CHEMISTRY JOURNAL OF MOLDOVA. General, Industrial and Ecological Chemistry. 2020, 15(2), 88-98
ISSN (p) 1857-1727 ISSN (e) 2345-1688
http://cjm.asm.md
http://dx.doi.org/10.19261/cjm.2020.695

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

Roman Rusnac [a*], Maria Botnaru [a], Nicanor Barba [a], Peter Petrenko [b], Yurii Chumakov [b,c], Aurelian Gulea [a]

[a]*State University of Moldova, 60, Mateevici str., Chisinau MD 2009, Republic of Moldova*
[b]*Institute of Applied Physics, 5, Academiei str., Chisinau MD 2028, Republic of Moldova*
[c]*Department of Chemistry, Faculty of Science, Gebze Technical University, Gebze 41400, Kocaeli, Turkey*
[*]*e-mail: roman.rusnac@usm.md; phone: (+373) 069 43 49 10*

Abstract. The research is devoted to the study of unexpected products that formed as a result of the condensation reaction between 2-acetylpyridine and 2-formylpyridine under the Claisen-Schmidt reaction conditions. The structure of the compounds was determined and confirmed using FTIR, ^{1}H and ^{13}C NMR spectroscopy, and X-ray diffraction technique. As a result, a sequence of reactions leading to the following compounds has been proposed: 1,3-bis(pyridin-2-yl)prop-2-en-1-one (**3**); 1,3,5-tri(pyridin-2-yl)pentane-1,5-dione (**4**); (2,4-dihydroxy-2,4,6-tri(pyridin-2-yl)cyclohexyl)(pyridin-2-yl)methanone (**5**) and (4-hydroxy-2,4,6-tri(pyridin-2-yl)cyclohexane-1,3-diyl)bis(pyridin-2-ylmethanone) (**6**) as well as 2-formylpyridine (**1**) and 2-acetylpyridine (**2**). The plausible mechanisms of these chemical transformations and synthetic methods for obtaining substituted cyclohexanol derivatives are also presented. The synthesized compounds were tested for antimicrobial and antioxidant activity. The obtained results show that the compounds **4**-**6** have moderate antifungal activity. The activity of compound **6** is nine times higher towards *Cryptococcus neoformans* than the activity of Nistatin that is used in medical practice. The present experimental results show that compound **6** has potential application in antibacterial and antifungal areas.

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

Received: 10 June 2020/ Revised final: 11 October 2020/ Accepted: 21 October 2020

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 used as pharmacological agents exerting antibacterial [1], antifungal [2], antimalaric [3], antioxidant [4], antitumoral [5] and anti-inflammatory [6] activities. The interest in studying these compounds is determined by their vast applications. In addition to being useful as a starting material for the synthesis of biologically active heterocyclic agents, these compounds can be used as pharmacological agents.

Studies have shown that biological activity is enhanced by the keto-ethylene moiety, the nature of the substituents in the aromatic rings, as well as the nature of the heterocycles [7-14]. Azachalcones exhibit biological properties similar to those of chalcones [15]. Therefore, the goal set to this study was to synthesize target azachalcones from 2-acetylpyridine with a series of halobenzaldehydes under Claisen-Schmidt condensation. As a result, not only the target azachalcones were obtained, but also unexpected products, which were confirmed to be unusual penta-substituted cyclo-hexanols by ^{1}H and ^{13}C NMR spectroscopy, and X-ray diffraction technique. It must be mentioned that there have been very few reports that mention the obtained cyclohexanol derivatives [16].

Experimental

Generalities

The reagents were purchased from Sigma-Aldrich (Merck), Alfa Aesar and Acros Organics, and used without purification. The purity of substances was monitored by thin-layer chromatography.

Nuclear magnetic resonance analysis was performed on a Bruker Avance DRX 400

spectrometer, at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR; the spectra were recorded in deuterated chloroform ($CDCl_3$) and dimethyl sulphoxide-d_6 (DMSO-d_6), using tetramethylsilane (TMS) as internal standard. The obtained spectra were processed using MestReNova v. 14.1.2 or SpinWorks v. 4.2.4 software.

Fourier-transform infrared (FTIR) spectroscopic analysis was performed on a Bruker ALPHA apparatus, in the wavelength range of 4000-360 cm^{-1}. The spectral results were processed using OPUS v. 7.5 or OMNIC v. 9.1 software.

X-ray diffraction measurements of compounds **5** and **6** were carried out on an Oxford-Diffraction XCALIBUR E CCD diffractometer (Santa Clara, CA, USA) equipped with graphite-monochromated MoKα radiation. The unit cell determination and data integration were carried out using the CrysAlis package of Oxford Diffraction [17]. All structures were solved by direct methods using SHELXS-97 and refined by full-matrix least-squares on Fo2 with SHELXL-97. 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 2. The geometric parameters were calculated and the figures were drawn with the use of the PLATON program [17]. The hydrogen atoms that are not involved in the hydrogen bonding were omitted from the generation of the packing diagrams [17].

Crystal structure data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. 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.

