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Original article

Synthesis, analgesic activity and computational study of new isothiazolopyridines of Mannich base type

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Abstract

A series of new 4-arylpiperazine derivatives of isothiazolopyridine of Mannich base type and their non-4-arylpiperazine analogues (**3** and **4**) were synthesized and assayed as potential analgesic agents. Pharmacological assay demonstrated that all the compounds prepared, without exception, displayed significant activity in the mouse writhing assay. The analgesic action, expressed as ED_{50}, was found to be 2–10 times more potent than that of acetylsalicylic acid and 1.5–10 times weaker than that of morphine, these being used as standards. The toxicities (LD_{50}) of the investigated derivatives varied and ranged from 250 to 2000 mg/kg. Additionally, the computational investigations were performed in order to find correlation between molecular structure and biological effects (toxicity, analgesic action) of discussed compounds. Useful model was found for toxicity assessment.

Keywords: Isothiazolopyridines; Analgesic activity; Structure–toxicity relationships

1. Introduction

It is known from literature [1–3], that benzoxazolinones and oxazolopyridinones of Mannich base type with a pharmacophoric 4-arylpiperazine moiety (**I**; Fig. 1) posses significant analgesic activity as evidenced in the mouse during a writhing test and very little or no antiinflammatory action in the acute carrageenan-induced paw edema test in rats.

In this context, recently we described the synthesis and analgesic action of a series of related derivatives of isothiazolo[5,4-*b*]pyridine of the general structure **II** (Fig. 1) [4]. In our studies we revealed that the potency of the analgesic effect is highly influenced by the nature of the substituent R present at the aromatic ring of the 4-arylpierazine substructure of **II** (Fig. 1). The best analgesic results were obtained especially with the *o*-chlorophenylpiperazine derivative **IIa** [ED_{50} = 16.9 mg/kg], however, the good analgesic action of this compound was accompanied by significant toxicity (LD_{50} = 83 mg/kg). In contrast, the use of the electron-donating *o*-methoxy group (**IIc**; Fig. 1) leads to compound practically inactive in this test. Replacement of the 4-arylpiperazine in **II** with 4-benzylpiperazine or 1,2,3,4-tetrahydroisoquinoline produces a similar effect. On the other hand, the analgesic action was retained after replacement of the 4-arylpiperazine pharmacophore with 4-hydroxy-4-phenylpiperidine or 4-benzylpiperidine (**III**: ED_{50} ~ 16 mg/kg, LD_{50} ~ 320 mg/kg) [4].

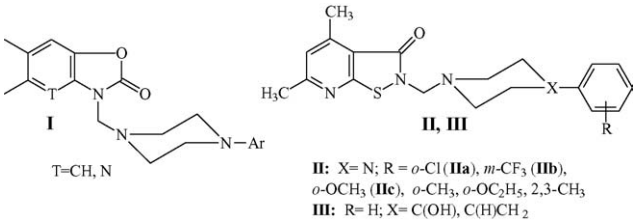

Fig. 1.

Due to the considerable analgesic potency exhibited by some isothiazolopyridins of type **II** and **III** (Fig. 1), the primary purpose of the present experiments was to synthesize and evaluate the toxicity and analgesic action of a series of related compounds. Most of these compounds were prepared

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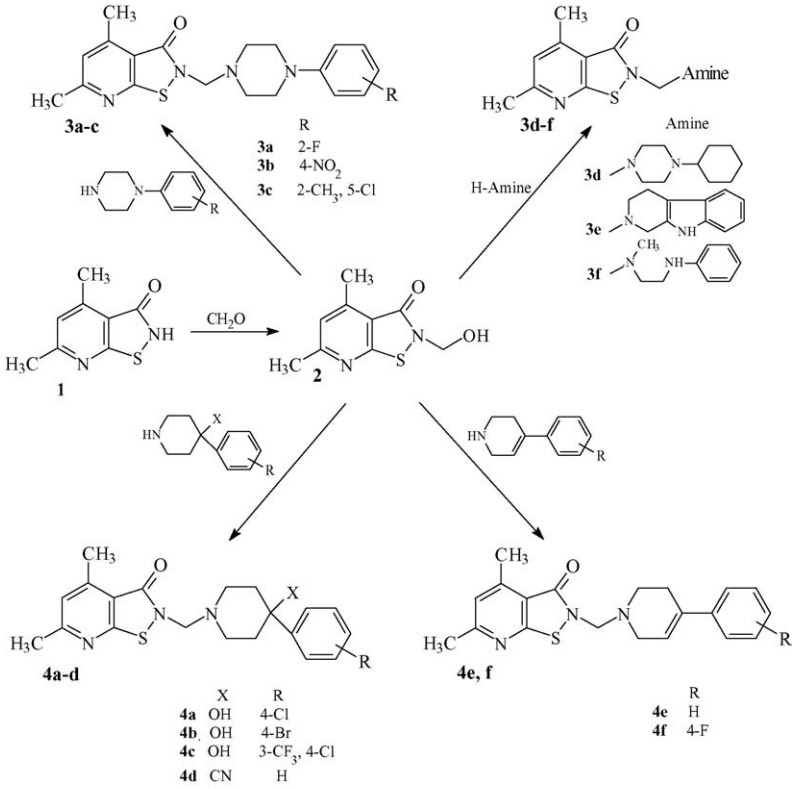

