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# Investigations on the synthesis and properties of *N*-phenyl derivatives of 1,4-dioxo(1,4,5-trioxo)-1,2,3,4-tetra(1,2,3,4,5,6-hexa)hydropyrido-[3,4-d]pyridazines and allied compounds

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**Abstract**

2-(1-Piperidino)- and 2-(4-methyl-1-piperazinyl)-6-methyl-3,4-pyridinedicarboximides (**1**, **2**) react with *N*-phenylhydrazine yielding *N*-phenylamino-3,4-pyridinedicarboximides (**7**, **8**). The same reaction with 1,6-dimethyl-2-oxo-1,2-dihydro- and 2-chloro-6-methyl-3,4-pyridinedicarboximides (**3**, **17**) gives the salts of the corresponding *N*-phenylpyridopyridazines with phenylhydrazine (**13**, **18**), which transform into *N*-phenylaminoimides (**14**, **19**) during boiling in 80% acetic acid. Compounds **7**, **8** and **14** isomerize to the corresponding 2-phenyl-1,4-dioxo(1,4,5-trioxo)-1,2,3,4-tetra(1,2,3,4,5,6-hexa)hydropyrido[3,4-d]pyridazines (**9**, **10**, **15**) under the influence of heating in alcoholic solution of $C_2H_5ONa$ or $CH_3ONa$. Only in the case of imide **19** are 2- and 3-phenyl isomers (**20** and **21**) formed under these conditions. Some of the obtained compounds were pharmacologically active. 

*Keywords:* *N*-Phenyl derivatives; Pyrido[3,4-d]pyridazines

## 1. Introduction

It was stated previously [1] that 2-(1-piperidino)- and 2-(4-methyl-1-piperazinyl)-6-methyl-3,4-pyridinedicarboximides (**1**, **2**) reacted with $CH_3NHNH_2$ yielding 3-methyl derivatives of 1,4-dioxo-1,2,3,4-tetrahydropyrido[3,4-d]-pyridazines (**4**, **5**). The same reaction with 1,6-dimethyl-2-oxo-1,2-dihydro-3,4-pyridinedicarboximide **3** afforded the 2-methyl derivative of 1,4,5-trioxo-1,2,3,4,5,6-hexahydro-pyrido[3,4-d]pyridazine **6**. Some of the *N*-aryl(hetero-aryl)piperazinylalkyl(hydroxyalkyl) derivatives of compounds **4** and **6** proved to be pharmacologically active (Fig. 1) [1,2].

Continuing our previous search [1–3] for biologically active pyrido[3,4-d]pyridazines and allied compounds we performed the condensation of imides **1–3** and **17** with *N*-phenylhydrazine and established the reaction course as well as the structures and properties of the formed compounds. The results obtained are described in this paper.

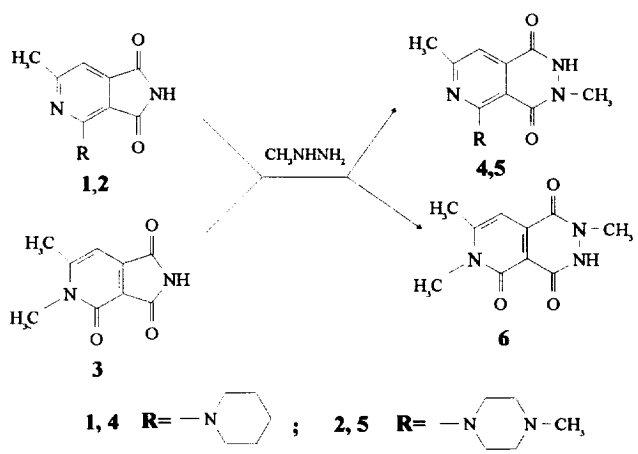


Fig. 1.

## 2. Chemistry

The reaction of imides **1–3** and **17** with an excess of *N*-phenylhydrazine was performed in boiling ethanol. Analysis of the reaction products showed that only one compound was formed in every case. The structure of *N*-phenylamino-3,4-

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pyridinedicarboximide (**7, 8**) was ascribed to this compound when using imides **1** and **2**, containing cyclic amines in position 2. Under these conditions, the 2-oxo- and 2-chloro-3,4-pyridinedicarboximides (**3**, **17**) gave the salts of the appropriate *N*-phenylpyrido[3,4-d]pyridazines with $H_2N$–$NHC_6H_5$ (**13** or **13a** and **18** or **18a**) (Fig. 2).

