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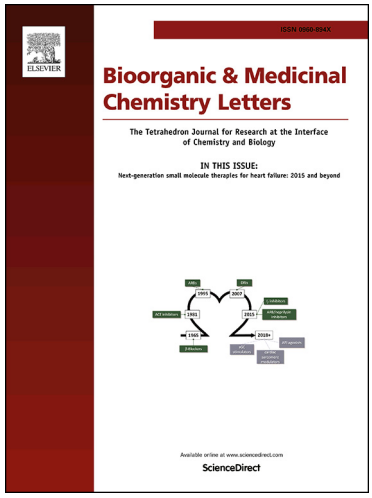


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# Synthesis, biological evaluations and computational studies of *N*-(3-(-2-(7-Chloroquinolin-2-yl)vinyl) benzylidene)anilines as fungal biofilm inhibitors

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## ABSTRACT

In the present investigation, new chloroquinoline derivatives bearing vinyl benzylidene aniline substituents at 2nd position were synthesized and screed for biofilm inhibitory, antifungal and antibacterial activity. The result of biofilm inhibition of *C. albicans* suggested that compounds **5j** ($IC_{50}$ value= 51.2 μM) and **5a** ($IC_{50}$ value= 66.2 μM) possess promising antibiofilm inhibition when compared with the standard antifungal drug fluconazole ($IC_{50}$= 40.0 μM). Two compounds **5a** (MIC= 94.2 μg/mL) and **5f** (MIC= 98.8 μg/mL) also exhibited good antifungal activity comparable to standard drug fluconazole (MIC= 50.0 μg/mL). The antibacterial screening against four strains of bacteria *viz. E. coli, P. aeruginosa, B. subtilis, and S. aureus* suggested their potential antibacterial activity and especially all the compounds except **5g** were found more active than the standard drug ciprofloxacin against *B. subtilis.* To further gain insights into the possible mechanism of these compounds in biofilm inhibition through the agglutinin like protein (Als), molecular docking and molecular dynamics simulation studies were carried out. Molecular

modeling studies suggested the clear role in inhibition of this protein and the resulting biofilm inhibitory activity.

**Keywords:** Quinoline; Schiff bases; fungal biofilm inhibition; antibacterial activity; molecular docking; molecular dynamics

Nitrogen bearing heterocyclic compounds has been the largest class of organic heterocyclic compounds possessing diverse biological properties and thus continually being exploited in unmet challenges in drug discovery and design. Quinoline is an important nitrogen containing heterocyclic ring and its derivatives possess diverse bioactivities such as antimicrobial [1], anticancer,[2] antileishmania [3]. Antimycobacterial [4], Anticonvulsant [5], anti-inflammatory and analgesic [6], antiallergenic [7], anti-alzheimer [8], antihypertensive [9], antifungal [10] and antidiabetic activities [11]. Some of the quinoline ring based antimicrobial agents currently being used as therapeutic agents are presented in **Figure 1.**

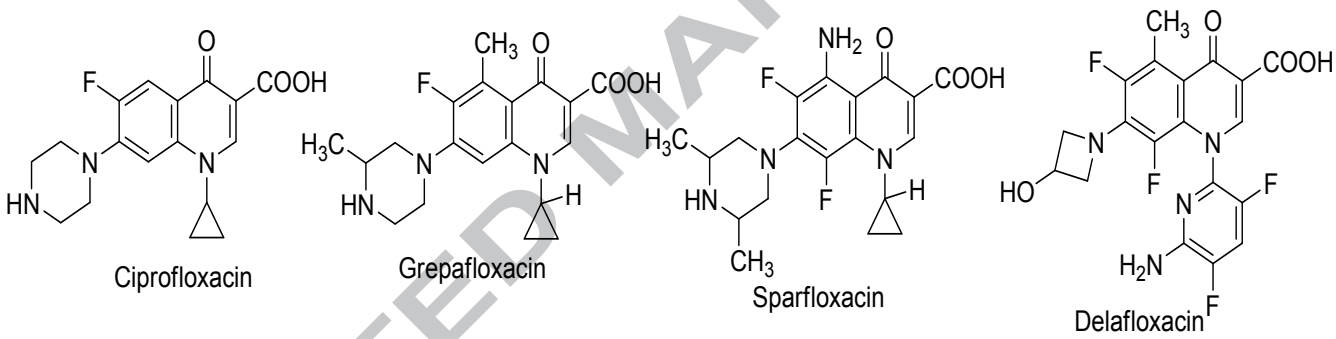



**Figure 1.** Currently used quinoline derivatives as antimicrobial agents

Microorganisms are continually evolving with newer means to cope up with antibiotics, antibacterials and toxins [12]. The process of cell to cell communication, called Quorum Sensing (QS) in bacteria, results in altered gene expression and subsequent survival and pathogenesis of bacteria [13-14]. Quorum sensing mediates formation of biofilm which is an association of micro-organisms where cells adhere to each other within matrix of extracellular polymeric substances [15]. Thus the QS results in release of variety of chemical signals, formation of pores and formation of water channels conferring the resistance in microbial colonies [16-17]. Among the different microbes, the Gram negative bacterium *Pseudomonas aeruginosa* is responsible for biofilm growth and has gained much attention, since they can form biofilms in urinary tract, kidney, lungs, leading to inflammations and septic shock. Thus, to study bacterial biofilm inhibition, *P.*

*aeruginosa* can be used as model organism [18]. In immune-compromised patients *Candida albicans* can cause superficial and systemic infections [19-20]. Further, in hospitalized patients, it is the major cause of morbidity and mortality [21]. The QS support the formation of complex biofilm from the budding state and subsequent colonization, entry in blood stream and virulence. Thus, the biofilm formation has aggrieved the problem of resistance in *C. albicans* infections [22]. All these issues suggest that there is strong need to develop more effective antimicrobial treatment mainly against infections associated with biofilm formation. At molecular level it has been reported that the cell surface glycoproteins from Agglutinin-like protein family (Als1–Als7 and Als9) found in the fungi like *C. albicans*, are involved in adhesion of the fungus to host cells and biofilm formation [23]. A recent report suggests that the inhibitors of Als proteins can be potential biofilm inhibitors [24]. It has been also established that the signaling molecules like 2-n-heptyl-3-hydroxy-4(1H)-quinolone, called Pseudomonas Quinolone Signal (PQS) and 2-n-heptyl-4-hydroxyquinoline (HHQ) derived from AnthraniloylCoA (ACoA) are responsible for biofilm formation [25]. Considering all above facts and in continuation of our ongoing efforts for synthesis of antimicrobial compounds acting on different targets [26-29], we are herewith reporting the synthesis of some new quinoline analogues which have been evaluated for their inhibitory activity against biofilm from *C.albicans*. These new quinoline analogues were designed on the basis of key signaling molecules, PQS and HHQ as shown in **Figure 2**. The possible binding mode and mechanism of inhibition of the Als protein, molecular docking and dynamics was carried out and reported to augment the biofilm inhibitory potential of these molecules.

