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Synthesis, spectroscopic and conformational analysis of 1,4-dihydroisonicotinic acid derivatives

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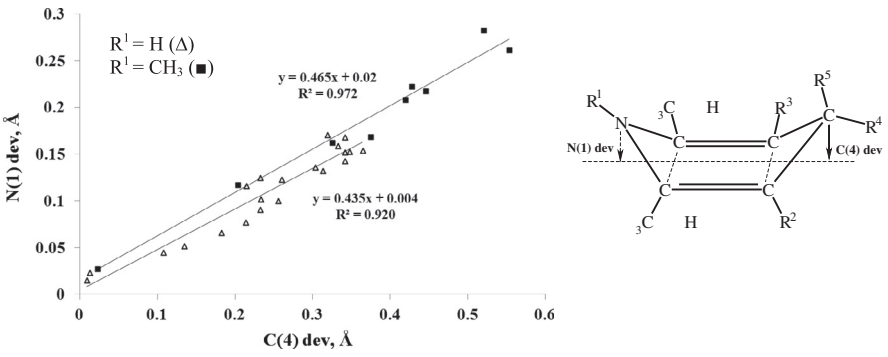
HIGHLIGHTS

- The present work offers methods for the synthesis of novel 1,4-DHINA derivatives.
- Crystals suitable for X-ray were obtained by slow evaporation from saturated solvents.
- Conformational properties have been investigated by X-ray, NMR and theoretical calculations.

GRAPHICAL ABSTRACT



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ABSTRACT

Structural and conformational properties of 1,4-dihydroisonicotinic acid derivatives, characterized by ester, ketone or cyano functions at positions 3 and 5 in solid and liquid states have been investigated by X-ray analysis and nuclear magnetic resonance and supported by quantum chemical calculations. The dihydropyridine ring in each of the compounds exists in flattened boat-type conformation. The observed ring distortions around the C(4) and N(1) atoms are interrelated. The substituent at N(1) has great influence on nitrogen atom pyramidality. The ^{1}H, ^{13}C and ^{15}N NMR chemical shifts and coupling constants are discussed in terms of their relationship to structural features such as character and position of the substituent in heterocycle, *N*-alkyl substitution and nitrogen lone pair delocalization within the conjugated system.

Introduction

1,4-Dihydroisonicotinic acid derivatives (1,4-DHINA) are important precursors for the synthesis of biologically active 1,4-dihydropyridines (1,4-DHPs). The parental compound, 2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydroisonicotinic acid sodium salt, as well as 1,4-DHINA coupled with glutamic acid residue – glutapyrone, both possess antimutagenic properties [1–3]. Besides, glutapyrone shows a wide variety of pharmacological properties, amongst which the neuromodulatory one is the most pronounced [4,5]. Taurine derivative of 1,4-dihydroisonicotinic acid (tauropyrone) shows anti-platelet properties and is active as an anti-aggregant at concentrations that are six times lower than those for taurine [6].

Since the discovery of pharmacological effects of this class of substances, it has been of interest to know which conformation produces the optimum effect. The conformation of the dihydropyridine ring, the nature of the substituents and their mutual

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orientation play a key role for the biological activity of 1,4-DHPs [7]. A number of studies have been devoted to the conformational analysis of numerous 4-phenyl and 4-heteroaryl substituted 1,4-DHP derivatives [8–19], however the crystal structures of 1,4-DHINA derivatives are not analysed in literature until now.

In the course of studies on the chemistry of 1,4-DHINA we focused on conformation and spectroscopic properties of several symmetrically and unsymmetrically substituted derivatives (Table 1).

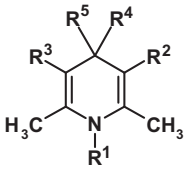
Experimental section

General information

Previously we have reported the synthesis and X-ray analysis of compounds **1d**–**1f** [20] and **5a**, **5b**, **5d** and **5e** [21]. The symmetrical 4-phenyl substituted 1,4-DHP derivatives **1c**–**3c** were synthesized at Latvian Institute of Organic Synthesis and obtained as stable solids with good yields.

All chemicals were purchased from commercial sources (Sigma–Aldrich and Acros) and used without further purification. Reactions were monitored using analytical TLC plates (Merck, silica gel 60 F_{254}), and visualized with ultraviolet light (254 nm). Column chromatography was carried out using Acros silica gel (particle size 0.035–0.070 mm). IR spectra were recorded on a IRPrestige-21 Shimadzu spectrometer in Nujol. Melting points were determined with a SRS Stanford Research Systems OptiMelt Automated Melting Point System instrument. Elemental analysis was carried out on EA 1106 (Carlo Erba Instruments) automatic analyzer.

Table 1
Chemical structures for 1,4-DHP derivatives.



1,4-DHP	R^1	R^2	R^3	R^4	R^5
1a	H	$COOCH_3$	$COOCH_3$	H	COOH
1b	H	$COOCH_3$	$COOCH_3$	H	$COOCH_3$
1c	H	$COOCH_3$	$COOCH_3$	H	C_6H_5
1d	CH_3	$COOCH_3$	$COOCH_3$	H	$COOCH_3$
1e	CH_3	$COOCH_3$	$COOCH_3$	CH_3	$COOCH_3$
1f	CH_3	$COOCH_3$	$COOCH_3$	$CH(CH_3)_2$	$COOCH_3$
2a	H	$COCH_3$	$COCH_3$	H	COOH
2b	H	$COCH_3$	$COCH_3$	H	$COOCH_3$
2c	H	$COCH_3$	$COCH_3$	H	C_6H_5
2d	CH_3	$COCH_3$	$COCH_3$	H	$COOCH_3$
3a	H	CN	CN	H	COOH
3b	H	CN	CN	H	$COOCH_3$
3c	H	CN	CN	H	C_6H_5
3d	CH_3	CN	CN	H	$COOCH_3$
3e	CH_3	CN	CN	CH_3	$COOCH_3$
4a	H	$COOCH_3$	CN	H	COOH
4b	H	$COOCH_3$	CN	H	$COOCH_3$
4c	H	$COOCH_3$	CN	H	C_6H_5
4d	CH_3	$COOCH_3$	CN	H	$COOCH_3$
5a	H	$COCH_3$	CN	H	COOH
5b	H	$COCH_3$	CN	H	$COOCH_3$
5c	H	$COCH_3$	CN	H	C_6H_5
5d	CH_3	$COCH_3$	CN	H	$COOCH_3$
5e	CH_3	$COCH_3$	CN	CH_3	$COOCH_3$
6a	H	$COOCH_3$	$COCH_3$	H	COOH
6b	H	$COOCH_3$	$COCH_3$	H	$COOCH_3$
6c	H	$COOCH_3$	$COCH_3$	H	C_6H_5
6d	CH_3	$COOCH_3$	$COCH_3$	H	$COOCH_3$

Synthesis

2,6-Dimethyl-1,4-dihydropyridine-3,4,5-tricarboxylic acid 3,5-dimethyl ester (1a) was synthesized by the procedure given in literature [22]. Yield 38%, colourless solid, mp 233–234 °C (from MeOH). ^{1}H NMR δ 2.22 (s, 6H, 2,6-CH_3), 3.60 (s, 6H, 3,5-$COOCH_3$), 4.58 (s, 1H, 4-H), 8.93 (s, 1H, NH), 11.95 (s, 1H, 4-COOH). ^{13}C NMR 17.85, 39.16, 50.79, 96.98, 146.17, 167.24, 174.42. Found: C, 53.52; H, 5.54; N, 5.16. Calc. for $C_{12}H_{15}NO_6$: C, 53.53; H, 5.62; N, 5.20%. IR spectrum, ν_{max}/cm^{-1}: 3346 (NH), 1700 (CO), 1662 (CO).

2,6-Dimethyl-1,4-dihydropyridine-3,4,5-tricarboxylic acid trimethyl ester (1b) was synthesized by the procedure given in literature [23]. Yield 93%, colourless solid, mp 158–159 °C (from MeOH). ^{1}H NMR δ 2.23 (s, 6H, 2,6-CH_3), 3.50 (s, 3H, 4-$COOCH_3$), 3.62 (s, 6H, 3,5-$COOCH_3$), 4.69 (s, 1H, 4-H), 9.01 (s, 1H, NH). ^{13}C NMR 17.89, 39.38, 50.89, 51.75, 96.30, 146.83, 166.97, 173.35. Found: C, 55.17; H, 5.98; N, 4.82. Calc. for $C_{13}H_{17}NO_6$: C, 55.12; H, 6.05; N, 4.94%. IR spectrum, ν_{max}/cm^{-1}: 3338 (NH), 1709 (CO), 1647 (CO).

3,5-Diacetyl-2,6-dimethyl-1,4-dihydropyridine-4-carboxylic acid (2a) was synthesized by the procedure given in literature [22]. Yield 25%, yellow solid, mp 173–174 °C (from MeOH) (lit. [22], 170 °C). ^{1}H NMR δ 2.23 (s, 6H, 2,6-CH_3), 2.27 (s, 6H, 3,5-$COCH_3$), 4.61 (s, 1H, 4-H), 8.91 (s, 1H, NH), 12.11 (s, 1H, 4-COOH). ^{13}C NMR 18.75, 29.65, 40.47, 107.67, 144.84, 174.41, 196.42. Found: C, 60.55; H, 6.36; N, 5.80. Calc. for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90%. IR spectrum, ν_{max}/cm^{-1}: 3318 (NH), 1715 (CO), 1601 (CO).