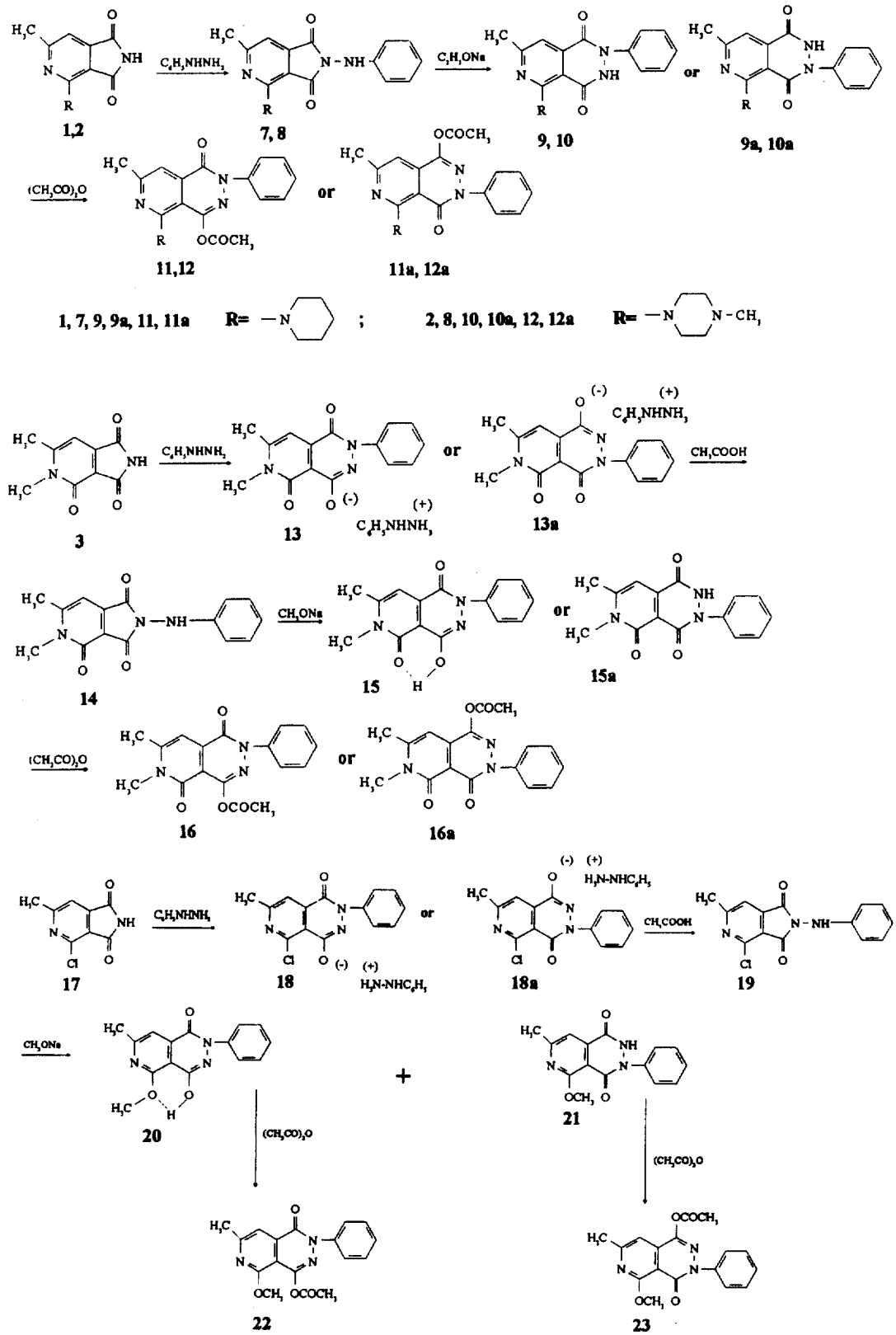


Fig. 2.

