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3D QSAR pharmacophore model based on diverse IKKβ inhibitors

Shanthi Nagarajan · Asif Ahmed · Hyunah Choo · Yong Seo Cho · Kwang-Seok Oh · Byung Ho Lee · Kye Jung Shin · Ae Nim Pae

Abstract The inhibitor kappaB kinase β (IKKβ) is a serine-threonine protein kinase that is critically involved in the activation of the transcription factor nuclear factor kappa B (NF-κB) in response to various inflammatory stimuli. IKKβ-selective inhibitors could prove useful for the treatment of inflammatory diseases. In the absence of structural information, a ligand-based approach can serve as an alternative to the virtual screening of large databases. We have developed a 3D QSAR pharmacophore model based on 23 IKKβ inhibitors with 3 nM≤IC_{50}≤50000 nM. A four-feature pharmacophore containing a hydrophobic (Hy) feature, two ring aromatic (RA) features, and a hydrogen bond donor (D) feature was constructed. It yielded a correlation coefficient of 0.93 with experimentally determined activity data, and a correlation coefficient of 0.77 with training set activity data. The best hypothesis, Hypo 1, was validated by estimating the activities of 136 compounds in a test set. As well as the correlation analysis and test set activity estimation, a Fisher's validation test was conducted at the 95% confidence level. The pharmacophore model's specificity and selectivity were determined in an exhaustive enrichment study.

Keywords IKK · Inhibitor kappa B kinase · Pharmacophore · HypoGen · Virtual screening · Enrichment

S. Nagarajan · A. Ahmed · H. Choo · Y. S. Cho · K. J. Shin · A. N. Pae (✉)
Center for Chemoinformatics Research, Life Sciences Division, Korea Institute of Science and Technology,
P.O. Box 131, Cheongryang,
Seoul 130-650, Republic of Korea
e-mail: anpae@kist.re.kr

S. Nagarajan · A. Ahmed
School of Science, Korea University of Science and Technology,
52 Eoeun dong, Yuseong-gu,
Daejeon 305-333, Republic of Korea

A. Ahmed
Biotechnology and Genetic Engineering Discipline,
Khulna University,
Khulna 9208, Bangladesh

K.-S. Oh · B. H. Lee
Drug Discovery Division,
Korea Research Institute of Chemical Technology,
Daejeon, Republic of Korea

Introduction

Transcription factors of the nuclear factor kappa B (NF-κB) Rel family are critical regulators of genes that function in inflammation, cell proliferation, and apoptosis [1]. NF-κB plays a central role in the autoimmune, inflammatory, and destructive mechanisms that drive the progression of diseases such as rheumatoid arthritis [2]. One key role of such transcription factors is to induce and coordinate the expression of a broad spectrum of proinflammatory genes, including cytokines, chemokines, interferons, MHC proteins, growth factors, and cell adhesion molecules [3].

The IκB kinase (IKK) is essential for transducing the signal-inducible activation of the transcriptional factor NF-κB in response to proinflammatory stimuli [4]. IKKβ is a serine–threonine protein kinase that is critically involved in the activation of the transcription factor NF-κB in response to various inflammatory stimuli [5]. The IKK complex consists of three subunits: the catalytic subunits IKKα and IKKβ, and the regulatory subunit IKKγ (also known as NEMO) [6]. Although IKKα and

IKKβ are highly homologous and contain similar structural domains, IKKβ has a 20- to 50-fold higher level of kinase activity for IκB than does IKK-1 [7]. Among the two catalytic subunits (IKKα and IKKβ), the most important one for activating the classical NF-κB signaling pathway is IKKβ [6].

IKKβ inhibitors could be useful for the treatment of inflammatory diseases [6]. They could potentially be used in diseases such as asthma, atopic dermatitis, allergic rhinitis, and rheumatoid arthritis [5]. So far, there is no drug on the market that acts on the IKKβ system; however, TPCA1, BMS345541, and ML120B (Fig. 1) have all been efficacious in preclinical models of arthritis, as each of these inhibitors seems to show selectivity for IKKβ over IKKα [2]. Leo Pharmaceuticals is the only company which has confirmed that it has progressed to human clinical trials with a compound (CHS-828) that is a potent inhibitor of IKKβ [7]. Research attempting to identify IKKβ inhibitors by various methods is ongoing, but the area is still relatively young [7]. Virtual screening (VS) of molecular libraries has emerged as a powerful method for the discovery of novel lead compounds for drug development [8].

