Department of Chemistry of Drugs[1], Wrocław Medical University, and Department of Pharmacology[2], Medical Academy, Lublin, Poland

Synthesis of some *N*-substituted 3,4-pyrroledicarboximides as potential CNS depressive agents

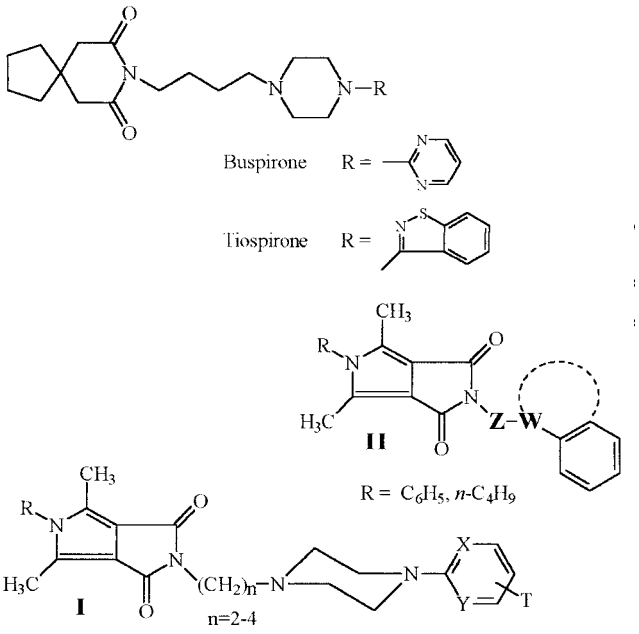
W. Malinka[1], M. Sieklucka-Dziuba[2], G. Rajtar[2], R. Rejdak[2], K. Rejdak[2] and Z. Kleinrok[2]

As a continuation of our work on *N*-[4-aryl(heteroaryl)piperazin-1-ylalkyl]-3,4-pyrroledicarboximides, which were characterized by strong analgesic activity and CNS depressive action, several novel *N*-substituted 3,4-pyrroledicarboximides were prepared and eleven representatives were examined in a series of *in vivo* CNS tests. A few of these compounds displayed a similar profile of biological selectivity to that of 3,4-pyrroledicarboximides described previously; their structure-activity relationships are discussed.

1. Introduction

Several derivatives of *N*-[4-heteroaryl(aryl)piperazin-1-ylalkyl]-imides have been described as compounds with pharmacological effects which result from activation of different central receptor systems [1–7]. Some of them represent a new generation of anxiolytic (i.e. buspirone) or antipsychotic (i.e. tiospirone) agents with reduced side effects associated with the corresponding "classical" drugs. In recent papers [8–10] we described the preparation and pharmacological properties of a series of 3,4-pyrroledicarboximides, all related to the structure **I**.

Our pharmacological studies had established that most of the compounds **I** show moderate acute toxicity, suppress spontaneous and amphetamine-induced locomotor activity in mice [CNS (central nervous system) depressive action]. Some of them were additionally active as analgesic agents. Furthermore, in preliminary screening none of the investigated compounds had anxiolytic or anticonvulsive properties, influenced arterial blood pressure or pulse in rats. Investigation of structure-activity relationships (S-A) demonstrated that compounds of series **IA** lack analgesic activity and *N*-phenylpiperazinyl derivatives of series **IA** (X=Y=CH) tend to exhibit stronger CNS depressive action then their *N*-heteroarylpiperazinyl analogues. For example, these compounds inhibited the spontaneous locomotor activity of mice 2–4 times more than corresponding pyridyl (**IA**: X=N, Y=CH) or pyrimidinyl (**IA**: X=Y=N) 3,4-pyrroledicarboximides [8, 9]. In another paper [10] we showed that the introduction of a substituent T in the aromatic ring of the *N*-phenylpiperazine grouping leads to compounds **IB** which revealed simultaneously CNS-depressive action and significant analgesic effect. Analgesic action was observed both in the writhing test (1/10-1/640 LD_{50}) and in the hot plate test (1/10-1/80 LD_{50}) and was practically independent of the length of the central alkanyl chain (C_2-C_4). This suggests that the flexible bridging chain lets molecules **I** to adopt a bioactive conformation quite easy. To increase CNS properties and to continue our systematic S-A studies in the class of compounds **I** we synthesized a series of new 3,4-pyrroledicarboximides related to **I** (Table 1). Modification of the side chain R_1 was concentrated on the portions **Z**, **W**(II), bridging the imide nitrogen and terminal aromatic ring. Substituent R was practically represented by the phenyl or n-butyl residue only, because our previous experiments showed that such pyrroledicarboximides **I** possess lower acute toxicity when compared to their R-heteroaryl analogues (**IC**) [10].



IA R = Ar, *n*-C_4H_9; X,Y = CH or N; T = H
IB R = Ar, *n*-C_4H_9; X = Y = CH; T = *o*-Cl, *o*-CH_3O, *m*-Cl, *m*-CF_3
IC R = Hetar.; X = N, Y = N or CH, T = H

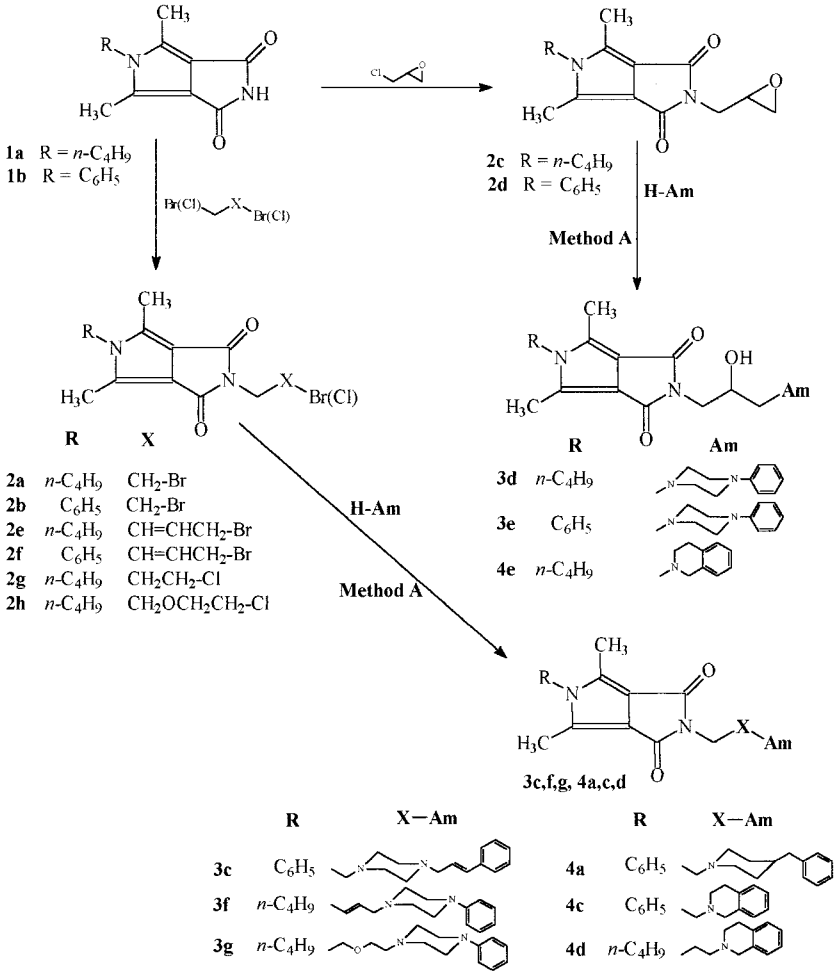
In modification of the bridging alkanyl chain (unit **Z**) our intention was to introduce a carbonyl group (**3b**), a hydroxyl group (**3d**, **e**, **4e**), an O-ethereal atom (**3g**) or a double bond (**3f**), which additionally restricts molecular flexibility. In this context we also shortened the central alkanyl chain to the methylene group (**3a**, n = 1) or bridging portions **Z**, **W** were incorporated in a cyclc structure (**4b**). We also examined the effect of replacement of the piperazine ring (unit **W**) with a part possessing one basic N-atom or with a fragment which does not possess a basic center on the behavioral response. For this purpose terahydoquinoline (**4c–e**), piperidine (**4a**, **b**) and compounds of simplified structure in which both nitrogen atoms of the phenylpiperazine group were replaced by O-ethereal or by O-ethereal and S-thioethereal atoms as is seen in **5a** and **5b**, respectively, were synthesized.

