

Proceeding Paper

Synthesis and Biological Activity of New Derivatives of Isoindoline-1,3-dione as Non-Steroidal Analgesics †

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Abstract: A simple method for the synthesis of new derivatives of isoindoline-1,3-dione is developed in this study, which consists of the interaction of *N*-arylbenzenecarboximidamides with phthalic anhydride in benzene at reflux. It was found that carrying out the reaction without heating leads to the formation of monoacylation products—phthalic acid amides. The structure of the isolated substances was proved using ¹H and ¹³C NMR spectroscopy. Acute toxicity and biological activity in silico were studied for all of the obtained compounds using the online programs GUSAR and PASS. For 2-(phenyl(phenylimino)methyl)isoindoline-1,3-dione and 2-((phenyl(phenylimino)methyl)carbamoyl)benzoic acid, an acute toxicity and biological activity in vivo was studied in laboratory mice. It was shown that the results of in silico and in vivo methods are comparable and indicate the low toxicity of the obtained compounds. It was revealed that 2-(phenyl(phenylimino)methyl)isoindoline-1,3-dione has a high analgesic activity, 1.6 times higher than the activity of the reference drug metamizole sodium.

Keywords: isoindoline-1,3-diones; 2-(phenyl(phenylimino)methyl)isoindoline-1,3-dione; analgesic activity; *N*-substituted derivatives of phthalimide; non-steroidal analgesic



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1. Introduction

Today, there are many pain relievers available; however, the search continues for more effective and less toxic organic molecules with a non-steroidal structure. Non-opioid analgesics are not addictive; however, the strength of their action is significantly lower than opioids. At the same time, the use of most active drugs leads to problems with the gastrointestinal tract [1]. Based on the results of the literature review, we decided to synthesize compounds containing a substituted *N*-phthalimide fragment in their structure, which is present in the molecule of such drugs as thalidomide, lenalidomide, amphotalide, pomalidomide, taltrimide and talmethoprim. Known *N*-substituted derivatives of phthalimide have analgesic [2,3], hypolipidemic [4], anti-inflammatory [5], anticonvulsant [6] activities. Compounds containing aminoacetylenic and isoindoline-1,3-dione moieties have been shown to have anti-inflammatory and analgesic effects without causing gastric lesions in the stomach of experimental animals [7,8]. Thus, the development of new derivatives of isoindoline-1,3-dione is relevant. There are several main approaches to the synthesis of *N*-substituted phthalimide (Figure 1). In this case, the simplest and most popular is the synthesis method, which consists of the interaction of phthalic anhydride with primary amines [9–11].

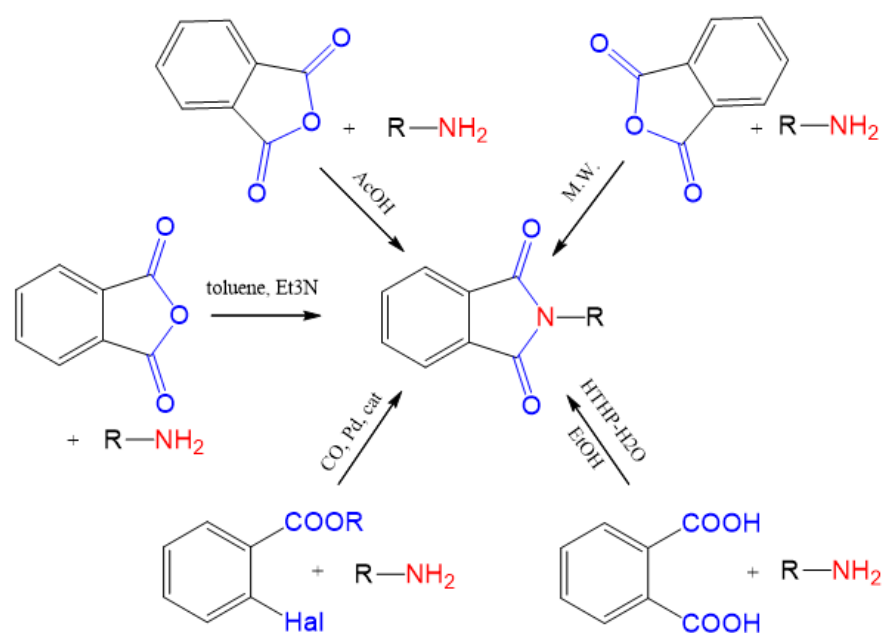


Figure 1. Known methods for the synthesis of *N*-substituted derivatives of phthalimide.

