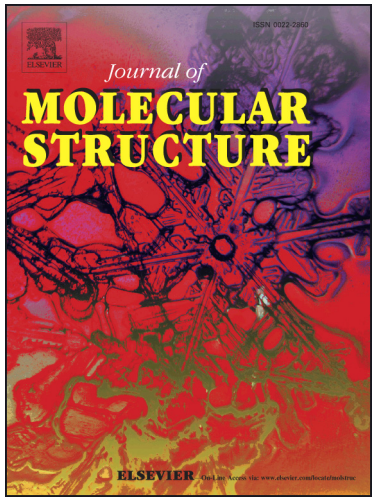

Synthesis, structural, conformational and dft studies of *n*-3 and *o*-4 alkylated regioisomers of 5-(hydroxypropyl)pyrimidine

Mirsada Salihović, Amar Osmanović, Selma Špirtović-Halilović, Sunčica Roca, Andrijana Meš čić, Ljubodrag Vujisić, Snežana Trifunović, Davorka Završnik, Emin Sofić

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SYNTHESIS, STRUCTURAL, CONFORMATIONAL AND DFT STUDIES OF *N*-3 AND *O*-4 ALKYLATED REGIOISOMERS OF 5-(HYDROXYPROPYL)PYRIMIDINE

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ABSTRACT

Because of the great pharmacological potential of the pyrimidine motif, novel C-5 substituted *N*-3 acyclic and *O*-4 acyclic pyrimidine derivatives were prepared as an interesting class of compounds for biological evaluation. Introduction of the 2,3-dihydroxypropyl (DHP) and penciclovir (PCV)-like side chains to 2-methoxypyrimidin-4-one (**2**) afforded a mixture of *N*- and *O*-acyclic pyrimidine nucleosides in the ratio of 54 : 29 (**3** : **4**) and 57 : 21 (**5** : **6**) with *N*-3 isomer being dominant. Distinction between *N*- and *O*- alkylated pyrimidine moiety was deduced from extensive experimental FT-IR, HPLC-MS and 1D (^{1}H, ^{13}C) and 2D (COSY, HMQC and HMBC) NMR analyses. The *N*-, *O*- regioisomers were also examined by computational method at density functional theory (DFT) RB3LYP/6-31G(d), 6-31G** and 6-31+G* levels. DFT global chemical reactivity descriptors (total energy, chemical hardness, electronic chemical potential and electrophilicity) were calculated for the isomers and used to predict and describe their relative stability and reactivity. The chemical reactivity indices were related to the C_2-N_3-C_4 bond angle. Theoretical predictions can be used to compare chemical reactivity and stability with future biological evaluation and behavior of these compounds.

Keywords: *N*-, *O*-acyclic pyrimidine nucleosides, 2,3-dihydroxypropyl (DHP), 4-hydroxy-(3-hydroxymethyl)butyl (PCV), NMR, FT-IR, DFT calculation.

1. INTRODUCTION

The development of new chemotherapeutic agents is becoming the major interest in many academic and industrial research laboratories all over the world with the aim to discover newer, more potent molecules with higher selectivity and reduced toxicity than the existing ones. Large arrays of uracil non-nucleoside derivatives possess variety of chemotherapeutic properties [1] including anticancer [2–6], antiviral [7–15] and antimicrobial activities [16]. Moreover, many purine and pyrimidine nucleoside analogues have been the object of intensive chemical and pharmacological investigation due to their potential anti-HIV activity [17]. Antiviral nucleoside analogues are known to localize selectively in *Herpes simplex* virus (HSV) infected cells because of monophosphorylation catalysed by virus-encoded thymidine kinase (TK). Among these, acyclovir, ganciclovir and penciclovir are reported to be potent against HSV types 1 and 2 [18].

Having in mind the significant pharmacological potential of the cited class of compounds, new synthetic pathways for C-5 substituted pyrimidines bearing an acyclic side chain have been developed, leading to novel nucleoside mimetics. We report here the synthesis of four novel C-5 hydroxypropyl *N*-acyclic and *O*-acyclic pyrimidine nucleosides (**3**, **4**, **7** and **8**) and their structural characterization. Their structure and stereochemistry were deduced from experimental FT-IR, HPLC-MS and 1D (^{1}H, ^{13}C) and 2D (COSY, HMQC and HMBC) NMR analyses and theoretical methodology based on global chemical reactivity indices calculated using the RB3LYP/6-31G(d), 6-31G** and 6-31+G* levels of theory.

Considering that these molecules will be used for biological evaluation, as potential new antiproliferative and antiviral agents, it is important to make theoretical studies of reactivity descriptors that could help in understanding their chemical behaviour [19]. In the future, these calculated parameters could be useful in understanding and predicting the behaviour of structurally similar molecules of unknown reactivity.

2. EXPERIMENTAL

2.1. General

Melting points (uncorrected) were determined with BŰCHI Melting Point B-545. Precoated silica gel 60F-254 plates were used for thin layer chromatography and the spots were detected under UV light (254 nm). Column chromatography was performed using silica gel (0.063–0.2 mm); glass column was slurry-packed under gravity.

NMR spectra were recorded on Bruker Avance 600 spectrometer, operating at 600.130 MHz for the ^{1}H nucleus and 150.903 MHz for the ^{13}C nucleus. Samples were measured from DMSO-d_6 solutions at 303 K in 5 mm NMR tubes and processed with the TopSpin NMR software. Chemical shifts, in ppm, are referred to tetramethylsilane (TMS) as internal standard. Proton spectra with spectral with of 12019 Hz and a digital resolution of 0.37 Hz per point were measured with 16-48 scans. Transmitter frequency offset was set at 5401 Hz. ^{13}C APT spectra with spectral widths of 39370 Hz and a digital resolution of 0.60 Hz per point,

respectively, were collected with 860-11000 scans. Carbon transmitter frequency offset was set at 15089 Hz.

