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An Efficient Synthesis of Novel 3-[(Heteroaryl-2-ylimino)-methyl]-4-hydroxy-chromen-2-ones and Analogue of Tetrazole Derivatives and Their Antibacterial Activity

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Abstract: Synthesis of a series of the substituted [(pyridinyl and pyrimidin-2-ylimino)-ethyl]-4-hydroxy-chromen-2-ones and their tetrazole derivates is presented in this study. By catalytic condensation of 4-hydroxy-3-acetylcoumarine 2 and 2-aminopyridines **3(a-d)**, 3-[(pyridin-2-ylimino)-ethyl]-4-hydroxy-chromen-2-ones **4(a-d)** are synthesized in high yield. During the condensation reaction of 2 and 4-amino-2,6-dihydroxypyrimidine **3e**, 3-[1-(2,6-Dihydroxy-pyrimidin-4-ylimino)-ethyl]-4-hydroxy-chromen-2-one **4e** as condensation products is synthesized. In following series, by cyclization reactions of compounds **4 (a-e)** with sodium azide, analogue 3-substituted pyridin-2-yl and pyrimidin-2-yl-5-methyl-2,5-dihydro-1H-tetrazol-5-yl]-4-hydroxy-chromen-2-one **5(a-e)** are synthesized the products. Structural characterization of the synthesized products is done on the basis of spectrometric data. Antibacterial activity of the compounds **4(a-e)** and **5(a-e)** against *S. aureus*, *E. coli* and *Klebsiella* was examined by measuring the inhibition zones around the disks marked with the corresponding products solution. The impact of substitutions in antimicrobial is also explored. Compounds with polar groups have shown significant antibacterial activity against these microorganisms.

Keywords: 4-hydroxy-chromen-2-one; condensation; cyclization; antibacterial; zones of inhibition

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1. Introduction

Coumarin (2H-Chromen-2-one) derivatives are a large class of oxygen-containing heterocyclic compounds with particular importance. Many coumarins are found as ingredients of the plant world [1]. Most of them play an important role in various life processes. Some coumarins are involved in various enzymatic reactions because their benzopyran-2-one structure enables them to interact with diversity of enzymes in organisms, thereby exhibiting wide potentiality as bioactive drugs [2]. Coumarin derivatives first were known as important agents for the treatment and prevention of various diseases [3]. Some naturally occurring coumarins such as novobiocin, chlorobiocin and coumermycin are an unprecedented class of antibiotics, showing expressed activity against Gram-positive bacteria [4]. Many of the compounds being in this class exhibit various biological activities, such as antimicrobial [5,6], antifungal [7,8] and antimalarial [9]. It was reported that some coumarin analogues also exhibited antioxidant [10,11], cytotoxic [12,13] and anti-tubercular activity [14]. A significant number of substituted coumarins also show sedative, analgesic, anticoagulant [15], anti-HIV [16,17], antidiabetic [18] and hepatoprotective activity [19]. The dynamics of the anticoagulant action of warfarin in the human body has also been investigated. Several warfarin analogues are currently used as rodenticides [20]. Some synthesized chromen-2-ones reported as anticancer agents [21,22]. For this reason, many

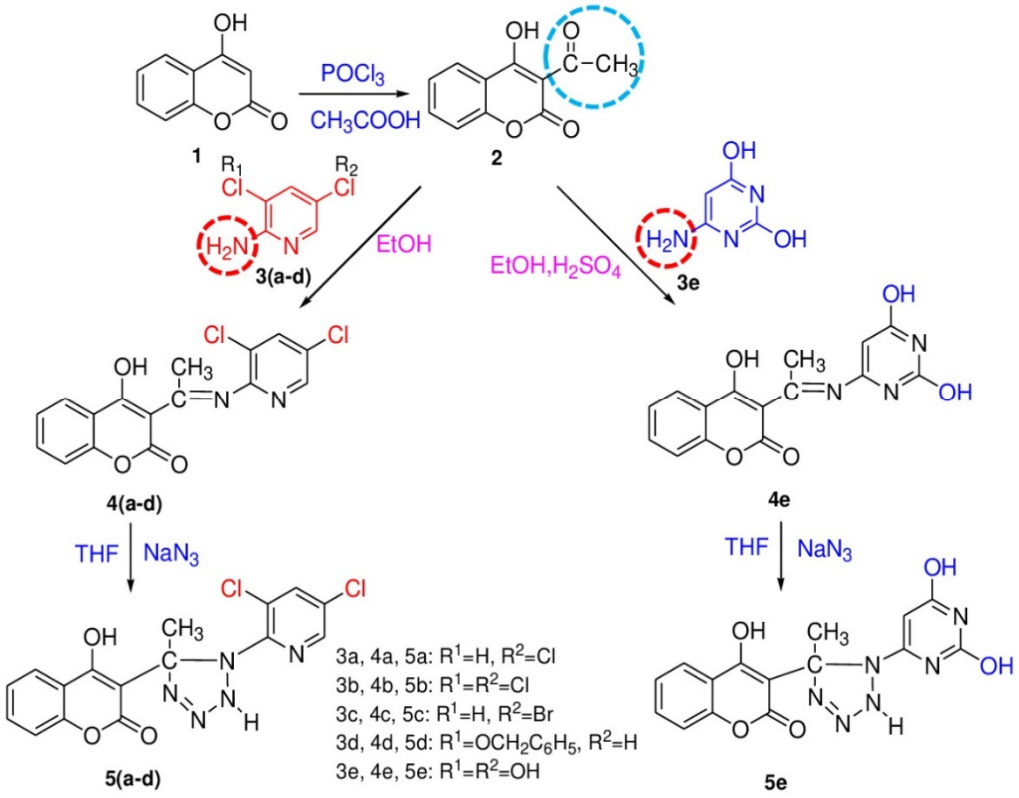
of coumarin derivatives have found widespread usage in pharmacies. On the other hand, tetrazoles and their heterocyclic analogues are useful drugs with a wide range of applications in medicine, agriculture and material sciences. Many tetrazoles are reported as important pharmacophores that demonstrate a wide range of pharmacological activities and are widely used in medicinal chemistry. Some tetrazole analogues as transporter inhibitors also are reported [23]. Many of them are reported to exhibit antihypertensive [24], anti-analgesic [25], anti-ulcer and anti-inflammatory activity [26]. The extraordinary biological importance of such derivatives on the basis of chromen-2-one has generated a constant interest in their synthesis and research. The biological activity of coumarin derivatives is conditioned by their structure. The type and potency of biological activity are conditioned by the presence of various substituents in the benzopyronic moiety. However, despite continuous efforts, the structure and biological activity relationship of these derivatives so far has not yet been sufficiently clarified. We have previously reported on the synthesis and antibacterial activity of some substituted chromen-2-one derivatives [27–29]. In continuation of our previous studies, in this paper our aim is to report about the synthesis of some new substituted 4-hydroxy-chromene-2-one derivatives through condensation reactions of 4-hydroxy-3-acetylcoumarin with substituted aminopyridines and aminopyrimidines, as well as some tetrazole analogues through cyclization reactions of these derivatives with sodium azide, for which so far there are no data from the literature. Following this work, their antibacterial activity against S. aureus, E. coli and Klebsiella also are screened. Based on the obtained results, we think that these compounds could find use as pharmaceutical products.

