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Synthesis, Characterization, Biological and Molecular Docking Studies of 1,3-Diphenyl-1*H*-pyrazole Terminated Imino Derivatives

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Herein, the novel pyrazole derivatives with imino moiety were synthsized, characterized using IR, NMR, mass spectroscopy, elemental analysis and screened their biological activities. Molecular docking studies were also carried out by the software Arguslab 4.0.1. The synthesized compounds showed moderate to good biological activities both experimentally and theoretically.

Keywords: Pyrazole derivatives, Vilsmeier-Haack reaction, Biological activities, Molecular docking.

INTRODUCTION

Compounds containing pyrazole moiety have been found large applications in pharmaceuticals [1-5]. *N*-Phenyl pyrazole derivatives show effective inhibitory activity as well as favourable selectivity ratio in clinical applications. These high-profile applications of *N*-phenyl pyrazole derivatives have encouraged the extensive studies of their synthesis and screen them for their potential biological activities.

From the literature, it was observed that the presence of pyrazole ring plays an important heterocyclic pharmacophore in the discovery of drug and the scientists are still trying to synthesize its derivatives of better biological and medicinal activities. These compounds with pyrazole ring are having more interest due to their broad spectrum of biological activities against NOS inhibitor [6], monoamine oxidase inhibitor [7], antibacterial [8], antiamoebic [9], anti-inflammatory [10], antiviral [11], anti-tumor [12], anti-depressant, anti-convulsant [13], antimicrobial [14] antibacterial, antifungal [15], anticancer [16], antihistaminic activities, proton pump inhibitor, antioxidant, anti-hypertensive and anticoagulant properties [17]. Moreover, *N*-phenyl pyrazole derivatives play an important role in antitumor screening as well as potent antimicrobial activity [18]. The pyrazole derivatives play important roles in the development of pesticides and medicines and found application as fungicides, antiviral agents, analgesic agents, insecticides and herbicides [19].

Herein, we synthesized *N*-phenyl and *C*-phenyl pyrazole terminated imino derivatives. It is expected that the presence of the different substituents on imino moiety may have an effect in the *in vitro* antibacterial and antifungal activities of these potential chemotherapeutics. In present work, directed towards the development of simple and efficient synthesis of biologically active pyrazole compounds, herein we report the synthesis of some pyrazole derivatives from pyrazole-4-caboxaldehyde by treating acetophenone with DMF/$POCl_3$ complex and functionalized by substituted amino compounds giving corresponding azomethane derivatives and their antimicrobial activities. All the synthesized compounds were characterized using IR, NMR, mass spectroscopy and elemental analysis. Biological activities like antimicobial, antibacterial and antifungal were also assessed.

EXPERIMENTAL

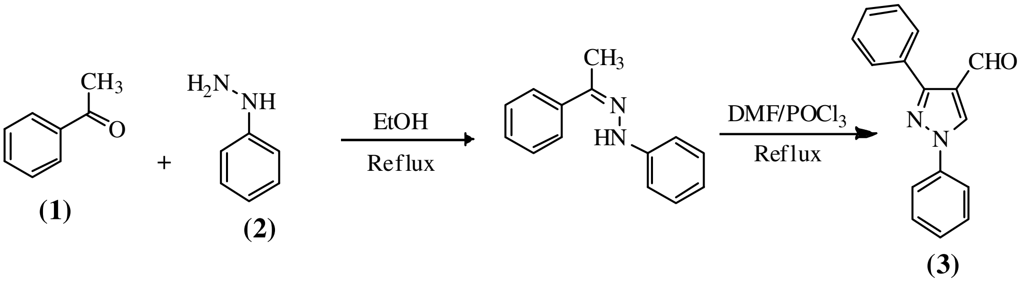
Melting points of the synthesized compounds were taken on a Yanaco MP-S3 microscopic melting point apparatus. The IR spectra were recorded in KBr pellets on a Brucker Equinox-55 FT-IR apparatus. The ^{1}H & ^{13}C nuclear magnetic resonance spectra were recorded on a NMR-JEOL GSX-400 spectrophotometer. Tetramethyl silane was used as the internal reference and all the spectra were recorded in $CDCl_3$ solvent. Mass spectra were recorded on an HP 1100 LC-MS (ESI). All the reagents used were commercial products of analytical grade and can be used without further purification except where they especially noted.

