

A Synthesis of Substituted 1,3,4-oxadiazole, pyrazole derivatives and its biological activities

ABSTRACT

Purpose: Nicotine Substituted 1,3,4 Oxadiazole and pyrazole moieties were integrated by utilizing hydrazide as half way. Oxadiazole were acquired by two techniques one is by utilizing POCl_3 within the sight of fragrant corrosive, other is by utilizing carbon disulphide in the presence of potassium hydroxide as an impetus. Pyrazoles were likewise gotten by ethylacetoacetate we acquired unrefined item and again it was recrystallized by utilizing alcohol. All incorporated mixtures were evaluated for Antibacterial activity and enaminones showed promising action against standard medication ciprofloxacin.

Methods : TLC on silica gel G was used to check for homogeneity of the title compound. H NMR, Mass, and IR Spectra were used to characterise these compounds, and their antibacterial activity was tested.

Results : All of these compounds, including nicotine substituted ester, hydrazide, 1,3,4 Oxadiazole, and pyrazole, showed antibacterial activity. The maximum activity of enaminones was comparable to that of the standard drug ciprofloxacin (5mcg). Compound 1 showed potent antimicrobial activity.

Conclusion : In the zone of inhibition studies, all six samples at MIC concentrations showed reasonable antimicrobial activity. Compound 1 showed very good antimicrobial activity.

Keywords: Enaminones, Nicotinic Hydrazide, Nicotinic Substituted Oxadiazole, Nicotinic Substituted Pyrazole.

1. Introduction

Among the wide range of heterocycles Oxadiazole and pyrazole have been explored to develop new therapies [1-7]. Pyrazole and oxadiazole have attracted lot of attention to medical chemists. Wide spread use of Oxadiazole and pyrazole scaffolding a medical use.

Oxadiazoles are five-membered heterocyclic compounds containing one oxygen and two nitrogen atoms (historically, they were also known as furadiazoles). Depending on the position of nitrogen atoms, oxadiazoles may occur in the form of four different isomers: 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole and 1,3,4-oxadiazole (Figure 1). Amongst the isomers, the greatest interest is involved with 1,3,4-oxadiazoles. Their high importance is highlighted by a large number of applications in various scientific areas, e.g.,

pharmaceutical industry, drug discovery, scintillating materials as well as dyestuff industry [8]. It is also worth noting that compounds containing 1,3,4-oxadiazole unit exhibit a wide range of biological activities such as anticancer, antiparasitic, antifungal, antibacterial, antidepressant, anti-tubercular and anti-inflammatory [9].

According to the Web of Science data the scientific attention of 1,3,4-oxadiazoles application is continuously rising since the year 2000. On the other hand, 1,2,5-oxadiazole derivatives found application mainly as High Energy Density Materials (HEDMs) as well as biologically active compounds with cytotoxic properties. Due to the instability and ring-opening of 1,2,3-oxadiazole heterocycle, resulting in substituted diazomethanes formation, this isomer of oxadiazole is least of all explored.

Importantly, the heterocycle demonstrates bioisosteric equivalence with ester and amide moieties due to the possibility of creation specific interaction (e.g., hydrogen bonding). It is a particularly useful alternative when the instability of those groups is observed (e.g., when the hydrolysis may appear) [10]. Nowadays, there are a few commercially available drugs containing 1,2,4-oxadiazole nucleus such as Oxolamine, Prenoxdiazine (cough suppressant) Butalamine (vasodilator), Fasiplon (nonbenzodiazepine anxiolytic drug, Pleconaril (antiviral drug), Ataluren (Duchenne muscular dystrophy treatment drug and Proxazole a drug used for functional gastrointestinal disorders. It is worth noting that 1,2,4-oxadiazole ring, as the only one of all oxadiazole isomers, occurs in the structures of natural products. For example, in 2011 Carbone M. et al. isolated two indole alkaloids Phidianidine A and Phidianidine B from sea slug Opisthobranch Phidiana militaris [11].

As a result, oxadiazole has a diverse set of biological properties, including antibacterial activity, [12] antifungal [13] antitumor [14] antitubercular, [14] anticonvulsant [15] and HIV-I inhibitory activities. [16]

The same range of medicinal significance is associated with compounds containing pyrazole. This article is an attempt to synthesize five membered heterocycles by using hydrazide bridge.

2. MATERIALS & METHODS

The open capillary method was used to determine the melting points, and the results are uncorrected. TLC on silica gel G was used to check for homogeneity of the compounds. Synthesized compounds were confirmed by ¹HNMR, Mass and IR spectra.

