Adenosine Kinase Inhibitor Design Based on Pharmacophore Modeling

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Adenosine kinase (AK) is a ubiquitous intracellular enzyme, which catalyzes the phosphorylation of adenosine (ADO) to adenosine monophosphate (AMP). AK inhibitors have therapeutic potential as analgesic and anti-inflammatory agents. A chemical feature based pharmacophore model has been generated from known AK inhibitors (26 training set compounds) by *HypoGen* module implemented in *CATALYST* software. The top ranked hypothesis (Hypo1) contained four features of two hydrogen-bond acceptors (HBA) and two hydrophobic aromatics (Z). Hypo1 was validated by 124 test set molecules with a correlation coefficient of 0.905 between experimental and estimated activity. It was also validated by *CatScramble* method. Thus, the Hypo1 was exploited for searching new lead compounds over 238,819 chemical compounds in NCI database and then the selected compounds were screened based on restriction estimated activity and Lipinski's rules to evaluate their drug-like properties. Finally we could obtain 72 new lead candidates and the two best compound structures from them were posted.

Key Words : Adenosine (ADO), Adenosine kinase (AK) inhibitors, Pharmacophore hypotheses, New lead search, Computer-aided drug design

Introduction

Adenosine (ADO) is an extracellular signaling agent within the central and peripheral nervous system.[1,2] It is a purine nucleoside released from the cells or formed extracellularly, and it diffuses to the cell membrane of surrounding cells and binds to adenosine receptors.[2,3,4] During cellular stress, local intra- and extracellular concentrations of adenosine markedly increase, which is followed by the active transport of ADO out of the cell and subsequent activation of adenosine receptor subtypes.[2,5] Activation of ADO receptors produces a variety of homeostatic inhibitory cellular events that contribute to anti-nociceptive and anti-inflammatory actions *in vivo*.[6] Strong evidence suggests that this protective pathway is involved in pathological processes including neurodegeneration, seizures, ischemia, inflammation and pain.[7]

Adenosine kinase (AK) is a ubiquitous intracellular enzyme, which catalyzes the phosphorylation of adenosine to adenosine monophosphate, and therefore is a key enzyme in the control of cellular concentrations of ADO.[8] It rapidly phosphorylates ADO, maintaining intracellular ADO concentrations at low levels. Since ADO uptake is driven by its concentration gradient, AK inhibition reduces the cellular uptake of ADO,[9] thus potentiating the local concentration of ADO in the extracellular compartment as well as increasing the local concentration of ADO in the intracellular site. AK inhibitors, therefore, have therapeutic potential as analgesic and anti-inflammatory agents.

Until recently all of the reported AK inhibitors contained adenosine-like structural motif which resemble the natural substrate ADO. Nucleoside analogues in general are highly polar and rapidly metabolized. Our interest has been to discover non-nucleoside AK inhibitors free of mentioned side affects. The goal of this study is to construct a pharmacophore model based on common chemical features of existing AK inhibitors by using the *HypoGen* module implemented in *CATALYST* software.[10] The pharmacophore modeling is a very effective method that allows scientists to gain valuable information of how ligands bind to the protein active site.[11] It is expected to provide useful knowledge for developing new potentially active candidates targeting the AK. Hence, the best pharmacophore model was selected along with established protocols carefully and then it was validated by two methods. New compounds with similar features were retrieved from chemical database and they were screened based on their estimated activity and calculated drug-like properties.

