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Research Article

THE COMPARISON OF INSILICO PHARMACOLOGICAL AND TOXICOLOGICAL STUDIES OF AMOXICILLIN, AMPICILLIN AND NOVEL SEMISYNTHETIC PENICILLIN *GARCICILLIN*

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Abstract:

The introduction of computers and softwares clearly reduced the time for the drug discovery and now much focusing on the insilico prediction of toxicological parameters. The pharmacological and toxicological parameters were once believed to be the monopoly of the pharmacologists. Up to certain extent this is true even now as the experiments were based on the living species in a well equipped animal house maintained in a well ethical manner. Now a days the new generation software came with a solution for the present problems by keenly observing the parameters and conditions and developed the methods accurately to an acceptable manner. This can surely minimize the laborious animal trials and the time consuming procedures. The present paper concentrates on the comparison of the pharmacological and toxicological parameters both insilico predictive manner and the actual laboratory values. Based on these results the insilico studies for the newly designed and synthesized novel semisynthetic penicillin named *Garcicillin* were performed and compared.

Key words: Penicillins, Amoxicillin, Ampicillin, *Garcicillin*, Insilico Toxicological studies, Docking, ArgusLab, Drug Discovery and Development

Introduction

The structures of the chemicals that showed druggability were getting more attention of scientists and they were trying to correlate the activity with the structure of the drugs for many years. The medicinal scientists were tirelessly in search of predicting the medicinal usefulness of the chemicals or drugs according to their structure[1]. Many of the scientists

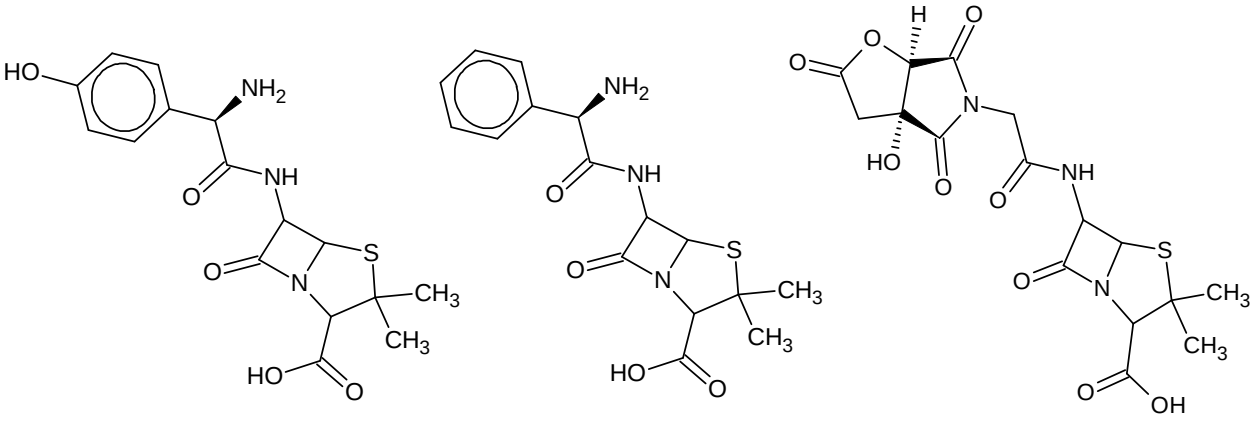
came with many fascinating results and correlations and none was the perfect one with all acceptable values or parameters. They tried to define the term drug in different ways and the recent one that accepted according to IUPAC is that chemicals that prevent disease or assist in restoring health to diseased individuals[2]. Animal experimentation has long been one of the cornerstones with crown valuable of biological and biomedical research. In fields from surgery to physiology and from pathology to pharmacology, *in vivo* models have been dominant for well over a century. The pharmacological and toxicological parameters were once believed to be the monopoly of the pharmacologists and toxicologists. Up to certain extent this is true even now as the experiments were based on the living species in a well equipped animal house maintained in a well ethical manner. Very recently the alternatives for animal experiments were found developing and will replace the time consuming animal studies which are bound with more ethical problems. No one can deny the sufferings of animals those played the silent but important roles including the precious sacrifices behind the success of many inventions and discoveries in the field of medicinal chemistry. Unfortunately we are neglecting or not bothering these sufferings and sacrifices in the success or failure of the invention of drugs. This is the time to come with alternatives to minimize these silent sacrifices and is our role to do research to find the better alternatives and let they must be cheaper, quicker, better controlled and more relevant than traditional animal models[3]. In short, considering such as system may enable a researcher to do more science and better science. For this the successful insilico methodologies with well acceptable values when compared to the in vivo values must be developed and we have to concentrate on the same. The present paper concentrates on the penicillins like Amoxicillin and ampicillin with the insilico predictions with the reported in vivo values. Based on these values the same methodology was adopted to the design and development of the novel semisynthetic penicillin termed *Garcicillin* which is a hybrid form of two natural products[4].

