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MOLECULAR DOCKING STUDY OF 2-AMINOIMIDAZOLE SUBSTITUTED LIGAND AS INHIBITORS AS CYTOCHROME P450-DEPENDENT LANOSTEROL 14α-DEMETHYLASE (P45014DM, CYP51) IN THE ERGOSTEROL-BIOSYNTHESIS

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ABSTRACT

Molecular docking study of Azoles act by inhibiting cyctochrome P450 14 α demethylase is a key enzymes in the fungal ergo sterol biosynthesis. Azoles antifungal now represents a successful strategy for antifungal development. A universal step in the biosynthesis of membrane sterol and steroid hormones is the oxidative removal of the 14 α methyl group from sterol precursors by sterols 14 α demethylase. This enzyme is a primary targets in the treatment of fungal infection in organism ranging from human to plants and development of more potent and selective CYP51 inhibitors (azoles) is an important biological objective. In study molecular docking via MOE-Dock program was used to evaluate binding interactions of ligands at target enzymes. We have recently described the synthesis and antifungal activity of series of 2-animoimidazoles derivatives. Here we reports set of our molecules in a 3D model of CYP51 of c*andida-albicans*. The docking and experimental results were found in good correlation.

KEYWORDS: Molecular docking, cyctochrome P450 14 α demethylase, Azoles antifungal.

1. INTRODUCTION

The incidence of primary and opportunistic fungal infections continues to increase rapidly because of the increased number of immune compromised patients (AIDS, cancer and transplants).[1] *Candida* sp. is one of the most well-known fungal pathogens which accounts for majority of opportunistic fungal infections occurring worldwide (Odds, 1996). The class of azoles (imidazole and triazole derivatives) has supplied many effective antifungal drugs currently in clinical use.[2] Resistance to wide spectrum antifungal agents has initiated the search for new therapeutic agents, including those produced by the modification of existing antifungal drugs.[3] The azoles ring has been demonstrated to be one of the most important pharmacophores for antifungal activity, and both the toxicity and activity of azoles antifungal agents are mainly attributed to coordination binding of the nitrogen atom of the azoles ring to the iron atom of heme.[4] These facts led us to search for novel azoles lead compounds with more structural specificity for fungal enzymes in order to separate their activity from their toxicity.

Molecular docking may be defined as an optimization problem, which would describe the "best-fit" orientation of a ligand that binds to a particular protein of interest and is used to predict the structure of the intermolecular complex formed between two or more molecules. The most interesting case is the protein ligand interaction, because of its applications in medicines. Ligand is a small molecule, which interacts with protein's binding sites. There are several possible mutual conformations in which binding may occur. These are commonly called binding modes[5] in modern drug designing, molecular docking is routinely used for understanding drug-receptor interaction. Molecular docking provides useful information about drug receptor interactions and is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. Heterocyclic containing an azoles ring system are found to exhibit a wide spectrum of biological activities, including antibacterial and antifungal properties. Imidazole and its derivatives have gained remarkable importance due to their widespread biological activities and their use in synthetic chemistry. Imidazole derivatives possess a broad spectrum of pharmacological activities such as, anti-inflammatory[6], analgesic, anti-conversant[7], ant tubercular[8], antimicrobial, anticancer and anti-Parkinson activities.[9] Imidazole and its derivatives are of great significance due to their important roles in biological systems, particularly in, enzymes as proton donors and/or acceptors, coordination system ligands and the base of charge–transfer processes. The imidazole nucleus appears in a number of naturally occurring products like,

amino acids histidine and purines, which comprise many of the most important bases in nucleic acids.

As part of our research project, we studied how our molecules may be interacting at target enzyme. Indeed, the understanding of the molecules basis of such interaction should help in the design of more active inhiditors. Here, we reports a docking study of a set our azoles inhibitors in a 3D model of CYP51 of Candida albicans (CA-CYP51). The modal was constructed on the basis of sequence homology relationship with the recently reported crystal structure of the CYP51 of Mycobacterium tuberculosis (MT-CYP51) pdb code 1ea1, 1e9x

2. EXPERIMENTAL

Computational Evaluation: Docking Study

All molecular techniques used in this manuscript were performedon Argus Lab ver. 4.0-work system. The starting 3Dstructure (Boscott and Grant, 1994) of the cytochromeP450sterol 14DM CPY51 of C. albicans was downloaded fromthe Protein Data Bank (http://www.rcsb.org) as PDB files(PDB entry: 1e9x).

The file containing the crystal structure of cytochromeP450a-sterol demethylase (14DM) with its selective inhibitori.e.4-Phenyl-1H-Imidazolesin the active site (PDB entry 1e9x, six ligand) was downloaded. It is monomer structure with onlychain A consisting of 449 residues. This chain A has 470YPF, water and 1 heme (HEM) groups. The chain A with residues, water and the hetero groups (HEM) within a radius of 5 A˚ was refined and cleaned by checking the hybridization, valence ofthe ligand and introducing H-atoms to the protein residues.1e9x carries net charge 2 and 3590 atoms.

