

Annonaceous acetogenins: A computational study into their antitumor activity in multidrug resistant enzymes

Abdullah Alanzi

King Saud University

Ashaimaa Y. Moussa (✉ Ashaimaa_yehia@pharma.asu.edu.eg)

Ain shams University

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Abstract

Natural products that could reverse tumor drug resistance are promising and highly appreciated; especially, if they are part of combination therapy with conventional anti-cancer drugs. Acetogenins, the long chain polyketide derived fatty acids, from *Annona squamosa* and *muricata* were screened in the last 20 years and promising compounds were selected to investigate their activity by computational studies inside the binding pockets of four major MDR enzymes (EGFR, MCL-1, BCL-2, and NQO1). Using virtual screening and molecular docking, compounds were ranked according to their fitting scores and squamostanin A, annupupuricin, Squamocin IV were the best fitting molecules with favorable non covalent interactions with key amino acids compared to native ligands and standards. Subsequently, these compounds in their prepared enzyme complexes were subjected to molecular dynamics stability study to investigate the complex characteristics. The key interactions were confirmed in the molecular dynamic study, which manifested no noticeable unfolding of the binding complexes. The physicochemical and drug likeness properties of all the compounds were evaluated through the QikProp module indicating their suitable ADMET properties within the recommended ranges.

Introduction

Cancer remains the leading cause of death according to the WHO records in 2022 with breast, colon and prostate cancer dominating the cases. (WHO fact sheet. 2022). Even though chemotherapy is a core strategy to treat and curb metastatic cancer, it was largely combated by drug resistance that emerged in most cancer types due to the continuous mutations encountered in tumor cells and the efflux of drugs resulting in a gradual decrease in therapeutic agents' concentrations (Liu et al. 2016). Multidrug resistance in cancer cells with the sequential fast chemotherapeutic failure is responsible for the high mortality rate related to cancer (Li et al. 2017). Drugs with varying mechanistic stages and chemical structures rarely sustain longer than few months before being confronted by cellular resistance (Guièze et al. 2019)

In the mitochondrial intrinsic pathways, one of the principal regulators, subjected to multi-drug resistance, of tumor biology is the B cell lymphoma-2 (Bcl-2) family, whose increased production inhibit the apoptotic pathway and leads to chemoresistance. Bcl-2 proteins are classified into anti-apoptotic and proapoptotic components integrating together to role the apoptotic system. While apoptotic activators as Bim, tBid and Puma bind to the anti-apoptotic proteins involving Mcl-1, Bcl-XL and Bcl-w, the effectors play a vital role by releasing the two interacting proteins from each other thus, delivering apoptosis inducing factor (AIF) and cytochrome c, which turn on the mitochondrial outer membrane permeabilization (MOMP) and the apoptotic vehicle (Xu et al. 2022). One of the major Bcl-2 inhibitors is venetoclax used in hematological cancers. Venetoclax binded to the Bcl2 binding pocket and induced the mitochondrial apoptotic pathway. Yet, as most of the antitumor agents, resistance developed against venetoclax as soon as it was used in a wider range. This was mainly through the enhanced production of antiapoptotic proteins as BCL-X and MCL-1, which bind to BIM; therefore, counteracting the apoptotic machinery. These resistance-related cellular changes are based on structural mutations in Bcl2 and BAX genes and others leading subsequently to the compromised therapeutic effect of venetoclax. (Xu et al. 2022).

Epidermal growth factor receptor protein (EGFR) involved in many dermatological cellular processes as proliferation and growth is elevated in patients with ovarian cancers as well as several downstream regulators in its pathway. (Tripathi et al. 2020). Besides K-RAS mutations, primary secondary or tertiary mutations in EGFR gene are the major cause of lung cancer. Even for patients receiving the EGFR tyrosine kinase inhibitors, the incidence of resistance is within few months (Russo et al. 2015). The EGFR inhibitors and their encountered resistance, therefore, demanded more novel compounds to target the EGFR tyrosine kinases; particularly, the T790M mutations causing around half of the drug resistance cases (Shi et al. 2022). Similarly, NAD(P)H: quinone oxidoreductase (NQO) cytosolic enzymes family provided chemoprotection through one electron reduction processes for the quinone endogenous and exogenous bio reductive substances to yield hydroquinones. Recently, the redox resetting of tumor cells has contributed to the resistance development where quinones and hydroquinone metabolic products were employed to combat cancer by killing tumor masses enriched with antioxidant enzymes. NQO1 role as a two-edged sword acted both for detoxification and toxicity induction presenting a promising target for cancer drug design. in brief, quinone conjugates were produced to trigger the formation of cytotoxic compounds inside the tumor masses; consequently, the role of NQO1 cannot be more emphasized as a receptor model in need of modulation for further better activity (Lee et al. 2021).

Annonaceous acetogenins are long chain polyketide derived fatty acids with a C-35-37 nucleus found almost exclusively in genus *Annona*. Uvaricin was the first reported before the introduction of more than 500 acetogenins from different organs of the plants belonging to family Annonaceae. (Lima et al. 2022). Acetogenins antitumor activity was attributed to key structural features, among which are the γ -lactone methyl-alpha beta-unsaturated ring in the terminal side and the tetrahydrofuran or the tetrahydropyran ring combinations believed to contribute to most of the pharmacological activities (Antony et al. 2016). These lipophilic molecules are classified based on their structural features, number of THF rings, and shapes of the lactone ring into type-A AAGs with a single THF ring and Type-B AAGs with bis-THF rings adjacently attached in the aliphatic chain. While subtypes 1a denoted the α , β -unsaturated γ -methyl- γ -lactone, subtypes 1b indicated a C-4 hydroxy lactone ring.

Studies indicated that the potent anticancer activity of AAGs involved a mechanism of action related to mitochondrial complex I inhibitory activity and apoptosis induction. Whereas autophagy induction with cell cycle arrest was manifested *in-vitro* studies, tumor suppression was

noted *in-vivo*, and the principal SAR feature was the presence of one or two hydroxy groups with either mono or bis THF rings (Jacobo-Herrera et al. 2019). The neurotoxic effect of acetogenins was reported when annonacin, isolated from *Annona muricata*, induced neurodegenerative changes with symptoms close to neuropathology or Parkinson disease in Caribbean tropical islands (Lima et al. 2022). Acetogenins intake at 6mg/mL recorded mesencephalic toxicity; accordingly, their use as anticancer agents was never pursued. (Lannuzel et al. 2002). Acetogenins effect on MDR cancers were only investigated in few studies and none discussed the binding affinity nor molecular interactions in the targeted enzymes binding pockets. Computational studies can provide an overview on the effect of mutations in these enzymes prone to resistance and give a prospect about the possible efficacy in drug resistant cancer cells in a cost and time effective manner. The use of low concentration acetogenins in antitumor drug combinations to reverse drug resistance is a promising approach still unappreciated. (Manoharan et al. 2021).

Virtual screening and *in-silico* molecular docking enable the prediction of bioactivities of natural compounds either from chemical libraries or database literature search based on preset criteria to save time, cost and effort that might be wasted doing *in-vitro* or cellular assays on unpromising entities. Molecular docking results verified by molecular dynamics can accurately estimate the binding interactions and stability of the selected molecules inside enzymes binding pockets; thus, being left with fewer hits than enable better investigation and faster further advancing to lead discovery. In this study, four vital cancer target enzymes NQO1, EGFR, BCL-2 and MCL-1 characterized by their high drug resistant rate were utilized herein to point out acetogenins mode of binding, strength, and molecular interactions. Selected acetogenin compounds from *Annona muricata* and *squamosa* were assigned to conduct the virtual screening and molecular docking. A molecular dynamics study (MD) in 100ns was conducted to illustrate the stability of binding of the most promising compounds. Furthermore, the ADME (absorption, distribution, metabolism, excretion, and toxicity) properties were measured for the chosen acetogenins to evaluate their physicochemical properties as well as pharmacokinetics, pharmacodynamics and toxicity (Periyasamy et al. 2021).

Selected Acetogenins Scaffold:

The chemical skeleton of acetogenins was investigated in this study through the selection eighteen compounds isolated from *Annona squamosa* and *muricata*. These were classified as: non-adjacent bis-tetrahydrofurans (THFs) as squamostanin A and B, adjacent -bis THF as annosquacin I, or mono THF derivatives. The latter was categorized according to its structure activity features and the number of hydroxyl groups, their positions, and stereochemistry. Moreover, annocherimolin and the C-37 muricenin were included as two acetogenins with C21-C22 double bonds.

