Research Article

Synthesis and molecular docking studies on biologically active N-((3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl) methylene) aniline derivatives

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Received: 28 September 2021 Revised: 30 October 2021 Accepted: 1 November 2021

Abstract

Objective: Pyrazoles and its derivatives have been explored in past and are still in practice for a variety of biological applications which make this scaffold as an interesting moiety for scientists especially in synthesis and designing new broad-spectrum pharmacophore. The objective of present study was to introduce a simple and efficient method to synthesis the compounds containing both substituted imino phenyl and pyrazole ring and investigate their biological activities both experimentally and theoretically. **Materials and methods**: The five membered N containing heterocyclic compounds have depicted various admirable biological properties in form of antioxidant, analgesic, antibacterial, antifungal activities. Clubbing imino or azo moiety to this compound has given a positive impact on its biological properties. Here we focused to synthesis pyrazole terminated imino phenyl derivatives and all the compounds were characterized by different spectroscopic techniques. The biological activities of the synthesized compounds were screened. The computational studies such as molecular docking with protein and DNA were also carried out. **Results**: All the synthesized compounds displayed moderate to good biological activities both experimentally and theoretically. **Conclusion:** From our compounds, pyrazole derivatives with electron withdrawing group at the 4th position of imino phenyl ring were showing more biological activities and molecular docking studies supported this finding.

Keywords: Vilsmeir -Haack reaction, pyrazole derivatives, antibacterial, antifungal, alamar blue assay, molecular docking, DNA binding

Introduction

Heterocycles are well known for displaying a wide range of biological properties (Monica Arora et al., 2013). The structural diversity and biological importance of nitrogen containing heterocycles have made them attractive synthetic targets over many years (Azam et al., 2007). In general, well-known five–membered heterocyclic compounds are oxadiazoline, pyrazole and pyrazoline derivatives. Compounds with pyrazole ring are of interest due to their broad spectrum of biological activities against NOS inhibitor (Carrión et al., 2008), monoamine oxidase inhibitor (Gökhan-Kelekçi et al., 2009), antibacterial (Faria et al., 2017), antiamoebic (Abid et al., 2009). Moreover, *N*-phenyl pyrazole derivatives play an important role in antitumor screening (Naito et al, 2002) as well as potent antimicrobial activity (Farag et al., 2008a). Furthermore, several hydrazide-hydrazone derivatives also have been claimed to possess interesting bioactivity such as antifungal (Karrouchi et al., 2018), anticonvulsant (Abdel-Aziz et al., 2009), antiinfloammatory (Farag et al., 2008b), anticancer activities (Chalina et al., 1998)1, antiviral (Shih SR et al., 2010), and anticoagulant (Michon et al., 1995). The pyrazole derivatives play important roles in the development of pesticides, insecticides, and herbicides (Saad et al., 2011).

From the literature, it was observed that the compounds with imino group are exhibiting excellent biological activities such as inhibition of DNA and RNA, protein synthesis, carcinogenesis (Balbi et al., 2006), and nitrogen fixation. The imino compounds are also having application in the field of hypnotic drugs for nervous system as well as showing

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DOI: https://doi.org/10.31024/ajpp.2021.7.5.2

biological activities against bacteria and fungus (Dai et al., 2014).

Herein, we focused to synthesis N-phenyl and C-4-methoxyphenyl pyrazole terminated imino phenyl derivatives, recognizing the importance of both pyrazole ring and imino moiety in biological activities. It is expected that the presence of methoxy phenyl group on pyrazole and different substituents on imino phenyl group may have an effect in the in vitro antibacterial and antifungal activities of these potential chemotherapeutics. In this work, we developed a simple and efficient method for the synthesis biologically active pyrazole terminated imino phenyl derivatives, all the synthesized compounds were characterized using IR, NMR, mass spectroscopy and elemental analysis and screened their biological activities.

