4.1 Introduction

Pyrazole chemistry has reached significant value in the past few decades due to the discovery of interesting properties exhibited by a great number of pyrazole derivatives. The term pyrazole was given by German chemist Ludwig Knorr in 1883. Pyrazole, a five-membered aromatic heterocyclic ring comprises three carbon atoms and two adjacent nitrogen atoms (4.01) is a motif found in number of molecules that possess a wide range of pharmaceutical and agricultural usages. Moreover, some pyrazole derivatives have been used in food chemistry, supramolecular and polymer chemistry, as cosmetic colouring agents, UV stabilizers and some are the part of liquid crystal properties.

Many pyrazole derivatives are considered as alkaloids although they are rare in nature. 1-Arylpyrazole fragment is found to be present in drugs such as Cox-2 (cyclooxygenase-2) inhibitors and protein kinase inhibitors, in antifungal compounds and in complexes with phosphorescent properties. Some 1,5-diarylpyrazole derivatives have shown the inhibition of HIV-1 reverse transcriptase, whereas 1,3,5-triaryl-4-alkylpyrazoles are proved to be potent ligands for estrogen receptor. Several substituted pyrazoles have also been used as ligands for transition metal-catalysed reactions. Accordingly, chemical, pharmaceutical and agrochemical industries have a great interest in the synthesis of pyrazole derivatives.

Pyrazole is the only one among 1,2-diazoles to be solid at room temperature and also has higher boiling point (187°C) which reflects in the intermolecular hydrogen bonding. This association probably takes the form of dimers, trimers and oligomers (4.02). Due to rapid tautomerism of hydrogen atom from one nitrogen to the other, substituted pyrazoles inevitably exist in mixtures, for example; 3(5)-methylpyrazole (4.03, 4.04).
The acid $pK_a$ value of pyrazole in water is 14.2 and its basic $pK_a$ is 2.5. Hence, pyrazole is a weak acid compared to aliphatic acids and is also a weaker base than pyridine or simple aromatic amines. Alkylation and arylation of pyrazole ring changes these values by no more than $\pm 2$ $pK$ units. However, strongly electron withdrawing substituents have a much more pronounced influence on the pyrazole ring.

Pyrazoles undergo many electrophilic reactions such as addition at nitrogen, alklylation and acylation of nitrogen, substitution at carbon, halogenation and with bases it undergoes deprotonation of $N$-hydrogen. The reaction of $N$-metallated, $C$-metallated pyrazoles and reaction with radicals are also reported.

### 4.2 Synthetic aspects

The most widely used conventional approach for the preparation of substituted pyrazoles involve either the construction of two C-N bonds by condensation of substituted hydrazines with 1,3-dicarbonyl compounds or their 1,3-dielectrophilic equivalents (Scheme 1, via a) or the intermolecular [3+2] cycloadditions of 1,3-dipoles and to dipolarophiles (Scheme 1, via b).  

![Scheme 1](image-url)
three carbon atom unit possessing two electrophilic carbons (Scheme 2), such as 1,3-dicarbonyl (I), α,β-unsaturated carbonyl compound (II) and β-enynes (III) and related compound (IV).

Similarly, condensation of 1,3-diketones, β-ketoesters and 2,4-diketoesters with hydrazines has been widely used in the preparation of N-substituted and N-unsubstituted 3,5- and 3,4,5-alkyl/(het)arylpyrazoles (Scheme 3, 4.05), alkoxy pyrazoles (Scheme 3, 4.06) and pyrazole carboxylic acid esters respectively (Scheme 3, 4.07).
Panda et al\textsuperscript{13} developed iron-catalyzed route for the regioselective synthesis of 1,3- and 1,3,5-substituted pyrazoles (4.08) using diarylhydrazones and vicinal diols (Scheme 4).

\[ \text{Ph} \equiv \text{N} \quad \text{Ph} + \text{HO} \equiv \text{OH} \xrightarrow{\text{5 mol\% FeCl}_3 \quad 2 \text{ equiv. acac}} \quad \text{Ph} \quad \equiv \text{N} \quad \text{Ph} \]

\text{Scheme 4}

Kong et al\textsuperscript{14} synthesized 1,3,5-trisubstituted pyrazoles (4.09) from N-alkylated tosylhydrazones and terminal alkynes. This methodology was proved to offer complete regioselectivity (Scheme 5).

\[ \text{Ph} \equiv \text{N} \quad \text{Ts} + \equiv \text{Ph} \xrightarrow{\text{2.1 equiv. KOTBu \quad 0.5 equiv. 18-crown-6 \quad pyridine \quad 0 \circ C, 15 min}} \quad \text{Ph} \quad \equiv \text{N} \quad \text{Ph} \]

\text{Scheme 5}

Highly efficient \textsuperscript{3}Bu\textsubscript{3}P-catalyzed desulfonative [3+2] cycloadditions of allylic carbonates with arylazosulfones (Scheme 6) were developed for the synthesis of 1,4,5-trisubstituted pyrazole derivatives (4.10) by Zhang et al\textsuperscript{15}.

\[ \text{BocO} \equiv \text{COOEt} + \text{Ph} \equiv \text{N} \equiv \text{N} \quad \text{Ts} \xrightarrow{\text{20 mol \% \textsuperscript{3}BuP \quad CH}_2\text{Cl}_2, \text{rt, 12 h}} \quad \text{Ph} \quad \equiv \text{N} \equiv \text{EtOOC} \]

\text{Scheme 6}

Raghunadh et al\textsuperscript{16} reported synthesis of unsymmetrically substituted 1,3-pyrazole derivatives (4.11) \textit{via} one-pot three-component coupling reaction involving 3-(dimethylamino)-1-phenylprop-2-en-1-one, hydrazine and aryl halides. The reaction proceeded \textit{via} a sequential series of reactions such as
Michael addition, heterocyclization, dehydration and Ullmann cross-coupling (Scheme 7).

\[
\begin{align*}
\text{Ph} \mathcal{\equiv} \text{N} & \quad + \quad \text{Ph} \quad + \quad \text{NH}_2 \quad + \quad \text{NH}_2 \\
\text{Cu(OAc)}_2 & \quad \text{Cs}_2\text{CO}_3 \quad \text{DMF} \\
\end{align*}
\]

\[
\text{Ph} \quad \text{N} \quad \text{N} \quad \text{N} \quad \text{Ph}
\]

**Scheme 7**

Ivonin *et al*\(^{17}\) used \(N\)-alkylhydrazones of aliphatic ketones with Vilsmeier-Haack reagent (DMF-POCl\(_3\)) for the synthesis 1,3,4-trisubstituted pyrazoles (4.12).

\[
\begin{align*}
\text{HN} \quad \text{N} & \quad \text{N} \quad \text{HN} \\
\text{DMF} & \quad \text{POCl}_3 \\
\end{align*}
\]

**Scheme 8**

Kumari *et al*\(^{18}\) applied microwave radiation for the synthesis of functionalised pyrazoles (4.13) from the reaction of phenyl hydrazine, aldehydes and ethyl acetoacetate in the presence scandium triflate catalyst under solvent free conditions (Scheme 9).

\[
\begin{align*}
\text{Ph} \quad \text{NHNNH}_2 & \quad + \quad \text{Ph} \quad \text{CHO} \\
\quad & \quad + \quad \text{HC} \quad \text{O} \quad \text{OEt} \\
\quad & \quad + \quad \text{OEt} \quad \text{OEt} \\
\text{5 mol \% Sc(OTf)}_3 \quad \text{MWI, 200 W, 100 °C} & \quad 3-6 \text{ min} \\
\end{align*}
\]

**Scheme 9**

Kumar *et al*\(^{19}\) reported regioselective route for the synthesis of unsymmetrically substituted pyrazoles (4.14) by the reaction of active methylene compound, 1,3-bisaryl-monothio-1,3-diketone and arylhydrazines (Scheme 10).
Scheme 10

Deng et al\textsuperscript{20} reported regioselective synthesis of 1,3,4-trisubstituted pyrazoles (4.15) by the reaction of hydrazones and nitroolefins mediated with strong base (Scheme 11).

