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Original Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NOVEL SCHIFF BASES OF 2-QUINOLONES

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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, antioxidant and cytotoxicity activities. The test 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 2, 3, 5, 6, 7, 11 and 12 showed good antibacterial activity compared to the standard drug amoxicillin. Compounds 1, 2, 4, 5, 6, 8, 9, 10 and 12 showed moderate antifungal activity compared to the standard drug fluconazole.The antioxidant activity of the synthesised test compounds was done using DPPH radical scavenging method. Compounds 2 and 6 showed values at 85.78 and 13.41 respectively when compared to that of ascorbic acid at IC_{50} value of 3.69µg/ml and showed inhibitory concentration for 50% inhibition below 100 µg/ml. The final synthesized compounds were screened for their *in vitro* cytotoxicity activity against Ehrlich Ascites Carcinoma cells (EAC) by Trypan blue exclusion method. Compounds 2, 6 and 7 induced the greatest effect on EAC cells with an activity more than 60% at a concentration of 250µg/ml. Hence novel schiff bases synthesized from 2-quinolones are found to be interesting lead molecules as antimicrobial, antioxidant and cytotoxicity agents.

Keywords: 2-Quinolones, Schiff base, Antibacterial activity, Antifungal activity, Antioxidant activity, Cytotoxicity 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 all the synthesized compounds were evaluated for their antimicrobial, antioxidant and cytotoxicity 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 (1a-12a) [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 (1-12) [14]

A mixture of substituted 3-acetyl-1-amino-quinolin-2-one (0.01 mol) 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.

Spectral data

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

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 (1)

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 (2)

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 (9)

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)

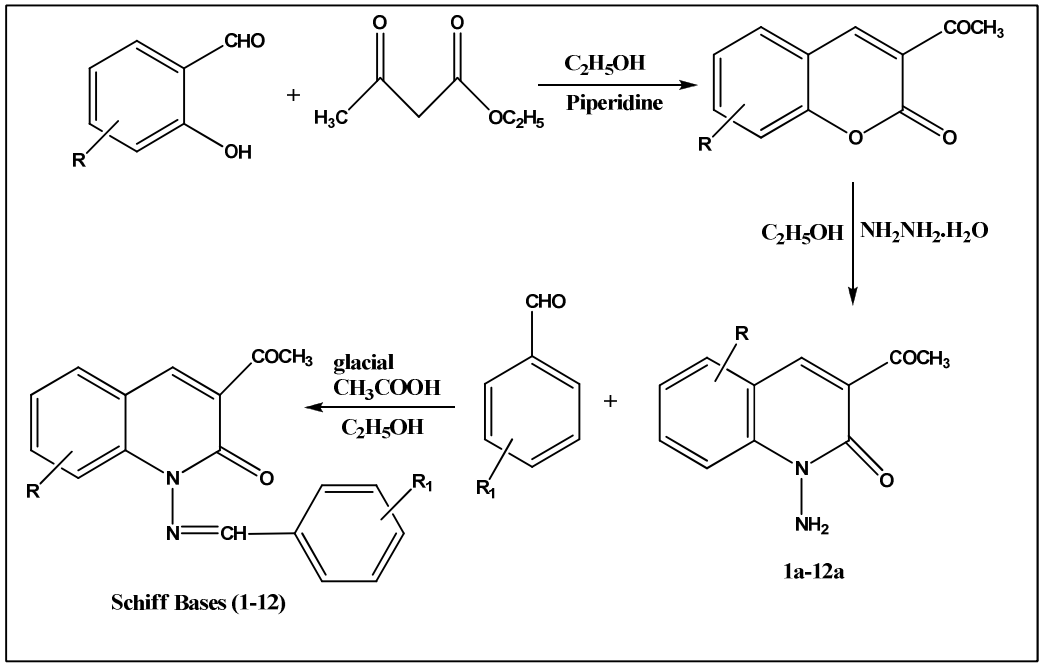

Fig. 1: General Scheme of Synthesis, R: H, 6-NO_2, 6-Cl, R_1: 3-NO_2, 3,4,5-OCH_3, 4-CH_3, 4-OH, 2-Cl, 2-NO_2

RESULTS

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 [15]. The synthesized test compounds were tested at a concentration of 100 µg/50 µl and the standard compound i.e. Ciprofloxacin 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°C for 24 hrs and 25°C for 48 hrs, 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.

Antioxidant activity

Antioxidant activity is a prerequisite for performing many related biological activities, including anticancer, antiallergic, anti inflammatory, antidiabetic etc. The antioxidant activity of the synthesised test compounds was done using DPPH (1, 1-diphenyl-2-picryl hydrazyl) radical scavenging method [16]. The sample size that can lower the initial absorbance of DPPH solution by 50% has been chosen as the endpoint for measuring the antioxidant activity. The assay was carried out in a 96 well microtitre plate. To 100 µl of methanol solution, 100 µl of each of the test sample or the standard ascorbic acid solution was added separately in wells of the microtitre plate in triplicate. Control was prepared by adding 100 µl methanol in 100 µl DPPH solution. The plates were incubated at 37°C for 20 minutes and the absorbance of each solution was measured at 540 nm using ELISA reader against the corresponding test and standard blanks and the remaining DPPH was calculated.IC_{50} is the concentration of the sample required to scavenge 50 % of DPPH free radicals.

$$\% \text{ inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Cytotoxicity Activity

All the test compounds were studied for short term *in vitro* cytotoxicity against Ehrlich Ascites Carcinoma cells (EAC) cells. The tumor cells aspirated from peritoneal cavity of tumor bearing mice was washed thrice with normal saline and checked for viability using Tryphan blue exclusion method [17]. The cell suspension (1 million cells in 0.1 ml) was added to tubes containing various concentrations of the test compounds and volume was made upto 1 ml using phosphate buffered saline. Control tubes contained only cell suspension. The assay mixtures were incubated for 3 h, at 37°C and then percent of dead cells were evaluated by tryphan blue exclusion method.

