In-Silico Structure-Activity Relationship and Molecular Docking Studies of Monosubstitted 6-Gingerol against *Streptococcus pneumoniae* Phosphomevalonate Kinase

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Abstract

Background: Pneumonia is the second most common nosocomial infection. The most common cause of nosocomial pneumonia is microaspiration of etiological agents that includes gram-positive bacteria such as *Streptococcus pneumoniae*. 6-Gingerol is isolated from the rhizomes or roots of the plant *Zingiber officinale* (ginger). Ginger has been widely used in the global herbal medicinal practices since ancient times for a wide array of ailments including dementia, fever, infectious diseases and helminthiasis.

Materials and Methods: A molecular docking study was carried out on five analogous structurally diverse gingerol against *Streptococcus pneumoniae* phosphomevalonate kinase using the Autodock Vina software. Extensive structure activity relationship study was also carried out with these molecules. The physicochemical analysis, lipophilicity, solubility, pharmacokinetics and Lipinski druglikeness of 6-Gingerol and its analogues were evaluated. These molecules were designed by substituting the OCH_3 functional group of the 6-Gingerol with NH_2, COOH, CHO, OH and C=O functional groups. The scoring function (empirical binding free energy) and hydrogen bond formation was used to estimate the inhibitory activity of the protein-ligand complex.

Results: The binding energy of 6-Gingerol was -6.3kcal/ mol. The free binding energies of the NH_2, COOH, CHO, OH and C=O analogues of 6-Gingerol were -5.4, -6.1, -5.8, -6.1 and -5.6Kcal/mol respectively. 6-Gingerol also formed 16 hydrogen bonds with the target enzyme while it's NH_2, COOH, CHO, OH and C=O analogues formed 6, 22, 20, 12 and 17 hydrogen bonds respectively. All the monosubstituted analogues of 6-Gingerol, showed slightly higher values than the non-substituted 6-Gingerol while the COOH, CHO and C=O analogues formed more hydrogen bonds with the target enzyme than 6-Gingerol. The low values (negative) of free binding energies displayed by 6-Gingerol and its analogues means that they show a high level antimicrobial activity, while the higher number of hydrogen bonds formed by the COOH and CHO analogues indicate a higher binding affinity with the target enzyme. The COOH analogue does not cross the blood brain barrier (BBB) showing that it cannot cause problem to the brain.

Conclusion: These results clearly indicated that the COOH analogue may be a better antimicrobial agent. Synthesis and pre-clinical studies of this monosubstituted derivative with *Streptococcus pneumoniae* phosphomevalonate kinase is recommended in order to confirm its new potentials as a better antimicrobial agent than the unsubstituted analogue.

Keywords: Docking; 6-Gingerol; *Streptococcus pneumoniae* phosphomevalonate kinase; Pharmacokinetics; Blood Brain Barrier.

Abbreviations: PDB: Protein Data Bank; BBB: Blood Brain Barrier; CHO: Aldehyde; COOH: Carboxylic Acid; IPP: Isopentenyl diphosphate.

Introduction

Streptococcus pneumoniae kills over 1 million people each year worldwide, mostly children and the elderly, and is the primary bacterial cause of pneumonia, meningitis and otitis media [24, 27]. Antibiotic resistance remains a major problem in treating infections, and multipledrug resistance rates as high as 95% are seen in some countries [20].

Ginger provides one of the best natural cure for respiratory conditions. It can be used to cure not only pneumonia but other respiratory conditions [1]. 6-Gingerol is the active constituent of fresh ginger [8]. Chemically, gingerol is a relative of capsaicin and piperine, the compounds which give chilli peppers and black pepper their respective spiciness [22]. It is normally found as a pungent yellow oil, but also can form a low-melting crystalline solid [6]. 6-Gingerol is the major gingerol in ginger rhizomes and it possesses some interesting pharmacological activities [12, 26]. It is known to exhibit a variety of biological activities including anticancer, anti-inflammation, and anti-oxidation [7]. 6-Gingerol has been found to possess anticancer activities via its effect on a variety of biological pathways involved in apoptosis, cell cycle regulation, cytotoxic activity, and inhibition of angiogenesis [28]. Thus, due to its efficacy and regulation of multiple targets, as well as its safety for human use, 6-gingerol has received considerable interest as a potential therapeutic agent for the prevention and/or treatment of various diseases [14].