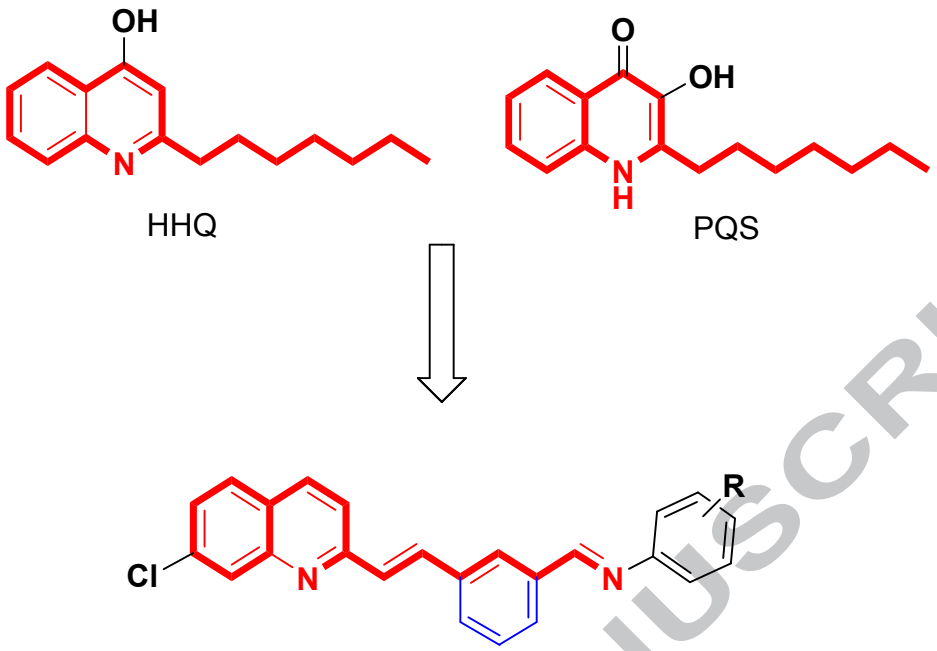
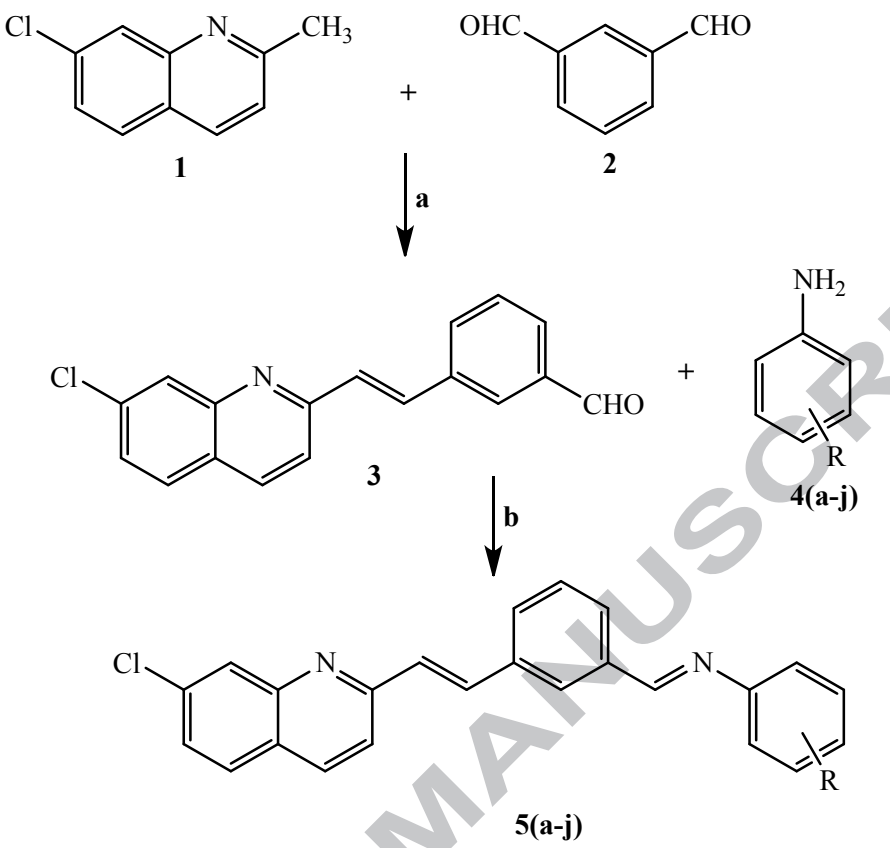



**Figure 2**. Strategy of design of fungal biofilm inhibitors.

The 2-substituted quninoline derivatives **5(a-j)** were synthesized as per the scheme outlined in **Scheme 1.** Sigma or Avra grade of compounds and solvents were used in the synthesis of titled compounds and used without further purification.




