New 3,5-disubstituted-4,5-dihydroisoxazole derivatives: Synthesis, antimicrobial, antioxidant and docking study against glucosamine-6-phosphate synthase

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Abstract. A novel derivative of 3,5-disubstituted-2-isoxazolines (3-11) have been synthesized and characterized by spectral analysis. All the synthesized derivatives were screened qualitatively for their antioxidant property using TLC technique and the percent DPPH radical scavenging activity of the potent derivatives (5, 11) were evaluated. The 2-pyrazolines (3-11) were in vitro screened against different bacterial strains as well as candida albicans and found exhibiting moderate to potent activity. Docking study of 2-isoxazoline derivative 11 against glucosamine-6-phosphate synthase, the target enzyme for the antimicrobial agents, was explored to study the interactions of the discovered hit (11) within the binding pocket residues of the enzyme.

Keywords: 2-isoxazoline, antioxidant, antibacterial, antifungal, docking

1. Introduction

Antioxidants are molecules with unpaired of electron which have the ability to scavenge free radical through donating or accepting electrons. The antioxidant reacts with the free radical directly or indirectly to protect the human body form oxidative damage. Versatile natural antioxidants are commonly existing in vegetables, fruits, grains, spices and herbs [1]. Moreover, the synthesis of new antioxidant molecules have been widely considered by research group during the last decade [2]. On the other hand, the World Health Organization report (WHO) in 2012, indicated the intensive interest in discovering of novel antimicrobial agents due to limited number of efficacious drugs for treatment of infectious diseases worldwide [3]. Recently, considerable attention has been attracted to 3,5-disubstituted-2-isoxazolines due to their versatile
pharmaceutical activities like antioxidant, antimicrobial [4], antitumor [5], antidepressant [6], anti-inflammatory [7]. Therefore, in our study and based on the potent antimicrobial activity of fused heterocyclic compounds, we report an efficient approach of novel 3,5-disubstituted-2-pyrazolines. The obtained derivatives were evaluated for their scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical qualitatively using TLC autographic assay. The percent inhibition of the potent hits (5, 11) were determined quantitatively by spectrophotometric method. The synthesized derivatives (3-11) were in vitro screened against various bacterial species including gram +ve and gram –ve strains as well as Candida albicans. Docking study of the potent antimicrobial agent (11) against L-Glutamine: D-fructose-6-phosphate amidotransferase, (GlcN-6-P) have been investigated [8], the target enzyme for the antimicrobial agents, was achieved to study the interactions of the discovered hit within the amino acid residues of enzymatic binding site using Autodock 4.2 package.

2. Experimental part

2.1 Synthesis

All starting materials and solvents were purchased from Sima-Aldrich and Fluka and used without further purification. Melting points were determined on an electro-thermal capillary apparatus and are uncorrected; FT-IR measurements were recorded on a Shimadzu model FTIR-8400S. Mass spectra were recorded on a Shimadzu GCMS-QP2010 Ultra apparatus. 1H NMR spectra were obtained with a Bruker spectrophotometer model ultra-shield at 300 MHz in DMSO-d6 and CDCl3 solution with the TMS as internal standard.

2.1.1 Synthesis of (E)-1-(4-aminophenyl)-3-(thiophen-2-yl) prop-2-en-1-one) (1). This compound was prepared according to the procedure described in reference [9]. To a solution of 4-aminoacetophenone (1 mmol) in ethanol (10 mL), sodium hydroxide (40%, 1ml) was added and the mixture was stirred for 30 minutes. After that 2-thiophenecarboxaldehyde (1 mmol) added and the reaction mixture was stirred overnight. The reaction mixture was allowed to stand at room temperature. The precipitated solid was dried and recrystallized from ethanol. Yellow powder, yield 96%, m.p 110-113°C; IR (υ cm⁻¹): 3427, 3317 (NH2), 3050 (aromatic C-H), 1631(C=O), 1597 (CH=CH), 1548 (aromatic C=C). 1H-NMR (300MHz, DMSO-d6) δ (ppm): 6.12 (s, 2H, NH2), 6.61 (d, 2H, Ar-H, j=8.56 Hz), 7.15-7.7 (m, 5H, Ar-H thiophen, -CH=CH) 7.8 (d, 2H, Ar-H). GCMS (NCI) m/z: 229 M+ for C13H11NOS.

