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Synthesis and pharmacological screening of some *N*-(4-substituted-piperazin-1-ylalkyl)-3,4-pyrroledicarboximides

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Abstract

As an extension of our previous work we describe the synthesis and pharmacological investigation of a new series of derivatives of pyrrole-3,4-dicarboximide possessing the 4-substituted-piperazin-1-ylalkyl group linked to the imide nitrogen. The products were evaluated for acute toxicity, and effectiveness in a series of CNS and arterial blood pressure tests. The preliminary pharmacological screening was determined in animal models. Several compounds demonstrated moderate to high analgesic activity in the 'writhing syndrome' test (**5f**-1/640 LD_{50}). Some of the structure–activity relationships are also discussed.

Keywords: 3,4-Pyrroledicarboximide derivatives; Analgesic activity

1. Introduction

N-[4-Aryl(heteroaryl)piperazin-1-ylalkyl] cyclic imides or heterocyclic systems produce a variety of pharmacological effects on the central nervous system (CNS) and behavioural responses which result from action on different receptor systems. Most of them are characterized by antianxiety activity and belong to the class of structural analogues of buspirone (Fig. 1) [1–3], the well-known nonbenzodiazepine anxiolytic drug [4]. Additionally, such compounds were also considered as analogues of antidepressive trazodone (Fig. 1, [4]) [5–7], atypical antipsychotics [8–12], neuroleptics [13] and analgesic [14,15], antihypertensive [16] or anorectic [17] agents.

In our previous papers [18,19] we described a series of derivatives of *N*-substituted pyrrole-3,4-dicarboximide with general structure **I** (Fig. 2). The compounds were characterized by the presence of an aromatic or aliphatic (*n*-butyl) substituent on the nitrogen atom of the pyrrole ring (R, Fig. 2) and of the *N*-[4-aryl-(heteroaryl)piperazin-1-ylalkyl] group. The pharmacological effectiveness of these compounds was defined with the use of behavioural tests in animal models (rats and mice). The pyrroledicarboximides **I** (Fig. 2) were found to produce a general depressive action on the CNS, whereas no anxiolytic, antidepressant, anticonvulsant or analgesic activity was shown [18,19]. For example, the most active compound (Fig. 2: $n = 0$, $R = n\text{-}C_4H_9$, $X = CH$, $Y = C–Cl$) inhibited spontaneous locomotor activity and decreased body temperature in mice up to a dosage of 1/80 LD_{50}. Furthermore, among the compounds with the two-carbon central chain, *N*-(4-phenylpiperazine) derivative (Fig. 2: $n = 0$, $R = n\text{-}C_4H_9$, $X = Y = CH$) and its *o*-chloro analogue (Fig. 2: $n = 0$, $R = n\text{-}C_4H_9$, $X = CH$, $Y = C–Cl$) were more active as depressant agents than their pyrido- or pyrimidopiperazine analogues ($X = N$, $Y = CH$ or $X = Y = N$).

Taking into account the pharmacological properties of compounds **I** (Fig. 2), we prepared a new series of pyrrole-3,4-dicarboximides related to series **I** for a screening in a series of CNS and arterial blood pressure tests. All compounds prepared for this study (**3**–**5**), bearing different combinations of substituents R and R_1, have been listed in Table 1.

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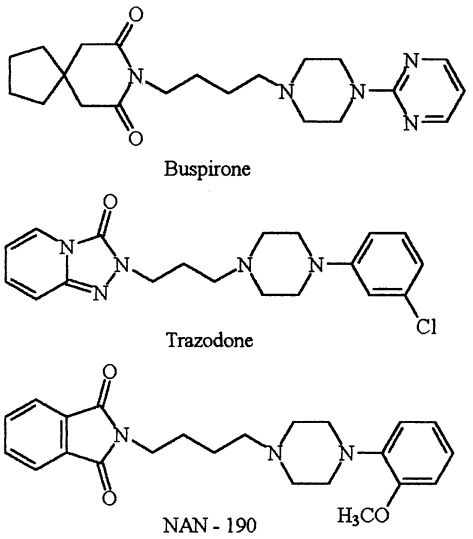


Fig. 1.

Compounds **3** were related to the most active agent of series **I** (Fig. 2: $n = 0$, R = n-C_4H_9, X = CH, Y = C–Cl) [19] with regard to the length of the central chain; they are characterized by the presence in their structure of the 2-[4-(o-CH_3O- or o-Cl-phenyl)piperazin-1-yl]ethyl fragment and a different substitution of the pyrrole nitrogen atom. Also prepared for the biological study were pyrroledicarboximides possessing the 3-[4-(m-Cl- or m-CF_3-phenyl)piperazin-1-yl]propyl fragment (**4**; Table 1) or the 4-[4-(o-CH_3O-phenyl)piperazin-1-yl]butyl fragment (**5e**,**f**; Table 1). These side chains, are similar to the partial structure of trazodone (Fig. 1), a non-tricyclic antidepressant agent [4] or NAN-190 (Fig. 1), which may be regarded as a well-recognized antagonist of postsynaptic receptors 5-HT_{1A} [20], respectively. Finally we prepared a series of buspirone-like (Fig. 1) pyrroledicarboximides **5a**,**c**,**d** (Table 1) which differ from one another with respect to their substitution at the nitrogen atom of the pyrrole ring. Additionally, compounds **5a**,**c**,**d**, like **5b** and **3d** enabled us to examine the influence of the heteroaromatic substituent of the pyrrole ring on the profile of the CNS activity of such derivatives.

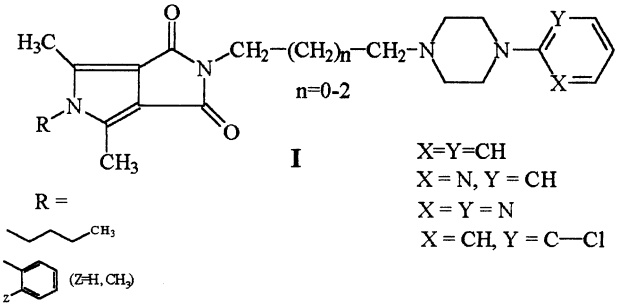
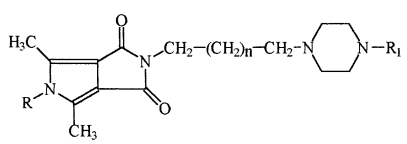


Fig. 2.

