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Synthesis and *In-Vitro* Antibacterial Activity of 2-Acetyl-4-Chlorophenyl Pentafluorobenzoate Derivatives

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

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

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ABSTRACT

Chromones and Pyrazole derivatives were reported wide range of biological activities. Hence, it was planned to synthesize and screen for their antibacterial (*in vitro*) activity. A series of novel 2-acetyl-4-chlorophenyl pentafluorobenzoate derivatives were synthesized and evaluated for *in- vitro* antibacterial activity. Chromones and Pyrazole derivatives like 6-chloro-2-(pentafluorophenyl)-4*H*-chromen-4-one, 4-chloro-2-[5-(pentafluorophenyl)-1*H*-pyrazol-3-yl]phenol were synthesized by a sequence of reactions starting 2-acetyl-4-chlorophenyl pentafluorobenzoate 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 was used as standard drugs for antibacterial. Characterization of all the compounds were performed by IR, 1H NMR and Mass spectroscopy. The anti-inflammatory data suggested that compounds BC, BD and BE showed significant activity. 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 Pentafluorobenzoic 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], antihypertensive[9], anticonvulsant and antiviral activity[10]. There are antifungal and antibacterial agent having different structure and used in the treatment of fungal and bacterial infection. They are known to possess variety of biological activities such as analgesic, anti-inflammatory, protein kinase C inhibitor[11-12]. Many Pyrazole 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].

MATERIALS AND METHODS

MATERIALS

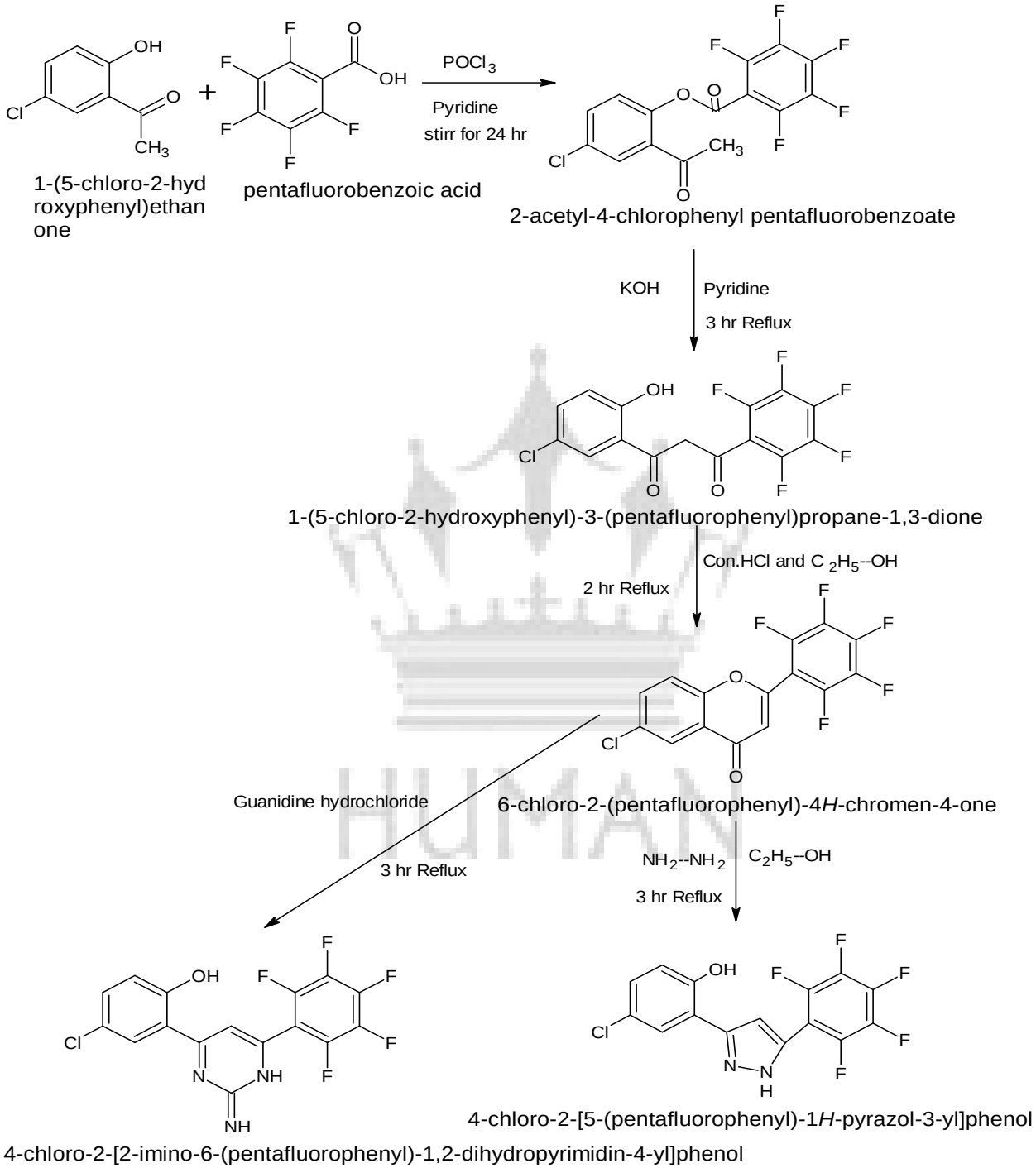
1-(5-chloro-2-hydroxy-4-methylphenyl) ethanone, Pentafluorobenzoic acid, Pyridine, Hydrazine Hydrate, Guanidine Hydrochloride, Ethanol, Con. Hydrochloric acid and Phosphorus oxychloride i.e. $POCl_3$ was used for the synthesis of Chromones and Pyrazole. All chemicals were of analytical grade. All Chromones and Pyrazole derivatives were synthesized by conventional method.

METHODS

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 are presented as m/z. The synthetic route for the title compounds was shown in Scheme 1.

Scheme of reaction:

Scheme 1:



Scheme 1: Synthesis of 6-chloro-2-(pentafluorophenyl)-4*H*-chromen-4-one (BC) and 4-chloro-2-[5-(pentafluorophenyl)-1*H*-pyrazol-3-yl] phenol (BD) derivatives (BA- BE).

Synthesis of 2-acetyl-4-chlorophenyl pentafluorobenzoate derivatives [17-20]:

Synthesis of 2-acetyl-4-chlorophenyl pentafluorobenzoate (BA): (Scheme 1)

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

Synthesis of 1-(5-chloro-2-hydroxyphenyl)-3-(pentafluorophenyl) propane-1, 3-dione (BB) :(Scheme 1)

A solution of 2-acetyl-4-chlorophenyl pentafluorobenzoate (BA) react with potassium hydroxide (0.5g) and pyridine (5ml) and reflux for 3 h and then completion of the reaction was confirmed on 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-(pentafluorophenyl) propane-1, 3-dione (BB).

