SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 2-(PIPERZIN-1-YL)-2, 5-DIHYDOPYRIMIDINE ANALOGUES CONTAINING [1, 3, 4] OXADIAZOLE RING

NAVEEN KUMAR KOTTAKKI
Department of Chemistry, GITAM University, Visakhapatnam, Andhra Pradesh.
naveen.kottakki@gmail.com

SANTHOSH KUMAR DHAVALA
Department of Physics, GVP College for Degree and PG Courses (A), Visakhapatnam, Andhra Pradesh

AMRUTHA SRI KONDURI
Andhra University Visakhapatnam..

Abstract

A modest and effective method for coupling of 1, 3, 4 oxadiazoles and 2-(piperazin-1-yl)-2, 5-dihydropyrimidine into 2-(piperazin-1-yl)-2,5-dihydropyrimidine-1,3,4-Oxadiazole analogues (PO1-PO6). All compounds were prepared at standard temperature and result was found with a good percentage of yields. The structures were definite using a ^{13}CNMR and ^{1}HNMR. Antibacterial study was done using gram negative (Vibrio cholera) and gram positive (Bacillus cereus) micro-organisms and disc diffusion method for evaluation of antibacterial activity. Compound containing chloro substitution and hydroxyl substitution (PO3 and PO 6) showed the highest effect, while compound PO1, PO4, PO5 showed the lowest.

***Keywords**: 2-(piperazin-1-yl)-2,5-dihydropyrimidine-1,3,4-Oxadiazole analogues analogues, Synthesis, Antimicrobial activity.*

INTRODUCTION

The heterocyclic compounds play vital parts in organic and pharmaceutical prepare. As a few drug molecules contain heterocyclic as centre structure, extraordinary endeavours have been made to create moved forward manufactured strategies for this structure and nitrogen-containing heterocyclic compounds have a diverse range of biological and pharmacological properties.

1,3,4-Oxadiazole ring is related with numerous sorts of organic properties such and anti-inflammatory[1-3], hypoglycaemic[4], antibacterial and antifungal[5-9] activities. The other oxadiazole isomers are well known and happen within the structure of numerous drugs, eg. Antitussive oxolamine[10], antimicrobial furamizoe[11], antiviral raltegravir[12] and others. Particularly essential are the subsidiaries of 1,3,4-oxadiazole. The 1,3,4-oxadiazole ring moreover acts as bioisosteres for carbonyl containing compounds such as esters, amides and carbamates. Oxadiazoles ring is utilized as significant portion of the pharmacophore which have capacity to lock in with ligand. In a few cases, it acts like a level fragrant linker to supply the suitable introduction of the atom[13-14]. From the point of view of natural movement, intertwined hetero aromatic frame works are frequently of much more noteworthy intrigued than the constituents monocyclic compounds. The appearance of subjectively modern properties of annulated atom, broadening of the plausibility of changing pharmacophore bunches in several positions of the atom and the capacity of the last mentioned to associate with a more extensive range of receptors embracing different confirmations are clearly of significant significance[15]. Pyridines are an important class of heterocyclic compounds, which possess a wide range of biological activities[16-20]. Within the sedate disclosure piperazine ring was far reaching platform with more number of natural applications

and it was the spine of many bioactive molecules it has flexible binding properties[21-23].

The incessant and common utilize of antimicrobial agents has brought about within the improvement of resistance to these drugs by pathogenic microorganisms from on there's a most necessity in an inventive course of drugs. In this way profound results in antimicrobial drugs revelation are, still required to create more hopeful and viable antimicrobial specialists for utilize with in the field of clinical inquire about indicted by the scope of the more current course of antimicrobial drugs and in the continuation of investigate on naturally dynamic heterocyclic in this see, the current work was proposed get ready novel 2-(piperazin-1-yl)-2,5-dihydropyrimidine-1,3,4-Oxadiazole analogues s and their natural thins about assessments

EXPERIMENTAL

Materials and Methods

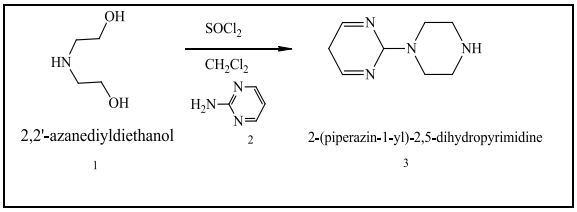
Analytical grade solvents used in all experiments, compounds synthesis were noticed by structural elucidations used ^{1}H-NMR (Bruker) 400 MHz spectrometer in $CDCl_3$. Softening focuses were decided on a WSR capillary dissolving point device and their maintenance times utilizing lean layer chromatography. IR spectra were recorded on Perkin element FTIR spectrophotometer utilizing KBR pellets and UV Spectra were recorded on perking Elmer spectrophotometer.

