Synthesis of mono and bis-substituted asymmetrical compounds, (1-(pyridin-2-yl)ethylidene)carbonohydrazide and 1-(2'-hydroxybenzylidene)-5-(1'-pyridylethylidene)carbonohydrazone: Structural characterization and antioxidant activity study

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ABSTRACT
Carbonohydrazide was used for synthesizing a new disymmetrical bis-substituted Schiff base 1-(2'-hydroxybenzylidene)-5-(1'-pyridylethylidene)carbonohydrazone (2). A mono substituted compound 1-(pyridin-2-yl)ethylidene)carbonohydrazide (1) was firstly prepared by condensation reaction of carbonohydrazide and 2-acetylpyridine in 1:1 ratio. Secondly, compound 2 was obtained by condensation reaction of compound 1 and salicylaldehyde in 1:1 ratio. The prepared compounds were characterized by elemental analysis, infrared and 1H and 13C NMR spectroscopy techniques, and the structure of compound 2 was determined by single-crystal x-ray diffraction study. The compound 2 (C15H15N5O2) crystallizes in the monoclinic space group P21/c with the following unit cell parameters: a = 8.36683(3) Å, b = 13.9986(4) Å, c = 12.1610(4) Å, β = 97.512(3)°, V = 1412.37(8) Å³, Z = 4, T = 100(2) K, μ(MoKα) = 0.098 mm⁻¹, Dcalc = 1.398 g/cm³, 6057 reflections measured (5.708° ≤ 2θ ≤ 54.962°), 6057 unique (Rsigma = 0.0395) which were used in all calculations. The final R = 0.0474 (I > 2σ(I)) and wR = 0.1971 (all data). The oxygen atom O1 and the azomethine nitrogen atom N5 adopt cis-configuration relative to the C8-N4 bond, while O1 adopts cis-configuration with the azomethine nitrogen atom N2 relative to C8-N3 bond. The crystal packing of compound 2 is stabilized by intramolecular O(phenol)-H···N(carbohydrazide) and intermolecular N(carbohydrazide)-H···O(carbohydrazide) hydrogen bonds which form layers parallel to [010] axis. Additional C-H···O hydrogen bond consolidate the structure. The carbonohydrazide moiety C=N=N-C(O)-N=C fragment and the phenyl ring are almost coplanar; with an angle of 1.73(1)° between their means plans. The dihedral angle between the mean planes of the phenyl and the pyridine rings is 22.67(2)°.

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KEYWORDS
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1. Introduction
Carbonohydrazide (H:NNHCONNH₂) is a compound with two identical moieties and very reactive with respect to carbonyl compounds. Controlling the ratio of carbonohydrazide/carbonyl allows to synthesize symmetrical or disymmetrical compounds by condensation reaction. Carbohydrazide and its derivatives have been largely investigated since 1894 when their syntheses were first reported [1]. Carbohydrazide derivatives were used as precursors for the synthesis of various heterocyclic compounds containing nitrogen atom in the cycle and/or in the free arms [2-4]. The reactivity as well as the biological properties of these derivatives have been investigated in the past decades for the development of drugs [5,6] or industrial applications [7,8]. They are known to possess a broad spectrum of biological activities such as antioxidant [9], analgesic [10], antiplatelet [11], antifungal [2], antimicrobial [12], anticonvulsant [13], antidepressant [14], anti-inflammatory [15], anti-tubercular [16], anti-HIV [5], anti-diabetic [10,17], and anticancer activities [3,18]. While most of the older work on carbohydrazide Schiff bases focused on their applicability in classical fields, nowadays these systems are increasingly acknowledged as supreme multitopic ligands for the targeted construction of original metal-organic architectures such as grids [19,20]. As part of our search for a suitable ligand for building grids of metal complexes, we have prepared 1-(2'-hydroxybenzylidene)-5-(1'-pyridylethylidene)carbonohydrazone (2), a...
vacuum over P2O5 (Scheme 1).

