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Synthesis of *N′*-(Substituted)-2-(4-(3-nitroimidazo[1,2-*b*]pyridazin-6-yl)piperazin-1-yl)acetohydrazides and their 1,3,4-Oxadiazole Derivatives: Characterization, Antimicrobial Activity and Molecular Docking Studies

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ABSTRACT

The growing pharmaceutical relevance of drug-resistant pathogens has necessitated the emergence of new treatment medicines. In this scenario, a novel series of *N′*-(substituted)-2-(4-(3-nitroimidazo[1,2-*b*]-pyridazin-6-yl)piperazin-1-yl)acetohydrazides and 1-(2-(substituted)-5-((4-(3-nitroimidazo[1,2-*b*]pyridazin-6-yl)piperazin-1-yl)methyl)-1,3,4-oxadiazol-3 (2*H*)-yl)ethan-1-ones have been synthesized and characterized by ^{1}H & ^{13}C NMR spectral data and screened for antimicrobial activity, as well as molecular docking studies. The synthesized compounds were tested against *Escherichia coli* (1668), *Bacillus cereus* (1272), *Candida albicans* (854) by using disc diffusion method. Among all the synthesized compounds **3c** and **4c** exhibit good potent antimicrobial activity against *Escherichia coli*, **3a**, **4c** against *Bacillus cereus* and **4a**, **4c** against *Candida albicans*. The Auto-Dock 4.2/ADT application was performed to investigate the binding interaction of the synthesized compounds with BAX protein. Among all the synthesized compounds **4e**, **4a**, **3e** and **3a** showed the highest binding affinity (-8.0, -7.5, -7.0 and -6.9 Kcal/mol) with BAX protein.

KEYWORDS

3-Nitro-6-(piperazin-1-yl)imidazo[1,2-*b*]pyridazine, Molecular docking, 1,3,4-Oxadiazoles, Antibacterial activity, Antifungal activity, BAX protein, Hydrazones.

INTRODUCTION

The increasing resistance to broad-spectrum antibacterial medicines has driven the development of novel antibacterial medications. Acquired resistance to one or more antimicrobial agents has been recognized as a consequence of enhanced over-use of antibiotics for extended periods of time. The evolution of multidrug-resistant bacterium strains is becoming an exceedingly major concern. Accordingly, developing novel antimicrobial drugs will continue to be a challenging task for medicinal chemists. Antimicrobial drugs are effective in the treatment of infectious diseases because of their selective toxicity and their ability to injure or kill an invading microorganism without harming the host [1,2].

The biological activities of hydrazones and 1,3,4-oxadiazole have been reviewed by many researchers. Hydrazone

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analoges shows a wide range of biological applications including, anticonvulsant [3,4], antitubercular [5,6], antioxidant [7,8], antiviral [9,10], vasodilator activity [11], antitumor activity [12,13], antimicrobial [6,14,15], antimycobacterial [16], antidepressant [17,18], antimalarial [2,19], antifungal [20,21], analgesic and anti-inflammatory activities [22-25]. Furthermore, some hydrazones have also been used as nematicides, herbicides, insecticides, rodenticides and plant growth regulators [26] as well as plasticizers, stabilizers for polymers also [9,27,28]. The hydrazones supported metal complexes exhibit broad spectrum of potential applications such as catalysts [29], luminescent probes [30], radio protective properties [31], enzyme inhibitors [32] and molecular sensors [33].

In the identification and development of novel promising molecules, combining the computational and experimental methodologies has proven to be extremely helpful. Molecular docking methods are widely utilized in modern drug design to investigate ligand conformations within macromolecular targets' binding sites. Pharmacokinetic parameters (ADMET: absorption, distribution, metabolism, excretion and toxicity) have also been explored to use these approaches in addition to pharmacodynamic data (*e.g.*, potency, affinity, efficacy, selectivity) [34]. The exploration of binding conformations, characterization of critical intermolecular interactions, identification of unknown binding sites, mechanistic studies and the clarification of ligand-induced conformational changes all benefit through structural descriptions of ligand-receptor complexes [35]. Because of its ability to predict the conformation of small-molecule ligands within the right target binding site with a high degree of certainty, molecular docking is one of the most usually applied methods in Structure Based Drug Design (SBDD) [36]. The binding modalities of small-molecule ligands within receptor binding sites are successfully predicted by the most of the molecular docking systems. Furthermore, molecular docking algorithms make quantitative estimates of binding energies, ranking docked molecules based on their ligand-receptor complexes' binding affinity [37,38].

BAX is a pro-apoptotic member of the Bcl-2 family that initiates apoptosis *via* the mitochondrial mechanism. The sensitivity of an apoptotic event during a stimulus is determined by the relative quantities of pro and anti-apoptotic proteins in the cell and resistance to apoptosis can be induced by an excess of anti-apoptotic proteins in the cell [13,39]. The formation of pores in the mitochondria and the release of cytochrome C and other pro-apoptotic molecules from the inter membrane space caused by disturbances in the normal function of pro- and anti-apoptotic Bcl-2 proteins [40]. All these signals in turn cause the activation of effector caspase, caspase 3 plays an important role in the process of the cleavage of chromosomal DNA into nucleosomal units by activating DNases, inhibiting DNA repair enzymes breaking down structural proteins in the nucleus [41]. Number of chemotherapeutic molecules or knock-down of certain cell cycle proteins can induce the BAX-dependent apoptosis in cancer cells [42]. Previous investigations found that the natural plant-derived gossypol, syringic acid and their derivatives showed antitumor activity in *in vitro* and animal models by modulating the Bcl-2 members *via* its BH3 mimetic properties [43].

Inhibiting anti-apoptotic Bcl-2 proteins has been employed in cancer therapy to selectively kill the cancer cells. In this study, *in silico* technique to evaluate various hydrazone analogues, 1,3,4-oxadiazoles, for drug ability, toxicity and binding affinity with BAX protein is used. By analyzing the binding mechanism of standard BAX activator, all compounds were further docked with BAX trigger site for the analysis of BH3 mimetic. The present study is emphasized on heterocyclic hydrazones and 1,3,4-oxadiazole derivatives that exhibited diverse pharmacological activities.

EXPERIMENTAL

Prior to use, the solvents were purified according to the conventional procedures and all commercial chemicals were used as they were received. Merck pre-coated plates (silica gel 60 F_{254}) were used for thin-layer chromatography (TLC) analysis and eluting solvents were indicated in the procedures. For the flash column chromatography, Merck silica gel 60 (230-400 mesh) was used. The Mel-temp apparatus was used to determine the melting point and the results are not corrected. At 400 MHz, Bruker and Jeol NMR instruments were used to record ^{1}H NMR spectra at room temperature. A VG 7070H mass spectrometer was used to record the mass spectra. All of the aldehydes required to develop compounds **3a-f** were purchased from the commercial sources.

Antimicrobial activity: The antibacterial and antifungal activities of the synthesized compounds were examined by disc diffusion method [44,45] against the bacterial strains *viz.* *Escherichia coli*, *Bacillus cereus* and fungus *Candida albicans* as compared to the standard drugs amoxycillin and amphotericin-B, respectively.