Scheme 1.

by incorporating the new 4-arylpiperazine or 4-arylpiperidine moiety possessing bromo, fluoro, trifluoromethyl or nitro substituents which, as R = Cl in compound **IIa**, are electron-attracting in nature (Scheme 1: **3a–c**, **4a–c**, **4f**). Additionally, analogues of **II** (Fig. 1) with the side chain, which represent a marked departure in the fragment of 4-arylpiperazine (**3d–f**) were prepared in order to extend the structure-activity (SAR) studies. Finally, we have undertaken computational study of the new compounds **3** and **4** to find correlation between their biological activity and molecular structure.

2. Experimental procedures

2.1. Chemistry

The new compounds **3** and **4** were synthesized as illustrated in Scheme 1, starting from 2*H*-2-hydroxymethyl-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-*b*]pyridine (**2**) [5]. The preparation of the final isothiazolopyridines **3** and **4** involved condensation of the substrate **2** and the appropriate derivatives of 1-arylpiperazine, 4-arylpiperidine or 1-cyclohexylpiperazine, 1,2,3,4-tetrahydro-*β*-carboline and N-methyl-N′-phenylethylenediamine. The arylpiperazine(piperidine) intermediates used are illustrated in Scheme 1.

The reactions were carried out under mild conditions as described in our previous paper [4] and gave, in several cases, the desired products in high yield (80%). The piperazine, piperidine and N-methyl-N′-phenylethylenediamine intermediates were commercially available; 1,2,3,4-tetrahydro-*β*-carboline was synthesized by the known method [6]. The structures of the final compounds **3** and **4** were elucidated by IR, ^{1}H NMR and microanalyses and all data were in accordance with the assigned structures. Melting points, yields and spectral data are shown in Section 4. The spectral data within the series of new compounds **3** and **4** did not show remarkable differences from those of the isothiazolopyridines of types **II** and **III** (Fig. 1), which we described earlier [4].

Finally, all compounds **3** and **4** were subjected to screening in an animal model to test their acute toxicity and analgesic action.

2.2. Pharmacology

Several structural changes were made at the 4-aryl(piperazine, piperidine) moiety of isothiazolopyridines **II** and **III** (Fig. 1) in order to improve their analgesic action and reduce their toxicity. The biological effects of the final twelve isothiazolopyridines **3** and **4** were measured using standard procedures (see Exp. part). The results of their bioactivity and the reference drugs (acetylsalicylic acid and morphine) are presented at Table 1.

2.2.1. Acute toxicity

The LD_{50} values of the investigated compounds **3** and **4** varied and ranged from ~250 to 2000 mg/kg (Table 1). Compounds **3e** and **4e** were not toxic (LD_{50} > 2000 mg/kg), and for these derivatives 200 mg/kg (1/10 LD_{50}) was taken as the initial dose in the further experiments. The most toxic compound was **3a** (LD_{50} = 255 mg/kg). The toxicity of the remain-

Table 1
Acute toxicity and influence of investigated compounds **3** and **4** on the "writhing syndrome" induced by phenylbenzoquinone in mice

