

Molecular Similarity between Gibberellic Acid and Gliotoxin: Unravelling the Mechanism of Action for Plant Growth Promotion by *Trichoderma harzianum*

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Abstract: Besides control of the fungal plant pathogens, another interesting aspect observed when plants are treated with *Trichoderma harzianum* include effects such as complete and even stand of plants, improved seed germination, increases in plant height and overall enhanced plant growth. No research has yet been conducted to elucidate the mechanism by which these effects occur. Improved seed germination, in particular, suggest that *Trichoderma harzianum* produces a metabolite that may mimic the plant growth hormone gibberellic acid. The metabolite gliotoxin, produced by *Trichoderma harzianum* appear to be structurally most similar to gibberellic acid. In this study, common pharmacophore generation and molecular ligand docking simulations were used to evaluate the molecular similarity between gibberellic acid, specifically GA₃, and gliotoxin. For the common pharmacophore evaluation, the structure of various gibberellic acids were used to construct a pharmacophore space to which gliotoxin was aligned, and during the molecular docking simulations the gibberellic acid receptor, GID1, served as ligand target for GA₃ and gliotoxin. During the common pharmacophore evaluation, gliotoxin was successfully aligned to the common pharmacophore model constructed from various gibberellic acids. Furthermore, molecular docking simulations of gliotoxin and GA₃ into the gibberellic acid receptor (GID1) yielded docking scores of -10.78 kcal/mol for the GA₃ molecule from Corina and a docking score of -10.17 kcal/mol for gliotoxin. The docking scores suggest that gliotoxin may be able to competitively occupy the receptor space for gibberellic acid, and as such elicit the similar physiological responses observed in literature.

Key words: Gibberellic acid, gliotoxin, GID1, molecular similarity, *Trichoderma harzianum*.

1. Introduction

As early as 1930, the potential of *Trichoderma* to serve as a biological control agent was recognised and research is increasing the list of diseases controlled by this genus of fungus. This has lead to the commercial production of several *Trichoderma* species and *Trichoderma*-based products in countries such as Israel, New Zealand, India, Sweden and South Africa

for crop-protection and growth enhancement [1].

Literature reports that certain *Trichoderma* strains are known to produce a variety of classes of bioactive metabolites such antibiotics of the peptaibols class, as well as inhibitors of fungal growth of a mainly terpenic nature [2]. Overall, the production of secondary metabolites in *Trichoderma* species is strain dependent and includes volatile and non-volatile antifungal substances such as 6-*n*-pentyl-6*H*-pyran-2-one (6PP or 6-pentyl- α -pyrone), gliotoxin, viridin, harziaopyridone, harziandione and peptaibols [3]. Gliotoxin was first described in 1934 and initially the compound was

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described as a “lethal principle” produced by *Trichoderma lignorum* [4]. By 1941 this “lethal principle” was characterised further and demonstrated to be toxic to both *R. solani* and *Sclerotinia americana* and named gliotoxin [1].

The mechanisms by which plants control growth are many faceted and complex. One of these mechanisms entails the so-called “plant growth substances” or plant hormones [5]. According to Hill [5], a plant growth substance can be defined as: “an organic substance which is produced within a plant and which will at low concentrations promote, inhibit or qualitatively modify growth, usually at a site other than its place of origin”. A further group of compounds is possible with this definition as a base. They are the “plant regulators” and they can be defined as compounds whose effects, when applied to plants, closely resemble that of the plant hormone. A variety of these compounds are known and some of them are chemical analogues of the endogenous plant hormones, though not all.

Gibberellic acids (GAs) (also referred to as gibberellins) are classified as tetracyclic diterpenoid plant growth regulators. According to current studies 126 GAs have been identified in higher plants, fungi and bacteria. Gibberellic acid regulates various developmental and growth processes in plants such as stimulation of seed germination; stimulation of stem elongation; flowering; stimulation of pathenocarpy, regulation of gene expression and stimulation of trichome development. The action of gibberellic acid is however antagonized by abscisic acid. Of the various known gibberellins only a few are biologically active in plants, these are GA₁, GA₃, GA₄ and GA₇ [6-10].

One particularly interesting phenomenon observed when seeds or crops are treated with *Trichoderma* species, is the complete and even stand of the treated plots compared to the uneven and random stand of untreated plots [1]. In a study of seed vigour of peas in potting soil, Zheng and Shetty [11] also showed that treatment with various *Trichoderma* species resulted

in increased and faster seed germination, increases in plant height, an increase in phenolic compound content in the seedlings and overall enhancement of plant growth [11-13]. During this particular study *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma pseudokoningii* were compared. During the particular study *Trichoderma harzianum* treatment of the peas resulted in the highest average plant height after 5 days, highest average weight of fresh seedlings and highest phenolic compounds in the seedlings relative to the other strains evaluated [11]. Another study by Vinale et al. [13] also demonstrated that secondary metabolites from *Trichoderma* have a role in activation of plant defences as well as plant growth regulation.

