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Prospecting molecular targets aiming topical treatment of human melanoma

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PREÂMBULO

PROSPECTING MOLECULAR TARGETS AIMING TOPICAL TREATMENT OF HUMAN MELANOMA

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Dedico esse trabalho aos meus avós Zélia Victor Peruzzo e Antonio Vicente Peruzzo (*in memoriam*), que mesmo sem compreender o papel do Químico Medicinal na sociedade, me incentivaram a continuar os estudos todo o tempo que estiveram aqui comigo.

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Muito obrigado!

Resumo

O melanoma humano é caracterizado por uma disfunção na regulação dos melanócitos, afetando o seu crescimento e proliferação. Quando não está em equilíbrio, a produção desenfreada de melanócitos pode causar tumores malignos e provocar metástases, sendo o melanoma tratado como o câncer de pele de pior prognóstico. Assim, a ideia de estudar a aplicação do medicamento diretamente no ambiente tumoral pode contribuir para o surgimento de novos protocolos de tratamento para doenças de pele. Para garantir que os medicamentos possam ser utilizados como tratamento tópico, é necessário conhecer os receptores e/ou proteínas expressos no ambiente tumoral que desempenham um papel no desenvolvimento do câncer. Com essas estruturas conhecidas, sua afinidade e interações com medicamentos podem ser estimadas por meio de docking molecular. Os resultados sugerem que os medicamentos anticâncer têm relativa afinidade por algumas enzimas produzidas nos melanócitos e que podem funcionar no tratamento tópico se forem capazes de inibir a atividade dessas proteínas.

Abstract

Human melanoma is characterized by a dysfunction in the regulation of melanocytes, affecting their growth and proliferation. When it is not in balance, the unrestrained production of melanocytes can cause malignant tumors and cause metastasis with melanoma being treated as the skin cancer with the worst prognosis. Thus, the idea of studying the application of the drug directly in the tumor environment may contribute to the emergence of new treatment protocols for skin diseases. To ensure that drugs can be used as topical treatment, it is necessary to know receptors and/or proteins expressed in the tumor environment that play a role in cancer development. With these known structures, their affinity and interactions for drugs can be estimated using molecular docking. The results suggest that anticancer drugs have a relative affinity for some enzymes produced in melanocytes and that they may work in topical treatment if they are able to inhibit the activity of these proteins.

SUMÁRIO

Prospecting molecular targets aiming topical treatment of human melanoma

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ABSTRACT

Human melanoma is characterized by a dysfunction in the regulation of melanocytes, affecting their growth and proliferation. When it is not in balance, the unrestrained production of melanocytes can cause malignant tumors and cause metastasis with melanoma being treated as the skin cancer with the worst prognosis. Thus, the idea of studying the application of the drug directly in the tumor environment may contribute to the emergence of new treatment protocols for skin diseases. To ensure that drugs can be used as topical treatment, it is necessary to know receptors and/or proteins expressed in the tumor environment that play a role in cancer development. With these known structures, their affinity and interactions for drugs can be estimated using molecular docking. The results suggest that anticancer drugs have a relative affinity for some enzymes produced in melanocytes and that they may work in topical treatment if they are able to inhibit the activity of these proteins.

1. Introduction

Cellular homeostasis is preserved when the processes of cell division, differentiation, aging and apoptosis are balanced. Cancer is characterized by dysregulation of this system with a set of genetic factors and epigenetic changes that allow uncontrolled neoplastic growth [1].

Melanoma, the classification of skin cancer with the worst prognosis among all others, occurs when melanocytes are constantly stimulated to cell growth and divide. Normally, melanocytes organize into a single cell layer; in melanoma, however, the overproduction of new melanocytes creates a stack of cells that range from the epidermis to the lymph nodes, in severe cases [2,3].

The growth and development of melanoma is induced by a stimulus and inhibition relationship between tumor cells and the environment. From this perspective, it is interesting to identify which biomolecules are involved in cell signaling processes and address them as therapeutic targets.

Currently, the treatments available for melanoma is chosen according to the stage of development of the cancer, ranging from surgical removal of the tumor to, in cases where the cells reach the lymphatic system and cause metastasis, immuno/chemotherapy [4]. Anti-cancer chemotherapy drugs such as dacarbazine, temozolomide, taxanes, fotemustine are usually given in oral or intravenous therapy. These treatments are constantly related to depression and anxiety, triggered by stress and fear of diagnosis. As a result, treatment adherence and compliance are impaired, and many patients give up on them [5,6]. Furthermore, these compounds often have cytotoxic effects on healthy cells as well. In the case of melanoma, the search for new routes of administration of chemotherapy that are less invasive, especially in the initial stages, is interesting, since the abnormal cells are in the superficial layer of the skin [2].

