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Synthesis, Antimicrobial Activities and Molecular Docking Studies of New *N*-Acylated Derivatives of 5-(2-Phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-amine

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Present study establishes a novel synthetic route of *N*-acetylated derivatives of 5-(2-phenyl-1,8-naphthyridine-3-yl)-1,3,4-oxadiazole-2-amine (**6a-j**), which was achieved in four steps with good yields. 2-Amino nicotinaldehyde and ethyl 3-oxo-3-phenylpropanoate on refluxing with triethylamine in ethanol undergoes Friedlander synthesis to furnish ethyl 2-phenyl-1,8-naphthyridine-3-carboxylate, which further converted into 2-phenyl-1,8-naphthyridine-3-carbohydrazide by reacting with hydrazine hydrate upon reflux, followed by cyclization with cyanogen bromide in the presence of water and 1,4-dioxane with sodium bicarbonate to afford 5-(2-phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-amine (**5**). Compound **5** was acetylated using numerous symmetrical anhydrides to synthesize novel *N*-acetylated derivatives (**6a-j**). The IR, ^{1}H, ^{13}C NMR and mass spectral analysis were used to characterize the structure of synthetic compounds. The synthesized compounds were evaluated for their antimicrobial efficiency against bacteria (*S. aureus* and *E. coli*) using ampicillin as a standard reference and against fungi (*C. albicans*) using fluconazole as a standard reference. Compound **6e** exhibited good antibacterial properties while compounds **6c** and **6e** had shown high antifungal activity, whereas remaining compounds shown moderate to weaker antimicrobial activity. The synthesized derivatives verified their docking strength against Mtb MurB (PDB ID: 5JZX) and showed significant docking activity, however, compound **6f** (-10.98 kcal/mol) and compound **6b** (-10.52 kcal/mol) had a strong binding affinity compared to the other synthesized compounds.

Keywords: 1,8-Naphthyridine, Oxadiazole, Antimicrobial properties, Docking study.

INTRODUCTION

1,8-Naphthyridine scaffold and their derivatives have attracted a lot of attention and have been explored to the discovery of a many proven broad ranges of pharmaceutically bioactive compounds [1-3]. Therefore, new approaches that allows the great research and optimization of new medicinal, pharmaceutical and agrochemical applications of 1,8-naphthyridine skeleton. 1,8-Naphthyridine structural skeleton emphasis on broad range of pharmacological submissions such as antihistaminic [4], anticonvulsant [5], antibacterial [6], antimalarial [7], anti-allergic [8], antidepressant [9], antioxidant [10], gastric anti-secretory [11], antitubercular [12], antimicrobial [13,14], potential diuretic [15], anticancer [16], HIV-1 integrase inhibitors [17], Alzheimer's disease [18], anti-inflammatory [19], telomerase and kinase inhibitors [20]. 1,8-Naphthyridines also found use in photophysical properties and in the study of bioorganic and progressions [21].

The presence of nitrogen heterocyclic compounds, which are found in many natural products like vitamins, hormones and alkaloids, makes them abundant in nature and essential to human life. Derivatives of 1,8-naphthyridine have been extensively employed as bidentate ligands in the complex formation with metal ions [22]. Nonetheless, fluorescent sensors for transition metals based on 1,8-naphthyridine derivatives are not common [23,24]. Due to their high fluorescence quantum yield, moderate fluorescence emission, perfect photostability and potential for structural modifications, 1,8-naphthalimide and its derivatives can be used as fluorophores in fluorescence sensing applications on a large scale [25,26]. 1,8-Naphthyridine motif with phenyl group at 2nd position have been interestingly showed great affinity towards adenosine receptors [27], anti-

bacterial activity [28], anti-tumour [29] activities and other wide range of applications.

In recent past several synthetic methods were employed for synthesis of biological potent derivatives of various oxadiazoles such as 1,2,4-oxadiazole 1,2,5-oxa-diazole and 1,3,4-oxa-diazoles. The structural motifs of 1,3,4-oxadiazole with a 2-amino substituent are useful building blocks for drug design. The biological and pharmacological activities of 2-amino-1,3,4-oxadiazole derivatives are widely distributed such as anticonvulsant sedative-hypnotic [30] antitubercular, antimicrobial [31], anticancer [32], antiepileptic [33] and muscle relaxing [34] properties. For the drug discovery community, developing synthetic techniques to obtain these 2-amino-substituted oxadiazoles is crucial. Numerous studies conducted by many researchers consistently demonstrated that 1,8-naphthyridine displays a high level of selectivity towards a specific biological therapeutic target when they are substituted with specific functional groups at different locations. The substitution of 1,8-naphthyridine at position 5 of 1,3,4-oxaiazole results in a compound with improved potency, decreased drug resistance, reduced toxicity and a wider range of therapeutic applications. Therefore, it is envisaged that the compounds containing phenyl group substituted on 2nd position of 1,8-naphthyridine moiety with 1,3,4-oxadiazole amine group in its molecular frame work to obtain 5-(2-phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-amines and their amide derivatives, which shows ameliorated biological activities.