General procedure

General procedure Synthesis of Acid hydrazide

In a one pot three-component heterocyclo condensation process, ethyl 2-methyl-6- ethyl nicotinate was obtained via the reaction of Enaminons again it react with ethylene acetoacetate and ammonium acetate in refluxing acetic acid and it is achieved via the hydrazinolysis of ester derivatives with refluxing hydrazine hydrate. [17]

Synthesis of compound 4:

A mixture of (0.01) acid hydroxide and (0.01) benzoic acid was dissolved in phosphorus oxide chloride and 18 to 22hrs . The reactive mixture was slowly poured onto crushed ice and stored all night. The separated solid mass was filtered, dried and purified by recrystallisation with ethanol.[18]

Synthesis of Compound 5:

A mixture of 0.01 moles of nicotinic acid hydrazide , (0.01) (0.56 g) of potassium hydroxide and 10 ml of carbon disulphide were relaxed in 50 ml of 95% ethanol for 12 hr. The resulting mixture was concentrated and cooled to RT. Then it was acidified with Dil. Hcl. The solid mass, thus separated out was filtered, dried and purified by recrystallization from ethanol. [19]

Synthesis of Compound 6

A mixture of 0.01 moles of Nicotinic Acid hydroxide and 0.1 moles (13 ml) of ethylaceto acetate was heated in a water bath for 2 hours with stirring from time to time with a glass rod. The resultant heavy reddish syrup was allowed to cool to RT. It was washed thoroughly with ether to remove colored impurities. The separated solid has been filtered, dried and purified by recrystallization using ethanol.[20]

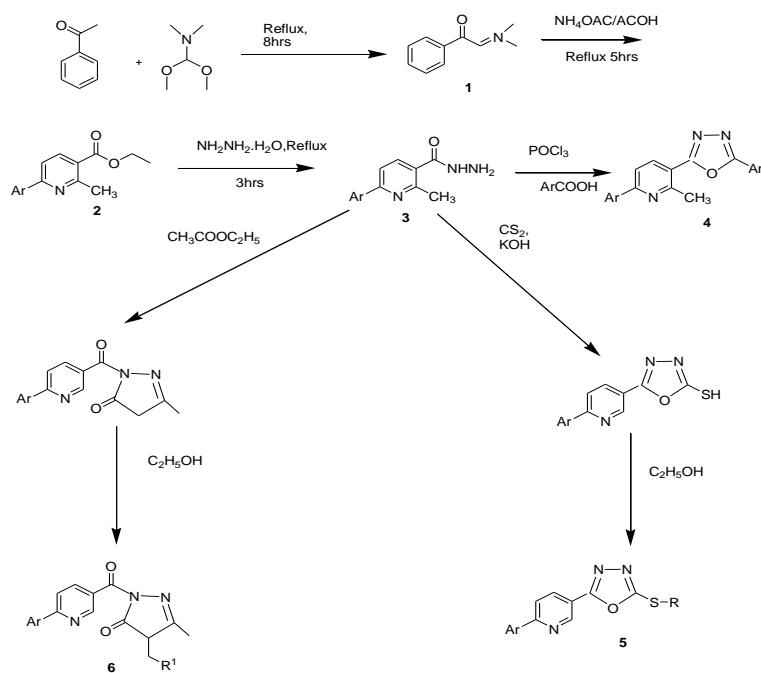


Figure 1: Schematic diagram of synthesis

Determination of Melting point

We utilized recrystallized Samples for the assurance of melting point by Guna apparatus. Firstly melting point Capillaries are loaded up with sample and kept inside a apparatus and noticed the liquefying point of the Sample .

Evaluation of NMR Spectra

Predominantly NMR spectra is utilized to discover the kind of protons present in the sample .Here additionally we utilized recrystallized Sample and TMS is utilized as Reference cores and we dissolved the sample by DMSO and kept in the sample holder and spectra were recorded

Instrument name :Bruker

Evaluation of Mass Spectra

Usually mass spectra is used to recognize obscure mixtures in a sample , and to clarify the design and compound properties of various atoms.of the sample .Here we used crude product for the experiment .

Instrument Used :Thermo Fisher Exactive Plus EMR Orbitrap LC-MS

Evaluation of IR spectra

It is used to find the functional group present in the sample.Here instrument used is Thermo Fisher Nicolet IS5 FTIR Spectrometer

Evaluation of antibacterial activity

Determination of minimum inhibitory concentration by tube dilution method

Antibacterial activity as MIC is evaluated by tube dilution method. The double strength Nutrient broth was prepared and sterilized. Then various concentrations of stock solution from 100 mcg/ml to 1.56 mcg/ml is prepared by serial dilution method. Take 2.5 ml of double strength nutrient broth in a sterile test tube add 2.5 ml of 200 mcg/ml soln of synthesised compound which makes the first solution as 100 mcg/ml of compound C1 mix the test tube well. same wise make the next test tube of 50 mcg/ml and label as C2 same way prepare the next 5 dilutions and add double strength nutrient broth.

Then Microorganism was inoculated *E. coli* in all test tubes were incubated at 37°C for 48h[22]

Strain used is *Escherichia coli* ATCC 25922

Determination of zone of inhibition by cup-plate method

Escherichia coli used for the determination of the antimicrobial activity of various synthesized compounds and standard cup plate methods used for the study. The nutrient agar solution Poured into the previously sterilized Petri dishes. Microorganism added to Petri dishes with a sterilized loop and spread on the petri plate by a sterile spreader then plates are allowed to solidify. In each Petri dish, four perforations made with a sterile metal cork borer to receive test samples. The standard ciprofloxacin and test materials added immediately into the wells and kept for incubation at 37°C for 24 h to allow the microorganism to grow and reagents to diffuse through the culture medium. At the end of the incubation, the zone diameter measured with the help of a zone reader [23]. All the experiments repeated 3 times to get accurate results.