2. Results and Discussion

2.1. Chemistry

The *3-acetyl-4-hydroxycoumarin* is synthesized by direct acetylation of 4-hydroxycouma rin with acetic acid in the presence of Phosphorous oxychloride as a catalyst, according to the procedure described in the literature [30]. By condensation reaction3-acety 4 hydroxycoumarin 2 and 2-amino-5-chloropyridine 3a, *3-[1-(5-Chloro-pyridin-2-ylimino)-ethyl]-4-hydroxy-chromen-2-one* **4a** is synthesized. Condensation reaction of compound *2 and 2-amino-3,5-dichloropyridine* **3b** resulted in formation of *3-[1-(3,5-Dichloro-pyridin-2-ylimino)-ethyl]-4-hydroxy-chromen-2-one* **4b**, whereas by condensation of 2 and 2-amino-5-bromopyridine **3c** afforded *3-[1-(5-Bromo-pyridin-2-yl)-methyl]-4-hydroxy-chromen-2-one* **4c**. Reaction of 2 and 2-amino-3-benzyloxypyridine **3d** gave *3-[1-(3-Benzyloxy-pyridin-2-ylimino)-ethyl]-4-hydroxy-chromen-2-one* **4d**, whereas condensation of 2 and 4-amino-2-6-dihydroxypyrimidine **3e** afforded *3-[1-(2,6-Dihydroxy-pyrimidin-4-ylimino)-ethyl]-4-hydroxy-chromen-2-one* **4e**. In the last series of reactions, by cyclization of the product **4(a-e)** with sodium azide, respective tetrazolyl analogues, *3-[1-(5-Chloro-pyridin-2-yl)-5-methyl-2,5-dihydro-1H-tetrazol-5-yl]-4-hydroxy-chromen-2-one* **5a**, *3-[1-(3,5-Dichloro-pyridin-2-yl)-5-methyl-2,5-dihydro-1H-tetrazol-5-yl]-4-hydroxy-chromen-2-one* **5b**, *3-[1-(5-Bromo-pyridin-2-yl)-5-methyl-2,5-dihydro-1H-tetrazol-5-yl]-4-hydroxy-chromen-2-one* **5c**, *3-[1-(3-Benzyloxy-pyridin-2-yl)-5-methyl-2,5-dihydro-1H-tetrazol-5-yl]-4-hydroxy-chromen-2-one* **5d** and *3-[1-(2,6-dihydroxy-pyrimidin-4-yl)-5-methyl-2,5-dihydro-1H-tetrazol-5-yl]-4-hydroxychromen-2-one* **5e** are synthesized (Scheme 1).

The synthesized products **4(a-e)** and **5(a-e)** are characterized on the basis of spectrometric data and their elemental analysis. In the IR spectrum of the product 4a a broad absorption mode at 3450–3100 cm^{-1} appeared due to ν(OH)str. Vibrations, while an absorption signal at 3071.08 cm^{-1} resulted from aromatic ν(CH)str. vibrations. The absorption peaks appeared at 1730.57, 1669.25 and 1612.46 cm^{-1} due to stretching ν(C=O), ν(C=N) and ν(C=C) of aromatic system, while the sharp peak at 765.33 cm^{-1} is due to aromatic δ(C-H) oop. vibrations.



