Synthesis of 3-{[2-(N^1-alkylidenehydrazinocarbonyl)-ethyl](4-alkoxyphenyl)amino}propanohydrazide derivatives and analysis of their isomer composition

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INTRODUCTION

N-substituted β-amino acids and their derivatives are structural units of numerous natural compounds such as coenzymes, alkaloids and antibiotics. As individual compounds, they are biologically active in a wide spectrum of applications [1–4]. They are useful synthons for the synthesis of various heterocyclic systems.

A simple and convenient synthesis method for N-substituted β-amino acids is nucleophilic addition of amines to acrylic acid [5]. The reaction proceeds depending on the basicity of the used amine; the more basic the amine is, the easier it forms products of double addition, which have not attracted the attention of researchers for a long time because of the absence of an active amino group. However, the interest in the compounds containing several identical functionalized fragments has been steadily increasing [6, 7].

The structural investigation of the compounds under study 3–11 by NMR spectroscopy is complicated. The results are not completely consistent with those of the studies of the compounds in which the molecules possess one side chain with amide and azomethine fragments [8–9]. Substantial differences between both such molecules are explained in terms of their steric effects, which can be understood from the optimized molecular models. In this work, a considerable interest was focused on the ability to detect the geometrical isomers originating from the azomethine group [10–17] and on the rotamer formation due to the restricted rotation in the amide group [10, 16–23]. The susceptibility of the molecules of the compounds to the intramolecular and intermolecular interactions in the solvents of different polarity was evaluated [11, 24–29]. The aim of this work was the synthesis of 3-{[4-alkoxyphenyl][2-(hydrazinocarbonyl)ethyl]amino}propanohydrazide derivatives and their total structural analysis on the basis of 1H, 13C NMR spectroscopy and computer molecular modeling. This in depth structural analysis allowed us to obtain strong evidence concerning the mechanism of the formation of possible isomers. We could also use the analysis to ascertain the cause of supererogatory spectral lines in the NMR spectra of the compounds under study.

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RESULTS AND DISCUSSIONS

3-[(2-carboxyethyl)(4-methoxyphenyl)amino]propanoic acids (4) were obtained from \( p \)-methoxy- (1) and \( p \)-ethoxyanilines (2), respectively, and double amount of acrylic acid – by keeping the reaction mixture at room temperature for 12 hours until the product crystallized.

The reaction of diacids 3 and 4 with hydrazine was carried out in refluxing toluene with the azeotropic separation of the formed water providing 3-[(2-hydrazinocarbonyl)ethyl](4-methoxyphenyl)amino)propanohydrazide (5) and 3-{(4-ethoxyphenyl)\{(2-hydrazinocarbonyl)ethyl\}amino}propanohydrazide (6).

The acid hydrazides form hydrazones in the reaction with aldehydes and ketones [7]. The reaction of dihydrazides 5 and 6 with cyclohexanone was very facile. \( N \)-Cyclohexylidene-3-[(2-(\( N \))-cyclohexylidenehydrazinocarbonyl)ethyl](4-methoxy- (7) and \( N \)-cyclohexylidene-3-[(2-(\( N \))-cyclohexylidenehydrazinocarbonyl)ethyl](4-ethoxyphenyl)amino)propanohydrazi (8) were synthesized in 90% yield having heated the reaction mixture for 1.5 hours. However, the reaction with acetone and ethyl methyl ketone at reflux temperature gave 9–11 only in up to 75% yield.

A detailed analysis of the structural features of compounds 3–11 with the total ascription of \(^1\)H and \(^{13}\)C NMR resonances is described in this work. The assignment of the NMR spectral lines was carried out using the chemical shift theory [16, 17], signal intensity arguments and multiplicities, and by a comparison with structurally related compounds with one side chain [8, 9]. The analysis data is presented in the Experimental section. Carbon atoms are marked arbitrarily according to the numbering given in Scheme.

The structural features of the investigated compounds were affected by stereo arrangement of the molecules and their ability to form interactions. These features have been thoroughly investigated by the combined use of NMR and molecular modeling. One example of a considerable interest is the molecular model of 5 (Fig. 1).