**Scheme 1**: Synthesis of *N*-(3-(-2-(7-Chloroquinolin-2-yl)vinyl) benzylidene)anilines **5(a-j)**.

Iron-catalyzed C(sp3)–H functionalization of methyl group substituted at 2nd position of 7-chloroquinoline was achieved through a analogues reported method [30]. Thus, the intermediate **3** 3-(2-(7-Chloroquinolin-2-yl)-vinyl)benzaldehyde was obtained by stirring a solution of 7-chloro-2-methylquinoline **1** (2.0 g, 11.26 mmol), isophthalaldehyde **2** (1.80 g, 13.4 mmol) and $Fe(OAc)_2$ (98 mg, 0.56 mmol) in AcOH (65 µL, 1.13 mmol) and toluene (12 mL) at 110 °C for 30 h. The progress of reaction was monitored by TLC. When the mixture was cooled to room temperature, it afforded a solid precipitate which was recovered by filtration and recrystallised by using EtOAc (25 mL) as a light-yellow solid (Yield: 60%). The mixture of compound **3** (1.0 mmol) and different aromatic amines **4(a-j)** (1.0 mmol) in absolute ethanol (15 mL) with catalytic amount of glacial acetic acid (3.0 mmol) was refluxed to obtain the titled compounds **5(a-j)**. The completion of reaction was monitored by TLC and the mixture was kept overnight. The crude solid was collected and recrystallized from ethanol. The physical data of the

synthesized compounds **5(a-j)** is given in **Table 1**. The purity of the synthesized compounds was determined by preparative TLC (Merck) with iodine vapor visualization technique. The melting points were measured in open capillary tubes and are uncorrected. $^{13}C$ NMR and $^{1}H$ NMR spectra were recorded on 400MHz BRUKER spectrometer. Chemical shifts for NMR studies are reported in parts per million (ppm), using TMS as an internal standard. Agilent technology 1200 series HPLC paired to a 6130 mass spectrometer with electron spray ionization (ESI) was used to record mass spectrum. The characterization data (Supplementary data) suggested the synthesis of proposed compounds.

**Table 1** Physical data for *N*-(3-(-2-(7-Chloroquinolin-2-yl)vinyl)benzylidene)anilines **5(a-j).**

| Entry | R | Mol. Formula | Yield (%) | Rf value | Mp ($^0$C) |
|---|---|---|---|---|---|
| **5a** | *p*-Cl | $C_{24}H_{16}Cl_2N_2$ | 87 | 0.71 | 110 |
| **5b** | *p*-$NO_2$ | $C_{24}H_{16}ClN_3O_2$ | 82 | 0.68 | 90-92 |
| **5c** | *m*-$NO_2$ | $C_{24}H_{16}ClN_3O_2$ | 90 | 0.65 | 220 |
| **5d** | *p*-$OCH_3$ | $C_{25}H_{19}ClN_2O$ | 85 | 0.60 | 110-112 |
| **5e** | *p*-$CH_3$ | $C_{25}H_{19}ClN_2$ | 83 | 0.70 | 80 |
| **5f** | -H | $C_{24}H_{17}ClN_2$ | 84 | 0.76 | 140-144 |
| **5g** | *o*-$CH_3$ | $C_{25}H_{19}ClN_2$ | 84 | 0.65 | 138-140 |
| **5h** | *o*-$CF_3$ | $C_{25}H_{16}ClF_3N_2$ | 84 | 0.68 | 110-114 |
| **5i** | *m*-$CF_3$ | $C_{25}H_{16}ClF_3N_2$ | 84 | 0.72 | 130-134 |
| **5j** | *p*-$CF_3$ | $C_{25}H_{16}ClF_3N_2$ | 85 | 0.70 | 140-142 |

The synthesized compounds **5(a-j)** were evaluated for *in vitro* biofilm inhibitory activity in *C. albican* strain. The reported method [31-32] of *in vitro* biofilm inhibitory activity was followed. The *N*-(3-(-2-(7-Chloroquinolin-2-yl)vinyl)benzylidene)anilines **5(a-j)** were evaluated for biofilm inhibition by 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay which is measure of metabolic activity of cells. *C. albicans* cells ($10^5$ cells/ well) were inoculated in RPMI medium and exposed to various concentrations (0-100 µM) of compounds **5(a-j)** and standard drug fluconazole and further incubated for 24 h at 30 $^o$ C. The media was removed and the biofilm formed was washed with phosphate buffer. Each well was further treated with 100 µL (1µg/µL) of MTT and the culture was further incubated for 3 h in dark. The

clear solution from each well was removed and dimethylsulfoxide (100 µL) was added. The optical density was recorded at 575 nm. From the growth inhibition curve, the concentration required to inhibit biofilms by 50 % ($IC_{50}$) was calculated [33-34]. The results of biofilm inbibition are given in **Table 2.**

**Table 2** *In vitro* Biofilm inhibition, antibacterial and antifungal activities of *N*-(3-(-2-(7-Chloroquinolin-2-yl)vinyl)benzylidene)anilines **5(a-j)**.

| Entry | Anti-biofilm activity ($IC_{50}$ µM) | Antifungal activity (MIC Values in µg/mL) | Antibacterial activity (MIC in µg/mL) | | | |
|---|---|---|---|---|---|---|
| | *C. albicans* (NCIM-3471) | *C. albicans* (NCIM-3471) | *E. coli* (NCIM-2256) | *P. aeruginosa* (NCIM-2036) | *B. subtilis* (NCIM-2063) | *S. aureus* (NCIM-2901) |
| 5a | 66.2 | 94.2 | 45 | 228.5 | 31.6 | 170.2 |
| 5b | 92.1 | 121.2 | 150 | 200.5 | 15.7 | 165.5 |
| 5c | 83.6 | 106 | 185 | 190 | 17.4 | 104.2 |
| 5d | 97.5 | 105 | 120 | 167 | 17.6 | 94.8 |
| 5e | 96.2 | 164.1 | 205 | 177 | 16.98 | 98.5 |
| 5f | 199.1 | 98.8 | 110 | 102 | 32.35 | 91.6 |
| 5g | 165.5 | 104.4 | 68.5 | 185 | 136.22 | 85.3 |
| 5h | 94.3 | 282 | 67.5 | 250 | 36.22 | 55.3 |
| 5i | 87.2 | 191 | 102 | 142 | 33.44 | 71.3 |
| 5j | 51.2 | 174.4 | 84.5 | 91.5 | 37.66 | 89.7 |
| FA | 40 | 50 | - | - | - | - |
| CP | - | - | 50 | 50 | 50 | 25 |

