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Synthesis, physical-chemical and biological properties of 1.8-disubstituted of theobromine. V. 8-Benzylidenhydrazino-1-p-methylbenzyltheobromines

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Key words: *Xanthine, Organic Synthesis, NMR-spectroscopy, Acute Toxicity, Antioxidant Effect.*

The problem of searching biologically active compounds amidst xanthine derivatives is a crucial one and is an issue for long-term investigation.

Aim. In order to find new biologically active compounds among xanthine derivatives, undescribed earlier 8-benzylidenhydrazino-1-p-methylbenzyltheobromines have been synthesized.

Methods and results. Reaction of 1-p-methylbenzyl-8-bromoxanthine with the excess of hydrazine hydrate in the aqueous dioxane is implemented through formation of 8-hydrazine-1(4-methylbenzyl)theobromine. Through the interaction of 8-hydrazinetheobromine with aldehydes in aqueous propan-2-ol respective 8-benzylidenhydrazino-1-p-methylbenzyltheobromines have been obtained. Structure of synthesized compounds has been definitely proved by NMR-spectroscopy.

Conclusions. Molecular and pharmacological descriptors of obtained substances have been calculated. The antioxidant activity of the obtained compounds has been explored. Priorities for further search of biologically active compounds in a range of xanthine derivatives have been set out.

Синтез, фізико-хімічні та біологічні властивості 1,8-дизаміщених теоброміну. V. 8-Бензиліденгідразино-1-п-метилбензилтеоброміни

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Проблема пошуку біологічно активних сполук серед похідних ксантину є актуальною та перспективною. З метою створення нових біологічно активних сполук похідних ксантину синтезували неописані в літературі 8-бензиліденгідразино-1-п-метилбензилтеоброміни. Реакцією 1-п-метилбензил-8-бромотеоброміну з надлишком гідразину гідрату в середовищі водного діоксану отримали 1-п-метилбензил-8-гідразинотеобромін. Взаємодія 8-гідразинотеоброміну з альдегідами в середовищі водного пропанолу-2 призвела до утворення відповідних 8-бензиліденгідразино-1-п-метилбензилтеобромінів. Структура сполук однозначно доведена методом ПМР-спектроскопії. Для синтезованих речовин розрахували молекулярні та фармакологічні дескриптори. Дані, що одержали, свідчать про доцільність наступних досліджень in vitro та in vivo. Вивчили антиоксидантну активність (in vitro). Встановили пріоритети для пошуку біологічно активних сполук в ряді похідних ксантину.

Ключові слова: *ксантин, органічний синтез, ПМР-спектроскопія, гостра токсичність, антиоксидантний ефект.*

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Синтез, физико-химические и биологические свойства 1,8-дизамещённых теобромина. V. 8-Бензилиденгидразино-1-п-метилбензилтеобромины

Д. Г. Иванченко

Проблема поиска биологически активных соединений среди производных ксантина актуальна и перспективна. С целью создания новых биологически активных соединений производных ксантина синтезировали неописанные в литературе 8-бензилиденгидразино-1-п-метилбензилтеобромины. Реакцией 1-п-метилбензил-8-бромтеобромина с избытком гидразин гидрата в среде водного диоксана получили 1-п-метилбензил-8-гидразинотеобромин. Взаимодействие 8-гидразинотеобромина с альдегидами в среде водного пропанола-2 привело к образованию соответствующих 8-бензилиденгидразино-1-п-метилбензилтеобромінов. Структура синтезированных соединений однозначно доказана методом ПМР-спектроскопии. Рассчитаны молекулярные и фармакологические дескрипторы. Полученные данные свидетельствуют о целесообразности дальнейших исследований in vitro и in vivo. Изучена антиоксидантная активность (in vitro). Установлены приоритеты для дальнейшего поиска биологически активных соединений в ряду производных ксантина.

Ключевые слова: *ксантин, органический синтез, ПМР-спектроскопия, острая токсичность, антиоксидантный эффект.*

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Oxygen is an abundant molecule in biological systems. Despite being a radical, it is sparingly reactive because its two unpaired electrons are placed in different molecular orbitals and demonstrate parallel spins. Thus, oxygen undergoes univalent reduction to form superoxide (O_2^-•) by means of enzymes such as the nicotinamide adenine dinucleotide (phosphate) oxidases and xanthine oxidases. Nonenzymatically, oxygen can also become O_2^-• by reacting with redox active compounds such as semiubiquinone of the mitochondrial electron transport chain [1]. Superoxide anion is dismutated enzymatically to become H_2O_2 through the action of superoxide dismutases (SODs) [2]. In biological tissues, O_2^-• can also undergo nonenzymatic transformation into H_2O_2 and singlet oxygen [3]. H_2O_2 can react with other radicals such as transition metal Fe^{2+} to produce highly reactive hydroxyl radicals (OH•); this is known as the Fenton reaction. These radicals are capable of destroying biomolecules through oxidation. When Fe^{3+} initially oxidizes O_2, molecular oxygen and Fe^{2+} are generated; the Fe^{2+} initiates the Fenton reaction and this regenerates Fe^{3+} which perpetuates the production of OH• [4].

