Design and Microwave Assisted Synthesis of Chalcone of N-(4,5-Dihydro-5-oxo-1H-Pyrazol-3-yl)Acetamide: Antimicrobial Properties evaluation and Docking study as Shikimate Kinase Inhibitors

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ABSTRACT

*Eight hybridized pyrazolone analogs were designed, docked, synthesized by microwave technique and evaluated for their in-vitro antimicrobial property against four Gram positive, four Gram negative bacterial strains and two fungal strains. Chalcone derivatives of pyrazolone moiety **5a, 5b, 5f, 5g** and **5h** showed good antibacterial activity aganist Gram positive strains (Percentage zone of inhibition range 68 to 100% with MIC range 12.5-50 µg/ml) compared with Ciprofloxacin (bacteria) and Clotrimazole (fungal). Compound **5h** showed good binding at the active site of the protein with docking score -8.69 and shows hydrogen bonding with Arg 58, Arg 136 and electronic interactions with Asp 34, Glu 38. All other compounds shows mild to moderate activity against all the screened micro-organisms tested.*

Key Words: *Pyrazolone, Antibacterial, Antifungal, Shikimate kinase inhibitor*

INTRODUCTION

Drug resistance, new and reemerging of microbial infections and technology improvements in bioengineering point to a need for accelerated drug discovery towards new class of chemotherapy with unique target. There are two basic approaches[1] to develop a new drugs: (a) synthesis of analogues, modification or derivatives of existing compounds for shortening and improving treatment and (b) searching for novel structures, that the microorganism/pathological condition has never been presented with before. Besides the exploitation of new targets, there is another approach of merging two or more pharmacophores into a single molecule. Therefore, a single molecule containing more than one pharmacophore, each with different mode of action, could be beneficial for the treatment of the above mentioned disorders. These 'merged' pharmacophores may be addressing the active site of different targets and offer the possibility to overcome drug resistance.

Shikimate Kinase[2] is an enzyme that catalyzes the adenosine triphosphate (**ATP**)-dependent phosphorylation of shikimate to form shikimate 3-phosphate (**Fig. 1**). This enzyme belongs to the family of the nucleoside monophosphate kinases (**NMP**). The NMP family is an important group of enzymes which catalyzes reversible phosphoryl transfer from nucleoside triphosphate to a specific nucleoside monophosphate, and the product of the reaction is subsequently phosphorylated, resulting in precursors of nucleic acids. The shikimate pathway is present only in bacteria, fungi and plants to synthesize the common precursor of essential aromatic amino acids[3] (phenylalanine, tyrosine, and tryptophan) and secondary metabolites (phenylpropanoids and alkaloids). In case of bacteria and fungi, enzymes of the shikimic pathway have been detected in their cytoplasm, whereas in case of plants-in plastids. Inhibitions of shikimate kinase lead to prevent the conversion of shikimate to form shikimate 3-phosphate, which is an important precursor for synthesis of aromatic amino acid. The absence of the pathway in all other genera has rendered the enzymes catalyzing these reactions potentially useful targets for the development of new antibiotics and herbicides.

Pyrazolones and its derivatives have attracted continuing interest because of their varied biological activities and also key structures for the development of new chemical class of chemotherapeutic agents,

which is not exposed as antimicrobial agent against any microorganism previously. On the other hand, literature showed amide[4] (-CONH-) and chalcones[5] (>CH=C) pharmacophores present in the structure (**compound I and II**) demonstrated significant antimicrobial activity against various microorganism at low concentration level Keeping in view the antimicrobial therapeutic[6,7] activities of pyrazolone derivatives and as part of our ongoing development of new class of antimicrobial agents[8,9]
Consequently, the combination of pyrazolone ring containing aromatic substituted group through -NH-CO-CH=C linkage, as promising approach in drug-like molecules design (**Fig. 2; Compound III**). In the present study, describes a design, docking, simple, novel, high yield, microwave methodology for synthesis of the title compounds and to screen them for their *invitro* antibacterial and antifungal activity.

MATERIALS AND METHODS

Docking Studies

The protein-ligand docking studies were performed on the basis of crystal structure of shikimate kinase in complex with ADP and Shikimate (2IYQ.pdb)[10]. The shikimate and solvent molecules were removed from the protein and polar hydrogen atoms were added using Molecular Operating Environment software package (MOE.2013.08). AutoDock 4.2[11,12] was used for flexible ligand docking into the protein structure. AutoDockTools[12] was used to add atomic partial charges. Three-dimensional scoring grids were computed within a user-specified three dimensional box of 60 × 60 × 60 grid points with a spacing of 0.375 Å centered on the co-crystallized ligand shikimate. Fifty independent docking runs for the ligand molecules were run into the binding site of shikimate kinase using the Lamarckian Genetic Algorithm in AutoDock 4.2. All the other parameters were set to their default values. Docking simulations with Autodock 4.2 successfully reproduced the crystallographic pose of shikimate with an RMSD value of 0.75Å (**Fig. 3**). On the basis of visual inspection of their interactions with the enzyme, high-scoring docking poses were selected as putative binding modes for the analysis and further designing of molecules.

