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SYNTHESIS OF IMIDAZOLE DERIVATIVES BEARING QUINOLINE NUCLEUS CATALYSED BY CAN AND THEIR ANTIMICROBIAL, ANTITUBERCULAR AND MOLECULAR DOCKING STUDIES

Pratik G. Shobhashana, Pratibha Prasad, Anirudhdha G. Kalola, Manish. P. Patel*

Department of Chemistry,

Sardar Patel University, Vallabh Vidyanagar, Gujarat, India

ABSTRACT: In present study highly efficient, one-pot, three-component synthesis 2,4,5-trisubstituted imidazole were synthesized from benzil, ammonium acetate, and 2-phenoxyquinoline-3-carbaldehyde using ceric ammonium nitrate as a catalyst in excellent yield. Structure of newly synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, IR, mass spectroscopy. The synthesized compounds were screened for the antimicrobial activity and anti-tubercular activity. Molecular docking studies were performed to exhibit the interaction with GlcN-6-P synthase enzyme.

KEYWORDS: Imidazole; multi component reaction, ceric ammonium nitrate, 2-phenoxyquinoline-3-carbaldehyde, Molecular docking, antimicrobial and antitubercular activity.

Corresponding Author: Dr. M. P. Patel

Department of Chemistry,

Sardar Patel University, Vallabh Vidyanagar, Gujarat, India

Email Address: patelpratik853@gmail.com; patelmanish1069@yahoo.com

1.INTRODUCTION

Human beings have been in common exposure to pathogens for many years. Microbial infections are major problem in the world. There are several antimicrobial agents currently available, but critical requirement to develop more effective antimicrobial agent with least side effects. Detail literature survey on antimicrobial activity of several type of compound indicate that the presence of certain pharmacophore such as imidazole in any molecule play a significant role in enhancing activity. It is well established that small variation in the structure are changing their biological character. The research article have been published during last decade showed that heterocyclic compound with imidazole moiety, fused hetero cyclic system with imidazole and their derivatives, posses a broad spectrum of pharmacological activities. Imidazoles are known for their use in synthetic chemistry and wide range of biological activities like, antitubercular activity[1,2], anticancer activity[3], antimicrobial[4-7], antifungal activity [8] etc. Many of Quinoline derivatives are useful in many applications including pharmaceuticals and also are existing as drugs today. Quinoline derivative also have wide range of biological activity [9,10].Multi-component reactions methodology has emerged as an efficient and powerful tool in modern synthetic organic chemistry [11]. These types of reactions have some advantages including lower costs, shorter reaction times, and high degrees of atom economy [12]. Also, the most appropriate protocol for this synthesis of organic compounds would be a one-pot reaction, there is a no need to isolate of intermediate. The catalyst Ceric ammonium nitrate (CAN) has recently received considerable attention as an inexpensive, nontoxic, solubility in organic solvent, commercially available catalyst for various organic reactions such as oxidation, oxidative addition, nitration, photo-oxidation, deprotection, graft polymerization etc. [13-18]. The most attribute aspect of CAN is that it acts as a water-compatible Lewis acid in aqueous solvents. The catalytic amount is sufficient for complete reaction in most cases of this catalyst. The use of ceric ammonium nitrate as a Lewis acid in C-C bond formation reaction. So, we have synthesized imidazole based quinoline derivatives using ceric ammonium nitrate by one pot multi-component reaction.Computational biology plays a significant role in designing the drug molecules [19]. Molecular docking studies were carried to find out the interaction of synthesized molecule with the protein in comparison to the standard drugs. The protein ligand interaction is the most interesting fact because of its applications in medicines. Glucosamine-6-phosphate is a new target for antimicrobial [20]. It is an important enzyme present in all kind of cell. The enzyme, namely glucosamine-6-phosphate synthase (GlmS, GlcN-6-P synthase, L-glutamine: D-fructose-6P amidotransferase, EC 2.6.1.16) also known under the trivial name of glucosamine-6-phosphate synthase, is a new target for antifungal [21]. GlcN-6-P synthase catalyzes the first step in hexosamine metabolism, convert fructose 6-phosphate into GlcN-6-P in the existence of glutamine. The reaction catalyzed by GlmS. The end product of this pathway, *N*-acetyl

