

Phenylalanine-Based Inactivator of AKT Kinase: Design, Synthesis, and Biological Evaluation

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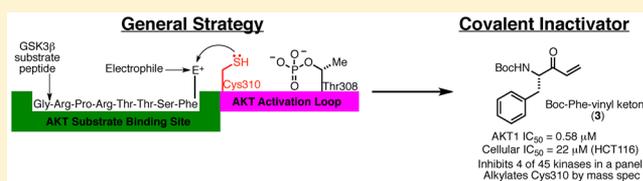
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S Supporting Information

ABSTRACT: Strategies to inhibit kinases by targeting the substrate binding site offer many advantages, including naturally evolved selectivity filters, but normally suffer from poor potency. In this work we propose a strategy to design and prepare covalent substrate-competitive kinase inhibitors as a method to improve potency. We have chosen AKT as the model kinase for this work. Using the AKT-GSK3 β cocrystal structure and a reactive cysteine near the substrate binding site, we have identified phenylalanine (Phe) as an appropriate scaffold for the covalent inactivator portion of these inhibitors. By synthesizing compounds that incorporate cysteine-reactive electrophiles into phenylalanine and testing these compounds as AKT inhibitors, we have identified Boc-Phe-vinyl ketone as a submicromolar inactivator of AKT. We also show that Boc-Phe-vinyl ketone (1) potently inhibits AKT1 and inhibits cell growth in HCT116 and H460 cells nearly as well as AKT inhibitors GSK690693 and MK-2206, (2) is selective for kinases that possess an activation loop cysteine such as AKT, (3) requires the vinyl ketone for inactivation, (4) has inactivation that is time-dependent, and (5) alkylates Cys310 of AKT as shown by mass spectrometry. Identification of Boc-Phe-vinyl ketone as a covalent inactivator of AKT will allow the development of peptide and small-molecule substrate-competitive covalent kinase inhibitors that incorporate additional substrate binding elements to increase selectivity and potency. This proof-of-principle study also provides a basis to apply this strategy to other kinases of the AGC and CAMK families.

KEYWORDS: AKT kinase, substrate competitive, covalent inhibitor



To date, the majority of kinase inhibitors that have been developed target the ATP binding site. This strategy, however, has several limitations. The ATP binding site is highly conserved across the kinome, making selectivity difficult to achieve.¹ ATP-competitive inhibitors can also bind to and modulate the activity of the ~1500 nonkinase enzymes that utilize ATP.^{2,3} Finally, ATP-competitive inhibitors must compete with high levels of intracellular ATP (~2–10 mM) to be effective.⁴

Substrate-competitive inhibitors offer many advantages over ATP-competitive inhibitors.⁵ The substrate binding site of kinases has evolved to recognize only specific substrate sequences.^{6,7} This natural selectivity filter allows a given kinase to recognize a limited number of substrates, an important feature for the control of cellular signaling. These substrates are typically present in the cell in much lower concentration than ATP. Taken together, these factors would highly recommend a substrate-competitive approach to kinase inhibitor development. However, while substrate peptides have high specificity for a given kinase, these sequences themselves are usually poor inhibitors due to lack of potency.⁵

Various strategies have been used to increase the potency of substrate-competitive inhibitors derived from natural substrate sequences including the synthesis and screening of combina-

torial libraries of peptides,^{7,8} inclusion of unnatural amino acids,⁹ and bisubstrate approaches linking the substrate peptide to ATP-competitive scaffolds.^{10,11}

Here we describe the development of *covalent* substrate-competitive kinase inhibitors from the substrate sequence as a new strategy to increase potency. Covalent inhibitors are known to have several advantages including increased biochemical efficiency due to irreversible binding, longer duration of action, increased efficacy achieved at lower drug concentrations, and the potential to avoid some drug resistance mechanisms.^{12,13} Despite the known drawbacks of lack of specificity and potential for immunogenic reaction to the protein–inhibitor adduct, there has been renewed and increasing interest in covalent inhibitors. The strategy reported here can potentially provide rapid access to inhibitors for kinases for which no inhibitor is currently known, derived solely from the structure of the kinase and known substrate sequences.

To develop this strategy, we have chosen AKT as a model because detailed knowledge of the structural interactions

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between the kinase and a fragment of its GSK3 β substrate are available.¹⁴ Our approach to combine the substrate peptide sequence with an electrophile to produce a covalent inhibitor (Figure 1a) requires that we (1) identify a reactive amino acid in the kinase and (2) identify the amino acid in the substrate that binds in close proximity.