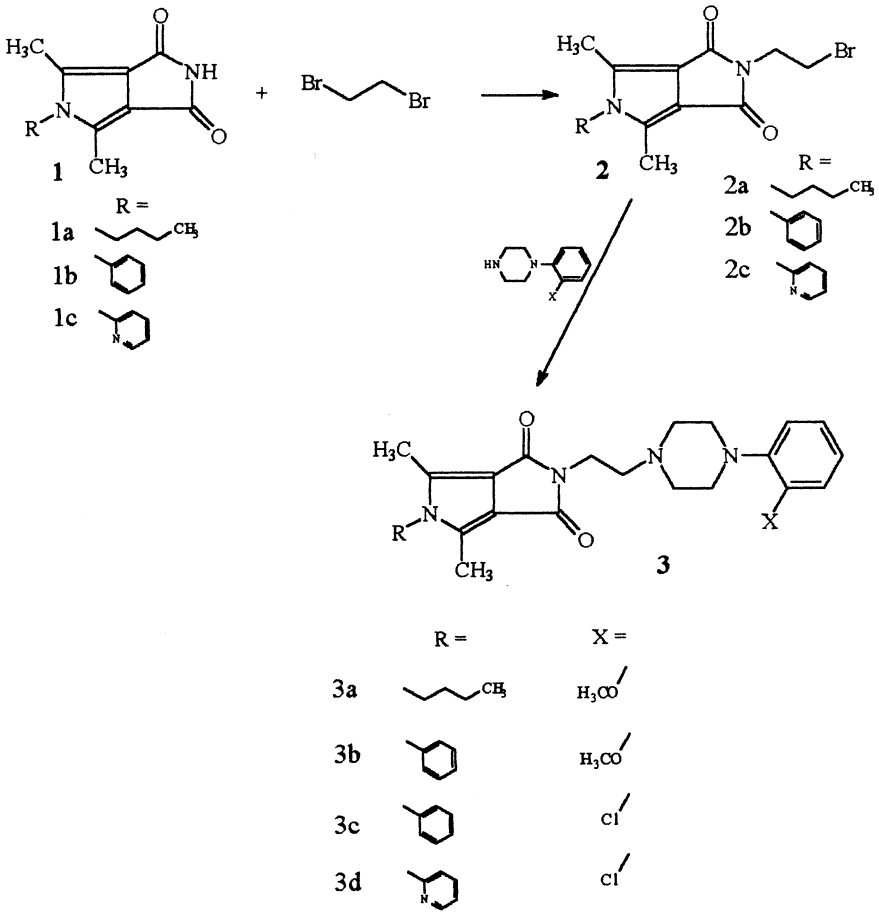
2. Chemistry

The methods used for the synthesis of compounds **3**–**5** (Table 1) were closely related to general procedures that we had already employed to prepare derivatives of pyrroledicarboximides of type **I** (Fig. 1) [18,19]. The key intermediates pyrroledicarboximides **1a**–**e** and **2a**–**e** (Schemes 1–3) were synthesized in five or six steps,

Table 1
Physical data of compounds **3**–**5**



Comp.	R	R_1	n	Mp (°C), crystallization solvent	Yield (%)	Formula (MW)	Log P_{calc}
3a	n-C_4H_9	o-CH_3O–C_6H_4	0	115–117, n-heptane	60	$C_{25}H_{34}N_4O_3$ (438.55)	3.64
3b	C_6H_5	o-CH_3O–C_6H_4	0	126–128, n-heptane	47	$C_{27}H_{30}N_4O_3$ (458.55)	
3c	C_6H_5	o-Cl–C_6H_4	0	141–143, EtOH	57	$C_{26}H_{27}ClN_4O_2$ (462.97)	4.88
3d	2-C_5H_4N	o-Cl–C_6H_4	0	45–48, EtOH	70	$C_{25}H_{26}ClN_5O_2$ (463.96)	
4a	n-C_4H_9	m-Cl–C_6H_4	1	125–127, methanol	47	$C_{25}H_{33}ClN_4O_2$ (457.01)	4.46
4b	C_6H_5	m-Cl–C_6H_4	1	117–119, methanol	65	$C_{27}H_{29}ClN_4O_2$ (477.00)	
4c	n-C_4H_9	m-CF_3–C_6H_4	1	83–85, n-heptane	21	$C_{26}H_{33}F_3N_4O_2$ (490.56)	
4d	C_6H_5	m-CF_3–C_6H_4	1	60–62, n-hexane	26	$C_{28}H_{29}F_3N_4O_2$ (510.55)	
5a	2-Pyridine	2-Pyrimidine	2	148–150, H_2O+EtOH	66	$C_{25}H_{29}N_7O_2$ (459.55)	2.80
5b	2-Pyridine	2-Pyridine	2	114–116, n-heptane	42	$C_{26}H_{30}N_6O_2$ (458.56)	3.64
5c	3-Pyridine	2-Pyrimidine	2	144–146, H_2O+EtOH	65	$C_{25}H_{29}N_7O_2$ (459.55)	2.10
5d	2-Thiazole	2-Pyrimidine	2	128–130, H_2O+EtOH	69	$C_{23}H_{27}N_7O_2S$ (465.57)	2.55
5e	n-C_4H_9	o-CH_3O–C_6H_4	2	71–73, n-hexane	54	$C_{27}H_{38}N_4O_3$ (466.62)	3.42
5f	C_6H_5	o-CH_3O–C_6H_4	2	132–134, cyclohexane	80	$C_{29}H_{34}N_4O_3$ (486.61)	3.89



Scheme 1.

according to known procedures [18,19,21]. Final compounds **3**–**5** were prepared from reagents **1** or **2** in different ways, depending on the length of the central alkanyl chain, using the methods shown in Schemes 1–3.

Compounds **3**, with the ethylene central chain (Scheme 1), were obtained by alkylation of the appropriate *N*-substituted piperazines (commercially available) with *N*-(2-bromoethyl)pyrroledicarboximides **2a**–**c** in yield ranging from 47 to 70%. The alkylation was conducted in refluxing acetonitrile in the presence of anhydrous K_2CO_3. The preparation of the *N*-(2-bromoethyl)imides **2a**,**b** was accomplished as already reported [18,19], starting from the corresponding pyrroledicarboximides **1** and 1,2-dibromoethane (Scheme 1). In the same way **2c** was also prepared. Target compounds **4a**,**b**,**d** with the propylene bridge (Scheme 2) were analogously obtained, starting from the chloropropylpyrroledicarboximides **2d**,**e**, obtained by the reaction of **1a**,**b** with 1-bromo-3-chloropropane [18,19]. Condensation of the substrates **2d**,**e** with twofold excess of the corresponding piperazines gave the expected compounds **4a**,**b**,**d** (Scheme 2, Method A). The compound bearing the *m*-CF_3-substituent (**4d**) was formed in low yield (about 24%). Synthesis of compounds **4b**,**c**,**d** through an alternative route, i.e. by reaction of the imides **1a**,**b** with the chloropropylpiperazines **6a**,**b** (Scheme 2, Method B), did not improve the yield of the products **4** (see Section 3).

Compounds **5** with the central butyl chain were obtained by reaction of the intermediates **1a**–**e** with 8-substituted-8-aza-5-azoniaspiro[4,5]decane bromides, represented by the structures **7**, in xylene solution (40–80% yield; Scheme 3). This one-step procedure was successfully used in our previous work where the synthesis of pyrroledicarboximides **I** with the central butyl chain was reported (Fig. 2, $n = 2$) [18,19]. The spirodecane bromides **7a**,**b** were obtained from the corresponding *N*-substituted piperazines and 1,4-dibromobutane according to the procedure described in our previous paper [18]. Synthesis of the new compound **7c** was achieved via a similar procedure (see Section 3).

