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ANTI - MICROBIAL ACTIVITY OF NOVEL ISOXAZOLE CONTAINING QUINAZOLINONE DERIVATIVES

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ABSTRACT

In this study, synthesis of 3-[4-(5-(3,4-disubstituted phenyl)-4,5-dihydro isoxazol-3-yl) phenyl]-2-substituted phenyl Quinolin-4(3H)-one derivatives has been described. Newly Isoxazole derivatives were prepared by reaction of quinazolinone derivatives with hydroxylamine hydrochloride in presence of pyridine. A total of eight derivatives were synthesized and the compounds were purified by chromatographic methods and identified by spectroscopic methods; FTIR, H1 NMR and also by measuring its melting point. The synthesized compounds were tested for antibacterial activity against four bacterial strains, of them two are positive strain *Staphylococcus aureus* and *Bacillus subtilis* and two gram negative strain *Escherichia coli* and *Pseudomonas aeruginosa*. The compounds were also evaluated for antifungal activity against two fungal strains and *Asperigillus niger* and *Saccharomyces cerevisiae*. The isoxazole compounds viz 3-(4-(5-(3-nitrophenyl)-4, 5-dihydroisozolyl-3-yl)- phenyl)-2-(4-nitrophenyl) quinazolin-4(3H)-one (4h), and 3-(4-(5-(3-chlorophenyl)-4, 5- dihydroisozolyl-3-yl)- phenyl)-2-(4- nitrophenyl) quinazolin-4(3H)-one (4f) derivatives were found to be quite superior in active against all organism employed in anti-bacterial action comparable to standard drug ampicillin. The compound 4b is quite superior in its anti-fungal action and also the activity was comparable to the standard drug griseofulvin.

KEYWORDS

Iso oxazole, Quinazolinone, Anti-bacterial and Anti-fungal.

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INTRODUCTION

Compounds containing heterocyclic ring systems continue to attract considerable interest due to the wide range of their biological activities. Amongst them five membered heterocyclic compounds occupy a unique place in the science of medicinal organic chemistry. Five membered heterocycles like isoxazoline have found wide application as pharmaceutical agents. In recent years, attention has increasingly been given to

the synthesis of isoxazoline derivatives as a source of new antibacterial agents. The synthesis of novel isoxazoline derivatives remain a main focus of medicinal research. Isoxazoline derivatives have been reported to possess antifungal, antibacterial¹ and anxiolytic².

It was observed that heterocycles that incorporated isoxazole derivatives in their molecules exhibited a wide range of biological properties such as antibiotic³, anticancer⁴, and antiviral⁵ activities. The quinazolinones are considered to be important versatile pharmacophore in the fields of pharmacy and biology. Quinazoline-4(3H)-ones are versatile nitrogen heterocyclic compounds, displaying a broad spectrum of biological and pharmacological activities such as anti-inflammatory and anticancer⁶. Prompted by all these observations work on the synthesis of some isooxazole and quinazolinone derivatives, herein we report the synthesis of some novel isooxazole contain quinazolinone derivatives, which have been found to possess an interesting profile of anti-microbial activity.

MATERIALS AND METHODS

All the chemicals and solvents required for the study were purchased from SD Fine, Kemphasol, Ranbaxy, Hay man Ltd, fisher and S.D. Fine Chem. Ltd. All the solvents procured were purified and dried. The solvent system used for Thin Layer Chromatography in Benzene and acetone (9:1). Iodine chamber and UV Lamps were used for visualization of TLC spots; Whatmann Filter Paper (No.1, England) was used for filtration (Vacuum or ordinary). H¹ NMR spectra were recorded on 300 MHz instruments and the Mass spectra were recorded on Joel SX102/Da-600. FT-IR was recorded in Shimadzu. Melting points were determined using Sulfuric acid bath which was uncorrected.

SYNTHESIS

Synthesis of 2-Substituted Phenyl-4H-Benzo-[1,3]-Oxazin-4-One 1_(a-b)

To a stirred solution of anthranilic acid (0.01 mole) in pyridine (50ml), substituted benzoyl chloride (0.01 mole) was added drop wise, maintaining the temperature near 80⁰ C for 2 hour. Reaction mixture was stirred for another 3 hours at room temperature. While stirring a solid product separates out. Whole

reaction mixture was neutralized with sodium bicarbonate solution. A pale yellow solid deposited which was filtered, washed with water and re-crystallized with sodium bicarbonate solution.