3,5-Diacetyl-2,6-dimethyl-1,4-dihydropyridine-4-carboxylic acid methyl ester (2b) was synthesized by the procedure given in literature [21]. Yield 2.4 g (63%), yellow solid, mp 151–153 °C (from MeOH). ^{1}H NMR δ 2.24 (s, 6H, 2,6-CH_3), 2.28 (s, 6H, 3,5-$COCH_3$), 3.50 (s, 3H, 4-$COOCH_3$), 4.74 (s, 1H, 4-H), 9.03 (s, 1H, NH). ^{13}C NMR 18.78, 29.74, 40.36, 51.75, 107.18, 145.43, 173.32, 196.06. Found: C, 61.87; H, 6.82; N, 5.52. Calc. for $C_{13}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.57%. IR spectrum, ν_{max}/cm^{-1}: 3307 (NH), 1728 (CO), 1675 (CO).

3,5-Diacetyl-1,2,6-trimethyl-1,4-dihydropyridine-4-carboxylic acid methyl ester (2d). To a stirred solution of compound **2b** (1.0 g, 0.004 mol) in anhydrous CH_3CN (20 mL) at r.t. was added sodium methoxide (0.3 g, 0.005 mol), as a result of which a vigorous reaction with hydrogen evolution was observed, and strongly colored anion, which gives characteristic orange fluorescence, was formed. At the end of hydrogen evolution CH_3I (1.2 mL, 0.02 mol) was added. Reaction mixture was stirred for 12 h and evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel (CH_2Cl_2-petroleum ether-acetone, 9:7:1) to give compound **2d**. Yield 0.6 g (57%), yellow solid, mp 106–108 °C (from CH_2Cl_2). ^{1}H NMR δ 2.31 (s, 6H, 2,6-$COCH_3$), 2.36 (s, 6H, 2,6-CH_3), 3.18 (s, 3H, N—CH_3), 3.51 (s, 3H, 4-$COOCH_3$), 4.73 (s, 1H, 4-H). ^{13}C NMR 16.27, 29.94, 33.97, 40.47, 51.89, 109.62, 148.99, 172.86, 196.77. Found: C, 63.42; H, 7.32; N, 5.21. Calc. for $C_{14}H_{19}NO_4$: C, 63.38; H, 7.22; N, 5.28%. IR spectrum, ν_{max}/cm^{-1}: 1736 (CO), 1649 (CO).

3,5-Dicyano-2,6-dimethyl-1,4-dihydropyridine-4-carboxylic acid monohydrate (3a) was synthesized by the procedure given in literature [22]. Yield 64%, colourless solid, mp 189–190 °C (from MeOH) (lit. [24], 190 °C). ^{1}H NMR δ 2.03 (s, 6H, 2,6-CH_3), 4.02 (s, 1H, 4-H), 9.62 (s, 1H, NH), 13.09 (s, 1H, 4-COOH). ^{13}C NMR 17.71, 40.57, 78.00, 119.03, 148.42, 172.01. Found: C, 54.21; H, 4.89; N, 18.86. Calc. for $C_{10}H_{11}N_3O_3$: C, 54.30; H, 5.01; N, 18.99%. IR spectrum, ν_{max}/cm^{-1}: 3245 (NH), 2211 (CN), 1714 (CO).

3,5-Dicyano-2,6-dimethyl-1,4-dihydropyridine-4-carboxylic acid methyl ester monohydrate (3b) was synthesized by the procedure given in literature [23]. Yield 3.2 g (86%), colourless solid, mp 176–178 °C (from MeOH) (lit. [24], 184 °C). ^{1}H NMR δ 2.04 (s, 6H, 2,6-CH_3), 3.70 (s, 3H, 4-$COOCH_3$), 4.24 (s, 1H, 4-H), 9.72 (s,

1H, NH). ^{13}C KMR 17.74, 40.45, 52.58, 77.30, 118.79, 148.95, 170.98. Found: C, 56.01; H, 5.42; N, 17.79. Calc. for $C_{11}H_{13}N_3O_3$: C, 56.16; H, 5.57; N, 17.86%. IR spectrum, ν, cm^{-1}: 3200 (NH), 2204 (CN), 1747 (CO).

3,5-Dicyano-1,2,6-trimethyl-1,4-dihydropyridine-4-carboxylic acid methyl ester (3d). To a stirred solution of compound **3b** (1.0 g, 0.005 mol) in anhydrous CH_3CN (20 mL) at r.t. was added sodium methoxide (0.3 g, 0.005 mol), as a result of which a vigorous reaction with hydrogen evolution was observed, and strongly colored anion, which gives characteristic green fluorescence, was formed. At the end of hydrogen evolution CH_3I (1.4 mL, 0.02 mol) was added. Reaction mixture was stirred for 2 h and evaporated under reduced pressure. The crude residue was treated with CH_2Cl_2 (20 mL), filtered and evaporated under reduced pressure. The residue was recrystallized from MeOH to afford compound **3d**. Yield 0.6 g (55%); colourless solid, mp 148–150 °C (from MeOH). ^{1}H NMR δ 2.24 (s, 6H, 2,6-CH_3), 3.16 (s, 3H, N—CH_3), 3.68 (s, 3H, 4-$COOCH_3$), 4.23 (s, 1H, 4-H). ^{13}C NMR 18.44, 34.52, 39.65, 52.58, 79.33, 119.33, 152.89, 170.89. Found: C, 62.23; H, 5.61; N, 18.17. Calc. for $C_{12}H_{13}N_3O_2$: C, 62.33; H, 5.67; N, 18.17%. IR spectrum, ν_{max}/cm^{-1}: 2195 (CN), 1762 (CO).

3,5-Dicyano-1,2,4,6-tetramethyl-1,4-dihydropyridine-4-carboxylic acid methyl ester (3e). To a stirred solution of compound **3b** (1.0 g, 0.005 mol) in anhydrous CH_3CN (20 mL) at r.t. was added sodium methoxide (0.5 g, 0.01 mol), as a result of which a vigorous reaction with hydrogen evolution was observed, and strongly colored anion, which gives characteristic green fluorescence, was formed. At the end of hydrogen evolution CH_3I (2.3 mL, 0.04 mol) was added. Reaction mixture was stirred for 12 h and evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel (CH_2Cl_2-petroleum ether-acetone, 9:7:1) to give compound **3e**. Yield 0.3 g (22%); beige solid, mp 103–105 °C (from MeOH). ^{1}H NMR δ 1.54 (s, 3H, 4-CH_3), 2.25 (s, 6H, 2,6-CH_3), 3.17 (s, 3H, N—CH_3), 3.68 (s, 3H, 4-$COOCH_3$). ^{13}C NMR 18.81, 25.19, 34.67, 41.98, 52.92, 85.47, 118.46, 151.09, 172.77. Found: C, 63.53; H, 6.07; N, 17.08. Calc. for $C_{13}H_{15}N_3O_2$: C, 63.66; H, 6.16; N, 17.13%. IR spectrum, ν_{max}/cm^{-1}: 2198 (CN), 1725 (CO).

5-Cyano-2,6-dimethyl-1,4-dihydropyridine-3,4-dicarboxylic acid 3-methyl ester monohydrate (4a). A mixture of methyl acetoacetate (5.4 mL, 0.05 mol) and glyoxylic acid monohydrate (4.6 g, 0.05 mol) in glacial acetic acid (10 mL) was stirred at 80 °C for 6 h. The reaction mixture was cooled and 3-aminocrotononitrile (4.1 g, 0.05 mol) was added under stirring. The mixture was stirred overnight and evaporated under reduced pressure. The remaining acetic acid was not fully removed by lyophilisation though. The crude residue was dissolved in anhyd CH_3CN (45 mL), and dicyclohexylamine (9.9 mL, 0.05 mol) was added. The mixture was stirred at r.t. for 1 h, filtered and washed with CH_3CN (10 mL). The residue was recrystallized from EtOH to afford **3-cyano-5-methoxycarbonyl-2,6-dimethyl-1,4-dihydro-pyridine-4-carboxylate dicyclohexylammonium salt**. Yield 8.2 g (39%), yellow solid, mp 227–229 °C (from EtOH). ^{1}H NMR δ 1.09–1.24 (m, 10H, CH), 1.57 (m, 2H, CH), 1.88 (m, 4H, CH), 1.69 (m, 4H), 1.97 (s, 3H, 2-CH_3), 2.16 (s, 3H, 6-CH_3), 2,78 (m, 2H), 3.53 (s, 3H, 5-$COOCH_3$), 3.74 (s, 1H, 4-H), 8.91 (s, 1H, NH). ^{13}C NMR 17.38, 17.97, 24.30, 25.24, 30.36, 41.99, 50.54, 51.80, 81.61, 98.63, 120.57, 143.73, 146.26, 167.66, 173.81. Found: C, 66.16; H, 8.45; N, 10.06. Calc. for $C_{23}H_{35}N_3O_4$: C, 66.19; H, 8.54; N, 10.23%. IR spectrum, ν_{max}/cm^{-1}: 3185 (NH), 2197 (CN), 1701 (CO), 1620 (CO).