2.1.2 Synthesis of 4-(5-(thiophen-2-yl)-4,5-dihydroisoxazol-3-yl)aniline (2). This compound was prepared according to the procedure described in reference [10]. To the chalcone derivative 1 (1mmol) dissolved in ethanol (10mL), a mixture of hydroxyl amine hydrochloride (1.5 mmol) and NaOH (40%, ml) was added and the crude reaction was refluxed for 15 h. The reaction was monitored by TLC using ethyl actate: hexane system (5:5). The product was precipitated by added crush ice then filtered and washed with water, dried and recrystallized from ethanol. Orange powder, yield 90%, m.p 100-103 °C; IR (υ cm⁻¹): 3416, 3229 (NH2), 3043 (aromatic C-H), 1631(C=O), 1597 (CH=CH), 1548 (aromatic C=C). 1H-NMR (300MHz, CDCl3) δ (ppm): 3.45-3.54 (m, 1H, CH- isoxazoline), 3.69-3.78 (m, 1H, CH- isoxazoline), 3.96 (s, 2H, NH2), 5.95 (m, 1H, CH- isoxazoline), 6.55-7.63 (m, 7H, Ar-H). GCMS (NCI) m/z: 244 M+ For C13H12N2SO, R.f = 0.65 (1:1, Hexane: Ethyl acetate).

2.1.3 Synthesis of Schiff bases (3-5). These compounds were prepared according to the procedure described in published reference [11]. To a solution of substituted benzaaldehyde (1mmol) in methanol (10mL) with few drops of glacial acetic acid, isoxazoline compound 2 (1mmol) was added. The mixture was refluxed for
10-12 h and the reaction was monitored by TLC using ethyl acetate:hexane system (5:5 and 3:7). The precipitate was filtered and washed with methanol, dried and recrystallized from ethanol.

**1-(4-methoxyphenyl)-N-(4-(5-(thiophen-2-yl)-4,5-dihydroisoxazol-3-yl)phenyl)methanimine (3).**

White powder, yield 65%, m.p 152-154 °C; IR (ῡ cm⁻¹): 3003 (aromatic C-H), 2964, 2835 (aliphatic C-H), 1626 (CH=N), 1599 (C=N), 1573 (aromatic C=C). ^1H-NMR (300MHz, CDCl₃) δ (ppm): 3.40-3.48 (dd, j= 8.1, 16.69 Hz, 1H, CH-isoxazoline), 3.68-3.77 (dd, j=10.64, 16.66 Hz, 1H, CH-isoxazoline), 3.83(s, 3H, OCH₃), 5.91 (m, 1H, CH-isoxazoline), 6.92-7.2 (m, 11H, Ar-H), 8.34 (s, 1H, CH=N). GCMS (NCI) m/z: 362 M⁺ for C₂₁H₁₈N₂O₂S, R.f=0.76 (1:1, Hexane: Ethyl acetate).

**N,N-dimethyl-4-(((4-(5-(thiophen-2-yl)-4,5-dihydroisoxazol-3-yl)phenyl)imino)methyl)aniline (4).**

Yellow powder, yield 86%, m.p 188-190 °C; IR (ῡ cm⁻¹): 3049 (aromatic C-H), 2935, 2848 (aliphatic C-H), 1624 (CH=N), 1599 (C=N), 1550 (aromatic C=C). ^1H-NMR (300MHz, CDCl₃) δ (ppm): 3.09 (s, 6H, 2CH₃), 3.47-3.55 (dd, j= 8.1, 16.5 Hz, 1H, CH-isoxazoline), 3.75-3.84 (dd, j=10.60, 16.56 Hz, 1H, CH-isoxazoline), 6.00 (m, 1H, CH-isoxazoline), 6.74-7.8 (m, 11H, Ar-H), 8.36 (s, 1H, CH=N). GCMS (NCI) m/z: 375 M⁺ for C₂₂H₂₁N₃OS, R.f=0.75 (1:1, Hexane: Ethyl acetate).

