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Facile synthesis of new pyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-ones via the tandem intramolecular Pinner–Dimroth rearrangement and their antibacterial evaluation

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Abstract: Some new 7-alkyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-ones were prepared through heterocyclization of 6-amino-1,4-dihydropyran[2,3-c]pyrazole-5-carbonitriles with aliphatic carboxylic acids in the presence of phosphoryl chloride under reflux in high yields. The suggested mechanism involves a tandem intramolecular Pinner–Dimroth rearrangement. The products were characterized on the basis of FT-IR, ^1H NMR, and ^{13}C NMR spectral and microanalytical data and evaluated for their antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) using the disk diffusion method.

Keywords: antibacterial; carboxylic acids; Pinner–Dimroth rearrangement; POCl_3 ; pyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine.

1 Introduction

The synthesis of nitrogen- and oxygen-containing heterocyclic compounds has received considerable attention due to their presence in a large number of natural products and wide biological and pharmaceutical activities [1–4]. The presence of different biologically active heterocyclic moieties in a single molecule may unite the properties of all structural motifs and enhance the biological activity. Therefore, the synthesis of new polycyclic heterocyclic

compounds formed from a combination of two or more heterocyclic scaffolds has been a subject of increasing interest.

Pyrazoles, pyrans, and pyrimidines have occupied unique positions in the design and synthesis of novel biologically active agents due to their broad spectrum of activities. Literature reports had already established that certain pyrazoles exhibit significant biological properties such as anti-HCV [5], antipyretic [6], antiviral [7], anti-inflammatory [8], antiangiogenic [9], and antitumor [10] activities. The success of COX-2 inhibitors containing a pyrazole moiety [11, 12] has highlighted the importance of this motif in medicinal chemistry [13]. A number of these compounds are also used as potential inhibitors of P38 kinase [14], hMAO-B [15], and TNF- α [16]. On the other hand, structures containing pyrimidine and pyran scaffolds have important biological activities such as antimalarial [17], anti-inflammatory [18], antitumor [19, 20], antibacterial [21], anti-osteosarcoma [22], and anti-proliferative [23] properties. Recently, pyrimidines have been known as inhibitors of tyrosine kinase [24], cyclin-dependent kinase 4 [25], and epidermal growth factor receptor tyrosine kinase [26]. Furthermore, a number of pyrans have been employed as apoptosis-inducing agents [27] and constitute the structural unit of a series of natural products [28]. Because of the importance of these heterocycles we became interested in the synthesis of some new heterocyclic compounds containing pyrazole, pyran, and pyrimidine scaffolds.

Among various pyrazolopyranopyrimidine scaffolds, pyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidines have been of more interest because of reported interesting biological properties such as antifungal [29], antiviral [30], and antimicrobial [31] activities. A number of methods have already been reported for the synthesis of these compounds starting from pyranopyrazoles or by a one-pot multi-component reaction [29–34].

In view of the above-mentioned properties, and in continuation of our previous studies on the synthesis of heterocyclic compounds with potential biological activities [35–44], we report here the synthesis of new 7-alkyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one derivatives **3a–3f** by the reaction of

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Scheme 1: Synthesis of new compounds **3a–3f** with yields given in parentheses.

6-amino-1,4-dihydropyranopyrazole-5-carbonitriles **1a–1d** with aliphatic carboxylic acid **2a** or **2b** in the presence of phosphoryl chloride (POCl₃) (Scheme 1). To the authors' knowledge, compounds **3a–3f** are new and have not been reported in the literature. The antibacterial assay of the synthesized compounds was also evaluated.

2 Results and discussion

6-Amino-1,4-dihydropyranopyrazole-5-carbonitriles **1a–1d** were prepared according to methods cited in the literature [45, 46]. At first, in a model reaction, 6-amino-4-(4-methylphenyl)-3-phenyl-1,4-dihydropyranopyrazole-5-carbonitrile **1a** (1 mmol) was allowed to interact with acetic acid **2a** (1 mL) under reflux without the addition of any catalyst and solvent. After 3 h, the product **3a** was obtained only in 27% yield. Next, the reaction was conducted in acetic acid (1 mL) in the presence of POCl₃ (0.2 mL) as the well-known chlorinating agent. After refluxing the reaction mixture for 3 h, monitoring with thin-layer chromatography (TLC) showed that the reaction efficiently proceeded in these conditions leading to 91% yield of the product identified as 7-methyl-4-(4-methylphenyl)-3-phenyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-*d*]pyrimidin-5(1*H*)-one **3a**. Consequently, all subsequent processes for the synthesis of compounds **3b–3f** were carried out under similar conditions. The structural elucidation of the isolated products **3a–3f** was based upon spectral and microanalytical data. For example, the ¹H NMR spectrum of compound **3a** in [D₆]dimethyl sulfoxide (DMSO) demonstrated two singlets at δ = 2.15 and 2.29 ppm for two methyl groups, a sharp singlet at δ = 5.33 ppm belonging to the CH in pyran ring, the characteristic signals at δ = 6.96–7.58 ppm for the aromatic protons, and two single broad bands at δ = 12.47 and 12.95 ppm for two NH groups, which indicated the formation of the tricyclic compound