The resulting mixture was heated under reflux for 4 h. The suspension was filtered, and the white precipitate obtained was washed with (2 × 10 mL) of hot methanol and dried under vacuum over P2O5 (0.949 g, 0.00777 mol) in 20 mL of methanolic solution was added. The mixture was heated under reflux for 30 minutes. Few drops of glacial acetic acid were added and immediately the suspension disappears. After 4 h under reflux, yellow clear solution was obtained. On cooling, white precipitate appears and was isolated by filtration. The solid was washed with cold methanol (2 × 10 mL) and dried under vacuum over P2Os. The filtrate was left under slow evaporation at room temperature. Few days later, colorless crystals suitable for X-ray diffraction were collected (Scheme 1).

2.2.2. Synthesis of mono-substituted precursor (1-(pyridin-2-yl)ethylidene)carbonohydrazide (1)

The procedure is inspired by the method reported by Novak et al. [23], with some modification. Herein, 2-acylpyridine was used instead of salicylaldehyde. To a mixture of 20 mL of methanol and 10 mL of distilled water was added carbonohydrazide (3 g, 0.0333 mol) at room temperature. A solution of 2-acylpyridine (4 g, 0.0330 mmol) dissolved in 20 mL of methanol was slowly dropwise over a period of one hour. The resulting mixture was heated under reflux for 4 h. The suspension was filtered, and the white precipitate obtained was washed with (2 × 10 mL) of hot methanol and dried under vacuum over P2Os (Scheme 1).

(1-(Pyridin-2-yl)ethylidene)carbonohydrazide (1): Color: Dark. M.p.: 221.8-222.5 °C. Yield: 86.37 %. FT-IR (ATR, v, cm⁻¹): 3306 (NH), 3086 (s=C-H), 1671 (C=O), 1634 (C=N), 1578 (C̃=N=C̃), 1506 (C̃=N=C̃), 1466 (C̃=N=C̃), 1441. 1H NMR (500 MHz, DMSO-d6, δ, ppm): 2.36 (s, 3H, CH3), 4.12 (s, 2H, NH2), 7.32-8.51 (m, 4H, Py-H), 8.19 (s, 1H, N-H), 9.64 (s, 1H, N-H), 12.9 (C̃, C-N). Anal. calcd. for C8H11N5O: C, 49.73; H, 5.09; N, 23.56. Found: C, 49.75; H, 5.05; N, 23.5%, M.p.: 189.8-190.4 °C. FT-IR (ATR, v, cm⁻¹): 3432 (OH), 3192 (=C-H), 1694 (C=O), 1619 (C=N), 1584 (C̃=N=C̃), 1536 (C̃=N=C̃), 1488 (C̃=N=C̃), 1462 (C̃=N=C̃), 1270 (C=O), 1146. 1H NMR (500 MHz, DMSO-d6, δ, ppm): 2.37 (s, 3H, CH3), 6.90-8.62 (m, 8H, PhH + PhPy), 8.52 (s, 1H, N=C-H), 11.34 (s, 1H, N-H), 10.99 (s, 1H, N-H), 10.25 (s, 1H, O-Ferrocene). 13C NMR (125 MHz, DMSO-d6, δ, ppm): 157.07 (C=O), 154.71 (PhCOH), 152.19 (Py), 148.37 (C=N), 147.47 (C̃), 136.71 (C̃), 116.29-136.13 (C̃), 11.05 (CH3). Anal. calcd. for C15H15N5O2: C, 60.60, H, 5.09, N, 23.56. Found: C, 60.58, H, 5.10, N, 23.53%.

2.3. Free radical scavenging antioxidant assay

Antioxidant capacities of compounds 1 and 2 are measured according to Ahkbar et al. [24] method with modifications. The methanol solution of 3.0 mL DPPH• (40 mg/L) was added to test compounds (200 µL) at different concentrations. The mixture was shaken vigorously and incubated in dark for 30 min at room temperature. After the incubation time, the absorbance of the solution was measured at 517 nm by using UV-vis spectrophotometer Perkin two. The DPPH+ radical scavenger effect was calculated using the Equation (1):

\[
\text{Scavenging activity (% control)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100
\]

where \(A_{control}\) is the absorbance of the control reaction and \(A_{sample}\) is the absorbance of the test compound. The tests were carried out in triplicate. Trolox was used as positive control.

