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SYNTHESIS AND *IN VITRO* STUDY OF ISOXAZOLE ANALOGUES BEARING 1, 2, 3-TRIAZOLE AS POTENT ANTIBACTERIAL AGENTS

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ABSTRACT

A new series of 3-(5-methyl-1-phenyl-1*H*-1,2,3-triazol-4-yl)-5-arylisoxazole **5(a-h)** have been synthesized and evaluated for their antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus, Micrococcus luteus*, *Proteus vulgaris, Salmonella typhimurium, Escherichia coli*. The antibacterial activity and the structure activity relationship revealed that, compounds that contain 4-methoxyphenyl (**5b**) and 2,6-difluorophenyl (**5c**) groups on isoxazole ring showed significant and equal activity against *B. subtilis, S. aureus, M. luteus and P. vulgaris* to that of standard drug. The other compounds also exhibited considerable antibacterial activities and therefore emerged as potential molecules.

Keywords: Isoxazole, Triazole, Synthesis, Antibacterial Activity.

1. INTRODUCTION

Isoxazole derivatives have played a crucial role in the history of heterocyclic chemistry and have been used extensively as important pharmacophore and synthons in the field of organic chemistry as well as in drug designing. Its derivatives have been widely investigated for therapeutic use like antiepileptic [1], PPAR-δ agonists [2], acetylcholine stearase (AchE) inhibitory [3], anti-inflammatory [4], acrosin inhibitory [5], antifungal [6], tools for Alzheimer's disease [7], protein tyrosine phosphate 1B inhibitory [8], antiviral [9], antihelmintics [10], antibacterial [11], anticonvulsant [12], insecticidal [13], antitubercular [14], immunomodulatory [15] and hypolipemics [16].

Similarly, the triazole ring is most important heterocycle which is most common and integral moiety of many natural products and medicinal agents. Its derivatives exhibited antitubercular [17], anti-HIV [18], antiallergenic [19], cytostatic [20], virostatic [21], anticancer [22], anticonvulsant [23], analgesic [24] and anti-inflammatory [25] activities. Triazoles are also being studied for the treatment of obesity [26] and osteoarthritis [27]. There are number of drugs, which are containing triazole nucleus, *viz*. Fluconazole [28], Isavuconazole [29], Itraconazole [30], Voriconazole [31], Pramiconazole [32] and Posaconazole [33], that have been used for the treatment of fungal infections.

In view of biological profile exhibited by the derivatives of isoxazole and triazole and in continuation of our work on the synthesis of new heterocyclic compounds [34-37], we designed a set of hybrid molecules with different heterocyclic scaffolds such as isoxazole and triazole with the aim of increasing their antibacterial activity. In the present study we report herein the synthesis of new series of 3-(5-methyl-1-phenyl-1*H*-1, 2,3-triazol-4-yl)-5-arylisoxazole **5(a-h)** and evaluation of their *in vitro* antibacterial activities.

2. MATERIALS AND METHOD

All reagents were of commercial grade and were used as supplied. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel F254 plates from Merck, and compounds visualized by exposure to UV light. Chromatographic columns 70-230 mesh silica gel for separations were used. IR spectra were recorded using KBr disk on a Perkin-Elmer FTIR spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Gemini spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C). Chemical shifts are reported in δ ppm units with respect to TMS as internal standard and coupling constants (*J*) are reported in Hz units. Mass spectra were recorded on a VG micro mass 7070H spectrometer.