SYNTHESIS OF 2-(4-(2, 5-DIHYDROPYRIMIDIN-2-YL) PIPERAZIN-1-YL)-N-(5-PHENYL-1,3,4-OXADIAZOL-2-YL)ACETAMIDE ANALOGUES

Preparation of 2-(piperazin-1-yl)-2,5-dihydropyrimidine

Diethanolamine (0.12 mol) was dissolved in dichloromethane (15 mL), and added to a solution of dichlorosulfoxide (30 mL) and stirred for 1 hr and pyrimidin-2-amine was added to the resulting mixture and refluxed for 2 hrs and The residue was cooled to room temperature and recrystallized from suitable solvents

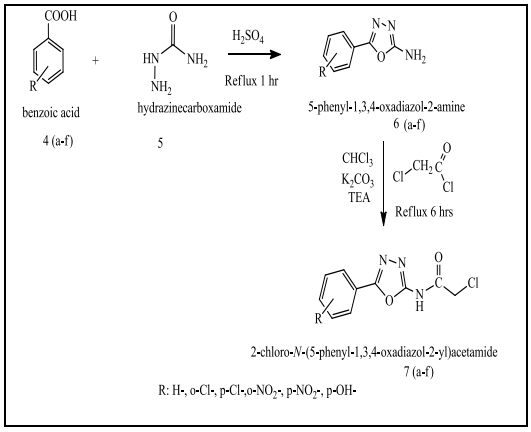
Scheme-1



Preparation of Thiadiazole Derivatives

To the benzoic acid (0.01moles) added hydrazinecarboxamide (0.01 moles), concentrated sulphuric acid (0.2 mL) and refluxed for 1hr, the resultant 5-(4-phenyl)-1, 3, 4-oxadiazole-2amine was poured into crushed ice and recrystallized with ethanol. 5-(4-phenyl)-1, 3, 4-oxadiazole-2-amine (0.01moles) dissolved in chloroform (25 mL) and added to a potassium carbonate (0.01 moles), chloroacetyl chloride (0.01 moles) and triethylamine (0.05 moles) and refluxed for 6 hrs. Cooled at room temperature and transferred into a crushed ice. The precipitate was filtered and dried. The residue was recrystallized with ethanol

Scheme-2



Preparation of 2-(4-(2,5-dihydropyrimidin-2-yl)piperazin-1-yl)-N-(5-phenyl-1,3,4-oxadiazol-2-yl)acetamide analogues.

2-chloro-N-(5-phenyl-1,3,4-oxadiazol-2-yl) acetamide (0.04 moles) and 2-(piperazin-1-yl)-2,5-dihydropyrimidine (0.05 moles) dissolved in chloroform (25 mL) and added triethylamine(0.02 moles). The mixture was refluxed for 3 hr. and cooled at room temperature. The residue recrystallized with ethanol

Scheme-3

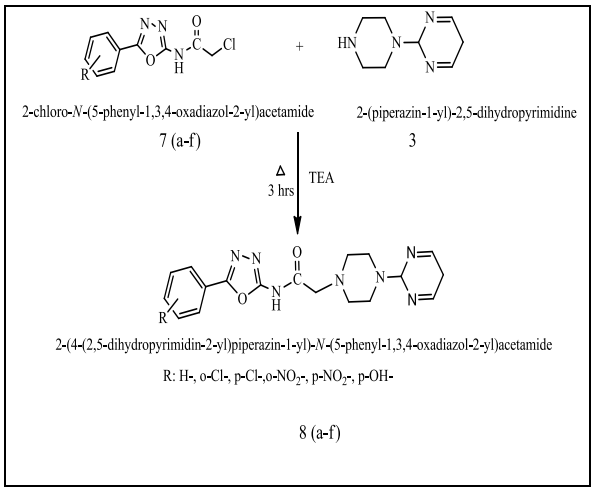
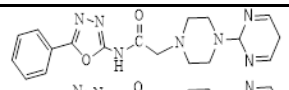
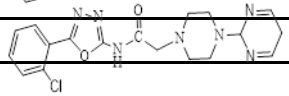
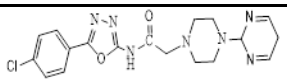
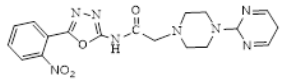
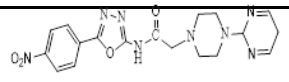
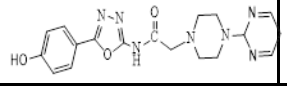


