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Conformational and spectral properties of newly synthesized compounds obtained by reaction of alrestatin with 3-aminocycloalkanespiro-5-hydantoins

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Dedicated to Acad. Ivan Juchnovski on the occasion of his 80th birthday

Synthesis of new heterocyclic compounds by reaction of alrestatin with 3-aminocyclopentanespiro-5-hydantoin and 3-aminocyclohexanespiro-5-hydantoin is presented. The structures of the products obtained are verified *via* IR, ^{1}H and ^{13}C NMR spectroscopy. Conformational analysis of the newly synthesized compounds is performed at the B3LYP/6-31G(d,p) level both in the gas phase and in solution (DMSO) in order to find the most stable conformers about all single bonds. We find that rotation about the torsion angles O=C-CH_2 and C=O-NH is important in the conformational search. The most stable structure has the all *trans*-conformation. Two more rotamers of comparable energy are located upon rotation about angle O=C-CH_2. Calculated energy differences and rotation barriers between the three most stable rotamers in DMSO show that they all should be present in gas phase and solution in fast equilibrium, their population being strongly dependent on solvent polarity. The theoretically predicted IR, ^{1}H and ^{13}C NMR spectra of the two compounds are close to experiment.

Key words: Conformational analysis; GIAO NMR computational; synthesis; alrestatin, 3-aminocycloalkanespiro-5-hydantoins

INTRODUCTION

Alrestatin /(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)acetic acid/ is the first orally effective aldose reductase inhibitor [1]. Different spirohydantoins are also known as compounds with a similar type of activity. Substituted indan, tetralin, chroman and thiochroman hydantoins [2] as well as (9'-fluorene)-spiro-5-hydantoin (spiro-(fluorene-9,4'-imidazolidine)-2',5'-dione) and its derivatives [3-5] should be mentioned in this regard.

The crystal structure and DFT calculations of the 3-aminocycloheptanespiro-5-hydantoin and 3-aminocyclooctanespiro-5-hydantoin [6] shows that two symmetrically nonequivalent molecules exist in the hydrogen bonding of the crystal lattice molecular packing due to the two different conformations of cycloheptane ring.

The aim of the current research is to elucidate the structure and conformational properties of two newly synthesized heterocyclic compounds with potential biological activity. The reaction of 3-aminocycloalkanespiro-5-hydantoins with alrestatin was studied for this purpose.

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COMPUTATIONAL AND EXPERIMENTAL DETAILS

Quantum-chemical calculations

All calculations have been performed using the Gaussian 09 software package, G09, [7] with default optimization criteria. Conformational analyses and geometry optimizations in the gas phase and dimethylsulfoxide (DMSO) have been done using the hybrid B3LYP functional, which combines the threeparameter exchange functional of Becke [8] with the LYP correlation [9], at the 6-31G(d,p) basis set. Solvent effects are accounted for using the Polarizable Continuum Model, as implemented in G09 [10]. Vibrational frequency calculations have been performed for each structure to obtain vibrational zero point and thermal energies and to validate that the located structures correspond to energy minima with no imaginary frequency or to transition structures with a unique imaginary frequency. The calculations have been carried out without symmetry constraints by the gradient procedure.

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The populations (p_i) of the conformers are calculated by formula 1:

$$p_i = e^{-\Delta G_i / RT} / \sum_i e^{-\Delta G_i / RT} \qquad (1)$$

In simulation of infrared (IR) spectra vibration frequencies are scaled by a factor of 0.945 to ensure better agreement with experimental values.

The methods currently used for prediction of proton and carbon chemical shielding in NMR spectra are *ab initio* (MP2, Hartee-Fock), and density functional (DFT) theory applying the gauge-including atomic orbitals (GIAO) approach [11,12] and PCM/B3LYP/6-31G(d) optimized geometry. Only the results at HF/6-31+G(2d,p) level of theory are presented in this paper because of the large deviations in chemical shift values (about 20 ppm) relative to experiment of the spiro-carbon atom obtained by all other methods. Isotropic magnetic shieldings are converted into chemical shifts by subtracting the corresponding isotropic magnetic shieldings of the reference compound tetramethylsilane (TMS): $\delta = \delta_{calc}(TMS) - \delta_{calc}$, calculated at the same level of theory.

