

Search of New Scaffolds against N-myristoyltransferase for Antifungal Activity

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Abstract. *N*-myristoyltransferase (NMT) has been an emerging target for the design of novel antifungal agents with new mode of action. A series of furan and imidazole containing heterocyclic derivatives have been designed using rational drug designing approach as NMT inhibitors. Newly Designed compounds were synthesized, characterized and evaluated for their antifungal activity against NMT. Synthesized compounds were characterized by IR, NMR and Mass. *In vitro* antifungal assay indicated that these derivatives have shown moderate to good antifungal activity against five fungal strains. A possible antifungal target was identified (NMT) and furan derivatives showed more favorable affinity than the imidazole ligands.

Keywords: N- myristoyltransferase, antifungal, antibacterial, molecular modeling, minimum inhibitory concentration.

1 Introduction

Invasive fungal infections have increased dramatically in recent years to become important causes of morbidity and mortality in hospitalized patients [1-3]. *N*-myristoyltransferase (NMT) has been a promising new target for the design of novel antifungal agents with new mode of action. NMT catalyzes the covalent co-translational attachment of the fatty acid, although some myristoylated proteins are cytosolic, many are associated with cellular membranes where myristoylation facilitates membrane attachment. The addition of myristate can also stabilize protein-protein interactions, and many acylated proteins require this modification for full expression of their biological function. Genetic studies have shown that the NMT gene is essential for the survival of *Saccharomyces cerevisiae* and the pathogenic fungi, *Candida albicans* and *Cryptococcus neoformans* [4]. The enzyme NMT is essential for viability of *C. albicans*, which is a major cause of systemic fungal infection in immunocompromised patients [5, 6]. Therefore it is worthwhile to develop new inhibitors against NMT. Imidazoles are important class of compounds that have shown multiple therapeutic actions and have been widely used including as antifungals [7, 8]. There is a report that compounds containing furan nucleus exhibited inhibitory effect on NMT of *C. albicans* [9, 10] and antimicrobial [11, 12] activities. In view of the potential pharmacophoric nature of imidazole and furan nucleus and affinity of furan nucleus with NMT [13], compounds containing both imidazole and furan nucleus were designed rationally with the aim to have synergistic effect on target, synthesized and evaluated for their antifungal activity. Docking studies were performed to get more insight on binding affinities of synthesized analogues to the target NMT.

2 Materials and Methods

All reagents were purchased from Sigma-Aldrich and were used without further purification. All melting points of synthesized compounds were determined by open capillary method and are uncorrected. IR spectra were recorded with Shimadzu FT-IR-8400S spectrophotometer on KBr. Elemental analysis (%C, %H, %N, %O and %S) of the synthesized compounds was done on Carlo Erba 1108 Heracus instrument at Central Drug Research Institute (CDRI), Lucknow, India. The ¹H NMR spectra were

recorded on an Advance - 400 MHz Bruker, using TMS as an internal reference and mass spectra were obtained on a JMS-T100LC, Accu TOF (DARTMS) spectrophotometer at CDRI, Lucknow, India.

2.1 Synthesis of Compound 1a, 1b

A solution of Salicylaldehyde (1 mmol) and substituted ketones (1 mmol) in 5-7 mL of ethanol was allowed to react with 3 mL of 60% KOH solution at 5-10 °C with stirring for 4 hrs. Reaction mixture was then diluted with water (10mL) and extracted with diethyl ether. The aqueous solution was acidified with dilute HCl. The solid obtained was filtered, washed thoroughly with water and dried. The crude product was purified by crystallization from benzene: MeOH (3:2) and resulted in 1a and 1b [13].

3-(2-hydroxyphenyl)-1-(1H-imidazol-1-yl)prop-2-en-1-one (1a) was obtained as cream colored crystals, mp 145-148 °C; ir: C=O 1706.1, C-O 1210, C-N 1221.8, C=C 1610, C=N 1312.6, OH 3295.8; ¹H NMR: 6.50 (s, 2H, ethylene), 5.01 (s, Ar-OH), 6.60-7.10 (Ar-H), 7.10-7.73 (Imidazole-CH); Mass m/z: 214 [M+]; *Anal.* Calcd. for C₁₂H₁₄N₂O₂: C, 66.65; H, 5.59; N, 12.96; O, 14.80. Found: C, 65.60; H, 5.10; N, 12.08; O, 13.90, yield-71%.

