Full Paper

Synthesis and Pharmacological Evaluation of *N*-(Dimethylamino)ethyl Derivatives of Benzo- and Pyridopyridazinones

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New *N*-(dimethylamino)ethyl derivatives of phthalazinones and pyridopyridazinones **7**, **9** were synthesized and assayed as potential analgesic agents in the hot-plate, tail-flick, and writhing tests. Pharmacological assay demonstrated that eight (in ten) of the newly synthesized compounds showed antinociceptive activity. Especially, 2-[2-(dimethylamino)ethyl]-4-phenyl-2*H*-phthalazin-1-one **7a** showed remarkably higher antinociceptive activity in all tests. This is connected with influence on supraspinal, spinal, and peripheral structures. The decreased sensitivity to the pain stimulus in the hot-plate was higher than that of metamizole.

Keywords: Hot-plate test / Phthalazinones / Pyridopyridazinones / Tail-flick test / Writhing test

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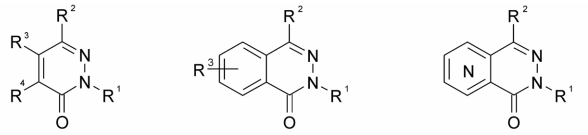
Introduction

During the last decade, a lot of attention has been given to the study focused on the construction of 2*H*-pyridazin-3-ones type **A** (Fig. 1) as well as their biological activity [1].

It has been observed that the derivatives of 2*H*-pyridazin-3-ones exhibit a number of different pharmacological activity *e.g.*, adenosine A_1 receptor antagonist [2], α-1 adrenoreceptors antagonist [3 – 5], anti-aggregation activity [6], antinociceptive agents [7 – 9], and nonprostanoid PGI_2 agonist [10]. It has been found that some of derivatives of phthalazinones exhibit potent inhibition of phosphodiesterase (PDE4) activity [11] and agonist activity to dopamine D_2 and serotonin 5-HT_2 receptors [12]. The research studies of the last years also show that a few of the analogues of phthalazinones inhibit HIV replication [13].



A **B** **C**

R^1, R^2, R^3, R^4=H, alkyl, aryl, heteroaryl, halogen, -$(CH_2)_n$-amine

Figure 1. Derivatives of 2*H*-pyridazin-3-ones type **A** and of benzo- **B** and pyridopyridazinones **C** types with a variety of substituents.

As part of the study for new analgesics and nonsteroidal antiinflammatory agents (NSAIDs), we initiated a research program based on the study related to synthesis of new derivatives of benzo- **B** and pyridopyridazinones **C** with variety of substituents. The well-documented antinociceptive activity of the systems **A** which are the core for the structures **B** and **C** (Fig. 1) dictated the selection of these compounds, with an idea to extent the knowledge concerned with their analgesic and NSAID pharmacological properties. Some of them were found to have analgesic and nonsteroidal antiinflammatory activity, as was shown in this preliminary communication.

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Abbreviation: percent of maximal possible effect (% MPE)

Table 1. Acute toxicity of compounds **7**, **8**, and **9** after *per-os* administration.

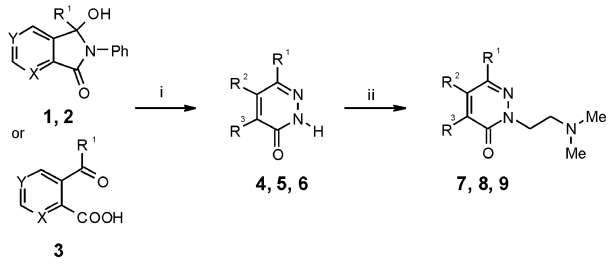
Compound	**7a**	**7b**	**7c**	**7d**	**7e**	**7f**	**7g**	**8**	**9a**	**9b**
LD_{50} (mg/kg b.w./*p.o.*)	200	400	400	750	500	375	150	600	1000	300

Results and discussion

Chemistry

N-[2-(Dimethylamino)ethyl] derivatives of 2*H*-pyridazin-3-one and benzo-, and pyrido- analogues (compounds **7**, **8**, and **9**) were synthesized as illustrated in Scheme 1. As starting materials for the synthesis of the amines **7**, **8**, and **9** the previously described 2*H*-phthalazin-1-ones **4** [14], pyridopyridazinones **6** [15] and commercially available 2*H*-pyridazin-3-one **5** were used.

