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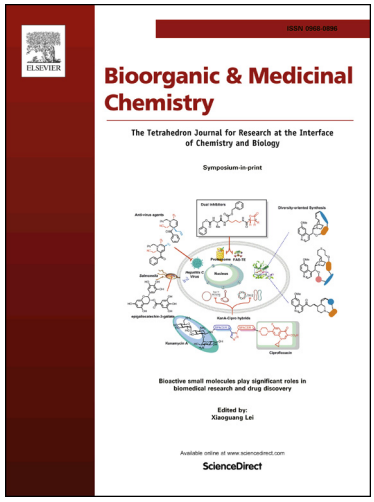

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1, 2, 4-triazole and 1, 3, 4-oxadiazole analogues: synthesis, MO studies, *in silico* molecular docking studies, antimalarial as DHFR inhibitor and antimicrobial activities

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Highlights

- Design and synthesis of 1, 2, 4-triazole and 1, 3, 4-oxadiazole analogues.
- *In vitro* antimicrobial and antimalarial activities.
- Molecules were subjected to DFT and PM6 calculations to explain the anti-bacterial activity.
- *In silico* molecular docking and DHFR inhibitors assay.

Abstract

1, 2, 4-triazole and 1, 3, 4-oxadiazole analogues are of interest due to their potential activity against microbial and malarial infections. In search of suitable antimicrobial and antimalarial compounds, we report here the synthesis, characterization and biological activities of 1, 2, 4-triazole and 1, 3, 4-oxadiazole analogues (**SS 1-SS 10**). The molecules were characterized by IR, mass, ^{1}H NMR, ^{13}C NMR and elemental analysis. The *in vitro* antimicrobial activity was investigated against pathogenic strains, the results were explained with the help of DFT and PM6 molecular orbital calculations. *In vitro* cytotoxicity and genotoxicity of the molecules were studied against *S. pombe* cells. *In vitro* antimalarial activity was studied. The active compounds were further evaluated for enzyme inhibition efficacy against the receptor Pf-DHFR computationally as well as *in vitro* to prove their candidature as lead dihydrofolate reductase inhibitors.

Keywords 1, 2, 4-triazole; 1, 3, 4-oxadizaole; DFT; PM6; anti-microbial activity; *S. pombe*; molecular docking; anti-malarial activity; DHFR

1. Introduction

Heterocyclic chemistry has become one of the most important fields of research in pharmaceutical industry due to their many fold applications. Amongst all, heterocyclic molecules containing nitrogen and oxygen have shown most potent biological activities [1]. It follows from the literature that depending on the type of substituent, the analogues of 1, 2, 4-triazole have a high potential for a wide range of biological activities such as antimicrobial [2-4], analgesic [4], anti-tumor [5], anti-inflammatory [6], anti-hypertensive [7], anti-cancer [8] and antiviral [9] activities. In a host of standard medicines 1, 2, 4-triazole moiety is present [10]. 1, 3, 4-oxadiazole is an essential core in heterocyclic chemistry and represents a key motif in medicinal chemistry due to their potential to exhibit bioactivities such as anti-HIV [11], analgesic [12, 13], anti-inflammatory [12, 13], anti-cancer [14, 15], antimalarial [16], antimicrobial [16] and anti-tuberculosis [16]. Medicines having 1, 3, 4-oxadiazole ring are plenty [10]. Schiff bases also have gained importance in the field of medicine due to their wide spectrum of biological activities such as antimicrobial [17-19], anti-tubercular [20-22], anti-HIV [22], anti-cancer [22], anti-tumor [23], anti-inflammatory [24], analgesic [24] and anticonvulsant [25, 26].

Considering the significant biological and medicinal importance of Schiff bases, 1, 2, 4-triazole and 1, 3, 4-oxadiazole, Schiff bases derived from 1, 2, 4-triazole and 1, 3, 4-

oxadiazole were synthesized and efficacy of these compounds as bioactive material was investigated. Different aldehydes having five members and six members (benzene) rings with various electron withdrawing and electron donating groups have been used to prepare the analogues. This was done to examine the effect of the ring size and substituent in the ring on the efficacy of the biological activities of these compounds.

Data from FTIR, NMR, mass and elemental analysis were used to characterize the compounds. The antibacterial and antifungal potency of these Schiff bases were investigated against certain standard strains. The activities of these compounds were compared with standard antibacterial and antifungal drugs. Molecular orbital calculations with DFT and PM6 have been done for the Schiff bases to correlate the antimicrobial activities with electronic parameters. The fission yeast *Schizosaccharomyces pombe* is an important model organism for the study of eukaryotic molecular and cellular biology [27]. As eukaryotes, these yeasts can be used to study processes that are conserved from yeast to humans but absent from bacteria, such as organelle biogenesis and cytoskeletal organization or to study mechanisms such as transcription, translation and DNA replication, in which the eukaryotic components and processes are significantly different from those of their bacterial counterparts [28]. Hence we studied the cytotoxic and genotoxic behaviors of compounds (**S 1- SS 10**) on *S. pombe* cells.

Malaria remains a major cause of public health problem in about 95 countries mainly located in the tropical zone of the globe (notably Africa, South-East Asia and also Eastern Mediterranean region) [29]. This parasitic disease is still estimated to affect over 212 million people and accounted for 429,000 deaths in 2015 [29]. Five species of protozoan parasites belonging to the *Plasmodium* genus, namely *falciparum*, *malariae*, *vivax*, *ovale* and *knowlesi* cause malaria in human beings and *Plasmodium falciparum* is the most dangerous of these species [30, 31]. The increasing prevalence of multiple drug resistant *P. falciparum* has significantly reduced the efficacy of the current anti-malarial drugs. Also, the resistance against *P. falciparum* is associated with mutations in the dihydrofolate reductase (DHFR) domain [32]. Hence, DHFR enzyme has shown to be reliable and the best target to design new antimalarial drugs. Accordingly, we studied antimalarial activity against *P. falciparum* strain for the compounds (**S 1-SS 10**) and obtained IC_{50}. Of these compounds, **SS 2**, **SS 3**, **SS 4** and **SS 9** were found to be significantly active. These compounds along with standard drugs (**Chloroquine** & **Pyrimethamine**) were docked with wild-type *Plasmodium falciparum*

dihydrofolate reductase-thymidylate synthase (pfdhfr-ts) complex (PDB ID: **4DPD**) and their *in vitro* DHFR enzyme inhibition activity was also studied.

2. Materials and methods

All starting materials and reagents were purchased from Sigma-Aldrich, Merck and Loba Chemie. Thin layer chromatography (TLC) was performed on silica gel G_{60} F_{254} (Merck) plates and eluted with the mobile phases *n*-hexane: ethyl acetate and methanol: chloroform (70:30 % v/v). Melting points were recorded on automated melting point system and were uncorrected. Nicolet 6700 spectrophotometer (Thermo Scientific) was employed to record IR spectra using KBr pellet. NMR spectra were recorded on Bruker 400 MHz using DMSO-d6 as solvent and TMS as an internal standard. Chemical shifts are reported in parts per million (δ in ppm). Mass spectra were recorded on Waters Micromass Q-Tof Micro. Elemental analysis was performed on the Thermo Scientific Flash 2000.

2.1 Preparation and characterization of compounds (A 1-5, S 1, S 2 and SS 1-10)

2.1.1 Synthesis of methyl-4-aminobenzoate (A 1): Concentrated H_2SO_4 (0.131 mol) was added dropwise to a solution of *p*-aminobenzoic acid (0.131 mol) in methanol (200 ml). The contents were refluxed for 3 h (**Scheme 1**) and then cooled to room temperature. Excess methanol was distilled out and the solution poured into cold water. The mixture was made alkaline by adding 10% NaOH solution. The precipitate of **A 1** obtained was filtered, washed with cold water and recrystallized from 1:1 methanol: water. (17.13 g, 86.40%); white solid; M.P.: 109 °C; IR (KBr) (cm^{-1}):3466, 3370, 3069, 2902, 2845, 1681, 1657, 1566, 1458, 836; EIMS (m/z): 152 (M+1); ^{1}H NMR (DMSO-d6, 400 MHz) δ (ppm): 7.696 (d, 2H, Ar-H), 6.625 (d, 2H, Ar-H), 5.945 (s, 2H, -NH_2), 3.745 (s, 3H, -OCH_3); ^{13}C NMR (DMSO-d6, 100 MHz) δ (ppm): 166.4, 153.4, 131.0, 115.8, 112.6, 51.0; Anal Calcd. for $C_8H_9NO_2$: C, 63.56; H, 6.00; N, 9.27%. Found: C, 63.58; H, 6.01; N, 9.30%.

2.1.2 Synthesis of methyl-4-acetamidobenzoate (A 2): A mixture of acetic acid (0.093 mol) and acetic anhydride (0.093 mol) was taken in a RBF and compound **A 1** (0.093 mol) was added pinch wise to the mixture with stirring (**Scheme 1**). After addition the content in the RBF was refluxed for 2 h and then poured into crushed ice. The solid was filtered, washed with cold water and recrystallized from methanol. (16.34 g, 91.32%); white solid; M.P.: 129 °C; IR (KBr) (cm^{-1}): 3361, 3071, 2927, 2851, 1686, 1611, 1597, 1525, 1442, 1368, 833; EIMS (m/z): 193.9 (M+1); ^{1}H NMR (DMSO-d6, 400 MHz) δ (ppm): 10.271 (s, 1H, -N<u>H</u>$COCH_3$), 7.886 (d, 2H, Ar-H), 7.734 (d, 2H, Ar-H), 3.783 (s, 3H, -OCH_3), 2.090 (s, 3H, -

NHCOC<u>H</u>$_3$); ^{13}C NMR (DMSO-d6, 100 MHz) δ (ppm): 168.8, 165.7, 143.6, 130.0, 123.6, 118.1, 51.5, 23.9; Anal Calcd. for $C_{10}H_{11}NO_3$: C, 62.17; H, 5.74; N, 7.25%. Found: C, 62.08; H, 5.76; N, 7.23%.