General procedure: 1,3-Diphenyl pyrazol-4-carboxaldehyde (**3**) intermediate was synthesized from acetophenone (**1**) and phenyl hydrazine (**2**) followed by Vilsmeir-Haack reaction as shown in **Scheme-I**. The formed pyrazole-4-carboxaldehyde was condensed with amino compounds to yield *C*-phenyl *N*-phenyl pyrazole terminated imino derivatives (**4a-e**) (**Scheme-II**).

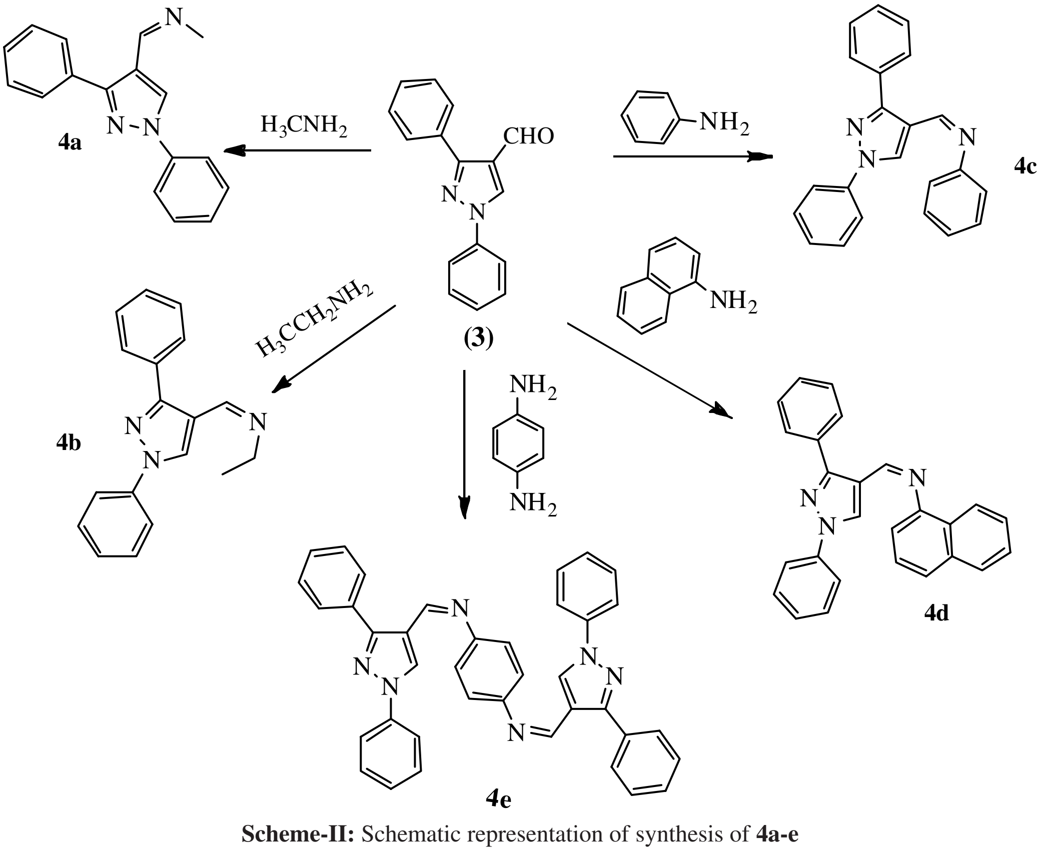
Synthesis of *N*-((1,3-Diphenyl-1*H*-pyrazol-4-yl)methylene)methanamine (4a): The compound was obtained by the reaction between 1,3-diphenyl pyrazol-4-carboxaldehyde with methylamine. Yield: 81%, pale yellow powder, m.p.: 112 ºC; IR (KBr, ν_{max}, cm^{-1}): 3047 (Ar-CH *str.*), 1459 (ArC=C *str.*), 1628 (C=N *str.*), 1231 (C-N *str.*); ^{1}H NMR (δ ppm): 8.62 (s, 1H, HC=N), 7.86 (s, 1H, CH of pyrazole ring), 6.76-8.02 (m, 10H, aromatic H),0.68 (s, 3H, CH_3); ^{13}CNMR (δ ppm): 109-141 (all aromatic carbons), 161.08 (HC=N, imino carbon), 46.54 (CH_3); MS: *m/z* 261(M$^+$).