In response to growth factors and cytokines, and during normal metabolic events such as respiration and phagocytosis, eukaryotic cells produce oxidants. To compensate this, the cells have evolved both enzymatic and nonenzymatic mechanisms to protect against oxidants' toxic effects. The enzymatic mechanisms include the actions of enzymes such as SOD, catalase, and glutathione peroxidase. The nonenzymatic antioxidants include glutathione, ascorbate, and α-tocopherol. However, in pathophysiologic circumstances, an excess of oxidants can overwhelm the scavenging capacity of cellular antioxidant systems. The subsequent oxidative stress damages the cell's lipids, membranes, proteins, and DNA.

Thus, creation of new modern drugs, that have antioxidant properties, is important and promising trend.

We have previously shown prospective search of antioxidants among derivatives of theobromine [5–7].

The aim of this paper is to elaborate unique methods unspecified in scientific papers earlier, to synthesize 8-benzylidenhydrazino-1-p-methylbenzyltheobromines, which are potential bioactive compounds, and to study their physical, chemical and biological properties.

Materials and methods of research

The melting point has been determined with the help of an open capillary method with PTP-M device. Elemental analysis has been performed with the help of the instrument Elementar Vario L cube, NMR-spectra have been taken on a spectrometer Bruker SF-200 (operating frequency of 200 MHz, solvent DMSO, internal standard – TMS). These data correspond to the calculated elemental analysis.

Analytical data of synthesized compounds are shown in *Tables 1, 2*.

Table 1

Physical-chemical properties of the synthesized compounds (3–7)

Compound	$T_{melting}$, °C	The empirical formula	Yield, %
3	234–235	$C_{23}H_{24}N_6O_3$	32,4
4	238–239	$C_{24}H_{26}N_6O_3$	89,7
5	230–231	$C_{23}H_{24}N_6O_4$	78,1
6	170–171	$C_{25}H_{28}N_6O_2$	97,4
7	232–233	$C_{24}H_{27}N_7O_2$	89,3

Synthesis of 8-bromo-1-p-methylbenzyltheobromine (1), 8-hydrazino-1-p-methylbenzyltheobromine (2) is described in works [5], respectively.

Synthesis of 8-benzylidenhydrazino-1-p-methylbenzyltheobromines (3-7). The solution of 1.57 g (0.005 mol) 8-hydrazino-1-p-methylbenzyltheobromine (2), 0.006 mol of corresponding aldehyde, 20 ml H_2O, 20 ml propan-2-ol and 5 drops of HCl was boiled for 10 minutes, cooled. The precipitate was filtered off, washed with water and crystallized from aqueous propan-2-ol.

Molecular descriptors have been calculated using the computer programs ALOGPS and DRAGON, whereas biological properties of the synthesized compounds have been calculated with the help of GUSAR and ACD / Percepta Platform. Antioxidant activity (AOA) has been studied in vitro applying the method of nonenzymic initiation of free-radical oxidation [8]. As reference substances for comparison Ascorbic acid and Thiotriazolinum have been used. Data of the biological effects of 8-benzylidenhydrazino-1-p-methylbenzyltheobromines have been shown in *Table 3*.

Table 3

Biological activity of synthesized compounds

Compound	AOA, %		
	$C=10^{-3}$ mol/L	$C=10^{-5}$ mol/L	$C=10^{-7}$ mol/L
3	75,61	26,83	14,63
4	75,61	14,63	17,07
5	80,0	41,77	31,77
6	80,52	35,07	29,87
7	88,51	37,93	31,04
Ascorbic acid	65,31	39,13	43,59
Thiotriazolinum	33,90	22,60	7,63

