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Synthesis, crystal structure and antibacterial studies of 2,4,6-trimethoxybenzaldehyde based dihydropyrimidine derivatives

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Abstract

We report herein the synthesis of new derivatives of dihydropyrimidine on the basis of 2,4,6-trimethoxybenzaldehyde, which was further regioselectively oxidized in the presence of cerium ammonium nitrate (CAN) with the formation of 5-acetyl-6-methyl-4-(2,4,6-trimethoxyphenyl)pyrimidin-2(1H)-one. The structures of both novel compounds were investigated by NMR, mass spectroscopy methods. The structure of the dihydropyrimidine compound **4** was also investigated by X-ray single crystal diffraction method. In order to understand the molecular interactions in its crystal packing, the Hirshfeld surface and contacts enrichment analyses were performed. Biological activities of the synthesized compounds were studied against gram-negative *A. baumannii*, *E. coli*, *P. aeruginosa* and *K. pneumoniae* and gram-positive *S. aureus* bacteria.

Key words: Biginelli reaction, pyrimidine, cerium ammonium nitrate, Hirshfeld surface analysis, crystal structure, antibacterial activity.

1. Introduction

Biginelli reaction as a typical representative of one-pot multicomponent reactions is an extremely potent synthetic strategy and highly flexible tool for the design and construction of dihydropyrimidines – a unique class of heterocyclic compounds with two nitrogen atoms in the cycle [1, 2]. The potency of this reaction is caused by high complexity and a large diversity of the product despite using simple starting materials such as aldehyde, urea derivative and methylene active compound. The evidence of the widespread interest of the scientific community to Biginelli reaction is the numerous research articles as well as reviews published so far on this topic, which in turn led to dihydropyrimidines being known in medicine and brought them enormous fame. The reason of such reputation is caused by the broad spectrum of biological activities of dihydropyrimidines [4-6]. According to the umpteen investigations, these compounds demonstrate anti-leishmanial [7], antitumor [8-13], antiviral [14, 15], antifungal [16], antiproliferative [17, 18], antibacterial [19-21], anti-inflammatory [22-24], anti-hypertensive [25-27], antiepileptic [28], anti-HIV [29], antidiabetic [30, 31], anti-malarial [32], mPGES-1 inhibitors [33], antitubercular [34], miscellaneous [35-37] activities, as well as calcium [38] and potassium channels [39-41] and α_1 adrenergic antagonists [42]. However, the mentioned broad spectrum of activities is not an application limit of dihydropyrimidines in medicine. A class of compounds can show extremely good results against various microbes, but because of a number of factors (toxicity, solubility, absorbability and so on) it cannot be a drug [43-45]. A uniqueness of dihydropyrimidines is that different drugs were synthesized on the basis of them (Fig. 1). Compounds which found their application in medicine include batzelladine A and B against HIV [29], (S)-monastrol, (S)-enastron, mon-97, (R)-fluorastrol against cancer [8-13], terazosin for treating of benign prostatic hyperplasia (prostate enlargement) and high blood pressure [36].

Figure 1. Acclaimed drugs on the basis of dihydropyrimidines

On the other hand, various procedures have been developed in order to modify the structure of dihydropyrimidines allowing introducing various pharmacophoric groups in their structure [36, 46-48]. One of such type modifications is the regioselective oxidation of dihydropyrimidine ring. This leads to the disappearing of one amine group, leaving another amine group as the only nucleophilic center that in turn impact on selectivity of the targeted N-modified product. Another advantage of dihydropyrimidine ring oxidation is its solubility. The point is that dihydropyrimidines, on the basis of aromatic aldehydes, are mostly soluble only in high polar

solvents such as methanol, ethanol, acetonitrile and DMSO. Some of them are only soluble just in DMSO, whereas oxidized products are also soluble in solvents with low polarity, such as chloroform, dichloromethane, ethyl acetate, which in turn expands their application area. Furthermore, in some cases, oxidized dihydropyrimidines are more biologically active than initial dihydropyrimidines [49-52].

Concerning the biological activity of dihydropyrimidine core, a new dihydropyrimidine, compound **4**, was synthesized by Biginelli reaction in microwave condition in the presence of copper triflate on the basis of 2,4,6-trimethoxybenzaldehyde. Taking into account the above mentioned perspectives of dihydropyrimidine ring oxidation reaction, the obtained dihydropyrimidine **4** was regioselectively oxidized with the formation of a novel pyrimidine **5**. As crystals of the dihydropyrimidine **4** were obtained, its structure was investigated by X-ray single crystal diffraction. In addition, the Hirshfeld surface and contacts enrichments analysis was done to quantify the molecular interactions and understand their importance in the crystal packing. Due to the fact that dihydropyrimidines demonstrate a wide spectrum of biological activities, the antibacterial activity of novel synthesized compounds **4** and **5** was analyzed against *A.baumannii*, *E.coli*, *P.aeruginosa*, *K.pneumoniae* and *S.aureus* bacteria and promising results were obtained.

