Biocatalysis and Agricultural Biotechnology 18 (2019) 101059

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Activity of antibacterial compounds from *Bacillus subtilis* against cellular oncoproteins by *in silico* approach

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ARTICLE INFO

Keywords:
Oncoproteins
Apoptosis
Anticancer
Antibacterial compounds
Molecular docking

ABSTRACT

The cellular oncoproteins Caspase-3, Caspase-8 and Nuclear Factor-kappa B (NF-κB) are the factors implicated in several cancers. Synthetic and natural compounds were demonstrated to interact with these oncoproteins. Though bacteria are the source of novel compounds including antimicrobial compounds, their anticancer activity remains elusive. Hence in this study, 10 antibacterial compounds of *Bacillus subtilis* retrieved from the pubchem database and literature were checked for the ability to bind with the chosen oncoproteins *in silico*. Among those, the compounds Bacilosarcin C, Lipoamicoumacin D and Lipoamicoumacin C showed higher binding GLIDE scores with Caspase-8 (−13.406), Caspase-3 (−9.955) and NF-κB (−8.702) respectively. The strength of hydrogen bond interactions for Bacilosarcin C, Lipoamicoumacin D and Lipoamicoumacin C with the corresponding oncoproteins were 2.62–3.35 Å, 2.05 to 3.15 Å and 2.59 to 3.21 Å respectively. In the binding of these 10 compounds of *Bacillus* with NF-κB, Thr, Ser and Gly were commonly involved. Similarly, Asn and Phe with Caspase-3 and Arg and Ser with Caspase-8. It is demonstrated that microbial compounds could be used to design novel drugs against oncoproteins to combat cancer.

1. Introduction

Cancer is the second leading cause of death worldwide and estimated to cause 9.6 million deaths in 2018. Several therapeutic approaches were investigated including the use of nanoparticles (Pugazhendhi et al., 2018). Approximately, 70% of deaths from cancer occur in low- and middle-income countries (Bray et al., 2018). It is characterized by an uncontrolled growth of abnormal cells in the body and exhibit deregulation of many cell signalling pathways (Khalil et al., 2015). In addition, alterations in apoptosis can lead to carcinogenesis (Sharma et al., 2016). The endogenous cell death pathways are likely to play an important role against malignant transformation (Wong, 2011). Deregulation of apoptosis can leads to many diseases including cancer (Liu et al., 2013).

Nuclear Factor-kappa B (NF-κB) is a major transcription regulating factor, which is involved in a variety of important cellular and physiological responses, including modulation of cell survival and the coordination of immune responses (Li and Verma, 2002). It has been identified as a key player in resistance mechanisms (Godwin et al., 2013). In addition, NF-κB plays an important role in the regulation of apoptotic signalling pathways by regulating transcription of growth-promoting and anti-apoptotic genes (Park and Hong, 2016). Several types of cancer cells showed higher levels of NF-kB and deregulation of apoptotic signalling pathway (Lee et al., 2006), which is one of the vital hallmarks of cancer. Moreover, aberrant NF-κB expression and regulation is involved in the development of many different cancer types, where it mediates the fine balance between cellular survival and death. NF-kB activity is modulated by some members of the Caspases like Caspase-3 and Caspase-8 (Bagnoli et al., 2010; Kalia and Kukol, 2011; Pu et al., 2017). Caspase-3 is believed to be the central executioner of the apoptotic pathway (Liu et al., 2013). Development of non-peptide Caspase-3 inhibitors were desired due to the problem of poor cell permeability and low metabolic stability associated with peptide inhibitors (Sakai et al., 2008; Sharma et al., 2013). Caspase-8 is pivotal in the apoptotic cell death (Ahmad et al., 2014).

Bacillus subtilis was known to produce the antimicrobial compounds like amicoumacins and bacilosarcin (Li et al., 2012). *Bacillus* spp. were demonstrated to possess antimicrobial and anticancer activities (Ramasubburayan et al., 2015). However, the beneficial role of antibacterial compounds in apoptosis induction and inhibition of proliferation of cancer cells is crucial in anticancer therapy (Chu et al., 2015; Felicio et al., 2017). Therefore, molecular docking approach was used to find out potential compounds from *Bacillus subtilis* for the inhibition of cellular oncoproteins (NF-κB, Caspase-3 and Caspase-8)

