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Synthesis and molecular docking study of new 1,3-oxazole clubbed pyridyl-pyrazolines as anticancer and antimicrobial agents

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ABSTRACT

The present investigation is in the quest of some novel biologically potent heterocyclic compounds 1-aryl-3-(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-propenones **6(a-e)** and [5-aryl-3-(2-{(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)}-4,5-dihydro-1H-pyrazol-1-yl)]-(pyridin-4-yl)methanone **7(a-e)** incorporated with biologically active heterocyclic entities namely oxazole, pyrazoline and pyridine. The structures of all the compounds were elucidated using various spectroanalytical techniques such as FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. The synthesized compounds were studied for their anticancer activity at the National Cancer Institute (NCI, USA) against 60 cancer cell line panel. Data of anticancer activity study revealed that the compound **6**(**d**) has the highest potency. Furthermore, all the compounds were studied for their *in vitro* antibacterial and antifungal activities. As documented, all the prepared compounds performed well against these pathogenic strains. Moreover, data acquired from the molecular docking studies are very inspiring with respect to the potential utilization of these compounds to help overcome microbe resistance to pharmaceutical drugs.

1. Introduction

Regardless of substantial advancement made in the treatment of infectious diseases caused by various bacteria and fungi, it remains a foremost worldwide health issue due to rapid development of resistance against the existing antimicrobial drugs. Designing and developing novel antimicrobial agents with diverse modes of action than that of the present drug candidates is one of the major tasks to overcome the antimicrobial resistance. In view of these circumstances, it is necessary to design and develop more effective antimicrobial agents. Hence, the design, synthesis and discovery of more effective antimicrobial drugs have been intensively considered during the last decade. Various heterocyclic compounds comprising oxygen, nitrogen and/or sulfur as hetero atom(s) have been explored in this regard. Uncontrolled cell growth in cancer results in an invasion in surrounding tissues and in further dissemination to other parts of the body. According to the world cancer report released by WHO, in the upcoming years, the death rates due to cancer will increase two folds its present-day percentage [1]. Subsequently, discovery and development of new effective and selective drug candidates for the treatment of cancer have become a pressing need and hence search, design and development of new anticancer agents having heterocyclic core are intensively pursued worldwide at several research laboratories [2–7].

Chalcone or 1,3-diphenyl-2-propenone is a precursor in the biosynthesis of flavonoids and isoflavonoids and is an open chain intermediate in the synthesis of flavone [8,9]. The chemistry of chalcone family has attracted a greater interest not only from the synthetic and biosynthetic perspectives but also due to the wide range of biological activities they possess. The privileged scaffold chalcone has been an attraction among chemists due to its ease of synthesis, diversity of substituents they may possess and also among biologists due to a wide range of biological activities [10–16] they exhibit. Chalcone is a core unit in many biologically essential compounds from natural sources [17].

With an aspiration to develop new drug agents holding vibrant, versatile and advantageous properties, pyrazoline was found to be of interest among the researchers. Pyrazoline containing compounds extensively occur in nature in the form of alkaloids, vitamins, pigments and as constituents of animal and plant cells. A wide range of synthetic compounds containing pyrazoline moiety have been reported so far demonstrating a diverse biological activities [18–24]. Furthermore, the six member nitrogen heterocycle, pyridine is also an important heterocycle found in a large number of naturally occurring compounds. Pyridine based compounds are extensively used as industrial, pharmaceutical and agricultural

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chemicals. In addition to this, there are many reports on the compounds holding pyridine scaffold and having a wide range of biological activities [25–29].

Computational biology and bioinformatics play the most important role in developing the drug candidates. Molecular docking studies of the new candidates into the active site of receptor provides an essential information regarding drug receptor interactions which is frequently used to find out the orientation of drugs to their targets in order to predict the affinity and activity. Glucosamine-6-phosphate (GlcN-6-P) synthase has been proposed as one of the most potential molecular targets for antimicrobial therapy [30]. GlcN-6-P synthase catalyses the biosynthesis of D-glucosamine-6-phosphate, which is a precursor of various amino sugar-containing macromolecules, including chitin and mannoproteins in fungi, peptidoglycan and lipopolysaccharides in bacteria and glycoproteins in mammals [30]. Therefore, specific inhibitors of GlcN-6-P synthase activity may also be regarded as new therapeutic agent for type II diabetes [31].

Keeping the therapeutic significance of heterocyclic compounds in mind and in continuation of our research efforts on the synthesis of biologically potent molecules [32–35], herein we report the synthesis, antimicrobial and anticancer evaluation and molecular docking study of new 1,3-oxazole clubbed pyridyl-pyrazoline derivatives.

2. Experimental

2.1. General

The chemicals were used as received from the local companies without further purification. Solvents were purified by distillation prior to use. Column chromatography was carried out using silica gel (60–120 mesh). Thin layer chromatography was performed on the pre-coated silica gel 60 F_{254} aluminum sheets. Melting points were determined in open capillary and are uncorrected. Infrared spectra were recorded on Perkin Elmer FTIR spectrometer between 4000 to 400 cm^{-1} in solid state as KBr discs. The NMR spectra were recorded on 400 MHz Bruker Avance-III instrument and the chemical shifts are given in parts per million. In the NMR data for ^{19}F decoupled ^{1}H NMR experiments, the data for the affected signals only are included. ^{19}F chemical shift values are of ^{1}H decoupled ^{19}F signals. Mass spectra were recorded on Waters Xevo G2-XS QToF at the Zydus Research Center, Ahmedabad (India).

2.2. Synthesis of 2-(4-chlorophenyl)-5-methyl-1,3-oxazole-4-carbaldehyde, (4)

2-(4-Chlorophenyl)-5-methyl-1,3-oxazole-4-carbaldehyde **(4)** was prepared from 4-chlorobenzaldehyde and diacetyl monoxime as follows [36,37]. To an ice-cold mixture of 4–chlorobenzaldehyde **(1)** (142 mmol, 1 eq) and diacetyl monoxime (142 mmol, 1 eq) in acetic acid (30 ml, 3 fold), dry HCl gas was passed for 3 h at 0 °C. The reaction mixture was than diluted with diethyl ether (70 ml, 6 fold). Separated solid was filtered, washed with diethyl ether and dried under vacuum to obtain 2-(4-chlorophenyl)-4,5-dimethyl-1,3-oxazole-N-oxide **(2)** as a white solid. Yield = 71%. To an ice cold suspension of 2-(4-chlorophenyl)-4,5-dimethyl-1,3-oxazole-N-oxide **(2)** (45 mmol, 1 eq) in dichloro ethane (DCE) (35 ml, 5 fold) was added $POCl_3$ (49 mmol, 1.1 eq) dropwise over a period of 2 h at 10 °C. The reaction mixture was slowly heated to 60 °C and stirred at that temperature for 3 h [36]. The reaction mixture was cooled to room temperature, poured into ice cold water and extracted with DCE. The combined organic extracts were washed with water, dried over $CaCl_2$ and concentrated under vacuum to furnish 4-(chloromethyl)-2-(4-chlorophenyl)-5-methyl-1,3-oxazole **(3)** with an excellent yield of 74%. Homogeneous solution of 4-(chloromethyl)-2-(4-chlorophenyl)-5–methyl-1,3-oxazole **(3)** (10 mmol, 1eq) and bis-tetrabutyl ammonium dichromate [37] (6 mmol, 0.6 eq) in chloroform (7.5 ml) was heated under reflux for 3 h. The crude product was filtered through silica gel to eliminate the TBA salts. The silica was then washed with diethyl ether (100 ml). Evaporation of the combined organic layer afforded the desired 2-(4-chlorophenyl)-5–methyl-1,3-oxazole-4-carbaldehyde **(4)**. Yield: 1.32 g, 59%; white solid. IR (KBr) cm^{-1}: 2921, 2853, 1687, 1596, 1066, 829; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.73 (3H, s, $-CH_3$), 7.46 (2H, d, J = 6.8 Hz, Ar-H), 7.90 (2H, d, J = 6.8 Hz, Ar-H), 10.02 (1H, s, -CHO); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 11.8 ($-CH_3$), 124.8, 127.3, 127.8, 129.0, 129.2, 135.9, 137.4, 156.7, 159.5, 185.3 (>C=O). ESI-MASS: (m/z) 221.95 $(M + H)^+$ for $M = C_{11}H_8ClNO_2$.

