

RESEARCH ARTICLE

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Synthesis, Characterization, Quantum-Chemical Calculations and Cytotoxic Activity of 1,8-Naphthalimide Derivatives with Non-Protein Amino Acids

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Abstract: Background: The 1,8-Naphthalimides constitute an important class of biologically active, DNA-binding compounds. There are no available data on the synthesis of 1,8-naphthalimide derivatives with non-protein amino acids and their biological activity. The aim of this paper was to determine the synthesis, structural characterization and cytotoxic activity of new 1-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)cycloalkane-1-carboxylic acids with 5-, 6-, 7-, 8- and 12-membered rings as well as 2-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)adamantane-2-carboxylic acid and 1-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-1,2,3,4-tetrahydronaphthalene-1-carboxylic acid.

Methods: The target compounds were obtained by an interaction of 1,8-naphthalic anhydride with a series of non-protein amino acids. The optimized geometry and harmonic vibrational frequencies have been calculated by DFT employing B3LYP functional using 6-31G(d,p) basis set. An *ab initio* (MP2 and Hartree-Fock) and DFT (different functionals) using several basis sets have been applied for NMR calculations. The cytotoxic effects of the synthesized compounds are assessed against two human tumor cell lines, namely K-562 (chronic myeloid leukemia) and HUT-78 (cutaneous T-cell lymphoma) after 72 h exposure, using the MTT-dye reduction assay. The apoptogenic effects and the ability to modulate the NFκB-signaling pathways were determined using commercially available ELISA kits.

Results: All compounds inhibited the growth of malignant cells at micromolar concentrations whereby compound **4b** (1-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)cyclohexane-1-carboxylic acid) demonstrated superior activity in both cell lines with IC₅₀ values comparable to those of the reference anticancer drug melphalan.

Conclusion: New 1,8-naphthalimide derivatives with non-protein amino acids were successfully synthesized. Quantum-chemical calculations were performed to elucidate the structure of the newly synthesized compounds. There is a proper alignment between theoretical and experimental results. The cytotoxicity of the synthesized products against two human tumor cell lines, namely K-562 and HUT-78 was evaluated. All compounds inhibited the growth of malignant cells at micromolar concentrations. The pharmacodynamics evaluation of compound **4b** showed that its cytotoxicity is mediated by induction of apoptosis and inhibition of NFκB-signaling.

Keywords: 1,8-Naphthalimides, Non-protein amino acids, Synthesis, DFT, GIAO, Cytotoxicity, MTT.

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1. INTRODUCTION

The 1,8-Naphthalimides constitute an important class of biologically active, DNA-binding compounds, and their synthesis has been in the focus of considerable attention in synthetic organic as well as in medicinal chemistry [1]. The development of functional 1,8-naphthalimide derivatives as DNA targeting, anticancer and cellular imaging agents is a fast growing area. Several such compounds have been involved into clinical trials [2, 3].

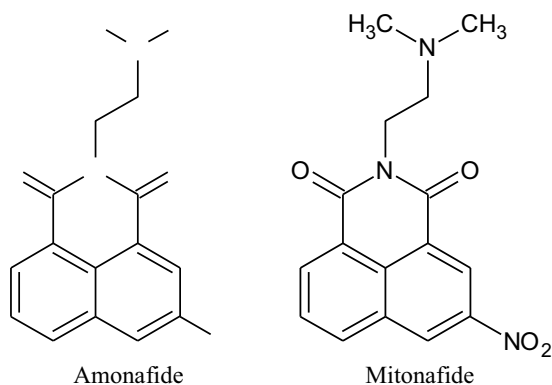
Data showing that 1,8-naphthalimides (benz[*de*]isoquinolin-1,3-diones) possess high antitumour activity towards various human

and murine cells have been published by other authors [4-6]. Clinical studies have shown that two leading members of this family amonafide (a 3-amino-1,8-naphthalimide) and mitonafide (a 3-nitro-1,8-naphthalimide), exhibit high antitumour activity against HeLa cell lines [4, 7, 8]. These compounds have also been found to stabilise double stranded DNA against heat denaturation [5, 7]. Both amonafide and mitonafide can bind to DNA *via* intercalation and inhibit topoisomerase II activity [9, 10].

Qian *et al.* have synthesized derivatives of 1,8-naphthalimide conjugated with a leucine amino acid [11]. It was found that these naphthalimide-leucine conjugates possess significant cytotoxicity against a wide range of tumour cells. Moreover, they exhibited strong interaction with bovine serum albumin [11].

In addition, several derivatives of naphthalimide with amino acids have been synthesized [3, 12, 13].

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Some representatives of 1,8-Naphthalimides have luminescent properties and participate in the construction of dendritic systems [14-16]. Their photophysical properties make them excellent substances for application as dual therapeutic and fluorescence imaging agents.

The uses of the 1,8-naphthalimide core extend beyond their application as DNA-binding motifs and anticancer agents. They have been extensively used within the field of supramolecular chemistry. Different naphthalimides are used for colouring of polymeric materials [17], as laser active medium [18], photosensitive potential biological units [19], fluorescent markers in biology [20], LEDs [21], photoinduced electron transfer sensors [22], electroluminescent materials [23], liquid crystal displays [24], ion probes [25], as analgesic and anti-rheumatic drugs [26].

On the other hand, it is well known that some non-protein cyclic amino acids have antitumor activity [27-34]. Our team has already investigated and presented the fungicidal [35] and insecticidal activity of 1-aminocyclopentane-1-carboxylic acid [36]. In previous works of ours, different non-protein cyclic amino acids have been synthesized by an alkaline hydrolysis of the corresponding spirohydantoin with barium hydroxide [37, 38].

