with duplex DNA depends on the relation $K^2_{\mathrm{h(THP)}}/K^2_{\mathrm{h(CAF)}}$ between the hetero-association constants (from Table 1) because the DNA–THP/CAF complexation constants are essentially equal. Computations using the data in Table 1 indicate that the effect of THP on the biological activity of DAU, EB and NOR ligands should be more pronounced than similar action of CAF, that the effects with PF are similar for THP and CAF and that CAF should remove the antibiotic NOV from DNA more effectively than theophylline. Applying similar logic to the complexation of Acridine Orange with chicken erythrocyte chromatin in the presence of CAF and THP (see [31]), one might expect that CAF should be at least $(256/157)^2 = 2.7$ times more effective than THP, which qualitatively agrees with experimental data reported.

4 Conclusions

In this work, we first made a decomposition of the binding of aromatic drugs (daunomycin, novantrone, ethidium bromide, proflavine, norfloxacin) with DNA in the presence of theophylline into interceptor (hetero-association) and protector (competition for DNA-binding sites) processes and provided a quantitation of these effects in terms of their influence on biological activity of the drugs. The results of this work suggest a method to anticipate relative sensitivity of certain aromatic drugs towards addition of THP using simply a set of equilibrium constants of intermolecular interaction. The utilisation of such approach for other methylxanthines is under way in our laboratory.

References

1. Lelo, A., Miners, J.O., Robson, R., Birkett, D.: Assessment of caffeine exposure: caffeine content of beverages, caffeine intake and plasma concentration of methylxanthines. Clin. Pharmacol. Ther. **39**, 54–59 (1986)
2. Baron, J.J., Roberts, H.: Caffeine. Springer, Berlin (1981)
3. Dews, P., Grice, H.C., Neims, A., Wilson, J., Wurtman, R.: Report of fourth international caffeine workshop. Food Chem. Toxicol. **22**, 163–169 (1984). doi:10.1016/0278-6915(84)90098-X
4. D'Ambrosio, S.M.: Evaluation of the genotoxicity data on caffeine. Regul. Toxicol. Pharmacol. **19**, 243–281 (1994). doi:10.1006/rtph.1994.1023
5. Sawynok, J.: Pharmacological rationale for the clinical use of caffeine. Drugs **49**, 37–50 (1995)
6. Traganos, F., Kapuscinski, J., Darzynkiewicz, Z.: 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 analague AD198. Cancer Res. **51**, 3682–3689 (1991)
7. Piosik, J., Zdunek, M., Kapuscinski, J.: 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 (2002). doi:10.1016/S0006-2952(01)00903-0