To a stirred solution of compound **3-cyano-5-methoxycarbonyl-2,6-dimethyl-1,4-dihydropyridine-4-carboxylate dicyclohexyl-ammonium salt** (8.2 g, 0.02 mol) in H_2O (200 mL) at 70 °C was added dropwise 1 N NaOH until the mixture became basic (pH 8). It was then extracted with Et_2O (3 × 200 mL). The aqueous layer was separated and acidified with concentrated HCl to pH 3. The solution was extracted with EtOAc (3 × 200 mL). The combined organic extracts were dried (Na_2SO_4), and concentrated under reduced pressure. The residue was washed with H_2O, filtered and dried to give the desired product **4a**. Yield 3.4 g (68%), colourless solid, mp 202–203 °C (from EtOH). ^{1}H NMR δ 2.02 (s, 3H, 6-CH_3), 2.23 (s, 3H, 2-CH_3), 3.57 (s, 3H, 3-$COOCH_3$), 4.04 (s, 1H, 4-H), 9.24 (s, 1H, NH), 12.50 (s, 1H, 4-COOH). ^{13}C NMR 17.49, 18.17, 41.21, 50.91, 79.31, 96.64, 119.67, 146.05, 148.28, 167.00, 173.47. Found: C, 52.02; H, 5.46; N, 10.84. Calc. for $C_{11}H_{14}N_2O_5$: C, 51.97; H, 5.55; N, 11.02%. IR spectrum, ν_{max}/cm^{-1}: 3419 (NH), 2213 (CN), 1699 (CO).

5-Cyano-2,6-dimethyl-1,4-dihydropyridine-3,4-dicarboxylic acid dimethyl ester (4b) was synthesized by the procedure given in literature [23]. Yield 1.9 g (92%), colourless solid, mp 141–143 °C (from CH_2Cl_2). ^{1}H NMR δ 2.02 (s, 3H, 6-CH_3), 2.24 (s, 3H, 2-CH_3), 3.57 (s, 3H, 3-$COOCH_3$), 3.62 (s, 3H, 4-$COOCH_3$), 4.17 (s, 1H, 4-H), 9.31 (s, 1H, NH). ^{13}C NMR 17.53, 18.14, 41.18, 51.06, 52.11, 78.21, 95.53, 119.04, 146.99, 149.12, 166.58, 172.19. Found: C, 57.66; H, 5.55; N, 11.11. Calc. for $C_{12}H_{14}N_2O_4$: C, 57.59; H, 5.64; N, 11.19%. IR spectrum, ν_{max}/cm^{-1}: 3222 (NH), 2211 (CN), 1742 (CO).

5-Cyano-2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3-carboxylic acid methyl ester (4c). A mixture of 3-phenyl-2-propionyl-acrylic acid methyl ester (0.8 g, 0.004 mol) and 3-aminocrotononitrile (0.3 g, 0.004 mol) in EtOH (10 mL) was stirred at 60 °C for 10 h and evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel (CH_2Cl_2-petroleum ether-acetone, 9:7:2) to give compound **4c**. Yield: 0.8 g (77%), yellow crystals, mp 165–167 °C (from acetone). ^{1}H NMR δ 2.01 (s, 3H, 6-CH_3), 2.27 (s, 3H, 2-CH_3), 3.47 (s, 3H, 3-$COOCH_3$), 4.44 (s, 1H, 4-H), 7.17–7.31 (m, 5H, C_6H_5), 9.19 (s, 1H, NH). ^{13}C NMR 17.43, 18.37, 41.61, 50.72, 84.32, 99.52, 119.92, 126.74, 126.96, 128.49, 145.68, 146.24, 146.37, 167.02. Found: C, 71.49; H, 6.04; N, 10.29. Calc. for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44%. IR spectrum, ν_{max}/cm^{-1}: 3301 (NH), 2192 (CN), 1696 (CO).

5-Cyano-1,2,6-trimethyl-1,4-dihydropyridine-3,4-dicarboxylic acid dimethyl ester (4d). To a stirred solution of compound **4b** (1.0 g, 0.004 mol) in anhydrous CH_3CN (20 mL) at r.t. was added sodium methoxide (0.3 g, 0.005 mol), as a result of which a vigorous reaction with hydrogen evolution was observed, and strongly colored anion, which gives characteristic green fluorescence, was formed. At the end of hydrogen evolution CH_3I (1.3 mL, 0.02 mol) was added. Reaction mixture was stirred for 2 h and evaporated under reduced pressure. The crude residue was treated with CH_2Cl_2 (20 mL), filtered and evaporated under reduced pressure. The residue was recrystallized from MeOH to afford compound **4d**. Yield: 0.6 g (57%), colourless crystals, mp 103–105 °C (from MeOH). ^{1}H NMR δ 2.22 (s, 3H, 6-CH_3), 2.45 (s, 3H, 2-CH_3), 3.17 (s, 3H, N-CH_3), 3.61 (s, 6H, 3,4-$COOCH_3$), 4.23 (s, 1H, 4-H). ^{13}C NMR 15.84, 18.30, 34.14, 40.17, 51.41, 52.22, 79.59, 98.11, 119.77, 150.65, 152.78, 166.62, 171.75. Found: C, 58.92; H, 6.09; N, 10.41. Calc. for $C_{13}H_{16}N_2O_4$: C, 59.08; H, 6.10; N, 10.60%. IR spectrum, ν_{max}/cm^{-1}: 2201 (CN), 1743 (CO).

5-Acetyl-2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3-carbonitrile (5c). A mixture of 3-benzylidene-2,4-pentanedione (1.8 g, 0.01 mol) and 3-amino-crotononitrile (0.8 g, 0.01 mol) in glacial acetic acid (10 mL) was stirred for 12 h and evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel (CH_2Cl_2-petroleum ether-acetone, 9:7:1) to give compound **5c**. Yield: 1.1 g (46%), yellow crystals, mp 54–56 °C (from diethyl ether). ^{1}H NMR δ 1.99 (s, 3H, 2-CH_3), 2.00 (s, 3H, 5-$COCH_3$), 2.27 (s, 3H, 6-CH_3), 4.58 (s, 1H, 4-H), 7.17–7.34 (m, 5H, C_6H_5), 9.13 (s, 1H, NH). ^{13}C NMR 17.37, 19.18, 29.83, 40.74, 85.21, 108.87, 119.93, 126.83, 127,03, 128.67, 144.59, 145.84, 145.93, 196.88. Found: C, 75.92; H, 6.44; N, 10.94. Calc.

for $C_{16}H_{16}N_2O$: C, 76.16; H, 6.39; N, 11.10%. IR spectrum, ν_{max}/cm^{-1}: 3286 (NH), 2197 (CN), 1667 (CO).

5-Acetyl-2,6-dimethyl-1,4-dihydropyridine-3,4-dicarboxylic acid 3-methyl ester (6a). A mixture of methyl acetoacetate (5.4 mL, 0.05 mol) and glyoxylic acid monohydrate (4.6 g, 0.05 mol) in glacial acetic acid (10 mL) was stirred at 80 °C for 6 h. The reaction mixture was cooled and acetylacetonamine (7.4 g, 0.07 mol) was added under stirring. The mixture was stirred overnight and evaporated under reduced pressure. The crude residue was treated with H_2O (30 mL), the precipitate was removed by filtration, washed with H_2O, and dried to give the product **6a**. Yield: 6.8 g (53%), yellow solid, mp 189–190 °C (from MeOH). ^{1}H NMR δ 2.19 (s, 3H, 6-CH_3), 2.25 (s, 3H, 2-CH_3), 2.26 (s, 3H, 5-$COCH_3$), 3.61 (s, 3H, 3-$COOCH_3$), 4.58 (s, 1H, 4-H), 8.91 (s, 1H, NH), 12.06 (s, 1H, 4-COOH). ^{13}C NMR 17.88, 18.71, 29.44, 40.06, 50.88, 97.63, 106.83, 144.74, 146.21, 167.15, 174.38, 196.75. Found: C, 56.88; H, 5.86; N, 5.49. Calc. for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53%. IR spectrum, ν_{max}/cm^{-1}: 3310 (NH), 1734 (CO), 1672 (CO).

5-Acetyl-2,6-dimethyl-1,4-dihydropyridine-3,4-dicarboxylic acid dimethyl ester (6b) was synthesized by the procedure given in literature [21]. Yield 4.0 g (87%), yellow solid, mp 158–160 °C (from MeOH). ^{1}H NMR δ 2.21 (s, 3H, 6-CH_3), 2.25 (s, 3H, 2-CH_3), 2.26 (s, 3H, 5-$COCH_3$), 3.50 (s, 3H, 4-$COOCH_3$), 3.62 (s, 3H, 3-$COOCH_3$), 4.71 (s, 1H, 4-H), 8.99 (s, 1H, NH). ^{13}C NMR 17.89, 18.79, 29.58, 40.05, 50.95, 51.76, 96.98, 106.42, 145.35, 146.78, 166.95, 173.32, 196.22. Found: C, 58.35; H, 6.36; N, 5.07. Calc. for $C_{13}H_{17}NO_5$: C, 58.42; H, 6.41; N, 5.24%. IR spectrum, ν_{max}/cm^{-1}: 3344 (NH), 1707 (CO), 1676 (CO).

