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

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Abstract: A simple method for the synthesis of new derivatives of isoindoline-1,3-diones has been developed, which consists in 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 ^{1}H and ^{13}C NMR spectroscopy. Acute toxicity and biological activity in silico was studied for all 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 of non-steroidal structure. Based on the results of the literature review, it was 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, taltrimide and talmethoprim. Known *N*-substituted derivatives of phthalimide, which have analgesic [1,2], hypolipidemic [3], anti-inflammatory [4], anticonvulsant [5] activities. 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 in the interaction of phthalic anhydride with primary amines [6–8].

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.

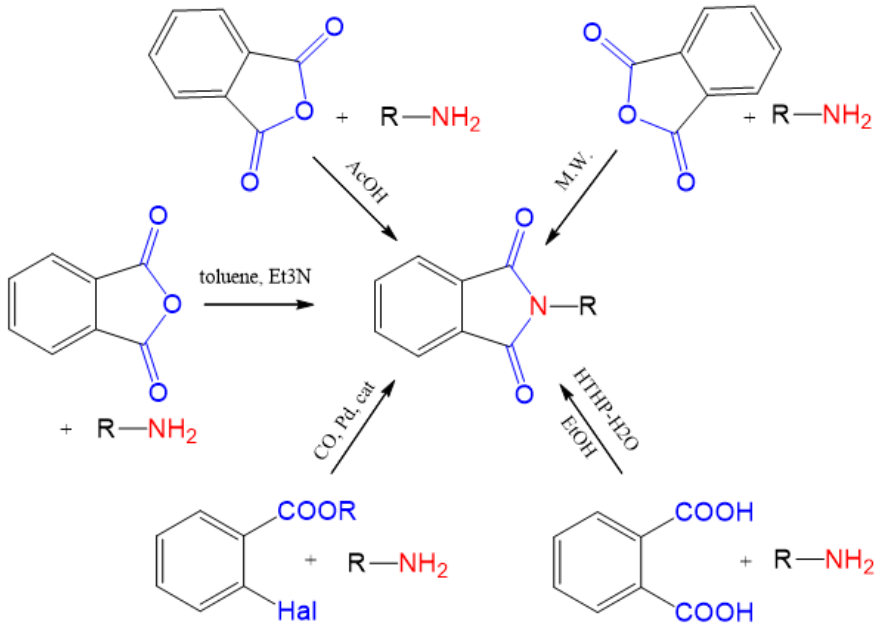

Figure 1. Known methods for the synthesis of *N*-substituted derivatives of phthalimide.

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)isoindline-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).

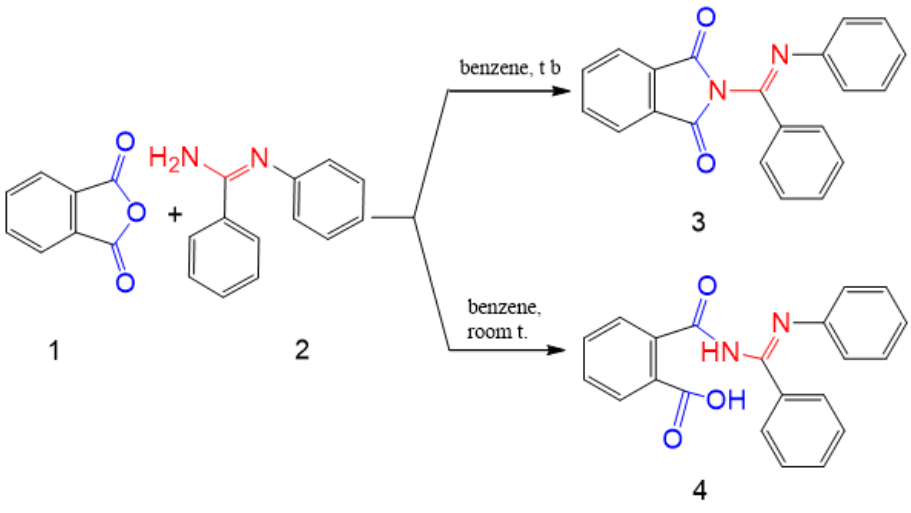

Figure 2. Carrying out the reaction of interaction of *N*-arylbenzenecarboximidamide **2** with phthalic anhydride **1** in benzene.