General procedure of Claisen-Schmidt condensation reaction

To the mixture of 2-formylpyridine (**1**) (2.14 g, 20 mmol) dissolved in 10 mL ethanol (EtOH) was added while stirring 2-acetylpyridine (**2**) (2.42 g, 20 mmol). The reaction mixture was cooled to 0-50°C, then vigorously stirred while adding NaOH (1.2 g, 30 mmol) dissolved in 15 mL of distilled water. Compounds were stirred at this temperature for 1 h, then another 6 h at room temperature. Initially the solution turned yellow, then became bright orange. Chromatographic control indicates the presence of three substances that differ in R_f from the initial compounds **1** and **2**. The end of the reaction was confirmed chromatographically on the silufol plate (EtOAc), after the consumption of 2-acetylpyridine (**2**). The reaction mixture was poured into cold water acidified with HCl (pH 5), and the reaction products were extracted with CH_2Cl_2. The extract was dried over anhydrous Na_2SO_4. After solvent removal, the mixture of products was separated on the silica gel column. Elution of the column with CH_2Cl_2:hexane (1:3) allowed the removal of a crystalline product with R_f= 0.6 (EtOAc) on the silufol plate, which corresponds to compound **4** with m.p.= 130-132°C [13]. Elution of the column with the mixture of solvents EtOAc:hexane (1:1) brought to the release of the crystalline product with R_f= 0.4 (EtOAc) with m.p.= 164-166°C corresponding to compound **5** [14]. Elution of the column with the EtOAc:EtOH (1:1) solvent allowed the separation of a crystalline product with R_f= 0.2 (EtOAc) with m.p.= 220-222°C, corresponding to compound **6**.

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

A mixture of **2** (1.21 g, 10 mmol) and **1** (1.07 g, 10 mmol), with Na_2CO_3 (0.26 g, 2.5 mmol) and water (300 mL) was subjected to microwave irradiation at 480 W for 5 min. When cooling the reaction mixture, a yellowish oil deposited on the bottom of the flask, out of which crystallized compound **3** (TLC monitoring: EtOAc). The crystals were separated by filtration, washed on the filter with water and air dried. A quantity of 1.26 g (60%) of the reaction product **3** was obtained with m.p.= 66-67°C, R_f= 0.6 (EtOAc), which corresponds to literature data [12]. FTIR (ν_{max}, cm^{-1}): (C-H, sp^2) (alkene) 3059(m), 3085(m); (C-H)py (heterocyclic) 3008(m), 2929(m); (C=O) 1670(s); (C=C) conjugated alkenes-1,2-substituted 1609(m); (C-C) in the aromatic ring 1579(s); (C-H in-plane bending 1090(m); alkenes-*trans*-1,2-substituted 983(s); the pyridine ring in the plane 618(m). ^{1}H NMR ($CDCl_3$): δ 8.82-8.65 (m, 3H); 8.13-8.04 (m, 2H); 7.93-7.79 (m, 3H); 7.80 (d, 1H, H$_\beta$, J= 15.6 Hz); 7.73-7.69 (m, 1H); 7.71 (m, 1H); 7.45 (d, 1H, H$_\alpha$, J= 15.6 Hz); 7.47-7.42 (m, 1H). ^{13}C NMR ($CDCl_3$) δ: 189.57 (C=O); 153.70; 153.09; 150.65; 149.75; 143.12; 138.26; 137.79; 128.28; 126.16; 125.46; 124.70; 122.98.

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

Method A. A mixture of **2** (2.42 g, 20 mmol) and **1** (1.07 g, 10 mmol), NaOH

(0.80 g, 20 mmol) and EtOH (30 mL) was stirred at room temperature for 4 h. The red-orange mixture was neutralized dropwise with 1 M HCl to pH 6-7, when a colourless microcrystalline substance deposited. The crystals were filtered and washed on the filter with cold EtOH to give 1.49 g (45%) of the reaction product **4**, m.p.= 130-132°C, corresponds to previously reported data [13].

Method B. A mixture of **2** (0.97 g, 8 mmol) and **1** (0.43 g 4 mmol) was dissolved in EtOH (15 mL). The reaction mixture was cooled to 0°C and 2 mL of HCl (36% by weight) were added dropwise while vigorously stirring. Furthermore, the mixture was stirred for 1 h at 0-5°C, then another 5 h at room temperature, afterwards neutralized with 10% NaOH solution. The reaction product was separated by filtration and washed on the filter with cold water and recrystallized from EtOH. Product yield 0.72 g (53%), m.p.= 130-132°C. IR (ν_{max}, cm^{-1}): (C-H)py 3090(w), 3019(w); (C-H) from (CH_2) 2914(as), 2882(sy); (C=O) 1692(s); the pyridine ring in the plane 617(m). ^{1}H NMR (DMSO-d_6): δ 8.67 (C-H, d, 2H, *J*= 7.58 Hz); 8.36-8.27 (C-H, m, 1H); 7.98-7.94 (C-H, m, 2H); 7.87 (C-H, d, 2H, *J*= 7.58 Hz); 7.67-7.61 (C-H, m, 3H); 7.35 (C-H, d, 1H, *J*= 4.2 Hz); 7.14-7.11 (C-H, m, 1H); 4.06 (C-H, p, 1H); 3.87-3.81(C-H, m, 4H). ^{13}C NMR (DMSO-d_6): δ 200.42 (C=O); 163.33; 153.15; 149.60; 149.13; 138.00; 136.82; 128.22; 123.64; 121.93; 121.73.

