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Molecular docking of *Vernonia conferta* stem bark isolated triterpenes on MRCK alpha- and beta-protein-mediated skin cancer

Arrimage moléculaire des triterpènes isolés de l'écorce de Vernonia conferta sur le cancer de la peau médié par les protéines MRCK alpha et bêta

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Mots-clés :MRCKα – MRCKβ – Triterpènes – *Vernonia conferta* – Cancer de la peau.

ABSTRACT

Introduction: Over the last thirty years, the number of new cases of skin cancer has more than tripled. This observation is a warning sign for the increasing prevalence of cancer in general and skin cancer in particular. Current drug treatments offer some solutions. However, there are still many toxic effects. In an attempt to provide new therapeutic solutions for treating skin cancer with an improved risk-benefit ratio, we used theoretical chemistry to investigate the possible molecular interactions that could be established between triterpenes isolated from the stem bark of *Vernonia conferta* and MRCKα/MRCKβ, two proteins involved in the development of skin cancer.
Materials and methods: Phytochemicals were isolated by column chromatography and structures determined by spectroscopic analysis (^{1}H and ^{13}C NMR) and TLC comparisons. Triterpene skeletons were selected, drawn and molecular docked using dedicated software.
Results: Six triterpenes (1-6) have been isolated from the stem bark of *V. conferta*. Molecular docking showed that lupane-type triterpenes (A) would be better ligands for the inhibition of the action of MRCKα and MRCKβ protein kinases, which are involved in the development of certain skin cancers.
Conclusion: We investigated the possible interactions between triterpenes isolated from the stem bark of *Vernonia conferta* and MRCKα/MRCKβ, two proteins involved in skin cancer development. It appears that lupan-type (A) triterpenes would be better ligands for inhibiting the action of MRCKα and MRCKβ protein kinases.

RESUME

Introduction : Au cours des trente dernières années, le nombre de nouveaux cas de cancer de la peau a plus que triplé. Cette observation est un signe d'alerte quant à l'augmentation de la prévalence du cancer en général et du cancer de la peau en particulier. Les traitements médicamenteux actuels offrent quelques solutions, cependant, les effets toxiques restent nombreux. Dans le but donc de tenter d'apporter de nouvelles solutions dans la prise en charge du cancer de la peau avec un meilleur rapport bénéfice/risque, nous avons à l'aide de la chimie théorique étudié les interactions moléculaires qui pourraient s'établir entre les triterpènes isolés de l'écorce de tronc de *Vernonia conferta* et MRCKα/MRCKβ, deux protéines impliquées dans le développement du cancer de la peau
Matériel et méthodes : Les molécules ont été isolées des écorces de tronc de *V. conferta* grâce à la chromatographie sur colonne. Leurs structures chimiques ont pu être élucidées grâce à la RMN (^{1}H et ^{13}C) ainsi qu'à la comparaison sur plaques de CCM avec des échantillons authentiques. Enfin, sur la base de leurs structures, les triterpènes ont été triés, modélisés et soumis au *docking* moléculaire via des logiciels spécialisés.
Résultats : Six triterpènes (1-6) ont pu être isolés de l'écorce de tronc de *V. conferta*. Le docking moléculaire a montré que les triterpènes de type lupane (A) seraient de meilleurs ligands pour inhiber l'action des protéines kinases MRCKα et MRCKβ, impliquées dans le développement de certains cancers de la peau.
Conclusion : Dans la recherche de nouveaux traitements contre les cancers de la peau, il semble que les triterpènes de type lupane (A) seraient des meilleurs candidats pour développer de nouveaux anticancéreux.

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Introduction

The skin is the largest and heaviest organ in the human body [1]. Cancer is a disease caused when cells become abnormal and multiply excessively [2]. Over the last thirty years, the number of new cases of skin cancer has more than tripled [1], a warning sign that the prevalence of cancer in general is on the rise. Current drug treatments offer some solutions but are also the source of numerous toxic effects [3]. In an attempt to provide new potentially less toxic therapeutic solutions to treat skin cancer, we investigated the possible interactions between triterpenes isolated from the stem bark of *Vernonia conferta* and MRCKα/MRCKβ, two proteins involved in skin cancer development [4].