In present work, our focus is to synthesize 2-phenyl substituted 5-(1,8-naphthyridin-6-yl)-1,3,4-oxadiazol-2-amine and its amide derivatives (**6a-j**) with view to screen them for certain biological activities. The molecular structures were also established by mass, FTIR, ^{1}H & ^{13}C NMR spectral data. Furthermore, the molecular docking studies were also performed.

EXPERIMENTAL

All the laboratory grade, chemicals, reagents and solvents were purchased from the commercial sources. Aluminum plates coated with silica gel were used for thin-layer chromatography (TLC), with UV light being used to visualize the constituent parts. The NMR spectra of ^{1}H and ^{13}C were captured with the help of Bruker AVANCE 300 III using DMSO-d_6 or $CDCl_3$ solvent. An FT-IR spectrometer, a Nicolet 380, was used to evaluate FTIR spectra through ATR trials with frequency range of 4000-500 cm^{-1}. Using Shimadzu LCMS 2010 spectrometer, the mass spectra were scanned. The melting point of the synthesized compounds were measured with the help of Stuart SMP 10 melting point equipment and are uncorrected.

Synthesis of ethyl 2-phenyl-l,8-naphthyridine-3-carboxylate (3) and 2-phenyl-1,8-naphthyridine-3-carboxylic acid hydrazide (4): Ethyl 2-phenyl-l,8-naphthyridine-3-carboxylate was obtained by the reaction of 2-amino nicotinaldehyde (1 mmol) with ethyl benzoyl acetate (1.2 mmol) in ethanol (20 mL) in the presence of piperidine for 6 h in an a round bottomed flask. The obtained product was filtered and recrystallized from ethanol. Ethyl 2-phenyl-1,8-naphthyridine-3-carboxylate (**3**) was then transformed into 2-phenyl-1,8-naphthyridine-3-carboxylic acid hydrazide by using excess of 85% hydrazine hydrate under reflux for 4 h and then recrystallized from ethanol [35].

Ethyl 2-phenyl- l,8-naphthyridine-3-carboxylate (3): Yield: 86%. ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 1.1 (t, 3H, -CH_3), 4.24 (q, 2H, -CH_2-), 7.22 (s, solvent peak), 7.46 (m, 3H, aromatic-H), 7.58 (m, 1H, aromatic-H) 7.7 (m, 1H, aromatic-H), 7.76 (m, 1H, C_6-H aromatic-H), 8.32 (dd, 1H, C_5-H), 8.68 (s, 1H, C_4-H), 9.42 (m, 1H, C_7-H). LC-MS: *m/z* 279.0 (M^++1). Elemental analysis of $C_{17}H_{14}N_2O_2$, calcd. (found) %: C, 73.42 (73.37); H, 5.09 (5.07); N, 10.09 (10.07); O, 11.54 (11.50).

