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Synthesis, Brain Antihypoxic Activity and Cell Neuroprotection of Ester Derivatives of 7-Theophylline acetic acid

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Abstract

*The synthesis of three ester derivatives of 7-theophylline-acetic acid and hydroxyl group containing compounds was studied by DCC/4-DMAP – mediated esterification under mild conditions. The structures of synthesized compounds were proved by microanalyses, UV-, IR- and ^{1}H-NMR data. Acute toxicity assessment of the compounds in mice shows less toxicity than standard theophylline. Compounds **3a-c** posses depressive activity on CNS (increasing of pentobarbital sleeping time and decreasing of spontaneous locomotor activity). In in vivo experiments of brain anoxic hypoxia, compounds **3a** and **3c** in a dose $^1/_{10}$ of LD_{50}, result in increase of the mean survival time of mice. These results indicate that compounds **3a** and **3c** are very prospective for further pharmacological and biochemical experiments in relation to the treatment of brain neurodegenerative disorders.*

Key words: 7-Theophylline acetic acid, esters, acute toxicity, brain antihypoxic activity

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INTRODUCTION

Dementia is the most important psychiatric syndrome associated with degenerative brain disease. Alzheimer's disease (AD) is the most common cause of dementia in the elderly, and its main clinical symptom is global deterioration of cognitive functions. AD appears to result from multiple pathogenic events, including inflammation, hypoxic / ischemic lesions, the symptoms of which may closely mimic primary brain degeneration. The pathogenic role of β-amyloid is also widely accepted [1-4]. Therapeutic approaches based on these pathogenic mechanisms are developing very rapidly in the last decade. One of the recent pharmaceutical research strategies is searching of possible therapeutic agents in the group of purine and xanthine derivatives. According to some data in the literature the asymmetrically substituted xanthines show activity

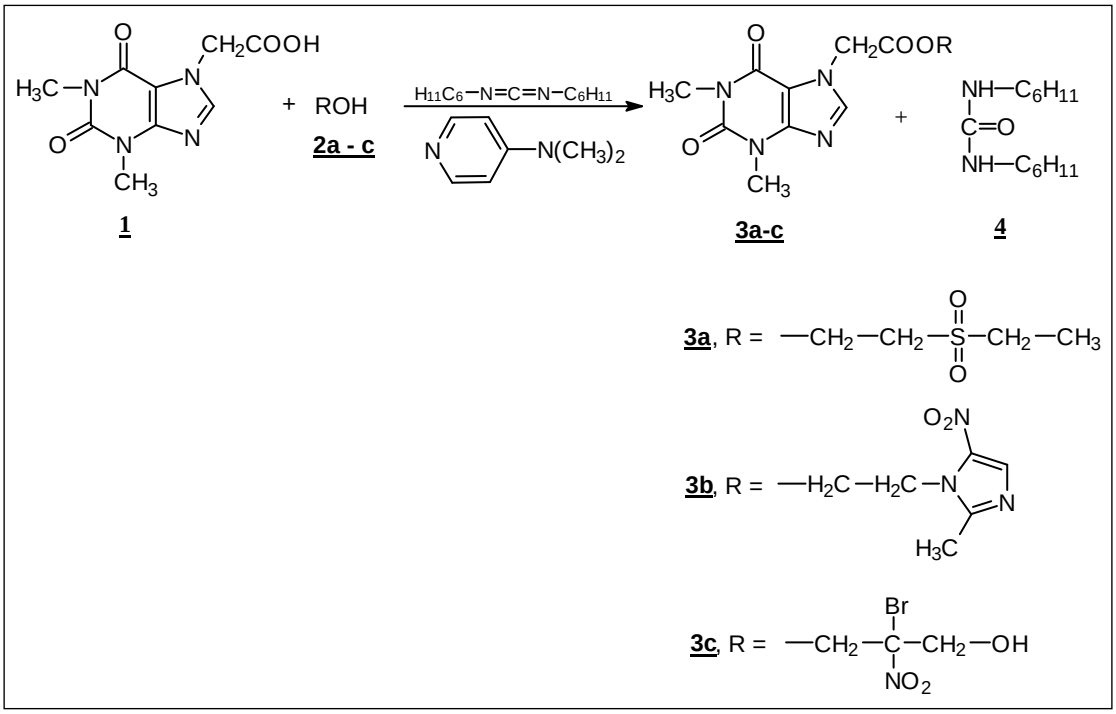
in animal models towards neurodegenerative diseases including Alzheimer's type dementia [5]. For example, recently a new xanthine derivative, Propentophylline, was introduced as a drug with neuroprotective properties for treatment of brain dementia [6], boosting the search for new xantine derivatives with neuroprotective actions. In previous studies 7- substituted-1,3-dimethylxanthines and 1-substituted-3,7-dimethylxanthines show brain antihypoxic activity, especially on model of anoxic hypoxia [7, 8].

