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# $^1$H NMR study of the complexation of aromatic drugs with dimethylxanthine derivatives

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ABSTRACT

With an aim of searching efficient interceptors of aromatic drugs, the self- and hetero-association of dimethylxanthine derivatives with different structures, selected according to Strategy 1 (variation of the position of methyl groups) and Strategy 2 (variation of the length of —$(CH_2)_n$—COOH group), with aromatic drug molecules: Ethidium Bromide, Proflavine and Daunomycin, were studied using $^1$H NMR spectroscopy. It was found that the association proceeds in a form of stacking-type complexation and its energetics is relatively independent on the structure of the dimethylxanthines. However, on average, the dimethylxanthines possess higher hetero-association constant and, hence, higher interceptor ability as compared to the trimethylxanthine, Caffeine, used during the past two decades as a typical interceptor molecule.



## 1. Introduction

Xanthine-type molecules, in particular, Caffeine and its derivatives, have long been known to interfere with biological action of DNA-targeting aromatic drugs, leading in some cases to lowering of their toxicity *in vitro* [1–5]. This effect may potentially have a very important application in clinical practice, *e.g.* for rapid regulation of the toxicity level after overdosing [6]. It appears as being generally accepted now that the mechanism of such synergistic action in drug-xanthine systems can be explained in terms of two basic molecular processes acting simultaneously, *viz.* the interceptor (hetero-association of the xanthine and the drug) and protector (removal of the drug from DNA due to xanthine–DNA binding) [for review see Ref. [6]]. Although there is still some debate on the interrelation of these two processes, in both cases on addition of xanthine molecules to drug-containing biological system, the concentration of drug–DNA complexes decreases resulting in alteration of the net biological effect of the drug. The binding density of the aromatic drug on DNA has been shown to correlate with xanthine–drug hetero-association constant [7,8], which suggests the principal possibility of amplifying the interceptor action and subsequent decrease in the net drug's toxicity by means of directed search of xanthine molecules which specifically target certain aromatic drugs and feature high xanthine–drug hetero-association constant.

Within the whole group of xanthine derivatives, the dimethylxanthines, containing two methyl groups in positions 1, 3 or 7 of the xanthine chromophore (Fig. 1), act as physiological derivatives (metabolites) of Caffeine [9], and for some of them the interceptor action towards aromatic drugs has been reported [3,5,10]. With an aim of seeking the relation between the structure of dimethylxanthine molecules and the hetero-association constant with aromatic drugs under conditions close to physiological (pH ≈ 7), in the present work we employed two strategies:

(i) Strategy 1, *viz.* the variation of the position of two methyl groups in xanthine chromophore, giving Theophylline (THP, 1,3-dimethylxanthine), Theobromine (THB, 3,7-dimethylxanthine) and Paraxanthine (PARA, 1,7-dimethylxanthine) (Fig. 1). The rationale behind this strategy is an assumption that the alteration of the position of the methyl groups may modulate the hydrophobic contribution, known as one of the principal determinants of the hetero-association of aromatic molecules [11], and
(ii) Strategy 2, *viz.* the introduction of —$(CH_2)_n$—COOH group to position 7 of the xanthine chromophore, giving Theophylline-7-acetic acid (AA, $R = CH_2$—COOH), Theophylline-7-propionic acid (PA, $R = (CH_2)_2$—COOH) and Theophylline-7-butyric acid (BA, $R = (CH_2)_3$—COOH) (Fig. 1). The rationale