Materials And Methods

Ligand selection and preparation

For anti-cancer screening, 114 compounds belong to annonaceous acetogenins were selected based on available literature. Their 2D structures were retrieved from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) and the ligands were prepared by using the LigPrep tool of Maestro (LigPrep. 2018). The geometries of the all ligands were optimized, and for each, 32 conformers were generated. The OPLS_2005 forcefield was used to minimize the energy of all prepared ligands (Shivakumar et al. 2012). The 3D structures of the prepared ligands were then saved as '.mae' files in Maestro Molecular Model Format (Schrödinger, LLC.) for the docking analysis.

Protein Preparation

As this study assesses the molecular interactions of natural compound with human NAD[P]H-Quinone Oxidoreductase (PDB ID: 1H69), epidermal growth factor receptor (EGFR) tyrosine kinase (PDB ID: 1M17) Bcl2-xL (PDB ID: 2W3L), and human Mcl-1 (PDB ID: 6UDV) were retrieved from Protein Data Bank (<https://www.rcsb.org>). The 3D structures of the targets were prepared using Protein Preparation Wizard in Schrodinger Maestro (Schrödinger. 2017). The targets were further processed by addition of hydrogens, creating disulfide bonds, zero order bonds to metals and assigning bond orders, as well as by removing any additional ligands and water molecules. The hydrogen bonds were optimized at pH 7.0 by using PROPKA (Kim et al. 2013). Finally, their energies were minimized (OPLS_2005 forcefield), and a 3D grid was generated at reported specific site of each target protein to conduct site- specific docking. The X, Y, and Z coordinates of grid box was in following order: (1H69: X = 19.8, Y = -9.23, Z = 5.54), (1M17: X = 21.29, Y = 0.53, Z = 52.37), (2W3L: X = 39.81, Y = 26.94, Z = -12.41), and (6UDV: X = 6.21, Y = 2.92, Z = 99.83).

Molecular Docking (Md) Analysis

The prepared ligands were docked against the target proteins at specific sites by using Glide docking module in Standard Precision (SP) mode (Friesner et al. 2004). The docked ligands were analyzed and selected based on the glide scores.

Physicochemical Properties Prediction (Dup: Abstract ?)

The selected ligands were subjected to calculate the physicochemical properties, such as molecular weight, hydrogen bond donors and hydrogen bond acceptors, and the relationship between their polar surface area (PSA) and LogP were predicted by using the QikProp tool of Maestro (Mali & Chaudhari. 2018).

Binding Modes Analysis

The binding modes of all selected ligands were analyzed and one compound from each protein were selected based on best plausible binding modes for protein-ligand stability analysis by MD Simulation in following step.

Md Simulation (Dup: Abstract ?)

The docking results of targeted proteins were analyzed and one compound for each study were selected for protein-ligand stability analysis at 100 ns MD Simulation using VMD (Humphrey, Dalke, & Schulten, 1996) and NAMD (Phillips et al., 2020) tools. The input files required for simulation were generated in Ambertools 21 (Case et al. 2021), therein the Antechamber was used to generate the ligand topology files while the missing hydrogens were added by using LeaP program (Case et al. 2005). Each ligand-protein complex was then solvated in a solvation box of 10 Å containing TIP3P water model (Jorgensen & Chandrasekhar. 1983). To further neutralize the systems, counter Na⁺ and Cl⁻ ions were added to the complexes. To remove clashes, the complexes were minimized by using ff14SB and GAFF forcefields for protein and ligand, respectively (Duan et al. 2003). Finally, the solvation systems were subjected to three additional equilibrations at 200, 250, and 300 K. The processed ligand-protein complexes were then subjected to 50 ns simulation, and the MD trajectories were saved at every 2 ps interval. The trajectories were analyzed by CPPTRAJ (Weinzierl. 2021) and R package (Grant, Skjærven, & Yao. 2021).

Results

Molecular docking

The selected compounds were docked against targeted proteins to find their virtual binding affinities. All the ligands were ranked based on the glide scores. Based on their binding affinities, 20 compounds for each target protein were selected (Table 2). The binding affinities of the selected ligands were in the range of -11.098 to -9.261 Kcal/mol for with human NAD[P]H-quinone oxidoreductase; -7.273 to -6.175Kcal/mol for epidermal growth factor receptor tyrosine kinase; -6.334 to -5.583 Kcal/mol for Bcl2-xL; and - 7.122 to -6.217 Kcal/mol for human Mcl-1.

Table 2
The Glide score of the selected acetogenins in the four docking enzymes.

The Glide score of the selected acetogenins in the four docking enzymes.								
Human NAD[P]H-quinone oxidoreductase Co			Epidermal growth factor receptor tyrosine kinase				Bcl2-xL	Human Mcl-1
No.	Compound	Glide score (Kcal/mol)	Compound	Glide score (Kcal/mol)	Compound	Glide score (Kcal/mol)	Compound	Glide score (Kcal/mol)
1	Squamostanin-A	-11.098	Squamostanin-A	-7.273	Annopurpuricin A	-6.334	Squamocin IV	-7.01
2	Squamocin-P	-10.66	Montalicin G	-7.213	Muricin G	-6.14	Muricenin	-6.766
3	Muricin D	-10.5	Squadiolin B	-7.029	Squamocin III	-6.046	Squamostanin-B	-6.72
4	Annopurpuricin D	-10.461	Annomontacin	-6.835	Annosquatin IV	-5.932	Rollicosin	-6.569
5	Calamistrin F	-10.241	Anmontanin B	-6.698	Squadiolin A	-5.827	Squamostanin-A	-6.543
6	Squamostanin-B	-10.157	Muricin E	-6.668	Annosquatin B	-5.717	Muricin F	-6.512
7	Squamotin A	-10.071	Monlicin A	-6.65	Muricin F	-5.707	Annocatacin B	-6.505
8	Annopurpuricin C	-9.98	Montalicin B	-6.473	cis-montacin	-5.698	Anmontanin B	-6.474
9	Tucupentol	-9.79	Annosquacin-I	-6.46	Annosquatin-I	-5.693	Annonamuricin C	-6.453
10	Cohibin D	-9.789	Annosquacin C	-6.436	Annosquacin C	-5.669	Annocherimolin	-6.408
11	Annosquacin B	-9.745	Annocherimolin	-6.436	Asitrilobin C	-5.631	Squamocin-P	-6.38
12	Montalicin E	-9.698	Muricenin	-6.431	Annosquatin A	-5.62	Annonamuricin B	-6.352
13	Muricin G	-9.489	Squamostanin-B	-6.424	Squamostanin-A	-5.613	Annonamuricin A	-6.325
14	calamistrin D	-9.464	Annosquatin IV	-6.373	Squamocin-P	-5.504	Annonamuricin D	-6.319
15	Annosquacin D	-9.443	Annonamuricin A	-6.367	Squamostanin-B	-5.477	Calamistrin E	-6.305
16	Montalicin H	-9.438	Tucupentol	-6.3	(2,4)-cis-asitrocinone	-5.467	Dotistenin	-6.26
17	Annopurpuricin B	-9.426	Asitrilobin C	-6.215	Muricin H	-5.467	Annosquatin B	-6.242
18	Annosquatin-II	-9.419	Annonacin	-6.209	Annocherimolin	-5.303	Squafosacin B	-6.217

Physicochemical Properties Prediction

The physicochemical properties of the selected ligands were predicted by using the QikProp tool of Maestro. The predicted properties included molecular weight, hydrogen bond donors, hydrogen bond acceptor, LogP, and PSA (Table 1). The acceptable range for the molecular weight was < 500 g/mol, < 10 hydrogen bond acceptors and < hydrogen bond donors whereas the value of logP was considered < 5. Notably, with few exceptions, all compounds met the criteria of Lipinski's rule of five.

