Article

Bioresearch of New 1H-pyrrolo[3,4-c]pyridine-1, 3(2H)-diones

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Abstract: The subject of the work was the synthesis of new derivatives of 1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione with potential analgesic and sedative activity. Eight compounds were received. The analgesic activity of the new compounds was confirmed in the “hot plate” test and in the “writhing” test. All tested imides 8–15 were more active in the “writhing” test than aspirin, and two of them, 9 and 11, were similar to morphine. In addition, all of the new imides inhibited the locomotor activity in mice to a statistically significant extent, and two of them also prolonged the duration of thiopental sleep. On the basis of the results obtained for the previously synthesized imides and the results presented in this paper, an attempt was made to determine the relationship between the chemical structure of imides and their analgesic and sedative properties.

Keywords: 3,4-pyridinedicarboximide; analgesic and sedative activity

1. Introduction

Pain is an important signal of the body, an element of the self-preservation instinct, but it is also a source of suffering. Despite the availability of pain medications, daily pain affects a large proportion of the world’s population and limits all aspects of a person’s life. Therefore, there is still a need to look for effective and safe tools to fight pain management, especially when current treatments are impossible (due to side effects or drug interactions) or ineffective. In order to improve the quality of life of patients suffering from idiopathic and chronic pain, medicine uses, among others, gene therapy (enzyme fatty acid amide hydrolase FAAH and “pseudogene” FAHH-OUT of SCN9A gene) [1–3] and virtual reality (VR) methods, as well as new drug mechanisms to fight pain—e.g., small molecule prototypes of the peripheral sodium channel, NaV1.7 antagonists [3]. The search for molecules with potential analgesic activity is constantly ongoing.

So far, no mechanism for inducing an analgesic effect is known for 1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione derivatives, but their high activity in the “writhing” test, accompanied by little or no toxicity compared to morphine and aspirin (ASA), is a good starting point for the search for an ideal antinociceptive drug [4].

The current paper presents the next stage of research on the synthesis and investigation of pharmacological properties of new 1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione derivatives.
As established earlier, the strongest analgesic and sedative properties in the studies were observed for derivatives 1A and 2A (Figure 1 and Figure S1), which were considered model compounds. They have become a point of reference to determine the relationship between the chemical structure and biological activity of pyrrolopyridine derivatives. We have obtained a series of successive derivatives, taking into account the following modifications in the structure of the basic system and the alkyarylamine linker:

1. The type and size of the alkoxy substituent in the 2-position of pyridine, 4-alkoxy derivatives have shown stronger analgesic properties than ethoxy analogs.
2. The role of the alkyl linker connecting the basic center of the arylamine with the cyclic imide system, 2-hydroxypropyl derivatives being the most active.
3. The importance of pharmacophoric groups in the phenyl substituent at N-4 of the piperazine ring for the direction and strength of their biological action. Phenyl homologs, unsubstituted, and also containing electron-withdrawing groups such as −CF₃, −F, −Cl and −OCH₃ were obtained. In some cases, the aryl ring has been replaced by a bioisosteric tetrahydroisoquinoline moiety and the piperazine ring replaced by another cyclic amine.

![Figure 1. Structure of imides 1A and 2A. The figure shows the sections of the molecule subject to modification discussed in the text. (1) 3,4-pyridinedicarboximide, (2) linker, (3) arylamine.](image)

The results were discussed in detail in our previous works [5–14]. As previously established, the shortening of the alkyl linker between the basic center of the arylamine and the cyclic imide moiety resulted in derivatives with similar biological properties [8]. Mannich base-type imides were active in the “writhing” test, more than aspirin, were additionally endowed with sedative properties, inhibited the spontaneous locomotor activity in mice and statistically significantly extended the duration of thiopental anesthesia [8].

To determine the effect of the length of the alkyl linker connecting the arylamine with the pyrrolo[3,4-c]pyridine-1,3(2H)-dione ring, we decided to supplement the study with a group of N-methylene and N-ethylene derivatives, which are the subject of ongoing analysis. The methods and effects of the synthesis will be supplemented with behavioral studies. Based on the obtained results of biological tests, the emerging relationships between the activity and chemical structure of homologs are discussed.

2. Results

2.1. Chemistry

The starting materials for the synthesis of compounds 9–15 were 4-methoxy- and 4-ethoxy-6-methyl-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-diones(3,4), and in the case of imide 8, it was an intermediate 4-methoxy-2,3-dihydro-6-methyl-2-(4-bromobutyl)-1,3-dioxo-1H-pyrrolo[3,4-c]pyridine (5) synthesized previously [5,8].
2.1.1. N-Substituted Derivatives of 4-alkoxy-6-methyl-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-diones (5, 6a, 6b, 7a, 7b)

The synthesis of the final compounds was carried out in two stages (Figure 2).

The starting reagents in the first stage of the synthesis, 4-methoxy/4-ethoxy-6-methyl-1H-pyrrolo[3, 4-c]pyridine-1,3(2H)-diones [8] and 1,2-dibromoethane (6a, 6b), 1-bromo-2-chloroethane (7a, 7b), 1,2-dibromobutane (5) [5], were used. The chemical structure of the obtained final products is presented in Table 1 and Figure 2.

The reaction with 1,2-dibromoethane and 1-bromo-2-chloroethane was carried out at the reflux temperature of acetonitrile against anhydrous potassium carbonate to obtain intermediate 6 and 7, respectively. The potassium salts of 2-methoxy-6-methyl-3,4-pyridinedicarboximide (3a) were condensed with 1,4-dibromobutane, used in a fourfold excess, which was carried out with dimethylformamide (DMF) in room temperature, with the aim to obtain N-bromobutyl derivative 5.
1,4-dibromobutane, which allowed the imide substitution of only one halogen atom and a small amount of by-products (symmetrically substituted alkyl imide), was separated in all cases by fractional crystallization.

In the synthesis of intermediates 5 and 6, a large excess of 1,2-dibromoethane and 1,4-dibromobutane, which allowed the imide substitution of only one halogen atom and a small amount of by-products (symmetrically substituted alkyl imide), was separated in all cases by fractional crystallization.

In the next step, N-halogenalkylimides 5–7, were condensed with the appropriate cyclic amines: morpholine (8), phenylpiperazine (9,10), 2-methoxyphenylpiperazine (11,12).

The β-bromoethyl and γ-bromobuthyl derivative reaction was carried out again at the boiling point of acetonitrile (method A) and the β-chloroethyl derivative (method B), during heating to boiling in xylene, in both cases with anhydrous potassium carbonate.

The physicochemical properties of the N-aminoethyl final derivatives of 3,4-pyridinedicarboximide formed on both routes (Method A and B) were identical, however, the condensation of 2-bromoethyl derivative had, as expected, higher yields, therefore, when obtaining imides 9–12, this method was used.

