Development and Experimental Validation of a Docking Strategy for the Generation of Kinase-Targeted Libraries

Rafael Gozalbes,*,† Laurence Simon,‡ Nicolas Froloff,† Eric Sartori,† Claude Monteils,† and Romuald Baudelle†

Cerep, 19 avenue du Québec, Courtaboeuf-1, Villebon sur Yvette, 91951 Courtaboeuf Cedex, France, and Cerep, Le bois l'Evêque, 86600 Celle l'Evescault, France

Received October 31, 2007

A high-throughput docking strategy for the filtering of in silico compounds and the generation of kinase-targeted libraries is described. Systematic docking and scoring in three kinase crystal 3D structures of 123 structurally diverse kinase ligands led to the determination of six thresholds for each kinase. These thresholds were used as filters for the virtual screening of two collections of compounds: a collection of more than 2500 drugs and drug-like compounds (negative control) and a kinase-targeted library of 1440 compounds. This strategy was then experimentally validated by testing 60 compounds from the kinase-targeted library on 41 kinases from five different families. The 60 compounds were split into those passing all the thresholds and the others (30 compounds in each group). The overall hit enrichment was 6.70-fold higher in the first group, validating our approach for the generation of kinase-targeted libraries and the identification of scaffolds with high kinase inhibitory potential.

Introduction

Phosphorylation of proteins, catalyzed by a kinase, was discovered in 1954 by Burnett et al.,[1] and since then, there has been strong interest in the role of protein phosphorylation in regulating protein function. Protein kinases play a crucial role in cellular proliferation, differentiation, or inflammation.[2] In addition, the most common regulatory mechanisms of protein kinases include protein localization, ligand-coupled allosteric activation or inhibition, and reversible conformational changes at the catalytic site that are controlled by phosphorylation or dephosphorylation.[3] Their improper activation or inhibition can be highly disruptive to the cell and be a major cause of cancer, and as a consequence, protein kinases have emerged as an extremely important set of targets for drug development.[4] Initially, a large number of natural compounds were found to be rather potent inhibitors of protein tyrosine kinases. Although many showed initial promise, they were found to be highly promiscuous and toxic;[5] therefore, many efforts have been devoted to the discovery of more specific compounds. Development and approval of imatinib (for chronic myeloid leukemia) and of gefitinib and erlotinib (for nonsmall cell lung cancer) have provided proof-of-principle that small molecule kinase inhibitors can be effective drugs.[6]

Combinatorial chemistry has now gained general acceptance as a tool to speed up drug discovery. To increase its efficiency, interest in library design has been recently shifted toward the generation of target-class focused libraries.[7] The possibility to design kinase targeted libraries is based on the existence of numerous docking data and on common structural features present in most kinase inhibitors.[8]

The first published crystal structure of a protein kinase was the catalytic site domain of the cyclic-AMP-dependent kinase.[9] All kinases with known structures adopt essentially the same fold consisting of an N-terminal and C-terminal domain connected through a short strand (the "hinge region"), with adenosine triphosphate (ATP) binding in the cleft between the two domains. Even when there is a low overall sequence identity in the ATP binding sites, such as for the tyrosine kinase Src and the serine threonine kinase CDK2,[a] the backbone structure is very similar.[10] It is then possible to identify a common pattern for most known kinase inhibitors, which consists of a heteroaromatic core including a hydrogen bond acceptor that interacts with the backbone NH of the "hinge" region and a variable hydrophobic pocket responsible for selectivity.

Intense activity of structural biologists in the field of kinases has given access to hundreds of crystal structures available for docking studies. Different approaches have been used for the identification of new promiscuous kinase scaffolds leading to the synthesis of kinase-targeted libraries, particularly structure and pharmacophore-based approaches. However, most published examples describe libraries designed for a single target and are not suited for the generation of class targeted libraries.[7a,11]

We describe here the development of a high-throughput docking and scoring strategy adapted to the filtering of in silico compounds and the subsequent generation of kinase targeted libraries.

Material and Methods

Crystal Structures of the Receptors. Three kinase crystal 3D structures cocrystallized with a kinase inhibitor in the ATP site were selected from the Protein Data Bank (PDB)[12] for docking (Figure 1):

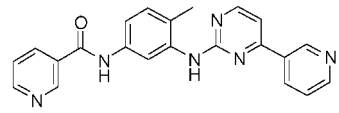
* To whom correspondence should be addressed. Current address: Structural Biology Laboratory, Medicinal Chemistry Department, Centro de Investigación Príncipe Felipe (CIPF), Avda. Autopista del Saler 16, 46012 VALENCIA, Spain. Phone: +34 963289681. Fax: +34 963289701. E-mail: rgozalbes@cipf.es.

† Cerep, Courtaboeuf Cedex, France.

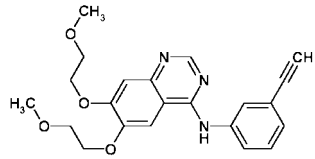
‡ Cerep, Celle l'Evescault, France.

[a] Abbreviations: Abl, Abelson tyrosine kinase; AGC, protein kinases A, G, and C (cyclic nucleotide regulated and phospholipid-regulated kinases and ribosomal S6 kinases); BSA, bovine serum albumin; CaMK, Ca^{2+}/calmodulin-dependent kinases; CDK, cyclin dependent kinases; CGMC, cyclin-dependent and mitogen-activated kinases; CTK, cytosolic tyrosine kinases; DIEA, *N*,*N*-diisopropylethylamine; DTT, 1,4-dithiothreitol; EDTA, ethylene-diamine-tetracetic acid; EGFR, epidermal growth factor receptor kinase; Fak, focal adhesion kinase; HTRF, homogeneous time-resolved fluorescence assays; Lck, lymphocyte-specific protein tyrosine kinase; PDB, Protein Data Bank; PDGFR, platelet derived growth factor receptor; PLP, piecewise linear potential; PMF, potential of mean force; RTK, receptor tyrosin kinases; Src, sarcoma tyrosine kinase; TBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; THF, tetrahydrofuran.

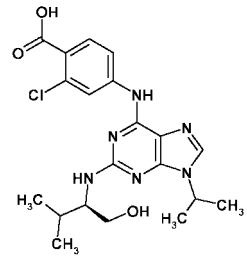
10.1021/jm701367r CCC: $40.75
Published on Web 05/15/2008



N-[4-methyl-3-[(4-pyridin-3-ylpyrimidin-2-yl)amino]phenyl]pyridine-3-carboxamide (a variant of imatinib)

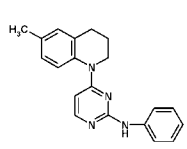


[6,7-bis(2-methoxy-ethoxy)quinazoline-4-yl]-(3-ethynylphenyl)amine (Erlotinib)



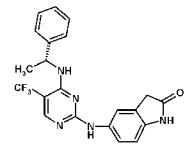
Purvalanol B

Figure 1. Kinase inhibitors cocrystallized with the three kinase crystal 3D receptors selected from the Protein Data Bank.



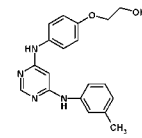
Lck inhibitor

(WO 0140215, Pfizer)



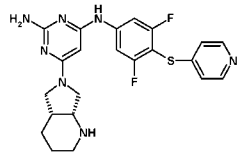
Fak inhibitor

(WO 040580, Pfizer)



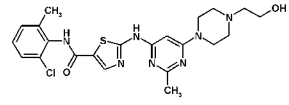
EGFR/PDGFR inhibitor

(WO 9515952, Zeneca)



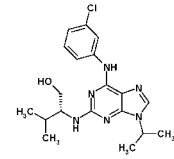
Rho inhibitor

(WO 01006450, Bayer)



Src/Abl inhibitor

(Dasatinib, BMS)



CDK1 inhibitor

(Purvalanol A)

Figure 2. Some examples of diaminopyrimidine and related kinase inhibitors.