Chemistry

Microwave synthesis was carried out using Biotage microwave initiator, Sweden. The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC) on silica gel-G (Merck) coated aluminium plates, visualized by iodine vapor and UV light. Melting points were determined in open end capillary tubes on a Buchi 530 melting point apparatus and were uncorrected. Infrared (IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded for the compounds on JASCO FTIR Report 4100 (KBr) and Brucker Avance (300 MHz) instruments, respectively. Chemical shifts are reported in parts per million (ppm) using tetramethyl silane (TMS) as an internal standard. All exchangeable protons were confirmed by the addition of D_2O. ^{13}C NMR spectra were recorded on Bruker AC 200/DPX 400 MHz. The mass spectra were recorded on JEOL GCMATE instrument. The mass of the compounds are expressed in *m/z* values. Elemental analyses (C, H, and N) were undertaken with Perkin Elmer model 240C analyzer and all analyses were consistent with theoretical values (within ± 0.4%) unless indicated.

Synthesis of 5-amino-2,4-dihydro-3H-pyrazol-3-one (3)

Microwave irradiation of ethyl cyanoacetate (0.01mol) and hydrazine hydrate (0.01mol) mixture in an Erlenmeyer flash for 2mins at 300W power with intermittent radiation of 30 sec interval. The progress of the reaction was examined by TLC, the mixture was poured onto the ice cold water, and the crude product (**3**) was filtered, dried and used without further purification.

Synthesis of N-(5-oxo-4, 5-dihydro-1H-pyrazol-3-yl)acetamide (4)

Acetyl chloride (0.03 mol) and triethylamine (0.03 mol) were added to a solution of compound **3** in anhydrous benzene and reaction mixture was stirring for 4-5 h on the magnetic stirrer using cold water both. The crude product (**4**) was obtained, filtered, dried and recrystallized.

Synthesis of chalcone of N-(5-oxo-4, 5-dihydro-1H-pyrazol-3-yl)acetamide (5 a-h)

Compound **(4)** (0.001mol), appropriate aromatic aldehydes (**5a-d**) or aromatic ketones (**5e-h)** (0.001mol), sodium hydroxide (0.004 mol) and ethanol (5 mL) was taken in a quartz tube and inserted into Teflon vial

with screw capped and subjected to microwave irradiation at 70°C for 5mins. After completion of reaction as indicated by TLC, the reaction mixture was poured on to crushed ice and neutralized with dilute hydrochloric acid. The solid separated was filtered and recrystallized from ethanol.

(2)-3-(2,4-dichlorophenyl)-N-(5-oxo-4,5-dihydro-1H-pyrazol-3-yl)prop-2-enamide (5a)
MP(130-132°C); Yield (81%); R_f (0.69); IR (cm^{-1}): 3418.21 (Ar C-H stretching), 1672.96 (C=O stretching), 1358.6 (pyrazolone ring stretching), 1024.02 (C=C stretching), 845.06 (C-Cl stretching); ^{1}H NMR (d ppm): 10.50 (H, s, NH), 9.09 (1H, s, NH- pyrazolone ring), 7.9 and 8.1 (2H, d, CH=CH-), 6.9 - 7.6 (3H, m, Ar-H), 2.8 (2H, s, -CH_2 pyrazolone ring); ^{13}C-NMR 172.5, 169.2, 154.2, 140.6, 133.5, 132.1, 131.6,130.2, 129.6, 127.1, 115.1, 71.9; M.wt: 298.12 MS: m/z: 299.15 $[M]^+$; Anal. Calcd, (found) for $C_{12}H_9Cl_2N_3O_2$: C, 48.34, (47.98); H, 3.04 (2.97); N, 14.09 (13.89).

(2)-3-(4-chlorophenyl)-N-(5-oxo-4,5-dihydro-1H-pyrazol-3-yl)prop-2-enamide (5b)
MP(128-130°C); Yield (80%); R_f (0.72); IR (cm^{-1}): 3194.51 (Ar C-H stretching); 1683.55 (C=O stretching); 1385.6 (pyrazolone ring stretching); 1093.44 (C=C stretching); 833.09 (C-Cl stretching); ^{1}H NMR (d ppm): 10.56 (H, s, NH), 9.12 (1H, s, NH- pyrazolone ring), 7.7 and 8.0 (2H, d, CH=CH-), 6.8 - 7.4 (4H, m, Ar-H), 2.7 (2H, s, -CH_2 pyrazolone ring); ^{13}C-NMR 172.4, 169.3, 154.3, 140.5, 132.3, 131.5,130.3, 129.5, 127.3, 126.5, 115.3, 71.5; M.wt: 263.68 MS: m/z: 264.59 $[M]^+$; Anal. Calcd, (found) for $C_{12}H_{10}ClN_3O_2$: C, 54.66, (47.98); H, 3.82 (3.78); N, 15.94 (15.69).

(2)-3-(3,4-dimethoxyphenyl)-N-(5-oxo-4,5-dihydro-1H-pyrazol-3-yl)prop-2-enamide (5c):
MP(132-133°C); Yield (82%); R_f (0.68); IR (cm^{-1})**:** 3108.69 (Ar C-H stretching), 1683.55 (C=O stretching),1347 (pyrazolone ring stretching), 1204 (C=C stretching); ^{1}H NMR (d ppm): 10.71 (H, s, NH), 9.11 (1H, s, NH- pyrazolone ring), 7.5 and 8.1 (2H,d, CH=CH-), 6.8 -7.4 (4H, m, Ar-H), 3.21 (3H, S, -OCH_3), 2.6 (2H, s, -CH_2 pyrazolone ring); ^{13}C-NMR 172.4, 169.3, 157.4, 154.3, 140.5, 132.3, 131.5,130.3, 127.3, 126.5, 115.3, 71.5, 50.2; M.wt: 259.26 MS: m/z: 260.46 $[M]^+$; Anal. Calcd, (found) for $C_{13}H_{13}N_3O_3$: C, 60.22, (60.03); H, 5.05, (4.98); N, 16.21, (16.32).