Our primary goal in the work described in this article was to develop a quantitative 3D-QSAR pharmacophore model that could be useful in the virtual screening of commercial databases. The model was developed based on features that IKKβ inhibitors have in common. The pharmacophore model was meticulously validated using 136 internal test set compounds. Besides the test set prediction, an extensive enrichment study was conducted to prove the sensitivity and selectivity of the pharmacophore model. Hence, chemical features needed for the activity-integrated pharmacophore model can be searched for in commercial databases, and this is expected to provide useful information for developing new potentially active candidates that target IKKβ.

Materials and methods

Pharmacophore modeling environment

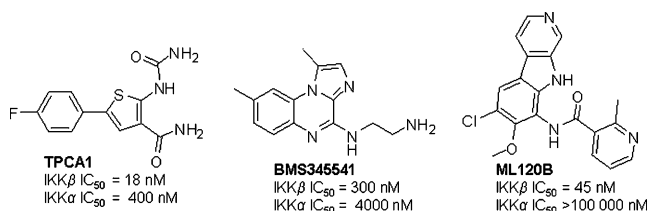Molecular modeling was performed on a Silicon Graphics Origin 300, eight-processor (600 MHz) MIPS R14000,

4 GB RAM server running on an Irix 6.5 operating system (SGI, Mountain View, CA, USA). Pharmacophore hypothesis modeling was performed using the Catalyst 4.11 software package (Accelrys, San Diego, CA, USA).

Data collection

All of the compounds used in the modeling study, and the corresponding biological activity data, were obtained from the literature [9–13]. The IKKβ-inhibiting potency of each compound was assessed in a homogeneous assay that measured the degree of phosphorylation of glutathione S-transferase (GST)-IκB-α. Furthermore, to facilitate the modeling, the compounds were divided into four groups according to their activity data (expressed as IC_{50} values). Compounds with activity values of <100 nM were classified as highly active (+++); those with activities of between >100nM and <1 μM were defined as active (++); compounds with activities of >1 μM to <10 μM were classified as moderately active (+); while compounds with IC_{50} values of >10 μM were classed as inactive (−). This classification is highly beneficial when training the pharmacophore model with a broad range of activities, and also to access the estimation accuracy of pharmacophore quickly. During the experiment, the activities of the IKKβ inhibitors were represented by their IC_{50} values in nM.

Training and test set

A database of IKKβ inhibitors containing 159 compounds was developed using the Catalyst 2D/3D sketcher and minimized to the closest local minima. Possible conformers for all of the training and test set compounds were generated using the ConFirm module, which uses the "Poling" algorithm [14–16] to generate reasonable conformers. A maximum of 250 conformers were generated for each molecule within an energy threshold of 20.0 kcal/mol above the global energy minimum using the CHARMM [17] force field. A "best quality" conformational analysis method was used to generate the conformers; this method considers the spatial arrangement of chemical features rather than simply the arrangement of the atoms. Instead of using just the lowest-energy conformation of each compound, all conformational models of each molecule in the training set were used in Catalyst for pharmacophore hypothesis generation.