Compound		Dose		Mean number of writhings ± S.E.M.	ED_{50} (mg/kg)
	LD_{50}	Part of LD_{50}	mg/kg		
Control				20.1 ± 1.9	
3a	255 (201–322)	1/40	6.4	5.4 ± 1.7*	3.4
		1/80	3.2	9.9 ± 2.5**	(2.8–4.1)
		1/160	1.6	16.1 ± 3.2	
3b	524 (392–687)	1/40	13.1	5.5 ± 2.5*	6.8
		1/80	6.5	9.1 ± 3.1***	(5.7–8.2)
		1/160	3.25	13.6 ± 2.5	
3c	510 (401–621)	1/40	12.2	4.7 ± 2.5*	5.9
		1/80	6.1	9.8 ± 3.1***	(4.2–8.3)
		1/160	3.05	14.9 ± 2.8	
3d	604 (524–720)	1/40	15.1	7.8 ± 2.4**	7.8
		1/80	7.5	10.0 ± 1.2***	(5.6–10.9)
		1/160	3.75	15.4 ± 3.7	
Control				19.8 ± 2.0	
3e	> 2000	1/40	50	7.0 ± 2.6*	4.9
		1/80	25	7.2 ± 3.2*	(0.3–86.0)
		1/160	12.5	7.5 ± 3.3**	
		1/320	6.25	10.2 ± 3.6	
Control				20.1 ± 1.9	
3f	668 (417–1069)	1/40	16.7	4.2 ± 1.7*	
		1/80	8.35	6.2 ± 2.6**	5.0
		1/160	4.17	10.0 ± 1.9***	(2.6–9.6)
		1/320	2.9	14.5 ± 2.8	
4a	751(688-821)	1/40	18.8	4.4 ± 1.2*	9.9
		1/80	9.4	9.6 ± 3.2**	(7.1–13.9)
		1/160	4.7	14.2 ± 4.8	
4b	522 (392–684)	1/40	13.0	7.5 ± 2.2*	6.6
		1/80	6.5	10.2 ± 3.1**	(5.5–7.9)
		1/160	3.25	14.6 ± 4.7	
4c	975 (842–1181)	1/40	24.4	5.9 ± 2.1*	11.6
		1/80	12.2	9.2 ± 2.1**	(8.9–15.1)
		1/160	6.1	12.2 ± 6.7	
Control				19.8 ± 2.0	
4d	902 (638–1658)	1/40	50	5.2 ± 3.1*	8.4
		1/80	25	9.0 ± 3.1***	(2.9–24.3)
		1/160	12.5	11.4 ± 6.8	
4e	> 2000	1/40	50	8.0 ± 2.2*	23.9
		1/80	25	9.0 ± 3.3**	(4.0–141.5)
		1/160	12.5	12.0 ± 5.1	
Control				20.1 ± 1.9	
4f	510 (401–621)	1/40	12.7	7.6 ± 2.1*	6.2
		1/80	6.4	9.9 ± 2.4***	(5.2–7.4)
		1/160	3.2	13.8 ± 2.1	
Control				19.2 ± 3.2	
Acetylsalicylic acid		–	100	3.2 ± 3.2*	39.15
		–	50	8.5 ± 1.3**	(29.1–48.4)
		–	30	11.2 ± 2.1	
Morphine		–	10	1.2 ± 0.8*	2.44
		–	3	7.5 ± 2.9**	(1.18–5.02)
		–	1	16.2 ± 3.5	

Each group consisted of six to eight animals. * $P < 0.01$; ** $P < 0.02$; *** $P < 0.05$.

ing compounds fluctuated between 510 and 975 mg/kg. Toxic doses of all the tested compounds caused sedation and depression of locomotor activity of mice.

Comparing the toxicities of the isothiazolopyridines **II** and **III** tested previously [4] and their new analogues **3** and **4**, we can observe that, in general, the new compounds are significantly less toxic than their precursors.

2.2.2. Analgesic properties

The analgesic activities of the compounds obtained in this study were measured using the phenylbenzoquinone-induced writhing assay and the hot plate test in mice. The data shown in Table 1 clearly indicate that all the newly synthesized isothiazolopyridines **3** and **4** demonstrated, without exception, strong analgesic action in the writhing assay. The ED_{50} values for 11 of the 12 tested compounds ranged between 3.4 and 11.6 mg/kg. The most potent effect was produced by *o*-fluorophenylpiperazine-**3a** and the 1,2,3,4-tetrahydro-*β*-carboline-**3e** derivatives, with values of ED_{50} = of 3.4 and 4.9 mg/kg, respectively. It should be noted that **3a** was the most toxic (LD_{50} = 255 mg/kg) while **3e** the least toxic (LD_{50} > 2000 mg/kg) derivative within the series of compounds investigated. The weakest analgesic action was shown by **4e** with the 4-phenyltetrahydropyridine moiety (ED_{50} = 23.9 mg/kg). By comparing the ED_{50} values of compounds **3** and **4** and the reference drugs, we can see that a similar analgesic action is observed with acetylsalicylic acid after application at a dose of 39.15 mg/kg and with morphine at a dose of 2.44 mg (Table 1). This indicates that the analgesic effects of the compounds **3** and **4** tested were ~2–10 times more potent than that of acetylsalicylic acid and ~1.5–10 times weaker than that of morphine.

