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Computer assisted designing (CADD), *insilico* pharmacological studies followed by synthesis and characterization of *garcinosporin* a novel semisynthetic β-lactam antibiotic

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ABSTRACT

The drug discovery and designing team is always being keen to find out newer and newer remedies for the industry but they have to cross many hurdles as the development of new drugs with potential therapeutic applications is one of the most complex and difficult process in the pharmaceutical industry. The emergence of superbugs and antibiotic resistance due to the incorrect usage and dosage of antibiotics is one of the serious threats in front of the team for many reasons. One of the solutions that can be adopted to overcome this hurdle is the designing of the new drug based on the natural product of proven therapeutic value and other one is the suitable derivatization of the existing drugs. The present research also looking to the world of natural products to make a new antibiotic based on both traditional and modern knowledge. The starting materials selected were Garcinia acid (Hydroxy citric acid) an active ingredient of many Ayurvedic drugs and 7-ACA the core part of Cephalosporins. The selected lead was modified and designed with simple application of computer assisted drug designing (CADD) and insilico pharmacological and toxicological studies to understand the druggability of the lead selected. This was followed by actual synthesis and characterization using spectroscopic methods. The resulted novel antibiotic is named as Garcinosporin belong to the class of semisynthetic β-lactam cephalosporin type.

Key words: *Garcinosporin*, Garcinia acid anhydride, 7-Aminocephalosporinic acid (7-ACA), Docking, ArgusLab, Toxicity estimation, antibacterial activity, Computer Assisted Drug Designing (CADD).

INTRODUCTION

The process of knowledge widening is important to any interdisciplinary fields like Drug Designing and Discovery and the knowledge irrespective of its origin is to be accepted and acknowledged without any presumptions for achieving the goals. The interdisciplinary field of medicinal chemistry is now widening the treasure of knowledge from all sources including traditional alternative medicines in addition to the modern medicinal chemistry. The drug discovery and designing team is always being keen to find out newer and newer remedies for the industry but they have to cross many hurdles as the development of new drugs with potential therapeutic applications is one of the most complex and difficult process in the pharmaceutical industry [1]. The emergence of superbugs and antibiotic resistance due to the incorrect usage and dosage of antibiotics is one of the serious threats in front of the team for many reasons [2-3]. One of the solutions that can be adopted to overcome this hurdle is the designing of the new drug based on the natural product of proven therapeutic value and other one is the suitable derivatization of the existing drugs. The present research also looking to the world of natural products to make a new antibiotic based on both traditional and modern knowledge. The introduction of computers and well developed softwares made the process more precise and accurate one. The use of computers and apt softwares reduced the time for designing and developing the drugs when used by the expert scientists with good imagination and creativity. The designing of drugs are controlled by many rules and found relevant for many active drugs to considerable extent but with many exemptions. It is worthwhile to think on the idea that “All molecules are not drugs but all drugs are molecules” and

because of this scientists are behind the problem to correlate physical properties to the medicinal activity and became the central objective of chemistry. The field of medicinal and pharmaceutical chemistry research we have to give much importance for the usefulness of the molecule to the humankind by considering the saying that the product of science is more important than the science behind it. Antibacterial herbs and formulations were known to humans from ancient times to treat wounds, carbuncles, boils and other infections successful to certain extent keeping the science behind the same as secret even now. The modern researchers developed herbal products like *Septilin* and proved to be a valuable adjuvant that enhances the activity of antibiotics clinically when co-prescribed with antibiotics [4]. The present study concentrates more on the semisynthetic antibiotics of antibacterial nature because the methods are comparatively simpler than other types and achievable in laboratory with limited facilities. Drugs come under the category antibiotics can be subdivided in to β-lactams, Tetracyclines, Aminoglycosides, Macrolides, Polypeptides, Polyenes, Phenylpropane diol derivatives, Sulfonamides, Quinalone carboxylic acids etc. This present study is focusing on a newly designed synthesized drug termed *Garcinosporin* which comes under the group of β-lactams with cephalosporin moiety. The designing of the drug and insilico pharmacological and toxicological studies were performed before to make sure the druggability. The actual synthesis based on common methods with characterization followed by the antibacterial screening was performed for the drug candidate *Garcinosporin* which is a chemical hybrid of natural products of proven therapeutic values.

