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Computational ligand-based molecular analysis of kaurenoic acid and kaurane diterpene derivatives as NF-κB pathway inhibitors

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ABSTRACT

Kaurenoic acid derivatives and Kaurane diterpenes are known to be inhibitors of the NF-kB pathway wherein both compounds share the same tetracyclic structure. Despite sharing close structural homology, Kaurenoic acid derivatives exert their activity through IKK inhibition whereas Kaurane diterpenes inhibit the p50 protein. Thus, a ligand-based similarity analysis was conducted in order to explain the observed target differences between the two sets of compounds through the calculation of chemical descriptors derived using the AM1 Hamiltonian. The calculated molecular descriptors for each set of molecules were then statistically compared in order to determine which among them may explain the observed target difference. Results indicated that size, hydrophobicity, and thermodynamic variables associated with binding were the points of differentiation for the observed target differences between the two sets of compounds.

Keywords: Similarity analysis; semi-empirical calculation; NF-kB inhibition; kaurane diterpenes

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INTRODUCTION

Nature derived compounds are considered to be a valuable resource for pharmaceutical research because they serve as a source for many biologically active compounds which can be used to treat inflammation and cancer, among other illnesses. Ent-kaurenoic acid is a plant derived compound known to effectively modulate inflammation through the inhibition of the NF-κB (Nuclear Factor-κB) pathway [1]. The NF-κB pathway is a transcription factor primarily involved in the regulation of the transcription of various genes which plays an important role in the immune, inflammatory and apoptotic responses [2, 3]. Another plant derived class of molecule known as Kaurane diterpene exhibits the same effect on the NF-κB pathway but, very little information exists on its mechanism [4]. Despite sharing the same tetracyclic framework, both sets of compounds exert their inhibitory activity through different targets. Ent-kaurenoic acid and its derivatives are directed towards the inhibition of IKK [1] while Kaurane diterpenes are directed towards the inhibition of p50 [4]. The study presented herein aims to establish properties which would provide information that would account for the observed difference for target preference. This study also intends to recognize the similarities and differences existing between the two classes of compounds (Figure 1) through the calculation of their corresponding chemical descriptors. Information derived from this study will be useful for lead optimization studies involving these natural products that can increase their potency and selectivity.

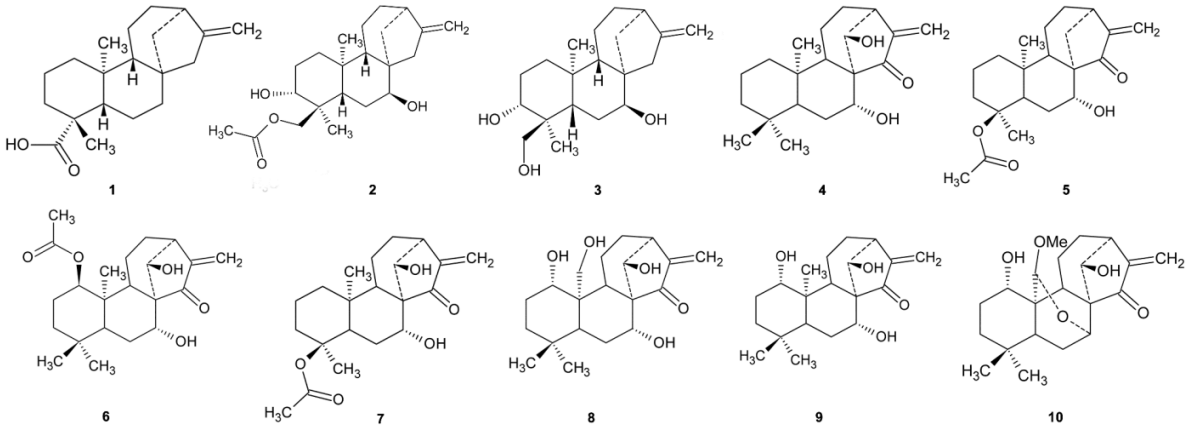

Figure 1. Skeletal structures of Kaurenoic acid derivatives (1-3) and Kaurane diterpenes (4-10).