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

A solution of 1-(5-chloro-2-hydroxyphenyl)-3-(pentafluorophenyl) propane-1, 3-dione (BB) (0.5g) react with con. Hydrochloric acid (5ml) and ethanol (5ml), and reflux for 2h and then completion of the reaction were confirmed on 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-(pentafluorophenyl)-4*H*-chromen-4-one (BC).

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

A solution of 6-chloro-2-(pentafluorophenyl)-4*H*-chromen-4-one (BC) (0.5g) react with hydrazine hydrate (5ml) and ethanol (10ml) and reflux for 3h and then completion of the reaction was confirmed on 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-[5-(pentafluorophenyl)-1*H*-pyrazol-3-yl] phenol (BD).

Synthesis of 4-chloro-2-[2-imino-6-(pentafluorophenyl)-1, 2-dihydropyrimidin-4-yl] phenol (BE): (Scheme 1)

A solution of 6-chloro-2-(pentafluorophenyl)-4*H*-chromen-4-one (BC) (0.5g) react with guanidine hydrochloride (5ml) and it was refluxed for 3 hrs and then completion of the reaction was confirmed on 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-(pentafluorophenyl)-1, 2-dihydropyrimidin-4-yl] phenol (BE).

Table 1: Physical Data for 2-acetyl-4-chlorophenyl pentafluorobenzoate derivatives

S. No	Compounds	Molecular Formula	Melting Point ^{0}C	% yields	Molecular Weight
1	BA	$C_{15}H_6O_3F_5Cl$	318-320°C	70.27 %	364
2	BB	$C_{15}H_6O_3F_5Cl$	340-342°C	88.23%	364
3	BC	$C_{15}H_4O_2F_5Cl$	318-320°C	75.78%	346
4	BD	$C_{15}H_6ON_2F_5Cl$	330-332°C	88.46%	360
5	BE	$C_{15}H_7ON_3F_5Cl$	310-312°C	83.17%	375

Characterization

2-acetyl-4-chlorophenyl pentafluorobenzoate (BA):

% Yield: 70.27 %; Melting point (^{0}C) :318-320°C; R_f Value: 0.86, chloroform: methanol (8:2); FTIR (KBr) ν cm^{-1} : 3074 (Ar C-H), 1642 (Ar C=C), 881 (Ar C-H def), 1234 (Ar C-F), 726 (Ar C-Cl), 1715 (Ester C=O), 1319 (C-O); ^{1}H NMR (500 MHz CDCl3 δ ppm) : 7.18-7.75 (m, 3H, aromatic protons), 2.34 (s, 3H, CH_3); JEOL GCMATE II GC-MS (m/z): 363 (M^+), 364 (M^++1), Mol. Wt.:364.

1-(5-chloro-2-hydroxyphenyl)-3-(pentafluorophenyl) propane-1, 3-dione (BB):

% Yield: 88.23%; Melting point (^{0}C) : 340-342°C; R_f Value:0.85 chloroform: methanol (8:2); FTIR (KBr) ν cm^{-1} :3011 (Ar C-H), 1616 (Ar C=C), 836 (Ar C-H def), 1158 (Ar C-F), 620 (Ar C-Cl), 1660 (Aryl Ketone C=O), 1221 (C-O), 3622 (Ar OH); ^{1}H NMR (500 MHz CDCl3 δ

ppm): 3.81(s, 2H, CH_2), 5.35 (s, 1H, OH), 7.02-7.57 (m, 3H, aromatic protons); JEOL GCMATE II GC-MS (m/z): 363(M^+), 364 (M^++1), Mol. Wt.:364.

6-chloro-2-(pentafluorophenyl)-4*H*-chromen-4-one (BC):

% Yield: 75.78%; Melting point (^{0}C) : 318-320°C; R_f Value: 0.90 chloroform: methanol (8:2); FTIR (KBr) ν cm^{-1} : 3029 (Ar C-H), 1530 (Ar C=C), 831 (Ar C-H def), 1217 (Ar C-F), 688 (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 JEOL GCMATE II GC-MS (m/z): 345 (M^+), 346 (M^++1) Mol. Wt.:346.

4-chloro-2-[5-(pentafluorophenyl)-1*H*-pyrazol-3-yl] phenol (BD):

% Yield: 88.46%, Melting point (^{0}C): 330-332°C, R_f 0.84 chloroform: methanol; FTIR (KBr) ν cm^{-1} : 3045 (Ar C-H), 1631 (Ar C=C), 728 (Ar C-H def), 1155 (Ar C-F), 749 (Ar C-Cl), 3538 (Ar OH), 1347 (C-O), 3385 (N-H); ^{1}H NMR (500 MHz CDCl3 δ ppm): 7.85-7.96 (m, 3H, aromatic protons), 5.35(s, 1H, O-H), 6.81 (s, 1H, C-H),12.62 (s, 1H,N-H), 2.34 (s, 3H, CH_3); JEOL GCMATE II GC-MS (m/z): 359 (M^+), 360 (M^++1). Mol. Wt.:360.

4-chloro-2-[2-imino-6-(pentafluorophenyl)-1, 2-dihydropyrimidin-4-yl] phenol (BE):

% Yield: 83.17%; Melting point (^{0}C): 310-312°C; R_f 0.94 chloroform: methanol; FTIR (KBr) ν cm^{-1} : 3028 (Ar C-H), 1656 (Ar C=C), 781 (Ar C-H def), 1278 (Ar C-F), 758 (Ar C-Cl), 3521 (Ar OH), 1320 (C-O), 3362 (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); JEOL GCMATE II GC-MS (m/z): 374 (M^+), 375 (M^++1). Mol. Wt.:375.

