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Synthesis, crystal structure and antibacterial studies of dihydropyrimidines and their regioselectively oxidized products†

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The syntheses and investigations of new biologically active derivatives of dihydropyrimidines by Biginelli reaction in the presence of copper triflate are reported. Due to the fact that salicylaldehyde and its derivatives under Biginelli reaction conditions can lead to the formation of 2 types of dihydropyrimidines, the influence of copper triflate on product formation was also investigated. In addition to this, regioselective oxidation of dihydropyrimidines was performed in the presence of cerium ammonium nitrate and novel oxidized dihydropyrimidines were obtained. Single crystals of some of them were obtained and as a result, the structures of them were investigated by X-ray diffraction method, which allows determining the presence of hydrogen bonds in their structures. In addition to this, the presence of hydrogen bonds in their structures affects the formation of the corresponding tautomer during oxidizing of dihydropyrimidines. Since dihydropyrimidines are claimed to be biologically active compounds, activities of the synthesized compounds were studied against *Acinetobacter baumannii*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* bacteria.

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Introduction

The three-component one-pot Biginelli reaction, which was discovered in 1893 by Pietro Biginelli by condensation of an aldehyde, urea and 1,3-dicarbonyl compound in an acid medium,¹ is one of the most important and oldest multicomponent reactions. It is still in trend, due to the fact that this flexible and multilateral tool allows construction of an astonishing class of compounds with a broad spectrum of application area, namely dihydropyrimidines (H₂Py).^{2–8} First of all, it must be mentioned that these marvelous compounds are important heterocyclic systems which play a crucial role as a building block of biomolecules such as DNA and RNA.⁹ Among other applications of these amazing compounds, the synthesis and development of materials with novel optical properties,¹⁰ design

of polymers,¹¹ dyes¹² and adhesives¹³ can be mentioned. But the most widespread application area of H₂Py is medicine due to their broad spectrum of biological activities. A multicomponent nature of Biginelli reaction provides large product diversity bearing different pharmacophoric groups in the structure of dihydropyrimidines which ensure the optimal supramolecular interactions with a specific biological target (molecular recognition) and trigger or block its biological response.^{3,14–17} Investigations through molecular manipulations reveal that this class of compounds demonstrate antiviral,³ anti-filarial,^{18,19} antifungal,^{3,15} analgesic,²⁰ anti-leishmanial,²¹ antiproliferative,²² antitumor,^{23–28} anti-convulsant,²⁹ antibacterial,^{30–33} anti-inflammatory,^{34–36} anti-hypertensive^{37–41} activities, melanin concentrating hormone 1 receptor antagonist,⁴² anti-HIV,⁴³ antiepileptic,⁴⁴ antidiabetic,⁴⁵ anti-SARS,⁴⁶ anti-malarial,⁴⁷ mPGES-1 inhibitors,⁴⁸ anti-hyperglycemic, antitubercular,⁴⁹ TRPA1 antagonist,⁵⁰ miscellaneous,^{51–53} potassium^{54–56} and calcium channels⁵⁷ and α_{1a} adrenergic antagonists.⁵⁸ As a result of these investigations, different drugs based on H₂Py such as riboflavin, idoxuridine, aminophylline, emivirine, 5-fluorouracil, methylthiouracil, batzelladine A and B *etc.* have found their application in medicine.⁹ Due to the utmost importance of H₂Py in pharmaceuticals, robust, efficient, cost-effective and “green” chemical synthesis and transformations of dihydropyrimidine scaffolds were developed.^{3,14,59–63}

Among important transformations of H₂Py, the oxidation reactions are one of the crucial directions due to the fact that

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the solubility of oxidized products in polar solvents is higher. Moreover, the introduction of various functional groups, as well as the formation of new C–C, S–S and other types of bonds is easier resulting in extending their application area. In addition to this, in some cases, oxidized H₂Py are more biologically active than initial H₂Py.^{63–66}

Concerning biological activity of H₂Py, novel molecules were synthesized by Biginelli reaction in microwave conditions in the presence of copper triflate on the basis of urea, methyl acetoacetate and aldehyde scaffolds. Due to the fact that the Biginelli reaction of salicylaldehyde derivatives can lead to the formation of 2 types of H₂Py, we also studied the effect of microwave conditions on product formation. Further, regioselective oxidation of H₂Py in the presence of CAN was performed and novel oxidized H₂Py were obtained. Moreover, crystals of some H₂Py as well as their oxidized products were received and as a result, their structures were investigated by X-ray single crystal diffraction method. Due to the fact that H₂Py demonstrate a wide spectrum of biological activities, their activity was analyzed against *A. baumannii*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *S. aureus* bacteria and promising results were obtained.

Results and discussion

Chemical synthesis

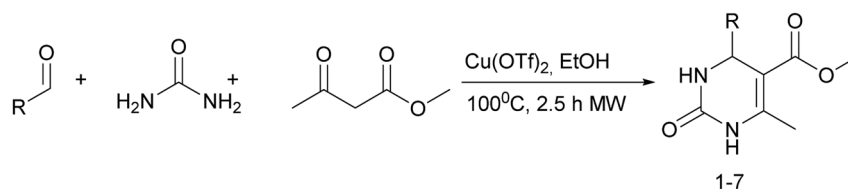
It is known that a lot of protocols were developed for performing Biginelli reaction, leading to the synthesis of H₂Py with high yields under “green” conditions and simple work-up procedure. Using catalyst types such as I₂/MWI,⁶⁷ Yb(PFO)₃,⁶⁸ TMSCl/CAN,⁶⁹ [Hmim]HSO₄–NaNO₃,⁷⁰ CAN,⁷¹ GaI₃,⁷² HBF₄–SiO₂,⁷³ Sm(ClO₄)₃,⁷⁴ H₃PMo₁₂O₄₀,⁷⁵ La(OTf)₃,⁷⁶ Sc(OTf)₃,⁷⁷ In(OTf)₃,⁷⁸ RuCl₃,⁷⁹ MgCl₂·6H₂O,⁸⁰ CeCl₃·7H₂O,⁸¹ acetic acid, NH₄Cl¹⁷ and others,^{3–14} it was

not possible to perform a Biginelli reaction on the basis of all used aldehyde scaffolds, urea and methylacetoacetate. In addition to this, performing the synthesis in microwave reactor becomes more popular over the conventional method, due to the fact that microwave method increases speed, reproducibility and scalability of the reaction.^{82,83} Taking into account the positive side of microwave assisted organic synthesis, improving the⁸⁴ procedure by performing the reaction in the presence of Cu(OTf)₂ in microwave conditions allowed us to obtain targeted H₂Py 1–7 (Scheme 1 and Table 1). The positive side of this procedure is the almost total absence of work-up stage during the reactions realized for obtaining H₂Py (1–7) – the precipitate was just washed with distilled water. Another positive side of using Cu(OTf)₂ as a catalyst is that it is an excellent triflate surrogate to other metal triflates because it is cheap, demonstrates high activity and low toxicity making the process more environmentally friendly. The structures of all synthesized novel H₂Py's were determined by ¹H, ¹³C NMR, mass spectroscopy and elemental analysis, whereas the spectroscopic data of known compounds were compared with reported in the literature.

It was also possible to obtain single crystals of compounds 2, 3 and 7 (Fig. 1).

According to the literature data, the participation of 2-hydroxybenzaldehyde (salicylaldehyde) or different substituted 2-hydroxybenzaldehyde derivatives, methylene active compound and urea or thiourea under Biginelli reaction conditions^{85–97} leads to the formation of oxygen-bridged tricyclic pyrimidine derivative **B** instead of simple dihydropyrimidine **A** (Scheme 2).

Considering this fact, it was interesting to check the activity of copper triflate in the presence of different substituted 2-hydroxybenzaldehyde derivatives. A small modification of the



Scheme 1 Synthesis of dihydropyrimidines in the presence of Cu(OTf)₂.

Table 1 Synthesized dihydropyrimidines by Biginelli reaction

	1	2	3	4	5	6 ^a	7
Product							
Yield%	56	74	74	79	72	60	54

^a The synthesis of initial aldehyde which has been used for obtaining of corresponding H₂Py was given in the experimental part.



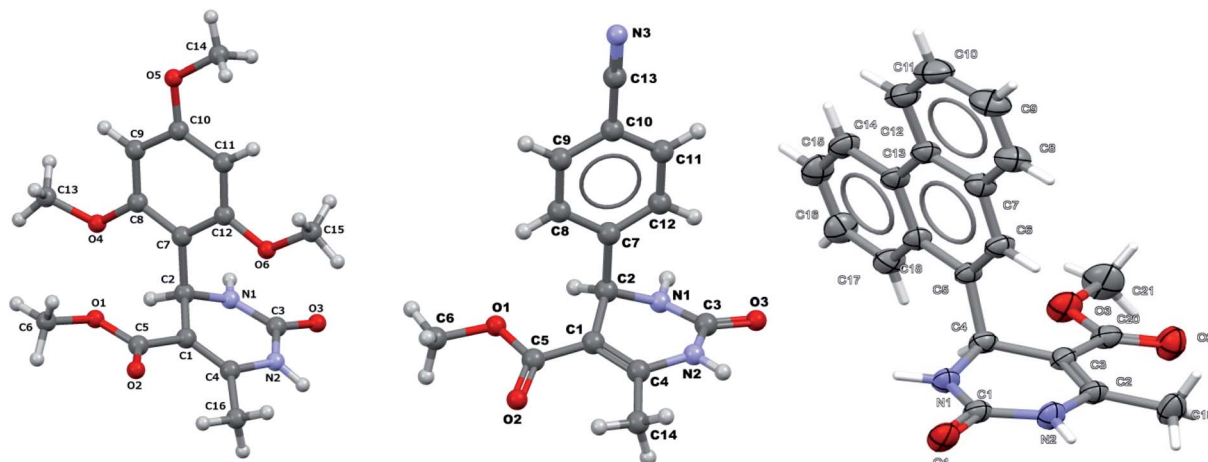
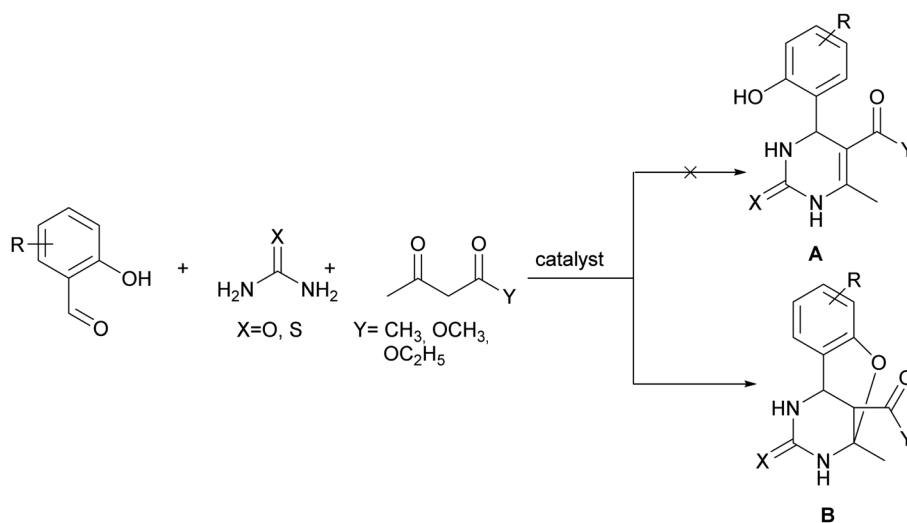


Fig. 1 X-ray structure of compounds 2, 3 and 7.

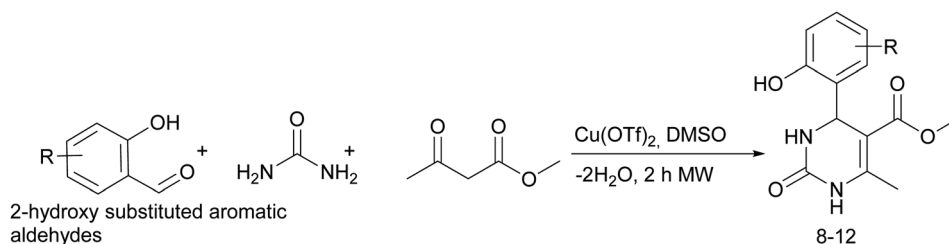


Scheme 2 The formation of oxygen-bridged tricyclic pyrimidine derivative.

initial procedure allowed to obtain various A types of H₂Py 8–12 (Scheme 3 and Table 2).

