Chemistry and Materials Research
ISSN 2224- 3224 (Print) ISSN 2225- 0956 (Online)
Vol.6 No.10, 2014

Synthesis and Antimicrobial Activity of Novel of 2,3-Disubstituted Quinazolin 4(3H)- One Derivatives

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Abstract
Aseries of novel derivatives of 2,3-disubstituted quinazolin4(3H)-ones have been synthesized from anthranilic acid and p-methoxybenzoylchloride.The structures of the newly synthesized compounds have been established on the basis of their m.p., TLC, IR,UV,Vis and 1H NMR data.These synthesized compounds were evaluated for their antimicrobial activity.The results showed that some of these derivatives have good antimicrobal activities when compared with standard antibiotic .
Keywords: Quinazolinone, Antimicrobial activity,Azo compound , Azetidinone

Introduction

Quinazolinone derivatives represent one of the most classes of heterocyclic compounds possessing a wide spectrum of biological activity. Medicinally it has been used in various areas as an analgesic and anti-inflammatory [1-3], antioxidant[4-5], Antimicrobial[6-7], antitubercular[8], anticonvulsant[9], anticancer[10], antimur[11] .

Chalcones, precursors of open chain flavonoids and isoflavonoids present in edible plants, and their derivatives have attracted increasing attention due to numerous potential pharmacological applications [12,13].

Heterocyclic azo compounds are well known for their medicinal importance and used in advanced organic synthesis [14,15]. The dyes containing heterocyclic moiety like quinazolinone have been found to give a wide range of color shades (yellow to red) with very good depth and levelness on fabrics and also showed excellent brightness and good fastness properties like sublimation, washing and light and also show high thermal stability[16].

Furthermore,compounds containing an azomethine group (imine) are a class of important compounds in medicinal and pharmaceutical field. The biological applications of these compounds have attracted remarkable attention [17].

Literature revealed that 2-azetidinone derivatives occupy an important place in medicinal chemistry as they show a variety of microbiological activity [18 -19].

Experimental

Materials and physical measurements

All reactants and solvents used in this study were reagents grade and they are available from Sigma - Aldrich and Fluka companies. Melting points were determined on Electro - thermal capillary apparatus and are uncorrected. Purity of the compounds was checked on silica coated Merck-TLC plates using water, chloroform benzene and acetone as mobile phase. FTIR measurements were recorded on Shimadzu model FT-IR-8400S. The UV-Visible spectra were measured in ethanol using Shimadzu UV-Vis. 160 a spectrophotometer.
Proton NMR spectra ^{1}H-NMR spectra were obtained with a Bruker spectrophotometer model Ultra Shield at 300 MHz in DMSO-d_6 solution with the TMS as internal standard.

Synthesis of 2-(4-methoxyphenyl)-4H-benzo[d][1,3]oxazin-4-one

This has been synthesized by following the reported procedures [20].
P- methoxybenzylchloride (0.01 mol) was added dropwise to stirred solution of anthranilic acid (0.01 m ol) in pyridine (20 ml) ,then the mixture was stirred at room tempearture for 2 h . poured into ice-water. The solid was filtered washed with water and recrystallized from ethanol . Yield: 50%. M.p143-144°C. UV (λmax,nm) 219, 306. FTIR (KBr, ν, cm-1):1757 (C=O); 1600 (C=N),1253 (C-O-C);
^{1}H- NMR (300 MHz, DMSO-d6, δ, ppm): 8.3-7.5 (m, 4H, Ar-H),) 7.70-7.78 (dd ,2H, Ar-H 7.29-7.20 (dd,2H,Ar-H) , 2.4 (s, 3H, CH_3).

Synthesis of 3-(4-acetylphenyl)-2-(4-methoxyphenyl)quinazolin-4(3H)-one

A mixture of compound I (0.01 mol) and p-aminoacetophenone (0.01 mol) was heated together upon fusion at 150˚C on oil bath for4 h. After cooling, the crude mass was crystallized from ethanol twice to give brown crystal Yield: 55 %;
M.p :198-200 °C; UV (λmax,nm) : 208, 285; FTIR (KBr, ν, cm-1): 1670 (C=O), 1681 (C=O of acetyl); 1H

NMR (300 MHz, DMSO-d6, δ, ppm): 8.42-7.22 (m, 4H, quinazolinone-H), 7.80-7.88 ,7.32-7.29 (dd ,8H, Ar-H) ,3.7 (s, 3H, O-CH_3), 2.6 (s, 3H, acetyl-H).

