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

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Keywords: Anti-bacterial activity; Conventional Method; Gentamycin; 2,3,4,5-tetrafluorobenzoic acid; 1-(5-chloro-2-hydroxy-4-methylphenyl) ethanone

ABSTRACT

Chromones derivatives and pyrazole derivatives were synthesized and screened for antibacterial activity. Some chromones and Pyrazole derivatives like 6-chloro-7-methyl-2-(2,3,4,5-tetrafluorophenyl)-4*H*-chromen-4-one, 4-chloro-5-methyl-2-[5-(2,3,4,5-tetrafluorophenyl)-1*H*-pyrazol-3-yl]phenol were synthesized by a sequence of reactions starting from 2-Acetyl-4-Chloro-5-Methylphenyl 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 compounds was performed by FTIR, ^{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

Heterocyclic compounds containing N and O are found to display variety of biological activities; antimicrobial activity [1]. Chromones, pyrazole and its derivatives are important heterocyclic in organic, biochemistry and have been found in many chromones containing natural products such as Khellin, sodium cromoglycate, diosmin, flavones, and flavonoids etc. Chromones and pyrazole derivatives play an important role in medical field with many pharmacological activities such as antimicrobial, antiviral, antidiabetic and anticancer activity[2-3]. Therefore substituted Chromones and pyrazole have attracted the interest of various research groups, especially since it has been reported that the influence of the substitution at 2 and 5-positions is very important for their pharmacological effect [5-6]. Chromones and pyrazole are belongs to heterocyclic compound, which has a wide range of biological and pharmacological properties such as antimicrobial activity (antifungal, antibacterial), antitumor, anti-inflammatory, anti-HIV, antihypertensive, hydantoin exhibits diverse biological activities, such as anticonvulsant, antifungal activities, anti-thyroidal, antiviral, antitubercular, antiarrhythmic and anticonvulsant [7-12]. Chromones and pyrazole nucleus were chosen because certain 2- amino Chromones and pyrazole were found to possess some anti-viral activity. It reveals that chromones and pyrazole possess broad spectrum activity such as antimicrobial[3-6], anti-inflammatory[7], analgesic[8], antitumorial[9], antihypertensives[10], anticonvulsant and antiviral[11]. There are antifungal and antibacterial agent 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

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. Conventional method was used for preparing 2-Acetyl-4-Chloro-5-Methylphenyl 2,3,4,5-Tetrafluorobenzoate derivatives.

Experimental Section: [16-20]

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 an internal standard in DMSO-d_6/$CDCl_3$ and mass spectra was obtained on JEOL GCMATE II GC-MS are presented as m/z. The synthetic route for the title compounds is shown in Scheme 1.

Synthesis of 2-acetyl-4-chloro-5-methylphenyl 2,3,4,5-tetrafluorobenzoate (CF):(Scheme 1)

A mixture of 1-(5-chloro-2-hydroxy-4-methylphenyl) 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-chloro-5-methylphenyl 2,3,4,5-tetrafluorobenzoate (CF).

Synthesis of 1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(2,3,4,5-tetrafluorophenyl)propane-1,3-dione (CG): (Scheme 1)

A solution of 2-acetyl-4-chloro-5-methylphenyl 2,3,4,5-tetrafluorobenzoate (CF) reacts with potassium hydroxide (0.5g) and pyridine (5 ml) and reflux for 3 h 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 1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(2,3,4,5-tetrafluorophenyl)propane-1,3-dione (CG).

Synthesis of 6-chloro-7-methyl-2-(2,3,4,5-tetrafluorophenyl)-4*H*-chromen-4-one (CH): (Scheme 1)

A solution of 1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(2,3,4,5-tetrafluorophenyl)propane-1,3-dione (CG) reacts with con. Hydrochloric acid (5ml) and ethanol (5ml), and reflux for 2 h 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-7-methyl-2-(2,3,4,5-tetrafluorophenyl)-4*H*-chromen-4-one (CH).

