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Der Pharma Chemica, 2012, 4(5):1991-1996
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Computer assisted designing (CADD), insilico pharmacological studies followed by actual synthesis and characterization of *Garcicillin* a novel semisynthetic antibiotic

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ABSTRACT

The present world of medicinal chemistry is in a swift boom for the immediate development. Every medicinal chemist is trying to synthesize a new antibiotic and which is not easy as we all know. The most fearful treat facing by the drug discovery and developing team is the increased antibiotic resistance shown by the pathogens of microbial world even to the new generation antibiotics. The immediate remedy is the herbal supplements as the starting materials as per the experts. The present research is again looking to the world of natural products to make a new antibiotic. In this present paper the simple application of computer assisted drug designing (CADD) and insilico pharmacological and toxicological studies followed by actual synthesis and characterization using spectroscopic methods of a novel antibiotic which is named as Garcicillin belong to the class of semisynthetic β-lactam penicillin type. The proposed antibiotic Garcicillin finds its starting materials from the natural products of medicinal importance.

Key words: *Garcicillin*, Garcinia acid anhydride, amino penicillanic acid (6-APA), Docking, ArgusLab, Insilico Toxicity estimation, antibacterial activity, β-lactam antibiotics, Computer Assisted Drug Designing (CADD)

INTRODUCTION

The field of medicinal chemistry and drug designing is in a state of swift development and is at present becoming more and more interdisciplinary in nature with the introduction of well developed softwares and super computers. The art of drug discovery and development needs the knowledge and skill of both the scientist and an artist. The scientific knowledge of a scientist and imagination and creativity of an artist are the most important qualities for a scientist working in the field of drug discovery. 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[1-2]. Drugs are normally low molecular weight chemicals that interact with macromolecular targets in the body to produce a beneficial or harmful pharmacological effect and well controlled by certain limiting laws[3]. It is worthwhile to think on the idea that "All molecules are not drugs but all drugs are molecules" because of this scientists are behind the problem to correlate physical properties to the medicinal activity. Thus the central objective of chemistry became how to explain the relationship between chemical structure and molecular properties that will directly give the idea of the usefulness of the molecule to humankind. Hence especially in the field of medicinal and pharmaceutical chemistry research we have to give much importance for the same so that the molecule can lead to drug likeness[4]. Antibacterial herbs and formulations were known to humans from ancient times to treat wounds, carbuncles, boils and other infections. 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. The emergence of pathogens with more antibiotic resistance is one of the majour challenges in front of the medicinal scientists all over the world and we know it is not easy to come with every time a new and specific drug for each disease[5-8]. This present study is focusing on a newly synthesized drug

termed *Garcicillin* which is a broad spectrum antibiotic comes under the group of β-lactams. The designing of the drug and insilico pharmacological and toxicological studies were performed before the actual synthesis and characterization followed by the antibacterial screening.

MATERIALS AND METHODS

The starting materials selected for the proposed drug are 6-aminopenicillanic acid (6-APA) the β-lactam part of penicillin and Garcinia acid (GA) the active ingredient of the fruits of *Garcinia cambogia* traditionally in Sanskrit known as *Vrukshamla* is one of the important and necessary part of many *Ayurvedic* drugs used by traditional practitioners in India for many centuries. 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 undeerstand the potency and drug likeness of the proposed drug candidate using ArgusLab. The insilico toxicological studies were performed to understand the druggability of the proposed drug candidate with well accepted technologies introduced by American Environment Protection Agency. After these the actual wet lab (laboratory) synthesis and antibacterial screening against a set of pathogens were performed using many methods. The main raw materials used were commercially available 6-amino penicillanic acid (6-APA), Garcinia acid (GA) isolated from *Garcinia cambogia* fruits and common organic solvents and reagents.

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 (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. 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 class of antibacterials was selected for easiness to achieve in the wet lab studies with limited facilities. 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 studies on APA already proved that it as the main portion of the structure of the drugs like penicillin, semisynthetic penicillins like ampicillin and amoxicillin, cephalosorins etc. which are proven antibaterials. The GA is the active principle of the Garcinia and can be extracted easily 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 GA can be extracted from the fruits in many methods. The aim was set to coin these two natural products to achieve the penicillin like novel antibacterial. The drug is designed well using both the principles of Drug Discovery & Development based on both the modern and traditional knowledge. The proposed drug is termed artistically as *Garcicillin* by joining the names of the starting material GA and the belonging class penicillin for easy recalling and mentioning.

