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Synthesis, Stability and Computational Study of some Ester Derivatives of Theophylline-7-acetic Acid with Antiproliferative Activity

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ABSTRACT

*Two new esters of theophylline-7-acetic acid were synthesized and their structures were confirmed by elemental analysis, FTIR, ^{1}H NMR spectral data. Structural analysis was also carried out using computational methods. The performed investigations on the spectral behaviour of the tested compounds show, that in comparison with the DFT calculations, the application of PM6 method is mainly limited to vibrations under 3000 cm^{-1}. From the performed stability evaluations was established that compounds **3a-b** are stable in acidic and weakly alkaline pH. In strong alkiline solution **3a-b** hydrolize and the corresponding rate constants were established. The analysis of the calculated molecular descriptors defined by Lipinski prove that the studied compounds obey "rule of five" and is very probable to expect good biological activity manifestation after peroral administration.The pharmacological screening in three human tumor cell lines show, that **3a** causes reduction of cellular viability at lower concentration as compared to **3b**.*

Key Words: antiproliferative activity, computational study, esters, stability, vibrational spectra.

INTRODUCTION

The purine system represents a versatile structural scaffold, whose extensive exploitation in drug design and discovery, has led to the identification of numerous lead compounds and subsequently commercialization of drugs with diverse biological effects including antineoplastic and immunosuppressive antimetabolites, adenosine receptor antagonists, xanthine oxydase and phosphodiesterase enzyme inhibitors etc. Among the purine-based drugs and lead compounds much attention has been paid to the structural modification of methylxanthines, due to their diverse pharmacological effects. Apart from the well established effects of methylxanthines on

CNS and cardiovascular function, bronchial muscle there is a compelling evidence for beneficial effects and augmentation of the cytotoxicity when co-administered with anticancer drugs [1-3]. Acting as G1 check point abrogators methylxanthines have been found to enhance the cytotoxicity of antineoplastic drugs and to restore sensitivity in resistant tumor models [4]. Methylxanthines inhibit DNA-repair and thus enhance the cytotoxicity of DNA-damaging cytotoxic agents such as cisplatin and alkylating drugs [5-9]. Additionally penthoxyphylline and its derivatives have been found to inhibit P-gp mediated multidrug resistance in cancer cells and thus to improve their responsiveness to chemotherapy [10]. Radiosensitizing activity has been also encountered for different methylxanthines [9, 11-13]. While these effects have been largely attributable to modulation of DNA-repair, xenobiotics efflux transporters, cell cycle check pint regulation and so on, there is also evidence for some intrinsic cytotoxic activity of methylxanthines, or their metabolites in tumor cells [14]. In this paper we explored the possibilities for attaining cytotoxic compounds via structural functionalization of theophyllin acetic acid, used as starting non-cytotoxic compound.

RESULTS AND DISCUSSION

2.1. Chemistry

Theophylline-7-acetic acid (**1**) was synthesized by method reported in the literature [15, 16]. The ester derivatives 2-(dibenzylamino)ethyl 2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetate (**3a**) and diphenylmethyl 2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetate (**3b**) were obtained by esterification of the acid with N,N-dibenzylaminoethanol (**2a**) and 1,1-diphenylmethanol (**2b**) in the presence of N,N'-dicyclohexylcarbodiimide (DCC) and 4-(N,N-dimethylamino)-pyridine (DMAP) as a catalyst (Fig. 1.). The reaction was carried out in anhydrous dichloromethane. The progress of reaction was monitored by TLC (until exhaustion of **1**).

All shown products proved to be homogeneous by TLC. No efforts were made to optimise yields. The obtained products **3a-d** are crystal compounds with white colour, soluble in chloroform and DMF, sparingly soluble in alcohol and insoluble in water.

Structures of **3a-b** were established by elemental analysis, UV, FTIR and ^{1}H NMR spectral data. The results were consistent with the assigned structures.

The FT-IR spectra of the studied theophylline-7-acetates **3a,b** in the region of the 4000 – 400 cm^{-1} exhibit several characteristic bands. The two strong bands at about 1662 to 1710 cm^{-1} are ascribable to the streching vibration of two carbonyl groups in the xanthine ring. The vibrational band recorded at 1738 – 1746 cm^{-1} belongs to the carbonyl in the ester moiety in the side chain. There is one characteristic band at about 1207 – 1218 cm^{-1}, ascribable to streching vibration of C—O—C atomic fragment in the ester group. The band at about 1550 cm^{-1} belongs to the streching vibration of C=C bonds in xanthine ring.