In the case of γ-bromobutyl(5), in the 1H NMR spectrum, signals from protons were observed at the same wavelength (δ) as before [5]. Respectively: three singlets (δ = 2.62 ppm—CH3, 4.13 ppm—OCH3, 7.18 ppm—the pyridine ring proton); multiplet of methylene protons 2 x CH2 β and γ of butyl (δ = 1.81–1.93 ppm); two triplets of CH2 (δ = 3.35–3.51 ppm) and CH2 α (δ = 3.61–3.77 ppm). The lack of a wide proton band of the N-H imide group in the low field (δ = 10.74 ppm) confirms the assumed condensation course. Their structures were confirmed by spectral (IR, 1H NMR) and elemental analyses (synthesis and properties of 8–12).

### 2.1.2. Synthesis of N-Aminomethyl Derivatives with Mannich Base Character (13–15)

Imides 13–15 were synthesized in a Mannich reaction by boiling 2-methoxy-2-ethoxy-6-methyl-3,4-pyridinedicarboximide (3,4) with 33% formaldehyde and the 2- or 3-chlorophenyle-1-piperazine in tetrahydrofuran (THF) solution (Figure 3).

| Compound | R | n | Amine | Compound | R | n | Amine |
|----------|---|---|-------|----------|---|---|-------|
| 8        | CH3 | 4 | ![Structure](image1) | 11 | CH3 | 2 | ![Structure](image2) |
| 9        | CH3 | 2 | ![Structure](image3) | 12 | C2H5 | 2 | ![Structure](image4) |
| 10       | C2H5 | 2 | ![Structure](image5) |  |

**Table 1. Structure of imides 8–12.**

**Figure 3. Synthesis of Mannich bases 13–15.**
2.2. Pharmacology

2.2.1. Toxicity

The LD₅₀ values of the investigated compounds after their intraperitoneal administration in mice are presented in Table 2.

Table 2. Acute toxicity of test compounds and Aspirin (ASA) and Morphine after intraperitoneal administration according to Litchfield and Wilcoxon [15].

| Compound | LD₅₀(mg/kg) |
|----------|-------------|
| 8        | >2000       |
| 9        | 1500 (1395.0–1710.0) |
| 10–15    | >2000       |
| ASA [15] | 167.0       |
| Morphine [16] | 140.0   |

The data are median lethal doses with 5% confidence limits in parentheses (n = 6).

Imides 8, 10–15 were not toxic (LD₅₀ > 2000 mg/kg). One of them, imide 9, showed a higher toxicity (LD₅₀ = 1500 mg/kg). It should be noted that the analgesic efficacy of compound 9 requires the use of just 1/408th of the lethal dose or less (ED₅₀ ≈ 1/408 LD₅₀; Table 3). It was much safer for laboratory animals than model analgesics, aspirin or morphine, 1/40 LD₅₀ or 1/57 LD₅₀, respectively [16, 17].

Table 3. Influence of the compounds investigated on the pain reaction in the “writhing” test in mice.

| Compounds | R   | n | Amine          | ED₅₀ (mg/kg) ± SEM |
|-----------|-----|---|----------------|--------------------|
| 8         | CH₃ | 4 | \(\text{AMINE}\) | 14.5 ± 0.03 (11.15–11.28) |
| 9         | CH₃ | 2 | \(\text{N} \)     | 3.67 ± 0.49 (2.82–4.77)  |
| 10        | C₂H₅ | 2 | \(\text{N} \)     | 15.8 ± 0.91 (14.1–17.7)  |
| 11        | CH₃ | 2 | \(\text{N} \)     | 3.25 ± 0.80 (2.01–5.16)  |
| 12        | C₂H₅ | 2 | \(\text{N} \)     | 14.9 ± 2.01 (11.5–19.4)  |
| 13        | CH₃ | 1 | \(\text{N} \)     | 14.8 ± 1.40 (12.4–17.9)  |
| 14        | C₂H₅ | 1 | \(\text{N} \)     | 18.4 ± 1.73 (15.3–22.1)  |
| 15        | CH₃ | 1 | \(\text{N} \)     | 19.2 ± 2.14 (14.3–22.7)  |
| ASA       |     |   |                | 39.15 ± 4.84 (29.1–48.1) |
| Morphine  |     |   |                | 2.44 ± 0.97 (1.18–5.02)  |
2.2.2. Analgesic and Sedative Activity

In order to screen the compounds for analgesic activity, two screening methods were chosen: the “writhing” test (Table 3 and Table S1) and the “hot plate” test (Table 4).

Table 4. Influence of the compounds investigated on the pain reaction in the “hot plate” test in mice.

| Compounds | Dose (mg/kg) | Prolonged Time (%) | Time of Reaction to Pain Stimulus (s) ± SEM |
|-----------|--------------|--------------------|------------------------------------------|
| Control   | 0            |                    | 9.57 ± 1.8                               |
| 8         | 200          | 27.48              | 12.2 ± 1.4                               |
|           | 100          | 3.87               | 10.2 ± 1.8                               |
| Control   | 0            |                    | 19.5 ± 2.6                               |
| 9         | 300          | 105.1 ****         | 40.0 ± 4.4 ****                          |
|           | 150          | 101.0 ***          | 39.2 ± 5 ***                             |
|           | 75           | 55.38*             | 30.3 ± 2.7 *                             |
| Control   | 0            |                    | 17.2 ± 2.1                               |
| 10        | 200          | 34.88              | 23.2 ± 2.6                               |
|           | 100          | 6.98               | 18.4 ± 1.7                               |
| Control   | 0            |                    | 19.5 ± 2.6                               |
| 11        | 400          | 105.1**            | 40.0 ± 8.5 **                            |
|           | 200          | 50.7               | 29.4 ± 5.0                               |
|           | 100          | 23.0               | 24.0 ± 5.0                               |
| Control   | 0            |                    | 17.2 ± 2.1                               |
| 12        | 200          | 52.33              | 26.2 ± 3.1                               |
|           | 100          | 28.46              | 22.1 ± 2.7                               |
| Control   | 0            |                    | 17.2 ± 2.1                               |
| 13        | 200          | 11.62              | 19.2 ± 1.8                               |
|           | 100          | 4.07               | 17.9 ± 2.4                               |
| 14        | 200          | 16.82              | 20.1 ± 2.3                               |
|           | 100          | 2.32               | 17.6 ± 2.4                               |
| 15        | 200          | 15.69              | 19.9 ± 3.9                               |
|           | 100          | 17.0 ± 2.8         |                                           |
| Control   | 0            |                    | 14.5 ± 3.6                               |
| ASA       | 400          | 115.86**           | 31.3 ± 1.2 **                            |
|           | 200          | 35.17              | 19.6 ± 4.1                               |
|           | 100          | 11.72              | 16.2 ± 4.9                               |
| Morphine  | 6            | 111.10**           | 30.6 ± 3.9 **                            |
|           | 3            | 104.13*            | 29.6 ± 6 *                               |
|           | 1            | 33.79              | 19.4 ± 2.1                               |

Each group consisted of six to eight animals. **** p < 0.001, *** p < 0.01, ** p < 0.02, * p < 0.05.

