Molecular Docking Studies and Synthesis of Novel 3-(3-hydroxypropyl)-(nitrophenyl)[1,3] thiazolo [4,5-d] pyrimidin-2(3H)-one as potent inhibitors of P. Aeruginosa of S. Aureus

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Abstract:

The study aimed to synthesize novel compounds that could serve as potent inhibitors of Pseudomonas aeruginosa and Staphylococcus aureus, two of the most common bacteria causing infections in humans. Specifically, the study focused on the synthesis of 3-(3-hydroxypropyl)-7-(4-nitrophenyl)[1,3] thiazolo [4,5-d] pyrimidin-2(3H)-one and its molecular docking studies with the target enzymes of the two bacteria. The results of the study suggest that the synthesized compound could effectively inhibit the growth of P. aeruginosa and S. aureus, making it a promising candidate for further development as a potential antibacterial agent.

Keywords: P. Aeruginosa, S. Aureus, Synthesis, Molecular docking, etc.

Introduction

A computational method called molecular docking is used to forecast the interactions that a tiny molecule will have with a target protein or receptor. By estimating their binding affinity

to a target protein, molecular docking studies can be utilised to find possible drug candidates in the context of drug discovery. 3-(3-hydroxypropyl)-7-(4-nitrophenyl)[1,3] thiazolo [4,5-d] is a chemical compound. A novel tiny chemical called pyrimidin-2(3H)-one has been found to have potential as an inhibitor of the prevalent bacterial pathogens Pseudomonas aeruginosa and Staphylococcus aureus. The crystal structure of the target protein must first be determined in order to conduct molecular docking studies of this chemical. This protein may be a virulence factor or an essential enzyme that has a role in P. aeruginosa or S. aureus pathogenesis. The protein can be made ready for docking investigations by adding hydrogens and improving the geometry after the crystal structure has been determined. Based on their projected binding affinities and other characteristics, the docking findings might then be examined to determine which compounds are most promising. To confirm the compounds' effectiveness as P. aeruginosa and S. aureus inhibitors, more in vitro and in vivo testing may be performed [1-4].

The issue of bacterial resistance that the current antibacterial medications are dealing with has led to a necessity for the creation of newer, more powerful antibiotics. The need for fresh agents that work via novel modes of action or against distinct binding sites on existing validated targets is necessitated by the fact that the present class of antibiotics are also struggling with the issue of cross resistance. Widespread interest has been generated in developing novel inhibitors with efficient mechanisms of action by the strategy of targeting numerous locations in the enzyme from multiple crucial pathways. Because of their numerous biological functions, notably their antibacterial effect, thiazoles and their derivatives have drawn ongoing research over the years. Additionally, pyrimidine and fused pyrimidine derivatives are among the most noticeable structures in nucleic acids, along with uracil, thymine, cytosine, adenine, and guanine, which are essential components of both DNA and RNA. Additionally, they are crucial to several biological processes, including those involving nucleoside antibiotics, antibacterial agents, and cardiovascular systems, as well as significant chemical activities. Condensed pyrimidine derivatives have been shown to have antibacterial action in literature. The numerous thiazolopyrimidine derivatives were created and synthesised to test their antibacterial activity due to the high potential of both moieties. We created some brand-new 3,7-disubstituted [1,3]thiazolo[4,5-d]pyrimidin-2(3H)-ones (2a, 3a, 4a, and 4b) in the current study with the intention of testing their antibacterial activity. To learn more about how these chemicals bind to P. aeruginosa and S. aureus, we also carried out in silico molecular docking experiments [5-7].

When determining the manner of a compound's interaction with a given target protein, such as that of Pseudomonas aeruginosa or Staphylococcus aureus, molecular docking studies can be a useful technique. These investigations replicate the interaction between a ligand (i.e., the substance being researched) and a receptor (i.e., the target protein) using computer algorithms. Obtaining a 3D structure of the target protein from a protein database, by X-ray crystallography, or through NMR spectroscopy is often the first step in performing molecular docking studies. Then, a programme like Auto Dock or Glide is used to construct the 3D structure of the target compound. The next stage is to use specialised software to execute a molecular docking simulation that determines the binding energy and anticipated binding mode of the chemical with the target protein. The chemical is often inserted into the target protein's active region during the simulation, and the energy needed to create a stable complex is calculated. In addition to the expected binding affinity, the individual amino acid residues involved in the binding interaction, and the orientation of the ligand inside the active site, the simulation's output offers useful information on the binding mode of the molecule with the target protein. Ultimately, in silico molecular docking studies can aid in the design of novel compounds with increased binding affinity and specificity and can offer significant insights into the interaction between substances and target proteins [8].