Scheme 11

Hu et al\textsuperscript{21} developed an unprecedented ruthenium catalyzed (II) oxidative C-N coupling that enabled a facile intramolecular synthesis of 1,3,4-trisubstituted pyrazoles (4.16) in presence of oxygen as oxidant (Scheme 12).

Scheme 12

One-pot synthesis of 4-substituted 1,5-diaryl-1H-pyrazole-3-carboxylic acid (4.17) has been developed by Jiang et al\textsuperscript{22} via MeONa/LiCl mediated sterically hindered Claisen condensation, Knorr reaction and hydrolysis sequence (Scheme 13).

Scheme 13
Carbethoxy substituted pyrazoles (4.18) from phenylhydrazones and dimethyl ethylenedicarboxylate using copper catalyst have been prepared (Scheme 14).

![Scheme 14](image_url)

4.3 Biological Significance

Literature survey revealed that N-substituted pyrazoles are proved to exhibit wide range of biological properties such as antiproliferative, antiangiogenic, DNA binding, anti-inflammatory, antibacterial, antidepressant, anticonvulsant, hypoglycemic and antitubulin properties.

Pathak et al synthesized various substituted 1-(3,5-diaryl-4,5-dihydro-1H-pyrazol-1-yl)ethanone derivatives (4.19) and evaluated for their in vitro antitubercular activity against Mycobacterium tuberculosis H37Rv strain. Hwang et al prepared 1,3,4-trisubstituted pyrazole derivatives (4.20) and reported as potent hepatitis C virus (HCV) entry inhibitors.

![4.19](image_url)

![4.20](image_url)

Ragab et al prepared 1,3,4-trisubstituted pyrazoles (4.21) and screened for their anti-inflammatory, analgesic as well as their ulcerogenic liability.

![4.21](image_url)
A series of 1,3-diphenyl-1H-pyrazoles functionalized with phenylalanine derived rhodanine derivatives (4.22) were synthesized and evaluated for their antibacterial activity by Zheng et al.34 Warshakoon et al35 have reported pyridine substituted with pyrazole 4-carboxylic acid (4.23) as Hypoxia Inducible Factor (HIF)-1α propyl hydroxylase inhibitors.

Tyagarajan et al36 synthesized a series of low molecular weight biaryl substituted pyrazole carboxamides (4.24) as sodium channel blockers. Schulz et al37 synthesized pyrimidine substituted pyrazoles (4.25) which are active as anti-depressive and anti-anxiety agents.

4.4 Present work

Pyrazole is a privileged heterocyclic scaffold which is an important constituent of agro-chemicals, polymeric materials and many drugs such as celecoxib, fipronil, lonazolac, viagra and many others. In view of this, we have synthesized 1,3-di and 1,3,4-trisubstituted pyrazoles 3-9 (a-c) starting from 3-arylsydnone (1a-c).

The antifungal activity of the newly synthesized compounds was hypothesized by in silico molecular docking studies to analyze the binding mode and structural orientation of the title compounds with β-(1,3)-endoglucanase protein of *A. fumigatus*. The docking results were further substantiated by the in vitro antifungal activity of title compounds against *A. fumigatus* and *C. albicans* fungal strains.
The synthetic routes for the synthesis of title compounds have been depicted under **Scheme 15**.
4.5 Results and discussion

4.5.1 Chemistry

3-Arylsydnone (1a-c) was ring transformed into 1-aryl-1\(H\)-pyrazole-3-carbonitrile (2a-c) when reacted with acrylonitrile in presence of chloranil (2,3,5,6-tetrachloro-\(p\)-benzoquinone). Here, the reaction is initiated by [3+2] cycloaddition reaction of 3-arylsydnone with acrylonitrile with the evolution of carbon dioxide (4.26), followed by rearomatization of the intermediate (4.27) by chloranil resulting in the formation of 2a-c (Scheme 16).

Cyanopyrazole (2a-c) undergone [3+2] cycloaddition with sodium azide in presence of triethylamine hydrochloride and afforded 5-(1-aryl-1\(H\)-pyrazol-3-yl)-1\(H\)-tetrazoles (3a-c). The process is generally known to occur by a concerted 1,3-dipolar cycloaddition reaction, in which nitrile (2a-c) serves as dipolarophile toward azide, which acts as 1,3-dipolar species. Further, cycloaddition through 4.28 leads to the tautomeric tetrazolium anions (4.29 and 4.30), which can be represented as the delocalised form 4.31. Protonation of 4.31 leads to the formation of 5-substituted tetrazole (3a-c, Scheme 17).

Scheme 16 Mechanism of [3+2] cycloaddition
When 3\textsubscript{a-c} was reacted with acetic anhydride under dry conditions gave 2-methyl-5-(1-phenyl-1\textsubscript{H}-pyrazol-3-yl)-1,3,4-oxadiazole (4\textsubscript{a-c}). The reaction is initiated with acetylation of 2\textsubscript{H}-tetrazole (3\textsubscript{a-c}) which further underwent ring opening followed by elimination of nitrogen molecule to form a carbene intermediate (3.32). Carbene insertion across the carbonyl carbon afforded 1,3,4-oxadiazole (4\textsubscript{a-c}, Scheme 18).

Scheme 17

When 2\textsubscript{a-c} was heated with 70\% HCl at 100°C, nitrile group was hydrolyzed to carboxylic acid affording 1-aryl-1\textsubscript{H}-pyrazole-3-carboxylic acids (5\textsubscript{a-c}). The amide derivative 6\textsubscript{a-c} was then prepared from carboxylic acid derivative (5\textsubscript{a-c}) by the refluxing 5\textsubscript{a-c} with thionyl chloride followed by addition of ammonia. Also, 1-aryl-1\textsubscript{H}-pyrazole-3-carboxamide (6\textsubscript{a-c}) was
synthesized directly from 3-arylsydnone by [3+2] cycloaddition reaction of 1a-c with acrylamide in presence of chloranil (Scheme 19).

