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EFFICIENT SYNTHESIS AND ANTIBACTERIAL EVALUATION OF A SERIES OF PYRAZOLYLBISCOUMARIN AND PYRAZOLYLXANTHENEDIONE DERIVATIVES

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Efficient synthesis of a series of pyrazolylbiscoumarin (**4a – 4e**) and pyrazolylxanthenedione derivatives (**5a – 5e**) is described. The structures of the synthesized compounds were confirmed by their spectral data (IR, ^{1}HNMR and ^{13}CNMR). All the synthesized compounds **4** and **5** were screened for their antibacterial activity against Gram-positive bacteria *S. aureus* and Gram-negative bacteria *K. pneumoniae*. The tested compounds exhibited varying degree of antibacterial activity, showing the inhibition zone size ranging from 6 to 21 mm.

Keywords: biscoumarin, xanthenedione, 1H-pyrazole-4-carbaldehyde, antibacterial evaluation.

INTRODUCTION

During the past few decades, the biological activity of coumarins and their derivatives have drawn much research interest in their synthesis and biological studies. Functionalized coumarins play a prominent role in medicinal chemistry and have been intensively used as scaffolds for drug development. A number of biological effects such as antimicrobial, antiviral, anticancer, anti-inflammatory, and anticoagulant have been attributed to 4-hydroxycoumarins [1]. Biscoumarin derivatives are essential for several biological and pharmaceutical applications such as anticoagulants, rodenticides, anti-inflammatory, urease inhibitors, and HIV-1 integrase inhibition [2 – 7]. In addition, pyrazole derivatives are also of particular interest because of their broad pharmacological profile [8 – 14].

Therefore, integration of these two moieties into pharmacy can be expected to yield compounds of potential medicinal interest. These observations prompted us to synthesize certain pyrazolylbiscoumarin derivatives (**4a – 4e)**. Synthesis of the title compounds was achieved by simple acid-catalyzed condensation reaction of 4-hydroxycoumarin and the corresponding 1H-pyrazole-4-carbaldehydes (**3a – 3e)**. In addition, xanthenedione derivatives have attracted considerable interest in recent years because of their important biological properties, including antibacterial [15], antiviral [16], and anti-inflammatory [17], as well as their action as positive allosteric modulators of metabotropic receptors[18] and potent nonpeptide inhibitors of recombinant human calpain I [19], and the efficiency in photodynamic therapy [20]. Keeping these points in mind, we extended our strategy toward the synthesis of some new pyrazolylxanthenedione derivatives (**5a – 5e**). All the synthesized compounds **4** and **5** were screened for their antibacterial activity against Gram-positive bacteria *S. aureus* and Gram-negative bacteria *K. pneumoniae.* Most of these compounds exhibited varying antibacterial properties, showing the inhibition zone size ranging from 6 to 21mm.

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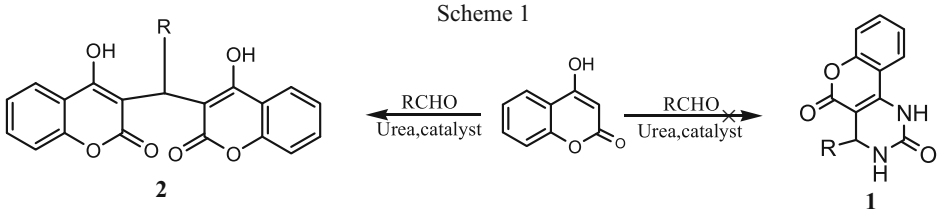
1. RESULTS AND DISCUSSION

1.1. Chemistry

Fused pyrimidocoumarin is the reported framework with a number of pharmacological activities. We initially started our present study by using the well-known multi-component Biginelli reaction in order to synthesize benzopyranopyrimidines and tried to synthesize pyrimidocoumarins (**1**) via multicomponent Bignelli condensation of urea, benzaldehyde, and 4-hydroxycoumarin in the presence of various catalysts, including $FeCl_3 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, HCl, and *p*-TsOH. However, all attempts to prepare the tricyclic target **1** by multicondensation at various reaction conditions, including those previously reported, failed. We were only able to isolate adducts **2** from the condensation of benzaldehyde