This suggests that treatment with *Trichoderma* affect not only plant pathogens, but at the same time the plant itself is also affected. Of all the mechanisms employed by *Trichoderma*, the secretion of antibiotic substances seems the most probable route. It is suggested that some of the secondary metabolites excreted by *Trichoderma* may function as a homologue for a plant growth controlling substance or hormone. In a review by Vinale et al. [13], studies are cited where koniginin A and 6PP were evaluated for plant growth promotion. At high concentrations (10⁻³ M) growth inhibitory effects were observed, however at concentrations in the range of 10⁻⁵ M and 10⁻⁶ M these compounds exhibited optimal auxin-like effects.

However, the auxin-like effects observed for koniginin A and 6PP does not account for all the effects observed from *Trichoderma* treatments. In particular, the complete and even stand, but more indicatively, the earlier germination of treated seeds following *Trichoderma* treatment, suggests that the effect of the plant hormone gibberellic acid, or gibberellin, is being mimicked. Evaluation of the various secondary metabolites secreted by *Trichoderma harzianum* suggests that gliotoxin is the most likely candidate to elicit these physiological responses in plant systems.

Tools such as X-ray crystallography, nuclear magnetic resonance and computational chemistry and modelling are providing researchers with valuable data to design and study ligand/substrate and protein interactions in the fields of chemistry, biochemistry and pharmacology [14]. Parallel to this, there has been a great increase in the number of high-resolution protein structures deposited in the Brookhaven Protein Databank (PDB). This has enabled successful drug design and evaluation in particularly the field of pharmacology [15]. In the field of computer aided drug design, ligand-protein interactions are a useful tool to design and evaluate potential ligands against a protein of interest [16, 17].

Docking-ligand studies can be described as a target-based method [18]. During docking, various interactions between the ligand and the protein must be considered such as shape complementarity, charge-charge interactions, solvation-desolvation interactions, hydrophobic interactions and hydrogen bonding [14]. Potentially suitable ligands are usually selected based on a molecular binding scoring function [16]. In general, lower energy scores indicate better protein-ligand bindings when compared to higher energy scores. As a result, in most cases the docking is an attempt to optimise the computations to find the lowest binding energy [19].

A pharmacophore represents a qualitative prediction of binding by specifying the spatial arrangement of a small number of atoms of functional groups or in other words a 3D arrangement of atoms or functional groups necessary to bind to a given receptor [17, 18, 20]. Various critical interactions, relatable to chemical features of the compound, include hydrogen bonding, charge transfer, steric and electrosteric properties, as well as lipophilic interactions [18]. The advantage of using this approach is that prediction and screening can be performed on large databases as the pharmacophore serves as a guide for searching for compounds or the synthesis of new compounds and has been

successfully applied to a multitude of drug development programs [17].

Due to the hydrophobic properties of gibberellic acid, it has been postulated that gibberellic acid may have both membrane-bound and soluble receptors in plant cells [21]. Until recently research has as yet not completely homed in on the specific receptors for gibberellic acid, however the list of intracellular GA signal transduction elements has been expanded to include G-proteins and protein kinases [8]. However, in the past decade various factors have been identified through studies of rice (*Oryza sativa*) and *Arabidopsis* mutants [22, 23]. Recently, Gibberellin-Insensitive Dwarf1 (GID1) has been identified as a soluble receptor for GA in both rice and *Arabidopsis* [21].

The GID1 proteins display a close structural similarity to hormone sensitive lipases such as those found in higher animals, being a globular protein and containing a pocket for the substrate. Gibberellic acid functions as an allosteric activator in GID1, allowing structural changes that enable the receptor to associate with DELLA proteins, however GA does not interact with DELLA proteins by itself [10, 24-26].

In this study molecular docking and common pharmacophore evaluation has been applied to evaluate possible molecular similarity between gibberellic acid and gliotoxin.

2. Materials and Methods

2.1 Molecular Modelling and Docking

All the proteins used for this study were acquired from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Databank (www.rcsb.org). The Protein Data Bank (pdb) file 3ED1 (Crystal structure of rice GID1 complexed with GA₃) was used in the docking studies for GID1 and GA₃ and gliotoxin [26]. The structure files for GT, GA₁, GA₃, GA₄, GA₅, GA₇ and GA₈ created online using CORINA [27] from its SMILES (simplified molecular-input line-entry specification) notation and saved as a pdb file.

