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RESEARCH ARTICLE

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

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ABSTRACT

Chromones derivatives and pyrazol derivatives were synthesized and screened for antibacterial activity. Some chromones and Pyrazol derivatives like 6-chloro-7-methyl-2-(pentafluorophenyl)-4*H*-chromen-4-one, 4-chloro-5-methyl-2-[5-(pentafluorophenyl)-1*H*-pyrazol-3-yl] phenol were synthesized by a sequence of reactions starting from 2-acetyl-4-chloro-5-methylphenyl pentafluorobenzoate, and were mentioned in scheme 1. The 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, 1H NMR and Mass spectrum. The compounds bearing nitro and oxygen groups have shown prominent activity when compared to compounds without these groups.

KEYWORDS

Anti-bacterial activity, Conventional Method, Gentamycin, Pentafluorobenzoic Acid, 1-(5-chloro-2-hydroxy-4-methylphenyl) Ethanone

INTRODUCTION

Chromones and pyrazol and its derivatives are important heterocyclic in organic and biochemistry and have been found in many chromones containing natural products such as Khellin, sodium cromoglycate, diosmin, flavones, and flavonoids etc. There are deep studies on the synthesis and reactivity of Chromones and pyrazol derivatives. Heterocyclic compounds containing N and O are found to display 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[2]. Some Chromones and pyrazol derivatives are prepared by using 1-(5-chloro-2-hydroxy-4-methylphenyl) ethanone, pentafluorobenzoic acid reagent. These pyrrole derivatives and chromones are screened for antibacterial activity and antifungal activity. It reveals that chromones and pyrazol posses broad spectrum activity such as antimicrobial[3-6], anti-inflammatory[7], analgesic[8], antitumorial[9], antihypertensive[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 gives variety of biological activities

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such as analgesic, anti-inflammatory, protein kinase C inhibitor[12].

Many Pyrazol derivatives possess activity like Antiepileptic and Antimicrobial[13], Antiamoebic[14] and Antiandrogenic activities[15]. 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[16].

MATERIAL AND METHODS

Chemical and Reagent

1-(5-chloro-2-hydroxy-4-methylphenyl) ethanone, Pentafluorobenzoic 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.

Experimental Section[17-20]

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 by 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 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 pentafluorobenzoate (CA):(Scheme 1)

A mixture of 1-(5-chloro-2-hydroxy-4-methylphenyl) ethanone (0.5g) and Pentafluorobenzoic 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 pentafluorobenzoate (CA).

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

A solution of 2-acetyl-4-chloro-5-methylphenyl pentafluorobenzoate (CA) react 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 The precipitate formed was washed with water and recrystalized from ethanol and then it gives1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(pentafluorophenyl) propane-1,3-dione (CB).

Synthesis of 6-chloro-7-methyl-2-(pentafluorophenyl)-4*H*-chromen-4-one (CC): (Scheme 1)

A solution of 1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(pentafluorophenyl) propane-1,3-dione (CB) react with con. Hydrochloric acid (5 ml) and ethanol (5 ml), 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 recrystalized from ethanol and then it gives 6-chloro-7-methyl-2-(pentafluorophenyl)-4*H*-chromen-4-one (CC).