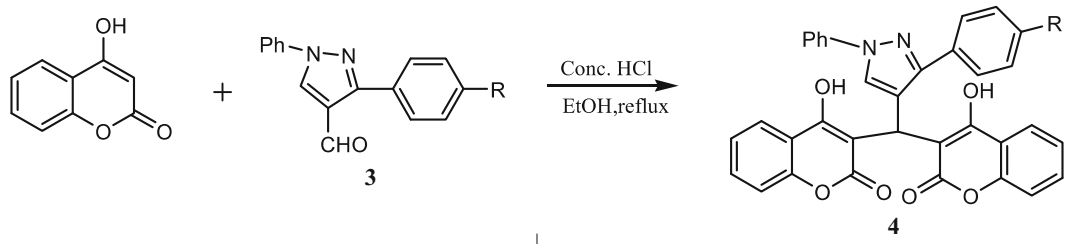
Scheme 1



2 **1**

where 2a R=C_6H_4Cl
2b R=$C_6H_4OCH_3$

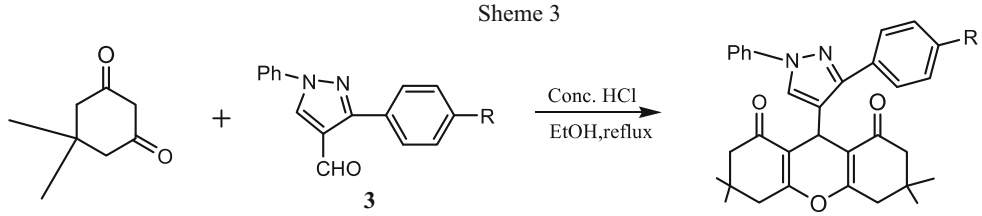
Scheme 2



3 **4**

Compounds **3, 4**	R	Compounds **3, 4**	R
3a, 4a	H	3d, 4d	4-Br
3b, 4b	4-CH_3	3e, 4e	4-NO_2
3c, 4c	4-OCH_3		

Sheme 3



3 **5**

Compounds **3, 5**	R	Compounds **3, 5**	R
3a, 4a	H	**3d, 4d**	4-Br
3b, 4b	4-CH_3	**3e, 4e**	4-NO_2
3c, 4c	4-OCH_3		

with 4-hydroxycoumarin, as proved by the physicochemical and spectral characteristics of products (Scheme 1). The treatment of 4-hydroxycoumarin with urea and benzaldehyde in the presence of acid catalysts led to compounds identical to those obtained umder the same reaction conditions in the absence of urea. Similar observations were reported by Matache, et al. [21]. Keeping in mind these points and the excellent biological profile associated with biscoumarin and pyrazoles, we diverted our study toward the synthesis of 3,3′-[(3-aryl-1-phenyl-1*H*-pyrazol-4-yl)methylene]bis(4-hydroxy-2*H*-chromen-2-ones) **4a – 4e** via the acid-catalyzed condensation reaction of 4-hydroxycounarin and 1*H*-3-aryl-1-phenylpyrazole-4-carbaldehydes **3a – 3e** (Scheme 2).