The antimicrobial activities were determined by disc diffusion method using nutrient agar medium (NAM) for bacterial and potato dextrose agar (PDA) medium for fungal culture, respectively. Nutrient agar medium (NAM) was prepared with beef extract (3 g), peptone (5 g), NaCl (5 g) and agar-agar (15 g) in 1000 mL distilled water and pH was adjusted to 7. Potato dextrose agar (PDA) was prepared by adding dextrose (20 g), agar-agar (15 g) to potato infusion (1000 mL) and pH was adjusted to 5.5. Potato infusion was made by boiling 200 g of sliced potatoes in distilled water for 0.5 h and then filtered through Whatman No.1 filter paper and filtrate was made up to 1 L with distilled water. Both the media were sterilized in an autoclave at 121 ºC, 15 lbs pressure for 0.5 h. After sterilization, approximately 20 mL of molten and cooled media was poured in sterilized Petri dishes. The plates were leftover for 30 min. The test compounds were dissolved in DMSO at a concentration of 100, 400, 600, 800 and 1000 µg/mL. The stock solutions were applied to each sterilized filter paper discs of 5 mm. Discs were dried and preserved for antimicrobial studies. The discs were placed on NAM and PDA inoculated with bacteria or fungi and NAM plates were incubated at 37 ºC and PDA plates at 30 ºC for 24-30 h. The plates were examined to measure the zone of inhibition at 400 µg/mL. The studies were repeated three times and the radius of the zone of inhibition was measured on each occasion.

Molecular docking: The optimized structures of the BAX, hydrazones and 1,3,4-oxadiazole derivatives were used for

molecular docking calculations. Molecular docking was carried out with the automated docking program, AutoDock 4.2 [46-48]. All the components such as BAX, hydrazones and 1,3,4-oxadiazole derivatives were optimized before conducting docking and the results were analyzed using binding energy. For each ligand, a docking experiment consisting of 100 stimulations performed and the analysis was based on binding-free energies and root-mean-square deviation (RMSD) values. Docking with all the newly synthesized compounds was performed onto BAX with the same parameters and PMV 1.4.5 viewer was then used to observe the interactions of the docked compound to the BAX protein, further docking analysis was carried out on PyMol software.

Synthesis of ethyl 2-(4-(3-nitroimidazo[1,2-*b*]pyridazin-6-yl)piperazin-1-yl)acetate (1): 3-Nitro-6-(piperazin-1-yl)-imidazo[1,2-*b*]pyridazine was dissolved in dry DMF (10 vol.) and cooled to 0 ºC in ice cold water. Ethyl bromo acetate 1.1 eqiv. was added dropwise and kept at room temperature for 16 h. TLC confirmed the completion of the reaction and reaction mass was poured into saturated ammonium chloride solution. The product was extracted with ethyl acetate (twice). Organic layer was dried over anhydrous Na_2SO_4 and evaporated to afford the crude product. The crude product was purified by silica gel column chromatography eluted with 2% methanol in chloroform to afford the product as light-yellow solid (82%). Light yellow solid; Yield: 82%, m.p.: decomposed at 175 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.190, 1.207, 1.225 (t, 3H, J = 6.8 Hz, CH_3), 2.670, 2.681, 2.692 (t, 4H, J = 4.4 Hz, piperazine ring), 3.363 (s, 2H, CH_2), 3.619, 3.631, 3.641 (t, 4H, J = 4.4 Hz, piperazine ring), 4.081, 4.099, 4.116, 4.134 (q, 2H, J = 6.8 Hz, CH_2), 7.568, 7.593 (d, 1H, J = 10.0 Hz, pyridazine ring), 8.102, 8.127 (d, 1H, J = 10.0 Hz, pyridazine ring), 8.514 (s, 1H, imidazole ring). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 14.14, 45.07, 51.26, 58.14, 59.91, 114.87, 126.65, 133.84, 134.89, 138.19, 155.68, 169.87. MS *m/z*: 335.2 [M+H], (334.14); Anal. calcd. (found) % for $C_{14}H_{18}N_6O_4$: C, 50.29 (49.40); H, 5.43 (5.31); N, 25.14 (24.81). IR (ν, cm^{-1}): 3111.82 (C-H *str.* in heterocyclic ring), 2855.47 (C-H *str.* in aliphatics), 1733.05 (C=O *str.* in esters), 1444.50 (C-H bending in aliphatics).

Synthesis of 2-(4-(3-nitroimidazo[1,2-*b*]pyridazin-6-yl)piperazin-1-yl)acetohydrazide (2): Ethyl 2-(4-(3-nitro-imidazo[1,2-*b*]pyridazin-6-yl)piperazin-1-yl)acetate (**1**) (1 equiv.) was dissolved in ethanol (15 vol.). Three equivalent of hydrazine hydrate was added and the reaction mass was heated at reflux for 6 h. Reaction completion was confirmed by TLC and evaporated to crude. The crude product was suspended in ethyl acetate, washed with water and brine. Organic layer was dried over Na_2SO_4 and evaporated to afford the pure product as off-white solid, which was directly used for the next step without further purifications (76%). Off-white solid; Yield: 76%, m.p.: decomposed at 178 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.578, 2.590, 2.602 (t, 4H, J = 4.8 Hz, piperazine ring), 3.000 (s, 2H, CH_2), 3.639, 3.651, 3.664 (t, 4H, J = 5.0 Hz, piperazine ring), 4.239 (s, 2H, NH_2), 7.571, 7.596 (d, 1H, J = 10 Hz, pyridazine ring), 8.110, 8.136 (d, 1H, J = 10.4 Hz, pyridazine ring), 8.523 (s, 1H, imidazole ring), 8.989 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.017, 52.106, 59.571, 114.873, 126.633, 133.832, 134.867, 138.178, 155.652, 168.028. MS *m/z*: 321.2 [M+H], (320.13); Anal. calcd. (found) % for $C_{12}H_{16}N_8O_3$: C, 45.00 (44.65); H, 5.03 (4.81); N, 34.98 (34.60). IR (ν, cm^{-1}): 3287.47 (N-H stretching in hydrazide), 3128.76 (C-H stretching in heterocyclic ring), 2947.77, 2841.84 (asymmetric/symmetric C-H stretching in aliphatics), 1656.36 (C=O stretching in hydrazide), 1453.67 (C-H bending in aliphatics).