Experimental

General experimental procedures

NMR spectra were obtained using Bruker® DRX 600 NMR spectrometers. Column chromatography was carried out on silica gel 230-400 mesh and silica gel 70-230 mesh, Merck®. Thin layer chromatography (TLC) was performed on Merck® precoated silica gel 60 F254 aluminium foil, and spots were revealed using a UV lamp (254-365 nm) and 10% H_2SO_4, followed by heating. Different mixtures of *n*-hexane, EtOAc and MeOH were used as eluting solvents.

a. Plant material

V. conferta stem bark was harvested at Nkol-Afamba (N3° 51′ 16″; E11° 39′ 55″), Center Region, Cameroon, in March 2021 and authenticated at the Cameroon National Herbarium (29458/HNC). The freshly collected material was thoroughly washed with running tap water followed by distilled water to remove all surface contaminants and roughly cut.

b. Extraction and isolation

The air-dried and powdered stem bark of *V. conferta* (4.8 kg) were macerated in 95% MeOH (10.0 L) two times each for three days at room temperature. The solvent was evaporated, using Heidolph® rotary evaporator under reduced pressure to yield 46.5 g of crude extract. An 36.0 g portion was subjected to chromatography on silica gel 60 (70-230 mesh) using a gradient of *n*-hexane, ethyl acetate and methanol. A total of 412 fractions (approximately 100 ml each) were collected and grouped into five sub-fractions (F1 to F5) based on TLC analysis (**Figure 1**). After purification, eight non-terpenoids compounds were obtained from sub-fractions F_1 and F_5. Only sub-fractions F_2 to F_4 yielded triterpenes (1-6). F_2 was purified on silica gel column (230-400 mesh, 3.5 x 50.0 cm) to yield *β*-amyrin (1) (8.6 mg) by elution with an *n*-hexane/EtOAc gradient (95:5, *v*/v); and *β*-amyrin acetate (2) (12.8 mg) by elution with an *n*-hexane/EtOAc gradient (90:10, *v*/v). F_3 was purified on silica gel column (230-400 mesh, 3.5 x 50.0 cm) to yield lupeol (3) (7.5 mg) by elution with an *n*-hexane/EtOAc gradient (95:5, *v*/v); lupeol acetate (4) (9.8 mg) by elution with an *n*-hexane/EtOAc gradient (90:10, *v*/v) and betulin (5) (10.4 mg) by elution with an *n*-hexane/EtOAc gradient (85:15, *v*/v). Finally, F_4 was purified on silica gel column (230-400 mesh, 3.5 x 50.0 cm) to yield betulinic acid (6) (11.0 mg) by elution with an *n*-hexane/EtOAc gradient (75:25, *v*/v).

General computerized analysis

Computational analysis was performed using HP Intel® Core™ i5 6200U CPU @ 2.30GHz × 4; 6.0GiB RAM, O.S: Ubuntu 22.04 LTS and Windows®10 Professional in dual-boot mode. Docking studies performed using AutoDockTools® 1.5.6 enabled us to calculate the binding energies and assess the nature of the interactions which might occur between the selected ligands and the docking proteins. Visualisation of protein-ligand interactions was performed using Discovery Studio Visualizer v17.2.0.16349. ChemDraw® online server was used to draw the two-dimensional structure of the ligand and SwissPdbViewer® v4.128 was used to minimise the protein energy.

a. Ligands selection and preparation

Ligands have been selected based on their triterpene skeleton [5]. Their 2D structures were plotted using ChemDraw® 15.0 software, and their corresponding backbone structures were also drawn to compare. Ligand structures (phytochemicals and backbones) were optimized for energy minimisation using MMFF94 force field [6].

b. Proteins preparation

3D structures of MRCK alpha (PDBID: 4AW2) and MRCK beta (PDBID: 3QFV) proteins were obtained from the Protein Data Bank at 1.70Å and 2.65Å RMSD resolution, respectively. Targets were prepared using Discovery Studio Visualizer® v17.2.0.16349 and AutoDockTools® 1.5.6; if