Materials and Methods

The penicillins constitute the main antibacterial drugs with the recently developed semisynthetic ones known as aminopenicillins. The aminopenicillins amoxicillin and ampicillin are widely prescribing ones in many formulations by the medical practitioners recently. The structure of the penicillin's like amoxicillin and ampicillin are well known. The

green pharmacology concepts and methods were followed in this study. For in vivo studies the physician's samples of amoxicillin and ampicillin and the newly designed and synthesized semisynthetic penicillin *Garcicillin* were used.

Figure 1: The drugs selected for study- Amoxicillin, Ampicillin and *Garcicillin*.



Amoxicillin Ampicillin Garcicillin

Amoxicillin: This is a semisynthetic penicillin type antibiotic prescribing in the present times and chemically (2*S*,5*R*,6*S*)-6-{[(2*S*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid.

Ampicillin: This is a semisynthetic penicillin type antibiotic prescribing in the present times and chemically 6-{[(2*R*)-2-amino-2-phenylacetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

Garcicillin: The newly synthesized semisynthetic penicillin Garcicillin is chemically 6-({[(3a*R*,6a*R*)-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.

The drug structures without considering the water hydration were selected purposefully for the study and presented the figure-1.

Experimental

This portion can be divided into portions viz. (1) Computer Assisted Drug Designing (CADD), (2) Insilico Pharmacological Studies and Docking, (3) Insilico Toxicological Studies and (4) Systematic Antibacterial screening.

1. **Computer Assisted Drug Designing (CADD):** The structures of Amoxicillin, Ampicillin and *Garcicillin* were

studied using well accepted methods. The reported structures were drawn and screened with the drug defining parameters. The newly designed and developed antibiotic *Garcicillin* was studied with more theoretical views.

2. **Insilico Pharmacological Studies and Docking**: The insilico pharmacological studies and docking were performed by using ArgusLab[5]. The docking is the best method to predict the activity of the drug candidate and are many approaches and methods available[6-7]. The drug which being the penicillin type the docking was performed over the bacterial protein which is betalactamase in nature[8-9]. 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[10].

3. **Insilico Toxicological Studies**: The insilico toxicological studies were performed after the docking studies for Amoxicillin, Ampicillin and the new *Garcicillin*. The studies were performed using many methods accepted and developed by the American Environment Protection Agency's guidelines[11].

4. **Systematic Antibacterial Screening**: The drugs understudy being proven antibacterials the screening was found necessary. The antibacterial screening for Amoxicillin, Ampicillin and Garcicillin was performed against a set of common pathogens using well accepted methods[12].

Results and Discussions

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

CADD and Docking Studies: The drugs Amoxicillin, Ampicillin and Garcicillin were optimized and docked using ArgusLab. The predicted Log P values of Amoxicillin and Ampicillin were (0.61+/- 0.33) and (1.35+/- 0.32) respectively. The actual values reported for Amoxicillin and Ampicillin were found comparable. The *Garcicillin* showed a calculated Log P value (-1.11+/- 0.62) which comes between common antibacterial oral drugs. The formula weight of anhydrous Amoxicillin and Ampicillin are 365.40 and 349.40 units respectively. The formula weight of the anhydrous drug *Garcicillin* is 427.38 units. The hydrophobic and hydrophilic values are also of acceptance range. These values support the Lipinski rules and other controlling rules for the acceptance of druglikeness. 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 protein structure was downloaded from the protein data bank and used as such. The docking results obtained for the drugs amoxicillin, ampicillin and *Garcicillin* with the betalactamase class bacterial protein is tabulated and presented in the table1.

