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Pharmacophore modeling study based on known Spleen tyrosine kinase inhibitors together with virtual screening for identifying novel inhibitors

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ABSTRACT

In this investigation, chemical features based 3D pharmacophore models were developed based on the known inhibitors of Spleen tyrosine kinase (Syk) with the aid of HIPHOP and HYPOREFINE modules within CATALYST. The best quantitative pharmacophore model, Hypo1, was used as a 3D structural query for retrieving potential inhibitors from chemical databases including Specs, NCI, MayBridge, and Chinese Nature Product Database (CNPD). The hit compounds were subsequently subjected to filtering by Lipinski's rule of five and docking studies to refine the retrieved hits. Finally 30 compounds were selected from the top ranked hit compounds and conducted an in vitro kinase inhibitory assay. Six compounds showed a good inhibitory potency against Syk, which have been selected for further investigation.

Spleen tyrosine kinase (Syk), a type of cytosolic non-receptor tyrosine kinase, has been demonstrated to play critical roles in the intracellular signal propagation of hematopoietic cells.[1–3] For example, Syk was shown to be an important regulator of FcεRI signaling in mast cells and basophils, suggesting that it could be a potential target for treating type I hypersensitivity reactions including allergic rhinitis, asthma, and anaphylaxis.[4] Further, the significant roles of Syk in B-cell receptor signaling on autoreactive B cells have also been realized, which uncovered an opportunity to suppress the formation of autoantibodies involved in a number of immune complex (IC) diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and multiple sclerosis (MS).[5,6] All of these prompt that Syk inhibitors might be effective in the treatment of allergic or immunological disorders. Indeed, recently a Syk inhibitor, R788 (tamatinib fodium), has been in phase II clinical trial of treating RA and already shown very good efficacy.[7] R788 is thought as a direct challenger to the tumor necrosis factor-alpha (TNF-α) inhibitor biologics.[8] Study of using R788 in the treatment of SLE is also in progress.[9]

Due to the potential therapeutic value of Syk inhibitors in the treatment of allergic and autoimmune diseases, some pharmaceutical companies including Rigel, Pfizer, ZaBeCor,[6] as well as many academic institutions have been involved in the development of small molecule kinase inhibitors of Syk. As far as we know, more than one hundred small molecule inhibitors against Syk have been reported publicly at present. These provide a good basis for elucidating the structure–activity relationship (SAR) of these diverse compounds, and hence facilitate identifying more new Syk inhibitors.

The goal of this account is to develop 3D pharmacophore models based on the known Syk inhibitors, which can correctly reflect the structure–activity relationship (SAR) of the existing Syk inhibitors. Then this model will be used as 3D search queries for searching several chemical databases, including Specs, NCI, MayBridge and Chinese Nature Product Database (CNPD) for identifying new inhibitors of Syk. The hit compounds will be subsequently subjected to filtering by Lipinski's rule of five[10] and docking studies to refine the retrieved hits.[11,12] Finally some of the refined hit compounds were purchased from the market and conducted an in vitro inhibitory assay against recombinant Syk protein kinase. By the way, as far as we know, this is the first report on the pharmacophore modeling even the first virtual screening study of Syk inhibitors.

Training set selection and conformational modeling. A set of 141 Syk inhibitors were collected from different literature resources.[1,13–26] From which, 23 compounds were carefully chosen to form a training set, which was based on the principles of structural diversity and wide coverage of activity range. The IC_{50} values of the inhibitors in training set span a range of five orders of magnitude (IC_{50} values range from 3 nM to 76,400 nM). Structures and biological activities of the training set compounds are shown in Chart 1. The remaining compounds were used as a test set (see Table S1 in Supplementary data).

