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Investigations on the synthesis and properties of 4-aminosubstituted 2,6,7-trimethyl-1,5-dioxo-1,2,5,6-tetrahydropyrido[3,4-d]pyridazines

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Abstract

Synthesis of 4-aminosubstituted 2,6,7-trimethyl-1,5-dioxo-1,2,5,6-tetrahydropyrido[3,4-d]pyridazines **2–11** and the results of the preliminary pharmacological screening of the selected compounds **2**, **4**, **5**, **10**, **11** are described in this paper.

Keywords: 4-Aminosubsituted 2,6,7-trimethyl-1,5-dioxo-1,2,5,6-tetrahydropyrido[3,4-d]pyridazines

1. Introduction

It was stated previously [1] that some *N*-arylpiperazinylalkyl derivatives of 1,4-dioxo(1,4,5-trioxo)-1,2,3,4-tetra (and 1,2,3,4,5,6-hexa)hydropyrido[3,4-d]pyridazines (**Ia,b**, **II**) significantly inhibited the spontaneous locomotor activity (**Ia,b**), decreased the excitatory action of amphetamine and exerted analgesic properties in mice (Fig. 1).

N-3-[4-Phenyl(2-pyrimidynyl)-1-piperazinyl]-2-hydroxypropyl derivatives of the appropriate pyrido[3,4-d]pyridazines (**IIIa,b**, **IV**) also had a general depressive effect on the central nervous system (CNS) in mice [2]. Contrary to the compounds **I** and **II** the latter were devoided of analgesic action. A strong analgesic effect and significant suppression of spontaneous locomotor activity were noted after administration of the compounds **V** and **VI** in mice [3]. The encouraging results of the pharmacological tests led us to undertake the synthesis of a new series of compounds, the 4-amino derivatives of the appropriate 1,2,5,6-tetrahydropyrido[3,4-d]pyridazine **2–11** in order to get further information concerning the structure–activity relationships. The motivation for this kind of investigation follows from, among others, Refs. [4–6] indicating hypotensive and diuretic effects of some 1,4-diaminosubstituted pyrido[3,4-d]pyridazines.

Also, the introduction of amino groups in positions 1 and (or) 4 of the pyridazine ring of allied heterocyclic systems, i.e. tieno[2′,3′:5,6]pyrido[2,3-d]pyridazine and 1,2,3-triazolo[4,5-d]pyridazine gives compounds which are pharmacologically active. The first of them is a new class of potential antitumor agents [7]; the second derivatives show binding affinity towards A_1-adenosine receptors [8].

2. Chemistry

The starting material in the above-mentioned synthesis was 2,6,7-trimethyl-1,4,5-trioxo-1,2,3,4,5,6-hexahydropyrido[3,4-d]pyridazine [1] which, in the reaction with $POCl_3$, was transformed into 4-chloro-2,6,7-trimethyl-1,5-dioxo-1,2,5,6-tetrahydropyrido[3,4-d]pyridazine **1** (Fig. 2).

Boiling the compound **1** with an excess of the suitable amines yielded the corresponding 4-amino derivatives of pyrido[3,4-d]pyridazine (**2–5**, **10**, **11**). Synthesis of the compounds **6–9** was performed analogously but in boiled anhydrous xylene.

The structures of all the obtained compounds were confirmed by elemental and spectral (IR, ^{1}H NMR) analyses.

3. Experimental

3.1. Chemistry

All the results of the C, H, N determinations were within ±0.4% of the values calculated from the corresponding for-