2. Materials and methods

2.1 General Information

All the solvents and reagents were purchased from commercial suppliers and were of analytical grade and used without further purification. The control of the reactions progress and the determination of the synthesized compounds purity were done by thin layer chromatography (TLC) on Merck silica gel plates (60 F254 aluminium sheets) which were visualized under UV light. Melting points were recorded in open capillary tubes on a Buchi B-540 apparatus and were uncorrected. Elemental analysis was performed on the Carlo Erba 1108 analyzer.

2.2 Experimental synthesis procedure

2.3

2.2.1 Synthesis of 5-acetyl-6-methyl-4-(2,4,6-trimethoxyphenyl)-3,4-dihydropyrimidin-2(1H)-one (**4**) by Biginelli reaction

Scheme 1. Synthesis of targeted dihydropyrimidine **4**.

0.5 mmol of 2,4,6-trimethoxybenzaldehyde, 0.75 mmol (45 mg) of urea and 0.027 mmol (10 mg) of copper-triflate $\text{Cu}(\text{OTf})_2$ were added to a microwave vial with a magnetic stirrer and dissolved in 1 ml of ethanol (Scheme 1). Subsequently, 0.49 mmol (50 μl) of acetylacetone was added to a vial, which was sealed and irradiated at 100°C in a microwave reactor for 2.5 h at a maximum power of 200W (CEM DiscoverTM System). At the end of reaction time, the precipitate was formed, filtered, washed with distilled water and dried. Single crystals of compound (4) were obtained by crystallization in methanol. Yield 80 %. M.p. $249\text{--}250^\circ\text{C}$. ¹H NMR spectrum: (DMSO- d_6 , δ , ppm), 1.91 s (3H, CH_3), 2.11 s (3H, CH_3), 3.72 s (6H, 2OCH_3), 3.76 s (3H, OCH_3), 5.79 s (1H, CH), 6.22 s (2H, 2C_{ArH}), 7.03 s (1H, NH), 8.95 s (1H, NH). ¹³C NMR spectrum: (DMSO- d_6 , δ , ppm), 18.8 (CH_3), 28.9 (CH_3), 45.78 (CH), 55.19 (OCH_3), 55.74 (2OCH_3), 91.26 (2C_{ArH}), 105.91 (C), 113.22 (C), 146.9 (C_{Ar}), 151.76 (C_{Ar}), 158.78 (2C_{Ar}), 160.3 (CO), 195.09 (CO). HRMS (ESI-MS): 343.17 [$\text{M}^+ + \text{Na}^+$]. Elemental analysis calcd. for $\text{C}_{16}\text{H}_{20}\text{N}_2\text{O}_5$, %: C, 59.99; H, 6.29; N, 8.74. Found, %: C, 59.94; H, 6.37; N, 8.79. This compound can also be obtained by the following procedure. To a solution of 2,3 mmol (0.45 gr) of 2,4,6-trimethoxybenzaldehyde in 30 ml of ethanol were added 10 mmol (0.6 gr) of urea, 9.75 mmol (1ml) of acetylacetone and 8 ml of acetic acid. The reaction mixture was refluxed for 10 h. At the end of reaction time, the mixture was cooled with ice and precipitate was formed, which was filtered, washed with distilled water and dried. Yield 58%.

2.2.2 Synthesis of 5-acetyl-6-methyl-4-(2,4,6-trimethoxyphenyl)pyrimidin-2(1H)-one (5) by regioselective oxidation in the presence of CAN

Scheme 2. Synthesis of pyrimidine **5** in the presence of CAN.

0.30 mmol of dihydropyrimidine **4** was dissolved in a solvent mixture consisting of 4 ml DMSO and 4 ml acetone. To this solution, 2 mmol of sodium bicarbonate (NaHCO_3) was added. The temperature of the reaction mixture was decreased to -5°C . Then, 1.2 mmol of CAN was dissolved in 2 ml of water and added to the solution of dihydropyrimidine under argon (Ar) atmosphere (the color changed from orange to a pale yellow). The reaction mixture was stirred 1 hour at -5°C and 20 hours at room temperature. At the end of reaction time, the reaction mixture was decanted with chloroform. Subsequently, the organic layer was washed with brine and dried over anhydrous sodium sulphate (Na_2SO_4). Removing of chloroform gave a precipitate, which further purification with the Biotage Isolera One Flash Chromatography System (ethyl acetate-methanol: 9:1) allowed obtaining of pure oxidized dihydropyrimidine **5**. Yield 36.4 %. M.p. 265--

266°C. ¹H NMR spectrum: (CDCl₃, δ, ppm), 1.95 s (3H, CH₃), 2.54 s (3H, CH₃), 3.75 s (6H, 2OCH₃), 3.87 s (3H, OCH₃), 6.16 s (2H, 2C_{Ar}H). ¹³C NMR spectrum: (CDCl₃, δ, ppm), 23.28 (CH₃), 30.05 (CH₃), 55.41 (OCH₃), 55.7 (2OCH₃), 90.82 (2C_{Ar}H), 102.9 (C), 120.84 (C), 156.85 (C_{Ar}), 158.56 (2C_{Ar}), 164.29 (CO), 171.17 (C), 171.34 (C_{Ar}), 198.7 (CO). HRMS (ESI-MS): 319.17 [M⁺+H⁺], 341.17 [M⁺+Na⁺]. Elemental analysis calcd. for C₁₆H₁₈N₂O₅, %: C, 60.37; H, 5.7; N, 8.8. Found, %: C, 60.31; H, 5.76; N, 8.89.