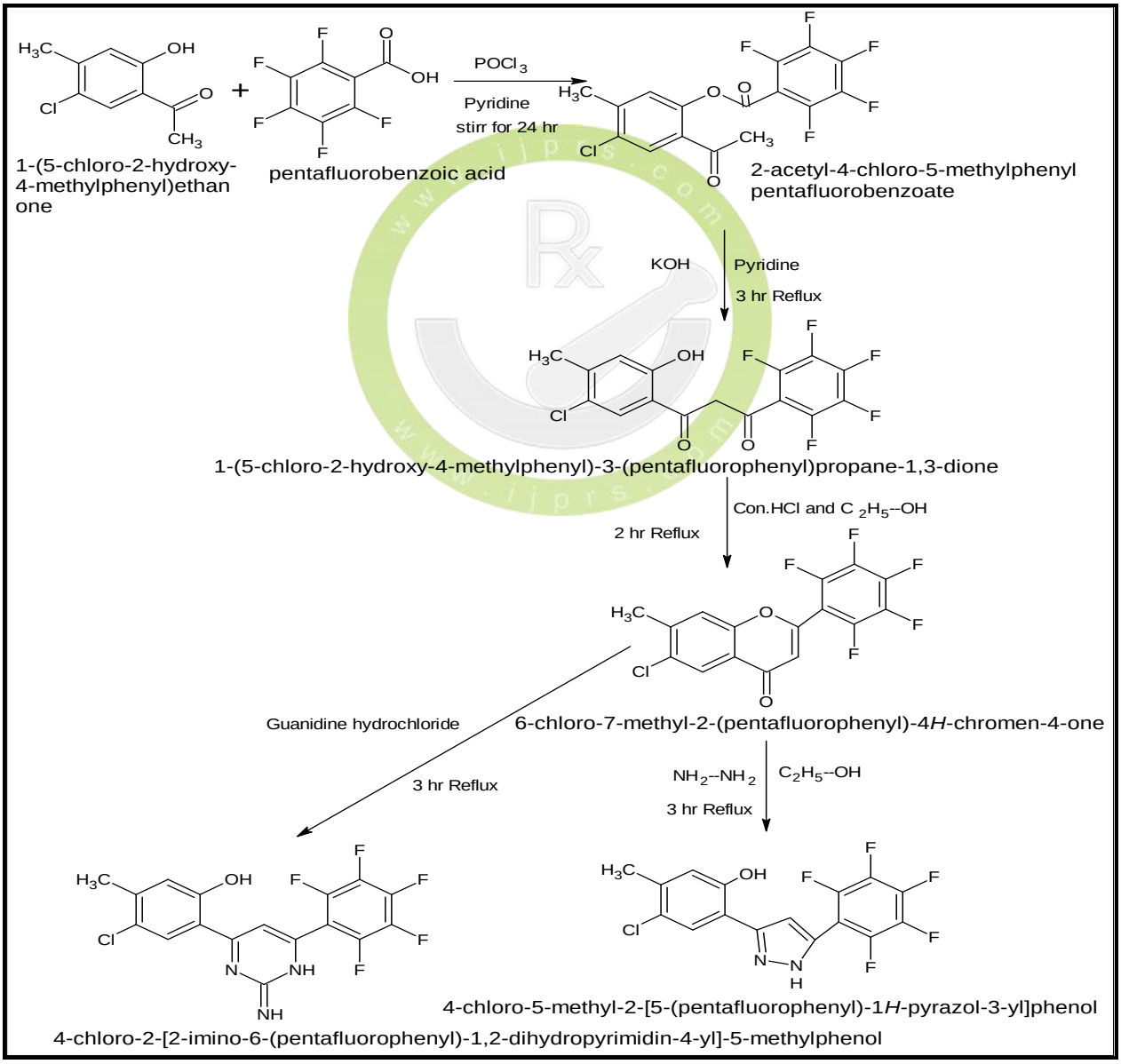
Synthesis of 6-chloro-7-methyl-2-(pentafluorophenyl)-4*H*-chromen-4-one (CD): (Scheme 1)

A solution of 6-chloro-7-methyl-2-(pentafluorophenyl)-4*H*-chromen-4-one (CC) react with hydrazine hydrate (5 ml)and ethanol(10 ml) 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 recrystalized from ethanol and then it gives4-chloro-5-methyl-2-[5-(pentafluorophenyl)-1*H*-pyrazol-3-yl] phenol