The molecular model of this compound was fully optimized without symmetry constraints (on the contrary, the side chains of the molecular models of 3 and 4 are symmetrically located with respect to the benzene ring). It was clearly seen that the side chains of the molecular model of 5 were arranged with the trend to maintain close contacts between the oxygen atom of the CO group and the nitrogen atom of the NH group. The implication

**Scheme.** Synthesis of \( N \)-alkylidene-3-[(2-(\( N \))-alkylidenehydrazinocarbonyl)ethyl](4-alkoxyphenyl)amino)propanohydrazides

**Fig. 1.** The view of optimized molecular model of 5. The total steric energy is \(-6.46 \text{ kJ/mol}\)
was that the H-bonding interaction was formed in this case. The rotation around the NH–CO bond in the amide group was not completely restricted. Therefore, the rotamers were not observed in the NMR spectra of 5 and 6.

The amide fragments investigated in this work are especially suited and of particular interest for detailed structural studies. It is known that amides are rather sensitive to the polarity of the medium and, in particular, the hydrogen bond-donor ability of the solvent. It was unexpected that the NMR spectra of compounds 7–11 were more complicated when using CDCl₃ as a solvent than when using d₆-DMSO. The molecules of 7 and 8 possess the azomethine group included in the cyclohexanone moiety. ¹³C NMR spectra revealed that the cyclohexanone ring carbons were affected by the lone pair of the nitrogen atom in the azomethine group. The distribution of the resonances of the cyclohexanone ring carbons showed a similar trend to the
ones of the alkylene substituents of the azomethine group in compounds 9–11. The difference in the chemical shifts of cyclo-
hexanone ring carbons C-2’ and C-6’ was about 8 ppm, and the difference between C-3’ and C-5’ was about 2.0 ppm. Likewise, the difference in carbon resonances of cis/trans CH$_1$ groups in
9–10 was about 7.5 ppm.

$^1$H NMR spectra of 7 and 8 in the d$_6$-DMso solution displayed the usual view of double sets of resonances (0.49 : 0.51) of NH protons, assigned respectively to $Z$ / $E$ isomers (Fig. 2). Unfortunately, it was difficult to understand $^1$H NMR spectra in the CDCl$_3$ solution, where three sets of resonances were displayed. An unexpected deshielding of the corresponding hydrogens and some of the carbon atoms was observed comparing to the ones in the d$_6$-DMso solution. Exclusively, NH group protons showed the opposite trend of changes in the chemical shifts. It was evidently seen that the molecules underwent an essential reorientation with the change of the solvent. The problem was resolved by the interpretation of these NMR spectra with a strong support from molecular modeling data.

The arrangement of side chains $Z$ (value of the total steric energy – 40.04 kJ/mol), $E$ (value of the total steric energy – 38.24 kJ/mol), and ($E$ + $Z$) (value of the total steric energy – 38.87 kJ/mol) of 7 determined by the specific intramole-
cular hydrogen bonds between CO and NH groups, was taken into account. The possibility of two close contacts arising be-
tween the oxygen atom of the CO group and the nitrogen atom of the NH group in each $E$ location of the side chain was detect-
ed. One of such contacts was observed for isomers with mixed ($E$ + $Z$) side chains, and there were no such contacts determined for isomers with $Z$ type side chain location. This observation led to a conjecture concerning the more stabilized $E$ type dimeric structure. The intensities of the NH resonances were consistent with the findings mentioned above. The same considerations may also be applied to the studies of the solutions of compounds 8–11. The views of the optimized molecular models of the isomers $E$($trans$) and $Z$($trans$) of 11 are presented in Fig. 3 a and 2 b, respectively, as the most characteristic case in this study.

The structure of 9 was investigated with respect to the rela-
tive stability of two possible $E$ / $Z$ rotamers formed in both solu-
tions. The presence of the azomethine group in these compounds affected the suggested structural features and specifically determined the distribution of the resonances in the NMR spectra (Fig. 2). Despite the identical substitution of the azomethine group by CH$_1$, each substituent felt different influence from the lone pair of the nitrogen atom.

The $^1$H and $^{13}$C NMR signals of the methyl groups in the azomethine fragment were observed as two sets of resonance due to the possible cis and trans arrangement in the molecule. $^1$H NMR spectra in the d$_6$-DMso solution showed the CH$_1$ group in the cis position experiencing the distinct shielding of ambi-
ence, because the set of spectral lines attributed to cis was split.