2. Investigations, results and discussion

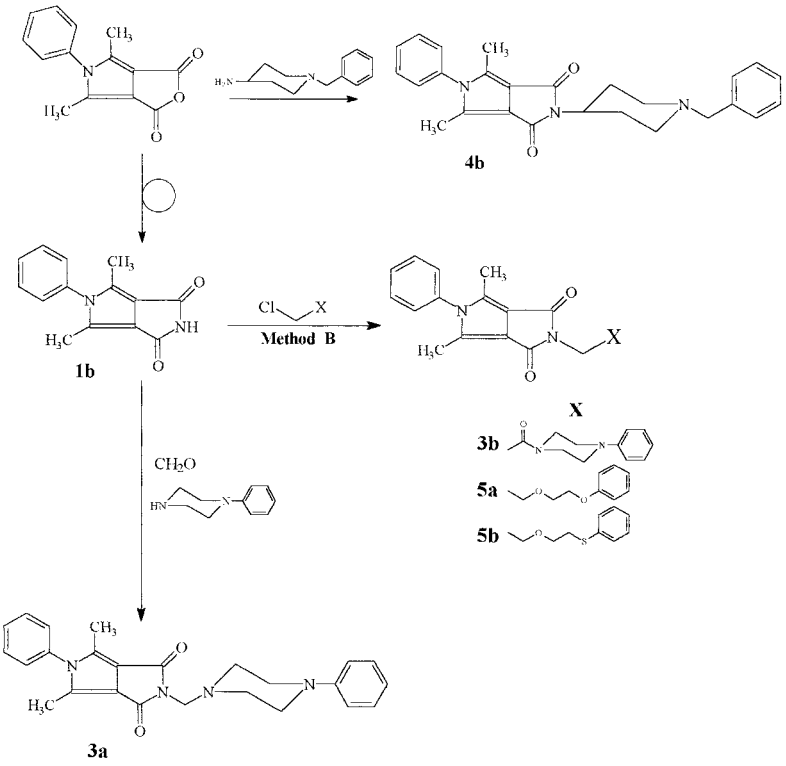
2.1. Synthesis of compounds

The procedures for preparation of the target *N*-substituted 3,4-pyrrolecarboximides **3–5** are shown in Schemes 1 and 2.

Scheme 1



Scheme 2



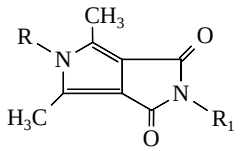
The final compounds were generally synthesized by nucleophilic substitution of the corresponding cyclic amines with appropriate 3,4-pyrroledicarboximide intermediates **2** (Method A, Scheme 1) or, alternatively, in reaction of the 3,4-pyrroledicarboximides **1** [11] with chlorides Cl−CH_2−X, as shown in Scheme 2 (Method B).

Method A involved alkylation of the corresponding, commercially available 4-substituted-piperazines(piperidines) or 1,2,3,4-tetrahydoisoquinoline with *N*-haloalkyl- or *N*-epoxypropyl-3,4-pyrroelidicarboximides **2** (Scheme 1). The target compounds **3c**–**g** and **4a**, **c**–**e** were obtained in 37–65% yield (Table 1). The epoxide intermediates **2c**, **d** were prepared by reactions of epichlorohydrin and 3,4-pyrroledcarboximides **1** in the presence of NaH/DMF. The N-haloalkyl intermediates **2a**, **b**, **e**–**h** were obtained by reacting 3,4-pyrroledicarboximides **1** with corresponding dihalides in the presence of K_2CO_3 in acetonitrile (**2e**, **f**) or NaH/DMF (**2h**) or by a previously reported method (**2a**, **b**, **g**) [8, 9].

Method B was direct alkylation of 3,4-pyrroledicarboximides **1** with the chloride intermediates Cl−CH_2−X (Scheme 2). The reaction was performed in the presence of NaH/DMF and the final products **3b**, **5a**, **b** were obtained in varying yield (Table 1). The chloride intermediates [Cl−CH_2−X: 1-chloroacetyl-4-phenylpiperazine and chloroethoxyethyl(phenol, thiophenol)] were prepared according to published procedures [12, 13]. Scheme 2 also illustrates other specific methods, described in the experimental section, leading to the target compounds **3a**, **4b**.

