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Synthesis and Biological Activity of New Schiff Bases Containing 4(3H)-Quinazolinone Ring System

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ABSTRACT: A series of new compounds **(3a-e)** were prepared by condensation reaction of 3-amino-2-methyl-4(3H)quinazolinone (AMQ) with different substituted aromatic aldehydes in methanol. The structures of the newly prepared compounds were confirmed by elemental analysis and spectrometric (IR, ^{1}H-NMR and MS) data. The compounds were also evaluated for their antimicrobial, anthelmintic and antioxidant activities. The results suggest that the compounds possess broad spectrum of *in vitro* antimicrobial activity. An anthelmintic result reveals that the compound **3c** is potentially active against earth worms. Antioxidant results obtained in the present study indicate that few of the synthesized compounds show moderate to better scavenging activity.
KEYWORDS: 3-amino-2-methyl-4(3H)quinazolinone, antimicrobial, anthelmentic, antioxidant.

INTRODUCTION

Over the past decade, the synthesis of privileged classes of heterocyclic molecules has become one of the main areas of interest in synthetic chemistry [1, 2]. These important structures have gained much attention, owing to their potential role as ligands, which are capable of binding multiple biological targets [3]. Among nitrogen-containing privileged class of molecules, substituted quinazolinones and quinazolines are considered as important therapeutic scaffolds [4, 5]. Quinazolinon-4(3H)-ones and its derivatives are versatile nitrogen heterocyclic compounds which have long been known as a promising class of biologically active compounds [6]. Compounds containing 4(3H)-quinazolinone ring system have been reported to possess different biological activities such as antibacterial [7], antifungal [8], antitubercular [9], antiviral, anticancer [10] and anticonvulsant activity depending on the substituents in the ring system. Because of this established biological activities of new 4(3H)-quinazolinone derivatives, the present paper reports the synthesis, characterization and biological activity of new series of Schiff bases using 3-amino-2-methyl-4(3H)quinazolinone.

EXPERIMENTAL

MATERIALS AND METHODS

All the chemicals and solvents were of AnalaR grade. The spectroscopic grade solvents were used as supplied by commercial sources without any further purification. All melting points (m.p.) were determined with a Büchi 530 melting point apparatus in open capillaries and are uncorrected. Elemental analyses were performed on a Carlo-Erba 1160 elemental analyzer. IR spectra were recorded on KBr discs, using a Perkin-Elmer Model 1600 FT-IR spectrometer. ^{1}H-NMR spectra were obtained on a Bruker AC 200 spectrometer. EIMS were determined on VG Zab spec (70 eV) mass spectrometer.

BIOLOGY

ANTIMICROBIAL ACTIVITY

The *in vitro* antimicrobial screening effects of the synthesized compounds were evaluated against four bacterial strains namely *Bacillus Subtilis, Escherichia coli, Staphylococcus aureus* and *Ralstonia solanacearum* and three fungal strains namely *Aspergillus niger, Aspergillus flavus* and *Alternaria solani* by disc diffusion method using nutrient agar

medium for antibacterial studies and potato dextrose agar medium for antifungal studies [11, 12].

The bacteria and fungi were sub-cultured in the agar and potato dextrose agar medium and were incubated for 24 h for bacteria and 48 h for fungi at 37 ºC. Standard antibacterial drug (Chloramphenicol) and antifungal drug (fluconazole) were used for comparison. The discs having a diameter of 4 mm were soaked in the test solutions and were placed on an appropriate medium previously seeded with organisms in petri plates and stored in an incubator at the above mentioned period of time. The inhibition zone around each disc was measured and the results have been recorded in the form of inhibition zones (diameter, mm) showed in Table 1. In order to clarify any effect of DMF on the biological screening, separate studies were carried out with solutions alone of DMF and they showed no activity against any microbial strains. The stock solution (1 mg/ml $^{-1}$) of the test compounds was prepared in DMF. Each test was performed in triplicate in individual experiments and the average is reported.

