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Synthesis and properties of 2-(4-substituted)butyl derivatives of some 2,3-dihydro-1,3-dioxo-1*H*-pyrrolo[3,4-*c*]pyridines

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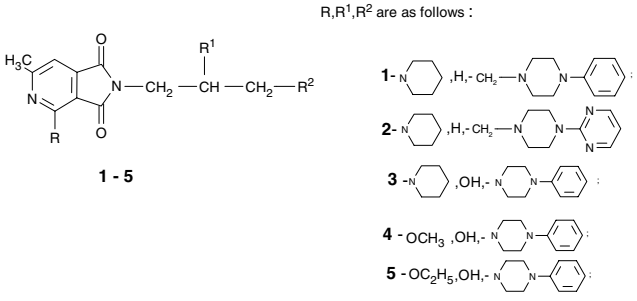
The synthesis of 2-(4-substituted)butyl derivatives of 4-alkoxy-2,3-dihydro-6-methyl-1,3-dioxo-1*H*-pyrrolo[3,4-*c*]pyridine (**10**–**15**) and the results of preliminary pharmacological screening are described in this paper. All the compounds tested showed a strong analgesic action, suppressed spontaneous locomotor activity and prolonged barbiturate sleep. Except **10**, all significantly decreased systolic and diastolic blood pressure.

1. Introduction

It has been reported previously [1, 2] that 2,3-dihydro-1,3-dioxo-1*H*-pyrrolo[3,4-*c*]pyridine (3,4-pyridinedicarboximide) derivatives **1** and **3** significantly suppressed the spontaneous locomotor activity of mice. **1**–**3** caused hypothermia in normothermic mice and significantly decreased their amphetamine induced hyperactivity. Compound **3** also displayed a weak analgesic action.



In continuing research on this series we reported recently [3] that replacement of the piperidino group in **3** by an alkoxy one gave the non-toxic substances **4**, **5** ($LD_{50} > 2000$ mg/kg) which have very strong analgesic properties. In this respect, methoxy derivative **4** proved to be particularly interesting. In pursuit of these studies we have now synthesized (independently of the investigations in the group of 2-hydroxy-3[4-aryl(heteroaryl)-1-piperazinyl)]-propyl-2,3-dihydro-1,3-dioxo-1*H*-pyrrolo[3,4-*c*]pyridines) 2-aryl(heteroaryl)piperazinylbutyl derivatives of 2,3-dihydro-4-methoxy(ethoxy)-6-methyl-1,3-dioxo-1*H*-pyrrolo[3,4-*c*] pyridine (**10**–**14**), combining certain structural elements of **1**, **2** with those of **4**, **5**.

In compound **15** we introduced a 1,2,3,4-tetrahydroisoquinolinyl substituent instead of a piperazinyl group at the butyl chain. According to Mokrosz et al. [4] replacement of the 4-substituted piperazine in the side – chain of Buspirone by the 1,2,3,4-tetrahydroisoquinolinyl group did not change its pharmacological profile. Given this, we wanted to know whether this replacement would also be possible in this group of compounds. We expected that the compounds obtained would exhibit potent analgesic activity.