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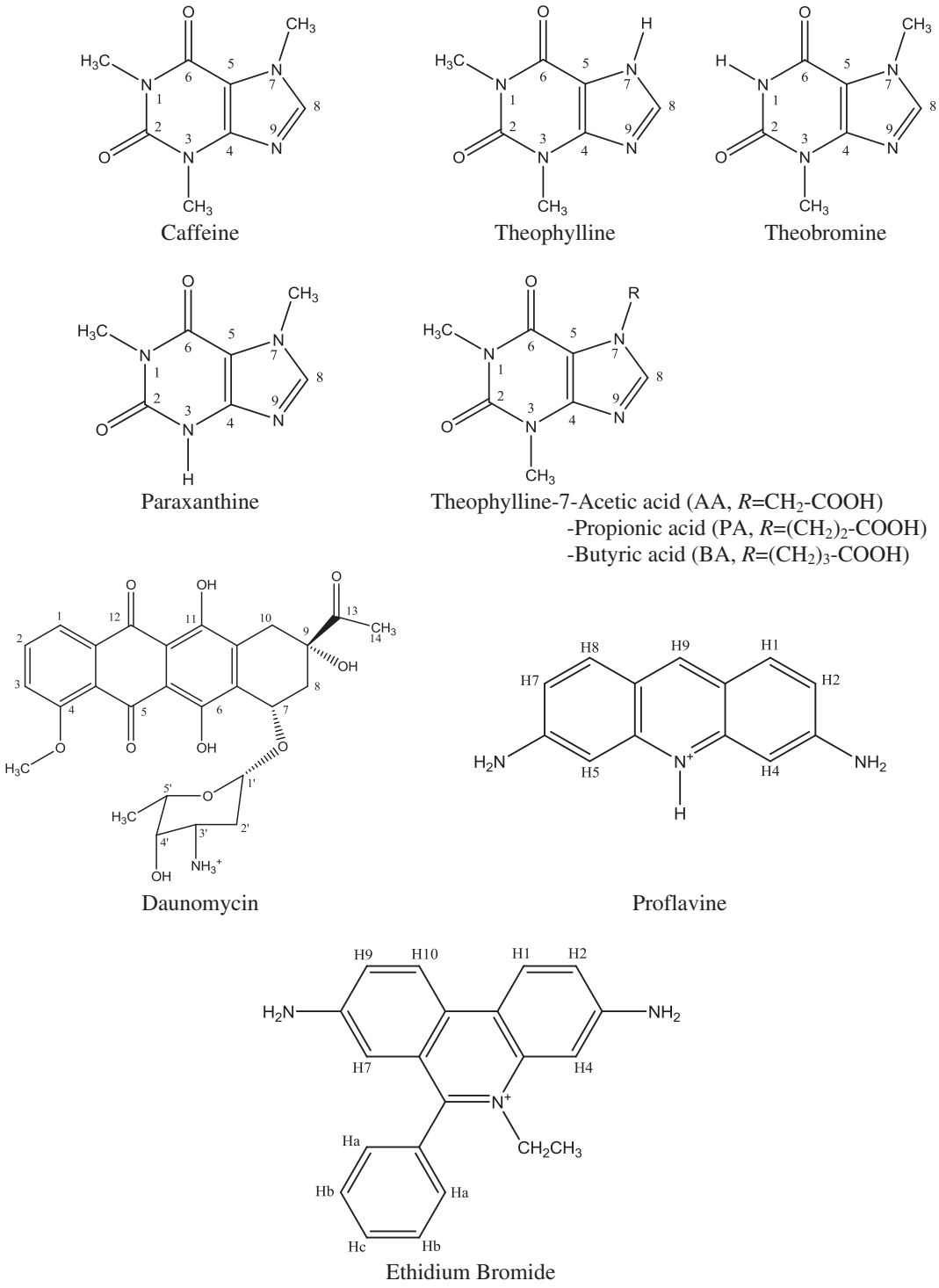


**Fig. 1.** Structures of the dimethylxanthines and aromatic drugs.

behind this strategy is an assumption that —$(CH_2)_n$—COOH moiety, which is flexible and contains hydrogen donor/acceptor groups, may form intermolecular hydrogen bond to aromatic drug molecule within the hetero-complex. The intermolecular H-bonds are known to provide additional stabilization of the hetero-complexes of aromatic molecules in solution which is monitored by increased magnitude of the hetero-association constant [12,13].

Antibiotic Daunomycin (DAU), mutagens, Ethidium Bromide (EB) and Proflavine (PF) (Fig. 1), which were used in the past to testify the interceptor action of the xanthines *in vitro* [for review see Ref. [6]], were taken as typical representatives of aromatic drugs. DAU, EB and PF ligands are known as being charged under neutral pH (see the charge localisation in Fig. 1 [6]), whereas the —$(CH_2)_n$—COOH group in THP derivatives is ionised and bears negative charge. The latter provides alternative to the formation of the

H-bond mechanism of possible additional stabilization of the hetero-complexes within the Strategy 2 due to specific electrostatic attraction.

