ABSTRACT

Pseudomonas aeruginosa is an opportunistic pathogen that causes fatal effects in patients with cystic fibrosis and immunocompromised individuals. The quorum sensing (QS) mechanism of *Pseudomonas aeruginosa* plays a major role in biofilm formation and expression of virulent genes. QS inhibition is a promising approach to circumvent its infections as they are antibiotic-resistant. Targeting LasR in the QS network serves beneficial as it holds the top position in the cascade. In the present study, high-throughput virtual screening was applied to identify a new class of LasR inhibitors. Three-tire structure-based virtual screening was performed on the Schrödinger small molecule database. Twelve hits with docking scores <-11.0 kcal/mol were retrieved. Top three of these compounds (3-[2-(3,4-dimethoxyphenyl) -2- (1H-indol-3-yl) ethyl] -1- (2-fluorophenyl) urea) (C1), (3-(4-fluorophenyl)-2-[(3-methylquinoxalin-2-yl)methylsulfanyl]quinazolin-4-one) (C2), and (2-({4-[4- (2- methoxyphenyl) piperazin-1-yl] pyrimidin-2-yl} sulfanyl) -N-(2,4,6-trimethylphenyl)acetamide) (C3) were selected. All three selected compounds were found to pass the ADMET properties. Molecular dynamics revealed that they were found to be in stable contact with LasR over the simulation of 100 ns. Cytotoxicity analysis showed that the compounds possessed lower cytotoxicity in peripheral blood lymphocyte cells. The minimum inhibitory concentration was found to be 1000 μM (43.3 μg/ml) for C1, 1000 μM (42.8 μg/ml) for C2, and 500 μM (23.8 μg/ml) for C3. At ½ and ¼ MIC, the compounds inhibited biofilm formation, disrupted preformed biofilms, retarded matrix materials and decreased viable cells of biofilm, which proved their antibiofilm properties. Inhibition of violacein production in *Chromobacterium violaceum*, suppression of swimming and swarming motilities in *Pseudomonas aeruginosa PAO1* confirmed their antiquorum sensing properties. The compounds hindered the production of virulence factors, namely pyocyanin, rhamnolipids, total protease, *lasB* elastase, alkaline protease, and lipase. FESEM showed a reduction in the biofilm attached to the surface, which further validated them as potential biofilm disruptors. Gene expression analysis of the QS regulatory genes, namely *lasI, lasR, rhlI, rhlR, pqsA,* and *pqsR,* showed significant downregulation. Finally, the antagonistic effect against LasR was confirmed through β-galactosidase reporter gene assay. A significant reduction in the β-galactosidase activity at 100 nM was observed for all compounds. Thus, the present study provides strong evidence that the selected compounds could serve as probable leads and be employed to treat *Pseudomonas aeruginosa*-associated infections.