The details of the preparation of the undescribed intermediates **2c**, **6b** and **7c** as well as the general description of the synthesis of the final compounds **3**–**5** are reported in Section 3. The analytical data (elemental analyses, IR and ^{1}H NMR spectra) of the new intermediates and compounds **3**–**5** are in agreement

with the assigned structures. IR data showed coupled imidic carbonyl bands at ca. 1750 and 1700 cm^{-1}, typical for five-membered imides. The ^1H NMR spectra revealed (from high to low field) signals consistent with alkyl protons of the central chain and of the piperazine ring, the signal of the methyl groups of the pyrrole ring (singlet, 6H) and the signals of the aromatic (heteroaromatic) protons (see Section 3). Table 1 summarizes the physical data of the pyrroledicarboximides **3**–**5**.

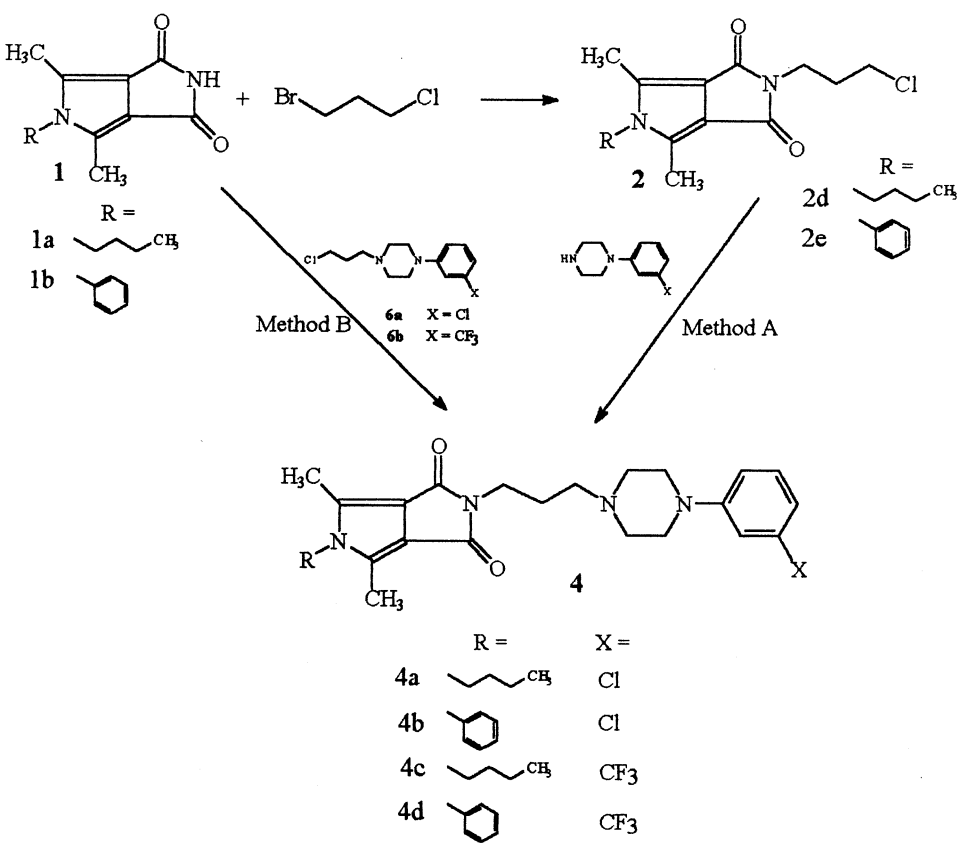
Finally, for each compound tested in the preliminary pharmacological screening (**3a**,**c**, **4a** and **5a**–**f**) the log of the octanol–water partition coefficient was calculated (log P_{calc}, Table 1). The log P values were calculated for the free bases, using the ChemPlus program from Hypercube, Inc., IBM PC version.

3. Chemical experimental

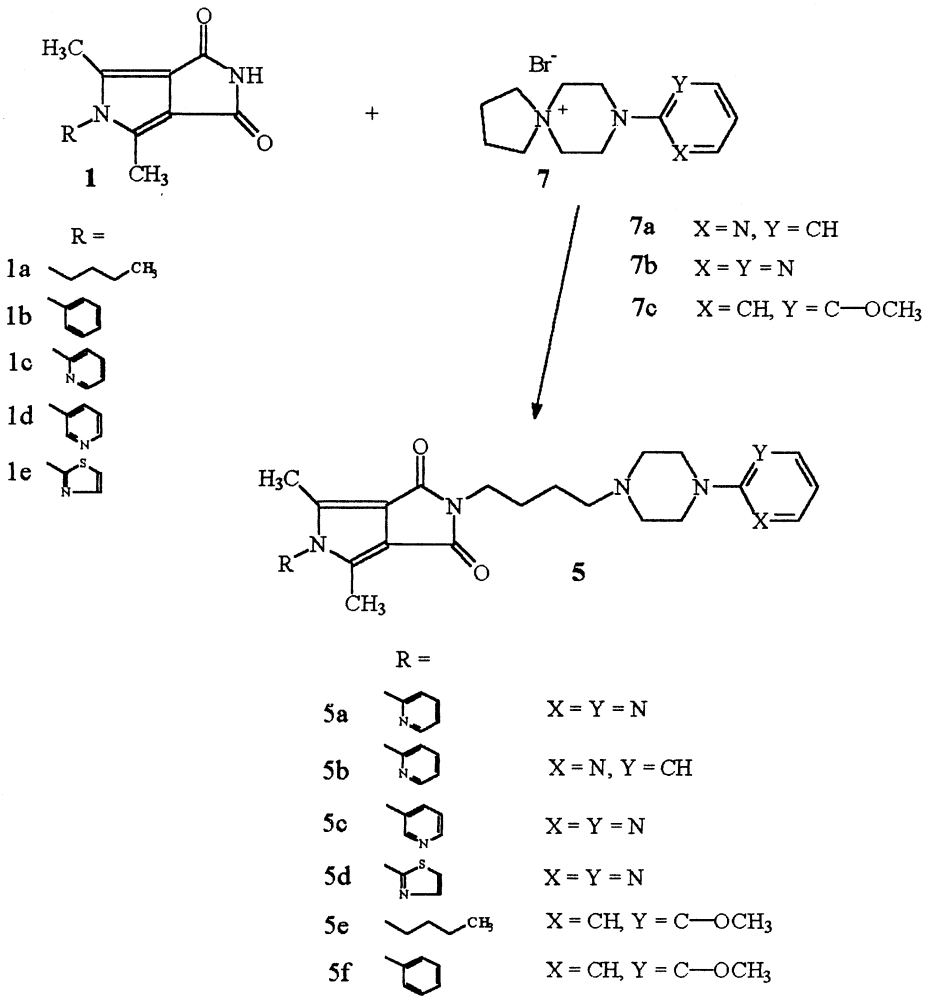
Melting points are uncorrected. ^1H NMR spectra were obtained with an 80 MHz Tesla spectrometer in $CDCl_3$; the chemical shifts are reported in δ (ppm). IR spectra were recorded on a Specord-75 IR spectrometer. Elemental C,H,N analyses were run on a Carlo Erba NA-1500 analyser. All the results of the C, H, and N determinations were within $\pm 0.4\%$ of the values calculated for the corresponding formulae. Chromatographic separations were performed on a silica gel column (Kieselgel 60 (70–230 mesh), Merck). Analytical thin-layer chromatography was carried out on Merck silica gel-60F_{254}, supported on alufolien and examined by UV light.