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

A mixture of **2** (1.82 g, 15 mmol), **1** (0.53 g, 5 mmol), NaOH (1.60 g, 40 mmol) and MeOH (8 mL) was heated at reflux for 6 h; the reaction progress was monitored by TLC. The reaction mixture was neutralized dropwise with 1M HCl to pH 7, then the reaction mixture was concentrated 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 1.08 g (48%) of a colourless crystalline substance of m.p.= 164-166°C, corresponds to [14]. IR (ν_{max}, cm^{-1}): (O-H) 3341, 3325 (m); (C-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 8.53-8.51 (C-H, m, 1H); 8.41-8.39 (C-H, m, 1H); 8.24-8.22 (C-H, m, 2H); 7.78 (C-H, d, 1H, *J*= 8.0 Hz); 7.69 (C-H, t, 1H, *J*= 5.3 Hz); 7.61 (C-H, d, 1H, *J*= 7.8 Hz); 7.50-7.46 (C-H, m, 2H); 7.42-7.37 (C-H, m, 2H); 7.16-7.12 (C-H, m, 2H); 6.86-6.83 (C-H, m, 2H); 6.47 (C-H, s, 1H); 6.23 (C-H, s, 1H); 5.54 (C-H, d, 1H, *J*= 12.0 Hz); 4.42-4.34 (C-OH; m, exchange with HOH); 3.14 (C-H, d, 1H, *J*= 12.0 Hz); 2.88 (C-H, t, 1H, *J*= 12.8 Hz); 2.22-2.02 (C-H, m, 2H). ^{13}C NMR ($CDCl_3$): δ 204.24 (C=O); 165.03; 162.43; 162.02; 153.97; 148.72; 148.06; 147.47; 136.73; 136.22; 136.06; 136.03; 125.82; 123.09; 121.88; 121.28; 120.54; 119.44.

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

Method A. Under vigorous stirring, **2** (1.09 g, 9 mmol) was added dropwise to the mixture of **1** (0.64 g, 6 mmol) in EtOH (30 mL) and NaOH (1.20 g, 30 mmol) in water (10 mL). The reaction mixture was stirred at room temperature for 6 h, then left for additional 60 h (TLC monitoring: EtOAc). The reaction mixture was neutralized with 1M HCl solution 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 with the EtOA:MeOH (1:1). Yield: 0.78 g (24%) of a colourless 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, 1.5 mmol) and 1,3-bis(pyridin-2-yl)prop-2-en-1-one (**3**) (0.32 g, 1.5 mmol) was dissolved in EtOH (6 mL). A solution containing NaOH (0.26 g, 6.5 mmol) in water (3 mL) was added to the resulting mixture and stirred at room temperature for 5 h, then left for 48 h (TLC monitoring: EtOAc). The 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 (ν_{max}, cm^{-1}): (O-H) 3350 (w); (C-H)py 3045 (w), 3004(w); (C-H) from CH_2 2925(as), 2852(sy); (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 8.58-8.53 (C-H, m, 2H); 8.41 (C-H, d, 1H, *J*= 8.8 Hz); 8.25-8.19 (C-H, m, 2H); 8.13-8.05 (C-H, m, 2H); 7.88 (C-H, t, 1H, *J*= 8.0 Hz); 7.67-7.60 (C-H, m, 2H); 7.54-7.40 (C-H, m, 6H); 7.19-7.16 (C-H, m, 2H); 7.00 (C-H, t, 1H, *J*= 7.6 Hz); 6.87 (C-H, t, 1H, *J*= 7.6 Hz); 5.86 (C-H, d, 1H, *J*= 12.6 Hz); 5.33 (C-OH, s, 1H); 4.87 (C-H, d, 1H, *J*= 12.0 Hz); 4.17-4.12; 4.1 (C-H, m, 1H); 3.44 (C-H, t, 1H, *J*= 3.2 Hz); 2.21-2.17 (C-H, m, 2H). ^{13}C NMR ($CDCl_3$):

δ 201.70 (C=O); 201.05 (C=O); 167.24; 162.54; 161.75; 154.25; 153.99; 148.33; 148.21; 148.01; 146.87; 137.77; 136.72; 136.35; 136.06; 135.80; 126.24; 125.97; 124.20; 122.60; 122.11; 121.88; 120.93; 120.79; 120.23; 75.44; 49.00; 46.40; 45.59; 42.38; 38.62.

Antimicrobial activity evaluation

The antibacterial activity of the synthesized compounds was tested against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *KIebsiella pneumonae* ATCC 700603. The antifungal activity was evaluated against *Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 22019, *Cryptococcus neoformans* CECT 1043 strains.

The antimicrobial activity of the synthesized compounds was assessed using the microdilution broth test, which allows the determination of the minimum inhibitory concentration (MIC) and minimum bactericide/fungicide concentration (MBC/MFC) [19,20].