Table 1 summarizes the physical data of the new intermediates **2c**–**f**, **h** as well as the final compounds **3**–**5**. The analytical data (elementary analyses, IR and ^{1}H NMR spectra) of the new products **2**, **3**–**5** are in agreement with the assigned structures. The IR spectra of all the new compounds **2**, **3**–**5** revealed coupled imidic carbonyl absorption (1740 and 1700 cm^{-1}) typical of 5-member cyclic imides [14]. The ^{1}H NMR spectra showed (from low to

Table 1: Physical data of 3,4-pyrroledicarboximide derivatives 2–5



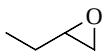
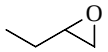
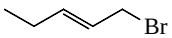
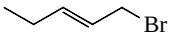
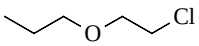
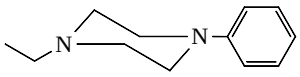
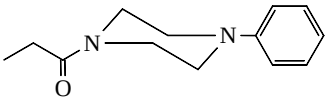
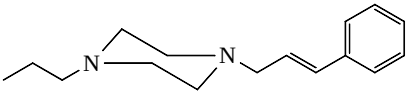
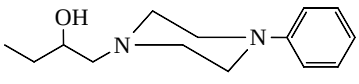
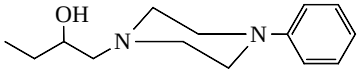
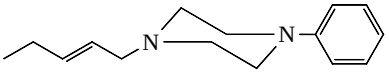
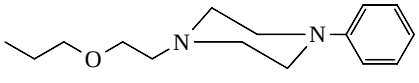
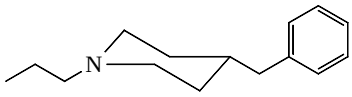
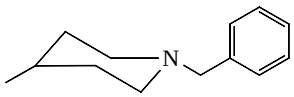
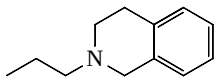
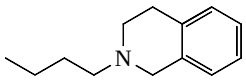
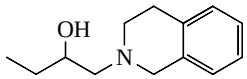
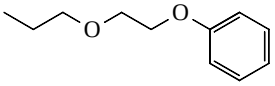
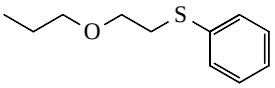
Compd.	R	R_1	Formula m.w.	M.p. (°C) Cryst. solv.	Log P (calc.)	Yield (%)
2c	*n*-C_4H_9		$C_{15}H_{20}N_2O_3$ 276.3	131–133 Ethanol		90
2d	C_6H_5		$C_{17}H_{16}N_2O_3$ 296.3	135–137 Cyclohexane		84
2e	*n*-C_4H_9		$C_{16}H_{21}BrN_2O_2$ 353.2	118–121 Ethanol		68
2f	C_6H_5		$C_{18}H_{17}BrN_2O_2$ 353.2	11 Ethanol		35
2h	*n*-C_4H_9		$C_{16}H_{23}ClN_2O_3$ 326.8	91–93 *n*-Heptane		73
3a	C_6H_5		$C_{25}H_{26}N_4O_2$ 414.5	116–118 Ethanol	4.73	70
3b	C_6H_5		$C_{26}H_{26}N_4O_3$ 442.5	256–259 Ethanol	4.0	82
3c	C_6H_5		$C_{29}H_{32}N_4O_2$ 468.6	180–182 Acetonitrile	4.07	48
3d	*n*-C_4H_9		$C_{25}H_{34}N_4O_2$ 438.6	151–53 Ethanol	3.52	52
3e	C_6H_5		$C_{27}H_{30}N_4O_2$ 458.6	129–131 Methanol	3.99	64
3f	*n*-C_4H_9		$C_{26}H_{34}N_4O_2$ 434.6	113–115 Cyclohexane	4.98	62
3g	*n*-C_4H_9		$C_{26}H_{36}N_4O_3$ 452.6	124–126 *n*-Heptane	3.72	65

Table 1 (cont.)

Compd.	R	R_1	Formula m.w.	M.p. (°C) Cryst. solv.	Log P (calc.)	Yield (%)
4a	C_6H_5		$C_{28}H_{31}N_3O_2$ 441.6	143–145 Ethanol	5.07	37
4b	C_6H_5		$C_{26}H_{27}N_3O_2$ 413.5	211–213 Cyclohexane	5.96	55
4c	C_6H_5		$C_{25}H_{25}N_3O_2$ 399.5	81–83 *n*-Hexane	4.17	50
4d	*n*-C_4H_9		$C_{24}H_{31}N_3O_2$ 393.5	73–75 *n*-Hexane		42
4e	n-C_4H_9		$C_{24}H_{31}N_4O_3$ 409.5	140–142 AcOEt		35
5a	C_6H_5		$C_{24}H_{24}N_2O_4$ 404.5	89–91 Cyclohexane	3.88	75
5b	C_6H_5		$C_{24}H_{24}N_2O_3S$ 420.5	71–73 Ethanol		37

high field) signals of aromatic protons (7.6–6.75 ppm; exception **2c**, **e**, **h**), the absorption consistent with alkyl (alkenyl) protons of the central hydrocarbon chain and of the piperazine (piperidine) ring (1.5–4.6 ppm, CH_2; 5.6 to 6.6 ppm CH=CH) and singlets (6H) of methyl groups of the pyrrole ring (2.15–2.4 ppm).

Finally, for each compound tested at the preliminary pharmacological screening (**3a**–**g**, **4a**–**c**, **5a**), the log of the octanol-water partition coefficient was calculated (log $P_{calc.}$). The calculations of the log $P_{calc.}$ values were made for the free bases, using the ChemPlus program from Hypercube, Inc., IBM PV version. The calculated lipophilicity of these compounds are shown in Table 1.

2.2. Pharmacological investigations

2.2.1. Acute toxicity

The LD_{50} value of the investigated compounds after their i.p. administration to mice are presented in Table 2. The most toxic compound was **3g** with a LD_{50} of 252 mg/kg.

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

Compd.	LD_{50} (mg/kg i.p.)	Confidence limit
3a	>2000	
3b	>2000	
3c	590.4	[487.1–715.6]
3d	>2000.0	
3e	1501.4	[913.4–2468.0]
3f	688.1	[569.2–832.0]
3g	252.0	[130.0–487.0]
4a	>2000	
4b	>2000	
4c	721.1	[609.0–853.9]
5a	>2000	

Compounds **3c**, **3f** and **4c** were quite toxic with LD_{50} 590.4, 688.1 and 721.1 mg/kg, respectively. Compound **3e** showed moderate toxicity (LD_{50} 1501.4 mg/kg). The other compounds **3a**, **3b**, **3d**, **4a**, **4b** and **5a** were not toxic with LD_{50} values over 2000 mg/kg.

2.2.2. Motor coordination

All investigated compounds at the doses equivalent to 1/10 LD_{50} had no neurotoxic properties as they did not affect the motor coordination in the rota-rod test.

2.2.3. Locomotor activity

Only compounds **3g** and **5a** did not affect spontaneous locomotor activity in mice. All other compounds supressed the spontaneous locomotor activity during an 1 h observation period, **4c** decreased spontaneous locomotor activity only at the dose of 1/10, **3a**, **3c**, **3e** and **4b** up to the dose equivalent to 1/20 of LD_{50}, **3b** and **4a** up to 1/40, **3d** and **3f** up to the dose of 1/80 of LD_{50} (Table 3).