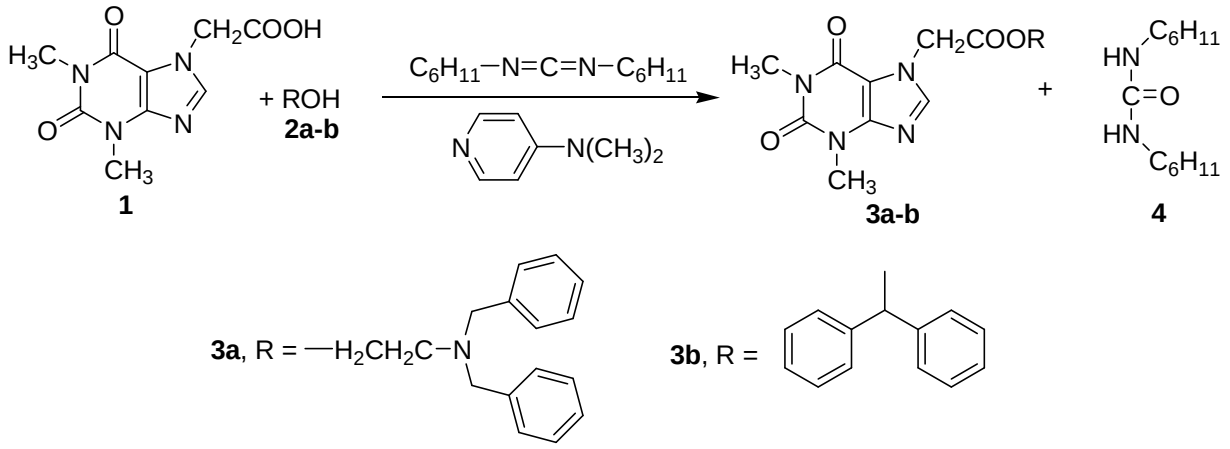


Figure 1. General reaction scheme for the synthesis of ester derivatives 3a-b.

More detailed information about the structure of compounds **3a,b** was provided by the ^{1}H NMR spectra. Thus, the strong singlets at 3.35 and 3.58 ppm in the spectrum of **3a** (3.31 and 3.58 ppm in the spectrum of **3b**) correspond to N-methyl protons at position 3 and 1. The signal of the N-methylene group from theophylline-7-acetic acid at position 7 appears at 4.99 ppm in **3a** (5.20 ppm in **3b**) as broad singlet. The signal of the C8-H was observed clearly in the spectra of **3a,b** at 7.51 ppm and 7.59 ppm respectively. These facts are in good correlation with the previously published data [17, 18].

The signals of methylene protons from the ethyl side chain of **3a** form two triplets at 4.27 ppm and 2.75 ppm with ^{3}J constants of 5.8 Hz. The signals of methylene protons from the benzyl residue of **3a** form strong singlet at 3.60 ppm. The methyne proton from the benzhydryl residue forms a clear strong singlet at 6.94 ppm. The signals of the aromatic protons in the spectra of **3a,b** correspond to complicated multiplets between 7.23 and 7.35 ppm, but the integral curves correspond to the exact number of the protons. The values of the chemical shifts of the protons registered by ^{1}H NMR spectra were compared with simulated values. We observed only small deviations of computed from experimental values, due to an impossibility to render an account of influence of the solvent. Regardless, the simulated ^{1}H NMR spectra are in good correlation with experimental ones.

2.2. Comparison of the theoretical and experimental Infrared spectra of theophylline, theophylline-7-acetic acid (1) and compounds 3a,b.

2.2.1. Molecular geometry

The calculated vibrational spectra have no imaginary frequency, which indicates that the optimized geometry is located at the minimum point on the potential energy surface. According to the theoretical calculations, all studied compounds (theophylline, **1** and **3ab)** have assumed to possess a planar *C1* point group symmetry.

Selected bond lengths and angles of calculated structural parameters of theophylline residue in **3a,b** in comparison with these of theophylline and theophylline-7-acetic acid (**1**) are given in Table 1, in accordance with the atom numbering scheme on Fig. 2.

The theophylline ring in compounds under consideration **3a,b** is essentially planar as observed in theophylline [19] and theophylline-7-acetic acid [20]. The acetic fragment is planar also [20].
The bond lengths, bond and torsion angles in **3a,b** are generally within the expected ranges, but some significant differences between the corresponding bond lengths and angles in theophylline

and theophylline-7-acetic acid **1** are observed. As it is seen, the benzhydryl group in **3b** brings about such significant differences in theophylline residue, probably due to electronic effects of conjugation. In favour of this assertion is the fact that the C – N bond lengths in theophylline ring range from 1.345 Å to 1.398 Å, which deviates from the values, observed in crystal structure of theophylline-7-acetic acid [20].

Table 1. Selected bond lengths (Å), bond angles (º) and torsion angles (º).

	Theophylline [19]	**1**	**3a**	**3b**
N1 – C1	1.413	1.420	1.420	1.398
N2 – C2	1.359	1.385	1.385	1.349
N4 – C3	1.388	1.402	1.402	1.344
N3 – C5	1.317	1.356	1.357	1.345
N1 – C6(CH_3)	1.488	1.445	1.445	1.451
O2 – C1 – N1	120.1	119.7	119.6	120.8
O2 – C1 – N2	122.4	120.6	119.7	128.8
O1 – C4 – N1	121.2*	121.5	121.4	120.8
O1 – C4 – C3	126.5*	124.6	124.6	119.2
C3 – N4 – C8 – C9		– 107.4	139.8	53.2

** The values are taken from [21].*

2.2.2. Vibrational spectra

The results of calculations of normal mode vibrations of some bonds in the molecules of theophylline, **1** and **3ab** at PM6 and B3LYP levels using 6-311+G(d,p) basis set, as well as experimental ones were shown in Table 2.