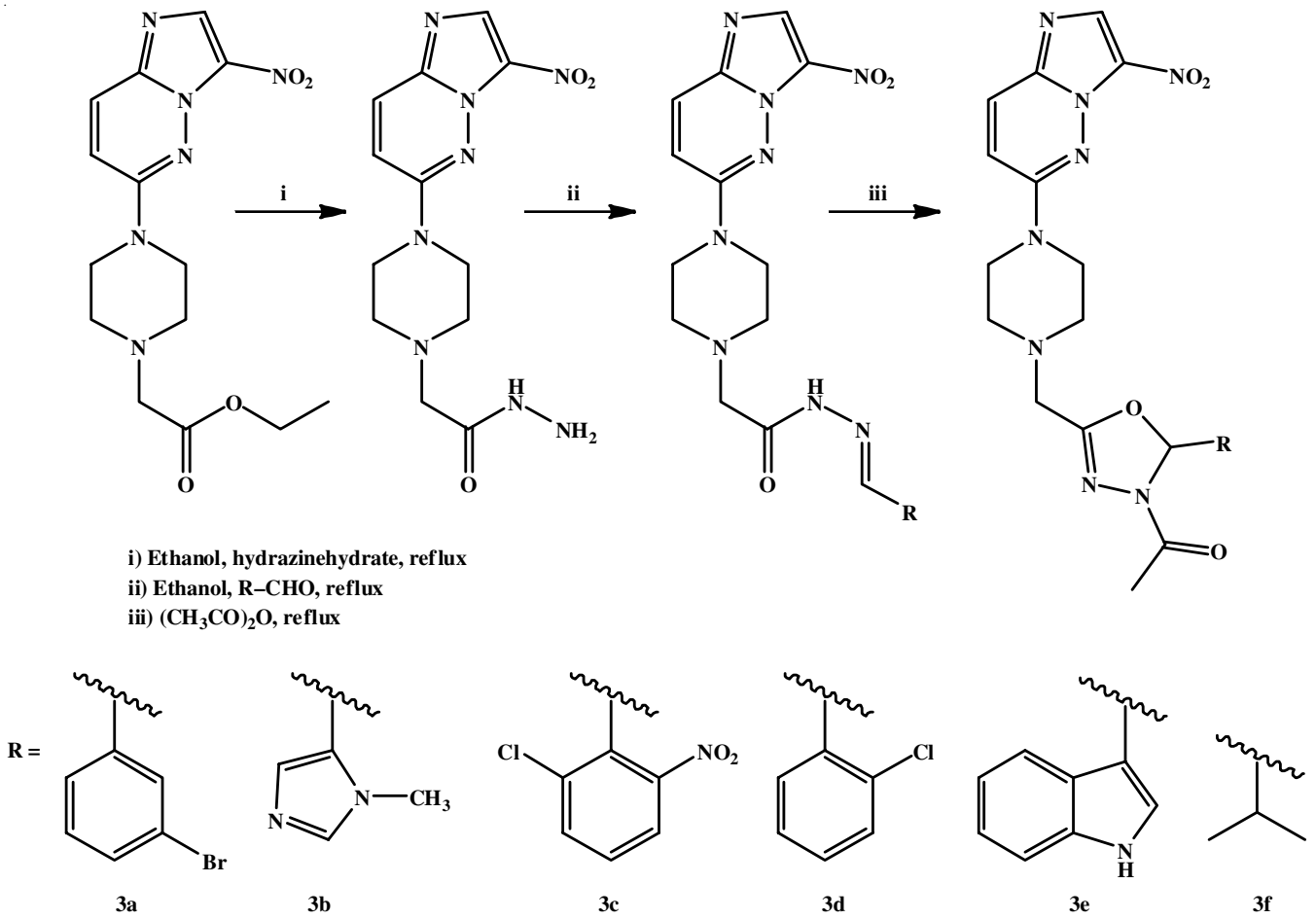
Synthesis of *N′*-(substituted)-2-(4-(3-nitroimidazo[1,2-*b*]-pyridazin-6-yl)piperazin-1-yl)acetohydrazides (3a-f): 2-(4-(3-Nitroimidazo[1,2-*b*]pyridazin-6-yl)piperazin-1-yl)aceto-hydrazide (**2**) (1 equiv.) and aldehyde (1.1 equiv.) was taken in ethanol (15 vol.). The reaction mass was heated to reflux for 6 h. The reaction progress was monitored with the TLC. After completion of the reaction ethanol was evaporated to 2 volumes and cooled to 10 ºC. The precipitate was collected and dried on filter paper to afford the product (71%) (**Scheme-I**).

***N′*-(3-Bromobenzylidene)-2-(4-(3-nitroimidazo[1,2-*b*]-pyridazin-6-yl)piperazin-1 yl)acetohydrazide (3a):** Light yellow solid; Yield: 75%, m.p.: decomposed at 165 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.702 (s, 2H, CH_2), 3.361 (s, 4H, piperazine ring), 3.704 (s, 4H, piperazine ring), 7.396-7.507 (m, 1H, pyridazine ring), 7.612-7.745 (m, 3H, phenyl ring), 7.882-7.969 (m, 1H, phenyl ring), 8.071-8.149 (m, 1H, pyridazine ring), 8.298 (s, 1H, =CH); 8.532 (s, 1H, imidazole ring), 11.476-11.643 (singlet distorted, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 44.95, 51.69, 52.06, 114.94, 122.13, 122.19, 126.17, 126.71, 128.85, 129.07, 131.00, 133.87, 134.93, 136.74, 138.23, 145.41, 155.69. MS *m/z*: 487.1 [M+H], (486.08); Anal. calcd. (found) % for $C_{19}H_{19}BrN_8O_3$: C, 46.83 (45.78); H, 3.93 (3.81); N, 22.99 (22.56).

***N′*-((1-Methyl-1*H*-imidazol-5-yl)methylene)-2-(4-(3-nitroimidazo[1,2-*b*]pyridazin-6-yl)piperazin-1-yl)aceto-hydrazide (3b):** Yellow solid; Yield: 82%, m.p.: decomposed at 155 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.669-2.771 (m, 4H, piperazine ring), 3.168 (s, 2H, CH_2), 3.657-3.694 (m, 4H, piperazine ring), 3.803 (singlet, 3H, N-CH_3), 7.585-7.641 (m, 3H, pyridazine ring, imidazole ring), 8.111-8.144 (m, 1H, imidazole ring), 8.258 (s, 1H, =CH), 8.529 (s,1H, imidazole ring), 11.129-11.233 (singlet distorted, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 45.04, 51.94, 52.13, 55.29, 56.97, 60.18, 114.31, 114.93, 126.80, 128.29, 133.87, 134.92, 138.23, 147.10, 155.72, 160.58, 165.19. MS *m/z*: 413.2 [M+H], (412.17); Anal. calcd. (found) % for $C_{17}H_{20}N_{10}O_3$: C, 49.51 (49.32); H, 4.89 (4.71); N, 33.96 (33.65).

***N′*-(2-Chloro-6-nitrobenzylidene)-2-(4-(3-nitro-imidazo[1,2-*b*]pyridazin-6-yl)piperazin-1-yl)aceto-hydrazide (3c):** White solid; Yield: 70%, m.p.: 172-174 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.901 (s, 2H, CH_2), 3.217-3.968 (broad, 8H, piperazine ring), 7.663-7.688 (d, 1H, J = 10.0 Hz, pyridazine ring), 7.766-7.788 (d, 1H, J = 8.8 Hz, pyridazine ring), 8.095-8.226 (m, 3H, phenyl ring), 8.264 (s, 1H, =CH), 8.494 (s, 1H, imidazole ring), 8.960 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 42.50, 51.36, 55.66, 113.85, 127.09, 127.34, 134.01, 135.29, 138.68, 155.36, 157.87, 158.25, 158.62, 159.00, 162.41, 163.04, 166.66. MS *m/z*: 488.2 [M+H], (487.11); Anal. calcd. (found) % for $C_{19}H_{18}ClN_9O_5$: C, 46.78 (46.52); H, 3.72 (3.59); N, 25.84 (25.59).