3a. The IR spectrum of **3a** was devoid of the CN absorption band at 2199 cm⁻¹ of the precursor **1a**, which shows the inclusion of the nitrile moiety in the cyclization process. Furthermore, this compound gave satisfactory ¹³C NMR spectrum and elemental analysis data corresponding to the molecular formula C₂₂H₁₈N₄O₂ (Experimental section and Supplementary Information).

On the basis of similar transformations [47–50], a plausible pathway for the formation of compounds **3a–3f** is proposed, as depicted in Scheme 2. Formation of these compounds probably proceeds via the tandem intramolecular Pinner–Dimroth rearrangement. Nucleophilic attack of the amino group in compounds **1a–1d** onto the activated carbonyl group of acyl chloride [I], which had been obtained by chlorination of carboxylic acid **2a** or **2b** with POCl₃, afforded the intermediate [II]. This intermediate undergoes an intramolecular Pinner reaction to give the oxazine intermediate [III]. The subsequent Dimroth rearrangement of the latter intermediate gave the final tricyclic products **3a–3f**. Attempts to isolate the proposed intermediates failed even after careful monitoring of the reactions.

Finally, we were interested in studying the role of the amount of POCl₃ in the reaction. For this purpose, the model reaction was again tested in the presence of excess POCl₃ (1 mL) under reflux. During monitoring of the reaction mixture by TLC, it was observed that a product, with different R_f's of that expected for compound **3a**, was forming. During workup and identification, it was established that *O*-acylation of the amidic group in the pyrimidine ring had occurred and the product 5-acetoxy-7-methyl-4-(4-methylphenyl)-3-phenyl-1,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-*d*]pyrimidine **5** was isolated. As is shown in Scheme 3, the formation of compound **5** is assumed to be the result of a cascade reaction by formation of the intermediate **4** followed by aromatic nucleophilic displacement with acetic acid. However, under

Scheme 2: Plausible mechanism for the formation of compounds 3a-3f.

Scheme 3: Formation of compound 5.

these conditions, attempts to isolate the intermediate **4** failed. Compound **5** can also be formed through the reaction of previously isolated compound **3a** with **2a** in the presence of excess POCl₃ under reflux. The ester hydrolysis of compound **5** in H₂O-EtOH containing NaOH under reflux afforded the product **3a** in high yield (Scheme 3).

The synthesized compounds **3a-3f** and **5** were screened for their antibacterial activity against reference strains of *Staphylococcus aureus* (*S. aureus*, American Type Culture Collection 6538), and *Staphylococcus epidermidis* (*S. epidermidis*, ATCC 12228) as Gram-positive bacteria and

Escherichia coli (*E. coli*, ATCC 8739) and *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC 9027) as Gram-negative bacteria. Gentamicin was used as the reference compound in the evaluation of antibacterial activity. None of the compounds had an antibacterial effect against Gram-negative bacteria, *E. coli*, and *P. aeruginosa*, in the primary screening test. The results revealed that compound **3f** has antibacterial activity against *S. aureus* and *S. epidermidis* in disks containing 3 mg per disk with inhibition zones of 11.00 ± 0.00 mm, while compound **3e** showed an antibacterial effect against only *S. aureus* bacterium in disk containing 3 mg per disk

with the inhibition zone of 13.00 ± 0.00 . The minimum inhibitory concentration (MIC) was 3 mg mL^{-1} for compound **3f** against tested Gram-positive bacteria and 2 mg mL^{-1} for compound **3e** against *S. aureus*. Growth inhibition of *S. aureus* and *S. epidermidis* was also observed against gentamicin ($10 \mu\text{g}$ per disk), which was 20.00 ± 0.00 and $22.00 \pm 0.00 \text{ mm}$, respectively. Thus, the antibacterial activity of compounds **3e** and **3f** against tested bacteria was satisfactory but less than those of the standard.