Table 1: Physical Data for Synthesized compounds

Sr. No.	Compounds	Molecular Formula	Melting Point^{0}C	% yields	Molecular Weight
1	CA	$C_{15}H_8O_3F_5Cl$	334-336°C	74.73%	366
2	CB	$C_{16}H_8O_3F_5Cl$	348-350°C	61.53%	378
3	CC	$C_{16}H_6O_2F_5Cl$	318-320°C	63.82%	360
4	CD	$C_{16}H_8ON_2F_5Cl$	320-322°C	86.20%	374
5	CE	$C_{16}H_9ON_3F_5Cl$	366-368°C	87.03%	395

Scheme of Reaction: Scheme 1:



Scheme 1:6-chloro-7-methyl-2-(pentafluorophenyl)-4*H*-chromen-4-one (CC) and 4-chloro-5-methyl-2-[5-(pentafluorophenyl)-1*H*-pyrazol-3-yl]phenol (CD) derivatives (CA- CE)

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

A solution of 6-chloro-7-methyl-2-(pentafluorophenyl)-4*H*-chromen-4-one (CC) react with guanidine hydrochloride (5 ml) and it was 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 recrystalized from ethanol and then it gives then it gives 4-chloro-2-[2-imino-6-(pentafluorophenyl)-1,2-dihydropyrimidin-4-yl]-5-methylphenol (CE).

RESULTS

Spectral Data

2-acetyl-4-chloro-5-methylphenyl pentafluoro-benzoate (CA)

%Yield :74.73%, Melting point (^{0}C):334-336°C; R_fValue:0.89,Chloroform: Methanol (8:2); FTIR (KBr) ν cm^{-1} :3062 (Ar C-H), 1641 (Ar C=C), 797 (Ar C-H def), 1172(Ar C-F), 796(Ar C-Cl),1730 (Ester C=O), 1366 (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); JEOL GCMATE II GC-MS (m/z) : 365(M^+), 366 (M^++1) Mol. Wt.:366.

1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(pentafluorophenyl) propane-1,3-dione (CB)

%Yield :61.53%, Melting point (^{0}C):348-350°C; R_f Value:0.79,Chloroform: Methanol (8:2); FTIR (KBr) ν cm^{-1} :3061 (Ar C-H str), 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); JEOL GCMATE II GC-MS (m/z) : 377(M^+), 378 (M^++1) Mol. Wt.:378.

6-chloro-7-methyl-2-(pentafluorophenyl)-4H-chromen-4-one (CC)

%Yield :63.82%, Melting point (^{0}C): 318-320°C; R_fValue:0.96,Chloroform: Methanol (8:2); FTIR (KBr) ν cm^{-1} :3028 (Ar C-H), 1529 (Ar C=C), 846 (Ar C-H def), 1108 (Ar C-F), 769(Ar C-Cl), 1668 (Aryl Ketone C=O), 1350 (C-O); ^{1}H NMR (400 MHz CDCl3 δ ppm) :6.54 (s, 1H, C-H), 2.34 (s, 3H, CH_3), 7.10-7.52 (m, 2H, aromatic protons); JEOL GCMATE II GC-MS (m/z) : 359(M^+), 360 (M^++1). Mol. Wt.:360.

4-chloro-5-methyl-2-[5-(pentafluorophenyl)-1H-pyrazol-3-yl] phenol (CD)

%Yield: 86.20%, Melting point (^{0}C):320-322°C; R_fValue:0.80,Chloroform: Methanol (8:2); FTIR (KBr) ν cm^{-1} 3025 (Ar C-H), 1645 (Ar C=C), 755 (Ar C-H def), 1273(Ar C-F), 652(Ar C-Cl),3535 (Ar OH), 1378 (C-O), 3477 (N-H); ^{1}H NMR (400 MHz CDCl3 δ ppm): 6.97-7.72 (m, 2H, aromatic protons), 2.34 (s, 3H, CH_3), 5.35(s, 1H, O-H), 6.81 (s, 1H, C-H),12.62(s, 1H,N-H); FABMS (m/z): 373(M^+), 374 (M^++1). Mol. Wt.:374.

