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Synthesis and biological evaluation studies of Some quinazolinone derivatives as antimicrobial and antioxidant agents

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Abstract

New quinazolineone derivatives comprising pyridine, Schiff base, 2- azetidinone, 4-thiazolidinoneand moieties are proved. The structures of the compounds have been confirmed by FT-IR and ¹H-NMR spectra and element analysis.. The synthesized derivatives have been screened for antimicrobial and *in vitro* antioxidant properties. The results of this investigation revealed that the newly synthesized compounds are potent antimicrobial and antioxidant agent.

Keywords: quinazolineone 2-Azetidinone, 4-thiazolidinone.Antioxidant

INTRODUCTION

The quinazolinone skeleton is a frequently encountered heterocycle in medicinal chemistry literature with applications They have drawn much attention due to their broad range of pharmacological properties [1], which include anticancer[2], anti-inflammatory[3], anticonvulsant[4] and antidiuretic [5] activities.
Little number of quinazolinones was reported as potent chemotherapeutic agents in the treatment of tuberculosis. For example 3-aryl-6, 8-dichloro-2H-1, 3-benzoxazine-2, 4(3H)-diones and 3-arylquinazoline-2, 4(1H, 3H)-diones as antimycobacterial agents, quinazolinone derivatives as antitubercular agents.[6] Compounds of both synthetic and natural origin comprising a diverse group of chemical structure have been reported as antileishmial agents. These include mostly nitrogen heterocyclic such as quinolines, purine, pyrimidine, acidine, phenothiazines, bisbenzamides, pyrazolol, pyridine, benzothiazole, imidazolines.[7]

MATERIALS AND METHODS

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,benzene, chloroform and acetone as mobile phase. FTIR measurements were recorded on Shimadzu model FT-IR-8400S. ^{1}HNMR spectra were obtained with a Brukerspectrophotometer model Ultra Shield at 300 MHz in DMSO-*d*6 solution with the TMS as internal standard.

Synthesis 1- mercapto-3-(pyridin-2-yl)quinazolin-4(3H)-one (1)

Carbon disulphide (12ml) was added drop wise to an ice cold solution of potassium hydroxide (0.015 mol) in absolute ethanol(25 ml) containing solution of anthranilic (0.01mol)and 0.01mol) 2-aminopyridine .The mixture *refluxed for 8 hours* then . poured into ice-water. The solid wasfiltered washed with water and recrystallized from ethanol. Yield: 78%. M.p.180-182°C, FT-IR spectrum (ν, cm^{-1}): 3367 υ(N-H) , 1685υ(C=O),1614υ(C=S),¹ H-NMR spectrum (δ, ppm): 9.23(s, 1H, NH), 8.46-6 .4 5 (m, 8H, Ar-H).

Ethyl 2-((4-oxo-3-(pyrdin-2-yl)-3,4-dihydroquinazolin-2-yl)thio) acetate (2)

A mixture of compound (1) (0.03mol) anhydrous K_2CO_3 (0.07mole, 10g) bromoethylacetate (0.035mol, 6g) in dry acetone (30mL) as solvent. was refluxed for8hrs. Acetone was removed under reduced pressure, the products were collected and recrystallization from ethanol. Yield: 79%.M.p.208-210°C, FT-IRspectrum (ν, cm^{-1}): 2980υ,2830υ(C-H), 1734υ(C=O), 1215υ(C-O),¹ H-NMR spectrum (δ, ppm): 7.89-6 .57 (m, 8H, Ar-H),4.5(q,2H,OCH_2),4.0(s,2H.CH_2),1.34(t,3H,CH_3).

2-((4-oxo-3-(pyridin-2-yl)-3,4-dihydroquinazolin-2-yl)thio) acetohydrazid (3)

A mixture of hydrazine hydrate 80% and Compound (**2**) (0.093 mol) were refluxed for 10 hrs. in (20mL) of absolute ethanol as solvent, the precipitate was formed in the mixture filtered and washed with cold water, dried and purification from ethanol.85%.M.p. 199-201 °C, FT-IR spectrum (ν, cm^{-1}): 3360,3178υ (NH_2),1653υ(C=O),¹ H-NMR spectrum (δ, ppm):9.35 (s,1H, NH), 8.32-6 .67 (m, 8H, Ar-H), 7.67(s, 1H, NH),4.62(s,2H,NH_2),3.82(s,2H,CH_2).

