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NFκβ INHIBITION MECHANISM OF DEOXYELEPHANTOPIN AND ISODEOXYELEPHANTOPIN WITH QSAR AND MOLECULAR DOCKING

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ABSTRACT: Deoxyelephantopin and isodeoxyelephantopin are member of a group of compounds, sesquiterpene lactones, that include proven anticancer agents against many types of cancer cells. Many sesquiterpene lactones suppress cancers by inhibiting NFκβ. This study explores the inhibitory interaction between deoxyelephantopin and isodeoxyelephantopin on NFκB using QSAR and molecular docking. Physicochemical properties and NFκβ (pIC_{50}) inhibition values of a number of sesquiterpene lactone compounds from *in-vitro* studies were used as reference data in constructing the QSAR model equation. This model was used to predict the inhibition potential of deoxyelephantopin and isodeoxyelephantopin on NFκβ. This result was confirmed with molecular docking. The potential NFκβ inhibition of deoxyelephantopin and isodeoxyelephantopin obtained were 59.1037 μM and 62.0321 μM, respectively, with RMSE = 0.07831 R^2 = 0.95465 and Q^2 = 0.67719. Both test compounds showed affinity for the Lys 122 receptor residue (PDB ID: 1NF1) with a docking score of -9.5318 kcal/mol and -8.4429 kcal/mol, respectively.

INTRODUCTION: Cancer is one of the leading causes of death worldwide. Lung, liver, colorectal and breast cancer are the biggest causes of cancer deaths every year [1]. Indonesian plants "tapak liman" known as the scientific name *Elephantopus scaber* Linn. is one of the potential plants developed as anticancer [2]. This plant extract was reported to increase apoptosis and inhibit multi-drug resistance transporters in human epithelial cancer cells [3]. Active metabolites which are widely reported to be anticancer activities are deoxyelephantopin and isodeoxyelephantopin, a compound group of sesquiterpene lactone [4, 5].

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Deoxyelephantopin (DEO) and isodeoxyelephantopin (IDEO) are two isomeric compounds that have potential anticancer agents with effects on multiple signaling pathways [6]. Deoxyelephantopin from *Elephantopus scaber* Linn. inhibits HCT116 human colorectal carcinoma cell growth through apoptosis and cell cycle arrest [7]. Deoxyelephantopin also induces apoptosis in HepG2 cells *via* oxidative stress, Nfkβ inhibition and mitochondrial dysfunction [8].

Likewise the isomeric compound, isodeoxyelephantopin (IDEO) has been reported to have a strong cytotoxic effect on cell line T47D breast cancer and lung carcinoma A549 cells [9], nasopharyngeal carcinoma cell line [10], murine fibroblast cell line, L-929 and Human colon carcinoma cell line, HCT 116. [11] Isodeoxyelephantopin also induces protective autophagy in lung cancer cells [12].

However, DOE and IDEO have been reported to have weak toxicity in normal lymphocytes cells [11]. DEO is known to reduce cell proliferation by increasing the expression of CDK inhibitors p21 and p53, instead of decreasing the expression of cd2-cyclin B so triggering the cell cycle arrest process. DEO also reduces the spread of cancer cells by decreasing the expression of mRNA matrix metalloproteinase (MMP-2 and MMP-9) and increasing the expression of the tissue inhibitor of matrix metalloproteinase (TIMP1 and TIMP2). It triggers the activation of JNK and p38, suppresses the expression of ERK, PI3K, Akt, and mTOR and increases ROS levels, and activates pro-apoptotic proteins, and caspase protein 9, 8, 3 which triggers apoptosis. DEO is also able to activate the β-Catenin pathway to trigger apoptosis by suppressing the expression of Cyclin and Cyclin D.[13] While the molecular mechanism of IDEO is to induce apoptosis through increased expression of caspase-3 and cell cyclus arrest through G2/M phase induction [9].

