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RESEARCH ARTICLE

Synthesis and antibacterial activity of a new series of 3-[3-(substituted phenyl)-1-phenyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one derivatives

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Abstract

A series of 3-[3-(substituted phenyl)-1-phenyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one (**4a–k**) were synthesized by reaction of 3-[2,3-dibromo-3-(substituted phenyl)propanoyl]-2*H*-chromen-2-one (**3 a-k**) with phenyl hydrazine in presence of triethylamine in absolute ethanol, characterized by spectral data and screened for their *in vitro* antibacterial activity against *gram*-positive and *gram*-negative bacteria. Among the series, compounds **4d, 4h** and **4i** displayed an encouraging antibacterial activity profile as compared to reference standard drug ciprofloxacin against tested bacterial strains.

Keywords: Antibacterial activity, ciprofloxacin, coumarin, pyrazole

Introduction

A continuous increase in the number of infections caused by bacteria resistant to one or multiple antibiotic classes poses a significant threat[1,2] as it may lead to treatment failures and complication. One of the main driving forces for development and spread of resistance is high antibiotic consumption, reflected in resistance rates directly correlating with the prescription of antimicrobial drugs[3]. To overcome resistance, in addition to prudent use of available drugs, a constant effort to discover and develop new agents with an improved spectrum of activity is required.

In drug-designing programmes, an essential component of the search for new leads is the synthesis of molecules, which is novel yet resembles known biologically active molecules by virtue of the presence of critical structural features. Certain small heterocyclic molecules act as highly functionalized scaffolds and are known pharmacophore of a number of biologically active and medicinally useful molecules[4,5].

Widespread interest in the chemistry of coumarins in a large number of natural products has attracted the interest due to their biological activities and their potential applications as pharmacological agents. Several coumarin ring systems bearing various substituents at the C-3 position are widely distributed in nature and have been reported to have anti-viral, anti-oxidant and anti-fungal activities. Furthermore, most of compounds prepared from 3-acetyl coumarin have anti-microbial, anti-inflammatory and anti-oxidant activities[6–10]. The heterocyclic systems encompassing pyrazoles are explored to the maximum extent owing to their wide spectrum of pharmacological activities, such as analgesic, anti-inflammatory, antipyretic, anti-depressant, hypotensive, anti-cancer, antibacterial, anti-parasitic, and anti-androgenic agents[10–17]. Special attention is warranted towards the synthetic design and development of pyrazoles because of their high demand in academic and pharmaceutical sectors, and in continuation of our previous work in the synthesis of biologically active heterocycles[6,10] we report herein synthesis of some new 3-[3-(substituted phenyl)-1-phenyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one **4a–k,** for investigation of their antibacterial profile.

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Experimental procedure

All research chemicals were purchased from Sigma–Aldrich (St. Louis, Missouri, MO, USA) or Lancaster Co. (Ward Hill, MA, USA) and used as such for the reactions. Solvents except laboratory reagent grade were dried and purified according to the literature when necessary. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel plates from E. Merck and Co. (Darmstadt, Germany). Melting points of synthesized compounds were determined in Thermonik (Mumbai, India) melting point apparatus and are uncorrected, UV spectra were recorded on Thermospectronic (Rochester, NY, USA) and IR spectra were recorded on Thermo Nicolet IR200 FT-IR Spectrometer (Madison, WI, USA) by using KBr pellets. The ^{1}HNMR were recorded on Bruker AVANCE 300 (Bruker, Rheinstetten/Karlsruhe, Germany) using DMSO-d_6 as solvent. Chemical shifts are reported in δ ppm units with respect to tetramethylsilane (TMS) as internal standard. The purity of compounds was examined by TLC on silica gel plate using chloroform and methanol (10:1) as mobile phase and iodine vapours as visualizing agent. The starting material 3-acetyl coumarin **1** was synthesized by a method reported earlier[18].

