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[Research article]

Synthesis, Characterization and Biological evaluation of substituted Pyrazole derivatives

[*1]Siddiqui Shakeel Ahmed, [2]Sudheendra G, [1]Md.Younus Ali, [1]Md.Hasnuddin.

[1] KCT College of Pharmacy, Gulbarga. Karnataka, India.

[2] Luqman College of Pharmacy, Gulbarga. Karnataka, India.

ABSTRACT

The work presented in this article consists of synthesis, characterization and biological evaluation of substituted pyrazole derivatives. Pyrazole derivatives have been shown to have wide variety of pharmacological activities like antimicrobial, antiviral, antihistaminic, antitumor, antipyretic, anti-inflammatory, antidepressant and anticonvulsant. As combination of biologically active moieties into one molecule and synthesis of totally newer moieties have been the methods of research, we present here synthesis of some novel pyrazole derivatives incorporating various biologically active aryl/aryloxy acid derivatives such as ibuprofen, diclofenac, aceclofenac as well as potent antibacterial quinolones, norfloxacin and ciprofloxacin. All the compounds synthesized were evaluated for their antimicrobial activity.

Keywords: Pyrazoles, anti-inflammatory, anti-microbial, cup-plate method, quinolones, derivative

INTRODUCTION

A considerable amount of research activity is directed towards the synthesis of potent, more specific and less toxic compounds. Substituted pyrazoles have received considerable attention during last few decades as they are endowed with variety of biological activities and have wide range of therapeutic properties. A Literature survey indicates that pyrazole derivatives possess different pharmacological and biological activities; which of most potent activity are anti-microbial and anti-inflammatory activities.

In the forgoing survey of literature, it is seen that the drug design by molecular manipulation is a productive source of new drugs. Synthesis of compounds to explore the potential biologically active agents still draws continued interest. Pyrazole derivatives have been shown to have wide variety of pharmacological activities like antimicrobial[1], antiviral[2,3], antihistaminic[4], antitumor[5], antipyretic[6], anti-inflammatory[7], antidepressant[8] and anticonvulsant[9]. Molecular manipulation, combination of biologically active moieties into one molecule and synthesis of totally newer moieties have been the methods of approach. Hence, we present here synthesis of some novel pyrazole derivatives incorporating various biologically active aryl/aryloxy acid derivatives such as ibuprofen, diclofenac, aceclofenac as well as potent antibacterial quinolones, norfloxacin and ciprofloxacin.

MATERIALS AND METHOD

General method of preparation of hydrazide I (a-h)

The mixture of aryl/aryloxy acid (R) (0.1mol) and ethanol (50ml) were taken with a few drop of

*** Corresponding author: Siddiqui Shakeel Ahmed**
E-mail address: siddiquishakeel.313@gmail.com

concentrated sulphuric acid and it was refluxed for 6 hours. The reaction mixture was concentrated by distilling off the excess of ethanol under reduced pressure and treated with a saturated solution of sodium bicarbonate. The ester obtained was used for the preparation of hydrazides directly. The ester (0.1 mole) was dissolved in appropriate quantity of ethanol and to this hydrazine hydrate (0.1 mole) was added. The reaction mixture was taken in a round bottomed flask and refluxed for a period of 12-18 hours. Excess of ethanol was distilled off under reduced pressure. It was then poured into ice cold water and the solid obtained was filtered. It was recrystallised from suitable solvent.

The following hydrazides were prepared.

- 2-hydroxy-1-benzenecarbohydrazide
- 2-phenylethanohydrazide
- 2-Pheoxyethano hydrazide
- 2-(4-isobutylphenyl)propanohydrazide
- 1-ethyl-6-fluoro-4-oxo-7-piperazino-1,4-dihydro -3-quinoline
- carbohydrazide
- 2-[2-(3,5-dichloroanilino)phenyl]ethanohydrazide
- 2-hydrazino-2-oxoethyl2-[2-(2,6-dichloroanilino)phenyl]acetate
- 1-cyclopropyl-6-fluoro-4-oxo-7-piperazino-1,4-dihydro-3-
- quinolinecarbohydrazide.