Synthesis of (E)-2-(4-methoxyphenyl)-3-(4-(3-(3-nitrophenyl) acryloyl) phenyl) quinazolin-4(3H)-one
To mixture of (0.01 mol) of compound **2** and m-nitrobenzaldhyde dissolved in 25mL CHCl3 and 10 drops of piperidine stirring till solving then refluxed for 8 h. with stirring , after having evaporated the solvent, and then recrystallized from ethanol. Yield: 50%; M. p: 156-158 °C; UV (λmax,nm) :242, 355 ;
FTIR(KBr,ν,cm^{-1}):1697(C=O), 1666(C=O of chalcon),1537, 1363(NO_2);
^{1}H-NMR (300 MHz, DMSO-d6, δ, ppm): 8.4-7.4 (m,16 H, Ar-H), 7.2 (d,1H,CH=) ,6.8 (d,1H, O=C-CH) ,3.7 (s,3H, O-CH_3), 2.6 (s, 3H, O=C-CH_3).

Synthesis of 2-amino-6-(4-(2-(4-methoxyphenyl)-4-oxoquinazolin- 3(4H) yl)phenyl)-4-(3-nitrophenyl)-2H-pyran-3-carbonitrile
A mixture of (0.01mol chalcone 3 and malononitrile (0.01mol) in the abs. ethanol containing few drops of piperidine as a catalyst was refluxed for 8 h. The resulting crude product filtered off, dried and recrystallized from methanol. Yield: 65 % ; M.p :173-175°C ; UV (λmax, nm) :206, 336; FT-IR (KBr, ν, cm^{-1}):1683 (C=O) ,2191 (C≡N), 3444,33350 (NH_2) ; ^{1}H -NMR (300 MHz, DMSO-d6, δ, ppm): 8.31-6.55(m ,16H ,Ar-H),5.32 (s ,2H, NH_2),3.75 (s ,3H ,O-CH_3) .

Synthesis of 2-(4-methoxyphenyl)-3-(4-(6-(3-nitrophenyl)-2-thioxo-2,3- dihydropyrimidin-4-yl)phenyl)quinazolin-4(3H)-one
Chalcone 3 (0.01mol) was added to a mixture of thiourea (0.01mol) in ethanol (20ml) and concentrated HCl (0.5ml), then refluxed for 6 h. The mixture was concentrated to half its volume, cooled and neutralized with ammonium hydroxide. The precipitate solid was filtered off, washed with water, dried and recrystallized from ethyl acetate. Yield: 76 % ; M.p: 102-104 °C ; UV (λmax, nm): 214 ,316 ; FTIR (KBr, ν, cm^{-1}): 1240 (C=S), 2660 (SH), 3281 (NH); ^{1} H- NMR (300 MHz, DMSO-d6, δ, ppm): 13.4 (s,1H,SH), 8.5 -6.4(m ,16H ,Ar-H)) , 3.8 (s,3H,O-CH_3), 2.1 (d,2H,CH_2 of pyrimidine), 4.0 (t,1H,CH of pyrimidine).

Synthesis of 3-(4-aminophenyl)-2-(4-methoxyphenyl)quinazolin-4(3H)one
Compound 1(0.01 ml) was added to a mixture of p-aminoaniline (0.01 mL) in pyridine (20mL), then refluxed for 12 h . The reaction mixture then poured into ice-water. The solid was filtered and recrystallized from ethanol.Yield: 70% ; M.p:164 -166 °C ; UV (λmax, nm): 205, 354 ; FTIR (KBr, ν,cm^{-1}):1675 (C=O), 3323 ,3215 (NH_2); ^{1} H -NMR (300 MHz, DMSO-d6, δ, ppm): 6.32 (s,2H, NH_2) , 8 .25-6.74 (m,12H , Ar-H), 3.70 (s,1H,O-CH_3)