(2)-3-(4-methylphenyl)-N-(5-oxo-4,5-dihydro-1H-pyrazol-3-yl)prop-2-enamide(5d):
MP(130-133°C); Yield (82%); R_f (0.71); IR (cm-1): 3422.06 (Ar C-H stretching), 1684.48 (C=O stretching), 1348 (pyrazolone ring stretching); 1240 (C=C stretching); ^{1}H NMR (d ppm): 10.56 (H, bs, NH), 9.12 (1H, s, NH- pyrazolone ring), 7.7and 8.0 (2H,d, CH=CH-), 6.8 -7.3 (4H, m, Ar-H), 3.2 (3H, s, -CH_3) 2.7 (2H, s, -CH_2 pyrazolone ring); ^{13}C-NMR 173.1, 169.5, 154.1, 141.1, 132.6, 131.2,130.5, 129.8, 126.9, 125.9, 116.3, 72.5; M.wt: 243.26 MS: m/z: 244.49 $[M]^+$; Anal. Calcd, (found) for $C_{13}H_{13}N_3O_2$: C, 64.19, (64.01); H, 5.39, (5.26); N, 17.27, (17.12).

(2)-N-(4,5-dihydro-5-oxo-1H-pyrazol-3-yl)-3-(4-methoxyphenyl)but-2-enamide (5e):
MP(130-133°C); Yield (76%); R_f (0.75); IR (cm^{-1}): 3418.21 (Ar C-H stretching); 1672.95 (C=O stretching); 1601.59 (C-O stretching); 1358.6 (pyrazole ring stretching); 1024.02 (C=C stretching); ^{1}H NMR (d ppm): 10.26 (H, bs, NH), 9.08 (1H, s, NH- pyrazolone ring), 8.0 (H,d, CH=C-), 6.8 -7.2 (4H, m, Ar-H), 3.4 (2H, s, -OCH_3), 2.6 (2H, s, -CH_2 pyrazolone ring), 2.1 (3H, s, -CH_3); ^{13}C-NMR 172.3, 169.2, 157.4, 154.1, 152.7, 132.6, 125.2, 112.1, 112.8, 112.6, 109.2, 72.5, 52.3, 09.3; M.wt: 273.29 MS: m/z: 274.29 $[M]^+$; Anal. Calcd, (found) for $C_{14}H_{15}N_3O_3$: C, 61.53, (61.23); H, 5.53, (5.42); N, 15.38, (15.09).

(2)-3-(4-chlorophenyl)-N-(4,5-dihydro-5-oxo-1H-pyrazol-3-yl)but-2-enamide(5f):
MP(130-133°C); Yield (76%); R_f (0.67); IR (cm^{-1}): 3194.51 (Ar C-H stretching); 1983.55 (C=O stretching); 1385.6 (pyrazolone ring stretching); 1093.44 (C=C stretching); 838.12 (C-Cl stretching); ^{1}H NMR (d ppm): 10.32 (1H, bs, NH), 9.13 (1H, s, NH- pyrazolone ring), 8.1(H,d, CH=C-), 6.4 -7.1 (4H, m, Ar-H), 2.7 (2H, s, -CH_2 pyrazolone ring), 2.2 (3H, s, -CH_3); ^{13}C-NMR 172.1, 169.5, 154.6, 152.3, 132.2, 131.5, 125.8, 112.3, 111.6, 110.2, 72.1, 53,0, 09.4; M.wt: 277.71 MS: m/z: 278.68 $[M]^+$; Anal. Calcd, (found) for $C_{13}H_{12}ClN_3O_2$: C, 56.22, (55.94); H, 4.36, (4.12); N, 15.13, (15.01).

(2)-N-(4,5-dihydro-5-oxo-1H-pyrazol-3-yl)-3-m-tolylbut-2-enamide(5g):
MP(128-130°C); Yield (74%); R_f (0.75); IR (cm^{-1})**:** 3108.69 (Ar C-H stretching); 1683.55 (C=O stretching); 1347 (pyrazolone ring stretching); 1204 (C=C stretching); ^{1}H NMR (d ppm): 10.11 (H, s, NH), 9.15 (1H, s, NH- pyrazolone ring), 8.2 (H, d, CH=C-), 6.5 -7.6 (4H, m, Ar-H), 3.3 (3H, s, -CH_3), 2.6 (2H, s, -CH_2 pyrazolone ring), 2.2 (3H, s, -CH_3); ^{13}C-NMR 172.1, 169.5, 154.6, 152.3, 132.2, 131.5, 125.8, 125.3, 112.3, 111.6, 110.2, 72.1, 53,0, 09.1; M.wt: 257.29 MS: m/z: 258.48 $[M]^+$; Anal. Calcd, (found) for $C_{14}H_{15}N_3O_2$: C, 65.35, (65.44); H, 5.88, (5.52); N, 16.33, (16.12).

