

SYNTHESIS OF AMINOPHTHALIDES BASED ON MORPHOLINE AND CYTISINE ALKALOID

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Abstract. This work describes the investigation of the reactions between *o*-formylbenzoic acid and morpholine, as well as the alkaloid cytosine. This study is aimed at the synthesis of new aminophthalide derivatives as compounds of potential interest for biological evaluation. The interactions between the starting reagents were studied, and optimal synthesis conditions were determined to ensure high the amounts of desired compounds obtained. The structures, electronic properties of the initial substances, intermediates, and synthesized compounds were analyzed using quantum-chemical computations are based on density functional theory using a quantum-chemical research method. The molecular constitution of the obtained products were established based on their Infrared spectral data, one-dimensional ¹H, ¹³C and two-dimensional Nuclear Magnetic Resonance spectroscopy (COSY, HMQC, HMBC), and mass spectrometry. A possible reaction pathway accounting for the formation of aminophthalides is presented, involving the nucleophilic addition of secondary amines to the carbonyl group of *o*-formylbenzoic acid, followed by intramolecular cyclization. The spectral analysis is fully consistent with the proposed structure of the obtained compounds. The findings reveal the effectiveness of the established procedure for synthesizing aminophthalides based on morpholine and cytosine and provide the basis for further studies of their reactivity, physicochemical characteristics, and potential biological activity.

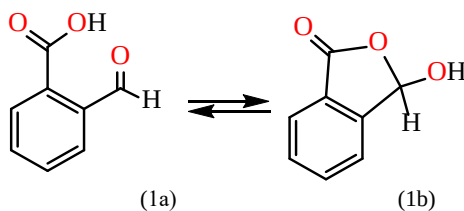
Keywords: *o*-formylbenzoic acid, morpholine, cytosine, aminophthalides, quantum chemical calculations, NMR spectroscopy, mass spectrometry

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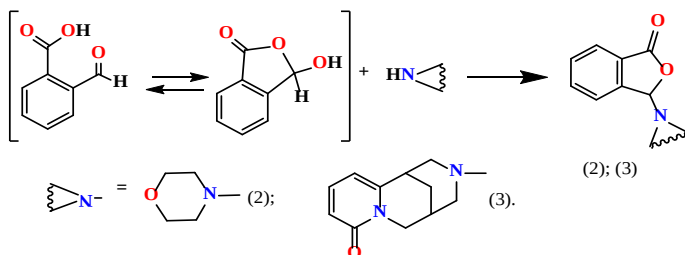
1. Introduction

The phthalide moiety serves as a key structural scaffold for compounds of practical importance, such as dyes, fungicides, natural oils (butylphthalide), and pharmaceuticals (isoxepac, vatalanib, hydralazine) [1-3]. Due to its broad spectrum of biological activity, the phthalide moiety is considered a promising pharmacophore platform. Of particular interest is the design of hybrid molecules ("dual drugs") containing two pharmacophore fragments, a strategy that can yield novel biological activity [4]. Aromatic compounds bearing active *ortho*-substituents are widely used in the synthesis of fused heterocyclic systems [5-7]. The presence of aldehyde and carboxyl groups in the *ortho*-position of such systems facilitates intramolecular cyclization [8]. A prime example is 2-formylbenzoic acid, which exists predominantly in the closed form in the solid state, whereas in solution, it exists in equilibrium between the open form (1a) and the cyclic 3-hydroxyphthalide form (1b)-an instance of ring-chain tautomerism. With the advancement of combinatorial chemistry, the scope of application for this compound continues to expand [9-12].