2.1. Synthesis of 1-(5-methyl-1-phenyl-1*H*-1,2,3-triazol-4-yl)-1-ethanone (3)

To a mixture of phenylazide **1** (0.01 mol), acetylacetone

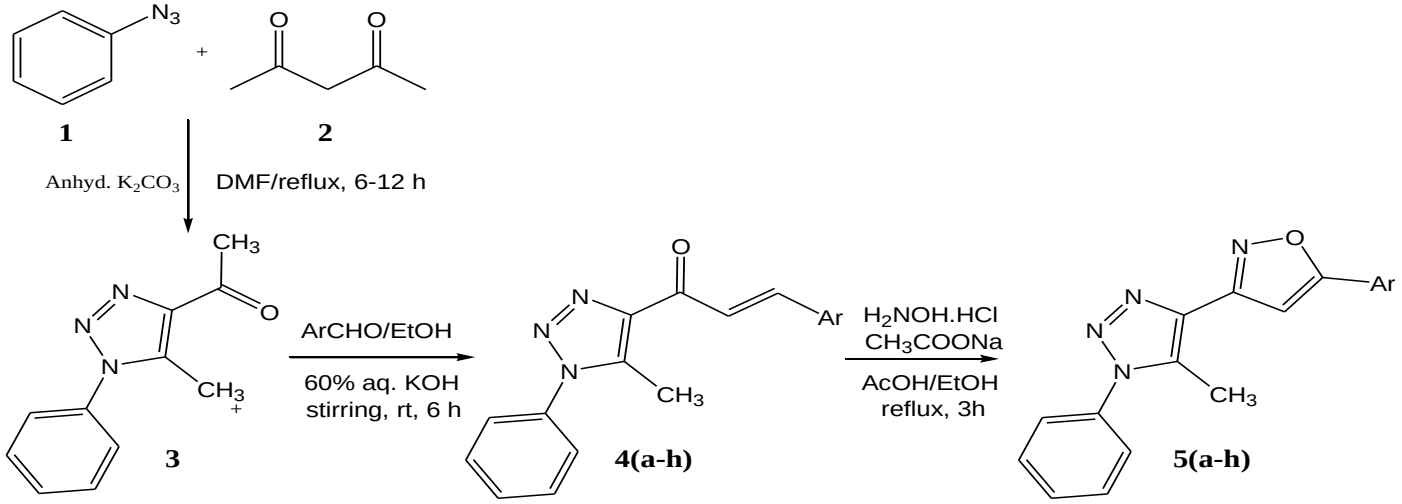
2 (0.02 mol) in DMF (30mL), anhydrous K_2CO_3 (0.06 mol) was added and with stirring at reflux temperature for 6-12 hours. The reaction mixture was cooled and the solvent was removed, poured into ice water and then neutralized with 5% hydrochloric acid and extracted with dichloromethane followed by purification by chromatographic column on silica gel using petroleum ether/ethyl acetate (8:1-6:1), afforded pure compound **3**. Yield 81%; IR (KBr) ν_{max}: 3057 (CH-Ar), 2978 (CH-Ali), 1714 (C=O), 1619 (C=N), 1548 (N=N), 1467 (C-N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.32 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 7.40-7.50 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.9, 29.6, 114.7, 128.8, 129.1, 134.3, 139.0, 139.9, 193.1; MS: *m/z* 199 (M^+).

2.2. General procedure for synthesis of (*E*)-1-(5-methyl-1-phenyl-1*H*-1,2,3-triazol-4-yl)-3-aryl-2-propen-1-one 4(a-h)

Compound **3** (0.01 mol) was dissolved in ethanol (20mL), to this solution an aryl aldehyde (0.01 mol) and potassium hydroxide (60%, 20mL) and stirred under room temperature for 6 hours. The reaction mixture was extracted with diethyl ether (3 x 20mL), the aqueous layer was neutralized with dilute hydrochloric acid and the solid obtained was purified by crystallization from benzene: methanol (3: 2), afforded corresponding compounds **4(a-h)** in 63-71% of yields.