Table 1
Properties of the investigated compounds

| Comp. | Formula (molecular wt.) | M.p. (°C) (solvent) | Yield (%) | IR absorptions in KBr ($cm^{-1}$) | | | |
|---|---|---|---|---|---|---|---|
| | | | | CO | –NH(OH), $H_3N^+$ | Monosubst. benzene | C–O–C |
| **7** | $C_{19}H_{20}N_4O_2$ (336.38) | 202–205 (ethanol) | 55 | 1720, 1775 | 3180, 3280 | 700, 750 | – |
| **8** | $C_{19}H_{21}N_5O_2$ (351.40) | 168–171 (ethanol) | 35–40 | 1720, 1770 | 3190, 3300 | 690, 740 | – |
| **9** | $C_{19}H_{20}N_4O_2$ (336.38) | 207–209 (ethanol) | 90 | 1640, 1670 | 2700–3100 | 700, 750 | – |
| **10** | $C_{19}H_{21}N_5O_2$ (351.40) | 225–228 (ethanol) | 65 | 1660 | 2800–3320 | 705, 770 | – |
| **11** | $C_{21}H_{22}N_4O_3$ (378.42) | 151–154 (ether/pet. ether) | 88 | 1670, 1775 | – | 690, 750 | 1070–1080, 1170–1180 |
| **12** | $C_{21}H_{23}N_5O_3$ (393.40) | 196–198 (ether/pet. ether) | 67 | 1670, 1770 | – | 680, 750 | 1080–1090, 1160 |
| **13** | $C_{21}H_{21}N_5O_3$ (391.42) | 209–210 (ethanol) | 90 | 1660, 1690 | 2900–3320 | 690, 745 | – |
| **14** | $C_{15}H_{13}N_3O_3$ (283.28) | 256–259 (without crystall.) | 70 | 1730, 1780 | 3300 | 700, 770 | – |
| **15** | $C_{15}H_{13}N_3O_3$ (283.28) | 235–236 (ethanol) | 71 | 1650, 1680 | 2900–3300 | 695, 760 | – |
| **16** | $C_{17}H_{15}N_3O_4$ (325.31) | 230–231 (anhydrous benzene) | 44 | 1650 (broad), 1770 | – | 700, 750 | 1100, 1190 |
| **18** | $C_{20}H_{18}N_5O_2Cl$ (395.84) | 197–199 (ethanol) | 90 | 1660 | 2920–3400 | 700, 750 | – |
| **19** | $C_{14}H_{10}N_3O_2Cl$ (287.71) | 172–174 (ethanol) | 55 | 1730, 1780 | 3300 | 700, 750 | – |
| **20** | $C_{15}H_{13}N_3O_3$ (283.28) | 245–247 (methanol) | 25 | 1630, 1670 | 2800–3350 | 700, 735 | – |
| **21** | $C_{15}H_{13}N_3O_3$ (283.28) | 255–256 (without crystall.) | 10–15 | 1600–1615, 1660 | 2600–3260 | 685, 700, 750 | – |
| **22** | $C_{17}H_{15}N_3O_4$ (325.31) | 198–199 (without crystall.) | 44 | 1680, 1770 | – | 690, 740–750 | 1090, 1170–1200 |
| **23** | $C_{17}H_{15}N_3O_4$ (325.31) | 167–168 (without crystall.) | 44 | 1700, 1765 | – | 680-695, 750 | 1050, 1150, 1180 |

The structures of all the above-mentioned compounds were confirmed by elemental and spectral analyses (IR, $^1$H NMR). In the IR spectrum, the *N*-phenylamino-3,4-pyridinedicarboximides (**7, 8**) exhibited two carbonyl bands at almost the same frequencies as those of the starting imides: 1720, 1775 (**7**), 1720, 1770 (**8**) $cm^{-1}$, while in the salts the carbonyl peaks were, as expected, shifted towards longer waves and occurred at 1660, 1690 (**13** or **13a**), 1660 (**18** or **18a**) $cm^{-1}$. The latter supported the fact that the corresponding pyrido[3,4-d]pyridazines took part in the formation of the salts. Furthermore, IR and $^1$H NMR spectra indicated the presence of the phenyl substituent in all investigated compounds (see Section 3). During boiling in 80% acetic acid, the salts were transformed into *N*-phenylaminoimides (**14, 19**), probably as a result of isomerization of the intermediary pyrido[3,4-d]pyridazines in acid medium. The structures of compounds **14** and **19** were assumed on the basis of the above-mentioned criteria. The *N*-phenylaminoimides **7, 8, 14** and **19** isomerized to the corresponding *N*-phenyl derivatives of the 1,4-dioxo(1,4,5-trioxo)-1,2,3,4-tetra(1,2,3,4,5,6-hexa)hydropyrido[3,4-d]pyridazines (**9** or **9a**, **10** or **10a**, **15** or **15a**, **20** and **21**) during boiling in ethanol or methanol solution of sodium ethoxide (**7, 8**) or methoxide (**14, 19**). Under these conditions the imides **7, 8** and **14** gave one compound among two possible ones from the theoretical point of view (phenyl in position 2 or 3 of the pyridazine ring). Only in the case of imide **19** was the formation of two isomers (**20** and **21**), having a methoxyl group in position 5, observed when 2 mol of $CH_3ONa$ were used for 1 mol of **19**. This indicated that during the heating the exchange of a chlorine atom for the methoxyl group also took place. The first isomer (m.p. 245–247°C) was isolated mainly by the crystallization of the reaction product from methanol. The second (m.p. 255–256°C) and the rest of the first isomer were separated by means of column chromatography. The pyridopyridazine structure of all the compounds obtained confirmed, among others, the location of the carbonyl peaks in their IR spectra, given in Table 1.

