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Synthesis, structure elucidation and antibacterial screening of some novel 1,3,4-oxadiazoline derivatives

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Anovel sequence of 1,3,4-oxadiazoline derivatives has been synthesized with an endeavour to explore their consequence on *in vitro* growth of microbes causing the microbial contagion. *In vitro* antimicrobial activity has been performed against the *Escherichia coli* (*E. coli*) and *Proteus mirabilis* (*P. mirabilis*) which are Gram-negative (Gram-ve) and *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermis*) which are Gram-positive (Gram+ve) by using disk diffusion method. The minimum inhibitory concentration (MIC) has been distinguished by employing the double dilution method. The result of percent inhibition area/µg of the compounds has been differentiated with the standard drug "Ciprofloxacin". Several compounds portray excellent activity as compared to the standard drug Ciprofloxacin while some of them presented a considerable zone of inhibition. The evaluated compounds for cytotoxicity effects *via* Human hepatocellular carcinoma (HepG2) cell line by MTT-assay and findings reveal that the experimental compounds display a viability of ≥80% at 100 µM. In molecular docking studies, the 1,3,4-oxadiazoline derivatives demonstrate the ligand-receptor interaction with amino acids which exist on the active sites of the peptide deformylase and the 1,3,4-oxadiazoline derivatives exhibit their antibacterial potential as peptide deformylase inhibitors.

Keywords: Synthesis, 1,3,4-oxadiazoline, antimicrobial activity, MTT-assay

Contagious diseases are nowadays the second vital reason for widespread death and the third significant reason for death in developed countries (WHO 2002; Nathan 2004). The advancement of microbes concerning antimicrobial resistance (AMR) albeit an unavoidable aspect of the general evolution of bacteria is a major public health concern as it is extremely difficult to overcome (Courvalin 2005). Multidrug resistance (MDR) Gram-positive (Gram+ve) bacteria, as Methicillin-resistant *Staphylococci aureus* (MRSA), Penicillin resistant *Streptococcus pneumoniae* (PRSP), and Vancomycin-resistant *Enterococci* (VRE), produced difficulties in the therapeutics (K.R Babu *et al*; 2008; A. Dalhoff 1994). Compounds containing oxadiazole ring possess a variety of therapeutic potentials like anti-inflammatory, antiviral, analgesic, antineoplastic, anti-microbial, fungicidal, anticonvulsant, anti-proliferative, anti-mycobacterial, anti-protozoal, anti-diabetic, anthelmintic, hypoglycaemic, antiallergic, enzyme inhibitors, insecticidal, inhibition of tyrosinase, cathepsin K and anticancer activities (B. Shivi & M. K. Gupta 2011; M.O. Ahmed *et al*; 2002; R. Chawla *et al*; 2010; N.N. Farshori *et al*; 2010; N. Bhardwaj *et al*; 2009; B. Chandrakantha *et al*; 2010; M. Shaharyar *et al*; 2010; H.S. athirajan *et al*; 2010; O. Parkash *et al*; 2010; M.M. Kumar *et al*; 2010; Y. Li *et al*; 2006; C.J. Chen *et al*; 2007; M. Islam *et al*; 2006; F. Liu *et al*; 2008; M.S. Karthikeyan *et al*; 2008; N.P. Rai *et al*; 2009; A. Husain & Ajmal 2009; S.G. Kucukguzel *et al*; 2002; O. Ates *et al*; 1998; V. Jakubkiene *et al*; 2003; M. Amir *et al*; 2004; T.M.C. Tan *et al*; 2006; A.S. Aboraia *et al*; 2006; Y. Li *et al*; 2006; M.T. Khan *et al*; 2005; J.T. Palmer; *et al*; 2006; K.C.N Chaluvaraju *et al*; 2011). The inductive effect of extra heteroatom is responsible for the basic nature of oxadiazole on the other hand two pyridine type nitrogen in the structure diminishes the aromaticity to the point where oxadiazole exerts the resemblance with conjugated diene. The nucleus participates in a variety of chemical reactions like thermal photochemical and substitution (electrophilic &

Abbreviations: Antimicrobial resistance (AMR); Multidrug resistance (MDR); Methicillin-resistant *Staphylococci aureus* (MRSA); Penicillin resistant *Streptococcus pneumoniae* (PRSP); Vancomycin-resistant *Enterococci* (VRE).

nucleophilic), (R.N. Warrener *et al*; 2000). There are furthermore valuable intermediates in organic synthesis (M. Guan *et al*; 2003) and extensively working as electron transporting and hole-blocking resources (C.R.W. Guimaraes *et al*; 2005). In addition, 1,3,4-oxadiazole heterocycles are especially good quality bioisosteres of amides and esters, which know how to contribute substantially to rising pharmacological activity by participating in hydrogen bonding interactions with the receptors (Guimaraes *et al*; 2005). Recently Liu *et al*. reported the antibacterial potential of 3-((5-phenyl-1,3,4-oxadiazol-2-yl) methyl)-2-thioxothiazolidin-4-one derivatives (Liu *et al*; 2014). Ranjith *et al*. synthesized N-(4-(4-chloro-1H-imidazol-1-yl)-3-methoxyphenyl) amide/sulfonamide derivatives and evaluated them for antimicrobial activity (P.K. Ranjith *et al*; 2014; C. Congio *et al*; 2008). On the other hand, the high biologicalpotential of the imidazole nucleus possessing drugs prompts scientists to produce better therapeutic compounds. Pharmacological potential of imidazole derivatives comprises anticancer (G. Aridoss *et al*; 2006) antimicrobial (L. Nagarapu *et al*; 2008; K. Bhandari *et al*; 2009; S. Emami *et al*; 2008) and antioxidant (R.C. Smith *et al*; 1987). It is known that clinically useful drugs such as miconazole, econazole and oxiconazole having imidazole moiety exhibits strong antifungal activity. Metalloprotease subfamily enzyme, peptide deformylase from *E. coli* is an extremely conserved enzyme which PDB ID is 1G2A. Peptide deformylase is a key factor for developing a new antibacterial agent (R. S Kumar *et al*; 2011). Computational studies are a key factor for drug design. There is an enormous area of drug designing but one of them is the relationship between protein and chemical structure, which is possible by Docking studies, it determines probable by ligand-receptor binding manner (M. A Beg *et al*; 2018). Therefore, the biological importance of 1,3,4-oxadiazole and substituted nitroimidazole nucleus impelled us to aim and synthesize some new moieties which cover mutually the functional nucleus, as well as their combination, will be essential for enhancing the anti-bacterial activity.

Experimental Protocol

Chemistry

All required components to complete the preparation of oxadiazoline derivatives were bought from Sigma, Merck, SRL and were consumed following the purification. Melting point (M.P) were analysed employing the instrument Melt-Tem.p.and were recorded as received. HeraeusVario EL III analyzer at CDRI, Lucknow, India was applied to carry out the Elemental analyses. IR, NMR (^{1}H and ^{13}C) spectra are recorded on instrument Perkin-Elmer model 1600" FT-IR RX1 and Bruker AVANCE"300 MHz spectrometers. The following abbreviations were applied as a singlet, s; doublet, d; double doublet, dd; triplet t; multiple, m; chemical shift, δ ;' MICROMASS" QUATTRO" II triple quadrupole mass spectrometer was applied to mass spectrum. Pre-coated silica gel aluminium sheets were employed for testing the purity and status of the reaction and observed under UV radiation.

General procedure for the synthesis of compound (A1-A2)

The concoction of 4-NO_2-$C_3H_4N_2$ or 2-CH_3-4(5)-NO_2- $C_3H_4N_2$ (1 mmol), ECA ($C_4H_7ClO_2$) (1 mmol) and K_2CO_3 (1.5 mmol) in Dry-C_3H_6O (5-10 mL) was refluxed for one day. The reaction concoction was strained hot, and the liquid was removed by the filtration. The crude product thus received followed by the purification of recrystallization.

Ethyl 2-(3,4-(NO_2)$_2$-1*H*-pyrazol-1-yl)acetate (A1): Yellow crystal (This compound was synthesized by the reaction between 4-nitroimidazole, ethylchloroacetate and K_2CO_3 under reflux condition Yellow, Yield 80%); IR: 1690, 1620; ^{1}H NMR (DMSO-d_6,500 MHz): δ 5.67 (2H,s, CH_2), 4.92 (2H,q, CH_2), 2.70 (3H,s, CH_3), 1.35 (3H, t, CH_3).

Ethyl 2-(5-CH_3-3,4--(NO_2)$_2$-1*H*-pyrazol-1-yl)acetate(A2): Yellow crystal (This compound was synthesized by the reaction between 2-methyl-4(5)-nitroimidazole, ethylchloroacetate and K_2CO_3 under reflux condition Yellow, Yield 86%); IR: 1686, 1626 cm^{-1}; ^{1}HNMR (DMSO-d_6,500 MHz): δ 7.7 (1H, s, CH), 5.63 (2H, s, CH_2), 4.90 (2H, q, CH_2), 1.37 (3H, t, CH_3).

General procedure for the synthesis of compound (B1-B2)

A concoction of compound A1 or A2 (2 mmol) and $NH_2NH_2.H_2O$ (2 mmol) in C_2H_5OH (10 mL) was refluxed for 10 h. A concrete mass of hydrazide B1-B2 was received followed by cooling, straining and recrystallization.

Ethyl 2-(3,4-dinitro-1*H*-pyrazol-1-yl) acetohydrazide (B1): White crystal (This compound was synthesized by the reaction between A1 and hydrazide in ethanol under reflux condition White, Yield 80%); IR: 3312, 3214, 1693 cm^{-1}; ^{1}H NMR (DMSO-d_6,500 MHz): δ

9.62 (1H, bs, NH), 4.82 (2H, bs, NH_2), 2.94 (3H, s, CH_3), 2.92 (3H, s, CH_3).

Ethyl 2-(5-methyl-3,4-dinitro-1*H*-pyrazol-1-yl) acetohydrazide (B2): White crystal (This compound was synthesized by the reaction between A2 and hydrazide in ethanol under reflux condition White, Yield 84%); IR: 3330, 3214, 1698 cm^{-1}; ^1H NMR (DMSO-d_6,500 MHz): δ 9.64 (1H, bs NH), 4.84 (2H, bs, NH_2), 2.94 (3H, s, CH_3).