2.1.3 Synthesis of N-(4-(hydrazinecarbonyl) phenyl)acetamide (A 3): Compound **A 2** (0.078 mol) was dissolved in a mixture of hydrazine hydrate (0.233 mol) and 50 ml methanol taken in a 150 ml RBF. The contents were refluxed for 3 h (**Scheme 1**). During reflux the solid residue of **A 3** was obtained. It was filtered, washed with methanol and dried. (13.81 g, 92.07%); white solid; M.P.: 255 °C; IR (KBr) (cm^{-1}): 3367, 3314, 3055, 2933, 2852, 1670, 1618, 1590, 1435, 1370, 835; EIMS (m/z): 194.1 (M+1); ^{1}H NMR (DMSO-d6, 400 MHz) δ (ppm): 10.129 (s, 1H, -N<u>H</u>COCH$_3$), 9.627 (S, 1H, -CON<u>H</u>NH$_2$), 7.780 (d, 2H, Ar-<u>H</u>), 7.642 (d, 2H, Ar-<u>H</u>), 4.436 (s, 2H, -CONHN<u>H</u>$_2$), 2.069 (s, 3H, -NHCOC<u>H</u>$_3$); ^{13}C NMR (DMSO-d6, 100 MHz) δ (ppm): 168.4, 165.6, 141.7, 127.6, 118.0, 112.5, 24.0; Anal Calcd. for $C_9H_{11}N_3O_2$: C, 55.95; H, 5.74; N, 21.75%. Found: C, 56.04; H, 5.72; N, 21.73%.

2.1.4 Synthesis of N-(4-(4-amino-5-mercapto-4H-1, 2, 4-triazol-3-yl) phenyl)acetamide (A 4): The compound **A 3** (0.031 mol) and carbon disulphide (0.031 mol) were added to solution of sodium hydroxide (0.031 mol) in methanol (30 ml) with constant stirring. The reaction mixture was stirred for 6 h at room temperature (**Scheme 1**). The precipitate of sodium dithiocarbazinate was collected by filtration, washed with ether and dried under vacuum. The sodium salt was obtained in quantitative yield and was used in the next step without further purification.

A suspension of the sodium salt, hydrazine hydrate (0.062 mol) and water (30 ml) was refluxed for 2 h. Hydrogen sulphide evolved and homogenous solution resulted, which was diluted with ice cold water and subsequent acidification with dilute HCl gave a white precipitate of **A 4**, which was filtered, washed with water and recrystallized from methanol. (6.95 g, 89.85%); white solid; M.P.: 207 °C; IR (KBr) (cm^{-1}): 3394, 3314, 3055, 2933, 2852, 1670, 1618, 1590, 1435, 1370, 835; EIMS (m/z): 250.1 (M+1); ^{1}H NMR (DMSO-d6, 400 MHz) δ (ppm): 13.772 (s, 1H, -S<u>H</u>), 10.132 (s, 1H, -N<u>H</u>COCH$_3$), 7.958 (d, 2H, Ar-<u>H</u>), 7.706 (d, 2H, Ar-<u>H</u>), 5.702 (s, 2H, -N<u>H</u>$_2$), 2.070 (s, 3H, -NHCOC<u>H</u>$_3$); ^{13}C NMR (DMSO-d6, 100 MHz) δ (ppm): 168.8, 166.2, 148.9, 141.0, 128.2, 120.0, 118.4, 23.9; Anal Calcd. for $C_{10}H_{11}N_5OS$: C, 48.18; H, 4.45; N, 28.09; S, 12.86%. Found: C, 48.20; H, 4.44; N, 28.02; S, 12.90%.

2.1.5 Synthesis of N-(4-(5-mercapto-1, 3, 4-oxadiazol-2-yl)phenyl)acetamide (A 5): To a mixture of NaOH (0.031 mol) and methanol (30 ml), **A 3** (0.031 mol) was added pinch wise with stirring. After complete addition, CS_2 (0.031 mol) was poured in reaction mixture and stirred for 6 h at room temperature (**Scheme 1**).The contents were then poured in crushed ice and acidified with dilute HCl. Precipitate of **A 5** thus obtained was filtered, recrystallized from methanol and dried (6.77 g, 92.70%); white solid; M.P.: 230 °C; IR (KBr) (cm^{-1}): 3307, 3052, 2930, 2573, 1679, 1613, 1588, 1351, 838; EIMS (m/z): 236.1 (M+1); ^{1}H NMR (DMSO-d6, 400 MHz) δ (ppm): 14.649 (s, 1H,-S<u>H</u>), 10.331 (s, 1H, -N<u>H</u>COCH$_3$), 7.881 (m, 4H, Ar-<u>H</u>), 2.091 (s, 3H, -NHCOC<u>H</u>$_3$); ^{13}C NMR (DMSO-d6, 100 MHz) δ (ppm): 177.1, 168.7, 160.3, 142.7, 126.8, 118.9, 116.5, 24.0; Anal Calcd. for $C_{10}H_9N_3O_2S$: C, 51.05; H, 3.86; N, 17.86; S, 13.63%. Found: C, 50.89; H, 3.87; N, 17.92; S, 13.67%.

2.1.6 Synthesis of 4-amino-5-(4-aminophenyl)-4H-1, 2, 4-triazole-3-thiol (S 1) and 5-(4-aminophenyl)-1, 3, 4-oxadiazole-2-thiol (S 2): Compound **A 4** (0.020 mol) in 15 ml 2.5 N HCl was refluxed for 1 h in round bottom flask. The refluxed mixture was poured in finely crushed ice and made alkaline using 10% $NaHCO_3$ solution; the residue of **S 1** was filtered, washed with cold water and recrystallized from methanol to get final product (**Scheme 1**). The same procedure was repeated with **A 5** (0.020 mol) to obtain **S 2** compound (**Scheme 1**).

4-amino-5-(4-aminophenyl)-4H-1, 2, 4-triazole-3-thiol (**S 1**): (3.46 g, 83.20%); white solid; M.P.: 218 °C; IR (KBr) (cm^{-1}): 3351, 3269, 3095, 2592, 1614, 1582, 1451, 846; EIMS (m/z): 208.1 (M+1); ^{1}H NMR (DMSO-d6, 400 MHz) δ (ppm): 13.577 (s, 1H, -S<u>H</u>), 7.742 (d, 2H, Ar-<u>H</u>), 6.640 (d, 2H, Ar-<u>H</u>), 5.665 (s, 2H, -N<u>H</u>$_2$), 5.543 (s, 2H, -N<u>H</u>$_2$); ^{13}C NMR (DMSO-d6, 100 MHz) δ (ppm): 165.7, 150.7, 149.8, 128.9, 113.0, 112.5; Anal Calcd. for $C_8H_9N_5S$: C, 46.36; H, 4.38; N, 33.79; S, 15.47%. Found: C, 46.41; H, 4.39; N, 33.70; S, 15.50%.

5-(4-aminophenyl)-1, 3, 4-oxadiazole-2-thiol (**S 2**): (3.26 g, 79.30%); white solid; M.P.: 215 °C; IR (KBr) (cm^{-1}): 3447, 3351, 3093, 2587, 1629, 1605, 1565, 1440, 836; EIMS (m/z): 194.1 (M+1); ^{1}H NMR (DMSO-d6, 400 MHz) δ (ppm): 14.375 (s, 1H,-S<u>H</u>), 7.542 (d, 2H, Ar-<u>H</u>), 6.670 (d, 2H, Ar-<u>H</u>), 6.021 (s, 2H, -N<u>H</u>$_2$); ^{13}C NMR (DMSO-d6, 100 MHz) δ (ppm): 176.5, 161.5, 152.5, 127.5, 113.4, 108.4; Anal Calcd. for $C_8H_7N_3OS$: C, 49.73; H, 3.65; N, 21.75; S, 16.59%. Found: C, 48.78; H, 3.66; N, 21.72; S, 16.55%.

2.1.7 General procedure for the synthesis of Schiff Bases (SS 1-10): Compounds **S 1** (0.002 mol)/ **S 2** (0.003 mol) were dissolved in 5 ml DMF followed by addition of different

aldehydes (0.005 mol for **S 1** and 0.003 mol for **S 2**) (**Schemes 2** and **3**) and catalytic amount of glacial acetic acid. The mixture was stirred at room temperature for 4 h. The reaction mixture was then poured into crushed ice, the residues of **SS 1-10** were obtained, which were filtered, washed with cold water and recrystallized from methanol to get purified products.

4-((thiophen-2-ylmethylene)amino)-5-(4-((-thiophen-2-ylmethylene)amino)phenyl)-4H-1, 2, 4-triazole-3-thiol (**SS 1**): (0.70 g, 73.62%); light yellow solid; M.P.: 181 °C; IR (KBr) (cm^{-1}): 3100, 2565, 1616, 1588, 1425, 838, 647; EIMS (m/z): 396 (M+1); ^{1}H NMR (DMSO-d6, 400 MHz) δ (ppm): 14.132 (s, 1H, -S<u>H</u>), 10.036 (s, 1H, Schiff Base), 9.948 (s, 1H, Schiff Base), 7.928 (m, 2H, thiophene-<u>H</u>), 7.879 (m, 2H, thiophene-<u>H</u>), 7.689 (m, 2H, thiophene-<u>H</u>), 7.578 (d, 2H, Ar-<u>H</u>), 7.363 (d, 2H, Ar-<u>H</u>); ^{13}C NMR (DMSO-d6, 100 MHz) δ (ppm): 165.8, 162.3, 161.5, 152.5, 149.0, 136.4, 136.3, 132.5, 132.3, 131.6, 131.4, 129.2, 129.0, 128.0, 122.7, 121.1; Anal Calcd. for $C_{18}H_{13}N_5S_3$: C, 54.66; H, 3.31; N, 17.71; S, 24.32%. Found: C, 54.70; H, 3.30; N, 17.76; S, 24.24%.