2.1Docking and binding evaluation

In the automated module of Argus Lab ver. 4.0 work systems, the ligand was docked into the active site of cytochrome P45014a-sterol demethylase (14DM) from C. albicans using Argus dock with a fast, simplified potential of mean force (PMF).The docking is carried with flexible ligand into a rigid protein active site. The general procedure for docking process starts with the addition of energy minimized target ligand on the enzyme. The active site and the ligand were specified in the program. The different starting parameters were optimized by using 15· 15· 15 box located at the centre of the target active site using a united atom (explicit hydrogen are not considered)potential of mean force (PMF) with a docking algorithm that has a population of 50 chromosomes and runs for 6000 generations. The process of docking is repeated until a constant value of docking score is reached. This takes about 12,000–18,000 generation. The final results are parameterizedin terms of docking score in kcal/mol. The docked ligand–P450 (14DM) CPY51 complex is interpreted by looking at the H-bonding or hydrophobic interaction of the ligand with the amino acid residues in the active site. The same procedure was followed for docking of different substituted 4, 5-imidazolylpyrimidine into the active site of cytochrome P450 14a-steroldemethylase CPY51 enzymes.

2.2 Validation of PMF method

To ensure the validation of the programme, before docking the test compounds, the docking of Fluconazole (470TPF) into the active site of P450 14DM was performed. This selective inhibitor of cytochrome P450 binds in the active site with a binding score of -8.86cal/mol.

P450 demethylase inhibitors into the active site of 1e9x.The behaviour of Ketanozoles, Miconazole, Griesofulv in and Fluconazole to ward scytochrome P450 (14DM) CY51 is in reasonable agreement with their binding energy (docking score) with 1e9x as calculated from the docking of these compounds in 1e9x active site. During binding of this known antifungal drug in the binding pocket of cytochrome P450, the conformational placement of amino acid residues in the active site is observed.

2.3 Spectral data of synthesized imidazole (3a-p)

2.3.1. 2-bromo -4-6-(2, 5-dihydro-2-imino-1H-imidazol-4-yl) phenol (3a)

White solid,m.p.:290^{0}C; Yield:89%; IR (KBr, λ_{max}/cm^{-1}): 3541 (OH), 2870 (-NH), 1509 (C=N); ^{1}H NMR (400MHz, DMSO-d_6) δ : 1.25 (s, 1H, NH), 1.65 (s, 2H,-CH_2), 6.98 (s, 1H, NH), 7.19-7.17 (s, 2H, Ar-*H*), 10.83 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 68.5, 115.7, 124.6, 127.2, 129.6, 131.0, 159.3, 163.2, 164.6; MS: *m/z* = 288 (M^+); Anal. Calcd for.$C_9H_7BrClN_3O$: C, 37.46; H, 2.65; N, 15.19. Found: C, 37.24; H, 2.47; N, 14.05 %.

2.3.2. 4-chloro-2-(2, 5-dihydro2-imimo2-amino-1H-imidazol-4-yl)-6-nitrophenol (3b)

White solid,m.p.: 225^{0}C; Yield: 85 %; IR (KBr λ_{max}/cm^{-1}): 3385 (OH), 3105 (-NH), 1523 (C=N); ^{1}H NMR (400MHz, DMSO-d_6) δ: 1.31 (s, 1H, NH); 1.45(s, 2H, -CH_2), 6.91 (s, 1H, NH), 7.96 (s, 1H, Ar-*H*), 7.72 (s, 1H, Ar-*H*), 10.73 (s, 1H, OH); ^{13}C NMR (100 MHz,$CDCl_3$): δ 52.6, 109.0, 122.6, 125.4, 128.3, 134.0, 137.5, 163.2, 164.2; MS*: m/z* =254 (M^+); Anal. Calcd for $C_9H_7ClN_4O_3$: C, 42.45; H, 2.77; N, 22.19. Found: C, 42.24; H, 2.65; N, 22.02 %.

2.3.3. *4-chloro-2-(2, 5-dihydro-2-imino-1H-imidazol-4-yl)- 6-iodophenol (3c)*

White solid,m.p.:276^{0}C; Yield:82%; IR (KBr λ_{max}/cm^{-1}): 3380 (OH), 3099 (-NH), 1521(C=N); ^{1}H NMR (400MHz, DMSO-d_6) δ : 1.34(s, 1H,N*H*); 1.54 (s, 2H, -CH_2), 6.21 (s, 1H, N*H*), 7.14 (s, 1H, Ar*H*), 7.51 (s, 1H, Ar-*H*), 10.22 (s,1H,OH); ^{13}C NMR (100MHz,$CDCl_3$): δ 68.9, 89.7,120.0, 127.2, 129.6, 140.5, 159.5, 161.8, 162.8; MS: *m/z* =334(M^+); Anal. Calcd for $C_9H_7ClN_3OI$: C, 32.22; H, 2.10; N, 12.52. Found: C, 31.90; H, 1.99; N, 12.40 %.

