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Steroidal Plant Growth Promoters vs. Phytopathogens, via Enzymatic Regulation; An *in Silico* Approach

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Abstract

Steroidal plant growth promoters (SPGP) have been continuously studied due to their high activity increasing biomass and resistance to diverse stress factors. In our hands, a new SPGP family of 22-oxocholestanic compounds stands out at a comparative level to brassinosteroids (BSs). The potential activity of new SPGP against phytopathogens was studied through *in silico* molecular docking, these assays were performed with relevant ensymes of phytopatogens Chitinase B and 1,3-β-Glucanase. Nine Chitinase B inhibitors and two 1,3-β-Glucanase inhibitors were proposed. The launched study analyzed the interactional and spatial level, determining the presence of interactions with key amino acids in receptors in comparison to reference inhibitors. Even more, the AVR4 and ECP6 effectors were also examined. No compound that blocks ECP6 was found; due to, probably, the influence of its highly hydrophilic environment. In the case of AVR4, two SPGP showed a better docking score (DS) than a chitin fragment (endogenous ligand); this fact demonstrates the latent potential of the 22-oxocholestanic derivatives against phytopathogens, with a specific regulation via proliferation inhibition. Moreover, this SPGP does not affect the symbiotic fungi that are beneficial for the natural plant system.

Keywords

22-Oxocholestanes, Brassinosteroids, Chitinase B, 1,3-β-Glucanase, ARV4, ECP6

1. Introduction

Plant growth promoters are compounds commonly called phytohormones, which are responsible for important roles, as regulation processes for increasing biomass, flowering, resistance to stress conditions, among others. A highly active group of growth promoters are Brassinosteroids; for example, brassinolide (**1**) has shown a huge promoter effect than other kinds of phytohormones and resistance for abiotic stresses, this discovery has led to an increased interest in the synthesis of diverse BSs analogues, but in most cases low yields have been reported [1]-[8]. Compounds of the novel family of 22-oxocholestanes (**SPGP1**, **SPGP3**, **SPGP5**), (**Figure 1**) have been positioned as SPGP alternative due to its comparable or even more potent activity to that of BSs and moreover, can be generated through higher reaction yields. Some 22-oxocholestanes have been successfully tested in rice, red-beans, and maize [9] [10] [11]. Although these compounds have demonstrated an exceptional plant growth promoting effect in *in vitro* and greenhouse assays, for field tests and future applications it is necessary to predict synergistic effects on beneficial/non-beneficial phytopathogens and to study their effect on the intrinsic defense system of vegetal models.

Plants have developed several mechanisms to recognize microbial infections and respond appropriately by activating defense responses [12]. Therefore, the knowledge of the relationship between plants and the pathogenic hosts is crucial for their control [13]. It has been found that the interaction that takes place between guest and host is carried out by means of special proteins, which have effects on both cells and phenotypes of hosts [14] (**Table 1**). Fortunately, all plant cells possess a sophisticated surveillance system that can register and distinguish many signals of different origins, which manages to induce a more efficient and

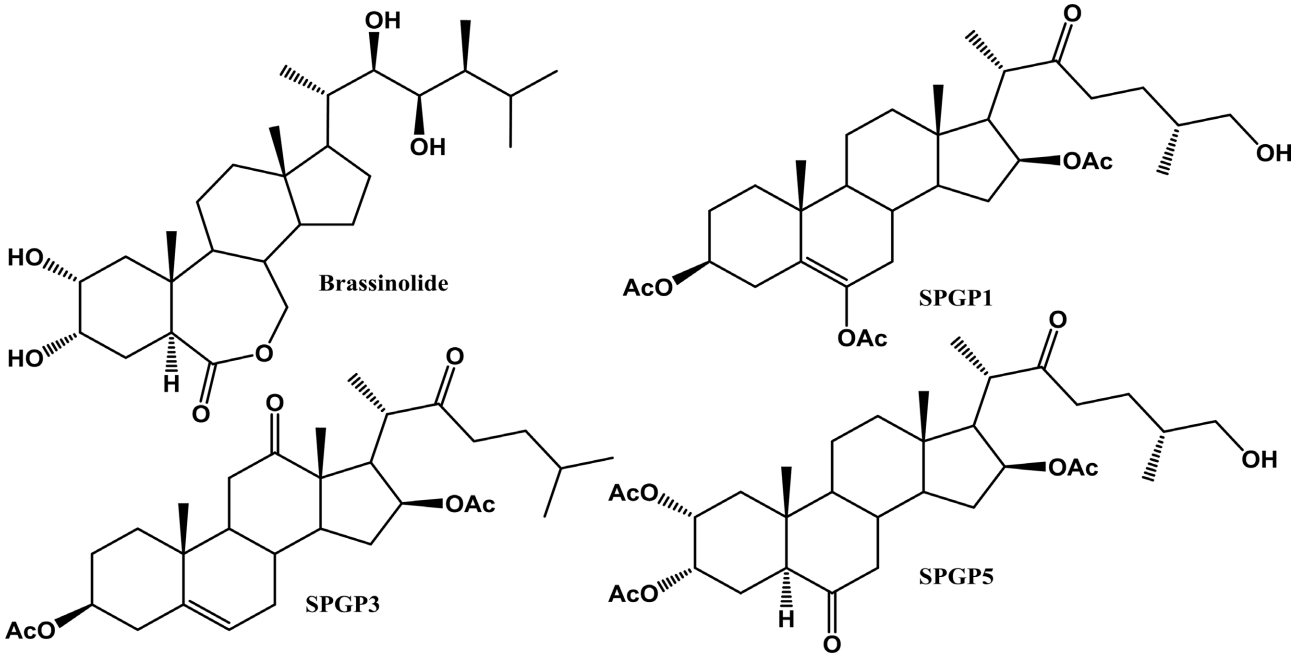

Figure 1. Steroidal derivatives providing plant growth promotion effect.

Table 1. Proteins associated with the proliferation of phytopathogens.

Enzyme/Protein	Function	Reference compound	Hosts	Ref.
Chitinase B	To hydrolyze the polymeric chitin of N-acetylglucosamine framework. Together with the β-1,3-glucanases plays an indirect defensive role: the oligosaccharides of 1,3-β-/1,6-glucan are destroyed in pathogens cell walls. Inhibition of the fungi growth is produced, and a wide range of defense responses are induced in plants.	Chitin (S) F0O (I)	*Alternaria brassicicola* *Sclerotinia sclerotiorum* *Rhizoctonia solani*	[19] [20] [21]
1,3-β-Glucanase	To perform their protective function by a hydrolysis of the 1,3-β-/1,6-glucan bond present in the pathogens cell walls.	1,3-β-/1,6-glucane (S) Apegin (I)	*Fusarium moniliforme* *F. verticillioides* *Aspergillus Flavus* *Alternaria solani* *Colletotrichum fragariae* *C. acutatum*	[22] [23] [24] [25]
ECP6	Recognition of specific sequences. It encodes three domains of lysine that disrupt chitin-triggered immunity.	Chitin (L)	*Cladosporium. fulvum*	[18]
AVR4	A chitin-binding lectin that protects fungal cell walls from plant chitinases, providing a defensive role during infection. It inhibits the Cys-protease Rcr3 secreted by tomato plants and binds chitinases at the fungus cell wall.	Chitin (L)	*Cladosporium. fulvum*	[26]

effective defense response at the infection site [15].