Assignment of ^{1}H and ^{13}C NMR signals was performed using gradient-selected two-dimensional homo- and heteronuclear correlation experiments (COSY, HMQC and HMBC). The COSY with standard $\pi/2$ pulse sequence was measured using 2048 points in F2 dimension and 512 increments in F1 dimension. The latter was subsequently zero-filled to 1024 points. Increments were obtained by 4 scans each, 12019 Hz spectral width and a relaxation delay of 1.0 s. Digital resolution was 5.87 and 23.44 Hz per point in F2 and F1 dimensions, respectively. Applied acquisition time was 0.085 s. The HMQC spectra ($^{1}J_{C,H}$ was set to 145 Hz) were recorded with 2048 points in F2 dimension and 187 increments in F1 dimension, subsequently zero-filled to 1024 points. For each increment 16 scans were collected, using relaxation delay of 1.3 s. The spectral widths were 9615 Hz (F2) and 36216 Hz (F1), with corresponding resolution of 4.69 and 193.48 Hz per point in F2 and F1 dimensions, respectively. Applied acquisition time was 0.106 s. The HMBC spectra were measured with 2048 points and 12019 Hz spectral width in F2 dimension and relaxation delay of 1.0 s. The additional delay of 0.065 s was used for detecting the long-range C-H couplings. The spectral width in F1 dimension was 36216 Hz, while 243 increments were recorded, each by 48 scans. The FID resolution was 5.87 and 149.05 Hz per point in F2 and F1 dimensions, respectively. Applied acquisition time was 0.085 s. The 2D NMR spectra were measured in pulsed field gradient mode (*z*-gradient).

Infrared absorption spectra were recorded using KBr pellets with an ABB Bomem FT model MB102 spectrometer, in the 4000–400 cm^{-1} region. Elemental analyses were performed on a Perkin–Elmer, series II, CHNS analyser 2400. Mass spectra were carried out with an Agilent 6410 instrument equipped with electrospray interface and triple quadrupole analyser (LC/MS/MS). High performance liquid chromatography was performed on an Agilent 1100 series system with UV detection (photodiode array detector) using Zorbax C18 reverse-phase analytical column (2.1 x 30 mm, 3.5 μm). All compounds showed >95% purity in this HPLC system.

2.2. Procedures for the preparation of compounds

The starting compound **1** was synthesized according to the known procedure given in the literature [20].

*2.2.1. 5-(3-Hydroxypropyl)-2-methoxypyrimidin-4-one (**2**)*

The reaction mixture of compound **1** (1.0 g, 6.02 mmol) in 1M NaOH (90 ml) was stirred at room temperature for 24 h and then neutralized with HCl. After evaporation to dryness and column chromatography (CH_2Cl_2 : CH_3OH = 5 : 1), white crystals of compound **2** were obtained (643.0 mg, 58%, m.p.=136°C).

2: IR: (ν) 3447, 1669, 1318, 1055 cm^{-1}. ^{1}H NMR: (δ) 12.28 (1H, br. s., NH), 7.56 (1H, s, H-6), 4.43 (1H, t, OH, J = 5.4 Hz), 3.84 (3H, s, OCH_3), 3.39 (2H, m, H-9), 2.28 (2H, t, H-7, J = 7.5 Hz), 1.60 (2H, m, H-8) ppm. ^{13}C NMR: (δ) 161.7 (C-4), 157.1 (C-2), 155.96 (C-6), 122.8 (C-5), 60.6 (C-9), 55.0 (OCH_3), 31.8 (C-8), 23.5 (C-7) ppm. MS (ESI): *m/z* = 185.1 ([M

+ H]$^+$). Anal. Calcd. (%) for $C_8H_{12}N_2O_3$: C, 52.17; H, 6.57; N, 15.21. Found: C, 52.14; H, 6.59; N, 14.99.

*2.2.2. N-3-(2,3-Dihydroxypropyl)-5-(3-hydroxypropyl)-2-methoxypyrimidin-4-one (**3**) and 4-(2,3-dihydroxypropoxy)-5-(3-hydroxypropyl)-2-methoxypyrimidine (**4**)*

To a stirred solution of compound **2** (200.0 mg, 1.09 mmol) and K_2CO_3 (225.1 mg, 1.63 mmol) in anhydrous DMF (6 ml), 3-chloro-1,2-propanediol (0.35 ml, 4.19 mmol) was added. The reaction mixture was stirred at 60°C overnight and then evaporated to dryness. After column chromatography (EtOAc : CH_3OH = 5 : 1) compounds **3** (151.7 mg, 54%) and **4** (81.2 mg, 29%) were isolated as colourless oils.

3: IR: (ν) 3406, 1662, 1292, 1051 cm^{-1}. ^{1}H NMR: (δ) 7.56 (1H, s, H-6), 4.71 (1H, d, OH-11, J = 5.7 Hz), 4.55 (1H, t, OH-12, J = 5.1 Hz), 4.38 (1H, t, OH-9, J = 4.9 Hz), 3.97 (1H, dd, H-10, J_1 = 13.1 Hz, J_2 = 8.1 Hz), 3.90 (4H, m, OCH_3 and H-10'), 3.76 (1H, m, H-11), 3.40 (2H, m, H-9), 3.35 (2H, m, H-12), 2.32 (2H, t, H-7, J = 7.4 Hz), 1.62 (2H, m, H-8) ppm. ^{13}C NMR: (δ) 162.3 (C-4), 155.8 (C-2), 148.0 (C-6), 119.6 (C-5), 68.5 (C-11), 64.2 (C-12), 60.1 (C-9), 55.3 (OCH_3), 44.3 (C-10), 31.2 (C-8), 23.6 (C-7) ppm. MS (ESI): *m/z* = 259.2 ([M + H]$^+$). Anal. Calcd. (%) for $C_{11}H_{18}N_2O_5$: C, 51.15; H, 7.02; N, 10.85. Found: C, 51.2; H, 7.09; N, 11.0.

4: IR: (ν) 3420, 1305, 1037 cm^{-1}. ^{1}H NMR: (δ) 7.36 (1H, s, H-6), 5.05 (1H, d, OH-11, J = 5.8 Hz), 4.78 (1H, t, OH-12, J = 5.4 Hz), 4.43 (1H, t, OH-9, J = 5.4 Hz), 3.97 (1H, dd, H-10, J_1 = 13.8 Hz, J_2 = 3.6 Hz), 3.85 (3H, s, OCH_3), 3.69 (1H, m, H-11), 3.56 (1H, dd, H-10', J_1 = 13.9 Hz, J_2 = 8.7 Hz), 3.38 (4H, m, H-9 and H-12), 2.23 (2H, t, H-7, J = 7.4 Hz), 1.58 (2H, m, H-8) ppm. ^{13}C NMR: (δ) 170.2 (C-4), 155.7 (C-2), 140.6 (C-6), 118.6 (C-5), 69.3 (C-11), 63.5 (C-12), 60.1 (C-9), 54.9 (OCH_3), 52.5 (C-10), 30.9 (C-8), 23.5 (C-7) ppm. MS (ESI): *m/z* = 259.2 ([M + H]$^+$). Anal. Calcd. (%) for $C_{11}H_{18}N_2O_5$: C, 51.15; H, 7.02; N, 10.85. Found: C, 51.19; H, 7.09; N, 10.9.