General

All used chemicals have been purchased from Merck and Sigma-Aldrich. The melting points are determined by a SMP-10 digital melting point apparatus. The purity of the compounds has been checked by thin layer chromatography on Kieselgel 60 F_{254}, 0.2 mm Merck plates, eluent system (vol. ratio): ethyl acetate : petroleum ether = 1 : 2. The elemental analysis data are obtained with an automatic analyzer Carlo Erba 1106. The IR spectra are taken on a Perkin-Elmer FTIR-1600 spectrometer in KBr discs. The NMR spectra are recorded on a Bruker Avance II + 600 MHz spectrometer, operating at 600.130 and 150.903 MHz for ^{1}H and ^{13}C, respectively, using the standard Bruker software. The chemical shifts are referenced to tetramethylsilane (TMS). The measurements in DMSO-d_6 solutions are carried out at ambient temperature

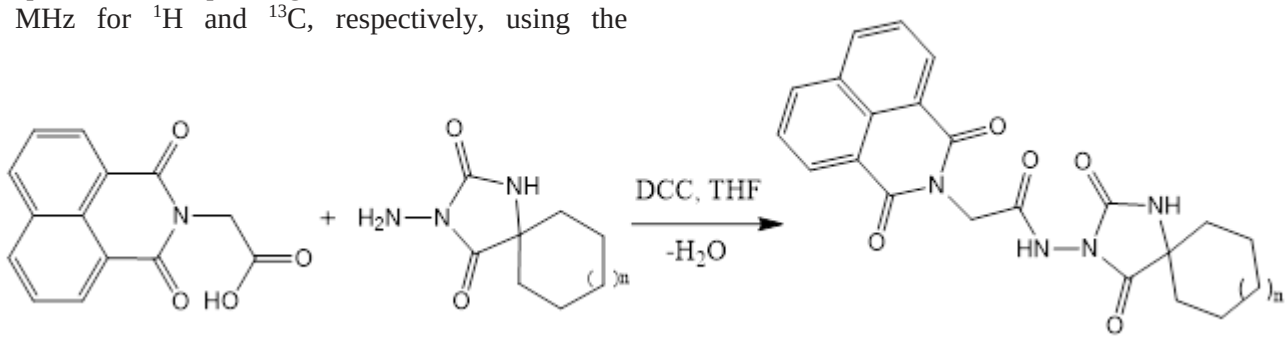
Synthesis of alrestatin derivatives with 3-aminocycloalkanespiro-5-hydantoins (Scheme 1):

A mixture of 2.55 g (0.01mol) of alrestatin (**1**) and 0.01 mol of the corresponding 3-aminospirohydantoins (**2a** and **2b**) has been dissolved in 50 ml of tetrahydrofuran with stirring at room temperature. *N,N'*-dicyclohexylcarbodiimide (DCC, 2.06 g, 0.01 mol) is added to the reaction mixture and the latter is left overnight. The *N,N'*-dicyclohexylcarbamide formed has been filtered off and 1 ml of glacial acetic acid is added to the filtrate for removing of the unreacted reagent. After filtration, the solvent is evaporated to dryness and the products obtained: 2-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-*N*-(2,4-dioxo-1,3-diazaspiro[4.4]nonan-3-yl)acetamide, **3a** (Yield = 58 %, M. p. = 206-207 ºC, R_f = 0.57) and 2-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-*N*-(2,4-dioxo-1,3-diazaspiro[4.5]decan-3-yl)acetamide, **3b** (Yield = 69 %, M. p. = 189-190 ºC, R_f = 0.63) are recrystallized from ethanol.

RESULTS AND DISSCUSION

3-aminocycloalkanespiro-5-hydantoins **3a** and **3b** can exist as mixtures of conformers. Since the used spectral methods give no possibility for identification of the different conformers, we perform quantum-chemical calculations to elucidate the structure of the newly synthesized compounds.

