Open Access Contents
Int. J. Drug Dev. & Res.
| April - June 2014 | Vol. 6 | Issue 2 | ISSN 0975-9344 |
www.ijddr.in

Full Length Original Research Paper

Synthesis, Antimicrobial and Anti-Inflammatory studies of some novel Schiff Base Derivatives

Kumar Abhishek*

Fernandes Jennifer

Kumar Pankaj

Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences , Nitte University, Paneer, Deralakatte-575018, Mangalore, Karnataka.

Corresponding Authors:
Email: abhi12bunty@gmail.com

Abstract:
A series of novel substituted 3-acetyl-1-(benzylideneamino) quinolin-2(1*H*)-one (1-12) have been synthesized by condensing different substituted 3-acetyl-1-amino-quinolin-2-one and aromatic aldehydes in alcohol medium. 3-acetyl-1-amino-quinolin-2-one were synthesized from substituted 3-acetyl coumarin upon refluxing with hydrazine hydrate and ethanol. The structures of the final synthesized compounds were confirmed by IR, ^{1}H NMR and mass spectra.
The synthesized compounds were screened for their antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*, Aspergillus niger respectively by tube dilution method. Most of the compounds showed good minimum inhibitory concentration compared to the standard drug amoxicillin and fluconazole respectively. Anti-inflammatory studies were carried out by carrageenan induced paw edema method. Anti-inflammatory studies showed statistically significant activity when compared to control.

Keywords: 2-Quinolones, schiff base, antimicrobial activity, minimum inhibitory concentration, 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[5] and antidepressant activity. Many substituted quinolin-2-one derivatives have recently craned great interest in chemotherapy as antitumor drugs[6]. Compounds containing an azomethine group (-CH=N-) known as Schiff bases are formed by the condensation of a primary amine with a carbonyl compound. Schiff bases of aliphatic aldehydes are relatively unstable and are readily polymerized while those of aromatic aldehydes having an effective conjugation system are more stable. Schiff bases and their complexes are largely studied because they interested and important properties such as their ability to bind reversibly oxygen[7] redox systems in biological systems and oxidation of DNA. Many biological important Schiff bases ligands have been reported which possess antibacterial, antifungal[8], antimicrobial[9], anticonvulsant, antioxidant[10], anti-inflammatory[11] and antitumor activity[12].

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 Schiff bases with substituted 3-acetyl-1-amino-quinolin-2-one and to probe how this combination could influence the biological activity. Hence 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 were checked on silica gel-G coated plate by using Chloroform: Methanol (8:2) 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 was recorded by LCMS method.

General Procedure:

Synthesis of substituted 3-acetyl-1-amino-quinolin-2-one (AJQ1-AJQ12) [13]

Substituted 3-acetyl coumarin (0.01 mol) with excess hydrazine hydrate 99% (0.1 mol) in 25 ml ethanol was refluxed for 12 hours. It was then cooled and poured into crushed ice with stirring. The solid product formed was filtered and recrystallised from ethanol.

Synthesis of Substituted 3-acetyl-1-(benzylideneamino) quinolin-2(1*H*)-one (AJS1-AJS12) [14]

A mixture of substituted 3-acetyl-1-amino-quinolin-2-one (0.01mol) and substituted benzaldehyde (0.01 mol) was refluxed for 4-5 hours with continuous stirring in presence of few drops of glacial acetic acid as catalyst. The reaction mixture was monitored by TLC. It was then cooled and added to ice cold water. The precipitated solid was filtered and recrystallised from ethanol.