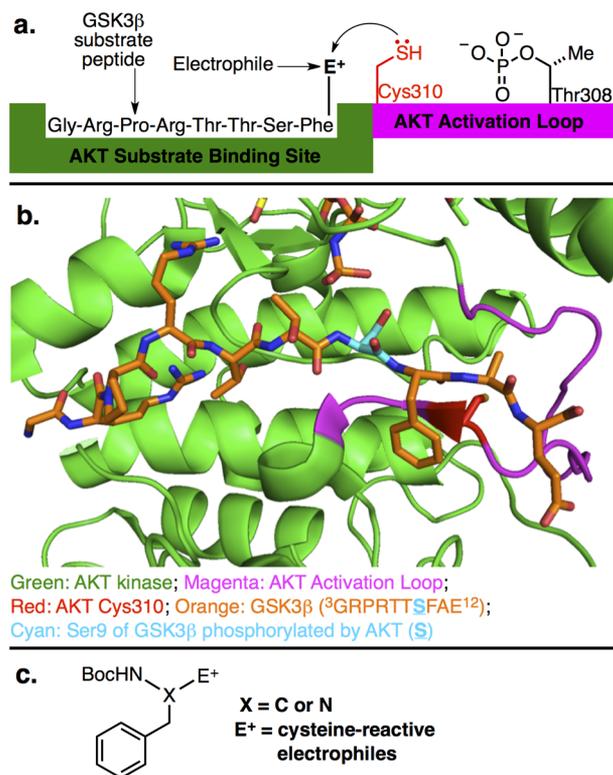


Figure 1. (a) Strategy for peptide-based substrate-competitive covalent inhibitors of AKT. (b) Co-crystal structure of AKT with a ten amino acid fragment of the substrate GSK3 β (PDB ID: 1O6K).¹⁴ (c) General design of phenylalanine-based covalent inactivators of AKT.

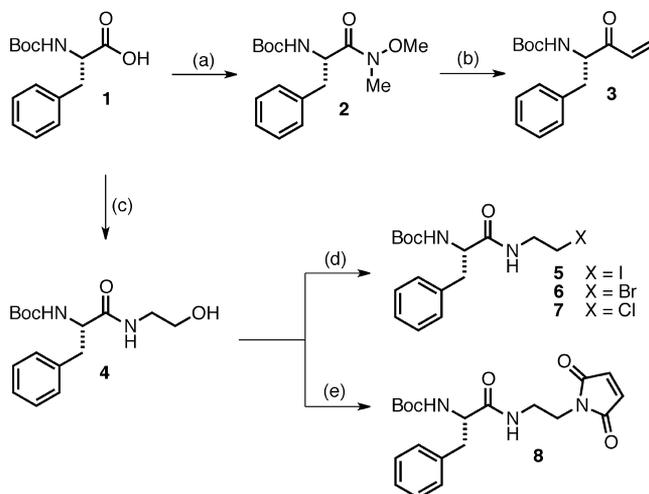
Several compounds have been reported to covalently modify Cys310 of the AKT activation loop. These include the pyranonaphthoquinone (PNQ) lactone natural products,^{15–17} the endogenous α,β -unsaturated aldehyde 4-hydroxynonal (4-HNE),¹⁸ and *N*-ethylmaleimide.¹⁹ Alkylation of Cys310 of AKT by the Michael acceptor in the PNQ lactone and 4-HNE has been demonstrated by mass spectrometry.^{17,18}

The cocrystal structure of AKT with a ten amino acid fragment of the substrate GSK3 β has been reported (PDB ID: 1O6K).¹⁴ As can be seen in Figure 1b, Cys310 is located on the C-terminal end of the AKT activation loop and is located adjacent to the substrate binding site. Phe10 of the GSK3 β peptide binds in close proximity to Cys310. The C $^\alpha$ to C $^\alpha$ distance between these two residues is ~ 5.5 Å. On the basis of this proximity, we chose phenylalanine as the scaffold for our covalent inactivator of AKT.

As the first step in a program to develop peptide-based substrate-competitive covalent inhibitors of AKT, we have designed, synthesized, and evaluated a set of phenylalanine derivatives that contain a C-terminal cysteine-reactive electrophile. The general design of these inactivators is shown in Figure 1c. The electrophiles that we evaluated in this set of compounds include a vinyl ketone, alkyl halides, maleimides, α -halo acetamides, and a vinyl amide.