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https://doi.org/10.1016/j.bcab.2019.101059
Received 1 January 2019; Received in revised form 7 February 2019; Accepted 23 February 2019
Available online 28 February 2019
1878-8181/

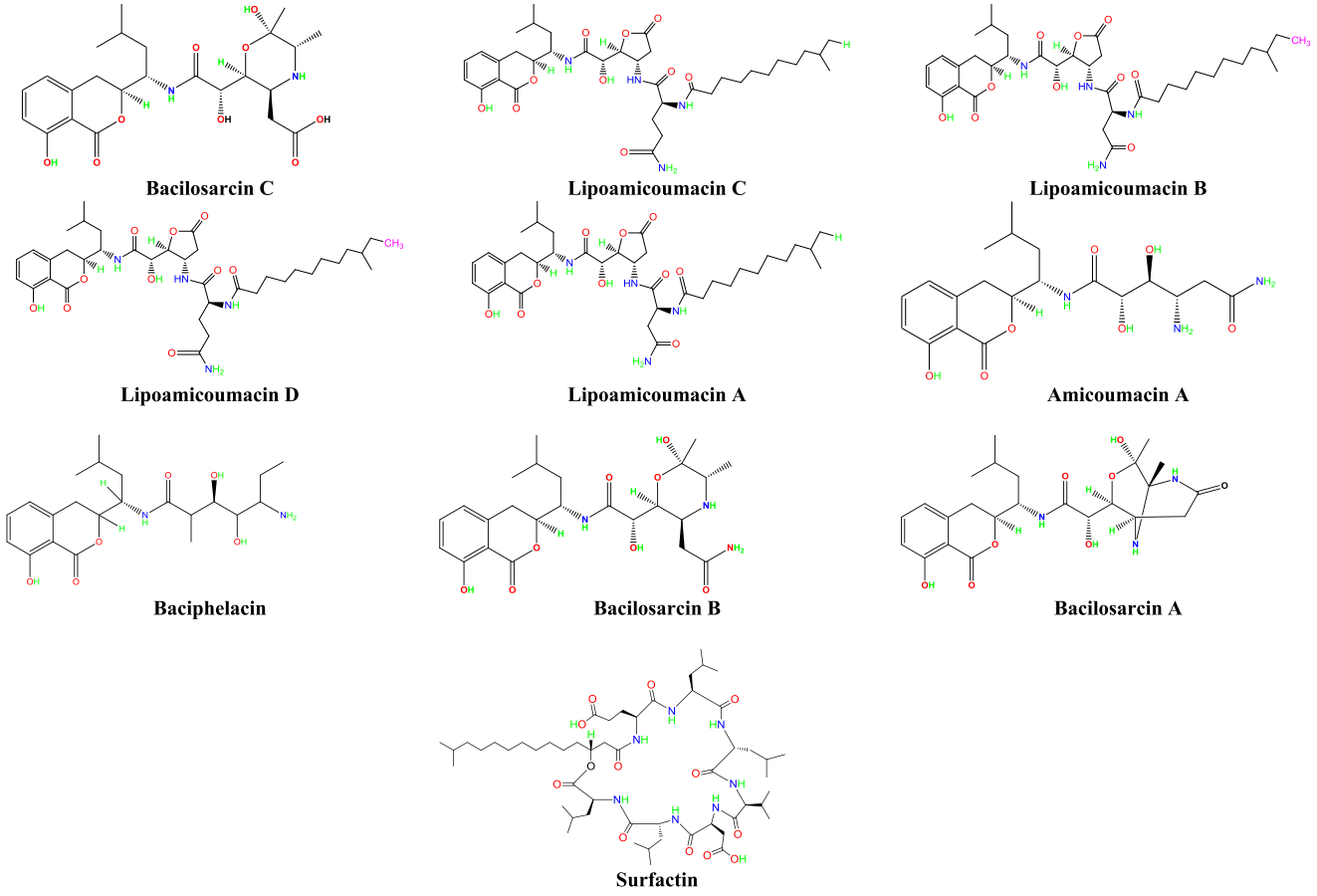

Fig. 1. Structure of antimicrobial compounds from *B. subtilis* used for molecular docking.

Table 1
GLIDE score of the best docking conformation of compounds from *Bacilus subtilis* with NF-κB, Caspase-3 and Caspase-8.