2.3.4. 2-(2-amino-1H-imidazol-4-yl)-4-chlorophenol (3d).

White solid,m.p. 270^{0}C; Yield: 90 %; IR (KBr λ_{max}/cm^{-1}): 3568 (OH), 2924 (-NH), 1500 (C=N); ^{1}H NMR (400MHz, DMSO-d$_6$) δ: 1.54 (s, 1H, NH), 1.48(s,2H,-CH$_2$), 6.56 (s,1H,NH), 7.11-7.47 (s, 2H, Ar-*H*), 7.98 (s, 1H, Ar-H), 10.02 (s, 1H, OH); ^{13}CNMR (100MHz,CDCl$_3$): δ 68.2, 117.7, 120.0, 122.0, 127.2, 129.6, 131.0, 159.7, 163.9, 165.8; MS: *m/z* =209 (M$^+$); Anal. Calcd for $C_9H_7ClN_3O$: C, 51.56; H, 3.85; N, 20.04. Found: C, 51.40; H, 3.72; N, 19.65 %.

2.3.5. 2-bromo-6-(2,5-dihyro-2-imino-1H-imidazol-4-yl)-4-methylphenol (3e)

White solid,m.p.: 285^{0}C; Yield: 75 %; IR (KBr λ_{max}/cm^{-1}): 3588 (OH), 3568 (NH), 1531 (C=N); ^{1}H NMR (400MHz, DMSO-d$_6$) δ: 2.11 (s, 3H, C*H*$_3$), 1.38(s, 2H, -CH$_2$), 7.16 (s, 1H, Ar-H), 7.01 (s, 1H, Ar-H), 10.83 (s, 1H, OH); ^{13}C NMR (100MHz,CDCl$_3$) δ: 26.7, 47.8, 114.2, 120.0, 122.3, 153.0, 153.9, 163.0, 164.8; MS: *m/z* =267 (M$^+$); Anal. Calcd for $C_{10}H_{10}BrN_3O$: C, 44.80; H, 3.76; N, 15.67. Found: C, 44.62; H, 3.22; N, 15.35%.

2.3.6. 2-(2,5-dihydro-2-imino-1H-imidazol-4-yl)-4-methyl-6-nitrophenol (3f)

White solid,m.p:220^{0}C; Yield: 80 %; IR (KBr λ_{max}/ cm^{-1}): 3543 (OH), 3105 (-NH),1512 (C=N); ^{1}H NMR (400MHz, DMSO-d$_6$) δ:1.32 (s, 1H,NH), 1.45 (2,2H,-CH$_2$), 2.11 (s, 3H, CH$_3$), 6.84 (s, 1H, NH),7.79 (s, 1H, Ar-H), 8.00 (s, 1H, Ar-H); ^{13}C NMR (100 MHz,CDCl$_3$) δ: 26.7, 48.3,114.2, 120.0, 121.4, 136.3, 147.8, 154.5, 164.1,166.0; MS: *m/z* =234 (M$^+$); Anal. Calcd for $C_{10}H_{10}N_4O_3$: C, 51.28; H, 4.30; N, 23.92. Found: C, 50.94; H, 3.87; N, 23.35%.

2.3.7. 2-(2,5-dihydro-2-imino-1H-imidazol-4-yl)-6-iodo-4-methylphenol (3g)

White solid,m.p.: 235^{0}C; Yield:82%; IR (KBr) λmax/ cm^{-1}: 3585 (OH), 3124 (-NH), 1558 (C=N); ^{1}H NMR (400MHz, DMSO-d$_6$) δ : 1.18 (s, 1H, NH), 1.37 (s, 2H,-CH$_2$), 2.33 (s, 3H, CH$_3$), 6.7 (s, 1H, NH), 7.16 (s, 1H, Ar-H), 10.8 (s, 1H, OH); ^{13}CNMR (100 MHz, CDCl$_3$) δ: 26.7, 50.0, 87.2, 120.0, 130.9, 133.7,143.5,157.1, 161.0,163.2; MS: *m/z* = 314 (M$^+$) Anal. Calcd for $\cdot C_{10}H_{10}IN_3O$: C, 38.12; H, 3.20; N, 13.34; Found: C, 37.96; H, 2.80; N, 13.15%.