General procedure for the synthesis of compound (C1-C2)

On a stirrer the solution of hydrazide B1 or B2 (1 mmol) and 3-hydroxy-4-methoxy-benzaldehyde (1 mmol) in C_2H_5OH (25 mL) was further added, followed by the drop wise adding up of glacial acetic acid (0.2 mL). The final concoction was refluxed for 5 h, stirred, precipitated, assembled following the straining, drying and recrystallization to yield C1-C2 in varying yields.

(E)-N-(3-hydroxy-4-methoxybenzylidene)-2-(3,4-dinitro-1*H*-pyrazol-1-yl)acetohydrazide (C1): White crystal (This compound was synthesized by the reaction between B1 and 3-hydroxy-4-methoxy-benzaldehyde in ethanol under reflux condition White, Yield 82%); IR: 3350, 3212, 1693, 1617 cm^{-1}; ^1H NMR (DMSO-d_6,500 MHz): δ 9.62 (1H, broad s, NH), 8.12 (1H, s, CH=N), 7.64 (1H, m, CH-Ar), 5.76 (2H, s, CH_2), 5.30 (1H, s, OH), 3.79 (3H, s, OCH_3), 2.93 (3H, s, CH_3), 2.90 (3H, s, CH_3).

(E)-N-(3-hydroxy-4-methoxybenzylidene)-2-(5-methyl-3,4-dinitro-1*H*-pyrazol-1-yl) acetohydrazide (C2): White crystal (This compound was synthesized by the reaction between B2 and 3-hydroxy-4-methoxy-benzaldehyde in ethanol under reflux condition White, Yield 86%); IR: 3360, 3218, 1692, 1620 cm^{-1}; ^1H NMR (DMSO-d_6,500 MHz): δ 9.64 (^1H, broad s, NH), 8.14 (^1H, s, CH=N), 7.64 (^1H, m, CH-Ar), 5.32 (^1H, s, OH), 5.70 (^2H, s, CH_2), 3.78 (^3H, s, OCH_3), 2.92 (^3H, s, CH_3).

General procedure for the synthesis of compound (D1-D2)

In an RB (Round bottomed flask) (D1 or D2(1 mmol) and 20 mL of acetic anhydride was reacted for 12 h under reflux. After this the concoction was kept cooling and the poured to ice cooled water and stirred to get precipitate now the straining, purification and recrystallization is performed to obtain the product (D1-D2).

1-(2-(3-hydroxy-4-mrthoxyphenyl)-5-((3,4-dinitro-1*H*-pyrazol-1-yl)methyl)-1,3,4-oxadiazol-3(2*H*)-yl)ethanone (D1): Yellow crystal (This compound was synthesized by the reaction between C1 and acetic anhydride under reflux condition Yellow, Yield 70%); IR: 3410, 1716, 1617, 1258 cm^{-1}; ^1H NMR (DMSO-d_6,500 MHz): δ 7.80 1H, (m, CH-Ar), 5.32 (s, 1H, OH), 3.80 (3H, s, OCH_3), 2.96 (3H, s, CH_3), 2.98 (3H, s, CH_3), 2.52 (3H, s, $COCH_3$).

1-(2-(3-hydroxy-4-mrthoxyphenyl)-5-((5-methyl-3,4-dinitro-1*H*-pyrazol-1-yl)methyl)-1,3,4-oxadiazol-3(2*H*)-yl)ethanone (D2): Yellow Crystals (This compound was synthesized by the reaction between C2 and acetic anhydride under reflux condition Yellow, Yield 75%); IR: 3400, 1715, 1620, 1255; ^1H NMR (DMSO-d_6,500 MHz): δ 7.62 (1H, m, CH-Ar), 5.34 (1H, s, OH), 3.82 (3H, s, OCH_3), 2.92 (3H, s, CH_3), 2.54 (3H, s, $COCH_3$), 2.52 (3H, s, $COCH_3$).

Common approach for the preparation of compound (1-14)

A concoction of compound D1 or D2 (1 mmol) and corresponding sulfonyl chloride (1 mmol) in acetone (20 ml) was stirred at 0-5°C for 10 h. The solid precipitate obtained was filtered out and dried under vacuum.

4-(3-acetyl-2,3-dihydro-5-(4-nitro-1*H*-imidazol-1-yl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenylbenzene sulfonate (1)

White crystals (This compound was synthesized by the reaction between D1 and benzene sulphonyl chloride in acetone at 0-5°C and obtained as white crystal, White, Yield 75%) m.p.250-252°C; IR: 3068,1675, 1648, 1255,1158 cm^{-1}; ^1H NMR (DMSO-d_6,500 MHz): δ 8.660 (s, 1H, Ar-H), 8.218 (1H, s, Ar-H), 8.212 (1H, s, Ar-H), 7.930 (1H, s, Ar-H), 7.851 (1H, d, J=7.5, Hz, Ar-H), 7.805 (1H, d, J=21, Hz, Ar-H), 7.653-7.703 (1H, m, Ar-H), 7.552 (1H, s, Ar-H), 7.462 (1H, d, J=8.4, Ar-H), 7.283 (1H, d, J=8.1, Ar-H), 3.838 (3H, s, OCH_3), 2.508 (3H, s, $COCH_3$); ^{13}C NMR (DMSO-d_6,500 MHz): δ 161.20 (CO), 151.90 (C=N), 140.50, 139.87, 134.49, 134.16, 130.66, 130.16, 124.72, 122.26, 112.26, 56.09 (OCH_3), 24.21 (CH_3). ESI-MS: *m/z* [M^++1], 487.04 (Calcd 487.07). Anal. calc. for $C_{20}H_{17}N_5O_8S$: C, 49.28; H, 3.52; N, 14.37. Found: C, 49.32; H 3.48; N, 14.40%.

4-(3-acetyl-2,3-dihydro-5-(4-nitro-1*H*-imidazol-1-yl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl-4-meythyl-benzenesulfonate (2)

Creamy white crystals (This compound was synthesized by the reaction between D1 and p-

methylbenzene sulphonyl chloride in acetone at 0-5°C and obtained as creamy White crystals, creamy White crystals, Yield 78%); m.p.246-248°C; IR: 3030, 1670, 1650 1258, 1160 cm^{-1};^{1}H NMR (DMSO-d_6,500 MHz): δ 8.639 (1H, s, Ar-H), 8.222 (1H, s, Ar-H), 8.216 (1H, s, Ar-H), 7.724 (2H, d, J=8.1, Hz,Ar-H), 7.533 (1H, s, Ar-H), 7.459 (2H, d, J=6.9, Hz,Ar-H), 7.431 (1H, d, J=2.7, Hz,Ar-H), 7.219 (1H, d, J=8.4, Hz, Ar-H), 3.800 (3H, s, OCH_3), 2.501 (3H, s, COCH_3), 2.43 (1H, s, CH_3); ^{13}C NMR (DMSO-d_6,500 MHz): δ 161.25 (CO); 151.94 (C=N), 139.83, 140.52, 134.18, 134.52, 23.46 (CH_3), 130.64, 130.18, 124.70, 122.24, 112.23, 56.14 (OCH_3), 24.22 ($COCH_3$); ESI-MS: *m/z* [M^++1], 502.14 (Calcd 502.10). Anal. calc. for $C_{21}H_{19}N_5O_8S$: C, 50.30; H, 3.82; N, 13.97. Found: C, 50.25; H, 3.84; N, 14.00%.

4-(3-acetyl-2,3-dihydro-5-(4-nitro-1*H*-imidazol-1-yl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl-4-methoxybenzenesulfonate (3)

White crystals (This compound was synthesized by the reaction between D1 and 4-methoxybenzene sulphonyl chloride in acetone at 0-5°C and obtained as white crystal, White crystal, Yield 80%); m.p.256-258°C; IR: 3022, 1660, 1640, 1260,1152 cm^{-1}; ^{1}H NMR (DMSO-d_6,500 MHz): δ 8.661 (1H, s,Ar-H), 8.451 (2H, d, J=8.4, Hz,Ar-H), 8.224 (1H, s, Ar-H), 8.216 (1H, s, Ar-H), 8.136 (2H, J d, =8.4, Hz,Ar-H), 7.560 (1H, s, Ar-H), 7.471 (1H, d, J=8.1, Ar-H), 7.321 (1H, d, J=8.4, Ar-H), 3.839 (3H, s, OCH_3), 3.838 (3H, s, OCH_3), 2.508 (3H, s, COCH_3); ^{13}C NMR (DMSO-d_6,500 MHz): δ 161.26 (CO), 151.92 (C=N), 140.54, 139.87, 134.45, 134.19, 130.67, 130.19, 124.68, 122.30, 112.28, 56.14 (OCH_3), 24.5 (CH_3); ESI-MS: *m/z* [M^++1], 518.1 (Calcd 518.09). Anal. Calcd for $C_{21}H_{18}N_5O_9S$: C, 48.84; H, 3.51; N, 13.56. Found: C, 48.14; H, 3.10; N, 13.80%.

4-(3-acetyl-2,3-dihydro-5-(4-nitro-1*H*-imidazol-1-yl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl-4-chloro-benzenesulfonate (4)

Light yellow crystals (This compound was synthesized by the reaction between D1 and 4-chlorobenzene sulphonyl chloride in acetone at 0-5°C and obtained as Light yellow crystal, Light yellow, Yield 74%); m.p.250-252°C; IR: 3074, 1670, 1650, 1266,1150 cm^{-1}; ^{1}H NMR (DMSO-d_6,500 MHz): δ 8.660 (1H, s, Ar-H), 8.219 (1H, s, Ar-H), 8.215 (1H, s, Ar-H), 7.887 (2H, d, J=9.3, Hz,Ar-H), 7.767 (2H, d, J=9.3, Hz, Ar-H), 7.553 (1H, s, Ar-H), 7.463 (1H, d, J=9.6, Hz,Ar-H), 7.282 (1H, d, J=8.4, Hz,Ar-H), 3.839 (3H, s, OCH_3), 2.504 (3H, s, COCH_3); ^{13}C NMR (DMSO-d_6,500 MHz): δ 161.29 (CO), 151.95 (C=N), 140.57, 139.89, 134.48, 134.16, 130.65, 130.13, 124.75, 122.27, 112.29, 56.14 (OCH_3), 24.3 (CH_3); ESI-MS: *m/z* [M^++1], 522.08 (Calcd 522.04). Anal. Calcd for $C_{20}H_{15}ClN_5O_8S$: C, 46.12; H, 2.90; N, 13.45. Found: C, 46.08; H, 3.12; N, 13.40%.