MATERIALS AND METHODS

The starting materials selected for the proposed drug are 7-aminocephalosporinic acid (7-ACA) which is the product of chemical hydrolysis of the β-lactam part of cephalosporins and Garcinia acid (GA) the active ingredient or active principle of the fruits of *Garcinia cambogia* traditionally in Sanskrit known as *Vrukshamla* which is one of the important and necessary part of many *Ayurvedic* drugs used by traditional practitioners in India for many centuries. The traditional knowledge of medicine with the modern synthetic strategies can achieve better results in the field of medicinal chemistry. Before the synthesis the designing of the proposed drug was achieved by applying the basics of CADD. The stereochemical and docking studies were performed to understand the potency and drug likeness of the proposed drug candidate using ArgusLab free software [5]. The insilico toxicological studies were performed to study the druggability of the proposed drug candidate with well accepted technologies introduced by American Environmental Protection Agency. After these the actual wet lab (laboratory) synthesis and antibacterial screening against a set of clinically isolated pathogens were performed using many modern methods.

Experimental

This portion can be divided into five portions viz. (1) Computer Assisted Drug Designing (CADD), (2) Insilico Pharmacological Studies and Docking, (3) Insilico Toxicological Studies, (4) Laboratory Synthesis and Characterization and finally (5) Systematic Antibacterial Screening.

(1) Computer Assisted Drug Designing (CADD): The proposed drug was designed by considering the concepts of CADD which are well known to the scientists of the drug discovery and development team [6-7]. The main aim was to utilize the method with minimum time with target specific drug designing. Usually the time required for designing the drug is much long and this has to be reduced by using the traditionally proven drug ingredients as starting material. The starting materials selected were of both pharmacological important and are natural products with sited medicinal values either in traditional or in modern systems of medicine. The GA is the active principle of the *Garcinia cambojia* and can be extracted easily with many methods from the dried fruits of the tree that finds importance in many traditional *Ayurvedic* drugs which also have antibacterial in addition to antiobesity activities. The aim was set to coin these two natural products to achieve a novel antibacterial. The drug is designed well using both the principles of the Computer Aided Drug Designing and Discovery (CADD) based on both the modern and traditional knowledge. The proposed drug is termed artistically as *Garcinosporin* by coining the names of the starting material GA and the belonging class cephalosporins for easy recalling and mentioning.

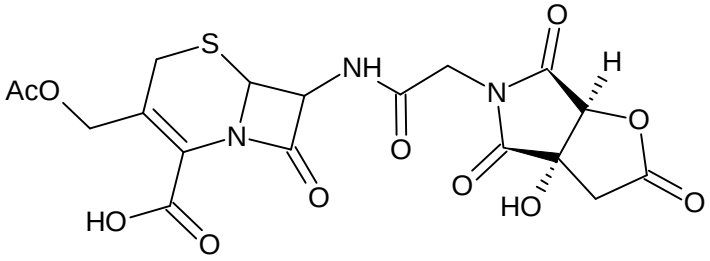
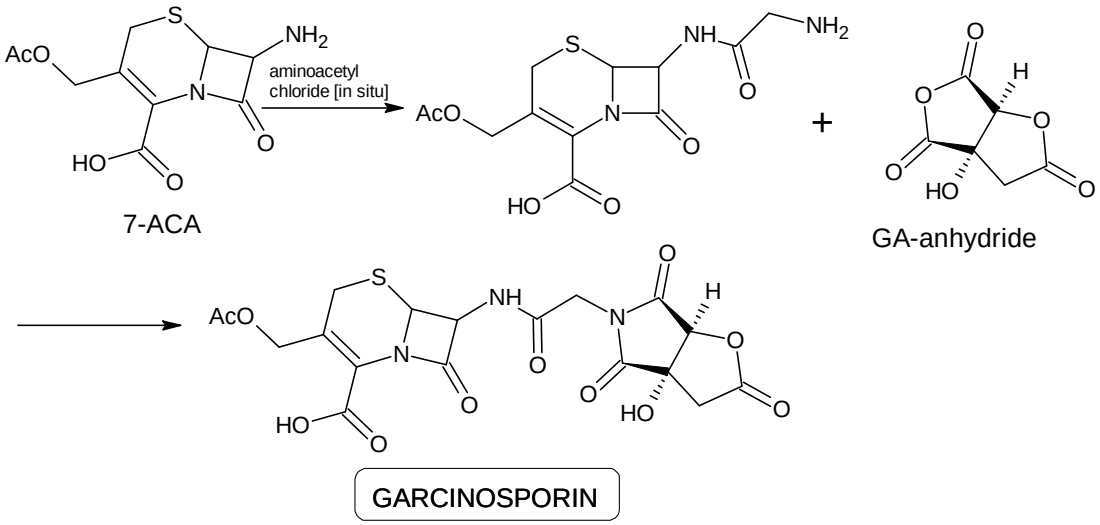