4-chloro-2-[2-imino-6-(pentafluorophenyl)-1,2-dihydropyrimidin-4-yl]-5-methylphenol (CE)

%Yield: 87.03%, Melting point (^{0}C):366-368°C; R_fValue:0.83,Chloroform: Methanol (8:2); FTIR (KBr) ν cm^{-1} : 3025 (Ar C-H), 1631 (Ar C=C), 817 (Ar C-H def), 1118 (Ar C-F), 752(Ar C-Cl), 3385 (Ar OH), `1319(C-O),3364 (N-H); ^{1}H NMR (400 MHz CDCl3 δ ppm): 6.92-7.62 (m, 2H, aromatic protons), 2.34 (s, 3H, CH_3), 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^+), 394 (M^++1). Mol. Wt.:395.

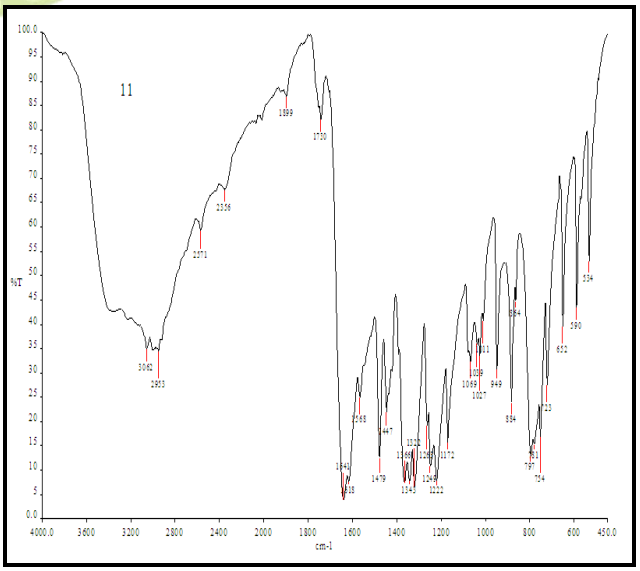


Figure 1: FTIR (KBr) ν cm^{-1} of 2-acetyl-4-chloro-5-methylphenyl pentafluorobenzoate (CA)

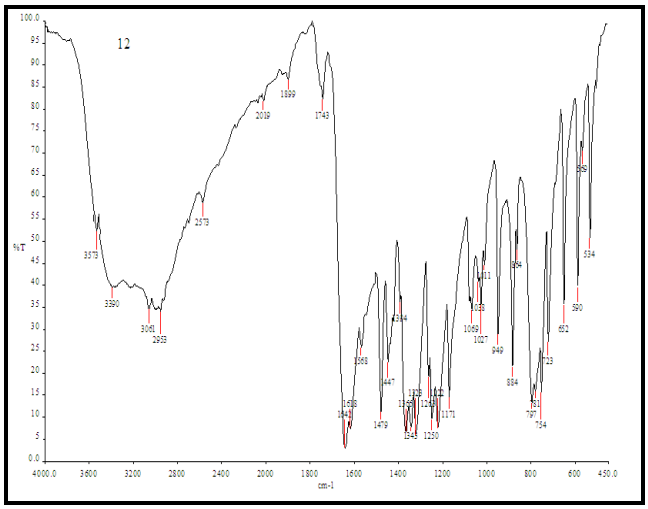


Figure 2: FTIR (KBr) ν cm^{-1} of 1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(pentafluorophenyl) propane-1,3-dione (CB)

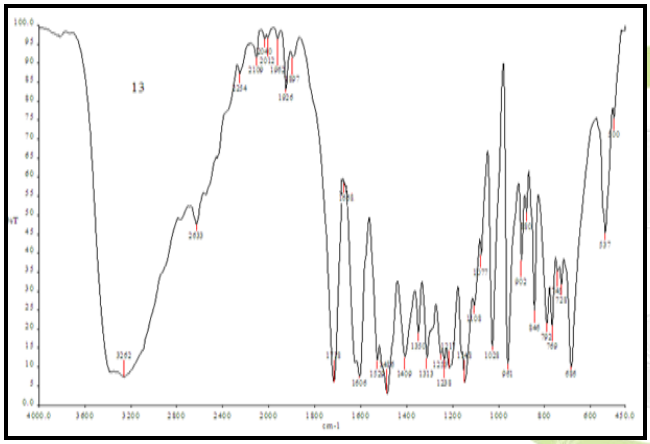


Figure 3: FTIR (KBr) ν cm^{-1} of 6-chloro-7-methyl-2-(pentafluorophenyl)-4*H*-chromen-4-one (CC)