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

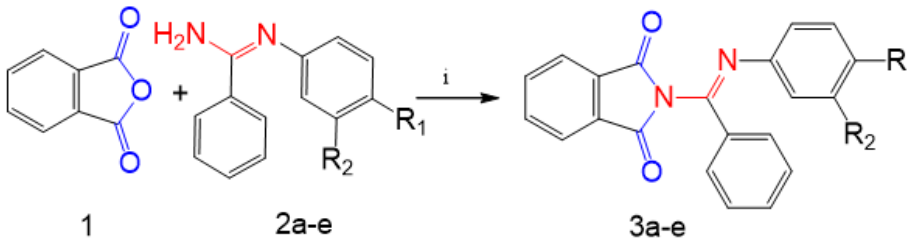

Figure 2. Scheme for the preparation of isoindoline-1,3-diones **3a–e**; i: benzene, Δ, 4–7 h.

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 ^{1}H, ^{13}C NMR method (p. 3.1).

For all 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	LD50, mg/kg, in silico	LD50, 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). Approbation 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, s.	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 ^{1}H, ^{13}C solutions of compounds in DMSO-d6 were recorded on a Bruker Avance III spectrometer (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C) relative to TMS (^{1}H, ^{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), eluent ethyl acetate-hexane (2:1), 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 of Isoindoline-1,3-Diones **3a–e**

In a flat-bottom 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), 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**

$C_{21}H_{14}N_2O_2$; Cream solid; Yield 84%; mp. 144–146 °C. NMR ^{1}H δ, ppm: 6.85 (d, J = 7.4 Hz, 2H), 7.03 (t, J = 7.4 Hz, 1H), 7.26 (t, J = 7.9 Hz, 2H), 7.53 (t, J = 7.6 Hz, 3H), 7.63 (t, J = 7.4 Hz, 1H), 7.93–7.82 (m, 4H), 8.04 (d, J = 7.3 Hz, 2H). NMR ^{13}C δ, ppm: 135.78, 133.76, 133.04, 131.21, 129.47, 128.85, 128.79, 125.38, 124.57, 119.31, 146.96, 148.16, 166.33.

2-(phenyl(phenylimino)methyl)isoindoline-1,3-dione **3b**

$C_{22}H_{16}N_2O_3$; Brown solid; Yield 75%; mp. 126–128 °C. NMR ^{1}H δ, ppm: 3.64 (s, 3H), 6.80 (m, 4H), 7.51 (t, 2H, J = 7.6 Hz), 7.61 (t, 1H, J = 7.4 Hz), 7.91 (m, 4H), 7.98 (d, 2H, J = 7.4 Hz). NMR ^{13}C δ, ppm: 29.21, 55.60, 55.69, 114.77, 121.09, 128.44, 129.35, 132.64, 133.51, 140.71, 146.68, 157.30, 176.39.

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

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

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 ^{1}H δ, ppm: 6.80 (d, 2H, J = 8.6 Hz), 7.46 (d, 2H, J = 8.6 Hz), 7.53 (t, 2H, J = 7.7 Hz), 7.64 (t, 1H, J = 7.4 Hz), 7.90 (m, 4H), 8.03 (d, 2H, J = 7.3 Hz). NMR ^{13}C δ, ppm: 29.21, 117.89, 121.65, 128.78, 129.46, 132.44, 132.94, 133.17, 146.97, 148.25, 176.27.

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

$C_{21}H_{13}ClN_2O_2$; Cream solid; Yield 78%; mp. 148–150 °C. NMR ^{1}H δ, ppm: 6.76 (d, 1H, J = 7.9 Hz), 6.90 (s, 1H), 7.10 (d, 1H, J = 8.0 Hz), 7.27 (t, 1H, J = 8.0 Hz), 7.54 (t, 2H, J = 7.7 Hz),

7.65 (t, 1H, J = 7.3 Hz), 7.90 (m, 4H), 8.04 (d, 2H, J = 7.6 Hz). NMR ^{13}C δ, ppm: 29.20, 117.63, 119.55, 119.61, 125.30, 128.84, 128.89, 129.49, 131.27, 132.74, 133.33, 133.78, 148.86, 176.25.

