SYNTHESIS AND MOLECULAR DOCKING STUDIES ON BIOLOGICALLY ACTIVE PYRAZOLE TERMINATED IMINO NAPHTHYL DERIVATIVES

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(Received 20 October 2021) (Accepted 02 August 2022)

ABSTRACT

In this present study, a series of pyrazole terminated imino naphthyl derivatives were synthesized by the condensation reaction between 1,3 - diphenyl pyrazol-4-carboxaldehyde with substituted aromatic amines. The compounds were analyzed for their *in vitro* antimicrobial activities against some bacterial and fungal species and compared with the standard drugs. The molecular docking studies with protein and DNA were also carried out and revealed that all the synthesized compounds showed moderate to good biological activities, both experimentally and theoretically.

Keywords: Pyrazole derivatives, imino naphthyl derivatives, biological activities, molecular docking, DNA binding

INTRODUCTION

Amongst heterocyclic compounds, nitrogen-containing heterocycles have striking structural features and they are widely observed in natural products such as vitamins, hormones and alkaloids[1,2]. Pyrazole is one of the most important nitrogen-containing heterocyclic compounds exhibiting a broad spectrum of biological profiles such as NOS inhibitor[3], monoamine oxidase inhibitor[4], antibacterial[5], antiamoebic[6], anti-inflammatory[7], anti-viral[8], anti-tumor[9], anti-depressant, anti-convulsant[10], antimicrobial[11], antifungal[12], anti-cancer[13], antihistaminic activities, proton pump inhibitor, antioxidant, anti-hypertensive, anticoagulant[14] and agrochemical agent[15]. Moreover, *N*-phenyl pyrazole derivatives show an increased activity among phenyl derivatives against antitumor screening as well as antimicrobial activity[16]. These pyrazole derivatives also have many applications in developing pesticides, insecticides and herbicides[17]. These activities treat pyrazole attached derivative as an important compound in the novel drug development process.

From the literature, the compounds with imine or azomethine groups present in various natural, naturally derived, and non-natural compounds are also exhibiting a broad spectrum in pharmacological activities such as inhibition of DNA and RNA, protein synthesis, carcinogenesis[18] and nitrogen fixation. Also the imino compounds have application in the field of hypnotic drugs for the nervous system as well as having biological activities against bacteria and fungi[19].

Moreover, the presence of pyrazole ring together with imino moiety may influence the *in vitro* antibacterial and antifungal activities. In continuation of our research work, we developed a simple and efficient method for the synthesis of biologically active pyrazole terminated imino naphthyl derivatives. It is also expected that the presence of different substituents on imino naphthyl ring also affects its potential as chemotherapeutics. Herein, we synthesized *N*-phenyl-C-phenyl terminated imino naphthyl derivatives, characterized using IR, NMR, mass spectroscopy and elemental analysis techniques. Biological activities like antimicrobial, antibacterial and antifungal were also investigated both experimentally and theoretically.

MATERIALS AND METHODS

Yanaco MP-S3 microscopic melting point apparatus was chosen for finding the melting points of the synthesized compounds. The IR spectra were taken in KBr pellets on a Brucker Equinox 55 FT-IR apparatus. The ^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded on NMR-JEOL GSX-400 spectrophotometer, tetramethyl silane was used as the internal reference

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https://doi.org/10.53879/id.59.09.13227

