# Optimization of a Dibenzodiazepine Hit to a Potent and Selective Allosteric PAK1 Inhibitor

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# **(5)** Supporting Information



**ABSTRACT:** The discovery of inhibitors targeting novel allosteric kinase sites is very challenging. Such compounds, however, once identified could offer exquisite levels of selectivity across the kinome. Herein we report our structure-based optimization strategy of a dibenzodiazepine hit 1, discovered in a fragment-based screen, yielding highly potent and selective inhibitors of PAK1 such as 2 and 3. Compound 2 was cocrystallized with PAK1 to confirm binding to an allosteric site and to reveal novel key interactions. Compound 3 modulated PAK1 at the cellular level and due to its selectivity enabled valuable research to interrogate biological functions of the PAK1 kinase.

**KEYWORDS:** PAK1, p21 activated kinase, allosteric inhibitor, dibenzodiazepine, tool compound

PAKs or p21-activated kinases are a family of serine/ threonine protein kinases that can be divided into two subgroups: group I consisting of PAK1-3 and group II consisting of PAK4-6.1 They are effectors of Rac/Cdc42 GTPases and play an important role in cell proliferation, survival, motility, and angiogenesis.<sup>2</sup> Group I PAKs are directly activated upon binding of GTPases, while the activity of group II PAKs is independent of GTPase binding.<sup>3</sup> Most PAK isoforms (in particular PAK1 and PAK4) have attracted a lot of interest and are considered as promising oncology targets because of their overexpression, amplification, and/or activation in many cancers.<sup>4,5</sup> In addition, there are reports suggesting that group I PAK inhibitors have potential in treating Alzheimer and Huntington diseases<sup>6</sup> and FXS (fragile X syndrome).<sup>7</sup> PAK1 is one of the most studied family members, and there are reports implicating dysregulation of PAK1 in breast and squamous NSCLC cancers<sup>8</sup> and upregulation of PAK1 in pancreatic cancers.9 It is also described that PAK1 regulates cytoskeletal signaling, promotes oncogenic transformation and survival,

affects chromatin and nuclear signaling, and promotes hormone independence.<sup>10</sup> Unfortunately, despite enormous interest in further elucidating PAK1 biology there is still no potent and selective inhibitor targeting exclusively the PAK1 isoform that would be suitable as a tool compound. There have been several pan-PAK inhibitors reported to date.<sup>11</sup> Most of them such as **PF-3758309**,<sup>12</sup> **FRAX486**, **FRAX597**,<sup>13,14</sup> and compound 4<sup>15</sup> bind in the active site (Figure 1). Despite targeting the same site, they exhibit different levels of selectivity across PAK isoforms and across the kinome in general. For example, **PF-3758309** is a pan-PAK inhibitor of both group I and group II isoforms, while FRAXs are highly selective for group I, and compound 4 displays group II selectivity (Table 1).

However, due to the fact that they target the highly conserved ATP site, selectivity over other kinases remained

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Figure 1. Known PAK inhibitors.

hard to achieve. Only the recently published compound 4, which extends into the back pocket from the ATP site, has been shown to be specific and can be considered as a good tool to study PAK group II biology. In general, allosteric kinase inhibitors are expected and have been shown to possess excellent selectivity across the kinome.<sup>16</sup> The field is rapidly growing and recent examples of allosteric inhibitors of Akt1,<sup>17</sup> LIMK2,<sup>18</sup> and RIP1<sup>19</sup> have been reported. Not surprisingly, there have also been efforts directed toward finding allosteric PAK1 inhibitors. For example, screening of a small library of compounds against full-length PAK1 resulted in the discovery of **IPA-3**, which binds to PAK1 in the regulatory domain and prevents its activation by Cdc42.<sup>20,21</sup>

It inhibits PAK1 with moderate potency (IC<sub>50</sub> 2.5  $\mu$ M), is noncompetitive with ATP, and was found to be selective over group II PAKs and other kinases (Figure 1, Table 1). However, it lacks sufficient potency, and further optimization is required to develop it into a compound to explore PAK1 biology on a cellular level.