Topical application is a viable alternative for patients with early-stage melanoma who are not susceptible to surgery and may increase the chances of disease remission when combined with conventional intravenous chemotherapy. Already described in the literature, intralesional injection of T-VEC (biopharmaceutical) and topical imiquimod cream (5%) are proposed applications for topical and transdermal application for the treatment of melanoma [7,8]. Topical treatment is effective for several skin diseases such as eczema, acne and dermatitis, and can be implemented in skin cancer as it reduces the frequency of administration and the occurrence of systemic effects [9–11]. Furthermore, formulations containing nanomaterials made from Realgar (a mineral of arsenic combined with turmeric) have been reported to deliver transdermal drugs to treat skin cancer, such as nanoparticles [12], nanogels [13] and microemulsions that, in addition to ensuring drug stability, help with diffusion through the stratum corneum, a natural barrier present in the superficial layer of the skin and hinders drug delivery [14,15].

Biomaterials have been used to treat injuries since ancient medicine. There are reports of the use of honey, fat and vegetable fibers in wounds [16]. Currently, the development of self-sufficient materials has been improved, allowing drugs to be administered in a controlled manner directly at the wound site [17]. The Human Amniotic Membrane (HAM) is the innermost layer of the placenta and although it has been a tissue for hospital disposal, it is useful in the treatment of superficial corneal injuries, burns and other dermatitis, with medical applications reported in Argentina, the United States of America and Spain [18–23]. Therefore, the application of HAM as a functionalized biological dressing can be an alternative and/or a complement to conventional chemotherapy, delivering drugs topically, adhering them to HAM, for example.

To sort the compounds to be integrated into the membrane, in silico studies can be performed, predicting the affinity of these molecules for the therapeutic targets of interest to treat melanoma. In this case, knowing the receptors involved in cell signaling processes and disease development, Structure-Based Drug Design (SBDD) and Fragment-Based Drug Design (FBDD), for example, support the docking studies of these targets [24] and, once the best candidates are selected, they can be incorporated into the HAM.

2. Materials and Methods

2.1 Prospection of targets

Targets available in databases which have defined crystallographic structures and play a role in the progression of melanoma skin cancer, were prospected. **Table 1** shows the studied structures for which in the database (RCSB Protein Data Bank), ligands are associated with. Structure **1** is an enzyme excreted in the extracellular matrix, playing the role of inhibiting the adhesion of

tumor cells, allowing them to move and cause metastasis [24–26]. Meanwhile, Structure **2** acts on cell pigmentation and signals for cell proliferation of melanocytes [27,28]. Similarly, Structure **3** signals for division in melanocytes that express kinases [29,30].

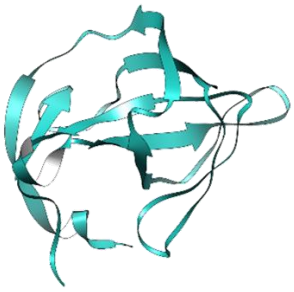
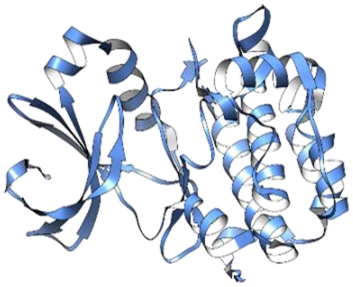
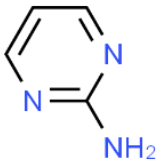
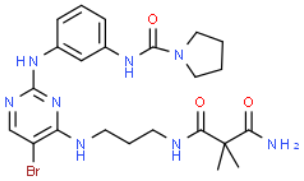
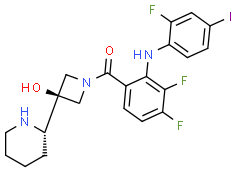
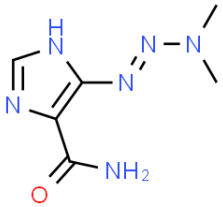
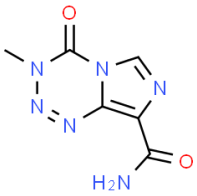
	PDB ID	1i1j	1z5m	4lmn
(I) Structures		 *Structure 1: Melanoma Inhibitory Activity*	 *Structure 2: 3-Phosphoinositide-dependent Kinase-1*	 *Structure 3: Mitogen-Activated Protein Kinase 1*
	PubChem ID	**7978**	**571329**	**17349374**
(II) Ligands		 *Ligand A: Pyrimidin-2-amine*	 *Ligand B: LI8*	 *Ligand C: Cobimetinib*
	Pubchem ID	**10481959**	**5201**	
(III) Drugs		 *Ligand D: Dacarbazine*	 *Ligand E: Temozolomide*	

Table 1: Structures and ligands used on (re)docking. (I) 3D illustration of structures from PDB; (II) Ligands described in the PDB entrys; (III) Drugs used on docking studies.