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complexed with ligands, water molecules or ions, targets were removed using Discovery Studio Visualizer [7].

c. Molecular docking experiments

Molecular docking was performed in-house by manually docking 'one ligand at a time to the protein' using AutoDock® 4.2. [7,8]

d. Initialising and preparation of PDBQT files

Prior to docking, the start directory was set to the desired folder. The processed protein targets were imported into AutoDock® 4.2. The polar hydrogen atoms were added and the Kollman and Gasteiger charges were calculated. The Ligand torsion tree was defined, then the ligand and protein were imported into the workspace.

e. Grid parameters

Active site coordinates were defined from the co-crystallised ligand coordinates of each protein. Grid spacing was set to 0.375 Å (default) and the center grid box values were set to x=00,000, y=00,000 and z=00,000. The number of grid points along the x, y and z directions were defined as 00 x 00 x 00.

f. *Running AutoGrid® and AutoDock®*

AutoGrid® was first run by entering the AutoGrid® executable and grid parameter file (GPF) into the grid log file (GLG). After successful execution of AutoGrid®, the Lamarckian Genetic Algorithm was used, and then AutoDock® was run, initiating docking. The results were analysed, ranked by binding energy, and saved.

g. Visualising interactions

Discovery Studio Visualizer® v17.2.0.16349 were used to visualise and study the 2-D, 3-D, and surface annotation of ligand interactions with the proteins.

h. Docking validation

The docking procedure was validated by re-docking the co-crystallised ligands and calculating the root mean square deviation (RMSD). Co-crystallised ligands were removed from MRCKα and MRCKβ then redocked to their respective active sites using AutoDock® 4.2. No changes were made to this protocol, including grid parameters. The re-docked complex was then superimposed on the reference co-crystallised complex using Discovery Studio Visualizer® v17.2.0.16349 and the RMSD was calculated. The superimposed structures of the co-crystallised and redocked ligands were then highlighted in the 3-D image.

Results and discussion

Chemistry

The crude extract obtained after MeOH extraction was subjected to column chromatography on silica gel and preparative thin layer chromatography (p-TLC). A total of fourteen compounds were obtained, of which six terpenoids (1-6), identified as *β*-amyrin (1)[9], *β*-amyrin acetate (2), lupeol (3), lupeol acetate (4), betulin (5)[10], betulinic acid (6) [11].

Computer analysis

In general, the molecular docking results show that ligands 1 to 6, as well as the lupan (A) and friedelan (B) cores, establish much more hydrophobic interactions with the MRCKα and MRCKβ proteins (**Table I**). This could be explained by the fact that MRCKα and MRCKβ belong to the kinase family [8]. Furthermore, as indicated by the very low Ki values (**Table I**), the hydrophobic ligand-receptor interactions observed in the majority of cases were very potent. In addition, compounds with acetate groups generally interacted better with proteins: ligand 2 (-8.52 kcal.mol^{-1}) versus ligand 1 (-8.09 kcal.mol^{-1}) with MRCKα; ligand 2 (-10.64 kcal.mol^{-1}) versus ligand 1 (-9.92 kcal.mol^{-1}), ligand 4 (-11.13 kcal.mol^{-1}) versus ligand 3 (-10.13 kcal.mol^{-1}) with MRCKβ.

Interactions of ligands 1-6 and cores A and B with MRCKα protein

Regarding the interactions between rings A and B with MRCKα, it appears that ring A (binding energy = -10.17 kcal.mol^{-1}) is more favourable for the stability of ligand-receptor interactions than ring B (binding energy = -8.78 kcal.mol^{-1}) (**Table I**). Since the difference between these two rings lies in the terminal ring (cyclopentane in A and cyclohexane in B), we can conclude that the pentacyclic configuration of this terminal ring would, in the first place, increase the stability of the ligand-receptor interactions [12].