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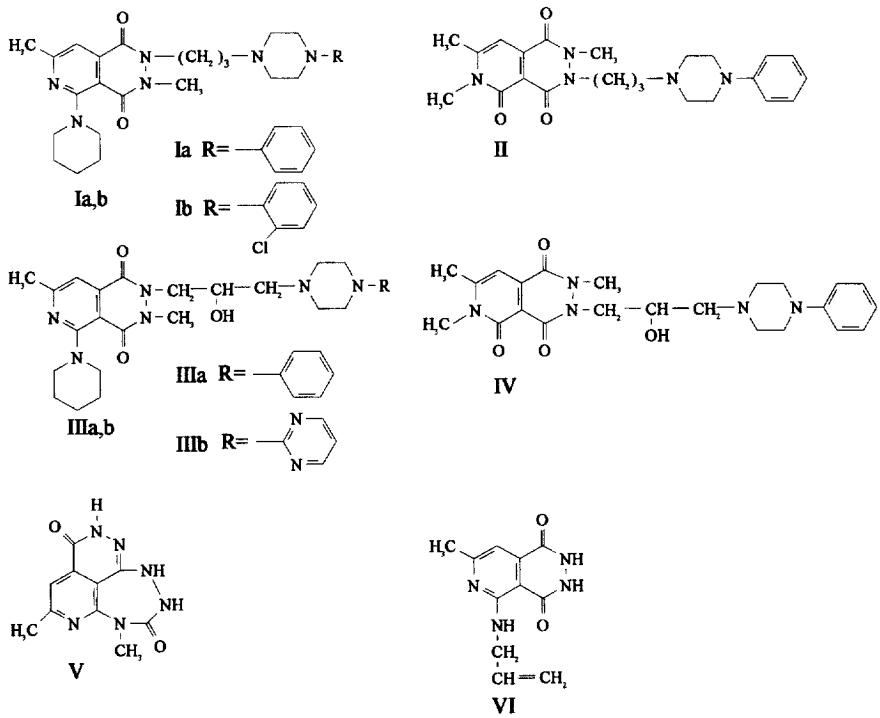

Fig. 1.

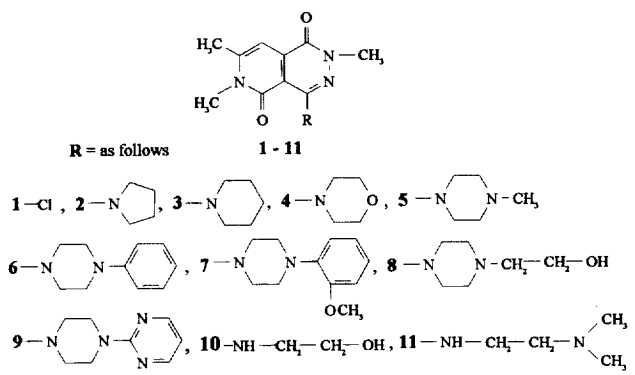

Fig. 2.

mulae. All melting points are uncorrected. IR absorption spectra were determined in KBr pellets, and ^{1}H NMR spectra were recorded in $CDCl_3$ using TMS as internal standard.

3.1.1. 4-Chloro-2,6,7-trimethyl-1,5-dioxo-1,2,5,6-tetrahydropyrido[3,4-d]pyridazine 1

3.15 g of 2,6,7-trimethyl-1,4,5-trioxo-1,2,3,4,5,6-hexahydropyrido[3,4-d]pyridazine and 63 ml of $POCl_3$ were refluxed on an oil bath (98–100°C) for 6 h. Then the excess of $POCl_3$ was distilled off under diminished pressure. The residue was treated with 32 g of crushed ice. The separated product was collected on a filter and purified by crystallization from water.

