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Synthesis, Characterization Of Piperidin-4-One Oxime and its effect on Pathogenic Bacteria isolated from hospitals

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ABSTRACT

Heterocyclic compounds containing piperidine moiety are gaining pharmacological importance due to their bioactive alkaloids. Hence the present study had been focused on organically synthesized N-substituted 2, 6-diarylpiperidin-4-one compounds and 2,6-diaryl-1-(2-(thiophene-2-yl)acetyl) piperidin-4-one oxime. The target piperidineoxime compound had been characterized by ^{1}H NMR, ^{13}C NMR and experimented against the wound infecting bacteria. In IR spectral studies, absence of carbonyl stretching frequency around 1710 cm^{-1} and presence of C=N frequency at 1629 cm^{-1} and OH stretching frequency at 3247 cm^{-1} were the supporting evidence for the formation of target compound. Bacterial pathogens such as Bacillussp, Escherichia coli, Streptococcus sp, Pesudomonasaeruginosa, Staphylococcus aureus,Enterococcussp,Klebsiellaspwere isolated from diverse environment of hospitals and maintained in asceptic conditions. Pharmacological activity of synthetic piperidineoxime at 25,50,75,100 and 125 µg/ml concentrations against bacterial isolates in terms of zone of inhibition in mm was resulted in the following order:Escherichia coli >Streptococcus pyogens>Staphylococcus aureus>Pseudomonas aeurigenosa>Enterococcussp>Bacillussp>Klebsiellasp

Key words: Piperidine 4-one oxime, NMR spectral study, hospital bacterial isolates, antibacterial activity

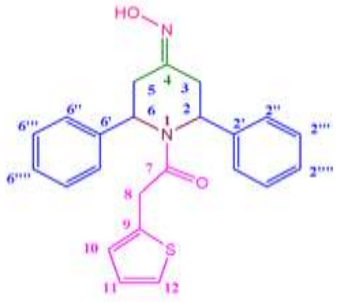
I. INTRODUCTION

Vital role of natural and synthetic piperidine in pharmaceutical industry is ubiquitous. Heterocyclic ring systems having piperidin-4-one nucleus have treated as pharmacophore due to their wide variety of bioactive alkaloids. Piperine and piperidine based drugs have been designed either based on natural compounds or synthetic routes[1-6].

Multi-component reactions are efficiently evolved in organic synthesis to generate yield in a single synthetic operation. Thus heterocyclic compounds having piperidin–4-one skeleton are the important target of organic synthesis owing to their pharmacological activity and their wide applications. Specifically many substituents at carbon C-2 and C-6 of the piperidin ring have been well documented as potent pharmacological agents [7-12].

With this back ground we have synthesized N-substituted 2, 6-diaryl piperidin-4-one oxime. The synthesized compound was characterized by ^{1}H NMR and ^{13}C NMR spectral studies. The target compound was further analyzed for antimicrobial studies. For this synthesized compound(Figure-1), the effect of substituent on the ring conformation and orientation of the substituent and the chemical shift of the carbon and their associated protons are discussed with the help of NMR Spectral data.

The synthesized compound further experimented for antibacterial potential against bacterial pathogens isolated from diverse environment of hospitals .



