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Synthesis and biological investigation of isoxazolo[4,5-e][1,2,4]triazine derivatives

Ajay N. Ambhore[1],Vishwash D. Surywanshi[1], Rahul D. Kamble[2], Shrikant V. Hese[2], Pratima P. Mogle[2], Shital S. Kadam[2] and Bhaskar S. Dawane*[2]

[1]S. M. Dr Bapuji Salunke College, Miraj. Dist Sangli (MS), India
[2]School of Chemical Sciences, SRTM University, Nanded (MS), India

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ABSTRACT

In present report we have successfully synthesized isoxazolo[4,5-e][1,2,4]triazine derivatives in quantitative yields using PEG-400 as green reaction medium. The synthesized compounds were screened for antimicrobial against selected bacterial strains. The results of anti-bacterial activity revealed that some of the compounds were found to be active antibacterial agent.

Keywords: isoxazolo[4,5-e][1,2,4]triazine, PEG-400, Antibacterial activity

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INTRODUCTION

Heterocyclic chemistry acquired a unique place in the field of medicine and pharmaceutical, which comprises wide number of organic compounds containing one or more heterocyclic ring imparting the various biological activities. There are hardly very few numbers of drugs which does not contain the heterocyclic compounds.

The triazine is a six-membered heterocyclic ring, analogous to the benzene ring but with three carbons replaced by nitrogens. The three isomers of triazine are distinguished from each other by the positions of their nitrogen atoms, and are referred to 1,2,3-triazine,1,2,4-triazine and 1,3,5-triazine [1] **Figure 1**.

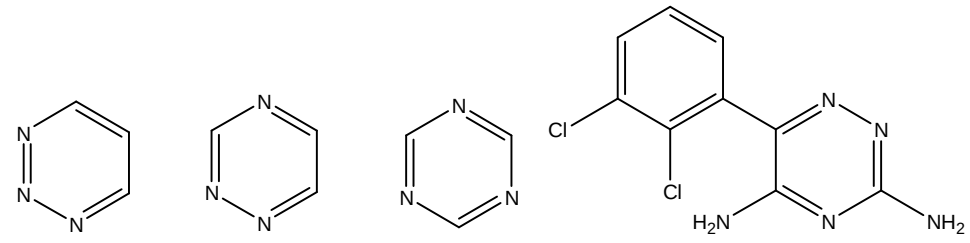


Figure 1: Isomeric forms of Triazinea-c, anti-anxiety drug d

Among the isomeric forms of the triazine, 1,2,4-triazine occupied unique position in the field of medicinal chemistry due to their biological interest [2].1,2,4-Triazines and their derivatives have been widely studied in terms of their syntheticmethodologies and reactivity since some of these derivatives were reported to have promising biological activities [3-5]. 1,2,4-Triazine derivatives have been reportedto possess a broad spectrum of biological activities, including antifungal [6, 7], anti-HIV [8], anticancer [9], antianxiety [10] anti-inflammatory [11], analgesic [12] and

antihypertensive activities [13]. Besides this, triazineswere used as herbicides, pesticides and dyes [14,15].Lamotrigine [16] (**Fig 1d**), is ananticonvulsant drug used in the treatment of epilepsy [17] and bipolar disorder contains 1,2,4-triazine scaffold.

These above observations prompted us to synthesize isooxazole derivatives of triazine derivatives as antimicrobial agents.

MATERIALS AND METHODES

Chemistry

All reagents were obtained from commercial suppliers and used without further purification. Reaction progress was monitored through thin layer chromatography (TLC) on pre-coated Merck alu-foil plate (silica gel 60F-254, 0.25 mm thickness) visualized by iodine vapors.Melting pointswere determined by open capillary method and areuncorrected.IR spectra were recorded (in KBr pallets) onSCHIMADZU spectrophotometer.^{1}H NMR spectra were recorded on an Avance/Bruker 300/400 MHz spectrophotometerusing TMS as an internal standard. All NMR spectra were obtained in DMSO d_6/deuterated chloroform ($CDCl_3$); chemical shifts are reported in parts per million, and coupling constant in hertz (Hz). Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), m (multiplet). The mass spectra wererecorded on GC–MS SHIMDZU (Q2010 PLUS) in EImode spectrometer and mass values are reported in m/z.