Strain used is *Escherichia coli* ATCC 25922

3. Results

Spectral Values of compounds

Compound1:

1. Melting point :245⁰c
2. Yield :70%
3. IR Spectral values (Cm⁻¹): (KBr): 1551, 1563(C=N)
4. ¹H NMR :(DMSO-d⁶): δ 7.21-8.78(m, 9H, Ar-H)
5. Mass spectral values(m/z) (%) : 223 (25), 199 (40), 162 (54), 147 (48), 118 (100)

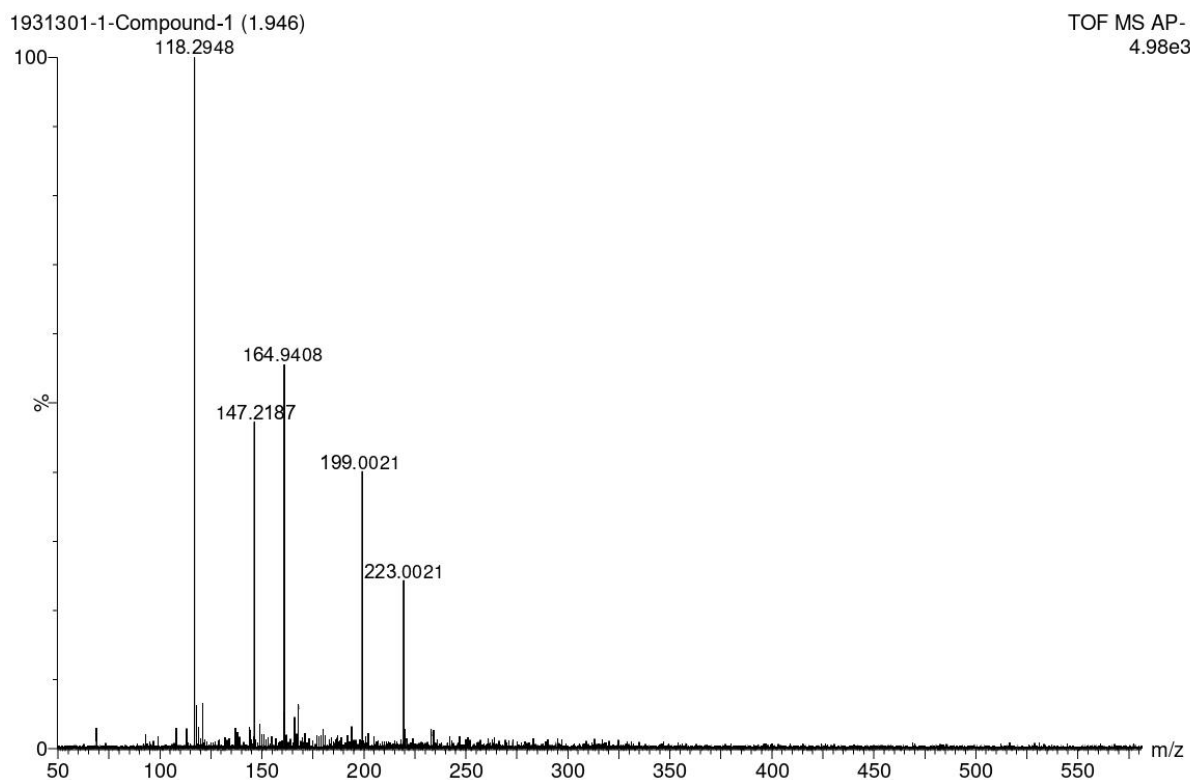


Figure 2: Mass Spectra of compound 1

Compound 2:

1. Melting Point :240⁰c

2. Yield:76%

3. IR Spectral Values (Cm-1):(KBr): 1123 (C-O-C), 1568, 1610 (C=N);

4. ¹H NMR (DMSO-d⁶): δ 3.79 (s, 2H, CH₂), 7.05-8.76 (9H, Ar-H).