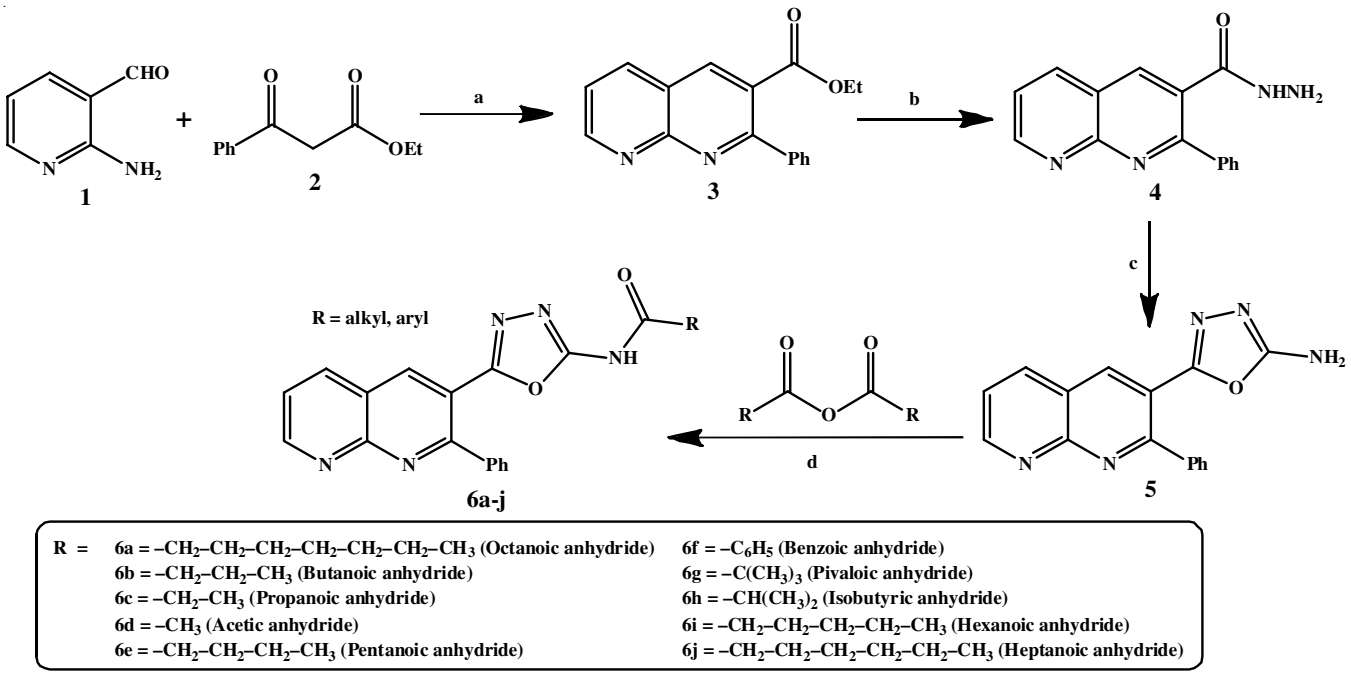
2-Phenyl-1,8-naphthyridine-3-carbohydrazide (4): Yield: 70%. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.24- 4.60 (br, 2H, -NH_2-), 2.5, 3.4 (solvent peak + water peak), 7.5 (m, 3H, aromatic-H), 7.7 (m, 1H, aromatic-H), 7.8 (m, 2H, aromatic-H), 8.52 (s, 1H, aromatic-H), 8.6 (d, 1H, aromatic-H), 9.36 (m, 1H, aromatic-H). 9.8 (br, 1H, -NH-) LC-MS: *m/z* 265.0 (M^++1). Elemental analysis of $C_{15}H_{12}N_4O$, calcd. (found) %: C, 68.24 (68.17); H, 4.62 (4.58); N, 21.23 (21.20); O, 6.08 (6.05).

Synthesis of 5-(2-phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-amine (5): Dioxane (15 mL) and water (10 mL) were refluxed for 18 h with 2-phenyl-1,8-naphthyridine-3-carbohydrazide (**4**) (1 mmol), cyanogen bromide (1.5 mmol) and sodium bicarbonate (1 g). The reaction mass was cooled to room temperature, filtered the solid precipitate and finally washed with of hexane followed by cold water to obtain 5-(2-phenyl-1,8-naphthyridin-3-yl)-2-oxadiazol-1,3,4-amine was obtained by recrystallization from ethanol. Yield 76%; m.p.: 220-221 ºC. IR (KBr, ν_{max}, cm^{-1}): 3282 (NH_2), 1652.82 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 4.5 (s, 2H, NH_2, D_2O exchangeable), 7.5 (m, 3H, aromatic-H), 7.7 (m, 1H, aromatic-H), 7.86 (m, 2H, C_6-H + aromatic-H), 8.6 (m, 1H, C_5-H), 9.18 (s, 1H, C_4-H), 9.8 (s, 1H, C_7-H). ^{13}C NMR (400 MHz, DMSO-d_6) δ: 167.05, 158.74, 154.88, 139.10, 138.02, 137.62, 130.18, 129.16, 128.76, 128.25, 122.90, 120.42. LC-MS: *m/z* 290 (M^{++}1). Elemental analysis of $C_{16}H_{11}N_5O$, calcd. (found) %: C, 66.51 (66.43); H, 3.89 (3.83); N, 24.25 (24.21); O, 5.57 (5.53).

General procedure for the synthesis of *N*-acetyl (5-(2-phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-yl)amine derivatives (6a-j): Symmetric anhydride (5 mmol) was added to a solution of 5-(2-phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-amine (1 mmol) (**5**) in pyridine (40 mL). Constant stirring was performed at 115 ºC for approximately 16 h and then the solid product was separated using ethyl acetate, dried and recrystallized from ethanol (**Scheme-I**).