2.3.8. 2-(-2, 5- dihydro 2-imino- 1H-imidazol-4-yl)-4-methylphenol (3h)

White solid,m.p.: 280^{0}C, Yield: 87%; IR (KBr λmax/cm^{-1}): 3432 (OH), 3076 (-NH), 1527 (C=N); ^{1}H NMR (400MHz, DMSO-d$_6$) δ: 1.09 (s, 1H, NH), 1.52(s, 2H, -CH$_2$), 2.72 (s, 3H, CH$_3$), 6.70 (s, 1H,NH), 7.35 (s, 1H, Ar-H), 7.11 (s,1H, Ar-H), 10.65 (d,1H,OH); ^{13}C NMR (100 MHz,CDCl$_3$) δ: 26.7, 49.7, 114.2, 120.0, 130.9, 131.1, 131.9, 156.0, 161.9, 164.6; MS: *m/z* = 191 (M$^+$) Anal. Calcd for $C_{10}H_{13}N_3O$: C, 62.81; H, 6.85; N, 21.97. Found: C, 62.54; H, 6.48; N, 21.11%.

2.3.9. 2-(2,5-dihyro-2-imino-1H-imidazol-4-yl)benzene-1,3-diol (3i)

White solid,m.p: 304^{0}C;Yield: 70%; IR (KBr λ_{max}/ cm^{-1}): 3527 (OH),1546 (C=N); ^{1}H NMR (400MHz, DMSO-d$_6$) δ: 1.89 (s, 1H, NH), 1.72(s, 2H,-CH$_2$), 6.91 (s, 1H, NH),7.96 (s, 1H, Ar-H), 8.06 (d, 2H, Ar-H),10.30 (s, 1H, OH), 11.02(s,1H, OH), ^{13}C NMR (100 MHz,CDCl$_3$) δ: 64.3, 104.6, 109.6, 115.7, 131.0, 162.0, 162.9, 164.7, 165.0; MS: *m/z* =191(M$^+$); Anal. Calcd for $\cdot C_9H_9N_3O_2$: C, 56.81; H, 4.7; N, 21.98. Found: C, 56.51; H, 7.40; N, 21.13%.

2.3.10. 4-(2, 5-dihydro-2-imino-1H-imidazol-4-yl)-6-iodobenzene-1,3-diol (3j)

White solid,m.p.: 310^{0}C; Yield: 79%; IR (KBr) λ_{max}/ cm^{-1} 3390 (OH), 1510 (C=N); ^{1}H NMR (400MHz, DMSO-d$_6$) δ: 1.47(s, 2H, -CH$_2$), 1.76 (s, 1H,NH), 6.17 (s, 1H, NH), 8.32 (s, 1H, Ar-*H*), 8.29 (s, 1H, Ar-*H*), 10.81 (s,1H, OH), 11.08 (s, 1H, OH), ^{13}C NMR (100 MHz, CDCl$_3$) δ: 63.5, 80.6, 104.6, 113.2, 140.0, 159.5, 162.0, 164.7, 166.9; MS: *m/z* = 316(M$^+$) Anal. Calcd for $C_9H_8IN_3O_2$: C, 62.81; H, 6.85; N, 21.97. Found: C, 62.54; H, 6.48; N, 21.11%.

2.3.11.4-(2,5-dihydro-2-imino-1H-imidazol-4-yl)-6-nitrobenzene-1,3-diol (3k)

White solid,.p.: 210^{0}C;Yield: 81%; IR (KBr λ_{max}/cm^{-1}): 3395 (OH), 1520 (C=N); ^{1}H NMR (400MHz,DMSO-d6) δ: 1.11 (s, 1H, NH), 1.36 (s,2H,-CH$_2$) , 5.81 (s, 1H, NH), 7.29 (s, 1H, Ar-*H*), 7.32 (s, 1H, NH), 9.20 (s, 1H, OH), 9.56 (s, 1H, OH); ^{13}C NMR (100MHz, CDCl$_3$) δ: 62.1, 102.9, 112.3, 125.9, 128.7, 160.0, 162.5, 167.0; MS: *m/z* = 236(M$^+$) ; Anal. Calcd for $C_9H_8N_4O_4$: C, 45.77; H, 3.41; N, 23.10. Found: C, 45.35; H, 3.18; N, 23.00%.

2.3.12. 4-bromo-6-(2,5dihyro-2-imino-1H-imidazol-4-yl)benzene-1,3-diol (3l)

White solid,m.p.: 268^{0}C; Yield: 88%; IR (KBr λ_{max}/ cm^{-1}): 3376 (OH),1590 (C=N); ^{1}H NMR (400MHz, DMSO-d$_6$) δ : 1.23(s,2H,-CH$_2$), 1.70 (s, 1H, NH), 6.09 (s, 1H, NH), 7.54 (s, 1H, Ar-H), 8.10 (s, 1H, NH), 11.53 (s, 1H, OH), 11.86 (s, 1H, OH); ^{13}C NMR (100 MHz, CDCl$_3$) δ : 60.9 , 106.4, 106.9, 115.3, 135.0, 163.0, 164.0, 165.9, 166.9; MS: *m/z* = 268 (M$^+$); Anal. Calcd for $C_9H_8BrN_3O_2$: C, 58.52; H, 5.40; N, 20.48. Found: C, 58.12; H, 5.23; N, 20.21%.