1-(furan-2-yl)-3-(2-hydroxyphenyl)prop-2-en-1-one 1(b) was obtained as white crystals, mp: 182-185 °C; ir: C=O 1679.3, C-O 1022.1, OH 3261.3, C=C Ar 1561.2, C=N 1251.1; ¹H NMR: 6.50-7.81 (s, 2H, ethylene), 5.04 (s, Ar-OH), 6.60-7.10 (Ar-H), 6.62-7.73 (CH, furon); Mass m/z: 214 [M+]; *Anal.* Calcd. for C₁₃H₁₀O₃: C, 72.89; H, 4.71; O, 22.41. Found: C, 72.29; H, 4.23; O, 22.11; yield-80%.

2.2 Synthesis of Compounds 2a, 2b

The compounds 1 (a, b) (1 mmol) and thiourea (1 mmol) in 5 mL ethanol was added with 0.5 mL of KOH solution (0.02 mol). The reaction mixture was refluxed for 4-5 hrs. The reaction mixture was then poured in 10 mL of 10% HCl solution (cold) and the precipitate was filtered, washed with water until free from acid and recrystallized from benzene-ethanol, which gave compound 2a & 2b [13].

2-(2-amino-4-(1H-imidazol-1-yl)-6H-1,3-thiazin-6-yl)phenol (2a) was obtained as white needle like crystals, mp: 201-203°C; ir: C-O 1169, C-N 1268.3, C=C Ar 1456, C=N 1201.8, OH 3339.5, NH 3666.2; ¹H NMR (DMSO-d₆, 300 MHz, ppm): 2.07 (s, NH₂), 4.58 (s, CH), 5.06 (s, Ar-OH), 6.35- (s, CH, ethylene), 6.67-7.18 (Ar-H), 7.10-7.70 (CH, Imidazole); Mass m/z: 273 [M+]; *Anal.* Calcd. for C₁₁H₁₁N₃OS: C, 56.91; H, 5.14; N, 20.42; O, 5.83; S, 11.69. Found: C, 56.41; H, 5.02; N, 20.82; O, 5.42; S, 11.10; Yield-76%.

2-(2-amino-4-(furan-2-yl)-6H-1,3-thiazin-6-yl)phenol (2b) was obtained as yellow crystalline compound, mp: 213-216 °C; ir: C-O 1169, C-N 1268.3, C=C Ar 1456, C=N 1201.8; O-H,3339.5, NH 3666.2; ¹H NMR (DMSO-d₆, 300 MHz, ppm): 2.04 (s, NH₂), 4.50 (s, CH), 5.21 (s, Ar-OH), 6.70 (s, CH, ethylene), 6.70-6.90 (Ar-H), 6.31-7.40 (CH, Imidazole); Mass m/z: 273 [M+]; *Anal.* Calcd. for C₁₁H₉N₃O₂S: C, 61.75; H, 4.44; N, 10.29; O, 11.75; S, 11.77. Found: C, 61.20; H, 4.34; N, 10.54; O, 11.30; S, 10.98; yield-68%.

2.3 Synthesis of Compounds 3a, 3b

A solution of 1a and 1b (0.001 mol) and guanidine hydrochloride (0.001mmol) in 5 mL ethanol was added with 0.5 mL of aqueous NaOH (0.02 mol). The reaction mixture was refluxed for 6hrs. The reaction mixture was poured in 5 mL of 10% HCl solution (cold) and the precipitate was filtered, washed with water until free from acid and recrystallized from benzene: ethanol, which gave compound 3a and 3b [13].

2-(2-amino-6-(1H-imidazol-1-yl)pyrimidin-4-yl)phenol (3a) was obtained as white powder, mp: 150-153 °C; ir: C-O 1032.3, C-N 1231.5, C=C Ar 1455.9, C=N 1231.5, C-H 2949.8, O-H 3240, NH 3364.8; ¹H NMR (DMSO-d₆, 300 MHz, ppm): 4.01 (s, Ar-CNH), 5.00 (s, Ar-OH), 6.72 (s, CH), 6.80-7.50 (Ar-H), 7.41-8.40 (CH, Imidazole); Mass m/z: 253 [M+]; *Anal.* Calcd. for C₁₃H₁₅N₅O: C, 60.69; H, 5.88; N, 27.22. Found: C, 60.68; H, 5.11; N, 27.34; yield-83%.