In addition to examining analgesia with the writhing test, compounds **3** and **4** were also evaluated with the hot plate test. With the exception of **3b**, **3e**, **4a** and **4c** none of the isothiazolopyridines investigated showed any activity at this assay. However, the effect of activity at the hot plate test for compounds **3b**, **3e**, **4a** and **4c** was observed only at the highest doses used, corresponding to 1/10 LD_{50}.

The writhing test in mice has been used by many investigators for measuring peripheral analgesic activity, whereas the hot plate test is used for evaluating central analgesia [7]. In this context one can say that the isothiazolopyridines **3** and **4** possess an analgesic action similar to that of acetylsalicylic acid, however, elucidation of the mechanism of their biological activity will require further investigation.

3. Results and discussion

*3.1. SAR of isothiazolopyridines **3** and **4** (Table 1)*

In spite of the limited number of compounds tested, on the bases of the results obtained with the writhing test (Table 1), we formulated some SAR. For SAR analyses, the new isothiazolopyridines were divided into two groups—derivatives of 4-arylpiperazine (**3**) and of 4-arylpiperidine (**4**). For the 4-arylpiperazine series we also introduced 1,2,3,4-tetrahydro-*β*-carboline-(**3e**) and N-methyl-N′-phenylethylenediamine-(**3f**) derivatives. The 1,2,3,4-tetrahydro-*β*-carboline substructure can be considered as a rigid analogue of the 4-phenylpiperazine moiety, whereas the side chain of compound **3f** can be regarded as its extremely flexible analogue.

- In a recent report [4] we revealed that within the class of isothiazolopyridines **II** (Fig. 1), the introduction of electron-withdrawing substituents, such as *o*-Cl or *m*-CF_3 to the aromatic ring of the 4-arylpiperazine moiety results in compounds which exhibit analgesic action (**IIa**, **b**) [4]. A similar trend is observed in the series **3a–c**, having another substituents (*o*-F, *p*-NO_2, *m*-Cl) which diminish the electron density on the aromatic ring of the side chain.
- Another interesting finding is that replacement of the 4-arylpiperazine substructure with 1,2,3,4-tetrahydro-*β*-carboline or N-methyl-N′-phenylethylenediamine (compounds **3e** and **3f**, respectively) did not diminish analgesic action. The above data show that the piperazine ring is not necessary as a specific spacer for the aryl ring in the side chain within the series **II** (Fig. 1) and **3**.
- Taking into account the structure of isothazolopyridines **I** and **II** (Fig. 1), we initially assumed that the presence of the terminal aromatic ring in the side chain is an essential requirement in eliciting analgesic action. Therefore, the specific object for developing the structure-activity relationship was the preparation of the 4-cyclohexylpiperazine derivative **3d**, which was also introduced for pharmacological evaluation. The pharmacological study of **3d** exhibited, unexpectedly, that such an alicylic replacement of the aromatic ring of the side chain results in an analgesic agent equipotent to its 4-arylpiperazine analogues **3a–c**. Hence, these data did not support our assumption and suggested that in the series of analgesic isothiazolopyridines of type **II** and **3**, even a very profound modification of the 4-arylpiperazine pharmacophore may be tolerated.
- In the series of 4-arylpiperidine derivatives **4**, compounds possessing an electron-attracting substituent on the aromatic ring of the 4-arylpiperidine moiety produce a more significant analgesic effect than their unsubstituted precursors (compare **4e** and **4f**). Introduction of a second withdrawing substituent does not cause an increase in activity (compare **4a** and **4c**).

3.2. Computational investigations

With the aim of establishing the role of the 2-substituent as a pharmacophore which can participate in the toxic and analgesic effects of isothiazolopyridines **3** and **4** (Scheme 1), we undertook a computational investigations of this set of compounds. From this study we excluded the recently described [4] isothiazolopyridines **II** and **III** (Fig. 1), because for these compounds and isothiazolopyridines **3** and **4** the writhing syndrome was induced by different chemical stimulants - 0.6% acetic acid for **II**, **III** and 0.02% phenylbenzoquinone for **3** and **4**.