With the aim of designing new and potent theophylline derivatives useful in the treatment of AD, the synthesis of new ester derivatives of 7-theophylline acetic acid is described. The acute toxicity and pharmacological assay of new compounds was also reported.

RESULTS AND DISCUSSION

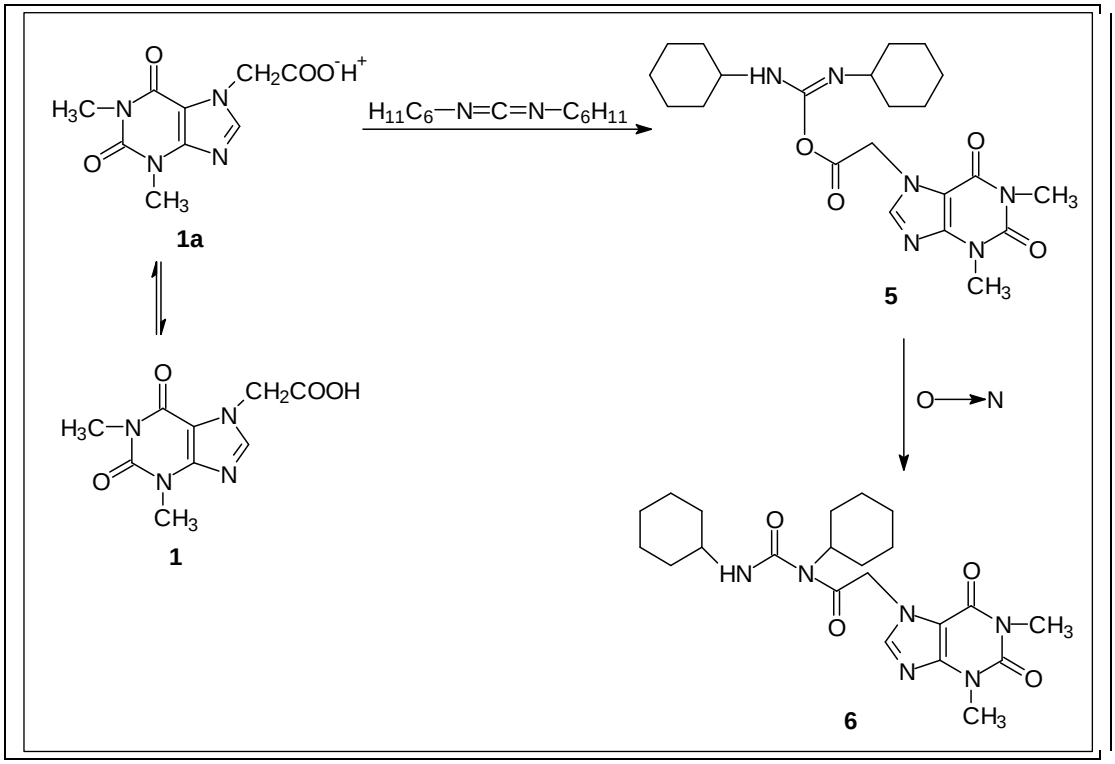
Chemistry

The 7-theophyllineacetates **3a-c** are synthesized according to the synthetic pathway shown in Scheme 1.