In order to determine the exact structures (phenyl in position 2 or 3) the obtained products were submitted to reaction with acetic anhydride. Crystalline substances were isolated in every case. In the $^1$H NMR spectrum they showed, among others, a three-proton singlet at $\delta = 2.4$ (**11** or **11a**), 2.39 (**12** or **12a**), 2.43 (**16** or **16a**), 2.37 and 2.44 (**22** and **23**), which according to the earlier findings [1] indicated the presence of the $-OCOCH_3$ group (not $N-COCH_3$) in the molecule of pyrido[3,4-d]pyridazine.

A further problem was the determination of the exact position of the $-OCOCH_3$ group (1 or 4). It was solved by means of $^1$H NMR spectroscopy. Similarly, as previously [1], we took into consideration the known fact that the value of the chemical shift for H-8 depends (among others) on the chemical character of the group in position 1. In the acetyl derivatives of compounds **9** or **9a**, **10** or **10a**, **15** or **15a**, **20** or **21** (m.p. 245–247°C), H-8 absorbed together with the aromatic protons the same as in the starting substances. Only in the $^1$H NMR spectrum of isomer, m.p. 255–256°C, was a high field shift of the signal for H-8 observed ($\delta = 6.8$ ppm). This

indicated the change in the chemical character of the group in position 1 and allowed us to assign the structure **23** for the acetyl derivative of the isomer having the higher melting point. According to the above, structures **11**, **12**, **16** and **22** could be ascribed for the other acetyl derivatives. In this way it was established that *N*-phenylaminoimides **7**, **8** and **14** isomerized in alkaline medium giving 2-phenyl isomers (**9**, **10**, **15**), while **19** formed a mixture of two isomeric compounds (**20** and **21**). The substance with the lower melting point contains phenyl in position 2 (**20**), whereas the isomer having the higher melting point is the 3-phenyl derivative of the corresponding pyrido[3,4-d]pyridazine (**21**).

The $^1$H NMR spectra of both isomers are similar. The only essential difference consists in the shift of the signal of the O*H* proton (enolic form of **20**) to a lower field ($\delta = 10.5$ ppm) in comparison with the same signal in **21** ($\delta = 8.91$ ppm).

This shift to a lower field may be explained by the engagement of this proton in the intramolecular hydrogen bond, as shown in Fig. 2. In like manner it can be interpreted as the low field absorption for the same proton in compound **15** ($\delta = 11.81$ ppm).

## 3. Experimental

### *3.1. Chemistry*

All the results of the C, H, N determinations were within $\pm 0.4\%$ of the values calculated for the corresponding formulae.

All melting points are uncorrected. IR absorption spectra were determined in KBr pellets; $^1$H NMR spectra were recorded using TMS as internal standard, in $CDCl_3$, when not otherwise indicated.

#### *3.1.1. General procedure for reaction of imides 1–3 and 17 with N-phenylhydrazine (compounds 7, 8, 13, 18)*

2 g of *N*-phenylhydrazine were added to 1 g of the appropriate imide (**1–3**, **17**) in 40 ml of anhydrous ethanol. The reaction mixture was refluxed for 22 h (imides **1** and **2**) and 6 h (**3** and **17**). Then, in the case of **1** and **2**, ethanol was distilled off to about 1/3 of its volume and left to crystallize. The separated product (**7** or **8**) was collected on a filter and purified by crystallization from the solvent indicated in Table 1.