Synthesis of *N*-((1,3-Diphenyl-1*H*-pyrazol-4-yl)methylene)ethanamine (4b): The compound was obtained by the reaction between 1,3-diphenyl pyrazol-4-carboxaldehyde with ethylamine. Yield: 79 %, pale yellow powder, m.p.: 115 ºC; IR (KBr, ν_{max}, cm^{-1}): 3041 (Ar-CH *str.*), 1452 (ArC=C *str.*), 1622 (C=N *str.*), 1225 (C-N *str.*); ^{1}H NMR (δ ppm): 8.73 (s, 1H, HC=N), 7.92 (s, 1H, CH of pyrazole ring), 6.82-8.11 (m, 10H, aromatic H), 0.92 (t, 3H, CH_3), 1.61 (q, 2H, CH_2); ^{13}C NMR (δ ppm): 112-149 (all aromatic carbons), 163.14 (HC=N, imino carbon), 16.09 (CH_3), 48.36 (CH_2); MS: *m/z* 275(M$^+$).

Synthesis of *N*-((1,3-Diphenyl-1*H*-pyrazol-4-yl)methylene)aniline (4c): The compound was obtained by the reaction between 1,3-diphenyl pyrazol-4-carboxaldehyde with aniline. Yield: 52 %, appearance, reddish orange coloured solids, m.p.: 176 ºC; IR (KBr, ν_{max}, cm^{-1}): 3057 (Ar-CH *str.*), 1463 (ArC=C *str.*), 1631(C=N *str.*), 1234 (C-N *str.*); ^{1}H NMR (δ ppm): 9.03 (s, 1H, HC=N), 8.56 (s, 1H, CH of pyrazole ring) 7.01-8.03 (m, 15H, aromatic H); ^{13}C NMR (δ ppm): 113-153.8 (all aromatic carbons), 163.24 (HC=N, imino carbon); MS: *m/z* 323(M$^+$).



Scheme-I: Schematic representation of synthesis of pyrazole carboxaldehyde



Scheme-II: Schematic representation of synthesis of **4a-e**

Synthesis of *N*-((1,3-Diphenyl-1*H*-pyrazol-4-yl) methylene)naphthalene-1-amine (4d): It was obtained when 1,3-diphenyl pyrazol-4-carboxaldehyde condensed with 1-naphthylamine. Yield: 48 %, appearance, red coloured solid, m.p.: 193 ºC; IR (KBr, ν_{max}, cm^{-1}): 3061 (Ar-CH *str.*), 1465 (ArC=C *str.*), 1636(C=N *str.*), 1240 (C-N *str.*); ^{1}H NMR (δ ppm): 10.31 (s, 1H, HC=N), 9.54 (s, 1H, CH of pyrazole ring), 7.36-8.15 (m, 17H, aromatic H), 0.92 (t, 3H, CH$_3$), 1.61 (q, 2H, CH$_2$); ^{13}C NMR: 113-150.6 ppm (all aromatic carbons), 164.24 (HC=N, imino carbon); MS: *m/z* 373(M$^+$).

Synthesis of *N*^{1},*N*^{4}-*bis*((1,3-diphenyl-1*H*-pyrazol-4-yl)-methylene)benzene-1,4-diamine (4e): It was obtained when 1,3-diphenyl pyrazol-4-carboxaldehyde condensed 1,4-diamino benzene (2:1). Yield: 35%, brownish black solid; m.p.: 246 ºC; IR (KBr, ν_{max}, cm^{-1}): 3046 (Ar-CH *str.*), 1452 (ArC=C *str.*), 1624 (C=N *str.*), 1231 (C-N *str.*); ^{1}H NMR (δ ppm): 10.06 (s, 2H, HC=N), 9.73 (s, 2H, CH of pyrazole ring), 7.48-7.83 (m, 20H, *N*-phenyl and *C*-phenyl protons), 6.45 (4H, core phenyl); ^{13}C NMR (δ ppm): 116-153.9 ppm (all aromatic carbons), 161.26 (HC=N, imino carbon); MS: *m/z* 568 (M$^+$).

