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NOVEL COMPOUNDS FROM CONDENSATION REACTION BETWEEN 2-ACETYLPYRIDINE AND 2-FORMYLPYRIDINE. SYNTHESIS, CRYSTAL STRUCTURE AND BIOLOGICAL EVALUATION

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Abstract

Two new carbonyl compounds (**5-6**) were synthesized and spectrally characterized. The reaction mechanism for obtaining the new carbonyl compounds (**5-6**) was proposed following the analisis retrosintetis reactions of the products and confirmed by ^{1}H and ^{13}C-NMR. Synthesized carbonyl compounds (**3-6**) can serve as precursors (carbonyls) in the synthesis of thiosemicarbazones, which exhibit a wide range of biological properties. Due the condensation reaction between 2-acetylpyridine and 2-formylpyridine, catalysed with Na_2CO_3 in aqueous solution and microwave irradiation at 480 W, the product 1,3-bis(pyridin-2-yl)prop-2-en-1-one (**3**) in a good yield is obtained. By separation on the silica gel column, three products (**4-6**) were removed. Characterization of novel condensation products between 2-acetylpyridine and 2-formylpyridine was performed by FTIR-ATR spectroscopy, ^{1}H and ^{13}C-NMR spectroscopy, and further confirmed by single crystal X-ray diffraction. Compound (**5**) crystallise in the space group P-1 and compound (**6**) in the space group $P2_{1/c}$. Based on the spectral data and X-ray crystallography analysis of the eliminated products, the succession of reactions resulting in compounds (**3-6**) from (**1**) and (**2**) were proposed. Finally, synthetic methods for the preparation of substituted cyclohexanol derivatives have also been proposed. The obtained compounds exhibit moderate antimicrobial and antifungal activity. Antioxidant activity of compounds (**4-6**) was also studied.

Keywords: 1,3-bis(pyridin-2-yl)prop-2-en-1-one, Claisen-Schmitd condensation, intramolecular aldol condensation, Michael addition, substituted cyclohexanol.

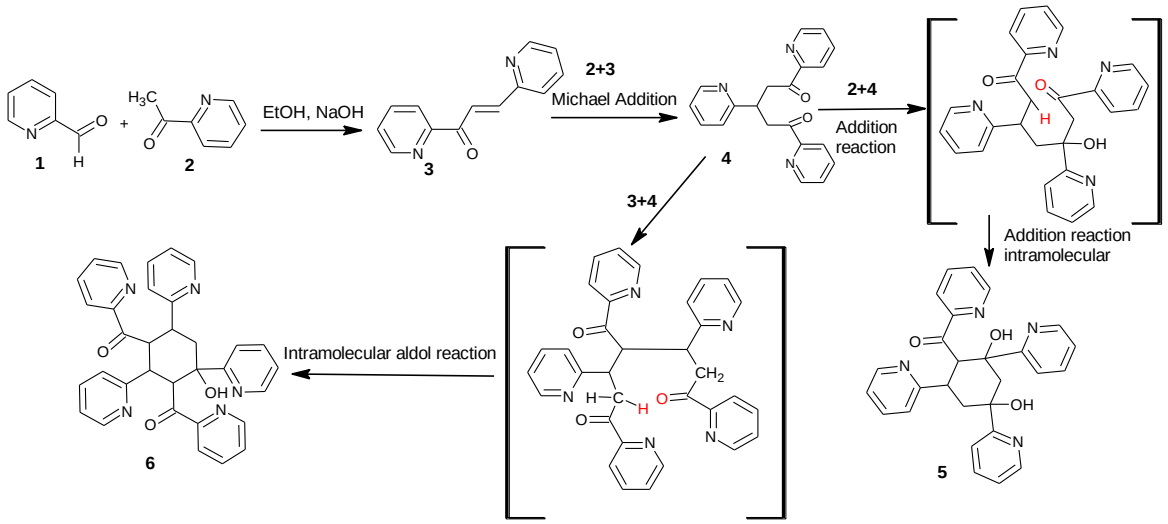
Introduction

Chalcones *E*-1,3-diaryl-prop-2-en-1-ones are considered precursors in the synthesis of flavonoid compounds which are very common in nature in various plant species. These compounds exhibit biological properties and can be as pharmacological agents which exhibit antibacterial [1], antifungals [2], antimalaric [3], antioxidant [4], antitumoral [5] and antiinflammatory [6] activities. The interest in studying these compounds is determined by their vast applications. In addition to being useful as a starting materials for the synthesis of biologically active heterocyclic agents, these compounds can be used as pharmacological agents. Scientific research has shown that biological activity is enhanced due to the keto-ethylene moiety in the structure of these compounds, the nature of the substituents in the aromatic rings, as well as the nature of the heterocycles [7-11]. **1,3-Bis(pyridin-2-yl)prop-2-en-1-one is the target compound for the given study, the chalconic fragment is the bridging bridge of the two pyridine rings. The creation of a conjugate system (single, double, single bond) leads to the creation of centers with different geometric conformation to each other, the appearance of cis or trans conformation to the double bond.** The interest in free radicals is determined by the fact that they are participating in the most important physiological processes in living organisms like aging process and different pathological processes in many diseases. Substances responsible to transfer free radicals in an inactive form called antioxidants, and knowledge of their activity is extremely important for medical science and practice. We used spectrophotometric measurement of the optical density of the solutions containing specific colored free radicals ABTS•+ and DPPH• of various concentrations for determination antioxidat ativity capacity.