General Procedure for preparation of 5a-j

A mixture of **4a-j** (1 mmol) and hydroxylamine hydrochloride (1.2 mmol) was refluxed in PEG-400as green reaction solvent (10 mL) in the presence of NaOH for 3 hours. After completion (TLC), the reaction mixture was poured in ice cold water, solid separated out. The separated solid was filtered; the crude product was recrystallized from ethanol to afford the pure product (**5a-l**). The remaining derivatives were also prepared by the same procedure.

General Procedure for preparation of 6a-j

A mixture of 5**a-j** (1 mmol) secondary amines and paraformaldehyde (excess) was refluxed in ethanol (10 mL) for 3 hours. After completion (TLC), the reaction mixture was poured in ice cold water, solid separated out. The separated solid was filtered; the crude product was recrystallized from ethanol-chloroform mixture to afford the pure product (**6a-l**). The remaining derivatives were also prepared by the same procedure.

Spectral data of some selected compounds

3-[3-(4-Chloro-phenyl)-1-phenyl-1H-pyrazol-4-yl]-5,6-diphenyl-6,7-dihydro-isoxazolo[4,3-e][1,2,4]triazine (**5a)** IR (KBr, vmax cm^{-1}); 3321, 3034, 1607, 754: ^{1}HNMR (DMSO d^6, 400 Hz) δ 8.45 (s, 1H, thiazole), 8.39 (s, 1H, NH), 7.23-7.57 (m, 19 H, Ar-H); ESMS for Molecular Formula$C_{31}H_{21}ClN_6O$; 528.

*3-[3-(4-Nitro-phenyl)-1-phenyl-1H-pyrazol-4-yl]-5,6-diphenyl-6,7-dihydro-isoxazolo[4,3-e][1,2,4]triazine***(5c)**IR (KBr, vmax cm^{-1}); 3331, 3031, 1607, 1532: ^{1}HNMR (DMSO d^6, 400 Hz) δ 8.47 (s, 1H, thiazole), 8.40 (s, 1H, NH), 7.20-7.66 (m, 19 H, Ar-H); ESMS for Molecular Formula $C_{31}H_{21}N_7O_3$;539.

*3-[3-(3-Nitro-phenyl)-1-phenyl-1H-pyrazol-4-yl]-5,6-diphenyl-6,7-dihydro-isoxazolo[4,3-e][1,2,4]triazine***(5d)**IR (KBr, vmax cm^{-1}); 3312, 3042, 1610, 1530: ^{1}HNMR (DMSO d^6, 400 Hz) δ 8.42 (s, 1H, thiazole), 8.40 (s, 1H, NH), 7.31-7.79 (m, 19 H, Ar-H); ESMS for Molecular Formula $C_{31}H_{21}N_7O_3$; 539.

*3-[3-(4-Methyl-phenyl)-1-phenyl-1H-pyrazol-4-yl]-5,6-diphenyl-6,7-dihydro-isoxazolo[4,3-e][1,2,4]triazine***(5f)**IR (KBr, vmax cm^{-1}); 3321, 3034, 2922, 1608: ^{1}HNMR (DMSO d^6, 400 Hz) δ 8.47 (s, 1H, thiazole), 8.40 (s, 1H, NH), 7.21-7.59 (m, 18 H, Ar-H), 2.31 (s, 3H, CH_3); ESMS for Molecular Formula $C_{32}H_{24}N_6O$; 508.

*3-[3-(4-Methoxy-phenyl)-1-phenyl-1H-pyrazol-4-yl]-5,6-diphenyl-6,7-dihydro-isoxazolo[4,3-e][1,2,4]triazine***(5g)** IR (KBr, vmax cm^{-1}); 3314, 3022, 2954, 1607, 1178: ^{1}HNMR (DMSO d^6, 400 Hz) δ 8.46 (s, 1H, thiazole), 8.41 (s, 1H, NH), 7.13-7.53 (m, 18 H, Ar-H), 3.41 (s, 3H, OCH_3); ESMS for Molecular Formula $C_{32}H_{24}N_6O_2$; 524.