glucosamine, is an important building block of bacterial and fungal cell wall. Structural difference between prokaryotic and human enzymes may be exploited to design specific inhibitor, which may serve as anti-fungal and anti-bacterial drugs [22]. Inspired from our previous research work and continuous efforts towards the development of novel class of antimicrobial agents [23], we have synthesized imidazole based quinoline derivatives. In continuation of that, we have performed molecular docking studies of the synthesized compounds for better understanding of the drug-receptor interaction. Also carried out antimicrobial and anti-tubercular activity of synthesized compound.

2. MATERIALS AND METHODS

2.1 Chemistry

The chemicals were used without any extra purification. All reactions were supervised by thin layer chromatography (TLC) on aluminum plates coated with silica gel 60 F254, of 0.25 mm thickness (Merck). Melting points were taken using the melting point apparatus ThermoCal10 (Analab Scientific Pvt. Ltd, India) and are uncorrected. Mass spectra were recorded using a Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan) purchased under the PURSE program of DST at Sardar Patel University, Vallabh Vidyanagar, India. ^{1}H and ^{13}C Nuclear Magnetic Resonance spectra were recorded in DMSO-d_6 on a Bruker Advance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd, Switzerland) using the residual solvent signal as an internal standard at 400 MHz and 100 MHz, respectively. The IR spectra were recorded using a FTIR MB 3000 spectrometer (ABB Bomem Inc., Canada/Agaram Industries, Chennai) using Zn–Se optics (490–8500 cm^{-1}) and only the characteristic peaks are reported in cm^{-1}.

2.2 General procedure for the synthesis of 2-(4-(un) substituted phenoxy)-3-(4,5-diphenyl-1H-imidazole-2-yl)-6-(un) substituted quinoline (5a-l)

A 50 mL round-bottom flask, fitted with a reflux condenser, was charged with a mixture of 2-(4-(un) substituted phenoxy)-6-(un) substituted quinoline-3-carbaldehyde **3a-f** (1 mmol), Benzil **4a** or Benzoin **4b** (1 mmol), ammonium acetate (5 mmol) and catalytic amount of ceric ammonium nitrate in acetic acid (10ml). If we used benzil **4a** then **5a-f** product was obtained and if we used benzoin **4b** then **5g-l** product was obtained. **5a-f** was similar to **5g-l** respectively. The mixture was heated under reflux for 3-3.5 h and the progress of the reaction was monitored by TLC. After the completion of reaction, the reaction mixture was cooled to room temperature and then filter. Ice-cold water was added into filtrate, and the precipitated products were separated by filtration. The resulting solid residue was purified by recrystallization from EtOH **(Scheme 1).**