4-(((4-(4-((4-hydroxybenzylidene)amino)-5-mercapto-4H-1, 2, 4-triazol-3-yl)phenyl)imino) methyl) phenol (**SS 2**): (0.72 g, 71.97%); light yellow solid; M.P.: 172 °C; IR (KBr) (cm^{-1}): 3318, 3019, 2598, 1601, 1580, 1437, 827; EIMS (m/z): 416 (M+1); ^{1}H NMR (DMSO-d6, 400 MHz) δ (ppm): 14.212 (s, 1H, -S<u>H</u>), 10.447 (b, 2H, -O<u>H</u>), 9.994 (s, 1H, Schiff Base), 9.810 (s, 1H, Schiff Base), 7.916 (m, 4H, Ar-<u>H</u>), 7.508 (m, 4H, Ar-<u>H</u>), 7.327 (d, 2H, Ar-<u>H</u>), 6.875 (d, 2H, Ar-<u>H</u>); ^{13}C NMR (DMSO-d6, 100 MHz) δ (ppm): 167.3, 163.9, 162.7, 159.39, 159.31, 150.8, 148.6, 132.4, 132.2, 130.8, 130.7, 128.3, 126.3, 126.2, 122.6, 121.4; Anal Calcd. for $C_{22}H_{17}N_5O_2S$: C, 63.60; H, 4.12; N, 16.86; S, 7.72%. Found: C, 63.70; H, 4.11; N, 16.80; S, 7.71%.

4-((3-nitrobenzylidene)amino)-5-(4-((3-nitrobenzylidene)amino)phenyl)-4H-1, 2, 4-triazole-3-thiol (**SS 3**): (0.86 g, 75.57%); light yellow solid; M.P.: 151 °C; IR (KBr) (cm^{-1}): 3030, 2605, 1614, 1602, 1580, 1532, 1443, 1313, 827, 740; EIMS (m/z): 474 (M+1); ^{1}H NMR (DMSO-d6, 400 MHz) δ (ppm): 14.300 (s, 1H, -S<u>H</u>), 10.159 (s, 1H, Schiff Base), 10.081 (s, 1H, Schiff Base), 8.860 (d, 2H, Ar-<u>H</u>), 8.534 (m, 2H, Ar-<u>H</u>), 8.441 (m, 2H, Ar-<u>H</u>), 7.985 (m, 2H, Ar-<u>H</u>), 7.560 (d, 2H, Ar-<u>H</u>), 7.468 (d, 2H, Ar-<u>H</u>); ^{13}C NMR (DMSO-d6, 100 MHz) δ (ppm): 168.1, 163.0, 162.3, 151.0, 148.2, 138.8, 138.7, 137.1, 136.9, 134.77, 134.71, 130.4, 130.3, 129. 5, 129.4, 128.7, 125.6, 125.5, 122.9, 122.0; Anal Calcd. for $C_{22}H_{15}N_7O_4S$: C, 55.81; H, 3.19; N, 20.71; S, 6.77%. Found: C, 55.93; H, 3.20; N, 20.63; S, 6.75%.

4-((4-chlorobenzylidene)amino)-5-(4-((4-chlorobenzylidene)amino)phenyl)-4H-1, 2, 4-triazole-3-thiol (**SS 4**): (0.69 g, 63.16%); light yellow solid; M.P.: 185 °C; IR (KBr) (cm^{-1}): 3095, 2568, 1610, 1592, 1425, 825, 728; EIMS (m/z): 452 (M+1); ^{1}H NMR (DMSO-d6, 400 MHz) δ (ppm): 14.192 (s, 1H, -SH), 10.001 (s, 1H, Schiff Base), 9.915 (s, 1H, Schiff Base), 7.903 (m, 4H, Ar-H), 7.558 (m, 4H, Ar-H), 7.378 (d, 2H, Ar-H), 6.644 (d, 2H, Ar-H); ^{13}C NMR (DMSO-d6, 100 MHz) δ (ppm): 169.6, 163.7, 162.2, 150.9, 148.3, 136.6, 136.5, 134.4, 134.2, 130.9, 130.7, 129.2, 129.1, 128.8, 122.9, 121.1; Anal Calcd. for $C_{22}H_{15}Cl_2N_5S$: C, 58.41; H, 3.34; N, 15.48; S, 7.09%. Found: C, 58.30; H, 3.33; N, 15.54; S, 7.11%.

4-((4-methoxybenzylidene)amino)-5-(4-((4-methoxybenzylidene)amino)phenyl)-4H-1, 2, 4-triazole-3-thiol (**SS 5**): (0.72 g, 67.39%); light yellow solid; M.P.: 173 °C; IR (KBr) (cm^{-1}): 3002, 2931, 2836, 2565, 1617, 1571, 1440, 1355, 1255, 831; EIMS (m/z): 444 (M+1); ^{1}H NMR (DMSO-d6, 400 MHz) δ (ppm): 14.097 (s, 1H, -SH), 9.862 (s, 1H, Schiff Base), 9.612 (s, 1H, Schiff Base), 7.945 (m, 4H, Ar-H), 7.575 (m, 4H, Ar-H), 7.321 (d, 2H, Ar-H), 6.637 (d, 2H, Ar-H), 3.850 (s, 6H, -OCH$_3$); ^{13}C NMR (DMSO-d6, 100 MHz) δ (ppm): 169.2, 162.9, 161.5, 150.8, 148.1, 131.6, 131.5, 130.6, 130.9, 129.0, 128.9, 128.6, 124.7, 124.3, 122.4, 121.0, 55.35, 55.31; Anal Calcd. for $C_{24}H_{21}N_5O_2S$: C, 64.99; H, 4.77; N, 15.79; S, 7.23%. Found: C, 65.02; H, 4.76; N, 15.82; S, 7.21%.

5-(4-((thiophen-2-ylmethylene)amino)phenyl)-1, 3, 4-oxadiazole-2-thiol (**SS 6**): (0.57 g, 77.24%); light yellow solid; M.P.: 160 °C; IR (KBr) (cm^{-1}): 3080, 3060, 2577, 1621, 1601, 1592, 1426, 1170, 838, 694; EIMS (m/z): 288 (M+1); ^{1}H NMR (DMSO-d6, 400 MHz) δ (ppm): 14.517 (s, 1H, -SH), 9.965 (s, 1H, Schiff Base), 8.156 (d, 1H, thiophene-H), 8.046 (d, 1H, thiophene-H), 7.922 (t, 1H, thiophene-H), 7.554 (d, 2H, Ar-H), 6.687 (d, 2H, Ar-H); ^{13}C NMR (DMSO-d6, 100 MHz) δ (ppm): 177.3, 161.5, 160.3, 152.6, 137.8, 134.6, 132.1, 128.9, 127.6, 113.8, 108.4; Anal Calcd. for $C_{13}H_9N_3OS_2$: C, 54.34; H, 3.16; N, 14.62; S, 22.31%. Found: C, 54.27; H, 3.17; N, 14.59; S, 22.38%.

4-(((4-(5-mercapto-1, 3, 4-oxadiazol-2-yl)phenyl)imino)methyl)phenol (**SS 7**): (0.54 g, 70.73%); light yellow solid; M.P.: 178 °C; IR (KBr) (cm^{-1}): 3381, 3096, 2589, 1631, 1606, 1575, 1440, 1175, 836, ; EIMS (m/z): 297 (M+1); ^{1}H NMR (DMSO-d6, 400 MHz) δ (ppm): 14.343 (s, 1H, -SH), 10.420 (s, 1H, -OH), 9.783 (s, 1H, Schiff Base), 7.792 (d, 2H, Ar-H), 7.548 (d, 2H, Ar-H), 6.929 (d, 2H, Ar-H), 6.687 (d, 2H, Ar-H); ^{13}C NMR (DMSO-d6, 100 MHz) δ (ppm): 176.6, 163.2, 161.4, 160.6, 152.3, 131.7, 128.2, 127.3, 113.4, 108.7; Anal

Calcd. for $C_{15}H_{11}N_3O_2S$: C, 60.59; H, 3.73; N, 14.13; S, 10.78%. Found: C, 60.65; H, 3.72; N, 14.18; S, 10.74%.

5-(4-((3-nitrobenzylidene)amino)phenyl)-1, 3, 4-oxadiazole-2-thiol (**SS 8**): (0.64 g, 76.22%); light yellow solid; M.P.: 189 °C; IR (KBr) (cm^{-1}): 3081, 2577, 1611, 1559, 1528, 1445, 1350, 1188, 833, 787; EIMS (m/z): 327 (M+1); ^{1}H NMR (DMSO-d6, 400 MHz) δ (ppm): 14.391 (s, 1H, -S<u>H</u>), 10.158 (s, 1H, Schiff Base), 8.705 (s, 1H, Ar-<u>H</u>), 8.517 (d, 1H, Ar-<u>H</u>), 8.364 (d, 1H, Ar-<u>H</u>), 7.876 (t, 1H, Ar-<u>H</u>), 7.542 (d, 2H, Ar-<u>H</u>), 7.467 (d, 2H, Ar-<u>H</u>); ^{13}C NMR (DMSO-d6, 100 MHz) δ (ppm): 176.6, 161.4, 159.8, 152.2, 137.8, 134.8, 134.6, 130.5, 128.2, 123.7, 113.4, 108.8; Anal Calcd. for $C_{15}H_{10}N_4O_3S$: C, 55.21; H, 3.09; N, 17.17; S, 9.82%. Found: C, 55.11; H, 3.10; N, 17.23; S, 9.79%.