Table -1: Molecular information of the six compounds

S. No	Compound	Structure	Molecular Formula
1	PO-1		C20H20N7O2
2	PO-2		C20H19ClN7O 2
3	PO-3		C20H19ClN7O 2
4	PO-4		C20H19N8O4
5	PO-5		C20H19N8O4
6	PO-6		C20H20N7O3

SPECTRAL DATA

2-(4-(2,5-dihydropyrimidin-2-yl)piperazin-1-yl)-N-(5-phenyl-1,3,4-oxadiazol-2-yl)acetamide (PO1): Melting Point: 194-198 °C, Mass (m/z): 413. (Yield-82 %). **FT**IR (KBr Cm^{-1})**:** 3374 (NH), 3025 (CH), 2882 (CH), 1666 (CO), 2256 (CN), 1435 (CC). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-8.40 (m, 5H, phenyl ring), δ 3.29 (s, 2H, CH2), δ 9.04(s, 2H, NH), δ 2.35, (s, 2H, CH2), δ 7.50-7.57 (s, 2H, CH2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.0, 168.5, 164.6, 129.2, 128.7, 127.5, 126.1, 110.5, 55.2, 47.7

N-(5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)-2-(4-(2,5-dihydropyrimidin-2-yl)piperazin-1-yl)acetamide (PT2): Melting Point: 230-235 °C, Mass (m/z): 401.14 (yield-78%). (Yield-82 %). **FT**IR (KBr Cm^{-1})**:** 3394 (NH), 3024 (CH), 2782 (CH), 2306 (CN), 1630 (CO), 1422 (CC)., ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55-7.83 (m, 5H, phenyl ring), δ 3.29 (s, 2H, CH2), δ 9.15 (s, 2H, NH), δ 2.35, (s, 2H, CH2), δ 7.50-7.57 (s, 2H, CH2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 168.5, 164.5, 163.6, 136.6, 130.1, 129.5, 128.4., 127.7, 110.5, 55.4, 47.8

N-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-2-(4-(2,5-dihydropyrimidin-2-yl)piperazin-1-yl)acetamide (PT3) : Melting Point: 185-190 °C, Mass (m/z): 401.14, (yield-75%). (Yield-82 %). **FT**IR (KBr Cm^{-1})**:** 3324 (NH), 3022 (CH), 2782 (CH), 2256 (CN), 1566 (CO), 1435 (CC).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72-7.55 (m, 5H, phenyl ring), δ 3.29 (s, 2H, CH2), δ 9.04(s, 2H, NH), δ 2.35, (s, 2H, CH2), δ 7.50-7.57 (s, 2H, CH2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 168.5, 164.6, 163.7, 129.7, 128.9, 124.1, 110.5, 55.2, 47.7

2-(4-(2,5-dihydropyrimidin-2-yl)piperazin-1-yl)-N-(5-(2-nitrophenyl)-1,3,4-oxadiazol-2-yl)acetamide (PT4) : Melting Point: 173-176 °C, Mass (m/z): 412. 16 (yield-65%). (Yield-82 %). **FT**IR (KBr Cm^{-1})**:** 3354 (NH), 3025 (CH), 2888 (CH), 2266 (CN), 1686 (CO), 1445 (CC). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90-8.02 (m, 4H, phenyl ring), δ 3.29 (s, 2H, CH2), δ 9.04(s, 2H, NH), δ 2.35, (s, 2H, CH2), δ 7.50-7.57 (s, 2H, CH2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 168.3, 163.7, 164.5, 146.9, 131.4, 132.6, 128.2, 110.5, 63.6, 55.3, 47.6, 21.3.

.

2-(4-(2,5-dihydropyrimidin-2-yl)piperazin-1-yl)-N-(5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)acetamide (PT5) : Melting Point: 260-265 °C, Mass (m/z): 412.16, (yield-85%). (Yield-82 %). **FT**IR (KBr Cm^{-1})**:** 3374 (NH), 3005 (CH), 2882 (CH), 2256 (CN), 1675 (CO), 1560 (NO), 1455 (CC). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (m, 4H, phenyl ring), δ 3.29 (s, 2H, CH2), δ 9.04(s, 2H, NH), δ 2.35, (s, 2H, CH2), δ 7.50-7.57 (s, 2H, CH2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 168.3, 163.7, 164.3, 130.4, 132.6, 128.2, 110.5, 63.7, 55.3, 47.6, 21.4

2-(4-(2,5-dihydropyrimidin-2-yl)piperazin-1-yl)-N-(5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)acetamide (PT6) : Melting Point: 241-244 °C, Mass (m/z): 383, (yield-60%). (Yield-82 %). **FT**IR (KBr Cm^{-1})**:** 3384 (NH), 3455 (OH), 3005 (CH), 2882 (CH), 1667 (CO), 2246 (CN), 1520 (NO), 1435 (CC). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.86-7.93 (m, 4H, phenyl ring), δ 3.29 (s, 2H, CH2), δ 5.36, (s, H, OH), δ 9.14 (s, 2H, NH), δ 2.35, (s, 2H, CH2), δ 7.50-7.57 (s, 2H, CH2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 168.2, 163.7, 164.3, 158.3 130.4, 132.6, 128.2, 116.4, 110.5, 63.7, 55.3, 47.6, 21.2.

