In silico spleen tyrosine kinase inhibitor screening by chooseLD

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Abstract: Background: Spleen tyrosine kinase (SYK) is a protein related to various diseases. Aberrant SYK expression often causes the progression and initiation of several diseases including cancers and autoimmune diseases. Despite the importance of inhibiting SYK and identification of candidate inhibitors, no clinically effective inhibitors have been reported to date. Therefore, there is a need for novel SYK inhibitors.
Results: Candidate compounds were investigated using *in silico* screening by chooseLD, which simulates ligand docking to proteins. Using this system, known inhibitors were correctly recognized as compounds with high affinity to SYK. Furthermore, many compounds in the DrugBank database were newly identified as having high affinity to ATP binding sites in the kinase domain with a similar affinity to previously reported inhibitors.
Conclusions: Many drug candidate compounds from the DrugBank database were newly identified as inhibitors of SYK. Since compounds registered in DrugBank are expected to have fewer side effects than currently available compounds, these newly identified compounds might be clinically useful inhibitors of SYK for the treatment of various diseases.

1. Introduction

Spleen tyrosine kinase (SYK) has been a drug target since it was identified as a disease-related non-receptor kinase [1]. SYK regulates many key-factor proteins that are involved in the initiation of progression of various diseases. The gendoo[2], [3] server lists many diseases reported to be related to SYK (Table 1). The deletion of SYK was reported to suppress the formation of immune complex arthritis[4]. SYK was also reported to be activated in diffuse large B-cell lymphoma [5]. Hypermethylation of the SYK gene promoter region was reported to be associated with oncogenesis and metastasis of gastric carcinoma[6]. Furthermore, the specific inhibition of SYK was reported to suppress leukocyte immune function and inflammation in animal models of rheumatoid arthritis [7]. We recently observed that the SYK gene promoter was often aberrantly methylated in three autoimmune diseases [8]. Thus, effective SYK inhibitors are urgently required for the treatment of numerous diseases.

There are several targets for SYK inhibition. SYK consists of a C-terminal kinase domain and two Src homology 2 (SH2) domains separated by a linker domain [9]. Inhibitors that target kinase domain mainly target ATP binding sites. For example, R112, R406, R788 and R343 are structurally related pyrimidine analogs that compete with ATP binding[9].

Alternatively, some inhibitors target the SH2 domains [10]. Many compounds have been reported to inhibit SYK[11]. The inhibition of protein complex formation was also proposed[12].

Despite these reported studies, no clinically effective SYK inhibitors have been established to date. Based upon the recent developments of computational methods, many trials have identified drug candidate compounds computationally. Li *et al* [13] tried to identify SYK inhibitors using machine learning methods. Kaur *et al* [14]investigated SYK inhibitors using 3D-Quantitative Structure Activity Relationship (QSAR) and Xie *et al* [15] used chemical features based on 3D pharmacophore models. Although listed numbers of drug candidate compounds were identified, they were based upon features extracted from candidate compounds, thus the estimation of drug ability was indirect. To our knowledge, there have been no comprehensive screens of compounds that target the kinase domain using docking-based prediction.

This study evaluated the docking affinity between SYK and over 1,000 compounds extracted from the DrugBank database and ranked these based on their binding affinity using chooseLD, a docking-based *in silico* drug-screening software. Top-ranked compounds were promising SYK inhibitor drug candidate compounds.

Materials and Methods

Tertiary structure prediction of SYK

Tertiary structure prediction of SYK was performed by Full Automatic Modeling System (FAMS)[16], [17]. The amino acid sequence of SYK (uniprot ID P43405.1) in fasta format obtained from uniprot[18] was uploaded to an isolated FAMS server. Then, the obtained top-ranked model proteins modeled using the Protein Data Bank (PDB) structure 3VF8_A (SYK) were regarded to be

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Table 1 Diseases reported to be related to SYK by gendoo server.