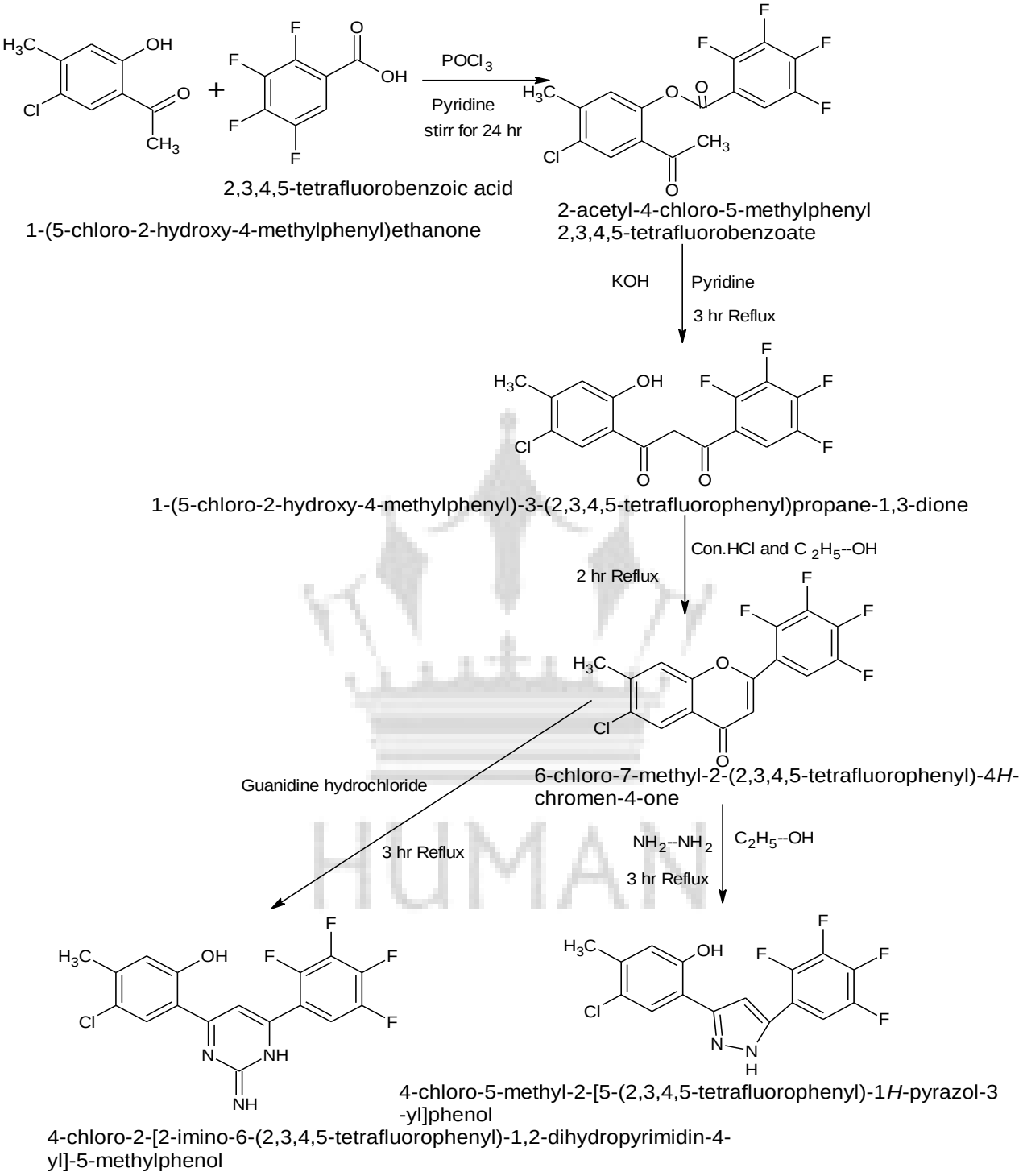
Synthesis of 4-chloro-5-methyl-2-[5-(2,3,4,5-tetrafluorophenyl)-1*H*-pyrazol-3-yl]phenol (CI): (Scheme 1)

A solution of 6-chloro-7-methyl-2-(2,3,4,5-tetrafluorophenyl)-4*H*-chromen-4-one (CH) reacts with hydrazine hydrate (5ml) and ethanol (10ml) and reflux for 3 h 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 4-chloro-5-methyl-2-[5-(2,3,4,5-tetrafluorophenyl)-1*H*-pyrazol-3-yl]phenol (CI)

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

A solution of 6-chloro-7-methyl-2-(2,3,4,5-tetrafluorophenyl)-4*H*-chromen-4-one (CH) reacts with guanidine hydrochloride (5 ml) and it was 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 4-chloro-2-[2-imino-6-(2,3,4,5-tetrafluorophenyl)-1,2-dihydropyrimidin-4-yl]-5-methylphenol (CJ).

Scheme of reaction: Scheme 1:



Scheme 1: 6-chloro-7-methyl-2-(2,3,4,5-tetrafluorophenyl)-4*H*-chromen-4-one (CH) and 4-chloro-5-methyl-2-[5-(2,3,4,5-tetrafluorophenyl)-1*H*-pyrazol-3-yl]phenol (CI)

Physical Data for Synthesized compounds given in Table 1:

Table 1: Physical Data for Synthesized compounds

Sr. No.	Compounds	Molecular Formula	Melting Point^{0}C	% yields	Molecular Weight
1	CF	$C_{16}H_9O_3F_4Cl$	338-340°C	76.68%	360
2	CG	$C_{16}H_8O_3F_4Cl$	318-320°C	65.30%	359
3	CH	$C_{16}H_7O_2F_4Cl$	310-312°C	82.92%	342
4	CI	$C_{16}H_9ON_2F_4Cl$	378-380°C	82.69%	356
5	CJ	$C_{17}H_{10}ON_3F_4Cl$	410-412°C	83.63%	395

Spectral Data:

2-acetyl-4-chloro-5-methylphenyl 2,3,4,5-tetrafluorobenzoate (CF):

% Yield : 76.68%; Melting point (^{0}C) : 338-340°C; R_f Value: 0.9, Chloroform: Methanol (8:2); FTIR (KBr) ν cm^{-1} :3036 (Ar C-H), 1592 (Ar C=C), 747 (Ar C-H def), 1115(Ar C-F), 796(Ar C-Cl),1750 (Ester C=O), 1334 (C-O); ^{1}H NMR (400 MHz CDCl3 δ ppm): 2.34 (s, 3H, CH_3), 7.44-7.81 (m, 2H, aromatic protons), 2.50 (s, 3H, CH_3) 7.6 (m, 1H, aromatic F protons); JEOL GCMATE II GC-MS (m/z) : 359(M^+), 360 (M^++1) Mol. Wt.:360.

1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(2,3,4,5-tetrafluorophenyl)propane-1,3-dione (CG):

% Yield : 65.30%; Melting point (^{0}C) : 318-320°C; R_f Value: 0.91, Chloroform: Methanol (8:2); FTIR (KBr) ν cm^{-1} :1568 (Ar C=C), 884 (Ar C-H def), 1171(Ar C-F), 723 (Ar C-Cl), 1641 (Aryl Ketone C=O), 1250 (C-O), 3573(Ar OH); ^{1}H NMR (400 MHz CDCl3 δ ppm): 3.81(s, 2H, CH_2), 5.35 (s, 1H, OH), 2.34 (s, 3H, CH_3), 7.02-7.57 (m, 2H, aromatic protons) 7.4 (m, 1H, aromatic F protons); JEOL GCMATE II GC-MS (m/z) : 358 (M^+), 359 (M^++1) Mol. Wt.:359.