5-Acetyl-2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3-carboxylic acid methyl ester (6c). A mixture of 3-benzylidene-2,4-pentanedione (1.8 g, 0.01 mol) and methyl-3-aminocrotonate (1.1 g, 0.01 mol) in EtOH (20 mL) was stirred at 60 °C for 6 h and evaporated under reduced pressure. The residue was recrystallized from MeOH to afford compound **6c**. Yield: 1.3 g (48%), yellow crystals, mp 172–173 °C (from MeOH) (lit. [18], 170–172 °C). ^{1}H NMR δ 2.12 (s, 3H, 5-$COCH_3$), 2.23 (s, 3H, 2-CH_3), 2.28 (s, 3H, 6-CH_3), 3.58 (s, 3H, 3-$COOCH_3$), 4.94 (s, 1H, 4-H), 7.11–7.21 (m, 5H, C_6H_5), 8.87 (s, 1H, NH). ^{13}C NMR 18.14, 19.08, 30.02, 38.78, 50.68, 102.48, 111.42, 125.98, 127.07, 128.13, 144.89, 145.12, 147.40, 167.39, 196.36. Found: C, 71.41; H, 6.81; N, 4.83. Calc. for $C_{17}H_{19}N_2O_3$: C, 71.56; H, 6.71; N, 4.91%. IR spectrum, ν_{max}/cm^{-1}: 3270 (NH), 1682 (CO), 1655 (CO).

5-Acetyl-1,2,6-trimethyl-1,4-dihydropyridine-3,4-dicarboxylic acid dimethyl ester (6d). To a stirred solution of compound **6b** (2.0 g, 0.008 mol) in anhydrous CH_3CN (40 mL) at r.t. was added sodium methoxide (0.5 g, 0.009 mol), as a result of which a vigorous reaction with hydrogen evolution was observed, and strongly colored anion, which gives characteristic orange fluorescence, was formed. At the end of hydrogen evolution CH_3I (1.2 mL, 0.04 mol) was added. Reaction mixture was stirred for 12 h and evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel (CH_2Cl_2-petroleum ether-acetone, 9:7:1) to give compound **6d**. Yield: 1.2 g (53%), colourless crystals, mp 79–81 °C (from MeOH). ^{1}H NMR δ 2.28 (s, 3H, 5-$COCH_3$), 2.33 (s, 3H, 6-CH_3), 2.45 (s, 3H, 2-CH_3), 3.17 (s, 3H, N-CH_3), 3.50 (s, 3H, 4-$COOCH_3$), 3.65 (s, 3H, 3-$COOCH_3$), 4.77 (s, 1H, 4-H). ^{13}C NMR 15.71, 16.37, 29.77, 33.98, 39.84, 51.26, 51.86, 99.32, 109.27, 148.76, 151.01, 166.85, 172.78, 196.96. Found: C, 59.69; H, 6.84; N, 4.87. Calc. for $C_{14}H_{19}NO_5$: C, 59.78; H, 6.81; N, 4.98%. IR spectrum, ν_{max}/cm^{-1}: 1742 (CO), 1692 (CO).

X-ray crystallography

Single crystals suitable for X-ray analysis were obtained by slow evaporation from saturated MeOH (**1a**-**3a**, **6a**, **1b**-**3b**, **6b**, **2c**, **6c**, **3d-4d**, **6d**, **3e**), EtOH (**1c**, **4a**), acetone (**3c**–**4c**), CH_2Cl_2 (**4b** and **2d**) and diethyl ether (**5c**), however suitable crystals for **6d** were not obtained.

X-ray crystal structure determination was performed on Bruker-Nonius KappaCCD automated diffractometer using graphite monochromated Mo Kα radiation (λ = 0.71073 Å). The crystal structures of compounds **1a**–**6c** were solved by the direct method and refined by full-matrix least squares. The details of X-ray structures, data collections and refinements are listed in Supplementary material Figs. 1–6, Table 1 and 2.

NMR spectroscopy

The NMR spectra were measured at 298 K. Sample concentrations were 0.02 M. The 1,4-DHINA derivatives were dissolved in deuterated dimethyl sulphoxide (DMSO-d_6) for NMR experiments. The ^{1}H chemical shifts were referenced to tetramethylsilane at 0.0 ppm, the ^{13}C chemical shifts to the solvent DMSO-d_6 at 39.5 ppm and the ^{15}N chemical shifts to liquid NH_3 at 0.0 ppm.

The isotropic ^{1}H, ^{13}C and ^{15}N chemical shifts were determined with an experimental error ±0.01 ppm, ±0.1 ppm and ±0.2 ppm accordingly; the experimental error in the determination of the $^1J(^{15}N—^1H)$ and $^1J(^{13}C_4—^1H)$ values was ±0.1 Hz.

The ^{1}H, ^{15}N and ^{13}C NMR experiments were carried out on *Varian INOVA 600 MHz* instrument operating at 599.9 MHz (^{1}H), 60.8 MHz (^{15}N) and 150.8 MHz (^{13}C) and equipped with cryoprobe. Accurate one bond $^1J(^{15}N—^1H)$ and $^1J(^{13}C—^1H)$ coupling constants were directly extracted from conventional 1D ^{1}H NMR spectra. The ^{1}H—^{15}N gradient-selected heteronuclear single quantum correlation (gHSQC) experiments were used to measure the $\delta(^{15}N)$ chemical shifts. Experiments were adjusted for a direct $^nJ(^{15}N—^1H)$ coupling constants of 3.0 Hz and 95.0 Hz. The spectral widths for ^{1}H and ^{15}N were 8000 and 20,000 Hz, respectively. Each ^{1}H—^{15}N gHSQC spectrum was collected with 2048 points in the direct ^{1}H dimension and 256 increments in the indirect ^{15}N dimension. Before Fourier transformation, the dataset was zero-filled to a 2048 × 2048 matrix and subjected to the application of cosine window function.

Theoretical calculations

For theoretical calculations the geometries of various conformations of symmetrically and unsymmetrically substituted 1,4-DHINA derivatives have been optimized at density functional theory (DFT) level using B3LYP with 6-31G* basis set method in both gas and liquid – $CHCl_3$ and DMSO (Poisson Boltzmann Finite element method) phases. The geometry optimization of 1,4-DHINA derivatives has been carried out with different starting points – several initial structures/conformations for each molecule. The starting structures of 1,4-DHINA derivatives have been based on some known typical values of bond lengths and angles. All quantum chemical calculations were performed by using Jaguar 8.0 program package [25]. The optimized geometries were used to obtain the Mulliken charges on nitrogen and carbon atoms in heterocycle. The ^{15}N and ^{13}C NMR chemical shifts were calculated with gauge-including atomic orbitals (GIAO) approach by subtracting the shieldings of the individual atoms from the shielding of the standard δ ($^{15}N_{NH3}$) = 254.4 ppm, δ ($^{13}C_{TMS}$) = 189.5 ppm calculated using the same method in the gas phase.

Results and discussion

Synthesis

The symmetrical 1,4-DHINA 1a-3a were prepared (25–64% yield) according to the modified Hantzsch condensation of one

Table 2
The X-ray geometric and puckering parameters of the 1,4-DHP ring in compounds **1a–6a, 1b–6b, 1c–6c, 1d–5d, 1e, 3e, 5e and 1f.**

1,4-DHP	C(4) dev[a], Å	N(1) dev[a], Å	φ_1, (°)	φ_2, (°)	*P*, (°)	τ, (°)
1a	0.233(9)	0.102(7)	167.5(5)	175.5(5)	69.3 (7)	79.2
1b	0.313(4)	0.132(3)	5.4(6)	−171.5(3)	91.9(4)	82.8
1c	0.341(9)	0.168(8)	176.6(6)	6.0(1)	102.0(8)	85.8
1d	0.520(7)	0.283(6)	162.4(6)	−15.0(1)	30.4(3)	85.6
1e	0.419(4)	0.209(3)	−26.9(6)	37.6(6)	122.4(5)	88.4
1f	0.325(3)	0.163(3)	−28.7(3)	−41.9(3)	94.6(3)	83.2
2a	0.341(1)	0.152(1)	167.7(8)	170.0(8)	102.0(1)	76.5
2b	0.332(3)	0.159(2)	−14.9(3)	171.0(2)	99.8(3)	82.3
2c	0.341(2)	0.143(2)	160.8(2)	−14.9(3)	99.7(2)	83.8
2d	0.553(3)	0.262(3)	−159.8(2)	−15.2(3)	161.9(2)	85.5
3a	0.319(1)	0.171(1)	–	–	95.8(2)	90.0
3b	0.134(3)	0.052(2)	–	–	38.5(3)	72.3
3c	0.107(4)	0.045(3)	–	–	31.1(3)	88.4
3d	0.374(3)	0.169(3)	–	–	109.4(3)	75.6
3e	0.203(2)	0.118(2)	–	–	61.6(2)	71.9
4a	0.255(4)	0.100(3)	175.9(3)	–	74.3(3)	80.4
4b mol A	0.182(4)	0.066(4)	2.5(5)	–	52.3(4)	80.3
4b mol B	0.232(4)	0.091(3)	2.7(5)	–	67.4(4)	79.9
4c mol A	0.214(3)	0.116(2)	4.7(4)	–	65.0(3)	76.9
4c mol B	0.260(3)	0.123(3)	2.3(4)	–	77.2(3)	76.1
4d	0.427(3)	0.223(2)	−21.5(3)	–	128.0(3)	79.0
5a	0.012(4)	0.023(3)	−174.5(5)	–	14.9(3)	72.9
5b mol A	0.213(3)	0.077(3)	−172.2(5)	–	61.4(4)	74.2
5b mol B	0.303(3)	0.136(3)	−168.4(5)	–	89.8(4)	79.6
5c	0.009(2)	0.015(2)	−32.4(2)	–	14.8(2)	87.7
5d	0.445(3)	0.218(3)	−33.3(4)	–	131.3(4)	86.2
5e	0.022(3)	0.028(3)	−134.1(4)	–	14.7(3)	89.5
6a	0.347(3)	0.153(3)	0.8(4)	169.9(2)	103.0(3)	86.3
6b	0.232(8)	0.125(6)	−3.4(7)	−30.7(7)	70.7(6)	84.2
6c	0.364(2)	0.154(2)	−3.3(3)	−174.8(2)	106.7(2)	85.0

[a] Deviation from the least-squares plane defined by C(2)—C(3)—C(5)—C(6).

equivalent of a glyoxylic acid with two equivalents of a methyl β-aminocrotonate, acetylacetone imine or 3-amino-crotononitrile, as reported in Scheme 1.

