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Synthesis, characterization and pharmacological evaluation of some tricyclic-theophylline derivatives

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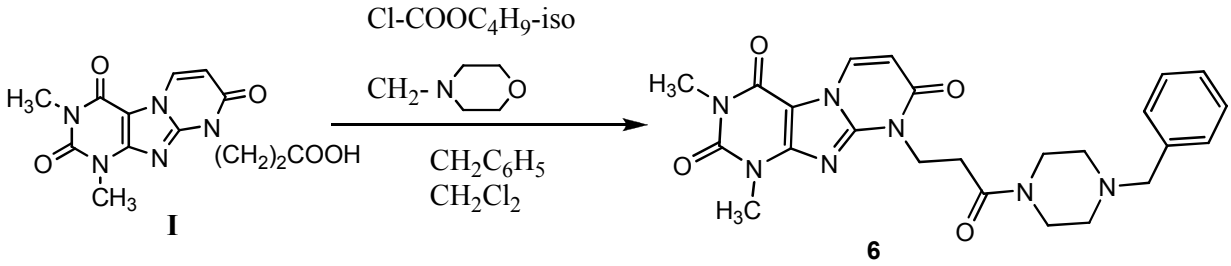
ABSTRACT

*New N4-substituted piperazides and morpholide of pyrimidin-8-on [2,1-f]-theophylline-9-propanoic acids amides **4-9** were synthesized and evaluated for their affinity at 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor subtypes. Compound **6** containing benzyl substituted nitrogen (N4) was the most active one.*

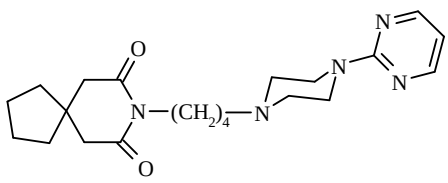


Key words: *5-HT$_{1A}$ ligand, 1-benzylpiperazines, pyrimido[2,1-f] theophyllines, Tricyclic theophylline, 5-HT$_{1A}$ receptor functional activity*

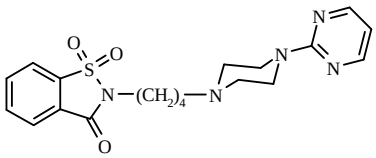
INTRODUCTION

1-Aryl piperazines constitute one of the most important classes of the 5-HT$_{1A}$ receptors ligands. The majority of them are classified as partial agonist of 5-HT$_{1A}$ receptor [1,2]. It was found that anellation of five-, six- or seven-membered ring at 7,8-position of theophylline changed its CNS activity profile. Pharmacological evaluation of the series of novel tricyclic theophylline derivatives with imidazo-, pyrimido- or diazepino[2,1- f] purine generally demonstrated their sedative effects on CNS [3-7] .

Several compounds from different chemical classes possess high affinity for 5-HT$_{1A}$ receptors. Among them, some 1-aryl piperazines linked with a terminal cyclic amide fragment via a long –chain, e.g. buspirone or ipsapirone are effective antianxiety and antidepressant drugs [7,8].

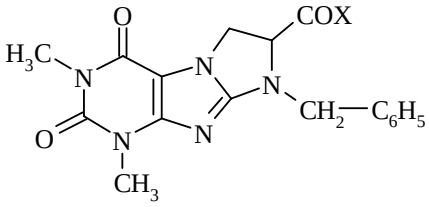


buspirone

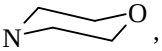


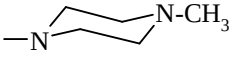
ipsapirone

Several N_8-phenyl piperazinopropyl-1, 3, 6, 7-tetrahydro-(8H)-imidazo[2,1-f] theophylline derivatives showed significant antiserotonin and long-lasting hypothermizing effects while other N_8-benzyl-1, 3, 6, 8-tetrahydro-imidazol-7-on[2,1-f]theophylline derivatives possessed anticonvulsant properties [9,10].