The presence of isomers of 9 in the CDCl$_3$ solution was specifically reflected in $^1$H NMR spectra. The distribution of the intensi-
Ties of resonances of the NH group (prevailing due to interactions of Z, E, and ($E$ + $Z$) side chains) followed the trend in the changes of the total steric energy values of suitable molecular models for 9. Total steric energy values of –17.11 kJ/mol, –2.34 kJ/mol, and –2.26 kJ/mol were reached for the optimized molecular models of $E$, $Z$, and mixed ($E$ + $Z$) side chains, respectively. Analogous evidence was displayed by resonances of the CH$_1$ group in the cis and trans locations. Both cis and trans spectral regions consisted of three lines related to $Z$, $E$, and ($E$ + $Z$) rotamers, respectively.

The different substitution of the azomethine group led to the formation of cis / trans geometrical isomers of 11. The stereo-
chemical orientation of the molecules of these isomers was governed by CH$_2$CH$_1$ group, which took priority over the CH$_1$ group. Each of these isomers underwent $E$ / $Z$ isomerization due to the amide group. Consequently, the existence of four different structures (Z($cis$), Z($trans$), E($cis$), and E($trans$)) was taken into account in the structural analysis of this type of compounds. Four resonances of the NH group were revealed by fine struc-
tural studies of the $^1$H NMR spectra of 11 in the d$_6$-DMso solution (Fig. 4).

The resonances of the characteristic CH$_1$CH$_1$ group were overlapping and not very informative; therefore, the signals of another substituent of the azomethine group, CH$_1$, were analyzed. $^{13}$C NMR spectra of 11 showed two sets of resonance with clearly different intensity (1 : 4) of substituents of the azomethine group. On the basis of the NMR assignment of the corresponding signals and the relative compounds [9], the conclusion was drawn that there existed predominant trans geometrical isomers of 11. The molecular modeling data was consistent with that as-
cription. The values of the total steric energy were –3.68 kJ/mol for E($cis$), 4.23 kJ/mol for Z($cis$), –3.18 kJ/mol for E($trans$), and 9.79 kJ/mol for Z($trans$). The above-mentioned information concerning the more predominant geometrical isomer was invoked to assign the resonances of the CH$_1$ group of this compound. As
the trans geometric isomers were present in larger amounts, the more intensive set of signals at higher magnetic field values was attributed to the trans location of the CH$_3$ group, while less intensive signals in a lower magnetic field were assigned to the cis location. Compound 11 exhibited a specific and more complex spectral view of the characteristic groups in the NMR spectra when dissolved in chloroform. The problem was to attribute four sets of resonances of NH group protons displayed in $^1$H NMR spectra to the corresponding isomers, because their differentiation was unexpectedly changed in comparison with the suitable spectral view in the d$_6$-DMSO solution.

There were two types of interactions observed in both solutions of compounds 7–11: the intramolecular interaction between the solute molecules (predominant in CDCl$_3$ solution) and the intermolecular interaction between the solute and solvent molecules (predominant in the d$_6$-DMSO solution). Based on these considerations, it was deduced that the molecules of the study compounds in CDCl$_3$ formed three available ($Z$, $E$, and ($E + Z$)) sterical structures for each cis and trans geometrical isomer.

Taking into account the above-mentioned values of the total steric energy for $Z$(cis), $Z$(trans), $E$(cis), and $E$(trans) and including the ones of ($E + Z$)(cis, cis), and ($E + Z$)(trans, trans) for mixed side chain isomers (6.94 kJ/mol and 8.41 kJ/mol, respectively) of 11, the distribution of the intensity of the corresponding resonances of the NH group protons in $^1$H NMR spectra was elucidated. Special attention was paid to the assignment of the CH$_3$ group resonances belonging to separate molecules of cis and trans isomers present in different amounts looked like the resonances of the CH$_3$ groups in trans and cis positions of the same molecule in the spectrum of compound 9 using CDCl$_3$ as a solvent. The displacement of the resonances termed trans / cis in compounds 11 and 9 was based on the different location of the uniform (CH$_3$) substituents with respect to the lone pair of the nitrogen atom in the azomethine group.