Procedure. A stock solution (10 mg/mL) of each tested compound was prepared in DMSO. This stock solution was then diluted in Muller Hinton Broth (MHB) for bacteria and RPMI 1640 medium with both L-glutamine and 0.165 M MOPS buffer and without sodium bicarbonate was provided (ready for use) for fungi. Further, serial dilutions of test compounds were carried out to achieve concentrations ranging from 500 to 0.24 μg/mL. A volume of 100 μL of each solution concentration was placed into a well (96-well microplate) containing 90 μL of RPMI or MHB, and 10 μL of inoculum (1×10^6 CFU/mL for bacteria and 1×10^5 CFU/mL for fungi) was added. Plates were covered and incubated on the shaker at 37°C for 24 h (bacteria), 48 h (*Candida* spp.), and 72 h (*Cryptococcus* spp.).

The MICs were assessed visually after the corresponding incubation period and were considered as the lowest sample concentration at which there was not registered a growth. For the MBC and MFC determination, 10 μL aliquots from each well that showed no growth of microorganism were plated on Mueller-Hinton agar or Sabouraud Dextrose agar and incubated at 37°C for 24 h (bacteria), 48 h (*Candida* spp.), and 72 h (*Cryptococcus* spp.). The lowest concentration that yielded no growth after subculturing was considered as the MBCs or MFCs. Furacilin for bacteria and Nistatin for fungi were used as positive controls and broth containing 20 μL of DMSO was used as a negative control. All the experiments were carried out in triplicates.

ABTS radical cation scavenging activity evaluation

The antioxidant activity by the ABTS$^{\bullet+}$ method was determined according to the method described by Re, R. *et al*. [19]. The ABTS$^{\bullet+}$ radicals formed through the reaction of 7 mM ABTS solution with 140 mM potassium persulphate solution, incubated in the dark at 25°C. When the ABTS$^{\bullet+}$ formed, the solution was diluted with acetate buffered saline (0.02 M, pH 6.5). For testing dilutions of synthesized compounds in DMSO were prepared. After that, a mixture of 20 μL of each sample solution with ABTS$^{\bullet+}$ radicals solution in dispenser module of hybrid reader (Biotek) were shacked during 15 s. The decrease in absorbance value at 734 nm was registered on a spectrophotometer (Biotek model) exactly after 30 min of incubation at 25°C. All the determinations were performed in triplicate. DMSO was used as blank sample. Trolox and Rutin were used as standard solution. The % inhibition was calculated according to Eq.(1):

$$IC_{50} = [(A_0 \cdot A_1)/A_0] \times 100\% \quad (1)$$

where, A_0 is the absorbance of the blank sample,

A_1 is the absorbance of the sample;

IC_{50} is the half maximal inhibitory concentration and was calculated using the statistical program GraphPad Prism 8.4.

Results and discussion

Following the research described above, it was decided to obtain 1,3-bis(pyridin-2-yl)prop-2-en-1-one (**3**), which would serve as a precursor in the synthesis of a series of thiosemicarbazones with potential biological activity. The classical method of obtaining chalcones described in the literature (Claisen-Schmidt reaction) did not allow to obtain this azachalcone [15]. It was found that the condensation reaction between 2-formylpyridine (**1**) and 2-acetylpyridine (**2**) under the conditions of the Claisen-Schmidt reaction does not end with a single product. Four substances with different chromatographic index (*Rf*), eluting with ethyl acetate, were detected on the silufol plate. The separation of the formed products was performed on the silica gel column (dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), methanol (MeOH)). The structure of the removed substances was performed by physical methods of analysis. Thus the structures of compounds **4**-**6** were proposed as follows: 1,3,5-tri(pyridin-2-yl)pentane-1,5-dione (**4**); (2,4-dihydroxy-2,4,6-tri(pyridin-2-yl)cyclohexyl) (pyridin-2-yl)methanone (**5**) and (4-hydroxy-

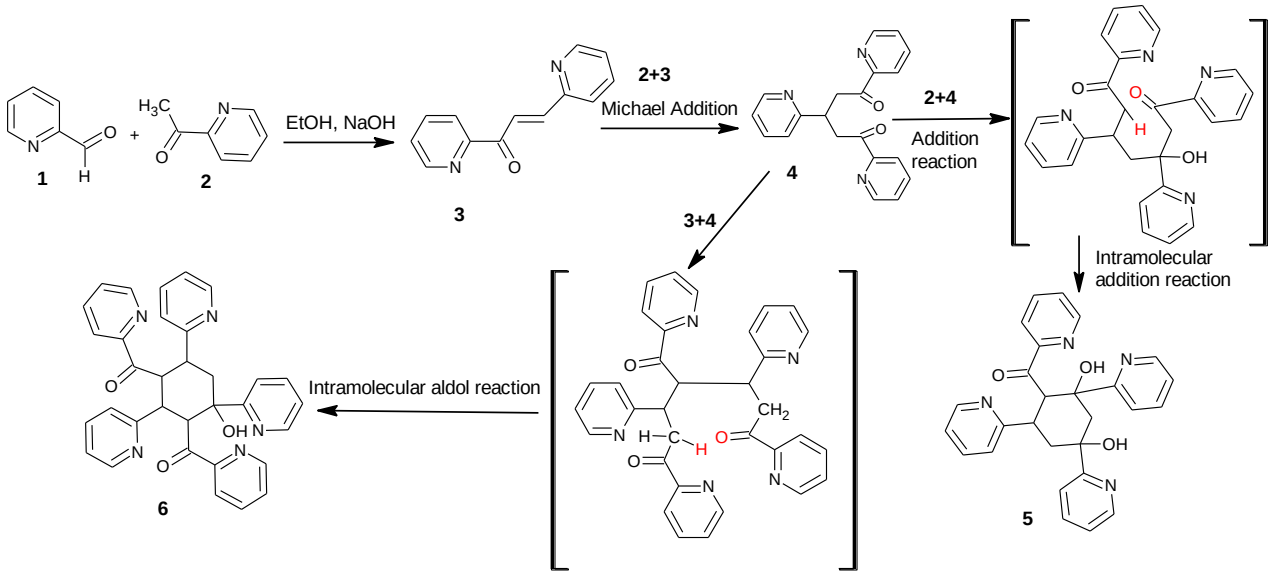
2,4,6-tri(pyridin-2-yl)cyclohexane-1,3-diyl)bis(pyridin-2-yl)methanone (**6**).