In the case of imides **3** and **17** the solid (**13**, **18**) was precipitated during heating of the reaction mixture. After cooling it was collected on a filter and washed with ethanol. For analysis, a small amount of the substances **13** and **18** was crystallized from ethanol. For further synthesis raw **13** and **18** were used.

The properties of compounds **7**, **8**, **13** and **18** are listed in Table 1 but the assignments in the $^1$H NMR spectra of **7**, **8** and **18** are presented below. $^1$H NMR of **7**: $\delta$ 1.69 (m, 6H, H of piperidine), 2.54 (s, 3H, C*H*$_3$ in 6), 3.79–3.95 (m, 4H, H of piperidine), 6.75–7.64 (m, 6H, arom. H and H in 8). $^1$H NMR of **8**: $\delta$ 2.33 (s, 3H, $CH_3$ in piperazine), 2.35–3.00 (m, 7H C*H*$_3$ in 6 and H of piperazine), 3.89–4.27 (m, 4H, H of piperazine), 6.48–7.47 (m, 6H, arom. H and H in 8), 8.92 (s broad, 1H, N*H*). $^1$H NMR of **18** ($CDCl_3$ + DMSO-$d_6$): $\delta$ 2.54 (s, 3H, C*H*$_3$ in 7), 6.75–7.51 (m, 11H, arom. H and H in 8), 10.07–10.47 (3 broad bands, $NH_3^+$).

The proton signal for the NH group was not found in the $^1$H NMR spectra of **7** and **18**.

#### *3.1.2. General procedure for synthesis of compounds 14 and 19*

2 g of compound **13** (or **18**) were refluxed in 30 ml of 80% acetic acid for 15 min after dissolution in this solvent. Then, in the case of **13**, acetic acid was distilled off under diminished pressure and the solid residue was boiled in ethanol. The insoluble product was collected on a filter. It was pure without recrystallization. In the case of compound **18** the separated product was collected on a filter and purified by crystallization from ethanol.

The properties of compounds **14** and **19** are given in Table 1 but the assignments in the $^1$H NMR spectrum of **19** are as follows: $\delta$ 2.75 (s, 3H, C*H*$_3$ in pyridine), 6.56–7.57 (m, 6H, arom. H and H in pyridine); the proton signal for the NH group was not found.

#### *3.1.3. General procedure for isomerization of N-phenylamino-3,4-pyridinedicarboximides 7, 8, 14 and 19 (compounds 9, 10, 15, 20, 21)*

0.2 g of sodium was dissolved in 140 ml of anhydrous ethanol and 2 g of compound **7** or 2.1 g of compound **8** were introduced to this solution. The reaction mixture was refluxed for 18.5 h. Then the ethanol was distilled off in a rotary evaporator. The solid residue was dissolved in distilled water and the aqueous solution was acidified with 80% acetic acid. The separated product (only in the case of **9**) was collected on a filter and after drying purified by crystallization from ethanol.

In the case of **10** the solvent was evaporated under diminished pressure from the acid filtrate and the residue was boiled in diethyl ether. After cooling, the separated product was collected on a filter, washed with water and dried. Then it was crystallized from ethanol. Isomerization of imides **14** and **19** was performed in a methanol solution of sodium methoxide using 0.47 g of sodium in 250 ml of anhydrous methanol for 5.8 g of imide **14** and 0.33 g of sodium in 70 ml of anhydrous methanol for 2 g of imide **19**. The reaction mixtures were refluxed for 5 h (**14**) and for 31 h (**19**). The solid substance was precipitated during the heating in both cases. Then the precipitate was collected on a filter and dissolved in a small amount of distilled water. The aqueous solution was acidified with 80% acetic acid (**14**) or concentrated hydrochloric acid (**19**). The separated product was filtered off, washed with water and dried. Compounds **15** and **20** were purified by crystallization from the solvents given in Table 1.