5. Mass Spectral values (m/z) (%) 237 (10), 227 (43), 189 (62), 147 (100).

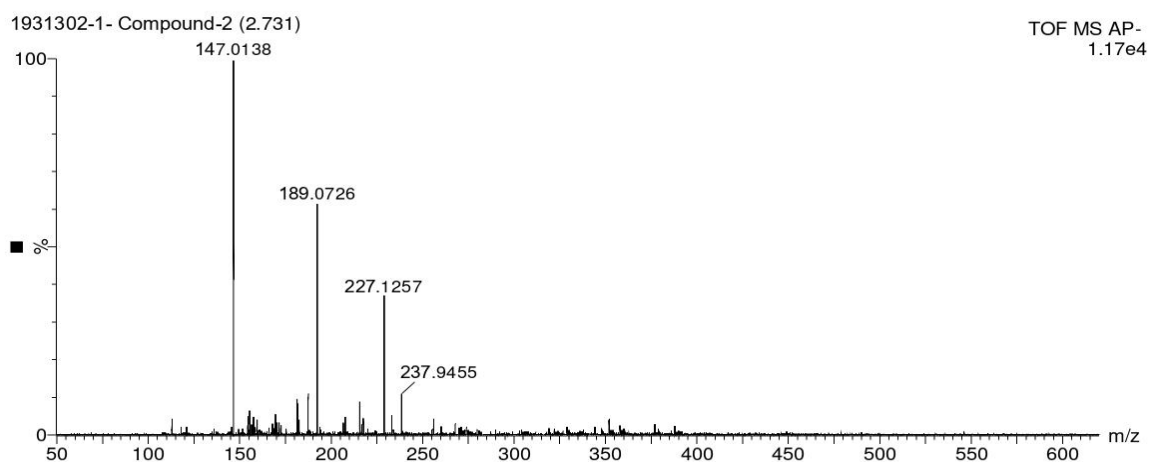


Figure 3: Mass Spectra of compound 2

Compound 3:

1. Melting point :281°C.

2. Yield :75%

3. IR Spectral values (Cm^{-1}): (KBr): 1146 (C-O-C), 1682, 1597 (C=N), 710 (C-Cl);

4. ^1H NMR (DMSO- d^6): δ 6.32-8.76(m, 8H, C₆H₅-H);

5. Mass Spectral values (m/z) (%) 259 (M+2, 15), 256 (19), 223 (31), 187 (42), 147(53), 118 (100).

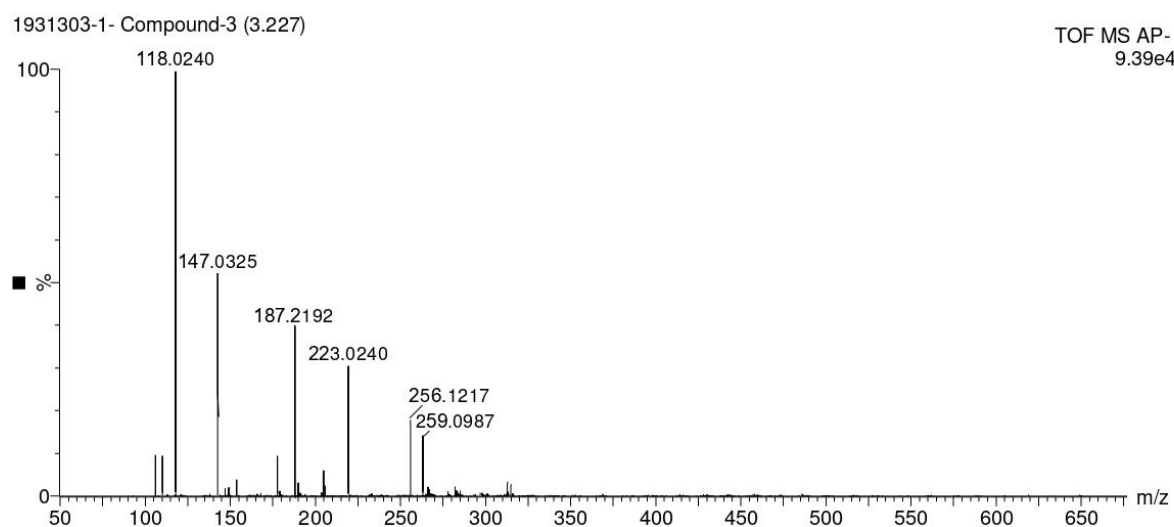


Figure 4: Mass Spectra of compound 3

Compound 4:

1. Melting point : 223°C.

2. Yield :73%

3. IR Spectral Values (Cm^{-1}): (KBr): 1155 (C-O-C), 1620, 1642 (C=N), 1560 (C-NO₂);

4. ^1H NMR Spectral Values (DMSO- d^6): δ 7.76-8.81 (m, 8H, Ar-H);