Diseases	*P*-values
Breast Neoplasms	3.92×10^{-9}
Arthus Reaction	5.09×10^{-7}
Lymphoma, B-Cell	1.79×10^{-6}
Neoplasm Metastasis	7.67×10^{-6}
Inflammation	8.28×10^{-5}
Agammaglobulinemia	2.38×10^{-4}
Lymphoma, Extranodal NKT-Cell	4.91×10^{-4}
Leukemia	5.00×10^{-4}
Neoplasm Invasiveness	5.38×10^{-4}
Purpura	9.13×10^{-4}
Gonorrhea	1.33×10^{-3}
Lymphoma, Large B-Cell, Diffuse	1.38×10^{-3}
Ehrlichiosis	1.47×10^{-3}
Lymphoma	3.86×10^{-3}
Leukemia, Lymphocytic, Chronic, B-Cell	3.33×10^{-3}
Lymphedema	3.86×10^{-3}
Lymphoma, T-Cell, Peripheral	5.46×10^{-3}
Leukemia, Basophilic, Acute	6.09×10^{-3}
Mediastinal Neoplasms	7.41×10^{-3}
Carcinoma, Ductal	7.56×10^{-3}
Urticaria	7.77×10^{-3}
Autoimmune Diseases	7.84×10^{-3}
Rhinitis	9.02×10^{-3}
Lymphoma, T-Cell, Cutaneous	9.23×10^{-3}
Synovitis	9.37×10^{-3}
Breast Neoplasms, Male	9.92×10^{-3}
Lymphoma, Large-Cell, Anaplastic	1.14×10^{-2}
Precursor B-Cell Lymphoblastic Leukemia-Lymphoma	1.31×10^{-2}
Leukemia, B-Cell	1.38×10^{-2}
Peritonitis	1.62×10^{-2}
Lymphatic Metastasis	1.69×10^{-2}
Nasal Polyps	1.80×10^{-2}
Bronchial Hyperreactivity	1.81×10^{-2}
Mammary Neoplasms, Animal	2.00×10^{-2}
Carcinoma, Intraductal, Noninfiltrating	2.12×10^{-2}
Arthritis, Rheumatoid	2.25×10^{-2}
Edema	2.34×10^{-2}
Arthritis, Experimental	2.42×10^{-2}
Vasculitis	2.67×10^{-2}
Wiskott-Aldrich Syndrome	2.80×10^{-2}
Stomach Neoplasms	3.09×10^{-2}
Melanoma	3.11×10^{-2}
Melanoma, Experimental	3.55×10^{-2}
Immunologic Deficiency Syndromes	3.63×10^{-2}
Hodgkin Disease	4.28×10^{-2}
Bacterial Infections	4.40×10^{-2}
Shock, Septic	4.70×10^{-2}

drug discovery template candidates.

Drug compound candidates

A total of 6,583 compounds included in DrugBank[19], [20] were downloaded. Of these, 6,510 tertiary structures were produced using Babel[21] software. Then 1,043 compounds with a Tanimoto index greater than 0.25, with at least one of 11 ligands that were reported to bind to one of 11 template proteins included in PDB, were selected as drug compounds candidates. The 11 template protein PDB IDs together with ligand IDs are listed in Table 2.

In silico **screening**

In silico screening was performed using a template-based ligand docking simulation program, chooseLD[22]. In chooseLD, the ligand affinity to SYK was evaluated based upon comparisons with 11 known ligand compounds (Table 2). If the ligands tested were well aligned with known ligand compounds, the ligand was given a high ranked score, i.e., FingerPrint Alignment Scores (FPAScores). Then, all tested compounds were ranked based upon their attributed FPAScores. During this simulation, atom types were modified to achieve more accurate FPAScores. Three independent trials were performed for each compound and the mean FPAScores were used to rank the compounds.