Dibenzodiazepines, such as 1 and 5, discovered in a fragment-based screen, were found to bind to PAK1 kinase in a novel allosteric site adjacent to the ATP binding site with the DFG loop adopting the "out" conformation (Table 2, Figure 2).

Typical for the DFG-out binders targeting the inactive conformation of the enzyme, such as type II kinase inhibitors, compounds 1 and 5 were found to be more active in the PAK1 dephosphorylated assay (compound 1, PAK1 phos/dephos IC<sub>50</sub> = 54/12  $\mu$ M; compound 5, PAK1 phos/dephos IC<sub>50</sub> = 9/ 0.9  $\mu$ M). Compounds 1 and 5 are usually referred to as type III allosteric inhibitors and are distinguished from type II inhibitors in that the ATP site is occluded (Figure 2) and canonical interactions with the hinge region are not formed. Several allosteric ligands binding to a similar site have been previously reported for other kinases (e.g., IGF1R,<sup>22</sup> FAK,<sup>23</sup> LIMK2,<sup>18</sup> etc.). Interestingly, we observed a shift in IC<sub>50</sub> upon increase of the ATP concentration for compound 5 (IC<sub>50</sub> 1, 6, and >25  $\mu$ M at 1.5, 15, and 150  $\mu$ M ATP concentration, respectively), suggesting that it may be ATP-competitive. This is likely an

indirect competition due to incompatibility of ATP binding with the DFG-out binding conformation of our allosteric hit.

Despite being a diverse and privileged scaffold among many receptors and targets, dibenzodiazepines are not particularly well-known as kinase inhibitors. There are only a few reports describing specific structural analogues such as dibenzodiazepine-ones as Chk1 inhibitors<sup>24</sup> and amino-pyrimido-diazepines as BMK1 inhibitors.<sup>25</sup> In these examples the compounds bind to the kinases in the active site and interact with the hinge either through the amide function present on the tricyclic core or via a fused amino-pyrimidine ring.

We reasoned that compounds 1 and 5 could serve as highly attractive starting points with the goal of improving biochemical potency while retaining exquisite kinase selectivity.

The synthesis of optimized PAK1 inhibitors is described in the Supporting Information. All compounds were first tested for potency in the primary biochemical assay using a more sensitive dephosphorylated form of the PAK1 protein. Their selectivity against other PAK isoforms was assessed at DiscoveRx Corporation (San Diego, CA, United States).

Both compounds 1 and 5, despite being very selective across a panel of kinases, turned out to modulate several GPCRs in the nanomolar range. For example compound 1 exhibited inhibition of the histamine receptor H1 ( $IC_{50} = 1 \text{ nM}$ ) and the muscarinic receptor M1 ( $IC_{50} = 6$  nM). Therefore, mitigation of GPCR liability was one of the key aspects of the optimization process. Our optimization began with probing the hydrophobic region in the northern part of the molecule. We introduced a bulkier and more hydrophobic ethyl substituent on the aniline nitrogen to result in inhibitor 11 (Table 2). This modification led to a 3-fold improvement in potency. Compound 11 was cocrystallized with PAK1 (Figure 3). The X-ray crystal structure of PAK1 and 11 confirmed an allosteric binding mode in the DFG-backpocket adjacent to the ATP binding site beside the gatekeeper (Met344) and beneath helix  $\alpha$ C. The DFG-motif and helix  $\alpha C$  are displaced from the active conformation (PDB code 1YHV) and resemble the autoinhibited inactive state of PAK1 (PDB code 1F3M). The binding is characterized by shape complementarity, a halogen carbonyl interaction (Thr406), and a salt bridge of the piperazine moiety with the catalytic amino acid Glu315 from helix  $\alpha$ C. The additional ethyl group of 11 causes a marked shift of the N-terminal lobe of the kinase compared to the structure of the original hit 1. Space is created between the two methionine side chains and Val328 by a further shift of the Chelix outward and a rotation of the beta-sheet domain down over the ATP site, along with a reordering of the DFG motif and activation loop, burying the ligand.