3 Conclusion

In summary, we have reported the synthesis of new 7-alkyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one derivatives **3a–3f** in high yields by the reaction of 6-amino-1,4-dihydropyrazolo[2,3-c]pyrazole-5-carbonitriles **1a–1d** with aliphatic carboxylic acid **2a** or **2b** in the presence of a limited amount of POCl_3 under reflux. Using excess amount of POCl_3 in a model reaction of **1a** with **2a**, the corresponding 5-acetoxy derivative **5**, instead of **3a**, was isolated. Compound **5**, which can also be formed through the reaction of **3a** with **2a** in the presence of excess POCl_3 under reflux, could be converted again to **3a** under hydrolysis conditions using NaOH in H_2O - EtOH at reflux temperature. An intramolecular Pinner–Dimroth rearrangement was also suggested for the formation of compounds **3a–3f**. All new products were characterized by FT-IR, ^1H , and ^{13}C NMR spectra and microanalytical data. Furthermore, the antibacterial assay of the synthesized compounds showed that two of them have satisfactory antibacterial effects on tested Gram-positive bacteria.

4 Experimental section

4.1 Chemicals and apparatus

All chemicals were purchased from Merck and Aldrich and used without additional purification. IR spectra were obtained with KBr pellets using a Bruker Tensor 27 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded with a Bruker 300 FT spectrometer at 300 and 75 MHz frequencies for ^1H and ^{13}C , respectively, using tetramethylsilane (TMS) as an internal standard. Elemental analysis was performed on a Thermo Finnigan Flash EA microanalyzer. Melting points were recorded on Stuart SMP3 melting point apparatus. Copies of the FT-IR and NMR spectra of compounds **3a–3f** and **5** are given as Supplementary Information available online.

4.2 General experimental procedure for the synthesis of compounds **3a–3f**

A mixture of 6-amino-1,4-dihydropyrazolo[2,3-c]pyrazole-5-carbonitriles **1a–1d** (1 mmol) and acetic acid **2a** or propionic acid **2b** (1 mL) in POCl_3 (0.2 mL) was heated under reflux for 3 h. Upon completion, the mixture was poured onto cold water (15 mL) and neutralized by 25% ammonia solution. Then, the precipitate was collected, washed with water and ethanol, and recrystallized from THF to afford compounds **3a–3f** in high yields.

4.3 Synthesis of compound **5**

4.3.1 Method A

A mixture of 6-amino-4-(4-methylphenyl)-3-phenyl-1,4-dihydropyrazolo[2,3-c]pyrazole-5-carbonitrile **1a** (1 mmol) and acetic acid **2a** (1 mL) in excess POCl_3 (1 mL) was heated under reflux for 3 h. After the completion of the reaction, the excess POCl_3 was evaporated *in vacuo*, and then ice water (15 mL) was added and the mixture was neutralized by 25% ammonia solution. The crude product was collected, washed with cold ethanol, and recrystallized from THF to afford 5-acetoxy-7-methyl-4-(4-methylphenyl)-3-phenyl-1,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine **5**.

4.3.2 Method B

A mixture of 7-methyl-4-(4-methylphenyl)-3-phenyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one **3a** (1 mmol) and acetic acid **2a** (1 mL) in excess POCl_3 (1 mL) was heated under reflux for 2 h. The reaction was monitored and the product was isolated as described above to give compound **5**.

4.4 Hydrolysis of compound **5**

5-Acetoxy-7-methyl-4-(4-methylphenyl)-3-phenyl-1,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine **5** (1 mmol) was dissolved in a mixture of H_2O (5 mL) and EtOH (5 mL) containing NaOH (1 mmol). The mixture was refluxed for 3 h. Upon completion, the reaction mixture was cooled to room temperature and neutralized with 1 N HCl . The crude product was collected, washed with H_2O (10 mL), and recrystallized from THF to afford compound **3a**.