Scheme

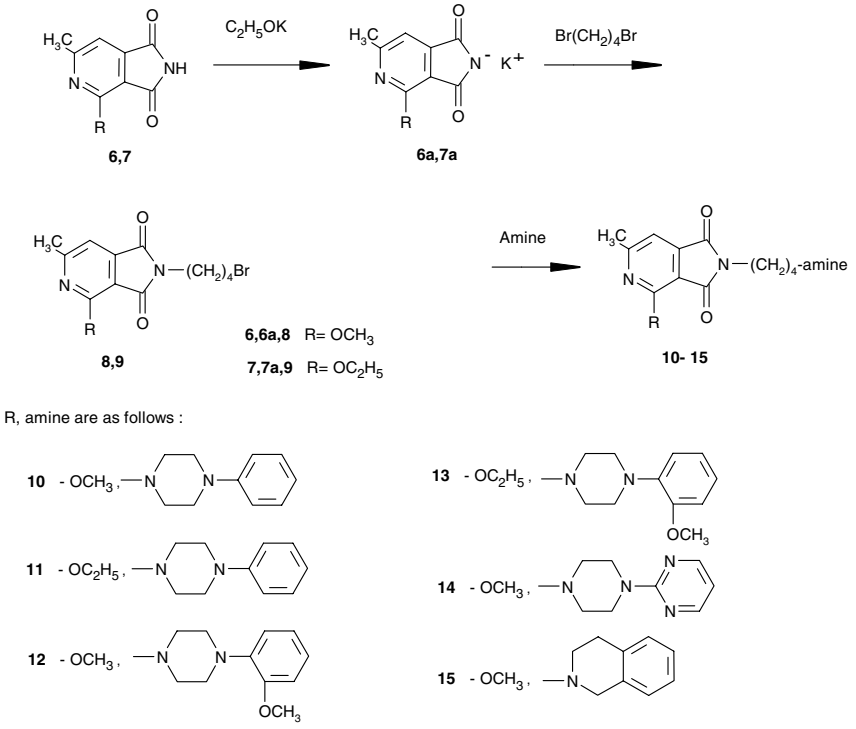


Table 1: Physical data of 3,4-pyridinedicarboximides 6–15

Compd.	Formula (Mol. wt.)	M.p. (°C) Solvent	Yield (%)	IR absorptions in KBr (cm^{-1})			
				CO	CH_2	mono- and disubst. benzene	NH
6	$C_9H_8N_2O_3$ (192.2)	249–251 ethanol	36	1720, 1765	–	–	3050, 3180
7	$C_{10}H_{10}N_2O_3$ (206.2)	216–218 ethanol	25	1730, 1775	–	–	3050, 3160
8	$C_{13}H_{15}BrN_2O_3$ (327.2)	125–127 ethanol	64	1705, 1765	2924–3000	–	–
9	$C_{14}H_{17}BrN_2O_3$ (341.2)	76–78 ether	45	1700, 1765	2820–2980	–	–
10	$C_{23}H_{28}N_4O_3$ (408.5)	130–133 ethanol	75	1710, 1770	2820, 2930	700, 770	–
11	$C_{24}H_{30}N_4O_3$ (422.5)	110–112 ether/petroleum ether	53	1700, 1760	2800, 2900	680, 750	–
12	$C_{24}H_{30}N_4O_4$ (438.5)	153–156 acetonitrile	80	1705, 1770	2820, 2920	760	–
13	$C_{25}H_{32}N_4O_4$ (452.5)	91,5–93 petroleum ether	61	1700, 1760	2800, 2900	752	–
14	$C_{21}H_{26}N_6O_3$ (410.5)	137–140 ethanol	80	1705, 1770	2950		–
15	$C_{22}H_{25}N_3O_3$ (379.4)	93–96 ethanol	61	1700–1710, 1770	2740–2920	750	–

2. Investigations and results

2.1. Chemistry

The starting materials for the synthesis of compounds **10**–**15** were 4-methoxy- and 4-ethoxy-2,3-dihydro-6-methyl-1,3-dioxo-1*H*-pyrrolo[3,4-*c*]pyridines (**6**, **7**) which were obtained by the reaction of 4-chloro-2,3-dihydro-6-methyl-1,3-dioxo-1*H*-pyrrolo[3,4-*c*]pyridine [5], with CH_3ONa or C_2H_5ONa in anhydrous methanol or ethanol, respectively. Their potassium salts (**6a**, **7a**) were condensed with 1,4-dibromobutane with the aim of obtaining 4-bromobutyl derivatives (**8**, **9**), which were transformed into the compounds **10**–**15** by heating with suitable amines (*N*-phenyl-, *N*-*o*-methoxyphenyl-, *N*-(2-pyrimidinyl)piperazines and 1,2,3,4-tetrahydroisoquinoline) in acetonitrile solution and in the presence of anhydrous potassium carbonate.

The compounds described in this paper are crystalline substances. Their structures were confirmed by spectral (IR, ^{1}H NMR) and elemental analyses (Table 1).

For pharmacological screening we chose four substances: **10**, **12, 14** and **15**. All are 4-methoxy derivatives of the appropriate pyrrolo[3,4-*c*]pyridine. From our earlier investigations [3] it follows that 4-methoxy-2-[2-hydroxy-3(4-phenyl-1-piperazinyl)]propyl-2,3-dihydro-6-methyl-1,3-dioxo-1*H*-pyrrolo[3,4-*c*]-pyridine (**4**) produced a considerably stronger analgesic effect than the corresponding 4-ethoxy derivative **5**. Compounds **10, 12** and **14** differ in the type of substituents at N-4 of the piperazine bound with the butyl chain (phenyl (**10**), *o*-methoxyphenyl (**12**), 2-pyrimidinyl (**14**)). By introducing them we wanted to verify their possible influence on CNS activity. For reasons mentioned above we also selected for testing compound **15** containing the 4-[2-(1,2,3,4-tetrahydroisoquinolinyl)butyl] substituent at the N atom.