The calculated frequencies are slightly higher than the observed values for the majority of the normal modes. The major factor which is responsible for these discrepancies between the experimental and computed value is related to the fact that the experimental value is an anharmonic frequency while the calculated value is harmonic frequency. Vibrational frequencies calculated at B3LYP level were scaled by 0.98 [22] and those calculated at PM6 level were not scaled.

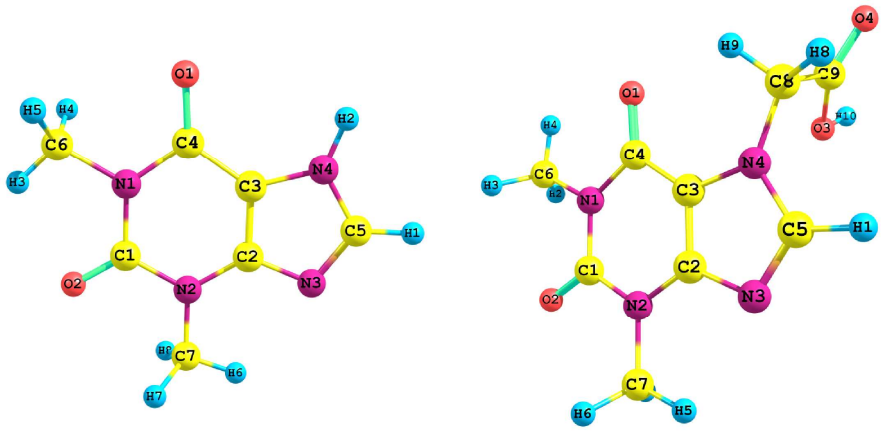


Figure 2. Numbering system adopted in this study.

CH and NH stretching vibrations

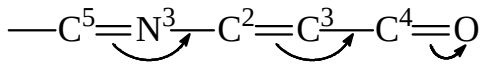
Most organic compounds have CH bonds; a useful rule is that absorption in the 2850–3000 cm^{-1} is due to sp^3–CH stretching whereas absorption above 3000 cm^{-1} is from sp^2 CH stretching.

Also the assignments of the CH_2 stretching bands are always ambiguous since they are coupled with the overtone and combination band of CH_2 bending at around 1450 cm^{-1}.

A good correlation of theoretical values with that of experimental evaluations was found in the symmetric and asymmetric stretching vibrations of aromatic CH and CH_2 moiety. A complex broad bands in the region 3100–2810 cm^{-1} were observed in the spectra of the studied compounds. These assignments are also supported by literary data [23]. We assigned these bands to the out-of-plane and in-plane asymmetric and symmetric —CH_2 and CH_3 stretching vibrations, respectively. To this region the aromatic CH vibrations in compounds **3a,b** and stretching vibration for C5-H1 bond belong, where the latter appears at around 3136 – 3062 cm^{-1} and has a higher vibration frequency in comparison to aromatic CH vibrations. The difference between the observed and the calculated by DFT method values, is at an average of 96 cm^{-1}, while the PM6 method gave poor results for this vibration mode. In addition, in theophylline, νN—H vibration occurs in the region 3200– 3100 cm^{-1}. The IR band appearing at 3121 cm^{-1} is assigned to this stretching mode of vibration. This vibration mode is calculated at 3593 cm^{-1} for DFT method. The difference between experimental and calculated νN—H stretching mode is about 84 cm^{-1}. This striking discrepancy may come from the difference between the solid and the gas phase, but the IR spectrum of theophylline was taken in KBr pellets while the theoretical calculation of molecule was performed in gas phase.

C=O and COOH streching vibrations

The carbonyl group exhibits a strong absorption band due to C=O stretching vibration and is observed in the region 1850–1550 cm^{-1}. The studied compounds contain two carbonyl groups in the meta position, which belong to a system conjugated on the principle of vinylogy:



Influence of N-2 p-electron lone pair on this system can't be excluded. Due to this conjugation the rank of multiple bonds decrease which leads to a decrease of their force constants and vibrations frequency. Such a decrease of vibration frequency of carbonyl groups is characteristic for α,β-unsaturated ketones [24]. Considering the above, the strong bands observed at 1698 – 1710 cm^{-1} and at 1662 - 1669 cm^{-1} in FTIR spectra are accounted to C=O asymmetric and symmetric stretching vibrations in xanthine.

On the other hand, it is a well known tendency of xanthines to associate in solid state. Investigations on self-association and complex formation of theophylline [25] show decrease of vibration frequency of carbonyl group. In this case we consider a formation of charge transfer complex, in which C-6 carbonyl group acts as □-electron acceptor while N-9 functions as □-electron donator. This self-complexing leads to additional decreasing of vibration frequency of carbonyl group to lower values of wave number.

The calculated wave numbers for stretching vibrations of carbonyl groups (□C=O) after scaling are in good correlation with experimental values. Our calculations show that greater contribution to the vibration band at 1698 – 1710 cm^{-1} have C^4-O^1 carbonyl group, while to the band at 1662 - 1669 cm^{-1} – C1-O2 carbonyl group. The side chain ester carbonyl group appears at 1746 cm^{-1} for **3a** and 1738 cm^{-1} for **3b**, while the same group in **1** appears at 1737 cm^{-1}. The calculated wavenumbers show positive deviation of 45 to 81 cm^{-1} in comparison with scaled DFT values and 66 to 104 cm^{-1} in comparison with PM6 values. OH stretching band is characterized by very broad complex band appearing near at about 3400 cm^{-1}. The band observed at 3441 cm^{-1} has its

origin in the O-H stretching vibration. The theoretically computed at DFT level value of 3644 cm^{-1} shows a good agreement with experimental results, while it is not the same at PM6 level.