Compounds	NF-κB		Caspase-3		Caspase-8	
	Glide g-score	Glide energy	Glide g-score	Glide energy	Glide g-score	Glide energy
Bacilosarcin C	−7.513	−48.191	−8.152	−43.981	−13.406	−50.786
Lipoamicoumacin C	−8.702	−62.913	−9.017	−56.537	−8.233	−64.347
Lipoamicoumacin B	−7.878	−64.528	−9.32	−60.938	−8.211	−70.248
Lipoamicoumacin D	−7.699	−66.612	−9.955	−54.919	−7.779	−63.688
Lipoamicoumacin A	−8.495	−63.64	−9.171	−61.126	−7.651	−67.859
Amicoumacin A	−8.585	−52.559	−8.37	−40.441	−7.212	−44.068
Baciphelacin	−6.766	−42.469	−8.452	−45.365	−7.119	−44.001
Bacilosarcin B	−7.757	−49.76	−9.43	−44.33	−7.117	−48.336
Bacilosarcin A	−7.241	−40.724	−8.505	−43.233	−6.677	−38.605
Surfactin	−4.272	−45.113	−6.7	−51.247	1.227	−40.778

which can be further used for designing anticancer therapeutics.

2. Materials and methods

2.1. Protein preparation

The three dimensional structure of NF-κB (PDB ID: 1NFK), Caspase-3 (PDB ID: 1GFW) and Caspase-8 (PDB ID: 1QTN) were retrieved from the Protein Data Bank (PDB). All water molecules and ligands were removed from the protein and the chemistry of the protein was corrected for missing hydrogen. Crystallographic disorders and unfilled valence atoms were corrected using alternate conformations and valence monitor options. Following the above steps of preparation, the protein was subjected to energy minimization using the OPLS force field (Rajmohamed et al., 2017).

2.2. Active site prediction

Active site from PDB complex was detected using Sitemap program. This software generates information on the binding characteristics using novel search and analytical facilities. A Sitemap calculation begins with an initial search step which identifies or characterizes, through the use of grid points, one or more regions on the protein surface that may be suitable for binding of ligands to the receptor. Then contour maps are generated, followed by hydrophobic and hydrophilic maps (Lauria et al., 2009).

2.3. Ligand preparation

Among the 10 chosen antimicrobial compounds, Bacilosarcin C (CID: 132549711), Lipoamicoumacin B (CID: 101884392),