Results and Discussion

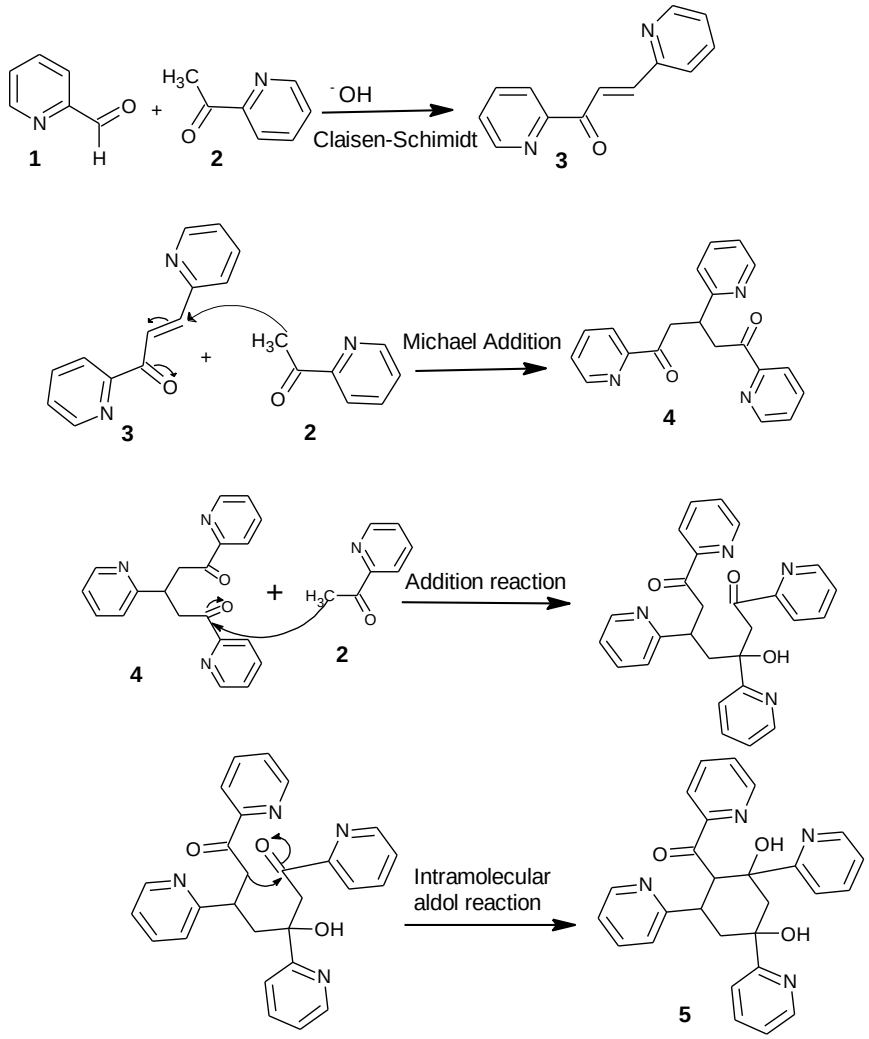
Research on the preparation of 1,3-bis(pyridin-2-yl)prop-2-en-1-one (**3**) by Claisen-Schmitd method did not result in the desired product. As a result of condensation reaction in basic ethanol solution, was obtained a three compounds:1,3,5-tri-(2-pyridinyl)pentane-2,4-dione (**4**), 2,4-dihydroxy-2,4,6-tri(pyridin-2-il)ciclohexil)(pyridin-2-il)metanon (**5**) and 4-hydroxy-2,4,6-tris(pyridin-2-yl)cyclohexane-1,3-diyl)bis(pyridin-2-yl)methanone (**6**) and the mixture was subjected to chromatographic separation on a column with SiO_2 using the eluent methanol to eliminated compounds (**4**-**6**). For the characterization of the final (**5**-**6**) and intermediate (**3**-**4**) products, monocrystals of (**4**-**6**) were obtained. The results of NMR spectroscopy and the sigle-crystal X-ray diffraction of the eliminated products suggested a series of intermediate product transformations which is formed in the reaction mixture.

Claisen-Schmitd condensation followed by the Michael addition of 2-acetylpyridine generates the diketone intermediate (**4**), which participates in the aldol double reaction with the third molecule of 2-acetylpyridine, forming an unstable intermediate which easily condenses with the formation of the cyclohexane ring (Scheme 1):



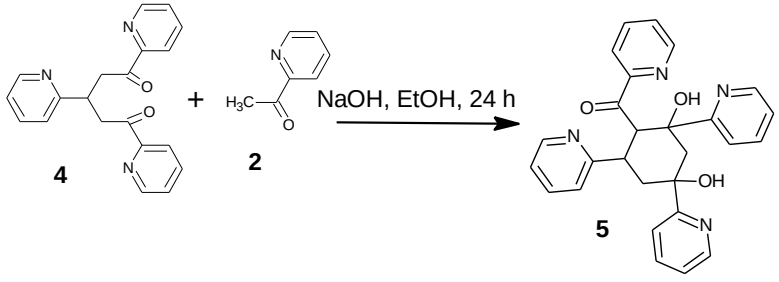
Scheme 1: The "domino" reaction of obtaining the condensation products (**1**) and (**2**) which terminates with the formation of the cyclohexane ring in compounds (**5**) and (**6**).

It should be noted that it was later found out that the Michael (**4**) addition product is described in the literature [12]. Note that compound (**5**) was reported by Chang Meng-Yang in 2012 with a yield of 65% and its structural formula was confirmed by NMR spectroscopy [13]. Their molecular structures were confirmed by FTIR-ATR, NMR spectroscopy and single-crystal X-ray diffraction. Substances similar to (**5-6**) which have been obtained as a result of condensation reactions between 2-acetylpyridine and different carbonyl compounds are described in the literature [14-23]. An acceptable mechanism formation of product (**5**) consists in the initial formation of the Michael addition intermediate (**4**), followed by the nucleophilic attack of the 2-acetylpyridine carbanion and the closing of the cyclohexane ring as a result of the intramolecular condensation reaction:



Scheme 2: The proposed mechanism of formation of compound (**5**).