5-(4-((4-chlorobenzylidene)amino)phenyl)-1, 3, 4-oxadiazole-2-thiol (**SS 9**): (0.57 g, 69.78%); light yellow solid; M.P.: 193 °C; IR (KBr) (cm^{-1}): 3057, 2593, 1651, 1611, 1560, 1491, 1185, 830, 729, ; EIMS (m/z): 316 (M+1); ^{1}H NMR (DMSO-d6, 400 MHz) δ (ppm): 14.439 (s, 1H, -S<u>H</u>), 10.015 (s, 1H, Schiff Base), 7.957 (d, 2H, Ar-<u>H</u>), 7.696 (d, 2H, Ar-<u>H</u>), 7.552 (d, 2H, Ar-<u>H</u>), 6.691 (d, 2H, Ar-<u>H</u>); ^{13}C NMR (DMSO-d6, 100 MHz) δ (ppm): 176.5, 162.2, 161.5, 152.6, 139.3, 134.7, 130.5, 129.3, 113.5, 108.4; Anal Calcd. for $C_{15}H_{10}ClN_3OS$: C, 57.05; H, 3.19; N, 13.31; S, 10.15%. Found: C, 56.94; H, 3.18; N, 13.36; S, 10.19%.

5-(4-((4-methoxybenzylidene)amino)phenyl)-1, 3, 4-oxadiazole-2-thiol (**SS 10**): (0.56 g, 69.03%); light yellow solid; M.P.: 165 °C; IR (KBr) (cm^{-1}): 3096, 2953, 2834, 2588, 1629, 1607, 1574, 1440, 1353, 1212, 1195, 832, ; EIMS (m/z): 312 (M+1); ^{1}H NMR (DMSO-d6, 400 MHz) δ (ppm): 14.349 (s, 1H, -S<u>H</u>), 9.867 (s, 1H, Schiff Base), 7.870 (d, 2H, Ar-<u>H</u>), 7.537 (d, 2H, Ar-<u>H</u>), 7.113 (d, 2H, Ar-<u>H</u>), 6.672 (d, 2H, Ar-<u>H</u>), 3.875 (s, 3H, -OC<u>H</u>$_3$); ^{13}C NMR (DMSO-d6, 100 MHz) δ (ppm): 176.6, 164.1, 161.4, 152.3, 131.5, 129.5, 127.3, 114.1, 114.0, 113.4, 108.7, 55.4; Anal Calcd. for $C_{16}H_{13}N_3O_2S$: C, 61.72; H, 4.21; N, 13.50; S, 10.30%. Found: C, 61.82; H, 4.22; N, 13.45; S, 10.26%.

3. Results and Discussion

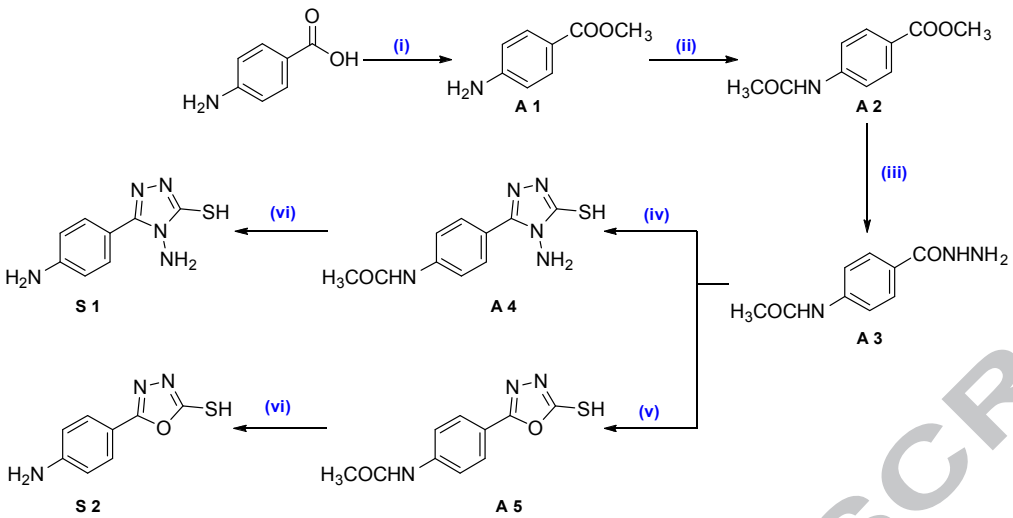
3.1 Chemistry

The syntheses of molecules (**A 1-5**, **S 1**, **S 2** and **SS 1-10**) are summarized in **Schemes 1-3**. **Table 1** shows the type of substituent present on the target molecules **SS 1-10**. The starting material methyl-4-aminobenzoate (**A 1)** was prepared according to Fischer-Speier esterification reaction. Methyl-4-aminobenzoate (**A 1)** was refluxed for 2 h with the mixture

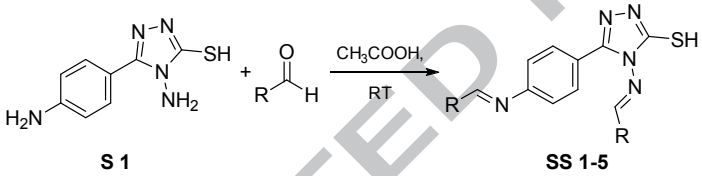
of acetic acid and acetic anhydride to get methyl-4-acetamidobenzoate (**A 2**). N-(4-(hydrazine carbonyl)phenyl)acetamide (**A 3**) was prepared by refluxing compound **A 2** and hydrazine hydrate in methanol. The compound **A 3** undergo cyclization reaction to form compounds **A 4** and **A 5** (**Scheme 1**). The parent molecules 1, 2, 4-triazole (**S 1**) and 1, 3, 4-oxadizaole (**S 2**) were obtained by hydrolysis of compounds **A 4** and **A 5** respectively. The analogues of 1, 2, 4-triazole and 1, 3, 4-oxadiazole (**SS 1-10**) were prepared by reacting **S 1** (**Scheme 2**) and **S 2** (**Scheme 3**) with different aldehydes in the presence of acetic acid as a catalyst.

The formation of synthesized compounds was confirmed by IR, mass spectra, ^{1}H NMR, ^{13}C NMR and elemental analysis. The IR spectra of all synthesized compounds (**A 1-5**, **S 1**, **S 2** and **SS 1-10**) showed peaks at ~3070 cm^{-1}, ~1570 cm^{-1} and ~1440 cm^{-1} were due to aromatic C-H, and C=C stretching. The peak at ~2570 cm^{-1} in **A 4**, **A 5**, **S 1**, **S 2** and SS 1-10 was attributed to stretching of -SH group. The molecules **SS 2** and **SS 7** showed a broad peak at ~3350 cm^{-1} due to -OH stretching. The compounds **SS 3** and **SS 8** showed two bands near ~1530 cm^{-1} and ~1340 cm^{-1} due to asymmetric and symmetric stretching of $-NO_2$ group. The peak at ~730 cm^{-1} in **SS 4** and **SS 9** was attributed to stretching of C-Cl group. The compounds **SS 5** and **SS 10** showed a peak at ~1230 cm^{-1} due to C-O-CH_3 stretching.

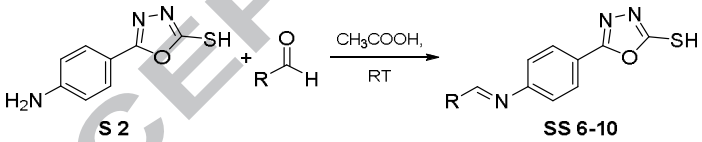
The mass spectrum of all the compounds showed molecular ion peak (M+1) corresponding to their respective molecular weights, which additionally confirmed by the molecular framework. The ^{1}H NMR resonances due to aromatic hydrogen in all synthesized compounds (**A 1-5**, **S 1**, **S 2** and **SS 1-10**) appeared between ~6.6-8.8 ppm. For compounds (**A 4, A 5, S 1, S 2** and **SS 1-10**), the thiol -SH proton appeared at ~13.8 ppm as a singlet. The two new peaks at ~9.8 ppm (singlet) and ~10.0 ppm (singlet) in the Schiff bases is due to -N=CH- for compounds **SS 1-5**. The peak at ~9.9 ppm (singlet) is attributed to Schiff base proton in compounds **SS 6-10**. The ^{13}C NMR of compounds (**A 1-5, S 1, S 2** and **SS 1-10**) showed peaks in the range of ~118-150 ppm due to the presence of aromatic C-atoms. The C-atoms of -N=CH- moiety in Schiff bases are seen between ~160-170 ppm for molecules **SS 1-10**.



Scheme 1. Method for the preparation of parent moieties i.e. 4-amino-5-(4-aminophenyl)-4H-1, 2, 4-triazole-3-thiol (**S 1**) and 5-(4-aminophenyl)-1, 3, 4-oxadiazole-2-thiol (**S 2**). (i) CH_3OH, conc. H_2SO_4, Reflux 3 h, 10% NaOH solution; (ii) $(CH_3CO)_2O$, CH_3COOH, Reflux 2 h; (iii) NH_2NH_2 H_2O, CH_3OH, Reflux 3 h; (iv) CS_2, NaOH, CH_3OH, RT for 6 h, NH_2NH_2 H_2O, Reflux 2 h, dil. HCl; (v) CS_2, 1 N NaOH, CH_3OH, RT for 6 h, dil. HCl; (vi) dil. HCl, Reflux 1 h, 10% $NaHCO_3$.

