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Synthesis and *In-Vitro* Antibacterial Activity of 2-Acetyl-4-Chlorophenyl 2, 3, 4, 5-Tetrafluorobenzoate Derivatives

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Rohit Bhor[1]*, Rahul Kunkulol[2]

*[1]*Department of Pharmaceutical Chemistry, PRES's College of Pharmacy Chincholi, Tal-Sinner, Dist-Nasik, 422103, Maharashtra, India.*

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Keywords: Anti-bacterial activity, Gentamycin, Pentafluorobenzoic acid, and 1-(5-chloro-2-hydroxyphenyl) ethanone

ABSTRACT

Chromones and Pyrazole derivatives were reported wide range of biological activities. Hence, it was planned to synthesize and screen for their antibacterial activity. A series of novel 2-Acetyl-4-Chlorophenyl 2, 3, 4, 5-Tetrafluorobenzoate derivatives were synthesized and evaluated for *in-vitro* antibacterial activity. Chromones and Pyrazole derivatives like 6-chloro-2-(2,3,4,5-tetrafluorophenyl)-4*H*-chromen-4-one, 4-chloro-2-[5-(2,3,4,5-tetrafluorophenyl)-1*H*-pyrazol-3-yl]phenol were synthesized by a sequence of reactions starting 2-acetyl-4-chlorophenyl 2,3,4,5-tetrafluorobenzoate and were mentioned in scheme 1. Antibacterial activities of chromones derivatives, pyrazole derivatives were tested by the disc diffusion method by using Mueller Hinton Agar (M173) medium against various microorganisms such as Gram-positive *Staphylococcus aureus,* Gram-negative *Escherichia coli, and Pseudomonas aeruginosa.* Gentamycin at 100µg/ml were used as standard drugs for antibacterial activities. Characterization of the compounds was performed by IR, ^{1}H NMR and Mass spectrum. The compounds bearing nitro and oxygen groups have shown prominent activity when compared to compounds without these groups.

INTRODUCTION

Chromones and Pyrazole and its derivatives are important heterocyclic in organic and biochemistry. There are many Chromones containing natural products such as Khellin, sodium cromoglycate, diosmin, flavones, and flavonoids etc. Fungal and bacterial infections are affecting millions of people worldwide. Heterocyclic compounds containing N and O give a variety of biological activities; antimicrobial activity[1]. Similarly, Chromones moiety constitutes the basic nucleus of flavones, which are most important and widespread natural product of plants and display a large number of biological activities. Some Chromones and Pyrazole derivatives are prepared by using and 1-(5-chloro-2-hydroxyphenyl) ethanone and 2, 3, 4, 5-Tetrafluorobenzoic acid reagent. These Pyrazole and Chromones derivatives are screened for antibacterial activity and antifungal activity. Chromones and Pyrazole give broad spectrum activity such as antimicrobial[2-5], anti-inflammatory[6], analgesic[7], antitumorial[8], antihypertensives[9], anticonvulsant and antiviral[10]. There are antifungal and antibacterial agents having different structure and used in the treatment of fungal and bacterial infection. They are known to give variety of biological activities such as analgesic, anti-inflammatory, protein kinase C inhibitor[11]. Many Pyrazole derivatives possess activity like Antiepileptic and Antimicrobial[12] Antiamoebic[13] and Antiandrogenic activities[14]. Particularly, compound having both electron withdrawing groups such chloro and fluoro attached with Chromones ring and Pyrazole showed more inhibitory potential against fungal strains and bacterial strains than standard drug[15].

MATERIALS AND METHODS

Materials

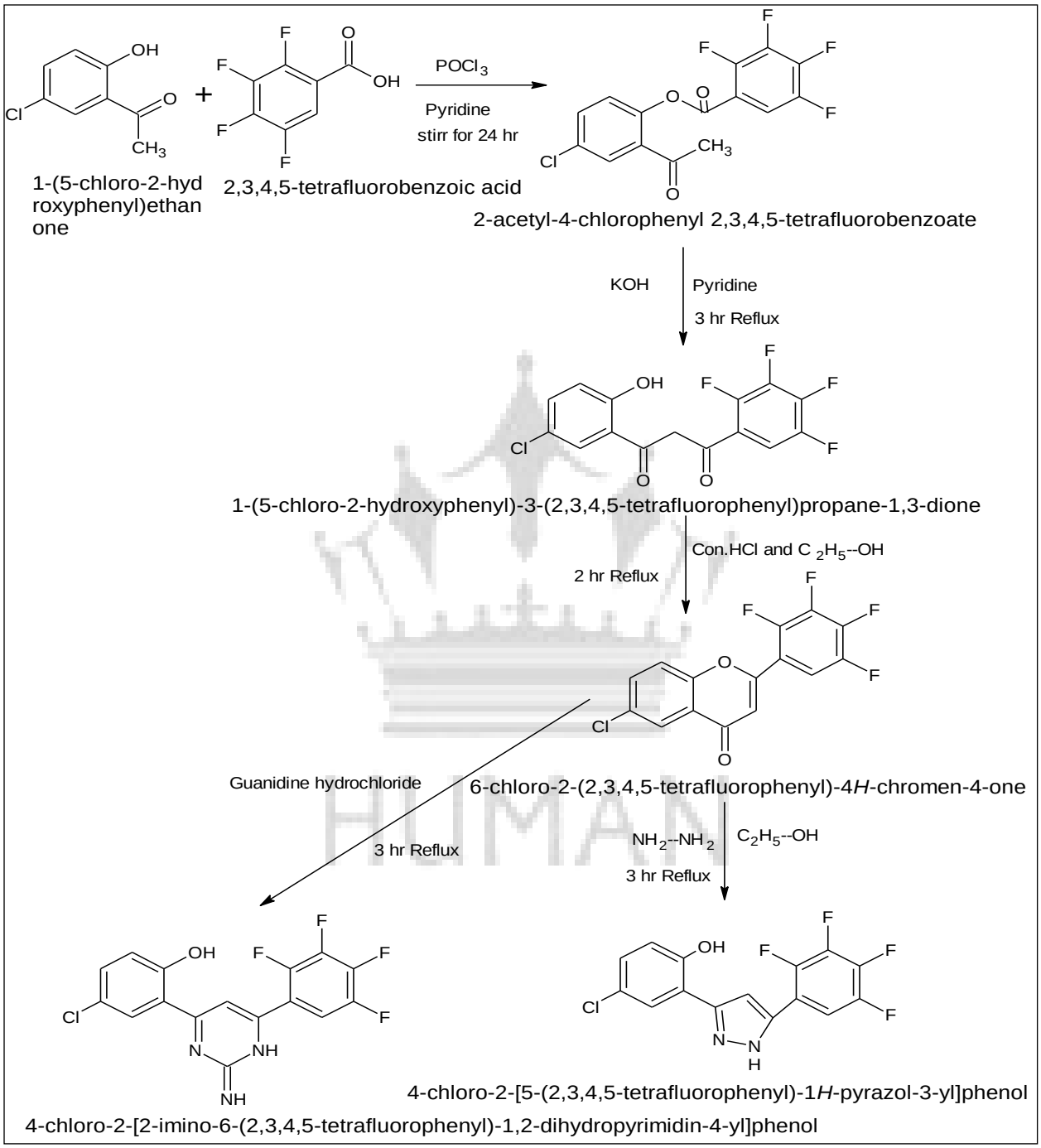
1-(5-chloro-2-hydroxy-4-methylphenyl) ethanone, 2, 3, 4, 5-Tetrafluorobenzoic acid, Pyridine, Hydrazine Hydrate, Guanidine Hydrochloride, Ethanol, Con.Hydrochloric acid and Phosphorus oxychloride i.e. $POCl_3$ were used for the synthesis of Chromones and Pyrazole. All chemicals were of analytical grade. All chemicals were of purchased from Modern Chemicals, Nashik and Atmaja Chemicals, Aurangabad.

