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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF 3-{6-[3-(SUBSTITUTED PHENYL)-1-PHENYL-1H-PYRAZOL-4-YL]-2-THIOXO-1,2,5,6-TETRAHYDROPYRIMIDIN-4-YL}-2H-CHROMEN-2-ONES

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Abstract
Reaction of synthon 3-acetyl-2H-chromen-2-one **(1)** with 3-substituted aryl-1-phenylpyrazol carboxaldehyde **(2a-d)** gave intermediate compounds 3-[3-(3-substituted aryl-1-phenylpyrazol-4-yl)acryloyl]-2H-chromen-2-ones **(3a-d)**. These chalcones upon cyclization with thiourea yielded desired target compounds **(4a-d)** in 46-62% yield. All the synthesized compounds were characterized by spectral data such as IR, ^{1}H NMR and mass spectra. These compounds were also screened for their antimicrobial and antioxidant activities.

Keywords: Coumarin, pyrimidine, pyrazole, antimicrobial activity, antioxidant activity

Introduction

Pyrimidine being an integral part of DNA and RNA, imparts to diverse pharmacological properties. Pyrimidine analogs have been used as antimicrobial[1], analgesic[2], antitumor[3], antiviral[4], anti-inflammatory[5] and acid pump antagonist[6] agents. Pyrazole derivatives have also been reported to exhibit hypoglycemic[7], fungicidal[8] properties and also some of these have been tested as potential cardiovascular drugs[9]. Coumarins are naturally occurring compounds, also known as benzopyrone consisting of fused benzene and α-pyrone ring. More than 1300 coumarins were identified from natural sources[10]. These natural compounds serve as important models for advanced design and synthesis of more potent analogues. Natural and synthetic coumarins were found to exhibit antioxidant, anti-inflammatory, anticoagulation, estrogenic, dermal photosensitizing, vasodilator and antiulcer activities[11,12]. Polyhydroxy (phenolic) coumarins are known to act as antioxidants in biological systems. The *o*-dihydroxy and *o*-diacetoxy substituted coumarins were demonstrated to be excellent radical scavengers[13,14]. In addition, dihydroxy and diacetoxy derivatives of thionocoumarin showed more potent antioxidant effects than corresponding coumarins[15]. In view of the facts mentioned above and as part of our efforts to discover potentially active newer agents, we herein report the synthesis and biological activities of coumarin bearing pyrazole and thiopyrimidine nucleus.

Results and Discussion

The starting material 3-acetylcoumarin **(1)** was condensed with different substituted pyrazole aldehydes **(2a-d)** in presence of ethanolic KOH adopting literature procedure[16]

furnished the desired corresponding 3-{3-[3-(substituted phenyl)-1-phenyl-1H-pyrazol-4-yl]acryloyl}-2H-chromen-2-ones (**3a-d)** in 45-69% yield **(Scheme-1).** Formation of these compounds were confirmed by spectral data.

Compound **3a** was obtained as a yellow coloured solid having m.p 138-140 ^{0}C in 69% yield. The IR (cm^{-1}) spectrum of compound **3a** shows characteristic absorptions at 3029 , 1718, 1615 are due to the presence of aromatic -CH, C=O and C=N group. ^{1}H NMR signals are at δ 10.06 (s, 1H, C_4-H of coumarin), 8.55 (s, 1H, C_5-H of phenyl pyrazole), 7.79 (d, 1H, -COCH), 7.37 (d, 1H, -CH), 7.26-7.84 (m, ArH). Further formation of compound **3a** was confirmed by mass spectral analysis, the molecular ion peak at m/z = 417. Cyclisation of 3-{3-[3(substituted phenyl)-1-phenyl-1H-pyrazol-4-yl]acryloyl}-2H-chromen-2-ones **(3a-d)** with thiourea in ethanol in presence of catalytic amount of NaOH furnished the target compounds (**4a-d)**.