(a)Abelson tyrosine kinase (ABL). PDB-code: 1FPU. Cocrystallized ligand: *N*-[4-methyl-3-[(4-pyridin-3-ylpyrimidin-2-yl)amino]phenyl]pyridine-3-carboxamide (a variant of imatinib).[13]

(b)Epidermal growth factor receptor kinase (EGFR). PDB-code: 1M17. Cocrystallized ligand: [6,7-bis(2-methoxy-ethoxy)quinazoline-4-yl]-(3-ethynylphenyl)amine (erlotinib).[14]

(c)Cyclin dependent kinase 2 (CDK2). PDB-code: 1CKP. Cocrystallized ligand: purvalanol B.[15]

These three kinases are validated major therapeutic targets, belonging to three different families according to a generally accepted classification derived from the "kinome" analysis by Manning[16] cytosolic tyrosine kinase (CTK) for Abl, receptor Tyrosine kinase (RTK) for EGFR, cyclin-dependent and mitogen-activated kinase (CMGC) for CDK2.

Collections of Compounds. Our strategy was based on the systematic docking of three sets of compounds in three kinase crystal 3D structures:

(a) A set of 123 old and recent kinase inhibitors described in publications and patents (see Supporting Information) and selected to exemplify a large variety of chemotypes which served to determine the best scoring function values.

(b) The BioPrint collection,[17] containing more than 2500 marketed drugs, reference compounds, and compounds that have failed in clinical trials which were selected for the purpose of our BioPrint pharmaco-informatics platform.[17] The hit rate of BioPrint compounds on a panel of kinases (inhibition >40% @ 10 μM) is around 2% (unpublished experimental results). This collection was used as a negative control for our molecular modeling study.

(c) A kinase-targeted library of 1440 compounds synthesized in-house based around 27 original scaffolds. This library was designed around a diaminopyrimidine central core, which can be considered as a privileged and a general structure for interacting with the ATP binding site of kinases. Some examples of diaminopyrimidines and related structures reported as kinase inhibitors

Table 1. (a) For Each Known Ligand of a Kinase, the Highest Scoring Value (Including All Conformations) Is Selected for Each Scoring Function,[a] and (b) For Each Kinase (ABL, CDK2, EGFR) and for Each Scoring Function, a Threshold Is Chosen in a Way That All Known Ligands of a Kinase Have a Score above This Threshold

a

Compound reference and conformation number	-PLP1	-PLP2	LIGSCORE1	LIGSCORE2	-PMF	JAIN
Reference_1, conformation_1	57.9	53.85	2.03	3.25	45.17	2.75
Reference_1, conformation_2	71.81	65.64	**2.36**	2.18	32.13	**3.66**
Reference_1, conformation_3	59.39	49.97	1.5	3.13	20.14	0.83
Reference_1, conformation_4	63.14	52.22	2.23	2.34	34.11	2.26
Reference_1, conformation_5	**78.32**	67.04	2.21	2.53	43.84	2.03
Reference_1, conformation_6	70.4	62.79	1.4	1.92	**51.12**	2.33
Reference_1, conformation_7	68.97	59.23	1.67	**3.35**	43.89	1.41
Reference_1, conformation_8	70.9	49.38	1.43	3.02	35.96	1.05
Reference_1, conformation_9	74.92	**68.69**	1.67	2.18	34.63	2.97
Reference_1, conformation_10	61.86	48.67	1.32	2.6	36.58	1.91
Reference_1	**78.32**	**68.69**	**2.36**	**3.35**	**51.12**	**3.66**

b

Compound reference (conformation independent)	-PLP1	-PLP2	LIGSCORE1	LIGSCORE2	-PMF	JAIN
Reference_2	119.66	105.83	4.82	5.96	63.33	9.35
Reference_3	99.97	95.41	4.24	5.53	96.34	9.21
Reference_4	103.73	96.02	4.14	4.51	73.67	9.2
Reference_5	98.35	91.55	3.51	4.94	84.51	9.12
Reference_6	110.93	103.62	4.6	5.87	66.93	9.02
Reference_7	95.63	86.09	3.01	4.5	80.06	8.96
Reference_8	115	108.68	3.31	5.44	98.8	8.95
Reference_9	94.88	90.53	3.22	3.99	50.25	8.93
Reference_11	110.06	100.71	4.34	5.71	**43.98**	8.91
Reference_1	**78.32**	**68.69**	2.36	3.35	51.12	3.66
Reference_10	99.23	97.79	**2.22**	**3.22**	87.63	**3.05**
Thresholds for one kinase	**78.32**	**68.69**	**2.22**	**3.22**	**43.98**	**3.05**

[a] This value becomes the score for each ligand and for each scoring function.

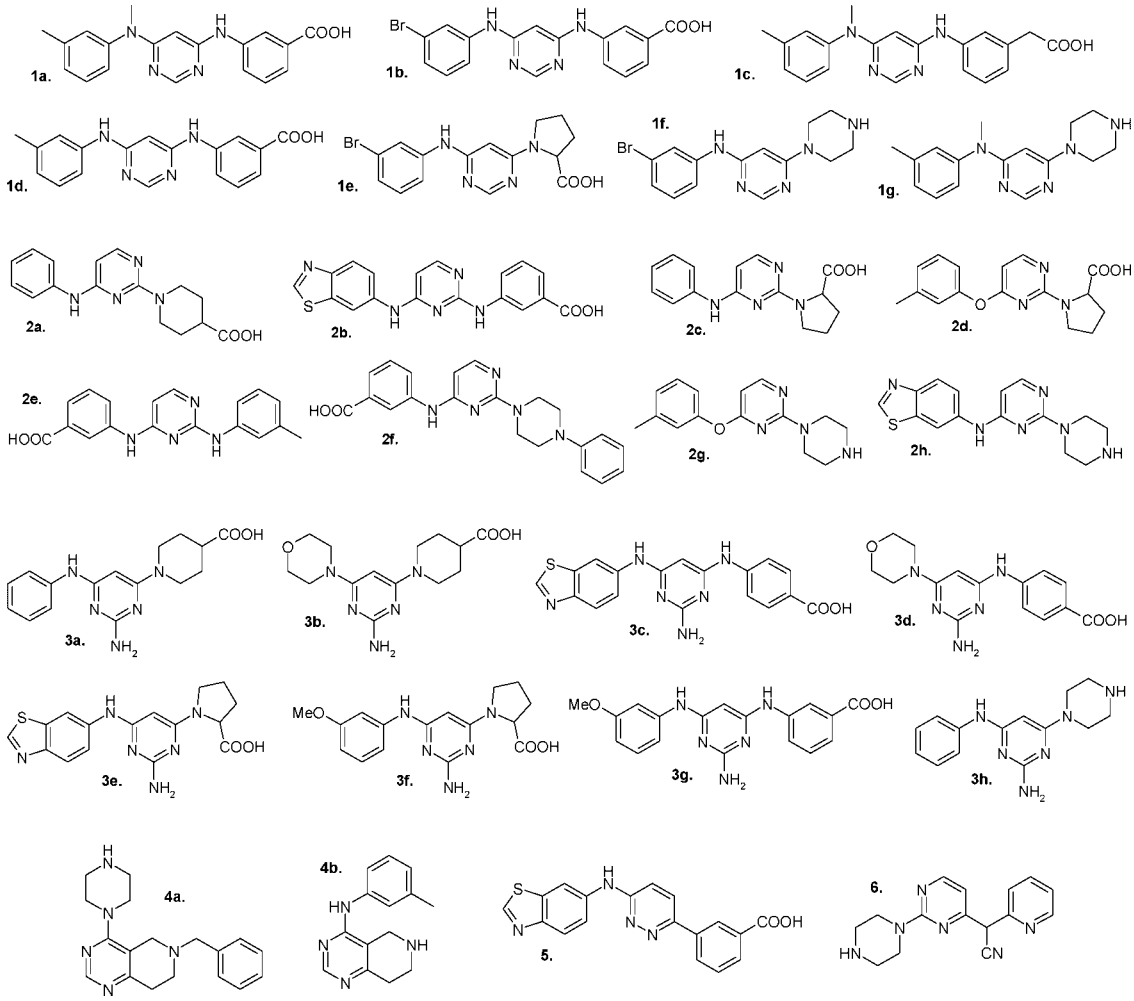
are shown in Figure 2: *N*-aryl-2,4-diaminopyrimidine derivatives, which have been described as inhibitors of Lck kinase[18a] and Fak (focal adhesion kinase);[18b] *N*-aryl-4,6-diaminopyrimidine derivatives, which have been reported to inhibit EGFR and PDGFR kinases,[18c] Rho kinase,[18d] and src and abl kinases;[18e] and diaminopurine derivatives such as Purvalanol A, which are inhibitors of cyclin dependent kinases (CDKs).[19]