*2.2.3. N-3-[4-Acetoxy-(3-acetoxymethyl)butyl]-5-(3-hydroxypropyl)-2-methoxypyrimidin-4-one (**5**) and 4-[4-acetoxy-(3-acetoxymethyl)butoxy]-5-(3-hydroxypropyl)-2-methoxypyrimidine (**6**)*

To a stirred solution of compound **2** (453.0 mg, 2.46 mmol) and K_2CO_3 (509.9 mg, 3.68 mmol) in anhydrous DMF (20 ml), 4-acetoxy-(3-acetoxymethyl)butyl iodide (910.0 mg, 2.88 mmol) was added. The reaction mixture was stirred at 60°C for 1 h and then evaporated to dryness. After column chromatography (CH_2Cl_2 : CH_3OH = 50 : 1) compounds **5** (518.2 mg, 57%) and **6** (198 mg, 21%) were isolated as yellowish oils.

5: IR: (ν) 3405, 1754, 1268, 1250, 1179 cm^{-1}. ^{1}H NMR: (δ) 7.56 (1H, s, H-6), 4.40 (1H, t, OH-9, J = 5.1 Hz), 4.36 (2H, t, OH-13 and OH-13', J = 5.1 Hz), 3.95 (2H, t, H-10, J = 7.5 Hz), 3.92 (3H, s, OCH_3), 3.40 (2H, m, H-9), 3.37 (4H, m, H-13 and H-13'), 2.32 (2H, t, H-7, J = 7.6 Hz), 1.60 (2H, m, H-8), 1.50 (3H, m, H-11 and H-12) ppm. ^{13}C NMR: (δ) 161.9 (C-4), 155.4 (C-2), 148.0 (C-6), 119.7 (C-5), 61.5 (C-13 and C-14), 60.2 (C-9), 55.5 (OCH_3), 41.4 (C-12), 39.4 (C-10), 31.2 (C-8), 26.8 (C-11), 23.6 (C-7) ppm. MS (ESI): *m/z* = 371 ([M +

H]$^+$). Anal. Calcd. (%) for $C_{17}H_{26}N_2O_7$: C, 55.13; H, 7.08; N, 7.56. Found: C, 55.2; H, 7.1; N, 7.9.

6: IR: (ν) 3405, 1301, 1235, 1230 cm^{-1}. ^{1}H NMR: (δ) 8.07 (1H, s, H-6), 4.46 (1H, t, OH-9, J = 5.1 Hz), 4.41 (2H, t, OH-13 and OH-13', J = 5.1 Hz), 4.39 (2H, t, H-10, J = 6.9 Hz), 3.84 (3H, s, OCH_3), 3.43 (4H, m, H-13 and H-13'), 3.39 (2H, m, H-9), 2.44 (2H, t, H-7, J = 7.6 Hz), 1.71 (2H, m, H-11), 1.64 (3H, m, H-8 and H-12) ppm. ^{13}C NMR: (δ) 168.5 (C-4), 163.5 (C-2), 157.0 (C-6), 114.6 (C-5), 64.8 (C-10), 61.5 (C-13 and C-14), 60.0 (C-9), 54.1 (OCH_3), 40.3 (C-12), 31.6 (C-8), 27.3 (C-11), 22.5 (C-7) ppm. MS (ESI): m/z = 371 ([M + H]$^+$). Anal. Calcd. (%) for $C_{17}H_{26}N_2O_7$: C, 55.13; H, 7.08; N, 7.56. Found: C, 55.19; H, 7.09; N, 7.6.

2.2.4. N-3-[4-Hydroxy-(3-hydroxymethyl)butyl)-5-(3-hydroxypropyl)-2-methoxypyrimidin-4-one (7)

Compound **5** (450.0 mg, 1.57 mmol) was dissolved in methanol (15 ml) and 0.1M $NaOCH_3/CH_3OH$ (3 ml) was added to the solution. The reaction mixture was stirred at room temperature for 1 h and then neutralized with HCl. After evaporation to dryness and column chromatography (CH_2Cl_2 : CH_3OH = 25 : 1) compound **7** was isolated (318.6 mg, 92%).

7: IR: (ν) 3421, 1664, 1283, 1200 cm^{-1}. ^{1}H NMR: (δ) 7.56 (1H, s, H-6), 4.40 (1H, t, OH-9, J = 5.1 Hz), 4.36 (2H, t, OH-13 and OH-13', J = 5.1 Hz), 3.95 (2H, t, H-10, J = 7.5 Hz), 3.92 (3H, s, OCH_3), 3.40 (2H, m, H-9), 3.37 (4H, m, H-13 and H-13'), 2.32 (2H, t, H-7, J = 7.6 Hz), 1.60 (2H, m, H-8), 1.50 (3H, m, H-11 and H-12) ppm. ^{13}C NMR: (δ) 161.9 (C-4), 155.4 (C-2), 148.0 (C-6), 119.7 (C-5), 61.5 (C-13 and C-14), 60.2 (C-9), 55.5 (OCH_3), 41.4 (C-12), 39.4 (C-10), 31.2 (C-8), 26.8 (C-11), 23.6 (C-7) ppm. MS (ESI): m/z = 286.16 ([M + H]$^+$). Anal. Calcd. (%) for $C_{13}H_{22}N_2O_5$: C, 54.53; H, 7.74; N, 9.78. Found: C, 54.19; H, 7.79; N, 9.61.

*2.2.5. 4-[4-Hydroxy-(3-hydroxymethyl)butoxy]-5-(3-hydroxypropyl)-2-methoxypyrimidine (**8**)*

Compound **6** (100.0 mg, 0.27 mmol) was dissolved in methanol (3.5 ml) and 0.1M $NaOCH_3/CH_3OH$ (0.7 ml) was added to the solution. The reaction mixture was stirred at room temperature for 1 h and then neutralized with HCl. After evaporation to dryness and column chromatography (CH_2Cl_2 : CH_3OH = 10 : 1) compound **8** was isolated (23.3 mg, 30%).