DEO suppresses NFκβ activation by preventing the localization of NFkβ/P65 and blocking the formation of P65-DNA complexes in the TNFα element binding regions through the 16th hydrogen carbonyl bond with Lys 122 NFkβ receptor residues [14]. This complex interaction is, as yet, poorly understood and the degree of NFκβ inhibition has yet to be quantified. In order to begin to address this gap, the authors have developed a model to predict the quantitative value of DEO and IDEO inhibition activity on NFkβ using QSAR and reference data from *in-vitro* studies of a number of sesquiterpenes lactone compounds that have been carried out by Villagomez *et al.* [15] The results of these predictions were then confirmed by molecular docking methods using MOE software.

MATERIALS AND METHODS:

Data Sets and Biological Activity: The sample used in this study is 10 analogs of NfkB inhibiting sesquiterpene lactone compounds synthesized by Villagomez, *et al.* [15] **Fig. 1** and **Table 1** shows the structure and IC_{50} of the compounds used. From 10 compounds, 5 compounds (sql_1, sql_2, sql_4, sql_10 and sql_15) used as data sets. The Others were removed because they have z-score > 2.5. The biological data obtained as IC_{50} were converted to pIC_{50} ($-\log IC_{50}$) values and used as dependent variables in the QSAR analysis.

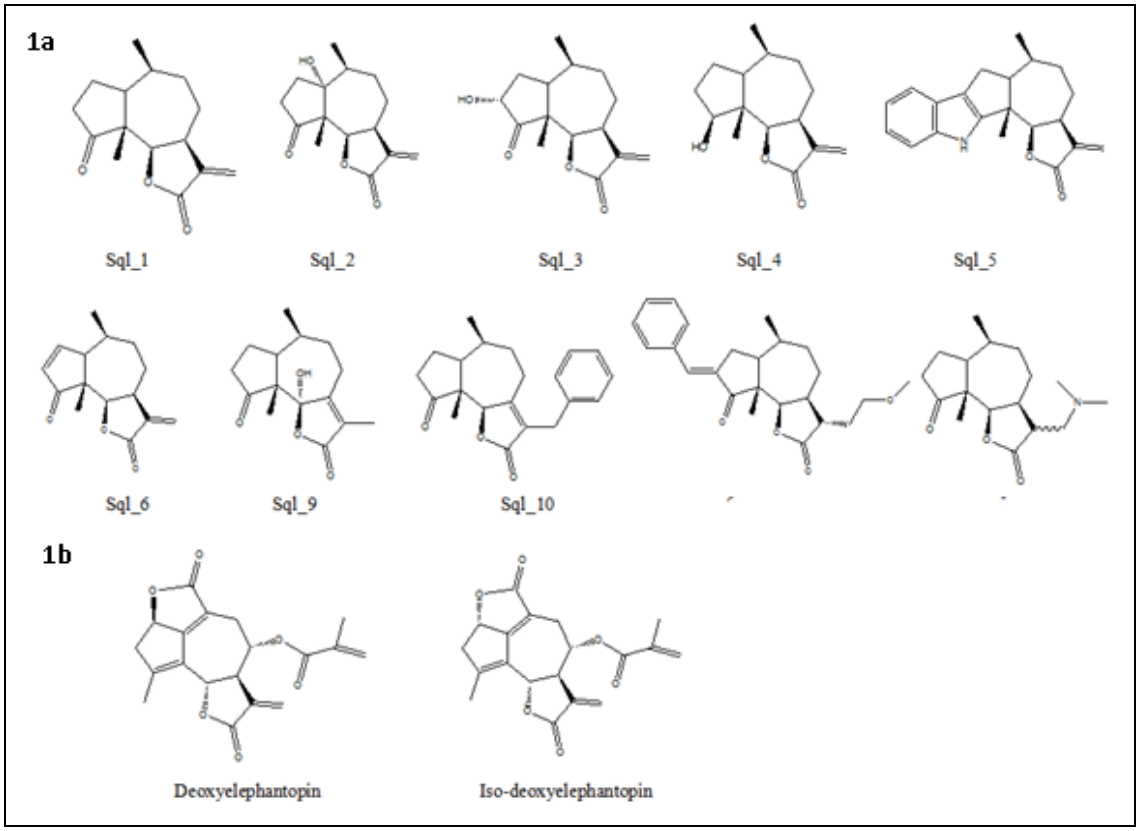


FIG. 1: A. STRUCTURE OF SESQUITERPENE LACTONE COMPOUNDS USED TO PREDICT DEO AND IDEO PROPERTIES [15]. B. STRUCTURE OF DEO AND IDEO USED IN THE DEVELOPED QSAR MODEL