(2)-N-(4,5-dihydro-5-oxo-1H-pyrazol-3-yl)-3-(4-nitrophenyl)but-2-enamide(5h):
MP(133-135°C); Yield (72%); R_f (0.65); IR (cm^{-1})**:** 3422.06 (Ar C-H stretching); 1685.48 (C=O stretching); 1587.13, 1513.85 (N-O stretching); 1348 (pyrazolone ring stretching); 1240 (C=C stretching); ^{1}H NMR (d ppm): 10.12 (H, s, NH), 9.11 (1H, s, NH- pyrazolone ring), 8.1 (H, d, CH=C-), 7.5 -8.1 (4H, m, Ar-H), 2.7 (2H, s, -CH_2 pyrazolone ring), 2.1 (3H, s, -CH_3); ^{13}C-NMR 172.5, 169.3, 154.2, 152.9, 142.6, 140.1, 121.8, 121.3, 118.7, 118.6, 110.2, 72.1, 09.2; M.wt: 288.26 MS: m/z: 289.29 $[M]^+$; Anal. Calcd, (found) for $C_{13}H_{12}N_4O_4$: C, 54.17, (54.44); H, 4.20, (4.12); N, 19.44, (19.14).

ANTIMICROBIAL ACTIVITY

Determination of Zone of inhibition[8]

All newly synthesized compounds (**5a-h**) were screened for their preliminary antibacterial activity against four Gram-positive strains: *Micrococcus luteus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Coryne bacterium*, four Gram-negative strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Klebsiella pneumoniae* and two fungal strains: *Candida albicans*, *Aspergillus parasites* by disc diffusion method. A standard inoculum (1–2 $\times 10^7$c.f.u./ml 0.5 McFarland standards) was introduced on to the surface of sterile agar plates and a sterile cotton swab was used for even distribution of the inoculum. The discs measuring 6.25 mm in diameter were prepared from what man No.1 filter paper and sterilized by dry heat at 140˚C for 1 h. The sterile discs previously soaked in a known concentration (100µg/ml) of the test compounds were placed in nutrient agar medium. The plates were inverted and incubated for 24 h at 37˚C±1˚C for bacteria and 72-96 h at 27˚C±1˚C for fungi. After the incubation zone of inhibition was measured. The media used was nutrient agar medium and sabouraud dextrose medium for antibacterial and antifungal activity respectively. Ciprofloxacin (5µg/disc) and Clotrimazole (5µg/disc) were used as standard drugs for antibacterial and antifungal activity respectively. Triplicate was maintained for all tested strains. Activity was determined by measuring the diameter of the zone showing complete inhibition. The average mean results of the antibacterial and antifungal studies are listed in **Tables 2**.

Determination of Minimum Inhibitory Concentration (MIC)[9]

The minimum inhibitory concentration (MIC) in µg/ml of the titled compounds was carried out by two-fold serial dilution method. The synthesized compounds **(5a-h)** were dissolved in dimethyl sulfoxide (DMSO) to obtain 1 mg/ml stock solution. Seeded broth (broth containing microbial spores) was prepared in nutrient broth (NB) from 24 h old bacterial cultures on nutrient agar at 37 ± 1°C while fungal spores from 1 to 7 days old Sabouraud agar slant cultures were suspended in Sabouraud Dextrose Broth (SDB). The colony forming units (cfu) of the seeded broth was determined by plating technique and adjusted in the range of 104-105 cfu/ml. The final inoculums size was 105 cfu/ml for antibacterial assay and 1.1-1.5 X 102 cfu/ml for antifungal assay. Testing is performed at pH 7.4 ± 0.2 for bacteria and at a pH 5.6 for fungi. Exactly 0.4 ml of the solution of test compound was added to 1.6 ml of seeded broth to form the first dilution. One ml of this was diluted with a further 1 mL of seeded broth to give the second dilution and so on till six of such dilutions are obtained. A set of assay tubes containing only seeded broth was kept as control. The tubes were incubated in BOD incubators at 37 ± 1°C for bacteria and 28 ± 1°C for fungi. The minimum inhibitory concentrations (MICs) were recorded through visual observations after 24 h (for bacteria) and 72-96 h (for fungi) of incubation. Ciprofloxacin was used as standard for bacterial studies and Clotrimazole was used as standard for fungal studies. The lowest concentration at which there was no visible growth was taken as MIC. The results of the MIC study are listed in **Tables 2.**