Table 2. Results of calculation of some normal mode vibrations of theophylline, 1 and 3a-b.

Compound	Assignment	Wave number [cm^{-1}]			
		Calculated PM6	Calculated DFT	Scaled DFT	Observed
3a	ν(C1-O2)	1756	1750	1715	1662
	ν (C4-O1)	1796	1790	1754	1710
	ν (C9-O4)	1812	1833	1796	1746
	ν (C2-C3)	1570	1643	1610	1551
	ν (C5-N3)	1407	1418	1389	1600
	ν (C9-O3)	1317	1296	1270	1218
	ν (C5-H1)		3262	3196	3116
	skeletal vibrations	1420	1437	1408	1435, 1444 s, 1454
3b	ν (C1-O2)	1755	1740	1705	1669
	ν (C4-O1)	1794	1790	1754	1698
	ν (C9-O4)	1806	1820	1783	1738
	ν (C2-C3)	1569	1643	1610	1550
	ν (C5-N3)	1407	1418	1389	1609
	ν (C9-O3)		1289	1263	1207
	ν (C5-H1)		3264	3198	3102
	skeletal vibrations	1421	1438	1409	1418, 1456, 1472
1	ν (C1-O2)	1757	1749	1714	1664
	ν (C4-O1)	1797	1791	1755	1710
	ν (C9-O4)	1841	1856	1818	1737
	ν (C2-C3)	1615	1644	1611	1554
	ν (C5-N3)	1407	1590	1558	1612
	ν (C9-O3)	1288	1392	1364	1244
	ν (O3-H10)	2533	3753	3677	3441
	ν (C5-H1)		3262	3196	3136
	skeletal vibrations	1422	1439	1410	1405, 1438, 1454 s
Theophylline	ν (C1-O2)	1757	1749	1714	1664
	ν (C4-O1)	1797	1791	1755	1710
	ν (C2-C3)	1841	1856	1818	1554
	ν (C5-N3)	1615	1644	1611	1612
	ν (N4-H2)	1407	1590	1558	3118
	ν (C5-H1)		3264	3198	3102
	skeletal vibrations	1397	1432	1403	1422, 1448, 1467

C=C, C-N and C=N vibrations

Pyrimidines absorb strongly in the region 1600–1500 cm^{-1} due to the C=C and C=N stretching vibrations. This is evident from the presence of the bands present at around 1600 cm^{-1} in the spectra of the compounds. In theophylline the band observed at 1566 cm^{-1} in FTIR spectra is assigned to be due to C=C stretching. The shoulders observed around 1600 - 1612 cm^{-1} are assigned to be due to C=N stretching vibration.

The absorption bands at about 1440 cm^{-1} are ascribable to skeletal vibrations of xanthine ring. These results are in accordance with those reported for the molecular structure of Vephylline metal complexes [26]. Our investigations show that the stretching vibrations of C^5–N^3, C^1–N^1

and C^1–N^2 bonds have a greatest contribution to these vibrations. Three bands of absorption were observed in this region of the spectra of studied compounds, which may due to solid – state effects.

From the above performed investigations of the spectral behaviour of the tested compounds may be concluded, that the DFT calculations give beter results in comparison with the PM6 method. Nevertheless, PM6 method may have its benefits and is certainly not always worse than DFT calculations, though application is mainly limited to vibrations under 3000 cm^{-1}.

2.2.3. Calculation of some quantum-chemical descriptors

It is well known, that in QSAR studies and rational drug design a number of quantum-chemical parameters are significant for the biological activity expressed as: interaction with receptor, penetration through the cell membrane and behaviour of the molecule during metabolic processes. As a measure of of molecular hydrophobicity the octanol-water partition coefficient known as logP is used. It has become a key parameter in studies of drug absorption, bioavailability, hydrophobic drug-receptor interactions, metabolism of molecules, as well as their toxicity. Molecular polar surface area (PSA) [27] is defined as a sum of surfaces of polar atoms (usually oxygens, nitrogens and attached hydrogens) in a molecule and is a very useful parameter for prediction of drug transport properties. This parameter has been shown to correlate very well with the intestinal absorption and blood-brain barrier penetration [28]. Rotatable bond (nrotb) is defined as any single non-ring bond, bounded to nonterminal heavy (i.e., non-hydrogen) atom and this simple topological parameter is a measure of molecular flexibility. High oral bioavailability is an important factor for the development of bioactive molecules as therapeutic agents. Passive intestinal absorption, reduced molecular flexibility (measured by the number of rotatable bonds), low polar surface area or total hydrogen bond count (sum of donors and acceptors) are found to be important predictors of good oral bioavailability [29, 30, 31]. Lipinski et al. [32] has used these molecular properties in formulating his “rule of five”. This rule reads that most molecules with good membrane permeability have logP □ 5, molecular weight □ 500, the number of hydrogen bond acceptors □ 10, and the number of hydrogen bond donor □ 5.