2,6-diphenyl-1-(2-(thiophen-2-yl)acetyl)piperidin-4-one oxime

Figure-1.Structure of synthetic piperidin-4-one oxime

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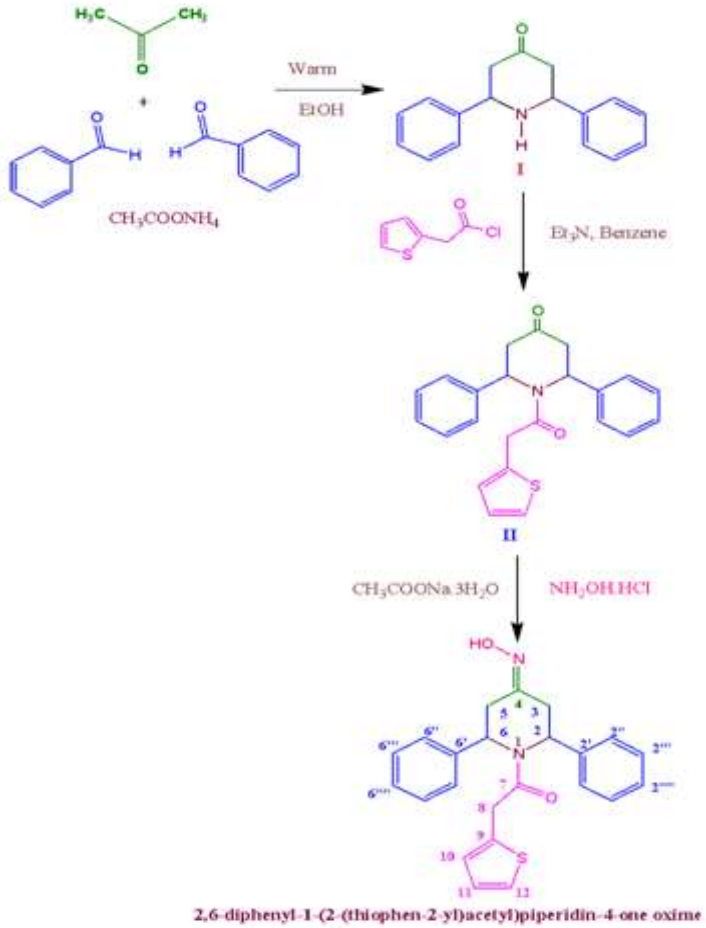
II. MATERIALS AND METHODS

2.1.Synthesis of 2,6-diaryl-1-(2-(thiophene-2-yl)acetyl) piperidin-4-one oxime

To a well-stirred solution of 2,6-diphenyl piperidin-4-one with triethylamine (1 mol) in 30ml of dry benzene, thiophene-2-acetyl chloride (1 mol) in 20 ml of benzene was added drop wise through the separating funnel for about half an hour. Stirring was continued with mild heating using magnetic stirrer. After completion of reaction, it was poured into water and extracted with ether in three 50 ml portions. The combined ether extract was washed well with 3% sodium bicarbonate solution and dried over anhydrous sodium sulphate. This upon evaporation and subsequent recrystallization in distilled ethanol (Scheme-1)



Scheme-1.Synthetic route for piperidin 4-one oxime

2.2.SPECTRAL MEASUREMENTS

The reagents used were purchased from commercial suppliers without further purification. Melting points were determined by using an open capillary method and are uncorrected. Thin layer chromatography (TLC) was performed with Aluminium sheet–silica gel 60F254 purchased from Merck. The column chromatography with silica gel (100-200 mesh) using Benzene: Petroleum ether(9:1) as eluent, NMR spectrum was run by BRUKER-400MHZ Spectrophotometer by using $CDCl_3$ as a solvent.

2.3 ANTIBACTERIAL STUDIES

2.3.1. Collection of microbes

Bacterial pathogens such as Bacillussp, Enterococcussp, Escherichia coli, Klebsiellasp , Pseudomonas aeurigenosa, Staphylococcus aureus and Streptococcus pyogens were collected from diverse environment of hospitals. The isolated

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microbes were maintained in nutrient agar broth and cultured in nutrient agar medium.

2.3.2.Preparation of medium and inoculum

Nutrient agar medium was prepared by dissolving 2.8g of nutrient agar in 100ml of distilled water. The solution was sterilized in an autoclave at 121°C for 15min. It was cooled and poured into sterile Petri dishes to solidify. Each wound infecting bacterium is inoculated on agar by streaking with the swab containing inoculums. Rotate the plate by 60° and repeat the rubbing procedure. This will ensure an even distribution of the inoculum. Whatman No.1 discs (6mm in diameter) were impregnated in the test compound dissolved in DMSO (25,50,75,100 and 125µg/ml) for about half an hour. Commercially available drug disc (kanamycin 10mg/disc) was used as positive reference standard. Negative controls were also prepared by impregnating the disc of same size on the inoculated agar plates and incubated at ±37˚C for about 18-24h.

All the tests were conducted in triplicates. The diameter of zone of inhibition was measured in mm. All the data obtained from the present study were analysed by SPSS -IBM for the statistical significance .