![Scheme 19](image)

The bis-carbethoxy pyrazole derivatives (7a-c) were prepared by [3+2] cycloaddition reaction of 3-arylsydnone (1a-c) with dimethyl acetylenedicarboxylate (DMAD). Upon alkaline hydrolysis (2N NaOH), compound 7a-c gave 1-aryl-1H-pyrazole-3,4-dicarboxylic acid (8a-c). The bis-amide derivative, 1-aryl-1H-pyrazole-3,4-dicarboxamide (9a-c) is synthesized by refluxing 8a-c with thionyl chloride followed by addition of ammonia.

All the newly synthesized compounds were characterized by ¹H and ¹³C NMR, mass spectral and elemental analyses.

### 4.5.2 Spectral characterization

In case of ¹H NMR studies, the OH and NH₂ protons of carboxylic acid and amide substituted pyrazole derivatives (5a-c, 6a-c, 8a-c, 9a-c) are resonated at δ11.98-12.96 ppm and δ7.45-9.00 ppm respectively. The C5 proton of pyrazole ring in all the derivatives has shown signal at δ8.55-9.06 ppm. The C4 proton of pyrazole ring in derivatives 3a-c, 4a-c, 5a-c and 6a-c has resonated at δ6.82-7.15 ppm. The C5 methyl protons of 1,3,4-oxadiazole derivatives (4a-c) gave signals at δ2.58-2.64 ppm. All other proton signals are in accordance with the proposed structure. In case of ¹³C NMR analysis, the
carbonyl carbons of carboxylic acid and amide derivatives have shown signals at $\delta$162-163 ppm. The C5 carbon in compounds 3a-c and 4a-c has shown signal at $\delta$149 and 163 ppm respectively. The C5 and C4 carbons of pyrazole ring have resonated around $\delta$127-131 ppm and $\delta$107-120 ppm respectively. The C5 methyl carbon of 1,3,4-oxadiazole derivatives (4a-c) gave signals at $\delta$10 ppm. All other carbon signals have resonated at expected regions. The mass spectral analyses of all the title compounds have shown the m/z values which correspond to their molecular mass.

4.6 Experimental

4.6.1 General procedure for the preparation of 1-aryl-1H-pyrazole-3-carbonitrile (2a-c)

A mixture of 3-arylsydnone (1a-c, 10.0 mM), chloranil (10.6 mM) and acrylonitrile (20 ml) in toluene (20 ml) was heated on water bath at 80°C till the evolution of CO$_2$ ceases. After completion of the reaction (monitored by TLC using hexane:ethylacetate (7:3) solvent mixture), the reaction mixture was cooled, treated with 2N NaOH and extracted using diethyl ether (20ml×3). The organic layer was collected and dried over anhydrous Na$_2$SO$_4$. The solvent is removed under reduced pressure to get crude 2a-c which was recrystallized from benzene-pet ether.

4.6.2 General procedure for the preparation of 5-(1-aryl-1H-pyrazol-3-yl)-1H-tetrazole (3a-c)

A mixture of nitrile derivative 2a-c (10 mM), sodium azide (44.8 mM) and triethylamine hydrochloride (44.8 mM) in toluene (20 ml) was stirred at 110°C for 48 h. After completion of the reaction (monitored by TLC using hexane:ethylacetate (7:3) solvent mixture), the reaction mixture was cooled and stirred for 30 min at room temperature. The product was extracted with water (15 ml) and 5% NaOH (5 ml). The aqueous layer was collected and acidified with dil. HCl. The solid separated was filtered, dried and recrystallized using acetone to get crystals of 3a-c.
4.6.3 General procedure for the preparation of 2-methyl-5-(1-aryl-1H-pyrazol-3-yl)-1,3,4-oxadiazole (4a-c)

5-(1-Aryl-1H-pyrazol-3-yl)-1H-tetrazole (3a-c, 1.0 g) was heated with acetic anhydride (10 ml) on water bath at 90°C under guard tube conditions for 4 h. The reaction was monitored by TLC using hexane:ethylacetate (7:3) solvent mixture. After completion of reaction, the reaction mixture was cooled and poured into ice cold water. The solid separated was filtered, dried and recrystallized using ethanol to get 4a-c.

4.6.4 General procedure for the preparation of 1-aryl-1H-pyrazole-3-carboxylic acid (5a-c)

Cyanopyrazole (2a-c, 1.0 g) was heated with 70% HCl at 100°C for 4 h. The reaction was monitored by TLC using hexane:ethylacetate (7:3) solvent mixture. After completion of reaction, the reaction mixture was cooled and the solid separated was filtered and dissolved in ethyl acetate. The compound was extracted with 10% NaHCO₃ solution. The aqueous layer was collected, cooled and acidified with dil. HCl to get analytically pure 5a-c.

4.6.5 General procedure for the preparation of 1-aryl-1H-pyrazole-3-carboxamide (6a-c)

A mixture of 3-arylsydnone (1a-c, 10.0 mM), chloranil (10.6 mM) and acrylamide (10 mM) in toluene (20 ml) was heated on water bath at 80°C till the evolution of CO₂ ceases. After completion of the reaction (monitored by TLC using hexane:ethylacetate (7:3) solvent mixture), the reaction mixture was cooled, treated with 2N NaOH and extracted with diethyl ether (20ml×3). The organic layer was collected and dried over anhydrous Na₂SO₄. The solvent is removed under reduced pressure to get crude 6a-c which was recrystallized from methanol.

4.6.6 Alternative procedure for the preparation of 1-aryl-1H-pyrazole-3-carboxamide (6a-c)

A mixture of 1-aryl-1H-pyrazole-3-carboxylic acid (5a-c, 1.0 g) was refluxed with thionyl chloride (10 ml) on water bath for 2 h. Excess of thionyl
chloride was expelled out and cooled. Ammonia was added to get white solid of 6a-c which was recrystallized using methanol.

The compound 7a-c is prepared according to the previously reported method.\textsuperscript{38}

4.6.7 General procedure for the preparation of 1-aryl-1H-pyrazole-3,4-dicarboxylic acid (8a-c)

Dimethyl-1-aryl-1H-pyrazole-3,4-dicarboxylate (7a-c, 1.0 g) was heated with 2N NaOH (10 ml) at 100°C for 2 h. After completion of the reaction (monitored by TLC using hexane:ethylacetate (7:3) solvent mixture), the reaction mixture was filtered, cooled and acidified with dil. HCl to get 8a-c which was recrystallized using methanol.

4.6.8 General procedure for the preparation of 1-aryl-1H-pyrazole-3,4-dicarboxyamide (9a-c)

1-Aryl-1H-pyrazole-3,4-dicarboxylic acid (8a-c, 1.0 g) was refluxed with thionyl chloride (10 ml) on water bath for 4 h. Excess of thionyl chloride was expelled out and cooled. Ammonia was added to get white solid of 9a-c which was recrystallized using methanol.