**1-(4-nitrophenyl)-N-(4-(5-(thiophen-2-yl)-4,5-dihydroisoxazole-3-yl)phenyl)methanimine (5).**

Yellow powder, yield 40%, m.p 180-182 °C; IR (ῡ cm⁻¹): 3032 (aromatic CH), 2995, 2883 (aliphatic CH), 1627 (CH=N), 1599 (C=N), 1516, 1350 (NO₂). ^1H-NMR (300MHz, CDCl₃) δ (ppm): 3.48-3.57 (dd, j=8.02, 16.49 Hz, 1H, CH-isoxazoline), 3.77-3.86 (dd, j=10.64, 16.78 Hz, 1H, CH-isoxazoline), 6.01 (m, 1H, CH-isoxazoline), 7.02-8.37 (m, 11H, Ar-H), 8.61 (s, 1H, CH=N). GCMS (NCI) m/z: 377 M⁺ for C₂₀H₁₅N₃O₃S, R.f=0.89 (1:1, Hexane: Ethyl acetate).

2.1.4 Synthesis of 2-azetidinone derivatives (6-8). These compounds were obtained according to a modified procedure in published work [12]. A mixture of Schiff bases (1mmol) and triethyl amine (2mmol) were dissolved in dry dioxane (25 mL) and stirred. To this well stirred solution chloroacetyl chloride (4 mmol) was added drop by drop for a period of 30 min at low temperature. The reaction was further stirred for 6-12 h (as monitored from TLC). The reaction mixture is poured into crushed ice and the resultant product was filtered and washed with water, dried and recrystallized from dioxane.

**3-chloro-4-(4-methoxyphenyl)-1-(4-(5-(thiophen-2-yl)-4,5-dihydro isoxazol-3-yl)phenyl)azetidin-2-one (6).**

White powder, yield 75%, m.p 148-150 °C; IR (ῡ cm⁻¹): 3012 (aromatic CH), 2918, 2877 (aliphatic CH), 1670 (C=O), 1599 (C=N), 1519 (aromatic C=C). ^1H-NMR (300MHZ, CDCl₃) δ (ppm): 3.37-3.45 (dd, j= 8.13, 16.46 Hz), 3.64-3.69 (dd, j=16.46 Hz, 1H, CH-isoxazoline), 3.74-3.86 (dd, j=10.64, 16.78 Hz, 1H, CH-isoxazoline), 4.15 – 4.17(m, 4H, CH-Cl, OCH₃), 5.90 (m, 1H, CH-isoxazoline), 6.63-8.26 (m, 11H, Ar-H). GCMS (NCI) m/z: 318, 210, 196, 111 M⁺, R.f=0.51 (1:1, Hexane: Ethyl acetate).

**3-chloro-4-(4-(dimethylamino)phenyl)-1-(4-(5-(thiophen-2-yl)-4,5-dihydroisoxazol-3-yl)phenyl)azetidin-2-one (7).**

Brown powder, yield 50%, m.p 143-145 °C; IR (ῡ cm⁻¹): 3099 (aromatic CH), 2926, 2856 (aliphatic CH), 1693 (C=O), 1604 (C=N). ^1H-NMR (300MHZ, CDCl₃) δ (ppm): 2.99-3.01 (m, 6H, N(CH₃)), 3.37-3.45 (dd, j= 7.76, 16.44 Hz), 3.69-3.64 (dd, 1H, CH-isoxazoline), 3.75-3.78 (d, 1H, CH-N, j=11.76 Hz), 4.16 (d, 1H, CH-Cl), 5.90 (m, 1H, CH-isoxazoline), 6.63-8.27 (m, 11H, Ar-H). GCMS (NCI) m/z: 318, 257, 208,196, 145, 110 M⁺, R.f= 0.70 (1:3, Hexane: Ethyl acetate).

