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Molecular docking approach to identify Bcl-xL inhibitory effect of sesquiterpene coumarins of *Ferula* species

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Abstract

Background and Aims: Due to the high mortality and morbidity rates associated with cancer, it is crucial to advance research in this area to develop new and effective agents. Natural product chemistry plays a crucial role in identifying potential lead molecules for the treatment of various cancers. Recently, several studies have demonstrated the selective and potent cytotoxic activity of sesquiterpene coumarins isolated from *Ferula* species against various cancer cell lines. In this study, we aimed to utilize *in silico* molecular docking studies to demonstrate the potential of sesquiterpene coumarins as inhibitors of Bcl-xL. For this purpose, 35 sesquiterpene coumarins were collected based on previous studies.

Methods: Molecular docking studies were conducted to examine the interactions of cytotoxic sesquiterpene coumarins with the active site of the Bcl-xL enzyme, using advanced computational techniques. Additionally, ADME (Absorption, Distribution, Metabolism, and Excretion) studies were performed to predict the pharmacokinetic properties and drug-likeness of sesquiterpene coumarins, ensuring their potential as viable therapeutic agents.

Results: As a result of this study, straight-chain sesquiterpene coumarin-derived compounds were more effectively positioned within the active site of the Bcl-xL enzyme and formed hydrogen bonds with amino acids. ADME analysis revealed favourable pharmacokinetic profiles, supporting their potential use in drug development.

Conclusion: These findings revealed promising Bcl-xL inhibitory activities of umbelliprene-type sesquiterpene derivatives, which might be a starting point for further structural optimisation to acquire novel compounds exhibiting more potent Bcl-xL inhibitory activity, paving the way for new therapeutic approaches in cancer treatment.

Keywords Bcl-xL · Cancer · *Ferula* · Molecular docking · Sesquiterpene coumarins

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INTRODUCTION

Ferula L., comprising approximately 227 species, is the largest genus of the Apiaceae family (Plants of the World Online – POWO). *Ferula* L. species are extensively distributed in the temperate regions of the European continent, surrounded by the Canary Islands in the west, China and India in the east, North Africa in the south, and Central Europe in the north (Pimenov & Leonov, 1993; Korovin, 1947). For centuries, *Ferula* plants have been traditionally used for both culinary and medicinal purposes (Mohammadhosseini et al., 2019; Iranshahy & Iranshahi, 2011). Two thousand years ago, Dioscorides documented the medicinal applications of several *Ferula* oleo gum resins in his De Materia Medica (Gunther, 1968). Avicenna meticulously described the use of *Ferula foetida* oleo gum resin for treating malignant tumours in his Canon of Medicine (Eisenman et al., 2013). Currently, *Ferula* resins are used in traditional medicine recipes, such as Benja Amarit, a Thai herbal concoction once used to cure colon and liver cancer (Yapasert et al., 2020). Furthermore, *Ferula* resins are used as complementary alternative medicine (CAM) by cancer patients in specific geographic areas (Ali-SHtayeh et al., 2016) .

Cancer is characterised by uncontrolled cell growth and division. Carcinogenesis, the process of cancer development, involves mutations in oncogenes or tumour-suppressor genes and chromosomal or epigenetic changes. Additionally, environmental stress often leads to the evasion of the programmed cell death process: apoptosis. Inhibited apoptosis is the hallmark of cancer (Sarkar et al., 2013). Within Bcl-2 family proteins, both proapoptotic proteins (Bax, Bak, Bad, Bim) and antiapoptotic proteins (Bcl-2, Bcl-xL, Mcl-1) interact dynamically, regulating intrinsic apoptosis pathways. These proteins play crucial roles in determining the survival of cells in various tumour types (Czabotar et al., 2014). Overexpression of Bcl-xL has been associated with numerous human cancers, including prostate, colon, lung, stomach, and kidney cancers, neuroblastoma, non-Hodgkin's lymphoma, acute leukaemia, and chronic leukaemia. Therefore, targeting the inhibition of the Bcl-xL enzyme is an important strategy for developing anticancer drugs (Kirkin et al., 2004; Tao et al., 2021).

Sesquiterpene coumarins, a class of compounds derived from sesquiterpenes, are found in plants belonging to the Rutaceae, Asteraceae, and Apiaceae families, and they have drawn the interest of natural products and medicinal chemists due to their various biological activities, including cytotoxicity. Several sesquiterpene coumarins that show notable cytotoxic activity have been identified through phytochemical studies of the species of *Ferula* genus (Eruçar et al., 2023a; Wang et al., 2023a; Eruçar et al. 2023b; Wang et al., 2023b; Tosun et al., 2019). Nevertheless, the precise mechanism underlying their cytotoxic activities has yet to be determined. Many studies that have focused on the elucidation of the mechanism of the cytotoxic activities of sesquiterpene coumarins have identified that sesquiterpene coumarins could also overcome drug resistance in cancer cells and augment the cytotoxic effects of existing anticancer drugs via p-gp inhibition (Barthomeuf, et al., 2015). In contrast, our recent study (Eruçar et al., 2023a) provided evidence that Bcl-xL, an enzyme targeted in cancer research, was inhibited by sesquiterpene coumarins isolated from *F. huber-morathii*. Thus, an alternative pathway for explaining the cytotoxic activity mechanism of sesquiterpene coumarins is suggested.