The selectivity of copper triflate in the above-mentioned reaction can be enlightened through considering the mechanism of formation of oxygen-bridged tricyclic pyrimidine derivative **B** (Scheme 4). According to the mechanism of the Biginelli condensation, the formation of oxygen-bridged

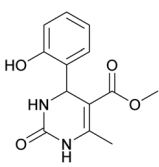
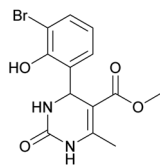
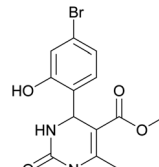
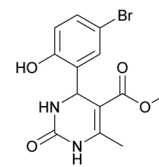
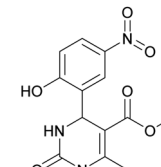
pyrimidine **B** occurs through the formation of **A**. After the formation of molecule **A** step, the double bond of **A** must react with the catalyst which will facilitate the attack of oxygen (hydroxyl group) on C-6 position (*). Taking into account the bulkiness of copper triflate molecule as well as the size of –CO–Y and –CH₃ groups, this interaction cannot occur because of the steric effect⁸⁷ (Scheme 4). As a result, the steric effect determines

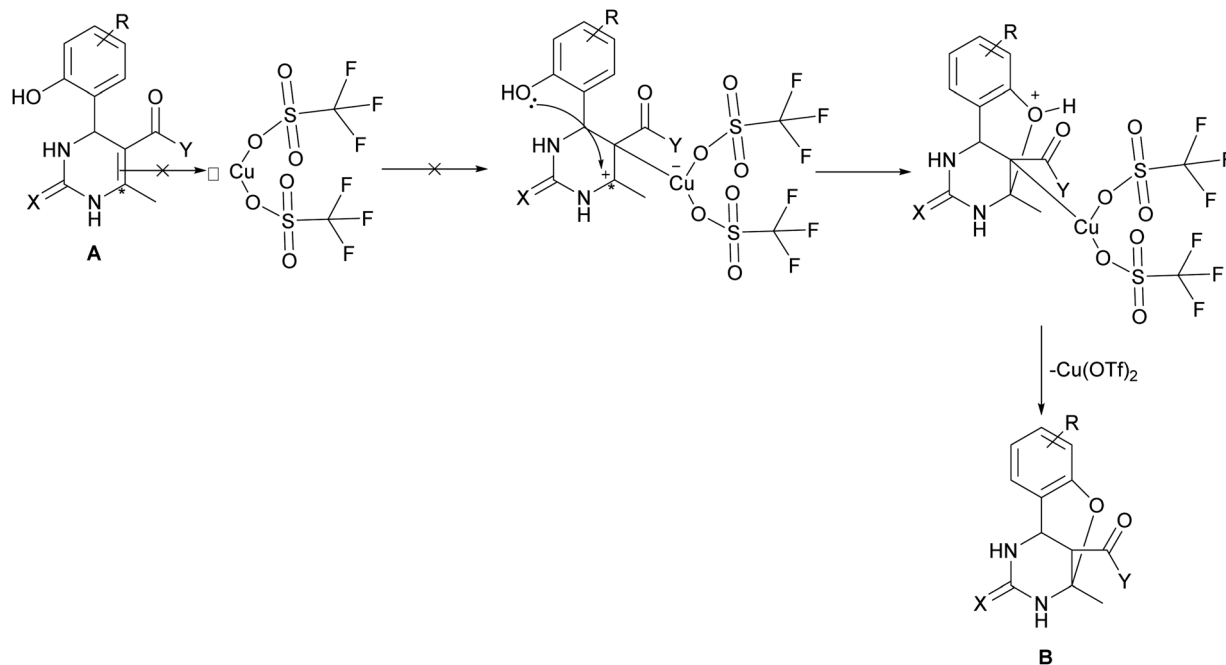


Scheme 3 Synthesis of dihydropyrimidines on the basis of 2-hydroxybenzaldehyde derivatives.

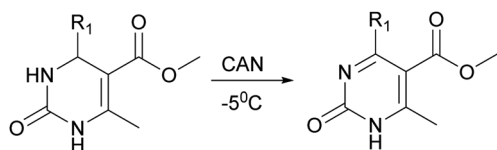


Table 2 Synthesized on the basis of 2-hydroxybenzaldehyde derivatives H₂Py

	8	9	10	11	12
Product					
Yield%	62	78	77	72	79



Scheme 4 A mechanism of the formation of oxygen-bridged pyrimidine B.

Scheme 5 Regioselective oxidation of H₂Py in the presence (CAN).

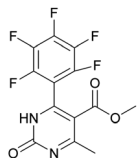
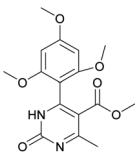
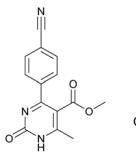
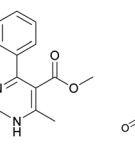
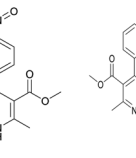
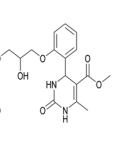
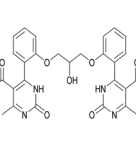
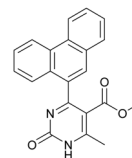
the selectivity of the Biginelli reaction in the presence of copper triflate towards product **A**. Another factor that supports this idea is the analysis of literature,^{85–97} according to which the formation of oxygen-bridged tricyclic pyrimidine derivative **B** in the one-step one-pot process occurs in the presence of Arrhenius acids (H⁺), which small size facilitates the formation of oxygen-bridged tricyclic compound **B**.

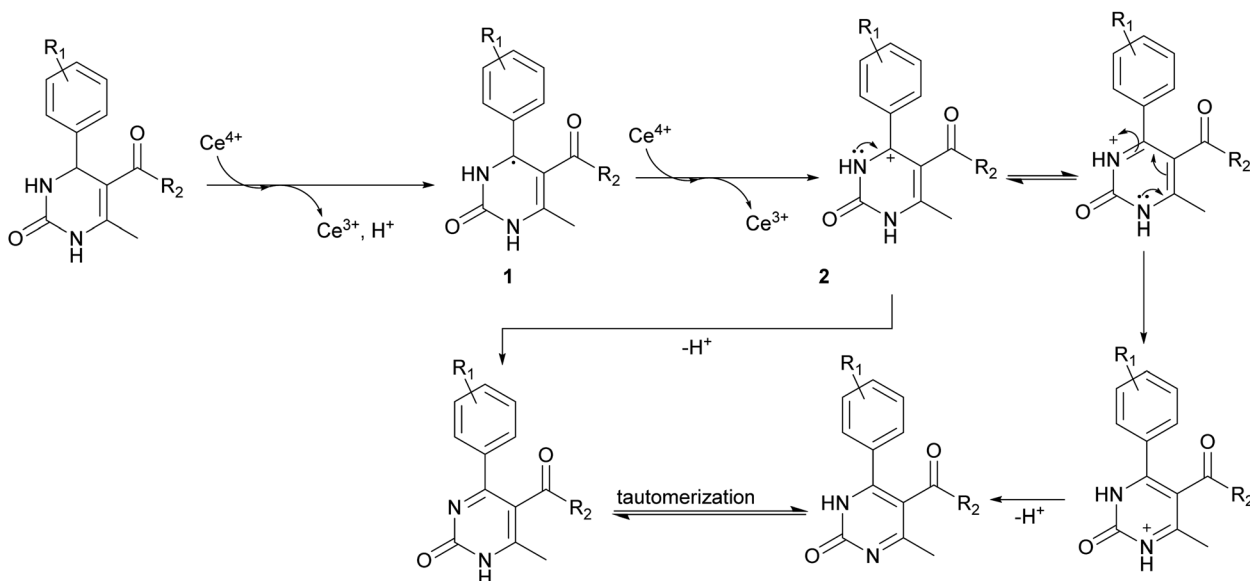
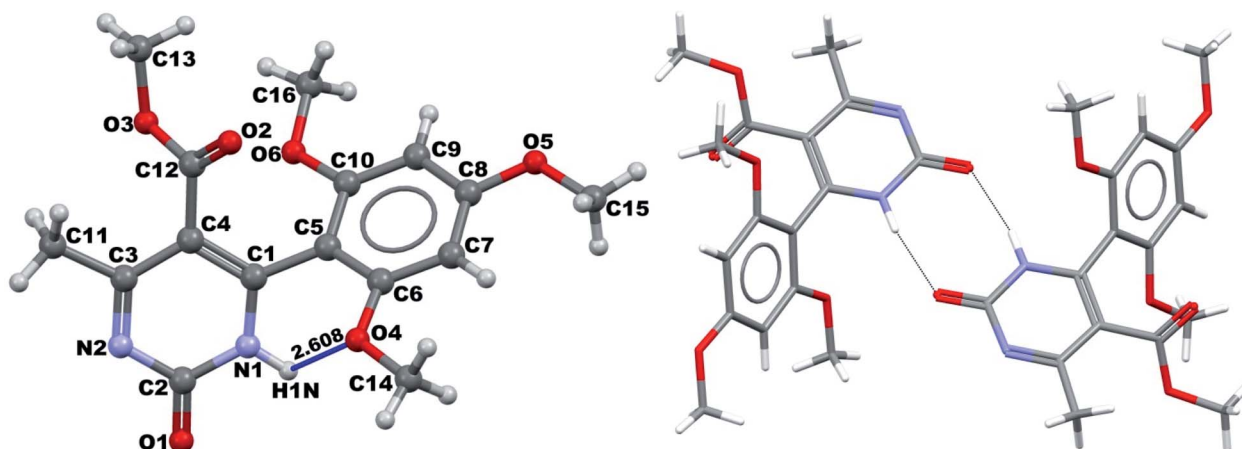
The next stage was performing of regioselective oxidation of H₂Py (**1–7**) in the presence of cerium ammonium nitrate (CAN). Modification of the procedure⁶⁴ allows us to obtain oxidized H₂Py **13–20** (Scheme 5 and Table 3).

The reason of the low yield (1%) of compound **13** can be explained through mechanistic consideration (Scheme 6) of this reaction. According to the mechanism of regioselective oxidation of H₂Py in the presence of CAN known in the literature,⁶⁴ this reaction is a one-electron oxidizing process and, at the first stage, carboradical **1** is formed. In case of compound **13**, all five positions in the benzene ring are substituted with fluorine atoms which draw electrons towards themselves resulting in decreasing the stability of the carboradical **1** as well as the subsequently formed carbocation **2** too (Scheme 6). As a result, it leads to the formation of the targeted product **13** with a low yield. This pattern also manifests itself in the case of other oxidation products. The yield of targeted oxidation products with electron enriched aromatic ring (**14**, **16** and **20**) is higher than that of oxidation products with electron-deficient aromatic ring (containing electron-withdrawing –NO₂ and –CN groups – **15** and **17**). This is due to the fact that the stability of the carboradical (as well as carbocation) in case of electron enriched



Table 3 Synthesized dihydropyrimidines by Biginelli reaction

	13	14	15	16	17	18	19	20
Product								
Yield%	1	40	27	39	28	15	5	35

Scheme 6 Mechanism of regioselective oxidation of H₂Py in the presence of CAN.Fig. 2 View of the X-ray structure of compound 14 and dimer formed *via* a double hydrogen bond around an inversion centre.

aromatic ring is higher than that of the electron-deficient aromatic ring.

It was possible to obtain single crystals of compounds 14 and 20 (Fig. 2 and 3). According to the literature data,⁶⁴ oxidized

products of H₂Py can exist in the solution in the form of various tautomers (Scheme 7).

According to the X-ray structure, compound 14 exists in the crystal structure in the form of tautomer II, whereas compound



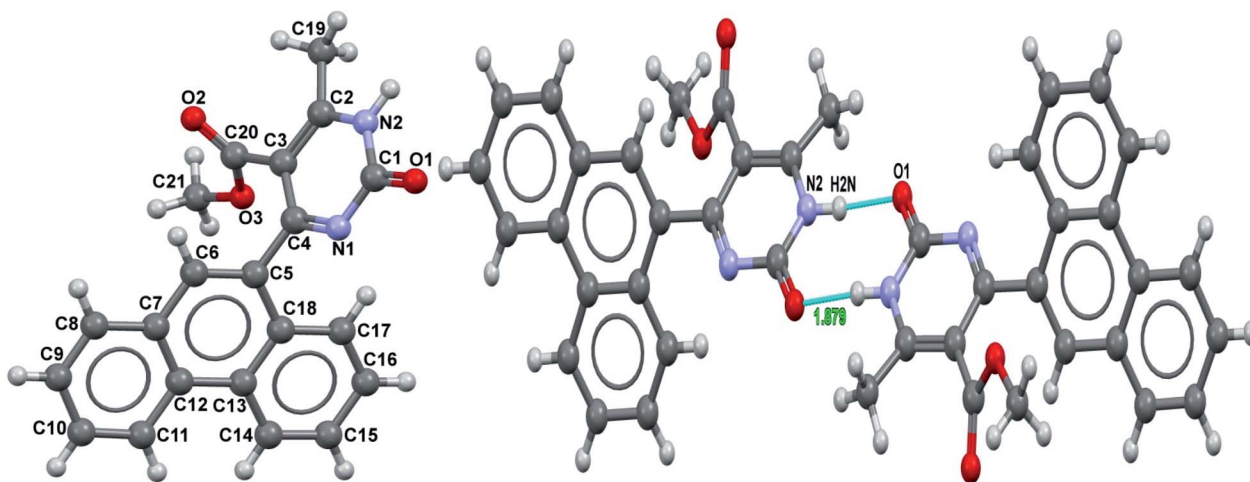
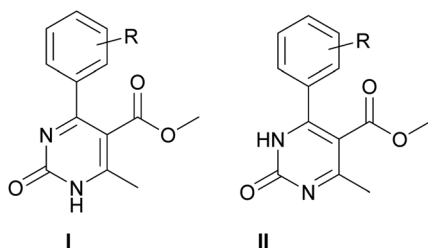


Fig. 3 View of the X-ray structure of compound 20 and dimer formed via a double hydrogen bond around an inversion centre.



Scheme 7 Two tautomers of pyrimidines.

20 is in the form of tautomer I (Fig. 2 and 3). The reason of existing of compound 14 in the form of tautomer II is caused by the presence of an intramolecular hydrogen bond between H1N and O4 of the methoxy group, which keeps the hydrogen at N1 position and stabilized the presence of tautomer structure II.

