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NITRO DERIVATI N-FENILSUKCINIMIDA: SINTEZA PRIMENOM MIKROTALASNE TEHNIKE I IN SILICO ODREĐIVANJE FARMAKOLOŠKE AKTIVNOSTI

**NITRO DERIVATIVES OF N-PHENYLSUCCINIMIDE:
A MICROWAVE-ASSISTED SYNTHETIC APPROACH
AND IN SILICO EVALUATION OF THE
PHARMALOGICAL POTENTIAL**

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Primena mikrotalasne tehnike u sintezi organskih molekula predstavlja efikasnu i održivu alternativu klasičnim metodama, jer omogućava kraće vreme reakcije, veći prinos i bolju selektivnost. Usaglašenost sa principima zelene hemije čini ovu tehniku posebno pogodnom za brzu i efikasnu sintezu različitih organskih jedinjenja. N-fenilsukcinimid i njegovi derivati privlače posebnu pažnju zbog širokog spektra bioloških aktivnosti, uključujući antikonvulzivno, antiinflamatorno i antimikrobno dejstvo. Strukturna raznovrsnost ovih jedinjenja čini ih perspektivnim u medicinskoj hemiji, naročito za razvoj agenasa koji imaju uticaj na centralni nervni sistem. Dalja funkcionalizacija može doprineti sintezi jedinjenja sa poboljšanim farmakološkim svojstvima. Polazeći od sukcininske kiseline i odgovarajućih o- i/ili p-nitro supstituisanih anilina, sintetisani su derivati N-fenilsukcinimida primenom mikrotalasnog zračenja kao brze i efikasne metode. Struktura sintetisanih jedinjenja potvrđena je primenom različitih spektroskopskih metoda. Njihov farmakološki potencijal procenjen je korišćenjem različitih empirijskih pravila i savremenih in silico metoda. Dobijeni podaci doprinose razvoju sintetskih strategija zasnovanih na mikrotalasnoj tehnologiji, usmerenih ka pronalaženju novih organskih molekula sa potencijalnom biološkom aktivnošću.

Microwave-assisted synthesis has emerged as a powerful and sustainable alternative to conventional methods, offering reduced reaction times, improved yields, and enhanced selectivity. Its alignment with green chemistry principles makes it particularly attractive for the rapid and efficient preparation of various organic compounds. N-Phenylsuccinimide and its derivatives have attracted

considerable attention due to their diverse biological activities, including anticonvulsant, anti-inflammatory, and antimicrobial effects. Their structural versatility makes them promising scaffolds in medicinal chemistry, particularly for the development of central nervous system active agents. Continued research into their functionalization may yield compounds with enhanced pharmacological profiles. Using succinic acid and corresponding *o*- and/or *p*-nitro substituted anilines as starting materials, *N*-phenylsuccinimide derivatives were efficiently synthesized under microwave irradiation. The obtained compounds were characterized by different spectroscopic methods. The pharmacological potential of these compounds was assessed using a combination of empirical rules and *in silico* methods. The obtained data contribute to the development of microwave-assisted synthetic strategies aimed at discovering new organic molecules with potential biological activity.

1. Introduction

Microwave-assisted synthesis has emerged as a valuable tool in modern chemistry, offering significantly reduced reaction time and improved efficiency compared to conventional methods. Its growing adoption across various chemical transformations highlights its potential for broader industrial application. In particular, the pharmaceutical industry increasingly relies on rapid and efficient synthetic methodologies to meet the demand for novel compounds, highlighting the importance of time-efficient and high-capacity experimentation [1].

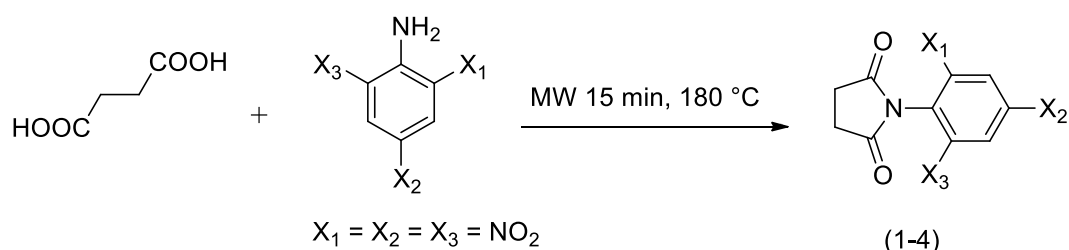
Beyond pharmaceutical synthesis, microwave technology has found widespread use in other fields as well. In the food industry, it is employed for processing and preservation, while in environmental science, it is applied to the pyrolysis of waste materials. Moreover, this technique is utilized in analytical chemistry for sample preparation, as well as in natural product research for the extraction of bioactive compounds. It also plays an important role in the hydrolysis of proteins and peptides, offering greater control over reaction conditions and reducing the need for excessive solvent use [1]. Given its versatility, efficiency, and alignment with green chemistry principles, microwave-assisted synthesis represents a powerful approach for the development of sustainable and scalable chemical processes. By enabling rapid and uniform energy transfer through electromagnetic waves, microwave irradiation significantly reduces the energy requirements compared to conventional heating methods [2,3]. Moreover, this technique not only shortens reaction times but also often results in higher yields and improved product purity [4].