Scheme 1. Synthesis of ester derivatives 3a-c

The main side reaction according to the proposed mechanism of the process [9] (Scheme 2) is the formation of N-acyl urea (**6**) by an intramolecular rearrangement of the O-acyl urea (**5**). The by-product was registered by TLC in the course of reaction. It was isolated as described elsewhere [10] in yields 29% to 38% as crude product. DCU (**4**) was isolated in yields 62% to 71%. These values show that the real yields of **3a-c** should be the same as these of DCU. The lower yields of isolated products were due to 15%-17% losses in isolation and recrystalization.



Scheme 2. Formation of N-acyl urea (6) by an intramolecular rearrangement of the O-acyl urea (5)

The structures of **3a-c** were supported by elemental analysis and UV, IR and ^{1}H-NMR spectral data. All the UV and IR spectra were consistent with the assigned structures. The ^{1}H-NMR spectra of **3a-c** were also in agreement with the structures cited. Thus, the strong singlets at 3.60 and 3.35 ppm correspond to N-methyl protons at position 3 and 1. The signal of the N-methylene group from theophylline acetic acid at position 7 appears at 5.04 ppm in as singlet. These facts are in good correlation with the previously published data [11-13]. The signals of the other methylene protons and C8-H were observed clearly in the spectra of **3b,c**, as well as methyl and methine protons at position 2 and 4 in imidazole ring of compound **3b** (2-(2-methyl-5-nitro-1H-1-imidazolyl)-ethyl 2-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-7-purinyl)-acetate). The signals of S-methylene protons from the side chain of **3a** (2-(ethylsulfonylethyl)-ethyl 2-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-7-purinyl) -acetate) form complicated multiplet at 3.83 – 3.67 ppm. However, the integral curves correspond to the exact number of the protons.

Pharmacology

Acute toxicity (LD_{50}) data of **3a-c** and 7-theophylline acetic acid **(1),** show statistically significant ($p \leq 0.05$) lower toxicity compared to the standard substance theophylline (Table 1).

Compound **3c** (2-bromo-3-hydroxy-2-nitropropyl 2-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-7-purinyl)-acetate), which have a bulky substituent in the side chain, had lowest acute toxicity. Compounds **3a** and **3b** had a comparable toxicity. Since molecular weights of the compounds were different with no more than 10%, according to us it will be correct to suggest that increasing of the volume and weight of the substituent decreased the acute toxicity. This fact is in good correlation with our previously published data [7, 8].

Table 1. Acute toxicity (LD_{50}) and influence on pentobarbital (PB) sleeping time of compounds 3a-c and 7-theophyllineacetic acid (1) in mice after intraperitoneal administration.

Compound	LD_{50} (Range of values) [mg/kg b.w.]	Doses [mg/kg b.w. i. p.]	Sleeping time (X + SD) min.
Control (PB)	-	40	30.8 ± 7.1
3a	1588* (1450 ÷ 1738)	150	48.2 ± 9.4*
3b	1528* 1400 ÷ 1668	150	59.0 ± 2.6*
3c	2523* 2320 ÷ 2744	250	46.5 ± 9.4*
1	2360* 2173 ÷ 2564	240	42.5 ± 8.1*
Theophylline	353 (301 ÷ 413)	35	29.0 ± 3.1

* $p \leq 0.05$ statistical significance in comparison with theophylline.

All tested compounds, when administered in a dose $^{1}/_{10}$ of $LD_{50,}$ significantly ($p \leq 0.05$) increased the pentobarbital sleeping time in comparison with theophylline (Table 1). The locomotor activity investigations show that tested compounds (**3a-c**) decreased the spontaneous locomotor activity in comparison with control group at doses $^{1}/_{10}$ of LD_{50}. Theophylline and 7-theophylline acetic acid increased the spontaneous locomotor activity (Fig. 1).