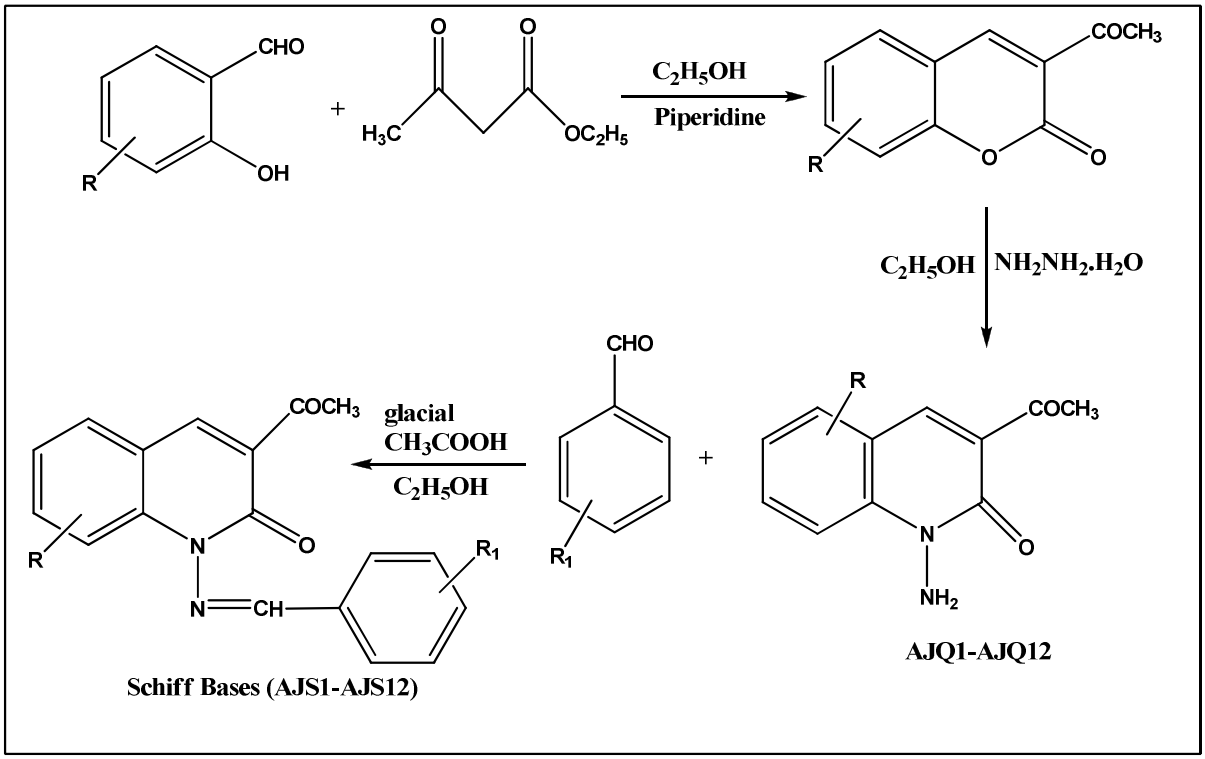

Fig. 1: General Scheme of Synthesis

Covered in Scopus & Embase, Elsevier Int. J. Drug Dev. & Res., April - June 2014, 6 (2): 165-171

R: H, 6-NO_2, 6-Cl

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

Spectral data

3-acetyl-1-aminoquinolin-2(1*H*)-one (AJQ1)

IR (cm^{-1}): 1506(Ar C=C str), 829 (Ar C-H bend), 2950(C-H aliphatic str), 1701 (C=O str), 3362, 3398 (N-H str).

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.25-8.27 (m, 5H, Ar-H), 3.73(s, 2H, NH_2), 2.59 (s, 3H, $COCH_3$).

Mass (m/z): 202 (M^+)

3-acetyl-1-(3-nitrobenzylideneamino) quinolin-2(1*H*)-one (AJS1)

IR (cm^{-1}): 1511(Ar C=C str), 816 (Ar C-H bend), 3083 (Ar C-H str), 1360 (Ar-NO_2), 1701 (C=O str), 1618 (C=N).

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.14-8.52 (m, 9H, Ar-H), 2.57 (s, 3H, $COCH_3$).

Mass (m/z): 335 (M^+)

3-acetyl-1-(3,4,5-trimethoxybenzylideneamino)quinolin-2(1*H*)-one (AJS2)

IR (cm^{-1}): 1504(Ar C=C str), 830 (Ar C-H bend), 3036 (Ar C-H str), 1223 (C-O str), 1698 (C=O str), 1611(C=N).

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.28-8.25 (m, 8H, Ar-H), 2.56 (s, 3H, $COCH_3$), 3.32 (s, 3H, OCH_3)

Mass (m/z): 380 (M^+)

3-acetyl-6-chloro-1-(4-methylbenzylideneamino)quinolin-2(1*H*)-one (AJS9)

IR (cm^{-1}): 1506(Ar C=C str), 832 (Ar C-H bend), 3030 (Ar C-H str), 1695 (C=O str), 776(C-Cl str), 1379(Ar-CH_3 C-H str), 1614(C=N).