***N′*-(2-Chlorobenzylidene)-2-(4-(3-nitroimidazo[1,2-*b*]-pyridazin-6-yl)piperazin-1-yl)acetohydrazide (3d):** Light yellow solid; Yield: 73%, m.p.: decomposed at 168 ºC. ^{1}H NMR



Scheme-I: Synthesis of *N′*-(substituted)-2-(4-(3-nitroimidazo[1,2-*b*] pyridazin-6-yl)piperazin-1-yl)acetohydrazides and their 1,3,4-oxadiazole derivatives

(400 MHz, DMSO-d_6) δ ppm: 3.570-3.989 (broad signal, 6H, piperazine ring), 4.125-4.424 (broad, 2H, piperazine ring), 4.703 (s, 2H, CH_2), 7.419-7.560 (m, 4H, phenyl ring), 7.663-7.688 (d, 1H, *J* = 10 Hz, pyridazine ring), 7.962-8.015 (singlet distorted, 1H, =CH), 8.262-8.287 (d, 1H, *J* = 10 Hz, pyridazine ring), 8.477 (s, 1H, imidazole ring), 12.491 (singlet distorted, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 42.40, 51.02, 51.25, 55.56, 144.13, 127.04, 127.28, 127.54, 127.78, 130.05, 130.87, 133.48, 133.95, 135.26, 138.43, 141.70, 144.75, 166.38. MS *m/z*: 443.2 [M+H], (442.13); Anal. calcd. (found) % for $C_{19}H_{19}ClN_8O_3$: C, 51.53 (51.16); H, 4.32 (4.21); N, 25.30 (24.81).

***N′*-((1*H*-Indol-3-yl)methylene)-2-(4-(3-nitroimidazo-[1,2-*b*]pyridazin-6-yl)piperazin-1-yl)acetohydrazide (3e):** Light yellow solid; Yield: 82%, m.p.: decomposed at 170 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.735 (s, 2H, CH_2), 3.179-3.394 (m, 4H, piperazine ring), 4.116-4.473 (m, 4H, piperazine ring), 7.132-7.261 (m, 2H, pyridazine ring and pyrrole ring), 7.332-7.512 (m, 1H, phenyl ring), 7.532-7.646 (m, 1H, phenyl ring), 7.791-7.959 (m, 1H, phenyl ring), 8.016-8.290 (m, 3H, pyridazine ring, phenyl ring and =CH), 8.417-8.557 (singlet distroted, 1H, imidazole ring), 11.162-11.559 (singlet distorted, 1H, NH), 11.657-11.773 (singlet distorted, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 44.82, 51.52, 52.03, 56.51, 114.97, 121.65, 121.90, 123.99, 124.31, 126.73, 133.89, 134.94, 137.01, 137.11, 138.26, 144.51, 155.67, 162.33. MS *m/z*: 448.2 [M+H], (447.18); Anal. calcd. (found) % for $C_{21}H_{21}N_9O_3$: C, 56.37 (55.90); H, 4.73 (4.59); N, 28.17 (27.91).

***N′*-(2-methylpropylidene)-2-(4-(3-nitroimidazo[1,2-*b*]-pyridazin-6-yl)piperazin-1-yl)acetohydrazide (3f):** Yellow solid; Yield: 75%, m.p.: 145-147 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.790-1.856 (m, 1H, CH), 2.737-2.898 (d, 6H, $2CH_3$), 3.218-3.390 (m, 8H, piperazine ring), 4.077 (s, 2H, CH_2), 8.134-8.213 (m, 2H, pyridazine ring), 8.533-8.564 (m, 2H, imidazole ring & =CH), 11.424 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 22.24, 22.29, 26.12, 43.48, 44.50, 51.22, 51.44, 51.87, 115.04, 126.99, 133.88, 134.97, 138.25, 151.27, 155.43, 162.33. MS *m/z*: 375.2 [M+H], (374.18); Anal. calcd. (found) % for $C_{16}H_{22}N_8O_3$: C, 51.33 (50.88); H, 5.92 (5.78); N, 29.93 (29.57).

1-(2-(Substituted)-5-((4-(3-nitroimidazo[1,2-*b*]pyridazin-6-yl)piperazin-1-yl)methyl)-1,3,4-oxadiazol-3(2*H*)-yl)-ethan-1-one (4a-f): A mixture of *N′*-(substituted)-2-(4-(3-nitroimidazo[1,2-*b*]pyridazin-6-yl)piperazin-1-yl)acetohydrazide (**3a-f**) (0.01) and acetic anhydride (5 mL) was refluxed for 3 h on a water bath. It was cooled to room temperature, poured into ice cold water and the solid separated was recrystallized from the suitable solvent (**Scheme-I**).

1-(2-(3-Bromophenyl)-5-((4-(3-nitroimidazo[1,2-*b*]-pyridazin-6-yl)piperazin-1-yl)methyl)-1,3,4-oxadiazol-3(2*H*)-yl)ethan-1-one (4a): Yellow solid; Yield: 76%, m.p.: 185 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.054 (s, 3H, CH_3), 2.800-2.896 (m, 4H, piperazine ring), 3.422 (s, 2H, CH_2),

3.761-3.833 (m, 4H, piperazine ring), 7.124 (s, 1H, oxadiazole ring), 7.600-7.629 (m, 1H, phenyl ring), 7.877-7.897 (m, 2H, pyridazine ring and phenyl ring), 8.429 (m, 1H, pyridazine ring), 8.533-8.587 (m, 1H, phenyl ring), 8.734 (s, 1H, imidazole ring). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 25.73, 40.45, 52.14, 58.66, 71.24, 116.83, 119.59, 126.26, 127.24, 129.11, 131.34, 139.21, 142.50, 145.67, 152.27, 155.08, 157.88, 165.80, 170.65. MS *m/z*: 529.09 [M+H], (528.09); Anal. calcd. (found) % for $C_{21}H_{21}BrN_8O_4$: C, 47.65 (47.38); H, 4.00 (3.81); N, 21.17 (21.01).

1-(2-(1-Methyl-1*H*-imidazol-5-yl)-5-((4-(3-nitroimidazo[1,2-*b*]pyridazin-6-yl)piperazin-1-yl)methyl)-1,3,4-oxadiazol-3(2*H*)-yl)ethan-1-one (4b): White solid; Yield: 78%, m.p.: 195 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.954 (s, 3H, CH_3), 2.688-2.708 (m, 4H, piperazine ring), 3.184 (s, 2H, CH_2), 3.699-3.719 (m, 4H, piperazine ring), 3.895 (singlet, 3H, N-CH_3), 6.998 (s, 1H, oxadiazole ring), 7.086-7.106 (d, 1H, *J* = 10 Hz, pyridazine ring), 7.284 (s, 1H, imidazole ring), 7.848-7.868 (d, 1H, *J* = 10 Hz, pyridazine ring), 8.064 (s, 1H, imidazole ring), 8.407 (s, 1H, imidazole ring). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 25.48, 36.78, 45.07, 57.89, 59.23, 82.12, 115.96, 118.54, 126.62, 132.58, 134.41, 139.78, 141.21, 145.01, 154.38, 163.72, 171.28. MS *m/z*: 455.18 [M+H], (454.18); Anal. calcd. (found) % for $C_{19}H_{22}N_{10}O_4$: C, 50.22 (50.03); H, 4.88 (4.69); N, 30.82 (30.69).