TABLE 1: THE IC_{50} VALUES OF SESQUITERPENE LACTONE COMPOUNDS USED TO PREDICT DEO AND IDEO PROPERTIES

Compound	Chemical name	IC_{50} experimental (µM)	pIC_{50} experimental
Sql_1	Damsin	7.2	-0.8573
Sql_2	Coronofilin	10.1	-1.0043
Sql_3	3-hydroxydamsin	76	-1.8808
Sql_4	4β-hydroxy-4-deoxydamsin	29	-1.4624
Sql_5	Indolo[3,2-c]-4-deoxydamsin	6	-0.77815
Sql_6	Ambrosin	0.5	0.30103
Sql_9	6α-hydroxyisodamsin	36	-1.5563
Sql_10	13-phenylisodamsin	76	-1.8808
Sql_13	(E)-11β,13-dihydro-13-ethoxy-3-(phenylmethylene)-damsin	34	-1.53148
Sql_15	epi-11,13-dihydro-13-(N,N-dimethylamino)damsin	31	-1.49136

Sql: Sesquiterpene lactone

QSAR: The data sets used to build the 2D and 3D structures of the QSAR equation building compound were generated using "Chemoffice 2002" software and saved in *pdb* format, while the structure of the DEO compound and its isomer IDEO were downloaded *via* PubChem in *sdf* format, then converted to a *pdb* format with "Open Babel" software. They were then optimized using the default system of MOE. Descriptor value calculations are also performed using MOE with 13 type descriptors selected which represented electronic, steric and hydrophobic parameters.

The QSAR model was built using the QuaSar-Model protocol available in the MOE software. The QSAR equation model obtained was validated using a leave one out cross validation technique (LOO) with cross-validation coefficient Q^2. According to Tropsha, [16] a QSAR model can be considered good if cross-validation $Q^2 > 0.5$.

Molecular Docking: The interaction between NFkB/P65 (receptor) and the sesquiterpenes of lactone (ligands) was studied using molecular docking using MOE software. The parameters observed were the change in free energy (ΔG) and the number of hydrogen bonds formed between the ligand with the receptor. All ligands and receptor were optimized by adding protons and conducting energy minimization processes. The docking process was performed on the binding site indicated by the default MOE system search results. The docking results were then visualized using LigPlot MOE for 2D structures and PyMOL for 3D structures.

TABLE 2: THE DESCRIPTOR VALUES OF SESQUITERPENE LACTONE COMPOUNDS USED TO PREDICT DEO AND IDEO PROPERTIES

Compound	AM1_Dipole	AM1-E	AM1_EeLe	AM1_HF	AM1_HOMO	AM1_LUMO	LogS
Sql_1	14.1182	-72272.656	-507915.56	112.2097	-13.6208	-7.3118	-1.8007
Sql_2	10.1773	-79677.093	-557923.12	56.7714	-13.4008	-5.8831	-1.5346
Sql_3	5.668	-80200.32	-568714.12	-151.5484	-10.2145	-0.1146	-2.8201
Sql_4	5.5274	-73459.718	-535807.56	-130.131	-10.4824	-0.1078	-3.0104
Sql_5	3.7467	-92061.539	-806451.18	-94.453	-8.1432	-0.0622	-5.2272
Sql_6	6.8789	-72808.906	-516548.87	-109.135	-10.209	-0.0527	-2.9046
Sql_9	6.6572	-80209.781	-575064.31	-161.0084	-10.2738	-0.4013	-2.7082
Sql_10	7.1555	-91788.484	-738879.06	-82.447	-9.549	-0.3675	-4.6768
Sql_13	8.0794	-109418.22	-918384.5	77.4856	-12.2661	-6.4293	-4.7503
Sql_15	17.2631	-85294.46	-673530.06	314.0127	-15.889	-9.6847	-1.2447