6-chloro-7-methyl-2-(2,3,4,5-tetrafluorophenyl)-4*H*-chromen-4-one (CH):

% Yield : 82.92%; Melting point (^{0}C) : 310-312°C; R_f Value: 0.90, Chloroform: Methanol (8:2); FTIR (KBr) ν cm^{-1} :3029 (Ar C-H), 1607 (Ar C=C), 846 (Ar C-H def), 1158 (Ar C-F), 688 (Ar

C-Cl), 1719 (Aryl Ketone C=O), 1349 (C-O); ^{1}H NMR (400 MHz CDCl3 δ ppm) :6.54 (s, 1H, C-H), 6.8 (m, 1H, aromatic F protons), 2.34 (s, 3H, CH_3), 7.10-7.52 (m, 2H, aromatic protons); JEOL GCMATE II GC-MS (m/z) : 341(M^+), 342 (M^++1). Mol. Wt.:342.

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

% Yield: 82.69%; Melting point (^{0}C) : 378-380°C; R_f Value: 0.90, Chloroform: Methanol (8:2); FTIR (KBr) ν cm^{-1} : 3073 (Ar C-H), 1645 (Ar C=C), 755 (Ar C-H def), 1312 (Ar C-F), 688 (Ar C-Cl),3565 (Ar OH), 1378 (C-O), 3383 (N-H); ^{1}H NMR (400 MHz CDCl3 δ ppm): 6.97-7.72 (m, 2H, aromatic protons), 2.34 (s, 3H, CH_3), 6.8 (m, 1H, aromatic F protons), 5.35 (s, 1H, O-H), 6.81 (s, 1H, C-H), 12.62 (s, 1H,N-H); FABMS (m/z): 355 (M^+), 356 (M^++1). Mol. Wt.:356.

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

% Yield: 83.63%; Melting point (^{0}C) : 410-412°C; R_f Value: 0.83, Chloroform: Methanol (8:2); FTIR (KBr) ν cm^{-1} : 3025 (Ar C-H), 1631 (Ar C=C), 817 (Ar C-H def), 1155 (Ar C-F), 755(Ar C-Cl), 3591 (Ar OH), `1319(C-O), 3363 (N-H); ^{1}H NMR (400 MHz CDCl3 δ ppm): 6.92-7.62 (m, 2H, aromatic protons), 2.34 (s, 3H, CH_3), 6.8 (m, 1H, aromatic F protons), 5.35 (s, 1H, O-H), 6.31 (s, 1H, C-H),13.86(s, 1H,N-H), 13.76 (s, 1H,N-H); JEOL GCMATE II GC-MS (m/z) : 394 (M^+), 395 (M^++1). Mol. Wt.:395.

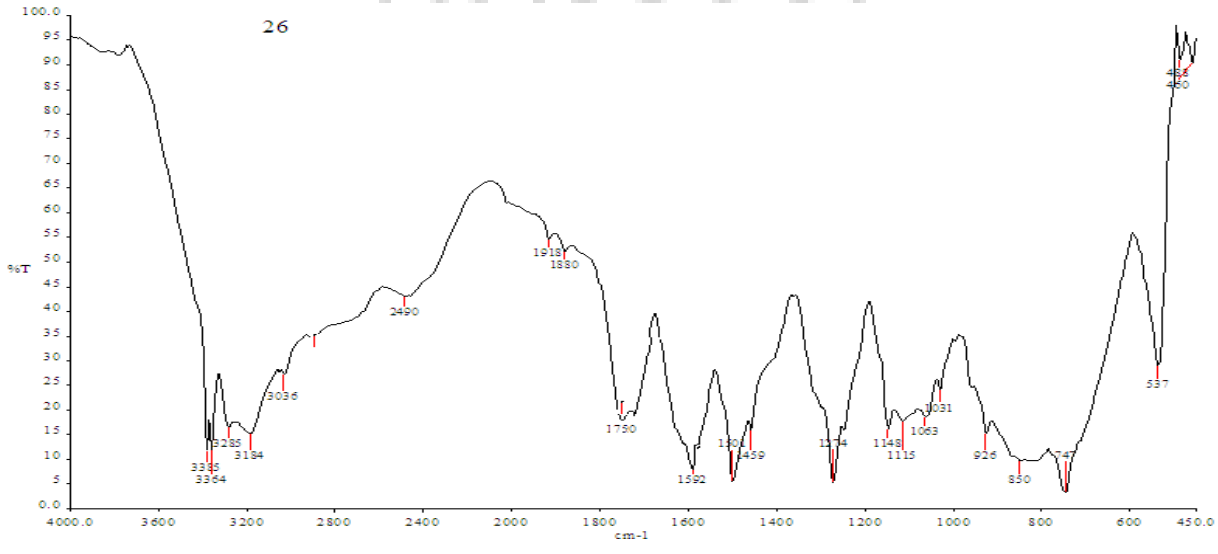


Fig. 1: FTIR (KBr) ν cm^{-1} of 2-acetyl-4-chloro-5-methylphenyl 2,3,4,5-tetrafluorobenzoate (CF)