Table 1

The virtual determination of physicochemical properties of selected compound MW: molecular weight; HBD: hydrogen-bond donor; HBA: hydrogen-bond acceptor; PSA: polar surface area; RO5: rule of five

No.	Compound	MW (g/mol)	HBD	HBA	QPlogP _{o/w}	PSA	RO5 (Violations)
1	Squamostanin-A	638.924	4	13	137.973	6.183	2
2	Squamocin-P	638.924	4	13	135.19	6.373	2
3	Muricin D	568.833	4	11	130.506	5.699	2
4	Annopurpuricin D	622.924	3	11	116.467	7.323	2
5	Calamistrin F	608.941	3	10	110.979	8.18	1
6	Squamostanin-B	638.924	4	13	140.098	6.188	2
7	Squamotin A	622.924	3	11	118.434	7.364	2
8	Annopurpuricin C	606.925	2	10	97.89	8.444	1
9	Tucupentol	612.886	5	13	152.242	5.214	2
10	Cohibin D	576.942	2	6	80.428	10.12	1
11	Annosquacin B	606.925	2	10	98.368	8.343	1
12	Montalicin E	622.924	4	11	133.303	6.919	2
13	Muricin G	594.871	4	11	125.718	6.09	2
14	Calamistrin D	608.941	3	10	110.446	8.198	1
15	Annosquacin D	606.925	2	10	97.607	8.386	1
16	Montalicin H	612.886	5	13	147.68	5.396	2
17	Annopurpuricin B	622.924	3	11	116.345	7.302	2
18	Annosquatin-II	638.924	4	13	136.76	6.404	2
19	Annocatacin B	578.871	2	9	95.064	7.817	1
20	Squamosten B	596.886	4	11	131.619	6.326	2
21	Montalicin G	612.886	5	13	149.605	5.45	2
22	Squamostanin-D	622.924	3	11	112.632	6.191	2
23	Squadiolin B	640.939	5	13	151.005	5.773	2
24	Annomontacin	624.94	4	11	130.696	7.275	2
25	Anmontanin B	610.87	3	12	156.63	6.042	2
26	Muricin E	568.833	4	11	130.377	5.674	2
27	Monlicin A	596.886	4	11	129.345	6.535	2
28	Montalicin B	596.886	4	11	129.345	6.535	2
29	Annosquacin-I	622.924	3	11	120.045	7.117	2
30	Annosquacin C	622.924	3	11	117.545	7.336	2
31	Annocherimolin	622.924	4	11	131.893	7.093	2
32	Muricenin	622.924	4	11	131.373	7.174	2
33	Annosquatin IV	638.924	4	13	135.616	6.071	2
34	Annonamuricin A	596.886	4	11	130.752	6.443	2
35	Asitrilobin C	624.94	4	11	129.77	7.296	2
36	Annonacin	596.886	4	11	129.614	6.525	2
37	Muricin-I	606.925	3	10	104.229	7.467	2

No.	Compound	MW (g/mol)	HBD	HBA	QLogP _{o/w}	PSA	RO5 (Violations)
38	Annopurpuricin A	622.924	3	11	116.889	7.269	2
39	Squamocin III	622.924	3	11	114.877	7.154	2
40	Squadiolin A	640.939	5	13	146.728	5.989	2
41	Annosquatin B	654.923	5	14	158.724	5.241	2
42	Muricin F	594.871	4	11	131.137	6.333	2
43	cis-montacin	610.87	3	12	155.104	5.945	2
44	Annosquatin-I	622.924	3	11	120.045	7.117	2
45	Plagionicin A	596.886	4	11	130.694	6.44	2
46	Annosquatin A	638.924	4	13	139.05	6.142	2
47	(2,4)-cis- asitrocione	596.886	3	11	137.861	6.456	2
48	Muricin H	580.887	3	10	109.565	7.523	2
49	Gigantecin	638.924	4	13	139.501	6.282	2
50	Squamocin IV	622.924	3	11	117.612	7.355	2
51	Rollicosin	396.523	2	10	121.541	2.938	0
52	Annonamuricin C	596.886	4	11	131.016	6.365	2
53	Annonamuricin B	596.886	4	11	130.773	6.408	2
54	Annonamuricin D	596.886	4	11	127.449	6.549	2
55	Calamistrin E	592.898	3	10	109.835	7.922	1
56	Dotistenin	562.872	2	8	91.94	8.513	1
57	Squafofacin B	624.94	4	11	130.36	7.159	2

Binding Pose Prediction

The binding poses of the docked ligands were analyzed and one compound from each complex was selected for protein-ligand stability analysis. For human NAD[P]H-quinone oxidoreductase, the selected ligand was Squamostanin-A whose interactions and binding pose are shown (Fig. 2A-B). It was observed that Squamostanin-A formed three hydrogen bonds with His11, Trp105, and Tyr12 whereas it was also involved in Pi-alkyl interaction with Ile50, Pro102, Phe106, His161, Phe178 and Met154. The binding pose showed that the ligand occupied specified space in the binding pocket of human NAD[P]H-quinone oxidoreductase.

For Epidermal Growth Factor Receptor tyrosine kinase, Squamostanin-A was selected. Therein, Squamostanin-A formed 3 hydrogen bonds with epidermal growth factor receptor tyrosine kinase residues Lys692, Asp776, and Asp831 while interacted through Pi-Alkyl bonds with Leu694, Val702, Ala719, Lys721, Cys773, Tyr777, and Leu821. The overall binding mode described that it occupied the residues with in the 3D grid box (Fig. 3A-B)

In the case of Bcl2-xL, Annopurpuricin-A was selected. Therein, Annopurpuricin-A formed 4 hydrogen bonds with Arg88, Glu95, and Asn102 as well as showed Pi-Alkyl interaction with Phe63, Arg66, Tyr67, Met74, Val92, Arg105, Val107, and Ala108. The interactions of Annopurpuricin-A with Bcl2-xl are shown in (Fig. 4A-B).

Lastly, for human Mcl-1, Squamocin_IV was selected. Therein, Squamocin_IV formed four hydrogen bonds with Lys234, His252, Arg263, and Thr266 including nine Pi-Alkyl interactions with Val274, Met250, Val253, Leu246, Leu267, Phe270, Val258, Val249 (Fig. 5A-B).

Md Simulation

The MD trajectories were analyzed to calculate the RMSD, RMSF, and Radius of gyration (Rg) of all selected ligand-protein complexes, separately. The RMSD plots of human NAD[P]H-quinone oxidoreductase -Squamostanin-A complex showed that the systems attained equilibrium at 10 ns with a RMSD value of ~ 1.5 Å (Fig. 6). After equilibration, the complex did not show any major deviation in the RMSD value

till 50 ns and after that the protein conformation deviated to ~ 1.75 Å and remained in the same range till the end of simulation which indicated that the protein did not undergo major conformational changes during simulation. The RMSD plot of Squamostanin-A exhibited that it was bound stably with protein throughout the simulation except for minor deviations during 80 to 90 ns but it attained the previous conformation towards the end of simulation. Similarly, RMSF analysis revealed that there were no major fluctuations in protein residues were observed. The higher RMSF value showed the loops regions while lower RMSF indicated the rigid secondary structures. The protein residues did not show major fluctuations except for C- and N-terminals. The Rg describes the compactness of protein structure where higher values infer that protein face some unfolding events during simulation. The Rg plots of human NAD[P]H-quinone oxidoreductase-Squamostanin-A complex showed that the system had a stable Rg value till 60 ns but then a deviation of ~ 0.2 Å was observed between 60–80 ns while it attained stability after 80 ns at ~ 23.1 – 23.2 Å. The overall results showed that human NAD[P]H-quinone oxidoreductase-Squamostanin-A complex did not face any unfolding event during simulation and remained compact.

The complex of epidermal growth factor receptor tyrosine kinase with Squamostanin-A was picked for the MD simulation. The RMSD plots of epidermal growth factor receptor tyrosine kinase backbone atoms showed that the RMSD values of the complex attained equilibrium at 10 ns and after that a sudden flip in the conformation was observed as the RMSD value exceeded to ~ 3.5 Å during 10 to 20 ns and then slightly decreased to ~ 2.5 Å during 20 to 30 ns. The RMSD value gradually increased to ~ 4.5 Å at 60 ns and then reduced to ~ 3 Å at 70 ns, The RMSD remained in the range of ~ 3 – 4 Å till the end of simulation after 70 ns. The RMSD analysis of Squamostanin-A showed it deviated throughout the simulation but at the end, it attained the equilibrated conformation (Fig. 7). The RMSF plots show that the major fluctuations were in the residues from 50 to 60 as this region contained loops the remaining residues did not show any notable fluctuation. Similarly, the Rg plots showed that protein bound to ligand remained compact as the Rg values did not show major variations and remained between ~ 19.75 – 20 Å during whole simulation time. This indicated that the ligand did not induce any unfolding event in the protein structure.

In the case of Bcl2-xL, the complex with Annopurpuricin-A was selected for MD simulation analysis. The RMSD of backbone atoms of Bcl2-xL complex did not show major deviations as it remained in the range of ~ 2 – 3 Å all the time (Fig. 8). While the RMSD of Annopurpuricin-A was thoroughly stable in the range of ~ 3 – 3.5 Å, but it showed a minor deviation towards the end of simulation. The RMSF analysis showed no major fluctuation in Bcl2-xL residues. Though some minor fluctuations were observed, the overall structure did not show flexibility. Similarly, the Rg plots showed that the protein remained in compact form when bound to ligand till 40 ns and then showed a major deviation of ~ 1 Å during 40 to 50 ns. The protein attained compactness during 50 to 80 ns but again showed a major deviation of ~ 2 Å during the period of 80 to 95 ns. The Rg plot showed that the protein might experience some unfolding events during the said time period.