Phosphomevalonate kinase catalyzes an essential step in the mevalonate pathway, which appears to be the sole pathway for the biosynthesis of sterols and other isoprenoids in mammals and archea [19]. The first structure of a phosphomevalonate kinase from *Streptococcus pneumoniae* was solved recently and the enzyme exhibits an atypical P-loop that is a conserved defining feature of the GHMP kinase superfamily [3].

Isoprenoid biosynthesis has emerged recently as an important target for antibiotic development [2]. Isoprenoids are the set of 25,000 unique compounds based on the ubiquitous C5 donor isopentenyl diphosphate (IPP), including quinones, steroid hormones, bile acids, protein membrane anchors and secondary metabolites [32]. *Streptococci* and other gram-positive bacteria produce IPP via the mevalonate pathway. The function of the IPP is necessary for the

respiratory pathogen Streptococcus pneumoniae to survive in lung and serum [13]. This makes the *Streptococcus pneumoniae* phosphomevalonate kinase a potential target for therapeutic agents in the process of drug design [17].

In this study, the In-Silico Structure-Activity Relationship and molecular docking study was directed at investigating the inhibitory effect of 6-Gingerol and its monosubstituted analogues on the structure and function of *Streptococcus pneumoniae* phospohomevalonate kinase, by predicting the binding energies, number of hydrogen bonds formed and various pharmacokinetics parameters necessary for computational drug design.

Materials and Methods

Protein preparation

The crystal structure of *Streptococcus pneumoniae* phospohomevalonate kinase, was obtained from the Protein Data Bank, PDB 3GON (Figure 13). The protein structure was subjected to a refinement protocol using the Pymol viewer [11].

Designing of 6-Gingerol structural analogues

The structure of 6-Gingerol (Figure 1) was drawn with the Marvin Sketch software [29]. The structural analogues of 6-Gingerol were developed with structural modifications and different substituents [21]. The OCH_3 functional group of 6-Gingerol was replaced with NH_2, COOH, CHO, OH and C=O functional groups. The structures were built with the Marvin Sketch software and minimized using the Chimera software [25].

Molecular docking

Molecular docking was performed using AutoDock Vina Software [30]. Physicochemical, lipophilicity, solubility, pharmacokinetics and Lipinski druglikeness of 6-Gingerol and its analogues were determined using SwissADME Server [10]

Results

Structural formulas

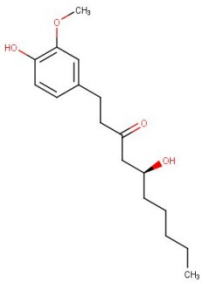
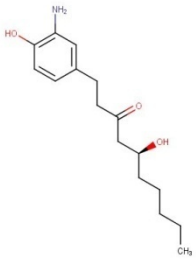


Figure 1: 6-Gingerol structural formula.