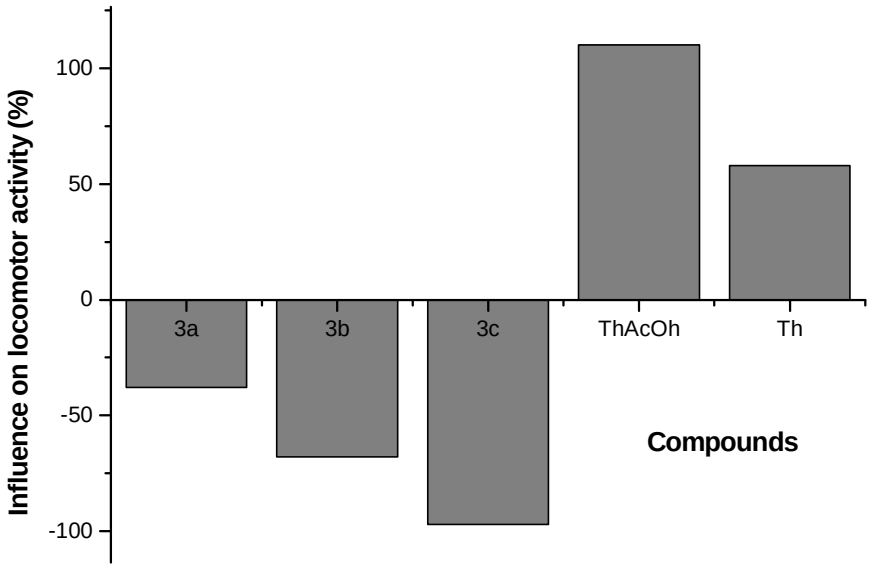


Figure 1. Influence on spontaneous locomotor activity of compounds 3a-c, 7-theophyllineacetic acid (ThAcOh) and theophylline (Th) in mice after i.p. administration expressed as percentage from contol values (0 %).

According to data of Ishii and co-workers [14], that medial temporal oxygen metabolism was markedly affected in patients with mild-to-moderate Alzheimer's disease, we analysed the effect of the xantine derivatives in model of provoked anoxic brain hypoxia. The results have shown that compounds **3a** and **3c** prolonged the survival time of mice in the model of anoxic hypoxia (Table 2).

Table 2. Brain antihypoxic activity of compounds 3a-c, 1, and theophylline on mice.

Compound	Doses mg/kg, b.w., i.p.	Survival time (X ± SD) min	% vs control
Control	30	20.4 ± 1.1	
3a	150	23.4 ± 4.0*	114.7
3b	150	18.6 ± 4.0	91.1
3c	250	24.4 ± 2.9*	119.6
1	240	21.5 ± 3.2	108.7
Theophylline	35	17.5 ± 4.3	

* $p \leq 0.05$ statistically significance in comparison with theophylline.

Several data in the literature [15] suggests that the main role in the mechanism of antihypoxic activity in this model play GABA, which have antihypoxic activity at doses 20 – 600 mg/kg b.w.. On the other hand, cerebrovascular drugs, such as Pentoxyphylline and Xanthinol nicotinate, show a most potent increasing of survival time of animals (more than with 20 – 30%) [16]. These drugs had a bulky substituents in the side chain too. The cited data are in good corelarion with effect observed in our experiments for compound **3a** and **3c**.

These results indicate that compounds **3a** and **3c** are very prospective for further pharmacological and biochemical experiments in relation to the treatment of brain neurodegenerative disorders connected with brain hypoxia.