The R-groups for the 27 diaminopyrimidine scaffolds were selected taking into account some of the most common groups described in scientific literature to be present in inhibitors of kinases, and the R-groups for partners were selected in order to respect Lipinski rules,[20] particularly concerning molecular weight.

Docking and Scoring Approach. A systematic docking of the three above-mentioned collections was performed with the three kinase structures extracted from the PDB by using the module "LigandFit" from Cerius2 (v. 4.10.).[21] This program is designed to dock molecules into a protein binding site. For each kinase, the residues around the cocrystallized ligands were selected, the cocrystallized ligands and water molecules were deleted, and the hydrogens of the site residues were optimized. For each of the 123 kinase inhibitors, 10 conformations were generated. They were selected for their adaptation to the shape of the site and then oriented into the binding site, and the energy was optimized. During the docking, the protein is rigid while the ligand remains flexible, allowing different conformations to be searched and docked. For each pose, six scoring functions implemented in LigandFit (PLP1, PLP2, LigScore1, LigScore2, PMF, Jain)[21] were calculated. For the known ligands of a kinase, the highest score of the six functions were saved. The lowest value of each function was then chosen as the threshold for this kinase (see Table 1). Following the same procedure for the three kinases, the 18 thresholds (six scoring functions per kinase) were then used as filters for the docking of BioPrint and the kinase-targeted library. A molecule was considered as predicted active on a kinase if the six scoring values were superior to the six thresholds.

Kinase Assays. To assay inhibitor activities on 41 kinases (selected from Cerep kinase platform so as to sample the diversity of kinase targets), homogenous time-resolved fluorescence (HTRF) assays were performed in 96 half-well plates. The following were combined in the reaction mixture: 4 μL of inhibitor (diluted in 100% DMSO at 50 μM), 8 μL of enzyme, and 8 μL of ATP/substrate mix (final concentrations around Km values). Enzymes, ATP, and substrate were previously diluted in kinase buffer to get final concentrations in the well: 50 mM Hepes/NaOH (pH 7.4), 40 μM Na_3VO_4, 0.005% Tween-20, 1 mM DTT, and various concentrations of $MnCl_2$ or $MgCl_2$. The assay was initiated by the addition of the ATP/substrate mix. The reaction was incubated for different times at room temperature. The reaction was stopped by addition of 55

Scheme 1. List of the 27 Building Blocks



μL of revelation mix. The revelation mix was composed by EDTA (0.33 M), specific europium-cryptate antibody antiphospho-substrate, and XL-665 with various tags (CisBio International). The mix was prepared in revelation buffer with final concentrations in the well of 50 mM Hepes/NaOH (pH 7.4), 0.1% BSA, and 150 mM KF. The reaction was incubated for 1 h or more at room temperature and was read with Rubystar (BMG, Germany).

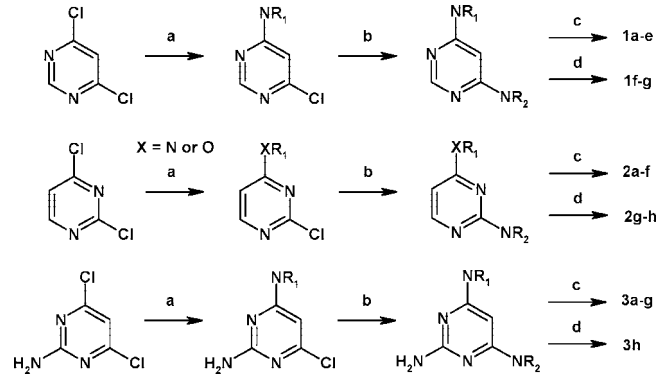
Kinase activities were expressed as a percentage of maximal activity (without inhibitor), resulting from $n = 2$ experiments.

Chemistry. Twenty-seven pyrimidine building blocks were selected and synthesized for the design of the kinase targeted library (Scheme 1).

The three main series (building blocks **1a**−**g**, **2a**−**h**, and **3a**−**h**) were obtained in three steps as represented in Scheme 2 from the appropriate dichloropyrimidine and sequential reaction with two electrophiles, one of them bearing a methyl ester or a Boc-amine to generate a functionalized building block. Using conditions well described in literature,[22] the selective substitution of the halides was achieved for the three scaffolds. In the second series (starting from 2,6-dichloropyrimidine), phenol was also used as a nucleophile for the first step. The protecting groups of the building blocks were then removed by hydrolysis with sodium hydroxide (for methyl esters) or treatment with methanolic hydrochloric acid (for Boc protection) to afford the 18 acid and the 5 secondary amine building blocks.

Four other scaffolds (**4a**, **4b**, **5**, and **6**) were also included in the library to increase its chemical diversity and its interest for the discovery of new kinase inhibitors. Compounds **4a** and **4b** were obtained in four steps starting from commercially available 1-benzyl-3-carbethoxy-4-piperidone (Scheme 3). Cyclization with formamidine, followed by chlorination with $POCl_3$ and substitution with the appropriate amine, and then catalytic hydrogenolysis or Boc-deprotection gave two secondary amine building blocks for the library.

Scheme 2. Synthesis of Diaminopyrimidine Scaffolds[a]

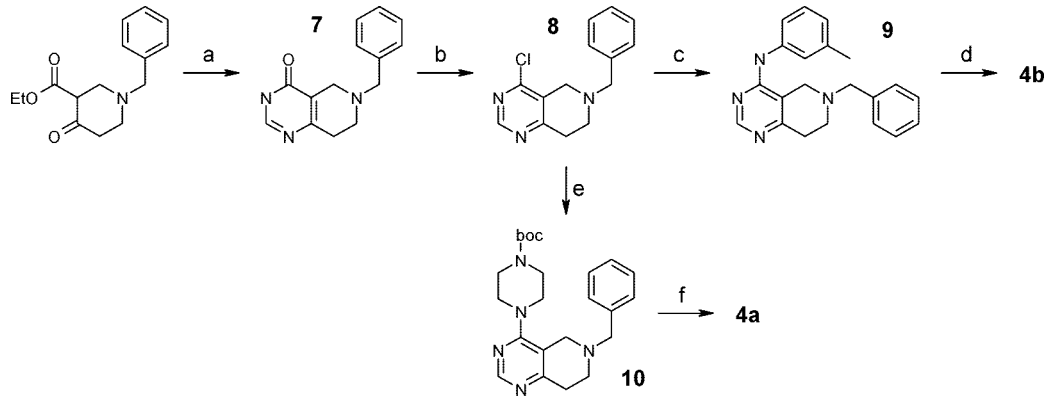


[a] Reagents: (a) appropriate amine or phenol, butan-1-ol, 120 °C; (b) appropriate amine, butan-1-ol, 120 °C; (c) NaOH, MeOH; and (d) HCl, MeOH.