There is a special class of proteins of low molecular mass, diverse chemical composition having a different hydrophilic character that is directly related to the pathogenic fungi activity, known as Pathogenesis-Related (PR or pathogenicity proteins), such as chitinases and 1,3-β-glucanases [16]. Another unique kind of proteins that acts as a defense mechanism in plants is named effectors, like AVR4 and EPC6 [17]. When these molecules are recognized by the plant, an unfavorable interaction between the plant and pathogens, therefore, the effectors' function has not been clearly clarified [18].

In the present work, fifteen 22-oxocholestanic SPGP, which previously showed a positive experimental results as growth promoters [9] [10] (**Figure 2**), were *in silico* studied on the effect in the two principal enzymes involved in fungal proliferation (Chitinase B and 1,3-β-Glucanase). Also, the influence against two effectors of infection in plant systems (AVR4 and ECP6) was studied, to prove the potential of SPGP in fungal pests.

2. Materials and Methods

2.1. Protein and Ligands Preparation

The enzymes used were obtained from the protein data bank (PDB) with codes

7CB1 (Chitinase B), 3N9K (1,3-β-Glucanase), 6BN0 (AVR4) and 4B8V (ECP6) [27] [28] [29] [30]. They were processed with the Protein Preparation Assistant [31] [32] using the Schrödinger Suite. Structural integrity was reviewed and adjusted; missing recurrences and loop segments near the active site were added by Prime. Hydrogen atoms were added to each protein to returned it to its original state. The protonation and tautomeric states of Asp, Glu, Arg, Lys and His were adjusted to a pH of 7.4. The water molecules were removed within a 5 Å sphere at the active site. The orientation of the hydrogen bonds was adjusted around the active site using PROPKA at a pH of 7.4. Finally, the protein-ligand complex was minimized using the OPLS4 force field [33], with a convergence of heavy atoms with an RMSD of 0.3 Å.

Reference ligands (substrates, inhibitors, endogenous ligands) were obtained from crystals. 22-oxocholestane steroidal derivatives were designed according to the 2D Sketcher program of the Master program in Schrödinger Suite, and converted to their most stable 3D conformer. For molecular docking, ligands were prepared using LigPrep [34] from Schrödinger Suite. After of 3D structures were generated, the OPLS4 force field and the loads were prepared in all stages. All possible protonated centers and ionization states were calculated for the scaffold using ionizer at pH 7.4. The stereoisomers were retained according to their original structures limited to 32 isomers for each ligand. Tautomeric states were generated for each group. The conformers with the best energy were selected for each ligand.

2.2. Docking Protocol

Molecular docking between catalytic sites and substrates was performed using the Glide module [35] [36] from Maestro 12.2 and the receptor grid for each target was prepared using OPLS4. Each grid was built based on co-crystallized reference inhibitors or ligands. The softening of the non-polar parts of the receivers was carried out by scaling the van der Waals radii by a 0.8 factor. Atoms were considered as nonpolar if their absolute partial atomic charge was determined to be <0.25. In flexibility, additional ligand rotations were allowed for the hydroxyl groups at Ser, Thr, and Tyr, and the thiol group at the Cys residues. Furthermore, the lowest binding position of each ligand was maintained. Slip coupling scores were performed in three high-throughput virtual detection modes (HTVS), standard precision (SP), and additional precision (XP). Before SPGP were coupling, the coupling with reference molecules of the respective target proteins was performed to validate the coupling protocol by limiting RMSE as heat cutoff < 2.0 Å in all proteins in the validation process. The XP mode to dock was applied.

3. Results and Discussion

The synthesized bioactive SPGP, SPGP1-SPGP15 (22-oxocholestane compounds) through an acetolysis opening of the spiroketal framework of spirostan steroids

has been reported by our research group [9] [11]. Various modifications at A, B and C rings and at C-26 were introduced (**Figure 2**): at C-3 an acetoxy, or an α,β-unsaturated ketone group is found. At ring B, a double bond at C-5 or a ketone group at C-6 is present. At ring C, the presence of a carbonyl group at C-12 was studied. Ring D was derivatized by the introduction of an acetoxy or a ketone group at C-16. The 22-oxocholestane side chain was modified introducing oxygenated groups at C-26 or introducing a trans diol at C-22 and C-23; similar function present in natural brassinosteroids [37].

Two enzymes associated with fungal proliferation were studied: chitinase B, which is responsible for the regulation of chitin in the fungus, and 1,3-β-glucanase [38]. Both enzymes are associated with the hydrolysis of 1,3- and 1,6-β-glucan (**Figure 2**). For the case of chitinase B, the chitin fragment has a better coupling energy than most plant growth promoters: SPGP13, SPGP7 and SPGP1 have better energy than the reference inhibitor (FO0), while SPGP12 presents the same level. For Glucanase, the SPGP15 compound has a greater affinity for the enzyme than 1,3-Glucane and Apegin, while SPGP7 has the same energy the substrate (**Figure 3**).

The AVR4 and ECP6 effectors are responsible for the intramolecular regulation and protection of chitin, so the bound to the active site of this is essential for fungal non-proliferation. For AVR4, SPGP1 and SPGP6 compounds have a higher energy than the Chitin fragment that binds at the site, while in the case of

