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Synthesis, Antimicrobial and Anti-inflammatory Activity of Newly Synthesized Isoxazoline Incorporated 2-Quinolones

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ABSTRACT
A series of novel substituted 1-amino-3-(5-phenyl-4,5-dihydroisoxazol-3-yl) quinolin-2(1*H*)-one (AJI1-AJI8) have been synthesized upon reaction with 1-amino-3-cinnamoyl-quinolin-2(1*H*)-one by using hydroxylamine hydrochloride as cyclising agent in alcohol medium. The intermediate chalcones 1-amino-3-cinnamoyl-quinolin-2(1*H*)-one (AJC1-AJC8) were synthesized by condensing 3-acetyl-1-amino-quinolin-2-one with different substituted benzaldehyde in presence of 40% ethanolic KOH. The title compounds obtained were characterized by IR, ^{1}H NMR, mass spectra and elemental analysis. The synthesized compounds were screened for their antimicrobial and anti-inflammatory activity.

Keywords: 2-Quinolones, chalcones, isoxazoline, antimicrobial, anti-inflammatory.

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-inflammatory [3], antiplatelet, antiulcer [4], antioxidant [5] and antidepressant activity. Many substituted quinolin-2-one derivatives have recently craned great interest in chemotherapy as antitumor drugs. [6] Isoxazolines are the dihydro derivatives of isoxazoles. From the literature survey, it was found that large numbers of isoxazoline derivatives of pharmacological significance were synthesized by the action of hydroxylamine hydrochloride on chalcones in presence of base. Synthesis of novel isoxazoline derivative remains a main focus of medicinal chemist, due to their diverse pharmacological activity. Cycloserine is the best known antibiotic drug that possess antitubercular, antibacterial activities and in treatment of leprosy. Acivicin is an antitumor, antileishmania drug, while isoxaflutole is used as herbicidal drug. Isoxazoline derivatives have been reported to possess antifungal [7], antibacterial [8]antioxidant, cytotoxicity [9] and anti-inflammatory [10] activity. In addition, isoxazoline derivatives have played a crucial role as intermediates in the organic synthesis of number of heterocyclic pharmacological active compounds.

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 isoxazolines with substituted 2-Quinolones and to probe how this combination could influence the biological activity.

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MATERIALS AND METHODS

All the chemicals were of analytical grade: substituted ethanol,
piperidine, glacial acetic acid, hydroxylamine hydrochloride and substituted benzaldehyde.

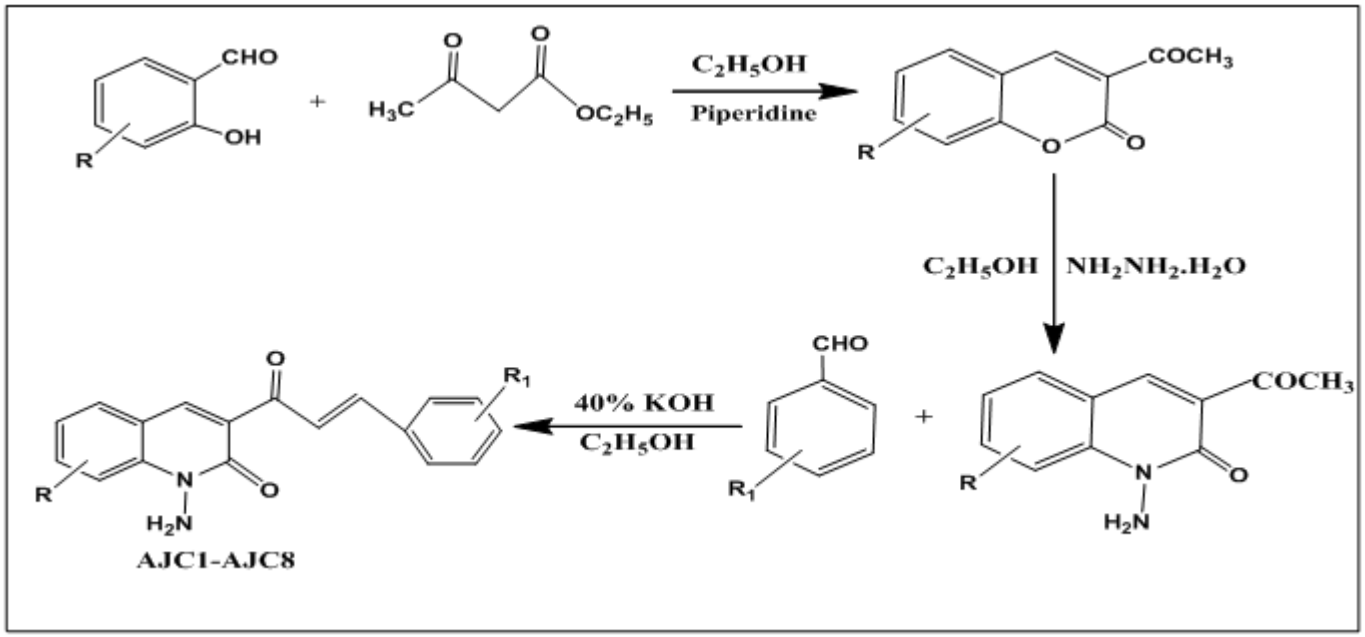
Melting points were determined by open capillary method and are uncorrected. The purity of the compounds was monitored by thin layer chromatography (TLC) using silica gel G plates. The spots were visualized under UV light and by the exposure to iodine vapors. The homogeneity of the compounds were checked on silica gel-G coated plate by using Chloroform: 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