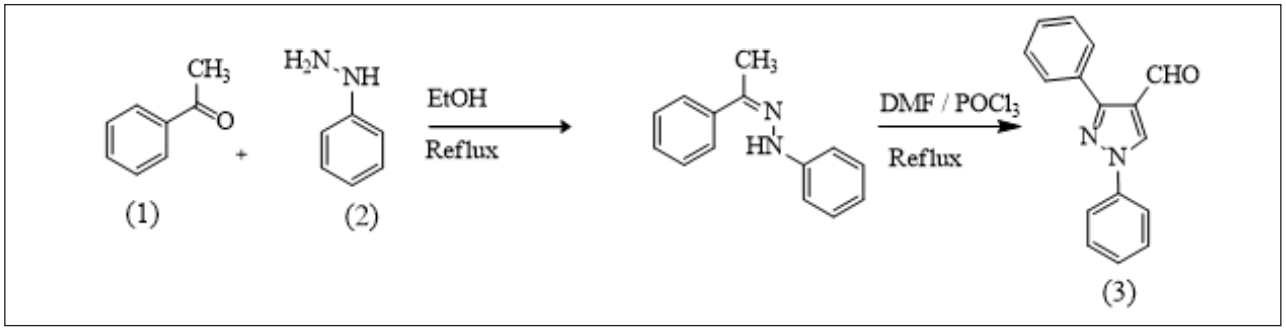

Fig. 1: Synthesis of 1,3-diphenyl-1*H*-pyrazol-4-carboxaldehyde

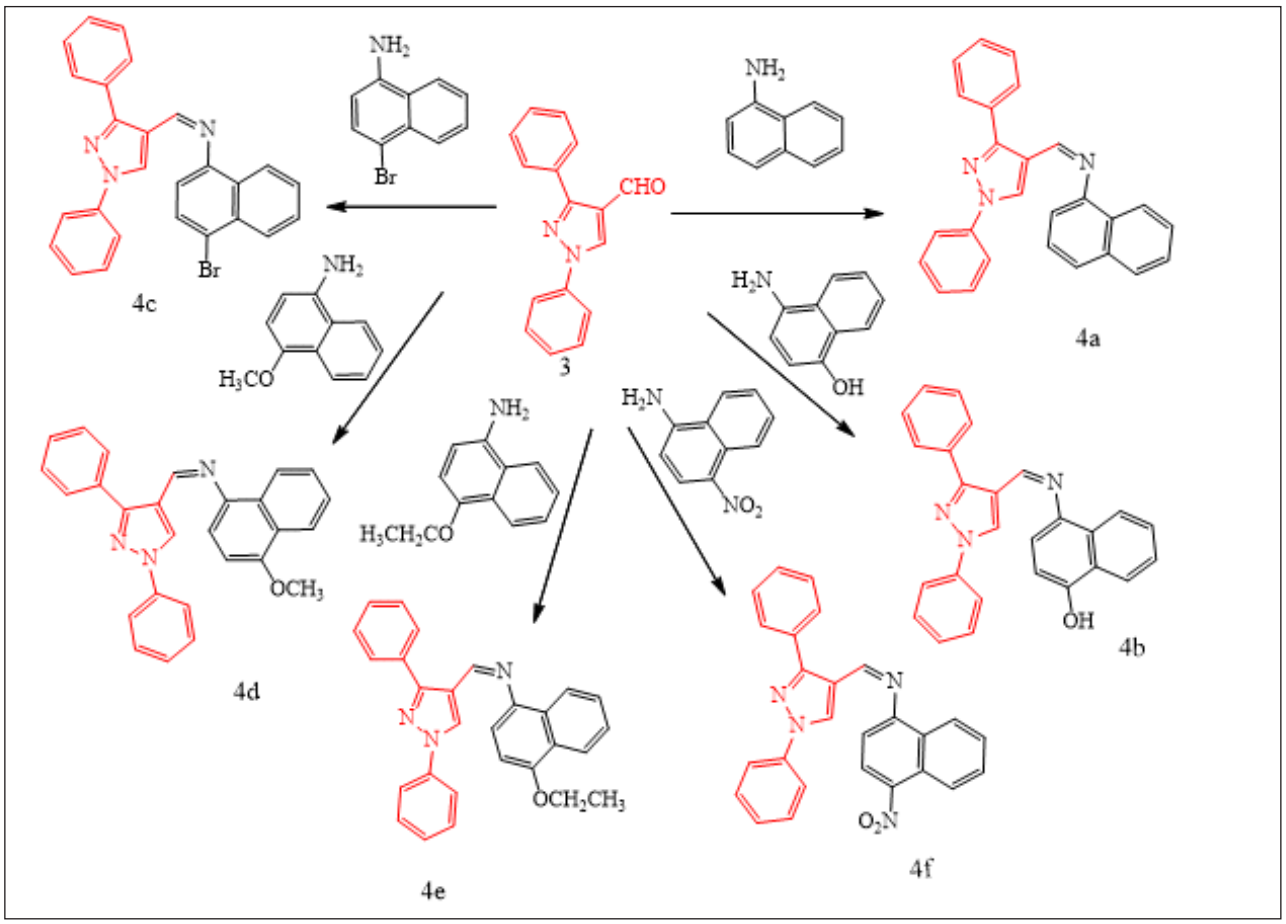

Fig. 2: Synthesis of pyrazole terminated imino naphthyl derivatives (4a-f)

and all the spectra were recorded in $CDCl_3$ solvent. Mass spectra were obtained on an HP 1100 LC-MS (ESI). All the reagents used for the present work were commercial products of analytical grade and were used without further purification except where they were especially needed.