Full conformational analysis has been performed for compound **3a** for rotation about the O=C-CH_2

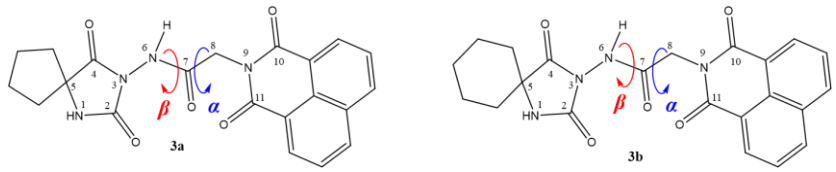


3a) n = 0; **3b)** n = 1

Scheme 1. Synthesis of alrestatin derivatives with 3-aminocycloalkanespiro-5-hydantoins

(α) and the C=O-NH (β) bonds as well as the two adjacent single bonds (Scheme 2) applying a step of 20° over a 360° range. The scans about the single bonds adjacent to the heterocyclic substituents show just a second, practically isoenergetic conformer at ca 180°. This result can be easily understood having in mind the symmetry of both heterocyclic substituents. On the other hand rotation about angle α (N9-C8-C7-N6) reveals the existence of three stable conformers. Located by the conformational scan minima have been further optimized to give 3*α1,* 3*α2* and 3*α3,* while located maxima have been optimized as transition structures. Rotation around angle β (N3-N6-C7-C8) and subsequent optimizations lead to the localization of two more conformers– 3*β1*, 3*β2*.

According to our calculations the relative stability of the conformers of compound **3a** in the gas phase decreases in the order 3a*α3* (Fig. 1 and Table 1), 3a*α2*, 3a*α1*. The energy difference between 3a*α3* and 3a*α2* is very small and they are practically isoenergetic, while 3a*α1* is less stable than 3a*α3* by 1.16 kcal mol^{-1}. Conformers 3a*β1* and 3a*β2* are of much higher energy and have not been considered further. That is why in the case of **3b** we have scanned only the rotation about the O=C-CH_2 bond, i.e. dihedral angle α (Figure 2). The stability sequence in the gas phase for compound **3b** is the same as that for **3a**: 3b*α3*>3b*α2*>3b*α1*. The relative free energy sequence of the conformers of compounds **3a** and **3b** in DMSO is changed in comparison to that in the gas phase (Table 1). The most stable structure of **3a** becomes conformer 3a*α1* followed by 3a*α2* (0.79 kcal mol^{-1}) and 3a*α3* (1.03 kcal mol^{-1}). The energy differences between the most stable conformer 3a*α1* and conformers 3a*β1* and 3a*β2* are insignificantly increased in comparison to the gas phase, being 1.73 and 5.81 kcal mol^{-1}, respectively. The percentage distribution of the conformers of **3a** in DMSO, calculated on the basis of their relative free energies using Equation 1, is as follows: 59.07% for 3a*α1*, 21.86% for 3a*α2*, 14.95% for 3a*α3*, 5.12% for 3a*β1* and 0.01% for 3a*β2*, respectively.



Scheme 2. Structural formulas and atom numbering of the investigated compounds. Rotation angles which are important in the conformational analysis are denoted as α and β.