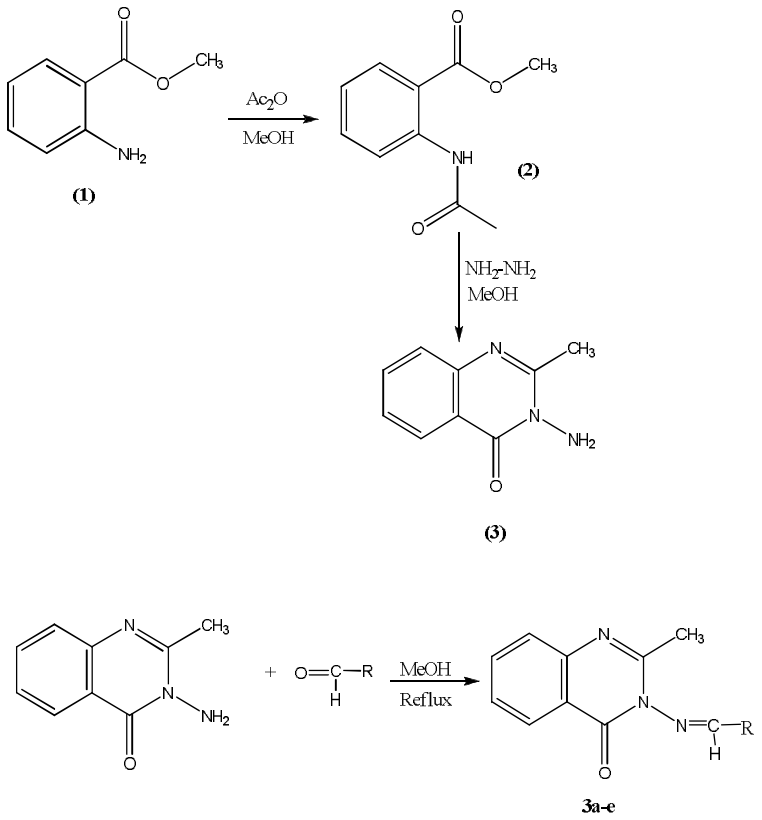
ANTHELMINTIC ACTIVITY

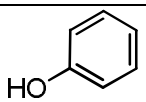
Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings [13, 14]. Six groups of six earthworms each were released in to 10 ml of solutions of piperazine citrate and test solution in DMSO (**3a-e**). Piperazine citrate was used as reference standard while DMSO as control (Table 2).

DPPH RADICAL SCAVENGING ACTIVITY

The free radical scavenging capacity of the synthesized Schiff bases **3a-e** was determined using DPPH. Schiff bases (**3a-e**) stock solution (1 mg/ml) was diluted to final concentrations of 20-100 µg in 2.5 ml methanol. Methanolic DPPH solution (1 ml, 0.3 mmol) was added to Schiff base solutions of different concentrations and allowed to react at room temperature. After 30 min, the absorbance values were measured at 518 nm and converted into the percentage antioxidant activity [15]. Methanol was used as the solvent and ascorbic acid as the standard. The trials were done in triplicate. The graph was plotted with percentage antioxidant activity (AA %) on the y-axis and concentration on the x-axis (Figure-2).

SCHEME-1



COMPOUND	R
3a	
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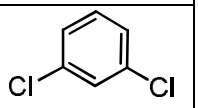
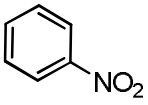
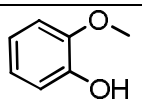
3c	
3d	
3e	

SYNTHESIS OF AMQ

A mixture of methyl 2-aminobenzoate (**1**) (10 mmol) and acetic anhydride (10 mmol) in methanol (20 ml) was refluxed for 2 h to form methyl 2-acetamidobenzoate (**2**) and it is then treated with hydrazine hydrate to form AMQ (**3**), which was then filtered, washed with H_2O and recrystallised from methanol.

SYNTHESIS OF SCHIFF BASES (3a-e)

Quinazolin-4(3H)-one Schiff bases were synthesized by the condensation of 3-amino-2-methyl-4(3H)quinazolinone with different substituted aromatic aldehydes in 1:1 ratio.

