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Research Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NOVEL PYRAZOLINE DERIVATIVES DERIVED FROM N-SUBSTITUTED QUINOLINYL CHALCONES

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ABSTRACT

A series of novel substituted 1-amino-3-(5-phenyl-4, 5-dihydro-1H-pyrazol-3-yl) quinolin-2(1H)-one (AJP1-AJP8) have been synthesized upon reaction with 1-amino-3-cinnamoyl-quinolin-2(1H)-one by using hydrazine hydrate as cyclising medium in alcohol medium. 1-amino-3-cinnamoyl-quinolin-2(1H)-one were synthesized by condensing 3-acetyl-1-amino-quinolin-2-one with different substituted benzaldehyde in presence of ethanolic KOH. The structures of the final synthesized compounds were confirmed by IR, ^{1}H NMR and mass spectra. The synthesized compounds were screened for their antibacterial and antifungal activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*, *Aspergillus niger* respectively by cup plate method. Compounds AJP1, AJP3, AJP4, AJP5, AJP6 and AJP7 showed good antibacterial activity compared to the standard drug amoxicillin. Compounds AJP1, AJP3, AJP5 and AJP7 showed moderate antifungal activity compared to the standard drug fluconazole. The synthesized compounds were screened for their anti-inflammatory activity by Carrageenan induced paw edema method. Compounds AJP1 and AJP7 showed significant anti-inflammatory activity compared to the standard drug diclofenac sodium.

Keywords: 2-Quinolones, Pyrazoline, antibacterial activity, antifungal activity, anti-inflammatory activity.

INTRODUCTION

2-Quinolones (carbostyrils or 1-aza coumarins) are isosteric with coumarins and isomeric to 4-quinolones could become the probable potential candidate for antibacterial activity[1]. 2-Quinolone derivatives were found to be associated with various biological activities such as antitumor[2], anti-inflammatoryl[3], antiplatelet, antiulcer[4], antioxidant activity[5] and antidepressant. Many substituted quinolin-2-one derivatives have recently craned great interest in chemotherapy as antitumor drugs[6]. Pyrazolines are the dihydro derivatives of pyrazoles and are well known five membered nitrogen containing compounds. Formation of pyrazolines has been reported by the action of nucleophile like hydrazine hydrate or phenyl hydrazine by using alcohol as solvent. Pyrazolines are associated with diverse biological and pharmacological activities like antimicrobial[7], antitubercular[7], anti-inflammatory[8], antidepressant[9]. Some other activities are also exhibited by them such as anticonvulsant, antitumor, analgesic and anti androgenic. The combinations of genetic versatility of microbes and widespread over use of antibiotics has lead for increasing clinical resistance of previously sensitive micro-organisms and the emergence of previously uncommon infections[10]. The discovery of new molecules exhibiting prominent activities against infectious micro-organisms such as toxogenic *Staphylococci, E. coli, Anaerobes, Pseudomonas,* various fungi and others showing no cross-resistance with the existing antibiotics would therefore be more welcome. Anti-inflammatory drugs presently available for the treatment of various inflammatory disorders have diverse and undesirable side effects. Hence there is a great need for developing new anti-inflammatory and anti-infective agents. By considering the above facts and their increasing importance in pharmaceutical and biological field, it was considered of interest to synthesize some new chemical entities incorporating the two active pharmacophores in a single molecular frame work and to evaluate their biological activities. Hence an attempt was made towards the incorporation of pyrazolines with quinolinyl chalcones and to probe how this combination could influence the biological activity. Hence all the synthesized compounds were evaluated for their antimicrobial and anti-inflammatory activities and compared with standard drugs.