To relieve the conformational strain evident in the X-ray structure of PAK1 with compound 11, we designed a series of amine derivatives with a preorganized bent conformation. To our surprise, the Boc-intermediate 13 maintained activity despite lacking the charged amine (and the associated salt

Table	1.	PAK	$IC_{50}$	for	Known	Inh	ibitors
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Compound	PAK1 $IC_{50}$ (nM)	PAK2 $IC_{50}$ (nM)	PAK3 IC <sub>50</sub> (nM)	PAK4 IC <sub>50</sub> (nM)	PAK5 $IC_{50}$ (nM)	PAK6 $IC_{50}$ (nM)
PF-3758309	$13.7 \pm 1.8$	190	99	$18.7 \pm 6.6$	$18.1 \pm 5.1$	$17.1 \pm 5.3$
FRAX486	8.3	39.5	55.3	779		
FRAX597	7.7	12.8	19.3	>10000		
4	5420	970	>10000	7.5	126	36
IPA-3	2500			>10000	>10000	>10000

# Table 2. SAR of PAK1 Inhibitors; Selectivity vs. Other PAK Isoforms<sup>a</sup>



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Compound	R1, X	R <sub>2</sub>	PAK1dephos	PAK1	PAK2	PAK3	PAK4	PAK6
			$IC_{50}(nM)$	Kd (nM)	Kd (nM)	Kd (nM)	Kd (nM)	Kd (nM)
1	H, F	1	12000					
5	CH <sub>3</sub> , Cl	Ň	900					
11	CH3CH2, Cl	N	323	340	>40000	>40000	>40000	>40000
13	CH3CH2, Cl	↓ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	190	130	>40000	>40000	>40000	>40000
2	CH3CH2, Cl		18	9.9	1100	>40000	>40000	
3	CHF2CH2, Cl		5.2	7	400			

<sup>a</sup>IC<sub>50</sub> values represent means of 2–4 measurements. Individual data points in each experiment were within 2-fold range with each other.



Figure 2. X-ray structure of 1 (green) binding in the novel allosteric site of PAK1.

bridge interaction with Glu315). Introduction of the hydrogen bond donor in the urea analogue 2 (Table 2) led to a further 10-fold improvement in potency. The X-ray cocrystal structure of PAK1 and ligand 2 (Figure 4) showed that the urea NH indeed interacts with Glu315, positioning the tertiary butyl group in a hydrophobic groove above the tricycle. The urea



**Figure 3.** Overlay of the X-ray structures of PAK1 (cyan and green, respectively) with 1 (cyan) and 11 (yellow) binding in the allosteric DFG-out pocket between the gatekeeper Met344, helix  $\alpha$ C (right), and the DFG motif (Asp407 and Phe408 are shown). Key residues are shown in stick form. Hydrogen bonds between PAK1, water molecules and 11 are shown as dashed red lines.

carbonyl is engaged in a water-mediated hydrogen bond network with Lys299. Finally, the optimization focused on improving the solubility of compound 2 (<0.004 mM at pH 6.8). This was achieved by replacing the *tert*-Bu group by the less hydrophobic *i*-Pr substituent. In addition, incorporation of two fluorine atoms to the ethyl group resulted in a slight improvement of the biochemical potency. Our optimization



**Figure 4.** Overlay of the X-ray structures of PAK1 (magenta and green, respectively) with the allosteric ligands **2** (yellow) and **11** (green) binding in the DFG-out pocket between the gatekeeper (Met344), helix  $\alpha$ C (right), and the DFG motif (Asp407 and Phe408 are shown). Key residues are shown in stick form. Hydrogen bonds between PAK1, water molecules, and **2** are shown as dashed red lines. Several water molecules were omitted for clarity as were the hydrogen bonds between water molecules and the protein.

efforts resulted in compound 3, which exhibited significantly improved aqueous solubility (0.055 mM at pH 6.8; >1 mM at pH 4.0). Compound 3 showed excellent activity in biochemical assays and an exceptional selectivity profile against other known kinases (Figure 5).