Materials and methods

All chemicals and reagents used in the synthesis were of analytical grade and purchased from Sigma-Aldrich and Merck. All reagents were commercial products of analytical grade and were used directly without purification except where they were especially noted. Melting points were taken on a Yanaco MP-S3 microscopic melting point apparatus. The FT-IR spectra were recorded in KBr pellets on a Brucker Equinox-55 FT-IR apparatus. The ^{1}H- NMR spectra were recorded on an INOVA-400 (using TMS as internal standard, DMSO-*d*6 as solvent).

General procedure for the synthesis of the title compounds

The general procedure for the synthesis of the intermediates and target compounds were illustrated in Figure 1 and 2. Intermediate 1-phenyl-3-methoxy phenyl pyrazol-4-carboxaldehyde (3) was prepared from 4-methoxy acetophenone (1) and phenyl hydrazine (2) and followed by Vilsmeir-Haack reaction as shown in scheme 1. The formed pyrazole-4-carboxaldehyde on condensation reaction with p-substituted aniline to afford the title compounds (4a-4f) illustrated in Figure 2.

Synthesis of 4-(((3-(4-methoxyhenyl)-1-phenyl-1*H*-pyrazol-4-yl) methylene)amino)phenol (4a)

The compound was obtained by the reaction between 3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazole -4-carboxaldehyde with 4-aminophenol. Yield 48 %, brown solid, mp 164 ^{0}C, IR cm^{-1}: 3420 (OH str), 3054 (ArCH str), 1471 (ArC=C str), 1646 (C=N str), 1239 (C-N str); ^{1}HNMR (δ ppm): 3.73 (s, 3H, OCH_3), 5.02 (s, 1H, OH), 8.95 (s, 1H, HC=N), 8.52 (s, 1H, CH of pyrazole ring), 6.98-8.13 (m, 13H, aromatic H), ^{13}CNMR (δ ppm): 107 -145 (all aromatic carbons), 19.03 (methoxy carbon)), MS: m/z 370 (M^+).

Synthesis of 4-bromo-N-((3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene) aniline (4b)

The compound was obtained by the reaction between 3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazole -4-carboxaldehyde with 4-bromoaniline.Yield 66 %, reddish brown solid, mp143 ^{0}C, IR cm^{-1}: 3038 (ArCH str), 1449 (ArC=C str), 1611 (C=N str), 1222 (C-N str), 744 (CBr str); ^{1}HNMR (δ ppm): 3.78 (s, 3H, CH_3), 9.16 1H, HC=N), 8.86 (s, 1H, CH of pyrazole), 7.08 -8.42 (m, 13H, aromatic H), ^{13}CNMR (δ ppm): 118 ppm -159 ppm (all aromatic carbons), 14.96 (methoxy carbon), MS: m/z 432 (M^+).

Synthesis of 4-(methoxy-N-((3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl) methylene) aniline (4c)

The condensation reaction was carried out between 3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazole -4-carboxaldehyde with 4-methoxyaniline to obtain 4c. Yield 54 %, yellow solid, mp 143 ^{0}C, IR cm^{-1} : 3035 (ArCH str), 1449 (ArC=C str), 1612 (C=N str), 1221 (C-N str), ^{1}HNMR (δ ppm): 3.01 (s, 6H, two OCH_3), 9.09 (s, 1H, HC=N), 8.93 (s, 1H, CH of pyrazole), 7.05-8.32 (m, 13H, aromatic H), ^{13}CNMR (δ ppm): 107 ppm -143 ppm (all aromatic carbons), 28 ppm (methoxy carbon) MS: m/z 383 (M^+).

Synthesis of 4-(ethoxy-N-((3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl) methylene) aniline (4d)

The condensation reaction was carried out between 3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazole -4-carboxaldehyde with 4-ethoxyaniline to obtain 4d. Yield 49 %, yellow powder, mp 143 ^{0}C, IR cm^{-1}: 3041 (ArCH str), 1453 (ArC=C str), 1620 (C=N str), 1228 (C-N str), ^{1}HNMR (δ ppm): 0.99 (t, 3H, CH_3), 3.27 (s, 3H, OCH_3), 4.34 (q, 2H, CH_2), 9.06 (s, 1H, HC=N), 8.89 (s, 1H, CH of pyrazole), 7.07-8.12 (m, 13H, aromatic H), ^{13}CNMR (δ ppm): 105 - 147 (all aromatic carbons), 68.5, 55.7 and 14.08 ppm (aliphatic carbons); MS: m/z 398 (M^+).