Biological investigation

Antibacterial activity: The synthesized compounds have been screened for the biological properties by adopting standard protocols available in the literature [20]. Antibacterial activity (*in vitro*) of the synthesized compounds were studied against the representative panel of bacterial strains such as *Escherichia coli* MTCC443 and *Pseudomonas aeruginosa* MTCC-1688 as Gram negative bacteria and *Staphylococcus aureus* MTCC-96 and *Streptococcus pyogenes* MTCC-442 as Gram positive bacteria. Ciprofloxacin was used as the standard antibacterial drug.

Antifungal activity: Antifungal activity was studied against two fungal species, *Candida albicans* MTCC-227 and *Aspergillus niger* MTCC-282, and fluconazole was used as the standard antifungal drug. The minimal inhibitory concentration (MIC) of all the synthesized compounds was measured by the broth micro-dilution method according to National Committee for Clinical Laboratory Standards (NCCLS) [21]. All the synthesized compounds were screened for their antibacterial and antifungal activities against bacteria and fungi used in the present protocol.

Antimycobacterial activity: The *in vitro* antitubercular activity of all the synthesized compounds was studied against *M. tuberculosis* H37Rv by microplate alamar blue assay method [22]. Isonicotinic acid hydrazide (INH) was used as the standard antituberculosis drug.

Molecular docking: Arguslab 4.0.1 version software was used for docking to prepare input files. Using this, binding conformations of the prepared compounds, its free energy of binding in the active site of the kinase domain of *c*-Met bound to XL880 [(GSK1363089) PDB Id: 3LQ8 downloaded from PDB database]. Molecular docking process was performed by assuming the protein to be rigid and ligand as flexible where all rotatable bonds of the ligand were considered. Before docking all the miscellaneous residues, water molecules and heterocyclic compounds were removed from the protein crystallographic structure to activate the binding site only for the synthesized compounds and subsequently adding hydrogen atoms to the protein. The structure of the ligand molecules was drawn and optimized using the above software. The calculation box was created and centered on the binding site residues with 60 × 60 × 60 grid points in XYZ direction. The molecular docking calculations were run with standard precision with default values. Normally, the docking setup was validated by ligand having protein binding with cells.

RESULTS AND DISCUSSION

The synthesis of these compounds was performed by the condensation of acetophenone with phenyl hydrazine followed by Vilsmeir-Hack reaction to yield pyrazole-4-carboxaldehyde, which on again undergo condensation with amino compound to form imino compounds (**4a-e**). The synthesized compounds were characterized by different spectroscopic techniques.

Biological studies

Antimicrobial activity: Among the synthesized compounds **3c**, **3d** and **3e** are active against *S. aureus* and **4a** and **4b** are weakly active (Table-1). All compounds shows moderate to highly active against *E. coli*. Compounds **4a**, **4b** and **4c** are active against *P. aerugivosa*, but compunds **4d** and **4e** are less active. *S. pyrogenes* is activity (moderate to high) against all the synthesized compounds. All the imino pyrazole derivatives displayed an outstanding antifungal activity with MIC 0.2 µg/mL against *A. niger* than fluconazole. In this work, the antifungal activity is found to be independent of the substituents. Against *C. albicans*, compounds **4c**, **4d** and **4e** exhibited good antifungal activity (MIC 50 µg/mL) than rest of the compounds.

TABLE-1
ANTIBACTERIAL, ANTIFUNGAL AND ANTITUBERCULOSIS ACTIVITIES OF COMPOUNDS **4a-e** (INHIBITION ZONE MEASURED IN mm)

Compound	Antibacterial activity				Antifungal activity		Antituberculosis activity
	S. aureus	*E. coli*	*P. aerugivosa*	*S. pyrogenes*	*A. niger*	*C. albicans*	*M. tuberculosis*
4a	9	14	12	14	0.2	100	30.2
4b	13	16	14	17	0.2	100	30.2
4c	15	19	13	14	0.2	70	100
4d	17	20	10	21	0.2	50	100
4e	15	22	11	21	0.2	65	100
Ciprofloxacin	24	22	23	23	–	–	–
Fluconazole	–	–	–	–	30	30	–
Isonicotinic acid hydrazide	–	–	–	–	–	–	0.4

Highly active-20-30, moderately active-15-20, weakly active -11-15, less than 11 inactive

However, they were less active than standard fluconazole (MIC 30 μg/mL). The imino pyrazoles with phenyl and naphthyl derivatives are displayed an outstanding antifungal activity with MIC 0.2 μg/mL against *A. niger* than fluconazole. This may be due to the presence of delocalized π-electrons which increase the lipophilicity of the molecule and thus cell permeation. Naphthyl derivative is found to have better activity than phenyl derivatives of pyrazole and the compound with both ends terminated with 1,3-diphenyl pyrazole moiety is still more active than naphthyl derivatives of iminopyrazole.