Computational investigations of correlation between biological activity and molecular structure started with derivation of descriptors values based on obtained geometries. On the basis of descriptors space the structure–toxicity and structure–analgesic activity models were obtained. The analyzed database contained 12 compounds so three-parameter equations were taken into account. The Fisher criterion threshold for this number of variables and compounds is 3.71 [8]. The equation describing structure–toxicity relationship contains two CPSA and one topographical descriptors and is presented below:

$\log LD_{50}$ = –1.533 (1.052) + 0.179 (0.032) *3D Structural Information Content (order2)* – 110.955 (30.092) *FPSA-3 Fractional PPSA (PPSA-3/TMSA)* + 48.029 (9.322) *FNSA-3 Fractional PNSA (PNSA-3/TMSA)*.

The obtained statistical parameters are: $R^2 = 0.826$, $F = 12.62$, $s^2 = 0.0157$.

The equation is reasonable and statistically significant for toxicity prediction for database of isothiazolopyridines **3** and **4**. The comparison of experimental and calculated toxic effect, represented by log LD_{50}, is given in Table 2.

The data from Table 2 demonstrated that the model is able to reproduce the toxicity quite accurately and may be useful for further toxicity prediction for a series of similar compounds.

In the next step correlation between analgesic activity and molecular structure was investigated using the same methodology. The obtained equation is presented as a result of the analysis:

$\log ED_{50}$ = –3.804 (1.649) + 0.296 (0.103) *Number of C atoms* + 0.483 (0.222) *Kier Shape Index (order 3)* – 0.161 (0.074) *Bonding Information Content (order 0)*.

The obtained statistical parameters are: $R^2 = 0.516$, $F = 2.84$, $s^2 = 0.031$.

On the basis of the Fisher criterion and low correlation coefficient R^2 value, the equation should be regarded as not significant statistically.

3.3. Conclusion

We prepared new 4-arylpiperazine derivatives of isothiazolopyridine of Mannich base type (**3a–c**), their non-4-arylpiperazine [4-cyclohexylpiperazine-(**3d**), 1,2,3,4-tetrahydro-*β*-carboline-(**3e**), N-methyl-N′-phenylethylenediamine-(**3f**)] and 4-arylpiperidine (**4a–f**) analogues. Pharmacological evaluation demonstrated that all the new compounds **3** and **4**, without exception, possess significant analgesic action in the writhing test. The analgesic action for 11 of the 12 compounds assayed was 4–10 times higher than that of acetylsalicylic acid and 1.5–5 times weaker than that of morphine used for reference. The relatively weak difference in analgesic potency of isothiazolopiyridines **3a–c** with 4-arylpiperazine pharmacophore and their non-4-arylpiperazine analogues **3d–f** suggests that a profound modification of the 4-arylpiperazine moiety may be tolerated. As an important result of computational analysis of isothiazolopyridnines **3** and **4**, the correlation between toxicity and molecular structure was found. Much worse correlation was observed between analgesic action and structural features. We hope that obtained result of the computational investigations of isothiazolopyridines **3** and **4** may be helpful in design of novel and more potent analgesic isothiazolopyridines of type **3** and **4** with reduced toxicity. Syntheses of such compounds are under consideration.

Table 2
Experimental and predicted toxicity for series of investigated compounds

Compound	Experimental $\log LD_{50}$	Calculated $\log LD_{50}$
3a	2.407	2.375
3b	2.719	2.682
3c	2.688	2.690
3d	2.781	2.855
3e	3.301	3.246
3f	2.825	2.892
4a	2.876	2.727
4b	2.718	2.887
4c	2.989	2.980
4d	2.955	3.080
4e	3.301	3.097
4f	2.708	2.756

4. Experimental

4.1. Chemical experimental section

Melting points are uncorrected. Proton NMR spectra were obtained with Tesla [80 MHz] and Bruker [300 MHz] spectrometers [$CDCl_3$, δ (ppm)]. IR (KBr) spectra were recorded on Specord M 80 (Carl Zeiss Jena). Elemental C, N, H analyses were run on a Carlo Erba NA-1500 analyzer. All the results of the C, N and H determinations were within ± 0.4% of the values calculated for the corresponding formulae.

4.1.1. General procedure for preparation of isothiazolopyridines *3a, 3c–f, 4a–f*

To a stirred mixture of 2.1 g (0.01 mol) of 2-hydroxymethylisothiazolopyridine **2** [5] in 40 ml of ethanol 0.01 mol of an appropriate amine was added and stirring was continued for 24 h at room temp. Than the precipitated, crude product (**3c–e**, **4a–e**) was filtered off and crystallized with charcoal from the appropriate solvent. In the case of compounds **3a**, **3f** and **4f** the ethanol solution was evaporated and the residue was crystallized from the appropriate solvent to give pure product.