Aminoalkylation of the appropriate lactams **4**, **5**, or **6** with the (2-chloroethyl)dimethylamine, via most commonly used methodology [16] afforded the final compounds **7**, **8**, **9** in good yields. IR, NMR, HRMS, and microanalyses confirmed the structures of the aminoethyl derivatives **7**, **8**, and **9**. Melting points, yields, and spectral data are shown in Experimental (Section 4). For pharmacological assays, compounds **7a–g**, **8**, **9a** and **9b** were converted to their water-soluble hydrochlorides.



i: $NH_2NH_2 H_2O$, propan-1-ol, Δ; ii: MeONa / MeOH, Δ; $ClCH_2CH_2NMe_2$ HCl, MeOH, Δ

For compounds **4**, **6**, **7**, **9** R^2, R^3=

1, **4**, **7** **a** R^1=Ph, X=Y=CH,
b R^1=2-(MeO)C_6H_4, X=CH, Y=C(OMe),
c R^1=2Py, X=CH, Y=C(OMe),
d R^1=3Py, X=CH, Y=C(OMe),
e R^1=4Py, X=CH, Y=C(OMe),
f R^1=Me, X=Y=CH,
g R^1=H, X=Y=CH;
3 **a** R^1=Ph, X=Y=CH, **b** R^1=Me, X=Y=CH;
5, **8** R^1=R^2=R^3=H;
2, **6**, **9** **a** R^1=H, X=N, Y=CH, **b** R^1=H, X=CH, Y=N.

Scheme 1. Synthesis of *N*-[2-(Dimethylamino)ethyl] derivatives of 2*H*-pyridazin-3-one and benzo-, and pyrido- analogues (compounds **7**, **8** and **9**).

Pharmacology

Acute toxicity

After a single *p.o.* administration of the compounds at various doses, the observations were carried out for a period of 14 days. The LD_{50} values of the investigated compounds **7a–g**, **8**, **9a**, **b** varied and ranged from ~150 to 1000 mg/kg (Table 1). The most toxic compound was **7g** (ALD_{50} = 150 mg/kg). The least toxic compound was **9a** (ALD_{50} = 1000 mg/kg). The toxicity of the remaining compounds fluctuated between 200 and 750 mg/kg. For all derivatives 1/10 LD_{50} was taken as the initial dose in the further experiments. Toxic doses of the all tested compounds caused sedation and depression of locomotive activity of mice.

Analgesic properties

The analgesic activities of the newly synthesized compounds: **7a–g**, **8**, **9a** and **9b** in this study were measured using the hot-plate and tail-flick tests, and the acetic acid-induced writhing assay in mice.

Effect of compounds on the hot-plate response

The % MPE (percent of maximal possible effect) obtained for the all compounds are presented in Table 2. The results of the hot-plate test after i.p. administration of compounds **7a**, **7b**, **7c**, **7e**, and **7g** are shown in Fig. 2.

Compound **7a**, at the dose of 20 mg/kg, significantly prolonged the latency to pain reaction after administration. The% MPE obtained for the compound **7a** was ranged 32.45 (measured after 60 min), 35.09 (measured after 120 min), and 39.84 (measured after 180 min). This result was significant ($p < 0.05$, see Table 2) compared to the zero measurement and comparable or higher than metamizole. Compounds **7b** and **7c**, at doses 40 mg/kg, significantly increased the time to pain reaction in animals only after 180 min (% MPE obtained for **7a** = 40.86, **7c** = 30.37, $p < 0.05$, see Table 2). Compound **7e**, at the dose of 50 mg/kg, prolonged significantly the latency of nociceptive reaction to the hot-plate test in mice at every measurement time. The% MPE obtained for the compound was ranged 16.00 (measured after 60 min), 24.59 (measured after 120 min), and 25.99 (measured after 180 min, Table 2). Compound **7g**, at the dose of 15 mg/kg, prolonged nociceptive reaction latency after 60 and 90 min (% MPE 18.95 and 16.05, respectively). This result was significant ($p < 0.05$, see Table 2) compared to the zero measurement. Compounds **7d**, **7f**, **8**, and **9b** did not demonstrate a significant effect in the test.

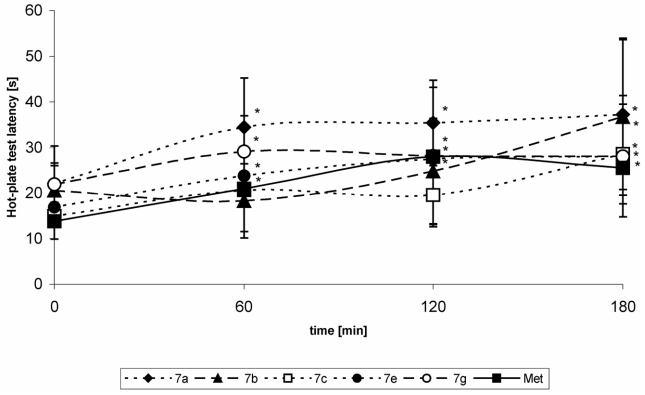


Figure 2. Results of the hot-plate test after i.p. administration of compounds **7a**, **7b**, **7c**, **7e** and **7g**.