N-Phenylsuccinimide derivatives represent an important class of compounds in both organic and medicinal chemistry, known for their structural stability and diverse biological activities. Due to the presence of an imide moiety connected to an aromatic ring, these molecules often exhibit pharmacologically relevant properties such as antimicrobial, anticancer, and anti-inflammatory effects. In addition, they serve as key intermediates in the synthesis of more complex heterocyclic systems and are frequently explored as scaffolds in drug development. The synthesis and structural modification of these derivatives—especially through efficient techniques such as microwave-assisted synthesis—remain an active area of research aimed at developing more selective and environmentally friendly synthetic strategies [5]. As part of the ongoing development of environmentally acceptable synthetic approaches, in this work, we synthesized four nitro *N*-phenylsuccinimide derivatives under microwave irradiation. All compounds are synthesized in good yields and fully characterized by various spectroscopic techniques. Prediction of potential pharmacological activity of investigated compounds is tested using *in silico* ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling.

2. Experimental part

2.1. General procedure for the synthesis of compounds 1-4

All of investigated *N*-phenylsuccinimide derivatives examined in this study were prepared by reacting succinic acid with substituted anilines under solvent-free conditions using microwave irradiation (MW) according to Scheme 1 [6]. All reactions were performed in a MW reactor Anton Paar Monowave 300. A mixture containing succinic acid (1 mmol, 295 mg) and a nitro substituted aniline (1.1 mmol) was stirred in a 25 mL microwave reactor at 180 °C under microwave irradiation for 15 minutes, without the use of a solvent. After the reaction was complete, the reaction mixture was cooled to room temperature. The resulting crude product was dissolved in ethyl acetate and sequentially washed with 10 mL of 5% aqueous HCl, 10 mL of saturated aqueous NaHCO₃, and twice with 10 mL portions of distilled water. The organic layer was dried over magnesium sulfate, and the solvent was evaporated under reduced pressure. The crude product was purified by recrystallization from ethanol. Their chemical structure was confirmed by melting points, elemental analysis, FT-IR and NMR spectroscopic methods.



Compound	X_1	X_2	X_3
1	NO ₂	NO ₂	H
2	NO ₂	H	NO ₂
3	H	NO ₂	H
4	NO ₂	H	H

Scheme 1 Synthetic pathway of the investigated compounds

The melting point of the synthesized compounds was determined by using the melting point system Stuart SMP30. ¹H and ¹³C NMR spectra of the compounds were recorded using a Varian Gemini 2000 (400 Hz and 100 Hz respectively) in DMSO-*d*₆. The FTIR spectra of the synthesized compounds were determined using a Nicolet™ iS™ 10 FT-IR Spectrometer (Thermo Fisher SCIENTIFIC) with Smart iTR™ Attenuated Total Reflectance (ATR) Sampling accessories in the range of 500–4000 cm⁻¹, with 32 scans per spectrum. Elemental analysis was performed on a Vario EL III elemental analyzer.

1-(2,4-dinitrophenyl)pyrrolidine-2,5-dione (**1**) Yellow crystalline, yield: 84%, m.p. 220.6–221.3 °C; FT-IR (ν/cm⁻¹): 3098, 2978, 2976, 1717, 1537, 1520, 1330, 1172, 848; ¹H NMR (400 MHz, DMSO-*d*₆, δ/ppm): 8.86 (d, *J* = 4 Hz, 1H), 8.76 (d, *J* = 2.4 Hz, 1H), 8.74 (d, *J* = 2.4 Hz, 1H), 2.95 (s, 4H); ¹³C NMR (100 MHz, DMSO-*d*₆, δ/ppm): 176.0, 150.3, 147.4, 145.4, 135.6, 129.1, 123.9, 29.2; Anal. Calcd. For C₁₀H₇N₃O₆: C, 45.29, H, 2.66, N, 15.85. Found: C, 45.46, H, 2.77, N, 15.34.