Methods

All Chromones and Pyrazole derivatives were synthesized by conventional method. Melting points were determined by open tube capillary method. The purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G) in chloroform: acetone (6:4) and chloroform: methanol (8:2) solvent systems, the spots were located under iodine vapors and UV light. IR spectra were obtained on a Perkin Elmer Spectrum1 FT-IR instrument (KBr pellets). Perkin Elmer Spectrum1 FT-IR instrument consists of globar and mercury vapor lamp as sources. ^{1}H-NMR spectra were recorded on a Bruker AVANCE III 500 MHz (AV 500) spectrometer using TMS as internal standard in DMSO-d_6/$CDCl_3$ and mass spectra was obtained on JEOL GCMATE II GC-MS is presented as m/z. The synthetic route for the title compounds is shown in Scheme 1.

Experimental work:

Chemistry: (Scheme I)



Scheme 1: Synthesis of 6-chloro-2-(2,3,4,5-tetrafluorophenyl)-4*H*-chromen-4-one (BH) and of 4-chloro-2-[5-(2,3,4,5-tetrafluorophenyl)-1*H*-pyrazol-3-yl]phenol (BI) derivatives (BF-BJ).

Synthesis of Chromones and Pyrazole derivatives [16-21]:

Synthesis of 2-acetyl-4-chlorophenyl 2, 3, 4, 5-tetrafluorobenzoate (BF): (scheme 1)

A mixture of 1-(5-chloro-2-hydroxyphenyl) ethanone (0.5g) and 2, 3, 4, 5-Tetrafluorobenzoic acid (0.5g) react with each other in the presence of $POCl_3$ (5 ml) and Pyridine (15 ml) and then stir on magnetic stirrer for 24 hrs, and then it gives solid product after addition of ice cold water and it gives 2-acetyl-4-chlorophenyl 2,3,4,5-tetrafluorobenzoate (BF).

Synthesis of 1-(5-chloro-2-hydroxyphenyl)-3-(2, 3, 4, 5-tetrafluorophenyl) propane-1, 3-dione (BG) :(Scheme 1)

A solution of 2-acetyl-4-chlorophenyl 2, 3, 4, 5-tetrafluorobenzoate (BF) reacted with potassium hydroxide (0.5g) and pyridine (5ml) and reflux for 3 hrs and then completion of the reaction was confirmed by TLC. The mixture was cooled by addition of ice. The precipitate formed was washed with water and recrystallized from ethanol and then it gives 1-(5-chloro-2-hydroxyphenyl)-3-(2, 3, 4, 5-tetrafluorophenyl) propane-1,3-dione (BG).

Synthesis of 6-chloro-2-(2, 3, 4, 5-tetrafluorophenyl)-4*H*-chromen-4-one (BH): (Scheme 1)

A solution of 1-(5-chloro-2-hydroxyphenyl)-3-(2, 3, 4, 5-tetrafluorophenyl) propane-1, 3-dione (BG) react with con. Hydrochloric acid (5ml) and ethanol (5ml), and reflux for 2 hrs and then completion of the reaction were confirmed by TLC. The mixture was cooled by addition of ice. The precipitate formed was washed with water and recrystallized from ethanol and then it gives 6-chloro-2-(2, 3, 4, 5-tetrafluorophenyl)-4*H*-chromen-4-one (BH).

Synthesis of 4-chloro-2-[5-(2, 3, 4, 5-tetrafluorophenyl)-1*H*-pyrazol-3-yl] phenol (BI): (Scheme 1)

A solution of 6-chloro-2-(2,3,4,5-tetrafluorophenyl)-4*H*-chromen-4-one (BH) reacts with hydrazine hydrate (5ml) and ethanol (10ml) and reflux for 3 hrs and then completion of the reaction were confirmed by TLC. The mixture was cooled by addition of ice. The precipitate formed was washed with water and recrystallized from ethanol and then it gives 2-[5-(2, 3, 4, 5-tetrafluorophenyl)-1*H*-pyrazol-3-yl] phenol (BI).

Synthesis of 4-chloro-2-[2-imino-6-(2, 3, 4, 5-tetrafluorophenyl)-1, 2-dihydropyrimidin-4-yl] phenol (BJ): (Scheme 1)

A solution of 6-chloro-2-(2, 3, 4, 5-tetrafluorophenyl)-4*H*-chromen-4-one (BH) react with guanidine hydrochloride (5ml) and it was refluxed for 3 hrs and then completion of the reaction was confirmed by TLC. The mixture was cooled by addition of ice. The precipitate formed was washed with water and recrystallized from ethanol and then it gives 4-chloro-2-[2-imino-6-(2, 3, 4, 5-tetrafluorophenyl)-1, 2-dihydropyrimidin-4-yl] phenol (BJ).