Scheme 1. Synthesis of imidazole derivatives 5a-l

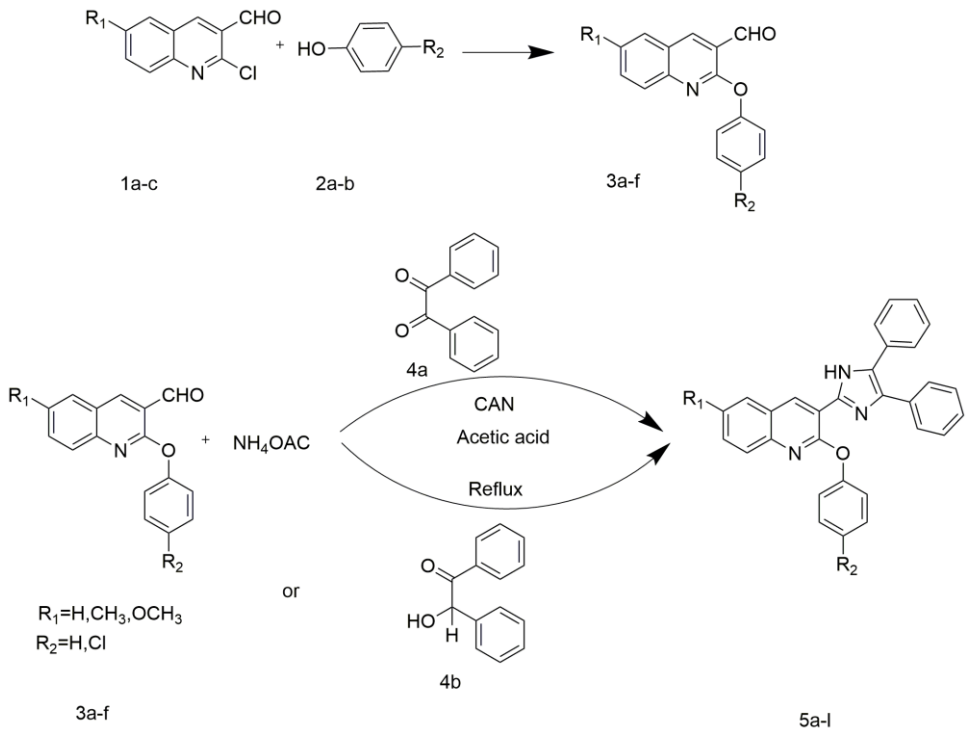


Table 1 % yield of Synthesized quinoline based imidazole derivatives 5a-l

Compound	R_1	R_2	Reacted with	%Yield
5a	-H	-Cl	4a	91
5b	$-CH_3$	-Cl	4a	79
5c	$-OCH_3$	-Cl	4a	89
5d	-H	-H	4a	76
5e	$-CH_3$	-H	4a	78
5f	$-OCH_3$	-H	4a	77
5g	-H	-Cl	4b	89
5h	$-CH_3$	-Cl	4b	77
5i	$-OCH_3$	-Cl	4b	88
5j	-H	-H	4b	76
5k	$-CH_3$	-H	4b	78
5l	$-OCH_3$	-H	4b	75

2.3 Biological Assay

2.3.1 Antimicrobial Assay

The in vitro antimicrobial activities of all the synthesized compounds were carried out by broth micro dilution method [24]. Antibacterial activity of synthesized compound was screened against three Gram-positive (*Bacillus subtilis* MTCC441, *Clostridium tetani* MTCC449, *Streptococcus pneumonia* MTCC1936) and three Gram-negative (*Escherichia coli* MTCC443, *Salmonella typhi* MTCC98, *Vibrio cholera* MTCC3906), each synthesized compound was diluted with dimethyl sulfoxide. The compounds found to be active in primary screening were further screened in a second

set of dilution at concentrations of 200, 100, 62.5, and 50 µg ml^{-1}. 10µl suspensions from each were further inoculated, and growth was noted after 24 and 48 h. Some compounds showed good to excellent antimicrobial and antifungal activity. In this study, Ampicillin, Ciprofloxacin and Chloramphenicol were used as standard antibacterial drugs, whereas Griseofulvin and Nystatin were used as standard antifungal drugs. The data are given in **Table 3**

2.3.2 Antituberculosis screening

In vitro antituberculosis activity of all the compound against *M. tuberculosis* $H_{37}Rv$ strain was determined using Lowenstein-Jensen medium (conventional method) as described by Rattan (2000) [25]. The Culture of *M. tuberculosis*$H_{37}Rv$ growing on Lowenstein-Jensen medium was harvested in 0.85% saline in bijou bottles. All test compound make solution of 250 mg ml^{-1} concentration of compound was prepared in DMSO. These tubes were then incubated at 37ºC for 24h followed by streaking of *M. tuberculosis* $H_{37}Rv$ (5 X 10^4 bacilli per tube). These tubes were then incubated at 37ºC. The growth of bacilli was seen after 12, 22, 28 days of incubation. Tubes having compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* $H_{37}Rv$. The screening results are summarized as % inhibition relative to standard drug isoniazid.