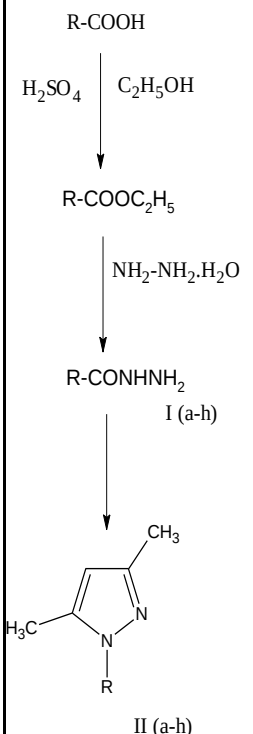
Preparation of 3, 5-dimethyl-1 *H*-1-substituted pyrazoles II (a-h)

The equimolar quantities of hydrazides I (a-h) and acetyl acetone was refluxed in methanol (25ml) containing few drops of concentrated HCl for 5-6 hours on water bath. The reaction mixture was cooled to room temperature and the solid separated was filtered, washed with petroleum ether, dried and recrystallized from suitable solvent.

EXPERMENTAL WORK

Pyrazoles derivatives are synthesized as shown in the scheme in figure 1. Melting Points were determined by using Toshniwal apparatus in open capillaries and are corrected. The purity of the compounds were checked by TLC on silica gel G plates using n-butanol, ethyl acetate (1:3) solvent system and UV lamp was used as a visualizing agent. IR spectra were recorded using KBr pellets on a Jasco FT/IR 5300 series spectrophotometer. ^{1}H NMR Spectra on an Avance 300MHZ spectrophotometer using DMSO d_6 as solvents and TMS as internal standard (chemical shift values are expressed in δ ppm). Mass Spectra were recorded by LCMS technique on a liquid chromatography mass spectrophotometer.

SCHEME:-



IIa -(2-hydrophenyl)methanone

IIb -2-phenyl-1-ethanone

IIc -2-phenoxy-1-ethanone

IId -2-(4-isobutylphenyl)-1-ethanone

IIe -3-carbonyl-1ethyl-6-fluro-7-piperazino-1,4-dihydro-4-quinolinone.

IIf -2-[2-(3,5-dichloroanilino)phenyl]-1-ethanone.

IIg -2-oxoethyl-1-{2-[2-(2,6-dichloroanilino)phenyl]}acetate.

IIh -[(1-cyclopropyl-6-fluro-7-piperazino)-3-carbonyl]-1,4-dihydro-4-quinolinone.

TABLE 1: Physical characteristic data of intermediates I(a-h):

Sr.No.	Compound code	R	Molecular formula	Mol.Wt	Melting point	Yield %
1	Ia	Salicyclic acid	$C_{17}H_8N_20_2$	152.152	$_{178}{}^0C$	70
2	Ib	Phenyl acetic acid	$C_8H_{10}N_20$	150.179	$_{121}{}^0C$	73
3	Ic	Phenoxy acetic acid	$C_8H_{10}N_20_2$	166.178	$_{110}{}^0C$	74
4	Id	2(4-isobutyl phenyl)Propionic acid	$C_{13}H_{20}N_20$	220.313	$_{72}{}^0C$	68
5	Ie	1-Ethyl-6-fluro-1,4dihydro -4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid	$C_{16}H_{22}FN_50_2$	335.380	$_{222}{}^0C$	65
6	If	[o-(2,6-dichloroanilino)phenyl]acetate	$C_{14}H_{13}\ Cl_2N_30$	310.182	$_{104}{}^0C$	60
7	Ig	2-({2-[(2,6-dichloroanilino)phenyl]acetyl}-oxy)acetic acid	$C_{16}H_{15}Cl_2N_30_3$	368.218	$_{145}{}^0C$	76
8	Ih	1-cyclopropyl-6-fluro-1,4dihydro-4-oxo-7-(1-piprazinyl)-3-quinolinecarboxylic acid	$C_{17}H_{22}FN_50_2$	347.391	$_{265}{}^0C$	70

TABLE 2: Physical characteristic data of synthesized compounds II (a-h)

