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Green Synthesis of 2-isonicotinoyl-4,6-dihydroimidazo[4,5-c] Pyrazol-5 (2H)-one Derivatives via One-pot Multicomponent Approach as a Potent Antifungal Agent

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Abstract

In the present research work, we made an effort to a green, efficient, and simple procedure for the one-pot multicomponent synthesis of novel 2-isonicotinoyl-4,6-dihydroimidazo[4,5-c] pyrazole-5 (2H)-one derivative in presence of environmentally sustainable PEG-400 reaction medium and recyclable catalyst bleaching earth clay (pH 12.5, 10% by weight). The benefits of this methodology include a quick reaction time, high product yield, and an easy work-up procedure. The antifungal potency was studied for synthesized derivatives using the agar well diffusion method against *Aspergillus niger* (MTCC280), *Aspergillus flavus* (MTCC3008), and *Penicillium citrinum* fungus strain. Among the tested compounds six from the nine compounds show promising antifungal activity among the tested compounds. The derivatives were characterized using ^{1}H NMR, ^{13}C NMR, Mass, and IR spectroscopic methods. Molecular docking study 2-isonicotinoyl-4,6-dihydroimidazo[4,5-c] pyrazole-5 (2H)-one derivative was conducted against the PDB : 3KHM, 14 α-demethylase (CYP51) from *Trypanosoma cruzi* in complex with inhibitor fluconazole protein and PDB : 6UW2 clotrimazole bound complex of *Acanthamoeba castellanii* CYP51.

Keywords

imidazolidine-2,4-dione**,** isonicotinic acid hydrazide, antifungal activity, molecular docking

1 Introduction

In tropical nations, infectious diseases are responsible for around half of all fatalities worldwide. Even if bacterial and fungal illnesses no longer account for a significant portion of mortality in industrialized nations, they do in poor nations. In addition, the number of immunocompromised patients (due to AIDS, cancer, and transplants) is rising quickly, contributing to an increase in primary and opportunistic fungal infections [1]. The widespread drug resistance and infectious diseases brought on by bacterial infections have emerged as a major public health issue. The species of *Penicillin*, *Aspergillus*, *Cryptococcus*, *Candida*, and *Pneumocystis* in particular are responsible for those deaths caused by fungi [2]. Even said, it appears that this sum is continuously rising in spite of the antifungal drugs that are currently available in the market.

Designing and discovering novel antifungal agents has emerged as one of humanity's most crucial challenges. The use of green solvents and viable catalyst resources are merely two fields where the concept of "green chemistry" has created significant effects. In order to reduce energy usage, increase atom economy, and increase reaction yields. environmentally friendly protocols have been investigated for heterocyclic synthesis. The traditional multistep synthetic approach to accessing such complex molecules generally involves several functional group interconversions, and each synthetic operation includes

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extraction and purification processes [3]. Multi-component reactions (MCRs) have recently gained prominence as a powerful synthetic tool for the creation of structurally complex molecular frameworks with appealing biological properties. The entire process is based on forming and breaking several carbon-carbon and carbon-heteroatom bonds in a single pot [4].

From heterocyclic compounds, hydantoin also known as imidazolidine-2,4-dione, is a non-aromatic five-membered heterocycle that is considered a valuable, privileged scaffold. The hydantoin moiety has the ability for two hydrogen bond acceptors and two hydrogen bond donors at each of its five potential substituent sites, therefore it has become a popular ring among scientists. The hydantoin moiety derivatives showed numerous biological properties such as anti-proliferative activity [5], antimicrobial activity [6], anti-convulsant activity [7], anti-inflammatory activity [8] and antioxidant activity [9].

In addition, pyrazoles have a five-membered heteroaromatic ring with two nitrogen atoms next to each other in the ring structure, in which one nitrogen atom acts as a proton donor while the other nitrogen atom acts as a proton acceptor (pyridine type). The type of their substituent groups has a significant impact on the potential of proton donor or proton acceptor strength of pyrazoles, which can function as weak bases or acids. The other three positions in the ring allow for structural variations starting with the appropriate precursors or after the pyrazole ring has been formed using post-functionalization reactions [10–12]. They are one of the most exhaustively studied groups of compounds in the azole family [13], and their derivatives showed numerous biological properties such as antibacterial [14], anticancer [15], anti-tubercular [16], anti-inflammatory [17], antidepressant [18], antifungal [19].

In recent decades, there has been a lot of interest in heterocyclic motifs that contain multiple structures in one molecule. Fused heterocycle, imidazopyrazole analogue has been such an active molecule that shows remarkable chemical, biological, and pharmaceutical activity. Imidazopyrazole analogues show pledge as selective Raf kinase inhibitors [20], anticancer [21], antibacterial [22], anti-inflammatory [23], anti-Alzheimer's activities few of medicinally relevant imidazole, pyrazole and imidazopyrazole drugs are shows in Fig. 1.