MATERIALS AND METHODS

Computational calculations

Computational calculations were performed using SPARTAN 08 v.1.2.0 (Wavefun, Inc.) wherein equilibrium conformation was established using MMFF Molecular Mechanics to generate the lowest energy conformer. The geometry of these conformers was optimized using AM1 semi-empirical quantum calculations from which their corresponding energy profiles, alignment scores, electronic and physical properties, and 3-D molecular electrostatic potential (MEP) maps were calculated [5, 6].

Model refinement

The resulting electronic and physical descriptors of the optimized structures were used as parameters for the Levene's Test using Statistica V.9 (StatSoft) with the significance level of 0.05. Levene's test was conducted to establish variance homogeneity within the two sets of compounds that would result to a p-value greater than 0.05. Thus, all chemical descriptors with equal variances were subjected for two-tailed t-test otherwise, they were discarded.

Comparative analysis

Two-tailed t-test was used to establish the points of differentiation of the two sets of compounds based on their molecular properties. The null hypothesis ($\mu_{\text{Kaurenoic acid derivatives}} = \mu_{\text{Kaurane diterpenes}}$) was tested and validated with the significance level of 0.05 and critical value of $t_{\alpha/2} = 2.306$. If the resulting t-value of a chemical descriptor lies within the critical region of $t_{\alpha/2} < t < - t_{\alpha/2,}$ then the null hypothesis would be rejected and this would deduce the point of differentiation between the two sets of compounds. Otherwise, the chemical descriptor would be discarded.

RESULTS AND DISCUSSION

Molecular similarity analysis

After geometry optimization, a quantification of similarity between the two sets of compounds was calculated by aligning the set of Kaurenoic acid derivatives with the set of Kaurane diterpenes. Its main goal is to establish structural similarity among the given compounds thus suggesting that the observed target preference may be attributed to other molecular properties. Possible alignment scores range from 0 to 1, considering 1 as the perfect alignment [7].

The derived data provides a numerical representation for the common structural framework among the given compounds and validates the assumption that perhaps the observed target difference may be in fact due to other molecular descriptors. In addition, this may suggest that the overall structure existing among the molecules meets the necessary requirement needed by the steric complementarities, such as molecular size and overall shape, which is a prerequisite for ligand-receptor recognition [5, 8].

Table 1. Alignment scores of Kaurenoic acid derivatives (1-3) with Kaurane diterpenes (4-10).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	1	1	1	0.93	1	0.92	0.92	1	1	0.91
2	1	1	1	0.93	1	0.94	0.93	1	0.93	0.92
3	1	1	1	0.93	1	0.93	0.93	1	0.93	0.92
4	0.93	0.93	0.93	1	0.93	0.99	1	0.93	1	0.98
5	1	1	1	0.93	1	0.93	0.93	1	0.93	0.92
6	0.92	0.94	0.93	0.99	0.93	1	1	0.94	0.99	0.99
7	0.92	0.93	0.93	1	0.93	1	1	0.94	1	0.98
8	1	1	1	0.93	1	0.94	0.94	1	0.94	0.93
9	0.93	0.93	0.93	1	0.93	0.99	1	0.94	1	0.97
10	0.91	0.92	0.92	0.98	0.92	0.99	0.98	0.93	0.97	1

Table 2. Summary of calculated descriptors of Kaurenoic acid derivatives (1-3) and Kaurane diterpenes (4-10).