2.4 Molecular docking study

The ligand were drawn in chembio3D Ultra 11.0 assign with proper 2D orientation and made PDB file. This PDB file was converted into mol2 file and made a minimum energy conformer in chimera 1.11.2. Molecular docking was performed on the active site of GlcN-6-P synthase as the target receptor. This receptor was obtained from Protein Data Bank (www.pdb.org), The PDB code was 2VF5. PDB file of receptor was also converted into mol2 file in chimera. All the water molecules were removed from the receptor. Molecular docking studies were carried out using AUTODOCK-VINA 1_1_2 software with the grid box size at 55, 45 and 45 Å for x, y and z respectively. The grid center was set at 23.46, 23.11 and 8.20 for x, y and z respectively. All the AUTODOCK docking runs were performed in AMD Athlon 64X2 Dual core processor 2 GHz with 2 GB RAM and 32 bits operating system.

3. RESULTS AND DISCUSSION

3.1 Chemistry

In this study, synthesis of target molecules is given in **Scheme 1.** From the literature procedure, the starting material 2-chloro-3-formyl quinoline **1a-c** was prepared by Vilsmeier-Haack reaction of acetanilide. **1a-c** reacted with different phenol **2a-b** in presence of DMF and K_2CO_3 gives **3a-f. 3a-f** reacted with Benzil or Benzoin, ammonium acetate and acetic acid as a solvent in presence of ceric ammonium nitrate as a catalytic amount. Then reflux at 90-100ºC temperature gives final product **5a-l**. The structures of all the synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and FT-IR spectrometry. In ^{1}H NMR of **5b** show signal for three protons of methyl at δ 2.48. For NH

proton (NH of imidazole ring) as a singlet at around δ 12.56, so we can hereby confirm that imidazole ring was formed. The aromatic protons resonate as multiplets at δ 7.23-8.89 ppm. The ^{13}C spectra show signal at 21.4 for Ar-CH_3. The signal at 116.0-157.5 for aromatic carbon atoms. Mass spectra of compound **5b** gave molecular ion peak at m/z 488 [M^+] corresponding molecular formula $C_{31}H_{22}ClN_3O$. so we confirmed molecular mass of the synthesized compound. (see supplementary file). Optimization reaction condition for the synthesis of **5a** compound was carried out and in this study, we observed the model reaction with and without of catalyst. All catalysts were used at reflux condition. When the reaction was carried out without adding of catalyst the product was obtained 45% with reflux condition. When we were used 5 mol % CAN the product was obtained 60%. When we were used NaOH, there was no product obtained. When we used $FeCl_3$ and p-TsOH 50% and 45% product was formed. When we used Iodine as a catalyst 55% product was formed. The highest yield 91% was obtained by using ceric ammonium nitrate. (mol %= 10). This process was found to be effectual for aromatic aldehyde for synthesis of targeted compound with enhanced yield. The simple work-up is beneficial aspect of this method, which includes the pouring of the reaction mixture over ice-water to get the precipitated solid which on filtration gave the sufficiently pure compound in good yield. The present method is better than other method in compare to yield and reaction time. The most attribute aspect of CAN is that it acts as a water-compatible Lewis acid in aqueous solvents. The catalytic amount is sufficient for complete reaction in most cases of this catalyst. The use of ceric ammonium nitrate as a Lewis acid in C-C bond formation reaction. So, we synthesized imidazole based quinoline derivatives using ceric ammonium nitrate by one pot multi-component reaction.

Table 2. Optimization Reaction condition for the synthesis of 5a compound

Entry[a]	Catalyst	Mol (%)	Temperature	Time (min.)	Yield(%)
1	NaOH	10	Reflux	190	-
2	$FeCl_3$	10	85	150	50
3	CAN	0	85	120	45
4	CAN	5	85	120	60
5	CAN	**10**	**85**	**120**	**91**
6	p-TsOH	10	85	140	45
7	Iodine	10	85	150	55

3.3 Antimicrobial activity

3.3.1 Gram positive bacteria

The values of the MIC against microorganisms tested are reported in **Table-3**. The examination existing that some of the compound showed outstanding activity and some of them comparable activity against Gram-positive and Gram-negative strains. Among them compound **5c** (MIC=62.5

μg ml^{-1}) show outstanding activity, compounds **5a**, **5f** (MIC=100 μg ml^{-1}) displayed excellent activity, compounds **5b**, **5d** (MIC=250 μg ml^{-1}) displayed comparable activity against Gram-positive bacteria *B.subtilis* as compared to standard drug Ampicillin (MIC=250 μg ml^{-1}). Against *C.tetani* compound **5d**, **5c** (MIC=100 μg ml-1) displayed excellent activity. **5a**, **5f** (MIC=250 μg ml^{-1}) exhibited comparable activity compare to ampicillin (MIC=250 μg ml^{-1}).