Synthesis of 4-(4-Oxo-2-Substituted Phenylquinazolin-3(4H)-yl) -benzaldehyde 2_(a-b)

Compound 1_(a-b) (0.01 mole) was dissolved in ethanol and 4-amino benzaldehyde (0.01 mole) in ethanol was added to it with a catalytic amount of pyridine. Then the reaction mixture was refluxed for 4 hours and after cooling a crystalline product was obtained. Then it was filtered and re-crystallized from ethanol to yield needle shaped shining white crystals.

Synthesis of Compound 2-Substituted Phenyl-3-(4-(3-(Substituted Phenyl-3-oxo Prop-1-enyl) Phenyl) Quiniazoline-4-One: 3_(a-h)

Equimolar quantities of compound 2 (a-b) and substituted acetophenone (0.01 mole) were dissolved in the minimum amount of alcohol. Then sodium hydroxide solution (0.02 mole) was added slowly and the mixture stirred for 3 hours until the entire mixture becomes very cloud and then the mixture was poured slowly in to 400 ml of water with constant stirring and kept in refrigerator for 24 hours. The precipitate obtained was filtered, washed and re-crystallized from ethanol.

Synthesis of 3-[4-(5-(3,4-disubstituted phenyl)-4,5-dihydro isoxazol-3-yl) phenyl]-2-substituted phenyl Quinolin-4(3H)-one derivatives: 4 (a-h)

A mixture of chalcone 3 (a-h) (0.02 mole), hydroxylamine hydrochloride (0.02 mole) and sodium acetate in ethanol (25 ml) was refluxed for 6 hr. Then the mixture was concentrated by distilling out the solvent under reduced pressure and poured in to ice water. The precipitate obtained was filtered, washed and re-crystallized

ANTI -MICROBIAL ACTIVITY

Anti-Bacterial Activity

All the newly synthesized compounds were screened for antibacterial activity against two Gram-positive organisms, *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus*, (ATCC 25923) and two Gram-negative organisms, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) by cup-plate method⁷. Antimicrobial activity is measured in

vitro in order to determine a) the potency of an antibacterial agent in solution b) the sensitivity of a given microorganism to know concentrations of the synthesized drug.

A suspension of the test organism was well mixed with 25 ml of sterile liquid nutrient agar media, at a temperature between 40-50⁰ C and poured immediately in to a pre-sterilized petri-dishes. The plates were left at room temperature to allow the solidification. In each plate four cups of 10 mm diameter were made with a sterile borer. Solutions of the test compounds were prepared by dissolving 10 mg of each in 100 ml dimethyl sulphoxide (AR grade) to get g/ml. final concentration of 100. A reference standard for gram-positive and gram-negative bacteria was made by dissolving accurately weighed quantity of g/ml of test solution was added to the Amphotericin in DMSO solution. Then, 100 cups, aseptically and labeled accordingly. The plates were kept undisturbed for at least 2 hrs at room temperature to allow diffusion of the solution properly 1° C for 24 hr into nutrient agar medium. After incubation of the plates at 37 the diameter of the zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader. All the experiments were carried out in triplicate. Simultaneously controls were maintaining employing 0.1 ml of dimethyl sulphoxide (DMSO) to observe the solvent effects and the results were shown in Table No.2.

Anti-Fungal Activity

All the compounds screened were also tested for their antifungal activity against the organism *Aspergillus niger* and *Saccharomyces cerevisiae* by cup-plate method⁷.

The test organisms were sub-cultured using potato dextrose agar medium. The tubes containing sterilized medium were inoculated with test fungi and after incubation at 25⁰C for 48 hr they were stored 4 in refrigeration. The inoculum was prepared by taking a loopful of stock culture to about 100 ml of nutrient broth, in 250 ml clean and sterilized flasks. The flasks were incubated at 25⁰C for 24 hr before use.

The solutions of test substances were prepared by similar procedure described under the antibacterial activity. A reference standard (0.1 mg/ml conc) was

prepared by dissolving 10 mg of Griseofulvin in 100 ml of DMSO to obtain a solution of 100 g/ml concentration.

The potato dextrose agar medium was sterilized by autoclaving at 121°C (15 lb/sq. inch) for 15 minutes. The petri plates, tubes and flask plugged with cotton plugs were sterilized in hot air oven at 150°C for an hour. Into each sterilized Petri-plate about 30 ml of each of molten potato dextrose agar medium inoculated with respective fungus (6ml of inoculum to 300 ml of potato dextrose agar medium) was transferred, aseptically. After solidification of the medium at room temperature four cups of 10 mm diameter were made in each plate with a sterile borer. g/ml. Accurately 0.1 ml (100 conc.) of test solution was transferred to the cups, aseptically and labeled, accordingly. The reference standard 0.1 g/ml conc.) was also added to the cups in each plate. The plates were ml (100 kept undisturbed for at least two hours at room temperature to allow diffusion of the solution properly, into potato dextrose agar medium. Then the plates were incubated at 25°C for 48 hr. The diameter of the zone of inhibition was read with help of an antibiotic zone reader. The experiments were performed in triplicate in order to minimize the errors and the results were shown in Table No.2.