4-(3-acetyl-2,3-dihydro-5-(4-nitro-1*H*-imidazol-1-yl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl-3-bromobenzenesulfonate (5)

White crystals (This compound was synthesized by the reaction between D1 and 3-bromobenzene sulphonyl chloride in acetone at 0-5°C and obtained as white crystal, white, Yield 78%)**;** m.p.256-258°C; IR: 3070, 1680, 1655, 1270, 1165 cm^{-1}; ^{1}H NMR (DMSO-d_6,500 MHz): δ 8.841 (1H, s, Ar-H), 8.363 (1H, d, J=13.5, Hz,Ar-H), 8.173 (1H, d, J=12.9, Hz,Ar-H), 8.015 (1H, d, J=7.8, Hz,Ar-H), 7.946 (1H, s, Ar-H), 7.850 (1H, s, Ar-H), 7.808 (1H, d, J=8.1, Hz,Ar-H), 7.408-7.505 (1H, m, Ar-H), 3.834 (3H, s, OCH_3), 2.505 (3H, s, COCH_3); ^{13}C NMR (DMSO-d_6,500 MHz): δ 161.29 (CO), 151.91 (C=N), 140.50, 139.81, 134.43, 134.15, 130.61, 130.15, 124.72, 122.29, 112.25, 56.10 (OCH_3), 24.27 (CH_3); ESI-MS: *m/z* [M^++1], 566.12 (Calcd 566.98). Anal. Calcd for $C_{20}H_{15}BrN_5O_8S$: C, 42.49; H, 2.67; N, 12.39. Found: C, 42.56; H, 2.68; N, 12.55%.

4-(3-acetyl-2,3-dihydro-5-(4-nitro-1*H*-imidazol-1-yl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl-4-bromobenzenesulfonate (6)

Creamy white crystals (This compound was synthesized by the reaction between D1 and 4-bromobenzene sulphonyl chloride in acetone at 0-5°C and obtained as white crystal, white, Yield 70%); m.p.252-254°C; IR: 3068, 1668,1658, 1245,1160 cm^{-1}; ^{1}H NMR (DMSO-d_6,500 MHz): δ 8.665 (1H, s, Ar-H), 8.223 (1H, s, Ar-H), 8.217 (1H, s, Ar-H), 7.885 (2H, d, J=8.4, Hz,Ar-H), 7.766 (d, J=8.7, 2H, Hz,Ar-H), 7.555 (1H, s, Ar-H), 7.462 (1H, d, J=8.4, Hz,Ar-H), 7.284 (1H, d, J=8.7, Ar- H), 3.832 (3H, s, OCH_3), 2.509 (3H, s, COCH_3); ^{13}C NMR (DMSO-d_6,500 MHz): δ 161.23 (CO), 151.90 (C=N), 140.51, 139.86, 134.43, 134.17, 130.60, 130.16, 124.68, 122.24, 112.26, 56.17 (OCH_3), 24.29 (CH_3); ESI-MS: *m/z* [M^++1], 566.20 (Calcd 566.98). Anal. calc. for $C_{20}H_{15}BrN_5O_8S$: C, 42.49; H, 2.67; N, 12.39. Found: C, 42.40; H, 2.88; N, 12.40%.

4-(3-acetyl-2,3-dihydro-5-(4-nitro-1*H*-imidazol-1-yl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl-4-nitro-

benzenesulfonate (7): Yellow crystals (This compound was synthesized by the reaction between D1 and 4-nitrobenzene sulphonyl chloride in acetone at 0-5°C and obtained as yellow crystal, yellow, Yield 95%)m.p.260-262°C; IR: 3030, 1672, 1656,1252, 1160 cm^{-1}; ^1H NMR (DMSO-d_6,500 MHz): δ 8.662 (1H, s, Ar-H); 8.211 (1H, s, Ar-H), 8.208 (1H, s, Ar-H), 7.888 (2H, d, J=8.1, Hz,Ar-H), 7.769 (2H, d, J=8.4, Hz,Ar-H), 7.556 (1H, s, Ar-H), 7.464 (1H, d, J=8.1, Hz,Ar-H), 7.285 (1H, d, J=8.1, Hz,Ar-H), 3.837 (3H, s, OCH_3), 2.507 (3H, s, COCH_3); ^{13}C NMR (DMSO-d_6,500 MHz): δ 161.22 (CO), 151.92 (C=N), 140.48, 139.85, 134.48, 134.12, 130.65, 130.13, 124.74, 122.26, 112.28, 56.10 (OCH_3), 24.22 (CH_3); ESI-MS: *m/z* [M^++1], 532.55 (Calcd 532.60). Anal. Calcd for $C_{20}H_{15}N_6O_{10}S$: C, 45.20; H, 2.84; N, 15.81. Found: C, 45.23; H, 2.58; N, 15.90%.

4-(3-acetyl-2,3-dihydro-5-(2-methyl-4,5-dinitro-1*H*-imidazol-1-yl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenylbenzenesulfonate (8):White crystals (This compound was synthesized by the reaction between D2 and benzene sulphonyl chloride in acetone at 0-5°C and obtained as white crystal, white, Yield 95%) m.p.248-250°C; IR: 3055, 1678, 1644, 1258,1164, cm^{-1}; ^1H NMR (DMSO-d_6,500 MHz): δ 8.662 (1H, s, Ar-H), 8.432 (2H, d, J=8.7, Hz,Ar-H), 8.110 (2H, d, J=8.7, Ar-H), 7.307-7.477 (5H, m, Ar-H), 3.839 (3H, s, OCH_3), 2.52 (3H, s, CH_3), 2.488 (3H, s, COCH_3); ^{13}C NMR (DMSO-d_6,500 MHz): δ 164.75 (CO), 152.23 (C=N), 140.58, 134.62, 131.64, 24.5 (CH_3), 126.56, 124.78, 122.34, 115.43, 112.12, 56.52 (OCH_3), 24.4 (CH_3); ESI-MS: *m/z* [M^++1], 546.1 (Calcd 546.07). Anal. Calcd for $C_{21}H_{17}N_6O_{10}S$: C, 46.24; H, 3.14; N, 15.41. Found: C, 46.60; H, 3.22; N, 15.60%.

4-(3-acetyl-2,3-dihydro-5-(2-methyl-4,5-dinitro-1*H*-imidazol-1-yl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl-4-methylbenzenesulfonate (9): White crystals (This compound was synthesized by the reaction between D2 and p-methylbenzene sulphonyl chloride in acetone at 0-5°C and obtained as white crystal, white, Yield 95%); m.p.254-256°C; IR: : 3030, 1673, 1652, 1252,1162 cm^{-1}; ^1H NMR (DMSO-d_6,500 MHz): δ 8.648 (1H, s, Ar-H), 7.726 (2H, d, J=7.8, Hz,Ar-H), 7.538 (1H, s, Ar-H), 7.461 (2H, d, J=7.8, Hz,Ar-H), 7.434 (1H, d, J=1.8, Hz, Ar-H), 7.221 (1H, d, J=8.1, Hz,Ar-H), 3.830 (3H, s, OCH_3), 2.508 (3H, s, COCH_3), 2.504 (s, 3H, CH_3), 2.433 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6,500 MHz): 164.52 (CO), 152.76 (C=N), 140.23, 134.82, 131.35, 126.41, 124.67, 122.88, 115.42, 112.13, 56.40 (OCH_3), 24.5 (CH_3), 24.3 (CH_3); ESI-MS: *m/z* [M^++1], 560.12 (Calcd 560.09). Anal. Calcd for $C_{22}H_{19}N_6O_{10}S$: C, 47.23; H, 3.42; N, 15.02. Found: C, 47.18; H, 3.65; N, 14.95%.

4-(3-acetyl-2,3-dihydro-5-(2-methyl-4,5-dinitro-1*H*-imidazol-1-yl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl-4-methoxybenzenesulfonate (10): White crystals (This compound was synthesized by the reaction between D2 and 4-methoxybenzene sulphonyl chloride in acetone at 0-5°C and obtained as white crystal, white, Yield 95%); m.p.258-260°C; IR: 3070, 1672, 1654, 1260,1162 cm-1; ^1H NMR (DMSO-d_6,500 MHz): δ 8.665 (1H, s, Ar-H); 7.846 (2H, d, J=8.7, Hz,Ar-H), 7.739 (2H, d, J=8.4, Hz,Ar-H), 7.546 (1H, s, Ar-H), 7.459 (1H, d, J=7.8, Hz,Ar-H), 7.280 (1H, d, J=8.4, Hz,Ar-H), 3.834 (3H, s, OCH_3), 3.832 (3H, s, OCH_3), 2.508 (s, 3H, COCH_3), 2.501 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6,500 MHz): δ 164.93 (CO), 24.62 (CH_3), 152.42 (C=N), 140.74, 134.63, 131.25, 126.19, 124.73, 122.57, 115.35, 112.08, 56.44 (OCH_3), 56.17 (OCH_3), 24.53 (CH_3); ESI-MS: *m/z* [M^++1], 576.1 (Calcd 576.08). Anal. Calcd for $C_{22}H_{19}N_6O_{11}S$: C, 45.92; H, 3.33; N, 14.60. Found: C, 46.00; H, 3.36; N, 14.56%.

4-(3-acetyl-2,3-dihydro-5-(2-methyl-4,5-dinitro-1*H*-imidazol-1-yl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl-4-chlorobenzenesulfonate (11):Light yellow crystals (This compound was synthesized by the reaction between D2 and 4-chlorobenzene sulphonyl chloride in acetone at 0-5°C and obtained as light-yellow crystal, yellow, Yield 95%); m.p.246-248°C;

IR: 3022, 1670, 1652, 1250,1160 cm-1; ^1H NMR (DMSO-d_6,500 MHz): δ 8.664 (1H, s, Ar-H), 7.844 (1H, d, J=8.7, Hz,Ar-H), 7.736 (2H, d, J=8.7, Hz,Ar-H), 7.548 (1H, s, Ar-H), 7.457 (1H, d, J=8.7, Hz,Ar-H), 7.282 (1H, d, J=8.7, Hz,Ar-H), 3.836 (3H, s, OCH_3), 2.506 (3H, s, COCH_3), 2.48 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6,500 MHz): δ 164.37 (CO), 152.53 (C=N), 140.91, 134.19, 131.04, 126.72, 124.48, 122.61, 115.37, 112.54, 56.41 (OCH_3), 24.52 (CH_3), 24.35 (CH_3); ESI-MS: *m/z* [M^++1], 580.06 (Calcd 580.03). Anal. Calcd for $C_{21}H_{16}ClN_6O_{10}S$: C, 43.49; H, 2.78; N, 14.49. Found: C, 43.52; H, 2.75; N, 14.48%.