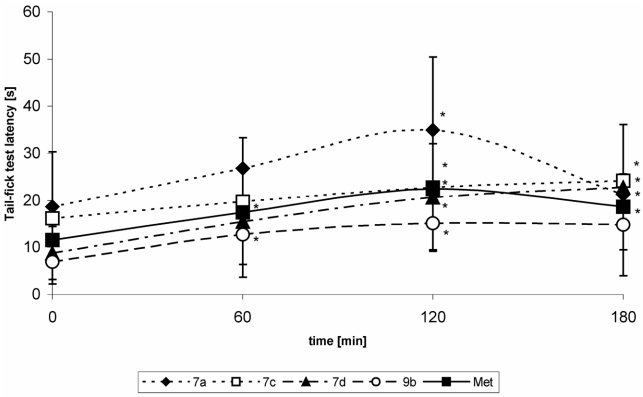


Figure 3. Results of the tail-flick test after i.p. administration of compounds **7a**, **7c**, **7d** and **9b**.

Table 2. The effects of compounds **7**, **8** and **9** in the mouse hot-plate test.

Compound	Dose (mg/kg b.w., i.p.)	Latency (% MPE) to paw licking or jumping		
		after 60 min	after 120 min	after 180 min
7a	20	32.45[a, b)]	35.09[a)]	39.84[a, b)]
7b	40	0	10.66	40.86[a, b)]
7c	40	12.86	10.42	30.37[a)]
7d	75	3.57	1.63	18.85[a)]
7e	50	16.00[a)]	24.59[a)]	25.99[a)]
7f	37.5	13.97	12.42	2.88
7g	15	18.95[a)]	16.05[a)]	16.00
8	60	0	0	0
9a	100	4.98	8.87	3.03
9b	30	1.10	3.29	5.27
Met	500	15.58[a)]	30.74[a)]	25.54[a)]

Each group consisted of eight to ten animals.
[a)] Significant differences versus initial value, $p < 0.05$.
[b)] Significant differences from the metamizole (Met)-treated group, $p < 0.05$.

Table 3. The effects of compounds **7**, **8** and **9** in the mouse tail-flick test.

Compound	Dose (mg/kg b.w., i.p.)	Latency (% MPE) to paw licking or jumping		
		after 60 min	after 120 min	after 180 min
7a	20	19.81	39.61[a, b)]	6.28
7b	40	4.05	10.45	11.72
7c	40	7.99	14.84[a)]	18.26[a)]
7d	75	13.08	23.05[a)]	27.34[a)]
7e	50	9.87	0	6.45
7f	37.5	3.58	5.26	13.26
7g	15	0	0	0
8	60	9.22	12.36	7.76
9a	100	5.69	14.22	8.94
9b	30	13.69[a)]	19.02[a)]	18.32[a)]
Met	500	12.16[a)]	22.68[a)]	14.63[a)]

Each group consisted of eight to ten animals.
[a)] Significant differences versus initial value, $p < 0.05$.
[b)] Significant differences from the metamizole (Met)-treated group, $p < 0.05$.

Effect of compounds on the tail-flick response

The % MPE obtained for all compounds are shown in Table 3. The results of the tail-flick test after i.p. administration of compounds **7a**, **7c**, **7d** and **9b** are presented in Fig. 3.

Compound **7a**, at the dose of 20 mg/kg significantly prolonged nociceptive reaction latency only 120 min after administration (% MPE 39.61, $p < 0.05$, Table 3) compared to the zero measurement. Compounds **7c** (40 mg/kg) and **7d** (75 mg/kg) prolonged nociceptive reaction latency after 120 and 180 min. The % MPE obtained for the compound **7c** was ranged 14.84 (measured after 120 min), 18.26 (measured after 180 min, $p < 0.05$, see Table 3). The% MPE obtained for the compound **7d** were 23.05 (measured after 120 min) and 27.34 (measured after 180 min, $p < 0.05$, Table 3). Compound **9b** at the dose of 30 mg/kg prolonged significantly the latency of nociceptive reaction to the tail-flick test in mice et every measurement time. The% MPE obtained for the compound was ranged 13.69 (measured after 60 min), 19.02 (measured after 120 min), and 18.32 (measured after 180 min). This result was comparable to metamizole (see Table 3). Compounds **7b**, **7e**, **7f**, **7g**, **8** and **9a** did not demonstrate a significant effect in the test.