The unsymmetrical 1,4-DHINA **4a** and **6a** were synthesized by condensing first glyoxylic acid in a Knoevenagel manner with methyl β-aminocrotonate. The condensation of Knoevenagel adduct with 3-aminocrotononitrile or acetylacetone imine gives 1,4-DHINA **4a** and **6a** in 68% and 53% overall yield (Scheme 2).

The esterification of the carboxylic acids **1a–4a** and **6a** was realized in a similar manner to the procedures described previously [21,23] in 63–93% yield.

The *N*-substituted and 4,4-disubstituted 1,4-DHINA derivatives **2d–4d**, **6d** (∼55% yield) and **3e** (22% yield) were obtained by the treatment of 1,4-DHINA methyl esters **2b–4b** or **6b** with sodium methoxide and methyl iodide.

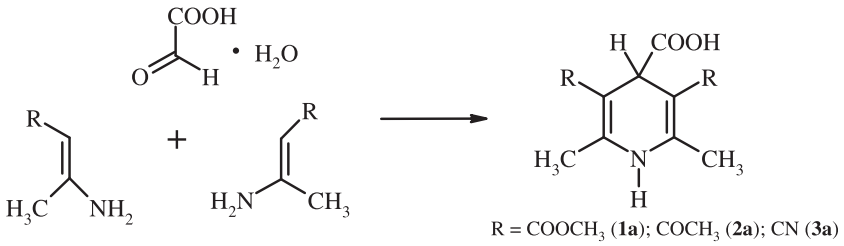
The unsymmetrical 4-phenyl-1,4-DHP derivatives **4c–6c** were prepared (48–77% yield) by the modified method of the Hantzsch condensation which involved Michael addition of a Knoevenagel adduct with an enamine.

The structures of the title compounds were confirmed by IR, ^{1}H, ^{13}C and ^{15}N NMR spectroscopy, X-ray and elemental analysis. All final products were pure and stable compounds. Chemical structures of the prepared compounds as well as compounds which were reported previously are summarized in Table 1.

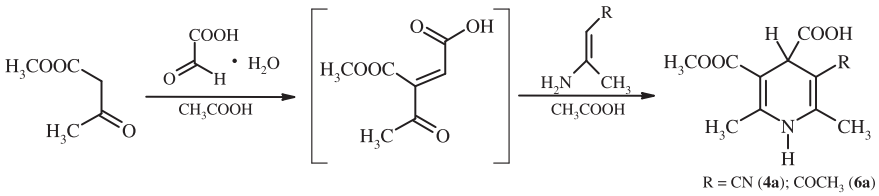
X-ray crystallography

Many X-ray structural analyses of 1,4-DHPs show that the dihydropyridine ring has a flattened boat conformation [8–19]. The X-ray structural studies of 4-phenyl substituted 1,4-DHPs indicate that the degree of puckering of the 1,4-DHP ring is related to pharmacological activity, the latter increasing with increasing ring planarity [8,26]. The sum of the absolute values of the ring internal torsion angles (*P*) was suggested as a quantitative measure for the evaluation of the flatness of the six-membered ring [9]. These values range from 14.7(3)° (**5e**) to 161.9(2)° (**2d**) in Table 2, indicating a significant amount of flattening from the ideal boat conformation (*P* = 90°). A mean value of 77(2)° was found previously for 1,4-DHP rings, although the *P* values generally vary over a wide range from 4° to 130° [27]. For example, in nifedipine, a calcium channel blocking drug, the value of *P* is 72° [8,26].

The crystal structures for the compounds studied (**1a–6a**, **1b–6b**, **1c–6c**, **1d–5d**, **1e**, **3e**, **5e** and **1f**) reveal that the 1,4-DHP ring exists in a boat form flattened at the N(1) and puckered at



Scheme 1. Synthesis of symmetrically substituted 1,4-dihydroisonicotinic acid **1a–3a**.



Scheme 2. Synthesis of unsymmetrically substituted 1,4-dihydroisonicotinic acid **4a** and **6a**.

the C(4) atoms as evidenced by the distances of N(1) and C(4) to the main plane C(2)—C(3)—C(5)—C(6) of the olefinic double bonds (Table 2). The C(4) puckering, which varies considerably among the compounds, is linearly related to that at the nitrogen (Fig. 1); the greater the displacement of C(4) from the ring, the larger is the ring puckering at N(1).

Comparative analysis of the data reported in Table 2 indicates that the deviations of the C(4) and N(1) atoms from the planarity follow the order $COCH_3$ > $COOCH_3$ > CN. So, the substitution with ester and acetyl groups at C(3) and C(5) positions induces more deviation on C(4) and N(1) atoms from the plane. A completely planar 1,4-DHP ring was found in the structures of **5a**, **5c** and **5e**, but N(1) substitution with methyl group (**1d**–**5d**) causes the 1,4-DHP ring to become more puckered (Table 2).

Comparative analysis with 4-phenyl group substituted 1,4-DHP derivatives **1c**–**6c** was carried out to elucidate the individual effect of 4-carboxyl and 4-methoxycarbonyl groups on the 1,4-DHP ring planarity. No relation between the nature of C(4) substituent and the puckering of the C(4) and N(1) atoms could be established.

Similarly to 4-phenyl substituted 1,4-DHP derivatives **1c**–**6c** the 4-carboxyl and 4-methoxycarbonyl groups in compounds **1a**–**6a**, **1b**–**6b**, **1d**–**5d** and **3e** prefer a pseudo-axial position on C(4). The dihedral angle (τ) between the least-squares plane of the 1,4-DHP ring and the 4-carboxyl or 4-methoxycarbonyl groups range from 71.9° (**3e**) to 90.0° (**3a**) indicating that both groups are almost perpendicular to the 1,4-DHP ring. It is worth noting that both 4-carboxyl and 4-methoxycarbonyl groups can rotate freely about their adjacent C(4)—CO bond.

However, the X-ray structures of 4,4-disubstituted 1,4-DHP derivatives **1e**, **1f** and **5e** in comparison with those of 4-monosubstituted **1d** and **5d** revealed drastic changes in conformation caused by the second 4-substitution. In 4-alkyl-4-methoxycarbonyl disubstituted 1,4-DHP (**1e**, **1f** and **5e**) 4-methoxycarbonyl group moved from pseudo-axial into the pseudo-equatorial conformation, as already observed in other 4-phenyl-4-methyl disubstituted 1,4-DHP structure [15]. It seems that the steric influence of alkyl group dominates the conformation.

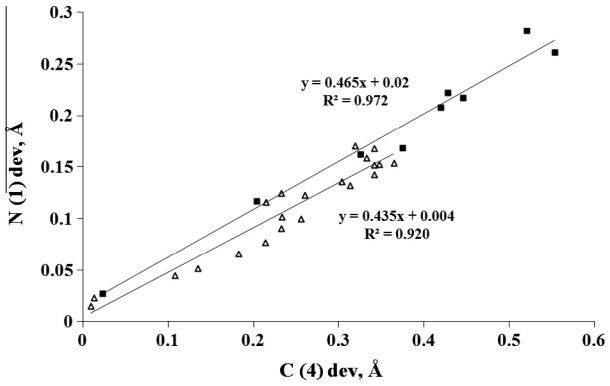


Fig. 1. The correlations of the NH (Δ) and NCH_3 substituted (■) 1,4-dihydropyridine ring distortion of C(4) with that at N(1).

The C(4) hydrogen substitution with alkyl group in compounds **1e**, **1f**, **3e** and **5e** causes the 1,4-DHP ring to become more flat (Table 2).

The carbonyl groups at the C(3) and C(5) positions of the 1,4-DHP ring are of crucial importance to the pharmacological effect, therefore important aspect is the rotation of the carbonyl group. It has been shown that unsymmetrical substituents at the C(3) and C(5) positions alter the activity [28].

Assuming a favoured coplanar arrangement of the ester or acetyl carbonyl with the double bound of the dihydropyridine ring, in principle, three different conformations at 3(5)-positions are possible for the carbonyl groups: *s-trans/s-trans*, *s-cis/s-cis*, and enantiomeric *s-cis/s-trans* and *s-trans/s-cis*. X-ray structural investigations, theoretical calculations and *in vitro* analyses of fused 1,4-DHPs indicate that, probably, at least one of the carbonyl must be in the *s-cis* arrangement making hydrogen bond to the receptor [28].

The orientation of ester and acetyl carbonyls is defined by torsion angles C(2)—C(3)—C(7)—O(8) (φ_1) and C(6)—C(5)—C(9)—O(10) (φ_2) in Table 2. An analysis of results of 22 X-ray structures shows a preference for the *s-trans/s-cis* arrangement in the crystal of 1,4-DHPs **1a**–**1f**, **2a**–**2d**, **6a**–**6c**, and a preference for the *s-cis* arrangement is found for unsymmetrical ones **4a**–**4d**, **5a**–**5e** (Table 2). Only in two cases of **1a** and **2a** both carbonyl groups have *s-trans/s-trans* arrangement. Examination of φ_1 and φ_2 (Table 2) revealed that ester and acetyl groups remain nearly coplanar with the plane of the 1,4-DHP ring due to the electron delocalization in the conjugated double bond system.