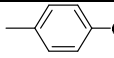


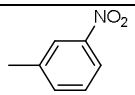
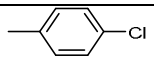
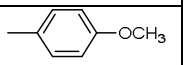
Scheme 2. Method for the preparation of Schiff Bases (**SS 1-5**)



Scheme 3. Method for the preparation of Schiff Bases (**SS 6-10**)

Table 1. Scheme followed to designate the molecules synthesized

Sample ID(s)	**R**
SS 1, SS 6	
SS 2, SS 7	

SS 3, **SS 8**	
SS 4, **SS 9**	
SS 5, **SS 10**	

3.2 *In silico* pharmacokinetic evaluation

The pharmacokinetic phase is important for an orally administrated drug. This phase includes absorption from gastrointestinal tract into the blood supply and various factors that affect drug's survival and processes as it travels to the target. The *in silico* study was performed by VlifeMDS 4.6 and ADMET software and the data obtained are presented in **Table 2** and **Table 3.**

Table 2. Prediction of Lipinski's Rule of Five and molecular properties of compounds

Compound ID(s)	Mol Wt.[a]	HBA[b]	HBD[c]	RotB[d]	ClogP[e]	logS[f] log(mol/l)	PSA[g] (°A^2)	Volume (°A^3)
S 1	207.06	3	5	1	1.06	-3.67	61.69	156.66
S 2	193.03	4	3	1	1.68	-3.60	50.44	148.97
SS 1	395.03	7	1	5	4.83	-7.99	44.24	349.21
SS 2	415.11	7	3	5	4.56	-8.39	77.44	373.59
SS 3	473.09	9	1	7	4.53	-9.51	118.73	404.21
SS 4	451.04	5	1	5	6.51	-10.59	42.21	387.18
SS 5	443.14	7	1	7	5.26	-9.19	57.29	416.48
SS 6	287.02	6	1	3	3.44	-5.94	38.78	243.74
SS 7	297.06	6	2	3	3.31	-6.14	55.37	256.08
SS 8	326.05	7	1	4	3.29	-6.70	76.02	271.24
SS 9	315.02	5	1	3	4.28	-7.24	37.76	262.73
SS 10	311.07	6	1	4	3.66	-6.54	45.30	277.38

a = Molecular Weight ≤ 500 (gm/mol) [33]; **b** = Hydrogen Bond Acceptor ≤ 10 [33]; **c** = Hydrogen Bond Donor ≤ 5 [33]; **d** = Rotatable Bonds ≤ 10 [33]; **e** = ClogP ≤ 5 [33]; **f**; Water Solubility range -0.5 to -6.5 (mol/l) [33]; **g** = Polar Surface Area ≤ 140°A^2 [33].

The values of physicochemical parameters (**Table 2**) such as molecular weight, HBA, HBD, RotB, PSA, volume and logP except for **SS 4** and **SS 5** analogues suggested that all of the

molecules (**S 1-SS 10**) had favorable Lipinski's rule of five (RO5) [33, 34]. The logS value for compounds **SS 1** to **SS 5** and **SS 7** to **SS 10** violated the RO5 suggested that these molecules were poorly absorbed and distributed.

Table 3. Prediction of ADMET parameters of compounds

Compound ID(s)	**BBB**	**Caco2 (nm/sec)**	**% HIA**	**%PPB**	**MDCK (nm/sec)**	**hERG inhibition**
S 1	0.1419	20.043	86.124	25.494	4.9436	Medium Risk
S 2	0.2459	20.456	89.611	67.574	32.3434	Low Risk
SS 1	0.2234	49.273	99.156	96.047	3.2930	Medium Risk
SS 2	0.0247	21.075	96.446	95.165	0.0688	Medium Risk
SS 3	0.1409	19.794	87.980	95.354	0.0567	Low Risk
SS 4	0.3170	45.947	97.704	99.999	2.5059	Medium Risk
SS 5	0.7745	34.712	99.572	92.199	0.0624	Medium Risk
SS 6	0.5181	44.420	97.115	94.517	3.9198	Medium Risk
SS 7	0.3553	7.7060	96.357	95.479	10.4598	Medium Risk
SS 8	0.2260	20.431	90.218	92.804	0.4744	Medium Risk
SS 9	1.7178	33.559	99.318	97.995	0.5745	Medium Risk
SS 10	0.6107	25.829	98.914	92.027	1.1258	Medium Risk

BBB (Blood Brain Barrier) [35]: High absorption CNS >2.0, Middle absorption CNS 2.0-0.1, Low absorption to CNS <0.1; **Caco2** [35]: High permeability >70, Middle permeability 4-70, Low permeability <4; **%HIA** (Human Intestinal Absorbance) [35]: Well absorbed compounds 70-100%, Moderately absorbed compounds 20-70%, Poorly absorbed compounds 0-20%; **%PPB** (Plasma Protein Binding) [35]: Strongly Bound >90%, Weakly Bound <90%, **MDCK** [35]: Higher permeability >500, Medium Permeability 25-500, lower permeability <25.

The parameters obtained from ADMET software are compared with the results reported in earlier work [35]. In comparison with 1, 2, 4-triazole (**S 1**) and 1, 3, 4-oxadiazole (**S 2**), **S 1** had lower absorption to CNS (**Table 3**). Also the analogues of triazole **SS 1-SS 5** had less absorption to CNS compared to oxadiazole **SS 6-SS 10** analogues, suggested that 1, 2, 4-triazole ring was more fit than 1, 3, 4-oxaadizole ring.

Compared with parent molecules **S 1** and **S 2**, the analogues **SS 1-SS 10** had good Caco2 permeability except **SS 2**, **SS 3**, **SS 7** and **SS 8**. Also all analogues **SS 1-SS 10** had higher human intestinal absorption compared to **S 1** and **S 2** (**Table 3**). In case of %PPB (Plasma protein binding), the parent molecule **S 1** weakly bound compared to all other molecules **S 2-SS 10**. Moreover all molecules **S 1-SS 10** had more or less MDCK permeability except **S 2** and **SS 7**. Additionally the lower to moderate risk to hERG inhibition of all molecules **S 1-SS 10** suggested that these molecules might be a good contender as a drug.

3.3 *In vitro* antimicrobial activity

The antibacterial and antifungal activities of the Schiff bases against certain bacterial and fungal strains were investigated. The activities of these compounds are compared with standard antibacterial and antifungal drugs. It is to be noted that all the compounds synthesized **S 1 - SS 10** in this study did not show any appreciable anti-fungal activities. Molecular orbital calculations have been done to correlate the anti-bacterial potency with electronic parameters.

The compound (**S 1**) is relatively a poor electron acceptor (energy of LUMO = -0.4870 eV from PM6 and = -1.0805 eV from DFT). When this is anchored to the Schiff bases, the resultant compounds become better electron acceptors. The compounds **SS 1**-**SS 5** are derivatives of **S 1** and as these molecules have stable LUMOs, we try to explain the anti-bacterial potency of these as electron acceptors (**Table 4**).

Amongst these compounds **SS 3** has most stable LUMO (energy = -1.9493 eV from PM6 and = -3.2750 eV from DFT) and therefore most potent electron acceptor in this series. It is gratifying to note that this molecule is the strongest antibacterial agent in the series. Of the two molecules **SS 4** (LUMO = -1.4605 eV from PM6 and = -2.5465 eV from DFT) and **SS 5** (LUMO=-1.0612 eV from PM6 and = -2.0412 eV from DFT), latter is a relatively poor electron acceptor, as expected **SS 4** is a slightly better antibacterial agent. Although, the compound **SS 1** (LUMO = -1.5232 eV from PM6 and = -2.5798 eV from DFT) appears to be a better acceptor than **SS 4** and **SS 5**, but is a less potent anti-bacterial agent than **SS 4** and **SS 5**. It is interesting to note that highest LUMO electron density in **SS 1** is 0.2031 (PM6) and 0.5144 (DFT), while the same in **SS 4** is 0.4017 (PM6) and 0.6930 (DFT) and in **SS 5** it is 0.4657 (PM6) and 0.7729 (DFT) (**Table 4**). The molecule **SS 2** (LUMO= -1.1897 eV from PM6 and = -1.8818 eV from DFT), although is a little more potent antibacterial agent than **SS**

1, is a poor electron acceptor compared to **SS 1**. Interestingly, the highest LUMO electron density in **SS 2** is 0.4530 (PM6).

Table 4. Energies of frontier orbitals from PM6 and DFT calculations

Compound ID(s)	**Energies (eV) from PM6**		**Energies (eV) from DFT**	
	HOMO	**LUMO**	**HOMO**	**LUMO**
S 1	-8.4879	-0.48707	-5.6950	-1.0805
SS 1	-8.8557	-1.5232	-5.7351	-2.5798
SS 2	-8.7632	-1.1897	-5.2632	-1.8818
SS 3	-9.1780	-1.9493	-6.1949	-3.2750
SS 4	-8.9797	-1.4603	-5.9242	-2.5465
SS 5	-8.6617	-1.0612	-5.5772	-2.0412
S 2	-8.6846	-0.8372	-5.9138	-1.4285
SS 6	-9.0245	-1.1450	-6.1237	-2.1926
SS 7	-8.9718	-1.0325	-5.9863	-1.8142
SS 8	-9.2490	-1.7531	-6.3546	-3.2811
SS 9	-9.0791	-1.1259	-6.1464	-2.2734
SS 10	-8.9176	-0.9810	-5.9411	-1.7684

It appears that for a good antibacterial agent, electron accepting ability and easy accommodation (high LUMO electron density) of an electron at the certain centre of the molecule are important. The compound **S 2** has LUMO energy (= -0.8370 eV from PM6 and = -1.4285 eV from DFT) and when this moiety is incorporated in Schiff bases (**SS 6**-**SS 10**), the LUMOs become more stable and consequently the analogues become better electron acceptor compared to **S 2**. In the series **SS 6**-**SS 10**, the molecules **SS 6**, **SS 8** and **SS 9** are relatively better anti-bacterial agents, compared to others in this series. The compound **SS 8** appears to be most potent anti-bacterial agent and a better electron acceptor (LUMO energy = -1.7531 eV, PM6 and = -3.2811 eV, DFT) in this series. The highest LUMO electron density in **SS 8** is 0.4706 from PM6 and 0.4763 from DFT. **SS 6** and **SS 9** are relatively poor electron acceptors as seen from their LUMO energies (**Table 4**).