2.3 NMR experiments

The NMR experiments were performed on a BRUKER FT NMR spectrometer AVANCE 300 (Bruker, Karlsruhe, Germany) (300 MHz for ¹H and 75 MHz for ¹³C) with a BVT 3200 variable temperature unit in 5 mm sample tubes using Bruker Standard software (TopSpin 3.1). Chemical shifts were given in ppm (δ) and were referenced to internal tetramethylsilane (TMS). Multiplicities are declared as follow: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet). Coupling constants *J* are given in Hz. The experimental parameters for ¹H are as follows: digital resolution=0.23 Hz, SWH=7530 Hz, TD=32 K, SI=16 K, 90° pulse-length=10 ms, PL1=3 dB, *ns*=1, *ds*=1, *dl*=1 s and for ¹³C as follows: digital resolution=0.27 Hz, SWH=17985 Hz, TD=64 K, SI=32 K, 90° pulse-length=9 ms, PL1=1.5 dB, *ns*=500, *ds*=2, *dl*=3 s. The NMR-grades DMSO-d₆ (99.7%, containing 0.3% H₂O), CDCl₃ were used for the solutions of synthesized compounds **4** and **5**.

2.4 Mass experiments

High-resolution mass spectrometry (HRMS) was performed using electrospray ionization (ESI) in positive-ion or negative-ion detection mode.

2.5 X-Ray analysis

X-Ray analyses were performed on Bruker SMART APEX II Single Crystal X-ray Diffractometer equipped with graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å) at 296(2) K, respectively. The crystal structure was solved by direct methods and refined on *F*² by full matrix least-squares using Bruker's SHELXTL-97 [53]. The details of the crystallographic data for synthesized compound are summarized in Table 1. Crystallographic data for the structural analysis have been deposited to the Cambridge Crystallographic Data Center under number CCDC 2014151.

2.6 Hirshfeld surface analysis

Hirshfeld surface and contact enrichment ratios were obtained with MoProViewer [54]. As X...Y and Y...X contacts yield similar contact surfaces and *E_{elec}* values in the context of this study, the reciprocal contacts were merged together.

For the electrostatic energy calculation, the charge density parameters were transferred from the ELMAM2 database of multipolar atoms [55]. The X-H bond lengths were elongated according to standard neutron diffraction distances [56]. The electrostatic energy was computed

with the VMoPro module of MoPro software [57] between pairs of atoms in close contact up to a cutoff of sum of van der Waals radii plus 0.5 Å. The average E_{elec} value was obtained by dividing the summation by the number of contacts. The energy of non-existent O...O contacts was computed at the cutoff distance.

2.7 Biological assay

The antibacterial activity of synthesized dihydropyrimidine derivatives **4** and **5** was studied against gram-negative *A. baumannii*, *E. coli*, *P. aeruginosa* and *K. pneumoniae* and gram-positive *S. aureus* bacteria by determining minimal inhibitory concentrations (MIC), which is the lowest concentration of compound that prevents the growth of bacterial cells after incubation. MIC was determined by the twofold micro-dilution method as described in [58]. The compounds were prepared according to CLSI guidelines and diluted in U-bottom 96 well microtiter plates which contained Muller Hinton Broth (MHB). The freshly prepared bacterial strains at about 10^5 CFU (colony forming unit) in MHB medium were added to each well of the microplate and incubated at 37°C for 24 hours. The concentration of the tested compounds ranged from 1000 to 7,8 µg/mL. The growth of the bacterial cells was determined by resazurin method. The solution of resazurin sodium salt (0.01%) was freshly prepared in sterile distilled water. After incubation, 30 µL of this solution was added in each microplate well and incubated again at the same condition for about 4 h. MIC was represented as the lowest concentration of the compounds which inhibited the color change from blue to pink since pink color indicated the growth of bacteria [58]. MIC of the studied compound was compared with MICs of pristine antibiotics (cefotaxime and ceftriaxone).