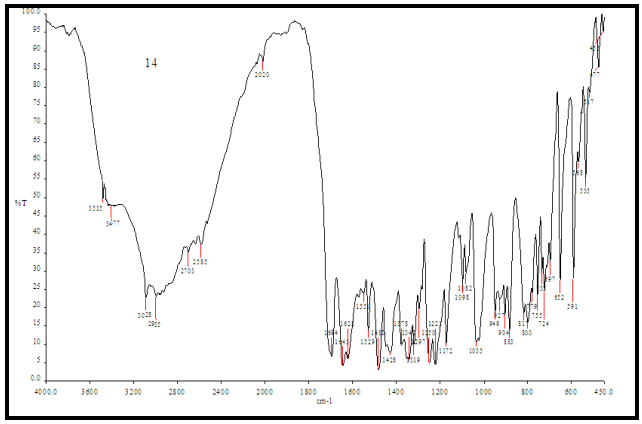


Figure 4: FTIR (KBr) ν cm^{-1} of 4-chloro-5-methyl-2-[5-(pentafluorophenyl)-1*H*-pyrazol-3-yl] phenol (CD)

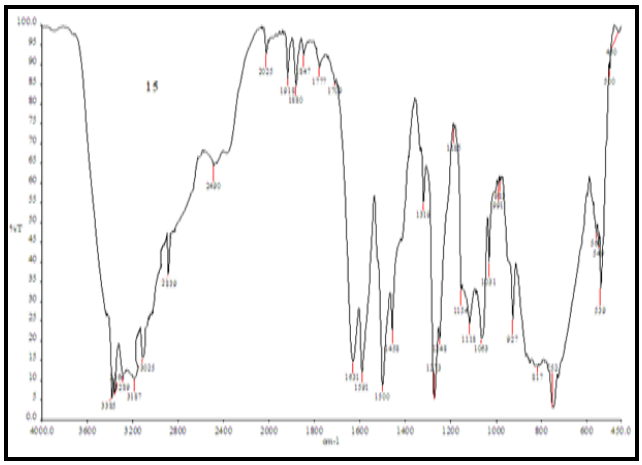


Figure 5: FTIR (KBr) ν cm^{-1} of 4-chloro-2-[2-imino-6-(pentafluorophenyl)-1,2-dihydropyrimidin-4-yl]-5-methylphenol (CE)

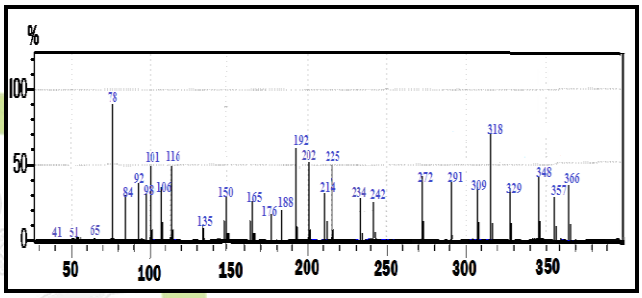


Figure 6: Mass spectrum of 2-acetyl-4-chloro-5-methylphenyl pentafluorobenzoate (CA)