*4.1.1.1. 2-[(4-o-Fluorophenylpiperazin-1-yl)methyl]-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine (**3a**).* Yield 1.85 g (50%) from **2** and 1-(*o*-fluorophenyl)-piperazine, m.p. 85–86 °C (*n*-hexane). ^{1}H NMR: 2.63 s (3H, CH_3), 2.77 s (3H, CH_3), 2.85–3.00 m (4H, $CH_{piperazine}$), 3.05–3.20 m (4H, $CH_{piperazine}$), 4.76 s (2H, CH_2), 6.90–7.15 m (5H, $H_{\beta\text{-pyridine}}$ and 4ArH). IR: 1660 (C = O).

*4.1.1.2. 2-{[4-(5-Chloro-2-methylphenyl)piperazin-1-yl]methyl}-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine (**3c**).* Yield 2.2 g (55%) from **2** and 1-(5-chloro-2-methylphenyl)piperazine, m.p. 139–142 °C (ethanol). ^{1}H NMR: 2.23 s (3H, CH_3), 2.64 s (3H, CH_3), 2.78 s (3H, CH_3), 2.90–3.00 m (8H, $CH_{piperazine}$), 4.79 s (2H, CH_2), 6.90–7.20 m (4H, $H_{\beta\text{-pyridine}}$ and 3ArH). IR: 1650 (C = O).

*4.1.1.3. 2-[(4-Cyclohexylpiperazin-1-yl)methyl]-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine (**3d**).* Yield 2.3 g (65%) from **2** and 1-cyclohexylpiperazine, m.p. 158–160 °C (ethanol). ^{1}H NMR: 1.10–1.90 m (10H, $CH_{cyclohexane}$), 2.15–2.30 m (1H, $CH_{cyclohexane}$), 2.59 s (1H, CH_3), 2.65–2.85 m (11H, 8$H_{piperazine}$ and CH_3), 4.65 s (2H, CH_2), 6.91 s (1H, $H_{\beta\text{-pyridine}}$). IR: 1660 (C = O).

*4.1.1.4. 2-[(1,2,3,4-Tetrahydro-β-carbolin-2-yl)methyl]-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine (**3e**).* Yield 3.1 g (85%), from **2** and 1,2,3,4-tetrahydro-β-carboline [6], m.p. 154–157 °C (methanol). ^{1}H NMR: 2.62 s (3H, CH_3), 2.79 s (3H, CH_3), 2.86 t (2H, CH_2, *J* ~6.5 Hz), 3.16 t (2H, N-CH_2, *J~6.5 Hz*) 3.95 s (2H, N-CH_2), 4.94 s (2H, CH_2), 6.93 s (1H, $H_{\beta\text{-pyridine}}$), 7.05–7.55 m (4H, ArH), 8.05 s [1H, NH (exchangeable D_2O)]. IR: 3280 (NH), 1650 (C = O).

*4.1.1.5. 2-{[N-(2-Anilinoethyl)-N-methylamino]methyl}-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine (**3f**).* Yield 2.7 g (80%) from **2** and N-methyl-N′-phenylethylenediamine, m.p. 74–6 °C (*n*-hexane). ^{1}H NMR: 2.54 s (3H, CH_3), 2.60 s (3H, CH_3), 2.78 s (3H, CH_3), 2.85–3.00 m (4H, NCH_2CH_2N), 4.82 s (2H, CH_2), 6,99 s (1H, $H_{\beta\text{-pyridine}}$), 7.30–7.45 m (5H, ArH). IR: 3100–2800 (NH), 1660 (C = O).

*4.1.1.6. 2-{[4-(p-Chlorophenyl)-4-hydroxypiperidin-1-yl]methyl}-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine (**4a**).* Yield 3.2 g (80%) from **2** and 4-(*p*-chlorophenyl)-4-hydroxypiperidine, m.p. 178–180 °C (toluene). ^{1}H NMR: 1.70–2.20 m (4H, 3′ and 5′ $CH_{piperidine}$), 2.35 br [1H, OH (exchangeable D_2O)], 2.58 s (3H, CH_3), 2.70 s (3H, CH_3), 2.80–3.05 m (4H, 2′ and 6′ $CH_{piperidine}$), 4.70 s (2H, CH_2), 6,91 s (1H, $H_{\beta\text{-pyridine}}$), 7.20–7.50 m (4H, ArH). IR: 3100–2930 (OH), 1670 (C = O).