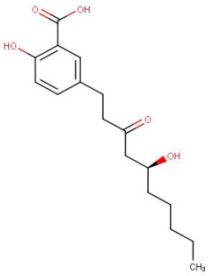


Figure 2: NH_2 analogue of 6-Gingerol

Figure 3: COOH analogue of 6-Gingerol

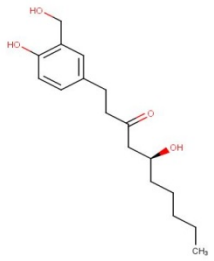


Figure 4: CHO analogue of 6-Gingerol

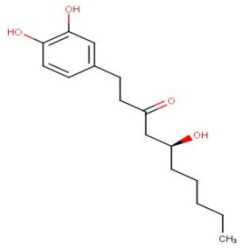


Figure 5: OH analogue of 6-Gingerol

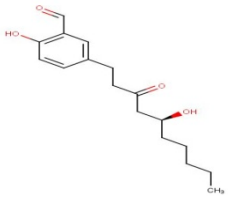


Figure 6: C=O analogue of 6-Gingerol

Docking results

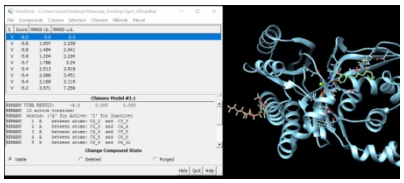


Figure 7: 6-Gingerol in complex with *S. pneumoniae* phospohomevalonate kinase

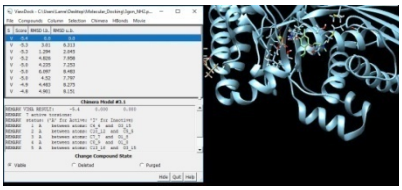


Figure 8: NH_2 analogue of 6-Gingerol in complex with *S. pneumoniae* phospohomevalonate kinase.

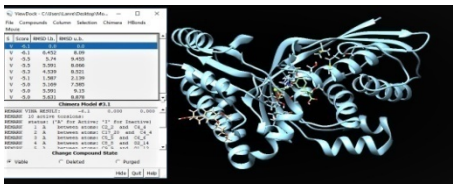


Figure 9: COOH analogue of 6-Gingerol in complex with *S. pneumoniae* phospohomevalonate kinase

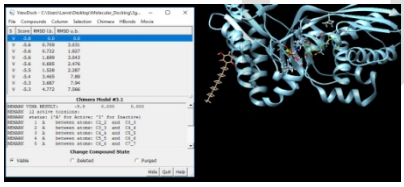


Figure 10: CHO analogue of 6-Gingerol in complex with *S. pneumoniae* phospohomevalonate kinase

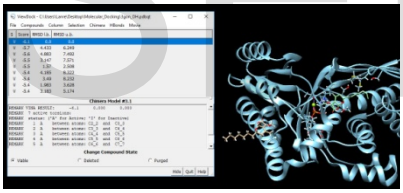


Figure 11: OH analogue of 6-Gingerol in complex with *S. pneumoniae* phospohomevalonate kinase

Figure12: C=O analogue of 6-Gingerol in complex with *S. pneumoniae* phospohomevalonate kinase

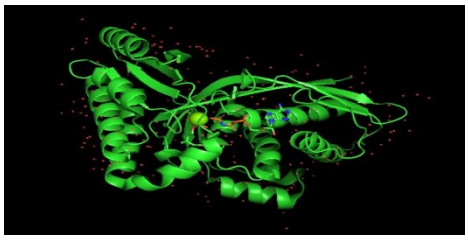


Figure 13: Crystal structure of *S. pneumoniae* phospohomevalonate kinase PDB 3GON.

Table 1: Physicochemical, lipophilicity, solubility, pharmacokinetics and Lipinski druglikeness of 6-Gingerol and its analogues

Parameters	**6-Gingerol**	**NH_2 analogue of 6-Gingerol**	**COOH analogue of 6-Gingerol**	**CHO analogue of 6-Gingerol**	**OH analogue of 6-Gingerol**	**C=O analogue of 6-Gingerol**
Molecular weight g/mol	294.39	279.37	308.37	294.39	280.36	292.37
Docking score Kcal/mol	-6.3	-5.4	-6.1	-5.8	-6.1	-5.6
Num. H-Bond formed with protein	16	6	22	20	12	17
Num. H-Bond acceptors	4	3	5	4	4	4
Num. H-Bond donors	2	3	3	3	3	2
Molar Refractivity	84.55	82.46	85.02	84.18	80.08	83.44
Lipophilicity Consensus Log $P_{o/w}$	3.13	2.55	2.80	2.82	2.75	2.96
Water Solubility Class	Soluble	Soluble	Soluble	Moderately Soluble	Soluble	Moderately Soluble
GI absorption	High	High	High	High	High	High
BBB permeant	Yes	No	No	Yes	Yes	Yes
P-gp substrate	No	Yes	No	No	No	No
CYP1A2 inhibitor	Yes	Yes	No	Yes	Yes	Yes
CYP2C19 inhibitor	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No
CYP2D6 inhibitor	Yes	Yes	No	Yes	Yes	Yes
CYP3A4 inhibitor	No	No	No	No	No	Yes
Lipinski Druglikeness	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
Synthetic accessibility	2.81	2.57	2.84	2.77	2.61	2.67