***N*-(5-(2-Phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-yl)octanamide (6a):** Yield: 65%; m.p.: 210-211 ºC. IR (KBr, ν_{max}, cm^{-1}): 3198 (NH), 1665 (C=O). ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 0.80 (m, 3H, CH_3), 1.3 (m, 10H, -CH_2), 2.20 (t, 2H, -CH_2), 7.5 (m, 3H, Ar-H), 7.7 (m, 1H, Ar-H), 7.96 (m, 2H, Ar-H), 8.65 (m, 2H, C_5-H & C_4-H), 9.2 (m, 1H, C_7-H), 10.06 (br, 1H, -NH-, D_2O exchang.), ^{13}C NMR (DMSO-d_6, 400 MHz, δ ppm): 171.59, 166.63, 155.12, 138.71, 138.49, 137.86, 129.12, 128.22, 123.04, 33.15, 31.19, 28.49, 25. 09, 22. 08, 13.99. Elemental analysis of $C_{24}H_{25}N_5O_2$, calcd. (found) %: C, 69.42 (69.38); H, 6.09 (6.06); N, 17.01 (16.86); O, 7.89 (7.70).

***N*-(5-(2-Phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-yl)butyramide (6b):** Yield: 62%; m.p.: 221-222 ºC; ^{1}H NMR



Reagents and conditions: (a) Et_3N, EtOH, reflux, (b) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, (c) cyanogen bromide $NaHCO_3$, 1,4-dioxane-H_2O, r.t., (d) pyridine, reflux

Scheme-I: Synthesis of the N-acetyl (5-(2-phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-yl)amine derivatives (**6a-j**)

(DMSO-d_6, 400 MHz, δ ppm): 0.91 (t, 3H, CH_3), 1.6 (q, 2H, -CH_2), 2.18 (t, 2H, -CH_2), 7.5 (m, 3H), 7.72 (m, 1H), 7.96 (m, 2H, Ar-H), 8.65 (m, 2H, C_5-H & C_4-H), 9.20 (m, 1H, C_7-H), 10.06 (br, 1H, -NH-). Elemental analysis of $C_{20}H_{17}N_5O_2$, calcd. (found) %: C, 66.87 (66.84); H, 4.79 (4.77); N, 19.52 (19.49); O, 8.92 (8.90).

***N*-(5-(2-Phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-yl)propionamide (6c):** Yield: 68%; m.p.: 244-245 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 1.08 (t, 3H, CH_3), 2.20 (q, 2H, -CH_2), 7.5 (m, 3H, aromatic-H), 7.7 (m, 1H), 7.96 (m, 2H), 8.64 (m, 2H), 9.18 (m, 1H), 10.06 (br, 1H, -NH-). Elemental analysis of $C_{19}H_{15}N_5O_2$, calcd. (found) %: C, 66.12 (66.08); H, 4.40 (4.38); N, 20.31 (20.28); O, 9.29 (9.27).

***N*-(5-(2-Phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-yl)acetamide (6d):** Yield: 64%; m.p.: 274-275 ºC. ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 1.95 (s, 3H, CH_3), 7.5 (m, 3H, Ar-H), 7.7 (m, 1H, Ar-H), 7.96 (m, 2H, Ar-H), 8.66 (m, 2H), 9.18 (m, 1H, C_7-H), 10.2 (s, 1H, -NH-). Elemental analysis of m.f.: $C_{18}H_{13}N_5O_2$, calcd. (found) %: C, 65.27 (65.25); H, 3.97 (3.95); N, 21.16 (21.14); O, 9.68 (9.66).

***N*-(5-(2-Phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-yl)pentanamide (6e):** Yield: 63%; m.p.: 211-212 ºC. ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 0.90 (m, 3H, -CH_2), 1.30 (m, 2H, CH_3), 1.56 (m, 2H, -CH_2), 2.20 (t, 2H, -CH_2), 7.5 (m, 3H, Ar-H), 7.7 (m, 1H, Ar-H), 7.90 (m, 2H, Ar-H), 8.70 (m, 2H), 9.2 (m, 1H, C_7-H), 10.1 (s, 1H, -NH-). Elemental analysis of $C_{21}H_{19}N_5O_2$, calcd. (found) %: C, 67.59 (67.55); H, 5.15 (5.13); N, 18.78 (18.76); O, 8.56 (8.57).

***N*-(5-(2-Phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-yl)benzamide (6f):** Yield: 59%; m.p.: 225-226 ºC. ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 5.87 (br, 1H, -NH-), 7.50 (m, 2H, Ar-H), 7.60 (m, 2H, Ar-H), 7.76 (m, 1H, Ar-H), 7.88 (m, 1H, Ar-H), 7.94 (m, 3H, Ar-H), 8.20 (m, 2H, Ar-H), 8.70 (m, 2H), 9.2 (m, 1H, C_7-H). Elemental analysis of $C_{23}H_{15}N_5O_2$, calcd. (found) %: C, 70.24 (70.22); H, 3.87 (3.84); N, 17.83 (17.80); O, 8.14 (8.13).