MATERIALS AND METHODS

All the chemicals were of analytical grade: Substituted salicylaldehyde, Ethylacetoacetate, Absolute ethanol, Piperidine, Glacial acetic acid, Hydrazine hydrate and Substituted benzaldehyde. Melting points were determined by open capillary method and are uncorrected. The purity of the compounds was monitored by thin layer chromatography (TLC) using silica gel G plates. The spots were visualized under UV light and by the exposure to iodine vapors. The homogeneity of the compounds was checked on silica gel-G coated plate by using Toluene: Acetone (9:1) as solvent. All IR spectra were recorded in Alpha Bruker using ATR method. ^{1}H NMR spectra were recorded on Bruker spectrophotometer (400 MHz) in DMSO-d_6 solvent using tetra methyl silane (TMS) as an internal standard. Mass spectra were recorded by LCMS method.

General Procedure

Synthesis of Substituted 1-amino-3-cinnamoyl-quinolin-2(1H)-one[11] (AJC1-AJC8)

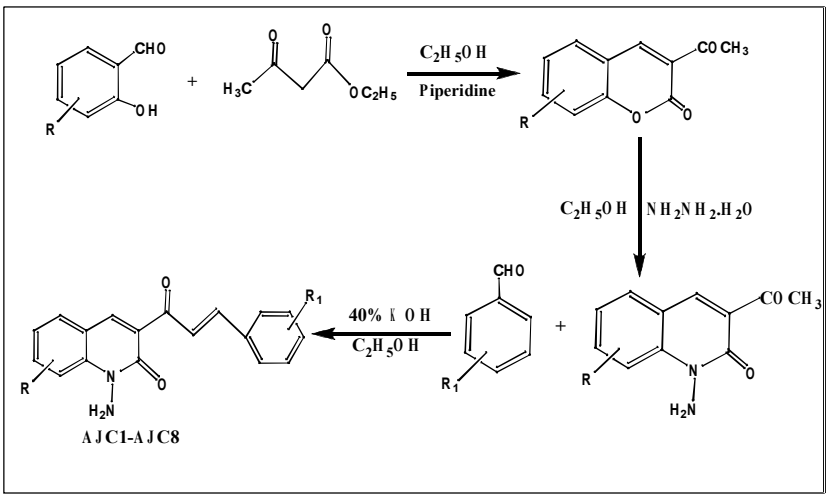
A mixture of 3-acetyl-1-amino-quinolin-2-one (0.01 mol) and different substituted benzaldehyde (0.01 mol) in 20 ml absolute ethanol was stirred together at room temperature for 24 hours in the presence of 40 % KOH. The completion of the reaction was monitored by TLC. The reaction mixture was then poured into crushed ice and acidified with 2N HCl with stirring. The product obtained was filtered, washed with water and re crystallized from ethanol.

Synthesis of Substituted 1-amino-3-(5-phenyl-4, 5-dihydro-1H-pyrazol-3-yl) quinolin-2(1H)-one[12] (AJP1-AJP8)

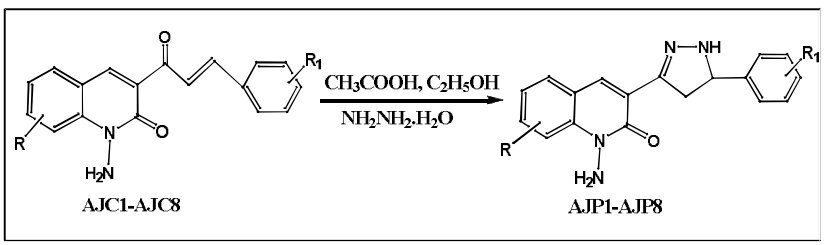
A mixture of substituted 1-amino-3-cinnamoyl-quinolin-2(1H)-one (0.1 mol) and hydrazine hydrate (0.01 mol) in 25 ethanol containing 2-3 drops of glacial acetic acid was refluxed for 5-6 hours. The reaction mixture was monitored by TLC. It was then cooled and added to ice cold water. The precipitated solid obtained was filtered, washed with water and re crystallized from ethanol.