Characterization:

Table 1: Physical Data of 2-acetyl-4-chlorophenyl 2,3,4,5-tetrafluorobenzoate derivatives:

Sr. No	Compounds	Color of Compounds	Molecular Formula	Melting Point ^{0}C	% yields	Molecular Weight
1	BF	Yellow	$C_{14}H_7O_3F_4Cl$	312-314°C	80.95%	334
2	BG	Yellow	$C_{15}H_6O_3F_4Cl$	346-348°C	61.53%	345
3	BH	Yellow	$C_{15}H_5O_2F_4Cl$	318-320°C	74.46%	328
4	BI	Yellow	$C_{15}H_7ON_2F_4Cl$	352-354°C	76.92%	342
5	BJ	Yellow	$C_{16}H_8ON_3F_4Cl$	324-326°C	87.50%	369

2-acetyl-4-chlorophenyl 2, 3, 4, 5-tetrafluorobenzoate (BF):

% yield: 80.95%; Melting point (^{0}C) : 312-314°C; R_f Value: 0.92 chloroform: methanol (8:2); FTIR (KBr) ν cm^{-1} : 3080 (Ar C-H), 1644 (Ar C=C), 881 (Ar C-H def), 1260 (Ar C-F), 726 (Ar C-Cl), 1751 (Ester C=O), 1319 (C-O); ^{1}H NMR (500 MHz CDCl3 δ ppm) : 7.93-8.11 (m, 3H, aromatic protons), 2.50 (s, 3H, CH_3), 7.53 (m, 1H, aromatic protons); JEOL GCMATE II GC-MS (m/z) : 333 (M^+), 334 (M^++1), Mol. Wt.:334.

1-(5-chloro-2-hydroxyphenyl)-3-(2, 3, 4, 5-tetrafluorophenyl)propane-1,3-dione (BG):

% yield: 61.53%; Melting point (^{0}C) : 346-348°C; R_f Value: 0.83 chloroform: methanol (8:2); FTIR (KBr) ν cm^{-1} : 1529 (Ar C=C), 844 (Ar C-H def), 1167 (Ar C-F), 680 (Ar C-Cl), 1639 (Aryl Ketone C=O), 1218 (C-O), 3622 (Ar OH); ^{1}H NMR (500 MHz CDCl3 δ ppm): 3.81(s,

2H, CH_2), 5.35 (s, 1H, OH), 7.00-7.69 (m, 3H, aromatic protons), 7.25 (m, 1H, aromatic protons); JEOL GCMATE II GC-MS(m/z): 377(M^+), 378 (M^++1), Mol. Wt.:378.

6-chloro-2-(2, 3, 4, 5-tetrafluorophenyl)-4*H*-chromen-4-one (BH):

% yield: 74.46%; Melting point (^{0}C) : 318-320°C; R_f Value: 0.89 chloroform: methanol (8:2); FTIR (KBr) ν cm^{-1} : 3061 (Ar C-H), 1568 (Ar C=C), 834 (Ar C-H def), 1171 (Ar C-F), 652 (Ar C-Cl),1719 (Aryl Ketone C=O), 1349 (C-O); ^{1}H NMR (500 MHz) CDCl3 δ ppm: 7.77-8.07 (m, 3H, aromatic protons), 6.54 (s, 1H, CH_2), 7.25 (m, 1H, aromatic protons); JEOL GCMATE II GC-MS (m/z): 327 (M^+), 328 (M^++1) Mol. Wt.:328.

4-chloro-2-[5-(2, 3, 4, 5-tetrafluorophenyl)-1*H*-pyrazol-3-yl] phenol (BI):

% yield: 76.92%; Melting point (^{0}C): 352-354°C; R_f Value**:** 0.9 chloroform: methanol (8:2); FTIR (KBr) ν cm^{-1} : 3032 (Ar C-H str), 1657 (Ar C=C), 758 (Ar C-H def), 1155 (Ar C-F), 729 (Ar C-Cl), 3449 (Ar OH), 1347 (C-O), 3336 (N-H); ^{1}H NMR (500 MHz CDCl3 δ ppm): 7.85-7.98 (m, 3H, aromatic protons), 5.35 (s, 1H, O-H), 6.81 (s, 1H, C-H),12.62 (s, 1H,N-H), 7.25 (m, 1H, aromatic protons JEOL GCMATE II GC-MS (m/z): 341 (M^+), 342 (M^++1). Mol. Wt.:342.

4-chloro-2-[2-imino-6-(2, 3, 4, 5-tetrafluorophenyl)-1,2-dihydropyrimidin-4-yl]phenol (BJ):

% yield: 87.50%; Melting point (^{0}C): 324-326°C; R_f Value: 0.88 chloroform: methanol (8:2); FTIR (KBr) ν cm^{-1} : 3028 (Ar C-H), 1656 (Ar C=C), 768 (Ar C-H def), 1273 (Ar C-F), 710 (Ar C-Cl), 3449 (Ar OH), 1320 (C-O), 3385 (N-H); ^{1}H NMR (500 MHz CDCl3 δ ppm): 6.96-7.74 (m, 3H, aromatic protons), 5.35 (s, 1H, O-H), 6.81 (s, 1H, C-H), 13.86 (s, 1H,N-H), 13.76 (s, 1H,N-H), 6.69 (m, 1H, aromatic protons); JEOL GCMATE II GC-MS (m/z): 368 (M^+), 369 (M^++1). Mol. Wt.:369.