4-(3-acetyl-2,3-dihydro-5-(2-methyl-4,5-dinitro-1*H*-imidazol-1-yl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl-3-bromobenzenesulfonate (12):Creamy white crystals (This compound was synthesized by the reaction between D2 and 3-

bromobenzene sulphonyl chloride in acetone at 0-5°C and obtained as creamy white crystal, creamy white, Yield 95%); m.p.262-264°C; IR: 3018, 1678, 1654, 1262,1155 cm-1; ^{1}H NMR (DMSO-d_6,500 MHz): δ 8.655 (1H, s, Ar-H), 7.933 (1H, s, Ar-H), 7.852-7.877 (1H, d, Ar-H), 7.806 (1H, d, J=7.2, Hz,Ar-H), 7.653 (1H, m, Ar-H), 7.534 (1H, s, Ar-H), 7.445 (1H, d, J=7.5, Hz,Ar-H), 7.248 (1H, d, J=8.1, Hz,Ar-H), 2.504 (3H, s, COCH_3), 3.833 (3H, s, OCH_3), 2.54 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6,500 MHz): δ 164.47 (CO), 152.97 (C=N), 140.55, 134.33, 131.74, 126.19, 124.80, 122.43, 115.67, 112.76, 56.42 (OCH_3), 24.72 (CH_3), 24.54 (CH_3); ESI-MS: *m/z* [M^++1], 625.2 (Calcd 625.98). Anal. calc. for $C_{21}H_{16}BrN_6O_{10}S$: C, 40.40; H, 2.58; N, 13.46. Found C, 40.42; H, 2.56; N, 13.52%.

4-(3-acetyl-2,3-dihydro-5-(2-methyl-4,5-dinitro-1*H*-imidazol-1-yl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl-4-bromobenzenesulfonate (13): White crystals (This compound was synthesized by the reaction between D2 and 4-bromobenzene sulphonyl chloride in acetone at 0-5°C and obtained as white crystal, white crystal, Yield 95%); m.p.256-258°C; IR: 3060, 1675, 1642, 1248, 1162 cm^{-1}; ^{1}H NMR (DMSO-d_6,500 MHz): δ 8.664 (1H, s, Ar-H), 7.760 (2H, d, J=9.9, Hz,Ar-H), 7.534 (2H, s, Ar-H), 7.435 (1H, d, J=9.9, Hz,Ar-H), 7.227 (1H, d, J=9.3, Hz,Ar-H), 7.112 (1H, d, J=22.8, Hz,Ar-H), 3.870 (3H, s, OCH_3), 2.501 (3H, s, CH_3), 2.506 (3H, s, COCH_3); ^{13}C NMR (DMSO-d_6,500 MHz): δ 164.26 (CO), 152.19 (C=N), 140.64, 134.21, 131.76, 24.29 (CH_3), 126.55, 124.34, 122.69, 115.43, 112.38, 56.49 (OCH_3), 24.25 (CH_3); ESI-MS: *m/z* [M^++1], 625.2 (Calcd 625.98). Anal. calc. for $C_{21}H_{16}BrN_6O_{10}S$: C, 40.40; H, 2.58; N, 13.46. Found: C, 40.44; H, 2.62; N, 13.40%.

4-(3-acetyl-2,3-dihydro-5-(2-methyl-4,5-dinitro-1*H*-imidazol-1-yl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl-4-nitrobenzenesulfonate (14): Yellow crystals (This compound was synthesized by the reaction between D2 and 4-nitrobenzene sulphonyl chloride in acetone at 0-5°C and obtained as yellow crystal, yellow, Yield 95%); m.p.264-266°C. Anal. calc. for $C_{21}H_{16}N_7O_{12}S$: C 42.72, H 2.73, N 16.61% found C 42.70, H 2.70, N 16.64%; IR: 3058, 1665, 1642, 1265,1166 cm-1; ^{1}H NMR (DMSO-d_6,500 MHz): δ 8.662 (1H, s, Ar-H), 7.762 (2H, d, J=8.7, Hz,Ar-H), 7.538 (1H, s, Ar-H), 7.438 (2H, d, J=10.2, Hz,Ar-H), 7.225 (1H, d, J=8.4, Hz,Ar-H), 7.115 (1H, d, J=2.1, Hz,Ar-H), 3.836 (3H, s, OCH_3), 2.501 (3H, s, CH_3), 2.507 (3H, s, COCH_3); ^{13}C NMR (DMSO-d_6,500 MHz): δ 164.67 (CO), 152.44 (C=N), 140.38, 134.56, 131.67, 126.59, 124.28, 122.45, 115.36, 112.27, 56.42 (OCH_3), 24.29 (CH_3), 24.24 (CH_3); ESI-MS: *m/z* [M^++1], 591.09 (Calcd 591.06). Anal. calc. for $C_{21}H_{16}N_7O_{12}S$: C, 42.72; H, 2.73; N, 16.61. Found: C, 42.70; H, 2.70; N, 16.64%.

Biological Activity

The disc diffusion plan following some improvement was employed to carry out the in vitro antibacterial assessment utilizing the bacterial culture of strains (*S. aureus*, *S. epidermidis*, *P. mirabilis*, and *E. coli*) that were cultivated consuming the agar media with incubation at 37°C for 18 h. Applying the McFarland protocol the cells were stretched for incubation in a solution of salt to yield a suspension of around 10^5 CFU/ml. Pouring the concoction (suspension 10 ml antibiotic agar at 40°C) onto a Petri-plate under the laminar flow cabinet and after this, the paper disks of 6.0mm was established on it. The compound to be screened (1 mg) disbanded in DMSO (100 ml) to produce a stock solution which was poured over the Petri-plate following further dilutions with different concentrations. In the estimation ciprofloxacin and DMSO were applied as positive and negative control respectively. Production of an inhibitory zone declared the sensitivity of the bacteria to the compounds studied after at 18 h on 36°C. The zone of inhibition for the oxadiazoline derivatives **(1-14).** The findings be matched among the Ciprofloxacin, and the inhibitory zone was elucidated at the lowest concentration that is responsible to prevent the growth of bacteria minimum inhibitory concentration (MIC). MIC was calculated by using the macro-dilution method applying for the benchmark inoculums of 10^5 CFL/ml. The serial dilutions of the compounds to be evaluated was assembled to the final concentrations of 400, 200, 100, 50, 12.5, 6.25 and 3.125µg/ml. The inhibition of the visible growth is observed after 18h incubation and the findings are recorded.

Cytotoxicity studies (MTT-assay)

Cell culture

Culturing of HepG2 cell line was performed into DMEM medium with FBS (10%), penicillin (100 units ml^{-1}), streptomycin (100 µg ml^{-1}), and amphotericin B (2.5 µg ml^{-1}) at 37°C under 95% air/5% CO_2 atmosphere (T. Mosmann 1983). To sustain the exponential growth HepG2 cell line were harvested when there is 80% confluence.

MTT assay

To observe the viability of cells, MTT [(3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl) tetrazolium bromide], the assay is performed that is a colorimetric method based on the principle that only the viable cells can split the tetrazolium salt by using mitochondrial dehydrogenase (T. Mosmann; 1983).

The harvesting of the cell monolayer was carried out by applying 0.25% trypsin to get single cell suspension followed by pipetting repeatedly. The cells that are growing exponentially were plated at 1.2×10^4 cells per well into 96-well microtiter plates following the incubation upto 48 hours before the addition of the tested compounds to attain the highest confluency. The compounds to be tested were dissolved in 10% (v/v) DMSO to produce a stock solution that was again diluted with the new medium to get a 1 M concentration. The incubation for the cells with different concentrations of reference as well as the compounds was done for 48 h at 37°C in 5% CO_2 in combination with the control. At selected time periods PBS was utilized to wash the cells, the cells were the treated with 50 µl MTT solution and entered the incubation for 4 h. After incubation, the medium was eliminated and fresh DMSO 150 µl was added to each well and subjected to evaluate the absorbance at 570 nm at a wavelength of 655 nm on a Microplate reader (iMark, BIORAD, S/N 10321). All the experiments were carried out in triplicate and viability (%) was calculated.

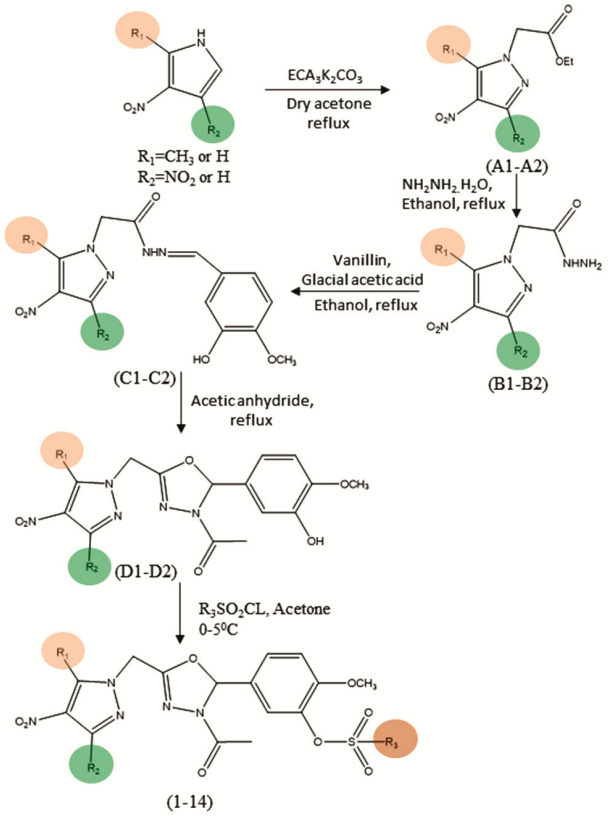
Molecular Docking Studies

The interaction analysis of the protein-ligand binding site was done by the molecular docking analysis where we are using the AutoDock tool (G. M Morris *et al*; 2009) and the 3D interaction analysis in between protein-compound is visualized by LigPlot$^+$ (R.A Laskowski, and M.B. Swindells; 2011). After the result of MIC and Zone inhibition test compounds which performed as good activity those docked. Our synthesized compound's structure was draw by using ChemBioDraw Ultra 14.0 and making 3D format by using Chem3D Pro 14.0 (K. R Cousins; 2011 and M.A. Beg *et al*; 2021). The target compounds docked with the *E. coli* protein-peptide deformylase which PDB ID_1G2A(S. Bala *et al*; 2014). The binding affinity of the interactive amino acids with the PDB ID_1G2A and compared their binding affinity with the standard drug Ciprofloxacin.