FTIR (KBr) ν cm^{-1} and ^{1}H NMR of 2-acetyl-4-chlorophenyl pentafluorobenzoate derivatives:

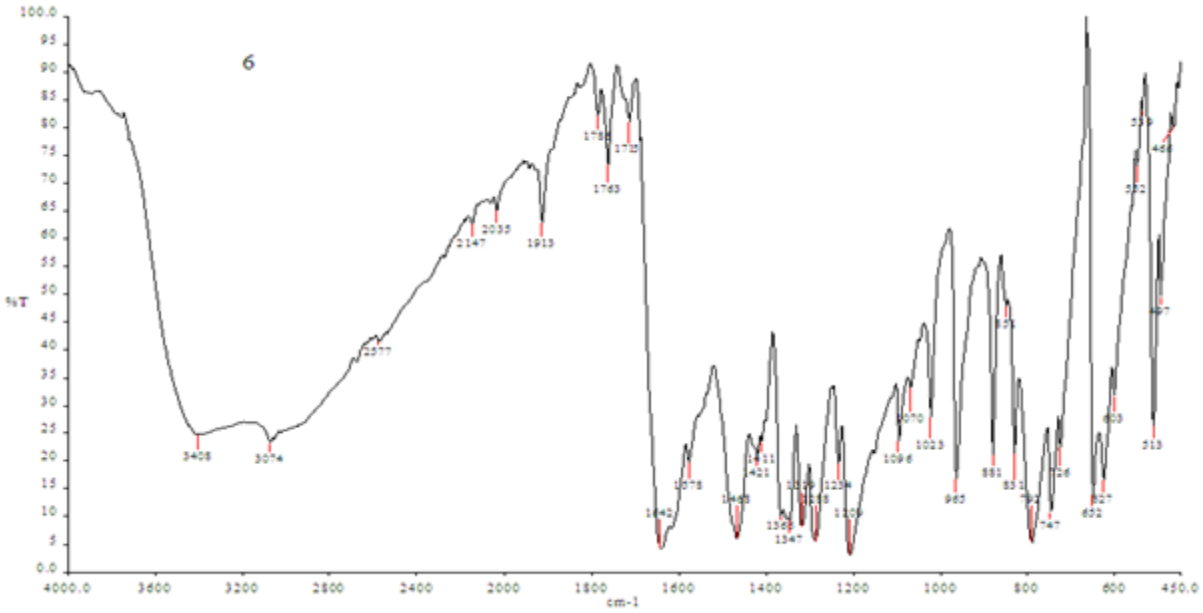


Fig. 1: FTIR (KBr) ν cm^{-1} of 2-acetyl-4-chlorophenyl pentafluorobenzoate (BA)

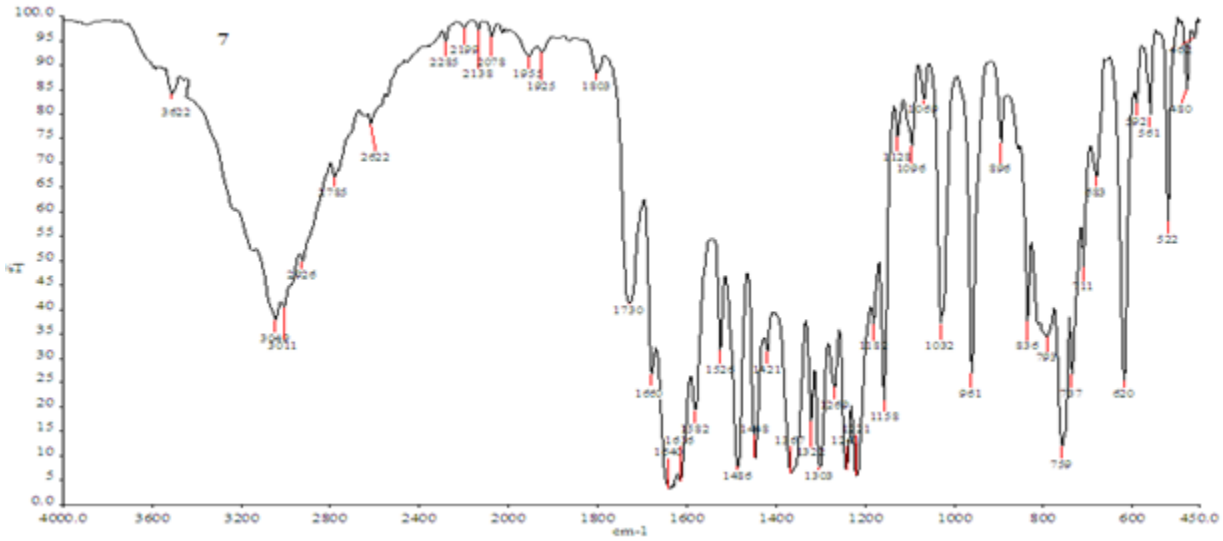


Fig. 2: FTIR (KBr) ν cm^{-1} of 1-(5-chloro-2-hydroxyphenyl)-3-(pentafluorophenyl) propane-1,3-dione (BB)

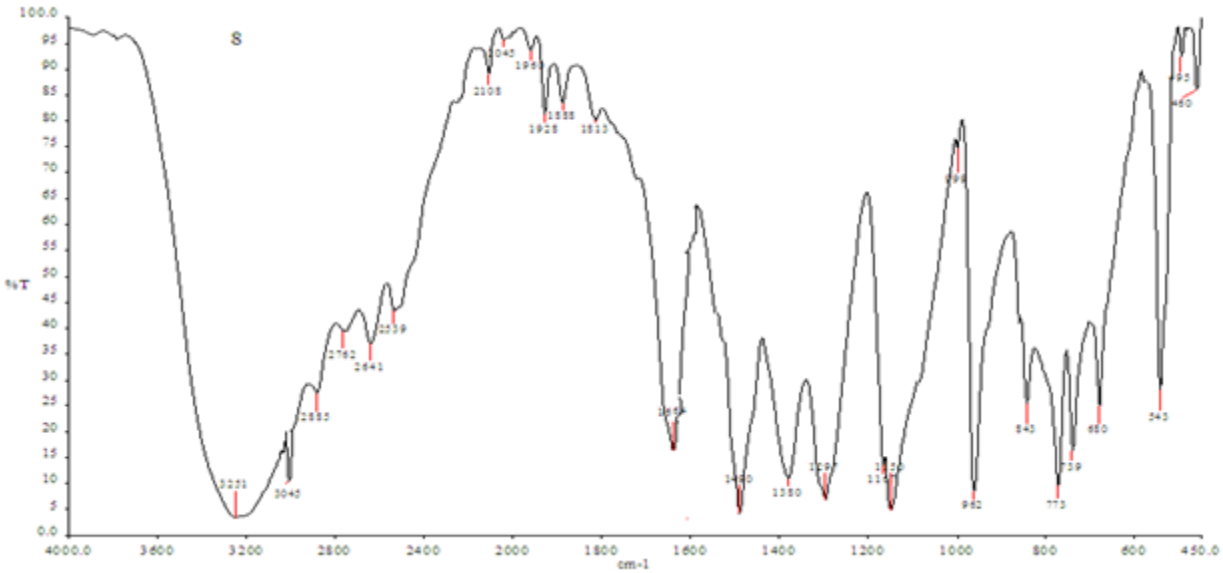


Fig. 3: FTIR (KBr) ν cm^{-1} of 6-chloro-2-(pentafluorophenyl)-4*H*-chromen-4-one (BC)