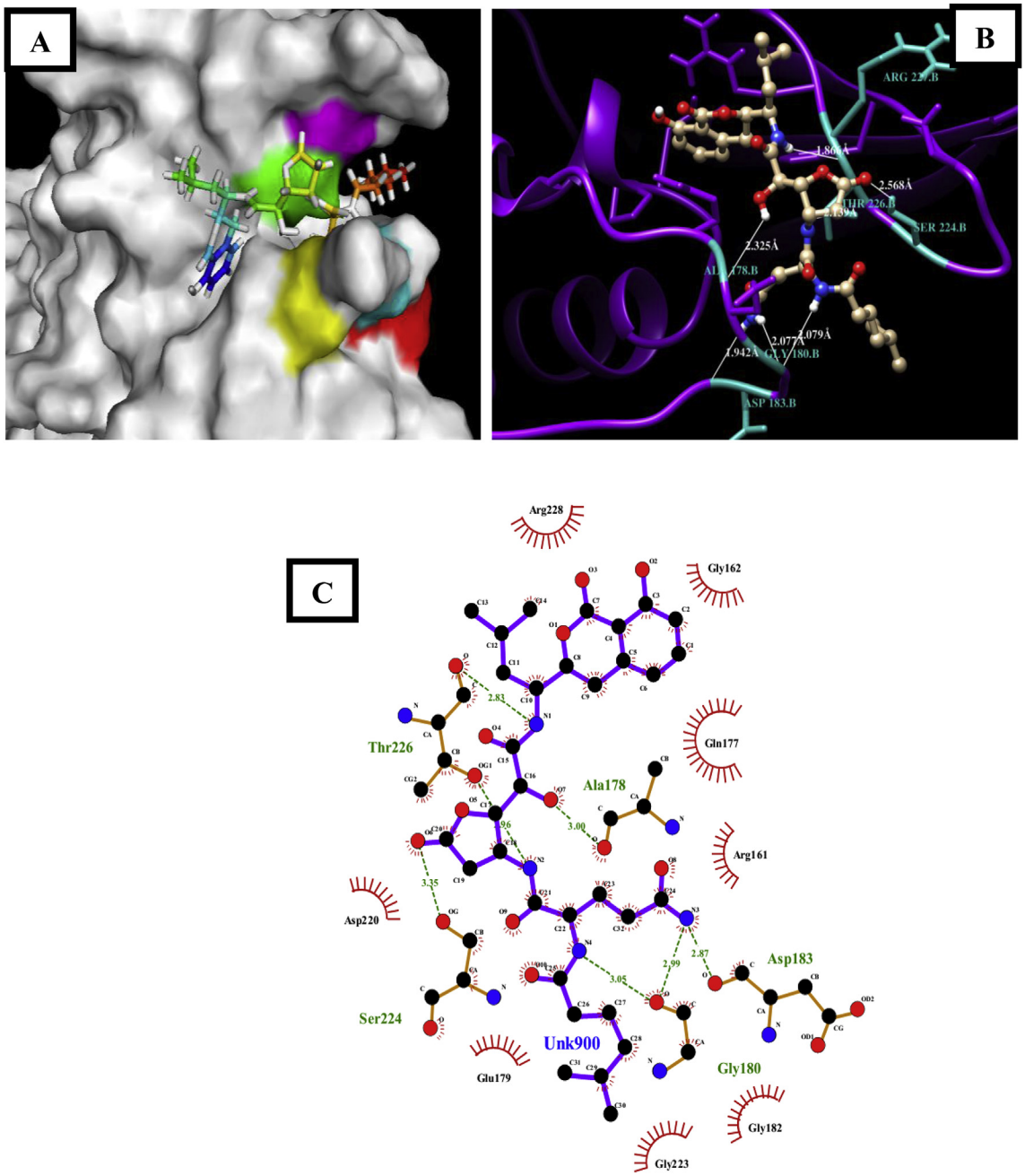

Fig. 2. Best docking pose conformation of the compound Lipoamicoumacin C with NF-κB. A. Surface view of binding of Lipoamicoumacin C in stick format with the binding pocket of NF-κB. B. Lipoamicoumacin C (ball and stick) binding with NF-κB (ribbon). C. Hydrogen bond interactions of Lipoamicoumacin C with NF-κB.

Amicoumacin A (CID: 25223631), Baciphelacin (CID: 171612), Bacilosarcin B (CID: 24905928), Bacilosarcin A (CID: 101439757) and Surfactin (CID: 65307) were obtained from pubchem database. Remaining 3 compounds namely, Lipoamicoumacin C, Lipoamicoumacin D and Lipoamicoumacin A were from literature (Li et al., 2012) and drawn using ChemDraw software. These ligands were processed with the LigPrep program to assign the suitable protonation states at physiological pH 7.0 ± 1.0. Conformer generation was carried out with the Conf Gen torsional sampling by using OPLS_2005 force field. The van der Waals radii were scaled using a default scaling factor of 0.80 and default partial cutoff charge of 0.15 to decrease the penalties.

2.4. Extra Precision (XP) docking

The ten extracellular antibacterial compounds of *Bacillus subtilis* were docked into the binding site of the Caspase-3, Caspase-8 and NF-κB oncoproteins using Grid Based Ligand Docking with Energetics (Glide) software from Schrodinger (Halgren et al., 2004; Friesner et al., 2004). The docking of *Bacillus subtilis* compounds with prepared oncoproteins were performed with OPLS_2005 force field using Extra Precision (XP) module of the Schrödinger Suite. The XP Glide Score scoring function was used to order the best ranked compounds. The specific interactions like hydrogen bonds were analysed using XP visualizer in Glide module. All the docked molecules were visualized using Pymol (The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC.), Chimera (Pettersen et al., 2004) and Ligplot^{+} (Laskowski and Swindells, 2011) software.