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.14-8.27 (m, 7H, Ar-H), 2.57 (s, 3H, $COCH_3$), 2.27 (s, 3H, CH_3)

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

Antimicrobial Activity

All the synthesized compounds were evaluated for their minimum inhibitory concentration by tube dilution method[15]. 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[16] model according to Winter *et al.*[17] 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

Covered in Scopus & Embase, Elsevier
Int. J. Drug Dev. & Res., April - June 2014, 6 (2): 165-171

was taken as the standard at a dose of 13.5 mg/kg body weight (p.o). The rat paw volume was measured after 1hr, 2hr, 3hr and 4hrs respectively after Carrageenan injection by using Plethysmometer. The difference between the paw volume at 4 hr and 0 hr 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

Table 1: Physicochemical data of the compounds AJS1-AJS12

Comp. code	R	R_1	Mol. formula	Mol. wt	M.P °C	R_f Value	% Yield
1	H	3-NO_2	$C_{18}H_{13}N_3O_4$	335	260-262	0.62	72
2	H	3,4,5-OCH_3	$C_{21}H_{20}N_2O_5$	380	226-228	0.72	68
3	H	4-CH_3	$C_{19}H_{16}N_2O_2$	304	240-242	0.52	70
4	H	4-OH	$C_{18}H_{14}N_2O_3$	306	272-274	0.68	75
5	H	2-Cl	$C_{18}H_{13}ClN_2O_2$	324	290-292	0.56	78
6	H	2-NO_2	$C_{18}H_{13}N_3O_4$	335	256-258	0.60	70
7	6-NO_2	3-NO_2	$C_{18}H_{12}N_4O_6$	380	268-270	0.74	60
8	6-NO_2	3,4,5-OCH_3	$C_{21}H_{19}N_3O_7$	425	236-238	0.68	58
9	6-Cl	4-CH_3	$C_{19}H_{15}ClN_2O_2$	338	222-224	0.58	62
10	6-Cl	3,4,5-OCH_3	$C_{21}H_{19}ClN_2O_5$	414	210-212	0.70	56
11	6-Cl	4-OH	$C_{18}H_{13}ClN_2O_3$	340	254-256	0.64	65
12	6-Cl	2-NO_2	$C_{18}H_{12}ClN_3O_4$	369	236-238	0.64	66

Table 2: Minimum inhibitory concentration of the compounds (AJS1-AJS12) by tube dilution method

Comp Code	Minimum inhibitory concentration (µg)					
	B. subtilis	*S. aureus*	*E.coli*	*P.aeruginosa*	*C.albicans*	*A.niger*
AJS1	3.2	12.5	1.6	25	12.5	100
AJS2	1.6	1.6	R	R	25	R
AJS3	R	R	1.6	1.6	R	R
AJS4	50	50	25	50	12.5	3.2
AJS5	25	R	6.25	0.8	50	6.25
AJS6	3.2	1.6	6.25	50	1.6	100
AJS7	3.2	6.25	25	12.5	R	100
AJS8	3.2	50	12.5	0.8	50	3.2
AJS9	100	50	50	100	3.2	6.25
AJS10	12.5	1.6	100	100	6.25	6.25
AJS11	25	50	6.25	1.6	100	100
AJS12	3.2	1.6	25	12.5	6.25	3.2
Amoxicillin	**1**	**2**	**2**	**1**		
Fluconazole					**16.6**	**8.3**

Covered in Scopus & Embase, Elsevier Int. J. Drug Dev. & Res., April - June 2014, 6 (2): 165-171

Table 3: Anti-inflammatory effect of Schiff base derivatives (AJS1-AJS12) using Carrageenin induced paw edema in rats.

Treatment	Dose mg/kg	Change in the paw volume in ml (% inhibition)			
		1h	2h	3h	4h
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)
AJS1	200	0.298±0.006* (30.69)	0.393±0.008* (46.53)	0.470±0.009* (42.04)	0.543±0.010* (37.94)
AJS2	200	0.295±0.007* (31.38)	0.368±0.013** (49.92)	0.465±0.007** (42.66)	0.525±0.009** (40.00)
AJS3	200	0.352±0.007 (18.14)	0.582±0.008 (20.80)	0.608±0.006 (25.03)	0.638±0.010 (27.08)
AJS4	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)
AJS7	200	0.246±0.006** (42.79)	0.353±0.006** (51.42)	0.443±0.010** (45.37)	0.451±0.007** (48.45)
AJS8	200	0.352±0.007 (18.15)	0.582±0.008 (20.81)	0.608±0.006 (25.03)	0.638±0.010 (27.08)
AJS11	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)
AJS12	200	0.315±0.004* (26.73)	0.433±0.004** (41.08)	0.480±0.003** (40.83)	0.540±0.005** (38.29)

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

Discussion

Antimicrobial Activity

All the synthesized compounds were evaluated for their minimum inhibitory concentration by tube dilution method. Compounds AJS2, AJS6, AJS7 and AJS12 showed significant antibacterial activity against gram +ve bacteria and compounds AJS3, AJS5 and AJS11 showed significant antibacterial activity against gram-ve bacteria compared to standard drug amoxicillin. Compounds AJS1, AJS4, AJS6, AJS9, AJS10 and AJS12 showed significant antifungal activity against *C.albicans* and compounds AJS4, AJS5, AJS8, AJS9, AJS10 and AJS12 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 are given in Table 3. Compounds AJS1, AJS2, AJS4, AJS7, AJS8 and AJS12 showed significant anti-inflammatory activity compared with respective control groups but the maximum inhibition of paw edema was shown by compounds AJS7 and

AJS8 at 4th hour when compared to the standard drug diclofenac sodium.

