Bioorganic & Medicinal Chemistry Letters 21 (2011) 3078–3083

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of a potent and highly selective PDK1 inhibitor via fragment-based drug discovery

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ARTICLE INFO

Article history:
Received 9 December 2010
Revised 8 March 2011
Accepted 9 March 2011
Available online 17 March 2011

Keywords:
PDK1
Selective
Tethering
Pyridinone
DFG-out
Fragment-based drug discovery

ABSTRACT

We report the use of a fragment-based lead discovery method, Tethering with extenders, to discover a pyridinone fragment that binds in an adaptive site of the protein PDK1. With subsequent medicinal chemistry, this led to the discovery of a potent and highly selective inhibitor of PDK1, which binds in the 'DFG-out' conformation.

The phosphatidylinositol-3 kinase (PI3K) signalling pathway could provide a compelling intervention point to treat cancer. When the pathway is activated, PI3K generates phosphatidylinositol 3,4,5-triphosphate, which causes protein 3-phosphoinositide-dependent protein kinase-1 (PDK1) to localize to the plasma membrane, where it first autophosphorylates and subsequently phosphorylates the protein Akt. This activates Akt, which in turn phosphorylates a number of substrates ultimately resulting in increased cell survival and resistance to apoptosis. Because the pathway is activated in most tumors, blocking the pathway may sensitize tumors to other anticancer agents.[1]

The importance of the PI3K signalling pathway is reflected in the number of programs targeting pathway members. However, although there have been considerable drug discovery efforts on PI3K and Akt, PDK1 has been relatively ignored. Indeed, when we first started working on the target, there were almost no inhibitors reported in the literature. Since then there have been some reports,[2–6] but most of these compounds are not specific for PDK1 over other kinases. In terms of validating the target, a potent and highly specific inhibitor would be a useful tool compound.

We used fragment-based drug discovery to search for such compounds. Fragment-based drug discovery builds drug leads from smaller component molecules, or 'fragments'.[7–12] Screening fragments rather than fully elaborated molecules can increase the efficiency of sampling chemical space, and can potentially increase the novelty of resulting molecules.

A previously reported fragment-based discovery technology called Tethering[13] uses reversible disulfide bonds between a cysteine residue in a protein and a thiol-containing fragment to enable the capture and identification of weak binding fragments by mass spectrometry.[14] A later version of the technology, Tethering with extenders, identifies companion fragments in the presence of a known binding moiety, or 'extender'.[15,16] An extender can both irreversibly modify a target cysteine residue and reversibly capture companion fragments that bind to an adjacent site. We have also reported a variant of this technology, Tethering with dynamic extenders, and used it to discover Aurora A inhibitors.[17] Here, we report the use of Tethering with extenders to discover highly selective PDK1 inhibitors.

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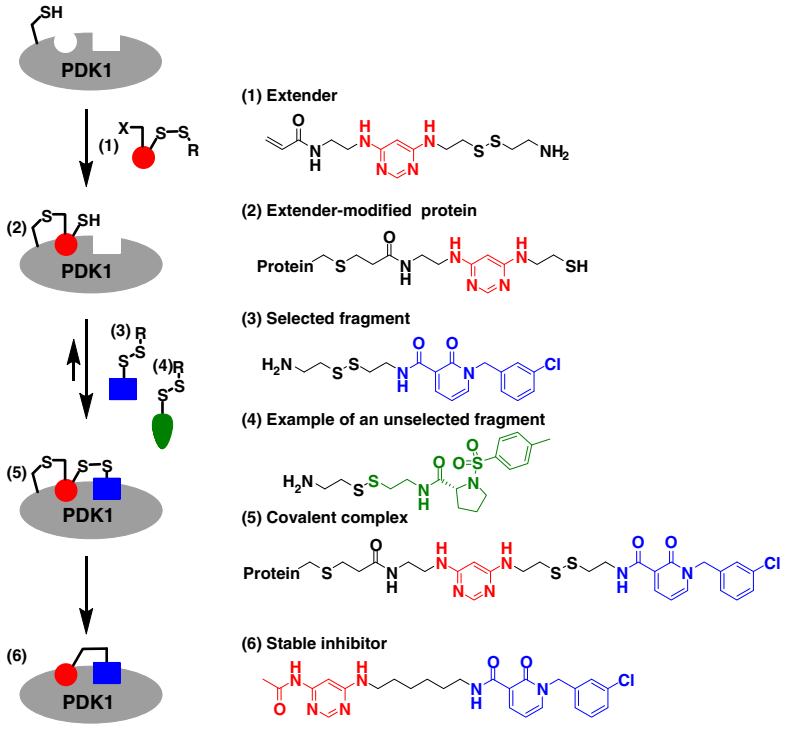
doi:10.1016/j.bmcl.2011.03.032