Table 2 Eleven template proteins with ligands used for *in silico* screening.

protein PDB	name	ligand ID	name
4DFN	SYK	0K1	3-amino-6-[3-(1-methyl-1H-pyrazol-4-yl)phenyl]-N-[(1R,2r,3S,5s,7s)-5-hydroxyadamantan-2-yl]pyrazine-2-carboxamide
3FQE	SYK	P5C	2-{[(1R,2S)-2-aminocyclohexyl]amino}-4-[(3- methylphenyl)amino]pyrimidine-5-carboxamide
1XBB	SYK	STI	IMATINIB
1XBC	SYK	STU	STAUROSPORINE
3VF8	SYK	0JE	3-[5-(5-ethoxy-6-fluoro-1H-benzimidazol-2-yl)-1H-pyrazol-4-yl]-1,1-diethylurea
3VF9	SYK	477	3-{2-[5-(difluoromethyl)-2H-thieno[3,2-c]pyrazol-3-yl]-1H-indol-6-yl}pentan-3-ol
3SRV	SYK	S19	GSK143
4DFL	SYK	0K0	3-amino-6-{3-[(methylsulfonyl)amino]phenyl}-N-(piperidin-4-ylmethyl)pyrazine-2-carboxamide
3FQH	SYK	057	N-(2-hydroxy-1,1-dimethylethyl)-1-methyl-3-(1H-pyrrolo[2,3-b]pyridin-2-yl)-1H-indole-5-carboxamide
3EMG	SYK	685	2-{2-[(3,5-dimethylphenyl)amino]pyrimidin-4-yl}-N-[(1S)-2-hydroxy-1-methylethyl]-4-methyl-1,3-thiazole-5-carboxamide

Evaluation set

To evaluate the performance of chooseLD in estimating the binding affinity to SYK, 12 compounds whose absolute inhibition constant Ki values were listed in ChEMBL[23], [24] were downloaded (see Table 3).

Table 3 Twelve compounds from ChEMBL used to evaluate the chooseLD performance to estimate ligands' binding affinity to SYK.

CHEMBL ID	Ki (nM)
CHEMBL553	1584.89
CHEMBL49120	79.43
CHEMBL340384	1584.89
CHEMBL422897	794.33
CHEMBL196363	1258.93
CHEMBL7064	63.1
CHEMBL211378	1258.93
CHEMBL213505	501.19
CHEMBL262433	398.11
CHEMBL379975	6.31
CHEMBL1421	199.53
CHEMBL243088	63.1
CHEMBL244378	10
CHEMBL396523	31.62

Validation of evaluation set using SwissDock

To evaluate the performance of chooseLD, compounds in the evaluation set was also tested by SwissDock [25], [26]. Compound structures were computed from canonical Simplified Molecular-Input Line-Entry System (SMILES) using open babel[21] and were uploaded to SwissDock as the ligand structure. For target protein structures, model protein structures inferred by FAMS using 3VF8_A as a reference protein were uploaded to

SwissDock. Minimum dGs for each compound were employed for evaluations.

Results

To perform *in silico* drug screening of SYK, the tertiary structure of the SYK protein must be determined. To infer the SYK tertiary structure, we used FAMS[16], [17]. SYK (uniprot ID P43405.1) has a length of 635 amino acids. Using 2OZ0_A (Flavocytochrome b2) as a reference protein, 625 amino acids of a total of 635 amino acids in SYK were successfully modeled (*E*-value obtained by Blast search was 1×10^{-170}), and the sequence similarity between 2OX0 and SYK was 50%. Amino acids 363-635 of SYK were modeled using 3VF8_A (SYK) (*E*-value obtained by blast search is 1×10^{-95}). Comparison of the model structures based on 2OZ0_A or 3VF8_A showed no significant difference within the commonly predicted regions of the protein. Because the ATP binding region was included in both models, we used a model protein structure based on 3VF8_A for *in silico* screening.

In addition, a binding ligand, 0JE (for more details, see Table 2) has been described for 3VF8_A. To use a tertiary structure as a template for ligand docking, a reference protein must have a ligand that binds to itself.

Following the procedure described in the Materials and Methods, we successfully obtained ranking for 1,043 compounds based on FPAScores (top-ranked 20 compounds are listed in Table 4. A full list of ranked compounds is available as additional file 1).