Table 1
Properties of the investigated compounds

Comp.	Formula (molecular wt.)	M.p. (°C) (solvent)	Yield (%)	IR absorptions in KBr (cm^{-1})		
				CO	NH or OH	Mono- and disubstituted benzene
1	$C_{10}H_{10}N_3O_2Cl$ (239.65)	208–211 (water)	50	1640, 1660–1670	–	–
2	$C_{14}H_{18}N_4O_2$ (274.32)	218–221 (ethanol)	83	1640, 1670	–	–
3	$C_{15}H_{20}N_4O_2$ (288.34)	213–216 (ethanol)	80	1640–1670	–	–
4	$C_{14}H_{18}N_4O_3$ (290.33)	239–241 (ethanol)	74	1650, 1670	–	–
5	$C_{15}H_{21}N_5O_2$ (303.36)	247–250 (acetonitrile)	85	1640, 1660	–	–
6	$C_{20}H_{23}N_5O_2$ (365.42)	230–232 (xylene)	53	1640–1670	–	700, 760
7	$C_{21}H_{25}N_5O_3$ (395.45)	239–240 (xylene)	40	1640–1660	–	735
8	$C_{16}H_{23}N_5O_3$ (333.38)	185–187 (xylene)	58	1640–1670	3360–3400	
9	$C_{18}H_{21}N_7O_2$ (367.15)	242–245 (xylene)	82	1640–1660	–	–
10	$C_{12}H_{16}N_4O_3$ (264.28)	206–208 (ethanol)	77	1630, 1670	3300, 3400	–
11	$C_{14}N_{21}N_5O_2$ (291.35)	196–197 (ethanol)	90	1630, 1670	3280	–

The properties of compound **1** are presented in Table 1, and the assignments in its ^{1}H NMR spectrum are presented below.

^{1}H NMR of **1**: δ=2.55, s-3H (CH_3 in 7); 3.61, s-3H (CH_3 in 6); 3.78, s-3H (CH_3 in 2); 6.82, s-1H (H-8).

3.1.2. General procedure for obtaining compounds 2–5 and 10, 11

2 g of compound **1** in 36 ml of the suitable amine (**2–5**) were refluxed for 10 h. In the case of **10**, 70 ml of β-hydroxyethylamine were used for 2.4 g of **1** and the mixture was boiled for 24 h. In the synthesis of compound **11**, 90 ml of *N,N*-dimethylethylenediamine and 1.75 g of **1** were heated under reflux for 22 h. Then the separated products (**2–5, 11**) were collected on a filter and purified by crystallization from the solvents given in Table 1. In the case of **10** only, an excess of amine was distilled off under diminished pressure and the residue was purified by crystallization from ethanol with simultaneous decolorization with charcoal.

The properties of compounds **2–5**, **10** and **11** are given in Table 1, and the assignments in their ^{1}H NMR spectra are presented below.

^{1}H NMR of **2**: δ=1.83–1.99, m-4H (H of pyrrolidine); 2.52, s-3H (CH_3 in 7); 3.30-3.46, tr-4H (H of pyrrolidine); 3.60, s-3H (CH_3 in 6); 3.71, s-3H (CH_3 in 2); 6.94, s-1H (H-8).

^{1}H NMR of **3**: δ=1.50–2.00, m-6H (H of piperidine); 2.50, s-3H (CH_3 in 7); 2.98–3.05, m-4H (H of piperidine); 3.60, s-3H (CH_3 in 6); 3.71, s-3H (CH_3 in 2); 6.93, s-1H (H-8).

^{1}H NMR of **4**: δ=2.52, s-3H (CH_3 in 7); 3.09-3.20, tr(distorted)-4H (H of morpholine); 3.61, s-3H (CH_3 in 6); 3.72, s-3H (CH_3 in 2); 3.87-3.98, tr(distorted)-4H (H of morpholine); 6.97, s-1H (H-8).

^{1}H NMR of **5**: δ=2.38, s-3H (CH_3 in piperazine); 2.53–2.72, m-7H (CH_3 in 7 and H of piperazine); 3.18–3.53, m-4H (H of piperazine); 3.62, s-3H (CH_3 in 6); 3.72, s-3H (CH_3 in 2); 6.94, s-1H (H-8).

^{1}H NMR of **10**: δ=2.51, s-3H (CH_3 in 7); 3.51–3.92, m-11H (CH_3 in 6, CH_3 in 2, $-CH_2-CH_2-OH$); 6.95, s-1H (H-8); 8.3, tr(distorted)-1H (N*H*).