X = OC_2H_5 , NH-$(CH_2)_3OCH_3$, NH-$(CH_2)_3OC_2H_5$,

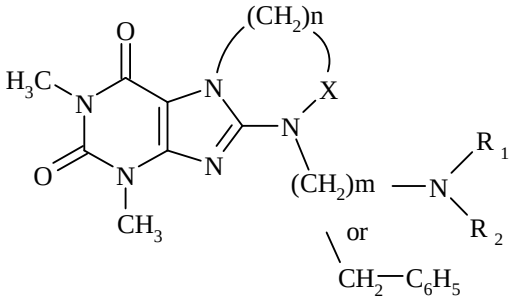
NH-(CH_2)n-N $(Alkyl)_2$, NH-(CH_2)n  ,

NH-(CH_2)n 

n = 2, 3

Fig.1. The structures of imidazo[2,1-f] theophylline derivatives

Lactam and non-lactam tricyclic theophylline derivatives with phenylpiperazino-alkyl substituent containing terminal pyrimidopurine or 1,3-diazepinopurine demonstrated post-synaptic antagonism towards 5-HT_{1A} receptors, whereas their pyrimido-piperazine analogs were classified as 5-HT_{1A} partial agonists [10]. 3-(Chloro or 2-methoxyphenylpiperazino)-propyl derivatives of those tricyclic systems acted as partial agonists or full agonists of pre - and postsynaptic 5-HT_{1A} receptors [11-13].



n = 1-3 or (CH_2)n = CH=CH; (CH_2)n = CH (CH_3)

X = C=O or CH_2

m= 2- 4

R_1 = R_2 = CH_3, C_2H_5, piperidine or N4-substituted piperazines

Fig.2. The structure of pharmacologically investigated imidazo, pyrimido and diazepino[2,1-f]

theophyllines

Based on the above findings, we have synthesized new N_4-substituted piperazides and morpholide of pyrimidin-8-on [2,1-*f*]-theophylline-9-propanoic acid derivatives structurally similar to the aforementioned ones with the aim to have 5-HT_{1A} and 5-HT_{2A} receptors affinity.

EXPERIMENTAL SECTION

Chemistry

Melting points were determined with a Gallenkamp (London, U.K.) melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on Varian 200 BB (200 MHz) with TMS as an internal standard in $CDCl_3$ for compounds **4-8**. MS spectra were acquired using mass spectrometer type AMD 604 with direct inlet, AMD Intectra, Germany (ionization energy 70 eV). The purity of the products was confirmed by TLC on Merck plates (Kieselgel 60 F_{254}) with the use of appropriate solvent, and the spots were visualized in the UV light. Elemental analyses were determined using Automatic Elemental Analyzer CHN Model 2400 Perkin Elmer (USA). All the results of elemental analyses (C, H, N.) were within ± 0.4 % of the theoretical values. *(Table 1)*.

The starting pyrimidin-8-on[2,1-f]theophylline-9-propanoic acid **I** was prepared according to the reported procedures [14,15].

General procedure for preparation of compounds 4-8

The mixture of pyrimidin-8-on[2,1-f]theophylline-9-propanoic acid **I** (2.23g; 0.007M) with N-methylmorpholine (NMM) (1.06g; 0.01M) was suspended in CH_2Cl_2 (20 ml) and stirred in room temperature until the clear solution was obtained (15-30 min). Then the mixture was cooled to ˉ5 ^oC, and iso-butylchloroformate (IBCF) (1.91g; 0.014M) was added. The temperature (ˉ5 ^oC) was kept during 15 min, then appropriate N-phenylpiperazine (2.26g; 0.014M) for **4,** N-2'-methoxy-phenylpiperazine (2.67g; 0.014M) for **5**, N-benzylpiperazine (2.45g; 0.014M) for **6**, 4'-fluoro-phenylpiperazine (2.51g; 0.014M) for **7**, N-methylpiperazine (1.41g; 0.014M) for **8,** was added. The reaction temperature was gradually raised to the room temperature and intensive stirring was continued during 12 h**.** The final products were isolated after evaporation under reduced pressure and treatment with water (20 ml). The obtained products were filtered off, washed several with times water and then recrystallized from aqueous ethanol.

