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EVALUATION OF NATURAL COMPOUNDS AS TARGETED ACTIVATORS AGAINST SYSTEMIN RECEPTOR 160 FOR DEFENSE GENES ACTIVATION IN THE SOLANACEAE FAMILY

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Abstract

Plants subjected to injury-causing substances are activating healing and other defense systems. Mechanical damage response involves either local, systemic or both, and consequently, wound signal production, translocation and detection, and transduction to trigger wound-inducible expression of genes. They become an effective target systemin receptor 160 (SR160) for the downstream process to activate the defense genes in the *Solanaceae* family. In-silico investigations of SR160 protein against natural chemicals have been presented. This study will create and anticipate potential modes of interaction and binding affinities between the natural substances and model SR160 as a 3-dimensional (3D) structure for SR160. The SR160 3D was designed utilizing the SWISS-MODEL system, while the InterBioScreen database provided the compound library. Maestro 10.5 was used for molecular docking. The binding energy ranged from -9.126 kcal to -12.813 mol in all tested compounds. Thus, active commercial defensive gene activators for wound healing can be further synthesized.

Key words: SR-160 receptor, Homology modeling, SWISS-MODEL, Docking and Wound healing.

Introduction

Plant resistance inducers (PRIs) are agents that lead to improved protection to pathogen attacks by inducing the plant's own defense mechanisms, so called induced resistance.

In current age of agriculture, few strategies such as resistance breeding and use of chemical pesticides have been applied against the crop pathogens. Based on the pathogenic impact on crop, the current conventional techniques are time consuming. It is due to rapid growth of pathogen (Alexandersson *et al*., 2016). Plants are sessile, rooted creatures to receive water and nutrients; therefore, there is a lack of all possible protection measures to prevent insect chewing or injury by larger herbivores (Howe & Jander, 2008; Wang *et al*., 2018). The plants are protected by physical obstacles which prevent damage by cuticular and tough woody surfaces, which are successfully resistant to the hostility of small herbivores, or others have the possibility of trichomas, spins and other specialized organizations (Leon *et al*., 2001). When damage occurs, specialist cells for wound healing, such as mammals, cannot be mobilized because plant cells are embedded inside stiff walls (Leon *et al*., 2001; Zhang *et al*., 2019). In this case, when plant are affected by the numerous kind of pathogens, its secreate their own plant restant inducer agents and improved the growth of the plants. Therefore, the plants have evolved to enable every cell to engage defense responses that depend greatly on the transcriptional expression of particular genes (Zhang *et al*., 2019) which ultimately leds to improve the plant defence system. These injury-activated processes are designed to cure the tissues harmed and activate protective mechanisms to prevent further damage (Schwab *et al*., 2016; Scandalios, 2005; Walters *et al*., 2006).

Many plant species are protected by defensive chemical synthesization from predators (Zvereva & Kozlov; 2016; Bowers, 1992). Plants create a polypeptide 18-amino acid hormone system, which activates defense genes (Pearce & Ryan, 2003; Rayan & Pearce, 2003). Systemin is released on tomato leaves a wound, where it interacts to the systemin 160 (SR160) surface receptor and initiates the downstream process of gene activation (Yadav *et al*., 2015). Systemin is required for defense signaling in tomatoes. It promotes the production of over 20 defense proteins, the majority of which are antinutritional proteins, signaling cascade proteins, and proteases (Coppola *et al*., 2015; Wasternack, & Hause, 2002). Prosystemin overexpression resulted in a significant reduction in larvae injury, implying that constitutive defense is preferable to mediated defense in plants (Degenhardt *et al*., 2010). In the present study, attempts were made to develop an SR160 model to analyze and bind natural compounds with SR160 using modern bioinformatics tools.

Materials and Methods

Systemin receptor 160 homology modeling and sequence alignment of the target template: As the systemin receptor 160 (SR160) crystal structure does not exist in the PDB (Protein Data Bank), its three-dimensional structure has been forecast (Burley *et al*., 2019). The target protein ID (SR160) was first obtained from UniProtKB (UniProt Knowledgebase) accession number AAM48285.1 (Dutta & Lahiri, 2017). The protein has been transferred to the SWISS-MODEL online service to produce a model with the required search sequence and identity coverage (Arnold *et al*., 2006). The most dependable 3D structure has been selected based on the GMQE and QMEAN values. The most reliable structure was chosen. Generally, the GMQE values range from 0 to 1. The more numerical, the more dependable the planned construction is, but a score less than 4.0 shows QMEAN confidence. Clustal Omega 1.2.1 was used to verify the identity of the amino acid sequence in an

SR160 homology model and the template design utilised for the homology model 9 (Sievers & Higgins, 2018).