Compound **5** was obtained using a Suzuki coupling on the substituted chloropyrazine **11** as depicted in Scheme 4.[24] Finally, compound **6** was obtained by first condensing 2-pyridylacetonitrile on 2,6-dichloropyrimidine to obtain **12**.[25] Halide substitution with excess piperazine gave the desired molecule.

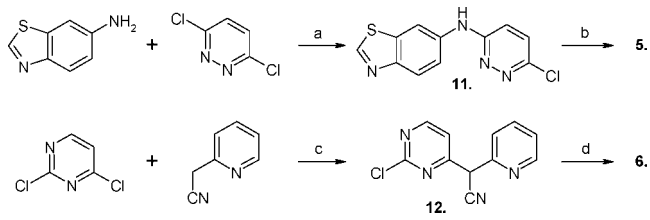
For the library synthesis, each acid building block was reacted with a diverse set of 80 amine partners in classical conditions

Scheme 3. [23] Synthesis of **4a** and **4b**[a]



[a] Reagents: (a) formamidine, NaOEt, EtOH, reflux; (b) $POCl_3$, reflux (c) *meta*-anisidine, propanol, reflux; (d) H_2, Pd/C, MeOH; (e) Boc-piperazine, propanol, reflux; and (f) HCl, MeOH.

Scheme 4. Synthesis of **5** and **6**[a]



[a] Reagents: (a) ethanol, reflux; (b) 3-carboxyphenylboronic acid, Na_2CO_3, $Pd(P(Ph)_3)_4$, ethanol/toluene, reflux; (c) $NaNH_2$, THF; (d) piperazine, propanol, reflux.

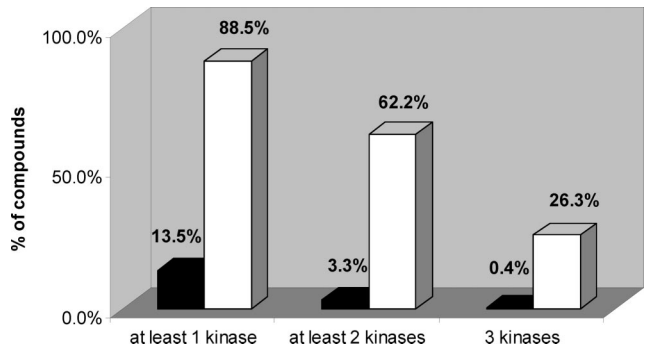


Figure 3. Percentages of compounds from each collection predicted to hit at least one, two or the three kinases. The BioPrint compounds are the black bars, and kinase-targeted compounds are white.

(TBTU, DIEA, DMF) to obtain the amides. Each amine scaffold was reacted with a diverse set of 80 acid partners in the same conditions and was also reacted with a diverse set of 40 halides in DMF in the presence of DIEA at 90 °C to obtain alkylated compounds. The compounds were extracted in ethyl acetate/Na_2CO_3 to remove salts and excess acidic reagents. All the compounds were analyzed by LC/MS. At this step, only the compounds with purities above 85% were kept for the final library. The compounds with insufficient purities were systematically purified by preparative LC/MS. The Tanimoto index[26] for this library using Isis keys as descriptors[27] was 0.61 which can be considered as indicative of a relatively high chemical diversity considering the limited number of building blocks.

Results

Docking of BioPrint and the Kinase-Focused Library. Figure 3 shows the percentage of compounds from each collection predicted to bind at least one, two, or the three kinases. As expected, the virtual hit rate is clearly higher for the diaminopyrimidine library when compared to BioPrint. Furthermore, this ratio is higher when the number of kinases considered increases: 6.6 for one kinase (88.5/13.5), 18.8 for two kinases (62.2/3.3), and 65.7 for the three kinases (26.3/0.4). For Bioprint, 0.4% of the compounds are able to pass all the filters for the three kinases. For the diaminopyrimidine library, more than 88% of the compounds were successfully docked in at least one kinase, but only 26.3% of the library was docked in the three kinases. We observed that the docking results were related to the building blocks (for example, 81% of the compounds built around scaffold **2e** were docked in the three kinases, versus only 5% for **1g**, scheme 1) but also to the partners reacted with the building blocks to generate the final compounds of the library. Even for kinase-oriented building blocks, the choice of the partners is crucial to obtain final compounds with kinase activities, and we expect our model to be able to correctly select them. For the experimental validation, in order to increase the hit rate and decrease the number of false positives, we decided to select the more drastic criterion and to consider as predicted active the compounds passing all the filters for the three kinases.

Experimental Validation. Sixty compounds from the diaminopyrimidine library were selected according to their docking scores: 30 compounds that passed the three filters were considered as predicted active, and 30 not passing the three filters were considered as predicted inactive. Compounds for experimental validation were selected (1) so as to represent all building blocks in the library, (2) so the number of compounds related to each building block was proportional to the number of these compounds predicted as active or inactive respectively, and (3) so the final compounds (building blocks coupled to a reagent) were randomly selected. The selection of compounds for the experimental screening was done randomly to avoid any bias in the selection. As a consequence, the selected compounds represent all the families included in the library (see Table 3 in the Supporting Information with the 60 structures of the compounds included in the screening). Experimental percentages of inhibition at 10 μM ($n = 2$) were determined for the 60 compounds on 41 kinases from five different families including the families related to ABL, CDK2, and EGFR (Table 2). By all means, the proportion of hits was found to be clearly much larger in the predicted active compounds group: the number of points of activity (percentage of inhibition superior to 40%) was 108 for the predicted active group against 22 for the predicted inactive group. Twenty compounds (67%) did inhibit at least

Table 2. Experimental Inhibition Results (at 10 μM) of 60 Compounds on 41 Kinases from Five Different Kinase Families (top 30: Predicted Active Compounds)[a]