It is worth noting that in the crystal structure of compounds **3a**, **3b** and **4a** a water molecule has been found, which forms an intermolecular hydrogen bond system with 1,4-DHP carboxylic acid and NH group. The lengths of these hydrogen bonds are O(12)—H···O(1w) 1.73(2) Å; O(12)···O(1w) 2.65(2) Å (**3a**); O(11)—H···O(1w) 1.70(2)···Å; O(11)···O(1w) 2.58(3) Å (**4a**) and N(1)—H···O(1w) 2.01(1) Å; N(1)···O(1w) 2.82(2) Å (**3b**). In **4b** and **4c** there are two independent molecules with similar conformation in the crystal asymmetric unit.

Quantum chemical conformational analysis

The prepared compounds as well as compounds which were reported previously were subjected to conformational analysis by quantum chemical calculations. The high level density functional theory (DFT) B3LYP method with 6-31G* basis set was used to obtain the optimum structures of the 1,4-DHINA derivatives in gas and liquid ($CHCl_3$ and DMSO) phases. The calculations in gas phase produced the same relative conformation patterns with respect to the liquid phase ($CHCl_3$ and DMSO) calculations (Supplementary material Tables 3–5). The results of calculations in gas phase are summarized in Table 3, and the optimized structures for some representative 1,4-DHINA derivatives **1b**–**6b** are demonstrated in Scheme 3.

The 1,4-DHP ring puckering analysis confirmed the known preference for a flattened boat conformation for all of the considered structures **1a**–**6a**, **1b**–**6b**, **1c**–**6c**, **1d**–**6d**, **1e**, **3e**, **5e** and **1f**. Stronger

Table 3
The gas phase optimized geometric and puckering parameters for the 1,4-DHP ring in compounds **1a–6a**, **1b–6b**, **1c–6c**, **1d–6d**, **1e**, **3e**, **5e** and **1f** using B3LYP/6-31G* method.

1,4-DHP	C(4) dev[a], Å	N(1) dev[a], Å	φ_1, (°)	φ_2, (°)	*P*, (°)	τ, (°)
1a	0.389	0.171	2.85	7.65	114.8	75.6
1b	0.400	0.176	2.45	7.50	118.0	75.7
1c	0.298	0.128	−1.60	1.27	87.1	89.9
1d	0.469	0.246	6.02	12.31	140.8	83.1
1e	0.494	0.252	−27.83	−145.99	143.2	90.0
1f	0.582	0.285	146.99	28.19	166.1	70.1
2a	0.429	0.184	−5.54	−4.78	125.8	76.0
2b	0.429	0.186	−5.16	−3.68	126.1	75.4
2c	0.349	0.145	5.99	−3.62	101.2	89.9
2d	0.492	0.244	−14.07	−5.76	145.9	88.8
3a	0.273	0.123	–	–	80.1	82.6
3b	0.278	0.119	–	–	81.0	83.8
3c	0.202	0.102	–	–	60.4	88.3
3d	0.354	0.165	–	–	103.4	85.5
3e	0.336	0.160	–	–	87.5	83.4
4a	0.262	0.116	5.18	–	76.9	79.3
4b	0.279	0.122	5.26	–	81.9	79.3
4c	0.252	0.120	−2.85	–	74.9	86.2
4d	0.382	0.195	−8.14	–	113.5	83.1
5a	0.307	0.120	0.38	–	88.6	86.9
5b	0.314	0.120	0.46	–	90.1	87.7
5c	0.269	0.117	4.06	–	78.6	82.7
5d	0.452	0.213	16.33	–	132.6	86.8
5e	0.401	0.182	132.34	–	115.0	70.9
6a	0.389	0.161	−7.25	−11.26	113.5	81.3
6b	0.397	0.163	−6.38	−11.53	115.7	81.3
6c	0.338	0.144	−3.85	1.91	98.6	86.1
6d	0.470	0.237	−7.71	−14.94	140.0	86.3

[a] Deviation from the least-squares plane defined by C(2)–C(3)–C(5)–C(6).

distortion from planarity around the C(4) was highlighted for all compounds (Table 3). The C(4) puckering is linearly related to that at the N(1) (Fig. 2).

The N(1) atom substitution with methyl group (**1d–5d**) causes the 1,4-DHP ring become more puckered (Fig. 2, Table 3). The quantum chemical calculations of these compounds show that

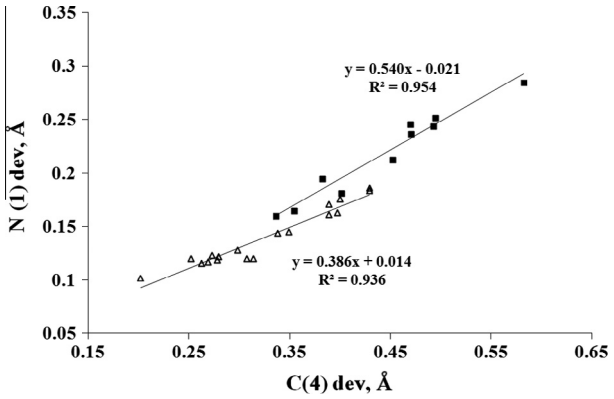


Fig. 2. The correlations of the NH (Δ) and NCH_3 substituted (■) 1,4-DHP ring distortion of C(4) with that at N(1) obtained by calculations in gas phase.

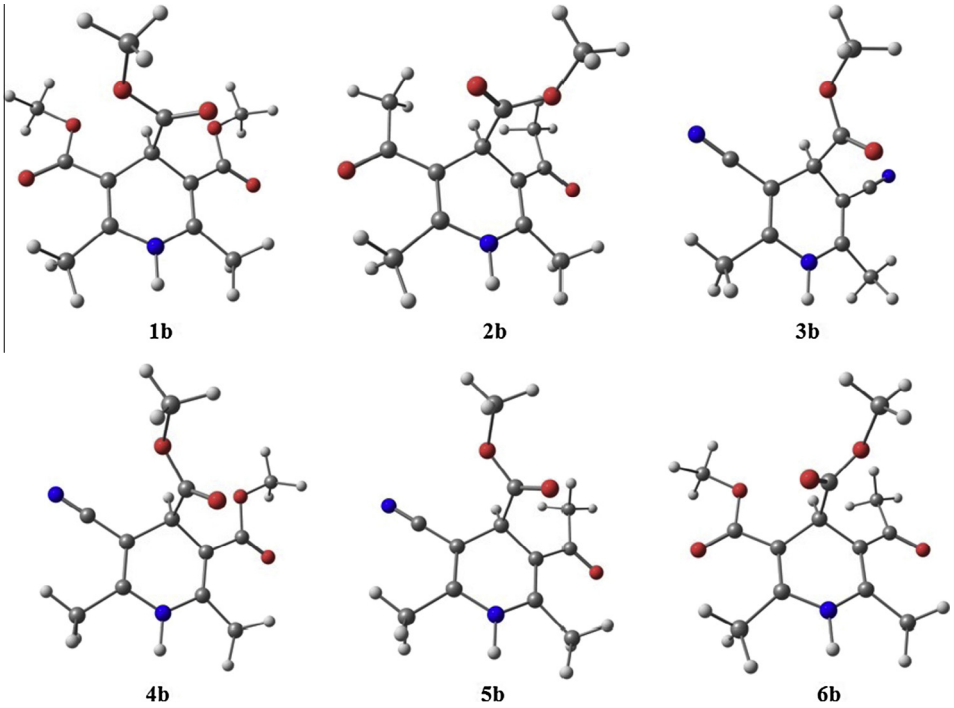
the *N*-methyl substituent occupies a pseudo-equatorial position that agrees with X-ray structural analysis.

The sum of the absolute values of the ring internal torsion angles (*P*) ranges from 60.4° to 166.1° in Table 3. These findings are in good agreement with the results of X-ray studies (Table 2).

Both X-ray structural analysis and quantum chemical calculations show that the change of the planarity of 1,4-DHP ring has small effect on bond lengths in 1,4-DHP heterocycle (Supplementary material Tables 2–5).

Regarding the orientation of the 4-carboxyl and 4-methoxycarbonyl groups with respect to the 1,4-DHP ring, the computational data confirmed the preference of C(4) substituent for pseudo-axial position. The pseudo-equatorial conformation of 4-methoxycarbonyl group was observed in 4-alkyl-4-methoxycarbonyl substituted 1,4-DHPs **1e**, **1f** and **5e** what is in agreement with the X-ray results.

The dihedral angle (τ) between the C(4) substituent and 1,4-DHP ring ranges from 70.1° to 90.0° which indicates that they are nearly perpendicular to each other (Table 3) as it was found by the X-ray analysis (Table 2).



Scheme 3. The DFT optimized structures of 1,4-DHINA derivatives **1b–6b**.

Unlike calculations reported in literature [29–32], the orientation of the carbonyl groups at the C(3) and C(5) positions of the studied 1,4-DHP derivatives **1a–1f**, **2a–2d** and **6a–6c**, the *s-cis/s-cis* arrangement seemed to be the preferred one both in gas and liquid phases, with the *s-cis/s-trans* arrangement showing up only in compounds **1e** and **1f** (Table 3). Interestingly, the *s-trans/s-trans* conformation was obtained for none of the 1,4-DHP derivatives. In the optimized structures of unsymmetrical 1,4-DHPs **4a–4d** and **5a–5d** the carbonyl group at C(3) or C(5) position is oriented in *s-cis* arrangement, only in **5e** the carbonyl group is oriented in *s-trans* conformation (Table 3). These small differences relative to X-ray studies (Table 2) could be explained by crystal packing effects in solids. Quite recently, it was found by quantum chemical calculations that C(3) and C(5) carboxyl group rotation around C(3,5)—CO has energy barrier 8–10 kcal/mol [33].