To a solution of 3-amino-2-methyl-4(3H) quina zolinone (5 mmol) in methanol (20 ml), required aromatic aldehyde (5 mmol) was added and the reaction mixture was then stirred for about 3-4 h and refluxed over night. The product obtained was filtered, washed with hot methanol and dried over $CaCl_2$. The general synthetic route of new series of Schiff base compounds **3a-e** are depicted in **scheme-I**.

3-(2-hydroxy benzylideneamino)-2-methylquinazolin-4(3H)-one (3a)

Yield: 71%; m.p.: 167 ^{0}C; IR (KBr cm^{-1}): 3067.3, 2974.6, 2367.3 (C-H)**,** 1656**.**0 (N-C=O), 1603 (C=N).; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.7(s, CH_3, 3H, C_8), 9.1 (s, CH, 1H, -N=CH-), 10.6 (s, OH, 1H), 7.31-8.12 (m, Ar-H, 8H, Aromatic protons); Mass (m/Z): 280 [M^+, 79 %]; Anal: Calcd. For $C_{16}H_{13}N_3O_2$: C 68.43, H 4.58, N 14.9, Found: C 68.5, H 4.61, N 15.02 %.

2-methyl-3-(pyridine-2-ylmethyleneamine)quinazolin-4(3H)-one (3b)

Yield: 63%; m.p.: 175 ^{0}C; IR (KBr cm^{-1}): 3088.4, 2924.5, 2161.8 (C-H), 1684.2 (N-C=O), 1597.0 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.72 (s, CH_3, 3H, C_8), 8.9 (s, CH, 1H, -N=CH-), 8.6 (d, CH, 1H, N-CH=CH-), 7.4-8.2 (m, Ar-H, 8H, Aromatic protons); Mass (m/z): 265 [M^+, 83 %]; Anal: Calcd. For $C_{15}H_{12}N_4O$: C 68.18, H 4.28, N 21.22, Found: C 68.27, H 4.3, N 21.32 %.

3-(2,4-dichlorobenzylideneamino)-2-methylquinazolin-4(3H)-one (3c)

Yield: 67%; m.p.: 159 ^{0}C; IR (KBr cm^{-1}): 3101.6, 3047.3, 2938.3 (C-H)**,** 1648.2 (N-C=O), 1616.2 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.49 (s, CH_3, 3H, C_8), 8.62 (s, CH, 1H, -N=CH-), 7.9 (s, CH, 1H, Cl-CH-Cl), 7.25-7.7.81 (m, Ar-H, 8H, Aromatic protons). Mass (m/z): 332 [M^+ 67 %]; Anal: Calcd. For $C_{16}H_{11}Cl_2N_3O$: C 57.85, H 3.34, N 12.65, Found: C 58.12, H 3.41, N 12.82 %.

2-methyl-3-(4-nitrobenzylideneamino)quinazolin-4(3H)-one (3d)

Yield: 57%; m.p.: 184 ^{0}C; IR (KBr cm^{-1}): 3057.3, 3012.7, 2876.4 (C-H)**,** 1637.0 (N-C=O), 1607.2 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6) δ; 2.82 (s, CH_3, 3H, C_8), 9.16 (s, CH, 1H, -N=CH-), 8.41 (s, CH, 1H, -CH-C-NO_2), 7.27-7.92 (m, Ar-H, 8H, Aromatic protons). Mass (m/z): 309 [M^+, 77 %]; Anal: Calcd. For $C_{16}H_{12}N_4O_3$: C 62.33, H 3.92, N 18.17, Found: C 62.81, H 3.98, N 18.32 %.