Figure 1: The structure of proposed drug *Garcinosporin*.

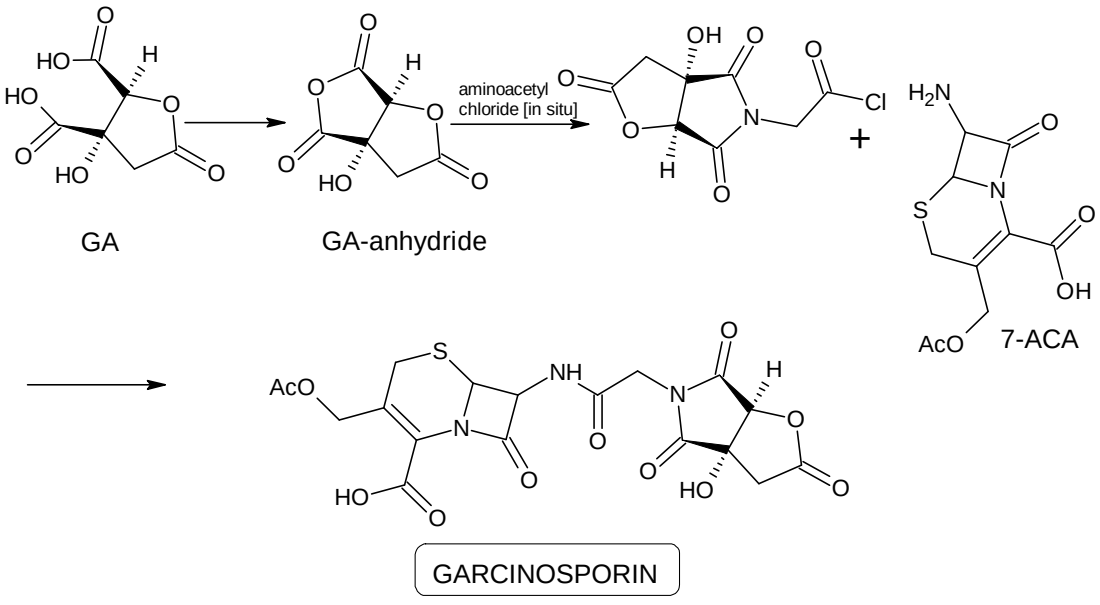
The structure proposed for the drug candidate *Garcinosporin* is as shown in Figure 1.

SCHEME: The two synthetic routes through which the Garcinosporin is synthesized.

ROUTE- I



ROUTE- II



The synthesized drug is chemically 3-[(acetyloxy)methyl]-7-({[(3a*S*,6a*S*)-3a-hydroxy-2,4,6-trioxohexahydro-5*H*-furo[2,3-*c*]pyrrol-5-yl]acetyl}amino)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid and named *Garcinosporin* for easy usage in the common parlance. The drug is separated and purified with common methods of separation and purification as commonly used for penicillin and cephalosporin type antibiotics. The characterization was carried out by using both laboratory methods as well as spectroscopic methods.