3.3.2 Gram Negative bacteria

Against Gram-negative bacteria *E.coli* compounds **5c** (MIC=62.5 μg ml^{-1}) expressed more active, compound **5a**, **5d** and **5f** comparable active as compare to standard drug ampicillin (MIC=100 μg ml^{-1}). Against *S.typhi* compound **5e** and **5f** (MIC=100 μg ml^{-1}) displayed comparable activity. Moreover, against *V.cholera* compounds 5c (MIC=100 μg ml^{-1}) showed similar activity.

3.4 Antifungal activity

Against fungal pathogen *C. albicans,* compounds **5b** and **5f** (MIC=500 μg ml-1) displayed equivalent activity compare to griseofulvin (MIC=500 μg ml-1).

3.5 Antitubercular activity

The result of synthesized compounds and standard drugs are reported in Table 4.The compound **5c** displayed good activity compare to another compound. Unfortunately, majority of compounds showed poor activity against *M. TB $H_{37}Rv$.*

Table 3 The in vitro antimicrobial activity of synthesized compounds5a-f

Compound	Minimum inhibitory concentration in μg/Ml							
	Gram positive bacteria			Gram negative bacteria			Fungi	
	B.S. MTCC441	C.T. MTCC449	S.P. MTC1936	E.C. MTCC443	S.T. MTCC98	V.C. MTCC3906	C.A. MTCC227	T.R. MTCC296
5a	**100**	**250**	500	**100**	250	250	>1000	1000
5b	**250**	500	250	250	200	500	**500**	>1000
5c	**62.5**	**100**	500	**62.5**	200	250	1000	1000
5d	**250**	**100**	125	**100**	125	**100**	1000	1000
5e	500	500	500	250	**100**	500	1000	>1000
5f	**100**	**250**	**100**	**100**	**100**	250	**500**	1000
Ampi.	250	250	100	100	100	100	-	-
Chlo.	50	50	50	50	50	50	-	-
Cipro.	50	100	50	25	25	25	-	-
Nyst.	-	-	-	-	-	-	100	500
Gre.	-	-	-	-	-	-	500	500

BS: *Bacillus subtilis*, CT: *Clostridium tetani,* SP: *Streptococcus pneumoniae*, EC: *Escherichia coli*,

ST: *Salmonella typhi*, VC: *Vibrio cholera*, CA: *Candida albicans*, TR: Trichophytonrubrum, Ampi.: Ampicillin; Chlo.: Chloramphenicol; Cipr.: Ciprofloxacin; Nyst.: Nystatin; Gri.: Griseofulvin. MTCC: Microbial Type Culture Collection Bolt value indicates compounds are more or equal potent than standard drug, - not tested

Table 4. Thein vitro antitubercular activity of synthesized compounds5a-f

METHOD	**L.J.MEDIUM(CONVENTIONAL METHOD)**
BECTERIA	$H_{37}RV$
CONCENTRATION	250 µg/ml
STANDARD DRUG	ISONIAZIDE
COMPOUND	%INHIBITION
5a	50%
5b	30%
5c	**84%**
5d	24%
5e	56%
5f	22%
ISONIAZIDE	99%