For the human Mcl-1 protein, squamocin_IV-complex was selected for stability analysis. The RMSD of human Mcl-1-squamocin_IV complex backbone showed that the complex was equilibrated at 10 ns, and then then attained stability in the range of ~ 2 – 2.5 Å till 95 ns. The RMSD value increased to ~ 3 Å towards the end of simulation (Fig. 9). The RMSD of Squamocin_IV was stable after being equilibrated at 10 ns and remained in the range of ~ 3 to 4 Å till 90 ns. It increases to 5 Å towards the end of simulation. The RMSF analysis showed that the human Mcl-1 residues did not show major fluctuations except for C-terminal. The Rg analysis showed that the Mcl-1 protein complex remained in the range of ~ 15.4 to 15.8 till 20 ns and then dropped to ~ 15.2 Å and remained in the same range till the end of simulation. The Rg plot of complex showed that the protein remained compact during simulation and did not show any conformational changes.

Discussion

More than thirty years ago, the first annonaceous acetogenin was discovered and named uvaricin with a nanomolar range minimum inhibitory concentration. These molecules were the subject of research as promising anticancer agents against several cancer cell lines, and over 500 analogues were reported ever since; nevertheless, the mode of action was not clearly understood and remained vague (Juang et al. 2016). Despite *in-vitro* and *in-vivo* studies suggesting the involvement of mitochondrial complex II disruption, cell cycle arrest, apoptosis or superoxide anion production, further advancing to produce pharmaceutical agents from acetogenins was hindered by the lack of a distinct mechanism (Juang et al. 2016). Moreover, the neurotoxicity manifested by the annonaceous acetogenins discouraged the attempts to proceed towards clinical trials (Lannuzel et al. 2002).

Today, multidrug resistant tumor cells are rising dramatically. Wherever a new chemotherapeutic agent was discovered and used, cancer cells respond with new resistance mechanism to maintain its survival in a vicious cycle that long existed and now realized to be more complex than ever perceived. Accordingly, discovering drug combinations with unique chemical components has never been more in demand (Liu et al. 2016).

Among the most chemo-resistant enzyme targets, EGFR and its inhibitors conferred a stromal micro environment and genetic factors largely hindering further cellular susceptibility (Garvey et al. 2020). Similarly, BCL-2 and MCL-1 encounter deletions or mutations in their genetic sequences, and abnormal pathway changes, which sustain proliferation and growth (Xu and Ye. 2022).

Based on the previous, we embarked on testing 18 acetogenins compounds using in-silico molecular docking with the aim of finding suitable molecules that could serve in low amounts in cancer therapeutic combination regimens to reverse the protein drug resistance.

A selected chemical scaffold of 18 acetogenin compounds were screened for their activity in four vital enzymes, human NAD[P]H-Quinone Oxidoreductase (PDB ID: 1H69), epidermal growth factor receptor (EGFR) tyrosine kinase (PDB ID: 1M17), Bcl2-xL (PDB ID: 2W3L), and human Mcl-1. In the EGFR, previous work of chen et al indicated the non-adjacent bis-THF were more effective than the mono THF derivatives, and the latter in turn was more powerful than compounds devoid of THF. The adjacent bis-THF acetogenins were of less efficacy than the non-adjacent with few carbon bonds between the rings. and the mono THF was less effective. This was clearly seen in our results where Squamostanin-A showed better binding than annosquacin C. Annosquatin IV was of better binding than tucupentol; even within the mono THF compounds, the position of the hydroxyl groups dictates the better binding as in annomontacin which scored lower Glidescore than monlicin A.

The range of ΔG free energy was between -7.273 and -6.175 Kcal/mol. compared to the native ligand -9.379 kcal/mol (AQ4). Squamostanin-A was the best fitting compound in the EGFR showing hydrogen bonds with residues Lys692, Asp776, and Asp831 and Pi-alkyl non covalent bonds with Cys773, Tyr777 and other non-polar amino acids. The AQ4 ligand simulated the same binding mode with Cys 773; moreover, it interacted with the mutation site Met 769. The lack of binding with this residue Met769 in squamostanin A is advantageous as it supported our hypothesis that acetogenins could affect the drug resistance EGFR binding pockets (Table 3). The stability of these interactions was verified by molecular dynamic simulation of the EGFR squamostanin A complex.

Table 3

Binding free energy and docking interactions of selected acetogenins in the binding pocket of human NAD[P]H-quinone oxidoreductase.

Human NAD[P]H-quinone oxidoreductase			
No.	Compound	Glide score (Kcal/mol)	Interactions
1	Squamostanin-A	-11.098	3 Hydrogen bonds: His11, Tyr126, Trp105 7 Hydrophobic interactions: Phe106, Tyr128, His161, Pro102, His161, Met154, Ile50
2	Squamocin-P	-10.66	6 Hydrogen bonds: Phe17, Asn18, Gly149, Gly150, Leu103, Trp105 9 Hydrophobic interactions: Phe106, Tyr128, His194, Pro68, Pro102, His11, His161, Met154, Ile192
3	Muricin D	-10.5	7 Hydrogen bonds: His11, Gly149, Trp105, Tyr67, Tyr155, Leu103, Phe106 5 Hydrophobic interactions: Ile50, Pro68, Tyr128, Met154, Tyr42 2 Pi-Sigma bonds: His11, Trp105
4	Annopurpuricin D	-10.461	5 Hydrogen bonds: Phe17, Gly149, Trp105, Tyr155, Gly150 10 Hydrophobic interactions: Ile50, Tyr67, Tyr126, Pro68, Pro102, Phe106, His161, Tyr128, Phe232, His194
5	Calamistrin F	-10.241	5 Hydrogen bonds: Phe17, Asn18, Trp105, Tyr155, Gly150 9 Hydrophobic interactions: Ile50, Tyr67, Tyr126, Pro68, Pro102, Phe106, Tyr128, Phe232, His11
6	Squamostanin-B	-10.157	5 Hydrogen bonds: Thr147, Gly150, Trp105, Phe106, Trp105 7 Hydrophobic interactions: Pro102, Pro68, Tyr126, His194, Ile192, Ala69, Phe232
7	Squamotin A	-10.071	4 Hydrogen bonds: Gly150, Tyr155, His11, Arg200 7 Hydrophobic interactions: Ile192, Met154, Phe106, Trp105, Leu103, Pro102, Pro68
8	Annopurpuricin C	-9.98	5 Hydrogen bonds: Phe17, Asn18, Gly150, Tyr151, His161 7 Hydrophobic interactions: Phe106, Tyr126, Tyr128, Phe232, His194, Pro68, Pro102
9	Tucupentol	-9.79	3 Hydrogen bonds: Leu103, Trp105, Phe17 5 Hydrophobic interactions: Met154, His161, Phe178, Pro68, Pro102 1 Sigma-bond: Tyr128
10	Cohibin D	-9.789	3 Hydrogen bonds: Tyr67, Tyr155, Gly150 10 Hydrophobic interactions: Ile50, Tyr126, Pro68, Trp105, Phe178, Tyr126, Pro102, Tyr128, Phe232, His11 1 Pi-Sigma bond: His161
11	Annosquacin B	-9.745	5 Hydrogen bonds: His11, Phe17, Asn18, Gly150, Tyr155 4 Hydrophobic interactions: Pro68, Val72, Phe106, Tyr126 1 Pi-Sigma bond: Tyr128
12	Montalicin E	-9.698	5 Hydrogen bonds: Thr147, Gln104, Tyr126, Phe120, Trp105 8 Hydrophobic interactions: Pro102, Tyr67, Pro68, Tyr128, His194, His11, His161, Ile50
13	Muricin G	-9.489	3 Hydrogen bonds: Gly150, Glu117, Trp105 8 Hydrophobic interactions: Tyr67, Ile50, Ile192, His61, Tyr128, Tyr126, Phe232, Phe106 1 Pi-Sigma bond: His194
14	calamistrin D	-9.464	4 Hydrogen bonds: Gly150, Tyr155, Gln104, Tyr126 8 Hydrophobic interactions: Phe17, Tyr67, Pro68, Pro102, Leu103, Trp105, Phe106, Met154

Human NAD[P]H-quinone oxidoreductase			
15	Annosquacin D	-9.443	5 Hydrogen bonds: His11, Phe17, Asn18, Gly150, Tyr155 6 Hydrophobic interactions: Phe106, Tyr126, Pro68, Tyr128, Ala69, Phe232
16	Montalycin H	-9.438	3 Hydrogen bonds: Thr147, His161, Trp105 5 Hydrophobic interactions: Ala69, Val72, Ile192, Pro68, Tyr128,
17	Annopurpuricin B	-9.426	7 Hydrogen bonds: His11, Phe17, Asn18, Leu103, Gly149, Gly150, Ser151 7 Hydrophobic interactions: Pro68, Val72, Phe106, Tyr128, Met154, His161, His194
18	Annosquatin-II	-9.419	2 Hydrogen bonds: Gln104, Tyr128, 10 Hydrophobic interactions: Pro102, Pro68, Ile50, Tyr67, Ile121, Phe106, Trp105, Phe178, Val72, Met154

In the NQO1, the compounds chosen scored between - 11.098 and -9.261 kcal/mol. Squamostanin-A was the best fitting compound in the NQO1 where it showed three hydrogen bonds to His11, Trp105, and Tyr12 and pi-alkyl interactions with six amino acids Ile50, Pro102, Phe106, His161, Phe178 and Met154 while occupying the chemical specified space of the pocket. This explained the better glide score of squamostanin A compared to the native ligand Arh - 3.184kcal/mol, which revealed one hydrogen bond to His161 and another interaction with His 194 at distance 2.14 and 4.71, respectively. The interactions were verified to be stable through the molecular dynamics' simulations. (Table 4)

Table 4

Binding free energy and docking interactions of selected acetogenins in the binding pocket of Epidermal growth factor receptor tyrosine kinase.