General procedure

The intermediate 1,3-diphenyl pyrazol-4-carboxaldehyde (3) was synthesized by the condensation reaction between acetophenone (1) and phenylhydrazine (2), followed by the Vilsmeier-Haack reaction, as shown in Fig. 1. The formed pyrazole-4-carboxaldehyde was condensed with naphthyl amino derivatives to yield C-phenyl *N*-phenyl pyrazole terminated imino naphthyl derivatives (4a-f) (Fig. 2).

Synthesis of *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl) methylene) naphthalen-1-amine (4a): The compound was obtained when 1,3-diphenyl pyrazol-4-carboxaldehyde was refluxed at 90 ^{0}C with 1-naphthylamine for 4 h. Yield: 52 %, appearance, red coloured solid, m.p.: 196 ^{o}C; 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): 10.34 (s, 1H, HC=N), 9.56 (s, 1H, CH of pyrazole ring), 7.32-8.19 (m, 17H, aromatic H); ^{13}C NMR (ppm): 113-150.6 (all aromatic carbons), 164.24 (HC=N, imino carbon); MS: m/z 373(M^+).

Synthesis of 4-(((1,3-diphenyl-1*H*-pyrazol-4-yl) methylene) amino) naphthalen-1-ol (4b): 1,3-Diphenyl pyrazol-4-carboxaldehyde undergoes condensation reaction with 4-aminonaphthol to yield 4b on reflux at 90 ^{0}C for 3.5 h. Yield: 36 %, yellow powder, m.p. 126 ^{0}C; IR (cm^{-1}): 3414 (OH str), 3039 (ArCH str), 1454 (ArC=C str), 1628 (C=N str), 1229 (C-N str); ^{1}HNMR (ppm): 4.97 (s, 1H, OH), 8.94 (s, 1H, HC=N), 8.47 (s, 1H, CH of pyrazole ring), 6.93-8.36 (m, 16H, aromatic H), ^{13}CNMR (ppm): 107 -146 (all aromatic carbons), 157 (HC=N, imino carbon), Ms: m/z: 389 (M^+).

Synthesis of 4-bromo-*N*-((1,3-diphenyl-1*H*-pyrazol-4-yl) methylene) naphthalen-1-amine (4c): The compound was synthesized by the condensation reaction between 1,3-diphenyl pyrazol-4-carboxaldehyde and 4-amino-1-bromonaphthalene at 90 ^{0}C for 4 h. Yield: 34 %, dark orange powder, m.p. 148 ^{0}C; IR (cm^{-1}): 3035 (ArCH str), 1447 (ArC=C str), 1618 (C=N str), 1225 (C-N str), 741 (C-Br str); ^{1}HNMR (ppm): 8.99 (s, 1H, HC=N), 8.47 (s, 1H, CH of pyrazole ring), 7.05-8.52 (m,16H, aromatic H); ^{13}CNMR (ppm): 109-142 (all aromatic carbons), 167.24 (HC=N, imino carbon);; Ms: m/z: 452 (M^+).

Synthesis of *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl) methylene)-4-methoxy naphthalen-1-amine (4d): The condensation reaction was carried out between 1, 3- diphenyl pyrazol-4-carboxaldehyde and 4-amino-1-methoxy naphthalene at 90 ^{0}C for 4 h to obtain 4d. Yield 30 %, dark yellow solid, m.p. 129 ^{0}C; IR (cm^{-1}): 3038 (ArCH str), 1459 (ArC=C str), 1619 (C=N str), 1231 (C-N str); ^{1}HNMR (ppm): 3.14 (s, 3H, OCH_3), 9.17 (s, 1H, HC=N), 8.48 (s, 1H, CH of pyrazole ring), 7.02-8.24 (m, 16H, aromatic H), ^{13}CNMR (ppm): 109-142 (all aromatic carbons), 162.8 (HC=N, imino carbon), 54 ppm (methoxy carbon); Ms: m/z: 403 (M+).

Synthesis of *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl) methylene)-4-ethoxy naphthalen-1-amine (4e): This was obtained by the condensation reaction between 1,3- diphenyl pyrazol-4-carboxaldehyde and 4-amino-1-ethoxynaphthalene at 90 ^{0}C for 4.5 h to obtain 4e. Yield 31 %, dark yellow solid, m.p. 131 ^{0}C; IR (cm^{-1}): 3041 (ArCH str), 1462 (ArC=C str), 1625 (C=N str), 1238 (C-N str); 1HNMR (ppm): 1.16 (t, 3H, CH), 4.54 (q,CH2), 9.17 (s, 1H, HC=N), 8.48 (s, 1H, CH of pyrazole ring), 7.02-8.24 (m, 16H, aromatic H), ^{13}CNMR (ppm): 109-144 (all aromatic carbons), 164.3 (HC=N, imino carbon), 59 and 76 ppm (ethoxy carbon); Ms: m/z: 417 (M^+).