2.2. Pharmacological investigations

2.2.1. Acute toxicity

The LD_{50} values for the compounds investigated are presented in Table 2. Toxic doses of all tested compounds caused sedation, decrease of locomotor activity and depression of respiratory movements. After intraperitoneal administration the most toxic compounds were **15** and **14**, while compounds **10** and **12** were not toxic ($LD_{50} > 2000$ mg/kg).

Table 2: Acute toxicity in mice of the compounds investigated, according to Litchfield and Wilcoxon [6]

Compd.	$LD_{50\,ip}$ (mg/kg)
10	> 2000
12	> 2000
14	1000 (820–1240)
15	920 (860–1080)

The data are median lethal doses with 5% confidence limits in parentheses (n = 6)

2.2.2. Locomotor activity

All compounds tested significantly suppressed the spontaneous locomotor activity of mice during a 30 min observation period. Compound **10**, given in doses of 1/20, 1/40 and 1/80 LD_{50}, inhibited spontaneous locomotor activity

Table 3: Influence of the compounds investigated on spontaneous locomotor activity in mice

Compd.	Dose (fraction of LD_{50})	No. of impulses ± SEM after 30 min
Control	–	217.3 ± 41.1
10	1/20	30.2 ± 18.6**
	1/40	71.4 ± 32.2*
	1/80	116.0 ± 39.5
12	1/20	38.4 ± 16.1**
	1/40	115.6 ± 37.0
	1/80	159.8 ± 21.8
14	1/20	27.6 ± 11.7**
	1/40	94.2 ± 19.6
	1/80	173.4 ± 21.9
15	1/20	27.2 ± 8.0**
	1/40	88.2 ± 18.3
	1/80	188.0 ± 23.7

Each group consisted of 6–8 animals. ** $p < 0.02$, * $p < 0.05$

Table 4: Influence of the compounds investigated on thiopental anesthesia

Compd.	Dose (fraction of LD_{50})	Duration of anaesthesia ± SEM (min)
Control	–	31.8 ± 6.7
10	1/20	108.8 ± 12.4****
	1/40	76.4 ± 13.6***
	1/80	57.5 ± 13.7
12	1/20	113.2 ± 27.2***
	1/40	82.4 ± 14.3***
	1/80	55.6 ± 14.8
14	1/20	137.0 ± 27.8***
	1/40	77.0 ± 9.5***
	1/80	54.6 ± 11.9
Control	–	35.6 ± 8.1
15	1/20	137.6 ± 25.6***
	1/40	71.2 ± 12.9*
	1/80	67.2 ± 17.0

Each group consisted of 6–8 animals. **** $p < 0.001$, *** $p < 0.01$, ** $p < 0.02$, * $p < 0.05$

in mice by 86 ($p < 0.02$), 67 (< 0.05) and 47%, respectively. The other compounds significantly decreased locomotor activity in mice, by 87–82% when administered in a dose of 1/20 LD_{50} (Table 3). Given in doses of 1/40 and 1/80 LD_{50} they reduced the spontaneous locomotor activity by 59–47% and 26–13%.

2.2.3. *Thiopental anesthesia*

All the compounds investigated, given in doses of 1/20 and 1/40 LD_{50}, significantly prolonged barbiturate sleep in mice by 330–242% and 186–140%, respectively (Table 4). Administered in doses of 1/80 LD_{50} they also prolonged the time of anesthesia by 72–89%, but this effect was not significant.

Table 5: Influence of the compounds investigated on the pain reaction in the "writhing syndrome" test in mice

Compd.	Dose (fraction of LD_{50})	Mean no. of writhings ± SEM	ED_{50} (mg/kg)
10	0	16.8 ± 1.2	4.5 (3.7–5.2)
	1/40	0	
	1/80	4.2 ± 2.2***	
	0	16.4 ± 1.8	
	1/160	4.4 ± 2.2***	
	1/320	5.8 ± 2.3***	
	1/640	10.4 ± 4.3	
12	0	16.8 ± 1.2	6.8 (6.2–7.4)
	1/40	0	
	1/80	1.0 ± 0.6****	
	0	21.1 ± 4.4	
	1/160	10.25 ± 3.0	
14	0	16.4 ± 1.8	11.9 (9.4–13.2)
	1/40	0	
	1/80	9.2 ± 2.8*	
	1/160	12.4 ± 2.5	
15	0	21.4 ± 4.4	0.72 (0.61–0.84)
	1/40	0	
	1/80	4.2 ± 2.2**	
	0	19.0 ± 5.9	
	1/160	4.2 ± 1.1*	
	1/320	6.0 ± 2.5	

Each group consisted of 6–8 animals. **** $p < 0.001$, *** $p < 0.01$, ** $p < 0.02$, * $p < 0.05$

2.2.4. *"Writhing syndrome" test in mice*

All compounds tested showed strong analgesic activity in this test. The most potent effect was produced by compound **10** which was active up to a dose of 1/320 LD_{50}. Compound **15** was effective in the "writhing syndrome" test in mice up to a dose of 1/160 LD_{50}, whereas compounds **12** and **14** had analgesic activity in doses up to 1/80 LD_{50} (Table 5, Fig.).