2.3. General procedure for the synthesis of 1-aryl-3-(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)- propenones, 6(a-e)

To a magnetically stirred mixture of 4-substituted acetophenones **(5)** (0.01 mol) in ethanol (95%, 80 ml) and NaOH (0.012 mol) in10 ml water in a 250 ml round bottom flask, a solution of 2-(4-chlorophenyl)-5-methyl-1,3-oxazole-4-carbaldehyde **(4)** (0.01 mol) in 20 ml ethanol was added drop wise using addition funnel during 20–30 min at room temperature. The reaction mixture was heated in water bath at 45–50 °C and the reaction was continued for further 2 h to complete the reaction (TLC: 10% EA/Pet ether). The reaction mixture was then poured into ice cold water to precipitate the product as yellow solid, which was then filtered, dried and crystallized from ethanol. Yield: 76–81%.

2.3.1. 3-(2-(4-Chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-1-phenyl-propenone, 6(a)

Yield = 0.57 g, 79%; Light Yellow Solid; M.P. = 148 °C; IR (KBr) cm^{-1}: 3057, 1659, 1626, 1568, 1110, 738; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.56 (3H, s, $-CH_3$), 7.47 (2H, m, Ar-H), 7.54 (2H, d, J = 8 Hz, Ar-H), 7.61 (1H, m), 7.72 (1H, d, -CH=CH-, J = 14.8 Hz), 7.84 (1H, d, -CH=CH-, J = 14.8 Hz), 8.03 (2H, m, Ar-H), 8.13 (2H, t(b), Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 10.7 ($-CH_3$), 121.8, 125.4, 145.8, 128.6, 128.6, 128.1, 131.8, 132.9, 134.2, 136.7, 138.0, 151.5, 159.6, 189.9 (>C=O); Mass (TOF MS ES+): m/z 324.0 $(M + H)^+$.

2.3.2. 3-(2-(4-Chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-1-(4-methylphenyl)-propenone, 6(b)

Yield = 0.58 g, 76%; Light Yellow Solid; M.P. = 136 °C; IR (KBr) cm^{-1}: 3055, 1662, 1616, 1581, 1117, 739; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.45 (3H, s, $-CH_3$), 2.55 (3H, s, $-CH_3$), 7.32 (2H, d, J = 8 Hz, Ar-H), 7.46 (2H, t, Ar-H), 7.70 (1H, d, -CH=CH-, J = 15.2 Hz), 7.84 (1H, d, -CH=CH-, J = 15.2 Hz), 8.03 (4H, m, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 10.7 ($-CH_3$), 21.7 ($-CH_3$), 121.8, 125.4, 145.7, 128.7, 128.1, 147.4, 131.4, 134.2, 135.4, 136.7, 143.8, 151.3, 159.5, 189.4 (>C=O); Mass (TOF MS ES+): m/z 338.0 $(M + H)^+$.

2.3.3. 1-(4-Chlorophenyl)-3-(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-propenone, 6(c)

Yield = 0.64 g, 80%; Light Yellow Solid; M.P. = 152 °C; IR (KBr) cm^{-1}: 3045, 1652, 1618, 1571, 1017, 729; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.56 (3H, s, $-CH_3$), 7.46–7.50 (4H, m, Ar-H), 7.71 (1H, d, -CH=CH-, J = 15.2 Hz), 7.78 (1H, d, -CH=CH-, J = 15.2 Hz), 8.05 (4H, m, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 10.7 ($-CH_3$), 121.1, 125.3, 145.7, 128.7, 128.9, 128.1, 130.0, 132.3, 134.1, 136.3, 136.8, 139.3, 151.8, 159.6, 188.5 (>C=O); Mass (TOF MS ES+): m/z 358.0 $(M + H)^+$.

2.3.4. 1-(4-Bromophenyl)-3-(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-propenone, 6(d)

Yield = 0.69 g, 76%; Light Yellow Solid; M.P. = 146 °C; IR (KBr) cm^{-1}: 3050, 1660, 1610, 1580, 1127, 739; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.57 (3H, s, -CH_3), 7.47 (2H, m, Ar-H), 7.67 (2H, m, Ar-H), 7.72 (1H, d, -CH=CH-, J = 14.8 Hz), 7.78 (1H, d, -CH=CH-, J = 14.8 Hz), 7.99 (2H, m, Ar-H), 8.04 (2H, m, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 10.8 (-CH3), 121.1, 125.3, 145.7, 128.0, 128.2, 130.1, 131.9, 132.3, 134.1, 136.7, 136.8, 151.8, 159.6, 188.7 (>C=O); Mass (TOF MS ES+): m/z 403.7 $(M + H)^+$.

2.3.5. 3-(2-(4-Chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-1-(4-fluorophenyl)-propenone, 6(e)

Yield = 0.62 g, 81%; Light Yellow Solid; M.P. = 160 °C; IR (KBr) cm^{-1}: 3025, 1642, 1636, 1561, 1017, 730; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.56 (3H, s, -CH3), 7.19 (2H, m, Ar-H), 7.47 (2H, dd, J = 6.8 Hz), 7.70 (1H, d, -CH=CH-, J = 14.8 Hz), 7.80 (1H, d, -CH=CH-, J = 14.8 Hz), 8.03 (2H, d, J = 6.8 Hz, Ar-H), 8.16 (2H, m, Ar-H); ^{19}F NMR (376 MHz, $CDCl_3$, δ ppm) : −105; ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 10.7 (-CH_3), 115.7 (d, $^2J_{CF}$ = 22 Hz), 121.3, 125.3, 125.7, 128.1, 131.1 (d, $^3J_{CF}$ = 9 Hz), 132.0, 134.1, 134.3 (d, $^4J_{CF}$ = 3 Hz), 136.8, 151.6, 159.6, 165.6 (d, $^1J_{CF}$ = 250 Hz), 188.2 (>C=O); Mass (TOF MS ES+): m/z 342.08 $(M + H)^+$.

2.4. General procedure for the synthesis of [5-aryl-3-(2-{(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl}-4,5-dihydro-1H-pyrazol-1-yl)] (pyridin-4-yl)methanone, 7(a-e)

An equimolar quantities of 1-aryl-3-(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-propenones **6** (**a-e**) (1.47 mmol) and isoniazid (1.47 mmol) were heated at 80–90 °C in glacial acetic acid (15 mL) for 10–12 h [20]. After the completion of the reaction (TLC), the reaction mixture was cooled, poured on to crushed ice and neutralized with dilute ammonia solution. Solid so obtained was filtered, washed and subjected to column chromatography to furnish the corresponding compounds **7** (**a-e**). Yield: 70–75%.