***N*-(5-(2-Phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-yl)pivalamide (6g):** Yield: 64%; m.p.: 253-254 ºC. ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 1.19 (s, 9H, 3-CH_3), 7.48 (m, 4H, Ar-H), 7.59 (m, 2H), 7.79 (m, 1H, Ar-H), 8.71 (m, 1H), 9.06 (s, 1H, -NH), 9.24 (m, 1H, Ar-H). LC-MS: *m/z* 374.20 ($M^{++}1$). Elemental analysis of $C_{21}H_{19}N_5O_2$, calcd. (found) %: C, 67.57 (67.55); H, 5.15 (5.13); N, 18.79 (18.76); O, 8.59 (8.57).

***N*-(5-(2-Phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-yl)isobutyramide (6h):** Yield: 61%; m.p.: 219-220 ºC. ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 1.06 (d, 6H, 2-CH_3), 2.50 (m, 1H, -CH), 7.49 (m, 3H, Ar-H), 7.57 (m, 2H, Ar-H), 8.79 (m, 1H), 8.70 (s, 1H, Ar-H), 9.04 (s, 1H, Ar-H), 9.24 (m, 1H, Ar-H), 11.65 (s, 1H, -NH-). LC-MS: *m/z* 360.15 (M^++1). Elemental analysis of $C_{20}H_{17}N_5O_2$, calcd. (found) %: C, 66.87 (66.84); H, 4.79 (4.77); N, 19.52 (19.49); O, 8.92 (8.90).

***N*-(5-(2-Phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-yl)hexanamide (6i):** Yield: 52%; m.p.: 231-232 ºC. ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 0.88 (m, 3H, -CH_2), 1.27 (m, 2H, CH_3), 1.47 (m, 2H, -CH_2), 1.62 (m, 2H, -CH_2), 2.14 (t, 2H, -CH_2), 7.41 (m, 3H, Ar-H), 7.57 (m, 1H), 7.81 (m, 2H, Ar-H), 8.54, (m, 2H), 9.01 (m, 1H, C_7-H), 9.89 (s, 1H, -NH-). LC-MS: *m/z* 388.28. (M^++1). Elemental analysis of $C_{22}H_{21}N_5O_2$, found (calcd.) %: C, 68.22 (68.20); H, 5.48 (5.46); N, 18.10 (18.08); O, 8.28 (8.26).

***N*-(5-(2-Phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-yl)heptanamide (6j):** Yield: 51%; m.p.: 218-219 ºC. ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 0.83 (m, 3H, CH_3), 1.25 (m, 6H, -CH_2), 1.52 (m, 2H, -CH_2), 2.51 (t, 2H), 7.48 (m, 3H, Ar-H), 7.57 (m, 2H, Ar-H), 7.79 (m, 1H), 8.71 (m, 2H, C_5-H & C_4-H),

9.07 (m, 1H, C_7-H), 9.23 (s, 1H, -NH-). LC-MS: *m/z* 402.31 (M^++1). Elemental analysis of $C_{23}H_{23}N_5O_2$, calcd. (found) %: C, 68.83 (68.81); H, 5.79 (5.77); N, 17.46 (17.44); O, 7.99 (7.97).

Antibacterial activity: The antimicrobial activity of synthesized compounds was evaluated using the Lysogeny broth Agar (LBA) dilution method [36,37] against both Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* bacteria. In brief, after dissolving in DMSO, the test compounds were added to the first concentration set. An LB agar plate was aseptically seeded with 20 µL of *S. aureus* and *E. coli* and then allowed to stand statically for 5 min. Finally, the plates were incubated for 12-16 h at 37 ºC.

Antifungal activity: Fungi strain (*Candida albicans*) was acquired from the MTCC and gene bank, CSIR-IMTECH in Chandigarh, India. The PDA medium was prepared by boiling 200 g of sliced, unpeeled potatoes in 1 L of distilled water for 30 min. The solution was filtered through cheesecloth to get potato infusion which is equivalent to 4.0 g of potato extract. It was then cooked to dissolve the infusion after mixing it with 29 g of glucose, 15 g of extracted agar and 1 L water. A 15-min autoclave was used to sterilize the solution at 121 ºC. In a subsequent step, the medium was acidified to a pH of 3.5 at 50 ºC with 10% tartaric acid. A 6 mm cork borer was used to pour the solidified plates. For fungal strains, novel 5-(2-phenyl-1,8-naphthyridine-3-yl)-1,3,4-oxadiazole-2-amine (**5**) and its amide derivatives (**6a-j**) showed a distinct zone of inhibition surrounding the well.

Docking studies: The Auto Dock Vina program was used to carry out the molecular docking [38,39] of the synthesized compounds. The RCSB protein data bank was used to obtain the crystal structures of the applied proteins [40]. First, all the proteins were cleaned and processed with the use of Auto Dock tools [41] and BIOVIA Discovery Studio 2020 [42]. The Gaussian09 software package is utilized to optimize each ligand structure [43], wehreas BIOVIA Discovery Studio 2020 was used to evaluate and depict the docked positions.