Methods

Training Set Selection and Conformational Search. The most important process in pharmacophore model generation is the selection of training set compounds. Over the last few years, a number of AK inhibitors have been identified, and thus we have collected a set of 381 molecules whose AK inhibitory activity data were taken from the literature and generated a database by using *MDL ISIS/Base*.[12] Among these molecules, whose activities span a range of 5 orders of magnitude, training set selection was based on the fact that each order of magnitude is represented by at least three compounds, including the most active and inactive ones. It is extremely important to include the most active compounds as they contribute more to form the chemical feature based model. Conformations for all training set molecules were generated by an energy constraint of 20 kcal/mol, using *Best Conformational Analysis* method and

Table 1. Molecular structures of the 26 training set compounds

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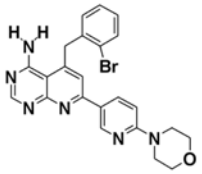
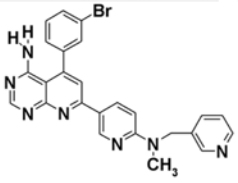
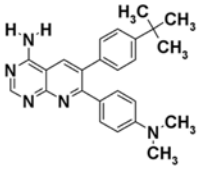
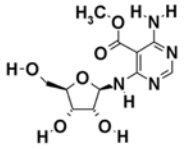
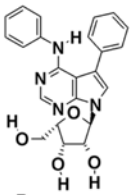
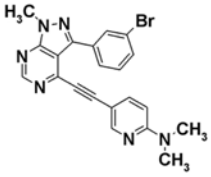
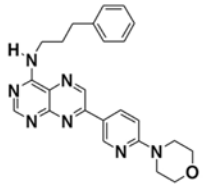
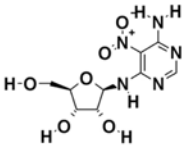
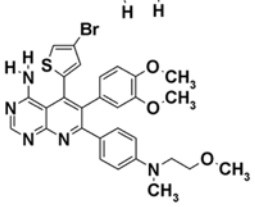
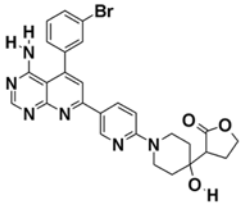
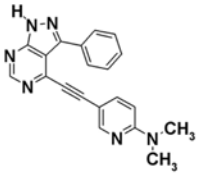
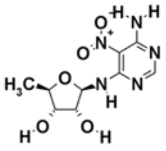
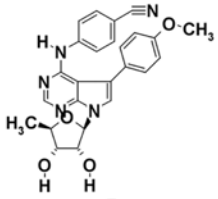
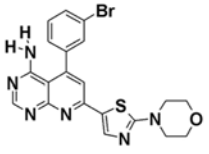
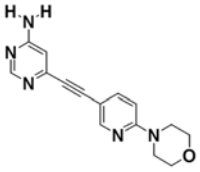
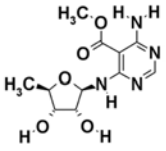
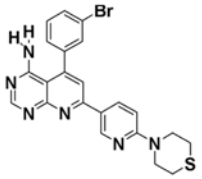
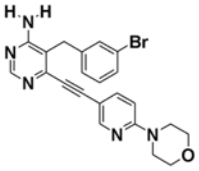
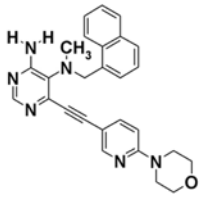
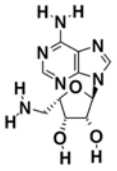
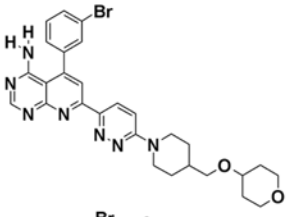
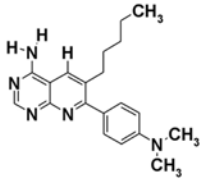
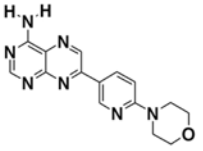
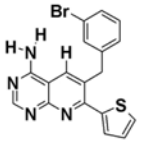
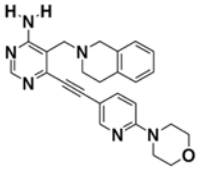
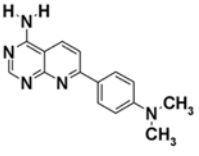
1		8		15		22	
2		9		16		23	
3		10		17		24	
4		11		18		25	
5		12		19		26	
6		13		20			
7		14		21			

Poling Algorithm[13] in *CATALYST*. A maximum of 250 conformations of each molecule were generated to ensure maximum coverage of the conformational space. The training set comprising of 26 compounds representing structural diversity and wide coverage of activity range (IC_{50} ranging from 0.17 nM to 10000 nM) was used to generate pharmacophore hypotheses (Table 1).[14-25] It comprises five scaffolds including nucleoside and non-nucleoside type AK inhibitors.