Scheme 1. Synthetic procedure to obtain the analogs of tetrazole derivatives.

In the ^{1}H-NMR spectrum, except characteristic peaks of aromatic protons, a singlet at 8.10 ppm and a doublet at 8.05 ppm appeared due to protons of pyridine moiety, whereas a singlet at 0.95 ppm resulted from methyl protons. The ^{13}C-NMR spectrum showed characteristic absorptions for 16 carbon atoms. On the other hand, elemental analysis supports the structure of compound **4a**.

In the IR spectrum of 4b, the broad absorption band at 3440–3100 cm^{-1} resulted due to ν(OH)stretching vibrations. The signal appeared at 3053.62 cm^{-1} corresponding to ν(CH)str. of the aromatic ring, while the sharp peak at 1734.85 cm^{-1} is characteristic for ν(C=O)str. vibrations. Absorption modes at 1651.02 and 1610.45 cm^{-1} appeared due to ν(C=N) str. and ν(C=C)str. of the aromatic system. The mode at 1093.23 cm^{-1} is also characteristic for stretching ν(C-O-C) vibrations of the lactonic moiety, while absorptions at 867.28 and 756.94 cm^{-1} appeared due to ν(C-Cl) str. and aromatic δ(C-H) oop. vibrations. On the other hand, except for characteristic signals for aromatic protons, a singlet at 1.1 ppm appeared due to methyl protons. ^{13}C-NMR spectrum also showed characteristic peaks for 16 carbon atoms and the elemental analysis is consistent with the composition of this compound.

In the IR spectrum of the compound 4d a broad absorption due to ν(OH)str. vibrations appeared at 3450–3100 cm^{-1} and peaks at 3067.46 and 2955.92 cm^{-1} are responsible for aromatic ν(CH)str. and methylene ν(CH)str. vibrations. The absorption modes at 1713.71, 1662.02 and 1610.56 cm^{-1} resulted from lactonic ν(C=O)str., ν(C=N)str. and ν(C=C)str. vibrations of this compound. A signal at 1043.07 cm^{-1} and another at 756.72 cm^{-1} are responsible for lactonic ν(C-O-C)str. and aromatic δ(C-H) oop. The ^{1}H-NMR spectrum of 4d showed aromatic protons appearing at 7.42 ppm as a triplet, at 6.99 ppm as a doublet and at 6.54 ppm as a doublet of a doublet. A proton singlet resulting from methyl protons appeared at 1.2 ppm. In the ^{13}C-NMR spectrum, methylene carbon appeared a signal at 75.2 ppm, whereas the elemental composition of product 4d is confirmed by elemental analysis.

IR spectrum of **4e** showed a broad band at 3360–3000 cm^{-1} which is responsible for ν(OH)str. vibrations. Signals at 1725.67, 1655.24 and 1584.73 cm^{-1} resulted from the ν(C=O)str., ν(C=N)str. and ν(C=C)str. vibrations, whereas characteristic modes at 1051.84 and 763.31 cm^{-1} appeared due to lactonic ν(C-O-C)str. and bending δ(CH) oop. vibrations of the aromatic ring. The ^{1}H-NMR spectrum of 4e, except doublets and doublet of doublets for aromatic protons, a singlet at 6.1 ppm for pyrimidine proton has appeared. The ^{13}C-NMR spectrum of **4e** showed characteristic signals for C-OH carbons appeared at 175.2 and 174.7 ppm, while C=N and C=O carbons displayed corresponding absorptions 167.4 and 160.5 ppm, respectively. Elemental analysis of this compound also confirmed their composition. Tetrazoles **5(a-e)** also are characterized on the basis of spectrometric data.

In the IR spectrum of compound **5a**, except others, the absorption peak at 3420.34 and 1669.84 cm^{-1} resulted from ν(NH)str. and ν(N=N)str. argue the presence of tetrazole ring. The ^{1}H-NMR spectrum of **5a** showed singlets at 8.2 ppm of N=C-H, at 3.2 ppm of N-H and another singlet at 1.3 ppm of methyl protons. The ^{13}C-NMR spectrum shows for 16 carbons, among which the characteristic ones are at 161.7 ppm from C-N of pyridine, a signal at 78.9 ppm from C-tetrazole, and the other signal at 20.2 ppm due to methyl carbon.

In the IR spectrum of product **5b** the characteristic signals appeared at 3391.65 and 1680.35 cm^{-1}, which resulted from ν(NH) and ν(N=N)str. vibrations of the tetrazole ring, respectively. Tetrazole residue was also confirmed by the presence of a singlet at 3.5 ppm from tetrazole. Another singlet at 1.4 ppm resulted from methyl protons. Tetrazole moiety also is confirmed from characteristic modes at 76.8 ppm in the ^{13}C-NMR spectrum, whereas absorption mode at 21,5 ppm resulted from methyl carbon.

Compound **5c** exhibited absorption mode from vibrations ν(N=N)str. at 1650.31 cm^{-1}, while in the ^{1}H-NMR spectrum the singlets at 2.1 ppm and at 1.4 ppm appeared resulting from tetrazole and methyl protons. The ^{13}C-NMR spectrum showed the characteristic signal for C-tetrazole at 76.1 ppm, as well as the characteristic signals for 15 other aromatic carbons.