Anti-Microbial Activity

Organisms:

Twomicro-organisms, used in the current study are collected from MTCC, Chandigarh, India. Those are *Bacillus subtilis* MTCC211 and *Escherichia coli* MTCC443.

Media:
Mueller Hinton Agar medium to be used for routine susceptibility testing of bacteria due to its acceptable reproducibility, satisfactory growth of most pathogens [24].

Agar-well diffusion testing:
The antibacterial activity was assessed for the synthesized drugs using agar well diffusion test method [25]. The method is basically on diffusion of the desired drug in a vertical cylinder well in an agar petri plate, which is pre-cultured with the testing bacterial strain. The activity of the compounds will be measured using the formation of zones around the wells [26-27]. In the current study, Muller-Hinton agar was used to culture the test micro-organisms on petri dishes. After solidification of agar, cotton swab was used to spread the testing bacterial strains and then 6mm wells were placed on agar plate with sterile steel borer. Then, 50µl of synthesized drugs (PT-O to PO-6) were tested on selected bacteria using vancomycin as standard drug (30µg) and dimethl sulphoxide (DMSO) as vehicle. After, placing the test compounds, standard, vehicle in wells placed the petri dishes aside for 1hr without disturbance for diffusion of compounds in wells. Then, plates were incubated for 24hrs at 37°C. After completion of incubation, the plates were used to measure the compounds

zones of inhibition around the wells using well reader (scale), the experiment was repeated thrice and the results were expressed as average in mm. Those compounds which were unable to exhibit inhibition zone (inhibition zone diameter less than 7 mm) were considered non-active.

Results and Discussion

2-(piperazin-1-yl)-2,5-dihydropyrimidine (3) was prepared by treated diethanolamine (1) with pyrimidin-2-amine (2) in presence of thionyl chloride scheme-1. 5-phenyl-1,3,4-oxadiazol-2-amine (6) was prepared by the reaction of compounds benzoic acid (1)and hydrazinecarboxamide (2) in the presence of conc. H_2SO_4, 2-chloro-N-(5-phenyl-1,3,4-oxadiazol-2-yl)acetamide (7) was obtained by 5-phenyl-1,3,4-oxadiazol-2-amine treated with triethyl amine 7(a-f) (Scheme-2). 2-(4-(2,5-dihydropyrimidin-2-yl)piperazin-1-yl)-N-(5-phenyl-1,3,4-oxadiazol-2-yl)acetamide (8) was prepared by treated 2-chloro-N-(5-phenyl-1,3,4-oxadiazol-2-yl) acetamide (7) and 2-(piperazin-1-yl)-2,5-dihydropyrimidine (3) in presence of triethylamine as coupling reagent 8 (a-f) Scheme-3.and the analogues **(PO1-PO6)** were prepared by using similar methods (Table 1).

The IR spectra peaks at 3300 and 1662 were corresponds to N-H of amide and C=O to the produced compound from methyl benzoate. The C-S stretch peaks at 698 cm^{-1} in IR spectra confirms of the synthesized potassium 2-benzoylhydrazinecarodithioate compound. The IR and ^1H NMR spectras of 4[amino]-5-phenyl-4H-1,2,4-triazole-3-thiol confirms its structure by appearance of N-C-S stretch at 943 cm^{-1} and N-C-C stretch peaks at 1278 cm^{-1} IR spectra and SH, NH peaks at 4.80 δ and 8.21 δ in NMR spectra. The compound synthesized from 4[amino]-5-phenyl-4H-1,2,4-triazole-3-thiol is (2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N-(3-mercapto-5-phenyl-4H-1,2,4-triazol-4-yl)penta-2,4-dienamide is

Confirmed by its IR spectra due to absence of NH peak and presence of -N-N-C.

Antibacterial activity of synthesis compounds on bacteria.