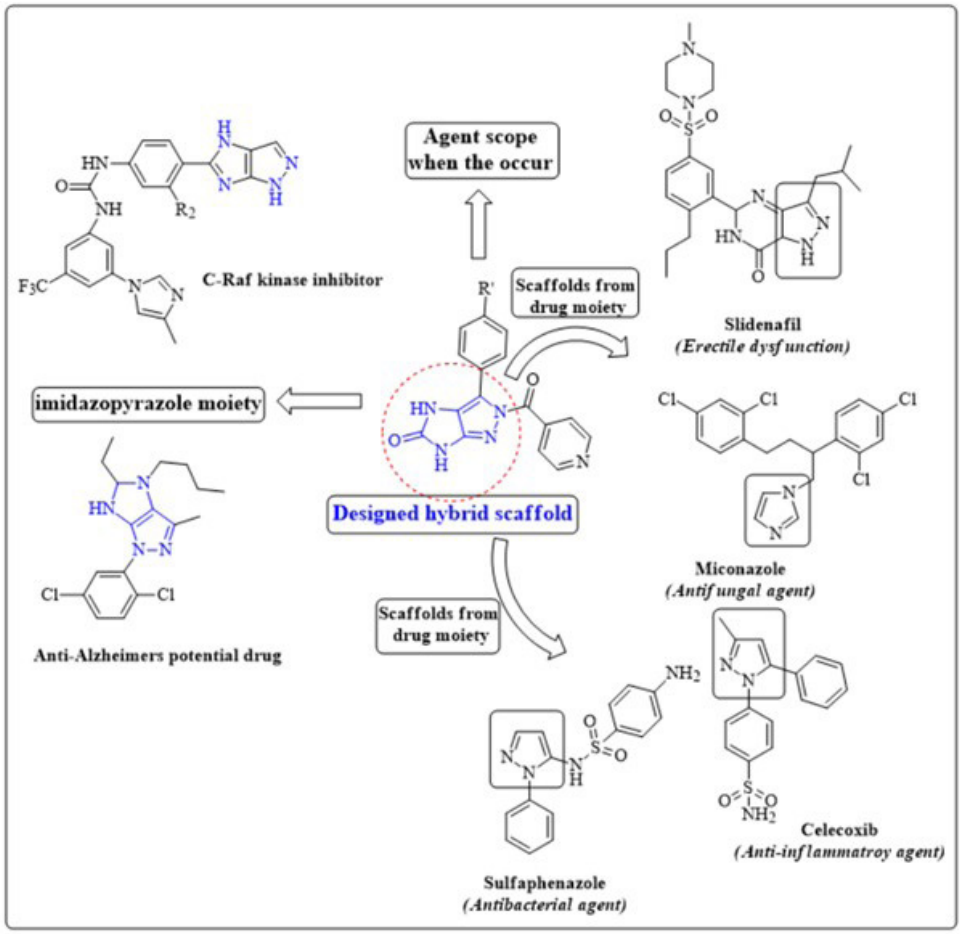


Fig. 1 Medicinally relevant imidazole, pyrazole and imidazopyrazole drugs

Considering the biological potential of dihydroimidazopyrazole derivatives, and as a part of our research into the design and synthesis of new heterocyclic compounds [24–27]. In present research work we have synthesized a new series of 2-isonicotinoyl-4,6-dihydroimidazo [4,5-c] pyrazol-5(2H)-one derivatives through single pot multi-component pathway. The molecular structure of the derivatives was confirmed by spectral data. They were explored for their antifungal activities and molecular docking application.

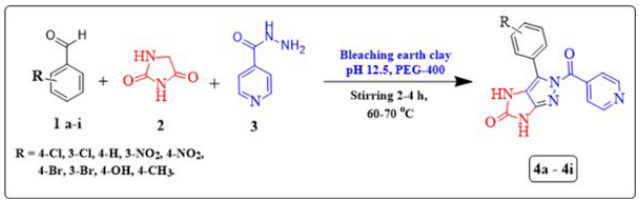
2 Results and discussion

2.1 Chemistry

In a progression of our efforts to develop eco-friendly methods for biologically active compounds, we introduce here a simple and handy method. In polyethylene glycol-400 (PEG-400), a bleaching earth clay (BEC) catalyzed procedure was utilized to synthesize 2-isonicotinoyl-4,6-dihydroimidazo[4,5-c] pyrazol-5(2H)-one derivatives.

The study starts with facile synthesis of 2-isonicotinoyl-4,6-dihydroimidazo[4,5-c] pyrazol-5(2H)-one derivatives (**4a-i**) via single pot, three-component combination of substituted aromatic aldehyde (**1a-i**), imidazolidine-2,4-dione (**2**) and isonicotinic acid hydrazide (**3**) one by one addition in environmentally friendly solvent PEG-400 and in presence of a catalytic amount of BEC (pH 12.5, 10wt%) as promotor through stirring at 60–70 °C for 3–4 h, (Scheme 1). To demonstrate the significance of green chemistry, the reaction was first carried out under catalyst-free conditions in PEG-400 at room temperature and higher temperatures (100 °C) for 60 min. Regrettably, no evidence of the desired product was observed (Table 1, entries 1, 2).

We investigated how some commonly used basic catalysts, such as K_2CO_3, KOH, and triethylamine (TEA), would affect PEG-400 transformation; surprisingly, none of these catalysts provided a sufficient yield (35–40% Table 1 entries 3, 4). The same reaction was then performed in a PEG-400 solvent with BEC acting as a catalyst to determine whether BEC was effective in producing



Scheme 1 Synthetic pathway of 2-isonicotinoyl-4,6-dihydroimidazo[4,5-c] pyrazol-5(2H)-one derivatives

Table 1 Optimization of the catalyst

Entry	Catalyst	Temp (°C)	Time (h)[a]	Yield (%)[b]
1	No Catalyst	RT	24	0
2	No Catalyst	90	>8	0
3	K_2CO_3 (1 mol%)	80–90	>6	38
4	KOH (1 mol%)	80–90	>6	40
5	BEC (1 wt.%)	60–70	>3–4	75
6	BEC (2 wt.%)	60–70	>3–4	78
7	BEC (5 wt.%)	60–70	>3–4	80
8	BEC (10 wt.%)	60–70	>3–4	90
9	BEC (20 wt.%)	60–70	4	80

[a] Reaction progress checked by TLC; [b] Yields refer to isolated yield.

the desired products (**4a-i**). We performed the model reaction in the presence of BEC and cross-examined the ideal reaction conditions using different catalyst amount ratios, namely 1, 2, 5, and 10 wt.% for this conversion, and clearly, a significant advancement was shown, increasing the yield to improve the reaction conditions. It was found that as catalyst amount increases, product yield rises to 75, 78, 80, and 90%, correspondingly (Table 1, entries 5–8). Further catalyst addition had no effect on yield due to the catalytic poisoning (Table 1, entry 9).

The result signified that 10 wt.% BEC (pH 12.5) was found to be sufficient to produce a yield of the product up to 90% with the successful completion of the reaction. The used catalyst can be recycled and reused without further purification for the next reaction. The catalytic activity of the catalyst was evaluated using the BEC for 5–6 runs, and it was noticed that the catalyst had good catalytic power for up to five runs with minimal loss of activity (Fig. 2).