Results and Discussion

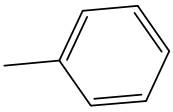
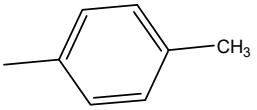
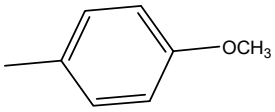
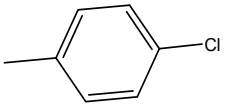
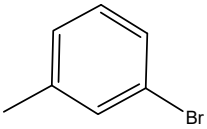
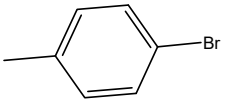
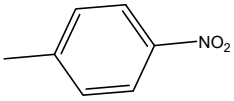
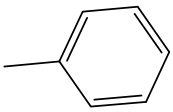
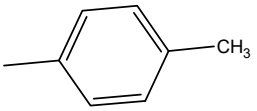
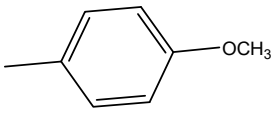
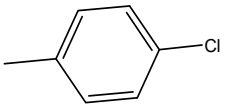
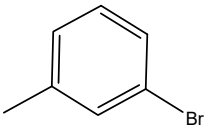
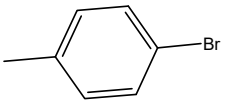
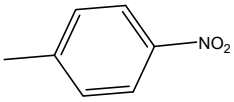
Chemistry

Substituted 4-(3-acetyl-2, 3-dihydro-5-(4-nitro-1*H*-imidazol-1-yl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenyl-4-benzenesulfonate **(1-14)** were synthesized by using a multi-step reaction process scheme as shown in Scheme I and Table I. Synthesis of the target compounds involves five steps, in the first step compounds **(A1-A2)** were obtained by the simple reaction of substituted nitroimidazole and ethyl chloroacetate in presence of potassium carbonate under reflux condition. Compounds **(A1-A2)** on reaction with hydrazine hydrate yields their corresponding hydrazides. **(B1-B2)** when reacted with 3-hydroxy-4-methoxy-benzaldehyde in ethanol yield compounds **(C1-C2)**. In the fourth step compounds **(C1-C2)** were refluxed with acetic anhydride to obtain oxadiazoline analogues **(D1-D2)**. In the final step, oxadiazoline analogues were treated with substituted sulfonyl chloride with stirring at 0-5°C. The compounds were obtained in high yields and were appropriately characterized. Spectral data like IR, ^{1}HNMR, ^{13}CNMR, ESI-MS and elemental analyses were found in accord



Scheme I — Schematic representation of the route adopted for the synthesis of oxadiazoline derivatives **1-14**, where "R_1, R_2 and R_3" correspond to different substituents as mentioned in Table 1.

Table I — Showing different substituent R_1, R_2 and R_3 indicated as in Figure 1

Compd	R_1	R_2	R_3
1	H	NO_2	
2	H	NO_2	
3	H	NO_2	
4	H	NO_2	
5	H	NO_2	
6	H	NO_2	
7	H	NO_2	
8	CH_3	NO_2	
9	CH_3	NO_2	
10	CH_3	NO_2	
11	CH_3	NO_2	
12	CH_3	NO_2	
13	CH_3	NO_2	
14	CH_3	NO_2	

with the anticipated structure of the compounds. The purity was established by the pointed melting-point and the elemental analysis. The selected diagnostic bands of the IR spectra of compounds (**1-14)** provided evidential support for constructing the expected structure. In addition to common bands which arise due to ν(C=C) of the aromatic region and benzene some other bands also appear in IR spectra which gives the key information about the conversion of reactants in a schematic representation shown in Scheme I. The compounds **(A1-A2)** showed stronger peaks in the regions near 1690-1686 cm^{-1} due to ν(C=O) vibration which provide strong evidence for the formation of ester. A peak in the region 3412-3330 cm^{-1} and 3212-3214 cm^{-1} due to the presence of NH_2 and NH confirmed the conversion of esters into their corresponding hydrazide **(B1-B2)**. Formation of compounds **(C1-C2)** was ascertained by the disappearance of the peak in the region 3412-3330 cm^{-1} due to NH_2 and by the presence of a peak in the range of 3350-3360 cm^{-1} assigned to a hydroxyl group. The presence of peak at around 1617-1620 cm^{-1} due to (HC=N) supports the formation of Schiff base. Structural confirmation for the compounds **(D1-D2)** is achieved by the presence of a peak in the region 1255-1258 cm^{-1} and 1715-1716 cm^{-1} dueto presence of (COC) and (C=O). The disappearance of a peak at around 1617-1620 cm^{-1} due to (HC=N) also provide strong confirmation. Structural confirmation for the compounds **(1-14)** was obtained by the disappearance of peak in the range of 3350-3360 cm^{-1} due to the presence of hydroxyl group as well as due to the presence of peak at around 1150-1172 cm^{-1} and 1245-1270 cm^{-1} due to the existence of (S=O) and (COC) further confirms the formation of compounds (**1-14)**. The structure of the oxadiazoline derivatives **(1-14)** compounds was further confirmed by ^{1}HNMR spectra. Presence of a triplet at around 1.35-1.37 ppm and a quartet at 4.90-4.92 ppm due to CH_3 and CH_2 strongly recommend the formation of compounds **(A1-A2)**. The formation of compounds **(B1-B2)** was confirmed by the absence of triplet and quartet due to CH_3 and CH_2 and by the appearance of broad singlet at 9.62-9.64 and 4.82-4.84 ppm due to NH and NH_2 respectively. The absence of broad singlet at 4.82-4.84 ppm due to NH_2 and presence of a singlet at 8.12-8.14 ppm due to CH=N reveal the formation of compounds **(C1-C2)**. Formation of compounds **(D1-D2)** was supported by the absence of a singlet at 8.12-8.14 ppm due to CH=N and a broad singlet at 9.62-9.64ppm due to NH. Formation of the compounds (**1-14)** was definite by the nonappearance of singlet at around 5.32-5.34 ppm due to OH. The structure of all compounds was further confirmed by ^{13}CNMR, and all the characteristic peaks are shown in experimental data.

Physicochemical properties, lipophilic efficiency, and PAINS rule analysis

In drug development failure there are many causing factors some of them are poor solubility and poor permeability. Therefore, determining physiochemical properties with a compound is important and it is done by using the SwissADME online server which use SDF file of the chemical structure (A. Dania *et al*; 2017). In generally we believed that the high molecular weight (M.W.) and high lipophilicity (C log P) have poor oral drug properties, but no doubt 1/3 FDA approved are high M.W. C log P values in between 2-3 considered as optimal for an oral drug. PAINS (pan-assay interference compounds) which describe seeming bioactive molecules that can interfere in readout done interaction with dissimilar biological targets PAINS must be recognised and evaded in biochemical and pharmacological assay instruction to avoid false outcomes (J. B. Baell and G. A. Holloway; 2010). In Table II there (**1-14**) derivatives of 1,3,4-oxidazole listed of these physicochemical properties, lipophilic efficiency and PAINS rule are given which suggested the active compounds is vital for drug discovery effort.

Biological Activity

Antibacterial therapeutic effect of the prepared compounds (1-14) was investigated utilizing the four distinct cultures Gram-ve (*E. coli* and*P. mirabilis*) and Gram+ve (*S. aureus* and*S. epidermidis*). MIC was calculated by diluting the compounds for study and the findings were compared with the referenced Table III. First, the compounds to be tested were dissolved in the DMSO, the serial dilutions were made to the concentrations of 400, 200, 100, 50, 12.5, 6.25, 3.125 $\mu g/ml^{-1}$ and added to the 24 h old inoculums. The effect was reported after 18 hr when the formation of the inhibition zone appears in Table IV. At MIC, the zone of inhibition was calculated in comparison with the standard and the percent are of inhibition was also estimated per microgram of the tested compounds and compared with standard drug Ciprofloxacin (Figure 1). When the zone of inhibition was calculated against standard drug it is found that some of the compounds were

Table II — Physicochemical properties, Lipophilic efficiency and PAINS rule analysis for **1-14** 1,3,4-oxadiazoline derivatives

Compd	Mol.Wt. (g/mol)	Hydrogen bonds acceptor	Hydrogen bonds donor	Rotatable bonds	Molar Refractivity	C log P	PAINS/ alert
1	546.47	12	0	10	138.63	1.23	Y
2	560.49	12	0	10	143.60	1.55	Y
3	576.49	13	0	11	145.12	1.18	Y
4	580.91	12	0	10	143.64	1.76	Y
5	625.36	12	0	10	146.33	1.74	Y
6	625.36	12	0	10	146.33	1.85	Y
7	591.46	14	0	11	147.45	0.54	Y
8	560.49	12	0	10	143.60	1.57	Y
9	574.52	12	0	10	148.57	1.88	Y
10	594.94	12	0	10	148.61	2.07	Y
11	594.94	12	0	10	148.61	2.07	Y
12	639.39	12	0	10	151.30	2.14	Y
13	639.39	12	0	10	151.30	2.15	Y
14	605.49	14	0	11	152.42	0.85	Y

Y: means zero alert (PAINS rule)

Table III — Representing minimum inhibitory concentration of oxadiazoline derivatives **1-14** Ciprofloxacin used as standard

Compd	Minimal Inhibitory Concentration (MIC) µg/ml			
	Gram positive (Gram^{+ve})		Gram negative (Gram^{-ve})	
	S. aureus	*S. epidermidis*	*P. mirabilis*	*E. coli*
1	>100	>100	>100	>100
2	>100	>100	>100	>100
3	6.25	3.125	6.25	>100
4	50	50	25	>100
5	25	100	25	12.5
6	>100	50	>100	>100
7	6.25	3.125	12.5	>100
8	12.5	3.125	100	>100
9	6.25	6.25	6.25	25
10	50	>100	>100	100
11	>100	>100	>100	12.5
12	100	25	>100	>100
13	6.25	3.125	12.5	>100
14	6.25	6.25	12.5	>100
Ciprofloxacin	6.25	3.125	6.25	12.5

[a] The value obtained in at least three separate assays done in triplicate.

found to show little to moderate activity in comparison to the standard drug. While some compounds (**3, 7, 8, 13 and 14**) showed 90% resemblance with the standard drug "Ciprofloxacin" against all the bacteria studied. On the other hand, compound **(9)** showed better activity than a standard drug in a case of *S. aureus* and found to possess significant activity against rest of the bacteria.The novel sequence of the oxadiazoline derivative was synthesized with a target that the newly synthesized compounds might be more potential therapeutic antibacterial agents. So, a complete investigation related to Cytotoxicity, antibacterial activity, and SAR (structure activity relationship) was carried out.