*3-[3-(4-Chloro-phenyl)-1-phenyl-1H-pyrazol-4-yl]-5,6-diphenyl-7-piperidin-1-ylmethyl-6,7-dihydro-isoxazolo[4,3-e][1,2,4]triazine***(6a)** IR (KBr, vmax cm^{-1}); 3032, 2958, 1609, 756: ^{1}HNMR (DMSO d^6, 400 Hz) δ 8.45 (s, 1H, thiazole), 7.23-7.57 (m, 19 H, Ar-H), 4.13 (s 2H, CH_2), 2.24 (t, 4H, CH_2), 1.50 (m, 6H, CH_2); ESMS for Molecular Formula for; $C_{37}H_{32}ClN_7O$; 626.

*3-[3-(4-Nitro-phenyl)-1-phenyl-1H-pyrazol-4-yl]-5,6-diphenyl-7-piperidin-1-ylmethyl-6,7-dihydro-isoxazolo[4,3-e][1,2,4]triazine***(6c)** IR (KBr, vmax cm^{-1}); 3029, 2930, 1605, 1530: ^{1}HNMR (DMSO d^6, 400 Hz) δ 8.45 (s, 1H,

thiazole), 7.26-7.69 (m, 19 H, Ar-H), 4.11 (s 2H, CH_2), 2.27 (t, 4H, CH_2), 1.48 (m, 6H, CH_2); ESMS for Molecular Formula; $C_{37}H_{32}N_8O_3$;636.

*3-[3-(3-Nitro-phenyl)-1-phenyl-1H-pyrazol-4-yl]-5,6-diphenyl-7-piperidin-1-ylmethyl-6,7-dihydro-isoxazolo[4,3-e][1,2,4]triazine***(6d)** IR (KBr, vmax cm^{-1}); 3032, 2943, 1609, 1532: ^{1}HNMR (DMSO d^6, 400 Hz) δ 8.46 (s, 1H, thiazole), 7.25-7.72 (m, 19 H, Ar-H) 4.14 (s 2H, CH_2), 2.23 (t, 4H, CH_2), 1.53 (m, 6H, CH_2); ESMS for Molecular Formula; $C_{37}H_{32}N_8O_3$;636.

*3-[3-(4-Methyl-phenyl)-1-phenyl-1H-pyrazol-4-yl]-5,6-diphenyl-7-piperidin-1-ylmethyl-6,7-dihydro-isoxazolo[4,3-e][1,2,4]triazine***(6f)** IR (KBr, vmax cm^{-1}); 3030, 2950, 1609: ^{1}HNMR (DMSO d^6, 400 Hz) δ 8.48 (s, 1H, thiazole), 7.26-7.69 (m, 19 H, Ar-H), 4.23 (s 2H, CH_2), 2.30 (s, 3H, CH_3), 2.27 (t, 4H, CH_2), 1.48 (m, 6H, CH_2); ESMS for Molecular Formula$C_{38}H_{35}N_7O$;605.

*3-[3-(4-Methoxy-phenyl)-1-phenyl-1H-pyrazol-4-yl]-5,6-diphenyl-7-piperidin-1-ylmethyl-6,7-dihydro-isoxazolo[4,3-e][1,2,4]triazine***(6g)** IR (KBr, vmax cm^{-1}); 3039, 2941, 1609, 1132: ^{1}HNMR (DMSO d^6, 400 Hz) δ 8.45 (s, 1H, thiazole), 7.15-7.52 (m, 19 H, Ar-H) 4.14 (s 2H, CH_2), 3.42(s, 3H, OCH_3), 2.23 (t, 4H, CH_2), 1.53 (m, 6H, CH_2); ESMS for Molecular Formula$C_{38}H_{35}N_7O_2$; 621.