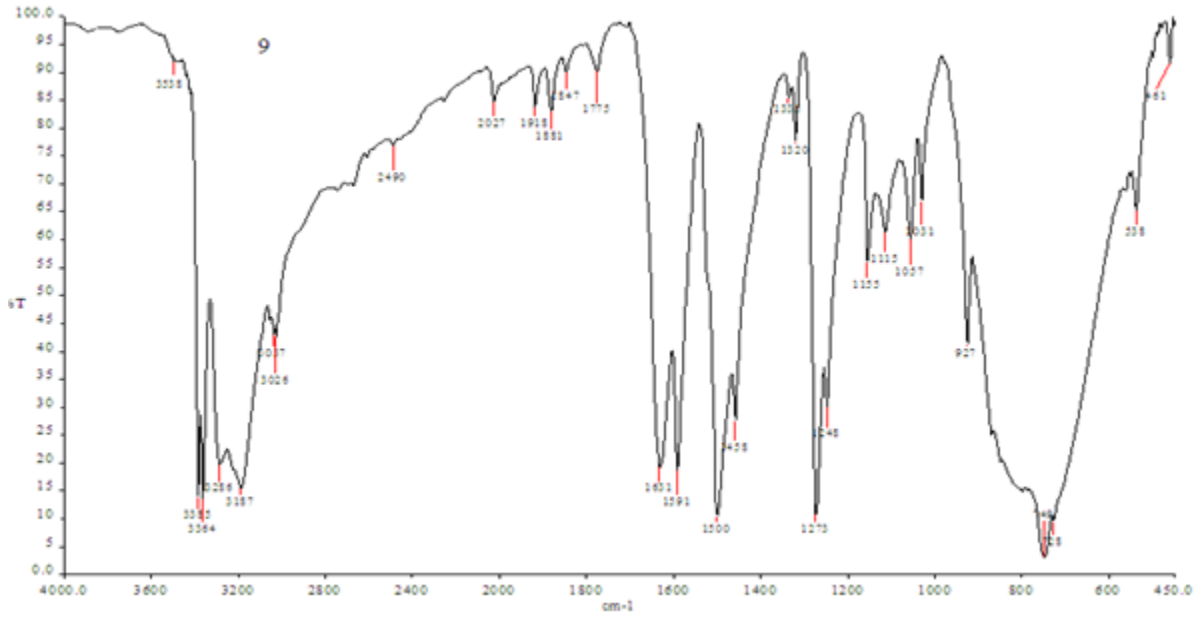


Fig. 4: FTIR (KBr) ν cm^{-1} of 4-chloro-2-[5-(pentafluorophenyl)-1*H*-pyrazol-3-yl] phenol (BD)

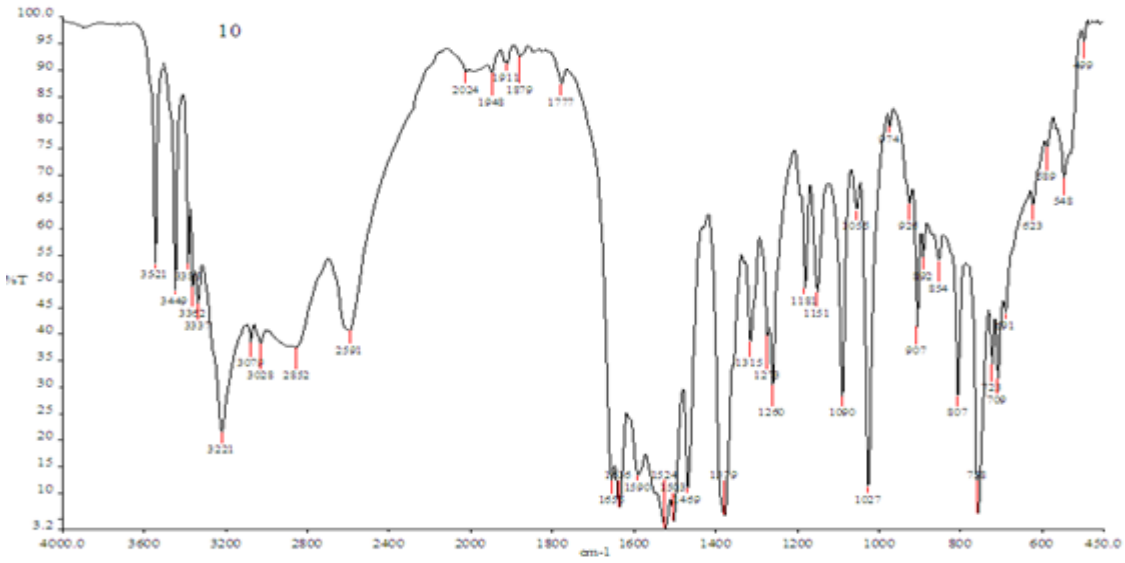


Fig. 5: FTIR (KBr) ν cm^{-1} of 4-chloro-2-[2-imino-6-(pentafluorophenyl)-1, 2-dihydropyrimidin-4-yl] phenol (BE)

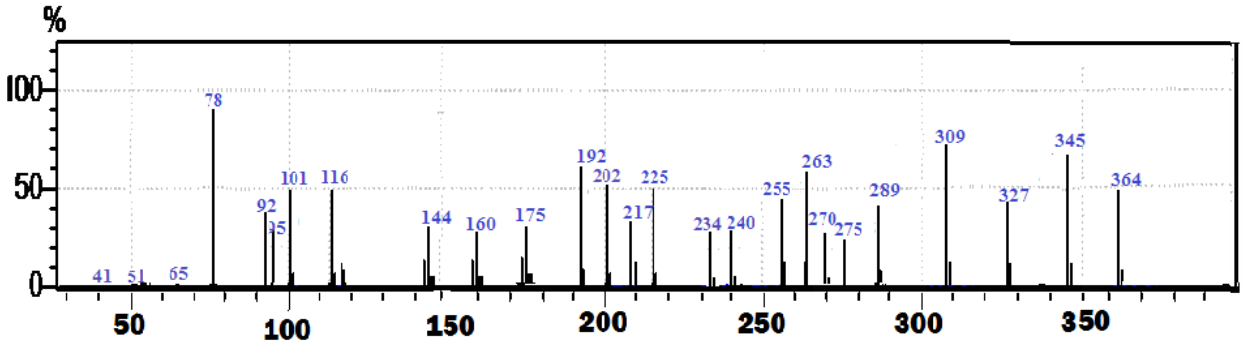


Fig. 6: Mass spectrum of 2-acetyl-4-chlorophenyl pentafluorobenzoate (BA)