**3-chloro-4-(4-nitrophenyl)-1-(4-(5-(thiophen-2-yl)-4,5-dihydroisoxazol-3-yl)phenyl)azetidin-2-one (8).**
Orange powder, yield 73%, m.p 150-152 °C; IR (ῡ cm⁻¹): 3034 (aromatic C-H), 2922, 2881 (aliphatic C-H), 1670 (C=O), 1599 (C=N), 1535 (NO₂). ¹H NMR (300 MHz, CDCl₃) δ (ppm): 3.37-3.45 (dd, 1H, CH-isoxazoline, j= 8.15, 16.6 Hz), 3.65-3.74 (dd, 1H, CH-isoxazoline, j= 10.53, 16.47 Hz), 4.09-4.17 (m, 2H, CH-N, CH-Cl), 5.91 (m, 1H, CH-isoxazoline), 6.63-8.27 (m, 11H, Ar-H). GCMS (NCI) m/z: 320, 305, 210, 196, 134 M⁺, R.f = 0.61 (1:3, Hexane: Ethyl acetate).

2.1.5 Synthesis of 2-(4-nitrophenyl)-3-(4-(5-(thiophen-2-yl)-4, 5-dihydroisoxazol-3-yl)phenyl)thiazolidin-4-one (9). These compounds were synthesized according to modified procedure in reported reference [13]. To a solution of Schiff base (5) (1 mmol) in benzene (25 mL) mercapto acetic acid was added (1.5mmol) with stirring and a few amount of anhydrous ZnCl₂ was added. The mixture was refluxed for 12h, after the completion of reaction, it was cooled and the solvent evaporated and poured into sodium bicarbonate (5%) solution to neutralize it. The solid product was filtered and washed with cold water.

Light yellow powder, yield 20%, m.p 123-125°C; IR (ῡ cm⁻¹): 3080 (aromatic C-H), 2964, 2854 (aliphatic C-H), 1687 (C=O), 1602 (C=N), 1519, 1346 (NO₂). ¹H NMR (300MHz, CDCl₃) δ (ppm): 3.34-3.42 (dd, j=7.7, 16.03 Hz, 1H, CH-isoxazoline), 3.63-3.72 (dd, j= 11.08, 16.11 Hz, 1H, CH-isoxazoline), 3.95 (m, 1H, CH-thiazolidine), 3.98 (m, 1H, CH-thiazolidine), 5.94 (m, 1H, CH-isoxazoline), 6.27 (s, 1H, CH-thiazolidine), 6.97-8.19 (m, 11H, Ar-H). GCMS (NCI) m/z: 451 M⁺ for C₂₂H₁₇N₃O₄S₂, R.f = 0.58 (1:1, Hexane: Ethyl acetate).

2.1.6 Synthesis 3-(N-substituted-4-aminophenyl)-5-substitutedaryl-isoxazoline derivatives (10, 11). These compounds were synthesized according to modified procedure in reported reference [14]. To a solution of isoxazoline derivative (2) (1mmol) and corresponding anhydrides (1mmol) (maleic or phthalic anhydride) in glacial acetic acid (3mL), anhydrous sodium acetate (1.2 mmol) was added and the mixture was allowed to reflux for 1 h. After completion of the reaction (as indicated by TLC) the mixture was added to a crushed ice and stirred. The solid separated was filtered and washed with water, dried and recrystallized from ethanol.

2-(4-(5-(thiophen-2-yl)-4, 5-dihydroisoxazol-3-yl) phenyl)isoindoline-1, 3-dione (10)

Gray powder, yield 73 %, m.p 254-256 °C; IR (ῡ cm⁻¹): 3088 (aromatic C-H), 2937, 2893 (aliphatic C-H), 1701, 1680 (C=O), 1604 (C=N), 1516 (aromatic C=C). GCMS (NCI) m/z: 374, 264, 110 M⁺ for C₂₁H₁₄N₂O₃S, R.f =0.84 (1:2.5, Hexane: Ethyl acetate).