2-(2-((4-oxo-3-(pyridin-2-yl)-3,4-dihydroquinazolin-2-yl) thio) acetyl)-N-phenylhydrazine-1-carbothioamide (4).

A mixture of compound **3** (0.01mole) and phenyl isothiocyanate (0.01mole) in (30ml) dry dioxan was refluxed for 8hrs. The reaction mixture was concentrated and the obtained solid was filtered off, and recrystallized from acetone.Yield: 83%.M.p. 145-147°C, FT-IR spectrum(ν, cm^{-1}): 3225, 3186, 3119 υ(N-H), 1683υ(C=O) , 1647υ(C=S).¹ H-NMR spectrum (δ, ppm);11.35(s,1H,NHph),9.34 (s, 1H, NHCS), 8,45--6 .65 (m, 13H, Ar-H,NHCO), 3.62(s,2H,CH_2).

Synthesis of Schiff bases (5,6)

To a solution of compound 2 (0.001mole) in (25 mL) of absolute ethanol, the aromatic aldehyde (0.001 mole) was added with 2-3 drops of glacial acetic acid. The mixture have been refluxed for 7 hours, reaction mixture was cooled then the mixturewasfiltered and recrystallized from chloroform.

N'-(4-nitrobenzylidene)-2-((4-oxo-3-(pyridin-2-yl)-3,4-dihydroquina- zolin-2-yl)thio)acetohydrazide (5).

Yield: 67%.M.p.270-272°C, FT-IRspectrum (ν, cm^{-1}): 3333 υ(N-H),1681υ(C=O),1523, 1343υ(NO_2). ¹ H-NMR spectrum (δ, ppm):10.43(s, 1H, NH), 8.87-6 .52 (m, 12H, Ar-H,CH=N).

N'-(2-chlorobenzylidene)-2-((4-oxo-3-(pyridin-2-yl)-3,4-dihydroquin- azolin-2-yl)thio)acetohydrazide(6).

Yield: 65%.M.p.225-227°C, FT-IR spectrum (ν, cm^{-1}): 3281 υ(N-H), 1695υ(C=O),1090υ(C-Cl). ¹ H-NMR spectrum (δ, ppm):10.45(s, 1H, NH) 8.67-6 .6 8(m, 12H, Ar-H,CH=N).

Synthesis of oxoazetidine derivatives (7,8)

The mixture of Schiff base(8,9) (0.01 mol) and triethyl amine (0.02 mol) was dissolved in dioxane (20 mL). Chloroacetyl chloride (0.02 mol) was added in portion wise with vigorous shaking at room temperature for 25 min. The reaction mixture was heated under reflux for 4 h and the content was kept at room temperature for 24 h and poured into ice-cold water. The product obtained was filtered,wasfiltered and recrystallized from chloroform.

N-(3-chloro-2-(2-chlorophenyl)-4-oxoazetidin-1-yl)-2-((4-oxo-3-(pyridin-2-yl)-3,4-dihydroquinazolin-2-yl)thio)acetamide (7).

Yield: 71%.M.p. 162-164°C, FT-IRspectrum(ν, cm^{-1}): 3210 υ(N-H), 1710υ(C=O),1560,1552υ(NO_2).¹ H-NMR spectrum (δ, ppm): 7.89-6 .55 (m, 8H, Ar-H),8.45(s, 1H, NH), 5.6 -5.0 (dd, 2H, CH)

N-(3-chloro-2-(4-Nitro)-4-oxoazetidin-1-yl)-2-((4-oxo-3-(pyridin-2-yl)-3,4-dihydroquinazolin-2-yl)thio)acetamide (8).

Yield: 69%.M.p. 178-180°C, FT-IRspectrum(ν, cm^{-1}): 3280 υ(N-H), 1734υ(C=O),1105υ(C-Cl).¹ H-NMR spectrum (δ, ppm): 7.80-6 .54 (m, 8H, Ar-H), 8.63(s, 1H, NH),5.3 -4.8 (dd, 2H, CH)

Synthesis of oxothiazolidinone derivateves (9,10)

A mixture of compounds(5,6) (0.001mol) was solved in 25mL chloroform with $ZnCl_2$ (0.01g) and (0.005mol) of thioglycolic acid was added to the mixture, the mixture was refluxed for 10hrs. The reaction completion was monitored by thin layer chromatography (TLC) using ethyl acetate : hexane system (3:7). The solvent was removed under reduced pressure, residue treated by solution of 10% $NaHCO_3$ to removed excess of mercapto acetic acid, washed with water, dried and recrystallization from chloroform.