Discussion

Evaluation of top-ranked compounds

The 20 top-ranked compounds shown in Table 4 were reported as kinase inhibitors in either the DrugBank or ChEMBL databases. Table 4 also includes four SYK inhibitors (DB07194, CHEMBL512172 (cmpd_648) ranked 3rd; DB04739, CHEMBL56904 (cmpd_507) ranked 4th; DB06834, CHEMBL1229525 (cmpd_550) ranked 12th; DB07545, CHEMBL383899 (cmpd_744) ranked 17th) excluding the known SYK inhibitor, imatinib, ranked 2nd. As a result, five of the 20 top-ranked compounds were identified as SYK inhibitors. Thus, the remaining 15 compounds were also expected to be SYK inhibitors.

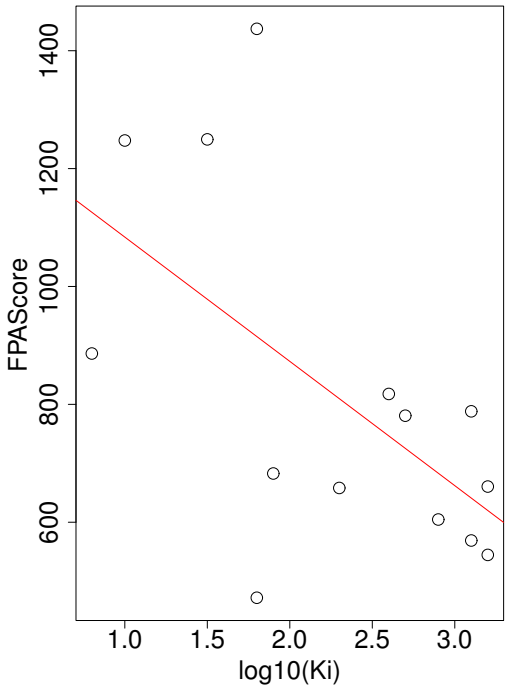
Comparisons with known SYK inhibitors' binding affinity

Although top-ranked compounds were candidate SYK inhibitors, it is important that ranking based upon FPAScores is validated using independent samples. For this purpose, we prepared a validation set of compounds (see Materials and Methods). Fig. 1 shows comparisons between the FPAScore and Ki values. Since smaller Ki values indicate a larger binding affinity, significant negative correlation observed shows that chooseLD correctly determined the binding affinity of compounds to SYK in the validation set.

In addition, the largest FPAScores attributed to compounds having the smallest Ki in the validation set (Fig. 1, vertical axis) were at most 1,400 to 1,500. As seen in Table 4, there were at least 10 compounds with FPAScores falling in this range, suggesting that the top-ranked compounds listed in Table 4 are promising SYK inhibitors.

Finally, to verify and demonstrate the superiority of chooseLD,

(a)



(b)

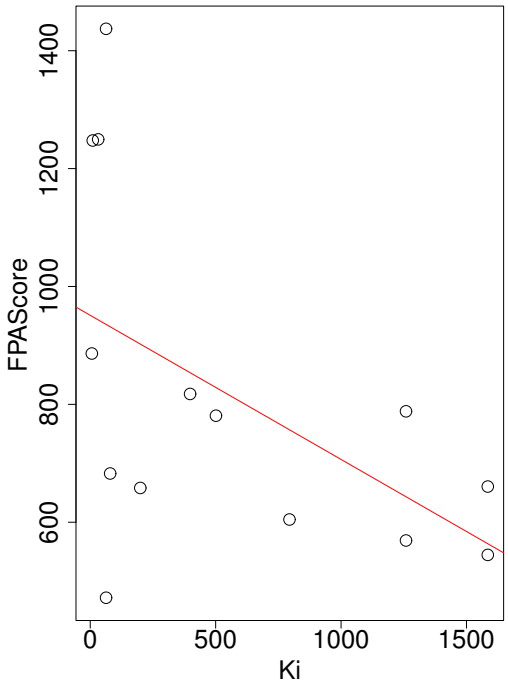


Fig. 1 Comparison between Ki and FPAscores (a) Vertical: FPAScores, horizontal: $\log_{10} Ki$. Pearson's correlation coefficient is -0.58 (P = 0.0278). (b) Vertical: FPAScores, horizontal: Ki. Spearman's correlation coefficient is -0.58 (P = 0.030). Red lines indicate regression line.