All tested derivatives (8–15) were active in the “writhing” test (ED$_{50}$ = 3.25–19.2 mg/kg), and their analgesic properties in this study exceeded the effect of aspirin (ED$_{50}$ = 39.15 mg/kg). In addition, for two imides, they were similar to morphine activity (9 = 3.25 mg/kg, 11 = 3.67 mg/kg, morphine = 2.44 mg/kg).
However, in the case of the “hot plate” test, the observed analgesic effects for the tested compounds 8, 10, 12–15 were not statistically significant. None of them had analgesic effects in the applied doses (Table 4). The derivative 9 exerted an analgesic effect in three doses: 300, 150, 75 mg/kg, and prolonged the latency time to nociceptive response by 105%, 102% and 55%, respectively. Compound 11 also prolonged the latency time by 105%, but in one 400 mg/kg dose only. For the tested derivatives, ED₅₀ values were not determined, due to the non-significant effect in this test (8, 10, 12–15) or activity that did not fulfill the requirements for the proper calculations of the value (derivative 9 and 11). The ED₅₀ values for the reference compounds were 266.7 mg/kg (±SEM = 98.26; 148.2–533.4) and 2.55 mg/kg (± SEM = 0.63; 1.59–4.08) for ASA and morphine, respectively.

As before [5,8], the tested derivatives showed sedative properties. Imides 8–15 significantly inhibited spontaneous locomotor activity in mice (Table 5 and Table S2) and the two strongest (9, 11) also extended the duration of thiopental anesthesia (Table 6).

Table 5. Influence of the compounds investigated on the spontaneous locomotor activity in mice.

| Compounds | R   | n | Amine | ED₅₀ (mg/kg) ± SEM |
|-----------|-----|---|-------|--------------------|
| 8         | CH₃ | 4 | N     | 34.2 ± 8.50 (21.37–54.72) |
| 9         | CH₃ | 2 | -     | 18.8 ± 4.00 (12.5–28.2)  |
| 10        | C₂H₅| 2 | -     | 84.0 ± 5.10 (75–95)     |
| 11        | CH₃ | 2 | -     | 19.7 ± 4.89 (12.3–31.5) |
| 12        | C₂H₅| 2 | -     | 85.0 ± 4.20 (77–93.5)   |
| 13        | CH₃ | 1 | -     | 164.0 ± 28.72 (117–229.6) |
| 14        | C₂H₅| 1 | -     | 98.0 ± 13.26 (75.4–127.4) |
| 15        | CH₃ | 1 | -     | 89.1 ± 4.46 (80–97.5)    |

The pilot biological studies for imides 9 and 11 were supplemented with the determination of the effect of the intraperitoneal administration of the test compounds on the duration of thiopental-induced sleep. The mechanism and the degree of crossing the blood–brain barrier has not been precisely defined. The observed sedative effects may indicate good penetration of compounds into the central nervous system, which, however, has not been confirmed.
Table 6. Influence of the compounds investigated on thiopental anesthesia.

| Compounds | Dose (mg/kg) | Prolongation (%) | Duration of Anesthesia ± SEM (min) |
|-----------|--------------|------------------|----------------------------------|
| Control   | 0            |                  | 51.5 ±11.2                       |
|           | 37.5         | 157.1 **         | 132.4 ±27.8 **                   |
| 9         | 18.75        | 96.5 *           | 101.2 ±28.4 *                    |
|           | 9.375        | 34.8             | 69.4 ±12                         |
| 11        | 50           | 140.8 ***        | 124 ±16.2 ***                    |
|           | 25           | 16.11 **         | 50.8 ±14.2 **                    |
|           | 12.5         | 36.3             | 70.2 ±24                         |

Each group consisted of six to eight animals. *** p < 0.01, ** p < 0.02. * p < 0.05.

3. Discussion

Based on our current and previous research [5,6,8,10], it is possible to determine the relationship between the structure of molecules and biological properties in the group of 3,4-pyridinedicarboximide derivatives. To this end, the impact on the animal test results of the following structural elements should be determined.

The basic modifications are: (I) an alkoxy substituent on the pyridine ring; (II) type of amino residue; (III) length of the alkyl link between these building elements. To this end, it is necessary to recall the previous conclusions (marked, to distinguish series 1 and 2), comparing the abovementioned modifications and the obtained results of experimental studies. (I) The type of alkoxy substituent on the pyridine ring has a decisive influence on the analgesic potency. The methoxyhomolog was more active in the tests. Only in halogenated derivatives was a deviation from this regularity observed. The intensification of hydrophobic properties by increasing the substituent to two carbon atoms weakened the analgesic properties, although their potential still remained noteworthy (II).

The type of amino residue, as the basic center of the molecule, is in most examples a phenylpiperazine moiety. The strongest analgesic effect was observed in derivatives with an unsubstituted benzene ring. The introduction of 2-OCH₃/3-CF₃ pharmacophoric moieties and halogen (Cl/F) atoms was not preferred, similarly in the case of replacing phenylpiperazine with another cyclic amine (morpholine, tetrahydroisoquinoline). The strongest analgesic properties in the tests performed showed phenylpiperazine derivatives in each series of homologs. Only in the case of the elimination of the OH group and the propyl linker did the presence of pharmacophores in phenyl significantly “compensate” this modification. These relationships were more analgetically active. The following thesis can be adopted that the analgesic potency of 1H-pyrrolo [3,4-c]pyridine-1,3 (2H) -dione derivatives decreases according to the following series: attitudes in the benzene ring H > OCH₃ ≥ CF₃ > Cl/F among all homologs.

A separate analysis should be done for the length of the alkyl linkage between the cyclic imide ring and the basic center of the amino residue (III). A significant influence of this element of the structure on the analgesic potency has been noticed. Compounds 1A,2A, considered by us as models [6], containing an OH group in the propyl linker (racemate), showed activity similar to that noted for aspirin (ED₅₀ in the “hot plate” test 10.6–96.8 mg/kg; ASA = 266.7 mg/kg) [6,8]. Not all derivatives obtained were active in this study, so we make a summary in the second, “writhing” test. In this test, the activity of the derivatives tested was similar or higher than that of morphine (ED₅₀ 0.4–2.80 mg/kg; morphine = 2.44 mg/kg, significantly higher than aspirin = 39.15 mg/kg, Table 7).

Elimination of the OH group weakened the analgesic effect. The ED₅₀ of propyl derivatives was 0.67–1.03–1.10–2.59 mg/kg, respectively.