In order to establish the significance of some quantum-chemical parameters and molecular descriptors, such as dipole moment, HOMO and LUMO energies, ionization potential, Log P, COSMO molecular volume, total polar surface area (TPSA), number of hydrogen bond acceptors (nON acceptors), number of hydrogen bond donors (nOHNH donors), number of rotatable bonds (nrotb) and charges a theoretical calculation of these parameters for compounds **3a-b** was made. The obtained values are presented in Tables 3 – 4, where Table 4 contains a calculated percentage of absorption (%ABS), molecular polar surface area (PSA), and Lipinski parameters of the compounds investigated. Extension of absorption is expressed by the percentage of absorption. Absorption percent was calculated [33] using the expression: %ABS = 109 – 0.345 PSA.

All of the investigated compounds are relatively small molecules, according to their COSMO molecular volume. As seen, **3a-b** are polar compounds, as they have high positive values of dipole moment. The values of HOMO and LUMO energies are important for evaluating the redox potential of the compounds. The lower negative HOMO energy and the negative values of LUMO energies show that these compounds have the properties of reductors. This information may be significant for drug metabolism, as the oxidation and the reduction are the main metabolic pathways for many drug molecules. As observed from the calculated Log P values, all of the compounds possess hydrophobic properties, which will contribute to good penetration through cell membrane, respectively to good absorption. High number of hydrogen bond donors

and acceptors in the **1** and **3a,b** resulted in its reduced absorption in comparison with the parent theophylline.

Table 3. Main quantum chemical descriptors of 3a-b compared to Theophylline and Theophylline-7-acetic acid

compound	□ [D]	$E_{(HOMO)}$ [eV]	$E_{(LUMO)}$ [eV]	Ionization Potential [eV]	COSMO Volume [Å^3]	LogP		
						MiLogP [34]	Ghose – Crippen [35]	Broto – Moreau [36]
3a	2.65	- 9.201	- 0.765	9.20	548.31	2.75	2.37	1.23
3b	4.26	- 9.201	- 0.73	9.20	465.58	3.10	2.59	1.29
Theophylline	3.12	-9.324	-0.738	9.32	198.05	0.06	-0.71	-0.97
1	3.16	-9.333	-0.840	9.33	256.22	-0.69	-1.21	0.548

Table 4. Calculated absorption (%ABS), polar surface area (PSA) and Lipinski parameters of the studied compounds

Compounds	% ABS	TPSA	nON acceptors	nOHNH donors	nrotb	Mw
3a	78	88.14	8	0	6	461.5
3b	77	91.38	9	0	10	404.4
1	75	99.14	8	1	2	238.2
theophylline	84	72.69	6	1	0	180.2

The tested compounds have several (8 for **3a** and 9 for **3d**) negative centres for hydrogen bond formation and v. d. Waals interactions with receptor. As it can be seen, compounds **3a,b** covered Lipinski's "Rule of 5" and a good biological activity manifestation may be expected.

2.3. Stability studies

In the buffer solution with pH=2 the hydrolysis starts spontaneously at the first minute after dissolving (90.10 % is area of theophylline-7-acetic acid). After 45 minutes the equilibrium between ester and **1** set in the slolution, due to esterification.

The esters are found to be stable in the buffer solution with pH=9 during the investigated time intervals of 300 min. The retention times (t_r) are unchanged and correspond to those of solutions A and B. The remaining concentration of compound is about 100% from theoretically estimated. Other peaks are not observed on the chromatograms.

Under the described conditions no detectable amounts of hydrolytic product were found in the first 2 h of the incubation period. These results showed that the synthesized esters were quite resistant against chemical hydrolysis and would be stable during the pass through the gastrointestinal tract. This high stability could be explained with the precence of steric hindered acid fragment.

In order to demonstrate that the esters can be converted to the parent compounds *in vitro*, kinetic studies at more drastic conditions were performed. Compounds **3a,b** underwent complete hydrolysis in 0.1 M sodium hydroxide solution (pH=13) ot 20°C. The complete hydrolitic degradation of **3a,b** in strong alkaline medium can be attributed to the immediate transformation of **1** obtained to the sodium salt. The exhaustion of ester was accompanied by progressive increase of concentration of free theophylline-7-acetic acid as revealed by HPLC analysis. The formation of **1** was found to be linear function of the initial ester concentration. This indicates

first order hydrolytic kinetics. The rate constants K for the hydrolysis were calculated from the slopes of semilogarithmic plots of C_∞–C / t curves, where C_∞ represents the concentration of **1** at the end of hydrolysis and C is the concentration of the product (**1**) at each of the time intervals. The corresponding half lives were calculated using the equation $t_{1/2} = \frac{\ln 2}{K}$. The results obtained are summarized on Fig. 3.