Table 5. *In vitro* antimicrobial activity of compounds **S 1-SS 10** (MICs, mM)

Compound	***Gram-positive bacteria***	***Gram-negative bacteria***	***Fungi***

ID(s)	*S.A.* MTCC 96	*S.P.* MTCC 442	*E.C.* MTCC 443	*P.A.* MTCC 1688	C.A. MTCC 227	A.C. MTCC 1323
S 1	1.207	1.207	1.207	0.966	4.830	2.415
S 2	0.324	0.518	0.518	1.295	2.590	1.295
SS 1	0.633	0.506	0.316	0.633	2.531	NA
SS 2	0.602	0.482	0.241	0.602	NA	NA
SS 3	0.264	0.132	0.423	0.528	1.057	2.114
SS 4	0.554	0.222	0.554	0.222	NA	NA
SS 5	0.564	0.226	0.282	0.564	1.128	1.128
SS 6	0.697	0.348	0.436	0.871	NA	NA
SS 7	0.673	0.673	0.421	0.842	NA	NA
SS 8	0.383	0.307	0.613	0.613	1.534	1.534
SS 9	0.794	0.397	0.635	0.397	NA	NA
SS 10	0.804	0.804	0.643	0.402	3.215	1.607
A	0.716	0.286	0.286	0.286	NT	NT
B	0.155	0.155	0.155	0.155	NT	NT
C	0.151	0.151	0.075	0.075	NT	NT
D	0.031	0.031	0.031	0.031	NT	NT
E	NT	NT	NT	NT	0.108	0.108
F	NT	NT	NT	NT	1.420	0.284

S.A.*:** *Staphylococcus aureus*; ***S.P.: *Streptococcus pyogenes*; ***E.C.***: *Escherichia coli*; ***P.A.***: *Pseudomonas aeruginosa*; ***C.A.***: *Candida albicans*; ***A.C.***: *Aspergillus clavatus*; **A**: Ampicillin; **B**: Chloramphenicol; **C**: Ciprofloxacin; **D**: Norfloxacin; **E**: Nystatin; **F**: Greseofulvin; NA: Not active; NT: Not tested

3.4 Cytotoxicity study

The cytotoxicity of compounds (**S 1-SS 10**) was tested using bioassay on *S. pombe* cells at the cellular level. The results obtained are shown in **Table 6**. From the result, it was easily monitored that cell death caused by toxic nature of the compounds (**S 1-SS 10**) by vital staining. The toxicity was found to vary with the type of substituent present and different concentrations of compounds (**S 1-SS 10**). It has been observed that cytotoxicity increases with increasing the concentration of compounds (**S 1-SS 10**). The incorporation of **S 1 /S 2** in

the Schiff bases (**SS 1-SS 10**) increased the cytotoxicity appreciably, except for SS **2** having 1, 2, 4-triazole (**S 1**) moiety. The compound **SS 5** containing 1, 2, 4-triazole (**S 1**) moiety and -OCH_3 group on *p*-position in benzene ring has maximum cytotoxicity against *S. pombe* cells. Most of the compounds can cause about 50% cell death.

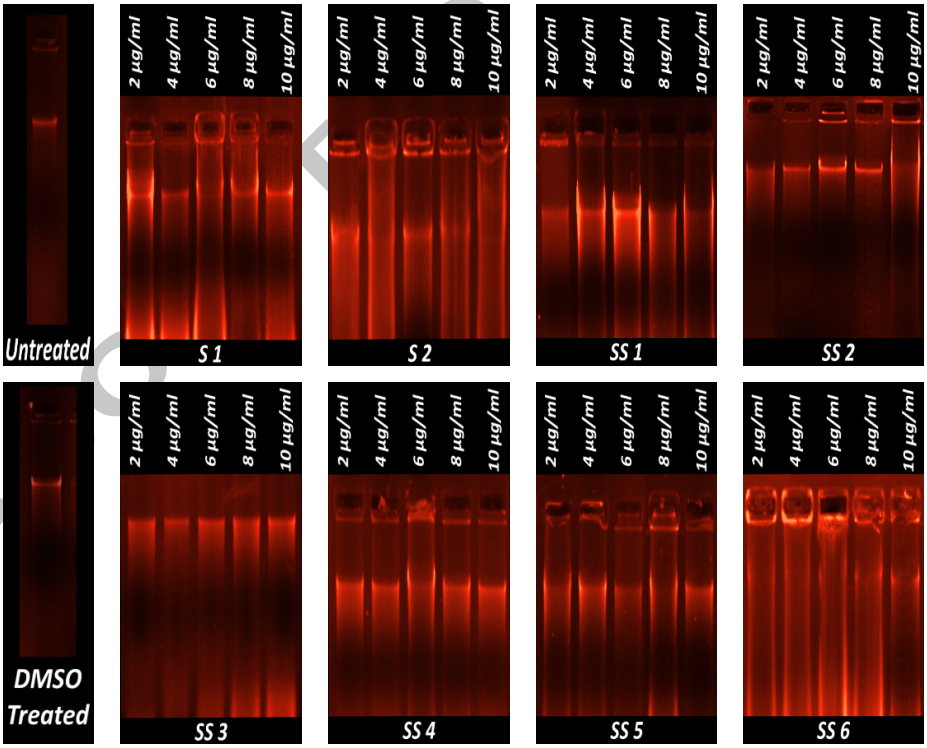
Table 6. Effect of compounds on percentage cell viability of *S. pombe* at different concentrations with standard deviation for three independent experiments

Compound ID (s)	Percentage cell viability at different concentrations					
		2 µg/ml	**4 µg/ml**	**6 µg/ml**	**8 µg/ml**	**10 µg/ml**
S 1	-	91.33 ± 0.58	85.67 ± 0.58	82.00 ± 1.00	75.67 ± 1.53	67.00 ± 1.00
S 2	-	87.33 ± 1.15	83.00 ± 1.00	76.00 ± 1.00	71.00 ± 1.00	68.33 ± 0.58
SS 1	-	87.67 ± 0.58	82.00 ± 2.00	75.33 ± 0.58	70.67 ± 1.15	63.33 ± 1.15
SS 2	-	84.67 ± 1.15	79.67 ± 0.58	78.67 ± 0.58	77.00 ± 1.00	72.33 ± 0.58
SS 3	-	87.00 ± 1.00	80.00 ± 1.00	73.67 ± 0.58	65.33 ± 1.53	59.67 ± 0.58
SS 4	-	83.33 ± 1.53	72.33 ± 0.58	62.33 ± 1.53	55.67 ± 1.53	47.00 ± 1.00
SS 5	-	79.00 ± 1.00	64.33 ± 0.58	59.00 ± 1.00	52.67 ± 1.53	38.67 ± 1.53
SS 6	-	74.33 ± 0.58	70.67 ± 0.58	66.33 ± 0.58	61.00 ± 1.00	52.67 ± 2.08
SS 7	-	82.33 ± 0.58	78.33 ± 0.58	72.00 ± 1.00	67.00 ± 1.00	45.33 ± 3.06
SS 8	-	78.33 ± 1.15	74.33 ± 0.58	65.67 ± 0.58	56.67 ± 1.15	46.33 ± 1.53
SS 9		81.33 ± 1.15	73.33 ± 2.52	64.67 ± 1.15	54.67 ± 0.58	47.00 ± 2.00
SS 10		80.33 ± 1.15	72.33 ± 0.58	68.00 ± 1.00	63.67 ± 1.53	49.00 ± 1.00

Untreated	94.67 ± 1.53	-	-	-	-	-
DMSO	93.67 ± 1.53	-	-	-	-	-

3.5 Genotoxicity study

Results of cytotoxicity encouraged us to find out whether compounds (**S 1-SS 10**) have any effect on the integrity of DNA or not. The effect of compounds (**S 1-SS 10**) on the DNA integrity of *S. pombe* cells was carried out by the isolation of DNA from treated and untreated *S. pombe* cells and isolated DNA was electrophoresed on 1% agarose gel followed by visualization. DNA damage was found as smeared in triazole and oxadiazole analogues (**S 1-SS 10**) treated *S. pombe* cells while in untreated *S. pombe* cells it was observed as an intact band without any smear (**Fig. 1**). The results showed that compounds (**S 1-SS 10**) affected the integrity of DNA of *S. pombe* cells. Smearing of DNA suggested that the damage has occurred due to the toxic nature of the compounds (**S 1-SS 10**). This result is in agreement with the results of cytotoxicity that triazole and oxadiazole analogues (**S 1-SS 10**) have entered into the cell and affected the integrity of DNA.



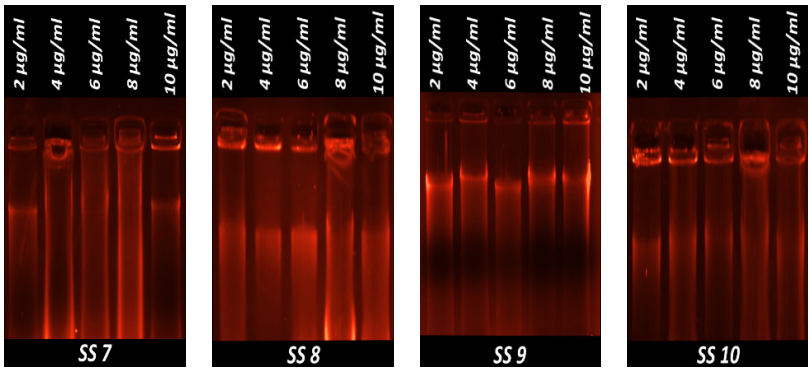


Fig. 1 Effect of the chemically synthesized compounds (**S 1-2** and **SS 1-10**) on the integrity of DNA isolated from *S. pombe* cells.