As mentioned, compounds containing the 2-OCH₃ pharmacophore in phenyl were more active. With the shortening of the distance to C-2 and C-1, activity decreased. Additionally, elongation of the C-4 linker (butyl derivatives) did not increase analgesic activity. To illustrate the above conclusions, Table 7 summarizes ED₅₀ values in the “writhing” test in mice of phenylpiperazine analogs (1H, 2H, 9,
1K), 2-methoxyphenylpiperazine analogs (1I, 1P, 11, 1L) and all amines (1N, 1M, 8, 1Q) or halogens (1J, 2J) in Table 8 [10].

Table 7. Influence of the 2-methoxy homologs on the pain reaction in the “writhing” test in mice/compounds obtained previously (series 1 and 2) [5,6,8,10] and new imides (8,9).

| Compound | R | n | ED50 (mg/kg) |
|----------|---|---|-------------|
| 1H |  | 3 | 1.03 |
| 1P |  | 4 | 4.5 |
| 9 |  | 2 | 3.67 |
| 1K |  | 1 | 2.55 |
| 1I | MeO | 3 | 0.67 |
| 1R | MeO | 4 | 6.8 |
| 11 | MeO | 2 | 3.25 |
| 1L | MeO | 1 | 6.53 |
| 1N |  | 4 | 0.72 |
| 1M |  | 1 | 12.7 |
| 8 |  | 4 | 1.5 |
| 1Q |  | 1 | 13.66 |

Modifications to the structure of the model 1A imide resulted in the occurrence of a calming effect in the test, determining the effect of the compounds tested on the spontaneous mouse mobility. Some of the derivatives obtained were additionally tested in a thiopental test.

All derivatives, obtained as a result of the modification of the imide structure 1A, statistically significantly inhibited the spontaneous locomotion activity of mice. The ED50 values of homologs are given in Table 9. Analyzing the relationship between the chemical structure of the molecule and the sedative activity of homologs, one can notice a large variation, depending on all introduced modifications. The strongest sedative properties were observed in the case of imide 1K (Mannich base character), while the effective dose values of other derivatives from the same group were much higher.
Some of the derivatives obtained were additionally tested in a thiopental test. Determining the effect of the compounds tested on the spontaneous mouse mobility.

The statistically significant analgesic activity of 3,4-pyridinedicarboximide derivatives, resulted in the occurrence of a calming effect in the test, determining the effect of the compounds tested on the spontaneous mouse mobility.

Modifications to the structure of the model molecule resulted in the occurrence of a calming effect in the test, determining the effect of the compounds tested on the spontaneous mouse mobility.

The strongest sedative properties were observed in the case of imide 1Q (Table 8.).

Morphine and tramadol (a centrally acting synthetic opioid analgesic and serotonin/norepinephrine reuptake inhibitor (SNRI), much simpler than opioids) have receptor affinity at concentrations several orders of magnitude higher.

Table 8. Influence of the halogen homologs on the pain reaction in the “writhing” test in mice, imides described previously (1J, 2J) and new imides 13,14 [10].

| Compound | R, n | ED50 (mg/kg) | % Inh. | Dose (mg/kg) | % Prolong. |
|----------|------|--------------|--------|--------------|------------|
| 1J       | CH3  | 3            | 8.8    |              |            |
| 2J       | C2H5 | 3            | 8.7    |              |            |
| 13       | CH3  | 1            | 14.8   |              |            |
| 14       | C2H5 | 1            | 18.4   |              |            |

Table 9. Comparison of sedative properties of selected homolog imides described previously (series 1) [5,6,8] and new derivatives 8,9,11.

| Compound | R, n | Influence on the Locomotor Activity | Thiopeptanl Anesthesia |
|----------|------|-------------------------------------|------------------------|
|          |      | ED50 (mg/kg) | Dose (mg/kg) | % Inh. | Dose (mg/kg) | % Prolong. |
| 1H       |      | 15.17       | 50          | 66.55 *** | 50          | 194 *** |
|          |      | 12.5        | 25          | 53.84 **  | 25          | 118 *  |
| 1P       |      | -           | 100         | 86.10 **  | 100         | 242.13 *** |
|          |      | 50          | 37.5        | 67.18 *** | 37.5        | 157.1 ** |
| 9        |      | 18.8        | 18.75       | 45.90 **  | 18.75       | 96.5 ** |
|          |      | 9.37        | 42.79 *     |           |             |         |
| 1K       |      | 2.28        | 4.5         | 61.77 *** | 4.5         | 199.1 ** |
|          |      | 2.25        | 49.23 **    |           |             |         |
| 1L       |      | 11.8        | 25          | 72.77 ****| 25          | 140.3 * |
|          |      | 12.5        | 53.91 **    | 12.5       | 123.3 *     |
| 1R       |      | -           | 100         | 82.32 **  | 100         | 255.97 *** |
|          |      |             |             |           |             |         |
| 1N       |      | 19.7        | 50          | 68.74 *** | 50          | 140.8 ** |
|          |      | 12.5        | 25          | 55.85 **  | 25          | 16.11 ** |
| 1L       |      | 25.2        | 50          | 68.25 *** | 50          | 184.3 ** |
|          |      | 12.5        | 25          | 40.84 *   | 25          | 133.2 ** |
| 1N       |      | 26.2        | 46          | 87.48 *   | 46          | 286.5 ** |
|          |      |             |             |           |             |         |
| 1O       |      | 29.5        | 50          | 59.21 *** | 50          | 195.1 ** |
|          |      | 25          | 47.25 **    | 25          | 146.6 **    |
| 8        |      | 34.2        | 50          | 61.64 ****| -           | Not tested |
|          |      | 12.5        | 35.36 **    |           |             |         |
| 1Q       |      | 44          | 42.65 *     | 44          | 141.7 **    |

Each group consisted of 6-8 animals. **** p < 0.001, *** p < 0.01, ** p < 0.02, * p < 0.05.
It is not possible to identify elements of the structure clearly responsible for the sedative effect. The results in Table 9 illustrate the significant variation in CNS inhibitory properties for the listed derivatives.

The statistically significant analgesic activity of 3,4-pyridinedicarboximide derivatives, discussed above, does not allow for determining the mechanism of the analgesic activity of new compounds and its relationship with the sedative effect. One direction of our further research was a pilot determination of the affinity of some imides for µ opioid receptors. To this end, our team performed an experiment to determine the ligand displacement ability of tritium-labeled dihydromorphine [3H-DHM] from binding sites of the µ receptor of the rat cerebral cortex (Table S3). Selected derivatives displaced the ligand at a concentration significantly exceeding 100 nM. Morphine and tramadol (a centrally acting synthetic opioid analgesic and serotonin/norepinephrine reuptake inhibitor (SNRI), much simpler than opioids) have receptor affinity at concentrations several times lower: 0.62 and 2.4 nM, respectively [18–20]. It can be concluded that the new derivatives that we synthesized and tested may become a new and useful class of analgesics with a unique mechanism of action, including cyclooxygenase (COX) inhibition at the lower doses and opioid receptor activation at higher ones. For this purpose, further studies will be needed to determine the affinity of 3,4-pyridinedicarboximides for the enzymes of the arachidonic acid pathway and for opioid receptors.