	ΔE (kJ/mol)	**CPK Area (Å²)**	**CPK Volume (Å³)**	**Dipole (debye)**	**PSA (Å²)**	**Molecular Wt.(amu)**	**ΔH (kJ/mol)**	**ΔS (J/mol)**	**ΔG (kJ/mol)**
1	-400.75	315.7	329.28	1.62	31.4576052	302.458	884.07	521.54	728.58
2	-811.43	353.59	365.19	2.14	50.3213215	348.483	602.64	636.33	412.92
3	-674.55	313.64	324.67	2.32	54.95735	306.446	629.96	565.39	461.39
4	-586.38	326.4	337.45	2.25	48.0052661	318.457	736.06	584.63	561.75
5	-702.09	346.68	360.1	6.33	53.647846	346.467	650.45	629.57	462.75
6	-912.44	372.84	385.16	2.92	65.3011509	376.493	535.29	677.8	333.2
7	-894.2	357.83	367.82	2.9	64.9426403	362.466	473.93	650.83	279.88
8	-960.77	330.76	350.39	4.42	77.0843964	350.455	395.17	611.69	212.79
9	-758.17	332.24	344.43	2.62	64.3863784	334.456	579.31	605.12	398.89
10	-896.82	346.38	362.55	2.38	56.9961887	362.466	478.71	616.36	294.94

	E HOMO (eV)	**E LUMO (eV)**	**Hardness**	**Electronegativity**	**Chemical Potential**	**Electrophilicity**	**LogP**
1	-9.81	1.34	5.575	4.235	-4.235	1.608540359	5.0446
2	-9.67	0.91	5.29	4.38	-4.38	1.813270321	2.4159
3	-9.61	1.44	5.525	4.085	-4.085	1.510156109	2.1864
4	-10	0.21	5.105	4.895	-4.895	2.346819295	3.731
5	-9.97	0.23	5.1	4.87	-4.87	2.325186275	3.2406
6	-10.04	0.16	5.1	4.94	-4.94	2.392509804	2.8689
7	-10.02	0.19	5.105	4.915	-4.915	2.366035749	2.2178
8	-10.05	0.14	5.095	4.955	-4.955	2.409423454	1.5779
9	-9.95	0.25	5.1	4.85	-4.85	2.306127451	2.6394
10	-10	0.16	5.08	4.92	-4.92	2.382519685	2.5001

Model optimization and validation

A series of statistical tests was conducted in order to reduce and identify which among the molecular properties play a significant role in the observed target differences of Kaurenoic acid derivatives and Kaurane diterpenes despite sharing a common structural framework.

Levene's test

Levene's test was conducted in order to establish the homogeneity of the variances of the two sets of compounds. This is important so that the study can assume that the two sets of compounds being compared came from the same group and reduces any error that may arise from a system exhibiting non-uniform variance. Nevertheless, the following p-values presented in Table 3 were calculated.

By convention, all chemical descriptors with p-values greater than 0.05 were assumed to have equal variances and were subjected to two-tailed t-test. However, chemical descriptors like E HOMO, E LUMO, hardness and electrophilicity, with P-value $\leq$ 0.05, were discarded since it failed to satisfy the necessary requirement for two-tailed t-test which is to possess equal variances.

Two-tailed t-test

For the two tailed t-test, the null hypothesis ($\mu_{\text{Kaurenoic Acid Derivatives}} = \mu_{\text{Kaurane Diterpenes}}$) will be rejected if the resulting t-value lies within the critical region of $t_{\alpha/2} < t < - t_{\alpha/2}$ or $2.306 < t < -2.306$ for the study. This deduced the point of

comparison of the two sets of compounds based on their molecular properties. From Table 4, we can infer that the observed target difference in the binding behavior of the two sets of compounds, Kaurenoic acid derivatives and Kaurane diterpenes, can be attributed to differences in their size (Molecular Weight, CPK Area & CPK Volume), hydrophobicity (PSA), and thermodynamic variables associated with binding (energy, enthalpy, entropy and Gibbs free energy).

Table 3. Chemical descriptors and their corresponding P-values obtained from Levene's test.

Chemical descriptor	P-value
ΔE (kJ/mol)	0.453270
CPK Area (Å²)	0.481209
CPK Volume (Å³)	0.445179
Dipole (debye)	0.126511
PSA (Å²)	0.574784
Molecular Wt.(amu)	0.521689
ΔH (kJ/mol)	0.511112
ΔS (J/mol)	0.252914
ΔG (kJ/mol)	0.438208
E HOMO (eV)	0.039041
E LUMO (eV)	0.001464
Hardness	0.000514
Electronegativity	0.057655
LogP	0.054808
Chemical Potential	0.057655
Electrophilicity	0.013572

Table 4. Chemical descriptors and their corresponding t-values from Two-tailed t-test.