General Scheme of Synthesis

STEP 1



STEP 2



R: H, 6-NO_2
R_1: 3-NO_2, 3, 4, 5-OCH_3, 4-CH_3, 4-OH, 2-Cl, 2-NO_2

Spectral data

1-amino-3-(3-nitrophenyl) acryloyl) quinolin-2(1H)-one (AJC1)

IR KBr (cm^{-1}): 1506 (Ar C=C str), 829 (Ar C-H bend), 2950 (C-H aliphatic str), 1701 (C=O str), 3398 (NH_2 str), 1350 (Ar-NO_2 str). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.12-8.28 (m, 9H, Ar-H), 4.81 (d, 2H of CH=CH), 3.73(s, 2H, NH_2). MS (M^+): m/z 235.

1-amino-3-(5-(3-nitrophenyl)-4, 5-dihydro-1*H*-pyrazol-3-yl) quinolin-2(1H)-one (AJP1)

IR (cm^{-1}): 1502 (Ar C=C str), 854 (Ar C-H bend), 2961 (C-H aliphatic str), 3422 (NH_2 str), 1360 (Ar-NO_2), 960 (N-N str). ^{1}H NMR (400 MHz, DMSO-d_6)**:** δ 7.13-7.26 (m, 9H, Ar-H), δ 4.5 (s, 2H, NH_2), δ 3.32-3.35 (m, 2H, CH_2), δ 7.65 (s, IH, NH). Mass (m/z): 349 (M^+)

1-amino-6-nitro-3-(5-(3, 4, 5-trimethoxyphenyl)-4, 5-dihydro-1H-pyrazol-3-yl) quinolin-2(1H)-one (AJP8)

IR (cm^{-1}): 1506 (Ar C=C str), 852 (Ar C-H bend), 2968 (C-H aliphatic str), 1250 (C-O str), 3435 (NH_2 str), 1365 (Ar-NO_2), 963 (N-N str). ^{1}H NMR (400 MHz, DMSO-d_6)**:** δ 7.18-7.42 (m, 6H, Ar-H), δ 4.32 (s, 2H, NH_2), δ 3.30-3.33 (m, 2H, CH_2), δ 7.68 (s, IH, NH). Mass (m/z): 439 (M^+)

Antimicrobial Activity

All the synthesized compounds were evaluated for their antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, *Bacillus subtilis* and antifungal activity against *Candida albicans* and *Aspergillus niger* using cup plate method[13]. The synthesized test compounds were tested at a concentration of 100 µg / 50 µl and the standard compound i.e. Amoxicillin and Fluconazole were tested at 25 µg / 50 µl. Dimethyl formamide (DMF) was used as control. In this technique, melted agar inoculated with microorganisms is poured into petridishes. Wells are made in the agar plate and a specific volume of the antimicrobial substances are placed in them, plates were incubated at a temperature of 37^0C for 24 h and 25^0C for 48 h, in case antibacterial and antifungal activity. The antimicrobial substance diffuses through agar around its well and produces a clear zone of inhibition. The diameter of this zone (mm) gives an estimation of the degree of activity of the antimicrobial substance.

Pharmacological screening

Acute toxicity studies

The preliminary pharmacological studies were conducted to assess the acute pharmacological effects and LD_{50} of the

drug. To assess the safety of the synthesized compounds under study acute toxicity studies were carried out as per OECD guidelines 425[14] in healthy adult Wistar rats by "up and down" method. The synthesized pyrazoline derivatives were given to rats in the form of suspension at a dose of 200 mg/kg body weight by oral route. The standard drug Diclofenac sodium was given to rats at a dose of 13.5 mg/kg body weight by oral route.