As mentioned above, ethanol solutions of 4-hydroxycoumarin and 1,3-diphenyl-1H-pyrazole-4-carbaldehyde (**3a**) were refluxed for 25 min in presence of HCl to give 3,3′-[(1,3-diphenyl-1*H*-pyrazol-4-yl)methylene]bis(4-hydroxy-2*H*-chromen-2-one) (**4a**) in 80% yield. To check for the feasibility of this reaction, we carried out the reaction of 4-hydroxycoumarin with different 3-aryl-1-phenylpyrazole-4-carbaldehydes (**3b – 3e**) under similar conditions (Scheme 2). It was found that this method afforded the desired pyrazolylbiscoumarins (**4b – 4e**) with excellent yield in each case. 3-Aryl-1-phenylpyrazole-4-carbaldehydes (**3a – 3e**) needed for the present study were prepared by the Vilsmeier – Haack reaction as reported in [22]. The struc-

TABLE 1. Physicochemical data for compounds **4** and **5**

Compound	Yield (%)	M.p. (°C)	Compound	Yield (%)	M.p. (°C)
4a	80	217 – 219 (lit. m.p. 222°C [23])	**5a**	83	95 – 96
4b	82	206 – 207 (lit. m.p. 210°C [23])	**5b**	82	185 – 186
4c	84	105 – 108	**5c**	86	135 – 140
4d	86	182 – 184	**5d**	80	224 – 225
4e	82	238 – 240	**5e**	84	202 – 204

tures of all compounds were confirmed by thorough analysis of spectral (IR, ^{1}HNMR and ^{13}CNMR) data. The purity of the synthesized compounds was checked by TLC.

As was mentioned above, xanthenedione derivatives are also associated with excellent biological profile. For this reason, the above scheme was also used to synthesize a series of pyrazolylxanthenedione derivatives (**5a – 5e**). The synthesis of 3,3,6,6-tetramethyl-9-(3-aryl-1-phenyl-1*H*-pyrazol-4-yl)-3,4,5,6,7,9-hexahydro-1*H*-xanthene-1,8(*2H*)-diones (**5a – 5e)** was achieved by refluxing dimedone with the corresponding 3-aryl-1-phenylpyrazole-4-carbaldehydes (**3a – 3e**) in the presence of concentrated HCl (Scheme 3). Some physicochemical data for these compounds are given in Table 1.

1.2. Biological Investigation and Results

All synthesized compounds **4** and **5** were evaluated *in vitro* for their antibacterial activity against G-positive bacteria *S. aureus* and Gram-negative bacteria *K. pneumoniae* in comparison to the well-known commercial antibiotic streptomycin. Most of the synthesized compounds exhibited variable antibacterial activities against test bacterial strains. Results of antibacterial evaluation are summarized in Table 2. These compounds showed varying inhibition zones with diameters ranging from 6 to 21mm. From overall results, it is evident that compounds **4a** and **5a** can be recognized as the most biologically active members of this study with good antibacterial profiles. However, none of the synthesized compounds were superior to the commercial reference antibiotic against the bacterial strains employed.

2. EXPERIMENTAL PART

2.1. Chemistry

The melting points have been determined in open capillaries and are uncorrected. The purity of compounds was checked by TLC. The ^{1}H-NMR and ^{13}CNMR spectra were measured in deuterated chloroform ($CDCl_3$) on a Brucker Advance II 400 Spectrometer. The IR spectra were recorded on a Bruker FT-IR spectrophotometer.

2.1.1. General procedure for the synthesis of 3,3′-[(3-aryl-1-phenyl-1*H*-pyrazol-4-yl)methylene]bis(4-hydroxy-2*H*-chromen-2-ones) (4a – 4e). To ethanol solution of the appropriate aldehyde (10 mmol) in a 100 mL round bottom flask were sequentially added 4-hydroxycomnarin (20 mmol) and a catalytic amount of concentrated HCl. The resulting solution was refluxed for 25 – 30 min to afford a yellow colored solid precipitate upon cooling. This solid was filtered and then washed with water and hot ethanol to obtain pure compound **4**.