Effect of compounds of the nociceptive response to acetic acid

Given orally at 20 mg/kg, compound **7a** caused an analgesic effect, statistically reducing the number of abdominal

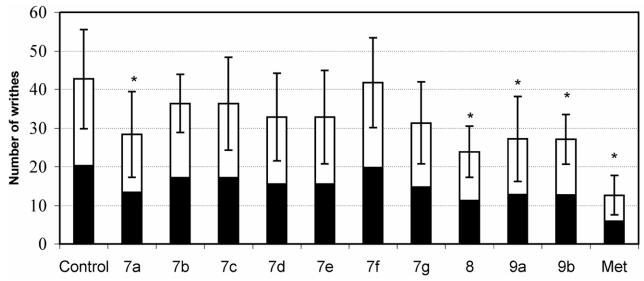


Figure 4. Effect of compounds in the writhing test.

constrictions induced by acetic acid. The most potent effect was produced by compounds **8** (60 mg/kg, *p.o.*), **9a** (100 mg/kg, *p.o.*), and **9b** (30 mg/kg, *p.o.*). The strongest action in the test was compound **8**, reducing nearly twofold the number of abdominal constrictions in comparison with animals receiving acetic acid (controls), however, two times weaker in comparison with mice, which received metamizole (Met). Compounds **7b**, **7c**, **7d**, **7e**, **7f**, and **7g** did not demonstrate a significant effect in the writhing test. The data are shown in Fig. 4.

The analgesic properties of the compounds obtained in this study were assessed using three tests. In the hot-plate test, the nociceptive reaction is coordinated predominantly by supraspinal structures, whereas the tail-flick test allows assessing the involvement of spinal cord structures in a nociceptive reaction [17]. The writhing test measures peripheral analgesia [18].

The efficiency of the tested compounds **7**, **8**, and **9** proved to be dependent upon their structures. From amongst of the compounds, **7a** appeared to be effective in all tests and exhibited the highest antinociceptive activity. In the hot-plate test, this compound proved to be even more active than the known metamizole. All other tested compounds proved to be effective in two tests or at least in one as systematized below:

Compound **7c** in the hot-plate and tail-flick tests, **9b** in tail-flick and writhing tests, **7b** and **7c** only in the hot-plate test, **7d** in the tail-flick test, **8** and **9a** in the writhing test. Two of the tested compounds, **7f** and **7g**, did not show any activity in the applied tests.

Conclusion

We can conclude the following: Eight (in a ten) of the newly synthesized compounds showed antinociceptive activity. Compound **7a** (2-[2-(dimethylamino)ethyl]-4-phenyl-2*H*-phthalazin-1-one) showed remarkably potent antinociceptive activity in all (three) tests. This is connected with influence on supraspinal, spinal, and peripheral structures as antinociceptive systems. The decreased sensitivity to the pain stimulus in the hot-plate was higher than to metamizole. Compound **7e** demonstrated a strong antinociceptive effect in the hot-plate test. Compounds **7d** and **9b** showed a significant effect in the tail-flick test. This result was comparable to metamizole.

Compounds **8**, **9a**, and **9b** exhibited an antinociceptive effect in the writhing test. Compound **8** showed here the strongest action.

Compound **7a** showed the highest activity of all newly synthesized compounds; this is probably due to the phenyl group attached to the 4-position of the phthalazinone ring. The observation that removal of the phenyl ring or replacing it by a methyl group or a pyridine nucleus caused a decrease of the antinociceptive activity suggests this point of view.

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The authors have declared no conflict of interest.

Experimental

Chemistry

The melting points were determined on a Boetius hot stage apparatus (VEB Analytic, Dresden, Germany) and are uncorrected. ^{1}H-NMR spectra were recorded at 200 MHz and ^{13}C-NMR spectra at 50 MHz on a Varian Gemini 200 BB spectrometer (Varian Inc., Palo Alto, CA, USA) with TMS as an internal reference. IR spectra were recorded on a Nexus FT-IR spectrometer (Thermo Nicolet Co., Madison, WI, USA). Mass spectra analyses were performed on a Mass Spectrometer MAT95 – Finnigan (Thermo Electron Corporation, Bremen, Germany). The analytical thin layer chromatography tests (TLC) were carried out on Merck silica gel plates (Kieselgel 60 F_{254}, layer thickness 0.2 mm; Merck, Darmstadt, Germany) and the spots were visualized using an UV lamp.