In this study, we aimed to utilize *in silico* molecular docking studies to explore the potential of sesquiterpene coumarins as inhibitors of Bcl-xL. For this purpose, molecular docking studies were conducted with the cytotoxic sesquiterpene coumarins isolated from various *Ferula* species Table 1 at the active site of the Bcl-xL enzyme, and the interactions between the sesquiterpene coumarins and the amino acids at the active site of the enzyme were examined.

MATERIALS AND METHODS

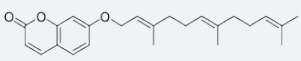
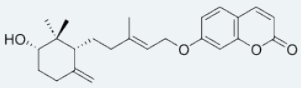
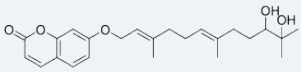
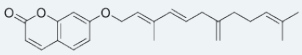
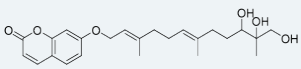
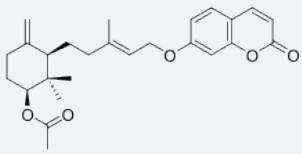
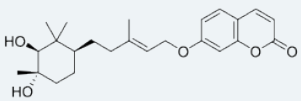
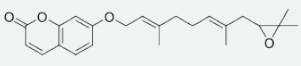
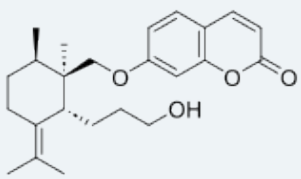
Ligand Preparation

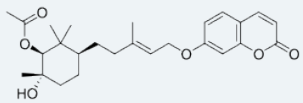
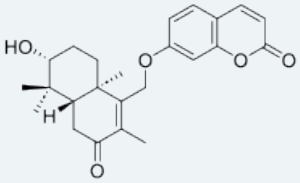
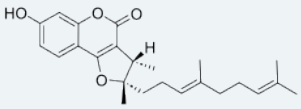
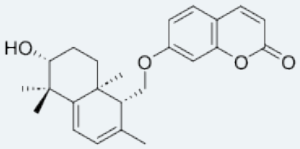
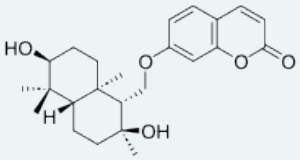
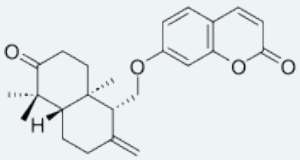
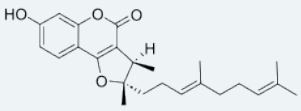
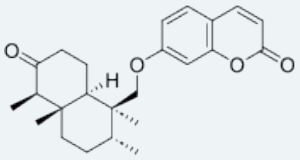
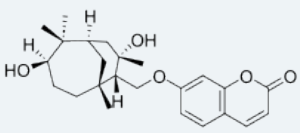
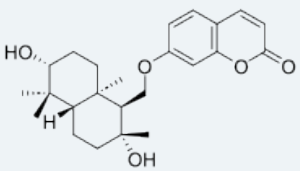
35 sesquiterpene coumarins obtained from previous studies, isolated from *Ferula* species, and showing cytotoxic activity against various cancer cell lines were drawn using the 2D Sketcher in Schrödinger Maestro. The LigPrep module of Schrödinger Suite (Schrödinger Release 2022-3: LigPrep, Schrödinger, LLC, New York, NY, 2022) was used to generate the energy-minimised conformations and tautomers at pH 7.0±2.0 using Epik. All the conformations and tautomers generated were minimised using the OPLS3 force field.

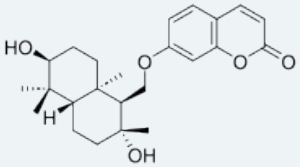
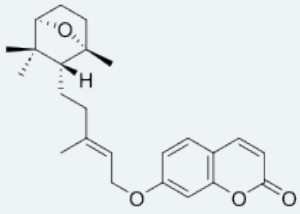
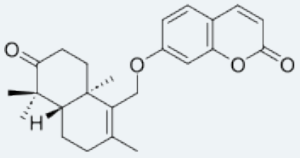
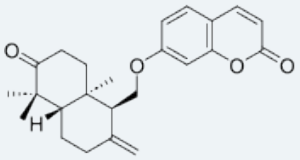
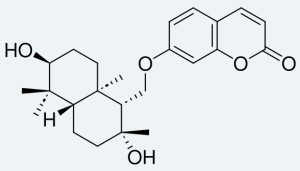
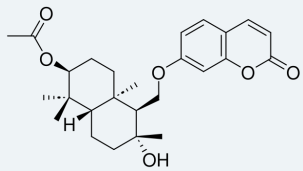
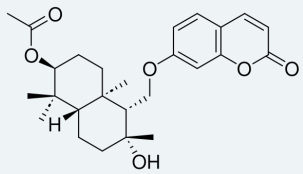
Protein Structure Preparation and Docking Experiments