2-(2-Amino-6-furan-2-yl-pyrimidin-4-yl)-phenol (3b) was collected as white crystalline solid, mp: 170-171 °C; ir: C-O 1032.3, C-N 1231.5, C=C Ar 1455.9, C=N 1231.5, C-H 2949.8, O-H 3239.5, N-H 3364.8; ¹H NMR (DMSO-d₆, 300 MHz, ppm): 4.04 (s, Ar-CNH); 5.08 (s, Ar-OH); 6.90 (s, CH); 6.80-7.51 (Ar-H).

6.30-7.44 (CH, Furon); Mass m/z: 253 [M+]. *Anal.* Calcd. for C₁₃H₁₄N₃O₂: C, 65.87; H, 5.13; N, 16.46; O, 12.54. Found: C, 65.07; H, 5.33; N, 16.24; O, 12.12; yield-79%.

2.4 Synthesis of Compound 4

Salicylaldehyde (2 mmol) and trioxane (3 mmol) in 6mL of glacial acetic acid was heated to a temperature of 90-95 °C under nitrogen atmosphere. 1mL of mixture of concentrated sulfuric acid and glacial acetic acid (1:45) was added drop wise. The temperature was maintained for 22hr while stirring was continued during the entire period. Subsequently, the reaction mixture was poured into 200mL of ice-water and allowed to stand overnight. The precipitated solid was filtered and extracted twice with n-hexane. Recrystallization from acetone resulted to compound 4 [14].

2.5 Synthesis of Compound 5a, 5b

A solution of 4 (1 mmol) and substituted compound (3 mmol) in 5-7 mL of ethanol was treated with 3 mL of 60% KOH solution at 5-10 °C. The reaction mixture was stirred at room temperature for 4 hrs. Reaction mixture was diluted with water (10mL) and extracted with diethyl ether. The aqueous solution was acidified with dil HCl. The solid obtained was filtered, washed thoroughly with water and dried. The crude product was purified by crystallization from benzene: MeOH (3:2) to obtain compound 5a and 5b [14, 15].

(2E,2'E)-3,3'-(5,5'-methylenebis(2-hydroxy-5,1-phenylene))bis(1-(1H-imidazol-1-yl)prop-2-en-1-one) (5a) was obtained as creamish white crystalline compound, mp: 159-161 °C; ir: C-O 1094.8, C-N 1231.5, C=C Ar 1634.6 and 1448.8, C=N 1363.9, C=O 1711.5, O-H 3333.1; ¹H NMR (DMSO-d₆, 300 MHz, ppm): 3.81 (CH₂), 5.03 (s, 2Ar-OH), 6.50-7.81 (s, CH), 6.51-6.70 (Ar-H), 7.31-7.62 (CH, imidazole); Mass m/z: 440 [M+]; *Anal.* Calcd. for C₂₅H₂₄N₄O₄: C, 67.55; H, 5.44; N, 12.60; O, 14.40. Found: C, 67.87; H, 5.20; N, 12.37; O, 14.21; yield-78%.

1-Furan-2-yl-3-{5-[3-(3-furan-2-yl-3-oxo-propenyl)-4-hydroxy-benzyl]-2-hydroxy-phenyl}-propenone (5b) was obtained as brown crystals, mp: 230-232 °C; ir: 1094.8; C-O, 1231.5; C-N, 1634.6 and 1448.8; C=C Ar, 1363.9; C=N, 1711.5; C=O, 3333.1; OH; ¹H NMR (DMSO-d₆, 300 MHz, ppm): 3.82 (CH₂), 5.01 (s, 2Ar-OH), 6.52-7.83 (s, CH), 6.50-6.91 (Ar-H), 6.60-7.71 (CH, furon), Mass m/z: 440 [M+]; *Anal.* Calcd. for C₂₇H₂₀O₆: C, 73.63; H, 4.58; O, 21.80. Found: C, 73.21; H, 4.43; O, 21.57; yield-76%.

2.6 Synthesis of Compounds 6a, 6b

A solution of 5a, 5b (0.01 mol) and thiourea (0.03 mol) in 10 mL ethanol was added with 5 mL alcoholic KOH (0.02 mol) and refluxed for 5 hrs. The reaction mixture was poured in 10 mL of 10% HCl solution (cold) and the precipitate was filtered, washed with water until free from acid and recrystallized from benzene-ethanol to obtain compound 6a and 6b [14].