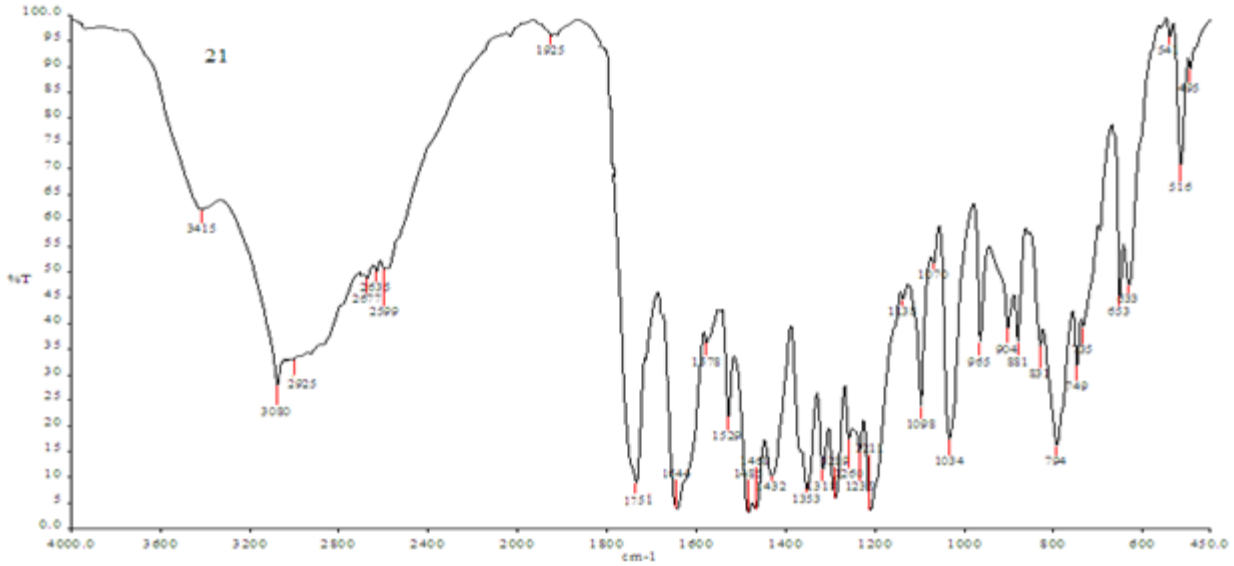


Fig. 1: FTIR (KBr) ν cm^{-1} of 2-acetyl-4-chlorophenyl 2,3,4,5-tetrafluorobenzoate (BF)

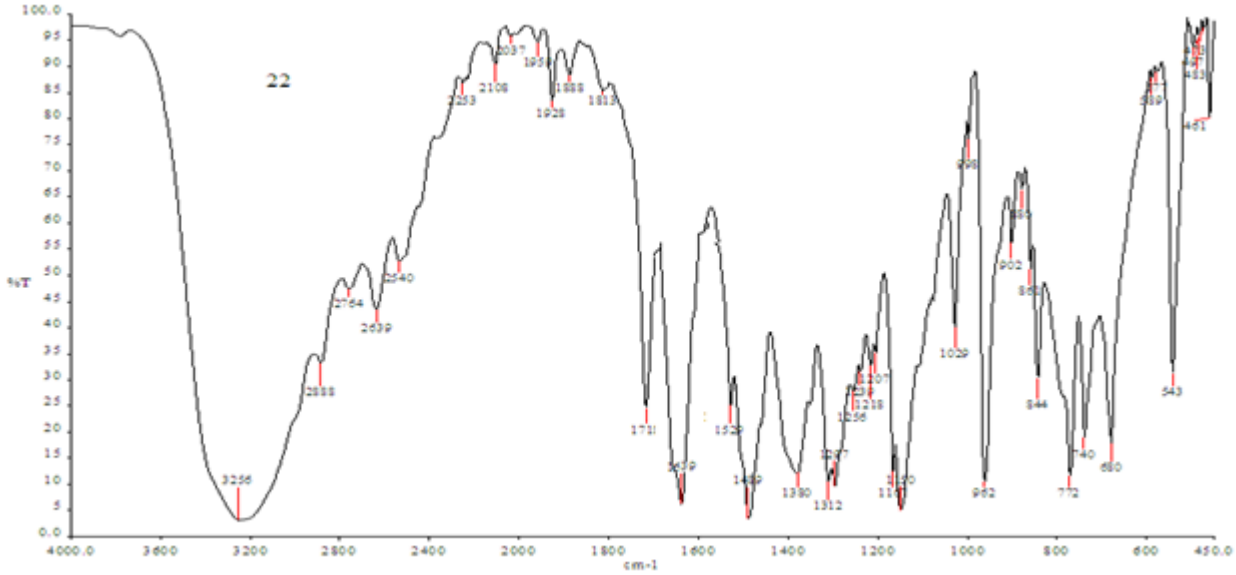


Fig. 2: FTIR (KBr) ν cm^{-1} of 1-(5-chloro-2-hydroxyphenyl)-3-(2,3,4,5-tetrafluorophenyl) propane-1,3-dione (BG)

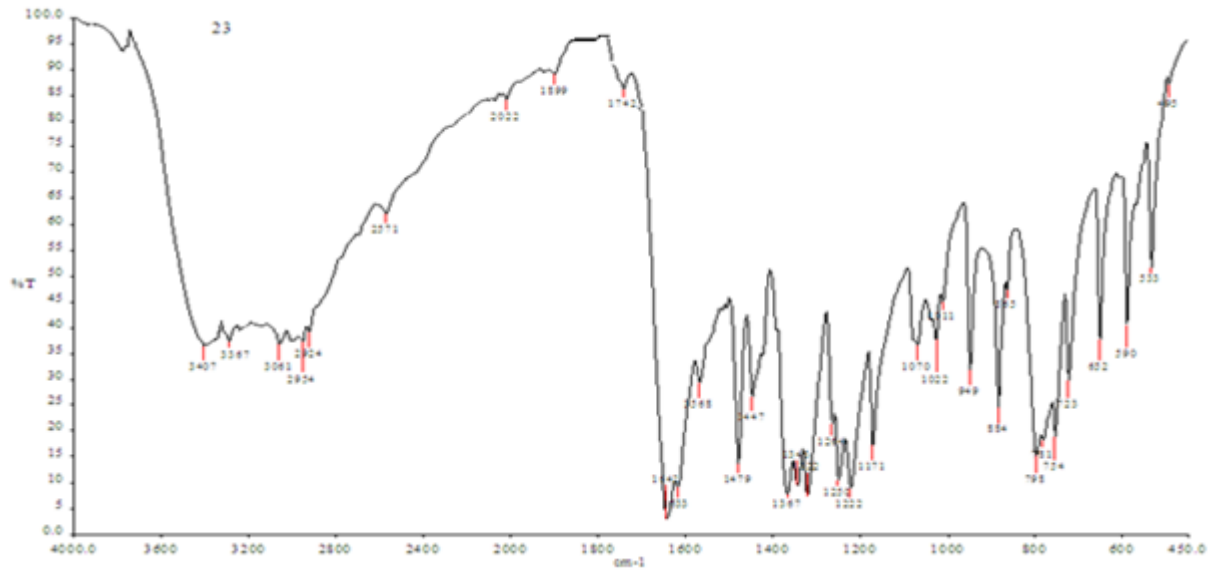


Fig. 3: FTIR (KBr) ν cm^{-1} of 6-chloro-2-(2, 3, 4, 5-tetrafluorophenyl)-4*H*-chromen-4-one (BH)