Conclusions

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

Acknowledgements

The authors are thankful to Nitte University for providing the necessary facilities to carry out this research. The authors are grateful to Sequent Research Ltd, Mangalore and Central Instrumentation Facility, MIT Manipal for providing spectroscopic data.

References

1) Milecki J, Baker SP, Standifer KM, Ishizu T, Chida Y, Kusiak JW. Carbostyril derivatives having potent β-adrenergic agonist properties. J Med Chem 1987; 30: 1563-1566.
2) Joseph B, Darro F, Behard A, Lesur B, Frydman A, Kiss R. 3-aryl-2-quinolone derivatives: Synthesis and characterization of *in vitro* and *in vivo* antitumor effects with emphasis on a new therapeutical target connected with cell migration. J Med Chem 2002; 45: 2534-2555.
3) Ukrainets IV, Mospanova EV, Davidenko AA, Tkach AA, Gorokhova OV. 4-hydroxy-2-quinolones. Synthesis, structure and anti-inflammatory activity of 4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl acetic acid and its derivatives. Chem Heterocycl Compd 2012; 46(8): 974-986.
4) Uchida M, Tabusa F, Komatsu M, Morita S, Kanbe T, Nakagawa K. Studies on 2(1*H*)-quinolinone derivatives as gastric antiulcer active agents. Synthesis and antiulcer activities of optically active alpha-amino acid derivatives of 2(1*H*)-quinolinone and oxindole. Chem Pharm Bull (Tokyo) 1987; 35(2): 853-856.
5) AL-Omar MA, EL-Azab AS, EL-Obeid HA, Abdel H. Synthesis of some new 4(3H)-quinazolinone analogues. J Sandi Chem Soc 2006; 10: 113.
6) Jin Y, Li HY, Lin LP, Tan JZ, Ding J, Luo XM. Synthesis and antitumor evaluation of novel-5-substituted-4-hydroxy-8-nitroquinazolines as EGFR signaling-targeted inhibitors. Bioorg Med Chem 2005; 13: 5613.
7) Yang DP, Ji HF, Tang GY, Siddiqi HM. How many drugs are catecholics? Molecules 2008; 12: 878-884.
8) Sridhar SK, Saravan M, Ramesh A. Synthesis and antibacterial screening of hydrazones Schiff and Mannich bases of isatin derivatives. Eur J Med Chem 2001; 36: 615-623.
9) Raman N, Kulandaisamy A, Thangaraja C. Redox and antimicrobial studies of transition metal (II) tetradendate Schiff base complexes. Trans Met Chem 2003; 28: 29-36.
10) Al-Amiery AA, Al-Majedy YK, Ibrahim HH, Al-Tamimi AA. Antioxidant, antimicrobial and theoretical studies of the thiosemicarbazone derivative Schiff base 2-(2-imino-1-methylimidazolidin-4-ylidene) hydrazinecarbothiamide (IMMC). Organic and Medicinal Chemistry Letters 2012; 2(4): 1-7.
11) Janos G, Tamas L. Macrophage Migration Inhibitory Factor (MIF) tautomerase inhibitors as potential novel anti-inflammatory agents:

Current Developments. Curr Med Chem 2009; 16: 1091-1114.

12) Billman JH, Schmidgall RL. Preparation and antitumor activity of some Schiff bases of 2-amino-4, 5-dichlorobenzene sulfonanilide and 2-amino-p-toulene sulfonamide. J Pharm Sci 2006; 59: 1191-1194.

13) Al-Bayati RIH, Radi MF. Synthesis of novel 2-quinolone derivatives. Afr JPure Appl Chem 2010; 4(10): 228-232.

14) Mobinikhaledi A, Foroughifar N, Khanpour M, Ebrahimi S. Synthesis of some novel schiff bases containing 1,2,4-triazole ring. Eur J Chem 2010; 1(1): 33-36.

15) Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother 2001; 48(S1): 5-16.

16) New OCED 425 guidelines. OCED guidelines for testing of animal. 2001, pp 1-26.

17) Winter CA, Risley EA, Nuss GW. Carrageenin-induced oedema in hind paw of the rats as an assay for anti-inflammatory drugs. Proc Soc Exp Bio Med 1962; 111: 544-547.

Article History: ----------

Date of Submission: 09-06-2014

Date of Acceptance: 29-06-2014

Conflict of Interest: NIL

Source of Support: NONE