Cytotoxicity studies

The mitochondria of viable cells possess succinate dehydrogenase which reduced MTT to insoluble purple formazan crystal that can be measured spectrophotometer after solubilisation (H. Garn *et al*;1994; S. M. Thom *et al*;1993). Only the active cells can produce formazan crystal, so the number of active cells is directly proportional to the amount of formazan formed (S.H. Kim *et al*; 2002; S.R. Kim *et al*; 2003; Y.R. Lina *et al*; 2003; M.K. Gupta *et al*; 2006). To find out the cytotoxicity effect, the active compounds were subjected to the MTT assay utilizing (HepG2) cell line.The studied compounds were taken in the range 3.125-100µm and the percent viability of

Table IV — Effect of compounds **1-14** on growth of microorganism by halo zone test. Ciprofloxacin used as standard drug and DMSO as negative control

Compd	Inhibitory effects of compounds on microorganisms			
	Gram positive (Gram^{+ve})		Gram negative (Gram^{-ve})	
	S. aureus	*S. epidermidis*	*P. mirabilis*	*E. coli*
1	8.81±0.06	9.97±0.12	11.54±0.17	12.60±0.10
2	8.24±0.18	8.59±0.28	9.68±0.51	7.59±0.40
3	20.86±0.64	22.27±0.31	22.06±0.17	23.05±0.15
4	11.85±1.44	14.19±0.92	12.71±0.14	9.56±0.21
5	14.94±0.34	10.93±0.18	10.39±0.16	10.97±0.2
6	8.61±0.18	15.61±0.40	9.09±0.21	8.79±0.74
7	19.43±0.21	20.63±0.08	18.79±0.71	19.39±0.19
8	18.24±0.18	21.40±0.30	19.50±0.14	18.52±0.24
9	21.88±0.50	19.44±0.59	20.59±2.05	20.38±0.28
10	12.39±0.15	8.94±0.94	8.07±0.14	12.54±0.18
11	8.81±0.22	8.84±0.08	11.56±0.16	8.94±0.44
12	10.24±0.18	16.43±0.45	12.61±0.08	9.43±0.21
13	21.14±0.23	20.68±0.51	18.51±0.16	21.56±1.06
14	19.46±0.28	18.41±0.25	19.58±0.34	21.45±0.23
Ciprofloxacin	21.46±0.31	22.64±0.54	22.24±0.30	23.82±0.47

[a] The value obtained in at least three separate assays done in triplicate, S.Da. (±) Standard deviation

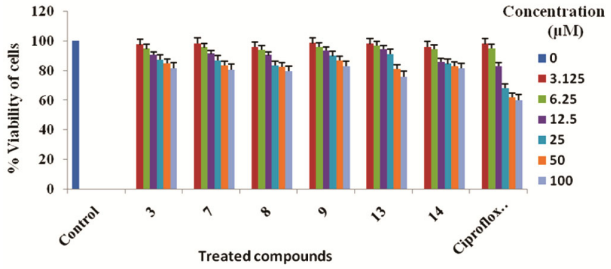


Figure 1 — The graph showing comparative percent area inhibition per µg of the compounds **1-14** and the Ciprofloxacin in case of gram-positive bacteria (*S. aureus and S. epidermidis*)and gram-negative bacteria (*P. mirabilis and E. coli*).

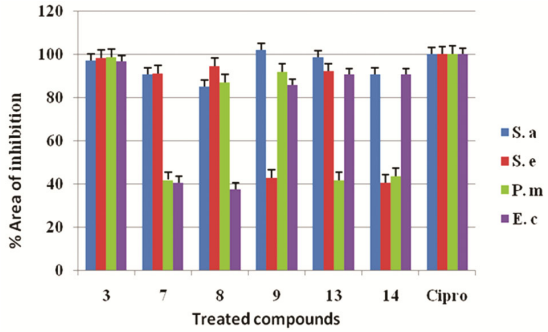


Figure 2 — Percent viability of cells after 48 h pre-treatment of HepG2 cell line with active compounds and Ciprofloxacin by MTT assay

the cells with regular exposure for 48 h is displayed in Figure 2.Findings revealed that the toxic effect depends on concentration, such as at3.125 µm the viability rages from 95-100% for all the active compounds including reference. As the concentration increases the viability decreases such as ≥90% (at 25µm) and ≥80% (at 50 & 100 µm) if the tested compounds did not exhibit remarkable toxic effects up to 100 µm as all exhibiting the viability of ≥80% at this concentration.

Molecular docking Studies

For the Molecular docking studies, the chemical structure of substituted 1,3,4-oxadiazoline derivatives drawn by using Chem3D Pro 14.0 (K. R Cousins; 2011) which is shown in Figure 3. 3D structure of 1,3,4-oxadiazoline derivatives docking outcome demonstrates that the synthesized 1,3,4-oxadiazole derivatives (Table I and Figure 4, Figure 5, Figure 6, Figure 7 and Figure 8) with *E. coli* protein peptide deformylase (S. Bala *et al*; 2014) shows the hydrogen bond interactions of amino acid residues and Chain ID. Docking studies (G. M Morris *et al*; 2009) shows the binding affinity of compound 3 is (-8.8) and interacting residues are His7(A), Ile44(A), Gly89(A), Leu91(A) and Glu95(A) in Figure 4, the binding affinity of compound 5 is -8.6 and interacting residues are Ile44(B), Gly45(B), His132(B) and Asp135(B) in Figure 5, the binding affinity of compound 11 is -8.6 and the interacting residues are Gly89(B), Arg97(B), Cys90(B) and Glu95(B) in Figure 6, the binding affinity of compound 13 is -9.3 and the interacting residues Ile44(B), Gly45(B) and Arg97(B) in Figure 7, the binding affinity of compound 14 is -9.1 and the interacting with B chain and residues are

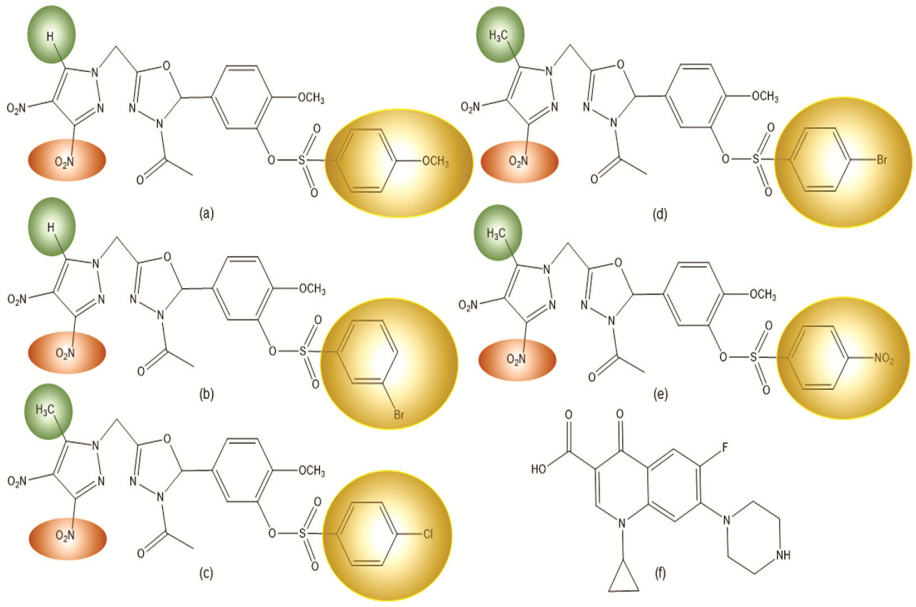


Figure 3 — Chemical structure of substituted oxadiazoline derivatives which shows in different colours green (R_1), red (R_2) and yellow (R_3): (a) 1,3,4-oxadizoline analogue **3** (b) 1,3,4-oxadizoline analogue **5** (c) 1,3,4-oxadizoline analogue **11** (d) 1,3,4-oxadizoline analogue **13** (e) 1,3,4-oxadizoline analogue **14**. (f) a standard drug Ciprofloxacin.

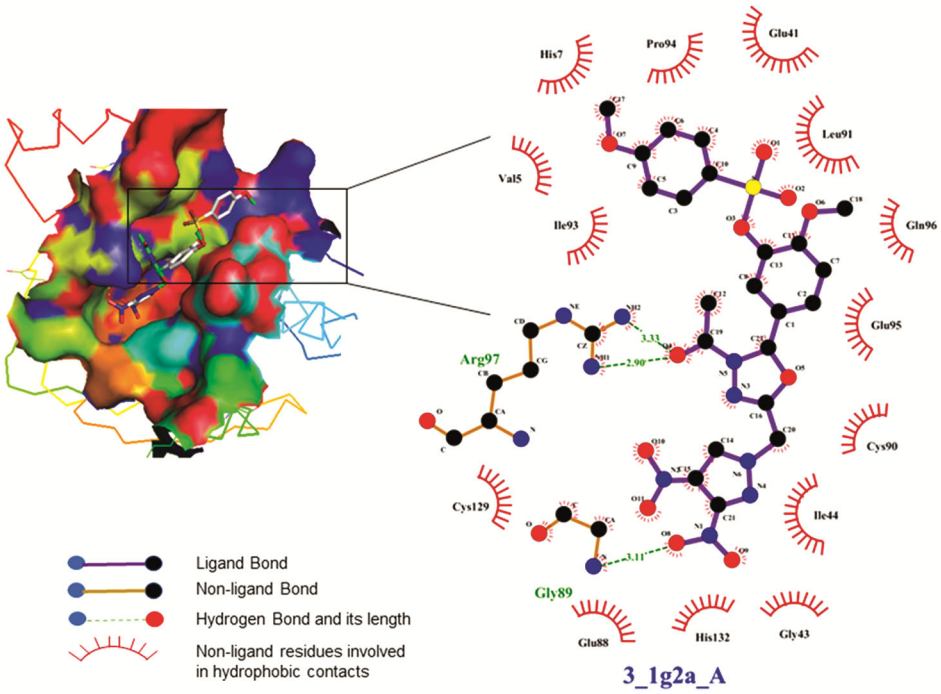


Figure 4 — Surface model of peptide deformylase in cavity compound **3** showing the protein compound interaction, where the interacting residues are His7, Ile44, Gly89, Leu91, Glu95 and Asp162.