RESULTS AND DISCUSSION

Chemistry

The synthetic pathway for the synthesis of the targeted compounds is illustrated in **Scheme 1**. The compound 5-amino-2, 4-dihydro-3H-pyrazol-3-one **(3)** and titled compounds (**5a-h**) were synthesized by Microwave method (eco-friendly) according to previously reported procedures with little modification[13,14]. The key intermediate compound ***N***-(5-oxo-4, 5-dihydro-1H-pyrazol-3-yl) acetamide (**4**) was synthesized by Conventional method (Traditional). Compound (**3)** was prepared by solvent free microwave irritation at 300 W for 5 mins with 30 sec interval, ethyl cyano acetate (**1)** under cyclization with hydrazine (**2)**. The intermediate pyrazolone (**3**) have free primary amine attached at 3th position of the ring react with acetyl chloride to form Compound (**4**). A series of chalcones (**5a-h**) were prepared by Claisen-Schmidt condensation of compound (**4)** containing methyl ketones with several aromatic aldehydes/ketones in presence of aqueous solution of sodium hydroxide using microwave irradiations. Microwave assisted techniques are found to be more effective in perspective of environment, reaction time, high yields, ease of work-up and isolation of products. More over microwave irradiation offers several advantages:[7] solvents are often expensive, toxic, difficult to remove in the case of aprotic dipolar solvents with high boiling point, and are environmentally polluting agents. Structure of the synthesized compounds (**5a-h**) was established on the basis of physicochemical, elemental analysis and spectral data (IR, ^{1}H-NMR, ^{13}C-NMR and Mass). Intermediate compounds (**3 and 4**) structure were confirmed by IR spectral data. Intermediate compound (**3**) shows an absorption band at 3495 cm^{-1}, corresponding to the vibration of the primary amino group and the key intermediate compound (4) shows carbonyl group of amide band at 1685.12 cm^{-1}. The IR spectrum of the titled compounds (**5a-j**) shows an absorption band at 1684.48-1672.95 cm^{-1}, corresponding to the vibration of the carbonyl group of amide in the chain, a band at 1385.6-1347 cm^{-1}, characteristic of the pyrazolone ring moiety (CONH), while the compounds (**5a, 5b and 5j**) spectra showed the appearance of the characteristic bands of the chlorine at 845.06, 833.09 and 838.12 respectively. The appearance of strong bands in the 3225 cm^{-1} region, attributed to NH group stretching. Proton assignments in ^{1}H-NMR spectra for synthesised compounds showed multiplet signals at δ 6.40- 8.10 for aromatic protons and singlet signals at δ 10.10 -10.71 for NH proton in open side chain. Pyrazolone ring NH proton signal appear at δ 9.08 -9.15. Compounds derived from ketone (**5e**, **5f**, **5g** and **5h**) showed methyl (CH_3) protons signal at δ 2.1-2.2. Formation of Chalcone (-CH_2=CH-) of pyrazolones (**5a-d)** confirmed doublet signals in the range δ 8.1-8.3 and 3.34-3.62 (CH_2) δ ppm. Compounds **5c** and **5e** showed the appearance of the characteristic signals for methoxy group signal appeared at δ 3.21 and 3.4 respectively. Compound **5d and 5g** containing aryl-methyl group signal appeared at δ 3.1 and 3.3 respectively. Further, the formation of title compounds was confirmed by recording their mass spectrums which were in full agreement with their molecular weights and the results of elemental analysis (carbon, hydrogen and nitrogen) were ± 0.4% of the theoretical values. In conclusion, we have synthesized eight chalcone of pyrazolone derivatives using microwave assisted techniques and are more convenient, environmentally safe as they require less volume of solvent, short reaction span and better yields.

Docking study and In-vitro Antimicrobial activity

The mechanism of transfering phosphate group from ATP to shikimate by shikimate kinase is reported as three-dimensional structure in apo and complex state. The shikimate kinase consists of three domains including SB (shikimate binding domain), a CORE domain containing highly conserved phosphate binding loop (P-loop) and the LID, a highly flexible domain in open and closed conformation. Among the reported structures, the complex with ADP and shikimate was selected for the docking study because the conformations of SB domain in both the states were similar. Shikimate is stabilized within the binding site through H-bond interactions with residues Asp 34, Arg 58, Gly 80 and Arg 136. Furthermore, residues Pro 11, Ile 45, Phe 49, Phe 57, Glu 61, Gly 79, Gly 81, Pro 118, and Leu 119 contributed within to form the binding site for shikimate. Binding pose of shikimate on the crystal structure of shikimate kinase along with ADP is given in **Fig. 3.**

All the compounds were explored with the interactions obtained from docking which revealed that the compounds were stabilized through hydrogen bonding interactions with the residues Asp 34, Glu 38, Arg 58, Gly 81, Arg 117 and Arg 136. Furthermore, residues Pro 11, Val 35, Ser 44, Ile 45, Ala 46, Phe 49,

Gly 80, Val 116, Pro 118, Leu 119 and Leu 132 contribute within to form the binding site for the compounds. The 2D view interaction of compounds **5a** and **5f** with amino acid residues at the SB of the shikimate kinase binding pocked site (**Fig. 4**). The binding energy of the compounds with the protein are given in **Table 1**. Compound **5h** showed good binding at the active site of the protein compared to other molecules with a docking score of -8.69. Analysing the docking of the compound **5h**, at the binding site, shows hydrogen bonding with Arg 58, Arg 136 and electronic interactions with Asp 34, Glu 38. Most of the other compounds of the series showed only hydrogen bond interactions with Arg 58, Arg 136 and other amino acid residues. All the compounds didn't fit properly in the active pocket except compound **5h**. The 2D interaction (**A**) and the diagram docked pose (**B**) of compound **5h** at the SB of the shikimate kinase (**Fig. 5**).