5. Mass Spectral Values (m/z) (%) 268 (18), 227 (28), 161 (63), 118 (100).

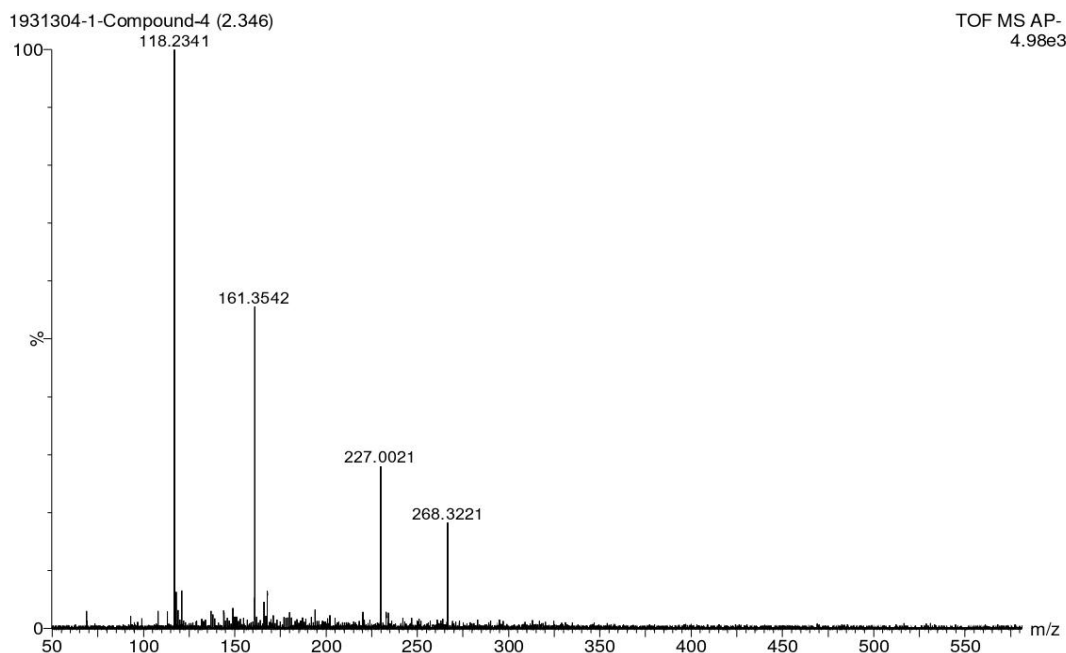


Figure 5: Mass Spectra of compound 4

Compound 5:

1. Melting point : 170⁰c

2. Yield : 72%

3. IR spectral Values (Cm⁻¹): (KBr): 2208 (CN), 1626 (C-C), 1238 (C-O-C), 754, 695 (SH)
2560-2600

4. ¹H NMR Spectral Values : (DMSO-d⁶): d: 5.41 (d, 1H), 5.81 (d, 1H), , (m, 13H, Ar-H),
8.00, 8.09 (2s, 2H,), 9.01 (s, 1H,).

5. Mass Spectral Values (m/z)(%): 589 [M⁺] (37), 509 (12.5), 470 (24.5), 259 (100), 236 (45),
145 (3.6), 117 (5.9), 77 (85).

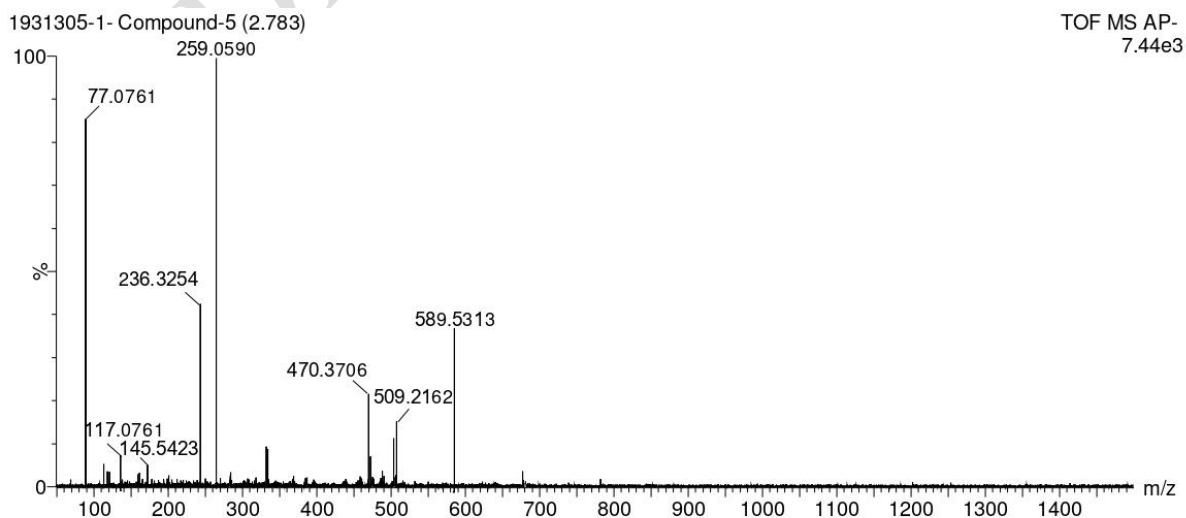


Figure 6: Mass Spectra of compound 5

Compound 6:

1. Melting Point :240⁰c

2. Yield :71%

3. IR spectral Values (Cm⁻¹):(KBr): 2958, 2873, 2202 (CN), 1622 (C-C), 759, 648.

4. ¹H NMR Spectral Values :(DMSO-d⁶): 7.11,7.91 (m, 14H, Ar-H and [CH=CH]) , 8.49, 8.79 (2s, 2H,), 9.59 (s, 1H,).

5. Mass Spectral Values (m/z)(%): 508 [M⁺] (12.5), 431 (35), 428 (12.9), 363 (17.8), 259 (45), 248 (18.9), 145 (12.6), 117 (64.6), 77 (100).