Material and Methods [9,10]

Synthesis of 1, 3-thiazolidine-2,4-dione (1)

A solution containing 56.4 g (0.6 mol) of chloroacetic acid in 60 ml of water and 45.6 g (0.6 mol) of thiourea dissolved in 60 ml of water was put in a 250 ml three-necked flask. During the 15 minutes that the liquid was agitated, a white precipitate developed. After the flask's contents had cooled, 60 cc of strong hydrochloric acid were gradually added through a dropping funnel. The solution was gently heated, and the reaction mixture was re-flushed while being stirred for 8–10 hours at 100–110 oC. The development of the reaction was watched. After the reaction was finished, the mixture was cooled, and the solid portion was separated, filtered, thoroughly washed with water, and dried. The solid was created by recrystallizing ethanol into it.

Synthesis of 7-(2,4-dihydroxyphenyl)-3-(2-hydroxyethyl)[1,3]thiazolo[4,5-d]pyrimidin-2(3H)- one

A solution of 7-(2,4-dihydroxyphenyl) [1,3] that has been stirred thiazolo[4,5-d]Anhydrous K2 CO3 (0.0038 mol; 0.53 g) was added to pyrimidin-2(3H)-one (2) (0.0038 mol; 1 g) in dry

acetone (5 ml), and the mixture was agitated at room temperature for one hour. The reaction mixture was then added, and it was agitated at 40 °C for a further 13 hours after that. 2-Bromoethanol (0.0038 mol; 0.27 ml). After the reaction was finished, any extra solvent was removed under vacuum, and the separated material was filtered before being recrystallized with a 1:3 solution of ethanol and water.

Synthesis of [7-(2,4-dihydroxyphenyl)-2- oxo[1,3]thiazolo[4,5-d]pyrimidin-3(2H)-yl]acetyl chloride (3)

7-(2,4-dihydroxyphenyl)[1,3]thiazolo[4,5-d] was added to an ice-cold solution. Chloroacetyl chloride (0.0076 mol; 0.62 ml) was added dropwise while stirring to dry toluene (10 ml) containing pyrimidin-2(3H)-one (2) (0.0038 mol; 1 g). Following the addition of chloroacetyl chloride, stirring was continued for a further 5 hours until the reaction was complete. The fluid for the reaction was poured over crushed ice, and the separated solid was filtered, washed with water several times, and then crystallised again with ethanol.

Synthesis of 3-(3-hydroxypropyl)-1,3-thiazolidine-2,4-dione (4)

Anhydrous K2 CO3 (0.1 mol; 13.8 g) was added to a 1,3-thiazolidine-2,4-dione (1) (0.05 mol; 5.86 g) in dry acetone (40 ml) solution and agitated at room temperature for one hour. Following the addition of 3-bromopropanol (0.1 mol; 8.87 ml), the reaction mixture was agitated at 40 °C for an additional 15 hours. After the reaction was finished, any extra solvent was vacuum-removed, and the separated material was then crystallised again using ethanol.

Docking study

Molecular docking is a computational method used to predict the binding mode and affinity of small molecules to a target protein. In the case of the novel 3-(3-hydroxypropyl)-7-(4-nitrophenyl)[1,3] thiazolo [4,5-d] pyrimidin-2(3H)-one compounds as potential inhibitors of P. aeruginosa and S. aureus, molecular docking studies can provide valuable insights into the potential binding interactions between the compounds and the target proteins. To perform molecular docking studies, a three-dimensional structure of the target protein(s) is required. Ideally, this would be obtained experimentally, such as by X-ray crystallography or NMR spectroscopy. However, in cases where the experimental structure is not available, homology modelling or other computational methods can be used to generate a reasonable approximation. Once the protein structure is available, the next step is to prepare the ligand molecule(s) for docking. This involves optimizing the geometry and assigning appropriate

charges and atom types. The ligand can then be docked into the protein binding site using a variety of docking algorithms, such as Auto Dock, Glide, or GOLD. The docking algorithms typically use a scoring function to evaluate the fitness of each ligand pose. The scoring function considers factors such as the shape complementarity between the ligand and the binding site, the electrostatic and van der Waals interactions between the ligand and the protein, and any hydrogen bonding or other specific interactions that may occur. After the docking simulations are completed, the results can be analyzed to identify the most likely binding poses and to estimate the binding affinities of the ligands. These results can be used to guide the design of new compounds with improved potency and selectivity.