Agar well-diffusion method was used to screen the antibacterial activity of synthesized compounds (PO1-PO6) at 50 µg and 100 µg and the compounds showed concentration-dependent activity **(Table 2)**. Antibacterial activity of the six selected compounds was varied with the microorganism tested in this study [*Bacillus subtilis* (Gram positive) and *Vibro cholera* (Gram negative)]. The results of the current study describe that tested compounds were more active against Gram positive bacteria compared to Gram negative bacteria. At 100 µg concentration PO3 showed highest (20 mm and 18 mm zone of inhibition) antibacterial activity against *Vibro cholera*. On the other hand, standard antibiotic vancomycine at 30 µg showed 30 mm and 28 mm zone of inhibition against *Bacillus subtilis* and *Vibro cholera*. Whereas PO6 also showed moderate results (20 mm and 14 mm zone of inhibition) against two pathogens, *Bacillus subtilis* and *Vibro cholera*. The other compounds are moderately active. PO5 showed 13 mm to 12 mm zone of clearance against pathogen *Bacillus subtilis* and *Vibro cholera*. PO4 exhibited 10 mm to 15 mm zone of clearance against the two bacteria. PO1 did n't have activity at 50 µg but showed 10 mm inhibition zone against *Vibro cholera* at 100 µg and PO2 showed 10 mm and 9 mm against *Bacillus subtilis* and *Vibro cholera* at 100 µg and 50 µg respectively.

.

Table 2: Mean value of zone of inhibition followed by standard error of antibacterial activity of selected compounds.

S. No	Compound Name	Inhibition zone (mm)			
		Gram negative *(Vibrio cholera)*		Gram positive *(Bacillus subtilis)*	
		50 µg	100 µg	50 µg	100 µg
1	PO 1	9	10	0	8
2	PO 2	8	9	9	10
3	PO 3	18	20	8	9
4	PO 4	12	15	10	11
5	PO 5	10	12	11	13
6	PO 6	12	14	14	20
	Vancomycine (30 µg)	28		30	

The results of the current study confirm that compounds PO-3 and PO-6 more active against the tested bacterial strains and was equivalent to standard drug. The structural similarity between them concludes that the existence of chloro at 3nd position on benzene and hydroxyl group at 4th position on benzene ring exhibited excellent antibacterial activity showing importance of halogen and hydroxyl groups in the compounds.

Conclusion

A new series of 2-(4-(2,5-dihydropyrimidin-2-yl)piperazin-1-yl)-N-(5-phenyl-1,3,4-oxadiazol-2-yl)acetamide analogues were synthesised with simple and efficient methods and were screened for antibacterial activity and their results concludes that the presence of chloro substituted derivatives possess good anti-microbial activity against gram positive bacteria, hydroxyl substituted derivatives possess good anti-microbial activity against gram negative bacteria and other substituted derivatives are moderately active.