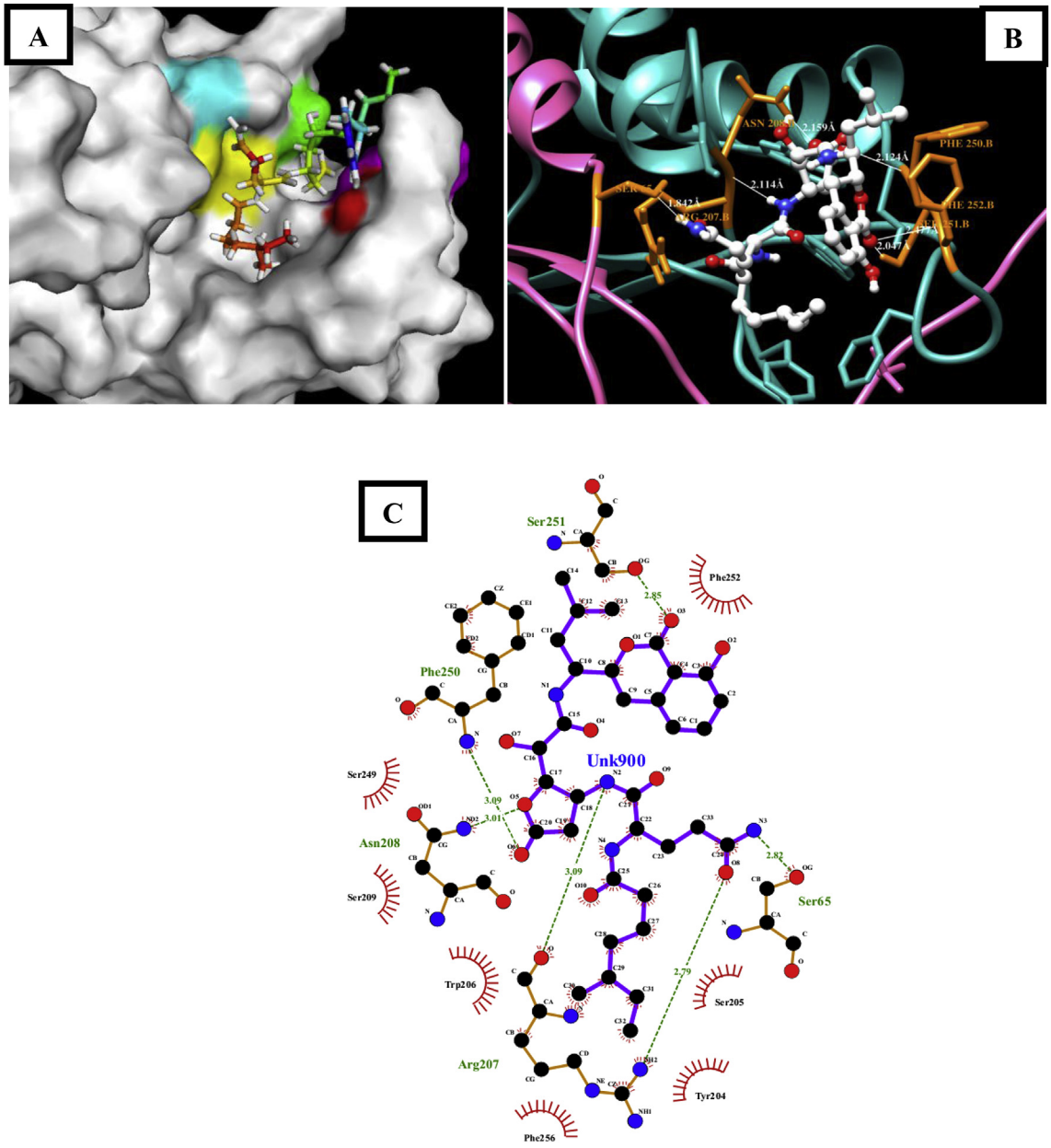

Fig. 3. Best docking pose conformation of the compound Lipoamicoumacin D with Caspase-3. A. Surface view of binding of Lipoamicoumacin D in stick format with the binding pocket of Caspase-3. B. Lipoamicoumacin D (ball and stick) binding with Caspase-3 (ribbon). C. Hydrogen bond interactions of Lipoamicoumacin D with Caspase-3.

3. Results and discussion

3.1. Molecular docking using GLIDE

The molecular docking of 10 bacteriocin compounds (Fig. 1) with NF-κB, Caspase-3 and Caspase-8 using Schrödinger software generated docking poses, glide score and interaction patterns. The best binding among the poses were selected and tabulated (Table 1). The docking pose of compounds with NF-κB, Caspase-3 and Caspase-8 in different modes such as three dimensional representations, buried surface area with ligand docked inside the binding cavity and two dimensional representation of protein-ligand complex with hydrogen bonds formed with residue and its distance expressed in Angstrom unit (Figs. 2–4).