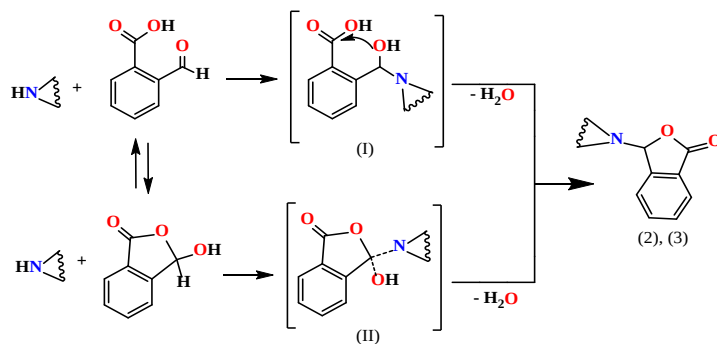


2. Results and discussion

In the search for new biologically active compounds, we investigated the reaction of *o*-formylbenzoic acid with morpholine and the alkaloid cytosine, yielding the corresponding 3-(3-oxo-1,3-dihydroisobenzofuran-1-yl)amines (2) and (3), respectively.



The formation of aminophthalides (2) and (3) can proceed via two mechanisms: through the nucleophilic addition of an amine to the aldehyde group of the open form of *o*-formylbenzoic acid, followed by intramolecular cyclization (I), or in a single step via the S_N^2 mechanism when an amine interacts with its cyclic form (II).



To study the geometric and electronic characteristics of the compounds under study, we carried out quantum chemical calculations using the density functional theory (DFT) method at the B3LYP/6-311G(d,p) level. Geometry optimization was performed without symmetry restrictions, and calculations were carried out in Gaussian 16W [13] with visualization of structures in GaussView 6.0 [14]. The resulting optimized structures are presented in Figure 1.

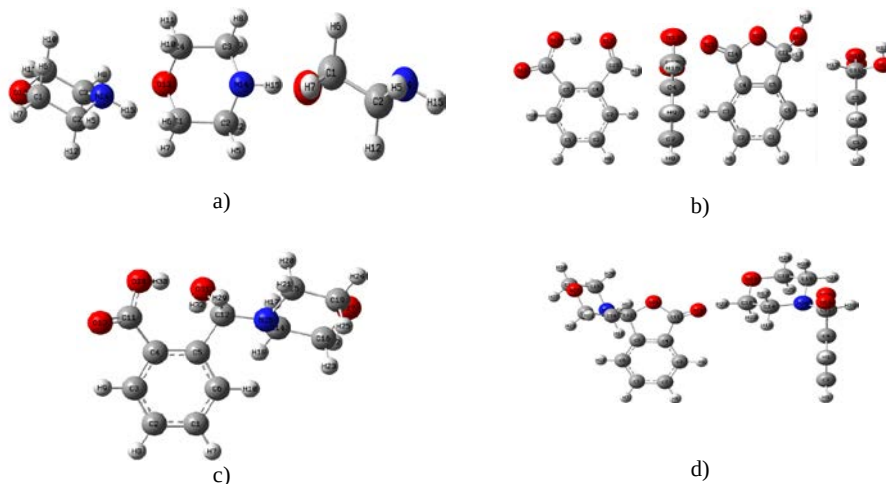


Figure 1 - Optimized 3D molecular configurations using the B3LYP/6-311G(d, p) method (top and side views; C atoms are shown in gray, Red - O, blue -N, white - H): a) morpholine; b) o-formylbenzoic acid; c) gem-amino alcohol; d) aminophthalide.

The calculated geometric parameters correspond to the energy minimum and are consistent with the standard values of chemical bonds and bond angles. For the systems under study, molecular characteristics have been determined, including the total energy (E_n), zero-point energy (E_0), ionization potential (IP), electron affinity (E_A), dipole moment (μ), entropy (S), and molar heat capacity (C_v).