General procedure of preparation of 3-aryl-1-(3-coumarinyl)prop-2-en-1-ones (2a–k)[19]

A mixture of 3-acetyl coumarin (**1**, 0.01 mol) and the various substituted aromatic aldehydes (0.012 mol) was dissolved in 10 mL of n-butanol under heating; then 0.3 mL of glacial acetic acid and the same quantity of piperidine were added. The reaction mixture was refluxed for 4 h and then the solvent was removed in vacuum. The residue was triturated with 10 mL of ethanol until a precipitate is formed. The precipitate was filtered off and crystallized from appropriate solvents.

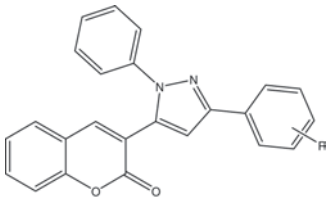
General procedure for preparation of 3-[2,3-dibromo-3-(substituted phenyl)propanoyl]-2H-chromen-2-one derivatives (3a–k)[20]

A 3-[(2E)-3-substituted-prop-2-enoyl]-2H-chromen-2-one (**2a–k**, 0.01 mol) was dissolved in chloroform (100 mL) and bromine (0.01 mol) in chloroform was added drop wise with constant stirring. After the complete addition of bromine solution, the reaction mixture was stirred for 12 h. Excess of chloroform was distilled off under reduced pressure. Thus obtained solid was filtered, dried and washed from hot ethanol.

General procedure for synthesis of 3-[3-(substituted phenyl)-1-phenyl-1H-pyrazol-5-yl]-2H-chromen-2-one (4a–k)

A mixture of 3-[2,3-dibromo-3-(substituted phenyl) propanoyl]-2*H*-chromen-2-one (**3a–k**, 0.01 mol) and phenyl hydrazine (0.01 mol) was dissolved in ethanol (150 mL) and triethylamine (10 mL). The reaction mixture was heated under reflux for 12 h. Excess of ethanol was distilled off under reduced pressure and residue was triturated with ice-cold water. The precipitated solid was filtered, dried and purified by column chromatography using silica gel (60–120) as stationary phase and chloroform:methanol (10:1) as mobile phase. The physico-chemical and spectral data of synthesized compounds 4a–k is summarized in Tables 1 and 2, respectively.

Table 1. Physico-chemical data of 3-[3-(substituted phenyl)-1-phenyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one derivatives **4a–k**.



(4a-k)

Compound	R[a]	Yield (%)	M. P. (^{0} C)	Rf [b]	Formula	M. W.
4 a	H-	53	158-260	0.38	$C_{24}H_{16}N_2O_2$	364
4 b	4-OMe-	83	120-122	0.27	$C_{25}H_{18}N_2O_3$	394
4 c	4-Cl-	76	98-100	0.48	$C_{24}H_{15}ClN_2O_2$	398
4 d	2,4-$(Cl)_2$-	92	126-130	0.24	$C_{24}H_{14}Cl_2N_2O_2$	433
4 e	4-NMe_2-	70	148-150	0.46	$C_{26}H_{21}N_3O_2$	407
4 f	3-NO_2-	67	100-102	0.25	$C_{24}H_{15}N_3O_4$	409
4 g	4-Me-	74	90-92	0.36	$C_{24}H_{18}N_2O_2$	378
4 h	3-OMe-	83	136-138	0.32	$C_{25}H_{18}N_2O_3$	394
4 i	4-F-	64	190-192	0.29	$C_{24}H_{15}FN_2O_2$	382
4 j	2-NO_2-	71	208-210	0.33	$C_{24}H_{15}N_3O_4$	409
4 k	4-OH-	76	182-184	0.42	$C_{24}H_{16}N_2O_3$	380

Note: [a]All compounds purified by column chromatography using chloroform:methanol (10:1) as mobile phase; [b]chloroform:methanol 10:1 as a mobile phase and iodine vapours as visualizing agent.

Antibacterial activity

Medium

The solid media Mullere Hinton agar (MHA; beef infusion 300 g/L, casein acid hydrolysate 17.5 g/L, starch 1.5 g/L, agar 17 g/L and distilled water 1000 mL, adjusted to pH 7.4) was used for the antibacterial activity.

