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QM/MM Docking Strategy and Prime/MM-GBSA Calculation of Celecoxib Analogues as N-myristoyltransferase Inhibitors

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Abstract

Developing chemicals that inhibit N-myristoyltransferase (Nmt) is a promising adjuvant therapeutic to improve the efficacy and selectivity of antifungal agents. Reliable prediction of binding-free energy and binding affinity of Nmt inhibitors can provide a guide for rational drug design. In this study, Quantum Polarised Ligand Docking (QPLD) strategy and Prime/Molecular Mechanics Generalized Born Surface Area (Prime/MM-GBSA) calculations were applied to predict the binding mode and free energy for a series of celecoxib analogues as Nmt inhibitors which were also found to have good anti-inflammatory activity. *In vitro* antifungal assay indicated that these derivatives were also acting as potent antifungal agents. Reliable docking results showed superior performance on both ligand binding pose and docking score accuracy. Then, the Prime/MM–GBSA method based on the docking complex was used to predict the binding-free energy. The combined use of QM/MM docking and Prime/MM-GBSA method gave a good correlation between the predicted binding-free energy and experimentally determined zone of inhibition and Minimum Inhibitory Concentration (MIC) values. The molecular docking combined with Prime/MM-GBSA simulation can not only be used to rapidly and accurately predict the binding-free energy of novel Nmt inhibitors but also provide a novel strategy for lead discovery and optimization targeting Nmt.

Keywords: N-myristoyltransferase inhibitors; Anti-inflammatory; QM/MM docking; Binding-free energy; Prime/MM-GBSA

Introduction

Fungi are responsible for various forms of diseases, ranging from superficial infections of the mucosal surfaces or skin to systemic infections, which in most cases are life threatening [1]. The incidence and mortality of invasive fungal infections are rising dramatically due to the increase in the number of immune compromised or immune suppressed individuals. The majority of these infections are caused by Candida species, with over 50% due to *Candida albicans* [2-5]. The difficulties in treating fungal infections are multifaceted including aspects such as the difficulty in correct diagnosis, leading to late diagnosis, and the lack of clinically established breakpoints for commonly used drugs [6].

Myristoyl-CoA: protein N-myristoyltransferase (Nmt) is a cytosolic monomeric enzyme that catalyzes the transfer of the myristoyl group from myristoyl-CoA to the N-terminal glycine of a number of eukaryotic cellular and viral proteins [7,8]. Myristoylation relates to diverse biological processes including signal transduction cascades and apoptosis. Genetic experiments have shown that Nmt is an essential enzyme for the viability of some important pathogenic fungi including *Candida albicans* and *Cryptococcus neoformans* [9,10]. Thus, Nmt has been a promising target enzyme for the development of novel fungicidal drugs having a broad antifungal spectrum [11]. Although Nmt also exists in humans, the differences in the protein substrate specificities of fungal and human Nmts have been utilized to develop species-selective inhibitors that are fungicidal and safe [11]. Till now, peptidomimetic molecules [12-15], myristic acid analogues [16,17], p-toluenesulfonamide derivatives [18], benzofuran analogues [19-22] and benzothiazole analogues [23] have been reported to be Nmt inhibitors. Among them, the benzofuran and benzothiazole inhibitors showed high selectivity over human Nmt and exhibited good antifungal activity.

The common antifungal agents currently used in clinic suffer from limited efficiency, narrow antifungal spectrum, drug related toxicity, severe drug resistance, nonoptimal pharmacokinetics and serious drug interactions. Therefore, there is an emergent need to develop novel fungicidal drugs with a new mode of action.

Co-administration of multiple drugs for treatment of inflammatory conditions associated with fungal infection is a major risk especially in the case of patients with impaired liver or kidney functions. A mono therapy of a drug with dual anti-inflammatory antifungal activity would be preferred from the pharmaco economic and patient compliance point of view. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) have been recognized as important therapeutic agents for the treatment of rheumatoid arthritis and its variants. 1,5-Diarylpyrazole class of drugs (celecoxib (1) Figure 1) has been explored to discover novel heterocyclic analogues as anti-inflammatory agents and selective cyclooxygenase-2 (COX-2) inhibitors. Two novel series of compounds 2 and 3 (Figure 1) analogous to the celecoxib class of vicinal diarylpyrazoles with bioisosteric replacement of the sulfonamide group with a cyano and carbothioamide moiety has been reported recently by our group as