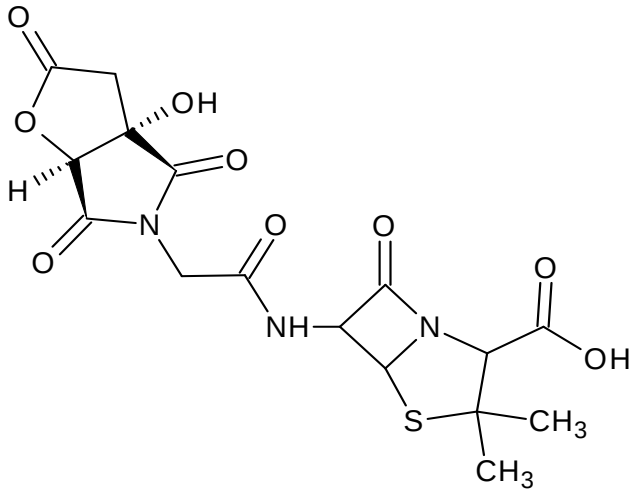


Figure 1: The structure of proposed drug *Garcicillin*.

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

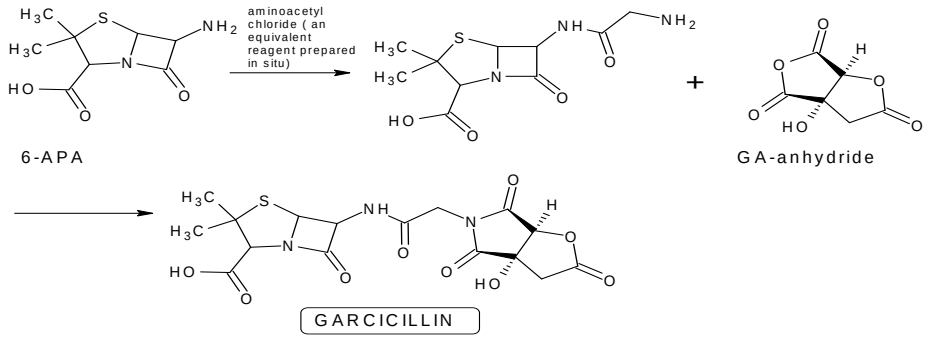
2.Insilico Pharmacological Studies and Docking: The insilico pharmacological studies and docking were performed by using ArgusLab. The docking is the best method to predict the activity of the drug candidate and are many approaches and methods available[9-10]. The drug which being the penicillin type the docking was performed over the bacterial protein which is betalactamase in nature[11-13]. 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.

3.Insilico Toxicological Studies: The insilico toxicological studies were performed after the docking studies gave hope in the proposed drug *Garcicillin*. The studies were performed using many methods accepted and developed by the American Environment Protection Agency's guidelines.

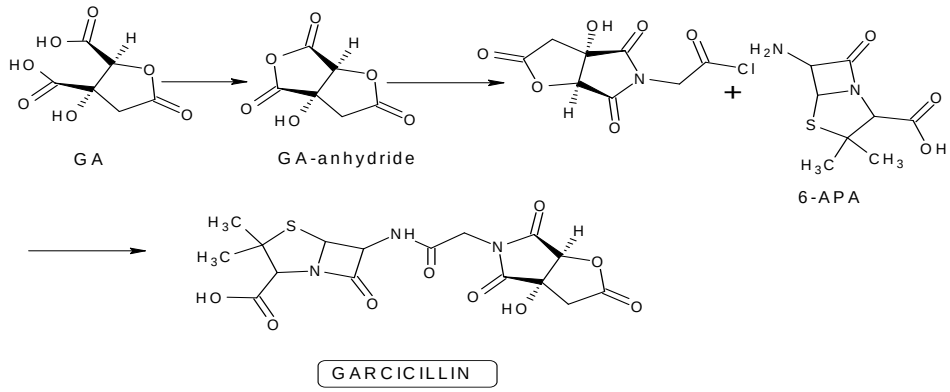
4.Laboratory Synthesis and Characterization: After completing the Docking and Toxicological studies the laboratory synthesis was started. The different methods for preparing the proposed drug were planned and executed in the laboratory. The main chemicals selected were commercially available 6-APA and Garcinia acid (GA) isolated from *Garcinia cambogia* fruits[14-15]. The Garcinia acid derivative was prepared with common methods of isolation and derivatization. The suitable derivative of 6-APA was prepared and coupled with GA-anhydride. Both the required derivative of 6-APA and the prepared GA-anhydride when coupled in equimolar portions produced *Garcicillin* (Route-I). When both the required derivative of GA and 6-APA coupled in equimolar proportions the proposed β-lactam antibiotic drug *Garcicillin* was obtained (Route-II). The scheme of syntheses with both the routes (Route-I and Route-II) is as shown below.