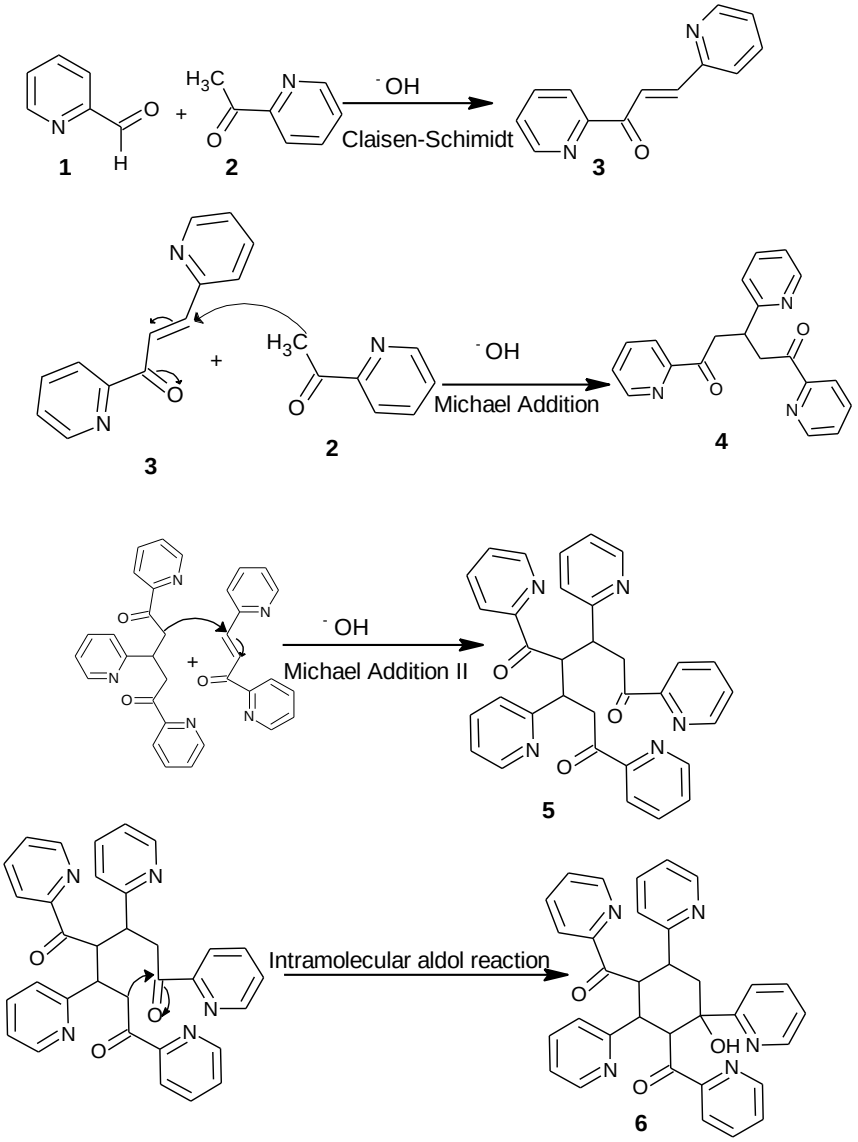
The mode of formation of compound (**5**) from compounds (**4**) and (**2**) present in the reaction mixture was confirmed by direct synthesis of the starting material, eliminated from the mixture by silica gel column (eluent: ethyl acetate) :



Scheme 3: Direct synthesis of (**5**) from (**4**) and (**2**).

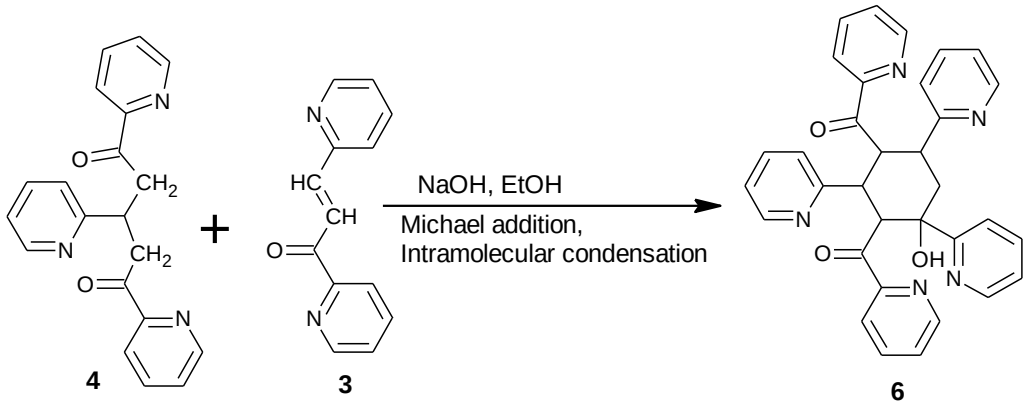
The structure of compound (**5**) was confirmed by single-crystal X-ray diffraction (Figure 2) and NMR spectroscopy. According to (Scheme 1), another cyclohexane ring product eluted on the silica gel column (eluent: methanol) from the reaction medium is compound (**6**), which also forms on the interaction of substances (**1**) and (**2**) in ethanolic solution NaOH. Compound

(**6**) crystallizes as colorless crystals with m.p. 220-222 ° C. The structure of compound (**6**) confirmed by spectral methods suggests that it was formed as a result of the interaction of diketonic intermediate (**4**) with compound (**3**) as a result of the Michael addition reaction followed by intramolecular condensation and subsequent cyclization (Scheme 4):



Scheme 4: The proposed mechanism of formation of compound (**6**).

To confirm formation of compound (6), from direct synthesis of the intermediaries (3) and (4) was obtain preventively in pure state (3) and (4) according to (Scheme 5):



Scheme 5: Direct synthesis of the formation of product (**6**) from compounds (**3**) and (**4**).

The structure of the product (**4**) was confirmed by single-crystal X-ray diffraction (Figure 3)

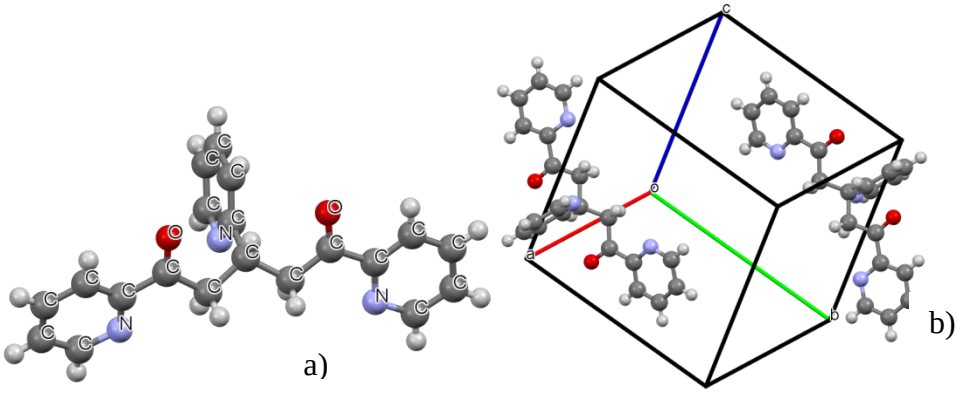


Figure 1: a- Structure crystalline of 1,3,5-tri(pyridin-2-yl)pentane-1,5-dione (**4**); b- Representation in the elemental cell. CCDC reference: 665647.