4,4'-methylenebis(2-(2-amino-4-(1H-imidazol-1-yl)-6H-1,3-thiazin-6-yl)phenol (6a) was obtained as white crystals, mp: 172-173 °C; ir: C-O 1091.2, C-N 1224.3, C=C Ar 1601.9, C=N 1191.3, O-H 3272.6, N-H 3303.7; ¹H NMR (DMSO-d₆, 300 MHz, ppm): 2.03 (NH₂), 3.81 (CH₂), 4.51 (CH), 5.00 (s, 2Ar-OH), 6.30 (CH), 6.53-7.81 (s, CH), 6.40-6.72 (Ar-H), 7.11-7.70 (CH, Imidazole); Mass m/z: 557 [M+]; *Anal.* Calcd. for C₇H₉S₂N₃O₂: C, 57.84; H, 5.03; N, 19.98; O, 5.71; S, 11.44. Found: C, 57.13; H, 5.44; N, 19.32; O, 5.22; S, 11.21; yield-73%.

4,4'-methylenebis(2-(2-amino-4-(1H-furan-1-yl)-6H-1,3-thiazin-6-yl)phenol (6b) was obtained as yellow crystals, mp: 179-181; ir: 1091.2; C-O, 1224.3; C-N; 1601.9; C=C Ar, 1191.3; C=N, 3272.6; OH, 3303.7; NH; ¹H NMR (DMSO-d₆, 300 MHz, ppm): 2.00 (NH₂), 3.80 (CH₂), 4.50 (CH), 5.01 (s, 2Ar-OH), 6.30 (CH), 6.50-7.81 (s, CH), 6.41-6.72 (Ar-H), 6.30-7.62 (CH, furan); Mass m/z: 556 [M+]; *Anal.* Calcd. for C₆H₉N₃O₂S₂: C, 62.57; H, 4.35; N, 10.06; O, 11.50; S, 11.52. Found: C, 63.07; H, 4.76; N, 10.23; O, 11.12; S, 11.14; yield-69%.

3 In Vitro Antifungal Activity

Evaluations of the susceptibility of fungal strains were performed using macro tube dilution method as per NCCLS guidelines [16]. The fungi used as inocula were grown overnight on sabouraud dextrose agar

(SDA) at $25\pm 1^\circ\text{C}$. To 5ml of SDA, 0.2 ml of culture was added and inoculated followed by incubation till it reached the turbidity equal to that of the standard 0.5 McFarland solution in 0.9 % (w/v) NaCl at 600 nm which was equivalent to 10^6 – 10^8 CFU/ml. A stock solution of 10 mg/mL was prepared in dimethyl sulfoxide (DMSO, Sigma) for the various synthesized compounds and for fluconazole (FLC), which was used as control. Two fold dilutions of test compounds from 256 to 0.25 $\mu\text{g}/\text{mL}$ were prepared with the suspension of the inoculum. The micro dilution tubes, which contained 0.1 mL of the serially diluted drug, were inoculated with 0.1 mL of the resulting suspension. The final inoculum concentration after dilution with the drug suspension was $10^3/10^4$ cells per mL. Two tubes containing the drug-free medium and inoculum were used as controls. The inoculated tubes were incubated at $35\pm 1^\circ\text{C}$ for 48h (*Candida* sp.) and 72 h (*A. niger*) in ambient air. The growth in each tube was then visually estimated. The minimum inhibitory concentrations (MIC) were determined visually, and were defined as the lowest concentration of a compound that inhibits growth of the organism as detected visually.

4 Antibacterial Activity

Evaluations of the susceptibility of bacterial strains were made by the macro tube dilution method as per NCCLS guidelines [16]. Mueller-Hinton broth was used as the test medium. An inoculum of approximately 5×10^5 CFU cm^{-3} was used. Serial twofold dilutions of the test compounds (256-0.25 $\mu\text{g}/\text{mL}$) and extra dilutions (0.12-0.015 $\mu\text{g}/\text{mL}$) for antibiotic standard (ciprofloxacin) was prepared. Tubes were incubated for 24 hrs at $37\pm 1^\circ\text{C}$ in an ambient air incubator. The lowest concentration of the test compounds inhibiting visible growth was taken as the MIC value.

5 Docking Studies

5.1 Software

Surflex 2.0 tools of Sybyl software were utilized to generate protomol, calculate dock score and evaluate conformers. The scoring function used by surflex is derived from the Hammerhead's empirical scoring function with a molecular similarity method (morphological similarity) to generate putative poses of ligand fragments. The active binding site region was defined as a spherical region which encompasses all protein of bound crystallographic ligand atom. Surflex is available free of charge to academic researchers for noncommercial use (see <http://jainlab.ucsf.edu> for details on obtaining the software).