## 2. Experimental

### 2.1. Materials

Daunomycin from 'Fluka', Proflavine, Ethidium Bromide, Theobromine, Paraxanthine, Theophylline-7-acetic acid from 'Sigma' (Fig. 1) were used without further purification. The samples were lyophilised 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 EDTA.

### 2.2. Preparation of Theophylline-7-propionic acid (PA) and Theophylline-7-butyric Acid (BA)

Forty eight milligrams (1 mmol) of sodium hydride (as 50% dispersion) were added to 180 mg (1 mmol) of Theophylline, dissolved in 5 ml of anhydrous dimethylformamide at room temperature, while stirring. After the evolution of hydrogen has ceased, 215 mg (1.1 mmol) of 4-bromobutyric acid ethyl ester were added under stirring and the mixture was then heated to 90° C for 4 h. Volatiles were removed *in vacuo*, and the residue was taken up in ethyl acetate, filtered and washed twice with water. After removing of solvent *in vacuo* and crystallization of the residue (iPrOH/petrol ether), 271 mg (92%) of ester were obtained. The purified ester (236 mg, 0.8 mmol) was dissolved in 3 ml of methanol/1 M NaOH solution and the solution was gently warmed for 2 h. After TLC-monitoring had shown no more starting material, the mixture was cooled down to 0 °C and acidified with diluted HCl. Precipitated white solid was filtered off, washed with water and crystallized from aqueous methanol to give 184 mg (86%) of pure Theophylline-7-butyric acid. The structure was confirmed by MS(ESI) and NMR data. Similarly, Theophylline-7-propionic acid was prepared.

### 2.3. NMR measurements

500 MHz $^1$H NMR spectra were recorded on Varian spectrometer with the residual water peak saturated during relaxation. Signal assignments of the non-exchangeable protons of the drugs were obtained using both two-dimensional homonuclear TOCSY and ROESY experiments. Chemical shift measurements of the non-exchangeable protons of the aromatic molecules were made as a function of concentration of the drug, maintaining the concentration of dimethylxanthine fixed ($y_0$ = 2 mM) – in the hetero-association analysis, and as a function of the dimethylxanthines concentration – in the self-association analysis. All measurements were made at $T$ = 298 K.

All sets of NMR measurements were made in the fast-exchange condition on the NMR timescale. 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-sulphonate), *i.e.* DSS = TMA + 3.178 (ppm).

### 2.4. Structure calculations

Calculations of the spatial structures of the 1:1 dimethylxanthine–drug complexes in an aqueous environment have been made by molecular mechanics methods using X-PLOR software with the Charmm22 force field, as previously [10,12,13]. Modelling of the aqueous environment was performed by water molecules in the form of TIP3P, placed in rectangular box (1100 molecules). Parameters of non-valent interactions between atoms corresponded to the MM3 force field. Prior to energy minimisations the initial structures of the dimethylxanthine dimers and 1:1 dimethylxanthine–drug hetero-complexes were built up from analysis of the induced chemical shifts of aromatic protons of the interacting molecules.

## 3. Results and discussion

### 3.1. The self-association of dimethylxanthines

The typical concentrations of aromatic molecules, used in NMR experiment, fall in millimolar concentration range which requires a consideration of indefinite self-association of dimethylxanthines and the drug molecules, as well as the indefinite hetero-association between the xanthines and the drugs. Hence, prior to the analysis of the hetero-association, it is necessary to get the full set of equilibrium parameters, describing the self-association, to be used further as an input data in the hetero-association analysis [10,14]. The self-association study of the dimethylxanthines was performed in the present work in similar solution conditions (pD 7.1, 0.1 M Na-phosphate buffer). The same conditions were also used before in the study of the self-association of DAU, PF, EB and Theophylline [15], so a comparative analysis of these systems is meaningful.

Experimental concentration dependences of chemical shifts of aromatic protons of the dimethylxanthines (data not shown) were fitted by means of indefinite self-association model based on common assumption that aromatic molecules aggregate with the equilibrium constant, $K$, independent on the number of molecules in aggregates (EK-model [16]):

$$\delta = \delta_m + 2(\delta_d - \delta_m)\left(\frac{2Kx_0 + 1 - \sqrt{(4Kx_0 + 1)}}{2Kx_0}\right), \quad (1)$$

where $\delta_m$, $\delta_d$ are proton chemical shifts in monomer and dimer forms (or on the edge of aggregates) of a dimethylxanthine in solution, respectively; $x_0$ is the total concentration.