2.4. Crystal structure determination

Crystals suitable for X-ray single crystal diffraction of the reported compound were grown by slow evaporation of MeOH solution of compound 2. Details of the X-rays crystal structure solution and refinement are given in Table 1. Diffraction data were collected using an ENRAF NONIUS Kappa CCD diffractometer with graphite monochromatized MoKα radiation (\(\lambda = 0.71073\) Å). All data were corrected for Lorentz and polarization effects. No absorption correction was applied. Complex scattering factors were taken from the program package SHELXTL [25].
The structures were solved by direct methods which revealed the position of all non-hydrogen atoms. All the structures were refined on F² by a full-matrix least-squares procedure using anisotropic displacement parameters for all non-hydrogen atoms [26]. The hydrogen atoms of water molecules and NH groups were located in the Fourier difference maps and refined. Others H atoms (CH and CH₃ groups) were geometrically optimized and refined as riding model by AFIX instructions. Molecular graphics were generated using ORTEP-3 [27].

### 3. Results and discussion

#### 3.1. Synthesis

The compound (1-(pyridin-2-yl)ethylidene)carbonohydrazide (1) was prepared by a condensation reaction of 2-acetoxybenzylidene and carbonohydrazide in methanol. The isolated product was used for the synthesis of the compound (1E,5E)-1-(2-hydroxybenzylidene)-5-(1-(pyridin-2-yl)ethylidene)carbonohydrazide (2). Salicylaldehyde and compound 1 were mixed in methanol under reflux (Scheme 1). The compounds yielded as soluble in polar organic solvents such as DMSO or DMF. The elemental analyses results are in accordance with the chemical formulae obtained from spectroscopic studies. Both infrared spectra of compounds 1 and 2 exhibit broad bands in the range 3310-3185 cm⁻¹ which are attributed to N-H stretching [28]. The vibration of the imine functions appears in the range 1634-1619 cm⁻¹ while the band due to C=O group is in the range 1694-1724 cm⁻¹ which form layers parallel to b-axis. Additional C=H-0 hydrogen bonds consolidate the structure. In the crystal, intra-molecular and intermolecular hydrogen bonds are simultaneously present.

| Parameters | Z       |
|------------|---------|
| Empirical formula | C₉H₁₁N₂O₂ |
| Formula weight | 297.32 |
| Temperature (K) | 100(2) |
| Crystal system | Monoclinic |
| Space group | P2₁/c |
| a (Å) | 8.3683(3) |
| b (Å) | 13.9986(4) |
| c (Å) | 12.1610(4) |
| α (°) | 90 |
| β (°) | 90 |
| γ (°) | 90 |
| Volume (Å³) | 1412.37(8) |
| Z | 4 |
| ρcalc (g/cm³) | 1.398 |
| μ (mm⁻¹) | 0.098 |
| F(000) | 6240 |
| Crystal size (mm³) | 0.175 x 0.06 x 0.025 |
| Radiation | MoKα (λ = 0.71075 Å) |
| 2θ range for data collection (°) | 5.708 to 54.962 |
| Index ranges | -10 ≤ h ≤ 10, -18 ≤ k ≤ 18, -14 ≤ l ≤ 15 |
| Reflections collected | 6057 |
| Independent reflections | 6057 [R(int) = 0.0395] |
| Data/restraints/parameters | 6057/0/210 |
| Goodness-of-fit on F² | 1.088 |
| Final R indexes [I>2σ(I)] | R₁ = 0.0474, wR₂ = 0.1475 |
| Final R indexes [all data] | R₁ = 0.0698, wR₂ = 0.1971 |
| Largest diff. peak/hole (e Å⁻³) | 0.28/0.23 |

3.2. Structure description of compound 2

The molecular structure of the compound 2 with atomic labelling scheme is shown in Figure 1. The asymmetric unit of compound 2 consists of one molecule of the disymmetric Schiff base ligand. Crystal structure reveals that the organic molecule adopts the keto form, as showed by bond length of 1.227(3) Å for C=O-01 which is double bond character [31]. Additionally, C6-N2 and C9-N5 have double bond character as shown by the distances values of 1.282(3) and 1.284(3) Å, respectively (Table 2). The values of 1.372(3) and 1.373(3) Å for C8-N3 and C8-N4 are indicative of single bond character [32,33]. Oxygen atom O1 and the azomethine nitrogen atom N5 adopt cis-configuration relative to the C8-N4 bond, while atom O1 and azomethine nitrogen atom N2 adopt trans-configuration relative to C8-N3 bond. The torsion angles C6-N2-C9-N5 adopt cis-configuration between their means plans, but the carbonohydrazide and the pyridine ring are not coplanar with dihedral angle value of 21.38(8)°. The dihedral angle value between the mean planes of the phenyl and the pyridine rings is 22.27(1)°.
Table 2. Selected bond lengths, bond angles and torsion angles for the compound 2.