	CTK									AGC									CaMK						CGMC							RTK									
Compound I.D.	Abl	Brk	FAK	Fes kinase	JAK3	Lyn kinase 6	Lck kinase	Src kinase 0	ZAP70 kinase	Akt1/PKBalpha	p70S6K	PDK1	PKA 0	PKCalpha	PKCgamma	ROCK2 0	RSK2 0	AMPKalpha	CaMKIIalpha 0	CaMKK	MAPKAPK2 0	CHK1	CHK2 0	CDK2	CDC2/CDK1 (cyc 0	ERK2 (P42mapkp	IKKbeta	JNK 1	JNK 2	p38alpha	p38gamma kinas	EGFR	EphB4 kinase 0	FGFR1 kinase	FLT-1 kinase (V 0	FLT-3 kinase	IGF1R kinase	IRK (InsR)	KDR kinase (VE	PDGFRbeta kina	TRKA
CER0262452	6	16	5	24	0	9	0	0	2	0	7	3	0	0	2	0	0	3	0	0	0	0	0	10	0	0	3	9	5	6	3	0	0	5	0	0	0	0	3	1	2
CER0262716	96	97	97	97	91	75	100	99	18	0	18	0	12	0	79	29	17	72	34	16	0	7	27	24	5	13	52	97	88	23	9	16	18	86	39	99	95	87	89	99	75
CER0262841	6	0	3	6	0	5	0	2	2	2	6	0	0	0	0	0	0	12	0	0	0	3	0	5	0	11	4	4	3	8	0	0	0	15	0	0	0	0	7	0	6
CER0262956	7	35	23	28	0	0	0	2	0	0	6	0	0	0	6	24	0	19	0	0	3	7	6	3	0	3	5	90	65	20	0	43	0	16	0	77	0	5	42	17	4
CER0262990	11	18	78	45	11	27	1	71	3	0	0	0	0	0	16	11	3	8	0	0	0	2	0	8	0	0	11	55	39	9	0	0	0	52	42	98	2	1	43	89	27
CER0263021	64	80	99	99	68	83	46	88	26	0	55	0	0	0	24	6	10	28	0	0	0	6	9	26	2	10	40	84	78	4	7	64	61	87	87	99	95	79	97	99	88
CER0262584	10	24	1	0	0	4	0	2	1	0	6	0	1	0	4	0	0	5	0	0	0	2	5	6	5	8	1	1	7	3	6	0	0	14	0	0	0	0	0	10	5
CER0262928	0	4	7	3	6	0	0	0	6	0	5	2	1	0	1	2	0	20	0	0	0	1	2	2	1	0	0	4	0	4	7	1	2	0	7	5	0	7	1	10	6	9
CER0262939	7	0	4	8	16	13	0	0	2	0	10	7	1	0	0	1	0	6	0	0	0	3	3	1	5	0	0	4	0	5	10	2	0	0	13	1	11	0	0	17	14	5
CER0262696	11	0	0	5	0	4	0	9	6	2	9	0	0	0	3	1	0	9	0	0	0	4	2	1	2	0	0	1	0	3	1	2	0	2	0	0	0	0	6	8	5
CER0263635	11	3	9	4	11	9	0	4	4	0	3	0	0	0	10	0	0	9	0	0	0	4	0	1	0	3	3	0	4	0	0	97	4	3	21	21	0	0	0	13	8
CER0263673	18	29	15	24	12	16	0	6	5	0	18	1	0	0	5	22	0	0	0	0	0	3	1	0	4	0	2	0	7	5	57	0	87	0	19	22	67	0	0	40	48	23
CER0263680	13	4	5	0	4	18	0	1	2	0	6	1	0	0	5	0	0	2	0	0	0	4	1	6	0	4	0	4	2	2	1	62	0	16	20	11	6	0	8	22	10
CER0263822	11	33	17	10	6	9	0	8	6	1	9	0	0	3	0	4	5	8	0	0	4	6	0	1	0	0	2	0	4	0	0	0	0	20	0	54	0	0	11	24	7
CER0263885	23	48	67	18	41	48	42	11	11	5	79	4	16	0	28	93	62	54	0	0	6	6	28	0	0	40	52	55	51	0	5	27	0	49	43	98	32	73	62	85	0
CER0263948	13	32	8	7	0	9	0	3	0	6	12	0	0	0	5	0	0	1	0	0	0	2	2	4	0	0	7	2	0	0	1	84	0	0	8	0	0	0	0	5	12
CER0264160	8	21	11	7	18	9	0	6	3	1	8	4	0	0	1	2	0	13	0	0	0	4	4	1	0	0	6	19	9	5	3	36	0	4	15	0	0	6	7	3	9
CER0264196	38	36	99	75	50	47	45	64	23	0	9	8	0	0	14	5	57	17	0	0	0	5	0	28	1	0	9	49	37	3	2	8	5	82	34	86	87	66	81	93	60
CER0264356	3	9	0	11	2	2	0	1	5	0	8	0	0	4	0	0	0	0	0	0	0	0	5	9	0	0	0	0	1	3	0	0	0	0	0	0	0	0	1	5	10
CER0264021	31	10	12	17	0	29	32	30	9	0	16	4	0	2	0	13	5	11	0	0	0	4	0	15	1	0	4	28	38	37	0	100	0	0	32	35	5	0	22	26	8
CER0263930	75	23	20	20	7	49	26	26	6	2	14	2	0	1	1	2	0	6	0	0	0	3	1	3	0	0	7	7	8	11	0	100	0	59	83	27	0	0	83	32	11
CER0263971	71	12	15	22	3	27	24	24	4	0	8	0	0	7	2	0	0	5	0	0	0	0	0	12	0	4	0	24	22	20	2	100	0	5	0	15	0	0	26	37	7
CER0263965	49	11	6	15	7	14	10	15	4	2	9	0	0	5	6	0	0	0	0	0	0	6	0	21	0	6	8	0	4	5	1	97	0	30	3	0	0	0	31	23	10
CER0263654	47	1	17	18	15	29	27	17	9	0	4	0	0	6	5	0	14	0	0	0	0	2	0	9	0	0	1	0	7	4	3	99	0	21	5	29	0	0	41	42	12
CER0263952	53	0	20	22	0	19	9	26	6	0	9	0	0	0	2	0	3	0	0	0	0	3	5	20	7	6	0	19	21	8	3	100	0	0	0	13	0	0	19	33	13
CER0263008	69	78	78	51	28	48	37	69	13	0	19	0	1	5	27	7	35	16	0	0	1	0	7	12	0	0	36	70	53	19	1	23	17	80	72	98	50	34	95	91	56
CER0264165	25	16	10	18	11	2	0	10	6	1	14	1	13	2	0	12	0	8	0	0	0	5	3	2	2	1	4	71	32	11	17	91	0	6	4	1	8	0	13	7	7
CER0262904	0	17	2	8	0	13	0	4	0	0	9	0	0	5	0	0	5	2	0	0	0	2	1	0	7	5	0	9	0	2	8	3	2	0	3	0	5	0	4	4	9
CER0262770	11	7	4	4	0	8	0	1	2	2	14	0	0	5	2	0	0	0	0	0	0	3	0	12	0	0	6	10	3	0	3	0	0	0	13	0	1	0	5	8	8
CER0263252	8	54	15	18	5	12	22	13	0	1	15	0	2	0	14	7	0	49	0	0	0	4	6	8	0	0	10	34	44	7	5	81	0	16	3	0	0	0	35	30	9
CER0262779	0	10	1	0	0	1	0	0	0	0	5	0	6	0	5	0	0	0	0	0	0	0	0	17	0	1	1	0	1	12	6	0	0	0	0	0	0	0	1	0	5
CER0263146	1	3	4	16	0	3	0	5	0	0	3	2	0	0	0	0	0	9	0	0	1	4	0	10	0	0	4	8	11	10	0	0	0	11	1	0	0	0	14	4	0
CER0264311	0	5	3	7	9	3	0	3	0	0	12	4	0	0	5	0	0	4	0	0	0	2	0	6	0	1	2	0	2	1	0	17	0	0	0	0	0	0	0	6	7
CER0264377	1	0	0	23	0	1	0	5	1	1	14	0	5	3	5	0	0	0	0	0	0	5	3	4	0	6	0	0	2	16	0	0	0	0	0	0	0	0	0	6	10
CER0264387	6	22	5	0	0	2	0	0	2	0	3	5	0	3	0	0	0	14	0	0	0	3	1	0	2	0	0	9	0	0	3	0	0	0	0	14	0	6	0	9	7	8
CER0262864	6	0	3	9	0	1	0	3	6	2	11	1	3	0	0	1	0	0	0	0	1	2	0	2	0	0	4	11	5	10	3	0	0	0	0	0	0	0	13	13	7
CER0262531	0	13	1	2	0	6	0	0	4	0	6	0	0	1	4	0	0	1	0	0	0	3	0	8	0	6	0	0	3	0	1	82	0	0	0	0	0	0	0	5	4
CER0262532	5	22	0	0	0	2	0	0	0	0	7	0	0	0	6	0	0	0	0	0	0	3	2	3	0	2	3	0	3	0	0	0	0	0	0	0	0	0	0	5	0
CER0262839	0	15	3	9	0	7	0	3	3	0	7	0	0	0	0	1	0	5	0	0	0	1	0	14	0	0	5	0	3	8	0	0	0	11	0	0	0	0	17	8	9
CER0262897	6	14	3	4	0	6	0	2	2	1	7	0	0	0	0	0	0	9	0	0	0	2	0	3	0	0	4	1	2	8	0	0	0	7	2	0	0	0	6	0	7
CER0263064	7	0	0	5	0	6	0	0	6	0	16	0	0	0	6	0	0	0	0	0	0	0	4	8	0	0	0	6	4	0	0	0	0	0	0	0	0	0	4	11	4
CER0263083	10	13	6	0	0	6	0	0	0	0	5	0	0	0	4	0	0	0	0	0	1	1	3	6	0	1	1	5	0	7	0	0	0	8	0	0	0	0	5	0	4
CER0263106	0	2	0	7	0	0	0	0	4	1	5	0	2	0	3	0	0	6	0	0	0	3	3	2	0	0	3	13	6	0	7	0	0	5	7	0	0	0	18	5	8
CER0263216	2	3	3	3	0	7	0	10	8	0	6	0	0	0	3	0	0	11	0	0	0	8	2	8	0	0	6	0	1	0	2	0	0	21	0	0	0	0	12	6	7
CER0263084	5	0	4	2	7	1	0	0	2	0	7	1	0	0	1	0	0	9	0	0	2	3	0	3	0	0	2	0	7	3	0	0	0	1	0	0	8	1	11	7	7
CER0262586	13	9	0	0	3	1	0	0	3	0	3	0	0	0	4	0	0	1	0	0	1	5	0	0	0	3	2	7	1	5	0	0	0	0	0	0	0	0	10	7	5
CER0262488	2	22	0	8	4	0	0	3	2	0	2	0	0	0	7	0	0	0	1	0	1	3	0	3	0	0	0	4	1	0	0	9	0	6	0	0	0	0	7	9	6
CER0262469	8	0	0	2	7	0	0	0	2	4	3	0	0	0	4	0	0	0	0	0	3	4	6	5	0	8	1	6	1	4	0	0	0	19	0	0	0	0	3	4	7
CER0262468	7	10	7	3	0	9	0	0	6	0	0	0	0	0	2	1	0	19	0	0	0	3	2	24	0	0	4	0	1	1	2	31	0	0	1	0	3	0	6	10	8
CER0263706	7	3	5	5	8	0	0	3	0	0	3	0	8	0	5	0	0	0	0	0	0	2	3	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	6	8
CER0263740	3	5	0	8	1	0	0	0	6	0	8	1	0	0	0	3	0	15	0	0	1	1	3	0	3	0	6	0	4	0	4	0	0	0	1	0	0	0	4	8	8
CER0263745	0	4	4	20	0	13	0	0	1	0	13	10	0	0	2	0	0	10	0	0	4	6	0	9	0	0	5	0	2	0	2	0	0	4	12	0	0	0	5	5	6
CER0263779	8	4	0	2	0	14	0	4	3	0	10	2	0	0	5	0	0	7	0	0	1	4	0	5	0	0	5	0	5	0	2	0	0	4	6	0	0	0	4	6	12
CER0263959	10	7	1	7	0	2	0	2	0	0	11	0	2	0	3	0	0	0	0	0	0	0	0	6	0	6	0	0	3	0	2	84	0	0	5	0	0	0	5	9	9
CER0264064	8	0	2	8	0	0	0	0	3	1	12	0	0	1	6	0	0	0	1	0	0	1	3	5	0	3	0	0	2	0	2	0	0	0	4	0	0	0	2	5	8
CER0264070	2	4	10	1	0	0	0	8	3	0	6	1	0	0	0	1	0	15	0	0	2	0	5	24	0	0	3	0	6	1	1	5	0	0	0	4	0	0	3	11	11
CER0264218	2	7	4	4	6	3	0	6	4	5	11	3	0	3	2	1	0	4	0	0	0	2	0	0	0	8	1	9	5	1	2	0	0	5	0	0	0	0	3	6	4
CER0264280	5	0	3	3	10	3	0	4	4	0	10	1	0	0	4	0	0	7	0	0	0	8	0	7	0	0	7	2	3	2	0	0	0	5	0	0	0	0	0	14	5
CER0264438	1	2	1	6	0	17	0	0	5	2	11	3	0	8	2	0	5	16	0	0	1	5	0	16	0	0	10	3	5	10	2	0	0	6	0	0	0	7	11	5	13
CER0262730	44	81	99	90	84	90	84	97	19	0	23	3	4	1	30	19	9	36	0	0	1	2	13	38	11	0	45	94	87	12	3	43	57	95	87	99	83	83	95	98	34