2.3. General procedure for synthesis of 3-(5-methyl-1-phenyl-1*H*-1,2,3-triazol-4-yl)-5-arylisoxazole 5(a-h)

To a mixture of hydroxylamine hydrochloride (0.03 mol) in 10mL of ethanol and sodium acetate (0.06 mol) in 10mL of hot acetic acid, was added a solution of corresponding compound **4** (0.01 mol) in 10mL of ethanol. The reaction mixture was refluxed for 3 h. It was then diluted with water (100 mL) and neutralized with dilute NaOH solution. The product separated after acidification with HCl, was extracted with ether. Removed the solvent and purified by crystallization from ethanol, afforded corresponding compounds **5(a-h)** in 47-63% of yields.



Scheme 1: Synthetic route of title compounds 5(a-h)

2.4. Antibacterial Assay

The *in vitro* antibacterial activity of compounds **5(a-h)** were evaluated against Gram +ve bacteria viz., *Bacillus subtilis*, *Staphylococcus aureus, Micrococcus luteus* and Gram -ve bacteria viz., *Proteus vulgaris, Salmonella typhimurium, Escherichia coli* by broth dilution method. The bacteria were grown overnight in Luria Bertani (LB) broth at 37°C, harvested by centrifugation, and then washed twice with sterile distilled water. Stock solutions of the series of compounds were prepared in DMSO. Each stock solution was diluted with standard method broth (Difco) to prepare serial two-fold dilutions in the range of 50 to 0.8μg/mL. Ten microliters of the broth containing about 10^5 colony-forming units (cfu)/mL of test bacteria were added to each well of a 96-well microtiter plate. Culture plates were incubated for 24 h at 37°C and the growth was monitored by visually and spectrophotometrically. The minimal inhibitory concentration (MIC, μg/mL) of the compounds were measured and compared with standard drug.

3. RESULTS AND DISCUSSION

3.1. Chemistry

The intermediate required for the synthesis of title compounds has been synthesized from phenylazide **1** on reaction with acetylacetone **2** in the presence of anhydrous potassium carbonate in dimethylformamide under stirring at 70°C 6-12 hours to yield 1-(5-methyl-1-phenyl-1*H*-1,2,3-triazol-4-yl)-1-ethanone **3** in 81% of yield. The condensation of compound **3** with corresponding aromatic aldehyde in ethanol in the presence of 60% aq. potassium hydroxide at 5-10°C under stirring at room temperature for 6 h, afforded corresponding (*E*)-1-(5-methyl-1-phenyl-1*H*-1,2,3-triazol-4-yl)-3-aryl-2-propen-1-one **4(a-h)** in 63-71% of yields. The corresponding compound **4** on cyclo-condensation with hydroxylamine hydro- chloride in the presence of sodium acetate in acetic acid under reflux temperature for 3 hours, afforded the corresponding 3-(5-methyl-1-phenyl-1*H*-1,2,3-triazol-4-yl)-5-arylisoxazole **5(a-h)** in 47-63% of yields (Scheme 1). The structure of the synthesized compound was confirmed by the interpretation of their IR, NMR and MS spectral analyses.

3.1.1. Characterization of (E)-1-(5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)-3-aryl-2-propen-1-one (4a):

Yield 66%; IR (KBr) ν_{max}: 3078 (CH-Ar), 2968 (CH-Ali), 1702 (C=O), 1612 (C=C), 1532 (N=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.78 (s, 3H, CH_3), 7.30-7.40 (m, 8H, ArH), 7.60-7.70 (m, 4H, ArH, CH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 24.3, 125.3, 128.7, 129.4, 130.1, 130.6, 131.2, 131.9, 133.9, 134.5, 137.9, 139.0, 145.6, 172.9; MS: m/z 289 (M^+).

3.1.2. Characterization of 3-(5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)-5-phenylisoxazole (5a)

Yield 49%; IR (KBr) ν_{max}: 3062 (CH-Ar), 2932 (CH-Ali), 1674 (C=N), 1538 (N=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.87 (s, 3H, CH_3), 7.40-7.50 (m, 8H, ArH), 7.60-7.65 (m, 3H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.4, 97.1, 126.9, 128.7, 129.3, 131.4, 137.8, 141.6, 143.7, 159.7; MS: m/z 302 (M^+).