Structure authentication of modeled protein: The web server SWISS-MODEL creates the QMEAN measurement feature, based on the protein model's geometry, interaction and solvent potential, to measure local and global model quality. It also generates a z-score that may be compared to the predicted value for any structure. PROCHECK verified the precision of the SR160 model 3D structure from SWISS-MODEL (Laskowski *et al*., 1993). The modeled SR160's.pdb file format was uploaded to the European Bioinformatics Institute's PDBsum website (Laskowski, *et al*., 2018). The Ramachandran plot and statistics were obtained by uploading the modeled SR160's.pdb file format to the server. The statistics on the Ramachandran Plot reflect the overall number of amino acid residues in favorable and acceptable regions, whereas the Ramachandran Plot evaluates the quality of an experimental or modeling protein. Verify 3D has also examined the model protein structure, a 3D structure compatible with amino acids was established, and the results were compared with recognized structures (Lüthy *et al*., 1992).

SR160 model alignment and structure of the template: PyMOL molecular viewer has been used to align the SR160 model and model structure with illustrating the intimate relationship between carbon atoms (DeLano *et al*., 2002). This is measured by the RMSD (root mean square deviation) between the site of the carbon atoms in the template and the alignment model. The smaller the RMSD, the related to the structures.

Prediction for active protein site: Active sites in the modeled protein structure have been predicted by onlne tools CASTp 3.0 9 (Tian *et al*., 2018). CASTp is an online tool for detecting and measuring voids in three-dimensional protein structure and the binding pockets.

Selection of Ligands and protein molecule: In this study, we chose ligand molecules from a natural compound library found in the Inter BioScreen database. As previously mentioned, the LigPrep module for the Maestro 10.5 app is used to configure ligands (Singh & Bast, 2014). Finally, using Maestro 10.5 Schrodinger and the Linux 64 package [Schrodinger, Version 10.5], ligands are ready for docking and binding modes with target SR160. The Maestro 10.5 optimization wizard was created based on previous research and was preceded by a molecular docking analysis.

Molecular docking: The Maestro 10.5 suite was used to perform molecular docking studies with selected ligand molecules, as previously described (Friesner *et al*., 2004; 2016; Tripathi *et al*., 2013). We compared approximately 25,000 compounds from InterBioScreen data to SR160 in this study. HTVS was used to dock InterBioScreen database compounds onto all selected protein molecules. Following that, the Glide-XP mode was applied to the particular diagnosis from the HTVS mode, resulting in a strong correlation between correct postures and high scores. We choose five compounds for GLIDE XP Molecular Docking based on the G-score. The most suited compounds for each target were chosen based on the ideal energy value responsible for the observable activity and interaction of the compounds (hydrogen bonds, pie-pie interactions, and hydrophobic interactions).

Results and Discussion

Homology modeling and sturture validations of systemin receptor 160: SWISS-MODEL was used to developed the 3D structure of SR160 with help of a GMQE of 0.42 and a QMEAN of 1.47 scores. Moreover, it was shown that the BRI1 Gly644-Asp (bri1-6) mutant of *Arabidopsis thaliana* has a similar template SR160, with 59.95% similarity and 0.50 identical sequence (PDB ID: 6FIF; resolution: 2.54). The model structure is confident and reasonable, as shown by 0.42 GMQE and 1.47 QMEAN scores. The multiple sequence alignment of the SR160 (UniProtKB ID: Q8L899) and BRI1 Gly644-Asp (bri1-6) mutants from Arabidopsis thaliana (PDB ID: 6FIF) amino acid sequences have shoen in (Fig. 1). The 59.95% homology model identity has been confirmed by a percentage identity matrix of 59.99%.

The estimated local target similarity was displayed in a graphic against the estimated 3D structure of the modeled protein's residue number (Fig. 2A). The majority of residues had values around 1, indicating that the predicted model's local residue quality assessment is correct. Residues of low quality were identified as those with a value of less than 0.4. The model protein structure is also covered by other crystal structures in PDB and implies that the protein structure is reliable (Fig. 2B). The protein confirmation has been analysed by online PDBsum server to developed the Ramachandran plot (Fig. 2C). According to the confirmation of the Ramachandran plot, 99.9% of residues of SR160 modeled 3D are in the most favored regions, 22.5% are additionally permitted areas, 1.1% are generously permitted regions and 0.0% in prohibited Ramachandrane areas. The 3D structure model is also confirmed to be of great quality. For structure validation, the Verify3D plot of the model protein was generated (Fig. 2D). The 3D environment profile reveals a 3D-1D score of 0.2 at 97.33 % of the residues showing the validity of the modeled protein.

The alignment calculated with the molecular PyMOL viewer resulted in the RMDS value of 0.104, showing a close connection between the structures (Fig. 3). The modeled protein structure is shown by cyan helices whereas the templete protein have shown by hot pink after the generation of model (Fig. 3A). According to the alignment, Chain A of the monomer template structure (6FIF) was Chain A of the protein model's monomer structure. Meanwhile, the structural alignment with the Arabidopsis thaliana BRI1 Gly644-Asp is the best structural homolog of the top-scoring SWISS-MODEL model (bri1-6) (PDB ID: 6FIF) (Fig. 3C).