Epidermal growth factor receptor tyrosine kinase			
No.	Compound	Glide score (Kcal/mol)	Interactions
1	Squamostanin-A	-7.273	3 Hydrogen bonds: Lys692, Asp776, Asp831 8 Hydrophobic interactions: Tyr777, Leu694, Leu768, Ala719, Leu820, Lys721, Val702, Cys773
2	Montalicens G	-7.213	3 Hydrogen bonds: Lys721, Asn818, Asp831 7 Hydrophobic interactions: Val702, Met742, Leu764, Lys721, Leu820, Ala719, Leu794
3	Squadiolin B	-7.029	2 Hydrogen bonds: Asn818, Asp831 9 Hydrophobic interactions: Ala731, Ile735, Leu820, Leu768, Met769, Pro770, Phe771, Cys773, Tyr777
4	Annomontacin	-6.835	6 Hydrogen bonds: Arg817, Asp813, Cys773, Leu694, Pro770, Gly833 5 Hydrophobic interactions: Val702, Leu820, Leu802, Leu768, Ala719
5	Anmontanin B	-6.698	3 Hydrogen bonds: Pro770, Gln704, Tyr728, 7 Hydrophobic interactions: Val702, Met742, Leu764, Lys721, Leu820, Ala719, Leu794
6	Muricin E	-6.668	2 Hydrogen bonds: Lys693, Pro770 5 Hydrophobic interactions: Leu768, Met769, Phe771, Cys773, Tyr777
7	Monlicin A	-6.65	3 Hydrogen bonds: Lys721 Asp831, Pro770 7 Hydrophobic interactions: Val702, Leu820, Leu694, Ala719, Phe699, Ile635, Cys773
8	Montalicin B	-6.473	4 Hydrogen bonds: Thr766, Pro770, Lys692, Asp831 8 Hydrophobic interactions: Val702, Leu820, Cys773, Leu768, Leu694, Lys721, Ala719, Met769
9	Annosquacin-I	-6.46	4 Hydrogen bonds: Thr830, Pro770, Leu694, Glu780 8 Hydrophobic interactions: Val702, Leu820, Cys773, Leu764, Leu768, Lys721, Ala719, Phe699
10	Annosquacin C	-6.436	3 Hydrogen bonds: Glu780, Pro770, Lys692 5 Hydrophobic interactions: Leu820, Cys773, Leu768, Ala719, Leu694
11	Annocherimolin	-6.436	4 Hydrogen bonds: Lys721, Asn818, Arg817, Asp831 7 Hydrophobic interactions: Val702, Leu820, Cys773, Leu694, Ile735, Leu794, Phe771
12	Muricenin	-6.431	3 Hydrogen bonds: Met769, Asp831, Pro770 6 Hydrophobic interactions: Val702, Leu820, Leu694, Ala719, Phe699, Ile635
13	Squamostanin-B	-6.424	4 Hydrogen bonds: Cys773, Asn818, Asp776, Asp831 6 Hydrophobic interactions: Tyr777, Leu775, Phe771, Pro770, Met769, Leu768
14	Annosquatin IV	-6.373	3 Hydrogen bonds: Glu780, Met769, Phe771 4 Hydrophobic interactions: Tyr777, Pro770, Leu768, Leu820
15	Annonamuricin A	-6.367	4 Hydrogen bonds: Lys792, Pro770, Asp831, Thr830 5 Hydrophobic interactions: Val702, Leu820, Leu694, Ala719, Lys721
16	Tucupentol	-6.3	2 Hydrogen bonds: Asp776, Asp831 8 Hydrophobic interactions: Phe699, Leu820, Leu768, Met769, Pro770, Phe771, Cys773, Tyr777
17	Asitrilobin C	-6.215	3 Hydrogen bonds: Lys692 Asp831, Leu694 5 Hydrophobic interactions: Val702, Leu820, Val693, Ala719, Phe699

Epidermal growth factor receptor tyrosine kinase			
18	Annonacin	-6.209	4 Hydrogen bonds: Asn818, Arg817, Leu694, Pro770
			7 Hydrophobic interactions: Val702, Leu820, Cys773, Leu794, Ala719, Leu764, Leu834

In the BCL-2, the chosen acetogenin compounds scored between - 6.334 and - 5.283 kcal/mol. Annopurpuricin revealed binding to Arg88, Glu95, and Asn102 through hydrogen bonds and to Phe63, Arg66, Tyr67, Met74, Val92, Arg105, Val107, and Ala108 through Pi alkyl bonds. The native ligand dro scored - 8.192kcal/mol with π - π stacking interaction to Phe63 (5.22) and Phe71(4.99) as well as salt bridge to Asp70 with a distance of 4.12Å and two hydrogen bonds through water bridging. Phe 63 and Val92 were the vital amino acids in the BCL-2 binding pocket (Xu et al. 2019) and its interactions were verified to be stable in the MD simulations.(Table 5)

Table 5
Binding free energy and docking interactions of selected acetogenins in the binding pocket of Bcl2-xL

Bcl2-xL			
No.	Compound	Glide score (Kcal/mol)	Interactions
1	Annopurpuricin A	-6.334	3 Hydrogen bonds: Arg88, Glu95, Asn102 9 Hydrophobic interactions: Val92, Met74, Ala108, Arg105, Tyr67, Arg66, Val107, Phe63, Tyr161
2	Muricin G	-6.14	4 Hydrogen bonds: Arg105, Glu95, Glu73, Leu96 7 Hydrophobic interactions: Val92, Met74, Ala108, Tyr67, Phe109, Phe112, Phe71
3	Squamocin III	-6.046	4 Hydrogen bonds: Arg105, Glu95, Glu73, Asn102 8 Hydrophobic interactions: Val92, Met74, Ala108, Tyr67, Phe109, Phe112, Phe71, Leu96
4	Annosquatin IV	-5.932	2 Hydrogen bonds: Glu95, Glu73 9 Hydrophobic interactions: Val92, Met74, Ala108, Tyr67, Phe71, Leu96, Val107, Ala59, Phe63
5	Squadiolin A	-5.827	3 Hydrogen bonds: Glu95, Arg88, Arg105 9 Hydrophobic interactions: Val92, Met74, Ala108, Tyr67, Phe71, Leu96, Phe112, Leu78, Phe109
6	Annosquatin B	-5.717	3 Hydrogen bonds: Glu95, Arg69, Asp70 6 Hydrophobic interactions: Val92, Met74, Tyr67, Phe71, Leu96, Phe63
7	Muricin F	-5.707	3 Hydrogen bonds: Glu95, Arg105, Asp99 8 Hydrophobic interactions: Val92, Met74, Tyr67, Phe71, Leu96, Phe71, Phe97, Ala108
8	cis-montacin	-5.698	3 Hydrogen bonds: Glu95, Arg105, Asp99 8 Hydrophobic interactions: Val92, Met74, Tyr67, Phe71, Leu96, Phe71, Phe97, Ala108
9	Annosquatin-I	-5.693	4 Hydrogen bonds: Gly77, Glu95, Arg105, Asn102 7 Hydrophobic interactions: Val92, Met74, Tyr67, Phe71, Leu96, Leu78, Ala108
10	Annosquacin C	-5.669	4 Hydrogen bonds: Arg88, Glu95, Arg105, Glu73 9 Hydrophobic interactions: Val92, Tyr67, Phe71, Phe109, Leu78, Ala108, Phe112, Met74
11	Asitrilobin C	-5.631	4 Hydrogen bonds: Arg88, Glu95, Arg105, Leu96 5 Hydrophobic interactions: Val92, Tyr67, Phe71, Ala108, Met74
12	Annosquatin A	-5.62	3 Hydrogen bonds: Arg88, Glu95, Arg105 9 Hydrophobic interactions: Val92, Tyr67, Phe71, Ala108, Met74, Phe109, Phe112, Leu96, Leu78
13	Squamostanin-A	-5.613	4 Hydrogen bonds: Asp70, Asp99, Arg105, Leu96 9 Hydrophobic interactions: Val92, Tyr67, Phe71, Ala108, Met74, Phe109, Phe112, Phe63, Leu96
14	Squamocin-P	-5.504	4 Hydrogen bonds: Arg88, Asp99, Arg105, Glu95 7 Hydrophobic interactions: Val92, Tyr67, Phe71, Ala108, Met74, Leu78, Leu96
15	Squamostanin-B	-5.477	3 Hydrogen bonds: Arg88, Gly77, Glu95 8 Hydrophobic interactions: Val92, Tyr67, Phe71, Ala108, Met74, Leu78, Leu96, Phe112
16	(2,4)-cis-asitrocinone	-5.467	3 Hydrogen bonds: Glu73, Gly77, Arg105 8 Hydrophobic interactions: Val92, Tyr67, Phe71, Ala108, Met74, Leu78, Phe112, Phe109