Antibacterial activity

The in vitro antibacterial activity (Desai et al., 2013) of the all the synthesized compounds have been investigated by adopting standard protocols available in the literature. The studies were carried on both gram-negative and gram-positive bacterial strains. Escherichia coli MTCC443 and Pseudomonas aeruginosa MTCC-1688 were selected as two different gram-negative bacteria and Staphylococcus aureus MTCC-96 and Streptococcus pyogenes MTCC-442 represented gram-positive bacterial strains. Ciprofloxacin was selected as the standard antibacterial drug.

Antifungal activity

Antifungal activity was screened against two fungal strains such as Candida albicans MTCC-227 and Aspergillus niger MTCC-282. Fluconazole was chosen as the standard antifungal drug. Here we adopted broth micro-dilution

method to measure the minimal inhibitory concentration (MIC) according to National Committee for Clinical Laboratory Standards (NCCLS) (Khalil and Collins, 2010).

Antimycobacterial activity

The in vitro antitubercular activity was carried out against M. tuberculosis H37Rv by microplate alamar blue assay method (Zhan et al., 2014). Isonicotinic acid hydrazide (INH) was chosen as the standard antituberculosis drug, and all the compounds were screened (Young et al., 2014).

Molecular docking

In the present study we used the software Arguslab 4.0.1 version for docking to set up input file. First, we optimized the structures of all the compounds, after that its binding conformation in the active site of the selected protein thymidylate kinase (TMPK) (PDB Id: 4QGG, downloaded from PDB database) was prepared. Before running the docking all the miscellaneous residues, water molecules, and heterocyclic compounds present in the crystallographic structure of the target protein were removed to activate the binding sites of each protein only for the synthesized compounds and subsequently hydrogen atoms were added to amino acids. The docking process was proceeded by assuming that the ligand molecule is flexible (all the rotatable bonds of ligands are considered) and the target protein is rigid. A calculation box was generated with 60 x 60 x 60 grid points in XYZ directions and placed at the centre of the binding site residues and the docking was started with standard precision with default values.

Results and Discussion

The chemistry behind the synthesis of the title compounds is that the condensation of methoxy acetophenone with phenyl hydrazine followed by Vilsmeir-Hack reaction to yield 3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazole -4- carboxaldehyde, which on again undergo condensation with p-substituted amino compound to form imino phenyl compounds (4a-d). All the synthesized compounds were characterized by different spectroscopic techniques.

Antimicrobial activity

From the antibacterial activity data illustrated in table 1, the compounds 4a and 4b are active against *S. aureus* and 4c and 4d are inactive. All the synthesised compounds showed an activity (weak to high activity) against E. coli. The compounds 4b is highly active against *P. aerugivosa,* 4a and 4c are showing weak activity and 4d is almost inactive. The compounds 4a and 4b are exhibiting moderate activity towards *S. pyrogenes* 4c and 4d are less active. All our compounds displayed an outstanding antifungal activity with MIC 0.2 µg/ mL against *A. niger* than the standard drug fluconazole and this activity is found to be independent of the substituents on imino phenyl ring. Moreover, the compounds 4a showed good antifungal activity (MIC 50 µg/mL) against *C. albicans* than rest of the compounds. However, they all were exhibiting less activity than standard drug fluconazole (MIC 30 µg/mL).