Generation of Pharmacophore Hypotheses with *HypoGen*. All training set compounds were structurally diverse and possessed certain common comparable inhibitory potencies, and chemical features. On the basis of the structural information from these known AK inhibitors, a set of features crucial for activity were considered to represent a pharmacophore hypotheses. The *HypoGen* module in *CATALYST* was used to generate pharmacophore hypotheses wherein it evaluates a collection of conformational models of molecules, and maps them to the selected chemical features (pharmacophore). The top ranked pharmacophore is expected to identify the common binding features and the hypothetical orientation of the active compounds interacting with the target enzyme, protein, or receptor.

Validation of Pharmacophore Hypothesis. Validation of pharmacophore hypothesis was done by two procedures[26]:

test set method and *CatScramble* method. The test set comprising of 124 compounds was collected from in-house database and conformers were generated in a similar way as that of training set compounds. Compounds which had only similar assay were included in the test set and their activities were estimated using the best ranked pharmacophore. The statistical validation based on Fischer's randomization test was also performed using the *CatScramble* program.[27] The goal of this type of validation is to check whether there is a strong correlation between the chemical structures and the biological activity and to generate pharmacophore hypotheses using a random reassignment of activity values among the molecules of the training set. In this statistical validation test, we selected 95% confidence level, and thus 19 spreadsheets were generated.

Database Screening. All the compounds with novel chemical structure and desired chemical features from NCI database consisting of 238,819 compounds were screened by the Hypo1 hypothesis. Database search was performed by the *Best Flexible Search Databases of Spread Sheets* method.[28]

Drug-like Property Calculation. Lipinski's rule-of-five is a simple model to forecast the absorption and intestinal permeability of a compound.[29] According to the rule, compounds are considered likely to be well-absorbed when they possess less than 5 of LogP, less than 500 of molecular weight, less than 5 of number of H-bond donors, less than 10 of number H-bond acceptors, and finally less than 10 of number of rotatable bonds. All these properties were calculated using *Molinspiration* online database.[30]

Results and Discussion

Pharmacophore Hypothesis Generation. During a *HypoGen* run, *CATALYST* distinguishes between alternatives of thousands of models by applying cost analysis and by searching for the simplest set of chemical functions that correlate best with the observed activity.[31] At the end of the run, it produces a set of 10 hypotheses using the data from the 26 training set compounds. Hypo1 is the most significant pharmacophore hypothesis in this study, characterized by the highest cost difference (73.824), lowest root-mean-square deviation (RMSD) (0.802), and the best correlation coefficient (0.957). The fixed cost represents the simple model that fits all data perfectly while the null cost presumes that there is no relationship in the dataset. The fixed cost and null cost are 102.47, 185.614 respectively. The total cost describes each of the pharmacophore hypothesis with a value of 111.79 for Hypo1 which is much below the null cost and closer to the fixed cost.

A meaningful pharmacophore hypothesis may result when the difference between null cost and fixed cost is large. A value greater than 60 bits for a pharmacophore hypothesis is an excellent chance the model represents a true correlation and a value of 40-60 bits may suggest that it has 75-90% probability of correlating the data. The cost values, correlation coefficients (r), RMSD, and pharmacophore features are listed in Table 2. From Table 2 we can see that all 10 hypotheses have common features of two hydrogen-bond acceptors (HBA) and two hydrophobic aromatics (Z) with the exception of only last hypothesis. The last hypothesis has different features with two HBAs, one Z and one ring aromatic (RA). The Hypo1 contains four features of two HBAs and two Zs. Two-dimensional (2D) distances between all features in Hypo1 are shown in Figure 1.

