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Bioorganic & Medicinal Chemistry Letters 16 (2006) 5973–5977
Bioorganic & Medicinal Chemistry Letters

# The development of 2-benzimidazole substituted pyrimidine based inhibitors of lymphocyte specific kinase (Lck)

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Received 7 June 2006; revised 30 August 2006; accepted 31 August 2006
Available online 25 September 2006

**Abstract**—This communication details the synthesis, biological activity, and binding mode of a novel class of 2-benzimidazole substituted pyrimidines. The most potent analogs disclosed showed low nanomolar activity for the inhibition of Lck kinase and a representative analog was co-crystallized with Hck (a structurally related member of the Src family kinases).


Lck is a 56-kD Src family protein tyrosine kinase (PTK) that plays a critical role in the development and activation of T cells including T-cell antigen receptor (TCR) phosphorylation (an event necessary for signal transduction in the T cell signaling cascade of the T-cell receptor).[1a,1b] Activation of this cascade ultimately results in the production of cytokines such as interleukin-2 (IL-2) and IFNγ.[1b,1c,1d] The production of these cytokines results in further activation and proliferation of T lymphocytes to generate an immune response. Unlike the widespread expression of some other Src family PTKs, Lck expression is restricted to T-cells and natural killer (NK) cells.[1d] As such the inhibition of Lck has been proposed as a potential treatment for a number of autoimmune diseases where T-cells are thought to play an important role such as rheumatoid arthritis (RA), inflammatory bowel disease (IBD), psoriasis, systemic lupus erythematosus (SLE), and organ graft rejection.[1e]

Screening efforts in our laboratories identified 4-benzo[*d*]isoxazole compounds **1a**–**b** as moderate Lck inhibitors (Fig. 1). Initial work directed at improving

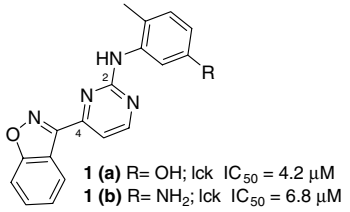


**Figure 1.** Initial benzo[*d*]isoxazole containing lead molecule.

the potency of these lead compounds led to the development of a facile SAR strategy incorporating benzimidazole substituted pyrimidines. This communication details the synthesis, biological activity, and binding mode of a novel class of 2,4,6-trisubstituted pyrimidine derivatives based on the initial lead benzo[*d*]isoxazole **1**. The binding mode of these trisubstituted pyrimidine inhibitors was also determined from X-ray co-crystallography experiments in the related hematopoietic cell kinase (Hck), a member of the Src family kinases.[1a,b]

The synthesis of the lead 2,4-disubstituted pyrimidines (**1a**–**b**) is outlined in Scheme 1. 4-Iodo-2-methylthiopyrimidine[2a] (**2**) was treated with isopropyl magnesium chloride followed by addition of 2-fluoro-benzaldehyde to give alcohol **3**.[2b,2c] Oxidation of this material with $MnO_2$ afforded the corresponding ketone which was condensed with hydroxyl amine resulting in oxime **4**.

*Keywords*: Kinase; Lck; Hck; Lymphocyte specific kinase; Hematopoietic cell kinase; Src family kinase; T cell.
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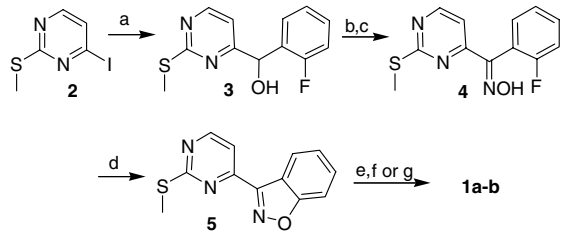
doi:10.1016/j.bmcl.2006.08.132

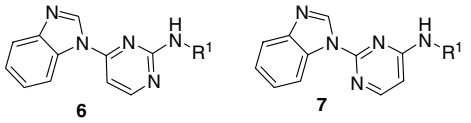


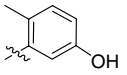
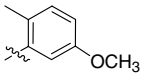
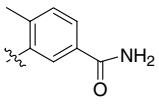
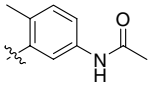
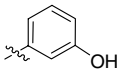
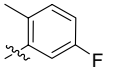