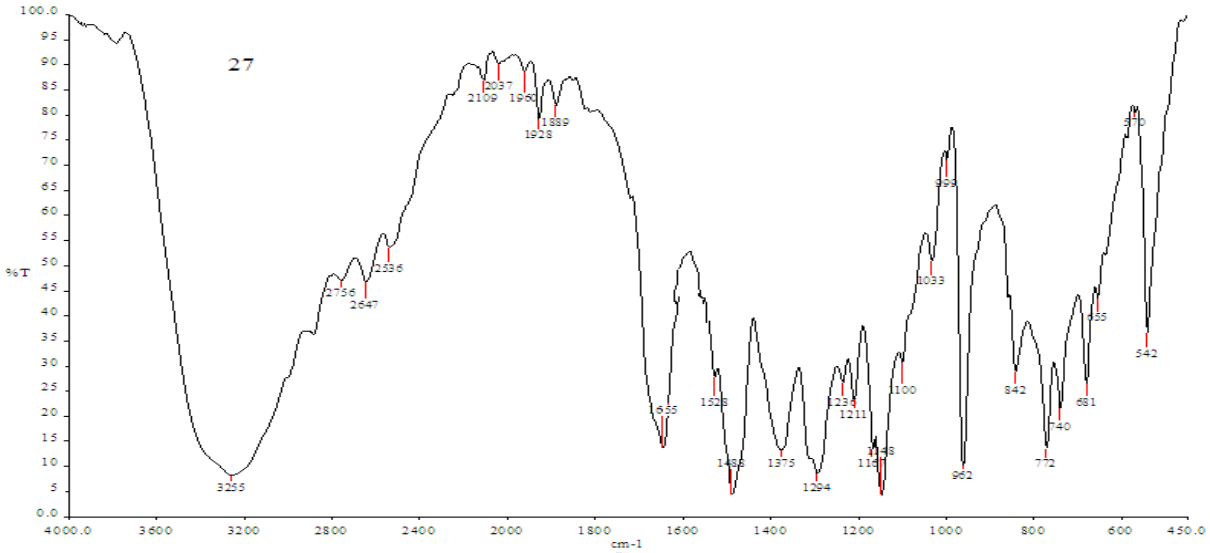


Fig. 2: FTIR (KBr) ν cm^{-1} of 1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(2,3,4,5-tetrafluorophenyl)propane-1,3-dione (CG)

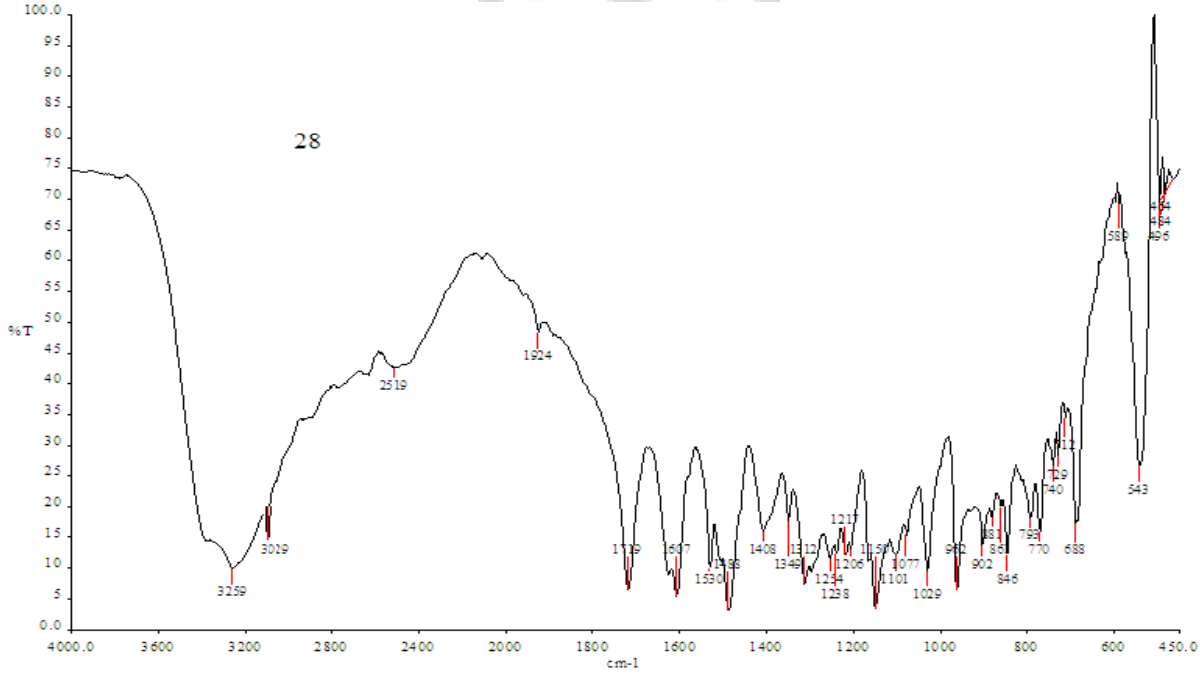


Fig. 3: FTIR (KBr) ν cm^{-1} of 6-chloro-7-methyl-2-(2,3,4,5-tetrafluorophenyl)-4*H*-chromen-4-one (CH)