After separation of the sodium salt of **20**, the methanol was evaporated to dryness and the solid was dissolved in distilled water and acidified with concentrated hydrochloric acid. The precipitate after filtering was chromatographed on a column filled with silica gel (70–230 mesh) using diethyl ether as eluant. Two fractions were collected. The first fraction ($R_f$=0.65) was evaporated to afford the rest of the compound **20** (m.p. 244–247°C) whereas the second fraction ($R_f$=0.48) gave **21** after evaporation (m.p. 255–256°C).

The properties of compounds **9**, **10**, **15**, **20** and **21** are listed in Table 1 but the assignments in their $^1$H NMR spectra are presented below.$^1$H NMR of **9**: δ 1.79–1.87 (m, 6H, H of piperidine), 2.71 (s, 3H, C*H*$_3$ in 7), 3.11–3.23 (m, 4H, H of piperidine), 7.27–7.66 (m, 6H, H arom. and H-8), 8.01 (s, 1H, O*H* in pyridazine).$^1$H NMR of **10**: δ 2.42 (s, 3H, C*H*$_3$ in piperazine), 2.70–2.78 (m, 7H, C*H*$_3$ in 7 and H of piperazine), 3.23–3.34 (m, 4H, H of piperazine), 7.26–7.65 (m, 6H, arom. H and H-8), 8.00 (s, 1H, O*H* in pyridazine).$^1$H NMR of **15**: δ 2.58 (s, 3H, C*H*$_3$ in 7), 3.7 (s, 3H, C*H*$_3$–N), 7.12–7.67 (m, 6H, arom. H and H-8), 11.81 (s, 1H, O*H* in pyridazine).$^1$H NMR of **20**: δ 2.62 (s, 3H, C*H*$_3$ in 7), 4.16 (s, 3H, OC*H*$_3$), 7.32–7.73 (m, 6H, arom. H and H-8), 10.5 (s, 1H, O*H* in pyridazine).$^1$H NMR of **21**: δ 2.60 (s, 3H, C*H*$_3$ in 7), 4.12 (s, 3H, OC*H*$_3$), 7.25–7.65 (m, 6H, arom. H and H-8), 8.91 (s, 1H, O*H* in pyridazine).

### *3.1.4. General procedure for obtaining acetyl derivatives **11, 12, 16, 22 and 23***

0.3 g of compound **9** (**10**, **15**, **20**, **21**) was treated with 10 ml of acetic anhydride and refluxed for 1 h. Then the excess of anhydride was evaporated under diminished pressure (compounds **11**, **12** and **16**) and a small amount of water was added to the residue. The crystalline product was collected on a filter and after drying purified by crystallization from the solvent given in Table 1. Compound **22** precipitated after cooling of the reaction mixture whereas **23** crystallized in a refrigerator after evaporation of the acetic anhydride to a small volume under diminished pressure. The solid substance was collected on a filter and washed with petroleum ether.

The properties of compounds **11**, **12**, **16**, **22** and **23** are given in Table 1 but the assignments in their $^1$H NMR spectra are presented below.$^1$H NMR of **11**: δ 1.70 (m, 6H, H of piperidine), 2.4 (s, 3H, –OCOC*H*$_3$), 2.59 (s, 3H, C*H*$_3$ in 7), 3.35 (m, 4H, H of piperidine), 7.26–7.70 (m, 6H, arom. H and H-8).$^1$H NMR of **12**: δ 2.35 (s, 3H, C*H*$_3$ in piperazine), 2.39 (s, 3H, –OCOC*H*$_3$), 2.40–2.58 (m, 7H, C*H*$_3$ in 7 and H of piperazine), 3.51–3.59 (m, 4H, H of piperazine), 7.32–7.73 (m, 6H, arom. H and H in pyridine).$^1$H NMR of **16**: δ 2.43 (s, 3H, –OCOC*H*$_3$), 2.53 (s, 3H, C*H*$_3$ in 7), 3.59 (s, 3H, C*H*$_3$–N), 7.00–7.62 (m, 6H, arom. H and H-8).$^1$H NMR of **22**: δ 2.37 (s, 3H, –OCOC*H*$_3$), 2.62 (s, 3H, C*H*$_3$ in 7), 4.09 (s, 3H, –OC*H*$_3$), 7.38–7.71 (m, 6H, arom. H and H-8).$^1$H NMR of **23**: δ 2.44 (s, 3H, –OCOC*H*$_3$), 2.61 (s, 3H, C*H*$_3$ in 7), 4.15 (s, 3H, –OC*H*$_3$), 6.8 (s, 1H, H in 8), 7.27–7.65 (m, 5H, arom. H).