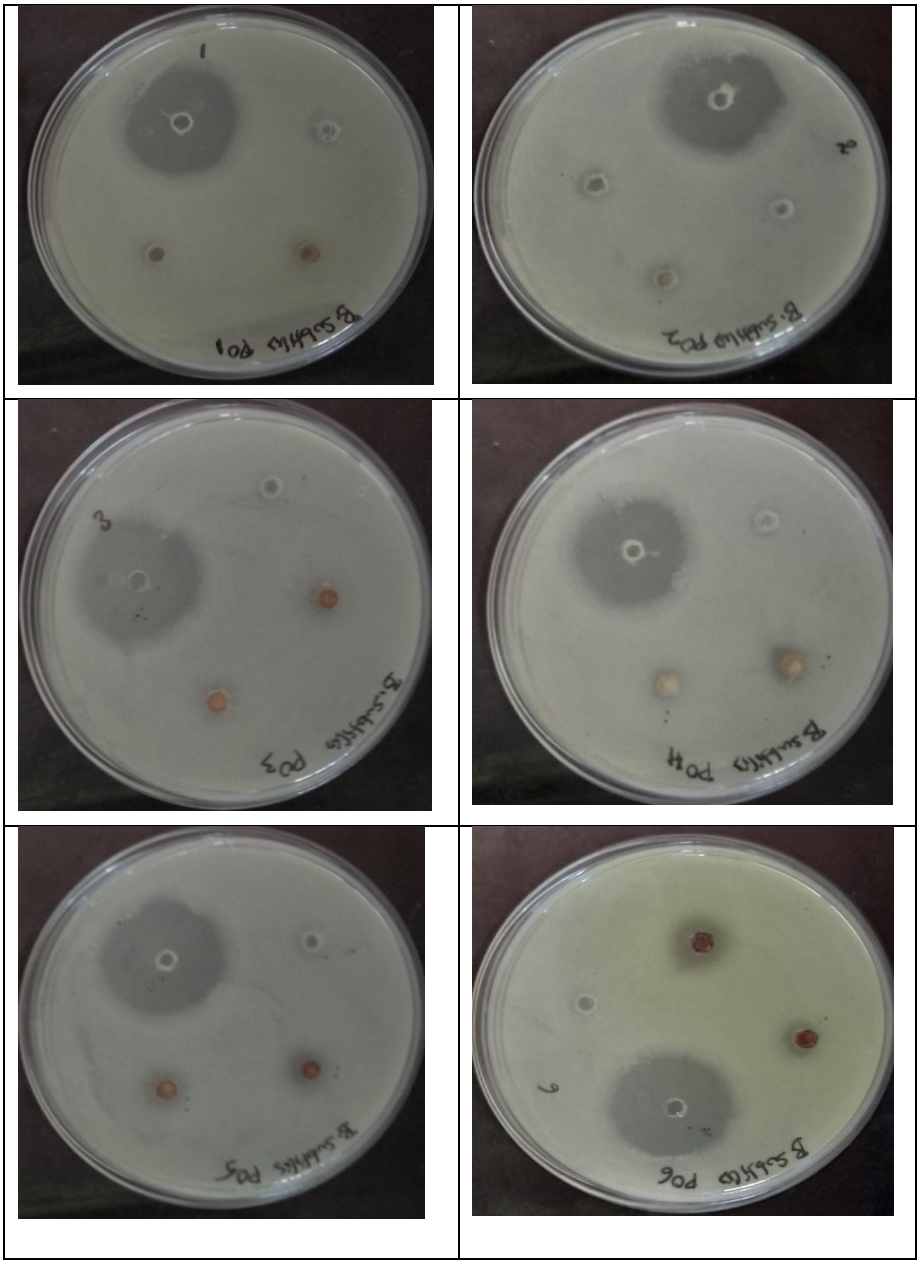
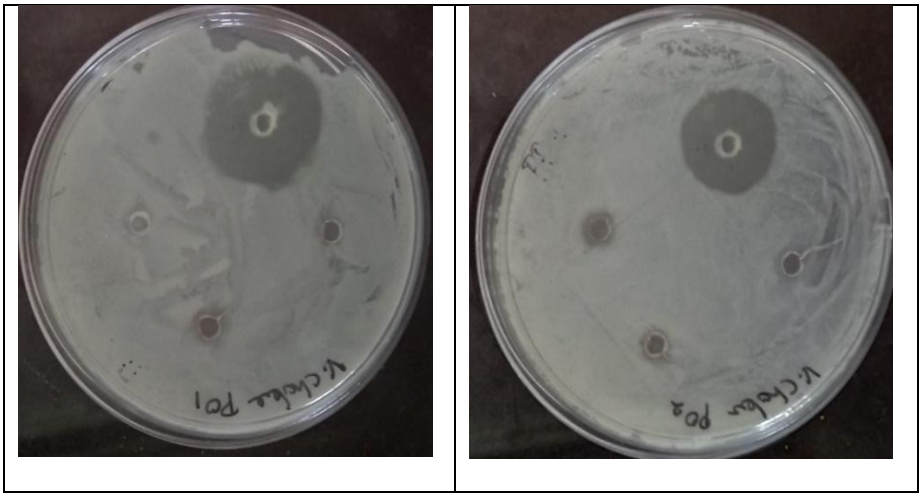


Figure 4: The zone of inhibitions of synthesized drugs against *Bacillus subtilis*