Test microorganisms

Two gram-positive bacteria namely, *Staphylococcus aureus* (ATCC-25923), *Bacillus subtilis* (ATCC 6633) and two gram-negative bacteria namely, *Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* (ATCC-27853) were used for the antibacterial activity.

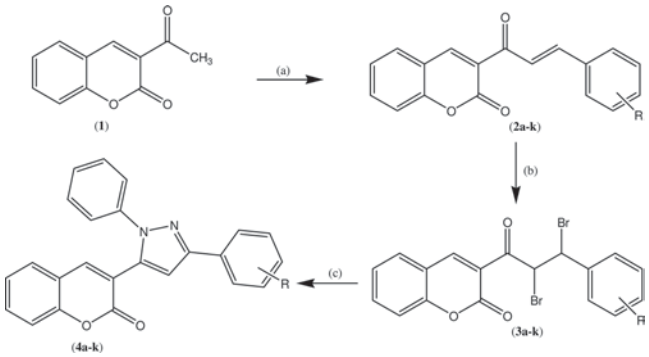
Minimum inhibitory concentration[21]

The *in vitro* antibacterial activity for newly synthesized compounds (**4a–k**) was evaluated using the conventional agar-dilution method. Two-fold serial dilutions of the compounds and reference drug (ciprofloxacin) were prepared in MHA. Drugs (10.0 mg) were dissolved in DMSO (1 mL) and the solution was diluted with water (9 mL). Further progressive double dilution with melted MHA was performed to obtain the required concentrations of 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.05 μg/mL. The bacterial inocula were prepared by suspending 24 h old bacterial colonies from MHA media in 0.85% saline. The inocula were adjusted to 0.5 McFarland Standard (1.5×10^8 CFU/mL)[22]. The suspensions were then diluted in 0.85% saline to give 10^7 CFU/mL. Petri dishes were spot-inoculated with 1 μL of each prepared bacterial suspension (10^4 CFU/spot) and incubated at 37°C for 24 h. At the end of the incubation period, minimum inhibitory concentration (MIC) was determined, which is the lowest concentration of the test compound that resulted in no visible growth on the plate. A control test was also performed with test medium supplemented with DMSO at the same dilutions as used in the experiment, in order to ensure that the solvent had no influence on bacterial growth.

Results and discussion

Synthesis

The synthesis of 3-[3-(substituted phenyl)-1-phenyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one (**4a–k**) was carried out as presented in Scheme 1. Starting material 3-acetyl-2-*H*-chromen-2-one **1** was synthesized by the reaction of salicylaldehyde with ethylacetoacetate in the presence of a catalytic amount of piperidine at room temperature following the literature procedure[18]. The 3-[(2*E*)-3-(substituted phenyl)prop-2-enoyl]-2*H*-chromen-2-one (chalcones, **2a–k**) were synthesized by Claisen–Schmidt condensation of 3-acetyl-2*H*-chromen-2-one **1** with various substituted benzaldehydes in the presence of a mixture of piperidine and n-butanol. Efforts to convert compounds **2a–k** into target molecules **4a–k** under a variety of conditions were not successful. Hence, an alternative method was adopted. This involved the bromination of chalcones **2a–k** and subsequent ring closure using



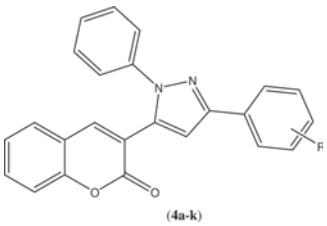
Scheme 1. Synthesis of 3-[3-(substituted phenyl)-1-phenyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one reagents and conditions: (a) Ar-CHO, piperidine/n-butanol, reflux, 4 h; (b) $Br_2/CHCl_3$, stir r.t. 12 h; (c) $PhNHNH_2$, TEA/ethanol, reflux, 12 h.

phenyl hydrazine. Bromination of chalcones **2a–k** was carried out in chloroform using bromine in chloroform to yield di-bromo compounds **3a–k,** which were cyclized with phenyl hydrazine in the presence of triethylamine in absolute ethanol to afford the 3-[3-(substituted phenyl)-1-phenyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one **4a–k**. The physical properties of the synthesized compounds are presented in Table 1. The structures and purity of the compounds were confirmed by spectral data and thin layer chromatography (Table 2).