The adjustable parameters in this model are $K/\delta_m/\delta_d$. Their values are presented in Table 1. The self-association parameters for the trimethylxanthine, Caffeine (CAF), are also presented here for the purposes of comparison. In numerical analysis of the self-, and hetero-association of AA/PA/BA (see below), we used only the aromatic protons directly attached to the xanthine chromophore (H8, 1Me, 3Me) and ignored the ethyl protons of the —$(CH_2)_n$—COOH group. This is because the latter are strongly affected by local shielding effects, which may not feature an additive property of magnetic shielding due to aromatic ring-current effect, put into basis of the models of self- and hetero-association used in the present work.

Due to relatively low solubility of THB ($\leqslant$2 mM) the concentration dependence of $\delta$ for this molecule within the available concentration range (0.05–2 mM) used in NMR experiment, was too weak to perform reliable self-association analysis (chemical shift changes were within 0.003–0.006 ppm). In such case the magnitudes of $\delta_m$ parameter for THB protons were obtained by extrapolation of the concentration curves down to zero concentration.

It is seen from Table 1 that the magnitudes of equilibrium self-association constants for the dimethylxanthines, PARA/THP/AA/PA/BA, are similar within the error limits which means that the position of the methyl groups in the xanthine chromophore and the distance of the —COOH group from the chromophore do not affect the energetics of the self-association. As discussed above, the value of $K$ for THB was not possible to determine from experimental data available, however, the insensitivity of the equilibrium constant to the position of the methyl groups allows to take the $K$ value for THB as intermediate between the $K$ for PARA and THP (see Table 1), which is required for further hetero-association analysis. It

**Table 1**
Calculated parameters of the self-association of dimethylxanthines (0.1 M Na-phosphate buffer in $D_2O$, pD 7.1, $T$ = 298 K).

| Xanthine | $\delta_m$ (ppm) | | | | $\delta_d$ (ppm) | | | | $K$ ($M^{-1}$)[a] |
|---|---|---|---|---|---|---|---|---|---|
| | H8 | 7Me | 3Me | 1Me | H8 | 7Me | 3Me | 1Me | |
| *Strategy 1 (variation of the position of methyl groups)* | | | | | | | | | |
| THB | 7.90 | 3.94 | 3.49 | – | – | | | | 7.2[b] |
| PARA | 7.83 | 3.94 | – | 3.32 | 7.80 | 3.89 | – | 3.26 | 7.1 ± 0.5 |
| THP [15] | 8.02 | – | 3.58 | 3.37 | 7.81 | – | 3.28 | 3.12 | 7.4 ± 0.4 |
| CAF [14] | 7.89 | 3.95 | 3.54 | 3.35 | 7.83 | 3.82 | 3.33 | 3.16 | 11.8 ± 0.3 |
| *Strategy 2 (variation of the length of —$(CH_2)_n$—COOH group)* | | | | | | | | | |
| AA ($n$ = 1) | 7.92 | – | 3.56 | 3.35 | 7.87 | – | 3.32 | 3.19 | 7.0 ± 3.0 |
| PA ($n$ = 2) | 7.93 | – | 3.53 | 3.34 | 7.90 | – | 3.32 | 3.16 | 7.8 ± 2.0 |
| BA ($n$ = 3) | 7.96 | – | 3.54 | 3.35 | 7.79 | – | 3.37 | 3.21 | 6.4 ± 1.0 |

[a] The errors for the equilibrium constant were calculated from the values of $K$, computed over individual protons.
[b] The value of $K$ for THB was taken as intermediate between the self-association constants for PARA and THP (see discussion in the text).

should, however, be noted that the trimethylxanthine, Caffeine, aggregates with the equilibrium constant, $K$, being higher than that for the dimethyxanthines. This result may be explained by the hydrophobic contribution from the additional methyl group in the structure of CAF as compared to the dimethylxanthines.