Scheme 1. Tethering with extenders applied to PDK1.

Our approach is shown schematically in Scheme 1. We first introduced a cysteine residue into PDK1 at position 166 (E166C). Next, we reacted the protein with an extender based on diaminopyrimidine (DAP), a moiety known to bind in the purine binding site, or hinge region.[17,18] The extender contains an acrylamide moiety that reacts with the introduced cysteine residue, as well as a disulfide linker. Under the labeling conditions, this disulfide is reduced to reveal a free thiol positioned for probing the adaptive binding site. We were able to obtain a crystal structure that reveals the extender binding as expected (Fig. 1), with the kinase in the active 'DFG-in' conformation.

We next performed a Tethering screen against a library of roughly 3000 compounds. Of these, one of the strongest hits was the pyridinone shown in Scheme 1. We used an unphosphorylated form of the protein for the initial screen; interestingly, when we repeated the screen with phosphorylated (active) PDK1, selection of the pyridinone was much weaker.

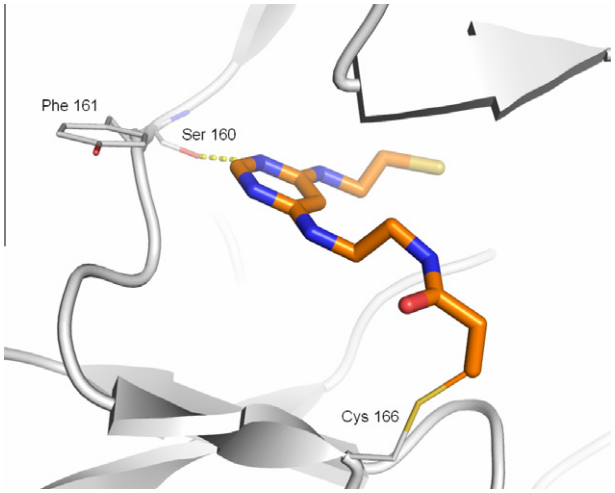


Figure 1. Crystal structure of complex **2**, (orange) extender-modified PDK1 (gray). Co-ordinates have been deposited with the protein data bank (PDB code: 3PWY).

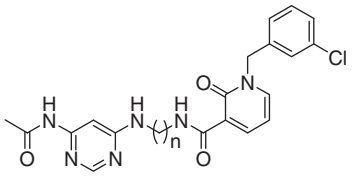
Subsequently, we replaced the disulfide linkage between the DAP moiety and the pyridinone with a simple alkyl carbon chain. We were pleased to find that the resulting compound **6** inhibited a cascade PDK1-Akt assay with an IC_{50} of 0.2 μM. Under the conditions of the assay, PDK1 autophosphorylates; when we repeated the assay, this time with pre-phosphorylated PDK1, we found that compound **6** was less potent, with an IC_{50} of 2.9 μM. This is consistent with the Tethering results, and both suggest that the inhibitor binds to the inactive form of the protein. Control experiments revealed that the compounds were completely inactive against Akt (data not shown).