MATHERIALS AND METHODS

Chemistry

Melting points were determined in °C on Büchi 535 apparatus and are corrected. UV spectra were recorded on a Hewlet Packard 8452A Diode Array Spectrophotometer equipped with a HP Vectra 386/25 computer in chloroform. IR spectra were recorded on a Shimadzu FTIR 8101M Spectrophotometer in nujol. ^{1}H–NMR spectra were recorded at ambient temperature on a Bruker–100 WP (100 MHz) spectrometer in $CDCl_3$. Chemical shifts are reported in ppm (δ) downfield from TMS as internal standard; the coupling constants are expressed in Hertz (Hz); standard abbreviations are used. Elemental analyses for N and Br were carried out in microanalytical laboratory (Institute of Polymers, Sofia) and were within ±0.4% of the theoretical values. Thin layer chromatography was performed on DC-Alufolien Kieselgel 60 F_{254} (Merck, Darmstadt, Germany) (0.20 mm) sheets with solvents: formic acid-chloroform-acetone-ethanol (1:3:3:4 volume parts). Detection at UV 254 nm. All products were shown to be homogeneous by TLC. The given yield is the yield of isolated TLC homogeneous product. No efforts were made to optimize yields. The starting materials were of commercially available research – grade chemicals, Fluka Chemie AG (Switzerland), and Sofarma (Sofia, Bulgaria).

General procedure for synthesis of 7-theophyllineacetates 3a-c

7-Theophyllineacetic acid (2.62 g, 0.011 mol), corresponding hydroxy derivative (0.01 mol) and 4-dimethylaminopyridine (DMAP, 0.3 g, 0.0025 mol) were dissolved in 100 ml of anhydrous dimethylformamide at 60°C. After complete dissolution, dicyclohexyl-carbodiimide (DCC, 2.7 g, 0.013 mol) dissolved in 15 ml of anhydrous dimethylformamide was added. The reaction mixture was stirred at room temperature for 38 h and the white precipitate of N,N-dicyclohexylurea (DCU) was filtered off. After concentration to 1/3 volume of the filtrate on rotary evaporator (Büchi Rotavapor R-114) cooled anhydrous ethanol was added and the solution was kept for 24 h at -5°C. The crystals (DCU) were filtered off and the filtrate was evaporated. The residue was crystallized from ethanol. The separated crystals were washed with petroleum ether and recrystallized from ethanol.

The structure, physical and spectral properties of compounds **3a-c** are given in Tables 3 – 4.

Pharmacological evaluation

The 7-theophyllineacetates **3a-c** were tested in *in vivo* screening in order to evaluate acute toxicity, influence on pentobarbital sleeping time, locomotor activity, and antihypoxic activity (Tables 1 – 2, Fig. 1).

Male albino mice, strain H, aged 5–7 weeks old (22–25) g, kept under standard conditions in animal house (water and food *ad libitum*, 12 h dark/light cycle) were used throughout the *in vivo* experiments. The ambient temperature of the room was maintained at 21±1ºC and the humidity was 50%. Animals were handled once daily, at least 3 days prior to testing. All subjects were experimentally naive and were used only once. Controls were treated with vehicle, in the same volume as the treated animals (0.1 mL/10 g i.p.). No effects of the vehicle were observed. All experimental procedures described herein were in accordance with the NIH guidelines of the Care and Use of Laboratory animals. For the *in vivo* screening, the test compounds were dissolved in saline (0.9% NaCl) with 1 - 2 drops of Tween 80.

Data analysis was done by factorial analysis of variance followed by Student's *t*-test with $p < 0.05$ chosen as the minimal level of significance. The results are expressed as mean (M) ± SD.

Acute toxicity (LD_{50}) in mice.

Acute i.p. toxicity (LD_{50}) was estimated by the Up-and-Down Procedure according to the OECD Test Guideline 425 (Up-and-Down Procedure, OECD 425) [17]. Animals were observed daily for clinical signs or mortality over a period of two weeks following the treatment.

Influence on pentobarbital sleeping time (PBST)

The studied compounds (0.1ml/10 g b.w.) were administered at doses $^1/_{10}$ of LD_{50} i.p. (8 per group). The same volume of solvent (0.9% NaCl) was administered to the controls. The solution of pentobarbital sodium at dose 40 mg/kg b.w. i.p. was administered 30 minutes after administration of the test compounds. Sleeping time was measured in minutes by observing the righting reflex recovery.

