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Synthesis and structure of new 1-cyanoacetyl-4-arylsemicarbazide derivatives with potential anticancer activity

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ABSTRACT

The aim of this work are experimental and computational structural studies of new derivatives of 1-cyanoacetyl-4-arylsemicarbazide with potential anticancer activity. We considered 10 possible tautomeric forms and ranked them according to their energy of stabilization using quantum chemical calculations with the single point B3LYP DFT method and the basis set 6-311++G(3df, 3pd) of Gaussian09 program. The order of tautomers was identical for all considered compounds with the exception of derivatives **1**, **5** and **10**. Furthermore, we computed HOMO and LUMO orbitals, electrostatic potential maps and non-covalent interaction maps for most stable tautomers. We confirmed the structures of the compounds with application of experimental and computed ^{1}H, ^{13}C NMR, IR and Raman spectra. In addition we performed PED analysis in order to assign IR vibrations and computed non-covalent interaction maps.

1. Introduction

The literature regarding semicarbazide derivatives is rather limited and focuses mainly on the role of semicarbazides as intermediates in the synthesis of new compounds with biological activity [1–6]. The substrates for the synthesis of semicarbazide derivatives are hydrazides of carboxylic acids and isocyanates. These reactions can be carried out in diethyl ether or *N*, *N*-dimethylacetamide (at room temperature) [7]. Good yields are obtained by heating the reactants in an oil bath at temperature of about 100 °C [8].

In spite of well-developed methods for the synthesis of semicarbazide a number of studies on the biological properties of these compounds is rather limited. Derivatives with anticonvulsant [9], antitumor [10,11] and antioxidant activity [12] are known. 1-Acetylo-4-substituted semicarbazide was tested for antitumor activity. The most promising results of the cytotoxic effect with respect to epithelial ovarian cancer cells (TOV 112D) was observed for 4-substituted 1-[(1-methylpyrrol-2-yl)]acetylsemicarbazide. The most active compound showed 85% inhibition of cancer cell line at a concentration of 50 ug/mL (0.14 mM) [13].

Saravanan et al. synthesized derivatives of semicarbazides bearing quinazoline system. These compounds showed antimicrobial activity against *S. aureus* with the MIC values in the range 7.81–31.25 mg/mL [14]. Similar compounds showed inhibition of growth of *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumoniae* at the concentration of 6.25 mg/mL [15]. Antibacterial activity of semicarbazide derivatives of the quinoline system depended on the presence of a methoxy group on the phenyl ring and two methyl groups. 1-Substituted derivatives of 4-(pyridin-4-ylmethyl)semicarbazide obtained in our group also deserve attention. These compounds showed inhibition of growth of *S. aureus*, *S. epidermidis*, *B. subtilis*, *M. luteus* at concentration of 15.63–31.25 mg/ml [15]. In

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the case of 1-(thiophen-2-ylmethyl)-4-(4-tolyl)semicarbazide significant degree of growth inhibition was observed with respect to *S. aureus*, *K. pneumoniae*, *P. aeruginosa* at concentration of 9.37 mg/mL [16]. Among bis-semicarbazide derivatives inhibition of growth of both bacteria and fungi were observed for substituted 1,6-bis-(semicarbazide)hexane with MIC = 7.50 mg/ml regardless of the type of the substituent. Only the modification consisting of the replacement of the diphenylmethylene for hexamethylene caused a significant decrease in the antimicrobial activity of [17]. It may be due to negative reinforcement structure and reducing the number of available conformations which limits the field of action of the compound.

It is generally known that the biological activity of chemical compounds depends on many physical and chemical factors directly related to the structure: molecular weight, molecular volume, surface area, geometric and conformational parameters, the distribution of loads on the atoms, dipole moment, polarizability, the conformation and configuration [18] An important parameter for the biological activity of chemical compounds is tautomerism. This issue is particularly important from the point of view of biochemical processes in living organisms, the mode of interaction with the molecular target and affect the performance and durability of the therapeutic dose of the drug substance. The phenomenon of tautomerism has been studied for many heterocyclic compounds [19,20]. Tautomerism resulting from the movement of protons in the direction of the carbonyl carbon may be present in semicarbazide derivative. This issue has not been thoroughly investigated. A relatively high number of tautomeric forms and few literature reports on the semicarbazide derivatives tautomerism constitutes the rationale of this work.

