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Molecular Design of Potential Anti-Tumor Drug Candidates via NF-κB Pathway Gene Inhibition

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ABSTRACT

Advancements in technology have provided many avenues for creating new compounds and improving existing ones. It has also allowed researchers to create drug lead molecules according to their preferences. Through this, trial and error method in drug design has been reduced. Furthermore, drug design is now less time consuming and cost-efficient with the use of computer aided drug design (CADD). In this study, CADD is used to create IKK and p50 inhibitor candidates deemed to possess better specificity and potency in targeting the NF-κB gene. The candidate inhibitors were designed utilizing ligand based drug design techniques in compliance with established confidence levels of their relevant chemical descriptors. The theoretical candidate inhibitors that were designed showed a high degree of possibility to be an anti-inflammatory drug and consequently, as anti-tumor agents.

Keywords: Kaurenoic acid derivatives, NF-kB inhibition, ligand-based drug design

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INTRODUCTION

Computer-aided drug design (CADD) allows the design of potential drug molecules to be less time consuming and cost-efficient since it lessens the trial and error in actual laboratory experiments [1]. In addition, CADD also allows researchers to create theoretical compounds according to their specifications from which after computation gives a conclusive result on whether the desired compound is logically and physically plausible or not. Furthermore, CADD presents itself as a promising tool since it allows computational chemists to highlight important properties of a drug lead molecule that can be used as the basis of creating drug candidates bearing enhanced affinity, higher degree of selectivity and better potency [1].

In a recent study, kaurenoic acid derivatives and kaurane diterpenes, known inhibitors of the IKK and p50 protein respectively, were subjected to a ligand-based molecular comparison [2,3]. Using AM1 Hamiltonian, their chemical descriptors were calculated from which the ideal physicochemical properties of being effective NF-κB gene inhibitors were established. Results indicated that size, hydrophobicity, and thermodyanamic variables associated

with binding were the significant molecular properties that affect the inhibition of the target protein [4]. The derived data are logical because these properties are the common prerequisite of the ligand-receptor recognition. As thus, this paper intends to utilize the CADD-derived data [4] to create and propose possible IKK and p50 inhibitor compounds which would serve as drug candidates with better potency and efficacy in targeting and inhibiting the NF-κB gene.

MATERIALS AND METHODS

Construction of confidence interval

The confidence interval of each significant chemical descriptor was calculated using all the data from a reported study [4]. The establishment of a confidence interval will serve as the parameter that will determine whether the proposed IKK and p50 inhibitor candidates are accepted or rejected. The study took consideration on the population used for the confidence interval, thus increasing the reliability and relevance of the data derived from the computations. All calculations were using the significance level of 0.05 and the formula [5]:

$$\bar{x} - t_{\alpha/2}\frac{s}{\sqrt{n}} < \mu < \bar{x} + t_{\alpha/2}\frac{s}{\sqrt{n}}$$

Equation 1. Confidence Interval.

wherein, $\bar{x}$ = mean of sample
s = standard deviation of sample
n = number of subjects in sample
$t_{\alpha/2}$ = t value with v = n-1 degrees of freedom

Calculation and construction of drug candidates

The construction of IKK and p50 inhibitor candidates and the calculation of their molecular properties were all done using SPARTAN 08 v.1.2.0 (Wavefun, Inc.). Wherein, the lowest energy conformer of the candidates was identified using MMFF Molecular Mechanics to establish equilibrium geometry. The generated lowest energy conformer was then optimized by employing AM1 semi-empirical calculations from which their corresponding molecular and energy profile were calculated.

RESULTS AND DISCUSSION

Confidence interval

The confidence interval of each significant chemical descriptor for the two sets of compounds was computed using the significance level of 0.05 to maintain the homogeneity from previously reported statistical calculations [4]. Homogeneity is a prerequisite for the study since it would serve as a bearing to ensure that the molecules being compared came from the same pool. Thus, the computed data of the chemical descriptors, found in Table 1, were used as a parameter in designing a suitable IKK and p50 inhibitor candidate.