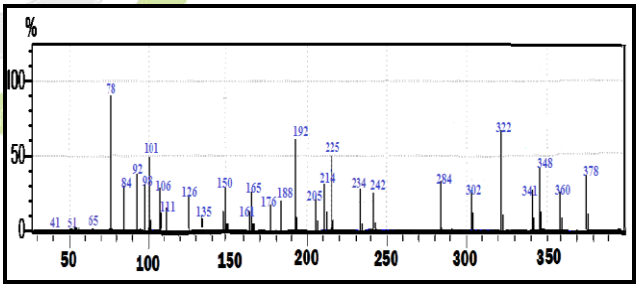


Figure 7: Mass spectrum of 1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(pentafluorophenyl) propane-1,3-dione (CB)

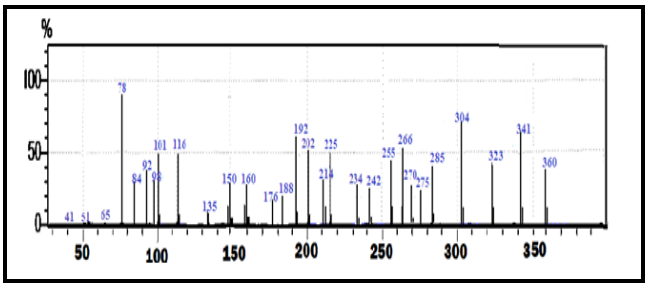


Figure 8: Mass spectrum of 6-chloro-7-methyl-2-(pentafluorophenyl)-4*H*-chromen-4-one (CC)

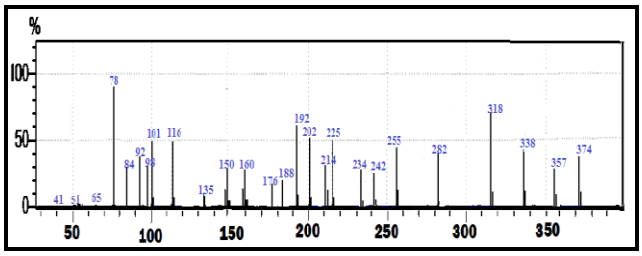


Figure 9: Mass spectrum of 4-chloro-5-methyl-2-[5-(pentafluorophenyl)-1*H*-pyrazol-3-yl] phenol (CD)

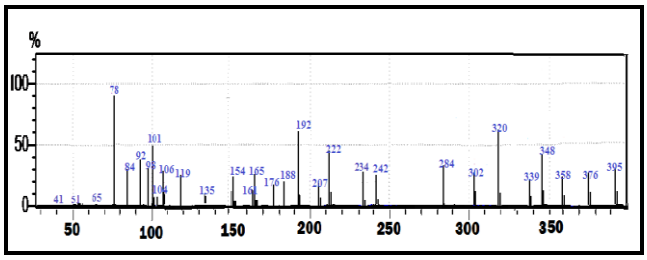


Figure 10: Mass spectrum of 4-chloro-2-[2-imino-6-(pentafluorophenyl)-1,2-dihydropyrimidin-4-yl]-5-methylphenol (CE)

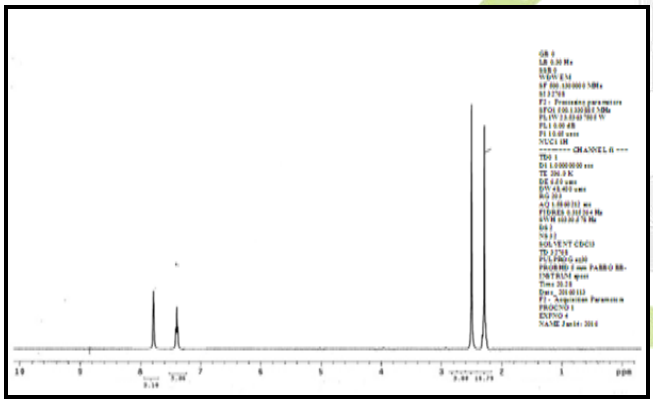


Figure 11: ^{1}H-NMR of 2-acetyl-4-chloro-5-methylphenyl pentafluorobenzoate (CA)