The aim of this work are computational and experimental studies of the structure, tautomerism and spectroscopic properties of new 1-cyanoacetyl-4-arylsemicarbazide derivatives with potential anticancer activity. In particular we performed PED analysis of computed IR spectra in order to assign computed IR frequencies and computed non-covalent interaction maps which is a relatively new technique.

2. Experimental

All chemicals used for the synthesis of new compounds were purchased from AlfaAesar, Merck and Sigma–Aldrich. Melting points (m.p.) for semicarbazide derivatives were determined in a Fisher-Johns block and are not corrected. NMR spectroscopic studies (^{1}H,^{13}C NMR) were obtained on a Bruker AVANCE III 300 MHz using commercially available solvents and tetramethylsilane as a reference. IR spectra were recorded on a Thermo Nicolet 6700 ATR technique in the range of 500–3500 cm^{-1}. FT–Raman spectra were recorded on a Reflex Raman microscopy Renishaw Invitrogen (UK) in the wave number range 200 cm^{-1}–3200 cm^{-1} at the excitation wavelength of the laser λ = 785 nm.

2.1. Synthesis of 1-cyanoacetyl-4-arylsemicarbazide derivatives (**1**–**10**)

New 1-cyanoacetyl-4-arylosemicarbazide derivatives were obtained in the reaction of cyanoacetic acid hydrazide (0.01 mol) and phenyl isocyanate or 2-or 3- or 4- halogen substituted phenylisocyanate (0.01 mol). The reaction carried out in anhydrous acetonitrile at room temperature for 48 h or in the methanol by heating under reflux for 0.5 h. After this time the reaction mixture was cooled (in case of methanol) and the precipitation was filtered off and recrystallized from acetonitrile-methanol (1:1).

2.1.1. 1-Cyanoacetyl-4-phenylsemicarbazide (**1**) [21]

Yield (80%), m.p. 165–167 °C. ^{1}H NMR (300 MHz, DMSO-d$_6$): δ = 3.74 (s, 2H, CH$_2$), 6.94–7.03 (m, 5H, CHarom), 8.26; 8.76; 10.06 (3s, 3H, 3NH). ^{13}C NMR (300 MHz, DMSO-d$_6$): δ = 24 (CH$_2$), 116 (CN), 118, 122, 128 (CHarom), 139 (Carom), 155 (C=O), 162 (C=O). FT–IR ATR (cm^{-1}): 3461, 3329, 3211 NH, 2264 CN, 1708, 1660C=O. FT–Raman (cm^{-1}): 3066, 2963, 2926 NH, 2264 CN, 1712, 1665 C=O.

2.1.2. 1-Cyanoacetyl-4-(2-fluorophenyl)semicarbazide (**2**)

Yield (79%), m.p. 180–181 °C. ^{1}H NMR (300 MHz, DMSO-d$_6$): δ = 3.74 (s, 2H, CH$_2$), 7.15–7.95 (m, 4H, CHarom), 8.51; 8.60; 10.15 (3s, 3H, 3NH). ^{13}C NMR (300 MHz, DMSO-d6): δ = 24 (CH$_2$), 115 (CN), 122, 124, 126 (CHarom), 151(Carom), 155 (C–F), 162 (C=O), 167 (C=O). FT–IR ATR (cm^{-1}): 3477, 3322, 3219 NH, 2263 CN, 1708, 1660 C=O. FT–Raman (cm^{-1}): 3066, 2979, 2932 NH, 2266 CN, 1713, 1665 C=O.

2.1.3. 1-Cyanoacetyl-4-(3-fluorophenyl)semicarbazide (**3**)

Yield (77%), m.p. 160–161 °C. ^{1}H NMR (300 MHz, DMSO-d$_6$): δ = 3.73 (s, 2H, CH$_2$), 6.78–7.45 (m, 4H, CHarom), 8.38; 8.99; 10.06 (3s, 3H, 3NH). ^{13}C NMR (300 MHz, DMSO-d$_6$): δ = 24 (CH$_2$), 105, 108, 114 (CHarom), 116 (CN), 130 (C–F), 144 (Carom), 155 (C=O), 164 (C=O). FT–IR ATR (cm^{-1}): 3350, 3279, 3036 NH, 2268 CN, 1717, 1658 C=O. FT–Raman (cm^{-1}): 3090, 2956, 2928 NH, 2271 CN, 1691, 1619 C=O.