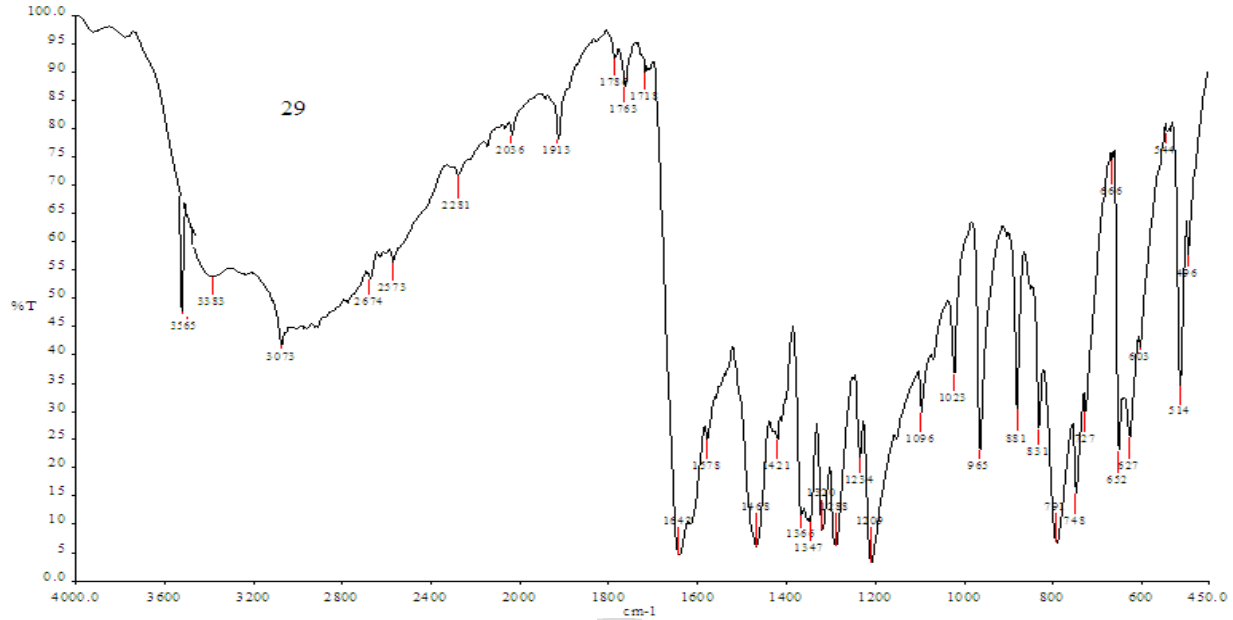


Fig. 4: FTIR (KBr) ν cm^{-1} of 4-chloro-5-methyl-2-[5-(2,3,4,5-tetrafluorophenyl)-1*H*-pyrazol-3-yl]phenol (CI)

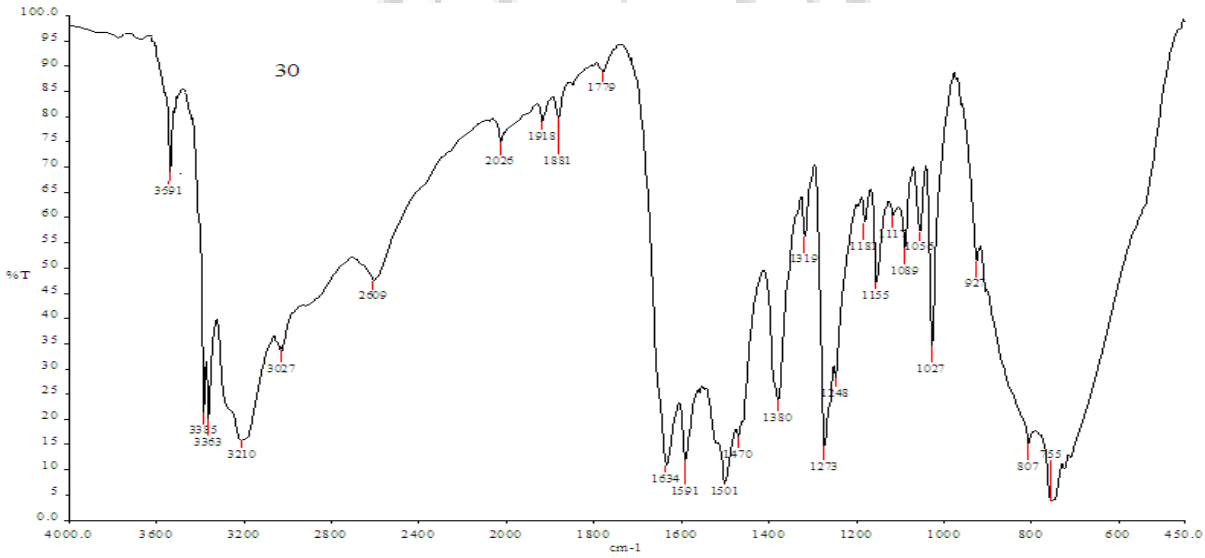


Fig. 5: FTIR (KBr) ν cm^{-1} of 4-chloro-2-[2-imino-6-(2,3,4,5-tetrafluorophenyl)-1,2-dihydropyrimidin-4-yl]-5-methylphenol (CJ)

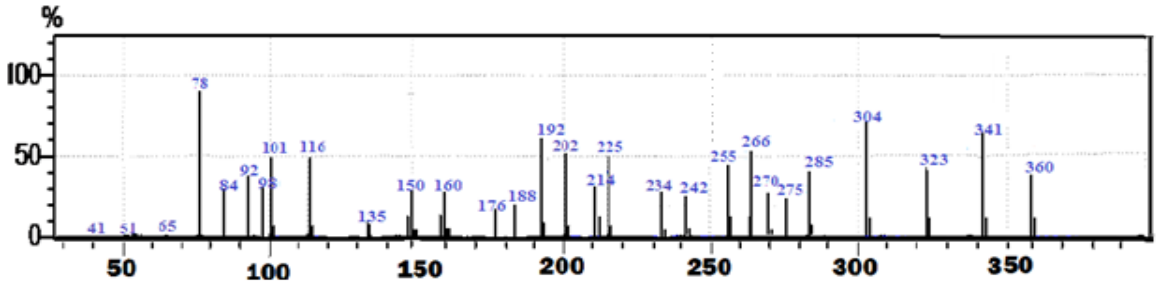


Fig. 6: Mass spectrum of 2-acetyl-4-chloro-5-methylphenyl 2,3,4,5-tetrafluorobenzoate (CF)