3.2. NF Kappa B (NF-κB)

Most of the compounds form hydrogen bonds with NF-κB and the average distance was approximately 2.59–3.21 Å. By analyzing the hydrogen bond and interaction of compounds docked with NF-κB, it was found that Lipoamicoumacin C has formed maximum number of hydrogen bonds i.e., seven hydrogen bonds. It was followed by Lipoamicoumacin D, Lipoamicoumacin B and Lipoamicoumacin A that formed six, five and four hydrogen bonds (Table 2) respectively. Among these compounds, Lipoamicoumacin C has a maximum glide score of −8.702 (Table 1) and formed hydrogen bond with the NF-κB aminoacid residues such as Ala 178, Thr 226, Ser 224, Gly 180 and Asp 183 (Table 2, Fig. 2a–c). The three compounds other than Lipoamicoumacin C formed an average of one to four hydrogen bonds with the residues of NF-κB (Table 2). Similar search for NF-κB ligands revealed the involvement of the aminoacid residues like Thr, Ser, Asp and Glu with

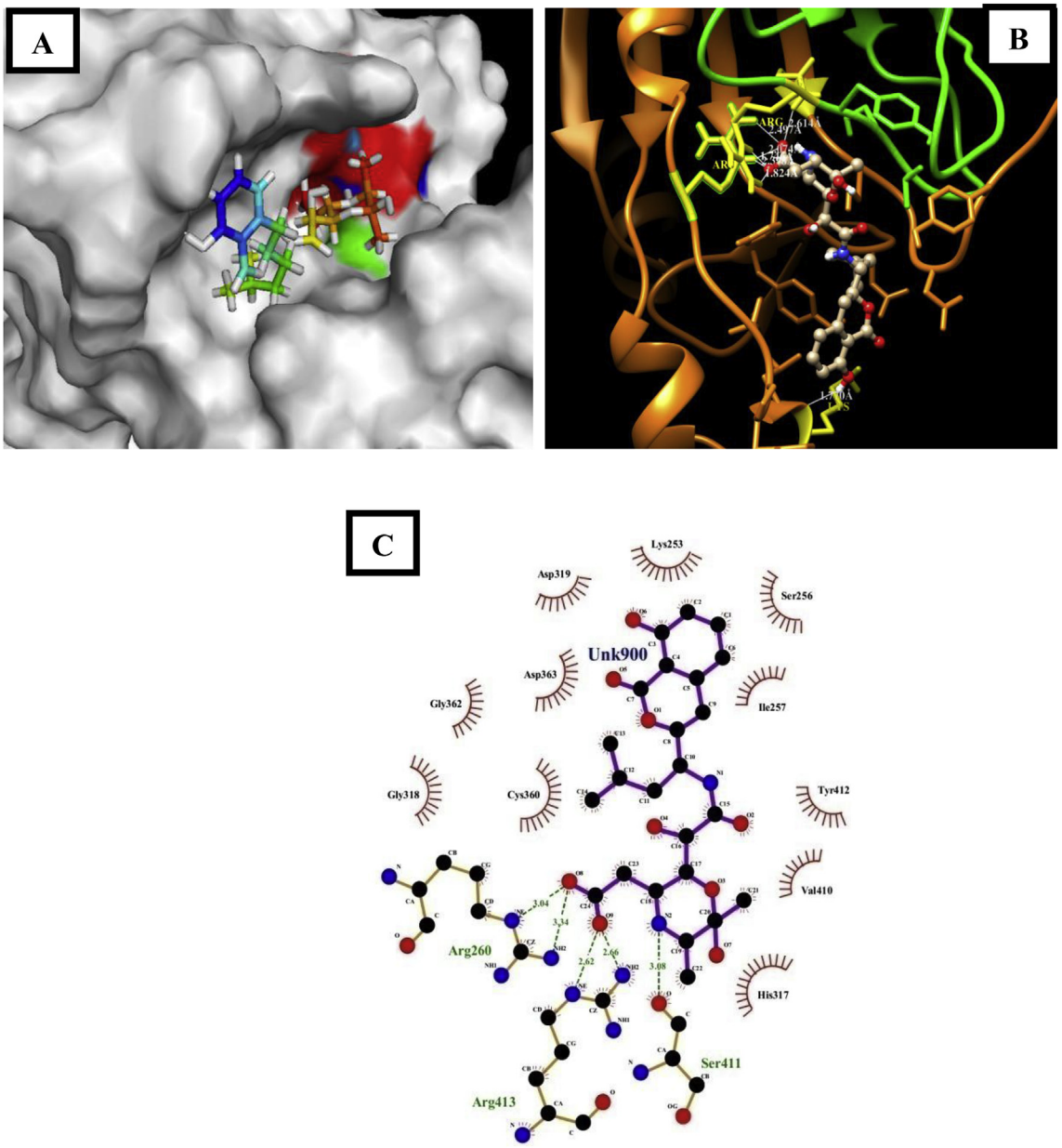

Fig. 4. Best docking pose conformation of the compound Bacilosarcin C with Caspase-8. A. Surface view of binding of Bacilosarcin C in stick format with the binding pocket of Caspase-8. B. Bacilosarcin C (ball and stick) binding with Caspase-8 (ribbon). C. Hydrogen bond interactions of Bacilosarcin C with Caspase-8.

other aminoacid residues for hydrogen bond interactions (Piccagli et al., 2008; Neelgundmath et al., 2015).

3.3. Caspase-3

Most of the compounds form hydrogen bonds with Caspase-3 with an average distance of 2.05–3.15 Å (Table 2). Among 10 bacteriocin compounds, Lipoamicoumacin D and B with glide score −9.955 and −9.32 formed five and six hydrogen bonds respectively. The compound Lipoamicoumacin