Chemical descriptor	t – value
ΔE (kJ/mol)	16.1832824
CPK Area (Å²)	-4.470888837
CPK Volume (Å³)	-4.899265187
Dipole (debye)	-2.178244549
PSA (Å²)	-5.578193545
Molecular Wt.(amu)	-7.611580164
ΔH (kJ/mol)	15.49494623
ΔS (J/mol)	-8.450245178
ΔG (kJ/mol)	16.31814174
Electronegativity	-2.293179232
logP	0.542321086
Chemical Potential	2.293179232

Points of Differentiation

The following molecular properties can possibly explain the observed target differences of the two sets of compounds being compared despite sharing a high degree of structural homology.

Size

In contrast to the set of Kaurane diterpenes, the set of Kaurenoic acid derivatives is relatively smaller and occupies lesser space. The large discrepancy in size of the two sets of compounds, represented in Figure 2, can be considered as a point of differentiation since steric complementarity is a requirement for ligand-receptor recognition [5, 6]. It means that a ligand can only bind with the receptor if it holds the molecule that contains a specific combination of size and shape. Therefore, only molecules with sterically similar binding surfaces can interact with the same receptor binding site [5, 6, 8].

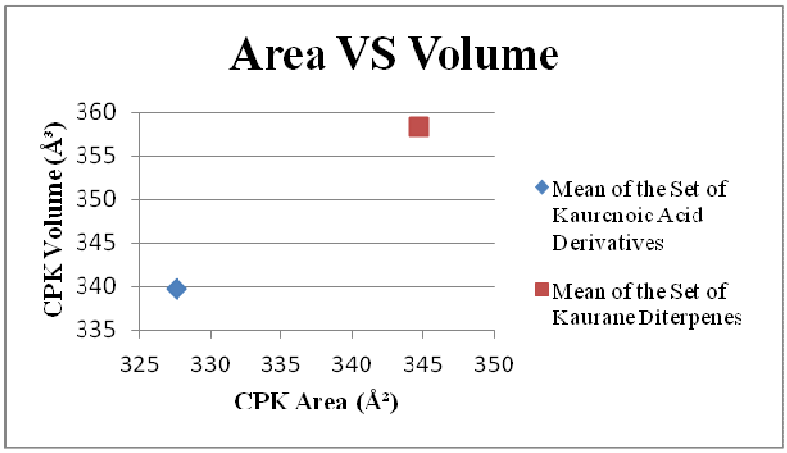

Figure 2. Two-descriptor model between the area and volume of Kaurenoic acid derivatives with Kaurane diterpenes.

In view to this, the discrepancy in size of the two sets of compounds might explain their observed target differences. Kaurane diterpenes cannot inhibit IKK since the compound is too big to fit in the active site of the enzyme. On the other hand, the adducts (ligand) formed by covalent bond between the Kaurenoic acid derivatives and the nucleophilic cysteine residues is not sufficient enough to fit the cavity (receptor) that it hinders the potency of the molecule to undergo nucleophilic attack [5].

Hydrophobicity

The significant difference in hydrophobicity between the two sets of compounds can also be noted as a point of differentiation because hydrophobic complementarity and interaction can influence the ligand receptor binding [5, 6, 8]. Hydrophobicity is a physical property of a molecule that allows itself to repel or not mixed with water. This molecular property also serves as the driving force that urges the ligand to leave the water in order for it to bind and interact with the receptor. Polar and non-polar regions of the ligand and receptor are preferred to be juxtaposed in such a way to prevent contact with water and to minimize the dehydration free energies for stability. In accordance to this, the set of kaurenoic acid derivatives and the set of kaurane diterpenes do not follow the same conformation since they have to match the hydrophobic region of their preferred target to achieve hydrophobic complementarity and stability in enzyme-substrate or ligand-receptor complexes [6, 8].