^{1}H NMR of **11**: δ=2.31, s-6H ($-N(CH_3)_2$); 2.50–2.65, m-5H (CH_3 in 7, $-CH_2-N(CH_3)_2$); 3.38–3.47, m-2H ($-CH_2-NH-$); 3.61, s-3H (CH_3 in 6); 3.69, s-3H (CH_3 in 2); 6.99, s-1H (H-8); 8.1, broad signal-1H (N*H*).

3.1.3. General method for obtaining compounds 6–9

0.45 g (1.88 mmoles) of 4-chloro-2,6,7-trimethyl-1,5-dioxo-1,2,5,6-tetrahydropyrido[3,4-d]pyridazine was dissolved in 20 ml of anhydrous xylene while boiling. After cooling, 11 mmoles of the corresponding derivative of piperazine were added to this solution. The reaction mixture was refluxed for 15 h. Then the precipitated product was collected on a filter, washed with xylene and purified by crystallization from the solvent given in Table 1.

The properties of compounds **6–9** are listed in Table 1, and the assignments in their ^{1}H NMR spectra are shown below:

^{1}H NMR of **6**: δ=2.50, s-3H (CH_3 in 7); 3.34–3.49, m-8H (H of piperazine); 3.6, s-3H (CH_3 in 6); 3.73, s-3H (CH_3 in 2); 6.94–7.27, m-6H (H arom.+H-8).

^{1}H NMR of **7**: δ=2.50, s-3H (CH_3 in 7); 3.24–3.33, m-8H (H of piperazine); 3.61, s-3H (CH_3 in 6); 3.73, s-3H (CH_3 in 2); 3.88, s-3H (OCH_3); 6.93–7.28, m-5H (H arom.+H-8).

^{1}H NMR of **8**: δ=2.50, s-3H (CH_3 in 7); 2.57–2.81, m-6H (H of piperazine+$-CH_2$); 3.11–3.22, m-4H (H of piperazine); 3.6–3.71, m-9H (CH_3 in 6+CH_3 in 2+$-CH_2-OH$); 6.97, s-1H (H-8).

^{1}H NMR of **9**: δ=2.51, s-3H (CH_3 in 7); 3.14-3.26, tr(distorted)-4H (H of piperazine); 3.62, s-3H (CH_3 in 6); 3.72, s-3H (CH_3 in 2); 3.98–4.13, tr(distorted)-4H (H of piperazine); 6.43–6.55, tr-1H (H-5 in pyrimidine); 6.97, s-1H (H-8); 8.30–8.35, d-2H (H-4 and 6 of pyrimidine).

3.2. Pharmacology

Compounds **2**, **4**, **5**, **10**, and **11** were investigated pharmacologically.

3.2.1. Materials and methods

The experiments were carried out on male and female albino Swiss mice (body weight of 20–25 g) and male Wistar rats (200–250 g). Investigated compounds were administered intraperitoneally (i.p.) as suspensions in 3% Tween 80 in a constant volume of 10 ml/kg in mice and 5 ml/kg in rats. The compounds were administered in doses equivalent to 1/10 or 1/20 of LD_{50}. Control animals received the equivalent volume of solvent. Each experimental group consisted of eight animals.

The following pharmacological tests were performed:

1. Acute toxicity in mice.
2. Motor coordination in the rota-rod test in mice.
3. Spontaneous locomotor activity in mice.
4. Amphetamine-induced locomotor hyperactivity in mice.
5. Pain reactivity in the 'writhing syndrome' test in mice.
6. Pain reactivity in the 'hot-plate' test in mice.
7. Anxiolytic properties in the 'four-plate' test in mice.
8. Pentetrazol-induced seizures in mice.
9. Maximal electric shock in mice.
10. Head twitches induced by 5-hydroxytryptophane in mice.
11. Arterial blood pressure in rats.

Acute toxicity was assessed by the methods of Litchfield and Wilcoxon [9] and presented as LD_{50} calculated from the mortality of mice after 24 h.

Motor coordination was measured according to the method of Gross and Tripod [10]. The mice were placed for 2 min on the rod rotating with the speed of 4 rpm. The effects were evaluated 15, 30, 45, 60, 75, 90 and 105 min after the administration of the investigated compounds.