Figure 5. Selectivity profile of compound 3 assessed at DiscoveRx. Image generated using TREE*spot* Software Tool and reprinted with permission from KINOME*scan*, a division of DiscoveRx Corporation.

It demonstrated a selectivity score (S10-score) of 0.003 when tested at 10  $\mu$ M against 442 kinases in the KinomeScan competition binding assay (DiscoveRx Corporation, San Diego, United States). In contrast, the reference inhibitor **PF-3758309** showed a selectivity score of 0.111 (hit 10 out of 96 kinases, screened at 1  $\mu$ M, data not shown). The optimized compound 3 had a biochemical PAK1 K<sub>d</sub> of 7 nM and a PAK2 K<sub>d</sub> of 400 nM. Selectivity over PAK2 was not anticipated at the beginning of the project since it is known that PAK1 and PAK2 share 93% of homology in the kinase domain.<sup>1</sup> Such excellent selectivity over PAK2 is hard to rationalize (our hypothesis is explained in the Supporting Information). It was pleasing to see that omitting the basic nitrogen resulted in a very clean profile in the off-target panel (22 targets with all IC<sub>50</sub>s > 10  $\mu$ M, for a list of off-targets see Supporting Information). Compound 3 possessed good physicochemical properties (solubility 0.055 mM at pH 6.8, HT logP 5.5, high PAMPA permeability cFA, calculated fraction absorbed = 99%, high Caco2 permeability  $P_{app}$  A-B 42.8 × 10<sup>-8</sup> cm/s, and efflux ratio 0.55). In addition compound 3 did not display significant inhibition of CYP450s (13.2  $\mu$ M for 3A4 the midazolam site, >20  $\mu$ M for 2D6 and 16.7  $\mu$ M for 2C9). Only relatively poor stability of compound 3 in rat liver microsomes (RLM) would limit its application for in vivo studies ( $t_{1/2}$  in RLM 3.5 min); however, our primary goal was to study PAK1 biology on the cellular level.

Having identified **3** as a potent and selective PAK1 inhibitor, we decided to validate the cellular potency in cell lines where both PAK1 and PAK2 are activated as measured by the autophosphorylation of Ser144 on PAK1 and Ser141 on PAK2. These phosphorylation sites prevent the autoinhibition by the p21-binding domain (PBD) of PAK1 and PAK2.<sup>26,27</sup> Given that there is no known downstream substrate of PAK1 that differs from PAK2, we believe that measuring PAK1 and PAK2 autophosphorylation is the best estimation of cellular potency of PAK1 and PAK2.

Several pancreatic cancer cell lines with mutated KRAS were shown by us to express high levels of activated PAK1 and PAK2 as measured by PAK1/2 phosphorylation on Ser144/141. One of them, Su86.86, was particularly sensitive to PAK1/2 shRNA mediated growth inhibition and was used as our cell line of choice for any downstream signaling analysis. As shown in Figure 6, incubation of 3 at increasing concentrations inhibited both PAK1 and PAK2 autophosphorylation.



Figure 6. Target modulation of PAK1/2 by 3 in the Su86.86 cell line.

Consistent with the observation that **3** is a more potent inhibitor of PAK1 than PAK2 measured biochemically using *in vitro* kinase assays and reflected in the biophysical binding  $K_d$  measurement (Table 2), the compound demonstrates higher inhibitory activity on PAK1 autophosphorylation than on PAK2.