The title quinoline derivatives **5(a-j)** exhibited good to moderate biofilm inhibition activity ($IC_{50}$ in the range 51.2-199.1 µM) against *C. albicans* strain. Compounds **5j** and **5a** with $IC_{50}$ values 51.2 and 66.2 µM respectively, were most active identified compounds in this series. The preliminary structure activity relationship (SAR) suggested that the compound **5j** ($IC_{50}$ = 51.2 µM) with *p*-trifluoromethyl substituent on the phenyl ring improves biofilm inhibitory activity as compared to the compound **5f** ($IC_{50}$ = 199.1 µM) which has unsubstituted phenyl ring. But this trifluoromethyl substituent at *ortho* and *meta* position as in compounds **5h** ($IC_{50}$ = 94.3 µM) and **5i** ($IC_{50}$ = 87.2 µM) found decreasing the biofilm inhibitory activity. This suggests that the small electronegative substituent like trifluoromethyl is more favaorable at *para* position of

phenyl ring. Hydrophobic substituent like *o*-methyl is less favorable as in compound **5g** ($IC_{50}$ = 165.5 μM). A halogen group like chloro at *para* position on pheyl ring as in compound **5a** ($IC_{50}$ = 66.2 μM) was also found increasing the biofilm inhibitory activity than the unsubstituted compound. The substituents like *p*-methoxy and *p*-nitro shows moderate antibiofilm activity in compounds **5b** ($IC_{50}$ = 92.1 μM) and **5d** ($IC_{50}$ = 97.5 μM). But the *m*-nitro substitution in compound **5c** ($IC_{50}$ = 83.6 μM) was found better than the *p*-nitro substitution. The structure activity relationship suggests that the trifluoromethyl group with significant electronegative characteristic exhibit the highest antibiofilm activity than the corresponding chloro substituted compound. The hydrophobic substituent's shows decrease in antibiofilm activity as compared to such electronegative substituents. The reference compound Fluconazole exhibited slightly better antibiofilm inhibition activity ($IC_{50}$ = 40.0 μM) than the series under investigation.

The synthesized compounds were also evaluated for *in vitro* antifungal activity. Standard agar method [35-36] as per the guidelines of The Clinical and Laboratory Standards Institute (CLSI) was employed. The solutions of title compounds and standard fluconazole were prepared in DMSO. The *C. albicans* was subcultured on the Sabouraud dextrose agar (SDA) and incubated at 25 $^0$C for 72 h. The resultant *C. albicans* cells were suspended in sterile distilled water to achieve $10^5$cells/mL dilution. This standardized *C. albicans* suspension was inoculated on control plates (plates without standard drug, fluconazole and plates for solvent control, DMSO) and test plates with title compounds. The plates were incubated at 25 $^0$C for 48 h. The lowest concentration of compounds (MIC) required to prevent growth of macroscopically visible colonies of *C. albicans* in control plates and test plates were measured by taking readings at 48 h and 72 h. The synthesized compounds exhibited good to moderate antifungal activity ($IC_{50}$ range = 94.2 – 282.0 μM). Compounds **5a** and **5f** exhibited good antifungal activity with $IC_{50}$ values of 94.2 and 98.8 μM, which are comparable to the reference compound Fluconazole with $IC_{50}$ value of 50 μM. All other compounds exhibited moderate antifungal activity as their $IC_{50}$ value is above 100 μM and below 300 μM. Interestingly the compound **5f** which is unsubstituted exhibited better antifungal activity than its antibiofilm inhibitory activity. In case of compound **5j** though the antibiofilm activity is higher the corresponding antifungal activity is moderate. Further, the substituent *p*-chloro improves the antifungal activity considerably suggesting that only electronegative substituent is favorable for antifungal activity.

The synthesized compounds were also evaluated for their *in vitro* antibacterial activity. The antibacterial potency in terms of MIC values was determined as per the guidelines of CLSI [35-36]. Nutrient Broth containing microbial spores was prepared from 24 h old bacterial cultures on nutrient agar (Hi-media) at 37±1 $^{o}$C. A concentration of 1× $10^{-4}$-$10^{-5}$ colony forming unit (CFU) in the bacterial suspension was adjusted with sterile saline solution. Two fold serial dilutions of the synthesized compounds and standard drug ciprofloxacin were prepared to obtain the concentrations of 400, 200, 100, 50, 25, 12.5, 6.25, and 3.13 µg/mL. The control and test samples were incubated in BOD incubators at 37± 1 $^{o}$C for bacteria. The MICs were measured after 24 h of incubation.

The title compounds **5(a-j)** showed good to moderate antibacterial activities against different bacterial strains. All the compounds **5(a-j)** (MIC range = 15.7 – 37.6 µg/mL) were found possessing better antibacterial activity than ciprofloxacin (MIC= 50 µg/mL) except compound **5g** (MIC= 136.2 µg/mL) against *B. subtilis* strain. This suggests that the hydrophobic methyl substituent decreases antibacterial activity against *B. subtilis.* The compound **5a** (MIC= 45 µg/mL) was found more active than the reference ciprofloxacin (MIC= 50 µg/mL) against *E. coli*. Compound **5g** (MIC= 68.5 µg/mL), **5h** (MIC= 67.5 µg/mL) and **5j** (MIC= 84.5 µg/mL) exhibited moderate antibacterial activity against *E. coli*. The MIC value for other compounds (**5b-5f** and **5i**) was found above 100 µg/mL, suggesting their decreased antibacterial activity against *E. coli*. All the compounds were found exhibiting less antimicrobial activity against *P. aeruginosa,* except compound **5j** (MIC= 91.5 µg/mL) as compared to standard drug ciprofloxacin (MIC= 50.0 µg/mL). This suggests that the synthesized compounds are less effective on gram negative organisms. The synthesized compounds exhibited moderate antimicrobial activity against *S. aureus* (MIC range= 55.3 – 170.2 µg/mL). Compound **5h** was found moderately active against *S. aureus* (MIC= 55.3 µg/mL), and compounds **5d-5j** have MIC value below 100.0 µg/mL, suggesting their moderate potency against the *S. aureus.* The results of antimicrobial activity also suggest that the compound **5j** is almost effective against all test organisms.

In order to gain insights of possible mode of inhibition of Als protein and mechanism of biofilm inhibition, molecular docking studies were performed. The crystal structure of Als-3 adhesin from *C. albicans* in complex with hepta-threonine (PDB ID: 5LEB) was retrieved from www.rcsb.org and used for docking with Autodock Vina [37] program. The 2D structures of compounds were drawn with Marvin Sketch 5.6.0.0, which were transformed to the 3D geometry and subsequently energy minimized in UCSF Chimera 1.8 [38] interface using steepest descent and conjugate gradient search criteria. The protein structure was refined by removing water and nonstandard residues and energy minimization with Amber ff12SB force field. Polar hydrogen were added in the protein structure and docking was carried out using the grid box of size 22 × 20 × 20 along x, y and z axis with 1 A° spacing. The key interactions of ligands with residues at active site and estimation of binding free energy in kcal/mol was considered for the analysis of results of docking studies.