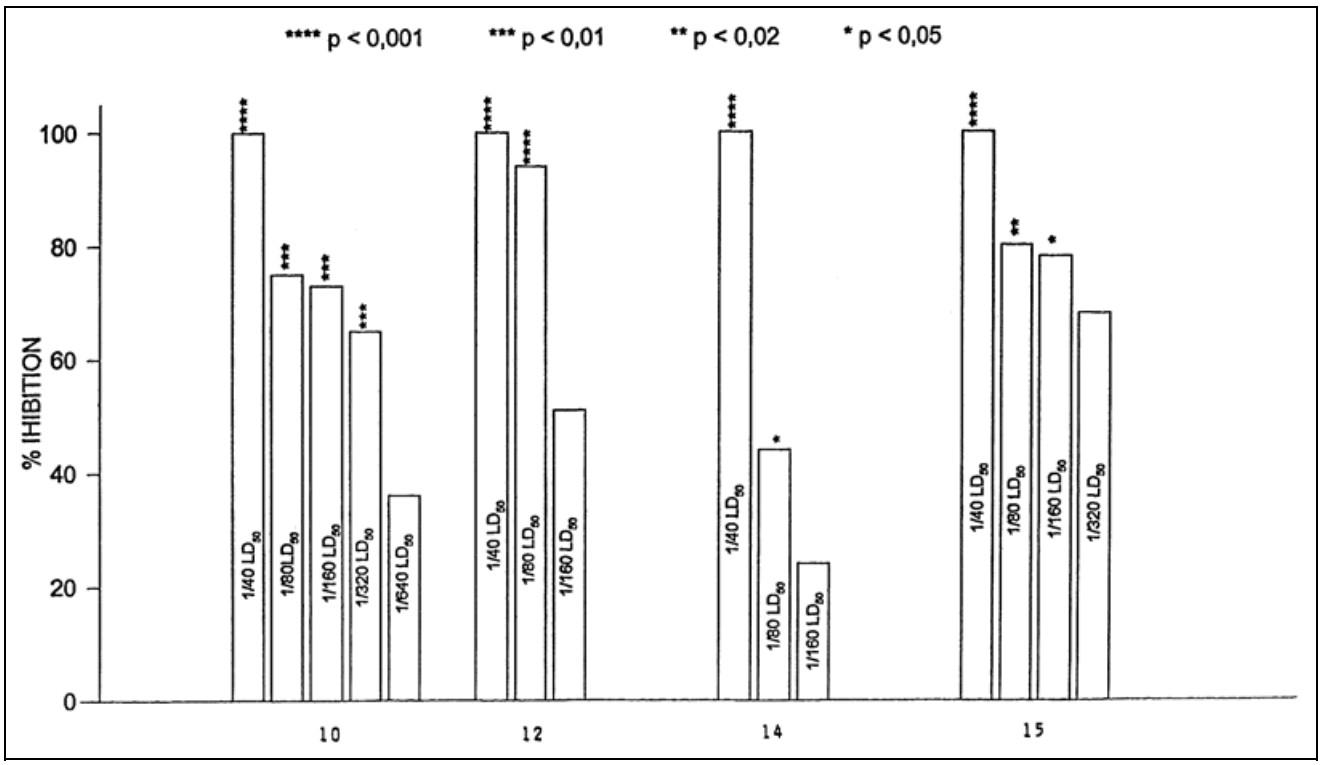


Fig.: Effect of investigated compounds in the "writhing syndrome" test in mice

Table 6: Influence of the compounds investigated on the pain reaction in the "hot-plate" test in mice

Compd.	Dose (fraction of LD_{50})	Time of reaction to pain stimulus ± SEM (s)
Control	–	9.57 ± 1.8
10	1/10	32.7 ± 13.2*
	1/20	25.0 ± 6.9*
	1/40	9.4 ± 2.1
12	1/10	34.8 ± 14.5*
	1/20	15.0 ± 5.2
	1/40	9.6 ± 1.7
14	1/10	13.4 ± 3.2
	1/20	8.4 ± 1.6
	1/40	7.6 ± 1.4
15	1/10	20.4 ± 5.3*
	1/20	13.8 ± 2.2
	1/40	10.6 ± 2.4