5-(1-Phenyl-1H-pyrazol-3-yl)-1H-tetrazole (3a)

\[
\begin{array}{c}
\text{Yield: 92%; m.p: 198-200°C; } ^1\text{H NMR (400 MHz, DMSO-}\delta_6, \delta \text{ ppm): (Spectrum No. 1) 8.73 (d, 1H, } J = 2.8 \text{ Hz, pyrazole C}_3\text{H), 7.92 (d, 2H, } J = 12.0 \\
\text{Hz, ArH), 7.59-7.37 (m, 3H, ArH), 7.15 (d, 1H, } J = 2.8 \\
\text{Hz, pyrazole C}_4\text{H); } ^1\text{C NMR (100 MHz, DMSO-}\delta_6, \delta \text{ ppm): (Spectrum No. 2) 149.95, 139.52, 139.09, 130.41, 129.58, 127.31, 119.03, 107.88 ; MS (m/z): (Spectrum No. 3) 212 (M), 184, 170, 157, 103, 77; Analysis Calcd. for C}_{10}\text{H}_8\text{N}_6 (212.21): C, 56.60; H, 3.80; N, 39.60; Found: C, 56.72; H, 4.01; N, 39.85.}
\end{array}
\]
**5-(1-(4-Chlorophenyl)-1H-pyrazol-3-yl)-1H-tetrazole (3b)**

Yield: 91%; m.p: 215-217°C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\), \(\delta\) ppm): 8.64 (d, 1H, \(J = 2.4\) Hz, pyrazole C\(_5\)H), 7.94 (d, 2H, \(J = 8.8\) Hz, ArH), 7.59 (d, 2H, \(J = 8.8\) Hz, ArH), 7.02 (d, 1H, \(J = 2.4\) Hz, pyrazole C\(_4\)H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\), \(\delta\) ppm): 152.55, 142.56, 139.91, 130.31, 129.55, 127.13, 120.33, 107.78; MS (\(m/z\)): 248 (M+2), 246 (M), 220, 218, 206, 204, 156, 113, 111, 75; Analysis Calcd. for C\(_{10}\)H\(_7\)N\(_6\)Cl (246.66): C, 48.69; H, 2.86; N, 34.07; Found: C, 48.91; H, 3.00; N, 34.17.

**5-(1-(4-Methoxyphenyl)-1H-pyrazol-3-yl)-1H-tetrazole (3c)**

Yield: 89%; m.p: 192-194°C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\), \(\delta\) ppm): 8.61 (d, 1H, \(J = 2.4\) Hz, pyrazole C\(_5\)H), 7.83 (d, 2H, \(J = 9.2\) Hz, ArH), 7.12 (d, 2H, \(J = 9.2\) Hz, ArH), 7.08 (d, 1H, \(J = 2.4\) Hz, pyrazole C\(_4\)H), 3.80 (s, 3H, CH\(_3\)); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\), \(\delta\) ppm): 158.39, 149.96, 139.10, 132.37, 128.48, 122.24, 114.69, 107.54, 59.49; MS (\(m/z\)): 242 (M), 196, 171, 154, 103, 77; Analysis Calcd. for C\(_{11}\)H\(_{10}\)N\(_6\)O (242.24): C, 54.54; H, 4.16; N, 34.69; Found: C, 54.81; H, 4.37; N, 34.87.

**2-Methyl-5-(1-phenyl-1H-pyrazol-3-yl)-1,3,4-oxadiazole (4a)**

Yield: 80%; m.p: 95-97°C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\), \(\delta\) ppm): 8.71 (d, 1H, \(J = 2.4\) Hz, pyrazole C\(_5\)H), 7.76 (d, 2H, \(J = 8.4\) Hz, ArH), 7.52-7.35 (m, 3H, ArH), 7.01 (d, 1H, \(J = 2.4\) Hz, pyrazole C\(_4\)H), 2.64 (s, 3H, CH\(_3\)); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\), \(\delta\) ppm): 163.45, 160.11, 141.26, 139.77, 131.20, 130.19, 129.68, 120.00, 107.78, 10.89; MS (\(m/z\)): 226 (M), 171, 155, 116, 104, 78; Analysis Calcd. for C\(_{12}\)H\(_{10}\)N\(_4\)O (226.23): C, 63.71; H, 4.46; N, 24.76; Found: C, 63.89; H, 4.71; N, 24.90.
2-(1-(4-Chlorophenyl)-1H-pyrazol-3-yl)-5-methyl-1,3,4-oxadiazole (4b)

Yield: 79%; m.p: 82-84°C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\), \(\delta\) ppm): 8.68 (d, 1H, \(J = 2.4\) Hz, pyrazole \(C_5\)H), 7.91 (d, 2H, \(J = 9.2\) Hz, ArH), 7.62 (d, 2H, \(J = 9.2\) Hz, ArH), 7.05 (d, 1H, \(J = 2.4\) Hz, pyrazole \(C_4\)H), 2.61 (s, 3H, \(CH_3\)); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\), \(\delta\) ppm): 163.23, 160.51, 141.87, 139.78, 130.24, 129.71, 129.47, 120.32, 107.86, 10.77; MS (\(m/z\)): 262 (M+2), 260 (M), 249, 182, 155, 102, 76; Analysis Calcd. for \(C_{12}H_9N_4OCl\) (260.68): C, 55.29; H, 3.48; N, 21.49; Found: C, 55.48; H, 3.71; N, 21.66.

2-(1-(4-Methoxyphenyl)-1H-pyrazol-3-yl)-5-methyl-1,3,4-oxadiazole (4c)

Yield: 79%; m.p: 114-116°C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\), \(\delta\) ppm): 8.60 (d, 1H, \(J = 2.4\) Hz, pyrazole \(C_5\)H), 7.82 (d, 2H, \(J = 9.2\) Hz, ArH), 7.06 (d, 1H, \(J = 2.4\) Hz, pyrazole \(C_4\)H), 3.81 (s, 3H, \(CH_3\)), 2.58 (s, 3H, \(CH_3\)); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\), \(\delta\) ppm): 163.56, 159.53, 158.35, 137.68, 132.59, 130.08, 120.55, 114.66, 107.60, 55.44, 10.46; MS (\(m/z\)): 256 (M), 201, 158, 130, 103, 78; Analysis Calcd. for \(C_{13}H_{12}N_4O_2\) (256.26): C, 60.93; H, 4.72; N, 21.86; Found: C, 61.20; H, 4.88; N, 21.98.

1-Phenyl-1H-pyrazole-3-carboxylic acid (5a)

Yield: 81%; m.p: 262-264°C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\), \(\delta\) ppm): 12.91 (s, 1H, OH), 8.57 (d, 1H, \(J = 2.4\) Hz, pyrazole \(C_5\)H), 7.88 (d, 2H, \(J = 9.6\) Hz, ArH), 7.55-7.36 (m, 3H, ArH), 6.94 (d, 1H, \(J = 2.4\) Hz, pyrazole \(C_4\)H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\), \(\delta\) ppm): 162.87, 145.17, 139.22, 130.16, 129.58, 127.26, 119.06, 110.14; MS (\(m/z\)): (Spectrum No. 9) 188 (M), 171, 143, 117, 104, 77; Analysis Calcd. for
C_{10}H_{8}N_{2}O_{2} (188.18): C, 63.82; H, 4.28; N, 14.89; Found: C, 63.99; H, 4.77; N, 15.01.