Using compound 3, we attempted to demonstrate inhibition of phosphorylation of the downstream substrate MEK1 Ser289. However, since both PAK1 and PAK2 mediate MEK1 phosphorylation, compound 3 is not sufficiently potent to inhibit this phosphorylation event at concentrations up to 2–6

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 $\mu$ M because PAK2 is not completely inhibited. We do observe significant inhibition of pMEK Ser289 with compound 3 at 6–20  $\mu$ M, the lowest tested concentrations that show complete and unambiguous inhibition of both pPAK1 and pPAK2. Consistent with this observation, compound 3 inhibited proliferation of Su86.86 cell line only above a concentration of 2  $\mu$ M. In contrast, by applying a mixture of compound 3 and PAK2 shRNA, we achieved inhibition of downstream signaling and cell proliferation at a significantly lower 0.21  $\mu$ M concentration (Supporting Information). Thus, for cell lines that are dependent upon and express both isoforms, we hypothesize that a dual PAK1/2 inhibitor would be necessary for efficient inhibition of cell proliferations) that are dependent upon and express that are dependent upon and express only PAK1 were not successful.

In summary, we have developed a potent and selective PAK1 inhibitor. Due to its allosteric binding mode, compound **3** demonstrates high selectivity for inhibition of PAK1 over other PAK isoforms and the kinome in general. In addition, the lack of activity on GPCRs combined with favorable physicochemical properties make it an excellent research compound to study biological functions of PAK1 on the cellular level. Furthermore, we have demonstrated that this compound shows target modulation of PAK1 in cells but does not result in inhibition of cell proliferation. Further studies concerning biological consequences of PAK1 inhibition will be reported later.

#### ASSOCIATED CONTENT

#### **S** Supporting Information

Complete experimental procedures, biochemical assays, crystallization, structure determination, rationale explaining selectivity over PAK1, inhibition of cell proliferation, and safety panel assays. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/ acsmedchemlett.5b00102.

#### **Accession Codes**

The coordinates for the PAK1:1, PAK1:2, and PAK1:11 complexes have been deposited in the Protein Data Bank with accession codes 4ZLO, 4ZJJ, and 4ZJI, respectively.

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# **Author Contributions**

The manuscript was written through contributions of all authors.

#### Notes

The authors declare no competing financial interest.

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# ABBREVIATIONS

ATP, adenosine 5'-triphosphate; DFG, aspartate-phenylalanineglycine loop; GPCR, G-protein coupled receptor; GTPase, guanosine 5'-triphosphatase; H1, the histamine H1 receptor; M1, the muscarinic M1 receptor; PAK, p-21-activated kinase

#### REFERENCES

(1) Jaffer, Z. M.; Chernoff, J. p21-Activated Kinases: Three More Join the Pak. *Int. J. Biochem. Cell Biol.* **2002**, *34*, 713–717.

(2) Ye, D. Z.; Field, J. PAK Signaling in Cancer. *Cell. Logistics* 2012, 105–116.

(3) Eswaran, J.; Soundararajan, M.; Kumar, R.; Knapp, S. UnPAKing the Class Differences among p21-Activated Kinases. *Trends Biochem. Sci.* **2008**, *33*, 394–403.

(4) Kumar, R.; Gururaj, A. E.; Barnes, C. J. P21-Activated Kinases in Cancer. *Nat. Rev. Cancer* 2006, *6*, 459–471.

(5) Radu, M.; Semenova, G.; Kosoff, R.; Chernoff, J. PAK Signalling during the Development and Progression of Cancer. *Nat. Rev. Cancer* **2014**, *14*, 13–25.

(6) Ma, Q.-L.; Yang, F.; Frautschy, S. A.; Cole, G. M. PAK in Alzheimer Disease, Huntington Disease and X-Linked Mental Retardation. *Cell. Logistics* **2012**, *2*, 117–125.

(7) Hayashi, M. L.; Shankaranarayana Rao, B. S.; Seo, J.-S.; Choi, H.-S.; Dolan, B. M.; Choi, S.-Y.; Chattarji, S.; Tonegawa, S. Inhibition of p21-Activated Kinase Rescues Symptoms of Fragile X Syndrome in Mice. *Proc. Natl. Acad. Sci. U.S.A.* **200**7, *104*, 11489–11494.