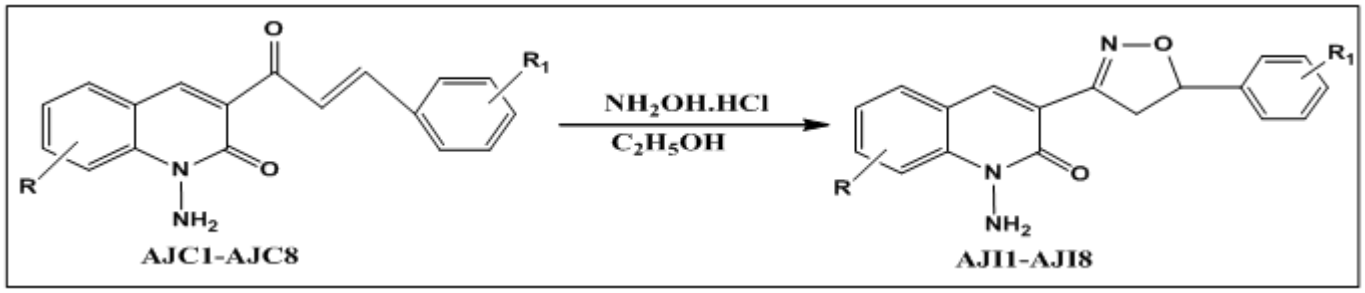
Synthesis of Substituted 1-amino-3-cinnamoyl-quinolin-2(1H)-one [11] (AJC1-AJC8)

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

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 recrystallised from ethanol.

Synthesis of Substituted 1-amino-3-(5-phenyl-4,5-dihydroisoxazol-3-yl)quinolin-2(1*H*)-one [12] (AJI1- AJI8)

A mixture of substituted 1-amino-3-cinnamoyl-quinolin-2(1H)-one (0.01 mol), hydroxylamine hydrochloride (0.01 mol) in 25 ethanol was refluxed for 4-5 hours in the presence of 30% KOH. It was then cooled and added to ice cold water and acidified with dilute HCl. The precipitated solid obtained was filtered, washed with water, dried and recrystallised from ethanol.

Spectral data

1-amino-3-(3-nitrophenyl)acryloyl)quinolin-2(1*H*)-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-dihydroisoxazol-3-yl)quinolin-2(1*H*)-one (AJI1)

IR KBr (cm^{-1}): 1512(Ar C=C str), 838 (Ar C-H bend), 2922 (C-H aliphatic str), 3393 (NH_2 str), 1339 (Ar-NO_2), 1276 (N-O str), 1649(C=N str).