The active site of Als-3, where hepta-threonine binds, has the surrounding residues Val172, Ser170, Gly297, Thr296, Arg294, Lys59, Gly27 and Tyr21. The interaction between the best docked conformer of ligands with these surrounding residues at this active site was analyzed. These results are shown in **Table 3**. The docking results and experimental results are concordant. The docking results suggest that the hydrogen bond interaction with the residue Tyr21 or Tyr226 is most crucial in deciding the potency of these compounds. The best docked conformers of compounds **5a**, **5b**, **5d** and **5j** produce such hydrogen bond interaction. The estimated binding free energy for these compounds is also lower (-7.8 to -8.6 Kcal/mol) as compared to other compounds suggesting their favorable interactions at the active site. The naphthyl ring forms hydrophobic π–π stacking interaction with residues like Leu293, Val161, Trp295, Tyr166 or Val172.

**Table 3: Results of docking studies**

| Compound ID | Estimated binding free energy in Kcal/mol | Interactions with key residues | |
|---|---|---|---|
| | | Hydrogen bond | Hydrophobic |
| 5a | -8.5 | Tyr21 | Val172, Leu193 |
| 5b | -8.1 | Tyr21 | Val172, Val162, Lys59, Trp295 |
| 5c | -8.2 | -- | Val161, Tyr166, Ser170 |
| 5d | -7.8 | Tyr21 | Val172, Trp295, Leu167, Val161 |
| 5e | -7.9 | -- | Pro29, Tyr226 |
| 5f | -7.4 | -- | Pro29, Tyr226 |
| 5g | -7.6 | -- | Ala19, Ala18, Pro29, Tyr226 |
| 5h | -8.0 | -- | Val161, Thr168, Val172 |
| 5i | -8.3 | -- | Val161, Ser170, Thr296, Trp295, Val268, Tyr271 |
| 5j | -8.6 | Tyr226 | Val161, Leu293, Arg171, Val172, Pro29, Lys59, Gly27 |

The extent and pattern of substitution on iminophenyl ring was found crucial in favorable binding at active site of Als-3. The chloro substitution in compound **5a** forms halogen bond with Val172. The trifluoromethyl substitution at *para* position in compound **5j** forms hydrophobic halogen bonds with Val172, Arg171, Pro29 and Gly27. Such hydrophobic halogen bond interactions in compound **5j** may be responsible for the highest biofilm inhibitory activity of this compound. The unsubstituted compound **5f** was not fond making any key interactions and was least active. The hydrophobic methyl substitution at *ortho* position in compound **5g** was also found unable to make above key interactions and also less active. The trifluoro methyl substitution at *ortho* position could not produce the key interactions in compound **5h** and this compound was found less active than compound **5j**. The central phenyl ring in all the compounds except compound **5j** could not form any hydrophobic π–π stacking interaction. In compound **5j** the central phenyl ring in a conformer was found oriented favaorably to form π–π. stacking interaction with Val161. **Figure 3** shows the key interactions at the active site for all the compounds.