III. RESULTS AND DISCUSSION

3.1.Spectral analysis of piperidin-4-one oxime

The synthetic method of preparation of 2,6-diphenyl-1-(2-(thiophen-2-yl)acetyl)piperidin-4-one oxime represented in Scheme 1. Synthesized compound was confirmed by their ^{1}H and ^{13}C NMR spectral studies.Types of spectrum recorded for the synthesized compounds are given in Table-1.

Elemental analysis of2,6-diphenyl-1-(2-(thiophen-2-yl)acetyl)piperidin-4-one oxime by NMR spectra revealed the following data:

Generally the signals due to the aromatic carbons can be very readily distinguished from that of other carbons due to their characteristic absorption in the region of 120-140 ppm. Aromatic and ipso carbon signals are observed more down field region compared to other carbon signals. Hence, in the present investigation the aromatic and ipso carbons can be identified by their characteristic absorption in the region of 142.1-125.0 ppm. The two signals of the target compound are exhibited in the most down field region 155.3 and 172.1 ppm. Among these two signals the lower frequency region signal (155.3ppm) is attributed to C=N carbon while the signal observed at in higher frequency region (172.1) is ascribed to amide (N-CO) carbonyl carbon. A signal observed with very low intensity at 58.1 ppm is assigned to C-2 and C-6 carbons of the piperidin ring. Whereas the signals observed at 27.5 ppm and 29.7 ppm is assigned to C-3 and C-5 carbons of the piperidin ring. Similarly, the high intensity signal observed at 36.3 ppm is characteristic for thiophene connected methylene carbon(C-8).

In the synthetic piperidine derivative, the substituted electron withdrawing thiophene acetyl group at the nitrogen site of 2,6-diphenyl piperidin-4-one ring is known to exert a minor change in the chemical shifts of the ring carbons and their attached protons. Heterocyclic benzylic protons at C-2 and C-6 position act as highly functionalized scaffolds and the piperidine moiety are used as pharmacophore for preparation of drugs that are in demand to treat various illnesses.

Experiments on functional scaffolds and their involvement in pharmacological activity were recorded by earlier researchers[13-17] .

3.2. Performance of Piperidin-4-one oxime against Pathogenic bacteria

Pathogenic inhibiting capacity of the drugs/biocomposites depends on the high intensive moieties like piperidine,oxime and thienewhich modulate the electronic effects on heterocyclic rings of the synthesized compound. So that is effected against molecular /metabolic control over bacteria. Hence the present investigation had been focused on the control of growth in terms of zone of inhibition(MIC) by synthetic oxime.

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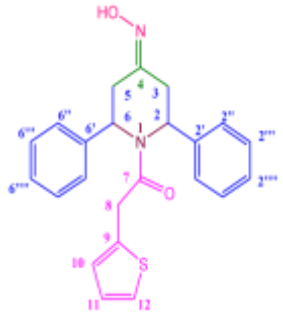
Table 1:Analytical and spectral data of piperidin-4-one oxime

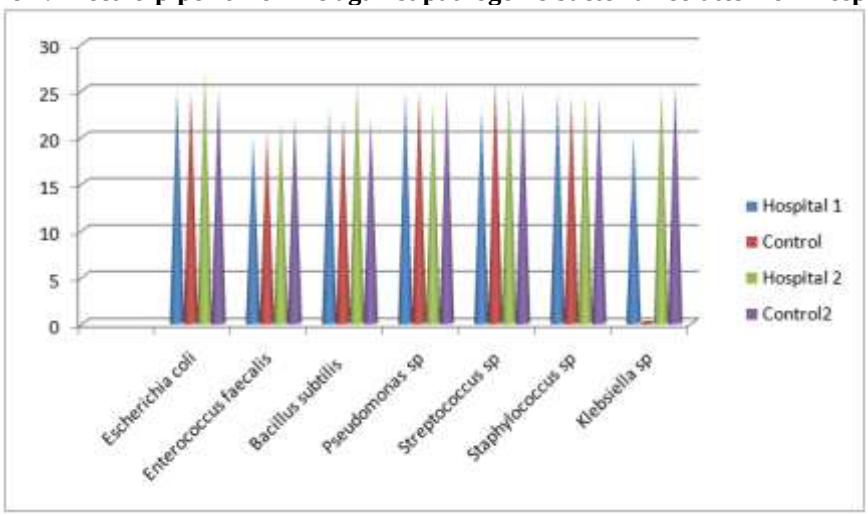
M.F.: $C_{23}H_{22}N_2O_2S$	**m.p. (^{0}C) :** 196-198	**Yield (%):** 50	**Structure**
^{1}H NMR($CDCl_3$, ppm);™: 2.82 (d, 2H, H-3), 2.61 (d, 1H, H-5a),3.43(d,1H,H5e) 3.80 (s, 2H, H-8),5.13 (s,1H,H-2) 6.30 (s, 1H, H-6), 10.00(s,1H,OH),6.69-7.73 (aromatic protons); **^{13}C NMR ($CDCl_3$, ppm);** ™: 36.3 (C-8), 27.5(C-3)29.7(C-5), 58.0 (C-2, C-6), 172.1 (C-7), 155.3 (C=N), 125.0-142.1 (aromatic carbons). **Mass : 390.5 (calculated),**			