Selection of the training set compounds is an important task because it contributes critical information to the pharmacophore model. To generate the model, 23 compounds in total (Fig. 2) were selected as a training set based on their scaffold diversity and their wide range of activity values (highly active, active, moderately active, and
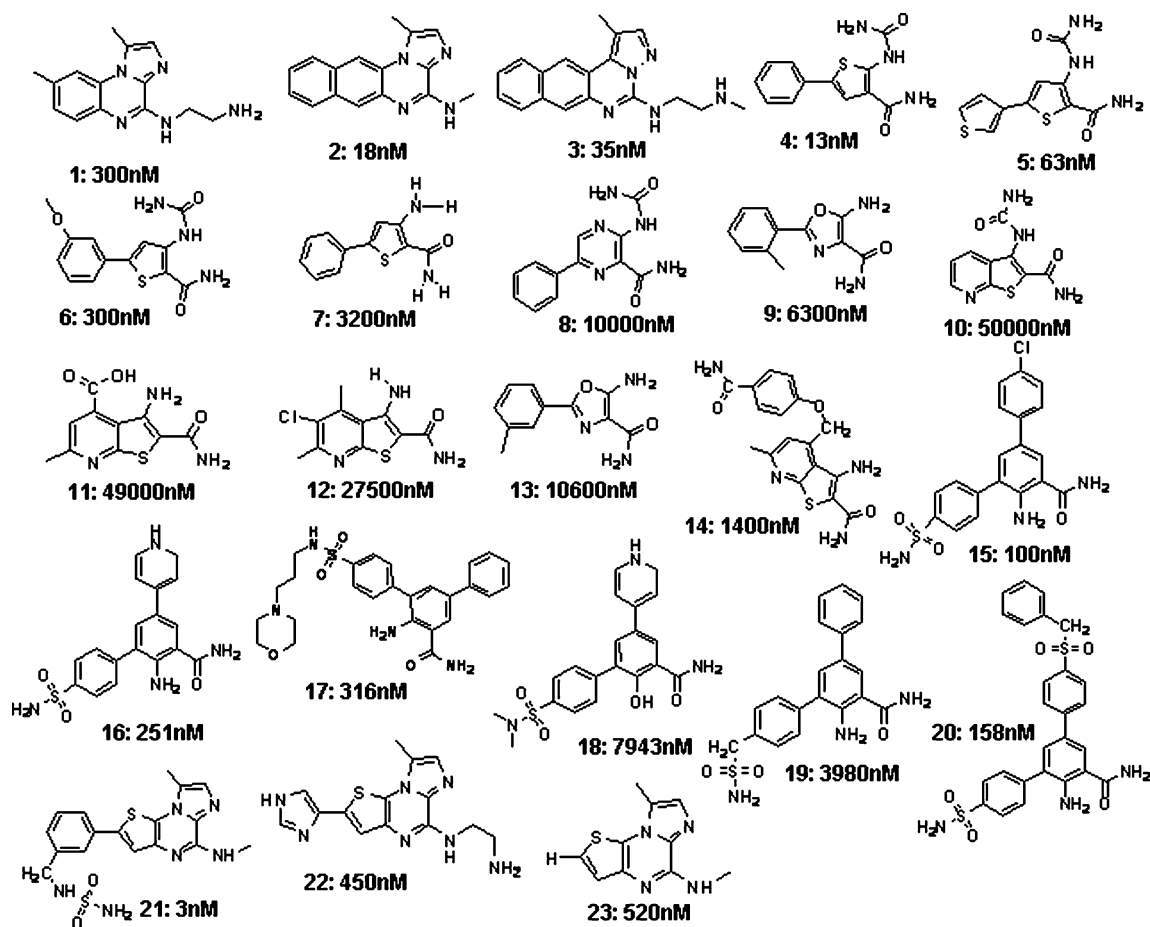

Fig. 1 IKKβ inhibitors tested in preclinical models of arthritis

TPCA1
IKKβ IC_{50} = 18 nM
IKKα IC_{50} = 400 nM

BMS345541
IKKβ IC_{50} = 300 nM
IKKα IC_{50} = 4000 nM

ML120B
IKKβ IC_{50} = 45 nM
IKKα IC_{50} >100 000 nM

Fig. 2 2D structures of the 23 training set compounds, along with their experimental IC_{50} values

inactive), spanning four orders of magnitude (3 nM≤IC_{50}≤ 50000 nM). Each compound selected added new information to the model, in order to avoid redundancy and bias, in terms of both structural features and activity range. The most active compounds were included to provide information on the most critical features required for a pharmacophore. Excluding the training set compounds, the remaining compounds were used as an internal test set to measure the efficiency of the pharmacophore model. These compounds also covered a wide range of activities: 4 nM≤IC50≤ 50000 nM.

Pharmacophore model generation

Prior to quantitative pharmacophore development, we conducted a common-feature (HipHop) pharmacophore modeling study in order to identify the features required for the pharmacophore model. Six highly active compounds were used as a training set; every compound was treated equally by setting the principal column to a value of 2 and the maximum number of omitted features to 0. A four-feature pharmacophore was

established using this method: ring aromatic, hydrophobic, hydrogen bond donor, and hydrogen acceptor features were found repeatedly in all of the pharmacophores generated.

The 23 training set compounds (Fig. 1, Table 1) were used along with their conformers to develop a 3D QSAR pharmacophore model. Based on the HipHop pharmacophore features, we selected hydrogen bond donor, hydrogen bond acceptor, ring aromatic, and hydrophobic features as the essential information for hypothesis generation. For each feature, the minimum number of instances was constrained to 0 and the maximum to 5, allowing the algorithm to generate an unbiased model. A spreadsheet containing the compounds and their corresponding activity data at the nanomolar scale with the aforementioned parameters was submitted to Catalyst.

Ten pharmacophore hypotheses were exported within a reasonable time frame, and all of these pharmacophores exhibited four features. Most of the pharmacophores had a hydrogen bond donor feature, two ring aromatic features, and a hydrophobic feature, whereas hypotheses 5 and 6 had a hydrophobic feature and hypotheses 7 and

Table 1 HypoGen output, showing fit values and the experimental and estimated activities of the training set compounds corresponding to pharmacophore hypothesis 1 (Hypo 1)