2.2 Molecular Similarity

The molecular similarity evaluation was only conducted between gliotoxin and GA₃, the primary molecules of interest in this study. Common feature pharmacophore generation using the HipHop algorithm of the Catalyst molecular modelling software suite version 4.8 [28] was applied to evaluate similarity between the molecules. During the pharmacophore model generation gliotoxin was tested against a model generated by using GA₁, GA₃, GA₄, GA₅, GA₇ and GA₈ as input.

2.3 Molecular Docking

For molecular docking the pdb file 3ED1 (Crystal structure of rice GID1 complexed with GA₃) served as the target with GA₃ and gliotoxin serving as ligands. The software package ArgusLab [29] was used for the molecular docking and scorings. The ArgusDock exhaustive search docking engine was used with a grid resolution of 0.25 Ångstrom, docking precision set to high precision and flexible ligand docking mode enabled.

3. Results and Discussion

3.1 Common Pharmacophore Modelling

For reference purposes the structures of gibberellic acid (GA₃) and gliotoxin (GT) is presented in Fig. 1a and Fig. 1b respectively.

The common pharmacophore model generated from the various gibberellic acid molecules identified three region types of interest for the pharmacophore alignment, namely the hydrogen bond donor regions

(represented as magenta), hydrophobic regions (represented as light blue, and hydrogen acceptor regions (represented as green). When aligning the GA₃ into this pharmacophore model it was observed that a hydrophobic region (light blue) is located to the C2 and C3 side of the structure and was situated in close proximity to the lactone ring between C4 and C10. Hydrogen bond acceptor areas (green) lie between C9 and C10, as well as C10 through C11. In relative close proximity to this, the hydrogen bond donor areas (magenta) are located in the vicinity of C13 through C14 (Fig. 2).

As is apparent from Fig. 3, gliotoxin was observed to successfully align in the common pharmacophore model that has been generated with the hydrogen bond acceptor regions (green) being located in the proximity of the carboxamide group and C12, while the hydrogen bond donor regions (magenta) were located in the proximity of C1 to C2 in relative close proximity the disulphide bridge. The hydrophobic region (light blue) was observed to align in the proximity of C7 to C8.

The structural analysis of GA₃ and GT separately within the common pharmacophore model suggests that GT and GA₃ might be perceived as being similar in a plant system. This perception in plant systems is significantly strengthened when the structures are overlaid (Fig. 4) onto each other within the pharmacophore model. The lactone ring of GA₃ overlays with the ring structure at C5 for GT, while the disulphide bridge at C1 and C11 overlays with the ring structure at C8-C13 in GA₃ (Refer to Fig. 1 for numbering scheme employed). The spatial occupation

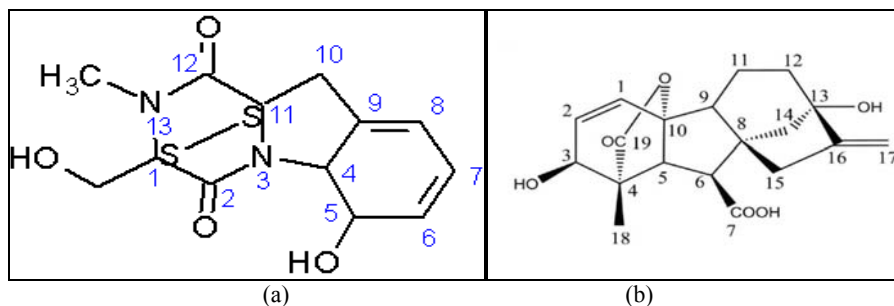


Fig. 1 Numbered structures for gibberellic acid 3 (a) and gliotoxin (b).