8: IR: (ν) 3366, 1311, 1089 cm^{-1}. ^{1}H NMR: (δ) 8.07 (1H, s, H-6), 4.46 (1H, t, OH-9, J = 5.1 Hz), 4.41 (2H, t, OH-13 and OH-13', J = 5.1 Hz), 4.39 (2H, t, H-10, J = 6.9 Hz), 3.84 (3H, s, OCH_3), 3.43 (4H, m, H-13 and H-13'), 3.39 (2H, m, H-9), 2.44 (2H, t, H-7, J = 7.6 Hz), 1.71 (2H, m, H-11), 1.64 (3H, m, H-8 and H-12) ppm. ^{13}C NMR: (δ) 168.5 (C-4), 163.5 (C-2), 157.0 (C-6), 114.6 (C-5), 64.8 (C-10), 61.5 (C-13 and C-14), 60.0 (C-9), 54.1 (OCH_3), 40.3 (C-12), 31.6 (C-8), 27.3 (C-11), 22.5 (C-7) ppm. MS (ESI): m/z = 286.16 ([M + H]$^+$). Anal. Calcd. (%) for $C_{13}H_{22}N_2O_5$: C, 54.53; H, 7.74; N, 9.78. Found: C, 54.13; H, 7.68; N, 9.84.

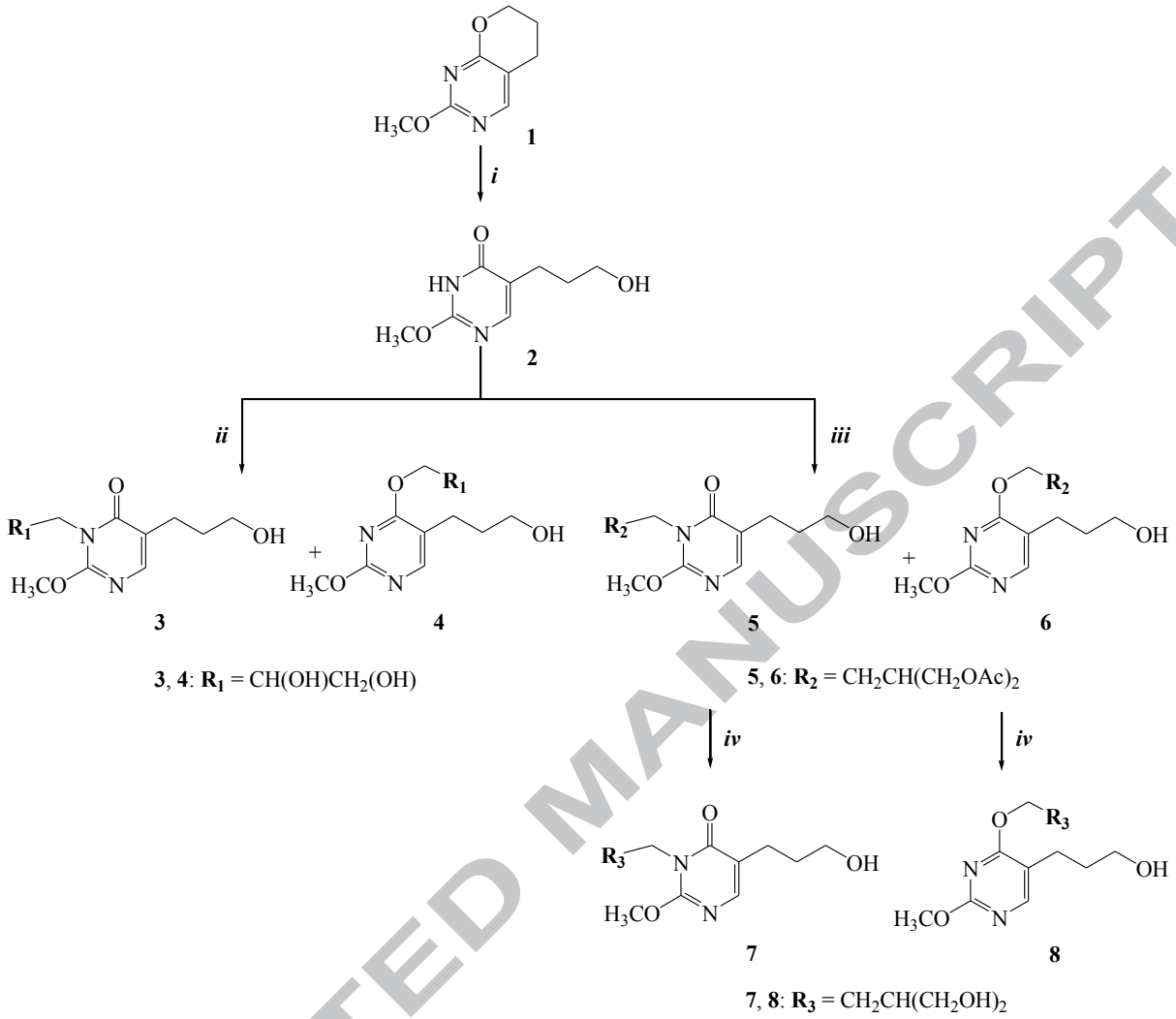
2.3. DFT methodology

The studied compounds **3**, **4**, **7** and **8** were subjected to geometry optimization using the density functional theory (DFT) with the Becke three-parameter exchange functional (B3) [21] and the Lee–Yang–Parr (LYP) correlation functional [22]. To help rationalize the experimental findings, a set of calculations were carried out for alkylated 5-(hydroxypropyl)pyrimidines. The most stable isomers **3**, **4**, **7** and **8** were obtained using the semi-empirical PM3 method [23] in the Spartan 10 mechanics program. The geometry of those regioisomers was then reoptimized using the method: RB3LYP basis sets: 6-31G(d), 6-31G** and 6-31+G*. Each optimized geometry was an isomer as a local minimum on the potential energy surface.