4. Materials and Methods

4.1. Chemistry

All the results of the C, H and N determinations (carried out by a Carlo Erba Elemental Analyzer model NA-1500, Carlo Erba, Thermo Scientific, Waltham, MA, USA) were within ± 0.4% of the theoretical values. All melting points are uncorrected. The IR spectra, in KBr pellets, were measured with a Zeiss Jena Specord model IR75 (Zeiss Jena, Uberlingen, Germany) and 1H NMR spectra were determined in CDCl3, if not otherwise indicated, on a Tesla 587 A spectrometer (80 MHz, Tesla, Brno, Czech Republic) using tetramethylsilane (TMS) as an internal standard.

4.1.1. Procedure for Obtaining 4-methoxy-6-methyl-4-(N-morpholino)-butyl-1H-pyrrolo[3,4-c]pyridine-1,3-(2H)-dion(8)

To 0.003 mol of compound 3 in 70 mL of anhydrous acetonitrile, 0.75 g of anhydrous potassium carbonate and 0.004 mol morpholine were added. The mixture was refluxed for 21 h. After filtration, the solvent was evaporated under reduced pressure and the residue was purified by crystallization from ethanol.

The properties of 8: Formula: C17H23N3O3; MW = 333.38; MP: 92–93°C; solvent: ethanol; yield: 51%; IR (cm⁻¹): C=O 1720, 1780; -CH2- 2800–2980; 1H NMR of 8: δ [ppm] = 1.34–2.22 (m-4H. Hβ + Hγ of butyl); 2.25–2.70 (m-9H. CH3 + -CH2-N(CH2)2); 2.34–4.20 (m-6H. Hα of butyl + -(CH2)2O); 4.13 (s-3H. OCH3); 7.18 (s-1H. H of pyridine).

4.1.2. Procedure for Obtaining 4-methoxy-/4-ethoxy-6-methyl-2-(2-bromoethyl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-diones (6a, 6b)

To 0.01 mol of compound 3 in 70 mL of anhydrous acetonitrile, 0.01 mol of anhydrous potassium carbonate and 0.004 mol morpholine were added. The mixture was refluxed for 21 h. After filtration, the solvent was evaporated under reduced pressure and the residue was purified by crystallization from ethanol.

The properties of 6a, 6b: Formula: C17H23N3O3; MW = 333.38; Melting point: 6a: 112°C; 6b: 118°C; Solvent: ethanol; Yield: 6a: 58%; 6b: 55%; IR (cm⁻¹): C=O 1720, 1780; -CH2- 2800–2980; 1H NMR of 6a, 6b: δ [ppm] = 1.34–2.22 (m-4H. Hβ + Hγ of butyl); 2.25–2.70 (m-9H. CH3 + -CH2-N(CH2)2); 2.34–4.20 (m-6H. Hα of butyl + -(CH2)2O); 4.13 (s-3H. OCH3); 7.18 (s-1H. H of pyridine).
H
(a rotary evaporator. The dry residue was crystallized from cyclohexane to give a light yellow product.

After hot filtration, the inorganic material was washed with acetonitrile. The solvent was completely evaporated on

\( \text{Method A} \)

| Compound | Formula (mol. wt.) | M.p. (°C) | Yield (%)/Method | IR Absorptions in KBr (cm\(^{-1}\)) |
|----------|--------------------|-----------|-------------------|-------------------------------------|
| 6a       | \( \text{C}_{11}\text{H}_{12}\text{BrN}_{2}O_{3} \) 299.13 | 113–114 n-hexane | 42 | C=O 1718 2920 CH\(_2\) 1771 2950 |
| 6b       | \( \text{C}_{12}\text{H}_{12}\text{BrN}_{2}O_{3} \) 313.10 | 100–102 Cyclohexane | 45 | C=O 1720 2900 CH\(_2\) 1770 2950 |
| 7a       | \( \text{C}_{11}\text{H}_{13}\text{ClN}_{2}O_{3} \) 254.67 | 110–112 Cyclohexane | 79 | C=O 1740 2920 CH\(_2\) 1780 2980 |
| 7b       | \( \text{C}_{12}\text{H}_{13}\text{ClN}_{2}O_{3} \) 268.69 | 102–103 Cyclohexane | 81 | C=O 1730 2900 CH\(_2\) 1780 2980 |
| 8        | \( \text{C}_{17}\text{H}_{12}\text{N}_{2}O_{4} \) 333.38 | 92–93 ethanol | 51 | C=O 1720 2800 CH\(_2\) 1780 2980 |
| 9        | \( \text{C}_{21}\text{H}_{22}\text{N}_{2}O_{3} \) 380.43 | 150–152 Cyclohexane | 51.7/A 28/B | C=O 1717 2820 CH\(_2\) 1770 2950 |
| 10       | \( \text{C}_{22}\text{H}_{24}\text{N}_{2}O_{3} \) 394.47 | 118–120 Cyclohexane | 55/A 35/B | C=O 1715 2840 CH\(_2\) 1770 2940 |
| 11       | \( \text{C}_{22}\text{H}_{24}\text{N}_{2}O_{4} \) 410.46 | 175–176 Cyclohexane | 48.5/A 32/B | C=O 1714 2930 CH\(_2\) 1769 2950 |
| 12       | \( \text{C}_{23}\text{H}_{24}\text{N}_{2}O_{4} \) 424.50 | 165–167 Ethanol/cyclohexane | 55/A 37/B | C=O 1715 2820 CH\(_2\) 1765 2940 |
| 13       | \( \text{C}_{20}\text{H}_{21}\text{ClN}_{2}O_{3} \) 400.5 | 157–160 n-heptane | 65 | C=O 1720 2970 CH\(_2\) 1770 - |
| 14       | \( \text{C}_{21}\text{H}_{21}\text{ClN}_{2}O_{3} \) 414.50 | 127–129 n-heptane | 70 | C=O 1715 2820 CH\(_2\) 1775 - |
| 15       | \( \text{C}_{20}\text{H}_{21}\text{ClN}_{2}O_{3} \) 400.5 | 162–164 n-heptane | 52 | C=O 1720 2970 CH\(_2\) 1770 - |

\(^{1}\text{H NMR of 6a: } \delta [\text{ppm}] = 2.62 (s-3\text{H}, \text{CH}_3); 3.34–3.74 (t-2\text{H}, \text{H}^\beta \text{ of ethyl}); 3.84–4.28 (m-5\text{H}, \text{OCH}_3 + \text{H}x \text{ of ethyl}); 7.11 (s-1\text{H}, \text{H} \text{ of pyridine}). \)