1,3-Dimethyl-9-(3-oxo-3-(4-phenylpiperazin-1-yl)propyl) pyrimido[1,2-f] theophylline -2,4,8 (1H, 3H, 9H)-trione (4)

M.P.: 258-260 ^oC; Yield: 91%; ^{1}H-NMR δ (ppm) 2.89-2.94(t, 2H, CH_2CO, J=8.0Hz); 3.16-3.21(m, 4H, $N(CH_3)_2N$-C_6H_5); 3.42(s,3H, N1CH_3); 3.60(s, 3H, N3CH_3); 3.63-3.81(t, 4H, N $(CH_2)_2$); 4.59-4.65(t,2H,N9CH_2); 6.31-6.89 (d,1H,C7-H,J=7.7 Hz) ; 6.88-6.93 (m, 2H,2'6' arom); 7.26-7.31(m, 3H, 3'4' 5' arom); 8.52-8.55(d, 1H, C6-H, J=7.7Hz . MS m/z: M^+, 463(76), 344(39), 302(32), 274(2), 248(40), 232(1), 214(4), 191 (5), 189(2), 161(11), 145(50), 132, (100), 120 (10), 104(7), 91(2), 77(3), 56(9), 42(2).

9-(3-(4-(2-methoxyphenyl)piperazin-1-yl)-3-oxopropyl)-1,3-dimethylpyrimido[1,2-f]theophylline-2, 4, 8 (1H, 3H, 9H) -trione (5)

M.P.: 168-170 ^oC; Yield: 88%; ^{1}H-NMR δ (ppm) 2.88-2.93(t,2H,CH_2CO,J=7.4Hz); 3.03-3.08 (m, 4H,$(\underline{CH_2})_2$N-C_6H_4; 3.67-3.84 (t, 4H, N$(\underline{CH_2})_2$); 3.42(s,3H, N3CH_3); 3.88(s, 3H, OCH_3); 3.63(s,3H,N1CH_3); 4.60-4.65(t, 2H, N9CH_2); 6.28-6.31(d,1H, C7-H, J=7.7Hz); 6.88-6.96(m, 4H, arom); 8.52-8.54 (d,1H, C6-H, J=7.7Hz)**.**MS m/z: M^+, 493(100), 476(2), 344(9), 331(2), 304(2), 302(13), 274 (1), 249 (4), 248 (26), 218(1), 191(9), 162(88), 149(26), 134 (11), 120(5), 91 (3), 82(1), 56(8), 42(3).

3-(4-benzylpiperazin-1-yl)-3-oxopropyl)-1,3-dimethylpyrimido[1,2-f]theophylline-2,4,8 (1H,3H,9H)-trione (6)

M.P.: 210-212 ^oC; Yield: 68%;^{1}H-NMR δ (ppm)**,** 2.81-2.85 (t, 2H, CH_2CO, J=4.1Hz); 2.42-2.46(m, 4H,$(\underline{CH_2})_2$N-CH_2-arom); 3.42(s, 3H, N1CH_3); 3.46(s, 2H,N$\underline{CH_2}$); 3.62(s, 3H, N3CH_3); 4.56-4.60(t, 2H, N9CH_2); 3.67-3.71(t,4H, N$(\underline{CH_2})_2$) 6.26-6.29(d, 1H, C7-H, J=7.7Hz); 8.50-8.52(d, 1H, C6-H, J=7.7Hz); 7.29-7.35 (m, 4H, arom). MS m/z: M^+ 477(66), 460(2), 386(13), 344(55), 332(1), 319(1), 302(43), 275(3), 274 (6), 248(100), 231(7), 217(5), 203(2), 191(9), 175(16), 146(74), 132(60), 126(5), 98(6), 91(72), 65(3), 55(12), 42(7).