(8) Ong, C. C.; Jubb, A. M.; Zhou, W.; Haverty, P. M.; Harris, A. L.; Belvin, M.; Friedman, L. S.; Koeppen, H.; Hoeflich, K. P. p21-Activated Kinase 1: PAK'ed with Potential. *Oncotarget* **2011**, *2*, 491– 496.

(9) Jagadeeshan, S.; Krishnamoorthy, Y. R.; Singhal, M.; Subramanian, A.; Mavuluri, J.; Lakshmi, A.; Roshini, A.; Baskar, G.; Ravi, M.; Joseph, L. D.; Sadasivan, K.; Krishnan, A.; Nair, A. S.; Venkatraman, G.; Rayala, S. K. Transcriptional Regulation of Fibronectin by p21-Activated Kinase-1 Modulates Pancreatic Tumorigenesis. *Oncogene* **2014**, 1–10.

(10) Eswaran, J.; Li, D.-Q.; Shah, A.; Kumar, R. Molecular Pathways: Targeting p21-Activated Kinase 1 Signaling in Cancer – Opportunities, Challenges, and Limitations. *Clin. Cancer Res.* **2012**, *18*, 3743–3749.

(11) Coleman, N.; Kissil, J. Recent Advances in the Development of p21-Activated Kinase Inhibitors. *Cell. Logistics* **2012**, *2*, 132–135.

(12) Murray, B. W.; Guo, C.; Piraino, J.; Westwick, J. K.; Zhang, C.; Lamerdin, J.; Dagostino, E.; Knighton, D.; Loi, C.-M.; Zager, M.; Kraynov, E.; Popoff, I.; Christensen, J. G.; Martinez, R.; Kephart, S. E.; Marakovits, J.; Karlicek, S.; Bergqvist, S.; Smeal, T. Small-Molecule p21-Activated Kinase Inhibitor PF-3758309 Is a Potent Inhibitor of Oncogenic Signaling and Tumor Growth. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 9446–9451.

(13) Dolan, B. M.; Duron, S. G.; Campbell, D. A.; Vollrath, B.; Shankaranarayana Rao, B. S.; Ko, H.-Y.; Lin, G. G.; Govindarajan, A.; Choi, S.-Y.; Tonegawa, S. Rescue of Fragile X Syndrome Phenotypes in Fmr1 KO Mice by the Small-Molecule PAK Inhibitor FRAX486. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 5671–5676.

(14) Licciulli, S.; Maksimoska, J.; Zhou, C.; Troutman, S.; Kota, S.; Liu, Q.; Duron, S.; Campbell, D.; Chernoff, J.; Field, J.; Marmorstein, R.; Kissil, J. L. FRAX597, a Small Molecule Inhibitor of the p21-Activated Kinases, Inhibits Tumorigenesis of Neurofibromatosis Type 2 (NF2)-Associated Schwannomas. *J. Biol. Chem.* **2013**, 288, 29105– 29114.

(15) Staben, S. T.; Feng, J. A.; Lyle, K.; Belvin, M.; Boggs, J.; Burch, J. D.; Chua, C.-C.; Cui, H.; Dipasquale, A. G.; Friedman, L. S.; Heise, C.; Koeppen, H.; Kotey, A.; Mintzer, R.; Oh, A.; Roberts, D. A.; Rouge, L.; Rudolph, J.; Tam, C.; Wang, W.; Xiao, Y.; Young, A.; Zhang, Y.; Hoeflich, K. P. Back Pocket Flexibility Provides Group II p21-Activated Kinase (PAK) Selectivity for Type I 1/2 Kinase Inhibitors. *J. Med. Chem.* **2014**, *57*, 1033–1045.

(16) Cowan-Jacob, S.; Jahnke, W.; Knapp, S. Novel Approaches for Targeting Kinases: Allosteric Inhibition, Allosteric Activation and Pseudokinases. *Future Med. Chem.* **2014**, 541–561.