3.6 Molecular docking

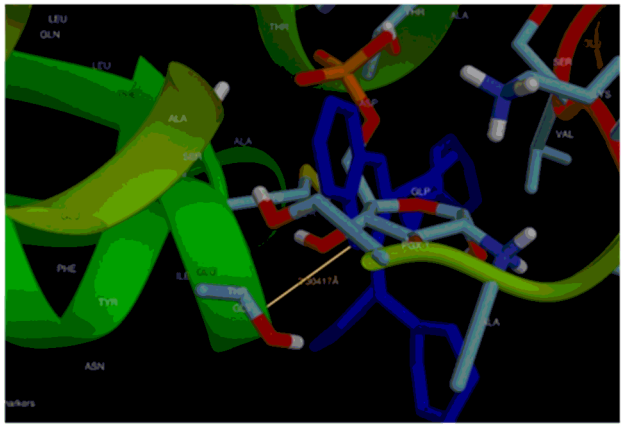
In Molecular docking predict the binding affinity and orientation at the active site of receptor. We have compare the binding affinity and hydrogen bond length of synthesized compound with the standard drugs. Automated docking was used to evaluate the orientation of ligand bound in the active pocket of GlcN-6-P synthase. This receptor was obtained from Protein Data Bank (www.pdb.org), The PDB code was 2VF5. The docking receptor of GlcN-6-P with synthesized ligand exhibited entrenched bonds with one or more amino acids in the receptor active pocket. The theoretical energies for binding of ligand and GlcN-6-P synthase were obtained to be -9.9 to -9.0 Kcal/mol, which are higher than those obtained for the binding of the standard drugs. The molecular docking of ligand molecules 5a, 5b, 5c, 5d, 5e and 5f with GlcN-6-P synthase. The synthesized ligand established hydrogen bond with Ser349, Thr302, Thr352, Ser401, Val399, Gly301 aminoacid. All docked ligand showed binding energy and hydrogen bonding with active pocket as show in Fig 1. The docked molecules 5a, 5c and 5d were create best docked confirmation with minimum binding affinity (-9.9, -9.3 and -9.0 kcal mol^{-1}) than standard drugs (-8.7 kcal mol^{-1}) used for docking (Table-5). Hydrogen bond length of best docked molecules 5a, 5c and 5d were 2.30 Å, 2.59 Å and 2.21 Å. Other molecule 5b, 5e and 5f minimum binding affinity (-8.6, -8.5 and -8.1 kcal mol^{-1}). H bond length with receptor were 2.20 Å, 2.41 Å and 2.54 Å.Fig1.represents the 3D binding conformation

of ligand-protein interactions.

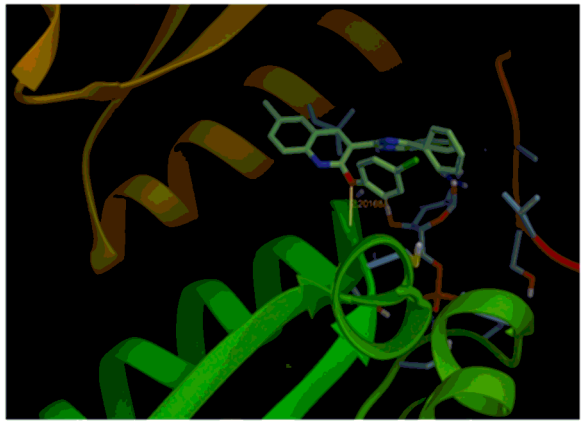
Table 5. Molecular docking result of synthesized compound5a-f

Ligand	Affinity (Kcal/mol)	H-bonds	H-bond length
5a	**-9.9**	1	2.30 Å
5b	-8.6	1	2.20 Å
5c	**-9.3**	1	2.59 Å
5d	**-9.0**	1	2.21 Å
5e	-8.5	1	2.41 Å
5f	-8.1	1	2.54 Å
Ampicillin	-8.7	4	2.24 Å, 2.53 Å, 2.48 Å, 1.97 Å