2.2. Molecular Docking

All mentioned structures will underwent the same molecular docking process, using DockThor [31]. First, the ligands were redocked with their original receptors registered in the PDB, forming pairs **1A**, **2B** and **3C**. Starting with the blind mode (Blind Docking), in which there is no binding site restriction, the software tests the docking positions over the entire exposed surface of the protein. Then, by defining the volume of the box that delimits the interaction zone Dockthor can inspect in better detail the binding pair over a second docking step. This process allows the determination of reference data using the affinity results of the original ligant-target pair as a parameter. Finally, with the region of the orthosteric site defined from the redocking, as well as the affinity parameters, the drugs proposed for the treatment (Dacarbazine and Temozolomide) were submitted to the same process and the docking results of these molecules were compared to the original ligands (**Table 2**)

2.3 Analysis of interaction sites

The next step was to compare the binding site reported by the original structure authors with that observed in the docking results. With PyMol (Version 2.0 – Free License for Students) visualization tools, it is possible to identify the site with which the drug interacts while LigPlot+ (Version 2.2 – Free License for Students) compare, in pairs, the amino acid residues that form the hydrophilic and hydrophobic portions of that site. Using the data obtained from the molecular docking of structures **1-3**, and using their respective inhibitors as a reference, it was possible to obtain the relative affinity of the drugs Dacarbazine and Temozolamide (**Table 2**) for the targets. Furthermore, this analysis allowed us to understand how inhibitors interact with the target structure and cause modulation.

2.4. Polymeric nanocapsules

The polymeric nanoparticle formulation used to encapsulate the best candidate was previously developed (and characterized) by Ghisio *et al.* [32], considering considering the physicochemical aspects that allow absorption of substances by the epidermis, in this case, mainly by the stratum corneum. Therefore, it is interesting that the nanoparticle can penetrate the superficial layers of the skin efficiently.

2.5 Characterization and Functionalization of the HAM

To characterize the physicochemical properties of HAM, it is necessary to know the permeability and porosity of the membrane. For this purpose, images obtained through electron microscopy techniques already described by Naasani et al. [33] will be used, and through fluorescent markers (FITC-Dextran 4kDa and 40kDa), which have a known size and will be used to estimatee the pore size of the HAM. To determine the pore size range, the Franz system for permeation studies (Franz diffusion cell illustrated in Fig.1) [34] will be used. In a buffered environment with controlled pH and temperature (pH 5 at 36ºC), simulating the epidermis [35], a slice of HAM will be positioned between the donor and the receptor compartments and the solution containing the fluorescent marker will be deposited on top of the membrane (donor compartment) and kept for 72h, under constant agitation. Periodically, 1mL aliquots will be collected from the receptor compartment and quantified by molecular fluorescence spectroscopy.

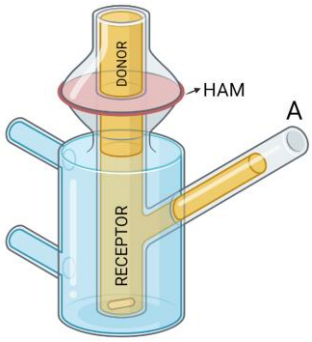


Fig. 1: Franz diffusion cell for permeation studies, pointing the donor and receptor pools, connected by a slice of Human Amniotic Membrane (HAM) with a collection pit (A). Rendered on Biorender.com

Using the same method for the study of antineoplastic release, the formulation of nanocapsules will be added to the donor compartment, over the HAM. At predetermined intervals, 1mL aliquots of the receptor medium will be collected and the drug will be quantified by high performance liquid chromatography (HPLC), following the validated method by Ghisio et al. [32].

3. Results

3.1 Molecular docking

Docking results (**Table 2**) estimate the affinity between ligand-receptor pairs by measuring different energy components. Knowing that Structures **1**, **2**, and **3** pair with Ligands **A**, **B** and **C**, respectively and have already been described in the literature as effective ligands, it is possible to use their docking results as a reference to compare with Ligands **D** and **E** docking.