Sl. no	Code	Fit	Activity (IC$_{50}$ in nM)		Activity scale[a]		Error	Reference
			Experimental	Estimated	Experimental	Estimated		
1	21	9.07	3	2.2	+++	+++	−1.4	[23]
2	4	7.12	13	190	+++	++	15	[9]
3	2	7.89	18	33	+++	+++	1.8	[10]
4	3	8.04	35	23	+++	+++	−1.5	[10]
5	5	7.23	63	150	+++	++	2.4	[9]
6	15	6.93	100	300	++	++	3	[11]
7	20	6.7	158	500	++	++	3.2	[11]
8	16	6.81	251	400	++	++	1.6	[11]
9	1	7.24	300	150	++	++	−2	[10]
10	6	7.21	300	160	++	++	−1.9	[10]
11	17	6.77	320	430	++	++	1.4	[11]
12	22	6.77	450	430	++	++	−1	[23]
13	23	6.56	520	700	++	++	1.3	[23]
14	14	5.89	1400	3200	+	+	2.3	[12]
15	7	5.96	3200	2800	+	+	−1.1	[9]
16	19	6.96	3980	280	+	++	−14	[11]
17	9	5.69	6300	5100	+	+	−1.2	[12]
18	18	5.89	7943	3300	+	+	−2.4	[11]
19	8	5.76	10000	4400	−	+	−2.3	[9]
20	13	5.62	10600	6000	−	+	−1.8	[12]
21	12	4.98	27500	27000	−	−	−1	[12]
22	11	4.9	49000	32000	−	−	−1.5	[12]
23	10	4.89	50000	33000	−	−	−1.5	[12]

[a] Highly active (<100 nM, +++); active (>100 nM to <1 μM, ++); moderately active (>1 to 10 μM, +); inactive (>10 μM, −)

10 had hydrogen bond acceptor features instead of a ring aromatic (Table 2). Statistical parameters such as cost values determined the significance of the models. Among the top ten hypotheses reported, hypothesis 1 (Hypo 1) had the best statistical parameters, a high correlation coefficient (r), and the lowest RMSD values. Further validations of pharmacophore models were carried out with the test set compounds and Fisher's randomization test.

Validation of the pharmacophore model

Cost analysis

The Catalyst software package provides statistical cost values in bit units in order to show the validity of the hypothesis. The cost of a theoretical ideal hypothesis is called the "fixed cost," which represents the simplest model

Table 2 The ten IKKβ inhibitor pharmacophore hypotheses generated by the HypoGen algorithm

Hypo. no	Total cost	Δ cost [a]	RMSD (Å)	Training set correlation (r)	Features[b]
1	105.068	40.21	0.899	0.93	DRRH
2	106.493	38.785	0.94	0.925	DRRH
3	106.641	38.637	0.948	0.923	DRRH
4	107.476	37.802	1.001	0.913	DRRH
5	110.645	34.633	1.164	0.878	DRHH
6	110.836	34.442	1.173	0.876	DRHH
7	113.771	31.507	1.283	0.849	DARH
8	114.979	30.299	1.308	0.844	DRRH
9	116.168	29.11	1.341	0.835	DRRH
10	116.228	29.05	1.369	0.826	DARH

[a] Δ cost = null cost – total cost; null cost=145.278, fixed cost= 94.6653; weight for hypothesis 1= 2.22678; configuration cost= 16.1773; all cost units are in bit units

[b] D, hydrogen bond donor; R, ring aromatic; H, hydrophobic feature; A, hydrogen bond acceptor

Table 3 Compounds used to asses the sensitivity and specificity of the pharmacophore model

	Drug targets included in the database	Number of compounds
	Kinase	
1	IKK	330
2	Aurora	210
3	Bcr-Abl	73
4	CDK	22
5	GSK	370
6	MAPK	1272
7	PKC	628
8	JAK	68
9	EGFR	585
	Non-kinase	
10	COX	701
11	GPCR	1417
	Total	5676

that fits all data perfectly. The "null cost" is the highest cost of a pharmacophore with no features and with an estimated activity that is the average of the activity data of the training set molecules. Statistically reliable pharmacophore models should not exhibit a difference between these two values (null cost and fixed cost) of >20. "Configuration/ entropy cost" is a cost that depends on the complexity of the hypothesis space being optimized. Therefore, as the cost of the hypothesis decreases, fewer bits are required to generate it, and the model becomes simpler.

Test set activity estimation

The pharmacophore model's efficiency at estimating the activities of new compounds must be confirmed by applying it to test set compounds. The compounds that were not used in model generation were used as the internal test set. The activities of these 136 test set compounds were estimated by the pharmacophore model (see the "Electronic supplementary material"). The best pharmacophore (Hypo 1), which had a high correlation

coefficient (r), the lowest total cost, and the lowest RMSD value, was utilized for test set activity estimation based on a "score hypothesis" method. Each test set compound activity was estimated based on a fit value calculation. In the HypoGen model, the fit function not only checks the function mapping; it also considers the distance term, which measures the distance from the function on the molecule to the centroid of the function on the pharmacophore hypothesis.