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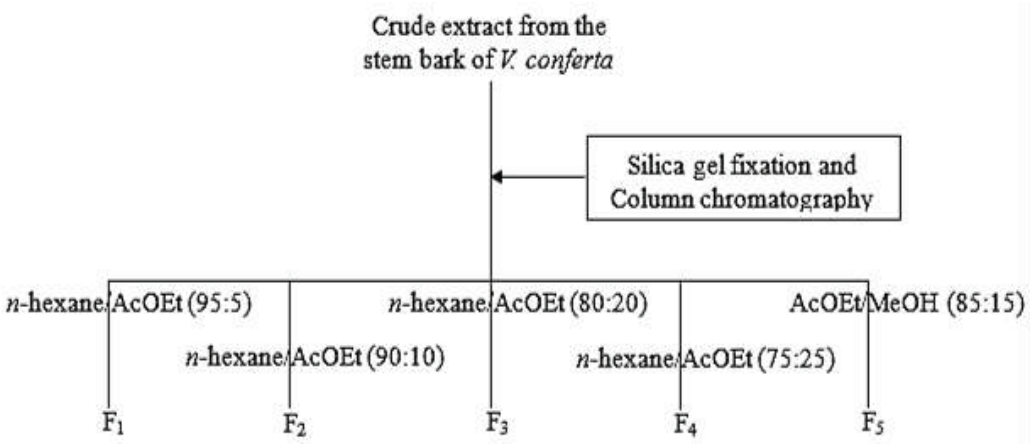

Figure 1: *General process leading to sub-fractions F_1 to F_5*

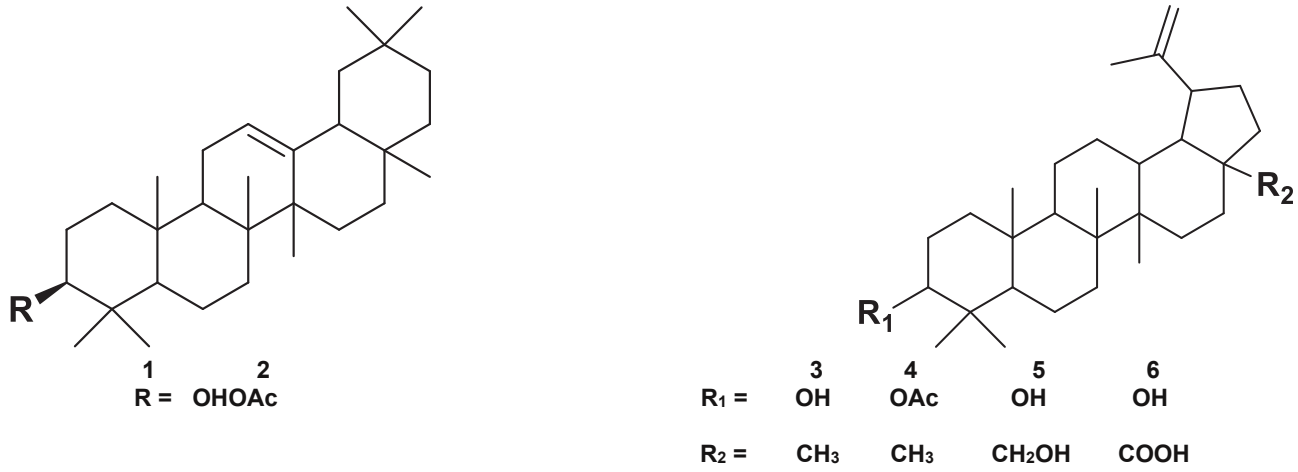

Figure 2: *Chemical structures of V. conferta isolated triterpenes*

Figure 3: *Lupan-type Ring (A) and friedelan-type Ring (B)*

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Table I : *Summary of MRCKα and MRCKβ proteins interactions with lupan-type, friedelan-type rings, and ligands 1 to 6*