1-(4-(5-(thiophen-2-yl)-4, 5-dihydroisoxazol-3-yl) phenyl)-1H-pyrrole-2, 5-dione (11)

Brown powder, 76%, m.p 135-137 °C; IR (ῡ cm⁻¹): 3020 (aromatic C-H), 2928, 2856 (aliphatic C-H), 1714, 1670 (C=O), 1595 (C=N), 1518 (aromatic C=C). GCMS (NCI) m/z: 423, 286, 134, 110 M⁺ for C₁₇H₁₂N₃O₃S, R.f = 0.24 (1:2.5, Hexane: Ethyl acetate).

2.2 Antimicrobial studies

The synthesized isoxazoline derivatives (3-11) were tested for their antimicrobial activity against Escherichia coli, Pseudomonas aeruginosa (gram -ve), Staphylococcus aureus, Streptococcus SPP (gram +ve) as well as C. albicans using the well diffusion method (Table 1) [15]. DMSO was run as a control and the test was performed at 2 mg/mL concentration using DMSO solvent. Amoxicillin were used as standard drugs. Each experiment was conducted in triplicate and the average reading was taken.

2.3 Antioxidant study (DPPH. radical scavenging assay)
2.3.1 TLC autographic assay: Few milligrams of isoxazoline derivatives (3-11) dissolved in methanol were added to the TLC plate by extremely small capillary. After drying, TLC plates were sprayed with methanolic solution of 0.2 % DPPH. The plates were examined 30 min after spraying. Active compounds appear as yellow or blue spots against a purple background [16].

2.3.2 Spectrophotometric (DPPH) assay: The scavenging activity to some of the synthesized derivatives compounds were examined in vitro using DPPH radical as described by Shimada et al. [17] with slight modification. One mL of the samples at concentration of 100, 300 and 500 μg/ mL was mixed with 0.5 mL of DPPH solution (1.3 mg DPPH / mL methanol) and the volume was complete to 3 mL with methanol. The reaction mixture was left to stand for 30 min in dark place. The control contained all reagents without the sample while gallic acid used as standard. The DPPH radical scavenging activity was determined by measuring the absorbance at 517 nm against the blank. The capability to scavenge the DPPH radical was calculated using the following equation: DPPH scavenging effect (%) = \( \frac{A_0 - A_1}{A_0} \times 100 \), where \( A_0 \) is the absorbance of the control reaction, and \( A_1 \) is the absorbance in the presence of the samples or standards.

2.4 Docking study
AutoDock 4.2 package software was used to investigate the affinity of the potent isoxazoline derivatives (11) to the binding pocket of GlcN-6-P synthase as described by the reported [18, 19]. The pdb file format of enzyme as receptor was obtained from the RCSB Protein Data Bank (PDB code 1MOQ) and used as a rigid molecule. All the water molecules were eliminated and hydrogens were added to the amino acid residues. The docked compounds were drawn using ChemDraw ultra 7.0 as mol file and the open Babel 2.3.1 software was used to constructing the pdb file. The docking study was achieved using grid dimensions 30.5, 17.5 and -2.2, respectively. Docking algorithm using Lamarckian Genetic was employed with 10 runs, 150 population size, 2,500,000 maximum number of energy evaluations and 27,000 maximum number of generations.

3. Results and discussion

3.1 Synthesis
Chalcone derivative 1 and the isoxazoline compound 2 were prepared and characterized as described by our previous work [20]. Shiff bases (3-5) were synthesized from the reaction of compound 2 with different aromatic aldehydes in acidic methanolic solution (Scheme 1).
Scheme 1: (a) p-methoxybenzaldehyde MeOH (b) 4-N,N-dimethylbenzylaldehyde, EtOH (c) p-nitrobenzaldehyde, EtOH (d) phthalic anhydride or maleic anhydride, glacial acetic acid (f) chloroacetyl chloride, triethyl amine, 1,4-dioxane (g) thioglycolic acid, benzene, anhydrous zinc chloride.