*3.1. N-(2-Bromoethyl)-2,5-dimethyl-1-(2-pyridyl)-3,4-pyrroledicarboximide (**2c**)*

A mixture of 1.05 g (0.005 mol) of pyrroledicarboximide **1c** [21], 0.7 g (0.005 mol) of anhydrous K_2CO_3 and 2.9 g (0.015 mol) of 1,2-dibromoethane in acetonitrile was refluxed with stirring for 10 h. The reaction mixture was then filtered and evaporated. The resulting crude **2c** was purified by crystallization from cyclohexane to give 1.25 g (72% yield, m.p. 141–143°C) of the product. *Anal.*: $C_{15}H_{14}BrN_3O_2$ (348.19) (C,H,N); ^1H NMR: 2.25 (s, 6H, $2CH_3$), 3.37–3.67 (m, 4H, $2CH_2$), 7.24–7.55 (m, 2H, $2H_\beta$), 7.85–8.06 (m, 1H, H_γ), 8.61–8.76 (m, 1H, H_α).



Scheme 2.



Scheme 3.

3.2. *N*-{2-[4-(*o*-Chloro or *o*-methoxyphenyl)piperazin-1-yl]ethyl}-1-substituted-2,5-dimethylpyrrole-3,4-dicarboximides (**3a–d**)

N-(2-Bromoethyl)imide (**2**, 0.01 mol), 1.4 g (0.01 mol) of anhydrous K_2CO_3 and 0.01 mol of the corresponding *N*-substituted piperazine in acetonitrile were refluxed with stirring for 15 h; **3a** and **3b** were obtained from *N*-(*o*-methoxyphenyl)piperazine and **2a** [18] or **2b** [19], respectively; *N*-(*o*-chlorophenyl)piperazine and **2b** or **2c** gave **3c** and **3d**, respectively. The hot reaction mixture was filtered, purified with charcoal and evaporated to dryness. The oily residue was crystallized to give the products **3a–c** (Table 1), whereas the purification of **3d** was achieved by column chromatography, eluting with EtOAc ($R_f = 0.58$). Further purification of **3d** was obtained by crystallization (Table 1).

3a ^{1}H NMR: 0.87–1.05 (m, 3H, CH_3), 1.3–1.85 (m, 4H, $2CH_2$), 2.38 (s, 6H, $2CH_3$), 2.55–2.8 (m, 6H, $N(CH_2)_3$), 2.9–3.15 (m, 4H, Ar–$N(CH_2)_2$), 3.35–3.95 (m, 7H, $N_{pyrrole}$–$CH_2 + N_{imide}$–$CH_2 + OCH_3$), 6.8–7.15 (m, 4H, ArH).

3b ^{1}H NMR: 2.17 (s, 6H, $2CH_3$), 2.47–2.85 (m, 6H, $N(CH_2)_3$), 3.06–3.32 (m, 4H, Ar–$N(CH_2)_2$), 3.75 (t, 2H, $N_{imide}CH_2$, $J = 6.8$ Hz), 3.86 (s, 3H, OCH_3), 6.8–7.6 (m, 9H, ArH).

3c ^{1}H NMR: 2.17 (s, 6H, $2CH_3$), 2.69–2.83 (m, 6H, $N(CH_2)_3$), 3.0–3.15 (m, 4H, Ar–$N(CH_2)_2$), 3.75 (t, 2H, $N_{imide}CH_2$, $J = 6.8$ Hz), 6.9–7.6 (m, 9H, ArH).

3d ^{1}H NMR: 2.26 (s, 6H, $2CH_3$), 2.58–2.77 (m, 6H, $N(CH_2)_3$), 2.97–3.13 (m, 4H, Ar–$N(CH_2)_2$), 3.75 (t, 2H, $N_{imide}CH_2$, $J = 6.8$ Hz), 6.81–7.53 (m, 6H, 4ArH + $2H_\beta$), 7.84–8.06 (m, 1H, H_γ), 8.61–8.71 (m, 1H, H_α).

3.3. *N*-{3-[4-(*m*-Chloro or *m*-trifluoromethylphenyl)-piperazin-1-yl]propyl}-1-substituted-2,5-dimethylpyrrole-3,4-dicarboximides (**4a–d**)

3.3.1. Method A (compounds **4a,b,d**)

A solution of 0.01 mol of *N*-(3-chloropropyl)imide **2d(e)** and 0.02 mol of *N*-(*m*-chloro or *m*-trifluoro-

methylphenyl)piperazine in xylene was refluxed for 25 h. In this way **4a**, **4b** and **4d** were obtained from *N*-(*m*-chlorophenyl)piperazine and **2d** [18], *N*-(*m*-chlorophenyl)piperazine and **2e** [19], *N*-(*m*-trifluoromethylphenyl)piperazine and **2e**, respectively. After filtration the solvent was evaporated to dryness and the residue was purified. Pure **4a** was obtained after crystallization from methanol, whereas **4b** and **4d** were purified with column chromatography, eluting with EtOAc (**4b**: $R_f = 0.73$, 42% yield; **4d**: $R_f = 0.61$, 26% yield). Further purification of **4b** and **4d** was accomplished by crystallization from the appropriate solvent (Table 1).

3.3.2. Method B (compounds *4b–d*)

1-(3-Chloropropyl)-4-(*m*-Cl or *m*-CF_3-phenyl)piperazine **6** (0.015 mol) was added to a stirred solution of 0.01 mol of pyrroledicarboximide **1a** [21] or **1b** [21] and 0.01 mol of sodium hydride (60% dispersion in mineral oil) in anhydrous DMF. In this way **4b–d** were obtained from 1-(3-chloropropyl)-4-(*m*-chlorophenyl)piperazine (**6a**) and **1b**, 1-(3-chloropropyl)-4-(*m*-trifluoromethylphenyl)piperazine (**6b**) and **1a**, **6b** and **1b**, respectively. The reaction mixture was refluxed for 5 h and after cooling was evaporated to dryness. The residue was treated with 5% HCl (50 ml) and charcoal, filtered and alkalized with Na_2CO_3 solution. The mixture was filtered to separate crude product **4**. The compound **4b** was purified by crystallization (Table 1), whereas **4c** and **4d** were isolated by column chromatography, eluting with EtOAc (**4c**: $R_f = 0.56$, 21% yield; **4d**: $R_f = 0.61$, 24% yield). Further purification of **4c** and **4d** was achieved by crystallization from the appropriate solvent (Table 1).