Furthermore, the efficiency of the synthesized dihydropyrimidine against the above-mentioned bacteria was also tested by disc-diffusion method as described by Mayrhofer [59]. In details, the surface of the nutrient medium (meat-peptone agar, Potato Dextrose Agar) was stratified with 1 mL of the diurnal suspension of the test culture (10^5 CFU/mL), which was used during 15 min after preparation. Previously prepared discs with certain concentrations were stratified on the surface of the nutrient medium by the sterile tweezers. Dishes were incubated at 37°C during 24 h. DMSO was used as a solvent. Record of the results was carried out, compared with control dishes without compound and with the known drugs cefotaxime and ceftriaxone. The biological assays were carried out in triplicate. Standard deviations data were also calculated.

3. Results and discussion

3.1 Chemical synthesis.

First of all, the synthesis was started by performing Biginelli reaction on the basis of 2,4,6-trimethoxybenzaldehyde, urea and acetylacetone in the presence of acetic acid [16] leading to the obtaining of new dihydropyrimidine **4** with 58% yield. The positive side of this protocol was simple work-up procedure – during cooling of the reaction mixture, the formed precipitate was washed just with distilled water. Among disadvantages, the high reaction time (10 h) and not so high yield of the desired product can be mentioned. This promoted us to search for a new procedure for this reaction. Performing Biginelli reaction in the presence of Yb(PFO)₃ [60], I₂/MWI [61],

[Hmim]HSO₄-NaNO₃ [62], TMSCl/CAN [63], GaI₃ [64], CAN [65], Sm(ClO₄)₃ [66], La(OTf)₃ [67], RuCl₃ [68], HBF₄-SiO₂ [69], H₃PMo₁₂O₄₀ [70], Sc(OTf)₃ [71], MgCl₂×6H₂O [72], In(OTf)₃ [73] and NH₄Cl [16] did not lead to the desired effect. Only improving the [74] procedure by performing the reaction in the presence of Cu(OTf)₂ in microwave conditions allowed us to obtain targeted dihydropyrimidine with 80% yield. Another positive side of this procedure is that the reaction time was decreased four times from 10 to 2.5 hours (Scheme 1). The structure of synthesized novel dihydropyrimidine was determined by ¹H, ¹³C, dept 135 NMR, mass spectroscopy and elemental analysis. As it can be seen from ¹H NMR spectrum, the signals from methyl and methoxy groups are observed at 1.91, 2.11, 3.72 and 3.76 ppm correspondingly, whereas the position of dihydropyrimidine core CH group is at 5.79 ppm. Their positions on ¹³C NMR spectra are at 18.8, 28.9, 45.78, 55.19 and 55.74 ppm. The signals from amine groups of dihydropyrimidine core are observed at 7.03 and 8.95 ppm respectively (Supplementary material).

The next step of synthesis was to perform regioselective oxidation of the synthesized dihydropyrimidine **4** in the presence of CAN. A small modification of the procedure [50] allowed the synthesis of the new pyrimidine **5** (Scheme 2). The structure of synthesized novel pyrimidine **5** was determined by ¹H, ¹³C, dept 135 NMR, mass spectroscopy and elemental analysis (Supplementary material). As it can be seen from ¹H NMR spectrum, the signals from the amine group and CH group of pyrimidine core have disappeared, which is a strong evidence of product **5** formation. The signals from methyl and methoxy groups are shifted to 1.95, 2.54, 3.75 and 3.87 ppm correspondingly. Their positions on ¹³C NMR spectra are at 23.28, 30.05, 55.41 and 55.7 ppm respectively.

3.2 Structure description.

It was also possible to obtain single crystals of the compound **4** by crystallization in methanol-ethyl acetate solution (9:1). The title compound crystallized in the triclinic system with space group P -1 and Z = 2. The molecule of the title compound is built up from a 3,4-dihydropyrimidin-2(*1H*)-one ring linked to an acetyl group (CH₃CO), a methyl group and a 2,4,6-trimethoxyphenyl ring (Fig. 2). Crystallographic data and detailed refinement results of the compound **4** are presented in Table 1. The bond lengths and angles are given in Table S1, while hydrogen bonds present in the structure are shown in Table S2 (Supplementary material).

Figure 2. A view of the asymmetric unit of 5-acetyl-6-methyl-4-(2,4,6-trimethoxyphenyl)-3,4-dihydropyrimidin-2(1H)-one, showing the atom labeling. Displacement ellipsoids are drawn at the 50% probability level.

The dihydropyrimidine ring (atoms N5-C4-N3-C2-C1-C6) adopts a boat conformation, as indicated by the puckering parameters: $Q_2 = 0.143$ (4) Å, $\theta = 72.53$ (2)° and $\varphi = 143.58$ (2)°. The mean plane through the ring is nearly perpendicular to the 2,4,6-trimethoxyphenyl ring, with which it makes a dihedral angle of 88.72° (Fig. 3, Table S1).