There are no available data on the synthesis of 1,8-naphthalimide derivatives with non-protein amino acids and their antiproliferative effects. Taking into account the fact that the aminocyclopentane-1-carboxylic acid, cycloleucin, exerts antineoplastic effects [27], we synthesized a series of new 1-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)cycloalkane-1-carboxylic acids with 5-, 6-, 7-, 8- and 12-membered rings as well as 2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)adamantane-2-carboxylic acid and 1-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)-1,2,3,4-tetrahydronaphthalene-1-carboxylic acid. Therefore, the purpose of this work is to carry out the synthesis and structural characterization of these compounds, as well as to study of their biological activity including the role of the ring size in the molecule.

2. MATERIALS AND METHODS

All chemicals used were purchased from Merck and Sigma-Aldrich. The melting points were determined by a SMP-10 digital melting point apparatus. The elemental analysis data were obtained with an automatic analyzer Carlo Erba 1106. The purity of the compounds was checked by thin layer chromatography on Kieselgel 60 F₂₅₄, 0.2 mm Merck plates, eluent system (vol. ratio): pyridine : n-butanol : glacial acetic acid : water = 10 : 15 : 3 : 12. The IR spectra were recorded on a spectrometer Perkin-Elmer FTIR-1600 in KBr discs. The NMR spectra were recorded on a Bruker Avance II + 600 MHz spectrometer, operating at 600.130 and 150.903 MHz for ¹H and ¹³C, respectively, using the standard Bruker software. The chemical shifts were referenced to tetramethylsilane (TMS). The measurements in DMSO-*d*₆ solutions were carried out at ambient temperature (300 K). Typical condi-

tions for ¹H NMR spectra were: pulse width 30°, 1s relaxation delay, 16K time domain points, zero-filled to 64K, hard pulse with 90° pulse width of 11.8μs; ¹³C NMR spectra: pulse width 30°, 1s relaxation delay, 16K time domain points, zero-filled to 32K, hard pulse with 90° pulse width of 6.4 μs at a power level of 3 dB below the maximum output.

2.1. Synthesis of 1,8-naphthalimide Derivatives (4a-4k) with Non-protein Amino Acids (3a-3k) (Scheme 1)

A mixture of 1.78g (0.009mol) of 1,8-naphthalic anhydride (1,8-NA, Fig. 1) and 0.0135mol of the corresponding non-protein amino acid (3a-3k) was refluxed for five hours in a solution of 20ml of *N,N*-Dimethylformamide (DMF) and 20ml of glacial acetic acid. After cooling down to room temperature, the resulting solution was poured into 100ml of cold water and was left overnight. The crystalline products obtained (4a-4k) were filtered off and recrystallized from glacial acetic acid.

2.1.1. 1-(1,3-Dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)cyclopentane-1-carboxylic acid (4a)

Yield 74%; M.p. 268-269°C; R_f = 0.51; Anal. calcd. for C₁₈H₁₅NO₄: C, 69.89; H, 4.89; N, 4.53; found: C, 69.61; H, 4.67; N, 4.39%.

2.1.2. 1-(1,3-Dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)cyclohexane-1-carboxylic acid (4b)

Yield 78%; M.p. 261-262°C; R_f = 0.43; Anal. calcd. for C₁₉H₁₇NO₄: C, 70.58; H, 5.30; N, 4.33; found: C, 70.41; H, 5.22; N, 4.08 %.

2.1.3. 1-(1,3-Dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)-2-methylcyclohexane-1-carboxylic acid (4c)

Yield 75%; M. p. 236-237°C; R_f = 0.54; Anal. calcd. for C₂₀H₁₉NO₄: C, 71.20; H, 5.68; N, 4.15; found: C, 70.95; H, 5.44; N, 3.99 %.

2.1.4. 1-(1,3-Dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)-4-methylcyclohexane-1-carboxylic acid (4d)

Yield 62%; M. p. 261-262°C; R_f = 0.45; Anal. calcd. for C₂₀H₁₉NO₄: C, 71.20; H, 5.68; N, 4.15; found: C, 71.00; H, 5.51; N, 3.92 %.

2.1.5. 1-(1,3-Dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)-4-ethylcyclohexane-1-carboxylic acid (4e)

Yield 61%; M. p. 268-269°C; R_f = 0.48; Anal. calcd. for C₂₁H₂₁NO₄: C, 71.78; H, 6.02; N, 3.99; found: C, 71.53; H, 5.88; N, 3.74 %.

2.1.6. 1-(1,3-Dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)-4-propylcyclohexane-1-carboxylic acid (4f)

Yield 69%; M. p. 257-258°C; R_f = 0.39; Anal. calcd. for C₂₂H₂₃NO₄: C, 72.31; H, 6.34; N, 3.83; found: C, 72.05; H, 6.11; N, 3.67%.

2.1.7. 1-(1,3-Dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)cycloheptane-1-carboxylic acid (4g)

Yield 75%; M. p. 257-258°C; R_f = 0.33; Anal. calcd. for C₂₀H₁₉NO₄: C, 71.20; H, 5.68; N, 4.15; found: C, 70.96; H, 5.57; N, 3.98%.

2.1.8. 1-(1,3-Dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)cyclooctane-1-carboxylic acid (4h)

Yield 76%; M. p. 247-248°C; R_f = 0.40; Anal. calcd. for C₂₁H₂₁NO₄: C, 71.78; H, 6.02; N, 3.99; found: C, 71.49; H, 5.87; N, 3.81%.

2.1.9. 1-(1,3-Dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)cyclodecane-1-carboxylic acid (4i)

Yield 81%; M.p. 218-219°C; R_f = 0.36; Anal. calcd. for $C_{25}H_{29}NO_4$: C, 73.68; H, 7.17, N, 3.44; found: C, 73.44; H, 7.11, N, 3.19%.

2.1.10. 2-(1,3-Dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)adamantane-2-carboxylic acid (4j)

Yield 57%; M.p. 259-260°C; R_f = 0.29; Anal. calcd. for $C_{23}H_{21}NO_4$: C, 73.58; H, 5.64; N, 3.73; found: C, 73.29; H, 5.38, N, 3.47%.