In the IR spectrum of the **5d** compound, except aromatic ν(CH)str. vibrations at 3079.55 cm^{-1}, the ν(CH)str. mode of the methyl group at 2947.48 cm^{-1} are also observed, while a signal displayed at 1665.82 cm^{-1} resulted from ν(N=N)str. vibrations. The ^{1}H-NMR spectrum shows the corresponding a signal at 2.2 ppm of the tetrazole proton, as well as a singlet at 1.4 ppm from the CH_3 protons. Absorption mode at 78.8 ppm in the ^{13}C-NMR spectrum argues the presence of tetrazole moiety.

A broad band in the IR spectrum of **5e** displayed at 3420–3100 cm^{-1} has resulted from ν(OH)str. vibrations, while the peak at 3450.05 appeared due to ν(NH)str. vibrations. On the other hand, absorption mode at 1665.82 cm^{-1} reflects the ν(N=N)str. vibrations of the tetrazole ring. In support of confirmation of tetrazole ring is also the singlet displayed at 2.2 ppm in the ^{1}H-NMR spectrum, as well as the signal at 78.8 ppm in the ^{13}C-NMR spectrum. The structure of compounds **5(a-e)** has also been confirmed by its elementary analysis (Table 1).

Table 1. Physical properties of compounds **4(a-e)** and **5(a-e)** and their elemental analysis.

Nr	Molecular Formulas	Molecular Mass	Elemental Analysis % Calc/Found	mp/°C	Yield %
4a	$C_{16}H_{11}N_2O_3Cl$	314.71	(C-61.06; H-3.52; N-8.90; O-15.25; Cl-11.26) (C-61.02; H-3.51; N-8.85; Cl: 11.28)	204–206	66
4b	$C_{16}H_{10}N_2O_3Cl_2$	349.15	(C-55.03; H-2.89; N-8.02; O-13.75; Cl-20.31) (C-49.99; H-2.86; N-8.04; Cl-20.27)	212–214	72
4c	$C_{16}H_{11}N_2O_3Br$	359.16	(C-53.50; H-3.09; N-7.80; O-13.36; Br-22.25) (C-53.48; H-3.12; N-7.76; Br-22.21)	225–227	75
4d	$C_{23}H_{18}N_2O_4$	385.38	(C-71.68; H-4.70; N-7.27; O-16.60) (C-71.72; H-4.67; N-7.24)	305–307	38

Table 1. *Cont.*

Nr	Molecular Formulas	Molecular Mass	Elemental Analysis % Calc/Found	mp/°C	Yield %
4e	$C_{15}H_{11}N_3O_5$	313.26	(C-57.51; H-3.54; N-13.41; O-25.54) (C-57.47; H-3.56; N-13.38)	302–304	62
5a	$C_{16}H_{12}N_5O_3Cl$	357.74	(C-53.71; H-3.38; N-19.57; O-13.42; Cl-9.91) (C-53.66; H-3.42; N-19.60; Cl-9.87)	211–213	65
5b	$C_{16}H_{11}N_5O_3Cl_2$	392.18	(C-48.99; H-2.83; N-17.86; O-12.24; Cl-18.08) (C-49.03; H-2.79; N-17.84; Cl-18.03)	208–211	73
5c	$C_{16}H_{12}N_5O_3Br$	402.19	(C-47.78; H-3.01; N-17.41; O-11.93; Br-19.87) (C-47.82; H-2.98; N-17.38; Br-19.90)	120–122	70
5d	$C_{23}H_{19}N_5O_4$	429.41	(C-64.33; H-4.46; N-16.31; O-14.19) (C-64.29; H-4.44; N-16.28)	224–226	67
5e	$C_{15}H_{12}N_6O_5$	356.29	(C-50.56; H-3.39; N-23.59; O-22.45) (C-50.58; H-3.43; N-23.62)	223–225	45

2.2. *Antibacterial Activity of the Products* **4(a-e)** *and* **5(a-e)**

In the following, this study includes results of antibacterial activity of products **4(a-e)** and **5(a-e)**. Our research has focused on testing the antibacterial activity of these compounds against the bacteria *S. aureus*, *E. coli* and *Klebsiella*. Screening of antibacterial activity was done on the basis of Kirby Bayer method [30–32], by measuring the zones of inhibition around the standard discs that have previously been marked with solutions of the products in N,N-DMF with concentrations of 2 mg/mL, 4 mg/mL and 6 mg/mL. The bacterial cultures have incubated for 48 h at 37 °C. The biological evaluation also is based on previous studies [33,34].

The results in Figures 1–3 show that compounds of series 4 and 5 showed exhibited considerable activity against these bacteria. Compounds **4b** and **5e** expressed the significant activity against *S. aureus*, while **5b** and **4e** were most active against *E. coli*, and **5b** and **5e** were more active against *Klebsiella*. These compounds expressed both bactericide and bacteriostatic activity against *S. aureus*. Furthermore, antibacterial activity against *E. coli* and *Klebsiella* is displayed on a large-scale as bactericide activity. In general, the bacteriostatic activity is exhibited in a large range (+4 mm), whereas bactericide activity showed in small diameter. The influence of polar groups was quite pronounced. while tetrazole moiety appeared low impact on antimicrobial activity. The impact of chlorine is particularly noted which has affected the increase of antibacterial activity. The pyrimidine hydroxyl groups **5e** has shown a significant impact on the range of antibacterial activity against the bacterial culture *Klebsiella*. Based on these results we consider that antibacterial activity may result as a consequence of the involvement of these compounds in enzymatic reactions during bacterial reproduction. It is believed that these compounds may have caused enzymatic inhibition by binding to the active center of enzymes that participates in the cellular wall construction of these bacteria. However, the mechanism of inhibition is not yet fully studied. In general, antimicrobial activity increased by increasing the concentration of the products.