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

ROUTE- I



ROUTE- II



The synthesized drug is chemically 6-({[(3a*S*,6a*S*)-3a-hydroxy-2,4,6-trioxohexahydro-5*H*-furo[2,3-*c*]pyrrol-5-yl]acetyl}amino)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid and named *Garcicillin* for easy usage. The drug is separated and purified with common methods of separation and purification as commonly used for penicillin type antibiotics. The characterization was carried out by using both laboratory methods as well as spectroscopic (UV, FT-IR and NMR) methods.

5.Systematic Antibacterial Screening: The drug *Garcicillin* was screened to many pathogens to understand the actual antibacterial activities of the proposed drug *Garcicillin* using semisynthetic penicillin drug amoxicillin physician's sample as the control[16].

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 *Garcicillin* showed a calculated Log P value (-1.11+/- 0.62) which comes between common antibacterial oral drugs. The formula weight of the anhydrous drug *Garcicillin* is 427.38 units. The hydrophobic and hydrophilic values are also of acceptance range.

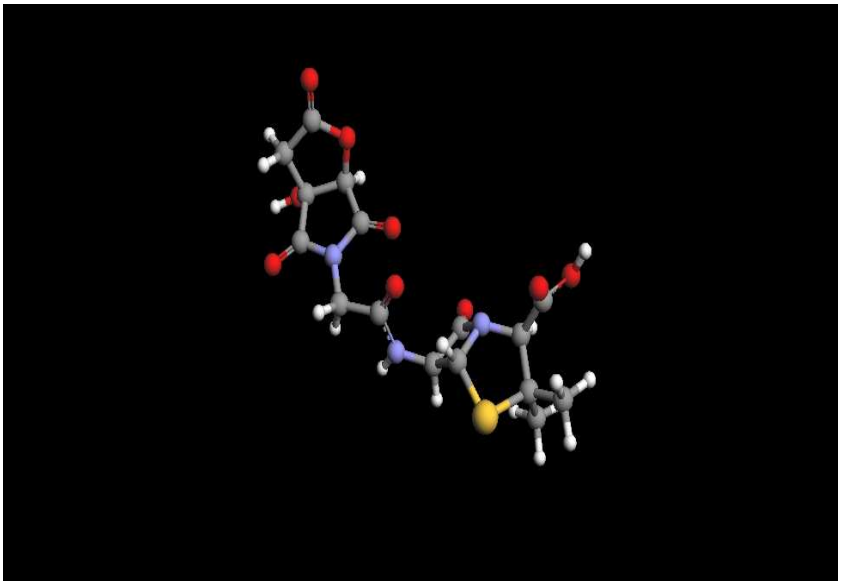


Figure 2: The 3D structure of the *Garcicillin*.

It was interesting to calculate the number of hydrogen bond acceptors (HBA) and the number of hydrogen bond donors (HBD). The values computed were found strictly following the governing rules like Lipinski rules, Weber rules etc. This gave hope to do with the rest of the discovery related works on this proposed drug. The most probable three dimensional structure designed using CADD methods that further used for docking and insilico studies is shown in Figure 2. The Highest Occupied Molecular Orbital (HOMO) and the Lowest Unoccupied Molecular Orbital (LUMO) were found away from the betalactamic carbonyl group and N-atom. This gave hope to further studies as these being the most reactive parts of the molecule. The HOMO was found mainly over the S-atom of the betalactam portion and the LUMO was found mainly over the N-atom and its immediate environment that constitute the GA-part. The docking studies of the proposed drug *Garcicillin* were performed using the software called ArgusLab over the active site of 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 of -5.55 kcal/mol and is comparable with that of amoxicillin a widely prescribing β-lactam antibiotic.

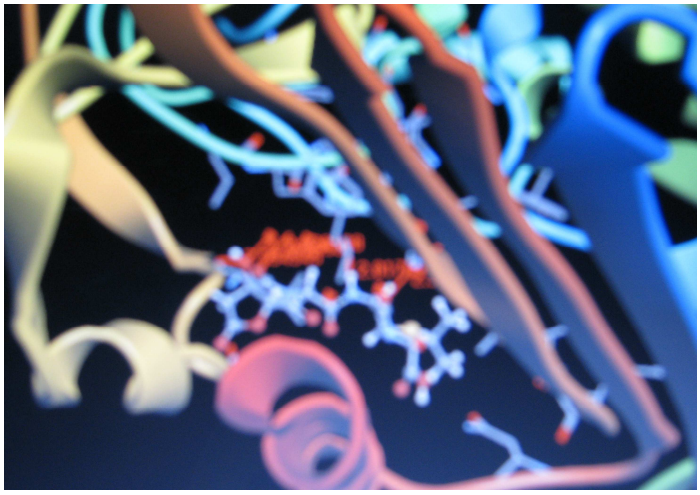


Figure 3: The *Garcicillin* docked on the binding site of betalactamase (1LL9.pdb)

The proposed drug candidate *Garcicillin* docked on 1LL9.pdb with its binding site is shown in Figure 3.