X-ray diffraction investigation of compound 20 did not reveal the presence of any intramolecular hydrogen bond in the structure, which makes possible existing of this molecule in the form of tautomer I in crystal. In addition to this, X-ray analysis revealed the presence of compounds 14 and 20 in the form of dimers (Fig. 2 and 3), due to the presence of double

Table 4 Crystallographic data and details of refinement for compounds 2, 3, 7, 14, 20

	Compound 2	Compound 3	Compound 7	Compound 14	Compound 20
Chemical formula	C ₁₆ H ₂₀ N ₂ O ₆	C ₁₄ H ₁₃ N ₃ O ₃	C ₂₁ H ₁₈ N ₂ O ₃	C ₁₆ H ₁₈ N ₂ O ₆	C ₂₁ H ₁₆ N ₂ O ₃
Formula weight (M)	336.34	271.27	346.37	343.33	344.36
Crystal system	Monoclinic	Tetragonal	Monoclinic	Triclinic	Monoclinic
Space group	<i>P</i> ₂ / <i>c</i>	<i>P</i> ₄ / <i>n</i>	<i>C</i> ₂ / <i>c</i>	<i>P</i> $\bar{1}$	<i>P</i> ₂ / <i>c</i>
<i>a</i> (Å)	11.6354(5)	23.7495(15)	25.515(5)	8.1493(12)	9.918(2)
<i>b</i> (Å)	14.5969(5)	23.7495(15)	7.710(3)	9.6420(17)	13.013(3)
<i>c</i> (Å)	9.3401(3)	4.8467(3)	18.240(4)	11.4398(10)	12.9630(18)
α (°)	90.00	90.00	90.00	74.396(10)	90.00
β (°)	98.173(2)	90.00	108.277(17)	88.752(10)	91.984(18)
γ (°)	90.00	90.00	90.00	78.900(17)	90.00
<i>V</i> (Å ³)	1570.22(10)	2733.7(4)	3407.2(17)	849.1(2)	1672.0(6)
<i>Z</i>	4	8	8	2	4
Temperature (K)	100(2)	100(2)	298(2)	293(2)	298(2)
Crystal size	0.320 × 0.290 × 0.145	0.320 × 0.054 × 0.040	0.650 × 0.360 × 0.220	0.580 × 0.430 × 0.320	0.300 × 0.220 × 0.060
Density (g cm ⁻³)	1.423	1.318	1.350	1.343	1.368
μ (Mo K α) (mm ⁻¹)	0.110	0.095	0.091	0.105	0.093
<i>F</i> (000)	712	1136	1456	362	720
Goodness of fit on <i>F</i> ²	1.005	1.005	1.101	1.098	1.131
<i>R</i> ₁ , <i>wR</i> ₂ ^a , [<i>I</i> > 2 σ (<i>I</i>)]	0.0544, 0.1152	0.0524, 0.1147	0.0637, 0.1320	0.0541, 0.1526	0.1089, 0.1743
<i>R</i> ₁ , <i>wR</i> ₂ (all data)	0.0817, 0.1240	0.0663, 0.1201	0.0894, 0.1529	0.0641, 0.1676	0.1932, 0.2282
Residual electron density (max, min) e Å ⁻³	0.275, -0.276	0.254, -0.230	0.199, -0.156	0.394, -0.184	0.254, -0.262

$$^a R = \frac{\sum ||F_o| - |F_c||}{\sum |F_o|}; wR(F^2) = \frac{[\sum w(|F_o|^2 - |F_c|^2)^2 / \sum w|F_o|^4]^{1/2}}{\sum w|F_o|^4}.$$



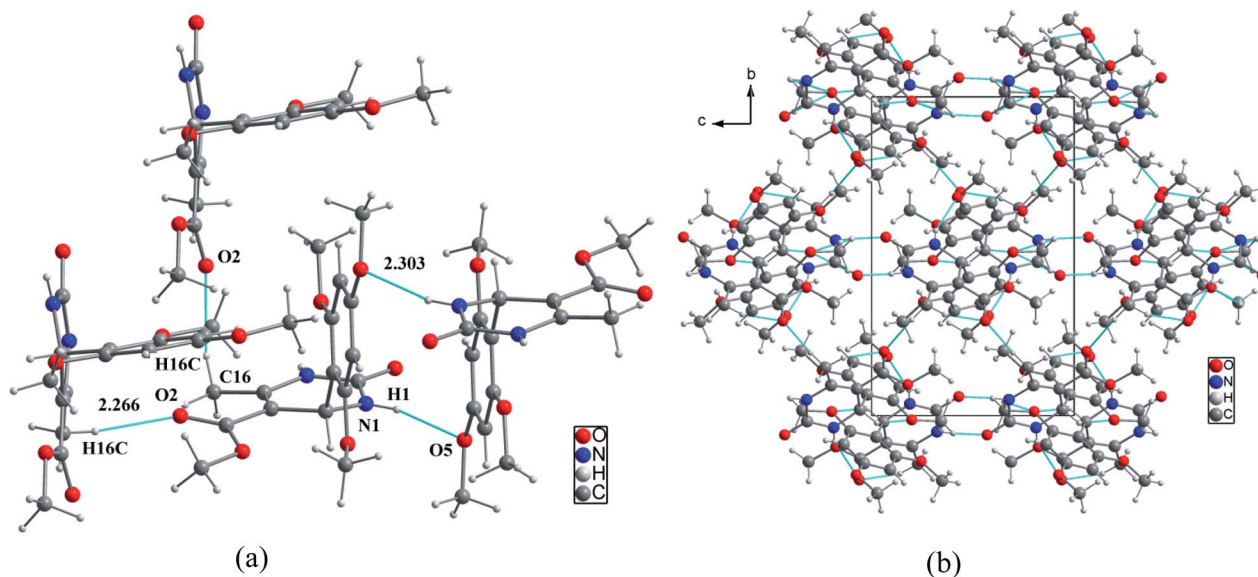


Fig. 4 (a) Representation of hydrogen bonds around the organic molecular, (b) projection of the structure of compound 2 along the *a*-axis.

intermolecular N–H···O hydrogen bonds between O=C–N–H groups. There is a H1N···O1 hydrogen bond in case of compound 14 and H2N···O1 in case of compound 20. Considering this fact, we can say that H1N of compound 14 takes part in the formation of both intramolecular and intermolecular hydrogen bonds (bifurcated hydrogen bond with two acceptors) stabilizing the crystal structure of the compound. Furthermore, it is possible to conclude that in case if there is a heteroatom group on the benzene ring which is able to participate in the formation of an intramolecular hydrogen bond with the amine group of the dihydropyrimidine ring, then the oxidized product will be in the form of tautomer II, otherwise in the form of tautomer I.

As a result, it is possible to predict that compound 13, as well as 18 and 19, will be in the form of tautomer II.

Structure description

It was also possible to obtain single crystals of compounds 2, 3, 7, 14 and 20, which crystallographic data and details of refinement were given in Table 4.

In the structural arrangement of compound 2, the organic molecules are arranged on top of each other to form pillars running along the *a*-axis direction at $(0, 0, 0)$ and $(0, \frac{1}{2}, \frac{1}{2})$ (Fig. 4). Furthermore, each organic molecule is linked to the three nearest neighboring molecules by N–H···O and N–H···N hydrogen bonds so that the resulting network assumes a three-dimensional form.

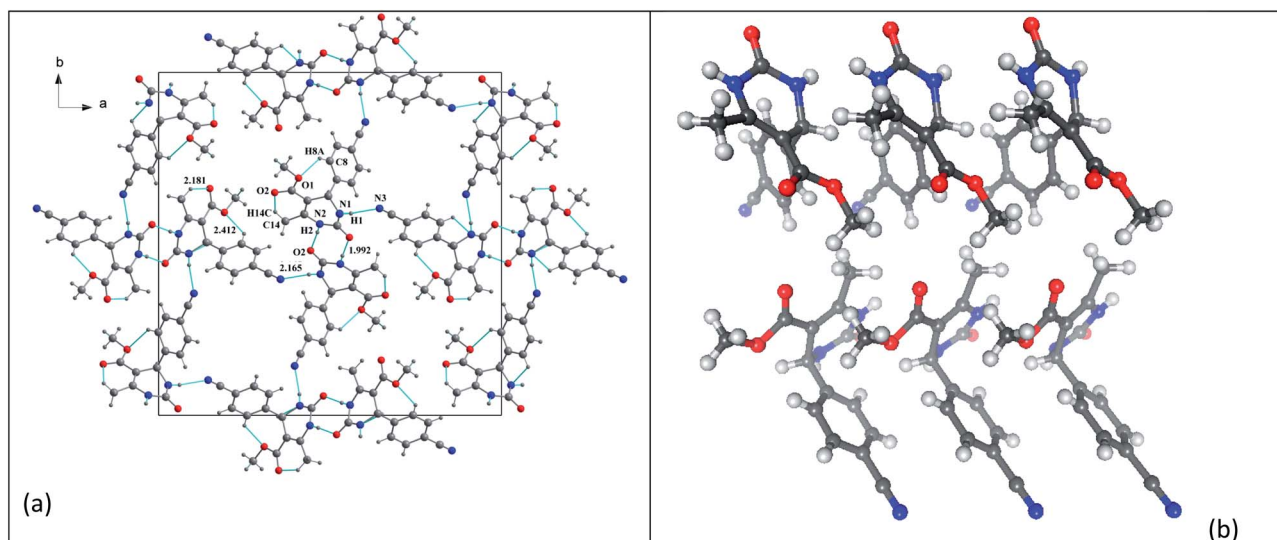


Fig. 5 Compound 3 (a) projection of the structure of along the *c*-axis. (b) Crystallographic autostereogram,⁹⁸ the horizontal translations are along the short *c* axis.



In the structure of compound **3** (Fig. 5), the organic molecules are linked together by C–H···O hydrogen bonds to form a three-dimensional network containing channels centered at $(\frac{1}{4}, \frac{1}{4}, 0)$ and $(\frac{3}{4}, \frac{3}{4}, 0)$ and extending along the *c*-axis. This structure also has intramolecular interactions such as C8–H8A···O1 and C14–H14C···O2.

For compound **7** the organic molecules are interconnected by two hydrogen bonds N1–H1N···O2 and N2–H2N···O1 generated by the imine group of the pyrimidine ring to form infinite chains running along the *b*-axis direction (Fig. 6a). This way the pyrimidine rings and the ester groups (–COOCH₃) are developed in planes parallel to the plane (*b*, *c*) at $x = \frac{1}{2}n$ between which the phenanthryl groups are segregated (Fig. 6b).

The crystal packing of compound **2** is also stabilized by intermolecular aromatic stacking interactions between neighboring pyrimidine rings, with a face-to-face distance of 3.712 Å (Fig. 7a). For the two compounds **3** and **7**, the crystal packing is also stabilized by intermolecular interactions C–H···π between hydrogen atoms of the ester groups –COOCH₃ and the neighboring aromatic rings, with H···π distance of 3.884 Å for compound **3** and 2.802 Å for compound **7** (Fig. 7b and c).

Compound **14** is a monohydrate where the water molecule occupies a void which is 74% hydrophobic as surrounded by C and H–C atoms. This more hydrophilic molecule has two strong C=O···H–N hydrogen bonds and five C–H···O/N weak hydrogen bonds.

Compound **20** is characterized by C···H, H···H and C···C contacts which represent up to 71% of the contact surface due to cycle stacking and C–H···π interactions. A double C=O···H–N hydrogen bond occurs within the dimer resulting from an inversion centre.

Hirshfeld surface analysis

Analysis of intermolecular interactions using the Hirshfeld surface was undertaken to gain a better understanding of the

three-dimensional crystal packings. The Hirshfeld surface and the decomposed two-dimensional fingerprint plots (ESI Fig. 58–62†) were calculated on the determined crystal structures using Crystal Explorer 3.1 software.⁹⁹

All the five compounds show two spikes at short distances due to the O–H···O hydrogen bonds constituting between 17 and 33% of the contact surface. Only compound **2** shows spikes at short distance for H···N hydrogen bonds. H···H contacts are always the most abundant contacts representing between 30 and 53% of contacts. The amount C···C contacts is low except for compound **20** where it reaches 6.2% due to aromatic stacking. In all cases, the most abundant contact is of type H···H whose proportion is between 30 and 53%. C···H contacts occur in the 12.8–29.1% range, with compound **7** display the highest amount of C–H···π weak hydrogen bonds.

Biological assays

The antimicrobial activity of the synthesized dihydropyrimidines (**1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, **14**, **15**, **16**, **17**, **19**, **20**) was investigated against Gram positive and Gram negative bacterial strains (*S. aureus*, *E. coli*, *A. baumannii*, *P. aeruginosa* and *K. pneumonia*). The investigations were started with the determination of the minimum inhibitory concentration (MIC) of the studied compounds by the twofold micro-dilution method.^{100–102} Obtained results were compared with the results of the pristine antibiotics (cefotaxime and ceftriaxone). As shown in Table 5, compound **10** and **16** showed the highest inhibitory effect against *A. baumannii* and *S. aureus* in value of 62.5 μg ml^{−1}. No inhibition was detected for compound **12** against all bacterial strains, while the compound **15** showed the similar activity against all tested cultures. So, the microbial cultures were more susceptible to compound **10**, **14**, **16** and **20** than other compounds. The lowest MIC in case of *E. coli*, *P. aeruginosa*, *S. aureus*, *A. baumannii*, *K. pneumonia* was 125 μg ml^{−1} (compounds **14**), 250 μg ml^{−1} (compounds **14** and **16**),

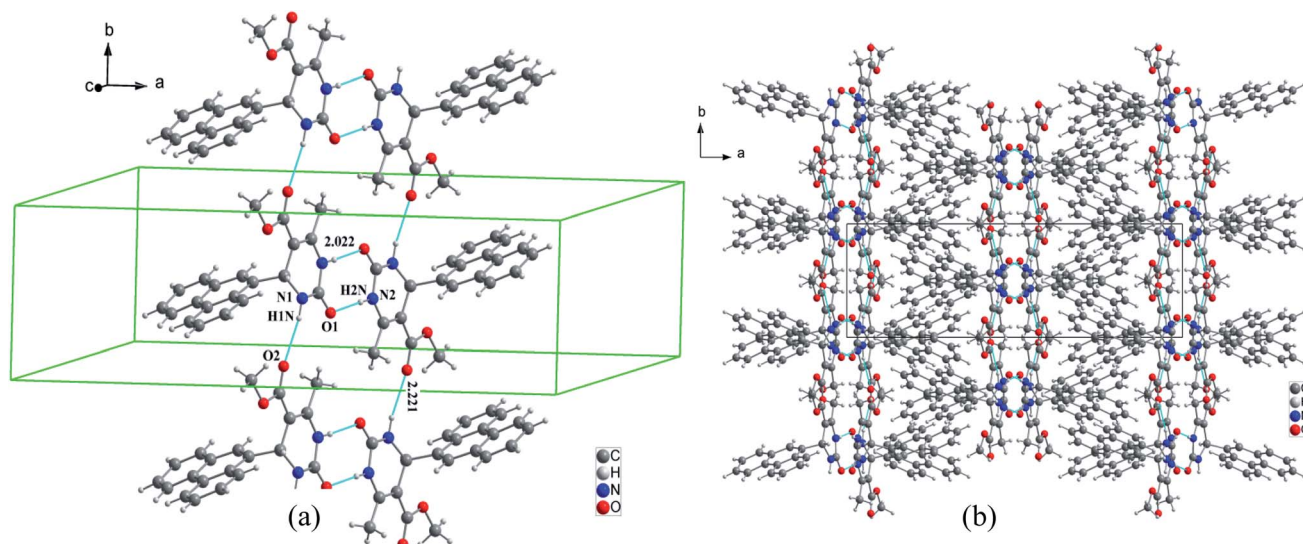


Fig. 6 Crystal packing of compound **3** (a) view of the 1D chain parallel to the *c*-axis for, (b) projection along the *b*-axis. N–H···O hydrogen bonds are represented as light blue lines.