\(^{1}\text{H NMR of 6b: } \delta [\text{ppm}] = 1.32–1.65 (t-3\text{H}, -\text{OCH}_2\text{CH}_3); 2.61 (s-3\text{H}, \text{CH}_3); 3.47–3.71 (t-2\text{H}, \text{H}^\beta \text{ of ethyl}); 3.92–4.23 (t-2\text{H}, \text{H}x \text{ of ethyl}); 4.42–4.78 (q-2\text{H}, -\text{OCH}_2\text{CH}_3); 7.17 (s-1\text{H}, \text{H} \text{ of pyridine}). \)

4.1.3. Procedure for Obtaining 4-methoxy-/4-ethoxy-6-methyl-2-(2-chloroethylo)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-diones (7a, 7b)

To 0.01 mol of compound 3 in 70 mL of anhydrous acetonitrile, 0.01 mol of anhydrous potassium carbonate were refluxed for 0.5 h. Next, 0.01 mol 1-bromo-2-chloroethan were added. The mixture was refluxed for 17 h. After hot filtration, the inorganic material was washed with acetonitrile. The solvent was completely evaporated on a rotary evaporator. The dry residue was crystallized from cyclohexane (7a,7b) to give a light yellow amorphous product. The physicochemical properties are given in Table 10.

\(^{1}\text{H NMR of 7a: } \delta [\text{ppm}] = 2.63 (s-3\text{H}, \text{CH}_3); 3.56–4.06 (m-4\text{H}, \text{H}x + \text{H}^\beta \text{ of ethyl}); 4.13 (s-3\text{H}, \text{OCH}_3); 7.20 (s-1\text{H}, \text{H} \text{ of pyridine}). \)

\(^{1}\text{H NMR of 7b: } \delta [\text{ppm}] = 1.31–1.78 (t-3\text{H}, -\text{OCH}_2\text{CH}_3); 2.61 (s-3\text{H}, \text{CH}_3); 3.75–3.86 (t-2\text{H}, \text{H}x \text{ of ethyl}); 3.87–4.18 (t-2\text{H}, \text{H}^\beta \text{ of ethyl}); 4.24–4.84 (m-2\text{H}, -\text{OCH}_2\text{CH}_3); 7.18 (s-1\text{H}, \text{H} \text{ of pyridine}). \)

4.1.4. General Procedure for Obtaining Compounds 9–12

Method A

To 0.01 mol of compound 6a or 6b and 0.01 mol of anhydrous potassium carbonate, 70 mL of acetonitrile and 0.02 mol of suitable amine (4-phenyl-1-piperazine; 4-(2-methoxyphenyl-1-piperazine) in 10 mL of acetonitrile were added. The mixture was refluxed for 17 h. After hot filtration (simple paper filter), the inorganic material was washed with acetonitrile. The solvent was completely evaporated on a rotary evaporator. The dry residue was crystallized from cyclohexane to give a light yellow product.
The physicochemical properties of compounds 9–12 are given in Table 10.

Method B

To 0.01 mol of the compound, suspended in 40 mL of dried xylene, 0.01 mol of anhydrous potassium carbonate and 0.02 mol of the corresponding amine were added. The whole mixture was heated to reflux with constant stirring for 15 h. Then it was filtered and the inorganics were washed with solvent and evaporated completely. The resulting oil was crystallized from cyclohexane or a mixture with ethanol (properties of compounds 9–12 in Table 10, method B).

\[ ^1H \text{ NMR of 9: } \delta [\text{ppm}] = 2.34–2.86 \text{ (m-9H, CH}_3+\text{-CH}_2\text{-N-(CH}_2\text{)}_2\text{-N-); 2.88–3.27 \text{ (m-4H, -N(CH}_2\text{)}_2\text{-N-); 3.62–3.94 (t-2H, H}_\alpha\text{ of ethyl); 4.11 (s-3H, OCH}_3\text{); 6.62–7.40 (m-6H, H arom.).} \]

\[ ^1H \text{ NMR of 10: } \delta [\text{ppm}] = 1.13–1.63 \text{ (t-3H, -OCH}_2\text{CH}_3\text{); 2.21–2.86 \text{ (m-9H, CH}_3+\text{-CH}_2\text{-N(CH}_2\text{)}_2\text{-N-); 3.05–3.51 (distorted t-4H, H-piperazine); 3.64–4.02 \text{ (t-2H, H}_\alpha\text{ of ethyl); 4.37–4.84 (q-2H, -OCH}_2\text{CH}_3\text{); 6.59–7.47 (m-6H, H arom.).} \]

\[ ^1H \text{ NMR of 11: } \delta [\text{ppm}] = 2.54–2.88 \text{ (m-9H, CH}_3+\text{-CH}_2\text{-N-(CH}_2\text{)}_2\text{-N-); 2.88–3.16 (m-4H, -(CH}_2\text{)}_2\text{-N-); 3.73–3.98 (m-5H, OCH}_3+\text{H}_\alpha\text{ of ethyl); 4.13 (s-3H, OCH}_3\text{); 6.73–7.38 (m-5H, H arom.).} \]

\[ ^1H \text{ NMR of 12: } \delta [\text{ppm}] = 1.27–1.62 \text{ (t-3H, -OCH}_2\text{CH}_3\text{); 2.44–2.84 \text{ (m-9H, CH}_3+\text{-CH}_2\text{-N(CH}_2\text{)}_2\text{-N-); 2.90–2.96 (m-4H, H-piperazine); 3.34–4.01 \text{ (m-5H, -OCH}_3+\text{H}_\alpha\text{ of ethyl); 4.38–4.75 (q-2H, -OCH}_2\text{CH}_3\text{); 6.72–7.33 (m-5H, H arom.).} \]

4.1.5. General Procedure for Obtaining Compounds 13–15

To 0.002 mole of 2-methoxy or 2-ethoxy-6-methyl-3,4-pyridinedicarboximide (3,4), suspended in 40 mL tetrahydrofuran, 1 ml 33% formalin was added. The reaction mixture was heated for 0.5 h, then 0.0022 mol of the corresponding amine (2- or 3-chloro-4-phenyl-1-piperazine) were added again to reflux for 10 h. The whole mixture was evaporated completely on a rotary evaporator and the remaining oil crystallized from suitable solvents (Table 10).