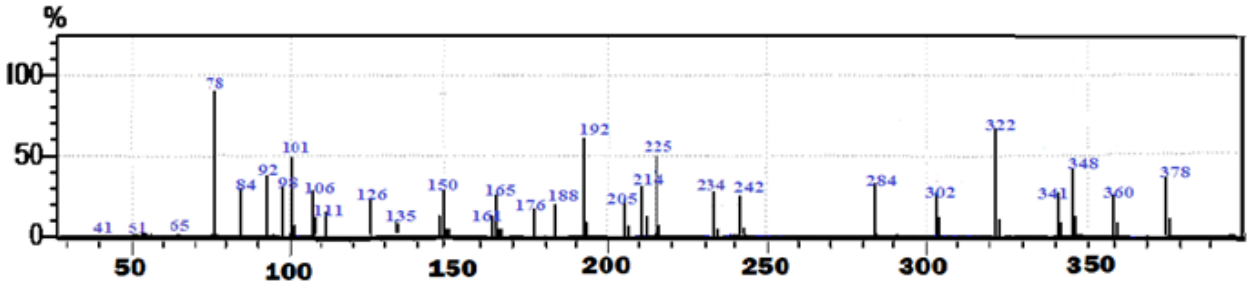


Fig. 7: Mass spectrum of 1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(2,3,4,5-tetrafluorophenyl) propane-1,3-dione (CG)

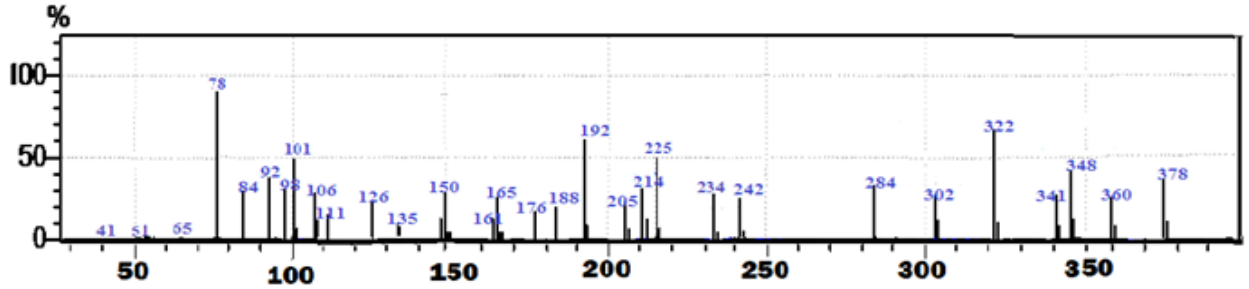


Fig. 8: Mass spectrum of 6-chloro-7-methyl-2-(2,3,4,5-tetrafluorophenyl)-4*H*-chromen-4-one (CH)

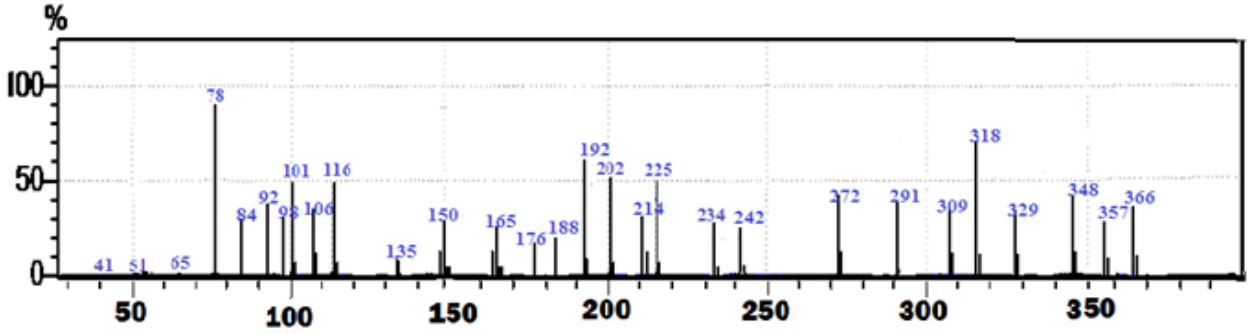


Fig. 9: Mass spectrum of 4-chloro-5-methyl-2-[5-(2,3,4,5-tetrafluorophenyl)-1*H*-pyrazol-3-yl]phenol (CI)

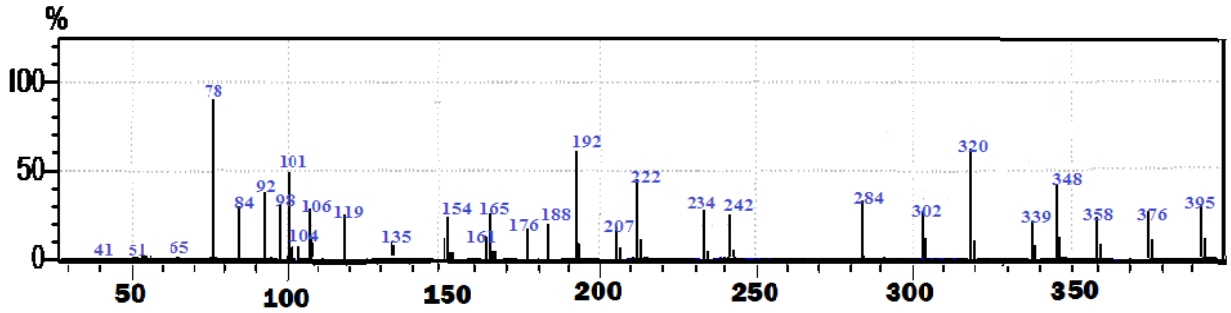


Fig. 10: Mass spectrum of 4-chloro-2-[2-imino-6-(2,3,4,5-tetrafluorophenyl)-1,2-dihydropyrimidin-4-yl]-5-methylphenol (CJ)