Butyl lithium solution in hexane (Sigma-Aldrich, Poznan, Poland) was each time titrated before use. Commercially available hydrazine monohydrate, *N*,*N*,*N*',*N*′-tetramethyl-ethylenediamine, *N*,*N*-dimethylethanolamine, 2-benzoilbenzoic acid **3a**, 2-acetylbenzoic acid **3b**, 2*H*-phthalazin-1-one **4g** (all substances Sigma-Aldrich) and 2*H*-pyridazin-3-one **5** (Fluka, Poznan, Poland) were used without further purification. 3-Hydroxy-1*H*-isoindolin-1-ones **1b**–**h**, pyrrolopyridinones **2a**–**b**, 2*H*-phthalazin-1-ones **4a**–**g**, pyridopyridazinones **6a**–**b**, and (2-chloroethyl)dimethylammonium chloride were prepared according to an already reported procedure [14, 15].

*General procedure for preparation of N-[2-(dimethylamino)ethyl]-derivatives **7, 8, 9***

To the solution of sodium methoxide (1.975×10^{-2} mol) in dry methanol (50 mL) was added 2*H*-phthalazin-1-one **4** or 2*H*-pyrida-

zin-3-one **5** or pyridopyridazinone **6** (7.897×10^{-3} mol) and (2-chloroethyl)dimethylammonium chloride (1.185×10^{-2} mol). The mixture was heated at reflux for 6 hours. Afterwards, the inorganic material was collected by filtration, washed with dry methanol and, the filtrate was evaporated to dryness. The residue was subjected to column chromatography to give the pure product.

Alternatively, in order to separate the starting lactam, the residue was triturated with hydrochloric acid (c = 2 mol/L, 20 mL). The filtrate was neutralized with aqueous solution of sodium hydroxide and extracted with dichloromethane (3 × 20 mL). The combined extracts were dried over $MgSO_4$ and concentrated to dryness. The amines were separated by column chromatography.

*2-[2-(Dimethylamino)ethyl]-4-phenyl-2H-phthalazin-1-one **7a***

Yield 65%; m.p.: 99 – 102°C. FT-IR (KBr) cm^{-1}: 1653 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.49 – 8.54 (m, 1H, 8-ArH), 7.70 – 7.80 (m, 3H, ArH), 7.49 – 7.60 (m, 5H, ArH), 4.48 (t, *J* = 6.8, 2H, CH_2), 2.84 (t, *J* = 6.8, 2H, CH_2), 2.35 (s, 3H, Me). ^{13}C-NMR ($CDCl_3$) d: 159.1 (C=O), 147.0, 135.2, 132.7, 131.3, 129.4, 129.1, 128.6, 128.2, 127.2, 126.6, 57.1 (CH_2), 48.6 (CH_2), 45.4 (Me). Anal. Calc. ($C_{18}H_{19}N_3O$): C, 73.70; H, 6.53; N, 14.32. Found: C, 73.52; H, 6.55; N, 14.29.

*2-[2-(Dimethylamino)ethyl]-6-methoxy-4-(2-methoxyphenyl)-2H-phthalazin-1-one **7b***

Yield 56%; m.p.: 110 – 113°C. Eluent: AcOEt / MeOH = 2 : 1. R_f = 0.26. FT-IR (KBr) cm^{-1}: 1651 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.40 (d, *J* = 8.9, 1H, 8-ArH), 7.45 – 7.53 (m, 1H, PhH), 7.37 (dd, *J* = 7.5, 1.7, 1H, PhH), 7.28 (dd, *J* = 8.8, 2.5, 1H, 7-ArH), 7.03 – 7.15 (m, 2H, PhH), 6.66 (d, *J* = 2.5, 1H, 5-ArH), 4.25 – 4.58 (m, 2H, CH_2), 3.76 (s, 3H, OMe), 3.75 (s, 3H, OMe), 2.82 (d, *J* = 7.0, 2H, CH_2), 2.34 (s, 6H, Me). ^{13}C-NMR ($CDCl_3$) d: 162.9, 159.4, 157.6, 145.0, 132.0, 131.5, 130.9, 128.9, 124.5, 121.7, 121.2, 119.9, 111.3, 108.3, 57.4, 55.51, 55.47, 48.6, 45.6. Anal. Calc. ($C_{20}H_{23}N_3O_3$): C, 67.97; H, 6.56; N, 11.89. Found: C, 67.75; H, 6.68; N, 11.80.