Biology

The antimicrobial activities of the synthesized compounds (IIIa–p) were determined by agar diffusion method as recommended bythe National Committee for Clinical Laboratory Standards, (NCCLS)[18–20]. The compounds were evaluated for antimicrobial activity against bacteria viz. *Escherichia coli* (MTCC 2939), *Salmonella typhi*(MTCC 98), *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 441). The antibiotic Tetracycline (25 mg/mL) is used as reference antibacterial drug for comparison. Dimethylsulphoxide (1%, DMSO) was used a control.

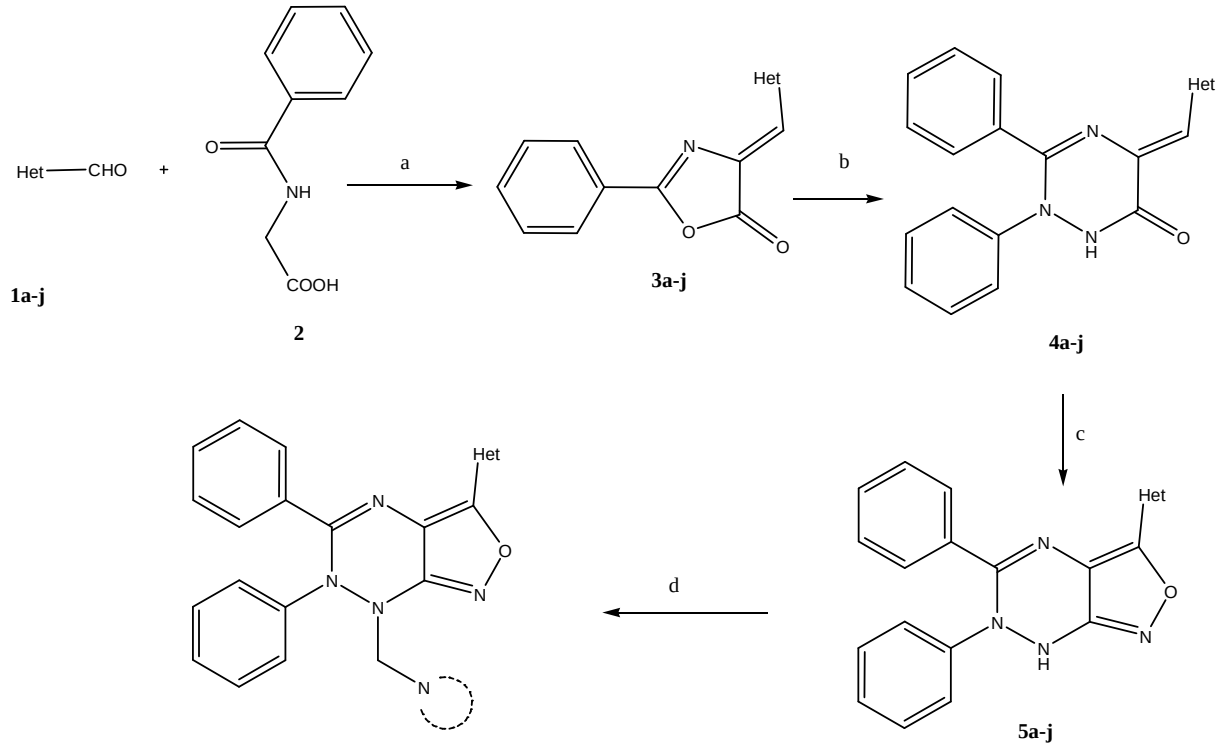
The culture strains of bacteria were maintained on nutrient agar slant at 37±0.5˚C for 24 h. The antibacterial activitywas evaluatedusing nutrient agar plate seeded with 0.1 mL of respective bacterialculture strain suspension prepared in sterile saline (0.85%) of105 CFU/mL dilutions. The wells of 6 mm diameter were filled with0.1 mL of target compound dilution ranging from 25 to 250 mg/mLseparately for each bacterial strain. All the plates were incubated at 37±0.5˚C for 24 h. Zone of inhibition and minimum inhibitoryconcentrations (MICs) were noted. The results of antibacterialstudies are given in Table 2.