The interaction behaviour of titled derivatives combining with *S. aureus* enzyme MurB was carried out in this work. Serving as an initiator for over dozen biosynthetic conversions, the Mur protein like Mur A-F, Y and G family is known to contribute to the development of the bacterial cell wall's peptidoglycan coating layer [44]. MurB, an enzyme that reduces UDP-N-acetylglucosamine to nolpyruvate, is crucial for binding NADPH in *E. coli* or EP-UDPGIcNAc. The residue-bound protein-ligand complexes with the lowest binding energies were calculated.

RESULTS AND DISCUSSION

In view of varied biological activities of 1,3,4-oxadiazole and 1,8-naphthyridines, it is considered of interest to combine these moieties to obtain novel compounds, which might exhibit enhanced biological activities. In present work, the synthesis of *N*-acylated derivatives of 5-(2-phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-amine (**6a-j**) was achieved *via* four steps. Initially, 2-amino nicotinaldehyde (**1**) was reacted with ethyl-3-oxo-3-phenylpropanoate (**2**) in the presence of triethylamine under refluxing condition to give ethyl-2-phenyl-1,8-naphthyridine-3-carboxylate (**3**) in 86% yield (**Scheme-I**). The infrared spectrum of compound **3** displays an absorption band at 1707 cm^{-1}, which is due to the presence of a ester carbonyl group. The ^{1}H NMR of compound **3** revealed a triplet at 1.23 ppm and a quartet at 4.24 ppm indicating the presence of CH_3 and CH_2 groups, respectively suggesting the production of acetate.

2-Phenyl-1,8-naphthyridine-3-carbohydrazide (**4**) was obtained by refluxing ethyl-2-phenyl-1,8-naphthyridine-3-carboxylate (**3**) with hydrazine hydrate in ethanol by refluxing for 4 h yielding the product of about 70%. The principal signs of secondary amines (NH) and amines (NH_2) from compound **4**'s hydrazide (-NH-NH_2) were visible in the infrared spectrum at 3298 cm^{-1} and 3187 cm^{-1} suggests the reaction convert acetate (**3**) into carbohydrazide (**4**), were also disappeared at the region of 3000, 2838, 1677-1630, 1603, 1531 and 1291, 1231 cm^{-1}, respectively showed the completion of reaction in the expected manner. In the ^{1}H NMR spectra, the broad peak at 4.62 & 9.63 ppm also confirmed the conversion of acetate (**3**) into carbohydrazide (**4**). Further by reacting 2-phenyl-1,8-naphthyridine-3-carbohydrazide (**4**) with cyanogen bromide using dioxane and water mixture as solvent under refluxing condition to obtain 5-(2-phenyl-1,8-naphthyridin-3-yl)-2-oxadiazol-1,3,4-amine (**5**) at 70% yield. In this, the ^{1}H NMR spectrum the singlet at 4.54 ppm is due to NH_2 group which further evidence by D_2O exchange. IR spectra frequencies was observed at 3282 (-NH_2) group, 3178, 3055, 2927, 1652.82 (-C=N) group, 1622, 1603, 1550, 1519, 1462, 1343, 1300, 1234, 1185, 1112, 1083, 783, 731, 701, 673, 620 cm^{-1} respectively supports the structure of compound **5**. The N-acylated derivatives of 5-(2-phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-amine (**6a-j**) were finally synthesized from the reaction with compound **5** and symmetrical anhydride in the presence of pyridine (**Scheme-I**). The distinctive singlet primary amine -NH_2 protons can be seen at 4.5 ppm in the ^{1}H NMR spectra. These protons disappear when D_2O was added in compound **5**, the obtained compound **6a** on acetylation, the distinguishing secondary amine –NH– proton is observed as a broad peak in the NMR spectra at 10.06 ppm and protons disappeared with the D_2O exchange spectra. At 2.22 ppm and 1.3 ppm, the protons were visible as a singlet for characteristic –$COCH_2$ and –CH_3 groups respectively. Where as in ^{13}C NMR spectra, signals appeared in the 158.74-167.05 ppm range for 1,3,4-oxadiazole ring to the corresponding asymmetric 2 and 5 carbons of compound **6a**. The formation of -NH_2 was confirmed due to the formation of peak at 3282 cm^{-1} vibrations in infrared spectra. The formation of 1,3,4-oxadiazole ring was confirmed by the IR absorption groups at 1343 and 1652 cm^{-1}, which were ascribed to the C–O, C–N bond vibrations, respectively, there was evidence of the distinctive C-O-C at 1185 and 3178 cm^{-1}. Further it forms secondary amine which shows a single weak band at 3198 cm^{-1} and -C=O was observed at 1665 cm^{-1}.