*2-[2-(Dimethylamino)ethyl]-6-methoxy-4-(pyridin-2-yl)-2H-phthalazin-1-one **7c***

Yield 64%; m.p.: 87 – 89°C. Eluent: AcOEt / MeOH = 1 : 1. R_f = 0.26. FT-IR (KBr) cm^{-1}: 1662 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.75 – 8.78 (m, 1H, 6-PyH), 8.43 (d, *J* = 8.8, 1H, 8-ArH), 7,96 (d, *J* = 2.6, 1H, 5-ArH), 7.88 – 7.92 (m, 2H, PyH), 7.38 – 7.45 (m, 1H, PyH), 7.33 (dd, *J* = 8.8, 2.4, 1H, 7-ArH), 4.47 (t, *J* = 6.9, 2H, CH_2), 3.90 (s, 3H, OMe), 2.88 (t, *J* = 6.9, 2H, CH_2) 2.39 (s, 6H, Me). ^{13}C-NMR ($CDCl_3$) δ: 162.8, 159.1, 154.9, 148.3, 143.3, 137.0, 128.8, 124.2, 123.4, 121.9, 120.1, 108.6, 57.2 (CH_2), 55.4 (OMe), 48.7 (CH_2), 45.4 (Me). MS *m/z* (%): 324 $[M]^+$ (1), 254 (88), 58 (100). HRMS $[M]^+$: calcd. for $C_{18}H_{20}N_4O_2$: 324.15862, found: 324.15897.

*2-[2-(Dimethylamino)ethyl]-6-methoxy-4-(pyridin-3-yl)-2H-phthalazin-1-one **7d***

Yield 60%; m.p.: 124 – 127°C. Eluent: AcOEt / MeOH = 1 : 1. R_f = 0.24. FT-IR (KBr) cm^{-1}: 1652 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.89 – 8.88 (m, 1H, 2-PyH), 8.76 (dd, *J* = 4.9, 1.7, 1H, 6-PyH), 8.46 (d, *J* = 8.9, 1H, 8-ArH), 7.92 – 7.98 (m, 1H, 4-PyH), 7.48 (ddd, *J* = 7.8, 4.9, 0.8, 1H, 5-PyH), 7.35 (dd, *J* = 8.9, 2.5, 1H, 7-ArH), 6.99 (d, *J* = 2.5, 1H, 5-ArH), 2.42 (t, *J* = 6.9, 2H, CH_2), 3.85 (s, 3H, OMe), 2.82 (t, *J* = 6.9, 2H, CH_2), 2.34 (s, 6H, Me). ^{13}C-NMR ($CDCl_3$) δ: 163.2, 158.9, 150.1, 143.3, 136.8, 131.5, 130.8, 129.7, 123.4, 122.0, 120.2, 107.4, 57.3 (CH_2), 55.6 (OMe), 48.7 (CH_2), 45.5 (Me). MS *m/z* (%): 324 $[M]^+$ (1), 254 (31), 71 (51), 58 (100); HRMS $[M]^+$: calcd. for $C_{18}H_{20}N_4O_2$: 324.15862, found: 324.15877.

*2-[2-(Dimethylamino)ethyl]-6-methoxy-4-(pyridin-4-yl)-2H-phthalazin-1-one **7e***

Yield 58%; m.p.: 149 – 152°C. Eluent: AcOEt / MeOH = 1 : 1. R_f = 0.24. FT-IR (KBr) cm^{-1}: 1644 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.81 (dd, *J* = 1.5, 4.4, 2H, PyH), 8.47 (d, *J* = 8.9, 1H, 8-ArH), 8.56 (dd, *J* = 1.5, 4.4, 2H, PyH), 7.36 (dd, *J* = 2.5, 8.9, 1H, 7-ArH), 7.02 (d, *J* = 2.4, 1H, 5-ArH), 4.18 (t, *J* = 6.8, 2H, CH_2), 3.86 (s, 3H, OMe), 2.81 (t, *J* = 6.8, 2H, CH_2), 2.34 (s, 6H, Me). ^{13}C-NMR ($CDCl_3$) d: 163.2, 158.9 (C=O), 150.3, 144.1, 143.0, 130.3, 129.7, 123.9, 123.7, 121.8, 120.3, 107.3, 56.8 (CH_2), 55. 7 (OMe), 47.9 (CH_2), 45.0 (Me). Anal. Calc. ($C_{18}H_{20}N_4O_2$): C, 66.65; H, 6.21; N, 17.27. Found: C, 66.30; H, 6.12; N, 17.15.