## *3.2. Pharmacology*

Compounds **9**, **14**, **16** and **19** were investigated pharmacologically.

### *3.2.1. Material and methods*

The experiments were carried out on male and female Albino-Swiss mice (body weight 20–25 g) and male Wistar rats (200–250 g). Investigated compounds were administered intraperitoneally (i.p.) as suspensions in 3% Tween 80 in a constant volume of 10 ml/kg in mice and 5 ml/kg in rats. The compounds were administered in doses equivalent to 1/10, 1/20, 1/40 or 1/80 of their $LD_{50}$. For compounds **9** and **14** which had $LD_{50}$ > 2000 mg/kg, 2000 mg/kg was taken to be the initial dose. 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 plates' test in mice.
8. Pentetrazol-induced seizures in mice.
9. Maximal electric shock in mice.
10. Head twitches induced by 5-hydroxytryptophane in mice.
11. Arterial blood pressure in rats.

Acute toxicity was assessed by the methods of Litchfield and Wilcoxon [4] and presented as $LD_{50}$ calculated from the mortality of mice after 24 h.

Motor coordination was measured according to the method of Gross et al. [5]. 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 administration of the investigated compounds.

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

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

Pain reactivity was measured by the 'writhing syndrome' test of Koster et al. [6]. 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 injection of 0.6% acetic acid.

Pain reactivity was also measured in the 'hot-plate' test according to the method of Eddy and Leimbach [7]. 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. The experiments were performed 30 min after administration of the investigated compounds.

Anxiolytic properties were assessed by the 'four plates' test in mice, according to Aron et al. [8], 60 min after administration of the investigated compounds in doses which had no effect on the spontaneous locomotor activity. Mice were placed in the cages with four plate floors (11×7 cm) with a 4 mm gap between each. After 15 s of adaptation the number of crossing 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.

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

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

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

Arterial blood pressure was determined according to the method of Gerold and Tschirky [10] 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.

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

## 4. Results and discussion

The $LD_{50}$ values (i.p. in mice) for the tested compounds **9**, **14**, **16** and **19** are listed in Table 2. The highest toxicity was shown by compound **19** ($LD_{50}$=232.1 mg/kg), while **9** and **14** were not toxic ($LD_{50}$>2000 mg/kg), and **16** was characterized by a moderate toxicity. None of the investigated compounds in doses equivalent to 1/10 $LD_{50}$ (200 (**9**), 200 (**14**), 144.2 (**16**) and 23.2 (**19**) mg/kg) showed neurotoxic properties, as they did not affect the motor coordination in the rota-rod test. Derivatives **9** and **14** strongly suppressed the spontaneous locomotor activity of mice during the 1 h observation period up to the dose of 1/40 $LD_{50}$ (50 mg/kg). **16** was active up to the dose of 1/20 $LD_{50}$ (72.1 mg/kg). Only compound **19** did not affect spontaneous locomotor activity (Table 3). Furthermore, **9** decreased the excitatory effect of amphetamine in mice at the highest dose used (1/10 $LD_{50}$=200 mg/kg). The other compounds were inactive in this test (Table 4). Pyrido[3,4-d]pyridazine derivatives **9** and **16** showed strong analgesic activity in the 'writhing syndrome' test up to the dose of 1/40 $LD_{50}$ (50 (**9**) and 36.05 (**16**) mg/kg). The analgesic action of **9** and **16** was also confirmed in the 'hot-plate' test; **9** was active up to the dose of 1/20 $LD_{50}$ (100 mg/kg); **16** was active only in the highest dose used (1/10 $LD_{50}$=144.2 mg/kg). Imide derivatives **14** and **19** were devoided of analgesic activity in both tests (Tables 5 and 6). In the remaining tests all the investigated compounds were inactive.