Sr. No.	Compound Code	R	Molecular Formula	Mol. wt	Melting point	Yield %	Rf
1	IIa	-(2-hydrophenyl)methanone	$C_{12}H_{12}N_20_2$	216.238	$_{154}{}^0C$	75	0.51
2	IIb	-2-phenyl-1-ethanone	$C_{13}H_{14}N_20$	214.268	$_{227}{}^0C$	72	0.63
3	IIc	-2-phenoxy-1-ethanone	$C_{13}H_{14}N_20_2$	230.265	$_{135}{}^0C$	67	0.58
4	IId	-2-(4-isobutylphenyl)-1-ethanone	$C_{17}H_{22}N_20$	270.373	$_{220}{}^0C$	77	0.49
5	IIe	-3-carbonyl-1ethyl-6-fluro-7-piperazino-1,4-Dihydro -4-quinolinone.	$C_{21}H_{24}F\ N_50_2$	397.451	$_{245}{}^0C$	80	0.53
6	IIf	-2-[2-(3,5-dichloroanilino)phenyl]-1-ethanone.	$C_{19}H_{17}\ Cl_2N_30$	374.268	$_{177}{}^0C$	70	0.51
7	IIg	-2-oxoethyl-1-{2-[2-(2,6-dichloroanilino)phenyl]} acetate.	$C_{21}H_{19}\ Cl_2N_30_3$	432.304	$_{145}{}^0C$	74	0.76
8	IIh	-[(1-cyclopropyl-6-fluro-7-piperazino)-3-carbonyl] -1,4-dihydro-4-quinolinone.	$C_{22}H_{24}F\ N_50_2$	409.462	$_{269}{}^0C$	72	0.47

Anti-microbial Activity[10,11]

Antibacterial and antifungal activities is carried out by cup-plate method, using *Pseudomonous aeruginosa (ATCC-27853), Escherichia coli (ATCC-25923), Enterococcus Fecalis (ATCC-29212) and Bacillus substilis* organisms for antibacterial activity using Amoxycillin as a standard drug and *Aspergillus niger, Aspergillus flavus* organism for antifungal activity using clotrimazole as a standard drug. The antimicrobial potency of the synthesized compounds was determined against standard drug by measuring the zone of inhibition at the concentration of 50µg and 100µg respectively.

RESULT AND DISCUSSION

Spectral Data

IIb- Aromatic C-H was absorbed in the form of intense peak at 3100 cm^{-1}, Aliphatic C-H peaks are also obtained from 3032 cm^{-1} to 2843 cm^{-1}. The C=O absorption peak was seen at 1607 cm^{-1}. The ^{1}HNMR spectrum recorded in DMSO D_6 exhibited two identical peaks in the form of singlet at 2.3δ and CH_2 protons absorption has merged with DMSO protons at 3.5δ. The methyl proton and aromatic together have shown multiplet from 7.1δ to 8.3δ. The base peak is observed by Mass spectra is m/z 91.