2.2.4. Amphetamine-induced locomotor hyperactivity

Compounds **3a**, **3c**, **3g**, **4a**, **4c** and **5a** administered at the dose equivalent to 1/10 of LD_{50} did not affect the excitatory action of amphetamine in mice. Compounds **3b** and **4b** supressed amphetamine-induced hyperactivity at the dose of 1/10, **3f** up to the dose of 1/20, **3d** and **3e** up to the dose of 1/40 of LD_{50}.

2.2.5. Pain reactivity

In the "writhing syndrome" test (Table 4) compound **3d** was the most active and acted up to the dose of 1/80 of LD_{50}, compounds **3e** – 1/40, **3b**, **3f** and **4a** showed an-

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

Compd.	Dose (part of LD_{50})	Number of impulses ± SEM after time (min.)	
		30	60
Control	–	3060.6 ± 301.3	5497.7 ± 361.0
3d	1/10	987.6 ± 122.7***	113.2 ± 225.3***
	1/20	1293.3 ± 212.8***	2615.3 ± 317.5***
	1/40	1635.6 ± 132.1***	3174.8 ± 187.2***
	1/80	2084.3 ± 225.4*	3552.4 ± 341.7***
	1/160	3083.6 ± 328.3	5484.7 ± 437.5
3e	1/10	1773.1 ± 199.8***	2512.4 ± 320.9***
	1/20	2187.3 ± 233.9*	3250.3 ± 351.7***
	1/40	2898.4 ± 280.6	4938.7 ± 368.3
3g	1/10	2950.3 ± 251.1	4986.5 ± 394.1
5a	1/10	3269.1 ± 425.7	5642.7 ± 601.4
Control	–	3308.9 ± 269.0	5628.6 ± 440.7
3a	1/10	1662.0 ± 488.9***	2441.0 ± 534.7***
	1/20	1963.0 ± 126.6***	2738.4 ± 302.7***
	1/40	2723.2 ± 278.0	4698.5 ± 425.2
3b	1/10	1575.9 ± 269.0***	2467 ± 382.2***
	1/20	1879.2 ± 227.3***	2824.3 ± 358.2***
	1/40	2199.3 ± 199.0**	3333.4 ± 342.2***
	1/80	2633.1 ± 325.0	4370.5 ± 445.0
3c	1/10	1662.0 ± 488.9***	2457.0 ± 613.9***
	1/20	1899.9 ± 397.8**	3642.5 ± 445.3**
	1/40	3392.0 ± 425.0	4672.5 ± 489.3
3f	1/10	482.0 ± 75.6***	815.0 ± 105.7***
	1/20	896.2 ± 201.0***	1355.1 ± 313.2***
	1/40	1113.8 ± 330.6***	1691.6 ± 505.1***
	1/80	2352.2 ± 196.0*	3648.1 ± 321.2**
	1/160	3311.0 ± 354.2	5314.7 ± 475.7
4a	1/10	810.9 ± 90.2***	948.8 ± 102.3***
	1/20	1765.0 ± 304.0***	2557.1 ± 537.2***
	1/40	1825.9 ± 135.5***	3623.8 ± 257.2**
	1/80	3755.4 ± 793.0	5855.8 ± 974.3
4b	1/10	1283.1 ± 102.6***	1670.1 ± 202.5***
	1/20	1911.2 ± 168.0***	2688.4 ± 254.3***
	1/40	2987.0 ± 146.3	4446.0 ± 258.3
4c	1/10	1694.0 ± 253.7***	2744.3 ± 523.9***
	1/20	3279.7 ± 314.7	4952.8 ± 414.7

* $p < 0.05$, ** $p < 0.01$, *** $p > 0.001$

algesic activity in a dose equivalent to 1/10 and 1/20 of LD_{50}. In the "hot plate" test (Table 5) compounds **3d** and **3e** acted up to the dose of 1/20 of LD_{50}. Compounds **3a**, **3b**, **3f**, **4a**, **4b** and **4c** possesed analgesic activity when administered to mice in a dose equivalent to 1/10 of LD_{50}. Compounds **3c**, **3g** and **5a** did not show analgesic activity in both tests performed.

2.2.6. Head twitches

Only **3f** reduced the number of head twitches episodes induced by 5-HTP in mice at a dose of 1/10 of LD_{50}. All other compounds did not change the number of head twitches.

2.2.7. Other tests

None of compounds investigated increased the number of punished crossings in the "four plates" test, had anticonvulsive properties in pentetrazole-induced seizures test, showed protection against tonic seizures in maximal electric shock in mice or lowered the pulse rate and arterial blood preasure.

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

Compd.	Dose (part of LD_{50})	Mean number of writhings ± SEM
Control	–	9.24 ± 0.67
3g	1/10	9.12 ± 0.45
3d	1/10	0.75 ± 0.31***
	1/20	1.00 ± 0.32***
	1/40	1.37 ± 0.46***
	1/80	4.12 ± 1.00***
	1/160	6.00 ± 2.07
5a	1/10	10.6 ± 1.1
Control	–	10.62 ± 0.98
3e	1/10	1.62 ± 0.41***
	1/20	2.00 ± 0.73***
	1/40	5.00 ± 0.61***
	1/80	7.75 ± 1.15***
Control	–	8.2 ± 0.8
3a	1/10	5.3 ± 1.2
3b	1/10	0.6 ± 0.4***
	1/20	4.4 ± 0.5**
	1/40	6.8 ± 0.4
3c	1/10	7.4 ± 0.8
3f	1/10	0.4 ± 0.8***
	1/20	3.6 ± 0.8*
	1/40	5.8 ± 0.9
4a	1/10	1.6 ± 0.2***
	1/20	3.4 ± 0.6**
	1/40	5.4 ± 1.0
4b	1/10	5.8 ± 0.8
4c	1/10	5.7 ± 0.9

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

Table 5: Influence of the investigated compounds on the pain reactivity in "hot plate" test in the mice (n = 8)

Compd.	Dose (part of LD_{50})	Time of reaction on pain stimulus (S ± SE)
Control	–	4.44 ± 0.4
3d	1/10	9.51 ± 0.82***
	1/20	6.28 ± 0.50*
	1/40	4.13 ± 0.28
3e	1/10	7.50 ± 0.70**
	1/20	5.68 ± 0.28*
	1/40	4.50 ± 0.23
3g	1/10	4.80 ± 0.3
5a	1/10	5.21 ± 0.3
Control	–	3.91 ± 0.3
3a	1/10	8.50 ± 0.63***
	1/20	4.11 ± 0.31
3b	1/10	7.2 ± 0.8***
	1/20	5.1 ± 0.6
3c	1/10	5.4 ± 0.8
3f	1/10	6.11 ± 0.51***
	1/20	4.61 ± 0.38
4a	1/10	5.5 ± 0.4**
	1/20	4.3 ± 0.2
4b	1/10	6.2 ± 0.5*
	1/20	4.2 ± 0.2
4c	1/10	7.5 ± 0.6***
	1/20	4.7 ± 0.5

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

2.2.8. Summary of the pharmacological results

Based on the screening results reported in this paper, to the general conclusion about S-A relationships at series of 4-phenylpierazin-1-ylalkyl-3,4-pyrroledicarboximides **IA** (X=Y=CH), described in the introduction, the following may be added:

• Introduction of a substituent (carbonyl amidic group, hydroxyl group) or double bound to the central alkanyl chain of 4-phenylpiperazinyl-pyrroledicarboximides **IA** gives compounds **3b**, **d**–**f** which show, similarly to derivatives **IB** tested previously [10], both analgesic and depressive action. However, in no case improvement in activity was seen. For example, the most analgesic agent from series **IB** was effective at a dose 1/640 LD_{50} [10], whereas compounds from series **3** described here showed analgesic activity only at doses ranged from 1/10 to 1/80 LD_{50}.