3. RESULTS AND DISCUSSION

3.1. Chemistry

The synthesis of 5-substituted pyrimidine acyclic nucleosides **3**, **4**, **7** and **8** was performed as outlined in Scheme 1. The 2-methoxy-6,7-dihydro-5*H*-pyrano[2,3-*d*]pyrimidine (**1**) served as a starting compound and its synthesis was reported earlier [20]. The synthesis of 5-(3-hydroxypropyl)-2-methoxypyrimidin-4-one (**2**) as the precursor for *N*- and *O*-alkylations, was performed in a relatively good yield (58%) *via* base promoted ring opening reaction of the bicyclic compound **1** with 1M sodium hydroxide at room temperature. Further synthetic steps included the introduction of the corresponding acyclic DHP or PCV-like side chain at *N*-3 and *O*-4 positions of compound **2** using 3-chloro-1,2-propanediol and 4-acetoxy-(3-acetoxymethyl)butyl iodide as alkylating agents and potassium carbonate as a base. DHP-chain bearing pyrimidine nucleosides **3** and **4** were isolated in moderate yields (54% and 29%, respectively) in the first synthetic route and two precursors of PCV-chain bearing pyrimidine nucleosides **5** and **6** with acetylated groups were isolated in similar yields (57% and 21%, respectively) following the second synthetic route. The target *N*-alkylated and *O*-alkylated 5-(3-hydroxypropyl)pyrimidines **7** and **8** were obtained in excellent yields (92% and 87%, respectively) after removal of the acetyl protecting groups using sodium methoxide in CH_3OH. Mass spectra showed the same molecular mass for compounds **3** and its structural congener **4**, as well as for the precursors **5** and its congener **6**, and later for the final derivatives **7** and **8**. Besides, NMR spectroscopic analysis revealed that compounds **4**, **6** and **8** contained the acyclic chain attached to the oxygen at C-4 instead of the *N*-3 position of the pyrimidine moiety as their corresponding *N*-3 alkylated regioisomers **3**, **5** and **7**.



Scheme 1. (i) 1M NaOH, rt, 24 h; (ii) 3-chloro-1,2-propanediol, K_2CO_3, DMF, 60°C, rt, 16 h; (iii) 4-acetoxy-(3-acetoxymethyl)butyl iodide, K_2CO_3, DMF, 60°C, rt, 1 h; (iv) 0.1M $NaOCH_3/CH_3OH$, rt, 1 h.

3.2. NMR and FT-IR analyses

Assignments of the ^1H and ^{13}C NMR signals of all the compounds were based on chemical shifts, signal intensities and multiplicity of resonances as well as on the spectra of gradient-selected 2D homo- and heteronuclear correlation experiments. ^1H-^1H COSY spectra enabled the assignment of ^1H resonances. The ^{13}C signals of the pyrimidine ring and substituted side chains were assigned using ^1H-^{13}C HMQC and ^1H-^{13}C HMBC spectra. All synthesized isomers showed very similar correlations in recorded 2D spectra. The most important correlations for determining the structures of the compounds are schematically shown in Fig. 1.

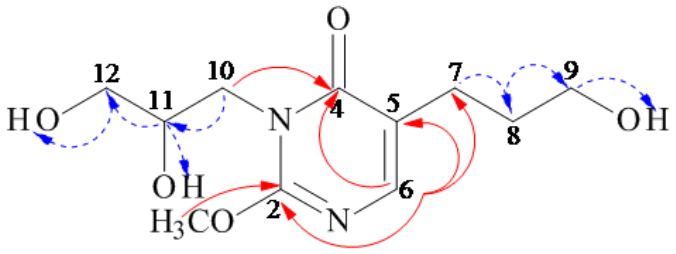


Fig. 1. The most important COSY (indicated with dash arrows) and HMBC (indicated with solid arrows) NMR correlations used for structure determination illustrated within compound **3.**

Cross peaks observed between all vicinal protons in COSY spectra (indicated with dash arrows in Fig. 1) made possible the assignment of all ^{1}H signals. In the HMBC spectrum of, i.e., compound **3** cross peaks were observed between δ_H 7.56 H-6 and δ_C 155.8 C-2, δ_C 162.3 C-4, δ_C 119.6 C-5 and δ_C 23.6 C-7, between δ_H 3.90 OCH_3 and δ_C 155.8 C-2 as well as between δ_H 3.97 H-10 and δ_C 162.3 C-4 (indicated with solid arrows in Fig. 1). Spin systems -CH_2(7)-CH_2(8)-CH_2OH(9) and -CH_2(10)-CHOH(11)-CH_2OH(12) were determined as well through HMBC experiments. HMBC spectra did not enable the identification of *N*- or *O*-alkyl regioisomers because the H10/C2 cross peak was not found while the H10/C4 was present in the HMBC spectra of all six synthesized isomers. This is shown in Fig. 2 where H10/C4 cross peaks are highlighted within dashed ovals. At the top edge of the HMBC plot complete ^{1}H NMR spectrum is shown and a portion of the ^{13}C NMR spectrum at the left hand edge.