Table 1. Confidence interval for IKK and p50 inhibitor candidates

Chemical Descriptor	Confidence Interval			
	IKK inhibitor		p50 inhibitor	
	Lower Limit	Upper Limit	Lower Limit	Upper Limit
ΔE (kJ/mol)	-928.842691	-328.977309	-863.588693	-768.088450
CPK Area (Å²)	295.379344	359.907323	338.926293	350.539422
CPK Volume (Å³)	307.894775	371.531891	352.701906	363.840952
PSA (Å²)	27.725576	63.431941	58.159468	64.801637
Molecular Wt.(amu)	282.554371	355.703629	343.392970	356.967030
ΔH (kJ/mol)	482.948996	928.164337	509.323299	590.368130
ΔS (J/mol)	491.335795	657.504205	614.318810	635.966904
ΔG (kJ/mol)	290.473308	778.120025	321.671430	405.242856

Drug design process

The 3D structures of IKK and p50 proteins are not known thus, the drug design procedure utilized known ligand structures that have been identified to exert on the activity of interest, a technique known as "ligand based drug design" [1]. The process takes into consideration the overall shape, electrostatic, and interaction points of the 2D or 3D structure of the given ligand when designing similar drug candidates [1]. Hence, the design process used all the information derived from the molecular comparison conducted between kaurenoic acid derivatives and kaurane diterpenes [4].

Molecular Structure

The molecular design process started by creating a compound that will have a high degree of activity towards the target [1]. The study utilized the structures of kaurenoic acid derivatives and kaurane diterpenes since they have already been established to inhibit IKK and p50 proteins respectively [2-3]. Thus, their common tetracyclic backbones (Figure 1) were used and modified through the addition of functional groups which are supposed to enhance bioavailability and activity without sacrificing toxicity [1].

IKK tetracyclic backbone **p50 tetracyclic backbone**

Figure 1. Tetracyclic backbones of IKK and p50 inhibitor

Size

The target of the IKK and p50 inhibitor candidates is a protein [2-3] so there is a high probability that they will act through a competitive inhibition [1]. Wherein, the drug candidate competes with the native substrate in gaining full access of the target's active site [7]. But for a drug to fit in and interact with the active site, it must have the precise size and shape; thus, imposing the idea of lock and key theory of drug interaction (Figure 2) [1,4,7]. Hence, IKK inhibitor candidates were designed to be smaller in size than p50 inhibitor candidates for steric complementarity purposes and to comply with the calculated parameters [4].

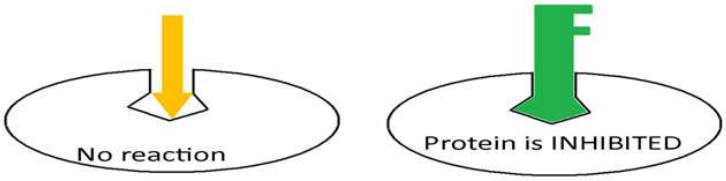


Figure 2. Lock and key theory of drug interaction

Binding activity

The binding activity of the IKK and p50 inhibitor candidates against their target's active site depends on the positioning of the functional groups attached to their tetracyclic backbone [1]. For example, the positively-charged group/part of the drug candidate must be aligned to its corresponding counterpart for an interaction to happen; thus imposing the idea of electrostatic complementarity (Figure 3). However, since the crystal structure of the target is unknown, the functional groups added to the tetracyclic backbone of the drug candidates depended on the data from the reported previous study as well as on the constructed parameters therein [4].

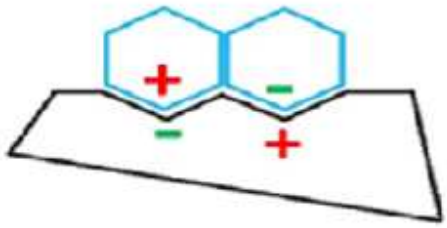
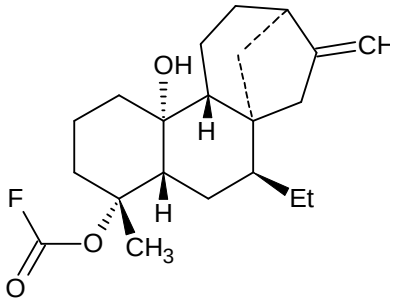
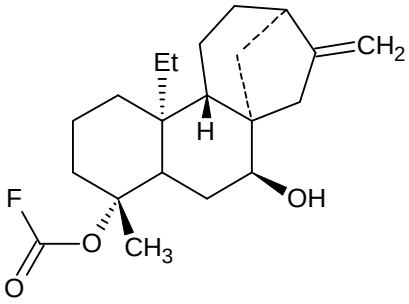