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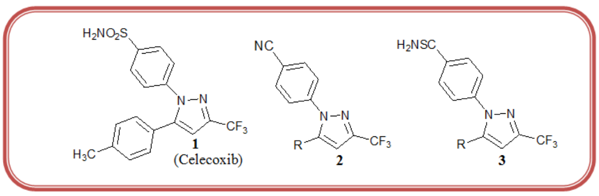
selective COX-2 inhibitors and anti-inflammatory drugs [24]. With the idea/concept of monotherapy of a drug, it was considered worthwhile to explore or evaluate the synthesized compounds for *in vitro* antifungal activity and their mode of action.

Growth inhibition assays targeting specific biochemical pathways provide additional information but still lack the resolution required to identify the molecular target [25]. Accurate and reliable prediction of protein–ligand interaction and binding-free energy (or binding affinity) is of vital importance in many fields such as structure-based drug design and lead optimization [26-30]. In drug design and discovery process, one is often interested in fast ranking of ligands of potential pharmaceutical interest according to their binding-free energies (or affinity) toward a given protein. Reliable prediction of binding-free energy (or affinity) can provide a guide for rational drug design but continues to be a daunting challenge. Computational approaches at different levels of complexity and sophistication have been used to calculate binding-free energies in complex biomolecular systems. Scoring functions of molecular docking were often used to predict the protein–ligand interaction and binding-free energy in screening of large molecular databases of compounds to identify potential lead compounds [31]. Despite their rapidity, there are still some drawbacks that need to be improved to achieve high scoring accuracy, such as lack of treatment of the protein flexibility, improper accounting for solvation, entropy, and polarizability [32-36]. More rigorous methods have been successfully proposed for binding-free energy calculation, such as free energy perturbation [27] and thermodynamic integration [37]. Unfortunately, these approaches are computationally expensive, thus are not realistically available to rapidly rank the ligands. There are also some other approaches, such as linear interaction energy [38] and molecular mechanics Poisson–Boltzmann surface area [39]. Among them, the Prime/MM–GBSA method using a single minimized protein–ligand structure instead of ensembles of snapshots derived from MD trajectories, thus becomes an efficient method to rapidly refine and rescore docking screening results [40-42].

Initially, molecular docking study is performed to take insight the binding interaction between the inhibitors and Nmt. Considering the protein flexibility, and the effect of polarization, we aim to obtain reliable docking results by QM/MM docking method [43]. Finally, Prime/MM-GBSA [40] simulation based on the docked complex from QM/MM docking method is used to predict the binding-free energy of these Nmt inhibitors.



2,3	a	b	c	d	e	f	g	h	i
R	Ph	4-CH$_3$-Ph	4-F-Ph	4-Cl-Ph	4-Br-Ph	4-OCH$_3$-Ph	2-naphthyl	2-thienyl	2-furyl

Figure 1: Structure of celecoxib and its synthesized analogues.

Results and Discussion

QM/MM docking analysis

It is now well recognized that the accuracy of electronic charges plays a critical role in protein–ligand docking. The QPLD aims to achieve the docking accuracy through improving the description of partial charges on the ligand atoms. The polarization of the charges on the ligand by receptor is taken into account by replacing them with charges derived from quantum mechanical calculations in the field of the receptor, and redocking of the ligands with QM/MM modified charges can result in improved docking accuracy. The docking performance was also validated using the known X-ray structure of Nmt of *Candida albicans* (PDB ID: 1IYL) and *Saccharomyces cerevisiae* (PDB ID: 2P6G) in complex with the co-crystal ligand R64 and 3LP, respectively. Figure 2 shows the binding mode of the docked R64 and 3LP to Nmt of *Candida albicans* and *Saccharomyces cerevisiae*, respectively. It can be seen that there are hydrogen bonds between R64 and residues (Tyr 119, Asn 392) of CaNmt and between 3LP and residue (Tyr 349) of SaNmt active sites, respectively. The pose obtained from QPLD superimposes well with the crystal structure of ligands. The Root-Mean-Square Deviation (RMSD) between them is 0.21Å and 0.75Å for CaNmt and SaNmt, respectively. Figure 3 highlights the electrostatic potential surface obtained using ESP QM charges. The binding mode of the whole dataset was then explored by the QPLD protocol. The obtained Gscore values are listed in Table 1. The QPLD protocol gives a more accurate treatment of the electrostatic interactions, which results in an improvement of the docking accuracy.