On the basis of optimized conditions, we then tested the comprehensiveness and complexity of the prevailing MCRs. Interestingly, imidazolidine-2,4-dione underwent a reaction with different substituted aromatic aldehydes,

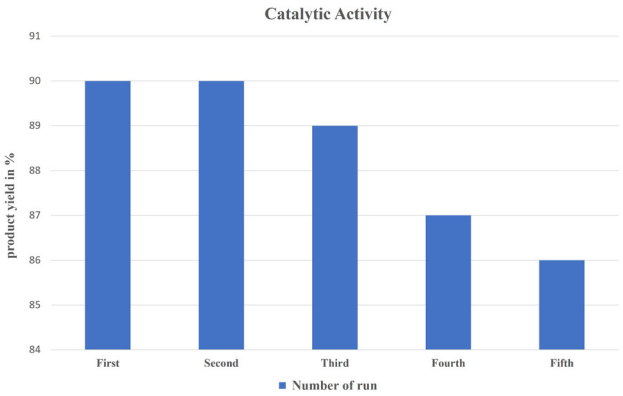


Fig. 2 Correlation between product yield and reusability of the catalyst

and isonicotinic acid hydrazide to give final products (**4a-i**) in good enough yield (Table 2). The purity of the synthesized compound was confirmed by TLC. All newly synthesized derivatives were well characterized and proved by spectral data (IR, ^{1}H NMR, ^{13}C NMR, and mass spectra). The IR spectrum of compound **4a** exhibited absorption band at 3228 cm^{-1} and 3277 cm^{-1} due to -NH (amidic or enolic OH stretching).

The absorption bands at 1665 cm^{-1} for Amidic C=O stretching and 1588.28 cm^{-1} corresponds to C=N stretching. The ^{1}H NMR spectrum of compound **4a** displayed a singlet at δ 10.921 ppm corresponds to two -NH group of imidazoline ring. The aromatic proton for 4-chloro phenyl and isonicotinoyl moiety appear around δ 6.38 to 8.71 ppm. The ^{13}C NMR spectra show the characteristic peak for the carbonyl carbon is 165 ppm and the peak for the amide carbonyl group appears at 160 ppm. Finally, the compound has been confirmed by taking mass spectrometry, it shows 339.1.

The Structure-Activity Relationship (SAR) study show that dihydroimidazo[4,5-c] pyrazole and isoniazid nucleus are essential for antifungal activity, it was observed that electron withdrawing group intensified the activity against fungal strain and electron donating group abridged the activity.

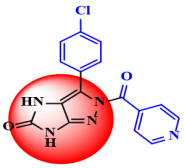
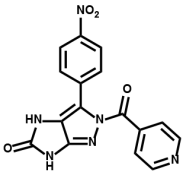
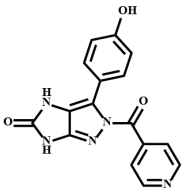
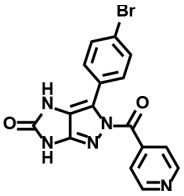
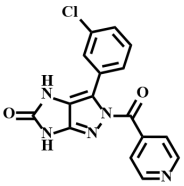
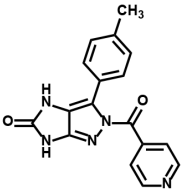
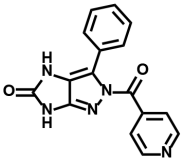
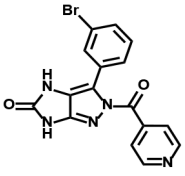
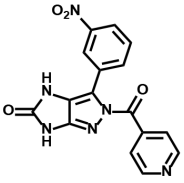
The imaginable pathway for the expected reaction is shown in Scheme 2. The reaction begins with the abstraction of the proton from imidazolidine-2,4-dione **i**) in presence of BEC (pH 12.5) and then reacts with substituted aromatic aldehydes **ii**), to form intermediate 5-(3-substituted benzylidene) imidazolidine-2,4-dione **iii**) which on reacting with isonicotinic acid hydrazide **iv**) undergoes cyclization with the formation of N-N bridgehead bond followed by dehydration and then aromatization to offer desired product 2-isonicotinoyl-4,6-dihydroimidazo[4,5-c] pyrazol-5(2H)-one derivatives **v**).