RESULTS AND DISCUSSION

Chemistry

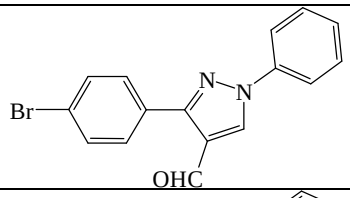
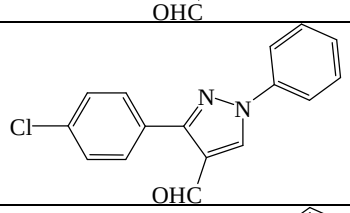
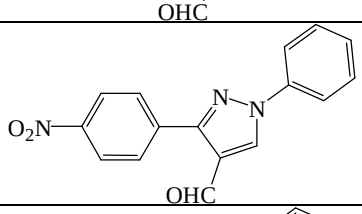
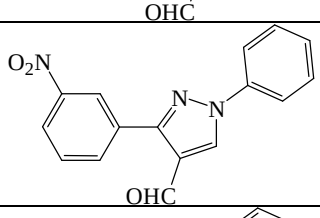
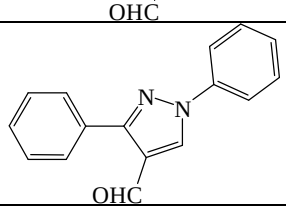
As part of our research programme, and in continuation of our work on the development of environmentally friendly methodologies for the preparation of biologically active compounds [21-24], herein we report an efficient synthesis ofisoxazolo[4,5-e][1,2,4]triazine derivatives. The title compounds were synthesized in three steps with good yield (**Table-1**). Initially, substituted heteroaldehydes**(1a-j)**were condensed with hippuric acid**(2)** in the presence of sodium acetate in acetic anhydride. The reaction follows the Knowengeal condensation followed by acid catalysed cyclization in the presence of acetic anhydride by the loss of the water molecule to afford **3a-j** in quantitative yields. Furthermore, the formation of **4a-j** was proceeding through the nucleophilic attack of phenyl hydrazine on**3a-j**which undergoes acid catalysed ring expansion to give **4a-j.** This**4a-j** was used as precursor for the synthesis of title compound which was achieved by the reaction of **4a-j** with hydroxyl amine hydrochloride under neutral condition.

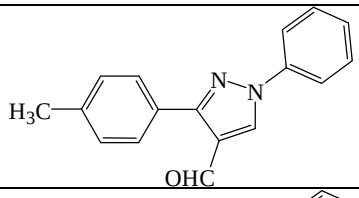
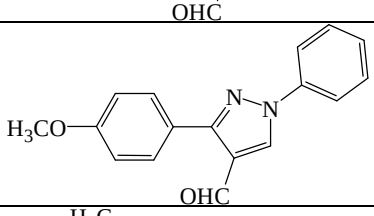
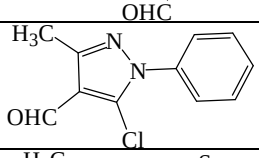
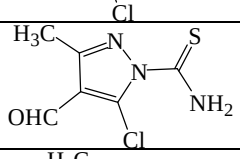
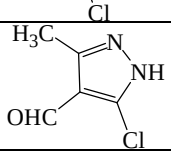
The structures of synthesized compounds were established on satisfactory spectral analysis. The formation of **4a-j** was confirmed by the IR, NMR and mass spectral analysis. The IR spectra of **4a** indicate presence of a sharp peak at 3342 cm^{-1} due to presence of –NH group, the peak at 1678 cm^{-1} was attributed for the presence of C=O group. Furthermore, ^{1}HNMR shows a down field peak at 8.43 δ ppm due to presence of –NH proton, a presence of sharp singlet at 7.12 δ ppm confirms the presence of C=C-H proton and all other protons are appeared at their respected region. Mass spectrum revealed that the molecular weight of the compound was corresponds to the molecular ion peaks. The formation of **5a-j** was confirmed by the IR spectra, the disappearance of peak from 1678 cm^{-1} confirms the formation of **5a-j.** The disintegration of singlet from 7.12 in ^{1}HNMR spectrum further supports formation of **5a-j.** Mass spectra were in accordance with molecular weight of the compounds.