Thermodynamic variables associated with binding

The binding affinity of a molecule is related to the strength of the association of the ligand being bound together with the receptor as well as its conformation. It is greatly affected by the free energy (ΔG) changes for binding which may occur with all possible net favorable combinations of enthalpic (ΔH) and entropic (ΔS) changes. However, for a series of related compounds with only slight variations in their structures, the entropy and enthalpy of binding do not vary independently, and introducing a slight structural variation in a compound may hardly change the Gibbs energy of binding, but the enthalpy and entropy may vary considerably. The prediction of binding affinity is greatly complicated by the sheer number of enthalpic and entropic effects that contribute to the observed free energy of binding [9, 10, 11]. Thus, the significant difference in the binding affinity of the two sets of compounds deduces that they follow different binding orientation to form a stable complex.

Molecular electrostatic potential (MEP) maps

MEP maps were used to visualize the overall charge of the compounds. It was also very helpful in predicting the compound's interaction with another compound [6, 7].

Potential Density Maps

Calculated Potential density maps, presented in Figure 3, showed multiple sites of red region found in the two sets of compounds being compared. Sites of red regions represent the area where electron density is concentrated [5, 7]. This describes the affinity of certain molecules towards its target since molecules react with another system through electrostatic potential. In relation, electrostatic interaction or complementarity is needed to allow maximum

interaction with the receptor. This can only happen when the charge distribution of the substrate is aligned with its corresponding counterpart at the binding site [5, 6].

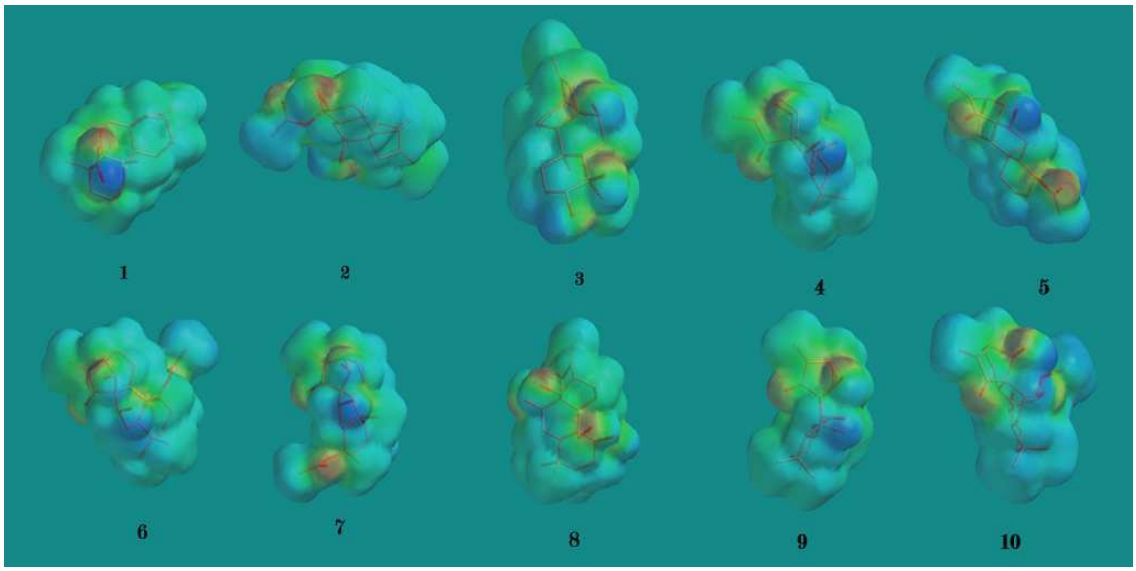

Figure 3. Potential density maps of Kaurenoic acid derivatives (1-3) and Kaurane diterpenes (4-10).