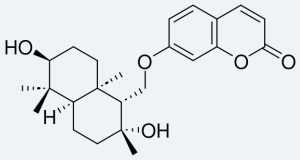
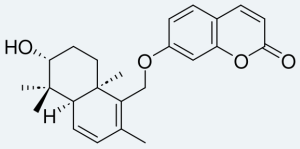
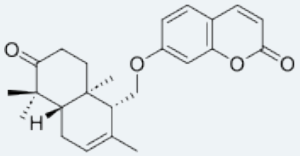
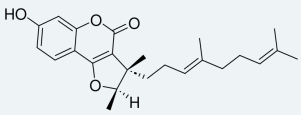
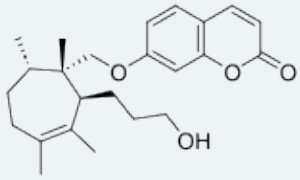
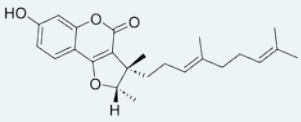
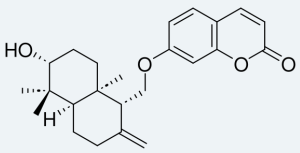
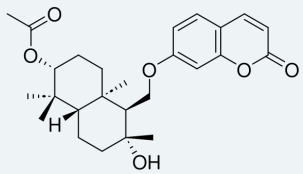
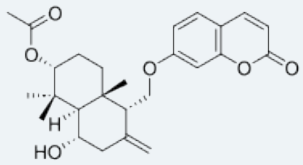
The crystal structure of Bcl-xL complexed with A1293102 (PDB ID: 7LH7, 1.40 Å) (Tao et al., 2021) was retrieved from the RCSB Protein Data Bank, and the crystal structure of the enzyme was prepared for docking studies using the multistep Protein Preparation Wizard module of the Schrödinger Software Suite (Sastry et al., 2013). The protein structure was optimized by adding missing hydrogen atoms and removing water molecules, heteroatoms, and co-factors, except for native ligands.

Table 1. Cytotoxic sesquiterpene coumarins used in this study and their references

No	Names	Compound	Species	Cytotoxicity	References
1	Umbelliprenin		*F. turcica*	Colo205: 49.5 µM HCT116:>50 µM A498:>50 µM UO31:>50 µM	(Eruçar et al., 2023b)
			F. sinkiangensis	HeLa: 202.2±1.2 µM K562: 141.6±1.1 µM AGS: 12.7±0.8 µM	(Li et al., 2015b)
			F. assa-foetida	MCF-7:>50 µg/ml PC3:>50 µg/ml NIH:>100 µg/ml	(Iranshahy et al., 2019)
			F. sinkiangensis	GES-1: 109.17±2.07 µM AGS: 13.67±1.73 µM HeLa: 75.83±2.66 µM A549:121.53±4.41 µM PC3:88.27±3.76 µM	(Zhang et al., 2015)
2	Farnesiferol B		*F. turcica*	Colo205: 42.3 µM HCT116: >50 µM A498: >50 µM UO31: >50 µM	(Eruçar et al., 2023b)
			F. samarcandica	HeLa: 31.71±1.58 µM HT-29: 8.33±0.34 µM A549: 28.73±0.90 µM	(Zhang et al., 2024)
			F. assa-foetida	MCF-7: 42.1±0.79 µg/ml PC3: 36.8±2.8 µg/ml NIH: >100 µg/ml	(Iranshahy et al., 2019)
3	Karatavicinol		*F .turcica*	Colo205: >50 µM HCT116: >50 µM A498: >50 µM UO31: 34.4 µM	(Eruçar et al., 2023b)
			F.seravschanica	Jurkat: 45.79±5.27 µM	(Shomirzoeva et al., 2021)
4	-		*F. seravschanica*	Jurkat: 60.11±6.43 µM	(Shomirzoeva et al., 2021)
5	-		*F. sinkiangensis*	HeLa: 48±1.3 µM MGC-803: >50 µM AGS: >50	(Wang et al., 2020)
6	Flabellilobin A		*F. pseudalliacea*	HeLa: 9.1±0.2 µM	(Dastan et al., 2014)
7	Sinkiangenorin F		*F. sinkiangensis*	HeLa: - K562: - AGS: 27.1±1.4 µM	(Li et al., 2015)
8	10',11'-epoxyumbelliprenin		*F. turcica*	Colo205: 44.4 µM HCT116: >50 µM A498: >50 µM UO31: >50 µM	(Eruçar et al., 2023b)
9	Fekrynol		*F. sinkiangensis*	HeLa: 35±1.6 µM MGC-803: 49±0.8 µM AGS: 20±0.5 µM	(Wang et al., 2020)
			F. sinkiangensis	HeLa: 142.7±3.2 µM K562: - AGS: -	(Li et al., 2015b)

No	Names	Compound	Species	Cytotoxicity	References
10	8-O-acetyl sinkiangenorin F		*F. sinkiangensis*	HeLa: - K562: - AGS: 62.7±2.5 μM	(Li et al., 2015a)
11	-		*F. samarcandica*	HeLa: 36.09±1.87 μM HT-29: - A549: -	(Zhang et al., 2024)
12	-		*F. ferulaeoides*	HepG2: 39.9±1.4 μM MCF-7: 37.7±1.3 μM C6: 16.0±1.0 μM	(Meng et al., 2013)
13	-		*F. seravschanica*	Jurkat: 58.35±5.10 μM	(Shomirzoeva et al., 2021)
14	Samarcandin		*F. assa-foetida* *F. huber-morathii*	MCF-7: >50 μg/ml PC3: >50 μg/ml NIH: >100 μg/ml COLO 205: 170.03±3.62 K-562: 143.03±1.67 MCF-7: 83.27±0.39 HUVEC: 169.16±2.68	(Iranshahy et al., 2019) (Eruçar et al., 2023a)
15	Badrakemone		*F.huber-morathii*	COLO 205: >200 K-562: >200 MCF-7: >200 HUVEC: >200	(Eruçar et al., 2023a)
16	-		*F. ferulaeoides*	HepG2: >100 μM MCF-7: 60.0±1.0 μM C6: 20.1±1.4 μM	(Meng et al., 2013)
17	kamolone		*F. turcica*	HCT116: 43.5 μM A498: >50 μM UO31: >50 μMColo205: >50 μM	(Eruçar et al., 2023b)
18	sinkiangenorin E		*F. sinkiangensis*	HeLa: 82.9±2.2 μM AGS: 12.7±2.5 μM	(Li et al., 2016)
19	Ferukrin		*F. huber-morathii*	COLO 205: >200 K-562: >200 MCF-7: 81.69±1.96 HUVEC: >200	(Eruçar et al., 2023a)