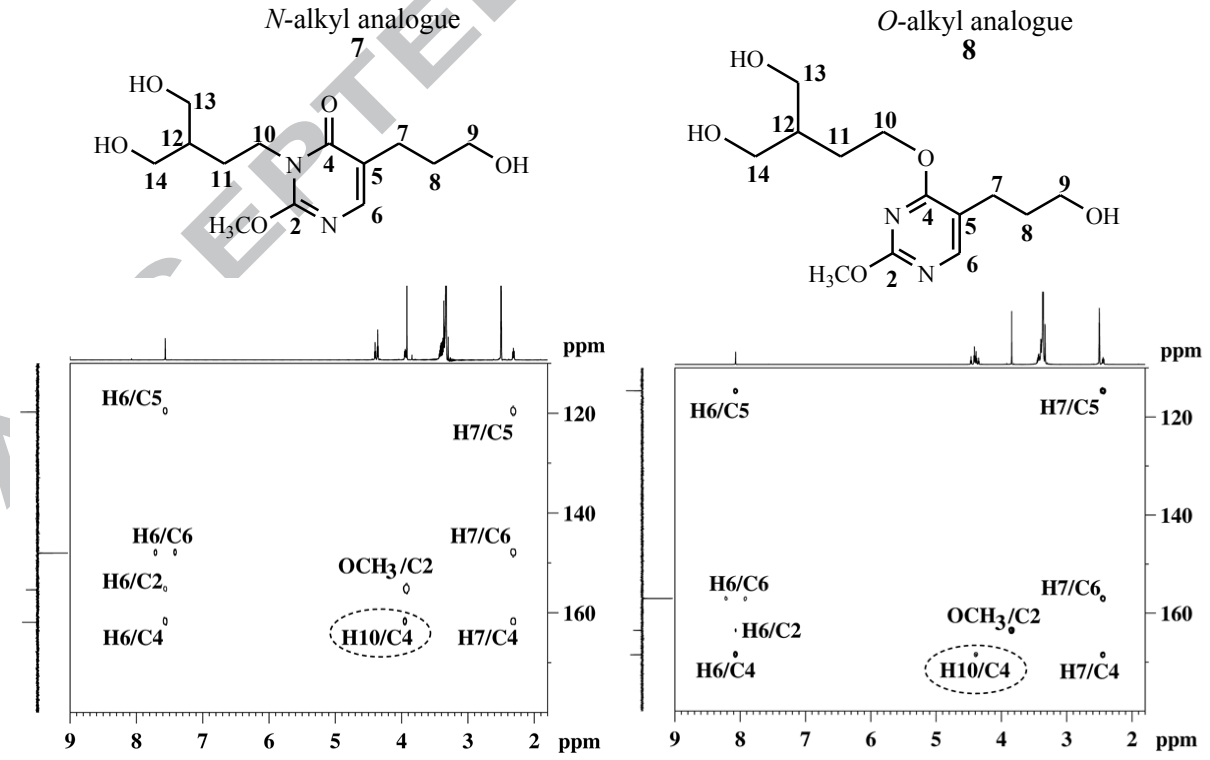


Fig. 2. Structures of *N*- (**7**) and *O*- (**8**) regioisomers with the atom-numbering scheme. ^{1}H-^{13}C HMBC spectra of *N*- and *O*-alkyl regioisomers in the same sub-region.

The chemical identities of the members of constitutional isomeric pairs **3**, **4**; **5**, **6** and **7**, **8** were confirmed from data obtained by APT NMR method.

O-acyclic pyrimidine nucleoside **4** exhibited around 8 ppm more deshielded C-4 and C-10 atoms and around 7 ppm more shielded C-6 atom than the corresponding atoms in *N*-alkylated regioisomer **3**. *O*-acyclic pyrimidine nucleosides **6** and **8** exhibited 25 ppm more deshielded C-10 atom as well as 6–9 ppm more deshielded C-2, C-4 and C-6 atoms than *N*-acyclic regioisomers. *N*-acyclic pyrimidine nucleosides **5** and **7** exhibited 5 ppm more deshielded C-5 atom than *O*-acyclic isomers. Distinctions in chemical shifts of ^{13}C signals between the isomeric pair **7**, **8** are presented in Fig. 3. C-10 resonances are left out because in *N*-alkyl analogue (**7**) spectra the signal is overlapped with the solvent and was thus assigned using ^{1}H-^{13}C HMQC spectra. These findings are in agreement with those of LaPlante et al. [24]

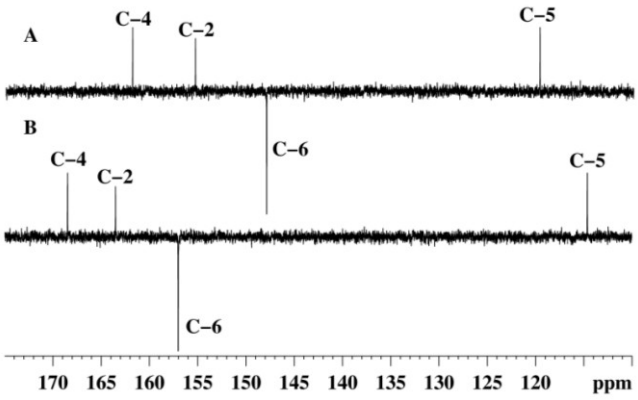


Fig. 3. Part of the ^{13}C NMR spectra of the *N*-alkyl isomer **7** (A) and the *O*-alkyl isomer **8** (B) in DMSO-d_6.

Distinctions among regioisomers were also characterized by FT-IR spectroscopy. Along with the evident bands for the common structure groups (pyrimidine moiety, hydroxyl, ether), a particularly important one was noticed. FT-IR spectrum of **3** showed a lactam's carbonyl C=O stretching band at 1662 cm^{-1}. The absence of this C=O stretching band in the spectrum of **4** confirmed the substitution at the oxygen atom. By the same analogy, the presence of C=O stretching bands on spectra of **5** and **7** (1754 cm^{-1} and 1664 cm^{-1}, respectively) and their absence on spectra of compounds **6** and **8** distinguished alkylating positions. The same stretching band of value 1669 cm^{-1} was evident on the spectrum of compound **2** as well.

3.3. Theoretical calculations

With the aid of models, the reactivity of the active sites of *N*-3 and *O*-4 alkylated pyrimidines can be elucidated by computation using global chemical reactivity indices. The chemical reactivity descriptors calculated using DFT were: total energy (E), chemical hardness (η), electronic chemical potential (μ), and electrophilicity (ω). Chemical hardness is associated with the stability and reactivity of a chemical system. In a molecule, it measures the resistance to change in the electron distribution or charge transfer. Based on frontier molecular orbitals, chemical hardness corresponds to the gap between the Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO) and can be approximated using Equation 1.

$$\eta = (\varepsilon_{LUMO} - \varepsilon_{HOMO})/2, \quad (1)$$

where ε_{LUMO} and ε_{HOMO} are the LUMO and HOMO energies. The larger the HOMO–LUMO energy gap, the harder and more stable/less reactive the molecule [25], Fig. 4.