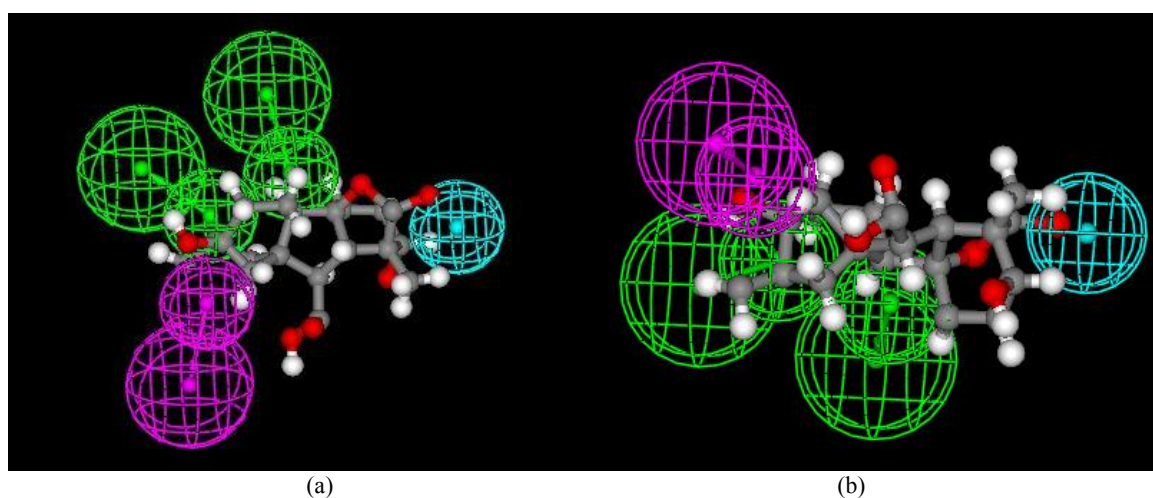


Fig. 2 Structure of GA₃ as aligned to the common pharmacophore model. (Colour coding employed by CATALYST: Magenta = hydrogen bond donor, Light Blue = hydrophobic region, Green = hydrogen bond acceptor).

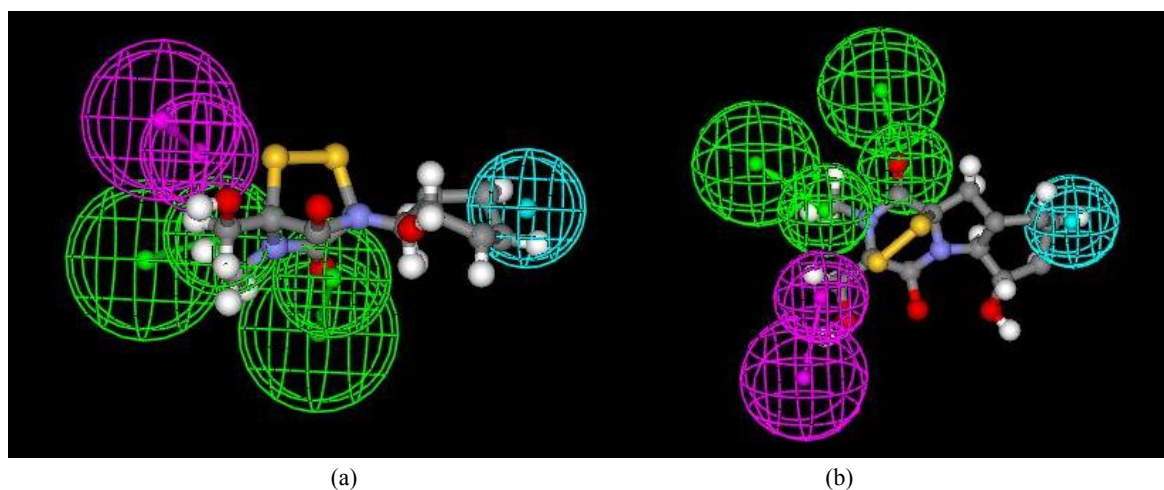


Fig. 3 Structure of gliotoxin as aligned to the common pharmacophore model. (Colour coding employed by CATALYST: Magenta = hydrogen bond donor, Light Blue = hydrophobic region, Green = hydrogen bond acceptor).

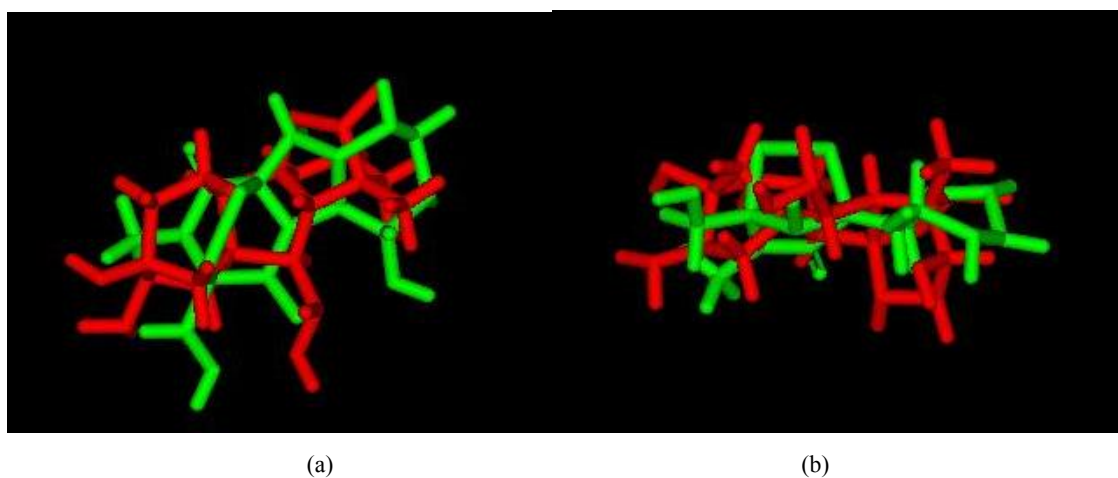


Fig. 4 Stick representation of GA₃ (red) and gliotoxin (green) as aligned to common pharmacophore model.