No	Names	Compound	Species	Cytotoxicity	References
20	Deacetylkellerin		*F. huber-morathii*	COLO 205: >200 K-562: >200 MCF-7: 47.62±0.40 HUVEC: >200	(Eruçar et al., 2023a)
21	Farnesiferol C		*F. sinkiangensis*	HeLa: 25±0.8 µM MGC-803: >50 µM AGS: >50 µM	(Wang et al., 2020)
			F. pseudalliacea	HeLa: 9.4±0.1 µM	(Dastan et al., 2014)
			F. sinkiangensis	HeLa: 86.9±1.1 µM K562: - AGS: 101.6±1.3 µM	(Li et al., 2015b)
			F.assa-foetida	MCF-7: 41.7±8.2 µg/ml PC3: 43.1±9.5 µg/ml NIH: >100 µg/ml	(Iranshahy et al., 2019)
22	Turcica ketone		*F. turcica*	Colo205: 37.3 µM HCT116: 37.1 µM A498: >50 µM UO31: 32.2 µM	(Eruçar et al., 2023b)
23	Mogoltadone		*F. turcica*	Colo205: 46.9 µM HCT116: >50 µM A498: >50 µM UO31: >50 µM	(Eruçar et al., 2023b)
			F. sinkiangensis	HeLa: 23±0.2 µM MGC-803: 49±1.1 µM AGS: 32±1.3 µM	(Wang et al., 2020)
			F. huber-morathii	COLO 205: 31.71±0.15 K-562: 21.11±0.85 MCF-7: 30.45±0.60 HUVEC: >200	(Eruçar et al., 2023a)
24	Feshurin		*F. samarcandica*	MV-4-11: - Mino: 7.88±0.60 µM	(Kamoldinov et al., 2021)
25	Kellerin		*F. huber-morathii*	COLO 205: 51.05±1.57 K-562: 78.14±3.13 MCF-7: 18.24±0.12 HUVEC: 99.39±1.63	(Eruçar et al., 2023a)
26	-		*F. samarcandica*	HeLa: 43.61±3.94 µM HT-29: - A549: 45.64±1.21 µM	(Zhang et al., 2024)

No	Names	Compound	Species	Cytotoxicity	References
27	-		*F. seravschanica*	Jurkat: >50 µM	(Shomirzoeva et al., 2021)
28	-		*F. seravschanica*	Jurkat: 37.68±5.65 µM	(Shomirzoeva et al., 2021)
29	Conferone		*F. samarcandica* *F. huber-morathii*	HeLa: 44.71±2.74 µM HT-29: 47.67±2.07 µM A549: 44.61±1.53 µM COLO 205: 27.63±0.69 K-562: 55.50±0.94 MCF-7: 34.02±0.68 HUVEC: 46.12±0.99	(Zhang et al., 2024) (Eruçar et al., 2023a)
30	-		*F. ferulaeoides*	HepG2: 53.5±2.2 µM MCF-7: 80.4±1.6 µM C6: 32.5±1.2 µM	(Meng et al., 2013)
31	Sinkiangenorin D		*F. sinkiangensis*	HeLa: 20.4±1.3 µM K562: 81.1±1.0 µM AGS: 104.8±1.2 µM	(Li et al., 2015b)
32	-		*F. ferulaeoides*	HepG2: 49.3±2.2 µM MCF-7: 66.0±1.9 µM C6: 35.3±1.4 µM	(Meng et al., 2013)
33	-		*F. seravschanica*	Jurkat: 20.70±2.31 µM	(Shomirzoeva et al., 2021)
34	Ferukrin acetate		*F.huber-morathii*	COLO 205: 105.72±1.35 K-562: 88.42±0.85 MCF-7: >200 HUVEC: >200	(Eruçar et al., 2023a)
35	-		*F. sinkiangensis*	HeLa: 16±0.8 µM MGC-803: >50 µM AGS: >50 µM	(Wang et al., 2020)