Synthesis of *N*-(1,3-diphenyl-1*H*-pyrazol) methylene)-4-nitronaphthalen-1-amine (4f):

The compound was obtained by the reaction between 1,3-diphenyl pyrazol-4-carboxaldehyde and

Table I: Antibacterial, antifungal and anti-tuberculosis activities of pyrazole clubbed phenyl derivatives (inhibition zone measured in mm)

Entry	*S. aureus* (mm)	*E. coli* (mm)	*P. aeruginosa* (mm)	*S. pyrogenes* (mm)	*A. niger* (mm)	*C. albicans* (mm)	*M. tuberculosis* (mm)
4a	7	9	11	12	0.2	100	100
4b	13	15	18	18	0.2	75	100
4c	19	21	22	22	0.2	100	30.2
4d	12	13	13	15	0.2	100	30.2
4e	12	8	10	13	0.2	100	100
4f	16	21	18	21	0.2	50	30.2
Ciprofloxacin	24	22	23	23	-	-	-
Fluconazole					30	30	-
INH							0.4

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

Table II: Binding energy of the compound and H-bond length calculated using ArgusLab 4.0.1

Entry	Binding energy (Kcal mol^{-1})	H bond length (A^0)
4a	-11.23	-
4b	-11.09	2.81 (301 SER with O of OH), 2.91 (274 ARG with O of OH)
4c	-10.87	-
4d	-10.77	2.92 (48 ARG with OCH_3)
4e	-10. 92	2.42 (70 ARG with O of OEt), 2.83 (97 SER with O of OEt) -
4f	-10.21	

4-nitronaphthylamine at 90 ^{0}C for 4.5 h. Yield: 42 %, reddish brown solid, m.p. 146 ^{0}C, IR (cm^{-1}): 3044 (ArCH str), 1449 (ArC=C str), 1616 (C=N str), 1214 (C-N str), 1399 and 1549 (NO stretching); ^{1}HNMR (ppm): 9.64 (s, 1H, HC=N), 8.35 (s, 1H, CH of pyrazole ring), 7.00-8.12 (m, 16H, aromatic H), ^{13}CNMR (ppm): 109-142 (all aromatic carbons), 164.3 (HC=N, imino carbon) Ms: m/z: 418(M^+).

Antibacterial activity

The antibacterial activity of all the synthesized pyrazole derivatives has been studied by adopting standard protocols available in the literature[20]. Antibacterial activity (*in vitro*) of the synthesized compounds was established against the representative panel of bacteria such as *Escherichia coli* MTCC 443*, Pseudomonas aeruginosa* MTCC-1688*, Staphylococcus aureus* MTCC-96 and *Streptococcus pyogenes* MTCC-442. Ciprofloxacin was selected as the standard antibacterial drug. Here we regulated the bacterial suspension with sterile saline to a concentration of $1*10^7$ CFU mL^{-1}. Different inoculums were made and were preserved at 4 ^{0}C for further use. Contamination in the inoculums was checked as all the dilutions were subjected to culture on a solid medium. Entire experiments were performed in triplicate stage with controls and repeated thrice.

Antimicrobial activities

Antifungal activity was exercised against two fungal species, *Candida albicans* MTCC-227 and *Aspergillus niger* MTCC- 282. Fluconazole was chosen as the standard antifungal drug. The stock solution of various samples taken out was made by taking a concentration of 1 mg mL^{-1}. Using the broth microdilution method, the minimal inhibitory concentration (MIC) of all the synthesized compounds was determined according to National Committee for Clinical Laboratory Standards (NCCLS)[21]. All the synthesized compounds were screened for antibacterial and antifungal activities against bacteria and fungi used in the present protocol and the results are tabulated in Table I.

Antimycobacterial activity

The *in vitro* antitubercular activity was screened against *M. tuberculosis* H37Rv by microplate alamar blue assay[22] and the results are tabulated in Table I. All our compounds showed very weak antitubercular activity at MIC 12.8 µg mL^{-1} vis-à-vis 0.4 µg mL^{-1} for standard drug INH. This less activity might probably be due to their lower lipophilicity and the reduced cell wall permeation.