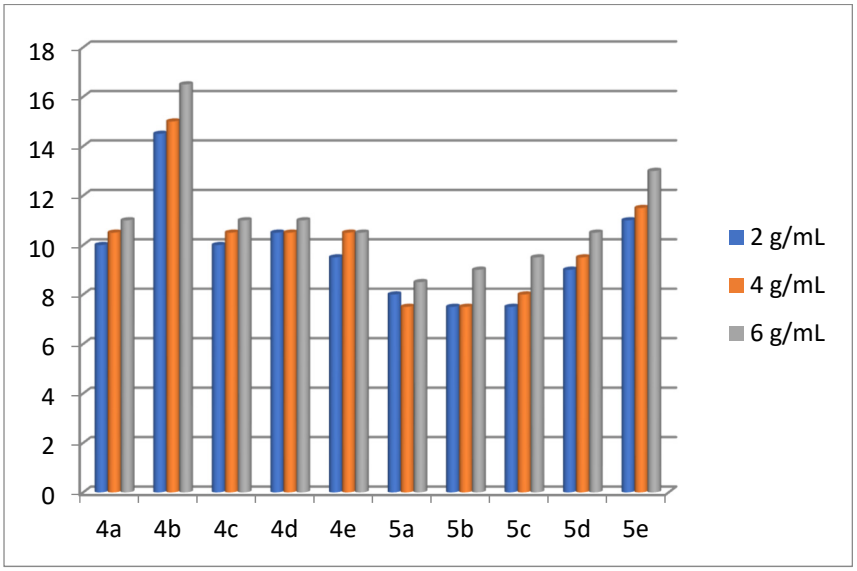


Figure 1. Graphical presentation of inhibition zone diameter (mm) against *S. aureus*.

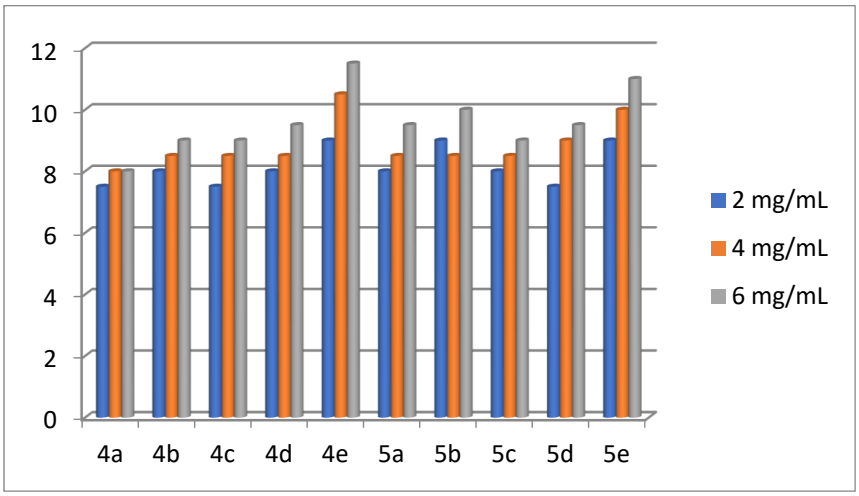


Figure 2. Graphical presentation of inhibition zone diameter (mm) against *E. coli*.

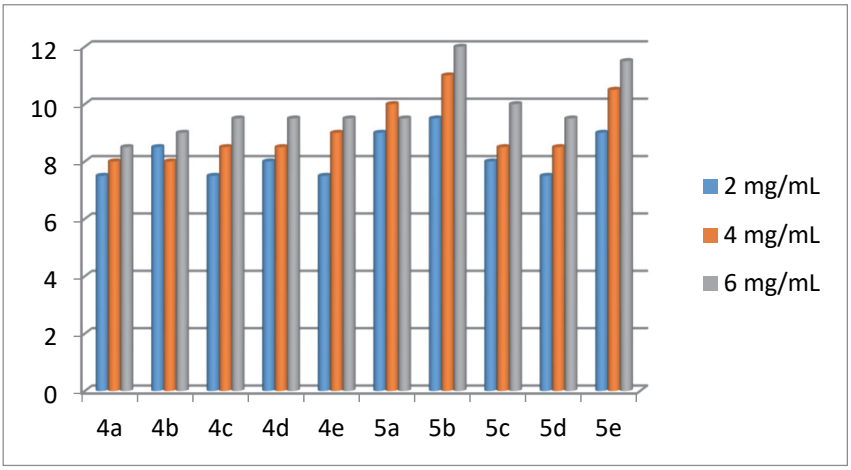


Figure 3. Graphical presentation of inhibition zone diameter (mm) against *Klebsiella*.