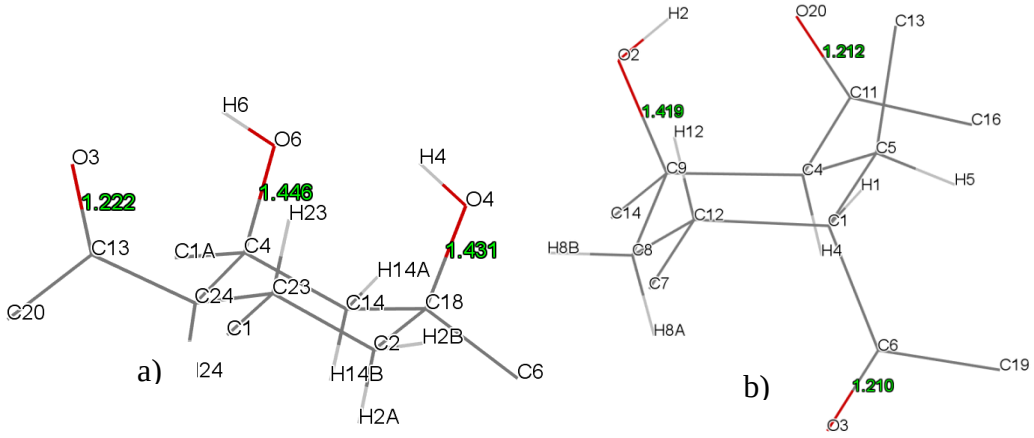


Figure 2: The molecular structure of the product (**5**), the representation of the stoichiometric configuration of the cyclohexane ring and the distance between C-O of the hydroxyl and ketone groups; b- Molecular structure of product (**6**), representation of the stance

conformation of the cyclohexane ring and the distance between C-O of the hydroxyl and ketone groups. (see Supporting Information File 2, Figures S-1.2; 2.1; 3.1).

Single-crystal X-ray diffraction of compounds 5 and 6.

Three pyridine-2-yl groups are attached to cyclohexane rings in (**5)** and (**6)** while one and two pyridine-2-ylmethanone groups are bound with cyclohexane in these compounds respectively (Figure 3). In both compounds the central cyclohexane ring adopts a chair conformation with puckering parameters [24] Q, θ, φ which are 0.5935 Å, 173.45°, 0.9° and 0.5414 Å, 169.64°, 110.4° for (**5)** and (**6**) respectively. In compounds of (**5**) and (**6**) the planes through the coplanar atoms (C1C(E)/C1D/C7/C8) form dihedral angles with pyridine cycles (N1/C2-C6) which are equal to 89.44, 59.51, 76.45, 39.82° and 83.86, 85.13, 84.02,45.12, 82.46° for rings *A*-*E* and *A*-*D* (Figure 4). The *bond lengths* and the *bond angles* the studied compounds are *consistent with those in* [25, 26]. The molecular structures of (**5)** and (**6**) are stabilized by intramolecular C—H···N, C—H···O and O—H···O hydrogen bonds (HB) (Table 4, Figure 5) and for **5** by π–π interaction between the pyridine rings *B* and *D* [centroid–centroid distance is equal to 3.775 Å]. In the crystal structure of (**5**) the molecules form the centrosymmetric dimers which are link by C(5C)-H…O(1B) HB. The dimers are joined into the chains along [1 1 0] direction through the hydrogen bonds C(1D)-H...O(1B) and O(1C)-H...N(1D) (Table 4, Figure 5). In the chains, molecules translated by a unit along the *y* axis, are linked *via* C(5D)-H…*Cg* (*Cg* is the pyridine *A* ring centroid) interactions with H… *Cg* distance equal to 2.77 Å. Between the chains in (**5**) the van der Waals interaction occurs. In the crystal structure of (**6**) the molecules are linked into a complex three-dimensional framework structure by a combination of C(5A)-H...N(1B), C(5E)-H...O(1B) and C(6B)-H...O(1A) hydrogen bonds (Table 4, Figure 5). The *A* and *C* pyridine rings related by symmery operator (1 - *x*, -*y*, -*z*) are stacked with a centroid–centroid separation of 3.986 Å. A C(5C)-H…π interaction is also observed for these cycles with H… *Cg* distance 2.97 Å. (see Supporting Information File 2, Tables S 1-3, page 6-9).

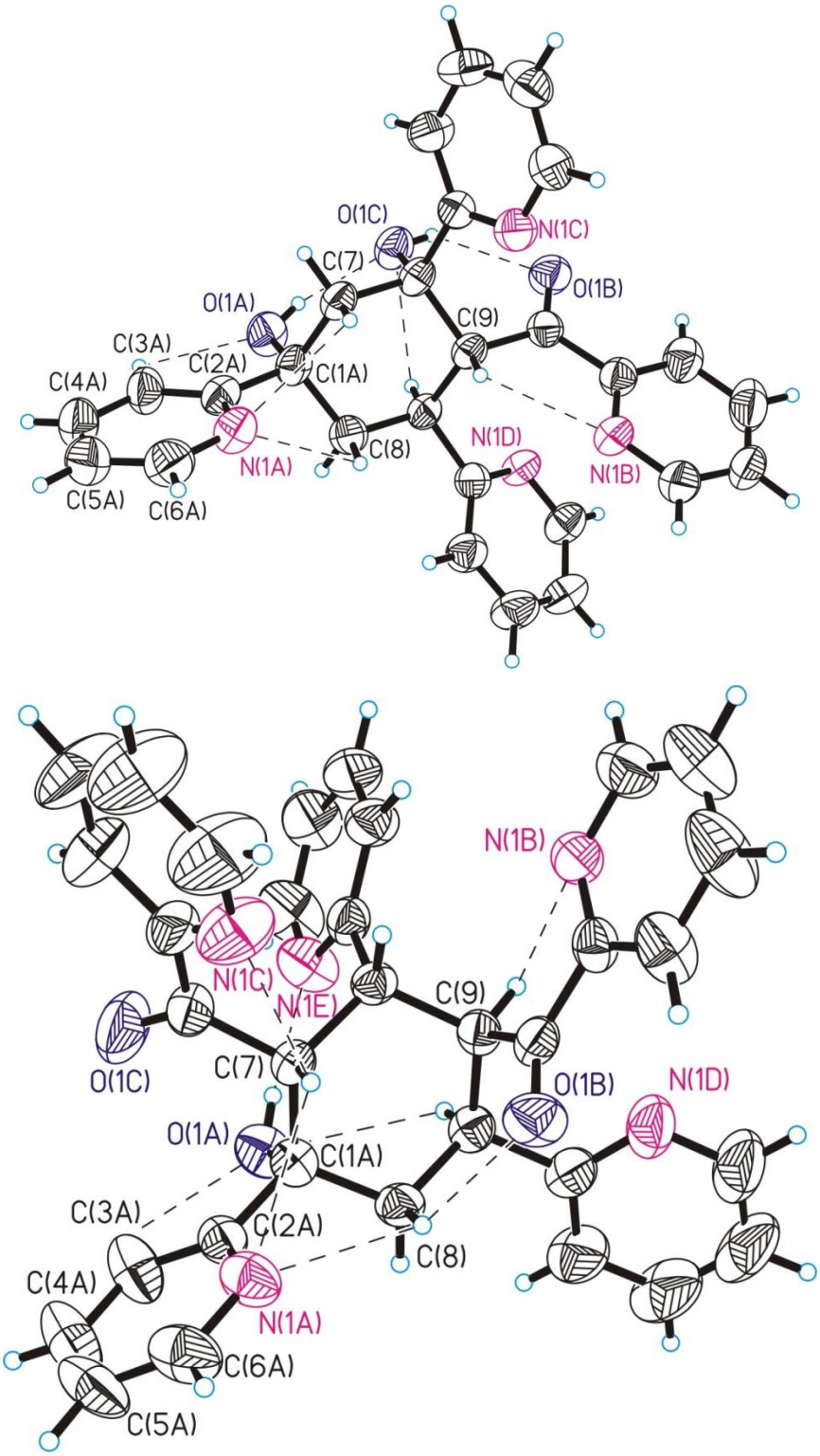


Figure 3: A *ORTEP* plot of (a) **5** and (b) **6**, showing 50% probability displacement ellipsoids and the atomic numbering.