The crystal packing of dihydropyrimidine **4** is formed via inter and intramolecular hydrogen bonding. There are five potential hydrogen bond donors, namely N3, N5, C2, C8 and C9 and five potential acceptors, namely the carbonyl oxygen atoms O2ⁱⁱ [(ii) $-x+1, -y, -z$] and O2ⁱⁱⁱ [(iii) $x-1, y, z$], the acetyl oxygen O1ⁱ ($x+1, y, z$) the methoxy oxygen O3.

Furthermore, the crystal packing of dihydropyrimidine **4** is formed by polar sheets with its functional groups involved in hydrogen bonds and by hydrophobic spacers which are formed by the phenyl moiety with its methoxy groups (Fig. 4). Dimeric motifs utilizing N3—H3 $\cdots$ O1ⁱ and C9—H9B $\cdots$ O2ⁱⁱⁱ hydrogen bonds can be described R₂²(10) graph-set motif connecting two adjacent molecules involving the carbonyl group and R₂²(8) which involved the ketone group in polar sheets along *a*-axis (Fig. 5, Table S2) [75].

Table 1. Crystallographic data and structure refinement for 5-acetyl-6-methyl-4-(2,4,6-trimethoxyphenyl)-3,4-dihydropyrimidin-2(1H)-one (**4**).

Chemical formula	C ₁₆ H ₂₀ N ₂ O ₅
<i>M_r</i>	320.34
Crystal system, space group	Triclinic, P -1
Temperature (K)	296
<i>a</i> , <i>b</i> , <i>c</i> (Å)	7.5197 (4), 8.4695 (4), 13.3399 (8)
<i>α</i> , <i>β</i> , <i>γ</i> (°)	96.231 (2), 100.613 (2), 99.357 (2)
<i>V</i> (Å ³)	815.50 (8)
<i>Z</i>	2
Radiation type	Mo <i>Kα</i>
Absorption <i>μ</i> (mm ⁻¹)	0.10
Crystal size (mm)	0.82 × 0.62 × 0.60
<i>T_{min}</i> , <i>T_{max}</i>	0.641, 0.746
<i>R_{int}</i>	0.024
sin <i>θ</i> _{max} / <i>λ</i> (Å ⁻¹)	0.682
<i>R</i> [<i>F</i> ² > 2σ(<i>F</i> ²)], <i>wR</i> (<i>F</i> ²), <i>S</i>	0.056, 0.169, 1.05
Δ <i>ρ</i> _{max} , Δ <i>ρ</i> _{min} (e Å ⁻³)	0.31, −0.27

Figure 3. Dihedral angle between the planes of 3,4-dihydropyrimidin-2(1H)-one ring and the 2,4,6-trimethoxyphenyl ring in the compound **4**.

Figure 4. Crystallographic autostereogram view of the dihydropyrimidine **4** crystal packing along the *b*-axis. Dotted lines indicate N-H...O and C-H...O hydrogen bonds.

Figure 5. Autostereogram view of packing crystal parallel to (*ab*) plane, showing molecules linked through C—H...O and N—H...O hydrogen bonds (dashed lines).

Within the dihydropyrimidin-2(*IH*)-one rings, an examination of the bond length data (Table S2) shows that N5-C6 [1.3754 (17) Å], N5-C4 [1.3727 (16) Å], N3-C4 [1.3383 (17)) Å], and N3-C2 [1.4725 (16) Å] can be attributed as having clear single bond character. The C1-C7 [1.4725 (18) Å], C1-C2 [1.5285 (17) Å], C2-C10 [1.5254(17) Å], C6-C9 [1.5007(18) Å], [C7-C8 1.505(2) Å] distances are in agreement with the presence of the C-C single bonds. Also, these phenyl moieties exhibit a regular spatial configuration with normal C-C distances and C-C-C angles. The average value of the C-C bond lengths of the phenyl rings is 1.397 (3) and 1.388 (3) Å which are between a single and double bond and agree with those in the literature [76, 77]. The conformation of the dihydropyrimidin-2(*IH*)-one ring defined by the torsion angles C4–N5–C6–C9, C6–N5–C4–O2, C6–C1–C7–O1 and C6–C1–C7–C8 of experimentally 170.03 (14)°, -176.62 (14)°, 11.1 (2)° and -170.10(4)°. The aromatic rings are planar with a r.m.s. (root-mean-square) deviation of 0.016 Å for the C10/C11/C12/C13/C14/C15 ring and -0.097 Å for the C1/C2/N3/C4/N5/C6 ring.

No stacking interactions are observed between neighboring aromatic rings in the molecular packing but the cohesion structure is ensured by weak C-H... π hydrogen bonds. Indeed, the distances between the H17B and H18C hydrogen atoms and the centroids Cg1 and Cg2 of two neighboring C10-C11-C12-C13-C14-C15 phenyl rings, at *x,y,z* and 1-*x*,1-*y*,1-*z* symmetry codes, vary between 3.786 Å and 3.852 Å (Fig. 6).

Figure 6. View of C-H ... π intermolecular interactions in the crystal unit cell of compound 4.