The following is the interpretation of \(^1\text{H NMR spectra of compounds 13–15.}\)

\[ ^1H \text{ NMR of 13: } \delta [\text{ppm}] = 2.63 \text{ (s-3H, CH}_3\text{); 2.67–2.86 \text{ (t-4H, -N-(CH}_2\text{)}_2\text{-N-); 3.00–3.26 \text{ (t-4H, -(CH}_2\text{)}_2\text{-N-); 4.13 (s-3H, OCH}_3\text{); 4.66 (s-2H, -CH}_2\text{); 6.66–7.27 (m-5H, H arom.).} \]

\[ ^1H \text{ NMR of 14: } \delta [\text{ppm}] = 1.45–1.55 \text{ (t-3H, -OCH}_2\text{CH}_3\text{); 2.62 \text{ (s-3H, CH}_3\text{); 2.73–2.95 \text{ (t-4H, -N(CH}_2\text{)}_2\text{-N-); 3.15–3.35 (t-4H, H-piperazine); 4.59–4.78 \text{ (m-4H, -CH}_2\text{-; +OCH}_2\text{CH}_3\text{); 6.70–7.25 (m-5H, H arom.).} \]

\[ ^1H \text{ NMR of 15: } \delta [\text{ppm}] = 2.64 \text{ (s-3H, CH}_3\text{); 2.73–2.86 \text{ (t-4H, -N-(CH}_2\text{)}_2\text{-N-); 3.06–3.36 \text{ (t-4H, -(CH}_2\text{)}_2\text{-N-); 4.14 (s-3H, OCH}_3\text{); 4.68 (s-2H, -CH}_2\text{); 6.88–7.43 (m-5H, H arom.).} \]

4.2. Materials and Methods of Pharmacology Experiments

4.2.1. Substances

Acetylic acid (polopiryina, Polpharma, Starogard Gda´nski, Poland). Morphine (morphinum hydrochloridum, Polfa-Kutno, Poland).

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 (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 6–8 animals each. The investigated compounds were administered intraperitoneally as a suspension in 0.5% methylcellulose in a constant volume of 10 mL/kg.
4.3. 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 [8,15]. The obtained 95% confidence limits were transformed to standard errors of the mean (SEM), as described previously [21].

4.4. Acute Toxicity

Acute toxicity was assessed by the methods of Litchfield and Wilcoxon [8,16] and presented as LD$_{50}$ calculated from the mortality of mice after 24 h.

4.5. Pain Reactivity

Pain reactivity was measured in two tests: “hot plate” test (according to the method of Eddy and Leimbach [9,22]) and “writhing” test in mice (according to Hendershot and Forsaith [8,23]).

4.5.1. “Hot plate” Test

Animals were placed individually on the metal plate, heated to 55 ± 1 °C. The latency time (s) to the pain reaction (licking of the hind paws or jumping) was recorded by a stop-watch. A cutoff time of 45 s was used to prevent tissue damage. The experiment was performed 30 min after the administration of the investigated compounds at graded doses of 4.5–100 mg/kg (1/160–1/20 LD$_{50}$ i.p.).

4.5.2. “Writhing” Test in Mice

Different doses of the compounds, ranging from 0.39 to 100 mg/kg (1/5120–1/20 LD$_{50}$ i.p.), were administered intraperitoneally. Then, after 25 min, the irritant (phenylbenzoquinone, 0.02% solution, ethanol-water, 5:95) was also administered intraperitoneally in a constant volume of 0.25 mL. Five min after the application of the irritant, a 10 min long period of observation started, in which the number of writhing episodes was counted.

The analgesic effect of individual doses was expressed in percent:

\[
\% \text{ Analgesic effect} = 100 - \frac{\sum \text{of writing incidents in experimental group}}{\sum \text{of writhing incidents in control group}} \times 100
\]

4.6. Sedative Effect

Spontaneous locomotor activity in mice was measured in circular photoresistoractometers (32 cm in diameter). The investigated compounds were injected intraperitoneally, at a dose range of 1.56–50 mg/kg. Thirty minutes after the injection of the investigated compounds, mice were placed in the actometers for 30 min. Each crossing of the light beam was recorded automatically. The amount of impulses was noted after 30 min.

5. Conclusions

The values of ED$_{50}$ in the spontaneous locomotor activity test are significantly higher than those obtained in the “writhing” test (the values were 5- and 6-fold higher for derivatives 9 and 11, respectively). These results show that the sedative effect of the tested compounds does not affect the analgesic effect in the “writhing” test. On the other hand, the analgesic effect in the “hot plate” test may result from the sedative activity of the compounds or may be in line with that activity. As it can be noticed for morphine, opioids are active in both tests within a similar range of doses, whereas drugs mainly active in the inflammatory pain, such as Nonsteroidal anti-inflammatory drugs NSAIDs (including aspirin), are active in the “writhing” test, while being much less active in the “hot plate” test. The results for the test compounds show that they are active in chemogenic inflammatory pain rather than in acute pain induced by a thermal stimulus. Thus, their pharmacological profile resembles...
the activity of NSAIDs and suggests that their mechanism of action may result from the inhibition of cyclooxygenase function. To confirm or exclude this hypothesis, some further biochemical studies of affinity for cyclooxygenase (COX) and opioid receptors should be performed. On this basis, it will be possible to determine the correlation between the effect confirmed in tests and the results of theoretical molecular docking.

**Supplementary Materials:** The following materials are available online. Figure S1. Structure of 1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione derivatives previously published and discussed in the text (1A–F, 2A–F). Part I. Figure S2. Structure of 1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione derivatives previously published and discussed in the text (1H–R, 2H–I). Part II. Table S1. Detailed data of the influence of the compounds investigated in the pain reaction in the “writhing” test in mice. Table S3. Detailed data of the influence of the compounds investigated in the spontaneous locomotor activity in mice. Table S2. Determination of the ability to displace ligands labeled with tritium dihydromorphine [3H-DHM] from the μ receptor binding sites of the rat cortex.

**Author Contributions:** D.S. performed the synthetic experiments, created the concept, designed the study, coordinated the work and wrote the manuscript. S.M. performed the biological tests and data analysis and wrote the manuscript. B.F. performed the biological tests and data analysis. J.S. performed the biological tests and data analysis. E.K. wrote the manuscript. P.´S.was responsible for coordination and was a project manager. All authors have read and agreed to the published version of the manuscript.