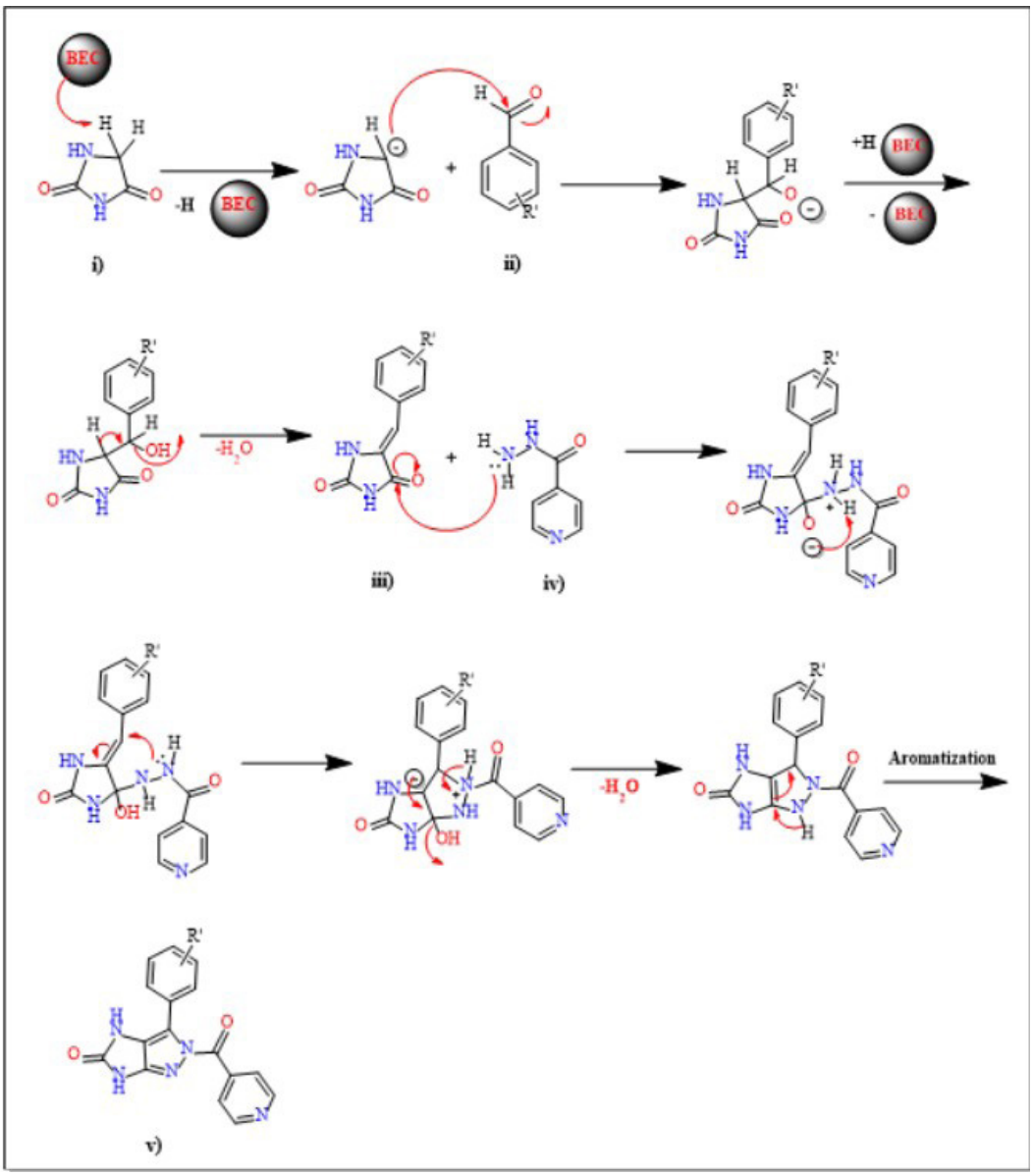
2.2 Biology: antifungal activity

The newly synthesized compounds (**4a-i**) were evaluated for their antifungal activity against three fungal strains viz. *Aspergillus niger* (MTCC280), *Aspergillus flavus* (MTCC3008) and *Penicillium citrinum* using disc diffusion assay and the results are shown graphically in Fig. 3, which clearly indicates the differential acuteness of fungal strains to the used test samples.

According to the results, the compounds **4a**, **4b**, **4d**, **4e**, **4h**, and **4i** had good antifungal activity against the used fungal strains *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium citrinum*. All of the remaining derivatives demonstrated good to moderate activity against the same fungal strains.

Table 2 Physico-chemical data of synthesized derivatives (**4a-i**)

Code	Structure of compounds	M.P. in °C	Yield %
4a		322–324	89%
4b		320–322	90%
4c		318–320	88%
4d		330–332	88%
4e		319–321	86%
4f		317–320	87%
4g		316–318	86%
4h		328–330	86%
4i		321–323	89%



Scheme 2 Plausible mechanistic way for the synthesis of 2-isonicotinoyl-4,6-dihydroimidazo[4,5-c] pyrazol-5(2H)-one derivatives (**4a-i**)

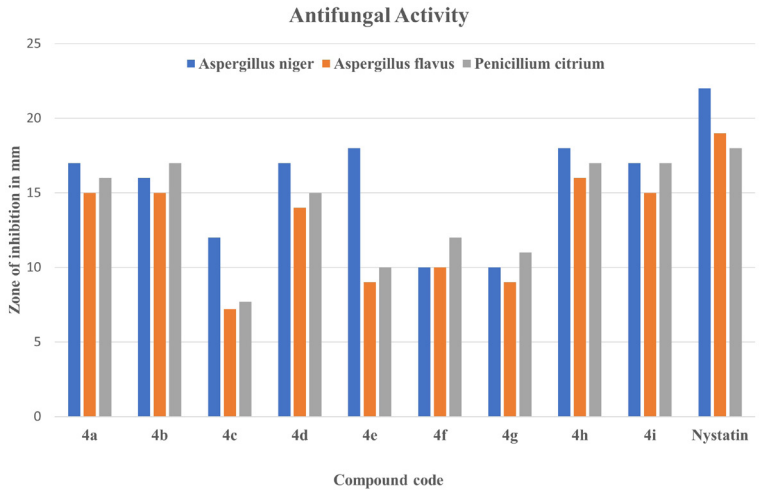


Fig. 3 Antifungal evaluation of 2-isonicotinoyl-4,6-dihydroimidazo[4,5-c] pyrazol-5(2H) one derivatives (**4a-i**)

According to the SAR, aromatic rings with chloro, bromo and nitro substituents have higher antifungal activity due to electron withdrawing substituents present on aromatic nucleus.

2.3 Molecular docking

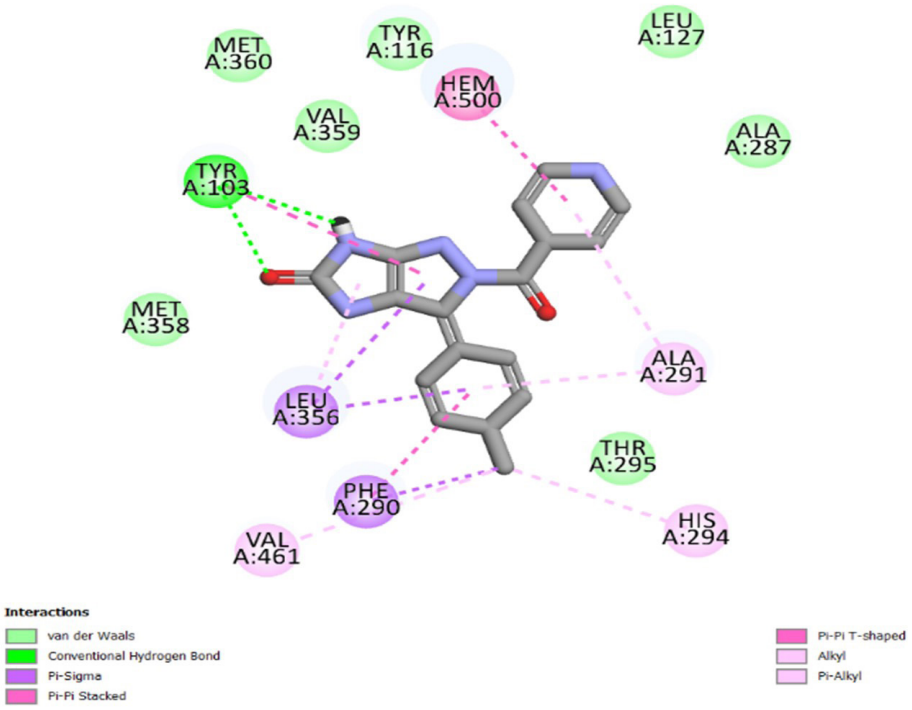
Using a molecular docking method, such as *in silico* docking, is an important way to identify the cellular target for various inhibitors. Exceptionally, such a tool can be used by scientists in the absence of advance laboratory to carry out enzymatic in vivo and in vitro examination. Herein, we have performed *in silico* study with our synthesized **4a-4i** imidazole pyrazole derivatives for the antifungal activity. This study gives rise to a clear idea about the synthesized molecule's potency with their ligand protein interactions.