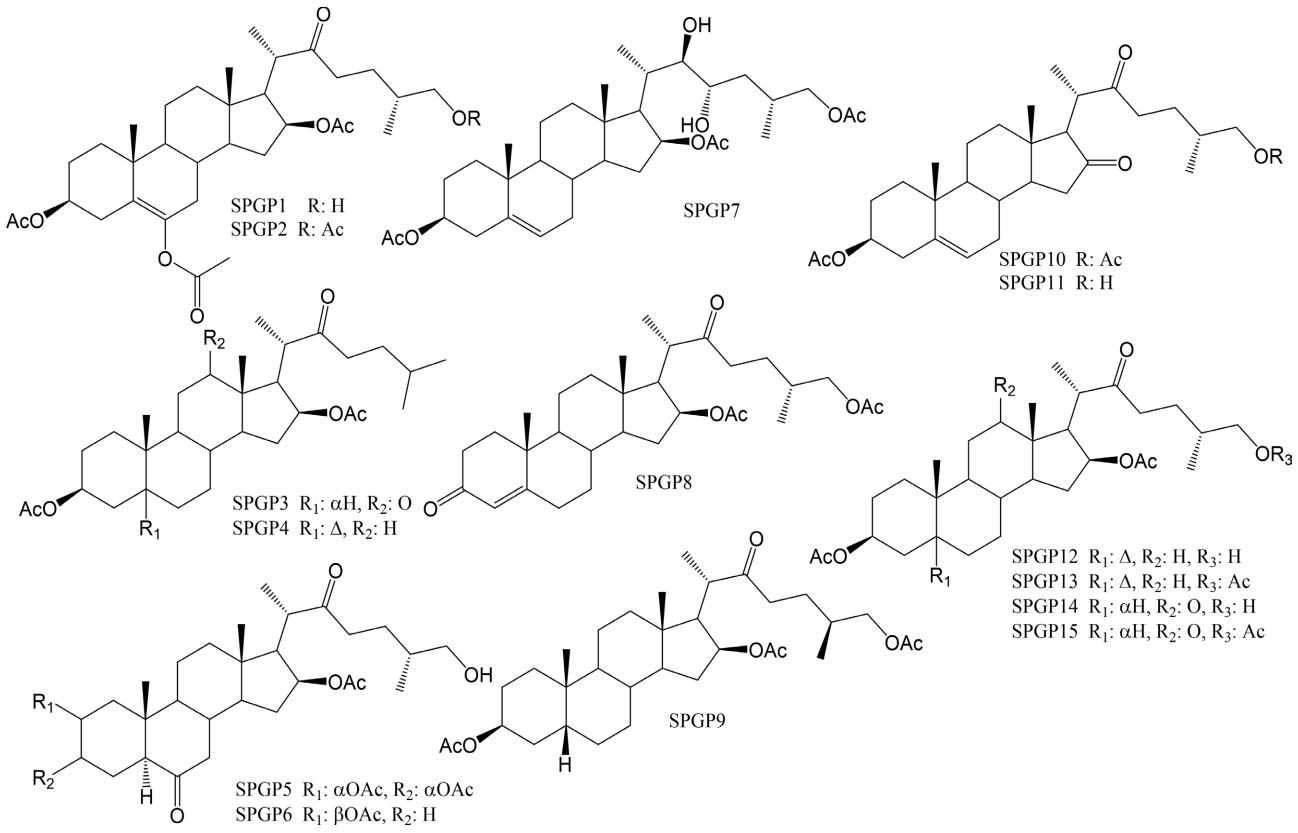

Figure 2. SPGPs evaluated *in silico* of antifungal targets.

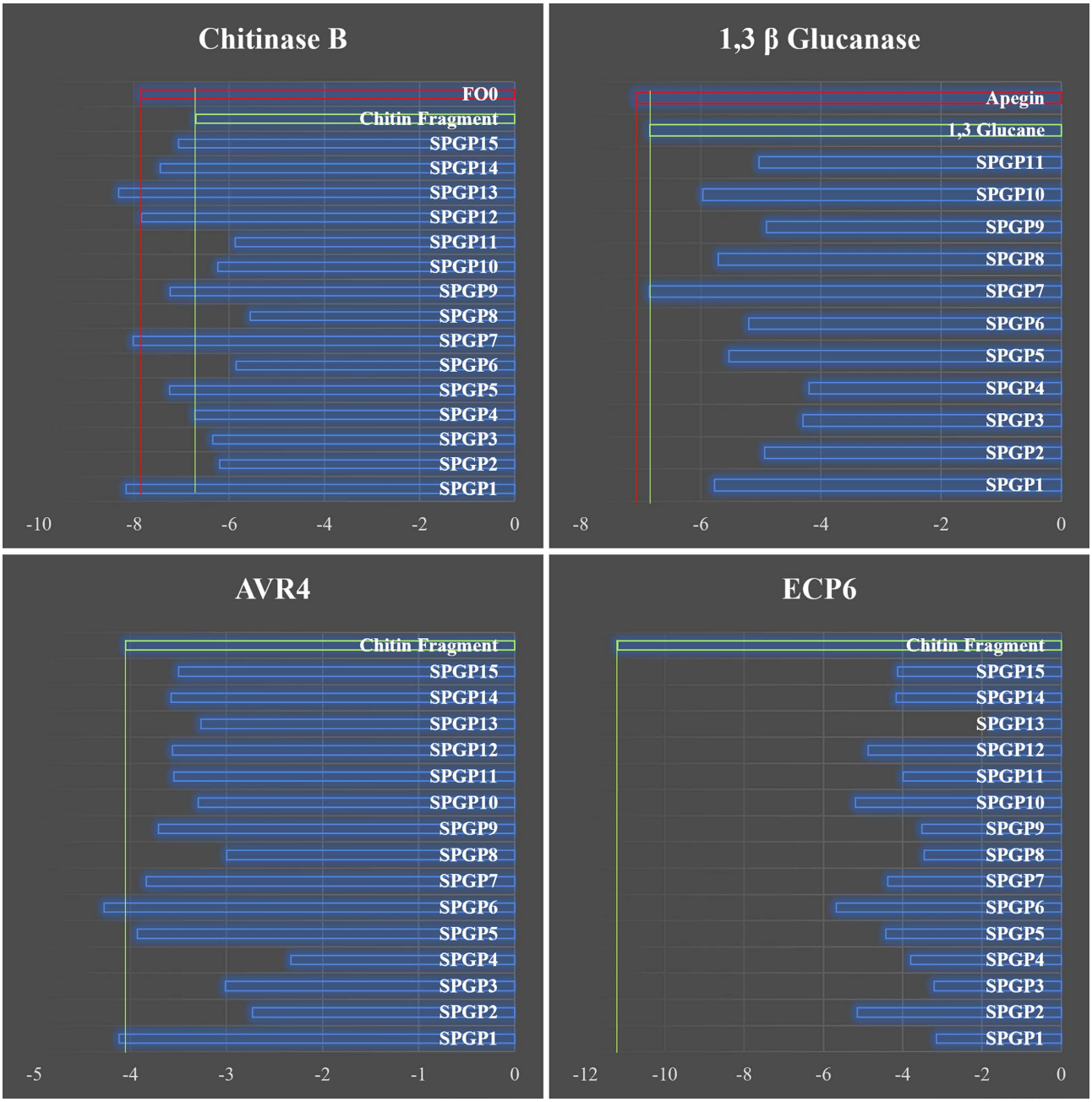

Figure 3. Docking score (kcal/mol) of SPGP in Chitinase B and 1,3-*β*-Glucanase enzymes and effectors AVR4 and ECP6.

ECP6, no steroidal derivative has the potential to bound to the active site of this protein. This study is at the energy level, which in the cases of the enzyme has previously established a direct relationship between the coupling energy and the enzyme inhibition constant [39], but they should be studied accordingly to the amino acid residues interact.

3.1. Chitinase B

Chitinase B is a chitinolytic enzyme responsible for the regulation of chitin levels in the invading fungus. Chitin is an essential protein (natural substrate) for fun-

gus proliferation. Herein, the first objective is to evaluate a DS between SPGP and Chitin, compared to the reference inhibitor, to delimit its potential. It was found that both Chitin and SPGP can be placed in the catalytic site; this fact validates the docking and the inhibitory potential of the steroid derivatives. The steroidal nuclei are hydrophobic compared to Chitin fragment one, as confirmed in Figure 4. The potential inhibitory effect can be observed with interacting amino acids, showing the formation of characteristic hydrogen bridges with TRP97, TRP145 and ASP215. These interactions can be observed in both the chitin fragment and the reference inhibitor. Most of the steroidal derivatives present hydrogen bridges with TRP97, even that the compounds with the best DS also present with TRP145, and in some cases with others such as GLH144 (Figure 4).