RESULTS AND DISCUSSION

Antimicrobial activity

All the newly synthesized 3-[4-(5-(substituted phenyl)-4,5-dihydro isoxazol-3-yl) phenyl]-2-substituted phenyl quinazolin-4(3H)-one derivatives 4_(a-h), were screened for their antibacterial activity against *B. subtilis* and *S. aureus* (Gram +ve), *E. coli* and *P. aeruginosa* (Gram -ve) and antifungal activity against *A. niger* and *C. albicans* by cup-plate method at a concentration of 100 µg / ml and measured the zone of inhibition in mm and the results were tabulated in table1. The reference drug used was Ampicillin and Griseofulvin at a concentration of 100 µg/ml for antibacterial and antifungal activity respectively. The isoxazole compounds viz 3-(4-(5-(3-nitrophenyl)-4, 5-dihydroisoxazolyl-3-yl)- phenyl)-2-(4-nitrophenyl) quinazolin-4(3H)-one (4h), and 3-(4-(5-(3-chlorophenyl)-4, 5- dihydroisoxazolyl-3-yl)- phenyl)-2-

(4- nitrophenyl) quinazolin-4(3H)-one (4f) derivatives were found to be quite superior in active against all organism employed in anti-bacterial action. The compound 4b is quite superior in its anti-fungal action and also the activity was comparable to the standard drug griseofulvin.

The sensitivity of microorganisms to the tested compounds is identified in the following manner:

Highly sensitive = Inhibition zone 30–40 mm

Sensitive = Inhibition zone: 20–30 mm

Slightly sensitive = Inhibition zone: 10–20 mm

Not sensitive = Inhibition zone: below 10 mm

Spectral Data

Compound 5a

IR (KBr) cm-1: 3092(C-H- str, Aromatic)., 2939(C-H Str, Alkyl), 1678 C=O str, Aromatic keto)., 1586 (C=N str).,2920 (CH str, Alkyl).1H NMR (DMSO) δ ppm: 6.964-7.899(m,18H,Ar-H),3.826-3.871(t,1H,-CH-CH2),3.247-3.269((d,2H,-CH-CH2), ESIMS (m/z): 443 (M+).

Compound 5b

IR (KBr) cm-1: 3101(CH- str,Aromatic)., 1670 (C=O str, Aromatic keto)., 1620 (C=C str).,1560(C=N str).,1506,1344(Aromatic Nitro).,1249(C-N str).,1103(C-O str).,

1H NMR (DMSO) δ ppm7.379-8.750(m,17H,Ar-H),4.283-4.330(t,1H,-CH-CH2),3.372-3.355(d,2H,-CH-CH2).ESIMS (m/z): 488 (M+).

Compound 5c

IR (KBr) cm-1: 3064(C-H- str,Aromatic).,2987(C-H str Methoxy)., 1678 (C=O str, Aromatic keto)., 1627(C=C str Aromatic).,1568 (C=N str).,1253,1166(C-O str).,

1H NMR (DMSO) δ ppm: 7.029-7.944(m,17H,Ar-H),4.328-4.370(t,1H,-CH-CH2),3.360-3.383(d,2H,-CH-CH2),3.92(s,3H,CO-CH3).ESIMS (m/z): 473 (M+).

Compound 5d

IR (KBr) cm-1: 3090(CH- str,Aromatic)., 1678 (C=O str, Aromatic keto).,1600(C=N str)., 1586 (C=N str).,1521,1304(Aromatic Nitro).,2939 (CH str, Alkyl).,1246(C-N str).,1182(C-O str, Methoxy).1H NMR

(DMSO) δ ppm: 7.019-7.964(m,16H,Ar-H).,4.229-4.274(t,1H,-CH-CH2).,2.820-2.843,(d,2H,-CH-CH2).,4.413,(s,3H, CO-CH3).ESIMS (m/z): 518 (M+).

Compound 5e

IR (KBr) cm-1: 3086(C-H str, Aromatic).,1658(C=O str, Aromatic Keto).,1586 (C=N str).,2920 (CH str

Alkyl)1H NMR (DMSO) δ ppm 6.709-6.806(m,17H,Ar-H).,4.687-4.730(t,1H,-CH-CH2).,3.018-3.041(d,2H,-CH-CH2).,ESIMS (m/z): 477 (M+).