Substitution on the piperidin-4-one by thiophene and amides are considered to be key factors in controlling the wound infecting pathogens. Thiophene and amides high intensive moieties which modulate the electronic effects on heterocyclic rings of the synthesized compound. These atoms may also influence the steric characteristics and the hydrophilic–hydrophobic balance of the molecule.

Similar trend of results were recorded in previous experiments[18-20] who synthesised (E)-1-(1H-indol-3-yl) ethanone O-benzyl oxime derivatives and studied antibacterial evaluation against MRSA and VRSA strains. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) exhibit a positive effect in controlling Mycobacterium tuberculosis.

1,2,4-triazolylchromanone oxime ethers were synthesized by [21] and tested for in vitro pharmacological activity against Candida albicans, Saccharomyces cerevisiae, Aspergillusniger and Microsporumgypseum.

Figure-2.Effect ofpiperidinoxime against pathogenic bacterial isolates from hospitals



The pharmacological activity in terms of maximum zone of inhibition by the Kanamycin Escherichia coli for is 19.8 mg/ml. Concentrations of 25,50,75,100 and 125 µg/ml of piperidineoxime when compared to control the compound has the maximum control over

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Streptococcus pyogens at the rate ranging from 20.6 mm 26.2 mm whereas the compound showed minimum activity the terms of zone of inhibition of 19 mm on Staphylococcus aureus and 19.4 mm on Pseudomonas aeruginosa , respectively (Table 2) and (Figure- 2).

The concentrations of piperidineoxime were tested from 10µg/ml to 125 µg/ml.There was no or less activity below 25µg/ml where as the concentrations ranging from 25 to 125 µg/ml of piperidineoxime were effectively control the wound pathogens.

Earlier experiments of [22-27] on synthesis of three series of oxime ethers viz, 2,6-diarylpiperidin-4-one O-benzyloximes 5a-o, 2,6-diaryl tetrahydropyran-4-one O-benzyloximes 7a-e and 2,6-diaryltetrahydrothiopyran-4-one O-benzyloximes 11a-b and 12a-c and stereochemistry was established.They had experimented the antibiotic activity which was moderate compared to Ciprofloxacin and Amphotericin B.

Table-2. Effect of Kanamycin and Piperidin 4 -one oxime against Bacterial pathogens

Bacterial Pathogens	Hospital 1	Control	Hospital 2	Control
	5.0mg/ml	10 mg/ml	5.0 mg/ml	10 mg/ml
Escherichia coli	25.2 ±0.01	25.0	26.9 ±0.03	25.0
Enterococcus faecalis	20.0 ±0.04	20.4	21.4 ±0.07	22.1
Bacillus subtilis	23.0 ±0.01	22.0	25.9 ±0.05	22.0
Pseudomonas sp	24.8 ±0.07	25.0	23.5 ±0.03	25.1
Streptococcus sp	23.1 ±0.04	26.0	25.0 ±0.04	24.9
Staphylococcus sp	24.8 ±0.04	24.2	24.5 ±0.04	24.3
Klebsiellasp	20.0 ±0.08	25..5	25.1 ±0.01	25.5

* Data represented as mean values ± standard derivation, Significance level at $p<0.05$

Literature recorded for the synthesised piperidine derived/substituted compounds and their pharmacological activities are as follows:

Hemaet. al.2005[2] reported the crystal structure of t-3-methyl-1-nitroso-r-2,c-6-diphenyl piperidin-4-one oxime monohydrate. They concluded the molecular formula of corresponding compound as $C_{18}H_{19}N_3O_2.H_2O$ and the piperidine ring adopts a distorted boat conformation.