[a] Orange: inhibition between 40% and 70%. Red: inhibition superior to 70%.

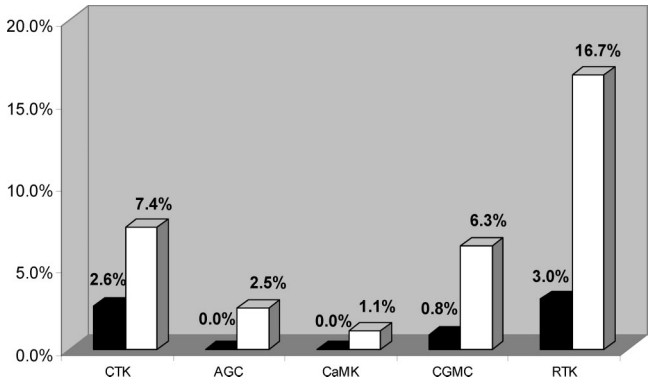


Figure 4. Experimental validation: hit rates by kinase family when comparing the compounds predicted as active (white) by the docking-scoring approach and those predicted as inactive (black).

one kinase of the panel (more than 40% inhibition at 10 μM), compared to three compounds (10%) for the inactive group. Our docking model was then able to generate a 6.7-fold enrichment, even when applied to a collection of closely structurally related compounds such as the diaminopyridine library. We found at least one hit for 28 kinases of the panel (68%) and for 21 kinases of the CTK, RTK, and CGMC families (85%). Interestingly, though our docking approach was based on these three families, some hits were also found for kinases belonging to other families (AGC and CaMK; Figure 4).

Our kinase-targeted library does not include purine structures similar to those of selective inhibitors of CDK2 like olomucin or roscovitine (purvalanol-type compounds). In consequence, it is not surprising that no activity was seen for any compound in the CDK2 assay. Nevertheless, we found to be very significant the fact that several active-predicted compounds showed activity to other targets of the CGMC family to which CDK2 belongs. This result is in perfect accordance with our initial objective, which was to implement a model able to identify some kinase-family related features not specific to one particular kinase.

Seventeen compounds of the predicted active group inhibited more than one kinase, compared to only one compound in the predicted inactive group. The case of this compound, (3-[4-(benzothiazol-6-ylamino)-pyrimidin-2-ylamino]-*N*-[(2-tetrahydrofuryl)-methyl]-benzamide (CER0262730), responsible for 20 of the 22 false negative results, was further investigated. While checking the six individual docking scores for each of the three kinases, it appeared that the molecule actually passed 15 out of the 18 filters (six for ABL, four for EGFR, and five for CDK2). Its experimental activity appears less surprising and reveals the stringent requirements of our model and the limits of a rapid docking strategy, since no deep analysis of the docking poses is done. Obviously the filters can be used in a less drastic manner (for example, a docking score superior to thresholds values for more than 9 out of the 18 scoring functions).