1-(2,6-dinitrophenyl)pyrrolidine-2,5-dione (**2**) Yellow crystalline, yield: 71%, m.p. 155.4–156.8 °C; FT-IR (ν/cm⁻¹): 3095, 2971, 2968, 1722, 1521, 1517, 1351, 1168, 836; ¹H NMR (400 MHz, DMSO-*d*₆, δ/ppm): 8.56 (d, *J* = 8.4 Hz, 2H), 8.06 (t, *J* = 8.4 Hz, 1H), 3.04 (s, 4H); ¹³C NMR (100 MHz, DMSO-*d*₆, δ/ppm): 175.8, 146.6, 141.3, 135.4, 134.5, 130.1, 119.6, 29.2; Anal. Calcd. For C₁₀H₇N₃O₆: C, 45.29, H, 2.66, N, 15.85. Found: C, 45.72, H, 2.41, N, 15.70.

1-(4-nitrophenyl)pyrrolidine-2,5-dione (3) Orange crystalline, yield: 89%, m.p. 215.3–216.8 °C; FT-IR (ν/cm^{-1}): 3082, 2954, 2944, 1704, 1520, 1504, 1346, 1162, 851; ^1H NMR (400 MHz, DMSO- d_6 , δ/ppm): 8.34 (d, $J = 8.8$ Hz, 2H), 7.60 (d, $J = 8.8$ Hz, 2H), 2.81 (s, 4H); ^{13}C NMR (100 MHz, DMSO- d_6 , δ/ppm): 176.9, 146.9, 138.8, 128.3, 124.6, 29.1; Anal. Calcd. For $\text{C}_{10}\text{H}_8\text{N}_2\text{O}_4$: C, 54.55, H, 3.66, N, 12.72. Found: C, 54.79, H, 3.48, N, 12.53.

1-(2-nitrophenyl)pyrrolidine-2,5-dione (4) Offwhite crystalline, yield: 76%, m.p. 159.3–160.5 °C; FT-IR (ν/cm^{-1}): 3093, 2992, 2942, 1711, 1522, 1483, 1354, 1172, 851; ^1H NMR (400 MHz, DMSO- d_6 , δ/ppm): 8.17 (d, $J = 8$ Hz, 1H), 7.91 (t, $J = 8$ Hz, 1H), 7.731 (t, $J = 8.4$ Hz, 1H), 7.56 (d, $J = 8$ Hz, 1H), 2.88 (s, 4H); ^{13}C NMR (100 MHz, DMSO- d_6 , δ/ppm): 176.5, 145.7, 135.1, 131.0, 130.7, 126.1, 125.8, 29.1; Anal. Calcd. For $\text{C}_{10}\text{H}_8\text{N}_2\text{O}_4$: C, 54.55, H, 3.66, N, 12.72. Found: C, 54.79, H, 3.48, N, 12.53.

2.2. In-silico assessment of physicochemical and ADMET properties

The relevant parameters of the studied compounds were determined using the SwissADME software (Swiss Institute of Bioinformatics, Switzerland) [7]. The physicochemical properties assessed include molecular weight (MW), number of hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), number of rotatable bonds (nrB), topological polar surface area (TPSA), octanol–water partition coefficient (logP), and molar refractivity (MR).

3. Results and discussion

Among the synthesized compounds, three have been previously reported in the literature, while compound **2** is novel. The conventional synthesis involves the reaction of succinic acid with the corresponding aryl amines in the presence of 86% polyphosphoric acid. For compounds **1** and **3**, the reaction was carried out at 80 °C for 12 hours [8], whereas for compound **4**, a reaction time of 3 hours at 90 °C was reported [9]. In this work, the microwave-assisted method significantly reduced the reaction time to just 15 minutes, offering an energy-efficient alternative to conventional heating while maintaining high yields of up to 70% and eliminating the need for corrosive agents. Figure 1 illustrates the power and temperature profiles recorded during the microwave-assisted synthesis of nitro-substituted *N*-phenylsuccinimides.

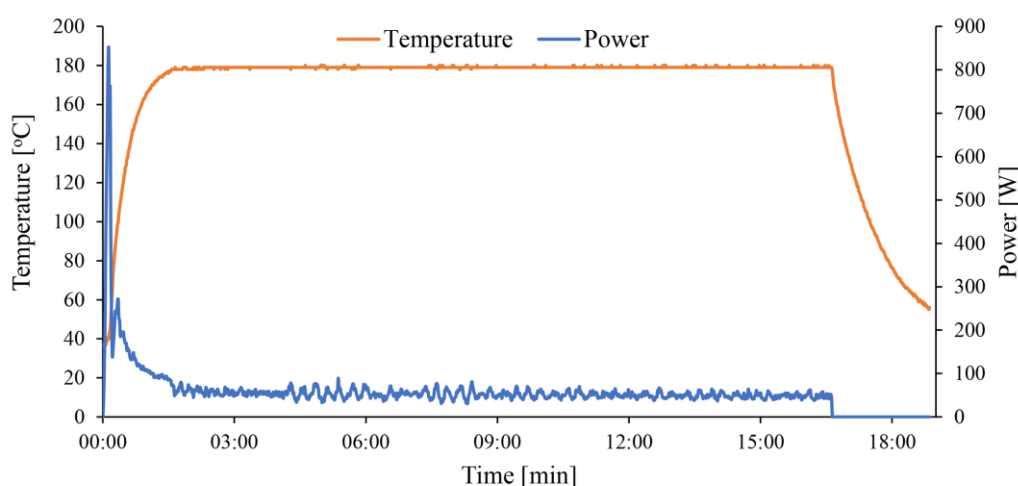


Figure 1. Energy input and temperature profile for the synthesis under MW irradiation