Analysis of the computed magnitudes of the monomer ($\delta_m$) and dimer ($\delta_d$) chemical shifts in Table 1 enables to draw certain conclusions on the influence of the structure of the dimethylxanthine molecule on the magnetic shielding of aromatic protons. The values of $\delta_m$ for 7Me group are nearly identical for THB and PARA, hence, the position of the second methyl group (in 1 or 3 position) does not affect the chemical shift of this group. On the other hand, H8 proton of THB and PARA appears to be sensitive to the presence of the 3Me or 1Me groups (*i.e.* $\delta_m$(H8) experiences 0.07 ppm shift in these dimethylxanthines) which is expected because of much lower electron density in the vicinity of H8 as compared to the methyl protons. At the same time the $\delta_m$ chemical shifts of 1Me group for THP and CAF are also close to each other suggesting that the existence of 7Me group does not affect the monomer chemical shift of the 3Me group. Hence, the variation of the position of two methyl groups in the dimethylxanthines (*i.e.* the Strategy 1) does not influence the magnetic shielding of the methyl groups, but affects the shielding of H8 proton. In general, similar conclusions can be made with respect to the introduction of acetic, propionic and butyric groups into the structure of Theophylline (*i.e.* the Strategy 2, see Table 1), *viz.* the changes of $\delta_m$ (3Me/1Me) are relatively small and the changes of $\delta_m$(H8) are relatively high as compared with the same protons of THP.

An important conclusion which comes from inspection of the data in Table 1 is that for the whole set of aromatic protons, without exceptions, the magnetic shielding effect is observed as a result of the self-association *i.e.* $\delta_m > \delta_d$. This effect has previously been reported for other xanthine derivatives [14,15] and interpreted as a consequence of a stacking-type aggregation when the xanthine chromophores in a complex are parallel to each other creating favourable conditions for the magnetic shielding of all aromatic protons. It thus may be stated that the mode of the self-association of all of the investigated dimethylxanthine derivatives is likely to be the stacking-type aggregation.

### 3.2. The hetero-association of dimethylxanthines with aromatic drugs

The typical concentration dependences of the proton chemical shifts for the drugs and the dimethylxanthines studied in the mixed solutions are given in Fig. 2. It is seen that on variation of the drugs' concentration the signals from aromatic protons of the dimethylxanthines move upfield. The latter indicates that the interaction between the drug and dimethylxanthine molecules occurs in solution and most likely proceeds in a form of the stacking-type association, also reported before for other drug–xanthine systems [14,15,17] and stated above for the self-association of the dimethylxanthines. Such concentration dependences were fitted employing the same model as used before in analysis of various drug–xanthine systems, which takes into account indefinite self- and hetero-association of $X$ (drug) and $Y$ (dimethylxanthine) molecules [14,15]:

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

where $\delta_h$ and $K_h$ are the proton chemical shift in hetero-complex and the equilibrium hetero-association constant, respectively, acting as search parameters; $x_1$, $y_1$ are the concentrations of monomers.

The magnitudes of $\delta_m$, $\delta_d$ and equilibrium self-association constants, $K_X$, $K_Y$, for PARA/THB/AA/PA/BA/DAU/EB/PF molecules are known from previous self-association studies (see Table 1 for the dimethylxanthines and Ref. [15] for DAU/EB/PF). Monomeric concentrations, $x_1$ and $y_1$, in Eq. (2) may be obtained from the solution of the system of mass balance Eq. (3) in each experimental point:

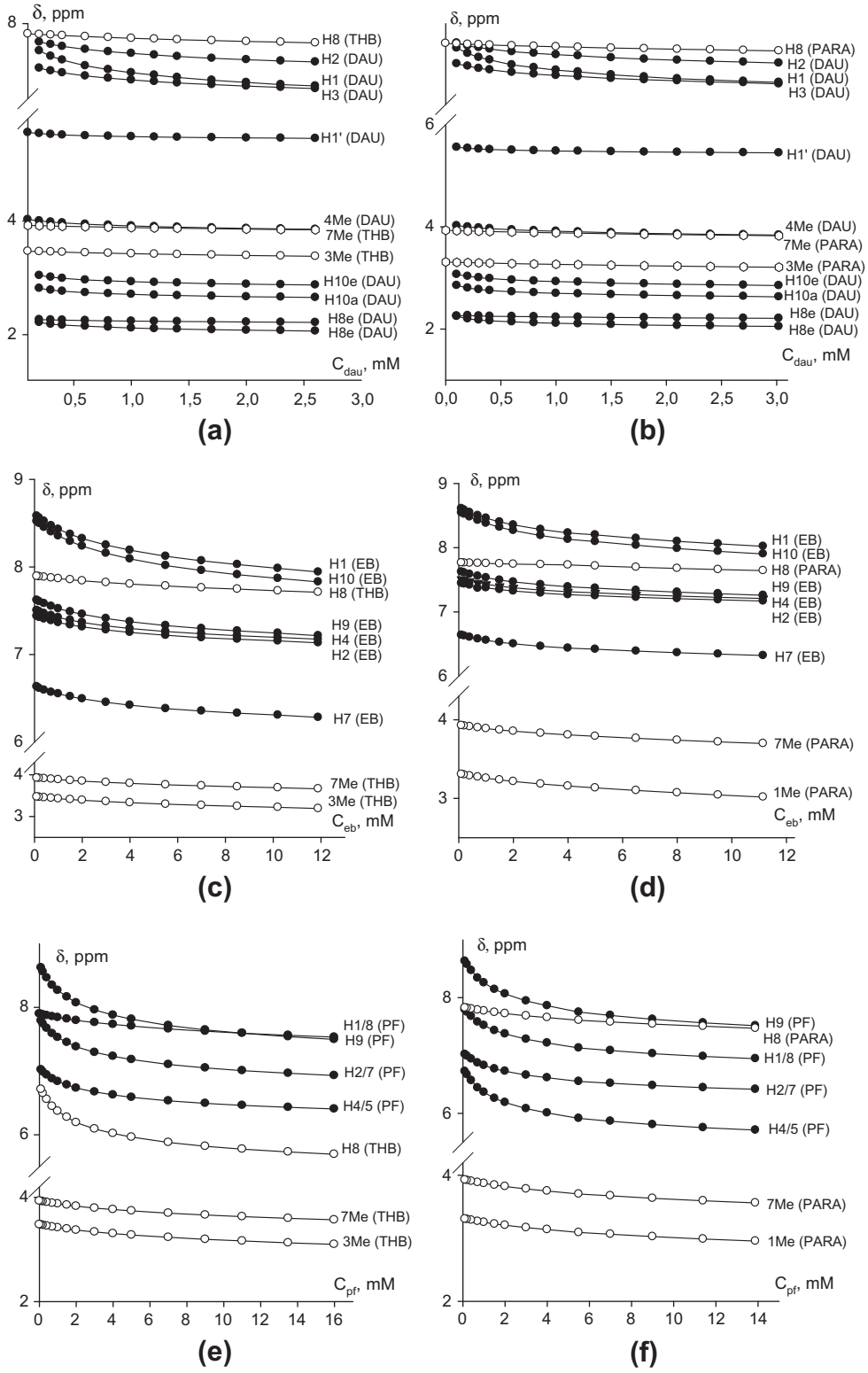


**Fig. 2.** Experimental dependences of the chemical shifts of aromatic protons of PARA and THB on EB/PF/DAU concentration in the mixed solutions: (a) THB–DAU, (b) PARA–DAU, (c) THB–EB, (d) PARA–EB, (e) THB–PF, (f) PARA–PF.

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

The results of computation of the hetero-association parameters for the systems studied are given in Tables 2 and 3.

Analysis of the computed values of equilibrium hetero-association constants in Table 2 suggests that there is a relatively insignificant change in $K_h$ on variation of the structure of dimethylxanthine molecules in either the Strategy 1 and Strategy 2. It was previously shown that the existence of apparent additional stabilization of the hetero-complexes of aromatic molecules via

**Table 2**
Calculated values of equilibrium constants ($M^{-1}$) of the hetero-association of dimethylxanthines with aromatic drugs (0.1 M Na-phosphate buffer in $D_2O$, pD 7.1, $T$ = 298 K).