Fisher's randomization test

The goal of this test is to check whether there is a strong correlation between chemical structures and biological activity. The test is based on randomly reassigning activity values to the molecules in the training set. This random reassignment of activity values is performed by the catScramble module of Catalyst. If the randomized data generate pharmacophores with similar or better cost values, RMSDs, and correlations than the original hypothesis, the original hypothesis is considered to have been generated by

Fig. 3 Pharmacophore model 1 (Hypo 1), showing the four different features as a solid model, and a distance map depicting inter-feature distances
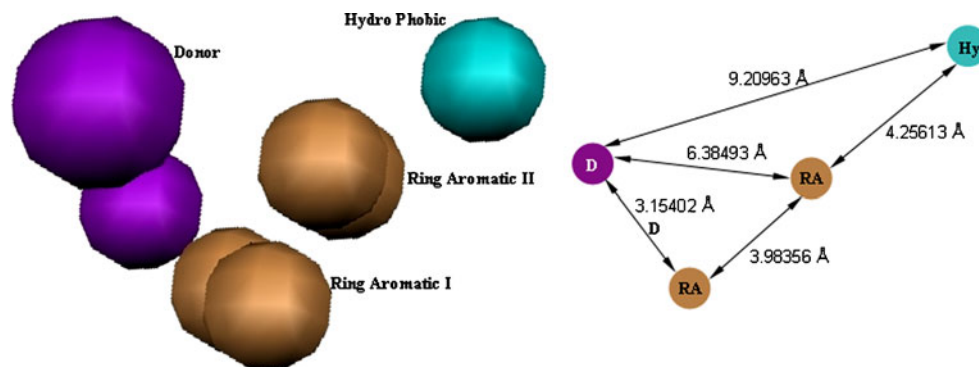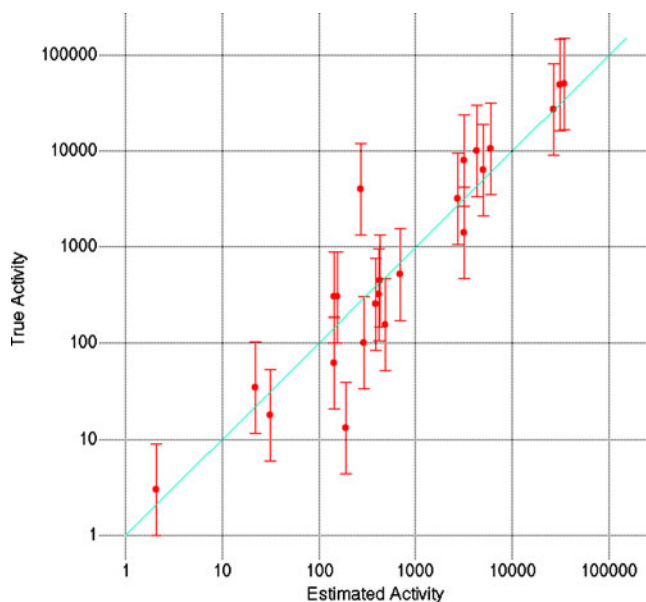
Fig. 4 Analysis of the correlation ($r=0.93$) between the experimental activities of the training set compounds and the activities estimated using hypothesis 1

chance. The statistical significance is given by the equation of significance: $= [1 - (1 + x)/y]$, where x is the total number of hypotheses that have total costs that are lower than the most significant original hypothesis, and y is the number of initial HypoGen runs plus random runs [18]. Nineteen different HypoGen runs were carried out at the 95% confidence level using the same features and parameters. Nineteen different hypotheses were generated with the scrambled activity data. Each hypothesis exhibited a correlation value, an RMSD, and other costs, as it was generated to unscramble the hypothesis.

IKKβ specificity and selectivity

The real use of the pharmacophore model is to pick IKKβ inhibitor-like molecules from a large database. Hence, to prove the specificity and selectivity of the pharmacophore model, we calculated the enrichment factor of the pharmacophore. A Catalyst database was created containing 5676 compounds collected from the Integrity database (http://www.prous.com/) which includes compounds that target IKKβ and eight different kinases along with the non-kinase compounds COX and GPCR. Enrichment factor calculations of the database can estimate the pharmacophore's specificity and selectivity towards the target. Hypo 1 was compared to the compounds in the multiconformer database. To assess its specificity and selectivity, various kinase inhibitors that exhibit close structural similarities to IKKβ inhibitors were also added to the database (Table 3). The pharmacophore model was compared to the compounds in this database, and the compounds were sorted according to how well they fitted the model. The top level of the database was sampled in certain intervals and the percentage of the enrichment factor (EF) was calculated as follows:

$$EF(\%) = \left(N_{active(\%)} \times N_{all}\right)/N_{(\%)} \times N_{active}\right) \tag{1}$$

$N_{active(\%)}$ is the proportion of actives found in $x\%$ of database sampled, N_{all} is the number of compounds used in the test, $N_{(\%)}$ is the $x\%$ of the compounds used in the calculation of EF (%), and N_{active} is the number of active compounds used in the calculation of the enrichment factor [19].