Antibacterial activity

All newly synthesized compounds **4a–k** were evaluated for their *in vitro* antibacterial activity against two gram-positive bacteria, namely *Staphylococcus aureus* (ATCC-25923) and *Bacillus subtilis* (ATCC 6633), and two gram-negative bacteria, namely *Escherichia coli* (ATCC-25922) and *Pseudomonas aeruginosa* (ATCC-27853) using conventional agar-dilution method[23]. Ciproflaxacin was used as a reference standard. The results of the *in vitro* antibacterial activity screening of the test compounds are summarized in Table 3. Among the series, three compounds (**4d, 4h** and **4i)** exhibited excellent antibacterial activity against both gram-positive and gram-negative bacteria, while the compound **4c** showed moderate antibacterial activity against the tested organisms. However, all other compounds in the series were found to have less or poor activity against both gram-positive and gram-negative bacteria as compared to standard. The MIC was recorded as the lowest concentration of a compound to inhibit growth of the tested micro-organisms. In comparing the MIC values with the standard (MIC = 0.5 μg/mL), compounds **4d, 4h** and **4i** exhibit the most potent *in vitro* antibacterial activity against all evaluated organisms. Compounds **4d** (MIC = 0.25–1 μg/mL), **4h** (MIC = 0.25–0.5 μg/mL) and **4i** (MIC = 0.25–1 μg/mL) especially showed high antibacterial activity, while compound **4c** (MIC = 0.5–2 μg/mL) showed respectable antibacterial activity. The investigation of the structure–activity relationship revealed that the compounds with p-fluoro, m-methoxy, p-chloro, and o, p-dichloro substituents at the third position in the aromatic ring of the pyrazole

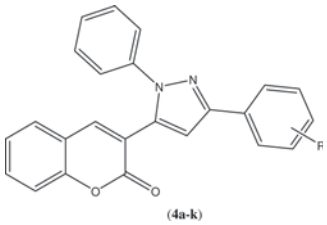
Table 2. Spectral data of 3-[3-(substituted phenyl)-1-phenyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one derivatives 4a–k.



(4a-k)