Structure Activity Relationship (SAR)

The biological studies point out that the presence of electron releasing or withdrawing group on any of the phenyl ring of the compound extensively increases the conjugation and this will enhance the bioactivity and thus exhibiting a broad-spectrum of antimicrobial activity. The bioactivity for these compounds may be due to the combination of the factors such as substituted phenyl group on the pyrazole ring, the presence of the imino moiety, steric hindrance, and substituents at the imino phenyl ring. SAR study helps to understand the effect of different substitutions on imino phenyl and electronic effect on microbial strain (Dai et al., 2016). The substituents have been carefully chosen to generate different electronic environment around the molecules. Methoxy and ethoxy groups were selected as electron donating groups and hydroxy and bromo groups are chosen as electron withdrawing groups (Pai et al., 2016). From the antibacterial data illustrated in table 1, the

Table 1. Antimicrobial activity of pyrazole clubbed phenyl derivatives (Inhibition zone measured in mm)

Compounds	*S. aureus*	*E. Coli*	*P. aerugi*	*S. pyrogenes*	*A. niger*	*C. albicans*	*M. tuberculosis*
4a	14	16	12	15	0.2	70	100
4b	18	21	21	19	0.2	100	51.2
4c	9	14	13	12	0.2	100	51.2
4d	6	11	9	7	0.2	100	51.2
Ciprofloxacin	24	22	23	23	-	-	-
Fluconazole	-	-	-	-	30	30	-
INH	-	-	-	-	-	-	0.4

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

compounds with electron withdrawing groups on imino phenyl ring show enhanced activity. Moreover, the hydrophobic substituents at 4th position of imino phenyl ring provide a positive impact on antimicrobial activity and its physiochemical properties. From our scanning the activity order follows as the compounds with Br> OH> OCH_3 > OCH_2CH_3 (Ravi et al., 2020). It was true in our case as the chain length increases the inhibitory activity generally decrease, compound with methoxy group was more active than corresponding ethoxy compounds (De Oliveira et al., 2007).

Antituberculosis activity

The antitubercular efficacy of the prepared pyrazole terminated imino compounds was explored against M. tuberculosis by microplate blue Almar assay as reported in the literature (Mustapha et al., 2018). All the title compounds showed poor antitubercular activity (Table 1) and may be due to the lower lipophilicity as indicated by their *C* log P values, which resulted in the reduced cell wall permeation.

Molecular docking studies with protein

Generally, the working of antibiotics may be by arresting the cell wall synthesis or inhibiting the protein synthesis or interrupting the nucleic acid synthesis or by anti-metabolism (Kapoor et al., 2017). That is the antibiotic attacks the specific proteins which is responsible for any of the above noted routes. Here we selected Thymidylate kinase (TMPK) as the target protein, which contains 50 monophosphate kinase, and the essential enzyme present in it catalyses the biosynthesis of DNA of bacterial cell. This protein TMPK generates dTTP for the above cell wall synthesis (Cui et al., 2013). The action of standard antibacterial drug ciprofloxacin is inhibiting the DNA gyrase which is necessary to separate the bacterial DNA and thus resulting the inhibition of the cell wall division. The H bond length and minimum binding energy of our compounds within the active site of the target protein were measured and tabulated in Table 2. The lowest energy conformation and its binding pose of all the compounds is shown in Figure 3.

The binding free energy calculation and its value help us to measure the accuracy of affinity between target protein and the docking models. The literature says if lower the value of binding energy more will be the binding strength of the ligand in the active site of the target protein and the binding energy values ranging from -8.19 to - 9.02 Kcal / mol. The predicted binding energy values of our compounds were not higher than 2.5 Kcal / mol, indicated those were well fitted

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 (A^0)
4a	-8.53	2.79 (265 THR with O of OH), 2.44 (270 PHE with O of OH), 1.96 (266 LEU with O of OH), 1.58 (269 LEU with O of OH)
4b	-9.02	-
4c	-8.19	2.93 (268 MET with O of OCH_3 of imino phenyl), 2.50 (270 PHE with O of OCH_3 of imino phenyl), 0.823 (269 LEU with O of OCH_3 of imino phenyl)
4d	-8.98	2.83 (270 PHE with O of OCH_2CH_3), 2.57 (268 MET with O of OCH_2CH_3), 0.366 (269 LEU with