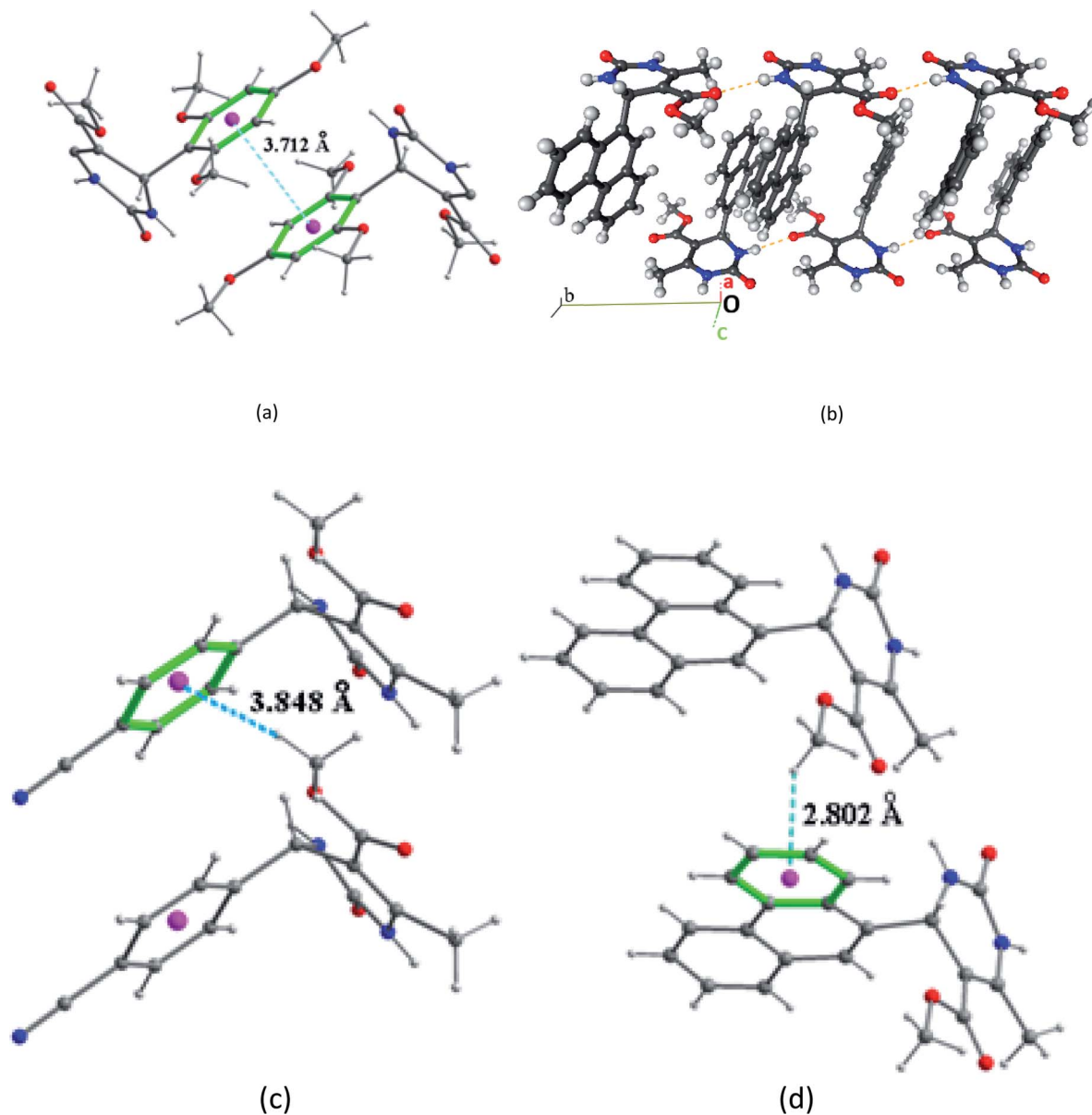


Fig. 7 Intermolecular aromatic stacking interactions between the neighboring aromatic rings: (a) in compound 2, (b) in compound 7 (autostereogram with horizontal translation along *b*). Intermolecular C–H... π interactions: (c) in compound 3, (d) compound 7.

62.5 $\mu\text{g ml}^{-1}$ (compounds **10** and **16**), 62.5 $\mu\text{g ml}^{-1}$ (compounds **10** and **16**) and 500 $\mu\text{g ml}^{-1}$ (compound **20**) respectively. Moreover, the antibacterial activity of the compound **1** was detected only in case of *P. aeruginosa* (1000 $\mu\text{g ml}^{-1}$). Compare with other microbial cultures *P. aeruginosa* was more susceptible against all of the compounds except **12** and **17**. Compound **1**, **2** and **12** were not affect the growth of *E. coli* but the MIC value of the other compounds was identical (1000 $\mu\text{g ml}^{-1}$) for this strain. *S. aureus*, *A. baumannii*, *K. pneumonia* was not susceptible when tested with the compounds **1**, **2**, **3** and **6**.

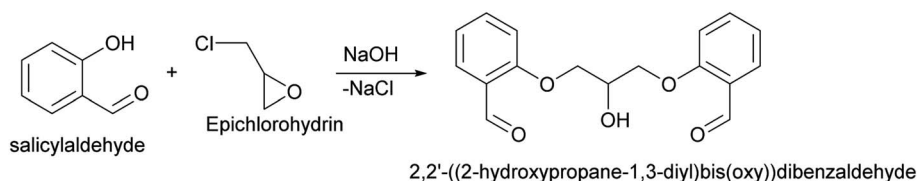
The obtaining results revealed that the MICs of compounds **14** in case of *E. coli* (125 $\mu\text{g ml}^{-1}$) and **10** and **16** in case of *S. aureus* and *A. baumannii* (62.5 $\mu\text{g ml}^{-1}$) were higher than MICs of both pristine antibiotics (250–500 $\mu\text{g ml}^{-1}$).

Taking into account the MIC of the compounds and pristine antibiotics, antibacterial activity of compounds **2**, **3**, **4**, **5**, **7**, **8**, **9**, **10**, **11**, **12**, **14**, **15**, **16** and **20** was also investigated by disc-diffusion method.¹⁰³ Results were compared with the antibacterial activity of pristine antibiotics. As it can be seen from the Fig. 65 (ESI),[†] the best antibacterial effect against *E. coli* in comparison with both antibiotics were demonstrated by the compounds **14**, **15** and **20**, whereas antibacterial effect of compound **8** was equal to ceftriaxone, but lower than cefotaxime. Looking at the inhibition zone in case of *P. aeruginosa*, compound **14** demonstrated the highest activity in comparison with both antibiotics, whereas the activity of compound **16** was higher than ceftriaxone and equal to cefotaxime (Fig. 63 – ESI[†]). In case of *S. aureus* and *A. baumannii* inhibition zone of



Table 5 Minimum inhibitory concentration (MIC, $\mu\text{g ml}^{-1}$) of the studied compounds

Investigated samples	Bacterial strains				
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>A. baumannii</i>	<i>K. pneumonia</i>
(1)	—	1000	—	—	—
(2)	1000	500	1000	1000	1000
(3)	1000	500	500	1000	—
(4)	500	1000	500	—	—
(5)	500	1000	250	1000	1000
(6)	1000	1000	—	—	—
(7)	1000	1000	250	1000	—
(8)	500	1000	250	1000	—
(9)	1000	500	500	500	1000
(10)	1000	1000	62.5	62.5	1000
(11)	1000	1000	1000	500	1000
(12)	1000	1000	500	—	—
(14)	125	250	500	1000	1000
(15)	250	500	250	1000	1000
(16)	1000	250	62.5	62.5	—
(17)	—	—	1000	—	1000
(19)	1000	1000	1000	—	1000
(20)	250	500	250	500	500
Cefotaxime	250	250	250	250	250
Ceftriaxone	500	500	500	500	500



Scheme 8 Synthesis of 2,2'-((2-hydroxypropane-1,3-diyl)bis(oxy))dibenzaldehyde.

compounds **10** and **16** was higher than that of both pristine antibiotics (Fig. 64 and 66 – ESI†). In general, the antibacterial activity of all compounds against *K. pneumonia* was low; only the effect of compound **20** was higher in comparison with others but lower than that of both cefotaxime and ceftriaxone, equal to 16, 32 and 24 mm respectively (Fig. 67 – ESI†). The pronounced antibacterial effect of the above-mentioned compounds may be related to their ability to enter the bacterial cytoplasm and even to cross the nuclear envelope. In addition to it, they may bind to different classes of enzymes, for example, dihydrofolate reductase, bacterial DNA gyrase, aminoacyl-tRNA synthetases and so on.¹⁰⁴

As DMSO was used as a solvent, 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.

Experimental

Materials and methods

All the solvents and reagents which were purchased from commercial suppliers 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 aluminum sheets) which were visualized under UV light. Melting points were recorded in open capillary tubes on a Buchi B-540 apparatus and are uncorrected. Elemental analysis was performed on the analyzer Carlo Erba 1108.

NMR experiments

The NMR experiments have been 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 = 0, d1 = 1 s and for ¹³C as follows: digital resolution = 0.27 Hz, SWH = 17 985 Hz, TD = 64 K, SI = 32 K, 90° pulse length = 9 ms, PL1 = 1.5 dB, ns = 100, ds = 2, d1 = 3 s. The NMR-grade DMSO-d₆



(99.7%, containing 0.3% H₂O), CDCl₃, CD₃OD was used for the solutions of synthesized compounds.

Mass experiments

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

X-ray analysis

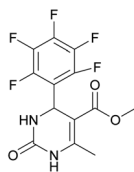
X-ray analyses have been performed on Bruker SMART APEX II Single Crystal X-ray Diffractometer equipped with graphite-monochromated Mo-K α radiation ($\lambda = 0.71073 \text{ \AA}$). The crystal structure was solved by direct methods and refined on F^2 by full matrix least-squares using Bruker's SHELXTL-97.¹⁰⁵ Crystallographic data for the structural analysis have been deposited to the Cambridge Crystallographic Data Center (CCDC 1998538 for 2, CCDC 1998557 for 3, CCDC 1998539 for 7, CCDC 1998546 for 14 and CCDC 1998547 for 20).

Experimental procedures

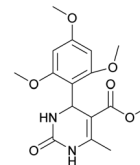
Synthesis of dihydropyrimidines by Biginelli reaction.

Dihydropyrimidines (1–7) were obtained by the known method⁸⁴ with small modifications (reaction time and catalyst concentration were increased) (Scheme 1):

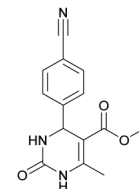
0.5 mmol of subsequent aldehyde, 0.75 mmol (45 mg) of urea and 0.08 mmol (30 mg) of Cu(OTf)₂ were added to a microwave vial with a magnetic stirrer and dissolved in 1 ml of ethanol. Subsequently, 0.46 mmol (50 μ l) of methyl acetoacetate was added to a vial, which was sealed and irradiated at 100 °C in a microwave reactor for 2.5 h at a maximum power of 200 W (CEM Discover™ System). At the end of reaction time, the precipitate was formed (in case if precipitate was not formed left it overnight), filtered, washed with distilled water and dried.



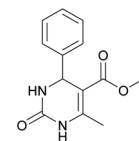
Methyl 6-methyl-2-oxo-4-(perfluorophenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1). The title compound was prepared according to the general procedure using 2,3,4,5,6-pentafluorobenzaldehyde to afford the title compound a colorless precipitate. Yield 56%. Mp 333–335 °C. ¹H NMR spectrum: (DMSO-d₆, δ , ppm), 2.23 s (3H, CH₃), 3.5 s (3H, OCH₃), 5.69 s (1H, CH), 7.7 s (1H, NH), 9.52 s (1H, NH). ¹³C NMR spectrum: (DMSO-d₆, δ , ppm), 18.26 (CH₃), 46.22 (CH), 51.15 (OCH₃), 94.08 (C), 118.61 (C), 135.47 (C_{Ar}), 143.19 (2C_{Ar}), 146.46 (C_{Ar}), 150.48 (2C_{Ar}), 151.01 (COO), 165.51 (CO). HRMS (ESI-MS): 359.21 [M⁺ + Na⁺]. Elemental analysis calcd for C₁₃H₉N₂O₃F₅, %: C, 46.44; H, 2.70; N, 8.33. Found, %: C, 46.40; H, 2.76; N, 8.31.



Methyl 6-methyl-2-oxo-4-(2,4,6-trimethoxyphenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2). The title compound was prepared according to the general procedure using 2,4,6-trimethoxybenzaldehyde to afford the title compound a light yellow precipitate. Yield 74%. Mp 252–254 °C. ¹H NMR spectrum: (DMSO-d₆, δ , ppm), 2.15 s (3H, CH₃), 3.39 s (3H, OCH₃), 3.72 s (6H, 2OCH₃), 3.75 s (3H, OCH₃), 5.74 s (1H, CH), 6.18 s (2H, 2C_{Ar}H), 6.9 s (1H, NH), 8.95 s (1H, NH). ¹³C NMR spectrum: (DMSO-d₆, δ , ppm), 17.78 (CH₃), 45.03 (CH), 50.14 (OCH₃), 55.1 (OCH₃), 55.7 (2OCH₃), 91.08 (2C_{Ar}H), 96.03 (C), 113.71 (C), 147.84 (C_{Ar}), 152.12 (C_{Ar}), 158.99 (2C_{Ar}), 159.91 (COO), 166.25 (CO). HRMS (ESI-MS): 359.17 [M⁺ + Na⁺]. Elemental analysis calcd for C₁₆H₂₀N₂O₆, %: C, 57.14; H, 5.99; N, 8.33. Found, %: C, 57.19; H, 5.96; N, 8.31. This compound can also be obtained by the following procedure: to a solution of 1 mmol of 2,4,6-trimethoxybenzaldehyde in 15 ml of ethanol were added 6.6 mmol of urea, 5.5 mmol of methyl acetoacetate and 4 ml of acetic acid. The reaction mixture was refluxed for 9 h. At the end of reaction time the reaction mixture was cooled with ice and precipitate was formed, which was filtered, washed with distilled water and dried (yield 68%).