SPECTRAS

Fig. No. 2 I.R. of Compound IIb

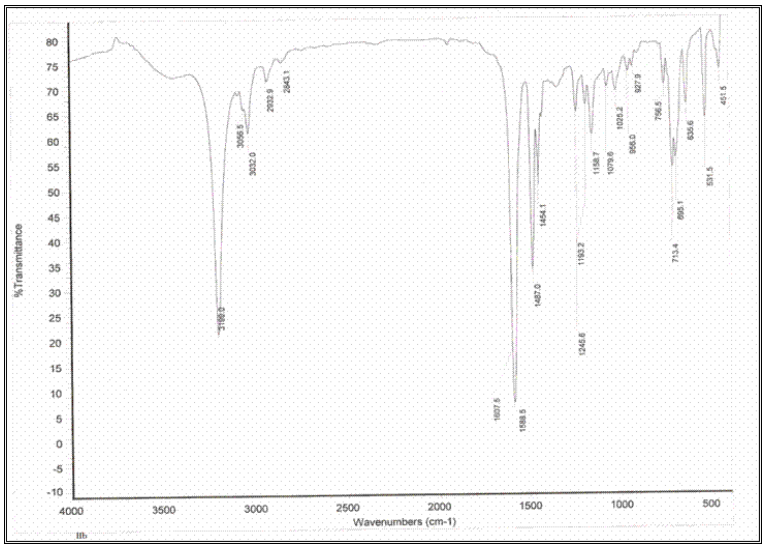


Fig. No.3 NMR. Of Compound IIb

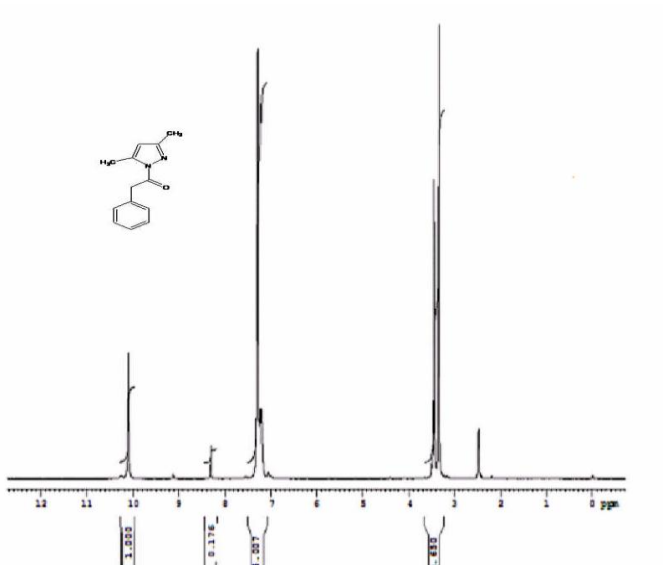


Fig. No. 4 NMR. Of Compound IIb

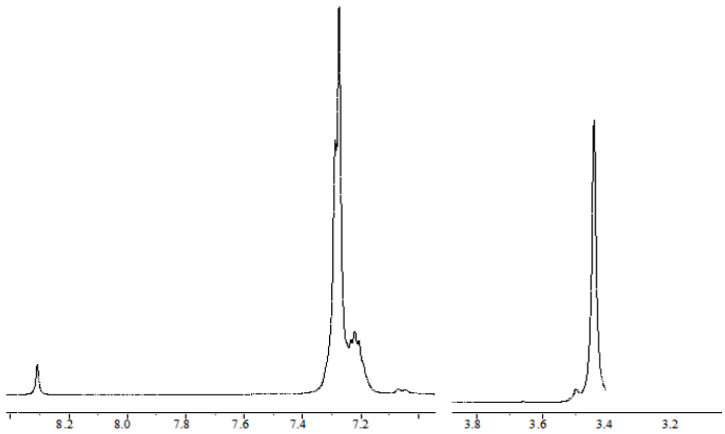
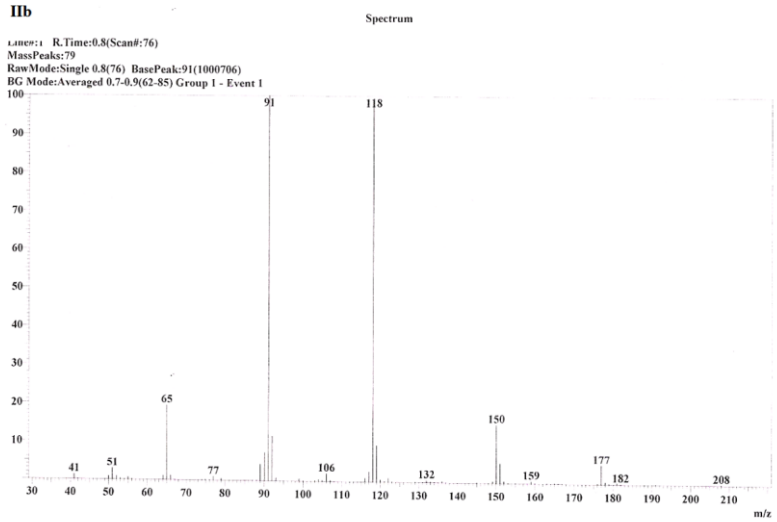


Fig. No. 5 MASS of Compound IIb



IIf-The N-H group present in the molecule sandwich between two phenyl molecules, exhibited a sharp peak at 3323 cm^{-1}, the aromatic and aliphatic C-H have exhibited an absorbance peak from 2854 cm^{-1} to 3078 cm^{-1}. The C=O group present in the molecule in the form of imine exhibited a peak at 1694 cm^{-1}. The ^{1}HNMR spectra of these molecules exhibits a broad peak at 3.3δ due to the presence of two CH_3 protons present in the molecule. The aromatic protons present in the molecule exhibited aromatic cluster from 6.8δ to 7.3δ in the form of a multiplet. The C-H peak of methylene appears to have merged with the aromatic cluster and the methylene protons sandwich between carbonyl group as well as phenyl moiety have been desheilded and gave a peak at 6.8δ. The H of N-H protons was resonated at 7.1δ. These measurement recorded are in concerns with proposed structure of the molecules. The base peak is observed by Mass spectra is m/z 214.