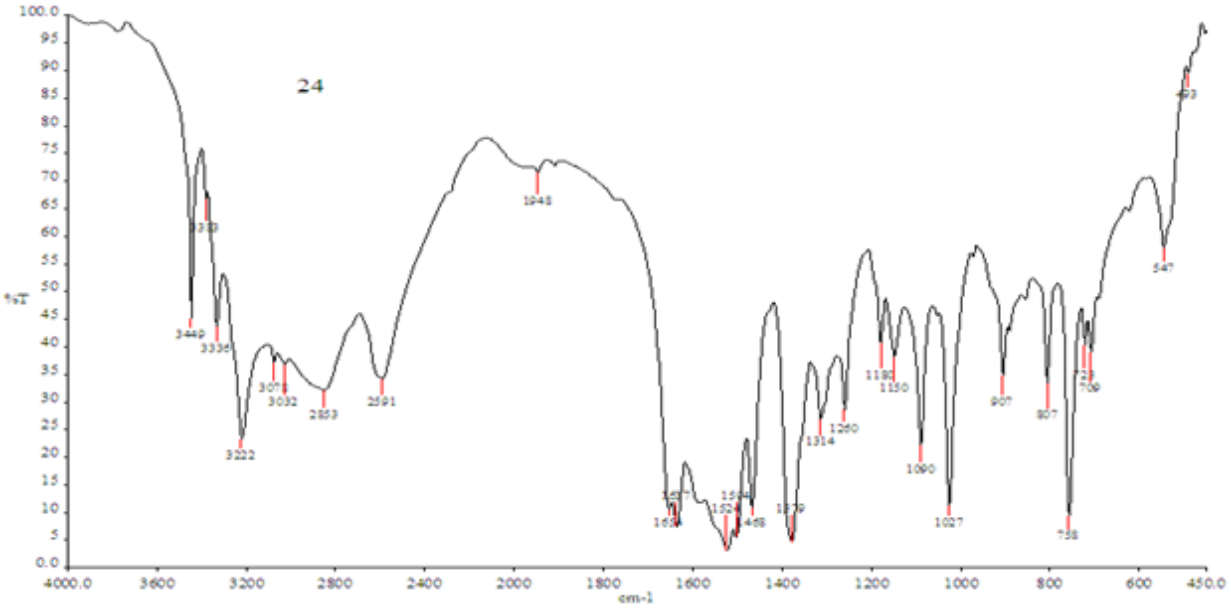


Fig. 4: FTIR (KBr) ν cm^{-1} of 4-chloro-2-[5-(2,3,4,5-tetrafluorophenyl)-1*H*-pyrazol-3-yl] phenol (BI)

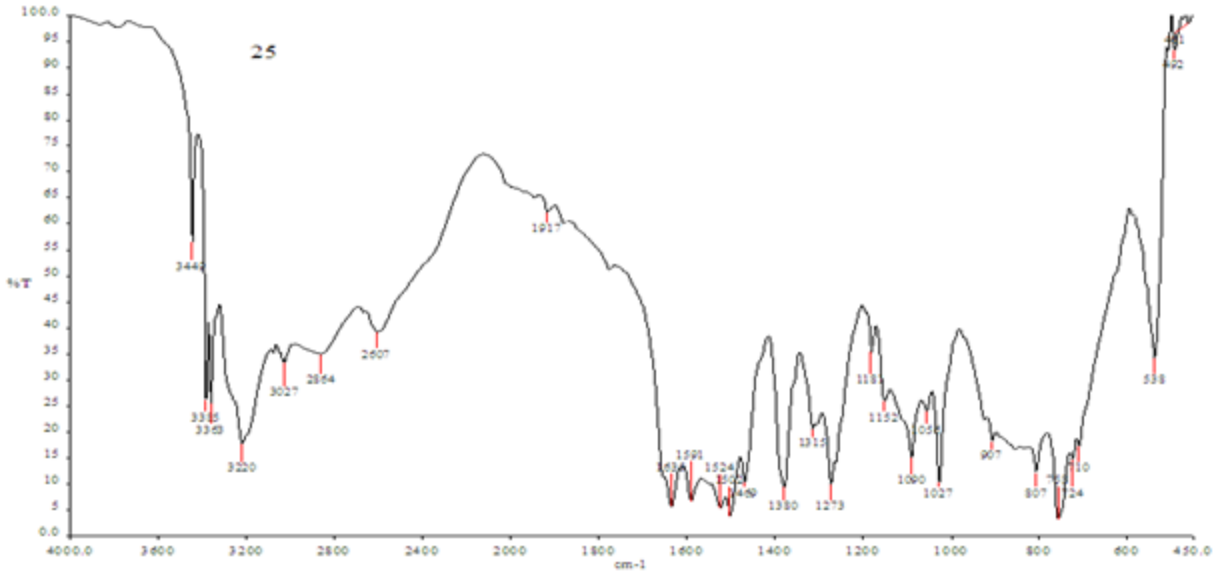


Fig. 5: FTIR (KBr) ν cm^{-1} of 4-chloro-2-[2-imino-6-(2,3,4,5-tetrafluorophenyl)-1,2-dihydropyrimidin-4-yl]phenol (BJ)

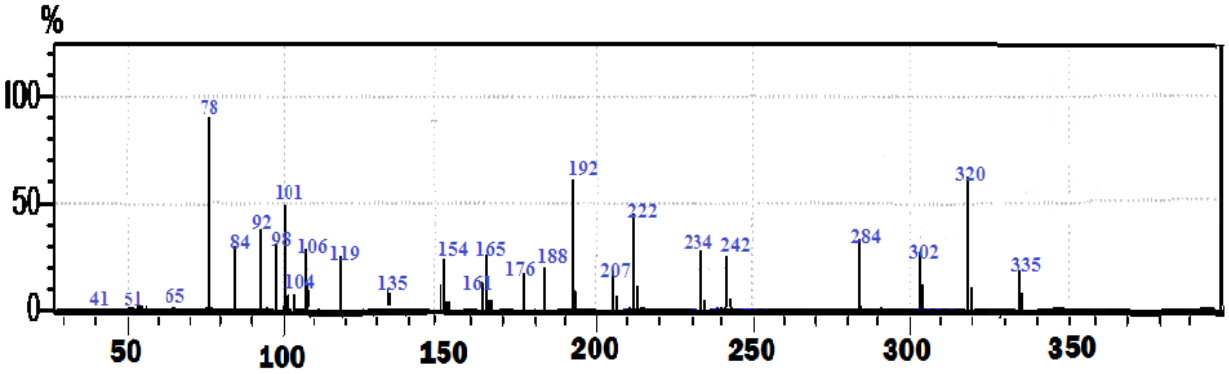


Fig. 6: Mass spectrum of 2-acetyl-4-chlorophenyl 2,3,4,5-tetrafluorobenzoate (BF)