We were interested to probe the linker-length dependence of this chemotype. We hypothesized that if the pyridinone is binding specifically to the protein, a linker, that is, either too long or too short would cause a loss in activity. Table 1 shows that there is in fact a pronounced linker length dependence, with 5 or 6 methylene units being optimal (compounds **10** and **6**), which is consistent with the linker length expected from Tethering. Furthermore, aware of the potential for artifacts among low-affinity binders, we tested some of our compounds at a high ATP concentration to ensure they were ATP-competitive. All the inhibitors are ATP-competitive, suggesting that the DAP element is contributing to the affinity.

Having established the optimal linker length, we next sought to improve the potency of our molecules by exploring replacements for the DAP. We made a small library of roughly 50 different purine mimetics, many of which had previously been reported as fragments of kinase inhibitors (Table 2).

We chose the five-methylene linker rather than the slightly more potent six-methylene linker to try to keep the molecules as compact as possible. Removing the DAP moiety (compound **12**) led to a complete loss of activity, and most amide (compound **13**) or sulfonamide (compound **14**) linked compounds were also inactive or only weakly active. On the other hand, a number of amino-heterocycles were tolerated, with compound **18** being slightly

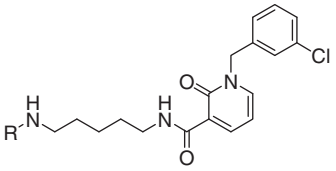
Table 1
Linker length-dependence of initial hit from Tethering with extenders

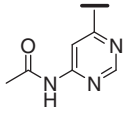
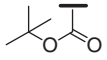
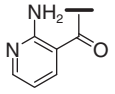
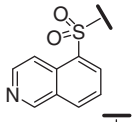
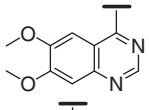
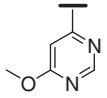
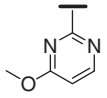


Compound	*n*	Unphosphorylated PDK-AKT IC_{50} (μM)	Phosphorylated PDK-AKT IC_{50} (μM)	Phosphorylated PDK-AKT, 20× K_m IC_{50} (μM)
7	2	ND	>50	ND
8	3	ND	>50	ND
9	4	ND	57	>200
10	5	0.64	10.8	111
6	6	0.2	2.9	28
11	7	ND	22	63

ND = Not determined.

Table 2
SAR around hinge-binding region

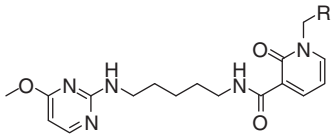


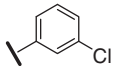
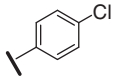
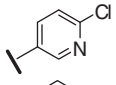
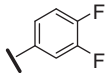
Compound	R	Unphosphorylated PDK-AKT IC_{50} (μM)	Phosphorylated PDK-AKT IC_{50} (μM)	Phosphorylated PDK-AKT, 20× K_m IC_{50} (μM)
10		0.64	10.8	111
12		>100	>200	>200
13		25	80	ND
14		ND	>50	ND
15		1.2	18	>200
16		2.0	31	>100
17		3.4	23	ND
18		0.78	5.3	45

more active than compound **10** as well as easier to work with synthetically.[20] Finally, the SAR suggested that a hydrogen-bond acceptor ortho to the exocyclic nitrogen was important for activity.

Having settled on the methoxyaminopyrimidine as a hinge-binder, we next explored changes to the pyridinone (Table 3). We found that the chlorine contributed modestly to the potency (compare compound **19** to compound **18**), and that a chlorine was also tolerated at the *para* position (compound **20**), but not at the *ortho* position (compound **21**). Heterocycles such as compound **22** were generally not tolerated, nor were alkyl substituents (com-

Table 3
SAR around pyridinone



Compound	R	Unphosphorylated PDK-AKT IC_{50} (μM)	Phosphorylated PDK-AKT IC_{50} (μM)
18		0.78	5.3
19		1.3	12
20		0.24	1.6
21		14	70
22		>100	>50
23		ND	>50
24		0.21	2.3