3.1.3. Characterization of 5(4-methoxyphenyl)3(5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl) isoxazole (5b)

Yield 54%; IR (KBr) ν_{max}: 3071 (CH-Ar), 2978 (CH-Ali), 1671 (C=N), 1533 (N=N), 1030 (C-O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.84 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 7.12 (d, J = 8.7 Hz, 2H, ArH), 7.40-7.50 (m, 8H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.9, 57.6, 89.7, 116.2, 125.9, 126.2, 128.1, 128.9, 129.9, 131.5, 137.1, 139.8, 141.0, 143.4. 160.1; MS: m/z 332 (M^+).

3.1.4. Characterization of 5(2,6-difluorophenyl)3(5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)isoxazole (5c)

Yield 63%; IR (KBr) ν_{max}: 3057 (CH-Ar), 2944 (CH-Ali), 1675 (C=N), 1531 (N=N), 1374 (C-F) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.84 (s, 3H, CH_3), 7.10-7.15 (m, 2H, ArH), 7.40-7.50 (m, 7H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.9, 96.3, 113.4, 114.1, 126.5, 128.6, 129.6, 129.9, 131.8, 137.1, 141.2, 143.5. 162.6, 168.7; MS: m/z 338 (M^+).

3.1.5. Characterization of 5-(4-chlorophenyl)-3-(5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl) isoxazole (5d)

Yield 59%; IR (KBr) ν_{max}: 3061 (CH-Ar), 2944 (CH-Ali), 1672 (C=N), 1535 (N=N), 689 (C-Cl) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.85 (s, 3H, CH_3), 7.40-7.50 (m, 8H, ArH), 7.75 (d, J = 8.6 Hz, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.7, 88.9, 126.2, 128.6, 128.9, 129.7, 130.8, 131.5, 132.8, 135.0, 137.2, 141.1, 143.8. 159.2; MS: m/z 336 (M^+).

3.1.6. Characterization of 3-(5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)-5-(4-nitrophenyl) isoxazole (5e)

Yield 55%; IR (KBr) ν_{max}: 3047 (CH-Ar), 2979 (CH-Ali), 1678 (C=N), 1578 (N=O), 1540 (N=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.83 (s, 3H, CH_3), 7.40-7.50 (m, 6H, ArH), 7.89 (d, J = 8.5 Hz, 2H, ArH), 8.45 (d, J = 8.5 Hz, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.2, 89.3, 124.9, 126.7, 128.6, 129.2, 129.8, 131.9, 134.9, 137.6, 141.7, 143.1, 146.0, 159.8; MS: m/z 347 (M^+).

3.1.7. Characterization of 3-(5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)-5-(2-nitrophenyl) isoxazole (5f)

Yield 51%; IR (KBr) ν_{max}: 3073 (CH-Ar), 2984 (CH-Ali), 1679 (C=N), 1577 (N=O), 1538 (N=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.84 (s, 3H, CH_3), 7.40-7.50 (m, 9H, ArH), 7.98 (d, J = 8.7 Hz, 1H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.1, 94.8, 120.8, 126.9, 127.0, 128.4, 128.9, 129.1, 130.9,

131.4, 134.2, 137.4, 141.3, 143.8, 147.8, 159.4; MS: *m/z* 347 (M^+).

3.1.8. Characterization of 5(4-methylphenyl)-3-(5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl) isoxazole (5g)

Yield 49%; IR (KBr) ν_{max}: 3084 (CH-Ar), 2982 (CH-Ali), 1674 (C=N), 1537 (N=N), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.48 (s, 3H, CH_3), 2.79 (s, 3H, CH_3), 7.10-7.20 (m, 4H, ArH), (d, *J* = 8.7 Hz, 2H, ArH), 7.40-7.50 (m, 6H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.1, 24.2, 90.3, 127.7, 128.5, 128.9, 129.0, 129.6, 130.8, 131.4, 137.8, 137.4, 141.2, 143.9. 160.7; MS: *m/z* 316 (M^+).