Synthesis of 3-(4-aminophenyl)-2-(4-2-hydroxy-5-((4-(2-(4 methoxyphenyl)-4-oxoquinazolin 3(4H)yl)phenyl)diazenyl)benzaldehyd
Compound **6** (1.78 mmol) was dissolved by heating and stirring in 16 mL of 85% phosphoric acid. The solution was cooled to 0˚C in an ice bath, and then concentrated nitric acid 8 mL and a solution of sodium nitrite (0 3.74 mmol) in 4 mL of water was added. The mixture was stirred vigorously and maintained at below 5˚C for 10 minutes. Afterwards salicylaldehyde (0.3.74 mmol) in 1 mL water was added dropwise with stirring. The dark brown solid was filtered, washed several times with water, then dissolved in 30 mL 10% NaOH, the solution filtered, the crude product precipitated during neutralization with 10% HCl, then filtered and washed with water several time .Yield: 40 % ; M.p: 200-202 °C ; UV (λmax,nm): 213 ,315 ; FTIR (KBr, ν, cm^{-1}): 1670 (C=O),1685(C=O of aldehyde) ,1521 (N=N),3452(O-H) ; ^{1} H -NMR (300 MHz, DMSO-d6, δ, ppm):10.5(s ,1H, aldehyde -H),5.63 (s ,1H, O-H), 8.53-6.21 (m, 15H, Ar-H), 3.80 (s, 3H,O-CH_3)

Synthesis of 4(3H)-of 3-(4-((3-acetyl-2-oxo-2H-chromen-6-yl) diazenyl) phenyl)-2-(4-methoxyphenyl)quinazolinone.
Compound **7** (0.01mol)in dry chloroform (20mL) were added ethylacetoacetate(0.01mol)and few droup of piperdine.the reaction mixture was heaed nuder reflux for 2 h. and left to cool after distilling off the excess solvent.The solid was filtered ,washed with cold water,dried and recrystallization from toluene.
Yield: 70% ; M.p: 123-125 °C ; UV (λmax , nm) : 313 ,315 ; FTIR (KBr, ν, cm^{-1}):1739 (C=O of chromen), 1653 (C=O of acetyl) ; ^{1} H -NMR (300 MHz, DMSO-d6, δ, ppm): 8.4-6.2 (m, 16H, Ar-H), 3.7 (s, 3H, O-CH_3), 2.6(s, 3H, acetyl-H) .

Synthesis of 2-(4-methoxyphenyl)-3-(4-((3 nitrobenzylidene) amino) phenyl)quinazolin-4(3H)-one.
A mixture of compound 3 (0.02 mol) and 3-nitro benzaldehyde (0.02 mol)) was refluxed in absolute ethanol (25 mL) for 7 h. The mixture was cooled and the product obtained recrystallized from of reaction mixture was then

stirred for 6 h, then poured into ice-water. The solid was filtered and recrystallized from dioxane. Yield: 64% ; M.p: 192-194 °C ; UV (λmax , nm)208 ,351 ; FTIR (KBr, ν, cm^{-1}):1670 (C=O), 1589 (C=N), 1506 ,1352 (NO_2) . ^{1}H- NMR (300 MHz, DMSO-d6, δ, ppm): 8.42-6.51 (m,17 H, Ar-H,CH=N), 3.75 (s, 3H,O-CH_3).

Synthesis of 3-(4-(3-chloro-2-(3-nitrophenyl)-4-oxoazetidin-1-yl) phenyl) -2-(4-methoxyphenyl)quinazolin-4(3H)-one

To a stirred solution of compound**9** (0.01mol) and triethyl amine (0.02 mol) in dry dioxin (15 mL), chloroacetyl chloride (0.02 mol) was added dropwise at (0-5 °C), the reaction mixture was then stirred for 6 h, then poured into ice-water. The solid was filtered and recrystallized from benzene . Yield: 59 % ; M.p275-277 °C ; UV (λmax ,nm) :202 ,354 ; FTIR (KBr, ν, cm^{-1}):1743 (C=O for β-lactam),763 (C-Cl) ; ^{1}H- NMR (300 MHz, DMSO-d6, δ, ppm): 8.32-6.55 (m,16 H, Ar-H), 5.6-5.6 (dd ,2H, azetidinyl- H) ,3.75 (s, 3H , O-CH_3).