Agar-diffusion method was used for the determination of the preliminary antibacterial and antifungal activity. Ciprofloxacin and Clotrimazole were used as reference drugs. The results were recorded for each tested compound in triplicate as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the disks in mm. The Minimum Inhibitory Concentration (MIC) measurement was determined for compounds showed growth inhibition zones >9 mm or 50% zone of inhibition compare with standard using twofold serial dilution method. The IZ diameters and MIC (µg/ml) values are recorded in **Table 2**. The results are describe in **Table 2** revealed that all tested compounds displayed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains as well as against fungal strains. Compound **5h** shows -8.69 docking score and demonstrated significant antimicrobial activity against all the tested bacterial and fungal stains with range between 65-100% zones of inhibition. All other synthesized compounds displayed mild to moderate antimicrobial activity against screened strains. Compounds **5a** and **5b** demonstrate significant activity against *C. bacterium* with 93% percentage zone of inhibition and 25 µg/ml of MIC.

The presence of electronegative element shows better interaction compared with other substitution in the series. Eventhough, the compounds **5a, 5b** and **5f** contain electronegative elements like Cl, they didn't show better interaction and docking score as compared with compound **5h**. The possible reason could be the lack of hydrogen bonding with Arg 58 and Arg 136 and also were out fitting inside the binding pocket. This could be the reason for better antimicrobial activity of compound **5h** and low antimicrobial activity profile of the other compounds of the series.

SAR of synthesized compounds

a. Docking studies with the protein revealed the necessity of an electronegative element and a hydrogen bond acceptor at para position to the phenyl ring attached with the pyrazolone nucleus as nitro group in the compound **5h**.
b. Compounds derived from ketone (**5f, 5g** and **5h**) demonstrate better antimicrobial activity than compound derived from aldehyde (**5c** and **5d**).
c. Compounds(**5a**, **5b, 5f** and **5h)** bearing elcctrons withdrawing at para position to the phenyl ring attached with the pyrazolone nucleus shows enhanced antimicrobial activity.

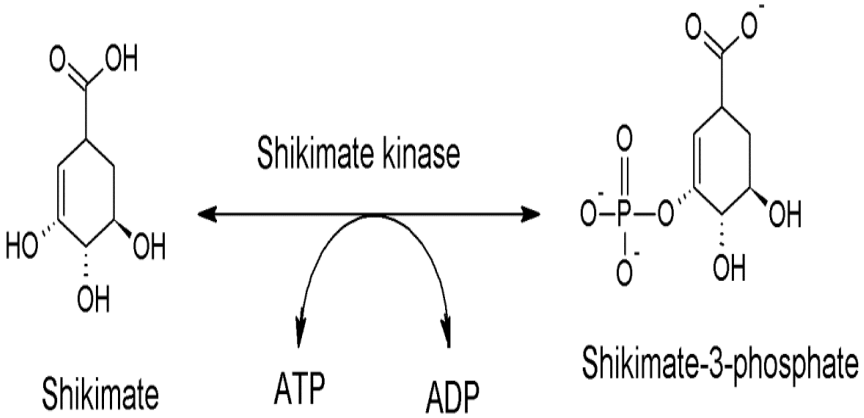


Fig. 1: Biological function of shikimate kinase

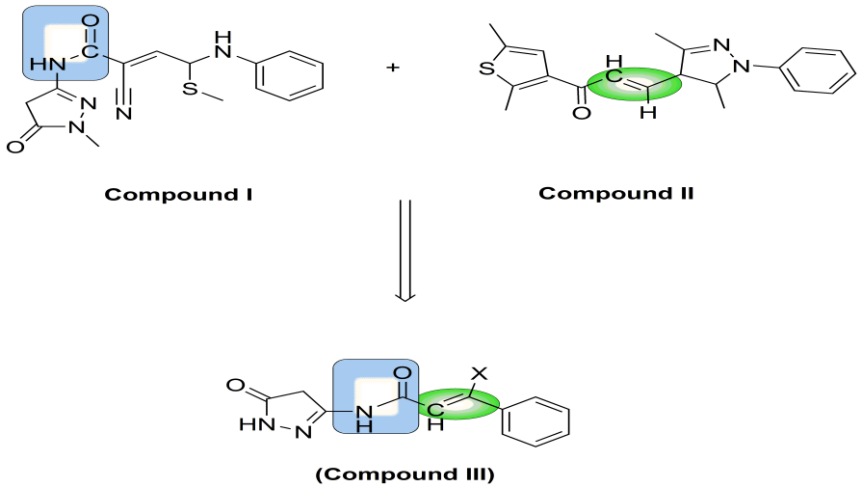
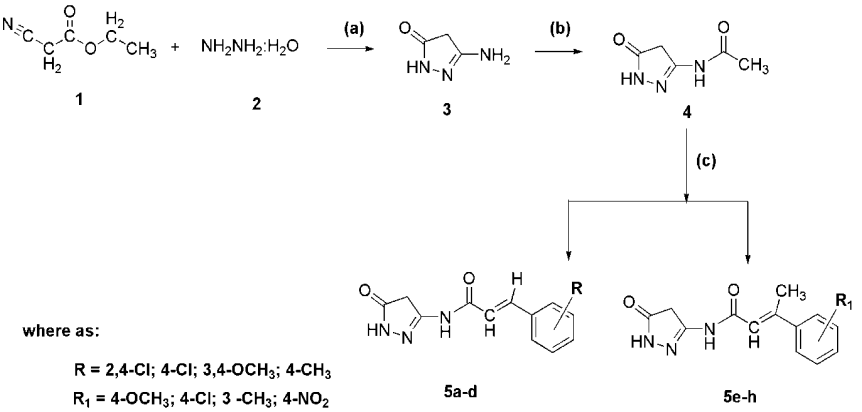
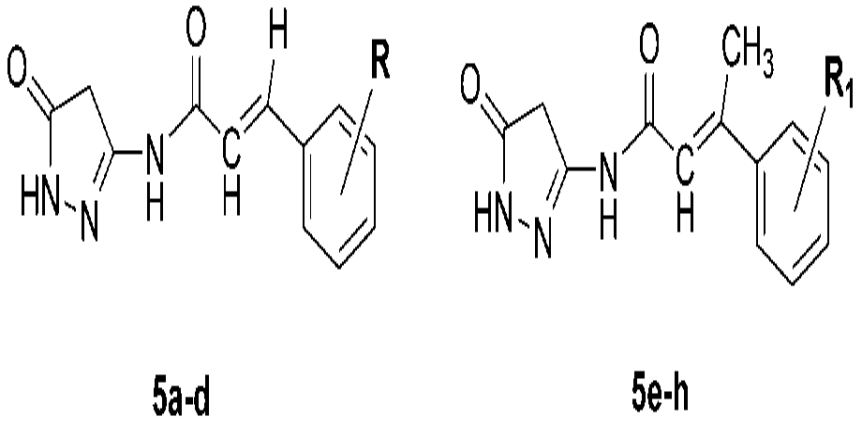


