Activity-Based Kinase Selectivity Profiling of Clinically Relevant Tyrosine Kinase Inhibitors using QuickScout™

Masaki Gouda*, Tetsuya Myojin, Yui Iwamae, Tokiko Asami, Yugo Narumi, Hiroshi Ohmoto, Kensaku Akita, and Koichi Yokota

Carna Biosciences, Inc., Kobe, Japan

Introduction

Protein kinases are crucial elements of inter- and intra-cellular signaling and, as such, have been important targets for the development of activity-modulating effectors. To date, the most relevant clinical application of using kinase inhibitors to disrupt cell signaling pathways has been in the area of oncology. In recent years, eight distinct tyrosine kinase inhibitors have been approved globally for adjunctive or primary cancer therapy. These molecular inhibitors were rationally designed to target specific tyrosine kinases, and most bind competitively, at or near the ATP pocket. Because of the competitive nature of inhibitor binding, changes in the concentration of ATP present in any such reaction may lead to unanticipated results. Here we describe the use of our QuickScout™ activity-based-kinase assay system to determine the full selectivity profiles of the eight commercially available, clinically-approved protein kinase inhibitors against our panel of 79 tyrosine, 197 serine/threonine, 24 mutant and 3 lipid kinases. In this study, all assays were performed at two different ATP concentrations - near the $Km^{App.}$ and at 1 mM ATP. This comparative analysis should provide useful insight for the development of additional protein kinase inhibitors as therapeutic agents.

Materials and Methods

All human recombinant protein kinases were made in Carna Biosciences, Inc. (Kobe, Japan) as indicated in its web site (http://www.carnabio.com). All kinase assays and profiling (% Inhibition and IC_{50} Determination) of each drug were performed according to the procedures indicated in the web site of Carna Biosciences, Inc. The kinase activities were determined by off-chip mobility shift assay using LabChip™3000 (Caliper LS) and fluorescence polarization assay using IMAP™ technology or ELISA. Clinically relevant tyrosine kinase inhibitors (right table) were used for the present experiments.

Approved Kinase Inhibitors

Name	Tradename (FDA)	Research name	Target kinases*
Dasatinib	Sprycel®	BMS354825	ABL, SRC family kinases, PDGFRβ, KIT, ephrin (EPH) receptor kinases
Erlotinib	Tarceva®	OSI774	EGFR
Gefitinib	Iressa®	ZD1839	EGFR
Imatinib	Gleevec®	STI571	ABL, PDGFRα, PDGFRβ, KIT
Lapatinib	Tykerb®	GW572016	EGFR, HER2
Sorafenib	Nexavar®	BAY 43-9006	C-RAF, B-RAF, KIT, FLT3, KDR, FLT4, PDGFRβ
Sunitinib	Sutent®	SU11248	PDGFRα, PDGFRβ, FLT1, KDR, FLT4, KIT, RET, FMS, FLT3
Nilotinib	Tasigna®	AMN107	ABL, KIT, PDGFRα, PDGFRβ

*) Target kinases were reported by Nielka P. van Erp *et al.*, Cancer Treatment Reviews (2009) 35, 692-706

Results

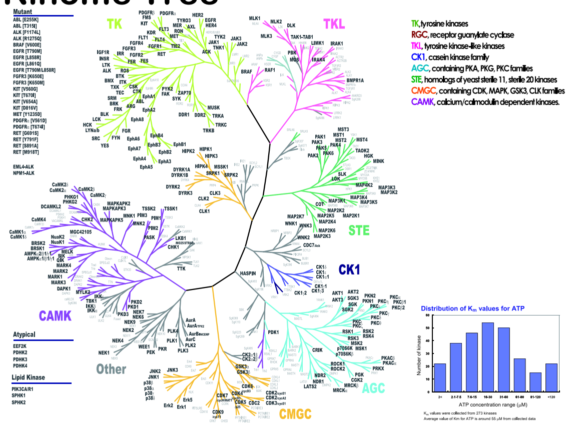
Each of the eight approved kinase inhibitors were screened at a concentration of 1 μM against the QuickScout™ panel of >300 human kinases, to identify candidate kinase targets (Primary Screen). Quantitative IC_{50} determinations were then made for each kinase-inhibitor against each kinase identified in the primary screen. Where inhibition > 40% was observed in these screens, additional testing was performed using approved inhibitors (and staurosporine, as positive control) at measured IC_{50} concentrations, and ATP at both $Km^{App.}$, and 1mM (physiological) concentrations. 'Kinome Clustering' results of these experiments are shown graphically in **Figure 1**.

In **Figure 2**, we show a novel approach for the visualization of inhibitor selectivity by ranking IC_{50} values obtained using physiological (1mM) ATP levels. Inhibitor selectivity was further analyzed using published pharmacokinetic parameters describing plasma concentration values, C_{min}. These results are shown in **Figure 3**.