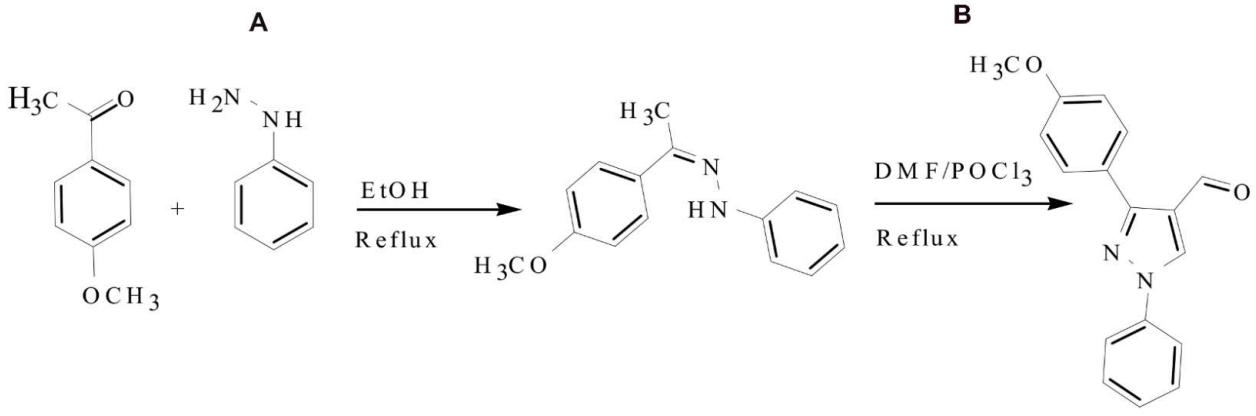

Figure 1. Schematic representation of synthesis of pyrazole carboxaldehyde

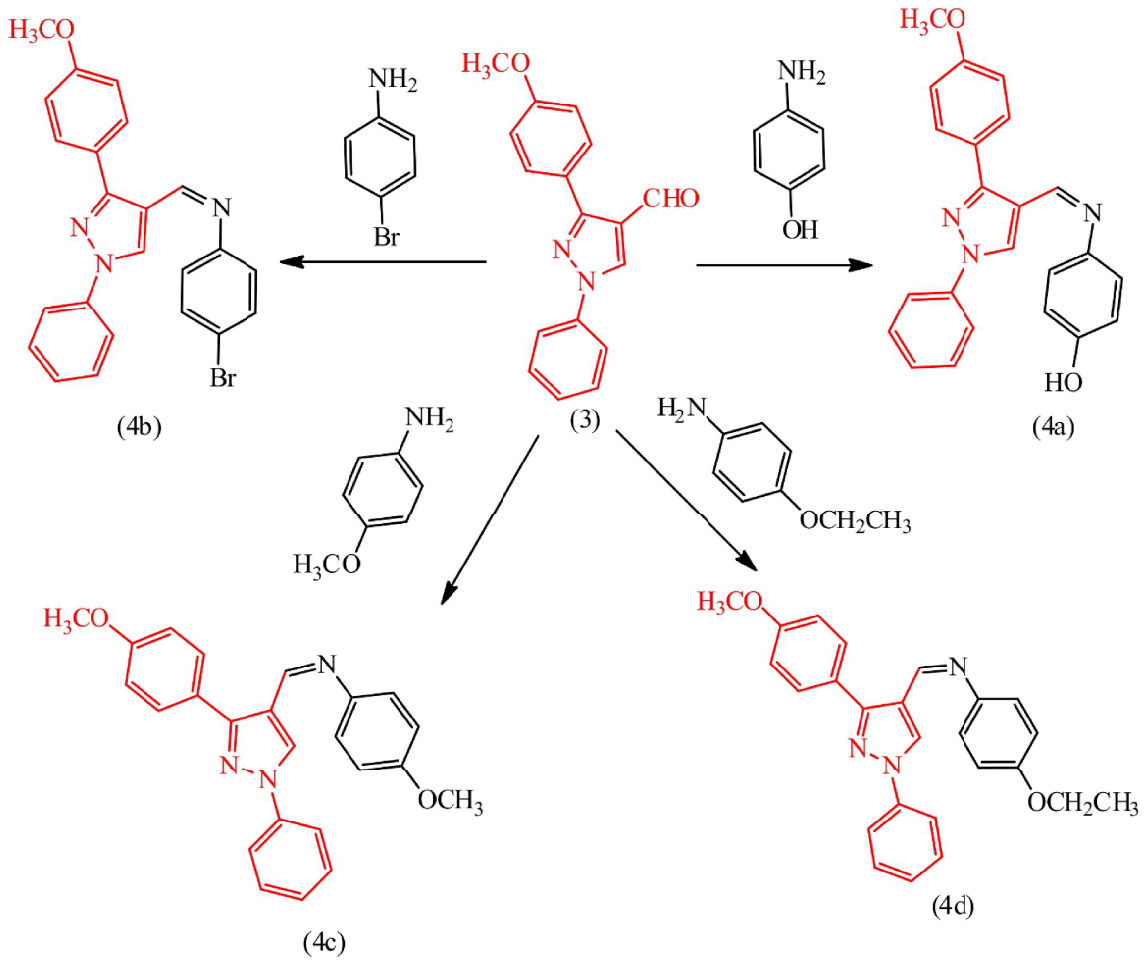

Figure 2. Schematic representation of synthesis of **4a-d**

in the active pocket of the targeted protein. The compound 4a is forming three different H bond interactions with the amino acid moiety 265 THR, 270 PHE, 266 LEU and 269 LEU with O of OH with bond length 2.79, 2.44, 1.96 and 1.58 A^0 respectively. In 4c, 268 MET, 270 PHE and 269 LEU amino acid moieties formed H bond with O of OCH_3 with bond length 2.93, 2.50, and 0.823 A^0 respectively. In compound 4d, there is three H bond interactions, 270 PHE, 268 MET and 269 LEU with O of OCH_2CH_3 with bond length 2.83, 2.57 and 0.366 A^0 respectively.

Molecular docking with DNA

Molecular docking technique is important for recognizing the nature of drug – DNA interaction in the drug design and discovery, in the development of new chemotherapeutic drug as well as in studying the binding mechanism of new molecule with the binding site of the DNA target specific region of the DNA mainly in a non-covalent manner (Rosh et al., 2005). Even though there are several factors which affect the binding modes, here we concerned only one factor, the shape of the molecule for DNA binding to bind either in major groove or manor groove as binding site. From the literature the forces responsible for the stability of DNA-intercalator complex are van der Waals, hydrogen bonding, hydrophobic, charge transfer and electrostatic complementarity (Baginski et al., 1997; Proudfoot et al., 2001). The efficiency of the molecule to act as a biologically active drug highly depends on its favorable conformation and binding location within the DNA. The binding conformations of 4a-d with DNA were performed with CT-DNA duplex of sequence d (CGCGAATTCGCG)2 dodecamer (PDB ID: 355D) and the most favorable docked poses are given in Figure 4. All our synthesized compounds could bind well with DNA in an interactive fashion near the minor groove. The planarity of the compound amplifies the binding via partial intercalation with DNA. From literature usually small molecules interacting with minor groove due to little steric hindrance (Corradini et al., 2007). Moreover, the presence of aromatic ring connected by single bonds allow for torsional strain to the molecule to enter the curvature of the groove with displacement of water molecules. Presence of heterocyclic rings in the molecule also make stacking interactions between DNA base pairs, resulting van der Waals interactions and hydrophobic contacts with DNA