Figure 2 shows that the Hypo1 aligned with the most

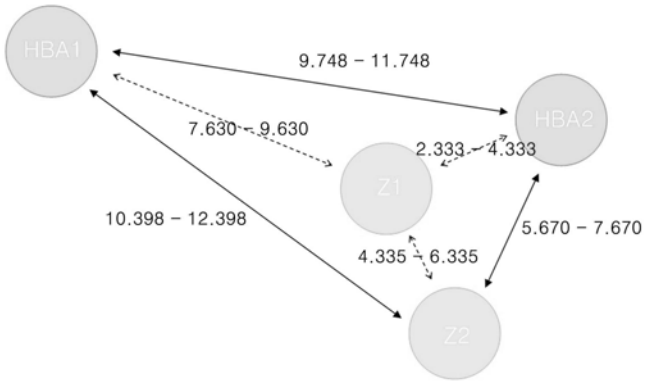


Figure 1. Two-dimensional representation of the top ranked hypothesis (Hypo1). All distances are in Å unit.

Table 2. Information of statistical significance and predictive power presented in cost values for top 10 hypotheses

Hypo No.	Features[a]	Training set				Test set correlation (r)
		Total cost	ΔCost	RMSD	Correlation(r)	
1	AAZZ	111.790	73.824	0.802	0.957	0.905
2	AAZZ	113.998	71.616	0.941	0.939	0.824
3	AAZZ	121.936	63.678	1.203	0.899	0.770
4	AAZZ	122.843	62.771	1.250	0.891	0.674
5	AAZZ	123.468	62.146	1.268	0.887	0.802
6	AAZZ	123.597	62.017	1.271	0.887	0.741
7	AAZZ	124.423	61.191	1.293	0.882	0.788
8	AAZZ	124.594	61.02	1.285	0.884	0.779
9	AAZZ	126.432	59.182	1.273	0.889	0.551
10	AAZR	126.601	59.013	1.331	0.876	0.685

Null cost of top-ten score hypotheses is 185.614 bits. Fixed cost is 102.47 bits. Configuration cost is 13.8914 bits. [a]Abbreviation used for features: A, hydrogen-bond acceptor; Z, hydrophobic aromatic; R, ring aromatic.

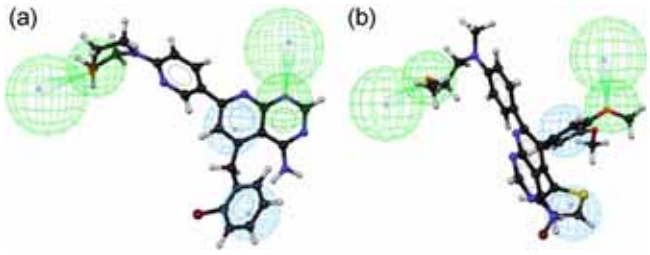


Figure 2. The Hypo1 mapping with training set compound 1 (a) and compound 3 (b). Pharmacophore features are color-coded: green for hydrogen-bond acceptor feature (HBA) and light blue for hydrophobic aromatic feature (Z).

active compound 1 (IC_{50} = 0.17 nM) and compound 3 (IC_{50} = 0.98 nM), among the training set molecules respectively. All compounds in this study were classified by their activity as highly active (IC_{50} < 10 nM, +++), moderately active (1000 > IC_{50} ≥ 10 nM, ++), and inactive (IC_{50} ≥ 1000 nM, +). The estimated inhibitory activities of the 26 molecules in the training set were evaluated using Hypo1 and then compared to the experimental data in Table 3. Compounds of all

Table 3. Experimental biological activity and the estimated activity for the training set molecules based on the top ranked hypothesis