This conformational flexibility of 1,4-DHINA derivatives could be of great relevance and could be exploited in the interaction process with the biological receptor.

Most conclusions about conformational characteristics of 1,4-DHP derivatives come from X-ray analysis and theoretical calculations, however these data may not reflect the conformation in solution. In order to improve and to complete the insight into the conformation of 1,4-DHPs, we present here NMR study of these compounds.

Spectral analysis

Several experimental studies have shown the usefulness of the ^{1}H, ^{13}C and ^{15}N NMR in structural and conformational studies of 1,4-DHP derivatives; in fact, the chemical shifts of these nuclei are highly influenced by structural changes [33–40]. In particular, ^{1}H and ^{13}C chemical shifts for a series of 4-phenyl substituted 1,4-DHPs have been studied [33–39], however, no systematic investigation has been made of substituent effects on NMR data for 1,4-DHINA derivatives.

The representative ^{1}H, ^{13}C, ^{15}N NMR chemical shifts and direct $^{1}J(^{15}N—^{1}H)$, $^{1}J(^{13}C_4—^{1}H)$ spin–spin coupling constants measured for 1,4-DHP derivatives **1a–6a**, **1b–6b**, **1c–6c**, **1d–6d**, **3e** and **5e** are summarized in Table 4 (fully in Supplementary material Tables 6 and 7).

The electronegativity of the substituents at C(3,5) positions of heterocycle has a straightforward effect on the $N^{1}H$ and $C_4—^{1}H$ proton chemical shifts (Table 4). The $N^{1}H$ chemical shifts in 3(5)-cyano-1,4-DHP analogues (**3a–3c**, **4a–4c** and **5a–5c**) are shifted downfield compared with 3(5)-acetyl- or 3(5)-methoxycarbonyl-ones (**1a–1c**, **2a–2c** and **6a–6c**) (Table 4). This indicates that both double bonds and the p-orbital of the nitrogen are in stronger conjugation in 3(5)-cyano-1,4-DHP analogues (**3a–3c**, **4a–4c** and **5a–5c**). Substituents at C(3,5) positions have a direct influence on the chemical shift of $C_4—^{1}H$, leading to upfield shifts for 3(5)-cyano-1,4-DHP derivatives (**3a–3c**, **4a–4c** and **5a–5c**) and downfield shifts for 3(5)-acetyl- or 3(5)-methoxy-carbonyl-ones (**1a–1c**, **2a–2c** and **6a–6c**) (Table 4). This effect can be attributed at least partially to the anisotropic effect of the C(3,5) substituents.

The chemical shift of the $C_4—^{1}H$ protons varies with the nature of the C(4) substituent (Table 4). In the case of the 4-phenyl substituted 1,4-DHPs **1c–6c** the signal corresponding to the $C_4—^{1}H$ atom in the 1,4-DHP ring is shifted downfield by 0.3–0.4 ppm as compared with 4-carboxyl substituted 1,4-DHP analogues (**1a–6a**), due to the influence of the anisotropic effect of the phenyl group.

The effect of the substituent at C(4) position of heterocycle on the $N^{1}H$ chemical shifts is small (Table 4).

The signals of carbon-13 resonance in the 1,4-DHP moiety were found in the range from 144.4 to 152.9 ppm for the C(2,6) atoms, in the range 77.3–112.5 ppm for the C(3,5) atoms, and in the range from 38.4 to 41.9 ppm for the C(4) atom (Table 4). As the electron-withdrawing characteristics of the C(3,5) substituents are increased in the series $COCH_3 < COOCH_3 < CN$ the signals corresponding to the C(2,6) atoms, as well as the C(4) carbon are shifted downfield. The upfield shift was observed for the carbons located at the C(3,5) positions of the 1,4-DHP ring (Table 4). In the case of *N*-methyl 1,4-DHP derivatives **1d–6d** the signals corresponding to the C(2,6) atoms are shifted downfield, while C(3,5) signals are shifted upfield, reflecting substantial polarization of the C(2,6)=C(3,5) double bonds under the influence of *N*-methyl group (Table 4). The introduction of a methyl group to the nitrogen

Table 4
The ^{1}H, ^{15}N and ^{13}C NMR chemical shifts and coupling constants $^{1}J(^{15}N—^{1}H)$, $^{1}J(^{13}C_4—^{1}H)$ in DMSO-d_6 for compounds **1a–6a**, **1b–6b**, **1c–6c**, **1d–6d**, **3e and 5e.**

1,4-DHP	δ, ppm								^{1}J, Hz	
	^{15}N	$N^{1}H$	$C_4—^{1}H$	C(2)	C(6)	C(3)	C(5)	C(4)	$^{15}N—^{1}H$	$^{13}C_4—^{1}H$
1a	139.7	8.93	4.58	146.2	146.2	97.0	97.0	39.2	−94.3	135.7
1b	140.2	9.01	4.69	146.8	146.8	96.3	96.3	39.4	−94.4	136.7
1c	138.9	8.87	4.88	145.7	145.7	101.5	101.5	38.5	−94.3	136.2
1d	126.5	–	4.83	150.9	150.9	99.1	99.1	38.9	–	135.8
2a	141.5	8.91	4.61	144.8	144.8	107.7	107.7	40.5	−93.8	131.8
2b	141.9	9.03	4.74	145.4	145.4	107.2	107.2	40.4	−93.8	133.1
2c	141.2	8.88	5.03	144.4	144.4	112.5	112.5	38.7	−93.6	132.2
2d	128.2	–	4.73	149.0	144.0	109.6	109.6	40.5	–	131.2
3a	132.3	9.62	4.02	148.4	148.4	78.0	78.0	40.6	−96.0	138.8
3b	133.0	9.72	4.24	148.9	148.9	77.3	77.3	40.5	−96.4	140.1
3c	130.5	9.52	4.39	146.7	146.7	82.7	82.7	40.9	−96.4	138.2
3d	123.3	–	4.23	152.9	152.9	79.3	79.3	39.7	–	138.7
3e	122.7	–	1.54	151.1	151.1	85.5	85.5	42.0	–	–
4a	134.8	9.24	4.04	146.1	148.3	96.6	79.3	41.2	−95.2	137.1
4b	134.9	9.31	4.17	147.0	149.1	95.5	78.2	41.2	−95.7	139.3
4c	133.7	9.19	4.44	145.7	146.4	99.5	84.3	40.6	−95.3	136.5
4d	124.7	–	4.23	150.7	152.8	98.1	79.6	40.2	–	137.4
5a	136.0	9.21	4.16	145.0	148.3	106.3	79.4	41.0	−94.9	136.0
5b	136.3	9.29	4.27	145.7	148.8	106.2	78.9	41.2	−95.2	137.6
5c	134.3	9.13	4.58	144.6	145.9	108.9	85.2	40.7	−94.9	134.9
5d	124.5	–	4.35	148.2	152.6	108.3	80.0	40.4	–	135.0
5e	122.0	–	1.25	148.9	150.6	116.7	86.3	45.4	–	–
6a	140.3	8.91	4.58	146.2	144.7	97.6	106.8	40.1	−94.4	133.8
6b	140.8	8.99	4.71	146.8	145.3	97.0	106.4	40.1	−94.1	134.9
6c	140.0	8.87	4.94	145.1	144.9	102.5	111.4	38.8	−93.8	134.0
6d	126.8	–	4.77	151.0	148.8	99.3	109.3	39.8	–	133.5

atom significantly changes the depth of the 1,4-DHP boat (X-ray data in Table 2) that reduces the role of the nitrogen unshared electron pair in the overall conjugation of the heterocycle. In the case of 4,4-disubstituted 1,4-DHP **3e** and **5e** introduction of methyl group instead of proton at C(4) position causes drastic downfield shift to the C(3,5) and C(4) resonances (Table 4). This effect could be probably explained by the shift of pseudo-equatorial into pseudo-axial conformation of 4-methoxycarbonyl group at C(4) position in **3e** and **5e** that could influence the ^{13}C chemical shifts.

The ^{13}C chemical shift values within the phenyl ring can be used to analyze the effect of 1,4-DHP ring on the phenyl ring itself. In the case of 4-phenyl substituted 1,4-DHP derivatives studied herein (compounds **1c**–**6c**) the electron withdrawing characteristics of the C(3,5) substituent are increased in row $COOCH_3$ < $COCH_3$ < CN, as the signals corresponding to the *meta*- and *para*-carbon atoms in the phenyl ring are shifted downfield (Supplementary material Table 7). The *meta*-carbon resonance is less affected, but the chemical shift of the carbon atom in *para* position varies significantly under the influence of the substituent at C(3,5). In the case of the *meta*-carbon resonance this effect can be explained in terms of inductive electron withdraw by the complex 4-(1,4-DHP) substituent, however the chemical shift of *para*-carbon atom could be related to the hyperconjugation and substantial polarization of π-electron system according to usual resonance concepts.

The computed atomic Mulliken charges and ^{13}C chemical shifts (Supplementary material Table 8) for the B3LYP/6-31G* geometry optimized structures of **1a**–**6a**, **1b**–**6b**, **1c**–**6c**, **1d**–**6d**, **3e** and **5e** do not show any correlations, probably due to the influence of the anisotropic effects of the ester, ketone or cyano functional groups at positions C(3,5).