As expected, the highest affinities are given to the ligand-receptor pairs described on PDB, highlighted on **Table 2**. However, the anticancer drugs **D** and **E** also showed encouraging results and may produce inhibitory response on the structures. Analyzing the orthosteric site by comparison, it is possible to understand whether the interaction zones occur on the same site and which residues are responsible for the interaction.

Structures	Ligands (A)	(B)	(C)	(D)	(E)	
(1)	**-6,822**	-8,274	-7,600	-6,372	-7,170	(i)
	-30,901	-13,292	52,304	-25,956	-7,255	(ii)
	-8,301	-27,547	-16,753	-12,801	-15,907	(iii)
	-6,914	-3,805	-16,823	-9,376	-4,197	(iv)
(2)	-6,414	**-8,217**	-7,762	-6,511	-7,259	(i)
	-32,956	**-29,446**	36,82	-28,725	-9,877	(ii)
	-6,216	**-32,336**	-16,551	-13,112	-14,746	(iii)
	-11,054	**-13,684**	-35,523	-12,605	-7,670	(iv)
(3)	-6,439	-7,560	**-8,438**	-6,147	-6,677	(i)
	-34,672	-26,847	**44,972**	-29,466	-13,808	(ii)
	-6,780	-29,497	**-24,959**	-4,969	-12,321	(iii)
	-12,206	-13,534	**-14,518**	-23,447	-14,021	(iv)

Table 2: Docking results (i)Affinity; (ii) Total Energy; (iii) Van der Waals; (iv) Electrostatic Energy presented in kcal/mol. Highlighted results correspond to ligand-receptor pair described on PDB.

3.2 Analysis of interaction sites

Using molecular visualization systems such as PyMol and LigPlot+, those regions where ligand and receptor interact can be illustrated. Tables **S1**-**S3** (suplementar material) compare interactions between all ligands used in the docking calculations, taking the original ligand from PDB as reference. Circled in red, the common residues suggest that interactions occurs in the same regions.

Structure 1: The interaction represented by PyMol and LigPlot+ shows the following residues: Lys10, Cys17, Phe49, Ser50, Gly61, Gly62, Leu76, Tyr78 and Asp103. Except for molecule C, all pairs show interaction with these residues. However, molecule C is interacting in the same site, with residues Glu16, Cys18 and Ala74, which also make up the binding site [24] but were not detected by LigPlot+ parameters.

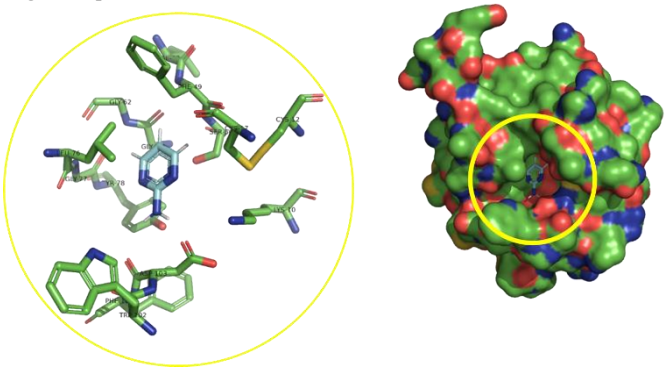


Fig. 2: 3D view of Interaction site of Structure 1 (PDB ID: 1i1j)

Structure 2: In the second structure, the residues involved are: Gly89, Glu90, Gly91, Ser92, Phe93, Ser94, Val96, Lys111, Lys123, Tyr126, Lys207, Asn210, Thr222, Asp223, Gly225, Thr226, Phe242, Val243 and Thr245. These residues are responsible for the hydrophilic and hydrophobic portions at the ligand site. In this perspective, all the molecules that participated in the docking showed some interaction with the amino acid residues, suggesting that they bind to the structure at the same site and may, therefore, have similar activity to the known inhibitor.