Results of the present study have shown that the aldol condensation reaction between 2-formylpyridine (**1**) and 2-acetylpyridine (**2**) is a 'domino' reaction with the formation of a series of compounds **3-6** which have been removed and identified from a single sample. Literature data on this type of condensation are scattered with the separate description of different components, and results of the present research have shown that all products are present simultaneously in the reactant.

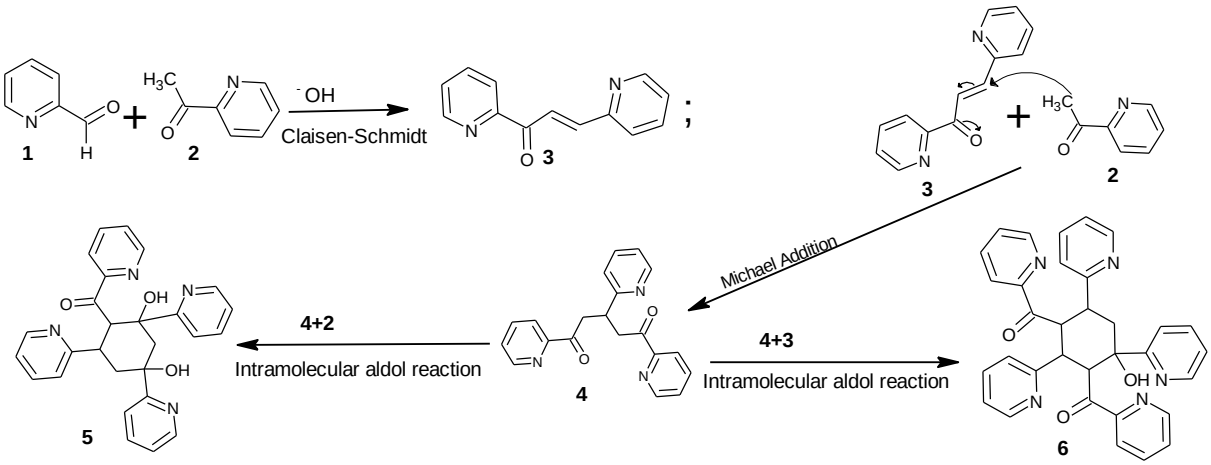
Synthesis and characterization

The research on the preparation of 1,3-bis(pyridin-2-yl)prop-2-en-1-one (**3**) by the Claisen-Schmitd method did not result in the desired product. As a result of condensation reaction between **1** and **2** in alkaline ethanol solution, three compounds were obtained:1,3,5-tri-(2-pyridinyl)pentane-2,4-dione (**4**), 2,4-dihydroxy-2,4,6-tri(pyridin-2-yl)cyclohexyl) (pyridin-2-yl)-methanone (**5**) and 4-hydroxy-2,4,6-tri(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 as eluent MeOH to eliminate the compounds **4-6**. The Claisen-Schmidt condensation between **1** and **2** followed by the Michael addition of 2-acetylpyridine (**2**) generates the diketone intermediate **4**, which participates in the aldol double reaction with the third molecule of 2-acetylpyridine (**2**), yielding an unstable intermediate which easily condenses forming the cyclohexane ring (Scheme 1).

An acceptable mechanism of formation of reaction products **5** and **6** is shown in Scheme 2.



Scheme 1. The "*domino*" reaction of obtaining the products of the condensation between 1 and 2, which ends with the formation of the cyclohexane ring in compounds 5 and 6.



Scheme 2. The proposed mechanism of formation of compounds 5 and 6.

As a result of the aldol condensation between 2-formylpyridine (**1**) and 2-acetylpyridine (**2**), 1,3-bis(pyridin-2-yl)prop-2-en-1-one (**3**) is formed which undergoes a rapid transformation into compound 1,3,5-tri(pyridin-2-yl)pentane-1,5-dione (**4**) as a result of the Michael addition of 2-acetylpyridine (**2**) to the conjugated double bond with the C=O group of the 1,3-bis(pyridin-2-yl)prop-2-en-1-one (**3**). The following 1,3,5-tri(pyridin-2-yl)pentane-1,5-dione (**4**) participates in two types of reactions: *a)* the nucleophilic centre of a carbonyl group of 1,3,5-tri(pyridin-2-yl)pentane-1,5-dione (**4**) is attacked by the 2-acetylpyridine carbanion (**2**), followed by intramolecular cyclization to form compound **5**; *b)* the addition of diketone (**4**) to the conjugate double bond of azachalcone (**3**) takes place, followed by cyclization with the formation of compound **6**, according to Scheme 2.