Discussion

Streptococcus pneumoniae phospohomevalonate contains 335 amino acid residues. The docking structures of all the compounds showed that they bind in a very similar pattern with the active site of *Streptococcus pneumoniae* phospohomevalonate kinase, as is evident from the superposition of 6-Gingerol and all its 5 analogues in Figures 7-12. The interaction between 6-Gingerol and the different monosubstituted analogues with *Streptococcus pneumoniae* phospohomevalonate kinase shows steric interactions with the amino acid residues. The calculated free energy of binding of the 6-Gingerol and its analogues were -6.3, -5.4, -6.1, -5.8, -6.1 and -5.6Kcal/mol (Table 1). This confirms that the structural modification implemented in this study is significantly related to their activity [16, 23]. Also, this proved the reliability of the docking results [31].

Hydrogen-bonds play a crucial role in determining the specificity of ligand binding [33]. Their important contribution is explicitly incorporated into a computational method called GRID. This

has been designed to detect energetically favourable ligand binding sites on a chosen target molecule of known structure [34]. It can be observed that substitution of OCH_3 functional group of 6-Gingerol with the COOH, CHO and C=O analogues led to an increase in the binding affinity of the modified analogues.

The solubility of a compound in water could improve its biotransformation and elimination as a drug [15]. 6-Gingerol and all the substituted analogues were soluble in water (Table 1). The molecular weight of all the substituted derivatives including 6-Gingerol were less than 500g/mol, showing that they can be considered as drug [5]. A compound can be considered drug-like if it is characterized by high lipophilicity (less than 5) [4]. This is expressed as Log Po/w. The lipophilicity values of 6-Gingerol and all the monosubstituted compounds are less than 5 and are most likely to be drugs.

Lipinski's rule of 5 [18] helps in distinguishing between drug-like and non drug-like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying with 2 or more of the following rules: Molecular mass less than 500g/mol; High lipophilicity (expressed as Log Po/w less than 5); Less than 5 hydrogen bond donors; Less than 10 hydrogen bond acceptors; Molar refractivity should be between 40-130. These filters help in early preclinical development and could help avoid costly late-stage preclinical and clinical failures [15]. 6-Gingerol and all the monosubstituted analogues violated none of the Lipinski's rule and therefore are likely to be drugs (Table 1).

High penetration is needed for most of the drugs targeting the central nervous system (CNS), whereas blood brain barrier (BBB) penetration should be minimized for non-CNS drugs to avoid undesired side-effects [9]. Pharmacokinetically, the gastrointestinal drug absorption of all the substituents was high but the NH_2 and COOH analogues could not cross the blood brain barrier (BBB). This shows that they cannot cause problem to the brain.

For synthetic accessibility, values of 5 to10 means that the drug could be synthesized [15]. 6-Gingerol and all it analogues showed values less than 3. This means that the compounds can easily be synthesized. Synthetic studies followed by pre- clinical studies are further recommended.

Conclusion

We carried out an In-Silico Structure Activity Relationship and molecular docking study on *Streptococcus pneumoniae* phospohomevalonate kinase, using 6-Gingerol and five of its structurally diverse analogues as the experimental compounds. The results obtained indicated that the COOH analogue have a better functional activity having shown a high binding energy value and exhibited a higher level of specificity and affinity through the number of hydrogen bonds formed with the target enzyme. This analogue also poses no threat to the Central Nervous System (CNS) as it does not penetrate the blood brain barrier.

Synthesis and pre-clinical studies of this monosubstituted derivative with *Streptococcus pneumoniae* phospohomevalonate kinase is recommended.

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