Results are expressed as a mean ± SEM, n = 6–8. * $p < 0.05$

2.2.5. *"Hot plate" test*

The analgesic action of compound **10** was also confirmed in the "hot plate" test in doses of 1/10 and 1/20 LD_{50}. Compounds **12** and **15** were active in this test only at the highest dose (1/10 LD_{50}). **14** did not significantly increase the times of appearance of the pain reaction (Table 6).

2.2.6. *Influence on the blood pressure*

Three compounds (**12**, **14** and **15**), injected intraperitoneally in a single dose corresponding to ED_{50} in the "writhing syndrome" test, significantly decreased the systolic and diastolic pressure in anesthetized normotensive rats. The peak effects were present within 20–40 min after administration. For all compounds the duration of action was more than 70 min (Table 7).

3. Discussion

From the data presented it follows that compounds **10** and **12** containing phenyl and *o*-methoxyphenyl at N-4 of piperazine were not toxic in contrast to imide **14**, with a 2-pyrimidinyl group substituted in this position. The toxicity of the latter compound was similar to that of **15** with the 1,2,3,4-tetrahydroisoquinolinyl substituent at the butyl chain. Imide **15** proved to be the most toxic of the compounds studied. Independently of the kind of the substituents at the alkyl chain all substances tested were active as analgesic agents in the "writhing syndrome" test but the strongest analgesic effect was produced by compound **10**. It was also the most active substance in the "hot plate" test. But in comparison with **12, 14** and **15**, derivative **10** was devoid of hypotensive activity. The tetrahydroisoquinolinylbutyl derivative **15** displayed the same pharmacological profile as the other imides studied. This indicates that replacement of the *N*-substituted piperazinyl group by the 1,2,3,4-tetrahydroisoquinolinyl group is possible in this series of compounds, apart of course from the increase in toxicity caused by this last substituent. But the same effect was observed with compound **14,** containing the 4-(2-pyrimidinyl)piperazinyl group. Further, it can be seen from the data presented that replacement of the piperidino group in compounds **1**, **2** by a methoxy one (imides **10, 14**) caused appearance of strong analgesic activity. At the same time, this change resulted in a non-toxic substance in the case of compound **10** (LD_{50} for **1** = 1750 mg/kg, for **10** > 2000 mg/kg) and a more toxic one in the case of **14** (LD_{50} for **2** > 2000 mg/kg, for **14**– 1000 mg/kg). Moreover, imide **14** in contrast to **2** significantly depressed the spontaneous locomotor activity of mice and displayed a hypotensive effect. The prolongation of the side-chain at the N atom to C_4 and elimination of the hydroxy group in imide **4** (compound **10**) decreased analgesic activity. **4** (the lead structure) was active in the "writhing syndrome" test up to a dose of 1/5120 $LD_{50} = 0.39$ mg/kg (3), whereas compound **10** was effective in this test up to the a of 1/320 $LD_{50} = 6.25$ mg/kg. These chemical changes did not influence the toxicity of these compounds (LD_{50} for **4** and **10** > 2000 mg/kg). In both cases analgesic action was associated with the suppression of the spontaneous locomotor activity of mice.

Compounds **10**, **12**, **14** and **15** were tested for analgesic activity in intraperitoneal administration (i.p.) in mice in terms of the inhibition of the "writhing syndrome" induced by phenylbenzoquinone and in the "hot plate" latency test. The calculated ED_{50} values which represent the dose producing 50% inhibition of "writhing" episodes suggest that special attention should be paid to compound

Table 7: Influence of compounds tested on blood pressure in anaesthetised normotensive rats