4a ^{1}H NMR: 0.87–1.05 (m, 3H, CH_3), 1.17–1.67 (m, 4H, $2CH_2$), 1.84 (t, 2H, CH_2, $J = 6.8$ Hz), 2.37 (s, 6H, $2CH_3$), 2.5–2.64 (m, 6H, $N(CH_2)_3$), 3.08–3.20 (m, 4H, $Ar–N(CH_2)_2$), 3.61–3.87 (m, 4H, $N_{pyrrole}CH_2 + N_{imide}CH_2$), 6.75–7.25 (m, 4H, ArH).

4b ^{1}H NMR: 1.85 (t, 2H, CH_2, $J = 6.8$ Hz), 2.15 (s, 6H, $2CH_3$), 2.42–2.62 (m, 6H, $N(CH_2)_3$), 3.05–3.2 (m, 4H, $Ar–N(CH_2)_2$), 3.65 (t, 2H, $N_{imide}CH_2$, $J = 6.8$ Hz), 6.67–6.84 (m, 3H, ArH), 7.02–7.17 (m, 3H, ArH), 7.45–7.55 (m, 3H, ArH).

4c ^{1}H NMR: 0.86–1.01 (m, 3H, CH_3), 1.17–1.67 (m, 4H, $2CH_2$), 1.82 (t, 2H, CH_2, $J = 6.8$ Hz), 2.37 (s, 6H, $2CH_3$), 2.43–2.61 (m, 6H, $N(CH_2)_3$), 3.06–3.22 (m, 4H, $Ar–N(CH_2)_2$), 3.50–3.78 (m, 4H, $N_{pyrrole}CH_2 + N_{imide}CH_2$), 6.96–7.09 (m, 3H, ArH), 7.18–7.34 (m, 1H, ArH).

4d ^{1}H NMR: 1.85 (t, 2H, CH_2, $J = 6.8$ Hz), 2.15 (s, 6H, $2CH_3$), 2.41–2.62 (m, 6H, $N(CH_2)_3$), 3.07–3.21 (m, 4H, $Ar–N(CH_2)_2$), 3.67 (t, 2H, $N_{imide}CH_2$, $J = 6.8$ Hz), 6.97–7.22 (m, 6H, ArH), 7.37–7.55 (m, 3H, ArH).

3.4. *N*-[4-(4-Substituted-piperazin-1-yl)butyl]-1-substituted-2,5-dimethylpyrrole-3,4-dicarboximides (*5a–f*)

A mixture of 0.01 mol of pyrroledicarboximide **1a–e** (Scheme 3), 0.011 mol of spiro[4,5]decane bromide **7a–c** and 1.7 g (0.012 mol) of anhydrous K_2CO_3 in xylene was refluxed with stirring for 20 h. In this way **5a–f** were obtained from **1c** [21] and **7b** [18], **1c** and **7a** [18], **1d** [21] and **7b**, **1e** [21] and **7b**, **1a** [21] and **7c**, **1b** [21] and **7c**, respectively. The hot reaction mixture was filtered and the solvent was evaporated to dryness. The resinous residue was dissolved in an excess of ethyl ether, the solution was treated with charcoal, filtered and the solvent was removed. The residue was crystallized from the solvent indicated in Table 1 to give **5a** and **5c–f**; **5b** was purified by column chromatography, eluting with EtOAc–methanol (7:3); $R_f = 0.73$. Further purification of **5b** was achieved by crystallization (Table 1).

5a ^{1}H NMR: 1.42–1.75 (m, 4H, $2CH_2$), 2.26 (s, 6H, $2CH_3$), 2.34–2.55 (m, 6H, $N(CH_2)_3$), 3.61 (t, 2H, $N_{imide}CH_2$, $J = 6.8$ Hz); 3.84 (t, 4H, $Ar–N(CH_2)_2$, $J = 4.9$ Hz), 6.44 (t, 1H, 5-$H_{pyrimidine}$, $J = 4.8$ Hz), 7.2–7.5 (m, 2H, $2H_\beta$), 7.84–8.04 (m, 1H, H_γ), 8.3 (d, 2H, 4- and 6-$H_{pyrimidine}$, $J = 4.8$ Hz), 8.62–8.68 (m, 1H, H_α).

5b ^{1}H NMR: 1.52–1.75 (m, 4H, $2CH_2$), 2.26 (s, 6H, $2CH_3$), 2.44–2.62 (m, 6H, $N(CH_2)_3$), 3.47–3.87 (m, 6H, $Ar–N(CH_2)_2 + N_{imide}CH_2$), 6.52–6.67 (m, 2H, $2H_\beta$), 7.21–7.54 (m, 3H, $2H_\beta + H_\gamma$), 7.84–8.06 (m, 1H, H_γ), 8.12–8.2 (m, 1H, H_α), 8.64–8.71 (m, 1H, H_α).

5c ^{1}H NMR: 1.54–1.75 (m, 4H, $2CH_2$), 2.18 (s, 6H, $2CH_3$), 2.32–2.56 (m, 6H, $N(CH_2)_3$), 3.61 (t, 2H, $N_{imide}CH_2$, $J = 6.8$ Hz), 3.84 (t, 4H, $Ar–N(CH_2)_2$, $J = 4.9$ Hz), 6.47 (t, 1H, 5-$H_{pyrimidine}$, $J = 4.88$ Hz), 7.55–7.61 (m, 1H, H_β), 7.95–8.07 (m, 1H, H_γ), 8.29 (d, 2H, 4- and 6-$H_{pyrimidine}$, $J = 4.88$ Hz), 8.5–8.62 (m, 1H, H_α), 8.7–8.82 (m, 1H, H_α).

5d ^{1}H NMR: 1.52–1.72 (m, 4H, $2CH_2$), 2.34 (s, 6H, $2CH_3$), 2.43–2.56 (m, 6H, $N(CH_2)_3$), 3.6 (t, 2H, $N_{imide}CH_2$, $J = 6.8$ Hz), 3.83 (t, 4H, $Ar–N(CH_2)_2$, $J = 4.9$ Hz), 6.47 (t, 1H, 5-$H_{pyrimidine}$, $J = 4.9$ Hz), 7.55 (d, 1H, $J = 3.8$ Hz), 7.85 (d, 1H, $J = 3.8$ Hz), 8.29 (d, 2H, 4- and 6-$H_{pyrimidine}$, $J = 4.9$ Hz).

5e ^{1}H NMR: 0.87–1.02 (m, 3H, CH_3), 1.23–1.71 (m, 8H, $4CH_2$), 2.37–2.45 (m, 8H, $2CH_3 + N_{piperazine}CH_2$), 2.54–2.67 (m, 4H, $N(CH_2)_2$), 2.97–3.13 (m, 4H, $Ar–N(CH_2)_2$), 3.42–3.92 (m, 7H, $N_{imide}CH_2 + N_{pyrrole}CH_2 + OCH_3$), 6.84–7.00 (m, 4H, ArH).

5f ^{1}H NMR: 1.5–1.8 (m, 4H, $2CH_2$), 2.17 (s, 6H, $2CH_3$), 2.38–2.52 (m, 2H, $N_{piperazine}CH_2$), 2.59–2.79 (m, 4H, $N(CH_2)_2$), 2.95–3.24 (m, 4H, $Ar–N(CH_2)_2$), 3.63 (t, 2H, $N_{imide}CH_2$, $J = 6.8$ Hz), 3.86 (s, 3H, OCH_3), 6.76–6.97 (m, 4H, ArH), 7.14–7.29 (m, 2H, ArH), 7.46–7.59 (m, 3H, ArH).