Structure activity relationship (SAR) studies: The antibacterial studies indicate that the incorporation of the aromatic fragment enhances the bioactivity of pyrazole compounds, when compared to the methyl and ethyl derivatives of pyrazole. The biological studies revealed that the presence of electron releasing phenyl and naphthyl groups extensively increases the conjugation in the molecule and this will result in the higher antimicrobial activity [23] and exhibit broad-spectrum of antimicrobial activity.

It is expected that bioactivity of these compounds may be due to the combination of the factors such as inclusion and substitutions on the pyrazole ring, the presence of the imino bond, steric hindrance and the presence of whether phenyl or naphthyl ring. The heterocyclic compounds enhance the pharmacological behaviour [24] and it is true in present case too. The substituents on pyrazole moiety were chosen carefully for establishing different electronic environment on the new molecules such as electron donating groups (+I) as methyl and ethyl, and phenyl and naphthyl groups as electron withdrawing (-I). The compounds with electron withdrawing groups like phenyl and naphthyl show enhanced biological activities. It is expected that more activity in the case of compound **4e**, seems to the dimer of compound **4c**, since it contains two 1,3-diphenyl pyrazole moiety. This decreased activity may be due to the bulkiness of the molecule. Compound **4e** shows almost similar activities like compound **4c**. As the chain length increases, the inhibitory activity generally decrease [25]. It was also noted that the naphthyl derivatives are biologically more active than corresponding phenyl derivative. It was also true in our case that compounds having N-atom in the heterocyclic system are better pharmacological agents than the compounds of simple benzene analogue. The antibacterial activity against *E. coli* improved when substitutions pattern was changed by the substitution of aromatic groups.

Antituberculosis activity: All the compounds exhibited a very weak tubercular activity (Table-1). The poor antitubercular activity of the title compounds may be because of their lower lipophilicity as indicated by their *C*log P values, which resulted in the reduced cell wall permeation.

Molecular docking studies: The synthesized compounds **4a-e** docked in the active site of the kinase domain of *c*-Met using Arguslab 4.0.1 software. Using autodock, active site-based docking was adopted; the ligands were made to bind inside the active site of *c*-Met proteins. These approaches were done to check the possibility of binding of ligand in the whole protein and specifically inside the protein binding site. The binding