Results and their discussion

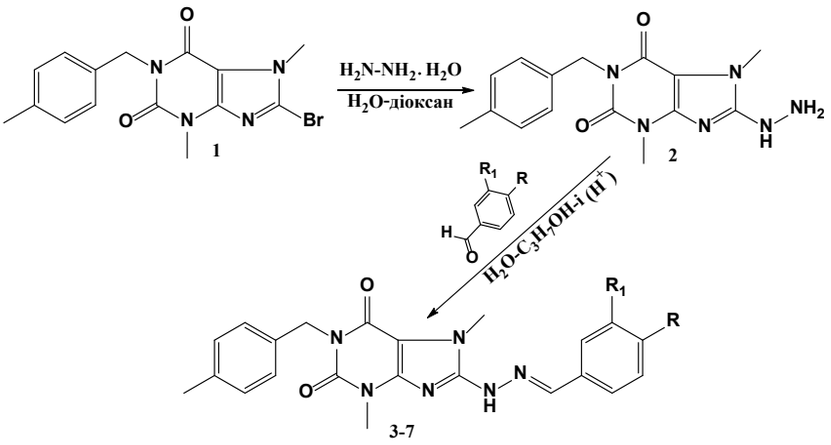
Through the interaction of bromoxanthine (2) with the excess of hydrazine hydrate in the aqueous dioxane an 8-hydrazine-1(4-methylbenzyl)theobromine (3) has been obtained. This compound under short-time heating up with aldehydes in aqueous propan-2-ol, also presented with equimolar amount of $HCl_{concentr.}$ forms respective benzylidenhydrazine derivatives of 1-(4-methylbenzyl)theobromine (3–7) (*Scheme 1*).

Table 2

NMR Spectra of Synthesized Compounds 3–7 (δ , ppm)

Compound	CH_{arom}	NH (s, 1H)	N=CH (s, 1H)	N_1CH_2 (s, 2H)	NCH_3 (s, 3H)	CH_{3arom} (s, 3H)	Other resonances
3	7.58 (d, 2H); 7.22 (d, 2H); 7.07 (d, 2H); 6.97 (d, 2H)	10.97	8.09	5.01	3.79; 3.39	2.24	3.93 (s, 3H) – OCH_3
4	7.56 (d, 2H); 7.17 (d, 2H); 7.07 (d, 2H); 6.95 (d, 2H)	11.29	8.03	4.96	3.82; 3.36	2.23	4.04 (q, 2H) – OCH_2; 1.31 (t, 3H) – CH_3
5	7.2-7.01 (m, 6H); 6.79 (d, 2H)	11.27	7.97	4.96	3.79; 3.36	2.23	9.41 (s, 1H) – OH; 3.91 (s, 3H) – OCH_3
6	7.56 (d, 2H); 7.44 (d, 2H); 7.18 (d, 2H); 7.07 (d, 2H)	11.36	8.06	4.97	3.92; 3.37	2.24	2.93-2.83 (m, 1H) – CH-Ar; 1.19 (d, 6H) – $(CH_3)_2$
7	7.45 (d, 2H); 7.18 (d, 2H); 7.07 (d, 2H); 6.72 (d, 2H)	11.09	7.96	4.97	3.91; 3.36	2.23	2.94 (s, 6H) – $(CH_3)_2$

Scheme 1



R= OCH_3, R_1= H (3); R= OH, R_1= OCH_3 (4); R= OC_2H_5, R_1= H (5); R= $CH(CH_3)_2$, R_1= H (6); R= $N(CH_3)_2$, R_1= H (7)

To prove the structure of synthesized compounds, their NMR spectra have been recorded and interpreted. The presence of benzene rings in position 1 and 8 of benzylidenhydrazine derivatives (3,4,6,7) are clearly demonstrated by 4 duplets of aromatic protons at 7.58–6.72 ppm with intensity in two proton units each. It should be noted that aromatic systems in a structure of 1-(4-methylbenzyl)-8-(4-ethoxybenzylidene)hydrazinotheobromine (5) are registered in a form of multiplet and duplet at 7.2–7.01 ppm (6H) and 6.79 ppm (2H) respectively. Methylene group protons, linked with a nitrogen atom in position 1 are recorded as intensive singlet at 4.98 ppm (2H). Protons of N-methyl groups in positions 3 and 7 of theobromine fragment are registered in a form of intensive singlets at 3.92–3.79 ppm (3H) and 3.39–3.36 ppm (3H) respectively.

Also, in NMR spectra of hydrazones 3–7, signals of NH_2-group protons in hydrazine residue are not detected. Meanwhile, signals of NH-group protons in position 8 are shifted dramatically in a low field and are registered there as singlets within the range of 11.36–10.97 ppm (1H). This is explained by the transition of a neighboring nitrogen atom to the sp^2-hybrid state, which in its turn leads to its electronegativity amplification. Methylidene protons are registered as singlets in a fairly small range of 8.09–7.96 ppm.