2.1.11. 1-(1,3-Dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)-1,2,3,4-tetrahydronaphthalene-1-carboxylic acid (4k)

Yield 59%; M.p. 273-274°C; R_f = 0.35; Anal. calcd. for $C_{23}H_{17}NO_4$: C, 74.38; H, 4.61, N, 3.77; found: C, 74.12; H, 4.42; N, 3.51%.

2.2. Computational Details

Full geometry optimization of the compounds synthesized was performed at B3LYP/6-31G(d) level of theory without symmetry constraints by the gradient procedure. The structure optimization of all compounds was carried out at B3LYP/6-31G(d,p) computational level using the Firefly QC package [39], which is partially based on the GAMESS (US) [40, 41] source code. The default gradient convergence threshold (1×10^{-4} hartree Bohr⁻¹) was used. Frequency calculations at the same theory level were carried out to confirm that the structures obtained correspond to energy minima.

NMR chemical shieldings were calculated using the GIAO (gauge-including atomic orbitals) approach [42, 43]. Proton and carbon chemical shieldings were calculated by *ab initio* (MP2, Hartree-Fock (HF)) and Density Functional Theory (DFT) methods using 6-31+G(2d,p) basis set. Solvent effects are accounted for using the Polarizable Continuum Model, as implemented in GAUSSIAN 09 [44, 45]. The inclusion of the solvent as dielectric in GIAO NMR calculations was used to estimate the effect of the medium dimethylsulfoxide (DMSO) on the chemical shifts of compounds **4a-4k**. In order to compare with the experimental data, the calculated absolute shieldings were transformed to chemical shifts using the reference compound tetramethylsilane (TMS): $\delta = \delta_{\text{calc}}(\text{TMS}) - \delta_{\text{calc}}$. Both $\delta_{\text{calc}}(\text{TMS})$ and δ_{calc} were evaluated with the same method and basis set.

2.3. Cell Lines, Culture Conditions and Cytotoxicity Evaluation

The cell line K-562 (chronic myeloid leukemia) was purchased from DSMZ (Germany), whereas Hut-78 (cutaneous T-cell lymphoma) was supplied by ATCC (USA). Cells were cultured in a controlled environment: 37°C in a 5% CO₂ humidified atmosphere, using RPMI-1640, supplemented with 2mM L-glutamine and 10% fetal calf serum as growth medium. The cellular viability was assessed using the MTT-dye reduction assay, as described by Mosmann, with minor modifications [46, 47]. In brief, exponentially proliferating cells were seeded in 96-well flat-bottomed microplates (100μL/well) at a density of 10^5 cells per mL and after 24h incubation at 37°C, they were exposed to serial dilutions of the tested compounds for 72h. Each exposure was run in at least 8 wells in parallel. After the exposure period, 10μL MTT solution (10mg/mL in PBS) aliquots were pipetted to each well and then the microplates were further incubated for 4h at 37°C. The MTT-formazan crystals were dissolved by the addition of 100μL/well 5% HCOOH-acidified 2-propanol. The MTT-formazan absorption was measured using a Beckman-Coulter DTX800 multimode microplate reader at 580 nm. Cell survival fractions were calculated as a percentage of the untreated control. In addition, IC₅₀ values were de-

rived from the concentration-response curves using non-linear regression analysis.

2.4. Apoptosis Assay

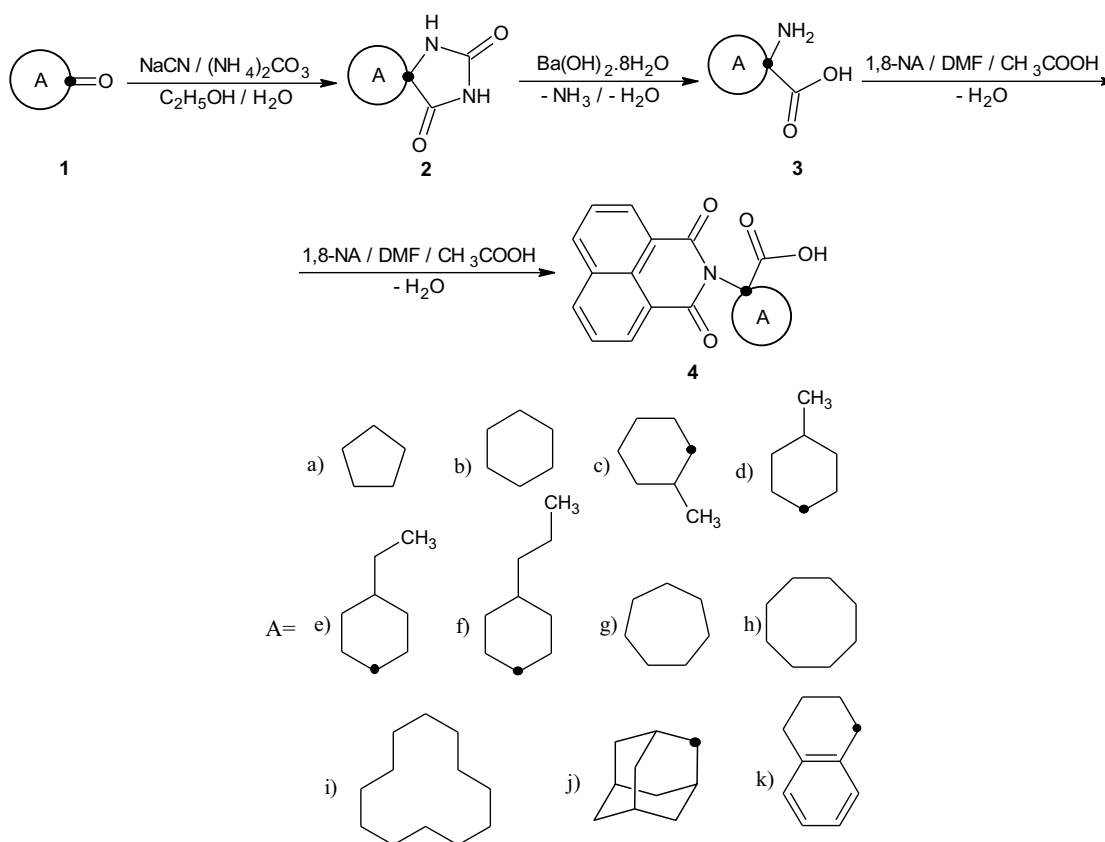
Exponentially proliferating K-562 cells were exposed to equieffective concentrations of melphalan or 4b for 24h. The control samples were exposed to RPMI-1640 medium only. After the treatment period of K-562 cells, the apoptotic pattern of DNA fragmentation was assessed by a commercially available ELISA kit (Roche Applied Science, Mannheim, Germany) according to the instructions of the manufacturer. The relative amounts of mono- and oligonucleosomes generated from the apoptotic cells were quantified using monoclonal antibodies directed against DNA and histones by ELISA. In brief, cytoplasmic fraction of the untreated controls and treated cells served as an antigen source in a sandwich ELISA, utilizing a primary anti-histone antibody-coated microplate and a secondary peroxidase-conjugated anti-DNA antibody. The spectrophotometric immunoassay was performed at 405 nm, using a multimode microplate reader (Beckman Coulter DTX800). The results are expressed as the oligonucleosome enrichment factor (calculated as the ratio between the absorption in the treated vs. the untreated control samples). Each data point was generated from 4 independent experiments. Curcumin was used as a positive control in this assay.