1-(4-Chlorophenyl)-1H-pyrazole-3-carboxylic acid (5b)
Yield: 78%; m.p: 204-206°C; $^1$H NMR (400 MHz, DMSO-$d_6$, $\delta$ ppm): 12.95 (s, 1H, OH), 8.60 (d, 1H, $J = 2.8$ Hz, pyrazole C$_5$H), 7.92 (d, 2H, $J = 10.0$ Hz, ArH), 7.59 (d, 2H, $J = 10.0$ Hz, ArH), 6.94 (d, 1H, $J = 2.8$ Hz, pyrazole C$_4$H); $^{13}$C NMR (100 MHz, DMSO-$d_6$, $\delta$ ppm): 162.74, 145.45, 138.02, 131.44, 130.89, 129.52, 120.69, 110.36; MS ($m/z$): 224 (M+2), 222 (M), 177, 142, 111, 75; Analysis Calcd. for C$_{10}$H$_7$N$_2$O$_2$Cl (222.63): C, 53.95; H, 3.17; N, 12.58; Found: C, 54.11; H, 3.25; N, 12.76.

1-(4-Methoxyphenyl)-1H-pyrazole-3-carboxylic acid (5c)
Yield: 75%; m.p: 192-194°C; $^1$H NMR (400 MHz, DMSO-$d_6$, $\delta$ ppm): 12.92 (s, 1H, OH), 8.61 (d, 1H, $J = 2.4$ Hz, pyrazole C$_5$H), 7.10 (d, 2H, $J = 12.4$ Hz, ArH), 7.01 (d, 1H, $J = 2.4$ Hz, pyrazole C$_4$H), 3.83 (s, 3H, CH$_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$, $\delta$ ppm): 163.57, 158.41, 145.06, 132.04, 130.61, 121.42, 114.54, 110.44, 59.52; MS ($m/z$): 218 (M), 175, 158, 130, 104, 78; Analysis Calcd. for C$_{11}$H$_{10}$N$_2$O$_3$ (218.21): C, 60.55; H, 4.62; N, 12.84; Found: C, 60.86; H, 4.91; N, 13.02.

1-Phenyl-1H-pyrazole-3-carboxamide (6a)
Yield: 71%; m.p: semisolid; $^1$H NMR (400 MHz, DMSO-$d_6$, $\delta$ ppm): 8.88 (s, 2H, NH$_2$), 8.55 (d, 1H, $J = 2.4$ Hz, pyrazole C$_3$H), 7.82 (d, 2H, $J = 10.8$ Hz, ArH), 7.60-7.34 (m, 3H, ArH), 6.97 (d, 1H, $J = 2.4$ Hz, pyrazole C$_4$H); $^{13}$C NMR (100 MHz, DMSO-$d_6$, $\delta$ ppm): 164.29, 145.78, 139.29, 130.09, 129.67, 128.32, 119.81, 110.22; MS ($m/z$): 187, 171, 105, 91, 77; Analysis Calcd. for C$_{10}$H$_8$N$_3$O (187.20): C, 64.16; H, 4.85; N, 22.45; Found: C, 64.38; H, 5.05; N, 22.64.
1-(4-Chlorophenyl)-1\textit{H}-pyrazole-3-carboxamide (6b)

Yield: 71%; m.p: semisolid; \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d\textsubscript{6}}, \(\delta\) ppm): (Spectrum No. 10) 9.03 (s, 2H, NH\textsubscript{2}), 8.58 (d, 1H, \(J = 2.8\) Hz, pyrazole C\textsubscript{5}H), 7.82 (d, 2H, \(J = 10.8\) Hz, ArH), 7.59 (d, 2H, \(J = 10.8\) Hz, ArH), 6.92 (d, 1H, \(J = 2.8\) Hz, pyrazole C\textsubscript{4}H); \textsuperscript{13}C NMR (100 MHz, DMSO-\textit{d\textsubscript{6}}, \(\delta\) ppm): (Spectrum No. 11) 162.27, 145.09, 139.46, 130.77, 129.83, 127.02, 119.27, 110.50; MS (m/z): (Spectrum No. 12) 224 (M\textsuperscript{+}2), 222 (M), 205, 178, 138, 111, 75; Analysis Calcd. for C\textsubscript{10}H\textsubscript{8}N\textsubscript{3}OCl (221.64): C, 54.19; H, 3.64; N, 18.96; Found: C, 54.25; H, 3.87; N, 19.11.

1-(4-Methoxyphenyl)-1\textit{H}-pyrazole-3-carboxamide (6c)

Yield: 68%; m.p: semisolid; \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d\textsubscript{6}}, \(\delta\) ppm): 8.75 (s, 2H, NH\textsubscript{2}), 8.43 (d, 1H, \(J = 2.0\) Hz, pyrazole C\textsubscript{5}H), 7.81 (d, 2H, \(J = 11.8\) Hz, ArH), 7.07 (d, 2H, \(J = 11.8\) Hz, ArH), 6.82 (d, 1H, \(J = 2.0\) Hz, pyrazole C\textsubscript{4}H), 3.80 (s, 3H, CH\textsubscript{3}); \textsuperscript{13}C NMR (100 MHz, DMSO-\textit{d\textsubscript{6}}, \(\delta\) ppm): 163.81, 159.20, 145.24, 131.63, 129.71, 121.46, 115.37, 108.04, 59.61; MS (m/z): 217 (M), 207, 175, 159, 108, 78; Analysis Calcd. for C\textsubscript{11}H\textsubscript{11}N\textsubscript{3}O\textsubscript{2} (217.22): C, 60.82; H, 5.10; N, 19.34; Found: C, 61.01; H, 5.26; N, 19.52.

1-Phenyl-1\textit{H}-pyrazole-3,4-dicarboxylic acid (8a)

Yield: 86%; m.p: 218-220\degree C; \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d\textsubscript{6}}, \(\delta\) ppm): 12.96 (s, 1H, OH), 12.02 (s, 1H, OH), 9.06 (s, 1H, pyrazole C\textsubscript{5}H), 7.92 (d, 2H, \(J = 11.2\) Hz, ArH), 7.56-7.39 (m, 3H, ArH); \textsuperscript{13}C NMR (100 MHz, DMSO-\textit{d\textsubscript{6}}, \(\delta\) ppm): 163.67, 163.36, 145.24, 138.56, 133.09, 130.90, 129.69, 119.43, 117.24; MS (m/z): 232 (M), 218, 188, 161, 144, 104, 78; Analysis Calcd. for C\textsubscript{11}H\textsubscript{8}N\textsubscript{2}O\textsubscript{4} (232.19): C, 56.90; H, 3.47; N, 12.06; Found: C, 57.03; H, 3.66; N, 12.31.
1-(4-Chlorophenyl)-1H-pyrazole-3,4-dicarboxylic acid (8b)
Yield: 90%; m.p: 265-267°C; \( ^1\text{H} \) NMR (400 MHz, DMSO-\( d_6 \), \( \delta \) ppm): 12.93 (s, 1H, OH), 11.98 (s, 1H, OH), 9.05 (s, 1H, pyrazole \( C_5 \)H), 7.91 (d, 2H, \( J = 10.8 \) Hz, ArH), 7.60 (d, 2H, \( J = 10.8 \) Hz, ArH); \( ^{13}\text{C} \) NMR (100 MHz, DMSO-\( d_6 \), \( \delta \) ppm): 163.77, 163.09, 145.06, 138.16, 133.80, 131.41, 129.12, 119.88, 118.31; MS (\( m/z \)): 268 (M+2), 222 (M), 204, 170, 138, 111, 75; Analysis Calcd. for C\(_{11}\)H\(_7\)N\(_2\)O\(_4\)Cl (266.64): C, 49.55; H, 2.65; N, 10.51; Found: C, 49.77; H, 2.72; N, 10.80.

