Protein-ligand docking study: diterpenes from *Juniperus brevifolia* as anticancer and antimicrobial agents

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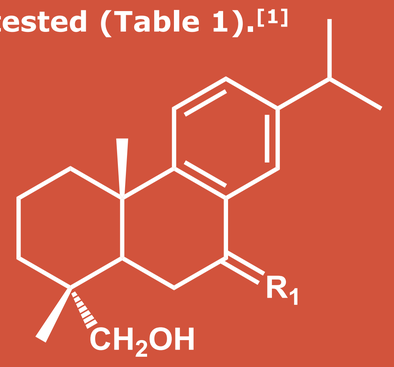
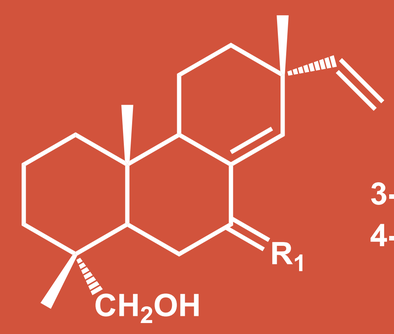
INTRODUCTION:

Several dehydroabietane and sandaracopimarane derivatives, (1-4) isolated from leaves of *Juniperus brevifolia*,[1] an endemic conifer from Azores, displayed antiproliferative activity against cancer cell lines (HeLa, A-549 and MCF-7) and bactericidal effect against *Bacillus cereus* at different concentrations tested (Table 1).[1]

1- R_1= H,H
2- R_1= O





3- R_1= H,H
4- R_1= O

Table 1: Cytotoxic (IC_{50} μM) and antimicrobial (μg/mL) activities of secondary metabolites from *J. brevifolia* leaves.

Comp.	HeLa		MCF-7		A-549		Vero		*B. cereus*	
	Lag phase	Log phase	Lag phase	Log phase	Lag phase	Log phase	Lag phase	Log phase	MIC	MBC
1	15.7	25.9	31.7	40.4	23.6	44.4	28.0	54.5	2.5 – 5	10 – 20
2	49.0	52.7	>60	>60	46.2	60.0	54.4	>60	-	-
3	46.5	52.1	58.5	>60	53.7	>60	>60	>60	20	20 – 40
4	49.6	>60	>60	>60	>60	>60	nd	nd	-	-

RESULTS:

In this work, protein-ligand docking was performed, using the FlexScreen program, in order to pick molecular targets, from a large set of common anticancer (63) and antimicrobial (39) targets, for the selected compounds 1-4.

The preferred targets for anticancer and antimicrobial compounds 1-4 and their interaction energies are shown in Tables 2 and 3.

- **The structural differences between the skeleton-type dehydroabietane and sandaracopimarane, do not seem to be relevant in the interaction with the targets**

Table 2: Preferred anticancer targets and interaction energies.

Comp.	Anticancer target			
	Target	PDB code	Energy (arbitrary units)	
			Sample 1	Sample 2
1	SHIP2	3RN8	-76,512	-76,156
2	PDF	3G5K	-86,313	-85,542
3	SHIP2	3RN8	-76,983	-76,831
4	TUBA	Model	-98,136	-97,943

Table 3: Preferred antimicrobial targets and interaction energies.

Comp.	Antimicrobial target			
	Target	PDB Code	Energy (arbitrary units)	
			Sample 1	Sample 2
1	RNAP	1EIK	-230,447	-212,083
3	RNAP	1EIK	-234,812	-234,152
1	PDF	2AIA	-76,970	-76,476
3	PDF	2AIA	-75,318	-72,259

Anticancer targets

- **The compounds 1 and 3 interact preferentially with phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 2, (SHIP2), a clinically relevant novel anticancer target. This enzyme has high therapeutic index given its over-expression in cancer and its dispensability for normal cell survival.[3]**
- **The hydroxyl groups and their ability to form H-bond, seem to play a significant role in these interactions since the H atom interacts with the carbonyl group of the aspartate residue and the oxygen atom interact with H-N of Arginine residue (Fig 1a).**
- **The compound 4 interact preferentially with α-tubulin (Fig 1b), the protein that makes up microtubules, not only by its OH group (H-bond with serine and tyrosine residue), but also by its carbonyl group (H-bond between N-H group of the alanine residue and oxygen atom from carbonyl group).**
- **The correlation between experimental and calculated data for anticancer targeta show that the compounds probably inhibited the targets TNKS-1, CDK1 and HIF1A in the assay against cancer cell line Vero. Against cancer cells lines HeLa, MCF-7 and A-59, the compounds probably inhibited PPAR-γ.**

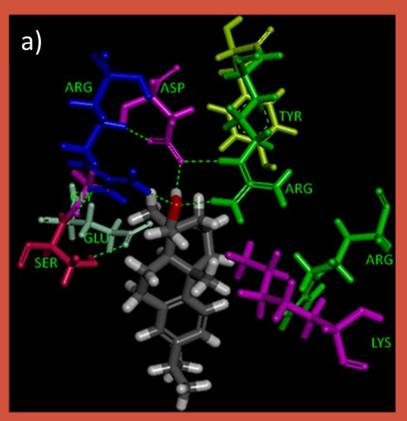


Fig 1. Representation of the obtained interactions between a) compound 1 and target SHIP2 and b) compound 4 and target TUBA.

Antimicrobial targets

- **Compounds 1 and 3 interact, both by H-bond, preferentially with RNA polymerase (RNAP), an enzyme that in bacteria catalyzes the synthesis of mRNA and ncRNA.**
- **The hydroxyl group of compound 3 interacts with carbonyl group of aspartate (ASP30) (Fig 2 a). The hydroxyl groups of the compound 1 and 3, also interact with the carbonyl group of lysine residue (LYS27) (Fig 2b).**

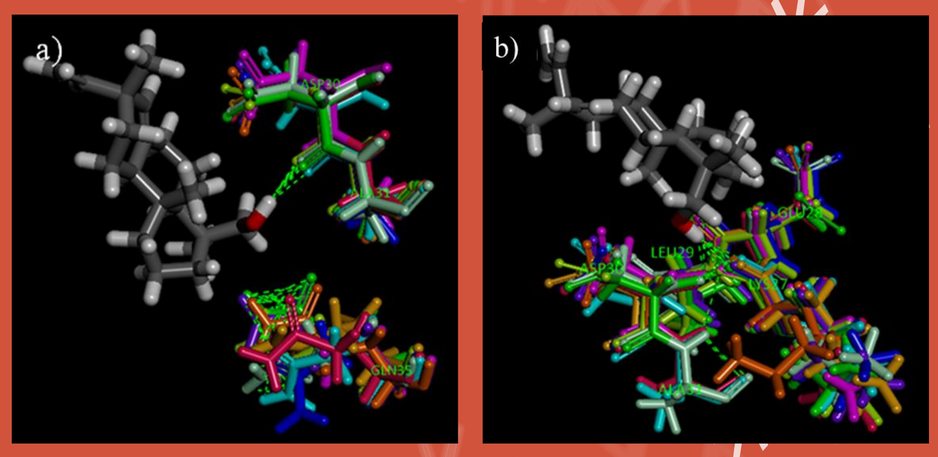


Fig 2. Representation of the obtained interactions between compound 3 and target RNAP a) interactions with ASP30, b) interactions with LYS27.

Acknowledgments

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References

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