5.2 Ligand Preparation

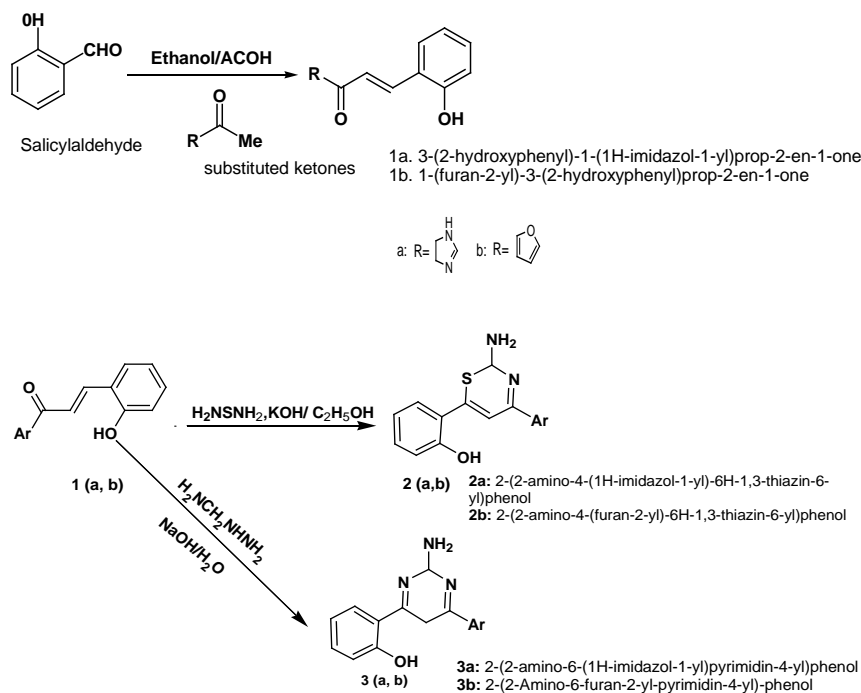
The structures of ten compounds (1a-6b) were converted into suitable chemical information using Chemdraw ultra 7.0 (Cambridge software), copied to Chem3D ultra 7.0 to create a 3D model using sybyl surflex-x 2.0 integral option.

5.3 Docking Method

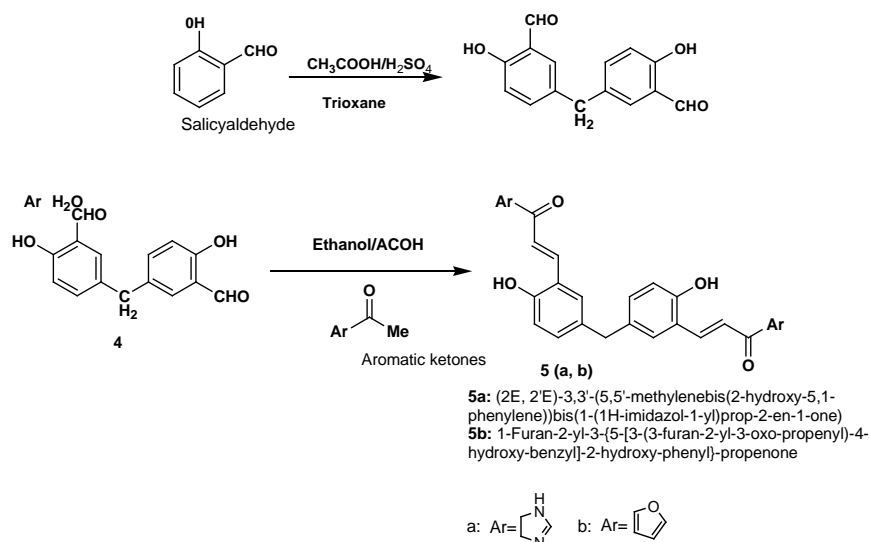
The crystal structure of target NMT was retrieved from the RCSB Protein Data Bank (PDB entry code: 4A33). The ligands were docked into corresponding protein's binding site by an empirical scoring function and a patented search engine in Surflex-Dock. All ligands and water molecules in target have been deleted and the polar hydrogen atoms were added to target. Protomol, a representation of a ligand making every potential interaction with the binding site, was applied to guide molecular docking. Then, the MOLCAD (Molecular Computer Aided Design) program was employed to visualize the binding mode between the protein and ligand. MOLCAD calculated and exhibited the surfaces of channels and cavities, as well as the separating surface between protein subunits. Moreover, Surflex-Dock total scores, which were expressed in $-\log_{10}$ (Kd) units to represent binding affinities, were applied to estimate the ligand-receptor interactions of newly designed molecules. The best poses of the studied compounds within the active sites of target enzymes are displayed in figure 2.