N-(2-(2-chlorophenyl)-4-oxothiazolidin-3-yl)-2-((4-oxo-3-(pyridin-2-yl)-3,4-dihydroquinazolin-2-yl)thio)acetamide (9).

Yield: 64%.M.p. 198-200°C, FT-IRspectrum(ν, cm^{-1}): 3319 υ(N-H), 1722υ(C=O),1562,1354υ(NO_2).¹ H-NMR spectrum (δ, ppm): 8.53-6.46 (m, 8H, Ar-H), 8.78(s, 1H, NH), 5.60 (s, 1H, CH-N), 3.75 (s, 2H, CH_2).

N-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl)-2-((4-oxo-3-(pyridin-2-yl)-3,4-dihydroquinazolin-2-yl)thio)acetamide(10).

Yield: 68%.M.p.228-230 °C, FT-IR spectrum (ν, cm^{-1}): 3328 υ(N-H), 1728υ(C=O),1095υ(C-Cl).¹ H-NMR spectrum (δ, ppm): 7.87-6 .35 (m, 8H, Ar-H), 7.98(s, 1H, NH)

Biological activities

In vitro antimicrobial testing effects of benzimidazole derivatives were estimated against four bacterial strains namely .The antimicrobial activity was determined using the agar well diffusion method [8]. Dimethyl sulfoxide worked as a control and the test was outright at 100mg/mL and by adding 50µl to each disc (i.e.5µg/disc) concentration using (DMSO) as solvent. The fungi and 4 bacteria was sub cultured in agar. The plates were incubated at 37 °C and checking after 24 hrs for bacteria and 27°C for 48 hrs for fungi Table 2.

Antioxidant activity

The free radical scavenging activity of compound **5** towards the radical (DPPH) 1,1-diphenyl-2-picryl hydrazyl wasmeasured as described by reference [9]. The 2-azetidinonestock solution (1 mg/mL) was diluted to final concentration of 20-100 µg/mL. Methanolic DPPH solution (1 mL, 0.3 mmol) was added to sample solution in DMSO (3 mL) at differentconcentration. The mixture was shaken vigorously and allowed to stand atroom temperature for 30 min. the absorbance was thenmeasured at 517 nm (As), using "Shimadzu175 spectrophotometer".

The methanol solution of DPPH was used as control sample Ac. The ability of scavenge the DPPH radical wascalculated using the following formula:

% Radical scavenging activity = 100 × (Ac-As)/Ac (1)

Methanol was used as the solvent and ascorbic acid as the standard.

Antimicrobial activities

In this work it has been used the Amoxicillin drug as standard antibacterial for comparison with the quinazolineone derivatives. Quinazolinone derivatives (13) showed high inhibition toward all kinds. The results of these studies are summarized in Table 1

Antioxidant activity

Hydrogen peroxide is generated in vivo by several oxidase enzymes and which product hydroxyl radical causes severe damage to biological systems. The antioxidant potential of compounds **4-10** was determined on the basis of its scavenging of the stable (DPPH) free radical. Figure 1 showed that the compound **5** exhibit excellent antioxidant properties, the potential is comparable with antioxidant activity of ascorbic acid activity. This may be due to the presence of -NH group.

RESULTS AND DISCUSSION

Compounds (1–10) were synthesized according to Scheme (1) and the purity of the synthesized compounds was confirmed by TLC, ^{1}HNMR. 1- mercapto-3-(pyridin-2-yl)quinazolin-4(3H)-one (1) was synthesized by heating anthranilic acid, carbon disulphide and 2-aminopyridine in ethanol containing a catalytic amount of potassium hydroxide, producing (1) in 78% yield. The FT-IR spectrum showed stretching bands at 3367 cm^{-1} for (N-H) group, 1685 cm^{-1} for (C=O),and 1614 cm^{-1} for (C=S). The ^{1}H NMR spectra revealed a singlet signal at 9.23 ppm corresponding to the NH group and multi signals at 6.45- 8.46 ppm for hydrogen aromatic. Ethyl 2-((4-oxo-3-(pyrdin-2-yl)-3,4-dihydroquinazolin-2-yl)thio) acetate (2) was obtained in 79 % yield through the reaction of compound (1) with ethyl bromoacetate in acetone. The FT-IR spectrum showed disappearance stretching bands of (N-H) , (C=S) and appearance stretching bands at 2980 ,2830 cm^{-1} for (C-H), 1734 cm^{-1} for (C=O) and 1215 cm^{-1} for (C-O). The ^{1}H NMR spectra showed a singlet signal at 9.23 ppm for SH group and multi signals at 6.57-7.89 ppm for hydrogen aromatic. Compound (2) was reacted with hydrazine hydrate in ethanol to produce 2- ((4-oxo-3-(pyridin-2-yl)-3,4-dihydroquinazolin-2-yl) thio) acetohydrazid (3) in 85% yield. The structure of compound (3) was confirmed via analysis of spectral data, The FT-IR spectrum showed stretching bands at 3360,3178 cm^{-1} for (NH_2) and 1653 cm^{-1} for (C=O), The ^{1}H NMR spectrum revealed quartet, singlet, and triplet signals corresponding to (OCH_2), (SCH_2), and (OCH_2CH_3), at 4.25, 4.02, and 1.31 ppm, respectively.