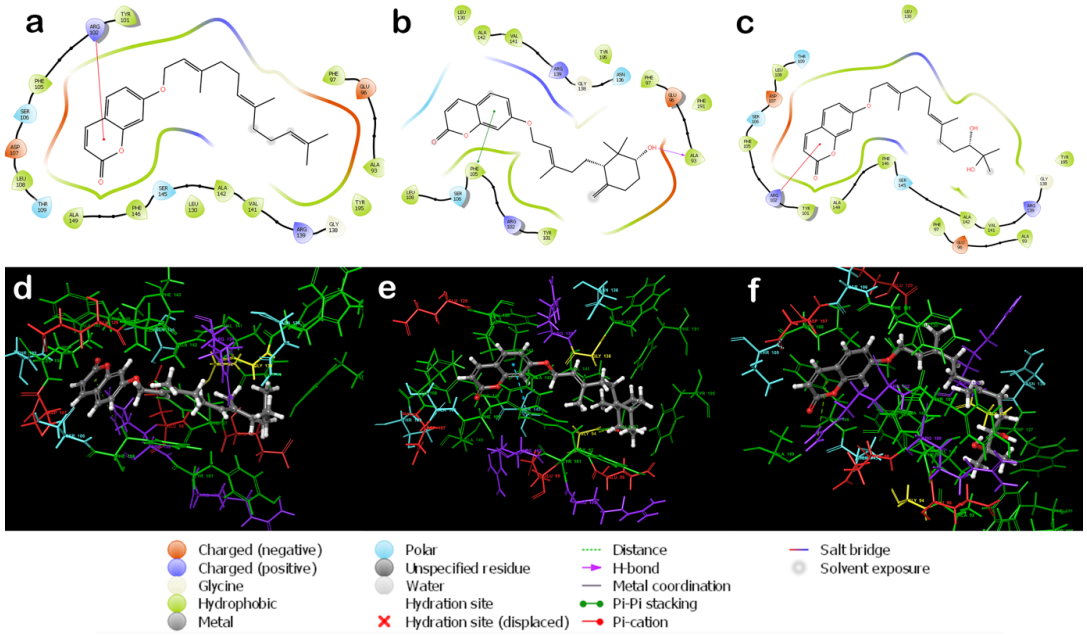


Figure 1. 2D interactions of umbelliprenin with the amino acids of the active site of Bcl-xL. **b)** 2D interactions of farnesiferol B with the amino acids of the active site of Bcl-xL. **c)** 2D interactions of karatavicinol with the amino acids of the active site of Bcl-xL. **d)** 3D interactions of umbelliprenin with the amino acids of the active site of Bcl-xL. **e)** 3D interactions of farnesiferol B with the amino acids of the active site of Bcl-xL. **f)** 3D interactions of karatavicinol with the amino acids of the active site of Bcl-xL.

The ligand binding site of the protein was determined by creating a receptor grid box based on the atomic coordinates of the native ligand using the Receptor Grid Generation implemented in Glide (Schrödinger Release 2022-3: Glide, Schrödinger, LLC, New York, NY, 2022) (Friesner et al., 2006; Halgren et al. 2004; Friesner et al., 2004). The compounds were docked to the binding site using Glide, and docking was performed using Standard Precision (SP) mode.

In silico ADME Studies

The QikProp module of Schrödinger Software (QikProp, Schrödinger, LLC, New York, NY, 2022) was used to predict the pharmacokinetic properties of sesquiterpene coumarins.

RESULTS

Sesquiterpene coumarins Table 1 were individually docked to the active site of Bcl-xL (PDB ID: 7LH7, 1.40 Å) (Tao et al., 2021) to examine their probable interactions with the amino acids in the binding site of Bcl-xL. The first three compounds that gave the best docking scores were umbelliprenin (**1**), Farnesiferol B (**2**), and karatavicinol (**3**), with docking scores of -7.114, -7.039, -7.021, respectively. While Umbelliprenin and Karatavicinol formed π-cation interaction with ARG102, Farnesiferol B formed a hydrogen bond with ALA 93 and π-π stacking interaction with PHE105 Figure 1. Docking scores and interactions of sesquiterpene coumarins within amino acids of the active site of Bcl-xL are shown in Table 2. Supplementary Data provides 2D and 3D representations of the docking positions of all sesquiterpene coumarins within the binding site of the Bcl-xL enzyme.

Since ADME studies have a critical role in optimising the pharmacokinetic properties of compounds in drug design, the pharmacokinetic properties of the sesquiterpene coumarins used in the molecular docking study were determined using the QikProp module of the Schrödinger Molecular Modelling software. Table 3 presents the predicted ADME descriptive values of sesquiterpene coumarins and their optimal value ranges (Lipinski, 2000; Lipinski, 2004).

DISCUSSION

Sesquiterpene coumarins Table 1 were individually docked to the active site of Bcl-xL (PDB ID: 7LH7, 1.40 Å) (Tao et al., 2021) to examine their probable interactions with the amino acids in the binding site of Bcl-xL. To verify the reliability of the docking study, the co-crystalised ligand (A1293102) of Bcl-xL was redocked to the active site of the protein, and the root-mean-square deviation (RMSD) was calculated. Depending on the ligand size, an RMSD value below 1.5 or 2 Å size is regarded to have performed successfully (Ece & Sevin, 2010). The RMSD was determined as 0.561, which was an acceptable value for docking model validation Figure 2.

The binding site of Bcl-xL contains two significant pockets: P2 and P4. Examining the interactions between the co-crystallised ligand (A1293102) and the active site of the Bcl-xL

Table 2. Docking scores and interactions of sesquiterpene coumarins within the aminoacids of active site of Bcl-xL