Compound **5** was reported for the first time in 2012 by Chang, M.-Y., with a yield of 65%, the structure was confirmed only by ^{1}H NMR spectroscopy [14]. In the present study, the molecular structure was confirmed by FTIR, NMR spectroscopy and single-crystal X-ray diffraction. Substances similar to **5** and **6** obtained as a result of condensation reactions between 2-acetylpyridine (**2**) and different carbonyl compounds have been previously described in the literature [15].

The formation of compound **5** from compounds **4** and **2**, and of compound **6** from compounds **4** and **3**, both present in the reaction mixture was confirmed by direct synthesis of the starting material, separated from the mixture by silica gel column (ethyl acetate). The structure of compound **4** was confirmed by single-crystal X-ray diffraction (Figure 1) as well as FTIR, and NMR spectroscopy.

According to the obtained results, the high concentration of carbanions formed in the alcoholic medium of 2-acetylpyridine (**2**) in the presence of strongly basic catalysts favours the "domino" condensation, which ends with the formation of the cyclohexane ring-based compounds **5** and **6**. This is the reason for the impossibility of eliminating 1,3-bis(pyridin-2-yl)prop-2-en-1-one (**3**) from the reaction mixture under the Claisen-Schmidt reaction conditions. The Michael addition reaction proceeds very rapidly with the formation of the diketonic intermediate **4**, which has pronounced nucleophilicity. In the literature, reactions have been proposed for the preparation of chalcones by condensation of aldehydes with ketones in alkaline medium by microwave irradiation with a significant increase in yield and reduction of the reaction time [20].

Condensation of 2-acetylpyridine (**2**) with sodium carbonate-catalysed pyridine-2-carboxaldehyde in aqueous medium and microwave irradiation (480 W) proved to be cost effective to produce 1,3-bis(pyridin-2-yl)prop-2-en-1-one (**3**). The product was isolated in practically pure form, after cooling of the aqueous mixture and by simple filtration on the Buchner funnel in a 60% yield. Compounds **5** and **6** were determined by infrared absorption bands of carbonyl groups C=O, at 1686-1676 cm^{-1}, which were similar to those reported in the literature [22-29]. The formation of compounds **1-6** was confirmed by NMR spectroscopy, for some ^{13}C NMR spectra DEPT 135 were performed. Carbonyl groups of **3** were identified at 189.57 ppm, whilst the grouping $HC_{\beta}=C_{\alpha}H-C=O$ were determined at 143.12 ppm (C_{β}) and 124.70 ppm (C_{α}) in accordance to [20]. Compound **4** is a symmetric molecule, so the groups C=O have the same chemical shift determined at 200.42 ppm.

Based on the experimental spectral data and the X-ray analysis of the obtained compounds **3-6**, optimized methods for the synthesis were proposed (Table 1).

Table 1

Optimized methods of synthesis of compounds 3-6.

Compound	*Molar ratio of reagents, (**1**):(**2**):catalyst*	*Solvent*	*Reactions conditions*	*Time*	*Yield, %*
3	1:1:0.25 (Na_2CO_3)	H_2O	MW, 480 W	5 min	60
4	1:2:2 (NaOH)	EtOH	20°C	4 h	45
	1:2:2 (HCl)	EtOH	0-5°C	5 h	53
5	1:3:8 (NaOH)	MeOH	60-65°C	6 h	48
6	2:3:10 (NaOH)	EtOH	20°C	60 h	24

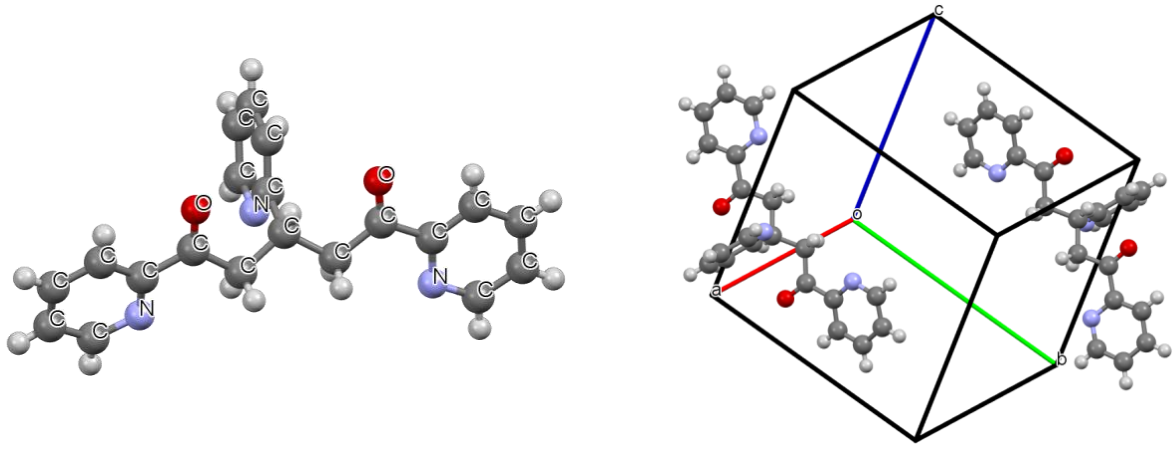
*All the isolated yields are based on the consumption of **1**.*

Single-crystal X-ray diffraction study

Monocrystals of **4-6** were obtained for the characterization of the final **5** and **6** and intermediate **3** and **4** compounds. The results of NMR spectroscopy and the single-crystal X-ray diffraction suggested a series of intermediate product transformations which occurred in the reaction mixture. The obtained crystallographic data of compounds **4**, **5** and **6** are presented in Figures 1, 2 and Table 2.