Compound 5f

IR (KBr) cm-1: 3097(C-H- str,Aromatic)., 2985(C-H str, Alkyl)., 1670 (C=O str, Aromatic keto).,

1639(C=O str, Aliphatic Amide keto)., 1604 (C=N str).,1539,1342(Aromatic Nitro)1477,1381(C-H Bending Alkyl).,1274,1213(C-N str).,1H NMR

(DMSO) δ ppm: 7.056-7.950(m,17H,Ar-H).,4.430-4.574(t,1H,-CH-CH2).,2.821-2.843(d,2H,-CH-CH2)., 2.095(s,3H, CO-CH3).ESIMS (m/z): 522 (M+).

Compound 5g

IR (KBr) cm-1: 3130(C-H- str,Aromatic)., 2874(C-H str, Alkyl)., 1651 (C=O str, Aromatic keto).,

1624(C=O str, Aliphatic Amide keto)., 1556 (C=N str).,1527,1402(Aromatic Nitro).,1242,(C-N str).,1H NMR

(DMSO) δ ppm: 6.989-7.925(m,17H,Ar-H).,4.496-4.549(t,1H,-CH-CH2).,2.726-7.747(d,2H,-CH-CH2)., 2.143(s,3H, CO-CH3).ESIMS (m/z): 488(M+).

Compound 5h

IR (KBr) cm-1: 3057(C-H- str,Aromatic)., 2978(C-H str, Alkyl)., 1669 (C=O str, Aromatic keto).,

1639(C=O str, Aliphatic Amide keto)., 1589 (C=N str).,1531,1325(Aromatic Nitro)1460,1381(C-H Bending Alkyl).,1284,1205(C-N str).,1H NMR

(DMSO) δ ppm 7.043-8.018(m,16H,Ar-H).,4.945-4.899(t,1H,-CH-CH2).,2.335-2.314(d,2H,-CH-CH2)., 2.055(s,3H, CO-CH3).ESIMS (m/z): 533 (M+).

Table No.1: Characterization data of synthesized compounds 4a-h

| S.No | Compound Code | Melting Point (°C) | % Yield | Molecular Formula | Molecular Weight |
|------|---------------|--------------------|---------|--|------------------|
| 1 | 4a | 169 °C | 75.17 % | C ₂₉ H ₂₁ N ₃ O ₂ | 443.50 |
| 2 | 4b | 154 °C | 73.94 % | C ₂₉ H ₂₀ N ₄ O ₄ | 488.49 |
| 3 | 4c | 158 °C | 75.48 % | C ₃₀ H ₂₃ N ₃ O ₃ | 473.52 |
| 4 | 4d | 172 °C | 78.68 % | C ₃₀ H ₂₂ N ₄ O ₅ | 518.52 |
| 5 | 4e | 155 °C | 74.13 % | C ₂₉ H ₂₀ N ₃ O ₂ Cl | 477.94 |
| 6 | 4f | 146 °C | 71.19 % | C ₂₉ H ₁₉ N ₄ O ₄ Cl | 522.94 |
| 7 | 4g | 151 °C | 76.42 % | C ₂₉ H ₂₀ N ₄ O ₄ | 488.49 |
| 8 | 4h | 166 °C | 75.29 % | C ₂₉ H ₁₉ N ₅ O ₆ | 533.49 |