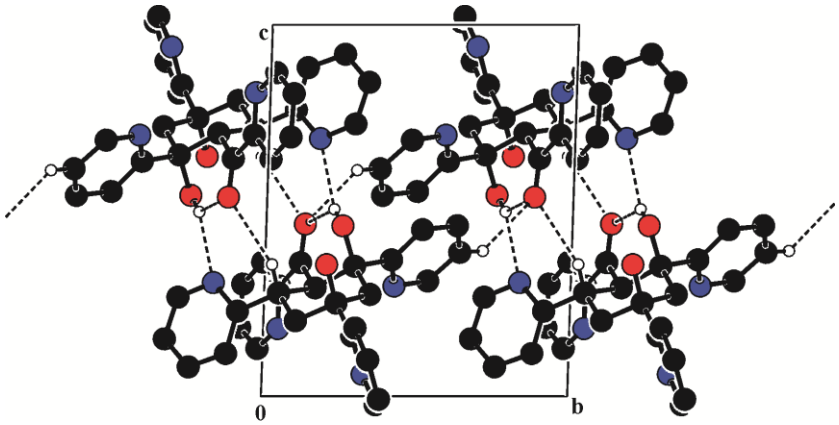


Figure 4: View of centrosymmetric dimmers in **5** which are aligned along [1 1 0] direction forming chains.

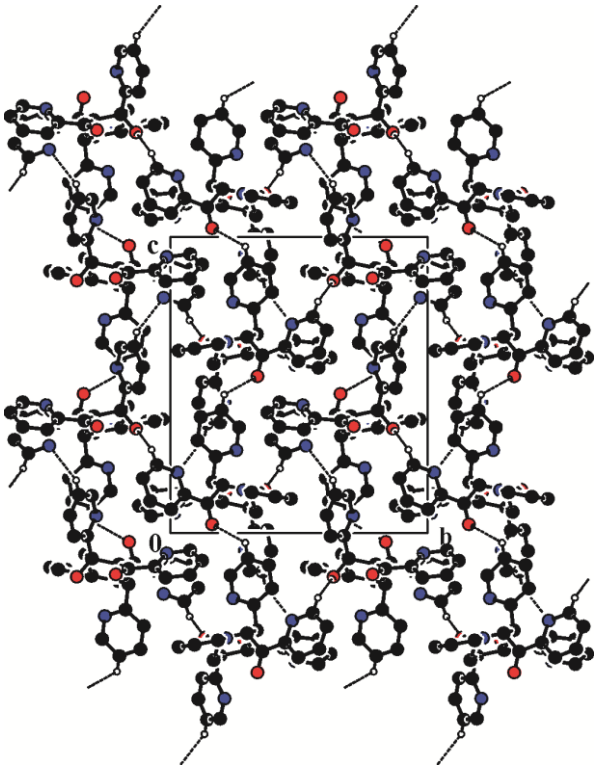


Figure 5: Packing of the molecules viewed down *a* axis showing the *3D* molecular network in **6**.

Analyzing the obtained experimental data, it has been found that the high concentration of carbanions formed in the alcoholic medium of 2-acetylpyridine in the presence of strongly basic catalysts favors the "domino" condensation, which ends with the formation of the cyclohexane (**5**) and (**6**) compounds. This is the reason for the impossibility of eliminating 1,3-di(pyridin-2-yl)prop-2-en-1-one from the reaction mixture under Claisen-Schmitd reaction conditions. The Michael addition reaction proceeds very rapidly with the formation of the diketonic intermediate (**4**) having pronounced nucleophilicity. In the literature, reactions for the preparation of chalcones by condensation of aldehydes with ketones in alkaline medium at microwave irradiation with a significant increase in yield and reduction of

the reaction time [26-27]. Condensation of 2-acetylpyridine with sodium carbonate-catalyzed pyridine-2-carboxaldehyde in aqueous medium and microwave irradiation (MW) proved to be cost effective to produce 1,3-di(pyridin-2-en-1-one. The product was isolated in practically pure form after cooling of the aqueous mixture by simple filtration on the Buchner funnel in a 60% yield.

Spectroscopy analysis

FTIR-ATR spectroscopy

Compounds **5** and **6** were determined by infrared absorption bands of carbonyl groups C=O, 1686-1676 cm^{-1} which were similar to those reported in the literature [22] (Figure S-3 from Supporting Information File 1).

^{1}H-NMR and ^{13}C-NMR spectroscopy

The formation of compounds **1**-**6** was confirmed by NMR spectroscopy, for some ^{13}C-NMR spectra DEPT 135 were performed. Carbonyl groups C=O of (**3**) are at 189.57 ppm, corresponding to source [27]. Grouping $HC_{\beta}=C_{\alpha}H$-C=O of (**3**) is at 143.12 ppm (Cβ) and 124.70 ppm (Cα) [27]. Compound (**4**) is a symmetric molecule, so the groups C=O have the same chemical shift and are at 200.42 ppm.

Antimicrobial (antibacterial and antifungal) activity of compound 4-6.

Our research has confirmed that compounds **4**-**6** exhibit antibacterial or antifungal properties. The bacteriostatic and bactericidal activity of the synthesized substances was investigated on three bacterial strains: *Escherichia coli* (G-); *Klebsiella pneumonia* (G-); *Staphylococcus aureus* (G +); four fungal strains: *Candida albicans; Candida krusei; Candida parapsilosis* and *Cryptococcus neoformans.* The minimum inhibitory concentration and bactericidal concentrations in mg/mL for compounds (**4**), (**5**) and (**6**) (Table 1.2) were determined, (see Supporting Information File 3, page 2).

Tabel 1: Results of the antimicrobial tests of compounds (**4**-**6**), (mg/mL).