The aim of the work was to develop a simple method for the synthesis of new non-steroidal analgesics containing an *N*-substituted phthalimide fragment in their structure.

2. Results and Discussion

When choosing the starting reagents, the already known methods of obtaining *N*-substituted derivatives of phthalimide were taken into account, and the decision was made to carry out the acylation of *N*-phenylbenzenecarboximidamide **2** with phthalic anhydride **1** for the first time.

When selecting the reaction conditions, such solvents as acetic acid, chloroform, ethyl alcohol, and benzene were considered. It was found that the optimal solvent for obtaining the target product **3** is benzene. The reaction of *N*-phenylbenzenecarboximidamide **2** with phthalic anhydride **1** in boiling benzene gives 2-(phenyl(phenylimino)methyl)isoindoline-1,3-dione **3** in 84% yield. When the reaction is carried out at ambient temperature, 2-(phenyl(phenylimino)methyl)carbamoyl)benzoic acid **4** is formed as the main product with a yield of 97% (Figure 2).

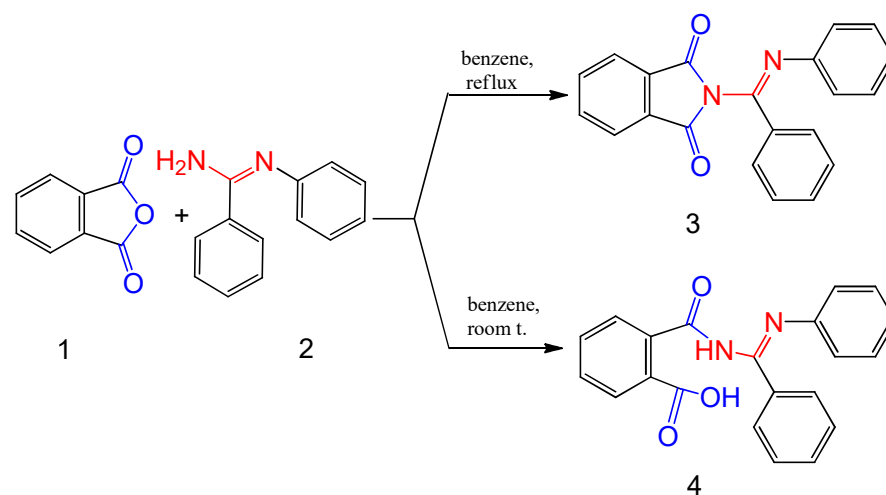


Figure 2. Carrying out the reaction of interaction of *N*-arylbenzenecarboximidamide **2** with phthalic anhydride **1** in benzene.

According to the developed method, five new derivatives of isoindoline-1,3-dione **3a–e** were synthesized with a yield of more than 75% (Figure 3, Table 1).

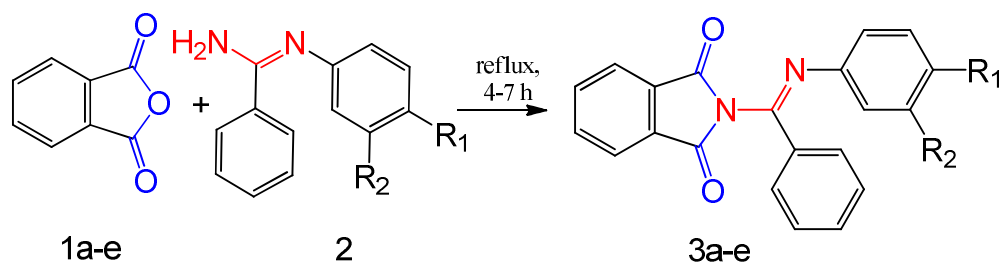


Figure 3. Scheme for the preparation derivatives of isoindoline-1,3-dione **3a–e**.