Bcl2-xL			
17	Muricin H	-5.467	3 Hydrogen bonds: Arg88, Glu95, Arg105 8 Hydrophobic interactions: Val92, Tyr67, Phe71, Ala108, Met74, Leu96, Phe112, Phe109
18	Annocherimolin	-5.303	3 Hydrogen bonds: Glu73, Asp70, Arg66 6 Hydrophobic interactions: Val92, Tyr67, Phe71, Met74, Leu96, Phe63

Squamocin IV recorded the lowest binding energy – 7.01 kcal/mol and the best stability in the MCL-1. The selected compounds scored between – 7.01 and – 6.217 kcal/mol compared to the native ligand whose score was – 16.595 kcal/mol. While squamocin IV formed four hydrogen bonds with Lys234, His252, Arg263, and Thr266, and nine alkyl interactions with hydrophobic residues, the Q51 native ligand formed ten H bonds with three amino acids: Thr266, Arg263, Ala227 with distances from 1.80 Å to 2.17 Å; additionally, a pi-stacking bond was detected with Phe270 in a distance of 4.68 Å. (Table 6) Binding to Thr266 and Arg263 featured a core interaction in the MCL1 chemical space (Osman et al. 2021) and its stability was confirmed further by the MD simulations.

Table 6
Binding free energy and docking interactions of selected acetogenins in the binding pocket of Human Mcl-1.

Human Mcl-1			
No.	Compound	Glide score (Kcal/mol)	Interactions
1	Squamocin IV	-7.01	4 Hydrogen Bonds: His252, Lys234, Arg263, Thr266 9 Hydrophobic interactions: Val274, Met250, Val253, Leu246, Leu267, Phe270, Val258, Val249
2	Muricenin	-6.766	1 Hydrogen Bonds: Arg263 8 Hydrophobic interactions: Phe270, Val274, Leu267, Val253, Phe254, Leu246, Met250, Val249
3	Squamostanin-B	-6.72	3 Hydrogen Bonds: Lys234, His252, Asp256 9 Hydrophobic interactions: Met231, Phe270, Val253, Val258, Phe254, Val249, Leu246, Met250, Ala227
4	Rollicosin	-6.569	1 Hydrogen Bonds: Thr266 9 Hydrophobic interactions: Ala227, Phe228, Met231, Phe270, Val253, Leu246, Met250, Leu267, Val249
5	Squamostanin-A	-6.543	3 Hydrogen Bonds: Asn260, Arg263, Thr266 11 Hydrophobic interactions: Ala227, Phe228, Met231, Phe270, Val253, Val258, Phe254, Val249, Leu246, Met250, Ala227
6	Muricin F	-6.512	1 Hydrogen Bonds: Arg263, Asn223 10 Hydrophobic interactions: Ala227, Phe228, Met231, Phe270, Val253, Leu267, Phe254, Met250, Val249, Val220
7	Annocatacin B	-6.505	1 Hydrogen Bonds: Asp256 10 Hydrophobic interactions: Ala227, Phe228, Met231, Phe270, Val253, Val258, His224, Arg263, Leu267, Met250
8	Anmontanin B	-6.474	2 Hydrogen Bonds: Arg263, Asn260 5 Hydrophobic interactions: Ala227, Phe228, Met231, Phe270, Val253
9	Annonamuricin C	-6.453	1 Hydrogen Bonds: Arg263 7 Hydrophobic interactions: Ala227, Phe228, Met231, Leu235, Phe270, Leu267, Val274
10	Annocherimolin	-6.408	5 Hydrogen Bonds: His252, Arg263, Thr266, Val253, Asp256 5 Hydrophobic interactions: Val249, His224, Ala227, Met250, Phe270
11	Squamocin-P	-6.38	1 Hydrogen Bonds: Arg263 10 Hydrophobic interactions: Ala227, Phe228, Met231, Val253, Phe254, Val249, Met250, Leu246, Val258, Leu235
12	Annonamuricin B	-6.352	1 Hydrogen Bonds: Asp256 5 Hydrophobic interactions: Val249, Val249, Ala227, Leu246, Arg263
13	Annonamuricin A	-6.325	2 Hydrogen Bonds: Asn260, Thr266 10 Hydrophobic interactions: Ala227, Phe228, Met231, Phe270, Val253, Phe254, Val274, Arg263, Leu246, Met250
14	Annonamuricin D	-6.319	10 Hydrophobic interactions: Ala227, Phe228, Met231, Phe270, Val253, Phe254, Val249, Met250, Leu246, Leu267
15	Calamistrin E	-6.305	1 Hydrogen Bonds: Arg263 9 Hydrophobic interactions: Ala227, Phe228, Met231, Val253, Leu246, Val249, Val258, Leu235, Met250
16	Dotistenin	-6.26	2 Hydrogen Bonds: Asn260, Thr266 11 Hydrophobic interactions: Ala227, Phe228, Met231, Val253, Leu246, Val249, Val258, Met250, Leu267, Phe270, Phe254

Human Mcl-1			
17	Annosquatin B	-6.242	2 Hydrogen Bonds: Asp256, Thr259 10 Hydrophobic interactions: Ala227, Phe228, Met231, Phe270, Val253, Phe254, Val274, Leu246, Val249, Val258
18	Squafofacin B	-6.217	2 Hydrogen Bonds: Arg233, Thr266 6 Hydrophobic interactions: Ala227, Phe228, Met231, Phe270, Leu267, Val265

Chen et al pointed out structural activity relationships for acetogenins bioactivity as anticancer agents to be due to the THF rings, a-b-unsaturated lactone ring, the number of carbons separating the THF units where non-adjacent bis-THF with few carbons' separation recorded the highest activity followed by adjacent bis-THF units then mono THF derivatives with the weakest effect before the non-THF acetogenins; moreover, the long alkyl flexible chain was essential for potency

(Chen et al. 2015) (Lima et al. 2022). It is worth mentioning that synthetic derivatives of acetogenins were not as effective as the natural ones and acetogenins flexibility rendered their structural prediction of activity challenging, which supported the use of computational studies to provide accurate molecular interaction data about compounds in a time and cost-effective way (Liao et al. 2016)

The most promising compounds were selected for further molecular dynamics study Squamostanin-A (Juang et al. 2016)

The molecular dynamic trajectories in the NADH (NQ01) enzyme complex with squamostanin A in a 100ns simulation showed stability of the Root Mean Square Deviation (RMSD) values with no major conformational changes (Fig. 4). The protein residues were analyzed through the root mean square fluctuation (RMSF) values, which measured the average deviation of a particle from a reference position, and were revealed to be devoid of major changes. The probability of protein unfolding was analyzed by measuring the Rg value, which designated the human NAD[P]H-quinone oxidoreductase-Squamostanin-A complex compactness and manifested the absence of unfolding during the simulation process.

Regarding the EGFR squamostanin A complex, a sudden flip was noted between 10 and 20ns as reflected in the RMSD values; meanwhile, the loop containing region of the protein complex demonstrated major conformational changes between 50 and 60 ns that reverted back to the normal range at the end of the 100ns. The stability of the Rg values confirmed the good binding of the squamostanin A inside the binding pocket with no induction of unfolding. Unfortunately, the molecular interactions with squamostanin A were not seen to be consistent and stable here.

Annopurpuricin-A complex with BCL2 enzyme was stable in the period 3-3.5A with a slight deviation at the end of the simulation. A tendency of unfolding was revealed at the end of the simulation time according to the measured RMSF values, and some unfolding was experienced during the time 80 to 95ns. Overall, the complex showed no flexibility and remained compact.