4.5 Spectral and microanalytical data

4.5.1 7-Methyl-4-(4-methylphenyl)-3-phenyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one (3a)

M.p. > 350°C. Yield 91%. – IR (KBr disk): ν = 3423 (overlapped NH groups), 1658 (C=O) cm^{-1} . – ^1H NMR (300 MHz, $[\text{D}_6]\text{DMSO}$, 25°C, TMS): δ = 2.15 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 5.33 (s, 1H, pyran CH), 6.96 (d, 2H, J = 7.1 Hz, aromatic H), 7.10 (d, 2H, J = 7.1 Hz, aromatic H), 7.32 (t, 1H, J = 6.7 Hz, aromatic H), 7.40 (t, 2H, J = 7.0 Hz, aromatic H), 7.58 (d, 2H, J = 6.8 Hz, aromatic H), 12.47 (s br., 1H, NH), 12.95 (s br., 1H, NH). – ^{13}C NMR (75 MHz, $[\text{D}_6]\text{DMSO}$, 25°C, TMS): δ = 20.98, 21.33, 34.50, 99.79, 102.00, 126.48, 128.38, 128.78, 128.97, 129.25, 129.40, 135.77, 138.26, 141.86, 157.43, 158.67, 161.53, 163.05. – $\text{C}_{22}\text{H}_{18}\text{N}_4\text{O}_2$ (370.40): Calcd. C 71.34, H 4.90, N 15.13; found C 71.57, H 5.01, N 14.99.

4.5.2 7-Ethyl-4-(4-methylphenyl)-3-phenyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one (3b)

M.p. > 350°C. Yield 89%. – IR (KBr disk): ν = 3433 (overlapped NH groups), 1658 (C=O) cm^{-1} . – ^1H NMR (300 MHz, $[\text{D}_6]\text{DMSO}$, 25°C, TMS): δ = 1.19 (t, 3H, J = 7.5 Hz, CH_3), 2.16 (s, 3H, CH_3), 2.56 (q, 2H, J = 7.5 Hz, CH_2), 5.33 (s, 1H, pyran CH), 6.96 (d, 2H, J = 7.9 Hz, aromatic H), 7.10 (d, 2H, J = 7.9 Hz, aromatic H), 7.32 (t, 1H, J = 7.2 Hz, aromatic H), 7.40 (t, 2H, J = 7.1 Hz, aromatic H), 7.58 (d, 2H, J = 7.3 Hz, aromatic H), 12.46 (s br., 1H, NH), 12.95 (s br., 1H, NH). – ^{13}C NMR (75 MHz, $[\text{D}_6]\text{DMSO}$, 25°C, TMS): δ = 11.35, 20.98, 27.63, 34.52, 99.76, 102.19, 126.46, 128.40, 128.78, 128.98, 129.25, 129.40, 135.78, 138.25, 141.83, 157.47, 161.69, 162.63, 163.08. – $\text{C}_{23}\text{H}_{20}\text{N}_4\text{O}_2$ (384.43): Calcd. C 71.86, H 5.24, N 14.57; found C 72.12, H 5.15, N 14.46.

4.5.3 4-(4-Chlorophenyl)-7-ethyl-3-phenyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one (3c)

M.p. > 350°C. Yield 93%. – IR (KBr disk): ν = 3427 (overlapped NH groups), 1658 (C=O) cm^{-1} . – ^1H NMR (300 MHz, $[\text{D}_6]\text{DMSO}$, 25°C, TMS): δ = 1.19 (t, 3H, J = 7.5 Hz, CH_3), 2.57 (q, 2H, J = 7.5 Hz, CH_2), 5.40 (s, 1H, pyran CH), 7.17–7.26 (m, 4H, aromatic H), 7.32 (t, 1H, J = 7.3 Hz, aromatic H), 7.40 (t, 2H, J = 7.1 Hz, aromatic H), 7.56 (d, 2H, J = 7.2 Hz, aromatic H), 12.52 (s br., 1H, NH), 13.00 (s br., 1H, NH). – ^{13}C NMR (75 MHz, $[\text{D}_6]\text{DMSO}$, 25°C, TMS): δ = 11.31, 27.64,

34.49, 99.11, 101.45, 126.58, 128.28, 128.90, 129.26, 129.48, 130.46, 131.28, 138.59, 143.60, 157.19, 161.76, 162.97, 163.08. – $\text{C}_{22}\text{H}_{17}\text{ClN}_4\text{O}_2$ (404.85): Calcd. C 65.27, H 4.23, N 13.84; found C 65.08, H 4.35, N 13.97.