| System | DAU | PF | EB |
|---|---|---|---|
| *Strategy 1 (variation of the position of methyl groups)* | | | |
| THB | 160 ± 40 | 250 ± 30 | 170 ± 20 |
| PARA | 110 ± 20 | 155 ± 15 | 102 ± 9 |
| THP[15] | 190 ± 30 | 180 ± 20 | 102 ± 6 |
| CAF[14] | 72 ± 4 | 160 ± 17 | 62 ± 4 |
| *Strategy 2 (variation of the length of —$(CH_2)_n$—COOH group)* | | | |
| AA | 180 ± 30 | 250 ± 30 | 125 ± 10 |
| PA | 160 ± 10 | 120 ± 15 | 120 ± 10 |
| BA | 150 ± 10 | 200 ± 20 | 170 ± 20 |

*Note*: the errors were calculated from the values of $K_h$, computed over individual protons.

intermolecular H-bond, charge-transfer interactions or specific electrostatic attraction is exerted through high magnitude of $K_h$ as compared to the self-association of *X* and *Y* molecules [12,13]. A formal criterion $f_h = \frac{K_h}{K_X+K_h+K_Y}$ was suggested in order to estimate relative importance of the hetero-association reactions in overall dynamic equilibrium in solution, and the threshold, $f_h \geqslant 35\%$, was established enabling to separate the systems which are likely stabilized by some additional factor such as H-bonds or charge-transfer [18]. The magnitude of the $f_h$ factor takes the maximum value, $f_h$ = 35%, only for EB-BA system and the lower values for all other systems. This result suggests that the studied systems most likely do not feature any additional stabilization of 1:1 hetero-complexes in solution and the hetero-association is governed by a balance of van der Waals, electrostatic and hydrophobic forces, as observed before for the majority of other hetero-associations of aromatic molecules [11]. The existence of intermolecular charge-transfer, H-bonding or specific electrostatic attraction, although being possible in principle, does not contribute significantly to the hetero-association of the dimethylxanthines with aromatic drugs. The highest, on average, value of $K_h$ is observed for PF-dimethylxanthine systems (Table 2) which is explained by the absence of bulky side chains in the structure of PF molecule as compared to EB and DAU (see Fig. 1), thus lowering the steric hindrance of the interacting molecules in the hetero-complexes. This conclusion is also supported by the fact that for all the drugs studied the $K_h$ values for the trimethylxanthine, CAF, are lower than the $K_h$ for any of the dimethylxanthines (see Table 2). This is likely a consequence of the presence of additional methyl group in the structure of CAF molecule providing additional steric hindrance in the hetero-complexes. It follows that the interceptor ability of the dimethylxanthines towards aromatic drugs is higher than that of CAF to the same drugs and is relatively unspecific to the drug itself.

The calculated magnitudes of the induced proton chemical shifts, $\Delta\delta_h = \delta_m - \delta_h$, in 1:1 hetero-complexes are given in Table 3. It is seen that the aromatic protons of THB and PARA are systematically shielded ($\Delta\delta_h > 0$) in the complexes with all drugs studied, which is in accord with the previously reported results on the hetero-association of THP and CAF with the same drugs [14,15]. It also confirms the conclusion, made above on the basis of analysis of experimental concentration dependences, on the stacking-type hetero-association of the dimethylxanthines with aromatic drugs. Similar shielding is also observed for PF and EB aromatic protons, but some of the DAU protons appear to be slightly deshielded ($\Delta\delta_h < 0$, see Table 3). The deshielding of the DAU protons in the hetero-complex with Theophylline was previously interpreted as the result of considerable difference in dimensions of the antibiotic and THP molecules [15], and positioning of the xanthine molecule above the two central rings of DAU. This leads to the situation when peripherial aromatic protons H2, H3, 4Me, H1′ of the antibiotic fall out of the shielding cone of the THP chromophore and results in the net deshielding (or nearly zero shielding) effect. It is possible to conclude that similar deshielding effect is the case for the drug-dimethylxanthine systems studied in the present work. Relatively high shielding of the H1 proton as compared to the neighbouring H2/H3 protons in the structure of DAU (see Fig. 1) is an artefact and is most likely due to local magnetic shielding from neighbouring polar C═O group of DAU, which was also noted before in THP-drug systems [15].

Based on the calculated magnitudes of $\Delta\delta_h$ in Table 3 it is possible to build initial structures of 1:1 hetero-complexes of the investigated systems for further energy minimisation by means of molecular modelling methods (see Supplementary material). As seen from the Table 3 the shielding profile for the drugs' protons is qualitatively similar for all dimethylxanthines studied, which suggests qualitatively similar structures of their hetero-complexes, also confirmed by the calculated structures. Relatively high shieldings of the dimethylxanthines' protons (mainly >0.5 ppm) means that they are located in close vicinity of the aromatic chromophores of the drugs.