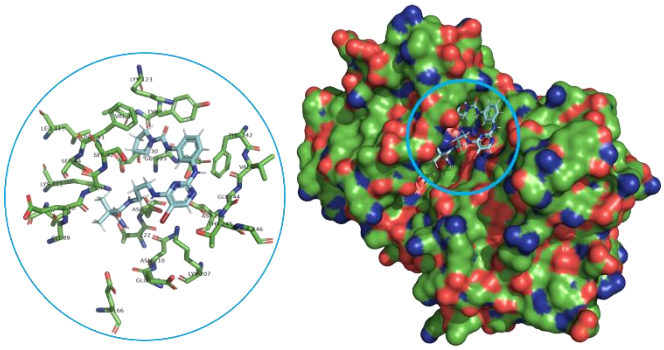


Fig. 3: 3D view of Interaction site of Structure 2 (PDB ID: 1z5m)

Structure 3: The third structure forms the binding site from: Leu74, Gly75, Ala76, Gly77, Asn78, Gly80, Val82, Lys97, Gly149, Ser150, Asp152, Gly153, Lys156, Lys192, Ser194, Asn195, Leu197, Asp208, Thr226, Tyr229, Glu255, Tyr261 and Ser288. The common interactions given by LigPlot+ are limited to the 2D view and only indicate whether these interactions exist or not. The 3D visualization represented in Fig. 5 shows that **A** and **E** Ligands (pink and yellow, respectively), despite not having the same interaction with the binding site, have affinity to the same region. Thus, it appears that all molecules interact at the same site.

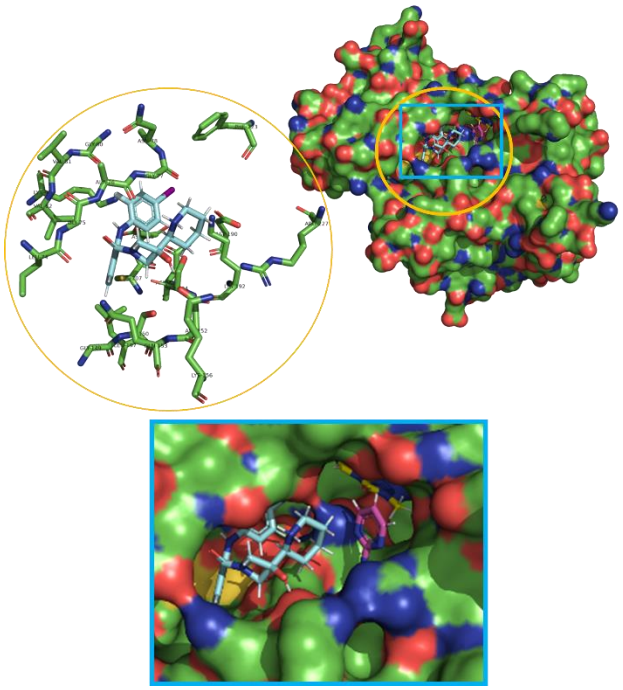


Fig. 4: 3D view of Interaction site of Structure 3 (PDB ID: 4lmn)

Due to the SARS-CoV-2 pandemic, the return to experiments has been halted. With that, the experimental design that involved nanoencapsulating and performing the characterization of HAM was left in the background, considering it for future work.

4. Conclusions

The results obtained in the docking studies suggest that the available anticancer drugs (Dacarbazine and Temozolomide) are promising for the topical treatment of human melanoma. The comparative data are similar to those obtained in the redocking process using the ligands described in PDB

The perspective for future work is to develop a biological scaffold that contains nanoencapsulated anticancer drugs to treat human melanoma topically/transdermally. However, permeation and characterization studies still need to be carried out to verify the applicability of this proposal.

Furthermore, it is important to highlight the innovative aspects of this study, which not only explores non-trivial pathways for the treatment of human melanoma, but also demonstrates why docking studies contribute to the rational development of new drugs by knowing the structure of their receptors.