Table 1 - Physicochemical characteristics of compounds (a-d) (method B3LYP/6-311G(d, p))

Characteristic	Optimized 3D molecular structures			
	a	b	c	d
E_p , a.u.	-287.8742681	-534.283849 (-534.283873)*	-822.1610836	-745.7149453
E_0 , a.u.	0.134636	0.1249707 (0.126386)	0.2640155	0.2365821
μ , D	1.561	6.726 (4.286)	5.420	4.908
E_{HOMO} , eV	-6.225	-7.242 (-7.576)	-6.820	-6.682
E_{LUMO} , eV	1.084	-2.423 (-1.687)	-1.575	-1.611
ΔE , eV	-	3.22 (4.33)	-	-
S , cal/mol*K	73.077	93.274 (89.443)	120.572	111.681
C_v , cal/mol*K	20.567	33.027 (32.297)	57.451	50.243

Note – Values in parentheses refer to the closed form of *o*-formylbenzoic acid

According to calculations, the stability of molecules is determined by their structure and composition. The closed form of *o*-formylbenzoic acid is 63 J/mol more energetically stable than the open form, which indicates the possibility of their equilibrium. The dipole moment characterizes the distribution of electron density, while the ionization potential and electron affinity reflect the electronic properties of molecules and are assessed based on the energies of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) in accordance with Koopmans' theorem.

In the reaction, morpholine acts as a nucleophile, and *o*-formylbenzoic acid acts as an electrophile. The reactivity is determined by the energy gap between the highest occupied molecular orbital (HOMO) of the nucleophile and the lowest unoccupied molecular orbital (LUMO) of the electrophile: a reduction in this gap promotes the reaction. For the open form of the acid, this gap is smaller, which indicates its higher reactivity and the preferential formation of a *gem*-amino alcohol.

As a result of the interaction between *o*-formylbenzoic acid and morpholine and cytosine, new aminophthalide derivatives (2) and (3) were synthesized. The structure of the synthesized compounds (2) and (3) has been confirmed by IR, ^1H NMR, ^{13}C NMR, COSY, HMQC, HMBC spectroscopy and mass spectrometry. In the IR spectra, aminophthalides are characterized by intense bands of carbonyl absorption (1715–1750 cm^{-1}) and C–O–C bond vibrations (1050–1096 and 847–900 cm^{-1}). In the ^1H and ^{13}C NMR spectra of compound (2), signals from the morpholine and phthalide fragments were detected, while compound (3) showed

characteristic signals of the cytosine and phthalide fragments, which confirms the structure of the obtained compounds.

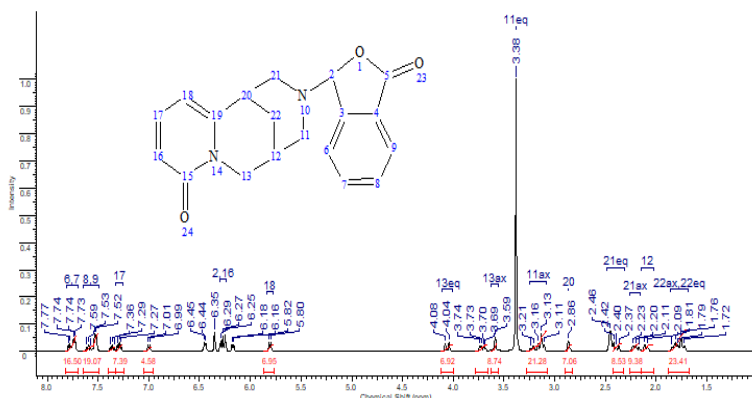


Figure 2 - ^1H NMR characteristics of compound (3)/

In the ^1H NMR spectrum of compound (3), the proton signals of the *bis*-piperidine fragment are observed in the region of 2.09–4.08 ppm, the aromatic protons of the cytosine fragment are observed at 5.80–7.31 ppm, and the phthalide fragment is observed at 7.51–7.79 ppm. In the ^{13}C NMR spectrum, the signals of the carbon atoms in the cytosine and phthalide fragments are located in characteristic ranges, which confirms the structure of the synthesized compound (3).