LUMO density maps

Nucleophilic addition of the sulfhydryl group of cysteine at the carbonyl carbon is the mechanism of inhibition of NF-kB [5]. However, the location of the carbonyl functionality of the set of Kaurenoic acid derivatives is different for the set of Kaurane diterpenes which can be seen in the LUMO density maps in Figure 4. The blue region in the LUMO density maps represents the area that is most susceptible to nucleophilic attack since it contains the minimum electron density [7]. Thus, this can also explain the differences in conformation exhibited by the two sets of compounds in order to match its corresponding target region.

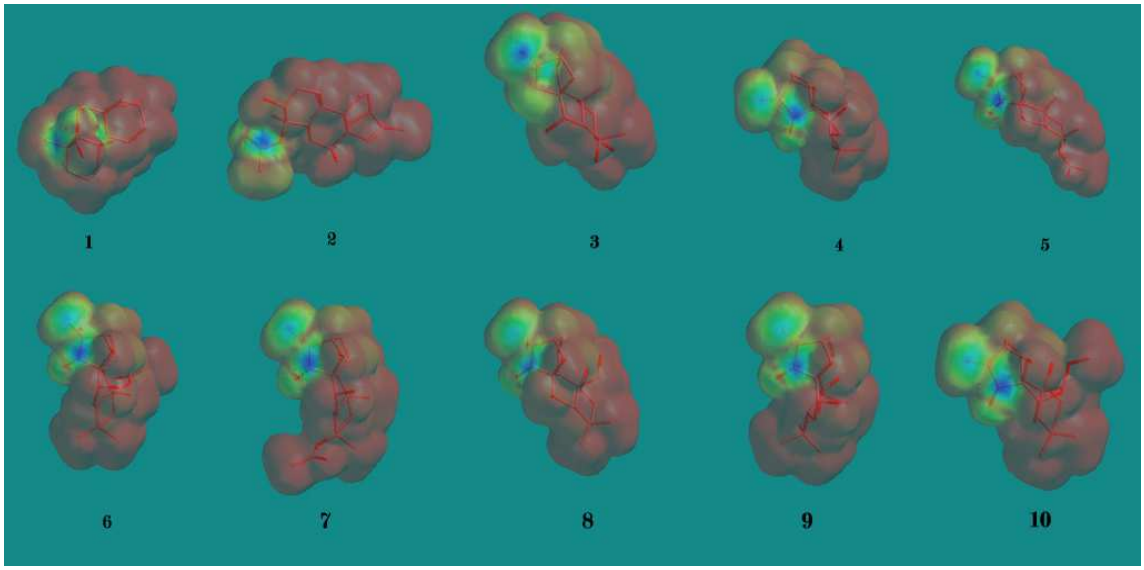

Figure 4. LUMO density maps of Kaurenoic acid derivatives (1-3) and Kaurane diterpenes (4-10).

CONCLUSION

The molecular similarity analysis conducted between the two sets of compounds, Kaurenoic acid derivatives and Kaurane diterpenes, unravels important molecular-level information which provides a better understanding of the

observed target differences despite sharing a common tetracyclic structure. Results showed that the two sets of compounds differ in size (CPK area, CPK volume, and molecular weight), hydrophobicity (PSA), and thermodynamic variables associated with binding (ΔE, ΔH, ΔS, and ΔG) which were common prerequisites for ligand-receptor recognition.

Size can affect the ligand-receptor binding because of steric complementarity. Wherein, only molecules with sterically similar binding surfaces can interact with the same receptor binding site. Thus, the large discrepancy in size explains the inability of Kaurane diterpenes to inhibit IKK since it is too big to fit into the active site of the enzyme while Kaurenoic acid derivatives is too small for the receptor of p50.

Second, hydrophobicity is also a factor since the polar and non-polar regions of the ligand and receptor were preferred to be juxtaposed in such a way to prevent contact with water and to minimize the dehydration free energies for the stability of the molecule. This deduces that the two sets of compounds follow different binding orientation to achieve hydrophobic complementarity in the ligand-receptor complex.

Lastly, thermodynamic variables associated with binding suggest that the two sets of compounds follow different binding conformation (related to the preferred mechanism of the molecule) whenever they interact with their corresponding enzyme to attain the most stable binding pose.

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