3.1.9. Characterization of 2-methoxy-4[3(5-methyl -1-phenyl-1H-1,2,3-triazolyl)5-isoxazolyl] phenol (5h)

Yield 58%; IR (KBr) ν_{max}: 3456 (O-H), 3088 (CH-Ar), 2976 (CH-Ali), 1671 (C=N), 1537 (N=N), 1078 (C-O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.86 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 4.92 (s, 1H, OH), 7.12 (s, 1H, ArH), 7.40-7.50 (m, 8H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.4, 57.8, 88.9, 112.6, 118.2, 119.0, 126.9, 128.7, 128.9, 129.9, 131.4, 137.2, 141.6, 143.1, 146.2, 147.7, 159.2; MS: *m/z* 348 (M^+).

in The IR spectrum of compound **3**, the absorption bands due to C=O and C=N are appeared at 1714, 1619 cm^{-1}. Its ^{1}H NMR spectra, the aromatic protons of phenyl groups were appeared as a multiple at δ 7.40-7.50 and protons of methyl groups appeared as singlet at δ 2.32 and 2.50 ppm. Its ^{13}C NMR specta, the signals of triazole ring appeared at δ 128.8 and 139.0 ppm. The structure of was further confirmed by the mass spectrum, which showed a molecular ion peak at *m/z* 199.

The IR spectra of compound **4a**, the characteristic stretching frequency of the enone C=O and C=C was observed at 1702 and 1612 cm^{-1}. The ^{1}H NMR spectrum showed, aromatic protons and α-CH, β-CH proton signal at δ 7.30-7.40 and 7.60-7.70 ppm as multiplets for eight and four protons in each, the signal at δ 2.78 as singlet, integrating three protons assigned for methyl group. ^{13}C NMR spectrum exhibit the signal at δ 134.5 and 133.9 for (C-5) and (C-4) carbons of triazole ring, the carbonyl carbon and ene carbons appeared at 172.9 (C=O) 130.6 (α-C) and (β-C) 145.6 ppm. The structure of was further confirmed by the mass spectrum, which showed a molecular ion peak at *m/z* 289.

The IR spectra of compound **5a**, N=N of triazole and C=N of isoxazole absorption bands appeared at 1674 and 1538 cm^{-1}. Its ^{1}H NMR spectrum showed a signal at δ 7.40-7.50 and 7.60-7.65 ppm as multiplets for eight and three protons in each and a signal at δ 2.87 as singlet, integrating three protons assigned for methyl group. Its ^{13}C NMR spectrum exhibit the signal at δ 129.3 (C-5) and 141.6 (C-4) for triazole ring carbons. The isoxazole ring carbons appeared at δ 143.7 (C-3), 97.1 (C-4) and 159.7 (C-5) ppm. The structure of was further confirmed by the mass spectrum, which showed a molecular ion peak at *m/z* 302.

3.2. Antibacterial Activity

The *in vitro* antibacterial activity of compounds **5(a-h)** were evaluated against Gram +ve bacteria (*Bacillus subtilis*, *Staphylococcus aureus, Micrococcus luteus*) and Gram -ve bacteria (*Proteus vulgaris, Salmonella typhimurium, Escherichia coli*) by broth dilution method [38]. The lowest concentration required to arrest the growth of bacteria was regarded as the minimum inhibitory concentration (MIC, μg/mL) was determined for all the compounds and presented in Table 1.