**Scheme 1.** Preparation of compounds **1a**–**b**. Reagents and conditions: (a) isopropyl magnesium chloride 2 M, THF −40 °C; then 2-fluorobenzaldehyde, 34%; (b) $MnO_2$, DCM, 24 h, 97%; (c) hydroxylamine·HCl, pyridine, 95 °C, 3 h; (d) NaH, DMF 165 °C, 0.5 h, 54% (2 steps); (e) *m*-CPBA, DCM, 0 °C, 0.25 h; (f) 3-amino-4-methylphenol, $CH_3CN$, 155 °C, microwave, 15% (2 steps **1a**); (g) 4-methylbenzene-1,3-diamine, $CH_3CN$, 150 °C, microwave, 0.5 h, HPLC separation of isomers, 1% (2 steps **1b**).

Treatment of this crude material with NaH followed by heat afforded intermediate **5**.[3] Oxidation of the thiogroup on compound **5** with Oxone® and displacement of the resultant sulfone/sulfoxide mixture generated the final products **1a**–**b**.[4]

To more quickly expand the SAR of pyrimidines **1** and to overcome synthetic difficulties with this scaffold, a benzimidazole group was substituted for the 4-benzo[*d*]isoxazole moiety. The resulting phenol **6a** (Table 1) proved to be a significantly more potent inhibitor (Lck $IC_{50}$ = 193 nM)[5a] compared to **1a**. Interestingly, the corresponding methyl ether **6b** and amides **6c**–**d** displayed greatly attenuated activity. Pyrimidine **6e** which did not contain the 4-methyl group on the C2 anilino substituent (compare **6a** vs **6e**) was devoid of activity.

**Table 1.** $IC_{50}$ values for derivatives **6a**–**m**, **7a**–**c**




| Compound | $R^1$ | Lck $IC_{50}$[a] (nM) |
|---|---|---|
| **6a** |  | 193 |
| **7a** | | 24 |
| **6b** |  | >10,000 |
| **7b** | | 7700 |
| **6c** |  | 5664 |
| **6d** |  | 8640 |
| **6e** |  | >10,000 |
| **7c** |  | >10,000 |

[a] $IC_{50}$'s were determined with a commercial Proflour assay (Promega corp., Cat. #1271).

Our attention subsequently focused on understanding the role of the pyrimidine ring nitrogen atoms (N1 and N3) in the potency observed. Regioisomeric analogs **7a**–**c** were synthesized using modified literature conditions and the results are presented in Table 1.[6]

Derivative **7a** which transposed the substituents at C2 and C4 relative to original compound **6a** displayed greater potency (Lck $IC_{50}$ = 24); however, the C4 C6 isomerically substituted compound (**8**, Fig. 2) was devoid of any Lck activity.[7]

With such a potent lead molecule (**7a**) we again attempted to introduce alternatively functionalized anilines at C4. However both the 5-methoxy (**7b**) and 5-fluoro aniline (**7c**) derivatives showed greatly attenuated activity (Table 1). The presence of both a 4-methyl and phenolic hydroxyl on the C2 anilino substituent appeared crucial for good activity.

Our efforts were next directed at adding functionality to improve the poor aqueous solubility (4 μg/ml) of scaffold **7a**.[8] A series of 2,4,6-trisubstituted pyrimidines were synthesized which maintained both the C2 and C4 groups found on **7a** while introducing various basic amine substituents to the C6 position of the pyrimidine core (Tables 2 and 3). We reasoned that basic amine substituents could be tolerated at position C6 after examining the active site of Lck in complex with inhibitors disclosed in the literature.[9] These groups may impart greater aqueous solubility and added potency through interactions with proximal acidic residues.