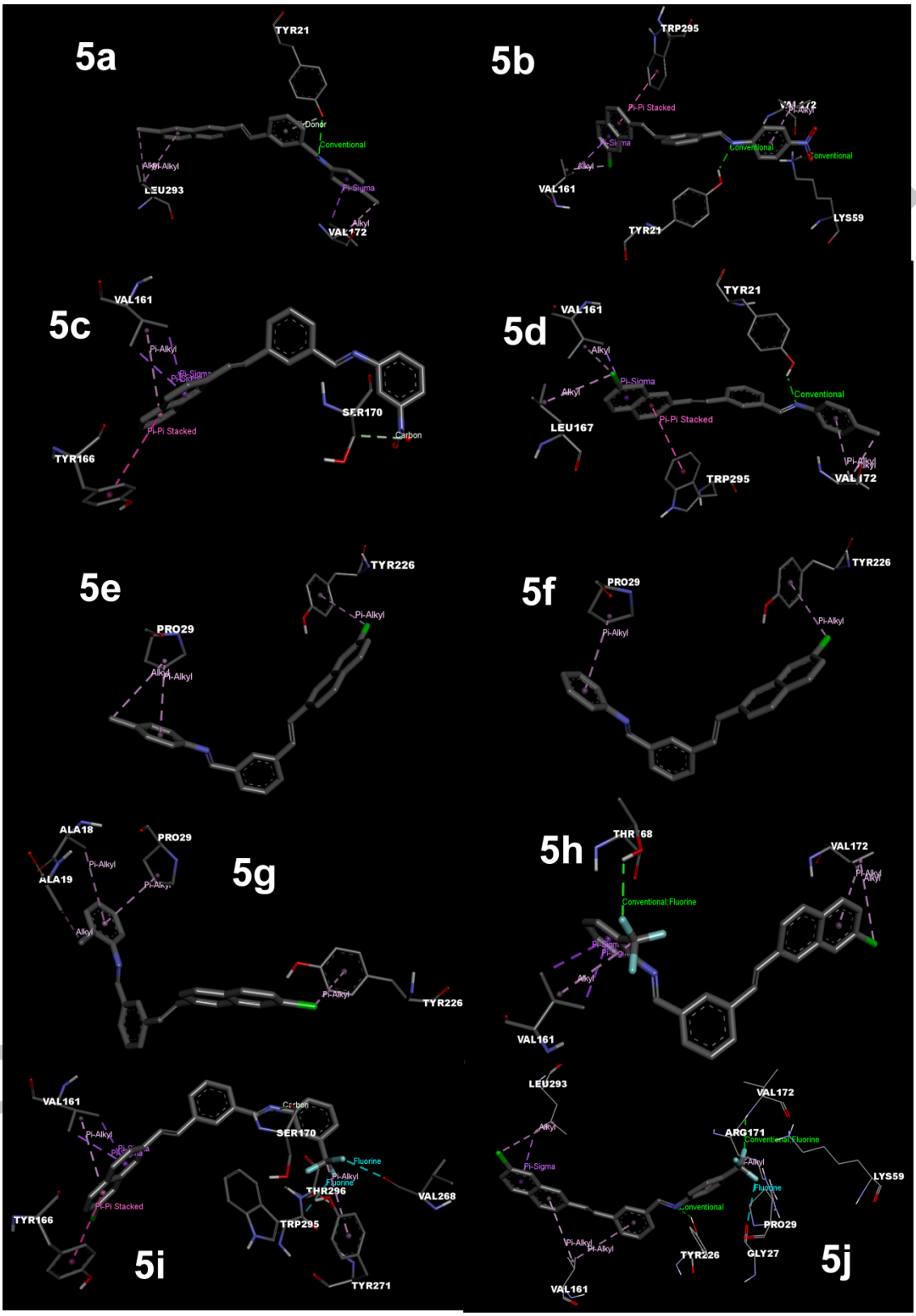



**Fig. 3.** Key interactions observed for all compounds in docking studies.

The docking simulation does not address the dynamic interaction between protein-ligand complexes. Further, the protein structure is kept rigid during the docking simulation, whereas proteins can undergo significant conformational changes while performing their function. Thus, molecular dynamics (MD) simulation was performed on Gromacs 5.1.4 [39] to augment the docking results and to gain deeper insights of the mode of action of ligands. The complex of Als-3 with best docked conformer of ligand was used during MD simulation. The complexes of two less active compounds **5f** and **5g** and two most active compounds **5a** and **5j** were selected for 1 nanoseconds MD simulation. The topology of protein was generated using CHARMM36 all-atom force field [40], where as ligand topology was generated using CHARMM General Force Field (CGenFF) [41] from CGenFF server available at https://cgenff.paramchem.org.

With topologies of protein and ligand the topology of complex was created. The complexes were solvated using simple point charge solvent model (SPC) 216 in a dodecahedral unit cell with minimum distance of 1 A° between the protein's outermost atoms and walls of the unit cell. Subsequently the systems were then neutralized by addition of counter ions. In order to remove the steric clashes the energy minimization was performed until the maximum force value on any atom is below 10.0 kJ/mol. The system was equilibrated with position restraint NVT and NPT at 300K for 100 ps each. Linear Constraint Solver (LINCS) algorithm was exploited to fix the covalent bonds of the system [42]. Long range electrostatic interactions were measured with Particle Mesh Ewald (PME) scheme [43]. The van der waals and coulomb interaction cutoff's were set with 12.0 A°. Different modules of GROMACS package were employed for the analysis of the results.

MD simulations of 1 ns each were performed for complexes of two less active compounds (compound **5f** and compound **5g**) and two more active compounds (compound **5a** and **5j**). MD simulation gave an impetus of the overall stability of these complexes. The overall stability in terms of root-mean-square-deviation (RMSD), Root-mean square fluctuation (RMSF), number of H-bonds and energy evaluation was evaluated.

Root mean square deviation (RMSD), which is the measure of the deviation of main chain atoms of the protein, help determines the dynamic stability of the system during the course of simulation. The complexes of more active compounds (compound **5a** and **5j**) were found to be stabilized during 1 ns simulation with average RMSD of 0.113 and 0.126. The complex of

compound **5g** stabilize with RMSD of 0.175 where as the complex of compound **5f** was found stabilize with RMSD of 0.693. As the RMSD values for these two complexes were found deviating these compounds require longer simulations and probably producing unfavorable interactions with the protein. **Figure 4** shows the RMSD of the backbone atoms during MD simulation.

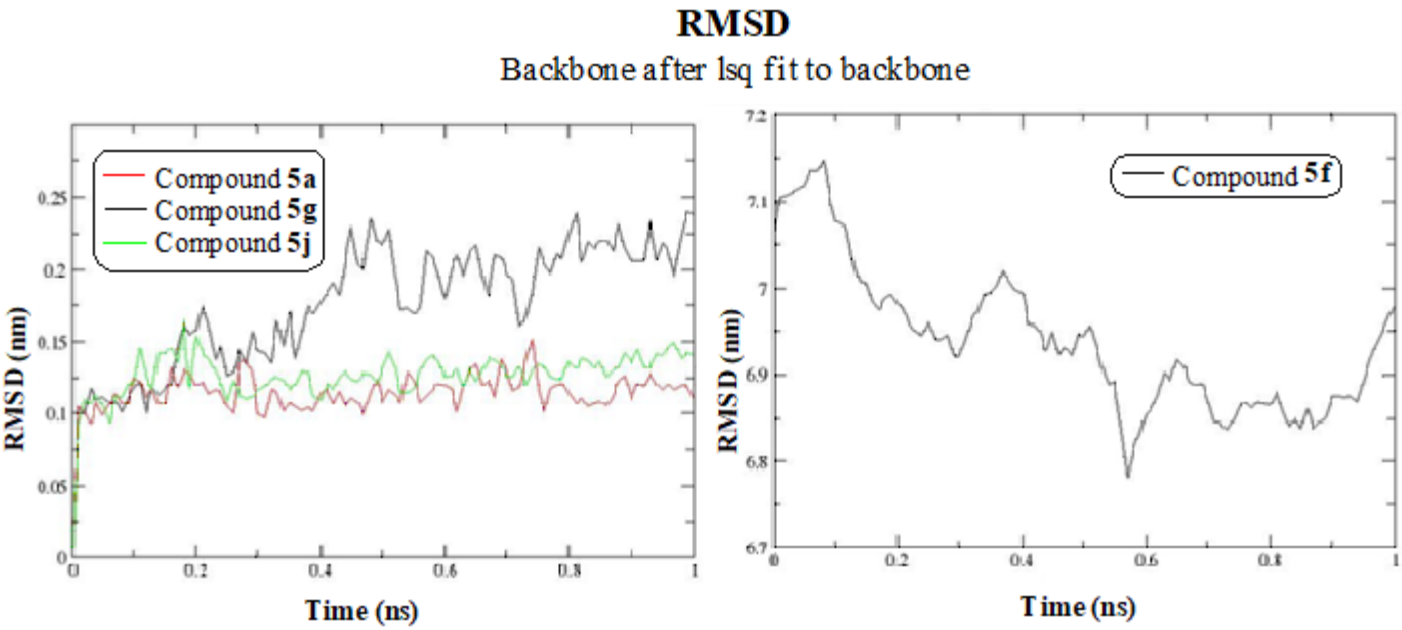



**Fig 4.** The RMSD of the backbone atoms of all complexes during MD simulations.

Thus the low RMSD of complexes of compound **5a** and **5j** shows the substitutions at *para* position of phenyl ring in these compounds was better stabilized whereas the unsubstituted compound **5f** display larger RMSD. Further the hydrophobic methyl substitution at *ortho* position in compound **5g** also shows slightly larger RMSD. This suggest that the importance of halogen substitution at *para* position of phenyl ring.