1-(4-Methoxyphenyl)-1H-pyrazole-3,4-dicarboxylic acid (8c)
Yield: 92%; m.p: 208-210°C; \( ^1\text{H} \) NMR (400 MHz, DMSO-\( d_6 \), \( \delta \) ppm): (Spectrum No. 13) 12.96 (s, 1H, OH), 12.00 (s, 1H, OH), 8.95 (s, 1H, pyrazole \( C_5 \)H), 7.83 (d, 2H, \( J = 8.8 \) Hz, ArH), 7.06 (d, 2H, \( J = 8.8 \) Hz, ArH), 3.80 (s, 3H, CH\(_3\)); \( ^{13}\text{C} \) NMR (100 MHz, DMSO-\( d_6 \), \( \delta \) ppm): (Spectrum No. 14) 163.77, 163.38, 158.78, 144.47, 132.95, 131.99, 121.09, 116.71, 114.65, 55.49; MS (\( m/z \)): (Spectrum No. 15) 262 (M), 203, 157, 121, 77; Analysis Calcd. for C\(_{12}\)H\(_{10}\)N\(_2\)O\(_5\) (262.22): C, 54.97; H, 3.84; N, 10.68; Found: C, 55.13; H, 3.98; N, 10.81.

1-Phenyl-1H-pyrazole-3,4-dicarboxamide (9a)
Yield: 86%; m.p: 278-280°C; \( ^1\text{H} \) NMR (400 MHz, DMSO-\( d_6 \), \( \delta \) ppm): 9.72 (s, 2H, NH\(_2\)), 8.87 (s, 1H, pyrazole \( C_5 \)H), 7.88 (d, 2H, \( J = 12.6 \) Hz, ArH), 7.56-7.38 (m, 3H, ArH), 7.43 (s, 2H, NH\(_2\)); \( ^{13}\text{C} \) NMR (100 MHz, DMSO-\( d_6 \), \( \delta \) ppm): 164.28, 163.44, 145.68, 138.43, 132.88, 130.33, 129.67, 119.21, 118.21; MS (\( m/z \)): 230 (M), 214, 196, 170, 104, 77; Analysis Calcd. for C\(_{11}\)H\(_{10}\)N\(_4\)O\(_2\) (230.22): C, 57.39; H, 4.38; N, 24.34; Found: C, 57.57; H, 4.56; N, 24.50.
1-(4-Chlorophenyl)-1H-pyrazole-3,4-dicarboxamide (9b)

Yield: 84%; m.p: 292-294°C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\), \(\delta\) ppm): (Spectrum No. 16) 9.75 (s, 2H, NH\(_2\)), 9.03 (s, 1H, pyrazole C\(_5\)H), 8.08 (d, 2H, \(J = 10.6\) Hz, ArH), 7.61 (d, 2H, \(J = 10.6\) Hz, ArH), 7.45 (s, 2H, NH\(_2\)); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\), \(\delta\) ppm): (Spectrum No. 17) 164.34, 162.01, 142.61, 137.28, 134.16, 131.99, 129.44, 121.07, 120.91; MS (\(m/z\)):

(Spectrum No. 18) 266 (M+2), 264 (M), 250, 248, 230, 138, 111, 75; Analysis Calcd. for C\(_{11}\)H\(_{10}\)N\(_4\)O\(_2\) (264.67): C, 49.92; H, 3.43; N, 21.17; Found: C, 50.20; H, 3.65; N, 21.35.

1-(4-Methoxyphenyl)-1H-pyrazole-3,4-dicarboxamide (9c)

Yield: 88%; m.p: 152-154°C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\), \(\delta\) ppm): 9.77 (s, 2H, NH\(_2\)), 8.88 (d, 2H, \(J = 4.8\) Hz, ArH), 7.41 (s, 2H, NH\(_2\)), 7.08 (d, 2H, \(J = 4.8\) Hz, ArH), 3.81 (s, 3H, CH\(_3\)); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\), \(\delta\) ppm): 164.52, 162.23, 158.64, 144.03, 132.58, 131.97, 122.53, 119.79, 114.54, 55.47; MS (\(m/z\)): 260 (M), 244, 226, 174, 134, 107, 78; Analysis Calcd. for C\(_{11}\)H\(_{10}\)N\(_4\)O\(_2\) (260.25): C, 55.38; H, 4.65; N, 21.53; Found: C, 55.56; H, 4.71; N, 21.68.
Spectrum No. 1: $^1$H NMR (DMSO-$d_6$) spectrum of compound 3a

Spectrum No. 2: $^{13}$C NMR (DMSO-$d_6$) spectrum of compound 3a
Spectrum No. 3: Mass spectrum of compound 3a

Spectrum No. 4: $^1$H NMR (DMSO-$d_6$) spectrum of compound 4c
Chapter 4

Synthesis and antifungal activity of pyrazole derivatives

Spectrum No. 5: $^{13}$C NMR (DMSO-$d_6$) spectrum of compound 4c

Spectrum No. 6: Mass spectrum of compound 4c

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$\text{m/z} = 256$
Chapter 4

Synthesis and antifungal activity of pyrazole derivatives

Spectrum No. 7: $^1$H NMR (DMSO-$d_6$) spectrum of compound 5a

![Spectrum No. 7: $^1$H NMR (DMSO-$d_6$) spectrum of compound 5a](image)

Spectrum No. 8: $^{13}$C NMR (DMSO-$d_6$) spectrum of compound 5a

![Spectrum No. 8: $^{13}$C NMR (DMSO-$d_6$) spectrum of compound 5a](image)
Chapter 4

Synthesis and antifungal activity of pyrazole derivatives

Spectrum No. 9: Mass spectrum of compound 5a

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DHARWAD

\[ m/z = 188 \]

Spectrum No. 10: \(^1\)H NMR (DMSO-\(d_6\)) spectrum of compound 6b

Cl

\[ \text{CONH}_2 \]
Chapter 4

Synthesis and antifungal activity of pyrazole derivatives

Spectrum No. 11: $^{13}$C NMR (DMSO-$d_6$) spectrum of compound 6b

![NMR Spectrum of Compound 6b]

Spectrum No. 12: Mass spectrum of compound 6b

![Mass Spectrum of Compound 6b]

m/z = 224 [M+2]

222
Spectrum No. 13: $^1$H NMR (DMSO-$d_6$) spectrum of compound 8c

Spectrum No. 14: $^{13}$C NMR (DMSO-$d_6$) spectrum of compound 8c
Chapter 4  
Synthesis and antifungal activity of pyrazole derivatives

Spectrum No. 15: Mass spectrum of compound 8c

Spectrum No. 16: $^1$H NMR (DMSO-$d_6$) spectrum of compound 9b
Chapter 4

Synthesis and antifungal activity of pyrazole derivatives

Spectrum No. 17: $^{13}$C NMR (DMSO-$d_6$) spectrum of compound 9b

Spectrum No. 18: Mass spectrum of compound 9b
4.7 Pharmacology

4.7.1 Background

The occurrence of fungal infections caused by opportunistic fungi are increasing especially in patients whose immune system is suppressed by AIDS, cancer, diabetes, aging and other causes. Among the human pathogenic fungi, *A. fumigatus* is the primary causative agent of human infections.