Table 1: Physicochemical data of the compounds 1-12

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: Antimicrobial Activity of the compounds (1-12) by cup plate method

Compound	Diameter of zone of inhibition (mm)					
	S.aureus	*B.subtilis*	*E.coli*	*P.aureginosa*	*C.albicans*	*A.niger*
1	08	07	-	-	12	10
2	18	16	11	13	08	15
3	13	11	17	21	05	06
4	06	-	08	07	13	10
5	20	17	10	14	14	16
6	18	15	17	23	12	15
7	17	15	12	16	-	-
8	-	-	07	08	13	14
9	07	06	-	-	14	10
10	06	-	-	09	09	13
11	18	16	13	16	-	06
12	12	10	18	22	11	14
Amoxicillin	26	23	25	30	-	-
Fluconazole	-	-	-	-	23	26
Control	-	-	-	-	-	-

Table 3: Antioxidant activity of the compounds (1-8) by DPPH radical scavenging method

Conc µg/ml	% DPPH Radical Scavenging							
	AJS 1	**AJS 2**	**AJS 3**	**AJS 4**	**AJS 5**	**AJS 6**	**AJS 7**	**AJS 8**
3.9	19.44	47.32	15.46	8.32	14.80	30.88	10.90	47.16
7.8	27.41	49.61	19.90	21.75	15.13	48.62	16.51	46.70
15.6	37.67	48.85	24.65	17.72	24.03	53.54	37.42	48.54
31.25	43.64	46.40	38.13	54.88	18.93	54.64	65.79	51.45
62.5	56.96	46.70	47.16	31.41	23.73	56.89	72.67	48.08
125	42.41	56.50	49.31	34.10	23.93	64.16	78.41	55.89
250	45.36	59.28	53.56	49.88	26.75	68.93	81.87	57.32
500	49.12	60.92	57.18	60.45	63.30	69.57	81.93	60.48
IC_{50}	**183**	**85.78**	**169**	**640**	**815**	**13.41**	**147**	**175**

Table 4: Cytotoxicity activity of compounds (1-12) by Trypan Blue exclusion method

Compounds	No. of dead cells (%) at different concentrations (µg/ml)			
	50	**100**	**200**	**250**
Control	-			
1	14	25	34	48
2	22	35	43	68
3	15	26	37	51
4	09	15	20	30
5	07	14	19	27
6	23	35	56	66
7	21	32	51	62
8	14	22	35	46
9	08	15	25	34
10	15	24	36	52
11	06	11	16	23
12	13	21	33	45
5-Fluorouracil	**36**	**51**	**88**	**96**

DISCUSSION

Antimicrobial activity

The *in vitro* antibacterial and antifungal activity of the synthesized compounds were determined by using cup-plate method. 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 2, 5, 6, 7 and 11 showed good antibacterial activity against gram +ve bacteria and compounds 3, 6, 11 and 12 showed good antibacterial activity against gram -ve bacteria respectively compared to the standard drug ciprofloxacin. Compounds 1, 4, 5, 6, 8, 9 and 12 showed moderate antifungal activity against *Candida albicans* and compounds 2, 5, 6, 8, 10 and 12 showed moderate antifungal activity against *Aspergillus niger* compared to the standard drug fluconazole. The results of the antimicrobial activity are summarized in Table 2. The presence of 2-quinolone moiety with electron withdrawing groups like nitro, chloro and electron donating groups like methyl, methoxy and hydroxy has accounted for their antimicrobial activity.

Antioxidant activity

The antioxidant activity of the synthesised test compounds was done using DPPH (1, 1-diphenyl-2-picryl hydrazyl) radical scavenging method. Out of the 8 synthesised isoxazoline derivatives, the test compounds such as, 2 and 6 showed values at 85.78 and 13.41 respectively when compared to that of standard ascorbic acid at IC_{50} value of 3.69µg/ml and showed inhibitory concentration for 50% inhibition below 100 µg/ml. The results of the antioxidant activity is presented in Table 3. The presence of 2-quinolone moiety with electron withdrawing group like nitro has accounted for their antioxidant activity.

Cytotoxicity Activity

The test compounds were subjected to *in vitro* cytotoxicity against Ehrlich Ascites Carcinoma (EAC) cells using Tryphan Blue exclusion method. The damaged cells are stained blue by Tryphan blue stain and can be distinguished from viable cells. Compound 2, 6 and 7 induced the greatest effect on EAC cells with an activity more than 60% at a concentration of 250µg/ml. The results of the cytotoxicity activity is presented in Table 4. The presence of 2-quinolone moiety with electron withdrawing group like nitro has accounted for their cytotoxic activity.

CONCLUSION

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

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