2.5. NFκB p65 ELISA Assay

NFκB p65 ELISA kit (Cat. # ADI-EKS 446) was obtained from Enzo Biochem Inc. The NFκB p65 ELISA kit is a reliable and assay to quantitatively assess p65 – the active form of the transcriptional factor NFκB. The assay set utilizes streptavidin-coated plates with bound NFκB biotinylated-consensus sequence to capture only p65. Thereafter the captured transcription factor is incubated with a specific NFκB p65 antibody, which is eventually detected using an horseradish peroxidase-conjugated secondary antibody. The procedure was carried out according to the instructions of the manufacturer. The luminescence was measured using a Beckman Coulter DTX 880 multimode microplate reader using specific chemiluminescent protocol for 96 wells plates. Each test was run in quadruplicate and the results were presented as percentage of the untreated control.

3. RESULTS AND DISCUSSIONS

The synthesis of the 1,8-naphthalimide derivatives (**4a-4k**) of non-protein amino acids (**3a-3k**) was performed in accordance with Scheme 1. The cycloalkanespiro-5-hydantoins (**2a-2i**) were obtained from the corresponding ketones (**1a-1i**) by the Bucherer-Lieb method [48]. As a result of this technique, the following spirohydantoins were prepared: 1,3-diazaspiro[4.4]nonane-2,4-dione (**2a**), 1,3-diazaspiro[4.5]decane-2,4-dione (**2b**), 6-methyl-1,3-diazaspiro[4.5]decane-2,4-dione (**2c**), 8-methyl-1,3-diazaspiro[4.5]decane-2,4-dione (**2d**), 8-ethyl-1,3-diazaspiro[4.5]decane-2,4-dione (**2e**), 8-propyl-1,3-diazaspiro[4.5]decane-2,4-dione (**2f**), 1,3-diazaspiro[4.6]undecane-2,4-dione (**2g**), 1,3-diazaspiro[4.7]dodecane-2,4-dione (**2h**) and 1,3-diazaspiro[4.11]hexadecane-2,4-dione (**2i**) [37, 49, 50]. The spiro(adamantane-2,4'-imidazolidine)-2',5'-dione (**2j**) was obtained from the adamantan-2-one (**1j**) in accordance with the procedure introduced by Nagasawa *et al.* [51]. The 3',4'-dihydro-2H,2'H,5H-spiro[imidazolidine-4,1'-naphthalene]-2,5-dione (**2k**) was synthesized from the 3,4-dihydronaphthalen-1(2H)-one (**1k**) as per the procedure described by Marinov *et al.* [52]. The spirohydantoins obtained (**2a-2k**) were subjected to an alkaline hydrolysis with barium hydroxide, resulting in the formation of the corresponding non-protein amino acids as follows: 1-aminocyclopentane-1-carboxylic acid (**3a**), 1-aminocyclohexane-1-carboxylic acid (**3b**), 1-amino-2-methylcyclohexane-1-carboxylic acid (**3c**), 1-amino-4-



Scheme 1. Synthesis of 1,8-naphthalimide derivatives with non-protein amino acids.

methylcyclohexane-1-carboxylic acid (**3d**), 1-amino-4-ethylcyclohexane-1-carboxylic acid (**3e**), 1-amino-4-propylcyclohexane-1-carboxylic acid (**3f**), 1-aminocycloheptane-1-carboxylic acid (**3g**), 1-aminocyclooctane-1-carboxylic acid (**3h**), 1-aminocyclododecane-1-carboxylic acid (**3i**), 2-aminoadamantane-2-carboxylic acid (**3j**) and 1-amino-1,2,3,4-tetrahydronaphthalene-1-carboxylic acid (**3k**) [37, 38].

The products obtained (**4a–4k**) have been characterized by physicochemical parameters, elemental analysis (see the “Materials and methods” part) and spectral data. The results obtained from the IR and NMR analyses are listed in Tables 1 and 2 respectively.