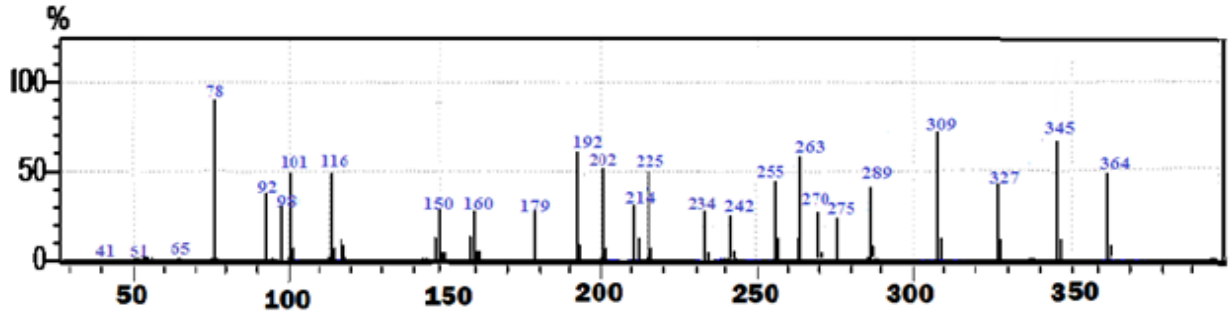


Fig. 7: Mass spectrum of 1-(5-chloro-2-hydroxyphenyl)-3-(pentafluorophenyl) propane-1,3-dione (BB)

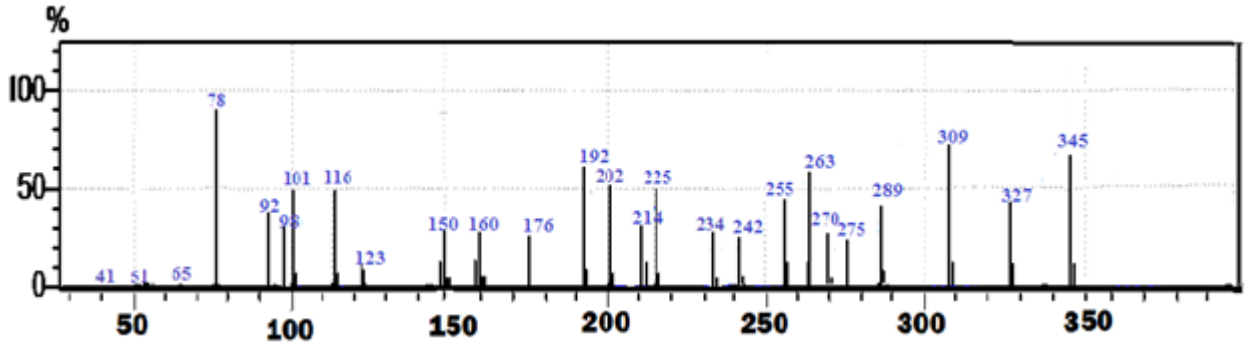


Fig. 8: Mass spectrum of 6-chloro-2-(pentafluorophenyl)-4*H*-chromen-4-one (BC)

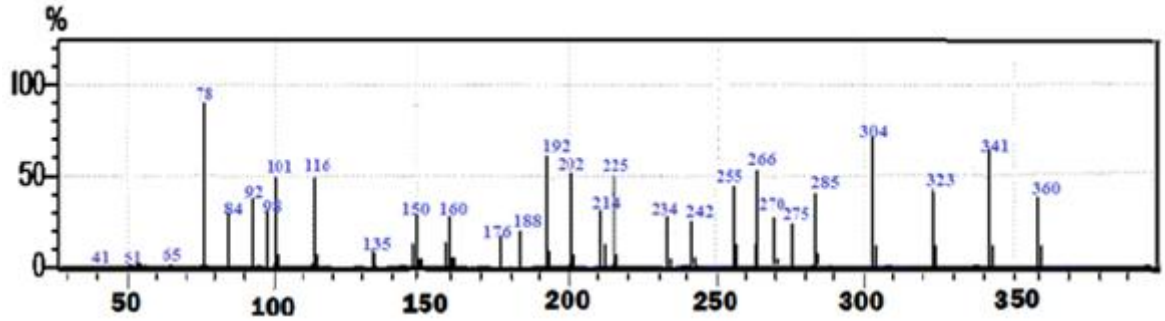


Fig. 9: Mass spectrum of 4-chloro-2-[5-(pentafluorophenyl)-1*H*-pyrazol-3-yl] phenol (BD)

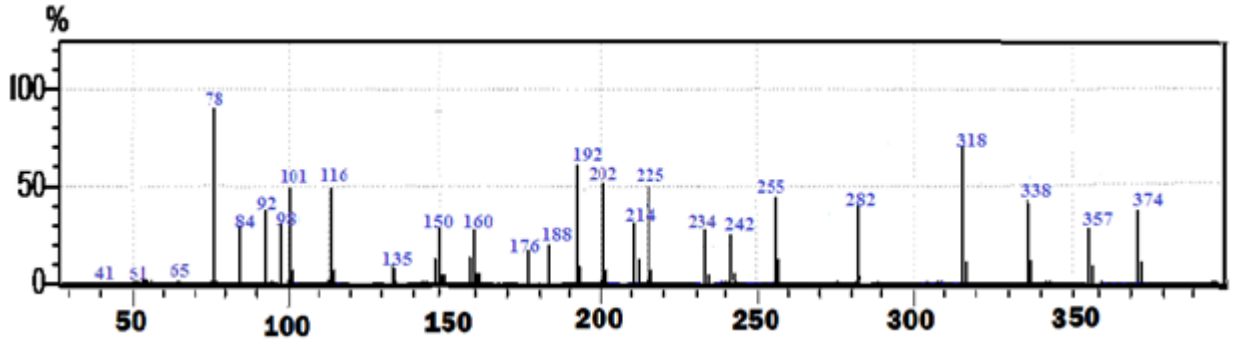


Fig. 10: Mass spectrum of 4-chloro-2-[2-imino-6-(pentafluorophenyl)-1, 2-dihydropyrimidin-4-yl] phenol (BE)