All compounds were built in 2D/3D Visualizer within CATALYST 4.11[27] and minimized to the closest local minimum using the CHARMm-like force field incorporated in the CATALYST program. A series of energetically reasonable conformational models which

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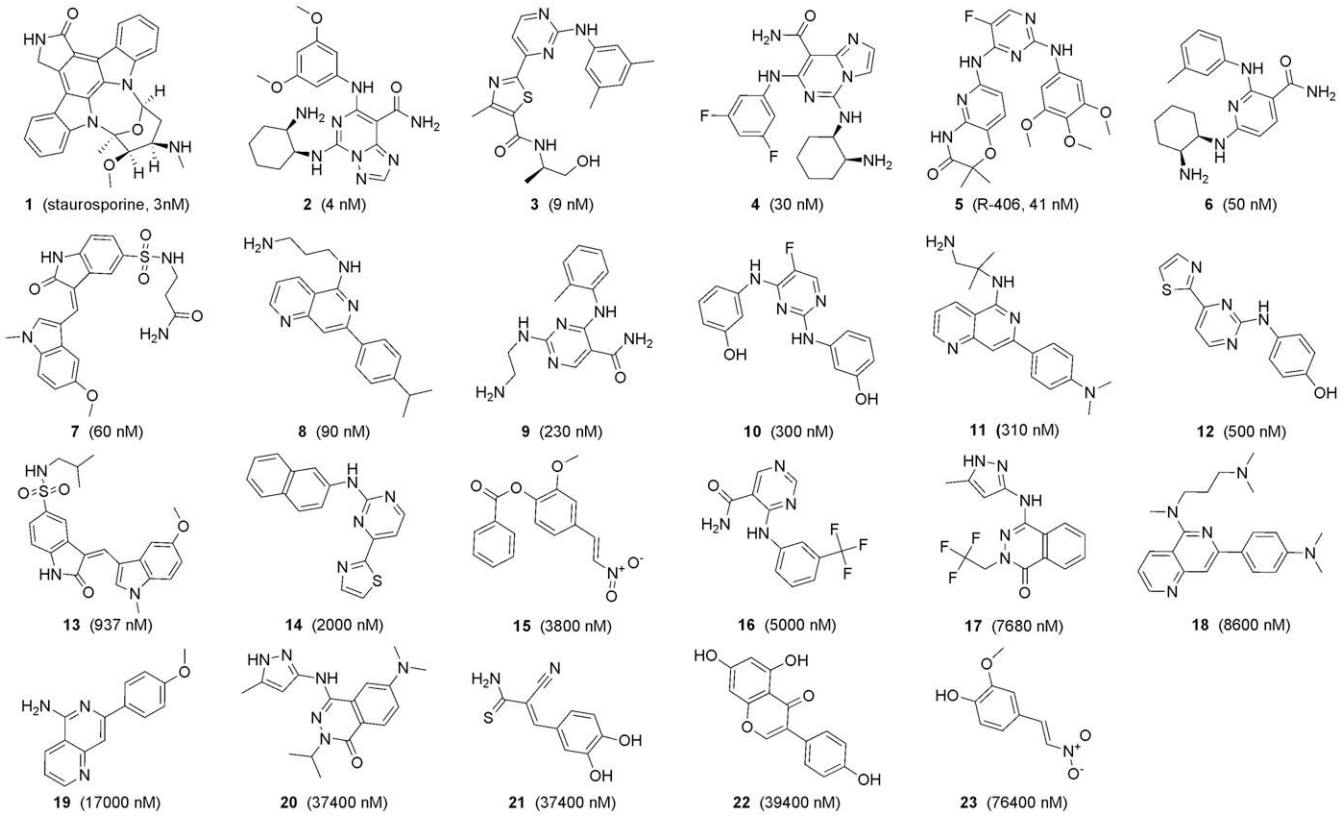

Chart 1. Chemical structures of Syk kinase inhibitors in training set (compounds **1**–**23**) together with their biological activity data (IC_{50} values, in parentheses).

represent the flexibility of each compound were generated by CatConf program within CATALYST. Conformations of all molecules were generated by using the 'Best conformer generation' with 20 kcal/mol as energy cutoff and 250 as maximum number conformers, while all other parameters were set to default.

Pharmacophore generation. Before performing quantitative pharmacophore modeling for the Syk kinase inhibitors, qualitative HIPHOP[28] models were first generated based on the six most-active compounds in the training set (compounds **1–6**, Chart 1), which purpose was to identify the common chemical features necessary for potent Syk kinase inhibitors, as well as to provide some information for the development of quantitative pharmacophore model. In the HIPHOP run, compound **1** was considered as reference molecule. The best HIPHOP model generated (not provided here) contains five types of chemical features, namely, hydrogen-bond donors (D), hydrogen-bond acceptor (A), ring aromatic (R), hydrophobic aromatic (Y), and hydrophobic aliphatic (Z) features. Following this information, the five chemical features were selected as the initial input of pharmacophore features in the subsequent quantitative pharmacophore modeling. The quantitative pharmacophore models were then generated by using the HYPOREFINE module within the CATALYST based on the training set (compounds **1–23**, Chart 1). The 'Uncertainty' values for all of compounds in the training set were set to 2.5, and default values for other parameters were employed.