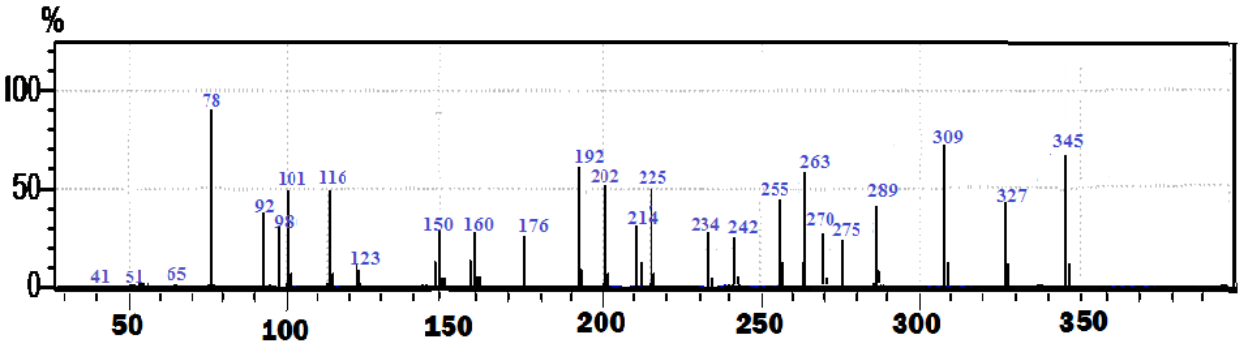


Fig. 7: Mass spectrum of 1-(5-chloro-2-hydroxyphenyl)-3-(2,3,4,5-tetrafluorophenyl) propane-1,3-dione (BG)

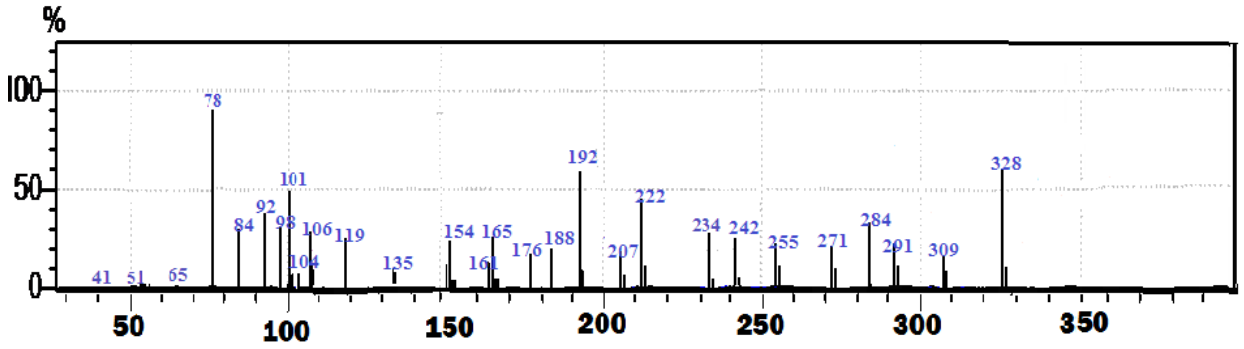


Fig. 8: Mass spectrum of 6-chloro-2-(2,3,4,5-tetrafluorophenyl)-4*H*-chromen-4-one (BH)

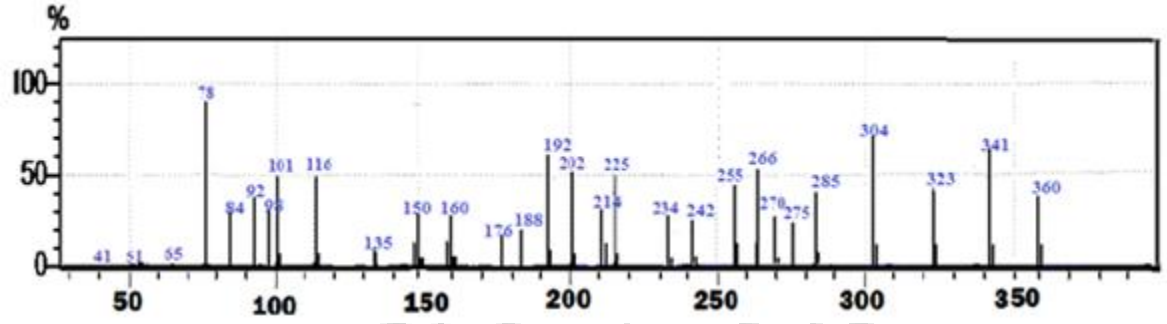


Fig. 9: Mass spectrum of 4-chloro-2-[5-(2,3,4,5-tetrafluorophenyl)-1*H*-pyrazol-3-yl]phenol (BI)

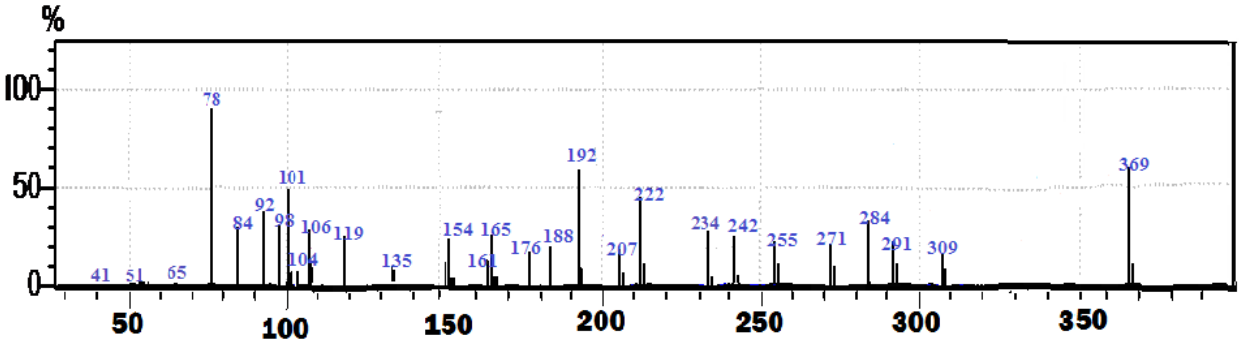


Fig. 10: Mass spectrum of of 4-chloro-2-[2-imino-6-(2,3,4,5-tetrafluorophenyl)-1,2-dihydropyrimidin-4-yl]phenol (BJ)