The docking analysis was carried out by Auto-dock software and the 2D results are visualized with discovery studio visualizer [26]. Based on the *in vitro* antifungal results of synthesized compounds, herein we have done the molecular docking against the PDB : 6UW2 the complex of *Acanthamoeba castellani* CYP51 with ligand the clotrimazole bound [27] and PDB : 3KHM protein having alpha-demethylase (CYP51) from *Trypanosoma cruzi* in complex with inhibitor fluconazole [28]. We have performed the in-silicon docking calculation with our prepared inhibitor with selected protein and found that all the ligands show a very good binding affinity with the active site of the protein and are well fitted in the active site of the enzyme. The docking results are shown in the form of the binding energy and their hydrogen bonding with van der Waals interaction in Table 3.

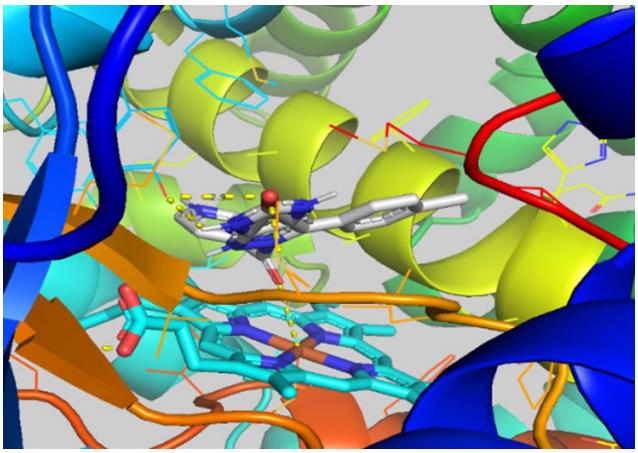
Table 3 The molecular docking results in bonding energy and interaction

Compounds	Protein PDB : 3KHM Docking score kcal/mol	Interactions	Protein PDB : 6UW2 Docking score kcal/mol	Hydrogen bonding
4a	−10.0	H-Bonds: TYR:103, van der Waals: MET:260, TYR:116, MET:358, THR:295, VAL:359, LEU:127, ALA:287; π-alkyl: HEM:500, ALA:291, LEU:356, VAL:461, PHE:290, HIS:294	−8.2	H-Bonds; TYR:114 van der Waals: ALA:294, PHE:293, LEU:216, SER:117, PHE:121, LEU:291, π-π- TYR:127, HIS:297; π-alkyl: ILE:141, ALA:290, VAL:126, LEU:363
4b	−8.7	H-Bonds: LAL:291 van der Waals: HEM:500, THR:295, PHE:290, TYR:116, MET:460, TYR:103, LEU:127, PHE:110, ALA:115; π-alkyl: LEU:356, MET:106	−7.6	H-Bonds: TYR;114, HIS:432, GLN:110, ARG:368 van der Waals: ALA:294, LEU:291, TYR:127; π-alkyl: LEU:363, ALA:290. VAL:126
4c	−8.5	H-Bonds TYR:103 van der Waals: ALA:287, THR:295, HIS:294, LEU:127, TYR:116, VAL:359, MET:460; π-alkyl: HEM:500, PHE:290, LEU:356	−7.8	H-Bonds: TYR:127, van der Waals: SER:117, ILE:141, LEU:291, ALA:294, VAL:366, LEU:216, PHE:293; π-alkyl: HIS:297, PHE:121, ALA:290
4d	−9.3	H-Bonds: van der Waals: VAL:359, ALA:115, THR:295, TYR:116, PHE:110, TYR:103, MET:469, HIS:294; π-sulphur: MET:106, π-alkyl: HEM:500, LEU:356, ALA:291, PHE:290.	−8.4	H-Bonds: PHE:293, TYR:114, TYR:127 van der Waals: VAL:366, THR:298, HIS:297, SER:117, GLN:110, ILE:141, LEU:138; π-alkyl: PHE:121, VAL:126, ALA:294, ALA:290
4e	−9.4	H-Bonds: TYR:116, van der Waals: VAL:359, MET:460, PHE:290, THR:295, HIS:294, THR:459; π-π alkyl: TYR:103, ALA:291, LEU:356, VAL:461; π-sulphur: MET:1065	−8.7	H-Bonds ARG: 368, HIS:432 van der Waals: GLY:433, LEU:138, ALA:290, ILE:141, ALA:294, VAL:366; π-alkyl: TYR:114, PHE:121, VAL:126
4f	−9.1	H-Bonds: TYR:103, HEM:500 van der Waals: MET:360, TYR:116, ALA:115, PHE:110, THR:295, VAL:359; π-alkyl: LEU:356, VAL:461, ALA:291, HIS:294, ALA:287, PHE:290	−8.6	H-Bonds: PHE:293, TYR:114, TYR:127 van der Waals: ALA:294, SER:117, GLN:110, PHE:121; π-alkyl: HIS:297, VAL:126, ALA:290
4g	−9.1	H-Bonds: TYR:103 van der Waals: MET:360, LEU:127, ALA:287, VAL:359, MET:358, ALA:287, TYR:116, MET:460, THR:295, HIS:294; π-alkyl: LEU:356, HEM:500, ALA:291, PHE:290, LEU:356	−8.6	H-Bonds: TYR:127, TYR:114 van der Waals: ALA:294, PHE293, LEU216, VAL:366, GLN:110, PHE:121; π-alkyl: HIS:297, LEU:138, VAL:126, ALA:290
4i	−9.9	H-Bonds TYR:103, ALA:291, TYR:116, PHE:290, van der Waals: VAL:359, THR:295, GLY:292, PHE:110, ALA:287; π-alkyl: LEU:356, HEM:500, VAL:461.	−8.5	H-Bonds: van der Waals: ALA:294, ALA:290, PHE:293, VAL:126, TYR:127, PHE:121, LEU:216, SER:117, MET:367, VAL:366