Ramalingan et al., 2006[3] synthesized and characterized series of substituted piperidin-4-one oxime ethers and their antimicrobial activity is closure to the standard drugs.

Letafat et al., 2007 [5]synthesised and investigated the antibacterial activity of new N-[2-(thiophen-3-yl)ethyl] piperazinyl quinolones.

The synthesis, in vitro antifungal activity, and molecular docking experiments of some oxime and oxime ether derivatives of azole 1,4-benzothiazine are reported by Milanese et al., 2007[17].

Sukhorukov and Ioffe,2011 [6]synthesized six-membered cyclic oxime ethers and experimented the antimicrobial activity.

Haideret al.2014 [18] synthesized 4-(1-Pyrrolidinyl) piperidinederivtaives and studied its effect on selected bacteria and fungi.

In the present study,compare to control Kanamycin (10mg/ml) the synthetic piperidine derivative (100µg/ml) was more efficient to inhibit the growth of bacterial pathogens.

The present study was supported by the previous investigations of[8],[13],[15],[23]studied the pharmacological profile of 6-Benzoyl-benzothiazol-2-one scaffold.Su et al.,2021 designed and synthesised penta-1,4-diene-3-one derivatives containing quinazoline and oxime ether fragments. Various Piperidine-4-One compounds and their derivatives were synthesised and characterized by NMR and it was experimented against the bacterial pathogens from the hospital environments[24-32].

IV. CONCLUSION

Pharmacological activity of synthetic piperidineoxime against bacterial isolates from hospitals in terms of zone of inhibition in mm was resulted in the following order:

Escherichia coli >Streptococcus pyogens>Staphylococcusaureus>Pseudomonas aeurigenosa>Enterococcussp>Bacillussp>Klebsiellasp

Hence from the present study it is confirmed that the heterocyclic benzylic protons at C-2 and C-6 position act as highly functionalized scaffolds including the piperidine moiety with oxime.These compounds are recommended as the potential candidates for controlling bacterial

pathogens and can be used as pharmacophore for the designing and preparation of drugs.

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Data Availability Statement
All experimental data of this study are available within the research article itself.

Conflict of Interests
There are no conflicts of interest.

REFERENCES

[1]. Akunuri R., Veerareddy V., Kaul G., Akhir A., Unnissa T., Parupalli R., Madhavi Y.V., Chopra S., Nanduri S. 2021.Synthesis and antibacterial evaluation of (E)-1-(1H-indol-3-yl) ethanone O-benzyl oxime derivatives against MRSA and VRSA strains. Bioorganic Chem. ;116:105288. doi: 10.1016/j.bioorg.2021.105288.

[2]. Hema, Ragavi&Parthasarathi, V. &Ravikumar, Kakara&Pandiarajan, Karuppiah&Muthukumaran, G.. (2005). t -3Methyl1-nitroso- r -2, c -6-diphenylpiperidin-4-one oxime monohydrate. ActaCrystallographica Section E-structure Reports Online - ACTA CRYSTALLOGR E-STRUCT REP. 61. 10.1107/S1600536805038444/ci6707Isup2.hkl.

[3]. Ramalingan C, Park YT, Kabilan S. 2006. Synthesis, stereochemistry, and antimicrobial evaluation of substituted piperidin-4-one oxime ethers. Eur J Med Chem. 2006 Jun;41(6):683-96. doi: 10.1016/j.ejmech.2006.02.005. Epub Apr 4. PMID: 16600438.

[4]. Tu S., Xie Y.-Q., Gui S.-Z., Ye L.-Y., Huang Z.-L., Huang Y.-B., Che L.-M. Synthesis and fungicidal activities of novel benzothiophene-substituted oxime ether strobilurins. Bioorganic Med. Chem. Lett. 2014;24:2173–2176. doi: 10.1016/j.bmcl.2014.03.024.

[5]. Letafat B., Emami S., Mohammadhosseini N., Faramarzi M.A., Samadi N., Shafiee A., Foroumadi A. 2007. Synthesis and antibacterial activity of new N-[2-(thiophen-3-yl)ethyl] piperazinyl quinolones. Chem. Pharm. Bull. ;55:894–898. doi: 10.1248/cpb.55.894.