A closer inspection of all other resonances in the NMR spectra of compounds 7–11 also revealed $E / Z$ isomer formation due to the restricted rotation of the amide group around the CO–NH bond. The existence of the $E / Z$ isomerization centre was proved by a decrease in the difference of the chemical shifts of the corresponding resonances of $E$ and $Z$ rotamers. In $^{13}$C NMR spectra, the differences in the chemical shifts for vari-

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*E, Z notation was chosen for isomers due to the restricted rotation in the amide group, while cis, trans notation was used for geometrical isomers of the azomethine group. The geometry of the whole molecule was called “cis” in the case of $R^1$ and $R^1$ in the cis location with respect to the double bond of $R^1N = CR^1CH_3$ group, and “trans” when $R^1$ and $R^1$ were located trans. $R^1 = CH_2CH_3$ and the rest of the moiety of the molecule in compound 11 is marked as $R^2$. 

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**Fig. 4.** $^1$H NMR spectral region of NH and = CH$_3$ resonances of compound 11

<table>
<thead>
<tr>
<th>NH signals in d$_6$-DMSO</th>
<th>CH$_3$ signals in d$_6$-DMSO</th>
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| $E$ (cis)  
$Z$ (trans)  
$E$ (trans)  
$Z$ (trans) | $E$ (trans)  
$Z$ (trans)  
$E$ (cis)  
$Z$ (cis) |
| ppm 10.150 10.100 10.050 10.000 9.950 | ppm 10.150 10.100 10.050 10.000 9.950 |
| 4.00 6.00 8.00 10.00 | 5.00 7.00 9.00 11.00 |

<table>
<thead>
<tr>
<th>NH signals in CDCl$_3$</th>
<th>CH$_3$ signals in CDCl$_3$</th>
</tr>
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</table>
| $E$ (trans)  
$Z$ (trans)  
$E$ (cis)  
$Z$ (cis) | $E$ (trans)  
$Z$ (trans)  
$E$ (cis)  
$Z$ (cis) |
| ppm 9.50 10.00 10.50 11.00 | ppm 2.00 2.50 3.00 3.50 |
| 0.00 1.00 2.00 3.00 | 0.00 1.00 2.00 3.00 |
ous groups located differently with respect to the isomerization center were as follows: 6 ppm for the CO group carbons, 4.5 ppm for the CH=N group carbons, 1.3 ppm for the methylene group carbons with differences in chemical shifts of about 0.2 ppm for the CH₂ CO fragment and about 0.1 ppm for the CH₃ N fragment; similarly, differences of 0.08 ppm for CO group carbons and 0.05 ppm for N-C group carbons were noted. The distributions of all other resonances in the NMR spectra of compounds 7–11 displayed evidence for the coexistence of isomeric structures and their relative stability using solvents of different polarity. The formation of the isomers of compounds 7–11 was reflected by characteristic splitting of the carbon resonances of the p-substituted benzene ring in ¹³C NMR spectra.

**EXPERIMENTAL**

The ¹H and ¹³C NMR spectra were recorded on a Brucker Avance DFX 400 (400 MHz) and a Varian Unity Inova (300 MHz) spectrometers operating in the Fourier transform mode. Chemical shifts (δ) are given from TMS (0 ppm) as an internal standard for ¹H NMR, and CDCl₃ (77.0 ppm) or d₄-DSMO (39.5 ppm) for ¹³C NMR. Melting points were determined on an automatic APA1 melting point apparatus and were uncorrected. Mass spectra were obtained using the chemical ionization (CI) mode.

The molecular modeling of the compounds under study was carried out by molecular mechanics method (MM2) using Chem 3D Ultra 9.0 (Licence Cambridge Software Package, Serial number: 031 406391 4800).