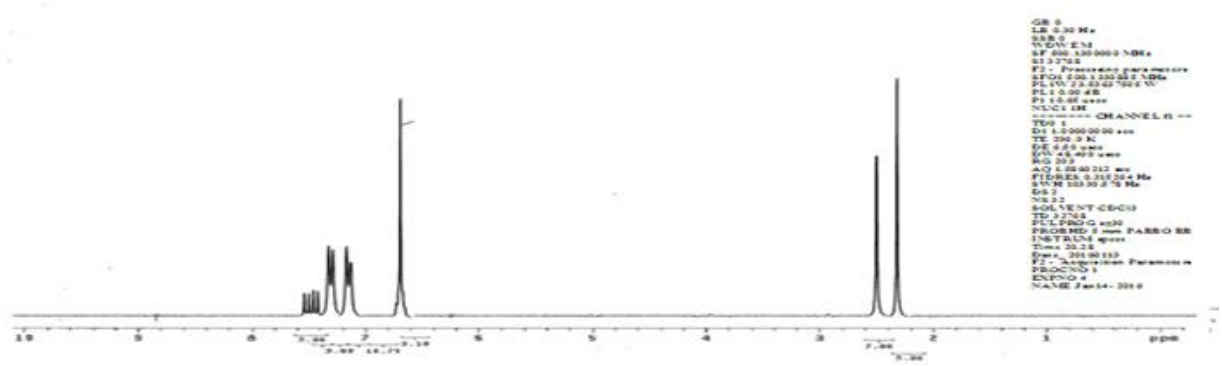


Fig. 11: ^{1}H-NMR of 2-acetyl-4-chloro-5-methylphenyl 2,3,4,5-tetrafluorobenzoate (CF)

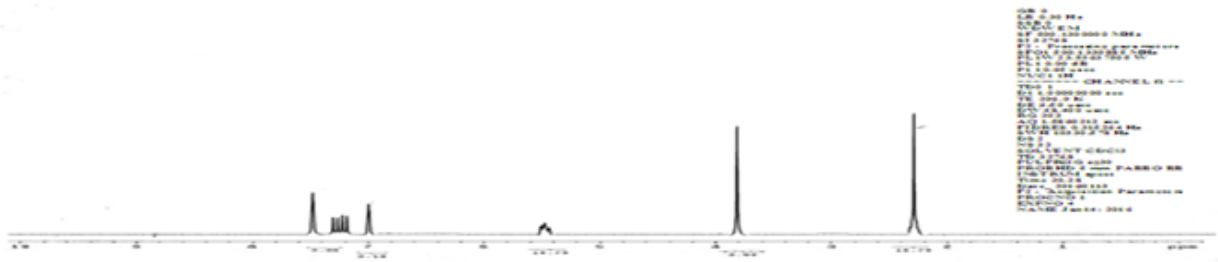


Fig. 12: ^{1}H-NMR of 1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(2,3,4,5-tetrafluorophenyl) propane-1,3-dione (CG)

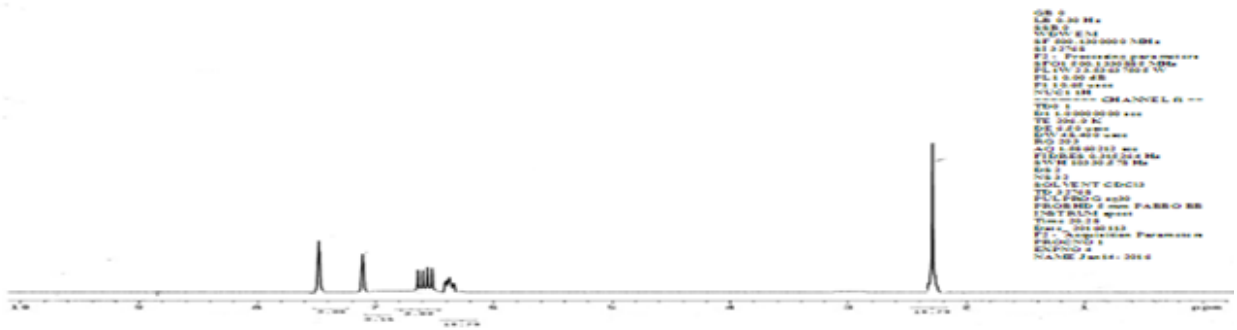


Fig. 13: [1]H-NMR of 6-chloro-7-methyl-2-(2,3,4,5-tetrafluorophenyl)-4*H*-chromen-4-one (CH)

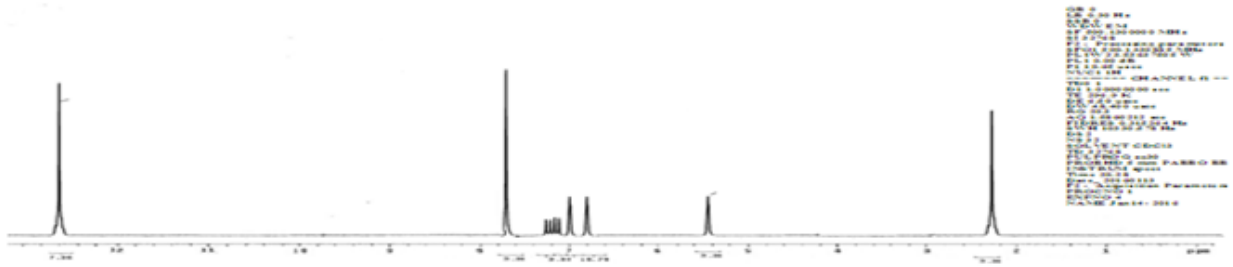


Fig. 14: [1]H-NMR of 4-chloro-5-methyl-2-[5-(2,3,4,5-tetrafluorophenyl)-1*H*-pyrazol-3-yl]phenol (CI)