A has the maximum hydrogen bond interactions; seven hydrogen bonds with aminoacid residues Asn 208, Phe 250, Ser 251, Trp 214, Gln 217 and Glide score is-9.171 (Tables 1 and 2). Lipoamicoumacin D binds with residues Asn 208, Phe 250, Arg 207, Ser 251 and Ser 65 and formed five hydrogen bonds with five aminoacid residues (Table 2, Fig. 3a–c) whereas Lipoamicoumacin B formed six hydrogen bonds with five aminoacid residues Asn 208, Phe 250, Arg 207, Ser 205, and Ser 251 (Table 2, Fig. 3a–c). In the virtual screening of non-peptide inhibitors as ligands against caspase 3, Arg 207, Ser 209 and Trp 214 in the active site were found to be involved (Lakshmi et al., 2009; Sharma et al., 2013). This study also indicated the involvement of same aminoacid residues in the same position for the binding of antimicrobial ligands with Caspase-3.

3.4. Caspase-8

All of the antibacterial compounds were docked with Caspase-8 and it formed one or more hydrogen bonds with the distance ranging between 2.62 and 3.35 Å (Table 2). Among 10 bacteriocin compounds, Lipoamicoumacin C formed nine hydrogen bonds, Lipoamicoumacin B and Lipoamicoumacin A formed seven and six hydrogen bonds respectively (Table 2). The Glide scores of Lipoamicoumacin C, B and A were −8.233, −8.211 and −7.651 respectively (Table 2). Though the Bacilosarcin C has maximum Glide score of −13.406 but it formed only five hydrogen bonds with 3 aminoacid residues Arg 260, Arg 413 and Ser 411. (Table 2, Fig. 4a–c). Remaining bacteriocin compounds showed one to four hydrogen bond interactions with Caspase-8. In the

Table 2
Hydrogen bond interactions of NF-κB, Caspase-3 and Caspase-8 upon docking with the compounds of *B. subtilis*.