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Supplementary Material

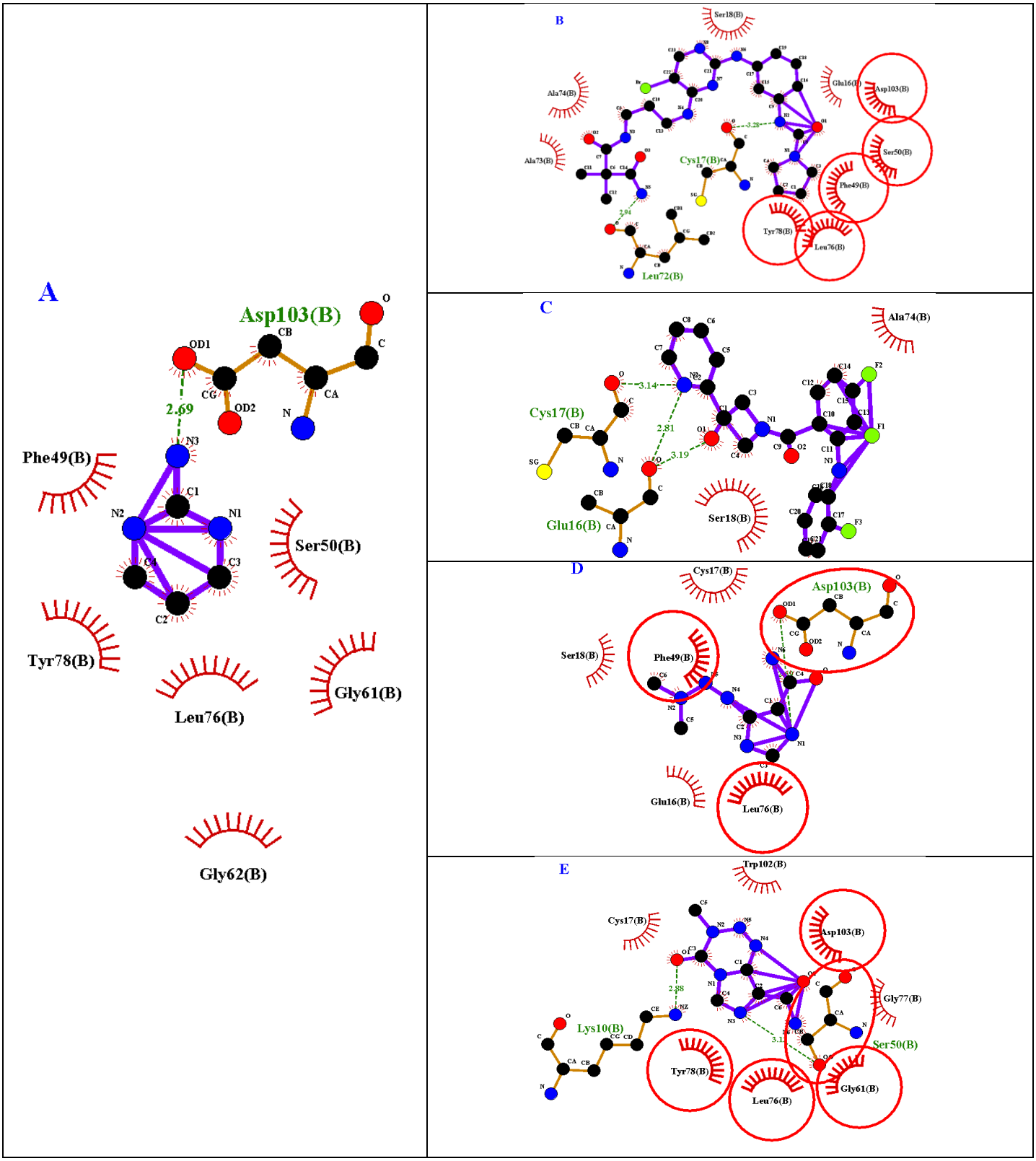

Table S1: Interaction zones of Structure 1. Amino acids residues produced interactions: (A) hydrophilic using Ser50, hydrophobic using Phe49, Gly61. Gly62 and Leu76, amphipathic using Tyr78 and hydrogen bonding using Asp103; (B) hydrophilic using Glu16, Ser18, Ser50 and Asp103, hydrophobic using Phe49, Ala73, Ala74 and Leu76, amphipathic using Tyr78 and hydrogen bonding using Cys17 and Leu72; (C) hydrophilic using Ser18, hydrophobic using Ala74 and hydrogen bonding using Glu16 and Cys17; (D) hydrophilic using Glu16, Cys17 and Ser18, hydrophobic using Phe49 and Leu76, and hydrogen bonding using Asp103; (E) hydrophilic using Cys17 and Asp103, hydrophobic using Gly61, Leu76 and Gly77, amphipathic using Tyr78 and Trp102, and hydrogen bonding using Lys10 and Ser50.