(2) Insilico Pharmacological Studies and Docking: The insilico pharmacological studies and docking were performed by using ArgusLab. The drug which being the β–lactam type the docking was performed over the bacterial protein which is betalactamase in nature. The betalactamase structure file was downloaded from protein data bank and the docking of the drug over it after achieving the minimum energy structure of the drug believing the stablest one [8].

(3) Insilico Toxicological Studies: The insilico toxicological studies were performed after the docking studies gave hope in the proposed drug *Garcinosporin*. The studies were performed using many methods accepted and developed by the American Environmental Protection Agency's guidelines [9].

(4) Laboratory Synthesis and Characterization: After completing the Docking and Toxicological studies the laboratory synthesis was started. The different methods for preparing the proposed drug were planned and executed in the laboratory. The 7-ACA was prepared by the chemical hydrolysis of Cephalosporin-C. This is also available from the Sigma Aldrich Company in a pure form which can be used without any purification. The Garcinia acid derivative was prepared with common methods of isolation and derivatization. The suitable derivative of 7-ACA was prepared and coupled with GA-anhydride. Both the required derivative of 7-ACA and the prepared GA-anhydride when coupled in equimolar portions produced *Garcinosporin* (Route-I). When both the required derivative of GA and 7-ACA coupled in equimolar proportions the proposed β-lactam antibiotic drug *Garcinosporin* was obtained (Route-II).

(5) Systematic Antibacterial Screening: The drug *Garcinosporin* was screened to many clinically isolated pathogens to understand the actual antibacterial activities of the proposed drug using amoxicillin as the control. The bacteria include include *Escherichia coli*, *Klebsiella sp*, *Pseudomonas sp*, *Staphylococcus aureus*, *Salmonella sp* etc. The cephalethin (physician's sample) was also co-screened to understand the activity in a better way. Many methods of screening were tried to understand the activity [10-11].

RESULTS AND DISCUSSION

The results of this present study are presented below in respective heads.

CADD and Docking Studies

The drug was designed as explained by considering the knowledge of both modern and traditional origin. The *Garcinosporin* showed a calculated Log P value (-1.23+/- 0.67) which comes between common antibacterial oral drugs. The formula weight of the anhydrous drug *Garcinosporin* is 483.40 units. The hydrophobic and hydrophilic values were also found obeying the governing rules of druggability. The docking studies of the proposed drug *Garcinosporin* were performed using the software called ArgusLab over the hypothetical binding site in the bacterial protein structure available from protein data bank. The bacterial protein of the type betalactamase was selected for docking believing the most active part would be the beta-lactam part of the proposed drug. The docking with the betalactamase class bacterial protein gave hopeful results with best pose energy value (-5.52 kcal/mol) found comparable with that of widely prescribing β-lactam antibiotic. The proposed drug candidate *Garcinosporin* docked on 1LL9.pdb is shown in Figure 2.

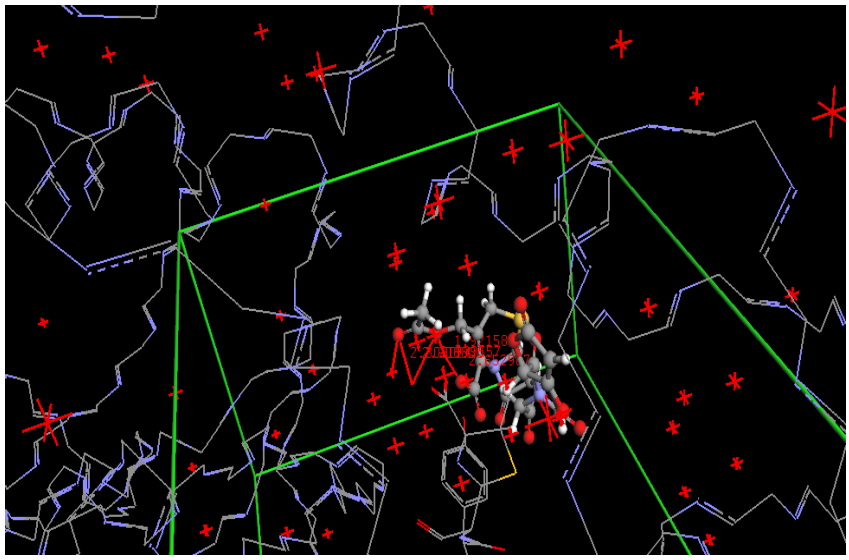


Figure 2: The *Garcinosporin* docked to β-lactamase 1LL9.pdb

The drug of the type cephalosporins were found inferior to many penicillins but showed proven enhanced activity to certain pathogens were penicillins were found inactive.