Antimicrobial activities: The synthesized 5-(2-phenyl-1,8-naphthyridine-3-yl)-1,3,4-oxadiazole-2-amine (**5**) and its amide derivatives (**6a-j**) were tested against one Gram-positive (*S. aureus*) and one Gram-negative (*E. coli*) bacteria and one fungal stain (*C. albicans*). Using LBA dilution method, the

TABLE-1
ANTIMICROBIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS **6a-j**

Compounds	Zone of inhibition (mm)											
	Antibacterial activity								Antifungal activity			
	Staphylococcus aureus				*E. coli*				*Candida albicans*			
	50 µg	100 µg	200 µg	300 µg	50 µg	100 µg	200 µg	300 µg	50 µg	100 µg	200 µg	300 µg
3	0	0	5	10	0	0	0	5	0	0	10	10
4	0	5	15	20	0	0	0	5	0	0	10	20
5	0	0	5	10	0	5	10	15	0	0	0	10
6a	0	5	5	15	0	0	0	5	0	0	5	15
6b	0	0	15	15	0	0	5	5	0	0	0	10
6c	0	0	5	10	0	0	5	10	0	0	15	27
6d	0	5	5	5	0	0	5	10	0	0	10	20
6e	0	5	10	25	0	5	10	15	0	5	10	25
6f	0	0	10	10	0	0	5	10	0	0	15	20
6g	0	0	11	12	0	0	10	12	0	0	10	13
6h	0	0	10	12	0	0	10	12	0	0	9	13
6i	0	0	13	15	0	0	9	12	0	0	12	13
6j	0	0	12	15	0	0	10	12	0	0	10	12
Ampicillin	10	20	21	21	14	17	19	23	–	–	–	–
Fluconazole	–	–	–	–	–	–	–	–	19	19	22	24

bioactivity of new compounds was examined and ampicillin served as standard reference. According to the data (Table-1), compound **6e** has shown good antibacterial action against *S. aureus* at 300 µg/mL with ZOI of 25 mm. Compound **4** demonstrated a ZOI at 20 mm at the same concentration. Compounds **5** and **6e** have shown strong antibacterial activity against *E. coli* with 15 mm at 300 µg/mL, whereas compounds **6g**, **6h**, **6i** and **6j** have exhibited the moderate antibacterial activity against *E. coli* with 12 mm at 300 µg/mL. According to the antifungal experiments (Table-1), compound **6c** exhibited a ZOI of 27 mm against *Candida albicans*, whereas compound **6e** demonstrated a ZOI of 25 mm.

Docking results: Molecular docking analysis of the synthesized compounds (**6a-j**) was conducted using the receptor UDP-N-acetylglucosamine-enol pyruvate reductase Mtb MurB (PDB ID: 5JZX) to ascertain their binding patterns. Among all the compounds, **6f** (-10.98 kcal/mol), **6b** (-10.52 kcal/mol), **6a** (-10.44 kcal/mol), **6j** (-10.34 kcal/mol) and **6h** (-10.30 kcal/mol) demonstrated good binding affinity with the minimum binding energies. The minimum binding energies range from -7.79 to -10.98 kcal/mol (Table-2). Compounds **6f** established 3 hydrogen bonds with an amino acid sequence ARG176, GLU361, ARG238, **6b** shown 2 hydrogen bonds with PRO128, SER70. Compound **6a** established 3 hydrogen bonds with an amino acid sequence ARG176, GLU361, ARG238 and Compounds **6j** established 3 hydrogen bonds with an amino acid sequence ARG238 (3) (Fig. 1). It was found that antifungal activity is enhanced by the presence of substituted phenyl ring in 5-(1,8-naphthyridin-6-yl)-1,3,4-oxadiazol-2-amine nucleus, which promotes binding interactions with receptors.

Conclusion

In this work, we reported a straightforward and effective method for sythesizing novel 5-(2-phenyl-1,8-naphthyridin-3-yl)-1,3,4-oxadiazol-2-amine (**5**) and its derivatives (**6a-j**). The antifungal and antibacterial properties of the newly prepared compounds were successfully evaluated and the results indicated that the phenyl group incorporating at 2-position in target compounds enhances their strength towards docking score and antibacterial and antifungal activity. Compounds **6c**, **6e** and **6f** have good activity against fungal strains, whereas compounds **6a**, **6b** and **6e** have significant antibacterial action against specific Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) strains. Studies using molecular docking demonstrated that compounds **6a**, **6b**, **6f**, **6h** and **6j** had more effective interactions with protein.