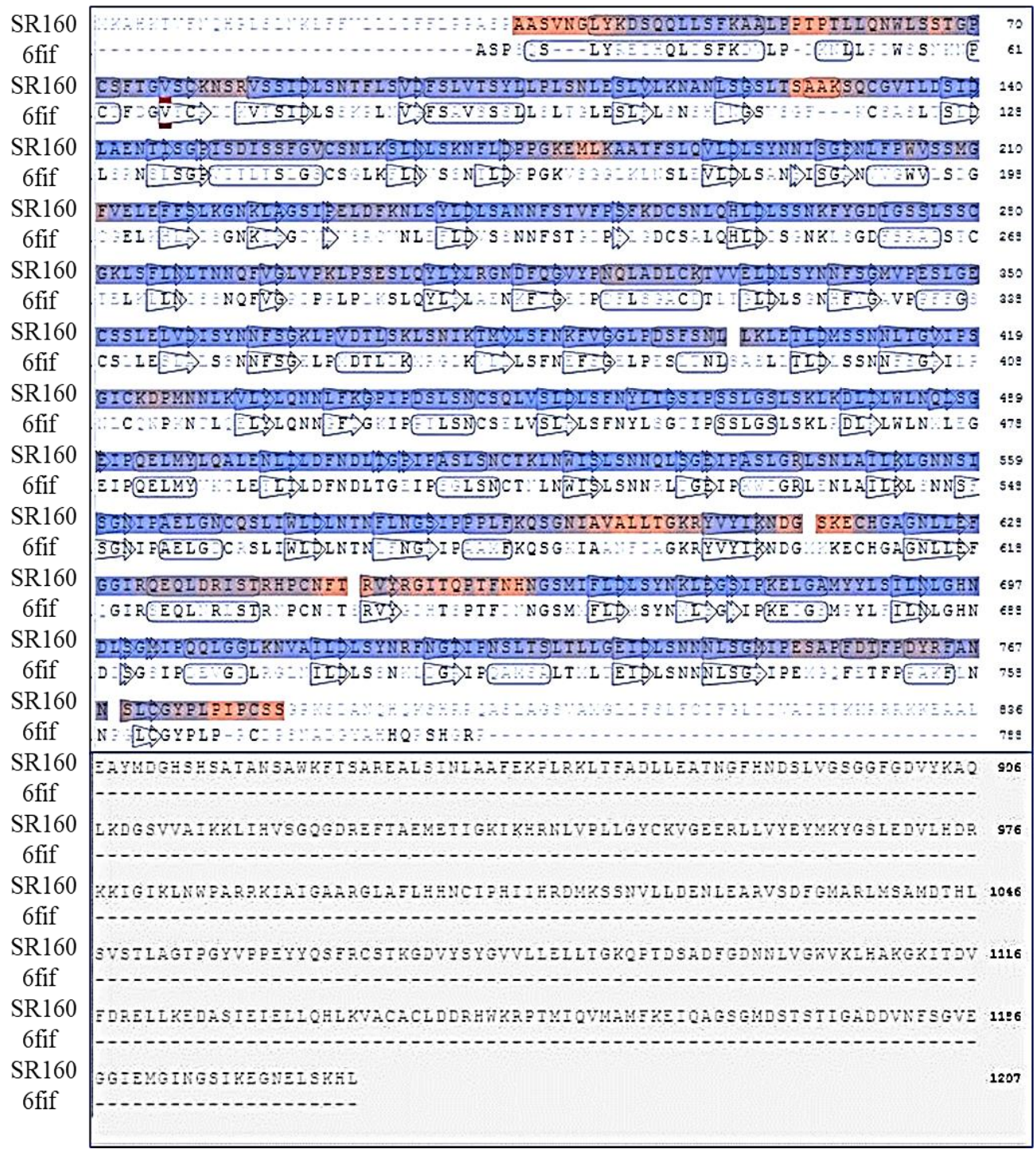

Fig. 1. Alignment of the systemin receptor 160 (SR160) amino acid sequences and 6FIF crystal structure.

Active site prediction and Molecular docking results: The active site forecast showed a pocket with a 2009.850% area (SA) and 7074.932 volume (SA). Actively, 1207 residues of the amino acid were predicted for the modeled protein. In SR160, all 85 binding categories were divided into 1.4 radii samples for finding the residue. The light color blue thus shows the residual amino acid in the field (Fig. 4A &4B).

The findings of these studies are reported in Table 1. For the positioning of the activators bound to the dynamic site of SR 160, Maestro 10.5 Suite has been utilized. The binding modes for using Discovery Studio 3.5 software for SR160 defense were examined as part of our efforts to build new activators (Ahmad *et al*., 2017; Tariq *et al*., 2017)).

The compounds establish a network of molecular interactions (H-bonds, Alkyl, Van der Waals [VdW], and Sulfur bonds) with active-site molecule residues, as depicted in the 2D plot in Figure 5. IBS_NC-0495 has established various binding interactions, including H-bonds (AsnA: 613, GlyA:615, ArgA:774), π-alkyl (AlaA:622, IleA:715), π-anion (GluA:739), and other interaction shown in 2D plot Fig. 5(A).