Compound	R	UV (CH_3OH)	IR (KBr, cm^1)	^{1}H-NMR (DMSO-d_6 δ, ppm)
4 a	H-	λ_{max} 290 (ε 14523)	1679.0 (coumarin, C=O), 1634.74 (C=N), 1491.00 (C=C).	7.72 (s, 1H, 4-H of coumarin), 7.02–7.48 (m, 14H,Ar-H), 6.7 (s, 1H, 4-H of pyrazole).
4 b	4-OMe-	λ_{max} 272 (ε 18673)	1681.97 (coumarin, C=O), 1624.74 (C=N), 1490.05 (C=C).	7.83 (s, 1H, 4-H of coumarin), 6.83–7.37 (m, 13H,Ar-H), 6.8 (s, 1H, 4-H of pyrazole), 3.73 (s, 3H, -OCH_3).
4 c	4-Cl-	λ_{max} 302 (ε 12657)	1682.05 (coumarin, C=O), 1614.43 (C=N), 1486.72 (C=C).	7.57 (s, 1H, 4-H of coumarin), 7.02–7.42 (m, 13H,Ar-H), 6.7 (s, 1H, 4-H of pyrazole).
4 d	2,4-$(Cl)_2$-	λ_{max} 286 (ε 16795)	1682.63 (coumarin, C=O), 1616.31 (C=N), 1484.97 (C=C).	7.58 (s, 1H, 4-H of coumarin), 7.02–7.36 (m, 12H,Ar-H), 6.56 (s, 1H, 4-H of pyrazole).
4 e	4-NMe_2-	λ_{max} 276 (ε 17987)	1673.65 (coumarin, C=O), 1604.94 (C=N), 1449.36 (C=C).	7.72 (s, 1H, 4-H of coumarin), 6.65–7.30 (m, 13H,Ar-H), 6.7 (s, 1H, 4-H of pyrazole), 2.85 (s, 6H, -$N(CH_3)_2$).
4 f	3-NO_2-	λ_{max} 286 (ε 15672)	1685.23 (coumarin, C=O), 1611.11 (C=N), 1449.36 (C=C).	7.82 (s, 1H, 4-H of coumarin), 7.02–8.41 (m, 13H,Ar-H), 6.65 (s, 1H, 4-H of pyrazole).
4 g	4-Me-	λ_{max} 282 (ε 17898)	1715.41 (coumarin, C=O), 1672.78 (C=N), 1552.98 (C=C).	7.72 (s, 1H, 4-H of coumarin), 7.02–7.36 (m, 13H,Ar-H), 6.76 (s, 1H, 4-H of pyrazole), 2.35 (s, 3H, -CH_3).
4 h	3-OMe-	λ_{max} 292 (ε 12893)	1681.97 (coumarin, C=O), 1624.94 (C=N), 1490.05 (C=C).	7.83 (s, 1H, 4-H of coumarin), 6.73–7.30 (m, 13H,Ar-H), 6.8 (s, 1H, 4-H of pyrazole), 3.63 (s, 3H, -OCH_3).
4 i	4-F-	λ_{max} 312 (ε 12331)	1682.05 (coumarin, C=O), 1614.43 (C=N), 1486.72 (C=C).	7.75 (s, 1H, 4-H of coumarin), 7.02–7.46 (m, 13H,Ar-H), 6.7 (s, 1H, 4-H of pyrazole).
4 j	2-NO_2-	λ_{max} 276 (ε 13892)	1689.61 (coumarin, C=O), 1609.40 (C=N), 1458.55 (C=C).	7.82 (s, 1H, 4-H of coumarin), 7.02–8.41 (m, 13H,Ar-H), 6.65 (s, 1H, 4-H of pyrazole).
4 k	4-OH-	λ_{max} 274 (ε 12891)	3208.00 (-OH), 1671.33 (coumarin, C=O), 1607.79,1505.92 (C=C).	7.72 (s, 1H, 4-H of coumarin), 6.79–7.31 (m, 13H,Ar-H), 6.65 (s, 1H, 4-H of pyrazole), 5.28 (s, 1H, -OH).

Table 3. *In vitro* antibacterial activity of 3-[3-(substituted phenyl)-1-phenyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one derivatives **4a–k (**MIC in μg/mL).



(4a-k)

		Gram-positive organisms			Gram-negative organisms
Compound	R	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
4 a	H-	32	64	> 128	> 128
4 b	4-OMe-	16	16	32	64
4 c	4-Cl-	0.5	1	1	2
4 d	2,4-$(Cl)_2$-	0.25	0.5	1	1
4 e	4-NMe_2-	32	64	> 128	> 128
4 f	3-NO_2-	2	2	4	8
4 g	4-Me-	4	8	2	1
4 h	3-OMe-	0.25	0.25	0.5	0.5
4 i	4-F-	0.25	0.5	1	1
4 j	2-NO_2-	32	64	64	64
4 k	4-OH-	16	64	32	64
Ciprofloxacin	-	0.5	0.5	0.5	0.5

nucleus gave better results. They have emerged as active antibacterial agents.

Conclusion

Herein, we have described an efficient and convenient synthesis of a novel series of 3-[3-(substituted phenyl)-1-phenyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one **4a–k**. These novel heterocyclic compounds containing both coumarin and pyrazole ring systems are prepared by the reaction of 3-[2,3-dibromo-3-(substituted phenyl) propanoyl]-2H-chromen-2-one **3a–k** with phenyl hydrazine in the presence of triethylamine in absolute ethanol. In general, the results of the *in vitro* antibacterial activity tests are also encouraging as out of 11 compounds tested, compounds **4d, 4h** and **4i** exhibited an antibacterial activity which is comparable or even more potent than that of the reference drug. The MIC values of these novel compounds evidenced that the presence of fluorine, methoxy and chlorine groups at the third position in the aromatic ring of the pyrazole nucleus gave rise to an increased antibacterial potency.

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Declaration of interest

The authors declare no conflict of interest.

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