Molecular docking

Molecular docking studies with protein were carried out with the help of the software ArgusLab 4.0.1 version. Binding conformations of the prepared compounds and their free energies of binding in the active site of the target protein thymidylate kinase (TMPK) (PDB Id: 4QGG downloaded from PDB database) were tabulated. Before running the docking with protein, all the miscellaneous residues, water molecules, and heterocyclic compounds were removed from the protein crystallographic structure, and subsequently, hydrogens were added to the amino acid residues of the protein to activate the binding site only for the synthesized compound. H - bond lengths were also tabulated (Table II, Fig. 3). A calculation box was created and placed at the centre of the binding site residues with 60 x 60 x 60 grid points in XYZ directions. The docking process was achieved by assuming that the protein is rigid, and the ligand molecule is flexible (all the rotatable bonds of ligands are considered) the analysis was carried out with standard precision with default values).

RESULTS AND DISCUSSION

The title compounds were synthesized successfully by the condensation of acetophenone with phenyl hydrazine followed by Vilsmeier-Haack reaction to yield pyrazol-4- carboxaldehyde. The formed C-phenyl *N*-phenyl pyrazol-4-carboxaldehyde again underwent condensation reaction with substituted naphthyl amines to form pyrazole terminated imino naphthyl derivatives and all were well characterized by different spectroscopic techniques.

Antimicrobial activity

Among the pyrazole terminated imino naphthyl derivatives, *S. aureus* is found to be more active against

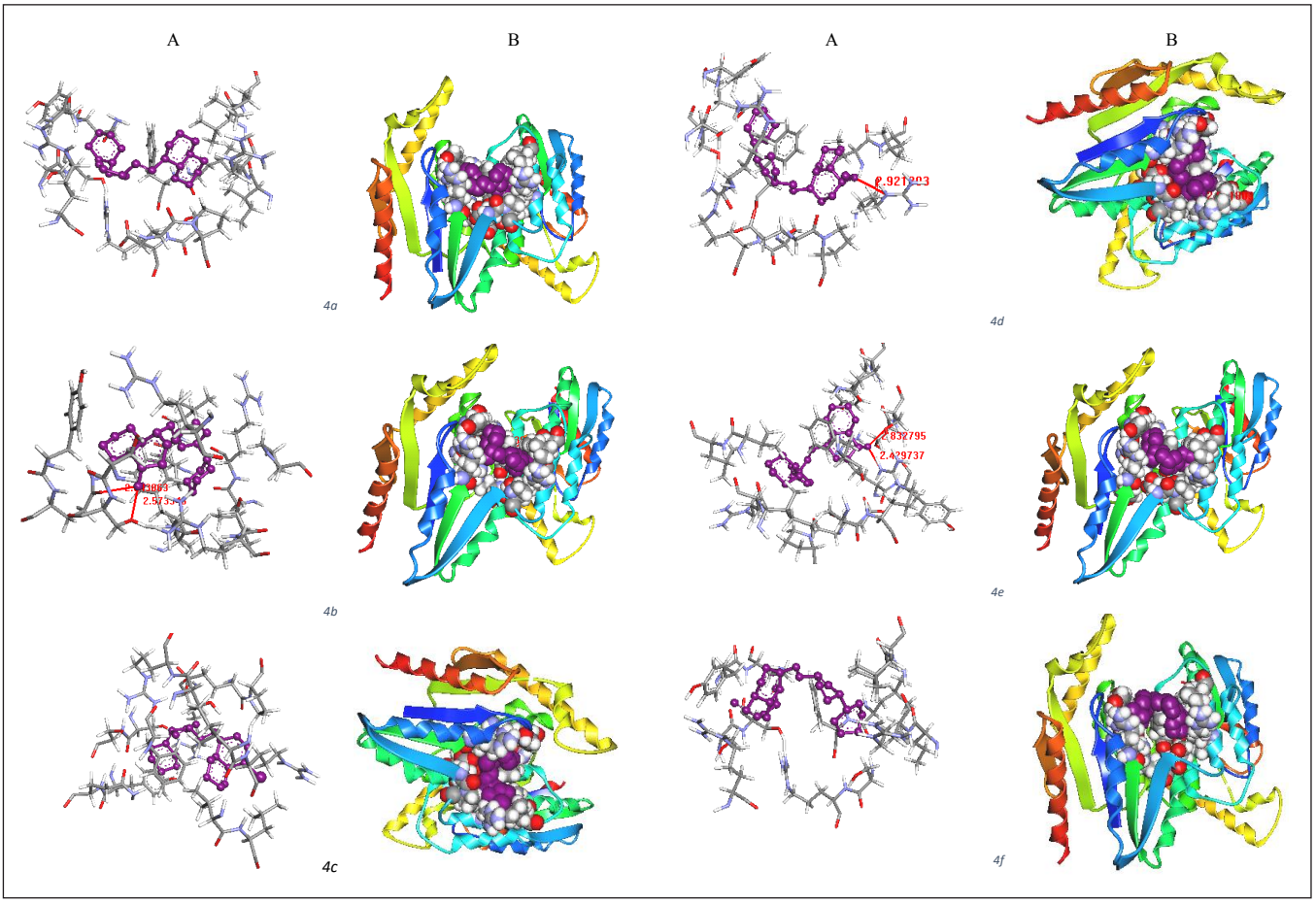

Fig. 3: The docked pose of the compounds (4a-f, purple colour) in the active site of the target protein grey and blue colour (A) and its cartoon view (protein as ribbon) (B)