*A. fumigatus* is the most prevalent airborne fungal pathogen responsible for fatal invasive aspergillosis (IA) in immunocompromised patients. The development of IA results primarily from dysfunction of host defence mechanism in combination with fungal growth during infection.\(^\text{38}\) It has been proved that the virulence and viability of fungal pathogens is due to its cell wall which is highly conserved and dynamic in structure.\(^\text{39}\) The cell wall is known to compose of many multifunctional molecules which help and promote the growth of *A. fumigatus* in the host during infection. Hence, the identification and study of these components is highly essential in order to lead new control measures for IA.

β-(1,3)-glucans is a predominant cell wall polysaccharide of many fungi including *A. fumigatus* and is thought to be responsible for the shape and rigidity of the fungal cell wall.\(^\text{40}\) It is evident that the organization of β-(1,3)-glucans in fungal cell wall is poorly known and the enzyme β-(1,3)-endoglucanase (ENGL1) is responsible for the continuous rearrangement of wall β-(1,3)-glucans during fungal growth.\(^\text{41}\) ENGL1 protein catalyzes the hydrolysis of the substrates by cleaving β-linkages at random sites along the polysaccharide chain releasing smaller oligosaccharides.\(^\text{42}\) The ENGL1 protein is believed to play multiple physiological and cell wall remodelling roles during growth and morphogenesis in filamentous fungi.

Despite extensive studies of β-(1,3)-endoglucanases, the structural information and their exact role during cell wall ontogeny have not been fully elucidated. It is clear that computer-based homology modelling and docking
studies can be useful in the identification of potent antifungal molecules for the regulation of enzyme activity.

In the present study, the homology modelling and docking analysis of a β-(1,3)-endoglucanases from *A. fumigatus* were performed and the results of molecular interactions of active site residue with synthesized molecules were further substantiated by *in vitro* antifungal activity against *A. fumigatus* fungal strain.

### 4.7.2 Methodology

**Sequence retrieval of target sequence**

The amino acid sequence of ENGL1 protein was obtained from the protein sequence database of NCBI and blasted against Protein Data Bank (PDB) entries to find similar sequences.\(^4^3\)

**Homology modelling**

Homology modelling was used to generate 3D model of ENGL1 protein by MODELLER 9.10.\(^4^4\) The obtained protein sequence was predicted using the online protein structure and function prediction server I-TASSER\(^4^5\) which uses threading technique to predict the reliable 3D structure. With the help of this server, 4 best models based on multiple-threading alignments and template fragment assembly simulations along with their confidence scores were generated. These models were visualized using Accelrys DS 2.0 software (Accelrys Inc., San Diego, CA, USA). Further the stereo chemical quality and accuracy of the predicted model was evaluated with PROCHECK\(^4^6\) by Ramachandran plot analysis\(^4^7\) (Table 1). The best model was selected on the basis of overall G-factor, number of residues in core, allowed and disallowed regions. The selected model was further analysed using VERIFY-3D\(^4^8\) analysis and finally the quality of the predicted structure was analyzed using ERRAT.
Table 1 Validation of modelled ENGL1 protein

<table>
<thead>
<tr>
<th>Analysis of modelled ENGL1 protein</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramachandran plot</td>
<td>72.3%</td>
<td>71.0%</td>
<td>72.5%</td>
<td>72.1%</td>
</tr>
<tr>
<td>(Residues of most favoured region)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VERIFY 3d</td>
<td>87.21%</td>
<td>86.52%</td>
<td>89.96%</td>
<td>86.24%</td>
</tr>
<tr>
<td>ERRAT</td>
<td>88.317</td>
<td>89.276</td>
<td>89.525</td>
<td>85.815</td>
</tr>
</tbody>
</table>

**Protein preparation**

In this process, polar hydrogens and Gasteiger charges were added to the modelled ENGL1 protein through http://mgltools.scripps.edu/.

**Ligand preparation**

All the synthesized compounds were drawn using Chem Draw Ultra 8.0 software. Using Argus lab, all the drawn structures were subjected to geometric cleaning and geometry optimization. Gasteiger charges were added and nonpolar hydrogen were merged, rotatable bonds were determined based on the nature of the ligand by using MGL tools.

**Grid preparation**

Grid maps were generated and spacing was adjusted to 1.0 Å to enable ligand binding. Grid dimension was adjusted to 104×82×80 points. AutoDock was used to obtain interacting maps for docking. Prior to the actual docking run, these maps were calculated by AutoGrid. For each ligand, the interaction energy between the ligand atom and the receptor was calculated for the entire binding site, which is discredited through a grid.\(^{49}\) The protein was embedded in a 3D grid and a probe was placed at each grid point. Interaction energy of the protein was assigned at each grid point and the affinity grid and electrostatic potential for each atom of the ligand was calculated. Electrostatic interaction was evaluated by interpolation.\(^{50}\)
Docking studies

Automated docking software AutoDock Vina was used to predict binding affinity of ligands with ENGL1 protein. Docking energy of all 10 ligand molecules were evaluated by using empirical free energy functions and Lamarkian genetic algorithm to calculate the binding free energy ($\Delta G$) in kcal/mol based on the different electrostatic, Van der Waal, hydrogen bonding and desolvation effects. At the end of docking, the best pose of the ligand was analyzed for its binding energy and interactions.