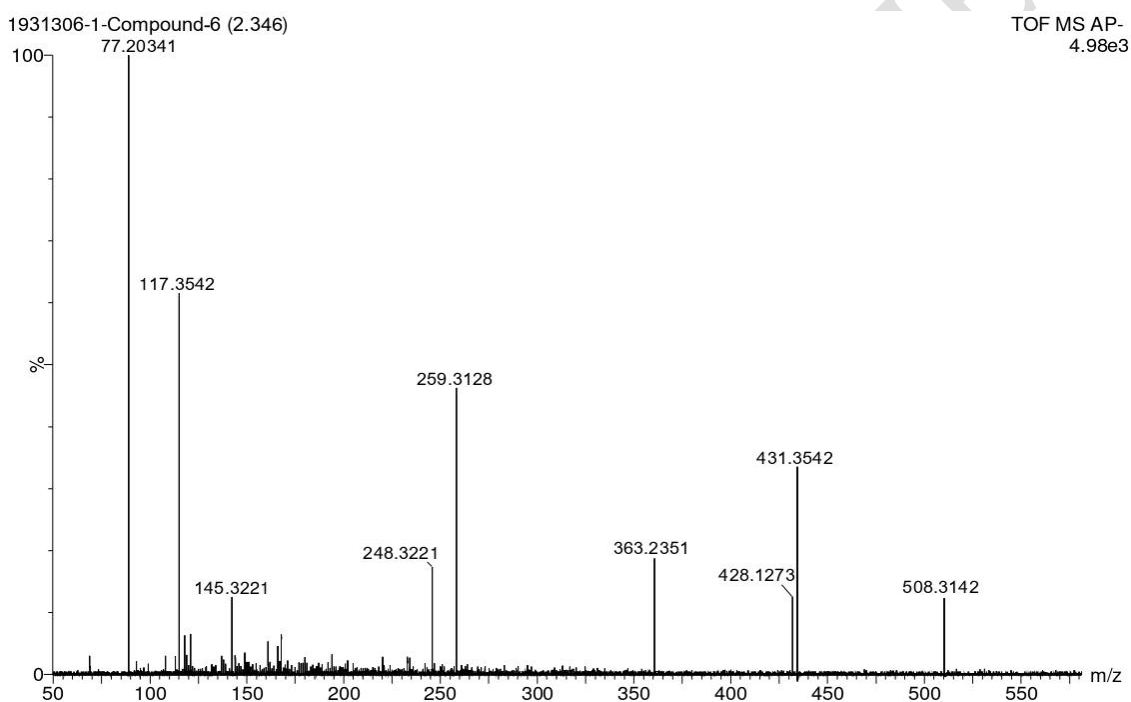


Figure 7: Mass Spectra of compound 6

Biological Activity :

1. Determination of minimum inhibitory concentration by tube dilution method.

Table :1 MIC of the the compounds

Compounds	Minimum Inhibitory Concentration (mcg/ml)
C1	12.5
C2	25
C3	25
C4	50

C5	50
C6	25

The antimicrobial activity of the compounds was promising. The compound minimum inhibitory concentrations were in the range of 12.5 to 50 mcg/ml. Compound 1 exhibited maximum activity with minimum inhibitory concentration (mic) of 12.5 mcg/ml as given in Table 1 and Figure 1 remaining three compounds C2, C3 and C6 showed MIC at 25 mcg/ml. The compounds C4 and C5 MIC was 50 mcg/ml. Compound1 was found to have potent antimicrobial activity.

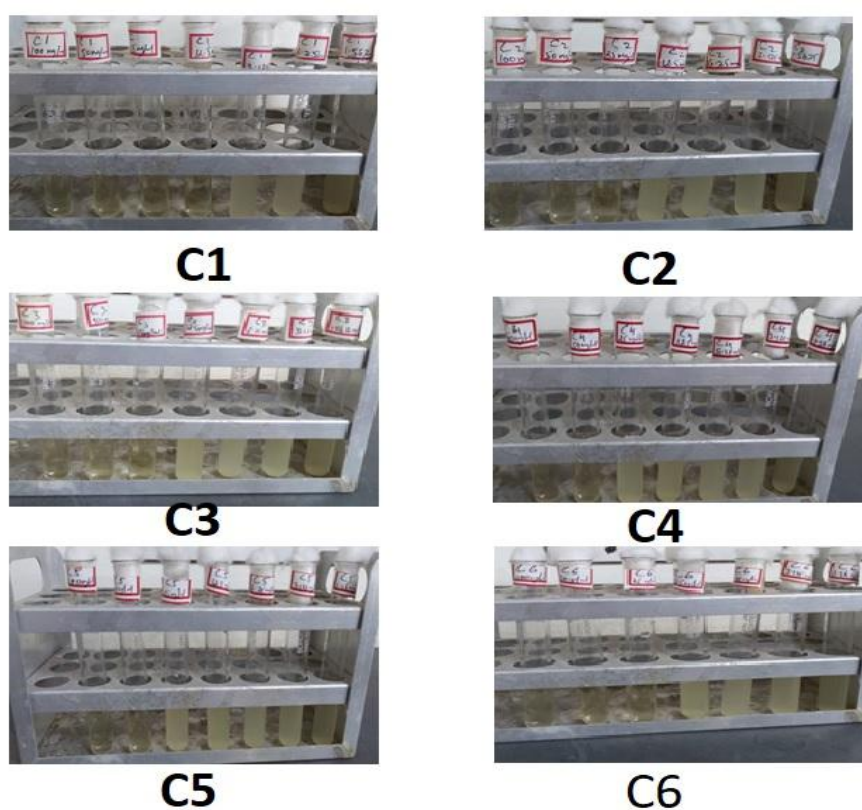


Figure 8: MIC of C1 to C6

3.Evaluation of antibacterial properties of the synthesized compounds were carried out against *E. coli*