The top 10 hypotheses as well as their statistical parameters obtained from the HYPOREFINE run are shown in Table 1. From Table 1, we can see that the best hypothesis corresponds to Hypo1, which was characterized by the lowest total cost value (98.56), the highest cost difference (101.62), the lowest RMS deviation (0.80), and the best correlation coefficient (0.971). Hypo1 contains four features, including one hydrogen-bond donor, one hydrogen-bond acceptor, one hydrophobic aromatic, and one ring aromatic features, as well as three excluded volumes (Fig. 1A). The 3D space and distance constraints of these pharmacophore features are shown in Figure 1B. Figure 1C and D presents the Hypo1 aligned with one of the most active compounds (compound **2**, IC_{50} = 4 nM) and the lowest active compound (**23**, IC_{50} = 76,400 nM) in the training set. All features of Hypo1 model were nicely mapped with the corresponding chemical functional groups on compound **2**. By contrast, the compound **23** just mapped two features while the other two features were not mapped.

Table 1
Statistical parameters of the top 10 hypotheses of Syk kinase inhibitors generated by HYPOREFINE program

Hypo. no.	Total cost	ΔCost[a]	RMSD	Correlation	Features[b]
1	98.56	101.62	0.80	0.971	ADYRE3
2	107.84	92.34	1.22	0.931	ADYRE1
3	108.10	92.08	1.24	0.928	ADYRE2
4	114.54	85.64	1.44	0.901	ADYR
5	116.32	83.86	1.37	0.914	ADY
6	117.05	83.13	1.39	0.910	ADY
7	117.32	82.86	1.54	0.887	DDYR
8	117.35	82.83	1.52	0.889	ADYR
9	118.90	81.28	1.49	0.895	ADDRE1
10	119.33	80.85	1.59	0.879	DDYR

[a] ΔCost = (null cost − total cost), where null cost = 184.29, fixed cost = 90.18, configuration = 15.86, all cost units are in bits.
[b] A = hydrogen-bond accept; D = hydrogen-bond donor; Y = hydrophobic aromatic; R = ring aromatic; E = excluded volumes; arabic numeral indicates the number of the excluded volumes.

To examine Hypo1's discriminating ability among Syk inhibitors in the training set with activity in different orders of magnitude, the training set compounds were roughly classified into three categories: highly active ($IC_{50} \leqslant 100$ nM, +++), moderately active (100 nM < $IC_{50} \leqslant 5000$ nM, ++), and low active ($IC_{50} > 5000$ nM,