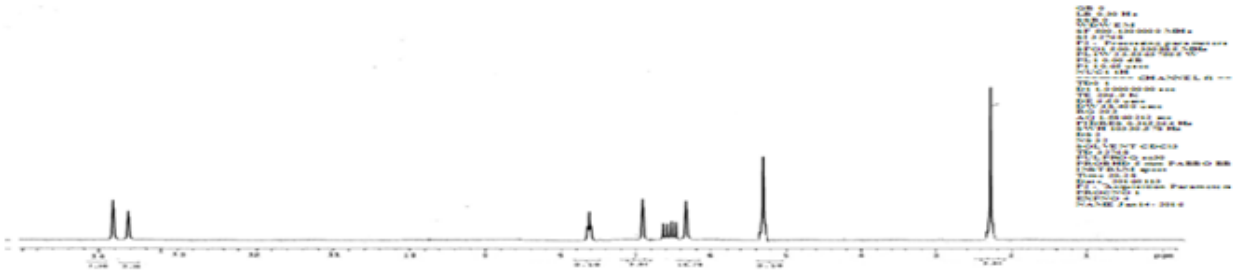


Fig. 15: [1]H-NMR of 4-chloro-2-[2-imino-6-(2,3,4,5-tetrafluorophenyl)-1,2-dihydropyrimidin-4-yl]-5-methylphenol (CJ)

PHARMACOLOGICAL STUDIES[21]

In vitro Antibacterial activity by disc diffusion method:

i) Antibacterial Activity**:**

The compounds like CF to CJ 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
CF	12	17	09
CG	10	11	10
CH	13	20	20
CI	15	24	21
CJ	16	21	19
Gentamycin	20	36	28

RESULTS AND DISCUSSION

The syntheses of compounds CF to CJ were undertaken as per the scheme 1. The required 2-acetyl-4-chloro-5-methylphenyl 2,3,4,5-tetrafluorobenzoate (CF) was prepared by mixture 1-(5-chloro-2-hydroxy-4-methylphenyl) ethanone (0.5g) and 2,3,4,5-tetrafluorobenzoic acid (0.5g) react with each other in the presence of $POCl_3$ (5ml) and Pyridine (15ml) 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-chloro-5-methylphenyl 2,3,4,5-tetrafluorobenzoate (CF). 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 an internal standard in DMSO-d_6/$CDCl_3$ and mass spectra was obtained on JEOL GCMATE II GC-MS are 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. The results indicated that the nitrogen and oxygen heterocyclic containing compounds having more antimicrobial activity. Moreover, the compounds CH, CI and CJ having the side chain showed higher activity than CF and CG, against *E.coli, S. aureus, Pseudomonas aeruginosa.* The replacement of oxygen to nitrogen resulted in 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 CH, CI and CJ 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 and Fig.16.

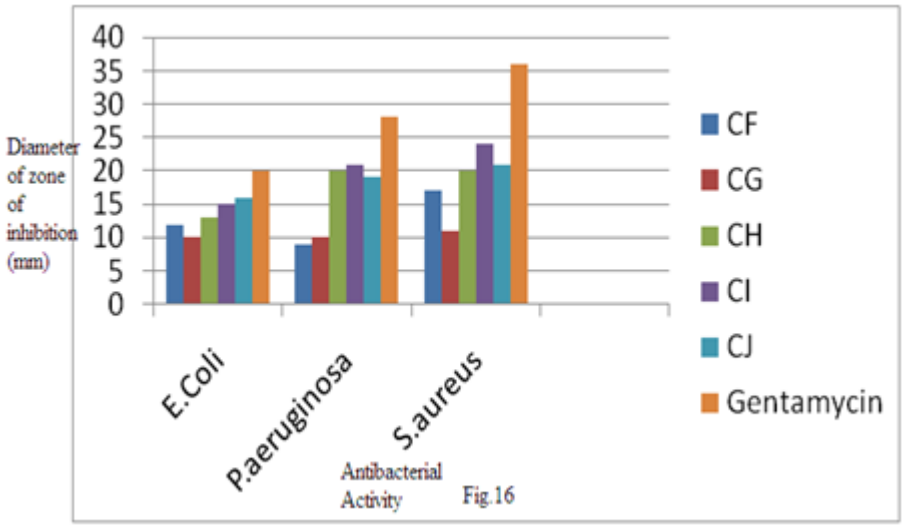


Fig 16: Antibacterial Activity of Chromones and Pyrazole derivatives.

CONCLUSION

Various 2-acetyl-4-chloro-5-methylphenyl 2,3,4,5-tetrafluorobenzoate (CF) was synthesized from a mixture of 1-(5-chloro-2-hydroxy-4-methylphenyl) ethanone (0.5g) and 2,3,4,5-tetrafluorobenzoic acid (0.5g). The structure antibacterial activity relationship of the synthesized compounds was based on the structure of final derivatives. These derivatives possess good antibacterial activity. The antimicrobial activities including antibacterial properties of the

synthesized derivatives showed a significant activity as compared with standard drugs like Gentamycin.

ACKNOWLEDGMENT

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