9-(3-(4-(4-fluorophenyl)piperazin-1-yl)-3-oxopropyl)-1,3-dimethylpyrimido[1,2-f]theophylline-2, 4, 8(1H, 3H, 9H) -trione (7)

M.P.: 216-218 ^oC; Yield: 53%;^{1}H-NMR δ (ppm)**,** 2.88-2.92(t, 2H, CH_2CO, J=5.3Hz); 3.06-3.12(m, 4H, $(\underline{CH_2})_2$N-arom); 3.42(s, 3H,N3CH_3); 3.62(s,3H,N1CH_3); 3.70-3.79 (t, 4H, N $(\underline{CH_2})_2$); 4.58-4.63(t, 2H, N9CH_2); 6.27-6.30 (d, 1H, C7-H, J= 5.5Hz); 6.86-6.98(m, 4H, arom); 8.52-8.54(d, 1H, C6-H, J= 4,2Hz).

Table 1. Physicochemical data of compounds 4-9

Comp.	M.p. ^oC	Recryst. Solvent	Yield %	Molecular formula (molecular weight)	Elemental analysis			R_f (solvent)
					%C	%H	%N	
4	258-260	80^o EtOH	91	$C_{23}H_{25}N_7O_4$ (463.49)	59,60 59,75	5,44 5,28	21,15 20,75	0,31(A); 0,95(B)
5	168-170	80^o. EtOH	88	$C_{24}H_{27}N_7O_5$ (493,48)	58,41 58,95	5,51 5,80	19,87 19,70	0,27(A); 0,94(B)
6	210-212	96^o EtOH	68	$C_{24}H_{27}N_7O_4$ (477,52)	60,37 60,71	5,70 5,88	20,53 20,06	0,32(A); 0,90(B)
7	216-218	80^o EtOH	53	$C_{23}H_{24}N_7O_4F$ (481,48)	57,24 56,74	5,01 4,95	20,31 20,21	0,33(A); 0,94(B)
8	220-222	96^o EtOH	43	$C_{18}H_{23}N_7O_4$ (401,42)	53,86 53,90	5,78 5,72	24,43 24,22	0,29(A); 0,81(B)
9	253-255	80^o EtOH	50	$C_{17}H_{20}N_7O_5$ (388,38)	52,57 52,59	5,19 5,34	21,64 21,44	0,29(A); 0,81(B)

Solvent system: A. benzene:acetone (7:3) , B. benzene:acetone:methanol (1:1:1)

1,3-dimethyl-9-(3-(4-methylpiperazin-1-yl)-3-oxopropyl)pyrimido[1,2-f]theophylline-2,4,8(1H,3H,9H)-trione (8)

M.P.: 220-222 ^oC; Yield: 43%;^{1}H-NMR δ (ppm)**,** 2.30(s, 3H, N4-$\underline{CH_3}$); 2.38-2.42(m, 4H, $(\underline{CH_2})_2$ N-CH_2); 2.80-2.86(t, 2H, CH_2CO, J= 4.1Hz); 3.41(s,3H,N3CH_3); 3.63 (s,3H, N1 CH_3); 3.63-3.71(t,4H,N$(\underline{CH_2})_2$) 6.26-6.29(d, 1H, C7-H, J= 7.7 Hz); 8.45-8.52(d, 1H, C6-H, J= 7.7Hz).

1,3-methyl-9-(3-morpholino-3-oxopropyl)pyrimido[1,2-f]theophylline-2,4,8(1H,3H, 9H)-trione (9)
To the mixed anhydride obtained in analogical manner as for compounds **4-8**, morpholine (1.20g; 0.014M) was added and the reaction was continued as before.