Compound	MR	ASA_H	Vdw_vol	Glob	Vol	LogP
Sql_1	6.7698	305.4478	342.812	0.3073	245.125	0.868
Sql_2	6.8746	273.4048	351.3342	0.2697	250.5	1.173
Sql_3	6.9448	276.1798	354.285	0.3008	254.75	1.345
Sql_4	6.8536	313.1144	353.1062	0.2985	253.25	2.617
Sql_5	9.7336	463.5106	499.9287	0.1895	357.25	4.34
Sql_6	6.8129	309.7274	345.7628	0.3028	247.375	2.006
Sql_9	6.9452	323.0038	354.285	0.2494	252.75	1.849
Sql_10	9.3362	441.3547	460.7367	0.2042	327	3.613
Sql_13	10.8496	509.7841	541.7348	0.1316	385.125	3.942
Sql_15	8.1205	356.6096	419.3593	0.1679	300.75	0.452

RESULTS AND DISCUSSION:

Calculation of Descriptor Values, QSAR Equation Analysis, and Validation: Descriptors used included lipophilicity (LogP), solubility (LogS), hydrophobic surface area (ASA_H), highest molecular orbitals filled with electrons (AM1_HOMO), lowest molecular orbitals that are electronless (AM1_LUMO), total energy (AM1_E), electronic energy (AM1_Eele), dipole moment (AM1_dipole), heat formation (AM1_HF), globularity (glob), van der Waals volume (Vol), and molar refractivity (Mr). The calculation results for the 13 compound descriptors used to build the QSAR equation are shown in **Table 2**.

We calculated the z-score of each compound from the initial QSAR model generated equation and if z- score was > 2.5 then that compound was excluded and a new QSAR equation created. Five sesquiterpen lactone compounds with z- score < 2.5 were used to build the QSAR model. The mathematical form of the resulting QSAR equation is shown in the equation and below,

pIC_{50} = − 1.58432＋0.00003 (AM1_dipole) - 0.00008 (AM1_E) + 0.00001 (AM1_Eele) + 0.00089 (AM1_HF) − 0.00001 (AM1_HOMO) − 0.00002(AM1_LUMO) − 0.00003(ASA_H) − 0.00001 (logP)

TABLE 3: THE PREDICTED pIC_{50} VALUES AND THE pIC_{50} EXPERIMENTAL VALUES SHOW VERY STRONG RELATIONSHIPS

Compound	Experimental pIC_{50} value	Predicted pIC_{50} value	Z-SCORE
Sql_1	-0.8573	-0.9814	1.5839
Sql_2	-1.0043	-0.9576	0.5962
Sql_4	-1.4624	-1.3803	1.0485
Sql_10	-1.8808	-1.9395	0.7497
Sql_15	-1.4914	-1.4374	0.6889

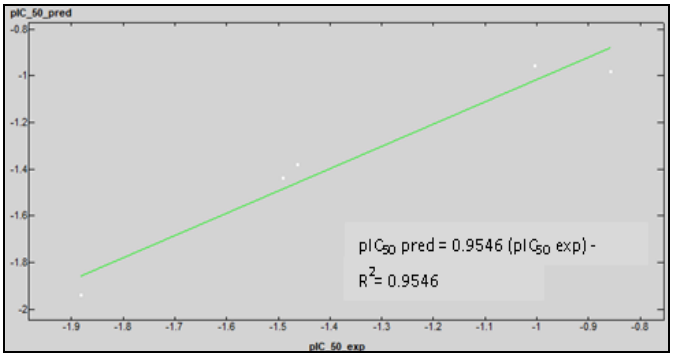


FIG. 2: SHOWS A GRAPH OF THE RELATIONSHIP BETWEEN THE pIC_{50} PREDICTED VALUES AND THE EXPERIMENTAL VALUES

The above QSAR equation model have Root Mean Square Error (RMSE) = 0.07831, regression correlation coefficient (R^2) = 0.95465 with 13 descriptor values that represent hydrophobic, electronic, and steric parameters. Furthermore, the cross-validation statistic (Q^2) is 0.67719 showing the model is capable of acceptably accurate predictions of the NFkB inhibition activity of original sesquiterpene lactone compounds [16].