^{1}H NMR (400 MHz, DMSO-d_6): δ 6.97-7.12 (m, 9H, Ar-H), 4.27 (s, 2H, NH_2), 3.61 (m, 2H, CH_2).

Mass (m/z): 350 (M^+)

1-amino-3-(5-(2-chlorophenyl)-4,5-dihydroisoxazol-3-yl)quinolin-2(1*H*)-one (AJI5)

IR KBr (cm^{-1}): 1516(Ar C=C str), 835 (Ar C-H bend), 2929 (C-H aliphatic str), 3390 (NH_2 str), 776 (C-Cl str), 1272 (N-O str), 1640(C=N str).

^{1}H NMR (400 MHz, DMSO-d_6): δ 6.95-7.16 (m, 9H, Ar-H), 4.20 (s, 2H, NH_2), 3.52 (m, 2H, CH_2).

Mass (m/z): 339 (M+1)

1-amino-6-nitro-3-(5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-3-yl)quinolin-2(1*H*)-one (AJI8)

IR KBr (cm^{-1}): 1510(Ar C=C str), 835 (Ar C-H bend), 2920 (C-H aliphatic str), 3383 (NH_2 str), 1128(C-O str), 1335 (Ar-NO_2), 1276 (N-O str), 1648 (C=N str).

^{1}H NMR (400 MHz, DMSO-d_6): δ 6.92-7.86 (m, 7H, Ar-H), 4.25 (s, 2H, NH_2), 3.38 (m, 2H, CH_2), 3.83(s, 3H, OCH_3).

Mass (m/z): 440 (M^+)

Antimicrobial Activity

All the synthesized compounds were evaluated for their minimum inhibitory concentration by tube dilution method. [13] The synthesized test compounds were tested at different concentrations and amoxicillin and fluconazole was used as standard. Serial dilutions of the test compound was made in a

liquid medium which was inoculated with a standardized number of organisms and incubated for 24 hrs. The lowest concentration of test compound preventing appearance of turbidity is considered to be the minimal inhibitory concentration (MIC). After preparation of different concentrations of the antimicrobial agent in brain heart infusion broth (by using the broth dilution method), we inoculate them with the tested organism. Then after incubation we can determine the MIC by choosing the lowest concentration in which no growth occurs.

Anti-inflammatory activity

The anti-inflammatory activity of the test compounds was carried out using carrageenan-induced rat paw edema [14] model according to Winter *et al*. [15] 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 min after Carrageenan injection. Naproxen 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,

$$\% \text{ Edema inhibition} = 100(1-V_t/V_c),$$

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.

Table 1: Physical data of the newly synthesized Isoxazoline derivatives (AJI1-AJI8)

Comp. code	R	R_1	Mol. formula	Mol. wt	M.P °C	R_f Value	% Yield
AJI-1	H	3-NO_2	$C_{18}H_{14}N_4O_4$	350	202-204	0.68	80
AJI-2	H	3,4,5-OCH_3	$C_{21}H_{21}N_3O_5$	395	142-143	0.58	78
AJI-3	H	4-CH_3	$C_{19}H_{17}N_3O_2$	319	160-161	0.60	75
AJI-4	H	4-OH	$C_{18}H_{15}N_3O_3$	321	172-174	0.52	79
AJI-5	H	2-Cl	$C_{18}H_{14}ClN_3O_2$	339	152-154	0.56	83
AJI-6	H	2-NO_2	$C_{18}H_{14}N_4O_4$	350	186-188	0.78	74
AJI-7	6-NO_2	3-NO_2	$C_{18}H_{13}N_5O_6$	395	211-213	0.48	57
AJI-8	6-NO_2	3,4,5-OCH_3	$C_{21}H_{20}N_4O_7$	440	150-152	0.72	52

Table 2: Minimum inhibitory concentration of substituted isoxazolines (AJI1-AJI8) by tube dilution method.