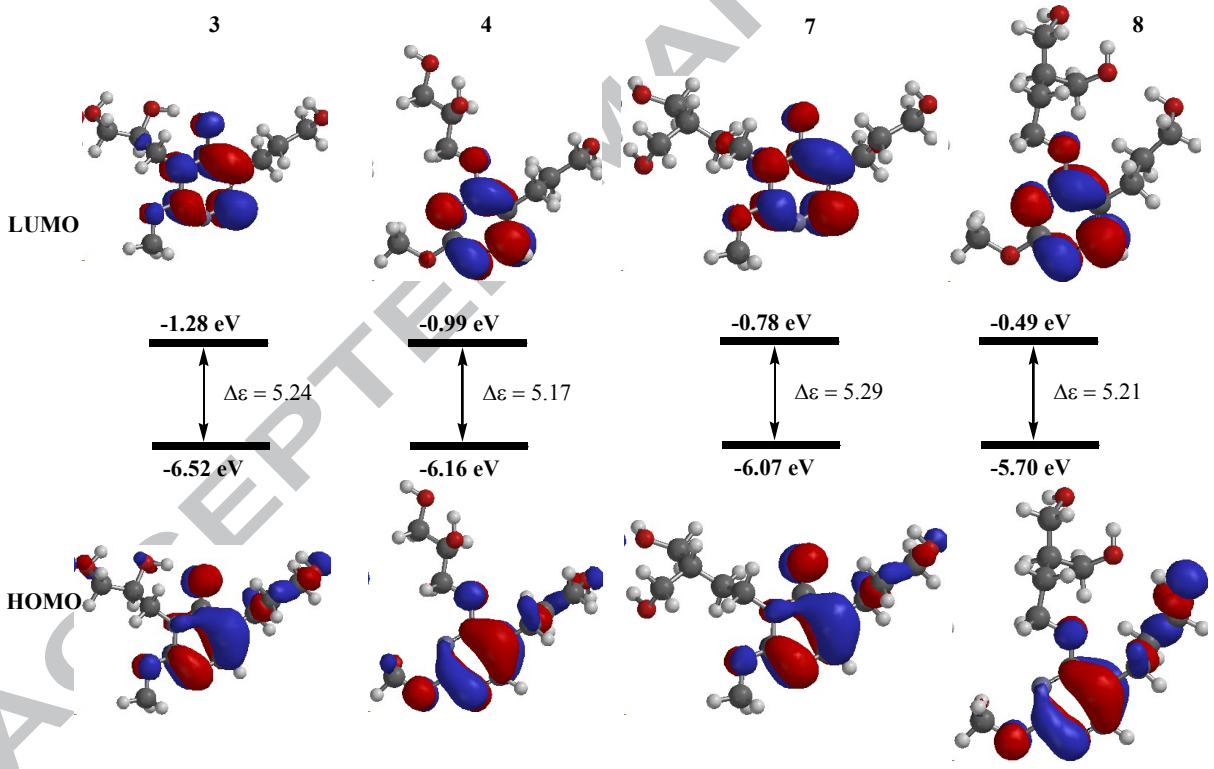


Fig. 4. Frontier molecular orbitals of isomers **3**, **4**, **7** and **8** at the 6-31G(d) level.

Tables 1, 2 and 3 (row 4) contain the computed chemical hardness values for compounds **3**, **4**, **7** and **8**. The results indicate that the isomer **3** is harder and therefore less reactive than the isomer **4** and isomer **7** is harder and less reactive than the isomer **8**.

Electronic chemical potential is defined as the negative of electronegativity of a molecule [25] and is determined using Equation 2.

$$\mu = (\varepsilon_{HOMO} + \varepsilon_{LUMO})/2, \quad (2)$$

Physically, μ describes the escaping tendency of electrons from an equilibrium system [25]. The values of electronic chemical potential for compounds **3**, **4** and **7**, **8** are presented in Tables 1, 2 and 3 (row 5). The trend in electronic chemical potential for the compounds are **4** > **3** and **8** > **7**. The greater the electronic chemical potential, the less stable or more reactive is the isomer. Therefore, we can conclude that the isomer **4** is more reactive than the isomer **3**, while the isomer **8** is more reactive than the isomer **7**.

Results of chemical hardness and electronic chemical potential of regioisomers showed that *N*-3 alkylated pyrimidines are harder and less reactive than *O*-4 alkylated derivatives. This is probably connected with a more stable C-N bond compared to a C-O bond in these molecules.

Computed energies (Tables 1, 2 and 3 (row 1)) at the DFT RB3LYP/6-31G(d), 6-31G** and 6-31+G* levels additionally confirm that compound **3** is more stable than **4** and that compound **7** is more stable than the compound **8**.

Global electrophilicity index (ω) is calculated using the electronic chemical potential and chemical hardness [25] as shown in Equation 3.

$$\omega = \mu^2/2\eta, \quad (3)$$

Electrophilicity index measures the propensity or capacity of a species to accept electrons [25]. It is a measure of the stabilization in energy after a system has accepted additional amount of electronic charge from the environment. When comparing the regioisomers, the electrophilicity values calculated at 6-31G(d) level indicate that compound **4** (2.47 eV) is a stronger nucleophile than the corresponding *N*-3 alkylated derivative **3** (2.9 eV) (Table 1, row 6). In addition, the *O*-alkylated derivative **8** (1.84 eV) is a stronger nucleophile than the corresponding *N*-3 alkylated derivative **7** (2.22 eV). Electrophilicity values calculated for isomers at 6-31G** and 6-31+G* levels (Tables 2 and 3, row 6) follow the same trend. The difference in nuchleophilicity is probably associated with the free *N*-3 atom in the *O*-alkylated derivatives **4** and **8**.

Table 1. Global chemical reactivity indices for compounds **3**, **4**, **7** and **8** at 6-31G(d) level

	3	**4**	**7**	**8**
E (Hatree)	-915.62	-915.60	-994.23	-994.22
ε_{HOMO} (eV)	-6.52	-6.16	-6.07	-5.70
ε_{LUMO} (eV)	-1.28	-0.99	-0.78	-0.49
η (eV)	2.62	2.59	2.65	2.61
μ (eV)	-3.90	-3.58	-3.43	-3.10
ω (eV)	2.90	2.47	2.22	1.84

Table 2. Global chemical reactivity indices for compounds **3**, **4**, **7** and **8** at 6-31G** level

	3	**4**	**7**	**8**
E (Hatree)	-915.64	-915.63	-994.27	-994.26
ε_{HOMO} (eV)	-6.52	-6.16	-6.07	-5.82
ε_{LUMO} (eV)	-1.28	-1.0	-0.79	-0.55
η (eV)	2.62	2.58	2.64	2.63
μ (eV)	-3.90	-3.58	-3.43	-3.18

ω (eV)	2.90	2.48	2.22	1.92

Table 3. Global chemical reactivity indices for compounds **3**, **4**, **7** and **8** at 6-31+G* level

	3	**4**	**7**	**8**
E (Hatree)	-915.66	-915.64	-994.28	-994.26
ε_{HOMO} (eV)	-6.55	-6.49	-6.40	-6.25
ε_{LUMO} (eV)	-1.67	-1.39	-1.19	-1.09
η (eV)	2.59	2.55	2.60	2.58
μ (eV)	-4.26	-3.94	-3.79	-3.67
ω (eV)	3.50	3.04	2.76	2.61

It was observed that electrophilicity values follow the hardness trend in violation of the minimum electrophilicity principle (MEP: for equilibrium geometries, ″more stable isomers correspond to lesser electrophilicity values″) [25]. This apparent violation may be attributed to the improper behavior of the chemical potential.

The HOMO and LUMO orbital energies are related to gas phase ionization energies (*I*) and electron affinities (*A*) of the isomers according to the Koopmans' theorem through Equations 4 and 5 [25].

$$A = -\varepsilon_{LUMO}, \quad (4)$$

$$I = -\varepsilon_{HOMO}, \quad (5)$$

Diagrams and energies of the HOMO and LUMO of compounds **3**, **4** and **7**, **8** are displayed in Fig. 4 and Tables 1, 2 and 3 (rows 2 and 3, respectively). This suggests that the *N*-3 and *O*-4 atoms of the alkylated pyrimidines may be involved in the reactivity of the isomers.