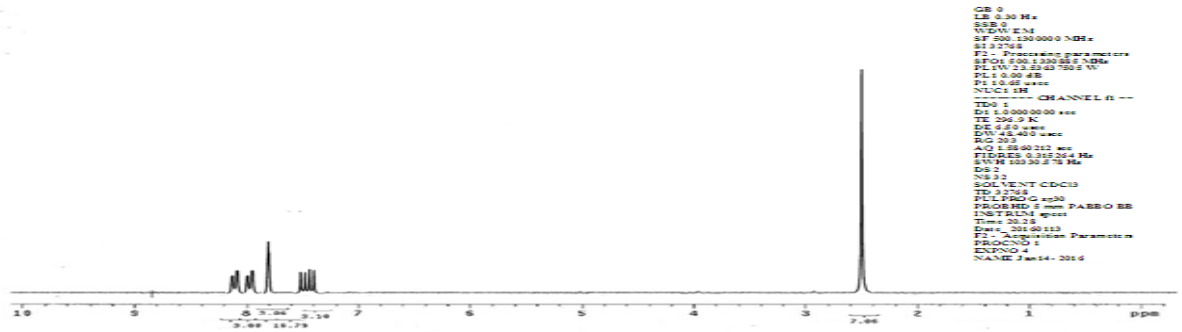


Fig. 11: ^{1}H-NMR of 2-acetyl-4-chlorophenyl 2,3,4,5-tetrafluorobenzoate (BF)

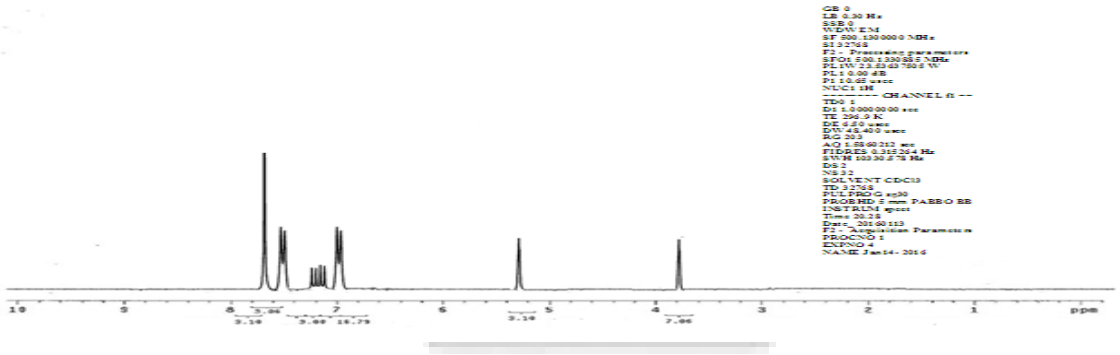


Fig. 12: ^{1}H-NMR of 1-(5-chloro-2-hydroxyphenyl)-3-(2,3,4,5-tetrafluorophenyl)propane-1,3-dione (BG)

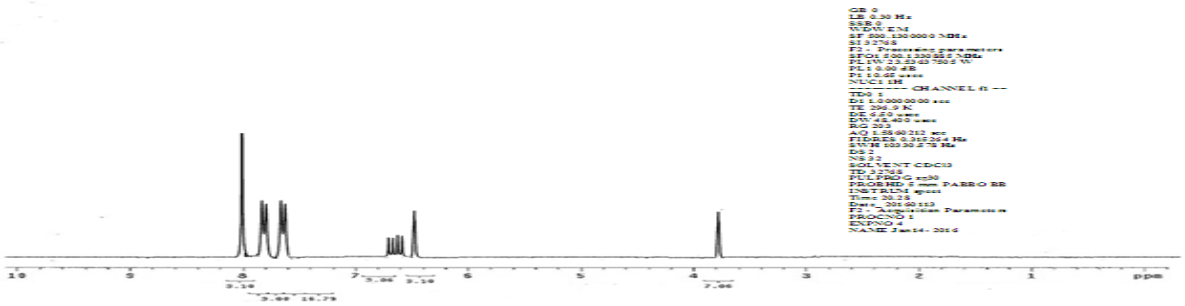


Fig. 13: ^{1}H-NMR of 6-chloro-2-(2,3,4,5-tetrafluorophenyl)-4*H*-chromen-4-one (BH)

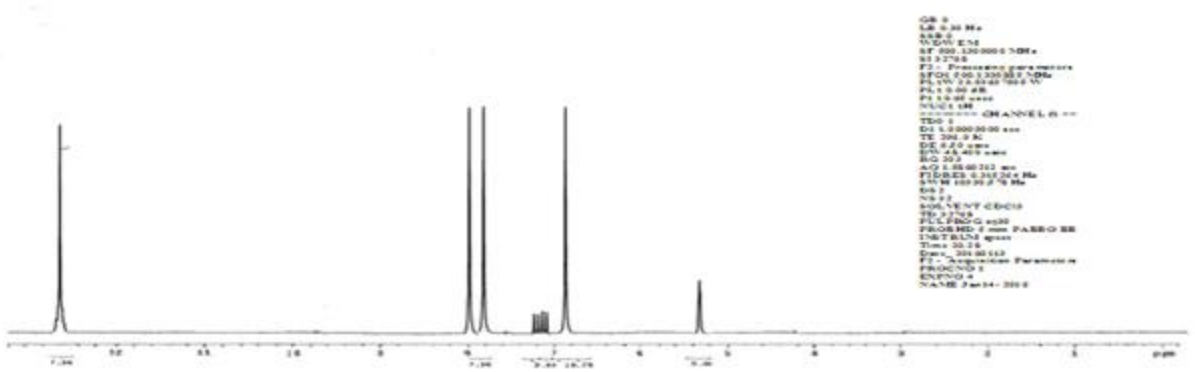


Fig. 14: ^{1}H-NMR of -chloro-2-[5-(2,3,4,5-tetrafluorophenyl)-1*H*-pyrazol-3-yl]phenol (BI)