Target protein receptor	Ligand identification	Binding energy (Kcal/mol)	Nb of hydrophobic interactions	Nb of Hbonds	Nb of salt bridges	Interaction amino acid residues	Ki (µM)
MRCKα ID: 4AW2	β-amyrin (1)	-8.09	04	---	---	ILE36 (02) ; LYS68 ; HIS396	1.18
	β-amyrin acetate (2)	-8.52	07	---	01	ILE36 (02); LYS68 (02); HIS73 (02); ASP146; HIS396	0.569
	Lupeol (3)	-9.00	08	---	---	ILE36 (02) ; LYS68 (02) ; ASP40 ; GLN69 ; GLN 145 ; ASP146	0.251
	Lupeol acetate (4)	-6.93	04	---	01	GLN69; ARG74; ASP146; ASP147; LYS68	8.32
	Betulin (5)	-7.88	06	02	---	ILE36; LYS68 (02); GLN69 (02); ARG71; HIS73; ASP147	1.67
	Betulinic acid (6)	-8.41	06	01	01	ILE36; LYS68 (02); GLN69 (02); ARG71; HIS73; ASP147	0.687
	Lupan ring (A)	-10.17	07	---	---	ILE36; LYS68; HIS73; ARG74; GLN145; ASP146; HIS396	0.035
	Friedelan ring (B)	-8.78	11	01	---	ASP33; ILE36 (03) ; ASP40 ; LYS68 (02) ; HIS73; ARG74; ASP146; HIS396; GLU75	0.367
MRCKβ ID: 3QFV	β-amyrin (1)	-9.92	11	01	---	ILE82 (02) ; VAL90 (02) ; ALA103 ; TYR155 ; TYR156 (02) ; LEU207 (02) ; PHE370 ; ASP371	0.053
	β-amyrin acetate (2)	-10.64	11	02	01	ILE82 ; VAL90 (02) ; ALA103 ; LYS105 (02) ; THR137 ; LEU162 ; ASP204 ; LEU207 (02) ; PHE219 (02) ; ASP218	0.016
	Lupeol (3)	-10.13	12	03	---	ILE82 (02) ; VAL90 ; TYR155 (02) ; LEU162 ; LEU207 (02) ; ALA217 ; PHE370 (03) ; THR163 ; ASP371 (02)	0.037
	Lupeol acetate (4)	-11.13	10	---	---	ILE82; VAL90; THR137; TYR155; TYR156 (02); ASP204; LEU207; PHE370 (02)	0.007
	Betulin (5)	-10.22	08	03	---	ILE82 (02) ; ASP204 ; LEU207 (02) ; ASP218 ; PHE370 (02) ; LYS105 ; ASP371 (02)	0.032
	Betulinic acid (6)	-9.77	07	---	01	VAL90 ; ALA103 ; TYR156 ; LEU207 (02) ; ASP218 ; PHE370 ; LYS105	0.069
	Lupan ring (A)	-9.19	13	02	---	VAL90 (02) ; ALA103 ; LYS105 ; GLU124 ; LEU128 (02) ; THR137 ; LEU207 ; ALA217 ; ASP218 ; PHE219 ; PHE370 ; ARG84 (02)	0.182
	Friedelan ring (B)	-9.38	16	02	---	ILE82 ; VAL90 (02) ; ALA103 ; THR137 ; MET153 ; TYR155 ; TYR156 (02); ASP204; LEU207 (02); PHE370 (04); ASP371 (02)	0.133

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Table II: *NMR data of isolated compounds 2, 3, 4 and 6*