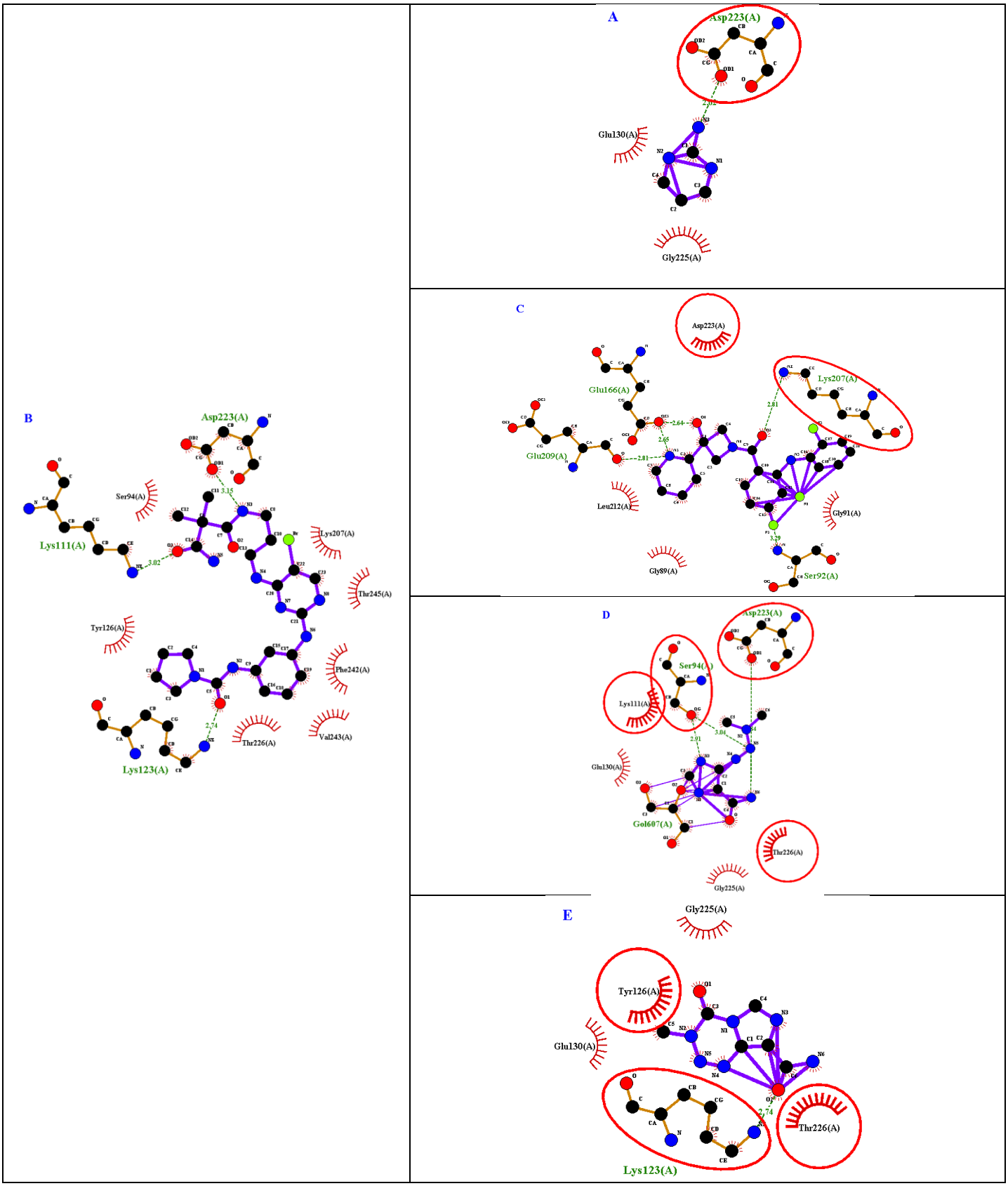

Table S2: Interaction site of Structure 2. Amino acids residues produced interactions: (A) hydrophilic using Glu130, hydrophobic using Gly225 and hydrogen bonding using Asp223; (B) hydrophilic using Ser94, Thr226 and Thr245, hydrophobic using Phe242 and Val243, amphipathic using Tyr126 and Lys207, and hydrogen bonding using Lys111, Lys123 and Asp223; (C) hydrophilic using Asp223, hydrophobic using Gly89, Gly91 and Leu212, and hydrogen bonding using Ser92, Lys207 and Glu209; (D) hydrophilic using Glu130, Thr226, , hydrophobic using Gly225, amphipathic using Lys111 and hydrogen bonding using Ser94 and Asp223; (E) hydrophilic using Glu130 and Thr226, hydrophobic using Gly225, amphipathic using Tyr126, and hydrogen bonding using Lys123.