M.P.: 253-255 ^{o}C; Yield:50%; MS m/z: 388(64), 358(6), 319(1), 302(50), 274(25), 248(100), 232(2), 217(3), 203 (1), 191(9), 175(2), 163(4), 149(1), 142 (20), 121 (3), 114 (3), 98(3), 86(13), 70(7), 56(4), 55(17), 41(8).

Pharmacology:
***In vitro* experiments:**
5-HT$_{1A}$ and 5-HT$_{2A}$ receptor binding assays
The affinity of the investigated compounds for 5-HT1A and 5-HT2A receptors was evaluated on the basis of their ability to displace [3H]-8-OH-DPAT [8-hydroxy- 2-(di-*n*-propylamino) tetralin; 222 Ci/mmol, Amersham] and [^{3}H]-ketanserin (66.4 Ci/mmol, NEN Chemicals), respectively. Radioligand binding experiments were performed on the rat brain using the following structures: the hippocampus for 5-HT1A receptors, and the cortex for 5-HT2A ones, according to the previously published procedures [13].

RESULTS AND DISCUSSION

The target compounds **4-9** were prepared as outlined in scheme 1. A reaction of pyrimidin-8-on[2,1-f]theophylline-9-propanoic acid **I** with iso-butylchloroformate led to the formation of anhydride intermediate which was allowed to react *in situ* with various piperazine derivatives or morpholine to obtain the novel N4-substituted piperazides **4-8** or morpholide **9** derivatives.

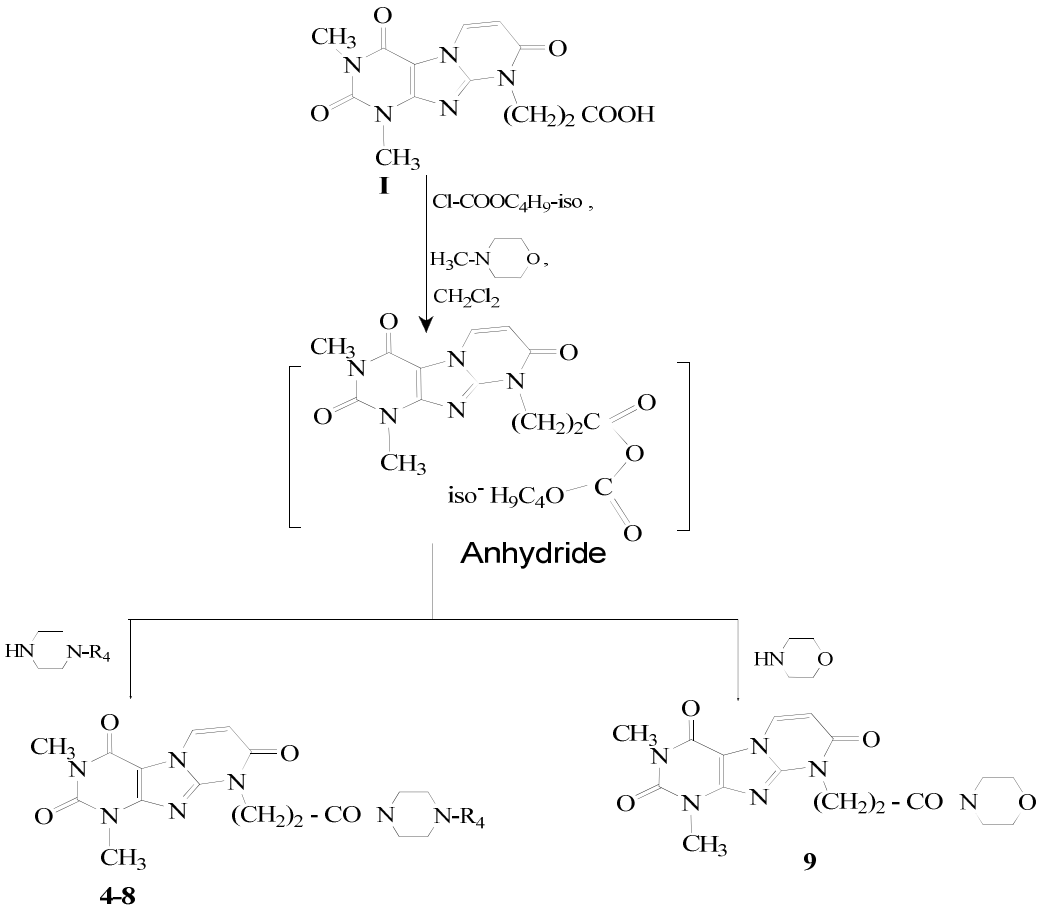
All the target N4-substituted piperazides and morpholide of pyrimidin-8-on [2,1-f]-theophylline-9-propanoic acid amides **4-9** were controlled using thin-layer chromatography (TLC) and melting point techniques. Both analytical and spectral data of all the compounds are in full agreement with the proposed structures.