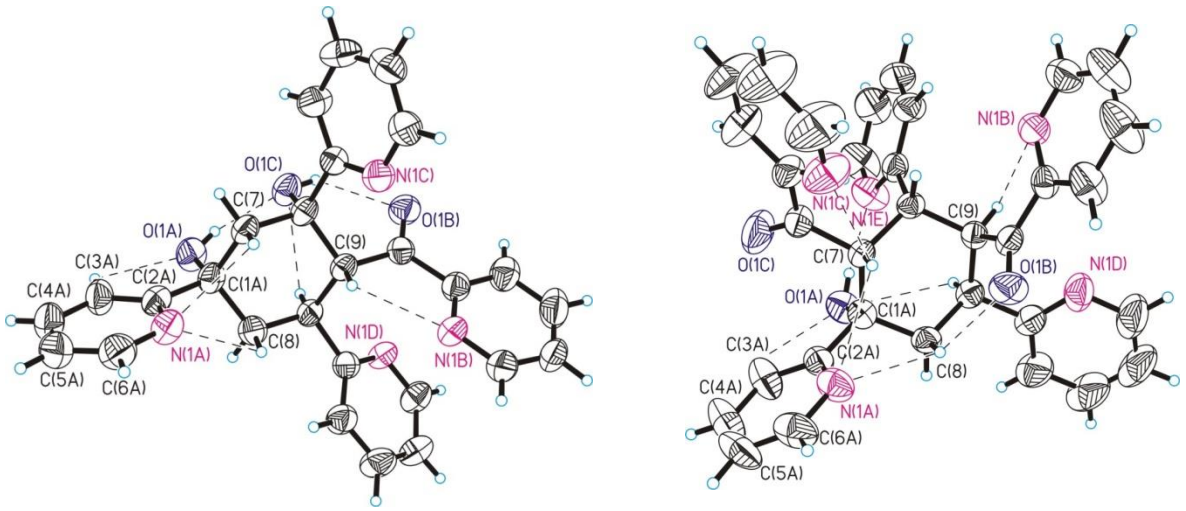
Three pyridine-2-yl groups are attached to cyclohexane rings in **5** and **6** while one and two pyridine-2-ylmethanone groups are bound with cyclohexane in these compounds respectively (Figure 2).

In both compounds, the central cyclohexane ring adopts a chair conformation with puckering parameters [20] Q, θ, φ equal to 0.5935 Å, 173.45°, 0.9° and 0.5414 Å, 169.64°, 110.4° for **5** and **6** respectively. In compounds **5** and **6**, the planes through the coplanar atoms (C1C(E)/C1D/C7/C8) form dihedral angles with pyridine cycles (N1/C2-C6) 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 2).



(a) ***(b)***

Figure 1. Crystalline structure of 1,3,5-tri(pyridin-2-yl)pentane-1,5-dione (4) *(a)* and the representation in the elemental cell *(b)*.



(a) ***(b)***

Figure 2. An ORTEP plot of compounds 5 *(a)* and 6 *(b)*, showing 50% probability displacement of ellipsoids and the atomic numbering.

Table 2

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

Parameters	***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 (°)	87.587(11)	90
β (°)	88.336(10)	121.940(2)
γ (°)	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 [$I > 2\sigma(I)$]	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

The bond lengths and angles in the studied compounds are consistent with those previously reported [20]. The molecular structures of compounds **5** and **6** are stabilized by intramolecular C—H···N, C—H···O and O—H···O hydrogen bonds (HB) (Table 2, Figure 2) 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 centrosymmetric dimers are linked by C(5C)-H…O(1B) HB. The dimers are joined into the chains along the [1 1 0] direction, through the hydrogen bonds C(1D)-H...O(1B) and O(1C)-H...N(1D) (Table 2, Figure 2). 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 the structure of compound **5**, van der Waals interactions 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 2, Figure 2). The A and C pyridine rings related by the symmetry 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.

Antimicrobial activity of compounds 4-6

The antimicrobial activity of the synthesized compounds was investigated on three bacterial strains: *Escherichia coli* (G-); *Klebsiella pneumonia* (G-); *Staphylococcus aureus* (G+); and four fungal strains: *Candida albicans*; *Candida krusei*; *Candida parapsilosis* and *Cryptococcus neoformans*. The minimum inhibitory concentration and minimum bactericidal concentrations (MIC and MBC) for compounds **4**-**6** (Tables 3 and 4) were determined.