• Shortening of the central alkanyl chain of 4-phenylpiperazinyl-3,4-pyrroledicarboximides **IA** to the methylene group (**3a**), replacement of the 4-phenyl substituent of piperazine with the cinnamyl residue (**3c**) or replacement of the 4-phenylpiperazine fragment with one-base residue [benzylpiperidine (**4a**, **b**) or tetrahydoisoquinoline (**4c**)] led to a marked decrease of depressive and analgesic activity, when compared with those of **3b**, **d**–**f**.

• Pyrroledicarboximides, which have an ethereal atom at the structure of the bridging portions **W**, **Z**, lack of biological activity (**3g**, **5a**) at pharmacological doses.

• It is difficult to simply correlate the log $P_{calc.}$ (Table 1) values of the compounds tested with the observed biological effects. For example, compounds **3d**, **e**, the most potent analgesic agents (Tables 4, 5), have log $P_{calc.} = 3.52$ and 3.99, respectively, while their ethereal analogue **3g** characterized by log $P_{calc.} = 3.72$ was devoid of any pharmacological activity. However, this conclusion needs a few words of comment. The procedure calculation of log P does not include, i.e. keto-enol equilibrium, molecular conformation, hydrogen bonds and in some cases it may be a reason of the difference between the values of log P as calculated and as experimentally determined.

In summary, pyrroledcarboximides, both **3** (exception **3g**) and **4**, generally possess the same biological selectivity as compounds **IB** tested previously (analgesic an CNS depressive action) [10], but lower effects were observed.

3. Experimental

3.1. Synthesis of the compounds

Melting points are uncorrected. ^{1}H NMR spectra were obtained with a Tesla spectrometer (80 MHz, $CDCl_3$, δ (ppm)). IR (KBr) spectra were recorded on a Specord-75 IR spectrometer. Elemental C, H, N analyses were run o a Carlo Erba NA-1500 analyzer. All the results of the C, H, and N determinations were within ±0.4% of the values calculated for the corresponding formulae. Chromatographic separations were performed on a silica gel column [Kieselgel 60 (70–230 msh), Merck]. Analytical TLC was carried on Merck silica gel – $60F_{254}$ and visualized by UV.

3.1.1. 1-Substituted-2,5-dimethyl-N-(2,3-epoxypropyl)-3,4-pyrroledicarboximides **2c**, **2d**

0.42 g of NaH (56–58% suspension in mineral oil) were added in portions to a solution of 0.01 mol of pyrroledicarboximide **1a** [11] or **1b** [11] in anh. DMF 20 (ml). After stirring at room temperature for 1 h, 3.1 ml (0.04 mol) of epichlorohydrin were added and stirring was continued at 60 °C for 5 h. The reaction mixture was then poured into cold H_2O and separated precipitate was filtered off and purified by crystallization with charcoal to give pure product **2c** or **2d** (Table 1).

2c: ^{1}H NMR: 0.98 (tr, 3 H, CH_3, J = 5.9 Hz), 1.17–1.78 (m, 4 H, $2 \times CH_2$), 2.39 (s, 6 H, $2 \times CH_3$), 2.59–2.81 (m, 2 H, CH_2), 3.04–3.25 (m, 1 H, CH), 3.61–3.99 (m, 4 H, $2 \times NCH_2$).

2d: ^{1}H NMR: 2.18 (s, 6 H, $2 \times CH_3$), 2.65–2.86 (m, 2 H, CH_2), 3.1–3.3 (m, 1 H, CH), 3.5–4.06 (m, 2 H, NCH_2), 7.16–7.27 (m, 2 H, ArH), 7.47–7.6 (m, 3 H, ArH).

3.1.2. trans-1-Substituted-N-(4-bromo-2-buten-1-yl)-2,5-dimethyl-3,4-pyrroledicarboximides **2e**, **2f**

A mixture of 0.01 mol of pyrroledicarboximide **1a** [11] or **1b** [11], 1.4 g of anh. K_2CO_3 and 6.4 g (0.03 mol) of *trans*-1,4-dibromo-2-butene in acetonitrile (50 ml) was refluxed with stirring for 15 h. After filtration the solvent was distilled off and the crude product **2e** obtained was treated with diethyl ether. Undissolved precipitate was filtered off and purified by crystallization (**2e** – Table 1). Purification of **2f** was carried out on silica gel column using benzene as eluent (**2f** – $R_f = 0.76$).

2e: ^{1}H NMR: 0.98 (tr, 3 H, CH_3, J = 5.9 Hz), 1.17–1.5 (m, 4 H, $2 \times CH_2$), 2.39 (s, 6 H, $2 \times CH_3$), 3.67–3.94 (m, 4 H, $N_{pyrrole}CH_2 + CH_2Br$), 4.1–4.17 (m, 2 H, $N_{imide}CH_2$), 5.79–5.91 (m, 2 H, CH=CH).

2f: ^{1}H NMR: 2.17 (s, 6 H, $2 \times CH_3$), 3.89–3.96 (m, 2 H, $N_{pyrrole}CH_2$), 4.15–4.2 (m, 2 H, CH_2Br), 5.82–5.94 (m, 2 H, CH=CH), 7.15–7.3 (m, 2 H, ArH), 7.51–7.59 (m, 3 H, ArH).

3.1.3. 1-n-Butyl-N-[2-(2-chloroethoxy)ethyl]-2,5-dimethyl-3,4-prroledicarboximide (**2h**)

0.42 g of NaH (56–58% suspension in mineral oil) were added in portion to a solution of 2.2 g (0.01 mol) of pyrroledicarboximide **1a** [11] in anh. DMF (7 ml). After stirring at room temperature for 1 h, 3.5 ml of 2-chloroethyl ether were dropped in and stirring was continued at 120 °C for 4 h. The reaction mixture was then poured into cold H_2O and the separated precipitate was filtered off and purified by crystallization (**2h** – Table 1).