4.7.3 Results

The docking studies of ligands with ENGL1 protein has been illustrated in Table 2. The docking complexes analyze the binding free energy of the ligands. The negative and low value of binding energy as well as maximum hydrogen bonding illustrated the occurrence of strong and most favourable binding between protein and ligand molecule. Docking results indicated that compounds $5b$, $6b$, $7b$, $9a$ and $9b$ have shown hydrogen bonds with amino acids of ENGL1 protein. From the table, it is clear that the docking complexes of $7b$ and $9b$ exhibited lowest free energy compared to others.
### Table 2 Docking results of synthesized compounds with ENGL1

<table>
<thead>
<tr>
<th>Entry</th>
<th>Binding free energy Kcal/mol</th>
<th>Amino acid interaction</th>
<th>H-bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td>3b</td>
<td>-5.5</td>
<td>Val464, Phe468, Leu 472, Asn 474, Asp475, His 476, Ala537, Lys538, Leu540, Phe541, Glu542, Ser543, Asp548, Glu549, Glu550, Ile614.</td>
<td>---</td>
</tr>
<tr>
<td>4a</td>
<td>-6.6</td>
<td>Ile3, Val4, Ser5, Phe6, Leu7, Phe19, Pro44, Leu139, Pro140, Ala270, Val271, Met272, Met285, Ser515, Ala516, Gly517, Phe524.</td>
<td>---</td>
</tr>
<tr>
<td>4b</td>
<td>-6.5</td>
<td>Val464, Gly465, Phe468, Gly469, Leu472, Asn474, Asp475, Phe478, Ala537, Gly539, Leu540, Phe541, Glu542, Glu550.</td>
<td>---</td>
</tr>
<tr>
<td>5a</td>
<td>-7.1</td>
<td>Thr43, Pro44, Val45, Glu46, Lys49, Thr51, Phe55, Pro138, Leu139, Pro140, Ser142, Pro523, Phe524.</td>
<td>---</td>
</tr>
<tr>
<td>5b</td>
<td>-6.8</td>
<td>Thr43, Pro44, Glu46, Lys49, Tyr51, Pro138, Leu139, Pro140, Ser142, Lys451, Asn514, Ala516, Gly517, Phe524.</td>
<td>Phe522…H-Pro523…H-</td>
</tr>
<tr>
<td>6a</td>
<td>-7.0</td>
<td>Thr43, Pro44, Val45, Glu46, Lys49, Tyr51, Cys52, Phe55, Pro138, Leu139, Pro140, Ser142, Gly517, Pro523, Phe524.</td>
<td>---</td>
</tr>
<tr>
<td>6b</td>
<td>-6.9</td>
<td>Ile3, Val4, Ser5, Phe6, Leu7, Asp17, Phe19, Leu139, Pro140, Ala270, Met285, Ser515, Ala516, Gly517, Phe524.</td>
<td>Val4…N-Ser5…N-Val271…O-Met272…O- Gly517…O-Pro523…O-</td>
</tr>
<tr>
<td>7b</td>
<td>-7.7</td>
<td>Thr43, Pro44, Glu46, Lys49, Tyr51, Pro138, Leu139, Pro140, Val271, Lys451, Asn514, Ser515, Ala516, Asp519, Pro520, Phe522, Pro523, Phe524.</td>
<td>Gly517…O-Pro523…O-</td>
</tr>
<tr>
<td>9a</td>
<td>-7.1</td>
<td>Pro44, Glu46, Tyr51, Lys49, Pro138, Leu139, Pro140, Ser142, Asn514, Ala516, Gly517, Phe522, Pro523, Phe524.</td>
<td>Thr43…N-Pro523…N-Gly517…O-</td>
</tr>
<tr>
<td>9b</td>
<td>-7.6</td>
<td>Thr43, Pro44, Tyr51, Glu46, Lys49, Pro138, Leu139, Pro140, Lys451, Asn514, Ala516, Asp519, Phe522, Phe524.</td>
<td>Pro523…N-Gly517…O-</td>
</tr>
</tbody>
</table>
Docking complexes of ENGL1 protein with synthesized compounds

**Figure 1** Docking complex of ENGL1 protein with compound 3b

**Figure 2** Docking complex of ENGL1 protein with compound 4a
Figure 3 Docking complex of ENGL1 protein with compound 5b

Figure 4 Docking complex of ENGL1 protein with compound 6a
Figure 5 Docking complex of ENGL1 protein with compound 7b

Figure 6 Docking complex of ENGL1 protein with compound 9b
4.7.4 In vitro antifungal activity

Azoles play an important role in the management of fungal diseases. Azoles block ergosterol synthesis by binding to the active site cavity of the enzyme and ligating the iron atom of the heme co-factor through nitrogen atom of azole. Molecular studies have shown that, majority of azole resistance in A. fumigatus is due to activity of cytochrome P450 sterol 14α-dimethylase enzyme (CYP51A gene). However, at present there are limited numbers of antifungal agents to overcome these infections and the situation has become more complicated due to the emergence of antifungal resistance and side-effects of antifungal drugs. In view of this, we have successfully attempted the in vitro antifungal activity of the synthesized pyrazole derivatives.

4.7.5 Methodology

Initially, 9 dilutions 100, 50, 25, 12.5, 6.25, 3.12, 1.6, 0.8, 0.4, 0.2 of each sample were prepared with Brain Heart Infusion (BHI) broth. In the initial tube 20microliter of sample was added into the 380 μl of BHI broth. For dilutions 200 μl of BHI broth was added into the next 9 tubes separately. Then from the initial tube 200 μl was transferred to the first tube containing 200 μl of BHI broth. This was considered as 10⁻¹ dilution. From 10⁻¹ diluted tube 200 μl was transferred to second tube to make 10⁻² dilution. The serial dilution was repeated up to 10⁻⁹ dilution for each drug. From the maintained stock cultures of required organisms, 5 μl was taken and added into 2ml of BHI broth. In each serially diluted tube 200microliter of above culture suspension was added. The tubes were incubated for 24 h and observed for turbidity. The minimum inhibition concentration (MIC) was noted down for each sample. Fluconazole was used as a standard drug.
### Table 3 MIC (µg/ml) results of synthesized compounds

<table>
<thead>
<tr>
<th>Entry</th>
<th>A. fumigatus</th>
<th>C. albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>25.0</td>
<td>12.5</td>
</tr>
<tr>
<td>3b</td>
<td>0.2</td>
<td>25.0</td>
</tr>
<tr>
<td>3c</td>
<td>0.4</td>
<td>100.0</td>
</tr>
<tr>
<td>4a</td>
<td>0.2</td>
<td>25.0</td>
</tr>
<tr>
<td>4b</td>
<td>1.6</td>
<td>50.0</td>
</tr>
<tr>
<td>4c</td>
<td>3.12</td>
<td>50.0</td>
</tr>
<tr>
<td>5a</td>
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Standard drug used: **Fluconazole**  
MIC for *A. fumigatus* – 8 µg/ml  
MIC for *C. albicans* – 16 µg/ml
4.7.6 Results

Table 3 depicts the MIC of the synthesized compounds against the fungal strains. From the table it is clear that, the compounds have shown significant activity against *A. fumigatus* strain compared against *C. albicans* strain.

**Activity against *A. fumigatus***

It is interesting to observe that compounds 3b, 3c, 4a, 5a, 5b, 5c, 6a, 6c, 7b, 9a and 9b have exhibited excellent antifungal activity at lower MIC values; 0.2-0.8 µg/ml which is lower than the standard drug Fluconazole (8 µg/ml). Compounds 4b, 4c, 6b, 7a and 9c with MIC values in the range 1.6-6.25 µg/ml have also shown lower MIC in comparison with standard drug. Derivatives 8a-c comprising of pyrazole ring substituted with bis-carboxylic acids have not shown significant activity.

**Activity against *C. albicans***

Compounds 8a, 8b and 9b have shown inhibition at 0.8-12.5 µg/ml which is lower than the standard drug Fluconazole (16 µg/ml). All other compounds did not show any significant activity.
References