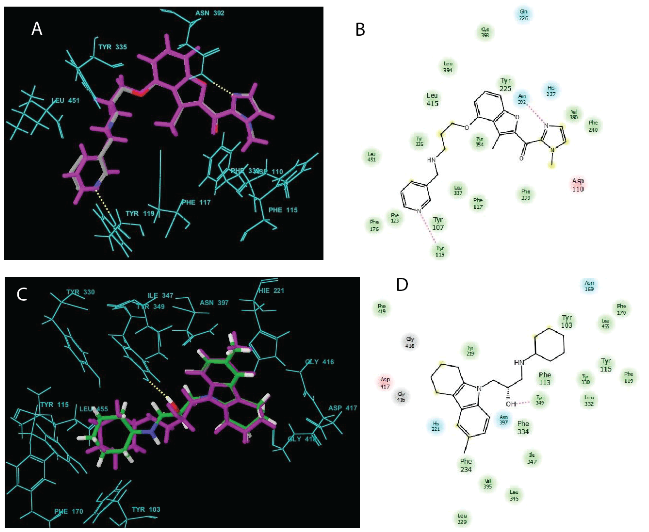


Figure 2: The QPLD docked inhibitor compared with its original structure in crystallographic complex by superimposing the coordinates of protein together. (2A) CaNmt R64 the crystal structure coloured magenta and the docked conformation coloured by atom colour, (2B) Schematic representation of interactions between compound R64 and CaNmt, (2C) SaNmt 3LP the crystal structure coloured magenta and the docked conformation coloured by green, and (2D) Schematic representation of interactions between compound 3LP and SaNmt.

S.No.	Compound	Zone of inhibition	MIC	Gscore	ΔGbind	Zone of inhibition	MIC	Gscore	ΔGbind
			Candida albicans			*Saccharomyces cerevisiae*			
1	2a	14.6	128	-8.234	-43.387	-	-	-	-
2	2b	14.6	128	-8.725	-48.456	-	-	-	-
3	2c	15.6	128	-8.536	-44.895	-	-	-	-
4	2d	14.3	128	-7	-41.021	-	-	-	-
5	2e	15.3	128	-7.978	-37.578	-	-	-	-
6	2f	14.3	128	-8.107	-39.456	-	-	-	-
7	2g	16.6	64	-7.75	-46.197	-	-	-	-
8	2h	18.6	32	-9.29	-49.558	-	-	-	-
9	2i	17.3	64	-8.049	-50.471	-	-	-	-
10	3a	18.3	64	-8.965	-48.667	15.6	64	-5.932	-29.366
11	3b	15.3	128	-7.209	-34.949	14.3	128	-5.442	-29.98
12	3c	20.6	32	-10.021	-56.531	18.3	32	-6.544	-35.63
13	3d	14.6	64	-7.538	-40.343	14.6	128	-5.267	-27.625
14	3e	17.6	64	-8.839	-47.524	16	64	-5.278	-32.326
15	3f	16	128	-7.695	-44.346	15.3	128	-5.438	-31.15
16	3g	16.6	64	-7.633	-45.567	15.3	128	-5.073	-24.919
17	3h	19	32	-9.117	-43.91	17.3	64	-7.531	-48.861
18	3i	19.6	32	-9.125	-49.962	18.6	32	-7.036	-44.328
19	Celecoxib	-		-	-	-		-	-
Std	Fluconazole	16.5	140			24.0	140		

Table 1: Zone of inhibition (mm), MIC (μg/ml), docking scores and predicted binding-free energies (kcal/mol) obtained by Prime/MM–GBSA. - no activity, Std-standard drug.