3-[(2-Carboxyethyl)-(4-methoxyphenyl)amino]propanoic acid (3). A solution of p-nitroaniline (1) (61.5 g, 0.5 mol) in toluene (100 ml) was heated up to 50 °C, and acetic acid (144 ml, 2 mol) was added. The reaction mixture was kept at room temperature for 12 h. The crystals formed were filtered off, washed twice with diethyl ether, and recrystallized from ethanol–diethyl ether mixture to give off, washed twice with diethyl ether, and recrystallized from 2-propanol. The crystals formed were filtered, washed with diethyl ether and recrystallized from 2-propanol to give (18.7 g, 63%). M. p. 143–144 °C. ¹H NMR (300 MHz, d₆-DSMO): δ: 2.23 (t, 4H, J = 6.9 Hz, CH₂ CO), 3.41 (t, 4H, J = 6.9 Hz, CH₂ N), 3.67 (s, 3H, OCH₃), 4.45 (br. s, 2H, NH). 6.68 (d, 2H, J = 9.2 Hz, 2.6-H₂), 6.80 (d, 2H, J = 9.2 Hz, 3.5-H₂), 9.02 (s, 1H, NH). ¹³C NMR (75.4 MHz, d₆-DSMO): δ: 31.58 (CH₂ CO), 47.59 (CH₂ N), 55.33 (OCH₃), 114.29 (C-2,6), 114.77 (C-3,5), 141.73 (C-1), 151.08 (C-4), 170.21 (CO). MS (15 V, m/z): 296.5 [M + H⁺] (90%). Elemental analysis data: found, %: C, 52.46; H, 6.98; N, 23.56; formula C₁₈H₁₆N₂O₃ (293.3409); calculated, %: C, 52.87; H, 7.17; N, 23.71.

3-[(4-Ethoxyphenyl)(2-hydrazinocarbonyl)ethyl]amino]propanoic acid (6). Prepared from 4 (28.1 g, 0.1 mol) similar as for 5 to obtain 6 which was recrystallized from 2-propanol (24.3 g, 79%). M. p. 158–159 °C. ¹³C NMR (75.4 MHz, d₆-DSMO): δ: 14.86 (CH₂ CH₃ O), 31.57 (CH₂ CO), 47.51 (CH₂ N), 63.30 (CH₂ CH₂ O), 114.20 (C-2,6), 114.58 (C-3,5), 141.67 (C-1), 150.23 (C-4), 170.17 (CO). MS (15 V, m/z): 310.2 [M + H⁺] (85%). Elemental analysis data: found, %: C, 54.01; H, 7.22; N, 22.19; formula C₁₈H₁₆N₂O₃ (309.3677); calculated, %: C, 54.35; H, 7.49; N, 22.64.

N-Cyclohexylidene-3-[(2-N¹-cyclohexylidene-hydrazinocarbonyl)ethyl]amino]propanoic acid (7). Hydrazide 5 (1.47 g, 5 mmol) was dissolved in methanol (20 ml), and cyclohexanone (0.98 g, 10 mmol) was added drop-wise under stirring. The reaction mixture was refluxed for 1.5 h and then cooled down. The crystals formed were filtered off and washed with diethyl ether to give 7 (2.05 g, 90%). M. p. 87.5–88.5 °C. ¹H NMR (300 MHz, d₆-DSMO): δ: 1.48–1.68 (br. m, 12H, (3’5’ CH₂ + (4’CH₃) CH₂), 2.18–2.44 (m, 8H, (2’5’ CH₂) + 0.49H, Z CH₂ CO), 2.67–2.77 (m, 0.51H, E CH₂ CO), 3.43–3.52 (m, 4H, CH₂ N), 3.66–6.83 (m, 4H, H), 10.14 (s, 0.49H, Z NH), 10.23 (s, 0.51H, E NH). ¹³C NMR (75.4MHz, d₆-DSMO): δ: 25.12 (C-3’, 25.59 (Z C( ‘C’)), 25.63 (E C( ‘C’)), 26.46 (C( ‘C’)), 28.88 (C( ‘C’)), 27.13 (E( ‘C’)), 30.66, 30.88 (Z CH₂ CO), 31.95, 32.19 (E CH₂ CO), 34.93 (Z C( ‘C’)), 35.11 (E( ‘C’)), 46.65, 46.76 (Z CH₂ N), 47.42 (E CH₂ N), 47.49 (E CH₂ N), 55.31 (OCH₃), 113.56, 113.97, 114.61 (C-2,6), 114.71 (C-3,5), 141.73, 141.81 (C-1), 150.72, 150.90, 151.17 (C-4), 155.59 (Z (N= C(’)), 160.49 (E (N= C(’))), 167.20, 167.26 (Z CO), 173.18 (E CO). ¹H NMR (300 MHz, CDCl₃): δ: 1.59–1.93 (br. m, 12H, (3’5’ CH₂ + (4’CH₃) CH₂), 2.20–2.40 (m, 8H, (2’5’ CH₂), 2.45–2.56 (m, 0.39H, Z CH₂ CO), 2.79–2.85 (m, 0.29H, (Z + E) CH₂ CO), 2.92–2.96 (m, 0.32H, E CH₂ CO), 3.44–3.49 (m, 0.34H, Z CH₂ N), 3.50–3.57 (m, 0.33H, (Z + E) CH₂ N), 3.63–3.68 (m, 0.33H, E CH₂ N), 3.75, 3.76 (2s, 3H, OCH₃), 6.80–6.93 (m, 4H, 289.1467).
trans-154.72 (m/z (C-2')), 26.92 (C-5'), 25.63 (Z (C-4')), 25.67 (E (C-4')), 26.48 (Z (C-2')), 26.92 (C-5'), 27.15 (E (C-2')).