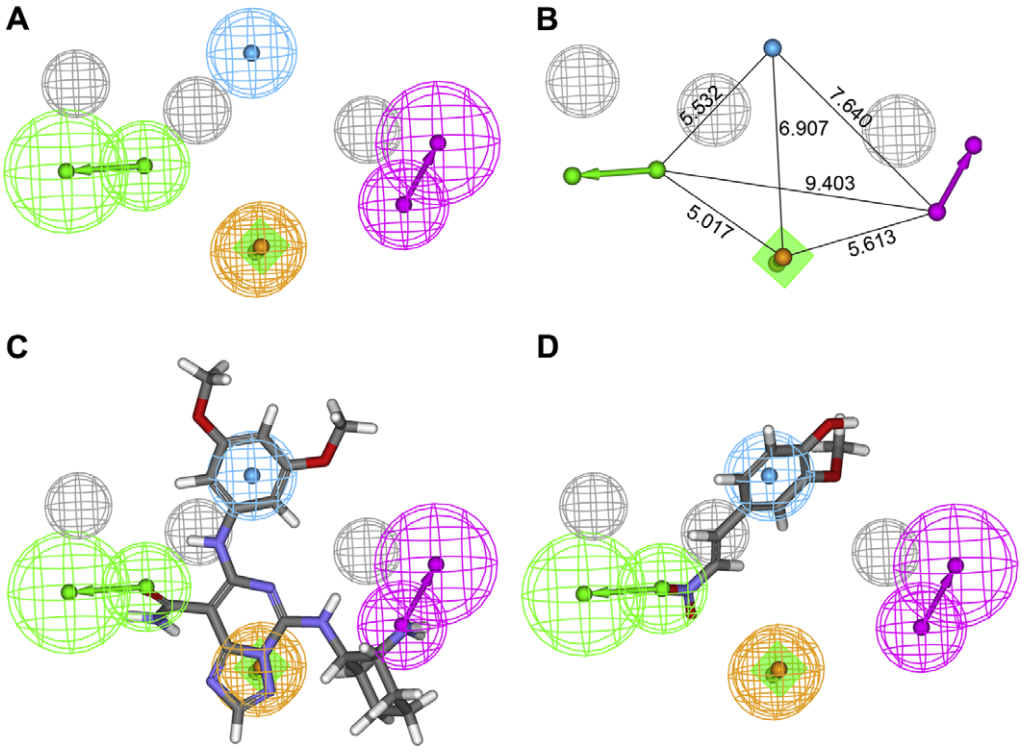

Figure 1. Pharmacophore model of Syk kinase inhibitors generated by HYPOREFINE. (A) The best HYPOREFINE model Hypo1. (B) 3D spatial relationship and geometric parameters of Hypo1. (C) Hypo1 mapping with one of the most active compound **2** (IC_{50} = 4 nM). (D) Hypo1 mapping with one of the least active compound **23** (IC_{50} = 76,400 nM). Pharmacophore features are color-coded with light-blue for hydrophobic aromatic feature, orange for ring aromatic feature, green for hydrogen-bond acceptor, magenta for hydrogen-bond donor, and grey for excluded volumes.

+). Table 2 shows the experimental and estimated inhibitory activities of the 23 training set compounds. Obviously, all compounds were predicted correctly except compounds **5** and **8**, which are highly active experimentally but are predicted to be moderately active. Additionally one may also notice that most of the absolute values of error costs, defined as the ratio between experimental and estimated activity, are below 3, indicating a considerable consistency between estimated and experimental IC_{50} values.

Table 2
Experimentally measured and predicted (by Hypo 1) activity (IC50 values, nM) for the training set compounds

Compound No.	Fit value	IC_{50} (nM)		Error cost[a]	Activity scale[b]	
		Pred.	Exptl.		Pred.	Exptl.
1	8.28	7.2	3	+2.4	+++	+++
2	8.68	2.9	4	−1.4	+++	+++
3	7.71	27	9	+3.0	+++	+++
4	8.23	8	30	−3.7	+++	+++
5	7.06	120	41	+2.9	++	+++
6	7.43	51	50	+1.0	+++	+++
7	7.62	32	60	−1.9	+++	+++
8	6.96	150	90	+1.6	++	+++
9	6.55	380	230	+1.7	++	++
10	6.30	680	300	+2.3	++	++
11	6.61	340	310	+1.1	++	++
12	6.18	900	500	+1.8	++	++
13	6.40	540	940	−1.7	++	++
14	6.05	1200	2000	−1.6	++	++
15	5.46	4800	3800	+1.3	++	++
16	6.07	1100	5000	−4.4	++	++
17	5.01	13,000	7700	+1.7	+	+
18	4.87	18,000	8600	+2.1	+	+
19	4.80	21,000	17,000	+1.3	+	+
20	4.91	17,000	37,000	−2.2	+	+
21	4.75	24,000	38,000	−1.6	+	+
22	4.89	17,000	39,000	−2.3	+	+
23	4.44	49,000	76,000	−1.6	+	+

[a] The error cost is computed as the ratio between the experimental IC_{50} and the estimated IC_{50} by the hypotheses. '+' 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}.
[b] Activity scale: +++, $IC_{50} \leqslant 100$ nM (highly active); ++, 100 nM < $IC_{50} \leqslant 5000$ nM (moderately active); +, $IC_{50} > 5000$ nM (low active).