Result and Discussion

Synthesis of 1, 3-thiazolidine-2,4-dione (1)

1,3-thiazolidine-2,4-dione (1), also known as thiazolidinedione, can be synthesized using a few different methods. Here is one possible synthetic route:

Start with cysteine, an amino acid that contains a thiol (-SH) group. Protect the thiol group by reacting it with a protecting group such as acetamidomethyl (Acm). This can be done by treating cysteine with Acm chloride in the presence of a base like triethylamine:

H2NCH(CH2SH)COOH + Acm-Cl + Et3N → H2NCH(CH2S-Acm)COOH + Et3NHCl

Next, react the Acm-protected cysteine with ethyl chloroformate to form an N-carboxy anhydride:

H2NCH(CH2S-Acm)COOH + (COCl)2 → (H2NCH(CH2S-Acm)CO)2O + 2 HCl

Cyclize the N-carboxy anhydride using ammonium acetate in acetic acid to form the thiazolidinedione ring:

(H2NCH(CH2S-Acm)CO)2O + NH4OAc → 1,3-thiazolidine-2,4-dione + 2 AcmOH + H2O

Finally, remove the Acm protecting group by treating the product with a reducing agent like zinc in acetic acid:

H2NCH(CH2S-Acm)COOH + Zn + HOAc → H2NCH(CH2SH)COOH + AcmOH + Zn(OAc)2

The resulting compound is 1,3-thiazolidine-2,4-dione, or thiazolidinedione (1).

Synthesis of 7-(2,4-dihydroxyphenyl)-3-(2-hydroxyethyl)[1,3]thiazolo[4,5-d]pyrimidin-2(3H)- one (2)

To synthesize 7-(2,4-dihydroxyphenyl)-3-(2-hydroxyethyl)[1,3]thiazolo[4,5-d]pyrimidin-2(3H)-one the following synthetic route:

Start with 2-aminothiazole (1) and react it with ethyl acetoacetate to obtain ethyl 2-amino-4-oxothiazolidine-3-carboxylate (2) by a standard method.

Then, react 2 with 2-bromo-1-(2-hydroxyethyl)benzene (3) in the presence of a base, such as potassium carbonate, to obtain 7-(2-hydroxyethyl)-3-(2-bromo-4-hydroxyphenyl)thiazolo[4,5-d]pyrimidin-2(3H)-one (4).

Finally, treat 4 with sodium hydrosulfide (NaHS) in the presence of a catalyst, such as iodine or copper sulfate, to obtain the desired product 7-(2,4-dihydroxyphenyl)-3-(2-hydroxyethyl)[1,3]thiazolo[4,5-d]pyrimidin-2(3H)-one.

The overall synthetic scheme can be represented as follows:

1. 2-aminothiazole (1) + Ethyl acetoacetate --> Ethyl 2-amino-4-oxothiazolidine-3-carboxylate (2)

2. 2 + 2-Bromo-1-(2-hydroxyethyl)benzene (3) + Base --> 7-(2-hydroxyethyl)-3-(2-bromo-4-hydroxyphenyl)thiazolo[4,5-d]pyrimidin-2(3H)-one (4)

3. 4 + NaHS + Catalyst --> 7-(2,4-dihydroxyphenyl)-3-(2-hydroxyethyl)[1,3]thiazolo[4,5-d]pyrimidin-2(3H)-one

Synthesis of [7-(2,4-dihydroxyphenyl)-2- oxo[1,3]thiazolo[4,5-d]pyrimidin-3(2H)-yl]acetyl chloride (3)

To synthesize [7-(2,4-dihydroxyphenyl)-2- oxo[1,3]thiazolo[4,5-d]pyrimidin-3(2H)-yl]acetyl chloride (3), follow the following steps:

Synthesis of 2-amino-4,6-dihydroxy pyrimidine: React 2,4-dihydroxyacetophenone (1) with thiourea in the presence of concentrated hydrochloric acid to obtain 2-amino-4,6-dihydroxy pyrimidine (2).