3. Material and Methods

3.1. Materials and Basic Measurements

The new compounds are synthesized under refluxing conditions using commercial reagents of Aldrich company as precursors with pro-analysis purity. Reaction flow was monitored by TLC using Merck Kieselgel-60 (F-254) as the stationary phase and mixture of benzene, toluene, glacial acetic acid (*v*/*v*/*v*, 80:10:10) as mobile phase, visualization with Uv lamp. Purification of the products is done by recrystallization from ethanol. Melting points of the compounds were determined in a paraffin oil bath with an open capillary tube and are uncorrected. IR spectra are recorded in KBr discs on Shimadzu 8400xFT-IR spectrometer with 4cm^{-1} resolution. ^{1}H-NMR and ^{13}C-NMR spectra are recorded in DMSO-d_6 on UNITYplus-500"NMR 1" spectrometer. Chemical shifts were reported in ppm down field from TMS as internal standard (δ0.00). Antibacterial activity of the compounds was examined on the basis of Kirby-Bayer's method, using standard discs (d = 5.0 mm, maximum capacity10 pg). Standard discs are previously saturated with compound solutionsin N,N-DMF concentrations 2 mg/mL, 4 mg/mL and 6 mg/mL.

3.2. 3-[Pyridin-2-ylimino)-ethyl]-4-hydroxy-chromen-2-ones **4(a-e)**, *General Procedure*

3-Acetyl-4-hydroxycoumarin 2 (0.82 g, 0.004 mol) is dissolved in 15 mL ethanol and 2–3 drops of sulphuric acid is added. The mixture is stirred at room temperature and an equimolar amount of corresponding 2-aminopyridine **3a-d** or 4-aminopyrimidine **3e** (0.004 mol dissolved in 10 mL ethanol) is added to the mixture in small portions, then the mixture is refluxed for 6–8 h. After cooling, the mixture is concentrated in the rotary evaporator, the residue is cooled and the crystalline product is filtered off under vacuum and washed with 2 × 2 mL of ethanol. The crystalline product is dried and recrystallized from ethanol.

3.3. 3-[1-(5-Chloro-pyridin-2-ylimino)-ethyl]-4-hydroxy-chromen-2-one

4a: mp = 204–206 °C reflux time 6 h, R= 66%. IR (KBr disc, cm^{-1}): 3450–3100, 3071.08, 1730.57, 1669.25, 1612.46, 1073.94, 765.33. ^{1}H-NMR; (δ, ppm) 8.10 (s, 1H Pyr), 8.05 (d, 1H, Pyr), 7.76 (d, 1H, Ar), 7.64–7.61 (t, 1H, Ar), 7.40–7.36 (m, 2H, Ar), 7.12 -7.07 (t, 1H, Ar), 0.95 (s, 3H, CH_3). ^{13}C-NMR; (δ, ppm) 170.6 (C-2, Pyr.), 163.6 (C=N), 163.2 (C=N), 162.4(C=O), 159,5 (C-OH), 151.4, 149.5, 134.6, 128.8, 128.2, 125,7, 125.2, 124.8, 123.6, 121.9, 11.6.

3.4. 3-[1-(3,5-Dichloro-pyridin-2-ylimino)-ethyl]-4-hydroxy-chromen-2-one

4b: mp = 212–214 °C reflux time 6 h, R = 72%. IR (KBr disc, cm^{-1}): 3440–3100, 3053.62, 1734.85, 1651.02, 1610.45, 1093.23, 867.28, 756.94. ^{1}H-NMR; (δ, ppm), 8.7 (s, 1H, Pyr), 8.1 (s, 1H, Pyr), 7.5–7.3 (m, 2H, Ar), 7.2–7.0 (m, 2H, Ar), 1.1 (s, CH_3). ^{13}C-NMR; (δ, ppm) 167.9 (C-2 Pyr), 163.6 (C=N), 160.8 (C=O), 151.2, 147.8, 134.5, 128.2, 127,8, 126,4, 125.2, 124.4, 123.0, 121.5, 120.2, 111.3, 11.2.

3.5. 3-[1-(5-Bromo-pyridin-2-ylimino)-ethyl]-4-hydroxy-chromen-2-one

4c: mp = 125–127 °C reflux time 7 h, R = 75%. IR (KBr disc, cm^{-1}): 3500–3110, 3068.35, 1739.44, 1683.37, 1022.41. ^{1}H-NMR; (δ, ppm), 8.75–8.8.73 (s, 1H Pyr), 8.18–8.14 (d, 1H, Pyr), 7.74–7.71 (t, 1H, Ar), 7.68–7.66 (d, 1H, Ar), 7.41–7.37 (m, 2H, Ar), 7.24 -7.21 (t, 1H, Ar), 1.20 (s, 3H, CH_3). $_{13}$C-NMR; (δ, ppm) 169.4 (C-2, Pyr.), 164.2 (C=N), 163.0 (C=N), 161.2 (C=O), 152.5, 150.1, 149.3, 137.0, 129.6, 128.2, 126.7, 125.3, 124.1, 123.5, 120.8, 10.8.