*4.1.1.7. 2-{[4-(p-Bromophenyl)-4-hydroxypiperidin-1-yl]methyl}-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine (**4b**).* Yield 3.1 g (70%) from **2** and 4-(*p*-bromophenyl)-4-hydroxypiperidine, m.p. 183–185 °C (ethanol). ^{1}H NMR: 1.70–2.30 m [5H, 3′ and 5′ $CH_{piperidine}$ and OH (exchangeable D_2O)], 2.59 s (3H, CH_3), 2.71 s (3H, CH_3), 2.84–3.13 m (4H, 2′ and 6′ $CH_{piperidine}$), 4.70 s (2H, CH_2), 6,92 s (1H, $H_{\beta\text{-pyridine}}$), 7.28–7.50 m (4H, ArH). IR: 3050–2900 (OH), 1650 (C = O).

*4.1.1.8. 2-{[4-(p-Chloro-m-trifluoromethylphenyl)-4-hydroxypiperidin-1-yl]methyl}-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine (**4c**).* Yield 3.1 g (65%) from **2** and 4-(*p*-chloro-*m*-trifluoromethylphenyl)-4-hydroxypiperidine, m.p. 175–177 °C (ethanol). ^{1}H NMR: 1.73–2.45 m [5H, 3′ and 5′ $CH_{piperidine}$ and OH (exchangeable D_2O)], 2.59 s (3H, CH_3), 2.72 s (3H, CH_3), 2.80–3.00 m (4H, 2′ and 6′ $CH_{piperidine}$), 4.72 s (2H, CH_2), 6.93 s (1H, $H_{\beta\text{-pyridine}}$), 7.45 d (1H, ArH, *J~9 Hz*), 7.65 d (1H, ArH, *J~9 Hz*), 7.86 s (1H, ArH). IR: 3100–2900 (OH), 1660 (C = O).

*4.1.1.9. 2-[(4-Cyano-4-phenylpiperidin-1-yl)methyl]-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine (**4d**).* Yield 3.2 g (85%,) from **2** and 4-cyano-4-phenylpiperidine, m.p. 177–179 °C (ethanol). ^{1}H NMR: 2.10–2.25 m (4H, 3′ and 5′ $CH_{piperidine}$), 2.62 s (3H, CH_3), 2.71 s (3H, CH_3), 2.85–3.35 m (4H, 2′ and 6′ $CH_{piperidine}$), 4.77 s (2H, CH_2), 6.98 s (1H, $H_{\beta\text{-pyridine}}$), 7.30–7.60 m (5H, ArH). IR: 2300 (CN), 1660 (C = O).

*4.1.1.10. 2-[(4-Phenyl-1,2,3,6-tetrahydropyridin-1-yl)methyl]-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine (**4e**).* Yield 1.75 g (50%) from **2** and 4-phenyl-1,2,3,6-tetrahydropyridine, m.p. 154–156 °C (ethanol). ^{1}H NMR: 2.55–2.70 m (5H, CH_3 and 2$CH_{piperidine}$), 2.78 s (3H, CH_3), 3.03 t (2H, $CH_{piperidine}$, *J* ~6 Hz), 3.49 d (2H, **CH_2**-CH = , *J~3 Hz*), 4.85 s (2H, CH_2), 6.10 t (1H, CH_2-**CH** = , *J~3 Hz*), 6.94 s (1H, $H_{\beta\text{-pyridine}}$), 7.25–7.45 m (5H, ArH). IR: 1650 (C = O).

*4.1.1.11. 2-{[4-(p-Fluorophenyl)-1,2,3,6-tetrahydropyridin-1-yl]methyl}-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine (**4f**).* Yield 2.6 g (70%) from **2** and 4-(*p*-fluorophenyl)-1,2,3,6-tetrahydropyridine, m.p. 106–107 °C (*n*-hexane). ^{1}H NMR: 2.55–2.70 m (5H, CH_3 and 2$CH_{piperidine}$), 2.76 s (3H, CH_3), 3.00 t (2H, $CH_{piperidine}$, *J* ~5.5 Hz), 3.44 d (2H, **CH_2**-CH = , *J~3 Hz*), 4.81 s (2H, CH_2), 6.00 t (1H, CH_2-**CH** = , *J~3 Hz*), 6.90–7.40 m (5H, $H_{\beta\text{-pyridine}}$ and 4ArH). IR: 1660 (C = O).