Influence on locomotor activity

Group of 6 animals was put on in electronic actometer (Activity Cage, Ugo Basile, Italy) and the locomotor activity in arbitrary units was determined at 10 minute intervals for 100 min. The tested compounds at dose $^1/_{10}$ of LD_{50} i.p. were administered to the animals and the results were compared to vehicle-treated group.

Table 3. Structures and chemical data of 7-theophyllineacetates 3a-c

Compd.	R	Mp [°C]	Cryst. solvent	Yield [%]	Analys calcd. (found) [%]					Molecular formula (Mw)
					C	H	N	S	Br	
3a	2-ethylsulfonyl-ethyl-	160 - 162	ethanol	45	43.57 (43.40)	5.06 (5.04)	15.63 (15.56)	8.95 (8.90)	-	$C_{13}H_{18}N_4O_6S$ (358.36)
3b	2-(2-methyl-5-nitroimidazol-1-yl)-ethyl-	142 - 144	ethanol	56	46.03 (45.85)	4.40 (4.38)	25.22 (25.30)	-	-	$C_{15}H_{17}N_7O_6$ (391.34)
3c	2-bromo-2-nitro-3-hydroxy-propyl-	133 - 136	ethanol	50	34.30 (34.17)	3.40 (3.38)	16.67 (16.61)	-	19.02 (18.98)	$C_{12}H_{14}N_5O_7Br$ (420.17)

Table 4. Spectral data of 7-theophyllineacetates 3a-c

Compd.	R	IR [cm^{-1}]	UV/VIS [nm] λ_{max}	^{1}H-NMR, δ ($CDCl_3$)
3a	2-ethylsulfonyl-ethyl-	1709 (νCO – ester); 1693, 1653 (νCO-xanthine); 1628, 1531 (νC=C, νC=N); 1256 (νC-O-C – ester); 1125 (ν_s SO_2).	242 276	7.97 (s, 1H, C8H); 5.04 (s, 2H, N7-CH_2-); 4.07 (t, 2H, O–CH_2, J = 6.8); 3.83 – 3.67 (m, 4H, 2 x CH_2–S), 3.59 (s, 3H, N3–CH_3); 3.35 (s, 3H, N1–CH_3); 1.22 (t, 3H, CH_3– side chain, J = 7.4).
3b	2-(2-methyl-5-nitroimidazol-1-yl)-ethyl-	1710 (νCO – ester); 1690, 1663 (νCO-xanthine); 1590 – 1530 with max. at 1560 (νC=C, νC=N, ν_{as} NO_2); 1259 (νC-O-C – ester).	244 278	7.97 (s, 1H, C8H); 7.59 (s, 1H, Im-4); 5.04 (s, 2H, N7-CH_2-); 4.49 (t, 2H, CH_2 – side chain, J = 5.8); 3.96 (t, 2H, CH_2 – side chain, J = 5.8), 3.60 (s, 3H, N3–CH_3); 3.35 (s, 3H, N1–CH_3); 2.52 (s, 3H, Im-2–CH_3).
3c	2-bromo-2-nitro-3-hydroxy-propyl-	3119 (νOH); 1709 (νCO – ester); 1693, 1674 (νCO-xanthine); 1653, 1628, 1531 (νC=C, νC=N, ν_{as} NO_2); 1255 (νC-O-C – ester).	248 275	7.97 (s, 1H, C8H); 5.04 (s, 2H, N7-CH_2-); 4.29 (s, 2H, CH_2–O); 4.06 (s, 2H, O–CH_2), 3.59 (s, 3H, N3–CH_3); 3.36 (s, 3H, N1–CH_3); 2.87 (s, 3H, OH– side chain).

Antihypoxic activity

The tested compounds were administered in doses $^{1}/_{20}$ and $^{1}/_{10}$ of LD_{50} i.p. 30 min prior to experiment. The mice (8 per group) were placed individually into hermetic bottles of 200 ml volume. The survival time (in min) was determined and compared with vehicle-treated group [18].

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