Comp. Code	Minimum inhibitory concentration (µg)					
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
AJI1	100	100	50	100	25	50
AJI2	0.8	3.2	6.25	6.25	50	25
AJI3	3.2	50	25	50	12.5	12.5
AJI4	50	50	50	25	R	100
AJI5	12.5	6.25	12.5	0.8	12.5	100
AJI6	R	100	R	100	50	50
AJI7	50	50	25	100	25	25
AJI8	0.8	1.6	3.12	1.6	50	25
Amoxicillin	**1**	**2**	**2**	**1**		
Fluconazole					**16.6**	**8.3**

Table 3: Anti-inflammatory effect of Isoxazoline derivatives (AJI1-AJI8) using Carrageenan induced paw edema in rats.

Treatment	Dose mg/kg	Change in the paw volume in ml (% inhibition)			
		1 h	2 h	3 h	4 h
Control	Vehicle	0.430±0.005	0.735±0.007	0.811±0.006	0.875±0.007
Diclofenac Sodium	13.5	0.213±0.003** (50.46)	0.343±0.011** (53.33)	0.410±0.007** (49.44)	0.426±0.008** (51.31)
AJI1	200	0.308±0.007* (28.37)	0.416±0.010* (43.40)	0.463±0.007* (42.90)	0.535±0.010* (38.85)
AJI2	200	0.255±0.006** (40.69)	0.336±0.008** (54.28)	0.426±0.010** (47.47)	0.455±0.007** (48.00)
AJI3	200	0.246±0.006** (42.79)	0.353±0.006** (51.43)	0.443±0.010** (45.37)	0.451±0.007** (48.45)
AJI4	200	0.298±0.006* (30.69)	0.393±0.015* (46.53)	0.470±0.009* (42.04)	0.543±0.010* (37.94)
AJI5	200	0.315±0.004* (26.74)	0.433±0.004** (41.08)	0.480±0.003** (40.81)	0.540±0.005** (38.28)
AJI6	200	0.295±0.007* (31.39)	0.368±0.013** (49.93)	0.465±0.007** (42.66)	0.525±0.009** (40.00)
AJI7	200	0.352±0.007 (18.14)	0.582±0.008 (20.81)	0.608±0.006 (25.03)	0.638±0.010 (27.08)
AJI8	200	0.335±0.006 (22.09)	0.516±0.008 (29.79)	0.568±0.004 (29.96)	0.602±0.009 (31.2)

All values are expressed as mean ± SEM (n = 6).

*P < 0.05 significant compared to control.

**P < 0.01 significant compared to control.

RESULTS AND DISCUSSION

Antimicrobial Activity

All the synthesized compounds were evaluated for their minimum inhibitory concentration by tube dilution method. Compounds AJI2, AJI3, AJI5 and AJI8 showed significant antibacterial activity against gram +ve bacteria and compounds AJI2, AJI5 and AJI8 showed significant antibacterial activity against gram-ve bacteria compared to standard drug amoxicillin. Compounds AJI3 and AJI5 showed significant antifungal activity against *C. albicans* and none of the compounds showed significant antifungal activity against *A. niger* compared to standard drug fluconazole. The results of the minimum inhibitory concentration are summarized in Table 2.

Anti-inflammatory activity

All the synthesized compounds were tested for their anti-inflammatory activity using Carrageenan induced rat paw edema method at a dose of 200 mg/kg of body weight using Diclofenac sodium as standard drug at the dose level of 13.5 mg/kg body weight. The percentage inhibition of edema volume was calculated by using the formula, % inhibition = $100(1- Vt/V_c)$, Where V_t and V_c are the relative change in the edema volume of paw after the administration of the test and control respectively. Percentage inhibition shown by tested compounds is given in Table 3. Compounds AJI1, AJI2, AJI3, AJI4, AJI5 and AJI6 showed significant anti-inflammatory activity compared with respective control groups but the maximum inhibition of paw edema was shown by compounds AJI2 and AJI3 at 4^{th} hour when compared to the standard drug diclofenac sodium.

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

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