Fig. 2: Designed hybridized analogue pyrazolone structure



Scheme 1: Synthesized pyrazolone derivatives



General structure for synthesized compounds

Table 1: Synthesized compounds substituted groups (R & R_1) and docking score

Compound code	R	R_1	Docking score
5a	2, 4-Cl	-	-6.58
5b	4-Cl	-	-6.27
5c	3,4-OCH_3	-	-6.35
5d	4- CH_3	-	-6.12
5e	-	4-OCH_3	-6.11
5f	-	4-Cl	-6.44
5g	-	3-CH_3	-6.52
5h	-	4-NO_2	-8.69

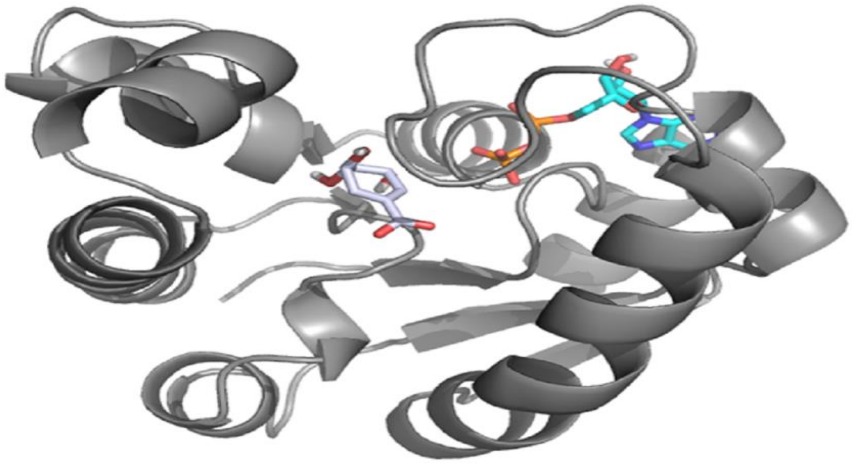


Fig. 3: Binding pose comparison of docked and crystal structure of shikimate on the shikimate kinase along with ADP. The crystal structure of shikimate kinase is represented in ribbon and colored in grey. Carbon atoms of AMP is colored in sky blue, crystal structure shikimate colored in silver ash. Oxygen atoms are colored in red, nitrogen atoms in blue, hydrogen in silver white and phosphor atoms in orange

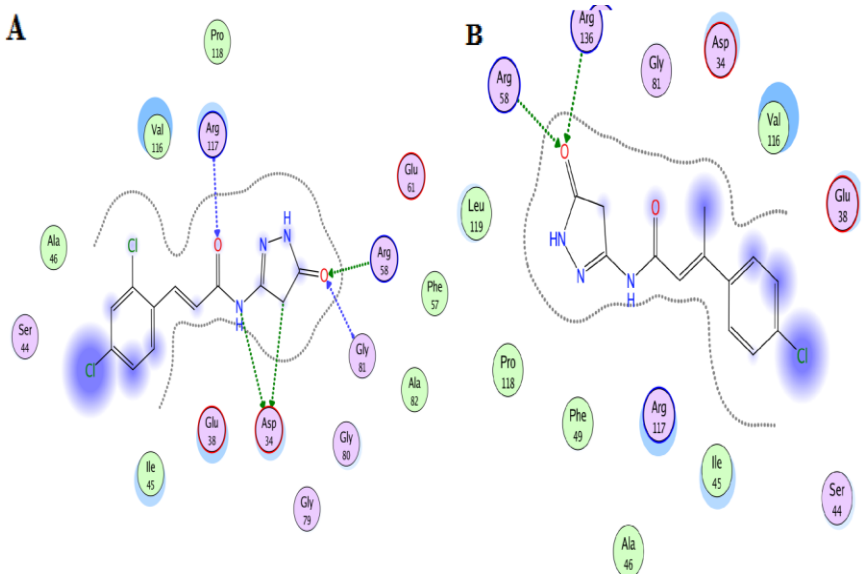


Fig. 4: The 2D view interaction of compounds 5a (A) and 5f (B) with amino acid residues at the SB of the shikimate kinase binding pocked site