In order to obtain additional information for the structure of the compounds presented in Fig. (2), we have performed DFT calculations in gas phase. Full geometry optimization of the structures has been carried out using B3LYP functional and 6-31G(d,p) basis set. Since two of the structures (**4c** and **4d**) are positional isomers (Fig. 2), the free Gibbs energies are calculated. It has been established that the **4d** isomer is more stable than the **4c** one by 3.69 kcal mol⁻¹. The different position of the CH₃-group in **4c** and **4d** compounds does not lead to any changes in bond lengths of the six-membered ring.

The bond length analysis of 1,8-naphthalimide derivatives shows that change of the substituent at carboximide carbon atom

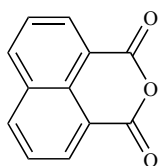


Fig. (1). 1,8-Naphthalic anhydride (1,8-NA). Systematic name: 1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione.

(C14) does not lead to any substantial alterations in naphthalimide moiety as well as in carboxyl group (Fig. 2 and Supplementary material). The alteration occurs only in C14-N and C14-C bond lengths from naphthalimide ring and carboxyl group, respectively. The C14-N bond is shorter for compounds **4a**, **4d** and **4k** - 1.499, 1.509 and 1.502 Å, respectively. The change of the substituent leads to lengthening of the bond from 0.003 to 0.022 Å and the bond is the longest in the **4c**, **4h** and **4j** compounds. This bond is in the range 1.512-1.518 Å in all rest compounds. The bond C14-C between carboximide carbon atom and carboxyl group is the shortest in **4d** (1.538 Å) and the longest in **4k** (1.556 Å).

The vibrational spectra of 1,8-naphthalimide derivatives (**4a–4k**) of non-protein amino acids have been computed at B3LYP/6-31G(d,p) level. Our assignments for the DFT calculated frequencies are based on the analysis of the corresponding vibrational eigenvectors. Available experimental data for the vibrational frequencies of the compounds in KBr are presented for comparison. All results are listed in Table 1. There is a good alignment between experimental and theoretical data. A slight deviation (57-129 cm⁻¹) has been observed for the calculated stretching frequency ν(O-H) due to the formation of a hydrogen bond in the carboxyl group during the geometric optimization of all compounds, probably. Experimentally identified values for characteristic valent frequencies ν(C=O) of two carbonyl group are about 1740 cm⁻¹ and 1700 cm⁻¹ while the theoretically predicted vibration frequencies ν(C=O) are about 1680 cm⁻¹ and 1645 cm⁻¹. The analysis of the theoretical IR spectra indicates that the change of the substituent at the carboximide carbon atom does not have a strong influence on the valent frequencies in the 1,8-naphthalimide derivatives molecules.

An *ab initio* (MP2, HF) and different DFT functionals (B3LYP, B3PW91, mPW1PW91, O3LYP, M062X) and basis sets (6-31+G(d,p), 6-31+G(2d,p), 6-311++G(2df,p), 6-311+G(d,p), DGDZVP)

Table 1. Experimental IR data (KBr, cm⁻¹) and selected frequencies calculated at B3LYP/6-31G(d,p) level (*italic*) for compounds 4a-4k. The calculated frequencies are given in the gas phase and are scaled by a factor of 0.945.

№	ν_{OH}	ν_{CH} (arom.)	$\nu_{as}(CH_2)$	$\nu_s(CH_2)$	$\nu_{C=O}(COOH)$	$\nu_{C=O}$	ν_{CC} (arom.)	ν_{CN} (imide)
4a	3481 3542	3058 3046	2940 2946	2865 2888	1781 1740	1742, 1708 1683, 1647	1581, 1511 1585, 1543	1384 1376
4b	3473 3530	3066 3058	2927 2931	2860 2889	1772 1728	1738, 1704 1675, 1641	1584, 1511 1584, 1544	1384 1376
4c	3444 3573	3065 3061	2934 2926	2860 2866	1770 1732	1741, 1713 1682, 1642	1580, 1511 1585, 1543	1384 1380
4d	3472 3544	3066 3046	2951 2940	2865 2866	1772 1744	1739, 1698 1683, 1647	1581, 1511 1584, 1543	1373 1377
4e	3474 3545	3072 3049	2927 2930	2863 2862	1773 1736	1738, 1698 1679, 1642	1581, 1512 1585, 1543	1384 1379
4f	3468 3545	3067 3050	2925 2933	2861 2862	1774 1736	1737, 1695 1680, 1641	1581, 1512 1586, 1544	1383 1379
4g	3468 3543	3065 3047	2933 2933	2862 2864	1771 1725	1741, 1703 1672, 1636	1584, 1511 1583, 1541	1379 1375
4h	3471 3581	3066 3058	2923 2928	2854 2863	1771 1735	1741, 1702 1674, 1638	1584, 1511 1585, 1543	1384 1378
4i	3465 3568	3065 3046	2925 2936	2857 2862	1771 1769	1741, 1701 1683, 1650	1584, 1511 1583, 1541	1384 1373
4j	3470 3532	3066 3047	2919 2913	2856 2862	1772 1728	1737, 1699 1683, 1646	1581, 1511 1583, 1544	1384 1377
4k	3471 3547	3066 3045	2934 2938	2857 2846	1771 1730	1741, 1701 1680, 1646	1580, 1511 1586, 1540	1384 1374

Table 2. GIAO ¹H and ¹³C chemical shifts (δ /ppm) in DMSO of compounds 4a-4k calculated at HF/6-31+G(2d,p) level (*italic*) and experimental data. The geometries are optimized at the B3LYP/6-31G(d,p) level.