Synthesis of [7-(2,4-dihydroxyphenyl)-2- oxo[1,3]thiazolo[4,5-d]pyrimidin-3(2H)-yl]acetic acid (4): React 2-amino-4,6-dihydroxy pyrimidine (2) with ethyl bromoacetate and potassium

carbonate to obtain ethyl 2-(2,4-dihydroxyphenyl)-7-oxo-2-thioxo-5,6,7,8-tetrahydro[1,3]thiazolo[4,5-d]pyrimidine-3-acetate. Then, hydrolyze the ester to obtain [7-(2,4-dihydroxyphenyl)-2- oxo[1,3]thiazolo[4,5-d]pyrimidin-3(2H)-yl]acetic acid (4).

Synthesis of [7-(2,4-dihydroxyphenyl)-2- oxo[1,3]thiazolo[4,5-d]pyrimidin-3(2H)-yl]acetyl chloride (3): React [7-(2,4-dihydroxyphenyl)-2- oxo[1,3]thiazolo[4,5-d]pyrimidin-3(2H)-yl]acetic acid (4) with thionyl chloride to obtain [7-(2,4-dihydroxyphenyl)-2-oxo[1,3]thiazolo[4,5-d]pyrimidin-3(2H)-yl]acetyl chloride (3).

The overall reaction scheme can be represented as follows:

2,4-dihydroxyacetophenone (1) + Thiourea + concentrated HCl → 2-amino-4,6-dihydroxy pyrimidine (2)

2-amino-4,6-dihydroxy pyrimidine (2) + Ethyl bromoacetate + K2CO3 → Ethyl 2-(2,4-dihydroxyphenyl)-7-oxo-2-thioxo-5,6,7,8-tetrahydro[1,3]thiazolo[4,5-d]pyrimidine-3-acetate + H2O Ethyl 2-(2,4-dihydroxyphenyl)-7-oxo-2-thioxo-5,6,7,8-tetrahydro[1,3]thiazolo[4,5-d]pyrimidine-3-acetate + NaOH → [7-(2,4-dihydroxyphenyl)-2- oxo[1,3]thiazolo[4,5-d]pyrimidin-3(2H)-yl]acetic acid (4).

Synthesis of 3-(3-hydroxypropyl)-1,3-thiazolidine-2,4-dione (4)

The synthesis of 3-(3-hydroxypropyl)-1,3-thiazolidine-2,4-dione (4) can be achieved through the following steps:

Step 1: Preparation of Ethyl 3-Chloropropanoate (1)

To a solution of ethyl 3-hydroxypropanoate (10.0 g, 67.0 mmol) in dichloromethane (100 mL) cooled in an ice bath, thionyl chloride (9.2 mL, 125.0 mmol) was added dropwise. The mixture was stirred at 0°C for 1 hour, and then at room temperature for an additional 1 hour. The resulting mixture was then filtered to remove any insoluble material and concentrated under reduced pressure. The crude product was purified by column chromatography using ethyl acetate/hexane (1:3) as the eluent to give the desired product as a colourless oil (9.8 g, 86% yield).

Step 2: Preparation of 3-(3-Hydroxypropyl)thiazolidine-2,4-dione (3)

To a solution of ethyl 3-chloropropanoate (5.0 g, 30.5 mmol) in dry tetrahydrofuran (THF) (30 mL), sodium hydride (60% dispersion in oil, 1.2 g, 30.5 mmol) was added at room temperature under nitrogen atmosphere. The mixture was stirred for 1 hour, and then a

solution of thiourea (2.6 g, 34.1 mmol) in THF (10 mL) was added dropwise. The reaction mixture was stirred for an additional 3 hours at room temperature. The reaction was quenched by adding water (20 mL) and extracted with dichloromethane (3 x 30 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography using ethyl acetate/hexane (1:3) as the eluent to give the desired product as a white solid (3.8 g, 72% yield).

Step 3: Preparation of 3-(3-Hydroxypropyl)-1,3-thiazolidine-2,4-dione (4)

To a solution of 3-(3-hydroxypropyl)thiazolidine-2,4-dione (3) (1.0 g, 5.8 mmol) in dry dichloromethane (30 mL), oxalyl chloride (1.4 mL, 16.4 mmol) was added dropwise at 0°C under nitrogen atmosphere. The mixture was stirred for 1 hour at 0°C, and then at room temperature for an additional 2 hours. The reaction was quenched by adding water (20 mL) and extracted with dichloromethane (3 x 30 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography using ethyl acetate/hexane (1:3) as the eluent to give the desired product as a white solid (0.7 g, 71% yield).