2.4.1. [(3-{(2-(4-Chlorophenyl)-5-methyl-1,3-oxazol-4-yl)}-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)](pyridin-4-yl)methanone, 7(a)

Yield = 0.47 g, 73%; Light Yellow Solid; M.P. = 142 °C; IR (KBr) cm^{-1}: 2924, 1636, 1445, 1091, 835, 755; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.59 (3H, s, -CH3), 3.69 (2H, dd, ^1J = 6.4, Hz, ^2J = 10.8), 5.74 (1H, dd, ^1J = ^2J = 10.8 Hz), 7.37 (2H, d, J = 6.8 Hz, Ar-H), 7.47 (3H, m, Ar-H), 7.76 (2H, m, Ar-H), 7.83 (2H, d, J = 6.8 Hz, Ar-H),7.88 (2H, d, J = 6.8 Hz, Ar-H), 8.73 (2H, d, J = 6.0 Hz, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 10.6 (-CH_3), 37.9 (-CH_2-), 53.1 (-CH-),123.8, 125.9, 126.8, 127.4, 128.1, 128.7, 128.8, 128.9, 130.7, 130.9, 133.5, 136.0, 141.9, 146.9, 149.3, 156.6, 159.0, 164.6 (>C=O); Mass (TOF MS ES+): m/z 443.13 $(M + H)^+$.

2.4.2. [(3-{(2-(4-Chlorophenyl)-5-methyl-1,3-oxazol-4-yl)}-5-(4-methylphenyl)-4,5-dihydro-1H-pyrazol-1-yl)](pyridin-4-yl)methanone, 7(b)

Yield = 0.50 g, 74%; Light Yellow Solid; M.P. = 148 °C; IR (KBr) cm^{-1}: 2937, 1640, 1430, 1090, 837, 745; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.42 (3H, s, -CH_3), 2.59 (3H, s, -CH_3), 3.67 (2H, dd, ^1J = 6.4, Hz, ^2J = 10.8), 5.72 (1H, dd, ^1J = ^2J = 10.8 Hz), 7.26 (2H, d(b), J = 8 Hz, Ar-H), 7.36 (2H, m, Ar-H), 7.65 (2H, d(b), J = 8.4 Hz, Ar-H), 7.87 (4H, m, Ar-H), 8.73 (2H, d, J = 5.2 Hz, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 10.6 (-CH_3), 21.6 (-CH_3), 38.0 (-CH_2-), 53.0 (-CH-),124.0, 125.9, 126.9, 127.4, 128.1, 128.7, 128.9, 129.5, 133.6, 136.0, 141.2, 142.3, 146.8, 148.9, 156.8, 159.0, 164.3 (>C=O); Mass (TOF MS ES+): m/z 457.15 $(M)^+$.

2.4.3. [(5-(4-Chlorophenyl)-3-{(2-(4-chlorophenyl)-5-methyloxazol-4-yl)}-4,5-dihydro-1H-pyrazol-1-yl)](pyridin-4-yl)methanone, 7(c)

Yield = 0.52 g, 75%; Light Yellow Solid; M.P. = 154 °C; IR (KBr) cm^{-1}: 2925, 1645, 1447, 1120, 833, 742; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.6 (3H, s, -CH_3), 3.66 (2H, dd, ^1J = 6.4, Hz, ^2J = 10.8), 5.74 (1H, dd, ^1J = ^2J = 10.8 Hz), 7.37 (2H, m, Ar-H), 7.43 (2H, m, Ar-H), 7.69 (2H, m, Ar-H), 7.79 (2H, m, Ar-H), 7.89 (2H, m, Ar-H), 8.73 (2H, d, J = 6 Hz, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 10.6 (-CH_3), 37.8 (-CH_2-), 53.2 (-CH-), 123.6, 125.9, 126.9, 127.4, 128.2, 128.9, 129.1, 129.5, 133.4, 136.0, 136.7, 141.6, 147.0, 149.6, 155.4, 159.1, 164.7 (>C = O); Mass (TOF MS ES+): m/z 476.8 $(M + H)^+$.

2.4.4. [(5-(4-Bromophenyl)-3-{(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)}-4,5-dihydro-1H-pyrazol-1-yl)](pyridin-4-yl)methanone, 7(d)

Yield = 0.54 g, 71%; Light Yellow Solid; M.P. = 150 °C; IR (KBr) cm^{-1}: 2926, 1645, 1428, 1090, 830, 744; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.6 (3H, s, -CH_3), 3.66 (2H, dd, ^1J = 6.4, Hz, ^2J = 10.8), 5.74 (1H, dd, ^1J = ^2J = 10.8 Hz), 7.37 (2H, d, J = 6.8 Hz, Ar-H), 7.61 (4H, m, Ar-H), 7.79 (2H, dd, J = 4.8 Hz, Ar-H), 7.88 (2H, d, J = 6.8 Hz, Ar-H), 8.74 (2H, d, J = 6.0 Hz, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 10.6 (-CH_3), 37.7 (-CH_2-), 53.2 (-CH-), 123.6, 125.1, 125.9, 127.4, 128.4, 128.9, 129.9, 132.0, 133.4, 136.0, 141.6, 147.0, 149.6, 155.5, 159.1, 164.7 (>C=O); Mass (TOF MS ES+): m/z 522.74 $(M + H)^+$.

2.4.5. [(3-{(2-(4-Chlorophenyl)-5-methyl-1,3-oxazol-4-yl)}-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)](pyridin-4-yl)methanone, 7(e)

Yield = 0.49 g, 72%; Light Yellow Solid; M.P. = 144 °C; IR (KBr) cm^{-1}: 2945, 1635, 1433, 1091, 830, 782; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.5 (3H, s, -CH3), 3.64 (2H, dd, ^1J = 6.4, Hz, ^2J = 10.8), 5.74 (1H, dd, ^1J = ^2J = 10.8 Hz), 7.15 (2H, m, Ar-H), 7.37 (2H, m, Ar-H), 7.76 (4H, m, Ar-H), 7.88 (2H, d, J = 6.8 Hz, Ar-H), 8.73 (2H, d, J = 6.0 Hz, Ar-H); ^{19}F NMR (376 MHz, $CDCl_3$, δ ppm) : −108; ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 10.5 (-CH_3), 37.9 (-CH_2-), 53.2 (-CH-), 115.8, 116.0, 123.6, 125.9, 127.4, 128.9, 129.0, 133.5, 136.0, 141.7, 146.9, 149.6, 155.4, 159.0, 164.7 (>C=O); Mass (TOF MS ES+): m/z 460.85 $(M)^+$.

2.5. Anticancer screening: methodology

Anticancer screening of the compounds **6**(**a-e**) and **7**(**a-e**) was carried out by the standard procedure followed by NCI (USA) for screening (http://dtp.nci.nih.gov). The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fatal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well µL plates in 100 µL at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line are fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing

50 µg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 µl of these different drug dilutions are added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations.