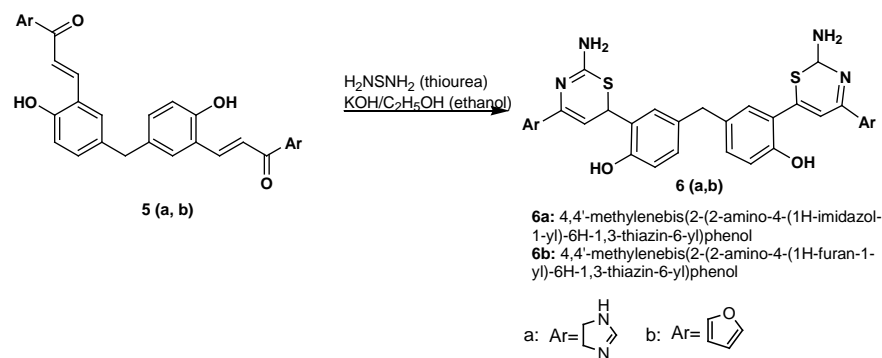
6 Results

Structural features responsible for antifungal activity have been collected such as imidazole, furan, thiazine, hydroxyl group at aromatic ring etc., and were used to design new molecules with the hope to have synergistic effect against fungal strains. Salicylaldehyde and thiourea and some substituted ketones were utilized as synthetic blocks for the synthesis of ten compounds as depicted in Scheme 1 and Scheme 2.



Scheme 1. Synthetic pathway for the target molecules.





Scheme 2. Synthetic pathway for the target molecules.

All synthesized compounds were evaluated for antifungal and antibacterial activity using macro tube dilution method. The results were indicated in Table 1 and Table 2. Docking studies were performed on synthesized compound to evaluate the binding affinity of all 10 compounds to the target and docking scores are shown in Table 3, binding affinities are shown in Figure 1 and 2.

Table 1. MIC values of synthesized compounds against fungal strains.

Sr. No.	Compound code	MIC value in $\mu\text{g/mL}$				
		A	B	C	D	E
1.	1a	16	64	64	64	32
2.	1b	8	8	16	16	16
3.	2a	32	8	8	8	32
4.	2b	8	8	16	8	8
5.	3a	16	32	32	32	32
6.	3b	8	8	8	8	16
7.	5a	16	16	8	8	32
8.	5b	2	2	2	2	16
9.	6a	16	16	8	8	64
10.	6b	2	2	2	2	32
11.	Fluconazole	1	2	2	1	2

* A = *A. niger* ATCC 9029, B= *C. albicans* ATCC 24433, C= *C. albicans* ATCC 10231, D= *C. tropicalis* ATCC 13803, E= *P. chrysogenum* ATCC 10002

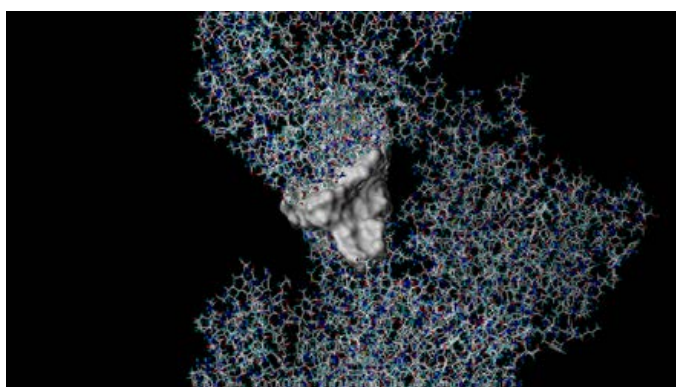
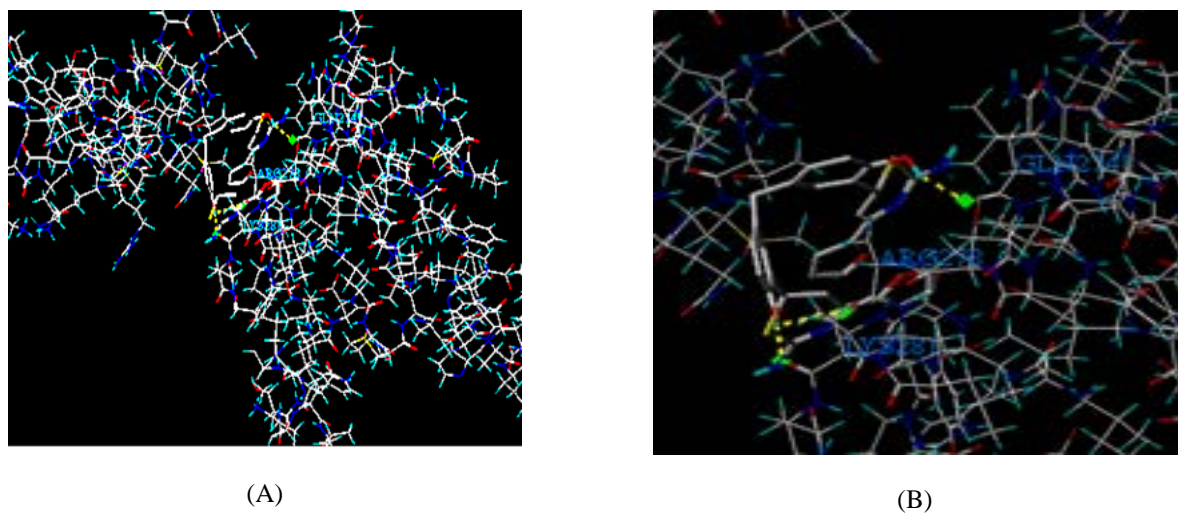
Table 2. MIC values of synthesized compounds against bacterial strains.