Compounds	*E. coli* (G-)		*K. pneumoniae* (G-)		*S. aureus* (G+)	
	*MIC	**MBC	MIC	MBC	MIC	MBC
4	500.00	500.00	500.00	500.00	2.00	4.00
5	500.00	500.00	250.00	500.00	0.50	1.00
6	100.00	200.00	100.00	200.00	15.00	30.00
Furacillinum	0.046	0.046	0.046	0.093	0.046	0.093

*MIC - minimum inhibitory concentration; **CMB - minimum bactericidal concentration.

Tabel 2: The results of the antifungal tests of compounds (**4**-**6**),(mg/mL).

Compounds	*Candida albicans*		*Candida krusei*		*Candida parapsilosis*		*Cryptococcus neoformans*	
	*MIC	**MBC	MIC	MBC	MIC	MBC	MIC	MBC
4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5	0.50	1.00	0.50	1.00	0.25	0.50	0.125	0.25
6	0.25	0.50	0.25	0.50	0.25	0.50	0.0039	0.0078
Nistatinum	0.032	0.064	0.032	0.064	0.032	0.064	0.032	0.064

*MIC - minimum inhibitory concentration; **CMB - minimum bactericidal concentration.

***In vitro* antioxidant activity.**

Tabel 3: The results of the *in vitro* antioxidant activity of the compounds (**4**-**6**),(IC_{50}, µM).

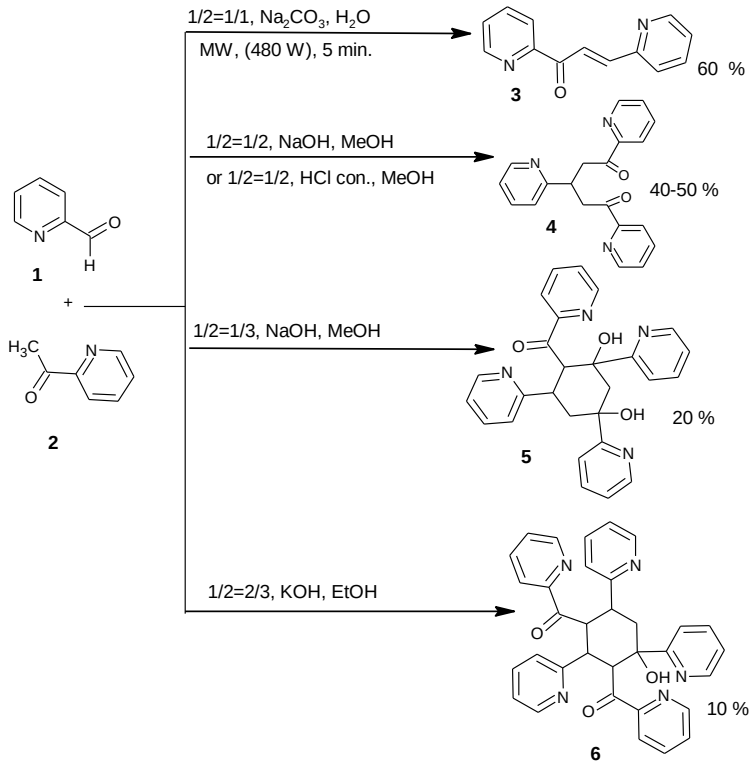
Compounds	ABTS radical cation scavenging activity
	IC_{50}, µM
4	24.23
5	>100
6	>100
Trolox	26.3
Rutin	20.7

In vitro antioxidant activity according to the described method (see Supporting Information File 3, page 3).

Conclusions

1- The succession of reactions resulting in the formation of compounds (**3**-**6**) begins with compounds (**1**) and (**2**) Claisen-Schmitd condensation, Michael addition, followed by double aldol reaction and following cyclization which finishes with cyclohexane ring formation as a synthetic method for the preparation of substituted cyclohexanol derivatives.

2- Practical methods of synthesis of the compounds eliminated and described according to the scheme:



Scheme 6: Practical methods of synthesis of products (**3**-**6**).

3- Bacteriostatic and bactericidal activities of compounds (**4**-**6**) against: *Escherichia coli* (G-); *Klebsiella pneumonia* (G-); *Staphylococcus aureus* (G +); *Candida albicans*; *Candida krusei*; *Candida parapsilosis*; *Cryptococcus neoformans*. Demonstrated that bacteriostatic and bactericidal concentrations, compound of (**4**) is within the range 2-500 mg/mL and for compound (**5**) bacteriostatic and bactericidal concentration is in the range 0.5-500 mg/mL, for compound (**6**) the bacteriostatic and bactericidal concentration is within the range (0.0039-0.5 mg/mL) and was studied a potential antioxidant activity of compounds (**4**-**6**).

4- Single-crystal X-ray diffraction results demonstrated that in both compounds the central cyclohexane ring adopts a chair conformation. Due to hydrogen bonds, the molecules of **5** form centrosymmetric dimers which are joined into chains along [1 1 0] direction while in the crystal structure of **6** the molecules are linked together by H-bonds to form a three-dimensional framework.

Experimental

General

The reagents were purchased from SigmaAldrich, Alfa Aesar or Acros Organics, and used without purification. NMR spectra: Bruker 400MHz (^{1}H NMR: 400 MHz, ^{13}C NMR: 100 MHz), the spectra were recorded in $CDCl_3$ and DMSO-d_6 using TMS as internal standard and are reported in ppm. The spectral results were interpreted using SpinWorks 3. FTIR-ATR spectra were recorded in powder form on the Bruker ALPHA apparatus in the wavelength range 4000-400 cm^{-1}. The spectral results were interpreted using OPUS version 7.5. Crystallographic measurements of compounds **5** and **6** were carried out with an Oxford-Diffraction XCALIBUR E CCD diffractometer (Santa Clara, CA, USA) equipped with graphite-monochromated Mo K_α radiation. The unit cell determination and data integration were carried out using the CrysAlis package of Oxford Diffraction (CrysAlisProAgilentTechnologies, Version1.171.34.49 (release20-01-2011 CrysAlis171.net; compiled Jan 20 2011,15:58:25)). All structures were solved by direct methods using SHELXS-97 and refined by full-matrix least-squares on F_o^2 with SHELXL-97 [25]. All atomic displacements for non-hydrogen, non-disordered atoms were refined using an anisotropic model. The main crystallographic data together with refinement details are summarized in Table 3. The selected bond lengths, angles and hydrogen bonds are presented in Table S 2 and Table S 3 respectively (see Supporting Information File 2, Tables S 2-3, page 7-9). The geometric parameters were calculated and the figures were drawn with the use of the PLATON program [26]. The hydrogen atoms that are not involved in the hydrogen bonding were omitted from the generation of the packing diagrams.