All other signals of proton substituents in position 8, their location, shape and intensity are fully consistent with their structure.

Further properties of the synthesized compounds have been calculated. It has been found that all benzylidenhydrazinotheobromines satisfy to the Rule of five [9], which means that the Lipinski index for all substances is 0 (*Table 4*). Further the Ghose filter has been used. It should be noted that compounds 6 and 7 in terms of polar surfaces do not satisfy to all criteria the Ghose filter [10].

The application of ACD/Percepta Platform has allowed calculating the absorption characteristics, permeability via blood-brain barrier, as well as establishing probable transport forms of blood of the synthesized compounds. Thus, it is assumed that synthesized substances stable in an acidic environment (pH <2) and passively absorbed in the small intestine. Lipoproteins are probable blood transport form of all synthesized compounds. Furthermore all derived substances are characterized by good permeability of the blood-brain barrier (*Table 5*).

Table 4

Molecular descriptors of synthesized compounds (3–7)

Compoud	M, Da	Number			LogP	TPSA, Å^2	Molar refractivity, m^3/mol
		Atoms	Donors of H^+	Acceptors of H^+			
3	433	56	1	5	3,88±0,39	95,44	123,327
4	447	57	1	5	4,25±0,45	95,44	128,075
5	449	57	2	6	3,45±0,51	115,67	125,021
6	445	61	1	4	5,09±0,52	86,21	131,055
7	446	60	1	4	4,07±0,42	89,45	131,292

Table 5

Pharmacological descriptors of synthesized compounds (3–7)

Compound	Absorption		Drug Binding to Plasma Proteins		Blood-Brain Barrier Transport		
	Pe, sm/s	Ka, min^{-1}	PPB, %	LogK_a^{HSA}	LogPS	LogPB	Log(PS*fu, brain)
3	$7{,}19\cdot10^{-4}$	0,049	96,44	3,48	-1,3	0,36	-3,1
4	$7{,}09\cdot10^{-4}$	0,049	96,81	3,52	-1,3	0,36	-3,1
5	$7{,}12\cdot10^{-4}$	0,049	96,32	3,21	-1,5	0,35	-3,3
6	$7{,}04\cdot10^{-4}$	0,048	97,81	3,62	-1,3	0,36	-3,1
7	$7{,}06\cdot10^{-4}$	0,048	96,07	3,45	-1,3	0,36	-3,1

Table 6

Acute toxicity of synthesized compounds *in silico*

Compound	LD_{50}, mg/kg					
	Oral rout of administration		Intraperitoneal rout of administration		Intravenous rout of administration	
	mice	rats	mice	rats	mice	rats
3	450	1875,0	380	659,1	71	194,0
4	470	1234,0	440	694,6	66	171,8
5	520	1787,0	340	758,1	74	222,3
6	380	1539,0	360	615,2	55	115,8
7	380	1280,0	340	499,7	60	174,3

Assisted by computer programs GUSAR and ACD / Percepta Platform, further on there the acute toxicity rate for rats and mice have been calculated. According to the data synthesized substances belong to Class IV of the toxicity (*Table 6*).

Thus the findings have shown the feasibility of further studies in vitro and in vivo.

The studies in vitro have shown that all the compounds in terms of AOA in concentration 10^{-3} mol / L exceed the standards of comparison (*Table 3*). The most active compound in this concentration is 8-(4-dimethylamino)benzylidenhydrazino-1-p-methylbenzyltheobromine (7).

Substitution dimethylamine radical in the para-position of the benzylidenehydrazine fragment for the isopropyl group leads to a decrease in antioxidant activity in concentrations of 10^{-3} mol / L only. It should be noted that the introduction of the hydroxyl group in position 3 of the benzylidenehydrazine fragment reduces antioxidant activity at a concentration of 10^{-5} mol / L only. Replacement of the methoxy group in the position 4 of the benzylidenehydrazine fragment for the ethoxy group leads to an increase AOA in all concentrations.

For the final conclusions we should conduct the additional researches. The work in this area is continued.

Conclusions

1. Accessible laboratory methods have been elaborated for synthesis of 8-benzylidenhydrazino-1-p-methylbenzyltheobromines, the structure of which has been proved by elemental analysis and NMR-spectroscopy data.

2. Molecular (LogP, TPSA, A) and pharmacological (Pe, Ka, PPB, $LogK_a^{HSA}$, LogPS, LogPB, Log(PS*fu)) descriptors have been calculated to prognosticate properties of obtained substances, in addition to index of acute toxicity.

3. Antioxidant activity of synthesized compounds has been studied and priorities for further research of biologically active compounds have been outlined.

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