| Atom-Atom | Bond lengths (Å) | Atom-Atom | Bond lengths (Å) |
|-----------|------------------|-----------|------------------|
| C8-O1     | 1.227 (3)        | N2-N3     | 1.304 (3)        |
| C8-N3     | 1.372 (3)        | N4-N5     | 1.354 (3)        |
| C8-N4     | 1.373 (3)        | C6-N2     | 1.282 (3)        |
| C5-C6     | 1.488 (3)        | C9-N5     | 1.284 (3)        |
| C6-C7     | 1.508 (3)        | C9-C10    | 1.451 (3)        |

Carbon atoms in the title compound were collected. Combining carbonohydrazone compounds gave several hits. The majority of these are symmetrically bis-substituted compounds. By using carbohydrazone, salicylaldehyde, and acetylpyridine as fragments, recodes representing symmetrical and dissymmetrical compounds, were collected. Combining carbonohydrazone and salicylaldehyde, symmetrical disubstituted 1,5-bis(salicylidene)carbohydrazone compounds were obtained: SAGXOP [34] and SAGXOP01 [35]. However, when carbohydrazone was combined with acetylpyridine in 1:2 ratio, disubstituted symmetrical bis(methyl-2-pyridylketone) carbonohydrazone compound was obtained: TIRYIC [36]. Dissymmetrical carbonohydrazone compounds are quite rare. However, it has been reported in CSD two dissymmetrical carbonohydrazone Schiff bases: AROLUP [37] and MILZ0Z [38].

3.3. Antioxidant activity

The method of scavenging the DPPH• radical is largely used to evaluate the antioxidant activity of organic or inorganic compounds [39–40]. The antioxidant activities of the two compounds 1 and 2 have been substantially investigated. Figure 4 shows the plots of DPPH• free radical scavenging activity (%) for Trolox, compounds 1 and 2. The DPPH• is a stable free radical and becomes a stable molecule when it accepts an electron or hydrogen radical.
The antioxidant molecules scavenge the DPPH• radical by hydrogen donating ability. For compounds 1 and 2, it is observed that the scavenging activity increases with increasing the concentration in the range tested (50-500 mmol/L). Compound 1 has scavenging activity between 7.21±0.42 and 32.86±0.01% within the investigated concentration range due to the NH groups which can react with DPPH• radical by the H- abstraction reaction to form a stable radical. Radical scavenging activity of compound 2 (5.62±0.12 - 29.96 ±0.12%) is slightly lower than that observed for compound 1 (Figure 4). Comparatively to the scavenging activity of Trolox (7.28±0.69 - 70.36±0.34%), the values observed for compound 1 are higher than those of Trolox for low concentration (50-200 mM) while those for compound 2 are comparable to those of Trolox. When increasing (300 to 500 mM) the concentration, the scavenging activity of Trolox increases rapidly while those of compounds 1 and 2 increase very slightly and do not exceed 33% for compound 1 and 30% for compound 2.

4. Conclusion

The disubstituted carbonohydrazide derivative namely, (1E, 5E)-1-(2-hydroxybenzylidene)-5-(1-(pyridin-2-yl)ethylidene) carbonohydrazide (2) was successfully synthesized from the mono substituted carbohydrazide derivative (1-(pyridin-2-yl)ethylidene)carbonohydrazide (1). The structures of the compounds were confirmed by elemental analysis and spectroscopic techniques (FT-IR, 1H and 13C NMR). The molecular structure of the newly (1E, 5E)-1-(2-hydroxybenzylidene)-5-(1-(pyridin-2-yl)ethylidene) carbonohydrazide was also determined using X-ray crystallography technique. Compounds 1 and 2 showed moderate antioxidant activity of about 30-33%.

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Supporting information

CCDC-2018491 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via https://www.ccdc.cam.ac.uk/structures/, or by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44(0)1223-336033.

Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest. Author contributions: All authors contributed equally to this work.

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