The synthesis of these 2,4,6-trisubstituted pyrimidines is outlined in Scheme 2. 4,6-Dichloro-2-methylsulfanyl-pyrimidine and 5-methoxy-2-methyl-phenylamine were heated at 140 °C to afford intermediate **10**. Oxidation of the thiol group in **10** with Oxone® followed by displacement of the resultant sulfone/sulfoxide mixture with sodium benzimidazolate and the subsequent phenol deprotection gave **11**. The 6-chloro group on this pyrimidine was then displaced with various amines and sodium alkoxides to give **12a**–**p**. Analogs **12q**–**r** resulted from Suzuki–Miyaura coupling of the corresponding vinyl heterocycle and **11**.[10]

The initially synthesized compounds **12a**–**e** containing simple substituents at the C6 position were somewhat

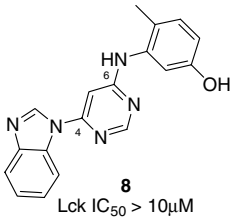
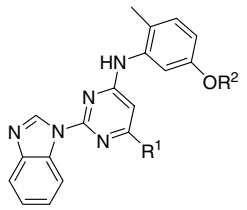
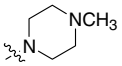
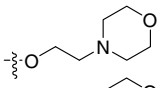
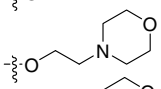
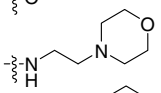
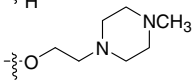
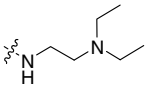
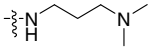
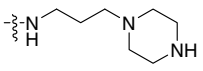
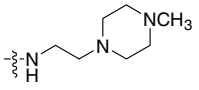
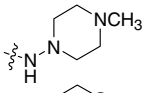
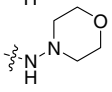
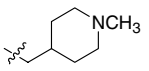
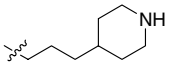



**Figure 2.** Compound **8**.

**Table 2.** Lck inhibition data for derivatives **12a–r**



| Compound | $R^1$ | $R^2$ | Lck $IC_{50}$[a] (nM) |
|---|---|---|---|
| **4** | –Cl | H | 1286 |
| **12a** | –NH2 | H | 294 |
| **12b** | $-NHCH_3$ | H | 79 |
| **12c** | $-N(CH_3)_2$ | H | 384 |
| **12d** | $-OCH_3$ | H | 71 |
| **12e** |  | H | 137 |
| **12f** |  | H | 3 |
| **12g** |  | H | 3 |
| **12h** |  | $-CH_3$ | 867 |
| **12i** |  | H | 17 |
| **12j** |  | H | 65 |
| **12k**[b] |  | H | 3 |
| **12l** |  | H | 44 |
| **12m** |  | H | 130 |
| **12n** |  | H | 45 |
| **12o** |  | H | 165 |
| **12p** |  | H | 519 |
| **12q** |  | H | 43 |
| **12r** |  | H | 333 |

[a] $IC_{50}$'s were determined with a commercial Proflour assay (Promega corp., Cat. #1271).

[b] Biological activity of **12k** in two related Src family kinases was determined in a similar fashion (Src $IC_{50}$ = 162 nM; Hck $IC_{50}$ = 46 nM).[5b,5c].

less potent inhibitors of Lck compared to the lead **7a** ($IC_{50}$ = 71–384 nM). The *N*-methyl piperazine analog **12f**, however, displayed better potency but possessed no aqueous solubility (sol $\leqslant$ 0.1 μg/ml at pH 7.4).

Introduction of an ethylmorpholine group at the C6 via an O (**12g**) or NH (**12i**) linker resulted in inhibitors with better potency in the enzyme assay (Lck $IC_{50}$ = 3 and 17 nM, respectively). Methylation on the phenolic hydroxyl (**12h**) dramatically reduced potency (compare **12g** vs **12h**).

Substitution of the *N*-methyl piperizine unit for morpholine (**12j** vs **12g**) gave the first analog with good aqueous solubility (sol = 70 μg/ml at pH 7.4) with only a slight decrease in activity (Lck $IC_{50}$ = 65 nM). Derivatives **12k–n** also displayed good kinase inhibition (Lck $IC_{50}$ = 3–130 nM). Hydrazino analogs **12o** and **12p** however displayed lower potency. Insertion of a methylene spacer (**12q**) produced an analog with better enzyme potency than the corresponding hydrazino analog **12o**. However insertion of an additional ethylene spacer (**12r**) in combination with a piperidine substituent (contrast **12q**, **12m**, and **12r**) reduced activity.