3.6. 3-[1-(3-Benzyloxy-pyridin-2-ylimino)-ethyl]-4-hydroxy-chromen-2-one

4d: mp = 305–307 °C reflux time 6 h, R = 38%. IR (KBr disc, cm^{-1}): 3450–3100, 3067.46, 2955.92, 1713.71, 1662.02, 1610.56, 1043.07, 756.72. ^{1}H-NMR; (δ, ppm), 8.3 (s, 1H, N=C-H, Pyr.), 7.74–7.73 (d, 1H), 7.68–7.67 (d, 1H), 7.42–7.41 (t, 1H), 6.99–6.97 (d, 1H), 6.87–6.70 (m), 6.54–6.51 (dd, 1H), 5.06 (s, 2H), 1.2 (s, 3H, CH_3). ^{13}C-NMR; (δ, ppm) 164.5 (C=N), 161.8 (C=O), 156.6, 151.2, 150,5, 146.7, 139.5, 129.8, 128.2, 127.6, 127,1, 126.7, 126,2, 125.9, 125.3, 123.4, 122.5, 121.6, 120.3, 110.6, 75.2, (O-CH_2).

3.7. 3-[1-(2,6-Dihydroxy-pyrimidin-4-ylimino)-ethyl]-chromen-2-one

4e: mp =302–304 °C reflux time 8 h, R = 62%. IR (KBr disc, cm^{-1}):3360–3000, 1725.67, 1655.24, 1584.73, 1051.84, 763.31. ^{1}H-NMR; (δ, ppm), 7.75 (d, 1H, Ar), 7.54 (dd, 1H), 7.24 (dd, 1H), 7.18 (d, 1H, Ar), 6.1 (s, 1H, Pyrim), 5.4 (s, 2H), 1.12 (s, 3H, CH_3). 13C-NMR; (δ, ppm) 175.2 (C-OH), 174.7 (C-OH), 167.4 (C-N), 165.2, 162.8, 160.5 (C=O), 148.6, 149.6, 129.7, 128.5, 126.5, 125.9, 121.6, 92.6, 12.4 (CH_3).

3.8. 3-[Pyridin-2-yl)-5-methyl-2,5-dihydro-1H-tetrazol-5-yl]-4-hydroxy-chromen-2-ones **5(a-e)**, *general procedure*

The mixture of compound **4(a-e)** (0.002 mol) and 0.2 g (0.003 mol) of sodium azide dissolved in 15 mL tetrahydrofuran refluxed for 6–8 h. The mixture is cooled in an ice bath and concentrated, then the crystalline product is filtered off under reduced pressure, then washed with 2 × 1 mL of ethanol and dried in the air. Compounds **5(a-e)** were purified by recrystallization from ethanol.

3.9. 3-[1-(5-Chloro-pyridin-2-yl)-5-methyl-2,5-dihydro-1H-tetrazol-5-yl]-4-hydroxy-chromen-2-one

5a:mp = 211–213 °C, R = 65%, IR (KBr disc, cm^{-1}): 3450–3100, 3420.34, 3051.48, 2983.65, 1708,77, 1669.84, 1612.27, 1603.57, 1082.38, 897.25, 758.84. ^{1}H-NMR; (δ, ppm), 8.2 (s, 1H, N = C-H), 7.6–7.4 (m, 3H, Ar), 7.3–7.2 (m, 2H, Pyr), 3.2 (s, ^{1}H), 1.3 (s, 3H, CH_3). ^{13}C-NMR; (δ, ppm) 166.1 C-OH, 162.8(C=O), 161.7 (C-N, Pyr.), 151,4, 148.3, 145.7, 139,2, 129.2, 128.3, 127.2, 126.6, 122.5, 121.0, 95.2, 78.9 (C-Tetraz.), 20.2 (CH_3). Please see Figure S2.

3.10. 3-[1-(3,5-Dichloro-pyridin-2-yl)-5-methyl-2,5-dihydro-1H-tetrazol-5-yl]-4-hydroxy-chromen-2-one

5b: mp = 208–211 °C, R = 73%, IR (KBr disc, cm^{-1}): 3420–3080, 3391.65, 3072.93, 2982.75, 1708,68, 1680.35, 1624.59, 1607.2, 1054.64, 912.42, 760.36. ^{1}H-NMR; (δ, ppm), 8.2 (s, 1H, N=C-H), 7.8 (s, 1H), 7.5–7.2 (m, 4H, Ar), 3.5, 1.4 (s, 3H, CH_3). ^{13}C-NMR; (δ, ppm) 163.9 (C-OH), 161.2 (C=O), 157.4 (C-N, Pyr.), 149,8, 138,3, 129.5, 128.4, 127,3, 127.0, 126,3, 125.2, 123.7, 122.7, 108.4, 76.8 (C-Tetraz.), 21.5 (CH_3). Please see Figure S3.

3.11. 3-[1-(5-Bromo-pyridin-2-yl)-methyl-2,5-dihydro-1H-tetrazol-5-yl]-4-hydroxy-chromen-2-one

5c: mp = 120–122 °C, R = 70%, IR (KBr disc, cm^{-1}): 3450–3100, 3068.38, 2974.38, 1711,46, 1650.31, 1602.26, 1600.31, 1049.39, 796.82, 764.32. ^{1}H-NMR; (δ, ppm) 8.2 (s, 1H, N=C-H), 7.8 (d, 1H), 7.5–7.7 (m, 3H, Ar), 7.1 (d, 1H), 3.5 (s, 1H). 1.4 (s, 3H,), ^{13}C-NMR; (δ, ppm) 166.4 (C-OH), 162.1 (C=O), 160.5 (C-N, Pyr.), 148.8, 145.6, 137,8, 129.2, 128.6, 127,3, 127.0, 125,1, 122.7, 120.8, 109.6, 76.1 (C-Tetraz.), 21.3 (CH_3).