The van der Waals and π-alkyl interactions are due to the acetyl groups of the substrate; this fact reveals importance highlighting the lipophilic character of the SPGP derivatives. The hydrophobic caracter of the rings let to observe interactions with with PHE191, TYR145, TRP97 and PHE190 in the case of SPGP13, SPGP7 and SPGP1 (Table 2). SPGP7 there is a hydrogen bridge with TRP220, as well as SPGP12, which has better DS than chitin but not than the reference inhibitor, demonstrating the importance of this residue.

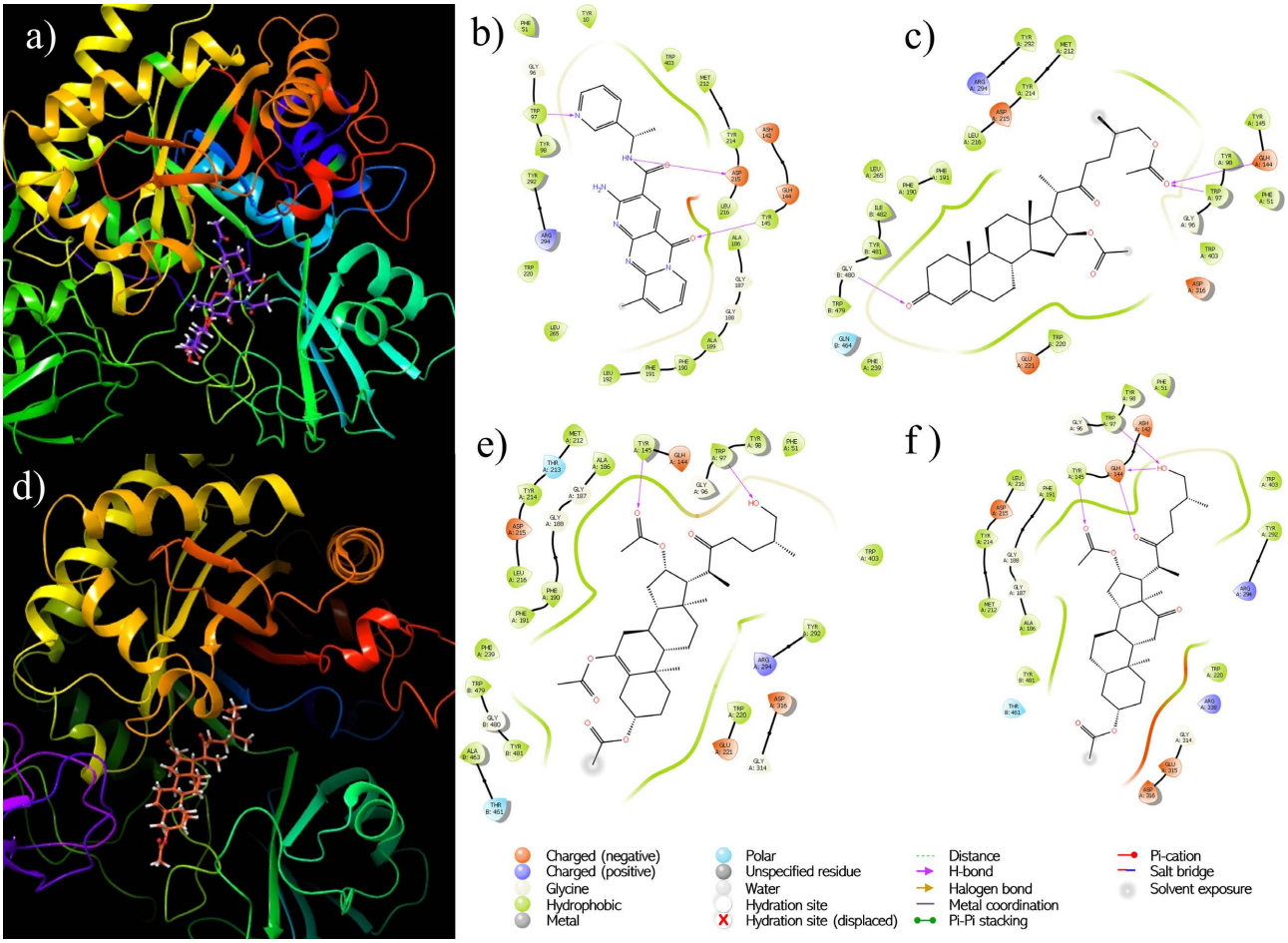

Figure 4. Binding mode in Chitinase B. (a) Chitin Fragment, (b) FO0, (c) SPGP8, (d) SPGP15, (e) SPGP1, (f) SPGP14.

Table 2. Interaction of amino acid residues to SPGP at the Chitinase B catalytic site.

Compound	Van der Waals and Pi-Alkyl	Conventional Hidrogen Bond	Others
Chitin Fragment	ALA A:145 **PHE A:191 TYR A:145 TRP A:97** LEU A:265, **PHE A:190** LEU A:216 GLU A:144 TYR A:214 MET A:212	GLY A:187 GLY A:188 **ASP A:215** TRP A:220	TYR B: 481
SPGP1	ALA A:186 ARG A:294 ASP A:215 ASP A:316 GLU A:315 GLY A:188 GLY A:314 ILE A:339 LEU A:216 MET A:212 **PHE A:190 PHE A:191** PRO A:317 **TRP A:97** TYR A:318 TYR B:481	TRP A:220 GLY A:187 ARG A:338	**TRP A:220**, ASP A:336
SPGP2	ARG A:294 TRP A:97 ASP A:215 TRP A:220 GLU A:144 GLY A:95 GLY A:96 MET A:212 PHE A:12 **PHE A:191** PHE A:51 PRO A:14 TYR A:145 TYR A:214 TYR A:98 TYR A:99 TYR B:481	**TRP A:97**	TRP A:403
SPGP3	ASP A:215 PHE A:12 GLU A:144 PHE A:51 GLY A:96 **TRP A:97** MET A:212 **PHE A:191** TYR A:214 TYR A:98 TYR A:99		TRP A:97, TRP A:403, **TRP A:220**
SPGP4	ARG A:194 ILE B:482 ASP A:316 **TRP A:220** GLU A:221 **TRP A:97** GLY A:314 TRP B:479 GLY B:480 TYR B:481 **PHE A:190** PHE A:239 THR B:483	TRP A:97	PHE A:191, TRP B:479
SPGP5	ALA A:186 ARG A:338 ASP A:215 ASP A:316 ASP A:336, GLY A:187 ILE A:339 LEU A:216 MET A:212 **PHE A:190**, **PHE A:191** PRO A:219 PRO A:313 TRP A:220 **TRP A:97** TYR A:145	ARG A:294	GLY A:188, GLY A:314
SPGP6	ALA A:186 **TRP A:220** ALA A:217 ARG A:294 GLU A:315 GLY A:188 GLY A:314 LEU A:216 LEU A:265 MET A:212 **PHE A:190 PHE A:191** TRP A:97 TYR A:145 TYR A:214 TYR B:481	**ASP A:215**, ASP A:316, **GLY A:187**	**TRP A:220**, GLU A:221
SPGP7	ASP A:215, GLU A:144, GLY A:95, GLY A:96, MET A:212, PHE A:12, **PHE A:191**, PHE A:51, TRP A:403, TYR A:214, **TRP A:97**, TYR A:98, TYR A:99,	**TRP A:220**	
SPGP8	ARG A:294 TRP A:220 ARG A:338 ASP A:215 ASP A:316, GLY A:314 GLY B:480 ILE B:482 **PHE A:190** THR B:483, TYR A:145 TYR B:481	ARG A:194 TRP B:479	PHE A:191 **TRP A:220**
SPGP9	ALA A:186 **TRP A:220** ARG A:294 ARG A:338 ASP A:215, ASP A:316 ASP A:336 GLU A:315 GLY A:188 GLY A:314 ILE A:339 LEU A:216 LEU A:265 MET A:212 **PHE A:190 PHE A:191**, **TRP A:97** TYR A:145 TYR B:481	GLY A:187, **TRP A:220**	**TRP A:220**
SPGP10	ASP A:215 **PHE A:191** GLU A:221 GLY A:96 GLY B:480, MET A:212 PHE A:51 **TRP A:220** TRP B:479 TYR A:214, TYR A:99 TYR B:481	TRP A:98	PHE A:190 TRP A:87 TRP A:403, GLU A:144
SPGP11	ALA B:491 TYR B:481 ASP A:316 ASP B:489 GLY B:480 ILE B:482 SER B:484 THR B:483 **TRP A:220** TRP B:479	ARG A:194 TRP A:97	PHE A:191, PHE A:190
SPGP12	ARG A:294, ASP A:215, ASP A:316, ASP A:336, GLY B:480, ILE A:339, **PHE A:190**, **PHE A:191**, **TRP A:97**, TRP B:479, TRP B:481, TYR A:481, PHE A:190, **TRP A:220**	ARG A:338, GLU A:221, **TRP A:220**	PHE A:190, **TRP A:220**
SPGP13	ARG A:294, ASP A:215, ASP A:316, ASP A:336, GLY B:480, ILE A:339, ILE B:482, **PHE A:190**, **PHE A:191**, THR B:483, **TRP A:97**, TYR B:481, **TRP A:220**	ARG A:194, ARG A:338, TRP B:479	
SPGP14	ASP A:215 **TRP A:97** GLU A:144 GLY A:96 MET A:212 PHE A:12 **PHE A:191** PHE A:51 TYR A:214 TYR A:98 TYR A:99	GLY A:95	**TRP A:220**, TRP A:403, TRP A:97
SPGP15	ASP A:215 **TRP A:97** GLU A:144 GLY A:95 GLY A:96 MET A:212 PHE A:12 **PHE A:191** PHE A:51 TRP A:403 TYR A:214 TYR A:98 TYR A:99		TRP A:97, **TRP A:220**