^{1}H NMR: 0.98 (tr, 3 H, CH_3, J = 6.16 Hz), 1.17–1.83 (m, 4 H, $2 \times CH_2$), 2.38 (m, 6 H, $2 \times CH_3$), 3.6–3.85 (m, 10 H, $N_{pyrrole}CH_2 + NCH_2CH_2OCH_2CH_2Cl$).

3.1.4. 2,5-Dimethyl-N-(4-phenylpiperazin-1-ylmethyl)-1-phenyl-3,4-pyrroledicarboximide (**3a**)

A solution of 2.4 g (0.01 mol) of pyrroledicaboximide **1b** [11], 1 ml of 37% formaldehyde (w/v) and 1.6 g (0.01 mol) of *N*-phenylpiperazine in ethanol (60 ml) was refluxed for 20 min. After cooling the separated precipitate was filtered off and purified by crystallization (**3a** – Table 1).

^{1}H NMR: 2.18 (s, 6 H, $2 \times CH_3$), 2.77–2.92 [m, 4 H, $N(CH_2)_2$], 3.1–3.26 [m, 4 H, $(CH_2)_2NAr$], 4.59 (s, 2 H, CH_2), 6.75–7.6 (m, 10 H, ArH).

3.1.5. 2,5-Dimethyl-1-phenyl-N-(4-phenylpiperdin-1-yl)-3,4-pyrroledicarboximide (**4b**)

A solution of 2.4 g (0.05 mol) of 2,5-dimethyl-1-phenyl-3,4-pyrroledicarboxylic acid anhydride [11] and 1.9 g (0.01 mol) of 4-amino-1-benzylpiperidine in anh. benzene (50 ml) was stirred at room temperature for 10 h. The precipitated solid was filtered off, dissolved in anh. $CHCl_3$ containing 2 ml of $SOCl_2$ and the solution was refluxed for 1 h. The solvent was distilled off and the resulting solid was treated with 2% $NaHCO_3/H_2O$ solution and filtered off. The crude product obtained was purified by crystallization (**4b**, Table 1).

^{1}H NMR: 1.52–3.09 (m, 14 H, CH_2), 3.65 (s, 2 H, NCH_2Ar), 3.9–4.2 (m, 1 H, $N_{imide}CH$), 7.15–7.6 (m, 10 H, ArH).

3.1.6. Method A

3.1.6.1. 1-Substituted-2,5-dimethyl-*N*-[2-hydroxy-3-(4-phenylpiperazin-1-yl)propyl]-3,4-pyrroledicarboximides **3d**, **3e**

A solution of 0.01 mol of epoxypropyl-pyrroledicarboximide (**2b** or **2c**) and 1.6 g (0.01 mol) of N-phenylpiperazine in absolute ethanol (50 mol) was refluxed for 3 h. The solvent was distilled off and the residue was purified by crystallization with charcoal to give pure product (**3d**, **3e** – Table 1).

3d: ^{1}H NMR: 0.95 (tr, 3 H, CH_3, J = 6.1 Hz), 1.26–1.76 (m, 4 H, $2 \times CH_2$), 2.29–2.82 [m, 12 H, $2 \times CH_3 + N(CH_2)_3$], 3.18 [tr, 4 H, $N(CH_2)_2Ar$, J = 4.9 Hz], 3.64–4.12 (m, 5 H, $N_{imide}CH_2 + N_{pyrrole}CH_2$ + CH), 6.75–6.96 (m, 3 H, ArH), 7.17–7.36 (m, 2 H, ArH). Position of the OH proton signal was not established. IR: 3390 (OH).

3e: ^{1}H NMR: 2.17 (s, 6 H, $2 \times CH_3$), 2.47–2.78 [m, 7 H, $N(CH_2)_3 + OH$ (D_2O exchangeable)], 3.12–3.23 [m, 4 H, $(CH_2)_2NAr$], 3.67–3.73 (m, 2 H, $N_{imide}CH_2$), 4.0–4.15 (m, 1 H, CH), 6.85–7.58 (m, 10 H, ArH).

3.1.6.2. *trans-N*-[4-[4-Phenylpiperazin-1-yl)-2-butenyl]-1-*n*-butyl-2,5-dimethyl-3,4-pyrroledicarboximide (**3f**)

trans-4-Bromobutenyl-pyrroledicarboximide **2e** (3.5 g, 0.01 mol), 1.4 g of anh. K_2CO_3 and 2.4 g (0.015 mol) of N-phenylpiperazine in CH_3CN (70 ml) were refluxed for 10 h. After filtration the solvent was evaporated and the resulting oil was treated with n-hexane. The solid formed was filtered off and purified by crystallization (**3f** – Table 1).

^{1}H NMR: 0.98 (tr, 3 H, CH_3, J = 5.9 Hz), 1.17–1.75 (m, 4 H, $2 \times CH_2$), 2.38 (s, 6 H, $3 \times CH_3$), 2.5–2.62 [m, 4 H, $N(CH_2)_2$], 2.99–3.05 (m, 2 H, $CH_2N_{piperazine}$), 3.12–3.25 [m, 4 H, $(CH_2)_2NAr$], 3.66–3.84 (m, 2 H, CH_2, $N_{pyrrole}CH_2$), 4.05–4.17 (m, 2 H, $N_{imide}CH_2$), 5.62–5.72 (m, 2 H, CH=CH), 6.75–6.97 (m, 3 H, ArH), 7.16–7.36 (m, 2 H, ArH).

3.1.6.3. 1-*n*-Butyl-*N*-{2-[2-(4-phenylpiperazin-1-yl)ethoxy]ethyl}-2,5-dimethyl-3,4-pyrroledicarboximide (**3g**)

Chloroethoxyethyl-pyrroledicarboximide **2h** (3.3 g, 0.01 mol) and 3.2 g (0.02 mol) of *N*-phenylpiperazine in xylene (100 ml) were refluxed for 15 h. After filtration, the solvent was distilled off and the resulting solid was purified by crystallization (**3g**, Table 1).
^{1}H NMR: 0.85–1.05 (m, 3 H, CH_3), 1.2–1.7 (m, 4 H, $2 \times CH_2$), 2.36 (s, 6 H, $2 \times CH_3$), 2.55–2.75 [m, 6 H, $N(CH_2)_3$], 2.8–3.2 [m, 4 H, $(CH_2)_2NAr$], 3.55–3.77 (m, 8 H, $N_{pyrrole}CH_2 + N_{imide}CH_2CH_2OCH_2$), 6.77–6.95 (m, 3H, ArH), 7.15–7.32 (m, 2 H, ArH).