Table 1: Antibacterial Activity of Compounds 5(a-h)

Compound	Minimum inhibitory concentration (MIC μg/mL)					
	B. subtilis	*S. aureus*	*M. luteus*	*P. vulgaris*	*S. typhimurium*	*E. coli*
5a	12.5	25.0	25.0	-	-	-
5b	1.56	3.12	1.56	6.25	25.0	25.0
5c	1.56	3.12	1.56	3.12	25.0	12.5
5d	6.25	12.5	12.5	25.0	-	-
5e	12.5	25.0	12.5	25.0	50.0	-
5f	12.5	50.0	25.0	-	12.5	12.5
5g	6.26	25.0	12.5	12.5	25.0	—
5h	6.25	12.5	12.5	25.0	—	50.0
Ampicillin	1.56	1.56	1.56	3.12	3.12	12.5

Note: - indicates, strains are resistant to the compound >50 μg/mL conc.

All assays included the solvent and reference controls, Ampicillin was used as standard drug. The investigation of antibacterial screening data revealed that all the tested compounds exhibited interesting biological activity, however, with a degree of variation.

The antibacterial activity and structure activity relationship (SAR) studies of compounds **5(a-h)** revealed that, compounds containing 4-methoxyphenyl (**5b)** and 2,6-difluorophenyl (**5c)** groups on isoxazole ring showed significant and equal activity against *B. subtilis, S. aureus, M. luteus and P. vulgaris* to that of standard drug. The other compounds also exhibited considerable antibacterial activities and therefore emerged as potential molecules for their further development.

4. CONCLUSION

A series of new 3-(5-methyl-1-phenyl-1*H*-1,2,3-triazol-4-yl)-5-arylisoxazole **5(a-h)** has been synthesized and screened for antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus, Micrococcus luteus, Proteus vulgaris, Salmonella typhimurium, Escherichia coli*. The antibacterial evaluation indicates, that compounds containing 4-methoxyphenyl (**5b)** and 2,6-difluorophenyl (**5c)** groups on isoxazole ring showed significant and equal activity against *B. subtilis, S. aureus, M. luteus and P. vulgaris* to that of standard drug.