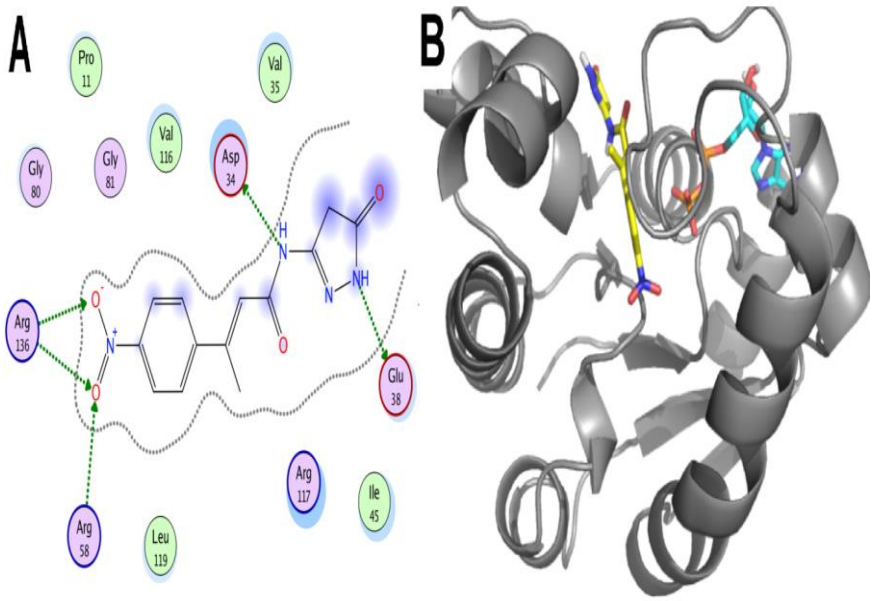


Fig. 5: The 2D interaction (A)and the diagram docked pose (B) of compound 5h at the SB of the shikimate kinase. The binding pose representation and other atoms are colored as described in Fig. 3.

Table 2: ***In-vitro*** **antimicrobial activity of synthesized compounds (5a-h)**

Cpd code	*M. luteus*		*S. aureus*		*B.subtilis*		*C.bacterium*		*E. coli*		*P.aeruginosa*		*V.cholerae*		*K.pneumoniae*		*C.albicans*		*A.parasites*	
	ZI (%)	**MIC**	**ZI**	**MIC**	**ZI**	**MIC**	**ZI**	**MIC**	**ZI**	**MIC**	**ZI**	**MIC**	**ZI**	**MIC**	**ZI**	**MIC**	**ZI**	**MIC**	**ZI**	**MIC**
5a	12(60)	50	12(80)	25	14(56)	50	14(93)	25	11(44)	NT	14(82)	50	15(58)	25	14(56)	50	10(50)	50	10(71)	50
5b	12(60)	50	12(80)	50	13(52)	50	14(93)	25	11(44)	NT	14(82)	25	14(54)	25	14(56)	50	10(50)	50	10(71)	50
5c	12(60)	50	10(67)	50	10(40)	NT	10(67)	50	15(60)	25	10(59)	50	-	-	10(40)	NT	09(45)	NT	-	-
5d	12(60)	50	10(67)	50	10(40)	NT	12(80)	50	12(48)	25	14(82)	50	15(58)	50	-	-	09(45)	NT	-	-
5e	12(60)	50	11(73)	50	13(52)	25	12(80)	50	12(48)	25	-	-	14(54)	25	12(48)	NT	10(50)	25	-	-
5f	14(70)	25	13(87)	25	14(56)	50	12(80)	50	12(48)	25	14(82)	25	14(54)	25	13(52)	25	15(75)	25	10(71)	50
5g	14(70)	25	13(87)	25	14(56)	25	12(80)	50	14(56)	25	10(59)	50	13(50)	25	14(56)	25	15(75)	25	12(86)	25
5h	15(75)	25	15(100)	12.5	17(68)	25	12(80)	25	19(76)	25	16(94)	25	17(65)	25	17(68)	25	15(75)	12.5	12(86)	25
Solvent	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Std	20*		15*		25*		15*		25*		17*		26*		25*		20**		14**	

ZI-Zone of Inhibition **(mm) % -(**percentage**); MIC-**Minimum inhibitory concentration **(µg/mL); Solvent-** DMSO; **Std**- *Ciprofloxacin and **Clotrimazole

CONCLUSION

Eight hybridized pyrazolone analogs were designed, docked, synthesized by microwave technique and evaluated for their in-vitro antimicrobial property against four Gram positive, four Gram negative bacterial strains and two fungal strains. Among the synthesized compounds, compound (**5h**) shows significant antimicrobial activity against screened strains. On the other hand, docking study of the compound **5h** shows good interactions with amino acid residues, reasonably fitting in the active pocket site compared with other compounds. Further modification is required in the structure to improve binding with shikimate kinase and antimicrobial activity at nanomolar concentration.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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