In **Table 1**, we show data comparing the results of our 'physiological' assays (performed using 1mM ATP) with reported results from cellular proliferation assays.

Finally, we report our inhibitory activity results as they relate to 'on-target' kinases and clinically significant mutant forms (**Table 2**).

Kinome Tree



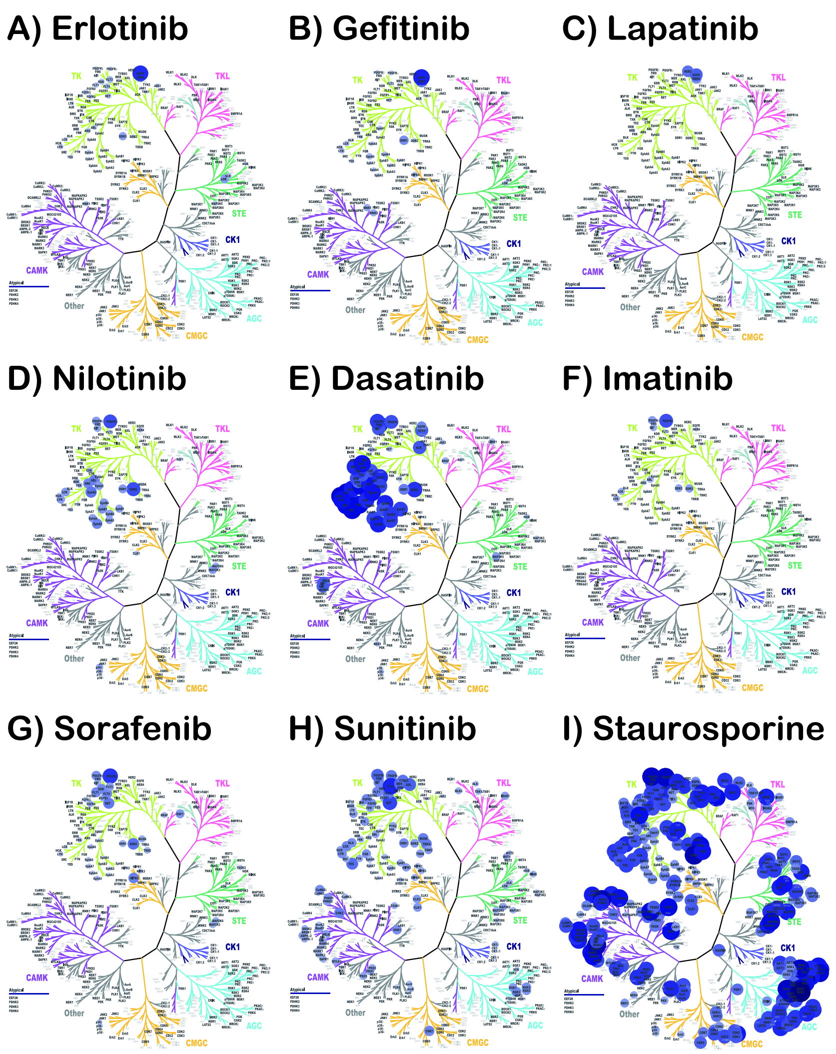


Figure 1

QuickScout™ profiling (ATP conc. = $K_m^{App.}$) results of approved 8 kinase inhibitors and staurosporine

Dendrograms were adapted to human kinome tree. Kinase inhibition maps for inhibitors,
A) erlotinib, B) gefitinib C) lapatinib D) nilotinib E) dasatinib F) imatinib G) sorafenib H) sunitinib and I) staurosporine, respectively.
Inhibition concentration (IC_{50}) values under 10,000 nM are marked with blue circles where larger circles indicated strongly inhibit to kinase.