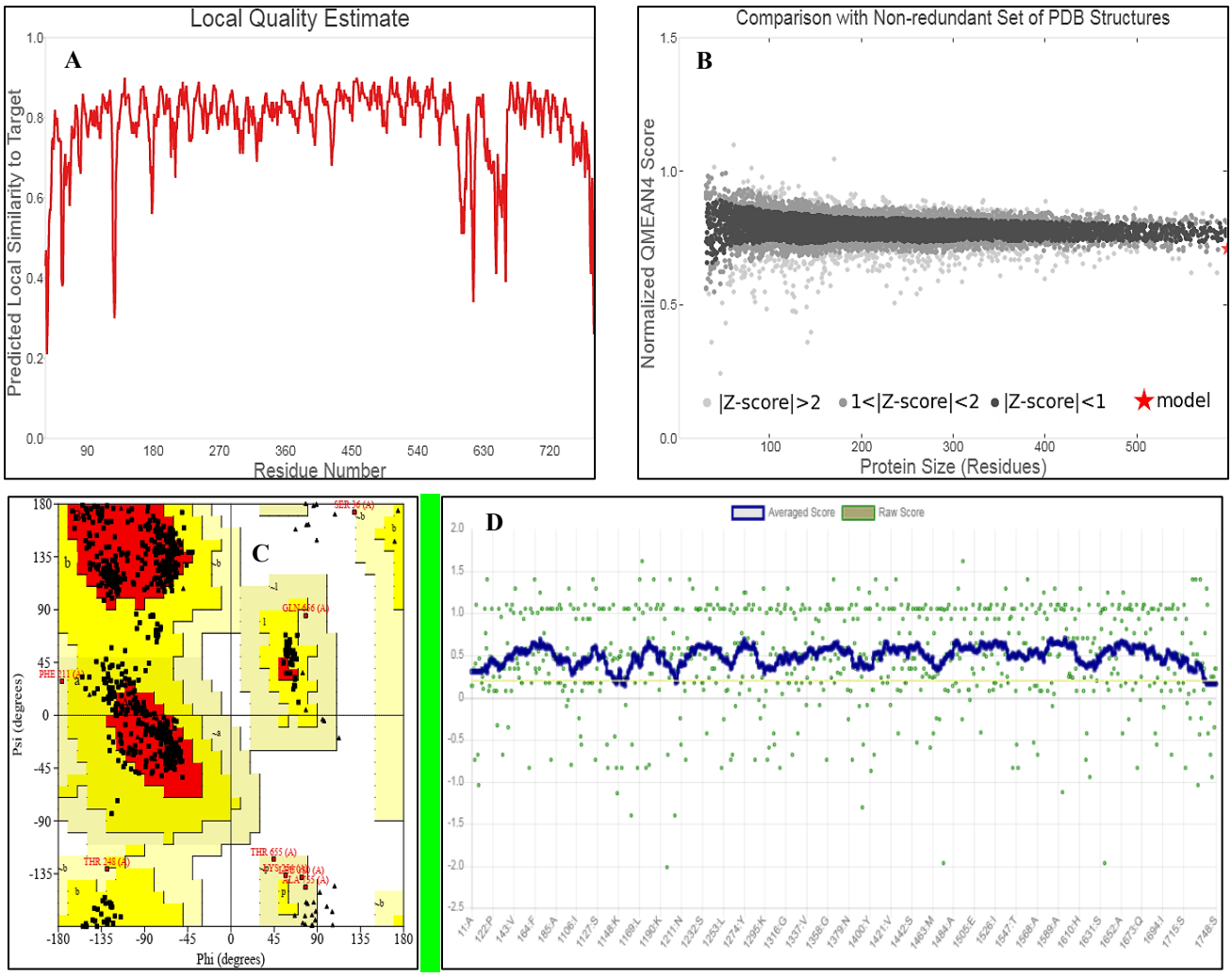

Fig. 2. Model SR160 structure validation: (A) Local quality estimate of the residues of the predicted SR160 model; (B) comparison of the predicted SR160 structure with a nonredundant set of PDB structures; (C) Ramachandran plot; and (D) Verify 3D.

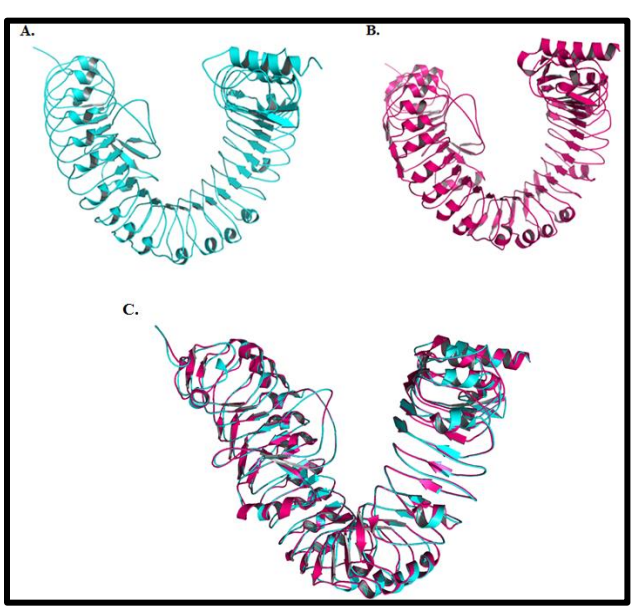

Fig. 3. (A) Three-dimensional structure of SR160 predicted by SWISS-MODEL shown in Cyan. (B) Three-dimensional structure of Template 6FIF predicted by SWISS-MODEL shown in hot pink. (C) Alignment of Structural Analog (hot pink) 6FIF in the PDB database of the query protein (cyan).