3.1.6.4. *N*-(2-Substituted-ethyl)-2,5-dimethyl-1-phenyl-3,4-pyrroledicarboximides **3c, 4a, 4c**

Bromoethyl-pyrroledicarboximide **2a** [9] (3.5 g, 0.01 mol), 1.4 g of anh. K_2CO_3 and 0.015 mol of the corresponding amine (*trans*-1-cinnamylpiperazine – for obtaining **3c**, 4-benzylpiperidine – **4a**, 1,2,3,4-tetrahydroisoquinoline – **4c**) were refluxed with stirring in CH_3CN (70 ml) for 10 h. After filtration the solvent was distilled off and the resulting crude products **3c** and **4a** were purified by crystallization (Table 1). Purification of **4c** was carried out on a silica gel column using a mixture of ethyl acetate-cyclohexane (1:1) as eluent ($R_f = 0.6$); an analytic sample of the compound was obtained after crystallization (Table 1).
3c: ^{1}H NMR: 2.16 (s, 6 H, $2 \times CH_3$), 2.47–2.69 [m, 10 H, $CH_2N(CH_2CH_2)_2N$], 3.15 (d, 2 H, $N_{piperazine}CH_2$, J = 5.4 Hz), 3.7 (tr, 2 H, $N_{imide}CH_2$), J = 7.4 Hz), 6.5–6.62 (m, 2 H, CH=CH), 7.12–7.37 (m, 7 H, ArH), 7.46–7.55 (m, 3H, ArH).
4a: ^{1}H NMR: 1.22–1.69 (m, 5 H, CH), 1.87–2.25 (m, 8H, $2 \times CH_3 + CH_2$), 2.47–2.65 (m, 4 H, $2 \times CH_2$), 2.92–3.06 (m, 2 H, CH_2Ar), 3.60–3.77 (m, 2 H, $N_{imide}CH_2$), 7.12–7.27 (m, 7 H, ArH), 7.42–7.57 (m, 3 H, ArH).
4c: ^{1}H NMR: 2.15 (s, 6 H, $2 \times CH_3$), 2.5–2.95 (m, 6 H, $CH_2NCH_2CH_2Ar$), 3.67–3.89 (m, 4 H, $N_{imide}CH_2 + NCH_2Ar$), 7.08–7.25 (m, 6 H, ArH), 7.42–7.56 (m, 3 H, ArH).

3.1.6.5. 1-*n*-Butyl-2,5-dimethyl-*N*-[3-(1,2,3,4-tetrahydroisoquinolin-2-yl)-propyl]-3,4-pyrroledicarboximide (**4d**)

N-(3-Chloropropyl)-pyrroledicarboximide **2g** [8] (2.2 g, 0.01 mol), and 2.7 g (0.02 mol) of 1-phenylpiperazine were refluxed in xylene for 20 h. After filtration the solvent was distilled off and the oily residue was purified on a silica gel column using ethyl acetate as eluent ($R_t = 0.5$). Further purification of **4d** was achieved by crystallization (Table 1).
^{1}H NMR: 0.96 (tr, 3 H, CH_3, J = 6.1 Hz), 1.27–1.75 (m, 4 H, $2 \times CH_2$), 1.84–2.1 (m, 2 H, CH_2), 2.33 (s, 6 H, $2 \times CH_3$), 2.47–2.94 (m, 6 H, $CH_2NCH_2CH_2Ar$), 3.35–3.77 (m, 6 H, $N_{imide}CH_2 + N_{pyrrole}CH_2 + NCH_2Ar$), 7.05 (s, 4 H, ArH).

3.1.6.6. 1-*n*-Butyl-2,5-dimethyl-*N*-[2-hydroxy-3-(1,2,3,4-tetrahydroisoquinolin-2-yl)propyl]-3,4-pyrroledicarboximide (**4e**)

A solution of 2.8 g (0.01 mol) of epoxypropyl-pyrroledicarboximide **2c** and 1.35 g (0.01 mol) of 1,2,3,4-tetrahydioisoquinoline in absolute ethanol (50 ml) was refluxed for 1.5 h. The solvent was distilled off and the resulting crude product **4e** was purified on a silica gel column using ethyl acetate as eluent ($R_f = 0.57$). Further purification of **4e** was achieved by crystallization (Table 1).
^{1}H NMR: 0.98 (tr, 3 H, CH_3, J = 5.9 Hz), 1.18–1.75 (m, 4 H, $2 \times CH_2$), 2.37 (s, 6 H, $2 \times CH_3$), 2.47–4.05 (m, 13 H, CH), 7.27 (s, 4 H, ArH). Position of the OH proton signal was not established. IR: 3390 (OH).

3.1.7. Method B

3.1.7.1. *N*-[2-(4-Phenylpiperazin-1-yl)-2-butenyl]-1-n-butyl-2,5-dimethyl-3,4-pyrroledicarboximide (**3b**)

0.42 g of NaH (56–58% suspension in mineral oil) were added in portions to a solution of 2.4 g (0.01 mol) of pyrroledicarboximide **1b** [11] in anh. DMF (15 ml). After stirring at room temperature for 1 h, 2.4 g (0.01 mol) of 1-(chloroacetyl)-4-phenylpiperazine [12] were added and stirring was continued at 120 °C for 5 h. The reaction mixture was then poured into cold water, the separated precipitate was filtered off and purified by crystallization with charcoal to afford **3b** (Table 1).
^{1}H NMR: 2.15 (s, 6 H, $3 \times CH_3$, 3.05–3.32 [m, 4 H, $(CH_2)_2NAr$], 3.57 to 3.82 [m, 4 H, $CON(CH_2)_2$], 4.42 (s, 2 H, $N_{imide}CH_2$), 6.88–7.56 (m, 10 H, ArH).

3.1.7.2. *N*-[2-(2-Phenoxyethoxy)ethyl]-2,5-dimethyl-1-phenyl-3,4-pyrroledicarboximide (**5a**) and *N*-[2-(2-Phenylthioethoxy)ethyl]-2,5-dimethyl-1-phenyl-3,4-pyrroledicarboximide (**5b**)

0.42 g of NaH (56–58% suspension in mineral oil) were added in portions to a solution of 2.4 g (0.01 mol) of pyrroledicarboximide **1b** [11] in anh. DMF (7 ml). After stirring at room temperature of 1 h, 0.015 mol of a corresponding chloroethoxy derivative [2-(2-chloroethoxy)ethoxybenzene [13] for obtaining **5a**, 2-(2-chloroethoxy)ethylthiobenzene [13] – **5b**] were added and stirring was continued under reflux for 5 h. The reaction mixture was then poured into cold H_2O and the separated precipitate was filtered off and purified by crystallization with charcoal to give pure product **5a** or **5b** (Table 1).
5a: ^{1}H NMR: 2.16 (s, 6 H, $2 \times CH_3$), 3.79–3.9 (m, 6 H, $2 \times CH_2$), 4.05 to 4.15 (m, 2 H, CH_2OAr), 6.82–7.58 (m, 10 H, ArH).
5b: ^{1}H NMR: 2.16 (s, 6 H, $2 \times CH_3$), 3.08 (tr, 2 H, CH_2SAr, J = 6.8 Hz), 3.59–3.75 (m, 6 H, $3 \times CH_2$), 7.12–7.58 (m, 10 H, ArH).