Compound **4a** was obtained as a pale yellow crystalline solid in 62% yield having M. P 130-132 ^{0}C. The IR spectrum of compound **4a** showed absorptions of NH and C=O at 3331 and 1735cm^{-1} respectively. ^{1}H NMR spectrum of compound **4a** showed signals at δ 10.36 (s, 1H, C_4-H of coumarin), 9.30 (s, 1H, -NH), 8.42 (s, 1H, C_5-H of phenyl pyrazole), 7.20-8.10 (m, ArH), 4.82 (s, 2H, -CH_2). Mass spectrum of compound **4a** showed molecular ion peak at m/z = 476. Physical constants of all the synthesized compounds are tabulated in **Table-1.**

Experimental

3-{3-[3-(substituted phenyl)-1-phenyl-1H-pyrazol-4-yl]acryloyl}-2H-chromen-2-ones (3a-d):

To a mixture of 3-acetylcoumarin **(1)** (0.01 mol) and pyrazole aldehyde **(2a-d)** (0.01 mol) in absolute ethanol (30 ml) was added 5 ml of 10% aq. potassium hydroxide with constant shaking maintaining a temperature of 5-10 ^{0}C. The mixture was stirred for 2 hours at room temperature and kept over night. The solid separated was collected by filtration and crystallized from ethanol gave 3-{3-[3-(substituted phenyl)-1-phenyl-1H-pyrazol-4-yl]acryloyl}-2H-chromen-2-ones **(3a-d)**.

Compound (3a): IR (cm^{-1}): 1718 (C=0); 3029 (CH), 1615 (C=N). ^{1}HNMR (DMSO-d_6): δ 10.06 (s, 1H C_4-H of coumarin), 8.55 (s, 1H, C_5-H of phenyl pyrazole), 7.79 (d, 1H, -COCH), 7.37 (d, 1H, -CH), 7.26-7.84 (m, ArH); Mass (m/z) = 417. *Anal* Calcd. For $C_{27}H_{18}N_2O_3$: C, 77.50; H, 4.34; N, 6.69. Found: C, 77.52; H, 4.30; N, 6.68%.

Compound (3b): IR (cm^{-1}): 1680 (C=0); 3068(CH), 1608 (C=N). ^{1}HNMR (DMSO-d_6): δ 10.20 (s, 1H, C_4-H of coumarin), 8.43 (s, 1H, C_5-H of phenylpyrazole), 7.65 (d, 1H, -COCH), 7.29 (d, 1H, -CH), 7.11-7.91 (m, ArH), 3.89 and 3.79 (s, 6H, -OCH_3). *Anal* Calcd. For $C_{29}H_{22}N_2O_5$: C, 72.79; H, 4.63; N, 5.85. Found: C, 72.80; H, 4.60; N, 5.82%.

Compound (3c): IR (cm-1): 1685 (C=0), 1599 (C=N), 3088 (CH), 1344 and 1529 (NO_2). ^{1}HNMR (DMSO-d6): δ 10.09 (s, 1H, C_4-H of coumarin), 8.58 (s, 1H, C_5-H of phenyl pyrazole), 7.57 (d, 1H, -COCH), 7.42 (d, 1H, -CH) 7.26-7.81 (m, ArH). *Anal* Calcd. For $C_{27}H_{17}N_3O_5$: C, 69.97; H, 3.70; N, 9.07. Found: C, 69.95; H, 3.73; N, 9.04%.

Compound (3d): IR (cm-1): 3136 (OH), 3050 (CH), 1694 (C=0), 1600(C=N). ^{1}HNMR (DMSO-d_6): δ 10.81 (s, 1H, C_4-H of coumarin), 8.0 (s, C_5-H, of phenyl pyrazole), 7.61 (d, 1H, -COCH),

7.24 (d, 1H, -CH), 7.21-7.90 (m, ArH), 2.73 (s, 1H, -OH). Mass (m/z) = 435. *Anal* Calcd. For $C_{27}H_{18}N_2O_4$: C, 74.64; H, 4.18; N, 6.45. Found: C, 74.68; H, 4.16; N, 6.41%.

Synthesis of 3-{6-[3-(substituted phenyl)-1-phenyl-1H-pyrazol-4-yl]-2-thioxo-1,2,5,6-tetrahydropyrimidin-4-yl}-2H-chromen-2-ones (4a-d).