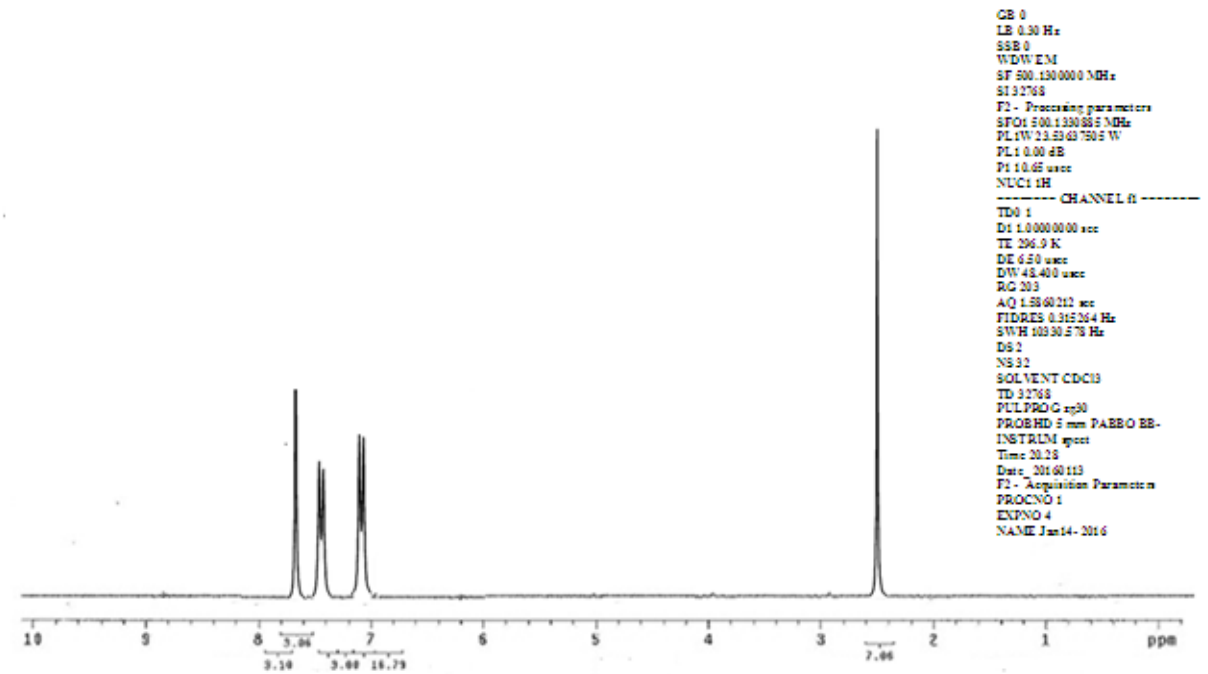


Fig. 11: ^{1}H-NMR of 2-acetyl-4-chlorophenyl pentafluorobenzoate (BA)

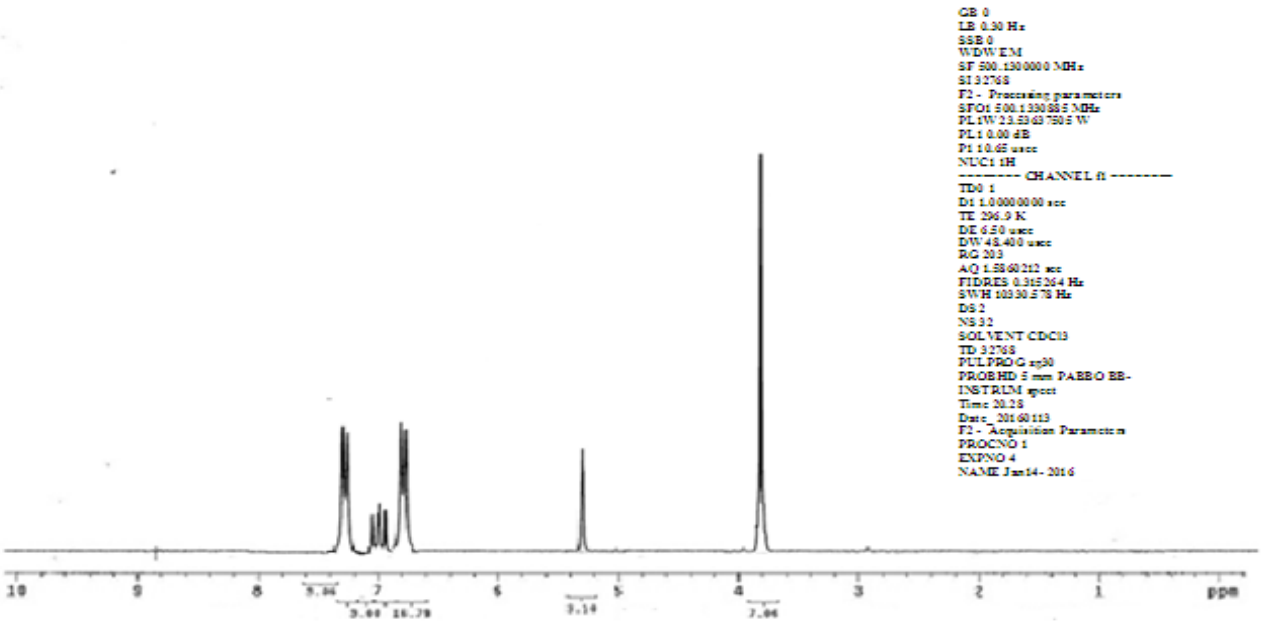


Fig. 12: ^{1}H-NMR of 1-(5-chloro-2-hydroxyphenyl)-3-(pentafluorophenyl) propane-1,3-dione (BB)

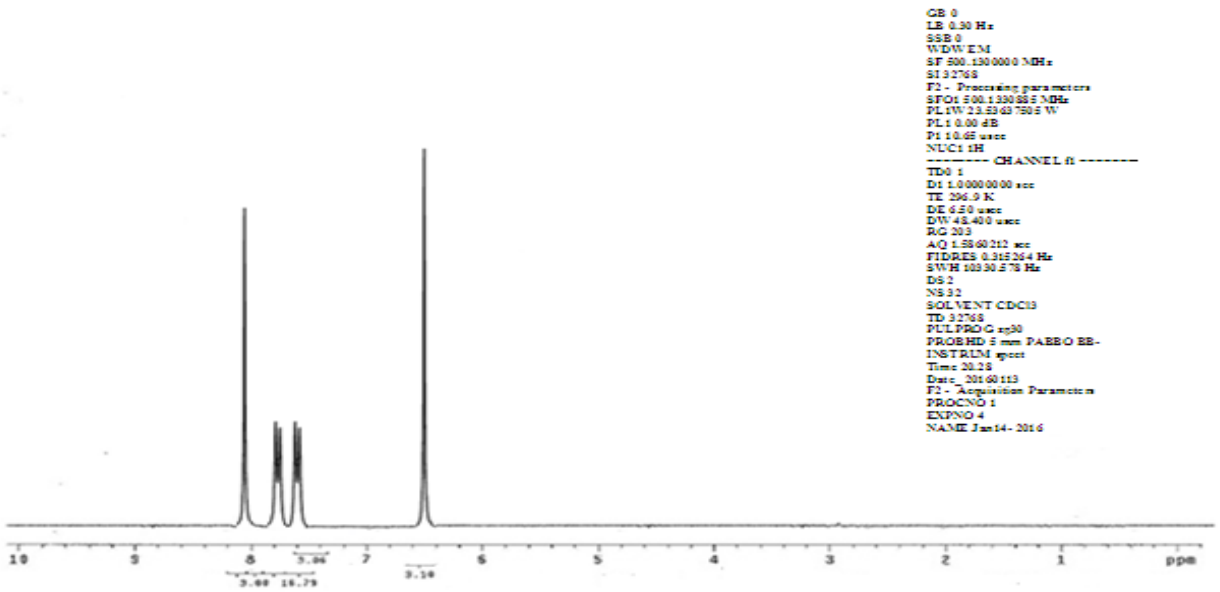


Fig. 13: ^{1}H-NMR of 6-chloro-2-(pentafluorophenyl)-4*H*-chromen-4-one (BC)