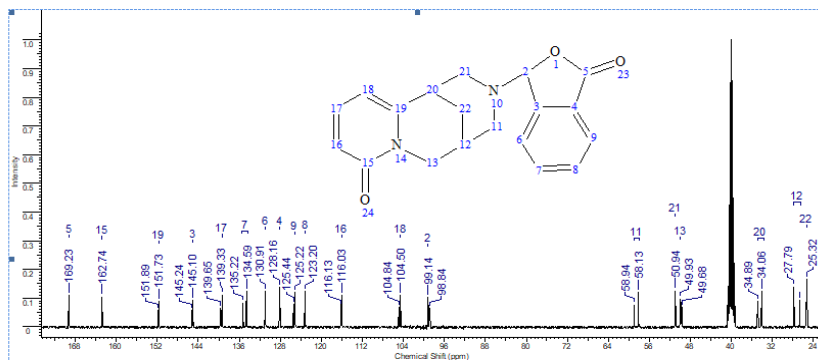


Figure 3 – NMR ^{13}C spectrum of compound (3).

The structure of compounds (2) and (3) was further confirmed using two-dimensional NMR spectroscopy methods: ^1H - ^1H COSY, ^1H - ^{13}C HMQC, and ^1H - ^{13}C HMBC. The identified short-range and long-range correlations between protons and carbon atoms correspond to the proposed structure of compound (2), confirming the relative arrangement of the morpholine, phthalide, and aromatic fragments. The ^1H - ^1H and ^1H - ^{13}C NMR connectivities determined by COSY, HMQC, and HMBC experiments for molecule (2) are depicted in Figure 4.

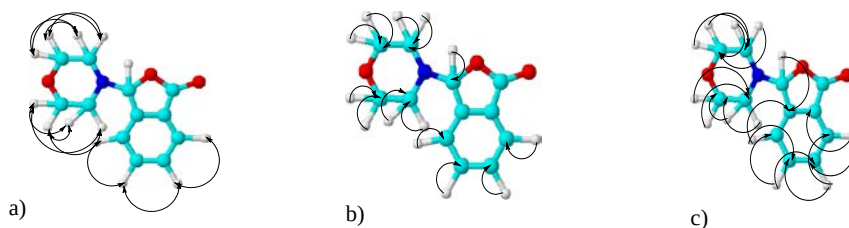


Figure 4 - Coupling diagram in the COSY (^1H - ^1H) (a), HMQC (^1H - ^{13}C) (b), and HMBC (^1H - ^{13}C) (c) spectra of compound (2).

The structure of compound (3) is additionally confirmed by data from two-dimensional NMR spectroscopy. In the ^1H - ^1H COSY and ^1H - ^{13}C HMQC spectra, characteristic correlations of protons with adjacent and directly bonded carbon atoms of the cytosine and phthalide fragments are established. The ^1H - ^{13}C HMBC method revealed distant correlations, confirming the relative arrangement of the functional groups and the correspondence of the obtained data to the proposed structure of compound (3). The observed COSY (^1H - ^1H), HMQC (^1H - ^{13}C) and HMBC (^1H - ^{13}C) NMR correlations within the molecule (3) shown in the Figure 5.

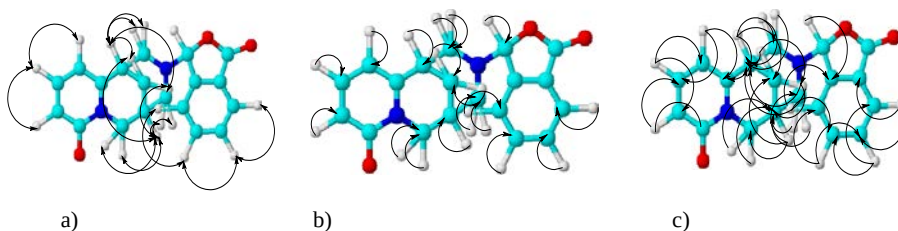


Figure 5 - COSY (a), HMQC (b), HMBC (c) correlation patterns observed for compound (3).