1-(2-(2-Chloro-6-nitrophenyl)-5-((4-(3-nitroimidazo-[1,2-*b*]pyridazin-6-yl)piperazin-1-yl)methyl)-1,3,4-oxadiazol-3(2*H*)-yl)ethan-1-one (4c): White solid; Yield: 72%, m.p.: 194-196 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.991 (s, 3H, CH_3), 2.215-2.967 (m, 4H, piperazine ring), 3.562-3.821 (m, 4H, piperazine ring), 3.900 (s, 2H, CH_2), 7.011 (s, 1H, oxadiazole ring), 7.553-7.588 (d, 1H, *J* = 10.0 Hz, pyridazine ring), 7.768-7.786 (d, 1H, *J* = 8.8 Hz, pyridazine ring), 8.205-8.299 (m, 3H, phenyl ring), 8.384 (s, 1H, imidazole ring). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 22.39, 43.96, 52.84, 63.74, 79.42, 119.20, 123.54, 126.82, 128.99, 132.00, 133.29, 134.76, 138.48, 139.84, 145.83, 148.31, 152.90, 158.36, 163.14. MS *m/z*: 530.12 [M+H], (529.12); Anal. calcd. (found) % for $C_{21}H_{20}ClN_9O_6$: C, 47.60 (47.42); H, 3.80 (3.67); N, 23.79 (23.62).

1-(2-(2-Chlorophenyl)-5-((4-(3-nitroimidazo[1,2-*b*]-pyridazin-6-yl)piperazin-1-yl)methyl)-1,3,4-oxadiazol-3(2*H*)-yl)ethan-1-one (4d): Light yellow solid; Yield: 76%, m.p.: 188 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.054 (s, 3H, CH_3), 2.545-2.820 (m, 4H, piperazine ring), 3.625-3.900 (m, 4H, piperazine ring), 4.023 (s, 2H, CH_2), 7.015 (s, 1H, oxadiazole ring),7.205-7.385 (m, 4H, phenyl ring), 7.443-7.468 (d, 1H, *J* = 10 Hz, pyridazine ring), 7.962-8.987 (d, 1H, *J* = 10 Hz, pyridazine ring), 8.196 (s, 1H, imidazole ring). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 23.66, 45.01, 52.45, 58.06, 75.24, 118.00, 125.83, 126.23, 127.24, 129.18, 131.34, 139.22, 142.80, 144.05, 155.59, 158.77, 162.27, 165.80, 179.65. MS *m/z*: 485.14 [M+H], (484.14); Anal. calcd. (found) % for $C_{21}H_{21}ClN_8O_4$: C, 52.02 (51.89); H, 4.37 (4.22); N, 23.11 (22.95).

1-(2-(1*H*-Indol-3-yl)-5-((4-(3-nitroimidazo[1,2-*b*]-pyridazin-6-yl)piperazin-1-yl)methyl)-1,3,4-oxadiazol-3(2*H*)-yl)ethan-1-one (4e): Yellow solid; Yield: 74%, m.p.: 190 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.001 (s, 3H, CH_3), 2.811-2.886 (m, 4H, piperazine ring), 3.338 (s, 2H, CH_2), 3.768-3.784 (m, 4H, piperazine ring), 6.954 (s, 1H, oxadiazole ring), 7.214-7.234 (d, 1H, *J* = 10 Hz, pyridazine ring), 7.511-7.539 (m, 1H, indol ring), 7.876-7.896 (d, 1H, *J* = 10 Hz, pyridazine ring), 8.020-8.053 (m, 2H, indol ring), 8.278 (s, 1H, indole ring), 8.418 (s, 1H, indole ring), 8.793 (s, 1H, imidazole ring), 10.252 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 24.13, 44.21, 53.89, 59.91, 84.32, 109.75, 112.09, 117.65, 118.45, 119.01, 120.14, 122.52, 125.96, 132.41, 136.25, 138.08, 142.53, 146.74, 148.80, 154.68, 169.19. MS *m/z*: 490.19 [M+H], (489.19); Anal. calcd. (found) % for $C_{23}H_{23}N_9O_4$: C, 56.44 (56.28); H, 4.74 (4.51); N, 25.75 (25.58).

1-(2-Isopropyl-5-((4-(3-nitroimidazo[1,2-*b*]pyridazin-6-yl)piperazin-1-yl)methyl)-1,3,4-oxadiazol-3(2*H*)-yl)-ethan-1-one (4f): White solid; Yield: 75%, m.p.: 178 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.236 (s, 3H, CH_3), 2.683-2.798 (m, 6H, $2CH_3$), 3.254-3.387 (m, 9H, piperazine ring and -CH), 3.977 (s, 2H, CH_2), 6.823 (s, 1H, imidazole ring), 8.132-8.215 (m, 2H, pyridazine ring), 8.566 (s, 1H, imidazole ring). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 14.06, 27.03, 33.68, 45.40, 55.81, 58.01, 80.20, 116.67, 125.83, 132.21, 138.51, 154.03, 157.72, 162.66, 168.81. MS *m/z*: 417.19 [M+H], (416.19); Anal. calcd. (found) % for $C_{18}H_{24}N_8O_4$: C, 51.92 (51.74); H, 5.81 (5.65); N, 26.91 (26.78).

RESULTS AND DISCUSSION

3-Nitro-6-(piperazin-1-yl)imidazo[1,2-*b*]pyridazine was dissolved in dry DMF and cooled to 0 ºC. Ethyl bromo acetate was added dropwise and kept at room temperature for 16 h. The product was poured into saturated ammonium chloride solution and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulphate and evaporated to afford the crude product. The crude product was purified by silica gel column chromatography eluted with 2% methanol in chloroform to afford ethyl 2-(4-(3-nitroimidazo[1,2-*b*]pyridazin-6-yl)piperazin-1-yl)acetate (**1**) as light-yellow solid.

The formation of compound **1** has been confirmed by the appearance of signals corresponding to the five protons of the ethyl group of the carboxylate with a triplet at δ 1.207 ppm for CH_3 group and a quartet at around δ 4.108 ppm for -CH_2 group along with signals for eight protons of the piperazine ring, three protons of the imidazopyridazine ring and a singlet for CH_2 at δ 3.363 ppm in proton NMR. The ^{13}C NMR spectral data are consistent with the structure of compound **1**. The mass spectrum of the compound **1** shows a peak at *m/z* 335.2 corresponding to its M+H ion. In the infrared spectrum, an absorption band appeared at 1733.05 cm^{-1} corresponding to C=O stretching in acetate.

Formation of 2-(4-(3-nitroimidazo[1,2-*b*]pyridazin-6-yl)-piperazin-1-yl)acetohydrazide (**2**) was confirmed by the disappearance of signals corresponding to the ethyl group of the carboxylate in compound **1** and appearance of signals corresponding to the three protons of -NH and -NH_2 groups in compound **2** at δ 8.989 and 4.239 ppm, respectively along with signals for eight protons of the piperazine ring, three protons of the imidazopyridazine ring and a singlet for CH_2 at δ 3.0 ppm in proton NMR. The ^{13}C NMR spectral data are consistent with the structure of compound **2**. The mass spectrum of the compound **2** showed a peak at *m/z* 321.2 corresponding

to its M+H ion. In the infrared spectrum, an absorption band appeared at 3287.47 cm^{-1} corresponding to NH stretching in hydrazides.

Finally, the title compounds were synthesized using 2-(4-(3-Nitroimidazo[1,2-*b*]pyridazin-6-yl)piperazin-1-yl)-acetohydrazide (**2**) and aldehyde in ethanol. The formation of hydrazone derivatives has been confirmed by the disappearance of signals corresponding to the two -NH_2 protons in hydrazide and appearance of signals corresponding to =CH proton at around δ 8.0 ppm. The ^{1}H NMR, ^{13}C NMR and mass spectral data are found consistent with the structures assigned to the compounds.