Representative compounds that showed promising Lck inhibition were tested for inhibition of IL-2 production in a Jurkat cellular assay.[11] Analogs displaying cellular potency were screened for in vitro metabolism[12] and progressed into PK studies (Table 3). Compounds **12g**, **12j**, and **12q** showed moderate to good IL-2 inhibitory activity (0.054, 0.400, and 0.163 μM, respectively). The morpholino compound **12g** however showed poor aqueous solubility. Metabolism studies of the compounds in Table 3 revealed moderate to high intrinsic clearance ($CL_{int}$ = 48–124 ml/min kg).[13] Inhibitors **12g**, **12h**, and **12q** were subsequently evaluated in a PK study to determine bioavailability and half-life. Both molecules **12g** and **12q** had no oral bioavailability. Only the less potent methoxy analog **12h** was orally bioavailable (% $F$ = 22; $T_{1/2}$ = 2.13 h).

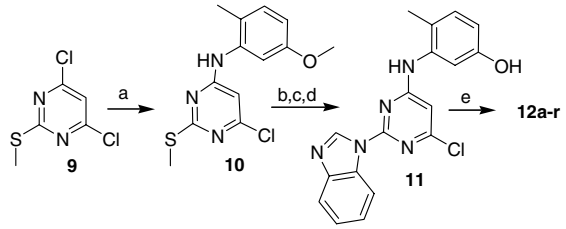
To delineate the binding mode of these molecules an X-ray crystallographic structure of **12k** with hck (a structurally related member of the Src family kinases) was obtained (Fig. 3).[14] Inhibitor **12k** orients itself in the hck enzyme such that the benzimidazole N hydrogen bonds to the amide N–H of Met319 and the phenolic OH hydrogen bonds to carboxyl of Glu 288. An additional interaction may occur between the O–H of the Thr316 (gatekeeper residue) and the aniline N–H on the phenol substituent. The 4-methyl group on the phenol substituent appears to sit in a small hydrophobic grove formed in part by the $CH_3$ of Thr316. This placement appears to position the phenolic OH for optimal interaction with Glu288.

In summary we have reported a class of trisubstituted pyrimidines that have shown nanomolar activity for inhibiting Lck kinase activity. The binding mode of this series of novel Lck inhibitors was determined through X-ray co-crystallographic studies in the structurally related Src family kinase Hck (PDB accession code 2HK5). Seven of the most potent analogs were further tested for inhibition of IL-2 cytokine production, showing a range of potencies (0.054–2.280 μM). The pharmacokinetic properties of these pyrimidine analogs were also evaluated. Compounds **12j–l**, **12n**, and **12q** all had

**Table 3.** IL-2 inhibition and pharmacokinetic data for select compounds

| Compound | IL-2[a] $IC_{50}$ (μM) | Solubility (μg/ml) | Met % loss[b] | $CL_{int}$[b] (ml/min kg) | $F$[d] (%) |
|---|---|---|---|---|---|
| **12g** | 0.054 | 3 | 48 | 124 | 0 |
| **12h**[c] | 1.270 | 27 | — | 48 | 22 |
| **12j** | 0.400 | 70 | 35 | 83 | — |
| **12k** | 0.549 | 127 | 45 | 107 | — |
| **12l** | 2.195 | 55 | 48 | 92 | — |
| **12n** | 2.281 | 54 | 39 | 93 | — |
| **12q** | 0.163 | 40 | — | 50 | 0 |

[a] Inhibition of IL-2 synthesis measured from Jurkat cells.
[b] % loss and intrinsic clearance after 1 h for compounds (1 μM concentration) tested in cryopreserved rat hepatocyte suspensions.
[c] Compound **12 h** had CL_plasma (ml/min kg) = 132, AUC-po (ng-h/ml) = 497, $C_{max}$ (ng/ml) = 76.
[d] Compounds dosed po and iv at 10 mg/kg.