2-((4-oxo-3-(pyridin-2-yl)-3,4-dihydroquinazolin-2-yl)thio)acetohyd - razid (3) was reacted with substituted benzaldehyde in ethanol to produce the Schiff bases (5,6) in 65–67% yield. The FT-IR spectra showed disappearance stretching bands of (NH_2), and appearance stretching bands at 3281-3333 cm^{-1} for (NH), 1681-1695 cm^{-1} for (C=O), 1343 cm^{-1} for (NO_2) and 1090 cm^{-1} for (CL). The ^{1}H NMR spectrum of compounds (5) and (6) showed singlet signal at 10.43 and 10.45 ppm corresponding to the NH group and multi signals at 6.52- 8.87 and 6.60- 8.67 ppm for aromatic hydrogen's and CH=N respectively.

To synthesis oxoazetidine derivatives (7,8) , the Schiff bases (5,6) were reacted with Chloroacetyl chloride in presence of triethyl amine as catalysis and dioxane as solvent. The FT-IR spectra showed stretching bands at 3210-3280 cm^{-1} for (NH), 1710-1734 cm^{-1} for (C=O), 1552, 1560 cm^{-1} for (NO_2) and 1105 cm^{-1} for (CL), the ^{1}H NMR spectrua of compounds (7 and 8) showed douplet douplet signal at 5.3 -5.0 ppm for CH , singlet signal for NH at 8.45-8.63 ppm and multi signals at 6.55-7.89 ppm for aromatic hydrogen.

The oxothiazolidinone derivateves (9,10) were synthesized by reaction compounds(5,6) with thioglycolic acid in presence anhydrous $ZnCl_2$ as catalyst. The FT-IR spectra showed stretching bands at 3319-3328 cm^{-1} for (NH), 1722-1728 cm^{-1} for (C=O), 1562, 1354 cm^{-1} for (NO_2) and 1095 cm^{-1} for (CL), The ^{1}H NMR spectrua of compounds (9 and 10) showed Singlet signal at 5.60 ppm for CH-N , Singlet signal at 7.98 - 8.78 ppm for NH and multi signals at 6 .35 - 8.53 ppm for aromatic hydrogen

Antioxidant activity Study:

Antioxidant screening (DPPH radical scavenging activity)

The scavenging activity results of some of synthetic compounds showed in table (1):

Table 1: Scavenging activity of some of synthetic compounds

Comp. No.	Scavenging activity % in (12.5 - 100) µg/ml
5	15-25 %
6	15-40 %
7	17-63 %
8	17-70 %
9	15-60 %
10	18-75 %

From the results in figures (1-6), the conc. (12.5) µg/ml is the most scavenging activity compared with other concentrations of compounds (7,8), while the conc. (100) µg/ml is the most scavenging activity for compound (10) because it's scavenging activity is more than scavenging activity of ascorbic acid in this concentration

DPPH radical scavenging is considered a good *in vitro* model and is widely used to conveniently assess antioxidant efficacy. In its radical form, DPPH has an absorbance at 515 nm which disappears when DPPH is reduced by an antioxidant compound or a radical species to become

a stable diamagnetic molecule. As a result, the color changes from purple to yellow. This color change is taken as an indication of the hydrogen donating ability of the tested compounds.