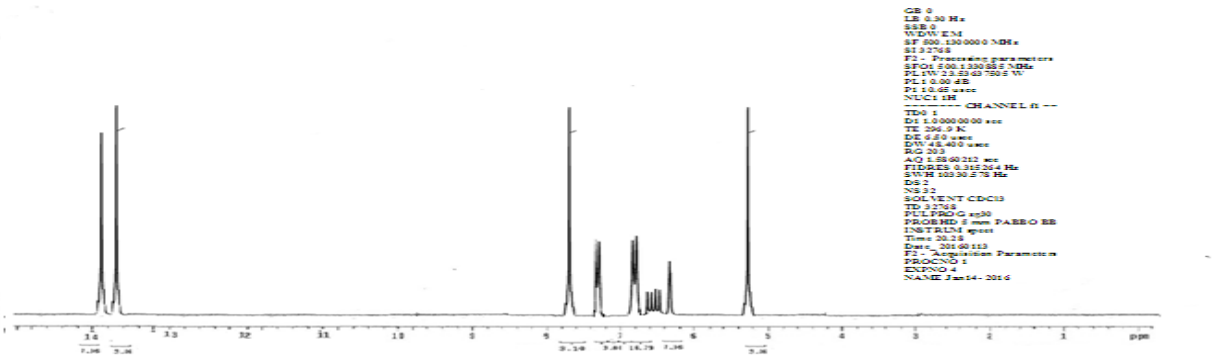


Fig. 15: ^{1}H-NMR of 4-chloro-2-[2-imino-6-(2,3,4,5-tetrafluorophenyl)-1,2-dihydropyrimidin-4-yl]phenol (BJ)

PHARMACOLOGICAL STUDIES[22]:

***In vitro* Antibacterial activity by disc diffusion method:**

i) Antibacterial Activity**:**

The compounds like BF to BJ were evaluated for their *in vitro* antibacterial activity against various microorganisms such as gram positive *Staphylococcus aureus*, gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* by *in vitro* method like disc diffusion method was performed using Mueller Hinton Agar (M173) medium. Each compound was tested at concentration 100µg/mL in DMSO. The zone of inhibition was measured after 24 h incubation at 37°C. Standard: Gentamycin (100µg/mL of DMSO).

Table 2- Antibacterial activity screening result of synthesized compound measuring the zone of inhibition in millimeter

Compound No.	Diameter of zone of inhibition (mm)		
	Escherichia coli	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
	ATCC 25922	ATCC 25923	ATCC 27853
BF	12	18	10
BG	08	15	09
BH	13	20	19
BI	14	22	20
BJ	14	21	21
Gentamycin	20	36	28

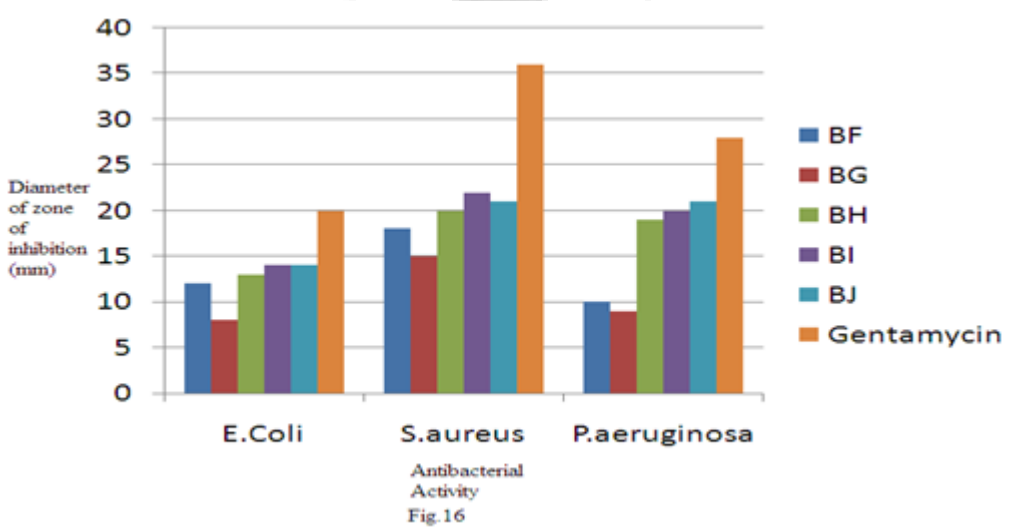
RESULTS AND DISCUSSION

The syntheses of compounds BF- BJ were undertaken as per the scheme 1. The required 2-acetyl-4-chlorophenyl 2,3,4,5-tetrafluorobenzoate (BF) was prepared by the action of 1-(5-chloro-2-hydroxyphenyl) ethanone and 2, 3, 4, 5-Tetrafluorobenzoic acid. IR spectra were obtained on a Perkin Elmer Spectrum1 FT-IR instrument (KBr pellets). Perkin Elmer Spectrum1 FT-IR instrument consists of globar and mercury vapor lamp as sources. ^{1}H-NMR spectra were recorded on a Bruker AVANCE III 500 MHz (AV 500) spectrometer using TMS as internal standard in DMSO-d_6/$CDCl_3$ and mass spectra was obtained on JEOL GCMATE II GC-MS is presented as m/z.

The results revealed that most of the synthesized compounds showed varying degrees of inhibition against the tested microorganisms. In general, the inhibitory activity against the tested gram-positive bacteria was higher than that of the gram-negative bacteria. Moreover, the compounds like BH, BI and BJ having the side chain showed higher activity than BF and BG against *S. aureus* and *Pseudomonas aeruginosa.* The replacement of oxygen to nitrogen resulted in a slightly increased antimicrobial activity. Our study revealed that all the compounds had

stronger antibacterial activity against Gram-positive bacteria when compared to Gram-negative bacteria. Antimicrobial activity revealed that newly synthesized compound BH, BI and BJ showed good significant activity. The results of the preliminary antimicrobial testing of the prepared compounds, the typical broad spectrum antibacterial drug like Gentamycin was shown in Table 2.

The synthesized compounds were screened for their antibacterial activity as showed in Fig.16. The derivatives like BH, BI and BJ showed highly active compound against *E. coli*, *Staphylococcus aureus* and *Pseudomonas aureus.* BH showed moderately active compound against *E. coli* and *S. aureus.* BI and BJ showed moderately active compound against *E. coli* and *S. aureus*. Standard (Gentamycin) showed highly active against *E. coli*, *Pseudomonas aeruginosa* and *S. aureus.*



Antibacterial Activity
Fig.16

CONCLUSION

Various 2-acetyl-4-chlorophenyl 2,3,4,5-tetrafluorobenzoate derivatives (BF) was synthesized from the action of 1-(5-chloro-2-hydroxyphenyl) ethanone and 2, 3, 4, 5-Tetrafluorobenzoic acid. The structure antibacterial activity relationship of the synthesized compounds was based on the structure of final derivatives. These derivatives possess good antibacterial activity. Antimicrobial activities including antibacterial properties of the synthesized derivatives showed a significant activity as compared with standard drugs like Gentamycin.

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