Compd	Experimental IC_{50} (nM)	Estimated IC_{50} (nM)	Error[a]	Fit value[b]	Activity scale[c]	Estimated activity scale[c]
1	0.17	0.19	+1.1	8.26	+++	+++
2	0.47	2.4	+5	7.17	+++	+++
3	0.98	0.9	–1.1	7.59	+++	+++
4	1	0.93	–1.1	7.58	+++	+++
5	1.3	5.4	+4.1	6.81	+++	+++
6	2.8	4.6	+1.7	6.88	+++	+++
7	3.8	9.3	+2.5	6.58	+++	+++
8	4.1	6.6	+1.6	6.73	+++	+++
9	7.5	18	+2.4	6.30	+++	++
10	8.1	6.6	–1.2	6.72	+++	+++
11	12	5.2	–2.3	6.83	++	+++
12	22	16	–1.4	6.35	++	++
13	30	160	+5.3	5.34	++	++
14	40	18	–2.2	6.28	++	++
15	63	110	+1.7	5.52	++	++
16	72	14	–5.1	6.40	++	++
17	100	21	–4.8	6.23	++	++
18	120	210	+1.8	5.22	++	++
19	250	78	–3.2	5.65	++	++
20	460	240	–1.9	5.17	++	++
21	770	340	–2.3	5.02	++	++
22	1000	1000	+1	4.54	+	+
23	2000	1600	–1.3	4.36	+	+
24	4000	3800	–1.1	3.97	+	+
25	6100	2600	–2.3	4.13	+	+
26	10000	18000	–1.8	3.28	+	+

[a]+ indicates that the estimated IC_{50} is higher than the experimental IC_{50}; – indicates that the estimated IC_{50} is lower than the experimental IC_{50}; a value of 1 indicates that estimated IC_{50} is equal to the experimental IC_{50}. [b]Fit value indicates how well the features in the pharmacophore overlap the chemical features in the molecule. [c]Activity scale: +++, IC_{50} < 10 nM (highly active); ++, 1000 > IC_{50} ≥ 10 nM(moderately active); +, IC_{50} ≥ 1000 nM (inactive).

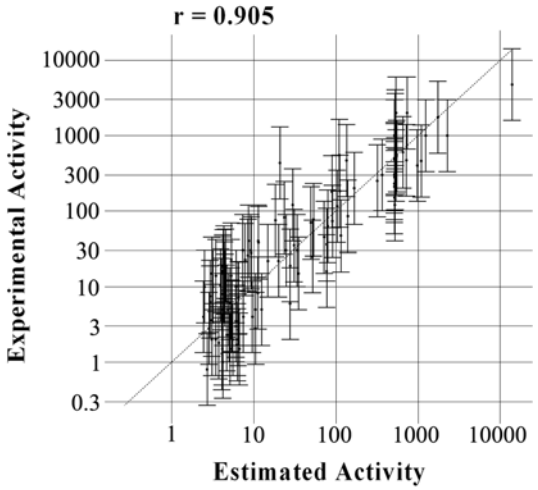


Figure 3. Correlation between experimental and estimated activity data over 124 test set compounds.

activity scale were predicted appropriately except for only two compounds. The compound 9 (highly active) was predicted to be moderately active and the compound 11 (moderately active) was predicted to be highly active.

Validation of the Pharmacophore Model. The Hypo1 gave a correlation coefficient 0.905 between experimental and estimated activity for 124 test set molecules (Figure 3) and it was the best correlation among all 10 hypotheses (Table 2). As represented in Figure 4a and 4b, the mapping of Hypo1 onto highly active compounds in the test set were fit appropriately and had a estimated activity of 5 nM (experimental IC_{50} = 5 nM) and 9.1 nM (experimental IC_{50} = 10 nM), respectively. The model was further validated by using *CatScramble* which generates random spreadsheets to create hypotheses using exactly the same features as used in generating the original pharmacophore hypothesis. The results from the 19 spreadsheets are listed in Table 4. The reasoning behind this procedure is that if the randomized data sets produced a hypothesis with a high correlation value than the original hypothesis, then the methodology of the pharmacophore generation is defective.