4.5.4 7-Methyl-1,3,4-triphenyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one (3d)

M.p. > 350°C. Yield 90%. – IR (KBr disk): ν = 3425 (NH), 1657 (C=O) cm^{-1} . – ^1H NMR (300 MHz, $[\text{D}_6]\text{DMSO}$, 25°C, TMS): δ = 2.32 (s, 3H, CH_3), 5.44 (s, 1H, pyran CH), 7.09 (t, 1H, J = 7.2 Hz, aromatic H), 7.19 (t, 2H, J = 7.6 Hz, aromatic H), 7.30–7.40 (m, 5H, aromatic H), 7.45 (t, 1H, J = 7.4 Hz, aromatic H), 7.63 (t, 2H, J = 7.9 Hz, aromatic H), 7.71 (d, 2H, J = 7.0 Hz, aromatic H), 7.92 (d, 2H, J = 7.8 Hz, aromatic H), 12.66 (s br., 1H, NH). – ^{13}C NMR (75 MHz, $[\text{D}_6]\text{DMSO}$, 25°C, TMS): δ = 21.26, 35.72, 99.71, 102.28, 121.65, 126.95, 127.02, 127.55, 128.41, 128.70, 128.91, 128.98, 129.98, 132.80, 137.86, 143.94, 146.60, 147.02, 159.38, 160.29, 162.98. – $\text{C}_{27}\text{H}_{20}\text{N}_4\text{O}_2$ (432.47): Calcd. C 74.98, H 4.66, N 12.95; found C 75.26, H 4.58, N 13.10.

4.5.5 4-(4-Chlorophenyl)-7-methyl-1,3-diphenyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one (3e)

M.p. > 350°C. Yield 93%. – IR (KBr disk): ν = 3430 (NH), 1659 (C=O) cm^{-1} . – ^1H NMR (300 MHz, $[\text{D}_6]\text{DMSO}$, 25°C, TMS): δ = 2.31 (s, 3H, CH_3), 5.47 (s, 1H, pyran CH), 7.22 (d, 2H, J = 8.3 Hz, aromatic H), 7.30–7.42 (m, 5H, aromatic H), 7.44 (d, 1H, J = 7.4 Hz, aromatic H), 7.61 (t, 2H, J = 7.7 Hz, aromatic H), 7.70 (d, 2H, J = 6.9 Hz, aromatic H), 7.92 (d, 2H, J = 7.9 Hz, aromatic H), 12.59 (s br., 1H, NH). – ^{13}C NMR (75 MHz, $[\text{D}_6]\text{DMSO}$, 25°C, TMS): δ = 21.26, 35.28, 99.15, 101.76, 121.58, 126.94, 127.52, 128.27, 128.73, 128.91, 129.93, 130.95, 131.55, 132.68, 137.85, 142.83, 146.53, 147.03, 159.57, 160.24, 162.98. – $\text{C}_{27}\text{H}_{19}\text{ClN}_4\text{O}_2$ (466.92): Calcd. C 69.45, H 4.10, N 12.00; found C 69.21, H 3.97, N 12.16.

4.5.6 7-Ethyl-1,3,4-triphenyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one (3f)

M.p. > 350°C. Yield 88%. – IR (KBr disk): ν = 3429 (NH), 1651 (C=O) cm^{-1} . – ^1H NMR (300 MHz, $[\text{D}_6]\text{DMSO}$, 25°C, TMS): δ = 1.19 (t, 3H, J = 7.5 Hz, CH_3), 2.58 (q, 2H, J = 7.5 Hz, CH_2), 5.45 (s, 1H, pyran CH), 7.10 (t, 1H, J = 7.2 Hz, aromatic H), 7.20 (t, 2H, J = 7.6 Hz, aromatic H), 7.28–7.41 (m, 5H,

aromatic H), 7.46 (t, 1H, $J=7.4$ Hz, aromatic H), 7.64 (t, 2H, $J=8.1$ Hz, aromatic H), 7.71 (d, 2H, $J=6.9$ Hz, aromatic H), 7.92 (d, 2H, $J=7.7$ Hz, aromatic H), 12.66 (s br., 1H, NH). – ^{13}C NMR (75 MHz, $[\text{D}_6]\text{DMSO}$, 25°C , TMS): $\delta=11.54, 27.73, 35.75, 99.68, 102.50, 121.87, 126.94, 127.04, 127.63, 128.42, 128.92, 129.04, 129.99, 132.82, 137.85, 143.93, 146.66, 146.99, 160.51, 162.79, 163.03, 163.37$. – $\text{C}_{28}\text{H}_{22}\text{N}_4\text{O}_2$ (446.50): Calcd. C 75.32, H 4.97, N 12.55; found C 75.59, H 5.05, N 12.42.