Table 4 Twenty top-ranked compounds based on FPAScores.

Rank	FPAScore	”Drug Name (cmpd No.)”	”DrugBank No.; CHEMBL No.”	Target/Activity
1	1583.5	”N-[(1S)-2-amino-1-phenylethyl]-5-(1H-pyrrolo[2,3-b]pyridin-4-yl)thiophene-2-carboxamide (cmpd_807)”	”DB07812; CHEMBL471034”	”—-”;”RAC-beta serine/threonine-protein kinase, Glycogen synthase kinase-3 beta, Inhibition of AKT1”
2	1481	”Imatinib (cmpd_55)”	”DB00619; CHEMBL941”	”—-”;”Inhibitor of BCR/ABL fusion protein isoform X9, Antagonist of Alpha and Beta platelet-derived growth factor receptor, Inhibitor of Proto-oncogene tyrosine-protein kinase ABL1, Inhibition of PTK, Abl, carbonic anhydrase, CSF1R, PDGFR α, LYN, LCK, FRK, 5HT2A, MAPK10, and BLK”
3	1468	”2-{2-[(3,5-dimethylphenyl)amino]pyrimidin-4-yl}-N-[(1S)-2-hydroxy-1-methylethyl]-4-methyl-1,3-thiazole-5-carboxamide (cmpd_648)”	”DB07194; CHEMBL512172”	”—-”;”Tyrosine-protein kinase **SYK**, Inhibition of **SYK**, ZAP70, ROCK, SRC, and CDK2”
4	1456.3	”4-[(4-methyl-1-piperazinyl)methyl]-N-[3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]phenyl]-benzamide (cmpd_507)”	”DB04739; CHEMBL56904”	”—-”;”Proto-oncogene tyrosine-protein kinase Src, Inhibition of **SYK**, v-Abl tyrosine kinase, c-Src-tyrosine kinase, and platelet-derived growth factor”
5	1436.4	”N-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]phenyl]-3-pyridinecarboxamide (cmpd_413)”	”DB03878; —”	”—-”;”Proto-oncogene tyrosine-protein kinase ABL, —-”
6	1436	”4-[4-(1-amino-1-methylethyl)phenyl]-5-chloro-N-[4-(2-morpholin-4-ylethyl)phenyl]pyrimidin-2-amine (cmpd_242)”	”DB02491; CHEMBL233209”	”—-”;”Fibroblast growth factor receptor 2, Inhibition of VEGFR2, CDK1, and HeLa, A375, HCT116 cells”
7	1432.1	”4-[4-(4-methyl-2-methylamino-thiazol-5-Yl)-pyrimidin-2-ylamino]-phenol (cmpd_472)”	”DB04407; CHEMBL47590”	”—-”;”Cell division protein kinase 2, Inhibition of Cyclin-dependent kinase 2 (CDK2), CDK4, and Plk1”
8	1418.1	”N-(2-methoxyethyl)-4-({4-[2-methyl-1-(1-methylethyl)-1H-imidazol-5-YL]pyrimidin-2-YL}amino)benzenesulfonamide (cmpd_799)”	”DB07790; CHEMBL478409”	”—-”;”Cell division protein kinase 2, Inhibition of Cyclin-dependent kinase 2 (CDK2), CyclinE, CDK4, MCF7 and LoVo cells”
9	1405.4	”4-(4-chlorobenzyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidin-4-aminium (cmpd_908)”	”DB08150; —”	”cAMP-dependent protein kinase inhibitor alpha”:” —-”
10	1391.6	”4-(2,4-dimethyl-thiazol-5-Yl)-pyrimidin-2-Yl]-(4-trifluoromethyl-phenyl)-amine (cmpd_294)”	”DB02915; CHEMBL48109”	”—-”;”Cell division protein kinase 2 and Cyclin-A2, Inhibition of Cyclin-dependent kinase 2 (CDK2), CDK4, and A549, HT-29, SaOS-2 tumor cells”