Chalcone **(1a-d)** (0.01 mol), thiourea (0.01 mol) and sodium hydroxide (0.4 g) in 25 ml of 80% aqueous ethanol was refluxed for 15 hours, then concentrated and cooled. The product was separated, filtered, washed with water and recrystallised from ethanol gave compounds **(4a-d).**

Compound (4a): IR (cm-1): 3331 (NH), 1735 (C=0). ^{1}HNMR (DMSO-d_6): δ 10.36 (s, 1H, C_4-H of coumarin), 9.30 (s, 1H, -NH), 8.42 (s, 1H, C_5-H of phenyl pyrazole), 7.20-8.10 (m, ArH), 4.82 (s, 2H, -CH_2). Mass (m/z) = 476. *Anal* Calcd. For $C_{28}H_{20}N_4O_2S$: C, 70.57; H, 4.23; N, 11.76. Found: C, 70.55; H, 4.24; N, 11.74%.

Compound (4b): IR (cm-1): 3316 (NH), 1674 (C=0). Mass (m/z) = 535. *Anal* Calcd. For $C_{30}H_{24}N_4O_4S$: C, 67.15; H, 4.51; N, 10.44. Found: C, 67.12; H, 4.55; N, 10.42%.

Compound (4c): IR (cm-1): 3316 (NH), 1673 (C=0). ^{1}HNMR (DMSO-d_6): δ 10.09 (s, 1H, C_4-H of coumarin), 8.58 (s, 1H, -NH), 8.16 (s, 1H, C_5-H of phenyl pyrazole), 7.24-7.90 (m, ArH), 4.17 (s, 2H, -CH_2). Mass (m/z) = 524. *Anal* Calcd. For $C_{28}H_{19}N_5O_4S$: C, 64.48; H, 3.67; N, 13.43. Found: C, 64.50; H, 3.64; N, 13.42%.

Compound (4d): IR (cm-1): 3616 (OH), 3141 (NH), 1681 (C=0). ^{1}HNMR (DMSO-d_6): δ 10.82 (s, 1H, C_4-H of coumarin), 9.87 (s, 1H, -NH), 8.00 (s, 1H, C_5-H of phenyl pyrazole), 6.87-7.68 (m, ArH), 4.22 (s, 2H, CH_2), 2.28 δ (s, 1H, -OH). *Anal* Calcd. For $C_{28}H_{20}N_4O_3S$: C, 68.28; H, 4.09; N, 11.37. Found: C, 68.26; H, 4.08; N, 11.35%.

Antimicrobial Activity

The antimicrobial activities were performed by cup plate method[17]. The sample was dissolved in DMF at the concentration of 1000 µg/ml. Antibacterial activity screened against, *Staphylococcus aureus, Bacillus substilus, Pseudomonas aeruginosa* and *Escherichia coli.* Antifungal activity was carried out against *Aspergillus flavus* and *Aspergillus niger* under aseptic conditions. Gentamycin and fluconazole were used as standard drug for antibacterial and antifungal activities respectively. The zone of inhibition was compared with standard drug after 24 hours of incubation at 25 ^{0}C for antibacterial activity and 48 hours at 30 ^{0}C for antifungal activity. Among the synthesized compounds compound **4d** showed good activity against gram +ve bacteria *Bacillus substilus* and *Staphylococcus aureus.* Compounds **3d, 4a** and **4c** showed good activity against gram –ve bacterial strain *Escherichia coli* and compounds **4a** and **4c** showed moderate activity against gram –ve bacterial strain *Pseudomonas aeruginosa* and remaining all the synthesized compounds showed poor activity against all the bacterial strains. Compounds **3b**, **3c**, and **4d** and compounds **4a** and **4c** were moderately active against fungus *Aspergillus flavus* and *Aspergillus niger* respectively. Remaining compounds showed poor activity.