3.5. 4-(m-Chloro or m-trifluoromethylphenyl)-1-(3-chloropropyl)piperazine (**6a**,**b**)

Compounds **6a** and **6b** were prepared according to the procedure described in Ref. [22], starting from 1-bromo-3-chloropropane and *N*-(*m*-chlorophenyl) piperazine or *N*-(*m*-trifluoromethylphenyl)piperazine, respectively. The crude products were purified by column chromatography, eluting with benzene–EtOAc (2:1), to obtain **6a** ([22], $R_f = 0.55$, yellow oil, 74% yield) or benzene–$CHCl_3$–methanol (2:2:1) to obtain **6b** ($R_f = 0.88$, yellow oil, 57% yield). These compounds were used in the subsequent reaction (Scheme 2) without any further purification.

6b ^{1}H NMR: 1.93 (t, 2H, CH_2, $J = 6.5$ Hz), 2.42–2.6 (m, 6H, $N(CH_2)_3$), 3.2 (t, 4H, Ar–$N(CH_2)_2$, $J = 4.9$ Hz), 3.59 (t, 2H, CH_2–Cl, $J = 6.5$ Hz), 6.98–7.41 (m, 4H, ArH).

3.6. 8-(o-Methoxyphenyl)-8-aza-5-azoniaspiro[4,5]-decane bromide (**7c**)

A mixture of 3.84 g (0.02 mol) of *N*-(2-methoxyphenyl)piperazine, 4.32 g (0.02 mol) of 1,4-dibromobutane and 5.53 g (0.04 mol) of anhydrous K_2CO_3 in 50 ml of 95% ethanol was refluxed with stirring for 24 h. After cooling the reaction mixture was filtered and evaporated to dryness. The oily residue was dissolved in chloroform (100 ml), dried over anhydrous $MgSO_4$, filtered and evaporated to dryness. The residue was treated with THF and the mixture was filtered to separate 5.4 g of **7c** as solid (m.p. 169–173°C, 79% yield). The analytical sample of **7c** was obtained after crystallization from acetone (m.p. 173–176°C). *Anal.*: $C_{16}H_{27}BrN_2O$ (343.13) (C,H,N); ^{1}H NMR: 2.27–2.52 (m, 4H, $2CH_2$), 3.33–3.54 (m, 4H, Ar–$N(CH_2)_2$), 3.8–4.08 (m, 11H, $N^+(CH_2)_4 + OCH_3$), 6.82–7.14 (m, 4H, ArH).

4. Pharmacological experimental

4.1. Material 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). Compounds were administered intraperitoneally (i.p.) as a suspension in 3% Tween 80 in a volume of 10 ml/kg in mice and 5 ml/kg in rats. The compounds were administered in doses equivalent to 1/10, 1/20, 1/40, 1/80, 1/160, 1/320, 1/640 or 1/1280 of LD_{50} (lethal dose-50, the dose that caused the death of 50% of animals). 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.

(1) Acute toxicity was assessed by the methods of Litchfield and Wilcoxon [23] and presented as LD_{50}, and the confidence limit calculated from the mortality of mice 24 h after the injection (Table 2).

(2) Motor coordination was measured according to the method of Gross et al. [24]. 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 compounds (Table 3).

(3) Spontaneous locomotor activity in mice was measured in circular photoresistor actometers (32 cm in diameter). After the injection of the investigated compounds, animals were placed in the actometers for 1 h. Each crossing of the light beam was recorded automatically. The amount of impulses was recorded after 30 and 60 min (Table 4).

(4) Amphetamine hyperactivity in mice was induced by D,L-amphetamine (2.5 mg/kg s.c.). Investigated compounds were injected 30 min before amphetamine. The locomotor hyperactivity was measured 30 and 60 min later in the photoresistor actometers (Table 5).

(5) Pain reactivity was measured by the 'writhing syndrome' test of Koster et al. [25]. The test was performed in mice by i.p. injection of a 0.6% solution of acetic acid in a volume of 10 ml/kg 60 min after the

Table 2
Acute toxicity ($n = 8$)[a]

Compound	LD_{50} (mg/kg i.p.)	Confidence limit
3a	386.9	240.4–622.5
3c	>2000.0	
4a	1345.3	1047.9–1727.1
5a	252.0	130.0–487.0
5b	69.5	52.8–92.6
5c	151.0	78.0–293.0
5d	1020.0	629.0–1655.0
5e	133.4	69.1–257.6
5f	383.2	277.9–528.4

[a] The LD_{50} values and confidence limits were calculated by the method of Litchfield and Wilcoxon [23].

Table 3
Influence of the compounds on motor coordination in the rota-rod test (4 rpm) in mice ($n = 8$)

Comp.	Dose (fraction of LD_{50})	Number of animals holding on rota-rod at time (min)						
		15	30	45	60	75	90	105
Control	–	8/8	8/8	8/8	8/8	8/8	8/8	8/8
3a	1/10	0/8***	0/8***	1/8***	1/8***	1/8***	1/8***	1/8***
	1/20	2/8***	3/8**	2/8***	2/8***	2/8***	2/8***	2/8***
	1/40	7/8	8/8	8/8	8/8	8/8	8/8	8/8
3c	1/10	8/8	8/8	6/8	7/8	8/8	7/8	7/8
4a	1/10	8/8	8/8	8/8	8/8	8/8	8/8	8/8
5a	1/10	5/8	6/8	8/8	8/8	8/8	8/8	8/8
5b	1/10	1/8***	2/8**	4/8*	4/8*	4/8*	5/8	5/8
	1/20	6/8	7/8	8/8	7/8	8/8	8/8	8/8
5c	1/10	7/8	6/8	7/8	6/8	8/8	7/8	7/8
5d	1/10	5/8	6/8	6/8	7/8	8/8	7/8	8/8
5e	1/10	8/8	8/8	8/8	8/8	8/8	8/8	8/8
5f	1/10	5/8	6/8	4/8*	3/8**	4/8*	4/8*	3/8**
	1/20	6/8	7/8	7/8	6/8	7/8	6/8	6/8

* $P<0.05$, ** $P<0.01$, *** $P<0.001$; exact Fischer's test.

administration of the compounds. The number of writhing episodes was counted for 30 min (Table 6).

(6) Pain reactivity was also measured in the 'hot plate' test according to the method of Eddy and Leinbach [26]. Animals were placed individually on the metal plate heated to 56°C. The time (s) of appearance of the pain reactivity (licking of the forepaws or jumping) was measured. Experiments were performed 60 min after the administration of the compounds (Table 7).

(7) Anxiolytic properties were assessed by the 'four plate' test in mice, according to Aron et al. [27], 60 min after the administration of the compounds at the doses which had no effect on the spontaneous locomotor activity. Mice were placed in the cages with four plates floor (11 × 7 cm) with 4 mm gape between each. After 15 s of adaptation the number of crossing was determined during 1 min. Each crossing was punished with direct current (180 V, 0.5 A) but no more often than every 3 s.