Figure 3. Electrostatic complementarity model

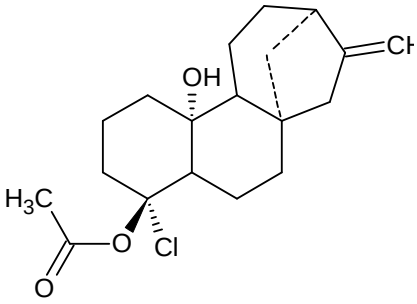
Moreover, electronegative groups attached to carbonyl groups were added to the tetracyclic backbone of IKK and p50 inhibitor candidates to enhance susceptibility to undergo nucleophilic attack to their respective receptor since nucleophilic addition of the sulfhydryl group of cysteine at the carbonyl carbon is the primary mode of NF-kB inhibition [4,6]. Non-polar groups were also added since the drug candidates were designed to be hydrophobic [2,4] to be able to enter the bloodstream through passive intestinal absorption [1]; as well as to achieve hydrophobic complementarity for the system to follow desired binding orientation [4,6]. Hence, the constructed candidate drug compounds are illustrated in Figure 4.



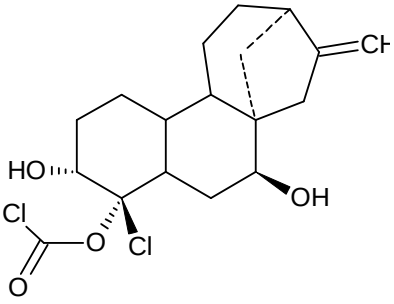
1-IKK



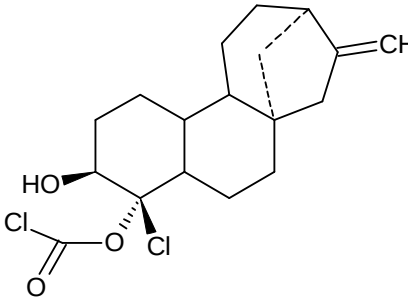
2-IKK



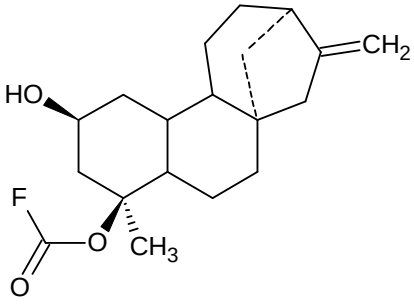
3-IKK



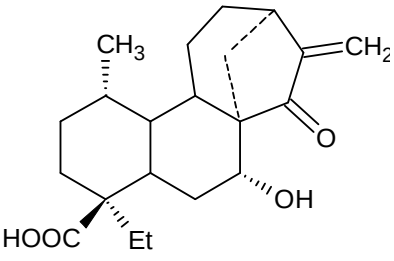
4-IKK

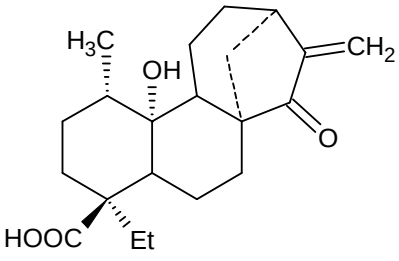


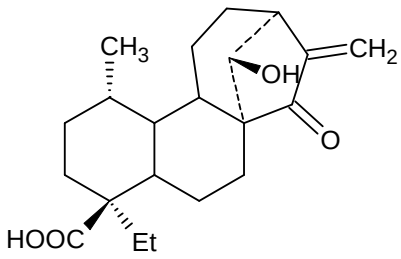
5-IKK



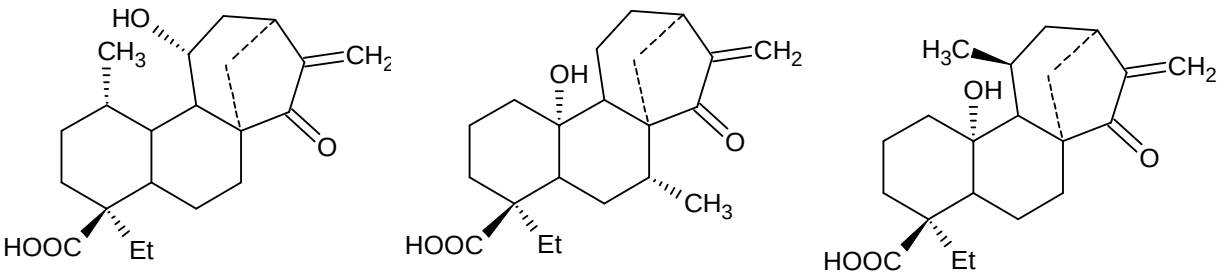
6-IKK







1-p50 **2-p50** **3-p50**



4-p50 **5-p50** **6-p50**

Figure 4. IKK and p50 Inhibitor Candidates.

Parameter testing

After designing the IKK and p50 inhibitor candidates, their chemical descriptors were calculated to make sure that it lies within the accepted range or else it will be rejected. Results are summarized in Table 2.

Table 2. Calculated chemical descriptors for IKK and p50 inhibitor candidates.

Inhibitor Candidate	ΔE (kJ/ mol)	CPK Area (Å^2)	CPK Volume (Å^3)	PSA (Å^2)	Mol. Weight (amu)	ΔH (kJ/ mol)	ΔS (J/ mol)	ΔG (kJ/ mol)
1-IKK	-886.22	357.3	363.57	30.38	363.57	496.44	634.2	307.35
2-IKK	-638.79	331.4	351.62	32.61	351.62	732.07	597.6	553.90
3-IKK	-617.08	333.6	337.65	34.46	337.65	601.75	593.8	424.70
4-IKK	-632.66	327.0	330.76	51.65	330.76	488.18	602.8	308.44
5-IKK	-458.82	319.1	323.34	33.05	323.34	646.96	581.9	473.46
6-IKK	-690.55	317.2	318.76	34.67	318.76	520.81	573.9	349.69
1-p50	-775.50	350.5	358.30	63.11	346.467	581.43	630.7	393.37