3.2. 1,3-β-Glucanase

The main function of 1,3-β-Glucanase lies in the hydrolysis of these glucans, necessary for the adaptation-proliferation process of phytpathogen. It is a so specific enzyme that there are only a few selective inhibitors, such as Apegin. The formation of a hydrogen bridge between the residue TYR29 and GLU192 is crucial in the catalytic process of the breakdown of b-glucan into monosaccharide units.

The hydrophobic nature of the steroid nuclei hinders the interaction with the catalytic site of the enzyme (Figure 5). SPGP7 and SPGP15 interact in a polar way with the amino acids GLH 27 and ASP 145 due to its polyoxygenated functions. The latter is important to be underlined in the glucanase hydrolysis process, having a mechanism like Apegin, with the advantage that these SPGP interact with PHE258 and ASN191. SPGP15 also interacts with PHE144. For the case of phytopathogen glucanase-dependent SPGP7 and SPG15, demonstrated the need for a high polarity to bind to this enzyme. Chitinase B, that Chitin, by having acetyl groups, the residues in the protein have a hydrophobic character.

The rest of SPGP (1 - 6, 8 - 14) have docking scores between −6.0 kcal/mol to −4.0 kcal/mol (Table 3). These values are not comparable to glucans or commercial inhibitors. This is not necessarily negative, since 1,3-β-Glucanase is present not only in phytopathogens, but also in beneficial fungi for plant development, which allows the use of these SPGPs with a plant growth promoting effect without altering the symbiosis with the kingdom fungi.

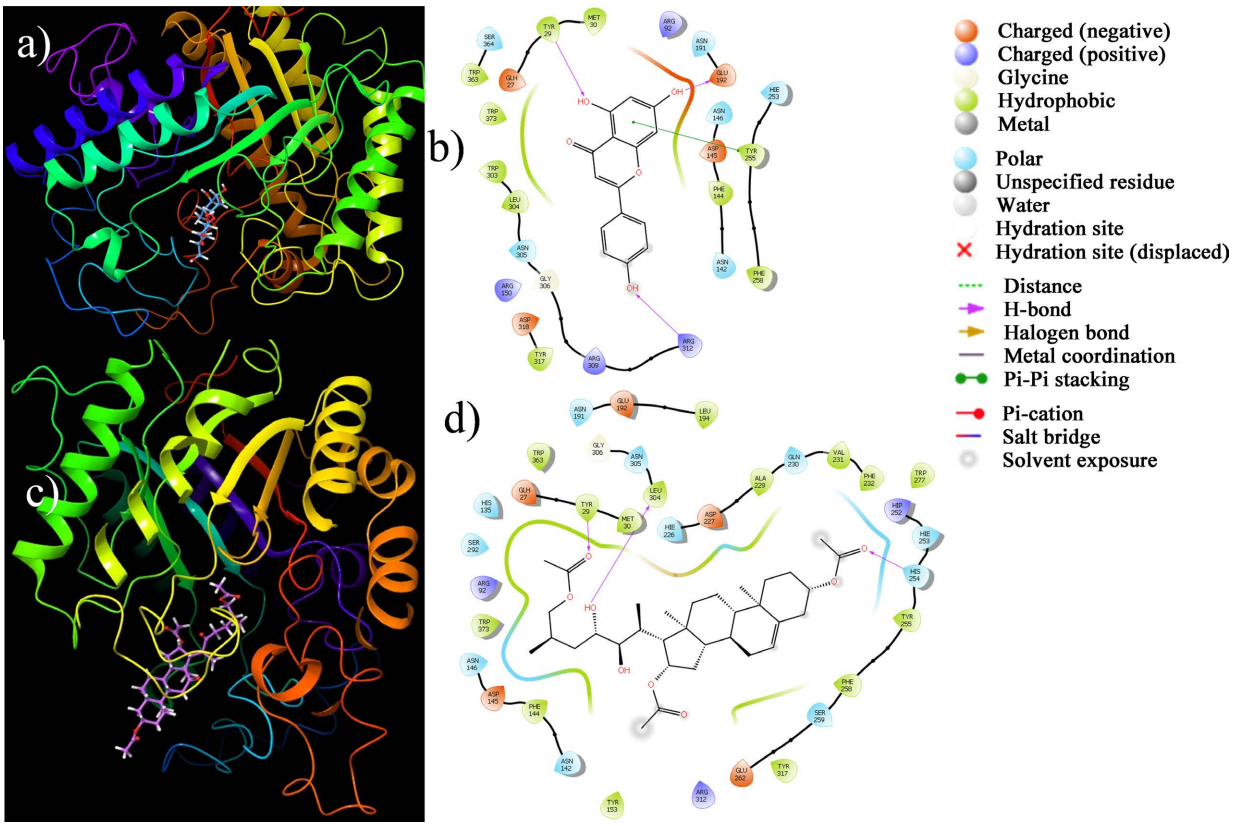

Figure 5. Interaction 1,3-β-Glucanase: (a) 1,3-β-Glucane, (b) Apegin, (c) SPGP15, (d) SPGP7.