(8) Pentetrazol seizures in mice were induced by administration of pentetrazol at the dose of 100 mg/kg s.c. 60 min after the investigated compounds. Animals were observed during 30 min and the number of mice developing clonic and tonic seizures as well as mortality were recorded.

(9) 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. [28]. The criterion of the convulsive response was the tonic extension of the hind limbs. The test was performed 60 min after the administration of the compounds.

(10) Head twitch behaviour was induced by the administration of 5-hydroxytryptophan (5-HTP) at the dose of 180 mg/kg i.p. 30 min after the investigated compounds. Animals were observed 60 min after 5-HTP administration [29].

Table 4
Influence of the compounds on the spontaneous locomotor activity in mice ($n = 8$)

Comp.	Dose (fraction of LD_{50})	No. impulses ± SEM after time (min)	
		30	60
Control	–	408.8 ± 52.4	517.0 ± 74.2
3a	1/10	93.4 ± 14.1***	195.2 ± 14.0***
	1/20	119.9 ± 22.1***	213.0 ± 23.2**
	1/40	332.0 ± 60.0	433.8 ± 77.0
3c	1/10	59.9 ± 16.2***	102.3 ± 19.8***
	1/20	130.0 ± 34.0***	195.3 ± 52.7*
	1/40	187.1 ± 36.1**	252.7 ± 58.9*
	1/80	237.1 ± 36.1*	299.2 ± 43.1*
	1/160	345.0 ± 81.4	441.1 ± 110.6
4a	1/10	114.3 ± 26.7***	132.1 ± 32.1***
	1/20	149.7 ± 36.4***	225.3 ± 23.5***
	1/40	154.3 ± 22.6***	255.7 ± 32.7***
	1/80	255.6 ± 30.3**	361.8 ± 40.7*
	1/160	313.2 ± 45.8	505.6 ± 39.5
5e	1/10	333.9 ± 151.4	437.6 ± 299.8
5f	1/10	166.3 ± 36.9**	232.6 ± 36.5**
	1/20	358.8 ± 115.6	472.6 ± 99.5
Control	–	346.5 ± 32.1	537.5 ± 53.6
5a	1/10	226.3 ± 30.1*	398.3 ± 31.9*
	1/20	328.0 ± 24.0	566.1 ± 35.5
5b	1/10	135.8 ± 10.9***	205.1 ± 9.9***
	1/20	289.7 ± 32.0	460.7 ± 38.3
5c	1/10	214.5 ± 29**	370.1 ± 44.7*
	1/20	370.3 ± 40.4	586.7 ± 59.3
5d	1/10	175.1 ± 33.8**	351.8 ± 34.0*
	1/20	285.0 ± 21.0	459.4 ± 31.3

* $P<0.05$, ** $P<0.01$, *** $P<0.001$; Student's t-test.

Table 5
Influence of the compounds on the amphetamine-induced locomotor hyperactivity in mice ($n = 6$–8)

Comp.	Dose (fraction of LD_{50})	No. impulses ± SEM after time (min)	
		30	60
Control	–	503.0 ± 75.9	797.5 ± 102.4
3a	1/10	81.2 ± 18.6***	116.0 ± 31.4***
	1/20	150.0 ± 23.5***	185.6 ± 34.1***
	1/40	410.0 ± 53.5	533.4 ± 76.6
3c	1/10	518.0 ± 80.5	602.0 ± 58.8
4a	1/10	271.4 ± 97.2	534.4 ± 92.5
5e	1/10	415.2 ± 97.2	553.6 ± 89.9
5f	1/10	296.2 ± 46.2*	261.6 ± 62.4***
	1/20	320.5 ± 26.8*	343.8 ± 102.3**
	1/40	514.0 ± 48.5	707.0 ± 40.51
Control	–	472.7 ± 58.7	947 ± 100.3
5a	1/10	192.0 ± 23.9***	369.4 ± 44.4***
	1/20	247.5 ± 28.4	443.3 ± 57.3**
	1/40	389.1 ± 21.2	774.2 ± 42.6
5b	1/10	421.5 ± 43.0	856.4 ± 62.3
5c	1/10	83.4 ± 23.4***	188.0 ± 42.1***
	1/20	240.9 ± 31.2**	493.7 ± 56.5***
	1/40	399.3 ± 25.3	803.4 ± 45.5
5d	1/10	470.5 ± 44.4	1036 ± 94.8

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; Student's t-test.

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

4.1.1. Statistics

Results obtained were presented as mean ± SEM and evaluated statistically using Student's t-test or exact Fischer's test. $P < 0.05$ was the significant criterion.

5. Results

5.1. Acute toxicity

LD_{50} values of the investigated compounds after their i.p. administration to mice are reported in Table 2. The most toxic compounds were **5b**, **5e** and **5c** with LD_{50} values 69.5, 133.4 and 151.0 mg/kg, respectively. Also compounds **5a**, **5f** and **3a** showed high toxicity, their acute toxicity being in a range from 252.0 to 386.9 mg/kg. Compounds **5d** and **4a** had moderate toxicity, with LD_{50} 1020 and 1345.3 mg/kg, respectively. Compound **3c** was not toxic ($LD_{50} > 2000$ mg/kg).

5.2. Motor coordination

Compounds **3a** up to the dose equivalent to 1/20 of LD_{50} as well as **5b** and **5f** at the dose 1/10 of LD_{50} affected the motor coordination in the rota-rod test. All other compounds at the doses equivalent to 1/10 of LD_{50} had no neurotoxic properties as they did not affect the motor coordination in the rota-rod test (Table 3).

5.3. Locomotor activity

Only compound **5e** did not affect the locomotor activity in mice. All the other compounds suppressed spontaneous locomotor activity during 1 h. This effect was produced by **5d**, **5c**, **5a**, **5b** and **5f** at the dose 1/10 of LD_{50}, compound **3a** up to the dose 1/20, compound **4a** and **3c** up to the doses 1/80 of LD_{50} (Table 4).

Table 6
Influence of the compounds on the pain reactivity in 'writhing syndrome' test in mice ($n = 6$–8)

Comp.	Dose (fraction of LD_{50})	Mean no. writhings ± SEM
Control	–	7.24 ± 0.67
3a	1/10	0.12 ± 0.12***
	1/20	1.00 ± 0.42***
	1/40	1.12 ± 0.51***
	1/80	1.62 ± 0.62***
	1/160	3.00 ± 1.05***
	1/320	2.50 ± 1.58*
	1/640	3.87 ± 1.82
3c	1/10	1.37 ± 0.56***
	1/20	1.50 ± 0.75***
	1/40	2.50 ± 0.98***
	1/80	4.25 ± 1.3**
	1/160	5.12 ± 0.85***
	1/320	8.87 ± 1.20
4a	1/10	1.00 ± 0.53***
	1/20	1.25 ± 0.47***
	1/40	5.87 ± 1.72
5e	1/10	0.62 ± 0.32***
	1/20	0.50 ± 0.26***
	1/40	1.00 ± 0.56***
	1/80	2.75 ± 0.97**
	1/160	4.62 ± 1.64
5f	1/10	0.25 ± 0.25**
	1/20	0.75 ± 0.41***
	1/40	1.75 ± 0.64***
	1/80	2.00 ± 0.46***
	1/160	5.25 ± 0.61***
	1/320	5.60 ± 1.2**
	1/640	6.00 ± 0.96**
	1/1280	8.75 ± 1.8
Control	–	7.01 ± 0.87
5a	1/10	7.17 ± 0.95
5b	1/10	4.58 ± 0.33*
	1/20	5.73 ± 0.61
5c	1/10	5.83 ± 1.06
5d	1/10	6.0 ± 0.69

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; Student's t-test.