IBS_NC-0816 established H-bond with TyrA:672, AspA:717, Arg:764, π-alkyl with LysA:617, VdW interactions with AsnA:613, GlyA:615, CysA:619, HisA:620, AsnA:693, HisA:696, TyrA:763, and other interaction bonds also shown in 2D plot Figure 5(B). IBS_NC-0822 is involved in various interactions including H-bonds (AsnA: 613, GlyA:615, AlaA:622, TyrA:672, AspA:717, GluA:739, ArgA:764), π-anion (HisA:696), and other interaction VdW, Amide-π-stacked also visualized in 2D plot Figure 5(C). The ligand IBS_NC-0712 was found to form H-bond with GlyA:621, SerA:671, Tyr A:672, GlyA:695, HisA;696, π-anion bonds with AspA:717 residue and VdW with AsnA:613, GlyA:615, SerA:616, LysA:617, HisA:620, AsnA:693, TyrA:763, ArgA:764 were found in 2D plot Figure 5(D). The IBS_NC-0064 established H-bonds (AsnA:613, GlyA:615, SerA:616, TyrA:672, SerA:719, AspA:741, TyrA:763), π-alkyl (LysA:617) and VdW (GlyA:619, HisA:620, GlyA:621, HisA:696, SerA:743, AsnA:744) in 2D plot Fig. 5(E). Molecular investigations of docking suggested that the abundant van der Waals, Pi alkyl, and carbon-hydrogen interactions are the important forces for binding compounds IBS_NC-0495, IBS_NC-0816, IBS_NC-0822, IBS_NC-0712 and IBS_NC-0064 together with the SR160.

Structure-activity relationship studies based on the observed dock score values of the compounds suggest that the presence of the interacting group, (H-bonds, Alkyl, Van der Waals [VdW], and Sulfur bonds), on the compounds could be responsible for the low binding energies and strong binding affinity (Table 1). All the compounds exhibited G-score values between−9.92 and −12.81kcal/mol, having lower binding energies which have been reported to be a potent defense-gene activator. The lowest dock score and the best interactions were used to ascertain the compound with the best conformation (Tarique *et al*., 2017).

Many plant species respond to herbivore attacks by synthesizing defensive chemicals which induce the plant defensive genes and protect them from predators (Rayan & Pearce, 1998). An 18-aminoacid polypeptide known as systemin was isolated from Tomato leaves, the key role in systemic wound signaling and a primary signal for systemic defense (Rayan & Pearce, 2003). The SR160 3D structure and the prediction of the functional binding complex of 'natural compounds-SR160' remain the point of research. Insilico dynamic simulation will help to understand the binding behaviour of the complex in a better way. Therefore, IBS_NC-0495, IBS_NC-0816, IBS_NC-0822, IBS_NC-0712, and IBS_NC-0064 had shown lower binding energy for SR160, and it may be identified in the Solanaceae group as a significant defense-gene activator.