Druglikeness is a crucial factor in the early stages of drug discovery, serving as an important criterion for compound selection [10]. It reflects the physicochemical and structural similarity of a

compound to known drugs, with properties like molecular weight, polarity, and lipophilicity influencing solubility, permeability, metabolic stability and transport. In order to evaluate pharmacological potential of the synthesized compounds, their physicochemical properties and druglikeness were assessed in relation Lipinski's Rule of 5 (RO5) [11]. According to Lipinski's Rule, an orally active compound must meet the following empirical criteria: i) the number of hydrogen bond donors is not greater than 5 (donors being N–H and O–H groups); ii) the number of hydrogen bond acceptors is maximum 10 (acceptors being N or O atoms); iii) a molecular weight is less than 500 and iv) lipophilicity (octanol/water partition coefficient – logP) is lower than 5. A compound that violates two or more of the specified rules is likely to have low absorption or bioavailability. The physicochemical parameters of the synthesized molecules are presented in Tables 1 and 2. According to bioavailability score (0.55 for all compounds) these compounds exhibit good bioavailability.

Table 1. Physico-chemical properties of the investigated compounds.

Compound	MW [g/mol]	NA	nrb	HBD	HBA	MR	TPSA [\AA^2]
1	285.2	19	3	0	6	69.1	129.0
2	285.2	19	3	0	6	69.1	129.0
3	220.2	16	2	0	4	60.3	83.2
4	220.2	16	2	0	4	60.3	83.2

Table 2. Values of the partition coefficient of the investigated compounds.

Compound	logPo/w (XLOGP3)	logPo/w (WLOGP)	logPo/w (MLOGP)
1	0.19	0.78	0.09
2	0.13	0.78	0.09
3	0.46	0.87	0.99
4	0.36	0.87	0.99

Furthermore, according to Veber's criterion, adequate oral bioavailability is achieved with molecules that have less than 10 rotatable bonds and a topological polar surface of less than 140 \AA^2 . According to the modified versions of these two concepts, for compounds whose physicochemical properties satisfy the following ranges: $160 \leq$ relative molecular mass ≤ 480 ; $-0.4 \leq$ WlogP < 5.6 ; $40 \leq$ molar refractivity ≤ 130 ; $20 \leq$ number of atoms ≤ 70 (Goose's criterion) and logP ≤ 5.88 , TPSA (topological polar surface area) $< 131.6 \text{ \AA}^2$ (Egan's criterion), there is a high probability of manifesting therapeutic effects.

Based on the values of molecular descriptors covered by the mentioned empirical rules (Tables 1 and 2), it can be concluded that all the examined compounds fulfill the all specified empirical criteria, indicating they meet the theoretical requirements for sufficient bioavailability and possess the appropriate pharmacological potential

Because logP is related to both the solubility and permeability of a molecule, it serves as a key physicochemical parameter for assessing the molecule's ability to transfer through the cell membranes. As shown in Table 2, the partition coefficient values varied for the same compound, reflecting differences in the mathematical algorithms used to calculate this parameter. Results indicate moderate lipophilicity of investigated compounds. The higher partition coefficients of compounds 3 and 4

(mono-nitro substituted) indicate a greater hydrophobic character, and thus an enhanced ability to permeate phospholipid cellular membranes compared to compounds **1** and **2**. Additionally, TPSA is recognized as a key descriptor of drug transport properties where TPSA below 140 Å² is generally favorable. The investigated compounds exhibit PSA values within this range, suggesting good potential for efficient transport across biological membranes.

4. Conclusion

Four nitro substituted *N*-phenylsuccinimides were successfully synthesized using MW irradiation. This method significantly reduced the reaction time compared to conventional procedures and was performed under solvent-free conditions, contributing to energy savings. The chemical structures of the synthesized compounds were confirmed by determination of melting point, FT-IR, ¹H and ¹³C NMR spectroscopy, and elemental analysis. *In silico* study revealed that all compounds comply with empirical rules indicating their good bioavailability. These findings provide a promising starting point for potential applications in the pharmaceutical industry.

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