of the molecules was also observed to be strikingly similar. Thus, from common pharmacophore modelling, the match between the molecules appear quite convincing, but it is important to remember that during common pharmacophore modelling only the functional characteristics are considered for model generation and alignment. In essence, gliotoxin should be able to bind to the same receptors as GA₃ does based on functional group interactions, but this does not guarantee that similar plant responses may be evoked.

3.2 Docking Simulation of GA₃(gibberellic acid 3) and GT (gliotoxin) into GID1

For reference purposes the structural basis of gibberellin (GA₃)-induced DELLA recognition by the gibberellin receptor (GID1) from Shimada et al. [26] are presented first (Protein file 3ED1 from RCSB). The crystallised structure of GA₃ is shown in Fig. 5a and 5b.

As stated in the study by Shimada et al. [26] the following apparent features were observed:

Arg244 and Ser116 and Ser191 and its orientation relative to the carboxylate group.

Ile126, Val239 and Val319 (Leu323 not included in the binding site defined by ArgusLab) and its orientation relative to the aliphatic rings of GA₃.

Ile24, Phe27 and Tyr31 (His119 not included in the

binding site defined by ArgusLab) are the N-terminal extension helices which are adjusted for GA ring recognition.

Tyr127 bond with the C3 hydroxyl group of GA₃.

Phe238 form a weak bond with C13 hydroxyl group of GA₃.

For further comparison of the possible structural similarity, a docking simulation was performed in ArgusLab, using a GA₃ molecule constructed from SMILES notation and optimised using CORINA.

The docking pose for the optimised GA₃ molecule obtained with CORINA was observed to be situated relatively higher than that for the GA₃ molecule crystallised within 3ED1 (Fig. 6 a and b as well as Fig. 7a and b). The orientation of the carboxylate group was similar. However, the orientation in terms of the lactone ring and hydroxyl groups appears to be flipped. A docking score of -10.78 kcal/mol was the optimal pose calculated. The change in the docking pose might be a result of the GA₃ from CORINA having a more sterical hindrance than that of the GA₃ molecule within 3ED1. The differences in the optimal pose calculated may also be due to differences between the optimal configuration calculated for the generated GA₃ and the structure of the GA₃ molecule within the receptor protein, a factor CORINA does not account for in the optimisation of the structure.

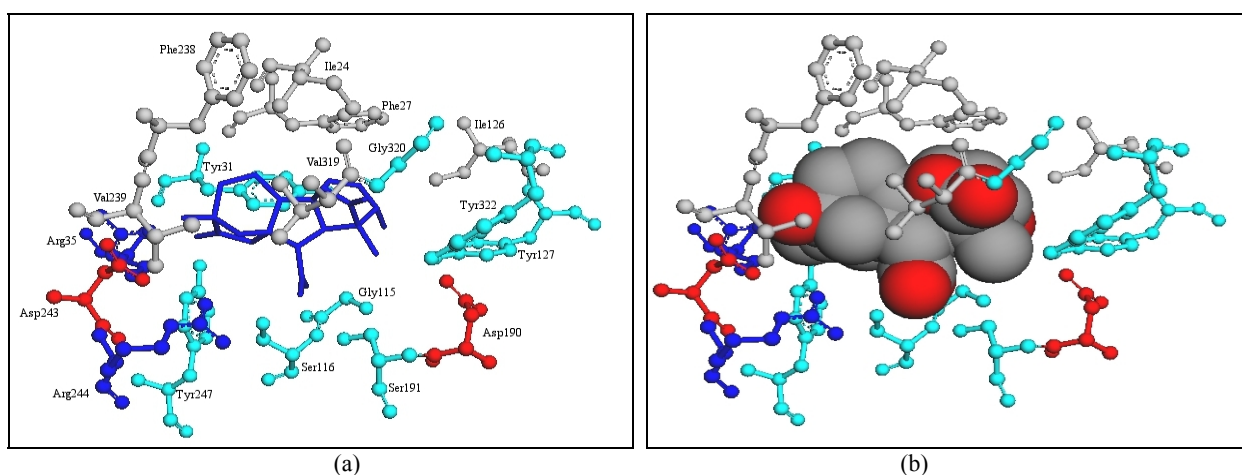


Fig. 5 GA₃ binding within the binding site of 3ED1. Stick representation (a), and the sphere representation (b).