Our docking strategy allowed the identification of several promiscuous inhibitors interacting with more than 10 kinases from the panel but also of relatively selective molecules with significant inhibition for 3 kinases or less, and it was observed that small structural modifications around a common core lead to considerable differences in selectivity (Figure 5). However, the screening concentration (10 μM) was adapted to hit seeking

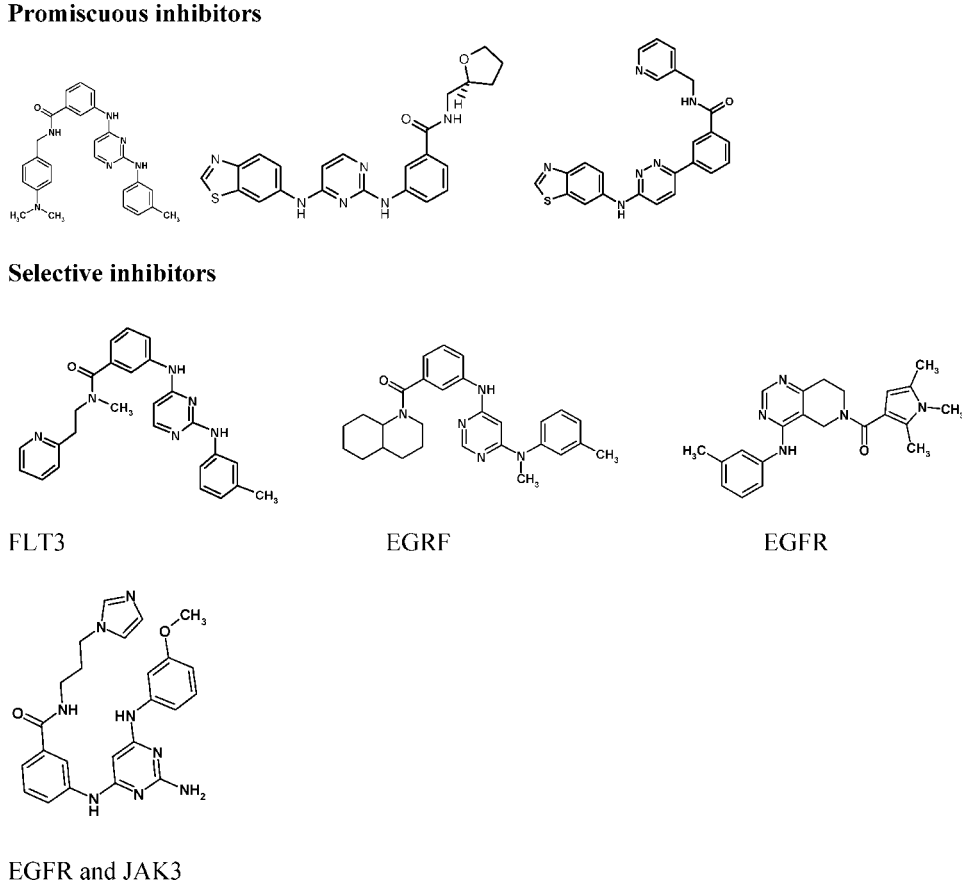


Figure 5. Promiscuous (more than 40% inhibition at 10 μM on more than 10 kinases) and selective (more than 40% inhibition at 10 μM on less than 3 kinases) inhibitors.

and not to specific lead finding so that promiscuity is certainly overestimated.

Conclusion

Our results confirm the feasibility of designing kinase targeted libraries by selecting privileged heteroaromatic central cores and taking advantage of the diversity generated by combinatorial chemistry to explore the hydrophobic pocket and fine-tune selectivity and potency. The method we have developed for kinase binding prediction is based on an original and rapid docking and scoring strategy using three different kinases and is particularly adapted for library design and virtual screening. This approach has shown its ability to identify promiscuous kinase targeted cores which, when subjected to combinatorial variations, can generate more or less selective hits for a large variety of kinases. It has been validated by an experimental HTS assay and could be applied to future libraries with a central core being a privileged structure for interacting with the kinase ATP site. Thanks to the rapidity and simplicity of the method, thousands of virtual compounds can be filtered, allowing an optimized selection of central heteroaromatic cores and of hydrophobic partners which will increase the hit rate of kinase-targeted libraries.

This efficient docking and scoring approach may easily be modified or extended by selecting other crystallized kinases for docking, and may be adapted to less drastic selections by, for example, picking up compounds passing only some of the filters.

Supporting Information Available: Set of 123 kinase inhibitors described in publications and patents which served to determine the best scoring function values; NMR spectroscopy of the 27 building blocks that were selected and synthesized for the design of the kinase targeted library; and structures of the 60 compounds from the diaminopyrimidine library that were assayed against 41 kinases. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Burnett, G.; Kennedy, E. P. The enzymatic phosphorylation of proteins. *J. Biol. Chem.* **1954**, *211*, 969–980.

(2) (a) Levitzki, A.; Gazit, A. Tyrosine kinase inhibition: an approach to drug development. *Science* **1995**, *267*, 1782–1788. (b) Traxler, P. Tyrosine kinases as targets in cancer therapy - successes and failures. *Expert. Opin. Ther. Targets.* **2003**, *2*, 215–34.

(3) (a) Gold, M. G.; Barford, D.; Komander, D. Lining the pockets of kinases and phosphatases. *Curr. Opin. Struct. Biol.* **2006**, *6*, 693–701. (b) Martin, B. T.; Strebhardt, K. Polo-like kinase 1: target and regulator of transcriptional control. *Cell Cycle* **2006**, *24*, 2881–2885. (c) Cowan-Jacob, S. W. Structural biology of protein tyrosine kinases. *Cell. Mol. Life Sci.* **2006**, *22*, 2608–2625.

(4) (a) Druker, B. J.; Lydon, N. B. Lessons learned from the development of an Abl tyrosine kinase inhibitor for chronic myelogenous leukaemia. *J. Clin. Invest.* **2000**, *105*, 3–7. (b) Drews, J. Drug discovery: a historical perspective. *Science* **2000**, *287*, 1960–1964. (c) Haluska, P.; Adjei, A. A. Receptor tyrosine kinase inhibitors. *Curr. Opin. Invest. Drugs.* **2001**, *2*, 280–286. (d) Fabbro, D.; Ruetz, S.; Buchdunger, E.; Cowan-Jacob, S. W.; Fendrich, G.; Liebetanz, J.; Mestan, J.; O'Reilly, T.; Traxler, P.; Chaudhuri, B.; Fretz, H.; Zimmermann, J.; Meyer, T.; Caravatti, G.; Furet, P.; Manley, P. W. Protein kinases as targets for anticancer agents: from inhibitors to useful drugs. *Pharmacol. Ther.* **2002**, *93*, 79–98.

(5) Fabian, M. A.; Biggs, W. H, 3rd; Treiber, D. K.; Atteridge, C. E.; Azimioara, M. D.; Benedetti, M. G.; Carter, T. A.; Ciceri, P.; Edeen, P. T.; Floyd, M.; Ford, J. M.; Galvin, M.; Gerlach, J. L.; Grotzfeld, R. M.; Herrgard, S.; Insko, D. E.; Insko, M. A.; Lai, A. G.; Lelias, J. M.; Mehta, S. A.; Milanov, Z. V.; Velasco, A. M.; Wodicka, L. M.; Patel, H. K.; Zarrinkar, P. P.; Lockhart, D. J. A small molecule-kinase interaction map for clinical kinase inhibitors. *Nat. Biotechnol.* **2005**, *23*, 29–36.

(6) (a) Capdeville, R.; Buchdunger, E.; Zimmermann, J.; Matter, A. Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. *Nat. Rev. Drug Discovery* **2002**, *1*, 493–502. (b) Ranson, M.; Mansoor, W.; Jayson, G. ZD1839 (IRESSA): a selective EGFR-TK inhibitor. *Expert Rev. Anticancer Ther.* **2002**, *2*, 161–168. (c) Smith, J. Erlotinib: small-molecule targeted therapy in the treatment of non-small-cell lung cancer. *Clin. Ther.* **2005**, *27*, 101513–101534.

(7) (a) Lowrie, J. F.; Delisle, R. K.; Hobbs, D. W.; Diller, D. J. The different strategies for designing GPCR and kinase targeted libraries. *Comb. Chem. High Throughput Screening* **2004**, *7*, 495–510. (b) Schnur, D.; Beno, B. R.; Good, A.; Tebben, A. Approaches to target class combinatorial library design. *Methods Mol. Biol.* **2004**, *275*, 355–378.

(8) (a) Trumpp-Kallmeyer, S.; Rubin, J. R.; Humblet, C.; Hamby, J. M.; Hollis Showalter, H. D. Development of a binding model to protein tyrosine kinases for substituted pyrido[2,3-d]pyrimidine inhibitors. *J. Med. Chem.* **1998**, *41*, 1752–1763. (b) Diller, D. J.; Li, R. Kinases, homology models, and high throughput docking. *J. Med. Chem.* **2003**, *46*, 4638–4647. (c) Naumann, T; Matter, H. Structural classification of protein kinases using 3D molecular interaction field analysis of their ligand binding sites: target family landscapes. *J. Med. Chem.* **2002**, *45*, 2366–2378.