The results of *CatScramble* clearly indicate that all values generated after randomization produced hypotheses with no predictive value similar to that of original hypothesis. Out of 19 runs, only three trials had a correlation value around 0.7, but the RMSD values were very high and the total cost values were almost equal to the null cost value, which is not

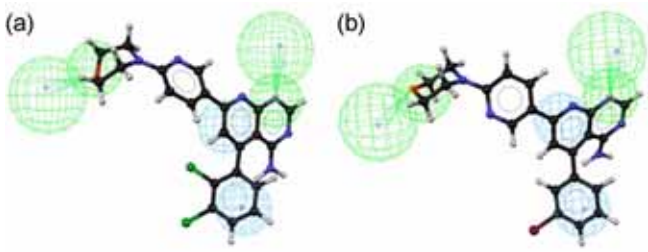


Figure 4. The Hypo1 mapping with two highly active compounds from the test set.

Table 4. Results from statistical validation using *CatScramble* implemented in *CATALYST* software

Validation No.	Total cost	ΔCost	Fixed cost	RMSD	Correlation (r)	Configuration cost
Results for unscrambled						
Hypo1	111.790	73.824	102.470	0.802	0.957	13.891
Results for scrambled						
Trial 1	156.787	56.820	99.967	2.073	0.658	11.388
Trial 2	171.106	71.240	99.866	2.335	0.528	11.288
Trial 3	159.052	56.206	102.846	2.078	0.655	14.267
Trial 4	151.878	46.109	105.769	1.868	0.734	17.191
Trial 5	163.257	60.886	102.371	2.163	0.617	13.792
Trial 6	170.052	68.621	101.431	2.289	0.554	12.852
Trial 7	182.615	88.544	94.071	2.584	0.400	5.492
Trial 8	149.323	50.973	98.350	1.946	0.709	9.771
Trial 9	180.660	78.335	102.325	2.454	0.450	13.747
Trial 10	174.147	71.153	102.994	2.314	0.545	14.415
Trial 11	151.442	47.106	104.336	1.899	0.723	15.757
Trial 12	166.759	68.206	98.553	2.287	0.554	9.974
Trial 13	161.561	60.668	100.893	2.134	0.633	12.314
Trial 14	166.107	63.009	103.098	2.195	0.602	14.519
Trial 15	180.235	83.708	96.527	2.532	0.390	7.948
Trial 16	162.488	61.093	101.395	2.166	0.616	12.817
Trial 17	161.746	61.797	99.949	2.170	0.614	11.370
Trial 18	176.539	78.064	98.475	2.450	0.453	9.896
Trial 19	159.889	63.516	96.373	2.208	0.596	7.794

Null cost of top-ten score hypotheses is 185.614 bits. Fixed cost is 102.47 bits. Configuration cost is 13.8914bits.

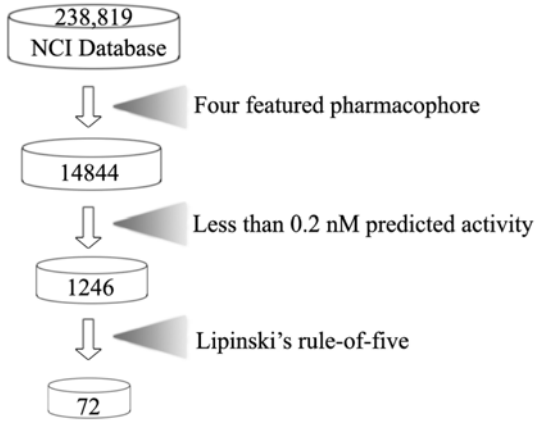


Figure 5. Flowchart of the screening procedure for new AK inhibitor design.