2.1.4. 1-Cyanoacetyl-4-(4-fluorophenyl) semicarbazide (**4**)

Yield (73%), m.p. 203–205 °C. ^{1}H NMR (300 MHz, DMSO-d$_6$): δ = 3.72 (s, 2H, CH$_2$), 7.10–7.45 (m, 4H, CHarom), 8.28; 8.78; 10.02 (3s, 3H, 3NH). ^{13}C NMR (300 MHz, DMSO-d$_6$): δ = 24 (CH$_2$), 115 (CN), 116, 120 (CHarom), 130 (Carom), 135 (C–F), 155 (C=O), 162 (C=O). FT–IR ATR (cm^{-1}): 3477, 3322, 3219 NH, 2263 CN, 1708 C=O, FT-IR ATR (cm^{-1}): 3477, 3322, 3219 NH, 2263 CN, 1691, 1635 C=O. FT–Raman (cm^{-1}): 3082, 2973, 2928 NH, 2267 CN, 1696, 1640 C=O.

2.1.5. 1-Cyanoacetyl-4-(2-bromofenyl)semicarbazide (**5**)

Yield (78%), m.p. 180–182 °C. ^{1}H NMR (300 MHz, DMSO-d$_6$): δ = 3.76 (s, 2H, CH$_2$), 6.99–7.62 (m, 4H, CHarom), 8.14; 8.95; 10.18 (3s, 3H, 3NH). ^{13}C NMR (300 MHz, DMSO-d$_6$): δ = 24 (CH$_2$), 115 (CN), 122, 125, 128, 155 (CHarom), 132 (Carom), 136 (C–Br), 162 (C=O), 167 (C=O). FT–IR ATR (cm^{-1}): 3399, 3310, 3211 NH, 2259 CN, 1736, 1690 C=O. FT–Raman (cm^{-1}): 3072, 2993, 2940 NH, 2261 CN, 1676, 1615 C=O.

2.1.6. 1-Cyanoacetyl-4-(3-bromophenyl)semicarbazide (**6**)

Yield (71%), m.p. 135–137 °C. ^{1}H NMR (300 MHz, DMSO-d$_6$): δ = 3.76 (s, 2H, CH$_2$), 7.16–7.83 (m, 4H, CHarom), 8.42; 8.96; 9.29 (3s, 3H, 3NH). ^{13}C NMR (300 MHz, DMSO-d$_6$): δ = 24 (CH$_2$), 115 (CN), 121, 130, 141 (CHarom), 154 (Carom), 155 (C–Br), 162 (C=O), 167 (C=O). FT–IR ATR (cm^{-1}): 3288, 3226, 3099 NH, 2269 CN, 1685, 1647 C=O. FT–Raman (cm^{-1}): 3067, 2974, 2934 NH, 2266 CN, 1682, 1641 C=O.

2.1.7. 1-Cyanoacetyl-4-(4-bromophenyl)semicarbazide (**7**)

Yield (69%), m.p. 105–107 °C. ^{1}H NMR (300 MHz, DMSO-d$_6$): δ = 3.47 (s, 2H, CH$_2$), 7.43–7.45 (m, 4H, CHarom), 8.26; 8.33; 10.05 (3s, 3H, 3NH). ^{13}C NMR (300 MHz, DMSO-d$_6$): δ = 23 (CH$_2$), 116 (CN), 116, 120, (CHarom), 138 (C–Br), 152 (Carom), 161 (C=O), 166 (C=O). FT–IR ATR (cm^{-1}): 3345, 3285, 3187 NH, 2261 CN, 1685, 1623 C=O. FT–Raman (cm^{-1}): 2983, 2931 NH, 2263 CN, 1714, 1675 C=O.

2.1.8. 1-Cyanoacetyl-4-(2-chlorophenyl)semicarbazide (**8**)

Yield (76%), m.p. 185–187 °C. ^{1}H NMR (300 MHz, DMSO-d$_6$):