Validation of the pharmacophore model. The purpose of the quantitative pharmacophore modeling is not just to estimate the activity of the training set compounds correctly but also to verify whether the model is capable of predicting the activity of external compounds of the test set series. The test set contains 118 compounds (see Table S1 in Supplementary data) structurally distinct from the training set molecules. All the test molecules were prepared by the same way as that for the training set molecules. Hypo1 was regressed against the compounds of test set, and a correlation coefficient of 0.817 was obtained. The results are presented in Figure 2. Obviously Hypo1 is capable of predicting the IC_{50} values correctly (the detailed information is provided in Supplementary data, see Table S1).

Furthermore, a cross validation that based on Fischer randomization test method[29] was used to evaluate the statistical relevance of Hypo1. With the aid of the CATSCRAMBLE program implemented in CATALYST, the spreadsheet of our training set was modified by randomly scrambling the experimental activities for all compounds, and the resulting training set was used for a HYPOREFINE run. As the confidence level was set to 95% in this study, the CATSCRAMBLE program generated 19 random spreadsheets, and then all the spreadsheets were used to construct hypotheses using exactly the same conditions as used in generating the original pharmacophore hypotheses. The total costs of pharmacophore models obtained in the 19 HYPOREFINE runs as well as the original HYPOREFINE run are presented in Figure 3. From Figure 3, one can see that the original hypothesis is far more superior to those of the 19 generated hypotheses after randomization. These results provide confidence on our pharmacophore model.

Comparison between Hypo1 and chemical features in the active site of Syk protein. The pharmacophore models developed here are all based on the known ligands of Syk protein. One may wonder whether the chemical features and their space arrangement described in the pharmacophore model are consistent with the actual protein–ligand interactions. Fortunately, there had already existed two crystal structures of Syk–ligand complexes in Protein Data

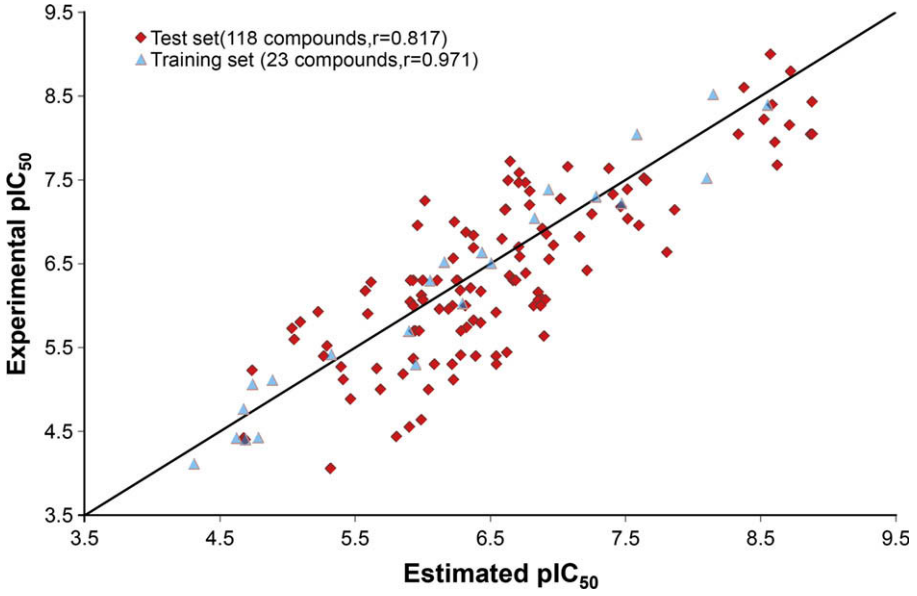

Figure 2. Plot of the correlation between the experimental activity and the predicted activity by Hypo1 for the test set compounds.