Ile44(B), Gly45(B), Arg97(B), His132(B), Asp135(B), and Asp162(A) in Figure 8 and the binding affinity of standard drug Ciprofloxacin is (-8.0) and the interacting residues Gln50(C), Leu91(C) and Glu133(C). Receptor-ligand binding interaction studies done by LigPlot (R.A Laskowski,M.B. Swindells; 2011 and I.I. Hejazi*et al*; 2021) with peptide deformylase protein as mentioned Table V. The receptor protein has 3 chains so, the compound 3 interacting with chain A, compound 5, 11, 13 and 14

interacts with chain B and the interaction of standard drug with chain C. The docking score of synthesis derivatives (Compounds 3, 5, 11,13 and 14) was found higher in comparison to standard drug ciprofloxacin. These results could be used for the development of effective antimicrobial agents. In between ligand and receptor more than 3.2˚A distance indicates frail hydrogen bonding, in between 2.6˚A–3.2˚A distance shows the virtuous hydrogen bonding, and less than 2.5˚ A indicates vigorous bonding.

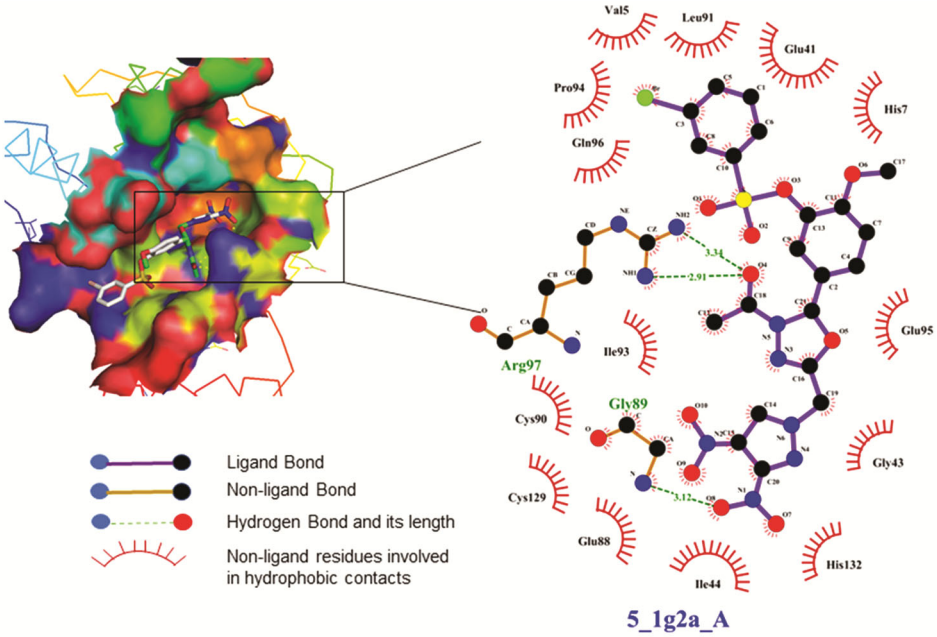


Figure 5 — Surface model of peptide deformylase in cavity compound **5** showing the protein compound interaction, where the interacting residues are Ile44, Gly45, His132 and Asp135.

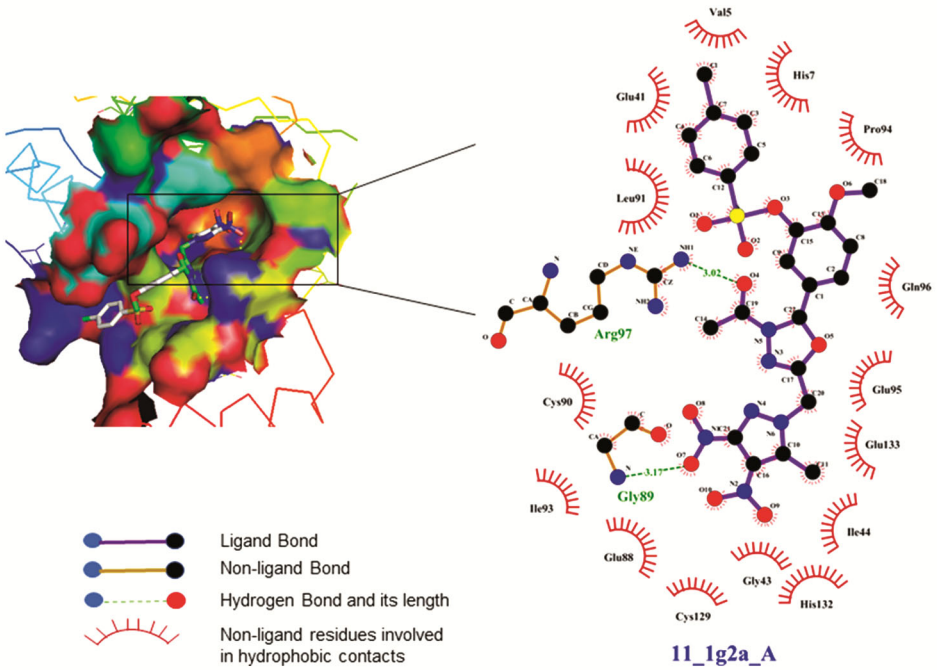


Figure 6 — Surface model of peptide deformylase in cavity compound **11** showing the protein compound interaction, where the interacting residues are Gly89, Arg97, Cys90 and Glu95.

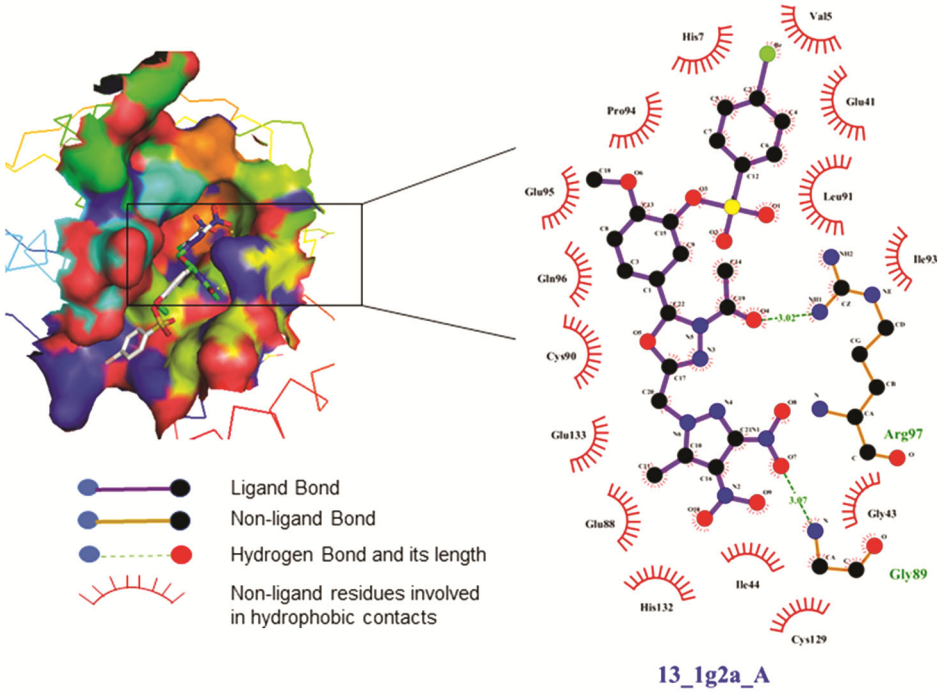


Figure 7 — Surface model of peptide deformylase in cavity compound **13** showing the protein compound interaction, where the interacting residues are Ile44, Gly45 and Arg97.

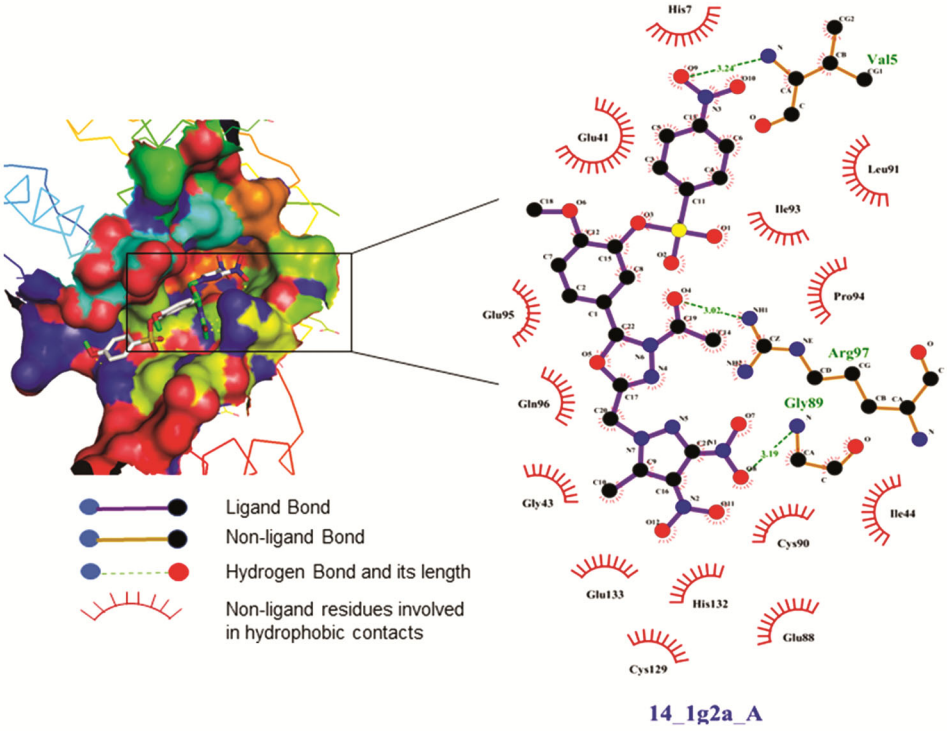


Figure 8 — Surface model of peptide deformylase in cavity compound **14** showing the protein compound interaction, where the interacting residues are Ile44, Gly45, Arg97, His132 and Asp135.