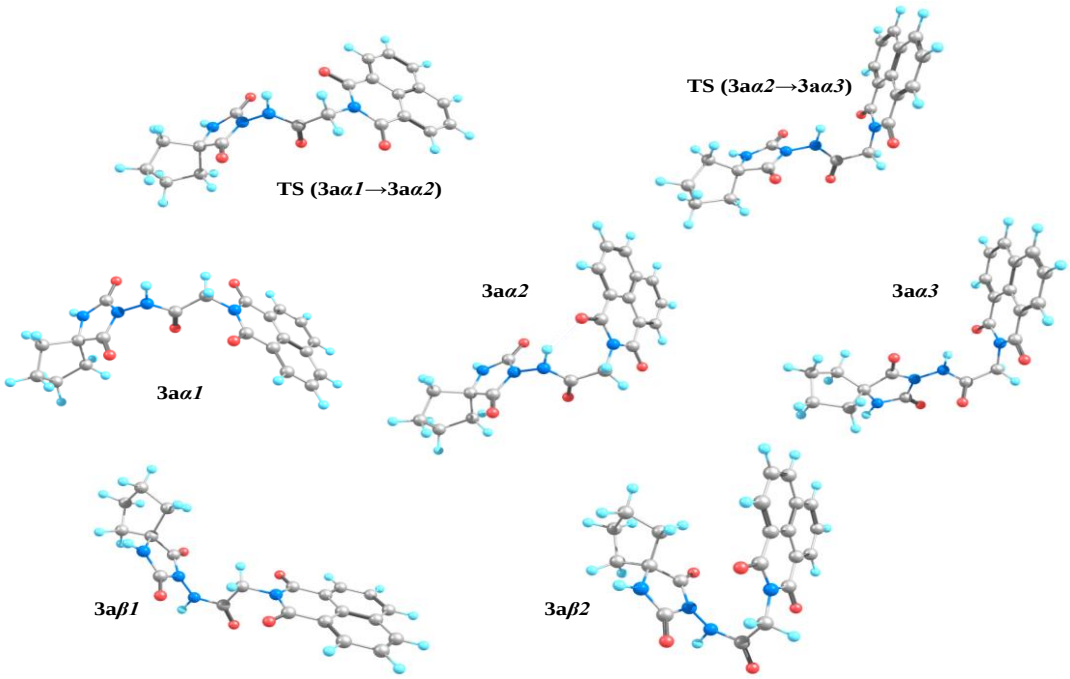


Fig. 1. PCM/B3LYP/6-31G(d,p) optimized structures of five conformers of compound **3a** in DMSO. The transition structures for rotation around angle α (Fig. 1) are presented as well.

The stability sequence of **3b** is also different in solution in comparison with the gas phase. The conformers are close in energy and at PCM/B3LYP/6-31G(d,p) level of theory conformer 3b*α1* became the most stable structure, followed by the 3b*α2* (0.31 kcal mol^{-1}) and the 3b*α3* (0.41 kcal mol^{-1}) ones. The calculated values of the rotation barriers for compound **3a** in DMSO relative to the most stable conformer 3a*α1* are given in Table 5. The free activation energy for the conversion 3a*α1*→3a*α2* is 3.54 kcal mol^{-1}, while that for the 3a*α2*→3a*α3* one is lower, 3.11 kcal mol^{-1} (Fig. 4). According to these results the three lowest energy conformers of compounds **3a** in DMSO exist in equilibrium. Based on the calculated populations and the low energy barriers of rotation it can be concluded that in DMSO compound **3a** could exist as four conformers - mainly 3a*α1* and in smaller concentrations of 3a*α2*, 3a*α3* and 3aβ*1*. The picture is similar for compound **3b**.

The synthesis of the target compounds (**3a** and **3b**) is shown in Scheme 1. Alrestatin (**1**) is obtained in accordance with ref. [13]. The 3-aminocyclopentanespiro-5-hydantoin (**2a**) and the 3-aminocyclohexanespiro-5-hydantoin (**2b**) are prepared by treatment of the corresponding cycloalkanespiro-5-hydantoins (obtained *via* the Bucherer-Lieb method [14]) with concentrated hydrazine hydrate in accordance with Marinov *et al*. [15]. The interaction between the above mentioned compounds following the DCC-method [16] leads to the formation of products **3a** and **3b**.

The elemental analysis, IR and NMR spectral data of the synthesized compounds (**3a** and **3b**) are listed in Tables 2-4 respectively. The structural formulas and atom numbering of the compounds synthesized are presented in Scheme 2.