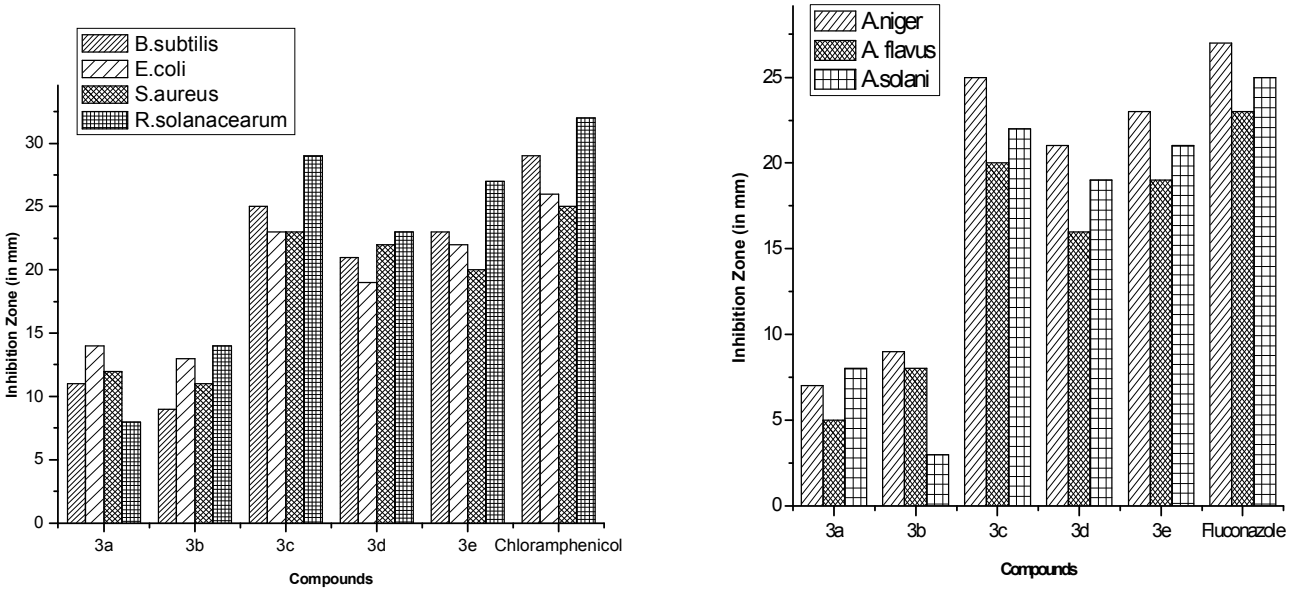
3-(4-hydroxy-3-methoxybenzylideneamino)-2-methylquinazolin-4(3H)-one (3e)

Yield: 61%; m.p.: 217 ^{0}C; IR (KBr cm^{-1}): 3063.1, 2987.4, 2912.7 (C-H)**,** 1650.1 (N-C=O), 1586.3 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.74 (s, CH_3, 3H, C_8), 9.0 (s, CH, 1H, N=CH), 3.91 (s, CH_3, 3H, meta to methoxy), 5.6 (s, OH, 1H), 6.9-7.4 (m, Ar-H, 7H, Aromatic protons). Mass (m/z): 310 [M^+, 61 %]. Anal: Calcd. For $C_{17}H_{15}N_3O_3$: C 66.01, H 4.93, N 13.58, Found: C 66.12, H 5.03, N 13.79 %.

RESULTS AND DISCUSSION

Quinazolin-4(3H)-one Schiff bases were synthesized by the condensation of 3-amino-2-methyl-4(3H)quinazolinone with different substituted aromatic aldehydes in 1:1 ratio. The structures of the newly prepared compounds were confirmed by elemental analyses, IR, ^{1}H-NMR and mass spectral studies.

The new compounds (**3a-e**) were assayed *in vitro* to assess their ability to inhibit the growth of selected species of bacteria and fungi. The data is summarized in Table 1. On the basis of observed zones of inhibition, it was found that, in general, all the prepared quinazolin-4(3H)-one derivative responded against all the tested bacterial and fungal strains significantly. It was found that compounds 3c, 3d and 3e exhibited more activity against all the selected microbial strains (Figures 1a and 1b).



(1a) **(1b)**

Figure 1: (a) antibacterial activity and (b) antifungal activity of Schiff bases (3a-e)

Helminthic infections of the gastrointestinal tract of human have been recognized to have adverse effects on health standards with a consequent lowering of resistance to other diseases. Earthworms have been used widely for the initial evaluation of anthelminthic compound *in vitro* because they resemble intestinal "worms" in their reaction to anthelminthics and easily available. From the results shown in Table 2, the predominant effect of piperazine citrate on the worms is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. After a brief stimulant effect, earthworms lost their motility on exposure to the test solutions (**3a-e**). From the observed results, it is concluded that all the synthesized compounds show moderate activity and particularly compound 3c has potent anthelminthic activity when compared to standard anthelminthic drug (piperazine citrate) due to the presence of chloro group which may enhance the conductance of worm muscle membrane that produces hyperpolarisation and reduces excitability that leads to muscle relaxation and flaccid paralysis. The results of anthelminthic activity are shown in Table 2.