The Mcl-1 protein complex with squamocin IV showed no conformational changes, and the fluctuations were minimal except for the C-terminal. The molecular interactions between the ligand and MCI-1, namely the four hydrogen bonds with Lys234, His252, Arg263, and Thr266 were stable during the simulation. The native ligand showed analogous binding to Arg263 and Thr266, which are core residues in the MCI-1 enzyme.

The ADME (Absorption, Distribution, Metabolism, Excretion) and drug likeness descriptors were calculated using the QikProp wizard for all the selected scaffold molecules (Table1). The filtering strategy was employed before conducting the molecular docking procedures to exclude the carbon skeletons with unpromising pharmacokinetics and pharmacodynamics. Twenty-five properties were used in the QikProp tool to indicate the molecules that lie within the 95% range of known drugs, and the rest are omitted. This favorable range is reflected through a value of #stars index, which should be between 0 and 5. All the compounds reported a suitable #Stars value in this study and were included for further analysis. Lipinski' rule of five expressed the weight of parameters like hydrogen bonds for donors and acceptors, with a value not exceeding 5 for the former and not exceeding 10 for the latter. The hydrophobicity ratio between octanol and water or the partition coefficient value must remain equal to or less than 5 together with a molecular weight not over 500Da. By assessing solubility prediction, permeability and liver first pass factors, compounds bioavailability was estimated using the Jorgensen's rule of three. While Caco-2 cell rate permeability (BIPcaco-2) should be greater than 22, the aqueous solubility (LogSwat) must be more than 5.7, and the metabolites number should not be over 7. In the same way, skin permeability characteristics (LogKp)together with human oral absorption (1–3) were considered.

The QPlogBB term expressed a value from 3.0 to 1.2 and indicated the absorption coefficient of the blood brain barrier. A satisfactory drug likeness was inferred as the QPlogKhsa coefficient denoting the plasma protein binding showed more than 90% binding to all of the compounds with an adequate blood brain absorption.

Conclusion

Molecular dynamics and molecular docking virtual tools are employed for structural based drug design. Herein we investigated the potential of a group of selected isolated acetogenins in the last twenty years to bind effectively in the binding sites of four drug resistant enzymes involved in the cytotoxic cascade inside the cell. Briefly, squamostanin A showed the best binding energy scores in both EGFR and NQO1 enzymes while exerting stable and consistent binding with core residues, other than the mutation active residues, in the chemical specified space as proved by the molecular dynamic's simulation. Annupurpuricin A manifested a better fitting into the enzyme pocket of BCL-2 than the native ligand with stable non covalent interactions, and squamocin IV revealed the best fitting score with MCL-1 -7.122 Kcal/mol through binding with Phe270 and three hydrophobic residues. These top scored compounds showed favourable drug likeness, pharmacokinetic and pharmacodynamic properties within 95% range of the known drugs; thus, could be utilised to reverse MDR tumour growth in future drug combinations.

Declarations

Consent for publication

Not applicable

Availability of data and materials

The datasets used or analyzed during the present study are available with the corresponding author upon reasonable request.

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Declaration of Competing Interest

The authors declare no conflict of interest

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Figures

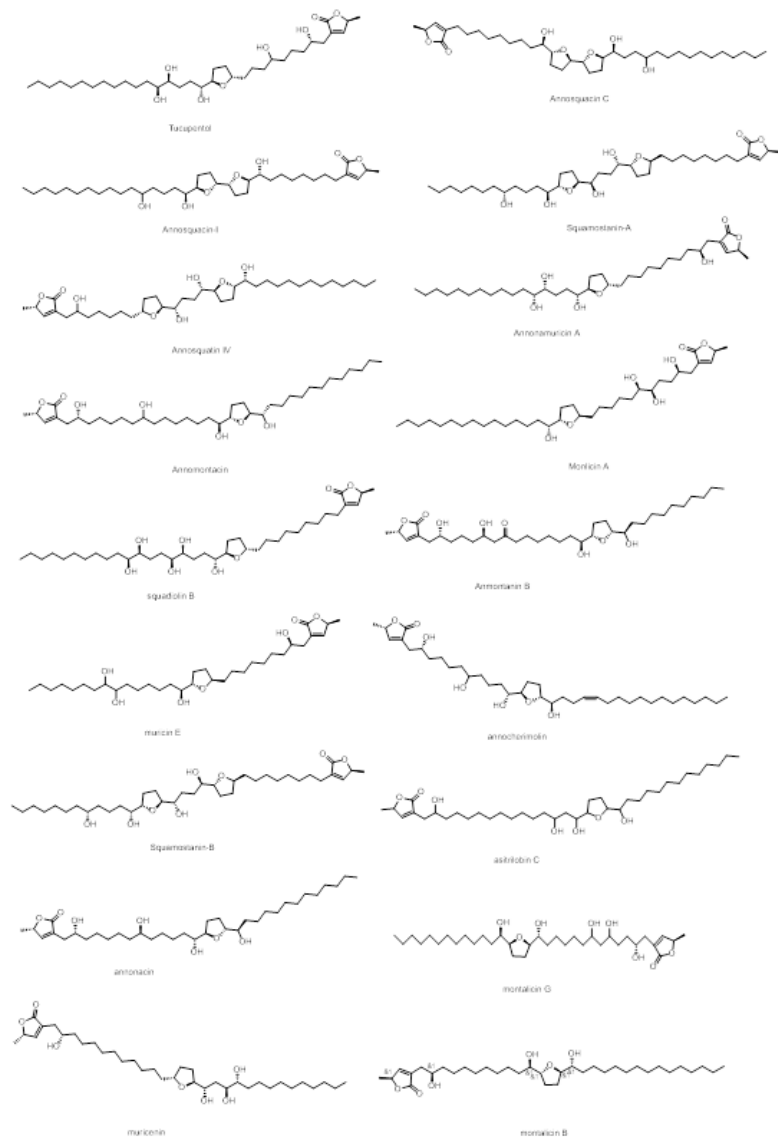


Figure 1

Some of the selected acetogenins for the *in-silico* molecular docking study.

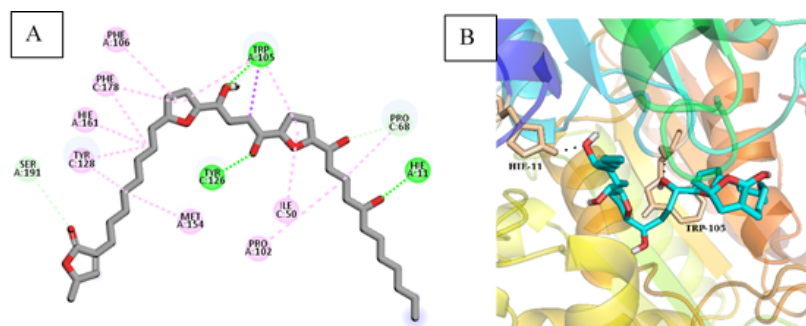


Fig 2A-B. (A) The binding interactions of human NAD[P]H-quinone oxidoreductase with Squamostatin-A. The interactions are shown in different colors. Hydrogen Bonding (Green), Hydrophobic (Magenta). (B) The binding pose of Squamostatin-A in protein binding pocket. The ligand is shown in cyan sticks.

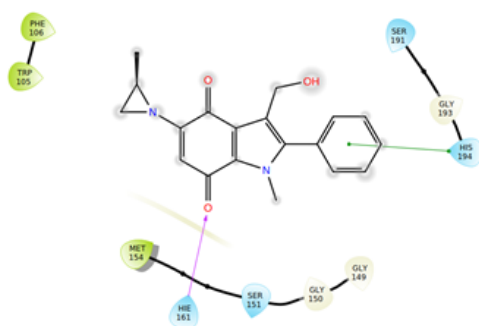


Fig 2C The binding 2D interactions of human NAD[P]H-quinone oxidoreductase with the native ligand Arh. The interactions are shown in different colors. Hydrogen bonding (green), hydrophobic (magenta).

Figure 2

See image above for figure legend.

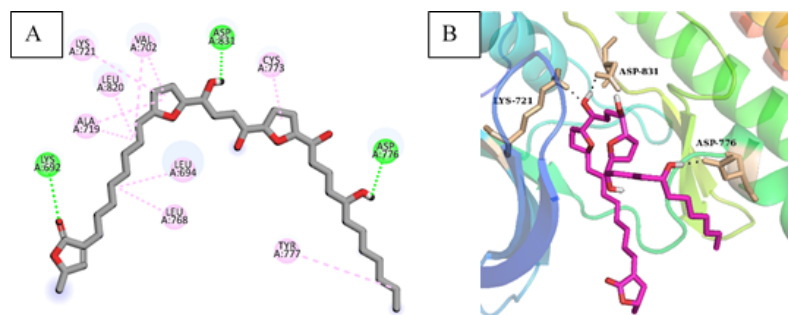


Fig 3A-B. (A) The binding interactions of epidermal growth factor receptor tyrosine kinase with Squamostatin-A. The interactions are shown in different colors. Hydrogen Bonding (Green), Hydrophobic (Magenta). (B) The binding pose of Squamostatin-A in protein binding pocket. The ligand is shown in magenta sticks.