2.1.2. General procedure for the synthesis of 3,3,6,6-tetramethyl-9-(3-aryl-1-phenyl-1*H*-pyrazol-4-yl)3,4,5,6,7,9-hexahydro-1*H*-xanthene-1,8(*2H*)-diones

TABLE 2. Antibacterial activities of compounds **4** and **5** against *S.aureus* and *K.pneumoniae*

Compound	Activity (zone of inhibition in mm) at various concentrations							
	S. aureus				*K. pneumonia*			
	50 ppm	100 ppm	150 ppm	250 ppm	50 ppm	100 ppm	150 ppm	250 ppm
4a	11	13	15	19	13	14	16	17
4b	7	9	11	12	9	11	12	14
4c	-	8	10	14	6	9	12	15
4d	6	8	11	13	8	11	13	16
4e	10	13	14	17	11	12	14	16
5a	13	17	18	19	16	18	20	22
5b	8	11	13	14	9	11	14	17
5c	-	7	9	12	-	8	9	11
5d	6	8	10	11	7	9	10	12
5e	-	7	9	13	-	8	12	14
Streptomycin	21				25			

(5a – 5e). To ethanol solution of the appropriate aldehyde (10 mmol) a 100 ml round bottom flask were sequentially added dimedone (20 mmol) added and a catalytic amount of concentrated HCl. The resulting solution was heated under reflux for 14 – 15 h., and then white solid precipitate was separated on cooling. This solid was filtered and then washed with water and hot ethanol to obtain pure compound **5.**

2.1.3. Characterization of 3,3′-[(3-aryl-1-phenyl-1*H*-pyrazol-4-yl)methylene]bis(4-hydroxy-2*H*-chromen-2-ones) (4) and 3,3,6,6-tetramethyl-9-(3-aryl-1-phenyl- 1*H*-pyrazol-4-yl)3,4,5,6,7,9-hexahydro-1*H*-xanthene-1,8-(*2H*)-diones (5).

4a: Yield: 80%; m.p. 217 – 219°C (lit m.p. 222°C [23]).

4b: Yield: 82%; m.p. 206 – 207°C (lit m.p. 210°C [23]).

4c: Yield: 84%; m.p. 105 – 108°C; IR (ν_{max}, cm^{-1}): 1655, 1603, 1567, 1503; ^{1}HNMR (400 MHz; δ, ppm): 3.9454 (s, 3H, OCH_3), 6.1733 (s, 1H, C_9-H), 7.0888 – 7.1055 (m, 2H), 7.2710 – 7.3360 (m, 3H), 7.4095 (m, 1H), 7.5267 – 7.6822 (m, 8H), 7.9046 – 7.9665 (m, 2H), 8.0993 – 8.1471 (m, 1H), 11.3014 (s, 1H, OH), 11.3261 (s, 1H, OH); ^{13}CNMR (100 Hz; δ, ppm): 29.33, 55.55, 104.29, 105.22, 115.67, 116.29, 116.71, 119.11, 124.67, 124.24, 124.62, 126.29, 126.67, 128.23, 129.32, 129.74, 130.27, 132.56, 137.43, 139.96, 137.43, 139.96, 152.12, 152.18, 152.34, 164.12, 164.49, 166.37, 168.75.

4d: Yield: 86%; m.p. 182 – 184°C; IR (ν_{max}, cm^{-1}): 1731, 1651, 1605, 1560; ^{1}HNMR (400 MHz; δ, ppm): 6.0893 (s, 1H, C_9-H), 6.7908 – 6.8282 (t, 1H), 6.9643 – 7.0027 (t, 2H), 7.2259 – 7.3555 (m, 5H), 7.4054 – 7.4449 (t, 2H), 7.5432 – 7.5822 (m, 2H), 7.7062 – 7.7254 (d, 2H, J= 7.52 Hz), 7.8201 (s, 1H), 7.8619 – 7.8853 (dd, 1H, J_1= 8.36 Hz, J_2= 1.44 Hz), 7.9462 – 7.9687 (dd, 1H, J_1=7.52 Hz, J_2= 1.04 Hz), 11.3144 (s, 1H, OH), 11.7606 (s, 1H, OH); ^{13}CNMR (100 Hz; δ, ppm): 28.3, 103.83, 115.58, 117.78, 119.08, 120.60, 123.40, 123.68, 125.61, 127.54, 129.10, 129.23, 129.41, 130.09, 130.37, 131.24, 131.53, 149.85, 151.94, 164.70.