Table 1. Dependence of the yield on various substituents.

Compounds Number	R1	R2	Yield (%)
3a	H	H	84
3b	OMe	H	75
3c	Me	H	94
3d	Br	H	78
3e	H	Cl	78

The structure of all compounds was confirmed using the ^1H , ^{13}C NMR method (p. 3.1).

For all of the obtained compounds, a computer study of acute toxicity (Table 2) and biological activity in silico (a package of online programs GUSAR and Pass) was conducted. According to the data obtained, compounds **3a–e** exhibit low toxicity and with a probability of 40–80% have analgesic activity. Moreover, the maximum activity (80%) is shown by compound **3a**. Compound **4** is also of low toxicity; however, according to the PASS program, it does not exhibit analgesic activity.

Table 2. Acute toxicity of compounds **3a–e** and **4**.

Compounds Number	LD ₅₀ , mg/kg, In Silico	LD ₅₀ , mg/kg, In Vivo
3a	722.6	1270.0
3b	572.4	1020.0
3c	407.6	930.0
3d	658.7	1160.0
3e	724.2	1270.0
4	861.5	1440.0

For 2-(phenyl(arylimino)methyl)isoindoline-1,3-diones **3a–e** and 2-((phenyl(phenylimino)methyl)carbamoyl)benzoic acid **4**, acute toxicity studies (Table 2) in vivo on laboratory mice were carried out. The computer predicted acute toxicity data are comparable to experimental data.

The study of analgesic activity in vivo in laboratory mice carried out for the most active compounds **3a**, according to in silico results (modeling of acetic acid “cramps”) showed that 2-(phenyl(phenylimino)methyl)isoindoline-1,3-dione **3a** possesses a high analgesic activity, exceeding 1.6 times the activity of the reference drug—metamizole sodium (Table 3). Approximation studies of analgesic activity in vivo for 2-((phenyl(phenylimino)methyl)carbamoyl)benzoic acid **4** indicated the absence of this activity, which is consistent with in silico data.

Table 3. Analgesic activity of compounds **3a** and **4** in comparison with metamizole sodium.

Group	Dose, mg/kg	Number of "Cramps" in 20 min, Abs.	Latent Time of Development, sec, Abs.	SPR, %
Control	-	74.30 ± 3.85	344.50 ± 18.35	-
Metamizole sodium	168.57	3.40 ± 0.77 *	911.80 ± 3.99 *	95.43
3a	100	3.40 ± 0.96 *#	837.6 ± 4.64 *#	95.43

* Differences with the "control" group are statistically significant $p \leq 0.01$. # Differences with the group "metamizole sodium" are statistically significant $p \leq 0.01$.

3. Experimental Part

The NMR spectra of ^1H , ^{13}C solutions of compounds in $\text{DMSO-}d_6$ were recorded on a Bruker Avance III spectrometer (400.13 MHz for ^1H and 100.62 MHz for ^{13}C) relative to TMS (^1H , ^{13}C) as an internal standard. Thin-layer chromatography to prove the identity of the compound and the completeness of the reaction was performed on plates of silica gel 60 F254 (Merck), with the eluent being ethyl acetate-hexane (2:1), assessing the manifestation of UV light. The melting point was determined by the capillary method and was not corrected.