For the correlation between the docking score/binding-free energy with the experimental zone of inhibition/MIC values to predict the relative binding affinities of these compounds, we performed correlation coefficients between QPLD Gscore values, and binding-free energy ΔGbind values calculated using Prime/MM-GBSA protocol with the experimental zone of inhibition and MIC values. The results of the correlations are summarized in Table 2. The correlation coefficient of QPLD Gscore or QPLD based MM-GBSA ΔGbind vs zone of inhibition or MIC values for CaNmt and SaNmt inhibitors are 0.77, 0.74, 0.60, 0.64 and 0.81, 0.76, 0.73, 0.66, depicted from the scatter plots , respectively (Figure 4). The QPLD protocol shows that the celecoxib analogues are having similar hydrogen bond interactions with the same active site residues as that of the co-crystal ligands in both CaNmt and SaNmt (Figure 5), respectively.

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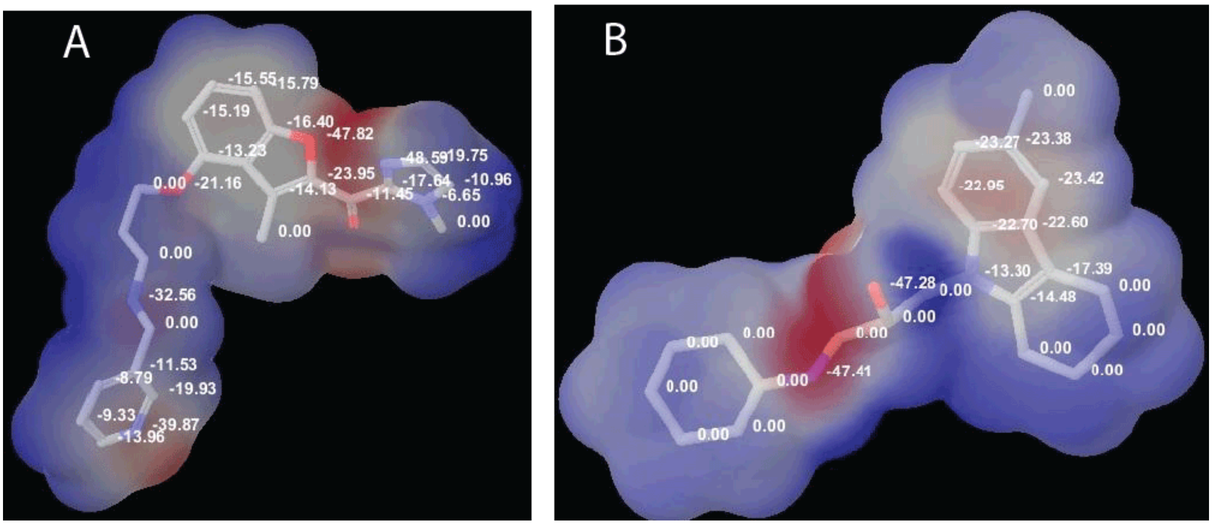

Figure 3: Electrostatic potential value plotted on the connolly surface using electrostatic potential fitting charge (ESP) atomic charges derived from a B3LYP/6-31G* for the crystal structure ligands, OPLS2005 (for the protein), QM/MM docking (electropositive charge blue, electronegative charge red and neutral white): (3A) CaNmt R64 and (3B) SaNmt 3LP.

Scoring Method	**Correlation Coefficient**	
	Candida albicans	*Saccharomyces cerevisiae*
QPLD Gscore vs Zone of inhibition	0.77	0.81
QPLD based MM-GBSA ΔG vs Zone of inhibition	0.74	0.76
QPLD Gscore vs MIC	0.60	0.73
QPLD based MM-GBSA ΔG vs MIC	0.64	0.66

Table 2: Comparison of the performance of four scoring functions in this study.

Considering the influence of different docking methods on the performance of Prime/MM–GBSA simulation for the binding-free energy calculation, we calculated the binding-free energy from the complexes obtained by QPLD docking strategy. This protocol calculates energy components including the minimized energy, solvation energy, and surface area energy of the complex, Nmt and free ligands. Corrections for entropic changes were not applied. Prime adopts a surface-generalized Born model using a Gaussian surface instead of a van der Waals surface for better representation of the solvent-accessible surface area [44]. The results of binding-free energy prediction using MM-GBSA are listed in Table 1. Table 2 shows the results from the correlation coefficient based on MM-GBSA binding-free energy from QPLD docking protocol. Analysis of the above study correlates well between experimental and in silico results.