*4.1.2. 2-{[4-(p-Nitrophenyl)piperazin-1-yl]methyl}-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-b]pyridine (**3b**).* A mixture of 2.1 g (0.01 mol) of 2-hydroxymethyl-isothiazolopyridine **2** [5] and 0.01 mol of 1-(*p*-nitrophenyl)piperazine in 40 ml of ethanol was refluxed with stirring for 6 h. Afterwards the solvent was distilled of and the residue was crystallized with charcoal from methanol to give pure product.

Yield 3.2 g (80%), m.p. 201–204 °C (methanol). ^{1}H NMR: 2.60 s (3H, CH_3), 2.74 s (3H, CH_3), 2.80–2.95 m (4H, $CH_{piperazine}$), 3.38–3.60 m (4H, $CH_{piperazine}$), 4.73 s (2H, CH_2), 6.75 s (1H, $H_{\beta\text{-pyridine}}$), 6.85–7.00 m (2H, 2′ and 6′-ArH), 8.00–8.25 m (2H, 3′ and 5′-ArH). IR: 1660 (C = O).

4.2. Pharmacological experimental section

Materials and methods.

4.2.1. Substances

Acetylsalicylic acid (Polopiryna, ZF Starogard Gdañski, PL), morphine (Morphinum hydrochloricum, Polfa-Kutno,

PL), phenylbenzoquinone (INC Pharmaceuticals, Inc., New York).

4.2.2. Animals

The experiments were carried out on male Albino-Swiss mice (body weight 18–26 g). All of the animals were housed at constant humidity (60%) and temperature (24–25 °C) and kept on a 12-h light/dark cycle. Animals were fed a standard pellet diet with free access to tap water. All procedures were conducted according to Animal Care and Use Committee guidelines, and approved by the Ethical Committee of Jagiellonian University, Kraków.

Control and experimental groups consisted of six to eight animals each. The investigated compounds were administered intraperitonelly as the suspension in 0.5% methylcellulose in constant volume of 10 ml/kg.

4.2.3. Statistical analysis

The statistical significance was calculated using a Student's *t*-test.

The ED_{50} values and their confidence limits were calculated according to the method of Litchfield and Wilcoxon [9].

4.2.4. Acute toxicity

Acute toxicity was assessed by the methods of Litchfield and Wilcoxon [9] and presented as LD_{50} calculated from the mortality of mice after 24 h.

4.2.5. "Writhing" syndrome in mice according to Hendershot and Forsaith [10]

Different doses of test compounds ranging from 1/320 to 1/40 $LD_{50\ i.p.}$ were administered intraperitoneally. Twenty-five minutes later, 0.02% phenylbenzoquinone was injected intrapertioneally in a constant volume of 0.25 ml. Five minutes after injection of the irritating agent, the number of "writhing" episodes in the course of 10 min was counted. The analgesic effect of individual doses was expressed in percent:

$$\%\ \text{analgetic effect} = 100 - \frac{\sum of\ writhing\ incidents\ in\ exerimental\ group}{\sum of\ writing\ incidents\ in\ control\ group} \times 100$$

The ED_{50} values and their confidence limits were estimated by the method of Litchfield and Wilcoxon [9].

4.2.6. Pain reactivity

Pain reactivity was measured in the "hot plate" test according to the method of Eddy and Leimbach [11]. 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 recorded by a stop-watch. The experiments were performed 30 min after administration of the investigated compounds at graded doses of 1/40 – 1/10 $LD_{50\ i.p.}$.

4.3. Computational methodology

Quantum-chemical calculations were performed for a series of investigated compounds. The Gaussian03 suite of programs [12] was used to build the molecules and optimize their geometry. The ab initio Hartree–Fock–Roothaan method and 6-31G(d,p) basis set were used [13,14]. The harmonic vibrational frequencies were calculated confirming that the obtained geometry corresponds to the minimum on the potential energy surface (PES). In the next step the descriptors were evaluated using computer software for calculation of molecular descriptors and derivation of QSAR/QSPR [15,16]. The obtained descriptor space contained 86 variables divided into five groups: constitutional, topological, topographical, geometrical and CPSA (charged partial surface area), as implemented in the program. In order to find correlation between biological activity and molecular structure stepwise selection of scales for the multiple linear regression (MLR) was used [15,16]. The correlation coefficient (R^2), Fisher criterion (F) and standard error of the MLR (s^2) gave information about the quality of the proposed models.

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