4.5.7 5-Acetoxy-7-methyl-4-(4-methylphenyl)-3-phenyl-1,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine (5)

M.p. $>350^\circ\text{C}$. Yield 88% in Method A and 92% in Method B. – IR (KBr disk): $\nu=3433$ (NH), 1743 ($\text{C}=\text{O}$) cm^{-1} . – ^1H NMR (300 MHz, $[\text{D}_6]\text{DMSO}$, 25°C , TMS): $\delta=2.15$ (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 4.96 (s, 1H, pyran CH), 6.63 (d, 2H, $J=7.7$ Hz, aromatic H), 6.85 (d, 2H, $J=7.7$ Hz, aromatic H), 7.17 (d, 2H, $J=7.0$ Hz, aromatic H), 7.30–7.43 (m, 3H, aromatic H), 12.61 (s br., 1H, NH). – ^{13}C NMR (75 MHz, $[\text{D}_6]\text{DMSO}$, 25°C , TMS): $\delta=21.00, 21.41, 23.64, 33.44, 101.18, 109.94, 127.64, 128.23, 128.90, 129.24, 129.38, 130.07, 135.84, 140.52, 142.04, 157.04, 159.42, 161.67, 162.80, 169.85$. – $\text{C}_{24}\text{H}_{20}\text{N}_4\text{O}_3$ (412.44): Calcd. C 69.89, H 4.89, N 13.58; found C 70.10, H 4.79, N 13.44.

4.6 Biological

The antibacterial activity of synthesized compounds was assessed by the disk diffusion method according to Clinical and Laboratory Standards Institute guidelines [51] against reference strains of *S. aureus* and *S. epidermidis* as Gram-positive and *E. coli* and *P. aeruginosa* as Gram-negative bacteria. Suspensions of the tested bacteria were prepared with turbidity equivalent to McFarland tube No. 0.5 (10^8 CFU mL^{-1}). Standard blank disks, containing 3 mg of the synthesized compounds, were prepared using DMSO as solvent and placed on a Mueller–Hinton agar pre-inoculated with 0.1 mL of bacterial suspension. Standard commercial disk of gentamicin (10 μg per disk) and disk containing DMSO were used as positive and negative controls, respectively. All disks were fully dried before the application on bacterial lawn. Plates were incubated for 18–24 h at 37°C , aerobically, and the diameter of inhibitory zones around the disks was measured in millimeter. All determinations were performed in triplicate, and the average value was reported as the inhibition zone (mean $\pm$ standard error of the mean). MIC was determined by agar dilution method using 24-well flat bottom tissue

culture plates. Different concentrations of compounds were prepared in melted Mueller–Hinton agar. After incubation, MIC was defined as the lowest concentration of compound that inhibited the growth of bacteria.

5 Supplementary information

Copies of the FT-IR, ^1H NMR, and ^{13}C NMR spectra as well as pictures of disks with the inhibition zones for the evaluation of the antibacterial activity are given as Supplementary information available online (<http://dx.doi.org/DOI:10.1515/znb-2018-0166>).