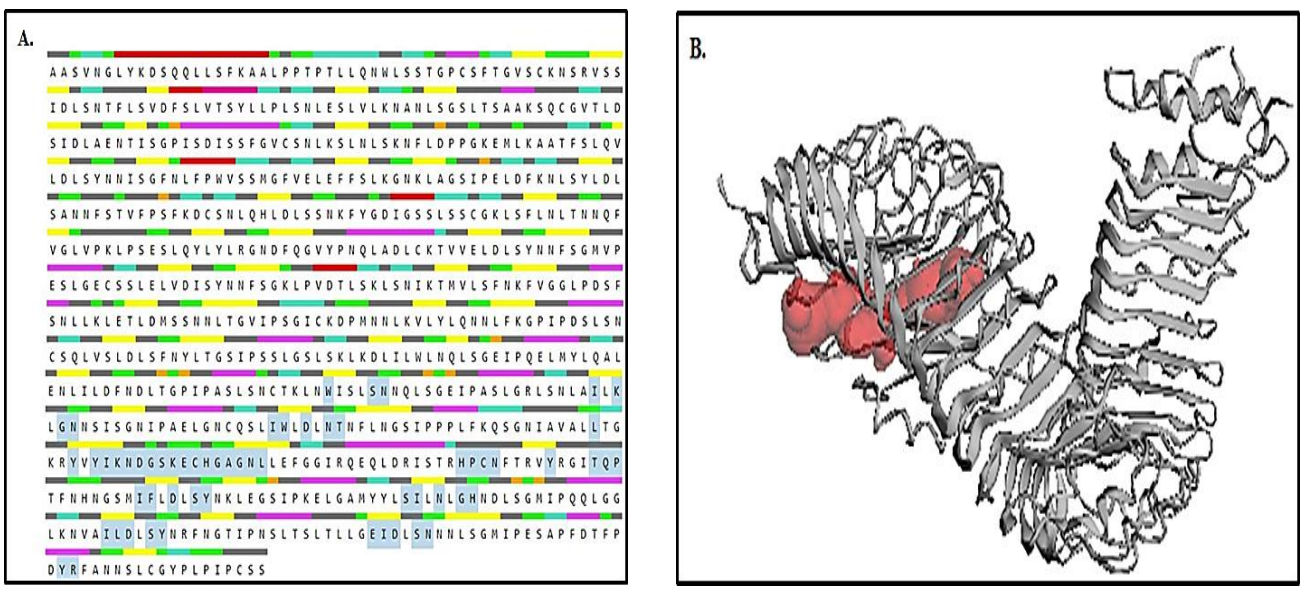

Fig. 4. Binding pocket prediction by CASTp server: (A) Light blue color boxes highlight the amino acid residues present in the binding site, (B) Show the binding sites of SR160.

Table 1. Lowest binding energy for the ligand-SR160 interaction, along with scores for various interaction types, as detected by GLIDE.

Compounds ID	G-Score (kcal/mol)	Lipophilic E vdw	H-bond	Protein ligands interaction
IBS_NC-0495	-12.813	-9.21	-2.57	LysA:612, AsnA:613, AspA:614, GlyA:615, SerA:616, AlaA:622, TyrA:672, AsnA:693, HisA:696, IleA:715, SerA:719, GluA:739, AspA:741, ArgA:774
IBS_NC-0816	-11.711	-8.19	-2.02	AsnA:613, GlyA:615, SerA:616, LysA:617, CysA:619, HisA:620, TyrA:672, AsnA:693, HisA:696, AspA:717, TyrA:763, Arg:764
IBS_NC-0822	-10.221	-6.83	-1.97	LysA:612, AsnA:613, AspA:614, GlyA:615, SerA:616, HisA:620, GlyA:621, AlaA:622, TyrA:672, AsnA:693, HisA:696, AspA:717, GluA:739, TyrA:763, ArgA:764
IBS_NC-0712	-9.923	-4.76	-0.94	AsnA:613, GlyA:615, SerA:616, LysA:617, GlyA:621, HisA:620, SerA:671, Tyr A:672, AsnA:693, GlyA:695, HisA;696, AsaA:717, TyrA:763, ArgA:764
IBS_NC-0064	-9.126	-4.11	-0.89	AsnA:613, GlyA:615, SerA:616, LysA:617, GlyA:619, HisA:620, GlyA:621, TyrA:672, HisA:696, SerA:719, AspA:741, SerA:743, AsnA:744, TyrA:763

GScore; Glide extra precision scores (kcal/mol)
Lipophilic E Vdw; Chemscore lipophilic pair term and fraction of the total protein-ligand vdw energy; H-Bond; Hydrogen-bonding term Protein ligands interaction; p–p stacking, p–cat interaction and hydrogen bond between the ligands and protein

Conclusion

SR160 is a possible therapeutic target for combating wound cure in the Solanaceae group in the defense gene activator. This study provides insight into forecasting possible interaction modes and binding affinities of IBS_NC-0495, IBS_NC-0816, IBS_NC-0822, IBS_NC-0712 IBS_NC-0064 compounds with homology modeled SR160. The highest dock value in the examined virtual ligands was IBS NC-0495 compound. The findings for docked data on all the virtually tested compounds show their safety in the ongoing research and production of active marketable wound-healing drugs. In order to further validate the protein target, experimental characterization is also required.