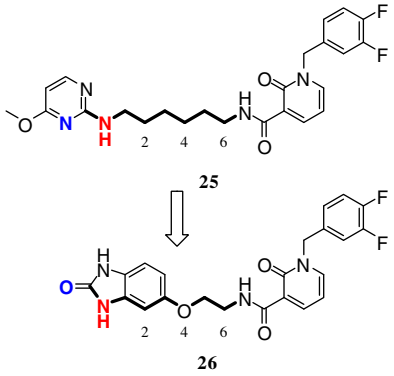
pound **23**). Compound **24**, with two fluorine atoms, was one of the more potent compounds obtained. As also seen above, all of these compounds show greater potency for unphosphorylated than pre-phosphorylated PDK1, consistent with binding to the inactive form of the protein.

At this point we were somewhat frustrated, as none of the substitutions had significantly improved potency over the initial compound **6**. We attempted to replace the flexible linker with a variety of aromatic and heteroaromatic spacers as we had done in previous programs,[15] but none of these improved activity, and indeed most of them decreased it (data not shown). Although we did not yet have a crystal structure of any of our compounds bound to PDK1, model-building suggested that the linker was buried far back in the active site, in a sterically congested region, and thus anything larger than a simple alkyl chain would not be accommodated. With this in mind, we designed a new molecule that would incorporate the hydrogen bond donor and acceptor that had been found to be important in the hinge-binding analysis. We mapped the six atoms between the hydrogen-bond donor and pyridinone amide of compound **25** onto compound **26**. Although the aromatic bond lengths in the latter compound would be shorter than their counterparts in compound **25**, we reasoned that since the five-atom linker of compound **24** was equipotent, the binding mode should be similar. Much to our delight, we found that compound **26** was more potent by roughly a factor of 50 in both the unphosphorylated and prephosphorylated assays (Scheme 2).

We examined the cellular activity of compounds in PC-3 cells. As mentioned above, Akt is a substrate for PDK1, and we used a cellular assay from Meso Scale Discovery (MSD) to measure the phosphorylation of residue Thr 308. We found that compound **26** was a weak but reproducible inhibitor, with an EC_{50} of 5.1 μM.

The ethanolamine linker of compound **26** is readily amenable to SAR studies using available chiral α-amino alcohols (Table 4). We found a strong stereochemical preference for substitution next to the nitrogen, with a 30-fold difference in potency between the methyl esters **27** and **28**. Hydrolyzing the methyl ester **27** to the free acid **29** led to a loss in potency, and substitution with either branched (compound **30**), unbranched (compound **31**), or benzyl substituents also reduced potency compared to compound **27**. However, compound **33**, with a phenyl substituent directly off the linker, proved to be a highly potent molecule with an IC_{50} of 2 nM in the pre-phosphorylated assay, and an EC_{50} of 400 nM in the cell assay. Synthesis of compound **33** is shown in Scheme 3.

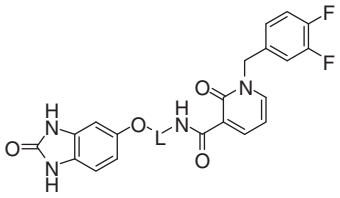
An X-ray crystal structure of compound **33** in complex with the PDK1 kinase domain is shown in Figure 2. As expected, the cyclic urea moiety lies in the ATP-binding pocket while the fragment identified through Tethering binds in the allosteric DFG-out pocket. The hinge interactions consist of a pair of hydrogen bonds between the amino group and the carbonyl of the cyclic urea that interact with, respectively, the carbonyl oxygen of Ser 160 and

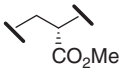
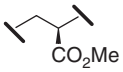
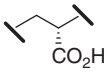
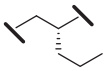
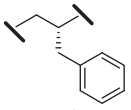
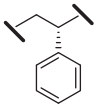


unphosphorylated PDK-AKT IC_{50}[μM]	phosphorylated PDK-AKT IC_{50}[μM]
0.32	2.6
<0.013	0.045

Scheme 2. Redesign of linker-hinge region. Note that the lower limit of detection for the unphosphorylated assay is 13 nM.