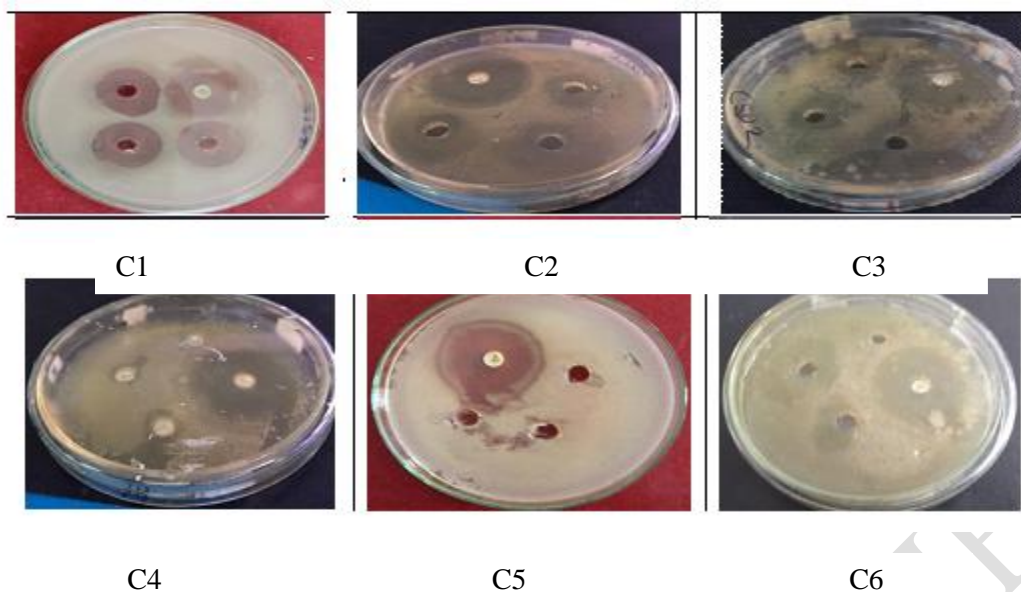


Fig 9 : Zone of inhibition of compound C1 - C6, with standard Ciprofloxacin

Table: 2 Zone of inhibition of compound C1 - C6, with standard Ciprofloxacin

Compound	Zone of inhibition in mm
Compound 1	31.01 ± 0.034
Compound 2	20.56 ± 0.106
Compound 3	19.28 ± 0.12
Compound 4	12.01 ± 0.135
Compound 5	10.75 ± 0.031
Compound 6	22.07 ± 0.65
Std ciprofloxacin 5 mcg	32.04 ± 0.044

In the zone of inhibition studies, all six samples at MIC concentrations showed antimicrobial activity. The maximum activity of compound 1 was comparable to that of the standard drug ciprofloxacin (5mcg).zone of inhibition was found to be 31.01 ± 0.034 for compound one with was potent as compared to standard ciprofloxacin $32 \text{ mm} \pm 0.044$.

So from this result compound C1 is having potent antibacterial activity.

DISCUSSION:

Utilizing corrosive hydrazide as a halfway, a progression of mixtures were integrated, including oxadiazole and pyrazole subsidiaries, and these mixtures were affirmed by its Dissolving point and by portrayed upsides of NMR.Compound 1,2,3,4,5 amd 6 Showed

HNMR signals in the scope of 7.21-8.78, 7.05-8.76, 6.32-8.76, 7.76-8.81, 7.26,7.89, 8.49 δ qualities and these mixtures were evaluated for antibacterial movement among that enamines showed most noteworthy action practically identical to standard medication ciprofloxacin(5mcg).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ACKNOWLEDGEMENT:

The authors are grateful to the authorities of Department of Chemistry, St.Philomena's College (Autonomous) Mysuru and, NGSM Institute of pharmaceutical Nitte Mangaluru, for providing all the necessary facilities. This work could not have been done without the excellent and valuable technical assistance of the following colleagues: Rector Dr.Bernard Prakash , Dr. Alphonsa D'Souza ,Dr.Prashanth Nayak Dr.Prasanna Sham Kandige . This work was supported by St.Philomenas college (Autonomous) Mysuru.

REFERENCES

1. Al-Haiza MA, El-Assiery SA, Sayed GH. Synthesis and potential antimicrobial activity of some new compounds containing the pyrazol-3-one moiety. *Acta Pharm.*; 51: 251-261, 2001.
2. Ibrahim MA, Saleh AM, Zohdi HF. Synthesis and antitumor activity of 5-trifluoromethyl-2,4-dihydropyrazol-3-one nucleosides. *Molecules*; 9: 109-119, 2004
3. Genin MJ, Biles C, Keiser BJ, Poppe SM, Swaney SM, Tarpley WG. Novel 1,5-diphenylpyrazole nonnucleoside hiv-1 reverse transcriptase inhibitors with enhanced activity versus the delavirdine-resistant p236l mutant: Lead identification and SAR of 3- and 4-substituted derivatives. *J Med Chem.*; 43: 1034-1040, 2000.
4. Kalla R, Zablocki J. Recent advances in adenosine receptor (AR) ligands in pulmonary diseases. *Annu Rep Med Chem.*; 44: 265-277, 2009.
5. Rostom SAF, Ashour HMA, El-Razik HAA, El-Fattah AEHA, El-Din NN. Azoleantimicrobial pharmacophore-based tetrazoles: Synthesis and biological evaluation as potential antimicrobial and anticonvulsant agents. *Bioorg Med Chem.*; 17: 2410-2422, 2009.