Spontaneous locomotor activity in mice was measured in circular photoresistor actometers (32 cm in diameter). After

injection of the investigated compounds, the animals were placed in the actometers for 1 h. Each crossing of the light beam was recorded automatically. The number of impulses was noted after 30 and 60 min.

Amphetamine hyperactivity in mice was induced by d,l-amphetamine 2.5 mg/kg s.c. The investigated compounds were injected 30 min before the amphetamine was administrated. The locomotor hyperactivity was measured 30 and 60 min later in the photoresistor actometers.

Pain reactivity was measured by the 'writhing syndrome' test of Koster et al. [11]. The test was performed in mice by the i.p. injection of a 0.6% solution of acetic acid in a volume of 10 ml/kg 60 min after administration of the investigated compounds. The number of writhing episodes was counted for 30 min after the injection of the 0.6% acetic acid.

Pain reactivity was also measured in the 'hot-plate' test according to the method of Eddy and Leimbach [12]. The animals were placed individually on the metal plate heating to 56°C. The time (s) of appearance of the pain reaction (licking of the forepaws or jumping) was measured. The experiments were performed 30 min after administration of the investigated compounds.

Anxiolytic properties were assessed by the 'four-plate' test in mice, following Aron et al. [13], 60 min after administration of the investigated compounds in doses which had no effect on the spontaneous locomotor activity. Mice were placed in cages with four-plate floors (11×7 cm) with a 4 mm gap between each. After 15 s of adaptation, the number of crossings was counted during 1 min. Each crossing was punished with direct current (180 V, 0.5 A) but no more frequently than every 3 s.

Pentetrazol seizures in mice were induced by the administration of pentetrazol at a dose of 100 mg/kg s.c., 30 min after administration of the investigated compounds. Animals were observed during 30 min and the number of mice developing clonic and tonic seizures as well as mortality was recorded in that period.

A maximal electric shock was induced by means of alternating current (50 Hz, 25 mA, 0.2 s) with the use of ear clip electrodes according to the method of Swinyard et al. [14]. The criterion for the convulsive response was the tonic extension of the hind limbs. The test was performed 60 min after administration of the investigated compounds.

Head twitch behaviour was induced by the administration of 5-hydroxytryptophane (5-HTP) at a dose of 180 mg/kg i.p., 30 min after administration of the investigated compounds. The animals were observed 60 min after the 5-HTP administration.

Arterial blood pressure was determined according to the method of Gerold and Tschirky [15] using the UGO-BASILE equipment (blood pressure recorder, Cat. No 8006). Systolic blood pressure on the tail artery was measured 30 min after administration of the investigated compounds.

3.2.2. Statistics

The results obtained were presented as means and evaluated statistically using Student's *t*-test or the exact Fisher test.

4. Results and discussion

The LD_{50} values of the investigated compounds after their i.p. administration in mice are presented in Table 2. They indicate that the least toxic compound was **10** (LD_{50} = 1180.4 mg/kg), and the most toxic one was **4** (LD_{50} = 202.8 mg/kg). The other amino derivatives were also toxic with LD_{50} between 415.1 and 565.5 mg/kg. None of the tested compounds had neurotoxic properties at the dose of 1/10 LD_{50} (56.6 (**2**), 20.3 (**4**), 54.4 (**5**), 118 (**10**) and 41.5 (**11**) mg/kg) as they did not affect the motor coordination in the rotarod test. Compounds **2** and **5** suppressed spontaneous locomotor activity during the 1 h observation period at the highest dose used (1/10 LD_{50}: 56.6 (**2**) and 54.4 (**5**) mg/kg) (Table 3). The remaining derivatives were inactive in this test. None of the investigated compounds affected the excitatory action of amphetamine.