Fig. No. 6 I.R. Of Compound IIf

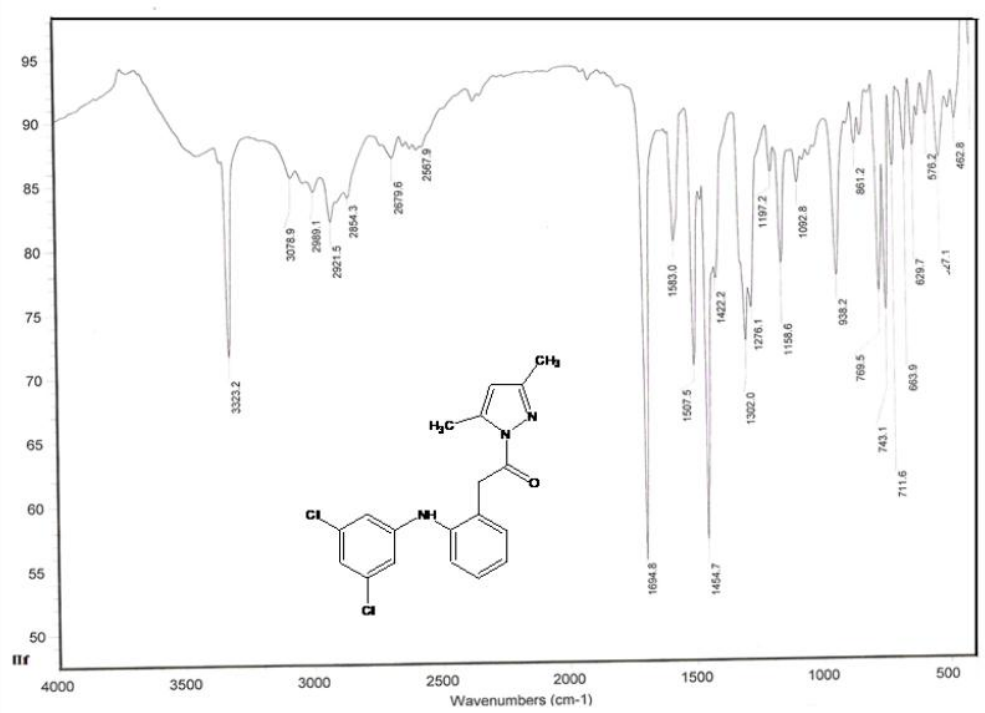


Fig. No. 7 N.M.R. Of Compound IIf

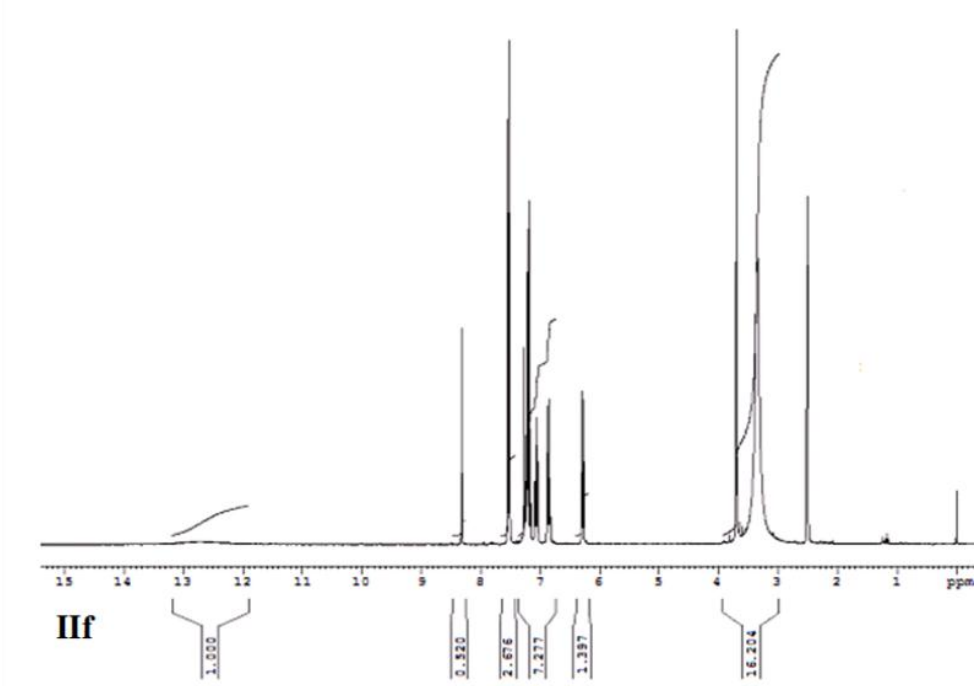


Fig. No. 8 N.M.R. Of Compound IIf

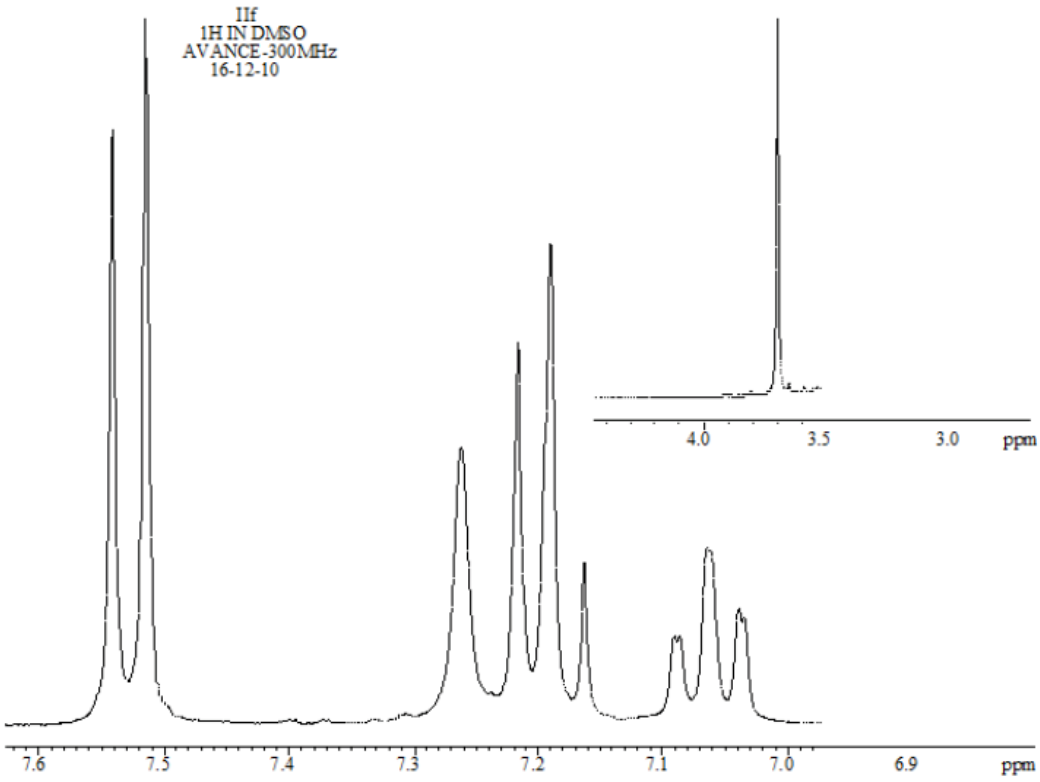


Fig. No. 9 MASS of Compound IIf

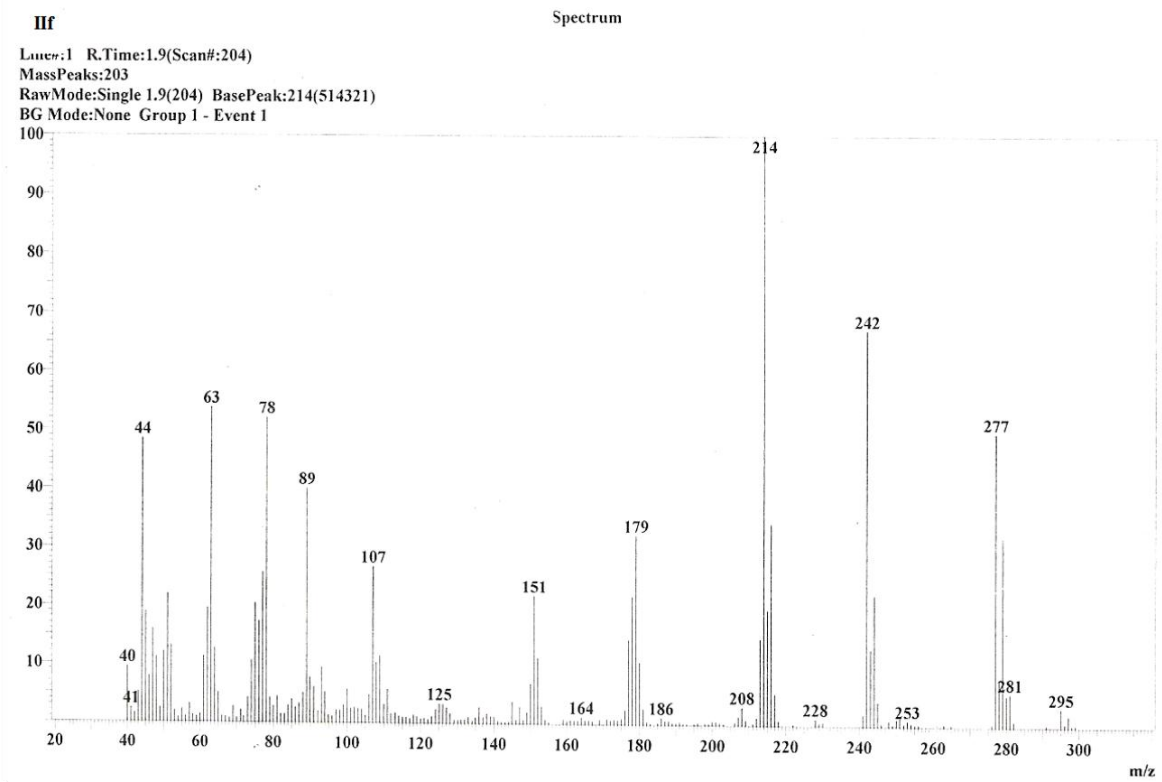


TABLE 3: Antimicrobial activity of newly synthesized Pyrazole derivatives

Sample Code	**Inhibition zone diameter in mm** (Average triplicate ± Standard deviation)											
	P. Aeruginosa		***E. Coli***		***E. Fecalis***		***B. Substilis***		***A. Niger***		***A Flavus***	
	50 µg	**100 µg**	**50µg**	**100 µg**	**50 µg**	**100 µg**	**50µg**	**100 µg**	**50µg**	**100 µg**	**50µg**	**100 µg**
IIa	9	13	11	16	9	11	8	12	7	9	8	10
IIb	13	17	14	17	15	18	14	18	12	15	12	14