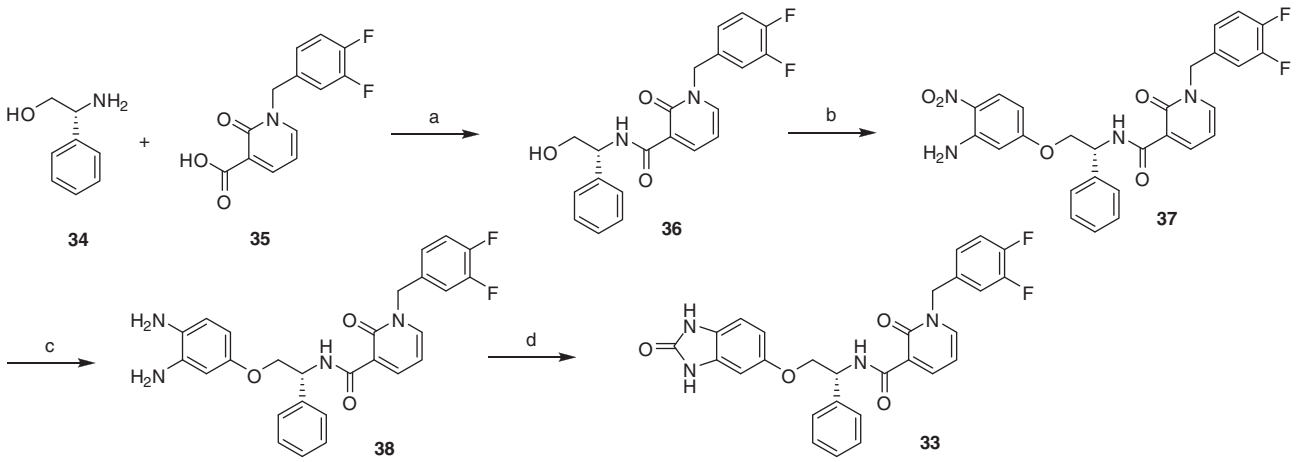
Table 4
SAR around linker region



Compound	L	Unphosphorylated PDK-AKT IC_{50} (μM)	Phosphorylated PDK-AKT IC_{50} (μM)	Cellular assay p308 (AKT) EC_{50} (μM)
26		<0.013	0.045	5.1
27		<0.013	0.006	2.1
28		0.045	0.18	>10
29		0.029	0.26	ND
30		0.077	0.5	2.6
31		0.024	0.083	2.7
32		0.042	0.46	5.4
33		<0.013	0.002	0.40

the backbone amide of Ala 162 of PDK1, which is reminiscent of the binding mode of the indole based inhibitors of PDK1 from Berlex.[4] The phenyl substitutent at the linker of the inhibitor engages in a key cation–π stacking interaction with the catalytic Lys 111. Interestingly, the central pyridinone core of **33** forms two hydrogen bonds, one being intramolecular, while the other is with PDK1 from the backbone amide of Asp 223 (DFG motif). In addition, the pyridinone ring is flanked on the opposite side by hydrophobic contacts with gatekeeper residue Leu 159 and Val 143. Finally, the terminal difluorobenzyl ring resides in the allosteric DFG-out pocket, that is, formed by mostly hydrophobic residues including Met 134, Leu 137, Phe 142, Leu 196 and Ile 221.

The compound **33** was profiled at Upstate Scientific at 10 μM concentration against what was at the time their entire panel of 241 kinases and showed no significant activity against any other kinases in the PI3K pathway. Moreover, it inhibited only one kinase



Scheme 3. Synthesis of compound **33**. Reagents and conditions: (a) EDC, HOBT, DIEA, DMF, rt, 16 h, 84%; (b) 5-fluoro-2-nitroaniline, NaH, DMF, rt, 16 h, 53%; (c) Zn, HCl, methanol–isopropanol, rt, 2 h, 100%; (d) 1,1′-carbonyl-diimidazole, DMF, 60 °C, 4 h.