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References

- [1] Z. U. H. Khan, A. U. Khan, P. Wan, Y. Chen, D. Kong, S. Khan, K. Tahir, *Nat. Prod. Res.* **2015**, 29, 933.
- [2] S. Vazirimehr, A. Davoodnia, S. A. Beyramabadi, M. Nakhaei-Moghaddam, N. Tavakoli-Hoseini, *Z. Naturforsch.* **2017**, 72b, 481.
- [3] A. H. Halawa, A. M. Fouda, A. A. M. Al-Dies, A. M. El-Agrody, *Lett. Drug Des. Discovery* **2016**, 13, 77.
- [4] H. Akrami, M. Safavi, B. F. Mirjalili, M. Dehghani Ashkezari, F. Dadfar, N. Mohaghegh, S. Emami, F. Salehi, H. Nadri, S. K. Ardestani, L. Firoozpour, M. Khoobi, A. Foroumadi, *Eur. J. Med. Chem.* **2017**, 127, 128.
- [5] S. A. F. Rostom, M. A. Shalaby, M. A. El-Demellawy, *Eur. J. Med. Chem.* **2003**, 38, 959.
- [6] N. Uramaru, H. Shigematsu, A. Toda, R. Eyanagi, S. Kitamura, S. Ohta, *J. Med. Chem.* **2010**, 53, 8727.
- [7] N. A. Hamdy, W. M. El-Senousy, *Acta. Pol. Pharm.* **2013**, 70, 99.
- [8] H. M. Faidallah, S. A. F. Rostom, *Arch. Pharm.* **2017**, 350, 1700025.
- [9] E. A. A. El-Meguid, M. M. Ali, *Res. Chem. Intermed.* **2016**, 42, 1521.
- [10] J. W. Tessmann, J. Buss, K. R. Begnini, L. M. Berneira, F. R. Paula, C. M. P. de Pereira, T. Collares, F. K. Seixas, *Biomed. Pharmacother.* **2017**, 94, 37.
- [11] T. D. Penning, J. J. Talley, S. R. Bertenshaw, J. S. Carter, P. W. Collins, S. Docter, M. J. Graneto, L. F. Lee, J. W. Malecha, J. M. Miyashiro, *J. Med. Chem.* **1997**, 40, 1347.
- [12] G. Steinbach, P. M. Lynch, R. K. S. Phillips, M. H. Wallace, E. Hawk, G. B. Gordon, N. Wakabayashi, B. Saunders, Y. Shen, T. Fujimura, L.-K. Su, B. Levin, L. Godio, S. Patterson, M. A. Rodriguez-Bigas, S. L. Jester, K. L. King, M. Schumacher, J. Abbruzzese, R. N. DuBois, W. N. Hittelman, S. Zimmerman, J. W. Sherman, G. Kelloff, *New Engl. J. Med.* **2000**, 342, 1946.
- [13] B. Zhong, X. Cai, S. Chennamaneni, X. Yi, L. Liu, J. J. Pink, A. Dowlati, Y. Xu, A. Zhou, B. Su, *Eur. J. Med. Chem.* **2012**, 47, 432.