**Table 3**
Induced chemical shifts of the protons of dimethylxanthines and aromatic drugs on hetero-association (0.1 M Na-phosphate buffer in $D_2O$, pD 7.1, $T$ = 298 K).

| System | DAU protons | | | | | | | Xanthine protons | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H2 | H1 | H3 | 4Me | H1' | H10e | H10a | H8e | H8 | 7Me | 3Me | 1Me |
| DAU + THB | −0.02 | 0.06 | −0.02 | −0.01 | −0.07 | 0.01 | −0.02 | 0.03 | 0.36 | 0.30 | 0.37 | – |
| DAU + PARA | −0.07 | −0.01 | −0.08 | −0.04 | −0.68 | −0.31 | −0.18 | 0.01 | 0.71 | 0.75 | – | 0.72 |
| DAU + THP [15] | 0.03 | 0.25 | 0.03 | 0.02 | −0.06 | 0.01 | −0.03 | 0.004 | 0.46 | – | 0.55 | 0.47 |

| | PF protons | | | | Xanthine protons | | | |
|---|---|---|---|---|---|---|---|---|
| | H9 | H1/8 | H2/7 | H4/5 | H8 | 7Me | 3Me | 1Me |
| PF + THB | 0.34 | 0.20 | 0.10 | 0.40 | 0.62 | 0.62 | 0.66 | – |
| PF + PARA | 0.31 | 0.17 | 0.06 | 0.35 | 0.86 | 0.89 | – | 0.85 |
| PF + THP [15] | 0.53 | 0.31 | 0.16 | 0.61 | 0.54 | – | 0.63 | 0.57 |

| | EB protons | | | | | | Xanthine protons | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | H1 | H10 | H4 | H2 | H7 | H9 | H8 | 7Me | 3Me | 1Me |
| EB + THB | 0.15 | 0.45 | 0.25 | 0.16 | 0.14 | 0.14 | 0.38 | 0.53 | 0.59 | – |
| EB + PARA | 0.35 | 0.39 | 0.22 | 0.16 | 0.13 | 0.16 | 0.54 | 0.68 | – | 0.85 |
| EB + THP [15] | 0.54 | 0.56 | 0.32 | 0.23 | 0.20 | – | 0.53 | – | 0.66 | 0.77 |

Analysis of the shielding profile of aromatic protons of the drugs and dimethylxanthines studied (data not shown) for the THP-7-AA/BA/PA systems (*i.e.* the Strategy 2) did not reveal any clear specificity in terms of some pattern in the dependence of $\Delta\delta_h$ on the length of —$(CH_2)_n$—COOH group. The latter is quite expected as the shielding of aromatic protons should be little affected by the length of the side chains attached to the chromophore. Analysis of the calculated spatial structures of the drug-AA/BA/PA hetero-complexes revealed no apparent hydrogen bonding between the —COOH group of the dimethylxanthines and the drugs (see Supplementary material) which is in accord with the same conclusion made above on the basis of the calculated values of equilibrium hetero-association constant and the $f_h$ factor.

## 4. Conclusions

The results of the present work suggest that the self- and hetero-association of dimethylxanthine derivatives with different structures, selected according to Strategy 1 (variation of the position of the methyl groups) and Strategy 2 (variation of the length of —$(CH_2)_n$—COOH group), with aromatic drug molecules proceeds in a form of a stacking-type complexation. The energetics of the self- and hetero-association was found to be relatively independent on the structure of the dimethylxanthines, which means that the introduction of a potential H-bond donor/acceptor —$(CH_2)_n$—COOH group into the structure of the xanthine chro mophore does not lead to additional stabilization of the hetero-complexes with aromatic drugs. However, it was also found that, on average, the dimethylxanthines possess higher hetero-association constant and, hence, higher interceptor ability as compared to the trimethylxanthine, Caffeine, used during the past two decades as a typical interceptor molecule. These results provide further step towards search of more efficient interceptors of aromatic drugs.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2011.11.045.

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