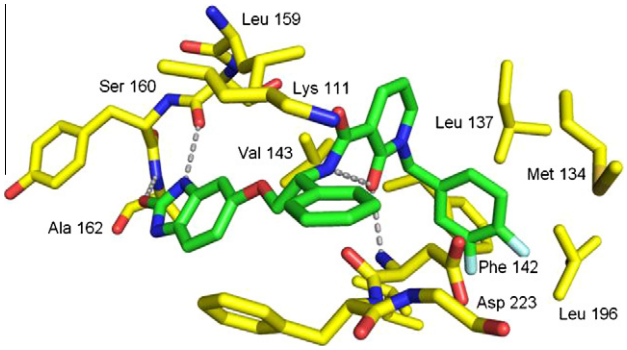


Figure 2. Co-crystal structure of compound **33** bound to PDK1, showing the interactions between the compound (green) and the protein (yellow). Figure made using PyMOL.[22] Co-ordinates have been deposited with the protein data bank (PDB code: 3QC4)

(Musk, 98% inhibition) besides PDK1 (92% inhibition) by more than 80%. With its high kinase selectivity and desirable cell potency, compound **33** was evaluated in a PC-3 prostate xenograft model. The compound was dosed intraperitoneally at 100 mg/kg to PC-3 tumor-bearing CB17 SCID mice and showed statistically significant 35% inhibition of phosphorylated Akt-T308 at 2 h but less inhibition at later time points, due to its poor pharmacokinetic properties, such as a low plasma exposure and high clearance.[19]

In conclusion, we have used Tethering with extenders to identify a fragment that binds in the adaptive region of PDK1 and is selective for the protein's inactive conformation. We used medicinal chemistry to advance this fragment to a low nanomolar inhibitor of PDK1 with activity in cells. An X-ray structure of the complex of this compound with PDK1 confirmed its unique DFG-out binding mode, which is to our knowledge the first reported DFG-out PDK1 inhibitor. This compound should prove a useful tool for probing PDK1 biology. In fact, after seeing the disclosure of this class of molecules,[20] researchers from Merck have re-synthesized and characterized compound **33**, confirming our findings.[21] Moreover, the promising in vivo PD activities demonstrated with this highly selective PDK1 inhibitor have prompted us to search for potent and selective PDK1 inhibitors that will have better pharmacokinetic properties for improved PD and efficacy activity.

Acknowledgments

We thank Monya L. Baker for helpful suggestions on the manuscript and Stuart Lam, Jacky Wang, and Liping Gao for preparatory HPLC and compound handling.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.032.