- [14] S. C. Abbot, R. J. Billedeau, N. J. Dewdney, T. Gabriel, D. M. Goldstein, K. L. McCaleb, M. Soth, T. A. Trejo-Martin, H. Zecic, *Heterocycles* **2009**, *78*, 2811.
- [15] R. Fioravanti, N. Desideri, M. Biava, L. Proietti Monaco, L. Grammatica, M. Yáñez, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5128.
- [16] M. Amina, N. K. Satti, N. M. Al Musayeib, S. Bani, T. Amna, *Biomed. Res.* **2017**, *28*, 4316.
- [17] K. Singh, T. Kaur, *MedChemComm* **2016**, *7*, 749.
- [18] E. P. D. S. Falcao, S. J. De Melo, R. M. Srivastava, M. T. Catanho, S. C. Nascimento, *Eur. J. Med. Chem.* **2006**, *41*, 276.
- [19] H. Atapour-Mashhad, Z. Tayaran-Najaran, A. Davoodnia, R. Moloudi, S. H. Mousavi, *Drug Chem. Toxicol.* **2011**, *34*, 271.
- [20] D. Kumar, P. Sharma, H. Singh, K. Nepali, G. K. Gupta, S. K. Jain, F. Ntie-Kang, *RSC Adv.* **2017**, *7*, 36977.
- [21] A. Saxena, L. Shastri, V. Sunagar, *Synth. Commun.* **2017**, *47*, 1570.
- [22] Q. Bi, W.-W. Ruan, L. Cao, Y.-J. Xu, *Biomed. Res. India* **2017**, *28*, 1290.
- [23] M. A. Mar'yasov, V. P. Sheverdov, V. V. Davydova, O. E. Nasakin, *Pharm. Chem. J.* **2017**, *50*, 798.
- [24] R. Chikhale, S. Thorat, R. K. Choudhary, N. Gadewal, P. Khedekar, *Bioorg. Chem.* **2018**, *77*, 84.
- [25] A. Pai, B. S. Jayashree, R. S. Jeyaprakash, S. G. Kini, R. Lobo, *Lat. Am. J. Pharm.* **2017**, *36*, 1568.
- [26] S. N. R. Mule, S. Nurbhasha, J. N. Kolla, S. S. Jadav, V. Jayaprakash, L. R. Bhavanam, H. B. Bollikolla, *Med. Chem. Res.* **2016**, *25*, 2534.
- [27] J.-L. Tian, G.-D. Yao, Y.-Y. Zhang, B. Lin, Y. Zhang, L.-Z. Li, X.-X. Huang, S.-J. Song, *Bioorg. Chem.* **2018**, *79*, 355.
- [28] C. D. Donner, M. Gill, L. M. Tewierik, *Molecules* **2004**, *9*, 498.
- [29] R. Qingyun, T. Xiaosong, H. Hongwu, *Curr. Org. Synth.* **2011**, *8*, 752.
- [30] A. H. Shamroukh, M. E. A. Zaki, E. M. H. Morsy, F. M. Abdel-Motti, F. M. E. Abdel-Megeid, *Arch. Pharm. Chem. Life Sci.* **2007**, *340*, 236.
- [31] A. H. Shamroukh, M. E. A. Zaki, E. M. H. Morsy, F. M. Abdel-Motti, F. M. E. Abdel-Megeid, *Arch. Pharm. Chem. Life Sci.* **2007**, *340*, 345.
- [32] M. E. A. Zaki, *Molecules* **1998**, *3*, 7179.
- [33] H. Hegde, C. Ahn, P. Waribam, N. S. Shetty, *J. Korean Chem. Soc.* **2018**, *62*, 87.
- [34] M. Bakherad, A. Keivanloo, M. Gholizadeh, R. Doosti, M. Javanmardi, *Res. Chem. Intermed.* **2017**, *43*, 1013.
- [35] M. Roshani, A. Davoodnia, M. Sh. Hedayat, M. Bakavoli, *Phosphorus Sulfur Silicon Relat. Elem.* **2004**, *179*, 1153.
- [36] A. Davoodnia, M. Bakavoli, M. Bashash, M. Roshani, R. Zhiani, *Turk. J. Chem.* **2007**, *31*, 599.
- [37] A. Davoodnia, R. Zhiani, N. Tavakoli-Hoseini, *Monatsh. Chem.* **2008**, *139*, 1405.
- [38] A. Davoodnia, M. Bakavoli, S. Mohseni, N. Tavakoli-Hoseini, *Monatsh. Chem.* **2008**, *139*, 963.
- [39] A. Davoodnia, M. Rahimizadeh, H. Atapour-Mashhad, N. Tavakoli-Hoseini, *Heteroat. Chem.* **2009**, *20*, 346.
- [40] M. Khashi, A. Davoodnia, J. Chamani, *Phosphorus Sulfur Silicon Relat. Elem.* **2014**, *189*, 839.
- [41] M. Khashi, A. Davoodnia, V. S. Prasada Rao Lingam, *Res. Chem. Intermed.* **2015**, *41*, 5731.
- [42] S. Vazirimehr, A. Davoodnia, M. Nakhaei-Moghaddam, N. Tavakoli-Hoseini, *Heterocycl. Commun.* **2017**, *23*, 65.
- [43] N. Hosseinasab, A. Davoodnia, F. Rostami-Charati, N. Tavakoli-Hoseini, A. Khojastehnezhad, *J. Heterocycl. Chem.* **2018**, *55*, 161.
- [44] M. Fattahi, A. Davoodnia, M. Pordel, S. A. Beyramabadi, N. Tavakoli-Hoseini, *Z. Naturforsch.* **2018**, *73b*, 557.
- [45] Y. Zou, Y. Hu, H. Liu, D.-Q. Shi, *J. Heterocycl. Chem.* **2013**, *50*, 1174.
- [46] I. S. A. Hafiz, M. A. M. A. Reheim, S. M. Baker, M. M. Ramiz, *J. Chem. Soc. Pak.* **2014**, *36*, 1133.
- [47] J. H. Tang, D. X. Shi, L. J. Zhang, Q. Zhang, J. R. Li, *Synth. Commun.* **2010**, *40*, 632.
- [48] H. Chai, J. Li, L. Yang, H. Lu, Z. Qi, D. Shi, *RSC Adv.* **2014**, *4*, 44811.
- [49] F. Khoramdelan, A. Davoodnia, M. R. Bozorgmehr, M. Ebrahimi, *Russ. J. Gen. Chem.* **2017**, *87*, 2961.
- [50] N. Karimi, A. Davoodnia, M. Pordel, *Heterocycl. Commun.* **2018**, *24*, 31.
- [51] Clinical and Laboratory Standards Institute, *Performance Standards for Antimicrobial Susceptibility Testing Twentieth Informational Supplement*, Clinical and Laboratory Standards Institute, Wayne, PA, CLSI document M100-S20. Available at: <http://www.clsi.org> (accessed 3 August 2018).

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