Results and discussion

Pharmacophore model

The HipHop model generated based on the six highly active compounds suggested features needed for IKKβ inhibition. HypoGen pharmacophores models were generated based on the 23 different training set compounds. In total, ten pharmacophore models were generated, along with their estimated activities, correlations between experimental and

Fig. 5 Mapping pharmacophore 1 to two training set compounds. **a** Mapping to the highly active compound **21** (imidazothieno-pyrazine derivative, 3 nM) gives a fit value of 9.1501. **b** Mapping to the inactive compound **10** (thienopyridine derivative, 50,000 nM) results in partial feature mapping and a reduced fit value of 4.5654
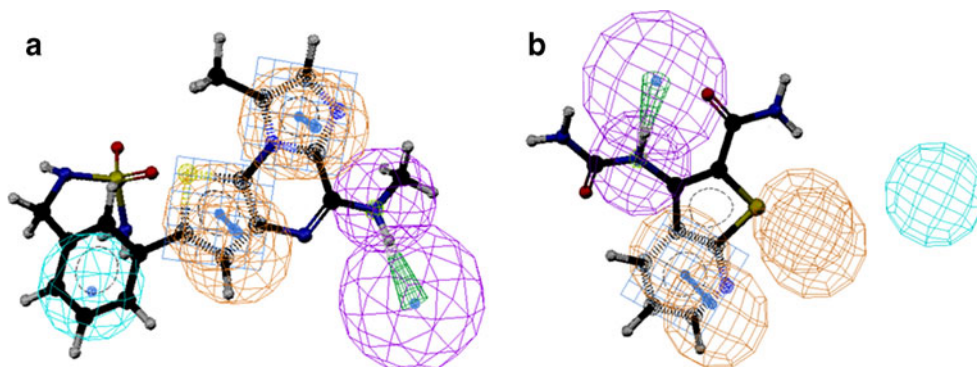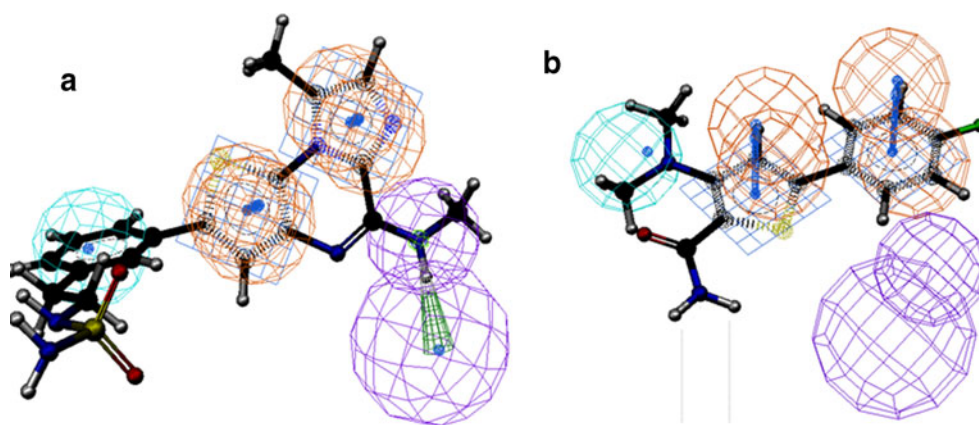
Fig. 6 Mapping pharmacophore 1 to two test set compounds. **a** Mapping to a highly active compound (imidazothienopyrazine derivative, 7 nM) gives a fit value of 9.12973. **b** Mapping to an inactive compound (thiophene derivative, 23,500 nM) results in partial feature mapping and a reduced fit value of 6.15365

estimated activities, RMSDs, and other cost values. The top ranked HypoGen models did not exhibit the entire four features suggested by the HipHop model. The most relevant pharmacophore exhibited one hydrogen bond donor feature, one hydrophobic feature, and two ring aromatic features (Fig. 3, Table 2). Hypotheses 7 and 10 exhibited all of the features of the HipHop model, but these models did not correlate well with the experimental data.