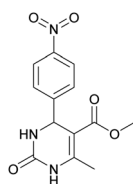
Methyl 4-(4-cyanophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3). The title compound was prepared according to the general procedure using 4-cyanobenzaldehyde to afford the title compound a colorless precipitate. Yield 74%. Mp 212–214 °C. ¹H NMR spectrum: (DMSO-d₆, δ , ppm), 2.26 s (3H, CH₃), 3.54 s (3H, OCH₃), 5.21–5.22 d (1H, CH, $J = 3 \text{ Hz}$), 7.41–7.44 d (2H, 2C_{Ar}H, $J = 9 \text{ Hz}$), 7.8–7.83 d (2H, 2C_{Ar}H, $J = 9 \text{ Hz}$), 7.86 s (1H, NH), 9.34 s (1H, NH). ¹³C NMR spectrum: (DMSO-d₆, δ , ppm), 18.23 (CH₃), 51.23 (CH), 54.03 (OCH₃), 98.39 (C), 110.49 (C), 119.08 (CN), 127.61 (2C_{Ar}H), 132.95 (2C_{Ar}H), 149.88 (C_{Ar}), 150.18 (C_{Ar}), 152.2 (COO), 165.95 (CO). Elemental analysis calcd for C₁₄H₁₃N₃O₃, %: C, 61.99; H, 4.83; N, 15.49. Found, %: C, 61.91; H, 4.87; N, 15.53. The data agrees with the reported literature values.¹⁰⁶



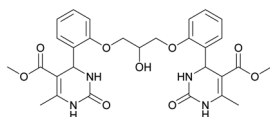
Methyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4). The title compound was prepared according to the general procedure using benzaldehyde to afford the title



compound a light yellow precipitate. Yield 79%. Mp 215–217 °C. ¹H NMR spectrum: (DMSO-d₆, δ, ppm), 2.24 s (3H, CH₃), 3.52 s (3H, OCH₃), 5.14 s (1H, CH), 7.20–7.31 m (5H, 5C_{Ar}H), 7.77 s (1H, NH), 9.23 s (1H, NH). ¹³C NMR spectrum: (DMSO-d₆, δ, ppm), 17.8 (CH₃), 50.7 (CH), 53.8 (OCH₃), 99 (C), 126.1 (2C_{Ar}H), 127.2 (C_{Ar}H), 128.4 (2C_{Ar}H), 144.6 (C), 148.6 (C_{Ar}), 152.2 (COO), 165.8 (CO). Elemental analysis calcd for C₁₃H₁₄N₂O₃, %: C, 63.40; H, 5.73; N, 11.38. Found, %: C, 63.47; H, 5.78; N, 11.33. The data agrees with the reported literature values.¹⁰⁷ This compound can also be obtained by the following procedure: to a solution of 2.5 mmol of benzaldehyde in 12 ml of ethanol were added 6.6 mmol of urea, 6 mmol of methyl acetoacetate and 2 ml of acetic acid. The reaction mixture was refluxed for 10 h. At the end of reaction time the reaction mixture was cooled with ice and precipitate was formed, which was filtered, washed with distilled water and dried (yield 76%).



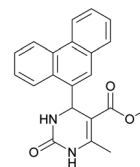
Methyl 6-methyl-4-(4-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (5). The title compound was prepared according to the general procedure using 4-nitrobenzaldehyde to afford the title compound a colorless precipitate. Yield 72%. Mp 235–237 °C. ¹H NMR spectrum: (DMSO-d₆, δ, ppm), 2.27 s (3H, CH₃), 3.54 s (3H, OCH₃), 5.28–5.29 d (1H, CH, *J* = 3 Hz), 7.5–7.53 d (2H, 2C_{Ar}H, *J* = 9 Hz), 7.88 s (1H, NH), 8.2–8.22 d (2H, 2C_{Ar}H, *J* = 6 Hz), 9.35 s (1H, NH). ¹³C NMR spectrum: (DMSO-d₆, δ, ppm), 18.25 (CH₃), 51.23 (CH), 53.91 (OCH₃), 98.39 (C), 124.19 (2C_{Ar}H), 127.93 (2C_{Ar}H), 147.11 (C), 149.94 (C_{Ar}), 152.13 (C_{Ar}), 152.16 (COO), 165.92 (CO). Elemental analysis calcd for C₁₃H₁₃N₃O₅, %: C, 53.61; H, 4.50; N, 14.43. Found, %: C, 53.66; H, 4.54; N, 14.40. The data agrees with the reported literature values.¹⁰⁸



Dimethyl 4,4'-(((2-hydroxypropane-1,3-diyl)bis(oxy))bis(2,1-phenylene))bis(6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate) (6). The title compound was prepared according to the general procedure using 2,2'-(2-hydroxypropane-1,3-diyl)bis(oxy)dibenzaldehyde to afford the title compound a colorless precipitate. Yield 60%. Mp 293–295 °C. ¹H NMR spectrum: (DMSO-d₆, δ, ppm), 2.32–2.33 d (6H, 2CH₃, *J* = 3 Hz), 3.38 s (6H, 2OCH₃), 4.04–4.1 m (1H, CH), 4.19–4.36 m (4H, 2OCH₂), 5.58 s (1H, CH), 5.78–5.8 d (1H, OH, *J* = 6 Hz), 6.87–6.92 t (2H, 2C_{Ar}H, *J* = 9 Hz), 7.01–7.08 q (4H, 4C_{Ar}H, *J* = 6 Hz), 7.22–7.28 t (2H, 2C_{Ar}H, *J* = 9 Hz), 7.32 s (1H, NH), 7.38 s (1H, NH), 9.2 s (2H, 2NH). ¹³C NMR spectrum: (DMSO-d₆, δ, ppm), 17.79 (2CH₃), 48.76 (2CH), 50.6 (2OCH₃), 67.65 (CH–OH), 69.67 (2CH₂O), 96.76 (2C), 112.54 (2C_{Ar}H), 120.51 (2C_{Ar}H), 126.55 (2C_{Ar}H),

128.79 (2C_{Ar}H), 131.04 (2C), 149.69 (2C_{Ar}), 152.27 (2C_{Ar}), 155.93 (2COO), 165.75 (2CO). HRMS (ESI-MS): 581.25 [M⁺ + H⁺], 579.25 [M⁺ – H⁺]. Elemental analysis calcd for C₂₉H₃₂N₄O₉, %: C, 59.99; H, 5.56; N, 9.65. Found, %: C, 59.94; H, 5.51; N, 9.69.

2,2'-((2-Hydroxypropane-1,3-diyl)bis(oxy))dibenzaldehyde. 2,2'-((2-Hydroxypropane-1,3-diyl)bis(oxy))dibenzaldehyde which was used in above mentioned synthesis, was obtained by following procedure (Scheme 8).¹⁰⁹ In details, to the solution of sodium hydroxide (110 mmol, 4.4 g) in 100 ml of water was added 109.4 mmol (11.65 ml) of salicylaldehyde. The solution was stirred and heated till 60 °C under N₂, after which 47.1 mmol (3.69 ml) of epichlorohydrin were added during 2 hours. Subsequently, the mixture was stirred at 60 °C for an additional 3 hours. At the end of the reaction time, the mixture was cooled and obtained precipitate was filtered, washed with distilled water and dried. In order to purify obtained dialdehyde, the crude product was recrystallized from methanol–water (8 : 1). 2,2'-((2-Hydroxypropane-1,3-diyl)bis(oxy))dibenzaldehyde was finally obtained as white needles. Yield 35%. Mp 109–110 °C. ¹H NMR spectrum: (CDCl₃, δ, ppm), 3.99–4.01 d (1H, OH, *J* = 6 Hz), 4.32–4.34 d (4H, 2OCH₂, *J* = 6 Hz), 4.52–4.56 m (1H, CH), 7.02–7.10 m (4H, Ar), 7.53–7.59 m (2H, Ar), 7.78–7.81 m (2H, Ar), 10.41 s (2H, 2COH). ¹³C NMR spectrum: (CDCl₃, δ, ppm), 68.34 (2OCH₂), 69.39 (CH), 112.94 (2C_{Ar}H), 121.43 (2C_{Ar}H), 124.97 (2C_{Ar}), 129.93 (2C_{Ar}H), 136.16 (2C_{Ar}H), 160.38 (2C_{Ar}), 189.89 (2COH). Elemental analysis calcd for C₁₇H₁₆O₅, %: C, 67.99; H, 5.37. Found, %: C, 67.93; H, 5.43. The data agrees with the reported literature values.¹⁰⁹

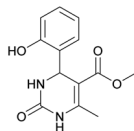


Methyl 6-methyl-2-oxo-4-(phenanthren-9-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (7). The title compound was prepared according to the general procedure using phenanthrene-9-carbaldehyde to afford the title compound a colorless precipitate. Yield 54%. Mp 263–265 °C. ¹H NMR spectrum: (DMSO-d₆, δ, ppm), 2.47 s (3H, CH₃), 3.39 s (3H, OCH₃), 6.1 s (1H, CH), 7.6–7.83 m (6H, 5C_{Ar}H + NH), 7.96–7.98 d (1H, C_{Ar}H, *J* = 6 Hz), 8.38–8.41 m (1H, C_{Ar}H), 8.8–8.83 d (1H, C_{Ar}H, *J* = 9 Hz), 8.89–8.92 m (1H, C_{Ar}H), 9.34 s (1H, NH). ¹³C NMR spectrum: (DMSO-d₆, δ, ppm), 17.97 (CH₃), 50.09 (CH), 50.77 (OCH₃), 98.08 (C), 122.7 (C_{Ar}H), 123.45 (C_{Ar}H), 124.35 (2C_{Ar}H), 126.62 (C_{Ar}H), 126.92 (2C_{Ar}H), 127.04 (C_{Ar}H), 128.76 (C_{Ar}H), 129.25 (C), 129.63 (C_{Ar}), 130.49 (C_{Ar}), 130.95 (C_{Ar}), 137.35 (C_{Ar}), 149.8 (C_{Ar}), 151.85 (COO), 165.8 (CO). HRMS (ESI-MS): 345.17 [M⁺ – H⁺]. Elemental analysis calcd for C₂₁H₁₈N₂O₃, %: C, 72.82; H, 5.24; N, 8.09. Found, %: C, 72.76; H, 5.29; N, 8.15.

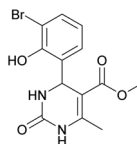
Synthesis of dihydropyrimidines (8–12) on the basis of 2-hydroxy substituted aromatic aldehydes (Scheme 3). 0.5 mmol of 2-hydroxy substituted aromatic aldehyde, 0.75 mmol (45 mg) of urea and 0.08 mmol (30 mg) of Cu(OTf)₂ were added to a microwave vial with a magnetic stirrer and dissolved in 1 ml of



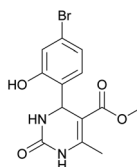
DMSO. Subsequently, 0.46 mmol (50 μ l) of methyl acetoacetate was added to a vial, which was sealed and irradiated at 100 $^{\circ}$ C in a microwave reactor for 2 h at a maximum power of 200 W (CEM DiscoverTM System). At the end of reaction time, the reaction mixture was poured on ice; the precipitate was formed, filtered, washed with distilled water and dried. Further purification of compounds was done by the Biotage Isolera One Flash Chromatography System (cyclohexane–ethyl acetate–methanol).



Methyl 4-(2-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (8). The title compound was prepared according to the general procedure using salicylaldehyde to afford the title compound a colorless precipitate. Yield 62%. Mp 230–232 $^{\circ}$ C. 1 H NMR spectrum: (CD₃OD, δ , ppm), 2.39 s (3H, CH₃), 3.58 s (3H, OCH₃), 5.66 s (1H, CH), 6.72–6.82 m (2H, 2C_{Ar}H), 6.99–7.13 m (2H, 2C_{Ar}H). Elemental analysis calcd for C₁₃H₁₄N₂O₄, %: C, 59.54; H, 5.38; N, 10.68. Found, %: C, 59.59; H, 5.31; N, 10.74. The data agrees with the reported literature values.¹¹⁰

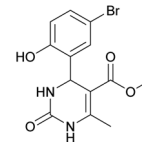


Methyl 4-(3-bromo-2-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (9). The title compound was prepared according to the general procedure using 3-bromo-2-hydroxybenzaldehyde to afford the title compound a yellow-green precipitate. Yield 78%. Mp 224–225 $^{\circ}$ C. 1 H NMR spectrum: (DMSO-d₆, δ , ppm), 2.29 s (3H, CH₃), 3.49 s (3H, OCH₃), 5.55 s (1H, CH), 6.75–7.42 m (5H, 3C_{Ar}H + NH + OH), 9.26 s (1H, NH). 13 C NMR spectrum: (DMSO-d₆, δ , ppm), 17.87 (CH₃), 49.22 (CH), 50.85 (OCH₃), 97.53 (C), 111.90 (C), 121.41 (C_{Ar}H), 126.59 (C_{Ar}H), 131.75 (C_{Ar}H), 133.64 (C_{Ar}), 149.45 (C_{Ar}), 150.70 (C_{Ar}), 151.95 (COO), 165.92 (CO). HRMS (ESI-MS): 341.11 [M⁺ + H⁺]. Elemental analysis calcd for C₁₃H₁₃N₂O₄Br, %: C, 45.77; H, 3.84; N, 8.21. Found, %: C, 45.71; H, 3.78; N, 8.28.