Following the drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed *in situ* by the gentle addition of 50 µl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

[(Ti - Tz)/(C - Tz)] x 100 for concentrations for which Ti>/=Tz
[(Ti - Tz) / Tz] x 100 for concentrations for which Ti<Tz

2.6. Antimicrobial activity

The synthesized quinolyl hydrazones were evaluated for antimicrobial activity against four bacterial and two fungal species using paper disk diffusion technique [38]. The Mueller-Hinton agar medium was sterilized (autoclaved at 120 °C for 30 min), poured at uniform depth of 5 mm and was allowed to solidify. The microbial suspension (105 CFU/mL; 0.5 McFarland Nephelometery Standards) was streaked over the surface of the medium using a sterile cotton swab to ensure even growth of the organisms. The test compounds were dissolved in dimethyl sulfoxide to give solutions of 3.12–125 μg/mL. Sterile filter paper discs measuring 6.25 mm in diameter (Whatman no. 1 filter paper), previously soaked in a known concentration of the respective test compound in dimethyl sulfoxide were placed on the solidified nutrient agar medium that had been inoculated with the respective microorganisms and the plates were incubated for 24 h at (37 ± 1) °C. A control disk impregnated with an equivalent amount of dimethyl sulfoxide without any sample was also used and did not produce any inhibition. Ciprofloxacin and Gresiofulvin (100 μg/disk) were used as control drugs for antibacterial and antifungal activity, respectively.

MIC of the test compound was determined by the agar streak dilution method [39]. A stock solution of a newly prepared compound (100 μg/mL) in dimethyl sulfoxide was prepared and classified amounts of the test compound were incorporated in a specified quantity of molten sterile agar that is nutrient agar for evaluation of antibacterial and sabouraud dextrose agar for antifungal activity, respectively. The medium containing the test compound was poured into a petri dish at a depth of 4–5 mm and allowed to solidify under sterile conditions. A suspension of the respective microorganism of nearly 105 CFU/mL was prepared and applied to plates with successively diluted compounds with concentrations in the range of 3.12–125 μg/mL in dimethyl sulfoxide and were incubated at (37 ± 1) °C for 24 h or 48 h. The lowest concentration of the substance that prevents the development of visible growth is considered to be the MIC value.

2.7. Molecular docking studies

All the synthesized molecules were screened against 2VF5 using CLC Drug Discovery Work Bench 3.0. All the molecules sketched using ChemDraw ultra 12, molecules in CDX format have been converted to .mol files using Open babel. Molecules were in .mol format having been imported into project tree, converted to 3D molecules by Balloon added on for CLC (://users.abo.fi/mivainio/balloon/index.php). All the ligands and proteins were optimized using Ligand and protein optimizer protocol of Qiagen CLC Drug Discovery Workbench 3. The docked ligands poses are in similarity to the binding pose of co-crystallized ligand with a root mean square deviation of 0.022_A.

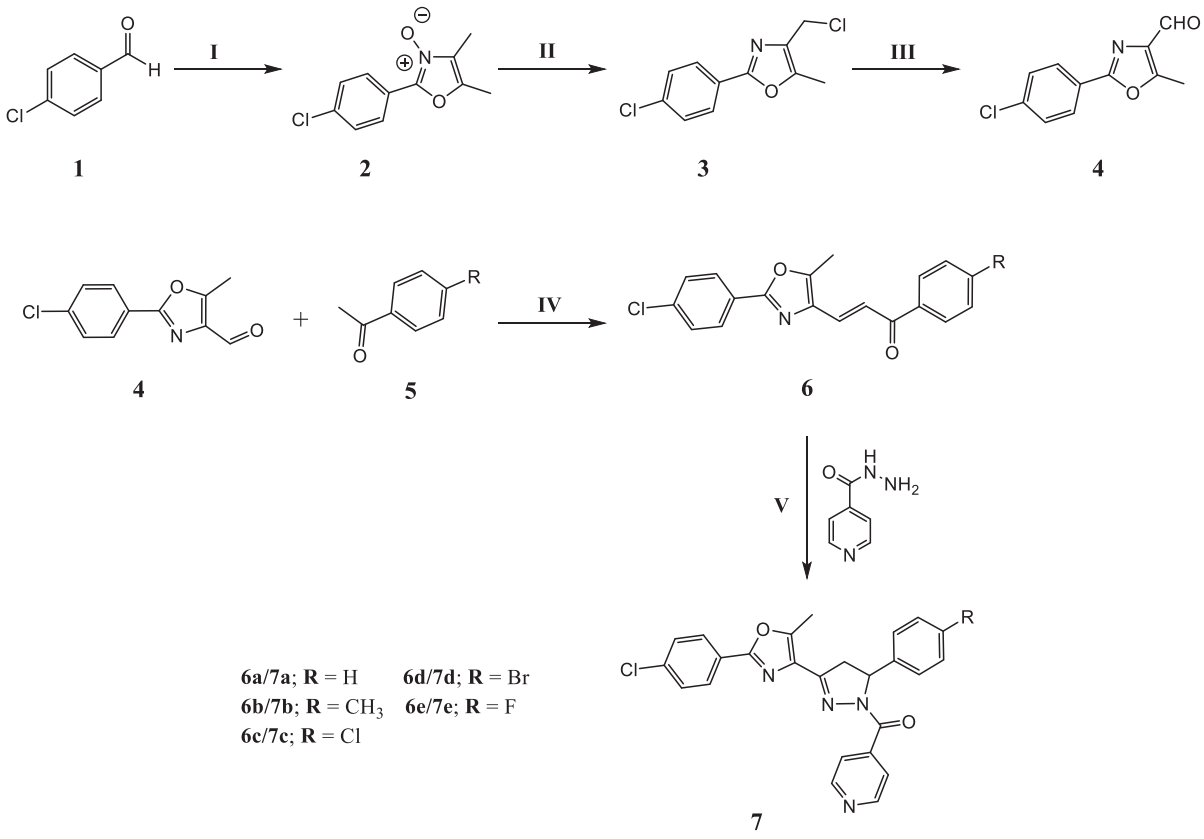
3. Results and discussion

3.1. Chemistry

Synthesis of the target molecules began with the preparation of 2-aryl-4-formyl-5-methyl-1,3-oxazole (**4**) (**Scheme 1**). Thus 4-chlorobenzaldehyde (**1**), was reacted with diaceyl monoxime in glacial acetic acid by passing dry HCl gas for 3 h at 0 °C to give 4-(chloromethyl)-2-(4-chlorophenyl)-5–methyloxazole-3-oxide (**2**) as a white solid which was converted to 4-(chloromethyl)-2-(4-chlorophenyl)-5–methyl-1,3-oxazole (**3**) on heating with $POCl_3$ in dichloroethane at 60 °C for 3 h [36]. 2-(4-Chlorophenyl)-5–methyl-1,3-oxazole-4-carbaldehyde (**4**) was prepared by oxidation of 4-(chloromethyl)-2-(4-chlorophenyl)-5–methyl-1,3-oxazole (**3**) with bis-TBAC (Bis-Tetrabutylammonium dichromate) in chloroform [37] (**Scheme 1**). For the synthesis of 1-aryl-3-(2-(4-chlorophenyl)-5–methyl-1,3-oxazol-4-yl)-propenones (**6**) the Claisen-Schmidt reaction between 2-(4-chlorophenyl)-5–methyl-1,3-oxazole-4-carbaldehyde (**4**) and various 4-substituted acetophenones **5(a-e)** was carried out in ethanol using aqueous alkali at 45–50 °C to afford the heteroaryl chalcones **6(a-e**) in good yields (**Scheme 1**). Further, employing the 3-(2-(4-chlorophenyl)−1,3-oxazol-4-yl-(1-aryl) propenones **6(a-e),** five new [5-aryl-3-{(2-(4-chlorophenyl)-5–methyl-1,3-oxazol-4-yl)}-4,5-dihydro-1*H*-pyrazol-1-yl)] (pyridin-4-yl)methanones **7(a-e)** were prepared [20] by reacting them with isoniazid in glacial acetic acid to afford the target molecules in good yields (**Scheme 1**). The summary of yields, melting points and molecular composition of the newly prepared compounds is presented in **Table 1**.