Antimicrobial activity: *In vitro* activity of synthesized compounds were tested against *Escherichia coli* (1668), *Bacillus cereus* (1272), *Candida albicans* (854) by disc diffusion method and the zone of inhibition of the synthesized compounds are shown in Figs. 1 and 2. Most of the compounds exhibit significant antimicrobial activity against the tested microorganism

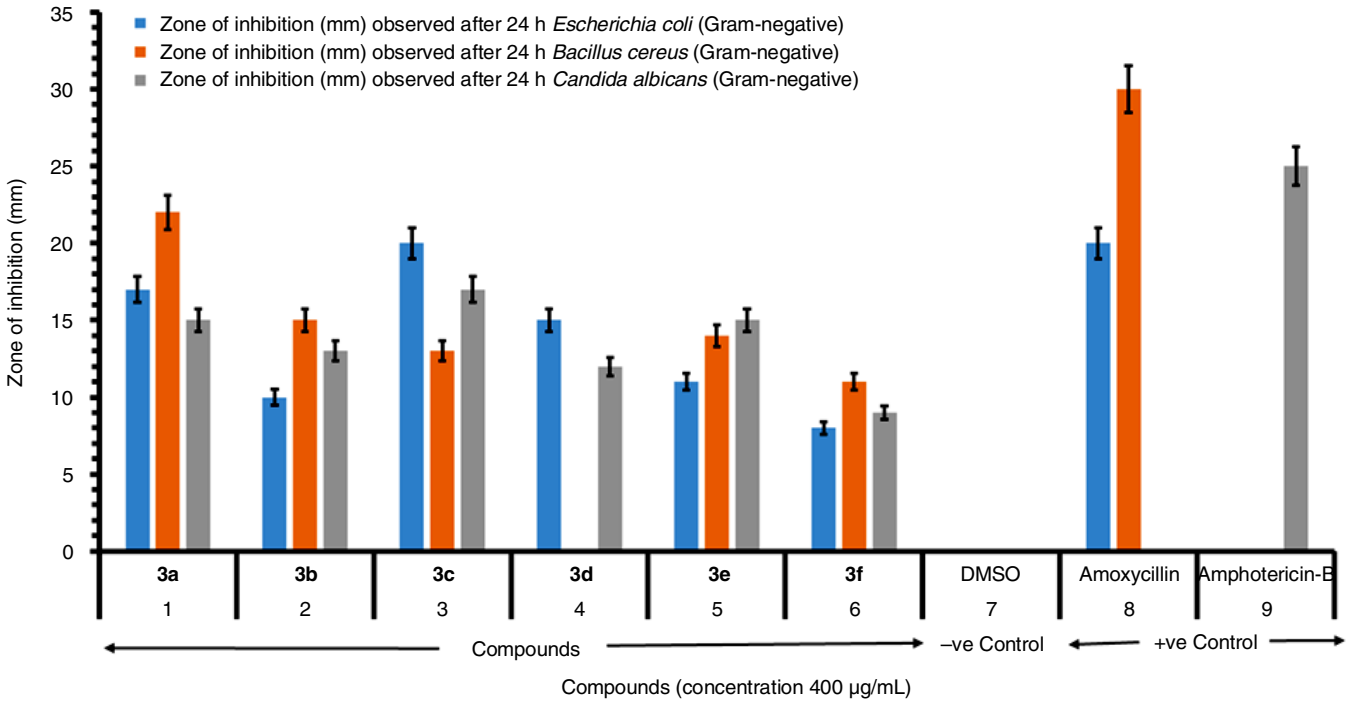


Fig. 1. Antimicrobial test results of *N′*-(substituted)-2-(4-(3-nitroimidazo[1,2-*b*] pyridazin-6-yl)piperazin-1-yl) acetohydrazide (**3a-f**)

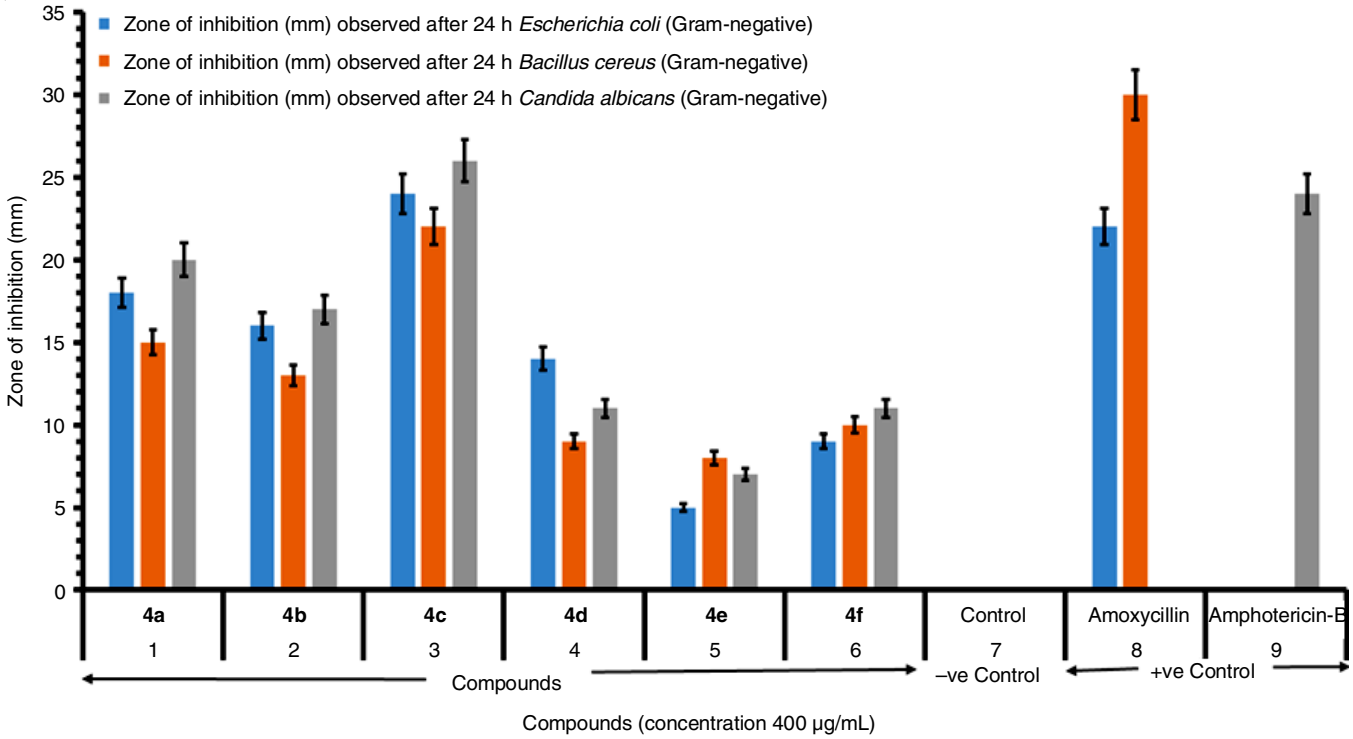


Fig. 2. Antimicrobial test results of 1-(2-(substituted)-5-((4-(3-nitroimidazo[1,2-*b*] pyridazin-6-yl) piperazin-1-yl)methyl)-1,3,4-oxadiazol-3(2*H*)-yl)ethan-1-one (**4a-f**)

and maximum zone of inhibition was observed with **4c**, **3c**, against *Escherichia coli*, **3a**, **4c** against *Bacillus cereus* and **4c**, **4a** against *Candida albicans*. Compared with base compound, synthesized compound **4c** has shown better results against test microorganisms. Among the tested compounds, **4c**, **3c** and **3a**, **4c** exhibits significant antimicrobial activity against Gram-negative and Gram-positive bacteria, respectively. Herein, compound **4c** exhibit better results against *Candida albicans* strains.