No	Compound	Docking Score	Hydrogen bond interaction	π-π stacking interaction	π-cation interaction
1	Umbelliprenin	−7.114	X	X	ARG 102
2	Farnesiferol B	−7.039	ALA 93	PHE 105	X
3	Karatavicinol	−7.021	X	X	ARG 102
4	-	−6.965	ARG 102	X	ARG 102
5	-	−6.850	ALA 93, ARG 102	X	ARG 102
6	Flabellilobin A	−6.744	X	PHE 105 (x2)	X
7	Sinkiangenorin F	−6.733	ALA 93	PHE 105	X
8	10',11'-epoxyumbelliprenin	−6.526	X	X	ARG 102
9	Fekrynol	−6.511	ALA 93	PHE 105 (x2)	X
10	8-O-acetyl sinkiangenorin F	−6.376	ALA 93	PHE 105	X
11	-	−6.319	ALA 93	PHE 105 (x2)	X
12	-	−6.243	ASN 136, ARG 139	PHE 105 (x2)	X
13	-	−6.198	X	PHE 105	X
14	Samarcandin	−6.066	ALA 93, ARG 139	PHE 105 (x2)	X
15	Badrakemone	−5.945	X	PHE 105 (x2)	X
16	-	−5.882	ASN 136, ARG 139	PHE 105	X
17	kamolone	−5.834	X	PHE 105	X
18	sinkiangenorin E	−5.809	ARG 139	X	X
19	Ferukrin	−5.808	ALA 93, ARG 139	PHE 105	X
20	Deacetylkellerin	−5.712	ALA 93, ARG 139	PHE 105	X
21	Farnesiferol C	−5.646	X	PHE 105	X
22	Turcica ketone	−5.572	ARG 139 (x2)	X	X
23	Mogoltadone	−5.561	ARG 139	PHE 105	X
24	Feshurin	−5.471	ARG 139	PHE 105 (x2)	X
25	Kellerin	−5.420	ALA 93, ARG 139	PHE 105	X
26	-	−5.395	X	PHE 105 (x2)	X
27	-	−5.367	ARG 139	PHE 105 (x2)	X
28	-	−5.354	ARG 139	PHE 105	X
29	Conferone	−5.280	ARG 139	PHE 105 (x2)	X
30	-	−5.271	X	X	X
31	Sinkiangenorin D	−5.262	GLU 96, ARG 139	PHE 105 (x2)	X
32	-	−5.252	X	TYR 195 (x2)	X
33	-	−5.232	ALA 93, ARG 139	PHE 105 (x2)	X
34	Ferukrin acetate	−5.028	ARG 139 (x2)	X	X
35	-	−4.988	ARG 139	PHE 105	X
	A1293102	−12.686	SER106, LEU 108, ASN 136, ARG 139	SER145, PHE 146	X

enzyme, it was observed that the benzothiazole group predominantly occupied the P2 pocket. The nitrogen within the benzothiazole ring forms a hydrogen bond with LEU108, and the hydrogen of the amide group forms a hydrogen bond with SER106. These interactions are crucial for the binding and selectivity of the molecule. The nitrogen atoms of the thiazole ring in the middle of the molecule form a hydrogen bond with ARG139, and the carbonyl oxygen atom forms a hydrogen bond with ASN 136. The remaining molecule part completely seals the P4 hydrophobic pocket and interacts with the amino acids ALA93, PHE97, TYR101, and VAL141 through van der Waals forces (Tao et al., 2021). The docking studies in this study supported the literature findings, which demonstrated that A1293102 formed identical interactions within the P2 and P4 binding regions of Bcl-xL enzyme Figure 3, Figure 4, and Figure 5.

Table 3. Predicted ADME properties of sesquiterpene coumarins using the QikProp module of Schrödinger Software.

Compound	MW[a]	LogPo/w[b]	Log BB[c]	PMDCK[d]	HOA%[e]	Rule of Five
1	366.499	6.229	−0.808	1069.075	100.00	1
2	382.499	4.540	−0.886	516.450	100.00	0
3	400.514	4.490	−1.436	320.640	100.000	0
4	364.483	6.155	−0.802	1069.075	100.000	1
5	416.513	3.515	−1.522	263.049	96.676	0
6	424.536	5.234	−1.008	449.958	100.000	1
7	400.514	4.18	−1.135	353.451	100.000	0
8	368.472	4.744	−0.683	1069.075	100.000	0
9	398.541	4.53	−1.141	291.181	100.000	0
10	442.551	4.79	−1.187	290.929	100.000	0
11	396.482	3.02	−1.13	149.051	89.694	0
12	382.499	5.222	−1.042	428.605	100.000	1
13	380.483	4.112	−0.679	453.66	100.000	0
14	400.514	3.568	−0.957	263.996	100.000	0
15	380.483	3.856	−0.681	418.45	100.000	0
16	382.499	5.201	−1.018	428.476	100.000	1
17	382.499	3.884	−0.679	403.221	100.000	0
18	414.541	3.913	−1.087	224.579	100.000	0
19	400.514	3.65	−1	253.705	100.000	0
20	400.514	3.667	−0.951	280.685	100.000	0
21	382.499	5.127	−0.533	1053.162	100.000	1
22	380.483	4.016	−0.716	419.84	100.000	0
23	380.483	3.971	−0.631	464.204	100.000	0
24	400.514	3.693	−0.966	288.787	100.000	0
25	442.551	4.429	−1.042	250.285	100.000	0
26	442.551	4.479	−1.068	265.473	100.000	0
27	400.514	3.563	−0.913	270.816	100.000	0
28	380.483	4.155	−0.689	461.354	100.000	0
29	380.483	3.875	−0.61	463.3	100.000	0
30	382.499	5.215	−0.915	552.584	100.000	1
31	384.514	4.277	−1.100	285.805	100.000	0
32	382.499	5.275	−0.839	662.359	100.000	1
33	382.499	3.941	−0.609	489.547	100.000	0
34	442.551	4.438	−1.085	248.18	100.000	0
35	440.535	3.949	−1.096	227.998	100.000	0
A1293102	972.116	6.078	−3.009	37.913	43.513	3

[a]: Molecular weight (recommended value: 150 to 500) [b]: Octanol/water partition coefficient (recommended value: −2 to 5) [c]: Brain/blood partition coefficient (recommended value: −3 to 1.2) [d]: Permeability Madin-Darby canine kidney (<25 is poor, >500 is great) [e]: HOA: Human oral absorption (≥80% is high, ≤25% is poor)