3.3 Hirshfeld surface analysis.

The Hirshfeld surface is representative of the region in crystalline space where molecules come into contact allowing the analysis of the chemical nature of intermolecular interactions. Table 2 shows the proportions C_{xy} of the different contact types. The enrichment ratio E_{xy} is obtained by comparing the actual contacts C_{xy} with those computed as if all contacts had the same probability to form. An enrichment ratio larger than unity indicates that the contact type is over-represented in the crystal [78, 79].

The fingerprint plot (Fig. 7, Supplementary material) shows two spikes at short distance corresponding the strong hydrogen bonds N-H...O=C. The other spike at shortest distance concern the H...H contacts with $d(\text{H,H})$ as low as 2.2 Å. The C...H weak hydrogen bonds appear in the fingerprint at longer distances $d > 2.8$ Å. The N...H contacts are not hydrogen bonds as the sp^2 nitrogen atoms have three covalent bonds and no lone pair.

The hydrophobic Hc atoms occupy more than half of the Hirshfeld surface and Hc...Hc contacts have the highest proportion at one third (Table 2) and are equi-represented ($E=1$). Globally hydrophobic atoms C and Hc represent nearly 80 % of the surface and their contacts at 63% are slightly over-represented ($E=1.03$).

The strongest possible hydrogen bond for this molecule is of C=O...H-N type. The two N-H donors and two C=O acceptors do form such a hydrogen bond and this contact type is the most enriched $E(\text{Hn,O=c})=4.9$. The crystal packing of the title compound is consistent with the often quoted Etter rule of the best donor–best acceptor pairing of hydrogen bonds [80]. On the other hand, the ether oxygen atoms Occ which are weaker acceptors are more interacting with the Hc hydrogen atoms and this contact is moderately over-represented ($E=1.34$).

The average electrostatic energy E_{elec} between the atom pairs in contact was computed for the different types. The scatterplot of E_{elec} and of contacts enrichment E (Fig. 8, Supplementary material) shows that the Oc...Hn interaction is an outlier in the graph with much higher E and E_{elec} values than the other contact types and is the major driving force of the crystal stabilization. The

weak H-bond Hc...Occ appears next in the region of high E values and most favorable E_{elec} values. The oxygen...carbon stacking contacts appears attractive according to the E_{elec} values although there are slightly under-represented.

Table 2. Statistical analysis of intermolecular contacts on the Hirshfeld surface. The second row shows the chemical content on the Hirshfeld surface. The middle part shows the proportions C_{xy} of the different contact types and the lower part shows the E_{xy} enrichment ratios of contact types. Reciprocal contact types X...Y and Y...X are merged. The main contacts and the most enriched ones are highlighted in bold. The hydrogen atoms bound to carbon Hc and to nitrogen Hn were distinguished as they have different charge character. The strong H-bond acceptor O=C oxygen carbonyl was also distinguished from the ether oxygen.

atom %	C	Hc	Occ	O=c	Hn
	21.2	58.3	4.2	8.9	6.2
C	5.1				
Hc	26.4	33.5			
Occ	1.3	6.2	0		
O=c	2.1	10.4	0.4	0.1	
Hn	0.6	5.2	0.2	5.7	0.5
C	1.17				
Hc	1.09	1.00			
Occ	0.79	1.34	0		
O=c	0.53	0.96	0	0.50	
Hn	0.24	0.72	0.33	4.9	1.14

3.4 Biological assays

The antibacterial activity of the synthesized dihydropyrimidine was investigated against gram-negative *A. baumannii*, *E. coli*, *P. aeruginosa* and *K. pneumoniae* and gram-positive *S. aureus* bacteria. Biological activity investigations were started with the determination of minimum inhibitory concentration (MIC) for the synthesized novel compound **4** and **5** and pristine antibiotics (cefotaxime and ceftriaxone) by the twofold micro-dilution method. As shown in Table 3, the synthesized dihydropyrimidine **4** activity was similar to the ceftriaxone one in case of *S. aureus* (31.2 $\mu\text{g}/\mu\text{L}$), whereas in case of *A. baumannii*, *E. coli*, *P. aeruginosa* and *K. pneumoniae* bacteria MICs were higher compared to both pristine antibiotics. MIC of compound (**5**) was similar to ceftriaxone result (62.5 $\mu\text{g}/\mu\text{L}$) in case of *K. pneumoniae*, whereas in case of all other bacteria it was two times lower. Comparative analysis of compound **5** MICs with cefotaxime demonstrated that just, in case of was *K. pneumoniae*, it was two times higher than cefotaxime (62.5 $\mu\text{g}/\mu\text{L}$),

whereas for all other bacteria, the results were similar to cefotaxime.

Table 3. Minimum inhibitory concentration (MIC, $\mu\text{g}/\mu\text{L}$) of the studied compounds.