Antioxidants can react with DPPH and produce 1,1-diphenyl-2-picryl-hydrazine. The reducing abilities of the examined compounds were determined by their interaction with the free stable radical 1,1- diphenyl-2-picryl-hydrazine (DPPH) at five different concentrations for 30 min. The highest scavenger activity observed in compound (10), this is probably due to the presence of nitro group and oxothiazolidin ring. Generally electron withdrawing substituent's deactivate aromatic ring and have no capability to bind the free radicals. From the results, nitro compound exhibited better activity than chloro and oxothiazolidin derivatives exhibited more activity than oxoazetidine derivative.

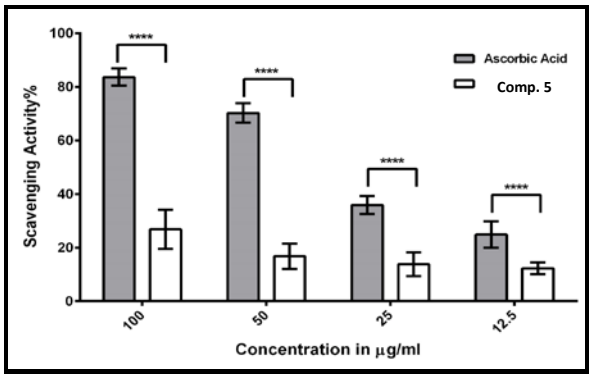


Figure 1: % Scavenging activity of the compound 5 using DPPH.

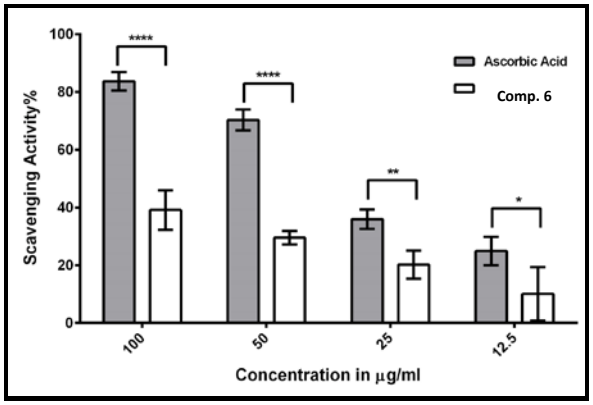


Figure 2: % Scavenging activity of the compound 6 using DPPH.

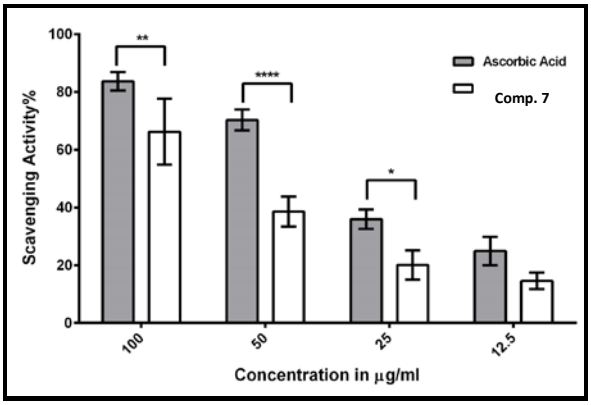


Figure 3: % Scavenging activity of the compound 7 using DPPH.

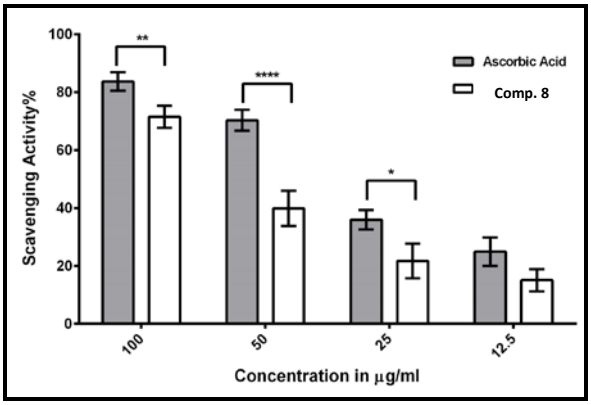


Figure 4: % Scavenging activity of the compound 8 using DPPH.