Scheme 1: Synthesis of isoxazolo[4,5-e][1,2,4]triazine derivatives; a) NaOAc/Ac_2O, 2-3 hrs; b) Phenyl hydrazine/ NaOAc/AcOH, 3 hrs, reflux; c) $NH_2OH.HCl$/NaOH/ PEG-400, 3 hrs, reflux. d) Pipyridine/ 1,2,4-triazine/ Morpholine, paraformaldehyde /EtOH, Reflux.

Table 1: Physical data of isoxazolo [4,5-e][1,2,4]triazine derivatives

Sr. No.	Product	Ar/Het	Yields in %	MP in ˚C
1	**6a**		82	168-171
2	**6b**		84	155-158
3	**6c**		80	175-178
4	**6d**		88	169-171
5	**6e**		79	140-143

6	**6f**		78	149-152
7	**6g**		86	160-163
8	**6h**		85	155-158
9	**6i**		84	162-165
10	**6j**		81	152-155

The IR spectrum of **6a-j** reveals the absence of peak at from 3342 cm^{-1} and singlet from 8.43 δ ppm in the ^{1}HNMR and the presence of singlet at 1.45 δ ppm in ^{1}HNMR for six proton of two $–CH_3$ group and singlet at 4.21 δ ppm confirms the presence of $–CH_2$- proton. The mass spectra of the compounds were in accordance with molecular weight of the compounds.

Biology

The synthesized compounds were screened for their antimicrobial potency against selected microbial strains. The results of *in vitro* antibacterial activities of compounds(**6a-j**) against various bacterial strains are summarized in Table 2.It has been observed that some of compounds exhibited interesting antibacterial activities. Compounds **6a**, **6b**, **6f**, **6g**, **6i** and **6j**showed effective activity against *E. coli*, and compounds **6a**, **6f**,**6g** and **6i** were displayed a good zone of inhibition against *B. subtilis*. Compounds **6b**, **6g** and **6j** displayed a slightly active towards *S. typhi* and *S. aureus*. Compounds **6c**, ***6e***, and **6h**were displayed less active against all tested bacteria. On the other hand, it was found that compounds **6a**, **6d**, **6g**and **6j** were showed stronger inhibitory activity against all bacteria than other compounds.

Table 2. Antibacterial Activity of isoxazolo[4,5-e][1,2,4]triazine derivatives

Compounds	EC	St	Sa	Bs
6a	18	12	16	18
6b	16	10	12	14
6c	20	16	18	21
6d	21	18	16	15
6e	14	09	12	-
6f	18	16	14	19
6g	15	12	11	18
6h	12	-	10	08
6i	16	14	18	16
6j	14	15	15	12
Tetracyclin	30	28	30	32

Zone of inhibition measured in mm; Ec-Escherichia coli; St-Salmonella typhi;Sa-Staphylococcus aureus; Bs-Bacillis subtilis;''–'' Indicates the concentration >100 mg/mL.

CONCLUSION

In summary, we have synthesized isoxazolo[4,5-e][1,2,4]triazine derivatives in quantitative yields. Furthermore, the antibacterial activity of compounds revealed that the compounds **6a, 6c, 6d, 6f,** and **6g** found to active against all selected bacterial strains. A cursory look at structure activity relationship revealed that for the manifestation of

antibacterial activity the groups like Cl, NO_2, methyl and methoxy have to incorporated with isoxazolo[4,5-e][1,2,4]triazine scaffold.