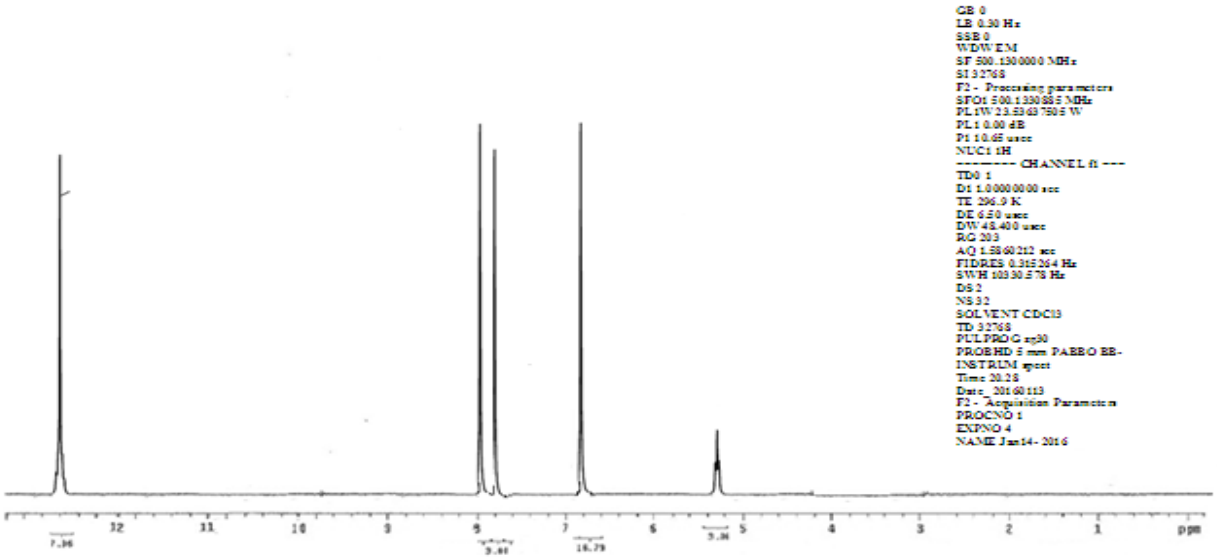


Fig. 14: ^{1}H-NMR of 4-chloro-2-[5-(pentafluorophenyl)-1*H*-pyrazol-3-yl] phenol (BD)

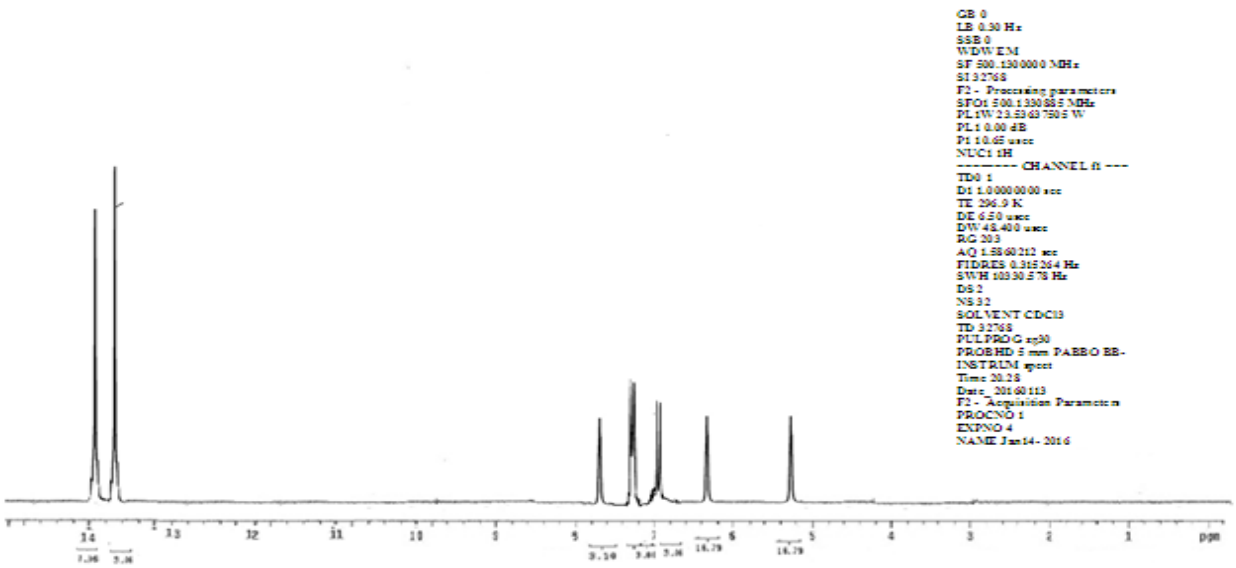


Fig. 15: ^{1}H-NMR of 4-chloro-2-[2-imino-6-(pentafluorophenyl)-1, 2-dihydropyrimidin-4-yl] phenol (BE)

PHARMACOLOGICAL ACTIVITY[8]:

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

i) Antibacterial Activity:

The compounds like BA to BE 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 24h 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
BA	12	17	12
BB	08	19	19
BC	13	20	19
BD	14	22	20
BE	14	21	21
Gentamycin	20	36	28

RESULTS AND DISCUSSION

The synthesis of compounds BA- BE were undertaken as per the scheme 1. The required 2-acetyl-4-chlorophenyl pentafluorobenzoate (BA) was prepared by the action of 1-(5-chloro-2-hydroxyphenyl) ethanone and Pentafluorobenzoic 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 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 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 like BC, BD and BE having the side chain showed higher activity than BA and BB against *S. aureus.* The replacement of oxygen to nitrogen resulted in a slightly increased antimicrobial activity. Our study revealed that all the compounds give stronger antibacterial activity against Gram positive bacteria when compared to Gram negative bacteria.

Antimicrobial activity revealed that newly synthesized compound BC, BD and BE showed good significant activity. The results of the preliminary antimicrobial testing of the prepared compounds, the typical broad spectrum antibacterial drug like Gentamycin (100µg/mL) were shown in Table 1.