4c and 4f and inactive against 4a and 4e. The compounds 4b, 4c, and 4d are active against *E. coli* and inactive against 4a and 4e. Compound 4c and 4f are more active against *P. aeruginosa* while 4e is less active. *S. pyrogenes* showed activity against 4b, 4c, and 4f and nil activity against 4a and 4e. The phenyl and naphthyl derivatives of pyrazoles exhibited an outstanding antifungal activity with MIC 0.2 µg mL^{-1} against *A. niger* than fluconazole and this activity is found to be independent of substituents. All exhibited a good antifungal activity against *C. albicans* (MIC 50 µg mL^{-1}) and also these compounds are less active than standard antifungal fluconazole (MIC 30 µg mL^{-1}).

Structure-activity relationship (SAR)

The presence of either electron releasing or electron withdrawing group on imino naphthyl ring extends the conjugation and this will affect the bioactivity and thus exhibit a broad spectrum of antimicrobial activity. It is expected that bioactivity for these compounds may be due to the combination of the factors such as differently substituted pyrazole rings, the presence of the imino linkage, steric hindrance, extension of conjugation, and the presence of aryl rings on pyrazole moiety. Most the heterocyclic compounds are exhibiting pharmacological behaviour and it was true in this study also. SAR studies were carried out to understand the effect of different substitutions on imino naphthyl ring and the electronic effect on microbial strain[22]. The substituents on imino naphthyl ring were selected carefully for establishing different electronic environments on the new molecules. Methoxy and ethoxy groups were selected as electron-donating groups to the aromatic ring and hydroxy, nitro, and bromo groups were electron-withdrawing groups from the aromatic system[23]. From our experiment, the compounds with electron-withdrawing groups (on imino naphthyl ring) showed a marked biological activity. Moreover, the presence of hydrophobic substituents at the 4th position of the naphthyl ring giving a positive impact on antimicrobial activity and its physiochemical properties. The activity order of the different pyrazole derivatives (substituent at the 4th position of the imino naphthyl ring) follows as NO_2>Br>OH>H>OCH_3>OCH_2CH_3[24]. From the literature,

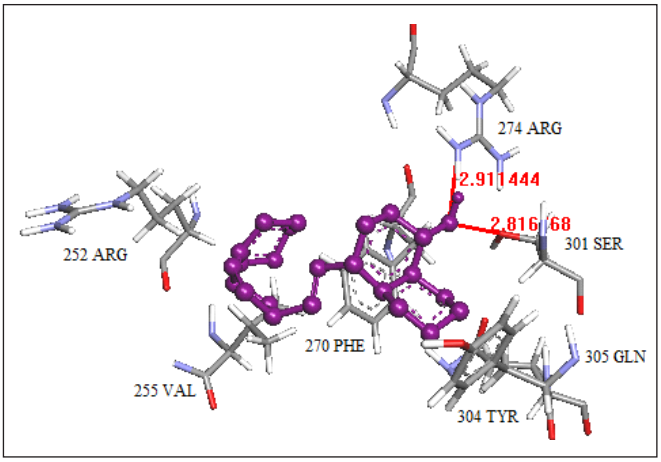

Fig. 4: The closer view of the docking complex 4b (purple colour) to justify the interaction behaviour in the binding pocket of the target protein (light grey and blue colour). The red colour bond is the H bond in A⁰. The above interactions contributed to the stability of the 4b docking complex

the chain length is inversely proportional to the inhibitory activity, generally decreasing, and with the synthesized compounds, the methoxy derivative was more active than the corresponding ethoxy compounds[25]. The *in vitro* activity against *E. coli* can be enhanced by the introduction of electron-withdrawing groups such as bromo, nitro, and hydroxy groups. Similarly, activity against *S. aureus*, electron-withdrawing groups at the 4th position of imino naphthyl ring showed good activity and inhibition (MIC= 12.5 µg mL^{-1}). From Table I, nitro, bromo, and hydroxy substituents at the 4th position of imino naphthyl groups are active against *S. pyogenes* at 50 µg mL^{-1} MIC.