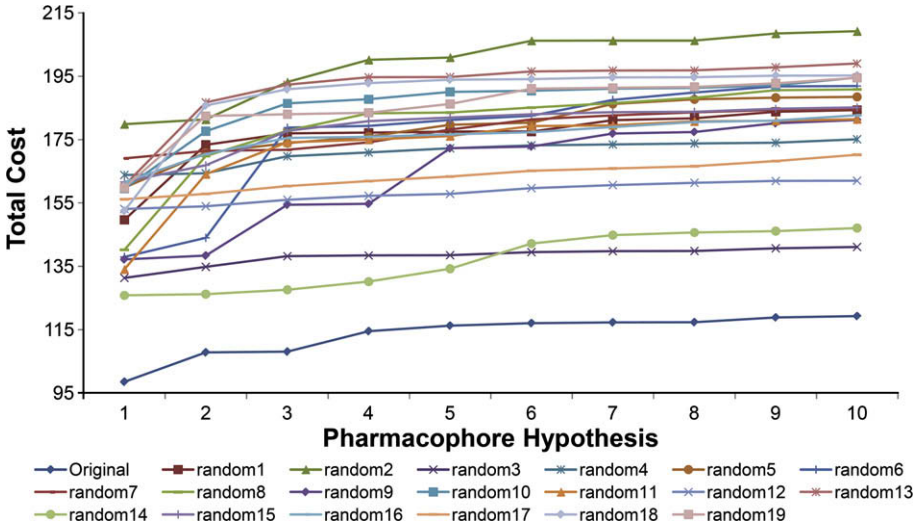

Figure 3. The difference in total cost of hypotheses between the initial spreadsheet and 19 random spreadsheets after CATSCRAMBLE run.

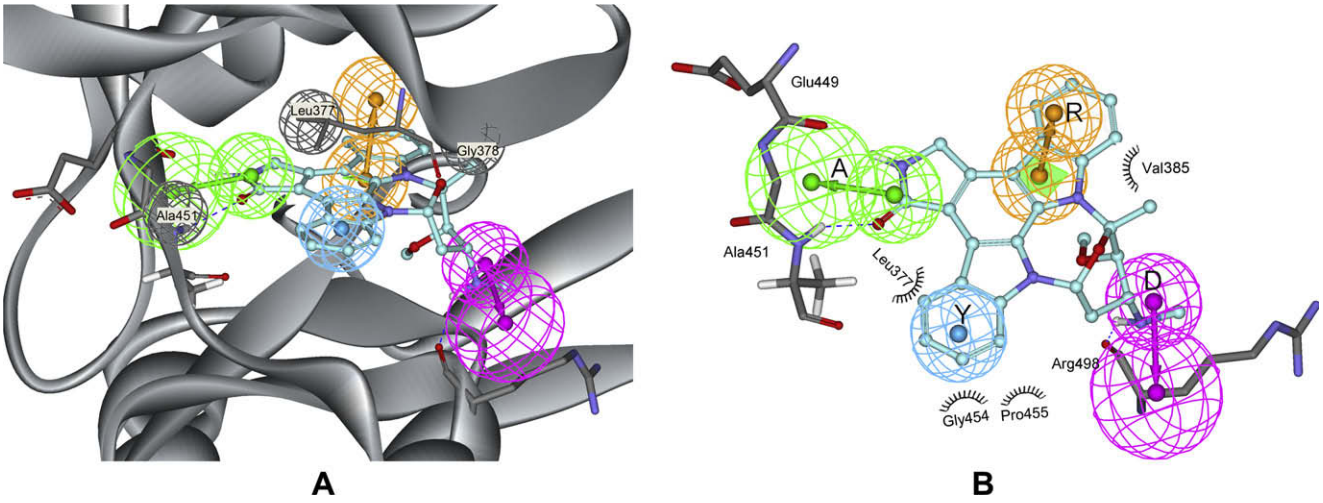

Figure 4. (A) The pharmacophore model Hypo1 positioned into the active site of Syk–Staurosporine complex (1XBC). (B) Enlargement of (A). The excluded volumes are ignored for clarity. Dashed lines indicate hydrogen bonds.

Bank at the time when we were preparing this manuscript: one is the Syk protein complexed with Staurosporine (compound **1** in the training set, PDB entry: 1XBC),[21] the other one is the Syk protein complexed with imatinib (PDB entry: 1XBB). Since Staurosporine has a very high inhibitory potency[26] (3 nM) against Syk, whereas imatinib just has a low activity (5000 nM), the comparison will be made between the pharmacophore model Hypo1 and chemical features in the active site of Syk–Staurosporine complex.