The descriptors that have the most influence on IC_{50} values are AM1_E and AM1_Eele. The values of AM1_E and AM1_Eele for deoxyelephantopin and isodeoxyelephantopin are from 1.2x to 2x greater than for the sesquiterpene lactone compounds used to build the QSAR model. These higher values are due to DEO and IDEO having larger structures containing 2 lactone rings attached to the core while the compounds used to construct the QSAR equation have only one.

One of the QSAR equation building compounds (sql_10) has a relatively large structure that has an additional substituent of one benzene ring, so the values of AM1_E and AM1_Eele are almost identical to DEO.

TABLE 4: THE PREDICTED INHIBITION RESULTS (IC_{50}) OF NFKB DEOXYELEPHANTOPIN AND ITS ISOMER ISODEOXYELEPHANTOPIN BASED ON THE ABOVE QSAR EQUATION

Compound	AM1_Dipole	AM1-E	AM1_EeLe	AM1_HF	AM1_HOMO	AM1_LUMO	Log S	Mr
DEO	6.0695	-106732.08	-805707.93	-156.5747	-9.9252	-0.6275	-3.1525	8.9517
IDEO	6.513	-106734.28	-810933.06	-159.6153	-10.0422	-0.602	-3.1525	8.9517
Compound	**ASA_H**	**Vdw_vol**	**Glob**	**Vol**	**Log P**	**Predicted pIC_{50}**	**Predicted IC_{50}**	
DEO	311.9521	448.276	0.1347	321.5	1.561	-1.522	59.1037	
IDEO	300.4768	448.276	0.2257	323	1.561	-1.5754	62.0321	

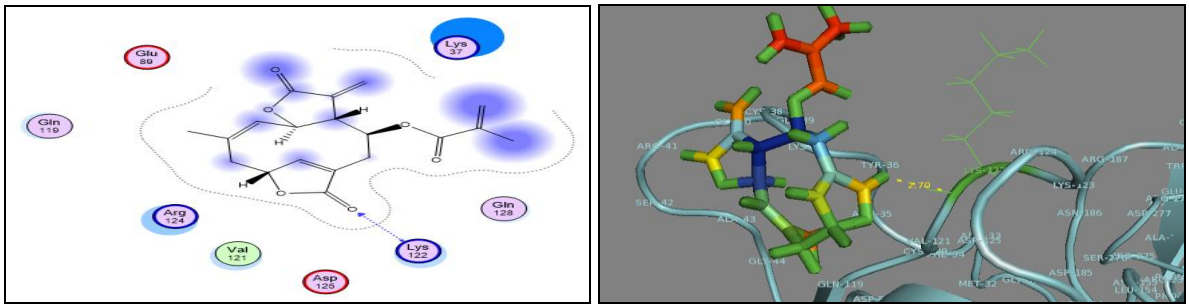
This compound has an experimental pIC_{50} of -1.8808 (IC_{50} 76 µM). In contrast, the sql_1 compound has a simpler structure, having a lower experimental pIC_{50} of -0.8573 (IC_{50} 7.2µM). The larger the structure of the sesquiterpene lactone compound the larger the AM1_E and AM1_Eele values and hence the larger the IC_{50} values and the weaker the potential for inhibition of NFkβ.

Furthermore, descriptors that have large coefficients such as ASA_H, and AM1_HF also affect IC_{50} values. A large ASA_H value causes an increase in the IC_{50} value. Conversely, a large AM1_HF value causes a decrease in IC_{50} value.