2-p50	-768.60	344.2	357.60	58.86	346.467	586.61	618.7	402.14
3-p50	-794.50	349.4	358.10	64.07	346.467	561.88	627.3	374.83
4-p50	-773.00	350.3	358.60	61.61	346.467	584.53	625.6	398.00
5-p50	-784.10	344.2	357.90	57.44	346.467	570.7	620.9	385.57
6-p50	-780.50	344.7	357.90	57.89	346.467	574.86	620.9	389.73

Based on the derived data, the designed IKK and p50 inhibitor candidates were ideal because their calculated molecular properties are within the parameters of the calculated confidence interval for each inhibitor.

Furthermore, the drug candidates displayed ideal Polar Surface Area (PSA) properties/data. PSA is considered to be a major requirement for bioavailability since it determines the degree of cellular permeability and intestinal absorptivity of a molecule. It has been long established that a completely absorbed drug would have a PSA of ≤ 60 (Å^2). Data revealed all candidates exhibit PSA values of ≤ 60 (Å^2) thus signifying that these potential compounds would be completely absorbed by the membrane [8]. In addition, the candidates also showed its potential in penetrating the blood brain barrier which is a requirement for targeting the central nervous system (CNS). The criterion for drugs penetrating the brain would be a molecular weight of < 450 and a PSA < 90 (Å^2), all of which are met by the candidates [9], thus making the designed drug candidates to be theoretically effective inhibitors of the NF-kB pathway and therefore can be used as anti-inflammatory agents, and consequently anti-tumor agents.

CONCLUSION

Using CADD, twelve (12) IKK and p50 inhibitor candidates were designed based on prescribed computational data parameter requirements. The inhibitor candidates were constructed utilizing ligand-based drug design technique in compliance with established confidence interval. When synthesized, these individual drug candidates are anticipated

to exert high degrees of specificity and potency in targeting the NF-κB pathway, hence, becoming potential anti-tumor agents.

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