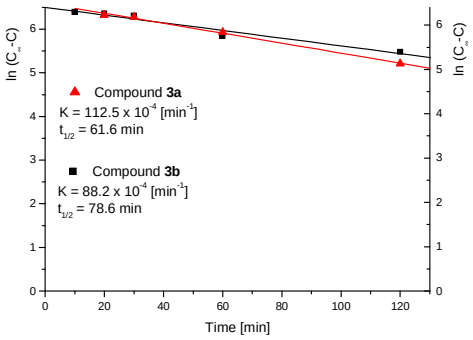


Figure 3. First order plot for the degradation of compounds 3a,b at pH=13 at 20 °C

2.4. Pharmacology

Compounds **1**, **3a** and **3b** were tested for antiproliferative activity against three human tumour cell lines, after 72 h continuous exposure, using the MTT-dye reduction assay. The results were normalized as percentage of the untreated control and fitted to sigmoidal concentration-response curves (Fig. 4).

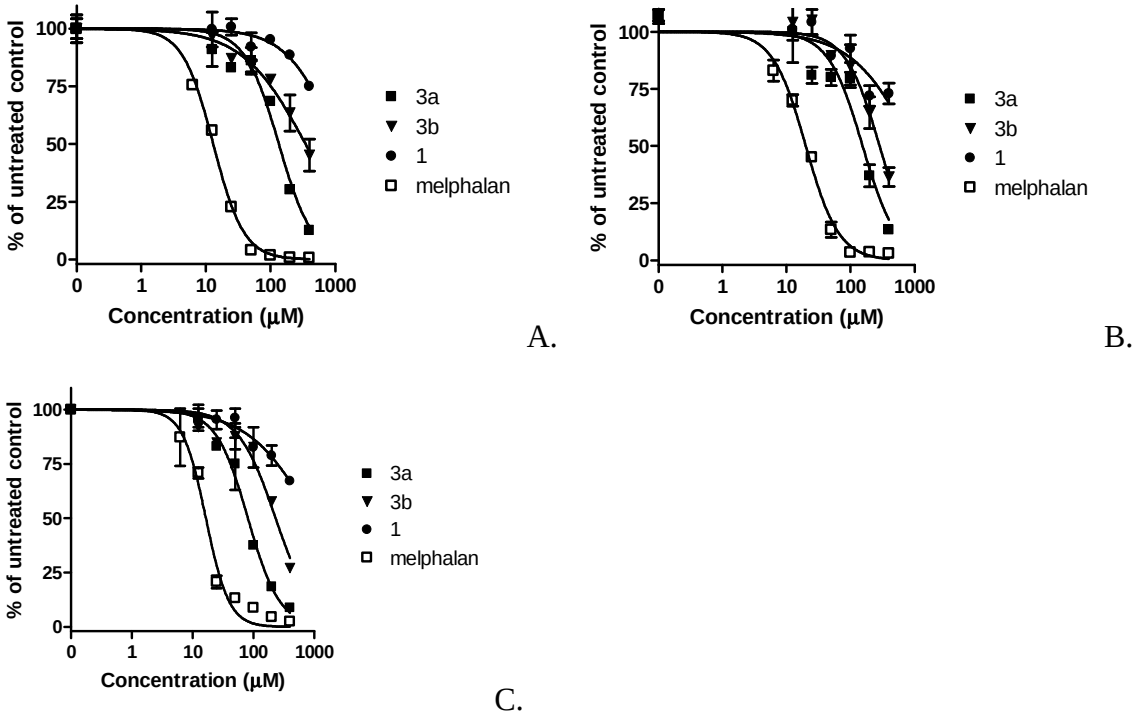


Figure 4. Antiproliferative activity of compounds 1, 3a and 3b, vs the reference antineoplastic agent melphalan against human tumor cell lines: HL-60 (A), BV-173 (B) and SKW-3 (C), after 72 h exposure (MTT-assay). Each data point represents the arithmetic mean (±sd) from 8 separate experiments.

The IC_{50} values were calculated using non-linear regression analysis, and summarized in Table 5.

Evident form the results obtained theophyllin acetic acid **1** was practically devoid of antiproliferative activity, failing to induce 50% reduction of cellular viability within the investigated range of concentrations (12.5-400 μM). The two ester derivatives **3a** and **3b** were more active than than **1**. Thereby throughout the tested cell lines 3a showed superior amntirpoliferative activity as compared to **3b**. These findings indicate that as far as antiproliferative activity is concenrned the introduction of an ester moiety is a structuralprerequisite for higher activity as compared to the non-estherifyed prototypic theophylline acetic acid. The bulkier dibenzylaminoethyl structural fragment present in **3a** affords superior activity as compared to the diphenylmethyl moierty in **3b**.

Table 5. Experimentally established IC_{50} for the target compounds.

Compound	IC_{50} (μmol/l)		
	HL-60	BV-173	SKW-3 (KE-37)
1	> 400.0	> 400.0	> 400.0
3a	134.2	155.3	81.3
3b	348.5	285.3	232.01
Melphalan	13.9	20.04	16.7