4e: Yield: 82%; m.p. 238 – 240°C; IR (ν_{max}, cm^{-1}): 1650, 1597, 1558; ^{1}HNMR (400 MHz; δ, ppm): 6.5251 (s, 1H, C_9-H), 7.2298 – 7.2506 (d, 2H, J= 8.32 Hz), 7.2925 – 7.3221 (m, 3H), 7.4295 – 7.4690 (t, 2H), 7.5242 – 7.5661 (m, 2H), 7.6544 – 7.6760 (d, 2H, J= 8.64 Hz), 7.7947 – 7.7.8985 (m, 7H), 8.1376 (s, 1H), 11.7428 (s, 1H, OH), 11.7576 (s, 1H, OH); ^{13}CNMR (100 Hz; δ, ppm): 29.31, 99.99, 116.44, 116.52, 119.34, 120.20, 122.61, 124.14, 124.25, 124.79, 125.14, 127.01, 127.46, 129.29, 129.51, 133.07, 133.27, 139.63, 149.61, 165.04.

5a: Yield: 83%; m.p. 95 – 96°C; IR (ν_{max}, cm^{-1}): 1707, 1587; ^{1}HNMR (400 MHz; δ, ppm): δ 1.253 (s, 6H, 2 CH_3), 1.5895 (s, 6H, 2 CH_3), 2.1743 – 2.4560 (m, 8H), 5.1991 (s, 1H), 7.3434 – 7.3635 (m, 2H), 7.6085 – 7.6352 (m, 3H), 7.6950 – 7.7271 (m, 4H), 7.8289 – 7.8498 (m, 2H); ^{13}CNMR (100 Hz; δ, ppm): 21.72, 69.69, 128.16 – 145.46 (all aromatic protons), 189.72 (C=O).

5b: Yield: 82%; m.p. 185 – 186°C; IR (ν_{max}, cm^{-1}): 1665, 1507; ^{1}HNMR (400 MHz; δ, ppm): 1.0029 (s, 6H, 2 CH_3), 1.0681 (s, 6H, 2 CH_3), 2.0094 – 2.2198 (m, 4H), 2.2920 – 2.3990 (m, 4H), 4.8781 (s, 1H), 7.2152 – 7.2521 (m, 1H), 7.3787 – 7.4179 (m, 2H), 7.5811 – 7.5979 (m, 2H), 7.6395 – 7.6591 (d, 2H, J= 7.8 Hz), 7.7614 (s, 1H), 7.7824 – 7.8016 (d, 2H, J= 7.8 Hz); ^{13}CNMR (100 Hz; δ, ppm): 22.44, 28.10, 28.60, 32.05, 40.85, 50.73, 115.23, 118.88, 121.98, 124.93, 126.18, 127.56, 129.26, 130.77, 131.21, 133.57, 139.91, 151.08, 161.86, 196.57 (C=O).

5c: Yield: 86%; m.p. 135 – 140°C; IR (ν_{max}, cm^{-1}): 1660, 1504; ^{1}HNMR (400 MHz; δ, ppm): 0.9831 (s, 6H, 2 CH_3), 1.04411 (s, 6H, 2 CH_3), 2.0666 – 2.2962 (m, 8H), 2.4027 (s, 3H, CH_3), 4.9371 (s, 1H), 7.2024 – 7.2493 (m, 3H), 7.3736 – 7.4127 (m, 2H), 7.5359 – 7.5558 (d, 2H, J= 7.96 Hz), 7.6766 – 7.6962 (d, 2H, J= 7.96 Hz), 7.9378 (s, 1H); ^{13}CNMR (100 Hz; δ, ppm): 21.34, 22.55, 28.32, 28.49, 31.93, 40.80, 50.75, 99.98, 114.81, 118.86, 123.78, 125.90, 128.68, 129.07, 129.16, 131.75, 137.23, 140.01, 161.85, 196.62 (C=O).