Molecular docking studies: All docking calculations were carried out using Auto Dock 4.2/ADT and the dlg files generated were analyzed for their binding conformations. Molecular docking analysis was performed to understand the molecular interaction between the all-synthesized compounds with BAX. Analysis was based on higher binding affinities/ lower docking scores and low inhibition constant values. Among the 12 derivatives of hydrazone (**3a, 3b, 3c, 3d, 3e, 3f, 4a, 4b, 4c, 4d, 4e** and **4f**) and 1,3,4-oxadiazole **4e, 4a, 3e** and **3a** showed highest binding energy (-8.0, -7.5, -7.0 and -6.9) with BAX protein (Fig. 3). All docked derivatives interacted by the same mode at the BAX protein binding site (Figs. 4 and 5). Compound **4e** shows three hydrogen bond interactions with

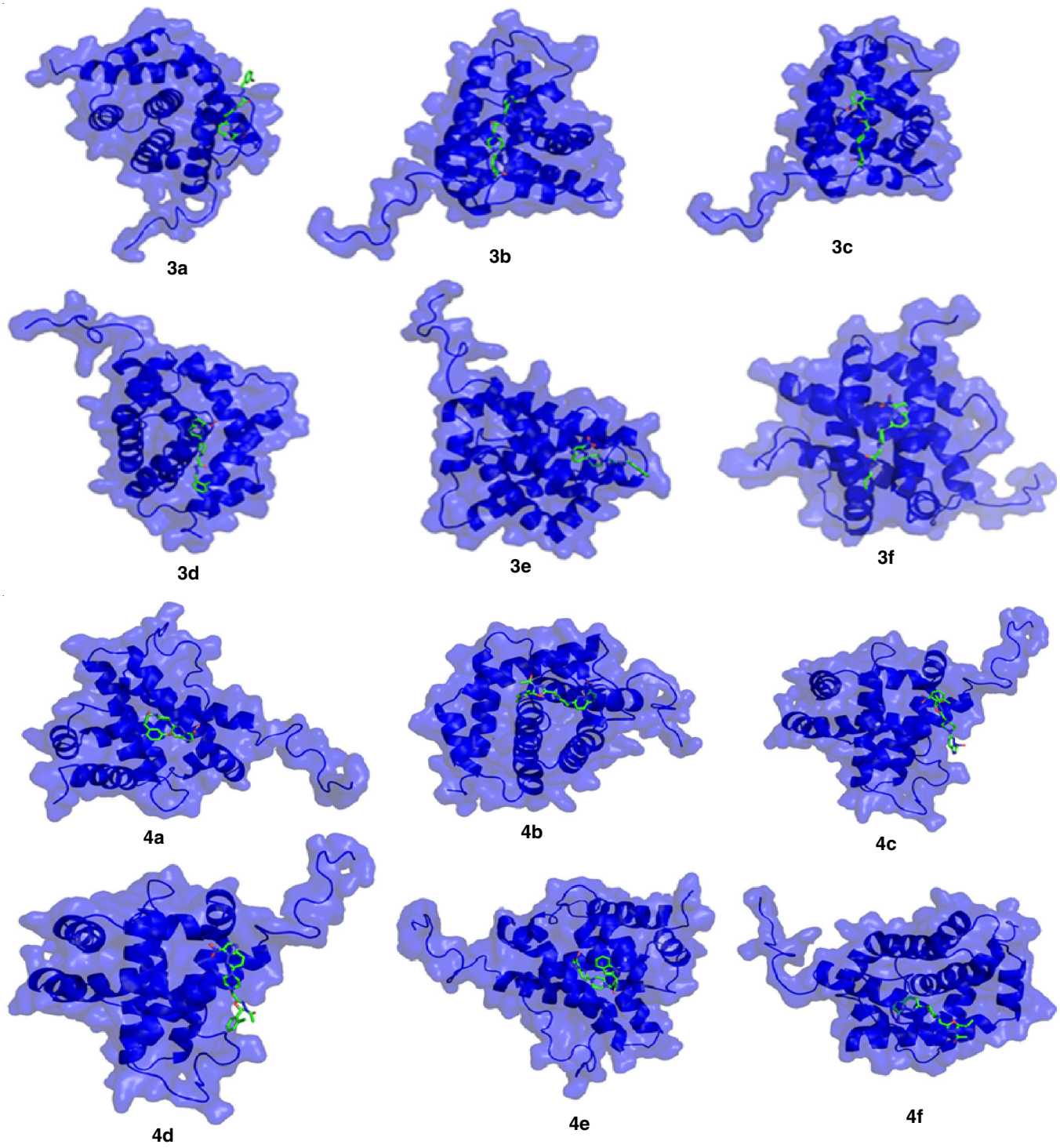


Fig. 3. 3D Confirmation for ligand binding pocket in BAX (PDB ID 1F16)