In the IR spectra of 3-(2-(4-chlorophenyl)−1-aryl-propenones **6(a-e),** aromatic C–H stretching vibrations are observed at ~3055 cm^{-1}. A characteristic band is observed at ~1670–1660 cm^{-1} for *>C=O* stretching as it is a part of extended conjugation. The aromatic *C=C* stretching bands are observed in the range of 1620–1480 cm^{-1} with variable intensity. In the IR spectra of (oxazolyl-pyrazolyl)-(pyridinyl)-methanones **7(a-e),** C–H stretching is observed at ~3055 cm^{-1}. The IR band for the carbonyl *>C=O* stretching for N-acyl group in pyrazolines **7(a-e)** was observed at ~1640 cm^{-1}.

Proton NMR spectra of 3-(2-(4-chlorophenyl)−1-aryl-propenones **6(a-e)** show a singlet at δ 2.5 ppm due to the CH_3 group protons on the oxazole ring. The ethylene protons (-CH=CH-) of the double bond appear at δ 7.72 ppm and 7.84 ppm as doublets with vicinal coupling constant J = 15 Hz due to *trans* stereochemistry. In the proton NMR spectra of (oxazolyl-pyrazolyl)-(pyridinyl)-methanones **7(a-e)** the CH_3 protons are observed at δ 2.59 ppm. The -CH_2 protons were observed at δ 3.68–3.69 ppm as a doublet of doublet with germinal coupling constant ^1J = 6.4 Hz and vicinal coupling constant ^2J = 10.8 Hz as both the diastereotopic protons couple with the neighbouring proton present in the pyrazole ring. The -CH proton is observed



Reagents and conditions: I) Diacetyl monoxime, AcOH, HCl (g); II) $POCl_3$, 1,2-dichloroethane; III) Bis-TBAC, $CHCl_3$, 3h; IV) 5% aqueous NaOH ethanol, 45-50 °C, 2h; V) Gla. AcOH, 80-90 °C, 10-12h.

Scheme 1. Synthesis of 3-(2-(4-chlorophenyl)−1,3-oxazol-4-yl)−1-aryl-propenones **6(a-e)** and oxazolyl-pyrazolyl-pyridinyl-methanones **7(a-e).**

Table 1
Yields, mp, and composition of the newly synthesized compounds.

ID	Substitution (**R**)	NSC code	Molecular Formula	Isolated Yield	Mp
6a	-H	D-806,539 / 1	$C_{19}H_{14}ClNO_2$	79%	148 °C
6b	-CH_3	D-806,536 / 1	$C_{20}H_{16}ClNO_2$	76%	136 °C
6c	-Cl	D-806,537 / 1	$C_{19}H_{13}Cl_2NO_2$	80%	152 °C
6d	-Br	D-806,535 / 1	$C_{19}H_{13}ClBrNO_2$	76%	147 °C
6e	-F	D-806,538 / 1	$C_{19}H_{13}ClFNO_2$	81%	160 °C
7a	-H	D-806,541 / 1	$C_{25}H_{19}ClN_4O_2$	73%	142 °C
7b	-CH_3	D-806,533 / 1	$C_{26}H_{21}ClN_4O_2$	75%	148 °C
7c	-Cl	D-806,534 / 1	$C_{25}H_{18}Cl_2N_4O_2$	74%	154 °C
7d	-Br	D-806,532 / 1	$C_{25}H_{18}ClBrN_4O_2$	71%	150 °C
7e	-F	D-806,540 / 1	$C_{25}H_{18}ClFN_4O_2$	72%	144 °C

at δ 5.74 ppm as a doublet of doublet with ^2J = 10.8 Hz as it couples differently with the diastereotopic vicinal protons. The ^{13}C NMR of 3-(2-(4-chlorophenyl)−1-aryl-propenones **6(a-e),** show the methyl carbon at δ 10.7 ppm. The Carbonyl carbon (>*C*=*O*) of all five compounds appear at δ ~189 ppm. All the other sp^2 carbons appear in between δ 120–160 ppm. In ^{13}C NMR of (oxazolyl-pyrazolyl)-(pyridinyl)-methanones **7(a-e),** methyl carbon is observed at δ 10.6 ppm and the carbonyl carbon (>*C*=*O*) is observed at slightly lower δ value (upfield) at δ 164.7 ppm.

3.2. Biological evaluation

3.2.1. in vitro *anticancer activity*

The anticancer activity screening of all the newly prepared compounds **6(a-e)** and **7(a-e)** was carried out under the screening project at the National Cancer Institute (NCI), USA. All the twelve submitted compounds were selected and were evaluated for primary *in vitro* one dose (10 μm) anticancer screening against the full NCI 60 cell line panels representing nine different kinds of cancer namely leukaemia, melanoma, lung, colon, brain, breast, ovary, kidney and prostate cancer following the standard protocol of the NCI, USA (http://dtp.nci.nih.gov). Results of each compound were reported as a mean graph of the percent growth of the treated cells when compared to the untreated control cells which gives both inhibition values (between 0 and 100) and cytotoxicity values (less than 0). The single dose screening results of all the ten molecules against sixty cancer cell lines were analyzed by COMPARE program (Results are included in supporting information). Average growth values of the dose-dependent antitumor activity against these cancer cell lines are as presented in **Table 2.**

The anticancer activity study of 3-(2-(4-chlorophenyl)−1-aryl-propenones **6(a-e)** showed noteworthy results against some of the

Table 2
Growth percentage of the tumor cell lines *in vitro* at 10^{-5} M concentration.