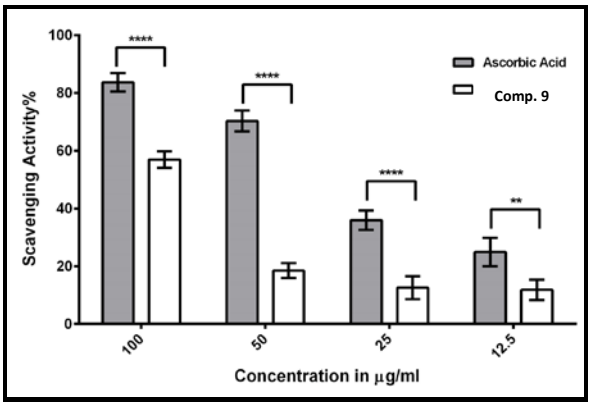


Figure 5: % Scavenging activity of the compound 9 using DPPH

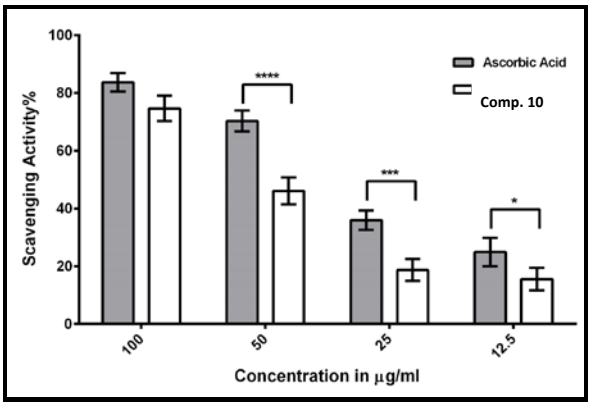
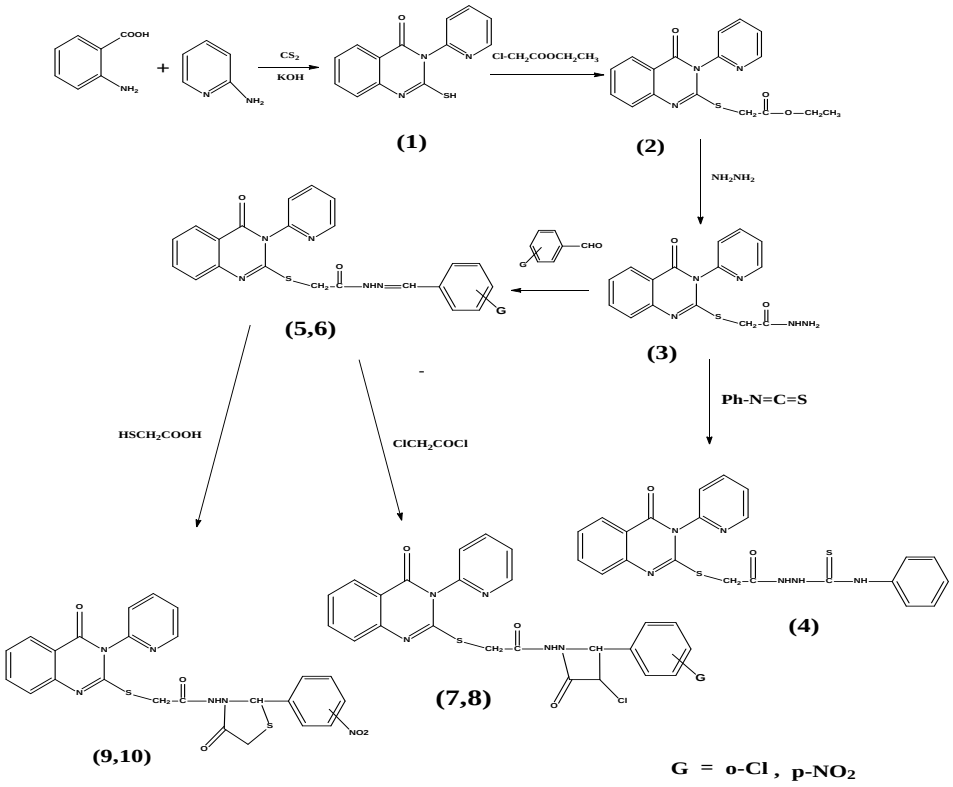


Figure 6: % Scavenging activity of the compound 10 using DPPH



Scheme 1

Table 2: Antimicrobial evaluation of compounds(3-10)

Heterocyclic Derivative	Inhibition zone (mm) at 100 mg/mL				
	Grampositive		Gram negative		Fungi
	S. aureus	*S.epidermidis*	*E.coli*	*Klebsiellaspp*	*C.albicanus*
3	10	15	13	13	14
4	15	14	15	14	15
5	13	10	-	14	18
6	14	10	15	-	15
7	16	12	20	13	12
8	18	12	18	10	18
9	21	15	19	19	13
10	19	13	18	18	19
Amoxicillin	20	17	19	20	21

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