3. EXPERIMENTAL

3.1. Chemistry

Melting points were determined on an electrothermal apparatus (Büchi 535, Switzerland) in an open capillary tube and are not corrected. UV spectra were recorded on a Hewlet Packard 8452A Diode Array Spectrophotometer equipped with an HP Vectra 386/25 computer. The IR spectra 400 – 4000 cm^{-1} were recorded on a Nicolet iS10 FT-IR Spectrometer in KBr. ^1H spectra were recorded at ambient temperature on a Bruker-250 WM (250-MHz) spectrometer (Germany) and were measured for approximately 0.03 M solutions using DMSO-d_6 as solvent and chemical shifts were expressed as □ values in ppm against TMS as an internal standard; the coupling constants were expressed in Hertz (Hz); standard abbreviations are used. The completion of reactions was monitored through TLC, which was performed on DC-Alufolien Kieselgel 60 F254 (Merck) (0.20 mm) sheets with solvents: ethanol–chloroform–acetone (4:3:3 volume parts). The spots were detected at UV 254 nm. Synthetic grade chemicals procured from Merck, Germany, were used for the synthesis of the target compounds, as received. Microanalyses were performed on a Perkin-Elmer 2400-II Element analyzer. All solutions were dried over anhydrous sodium sulfate and evaporated on a Büchi rotary evaporator at reduced pressure. All products were shown to be homogeneous by TLC. The given yield is the yield of TLC homogeneous product. No efforts were made to optimize the yields. Theophylline-7-acetic acid was obtained in accordance with ref. [15, 16].

3.2. Chromatographic conditions, apparatus and reagents

The HPLC system was consisted of a Shimadzu model LC-10A with a model SPD 10 AVvp – UV-VIS with fixed analytical wave lengths (273 nm analytical wave length), a manual injector module with a 20 μl loop and a Shimadzu model CBM-10A system controller (Shimadzu, Japan). HPLC analysis was performed on a chromatographic column LiChrosorb RP-18, 4.6 x 250 mm, ODS with particle size 5 μm (Merck, Germany). The mobile phase was a mixture of filtered and degassed acetonitrile: water 70:30 v/v respectively. The flow rate was 1 mL x min^{-1}. All analyses were carried out isocratically at temperature of 20°C. HPLC grade acetonitrile was used to prepare the mobile phase. Buffer reagents and all other chemicals were of analytical

grade quality. Buffer solution with pH = 2.0 and pH = 9.0 were prepared by European Pharmacopoeia VI. Theophylline-7-acetic acid (**1**) was used as reference substance.

3.2.1. Preparation of chromatographic solutions:
Solution A of **3a** was prepared by dissolving of adequate amount of substance in buffer solution to obtain solution with concentration 0.0030 g/ml. Solution B of **3b** was prepared by dissolving of adequate amount of substance in buffer solution to obtain solution with concentration 0.0030 g/ml. Mixture C was prepared by adding of equal volumes from solution A and solution containing appropriate amount of 7-theophylline acetic acid. Mixture D was prepared by adding of equal volumes from solution B and solution containing appropriate amount of 7-theophylline acetic acid (**1**).

3.3. Stability studies
To a sample containing about 30 mg of **3a** or **3b** was added 10.0 ml buffer solution with pH 2.0, 9.0 or 10.0 ml of 0.01 mol sodium hydroxide solution (pH = 13), respectively. The obtained test solution was maintained at fixed temperature of 37 ± 0.1 ^{0}C and continuously stirring. Samples of 0.1 ml from the investigated mixture were taken at appointed periods, dissolved to 10.0 ml with mobile phase and immediately injected in the chromatograph. The study was prolonged to the obtaining of unchangeable remainder. Quantitation of each compound was achieved with a reference to a suitably prepared calibration curve, using peak areas.

3.4. General procedure for synthesis of 7-theophyllineacetates 3a-b
7-Theophyllineacetic acid (2.38 g, 11 mmol), corresponding hydroxy derivative (11 mmol) and 4-dimethylaminopyridine (DMAP, 0.3 g, 2.5 mmol) were dissolved in 100 ml of anhydrous dichloromethane at room temperature. After complete dissolution, dicyclohexylcarbodiimide (DCC, 2.7 g, 0.013 mol) dissolved in 15 ml of anhydrous dichloromethane was added. The reaction mixture was stirred at room temperature for 1 h, then the white precipitate of N,N-dicyclohexylurea (DCU) was filtered off, and the solvent was evaporated (Büchi Rotavapor R-114) to give a pale yellow oil-like residue. A small amount of 2-propanol was added to the residue and it was kept for 24 h at -5 °C. Whitish crystals of crude product were obtained. The separated crystals were recrystallized from 2-propanol.

2-(dibenzylamino)ethyl 2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetate (**3a**). Yield – 2.95 g (58%). M. p.: 97 – 99 °C. IR: 3116 (νAr-H); 2800 – 3000 ($\nu^{s \text{ and } as}CH_3$, $\nu^{s \text{ and } as}CH_2$); 1746 (□CO – ester); 1710, 1662 (□CO-xanthine); 1551 (□C=C); 1378 (δ^sCH_3, δ^sCH_2); 1218 (□C-O-C – ester); 745, 701 (δAr-H); ^{1}H-NMR: 7.51 (s, 1H, C8H); 7.34 – 7.23 (m, 10H, aromatic); 4.99 (s, 2H, N7-CH_2-); 4.27 (t, 2H, CH_2 – side chain, J = 5.8); 3.60 (s, 4H, N–CH_2 – side chain), 3.58 (s, 3H, N3–CH_3); 3.35 (s, 3H, N1–CH_3); 2.75 (t, 2H, –CH_2–N– side chain, J = 5.8). UV ($CHCl_3$, nm): λ_{max} = 244, 268. Calculated $C_{25}H_{27}N_5O_4$ (461.52) requires: C, 65.06; H, 5.90; N, 15.17; found: C, 64.95; H, 5.95; N, 15.05.