A significant structural difference between *N*- and *O*-alkylated regioisomers of 5-(hydroxypropyl)pyrimidine is the C_2-N_3-C_4 bond angle. These bond angles are 120.17° for **3**, 116.13° for **4**, 120.68° for **7** and 116.04° for **8**.

The electronic chemical potential, chemical hardness and electrophilicity index increase with the increase in the C_2-N_3-C_4 bond angle. A larger angle between the target alkylation atoms corresponds to a more stable alkylated isomer. Hence, the bond angle C_2-N_3-C_4 of similar models may be used to predict relative chemical reactivity of pyrimidine derivatives.

4. CONCLUSIONS

The introduction of an acyclic chain to the pyrimidine moiety of the 5-(3-hydroxypropyl)-2-methoxypyrimidin-4-on (**2**) using alkyl halides and potassium carbonate, afforded a mixture of 5-(3-hydroxypropyl) *N*-acyclic (**3** and **5**) and *O*-acyclic (**4** and **6**) pyrimidine nucleosides, in the ratio of 54 : 29 (**3** : **4**) and 57 : 21 (**5** : **6**).

Distinction between *N*-alkylated and *O*-alkylated regioisomers was deduced from extensive experimental FT-IR and 1D (^{1}H, ^{13}C) and 2D (COSY, HMQC and HMBC) NMR analyses. Evidence of a specific lactam's carbonyl C=O stretching band in the IR spectra of compounds **2**, **3**, **5** and **7**, and its absence in the IR spectra of compounds **4**, **6** and **8** was convenient in elucidation of regioisomers.

A comparative DFT RB3LYP/6–31G(d), 6-31G** and 6-31+G* studies on *N*-3 and *O*-4 regioisomers of 5-(3-hydroxypropyl)pyrimidine has been accomplished. RB3LYP reactivity descriptors: E, η, μ, ω, were calculated consistently to predict the stability of regioisomers. A relationship between the geometric parameter, bond angle of C_2-N_3-C_4, and the chemical reactivity descriptors was observed, leading to the final conclusion that *N*-alkylated isomers are more stable and less reactive than the corresponding *O*-alkylated isomers. This study showed that the C_2-N_3-C_4 bond angle of similar models can be used to predict the relative chemical reactivity of similar pyrimidine derivatives.

5. ACKNOWLEDGMENTS

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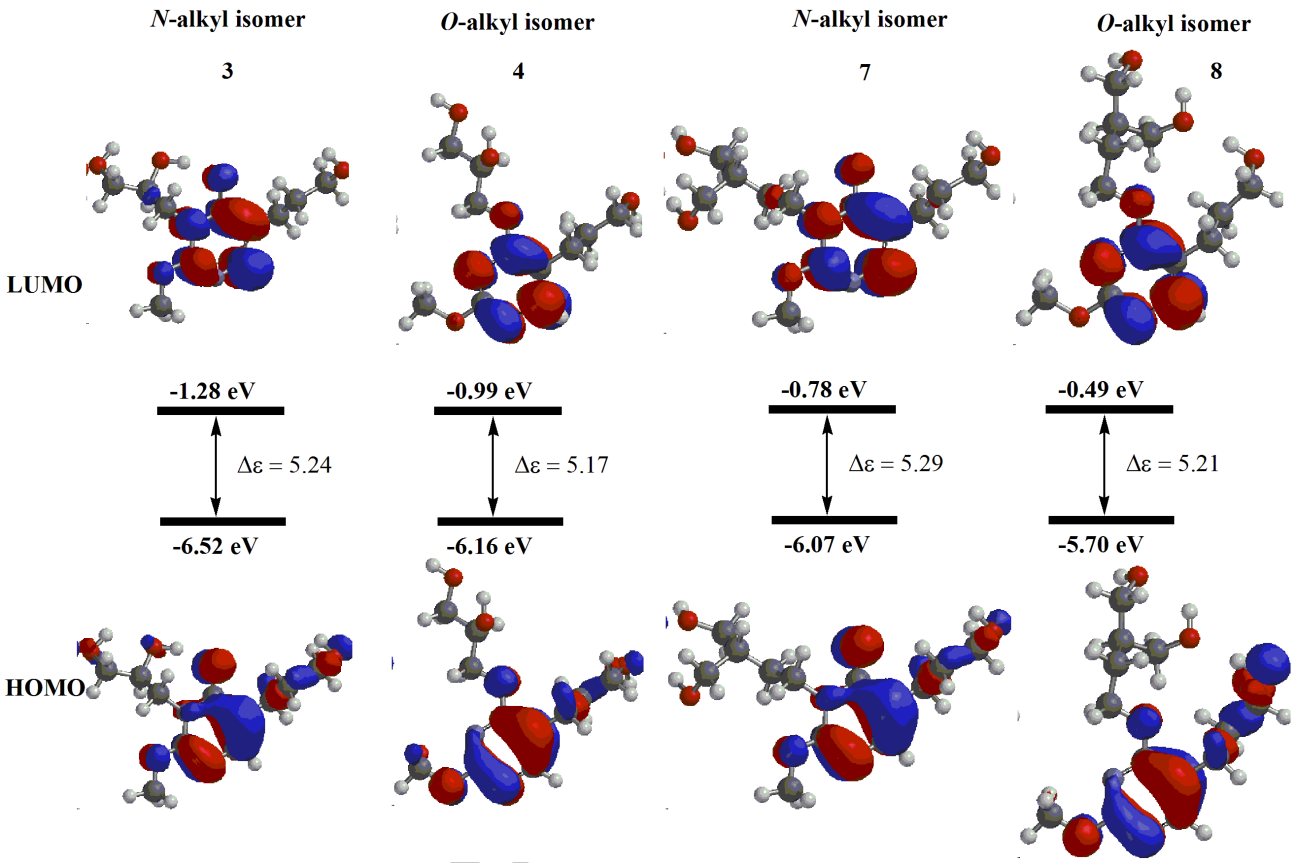
Graphical abstract



Highlights

-syntheses of *N*-3 acyclic and *O*-4 acyclic pyrimidine derivatives are presented

-DFT global chemical reactivity descriptors were calculated for the regioisomers

-descriptors were used to predict and describe stability and reactivity

-regioisomers were distinguished based on their NMR and FT-IR analyses