N°	Compound 3	Lupeol [15]	Compound 4	Lupeol acetate[16]	Compound 6	Betulinic acid[17]
1	38,8	38.7	39,0	38,6	38,3	38,7
2	27,3	27.4	26,5	21,7	27,3	27,4
3	79,0	78.9	82,2	81,2	78,2	78,9
4	38,8	38.8	38,0	38,8	38,7	38,8
5	55,4	55.3	55,1	55,6	55,2	55,3
6	18,4	18.3	16,9	18,4	18,2	18,3
7	34,3	34.2	34,0	34,4	34,2	34,3
8	40,9	40.8	44,2	41,0	40,5	40,7
9	50,5	50.4	50,1	50,5	50,3	50,5
10	37,1	37.1	37,0	37,3	37,1	37,2
11	21,0	20.9	21,0	21,1	20,7	20,8
12	25,2	25.1	28,0	24,0	25,2	25,5
13	38,1	38.0	38,5	36,2	38,2	38,4
14	42,8	42.8	44,0	43,0	42,3	42,4
15	27,4	27.4	25,0	25,3	306	30,5
16	35,5	35.5	36,1	35,8	32,0	32,1
17	43,1	43.0	48,1	43,2	56,2	56,3
18	48,2	48.2	48,3	48,5	46,7	46,8
19	47,9	47.9	48,2	48,2	49,1	49,2
20	151,0	150.9	151,0	151,2	150,0	150,3
21	29,9	29.8	41,0	40,2	29,5	29,7
22	40,1	40.0	40,1	30,0	36,9	37,0
23	28,1	28.0	24,5	28,2	27,7	27,9
24	15,5	15.4	24,3	27,6	15,1	15,3
25	16,2	16.1	16,8	16,2	15,9	16,0
26	16,0	15.9	17,1	14,7	16,0	16,1
27	14,6	14.5	16,5	18,2	14,6	14,7
28	18,1	18.0	17,1	16,7	177,6	180,5
29	109,4	109.3	109,0	109,6	109,4	109,6
30	19,3	19.3	22,0	19,5	19,2	19,4
$COCH_3$			171,2	171,3		
$COCH_3$			19	16,4		

Appendix: 1H and 13C NMR spectra of compounds 2, 3, 4 and (Compounds 1 and 5 were identified by comparison with authentic samples in our laboratory)

Secondly, we observe that this stability is better when only hydrophobic interactions are involved. This is confirmed by analysing the interactions of ligands 1 to 6 with the MRCKα protein. Here we can see that lupeol (3), which forms the most stable ligand-receptor complex with MRCKα, alone forms 08 hydrophobic interactions with this protein (more than any other isolated compound). This contrasts with lupeol acetate (4), which forms 01 salt bridges with MRCKα in addition to 04 hydrophobic interactions (**Table I**). Finally, the analysis shows that lupeol acetate (4) effectively forms the least stable ligand-receptor complex with the MRCKα protein of the series of isolated compounds (ligands). More specifically, the amino acids most likely to be involved in these interactions, which are both stable (low binding energy) and effective (very small Ki), are isoleucine 36 (ILE36) and lysine 68 (LYS68) [8] (**Table I**). Of the series of triterpenes isolated from *V. conferta* stem bark, lupeol (3) is thought to be the best ligand for inhibiting the activity of the MRCKα protein involved in the development of skin cancers. It acts by hydrophobic interactions between the aliphatic carbons of cyclopentane (and methyls neighbouring cyclopentane) of nucleus A

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(**Figure 4**), and the aliphatic carbons of isoleucine 36 and lysine 68, amino acids of the MRCKα protein.