In vitro Antifungal Activity

All the eighteen compounds were evaluated for their *in vitro* antifungal activity against two fungal strains, *C. albicans* (MTCC 227) and *S. cerevisiae* (MTCC 170) by agar well diffusion method [45]. Fluconazole was used as the reference drug and the data of antifungal tests are depicted in Table 1 and Figure 6. MIC of only those compounds was determined which were showing activity in primary screening.

From the activity results it has been revealed that most of the compounds possessed remarkable antifungal activity against *C. albicans* fungal strains. From the QPLD docking and the Prime MM-GBSA studies it is seen that most of the synthesised celecoxib analogues are showing hydrogen bond interactions with the active site residues and good binding energy (ΔGbind) for *Candida albicans*. Interestingly, nine compounds were found to be potent members showing greater activity against *C. albicans* than standard drug fluconazole (Figure 6). On the basis of zone of inhibition against *C. albicans*, three compounds 3c, 3h and 3i were found to be most potent showing the maximum zone of inhibition ≥ 19.0 mm as compared with standard drug fluconazole which showed the zone of inhibition 16.5 mm against *C. albicans*.

Similarly, on the basis of zone of inhibition studies, three compounds, 3c, 3h and 3i were found to be most effective against *S. cerevisiae* showing the maximum zone of inhibition ≥ 17.0 mm as compared with standard drug fluconazole (Figures 5 and 6) which showed the zone of inhibition 24.0 mm against *S. cerevisiae*. However, out of the eighteen compounds tested nine compounds belonging to cyanopyrazoles series (2a-2i) did not show activity against *S. cerevisiae* (Table 1). In the whole series, MIC ranged between 32 and 128 μg/ml. In case of *C. albicans*, three compounds 3c, 3h and 3i are the most potent members having MIC 32 μg/ml in comparison to standard drug having MIC of 140 μg/ml. Other compounds were showing good to moderate activity having MIC ranged between 64 and 128 μg/ml. However, two compounds 3c and 3i were found to be best against *S. cerevisiae* showing lowest MIC 32 μg/ml (Table 1) compared to standard drug having MIC of 140 μg/ml.

In general, carbothioamides 3a–3i showed better *in vitro* activity which is proved from the hydrogen bonding interactions and the higher binding energy for most of them than the corresponding cyanopyrazoles 2a-2i for *Candida albicans* whereas for *Saccharomyces cerevisiae* cyanopyrazoles did not show any activity. The compound in each series (2c, 3c) in terms of zone of inhibition against *C. albicans* contains a fluoro (F) substituent at position-4 of the phenyl ring that is attached to the C-5 of the pyrazole moiety. Interestingly, these compounds also showed excellent anti-inflammatory activity and good COX-2 selectivity [24]. However, among all the tested chemical compounds, compound 3c was found to be the best in inhibiting growth of both fungal pathogens. From the in silico and *in vitro* results of the anti-inflammatory and antifungal activity of the celecoxib analogues it is attributed that the presence of the fluoro and the –$CSNH_2$ moiety are responsible for the activity. Thus this compound can be further used as an anti-inflammatory antifungal agent in pharmaceutical industry, after testing its toxicity on human beings.