11	1379.7	”4-[(4-imidazo[1,2-a]pyridin-3-Ylpyrimidin-2-Yl)amino]benzenesulfonamide (cmpd_214)”	”DB02197; CHEMBL73303”	”—-”;”Cell division protein kinase 2, Inhibition of Cyclin-dependent kinase 2 (CDK2), 1GF1R, and MCF7 cells”
12	1352.2	”N-(2-hydroxy-1,1-dimethylethyl)-1-methyl-3-(1H-pyrrolo[2,3-b]pyridin-2-yl)-1H-indole-5-carboxamide (cmpd_550)”	”DB06834, CHEMBL1229525”	”—-”;”Tyrosine-protein kinase **SYK**, Inhibition of **SYK**”
13	1335.4	”3-[4-(2,4-dimethyl-thiazol-5-Yl)-pyrimidin-2-ylamino]-phenol (cmpd_486)”	”DB04518; CHEMBL47527”	”—-”;”Cell division protein kinase 2, Inhibition of Cyclin-dependent kinase 2 (CDK2) and CDK4”
14	1334	”(2R)-1-[(5,6-diphenyl-7H-pyrrolo[2,3-D]pyrimidin-4-YL)amino]propan-2-ol (cmpd_770)”	”DB07647; CHEMBL371415”	”—-”;”Serine/threonine-protein kinase Chk1, Inhibition of serine/threonine-protein kinase Chk1, cyclin-dependent kinase 1 (CDK 1), and protein kinase A (PKA)”
15	1312.7	”(2R)-3-{[(4Z)-5,6-diphenyl-6,7-dihydro-4H-pyrrolo [2,3-D] pyrimidin-4-ylidene]amino}propane-1,2-diol (cmpd_771)”	”DB07648, CHEMBL372247”	”—-”;”Serine/threonine-protein kinase Chk1, Inhibition of serine/threonine-protein kinase Chk1, cyclin-dependent kinase 1 (CDK 1), and protein kinase A (PKA)”
16	1311.1	”[4-(2-amino-4-methyl-thiazol-5-Yl)-pyrimidin-2-Yl]-(3-nitro-phenyl)-amine (cmpd_281)”	”DB02833; CHEMBL298445”	”—-”;”Cell division protein kinase 2 and Cyclin-A2, Inhibition of Cyclin-dependent kinase 2 (CDK2), CDK9, CDK4, CDK7, CDK1, GSK3-beta, Aurora A/B, and Abl Kinase”
17	1301.4	”N-{3-[(4-{[3-(trifluoromethyl)phenyl]amino}pyrimidin-2-YL)amino]phenyl}cyclopropanecarboxyamide (cmpd_744)”	”DB07545; CHEMBL383899”	”—-”;”Serine/threonine-protein kinase 6, Inhibition of Aurora Kinase A, Lck, Bmx, IGF1R, **SYK**, and EGFR”
18	1287.7	”K-252a (cmpd_209)”	”DB02152; CHEMBL281948”	”—-”;”Hepatocyte growth factor receptor and Dual specificity mitogen-activated protein kinase kinase 1, Inhibition of trka, VEGFR, protein kinase C, and myt1 kinase”
19	1279.9	”1-(dimethylamino)-3-(4-{{4-(2-methylimidazo[1,2-A]pyridin-3-YL)pyrimidin-2-YL]amino}phenoxy)propan-2-ol (cmpd_828)”	”DB07889, CHEMBL102926”	”—-”;”Cell division protein kinase 2, Inhibition of Cyclin-dependent kinase 1 (CDK1), CDK2, and CDK4”
20	1276.9	”2-{4-[4-({4-[2-methyl-1-(1-methylethyl)-1H-imidazol-5-yl]pyrimidin-2-yl}amino)phenyl]piperazin-1-yl}-2-oxoethanol (cmpd_854)”	”DB07982; CHEMBL477786”	”—-”;”Cell division protein kinase 2, Inhibition of CDK2, MCF7 cells and ERG”

SwissDock[25], [26] was used for validation (Fig. 2). Although dGs (the amount of Gibbs free energy reduction due to ligand binding) were negatively correlated to Ki as expected, the correlation coefficients were not significant. This suggests that candidate compounds identified by chooseLD were superior compared with those identified by SwissDock.