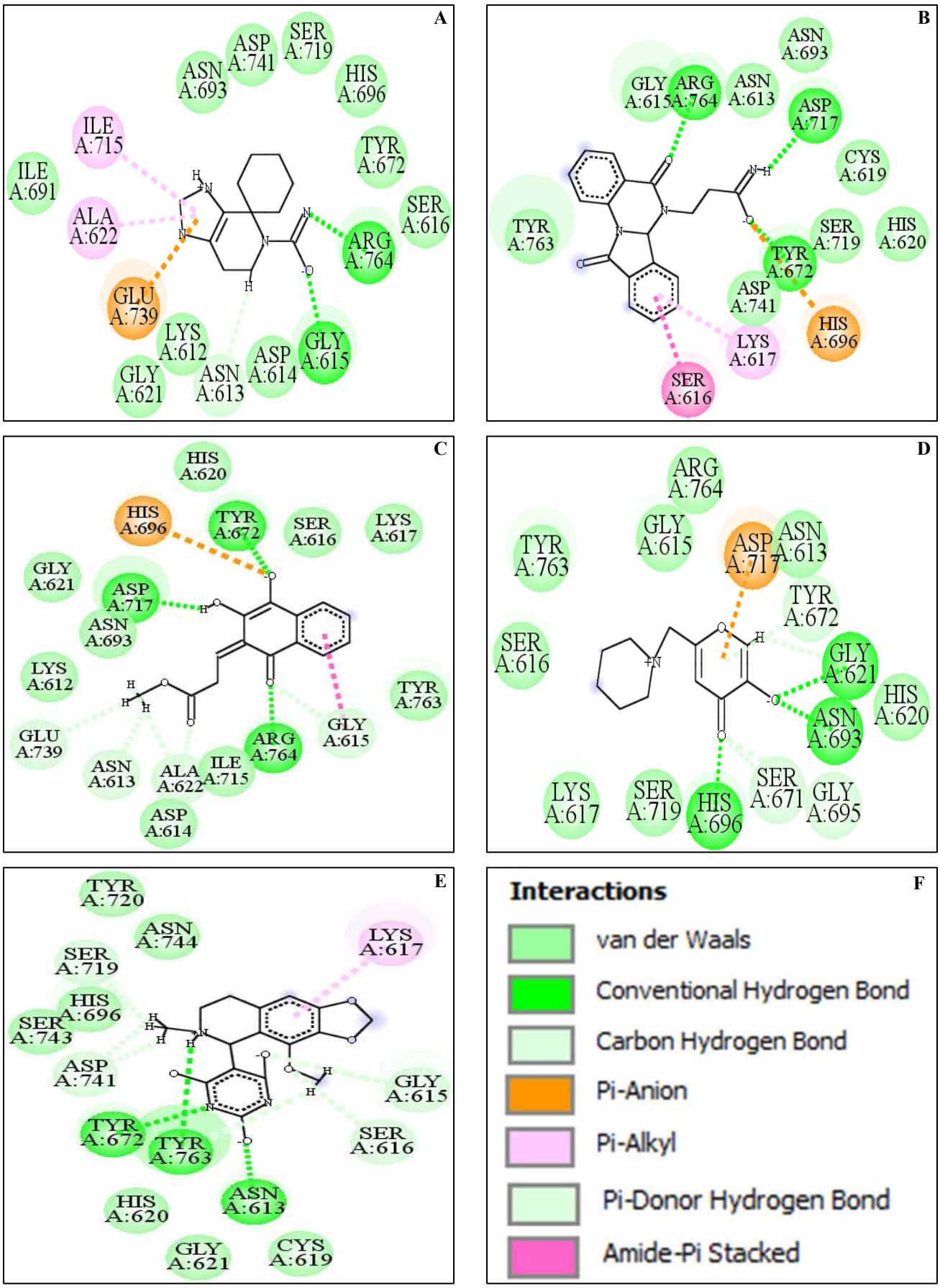

Fig. 5. 2D model of the interactions of compounds with SR160: (A) IBS_NC-0495; (B) IBS_NC-0816; (C) IBS_NC-0822; (D) IBS_NC-0712; (E) IBS_NC-0064. In different colours shown on the insert are displayed residues engaged in hydrogen, van der Waals interactions, carbon hydrogen and pi-alkyl.

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References

Ahmad, K., A.R. Bhat and F. Athar. 2017. Pharmacokinetic evaluation of callistemon viminalis derived natural compounds as targeted inhibitors against δ-opioid receptor and farnesyl transferase. *Lett. Drug Design Discov.*, 14: 488-499.

Alexandersson, E., M. Tewodros L. Åsa L. Erland and A. Erik. 2016. Plant resistance inducers against pathogens in *Solanaceae* species from molecular mechanisms to field application. *Int. J. Mol. Sci.*, 17: 1673. https://doi.org/10.3390/ijms17101673.

Arnold, K., L. Bordoli, J. Kopp and T. Schwede. 2016. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinform.*, 22: 195-201.

Bowers, M.D. 1992. The evolution of unpalatability and the cost of chemical defense in insects. *Insect chemical ecology: an evolutionary approach Chapman and Hall, Britain*.

Burley, S.K., H.M. Berman, C. Bhikadiya, C. Bi, L. Chen, L. Di Costanzo, C. Christie, K. Dalenberg, J.M. Duarte, S. Dutta, Z. Feng, S. Ghosh, D.S. Goodsell, R.K. Green, V. Guranović, D. Guzenko, B.P. Hudson, T. Kalro, Y. Liang and R. Lowe. 2018. RCSB Protein Data Bank: biological macromolecular structures enabling research and education in fundamental biology, biomedicine, biotechnology and energy. *Nucl. Acids Res.*, 47(D1): D464-D474.

Coppola, M., G. Corrado, V. Coppola, P. Cascone, R. Martinelli, M.C. Digilio, F. Pennacchio and R. Rao. 2015. Prosystemin overexpression in tomato enhances resistance to different biotic stresses by activating genes of multiple signaling pathways. *Plant Mol. Biol. Report*, 33(5): 1270-1285.

Degenhardt, D.C., S. Refi-Hind, J.W. Stratmann and D.E. Lincoln. 2010. Systemin and jasmonic acid regulate constitutive and herbivore-induced systemic volatile emissions in tomato, Solanum lycopersicum. *Phytochem.*, 71: 2024-2037.

DeLano, W.L. 2002. Pymol: An open-source molecular graphics tool. *CCP4 Newsletter on Protein Crystallography*, 40: 82-92.

Dutta, C.S. and A. Lahiri. 2017. Plant polypeptide hormone systemin prefers polyproline II conformation in solution. *ACS Omega*, 2: 6831-6843.