Comp.	Growth range (%)	The most sensitive cell line growth (%)
6a	49.88–114.84	79.41 (OVCAR-4/o), 49.88 (MCF7/b), 79.48 (T-47D/b)
6b	64.91–107.38	64.91 (MCF7/b), 73.88 (T-47D/b), 79.41 (OVCAR-4/o),
6c	63.05–131.05	63.05 (MCF7/b), 67.24 (T-47D/b), 65.99 (OVCAR-4/o), 72.23 (RPMI-8226/l)
6d	40.17–133.15	40.17 (MCF7/b), 47.60 (T-47D/b), 47.68 (OVCAR-4/o), 51.99 (SW-620/c), 49.41 (RPMI-8226/l), 55.29 (SR/l), 73.19 (SNB-19/c), 70.42 (PC-3/p), 69.96 (UO-31/r)
6e	75.22–111.72	75.22 (OVCAR-4/o), 78.66 (T-47D/b)
7a	59.52–114.05	59.52 (UO-31/r), 71.27 (IGROV1/o), 77.13 (MOLT-4/l), 76.74 (MDA-MB-231/ATCC/b), 72.93 (UACC-62/m)
7b	58.18–107.05	58.18 (UO-31/r), 64.67 (MOLT-4/l), 71.57 (MCF7/b), 73.19 (PC-3/p), 70.70 (IGROV1/o), 72.28 (UACC-62/m)
7c	60.13–107.74	60.13 (RXF-393/r), 63.80 (UO-31/r), 63.33 (SF-539/c), 64.96 (MDA-MB-231/ATCC/b), 75.82 (ACHN/r), 73.67 (CAKI-1/r)
7d	58.32–108.83	58.32 (A498/r), 60.35 (UO-31/r), 62.70 (RXF-393/r), 73.98 (HOP-92/n), 74.44 (HS 578T/b)
7e	54.12–119.14	54.12 (RXF-393/r), 60.86 (UO-31/r), 65.99 (MDA-MB-231/ATCC/b), 72.46 (A498/r)

l = leukemia, *s* = non-small-cell lung cancer, *c* = colon cancer, *n* = central nervous system cancer, *m* = melanoma, *o* = ovarian cancer, *r* = renal cancer, *p* = prostate cancer, *b* = breast cancer.

cancerous cell lines as shown in Table 2. Compound **6(a), 6(b), 6(c)** and **6(d)** showed 50%, 35%, 37% and 60% inhibition against MCF7 (breast cancer) cell line respectively. Compound **6(c)** also inhibited T-47D (breast cancer) and OVCAR-4 (ovarian cancer) to 32% and 34% respectively. The chalcone with fluoro substitution showed poor inhibition compared to the other chalcones. This indicates that inductive effect of the fluoro substitution has no significant effect on the anticancer activity of the new compounds. Compound **6(d)** showed highest potency among all the synthesized compounds with inhibition of 52% T47D (breast cancer), 55% against Ovarian cancer OVCAR-4 (renal cancer) cell line 50% against RPMI-8226 (Leukemia) cell line and 48% inhibition against SW-620 (colon cancer) cell line. The sixty human tumor cell line anticancer screening data at single dose assay (10-5- M concentration) of compound **6(d)** is shown in Fig. 1. The heteroaryl chalcone with methyl substitution did not show significant enhancement in the anticancer activity. The anticancer activity study of oxazolyl-pyrazolyl-pyridinyl-methanones **7(a-e)** showed moderate to good activity with the inhibition of several cancer cell lines as presented in Table 2. Compound **7(a)** (*R* = *H*) and **7(b)** displayed 40% and 41% inhibition against renal cancer UO-310 (renal cancer) cell line respectively. Compounds **7(c)** and **7(d)** showed highest inhibition against RXF-393 (40%) and 58.32 A498 (42%) respectively among the renal cancer cell lines. Compound **7(d)** also showed about 40% inhibition on UO-31 and 38% inhibition against RXF-393 of renal cancer cell lines correspondingly. From the anticancer results, it is observed that compounds with electron withdrawing substituents (*R* = Cl, Br) displayed better activity with greater inhibition of cancer cell lines (See supporting information).

3.2.2. Antimicrobial activity

All the newly synthesized compounds **6(a-e)** and **7(a-e**) were screened for antibacterial activity against (*S. aureus* (MTCC 96) and *B. subtilis* (MTCC 619) as Gram positive bacteria and *E. coli* (MTCC 739) and *P. aeruginosa* (MTCC 741) as Gram negative bacteria. The study of antifungal activity was carried out against *A. niger* (MTCC 282) and *C. albicans* (MTCC 183). The activity of the synthesized compounds was compared with ciprofloxacin and griseofulvin, effective antibiotics taken as a standard reference compound. The detailed experimental procedure is included in the supplementary material. Results of the antibacterial and antifungal activities are summarized in Table 3.

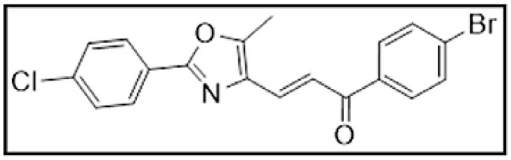
Among all the newly synthesized compounds, several compounds exhibited good to excellent antimicrobial activity. From 3-(2-(4-chlorophenyl)−1-aryl-propenones **6(a-e)**, compounds **6(b)** and **6(d)** displayed good inhibitory potency against *S. aureus* bacterial strain with MIC of 12.5 µg/ml and 25 µg/ml and showed good inhibition with MIC = 12.5 µg/ml against *B. subtilis*. Compound **6(b)** showed excellent inhibition (MIC = 6.25 µg/ml) against *E. coli*, whereas compounds **6(b)** and **6(e)** displayed inhibition at the concentration 12.5 µg/ml against *P. aeruginosa*.

In the case of (oxazolyl-pyrazolyl)-pyridinyl)-methanones **7(a-e),** only the compound **7(b)** showed greater inhibition with MIC = 6.25 µg/ml against *S. aureus* as compared to that of the parent chalcone **6(b),** while compound **7(e)** displayed good inhibition potency with MIC of 12.5 µg/ml against the same bacterial strain *S. aureus*. Highest inhibition was observed for compound **7(d)** with MIC = 6.25 µg/ml against *B. subtilis.* Compound **7(b)** showed good inhibition with MIC = 12.5 µg/ml against the same bacterial strain which is same as that of the parent chalcone **6(b)**. In case of Gram negative bacteria compound **7(b), 7(c)** and **7(d)** showed noteworthy inhibition against *E.coli* with MIC = 12.5 µg/ml which are higher than that of parent chalcones showed the higher potency of pyridyl-pyrazoline **7(d)** as compared to that of **6(d)**. Compounds **7(c)** and **7(e)** displayed inhibition at the concentration 12.5 µg/ml against *P. aeruginosa* which is comparable with that of the oxazolyl chalcones **6(c)** and **6(e)**.

In the antifungal activity of aryl-oxazolyl-propenones **6(a-e**), **6(c)** and **6(e)** displayed good inhibition (MIC = 12.5 µg/ml) against the fungal strain *C. albicans*. Whereas compound **6(c)** also exhibited good inhibition potency with MIC = 12.5 µg/ml against *A. niger* fungal strain. Antifungal activity of pyridyl-pyrazolines **7(a-e)**, showed that compound **7(d)** exhibited excellent inhibition with MIC = 6.25 µg/ml against *C. albicans* as compared to that of poor activity by **6(d)** (MIC = 100 µg/ml) while compound **7(c)** displayed good inhibition (MIC = 12.5 µg/ml) against the same fungal strain which is comparable as that of **6(c)**. Compounds **7(b), 7(d)** and **7(e)** were found to inhibit growth at MIC = 12.5 µg/ml of against *A. niger.* Against *A. niger,* compounds **7(d)** and **7(e)** (MIC = 12.5 µg/ml) showed greater potency than that of the parent chalcones **6(d)** and **6(e)** (MIC = 100 µg/ml). The remaining compounds of the series possessed noteworthy antimicrobial activity as shown in Table 3. The antimicrobial activity results revealed that pyridyl-pyrazolines **7(a-e)** have greater potency against bacterial and fungal strains then that of the parent oxazolyl-propenones **6(a-e)**. On comparing the effect of the substitutions on aromatic ring it was observed that halogen substituted compounds displayed greater antimicrobial activity.