3.12. 3-[1-(3-Benzyloxy-pyridin-2-yl)-5-methyl-2,5-dihydro-1H-tetrazol-5-yl]-4-hydroxy-chromen-2-one

5d: mp = 224–226 °C, R = 67%, IR (KBr disc, cm^{-1}): 3420–3100, 3055.28, 2957.72, 1711.34, 1694,32, 1616.38, 1233.86, 1120.1, 762.53. ^{1}H-NMR; (δ, ppm)7.9 (d, 1H), 7.5–7.8 (m, 4H, Ar), 7.3–7.1 (m, 5H), 7.0 (d, 1H), 6.8 (dd, 1H), 5.4 (s, 2H, OCH_2), 3.4 (s, 1H,), 1.4 (s, 3H, CH_3). ^{13}C-NMR; (δ, ppm) 165.4 (C-OH), 163.8 (C=O), 152.4 (C-N, Pyr.), 148.3, 144.8, 143.4, 131.5, 130.4, 129.3, 128,7, 127,6, 127.0, 126.5, 126.0, 125.6, 124.2, 122.7, 122.2, 120.8, 109.6, 96.5, 77.4 (C-Tetraz.), 19.8 (CH_3). Please see Figure S4.

3.13. 3-[1-(2,6-Dihydroxy-pyrimidin-4-yl)-5-methyl-2,5-dihydro-1H-tetrazol-5-yl]-4-hydroxy-chromen-2-one

5e: mp = 223–225 °C, R = 45%, IR (KBr disc, cm^{-1}): 3450.05, 3420–3100, 3079.55, 2947.48, 1727.86, 1665.82, 1660.24, 1054.26, 986.29, 761.25. ^{1}H-NMR; (δ, ppm)7.6–7.4 (m, 2H, Ar), 7.2–7.0 (m, 2H, Ar), 5.2 (s, 2H,), 4.5 (s, 1H), 3.5 (s, 1H), 2.2 (s, 1H), 1.4 (s, 3H, CH_3). ^{13}C-NMR; (δ, ppm) 172.6, 165.5, 163.4 (C=O), 161.0 (C-N, Pyr.), 151.2, 129.2, 128.8, 127.3, 126.2, 125.5, 122.6, 121.2, 102,4, 78.8 (C-Tetraz.), 21.3 (CH_3). Please see Figure S5.

Antibacterial activity is screened by measuring the zones of inhibition around the standard discs saturated with solutions of the products in N,N-DMF with concentrations of 2 mg/mL, 4 mg/mL and 6 mg/mL, incubation at 37 °C for 48 h.

4. Conclusions

Novel-substituted-[(pyridinyl-and-[(pyrimidin-2-ylimino)-ethyl]-4-hydroxy-chromen-2-ones **4(a-e)** and respective 3-substituted pyridin-2-yl and pyrimidin-2-yl-5-methyl-2,5-dihydro-1H-tetrazol-5-yl]-4-hydroxy-chromen-2-one **5(a-e)** are synthesized in the moderate and high yield. The synthesis was done stepwise with different reactants in different condition. Synthesis is carried off by refluxing the reaction mixture for a given period. Synthesized compounds were characterized on the basis of their spectrometric data. The impact of polar substituents in antibacterial activity was significant, so compounds with chlorine and hydroxyl substituents showed expressed activity against *S. aureus*, *E. coli*, and *Klebsiella* bacteria. Antibacterial activity is shown to be proportional to the concentration of these compounds. The compounds 4e and 5e have shown significant activity at *E. coli*. The compounds **5b** and **5e** showed very good antibacterial activity at Klebsiella, whereas compound 4b has shown good activity at *S. aureus*.

Supplementary Materials: The following are available online, all synthesized compounds are characterization by spectral methods, IR and NMR. Graphical presentation of inhibition zone diameter (mm) against *S. aureus.* Graphical presentation of inhibition zone diameter (mm) against *E. coli.* Graphical presentation of inhibition zone diameter (mm) against Klebsiella. Melting points are shown melting points for synthesized compounds **4(a-e)** and **5(a-e)**. Figure S1. **2.** 3-Acetyl-4-hydroxy-chromen-2-one. Figure S2. **5a.** 3-[1-(5-Chloro-pyridin-2-yl)-5-methyl-2,5-dihydro-1H-tetrazol-5-yl]-4-hydroxy-chromen-2-one. Figure S3. **5b.** 3-[1-(3,5-Dichloro-pyridin-2-yl)-5-methyl-2,5-dihydro-1H-tetrazol-5-yl]-4-hydroxy-chromen-2-one. Figure S4. **5d.** 3-[1-(3-Benzyloxy-pyridin-2-yl)-5-methyl-2,5-dihydro-1H-tetrazol-5-yl]-4-hydroxy-chromen-2-one. Figure S5. **5e.** 3-[1-(2,6-Dihydroxy-pyrimidin-4-yl)-5-methyl-2,5-dihydro-1H-tetrazol-5-yl]-4-hydroxy-chromen-2-one.

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