Table 7
Influence of the compounds on the pain reactivity in 'hot plate' test in mice ($n = 8$)

Comp.	Dose (fraction of LD_{50})	Time of reaction to the pain stimulus (s) ± SE
Control	–	4.44 ± 0.43
3a	1/10	11.10 ± 0.35***
	1/20	8.94 ± 0.55***
	1/40	6.34 ± 0.28**
	1/80	4.08 ± 0.32
3c	1/10	8.00 ± 0.28***
	1/20	7.52 ± 0.38***
	1/40	6.83 ± 0.51**
	1/80	5.00 ± 0.63
4a	1/10	7.44 ± 0.72**
	1/20	4.49 ± 0.27
5e	1/10	8.25 ± 0.62***
	1/20	6.95 ± 0.51**
	1/40	4.77 ± 0.53
5f	1/10	11.6 ± 0.90***
	1/20	9.62 ± 0.26***
	1/40	7.50 ± 0.23***
	1/80	5.89 ± 0.41*
	1/160	4.50 ± 0.26
Control	–	4.10 ± 0.3
5a	1/10	5.74 ± 0.23*
	1/20	5.12 ± 0.54
5b	1/10	5.43 ± 0.63
5c	1/10	5.43 ± 0.78
5d	1/10	6.00 ± 0.79*
	1/20	5.25 ± 0.65

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; Student's t-test.

5.4. Amphetamine-induced locomotor hyperactivity

Compounds **5d**, **5b**, **5e**, **4a** and **3c** did not affect the excitatory action of amphetamine in mice at the dose equivalent to 1/10 of LD_{50}. Compounds **5c**, **5a**, **3a** and **5f** reduced amphetamine-induced locomotor hyperactivity at the dose 1/20 of LD_{50} (Table 5).

5.5. Pain reactivity ('writhing syndrome' test)

Compounds **5a**, **5c** and **5d** did not possess analgesic activity. All the other compounds possessed analgesic activity. Compound **5b** was active at the dose 1/10 of LD_{50}, **4a** up to 1/20, **5e** up to the dose 1/80 of LD_{50}, **3c** up to the dose 1/160 of LD_{50}. The most potent compounds were **3a** and **5f** which showed analgesic activity up to the dose 1/320 and 1/640 of LD_{50}, respectively (Table 6).

5.6. Pain reactivity ('hot plate' test)

Most of the compounds showed analgesic activity. Compounds **5d**, **5a** and **4a** were active at the dose equivalent to 1/10 of LD_{50}, **5e** up to the dose 1/20, **3a** 1/40 and **5f** up to the dose 1/80 of LD_{50}. Compounds **5c**, **5b** and **3c** had no analgesic activity (Table 7).

5.7. Anxiolytic action

None of the compounds, administered at the doses which did not affect spontaneous locomotor activity, increased the number of punished crossings in the 'four plate' test in mice.

5.8. Pentetrazol-induced seizures

The compounds administered at the doses equivalent to 1/10 of LD_{50} had no anticonvulsant properties in the pentetrazol-induced seizure test in mice.

5.9. Maximal electric shock

The compounds administered at the dose of 1/10 of LD_{50} showed lack of protection against tonic seizures in maximal electric shock in mice.

5.10. Head twitches

Compounds **3a**, **3c**, **4a**, **5b**, **5c**, **5e** and **5f** did not change the number of head twitches induced by 5-HTP in mice. Compounds **5a** and **5d** decreased the number of head twitches at the dose 1/10 of LD_{50}.

5.11. Arterial blood pressure

Compound **3c**, administered at the dose 1/10 of LD_{50}, lowered the pulse rate and arterial blood pressure, compound **4a** lowered the blood pressure at the dose 1/10 of LD_{50}.

6. Discussion

Our previous structure–activity relationship studies on pyrroledicarboximides **I** (Fig. 2) revealed that, for the compounds with the central two-carbon chain, the higher depressant activity associated with lower acute toxicity was shown by compounds carrying the aromatic rather than heteroaromatic (pyrimidine, pyridine) substituent on the piperazine ring [19]. On the basis of the results obtained with the compounds **3a**, **3c**, **4a**, and **5a**–**f**, some additional observations can be made about the structure–CNS effects relationship for both series of pyrroledicarboximides **I** (Fig. 2) and **3**–**5**.

The data of the Pharmacological section show that the substitution of the pyrrole ring with heteroaromatic residues causes a significant reduction of the depressant activity. For example, **5a**,**c**,**d** are less active in the spontaneous locomotor activity test (1/10 of LD_{50}) than their 1-*n*-butyl analogues of series **I** (Fig. 2, $n = 2$, $R = n\text{-}C_4H_9$, $X = Y = N$; 0.028 LD_{50} [18]). However, the biological action of compounds **5a**–**d** changes considerably after replacement of the heteroaromatic sub-

stituent of the pyrrole ring with 1-*n*-butyl or 1-phenyl residue (compounds **5e** and **5f**) and replacement of the buspirone type side chain (Fig. 1) with the chain characteristic for NAN-190 (Fig. 1); analogously for **4a** having a 1-*n*-butyl substituent in the pyrrole ring and the trazodone-type side chain. As shown in Tables 4–7, this class of pyrroledicarboximides (**4a** and **5e**,**f**) was generally more active in CNS tests than compounds of series **I** (Fig. 2) and exhibited some analgesic action (Tables 6 and 7). Moreover, the analgesic action of pyrroledicarboximides with an *o*-methoxy substituent introduced at the aromatic ring of the phenylpiperazine residue is independent of the length of the central alkyl chain (compare **3a** and **5e**, Tables 6 and 7). Unfortunately, the most active compounds in the 'writhing syndrome' test (**3a** and **5f**) revealed a neurotoxic effect in the rota-rod test at doses 1/10 and 1/20 of LD_{50}, respectively.

It is interesting that compounds which exhibited analgesic action in the 'writhing syndrome' test (**3a**,**c**, **4a** and **5e**,**f**) were characterized by lipophilicity ($LogP_{calc.}$, Table 1) superior to that of compounds **5a**,**c**,**d**, which did not show such a profile of pharmacological activity. Compound **5b** is an exception because it shows low activity even if the value of log *P* is equal to that of **3a**. However, the knowledge acquired did not permit us to relate the observed analgesic activity of some pyrroledicarboximides **3**–**5** to their lipophilicity.

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