Compd.	Dose (mg/kg) (ED_{50})	Blood pressure (mm Hg)	Time (min)								
			0	**5**	**10**	**20**	**30**	**40**	**50**	**60**	**70**
10	4.5	systolic	136.7 ± 7.2	137.6 ± 7.7	137.3 ± 6.4	136.6 ± 6.8	136.3 ± 6.3	135.3 ± 3.9	132.6 ± 2.1	132.0 ± 1.5	133.0 ± 1.7
		diastolic	120.0 ± 7.6	121.3 ± 9.5	121.0 ± 9.5	120.3 ± 9.0	121.0 ± 8.3	117.6 ± 8.0	114.6 ± 6.8	114.0 ± 6.0	114.3 ± 6.9
12	6.8	systolic	138.5 ± 7.0	128.7 ± 5.6	128.7 ± 5.7	122.0 ± 6.4	115.5 ± 7.5**	118.2 ± 6.2*	120.5 ± 5.5*	123.2 ± 4.9	123.7 ± 5.1
		diastolic	118.7 ± 7.7	111.5 ± 5.0	111.2 ± 4.6	104.7 ± 1.6**	98.0 ± 3.3****	101.0 ± 2.9***	100.7 ± 2.2***	104.0 ± 0.7**	104.2 ± 1.4**
14	11.9	systolic	146.5 ± 7.2	131.7 ± 3.9	124.0 ± 8.7	118.0 ± 11.2*	114.7 ± 11.7**	120.5 ± 9.3*	120.5 ± 8.8*	119.5 ± 8.3*	122.5 ± 6.4
		diastolic	127.7 ± 9.3	113.2 ± 3.7	103.0 ± 6.6*	95.2 ± 10.0***	95.7 ± 8.1***	97.2 ± 7.5***	99.2 ± 6.3***	99.7 ± 5.3**	102.5 ± 4.6**
15	0.72	systolic	139.5 ± 1.5	139.2 ± 2.3	137.0 ± 3.0	132.7 ± 2.2	128.7 ± 3.7***	124.5 ± 1.1****	122.5 ± 1.7****	120.2 ± 3.0****	116.5 ± 4.0****
		diastolic	117.0 ± 4.6	116.2 ± 6.7	115.0 ± 5.7	112.0 ± 4.5	107.5 ± 5.6	104.7 ± 3.1	100.2 ± 1.7**	98.0 ± 3.1***	92.5 ± 4.2****

The data were means of 4–5 experiments ± S.E.M. Statistical analyses were performed using a one-way ANOVA test: * $p < 0.05$; ** $p < 0.02$; *** $p < 0.01$; **** $p < 0.001$

10 which also shows one of the strongest effects in the "hot plate" test. Furthermore, compound **10** significantly suppresses spontaneous locomotor activity and prolonged barbiturate sleep in mice. The analgesic effects generated by imide **10** and other compounds were, however, much weaker in comparison with those caused by 2,3-dihydro-2-[2-hydroxy-3-(4-phenyl-1-piperazinyl)]propyl-4-methoxy-6-methyl-1,3-dioxo-1*H*-pyrrolo[3,4-*c*]pyridine (**4**) (3). This indicates that in this series of compounds the structure of the side-alkyl chain influences the strength of the analgesic action.

4. Experimental

4.1. Chemistry

All the results of the C, H, and N determinations (carried out by a Carlo Erba Elemental Analyzer model NA-1500) were within ± 0.4% of the theoretical values. All m.p.'s are uncorrected. The IR spectra, in KBr pellets, were measured with a Zeiss Jena Specord model IR 75. ^{1}H NMR spectra were determined in $CDCl_3$, if not otherwise indicated, on a Tesla 587 A spectrometer (80 MHz) using TMS as an internal standard.

4.1.1. General method for synthesis of 4-methoxy- and 4-ethoxy-2,3-dihydro-6-methyl-1,3-dioxo-1H-pyrrolo[3,4-c]pyridines **6**, **7**

0.92 g (0.04 mol) of sodium was dissolved in 120 ml of anhydrous methanol (**6**) or anhydrous ethanol (**7**) and to this solution 3.93 g (0.02 mol) of 4-chloro-2,3-dihydro-6-methyl-1,3-dioxo-1 *H*-pyrrolo[3,4-*c*]pyridine were added. The mixture was refluxed for 25 h. Then the solvent was evaporated under reduced pressure. The dry residue was dissolved in 25 ml of distilled water and after filtration the solution was acidified with 80% acetic acid to pH = 6–7 and left for 1 h at room temperature. The precipitated product was collected and purified by crystallization.
The properties of compounds **6** and **7** are given in Table 1 and the assignments in their ^{1}H NMR spectra are given below:
^{1}H NMR of **6** (in $CDCl_3$ + 1 drop of DMSO-d_6): δ = 2.62 (s, [3 H], CH_3 at C-6); 4.12 (s, [3 H], OCH_3); 7.16 (s, [1 H], 7-H); 8.51 (s (broad), [1 H], NH).
^{1}H NMR of **7** (in $CDCl_3$ + 2 drops of DMSO-d_6): δ = 1.36–1.53 (t, [3 H], J = 6,8 Hz, OCH_2CH_3); 2.60 (s, [3 H], CH_3 at C-6); 4.46–4.72 (q, [2 H], J = 6,8 Hz; OCH_2CH_3); 7.12 (s, [1 H], 7-H); 11.19 (s (broad), [1 H], NH).