In the mass spectrum of compound (3), at $t = 23.95$ min, the molecular ion m/z 322.14 $[\text{M}]^+$ was detected, corresponding to the molecular mass of the compound. The most intense peak was observed at m/z 133.07. Characteristic fragment ions at m/z 146.08, 189.14, 207.05, 176.09, 160.09, 117.07, 105.07, and 77.10 were also observed, resulting from molecular fragmentation under electron impact. The obtained data confirm the structure and molecular mass of compound (3) (Fig. 6). Chromatographic-mass spectrometric analysis was carried out using a Trace GC Ultra-DSQ II gas chromatograph (Thermo Fisher Scientific, USA; 2006–2007).

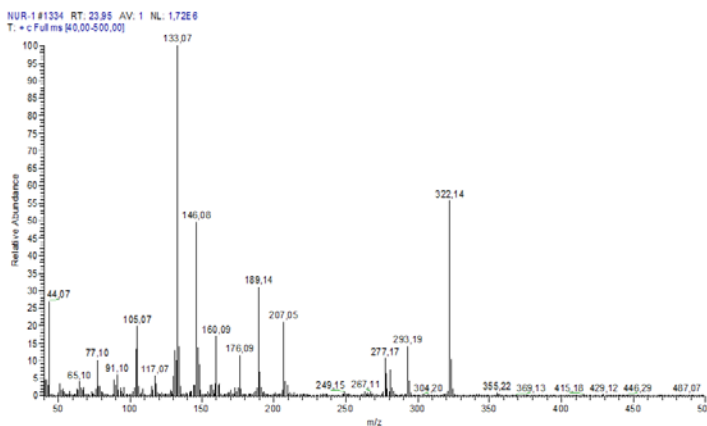


Figure 6 - Mass spectrum of compound (3), $t=23.95$

3. Experimental part

^1H and ^{13}C NMR measurements were carried out using a JNM-ECA Jeol 400 spectrometer operating at 399.78 and 100.53 MHz, respectively. DMSO- d_6 and CDCl_3 were employed as solvents. Chemical shifts were referenced to the residual proton and carbon resonances of the corresponding deuterated solvents. Melting points were measured with an «SMP10» apparatus. The synthesized products were monitored by thin-layer chromatography (TLC) on Silufol UV-254 plates using ethyl acetate as a solvent system and visualization of spots in iodine vapor. For the original compound, $R_f \approx 0.44$, whereas for the synthesized 3-morpholinoisobenzofuran-1(3H)-one, $R_f \approx 0.34$.

Methods for the synthesis of aminophthalides morpholine (2) and cytosine (3).

3-Morpholinoisobenzofuran-1(3H)-one (2). A mixture of 2-carboxybenzaldehyde (3.4 g, 0.02 mol) and morpholine (2.0 g, 0.02 mol) in isopropyl alcohol (25 ml) was stirred at 40 °C for 3 hours, then 1–2 drops of glacial acetic acid were added. After cooling, the precipitate was filtered out. Yield: 3.9 g (83%), mp 129–130°C.

3-(Cytisin-1-yl)isobenzofuran-1(3H)-one (3). To the solution of cytosine (1.3 g, 0.0068 mol) in 25 ml of dry benzene, 2-carboxybenzaldehyde (1.02 g, 0.0068 mol) was added, and the mixture was heated for 3 hours with a Dean-Stark trap. After distilling off 2/3 of the solvent, a white precipitate formed upon cooling. The yield was 2.02 g (92.5%), with a melting point of 170–173°C.

4. Conclusion

The reaction of 2-formylbenzoic acid with amines (morpholine and cytosine) occurs under mild conditions and leads to the formation of 3-morpholinoisobenzofuran-1(3H)-one and 3-(cytisin-1-yl)isobenzofuran-1(3H)-one derivatives in high yields. The proposed reaction mechanism involves the nucleophilic addition of secondary amines to o-formylbenzoic acid followed by

intramolecular cyclization. Quantum chemical DFT calculations at the B3LYP/6-311G(d,p) level of theory determined the energy characteristics, dipole moments, ionization potentials, and electron affinities of the studied compounds.