Staphylococcus aureus was the most vulnerable to the studied compounds. In this case, the values of MIC and MBC vary in the range of concentrations 4.0-0.15 mg/mL indicating a moderate activity of synthesized compounds.

Generally, compounds **4**-**6** present moderate antifungal activity. The activity of compound **6** is nine times higher towards *Cryptococcus neoformans* than the activity of Nistatin that is used in medical practice. The obtained results demonstrate that compound **6** has potential application as an antibacterial and antifungal drug.

Antioxidant activity of compounds 4-6

The antioxidant activity of the compounds was determined by the ABTS•$^+$ method. Experimental data presented in Table 5 revealed that compound **4** possesses five times higher antioxidant activity against ABTS•$^+$ with an IC_{50}= 24.2 μM, than compounds **5** and **6**.

Table 3

Antibacterial activity (MIC* and MBC) of compounds 4-6, expressed in mg/mL.**

Compounds	*Escherichia coli (G-)*		*Klebsiella pneumonia (G-)*		*Staphylococcus aureus (G+)*	
	MIC	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*
4	>10.0	>10.0	>10.0	>10.0	2.0	4.0
5	>10.0	10.0	0.25	>10.0	0.50	1.0
6	0.10	0.20	0.10	0.20	0.15	0.30
Furacillin	0.0046	0.0046	0.0046	0.0093	0.0046	0.0093

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

Table 4

Antifungal activity (MIC* and MFC) evaluation of compounds 4-6, expressed in mg/mL.**

Compounds	*Candida albicans*		*Candida krusei*		*Candida parapsilosis*		*Cryptococcus neoformans*	
	MIC	*MFC*	*MIC*	*MFC*	*MIC*	*MFC*	*MIC*	*MFC*
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	0.12	0.25
6	0.25	0.50	0.25	0.50	0.25	0.50	0.0039	0.0078
Nistatin	0.032	0.064	0.032	0.064	0.032	0.064	0.032	0.064

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

Table 5

The antioxidant activity of compounds 4-6 determined by *ABTS*.

Compounds	IC_{50}, *μM*
4	24.2
5	>100
6	>100
Trolox	26.3
Rutin	20.7

Conclusions

The aldol condensation between 2-formylpyridine (**1**) and 2-acetylpyridine (**2**), leads to the formation of 1,3-bis(pyridin-2-yl)prop-2-en-1-one (**3**), which cannot be removed under the conditions of the Claisen-Schmidt reaction. Subsequently, it has been shown that in the first stage azachalcone, as a result of Michael's addition of 2-acetylpyridine (**2**), generates the diketone intermediate 1,3,5-tri(pyridin-2-yl)pentane-1,5-dione (**4**) that participates in the double aldol reaction with the third molecule of 2-acetylpyridine (**2**), forming an intermediate that is easily cyclized into the cyclohexane ring **5**. Michael's addition of diketone **4** to the conjugated double bond of azachalcone **3** also leads to cyclization with the formation of (4-hydroxy-2,4,6-tri(pyridin-2-yl)cyclohexane-1,3-diyl)bis(pyridin-2-ylmethanone) (**6**).

Based on the experimentally obtained data of synthesized compounds, a plausible mechanism for the formation of compounds **3**-**6** was proposed and optimized methods for synthesis were suggested.

Condensation of 2-acetylpridine (**2**) with 2-formylpyridine (**1**), catalysed by Na_2CO_3 in aqueous medium and microwave irradiation (480 W), proved to be cost-effective to obtain **3**.

Single-crystal X-ray diffraction results demonstrated that the central cyclohexane ring adopts a chair conformation in compounds **5** and **6**. Due to hydrogen bonds, the molecules of **5** form centrosymmetric dimers which are joined into chains along the [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.

The antibacterial activity evaluation against *Escherichia coli* (G-); *Klebsiella pneumonia* (G-); *Staphylococcus aureus* (G+); *Candida albicans*; *Candida krusei*; *Candida parapsilosis*; *Cryptococcus neoformans* demonstrated that the bacteriostatic and bactericidal concentrations of compound **4** are within the range of 1-10 mg/mL, for compound **5** in the range 0.12-10 mg/mL and for compound **6** within the range 0.0039-0.5 mg/mL.

The antioxidant activity determined by the $ABTS^{\bullet+}$ method revealed that compounds **5** and **6** show moderate antioxidant activity with an $IC_{50} \geq 100$ μM, while compound **4** has a much higher antioxidant activity, $IC_{50} = 24.2$ μM.

Acknowledgements

The authors are thankful to Dr. Alic Barba (Institute of Chemistry, Chisinau, Republic of Moldova) for providing assistance with NMR analysis.

The authors would like to thank Olga Garbuz (State University of Moldova) for helping in performing the *in vitro* antioxidant activity.

The authors would like to thank Dr. Olga Burduniuc, (National Agency for Public Health, Chisinau, Republic of Moldova) and Dr. Greta Balan (State University of Medicine and Pharmacy "N. Testemitanu", Chisinau, Republic of Moldova) providing assistance in antibacterial and antifungal activity evaluation tests.

Funding

Ministry of Education, Culture and Research of the Republic of Moldova is greatly acknowledged for providing financial support of the Institutional Project no. 15.817.02.24F (2019).

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