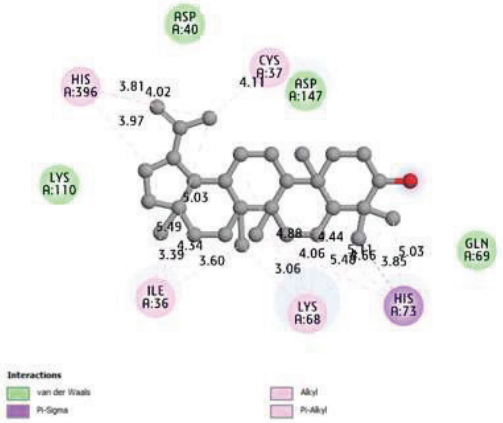

Figure 4: *2D interactions of lupeol with MRCKα*

Interactions of ligands 1-6 and cores A and B with MRCKβ protein

Regarding the interactions between rings A and B with MRCKβ, it would appear that ring A (binding energy = -9.19 kcal.mol^{-1}) is less favourable for the stability of ligand-receptor interactions than ring B (binding energy = -9.38 kcal.mol^{-1}) (Table I). In contrast to the MRCKα protein, it seems that it is the cyclohexane end of the friedelane core (B) that increases the stability of the ligand-receptor interactions compared to the cyclopentane in A. The number of hydrophobic bonds (16 for B; 13 for A) is also strongly implicated in this stability (**Table I**); a stability that is probably diluted by the presence of hydrogen bonds (unfavourable for kinase inhibition) in each ligand-receptor complex formed from the A and B cores with MRCKβ [8]. However, a closer look at the interactions between the isolated compounds (1-6) and the MRCKβ protein reveals a contradiction. Indeed, it appears that, as with the MRCKα protein analysed in the previous paragraph, it is a lupan-type compound (lupeol acetate: 4) that seems to form the most stable complex with the MRCKβ protein (Table I) [13]. This tends to confirm the effectiveness of cyclopentane and neighbouring methyls in providing stable hydrophobic interactions (binding energy 4 = -11.13 kcal.mol^{-1}) and efficiency (Ki 4 = 0.007 µM) rather than cyclohexane as seemed to be shown by the interactions of nuclei A and B with MRCKβ. The amino acids probably involved in all these interactions were leucine 207 (LEU207), isoleucine 82 (ILE82) and valine 90 (VAL90) [8] (Table I). Of the series of triterpenes isolated from the stem bark of *V. conferta*, lupeol acetate (4) is the best ligand for the inhibition of the activity of the MRCKβ protein, which is involved in the development of skin cancer. This ligand (4) acts through hydrophobic interactions between the aliphatic carbons of cyclopentane (and adjacent methyls) of core A (**Figure 5**) and the aliphatic carbons of LEU207, ILE82 and VAL90, three amino acids of the MRCKβ protein.

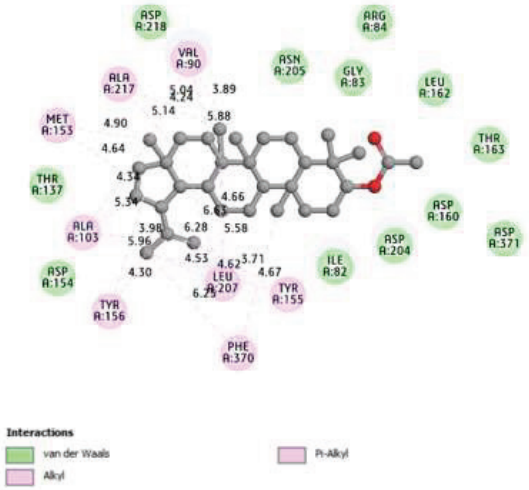

Figure 5: *2D interactions of lupeol acetate with MRCKβ*

Finally, it appears that lupan-type (A) triterpenes would be better ligands for the inhibition of the action of MRCKα and MRCKβ protein kinases, which are involved in the development of certain skin cancers. These observations are essential for identifying potential new plant-based drugs to treat skin cancer. *V. conferta*, like several other species of the *Vernonia* genus, would therefore be an important source of triterpenes in general and lupans in particular [14].

Conclusion

In an attempt to provide new, potentially less toxic therapeutic solutions for the treatment of skin cancer, we investigated the possible interactions between triterpenes isolated from the stem bark of *Vernonia conferta* and MRCKα/MRCKβ, two proteins involved in skin cancer development. It appears that lupan-type (A) triterpenes would be better ligands for inhibiting the action of MRCKα and MRCKβ protein kinases.

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Authors' contributions:

Nko'o M.H.J. and Soppo Lobe C.V. **conceived and designed the study**; Nko'o M.H.J., Foumane Maniepi J.S., Ngo Nyobe J.C. and Ngah D.K. **carried out the experiment, analysed and interpreted the results** with help of Kack Ngavom Saboke; Nko'o M.H.J. **drafted the manuscript** and Soppo Lobe C.V., Foumane Maniepi J.S., Benga Mekoulou F.C., Ndzié Maniben B.P., Obono Fouda Mballa P., Emanda Ekoudi M.G. **revised the manuscript** with help of Nyangono Ndongo M.; Ndom J.C., Nnanga Nga, Mpondo Mpondo E. and Foumane P. **supervised the work at all stages.**

Competing interest: None

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