МОРФОЛИН ЖӘНЕ АЛКАЛОИД ЦИТИЗИН НЕГІЗІНДЕГІ АМИНОФТАЛИДТЕРДІҢ СИНТЕЗІ

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Түйіндеме. Бұл мақалада *о*-формилбензой қышқылының морфолинмен және алкалоид цитизинмен реакциясын зерттеу нәтижелері ұсынылған. Бұл зерттеу болашағы зор биологиялық белсенді қосылыстар ретінде қызығушылық тудыратын жаңа аминфталид туындыларын синтездеуге бағытталған. Бастапқы, аралық және соңғы қосылыстардың геометриялық құрылымын, электрондық құрылымын және физика-химиялық сипаттамаларын зерттеу үшін B3LYP/6-311G(d,p) деңгейіндегі тығыздық функционалдық теориясын (ТФТ) қолданатын кванттық-химиялық есептеулер жүргізілді. Синтезделген қосылыстардың құрылымы ИК, бір өлшемді ¹H, ¹³C және екі өлшемді ЯМР спектроскопиясы (COSY, HMQC, HMBC) және масс-спектрометрия арқылы расталды. Бастапқы реагенттер арасындағы өзара әрекеттесулер зерттелді және мақсатты өнімдердің жоғары өнімділігін қамтамасыз ету үшін оңтайлы синтез жағдайлары анықталды. Аминфталидтердің түзілу механизмі ұсынылды, ол *о*-формилбензой қышқылының карбонил тобына екінші реттік аминдерді нуклеофильді қосуды, содан кейін молекулаішілік цикленуді қамтиды. Спектрлік талдау алынған қосылыстардың ұсынылған құрылымына толығымен сәйкес келеді. Алынған нәтижелер морфолин мен цитизин негізінде аминфталидтерді синтездеудің әзірленген әдісінің тиімділігін көрсетеді және олардың реактивтілігін, физика-химиялық сипаттамаларын және әлеуетті биологиялық белсенділігін одан әрі зерттеу үшін негіз болады.

Түйінді сөздер: *о*-формилбензой қышқылы, морфолин, цитизин, аминфталидтер, кванттық химиялық есептеулер, ЯМР спектроскопия, масс-спектрометрия

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СИНТЕЗ АМИНОФТАЛИДОВ НА ОСНОВЕ МОРФОЛИНА И АЛКАЛОИДА ЦИТИЗИНА

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Резюме. В работе представлены результаты исследования реакции *o*-формилбензойной кислоты с морфолином и алкалоидом цитизином, направленной на синтез новых аминоталидных производных, представляющих интерес в качестве перспективных биологически активных соединений. Для исследования геометрического строения, электронной структуры и физико-химических характеристик исходных, промежуточных и конечных соединений проведены квантово-химические расчёты проведены с использованием теории функционала плотности (DFT) на уровне B3LYP/6-311G(d,p). Структуры синтезированных соединений подтверждены данными ИК, одномерной ¹H и ¹³C и двумерной ЯМР-спектроскопии (COSY, HMQC, HMBC), а также масс-спектрометрии. Изучены особенности взаимодействия исходных реагентов и определены оптимальные условия проведения синтеза, обеспечивающие получение целевых продуктов с высокими выходами. Предложен механизм образования аминоталидов, включающий нуклеофильное присоединение вторичных аминов к карбонильной группе *o*-формилбензойной кислоты с последующей внутримолекулярной циклизацией. Выполненный спектральный анализ полностью согласуется с предложенным строением полученных соединений. Полученные результаты свидетельствуют об эффективности разработанного метода синтеза аминоталидов на основе морфолина и цитизина и создают основу для дальнейших исследований их реакционной способности, физико-химических характеристик и потенциальной биологической активности.

Ключевые слова: *o*-формилбензойная кислота, морфолин, цитизин, аминоталиды, квантово-химические расчеты, ЯМР-спектроскопия, масс-спектрометрия.

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