Figure 4 presents the mapping of Hypo1 with the active site of Syk–Staurosporine complex. Clearly the hydrogen-bond acceptor feature (A) of Hypo1 corresponds to the hydrogen-bond interaction between the backbone amino nitrogen of Ala451 (hydrogen donor) and the pyrrol-2-one motif (hydrogen acceptor); this type of hydrogen-bond interaction is commonly observed in other kinase-inhibitor complexes.[30] The hydrogen-bond donor feature (D) of Hypo1 corresponds to the hydrogen-bond interaction between the methylamine in the tail of Staurosporine and the backbone carbonyl oxygen of Arg498. The hydrophobic aromatic feature (Y) locates in the hydrophobic region surrounded by Leu377, Gly454, and Pro455. The ring aromatic feature (R) was mapped on the central pyrrole ring, which roughly locates in the adenine region.[31] Among the three excluded volumes, two are very close to the back-bone of the protein, and one is in the position of side chain of Leu377. Therefore, we can conclude here that the pharmacophore model Hypo1 developed from the small molecule inhibitors of Syk can correctly reflect the interactions between Syk and its ligands.

Database searching. The best quantitative pharmacophore model Hypo1 was used as a 3D structural query for retrieving potential inhibitors from chemical databases including NCI (136,000 molecules), MayBridge (59,652 molecules), Specs (202,487 molecules), and CNPD (43,055 molecules). A total of 3368 hit compounds were obtained from the first screening by restricting the minimum estimated activity to 5 μM. The hit compounds were further screened by using the Lipinski's rule of five to make them more drug-like. 1471 compounds passed the drug-like check.

Docking study. To further refine the retrieved hits, the 1471 hit compounds were docked into the ATP binding site of Syk by using LigandFit within Accelrys Discovery Studio 1.7.[32] The crystal structure of Syk complexed with staurosporine (PDB entry: 1XBC) was chosen as reference protein. The active site search was carried out in the shape-based mode based on the X-ray structure of receptor and the site was further modified to cover the co-crystal ligand. The docking protocol was validated through docking the bound ligand back into the protein. The best docked pose should differ only minimally from the position of the ligand in the crystal structure (Root mean square deviation is less than 0.5 Å). Conformations of each compound were created with Monte Carlo simulation (15,000 trials). Several scoring functions are available within LigandFit, including LigScore1, LigScore2, PLP1, PLP2, Jain, Ludi, and PMF. The Ludi scoring function was used as the sorting function since it performed better than others in a pre-evaluating process. A total of 30 compounds were carefully chosen from the top ranked hit compounds based on our experience and visual inspection. These compounds were purchased from the market and shifted for the subsequent in vitro assay. By the way, some new scaffolds that deviate from that of the training set molecules have been obtained in the final selected hit compounds.

In vitro kinase inhibitory assays. In vitro kinase inhibitory assays were performed against recombinant Syk kinase at the Km of ATP (15 μM) and at a fixed concentration of 10 μM of test compounds. Each assay was repeated twice. All the inhibitory assays against Syk were carried out through kinase profiling services provided by ProQinase (PanQinase, Freiburg, Germany), in which radiometric protein kinase assays were used. Among the selected 30 compounds, six compounds showed a good inhibitory potency again Syk at the concentration of 10 μM. Figure 5 presents two of the six compounds, AK-968/1209687 and AK-968/15361771, which afforded an inhibitory rate of 51% and 63%, respectively. All of the six compounds have been selected for further investigation.

In conclusion, 3D pharmacophore models of Syk inhibitors have been developed with the aid of HIPHOP and HYPOREFINE modules in CATALYST program packet. The pharmacophore models established were validated by test set and Fischer randomization test methods. And comparison between the pharmacophore model and chemical features in the active site of Syk protein was made, which shows that the pharmacophore model Hypo1 developed from the small mole-

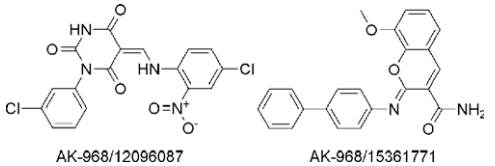

Figure 5. Two selected compounds showing a good inhibitory potency against the Syk protein kinase.

cule inhibitors of Syk can correctly reflect the interactions between Syk and its ligands. Furthermore, the best pharmacophore model Hypo1 was used as a 3D structural query for retrieving potential inhibitors from chemical databases including Specs, NCI, MayBridge, and CNPD. The hit compounds were subsequently subjected to filtering by Lipinski's rule of five and docking studies to refine the retrieved hits. Finally a total of 30 compounds were selected and conducted an in vitro kinase inhibitory assay. Six compounds showed a good inhibitory potency against Syk, which have been selected for further investigation.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.02.049.

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