Table 3. Amino acid residues interacting to SPGP at the catalytic site of 1,3-β-Glucanase.

Compound	Van der Waals and Pi-Alkyl	Conventional Hidrogen Bond	Others
1,3-β-Glucane	HIE 253 **ASN 191 PHE 258** ASN 305 LEU 304 **PHE 144** ASP 145	TYR 255 **GLU 192 TYR 29 GHL 27** ASN 149 **ARG 312**	ASP 145
Apegin	**ASN 191** ASN 146 **PHE144** ASN 142 GLY 306 **ASN 305 LEU 304** TRP 303 TRP 373	**TYR 29 GLU 192** ARG 211	ARG 92 **ASP 145** ARG 309 **GLH 27** TYR 255
SPGP1	GLY 261 SER 259 **PHE 258** VAL 257 GLN 256 TYR 255 HIE 253 PHE 232 VAL 231 GLN 230 ALA 229 ALA 228 HIE 226 TRP 373 HIS 135 SER 147 **PHE 144** GLY 143 ASN 142 TYR 153	ARG 312 HIS 254 **ASP 145** ASN 146	ARG 265 GLU 262 HIP 252 ASP 227
SPGP2	TYR 153 GLY 306 ASN 305 LEU304 TRP 303 MET 30 **ASN 191** LEU 194 PRO 196 HIE 253 TYR 255 GLN 256 VAL 257 **PHE 258** SER 259	ARG 312 TYR 29 **GLH 27** HIS 254	ARG 309 GLU 191 HIP 252 GLU 262 ARG 265
SPGP3	TYR 317 GLY 306 ASN 305 LEU 304 TRP 303 TRP 373 HIS 135 **ASN 191** LEU 194 PRO 196 HIE 253 HIS 254 TYR 255 GLN 256 VAL 257 **PHE 258** SER 259	ARG 162 TRP 277	ASP 318 ARG 309 GLU 192 GLU 262 ARG 265 ASP 318
SPGP4	TYR 153 ASN 146 **PHE 144** GLY 143 ASN 142 HIE 226 ALA 229 GLN 230 VAL 231 PHE 232 HIE 253 HIS 254 TYR 255 **PHE 258** SER 259	TRP 277	GLY 262 ARG 312 ARG 309 GLU 192
SPGP5	TYR 153 SER 147 **PHE 144** GLY 143 ASN 142 HIE 253 HIS 254 TYR 255 GLN 256 VAL 257 **PHE 258** SER 259	ASN 146 **ASP 145**	ASP 151 ARG 150 HIP 252 GLU 262 ARG 265
SPGP6	TYR 153 SER 147 ASN 142 HIE 253 HIS 254 GLN 256 VAL 257 **PHE 258** SER 259	ASN 146 **ASP 145** TRP 277	ARG 265 GLU 262 HIP 252 ARG 150 ASP 318
SPGP7	SER 259 **PHE 258** VAL 257 GLN 256 TYR 255 HIS 253 PHE 232 VAL 231 GLN 230 ALA 229 ALA 228 HIS 226 PRO 94 MET 30 TRP 303 **ASN 305** GLY 306	HIS 254 LEU 304 TYR 29	GLU 262 HIS 252 ASP 227 ARG 92 **GLH 27** ARG 309 ARG 312
SPGP8	ASN 146 **PHE 144** GLY 143 ASN 142 HIE 253 HIS 254 TYR 255 GLN 256 VAL 257 **PHE 258** SER259 TYR 153 MET 30 TYR 29 HIS 135 ASP 133	ARG 312 **GLH 27**	ARG 312 ARG 309 ARG 150 ARG 265 GLU 262 HIP 252 **ASP 145** ASP 133
SPGP9	TYR 153 GLY 306 ASN 305 LEU 304 TRP 303 PRO 28 MET 30 HIS 135 HIE 253 TYR 255 GLN 256 VAL 257 **PHE 258** SER 259	ARG 312 **TYR 29** HIS 254	ARG 309 **GLH 27** ARG 92 HIP 252 GLU 262 ARG 265
SPGP10	TYR 153 SER 147 **PHE 144** GLY 143 ASN 142 SER 364 TRP 363 HIE 253 VAL 257 **PHE 258 ASN 191** LEU 194 VAL 197	ASN 146 **TYR 255**	ASP 151 ARG 150 **ASP 145** GLU 192 ARG 92
SPGP11	TYR 317 VAL 307 GLY 306 ASN 305 LEU 304 TRP 303 HIE 253 HIS 254 TYR 255 GLN 256 VAL 257 **PHE 258** SER 259	**ASP 145** ARG 265	ARG 312 ARG 309 GLU 262
SPGP12	GLY 306 **ASN 305 LEU 304** TRP 303 PRO 28 TYR 29 MET 30 TRP 373 HIE 226 HIE 253 TYR 255 GLN 256 VAL 257 **PHE 258** SER 259	**ARG 312** HIS 254 **ASP 145**	ARG 309 **GLH 27** HIP 252 GLU 262 ARG 265
SPGP13	TYR 137 GLY 306 **ASN 305 LEU 304** TRP 303 TRP 373 HIE 253 HIS 254 **PHE 258** SER 259	TYR 255 **TYR 29** ARG 312	ARG 309 **GLH 27** ARG 92 ASP 227 GLN 262
SPGP14	TYR 153 GLY 143 **PHE 144** SER 157 TRP 373 TRP 363 HIE 253 HIS 254 TYR 255 GLN 256 VAL 257 PHE 258 SER 259 VAL 273 TYR 317	ASP 145 ASN 146 ARG 265	ASP 318 GLU 262 ARG 312
SPGP15	TRP 153 SER 147 ASN 146 **PHE 144** GLY 143 ASN 142 HIE 253 TYR 255 VAL 257 **PHE 258** SER 259	ASN 146 **GLH 27**	ASP 151 ARG 150 ASP 256 GLU 262

3.3. ECP6 and AVR4

Given that the regulation observed is via Chitinase B, it was interesting to study the effectors by the fungus necessary for fungal proliferation, with ECP6 and AVR4 being critical for the transport and proliferative process via Chitin, an indirect regulation option is by allosterically blocking the site. of transport that is located between two chains both in the ECP6 and the AVR4 (Figure 6(a) and Figure 6(d)). At the energy level, we can observe that only 2 compounds have a higher energy than the chitin fragment in the case of AVR4, but in ECP6 no compound has a competitive energy. At the spatial level we can see that the Chitin fragment in both cases is in the middle of the two chains, the SPGP in all cases bind at that site (Figure 6(b) and Figure 6(e)), but particularly in the case of ECP6 this region inter-chains are highly hydrophilic, so the steroidal nucleus being lipophilic does not allow a strong interaction with the site, although acetates and hydroxyls form hydrogen bonds is not enough to compare with chitin. In the case of AVR4, although chitin is hydrophilic, the site is not completely hydrophilic, presenting interaction with the nucleus and with a carbonyl or a enol at C-6 for SPGP1 and SPGP6 (Figure 6(c) and Figure 6(f)).