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**References**

1. Palmer, R.H.C.; McGeary, J.E.; Knopik, V.S.; Bidwell, L.C.; Metrik, J.M. CNR1 and FAAH variation and affective states induced by marijuana smoking. *Am. J. Drug Alcohol Abus.* 2019, 45, 514–526. [CrossRef] [PubMed]
2. Bang, S.; Yoo, J.; Gong, X.; Liu, D.; Han, Q.; Luo, X.; Chang, W.; Chen, G.; Im, S.-T.; Kim, Y.H.; et al. Differential Inhibition of Nav1.7 and Neuropathic Pain by Hybridoma-Produced and Recombinant Monoclonal Antibodies that Target Nav1.7: Differential Activities of Nav1.7-Targeting Monoclonal Antibodies. *Neurosci. Bull.* 2018, 34, 22–41. [CrossRef] [PubMed]
3. Chew, L.A.; Bellampalli, S.S.; Dustrude, E.T.; Khanna, R. Mining the Nav1.7 interactome: Opportunities for chronic pain therapeutics. *Biochem. Pharmacol.* 2019, 163, 9–20. [CrossRef] [PubMed]
4. Dziubina, A.; Szkatula, D.; Gdula-Argasińska, J.; Kotarńska, M.; Filipek, B. Antinociceptive, antiedematous, and antiallodynic activity of 1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione derivatives in experimental models of pain. *Naunyn-Schmiedeberg’s Arch. Pharmacol.* 2019, 393, 813–827. [CrossRef] [PubMed]
5. Śladowska, H.; Szkatula, D.; Filipek, B.; Maciąg, D.; Sapa, J.; Zygmun, M. ChemInform Abstract: Synthesis and Properties of 2-(4-Substituted)butyl Derivatives of Some 2,3-Dihydro-1,3-dioxo-1H-pyrrolo[3,4-c]pyridines. *Chem. Rev.* 2001, 32, 133–138. [CrossRef]
6. Śladowska, H.; Filipek, B.; Szkatula, D.; Sabiniarz, A.; Kardasz, M.; Potoczek, J.; Sieklucka-Dziuba, M.; Rajtar, G.; Kleinrok, Z.; Lis, T. Investigations on the synthesis and pharmacological properties of 4-alkoxy-2-[2-hydroxy-3-(4-aryl-1-piperazinyl)propyl]-6-methyl-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-diones. *Farmaco* 2002, 57, 897–908. [CrossRef]
7. Muszalska, I.; Śadowska, H.; Szkatula, D. A validated spectrophotometric and liquid chromatography method for determination and purity evaluation of 4-methoxy-2-[2-hydroxy-3(4-phenyl-1-piperazinyl)propyl]-2,3-dihydro-6-methyl-1H-pyrrolo[3,4-c]pyridine. *Farmaco* 2003, 58, 513–519. [CrossRef]
8. Śladowska, H.; Filipek, B.; Szkatula, D.; Sapa, J.; Bednarski, M.; Ciolkowska, M. Investigations on the synthesis and pharmacological properties of N-substituted derivatives of 4-alkoxy-6-methyl-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-diones. *Farmaco* 2005, 60, 53–59. [CrossRef]
9. Muszalska, I.; Śladowska, H.; Szkatula, D. Quantitative Determination of 4-Ethoxy-2-[2-Hydroxy-3-(4-Phenyl-1-Piperazinyl)]-Propyl-2,3-Dihydro-6-Methyl-1H-Pyrrolo-[3,4-c] Pyridine Applying High-Performance Liquid Chromatography Using UV Detection. Studies on Degradation Mechanism. *Chem. Anal.* 2005, 50, 875–886.
10. Śładowska, H.; Sabiniarz, A.; Szkatula, D.; Filipk, B.; Sapa, J. Synthesis and properties of 4-alkoxy-2-[2-hydroxy-3-(4-o.m.p-halogenoaryl-1-piperazinyl)propyl]-6-methyl-1H-pyrrolo-[3,4-c]pyridine-1,3(2H)-diones with analgesic and sedative activities. *Acta Pol. Pharm. -Drug Res.* 2007, 63, 245–254.

11. Muszalska, I.; Górski, P.; Śładowska, H.; Szkatula, D.; Sabiniarz, A. Chromatographic Separation of Derivatives of 4?Alkoxy?6?methyl?1 H ?pyrrolo[3,4-c]pyridine?1,3(2H)?dione by TLC and HPLC. *J. Liq. Chromatogr. Relat. Technol.* 2007, 30, 2103–2115. [CrossRef]

12. Krzyżak, E.; Szkatula, D.; Szczęśniak-Siega, B.M.; Malinka, W. Synthesis and DSC study a new pyridinedicarboximide diones derivatives, obtained under various conditions. *J. Therm. Anal. Calorim.* 2014, 120, 847–853. [CrossRef] [PubMed]

13. Muszalska, I.; Ciemniewski, M.P.; Lesniewska, M.A.; Szkatula, D.; Malinka, W. Forced Degradation and Photodegradation Studies of Pyrrolo[3,4-c]pyridine-1,3-dione Derivatives as Analgesic Active Compounds Using HPLC, UV and IR Spectrometry, and HPLC/MS Methods. *J. AOAC Int.* 2015, 98, 1248–1259. [CrossRef]

14. Krzyżak, E.; Szkatula, D.; Wiatrak, B.; Gębarowski, T.; Marciniak, A. Synthesis, Cyclooxygenases Inhibition Activities and Interactions with BSA of N-substituted 1H-pyrrolo[3,4-c]pyridine-1,3(2H)-diones Derivatives. *Molecules* 2020, 25, 2934. [CrossRef] [PubMed]

15. Litchfield, J.T.; Wilcoxon, F. A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.* 1949, 96, 99–113. [PubMed]

16. United States Patent Document. Vol. #4205173. Available online: http://chem.sis.nlm.nih.gov/chemidplus/ [morphine] (accessed on 12 December 2020).

17. Kleemann, A.; Engel, J.; Kutscher, B.; Reichert, D. *Pharmaceutical Substances, 5th Edition*, 2009: Syntheses, Patents and Applications of the Most Relevant APIs, 5th ed.; Thieme: Leipzig, Germany, 2009.

18. Hennies, H.H.; Friderichs, E.; Schneider, J. Receptor binding, analgesic and antitussive potency of tramadol and other selected opioids. *Arzneimittelforschung* 1988, 38, 877–880.

19. Raffa, R.B.; Friderichs, E.; Reimann, W.; Shank, R.P.; E Codd, E.; Vaught, J.L. Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an ‘atypical’ opioid analgesic. *J. Pharmcol. Exp. Ther.* 1992, 260, 275–285.

20. Gillen, C.; Haurand, M.; Kobelt, D.J.; Wnendt, S. Affinity, potency and efficacy of tramadol and its metabolites at the cloned human μ-opioid receptor. *Naunyn-Schmiedeberg’s Arch. Pharmacol.* 2000, 362, 116–121. [CrossRef]

21. Luszczki, J.J.; Borowicz, K.K.; Swiader, M.; Czuczwar, S.J. Interactions Between Oxcarbazepine and Conventional Antiepileptic Drugs in the Maximal Electroshock Test in Mice: An Isobolographic Analysis. *Epilepsia* 2003, 44, 489–499. [CrossRef]

22. Eddy, N.B.; Leimbach, D. Synthetic Analgesics. II. Dithienylbutenyl- and Dithienylbutylamines. *J. Pharmcol. Exp. Ther.* 1953, 107, 385–393.

23. Hendershot, L.C.; Forsaith, J. Antagonism of the frequency of phenylquinone-induced writhing in the mouse by weak analgesics and nonanalgesics. *J. Pharmcol. Exp. Ther.* 1959, 125, 237–240. [PubMed]

**Sample Availability:** Samples of the compounds 8–15 are available from the authors.

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