References and notes

1. Mora, A.; Komander, D.; van Aalten, D. M.; Alessi, D. R. *Semin. Cell Dev. Biol.* **2004**, *15*, 161.
2. Feldman, R. I.; Wu, J. M.; Polokoff, M. A.; Kochanny, M. J.; Dinter, H.; Zhu, D.; Biroc, S. L.; Alicke, B.; Bryant, J.; Yuan, S.; Buckman, B. O.; Lentz, D.; Ferrer, M.; Whitlow, M.; Adler, M.; Finster, S.; Chang, Z.; Arnaiz, D. O. *J. Biol. Chem.* **2005**, *280*, 19867.
3. Gopalsamy, A.; Shi, M.; Boschelli, D. H.; Williamson, R.; Olland, A.; Hu, Y.; Krishnamurthy, G.; Han, X.; Arndt, K.; Guo, B. *J. Med. Chem.* **2007**, *50*, 5547.
4. Islam, I.; Bryant, J.; Chou, Y. L.; Kochanny, M. J.; Lee, W.; Phillips, G. B.; Yu, H.; Adler, M.; Whitlow, M.; Ho, E.; Lentz, D.; Polokoff, M. A.; Subramanyam, B.; Wu, J. M.; Zhu, D.; Feldman, R. I.; Arnaiz, D. O. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3814.
5. Islam, I.; Brown, G.; Bryant, J.; Hrvatin, P.; Kochanny, M. J.; Phillips, G. B.; Yuan, S.; Adler, M.; Whitlow, M.; Lentz, D.; Polokoff, M. A.; Wu, J.; Shen, J.; Walters, J.; Ho, E.; Subramanyam, B.; Zhu, D.; Feldman, R. I.; Arnaiz, D. O. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3819.
6. Medina, J. R.; Blackledge, C. W.; Heerding, D. A.; Campobasso, N.; Ward, P.; Briand, J.; Wright, L.; Axten, J. A. *ACS Med. Chem. Lett.* **2010**, *1*, 439.
7. Erlanson, D. A.; McDowell, R. S.; O'Brien, T. *J. Med. Chem.* **2004**, *47*, 3463.
8. Rees, D. C.; Congreve, M.; Murray, C. W.; Carr, R. *Nat. Rev. Drug Disc.* **2004**, *3*, 660.
9. *Fragment-based Approaches in Drug Discovery*; Jahnke, W., Erlanson, D. A., Eds.; Wiley-VCH: Weinheim, Germany, 2006; 34,.
10. Erlanson, D. A. *Curr. Opin. Biotechnol.* **2006**, *17*, 643.
11. Hajduk, P. J.; Greer, J. *Nat. Rev. Drug. Disc.* **2007**, *6*, 211.
12. Congreve, M.; Chessari, G.; Tisi, D.; Woodhead, A. J. *J. Med. Chem.* **2008**, *51*, 3661.
13. Tethering is a registered service mark of Sunesis Pharmaceuticals, Inc. for its fragment-based drug discovery.
14. Erlanson, D.; Braisted, A.; Raphael, D.; Randal, M.; Stroud, R.; Gordon, E.; Wells, J. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 9367.
15. Erlanson, D. A.; Lam, J. W.; Wiesmann, C.; Luong, T. N.; Simmons, R. L.; DeLano, W. L.; Choong, I. C.; Burdett, M. T.; Flanagan, W. M.; Lee, D.; Gordon, E. M.; O'Brien, T. *Nat. Biotechnol.* **2003**, *21*, 308.
16. Erlanson, D. A.; Wells, J. A.; Braisted, A. C. *Annu. Rev. Biophys. Biomol. Struct.* **2004**, *33*, 199.
17. Cancilla, M. T.; He, M. M.; Viswanathan, N.; Simmons, R. L.; Taylor, M.; Fung, A. D.; Cao, K.; Erlanson, D. A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3978.
18. Bridges, A. *J. Chem. Rev.* **2001**, *101*, 2541.
19. PK parameters of compound **33** after intravenous injection in male rats at 1 mg/kg dose: AUC_{last} = 289 h × ng/mL, CL = 45.4 mL/min/kg, and MRT_{last} = 2.2 h.
20. Lind, K. E.; Cao, K.; Lin, E. Y.; Nguyen, T. B.; Tangonan, B. T.; Erlanson, D. A.; Guckian, K.; Simmons, R. L.; Lee, W.; Sun, L.; Hansen, S.; Pathan, N.; Zhang, L. WO 2008/005457, 2008.
21. Nagashima, K.; Shumway, S. D.; Sathyanarayanan, S.; Chen, A. H.; Dolinski, B.; Xu, Y.; Keilhack, H.; Nguyen, T.; Wiznerowicz, M.; Li, L.; Lutterbach, B. A.; Chi, A.; Paweletz, C.; Allison, T.; Yan, Y.; Munshi, S. K.; Klippel, A.; Kraus, M.; Bobkova, E. V.; Deshmukh, S.; Xu, Z.; Mueller, U.; Szewczak, A. A.; Pan, B. S.; Richon, V.; Pollock, R.; Blume-Jensen, P.; Northrup, A.; Andersen, J. N. *J. Biol. Chem.* **2011**, *286*, 6433.
22. DeLano, W. L. http://pymol.sourceforge.net/.