The molecular docking for the synthesized compound has been performed, and here we have found the compounds **4a** to **4i** show the excellent binding affinity with the protein PDB : 3KHM. The compounds **4a** (Fig. 4) indicate excellent bonding interaction with the selected protein and molecules were fitted in the binding pocket of the PDB : 3KHM protein with −10.0 kcal/mol. Fig. 4 (a) shows the 2D view of the **4a** in the active sites of the PDB : 3KHM protein possessing the desired hydrogen as well as van der Waals bond of interaction with the amino acid residues. The molecules **4a** show the hydrogen bond with the TYR:103 amino acid with the C=O-----H (3.2 Å) and H----N-H (2.3 Å) bond distance **4a** molecule. The molecules also display the van der Waals interaction with MET:260, TYR:116, MET:358, THR:295, VAL:359, LEU:127, ALA:287, amino acids as well as π-alkyl HEM:500, ALA:291, LEU:356, VAL:461, PHE:290, HIS:294. The Fig. 4 (a) indicates the molecules display the interaction with the HEM-500 via π-π interaction with the pyridine ring along with the C=O group of the inhibitor pointing towards the HEM-500 center with bond distance (3.5 Å) (Fig. 4 (b)). The molecules **4c** also display good *in vitro* laboratory results having 17 mm, 16 mm, and 16 mm inhibitory activity with *Aspergillus niger*, *Aspergillus flavus*, *Penicillium citrinum* compared with Nystatin standard drug.



(a)



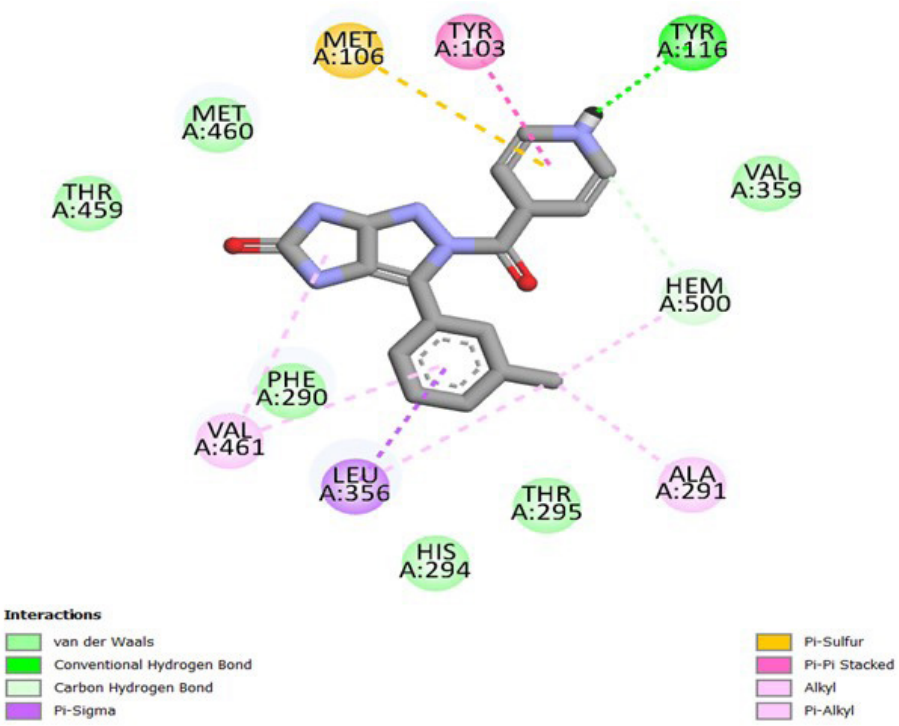
(b)

Fig. 4 (a) The 2D view of the **4a** derivative display H-Bonds and favourable interaction. (b) The cartoon views display the C=O group pointing towards the HEM-500 in the active sites of protein with **4a** ligand in PDB : 3KHM protein.

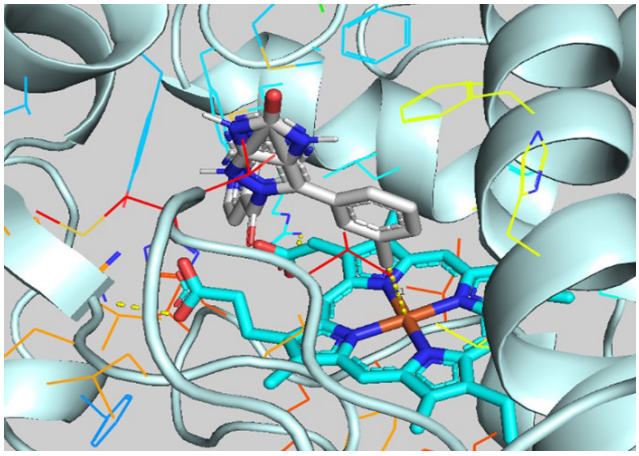
The molecules **4e** also display similar docking results and shows the hydrogen bonding with the TYR:113. The interesting facts about molecule **4e** is that N-H group of pyridine group forms hydrogen bonds with TYR-116 residues, while the Cl atom of the molecule is pointing towards the center of the HEM:500 and forming π-π interaction with Cl-----Fe (3.1 Å) (Fig. 5 (b)).

The molecules **4e** also display the van der Waals interaction with VAL:359, MET:460, PHE:290, THR:295, HIS:294, THR:459 π-π alkyl interaction with TYR:103, ALA:291, LEU:356, VAL:461 as well as π-sulphur interaction with MET:1065 residues (Fig. 5 (b)).