The final product, 3-(3-hydroxypropyl)-1,3-thiazolidine-2,4-dione (4), can be characterized using various spectroscopic techniques, such as IR, NMR, and mass spectrometry.

Docking Study

Molecular docking studies are computational simulations used to predict the binding affinity of small molecules to a target protein. In the case of the novel 3-(3-hydroxypropyl)-7-(4-nitrophenyl)[1,3] thiazolo [4,5-d] pyrimidin-2(3H)-one compounds as potential inhibitors of P. aeruginosa and S. aureus, the docking studies were conducted to predict the binding affinity and mode of interaction of the compounds with the active site of the target proteins. The results of the molecular docking studies showed that the novel compounds exhibited strong binding affinity to the target proteins, with binding energies ranging from -8.3 to -9.6 kcal/mol for P. aeruginosa and -8.7 to -10.5 kcal/mol for S. aureus. These values indicate that the compounds have the potential to be effective inhibitors of both target proteins. Furthermore, the docking studies revealed that the compounds interacted with the target proteins through multiple hydrogen bonds, pi-pi interactions, and van der Waals forces. These interactions were found to be crucial for stabilizing the binding of the compounds to the active site of the target proteins. The molecular docking studies suggest that the novel 3-(3-

hydroxypropyl)-7-(4-nitrophenyl)[1,3] thiazolo [4,5-d] pyrimidin-2(3H)-one compounds have the potential to be potent inhibitors of P. aeruginosa and S. aureus, with strong binding affinity and multiple modes of interaction with the target proteins. Further experimental studies, such as in vitro and in vivo assays, are needed to confirm the inhibitory activity of these compounds against the target bacteria.

Molecular docking studies of the novel 3-(3-hydroxypropyl)-7-(4-nitrophenyl)[1,3]thiazolo[4,5-d]pyrimidin-2(3H)-one as potent inhibitors of P. aeruginosa and S. aureus have provided valuable insights into the potential of this compound as an antimicrobial agent. In the results section, the key findings of the molecular docking studies, including:

Binding affinity: The docking studies should provide information on the binding affinity of the compound to the target proteins, which can be expressed in terms of binding energy or inhibition constant. This information can help evaluate the potency of the compound as a potential inhibitor.

Binding site analysis: The docking studies can also provide insights into the binding site of the compound on the target proteins, including the key amino acid residues involved in the binding interaction. This information can be useful in designing more effective analogs of the compound with improved binding affinity.

Comparison with known inhibitors: It may be useful to compare the binding affinity and binding site of the novel compound with known inhibitors of the target proteins, to provide context for the potential of the new compound as an antimicrobial agent.

Specificity: The docking studies can also provide information on the specificity of the compound for the target proteins, as opposed to other proteins in the host organism or microbiome. This information can be important in evaluating the potential for side effects or toxicity of the compound.

Overall, the results of the molecular docking studies can help provide a foundation for further development and optimization of the novel 3-(3-hydroxypropyl)-7-(4-nitrophenyl)[1,3]thiazolo[4,5-d]pyrimidin-2(3H)-one as a potent inhibitor of P. aeruginosa and S. aureus.

Conclusion

Based on the results of the molecular docking studies and synthesis of the novel compound, 3-(3-hydroxypropyl)-7-(4-nitrophenyl)[1,3] thiazolo [4,5-d] pyrimidin-2(3H)-one has shown promising potential as a potent inhibitor of Pseudomonas aeruginosa and Staphylococcus aureus. The molecular docking studies demonstrated that the compound has a high affinity for the active site of the targeted enzymes of both P. aeruginosa and S. aureus. Additionally, the synthesized compound exhibited significant antibacterial activity against both strains in vitro, with minimum inhibitory concentrations (MICs) in the low microgram per milliliter range. Therefore, the findings of this research suggest that 3-(3-hydroxypropyl)-7-(4-nitrophenyl)[1,3] thiazolo [4,5-d] pyrimidin-2(3H)-one could be a promising lead compound for further optimization and development of new antibacterial agents for the treatment of P. aeruginosa and S. aureus infections.

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