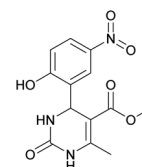


Methyl 4-(4-bromo-2-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (10). The title compound was prepared according to the general procedure using 4-bromo-2-hydroxybenzaldehyde to afford the title compound a yellow-green precipitate. Yield 77%. Mp 231–232 $^{\circ}$ C. 1 H NMR spectrum: (DMSO-d₆, δ , ppm), 2.27 s (3H, CH₃), 3.48 s (3H, OCH₃), 5.39 s (1H, CH), 6.91–6.96 m (3H, 3C_{Ar}H), 7.20 s (1H,

NH), 9.17 s (1H, NH), 10.16 s (1H, NH). 13 C NMR spectrum: (DMSO-d₆, δ , ppm), 17.76 (CH₃), 48.78 (CH), 50.70 (OCH₃), 96.99 (C), 118.01 (C_{Ar}H), 120.33 (C), 121.52 (C_{Ar}H), 128.98 (C_{Ar}), 129.37 (C_{Ar}H), 149.28 (C_{Ar}), 152.13 (C_{Ar}), 155.89 (COO), 165.74 (CO). HRMS (ESI-MS): 341.11 [M⁺ + H⁺]. Elemental analysis calcd for C₁₃H₁₃N₂O₄Br, %: C, 45.77; H, 3.84; N, 8.21. Found, %: C, 45.70; H, 3.88; N, 8.17.



Methyl 4-(5-bromo-2-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (11). The title compound was prepared according to the general procedure using 5-bromo-2-hydroxybenzaldehyde to afford the title compound a yellow-green precipitate. Yield 72%. Mp 215–217 $^{\circ}$ C. 1 H NMR spectrum: (DMSO-d₆, δ , ppm), 2.27 s (3H, CH₃), 3.49 s (3H, OCH₃), 5.39 s (1H, CH), 6.74–6.78 d (1H, C_{Ar}H, *J* = 12 Hz), 7.0 s (1H, C_{Ar}H), 7.20–7.27 m (2H, C_{Ar}H + NH), 9.20 s (1H, NH), 10.01 s (1H, NH). Elemental analysis calcd for C₁₃H₁₃N₂O₄Br, %: C, 45.77; H, 3.84; N, 8.21. Found, %: C, 45.73; H, 3.81; N, 8.25. The data agrees with the reported literature values.¹¹¹

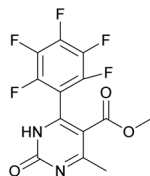


Methyl 4-(2-hydroxy-5-nitrophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (12). The title compound was prepared according to the general procedure using 2-hydroxy-5-nitrobenzaldehyde to afford the title compound a yellow-green precipitate. Yield 79%. Mp 239–240 $^{\circ}$ C. 1 H NMR spectrum: (DMSO-d₆, δ , ppm), 2.30 s (3H, CH₃), 3.49 s (3H, OCH₃), 5.48 s (1H, CH), 6.97 s (1H, C_{Ar}H), 7.44 s (1H, C_{Ar}H), 7.87 s (1H, C_{Ar}H), 8.06 s (1H, NH), 9.28 s (1H, NH), 11.36 br s (1H, NH). 13 C NMR spectrum: (DMSO-d₆, δ , ppm), 17.80 (CH₃), 49.38 (CH), 50.78 (OCH₃), 96.60 (C), 115.95 (C_{Ar}), 123.56 (C), 124.93 (C_{Ar}H), 139.25 (C_{Ar}H), 149.60 (C_{Ar}H), 151.94 (2C_{Ar}), 161.68 (COO), 165.63 (CO). HRMS (ESI-MS): 308.08 [M⁺ + H⁺], 330.08 [M⁺ + Na⁺], 306.08 [M⁺ – H⁺]. Elemental analysis calcd for C₁₃H₁₃N₃O₆, %: C, 50.82; H, 4.26; N, 13.68. Found, %: C, 50.88; H, 4.21; N, 13.61.

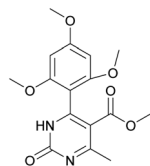
Regioselective oxidation of dihydropyrimidines in the presence of cerium ammonium nitrate (CAN) (Scheme 5). Regioselective oxidation of dihydropyrimidines in the presence of cerium ammonium nitrate (CAN) was done by using the known procedure⁶⁴ with small modifications. In details, 0.30 mmol of subsequent dihydropyrimidine was dissolved in a solvent mixture consisting of 4 ml DMSO and 4 ml acetone. To this solution, 2 mmol of sodium bicarbonate (NaHCO₃) was added. The temperature of the reaction mixture was decreased to –5 $^{\circ}$ C. Then, 1.2 mmol of CAN was dissolved in 2 ml of water



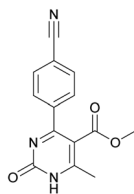
and added to the solution of dihydropyrimidine under argon (Ar) atmosphere (the color changed from orange to a pale yellow). The reaction mixture was stirred 1 hour at -5°C and 20 hours at room temperature. At the end of reaction time, the reaction mixture was decanted with chloroform. Subsequently, 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.



Methyl 6-methyl-2-oxo-4-(perfluorophenyl)-1,2-dihydropyrimidine-5-carboxylate (13). The title compound was prepared according to the general procedure using methyl 6-methyl-2-oxo-4-(perfluorophenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1) to afford the title compound a colorless precipitate. Yield 1%. Mp $311\text{--}312^{\circ}\text{C}$. ^1H NMR spectrum: (DMSO-d_6 , δ , ppm), 2.31 s (3H, CH_3), 3.43 s (3H, OCH_3). HRMS (ESI-MS): 357.08 [$\text{M}^+ + \text{Na}^+$]. Elemental analysis calcd for $\text{C}_{13}\text{H}_7\text{N}_2\text{O}_3\text{F}_5$, %: C, 46.72; H, 2.11; N, 8.38. Found, %: C, 46.78; H, 2.19; N, 8.44.

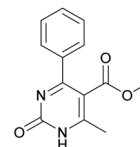


Methyl 6-methyl-2-oxo-4-(2,4,6-trimethoxyphenyl)-1,2-dihydropyrimidine-5-carboxylate (14). The title compound was prepared according to the general procedure using methyl 6-methyl-2-oxo-4-(2,4,6-trimethoxyphenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2) to afford the title compound a light yellow precipitate. Yield 40%. Mp $220\text{--}222^{\circ}\text{C}$. ^1H NMR spectrum: (CDCl_3 , δ , ppm), 2.58 s (3H, CH_3), 3.55 s (3H, OCH_3), 3.73 s (6H, 2OCH_3), 3.84 s (3H, OCH_3), 6.13 s (2H, $2\text{C}_{\text{Ar}}\text{H}$). ^{13}C NMR spectrum: (CDCl_3 , δ , ppm), 24.09 (CH_3), 51.54 (OCH_3), 55.25 (OCH_3), 55.63 (2OCH_3), 90.52 ($2\text{C}_{\text{Ar}}\text{H}$), 103.72 (C), 112.29 (2C), 157.73 (C_{Ar}), 158.2 (3C_{Ar}), 163.26 (COO), 165.96 (CO). HRMS (ESI-MS): 335.17 [$\text{M}^+ + \text{H}^+$], 357.17 [$\text{M}^+ + \text{Na}^+$]. Elemental analysis calcd for $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_6$, %: C, 57.48; H, 5.43; N, 8.38. Found, %: C, 57.55; H, 5.36; N, 8.31.

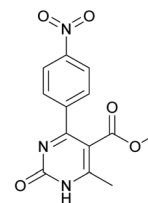


Methyl 4-(4-cyanophenyl)-6-methyl-2-oxo-1,2-dihydropyrimidine-5-carboxylate (15). The title compound was

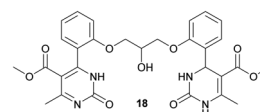
prepared according to the general procedure using methyl 4-(4-cyanophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3) to afford the title compound a colorless precipitate. Yield 27%. Mp $199\text{--}200^{\circ}\text{C}$. ^1H NMR spectrum: (CDCl_3 , δ , ppm), 2.68 s (3H, CH_3), 3.62 s (3H, OCH_3), 7.72–7.75 m (4H, $4\text{C}_{\text{Ar}}\text{H}$). ^{13}C NMR spectrum: (CDCl_3 , δ , ppm), 19 (CH_3), 52.41 (OCH_3), 110.88 (C), 114.35 (2C), 117.93 (2C, $\text{CN} + \text{C}_{\text{Ar}}$), 128.53 ($2\text{C}_{\text{Ar}}\text{H}$), 132.00 ($2\text{C}_{\text{Ar}}\text{H}$), 141.73 (C_{Ar}), 157.94 (COO), 165.50 (CO). HRMS (ESI-MS): 270.08 [$\text{M}^+ + \text{H}^+$], 292.08 [$\text{M}^+ + \text{Na}^+$]. Elemental analysis calcd for $\text{C}_{14}\text{H}_{11}\text{N}_3\text{O}_3$, %: C, 62.45; H, 4.12; N, 15.61. Found, %: C, 62.51; H, 4.17; N, 15.66.

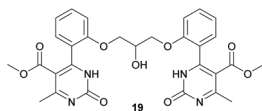


Methyl 6-methyl-2-oxo-4-phenyl-1,2-dihydropyrimidine-5-carboxylate (16). The title compound was prepared according to the general procedure using methyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4) to afford the title compound a light yellow precipitate. Yield 39%. Mp $193\text{--}194^{\circ}\text{C}$. ^1H NMR spectrum: (CDCl_3 , δ , ppm), 2.59 s (3H, CH_3), 3.58 s (3H, OCH_3), 7.43–7.61 m (6H, $5\text{C}_{\text{Ar}}\text{H} + \text{NH}$). Elemental analysis calcd for $\text{C}_{13}\text{H}_{12}\text{N}_2\text{O}_3$, %: C, 63.93; H, 4.95; N, 11.47. Found, %: C, 63.99; H, 4.89; N, 11.41. The data agrees with the reported literature values.¹¹²



Methyl 6-methyl-4-(4-nitrophenyl)-2-oxo-1,2-dihydropyrimidine-5-carboxylate (17). The title compound was prepared according to the general procedure using methyl 6-methyl-4-(4-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (5) to afford the title compound a colorless precipitate. Yield 28%. Mp $210\text{--}211^{\circ}\text{C}$. ^1H NMR spectrum: (DMSO-d_6 , δ , ppm), 2.46 s (3H, CH_3), 3.49 s (3H, OCH_3), 7.68–7.71 d (2H, $2\text{C}_{\text{Ar}}\text{H}$, $J = 9$ Hz), 8.30–8.33 d (2H, $2\text{C}_{\text{Ar}}\text{H}$, $J = 9$ Hz), 12.62 s (1H, NH). ^{13}C NMR spectrum: (DMSO-d_6 , δ , ppm), 28.99 (CH_3), 51.98 (OCH_3), 123.42 ($2\text{C}_{\text{Ar}}\text{H}$), 128.87 ($2\text{C}_{\text{Ar}}\text{H}$), 144.82 (C), 148.12 (C), 154.69 (C), 154.90 (C_{Ar}), 161.83 (C_{Ar}), 162.12 (COO), 165.60 (CO). Elemental analysis calcd for $\text{C}_{13}\text{H}_{11}\text{N}_3\text{O}_5$, %: C, 53.98; H, 3.83; N, 14.53. Found, %: C, 53.91; H, 3.89; N, 14.57. The data agrees with the reported literature values.¹¹³

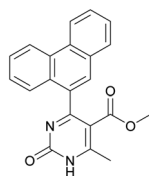




Methyl 4-(2-(2-hydroxy-3-(2-(5-(methoxycarbonyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl)phenoxy)propoxy)phenyl)-6-methyl-2-oxo-1,2-dihydropyrimidine-5-carboxylate (18) and dimethyl 4,4'-(((2-hydroxypropane-1,3-diyl)bis(oxy))bis(2,1-phenylene))bis(6-methyl-2-oxo-1,2-dihydropyrimidine-5-carboxylate) (19). The title compounds were prepared according to the general procedure using dimethyl 4,4'-(((2-hydroxypropane-1,3-diyl)bis(oxy))bis(2,1-phenylene))bis(6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate) (6) to afford the title compounds a colorless precipitate. Yield 15 and 5% respectively. In case if the amount of sodium bicarbonate and CAN was increased two times in comparison with the general oxidation procedure, then the obtaining of compound 18 was not observed. In this case the yield of compound 19 increased till 22% respectively. Mp 283–285 °C and 267–268 °C accordingly.

¹H NMR spectrum of compound 18: (CDCl₃, δ, ppm), 1.90 s (1H, NH), 2.33 s (3H, CH₃), 2.41–2.43 d (3H, CH₃, *J* = 6 Hz), 3.41–3.42 d (3H, OCH₃, *J* = 3 Hz), 3.46 s (3H, OCH₃), 3.87–3.93 t (1H, OH, *J* = 9 Hz), 4.07–4.20 m (5H, 2OCH₂ + CH), 5.56 s (1H, CH), 6.87–6.92 t (1H, C_{Ar}H, *J* = 9 Hz), 6.96–7.07 m (3H, 3C_{Ar}H), 7.11–7.14 m (1H, C_{Ar}H), 7.23–7.26 m (2H, C_{Ar}H + NH), 7.32–7.34 d (1H, C_{Ar}H, *J* = 6 Hz), 7.40–7.45 t (1H, C_{Ar}H, *J* = 9 Hz), 9.19 s (1H, NH). ¹³C NMR spectrum of compound 18: (CDCl₃, δ, ppm), 10.60 (CH₃), 17.82 (CH₃), 48.49 (CH), 50.70 (OCH₃), 51.27 (OCH₃), 69.65 (CH–OH), 69.94 (CH₂O), 70.48 (CH₂O), 96.75 (C), 109.66 (C_{Ar}H), 111.66 (C_{Ar}H), 112.30 (C_{Ar}H), 120.57 (C_{Ar}H), 126.35 (C_{Ar}H), 128.75 (C_{Ar}H), 129.56 (C_{Ar}H), 131.11 (C_{Ar}H), 131.31 (C), 149.72 (C_{Ar}), 149.77 (C_{Ar}), 152.26 (C_{Ar}), 152.32 (C), 155.12 (C), 155.16 (C), 155.80 (C_{Ar}), 156.06 (COO), 165.65 (COO), 165.67 (CO), 165.76 (CO). HRMS (ESI-MS): 579.33 [M⁺ + H⁺], 601.25 [M⁺ + Na⁺], 577.33 [M⁺ – H⁺]. Elemental analysis calcd for C₂₉H₃₀N₄O₉, %: C, 60.20; H, 5.23; N, 9.68. Found, %: C, 60.27; H, 5.28; N, 9.61.