2.3.13. 2-(2,5-dihyro-2-imino-1H-imidazol-4-yl)-5-methoxphenol (3m)

White solid,m.p.:230^{0}C; Yield: 70%; IR (KBr λ_{max}/cm^{-1}): 3380 (OH), 1545 (C=N); ^{1}H NMR (400MHz DMSO-d$_6$) δ: 1.05(s, 1H, NH), 1.30(s, 2H,-CH$_2$), 3.57 (s, 3H, OCH$_3$), 5.23 (s, 1H, NH), 7.21 (s, 1H, Ar-H), 7.30 (d, 2H, Ar-H), 10.83 (s, 1H, OH); ^{13}C NMR (100 MHz, CDCl$_3$) δ 47.1, 55.2, 102.2, 107.5, 112.5, 130.9, 163.3, 164.5, 165.6, 167.0; MS: *m/z* = 205(M$^+$); Anal. Calcd for $C_{10}H_{11}N_3O_2$: C, 40.02; H, 3.99; N, 15.56. Found: C, 39.35; H, 3.20; N, 15.01%.

2.3.14. 2-(2,5-dihyro-2-imino-1H-imidazol-4-yl)-4-iodo-5-methoxphenol (3n)

White solid,m.p.:260^{0}C; Yield: 86%; IR (KBr λ_{max}/ cm^{-1}) : 3190 (OH), 1550 (C=N); ^{1}H NMR (400MHz, DMSO-d_6) δ: 1.43(s, 1H, NH), 1.76 (s, 2H,-CH_2), 3.05 (s, 3H, OC*H*$_3$), 5.67(s, 1H, NH), 7.21 (s, 1H, Ar-H), 7.56 (s, 1H, Ar-H), 10.83 (s, 1H, OH); ^{13}C NMR (400 MHz, $CDCl_3$) δ : 49.2, 55.2, 102.5, 112.8, 140.5, 161.0, 163.5 ,164.0, 164.9; MS: *m/z* = 330(M^+); Anal. Calcd for $C_{10}H_{10}IN_3O_2$: C, 36.27; H, 3.04; N, 20.48. Found: C, 36.05; H, 2.90; N, 20.20%.

2.3.15.2-(2,5-dihydro-2-imino-1H-imidazol-4-yl)-5-methoxy-4-nitrophenol (3o)

White solid,m.p.: 260^{0}C; Yield: 75%; IR (KBr) λ_{max}/ cm^{-1}: 3263 (OH), 1581 (C=N); ^{1}H NMR (400MHz, DMSO-d_6) δ: 1.50 (s, 1H, NH), 1.64 (s, 2H,-CH_2), 3.37 (s, 3H, OC*H*$_3$), 5.49 (s, 1H, NH), 8.26 (s, 1H, Ar-*H*), 7.01 (s, 1H, Ar-H), 11.80 (s, 1H, OH); ^{13}C NMR δ: (100 MHz, $CDCl_3$) δ : 49.2, 55.2, 102.5, 113.0, 126.1, 126.5, 161.0, 162.3, 164.6, 168.0; MS: *m/z* = 250(M^+); Anal. Calcd for $C_{10}H_{10}N_4O_4$: C, 48.00; H, 4.03; N, 22.39; Found: C, 47.87; H, 3.77; N, 22.10%.

2.3.16. 4-bromo-(2,5-dihydro-2-imino -1H-imidazol-4-yl)-5-methoxphenol (3p)

White solid,m.p.: 309^{0}C;Yield: 73% IR (KBr) λ_{max}/ cm^{-1}: 3091 (OH), 1509 (C=N); ^{1}H NMR (400MHz, DMSO-d_6) δ : 1.26 (s, 1H, NH), 1.40 (s, 2H,-CH_2), 2.81 (s, 3H, OC*H*$_3$), 5.09 (s, 1H, NH), 7.14 (s, 1H, Ar-*H*), 7.32 (s, 1H, NH), 8.43 (s,1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 51.3,56.1, 106.4,106.9, 115.3, 135.0, 160.2, 161.0, 163.1, 165.1, MS*: m/z* = 283(M^+); Anal. Calcd for $C_{10}H_{19}BrN_3O_2$: C, 42.27; H, 3.55; N, 14.79. Found: C, 47.07; H, 3.21; N, 14.55 %

3. RESULT AND DISCUSSION

In the present study, all the ligands used were made using Chemdraw 3D Ultra 8.047. Before the docking calculation of the ligands, the structures were fully optimized. As details of the calculations used are available in the literature and therefore, they are not mentioned here. Argus Lab 4.048 was used to perform all the docking techniques. The crystal structure used for the present study was found to be complexed with inhibitor 4 phenyl-1H- imidazoles was downloaded from Protein Data Bank (http://www.rscb.org/) as PDB files**.**(PDB entry: 1e9x).