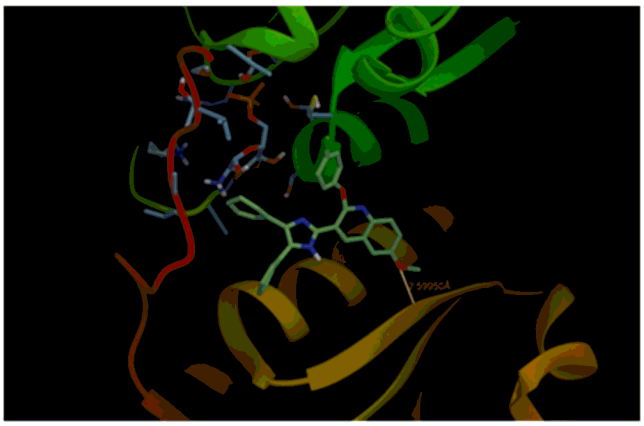
Fig1. Docking image of the targeted compound 5a-f with receptor 2VF5



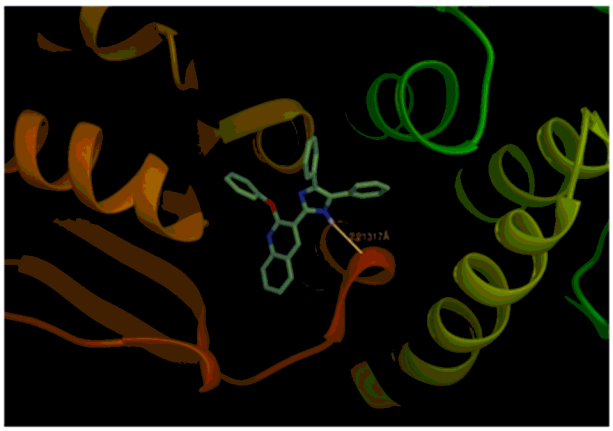
5a



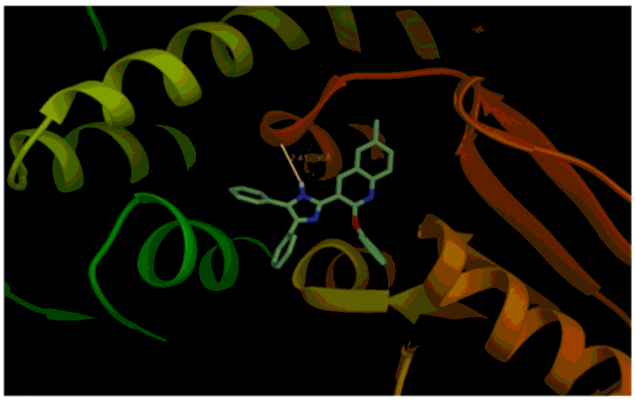
5b



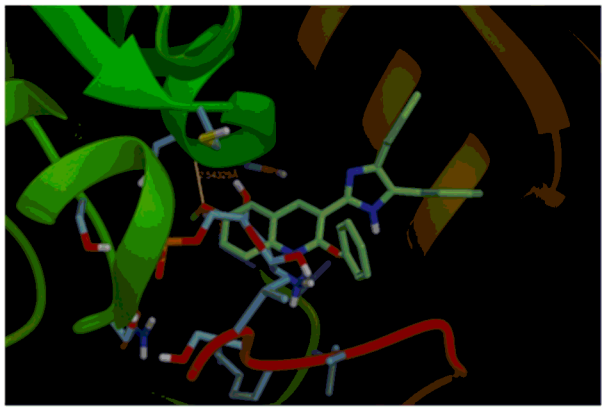
5c



5d



5e



5f

4. CONCLUSION

In conclusion, we have established a convenient method for the synthesis of imidazole based quinoline derivatives by applying a three-component reaction in the presence of an inexpensive, nontoxic, commercially available ceric ammonium nitrate. The simplicity of the reaction, easy workup, excellent yields of the products, and short reaction time make it an efficient route for synthesizing imidazole based quinoline heterocycles. Among the compounds, **5c** and **5d** were emerged as potentially active antimicrobial agents. In molecular docking studies, compound **5a**, **5c** and **5d** exhibited least binding energy. The compounds with least binding energy are responsible for more active antimicrobial agent with respect to standard drugs. The best dock conformation is one with minimum binding energy has the maximum affinity.

5. ACKNOWLEDGEMENT

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6. CONFLICT OF INTEREST

There is no conflict of interest

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