Toxicological Studies

The toxicology studies of every drug are much important and performed for the proposed drug *Garcicillin* also. The insilico toxicological studies were performed for the proposed drug and are presented for better understanding. As expected the designed drug *Garcicillin* was found to be developmental non-toxicant with negative mutangenicity. The bioaccumulation factor log value (1.46) was found comparable with presently prescribing penicillin class antibiotics. The LC_{50} value for *Daphnia magna* was found to be 3.50mg 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 767.28mg per Kg and is most comparable with penicillins. The value calculated for *T.pyriformis* is 145.95mg per litre. All these estimated values are par with penicillin type drugs and supported the druggability of the designed drug *Garcicillin*. On comparison with the original laboratory toxicity estimation values reported for the penicillin type antibiotics these predicted values could be acceptable and animal studies can be avoided to a certain extent.

Characterization

The product *Garcicillin* obtained was found to be shining yellow free flowing amorphous compound with characteristic type of odour like amoxicillin and ampicillin. The solubilities were checked for both organic and inorganic solvents. Found easily soluble in polar solvents than in non-polar solvents supported the polar nature of the drug *Garcicillin*. The melting point was recorded using traditional method and was found to be charring one between 200-205^{o}C ranges (uncorrected) with the evolution of vapours of 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. The IR spectrum showed the prominent peaks and those were assigned to all the majour plausible groups of *Garcicillin*. The IR showed the characteristics of β-lactam antibiotic and derivative of the GA with necessary changes in peak positions and found in support to the formation of the drug. The broad peak entered at 3266cm^{-1} is attributed to the carboxyl group. The sharp peaks observed at 1776cm-^{1}, 1686cm-^{1} can be assigned to the CO group. 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 two prominent singlet peaks of 3H each at 1.656 ppm and 1.673 ppm are of the two CH_3 groups of the β-lactam part which were initially at 1.408ppm and 1.533 ppm respectively in the 6-APA. The characteristic peaks at 4.201ppm, 4.878ppm and at 5.474 ppm observed in 6-APA were found shifted to 3.831ppm,5.221ppm and 5.221ppm in the product *Garcicillin*. The characteristic peak of the GA portion C**H** was observed as a doublet 2.896ppm and 2.942ppm respectively. The C-NMR also showed the characteristic peaks that can be attributed to the proposed structure. The **C**O groups of the *Garcicillin* showed characteristic peaks at 175.95, 173.43, 172.62, 170.70 and 169.97 respectively. The peaks at 173.43 and at 169.97 could be assigned to the **C**O groups of 6-APA when compared. The peaks at 26.93 and 25.48 were found that of two CH_3 carbons. This supported the presence of β-lactam ring the main active part of any penicillins in the product *Garcicillin*. The NMR studies showed the characteristic peaks for β-lactam and the portion of derivative of GA supported the characteristic nature of the synthesized drug. The complex nature of 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 series of pathogenic bacteria for the drug candidate *Garcicillin* with amoxicillin as control. The pathogens include *Klebsiella sp*, *Pseudomonas sp*, *Staphylococcus aureus*, *Salmonella sp* etc. In many cases the *Garcicillin* showed comparable results with amoxicillin. In some cases like in *Salmonella paratyphi* this showed more potency over the control.

CONCLUSION

The studies based on CADD for the proposed drug *Garcicillin* were found supportive to the limiting laws and further supported the druglikeness of the proposed *Garcicillin*. The insilico pharmacological and Docking studies were found similar to that of many semisynthetic penicillins. The insilico Toxicological studies showed the results that were found supportive to the druggability of the proposed drug *Garcicillin*. These gave hope to synthesise the drug in the laboratory and achieved the same. The wet lab studies for the antibacterial screening on a series of microbes supported the CADD and Docking results. The drug *Garcicillin* showed more potency in some cases and atleast the same activity to many when compared to the control. Further studies on the derivatives and metal salts of the *Garcicillin* are in progress and found positive in preliminary studies. The stability studies of the *Garcicillin* are to be carried out along with animal and clinical studies which are beyond the scope of this paper.

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