Antioxidant Activity

DPPH radical scavenging activity

Reactive oxygen species (ROS) are formed in living cells via both enzymatic and non-enzymatic mechanisms. Some ROS are required for the creation of specific physiological functions and some ROS formation is involved in the pathogenesis of a number of diseases[18]. Antioxidants may react with ROS and decrease their toxic actions, and for that reason many researchers are searching for novel natural and synthetic antioxidants. Various antioxidant methods have been used to monitor and compare antioxidant activity of foods. The main characteristic of an antioxidant is its ability to trap free radicals which is generally measured by using free radicals such as DPPH where free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) is supposed to abstract the proton from the sample and convert it into 1,1-diphenyl-2-picrylhydrazine. This reaction could be visualized by change in colour from deep violet to light yellow and is monitored spectrophotometrically at characteristic wavelength of 517 nm. All compounds were tested for their interaction with stable free radical DPPH by laboratory method described by Hatano's method[19] using butylated hydroxyl anisole (BHA), tertiary butylated hydroxyl quinone (TBHQ) and ascorbic acid (AA) as standards. The DPPH radical scavenging activity (RSA) of test compounds in methanolic solution at concentrations 25, 50, 75, and 100 µg/ml containing freshly prepared DPPH solution (0.004% w/v) was carried out and compared with standards BHA, TBHQ and AA. The percent inhibition was calculated from the following equation. The results are shown in Fig. 1& 2.

$$\% \text{ of inhibition RSA} = \frac{\text{Absorbance of control- Absorbance of test sample}}{\text{Absorbance of control}} \text{ x } 100$$

The results revealed that compounds **3c, 3d, 4c** and **4d** exhibited good RSA (76.41%, 65.09%, 74.86%, 71.90%) at 100 µg/ml respectively. Remaining compounds exhibited moderate or poor radical scavenging activity when compared with the standards BHA, TBHQ and AA.

Conclusion:

The present study revealed that compounds **4a, 4c** and **4d** were exhibited good antimicrobial activity against *B. Substilus, S. Aureus*, *A. Niger* and *A. Flavus*. Whereas antioxidant activity results suggested that nitro and hydroxyl substituted compounds **3c**, **3d, 4c,** and **4d** were the most active among the series exhibiting good radical scavenging activity compared to other compounds. This activity may be due to the better radical stabilizing ability of coumarin and thiopyrimidine systems.

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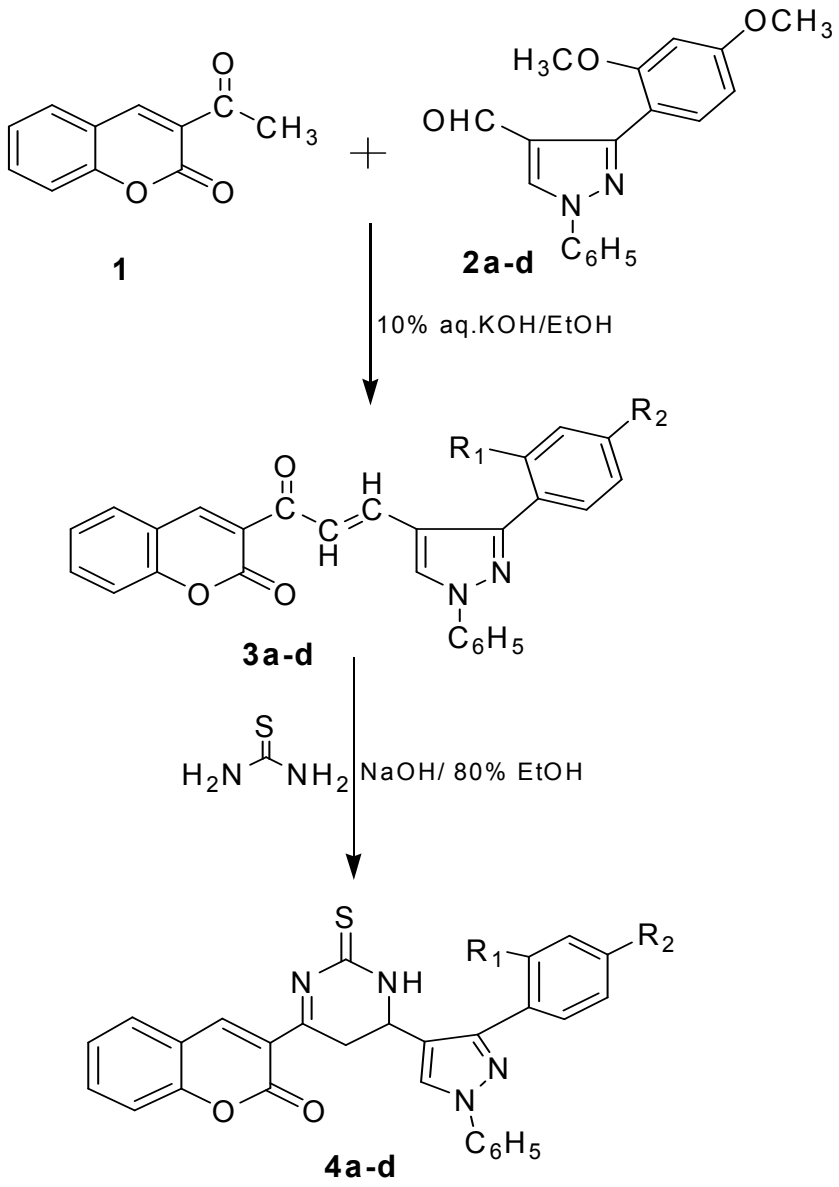