Bacteria	Minimum inhibitory concentration (MIC, $\mu\text{g}/\mu\text{L}$)			
	Compound (4)	Compound (5)	Cefotaxime	Ceftriaxone
<i>E. coli</i>	62.5	15.6	15.6	31.2
<i>P. aeruginosa</i>	500	31.2	31.2	62.5
<i>A. baumannii</i>	500	31.2	31.2	62.5
<i>K. pneumoniae</i>	500	62.5	31.2	62.5
<i>S. aureus</i>	31.2	15.6	15.6	31.2

Table 4. Antibacterial activity of novel dihydropyrimidine 4.

<i>E.coli</i>	compound (4)					cefotaxime	ceftriaxone
Concentration ($\mu\text{g}/\mu\text{l}$)	62.5	125	250	500	1000	1000	1000
Inhibition zone (mm)	3	7	15	22	30	40	32

<i>P.aeruginosa</i>	compound (4)					cefotaxime	ceftriaxone
Concentration ($\mu\text{g}/\mu\text{l}$)	500	1000	1500	2000	2500	2500	2500
Inhibition zone (mm)	2	6	14	20	28	45	45

<i>A.baumannii</i>	compound (4)					cefotaxime	ceftriaxone
Concentration ($\mu\text{g}/\mu\text{l}$)	500	1000	1500	2000	2500	2500	2500
Inhibition zone (mm)	2	5	9	15	24	45	45

<i>K.pneumoniae</i>	compound (4)					cefotaxime	ceftriaxone
Concentration ($\mu\text{g}/\mu\text{l}$)	500	1000	1500	2000	2500	2500	2500
Inhibition zone (mm)	3	8	13	20	31	45	45

<i>S.aureus</i>	compound (4)					cefotaxime	ceftriaxone
Concentration ($\mu\text{g}/\mu\text{l}$)	31.2	62.5	125	250	500	500	500
Inhibition zone (mm)	3	5	9	15	28	34	26

Taking into account the MIC of the compounds, antibacterial activity was also investigated by disc-diffusion method. Results were compared with the antibacterial activity of pristine antibiotics. As it can be seen from Table 4, the antibacterial activity of the novel dihydropyrimidine **4** against *S. aureus* was higher than that of pristine antibiotic ceftriaxone, equal to 28 and 26 mm inhibition disk radius correspondingly. The same tendency was not observed in case of all gram-negative bacteria, where the antibacterial activity of compound **4** was lower than that of both pristine antibiotics cefotaxime and ceftriaxone (Table 4).

In comparison to compound **4**, the antibacterial activity of compound **5** was higher against all mentioned bacteria. In case of *E.coli*, the antibacterial activity of compound **5** was higher than that of ceftriaxone but lower than cefotaxime, equal to 20, 17, 22 mm respectively. Looking at the inhibition zone of compound **5** in the case of *K.pneumoniae*, it was equal to ceftriaxone one (29 mm) but lower than cefotaxime (36 mm). A positive trend was observed in case of *P. aeruginosa*, *A. baumannii* and *S. aureus*, where the antibacterial activity of compound **5** was higher than that of the two pristine antibiotics cefotaxime and ceftriaxone (Table 5).

Table 5. Antibacterial activity of novel pyrimidine **5**.

<i>E.coli</i>	compound (5)					cefotaxime	ceftriaxone
Concentration (µg/µl)	15.6	31.2	62.5	125	250	250	250
Inhibition zone (mm)	3	5	9	14	20	22	17

<i>P.aeruginosa</i>	compound (5)					cefotaxime	ceftriaxone
Concentration (µg/µl)	31.2	62.5	125	250	500	500	500
Inhibition zone (mm)	2	5	14	23	30	28	21

<i>A.baumannii</i>	compound (5)					cefotaxime	ceftriaxone
Concentration (µg/µl)	31.2	62.5	125	250	500	500	500
Inhibition zone (mm)	5	13	20	30	38	35	29

<i>K.pneumoniae</i>	compound (5)					cefotaxime	ceftriaxone
Concentration (µg/µl)	62.5	125	250	500	1000	1000	1000
Inhibition zone (mm)	4	9	15	21	29	36	29

<i>S.aureus</i>	compound (5)					cefotaxime	ceftriaxone
Concentration (µg/µl)	15.6	31.2	62.5	125	250	250	250
Inhibition zone (mm)	5	11	18	25	31	28	21

Taking into account that DMSO, which was used as a solvent, is an example of a compound able to demonstrate biological activity, the record of the results was also carried out with control dishes without investigated compound. It was determined that DMSO does not influence on the above mentioned *gram*-positive and *gram*-negative bacteria.