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

| Comp. | $LD_{50}$ (mg/kg i.p.) | Confidence limits |
|---|---|---|
| **9** | >2000.0 | |
| **14** | >2000.0 | |
| **16** | 1442.2 | 1218.0–1707.8 |
| **19** | 232.1 | 157.0–343.2 |

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

| Comp. | Dose (part of $LD_{50}$) | No. of impulses ± SEM after | |
|---|---|---|---|
| | | 30 min | 60 min |
| Control | – | 291.5±27.7 | 406.6±42.2 |
| **14** | 1/10 | 96.7±18.6*** | 118.5±16.8*** |
| | 1/20 | 104.1±23.6*** | 143.2±19.7*** |
| | 1/40 | 150.4±23.2** | 175.8±33.2** |
| | 1/80 | 264.7±68.6 | 421.1±72.1 |
| **19** | 1/10 | 307.2±61.4 | 435.2±78.3 |
| Control | – | 346.0±47.4 | 552.6±69.3 |
| **9** | 1/10 | 172.6±46.2** | 242.0±74.7*** |
| | 1/20 | 103.4±11.8*** | 140.6±14.1*** |
| | 1/40 | 124.4±12.9*** | 175.6±21.9*** |
| | 1/80 | 244.1±52.5 | 430.7±75.2 |
| **16** | 1/10 | 134.3±26.7*** | 224.6±36.3*** |
| | 1/20 | 244.3±22.0* | 327.1±40.8** |
| | 1/40 | 270.1±84.0 | 479.1±99.8 |

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

Table 4
Influence of the investigated compounds on the amphetamine-induced locomotor hyperactivity in mice ($n$=6–8)

| Comp. | Dose (part of $LD_{50}$) | No. of impulses ± SEM after | |
|---|---|---|---|
| | | 30 min | 60 min |
| Control | – | 447.80±72.20 | 842.50±219.00 |
| **14** | 1/10 | 261.50±56.3 | 567.30±118.8 |
| **19** | 1/10 | 428.30±124.6 | 951.20±200.9 |
| Control | – | 404.75±62.92 | 672.62±116.91 |
| **9** | 1/10 | 170.00±23.04** | 378.67±47.91* |
| | 1/20 | 558.37±152.51 | 1216.12±304.32 |
| **16** | 1/10 | 417.50±137.54 | 884.12±283.15 |

*$p$<0.05, **$p$<0.01.

Table 5
Influence of the investigated compounds on the pain reactivity in the 'writhing syndrome' test in mice ($n=6$–8)

| Comp. | Dose (part of $LD_{50}$) | Mean no. of writhings ± SEM |
|---|---|---|
| **Control** | – | 5.37 ± 0.96 |
| **14** | 1/10 | 3.55 ± 0.41 |
| **19** | 1/10 | 3.89 ± 0.76 |
| **Control** | – | 5.25 ± 0.69 |
| **9** | 1/10 | 0*** |
| | 1/20 | 1.62 ± 0.86** |
| | 1/40 | 0.75 ± 0.53*** |
| | 1/80 | 3.37 ± 1.12 |
| **16** | 1/10 | 0.25 ± 0.25*** |
| | 1/20 | 2.37 ± 0.86** |
| | 1/40 | 1.12 ± 0.44*** |
| | 1/80 | 4.62 ± 0.62 |

**$p<0.01$, ***$p<0.001$.

Table 6
Influence of the investigated compounds on the pain reactivity in the 'hot-plate' test in mice ($n=8$)

| Comp. | Dose (part of $LD_{50}$) | Time of reaction on pain stimulus ± SEM (s) |
|---|---|---|
| **Control** | – | 5.44 ± 0.77 |
| **14** | 1/10 | 7.50 ± 1.16 |
| **19** | 1/10 | 5.19 ± 0.45 |
| **Control** | – | 4.19 ± 0.64 |
| **9** | 1/10 | 7.73 ± 0.47*** |
| | 1/20 | 8.19 ± 1.13** |
| | 1/40 | 5.69 ± 0.92 |
| **16** | 1/10 | 8.31 ± 0.84** |
| | 1/20 | 5.90 ± 0.91 |

**$p<0.01$, ***$p<0.001$.

The pharmacological results obtained clearly indicated that *N*-phenylamino-2-chloro-6-methyl-3,4-pyridinedicarboximide **19** had no biological activity in the tests performed and was the most toxic of the compounds investigated, while *N*-phenylamino-2-oxo-1,6-dimethyl-1,2-dihydro-3,4-pyridinedicarboximide **14** was non-toxic. It only suppressed spontaneous locomotor activity.

The pyrido[3,4-d]pyridazines **9** and **16** proved to be the relatively most pharmacologically active compounds. Contrary to imides, they exhibited analgesic properties in both tests and significantly inhibited the spontaneous locomotor activity of mice.

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