№	¹ H NMR (DMSO- <i>d</i> ₆), δ / ppm	¹³ C NMR (DMSO- <i>d</i> ₆), δ / ppm*
4a	1.53 1.50 (s, CH ₂), 2.51 2.93 (s, CH ₂), 7.91-8.55 8.27-9.70 (m, 6H, naphthalene core), 13.80 6.94 (s, OH)	21.7 23.0 (CH ₂), 39.3 36.7 (CH ₂), 60.1 68.1 (C), 137.8 123.4 (CH), 139.0 139.7 (CH), 139.5 140.0 (CH), 161.3 173.2 (C=O), 182.3 180.4 (C=O, carboxyl group)
4b	1.90 1.56 (s, CH ₂), 2.50 1.68 (s, CH ₂), 2.87 2.64 (s, CH ₂), 7.59-8.54 8.24-9.66 (m, 6H, naphthalene core), 12.50 7.09 (s, OH)	28.7 22.9 (CH ₂), 39.5 24.7 (CH ₂), 39.8 29.3 (CH ₂), 54.5 62.9 (C), 129.6 123.4 (CH), 137.8 139.6 (CH), 139.2 140.2 (CH), 161.2 173.4 (C=O), 181.5 180.1 (C=O, carboxyl group)
4c	1.17 1.02 (s, CH ₃), 1.54 1.50 (s, CH ₂), 1.68 1.78 (s, CH ₂), 2.51 2.23 (s, CH ₂), 2.87 2.60 (s, CH), 7.59-8.55 8.26-9.25 (m, 6H, naphthalene core), 10.61 6.42 (s, OH)	15.8 18.9 (CH ₃), 21.1 23.5 (CH ₂), 25.5 25.3 (CH ₂), 29.6 28.6 (CH ₂), 34.7 33.0 (CH ₂), 66.4 63.6 (C), 128.2 123.4 (CH), 133.1 140.3 (CH), 135.9 140.4 (CH), 161.2 173.4 (C=O), 178.9 177.8 (C=O, carboxyl group)
4d	1.88 1.13 (s, CH ₃), 2.48 1.46 (s, CH ₂), 2.50 1.56 (s, CH), 2.88 2.63 (s, CH ₂), 7.60-8.55 8.25-9.22 (m, 6H, naphthalene core), 10.75 6.87 (s, OH)	22.4 19.1 (CH ₃), 38.6 26.9 (CH ₂), 39.5 25.6 (CH), 40.1 26.9 (CH ₂), 54.8 63.7 (C), 128.1 123.5 (CH), 133.0 139.2 (CH), 135.9 139.8 (CH), 161.2 173.2 (C=O), 180.2 179.3 (C=O, carboxyl group)
4e	0.84 1.03 (s, CH ₃ , Et), 0.94 1.15 (s, CH ₂ , Et), 1.90 1.64 (s, CH ₂), 2.50 1.30 (s, CH), 2.88 2.48 (s, CH ₂), 7.60-8.54 8.24-9.62 (m, 6H, naphthalene core), 10.89 6.80 (s, OH)	13.7 14.5 (CH ₃), 25.8 25.9 (CH ₂), 27.8 24.9 (CH ₂ , Et), 29.7 26.9 (CH ₂), 38.5 31.4 (CH), 67.5 64.5 (C), 128.2 123.3 (CH), 133.1 139.9 (CH), 135.9 140.0 (CH), 161.2 173.8 (C=O), 177.5 180.2 (C=O, carboxyl group)
4f	0.83 1.16 (s, CH ₃ , Pr), 1.16 0.97 (s, CH ₂ , Pr), 1.51 1.27 (s, CH ₂ , Pr), 1.60 1.20 (s, CH), 1.62 1.42 (s, CH ₂), 1.90 2.47 (s, CH ₂), 7.51-8.01 8.25-9.75 (m, 6H, naphthalene core), 11.1 6.77 (s, OH)	14.1 16.5 (CH ₃), 21.3 22.4 (CH ₂), 24.2 28.9 (CH ₂), 31.5 34.6 (CH), 37.7 37.6 (CH ₂), 62.2 64.2 (C), 128.5 123.4 (CH), 135.3 139.6 (CH), 137.8 140.0 (CH), 163.6 172.6 (C=O), 179.3 180.2 (C=O, carboxyl group)
4g	1.46 1.69 (m, 8H, CH ₂), 1.87 2.72 (m, 4H, CH ₂), 7.60-8.44 8.25-9.69 (m, 6H, naphthalene core), 10.50 6.98 (s, OH)	22.6 21.6 (CH ₂), 29.6 23.0 (CH ₂), 35.8 31.2 (CH ₂), 61.2 67.2 (C), 128.1 123.4 (CH), 133.0 139.9 (CH), 135.9 140.2 (CH), 161.2 173.8 (C=O), 178.5 180.0 (C=O, carboxyl group)
4h	1.47 1.55 (m, 10H, CH ₂), 1.90 2.65 (m, 4H, CH ₂), 7.62-8.45 8.24-9.70 (m, 6H, naphthalene core), 10.48 6.53 (s, OH)	21.2 23.5 (CH ₂), 28.5 27.6 (CH ₂), 32.7 30.0 (CH ₂), 61.3 66.3 (C), 128.1 123.3 (CH), 133.0 139.9 (CH), 135.9 140.2 (CH), 161.2 173.4 (C=O), 179.6 176.7 (C=O, carboxyl group)
4i	1.43 1.55 (m, 22H, CH ₂), 7.58-8.51 8.26-9.66 (m, 6H, naphthalene core), 10.53 7.10 (s, OH)	21.6 25.6 (CH ₂), 28.5 27.5 (CH ₂), 31.3 31.6 (CH ₂), 64.8 66.5 (C), 127.3 123.4 (CH), 133.6 140.2 (CH), 136.5 140.8 (CH), 162.4 173.4 (C=O), 178.8 173.9 (C=O, carboxyl group)
4j	1.90 1.76 (m, 10H, CH ₂), 2.49 2.28 (d, 2H, CH), 2.51 2.66 (d, 2H, CH), 7.60-8.47 8.24-9.65 (m, 6H, naphthalene core), 10.75 7.19 (s, OH)	28.1 26.5 (CH), 30.3 32.1 (CH), 32.2 31.9 (CH ₂), 38.1 35.5 (CH ₂), 66.4 69.0 (C), 128.1 123.6 (CH), 137.2 138.9 (CH), 139.5 139.5 (CH), 161.2 174.7 (C=O), 178.8 181.4 (C=O, carboxyl group)
4k	1.64 2.22 (s, CH ₂), 2.03 2.83 (s, CH ₂), 2.88 3.03 (s, CH ₂), 6.95-7.14 7.57-8.46 (m, 4H, tetrahydronaphthalene core), 7.58-8.52 8.18-9.65 (m, 6H, naphthalene core), 10.66 6.89 (s, OH)	27.2 22.9 (CH ₂), 32.5 28.9 (CH ₂), 36.7 30.8 (CH ₂), 59.9 60.6 (C), 119.5 123.4 (CH), 128.1 127.4 (CH), 128.9 130.1 (CH), 137.4 139.4 (CH), 138.6 140.3 (CH), 161.2 172.8 (C=O), 180.4 178.8 (C=O, carboxyl group)