The structures of obtained compounds were confirmed by spectral analysis (see experimental section). The FT-IR spectra of compounds (3-5) showed the absorption bands at 1627-1624 cm\(^{-1}\), 1599 cm\(^{-1}\) regions due to the stretching vibrations of the CH=N and C=N groups, respectively. The disappearance of the NH\(_2\) stretching frequencies a good evidence of prepared target compounds. The mass spectra are consistent with the molecular ion peak values of the prepared compounds. The \(^1\)HNMR spectra of compound 3,4 and 5 showed singlet within the 8.34,8.36, 8.61 ppm regions due to CH=N protons with the absent of the singlet signal at 3.96 related to NH\(_2\) group in compound 2. The 2-azetidinone derivatives 6, 7 and 8 were obtained by the reaction of schiff bases (3-5) with chloroacetyl chloride in triethyl amine. IR spectrum of compounds (6-8) showed characteristic peak at 1693-1670 cm\(^{-1}\) due to C=O group. The \(^1\)HNMR spectra of compound 6, 7 and 8 showed doublet signal at 3.71-3.75, 3.75-3.78 ppm and multiplet at 4.09 ppm regions due to CH-N group and multiplet signal at 4.09-4.17 ppm due to CH-Cl group. The reaction between the isoxazoline derivative 2 with phthalic and maleic anhydride were carried out (Scheme1) and purified by recrystallization from ethanol to yield N-substitutedphthalimide 10 and N-substitutedmaleimide 11 in high yield. The structures of the N-substituteimide derivatives were confirmed using IR spectroscopy. The stretching of two carbonyl groups appeared at 1701, 1680 and 1714, 1670 cm\(^{-1}\) for compound 10 and 11, respectively. Further elucidation of molecular ion was confirmed via Mass spectroscopy.

3.2 Antimicrobial activity

The in vitro assay of the synthesized compounds (3-11) against several microbial species was achieved using 2 mg/mL concentration as illustrated by Table 1. The tested derivatives exhibited promising activity against different species. Compounds 10 and 11 were the potent agents against gram +ve, gram –ve as well as Candida Albicans.
Table 1: *In Vitro* antimicrobial inhibition zone (mm) of the synthesized compounds.

| Isoxazoline derivatives | Gram negative | Gram positive | Fungi |
|-------------------------|----------------|---------------|-------|
|                         | *E.coli* | *P.aeruginosa* | *S.aureus* | *Streptococcus SPP* | *C. albicans* |
| 3                       | 12      | 10            | 10     | 13                  | 14          |
| 4                       | 13      | 9             | 14     | -                   | 14          |
| 5                       | 12      | -             | 15     | 12                  | 14          |
| 6                       | 13      | 10            | -      | 16                  | 15          |
| 7                       | 11      | 9             | -      | 10                  | 16          |
| 8                       | 12      | 11            | -      | 16                  | 16          |
| 9                       | -       | -             | -      | 11                  | -           |
| 10                      | 10      | 10            | 15     | 14                  | 14          |
| 11                      | 13      | 9             | 17     | 13                  | 12          |
| **Amoxicillin**         | 20      | 15            | 33     | 21                  | 28          |

(-) exhibit no activity at specific concentration

3.3 Antioxidant activity
The scavenging properties of all the synthesized derivatives (3-11) were evaluated against DPPH radical using *TLC* autographic assay. The isoxazoline derivatives dissolved in methanol were transferred to the one end of a *TLC* plate using spotting capillary. After drying and spraying the DPPH solution, the active compounds (5, 11) appeared as yellow or blue spots with purple background. The scavenging activity of the lead derivatives (5, 11) was determined using spectroscopic method as described by the indicated [16]. The relationships between the *in vitro* percentage inhibition and the concentration of the potent hits (100, 200 and 300 μg/mL) are summarized in Figure 1.
3.4 Docking study

The docking study of the potent active isoxazoline derivative (11) toward antimicrobial species inside the active site of glucosamine-6-phosphate synthase, the potential target for antibacterial and antifungal agents was explored. As indicated by the X-ray, the binding pocket of the enzyme include the following residues, Cys 300, Gly 301, Thr 302, Ser 303, Ser 347, Gln 348, Ser 349, Thr 352, Val 399, Ser 401, Ala 602 and Lys 603 as shown in Figure 2 [21].
Figure 2. Ligplot of GlcN-6-P showing the binding of glucosamine-6-phosphate in an active site of enzyme.