Table 4. Crystallographic data, details of data collection and structure refinement parameters for compounds **5** and **6**.

Compound	**5**	**6**
CCDC codes	1880245	1880244
Chemical formula	$C_{27}H_{24}N_4O_3$	$C_{33}H_{27}N_5O_3$
M (g mol^{-1})	452.50	541.60
Temperature, (K)	293(2)	293(2)
Wavelength, (Å)	0.71073	0.71073
Crystal system	triclinic	monoclinic
Space group	*P-1*	*P2$_1$/c*
a (Å)	10.0138(12)	11.8346(4)
b (Å)	10.7521(15)	14.3760(6)
c (Å)	11.5927(14)	19.5697(7)
α (^{0})	87.587(11)	90
β (^{0})	88.336(10)	121.940(2)
γ (^{0})	9062.875(13)	90
V (Å^3)	1109.8(2)	2825.40(18)
Z, D_{calc} (g cm^{-3})	2, 1.354	4, 1.273
μ (mm^{-1})	0.090	0.084
F(0 0 0)	476	1136
Goodness-of-fit on F^2	0.906	1.067
Final R_1 , wR_2 [I > 2σ(I)]	0.0663, 0.1370	0.0504, 0.1199
R_1 , wR_2 (all data)	0.1351, 0.1735	0.0780, 0.1330
Largest difference in peak and hole (e Å^{-3})	0.374, -0.187	0.179, -0.154

Synthesis of 1,3-bis(pyridin-2-yl)prop-2-en-1-one (**3**).

A mixture of 2-acetylpyridine (1.21 g, 0.01 mol), and 2-formylpyridine (1.07 g, 0.01 mol), of Na_2CO_3 (1.06 g, 0.01) and 300 mL of water was subjected to microwave irradiation at 480 W for 5 minutes. On cooling the reaction mixture pale yellowish crystals and a yellowish oil is deposited on the bottom of the flask, which then crystallizes and represents a mixture of product (**3**) and (**4**). (TLC monitoring: EtOAc). The crystals were separated by filtration, washed on the filter with water and air dried. 1.26 g (60%) of the product (**3**) are obtained with m.p. = 66-67 °C, Rf = 0.6 (eluent: EtOAc), corresponds to literature [28]. IR (FTIR, ν_{max}, cm^{-1}): (C-H,sp^2) (alkene) 3059(m), 3085(m); $(C\text{-}H)_{py}$ (heterocyclic) 3008(m), 2929(m); (C=O) 1670 (s); (C=C) conjugated alkenes-1,2-subst. 1609(m); (C-C) in the aromatic ring 1579 (s); (C-H in-plane bending 1090 (m); alkenes-trans-1,2-subst. 983(s); the pyridine ring in the plane 618(m). ^{1}H-NMR ($CDCl_3$, 298 K) δ (ppm), 400 MHz: 8.825-8.655 (m, 3H); 8.139-8.040 (m, 2H); 7.934-7.793 (m, 3H); 7.809 (d, 1H); 7.735-7.691(m, 1H); 7.713 (m, 1H); 7.450 (m, 1H); 7.471-7.429 (m, 1H). ^{13}C-NMR ($CDCl_3$, 298 K) δ (ppm), 100 MHz: 189.574(C=O); 153.701(C^1); 153.095(C_1); 150.654(C^2); 149.754(C_2); 143.120(Cβ);

138.267(C_4); 137.791(C^4); 128.287(C_3); 126.167(C^3); 125.463(C^5); 124.709(Cα); 122.982(C_5).

Synthesis of 1,3,5-tri(pyridin-2-yl) pentane-1,5-dione (**4**).

Method A. A mixture of 2-acetylpyridine (2.42 g, 0.02 mol) and 2-formylpyridine (1.07 g, 0.01 mol) and NaOH (0.80 g, 0,02 mol) of and 30 mL of EtOH was stirred at room temperature for 4 hours. The red-orange mixture was neutralized with 10% HCl solution to pH = 6-7, when a colorless microcrystalline substance deposited. The crystals were filtered and washed on the filter with cold ethanol to give 2.98 g (45%) of the product (**4**), m.p. = 130-132 ° C, corresponds to [12].

Method B. A mixture of 2-acetylpyridine (0.97 g, 0.008 mol) and 2-formylpyridine (0.43 g 0.004 mol) of was dissolved in 15 mL of EtOH. The reaction mixture was cooled to 0 °C and 2 mL of HCl (con.) was added dropwise and vigorously. The mixture was further stirred for 1 hour at 0-5 °C, then another 5 hours at room temperature then neutralized with 10% NaOH solution. The reaction product was filtered and washed on the filter with cold, dried water and recrystallized from EtOH. Yield 1.40 g (53%), m.p. = 130-132 °C. IR (FTIR, ν_{max}, cm^{-1}): $(C\text{-}H)_{py}$ 3090(w), 3019(w); (C-H) from (CH_2) as 2914, sy 2882; (C=O) 1692(s); the pyridine ring in the plane 617(m). ^{1}H-NMR (DMSO-d6) δ (ppm), 400 MHz: 8.679(C16-H, C22-H, d, 2H); 8.364-8.278 (C6-H, d, 1H); 7.983-7.941 (C18-H, C24-H, m, 2H); 7.871(C17-H, C23-H, d, 2H); 7.677-7.613(C3-H, C19-H, C25-H, m, 3H); 7.353(C4-H, d, 1H); 7.143-7.112(C5-H, m, 1H); 4.065(C7-H, p, 1H); 3.879-3.814(C11-H, C8-H, m, 4H). ^{13}C-NMR (DMSO-d6) δ (ppm), 100 MHz: 200.428 (C9=O, C12=O); 163.334(C2); 153.156(C14, C20); 149.602(C16, C22); 149.132(C6); 138.009(C18, C24); 136.826(C4); 128.225(C23, C17); 123.645(C5); 121.939(C3); 121.737(C19, C25).

Synthesis of (2,4-dihydroxy-2,4,6-tri(pyridin-2-yl)cyclohexyl)(pyridin-2-yl)methanone (**5**).

A mixture of 2-acetylpyridine (0.36 g, 0.003 mol), 2-formylpyridine (0.11 g, 0.001 mol), NaOH (0.12 g, 0.003 mol) and MeOH (6 mL) was heated at reflux for 6 h, when the TLC analysis showed the completion of the reaction. The reaction mixture was neutralized with HCl until pH= 7, MeOH, the reaction mixture was concentrated to dryness under reduced pressure. The organic phase was extracted with EtOAc, separated and dried with anhydrous Na_2SO_4, followed by filtration and concentration. After removal of the solvent, the residue was purified by column chromatography on silica gel (eluent: EtOAc), yield 0.65 g (48%) of a colorless crystalline substance of m.p.= 164-166 °C, corresponds to [13].