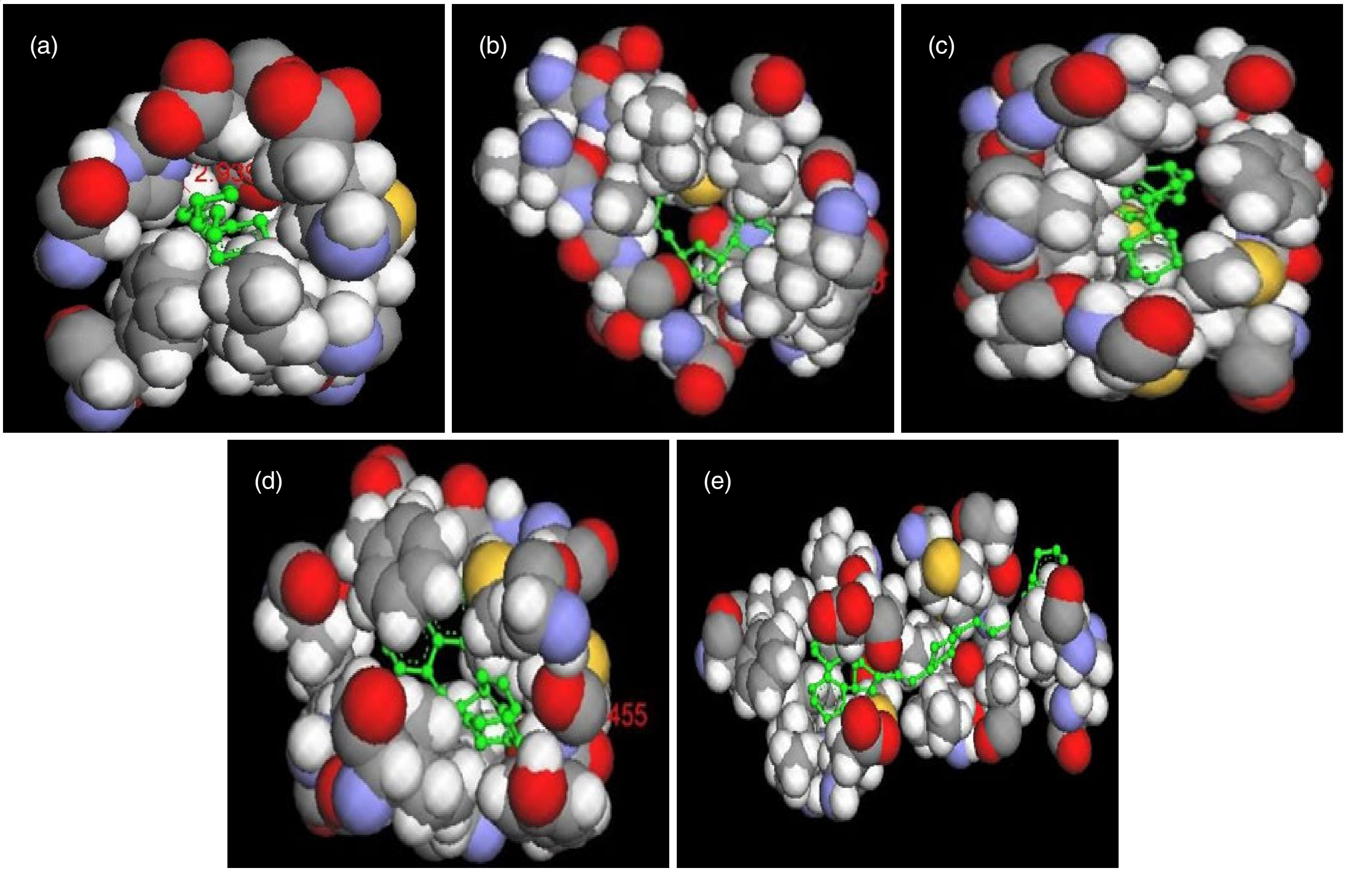


Fig. 1. Binding pose of the synthesized compounds (green in colour) in the active site of c-Met kinase target

site was selected based on the co-crystal ligand molecule in the target protein structure [26-28]. The conformation having the lowest binding energy and better interaction at active site residues is shown in Fig. 1. H-bond interaction and binding energy of the docked compounds within the active site were also analyzed and tabulated in Table-2. It is observed that all these compounds showing better binding energy in the range of -7 to -13 kcal/mol for *c*-Met kinase. It is observed that compounds **4a**, **4b** and **4d** formed H-bond interaction with the active residues. Compound **4a**, 1202 HIS formed H-bond with N of N-phenyl residue. The 1110 LYS formed H bonds with N^1 and N^2 of pyrazole entity of compound **4b**. H-bond interaction was formed in compound **4d** between 1160 MET and *N*-phenyl moiety of pyrazole (Fig. 2).

TABLE-2
BINDING ENERGY OF THE COMPOUND AND H-BOND LENGTH CALCULATED USING ARGUS LAB 4.0.1

Entry	Binding energy (Kcal/mol)	H bond length (Å)
4a	-7.49	2.93 (1202 HIS with N-phenyl)
4b	-8.79	2.94 (1110 LYS of N of pyrazole ring) 2.84 (1110 LYS with N-phenyl of pyrazole)
4c	-10.05	–
4d	-12.53	2.57 (1160 MET with N-phenyl of pyrazole)
4e	-13.43	–

Conclusion

In present work, 1,3-diphenyl pyrazole terminated imino derivatives were synthesized and characterized. The antibacterial, antifungal and antituberculosis activities of the syntehsized compounds were also evaluated. Most of the derivatives were found very active, which might due to the greater lipophilicity of phenyl group. It was observed that the phenyl and naphthyl groups on the title compounds showed excellent activity against all type of bacterial strains. Molecular docking study was carried out using the software Arguslab 4.0.1 and minimum ligand pose binding energy and H-bond information of all the synthesized compounds in *c*-Met kinase protein were also tabulated.