3-(4-Buten-2-ylidene)-3-((2-(N'-propan-2-ylidenydrazonecarbonyl)ethyl)amino)-N'-propanoyl-L-proline (11). Prepared from 6 (1.545 g, 5 mmol) according to the synthesis procedure of 9 to yield 11, which was recrystallized from acetonitrile–water mixture (1:2.5, 62%). M.p. 151.6–152.5°C. 1H NMR (300 MHz, d5-DMSO): δ 9.18 (s, 0.20H, (Z + E) NH). MS (25 V, m/z): 456.4 [M + H]+ (100%). Elemental analysis data: found: %: C, 60.18; H, 7.39; N, 18.25; for formula C19H30N6O3 (375.4701): calculated: %: C, 60.78; H, 7.78; N, 18.68.

3-{(4-Ethoxycarbonyl)[2-(N'-propan-2-ylidenydrazonecarbonyl)ethyl]amino}-N'-propanoyl-L-proline (10). Prepared from 6 (1.545 g, 5 mmol) according to the synthesis procedure of 9 to give 10, which was recrystallized from methanol (1:3, 67%). M.p. 131–132°C. 13C NMR (300 MHz, d5-DMSO): δ 26.48 (m, 0.19H, Z CH,CO), 2.64–2.75 (m, 0.53H, E CH,CO), 3.40–3.52 (m, 4H, CH, N), 3.90 (q, 2H, J = 6.9, CH2CH2O), 6.88–6.83 (m, 4H, H,), 10.17 (s, 0.49H, Z NH), 10.26 (s, 0.51H, E NH).

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9.64 (cis(Z) (C=CH2CH2)), 9.74 (cis(E) (C=CH2CH2)), 10.43, 10.58 (trans (Z) (C=CH2CH2)), 10.99, 11.06 (trans(E) (C=CH2CH2)), 14.83 (CH3CH2O), 14.92 (trans(Z) (C=CH2)), 14.94 (trans(E) (C=CH2)), 22.49, 22.63, 22.86, 23.61 (cis(Z, E) (C=CH2CH2) + cis(Z, E) (C=CH2CH2)), 30.14, 30.73, 31.07 (Z cis, trans) (CH2CO), 31.99, 32.04 (trans(Z) (C=CH2CH2)), 32.76, 33.16, 33.26 (E cis, trans) (CH2CO), 42.75 (Z cis, trans) (CH3N), 48.43, 48.74, 49.00, 49.46, 49.46, 49.67 (E cis, trans), (Z + E) (cis, trans) (CH3N), 63.72, 64.01 CH3CH2O), 113.96, 115.31, 115.62, 118.04, 119.17, 119.33, 119.43 (C-2,3,5,6), 141.16, 141.59, 141.77 (C-1), 150.52 (C-4), 153.40, 153.52, 153.73, 153.94, 154.26 (Z cis, trans) (C=N), 158.12 (E cis) (C=N), 158.71 (E cis, trans) (C=N), 168.34 (Z CO), 172.52 (E cis, trans) (C O), 174.18 (E cis) (CO); 174.27 (E cis, trans) (CO). MS (15 V, m/z): 418.6 [M + H]+ (100%). Elemental analysis data: found, %: C, 62.98; H, 8.14; N, 16.39; formula C22H39N5O5 (417.5505); calculated, %: C, 63.28; H, 8.45; N, 16.77.

CONCLUSIONS

3-[[2-({N'-alkylidenehydrazinocarbonyl}ethyl)({4-alkoxyphenyl})amino]propanohydrazide derivatives and analysis...