At the specific level for AVR4, the compounds interact at the particular site and are supported with an increase in van der Walls and π-alkyl interactions, as can be seen in Table 4, the amino acids for the chitin fraction are repeated in

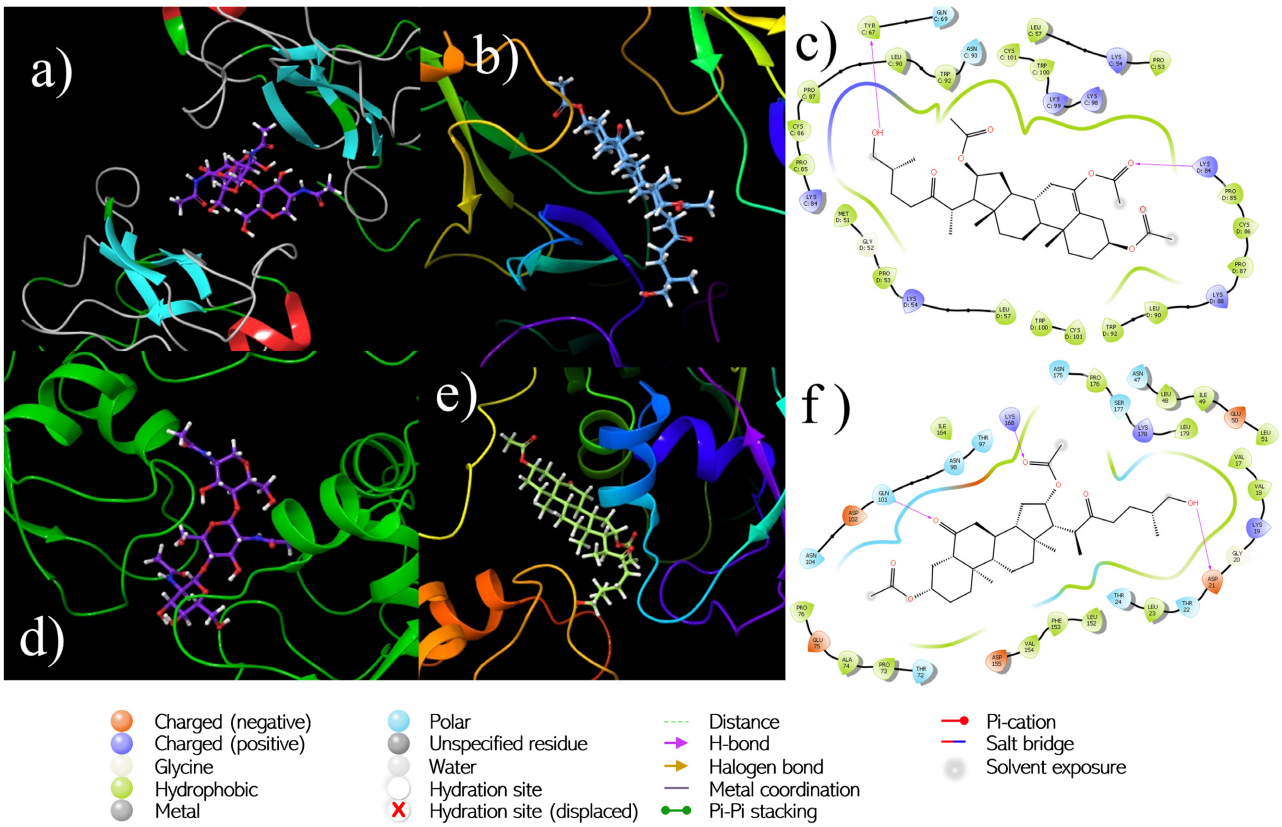

Figure 6. Binding mode for (a) Chitin Fragment vs. ECP6, (b) SPGP6 vs. ECP6, (c) SPGP1 vs. ECP6, (d) Chitin Fragment vs. AVR4, (e) SPGP1 vs. AVR4, (f) SPGP6 vs. AVR4.

Table 4. Amino acid residues with interaction of SPGP with the catalitic site of AVR4.