Molecular docking studies with protein

The antibacterial drugs generally act by mechanisms such as the inhibition of cell wall synthesis, inhibition of protein synthesis, inhibition of nucleic acid synthesis, and anti-metabolism[26]. That is its action is interaction with the specific proteins which are responsible for the above-mentioned routes. Thymidylate kinase (TMPK) is the selected protein that contains 50 monophosphate kinase, and the essential enzyme present in it catalyzes the biosynthesis of DNA of bacterial cells. This protein is generating dTTP for the above cell wall synthesis[27]. The standard antibacterial drug ciprofloxacin generally inhibits the DNA gyrase which is necessary for the separation of the bacterial DNA and thus inhibits the cell wall division.

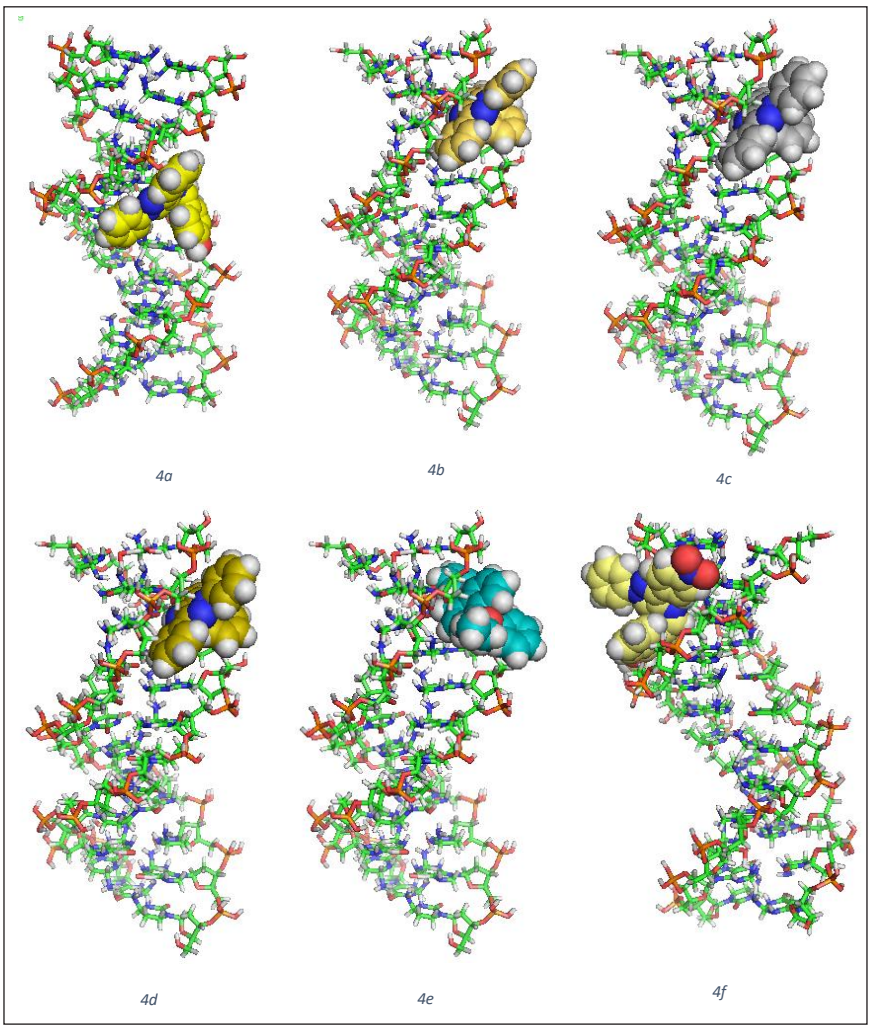

Fig. 5: Molecular docked model of 4a-f with DNA dodecamer duplex of sequence d(CGCGAATTCGCG)2(PDB ID: 1BNA)

The synthesized compounds 4a-4f docked in the active site of the target protein TMPK using the software ArgusLab 4.0.1 and are tabulated in Table II. Here the active site of docking was performed by autodock which made the ligands bind inside the active site of the protein TMPK. The H bond length of the synthesized compounds within the active site of the proteins is also tabulated.