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REFERENCES

[1] H. Schroeder, C.Grundmann, *J. Am. Chem. Soc.*,**1956**, 78, 2447.
[2] K.Sztanke, S.Fidecka, E. Kedzierska,Z. Karczmarzyk, K. Pihlaja, D. Matosiuk,*Eur. J. Med. Chem.*,**2005**, 40, 127.
[3] A. K.Mansour, M. M.Eid, S. A. M.Khalil,*Nucleosides, Nucleotides & Nucleic Acids*,**2003**, 22, 21.
[4] K.Sztanke,S.Fidecka,E.Kedzierska,Z.Karczmarzyk,K.Pihlaja,D.Matosiuk,*Eur. J. Med. Chem.*,**2005**, 40, 127.
[5] C.Nyffenegger,G.Fournet,B.Joseph,*Tetrahedron Lett.*,**2007**, 48, 5069.
[6] M.Kidwai, Y.Goel, R. Kumar,*Indian J. Chem.*,**1998**, 37B, 174.
[7] B.S.Holla, R.Gonsalves, B.S.Rao, S.Shenoy, H.N.Gopalakrishna,*Farmaco*,**2001** 56, 899.
[8] R.M.Abdel-Rahman, J.M.Morsy, F.Hanafy,H.A.Amene,*Pharmazie*,**1999** 54, 347.
[9] M.W.Partridge, M.F.G.Stevens,*J. Chem. Soc*,**1966,** 1127.
[10]P.Mulick, S. A.Khan, T.Begum, S.Verma, D.Kausik, O.Alam,*ActaPoloniae Pharmaceutica-Drug Research*,**2009,** 66, 379.
[11]E.I.Abd, Z.K.Samii,*J. Chem. Technol. Biotechnol*, **1992,** 53, 143.
[12]M.P.Hay, F.B.Prujin, S.A.Gamage, H.D.Liyanage, W.R.Wilson, *J. Med. Chem.*,**2004**, 47,475.
[13]W.P.Heilman, R.D.Heilman, J.A.Scozzie,R.J.Wayner, J.M.Gullo, Z.S.Ariyan,*J. Med. Chem.*,**1979**, 22, 671.
[14]J.G.Erickson, *Chem. Heterocycl Comp*., **1956**, 10, 44.
[15]R.L.Jones, J.R.Kershaw,*Rev. Pure Appl.Chem.*,**1971**, 21, 23.
[16] W.A. R.Richard, B. R.Peter, J. F. Theodore,&E. B.Royal, *J Med Chem*,**1972,**15, 859.
[17]B.Michel, M.Fabienne,& H. J.Martine,Psychiatr. Neurosci, **2005**,30, 275.
[18]National Committee for Clinical Laboratory Standards **1998,** Performance Standards For Antimicrobial Susceptibility Testing. Eighth Information Supplement, Villanova (PA), NCCLS; (Publication no NCCLS M100–58).
[19] National Committee for Clinical Laboratory Standards, **1997**, Methods forAntimicrobial Susceptibility Testing Anaerobic Bacteria, Approved Standard-Fourth Edition, Villanova (PA), NCCLS; (Publication no NCCLS M 11–A4).
[20] National Committee for Clinical Laboratory Standards, **1992,** Methods forDetermining Bactericidal Activity of Antimicrobial Agents: Tentative Guidelines,Villanova (PA), NCCLS; (Publication no NCCLS M 26-T).
[21]P.P.Mogle, R.D.Kamble, S.V. Hese, B.S. Dawane, *Res ChemIntermed, 2014,* DOI 10.1007/s11164-014-1842-z.
[22]G.G.Mandawad,R.D.Kamble,S.V.Hese, R.A.More,R.N.Gacche,K.M.Kodam, B.S.Dawane, *Med Chem Res*, **2014,** 23, 4455.
[23] R.D.Kamble, S.V.Hese, R.J.Meshram, J.R.Kote, R.N.Gacche, B.S.Dawane,*Med Chem Res*,**2015,** 24, 1077.
[24]B.S.Dawane, S.N.Kadam, B.M.Shaikh, *Der Pharmacia Lettre***, 2010**, 2(4), 126