Compound	Van der Waals and Pi-Alkyl	Conventional Hidrogen Bond	Others
SPGP1	CYS C:101, TRP C:100, LEU C:57, PRO C:53, PRO D:85, CYS D:86, PRO D:87, LEU D:90, TRP D:92, GLN D:69, TYR D:67, CYS D:101, TRP D:100, LEU D:57, PRO D:53, GLY D:52, MET D:51, PRO C:85, CYS C:86, PRO C:87, LEU C:98, TRP C:92, ASN C:93, TYR C:67, GLN C:69	LYS D:84, TYR C:67	LYS D:84, LYS C:54, LYS C:98, LYS C:99, LYS D:88, LYS D:54, LYS C:84
SPGP2	CYS C:50, MET C:51, GLY C:52, PRO C:53, LEU C:57, TYR D:67, GLN D:69, GLY C:97, TRP C:100, CYS C:101, PRO A:61, SER A:63, CYS A:64, TRP C:92, LEU C:90, GLY C:89, PRO D:53, PRO C:87, GLY D:52, CYS C:86, MET D:51, CYS D:50, PRO C:85, VAL C:83, VAL C:82, TYR C:67, GLN C:69	LYS C.84, TYR C:67, LYS C:54	LYS C:54, ASP C:55, LYS D:84, LYS C:98, LYS C:99, ASP A:62, CL A:208, ASP D:55, LYS D:54, LYS C:88, LYS C:84
SPGP3	PRO D:87, TRP D:100, CYS D:101, LEU D:90, LEU C:106, TYR C:103, ASN C:93, CYS C:101, TRP C:92, TRP C:100, LEU C: 90, MET D:51, PRO C:87, GLY D:52, CYS C:86, PRO D.53, PRO C:85, TYR C:67, GLN C:69, LEU C:57, RPO C:53, GLY C:52, TYR D:67, GLN D:69		LYS D:84, ASP C:102, LYS C:99, LYS C:98, LYS C:84, ASP C.55, LYS C:54
SPGP4	PRO D:87, LEU D:90, THR A:113, LEU C:106, ASN C:105, TYR C:103, TYR D:103, CYS C:101, TRP D:100, TRP C:100, MET D:51, GLY D:52, PRO D:53, PRO C:85, CYS C:86, PRO C:87, TYR C:67, LEU C:90, TRP C:92, ASN C:93		LYS A:112, ASP C:102, LYS C:98
SPGP5	TYR C:58, LEU C:57, THR C:65, THR C:66, TYR C:67, ILE C:68, GLN C:69, PRO C:53, GLY C:52, TRP C:100, CYS C:101, CYS D:86, GLN D:69, TYR D:67, PRO D:87, TYR C:103, PRO C:104, LEU C:106, TYR D:103, CYS D:101, TRP D:100, GLY D:97, ASN C:93, THR A:113, TRP C:92, GLN C:91, LEU C:90, GLY C:89, LEU D:57, VAL A:111, PRO D:53, PRO C:87, GLY D.52, CYS C:86, PRO C:85, MET D:51, CYS D:50, VAL C:83, VAL C:82, THR D:48	LYS C:84, MET D:51	ASP C:55, LYS C:54, LYS C:98, LYS C.99, LYS C:84, ASP C:102, ASP D:102, LYS C:88, LYS C:54, LYS D.49, LYS C:84
SPGP6	TYR D:67, GLN D:69, LEU C:57, PRO C:53, GLY C:52, CYS D:85, PRO D:87, LEU D:90, TRP D:100, CYS D:101, LEU C:106, TYR C:103, CYS C:101, TRP C:100, ASN C:93, TRP C:92, PRO D.53, GLY D:52, MET D:51, TYR C:67, LEU C:90, GLN C:69, PRO C:87, CYS C:86, PRO C:85	LYS C:98, ASP C:102, TYR C:67	LYS C:54, LYS D:84, ASP C:102, LYS C:99, LYS C:98, LYS C:84
SPGP7	VAL C:82, VAL C:83, PRO C:85, TYR C:67, GLN C:69, CYS C:86, CYS D:50, PRO C:87, MET D:51, GLY D:52, PRO D:53, LEU C:90, LEU C:57, TRP C:92, GLN D:69, TYR D.67, PRO C:53, LEU D:57, TRP D:92, LEU D:90, PRO D:87, CYS D:86, PRO D:85, CYS D:101. TRP D:100, TRP C:100, CYS C:101	LYS C:84, PRO C:85, MET D:51	LYS C:84, LYS C:88, LYS D:54, ASP D:55, LYS C:54, LYS D:84
SPGP8	GLN D:69, TYR D:67, PRO C:53, PRO D:87, TRP D:100, CYS D:101, LEU C:57, LEU D:90, TRP D:92, TRP C:100, ASN C:93, TRP C:92, CYS C:101, TYR C:67, LEU C:90, TYR C:103, LEU C.106, PRO C:87, CYS C:86, CYS D:50, MET D:51, GLY D:52, PRO D:53, LEU D:57	TYR D:67, LYS C:98	LYS D:84, LYS C:54, LYS D:99, LYS C:98, LYS C:99, ASP C:102, LYS D:54, ASP D:55
SPGP9	PRO D:85, CYS D:86, LEU D:106, PRO D:87, TYR D:103, TYR D:67, LEU D.90, CYS D:101, TRP D:100, TRP D:92, ASN D:93, THR A:113, TYR C:103, CYS C:101, TRP C:100, PRO C:85, CYS C:86, PRO C:87, LEU C:90, TYR C:67, MET D:51, GLY D:52, PRO D:53, TRP C:92, ASN C:93	CYS D:101, LYS D:98	ASP D:102, LYS D:99, LYS D:98, ASP C:102, LYS D:99, LYS D:54
SPGP10	GLN D:69, TYR D:67, PRO D:85, CYS D:86, LEU D:106, PRO D:87, TYR D:103, LEU D:90, TRP D:92, ASN D:93, CYS D:101, TRP D:100, TYR C:103, PRO D:53, LEU C:57, TRP C:100, LEU D:57, PRO C:53	LYS D:84, CYS D:101, LYS D:98	LYS D:84, ASP D:102, LYS D:99. LYS D:98, LYS D:54, LYS C:99, LYS C:98, LYS C:54

Continued

SPGP11	PRO D.85, PRO D:87, TYR D:67, GLN D:69, TRP C:92, LEU C:90, PRO C:87, CYS C:86, PRO C:85, PRO D:53, GLY D:52, MET D:51, CYS D:50, GLN C:69, TYR C:67, LEU C:57, PRO C:53, CYS C:101, TRP C:100, GLY C:97	LYS C:84, MET D:51	LYS D:84, LYS C:84, ASP C:102, LYS C:99, LYS C:54, LYS C:98
SPGP12	MET D:51, GLY D:52, PRO D:53, LEU D:57, TYR C:67, GLN C:69, PRO C:53, PRO C:85, CYS C:86, PRO C:87, LEU C:90, TRP C:92, GLN D:69, TRP D:92, CYS D:101, LEU C:57, PRO D:85, CYS D:86, PRO D:87,	LYS C:54, TYR D:67	LYS C:84, LYS D:54, ASP D:55, LYS C:54, LYS D:84, LYS C:98, LYS C:99, ASP C:102
SPGP13	MET D:51, GLY D:52, PRO D:53, PRO D:87, LEU D:106, LEU D:90, TYR D:103, ASN D:93, CYS D:101, TRP D:100, THR A:113, TYR C:103, TRP C:92, LEU C:90, CYS C:101, PRO C:53, TRP C:100, PRO C:87, CYS C:86, LEU C:57, PRO C:85, TYR C:67	LYS D:98	LYS D:54, ASP D:102, LYS D:98, ASP C:102, LYS C:99, LYS C:54, LYS C:98, LYS C: 84
SPGP14	TYR C:67, TRP C:100, CYS C:101, TYR C:103, PRO D:87, LEU D:106, LEU D:90, TYR D:103, ASN D:93, CYS D:101, TRP D:100, THR A:113, VAL A:111, PRO D:53, TRP C:92, LEU C:90, PRO C:87, CYS C:87, CYS C:88, PRO C:85	CYS C:101, ASP D:102, LYS D:98	LYS C:99, ASP C:102,, LYS A:112, LYS D:54, LYS C:84
SPGP15	PRO C:53, GLN D:69, TYR D:67, PRO D:85, LEU D:106, CYS D:86, PRO D:87, TYR D:103, LEU D:90, CYS D:101, TRP D:92, TRP D:100, ASN D:93, THR A:113, TYR C:103, PRO C:87, CYS C:101, TRP C:100, LEU C:90, LEU D:57, PRO D:53, GLY D:52	TYR D:67, LYS D:98	LYS D:84, ASP D:102, LYS D:99, LYS D:98, LYS D:54

interaction for derivatives SPGP, but in the formation of hydrogen bridges is where it is key for SPGP1 and SPGP6 since they interact with TYR D: 67, as well as annexing TYR C: 67, ASP C: 102 and LYS C: 98, which its increases the coupling energy allowing it to compete in the case of SPGP1 at the same energy level and for SPGP6 with a better DS than the endogenous ligand, thus allowing the AVR4 effector to be blocked.

4. Conclusion

SPGPs compounds have a huge plant growth promoting effect in various biological systems, and an effect against phytopathogens (specifically for Chitinase B and 1,3-β-Glucanase). Some SPGPs were studied *in silico* finding 5 competitive inhibitors better than Chitin and 4 preferred than FO0 (reference inhibitor). While for 1,3-β-Glucanase, 2 potential inhibitors were found (SPGP7 at the level of 1,3-β-glucane and SPGP15) having a better activity than Apegin (reference inhibitor). For the blockage of chitin effectors (AVR4 and ECP6), only an allosteric blockade against AVR4 was achieved, so the 22-oxocholestan studied compounds have a latent potential as inhibitors of fungal proliferation at the enzymatic level. In conclusion, SPGPs have a potential dual action, as promoters for plant growth and as antifungal against phytopagens.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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