*2-[2-(Dimethylamino)ethyl]-4-methyl-2H-phthalazin-1-one **7f***

Yield 55%; m.p.: 30 – 33°C. Eluent: AcOEt / MeOH = 1 : 1. R_f = 0.20. FT-IR (KBr) cm^{-1}: 1652 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.40 – 8.46 (m, 1H, 8-ArH), 7.67 – 7.82 (m, 3H, 5,6,7-ArH), 4.31 (t, *J* = 7.0, 2H, CH_2), 2.75 (t, *J* = 7.0, 2H, CH_2), 2.56 (s, 3H, Me), 2.32 (s, 6H, Me). ^{13}C-NMR ($CDCl_3$) δ: 159.3 (C=O), 143.7, 132.7, 131.2, 129.7, 127.6, 127.0, 124.7, 57.0 (CH_2), 48.2 (CH_2), 45.3 (Me), 18.8 (Me). Anal. Calc. ($C_{13}H_{17}N_3O$): C, 67.51; H, 7.41; N, 18.17. Found: C, 67.49; H, 7.35; N, 18.09.

*2-[2-(Dimethylamino)ethyl]-2H-phthalazin-1-one **7g***

Yield 60%; oil. Eluent: AcOEt / MeOH = 2 : 1. R_f = 0.24. FT-IR (film) cm^{-1}: 1648 (C=O). ^{1}H-NMR ($CDCl_3$) d: 8.40 – 8.46 (m, 1H, 8-ArH), 8.17 (s, 1H, 4-ArH), 7.66 – 7.83 (m, 3H, 5,6,7-ArH), 4.38 (t, *J* = 6.9, 2H, CH_2), 2.78 (t, *J* = 6.8, 2H, CH_2), 2.33 (s, 6H, Me). ^{13}C-NMR ($CDCl_3$) δ: 159.4 (C=O), 137.8, 132.9, 131.5, 129.6, 127.8, 126.6, 125.9, 57.2 (CH_2), 48.6 (CH_2), 45.4 (Me). MS *m/z* (%): 217 $[M]^+$ (5), 173 (14), 71 (20), 58 (100). HRMS $[M]^+$: calcd for $C_{12}H_{15}N_3O$: 217.12150, found: 217.12187.

*2-[2-(Dimethylamino)ethyl]-2H-pyridazin-3-one **8***

Yield 60%; oil. FT-IR (KBr) cm^{-1}: 1660. ^{1}H-NMR ($CDCl_3$) δ: 7.73 (dd, *J* = 3.9, 1,7, 1H, 6Ar-H), 7.13 (dd, *J* = 9.4, 3.8, 1H, 5Ar-H), 6.87 (dd, J = 9.4, 1.7, 1H, 4Ar-H), 4.25 (t, *J* = 6.7, 2H, CH_2), 2.69 (t, *J* = 6.8, 2H, CH_2), 2.26 (s, 6H, Me). ^{13}C-NMR ($CDCl_3$) δ: 160.4 (C=O), 135.9, 130.9, 129.6, 56.9, 49.3, 45.4. Anal. Calc. ($C_8H_{13}N_3O$): C, 57.47; H, 7.84; N, 25.13. Found: C, 57.16; H, 8.04; N, 24.99.

*7-[2-(Dimethylamino)ethyl]-7H-pyrido[2,3-d]pyridazin-8-one **9a***

Yield 65%; m.p.: 77 – 79°C. Eluent: AcOEt / MeOH = 1 : 1. FT-IR (KBr) cm^{-1}: 1655. ^{1}H-NMR ($CDCl_3$) δ: 9.11 (dd, *J* = 4.5, 1.5, 1H, 2Ar-H), 8.19 (s, 1H, 5Ar-H), 8.07 (dd, *J* = 8.1, 1.6, 1H, 4Ar-H), 7.73 (dd, *J* = 8.1, 4.5, 1H, 3Ar-H), 4.63 (t, *J* = 6.5, 2H, CH_2), 2.87 (t, *J* = 6.6, 2H, CH_2), 2.37 (s, 6H, Me). ^{13}C-NMR ($CDCl_3$) δ: 158.4 (C=O), 143.4, 135,9, 133.9, 127.1, 125.6, 57.0, 49.0, 45.4; Anal. Calc. ($C_{11}H_{14}N_4O$): C, 60.53; H, 6.47; N, 25.67. Found: C, 60.51; H, 6.67; N, 25.42.