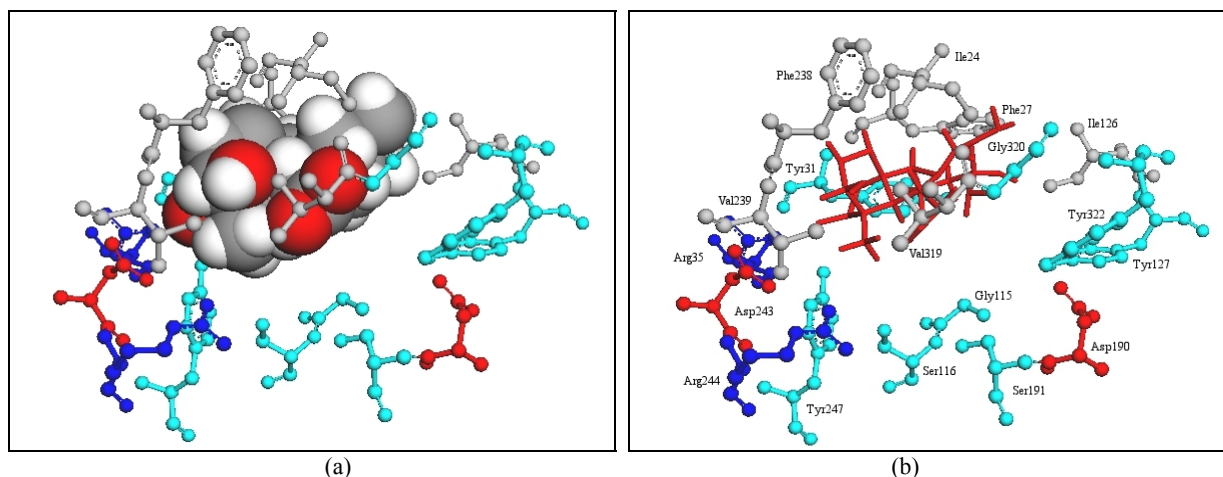


Fig. 6 GA₃ (Generated with CORINA) binding within the binding site of 3ED1. Stick representation (a) and sphere representation (b).

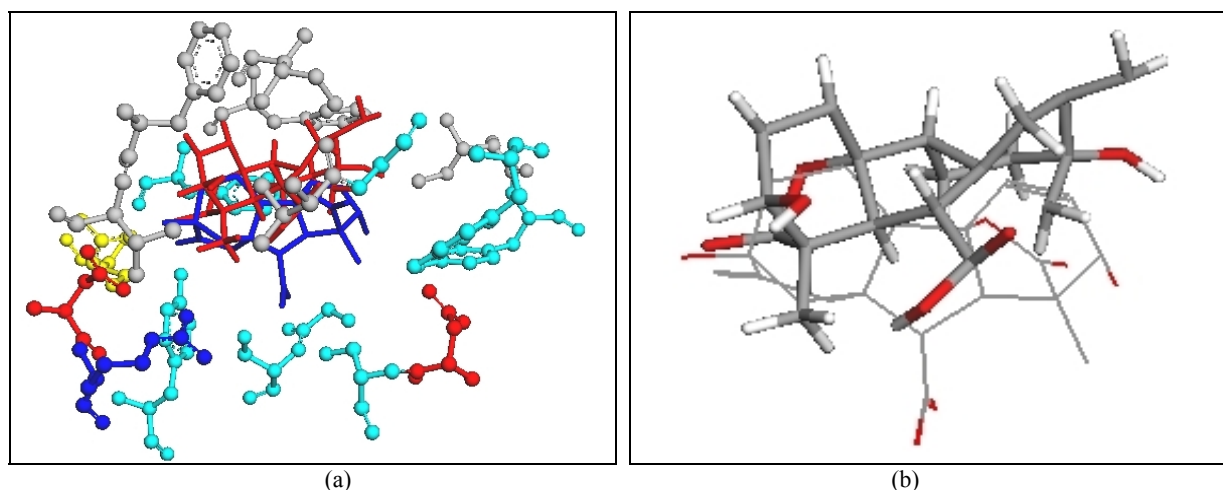


Fig. 7 Comparison of the docking poses for the GA₃ molecule crystallised with 3ED1 (blue) and the GA₃ molecule from CORINA. Docking poses with the binding site displayed (a) and docking poses with the binding site removed (b) for greater clarity.

In Fig. 7b, the GA₃ molecule within 3ED1 is represented as wireframe and the GA₃ from CORINA is represented by a stick representation.