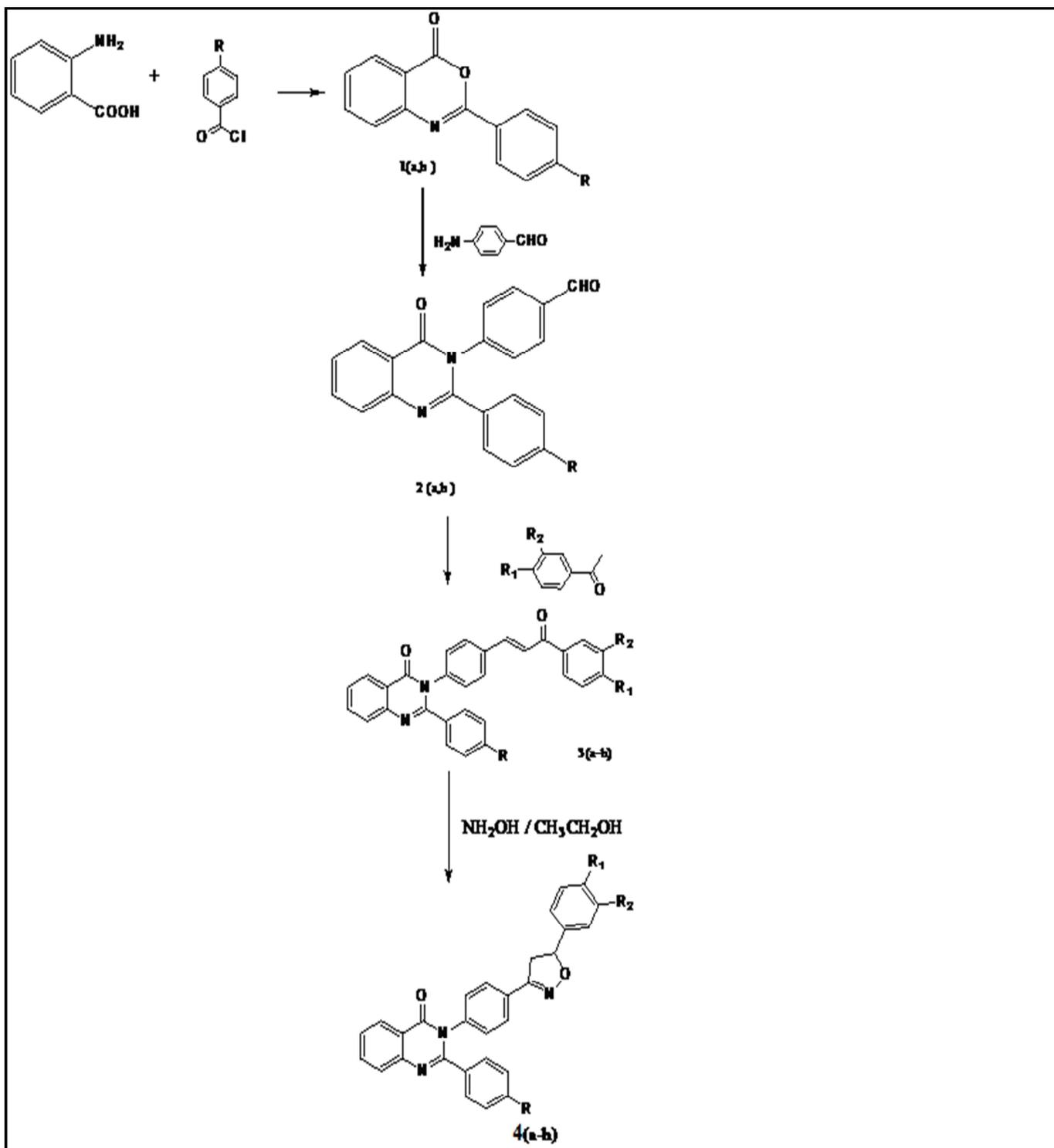
Table No.2: Results of Anti-Bacterial and Antifungal activity of synthesized Quinazolinone Derivatives

| S.No | Compounds | Zone of Inhibition (mm) | | | | | |
|------|-----------|-------------------------|-----------------|---------------|---------------------|-----------------|----------------------|
| | | Gram Positive | | Gram Negative | | Fungal strains | |
| | | <i>B.subtilis</i> | <i>S.aureus</i> | <i>E.coli</i> | <i>P.aeruginosa</i> | <i>A. niger</i> | <i>S. cerevisiae</i> |
| 1 | 4a. | 14 | 15 | 13 | 12 | 25 | 10 |
| 2 | 4b. | 15 | -- | 14 | -- | 30 | 33 |
| 3 | 4c. | 20 | 18 | 16 | 18 | 22 | 26 |
| 4 | 4d. | 16 | 12 | 14 | 12 | 14 | 27 |
| 5 | 4e. | 18 | 21 | 12 | -- | 29 | -- |
| 6 | 4f. | 23 | 22 | 21 | 18 | 15 | 18 |
| 7 | 4g. | 14 | 15 | 14 | 15 | 25 | 22 |
| 8 | 4h. | 23 | 26 | 21 | 22 | 17 | 22 |
| 9 | Std* | 27 | 30 | 32 | 28 | 34 | 35 |

Standard Drug Used

Antibacterial activity – Ampicillin

Antifungal activity - Griseofulvin



a=R, R¹, R² - H , b= R- NO₂, R¹, R² -H , c= R- H, R¹, -OCH₃, R² - H d= R- NO₂, R¹, -OCH₃, R² -H
 e= R, R¹, -H, R² - Cl f= R- NO₂, R¹, - H, R² - Cl g= R, R¹- H, R² - NO₂ h= R- NO₂, R¹-H, R² - NO₂

Physical data of synthesized compounds is given in Table No.1.