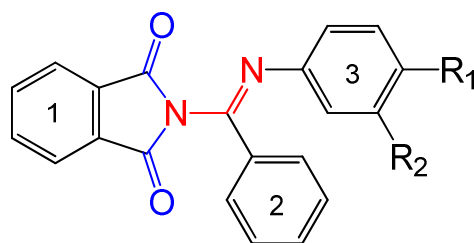
3.1. Synthesis of Compounds **3a–e** and **4**

3.1.1. General Procedure for the Synthesis Derivatives of Isoindoline-1,3-dione **3a–e**

Into a flat-bottomed flask with a volume of 100 mL was placed 2.6 mmol of *N*-arylbenzenecarboximidamide **2a–e**, 50 mL of benzene and 6.8 mmol of phthalic anhydride **1**. The reaction mixture was boiled for 4–7 h with a Dean–Stark trap, a reflux condenser and a calcium chloride tube until the initial *N*-arylbenzenecarboximidamide **2a–e** disappeared (TLC, ethyl acetate-hexane (2:1), development in UV light), and then the formed precipitate was filtered off, leaving mother liquor. The mother liquor was evaporated to dryness on a rotary film evaporator. The resulting precipitate was recrystallized from ethyl alcohol and dried at ambient temperature.

2-(Phenyl(phenylimino)methyl)isoindoline-1,3-dione **3a**

$\text{C}_{21}\text{H}_{14}\text{N}_2\text{O}_2$; cream solid; Yield 84%; mp. 144–146 °C. NMR ^1H δ , ppm: 6.85 (d, $J = 7.4$ Hz, 2H, Ph-3), 7.03 (t, $J = 7.4$ Hz, 1H, Ph-3), 7.26 (t, $J = 7.9$ Hz, 2H, Ph-3), 7.53 (t, $J = 7.6$ Hz, 3H, Ph-2), 7.63 (t, $J = 7.4$ Hz, 1H, Ph-2), 7.93–7.82 (m, 4H, Ph-1), 8.04 (d, $J = 7.3$ Hz, 2H, Ph-2). NMR ^{13}C δ , ppm: 119.31 (Ph-3), 124.57 (Ph-3), 125.38 (Ph-3), 128.79 (Ph-1), 128.85 (Ph-1), 129.47 (Ph-1), 131.21 (Ph-2), 133.04 (Ph-2), 133.76 (Ph-2), 135.78 (Ph-2), 146.96 (Ph-N-), 148.16 (-C=N-), 166.33 (-C=O) (Figure 4).



3a–e

Figure 4. Derivatives of isoindoline-1,3-dione **3a–e**.

2-(Phenyl[[4-methoxyphenyl]imino]methyl)isoindoline-1,3-dione **3b**

$\text{C}_{22}\text{H}_{16}\text{N}_2\text{O}_3$; brown solid; Yield 75%; mp. 126–128 °C. NMR ^1H δ , ppm: 3.64 (s, 3H, -OMe), 6.80 (m, 4H, Ar-3), 7.51 (t, 2H, $J = 7.6$ Hz, Ph-2), 7.61 (t, 1H, $J = 7.4$ Hz, Ph-2), 7.91 (m, 4H, Ph-4), 7.98 (d, 2H, $J = 7.4$ Hz, Ph-2). NMR ^{13}C δ , ppm: 55.51, 55.62 (-OMe), 114.77 (Ar-3), 121.09 (Ar-3), 128.44 (Ph-1), 129.35 (Ph-1), 132.64 (Ph-2), 133.51 (Ph-2), 140.71 (Ph-2), 146.68 (Ar-N-), 157.30 (-C=N-), 166.39 (-C=O) (Figure 4).

2-(Phenyl(p-tolylimino)methyl)isoindoline-1,3-dione **3c**

$C_{22}H_{16}N_2O_2$; cream solid; Yield 94%; m.p. 118–120 °C. NMR 1H δ , ppm: 2.16 (s, 3H, -Me), 6.73 (d, $J = 8.2$ Hz, 2H, Ar-3), 7.06 (d, $J = 8.1$ Hz, 2H, Ar-3), 7.52 (t, $J = 7.6$ Hz, 2H, Ph-2), 7.62 (t, $J = 7.3$ Hz, 1H, Ph-2), 7.89 (q, $J = 4.4$ Hz, 4H, Ph-1), 8.00 (d, $J = 7.4$ Hz, 2H, Ph-2). NMR ^{13}C δ , ppm: 20.87 (-Me), 119.32 (Ar-3), 123.85 (Ar-3), 127.71 (Ar-3), 128.72 (Ph-1), 129.99 (Ph-1), 131.23 (Ph-2), 133.89 (Ph-2), 134.55 (Ph-2), 135.83 (Ph-2), 145.56 (Ar-N-), 146.36 (-C=N-), 166.34 (-C=O) (Figure 4).