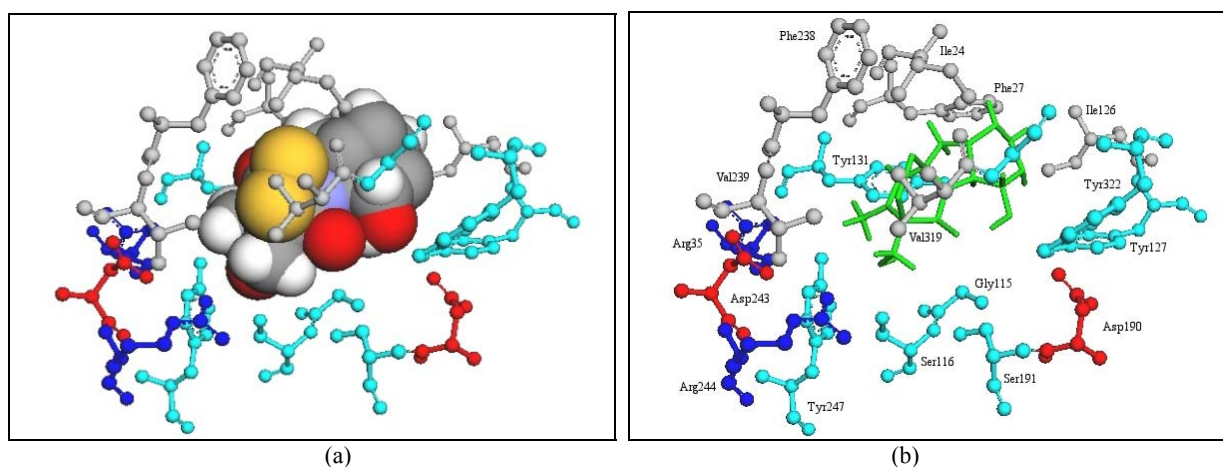


Fig. 8 GT (Generated with CORINA) binding within the binding site of 3ED1. Stick representation (a) and sphere representation (b).

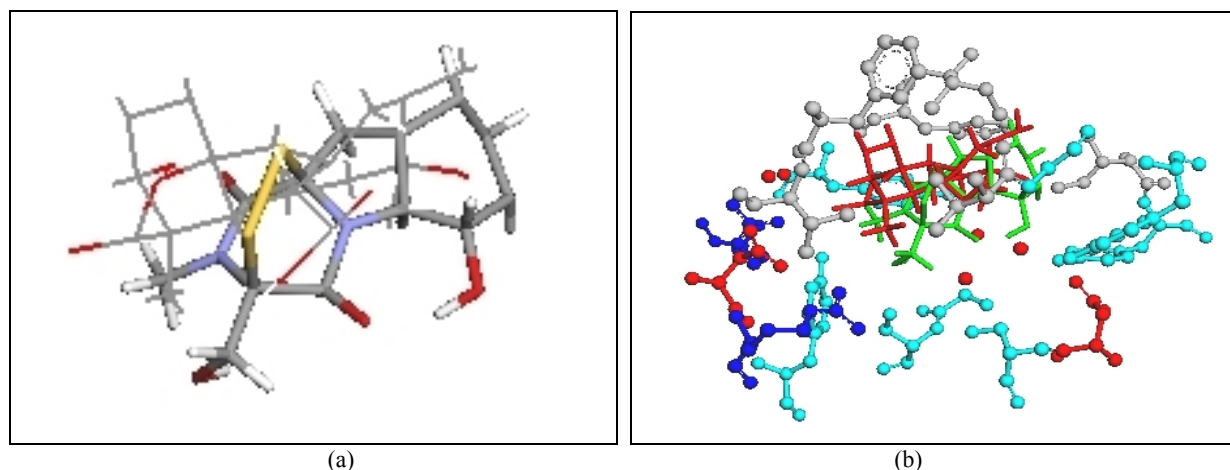


Fig. 9 Comparison of the docking poses for the GA₃ molecule crystallised with 3ED1 (red) and the GT molecule from CORINA (green). Docking poses with the binding site displayed (a) and docking poses with binding site not displayed (b) for greater clarity.

In Fig. 9b, the GA₃ molecule from CORINA is represented as wireframe and the GT from Corina is represented by a stick representation.