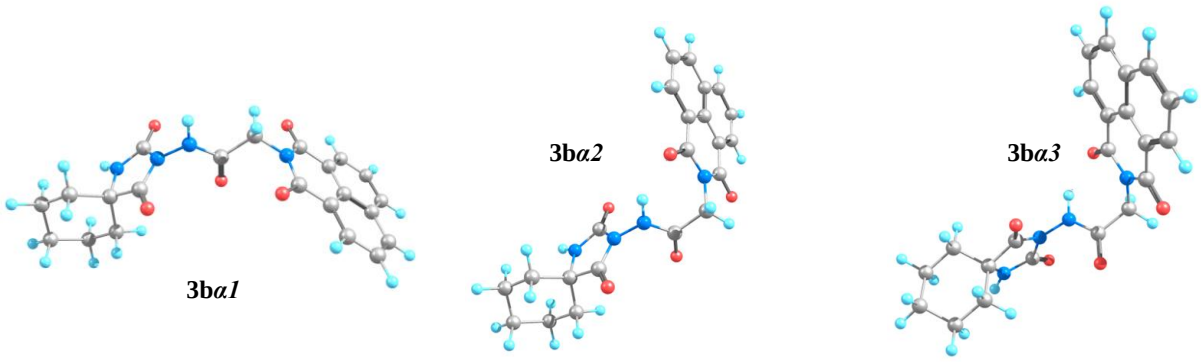


Fig. 2. PCM/B3LYP/6-31G(d,p) optimized structures of the three most stable isomers of compound **3b**.

Table 1. Relative free energies (kcal mol^{-1}) and torsion angles (º) of rotation about the CO-NH and CO-CH_2 bonds of the conformers of compounds **3a** and **3b** (Fig. 1 and Fig. 2.) calculated in the gas phase and in DMSO.

	PCM/B3LYP/6-31G(d,p)			B3LYP/6-31G(d,p)		
	ΔG_{298}	*β*	*α*	ΔG_{298}	*β*	*α*
3a*α1*	0.00	172.9	161.6	1.16	167.9	162.9
3a*α2*	0.79	174.1	-73.6	0.05	168.3	-80.6
3a*α3*	1.03	-179.8	65.8	0.00	-172.2	78.5
3a*β1*	1.73	-7.3	-170.6	1.59	17.4	-173.4
3a*β2*	5.81	-170.6	-47.8	4.10	-32.1	-47.4
TS (3a*α1*→3a*α2*)	3.54	-178.5	-130.4			
TS (3a*α2*→3a*α3*)	3.11	178.6	-10.4			
3b*α1*	0.00	173.8	163.3	1.43	167.9	162.6
3b*α2*	0.31	175.6	-70.8	0.13	168.3	-80.6
3b*α3*	0.41	-177.0	69.0	0.00	-172.6	78.1

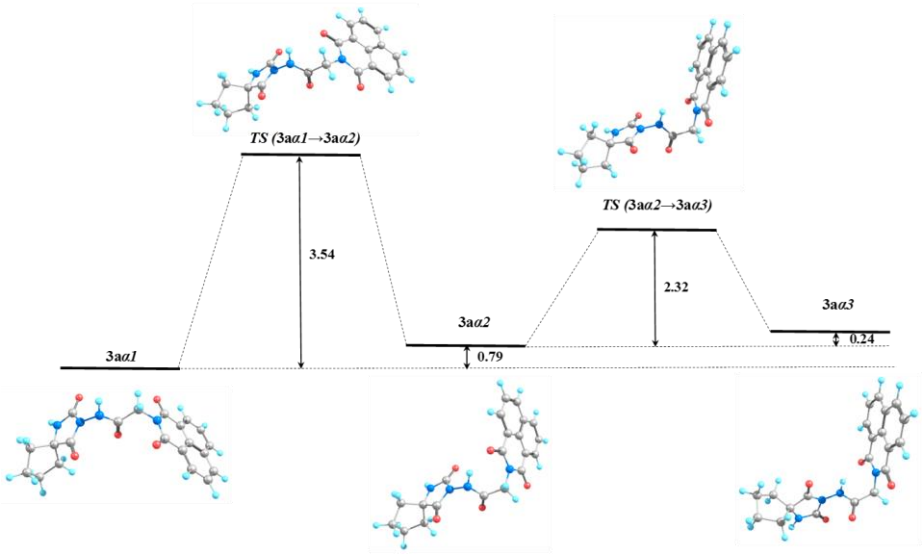


Fig. 3. PCM/B3LYP/6-31G(d,p) calculated free energy differences and rotation barriers (in kcal mol^{-1}) of the three most stable conformers of compound **3a** in DMSO.