¹H NMR spectrum of compound 19: (CD₃OD, δ, ppm), 3.41 s (6H, 2CH₃), 3.54 s (6H, 2OCH₃, *J* = 6 Hz), 3.99–4.02 d (1H, OH, *J* = 9 Hz), 4.13–4.28 m (5H, 2OCH₂ + CH), 7.16–7.21 t (5H, 4C_{Ar}H + NH, *J* = 9 Hz), 7.47–7.59 m (5H, 4C_{Ar}H + NH). ¹³C NMR spectrum of compound 19: (CDCl₃, δ, ppm), 23.88 (2CH₃), 52.62 (2OCH₃), 69.37 (CH–OH), 71.44 (2CH₂O), 113.00 (2C), 113.35 (2C_{Ar}H), 122.57 (2C_{Ar}H), 127.78 (2C), 131.19 (2C_{Ar}H), 133.14 (2C), 133.28 (2C_{Ar}H), 157.16 (4C_{Ar}), 158.96 (2COO), 167.97 (2CO). HRMS (ESI-MS): 577.29 [M⁺ + H⁺], 599.27 [M⁺ + Na⁺]. Elemental analysis calcd for C₂₉H₂₈N₄O₉, %: C, 60.41; H, 4.90; N, 9.72. Found, %: C, 60.45; H, 4.83; N, 9.79.



Methyl 6-methyl-2-oxo-4-(phenanthren-9-yl)-1,2-dihydropyrimidine-5-carboxylate (20). The title compound was prepared according to the general procedure using methyl 6-methyl-2-oxo-4-(phenanthren-9-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (7) to afford the title compound a colorless precipitate. Yield 35%. Mp 239–241 °C. ¹H NMR spectrum: (DMSO-d₆, δ, ppm), 2.55 s (3H, CH₃), 3.07 s (3H, OCH₃), 7.61–7.81 m (6H, 6C_{Ar}H), 8.04–8.07 d (1H, C_{Ar}H, *J* = 9 Hz), 8.87–8.93 t (2H, 2C_{Ar}H, *J* = 9 Hz). ¹³C NMR spectrum: (DMSO-d₆, δ, ppm), 20.00 (CH₃), 51.54 (OCH₃), 110.37 (C), 122.92 (C_{Ar}H), 123.31 (C_{Ar}H), 125.57 (2C_{Ar}H), 126.18 (C_{Ar}H), 127.04 (2C_{Ar}H), 127.11 (C_{Ar}H), 127.29 (C_{Ar}H), 127.88 (C), 128.77 (C_{Ar}), 129.07 (C_{Ar}), 129.53 (C_{Ar}), 129.97 (C_{Ar}), 130.28 (C_{Ar}), 133.84 (C), 154.62 (COO), 165.10 (CO). HRMS (ESI-MS): 343.17 [M⁺ – H⁺]. Elemental analysis calcd for C₂₁H₁₆N₂O₃, %: C, 73.24; H, 4.68; N, 8.13. Found, %: C, 72.29; H, 4.75; N, 8.17.

Biological assay

The antibacterial activity of the investigated compounds (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 19 and 20) against bacteria *A. baumannii*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *S. aureus* was assessed determining minimum inhibitory concentration (MIC) by twofold microdilution method as described in elsewhere.^{100–102} The compounds were prepared according to CLSI guidelines and diluted in U-bottom 96 well microtiter plates which contained Mueller–Hinton Broth (MHB). The freshly prepared bacterial strains about 10⁵ CFU (colony forming unit) in MHB medium were added to each well of the microplate and incubated at 37 °C for 24 hours. At the end of the experiment, the concentration of the tested compounds ranged from 1000 to 7.8 μg ml⁻¹. The growth of the bacterial cells was determined by resazurin method. The solution of the resazurin sodium salt (0.01%) was freshly prepared in sterile distilled water. After incubation, 30 μl of this solution was added in each well of microplate and incubated again at the same condition for about 4 h. MIC was represented as the lowest concentration of the compounds which inhibited the change color from blue to pink since pink color indicated the growth of bacteria. MIC of the studied compound was compared with MICs of pristine antibiotics (cefotaxime and ceftriaxone).

In addition to this, the antibacterial activity of the investigated dihydropyrimidines against the above-mentioned bacteria was also studied by disc-diffusion method as described by Mayrhofer.¹⁰³ 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 (10⁵ CFU ml⁻¹), 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.

Conclusion

The new derivatives of dihydropyrimidines were synthesized by Biginelli reaction in microwave conditions in presence of



copper triflate. Salicylaldehyde under Biginelli reaction conditions can lead to the formation of 2 types of dihydropyrimidines. This procedure was therefore also used for salicylaldehyde derivatives and the formation of hydroxy substituted dihydropyrimidines was demonstrated. Furthermore regioselective oxidation of dihydropyrimidine ring in the presence of CAN was also done. The structures of all synthesized compounds were investigated by NMR, mass spectroscopy methods and X-ray single crystal diffraction.

The crystal structures allowed determining structure-tautomer dependence. The oxidized products of dihydropyrimidines are in the form of tautomer **II**, in case they have a heteroatom group on the benzene ring that is able to participate in the formation of intramolecular hydrogen bond with amine group in the dihydropyrimidine ring. Otherwise, regioselectively oxidized products are in the form of tautomer **I**. Along with it, Hirshfeld surface analysis was carried out to gain insight into the crystal packing and molecular interactions. Considering that H₂Py can have an ability to act as antibacterial drug, they were tested for the biological activity against *A. baumannii*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *S. aureus* bacteria. In addition, their activity was also compared with that of pristine antibiotics. The results obtained are promising and suggest that the synthesized compounds are biologically active with antibacterial activity.

Author contributions

Alakbar E. Huseynzada: methodology, investigation, formal analysis, writing – original draft. Christian Jelch: formal analysis, writing – original draft, writing – review & editing. Haji Vahid N. Akhundzada: writing – review & editing. Sarra Soudani: formal analysis, writing – original draft. Cherif Ben Nasr: formal analysis, writing – original draft. Aygun Israyilova: formal analysis, writing – original draft. Filippo Doria: resources, data curation. Ulviyya A. Hasanova: writing – original draft, supervision, funding acquisition. Rana F. Khankishiyeva: writing – review & editing. Mauro Freccero: supervision, project administration.

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Conflicts of interest

The authors declare no conflict of interest.

References

- P. Biginelli, *Gazz. Chim. Ital.*, 1893, **23**, 360–416.
- G. C. Tron, A. Minassi and G. Appendino, *Eur. J. Org. Chem.*, 2011, 5541–5550.
- C. O. Kappe, *Acc. Chem. Res.*, 2000, **33**, 879–888.
- W. Jie-Ping and Y. Liu, *Synthesis*, 2010, **23**, 3943–3953.
- M. Kazemi, *Synth. Commun.*, 2020, **50**, 1409–1445.
- P. M. Kumar, K. S. Kumar, S. R. Poreddy, P. K. Mohakud, K. Mukkanti and M. Pal, *Tetrahedron Lett.*, 2011, **52**, 1187–1191.
- Y. U. Gadkari, N. T. Hatvate, B. S. Takale and V. N. Telvekar, *New J. Chem.*, 2020, **44**, 8167–8170.
- J. C. M. Willig, *et al.*, *RSC Adv.*, 2020, **10**, 3407–3415.
- R. Kaur, S. Chaudhary, K. Kumar, M. K. Gupta and R. K. Rawal, *Eur. J. Med. Chem.*, 2017, **132**, 108–134.
- J. C. Barrow, P. G. Nantermet, H. G. Selnick, K. L. Glass, K. E. Rittle, K. F. Gilbert, T. G. Steele, C. F. Homnick, R. M. Freidinger, R. W. Ransom, P. Kling, D. Reiss, T. P. Broten, T. W. Schorn, R. S. L. Chang, S. S. O'Malley, T. V. Olah, J. D. Ellis, A. Barrish, K. Kassahun, P. Leppert, D. Nagarathnam and C. Forray, *J. Med. Chem.*, 2000, **43**, 2703.
- A. C. Boukis, A. Llevot and M. A. R. Meier, *Macromol. Rapid Commun.*, 2016, **37**, 643.
- S. R. Patil, A. S. Choudhary, V. S. Patil and N. Sekar, *Fibers Polym.*, 2015, **16**, 2349.
- Y. Zhao, Y. Yu, Y. Zhang, X. Wang, B. Yang, Y. Zhang, Q. Zhang, C. Fu, Y. Weia and L. Tao, *Polym. Chem.*, 2015, **6**, 4940.
- S. S. Jagir, *Arkivoc*, 2012, 66–133.
- C. G. Wermuth, C. R. Ganellin, P. Lindberg and L. A. Mitscher, *Pure Appl. Chem.*, 1998, **70**, 1129–1143.
- K. S. Atwal, B. N. Swanson, S. E. Unger, D. M. Floyd, S. Moreland, A. Hedberg and B. C. O'Reilly, *J. Med. Chem.*, 1991, **34**, 806–811.
- A. M. Maharramov, *et al.*, *J. Mol. Struct.*, 2017, **1141**, 39–43.
- P. Murthy and R. Chatterjee, *Curr. Sci.*, 1999, **77**, 1084–1089.
- R. Gaur, S. Dixit, M. Sahoo, M. Khanna, S. Singh and P. Murthy, *J. Parasitol.*, 2007, **134**, 537.
- S. M. Sondhi, N. Singh, M. Johar and A. Kumar, *Bioorg. Med. Chem.*, 2005, **13**, 6158–6166.
- U. Rashid, *et al.*, *Eur. J. Med. Chem.*, 2016, **115**, 230–244.
- (a) X. Zhu, G. Zhao, X. Zhou, X. Xu, G. Xia, Z. Zheng, L. Wang, X. Yang and S. Li, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 299; (b) K. L. Dhumaskar, S. N. Meena, S. C. Ghadi and S. G. Tilve, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 2897; (c) T. G. M. Treptow, F. Figueir, E. H. F. Jandre, A. M. O. Battastini, C. G. Salbego, J. B. Hoppe, P. S. Taborda, S. B. Rosa, L. A. Piovesan, C. R. M. Doca, D. Russowsky and M. G. M. Doca, *Eur. J. Med. Chem.*, 2015, **95**, 552; (d) R. Chikhale, S. Menghani, R. Babu, R. Bansode, G. Bhargavi, N. Karodia, M. V. Rajasekharan, A. Paradkar and P. Khedekar, *Eur. J. Med. Chem.*, 2015, **96**, 30; (e) U. Rashid, R. Sultana, N. Shaheen, S. F. Hassan, F. Yaqoob, M. J. Ahmad, F. Iftikhar, N. Sultana, S. Asghar, M. Yasinza, F. L. Ansari and N. A. Qureshi, *Eur. J. Med. Chem.*, 2016, **115**, 230; (f) K. Singh and T. Kaur, *RSC Med. Chem.*, 2016, **7**, 749.
- E. Klein, S. DeBonis, B. Thiede, D. A. Skoufias, F. Kozielskib and L. Lebeau, *Bioorg. Med. Chem.*, 2007, **15**, 6474.
- H. Y. K. Kaan, V. Ulaganathan, O. Rath, H. Prokopcov, D. Dallinger, C. O. Kappe and F. J. Kozielski, *Med. Chem.*, 2010, **53**, 5676.