Binding energy evaluation

Evaluation of binding free energy of the ligand within the active site of the protein helps to understand the accuracy of binding affinity between the target protein and the docking models. From the literature, we understood to know that the lower the value of binding energy

more will be the binding strength of the ligand within the protein. To compute the binding strength of our synthesized compounds, the ligand - protein docked complexes were analyzed, based on the minimum binding energy values and ligand interaction (hydrogen / hydrophobic) pattern, and H-bond length was measured. The results showed that the synthesized compounds were possessing good binding energy values ranging from -10.21 to -11.23 Kcal mol^{-1} as mentioned in Table II. It was also observed from the literature that the predicted binding energy values were not higher than 2.5 Kcal mol^{-1} indicating the synthesized compounds were well fitted in the active pocket of the targeted protein.

Binding pocket and structure-activity relationship analysis

The docking studies revealed how the ligand is bonded within the active site of the target protein, as shown in Fig. 4. From the antibacterial activity studies, the compound 4b was found to be one of the active compounds and therefore it was selected to check its binding profile against the target protein. The docking study also supported that 4b firmly binds within the binding pocket of TMPK and thus inhibits the function of TMPK for DNA synthesis. The amino acid residues 270 PHE and 272 ARG binds with the ligand 4b through electrostatic interaction. Other amino acids like 255 VAL, 304 TYR, and 305 GLN bind with 4b through pi-sigma bonding. There are two H bond interactions, one with the amino acid 274 ARG and the other with 301 SER with OH moiety with bond distances 2.91 A^0 and 2.81 A^0 respectively. The molecular docking studies proved that the structure of our synthesized compounds could be used as a therapeutic agent for bacterial infections.

The compound 4d forms an H bond with the amino acid moiety 48 ARG with O of OCH_3 of the compound with a bond length of 2.92 A^0 and the compound 4e is forming two different H bonds with 97 SER and 70 ARG with a bond length of 2.83 and 2.42 A^0 respectively with O of OCH_2CH_3. The compounds 4a, 4c and 4f are not showing H bond interaction.

Molecular docking with DNA

Molecular docking analysis is an important approach to recognize the nature of drug– DNA interaction for the synthesis of the drug and its design as a new chemotherapeutic drug as well as in the study of its mechanism when a new molecule is introduced into the binding site of the DNA target specific region of the DNA mainly in a non-covalent manner[28]. Still, there are different structural properties to determine the binding modes; here we exercised the shape of the molecule for DNA binding to bind either in a major groove or manor groove as the binding site for the synthesized compounds. Literature reports reveal that the forces responsible for the stability of the DNA-intercalator complex include van der Waals, hydrogen bonding, hydrophobic, charge transfer and electrostatic interactions[29,30]. The strength of the molecule to act as a biologically active drug depends on its favorable conformation and binding location within the DNA. The DNA binding conformations for 4a-f were done with CT-DNA duplex of sequence d(CGCGAATTCGCG)2 dodecamer (PDB ID: 355D) and the most favourable docked poses are given in Fig. 5. It can be seen from Fig. 5 that all the compounds could bind with DNA in an interactive fashion near the minor groove. The planarity of the compounds strengthens the binding of these compounds via partial intercalation with DNA. From literature, small molecules prefer to interact with minor grooves due to little steric hindrance[31]. Moreover, the presence of an aromatic ring connected by single bonds allows for a torsional strain to facilitate the curvature of the groove with a displacement of water molecules. Also, the presence of a heterocyclic ring in the molecule makes favorable stacking interactions between DNA base pairs, resulting in van der Waals interactions and hydrophobic contacts with DNA functional groups that define the groove[32]. Thus, the molecular modeling studies focus on the binding modes through which these compounds interact with DNA.

CONCLUSION

A series of five new 1,3-diphenyl pyrazole-linked imino naphthyl derivatives were synthesized and characterized and investigated their antibacterial activity against gram-negative bacterial strains such as *E. coli* MTCC-443 and *P. aeruginosa* MTCC-1688 and two – gram-positive strains as *S. aureus* MTCC-96 and *S. pyogenes* MTCC-442. Antifungal and antituberculosis activities were also investigated. Most of the synthesized imino pyrazole derivatives were very active, which might be due to the greater lipophilicity of two different phenyl and one naphthyl group present in the molecule. It may be also noticed that the electron-withdrawing substituent present at the 4^{th} position of the imino naphthyl ring enhanced its biological activity. By running the molecular docking studies, minimum ligand poses binding energy and H-bond information of all the synthesized compounds in the protein TMPK were noted. The synthesized compounds bond somewhat firmly with the selected protein, and many of them also formed H-bond interactions which selectively hinder the action of the protein, acting as an antibiotic. The molecular docking with DNA also holds up the non-

covalent interactions into the groove binding mode and thus preventing its replication.

ACKNOWLEDGEMENTS

One of the authors (Sandhya P.V.) would like to acknowledge the financial support from the University Grants Commission, India.

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