Table 1: Physicochemical data of the compounds (AJP_1-AJP_8)

Comp. code	R	R_1	Mol. formula	Mol. wt	M.P °C	R_f Value	% Yield
AJP-1	H	3-NO_2	$C_{18}H_{15}N_5O_3$	349	212-214	0.68	77
AJP-2	H	3,4,5-OCH_3	$C_{21}H_{22}N_4O_4$	394	148-150	0.52	72
AJP-3	H	4-CH_3	$C_{19}H_{18}N_4O$	318	170-171	0.58	78
AJP-4	H	4-OH	$C_{18}H_{16}N_4O_2$	320	182-184	0.64	80
AJP-5	H	2-Cl	$C_{18}H_{15}ClN_4O$	338	156-158	0.56	74
AJP-6	H	2-NO_2	$C_{18}H_{15}N_5O_3$	349	192-193	0.72	77
AJP-7	6-NO_2	3-NO_2	$C_{18}H_{14}N_6O_5$	394	224-226	0.6	54
AJP-8	6-NO_2	3,4,5-OCH_3	$C_{21}H_{21}N_5O_6$	439	154-156	0.56	50

Table 2: Antimicrobial Activity of the synthesized compounds (AJP1-AJP8) by cup plate method

S. No.	Comp.	Diameter of zone of inhibition (mm)					
		S. aureus	*B. subtilis*	*E. coli*	*P. aureginosa*	*C. albicans*	*A. niger*
1	AJP-1	17	15	16	21	12	11
2	AJP-2	10	08	11	12	08	07
3	AJP-3	18	17	13	15	12	13
4	AJP-4	13	15	18	20	06	-
5	AJP-5	14	13	17	23	14	12
6	AJP-6	19	16	17	21	07	08
7	AJP-7	17	16	19	23	13	15
8	AJP-8	09	07	10	11	07	09
	Amoxicillin	26	23	25	30	-	-
	Fluconazole	-	-	-	-	23	26
	Control	-	-	-	-	-	-

Table 3: Anti-inflammatory effect of Pyrazoline derivatives (AJP1-AJP8) using Carrageenin induced paw edema in rats

Treatment	Dose mg/kg	Change in paw volume in ml (% inhibition)				
		0 h	1 h	2 h	3 h	4 h
Control	_	0.22 ± 0.03	0.28 ± 0.03	0.37 ± 0.03	0.38 ± 0.02	0.41 ± 0.02
Diclofenac Sodium	13.5	0.08 ± 0.02** (63.63)	0.10 ± 0.02** (64.28)	0.12 ± 0.02** (67.56)	0.13 ± 0.01** (65.57)	0.14 ± 0.01** (65.85)
AJP1	200	0.18 ± 0.01* (22.22)	0.19 ± 0.01* (32.14)	0.21 ± 0.01** (37.83)	0.23 ± 0.01** (39.47)	0.24 ± 0.02** (41.46)
AJP2	200	0.19 ± 0.01 (15.79)	0.21 ± 0.01 (25)	0.28 ± 0.01 (24.32)	0.27 ± 0.01 (28.94)	0.31 ± 0.03 (24.39)
AJP3	200	0.17 ± 0.03 (22.72)	0.19 ± 0.03 (32.36)	0.26 ± 0.01 (24.32)	0.24 ± 0.04 (36.84)	0.23 ± 0.03 (43.90)
AJP4	200	0.17 ± 0.01 (29.41)	0.20 ± 0.01 (28.57)	0.22 ± 0.02 (40.54)	0.26 ± 0.04 (47.36)	0.23 ± 0.01 (43.90)
AJP5	200	0.17 ± 0.01 (22.72)	0.19 ± 0.01 (32.36)	0.21 ± 0.02 (43.24)	0.22 ± 0.04 (42.10)	0.25 ± 0.04 (39.02)
AJP6	200	0.15 ± 0.02* (31.81)	0.18 ± 0.02* (35.71)	0.21 ± 0.01 (43.24)	0.23 ± 0.01 (39.47)	0.26 ± 0.01** (36.58)
AJP7	200	0.16 ± 0.01* (27.22)	0.18 ± 0.01* (35.71)	0.20 ± 0.01** (45.94)	0.19 ± 0.03** (50)	0.21 ± 0.02** (48.78)
AJP8	200	0.18 ± 0.01 (22.22	0.19 ± 0.01 (32.36)	0.21 ± 0.01 (37.83)	0.24 ± 0.02 (36.84)	0.25 ± 0.03 (39)