*These assignments are confirmed by the DEPT-135 spectral data.

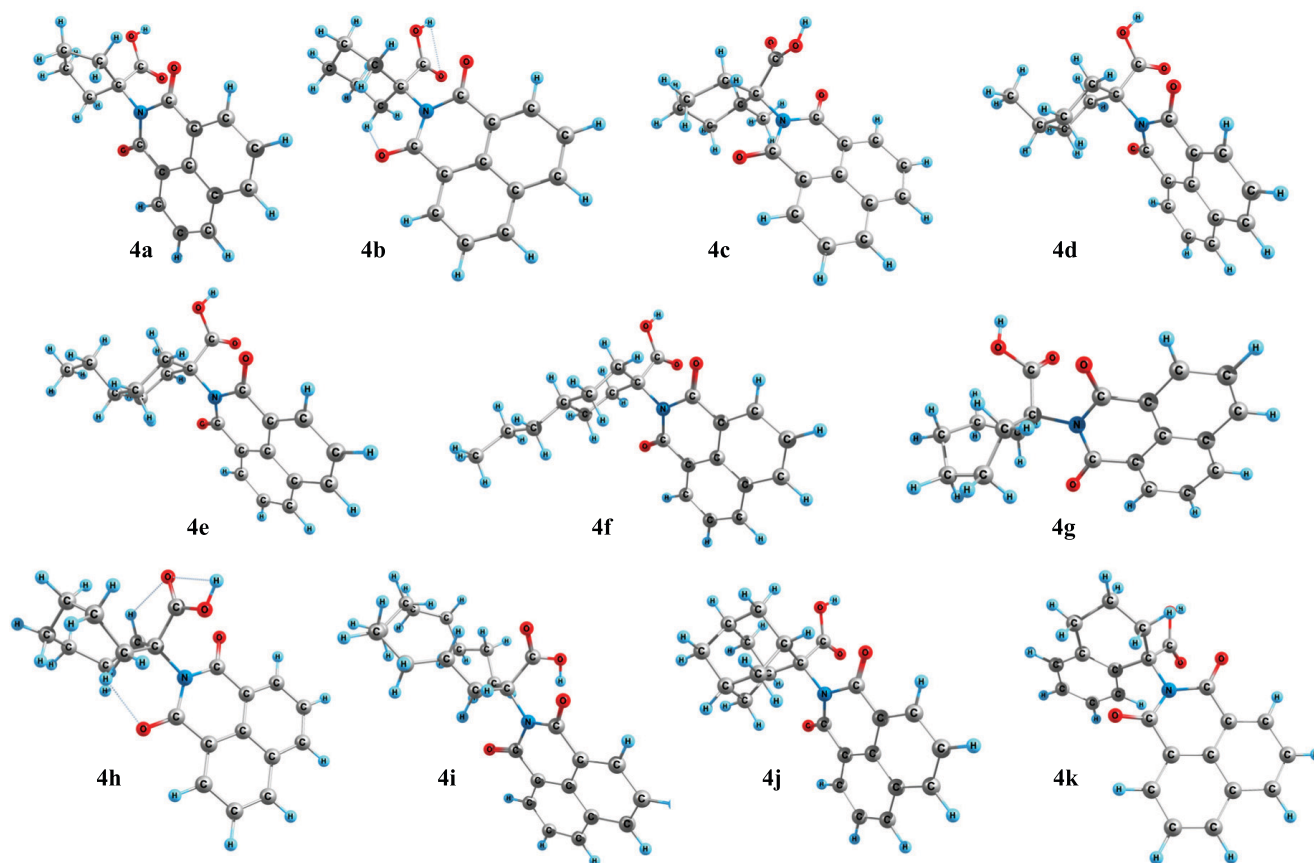


Fig. (2). B3LYP/6-31G(d,p) optimized structures of compounds 4a-4k.