Root mean square fluctuation (RMSF) is characteristic to the elasticity of the native protein structure which is investigated through the fluctuations in backbone atoms and atoms of ligand. The RMSF evaluation helps in investigating the accuracy and stability of a system during simulation. As shown in **Figure 5**, the fluctuations in atom 2000 onwards were observed for complexes of compound **5f** and **5g**, whereas such fluctuations are minimal for the complexes of

compounds **5a** and **5j**. The least fluctuations in compound **5a** and **5j** could be due to the most favorable substitution at para position of phenyl group which allows the ligand to properly orient within the active site.

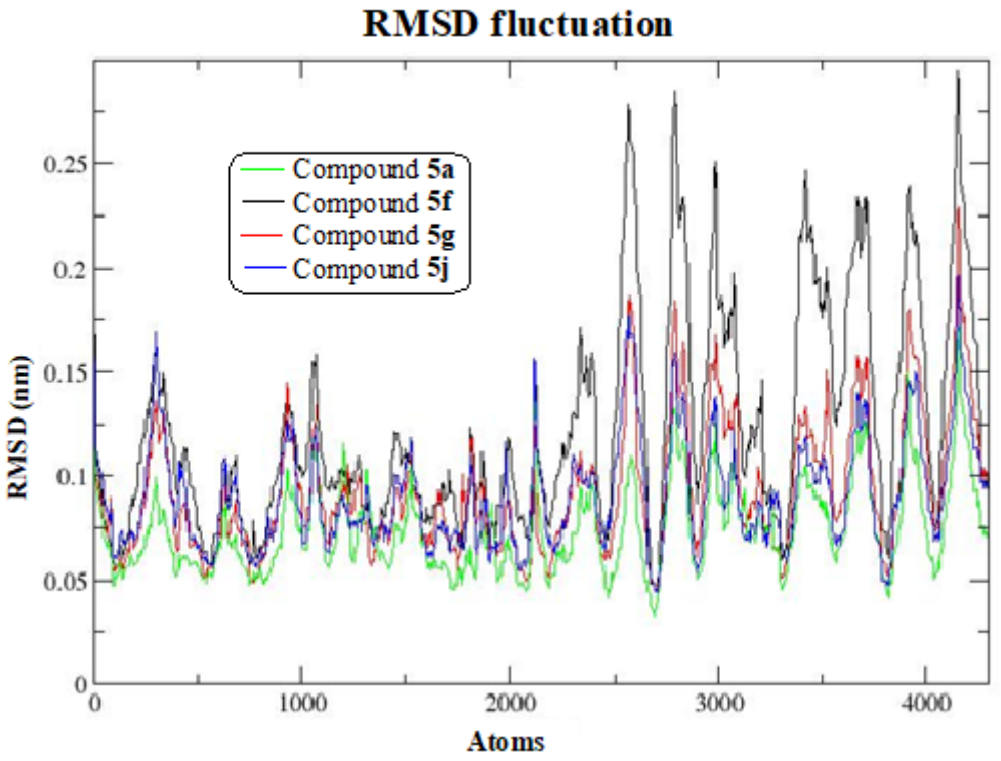



**Fig 5.** The RMSF of all complexes back bone atoms during MD simulations.

The binding affinity of ligands depends on capacity to form hydrogen bonding with key residues. The stability of system increases as the number of hydrogen bond interaction increases. **Figure 6** shows number of hydrogen bonds formed between ligands and active site residues during entire simulation.