Biological Activities

Antimicrobial Activity

Quinazolinone derivatives (2-10) were in vitro screened for antibacterial activity against two Gram positive *Staphylococcus aureus and Streptococcus pyogenes* and two gram negative bacteria microorganisms and two fungal strains namely *Candida albicans and Aspergillus niger* using well diffusion method [21]. Dimethyl sulfoxide DMSO was run as a control and the test was performed at 10mg/mL concentration using DMSO as solvent. The bacteria and fungi were sub cultured in agar and potato dextrose agar medium and these plates were incubated for 24 h for bacteria and 48 h for fungi at 37 °C. The zone inhibition observed around the cups after respective incubation was measured in mm .

Results and discussions

Synthesis

The new quinazolinone derivatives were prepared following the reaction sequences depicted in scheme 1. Compound 1 is the key intermediates for the compounds synthesized later in this work . It has been prepared by the condensation of the anthrnilic acid with p-methoxybenzoylchloride. 3-(4-acetylphenyl)-2-(4-methoxyphenyl)quinazolin-4(3H)-one was prepared by fusion mixture of compound 1 and p-aminoacetophenone .

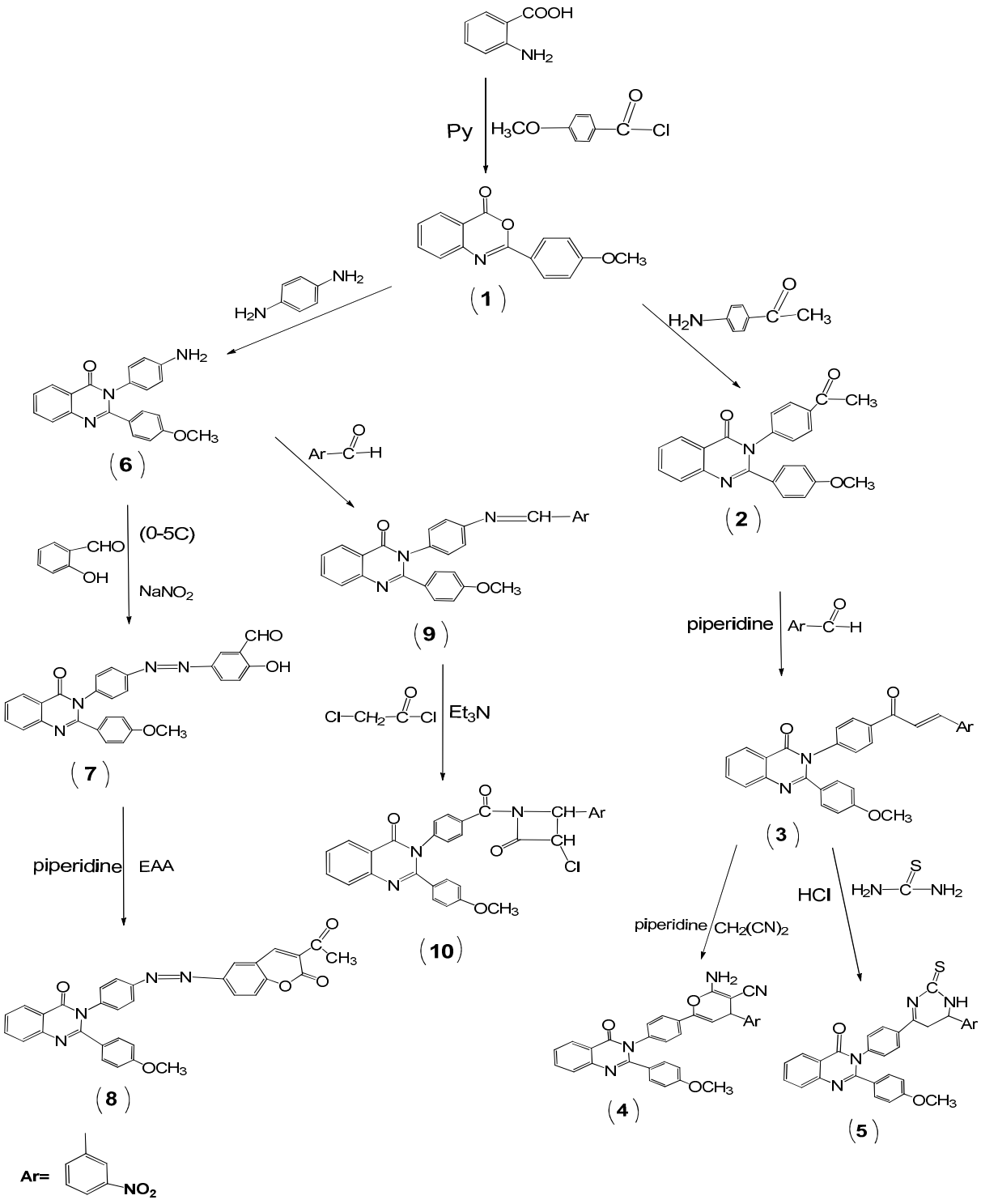
The structure of all compounds were proven based on the melting point (m.p), thin layer chromatography (TLC) and spectral data. FTIR spectrum of compound 2 showed the appearance of new characteristic bands at 1670 cm^{-1} and 1681 cm^{-1} due to C=O stretching of quinazolinone and carbonyl of acetyl group respectively .