The best pharmacophore model has the highest cost difference, the lowest RMSD, and the best correlation coefficient [20]. Pharmacophore 1 had the lowest RMSD value of 0.89 Å, and showed good correlation ($r=0.93$) between the experimental and estimated activity data of the training set (Fig. 4). The difference between the total cost and the null hypothesis cost was 40.21; thus, there is a good chance that the model is a true representation of the data (if the difference is 40–60 bits, there is a 75–90% chance that it represents the data well) [21]. The difference between the null and fixed costs was more than 50. The configuration cost was 16.1773, which is less than the maximum threshold of 17. Cost analysis confirms the statistical relevance of pharmacophore 1 as a reliable model that can precisely forecast IKKβ inhibitory activity.

During training set estimation, compounds **4** and **5** were actually highly active (+++) but were underestimated as active (++), **19** was actually moderately active (+) but was overestimated as active (++), and **8** and **13** actually fell into the inactive (−) category but were estimated to be moderately active (+). However, the differences between the actual and estimated activities were generally not large—around an order of magnitude. Figures 5 and 6 show how hypothesis 1 maps to representative highly active and inactive compounds from the training and test sets. All of the features are mapped perfectly for the highly active compounds, whereas the features of inactive compounds are not mapped well. The inactivity of these compounds may thus be due to the lack of one of the features present in the active compounds.

Estimating the activities of test set molecules

The best pharmacophore model, Hypo 1, was validated by estimating the activities of 136 compounds with an activity range spanning four orders of magnitude. The model estimated the activities of the compounds fairly well; the correlation (r) between the experimental and estimated values was 0.77. Thus, this pharmacophore can estimate over 77% of the true activity (see Fig. 7 and the "Electronic supplementary material"). The activities of some compounds were either overestimated or underestimated, which may be an artifact of the program, which uses a different number of degrees of freedom that can cause reduction to accuracy of the pharmacophore. However, the overall discrepancy between the estimated and actual activities observed for the test set compounds was not great.
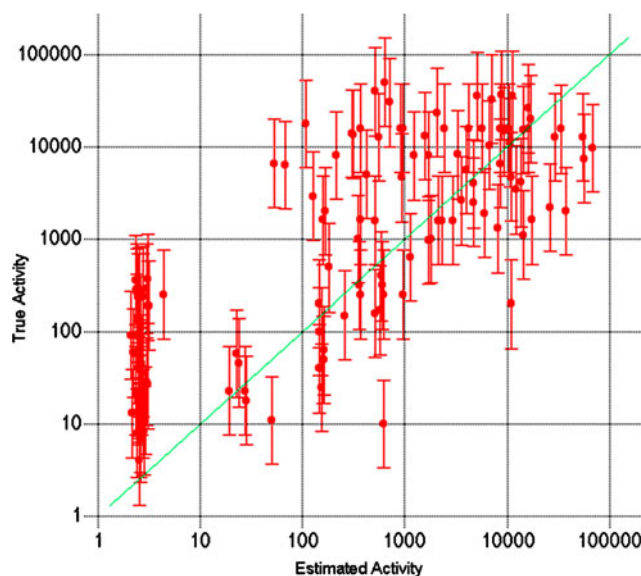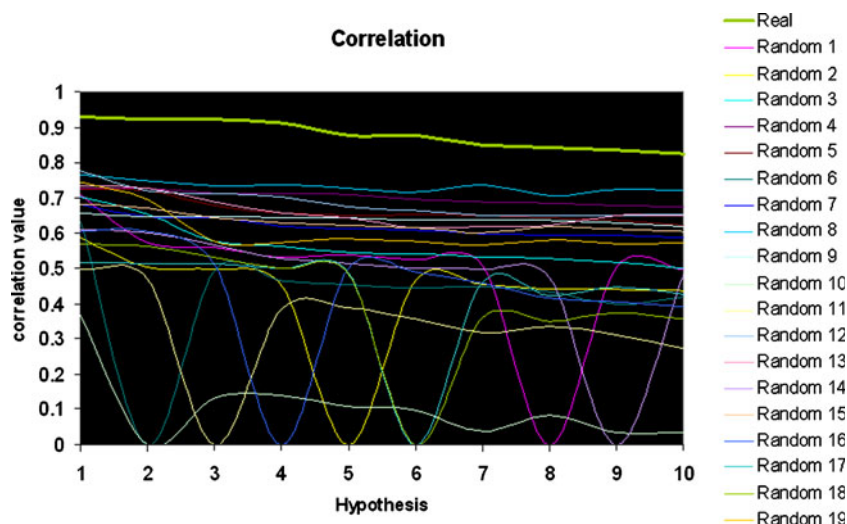

Fig. 7 Correlation ($r=0.77$) between the experimental activities of the test set compounds and the activities estimated by hypothesis 1