All values are expressed as mean ± SEM (n = 6), *P < 0.05 significant compared to control, **P < 0.01 significant compared to control

Anti-inflammatory activity

The anti-inflammatory activity of the test compounds was carried out using carrageenan-induced rat paw edema model according to Winter *et al*. by employing 1 % Carrageenan solution as phlogistic agent. Edema was induced in the left hind paw of Wistar rats (150-200 g) of either sex by the sub-plantar injection of 0.1 ml of 1 % Carrageenan in distilled water. Each group composed of six animals. The animals which were bred in our laboratory were housed under standard conditions and received a diet of commercial food pellets and water *ad libitum* during the maintenance but they were entirely fasted during the experiment period. Our studies were conducted in accordance with recognized guidelines on animal experimentation. The test compounds were given intraperitoneally 30 minutes after Carrageenan injection. Diclofenac sodium was taken as the standard at a dose of 13.5 mg/kg body weight (p.o). The rat paw volume was measured after 1 h, 2 h, 3 h and 4 h respectively after Carrageenan injection by using Plethysmometer. The difference between the paw volume at 4 h and 0 h measurement was calculated and taken as edema volume. Percentage inhibition in the paw edema was calculated by using the formula,

% Edema inhibition= 100 $(1-V_t/V_c)$,
Where V_t represents mean increase in paw volume of test and V_c represents mean increase in paw volume of control

Statistical Analysis

All experimental groups were composed of six animals. Data obtained from animal experiments were expressed as mean ± SEM. The statistical significance of difference between groups were assessed by means of analysis of variance (ANOVA) followed by Dunnet's test.

RESULTS AND DISCUSSION

Antimicrobial Activity

The *in vitro* antibacterial and antifungal activity of the synthesized compounds were determined by using cup-plate method[17]. The results of antibacterial and antifungal activity of newly synthesized compounds are reported against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and two fungi *Candida albicans* and *Aspergillus niger*. Compounds AJP1, AJP3, AJP6 and AJP7 showed good antibacterial activity against gram +ve bacteria and compounds AJP1, AJP4, AJP5, AJP6 and AJP7 showed good antibacterial activity against gram -ve bacteria respectively compared to the standard drug amoxicillin. Compounds AJP1, AJP3, AJP5 and AJP7 showed moderate antifungal activity against *Candida albicans* and compounds AJP3 and AJP7 showed moderate antifungal activity against *Aspergillus niger* compared to the standard drug fluconazole. The results of the antimicrobial activity are summarized in Table 2.

Anti-inflammatory activity

All the synthesized compounds were tested for their anti-inflammatory activity using Carrageenan induced rat hind paw edema method. Data of anti-inflammatory activity was expressed as mean ± SEM, and the student's t-test was applied to determine the significance of the difference between the control group and rats treated with the test compounds. The anti-inflammatory activity of the newly synthesized compounds (AJP1-AJP8) was compared with the standard Diclofenac sodium 13.5 mg/kg body weight, showing 64.52 % inhibition of rat paw edema whereas tested compounds showed inhibition ranging from 24.39 to 48.78 % after 120 minutes. Compounds AJP1 and AJP7 exhibited significant anti- inflammatory activity as compared to the standard drug diclofenac sodium. The results of the anti-inflammatory studies are summarized in Table 3.

CONCLUSION

The above results proved that novel pyrazoline derivatives synthesized from quinolinyl chalcones are found to be interesting lead molecules as antimicrobial and anti-inflammatory agents. The study reports the successful synthesis of pyrazoline derivatives with moderate yields. Most of the synthesized compounds showed good antimicrobial activity and few compounds showed significant anti-inflammatory activity. It can be concluded that pyrazoline derivatives containing 2-quinolone moiety certainly holds great promise towards the good activity leads in medicinal chemistry.

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