Figure No.1: Synthesized compounds

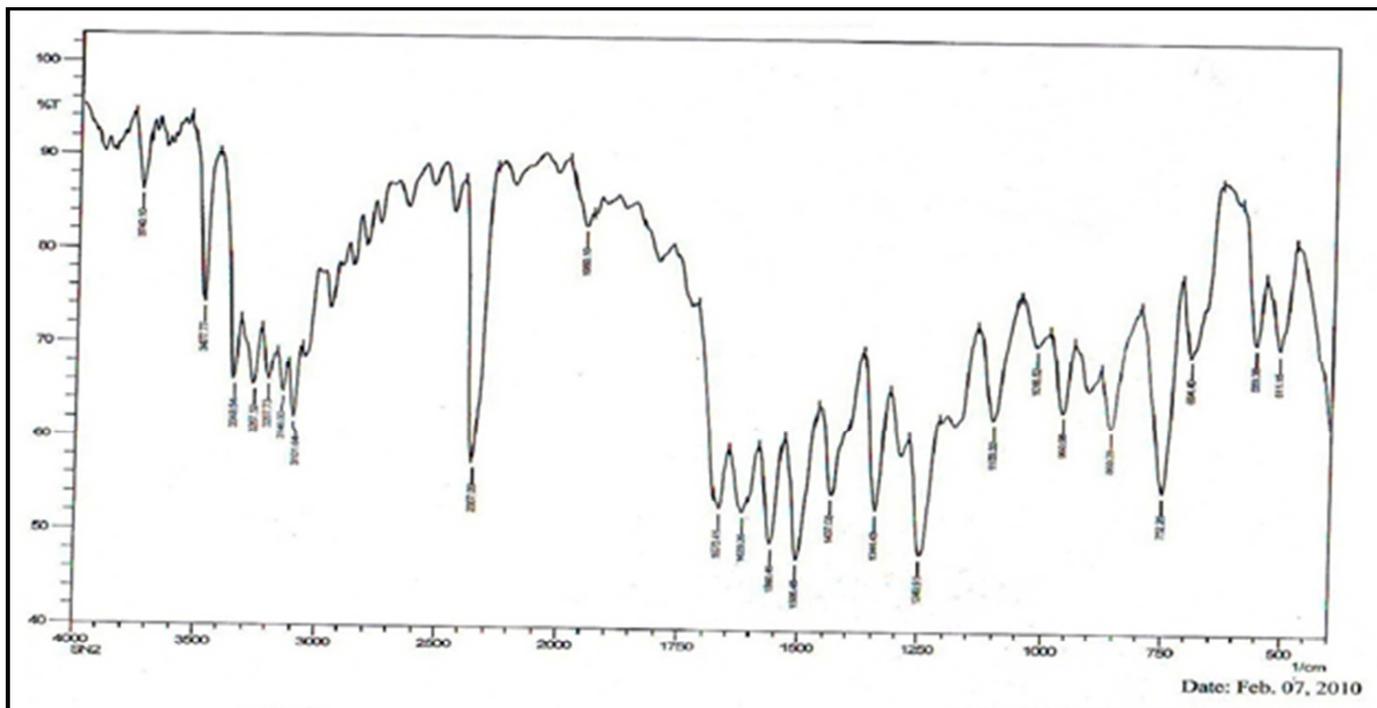


Figure No.2: IR Spectrum of Compound 4b

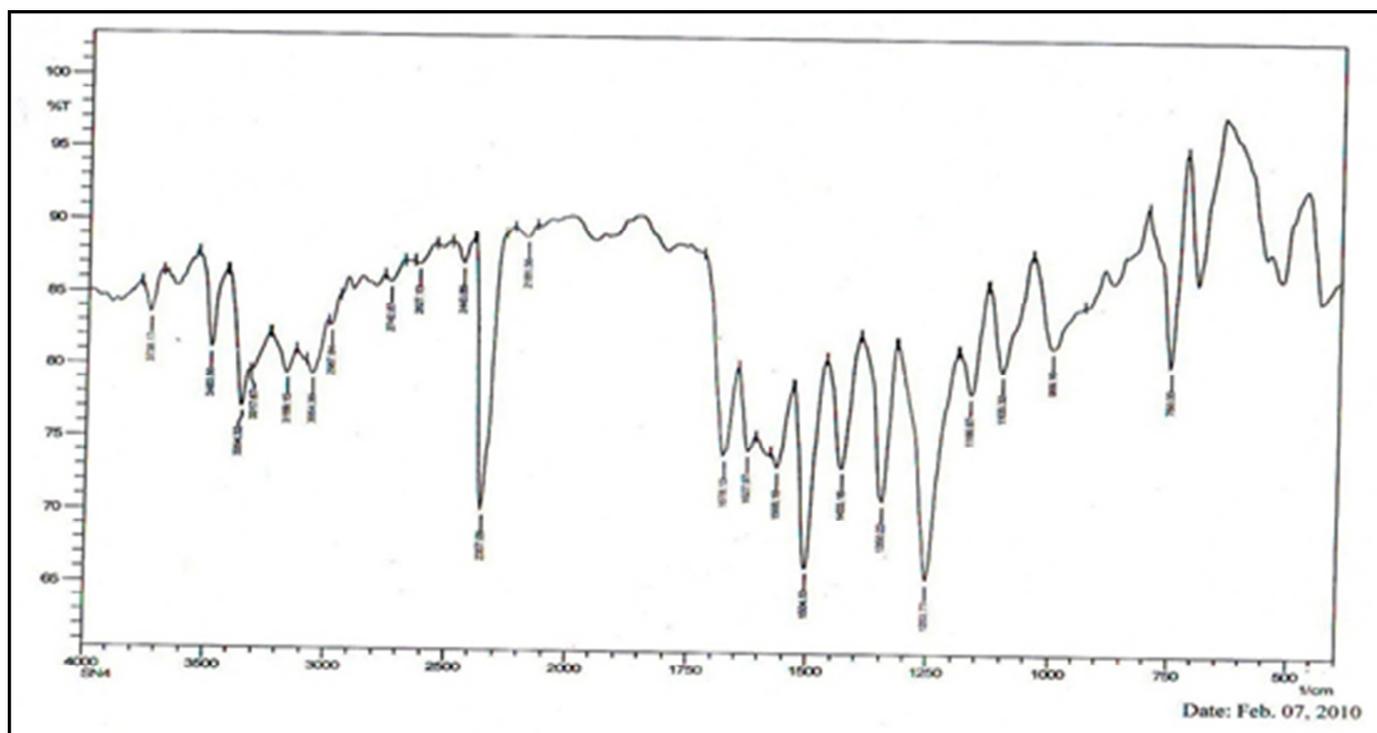


Figure No.2: IR Spectrum of Compound 4c

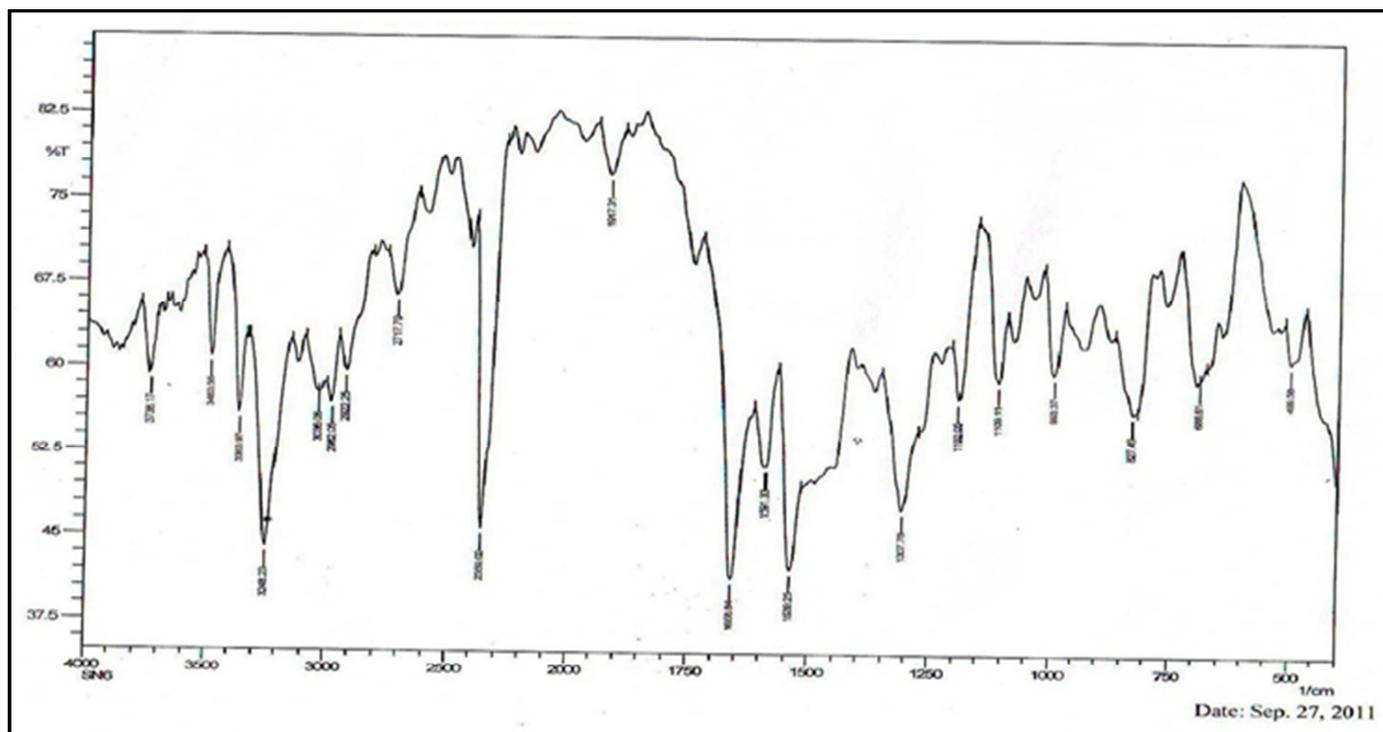


Figure No.2: IR Spectrum of Compound 4e

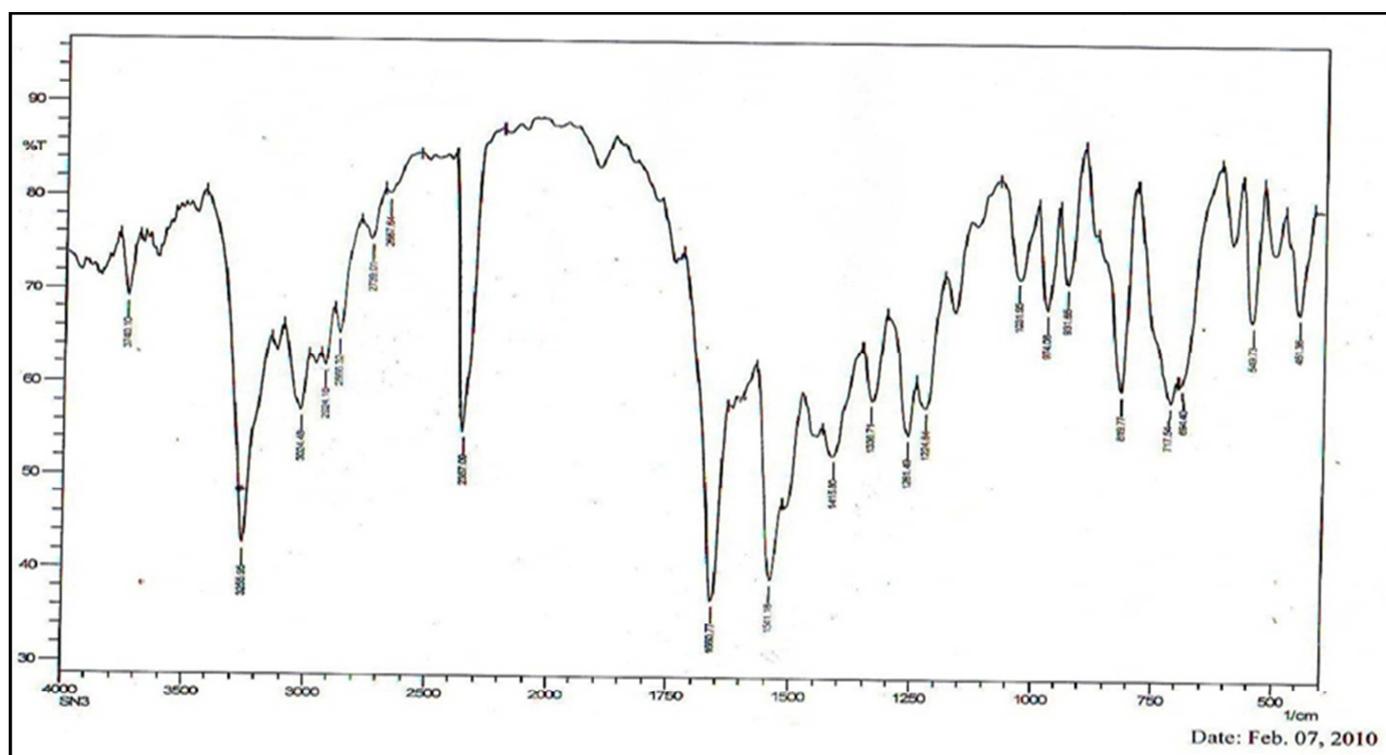


Figure No.2: IR Spectrum of Compound 4h

CONCLUSION

Eight novel isoxazole contain quinazolone derivative have been synthesized, characterized by IR, ¹HNMR and Mass spectral data and few novel selected compounds 4a-h are screened for their anti-Microbial activity Among the series of compounds, presence of two electron withdrawing groups such as nitro and chloro in isoxazole derivatives namely 4f and 4h showed more appreciable activity than other monosubstituted derivatives against all the four anti-bacterial strains and antifungal strains revealing that substitution by strong electron withdrawing groups in isoxazole and quinazolinone enhances the antimicrobial activity.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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