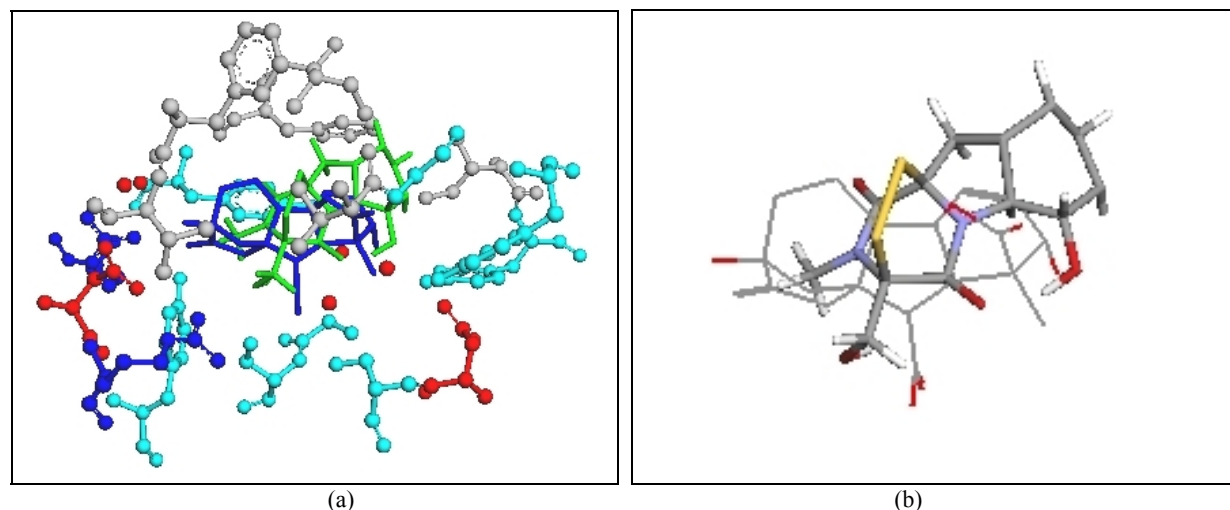


Fig. 10 Comparison of the docking poses for the GA₃ molecule crystallised with 3ED1 (blue) and the GT molecule from CORINA (green). Docking poses with the binding site displayed (a) and docking poses with binding site not displayed (b) for greater clarity. The GA₃ molecule within 3ED1 (Fig. 10 b) is represented as wireframe and the GT from CORINA is represented as a stick representation.

When comparing the docking of gliotoxin and the GA₃ molecule crystallised into the structure of GID1, the results appear to be more in line with the orientation calculated from pharmacophore generation. When the docking poses of both GA₃ (from the GID1 structure itself) and GT are examined, the docking pose for GT appears very close to that of the GA₃ within the GID1 protein structure (Fig. 10a). Also, when the molecules' docking poses were compared without the docking cage (Fig. 10b), the orientation

seemed to match that of the results during the common pharmacophore evaluation. In this docking, the lactone ring of GA₃ aligned with the ring structure at C5 (Refer to Fig. 1), while the disulphide bridge at C1 and C11 aligned with the ring structure at C8-C13 in GA₃. Furthermore, the COH group at C1 of GT aligned with the carboxylate group at C6 from GA₃ as well as with Arg244, Ser116 and Ser191 of the protein. The disulphide bridge of GT orientated with Val319 and a resulting docking score of -10.18 kcal/mol, was

calculated by ArgusLab for the optimal pose.

Overall, with GT docked within 3ED1, gliotoxin does appear to dock in a pose that is quite similar to that predicted by the common pharmacophore alignment. The COH group at C1 of GT appear to orientate in a similar manner to the carboxylate group of GA₃. Alignment to the other functional groups appears similar to that predicted from common pharmacophore alignment. Again, sterical hindrance might play a role in the alignment not being an exact overlay match. Another reason for the differences between the docking results obtained with regard to molecule poses and the common pharmacophore poses, might be that with the common pharmacophore modelling only the characteristics of the molecules are considered and aligned, whereas with docking, molecular charges and various interactions between the ligand and the binding site are allowed to affect the final calculated orientation. In terms of docking with the aid of ArgusLab, a docking score of -10.78 kcal/mol for the GA₃ molecule from CORINA and a docking score of -10.17 kcal/mol for GT was calculated. Since the docking scores are comparable for both compounds, this suggests that gliotoxin may compete with gibberellic acid for the binding site within GID1 [16].

Though the docking scores suggest good similarity between the molecules, differences in the structure and shape of the GA₃ within GID1 and the external GA₃ (constructed using CORINA), might contribute to the differences in docking poses observed. Thus, based on the data presented above and the docking scores obtained, it seems reasonable to deduce that GT will dock to the GID receptor protein and may elicit physiological responses similar to that of the natural GA₃ molecule.

Even though similarities between docking poses and docking scores may strongly suggest similar affinities for the ligands to the receptor, it still does not guarantee that similar physiological responses will be evoked. The evaluation of the physiological

responses was studied separately.

4. Conclusions

The results from the common pharmacophore evaluation and molecular docking suggests a high likelihood that gliotoxin may be perceived as gibberellic acid in plant systems, however, molecular similarity is not a guarantee that similar physiological responses will be elicited in plant systems. However, the approach may be valuable to screen candidate plant growth regulators prior to evaluating the physiological responses in plant systems.

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