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CONFLICT OF INTEREST

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

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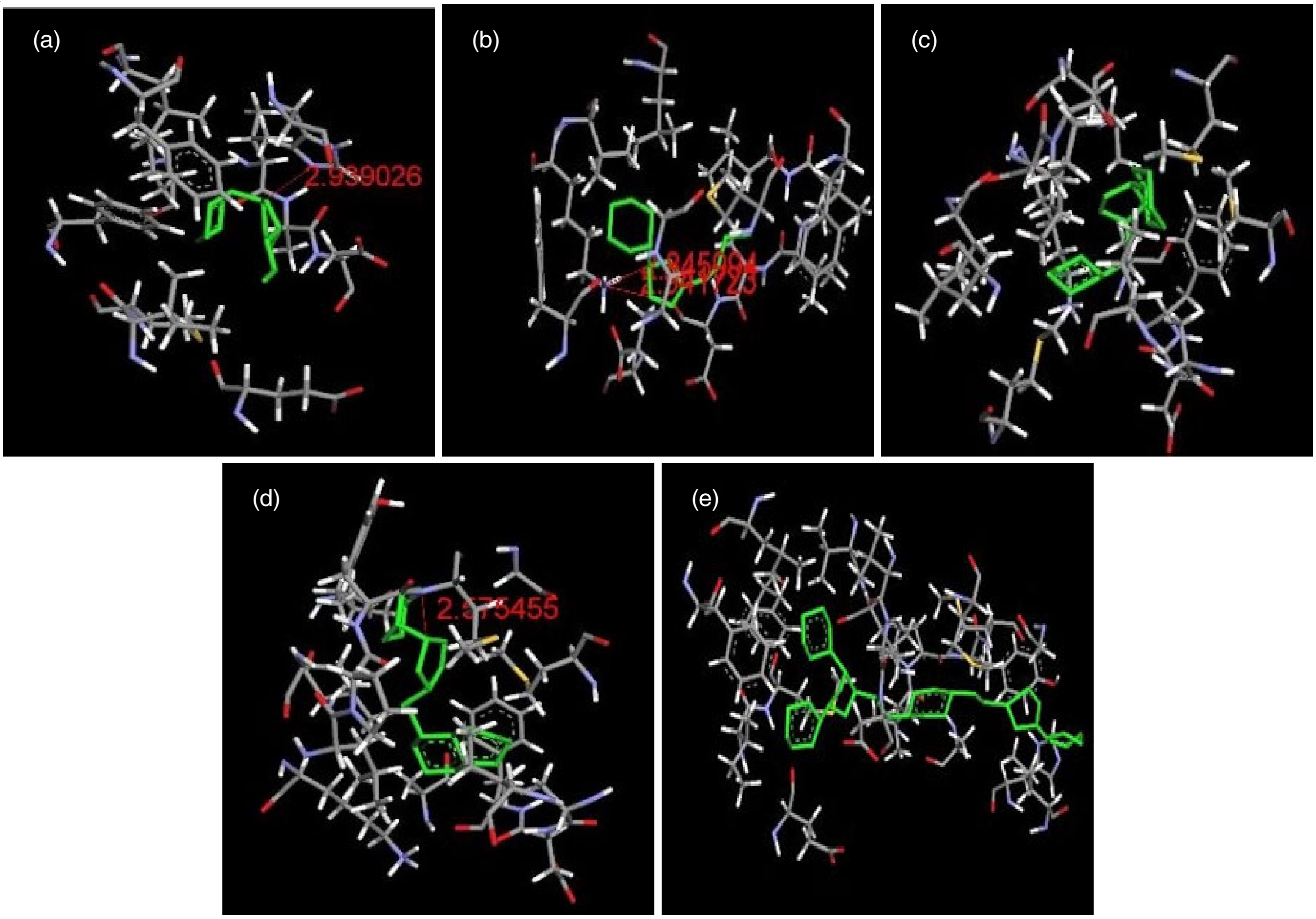

Fig. 2. Binding of the synthesized compound (green in colour) in the active site of c-Met target showing H-bond (red colour)

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