As previously mentioned, quantum-chemical studies are performed using B3LYP functional and 6-31G(d,p) basis set in the gas phase and including solvent effects, as described in the computational section. The vibrational spectra of the all conformers of **3a** and **3b** were computed at B3LYP/6-31G(d,p) level also. Because of the fast equilibrium between the three most stable rotamers and the relatively similar predicted frequencies in their IR spectra we present selected vibration frequencies only for the most stable conformer, 3a*α3* in the gas phase. Available experimental data for the vibrational frequencies of the two compounds in KBr are presented for comparison. All results are listed in Table 3. Our assignments for the DFT calculated frequencies are based upon analysis of the corresponding vibrational eigenvectors. Some modes such as NH and C=O stretching have been found to be characteristic. The analysis of the theoretically predicted spectra of the two compounds shows that they are in a good agreement with experimental data. The augmentation of the cycloalkane ring in the molecule of **3a** to **3b** does not lead to the changes in IR spectra.

To elucidate the structure of newly synthesized compounds NMR quantum-chemical study is performed. We carry out calculations using the ab-initio (MP2), Hartee-Fock and different density functionals with a wide range of basis sets to find that HF results are closest to experimental data. MP2 and DFT do not reproduce accurately the chemical shifts of the spiro-carbon atom, the deviation from the experiment being about 20 ppm. Therefore, we present only the HF results in GIAO NMR calculations in DMSO. Because of the sensitivity of ^{13}C NMR chemical shifts to the presence of polarization and diffuse functions in the basis set, the 6-31+G(2d,p) basis set is employed. GIAO NMR calculations in DMSO are performed for all conformers of the compounds studied.

Table 2. Elemental analysis data of compounds **3a** and **3b.**

Compound	Molecular formula	Elemental analysis, %					
		Calculated			Found		
		C	**H**	**N**	**C**	**H**	**N**
3a	$C_{21}H_{18}N_4O_5$	62.06	4.46	13.79	62.35	4.23	13.57
3b	C22H20N4O5	62.85	4.79	13.33	62.55	4.45	13.49

Table 3. Experimental IR data and selected frequencies calculated at B3LYP/6-31G(d,p) level (in italic) for compounds **3a** and **3b**. The calculated frequencies are given for the most stable isomer (**3a*α3***) of the two compounds in the gas phase and are scaled by a factor of 0.945.

№	νNH	νCH (arom.)	νas (CH2)	νs (CH2)	νC=O (amide)	νC=O	νCC (arom.)	νCN (imide)
3a	3328 3394	3065 3030	2933 3022	2854 2935	1698 1684	1775, 1660 1793, 1627	1590, 1537 1582, 1543	1380 1369
3b	3328 3396	3066 3030	2933 3022	2853 2935	1696 1684	1766, 1658 1792, 1627	1590, 1537 1582, 1543	1380 1368

Table 4. GIAO ^{1}H and ^{13}C chemical shifts (δ/ppm) in DMSO of the most stable conformers of compounds **3a** and **3b** calculated at HF/6-31+G(2d,p) level and experimental data. The geometries are optimized at the PCM/B3LYP/6-31G(d,p) level in DMSO.