have been applied for NMR calculations in order to confirm that HF results are the closest to the experimental data. MP2 and DFT did not reproduce accurately the chemical shifts of the carboximide carbon atom [53], the deviation from the experiment being about 20 ppm. Therefore, we have presented only the HF results in GIAO NMR calculations in DMSO with 6-31+G(2d,p) basis set because of the sensitivity of ^{13}C NMR chemical shifts to the presence of polarization and diffuse functions in the basis set [54, 55]. ^1H and ^{13}C NMR spectra of the compounds studied have been computed in solution by using the self-consistent reaction field method with the Polarizable Continuum Model (PCM) formalism. There is a good alignment between experimental and theoretical data. After consideration of the ^{13}C spectra of the newly synthesized compounds (Table 2), it can be seen that the type of substituent at the carboximide carbon atom affects the chemical shifts only of this atom. It can be seen from Table 2 that chemical shifts of carboximide carbon atom are 5-9 ppm downfield, both in experimental and in theoretically predicted spectra when this atom is part of a cycloalkane ring (4b, 4d and 4k). From theoretical ^1H NMR spectra of compounds 4a-4k (Table 2), it can be concluded that experimental isotropic chemical shifts for all molecules in DMSO were in excellent agreement with the predicted values, especially for CH_3 - and CH_2 -protons. Small deviations were observed in the calculated chemical shifts for the CH-protons from the naphthalimide moiety compared to the experimental ones (0.32-1.20 ppm). However, the difference between the theoretical and experimental results for chemical shifts of the proton from the OH-group is significant. The deviations are in the range of 3.43-6.86 ppm. The isotropic chemical shift of the proton of OH-group in 4i (7.10 ppm) calculated at HF/6-31+G(2d,p) level is closest to the experimental data (10.53 ppm), while for the chemical shift of OH-proton for 4a (6.94 ppm) there is a greatest deviation to the experiment (13.80 ppm). As in IR spectra, the de-

viations between experimental and theoretical results can be addressed to the formation of a hydrogen bond in the carboxyl group during the geometric optimization of all compounds.

The cytotoxic effects of the tested compounds were assessed against two human tumor cell lines, namely K-562 (chronic myeloid leukemia) and HUT-78 (cutaneous T-cell lymphoma) after 72h exposure, using the MTT-dye reduction assay (Table 3). All

Table 3. Cytotoxic effects of the tested series as assessed by the MTT-dye reduction assay.

Compound	IC ₅₀ (μM)	
	K-562	HUT-78
4a	24.81 ± 2.12	34.18 ± 3.32
4b	22.39 ± 2.73	29.55 ± 2.11
4c	24.48 ± 1.51	49.32 ± 3.10
4d	24.28 ± 3.62	36.82 ± 2.71
4e	41.78 ± 4.23	34.12 ± 1.97
4f	39.00 ± 2.02	33.69 ± 3.02
4g	55.45 ± 3.75	48.90 ± 3.43
4h	46.46 ± 2.92	35.84 ± 2.17
4i	49.58 ± 4.65	36.65 ± 2.65
4j	45.70 ± 4.02	31.43 ± 1.98
4k	78.11 ± 5.07	50.22 ± 4.75
Melphalan	17.21 ± 2.22	21.27 ± 1.04

^aEach data point is representative for a set of 8 independent experiments.

Table 4. Apoptotic DNA fragmentation following treatment with the tested compounds for 24 h as assessed by “Cell death detection ELISA”™ kit.

Treatment Series	Enrichment Factor
Untreated control	1.0 ± 0.1
4b (1/2 IC ₅₀)	2.3 ± 0.4*
4b (IC ₅₀)	2.9 ± 0.3*
melphalan (1/2 IC ₅₀)	3.2 ± 0.1*
melphalan (IC ₅₀)	3.7 ± 0.2*

*p ≤ 0.05 vs. the untreated control (paired Student's t-test).

Table 5. Modulation of NFκB signaling after treatment with **4b** for 24h as assessed by NFκB p65 ELISA kit.

Treatment Series	P65 Levels (%)
Untreated control	100.0 ± 21.1
4b (1/2 IC ₅₀)	63.4 ± 5.9*
4b (IC ₅₀)	17.6 ± 2.8*
Positive control [§]	9.4 ± 0.7*

*p ≤ 0.05 vs. the untreated control (paired Student's t-test); [§]Curcumin (25μM).

compounds inhibited the growth of malignant cells at micromolar concentrations where by compound **4b** demonstrated superior activity in both cell lines with IC₅₀ values comparable to those of the reference anticancer drug melphalan. The half-maximal inhibitory concentrations of the other compounds where with few exceptions lower than 50μM. Compound **4k** was the least potent cytotoxic agent of the series.

In order to elucidate the underlying mechanism of action, we sought to determine the ability of **4b** to trigger apoptotic cell death, using a commercially available “Cell death detection ELISA”™ kit. As evident from the data summarized in Table 4, the tested compound caused concentration-dependent increase in the content of histone-associated mono- and oligonucleosomal DNA fragments in K-562, which indicates that the induction of apoptotic cell death is implicated in the mechanism of cytotoxicity of the tested series of compounds.

The aforementioned compound was further assessed for its ability to modulate the NFκB-signaling using a commercially available ELISA kit for quantification of p65 - the active form of this transcription factor. As evident from the results summarized in Table 5 **4b** significantly decreased the levels of p65. These findings suggest that the inhibition of NFκB-dependent signaling pathways is at least partly involved in the cytotoxicity mode of action of the tested series.

CONCLUSION

New 1,8-naphthalimide derivatives with non-protein amino acids were successfully synthesized. The structures of the compounds obtained were proven by physicochemical parameters, IR, ¹H, ¹³C NMR spectroscopy and quantum-chemical calculations. A proper alignment between theoretical and experimental spectral data was established.

The cytotoxicity of the synthesized products against two human tumor cell lines, namely K-562 (chronic myeloid leukemia) and HUT-78 (cutaneous T-cell lymphoma) was evaluated. In our hands, the tested series inhibited the growth of malignant cells at micromolar concentrations. 1-(1,3-Dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)cyclohexane-1-carboxylic acid demonstrated superior activity in both cell lines. The mechanistic elucidation of this compound dem-

onstrated its ability to trigger programmed cell death through apoptosis and to modulate NFκB signaling.

LIST OF ABBREVIATIONS

DFT	=	Density Functional Theory
DMF	=	<i>N,N</i> -Dimethylformamide
DMSO	=	Dimethylsulfoxide
Et	=	Ethyl
GIAO	=	Gauge-Independent Atomic Orbital
HF	=	Hartree-Fock
MTT	=	3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide
Pr	=	Propyl
TMS	=	Tetramethylsilane

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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