TABLE-2
DOCKING RESULTS OF THE SYNTHESIZED COMPOUNDS **6a-j**

Compd.	Mtb MurB (PDB ID: 5JZX)		
	Binding energy (Kcal mol^{-1})	No. of H bonds	Bonding-related residues
3	-7.95	1	ARG238
4	-7.79	4	SER254, THR26, ARG238 (2)
5	-8.72	3	GLU361 (2), ARG238
6a	-10.44	3	ARG176, GLU361, ARG238
6b	-10.52	2	PRO128, SER70
6c	-9.85	3	GLY140, ARG238 (2)
6d	-9.89	3	GLY140, ARG238 (2)
6e	-10.13	3	ARG176, GLU361, ARG238
6f	-10.98	3	ARG176, GLU361, ARG238
6g	-10.04	2	SER70, PRO128
6h	-10.30	1	SER70
6i	-10.14	1	SER70
6j	-10.34	3	ARG238 (3)

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

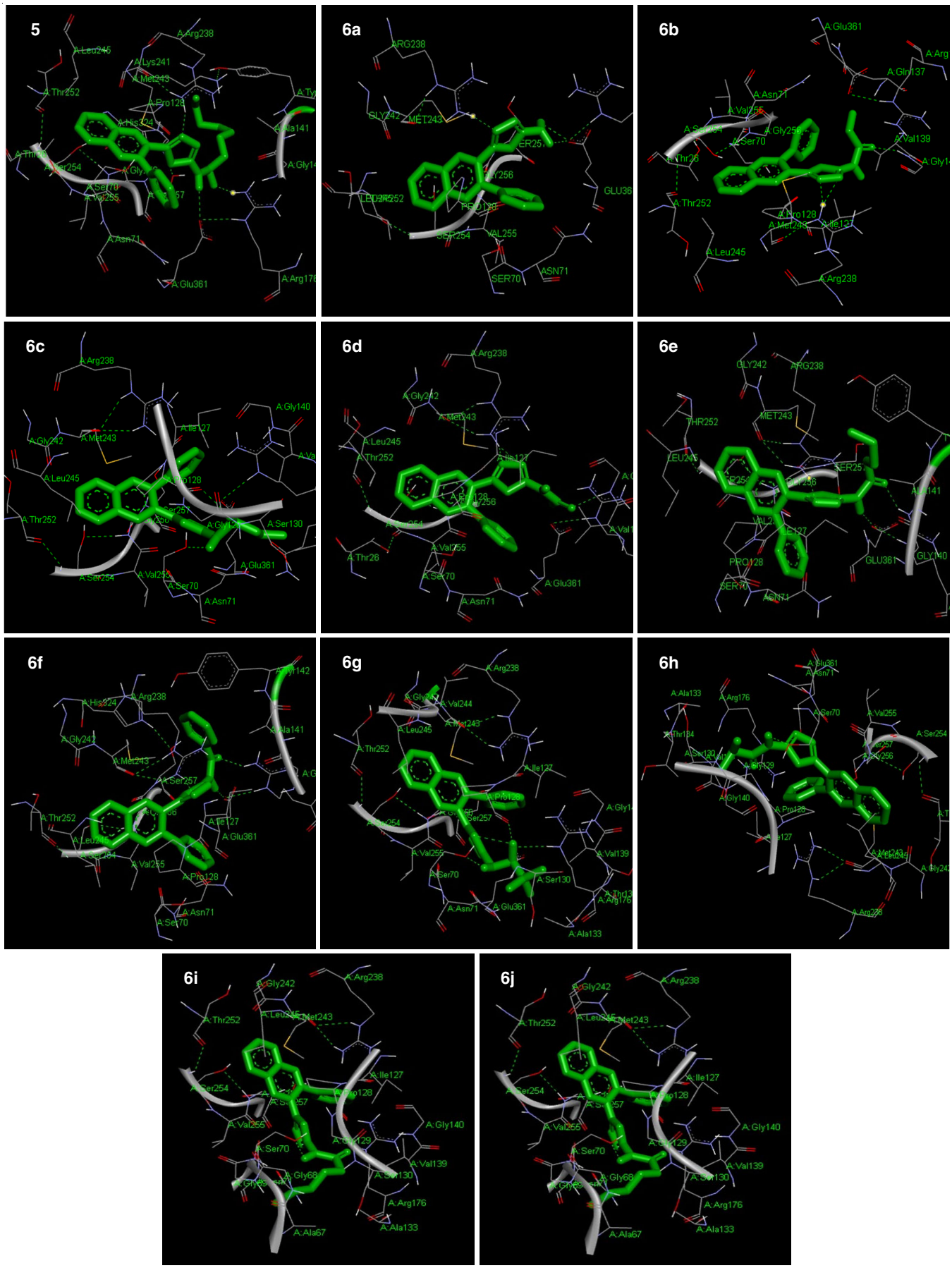

Fig. 1. Compound **5**, **6a-j** bind to the receptor UDP-N-acetylglucosamine-enol pyruvate reductase Mtb MurB by binding to its active site

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