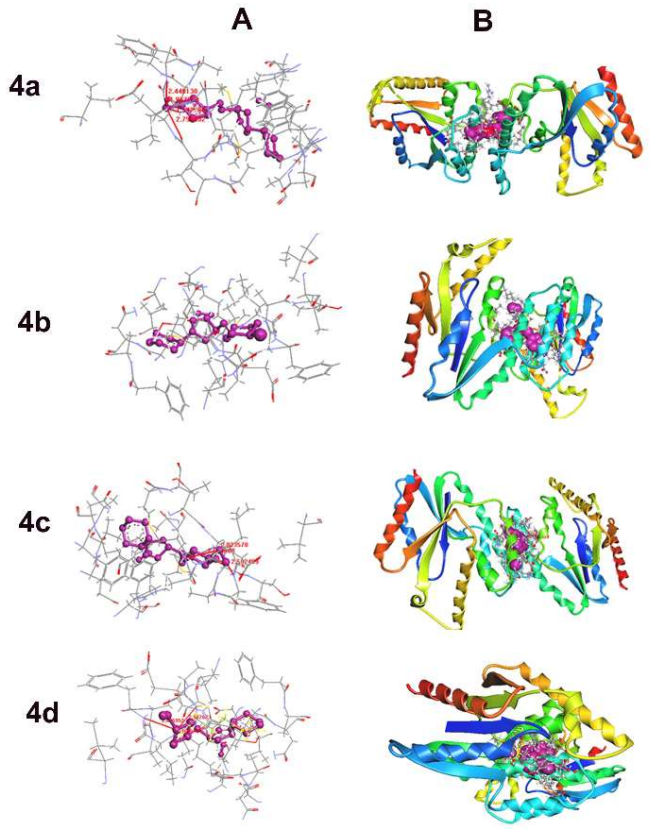

Figure 3. The docked pose of the compounds (4a – d, purple colour) in the active site of the target protein (grey and blue colour (A), H bondings in red colour and its cartoon view (protein as ribbon) (B)

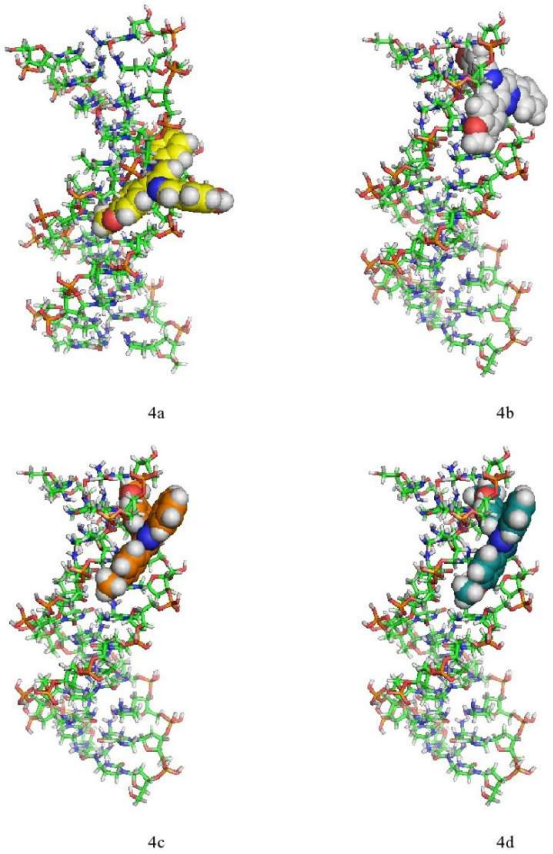

Figure 4. Molecular docked model of 4a-d with DNA dodecamer duplex of sequence d(CGCGAATTCGCG)2(PDB ID: 1BNA)

functional groups that define the groove (Hill et al., 2011; Lakshmipraba et al., 2015). Thus, our molecular modeling studies fall light on the binding modes through which these compounds interact with DNA.

Conclusion

A series of four new 1-phenyl-3- methoxy phenyl pyrazole linked imino phenyl derivatives were synthesized and characterized and investigated their antibacterial activity against gram negative bacterial strains such as Escherichia coli MTCC-443 and Pseudomonas aeruginosa MTCC-1688 and two - gram positive strains as Staphylococcus aureus MTCC-96 and Streptococcus pyogenes MTCC-442. Antifungal and antituberculosis activities were also tabulated. Most of our compounds were very active, which might be due to the greater lipophilicity of three different phenyl groups present in the molecule. It may be also noticed that the electron withdrawing substituent present at 4^{th} position of the imino phenyl ring and electron releasing group at 4^{th} position of C-phenyl of pyrazole moiety enhanced its biological activity. Carrying out the molecular docking studies, minimum ligand pose binding energy and H-bond information of all the synthesized compounds in the protein TMPK were tabulated. The molecular docking with DNA also supported the non-covalent interactions into the groove binding mode.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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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