The direct $^1J(^{13}C_4—^1H)$ spin–spin coupling constants measured for the 1,4-DHP derivatives **1a**–**6a**, **1b**–**6b**, **1c**–**6c** and **1d**–**6d** change considerably as the substituent at C(3,5) position was varied (Table 4). It was found that $^1J(^{13}C_4—^1H)$ coupling constants are larger for 3(5)-cyano-1,4-DHP derivatives (**3a**–**3d**, **4a**–**4d** and **5a**–**5d**) (Table 4). Supporting X-ray data have shown that 3(5)-cyano group is a more effective boat flattening substituent than 3(5)-methoxycarbonyl or 3(5)-acetyl groups in 1,4-DHPs. Such increasing of planarity at C(4) increases $^1J(^{13}C_4—^1H)$ values (Table 4).

There are two effects possible that could act on $\delta(^{15}N)$ in 1,4-DHPs: first, the change of the flattened boat conformation of the 1,4-DHPs upon the electronic effects of substituents at C(3,5) positions, and second, the change of pseudo-axial/pseudo-equatorial equilibrium on the nitrogen, depending on both the conformation of the flattened boat and the electronic effects of substituents at C(3,5) positions.

The 1,4-DHP derivatives bearing methoxycarbonyl and acetyl groups at C(3) and/or C(5) position of heterocycle (**1a**–**1c**, **2a**–**2c** and **6a**–**6c**) have ^{15}N resonating the farthest downfield relative to the ones bearing cyano group (**3a**–**3c**, **4a**–**4c** and **5a**–**5c**) (Table 4). *N*-methyl substitution results in an upfield shift as compared with NH analogues (Table 4). The changes in geometry and different ability of the lone pair delocalization in *N*-methyl derivatives must be considered as the dominant $\delta(^{15}N)$ shielding contributor.

The X-ray structures of *N*-methyl derivatives (**1d**–**6d**) show that the nitrogen atom has larger displacement from the base of the boat plane as compared with the NH analogues (**1b**–**6b**) (Table 2). The upfield shift of the *N*-methyl nitrogen may therefore be rationalized in terms of partial increase in sp^3 character of the *N*-methyl nitrogen relative to the NH analogues and preferred pseudo-equatorial conformation of *N*-methyl group.

The effect of the substituent at C(4) position of heterocycle on the $\delta(^{15}N)$ chemical shifts is very small (Table 4).

Our quantum chemical calculations show that the calculated $\delta(^{15}N)$ values (Supplementary material Table 8) are in good agreement with experimental values (Table 4) and confirm the shielding effect of methyl group (Fig. 3).

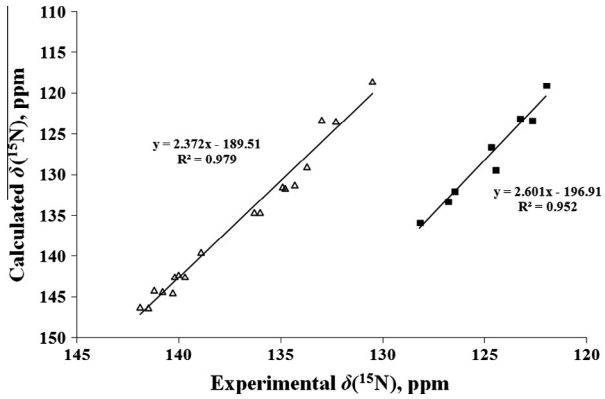


Fig. 3. Relationship between experimental (DMSO-d_6) and calculated (gas phase) ^{15}N chemical shifts of NH (Δ) and NCH_3 (■) 1,4-DHPs.

The computed atomic Mulliken charges (Supplementary material Table 8) for the B3LYP/6-31G* geometry optimized structures of **1a**–**6a**, **1b**–**6b**, **1c**–**6c**, **1d**–**6d**, **3e** and **5e** showed correlation with the experimental ^{15}N chemical shifts (Supplementary material Fig. 7).

The range of $^1J(^{15}N—^1H)$ values corresponds to the nearly planar arrangement around the nitrogen. It was found that direct $^1J(^{15}N—^1H)$ spin–spin coupling constants are larger for 3(5)-cyano-1,4-DHP derivatives (**3a**–**3c**, **4a**–**4c** and **5a**–**5c**) in comparison to 3(5)-acetyl- or 3(5)-methoxycarbonyl-ones (**1a**–**1c**, **2a**–**2c** and **6a**–**6c**) (Table 4). Substituents giving the flattened 1,4-DHP boat and/or pyramid at nitrogen lead to the larger absolute value of the $^1J(^{15}N—^1H)$ coupling constant that is observed for 3(5)-cyano-1,4-DHP derivatives (**3a**–**3c**, **4a**–**4c** and **5a**–**5c**).

It should be noted that the sign of $^1J(^{15}N—^1H)$ is negative [41]; this leads to the opposite appearance of the influence of the electronic effects in 1,4-DHP ring to $^1J(^{15}N—^1H)$ and $\delta(^{15}N)$, $^1J(^{15}N—^1H)$ and $\delta(N^1H)$ values in correlations (Figs. 4 and 5).

A regular dependence of the $^1J(^{15}N—^1H)$ coupling constants on the $\delta(^{15}N)$ and $\delta(N^1H)$ chemical shifts is observed (Figs. 4 and 5). The increase of $^1J(^{15}N—^1H)$ coupling constants by the introduction of cyano group in molecule demonstrates that 3(5)-cyano group is a more effective boat and/or nitrogen pyramid flattening substituent than 3(5)-methoxycarbonyl or 3(5)-acetyl groups in 1,4-DHPs probably due to its smaller volume.

In general the extensive NMR studies of ^1H, ^{13}C and ^{15}N chemical shifts of 1,4-DHINA derivatives, both concerning 1,4-DHP ring

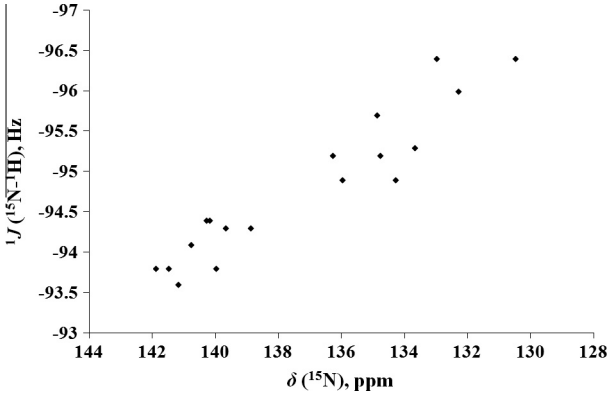


Fig. 4. A plot of the observed ^{15}N chemical shifts of 1,4-DHPs against $^1J(^{15}N—^1H)$ coupling constants in DMSO-d_6.

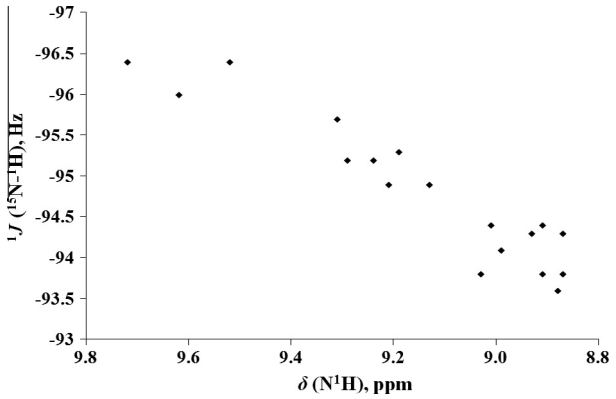


Fig. 5. A plot of the observed N^1H chemical shifts of 1,4-DHPs against $^1J(^{15}N—^1H)$ coupling constants in DMSO-d_6.

geometry and the substituents at the positions N(1), C(3,5) and C(4), have added useful information for the understanding of the features of this class of compounds.

Conclusions

The structural and conformational properties of a new series of 1,4-DHINA derivatives, characterized by ester, ketone or cyano functions at positions C(3) and C(5) have been investigated by X-ray crystallography, ^1H, ^{13}C and ^{15}N NMR spectroscopy and theoretical calculations.

The 1,4-DHP ring has a flattened boat conformation and the C(4) substituent occupies a pseudo-axial position, but, depending on other substituents, there is some flexibility around this preferred arrangement. The 1,4-DHP ring puckering at the nitrogen is linearly related to that at the tetrahedral C(4) carbon and it is influenced to a great extent by the alkyl substituent at the nitrogen. The carbonyl groups at C(3,5) positions of 1,4-DHP ring show a preference for the *s-cis*/*s-cis* conformation with respect to the adjacent C=C bond in the quantum chemically calculated structures. In X-ray structures the preferred carbonyl orientation is *s-trans*/*s-cis*. These small differences could be rationalized in terms of crystal packing effects in solids.

NMR studies of 1,4-DHINA derivatives clearly demonstrate that ^1H, ^{13}C and ^{15}N chemical shifts are a sensitive probes for structural modifications of heterocycle, whereas one-bond $^1J(^{15}N—^1H)$ and $^1J(^{13}C_4—^1H)$ couplings essentially reflect geometric distortions.

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Appendix A. Supplementary material

Supplementary crystallographic data for this paper can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; or E-mail: deposit@ccdc.cam.ac.uk). Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molstruc.2014.06.044.

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