It is monomer structure with only chain A consisting of 449 residues. This chain A has PIM, water and 1 heme (HEM) groups. The chain A with residues, water and the hetero groups (HEM) within a radius of 5Å was refined and cleaned by checking the hybridization, valence of the ligand and introducing H-atoms to the protein residues. 1e9x carries net charge 2 and 3590 atoms **Figure 1.**

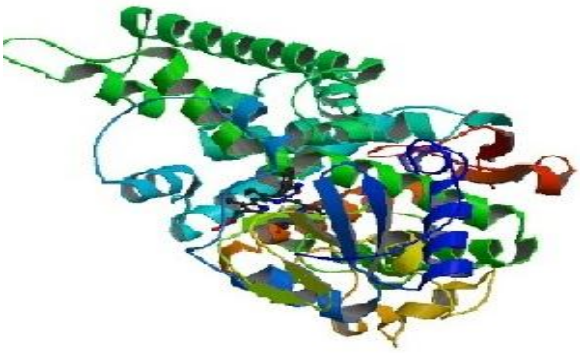

Figure 1: 3 D model of P450 (14DM) CPY51

Docking and binding evaluation

In the automated Argus Lab 4.0 system, using a generic algorithm with a fast-simplified Potential of Mean Force (PMF) carried docking of synthesized ligands into active site of 4- phenyl- imidazoles. All final geometry optimizations and energy minimizations were performed (using the AM1 Hamiltonian method) through the eigenvector (EF) routine on restricted Hartree-Fock (RHF) basis with maximum gradient 0.001-0.002 kcal/mol and 200 iterations without any conformational or symmetry restrictions. Since the exact geometries of all the molecules are not known experimentally, final geometry optimizations and energy minimizations was determined using AM1 method. The first step was the construction of ligands in Chemdraw 3D Ultra 8.047 followed by their optimization. It was assumed that the protein and the ligand docked non-covalently. The standard PMF implementation used UFF potential for this purpose. The docking was carried with both flexible and rigid ligand into a rigid protein active site. As many ligands failed to undergo docking procedure when they were considered to be flexible in nature; on the other hand while considering their rigid nature they underwent the whole procedure with ease. The general procedure for the docking process started with the addition of energy minimized target ligand on the enzyme. The active site and the ligands were specified in the programme. The different starting parameters were optimized by using 15x15x15 box located at the centre of the target active site using a united atom (explicit hydrogen are not considered) potential of mean force (PMF) with a docking algorithm that has a population of 50 chromosomes and runs for 6000 generations. The process of docking is repeated until a constant value of docking score is reached. This takes about 12000-18000 generation. The final results are parameterized in terms of docking score in kcal/mol. The docked ligand-p450 (14DM) CPY51 complex is interpreted by looking at the H-bonding or hydrophobic interaction of the ligand with the amino acid residues in the active site. The same procedure was followed for docking of different substituted into the active site of cytochrome P450 14 α - sterol demethylase CPY51 enzymes.

The non-selective behavior of Ketanozoles, Miconazole, Griesofulvin and Fluconazole towards cytochrome p450 (14DM) CY51 is quite in agreement with their binding energy (docking score) with 1e9x as calculated from the docking of these compounds in 1e9x active site **Table 1**. During binding of this known antifungal drug in the binding pocket of cytochrome p450, the conformational placement of amino acid residues in the active site is observed. The molecules **3a-p** show binding in the active site of enzymes with the binding scores ranging from -8.86 to- 6.47 kcal/mol.

Table 1. Binding Score of Known Drugs With P450 (14DM) CPY51.

Entry	Ligand	Binding score (Kcal/mol)
1	Ketanozoles	-9.39
2	Miconazole	-11.04
3	Griesofulvin	-8.71
4	Fluconazole	-8.38
5	4-Phenyl-1H-Imidazoles	-7.96

In our studies the Affinity method has proved to be a powerful docking tool for identifying key residues involved in azoles binding. Moreover, the inhibitor recognition and binding process is a process of "induced fit," and the conformations of both the target enzyme and the inhibitor would be changed during the enzyme-inhibitor interaction. The flexibilities of both the active site of the target enzyme and the inhibitor are taken into account in the Affinity docking Program. The calculated energies of interaction of azoles antifungal agents with CYP51 are given in **Table 2.** The most prominent bindings are observed in the case of ligands **3a, 3b, 3c, 3d, 3e, 3f, 3g,** and **3h** in compared 4-Phenyl-1H-Imidazoles. In case of **3h**, phenyl ring is surrounded by HIS259, PHE78, MET79, and PHE. Imidazole ring is surrounded by HIS259, ALA256, LEU321, and THR260. The docking score is 8.86 Kcal/mol because the docking model revealed that the addition of a methyl group on C-6 could form favorable hydrophobic interaction with the surrounding residues. Substitution of phenyl rings with a variety of substituent led to an increase in binding energy, but introduction of halogens in *ortho*- and *para*-position gave potent compounds.