While the ^{1}H-NMR showed singlet signal at 2.6 due to methyl proton of acetyl group and a singlet at3.7 related to three protons of p-substituted methoxy group while doublet of doublet at 7.80- 7.88 ppm and 7.32-7.29 ppm belong to (8H, 2ph), a multiplet signal at 7.22 -8.42 ppm due to four quinazolinone protons.

Reaction compound 2 with m-nitrobenzaldyde afforded chalcon deravtive 3 .

FT-IR spectrum of 3 shows a band at 1560cm^{-1} and 1666cm^{-1} due to (C=C and C=O) of α, β-unsaturated compound respectively.

The^1 H -NMR spectrum of 3 showed singlet signal at 2.6 ppm due to methyl proton of acetyl group and a singlet at 3.7 ppm related to three protons of p-substituted methoxy group and two doublets one at 6.8 ppm relate to(O=C- CH) another at 7.2 ppm due(CH of α,β unsaturated carbonyl), while amultiplet signals at 8 .4- 7.4 ppm due to aromatic protons.



Scheme1

Cyclization of chalcone 3 with malononitrile in the presence of piperidine afforded compound 4. The FTIR showed new absorption bands 2191cm^{-1} due to stretching vibration of C≡N and aband at 3444, 3350cm^{-1}due to stretching vibration of NH_2. The ^{1} H-NMR showed singlet signals 3.75 ppm assigned to three protons of p-substituted methoxy group and 5.32 ppm was attributed to NH_2 proton. The aromatic protons were appeared at 8.31-6.55 ppm.

Compound 5 was synthesized by reacting chalcone 3 with thiourea , in acidic medium. The FT IR spectrum showed NH stretching absorption in 3281cm^{-1} and C=S at 1240 cm $^{-1}$ with a weak absorption near 2660 cm^{-1} due to SH stretch because of thiol-thion-tautomeriism, while ^{1}H-NMR spectra of compound 5 show

doublet signal at 2.1 ppm and triplet at 4.0 ppm due to pyrimidine proton, singlet peak at δ 13.4 ppm belong to (SH-NH tautomeric)state.

Moreover, treatment of compound 1 with p-aminoaniline afforded compound 6.

The structure of compound 6 was confirmed by their FTIR spectra through the appearance of stretching vibration of NH_2 group, (asymmetrical and symmetrical) at 3323-3215 and C=O stretching vibrations at 1675 cm^{-1}.

^{1}H-NMR spectrum of compound 6 exhibited singlet signals 6.32 ppm was assigned to NH_2 proton and 3.70 ppm was attributed to three protons of p-substituted methoxy group. The aromatic protons were appeared at 8.25-6.74ppm.

The azo compound was synthesiszed by coupling between diazoniuim salt of aminoquinazolinone derivative with salicylaldehyde [22].