2-(((4-Bromophenyl)imino)(phenyl)methyl)isoindoline-1,3-dione **3d**

$C_{21}H_{13}BrN_2O_2$; cream solid; Yield 78%; mp. 118–120 °C. NMR 1H δ , ppm: 6.80 (d, 2H, $J = 8.6$ Hz, Ar-3), 7.46 (d, 2H, $J = 8.6$ Hz, Ar-3), 7.53 (t, 2H, $J = 7.7$ Hz, Ph-2), 7.64 (t, 1H, $J = 7.4$ Hz, Ph-2), 7.90 (m, 4H, Ph-1), 8.03 (d, 2H, $J = 7.3$ Hz, Ph-2). NMR ^{13}C δ , ppm: 117.89 (Ar-3), 121.65 (Ar-3), 128.78 (Ph-1), 129.46 (Ph-1), 132.44 (Ph-2), 132.94 (Ph-2), 133.17 (Ph-2), 146.97 (Ar-N-), 148.25 (-C=N-), 166.27 (-C=O) (Figure 4).

2-(((3-Chlorophenyl)imino)(phenyl)methyl)isoindoline-1,3-dione **3e**

$C_{21}H_{13}ClN_2O_2$; cream solid; Yield 78%; m.p. 148–150 °C. NMR 1H δ , ppm: 6.76 (d, 1H, $J = 7.9$ Hz, Ar-3), 6.90 (s, 1H, Ar-3), 7.10 (d, 1H, $J = 8.0$ Hz, Ar-3), 7.27 (t, 1H, $J = 8.0$ Hz, Ar-3), 7.54 (t, 2H, $J = 7.7$ Hz, Ph-2), 7.65 (t, 1H, $J = 7.3$ Hz, Ph-2), 7.90 (m, 4H, Ph-1), 8.04 (d, 2H, $J = 7.6$ Hz, Ph-2). NMR ^{13}C δ , ppm: 117.77 (Ar-3), 119.68 (Ar-3), 119.61 (Ar-3), 124.70 (Ar-3), 125.22 (Ar-3), 129.01 (Ph-1), 129.57 (Ph-1), 131.27 (Ph-2), 132.74 (Ph-2), 133.33 (Ph-2), 133.78 (Ph-2), 133.93 (Ph-2), 148.12 (Ar-N-), 149.49 (-C=N-), 166.23 (-C=O) (Figure 4).

3.1.2. Synthesis 2-((Phenyl(phenylimino)methyl)carbamoyl)benzoic Acid **4**

Into a flat-bottomed flask with a volume of 100 mL was placed 2.6 mmol of *N*-phenylbenzenecarboximidamide **2a**, 50 mL of benzene and 6.8 mmol of phthalic anhydride **1**. The reaction mixture was stirred for 4 h until the starting *N*-phenylbenzenecarboximidamide **2a** disappeared (TLC, ethyl acetate-hexane (2:1), development under UV light), and then the formed precipitate was filtered off and dried at ambient temperature.

$C_{21}H_{16}N_2O_3$; white solid; Yield 97%; m.p. 190–192 °C. NMR 1H δ , ppm: 7.42 (d, $J = 7.7$ Hz, 3H, Ph-3), 7.56–7.48 (m, 4H, Ph-1), 7.64 (t, $J = 7.7$ Hz, 2H, Ph-3), 7.74 (t, $J = 7.5$ Hz, 1H, Ph-2), 7.92 (d, $J = 7.7$ Hz, 2H, Ph-3), 8.17 (dd, $J = 6.0, 3.5$ Hz, 2H, Ph-3), NMR ^{13}C δ , ppm: 125.51, 128.11, 129.06, 129.34, 130.02, 130.41, 130.84, 132.77, 132.85, 133.79, 135.35, 136.95, 162.78 (-C=O), 168.67 (-COOH) (Figure 5).