Table2: **Binding Score of2 -(2-imino-1H-imidazol-4-yl) Derivatives with P450 (14DM) CPY51.**

Entry	Ligand	Binding energy (Kcal/mol)
1	3a	-8.55
2	3b	-8.14
3	3c	-8.67
4	3d	-8.42
5	3e	-8.34
6	3f	-8.10
7	3g	-8.38
8	3h	-8.86
9	3i	-7.01
10	3j	-6.47
11	3k	-7.76
12	3l	-7.75
13	3m	-7.09
14	3n	-7.37
15	3o	-7.26
16	3p	-7.67

This extensive investigation prompted us to explore structure-activity-relationships (SAR) **(Figure 2)** in this class of compounds. The acetophenones, easily accessible from phenacyl bromides and excess imidazole, were very good starting materials. The active site of enzyme contains iron proto-porphyrin IX bound in part by hydrophobic forces lined by hydrophobic residues. It renders, heme binding site as a highly hydrophobic pocket having tendency to show better binding energy with the highly electronegative substructure. Based on binding energies and hydrogen bonding ligands were selected for molecular dynamics simulation. On the basis of structural features essential for binding in the cavity, the scaffolds could be divided into two segments: phenyl ring occupied the hydrophobic pocket and imidazole ring located in polar binding cleft. (**Figure 2)**The supposed binding mode of the docked molecule **3a-p** suggests key hydrogen bonding interaction between the Hydroxyl group of the phenyl ring and the prorogated nitrogen of His259 side chain. NO_2 group at 4^{th} position of phenyl ring **(3a, 3f)** was found forming hydrogen bond with nitrogen of ALA256. The imidazole ring was found in polar pocket and N1 of imidazole exhibited interactions with ALA256 (2.75 A°) in **3e,** TYR 76(2.79 A°) in **3g**, HIS259(2.56 A°,2.99 A°) in **3l,3m** and HEME ($2.79A^0$). Inhibitors **3h** containing hydrogen bond donor NH_2 groups of imidazole ring bonded with HIS259 (2.56 A°) and ALA256 (2.79 A°).In the present docking studies, five hydrogen bonding interactions have been explored in **3o**, viz., hydrogen bonding between the N1-imidazole with HEME (2.1 A°), phenyl NO_2 with Fe of HEME (2.39A°). All the molecules in the series were placed well in the active site and demonstrated the above mentioned interactions. The only difference observed is the orientation of substituted phenoxy aromatic ring. From the above investigations, the hydroxyl group attached to C-2 of phenyl ring was important for antifungal activity, in the crystal structure of; fluconazole interacts with at least three water molecules, which bridge the interactions with the active site. [112] From the docking model, it is possible that the water molecules in the active site mediate the interaction between the hydroxyl group and the vicinal H320 (the P2 subsite), which is a highly conserved functional residue in the CYP51 family. Comparing the active sites of 1ea1 and 1e9x.The residues surrounding each imidazole ring and substituted phenyl molecule have been determined **Table 3.**

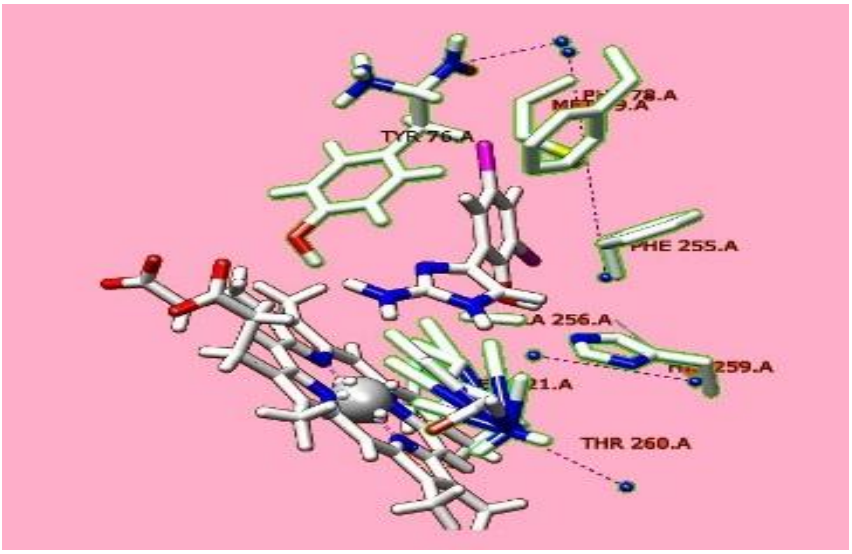

Figure 2: Docking of known selective and non selective cytochrome p450 demethylase inhibitors into the active site of 1e9x.