5d: Yield: 80%; m.p. 224 – 225°C; IR (ν_{max}, cm^{-1}): 1658, 1620, 1599, 1540; ^{1}HNMR (400 MHz; δ, ppm): 1.0089 (s, 6H, 2 CH_3), 1.0562 (s, 6H, 2 CH_3), 2.0758 – 2.1736 (m, 4H), 2.2566 – 2.3032 (m, 4H), 3.8588 (s, 3H, OCH_3), 4.9262 (s, 1H), 6.9747 – 6.9887 (m, 2H), 7.2293 – 7.4101 (m, 3H), 7.6545 – 7.80 (m, 5H); ^{13}CNMR (100 MHz; δ, ppm): 22.11, 27.10, 28.60, 31.08, 55.55 115.23, 117.88 – 155.08, 167.86, 189.98 (C=O).

5e: Yield: 84%; m.p. 202 – 204°C; IR (ν_{max}, cm^{-1}): 1657, 1599, 1513; ^{1}HNMR (400 MHz; δ, ppm): 1.0422 (s, 6H, 2 CH_3), 1.0956 (s, 6H, 2 CH_3), 2.1508 – 2.2522 (m, 4H), 2.3893 – 2.4421 (m, 4H), 4.8674 (s, 1H), 7.2724 – 7.2975 (m, 1H), 7.4057 – 7.4445 (m, 2H), 7.6313 – 7.6763 (m, 3H), 8.3102 – 8.3755 (m, 4H); ^{13}CNMR (100 MHz; δ, ppm): 22.36, 27.75, 28.87, 29.70, 32.22, 40.88, 50.67, 115.58, 118.98, 123.54, 126.61, 127.04, 129.40, 129.72, 129.86, 161.91, 196.63 (C=O).

2.2. Biological Assay

2.2.1. Medium. The biological testing was performed in nutrient agar medium (NAM) of the following composition: peptone, 10 g; yeast extract, 3 g; sodium chloride, 5 g; nutrient agar, 2% with the final volume adjusted to 1000 mL with sterile distilled water at pH 7.

2.2.2. *In vitro* antibacterial assay. The compounds were screened for their antibacterial activity against test bacterial cultures using the agar-well diffusion assay technique.

2.2.3. Primary screening [24]. The antibacterial activities of newly synthesized compounds were evaluated by the agar-well diffusion assay technique against Gram-positive bacteria *S. aureus* and Gram-negative bacteria *K. pneumoniae*. The bacterial cultures were maintained on the nutrient agar media by sub-culturing on fresh slants every 4 – 6

weeks and incubating at the appropriate temperature for 24 h. All stock cultures were stored at 4°C. For the evaluation of antimicrobial activity of the synthetic compounds, suspension of each test microorganism was prepared. A volume of 20 mL of agar media was poured into each petri dish, after which the agar dishes were swabbed with 100 µl inoculum volume of each test bacterium and kept for 15 min for the adsorption to take place. Using a punch, 8 mm diameter wells were bored in the seeded agar plates and 50 µl aliquot of each test compound reconstituted in DMSO was added to each dish. Pure DMSO was used as control for all the test compounds. After holding the plates at room temperature for 2 h to allow thr diffusion of test compounds into the NAM, the Perti dishes were incubated at 37°C for 24 h. The antibacterial activity was evalusted by measuring the inhibition zone diameter. The entire tests were made in triplicate and the mean diameter of inhibition was calculated. The antimicrobial activities of test compounds were compared to that of the reference drug streptomycin.

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Competing Interests

The authors declare that they have no competing interests.

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