**Scheme 2.** Preparation of compound **12a**–**r**. Reagents and conditions: (a) 5-methoxy-2-methyl-phenylamine, Hünig's base, DMF 140 °C, 85%; (b) Oxone® MeOH, $H_2O$, quantitative; (c) benzimidazole, NaH, DMF, 0 °C to generate sodium benzimidazolate then MW 60 °C, 67%; (d) $BBr_3$, DCM, 50%; (e) various amines, anilines, and alkoxides, NMP, MW 100 °C, 15 min.

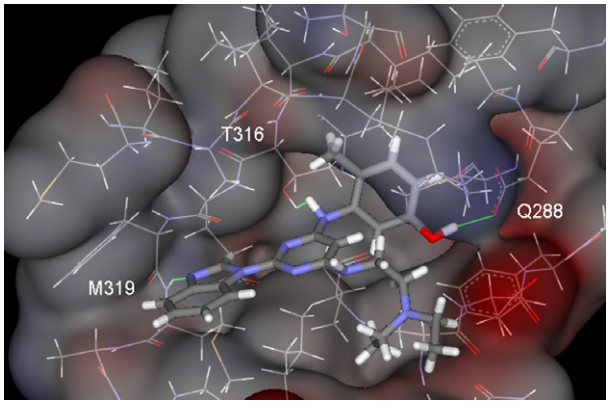



**Figure 3.** Key hydrogen bonds between **12k** and Hck.

good aqueous solubility (40–127 μg/ml). Metabolism studies of the compounds revealed moderate to high intrinsic clearance ($CL_{int}$ = 48–124 ml/min kg).[1] Subsequent PK studies revealed the phenol containing pharmacophore produced inhibitors with poor bioavailability. Only the methoxy derivative **12h** had good BA (% $F$ = 22, $T_{1/2}$ = 2.1 h).

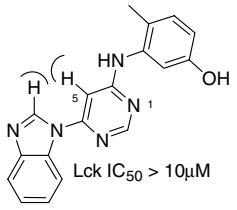
## Acknowledgments

We are grateful to A. L. Roe, C. A. Cruze, W. E. Schwecke, C. R. Dietsch for pharmacokinetic studies, and M. Buchalova for chemical stability and solubility studies.

## References and notes

1. (a) Molina, T. J.; Kishihara, K. I.; Siderovski, D. P.; van Ewijk, W.; Narendran, A.; Timms, E.; Wakeham, A.; Paige, C. J.; Hartmann, K. U.; Veillette, A.; Davidson, D.; Mak, T. W. *Nature* **1992**, *357*, 161; (b) Levin, D. S.; Anderson, S. J.; Forbush, K. A.; Perlmutter, R. M. *EMBO J.* **1993**, *12*, 1671; (c) August, A. *J. Biol. Chem.* **1996**, *271*, 10054; (d) Bolen, J. B.; Brugge, J. S. *Annu. Rev. Immunol.* **1997**, *15*, 371; (e) Kamens, J. S.; Ratnofsky, S. E.; Hirst, G. C. *Curr. Opin. Invest. Drugs* **2001**, *2*, 1213.
2. (a) Majeed, A. J.; Antonsen, O.; Benneche, T.; Undheim, K. *Tetrahedron* **1989**, *45*, 993; (b) Tobrman, T.; Dvorak, D. *Org. Lett.* **2003**, *5*, 4289; (c) Boudier, A.; Bromm, L. O.; Lotz, M.; Knochel, P. *Angew. Chem., Int. Ed.* **2000**, *39*, 4414.
3. Shutske, G. M.; Setescak, L. L.; Allen, R. C.; Davis, L.; Effland, R. C.; Ranbom, K.; Kitzen, J. M.; Wilker, J. C.; Novick, W. J. *J. Med. Chem.* **1982**, *25*, 36.
4. Oxidation with a single equivalent of Oxone® at 0 °C favored formation of sulfoxide. However, small amounts of the sulfone were formed. Both intermediates underwent nucleophilic displacement.
5. (a) The ability of compounds to inhibit human Lck enzyme ($IC_{50}$) was determined using the commercially available ProFlour Src-family Kinase Assay (Promega Corporation, Madison, WI; cat. #1271). The assay was performed according to manufacturer's instructions, with 2 nM recombinant human active Lck (Upstate Cell Signaling Solutions, Charlottesville, VA; cat. #14-442) at an ATP concentration of 10 uM. These IC50 assays were performed using 10-point dose–response curves, in duplicate (one curve on two separate plates), and on two separate days ($n$ = 2). Every assay plate included a 10 pt. dilution curve of 1 benchmark compound. Curve Fitting was performed using the Sigmoidal Dose Response Model (4 parameter logistic equation 205 in ExcelFit). 95% confidence intervals were calculated for each $IC_{50}$ value. The assay Quality Control Criteria inlcuded; Signal/Background: >4; Signal/Noise: >10; % Coefficient of Variance on No ATP Control (Max. Signal): <10.; (b) The ability of compounds to inhibit human Hck enzyme ($IC_{50}$) was determined using the commercially available ProFlour Src-family Kinase Assay (Promega Corporation, Madison, WI; cat. #1271). The assay was performed according to manufacturer's instructions, with 2.8 nM recombinant human active Hck (Invitrogen, Carlsbad, CA #P2908) at an ATP concentration of 10 mM.; (c) The ability of compounds to inhibit human Src enzyme ($IC_{50}$) was determined using the commercially available ProFlour Src-family Kinase Assay (Promega Corporation, Madison, WI; cat. #1271). The assay was performed according to manufacturer's instructions, with 14.3 nM