[6]. Sukhorukov AY, Ioffe SL. 2011.Chemistry of six-membered cyclic oxime ethers. Application in the synthesis of bioactive compounds. Chem Rev. 2011 Aug 10;111(8):5004-41. doi: 10.1021/cr100292w. Epub Mar 24. PMID: 21434602.

[7]. Emami S, Falahati M, Banifatemi A, Amanlou M, Shafiee A. 2004. (E)- and (Z)-1,2,4-triazolylchromanone oxime ethers as conformationally constrained antifungals. Bioorg Med Chem. Aug 1;12(15):3971-6. doi: 10.1016/j.bmc.2004.06.010. PMID: 15246073.

[8]. Hurtevent A., Le Naour M., Leclerc V., Carato P., Melnyk P., Hennuyer N., Staels B., Beucher-Gaudin M., Caignard D.-H., Dacquet C., 2020. Effect of 6-Benzoyl-benzothiazol-2-one scaffold on the pharmacological profile of α-alkoxyphenylproponic acid derived PPAR agonists. J. Enzym. Inhib. Med. Chem. ;35:524–538. doi: 10.1080/14756366.2020.1713771.

[9]. Surkau G., Böhm K.J., Müller K., Prinz H. 2010.Synthesis, antiproliferative activity and inhibition of tubulin polymerization by anthracenone-based oxime derivatives. Eur. J. Med. Chem. ;45:3354–3364. doi: 10.1016/j.ejmech.2010.04.019.

[10]. Park H.-J., Lee K., Park S.-J., Ahn B., Lee J.-C., Cho H., Lee K.-I. 2005. Identification of antitumor activity of pyrazoleoxime ethers. Bioorganic Med. Chem. Lett. ;15:3307–3312.doi: 10.1016/j.bmcl.2005.03.082.

[11]. Jindal D.P., Chattopadhaya R., Guleria S., Gupta R. 2003.Synthesis and antineoplastic activity of 2-alkylaminoethyl derivatives of various steroidal oximes. Eur. J. Med. Chem. ;38:1025–1034. doi: 10.1016/j.ejmech.2003.09.002.

[12]. Ajduković J.J., Jakimov D.S., Rárova L., Strnad M., Dzichenka Y.U., Usanov S., Škorić D.Đ., Jovanović-Šanta S.S., Sakač M.N. Novel alkylaminoethyl derivatives of adrostane 3-oximes as anticancer candidates: Synthesis and evaluation of cytotoxic effects. RSC

Adv. 2021;11:37449–37461.
doi: 10.1039/D1RA07613B.

[13]. Su S., Chen M., Li Q., Wang Y., Chen S., Sun N., Xie C., Huai Z., Huang Y., Xue W. Novel penta-1,4-diene-3-one derivatives containing quinazoline and oxime ether fragments: Design, synthesis and bioactivity. Bioorganic Med. Chem. 2021;32:115999.
doi: 10.1016/j.bmc.2021.115999.

[14]. Ma J., Ma M., Sun L., Zeng Z., Jiang H. Synthesis, herbicidal evaluation, and structure-activity relationship of benzophenoneoxime ether derivatives. J. Chem. 2015;2015:435219.
doi: 10.1155/2015/435219.

[15]. Zarenezhad E., Sadeghian S., Shekoohi K., Emami I., Ghasemian A.M., Zarenezhad A. Synthesis, Biological Evaluation and In Silico Studies of Oxime Ether Derivatives Containing a Quinoxaline Moiety. Russ. J. Bioorganic Chem. 2023;49:101–113.
doi: 10.1134/S1068162023010326.

[16]. Parthiban P., Rathika P., Ramkumar V., Son S.M., Jeong Y.T. Stereospecific synthesis of oximes and oxime ethers of 3-azabicycles: A SAR study towards antimicrobial agents. Bioorganic Med. Chem. Lett. 2010;20:1642–1647.
doi: 10.1016/j.bmcl.2010.01.048.

[17]. Milanese L, Giacchè N, Schiaffella F, Vecchiarelli A, Macchiarulo A, Fringuelli R. Oxime and oxime ether derivatives of 1,4-benzothiazine related to oxiconazole. ChemMedChem. 2007 Aug;2(8):1208-13. doi: 10.1002/cmdc.200700066. PMID: 17541993.