4.1.2. General procedure for obtaining compounds **8** *and* **9**

0.01 mol of potassium was dissolved in 100 ml of anh. ethanol and to this solution 0.01 mol of the appropriate imide (**6** or **7**) was added. The mixture was refluxed for 20 min. Then after cooling ethanol was distilled off under reduced pressure and the dry crystalline residue was treated with 12 g of 1,4-dibromobutane and 30 ml of anh. DMF. The suspension obtained was stirred at room temperature until the alkaline reaction disappeared. Then 200 ml of ether were added to the reaction mixture, and it was stirred again for 1 h. After filtration the solvents were evaporated completely under reduced pressure. The residue was treated with a small amount of distilled water and the crystalline substance was collected and purified.
The properties of compounds **8** and **9** are given in Table 1 and the assignments in their ^{1}H NMR spectra are given below:
^{1}H NMR of **8**: δ = 1.81–1.93 (m, [4 H], Hβ, γ of butyl); 2.62 (s, [3 H], CH_3 at C-6); 3.35-3.51 (t, [2 H], Hδ of butyl); 3.61–3.77 (t, [2 H], Hα of butyl); 4.13 (s, [3 H], OCH_3); 7.18 (s, [1 H], 7-H).
^{1}H NMR of **9**: δ = 1.38–1.56 (t, [3 H], J = 6,8 Hz; CH_2CH_3); 1.81–1.89 (m, [4 H], Hβ, γ of butyl); 2.61 (s, [3 H], CH_3 at C-6); 3.43–3.51 (t, [2 H], Hδ of butyl); 3.68–3.76 (t, [2 H], Hα of butyl); 4.56–4.65 (q, [2 H], J = 6,8 Hz; CH_2CH_3); 7.16 (s, [1 H], 7-H).

4.1.3. General procedure for obtaining compounds **10**–**15**

To 0.003 mol of compound **8** or **9** in 70 ml of anh. acetonitrile 0.75 g of anhydrous potassium carbonate and 0.004 mol of the suitable amine (*N*-phenyl-,*N*-*o*-methoxyphenyl-, *N*-(2-pyrimidinyl)piperazine and 1,2,3,4-tetrahydroisoquinoline) were added. The mixture was refluxed for 20 h (**10**, **11**, **14**, **15**), or 25 h (**12**, **13**).
After filtration the solvent was evaporated under reduced pressure and the residue was purified by crystallization from the solvent given in Table 1.
The properties of compounds **10**–**15** are given in Table 1 and the assignments in their ^{1}H NMR spectra are given below:
^{1}H NMR of **10**: δ = 1.59–1.64 (m, [4 H], H β, γ of butyl); 2.40–2.62 (m, [9 H), CH_3 at C-6 + $H_2C-N(CH_2)_2-$); 3.11–3.24 (m, [4 H], H of piperazine); 3.61–3.69 (t, [2 H], Hα of butyl); 4.13 (s, [3 H], OCH_3); 6.86–7.17 (m, [6 H], arom. H + 7-H).
^{1}H NMR of **11**: δ = 1.38–1.65 (m, [7 H], Hβ, γ of butyl + CH_2CH_3, J = 6,8 Hz); 2.40–2.63 (m, [9 H], CH_3 at C-6 + $H_2C-N(CH_2)_2-$); 3.12–3.25 (m, [4 H], H of piperazine); 3.60–3.76 (t, [2 H], Hα of butyl); 4.47–4.73 (q, [2 H], J = 6,8 Hz; CH_2CH_3); 6.85–7.35 (m, [6 H], arom. H + 7-H).
^{1}H NMR of **12**: δ = 1.57–1.64 (m, [4 H], Hβ, γ of butyl); 2.25–2.67 (m, [9 H], CH_3 at C-6 + $H_2C-N(CH_2)_2-$); 3.01–3.13 (m, [4 H], H of piperazine); 3.61–3.77 (t, [2 H], Hα of butyl); 3.85 and 4.13 (2s, [6 H], 2 × OCH_3); 6.91 (s (broad), [4 H], arom. H); 7.17 (s, [1 H], 7-H).
^{1}H NMR of **13**: δ = 1.38–1.80 (m, [7 H], Hβ, γ of butyl + CH_2CH_3, J = 7,3 Hz); 2.34–2.68 (m, [9 H], CH_3 at C-6 + $H_2C-N(CH_2)_2-$); 3.02–3.14 (m, [4 H], H of piperazine); 3.60–3.77 (t, [2 H], Hα of propyl); 3.85 (s, [3 H], OCH_3); 4.47–4.74 (q, [2 H], J = 7,3 Hz, CH_2-CH_3); 6.92 (s, (broad)), [4 H], arom. H); 7.15 (s, [1 H], 7-H).
^{1}H NMR of **14** : δ = 1.59–1.65 (m, [4 H], Hβ, γ of butyl); 2.32–2.73 (m, [9 H], CH_3 at C-6 + $H_2C-N(CH_2)_2-$); 3.61–4.04 (m, [6 H], Hα of butyl + H of piperazine); 4.13 (s, [3 H], OCH_3); 6.41–6.53 (t, [1 H] and 8.26–8.41 (d, [2 H], J = 4,4 Hz, H of pyrimidine); 7.18 (s, [1 H], 7-H).
^{1}H NMR of **15**: δ = 1.63–1.7 (m, [4 H], Hβ, γ of butyl); 2.44–2.87 (m, [9 H], CH_3 at C-6 + Hδ of butyl + H of tetrahydroisoquinoline); 3.59–3.77 (m, [4 H], Hα of butyl + H of tetrahydroisoquinoline); 4.12 (s, [3 H], OCH_3); 7.06–7.15 (m, [5 H], arom. H + 7-H).