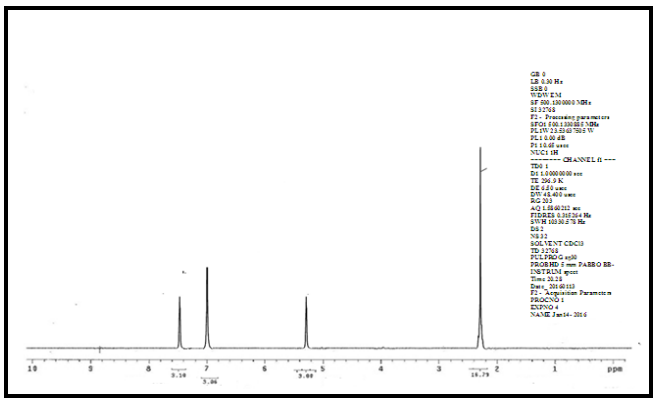


Figure 12: ^{1}H-NMR of 1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(pentafluorophenyl) propane-1,3-dione (CB)

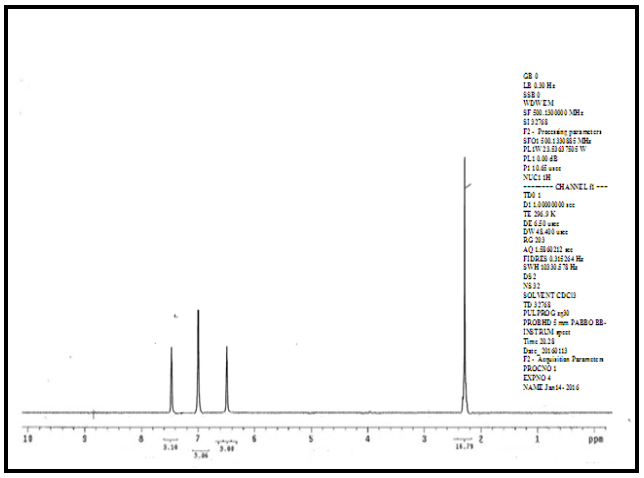


Figure 13: ^{1}H-NMR of 6-chloro-7-methyl-2-(pentafluorophenyl)-4*H*-chromen-4-one (CC)

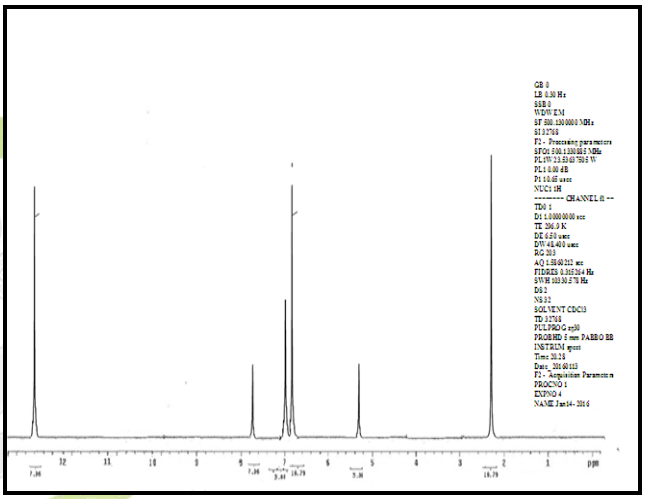


Figure 14: ^{1}H-NMR of 4-chloro-5-methyl-2-[5-(pentafluorophenyl)-1*H*-pyrazol-3-yl] phenol (CD)