Table V — Ligand-receptor interaction of synthesized 1,3,4-oxadiazole derivatives with *E. coli* peptide deformylase

Compd	Binding affinity	Amino acid	Distance (Å)	Group involved in Ligand-receptor interaction
3	−8.9	Arg97	3.33	Non-ligand Residues
		Arg97	2.90	
		Gly89	3.11	
5	−8.8	Arg97	3.34	Val5(A), Glu41(A), Glu42(A), Gly43(A), Glu88(A), Cys90(A), Pro94(A) and Arg97(A)
		Arg97	2.91	
		Gly89	3.12	
11	−9.5	Arg97	3.02	Val16(C), Glu42(B), Gly43(B), Ser63(B), Glu64(B), Arg66(B), Glu88(B), Gly89(B) and Cys129(B)
		Gly89	3.17	
13	−9.5	Arg97	3.02	Glu42(B), Gly43(B), Ile44(B), Ile86(B), Glu87(B), Glu88(B), Leu91(B), Pro94(B) and His132(B)
		Gly89	3.07	
14	−9.9	Arg97	3.02	Val16(C), Gly43(B), Ser63(B), Glu64(B), Arg66(B), Glu87(B), Glu88(B), Gly89(B), Leu91(B), Cys99(B), Leu125(B) and His132(B)
		Gly89	3.19	
Cipro.	−8.1	Ile44	3.20	Val16(C), Val38(), Glu42(B), Gly43(43), Ser63(B), Glu64(B), Arg66(B), Glu87(B), Gly89(B), Cys90(B) and Cys129(B)
		Gly45	2.74	
		Gly45	2.99	
		Arg97	3.27	
		His132	3.23	
		Asp135	3.29	

Cipro: Ciprofloxacin; Å: Angstrom; (A), (B), (C): Protein Chains

Almost all the active derivatives showed good hydrogen bonding with protein.

Conclusions

Novel library of 1,3,4-oxadiazoline analogues with low molecular weight was synthesized by the following few step reactions to make cost effectual chemical that can be used as an antibacterial agent and targeted the *S. aureus* and *S. epidermidis* (Gram+ve) and *P. mirabilis* and *E. coli* (Gram-ve) bacteria. Accounting for the pharmacological potential of both nucleus oxadiazoline and nitro-imidazole the scheme was planned in such a manner for the targeted compounds will be having both significant functional moieties. All the substituted analogues of 1,3,4-oxadizoline **(1-14)** were screened for their antibacterial activity against Gram+ve and Gram-ve bacteria discussed above. Several compounds showed outstanding activity evaluated with the standard drug Ciprofloxacin results are calculated in terms of zone of inhibition and MIC. Among them, some of the compounds performed good activity while some were found to have similar activity in a contrast to standard. Among all the synthesized compounds 3, 5, 11, 13 and 14were found to be the most potent peptide-deformylase inhibitor with the highest dock score for compound 13 is -9.3 than that of standard drug Ciprofloxacin is -8.0.

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Conflict of Interest

There is no confliction among the authors.

References

1 Bala S, Kamboj S, Kajal A, Saini V & Prasad D N, *Biomed Res Int*, 2014 (2014), 172791.
2 Dalhoff A, *Infection*, 22 (1994),S111-S121.
3 Husain A&Ajmal M.,*Acta Pharm*, 59 (2009), 223-233.
4 Aboraia AS, Abdel-Rahman HM, Mahfouz NM& El-Gendy MA.*Bioorg Med Chem*, 14 (2006), 1236-1246.
5 Chandrakantha B, Shetty P, Nambiyar V, Isloor N & Isloor AM, *Eur J Med Chem*, 45(2010), 1206-1210.
6 Cousins K R, *J Am Chem Soc*, 133 (2011), 8388.
7 CongiuC, Cocco M T& Onnis V,*Bioorg Med Chem Lett*, 18 (2008), 989-993.
8 Laskowski RA& Swindells MB, *J Chem Inf Model*, 51 (2011), 2778–2786.
9 Morris GM, Huey R& Lindstrom W, *J Comput Chem*, 30 (2009), 2785–2791.
10 Nathan C, *Nature*, 431 (2004), 899-902.
11 Chen C J, Song B A, Yang S, Xu G F, Bhadury P S, Jin L H, Hu D Y, Li Q Z, Liu F, Xue W, Lu P & Chen Z,*Bioorg Med Chem*, 15 (2007), 3981-3989.
12 Guimaraes C R, Boger D L& Jorgensen W L, *J Am Chem Soc*, 127 (2005), 17377-17384.
13 Liu F, Luo X Q, Song B A, Bhadury P S, Yang S, Jin L H, Xue W & Hu D Y, *Bioorg Med Chem*, 16 (2008), 3632-3640.
14 Aridoss G, Balasubramanian S, Parthiban P& Kabilan S, *Eur J Med Chem*, 41 (2006), 268-275.
15 Garn H, Krause H, Enzmann V& Drossler K, *J Immunol Methods*, 168 (1994), 253-256.
16 Hejazi II, Beg MA, Imam MA, Athar F& Islam A, *Food Chem Tox*, 150 (2021) 112057.

17 Mayekar N A, Yathirajan H S, Narayana B,Sarojini B K & Suchetha N K, *Int J Chem*, 2 (2010) 38-54.
18 Lin H Y, Juan S H, Shen S C, Hsu F L& Chen Y C, *Biochem Pharmacol*, 66 (2003), 1821-1832.
19 Liu J C, Zheng C J, Wang M X, Li Y R, Ma L X, Hou S P & Piao H R, *Eur J Med Chem*, 74(2014), 405-410
20 Palmer J T, Hirschbein B L, Cheung H, McCarter J, Janc J W, Yu Z W & Wesolowski G, *Bioorg Med Chem Lett*, 16 (2006), 2909-2914.
21 Bhandari K, Srinivas N, Shiva Keshava G B& Shukla P K, *Eur J Med Chem*,44 (2009), 437-447.
22 Nagaraj C K C, Niranjan M S &Kiran S, *Int J Pharm Pharm Sci*, 3 (2011), 9-16.
23 Babu K R, Eeshwaraiah B, Aravind D, Meshram H M,Raju R M, Bhattacharya A &Bandichhor R, *Monatshefte für Chemie*, 139 (2008), 179-181.
24 Nagarapu L, Satyender A, Rajashaker B, Srinivas K, Rupa R P, Radhika K & Subhashini G, *Bioorg Med Chem Lett*. 18(2008), 1167-1171.
25 Amir M& Shikha K, *Eur J Med Chem*, 39 (2004), 535-545.
26 Guan M, Bian Z Q, Zhou Y F, Li F Y, Li Z J& Huang C H, *Chem Commun (Camb)*, (2003), 2708-2709.
27 Islam M, Siddiqui A A, Rajesh R, Bakht A& Goyal S, *Acta Pol Pharm*, 65(2008), 441-447.
28 Karthikeyan M S, Prasad D J, Mahalinga M, Holla B S& Kumari N S, *Eur J Med Chem*, 43(2008), 25-31.
29 Yar SM, Siddiqui A A & Ali M A, *J Chin Chem Soc*, 54 (2007), 5-8.
30 Gupta M K, Neelakantan T V, Sanghamitra M, Tyagi R K, Dinda A, Maulik S, Mukhopadhyay C K & Goswami S K, *Antioxid Redox Signal*, 8(2006), 1081-1093.
31 Kumar M M, Gupta A K, Negi S & Bhatt M, *Int J Pharma Sci Res*, 1 (2010), 172-177.
32 Ahmed M O, Mahmud A, Hasan T & Mahmoud T, *Pol J Pharma*, 54 (2002), 55-59.
33 Khan M T, Choudhary M I, Khan K M, Rani M& Atta-ur-Rahman, *Bioorg Med Chem*, 13(2005), 3385-3395.
34 Bhardwaj N, Saraf P, Sharma S K & Kumar P, *E- J Chem*, 6 (2009), 1133-38.
35 Farshori N N, Banday M R, Ahmad A, Khan A U& Rauf A. *Bioorg Med Chem Lett*, 20(2010), 1933-1938.
36 Rai N P, Narayanaswamy V K, Shashikanth S & Arunachalam P N, *Eur J Med Chem*, 44(2009), 4522-4527.
37 Ateş O, Kocabalkanli A, Cesur N& Otük G, *Farmaco*, 53(1998), 541-544.
38 Prakash O, Kumar M, Kumar R, Sharma C& Aneja K R, *Eur J Med Chem*, 45(2010), 4252-4257.
39 Courvalin P, *Emerg Infect Dis*, 11(2005), 1503-1506.
40 Ranjith P K, Pakkath R, Haridas K R& Kumari S N, *Eur J Med Chem*, 71 (2014), 354-365.
41 Chawla R, Arora A, Parameswaran M K, Chan P, Sharma D, Michael S & Ravi T K, *Acta Pol Pharm*, 67 (2010), 247-253.
42 Smith R C, Reeves J C, Dage R C& Schnettler R A, *Biochem Pharmacol*, 36 (1987), 1457-1460.
43 Warrener R N, *Eur J Org Chem*, 65 (2000) 3363-3380.
44 Emami S, Foroumadi A, Falahati M, Lotfali E, Rajabalian S, Ebrahimi SA, Farahyar S & Shafiee A, *Bioorg Med Chem Lett*, 18 (2008), 141-146.
45 Küçükgüzel S G, Oruç E E, Rollas S, Sahin F& Ozbek A, *Eur J Med Chem*, 37 (2002), 197-206.
46 Shivi B & Gupta M, *J Chem Pharm Res*, 3 (2011), 137-147.
47 Kim S H, Zo J H, Kim M A, Hwang K K & Chae I H, *Nutr Res*, 23 (2003), 1671.
48 Thom S M, Horobin R W, Seidler E& Barer M R, *J Appl Bacteriol*, 74 (1993), 433-43.
49 Kim S R, Park M J, Lee M K, Sung S H, Park E J, Kim J, Kim S Y, Oh T H, Markelonis GJ & Kim Y C, *Free Radic Biol Med*, 32 (2002), 596-604.
50 Mosmann T, *J Immunol Methods*, 65 (1983), 55-63.
51 Tan T M, Chen Y, Kong K H, Bai J, Li Y, Lim S G, Ang T H & Lam Y, *Antiviral Res*, 71 (2006), 7-14.
52 Beg M A, Hejazi I I, Thakur S C & Athar F, *Biotechnol Appl Biochem*, *2021; 10.1002/bab.2109.*
53 Jakubkiene V, Burbuliene MM, Mekuskiene G, Udrenaite E, Gaidelis P& Vainilavicius P, *Farmaco*, 58 (2003), 323-328.
54 Li Y, Liu J, Zhang H, Yang X & Liu Z, *Bioorg Med Chem Lett*, 16 (2006), 2278-2282.