Scheme-1

a: $R_1 = R_2 = H$ b: $R_1 = OCH_3$, $R_2 = OCH_3$

c: $R_1 = H$, $R_2 = NO_2$ d: $R_1 = OH$, $R_2 = H$

Table – 1: Physical data of the Synthesized Compounds

Compd. No.	Substitution		Mol. Formula (Mol.wt.)	M. P. (^{0}C)	Yield (%)
	R_1	R_2			
3a	H	H	$C_{27}H_{18}N_2O_3$ (418)	138-140	69
3b	2-OCH_3	4-OCH_3	$C_{29}H_{22}N_2O_5$ (478)	130-132	65
3c	H	4-NO_2	$C_{27}H_{17}N_3O_5$ (463)	142-144	66
3d	2-OH	H	$C_{27}H_{18}N_2O_4$ (434)	160-162	45
4a	H	H	$C_{28}H_{20}N_4O_2S$ (476)	130-132	62
4b	2-OCH_3	4-OCH_3	$C_{30}H_{24}N_4O_4S$ (536)	174-176	58
4c	H	4-NO_2	$C_{28}H_{19}N_5O_4S$ (521)	106-108	49
4d	2-OH	H	$C_{28}H_{20}N_4O_3S$ (492)	120-122	46

Figure-1: Free radical scavenging activities of compounds (3a-d)

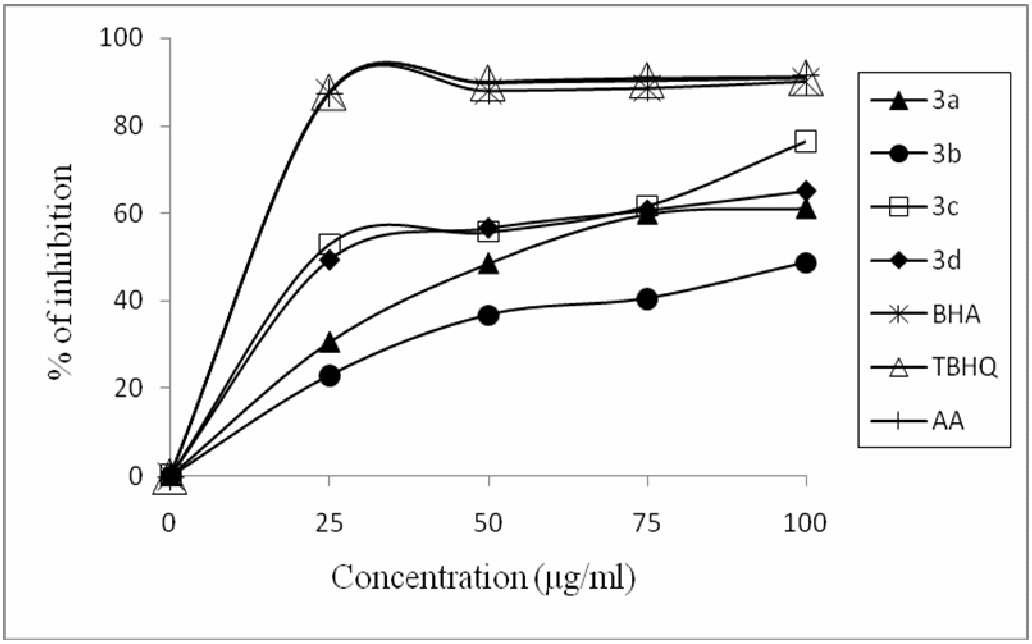

Figure-2: Free radical scavenging activities of compounds (4a-d)