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Declaration

The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

6. REFERENCES

1. Joseph L, George M, *Int J Pharm Res Health Sci*, 2016; **4**:1474-1477.
2. Epple R, Azimioara R, Russo R, Xie Y, Wang X, Cow C et al. *Bioorg Med Chem Lett*, 2006; **16**:5488-5492.
3. Margarita G, Maria FM, Tomas P, Jessica A, Gabriel V, Luis A, *J Pharm Pharmacol*, 2013; **65**: 1796-1804.
4. Archana K, Renu B, *Der Pharmacia Lettre*, 2016; **8**:127-134.
5. Zhao J, Tian W, Qi J, Lv D, Liu Y, Jiang Y et al. *Bioorg Med Chem Lett*, 2014; 24:2802-2806.
6. Chevreuil F, Landreau A, Seraphin D, Larcher G, Mallet S, Bouchara JP et al. *J Enzyme Inhib Med Chem*, 2007; 22:563-569.
7. Narlawar R, Pickhardt M, Leuchtenberger S, Baumann K Krause S, Dyrks T et al, *Chem Med Chem*, 2008; **3**:165-172.
8. Zhao H, Liu G, Xin Z, Srby MD, Pei Z, Szczepankiewicz BG et al, *Bioorg Med Chem Lett*, 2004; 14:5543-5546.
9. Schmidtke M, Wutzler P, Zieger R, Riabova OB, Makarov VA, *Antiviral Res*, 2009; **81**:56-63.
10. Carr JB, Durham HG, Hass DK, *J Med Chem*, 1977; **20**:934-939.
11. Panda SS, Chowdary PV, Jayashree BS, *Indian J Pharm Sci*, 2009; 71:684-687.
12. Eddington ND, Cox DS, Roverts RR, Butcher RJ, Edafiogho IO, Stables JP et al, *Eur J Med Chem*, 2002; **37**:635-648.
13. Sun R, Li Y, Xiong L, Liu Y, Wang Q, *J Agric Food Chem,* 2011; **59**:4851-4859.
14. Balaji NV, Haribabu B, Rao V U, Subbaraju GV, Nagasree KP, Kumar MMK, *Curr Top Med Chem*, 2019; **19**:662-682.
15. Zimecki M, Bachor U, Maczynski M, *Molecules*, 2018; **23**:2724.
16. Patra A, Batra S, Bhaduri AP, Khanna A, Chander R, Dikshit M, *Bioorg Med Chem*,2003;**11**:2269-2276.
17. Suresh Kumar GV, Rajendraprasad Y, Mallikarjuna BP, Chandrashekar SM, Kistayya, C, *Eur J Med Chem*, 2010; **45**:2063-2074.
18. Lazrek HB, Taourirte M, Oulih T, Barascut JL, Imbach JL, Pannecouque C et al. *Nucleos Nucleot Nucl*, 2001; **20**:1949-1960.
19. Buckle DR, Rockell CJ, Smith H, Spicer BA, *J Med Chem*, 1984; **27**:223-227.
20. de las Heras FG, Alonso R, Alonso G, *J Med Chem*, 1979; **22**:496-501.
21. El-Etrawy AS, Abdel-Rahman AAH, *Chem Heterocycl Comp*, 2010: **46**:1105-1108.
22. Hivarama Holla B, Veerendra B, Shivananda MK, Poojary B, *Eur J Med Chem*, 2003; **38**:759-767.
23. Chen J, Sun XY, Chai KY, Lee JS, Song MS, Quan ZS, *Bioorg Med Chem* 2007; **15**:6775-6781.
24. Almajan GL, Barbucenau SF, Almajan ER, Draghici C, Saramet G, *Eur J Med Chem*, 2009;**44**:3083-3089.
25. Palaska E, Sahin G, Kelicen P, Durlu NT, Altinok G, *Farmaco*, 2002; 57:101-107.
26. Poulsen SA, Wilkinson BL, Innocenti A, *Bioorg Med*

Chem Lett, 2008; **18**: 4624-4627.
27. Tullis JS, VanRengs JC, Natchus MG, Clark MP, De B, Hsieh LC, Janusz MJ, *Bioorg Med Chem Lett*, 2003; **13**:1665-1668.
28. Xu Y, Wang Y, Yan L, Liang RM, Dai BD, Tang RJ et al, *J Proteome Res,* 2009; **8**:5296-5304.
29. Pasqualotto AC, Denning DW, *J Antimicrob Chemother, 2010;* 61: i19-30.
30. Mellaerts R, Aerts A, Caremans TP, Vemant J, Van den Mooter G, Martens JA et al. *Mol Pharm*, 2010; **7**:905-913.
31. Smith J, Safdar N, Knasinski V, Simmons W, Bhavnani SM, Ambrose PG et al, *Antimicrob Agents Chemother*, 2006; **50**:1570-1572.
32. Geria AN, Scheinfeld NS, *IDrugs*, 2008; **11**:661-670.
33. Schiller DS, Fung HB, *Clin Ther*, 2008; **29**:1862-1886.
34. Reddy CS, Kumar GR, Devi MV, Nagaraj A, *Acta Chim Slov,* 2011; **58**:576-581.
35. Nagaraj A, Srinivas S, Ramesh Naik P, Neelofer R, *Asian J Org Med Chem*, 2019; **4**:101-108.
36. Sajeeva Reddy Ch, Nagaraj A, Yakub V, Kalyani B, *Asian J Chem,* 2019; **31**:1913-1918.
37. Sanjeeva Reddy Ch, Chandrashekar Rao D, Yakub V, Nagaraj A, *Acta Chim Slov* 2011, **58**:582-589.
38. National Committee for Clinical Laboratory Standards (NCCLS), *Nat Comm Lab Stands*, Villanova, 1982, pp 242.