*2-[2-(Dimethylamino)ethyl]-2H-pyrido[3,4-d]pyridazin-1-one **9b***

Yield 55%; oil. FT-IR (KBr) cm^{-1}: 1655. ^{1}H-NMR ($CDCl_3$) δ: 9.15 (s, 1H, Ar-H), 8.97 (d, *J* = 5.3, 1H, Ar-H), 8.30 (s, 1H, Ar-H), 8.20 (d, *J* = 5.3, 1H, Ar-H), 4.39 (t, *J* = 6.6, 2H, CH_2), 2.80 (t, *J* = 6.6, 2H, CH_2), 2.34 (s, 6H, Me). Anal. Calc. ($C_{11}H_{14}N_4O$): C, 60.53; H, 6.47; N, 25.67. Found: C, 60.48; H, 6.66; N, 25.31.

Pharmacology

The experiments were carried out with Balb/c male mice (18 – 25 g; Animal Quarters, Medical University of Lodz, Poland). The mice were kept in group cages under laboratory conditions at a temperature of 20 – 21°C, natural day / night cycle and they had free access to commercial chow food and water. All experiments were performed between 09.00 a.m. and 02.00 p.m. The synthesized compounds **7**, **8**, **9** were dissolved in distilled water with addition of one molar equivalent of hydrochloric acid and administrated 0.1 mL/10 g, body weight (b.w.).

All the procedures used in these studies were approved by the Ethics Committee of the Medical University of Lodz, Poland (licence 115, permission L/BD/199).

Determination of acute toxicity

The LD_{50} values as an indicator of acute toxicity of compounds were determined according to the Kärber method [19]. Results are given in Table l.

Nociceptive tests

Hot-plate test was performed according to the method of Eddy and Leimbach [20]

The compounds (**7a – g**, **8**, **9a**,**b**) in doses of 0.1 ALD_{50}, metamizole (Pyralginum, Polpharma, Poland), in a dose of 500 mg/kg b.w. were administered intraperitoneally (i.p.). The groups consisted of 8 – 10 mice each. A transparent plastic cylinder (14 cm diameter, 20 cm height) was used to confine the mouse on heated (52 ± 0.4°C) surface of the plate. The animals were placed on the hot-plate 1 min before (0 (zero) measurement), and 60, 120, 180 min after i.p. injection of compounds or metamizole, and latencies to paw licking or jumping were measured. A cut-off time of 60 s was used to avoid tissue injury. The data are presented in Fig. 1.

Tail-flick test of D' Amour and modified by Smith was used for mice [21]

The compounds (**7a – g**, **8**, **9a**,**b**) in doses of 0.1 ALD_{50}, or metamizole (Pyralginum, Polpharma, Poland), in dose of 500 mg/kg b.w. were administered intraperitoneally (i.p.). The groups consisted of 8 – 10 mice each. The latency of tail withdrawal was determined by focusing a radiant heat source on the tail, about 3 cm from the tip of the tail. The temperature of heat source was 70 ± 0.2°C and the maximum time of exposure was 60 s. This noxious stimulation did not cause tissue damage. The latency was measured 1 min before, (0 measurement), and 60, 120, 180 min after the injection of compounds or metamizole. Each group consisted of 8 – 10 mice. The data are presented in Fig. 2.

Writhing test of Collier et al. was used for mice [22]

The compounds (**7a – g**, **8**, **9a**,**b**) in doses of 0.1 ALD_{50} or metamizole in dose of 500 mg/kg b.w. were administered orally (*per os, p.o.*). Acetic acid (0.1 mL/10 g b.w. of a 0.6% v/v solution) was injected 60 min after compounds (or metamizole) into the peritoneal cavities of mice, which were placed in a large glass cylinder and the intensity of nociception was quantified by counting the total number of writhing occurring between 0 and 30 min after stimulus injection. Each group consisted of ten mice. The data are presented in Fig. 3.

Statistical analysis

Results are expressed as the mean ± SD. The normality of distribution was checked by means of Kolmogorov-Smirnov test with Lilliefors test. The statistical evaluation was performed using analysis of variance (ANOVA) and post hoc comparisons were performed by means of Least Significant Differences (LSD) test. If the data were not normally distributed, statistical evaluation was performed by using ANOVA (Kruskall-Wallis) and Mann-Whitney U test. Differences were considered significant when $p < 0.05$.

The analgesic effect in the hot-plate and tail-flick tests were expressed in percent of maximal possible effect (% MPE):

$$100\% \times \frac{(\text{latency after drug administration} - \text{basic latency})}{(\text{“cut off“} - \text{basic latency})}$$

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