As a result of the docking studies conducted in this study revealed the significance of sesquiterpene coumarins like umbelliprenin, karatavicinol, farnesiferol B, sinkiangenorin F, flabellilobin A, and epoxyumbelliprenin. These molecules share a common characteristic, which is the presence of farnesyl moiety bonded to the umbelliferon coumarin core through an ether bridge, resulting in the derivative formation. Consequently, these molecules become longer. The coumarin ring within these molecules constituted the more polar part of the molecule and resides within the P2 pocket, assuming the

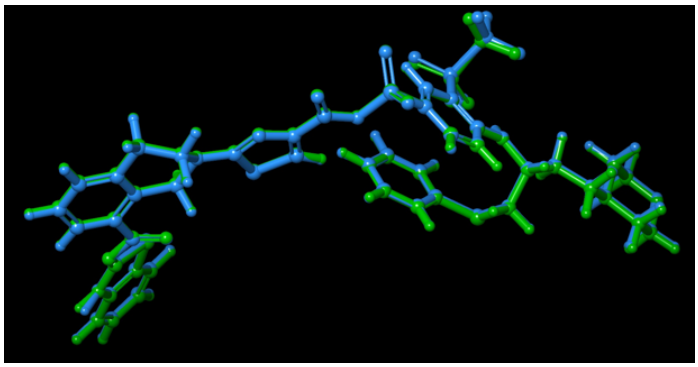


Figure 2. Validation of the docking models: Superposition of the docked pose (green) and experimental binding conformation (blue) of A1293102 in the binding site of Bcl-xL.

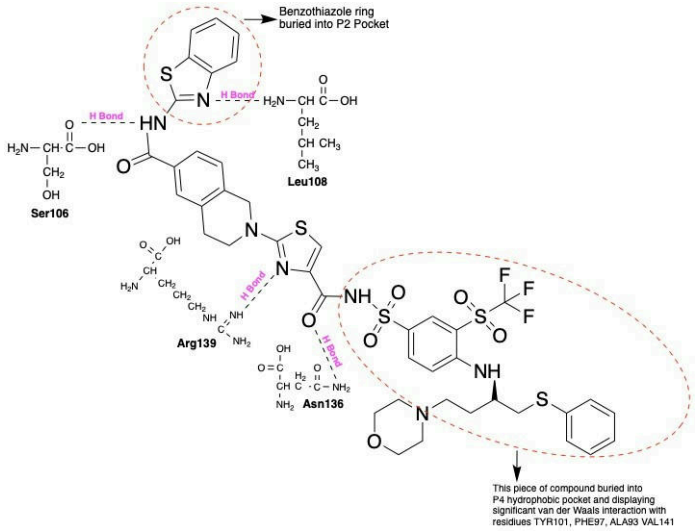


Figure 3. Significant interactions between A1293102 and amino acids within the active site of the Bcl-xL enzyme.

original role of the benzothiazole ring in the co-crystallised ligand (A1293102). As unsaturated hydrocarbon chains, farnesyls or their derivatives in these compounds stretch between the P2 and P4 pockets. They are anchored in place by forming van der Waals interactions with the hydrophobic amino acids in the P4 pocket. Figure 6 illustrates this by superimposing the binding poses of these molecules and A1293102. In a 2013 study, it was demonstrated that umbelliprenin obtained from the *Ferula szowitsiana* has the ability to inhibit Bcl-2, an antiapoptotic enzyme having a structure similar to Bcl-xL. This finding aligns with the outcomes of our docking studies (Gholami et al., 2013). Conversely, the docking scores of derivatives containing drimane-type sesquiterpenes, a significant group of sesquiterpene coumarins commonly isolated from *Ferula* species, tend to be higher than those of the umbelliprene-type derivatives, suggesting that their binding affinities may be weaker. Drimane-type sesquiterpenes cannot simultaneously access the two crucial binding sites of the enzyme, thereby limiting their ability to form adequate hydrophilic or hydrophobic interactions.

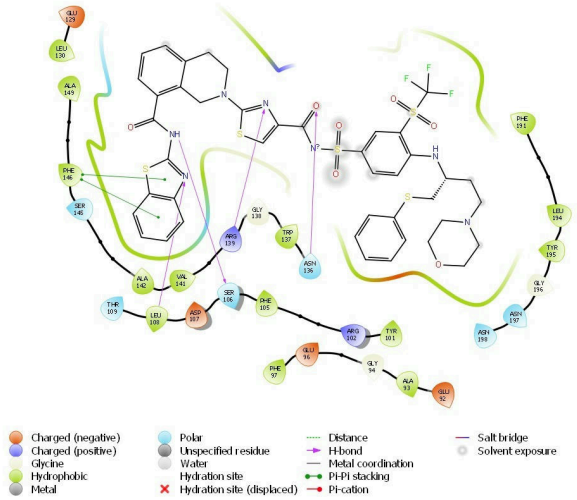


Figure 4. A1293102 and its 2D interactions with the active site of Bcl-xL (Docking Score: –12.686).