Compounds	NF-κB		Caspase-3		Caspase-8	
	Amino acid Residues	Distance Å	Amino acid Residues	Distance Å	Amino acid Residues	Distance Å
Bacilosarcin C	Arg 228	2.59, 2.84	Ser 209	3.10	Arg 260	3.04, 3.34
			Asn 208	2.81	Arg 413	2.62, 2.66
			Phe 250	2.90	Ser 411	3.08
Lipoamicoumacin C	Ala 178	3.00	Asn 208	2.77	Arg 413	3.22, 2.94
	Thr 226	2.83, 2.96	Phe 250	2.05, 3.02	Arg 260	2.97, 2.88
	Ser 224	3.35	Phe 252	3.16	Arg 258	3.11
	Gly 180	3.05, 2.99	Trp 214	2.86, 2.75	Ser 316	2.91
	Asp 183	2.87			Cys 360	3.35
					Gln 358	3.08
					Lys 253	2.95
Lipoamicoumacin B	Ala 178	2.97	Asn 208	3.15	Arg 413	2.98, 3.10
	Thr 226	3.10	Phe 250	3.02, 2.99	Arg 258	2.82
	Ser 224	3.11	Arg 207	2.88	Ser 256	3.00
	Gly 180	2.81, 2.88	Ser 205	2.79	Ser 411	2.84
			Ser 251	3.09	Cys 360	3.26
					Asp 455	2.65
Lipoamicoumacin D	Thr 226	3.21, 3.16, 3.27	Asn 208	3.01	Arg 260	2.95, 3.04
	Ser 224	2.74	Phe 250	3.09	Ser 316	2.81
	Gly 180	3.01	Arg 207	3.09	Lys 253	2.94
	Glu 179	2.97	Ser 251	2.85		
			Ser 65	2.82		
Lipoamicoumacin A	Thr 226	2.81	Asn 208	2.89	Arg 413	2.82
	Ser 224	2.93	Phe 250	2.90, 3.04, 2.79	Arg 258	2.95
	Gly 180	2.96	Ser 251	2.94	Arg 260	3.19
	Gly 162	2.96	Trp 214	2.87	Ser 411	2.73
			Gln 217	3.09	Tyr 365	2.86
					Asn 458	2.90
Amicoumacin A	Ala 178	2.86	Asn 208	3.05	Nil	Nil
	Thr 226	2.94	Phe 250	2.79		
	Gly 180	2.67	Ser 251	3.11		
			Ser 205	2.96		
			Arg 207	2.98		
Baciphelacin	Ala 174	3.16	Arg 207	2.93	Arg 413	3.13
			Tyr 204	2.80	Asp 319	2.64
			Gly 122	2.85, 3.06	Tyr 324	2.94
			Glu 123	3.03		
Bacilosarcin_B	Thr 226	3.01, 3.07	Asn 208	3.00	Arg 413	2.80, 3.13
	Gly 180	2.67	Phe 250	2.75, 2.99	Arg 260	3.00
	Gln 177	2.76	Ser 251	2.98	Ser 411	2.75, 2.99
			Arg 207	2.91, 2.84		
Bacilosarcin A	Thr 226	3.04	Phe 250	2.75	Arg 413	2.98
	Ser 224	3.05	Ser 251	2.75	Cys 360	3.13
			Ser 209	2.84		
			Arg 207	3.24		
Surfactin	Arg 228	2.88, 2.92	Arg 207	2.87	Asp 363	2.71
	Gln 177	3.20	Arg 64	2.97, 3.00		
			Cys 163	3.07, 3.14		

search for inhibitors of Caspase-8 from phytochemical ligands, it was observed that Arg 258 is crucial in hydrogen bond interaction in addition to Arg 260 and Cys 360 apart from other aminoacid residues (Ahmad et al., 2014). In this study also, the hydrogen bond interaction between antimicrobial compounds and Caspase-8 is critically mediated by the same aminoacid residues (Arg 258, Arg 260 and Cys 360).

Docking analysis indicated that the antimicrobial compounds have specific functional groups that can interact with specific amino acid residues to fit properly in the binding pocket of cellular oncoproteins. It was revealed that the compounds Lipoamicoumacin C, Lipoamicoumacin D, B, and A formed 4 to 9 hydrogen bonds with NF-κB, Caspase-3 and Caspase-8. Bacilosarcin C has got maximum glide score of −13.406 but it formed 5 hydrogen bonds with Caspase-8. It can be inferred that *Bacillus subtilis* antibacterial compounds particularly Lipoamicoumacin C, D, B, A and Bacilosarcin C can exhibit enhanced immune responses, bind to suppress inflammation markers and also helpful to protect apoptotic inducing genes such as Caspase-3 and Caspase-8.

4. Conclusion

These compounds could be used as leads to design potential regulators against cancer inducing cellular oncoproteins. Thus, *in silico* approaches revealed that the *Bacillus subtilis* strain and its antibacterial compounds may have the therapeutic potential for enhancing immune responses, suppression of inflammation marker genes and anti-apoptotic genes. Such non-peptide inhibitors can be advantageous than the peptide inhibitors due to the problem of permeability and stability. However, further investigations would strengthen the understanding on the therapeutic value of these antimicrobial compounds.

Declaration of interest

None.

Acknowledgements

Award of UGC-RGN Fellowship, Govt. of India to AV is acknowledged. Sincere acknowledgement to Bharathidasan University,

Tiruchirappalli, India for providing Grid Based Ligand Docking with Energetics (GLIDE) software from Schrödinger facility to carry out this work.

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