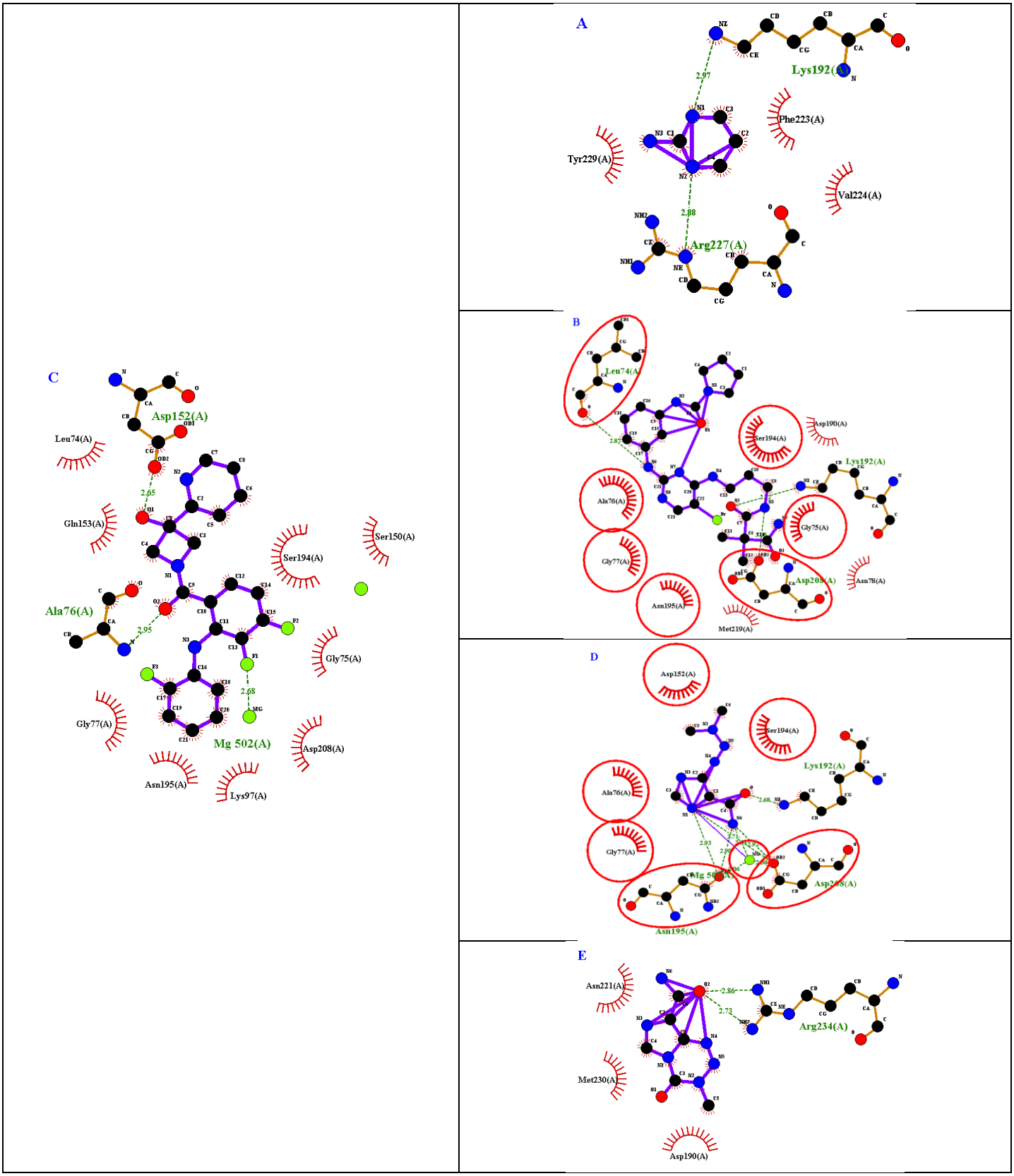

Table S3: Interaction site of Structure 3. Amino acids residues produced interactions: (A) hydrophobic using Phe223 and Val224, amphipathic using Tyr229 and hydrogen bonding using Lys192 and Arg 227; (B) hydrophilic using Asn78, Asp190, Ser194 and Asn195, hydrophobic using Gly75, Ala76, Gly77, amphipathic using Met219 and hydrogen bonding using Leu745, Lys192 and Asp208; (C) hydrophilic using Ser150, Gln153, Ser194, Asn195 and Asp208, hydrophobic using Leu74, Gly75 and Gly77, amphipathic using Lys97 and hydrogen bonding using Ala76 and Asp152; (D) hydrophilic using Asp152 and Ser194, hydrophobic using Ala76 and Gly77, and hydrogen bonding using Lys192, Asn195 and Asp208; (E) hydrophilic using Asp1909 and Asn221, amphipathic using Met230 and hydrogen bonding using Arg234.