diphenylmethyl 2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetate (**3b**). Yield – 2.50 g (56%). M. p.: 169 – 171 °C. IR: 3102 (νAr-H); 2800 – 3000 ($\nu^{s \text{ and } as}CH_3$, $\nu^{s \text{ and } as}CH_2$); 1738 (□CO – ester); 1698, 1669 (□CO-xanthine); 1550 (□C=C); 1374 (δ^sCH_3, δ^sCH_2); 1207 (□C-O-C – ester); 746, 699 with shoulder at 701 (δAr-H); ^{1}H-NMR: 7.59 (s, 1H, C8H); 7.35 – 7.26 (m, 10H, aromatic); 6.94 (s, 1H, O–CH); 5.198 (s, 2H, N7-CH_2-); 3.58 (s, 3H, N3–CH_3); 3.34 (s, 3H, N1–CH_3). UV ($CHCl_3$, nm): λ_{max} = 242, 276. Calculated $C_{22}H_{20}N_4O_4$ (404.43) requires: C, 65.34; H, 4.98; N, 13.85; found: C, 65.15; H, 5.05; N, 13.65.

3.5. Computational details

All names were generated by using structure-to-name and name-to-structure algorithms included with ChemBioDraw Ultra 11.0 (CambridgeSoft) [37]. The theoretical ^{1}H NMR spectra were calculated on ACD/HNMR Version 1.0 program [38]. The energetically preferred geometries were calculated by force field energy minimization. Force field calculations were performed with the program ChemBio3D Ultra 11.0 [37] using the parameter set of the Allinger's MM2 force field [39]. Then the obtained new geometries were completely optimised and vibrational frequencies and the force matrix were calculated, using the PM6 semiempirical Hamiltonian [40], as implemented in the MOPAC 2009 [41] package. All semiempirical calculations were performed with VEGAZZ ver. 2.3.1 program [42], as well as the quantum-chemical parameters: dipole moment, HOMO and LUMO energies, ionization potential, Log P and COSMO molecular volume. The range of Log P values was obtained by three theoretical methods – Ghose – Crippen [35], Broto – Moreau [36] and MiLogP [34]. The molecular structures, obtained after force field energy minimization, were also optimized by density functional using Becke's three-parameter hybrid method [43, 44] with the Lee, Yang and Parr [45] correlation functional methods (B3-LYP) with the standard 6-31G*(d,p) basis set [48]. The optimized structural parameters were used in vibrational frequency calculations on DFT level and 6-31G*(d, p) basis set was used for all elements. All DFT calculations were performed with the Gaussian 03 rev. E01 program package [47]. Molecular descriptors (total polar surface area (TPSA), number of hydrogen bond acceptors (nON acceptors), number of hydrogen bond donors (nOHNH donors) and number of rotatable bonds (nrotb) were calculated with Molinspiration free online chemoinformatics service tool [34].

3.6. Pharmacology

3.6.1. Cytotoxicity assessment (MTT-dye reduction assay).

The cell lines used in this study namely HL-60 (acute myelocyte leukemia), BV-173 (chronic myeloid leukemia) and SKW-3 (KE-37 derivative) (T-cell leukemia) were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ GmbH, Braunschweig, Germany).

Cellular viability after exposure to the tested compounds was assessed using the standard MTT-dye reduction assay as described by Mosmann [48] with some modifications [49]. Cell survival fractions were calculated as percentage of the untreated control. In addition IC_{50} values were derived from the concentration-response curves.

The cell survival data were normalized as percentage of the untreated control (set as 100% viability). The statistical processing of biological data included the Student's t-test whereby values of $p \leq 0.05$ were considered as statistically significant.

CONCLUSION

In the present study the synthesis of two ester derivatives of theophylline-7-acetic acid was described. The newly synthesized **3a-b** are crystal compounds with white to very pale-yellow colour, soluble in water, sparingly soluble in alcohol and insoluble in DMF. Structures of **3a-b** were established by elemental analysis, FTIR, ^{1}H NMR spectral data. Structural analysis was carried out using spectral data and computational methods. A very good correlation between the registered spectra and computed values was observed. A several number of quantum-chemical parameters were calculated for **3a-d**. The performed investigations of the spectral behaviour of the tested compounds show, that the DFT calculations give beter results in comparison with the PM6 method. Nevertheless, PM6 method may have its benefits and is certainly not always worse

than DFT calculations, though application is mainly limited to vibrations under 3000 cm^{-1}. The performed stability evaluations show that compounds **3a-b** are stable in acidic and weakly alkaline pH values. In strong alkiline solution **3a-b** hydrolize and the corresponding rate constants were established. The analysis of the molecular descriptors defined by Lipinski has shown that the compounds studied obey "rule of five" and it is very probable to expect a good biological activity manifestation after peroral administration. The pharmacological screening in three human tumor cell lines shows that while the prototype theophylline acetic acid **1** is devoid of antiproliferative activity the introduction of an ester function renders the compounds active. The juxtaposition between the two novel compounds shows that **3a** causes reduction of cellular viability at lower concentration as compared to **3b**.

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