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

In a flat-bottom 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), then the formed precipitate was filtered off and dried at ambient temperature. $C_{21}H_{16}N_2O_3$; White solid; Yield 97%; mp. 190–192 °C. NMR ^{1}H δ, ppm: 7.42 (d, J = 7.7 Hz, 3H), 7.56–7.48 (m, 4H), 7.64 (t, J = 7.7 Hz, 2H), 7.74 (t, J = 7.5 Hz, 1H), 7.92 (d, J = 7.7 Hz, 2H), 8.17 (dd, J = 6.0, 3.5 Hz, 2H), 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, 168.67.

3.2. 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 in each. Convulsions in animals were caused by intraperitoneal administration of 0.5% acetic acid solution. Compound **3a** were 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 compounds **3a** 40 min before the start of the experiment. Animals of 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 of 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:

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

4. Conclusions

A simple method has been developed for the synthesis of five new substituted isoindoline-1,3-diones **3a–e**, consisting in 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 ^{1}H and ^{13}C 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 a 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.

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References

1. Antunes, R.; Batista, H.; Srivastava, R.M.; Thomas, G.; Araújo, C.C.; Longo, R.L.; Magalhães, H.; Leão, M.B.C.; Pavão, A. Synthesis, characterization and interaction mechanism of new oxadiazolo-phthalimides as peripheral analgesics. *J. Mol. Struct.* **2003**, *660*, 1–13. https://doi.org/10.1016/S0022-2860(03)00418-6.
2. Reddy, Y.D.; Kumari, Y.B.; Dubey, P.K. Synthesis of a novel water soluble phthalimide derivative of acetaminophen as potential analgesic and antipyretic agent. *Indian J. Chem.* **2013**, *52B*, 691–693.
3. Sena, V.L.; Srivastava, R.M.; Silva, R.O.; Lima, V.L. Synthesis and hypolipidemic activity of *N*-substituted phthalimides. *Farm* **2003**, *58*, 1283–1288. https://doi.org/10.1016/S0014-827X(03)00185-X.
4. Machado, A.L.; Lima, L.M.; Araújo-Jr, J.X.; Fraga, C.A.M.; Gonçalves, Koatz, V.L.; Barreiro, E.J. Design, synthesis and anti-inflammatory activity of novel phthalimide derivatives, structurally related to thalidomide. *Biol. Med. Chem. Lett.* **2005**, *15*, 1169–1172. https://doi.org/10.1016/j.bmcl.2004.12.012.
5. Bailleux, V.; Vallee, L.; Nuyts, J-P. Comparative Anticonvulsant Activity and Neurotoxicity of 4-Amino-*N*-(2,6-dimethylphenyl)phthalimide and Prototype Antiepileptic Drugs in Mice and Rats. *Epilepsia* **1995**, *36*, 559–565. https://doi.org/10.1111/j.1528-1157.1995.tb02567.x.
6. Vamecq, J.; Bac, P.; Herrenknecht, C.; Maurois, P.; Delcourt, P.; James, P. Synthesis and Anticonvulsant and Neurotoxic Properties of Substituted *N*-Phenyl Derivatives of the Phthalimide Pharmacophore. *J. Med. Chem.* **2000**, *43*, 1311–1319. https://doi.org/10.1021/jm990068t.
7. Omran, F.; Talal, H.Z. Synthesis, characterization and pharmacological activity of some new phthalimide derivatives. *Der. Pharma Chem.* **2014**, *6*, 234–238.
8. Sharma, U.; Kumar, P.; Kumar, N.; Singh, B. Recent Advances in the Chemistry of Phthalimide Analogues and their Therapeutic Potential. *Mini-Rev. Med. Chem.* **2010**, *10*, 678–704. https://doi.org/10.2174/138955710791572442.