3.2.3. Molecular docking

In order to have understanding of the binding interactions of the ligands into the active site of enzyme, all the new molecules **6(a-e)** and **7(a-e**) were subjected to molecular docking with the active site of GlcN-6-P synthase (**PDB ID: 2VF5**). The receptor GlcN-6-P synthase in complex with glucosamine-6- phosphate was downloaded from RCSC PDB data bank (https://www.rcsb.org) and processed by SPDB software and by CLC Drug Discovery Work Bench.3 Evaluator version. The target validation of the receptor was done by re-docking the co-crystallized ligand on the receptor



(6d)

Developmental Therapeutics Program
One Dose Mean Graph

NSC: D-806535 / 1 **Conc:** 1.00E-5 Molar **Test Date:** Aug 20, 2018
Experiment ID: 1808OS96 **Report Date:** Sep 11, 2018

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	80.20
HL-60(TB)	78.08
K-562	79.46
MOLT-4	73.98
RPMI-8226	49.41
SR	55.29
Non-Small Cell Lung Cancer	
A549/ATCC	86.08
EKVX	104.64
HOP-62	96.75
HOP-92	80.09
NCI-H226	71.30
NCI-H23	81.05
NCI-H322M	81.48
NCI-H460	60.24
NCI-H522	81.86
Colon Cancer	
COLO 205	97.59
HCC-2998	86.01
HCT-116	93.41
HCT-15	84.14
HT29	98.48
KM12	84.49
SW-620	51.99
CNS Cancer	
SF-268	93.13
SF-295	96.41
SF-539	66.17
SNB-19	73.40
SNB-75	90.07
U251	76.14
Melanoma	
LOX IMVI	82.81
MALME-3M	98.13
M14	101.74
MDA-MB-435	100.62
SK-MEL-2	103.36
SK-MEL-28	98.58
SK-MEL-5	98.75
UACC-257	99.56
UACC-62	75.78
Ovarian Cancer	
IGROV1	80.99
OVCAR-3	71.14
OVCAR-4	47.68
OVCAR-5	109.27
OVCAR-8	86.47
NCI/ADR-RES	94.38
SK-OV-3	89.34
Renal Cancer	
786-0	97.38
A498	87.13
ACHN	95.22
CAKI-1	72.69
RXF 393	81.49
SN12C	88.87
TK-10	133.15
UO-31	69.96
Prostate Cancer	
PC-3	70.42
DU-145	97.83
Breast Cancer	
MCF7	40.17
MDA-MB-231/ATCC	87.62
HS 578T	91.08
BT-549	79.19
T-47D	47.60
MDA-MB-468	71.10
Mean	83.35
Delta	43.18
Range	92.98

Mean Growth Percent - Growth Percent

150 100 50 0 -50 -100 -150

Fig. 1. Sixty human tumor cell line anticancer screening data at single dose assay (10^{-5} M concentration) of compound **6(d)**.

pocket to validate the similar pattern docking with an RMSD of 0.002 A°. All the compounds exhibited worthy binding interactions with the amino acid residues of enzyme. The docking score and the interacting amino acids (H-bonded and steric interactions) are tabulated as **Table 4**. All the new compounds showed well established bonds with one or more amino acids in the receptor active pocket of 2VF5. Docking of synthesized ligands with GlcN-6-P exhibit bonds with amino acids present in the active pocket of the receptor. The active pocket was considered to be the site where glucosamine-6-phosphate complexes in GlcN-6-P of 2VF5. Representative docked images of compounds **7(a), 7(b), 6(a)** and **6(b)** are as shown in **Fig. 2**.

The potential of the ligands as antimicrobial agents was evaluated based on the docking scores. The docking scores with 2VF5 protein ranged from −65.354 to −50.172 as presented in **Table 4** and the highest negative value indicated the best docked ligand into the active site of receptor. According to the docking data, the most potent compound **7(a)** showed hydrogen bond interactions with Ser-347 & Gln 348 through the N atom of the pyridine ring and steric interactions with Val 399, Ala 602, Ser

Table 3
Antimicrobial activity results of the synthesized compounds **6(a-e)** and **7(a-e)**.

ID	Zone of inhibition in mm and (MIC in μg/mL)											
	Gram(+*ve*) bacteria				**Gram(-*ve*) bacteria**				**Fungi**			
	S. aureus		***B. subtilis***		***E. coli***		***P.aeruginosa***		***C. albicans***		***A. niger***	
	Zone (mm)	**MIC (μg/mL)**	**Zone (mm)**	**MIC (μg/mL)**	**Zone (mm)**	**MIC (μg/mL)**	**Zone (mm)**	**MIC (μg/mL)**	**Zone (mm)**	**MIC (μg/mL)**	**Zone (mm)**	**MIC (μg/mL)**
6a	19±0.2	125	20±0.3	100	22±0.1	50	23±0.3	25	18±0.2	125	20±0.3	100
6b	26±0.3	12.5	26±0.2	12.5	28±0.3	6.25	26±0.1	12.5	24±0.1	25	23±0.2	25
6c	21±0.2	100	22±0.3	50	24±0.1	25	23±0.2	25	25±0.3	12.5	26±0.2	12.5
6d	23±0.1	25	25±0.2	12.5	17±0.2	125	22±0.1	50	22±0.1	100	21±0.1	100
6e	21±0.1	100	18±0.2	125	24±0.1	25	25±0.13	12.5	25±0.2	12.5	22±0.1	100
7a	20±0.3	100	22±0.1	50	21±0.3	100	18±0.1	125	20±0.3	100	23±0.2	25
7b	45±0.2	6.25	25±0.1	12.5	26±0.2	12.5	23±0.2	25	24±0.1	25	25±0.3	12.5
7c	26±0.1	12.5	23±0.2	25	25±0.1	12.5	26±0.3	12.5	25±0.1	12.5	20±0.1	100
7d	24±0.2	25	26±0.3	6.25	25±0.1	12.5	24±0.1	25	28±0.2	6.25	26±0.1	12.5
7e	25±0.2	12.5	24±0.1	25	22±0.2	50	25±0.1	12.5	21±0.2	100	25±0.2	12.5
Ciprofloxacin	30±0.1	<3.12	31±0.2	<3.12	33±0.1	<3.12	32±0.2	<3.12	–		–	
Griseofulvin	–		–		–		–		33±0.1	<3.12	30±0.2	<3.12
DMSO	–		–		–		–		–		–	

MIC = Minimum inhibitory concentration.