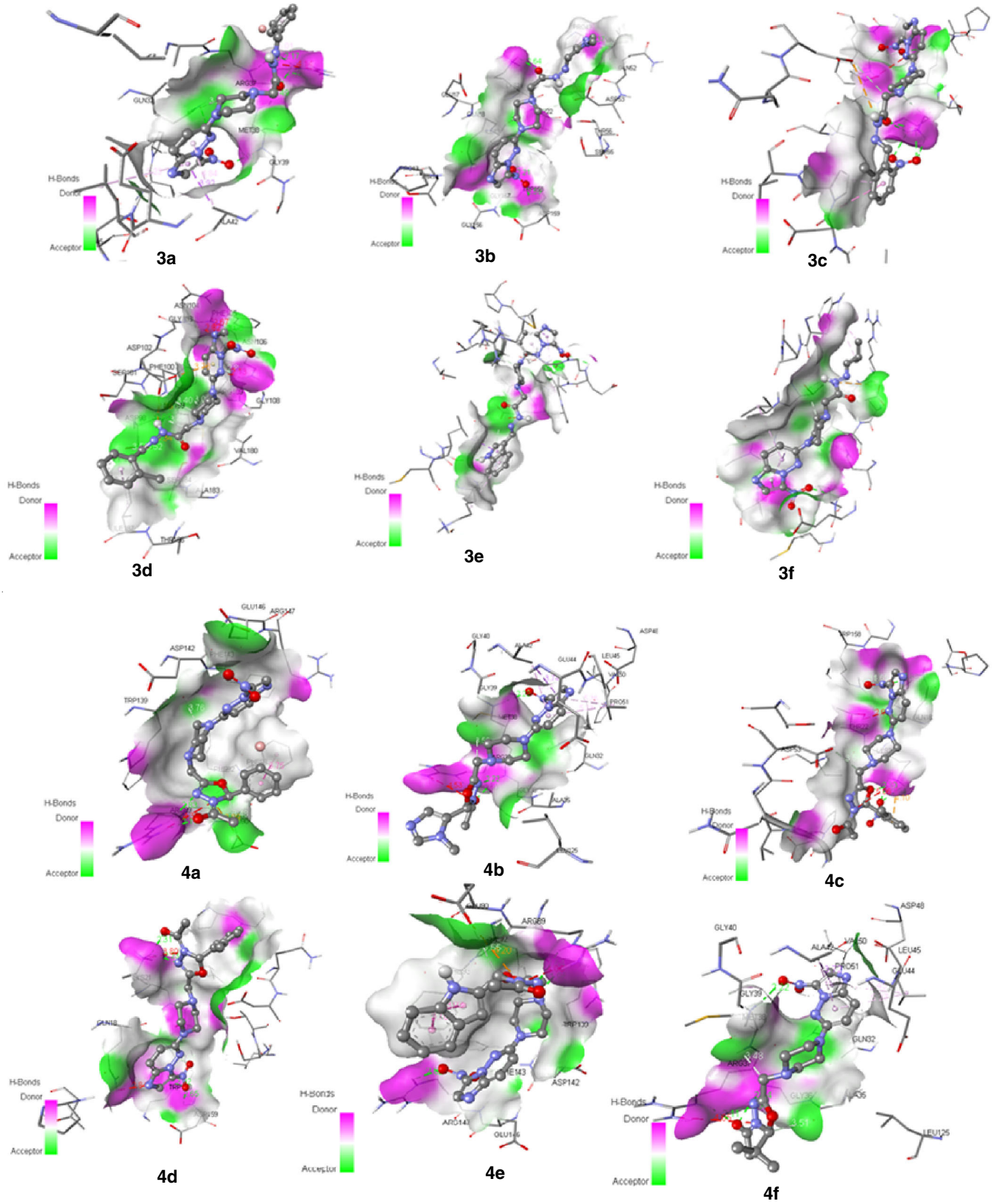


Fig. 4. 3D Protein - ligand interactions with respect to bond distance (≤ 4.0 Å) and amino acids interactions (pocket colour representation shows the acceptor and donor forms of binding region)

amino acids ARG89, ARG147 and PHE143; compound **4a** shows four hydrogen bonding interactions with amino acids MET38, LES45, ARG37 and GLY39; compound **3e** shows four hydrogen bond interactions with amino acids GLU41, LEU125, GLN32 and GLU44 and Compound **3a** shows four hydrogen bonding interactions with amino acids ARJ37, MET38, GLY39 and GLN32; for this reason it is concluded that greater the negative value of the binding energy better will

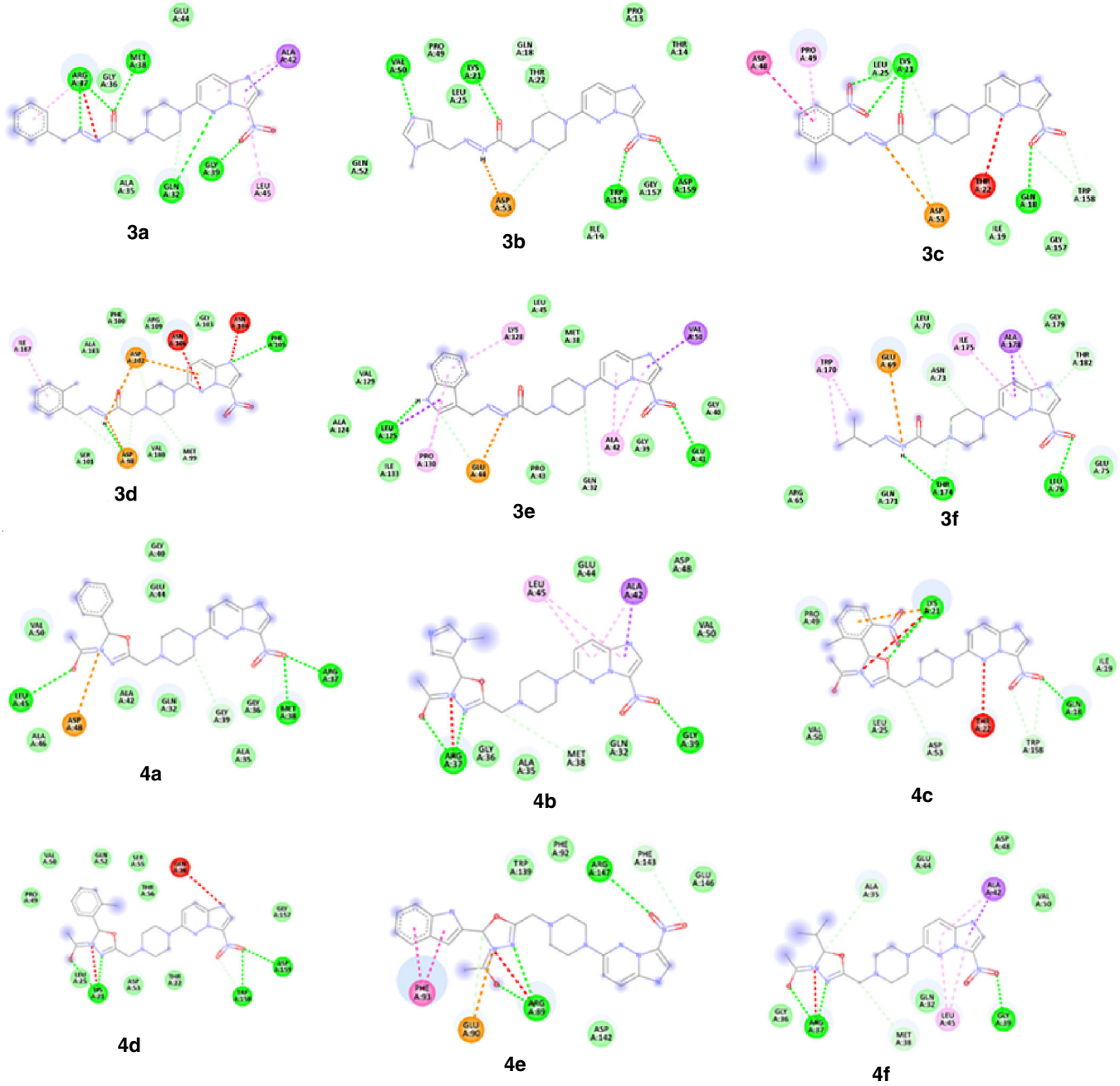


Fig. 5. 2D Protein - ligand interactions with respect to bond distance and amino acids interactions (pocket colour representation shows the acceptor and donor forms of binding region)

be the interaction towards target receptor of BAX protein as shown by compounds **4e** and **4a**.

Conclusion

The newly designed hydrazones and 1,3,4-oxadiazole compounds found to have good synthetic accessibility which indicates that these newly designed compounds can be easily synthesized in the laboratory. Majority of the compounds tested exhibit moderate to good antimicrobial activities. BAX protein interactions were done and the residues responsible for binding to the inhibitors of 1,3,4-oxadiazoles substrates with high binding affinity were identified. On the basis of the results obtained we conclude that these 1,3,4-oxadiazoles derivatives could be potential anticancer lead molecules for modulating the expression of BAX protein supports for experimental testing.

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