FTIR absoption bands of azo compound exhibited the disappearancs of two absorption bands due toNH_2 stretching of compound 6 together with the appearance of stretching band at 1521 cm^{-1} due to N=N group,which it also shows stretching abroad band around 3452 cm^{-1} due to the intramolecular hydrogen bonding of O-H group [23].^{1}H-NMR spectrum of azo compound exhibited two singlet signals 3.80 ppm was assigned to three protons of p-substituted methoxy group, 5.63 ppm was attributed to O-H proton, doublet of doublet at 7.40- 7.45and 7.61-7.69 ppm belong to (8H, 2ph),which is interference with the proton of quinazlinone and proton of salicylaldehyde ring , singlet at 10.5 ppm due to proton of aldehyde group.

The Knoevenagel condensation can be successfully applied to the synthesis of a number of coumarins, and the scope of the method is much broader. It is reported a very simple, fast and general procedure where the cyclization of azo compound with ethylacetoacetate in the presence of piperidine leads to coumarin dervative 8 [24].

The FTIR spectrum of compound 8 showed the disappearance of the O-H stretching frequency and C=O of aldehyde together with the appearance of bands at 1739 cm^{-1} assignable to C=O of chromen ring and C=O of acetyl group at 1653 cm^{-1}, are good evidence for the structure given to this compound.The ^{1} H-NMR spectrum of compound 8 exhibited two singlet signals: at 2.6 ppm corresponds to proron of acetyl group, 3.7 ppm was assigned to three protons of p-substituted methoxy group, doublet of doublet signals and a multiplet signals at 8.4- 6.2 ppm due to 16H aromatic proton.

Condensation of the amino group of derivative 6 with m-nitrobenzaldeyde in absolute ethanol gave the Schiff base 9. The formation of these Schiff base was indicated by the presence in their FTIR spectra of the (CH = N) stretching band at 1589 cm^{-1} combined with the disappearance of NH_2 stretching band of compound 6 and carbonyl group of m-nitrobenzaldehyde.

On the other hand, treatment of schiff base 9 with chloroacetyl chloride in triethylamine and dioxane yielded azetidinyl derivatives 10. The FTIR spectrum of compound 10 showed the disappearance bands of (CH=N) in the region 1589cm^{-1}, combined with the appearance of the absorption band at 1743 cm^{-1} (C=O for β-lactam).

The ^{1} H-NMR for compound 10 as representive case was showed the singlet signal: at 3.74 ppm was assigned to three protons of p-substituted methoxy group, doublet of doublet signals at 5.6-5.3 ppm due to proton of azetidinyl ring.

Antimicrobial activity

Standard antibacterial drug (Ampicillin) and antifungal drug (Fluconazole) were used for comparision. The experiments were performed in triplicate inorder to minimize errors.

The results of antimicrobial studies are given in Table 1. Compound 2-9 are potential antimicrobial.

Quinazolinone carrying chalcon ,pyrimdine, azo, coumarin, Schiff base moieties , which is responsible for antimicrobial activity . It seems that the compound 10 is very significant for activity against both bacterial and fungal species due to the presence of β-lactam ring. The increased activity of the new derivatives can be explained that act as more powerful and potent bactericidal agents, thus killing more of the bacteria . The π-electron delocalization over the new derivatives increases the lipophilic character and favours its permeation through the lipoid layer of the bacterial memberanes.

4 .CONCLUSION

Novel quinazolinone derivatives are prepared and are characterized on the basis of analytical and spectral data .Screening of these compounds against pathogenic microorganism reveals that these compounds have the capacity of inhibiting metabolic growth of some microorganisms to different extent. The antimicrobial activity of the compounds depends on the nature of substituent present on the quinazolinone ring.

Table(1) : Antimicrobial evaluation of compound (2-10)

Compound	Antibacterial Activity				Antifunal Activity	
	Zone of Inhibition in (mm)					
	Gram Negative		Gram Positive		Fungi	
	E. coli	*P. aeragines*	*S. aureus*	*S.pyogenes*	*C. albicans*	*A. niger*
2	50	0	0	30	60	50
3	80	50	0	50	70	50
4	80	60	0	50	70	55
5	100	60	10	60	80	60
6	80	50	10	50	70	50
7	110	70	10	50	70	60
8	120	100	10	70	80	80
9	100	70	10	70	70	50
10	150	140	10	80	75	80
Ampicillin	100	100	100	100	-	-
Fluconazole	-	-	-	-	100	100

Acknowlegement
This study supported by Department of Chemistry, College of Science, Al-Mustansiriyah University, Baghdad-Iraq.

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