3.6 *In vitro* antimalarial activity

The compounds **S 1-SS 10**, **Chloroquine** and **Pyrimethamine** were evaluated for their antimalarial screening against *P. falciparum* strain. All the experiments were performed in duplicate and a mean value of IC_{50} is reported in **Table 7**.

Table 7. *In vitro* antimalarial activity of triazole and oxadiazole analogues (**S 1-SS 10**) (IC_{50}, µM)

Compound ID(s)	IC_{50} (µM)
S 1	0.960 ± 0.031
S 2	0.810 ± 0.043
SS 1	0.815 ± 0.026
SS 2	0.282 ± 0.020
SS 3	0.245 ± 0.030
SS 4	0.230 ± 0.029
SS 5	0.835 ± 0.055
SS 6	0.802 ± 0.041
SS 7	0.822 ± 0.035
SS 8	0.806 ± 0.039
SS 9	0.301 ± 0.021
SS 10	0.790 ± 0.030
Chloroquine	0.063 ± 0.009
Pyrimethamine	1.005 ± 0.053

The IC_{50} values of all compounds (**S 1**-**SS 10**) showed improved potency, even better than reference compound **Pyrimethamine**. The result of *in vitro* assay suggested that the presence of 4-OH (**SS 2**), 3-NO_2 (**SS 3**) and 4-Cl (**SS 4** and **SS 9**) in phenyl ring exhibited best antimalarial activity (**Table 7**). The compounds containing thiophene ring (**SS 1** and **SS 6**), *p*-hydroxy benzene (**SS 7**), *m*-nitrobenzene (**SS 8**) and *p*-methoxybenzene (**SS 10**) were found to have enhanced potency, while **S 1** (1, 2, 4-triazole; IC_{50} = 0.960 ± 0.031 µM) and **SS 5** (*p*-methoxybenzene; IC_{50} = 0.835 ± 0.055 µM) showed poor activity against *P. falciparum*.

3.7 *In silico* docking study

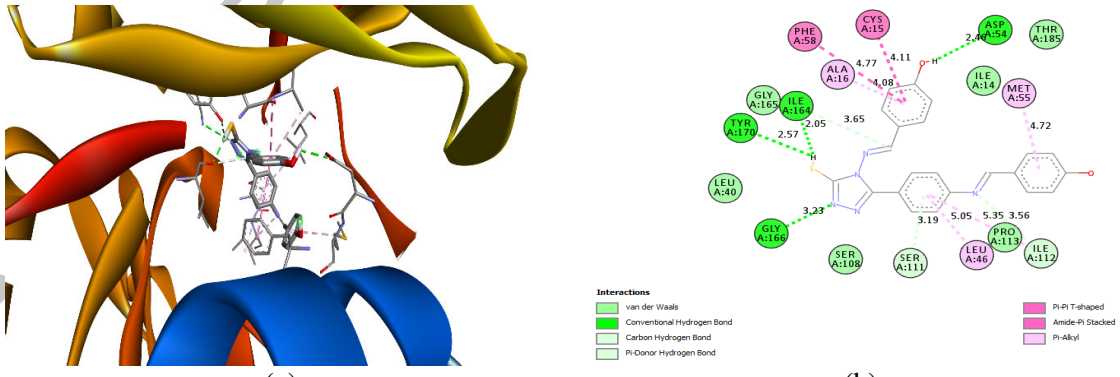
To rationalize the potency of the active compounds (**SS 2**, **SS 3**, **SS 4**, **SS 9**) as antimalarial agents, these compounds were docked against *P. falciparum* dihydrofolate reductase (PDB ID: **4DPD**) obtained from protein data bank (RCSB). The binding energies of drug-receptor complex were obtained for ligands (**SS 2**, **SS 3**, **SS 4**, **SS 9**) and standard drugs **Chloroquine** & **Pyrimethamine** are presented in **Table 8**.

Table 8. Docking results of triazole and oxadiazole analogues (**S 1-SS 10**) against *P. falciparum* (PDB ID: **4DPD**)

Compound ID(s)	Binding Energy (kcal/mol)	Intermol Energy (kcal/mol)	Internal Energy (kcal/mol)	Torsional Energy (kcal/mol)	Unbound Energy (kcal/mol)
SS 2	-7.70	-10.09	-0.93	2.39	-0.93
SS 3	-9.06	-11.45	-1.02	2.39	-1.02
SS 4	-8.78	-10.57	-0.95	1.79	-0.95
SS 9	-7.20	-8.39	-0.64	1.19	-0.64
Chloroquine	-4.24	-6.63	-1.06	2.39	-1.06
Pyrimethamine	-6.10	-7.00	-0.26	0.89	-0.26

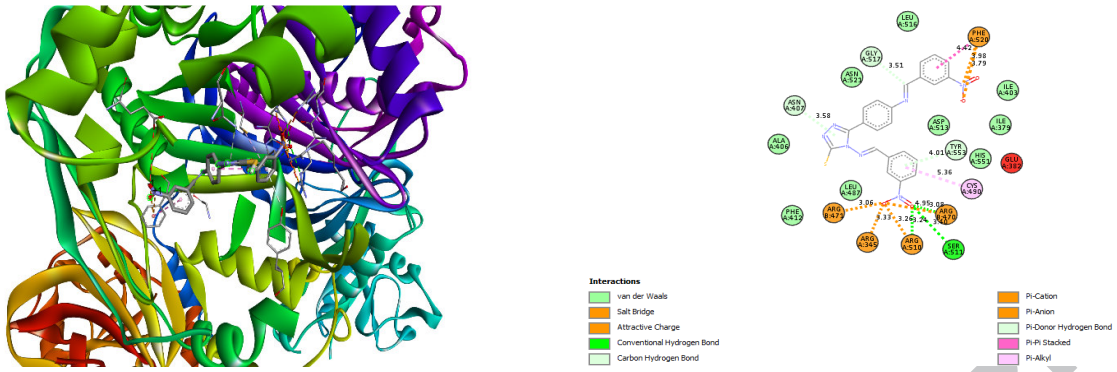
A potential interaction was observed between the active molecules and the Pf-DHFR enzyme. The 3D diagrams (**Fig. 2a-7a**) showed the binding sites of all the ligands within the receptor **4DPD**. The molecules interacted with the active sites (amino acids) of receptor **4DPD** through conventional hydrogen bonds, van der Waals, π-cation, π-anion, π-alkyl, π-donor hydrogen bond, π-sulphur, carbon-hydrogen bond, π-sigma and much more (**Fig. 2b-7b**). As shown in **Table 8**, binding energies of the molecules against the active sites indicated higher affinity of the molecules to bind to the protein leading to its inhibition. The binding energies

of the molecules (**SS 2**, **SS 3**, **SS 4**, **SS 9**) were found to be ranging from -7.20 to -9.06 kcal/mol and were better compared to standard drugs **Chloroquine** and **Pyrimethamine** (**Table 8**). The H-atoms of -OH and -SH in **SS 2** interacted with active sites of enzyme by forming H-bond with ASP A:54, ILE A:164 and TYR A:70 at distances of 2.46 °A, 2.05 °A and 2.57 °A respectively (**Fig. 2b**). Also, π-alkyl type of interaction observed between aromatic rings of **SS 2** and ALA A:16, LEU A:46, PRO A:113 and MET A:55 with distances 4.08 °A, 5.05 °A, 5.35 °A & 4.72 °A respectively. Hydrogen bonds were observed between the O-atom of -NO_2 group of **SS 3** and ARG A:345, SER A:511 & ARG B:470 with bond lengths of 3.14 °A, 3.20 °A & 3.08 °A respectively (**Fig. 3b**). In ligand **SS 4**, aromatic rings interacted with receptor by forming π-alkyl and alkyl bonds with LEU B:40 (4.84 °A), MET B:55 (5.31 °A), LEU B:46 (4.88 °A), ILE B:112 (4.64 °A) & PRO B:113 (5.27 °A) (**Fig. 4b**). Also, van der Waals, H-bond & π-suplhur type of interactions were indicated with different amino acids of chain A & B with **SS 4** (**Fig. 4b**). The oxadiazole ring in **SS 9** formed π-sigma & π-alkyl bonds with ILE B:567 (3.93 °A) & ILE B:314 (4.61 °A) respectively (**Fig. 5b**). The Cl-atom in **Chloroquine** (**Fig. 6b**) interacted through conventional H-bond with THR A:391 at distance 2.90 °A. Furthermore this Cl-atom formed π-alkyl bond with PHE A:42 (4.95 °A), TYR A:441 (5.49 °A) & TYR A:448 (4.59 °A). In the case of **Pyrimethamine,** van der Waals, carbon-hydrogen bond, π-alkyl, alkyl etc. type of interactions were observed between different amino acids present in chain A of **4DPD** receptor and ligand as shown in **Fig. 7b**. The results of docking study indicated the selectivity of the potent ligand molecules as DHFR inhibitors.