Autodock 4.2 was used to evaluate the binding energy of active compound inside the known 3D structure of target enzyme. The binding of the best generated conformers for compound 11 inside the binding pocket of target enzyme illustrated in Figure 3.

Figure 3. The docking of the best generated conformers of the potent discovered hits (11) inside the binding pocket of glucoseamine-6-phosphate synthase (GlcN-6-P).

As indicated by molecular docking parameters (Table 1), the high ranking binding energies of the generated conformer were -7.17 kcal mol\(^{-1}\) for compound 11. The docking results of all generated conformers of compounds within the binding pocket are strongly proportional to the antibacterial activities as shown in Table 1. Inhibition constant \(K_i\), intermolecular energy and hydrogen bonds were also determined and depicted in Table 2.
Table 2: docking parameters of isoxazole compounds (11).

| Compound | Bonding Energy (Kcal mol⁻¹) | Inhibition constant (µM) | Intermolecular energy (kcal mol⁻¹) | H-bonds | Bonding |
|----------|----------------------------|--------------------------|-----------------------------------|---------|---------|
| 11       | -7.17                      | 5.57                     | -8.06                             | 2       | LYS603:HZ3: LIG: O ALA602:HN: LIG: O |
| 2        | -7.15                      | 5.74                     | -8.05                             | 2       | LYS603:HZ3: LIG: O ALA602:HN: LIG: O |
| 3        | -7.15                      | 5.74                     | -8.05                             | 2       | LYS603:HZ3: LIG: O ALA602:HN: LIG: O |
| 4        | -7.13                      | 5.98                     | -8.02                             | 1       | LYS603:HZ3: LIG: O ALA602:HN: LIG: O |
| 5        | -7.09                      | 6.33                     | -6.33                             | 2       | LYS603:HZ3: LIG: O ALA602:HN: LIG: O |
| 6        | -7.03                      | 7.04                     | -7.92                             | 2       | LYS603:HZ3: LIG: O ALA602:HN: LIG: O |
| 7        | -6.84                      | 9.07                     | -7.73                             | 2       | GLY301:HN: LIG:O VAL605:HN: LIG:O |
| 8        | -6.82                      | 10.06                    | -7.71                             | 2       | GLY301:HN: LIG:O VAL605:HN: LIG:O SER401:HN: LIG:O |
| 9        | -6.73                      | 11.67                    | -7.62                             | 3       | ALA602: HN: LIG:O LIG:N: GLU488:OE2 SER401:HN: LIG:O |
| 10       | -6.68                      | 12.66                    | -7.58                             | 3       | ALA602: HN: LIG:O LIG:N: GLU488:OE2 |

4. Conclusions

The present work aims to explore of novel isoxazole derivatives as promising antimicrobial and antioxidant agents. Isoxazole were synthesized and used as starting materials to synthesized new Schiff base (3-5), amide (10-11), Azetidinone derivatives (6-8) and thiazolidinone derivative (9) were also synthesized form corresponding Schiff bases. The antimicrobial evolution of all synthesized compounds exhibited moderate to excellent activity against several antimicrobial species. TLC autographic assay was achieved to indicate the scavenging activity of the synthesized compounds toward DPPH radical followed by the spectroscopic method to evaluate the % inhibition for the potent derivatives (5, 11). Docking was studied to explore the binding affinity of the lead derivative (11) inside the binding pocket of glucosamine-6-phosphate synthase, the target enzyme for the antimicrobial agents.

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