6. Rostom SAF. Synthesis of some 1-(4-chlorophenyl)-4- hydroxy-1H-pyrazol-3-carbonyl derivatives linked to nitrogenous heterocyclic ring systems as potential antitumor agents. *Bioorg Med Chem.*; 18: 2767-2776, 2010.
7. Sujatha K, Shanthi G, Selvam NP, Manoharan S, Perumal PT, Rajendran M. Synthesis and antiviral activity of 4,4'-(arylmethylene)bis(1H-pyrazol-5-ols) against peste des petits ruminant virus (PPRV). *Bioorg Med Chem Lett.*; 19: 4501-4503, 2009.
8. Salahuddin.; Mazumder, A.; Yar, M.S.; Mazumder, R.; Chakraborty, G.S.; Ahsan, M.J.; Rahman, M.U. Updates on synthesis and biological activities of 1,3,4-oxadiazole: A review. *Synth. Commun.* 2017, 47, 1805–1847.
9. Bajaj, S.; Asati, V. Singh, J.; Roy, P.P. 1,3,4-Oxadiazoles: An emerging scaffold to target growth factors, enzymes and kinases as anticancer agents. *Eur. J. Med. Chem.* 2015, 97, 124–141.
10. Pitasse-Santos, P, Sueth-Santiago, V, Lima, M. 1,2,4- and 1,3,4-Oxadiazoles as Scaffolds in the Development of Antiparasitic Agents. *J. Braz. Chem. Soc.* 2017, 29, 435–456.
11. Carbone, M.; Li, Y.; Irace, C.; Mollo, E.; Castelluccio, F.; Di Pascale, A.; Cimino, G.; Santamaria, R.; Guo, Y.W.; Gavagnin, M. Structure and Cytotoxicity of Phidianidines A and B: First Finding of 1,2,4-Oxadiazole System in a Marine Natural Product. *Org. Lett.* 2011, 13, 2516–2519.
12. Kadi AA, El-Brollosy NR, Al-Deeb OA, Habib EE, Ibrahim TM, El-Emam AA. Synthesis, antimicrobial, and anti-inflammatory activities of novel 2-(1-adamantyl)-5-substituted-1,3,4-oxadiazoles and 2-(1-adamantylamino)-5-substituted-1,3,4-thiadiazoles. *Eur J Med Chem.*; 42: 235-242, 2007.
13. Xu W, He J, He M, Han F, Chen X, Pan Z. Synthesis and antifungal activity of novel sulfone derivatives containing 1,3,4-oxadiazole moieties. *Molecules*; 16: 9129-9149, 2011.
14. Bezerra NMM, De Oliveira SP, Srivastava RM, Da Silva JR. Synthesis of 3-aryl-5-decapentyl-1,2,4-oxadiazoles possessing anti-inflammatory and antitumor properties. *Farmaco*; 60: 955-960, 2005 .
15. Ali M, Yar MS. Oxadiazole Mannich bases: Synthesis and antimycobacterial activity. *Bioorg Med Chem Lett*; 17:3314-3316, 2007.
16. Zarghi A, Tabatabai SA, Faizi M, Ahadian A, Navabi P, Zanganeh V. Synthesis and anticonvulsant activity of new 2-substituted-5-(2-benzyloxyphenyl)-1,3,4-oxadiazole. *Bioorg Med Chem Lett.*; 15: 1863-1865, 2005 .

17. Ravichandran V, Shalini S, Sundram K, Sokkalingam AD. QSAR study of substituted 1,3,4-oxadiazole naphthyridines as HIV-1 integrase inhibitors. *Eur J Med Chem.*; 45:2791-2797, 2010.
18. Furniss B S, Hannaford A J, Smith P W G & Patchel A R, *Vogel's Textbook of Practical Organic Chemistry*. 5th edn (Pearson Education Pvt. Ltd, Singapore), p.1269, 1996.
19. Amir M & Shikha K, *Indian J Heterocyclic Chem*, 14, 51, 2004.
20. Amir M, Javed S A & Harish K, *Indian J Chem*, 46B, 1014, 2007.
21. Furniss B S, Hannaford A J, Smith P W G & Patchel A R, *Vogel's Textbook of Practical Organic Chemistry*. 5th edn (Pearson Education Pvt. Ltd, Singapore), p.1269, 1996.
22. Determination of minimum inhibitory concentrations. Andrews JM. *J Antimicrob Chemother.* Jul;48 Suppl 1:5-16, 2001.
23. Heatley N.G. A method for the assay of penicillin. *Biochem. J.*;38:61-65; 1944.