- 25 M. Wright Christine, *et al.*, *Bioorg. Med. Chem.*, 2008, **16**, 3291–3301.
- 26 O. C. Agbaje, O. O. Fadeyi, S. A. Fadeyi, L. E. Myles and C. O. Okoro, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 989.
- 27 B. R. P. Kumar, G. Sankar, R. B. N. Baig and S. Chandrashekar, *Eur. J. Med. Chem.*, 2009, **44**, 4192.
- 28 D. A. Ibrahim and A. M. El-Metwally, *Eur. J. Med. Chem.*, 2010, **45**, 1158.
- 29 S. G. Khanage, S. A. Raju, P. B. Mohite and R. B. Pandhare, *Adv. Pharm. Bull.*, 2012, **2**, 213–222.
- 30 (a) A. Wang, X. Liu, Z. Su and H. Jing, *Catal. Sci. Technol.*, 2014, **4**, 71; (b) B. K. Ghosh, S. Hazra and N. N. Ghosh, *Catal. Commun.*, 2016, **80**, 44.
- 31 (a) N. October, N. D. Watermeyer, V. Yardley, T. J. Egan, K. Ncokazi and K. Chibale, *ChemMedChem*, 2008, **3**, 1649; (b) S. Fatima, A. Sharma, R. Saxena, R. Tripathi, S. K. Shukla, S. K. Pandey, R. Tripathi and R. P. Tripathi, *Eur. J. Med. Chem.*, 2012, **55**, 195; (c) H. Kaur, M. Machado, C. Kock, P. Smith, K. Chibale, M. Prudencio and K. Singh, *Eur. J. Med. Chem.*, 2015, **101**, 266.
- 32 (a) T. N. Akhaja and J. P. Raval, *Eur. J. Med. Chem.*, 2011, **46**, 5573; (b) R. K. Yadlapalli, O. P. Chourasia, K. Vemuri, M. Sritharan and R. S. Perali, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 2708.
- 33 (a) K. T. Homan, K. M. Larimore, J. M. Elkins, M. Szklarz, S. Knapp and J. J. G. Tesmer, *ACS Chem. Biol.*, 2015, **10**, 310; (b) H. V. Waldschmidt, K. T. Homan, O. Cruz-Rodríguez, M. C. Cato, J. Waninger-Saroni, K. M. Larimore, A. Cannavo, J. Song, J. Y. Cheung, P. D. Kirchhoff, W. J. Koch, J. J. G. Tesmer and S. D. Larsen, *J. Med. Chem.*, 2016, **59**, 3793.
- 34 S. N. Mokale, S. S. Shinde, R. D. Elgire, J. N. Sangshetti and D. B. Shinde, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 4424.
- 35 S. S. Bahekar and D. B. Shinde, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 1733.
- 36 S. S. Bahekar and D. B. Shinde, *Acta Pharm.*, 2003, **53**, 223.
- 37 K. S. Atwal, G. C. Rovnyak, J. Schwartz, S. Moreland, A. Hedberg, J. Z. Gougoutas, M. F. Malley and D. M. J. Floyd, *Med. Chem.*, 1990, **33**, 1510.
- 38 I. S. Zorkun, S. Sarac, S. Celebib and K. Erolb, *Bioorg. Med. Chem.*, 2006, **14**, 8582.
- 39 R. V. Chikhale, R. P. Bhole, P. B. Khedekar and K. P. Bhusari, *Eur. J. Med. Chem.*, 2009, **44**, 3645.
- 40 O. Alam, S. A. Khan, N. Siddiqui, W. Ahsan, S. P. Verma and S. J. Gilani, *Eur. J. Med. Chem.*, 2010, **45**, 5113.
- 41 C. A. Sehon, G. Z. Wang, A. Q. Viet, K. B. Goodman, S. E. Dowdell, P. A. Elkins, S. F. Semus, C. Evans, L. J. Jolivet, R. B. Kirkpatrick, E. Dul, S. S. Khandekar, T. Yi, L. L. Wright, G. K. Smith, D. J. Behm and R. J. Bentley, *Med. Chem.*, 2008, **51**, 6631.
- 42 B. Borowsky, M. M. Durkin, K. Ogozalek, M. R. Marzabadi, J. Deleon, R. Heurich, H. Lichtblau, Z. Shaposhnik, I. Daniewska and T. P. Blackburn, *Nat. Med.*, 2002, **8**, 825–830.
- 43 A. D. Patil, N. V. Kumar, W. C. Kokke, M. F. Bean, A. J. Freger, C. Debrossi, S. Mai, A. Truneh, D. J. Faulkner, B. Carte, A. L. Breen, R. P. Hertzbery, R. K. Johnson, J. W. Westley and B. C. M. J. Potts, *Org. Chem.*, 1995, **60**, 1182.
- 44 R. W. Lewis, J. Mabry, J. G. Polisar, K. P. Eagen, B. Ganem and G. P. Hess, *Biochemistry*, 2010, **49**, 4841.
- 45 L. Figueroa-Valverde, F. Díaz-Cedillo, M. López-Ramos and E. Garcia-Cervera, *Int. J. PharmTech Res.*, 2010, 2075–2080.
- 46 R. Ramajayam, K.-P. Tan, H.-G. Liu and P.-H. Liang, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 3569–3572.
- 47 (a) K. Folkers and T. B. Johnson, *J. Am. Chem. Soc.*, 1933, **55**, 3784; (b) F. S. Sweet and J. D. Fissekis, *J. Am. Chem. Soc.*, 1973, **95**, 8741; (c) C. O. Kappe, *J. Org. Chem.*, 1997, **62**, 7201; (d) R. De Souza, E. T. Penha, H. M. S. Milagre, S. J. Garden, P. M. Esteves, M. N. Eberlin and O. A. C. Antunes, *Chem.–Eur. J.*, 2009, **15**, 9799; (e) L. M. Ramos, A. Y. P. L. Tobio, M. R. Santos, H. C. B. Oliveira, A. F. Gomes, F. C. Gozzo, A. L. Oliveira and B. A. D. J. Neto, *Org. Chem.*, 2012, **77**, 10184; (f) M. Puripat, R. Ramozzi, M. Hatanaka, W. Parasuk, V. Parasuk and K. J. Morokuma, *Org. Chem.*, 2015, **80**, 6959.
- 48 S. Terracciano, G. Lauro, M. Strocchia, K. Fischer, O. Werz, R. Riccio, I. Bruno and G. Bifulco, *ACS Med. Chem. Lett.*, 2015, **6**, 187.
- 49 A. R. Trivedi, V. R. Bhuvu, B. H. Dholariya, D. K. Dodiya, V. B. Kataria and V. H. Shah, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 6100.
- 50 H. J. Gijssen, D. Berthelot, M. A. De Cleyn, I. Geuens, B. Brone and M. Mercken, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 797–800.
- 51 B. K. Singh, M. Mishra, N. Saxena, G. P. Yadav, P. R. Maulik, M. K. Sahoo, R. L. Gaur, P. K. Murthy and R. P. Tripathi, *Eur. J. Med. Chem.*, 2008, **43**, 2717.
- 52 J. C. Barrow, P. G. Nantermet, H. G. Selnick, K. L. Glass, K. E. Rittle, K. F. Gilbert, T. G. Steele, C. F. Homnick, R. M. Freidinger, R. W. Ransom, P. Kling, D. Reiss, T. P. Broten, T. W. Schorn, R. S. L. Chang, S. S. O'Malley, T. V. Olah, J. D. Ellis, A. Barrish, K. Kassahun, P. Leppert, D. Nagarathnam and C. Forray, *J. Med. Chem.*, 2000, **43**, 2703.
- 53 X. Zhu, G. Zhao, X. Zhou, X. Xu, G. Xia, Z. Zheng, L. Wang, X. Yang and S. Li, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 299.
- 54 H. J. Finlay, *et al.*, *J. Med. Chem.*, 2012, **55**, 3036–3048.
- 55 J. Lloyd, *et al.*, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 5469–5473.
- 56 J. Lloyd, H. J. Finlay, W. Vacarro, T. Hyunh, A. Kover, R. Bhandaru, L. Yan, K. Atwal, M. L. Conder, T. Jenkins-West, H. Shi, C. Huang, D. Li, H. Sun and P. Levesque, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 1436.
- 57 G. C. Rovnyak, K. S. Atwal, A. Hedberg, S. D. Kimball, S. Moreland, J. Z. Gougoutas, B. C. O'Reilly, J. Schwartz and M. F. Malley, *J. Med. Chem.*, 1992, **35**, 3254–3263.
- 58 S. R. Patil, A. S. Choudhary, V. S. Patil and N. Sekar, *Fibers Polym.*, 2015, **16**, 2349.
- 59 M. Heravi, M. Ghavidel and B. Heidari, *Curr. Org. Synth.*, 2016, **13**, 569–600.
- 60 J. P. Wan and Y. Liu, *Synthesis*, 2010, **23**, 3943–3953.
- 61 P. Shanmugam and P. T. Perumal, *Tetrahedron*, 2007, **63**, 666–672.



- 62 D. S. Bose, F. Liyakat and H. B. Mereyala, *J. Org. Chem.*, 2003, **68**, 587–590.
- 63 A. R. Gholap, K. S. Toti, F. Shirazi, M. V. Deshpande and K. V. Srinivasan, *Tetrahedron*, 2008, **64**, 10214–10223.
- 64 P. Shanmugam and P. T. Perumal, *Tetrahedron*, 2006, **62**, 9726–9734.
- 65 K. Yamamoto, Y. G. Chen and F. G. Buono, *Org. Lett.*, 2005, **7**, 4673–4676.
- 66 M. Hayashi, K. I. Okunaga, S. Nishida, K. Kawamura and K. Eda, *Tetrahedron Lett.*, 2010, **51**, 6734–6736.
- 67 D. Prajapati, D. Bhuyan, M. Gohain and W. Hu, *Mol. Diversity*, 2011, **15**, 257–261.
- 68 M. Wu, J. Yu, W. Zhao, J. Wu and S. Cao, *J. Fluorine Chem.*, 2011, **132**, 155–159.
- 69 J. P. Wan, C. Wang and Y. Pan, *Tetrahedron*, 2011, **67**, 922–926.
- 70 V. P. Srivastava and L. D. S. Yadav, *Tetrahedron Lett.*, 2010, **51**, 6436–6438.
- 71 K. U. Sadek, F. Al-Qalaf, M. M. Abdelkhalik and M. H. Elnagdi, *J. Heterocycl. Chem.*, 2010, **47**, 284.
- 72 P. Gupta, S. Gupta, A. Sachar, D. Kour, J. Singh and R. L. Sharma, *J. Heterocycl. Chem.*, 2010, **47**, 324–333.
- 73 V. T. Kamble, D. B. Muley, S. T. Atkore and S. D. Dakore, *Chin. J. Chem.*, 2010, **28**, 388–392.
- 74 C. J. Liu and J. D. Wang, *Molecules*, 2010, **15**, 2087–2095.
- 75 Z. Pourghobadi and F. Derikvand, *Chin. Chem. Lett.*, 2010, **21**, 269–272.
- 76 M. Matache, *et al.*, *Tetrahedron*, 2009, **65**, 5949–5957.
- 77 S. K. De and R. A. Gibbs, *Synth. Commun.*, 2005, **35**, 2645–2651.
- 78 M. N. Godoi, H. S. Costenaro, E. Kramer, P. S. Machado, M. G. M. Doca and D. Russowsky, *Quim. Nova*, 2005, **28**, 1010.
- 79 S. K. De and R. A. Gibbs, *Synthesis*, 2005, **11**, 1748–1750.
- 80 G. L. Zhang and X. H. Cai, *Synth. Commun.*, 2005, **35**, 829–833.
- 81 A. E. Huseynzada, *et al.*, *J. Mol. Struct.*, 2020, 128581.
- 82 J. Jacob, *Int. J. Chem.*, 2012, **4**, 29–43.
- 83 C. O. Kappe and D. Dallinger, *Mol. Diversity*, 2009, **13**, 71.
- 84 K. K. Pasunooti, H. Chai, C. N. Jensen, B. K. Gorityala, S. Wang and X. W. Liu, *Tetrahedron Lett.*, 2011, **52**, 80–84.
- 85 Q. Cheng, Q. Wang, T. Tan, N. Chen and M. Shuaib, *J. Heterocycl. Chem.*, 2012, **49**, 1352–1356.
- 86 X. Jing, Z. Li, X. Pan, Q. Wang, C. Yan and H. Zhu, *Synth. Commun.*, 2009, **39**, 3796–3803.
- 87 J. Sve, L. Veizerová and V. Kettmann, *Tetrahedron Lett.*, 2008, **49**, 3520–3523.
- 88 Q. Liu, J. Xu, F. Teng, A. Chen, N. Pan and W. Zhang, *J. Heterocycl. Chem.*, 2014, **51**, 741–746.
- 89 O. Rosati, M. Curini, F. Montanari, M. Nocchetti and S. Genovese, *Catal. Lett.*, 2011, **141**, 850–853.
- 90 D. Kumarasamy, *et al.*, *Bioorg. Med. Chem. Lett.*, 2017, **27**, 139–142.
- 91 M. M. Ibrahim, H. S. El-Sheshtawy, M. El-Kemary, S. Al-Juaid, M. Youssef and I. H. El-Azab, *J. Mol. Struct.*, 2017, **1137**, 714–719.
- 92 R. Zheng, X. Wang, H. Xu and J. Du, *Synth. Commun.*, 2006, **36**, 1503–1513.
- 93 N. Y. Fu, Y. F. Yuan, Z. Cao, S. W. Wang, J. T. Wang and C. Peppe, *Tetrahedron*, 2002, **58**, 4801–4807.
- 94 D. S. Bose, M. V. Chary and H. B. Mereyala, *Heterocycles*, 2006, **68**, 1217–1224.
- 95 J. Zhu, M. Zhang, B. Liu and X. Li, *Chem. Lett.*, 2009, **38**, 56–57.
- 96 A. Kumar and R. A. Maurya, *Tetrahedron Lett.*, 2007, **48**, 4569–4571.
- 97 D. S. Bose, M. Sudharshan and S. W. Chavhan, *Arkivoc*, 2005, **228**, 236.
- 98 A. Katrusiak, *J. Mol. Graphics Modell.*, 2001, **19**, 363–367.
- 99 J. J. McKinnon, D. Jayatilaka and M. A. Spackman, *Chem. Commun.*, 2007, **37**, 3814–3816.
- 100 A. Martin, H. Takiff, P. Vandamme, J. Swings, J. C. Palomino and F. Portaels, *J. Antimicrob. Chemother.*, 2006, **58**, 327–331.
- 101 A. Israyilova, S. Buroni, F. Forneris, V. C. Scoffone, N. Q. Shixaliyev, G. Riccardi and L. R. Chiarelli, *PLoS One*, 2016, **11**, 1–17.
- 102 S. Hajiyeva, *et al.*, *Turk. J. Chem.*, 2019, **43**, 1711–1721.
- 103 S. Mayrhofer, *et al.*, *Appl. Environ. Microbiol.*, 2008, **74**, 3745–3748.
- 104 M. J. Ahmad, *et al.*, *Med. Chem. Res.*, 2016, **25**, 1877–1894.
- 105 G. M. Sheldrick, *SHELXTL V5. 1*, Bruker AXS Inc., 1997.
- 106 C. Mukhopadhyay and D. Arup, *J. Heterocycl. Chem.*, 2010, **47**, 136–146.
- 107 C. K. Khatri, S. R. Deelip and U. C. Ganesh, *New J. Chem.*, 2016, **40**, 10412–10417.
- 108 J. Safari and S. Gandomi-Ravandi, *New J. Chem.*, 2014, **38**, 3514–3521.
- 109 Z. Bin, *et al.*, *Polyhedron*, 1996, **15**, 1197–1202.
- 110 H. M. Savanur, *et al.*, *Tetrahedron Lett.*, 2016, **57**, 3029–3035.
- 111 Q. Cheng, *et al.*, *J. Heterocycl. Chem.*, 2010, **47**, 624–628.
- 112 S. Kamaljit Singh and S. Kawaljit, *Aust. J. Chem.*, 2008, **61**, 910–913.
- 113 D. S. Kolarović, V. Vladimir and L. Mladen, *Arkivoc*, 2016, 271–286.