Sr. No.	Compound	*Tested bacterial strains (MIC $\mu\text{g/mL}$)				
		A	B	C	D	E
1.	1a	64	64	64	64	32
2.	1b	64	64	64	64	64
3.	2a	64	64	64	64	64
4.	2b	64	64	64	64	64
5.	3a	64	32	32	32	64
6.	3b	64	64	64	64	64
7.	5a	64	>64	>64	64	64
8.	5b	64	32	16	64	64
9.	6a	64	64	64	64	64
10.	6b	64	64	64	64	64
11.	Ciprofloxacin	8	2	2	8	16

*A = *Pseudomonas aeruginosa* ATCC 9027, B= *Staphylococcus aureus* ATCC, C= *Staphylococcus aureus* ATCC 25923, D= *Serratia marcescens* ATCC 13880, E= *Escherichia coli* ATCC 8739

Table 3. Docking scores of synthesized compounds

Compound no.	Total Score	Cresh Score	Polar bond
6b	6.6574	-1.9554	1.1974
5b	5.8645	-1.3212	1.3283
5a	5.6161	-1.5472	2.3208
2a	4.8948	-0.9298	1.0850
3a	3.5286	-0.4377	1.7720
6a	3.3946	-1.7743	1.2591
1a	3.0508	-0.6627	1.1781
1b	2.9090	-0.3017	0.8928
3b	2.7736	-0.6045	1.3420
2b	2.5438	-0.4673	1.6022

**Figure 1.** Active binding site region of selected antifungal drug target N-myristoyltransferase (PDB entry code: 4A33)**Figure 2.** A. Superimposed binding orientation of docked compound (6b) forming three hydrogen bonds with ARG 278, LYS281, GLN274 shown by yellow colored lines. B. Superimposed binding orientation of docked compound (5b) forming three hydrogen bonds with ARG 278, LYS281, GLN274 shown by yellow colored lines.

7 Discussion

Ten compounds containing furan and imidazole and thiazine nucleus along with other structural features

were designed and synthesized using known procedures depicted in Scheme 1 and 2. Scheme 1 involved condensation reaction between substituted ketones and salicylaldehyde and resulted in the formation of E)-3-(2-hydroxyphenyl)-1-(1H-imidazol-1-yl)prop-2-en-1-one (1a) and E)-1-(furan-2-yl)-3-(2-hydroxyphenyl)prop-2-en-1-one 1(b). Compound 1a and 1b were further allowed to react by two ways, one was with thiourea in presence of KOH and methanol, which resulted in the formation of 2-(2-amino-4-(1H-imidazol-1-yl)-6H-1,3-thiazin-6-yl)phenol 2(a) and 2-(2-amino-4-(furan-2-yl)-6H-1,3-thiazin-6-yl)phenol 2(b), and the other was with guanidine hydrochloride under basic conditions, which resulted in 3a and 3b. Scheme 2 is characterized by reaction of salicylaldehyde with trioxane under acidic environment to yield compound 4. Compound 4 further reacted with substituted aromatic ketones and resulted in condensation products 5a and 5b. These compounds were further condensed with urea to form compound 6a and 6b.