Toxicological Studies

The toxicology studies of every drug are much important and performed for the proposed drug *Garcinosporin* also. As expected the designed drug *Garcinosporin* was found to be developmental non-toxicant with negative mutangenicity. The bioaccumulation factor log value (1.46) was found comparable with presently prescribing antibacterials. The LC_{50} value for *Daphnia magna* was found to be 2.40 mg per litre and was comparable to calculated value for amoxicillin and ampicillin. The insilico estimated value of LD_{50} values for mice is found to be 6582.39mg per Kg and is most comparable with reported values of β-lactams. All these estimated values are par with β-lactams drugs and supported the druggability of the designed drug *Garcinosporin*.

Characterization

The product *Garcinosporin* prepared was found to be light yellow amorphous hygroscopic compound with characteristic type of odour. The melting point was found using traditional method and was found to be charring one 200-205^{0}C range (uncorrected) with characteristic odour. The CHN studies showed the hygroscopic nature of the drug. The synthesized drug candidate was characterized with, UV, IR and NMR studies. The UV spectrum was found to be a characteristic of β-lactam antibiotic of cephalosporin type. The IR spectrum showed the prominent peaks and those were assigned to the majour plausible functional groups of *Garcinosporin*. The IR showed the characteristics of β-lactam antibiotic of cephalosporinic type and derivative of the GA with necessary and expected shifts in peak positions were in support to the formation of the drug. The peaks for COOH, OH and NH could be assigned to the broad band centred 2880cm^{-1} and 1798cm^{-1} (prominent and sharp peak) characteristic CO of β-lactams with cephalosporin moiety. The H-NMR spectra were recorded in three different solvents starting with DMSO-D_6, $CDCl_3$ and in D_2O to understand the progress of the reaction and for comparing with that of the starting materials. The characteristic peak observed nearly 10.8ppm singlet was found D_2O exchangeable and can be attributed to the H atom of attached COOH of cephalosporin ring. The doublets observed at 5.45 and 5.20 could be of the adjacent H-atoms of the four member ring of the β-lactam. The C-NMR also showed the characteristic peaks that can be attributed to the proposed structure. The CNMR studies showed the characteristic peaks for β-lactam of cephalosporins (~120ppm and ~130ppm for ring carbons with double bonds) and the portion of derivative of GA (173ppm, 178ppm, 172ppm for CO and ~92ppm for the central C atom that connected to O and CO) supported the characteristic nature of the synthesized drug. The complex nature of H-NMR to be solved using apt relaxation agents and is the subject matter of further research.

Antibacterial Screening

The antibacterial screenings were performed for a range of pathogenic bacteria for the drug candidate *Garcinosporin* with amoxicillin as control. The drug showed comparatively less activity than the control (amoxicillin physician's sample) but with comparable results to cephalethin (physician's sample). Most of the cephalosporins are less active the penicillins but with specific enhancement to some species over the other.

CONCLUSION

The studies based on CADD for the proposed drug *Garcinosporin* were found supportive. The insilico pharmacological and Docking studies were found similar to that of many semisynthetic β-lactam antibiotics. The insilico Toxicological studies showed the results that are found supportive to the druggability of the proposed drug *Garcinosporin*. These gave hope to synthesise the drug in the laboratory and achieved the same. The wet lab studies for the antibacterial investigation supported the CADD and Docking results. Further studies on the derivatives and metal salts of the *Garcinosporin* are in progress and found positive in preliminary studies. The stability studies of the *Garcinosporin* are to be carried out along with animal and clinical studies which are beyond the scope of this paper.

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