Fig. 8 Fisher's randomization test results. Correlation values for a particular pharmacophore across the tests correspond to a line. The *topmost line* shows the correlation values for the unscrambled pharmacophore

Randomization test

Fisher's randomization test of scrambled IC_{50} values of the test set compounds was conducted. Nineteen different randomizations at the 95% confidence level were performed, giving 19 different HypoGen models. The correlation values of these 19 models are plotted along with those of the true hypothesis in Fig. 8; interestingly, none of the HypoGen models generated with randomized activity data gave higher correlation values than those of the true pharmacophore hypothesis. Moreover, none of these 19 new hypotheses had lower cost values and RMSD values than the true hypothesis. This test shows that this pharmacophore hypothesis was not generated by chance, and that there is a probability of at least a 95% that it shows a valid correlation between structure and $IKK\beta$ inhibitory activity [22].

Enrichment calculation

Sampling the database at different levels allows us to explore the specificity and selectivity of a pharmacophore. Top-level sampling at 1% yielded 97% true positives; only one COX inhibitor was reported and no other kinase compounds were included. At 5% database sampling, two PKC inhibitors were included, along with a few COX and GPCR compounds. Figure 9 shows the enrichment factor (EF%) plotted at each level of database sampling. Most of the true compounds ($IKK\beta$ inhibitors) were retrieved from the database at the top level of database sampling. Therefore, this analysis shows that the pharmacophore has very high specificity and selectivity, meaning that it would be a very useful tool for virtual screening.

Conclusions

Research aimed at identifying novel and selective $IKK\beta$ inhibitors is ongoing in anti-inflammatory drug research. In the absence of structural information on the $IKK\beta$ protein, a ligand-based approach to virtual screening is the only alternative. The use of a pharmacophore hypothesis to estimate the activities of other compounds with similar receptor binding behaviors is a powerful concept. A useful hypothesis (i.e., model) allows prospective synthetic targets to be tested, and so the compounds that are most likely to possess desirable activities can be assessed before actually committing to synthesis. In the present work, we developed a 3D QSAR
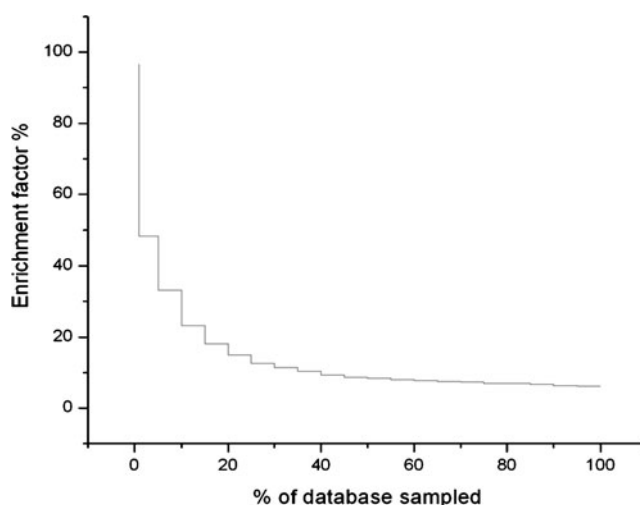

Fig. 9 The enrichment factor (in %) plotted at each level of the database sampled. Top-level database sampling (1%) yields the highest $IKK\beta$ inhibitor hit rate

pharmacophore model based on 23 IKKβ-selective inhibitors using a ligand-based computational approach. To our knowledge, this is the first quantitative pharmacophore model that has been developed for IKKβ using diverse chemical structures. This pharmacophore model consists of a hydrogen bond donor feature (D), a hydrophobic feature (Hy), and two ring aromatic (RA) features. Activities estimated with this four-feature pharmacophore show the highest correlation with experimental activity data for compounds in both the training set ($r=0.93$) and the test set ($r=0.77$). It also exhibits good accuracy in discriminating between various classes of IKKβ inhibitors. Pharmacophore validation results demonstrate that the hypothesis derived in this study can be considered to be a useful and reliable tool for identifying structurally diverse compounds with the desired biological activity. However, the inhibitory power of a molecule is not based solely on the features identified by the pharmacophore model; other moieties present in the compound also perform an important role in determining its activity. On the other hand, this pharmacophore model can guide the drug discovery process when used in combination with docking methods to further refine the hits obtained during virtual screening and increase the likelihood of selecting a compound that shows IKKβ inhibition. Applying the developed pharmacophore features to the virtual screening of a large database can result in a higher number of selective compounds and greatly reduce the number of false positives. Hence, the pharmacophore model generated in this work could be very helpful for identifying IKKβ-specific inhibitors.

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