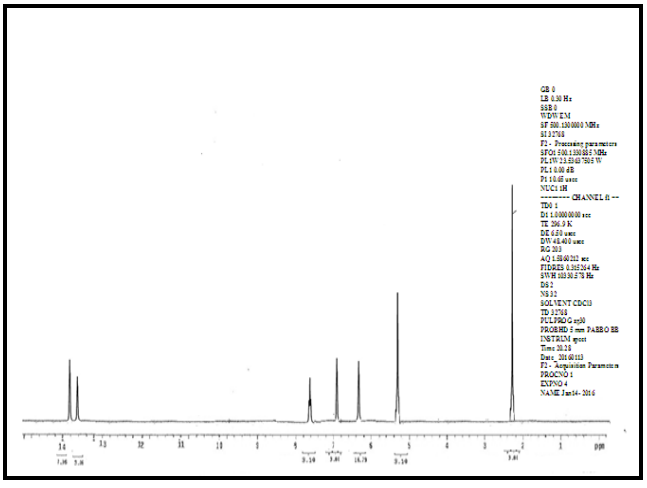


Figure 15: ^{1}H-NMR of 4-chloro-2-[2-imino-6-(pentafluorophenyl)-1,2-dihydropyrimidin-4-yl]-5-methylphenol (CE)

Pharmacological Studies[21]

In vitro Antibacterial activity by disc diffusion method:

Antibacterial Activity

The compounds like CA to CE 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 100 µg/mL in DMSO. The zone of inhibition was measured after 24 h incubation at 37^0C. Standard: Gentamycin (100 µg/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	***Staphylo-coccus aureus***	***Pseudomonas aeruginosa***
	ATCC 25922	ATCC 25923	ATCC 27853
CA	12	18	10
CB	08	15	09
CC	13	20	19
CD	14	22	20
CE	14	21	21
Genta-mycin	20	36	28

DISCUSSION

The syntheses of compounds CA to CE were undertaken as per the scheme 1. The required2-acetyl-4-chloro-5-methylphenyl pentafluorobenzoate(CA)was prepared by mixture of 1-(5-chloro-2-hydroxy-4-methylphenyl) ethanone (0.5g) and Pentafluorobenzoic 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 pentafluorobenzoate (CA).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 by 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 are presented as m/z.

The synthesis of compounds CA-CE was undertaken as per the scheme 1. The required 2-acetyl-4-chloro-5-methylphenyl pentafluorobenzoate (CA) was prepared by the action of 1-(5-chloro-2-hydroxy-4-methylphenyl) ethanone, Pentafluorobenzoic acid. The results revealed that majority 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 containing compounds, having more antimicrobial activity.

Moreover, the compounds CC, CD and CE, having the side chain showed higher activity than CA and CB, against *E.coli, S. aureus, 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. The antimicrobial activity revealed that newly synthesized compound CC, CD and CE, showed good significant activity.

The results of the preliminary antimicrobial testing of the prepared compounds, the typical

broad spectrum antibacterial drug like Gentamycinwas shown in Table 2 and Fig.16.

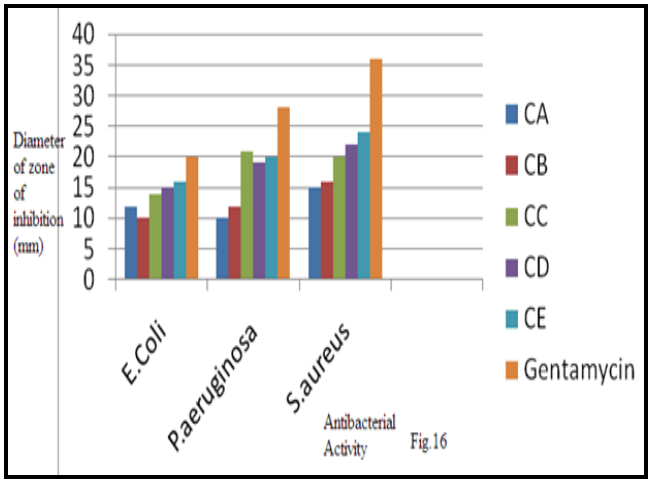


Figure 16: Anti Bacterial Activity of Chromones and Pyrazole derivatives

CONCLUSION

Various 2-acetyl-4-chloro-5-methylphenyl pentafluorobenzoate (CA) was synthesized from a mixture of 1-(5-chloro-2-hydroxy-4-methylphenyl) ethanone (0.5g) and Pentafluorobenzoic 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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