Nuclei	*Exptl.** *(DMSO-d_6)*	**3a*α1***	*Exptl.** *(DMSO-d6)*	**3b*α1***
CH2, cyclopentane/ cyclohexane	23.4, 32.6	26.1, 36.2	24.9-32.2	20.8-31.2
CH2, methylene	43.3	39.7	42.4	39.7
spiro C-atom	52.4	60.0	50.2	56.1
CH, naphthalene	128.2-138.5	122.9-141.7	127.8-135.2	123.0-141.2
C=O, spirohydantoin	159.2	157.2	158.2	157.4
C=O, naphthalene	165.5	171.4	163.6	171.6
C=O	171.2	175.0	169.4	174.9
C=O, spirohydantoin	209.6	182.9	207.5	180.8
m, 8H (10H), CH2	1.52-1.92	1.53-2.11	1.50-1.95	1.18-1.67
s, 2H, CH2	4.91	5.05	4.87	5.07
m, 6H, CH	7.59-7.94	8.23-9.56	7.68-7.90	8.23-9.65
s, 1H, NH	8.54	5.25	8.51	5.71
s, 1H, NH	11.12	7.28	10.82	7.31

*The assignments of ^{13}C chemical shifts are confirmed by the DEPT-135 spectral data.

Due to the fact that the chemical shifts of the different conformers are relatively close we present the chemical shifts only of the most stable conformers of **3a** and **3b** in DMSO, *α1*.

The augmentation of the cycloalkane ring in the molecule of **3b** relative to **3a** leads only to minor changes in ^{1}H and ^{13}C NMR spectra (Table 4). Our theoretical results are in agreement with the ^{1}H and ^{13}C NMR measurements in DMSO-d_6 solutions of compounds **3a** and **3b**. The exceptions are the chemical shifts of carbonyl group in spirohydantoin moiety as well as of the NH-protons, the theoretically predicted shifts being underestimated in comparison to the experimentally found ones.

CONCLUSIONS

New alrestatin derivatives with 3-amino cyclopentanespiro-5-hydantoin and 3-amino cyclohexanespiro-5-hydantoin are successfully synthesized. The structures of the products obtained have been proven by physicochemical parameters, IR, ^{1}H and ^{13}C NMR spectroscopy. The performed full computational analyses of the studied compounds about all single bonds show, that in the most stable conformers angle β is ca 180º while rotation about angle α leads to the three most stable conformerspopulation is strongly dependent on solvent polarity. Calculated free energies for rotation about angle α suggest that these three conformers are in rapid equilibrium. Computationally predicted IR, ^{1}H and ^{13}C NMR spectra of studied compounds are in good agreement with experiment.

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КОНФОРМАЦИОННИ И СПЕКТРАЛНИ СВОЙСТВА НА НОВОСИНТЕЗИРАНИ СЪЕДИНЕНИЯ, ПОЛУЧЕНИ ПРИ РЕАКЦИЯ НА АЛРЕСТАТИН И 3-АМИНОЦИКЛОАЛКАНСПИРО-5-ХИДАНТОИНИ

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(Резюме)

Представен е синтез на нови хетероциклени съединения чрез реакция между алрестатин и 3-аминоциклопентанспиро-5-хидантоин и 3-аминоциклохексанспиро-5-хидантоин. Структурите на получените продукти са потвърдени с помощта на ИЧ, ^{1}H и ^{13}C ЯМР спектроскопия. За да се локализират най-стабилните конформери на новосинтезираните съединения е направен конформационен анализ спрямо всички прости връзки на ниво B3LYP/6-31G(d,p) в газова фаза и в разтвор (диметил сулфоксид, ДМСО). Установено е, че въртенето около торзионните ъгли O=C-CH_2 и C=O-NH е важно при конформационния анализ. Най-стабилната структура има транс-конформация. Локализирани са още два конформера със сравнима енергия, при ротация около ъгъл O=C-CH_2. Изчислените в разтворител (ДМСО) енергетични разлики и бариери на ротация между трите най-стабилни ротамера показват, че те би трябвало да присъстват в разтвор, и се намират в бързо равновесие, като концентрациите им зависят от полярността на разтворителя. Теоретично предсказаните ИЧ, ^{1}H и ^{13}C ЯМР спектри на двете съединения са близки до експериментално установените.