Similarly, the molecular docking of **4a-4i** molecules has been done with the PDB : 6UW2 protein, the molecules also docked in the active sites of the enzyme, but the orientation of the carbonyl group of the inhibitor are opposite in direction with different configuration interaction. All the molecules display good to excellent docking interaction with both the protein PDB : 6UW2 and PDB : 3KHM, but the orientation of all in inhibitor **4a-4i** in the PDB : 3KHM enzyme are similar to the native ligands and C=O pointing towards the Heme protein (HEM)-500 residues. While the docking results in case of protein PDB : 6UW2, the orientation of all in inhibitor **4a-4i** is opposite to the native



(a)



(b)

Fig. 5 (a) The Cl atom of the molecules **4e** pointing towards the center of HEM-500. (b) 2D view of the configuration display the H-bond and van der Waals bond with π-alkyl interaction of molecule **4e** in the active sites of enzyme.

ligand and C=O groups are oriented in the opposite direction of the HEM-500 residues. Finally, the docking results indicates that all molecules are fitting in the pocket of PDB : 3KHM while the molecules **4a**, **4b**, **4d**, **4e**, **4h**, and **4i** could be the promising antifungal candidates from their orientation and docking scores.

3 Materials and methods

3.1 Chemistry

All the melting points were determined in an open capillary tube. The chemicals and solvents used were of laboratory grade and were purified. Completion of the reaction was monitored by thin layer chromatography and solvents used for the system is ethyl acetate and pet ether (30:70) for reaction on precoated aluminum silica sheets (Merck, Germany) using UV-chamber for detection. BEC (pH 12.5) was gifted from Supreme silicone Pune Pvt. Limited. IR spectra were recorded in KBr pellets on an FTIR Shimadzu spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in (DMSO)-d_6 with an Avance spectrometer (Bruker, Germany) at a 300-MHz frequency using TMS as an internal standard; chemical shifts were expressed in parts per million. Multiplicities are proclaimed as follows: *s* (singlet), *d* (doublet), *t* (triplet), *m* (multiplate). Mass spectra were recorded on an EI-Shimadzu QP 2010 PLUS GC-MS system (Shimadzu, Japan). Elemental analysis was performed on a Carlo Erba 106 Perkin-Elmer model 240 analyzer (Perkin-Elmer, USA).

3.2 General procedure for the synthesis of 2-isonicotinoyl-4,6-dihydroimidazo[4,5-c] pyrazol-5(2H)-one (4a-i)

In 100 mL round bottomed flask an equimolar mixture of hydantoin [imidazolidine-2,4-dione] (0.001 mol) and substituted aromatic aldehyde (0.001 mol) were taken in green solvent PEG-400 and stirred for about 2 h at 60–70 °C using bleaching earth clay (pH 12.5, 10 wt%). Subsequently to this reaction mixture add equimolar quantity of isoniazide [isonicotinic acid hydrazide] (0.001 mol) and again stirred for 2 h with continuous heating at 60–70 °C. After complete conversion as indicated by TLC, the catalyst was filtered out by simple filtration and the mother liquor poured onto ice-cold water, the solid separated out, neutralized, and filtered out the product. The resultant product was dried and recrystallized from methanol.

3.2.1 3-(4-chlorophenyl)-2-isonicotinoyl-4,6-dihydroimidazo[4,5-c] pyrazol-5(2H)-one (4a)

M.P. 322–324 °C; Yield, 89%; IR (KBr, cm^{-1}): 3228-3277 (amidic N-H), 1768 (C=O), 1665 (amidic C=O and N stretching), 1588 (C=N stretching); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, *δ*, ppm): 10.92 (s, 2H, NH), 8.71–6.38 (m, 8H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6, TMS, *δ*, ppm): 165.62, 160.58, 155.91, 136.00, 132.65, 132.02, 130.95, 130.00, 129.07, 128.67,106.54; EIMS: 339.1 [M+] Calculated: $C_{16}H_{10}ClN_5O_2$: 339.74; found: 339.1.

3.2.2 2-isonicotinoyl-3-(4-nitrophenyl)-4,6-dihydroimidazo[4,5-c] pyrazol-5(2H)-one (4b)

M.P. 320–322 °C; Yield, 90%; IR (KBr, cm^{-1}): 3220 (amidic N-H), 1721 (C=O), 1658 (amidic C=O and N stretching), 1552 (C=N stretching); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, *δ*, ppm): 10.79 (s, 2H, NH), 8.79–6.42 (m, 8H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6, TMS, *δ*, ppm): 161.51, 157.66, 152.28, 149.06, 140.45, 136.23, 129.89, 121.46, 118.96, 117.70, 110.68 ; EIMS: 349.3 [M+] Calculated: $C_{16}H_{10}N_6O_4$: 350.49; found: 349.3.

3.2.3 3-(4-hydroxyphenyl)-2-isonicotinoyl-4,6-dihydroimidazo[4,5-c] pyrazol-5(2H)-one (4c)

M.P. 318–320 °C; Yield, 88%; IR (KBr, cm^{-1}): 3423 (Ar-OH), 3237 (amidic N-H), 1723 (C=O), 1658 (amidic C=O and N stretching), 1551 (C=N stretching); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, *δ*, ppm): 12.12 (s, 1H, OH), 11.18 (s, 2H, NH), 8.79–6.37 (m, 8H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6, TMS, *δ*, ppm): 161.51, 157.66, 150.28, 149.06, 140.45, 135.23, 129.89, 121.46, 118.96, 117.70, 112.68; EIMS: 321.0 [M+] Calculated: $C_{16}H_{11}N_5O_3$: 321.29; found: 322.0.

3.2.4 3-(4-bromophenyl)-2-isonicotinoyl-4,6-dihydroimidazo[4,5-c] pyrazol-5(2H)-one (4d)

M.P. 330–332 °C; Yield, 88%; IR (KBr, cm^{-1}): 3237 (amidic N-H stretching), 1723 (C=O stretching), 1657 (amidic C=O and N stretching), 1575 (C=N stretching); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, *δ*, ppm): 10.86 (s, 2H, NH), 8.79–6.00 (m, 8H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6, TMS, *δ*, ppm): 161.63, 155.61. 150.29, 149.52, 147.72, 140.32, 133.27, 132.23, 131.95, 122.99, 121.46, EIMS: 385.4 [M+] Calculated: $C_{16}H_{10}BrN_5O_2$: 384.19; found: 385.4.