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

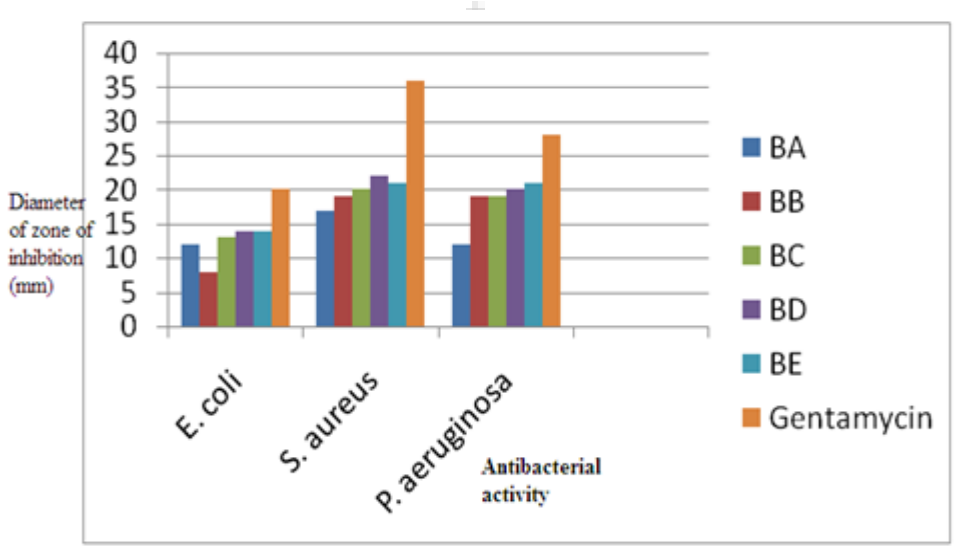


Fig. 16: Antibacterial activity of 2-acetyl-4-chlorophenyl pentafluorobenzoate derivatives

CONCLUSION

Various 2-acetyl-4-chlorophenyl pentafluorobenzoate (BA) was synthesized from the action of 1-(5-chloro-2-hydroxyphenyl) ethanone and Pentafluorobenzoic acid. The structure 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.

ACKNOWLEDGEMENT

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REFERENCES

1. J. A. Joule and K. Mills Heterocyclic Chemistry, Backwell publisher, Germany, 4th edition, 2000; 237,255.
2. R. K. Bansal, Heterocyclic Chemistry, New age international publisher, New Delhi, 4th edition, 2008; 152-159.
3. V. K. Ahluwalia R. K. Parashar,Organic Reaction Mechanism, Narosa publishing house, New Delhi, 3rd edition, 2007; 361.
4. Jamal Abdul Nasser, Synthesis of some new pyrrole derivatives and their antimicrobial activity, Der Pharma Chemica, 2011, 3 (4): 210-218
5. Jamal Abdul Nasser, Synthesis of some new pyrrole derivatives and their antimicrobial activity, Der Pharma Chemica, 2011, 3 (4): 210-218
6. Prativa B. S. Dawadi, Synthesis of Biologically Important Pyrrole Derivatives in Any 13C and 15N Isotope Enriched Form, Global Journal of Science Frontier Research Chemistry Volume 12 Issue 2 Version 1.0 February 2012,24-3
7. Ming-Chang P. Yeh, Synthesis of Pyrrole Derivatives Mediated by Dicobalthexacarbonyl, Tetrahedron Letters,Vol. 36, No. 16, pp. 2823-2826, 1995
8. M. S. Mohamed et al. Synthesis of certain pyrrole derivatives as antimicrobial agent, *Acta pharm*.2009; 59, 145-158.
9. M. S. Mohamed et al. New condensed pyrrole of potential biological interest Synthesis and structure activity relationship studies, *European Journal of Medicinal chemistry*, 2011; 46, 3022-3029.
10. M. A. Chowdhury,. et al. (2008), Synthesis of new 4-[2-(4-methyl (amino) sulfonylphenyl)-5-trifluoromethyl-2H-pyrazol-3-yl]-1,2,3,6-tetrahydropyridines: A search for novel nitric oxide donor anti-inflammatory agents,
11. *Bioorganic & Medicinal Chemistry*, 16, pp.8882–8888
12. M. A. Chowdhury,. et al. (2008), Synthesis of new 4-[2-(4-methyl (amino) sulfonylphenyl)-5-trifluoromethyl-2H-pyrazol-3-yl]-1,2,3,6-tetrahydropyridines: A search for novel nitric oxide donor anti-microbial agents, *Bioorganic & Medicinal Chemistry*, 16, pp.8882–8888
13. B. C. Daniel, et al. (2008), Non-steroidal Anti-microbial Agents – Benefits and New Developments for Cancer Pain , *Supportive Oncology*, pp. 18-22.
14. J.E Downing, et a.l (2005) Gastric and thymic assay of acute oral treatment of rats with nitric oxide esters of Gentamycin or indomethacin, *Biochem. Biophys. Res. Commun.*
15. O. O. Fadeyi, et al (2004) Antipyretic, analgesic, anti-inflammatory and antimicrobial effects of four derivatives of salicylic acid and anthranilic acid in mice and rats, *African Journal of Biotechnology*, 3(8), pp. 426-431
16. S. Fiorucci, J. L. Wallace, R. N. Dubois, et al.(2000) New antimicrobial agents: NO-NSAIDS and COX-2 inhibitors. *International conference on "Advances In Prostaglandin and Leukotriene Research: Basic Science and New Clinical Applications",* June (4-8), pp. 2.
17. S. Fioruccia, *et al.*(2004) Cooperation between 2-(Acetyloxy) benzoic acid 3-(nitrooxymethyl) phenyl ester (NCX-4016) Nitric oxide aspirin on neutrophile endothelial cell dherence, *The Journal of Pharmacology and Experimental Therapeutics*, 306, pp.1174-1182
18. W. (Foye, 2009,) *Principles of Medicinal Chemistry*. New Delhi: B. J. Waverly Pvt. Ltd., 4, pp.-1021-1023.
19. J.F. Gilmer, et al. (2001) Synthesis, hydrolysis kinetics and antimicrobial effects of isosorbide mononitrate derivatives of Gentamycin, *Euopean journal of Pharmaceutical Sciences*, 14, pp. 221-7.

20. R. A. Khaled, et al. (2007) Novel (E)-2-(aryl)-3-(4-methanesulfonylphenyl)acrylic ester prodrugs possessing a diazen-1-ium-1,2-diolate moiety: Design, synthesis, cyclooxygenase inhibition, and nitric oxide release studies, *Bioorganic & Medicinal Chemistry*, 15, pp. 6796–6801.