IR (FTIR, ν_{max}, cm^{-1}): **(**O-H) 3341, 3325 (m); $(C\text{-}H)_{py}$ 3050 (w), 3008(w); (C-H) from CH_2 as 2963, sy 2912; (C=O) 1686 (s); (C=C) 1470 (s); CH_2 bending 1431 (w); (C-O) 1219 (m); (C-

OH) axial 929 (s); the pyridine ring in the plane 1569, 618, 469 (m). ^{1}H-NMR ($CDCl_3$) δ (ppm), 400 MHz: 8.536-8.518(C31-H, m, 1H); 8.413-8.396(C23-H, m, 1H); 8.240-8.224(C17-H, C6-H, m, 2H); 7.787(C20-H, d, 1H); 7.694(C34-H, t, 1H); 7.615(C33-H, d, 1H); 7.504-7.465(C25-H, C4-H, m, 2H); 7.429-7.374(C3-H, C26-H, m, 2H); 7.160-7.127(C32-H, C19-H, m, 2H); 6.867-6.834(C18-H, m, 2H); 6.477(C24-H, s, 1H); 6.232(C5-H, s, 1H); 5.545(C12-H, d, 1H); 4.420-4.349(C7-OH; C9-OH, m, exchange with HOH); 3.144(C11-H, d, 1H); 2.885(C8-H, t, 1H); 2.228-2.021(C10-H, m, 2H). ^{13}C-NMR ($CDCl_3$) δ (ppm) 100 MHz: 204.244(C27=O); 165.031(C21); 162.436(C15); 162.025(C2); 153.971(C29); 148.725(C23); 148.068(C6, C17); 147.470(C31); 136.734(C33); 136.227(C4); 136.069(C25); 136.037(C19); 125.820(C32); 123.092(C18); 121.889(C20, C3, C26); 121.283(C24); 120.542(C5); 119.441(C34).

Synthesis of (4-hydroxy-2,4,6-tri(pyridin-2-yl)cyclohexane-1,3-diyl)bis(pyridin-2-ylmethanone) (**6**).

Method A. To the solution of 2-formylpyridine (0.64 g, 0.006 mol) in 30 mL of EtOH was added dropwise the solution formed from NaOH (0.36 g, 0.009mol) and 6 mL water. 2-Acetylpyridine 1.09 g (0.009 mol) was added dropwise to the reaction mixture by vigorous stirring. The reaction mixture was stirred at room temperature for 6 hours, then left for a additional 60 hours. The reaction time was determined by thin-layer chromatography (TLC) analysis showed the completion of the reaction as against 2-formylpyridine (eluent: EtOAc). The reaction mixture was neutralized with HCl until pH= 6-7. The reaction product was extracted with CH_2Cl_2, dried with anhydrous Na_2SO_4 after removing the solvent, the residue was purified on a silica gel column (eluent: EtOAc, then MeOH). Yield: 1.16 g (24%) of a colorless substance of m.p. 220-222 ° C.

Method B. A mixture of 1,3-bis(pyridine)1,3,5-tri(pyridin-2-yl)pentane-1,5-dione (**4**) (0.49 g, 0.0015 mol) and 0.32 g (0.0015 mol) 1,3-bis(pyridin-2-yl)prop-2-en-1-one (**3**) is dissolved in 6 mL of EtOH. To the mixture a solution of 0.26 g of NaOH and 3 mL of water was added. The mixture was stirred at room temperature for 5 hours, then left for 48 hours. The reaction time was determined by thin layer chromatography (TLC) analysis showed the completion of the reaction as against (**4**), (eluent: EtOAc). Purification of the product was performed as in method A. Yield: 0.36 g (44%) of a colorless substance, m.p. 220-222 °C. IR (FTIR, ν_{max}, cm^{-1}): **(**O-H) 3350 (w); $(C-H)_{py}$ 3045 (w), 3004(w); (C-H) from CH_2 as 2925, sy 2852; (C=O) 1676, 1583 (s); (C=C) 1470 (s); CH_2 bending 1438 (s); (C-O) 1232 (m); (C-OH) axial 937 (s); the pyridine ring in the plane 1449, 618, 505 (m). ^{1}H-NMR ($CDCl_3$) δ (ppm), 400 MHz: 8.583-8.531(C32-H, C38-H, m, 2H); 8.418(C16-H, d, 1H); 8.250-8.195(C24-H, C-6H, m,

2H); 8.139-8.055(C41-H, C35-H, m, 2H); 7.880(C3-H, t, 1H); 7.678-7.607(C40-H, C34-H, m, 2H); 7.543-7.402(C18-H, C4-H, C33-H, C39-H, C26-H, C27-H, m, 6H); 7.198-7.168(C19-H, C25-H, m, 2H); 7.002(C5-H, t, 1H); 6.876(C17-H, t, 1H); 5.869(C12-H, d, 1H); 5.339(C7-OH, s, 1H); 4.879(C10-H, d, 1H); 4.175-4.1234.1(C9-H, m, 1H); 3.446(C11-H, t, 1H); 2.217-2.176(C8-H, m, 2 H). ^{13}C-NMR ($CDCl_3$) δ (ppm) 100 MHz: 201.704(C20=O); 201.055(C28=O); 167.241(C14); 162.540(C22); 161.758(C2); 154.255(C36); 153.996(C30); 148.330(C24); 148.211(C16); 148.015(C38, C32); 146.878(C6); 137.778(C34); 136.729(C40); 136.354(C26); 136.064(C4); 135.808(C18); 126.246(C39); 125.975(C33); 124.202(C3); 122.605(C35); 122.118(C5); 121.884(C41, C25); 120.939(C19); 120.791(C27); 120.235(C17); 75.443(C7); 49.001(C12); 46.408(C10); 45.596(C11); 42.381(C8); 38.623(C9).

Crystal structure data Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number 1880245 and 1880244. The data can be obtained free of charge via http://www.ccdc.cam.ac.uk 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) 1223/336-033, Tel.: (+44) 1223/336-408.

Supporting Information

Supporting Information File 1

Experimental procedures and analytical of ^{1}H NMR, ^{13}C NMR, FTIR-ATR spectrums of compounds 3-6.

Supporting Information File 2

Crystallographic data for compounds 5 and 6 (additional figures and tables).

Supporting Information File 3

Antimicrobial (antibacterial and antifungal) activity and antioxidant activity.

Supporting Information File 4

CIF data for compound 5-6.

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