3.2. Pharmacology

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). The investigated compounds **3a–g**, **4a–c**, **5a** (Table 1) were administered intraperitoneally (i.p.) as a suspension 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, 1/20, 1/40, 1/80 or 1/160 of LD_{50}. Control animals received the equivalent volume of solvent. Each experimental group consisted of 8 animals.

3.2.1. Acute toxicity

Acute toxicity was assesed by the methods of Litchfield and Wilcoxon [15] and presented as LD_{50} calculated from the mortality of mice after 24 h.

3.2.2. Motor coordination

Motor coordination was measured according to the method of Gross and Tripod [16]. 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.

3.2.3. Spontaneous locomotor activity

After the injection of the investigated compounds animals were placed separately in plexiglass cages ($20 \times 20 \times 30$ cm) for 1 h. The test was performed by means of the DIGISCAN Optical Animal Activity Monitoring System (Omnitech Electronics, Inc. Columbus, Ohio, USA). The apparatus monitors animal locomotor activity via a grid of invisible infrared light beams which in an equal number traverse the animal cage from front to back and from left to right. Each crossing of the light beam was recorded automatically and subjects to rapid analysis by the Digiscan Analyzer using computer program Omni-Pro, version 2.40. The spontaneous activity were evaluated 30 and 60 min after administration of the investigated compound.

3.2.4. Amphetamine hyperactivity

Amphetamine hyperactivity in mice was induced by D,L-amphetamine administration (2.5 mg/kg s.c.). The investigated compounds were injected 30 min before amphetamine. The locomotor hyperactivity was evaluated 30 and 60 min after their administration by the DIGISCAN apparatus as described above.

3.2.5. Pain reactivity

Pain reactivity was measured by the "writhing syndrome" test of Koster et al. [17]. 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 acetic acid.
Pain reactivity was also measured in the "hot plate" test according to the method of Eddy and Leimbach [18]. Animals were placed individually on the metal plate heated to 56 °C. The time(s) of appearance of the pain reaction (licking of the forepaws or jumping) was measured. Experiments were performed 60 min after administration of the investigated compounds.

3.2.6. Anxiolytic properties

Anxiolytic properties were assesed by the "four plate" test in mice, according to Aron et al. [19], 60 min after administration of the investigated compounds at doses which had no effect on the spontaneous locomotor activity. Mice were placed in the cages with a four plates floor (11×7 cm) with 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 not more often than every 3 s.

3.2.7. Pentetrazol seizures

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

3.2.8. Maximal electric shock

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. [20]. The criterion of the convulsive response was the tonic extension of the hind limbs. The test was performed 60 min after administration of the investigation compounds.

3.2.9. Head twitches

Head twitches behaviour was induced by the administration of 5-hydroxytryptophan (5-HTP) at a dose of 180 mg/kg i.p. 30 min after the investigated compounds. Animals were observed 60 min after 5-HTP administration [21].

3.2.10. Arterial blood pressure

Arterial blood pressure was determined according to the method of Gerold and Tschirky [22] 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.

3.2.11. Statistics

Results obtained were presented as means and evaluated statistically using Student's t-test or exact Fischer's test.

References

1 New, J. S.; Yevich, J. P.; Eison, M. S.; Taylor, D. P.; Eison, A. S.; Riblet, L. A.; VanderMaelen, C. P.; Temple, D. L.: J. Med. Chem. **29**, 1476 (1986)
2 New, S. J.; Yevich, J. P.; Temple, D. L.; New, K. B.; Gross, S. M.; Schlemmer, R. F.; Eison, M. S.; Taylor, D. P.; Riblet, L.: J. Med. Chem. **31**, 618 (1988)
3 Abou-Gharbia, M.; Moyer, J. A.; Patel, U.; Webb, M.; Schiehser, G.; Andree, T.; Haskins, J. T.: J. Med. Chem. **32**, 1024 (1989)
4 Hirose, A.; Kato, T.; Ohno, Y.; Shimizu, H.; Tanaka, H.; Nakamura, M.; Katsube, J.: Japan. J. Pharmacol. **53**, 321 (1990)
5 Raghupathi, R. K.; Rydelek-Fitzgerald, L.; Teitler, M.; Glennon, R. A.: J. Med. Chem. **34**, 2633 (1991)
6 Romeo, G.; Ambrosini, G.; Guccione, S.; De Blasi, A.; Russo, F.: Eur. J. Med. Chem. **28**, 499 (1993)
7 Norman, M. H.; Minick, D. J.; Rigdon, G. C.: J. Med. Chem. **39**, 149 (1996)
8 Malinka, W.; Tatarczyńska, E.: Farmaco **48**, 933 (1993)
9 Malinka, W.; Sieklucka-Dziuba, M.; Robak, J.; Kleinrok, Z.: Farmaco **49**, 481 (1994)
10 Malinka, W.; Sieklucka-Dziuba, M.; Rajtar, G.; Rubaj, J.; Kleinrok, Z.: Farmaco **54**, 390 (1999)
11 Malinka, W.; Bodalski, T.: Polish J. Chem. **68**, 297 (1994)
12 Cyanamid Co.: US Patent, 2807617 (1955); Beil. **23**(19), 203 E III/IV
13 Cretcher, L. H.; Koch, J. A.; Pittenger, W. H.: J. Am. Chem. Soc. **47**, 1173 (1925)
14 Bellamy, L. J.: The Infrared Spectra of Complex Molecules, Methuen, London 1960
15 Litchfield, I. T.; Wilcoxon, F.: J. Pharmacol. Exp. Ther. **96**, 99 (1949)
16 Gross, F.; Tripod, J.; Meier, R.: Med. Wschr. **85**, 305 (1955)
17 Koster, R.; Anderson, M.; de Bear, E. J.: Fed. Proc. **18**, 412 (1959)
18 Eddy, N. B.; Leinbach, D.: J. Pharmacol. Exp. Ther. **107**, 385 (1953)
19 Aron, C.; Simon, D.; Larousse, C.; Boissier, J. R.: Neuropharmacology **10**, 459 (1971)
20 Swinyard, E. A.; Brown, W. C.; Goodman, L. S.: J. Pharmacol. Exp. Ther. **106**, 319 (1952)
21 Nakamura, M.; Fukushima, H.: J. Pharm. Pharmacol. **30**, 254 (1978)
22 Gerold, M.; Tschirky, H.: Arzneim.-Forsch. **18**, 1285 (1968)

Received December 16, 1998
Accepted March 3, 1999

Dr. habil. W. Malinka
Department of Chemistry of Drugs
Wrocław Medical University
ul. Tamka 1
50-137 Wrocław
Poland