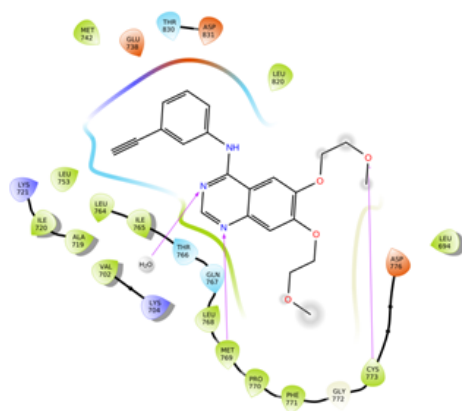


Fig 3C The binding interactions of epidermal growth factor receptor tyrosine kinase with the native ligand AQ4. The interactions are shown in different colors. Hydrogen bonding (green), hydrophobic (violet). (B) The binding pose of AQ4 in protein binding pocket.

Figure 3

See image above for figure legend.

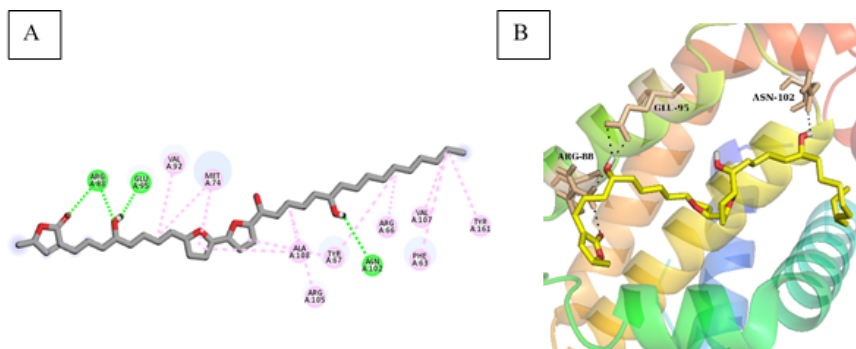


Fig 4A-B. (A) The binding interactions of Bcl2-xL with annopurpuricin-A. The interactions are shown in different colors. Hydrogen bonding (green), hydrophobic (magenta). (B) The binding pose of annopurpuricin-A in protein binding pocket. The ligand is shown in yellow sticks.

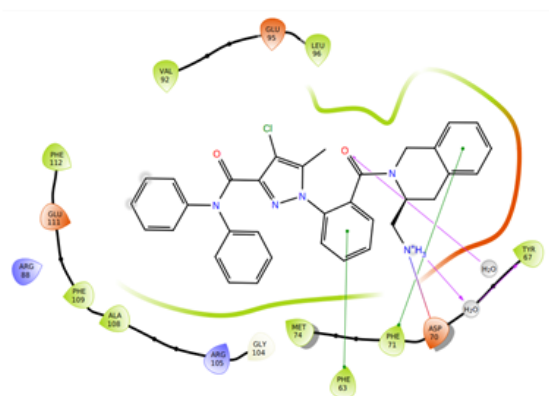


Fig 4C. The binding interactions of Bcl2-xL with the native ligand (Dro). The interactions are shown in different colors. Hydrophobic (green), hydrogen bonding (magenta), polar residues (red and blue).

Figure 4

See image above for figure legend.

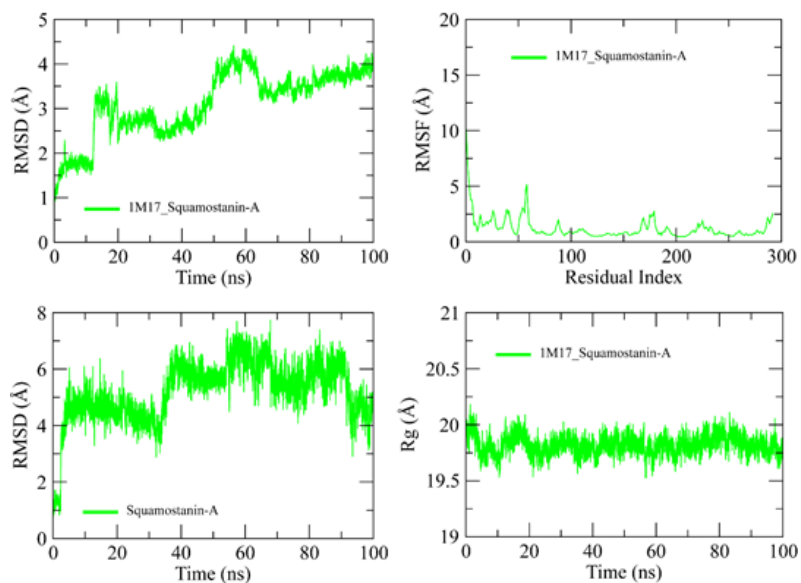


Figure 7

The protein-ligand stability analysis of epidermal growth factor receptor tyrosine kinase with Squamostanin-A.

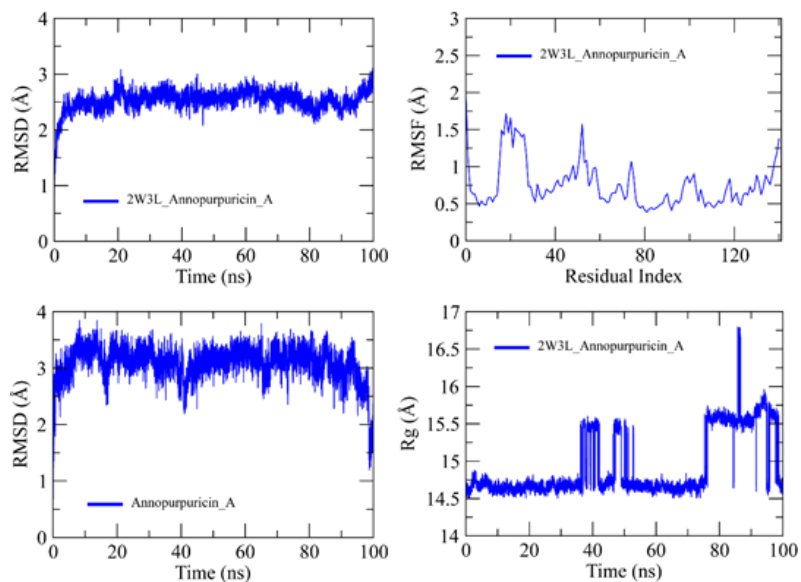


Figure 8

The protein-ligand stability analysis of Bcl2-xL, the complex with Annopurpuricin-A.

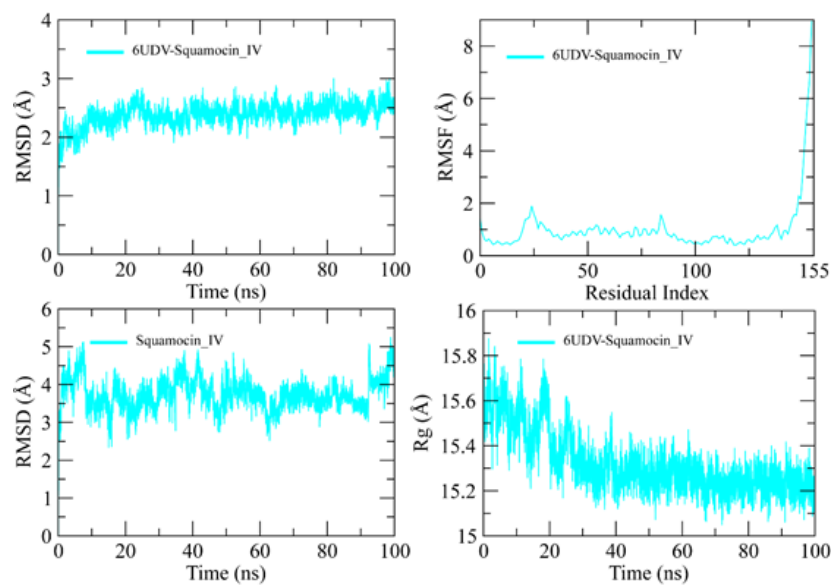


Figure 9

The protein-ligand stability analysis of human Mcl-1-squamocin IV complex.