(9) (a) Zheng, J.; Knighton, D. R.; Ten Eyck, L. F.; Karlsson, R.; Xuong, N.; Taylor, S. S.; Sowadski, J. M. Crystal structure of the catalytic subunit of cAMP-dependent protein kinase complexed with MgATP and peptide inhibitor. *Biochemistry* **1993**, *32*, 2154–2161. (b) Knighton, D. R.; Zheng, J. H.; Ten Eyck, L. F.; Ashford, V. A.; Xuong, N. H.; Taylor, S. S.; Sowadski, J. M. Crystal structure of the catalytic subunit of cyclic adenosine monophosphate-dependent protein kinase. *Science* **1991**, *253*, 407–414.

(10) Cheek, S.; Ginalski, K.; Zhang, H.; Grishin, N. V. A comprehensive update of the sequence and structure classification of kinases. *BMC Struct. Biol.* **2005**, *5*, 1–19.

(11) Honma, T.; Yoshizumi, T.; Hashimoto, N.; Hayashi, K.; Kawanishi, N.; Fukasawa, K.; Takaki, T.; Ikeura, C.; Ikuta, M.; Suzuki-Takahashi, I.; Hayama, T.; Nishimura, S.; Morishima, H. A novel approach for the development of selective Cdk4 inhibitors: library design based on locations of Cdk4 specific amino acid residues. *J. Med. Chem.* **2001**, *44*, 4628–4640.

(12) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28*, 235–242.

(13) Schindler, T.; Bornmann, W.; Pellicena, P.; Miller, W. T.; Clarkson, B.; Kuriyan, J. Structural mechanism for STI-571 inhibition of Abelson Tyrosine Kinase. *Science* **2000**, *289*, 1938–1942.

(14) Stamos, J.; Sliwkowski, M. X.; Eigenbrot, C. Structure of the Epidermal Growth Factor Receptor kinase domain alone and in complex with a 4-anilinoquinazoline inhibitor. *J. Biol. Chem.* **2002**, *277*, 46265–46272.

(15) Gray, N. S.; Wodicka, L.; Thunnissen, A. W. H.; Norman, T. C.; Kwon, S.; Hernan Espinoza, F.; Morgan, D. O.; Barnes, G.; LeClerc, S.; Meijer, L.; Kim, S-H.; Lockhart, D. J.; Schultz, P. G. Exploiting chemical libraries, structure, and genomics in the search for kinase inhibitors. *Science* **1998**, *281*, 533–538.

(16) Manning, G; Whyte, D. B.; Martinez, R.; Hunter, T.; Sudarsanam, S. The protein kinase complement of the human genome. *Science* **2002**, *298*, 1912–1934.

(17) (a) Krejsa, C. M.; Horvath, D.; Rogalski, S. L.; Penzotti, J. E.; Mao, B.; Barbosa, F.; Migeon, J. C. Predicting ADME properties and side effects: The BioPrint approach. *Curr. Opin. Drug. Discov. Devel.* **2003**, *6*, 470–480. (b) Froloff, N.; Hamon, V.; Dupuis, P.; Otto-Bruc, A.; Mao, B.; Merrick, S.; Migeon, J. In: Jacoby, E. (Ed.), *Chemogenomics Knowledge-based Approaches to Drug Discovery*, Imperial College Press, London, 2006, pp. 175−206. (c) http://www.cerep.fr/cerep/users/pages/ProductsServices/bioprintservices.asp.

(18) (a) Blumenkopf, T.; Mueller, E.; Roskamp, E. WO 0140215 (Pfizer, 2001). (b) Kath, J. C.; Luzzio, M. J. WO 04056786 (Pfizer, 2004) (c) Thomas, A. P. WO 9515952 (Zeneca, 1995). (d) Feurer, A.; Bennabi, S.; Heckroth, H.; Ergueden, J.; Schenke, T.; Bauser, M.; Kast, R.; Stasch, J. P.; Stahl, E.; Muenter, K.; Lang, D.; Ehmke, H. WO 03106450 (Bayer, 2003). (e) Luk, K. C.; Rossman, P. L.; Scheiblich, S.; So, S. S. U.S. Patent 7129351 (Hoffmann-La Roche Inc., 2004).

(19) (a) Haesslein, J. L.; Jullian, N. Recent Advances in Cyclin-Dependent Kinase Inhibition. Purine-Based Derivatives as Anti-Cancer Agents. Roles and Perspectives for the Future. *Curr. Top. Med. Chem.* **2002**, *1*, 1037–1050. (b) Breault, G. A.; Ellston, R. P. A.; Green, S.; James, S. R.; Jewsbury, P. J.; Midgley, C. J.; Pauptit, R. A.; Minshull, C. A.; Tucker, J. A.; Pease, J. E. Cyclin-dependent kinase 4 inhibitors as a treatment for cancer. Part 2: identification and optimisation of substituted 2,4-bis anilino pyrimidines. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2961–2966.

(20) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **2001**, *46*, 3–26.

(21) *Cerius*^{2}, version 4.10; Accelrys Software Inc, San Diego, CA, USA, 2004.

(22) (a) Beattie, J. F.; Breault, G. A.; Ellston, R. P. A.; Green, S.; Jewsbury, P. J.; Midgley, C. J.; Naven, R. T.; Minshull, C. A.; Pauptit, R. A.; Tucker, J. A.; Pease, J. E. Cyclin-dependant kinase 4 inhibitors as a treatment for cancer. Part 1: Identification and optimisation of substituted 4,6-bis anilino pyrimidines. *Bioorg. Med. Chem.* **2003**, *13*, 2955–2960. (b) Breault, G. A.; Ellston, R. P. A.; Green, S.; James, S. R.; Jewsbury, P. J.; Midgley, C. J.; Pauptit, R. A.; Minshull, C. A.; Tucker, J. A.; Pease, J. E. Cyclin-dependant kinase 4 inhibitors as a treatment for cancer. Part 2: Identification and optimisation of substituted 2,4-bis anilino pyrimidines. *Bioorg. Med. Chem.* **2003**, *13*, 2955–2960. (c) Aoyagi, T.; Yanada, R.; Bessho, K.; Yoneda, F. Synthesis of 2-amino-2-deoxy-5-deazaflavins and related compounds. *J. Heterocyclic Chem.* **1991**, *28*, 1537–1539.

(23) Sekiya, T.; Hiranuma, H.; Kanayama, T.; Hata, S. Pyrimidine derivatives III (1): synthesis of hypoglycemic 4-alkoxy-2-piperazino-5,6-polymethylenepyrimidines. *Eur. J. Med. Chem. Chim. Ther.* **1982**, *17*, 75–80.

(24) Maes, B. U. W.; Lemière, G. L. F.; Dommisse, R.; Augustyns, K.; Haemers, A. A new approach towards the synthesis of 3-amino-6-(hetero)arylpyridazines based on palladium catalyzed cross-coupling reactions. *Tetrahedron* **2000**, *56*, 1777–1781.

(25) Bernhart, C. A.; Condamines, C.; Demarne, H.; Roncucci, R.; Gagnol, J. P.; Gautier, P. J.; Serre, M. Synthesis and antiarrhythmic activity of new [(dialkylamino)alkyl]pyridylacetamides. *J. Med. Chem.* **1983**, *26*, 451–455.

(26) Flower, D. R. J. On the properties of bit string-based measures of chemical similarity. *J. Chem. Inf. Comput. Sci.* **1998**, *38*, 379–386.

(27) *MDL ISIS/HOST*; MDL Information Systems, Inc., San Ramón, CA, USA, 1991.

JM701367R