4.2. Pharmacology

4.2.1. Materials and methods

Substances used: Thiopental sodium (HEFA-FRENON Arzneimittel, Germany), phenylbenzoquinone (INC Pharmaceuticals, Inc. N.Y.).
The experiments were carried out on male Wistar rats (body weight 180–250 g) and male Albino-Swiss mice (body weight 18–26 g). Animals were housed in constant temperature facilities exposed to a 12 : 12 h light-dark cycle and maintained on a standard pellet diet and tap water was given *ad libitum*. Control and experimental groups consisted of 6–8 animals each. The compounds investigated were administered intraperitoneally as a suspension in 0.5% methylcellulose at a constant volume of 10 ml/kg (mice) and 1 ml/kg (rats). For compounds with LD > 2000 mg/kg 2000 mg/kg was taken to be the initial dose.
The statistical significance was calculated using a one-way ANOVA or Student's t-test.

4.2.2. Acute toxicity

The compounds investigated were injected intraperitoneally in increasing doses from 200 to 2000 mg/kg. Each dose was given to 6 animals. The number of dead mice was assessed 24 h after the injection. LD_{50} values were calculated according to the method of Litchfield and Wilcoxon [6].

4.2.3. Locomotor activity

The compounds investigated were injected intraperitoneally in doses equivalent to 1/80–1/20 LD_{50}. Thirty minutes later the mice were placed in cages with a photocell and the numbers of movements of the animals during the first 30 min were recorded.

4.2.4. Thiopental anesthesia

Thiopental in a narcotic dose (55 mg/kg) was injected intraperitoneally 30 min after administration of the tested compounds. Duration of narcotic sleep was taken as being from disappearance to return of the righting reflex.

4.2.5. "Writhing syndrome" test

The substances were tested in mice according to Hendershot and Forsaith [7]. The compounds investigated were administered intraperitoneally in doses corresponding to 1/640–1/40 LD_{50}.
Twenty five minutes later, 0.2% phenylbenzoquinone was injected intraperitoneally in a constant volume of 0.25 ml. Five minutes after injection of the irritating agent, the number of "writhing" episodes during a 10 min period was counted. Analgesic activity was expressed by the following formula:

$$100 - \frac{\sum \text{of writhing incidents in experimental group}}{\sum \text{of writhing incidents in control group}} \times 100 = \% \text{ inhibition}$$

The ED_{50} values and their confidence limits were calculated according to the method of Litchfield and Wilcoxon [6].

4.2.6. "Hot plate" test

The compounds were tested in mice according to Eddy and Leimbach [8]. Animals were placed individually on the metal plate heated to 55–56 °C. The time (s) of appearance of the pain reaction (licking or jumping) was recorded by a stop-watch. The experiments were performed 30 min after administration of the compounds investigated.

4.2.7. Influence on blood pressure

Arterial blood pressure in the common carotic artery of normotensive anesthetized rats was measured using a Datamax apparatus (Columbus Instruments). The compounds investigated were injected intraperitoneally in doses corresponding to ED_{50}. The effect on blood pressure was monitored for 1 h.

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