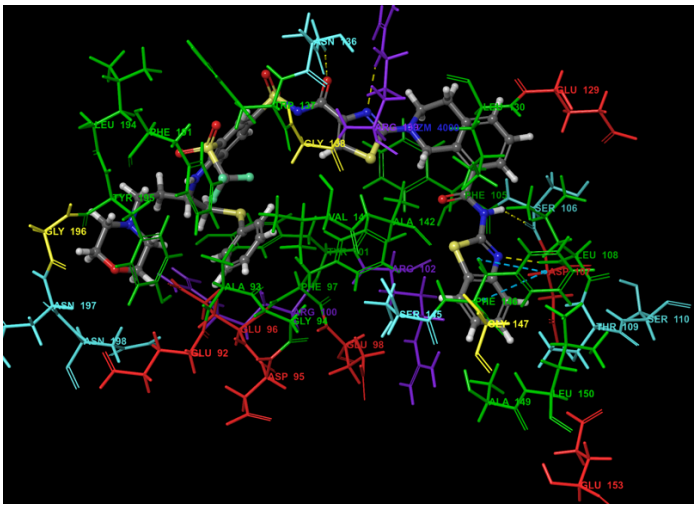


Figure 5. A1293102 and its 3D interactions with the active site of Bcl-xL (Docking Score: –12.686).

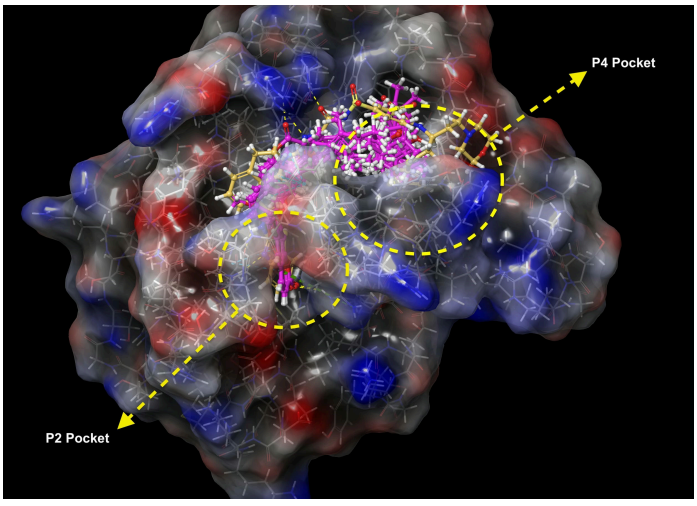


Figure 6. Superimposing the binding poses of umbelliprenin-type sesquiterpene coumarins and A1293102.

Molecular docking calculations in this study suggest that it is possible to enhance the potency and selectivity of umbelliferone-type sesquiterpene coumarins against Bcl-xL by modifying the coumarin ring to interact with the essential amino acids, SER106 and LEU108, which are crucial for binding and selectivity to the Bcl-xL enzyme. The findings of this study can serve as a guide for researchers in the development of specific umbelliprene-type sesquiterpene coumarins that exhibit selectivity towards the Bcl-xL enzyme.

Since ADME studies have a critical role in optimising the pharmacokinetic properties of compounds in drug design, the pharmacokinetic properties of the sesquiterpene coumarins used in the molecular docking study were determined using the QikProp module of the Schrödinger Molecular Modelling software. Table 3 presents the predicted descriptive ADME values of sesquiterpene coumarins and their optimal value ranges (Lipinski, 2000; Lipinski, 2004). All sesquiterpene coumarins have MW values ranging from 364.483 to 442.551 and follow Lipinski's rule of five as their MW is less than 500, while the molecular weight of A1293102 is 972.116. The LogPo/w, which indicates the solubility properties of the compounds, should be between −1 and 5. The LogPo/w values of sesquiterpene coumarins and A1293102 ranged from 3.02 to 6.229, and all values fell within the specified acceptable range. The LogBB parameter, which is used to evaluate the ability of compounds to cross the blood-brain barrier, should be between values of −3 and 1.2. The LogBB values of sesquiterpene coumarins and A1293102 varied between −3.009 and −0.533, within the specified acceptable range. The PMDCK (Permeability Madin Darby Canine Kidney) measures the apparent permeability of MDCK cells, represented in nm/s. MDCK cells are a reliable model for studying the blood-brain barrier. A higher PMDCK value signifies enhanced cell permeability. The PMDCK values of sesquiterpene coumarins ranged from 149.051 to 1069.075, which can be considered good values. The %HOA values of all sesquiterpene coumarins, except compounds **5** and **11**, are 100%, whereas the %HOA value of A1293102 is 43.513%.

CONCLUSION

The objective of this study was to investigate whether sesquiterpene coumarins can be used as inhibitors of Bcl-xL enzyme through the use of *in silico* molecular docking. To achieve this, molecular docking studies were conducted using various cytotoxic sesquiterpene coumarins isolated from different *Ferula* species. As a result of the docking studies, it was observed that the docking scores of compounds that contain drimane-type sesquiterpenes, which are a notable group of sesquiterpene coumarins generally found in *Ferula* species, were generally higher than those of strain-chain-type derivatives. This suggests that the binding affinity of the drimane-type derivatives might be weaker. Upon examining the interactions between the compounds and the enzyme's active site, it is evident that drimane-type sesquiterpene compounds are unable to simultaneously access two critical binding sites of the enzyme. Thus, their capacity to form adequate hydrophilic or hydrophobic interactions is restricted. Conversely, sesquiterpene compounds with linear chains, such as umbelliprenin, are capable of accessing these two crucial regions and forming bonds with amino acids. These findings revealed promising Bcl-xL inhibitory activities of umbelliprene-type sesquiterpene derivatives, which might be a starting point for further structural optimisation to acquire novel compounds exhibiting more potent Bcl-xL inhibitory activity.

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