Table-1: The values for Amoxicillin, Ampicillin and Garcicillin.

DRUG	MW	HBA	HBD	Log P (calculated)	Best Pose Energy (kcal/mol)
Amoxicillin	365.40	7	5	0.61+/- 0.33	-8.40
Ampicillin	349.40	6	4	1.35+/- 0.32	-8.36
Garcicillin	427.38	10	2	-1.11+/- 0.62	-5.55

The values calculated for Amoxcillin, Ampicillin and Garcicillin were found to be obeying drug designing rules like Lipinski rules, Weber rules etc. and were not crossed the limiting values. The pose energies observed from docking over β-lactamase 1LL9.pdb the bacterial protein of E.coli were also comparable ones.

Insilico Toxicological Studies: The insilico toxicological studies were performed for Amoxicillin, Ampicillin and *Garcicillin* as per the TEST methodologies accepted and developed by American Environment Protection Agency.

Amoxicillin: The drug Amoxicillin was found to be developmental non-toxicant with negative mutangenicity. The bioaccumulation factor log value (0.30) was found comparable with presently prescribing penicillin class antibiotics. The LC_{50} value for *Daphnia magna* was found to be 0.75mg per litre and the insilico estimated value of LD_{50} values for mice is found to be 2638.56mg per Kg and is most comparable with penicillins. The value calculated for *T.pyriformis* is 34.11mg per litre.

Ampicillin: The drug Ampicillin was found to be developmental non-toxicant with negative mutangenicity. The bioaccumulation factor log value (0.47) was found comparable with presently prescribing penicillins. The LC_{50} value

for *Daphnia magna* was found to be 0.19mg per litre and the insilico estimated value of LD_{50} for mice is found to be 5236.33mg per Kg and is most comparable with other penicillins. The value calculated for *T.pyriformis* is 32.61mg per litre.

Garcicillin: The toxicology studies of every drug are much important and performed for the proposed drug *Garcicillin* also. The novel semisynthetic drug *Garcicillin* was found to be developmental non-toxicant with negative mutangenicity. The bioaccumulation factor log value (1.46) was found large when comparable with presently prescribing penicillin class antibiotics amoxicillin and ampicillin. Both the starting materials of this drug being the natural products the higher value was expected and was in limits. 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. The value calculated for *T.pyriformis* is 145.95mg per litre. These support the non aqu-toxic nature of the drug. All these estimated values are par with penicillin type drugs and supported the druggability of the designed drug *Garcicillin*.

Systematic Antibacterial Screening: A systematic antibacterial screening for Amoxicillin, Ampicillin and Garcicillin were performed for a number of common clinically isolated pathogens like *Klebsiella sp*, *Pseudomonas sp*, *Staphylococcus aureus*, *Salmonella sp* etc. In most cases the *Garcicillin* was retaining the comparable results with Amoxicillin and Ampicillin. In the case of *Salmonella paratyphi* the *Garcicillin* showed more activity.

Conclusion

The predicted values for Amoxicillin and Ampicillin, both widely prescribing, were also found comparable to the estimated values in the laboratory. This suggests the acceptability of the methods and hence successfully applied to the novel semisynthetic penicillin *Garcicillin*. The other effects like anti obesity activity and anti ulcer activity may also present for the *Garcicillin* and yet have to be studied thoroughly. The traditional knowledge of *Ayurveda*, a leading system of Alternative medicine gives hope for the presence of these activities. On comparison with the original laboratory toxicity estimation values reported for the penicillin type antibiotics amoxicillin and ampicillin the values predicted for *Garcicillin* could be acceptable and laborious and expensive animal studies can be avoided to a certain

extent.

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