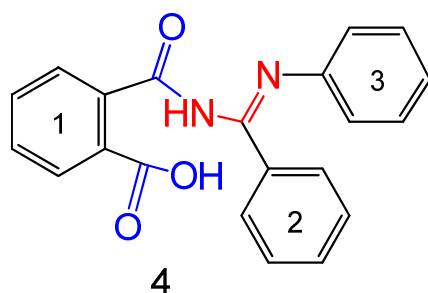


Figure 5. 2-((phenyl(phenylimino)methyl)carbamoyl)benzoic acid **4**.

3.2. Acute Toxicity

Acute toxicity was determined on white outbred male mice weighing 20 ± 2 g. Substances **3a–e**, **4** were administered once, intraperitoneally in the dose range from 600 to 1600 mg/kg in the form of suspensions in a mixture of dimethyl sulfoxide (DMSO): water for injection (1:5) using a suspension of hydroxy-leaded sorbitan—twin-80 as a stabilizer. The survival of animals was determined by observing them 24 and 48 h after administration of the drug. The animals were monitored for 72 h. The development of the main symptoms and the time of death of animals were recorded. The experimental average lethal dose (LD_{50}) was calculated using the installed software “STATISTICA 7.0”, which uses the least squares method for calculating LD_{50} .

3.3. Analgesic Activity

To simulate acetic acid “cramps”, white outbred male mice weighing 18–22 g were used, from which 3 groups of 5 individuals were formed. Convulsions in animals were caused by intraperitoneal administration of 0.5% acetic acid solution. Compound **3a** was dissolved in water for injection and administered intraperitoneally at a dose of 100 mg/kg. The comparison drug, metamizole sodium, was administered in the same way at a dose of 168.57 mg/kg. Animals of the first experimental group received intraperitoneal compound **3a** 40 min before the start of the experiment. Animals in the second experimental group received the comparison drug intraperitoneally 40 min before the start of the experiment. Only a solution of 0.5% acetic acid was administered intraperitoneally to individuals in the control group. The time of the onset of seizures and their number within 20 min were recorded. The analgesic activity of the studied compound was evaluated by a significant decrease in the number of convulsions in the group receiving the drug relative to the control group. The indicator of effectiveness was the coefficient of suppression of pain reaction (SPR), which was calculated by the formula:

$$\text{SPR, \%} = \left(1 - \frac{\text{average number of cramps per group}}{\text{average number of cramps in control}} \right) \times 100 \quad (1)$$

4. Conclusions

A simple method has been developed for the synthesis of five new substituted isoindoline-1,3-diones **3a–e**, consisting of the interaction of *N*-arylbenzenecarboxymidamides **2a–e** with phthalic anhydride **1** in a benzene medium during boiling. It was found that carrying out the reaction without heating leads to the formation of 2-((phenyl(phenylimino)methyl)carbamoyl)benzoic acid **4**. The structure of all isolated substances was proved using ¹H and ¹³C NMR spectroscopy. Acute toxicity study in vivo in laboratory mice showed low toxicity of all compounds, which is comparable to the results in silico (GUSAR online software package). Using the PASS online software package, it was predicted that isoindoline-1,3-diones **3a–e** have analgesic activity with a probability of 40–80%, while for 2-((phenyl(phenylimino)methyl)carbamoyl)benzoic acid **4**, this activity was not predicted. For the most bioactive compound—2-(phenyl(phenylimino)methyl)isoindoline-1,3-dione **3a**—and for 2-((phenyl(phenylimino)methyl)carbamoyl)benzoic acid **4**, the analgesic activity in vivo was studied in laboratory mice. It was found that compound **3a** has high analgesic activity, 1.6 times higher than the activity of the reference drug metamizole sodium, while compound **4** does not. The obtained experimental data are fully consistent with computer forecasts.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ecsoc-25-11773/s1>.

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Conflicts of Interest: The authors declare no conflict of interest.

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