All synthesized compounds were primarily characterized by R_f values, IR, NMR, Mass study and elemental analysis. The Infrared spectra (IR) of each of the synthesized compounds showed absorption bands which are characteristic of the anticipated structure of the synthesized compounds. Infrared measurements showed the presence of NH stretching (3303-3666 cm⁻¹) in compounds 2a, 2b, 3a, 3b, 6a and 6b. All compounds have exhibited O-H stretching (3210-3350 cm⁻¹), C=N stretching (1191-1363 cm⁻¹), C-N stretching (1221-1234 cm⁻¹), C-O stretching (1020-1094 cm⁻¹), C=C stretching (1448-1610 cm⁻¹). Compound 1a, 1b, 5a and 5b have shown C=O stretching at 1679-1711 cm⁻¹. All OH protons were appeared as singlet in ¹H NMR at δ 5.0- 3.2ppm. Peaks at δ 6.4-7.9 in all the compounds accounted for presence of aromatic groups through ¹H NMR. The ¹H NMR spectrum corroborated the proton and IR data, clearly displaying the expected signals (C=C, C=O, C-O, C=N, C-N, C-S, CH, NH and CH₂). Mass spectra of compounds (1-10) exhibited molecular ion peak at their expected m/z values.

All synthesized compounds were evaluated for antifungal and antibacterial activity using microtube dilution method. Fluconazole were used as positive control for various fungal strains and ciprofloxacin for bacterial strains for their MIC determination. Preliminary assay was performed for a total of five fungal strains and five bacterial strains (including both gram positive and gram negative strains). The included fungal isolates were *Candida albicans* (2 strains: ATCC 24433, ATCC 10231), *Candida tropicalis* ATCC 13803, *Aspergillus niger* ATCC 9029 and *Penicillium chrysogenum* ATCC 10002. The included bacterial isolates were *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus aureus* ATCC 25923, *Serratia marcescens* ATCC 13880, *Escherichia coli* ATCC 8739. Table 1 exhibited the antifungal activity of synthesized compounds against various fungal strains. Compound 1b, 2b, 3b, 5b and 6b have shown remarkable activity (2-8 µg/mL) against *A.niger* ATCC 9029. Compound 5b and 6b (2 µg/mL) have shown maximum activity against *C. Albicans* ATCC 24433, *C. Albicans* ATCC 10231 (2-8 µg/mL) and against *C. tropicalis* ATCC 13803. Only compound 2b have resulted in good activity (8µg/mL) against *P. chrysogenum* ATCC 10002. Among all derivatives, compound 5b and 6b have shown interesting activity against almost all the strains and shown to be equipotent (2µg/mL) to that of standard fluconazole against *C. albicans* ATCC 10231. All of the synthesized derivatives were also tested against various bacterial strains. The data reported in Table 2 indicated that compound 3a and 5b were found to have moderate activity against *S. aureus* ATCC 6538P and *S. aureus* ATCC 25923. All other compounds have shown antibacterial activity less than the reference drug. In general the compounds showed low antibacterial activity as compared to antifungal activity.

Docking scores of all 10 compounds are shown in Table 3. All the ligands showed good binding against NMT target. The docking results obtained showed hydrogen bond interaction of the best compound number 6b with total score value of 6.6574 and Cresh score of -1.955. This candidate showed appreciable interaction as compared to other molecules, and amino acids residues present around it exhibited reasonable interactions. Amino acid residues ARG 278, LYS281 and GLN274 were found to be involved in hydrogen bond interactions of compound 6b. Compound 5b exhibited hydrogen bonding with ARG 278, LYS281 and GLN274 amino acid residues of NMT with good total score and cresh score. The presence of the furan ring and electron-donating hydroxyl group (compounds 5b and 6b) seemed to confer higher antifungal potency than that of other substituted groups. Electronegative oxygen atoms facilitate hydrogen bonding and can be identified as another structural feature for antifungal activity through inhibition of NMT. The best poses of the studied compounds within the active sites of target enzymes are displayed in Fig. 2.

8 Conclusions

A series of ten compounds containing imidazole, furan heterocyclic rings were synthesized and screened for their antifungal activities using macro tube dilution method. Compound 5b and 6b with furan ring were found to be most active against most of the fungal strains used in the study. Docking studies further showed that compound 5b and 6b possessed good binding scores for fungal *N*-myristoyltransferase (NMT). More studies to optimize the structural feature and mechanism of action of this class of derivatives are required which could be helpful in designing more potent antifungal agents for therapeutic use.

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