3. Conclusion.

The new derivative of pyrimidine **5** was synthesized by the CAN mediated regioselective oxidation of novel dihydropyrimidine **4** which was obtained by Biginelli reaction in microwave conditions in presence of copper triflate. The structure of novel dihydropyrimidine **4** was investigated by X-ray single crystal diffraction. Along with it, Hirshfeld surface analysis was carried out to gain insight into the crystal packing and molecular interactions. Considering that the proposed substances **4** and **5** can have an ability to act as an antibacterial drug, they were tested for their biological activity against gram-negative *A. baumannii*, *E. coli*, *P. aeruginosa* and *K. pneumoniae* and gram-positive *S. aureus* bacteria. A comparison with the activities of pristine antibiotics cefotaxime and ceftriaxone was made. According to the antibacterial activity results, compound **5** demonstrates pronounced antibacterial activity in comparison to compound **4** and pristine antibiotics. In the future, by applying innovative technologies, they could lead to the creation of new effective antibacterial drugs.

Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Materials

Table of contents

1. NMR spectra.....	2
2. MS spectra.....	5
3. X-ray data.....	6
4. Hirshfeld surface analysis data.....	8

Table S1. Selected bond lengths (Å) and bond angles (°) for non-H atoms with *esd* values in parenthesis for the title compound.

O1—C7	1.2239 (18)	C1—C6	1.3560 (18)
O2—C4	1.2368 (16)	C1—C7	1.4725 (18)
O3—C11	1.3631 (17)	C1—C2	1.5285 (17)
O3—C16	1.4066 (19)	C10—C15	1.3920 (19)
O4—C13	1.3715 (18)	C10—C11	1.4049 (18)
O4—C17	1.421 (2)	C11—C12	1.390 (2)
O5—C15	1.3664 (16)	C12—C13	1.389 (2)
O5—C18	1.414 (2)	C2—C10	1.5254 (17)
N3—C4	1.3383 (17)	C6—C9	1.5007 (18)
N3—C2	1.4725 (16)	C7—C8	1.505 (2)
N5—C4	1.3727 (16)	C13—C14	1.383 (2)
N5—C6	1.3754 (17)	C14—C15	1.399 (2)
C11—O3—C16	119.15 (13)	O1—C7—C1	122.74 (13)
C13—O4—C17	118.41 (14)	O1—C7—C8	117.64 (13)
C15—O5—C18	119.08 (13)	O2—C4—N3	123.67 (12)
C4—N3—C2	125.92 (11)	O2—C4—N5	119.97 (12)
C4—N5—C6	124.03 (11)	O3—C11—C12	122.75 (12)
C4—N5—H5	114.7 (13)	O3—C11—C10	115.63 (12)
C6—N5—H5	120.3 (13)	O4—C13—C14	124.02 (15)
N3—C4—N5	116.30 (12)	O4—C13—C12	114.59 (14)
N5—C6—C9	112.49 (11)	O5—C15—C10	115.01 (12)
N3—C2—C10	110.27 (10)	O5—C15—C14	122.59 (13)
N3—C2—C1	110.64 (10)	C10—C15—C14	122.39 (12)
C1—C7—C8	119.61 (12)	C11—C10—C2	120.13 (11)
C1—C6—N5	120.85 (11)	C12—C11—C10	121.62 (13)
C1—C6—C9	126.65 (12)	C13—C12—C11	119.04 (13)
C6—C1—C7	121.09 (11)	C14—C13—C12	121.40 (13)
C6—C1—C2	119.83 (11)	C13—C14—C15	118.30 (13)
C7—C1—C2	119.07 (11)	C15—C10—C11	117.19 (12)
C10—C2—C1	114.90 (10)	C15—C10—C2	122.62 (11)

Table S2. Geometric details of hydrogen bonds (Å, °) (D-donor; A-acceptor; H-hydrogen).

D—H...A	D—H	H...A	D...A	D—H...A
N3—H3...O1 ⁱ	0.92	2.16	3.061 (2)	169
N5—H5...O2 ⁱⁱ	0.89	1.99	2.873 (2)	175
C2—H2A...O3	0.98	2.25	2.761 (2)	111
C8—H8A...O3	0.96	2.40	3.344 (3)	167
C9—H9B...O2 ⁱⁱⁱ	0.96	2.52	3.127 (2)	121

Symmetry codes : (i) x+1, y, z ; (ii) -x+1, -y, -z ; (iii) x-1, y, z

Figure S1. ¹H NMR spectrum of synthesized dihydropyrimidine 4

Figure S2. ¹³C NMR spectrum of synthesized dihydropyrimidine 4

Figure S3. dept135 NMR spectrum of synthesized dihydropyrimidine **4**

Figure S4. ^1H NMR spectrum of synthesized dihydropyrimidine **5**

Figure S5. ^{13}C NMR spectrum of synthesized dihydropyrimidine **5**

Figure S6. dept135 NMR spectrum of synthesized dihydropyrimidine **5**

Figure S7. MS spectrum of synthesized dihydropyrimidine 4

Figure S8. MS spectrum of synthesized dihydropyrimidine 5

Figure S9. Fingerprint plot of the main contacts at the Hirshfeld surface computed with program Crystal Explorer 17 [1].

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Figure S10.

Scatterplot of contacts enrichment and average electrostatic energy per contact type