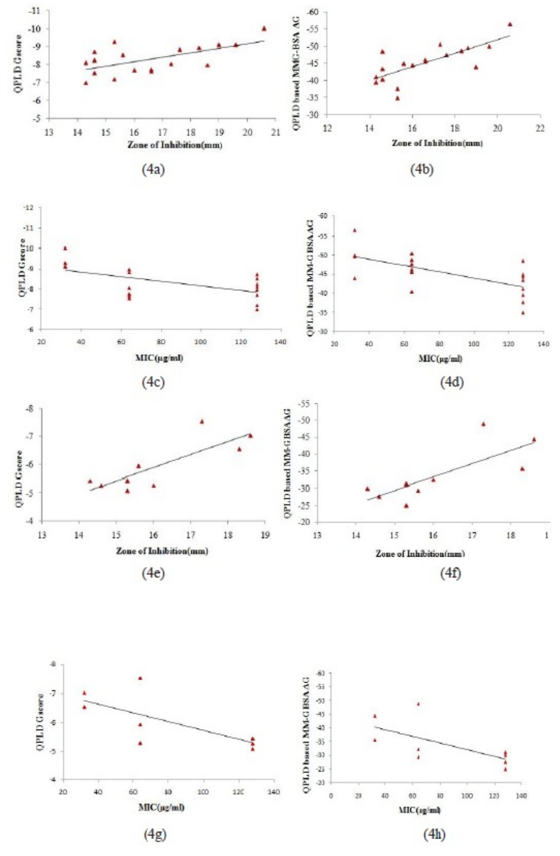

Figure 4: Correlation between experimental zone of inhibition or MIC against Nmt docking scores or estimated free energies of binding (kcal/mol) obtained by CaNmt (4a) QPLD Gscore vs Zone of inhibition, (4b) CaNmt QPLD based MM-GBSA ΔG vs Zone of inhibition, (4c) QPLD Gscore vs MIC, (4d) CaNmt QPLD based MM-GBSA ΔG vs MIC. SaNmt (4e) QPLD Gscore vs Zone of inhibition, (4f) CaNmt QPLD based MM-GBSA ΔG vs Zone of inhibition, (4g) QPLD Gscore vs MIC, (4h) CaNmt QPLD based MM-GBSA ΔG vs MIC.

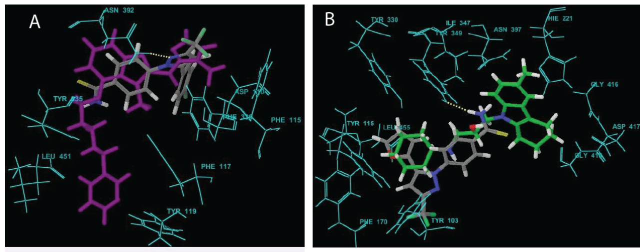

Figure 5: (5A) QPLD model of CaNmt inhibitor (3c) coloured by atom colour along with the crystal structure of ligand R64 coloured by magenta and (5B) QPLD model of SaNmt inhibitor (3i) coloured by atom colour along with the crystal structure of ligand 3LP coloured by green.

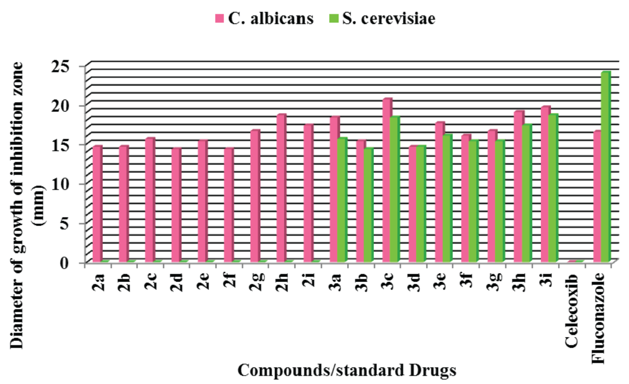

Figure 6: Comparison of diameter of growth of inhibition zone (mm) of synthesized compounds with celecoxib and standard drug fluconazole

Conclusions

In this work, synthesized celecoxib analogues which were found to be good anti-inflammatory agents [24] were tested for antifungal activity to explain the dual property of the compounds. QPLD docking method was used to study the binding mode of series of celecoxib analogues as Nmt inhibitors. *In vitro* antifungal assay results of celecoxib analogues were displaying more potent antifungal activity than celecoxib and fluconazole. The QPLD with the improved charge calculation model makes an improvement of the docking performance. To fully consider the influence of docking method on the performance of Prime/MM-GBSA simulation for the binding affinity calculation, the simulation was carried out based on the QPLD complex. The QPLD based MM-GBSA simulation showed a good correlation between the predicted energy and experimental zone of inhibition or MIC. The good performance of the combined strategy to predict the binding-free energy suggests that it can be used to lead discovery and optimization of Nmt. From the studies it reveals that the synthesized celecoxib analogues can be used as the dual anti-inflammatory and antifungal agents which can serve as a good starting point for further studies of structural diversity of the NMT inhibitors.