3.2.5 3-(3-chlorophenyl)-2-isonicotinoyl-4,6-dihydroimidazo[4,5-c] pyrazol-5(2H)-one (4e)

M.P. 319–321 °C; Yield, 86%; IR (KBr, cm^{-1}): 3228 (amidic N-H), 3277 (amidic N-H), 1768 (C=O), 1665 (amidic C=O and N stretching), 1588 (C=N stretching); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, *δ*, ppm): 10.92 (s, 2H, NH), 8.71–6.38 (m, 8H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6, TMS, *δ*, ppm): 165.62, 160.58, 155.91, 136.00, 132.65, 132.02, 130.95, 130.00, 129.07, 128.67,106.54; EIMS: 339.0 [M+] Calculated: $C_{16}H_{10}ClN_5O_2$: 339.74; found: 339.0.

3.2.6 2-isonicotinoyl-3-(p-tolyl)-4,6-dihydroimidazo[4,5-c] pyrazol-5(2H)-one (4f)

M.P. 317–320 °C; Yield, 87%; IR (KBr, cm^{-1}): 3300 (aromatic C-H), 3228 (amidic N-H), 3277 (amidic N-H), 1768 (C=O), 1665 (amidic C=O and N stretching), 1588 (C=N stretching); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, *δ*, ppm): 10.92 (s, 2H, NH), 2.3 (s, 3H,-CH_3) 8.33–6.82 (m, 8H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6, TMS, *δ*, ppm): 165.62, 160.58, 155.91, 136.00, 132.65, 132.02, 130.95, 130.00, 129.07, 128.67,106.54; EIMS: 340.0 [M+] Calculated: $C_{16}H_{13}N_5O_2$: 319.32; found: 340.0.

3.2.7 2-isonicotinoyl-3-phenyl-4,6-dihydroimidazo[4,5-c] pyrazol-5(2H)-one (4g)

M.P. 316–318 ℃; Yield, 86%; IR (KBr, cm^{-1}): 3237 (amidic N-H), 1723 (C=O), 1658 (amidic C=O and N stretching), 1551 (C=N stretching); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, *δ*, ppm): 11.18 (s, 2H, NH), 8.00–6.00 (m, 9H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6, TMS, *δ*, ppm): 161.51, 157.66, 150.28, 149.06, 140.45, 135.23, 129.89, 121.46, 118.96, 117.70, 112.68; EIMS: 306.1 [M+] Calculated: $C_{16}H_{11}N_5O_2$: 305.29; found: 306.1.

3.2.8 3-(3-bromophenyl)-2-isonicotinoyl-4,6-dihydroimidazo[4,5-c] pyrazol-5(2H)-one (4h)

M.P. 328–330 °C; Yield, 86%; IR (KBr, cm^{-1}): 3237 (amidic N-H stretching), 1723 (C=O stretching), 1657 (amidic C=O and N stretching), 1575 (C=N stretching); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, *δ*, ppm): 10.86 (s, 2H, NH), 8.79–6.00 (m, 8H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6, TMS, *δ*, ppm): 161.63, 155.61. 150.29, 149.52, 147.72, 140.32, 133.27, 132.23, 131.95, 122.99, 121.46; EIMS: 385.4 [M+] Calculated: $C_{16}H_{10}BrN_5O_2$: 384.19; found: 385.4.

3.2.9 2-isonicotinoyl-3-(3-nitrophenyl)-4,6-dihydroimidazo[4,5-c] pyrazol-5(2H)-one (4i)

M.P. 320–322 °C; Yield, 90%; IR (KBr, cm^{-1}): 3220 (amidic N-H), 1721 (C=O), 1658 (amidic C=O and N stretching), 1552 (C=N stretching); ^{1}H NMR (400 MHz, DMSO-d_6, TMS, *δ*, ppm): 10.79 (s, 2H, NH), 8.79–6.42 (m, 8H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6, TMS, *δ*, ppm): 161.51, 157.66, 152.28, 149.06, 140.45, 136.23, 129.89, 121.46, 118.96, 117.70, 110.68 ; EIMS: 349.3 [M+] Calculated: $C_{16}H_{10}N_6O_4$: 350.49; found: 349.3.

4 Biology: Antifungal activity

The Agar well diffusion method was employed to screen the newly synthesized compounds (**4a-i**) for antifungal activity. The three strains used for the antifungal evaluation of the compounds were *Aspergillus niger* (MTCC 282), *Aspergillus flavus* (MICC 3008), and *Penicillium citrinum*. Nystatin (20 µg/mL) was used as the reference medication. Using a potato dextrose agar (PDA) slant for 20–24 h, the fungal culture strains were kept at 27 ± 0.2 °C until sporulation took place. The strain's spores were then transferred into 5 mL of sterile distilled water along with 1% Tween-80 (to ensure proper spore suspension). A hemocytometer (106 CFU/mL) was used to measure the spores. On each and every sterile PDA plate made with 2% agar, the prepared fungal spore suspension (0.1 mL) was applied. These plates undergo a 10-hour incubation period at 27 ± 0.2 °C after being spread with suspension. Agar wells that had been previously prepared using a sterile cork borer were then filled with 0.1 mL of the compound solution at a fixed concentration of 10 µg/mL each and incubated. The plates were then incubated for 24 to 40 h at 27 ± 0.2 °C for diffusion after being chilled for 25 min. Following incubation, the compounds' zones of inhibition were measured in mm, and the standard and minimum inhibition concentrations were computed in µg/mL.

5 Conclusion

We have described a novel and efficient one-pot multi-component synthesis of 2-isonicotinoyl-4,6-dihydroimidazo[4,5-c] pyrazol-5 (2H)-one derivatives by reacting substituted aldehydes with hydantoin and isoniazid. In the current study, using a catalyst or solvent alone is ineffective for generating a high yield of product, but when we combine PEG-400 and basic bleaching earth clay catalyst, their cooperative effect has been seen on the reaction in the form of shorter reaction times and higher yields when compared to recently reported methods, giving the method a broad application in organic synthesis. The antifungal activity evaluated for dihydroimidazo[4,5-c] pyrazol-5 (2H)-one derivatives against three fungal strains *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium citrinum*. Among them compounds, **4a**, **4b**, **4d**, **4e**, **4h**, and **4i** show promising

antifungal activity against all fungal strains. These findings were supported by a theoretical molecular docking study, which found that among all compounds, **4a**, **4b**, **4d**, **4e**, **4h**, and **4i** derivatives of the proteins PDB : 6UW2 and PDB : 3KHM exhibit good to excellent docking interactions.

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