Table 3: Amino acid residues around all segment of phenyl and imidazole ring.

Ligand	No. of H bond	H Bond interaction	Hydrophobic interaction	
			Phenyl ring	**Imidazole ring**
3a	1	HIS259	HIS259, PHE255, TYR76, PHE78,	THR260, LEU321
3b	2	HIS 259, ALA256	ALA256, HIS259, LEU321. PHE255, THR260	TYR76, PHE78, MET79
3c	1	HIS259	HIS259, PHE255, THR260 PHE78	LEU321, TYR76, ALA256, MET79
3d	1	HIS259	HIS259, TYR76, MET79, PHE78	ALA256, HE255, THR260, LEU321.
3e	1	AlA256	LEU321, HIS259,MET79, PHE255	ALA256, THR260, TYR76, PHE78
3f	2	HIS259, ALA256	TYR76, ALA256, MET79, HIS256, PHE78	PHE255, THR260, LEU321,
3g	1	TRY76	PHE255, PHE78, MET79, LEU321	TRY76, HIS256, THR260, ALA256
3h	3	HIS259, ALA256	PHE255, MET79, PHE78, LEU321	HIS259, ALA256, EU321, THR260
3i	Nil	HIS259	HIS259, MET79, PHE78, LEU321, ALA256,	THR260, PHE255, TYR76
3j	1	HIS 259	HIS259, THR260, PHE255, TYR76	MET79, PHE78, LEU321, ALA256
3k	1	HIS259	HIS259, THR260, ALA256, PHE78	PHE78, TYR76, MET79
3l	2	HIS259, ALA256	HIS259, THR260, TYR76	ALA256, PHE255, MET79, PHE78, ALA26
3m	2	HIS259, HEME	THR260, TYR76	HIS259, PHE255, MET79, ALA256
3n	1	HIS 259	HIS259, THR260, TYR76	ALA256, PHE255, ALA256
30	5	HEME, Metal Fe, HIS259	HIS259, TYR76, ALA256	PHE255, MET79, PHE78, ALA26
3p	1	HIS 259	HIS259, MET79, PHE78,	TYR76, ALA256

1.4.5.3. Validation of the docking protocol

The most suitable method of evaluating the accuracy of a docking procedure is to determine how closely the lowest energy pose predicted by the scoring function resembles an experimental binding mode as determined by X-ray crystallography. In the present study, the Extra Precision Glide docking procedure was validated by removing 4-Phenyl-1H-Imidazoles from the binding site and re-docking it to the cytochrome p450 demethylase. We found very good agreement between the localization of the inhibitor4-Phenyl-1H-Imidazoles upon docking and from the crystal structure. The relative mean square deviations (RMSD) between the predicted conformation and the observed X-ray crystallographic conformation of 4 -Phenyl-1H-Imidazoles equaled 1.026 Å. This indicated the reliability of the docking method in reproducing the experimentally observed binding mode for cytochrome p450 demethylase. **Figure 3.**

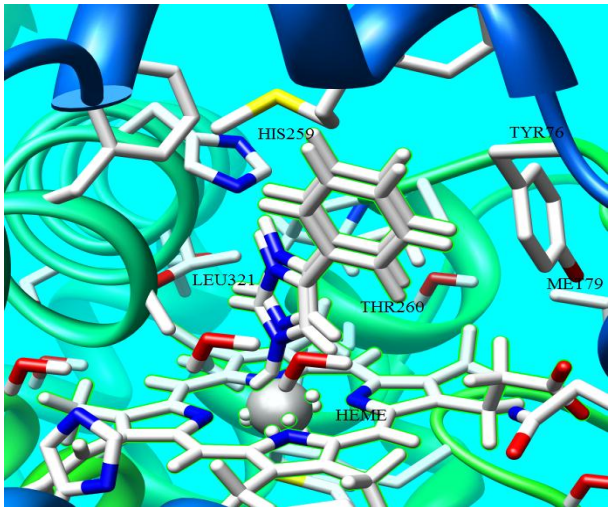

Figure 3: Close overlapping of 4-Phenyl-1H-Imidazoles in vicinity of amino acid residue

4. CONCLUSION

New imidazoles derivatives were synthesized in reasonably goog yields. They were characterized by 1H NMR, 13C NMR, mass spectrometry, IR studies and elements analyses. Finally the molecular docking studies of the synthesized compounds. The most prominent bindings are observed in the case of ligands **3a, 3b, 3c, 3d, 3e, 3f, 3g,** and **3h** in compared 4-Phenyl-1H-Imidazoles. Hence this study has widened the scope of developing these imidazole derivatives as promising antifungal agent

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