recombinant human active Src (Invitrogen, Carlsbad, CA #P3044) at an ATP concentration of 10 mM.
6. Luo, G.; Chen; Ling, P.; Graham, S. *Tetrahedron Lett.* **2002**, *43*, 5739.
7. The authors speculate that repulsive interaction between pyrimidine C5 proton and the proton on the imidazole ring forced an unfavorable orientation of this molecule toward the active site of Lck.




8. Solubility assay procedure: Solubility of analogs measured in 50 mM phosphate buffer, pH 7.4, ionic strength 0.15 M. The solubilities were determined by shake flask method after 24 h of equilibration. Concentrations in aqueous solutions were determined for supernatant of centrifuged and filtered samples by UV–vis spectrophotometry. Calibration solutions were prepared in acetonitrile/ buffer in 1:1 volume ratio. The samples were diluted with acetonitrile to obtain identical media with calibration standards.
9. Zhu, X.; Kim, J. L.; Newcomb, J. R.; Rose, P. E.; Stover, D. R.; Toledo, L. M.; Zhao, H.; Morgenstern, K. A. *Structure* **1999**, *7*, 651 (1QPC pdb, Research Collaboratory for Structural Bioinformatics).
10. Vice, S.; Bara, T.; Bauer, A.; Evans, C. A.; Ford, J.; Josien, H.; McCombie, S.; Miller, M.; Nazareno, D.; Palani, A.; Tagat, J. *J. Org. Chem.* **2001**, *66*, 2487.
11. IL-2 release was measured by stimulating the Jurkat E6-1 T cell line (human acute T cell leukemia, ATCC, Manassas, VA) with monoclonal anti-human CD3ε antibody and Phorbol Myristate Acetate (PMA) (Sigma, St. Louis, MO). Flat bottomed-96-well plates were pre-coated with 400 ng/well of anti-human CD3ε mouse antibody UCHT1 (R&D systems, Minneapolis, MN) and incubated for 2 h at 37 °C. Jurkat cells maintained in RPMI-1640 containing 10% fetal bovine serum and 1% antibiotics in the log growth phase ($2 \times 10^5$ to $1 \times 10^6$ cells/ml) were harvested and incubated in triplicate in 96-well plates for 30 min at 37 °C in the presence or absence of various concentrations of Lck inhibitors. The cell-inhibitor mixture was then transferred into the wells of the anti CD3ε-coated 96-well plates, and PMA was added to the wells at a final concentration of 10 ng/ml (1 ng/well). The plates were incubated overnight at 37 °C. The amount of IL-2 released into the culture media was measured by ELISA (R&D Systems) and the viability of the cells was determined using the MTS assay (Promega, Madison, WI).
12. (a) Measured as percent loss at 4 hours in rat hepatocytes; (b) In vitro metabolism assay procedure: In vitro metabolic stability of analogs in plated rat hepatocytes (Sprague–Dawley) obtained from Cedra Corporation. Metabolic activity was determined in triplicate using a total volume of 0.2 ml containing 0.25 μM NCE incubated in rat hepatocyte and Matrigel blank microtiter plates. The plates were maintained at 37 °C throughout the study. Samples were removed from wells at 0, 2, and 4 h, and NCE samples were analyzed by HPLC/MS/MS with reverse-phase chromatography. To improve analytical efficiency, compounds were grouped together (post-incubation) into a multi-compound assay. Samples from like time-points containing the different compounds were combined and an internal standard (1.1 ng/ml Stock) was added. Results for each compound were expressed as the ratio of the compound response area over the internal standard response area. Percent loss was calculated by dividing the 2 and 4 h ratios by the 0 h ratio.