Docking Analysis:

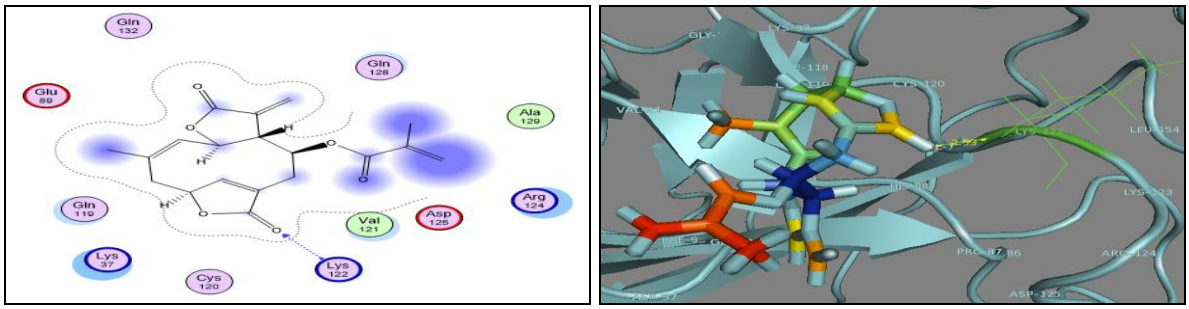
TABLE 5: THE RESULT OF DOCKING ANALYSIS OF ALL LIGANDS

Compound	Docking Scores (S) (kcal/mol)	H-Bound with residue	Bond distance (Å)
Sql_1	-10.1669	Lys 122 (16.8%)	2.86
Sql_2	-10.7651	Gln 128 (69%)	2.53
		Lys 122 (11.4%)	2.87
		Arg 124 (23.2%)	2.97
		Gln 128 (21.9)	2.57
Sql_4	-8.9107	Lys 122 (18.2%)	3.02
Sql_10	-9.0569	Lys 122 (60.6%)	2.77
Sql_15	-9.6482	Lys 122 (15.7%)	2.83
		Arg 124 (15.9%)	2.98
		Gln 128 (15.4%)	2.97
DEO	-9.5318	Lys 122 (67.7%)	2.7
IDEO	-8.4429	Lys 122 (76 %)	2.53



DEO 2D **DEO 3D**

FIG. 3A: THE INTERACTION OF LIGANDS 2D AND 3D "DEOXYELEPHANTOPIN



IDEO 2D **IDEO 3D**

FIG. 3B: 2D AND 3D ISODEOXYELEPHANTOPIN" ON RECEPTOR NFKB

The results of the docking scores for all compounds show they have an affinity for NFkβ/P65 receptors (PDB ID: INF1). All showed a tendency to release energy when forming a complex with NFkβ/P65 receptors, but the strength of each affinity is different. The more negative the value of the docking score obtained the stronger the affinity with the receptor. The sql_1 and sql_2 compounds exhibit the strongest affinity with docking scores of -10.1669 kcal/mol and -10,765 kcal/mol respectively. In contrast, DEO and isomer IDEO have weaker affinity with docking scores of -9.5318 kcal/mol and -8.4429 kcal/mol. However, all compounds used to build the QSAR model formed hydrogen bonds between the 16th carbonyl group and the Lys 122 residual N atom receptor when interacting with the receptors.

This preliminary bond is a pre-requisite for inhibitory activity against NFkβ where it is able to block nuclear translocation of P65 and DNA-binding elements from TNFα.[14] The interaction models for DEO and IDEO are visualized in 2D and 3D in **Fig. 3a** and **3b**.

Both these *in-silico* analysis methods (QSAR and Docking) are similar and support each other. QSAR equation predictions indicate sql_1 and sql_2 should strong inhibitory activity against Nfkβ. This has also been verified experimentally [15]. Likewise, the docking score of these two compounds shows they will have a stronger affinity for the NFkβ/P65 receptor than DEO and IDEO. However, the docking scores of DEO and IDEO also exhibit significant inhibitory activity against NFkβ.

CONCLUSION: QSAR and Molecular Docking methods confirm that DEO and its isomer IDEO have the potential to inhibit NFkβ activity. These results suggest that study is warranted to further investigate the properties of these compounds sourced from *Elephantopus scaber* L. (Asteraceae) and its potential as a natural source for a novel affordable cancer drug that would be accessible in the developing world.

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CONFLICT OF INTEREST: The authors have declared that there is no conflict of interest associated with this publication.

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