(17) Wu, W.-I.; Voegtli, W. C.; Sturgis, H. L.; Dizon, F. P.; Vigers, G. P. A.; Brandhuber, B. J. Crystal Structure of Human AKT1 with an

# **ACS Medicinal Chemistry Letters**

Allosteric Inhibitor Reveals a New Mode of Kinase Inhibition. *PloS One* **2010**, 5 (e12913), 1–9.

(18) Goodwin, N. C.; Cianchetta, G.; Burgoon, H. A.; Healy, J.; Mabon, R.; Strobel, E. D.; Allen, J.; Wang, S.; Hamman, B. D.; Rawlins, D. B. Discovery of a Type III Inhibitor of LIM Kinase 2 That Binds in a DFG-out Conformation. *ACS Med. Chem. Lett.* **2015**, *6*, 53–57.

(19) Xie, T.; Peng, W.; Liu, Y.; Yan, C.; Maki, J.; Degterev, A.; Yuan, J.; Shi, Y. Structural Basis of RIP1 Inhibition by Necrostatins. *Structure* **2013**, *21*, 493–499.

(20) Deacon, S. W.; Beeser, A.; Fukui, J. A.; Rennefahrt, U. E. E.; Myers, C.; Chernoff, J.; Peterson, J. R. An Isoform-Selective, Small-Molecule Inhibitor Targets the Autoregulatory Mechanism of p21-Activated Kinase. *Chem. Biol.* **2008**, *15*, 322–331.

(21) Viaud, J.; Peterson, J. R. An Allosteric Kinase Inhibitor Binds the p21-Activated Kinase Autoregulatory Domain Covalently. *Mol. Cancer Ther.* **2009**, *8*, 2559–2565.

(22) Heinrich, T.; Grädler, U.; Böttcher, H.; Blaukat, A.; Shutes, A. Allosteric IGF-1R Inhibitors. ACS Med. Chem. Lett. 2010, 1, 199–203.

(23) Iwatani, M.; Iwata, H.; Okabe, A.; Skene, R. J.; Tomita, N.; Hayashi, Y.; Aramaki, Y.; Hosfield, D. J.; Hori, A.; Baba, A.; Miki, H. Discovery and Characterization of Novel Allosteric FAK Inhibitors. *Eur. J. Med. Chem.* **2013**, *61*, 49–60.

(24) Wang, L.; Sullivan, G. M.; Hexamer, L. A.; Hasvold, L. A.; Thalji, R.; Przytulinska, M.; Tao, Z.-F.; Li, G.; Chen, Z.; Xiao, Z.; Gu, W.-Z.; Xue, J.; Bui, M.-H.; Merta, P.; Kovar, P.; Bouska, J. J.; Zhang, H.; Park, C.; Stewart, K. D.; Sham, H. L.; Sowin, T. J.; Rosenberg, S. H.; Lin, N.-H. Design, Synthesis, and Biological Activity of 5,10-Dihydro-dibenzo[b,e][1,4]diazepin-11-one-Based Potent and Selective Chk-1 Inhibitors. J. Med. Chem. 2007, 50, 4162–4176.

(25) Deng, X.; Yang, Q.; Kwiatkowski, N.; Sim, T.; McDermott, U.; Settleman, E.; Lee, J.; Gray, N. S. Discovery of a Benzo[e]pyrimido-[5,4-b][1,4]diazepin-6(11H)-one as a Potent and Selective Inhibitor of Big MAP Kinase 1. ACS Med. Chem. Lett. **2011**, *2*, 195–200.

(26) Chong, C.; Tan, L.; Lim, L.; Manser, E. The Mechanism of PAK Activation. Autophosphorylation Events in Both Regulatory and Kinase Domains Control Activity. *J. Biol. Chem.* **2001**, *276*, 17347–17353.

(27) Jung, J.-H.; Traugh, J. A. Regulation of the Interaction of Pak2 with Cdc42 via Autophosphorylation of Serine 141. *J. Biol. Chem.* **2005**, *280*, 40025–40031.