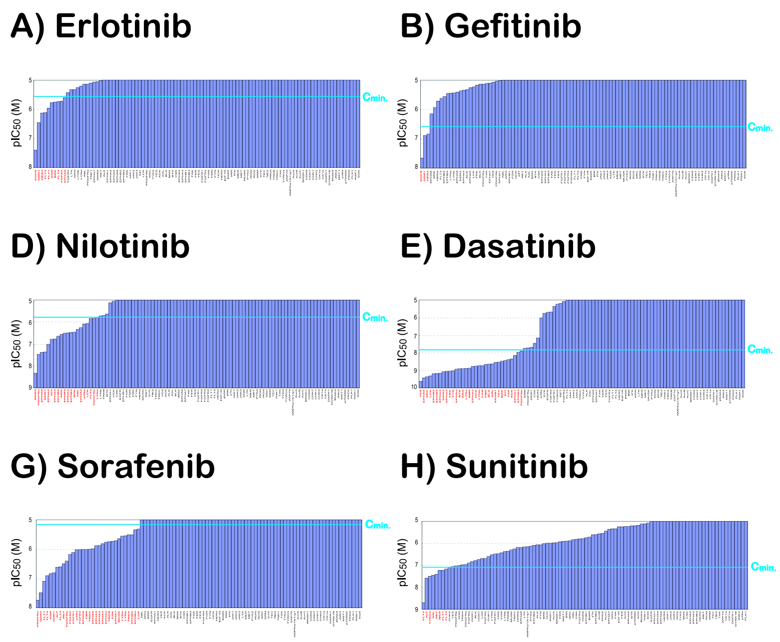


Figure 2

QuickScout™ profiling (ATP conc. = 1 mM) results of approved 8 kinase inhibitors

Top hit 100 kinases were selected from results in figure 1 and IC_{50} values were determined using 1 mM ATP.
Kinases were aligned in ascending order of the IC_{50} values. Mutant kinases were excluded from this analysis.
The blue line indicates the $C_{min.}$ plasma concentration (mol/L) of the drugs in patients.
These $C_{min.}$ concentrations were reported in Cancer Treatment Reviews (2009) 35. 692-706 (erlotinib, gefitinib, lapatinib, nilotinib, imatinib, and sunitinib), Journal of Clinical Oncology, (2008) 26. ASCO abstract #3590. (dasatinib) and Clinical Cancer Res. (2006) 12. 144-151 (sorafenib).

Table 1 IC_{50} competition with 1 mM ATP condition assay and On-target cellular assay

Inhibitor	Target kinase	IC_{50} (μM) 1 mM ATP	IC_{50} (μM) Cellular Proliferation*	
Erlotinib	EGFR	0.039	0.041 ± 0.001 [1]	NH5 cell EGFR over-expressed
Gefitinib	EGFR	0.022	0.032 ± 0.005 [1]	NH5 cell EGFR over-expressed
			0.825 ± 0.106 [1]	A431cell EGFR over-expressed
Lapatinib	EGFR	0.549	0.395 ± 0.156 [1]	A431cell EGFR over-expressed
Nilotinib	PDGFRα	0.034	0.023 [2]	FIP1L1-PDGFRα transformed Ba/F3 cells
	ABL	0.471	0.018 [3]	BCR/ABL expressed in Ba/F3 transfected cells
Dasatinib	ABL	0.002	0.0018 [3]	BCR/ABL expressed in Ba/F3 transfected cells
Imatinib	ABL	2.14	0.527 [3]	BCR/ABL expressed in Ba/F3 transfected cells
			5.66 ± 2.76 [4]	WT-Ba/F3 + IL3
Sorafenib	PDGFRβ	0.397	0.28 ± 0.14 [5]	PDGFRβ stimulated HAoSMC (0.1% BSA)
Sunitinib	PDGFRβ	0.094	0.039 ± 0.013 [6]	NIH-3T3 cells over-expressing PDGFRβ
	KDR	0.098	0.040 ± 0.02 [6]	VEGF-induced HUVECs
	PDGFRα	0.111	0.069 ± 0.015 [6]	NIH-3T3 cells over-expressing PDGFRα

* Cellular proliferation assay data resource.
1) Molecular Pharmacology (2008) 73, 338-348.
2) Blood (2005) 106, 3206-3213.
3) Journal of Clinical Oncology (2008), 469-471.
4) Cancer Research (2006) 66, 11314-11322.
5) Cancer Research (2004) 64, 7099-7109.
6) Clinical Cancer Research (2003) 9, 327-337.

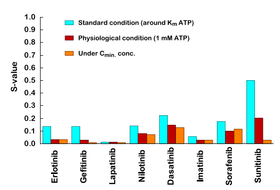


Figure 3

Selectivity as quantitative measure of specificity

Selectivity (S-values) calculated for kinases with IC_{50} values < 3 μM at both conditions of an around ATP Km (Standard, Blue bars) and 1 mM ATP (physiological, Red bars) with following equation:
Number of kinases (IC_{50} values < 3 μM) / tested kinase.
Orange bars indicate that S-values calculated from count of kinase in under $C_{min.}$ concentration per tested kinase, with follwing equation:
Number of kinases (IC_{50} values < $C_{min.}$) / tested kinase.
Mutant kinases were excluded from this analysis.