[18]. Ghabbour H.A., El-Bendary E.R., El-Ashmawy M.B., El-Kerdawy M.M. Synthesis, docking study and β-adrenoceptor activity of some new oxime ether
derivatives. Molecules. 2014;19:3417–3435. doi: 10.3390/molecules19033417.

[19]. Rastogi, S.; Rastogi, H. An efficient synthesis of some substituted piperidine-4-one thiosemicarbazone derivatives as potential anti convulsant under microwave irradiation. Indian J. Chem. 2010, 49, 547-553.

[20]. Chakkaravarthy, J.; Muthukumaran, G.; Pandiarajan, K. 2008. Conformational study of some N-acyl-2r,6cdiphenylpiperidin-4-one oximes using NMR spectra. J. Mol. Struct., 889, 297-307.

[21]. Rameshkumar, N.; Veena, A.; Ilavarasan, R.; Adirja, M.; Shanmugapandian, P.; Sridhar, S. K. Synthesis and biological activities of 2,6-diaryl-3-methyl-4-piperidone derivatives. Biol. Pharm. Bull. 2003, 26, 188-193.

[22]. Vimalraj, V.; Pandiarajan, K.; Ahmad Bhat , I.2010. Synthesis and structural study of 3t-isopropyl-2r,6cdiphenyl-4-oxo piperidinium nitrate and N-benzoyl-3t-isopropyl-2r,6c-diphenyl piperidine-4-one oxime. J.Mol. Struct., 975, 137-146.

[23]. Jayabharathi J, Manimekalai A, Vani TC, Padmavathy M (2007) Synthesis,stereochemistry and antimicrobial evaluation of t(3)-benzyl-r(2), c(6)-diarylpiperidin-4-one and its derivatives. Eur J Med Chem 42:593–605.

[24]. T Yasodha and R Venkateswara Moorthy.2021.Synthesis, Characterization and Antibacterial Evaluation of 3-Allyl 2,6-Bis(4-Fluorophenyl) Piperidine-4-One. Acta Scientific Pharmacology 2.8 **:** 48-54.

[25]. Abate G, Mshana RN, Miörner H (1998). Evaluation of a colorimetric assay based on 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) for rapid detection of rifampicin resistance in Mycobacterium tuberculosis. Int J Tuberc Lung Dis. 2(12):1011-6

[26]. Amr, A. E.-G. E.; Sabry, N. M.; Abdulla, M. M. Synthesis, reactions, and anti-inflammatory activity of heterocyclic systems fused to a thiophene moiety using citrazinic acid as synthon. Monatsh.Chem. 2007, 138, 699−707.

[27]. Seck R, Gassama A, Cojean S, Cave C (2020) Synthesis and antimalarial activity of 1,4-disubstituted piperidine derivatives. Molecules 25:21–26.

[28]. McArdell, C.S., Kovalova, L., Siegrist, H., Kienle, C., Moser R., Schwartz, T., 2011. 'Input and elimination of pharmaceuticals and disinfectants from hospital wastewater', EAWAG final project report, July 2011, 95p.

[29]. Verlicchi, P., Galleti, A., Petrovic, M., Barceló, D., 2010. 'Hospital effluents as a source of emerging pollutants: an overview of micropollutants and sustainable treatment options'. J. Hydrol., 389 (3-4), 416-428.

[30]. Datta PThakur A and Mishra B .2004. Prevalence of Clinical strains resistant to various beta-lactams in a tertiary care hospital in India. Ind. J Med Microbiol . 57:146 – 9.

[31]. Mullot, J., Karolak, S., Fontova, A., Levi, Y., 2010. 'Modelling of hospital wastewater pollution by pharmaceuticals: First results of Mediflux study carried out in three French hospitals'. Water Sci. Technol., 62 (12), 2912-2919.

[32]. Lindberg, R., Jarnheimer, P., Olsen, B., Johansson, M., Tysklind, M., 2004. 'Determination of antibiotic substances in hospital sewage water using solid phase extraction and liquid chromatography/mass spectrometry and group analogue internal standards'. Chemosphere, 57 (10), 1479–1488.