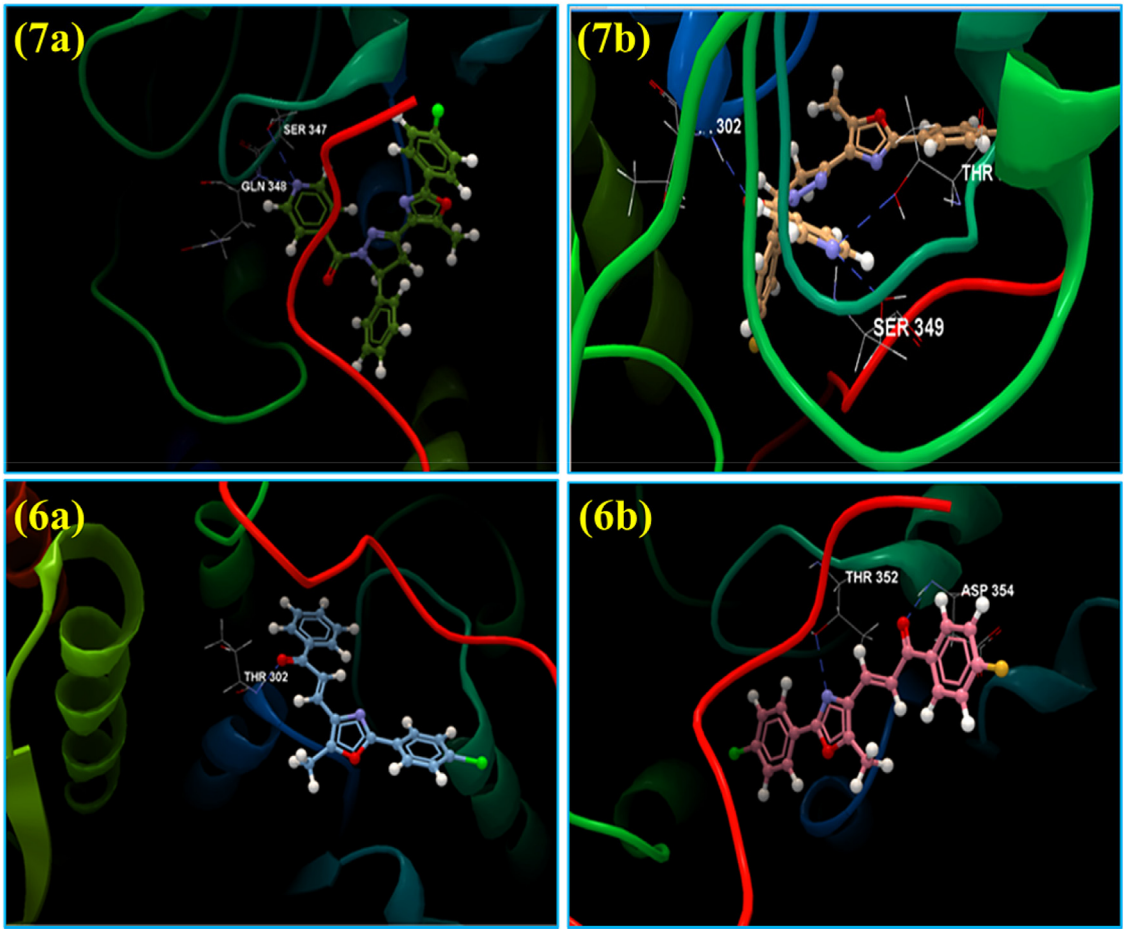


Fig. 2. Binding poses of the more active compounds **7(a), 7(b), 6(a)** and **6(b)** into the active pockets of GlcN-6-P synthase. The proteins are displayed by coloured ribbon. Hydrogen bonding interactions between docked ligands and GlcN-6-P synthase are indicated with blue dotted line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

303, Thr 352, Ser 349, Thr 352, Asp-354 amino acid residues. Compound **7(b)** and **7(d)** showed similar interaction pattern and displayed hydrogen bond interactions between Thr-352 & Ser-347 with nitrogen atom of the pyridine ring and between Thr-302 and O atom of carbonyl group. Compound **7(b)** and **7(d)** showed electrostatic forces of interaction with Ala 602, Val 399, Ser 303, Ser 349, Ser-347, Gln 348, Asp-354. Compound **7(c)** and **7(e)** interacted with Thr 302 Ala-602 respectively by forming two hydrogen bonds Compounds with O atom of carbonyl group and N atom of the pyridine ring. Compounds **6(b)** and **6(c)** also showed the similar interaction pattern and displayed hydrogen bonding between Thr352 residue and nitrogen atom of pyrazoline ring and

Table 4
Docking score and amino acid residue interactions of docked compounds **6**(**a-e**) & **7**(**a-e**) into the active sites of GlcN-6-P synthase.

Compound ID	CLC dock Score	Hydrogen bonds within 6A^0	Hydrophobic/Stearic interactions within 6A^0
6a	−53.211	Thr 302	Val 399, Ala 602, Ser 303, Thr352, Ser 349, Ser 349, Thr 352
6b	−52.263	Thr 352 & Asp 354	Val 399, Ala 602, Ser 303, Ser 349,Thr 302, Ser 349
6c	−50.172	Thr 352 & Asp 354	Val 399, Ala 602, Ser 303, Ser 349,Thr 302, Ser 349
6d	−51.554	Thr 302	Val 399, Ala 602, Ser 303, Thr 352, Ser 349, Ser 347, Asp-354
6e	−51.863	Thr 302	Val 399, Ala 602, Ser 303, Thr 352, Ser 349, Ser 347, Asp-354
7a	−65.354	Ser 347 & Gln 348	Val 399, Ala 602, Ser 303, Thr 352, Ser 349, Thr 352, Asp 354
7b	−62.113	Thr 302, Thr 352 & Ser 347	Ala 602, Val 399, Ser 303, Ser 349, Ser-347, Gln 348, Asp 354
7c	−60.600	Thr 302	Val 399, Ala 602, Ser 303, Thr 352, Ser 349, Ser-347, Asp 354
7d	−57.586	Thr 302, Thr 352 & Ser 347	Val 399, Ala602, Ser 303, Ser 349, Ser 349, Ser 349, Asp 354
7e	−60.464	Ala 602	Ser 349, Thr 352, Val 399, Ser 303, Thr 352, Ser 349, Asp 354

between Asp 354 and O atom of carbonyl group. Compounds **6(b)** and **6(c)** showed hydrophobic/steric interactions with Val 399, Ala 602, Ser 303, Ser 349, Thr 302, Ser 349 amino acids. Compounds **6(a), 6(e)** and **6(d)** interacted with Thr-302 by hydrogen bonding with O atom of carbonyl group and showed steric interactions with Val 399, Ala 602, Ser 303, Thr352, Ser 349, Ser 349, Thr 352 amino acid residues. The docking results support the higher *in vitro* antimicrobial potency of compounds **7(a-e)** as compared to that of compounds **6(a-e)**. Docking results showed that this kind of oxazole possessing pyridyl-pyrazolines hybrids were assumed precisely within the GlcN-6-P synthase binding site, signifying that they could be potential antimicrobial agents.

4. Conclusion

In summary, a series of new 1,3-oxazoles attached to pyridyl-pyrazoline moieties has been synthesized. The structures of all the newly prepared compounds were determined using IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. All the newly prepared compounds were screened for *in vitro* anticancer activity against 60 cancer cell lines at NCI, USA. Overall, based on the bio-activity evaluation results, the compound **6(d)** showed the best inhibition of some of the cancer cell lines and emerged as a potent anticancer agent among all. Furthermore, *in vitro* anti-microbial properties of all the new compounds were also evaluated using six pathogenic strains. Antimicrobial activity data revealed that some of the compounds exhibited good to excellent activity with MIC = 6.25 µg/ml. The molecular docking studies of all the new molecules were carried out and the results showed that the new hybrid heterocyclic compounds exhibited very good docking score and interactions with the targeted enzyme. Further investigations on this kind of hybrids could lead to new effective molecules as promising candidates for the development of some new drug molecules.

Credit author statement

It is to declare that names of the authors who have contributed in the scientific work leading to the present research paper have been included.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary materials

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