The pharmacological properties of the tested compounds were discussed in comparison with those of the three methylene-analogs (**1**–**3**) described earlier.

The *in vitro* radioligand binding studies with arylpiperazines containing a terminal pyrimido[2,1-*f*] theophylline fragment and a propanoic acid group (**4**–**9**) exhibited much lower affinity for 5-HT$_{1A}$ and 5-HT$_{2A}$ serotonin receptors in comparison with those of the three methylene-analogs (**1**–**3**) described earlier [11].

It is clear from *table 2*. That the carbonyl group directly bounded the piperazine ring led to change the basic character of the nitrogen atom (N1) in piperazine or morpholine which reflected on the absence of intermolecular ionic interactions between the receptors and the compounds. This become much clearer on comparison the affinity of compound **3** which containing phenyl substituted nitrogen (N4) and that of compound **4** with benzyl group for 5 -HT$_{1A}$ and 5-HT$_{2A}$ receptors. Compound **4** with benzyl group showed higher affinity for 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors than that of compound **3**. This may be attributed to the higher basic character of the benzyl substituted nitrogen (N4) than that in case of compound **3**.

On the structure-affinity relationships may also be drawn on the basis of 5-HT$_{1A}$ receptor binding data presented in table 2. The affinity depends on the structure of the substituent in the amide fragment. Elongation of the alkenyl chain between amide nitrogen and the basic centre plays an important role in 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors affinity.



Comp	R_4
4	- C_6H_5
5	-2(OCH_3)C_6H_4
6	- $CH_2C_6H_5$
7	- 4(F)C_6H_4
8	- CH_3

Scheme 1: Preparation of the new compounds 4-9

Table 2: 5-HT_{1A} and 5-HT_{2A} receptor affinities.

Comp	K_i ± SEM (nM)	
	5-HT_{1A}	5-HT_{2A}
1	25.0	4680
2	10.4	3882
3	32.8	1080
4	9120	11990
5	8130	28300
6	1690	3012
7	8420	31200
8	9310	26400
9	8320	29900

CONCLUSION

It could be concluded that the free acid **I** was a versatile starting material for the synthesis of some tertiary amides by acylation via mixed anhydride and acid chloride. From the investigated procedure the method via mixed anhydride was the reaction of choice for the synthesis of N4-substituted piperazides of pyrimidin-8-on [2,1-f]theophylline-9-propanoic acid **4-8** and morpholide of pyrimidin-8-on[2,1-f]theophylline-9-propanoic acid **9** because of high yield, purity of products and simple isolation procedure. The tertiary amides **4-9** were tested in 5-HT_{1A} and 5-HT_{2A} receptor-binding assay. They showed very weak affinity towards serotonin receptors which made

evidence that the basic nitrogen is necessary part of the receptor ligand, probably acting with carboxylic function of aspartic acid rest in the receptor binding site, according to the model described.

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