Friesner, R.A., J.L. Banks, R.B. Murphy, T.A. Halgren, J.J. Klicic, D.T. Mainz, M.P. Repasky, E.H. Knoll, M. Shelley, J.K. Perry, D.E. Shaw, P. Francis and P.S. Shenkin. 2004. Glide: A new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J. Med. Chem.*, 47: 1739-1749.

Friesner, R.A., R.B. Murphy, M.P. Repasky, L.L. Frye, J.R. Greenwood, T. A. Halgren, P.C. Sanschagrin and D.T. Mainz. 2006. Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein−ligand complexes. *J. Med. Chem.*, 49: 6177-6196.

Howe, G.A. and G. Jander. 2008. Plant immunity to insect herbivores. *Annu. Rev. Plant Biol.*, 59: 41-66.

Laskowski, R.A., J. Jabłońska, L. Pravda, R.S. Vařeková and J.M. Thornton. 2018. PDBsum: Structural summaries of PDB entries. *Prot. Sci.*, 27: 129-134.

Laskowski, R.A., M.W. MacArthur, D.S. Moss and J.M. Thornton. 1993. PROCHECK: a program to check the stereochemical quality of protein structures. *J. Appl. Crystallogr.*, 26: 283-291.

León, J.E., E. Rojo and J.J. Sánchez-Serrano. 2001. Wound signalling in plants. *J. Exp. Bot.*, 52: 1-9.

Lüthy, R., J.U. Bowie and D. Eisenberg. 1992. Assessment of protein models with three-dimensional profiles. *Nature*, 356: 83-85.

Pearce, G. and C.A. Ryan. 2003. Systemic signaling in tomato plants for defense against herbivores: isolation and characterization of three novel defense-signaling glycopeptide hormones coded in a single precursor gene. *J. Biol. Chem.*, 278: 30044-30050.

Ryan, C.A. and G. Pearce. 1998. Systemin: A polypeptide signal for plant defensive genes. *Ann. Rev. Cell Devel. Biol.*, 14: 1-17.

Ryan, C.A. and G. Pearce. 2003. Systemins: A functionally defined family of peptide signals that regulate defensive genes in Solanaceae species. *Proceed. Nat. Acad. Sci.*, 100: 14577-14580.

Scandalios, J. 2005. Oxidative stress: Molecular perception and transduction of signals triggering antioxidant gene defenses. *Brazil. J. Med. Biol. Res.*, 38: 995-1014.

Schwab, F., G. Zhai, M. Kern, A. Turner, J.L. Schnoor and M.R. Wiesner. 2016. Barriers, pathways and processes for uptake, translocation and accumulation of nanomaterials in plants-Critical review. *Nanotoxicology*, 10: 257-278.

Sievers, F. and D.G. Higgins. 2018. Clustal Omega for making accurate alignments of many protein sequences. *Prot. Sci.*, 27: 135-145.

Singh, P. and F. Bast. 2014. In silico molecular docking study of natural compounds on wild and mutated epidermal growth factor receptor. *Med. Chem. Res.*, 23: 5074-5085.

Tarique, M., M. Ahmad, M. Chauhan and R. Tuteja. 2017. Genome wide in silico analysis of the mismatch repair components of Plasmodium falciparum and their comparison with human host. *Front. Microbiol.*, 8: 130.

Tian, W., C. Chen, X. Lei, J. Zhao and J. Liang. 2018. CASTp 3.0: computed atlas of surface topography of proteins. *Nucl. Acids Res.*, 46:W363-W367.

Tripathi, S.K., R. Muttineni and S.K. Singh. 2013. Extra precision docking, free energy calculation and molecular dynamics simulation studies of CDK2 inhibitors. *J. Theor. Biol.*, 334: 87-100.

Walters, D.R., T. Cowley and H. Weber. 2006. Rapid accumulation of trihydroxy oxylipins and resistance to the bean rust pathogen Uromyces fabae following wounding in Vicia faba. *Ann. Bot.*, 97: 779-784.

Wang, L., E. Einig, M. Almeida-Trapp, M. Albert, J. Fliegmann, A. Mithöfer, H. Kalbacher and G. Felix. 2018. The systemin receptor SYR1 enhances *et al*. The systemin receptor SYR1 enhances resistance of tomato against herbivorous insects. *Nat. Plants*, 4: 152-156.

Wasternack, C. and B. Hause. 2002. Jasmonates and octadecanoids: signals in plant stress responses and development. *Prog. Nucl. Acid Res. Mol. Biol.*, 72: 165-221.

Yadav, M.K., S. Govil and S. Kumar. 2015. Interaction pattern of Systemin with SR 160 receptor for activation of wound defensive gene. *IJPTB.*, 2(3): 17-20.

Zhang, G., F. Zhao, L. Chen, Y. Pan, L. Sun, N. Bao, T. Zhang, C.X. Cui, Z. Qiu, Y. Zhang, L. Yang and L. Xu. 2019. Jasmonate-mediated wound signalling promotes plant regeneration. *Nat. Plants*, 5: 491-497.

Zvereva, E.L. and M.V. Kozlov. 2016. The costs and effectiveness of chemical defenses in herbivorous insects: A meta-analysis. *Ecol. Monogr.*, 86: 107-124.

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