Conclusion

This study performed comprehensive *in silico* drug screening for SYK using chooseLD software. Top-ranked drug candidate compounds were kinase inhibitors that included several reported SYK inhibitors. The performance of chooseLD was evaluated using an independent evaluation set and FPAScores inferred by chooseLD were significantly and negatively correlated to experimentally reported Ki values as expected. The significance of chooseLD was better than that of SwissDock, another *in silico* screening software. Thus, the predicted drug candidate compounds are promising new SYK inhibitors for the treatment of several diseases.

Acknowledgements

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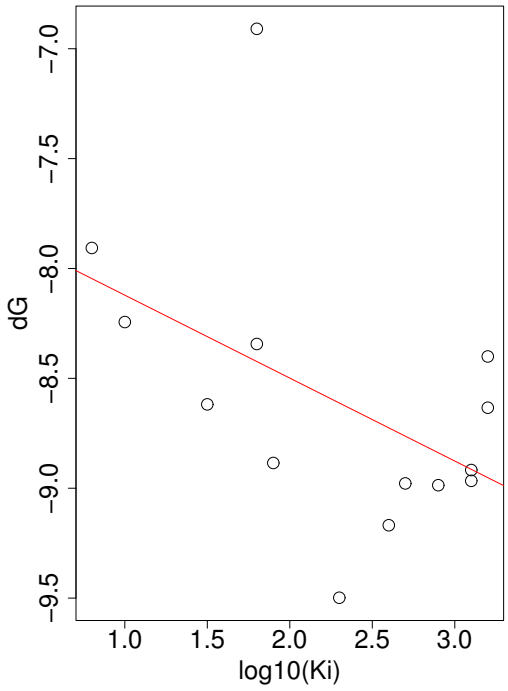
(a)



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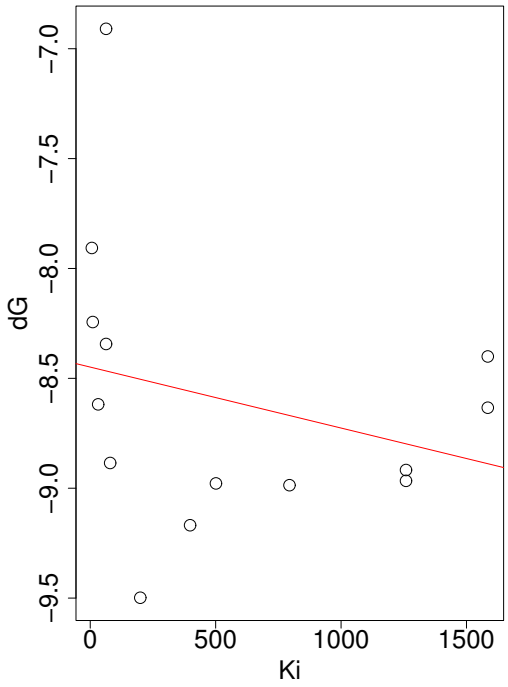

Fig. 2 Comparison between Ki and dG (a) Vertical: dG, horizontal: $\log_{10} Ki$. Pearson's correlation coefficient is -0.48 (P = 0.079). (b) Vertical: dG, horizontal: Ki. Spearman's correlation coefficient is -0.49 (P = 0.077). Red lines indicate regression line.

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Additional Files

Additional file 1 — Full list of FPAScores of drug candidate compounds

Mean FPAScores of three independent trials for 1,043 drug candidate compounds taken from DrugBank. http://dx.doi.org/10.6084/m9.figshare.1312839