DPPH assay has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances. The DPPH characterized as stable-free radical by virtue of the delocalization of the spare electron over the molecule; this delocalization gives rise to a deep violet colour, characterized by an absorption band in methanol solution centered at 518 nm. When a solution of DPPH is mixed with a substance, that can donate a hydrogen atom, this gives rise to the reduced form (diphenyl picrylhydrazine), with the loss of violet colour. The graph was plotted with percentage antioxidant activity (AA %) on the y-axis and concentration on the x-axis. The scavenging ability of the synthesized compounds was compared with ascorbic acid as a standard. Compounds 3a and 3e displayed better scavenging ability. This may be due to the presence of hydroxyl group. Rest of the compounds showed moderate antioxidant activity (Fig 2).Radical scavenging activity was expressed as a percentage and was calculated using the following formula:

$$\% \text{ Scavenging} = \frac{(A_{control} - A_{sample})}{A_{control}}$$

Where A_{sample} is the absorbance of the test sample and $A_{control}$ is the absorbance of the control.

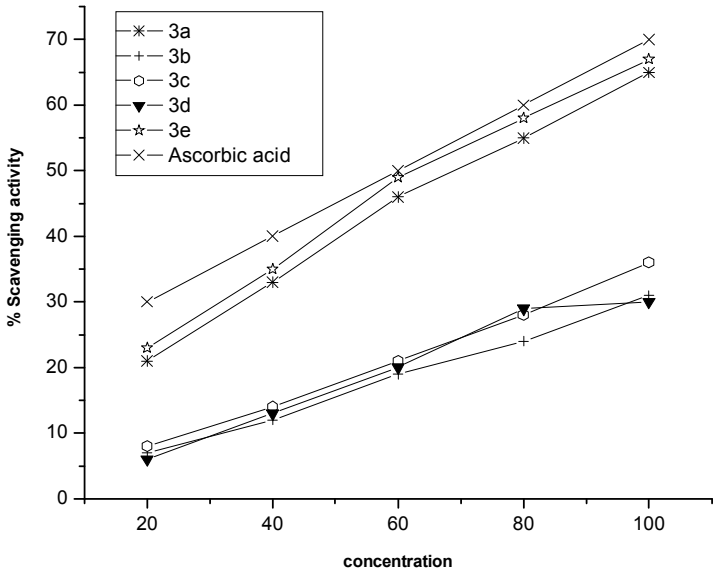


Figure 2. Scavenging activity of the Schiff bases (3a-e) using DPPH.

Table 1: *In vitro* Antimicrobial Activity of the Schiff bases (3a-e).

	Antibacterial activity				**Antifungal activity**		
	Zone of inhibition (in mm)						
Compound	*B.subtilis*	*E.coli*	*S.aureus*	*R.solanacearum*	*A.niger*	*A. flavus*	*A.solani*
3a	11	14	12	08	07	05	08
3b	09	13	11	14	09	08	03
3c	25	23	23	29	25	20	22
3d	21	19	22	23	21	16	19
3e	23	22	20	27	23	19	21
Chloramphenicol	29	26	25	32			
Fluconazole					27	23	25

Table 2: **Results of Anthelmentic Activity of Schiff bases (3a--e)**

Compound	**Concentration in mg/ml**	**Time taken for paralysis (in min)**	**Time taken for death (in min)**
Standard Piperzine citrate	5	10	10
3b	5	25	29
3a	5	27	32
3c	5	08	09
3d	5	21	24
3e	5	16	19

CONCLUSIONS

In conclusion, the synthesized new compounds 3-amino-2-methyl-4(3H)quinazolinone derivatives (3a-e) are characterized by spectral data and subjected them for biological assay. All the compounds are potential antibacterial, antifungal and anthelmintic agents. Compounds 3a and 3e are very good antioxidants due to the presence of hydroxyl group in them.

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