Derivatives **2**, **5** and **10** possessed analgesic activity assayed in the 'writhing syndrome' test. **5** was active up to the dose of 1/20 LD_{50} (27.2 mg/kg), **2** and **10** at the dose of 1/10 LD_{50} (56.6 (**2**) and 118 (**10**) mg/kg) (Table 4). This action was not confirmed in the 'hot-plate' test. The other compounds were inactive in both tests.

None of the investigated substances showed anxiolytic or anticonvulsive properties. Furthermore, compounds **2**, **5**, **10** and **11** did not change the number of head twitches induced by 5-HTP in mice. Only **4** decreased the activity of the serotoninergic system, reducing the number of head twitches (Table 5). In spite of expectations, none of the investigated compounds administered at the dose of 1/10 LD_{50} (56.6 (**2**),

Table 2
Acute toxicity of the investigated compounds (n = 8)

Comp.	LD_{50} (mg/kg i.p.)	Confidence limits
2	565.5	443.4–721.2
4	202.8	157.0–262.0
5	543.9	430.2–687.6
10	1180.4	961.4–1449.3
11	415.1	351.0–491.0

Table 3
Influence of the investigated compounds on the spontaneous locomotor activity in mice (n = 8)

Comp.	Dose (part of LD_{50})	No. of impulses ± SEM after:	
		30 min	60 min
Control	–	2519.2 ± 295.9	4230.0 ± 571.2
2	1/10	1790.8 ± 366.9	2414.5 ± 377.9*
	1/20	2148.2 ± 405.6	3761.1 ± 359.9
4	1/10	2464.3 ± 258.7	3605.6 ± 454.2
5	1/10	1698.6 ± 117.8*	2630.5 ± 314.4*
	1/20	2018.1 ± 103.8	3658.6 ± 258.6
10	1/10	2454.3 ± 355.6	3062.8 ± 436.5
11	1/10	2658.0 ± 197.8	4065.6 ± 286.0

*p < 0.05.

Table 4
Influence of the investigated compounds on the pain reactivity in the 'writhing syndrome' test in mice ($n=8$)

Comp.	Dose (part of LD_{50})	Mean no. of writhings ± SEM
Control	–	5.25 ± 0.69
2	1/10	2.10 ± 0.51**
	1/20	4.71 ± 0.71
4	1/10	8.00 ± 1.12
5	1/10	0.36 ± 0.21***
	1/20	3.11 ± 0.71*
	1/40	5.41 ± 1.39
10	1/10	0.37 ± 0.12***
	1/20	4.14 ± 1.03
11	1/10	4.00 ± 1.13

*$p<0.05$; **$p<0.01$; ***$p<0.001$.

Table 5
Influence of the investigated compounds on the number of head twitches induced by 5-HTP in mice ($n=8$)

Comp.	Dose (part of LD_{50})	No. of head twitches ± SEM
Control	–	7.11 ± 0.62
2	1/10	5.25 ± 1.68
4	1/10	2.14 ± 0.43**
	1/20	6.21 ± 2.12
5	1/10	6.50 ± 1.05
10	1/10	7.12 ± 1.06
11	1/10	5.04 ± 1.12

**$p<0.01$.

20.3 (**4**), 54.4 (**5**), 118 (**10**) and 41.5 (**11**) mg/kg) affected the pulse rate and arterial blood pressure in rats.

The above data indicate that compound **11**, containing the β-(dimethylamino)ethylamino group in position 4 of the pyrido[3,4-d]pyridazine system was inactive in all used tests. The 4-morpholino derivative **4** diminished the activity of the serotoninergic system and was the most toxic compound.

Only compounds **2** and **5** with the pyrrolidinyl and *N*-methylpiperazinyl groups, respectively, were active in two tests, whereas β-hydroxyethylamino derivative **10** proved to be the least toxic compound, showing analgesic activity in the 'writhing syndrome' test.

In view of these results we conclude that introduction of amino groups in position 4 of 2,6,7-trimethyl-1,5-dioxo-1,2,5,6-tetrahydropyrido[3,4-d]pyridazine gives pharmacologically less active compounds than those previously obtained.

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