IIc	15	18	14	17	15	18	14	18	12	17	14	17
IId	12	13	10	12	11	12	12	13	6	9	7	9
IIe	14	17	15	17	15	18	15	18	14	18	15	17
IIf	14	15	14	15	13	17	13	16	8	11	10	12
IIg	13	14	13	17	15	16	14	15	9	13	11	13
IIh	14	18	15	17	15	18	15	18	12	16	13	17
Amoxicillin	25	28	26	27	26	29	27	29	-	-	-	-
Clotrimazole	-	-	-	-	-	-	-	-	17	20	19	23
DMSO	-	-	-	-	-	-	-	-	-	-	-	-

CONCLUSION

During the present investigation, the new pyrazole derivatives have been successfully synthesized by linking two biologically active moieties, such as anti- inflammatory molecules, Ibuprofen, Diclofenac, Acelofenac and synthetic antibacterial agents like Ciprofloxacin and Norfloxacin with pyrazole moiety. This was done based on the observation that combination of biologically active moieties into one molecule and synthesis of totally newer moieties may result into compounds with improved potency and reduced toxicity. Even though the results obtained reveals that, few of the synthesized pyrazole derivatives are inferior to that of the standard drugs employed, while few of the compounds displayed encouraging results and were found to possess good anti microbial activity. All the above results establish the fact that, the obtained pyrazole moiety can be a rich source for further exploitation. Hence, in search for new generation of drugs with high potency, selectively and reduced toxicity, it may be worthwhile to explore the possibility in this area by fusing different moieties. If suitably exploited it may results in better compounds.

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