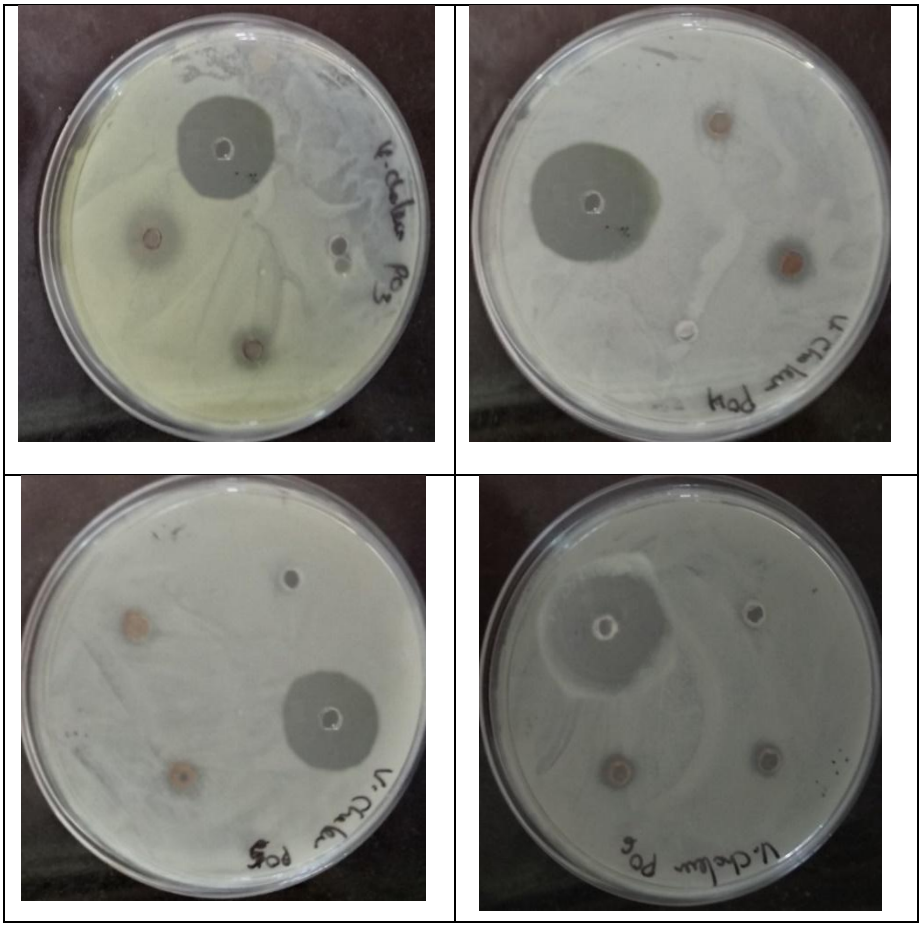


Figure 5: The zone of inhibitions of synthesized drugs against *Vibrio Chloriea*

References

1. *Mullican, M. D., Wilson, M. W., Conner, D. T., Kostlan, C. R., Schrier, D. J. and Dyer, R. D. (1993). Design of 5-(3, 5-di-tert-butyl-4-hydroxyphenyl)-1, 3, 4-thiadiazoles,-1, 3, 4-oxadiazoles, and-1, 2, 4-triazoles as orally active, nonulcerogenic antiinflammatory agents. Journal of medicinal chemistry **36**(8):1090-1099.*

2. *Boschelli, D. H., Connor, D. T., Bornemeier, D. A., Dyer, R. D., Kennedy, J. A., Kuipers, P. J. and Wright, C. D. (1993). 1, 3, 4-Oxadiazole, 1, 3, 4-thiadiazole, and 1, 2, 4-triazole analogs of the fenamates: in vitro inhibition of cyclooxygenase and 5-lipoxygenase activities. Journal of medicinal chemistry **36**(13): 1802-1810.*

3. *Raman, K., Singh, H. K. Salzman, S. K., and Parmar, S. S. (1993). Substituted thiosemicarbazides and corresponding cyclized 1, 3, 4-oxadiazoles and their anti-inflammatory activity. Journal of pharmaceutical sciences 82(2): 167-169.*

4. *Sahin, G., Palaska, E., Kelicen, P., Demirdamar, R. and Altinok, G. (2001). Synthesis of Some New 1-Acylthiosemicarbazides, 1, 3, 4-Oxadiazoles, 1, 3, 4-Thiadiazoles and l, 2, 4-Triazole-3-thiones and their Anti-inflammatory Activities. Arzneimittelforschung **51**(06), 478-484.*

5. *Husain, M. I., KUMAR, A. and Srivastava, R. C. (1986). Synthesis of N-(2-Naphthyloxyacetyl) thiosemicarbazides and 2-arylamino-5-(2-naphthyloxymethyl)-1, 3, 4-thiadiazoles/oxadiazoles as oral hypoglycemic agents. Current Science 644-646.*

6. *Giri, S., Singh, H. and Yadav, L. D. S. (1976). Studies in Oxadiazoles Synthesis of Some 2-Mercapto-l, 3, 4-oxadiazoles and Related Compounds as Potential*

Fungicides. Agricultural and Biological Chemistry ***40****(1): 17-21.*

7. *Singh, H. and Yadav, L. D. S. (1976). Synthesis of Some 5-Aryl-2-heteroaryl/heteroarylamino-l, 3, 4-oxadiazoles as Potential Fungicides. Agricultural and Biological Chemistry* ***40****(4): 759-764.*

8. *Misra, H. K. (1983). Synthesis of Some New Substituted 1, 3, 4-Oxadiazoles as Potential Insecticidal, Antibacterial and Anti-acetylcholine Esterase Agents. Archiv der Pharmazie* ***316****(6): 487-493.*

9. *Adhikari, V. A. and Badiger, V. V. (1988). Synthesis and Biological Activities of Isoxazolo (5, 4-d) pyrimidinyloxymethyl-thiadiazoles,-oxadi azoles and-triazoles. ChemInform* ***19****(47): no-no.*