Experimental Protocols

Protein preparation

The three-dimensional complex structure of *Candida albicans* (PDB ID: 1IYL) and *Saccharomyces cerevisiae* (PDB ID: 2P6G) were downloaded from the Protein Data Bank [46,47]. The structure was prepared with the Protein Preparation Wizard [48] workflow as follows: adding hydrogens, assigning partial charges using the OPLS-2005 force field, and assigning protonation states. Following this step, the structure underwent restrained minimization in vacuum. The co-crystal ligand was used to determine the location of a docking grid box and was then removed prior to grid generation in next step.

Ligand preparation

The 3D molecular structures of all compounds (18 celecoxib analogues, anti-inflammatory drug celecoxib, and antifungal drug fluconazole) were built with the Schrödinger software. The energy minimization was performed using the OPLS-2005 force field. Then, all the compounds were prepared by Ligprep module [49].

QM/MM docking study

The Quantum Mechanics/Molecular Mechanics (QM/MM) docking were performed by the Schrödinger QM-Polarized Ligand Docking Protocol (QPLD) [43]. The protocol was carried out as follows: (1) Rigid Receptor Docking (RRD) was performed using Glide [50]. In this step, the top five poses of each ligand in the initial RRD were used, (2) the polarizable ligand charges induced by the protein were calculated with QSite [51] at the B3LYP/6-31G* level, and (3) the ligands with QM/MM modified charges were redocked and five poses of each ligand were saved. The Gscore value was selected for scoring the poses.

Prime/MM-GBSA simulation

The Prime/MM-GBSA [52] method based on the docking complex was used to calculate the binding-free energy (ΔG_{bind}) of each ligand, using the following equation [40]

$$\Delta G_{bind=} \Delta E_{MM} + \Delta G_{SOL\,+} \Delta G_{SA}$$

where ΔE_{MM} is the difference in the minimized energies between the Nmt–inhibitor complex and the sum of the energies of the unliganded Nmt and inhibitor. ΔG_{SOLV} is the difference in the GBSA solvation energy of the protein–inhibitor complex and the sum of the solvation energies for the unliganded Nmt and inhibitor. ΔG_{SA} is the difference in surface area energies for the complex and the sum of the surface area energies for the unliganded Nmt and inhibitor. The simulation was performed based on the receptor–ligand complex structure obtained from molecular docking. The obtained ligand poses were minimized using the local optimization feature in Prime, whereas the energies of complex were calculated with the OPLS-2005 force field and Generalized-Born/Surface Area continuum solvent model. During the simulation process, the ligand strain energy was also considered.

Based on the docking score and MM/GBSA binding-free energy, we developed the correlation coefficient between the docking score or calculated binding-free energy and the experimental zone of inhibition values or MIC values.

Antimicrobial assay

Test microorganisms

Two yeasts, *Candida albicans* (MTCC 227), and *Saccharomyces cerevisiae* (MTCC 170) were screened for evaluation of antifungal activity of the chemical compounds. All the microbial cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh.

Antifungal activity (yeasts)

The antifungal activity of 18 chemical compounds was evaluated by the agar well diffusion method. All the microbial cultures were adjusted to 0.5 McFarland standards, which is visually comparable to a microbial suspension of approximately 1.5 × 108 cfu/ml. 20 ml of agar medium was poured into each petri plate and plates were swabbed with 100 µL inocula of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 µl volume with concentration of 4.0 mg/ml of each compound reconstituted in dimethylsulphoxide (DMSO). All the plates were incubated at 37°C for 24 h. Antimicrobial activity of each compound was evaluated by measuring the zone of growth of inhibition against the test organisms with zone reader (Hi Antibiotic zone scale). DMSO was used as a negative control whereas fluconazole was used as positive control for yeast. This procedure was performed in three replicate plates for each organism [53,54].

Determination of Minimum Inhibitory Concentration (MIC) of chemical compounds

MIC is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. MIC of the various compounds against yeast strains was tested through a modified agar well diffusion method [45]. In this method, two fold serial dilution of each chemically synthesized compound was prepared by first reconstituting the compound in DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 140 to 0.5 µg/ml. A 100 µl volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100 µl of standardized inoculum (106 cfu/ml) of the test microbial strain. All test plates were incubated aerobically at 37° C for 24 h and observed for the inhibition zones. MIC, taken as the lowest concentration of the chemical compound that completely inhibited the growth of the microbe, showed by a clear zone of inhibition, was recorded for each test organism. Fluconazole was used as positive control while DMSO as negative control.

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