8. Piosik, J., Ulanowska, K., Gwizdek-Wisniewska, A., Czyz, A., Kapuscinski, J., Wegrzyn, G.: Alleviation of mutagenic effects of polycyclic aromatic agents (quinacrine mustard, ICR-191 and ICR-170) by caffeine and pentoxifylline. Mutat. Res. **530**, 47–57 (2003). doi:10.1016/S0027-5107(03)00136-2
9. Ulanowska, K., Piosik, J., Gwizdek-Wisniewska, A., Wegrzyn, G.: Formation of stacking complexes between caffeine (1,2,3-trimethylxanthine) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine may attenuate biological effects of this neurotoxin. Bioorg. Chem. **33**, 402–413 (2005). doi:10.1016/j.bioorg.2005.07.004
10. Davies, D.B., Veselkov, D.A., Djimant, L.N., Veselkov, A.N.: Hetero-association of caffeine and aromatic drugs and their competitive binding with a DNA oligomer. Eur. Biophys. J. **30**, 354–366 (2001). doi:10.1007/s002490100150
11. Evstigneev, M.P., Khomich, V.V., Davies, D.B.: Complexation of anthracycline drugs with DNA in the presence of caffeine. Eur. Biophys. J. **36**, 1–11 (2006). doi:10.1007/s00249-006-0071-9
12. Evstigneev, M.P., Lantushenko, A.O., Evstigneev, V.P., Mykhina, Y.V., Davies, D.B.: Quantitation of the molecular mechanisms of biological synergism in a mixture of DNA-acting aromatic drugs. Biophys. Chem. **132**, 148–158 (2008). doi:10.1016/j.bpc.2007.11.001
13. Larsen, R.W., Jasuja, R., Hetzler, R., Muraoka, P.T., Andrada, V.G., Jameson, D.M.: Spectroscopic and molecular modelling studies of caffeine complexes with DNA intercalators. Biophys. J. **70**, 443–452 (1996)
14. Lyles, M.B., Cameron, I.L., Rawls, H.R.: Structural basis for the binding affinity of xanthines with the DNA intercalator acridine orange. J. Med. Chem. **44**, 4650–4660 (2001). doi:10.1021/jm9904708
15. Davies, D.B., Veselkov, D.A., Evstigneev, M.P., Veselkov, A.N.: Self-association of the antitumour agent novatrone (mitoxantrone) and its hetero-association with caffeine. J. Chem. Soc. Perkin Trans. **2**, 61–67 (2001). doi:10.1039/b007042o
16. Veselkov, A.N., Evstigneev, M.P., Rozvadovska, A.O., Mykhina, Y.V., Davies, D.B.: A structural and thermodynamic analysis of novatrone and flavin mononucleotide heteroassociation in aqueous solution by ^{1}H NMR spectroscopy. Russ. J. Bioorg. Chem. **31**, 453–459 (2005). doi:10.1007/s11171-005-0062-0
17. Evstigneev, M.P., Rybakova, K.A., Davies, D.B.: Complexation of norfloxacin with DNA in the presence of caffeine. Biophys. Chem. **121**, 84–95 (2006). doi:10.1016/j.bpc.2005.12.003
18. Nakano, N.I., Igarashi, S.J.: Molecular interactions of pyrimidines, purines, and some other heteroaromatic compounds in aqueous media. Biochemistry **9**, 577–583 (1970). doi:10.1021/bi00805a019
19. Aradi, F., Foldesi, A.: Equilibrium constants for association of caffeine and theophylline with aromatic salts in aqueous solutions studied by ^{1}H NMR chemical shift measurements. Magn. Reson. Chem. **23**, 375–378 (1985). doi:10.1002/mrc.1260230518
20. Aradi, F., Foldesi, A.: Hetero-association of caffeine and theophylline with purine and pyrimidine in aqueous solutions studied by ^{1}H NMR chemical shift measurements. Magn. Reson. Chem. **27**, 249–252 (1989). doi:10.1002/mrc.1260270311
21. Aradi, F., Foldesi, A.: Effect of methylation on the association ability of purine and pyrimidine in aqueous solutions. A ^{1}H NMR chemical shift study. Magn. Reson. Chem. **27**, 592–598 (1989). doi:10.1002/mrc.1260270613
22. Goodman, A.G., Rall, T.W., Nies, A.S.: The pharmacological basis of therapeutics, pp. 618–637. Pergamon, NY (1990)
23. Wijnands, W.J.A., Vree, T.B.: The clinical importance of the interaction between (fluoro)quinolones and the methylxanthines theophylline and caffeine. J. Drug Ther. Res. **16**, 15–17 (1991)
24. Johnson, I.M., Bhuvan Kumar, S.G., Malathi, R.: De-intercalation of ethidium bromide and acridine orange by xanthine derivatives and their modulatory effect on anticancer agents. J. Biomol. Struct. Dyn. **20**, 677–685 (2003)
25. Veselkov, A.N., Evstigneev, M.P., Veselkov, D.A., Davies, D.B.: A generalized NMR-derived statistical-thermodynamical model of hetero-association of aromatic molecules in aqueous solution. J. Chem. Phys. **115**, 2252–2266 (2001). doi:10.1063/1.1359767
26. Davies, D.B., Djimant, L.N., Veselkov, A.N.: ^{1}H NMR investigation of self-association of aromatic drug molecules in aqueous solution. Structural and thermodynamical analysis. J. Chem. Soc., Faraday Trans. **92**, 383–390 (1996). doi:10.1039/ft9969200383
27. Thakkar, A.L., Tensmeyer, L.G., Wilham, W.L.: NMR evidence for self-association of theophylline in aqueous solution. J. Pharm. Sci. **60**, 1267–1268 (1971). doi:10.1002/jps.2600600841
28. Davies, D.B., Eaton, R.J., Baranovsky, S.F., Veselkov, A.N.: NMR investigation of the complexation of daunomycin with deoxytetranucleotides of different base sequence in aqueous solution. J. Biomol. Struct. Dyn. **17**, 887–901 (2000)
29. Johnson, I.M., Bhuvan Kumar, S.G., Malathi, R.: RNA binding efficacy of theophylline, theobromine and caffeine. J. Biomol. Struct. Dyn. **20**, 687–692 (2003)

30. Zimmermann, G.R., Jenison, R.D., Wick, C.L., Simorre, J.-P., Pardi, A.: Interlocking structural motifs mediate molecular discrimination by a theophylline-binding RNA. Nat. Struct. Biol. **4**, 644–649 (1997). doi:10.1038/nsb0897-644
31. Lyles, M.B., Cameron, I.L.: Caffeine and other xanthines as cytochemical blockers and removers of heterocyclic DNA intercalators from chromatin. Cell Biol. Int. **26**, 145–154 (2002). doi:10.1006/cbir.2001.0810