Table 2

IC_{50} effective ratios at 1mM ATP condition assay
Wild type kinase vs Mutants

EGFR	Erlotinib IC50 (nM)	Erlotinib Ratio	Gefitinib IC50 (nM)	Gefitinib Ratio	Lapatinib IC50 (nM)	Lapatinib Ratio
W.T.	39.8	1.0	22.0	1.0	164.0	1.0
T790M	>10000	N.D.	>10000	N.D.	>10000	N.D.
T790M/L858R	>10000	N.D.	>10000	N.D.	>10000	N.D.
L858R	9.6	4.1	6.0	3.6	144.4	1.1
L861Q	27.7	1.4	12.5	1.8	52.2	3.1

ABL	Nilotinib IC50 (nM)	Nilotinib Ratio	Dasatinib IC50 (nM)	Dasatinib Ratio	Imatinib IC50 (nM)	Imatinib Ratio
W.T.	471.4	1.0	2.3	1.0	2144.6	1.0
E255K	>10000	N.D.	10.0	0.2	>10000	N.D.
T315I	>10000	N.D.	>10000	N.D.	>10000	N.D.

KIT	Nilotinib IC50 (nM)	Nilotinib Ratio	Dasatinib IC50 (nM)	Dasatinib Ratio	Imatinib IC50 (nM)	Imatinib Ratio
W.T.	166.4	1.0	3.7	1.0	168.1	1.0
V560G	6.0	28	0.4	9.3	9.5	18
V654A	>10000	N.D.	37.6	0.1	>10000	N.D.
T670I	7332.3	0.02	>10000	N.D.	>10000	N.D.
D816V	>10000	N.D.	50.4	0.07	>10000	N.D.

PDGFRa	Nilotinib IC50 (nM)	Nilotinib Ratio	Dasatinib IC50 (nM)	Dasatinib Ratio	Imatinib IC50 (nM)	Imatinib Ratio
W.T.	34.6	1.0	19.3	1.0	28.8	1.0
V561D	201.6	0.17	26.0	0.17	87.7	0.33
T674I	>10000	N.D.	>10000	N.D.	>10000	N.D.

RET	Sunitinib IC50 (nM)	Sunitinib Ratio	Sorafenib IC50 (nM)	Sorafenib Ratio
W.T.	322.5	1.0	39.1	1.0
G691S	448.3	0.72	50.3	0.78
Y791F	407.8	0.79	62.9	0.62
S891A	239.5	1.4	50.5	0.77
M918T	833.1	0.39	95.2	0.41

FGFR3	Sunitinib IC50 (nM)	Sunitinib Ratio	Sorafenib IC50 (nM)	Sorafenib Ratio
W.T.	974.6	1.0	128.6	1.0
K650E	725.8	1.3	264.1	0.49
K650M	1250.1	0.78	540.8	0.24

Ratio values were calculated [IC_{50} value of W.T.] / [IC_{50} value of mutant].
N.D. are not determined IC_{50} values (IC_{50} is obserbed over 10,000 nM).
Values shown in RED are > 5x MORE potent than wild-type.
Values shown in BLUE are > 5x LESS potent than wild-type.
Values shown in BLACK are SIMILAR to wild-type (within 5-fold range).

Conclusion

- Selectivity profiling was performed for each of the eight clinically approved kinase inhibitors against a panel containing 79 tyrosine-., 197 serine/threonine-, and 3 lipid kinases at ATP concentrations of $K_m^{App.}$, and 1 mM.
- Under standard profiling conditions (ATP concentration approximately Km), the **Selectivity Ranking** (number of kinases with IC_{50} < 3 μM/number of kinases tested) was:
  lapatinib < imatinib < erlotinib = gefitinib < nilotinib < sorafenib < dasatinib < sunitinib.
- Under physiological profiling conditions (ATP concentration = 1 mM), the **Selectivity Ranking** (number of kinases with IC_{50} < 3 μM/number of kinases tested) was:
  lapatinib < gefitinib < imatinib < erlotinib < nilotinib < sorafenib < dasatinib < sunitinib.
- When analysis incorporated the use of reported pharmacokinetic data with data from physiological profiling conditions (ATP concentration – 1 mM), the **Target Selectivity Index** (number of kinases with IC_{50} values < plasma concentration / number of kinases tested was:
  lapatinib = gefitinib < imatinib < sunitinib < erlotinib < nilotinib < dasatinib = sorafenib.
- Biochemical assays performed using 1mM ATP resulted in IC_{50} values very similar to those reported in Cellular Proliferation Assays for the approved kinase inhibitors.
- When tested against mutated target kinases at 1 mM ATP, all approved inhibitors showed less potency against gate-keeper mutants.
- Comparative selectivity profiling performed at both K_m and 1 mM concentrations of ATP provide useful insight for the assessment of on-target and off-target kinase inhibitors.

Acknowledgements; Yasuyuki Kirii, Eiji Nishiwaki, Ryoko Nakai, Masanori Nakamura, Mariko Hatakeyama, Yusuke Kawase, Yuko Uno, Daisuke Kitagawa, and Hiromi Akimichi

E-mail: info@carnabio.com www.carnabio.com