10. *Glomb, T., Szymankiewicz, K. and Świątek, P. (2018). Anti-cancer activity of derivatives of 1, 3, 4-oxadiazole. Molecules* ***23****(12): 3361.*

11. *Mutschler, E., Geisslinger, G., Kroemer, H. K., Ruth, P. and Schaefer-Korting, M. (2010). Farmakologia i toksykologia. MedPharm, Wrocław, 353-355.*

12. *Byler, S., Heerboth, S., Lapinska, K., Longacre, M., Snyder, N. and Sarkar, S. (2014). Drug Resistance in Cancer: An Overview.*

13. *Boström, J., Hogner, A., Llinàs, A., Wellner, E. and Plowright, A. T. (2012). Oxadiazoles in medicinal chemistry. Journal of medicinal chemistry* ***55****(5): 1817-1830.*

14. *Ahmed, M. N., Sadiq, B., Al-Masoudi, N. A., Yasin, K. A., Hameed, S., Mahmood, T. and Tahir, M. N. (2018). Synthesis, crystal structures, computational studies and antimicrobial activity of new designed bis ((5-aryl-1, 3, 4-oxadiazol-2-yl) thio) alkanes. Journal of Molecular Structure* ***1155****: 403-413.*

15. *Gadaginamath, G. S., Shyadligeri, A. S. and Kavali, R. R. (1999). Chemoselectivity of indole-dicarboxylates towards hydrazine hydrate: part III-synthesis and antimicrobial activity of novel 4-thiazolidinonylindoles.*

16. *Chambhare, R. V., Khadse, B. G., Bobde, A. S. and Bahekar, R. H. (2003). Synthesis and preliminary evaluation of some N-[5-(2-furanyl)-2-methyl-4-oxo-4H-thieno [2, 3-d] pyrimidin-3-yl]-carboxamide and 3-substituted-5-(2-furanyl)-2-methyl-3H-thieno [2, 3-d] pyrimidin-4-ones as antimicrobial agents. European journal of medicinal chemistry* ***38****(1): 89-100.*

17. *Mansour, A. K., Eid, M. M and Khalil, N. S. (2003). Synthesis and reactions of some new heterocyclic carbohydrazides and related compounds as potential anticancer agents. Molecules* ***8****(10): 744-755.*

18. *Thakur, A., Khan, S. I. and Rawat, D. S. (2014). Synthesis of piperazine tethered 4-aminoquinoline-pyrimidine hybrids as potent antimalarial agents. RSC Advances* ***4****(40): 20729-20736.*

19. *Jin, K., Sang, Y., De Clercq, E., Pannecouque, C. and Meng, G. (2018). Design and synthesis of a novel series of non-nucleoside HIV-1 inhibitors bearing pyrimidine and N-substituted aromatic piperazine. Bioorganic & Medicinal Chemistry Letters* ***28****(22): 3491-3495.*

20. *Geisen, K., Lang, H. J., Nimmesgern, H. and Weidmann, K. (1992). U.S. Patent No. 5,138,058. Washington, DC: U.S. Patent and Trademark Office.*

21. *Thakur, A., Khan, S. I. and Rawat, D. S. (2014). Synthesis of piperazine tethered 4-aminoquinoline-pyrimidine hybrids as potent antimalarial agents. RSC Advances* ***4****(40): 20729-20736.*

22. *Basavaraja, H. S., Jayadevaiah, K., Hussain, M. M., Kumar, V. and Basavaraj, P. (2010). Synthesis of novel piperazine and morpholine linked substituted pyrimidine derivatives as antimicrobial agents. Journal of Pharmaceutical Sciences and Research* ***2****(1): 5-12.*

23. *Thriveni, K. S., Padmashali, B., Siddesh, M. B. and Sandeep, C. (2014). Synthesis of pyrimidine incorporated piperazine derivatives and their antimicrobial activity. Indian journal of pharmaceutical sciences* ***76****(4), 332.*

24. *Atlas, R. M. (2010). Handbook of microbiological media. CRC press.*

25. *Bonev, B., Hooper, J. and Parisot, J. (2008). Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. Journal of antimicrobial chemotherapy* ***61****(6): 1295-1301.*

26. *Windson, J. J., Rafay, A. M., Shenoy, A. K., & Johnson, E. H. (1998). Incidence of Dientaboeba fragilis in faecal samples submitted for routine microbiological analysis. British journal of biomedical science* ***55****(3): 172.*

27. *Cappuccino, J. G. and Sherman, N. (2004). Microbiology, Laboratory manual. Person education. INC, New Delhi, 282-283.*

.