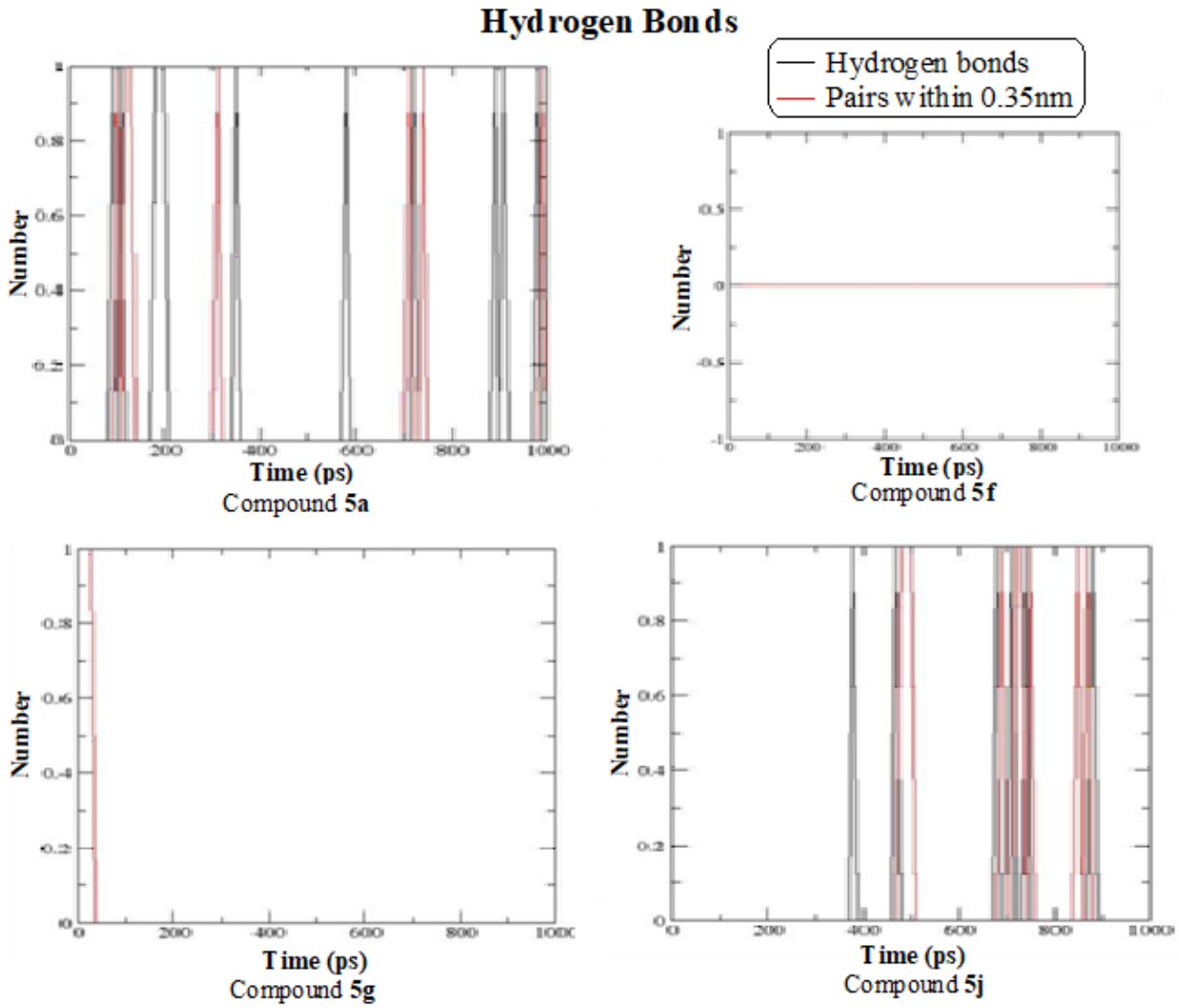



**Fig 6.** Hydrogen bond formation for Compounds **5a**, **5f**, **5g** and **5j.**

It is clearly evident that in complex of compound **5a**, the hydrogen bond interactions were formed throughout the simulation whereas in complex of compound **5j**, the hydrogen bond formation was observed after 300 ps simulation. Interestingly, in complex of compound **5f** no hydrogen bond formation was observed throughout the simulation and in complex of compound **5g** only one hydrogen bond interaction during initial phase of simulation was observed. This supports the experimental activities of these compounds against the target protein.

In conclusion, a series of quinoline ring bearing schiff's bases **5(a-j)** were designed and synthesized as promising fungal biofilm inhibitors and as antifungal and antibacterial agents. The compounds **5j** ($IC_{50}$= 51.2 µM) and **5a** ($IC_{50}$= 66.2 µM) with *o*-hydroxy and *p*-methoxy

substituents showed promising *C. albicans* biofilm inhibition activity in comparison with standard fluconazole ($IC_{50}$= 40.0 μM). All the compounds except compound **5g**, were also found possessing promising antimicrobial activity against *B. subtilis*, and are more potent than the standard drug ciprofloxacin. The synthesized compounds were found less active on the *P. aeruginosa* strain, suggesting the ineffectiveness on Gram-negative organisms. The docking study supports the experimental biofilm inhibition and suggests that for such activity the compounds require halogen substitution at *para* position of the phenyl ring. Hydrophobic substitutions on phenyl ring are unfavorable for binding of the conformers at the active site of Als-3 protein. Molecular dynamics also supports these finding suggesting that hydrogen bond formation is key in inhibition of the Als-3, and substitutions at *para* position of phenyl ring with halogens or groups involved in hydrogen bond formation could lead to promising biofilm inhibitors.

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## Graphical Abstract

# Synthesis, biological evaluations and computational studies of *N*-(3-(-2-(7-Chloroquinolin-2-yl)vinyl) benzylidene)anilines as fungal biofilm inhibitors

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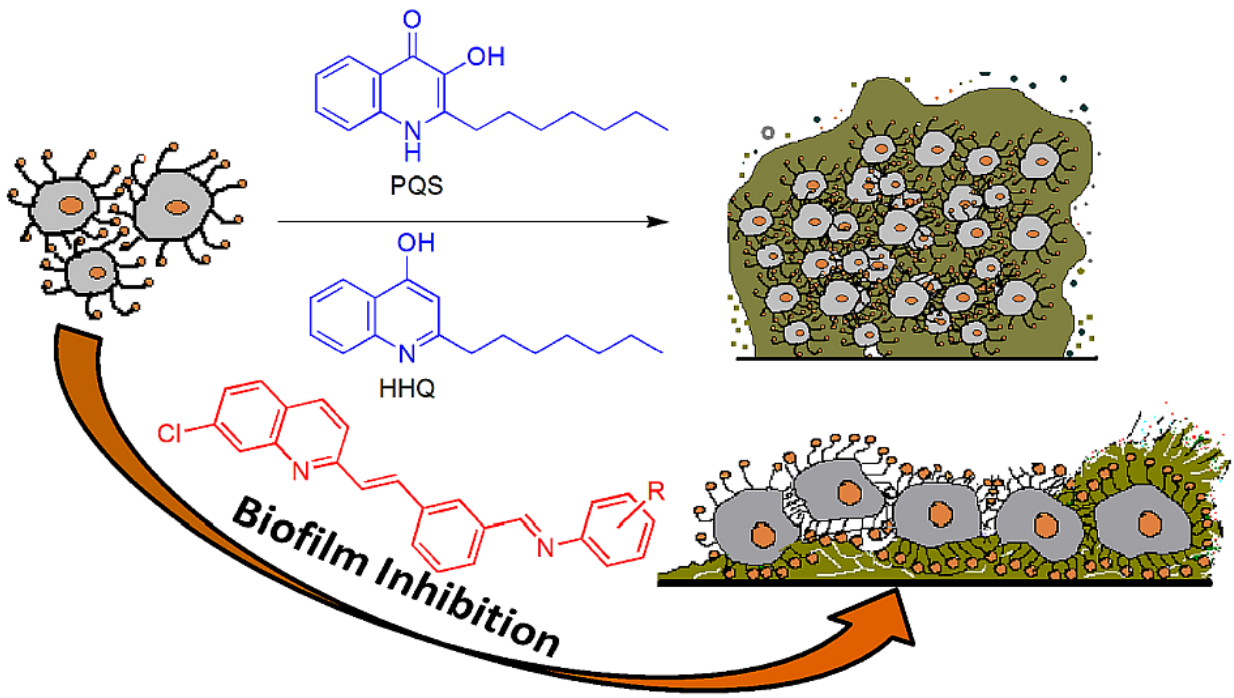


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