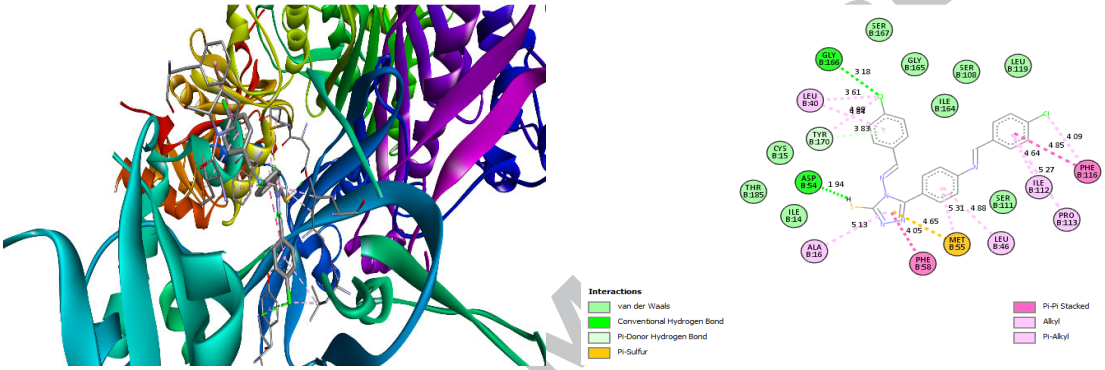
(a) (b)

Fig. 2 (a) 3D model of ligand **SS 2** bonded with active site of **4DPD** receptor. (b) 2D model of interaction between amino acid residues and **SS 2** with types of bond and bond lengths.



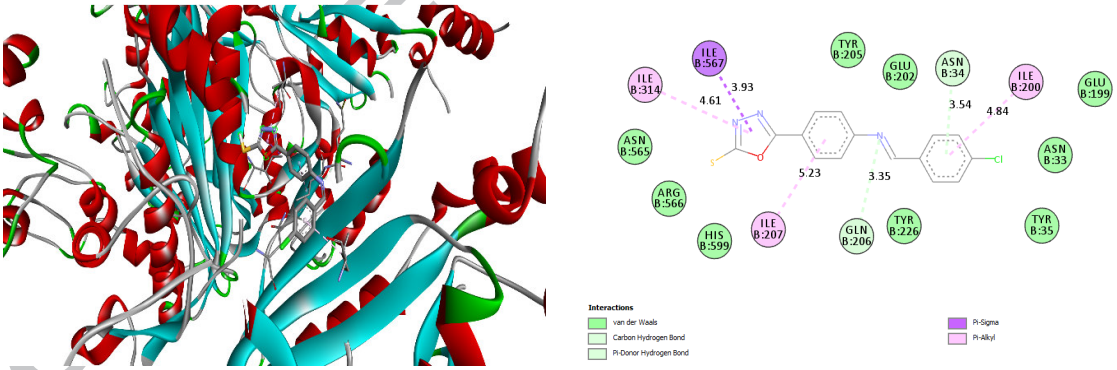
(a) (b)

Fig. 3 (a) 3D model of ligand **SS 3** bonded with active site of **4DPD** receptor. (b) 2D model of interaction between amino acid residues and **SS 3** with types of bond and bond lengths.



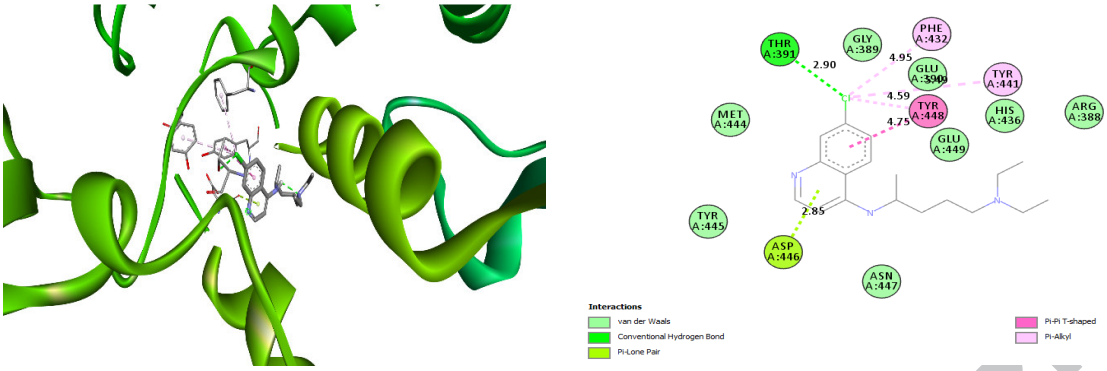
(a) (b)

Fig. 4 (a) 3D model of ligand **SS 4** bonded with active site of **4DPD** receptor. (b) 2D model of interaction between amino acid residues and **SS 4** with types of bond and bond lengths.



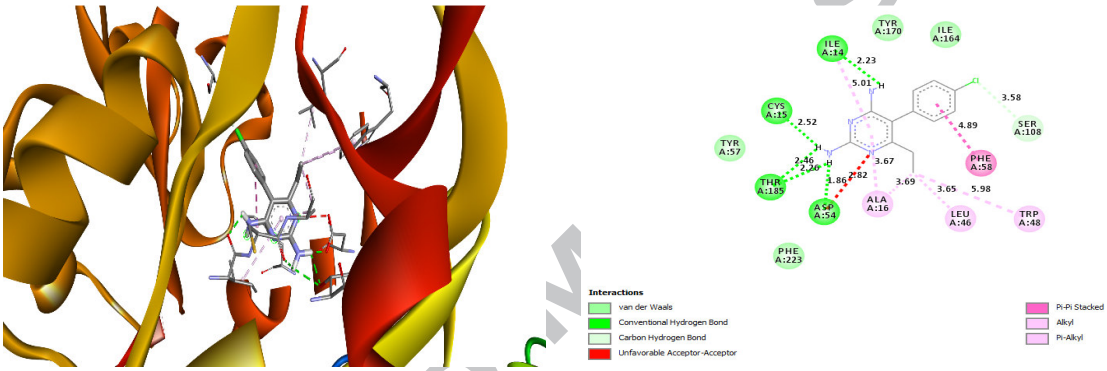
(a) (b)

Fig. 5 (a) 3D model of ligand **SS 9** bonded with active site of **4DPD** receptor. (b) 2D model of interaction between amino acid residues and **SS 9** with types of bond and bond lengths.



(a) (b)

Fig. 6 (a) 3D model of ligand **Chloroquine** bonded with active site of **4DPD** receptor. (b) 2D model of interaction between amino acid residues and **Chloroquine** with types of bond and bond lengths.



(a) (b)

Fig. 7 (a) 3D model of ligand **Pyrimethamine** bonded with active site of **4DPD** receptor. (b) 2D model of interaction between amino acid residues and **Pyrimethamine** with types of bond and bond lengths.

3.8 DHFR Inhibition assay:

The potent entities (**SS 2**, **SS 3**, **SS 4**, **SS 9**) found to be active against *P. falciparum* strain and standard drugs (**Chloroquine** & **Pyrimethamine**) were further evaluated for inhibitory efficacy against bovine liver DHFR enzyme. The average IC_{50} (µM) values are presented with standard deviation for three independent experiments in **Table 9**.

Table 9. DHFR enzyme inhibition assay

Compound ID(s)	IC_{50} (µM)
SS 2	0.0495 ± 0.009
SS 3	0.0259 ± 0.006
SS 4	0.0349 ± 0.004

SS 9	0.0450 ± 0.005
Chloroquine	0.0301 ± 0.005
Pyrimethamine	0.1007 ± 0.012

The IC_{50} values of **SS 2**, **SS 3**, **SS 4** & **SS 9** indicated that all compounds were potent DHFR inhibitors when compared to standard drugs (**Table 9**). The triazole motif attached with two *m*-nitrobenzene groups (**SS 3**; IC_{50} **=** 0.0259 ± 0.006 µM) was found to be most potent inhibitor, while the compound **SS 4** (IC_{50} **=** 0.0349 ± 0.004 µM) having two *p*-chlorobenzene ring attached to triazole motif had more or less inhibitory property similar to **Chloroquine** (IC_{50} = 0.0301 ± 0.005 µM). The presence of 4-OH (**SS 2**) and 4-Cl (**SS 9**) in phenyl ring were found to be poor inhibitors of enzyme compared to **Chloroquine** (**Table 9**). The results suggested that the analogues were active anti-malarial compounds inhibiting dihydrofolate reductase enzyme.

4. Conclusion

Analogues of 1, 2, 4-triazole and 1, 3, 4-oxadiazole have been designed, synthesized and characterized. To avoid late stage failure, it is important to study the preliminary pharmacokinetic parameters. The results of pharmacokinetic data suggested that, all molecules have tendency to be considered as drugs. So *in vitro* antimicrobial, cytotoxicity and genotoxicity on *S. pombe* cells and antimalarial on *P. falciparum* of these analogues have been investigated. The molecules were subjected to DFT and PM6 calculations to explain the anti-bacterial activity. It was found that the antibacterial activity could be correlated to energies of the LUMO and maximum LUMO electron density. All the compounds possessed moderate to good inhibitory potency against bacterial strains and did not show any anti-fungal activities. The compounds **SS 4**, **SS 5** and **SS 7**-**SS 10** were found to possess maximum toxicity against *S. pombe* cells at cellular level. All the compounds **S 1**-**SS 10** showed smearing type pattern on agarose gel due to toxic nature of these compounds. The anti-malarial activity of these compounds were studied. The compounds **SS 2** (two *p*-hydroxy benzene groups), **SS 3** (two *m*-nitrobenzene groups) and **SS 4** (two *p*-chlorobenzene groups) attached to 1, 2, 4-triazole ring and **SS 9** (*p*-chlorobenzene) attached to 1, 3, 4-oxadiazole ring were found to be potent against *P. falciparum* strain. The *in silico* docking showed that the potent molecules synthesized and the standard drugs inhibit the enzyme *P. falciparum* dihydrofolate reductase occupying the active binding pocket with great ease. The antimalarial efficacy was further proved by *in vitro* DHFR enzyme inhibition study. Hence, this study

identified new structural type antibacterial and antimalarial agents which could be used as lead molecules for further research and development of antibacterial and antimalarial agents.

Conflict of interest We wish to confirm that there are no known conflicts of interest associated with this publication.

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