13. (a) In vitro intrinsic clearance ($CL_{int}$) was calculated from the first-order depletion rate constant and appropriate biological scaling factors.[13b,13c]; (b) Davies, B.; Morris, T. *Pharm. Res.* **1993**, *10*, 1093; (c) Venkatakrishnan, K.; von Moltke, L. L.; Obach, R. S.; Greenblatt, D. J. *Curr. Drug Metab.* **2003**, *4*, 423.
14. (a) Crystallographic methods: The Hck protein herein described as used for the crystallographic studies is fully dephosphorylated human Hck kinase domain (HckKD), residues 225–504. The protein was co-expressed in *Escherichia coli* with the phosphatase PTPβ-90 to maximize production of soluble HckKD, which was otherwise toxic to the cells. The HckKD was cloned with a non-cleavable 6-His tag for purification purposes. Briefly, protein for crystallization studies was prepared by lysing biomass using a mixture of commercially available surfactant-based lysis buffers. The resulting lysate was clarified by centrifugation, loaded onto a Ni-affinity column (Sigma His-Select™), and eluted with a linear imidazole gradient (0.01–0.250 M). The resulting HckKD containing elution fractions were pooled and diluted 20× in an appropriate load buffer (20 mM Tris, pH 8.8, 10 mM DTT) for anion exchange chromatography (AEC) on MonoQ (GE Healthcare). A linear NaCl gradient in the AEC resulted in one large elution peak containing HckKD. Fractions from this peak were pooled and concentrated to 12–15 mg/ml and used for crystallization studies. Co-crystals with inhibitors were grown by incubating the concentrated HckKD with 0.5–1 mM compound for several hours before crystallization trials. Crystals suitable for X-ray diffraction grew in 3–7 days at 15 °C using either 1.9–2.2 M ammonium sulfate, 0.1 M Tris (pH 8.5) or 3.9–4.1 M sodium formate. Crystals could be improved by the addition of several additives to the above conditions, such as 5–10% glycerol or ethylene glycol. Optimal crystals were typically grown by streak seeding fresh drops using previously grown crystals. Crystals grew as hexagonal rods with average dimension $0.07 \times 0.07 \times 0.25$ mm (though crystals as small as $0.01 \times 0.01 \times 0.15$ were successfully used for data collection). X-ray data were collected at Southeast Regional Collaborative Access Team (SER-CAT) 22-ID beamline at the Advanced Photon Source, Argonne National Laboratory. Supporting institutions may be found at www.ser-cat.org/members.html. The crystals provided typical diffraction of better than 2.0 Å resolution. Crystals were indexed and X-ray data were processed in the $P6_5$ space group with approximate unit cell dimensions a = b = 96.4 Å, c = 70.4 Å. Structures were determined by molecular replacement using a structure previously determined in-house. Use of the Advanced Photon Source was supported by the US Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38; (b) The non-phos Hck construct used in these studies appears to place the activation loop in a conformation resembling activated Lck (3LCK, 1QPC; phosphorylated Y394); the authors speculate inhibitor **12k** is capable of inducing such a conformation through interaction with Glu288.