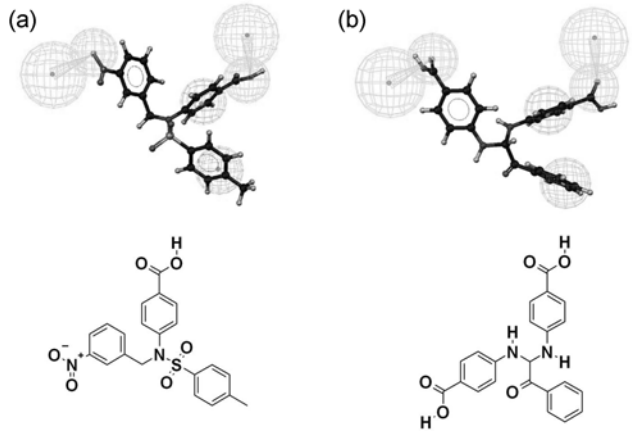


Figure 6. The Hypo1 mapping with new lead compound NCI0210803 (a) and NCI0109967 (b).

desirable for a good hypothesis. Therefore, the statistical validation results strongly support that the Hypo1 is not generated by chance since its values are far more superior to those of the 19 random hypotheses generated. These validations provide confidence on our pharmacophore model and thus it had been used for the next step, new lead search.

Database Search. The validated four feature pharmacophore, Hypo1 was used to screen molecules with similar features from the NCI2000 database which contains 238,819 compounds. The 14,844 lead compounds were obtained from the first 3D query. Their activities were estimated and were screened based on various criteria as shown in flowchart (Figure 5). Upon restricting the minimum estimated activity to 0.2 nM which is the activity threshold for the most active compounds, 1246 structures were left from the 14,844 compounds. Properties of the each compound like H-bond donors, H-bond acceptors, number of rotatable bonds, LogP values can be calculated based upon the structure. Though there are no specific rules for an ideal drug candidate, Lipinski's rule of five (LogP < 5, number of H-bond acceptors < 10, number of H-bond donors < 5) give us a basic idea about the fundamental properties to be a drug. Thus, molecular weight and number of rotatable bonds were calculated in *CATALYST* spreadsheet and compounds which had molecular weight less than 500 and numbers of rotatable bonds less than 10 were only considered. Only 152 leads were obtained which were further screened for compounds having H-bond acceptors less than 10 and H-bond donors less than 5 using *Molinspiration* software. Ultimately 72

lead compounds satisfied the Lipinski's rules indicating that they have ideal physiological properties. From the final 72 compounds, here we present only the two best candidate structures for new lead compounds, NCI0210803 and NCI0109967 (Figure 6). They showed an estimated activity of 0.01 nM and 0.012 nM, respectively and fitted well with the Hypo1. Thus, our pharmacophore model was able to retrieve few leads which had estimated inhibitory activity similar to most active compounds with acceptable calculated drug-like properties and therefore they could be recommended for further studies.

Conclusion

Our goal was to generate a predictive pharmacophore model that can be utilized to search 3D databases and screen them based on drug-like compounds to identify new non-nucleoside AK inhibitors. The 26 training set compounds were selected rationally and were used to generate pharmacophore hypothesis. The Hypo1, generated for AK inhibitors is characterized by four features: two HBAs and two Zs which complement the active site nature with a high correlation coefficient of 0.957. Our hypothesis was validated by the following two methods: first with a test set of 124 compounds; second by *CatScramble* method. The validated pharmacophore model was used for searching new lead compounds. Through the 3D query, 14,844 compounds were obtained from the 238,819 compounds of NCI database, and the number was reduced to 1246 when physico-chemical properties were considered. The new lead candidate compounds were screened based on Lipinski's rule to have drug-like properties and so finally we could obtain 72 compounds and two of them were posted in this article. Thus, our pharmacophore model was able to retrieve few leads which had estimated inhibitory activity similar to most active compounds with acceptable calculated drug-like properties and therefore they could be recommended for further studies.

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