The syntheses of compounds based on the phenylalanine are shown in Scheme 1. Starting from commercially available Boc-

Scheme 1. Synthesis of 3, 5–7, and 8^a

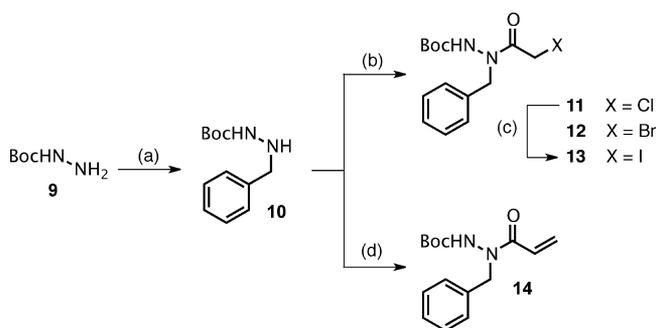


^aReagents and conditions: (a) MeON(H)Me-HCl, HOBt, EDCI, DCM, NMM, 0 °C to rt, overnight, 92%; (b) vinylmagnesium bromide, THF, –40 °C, 3 h, 70%; (c) ethanolamine, HOBt, EDCI, DCM, NMM, 0 °C, 3 h, 90%; (d) for 5, I₂, PPh₃, imid., DCM, rt, 15 min, 51%; for 6, CBr₄, PPh₃, DCM, 0 °C, 2 h, 50%; for 7, CCl₄, PPh₃, DCM, 0 °C, 2 h, 50%; (e) maleimide, DEAD, PPh₃, DCM, –78 °C to rt, overnight, 44%.

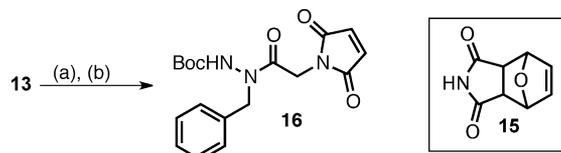
Phe-OH **1**, we prepared Weinreb amide **2** by *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbo-diimide (EDC) coupling and converted it to the vinyl ketone **3** by treatment with vinylmagnesium bromide. Also starting from Boc-Phe-OH **1**, we prepared amide **4** by EDC coupling. The primary alcohol of **4** was then converted to the halides **5–7** using triphenylphosphine and the appropriate halogen source. Alcohol **4** was also used to prepare maleimide **8** by Mitsunobu reaction.

To explore other electrophiles, such as α -halo-acetamides and the vinyl amide, and to vary the linker length of the maleimide, we prepared aza-phenylalanine analogues where the C $^\alpha$ has been replaced by nitrogen (Schemes 2 and 3). Starting from commercially available *N*-Boc-hydrazine, reductive

Scheme 2. Synthesis of 11–14^a



^aReagents and conditions: (a) i. PhCHO, THF, rt, 4 h; ii. NaBH₃CN, AcOH, THF, 0 °C to rt, overnight; iii. NaOH, MeOH, rt, 2 h, 82%; (b) for **11**, chloroacetyl chloride, Et₃N, THF, 0 °C to rt, overnight, 93%; for **12**, bromoacetyl chloride, Et₃N, THF, 0 °C to rt, overnight, 95%; (c) NaI, acetone, reflux, overnight, 86%; (d) acryloyl chloride, Et₃N, THF, 0 °C to rt, overnight, quantitative.

Scheme 3. Synthesis of 16^a

^aReagents and conditions: (a) **15**, K₂CO₃, MeCN, reflux, overnight, 55%; (b) PhOMe, 150 °C, 1.5 h, 62%.

amination with benzaldehyde afforded *N*-Boc-*N'*-benzylhydrazine **10**. Treatment of **10** with chloroacetyl chloride or bromoacetyl chloride afforded α -chloroacetamide **11** and α -bromoacetamide **12**, respectively. α -Chloroacetamide **11** was then further converted to the α -iodoacetamide **13** under Finkelstein conditions. Vinyl amide **14** was prepared by reaction of hydrazine **10** with acryloyl chloride. Finally, α -iodoacetamide **13** was treated with protected maleimide **15** followed by thermal retro-Diels–Alder deprotection to afford maleimide **16** (Scheme 3).

The phenylalanine-electrophile analogues prepared in Schemes 1–3 were tested in the Z'-LYTE assay (Invitrogen) for their ability to inhibit AKT1 (Table 1). This FRET-based

Table 1. Inhibition of AKT1 by Phenylalanine-Based Covalent Inactivators^a

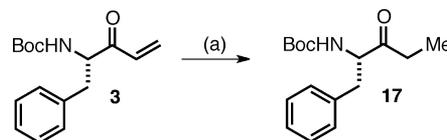
compd	AKT1 IC ₅₀ (μ M) ^b
3	0.58 (0.46–0.75)
5	>100
6	>100
7	>100
8	4.48 (3.93–5.10)
11	>100
12	24.66 (21.59–28.16)
13	16.13 (14.64–17.77)
14	>100
16	4.68 (3.29–6.65)
17	>100
GSK690693	0.0006 (0.0003–0.0009)
MK-2206	0.010 (0.006–0.016)

^aIC₅₀s were determined using Z'-LYTE assay kits (Invitrogen). ^bData represents the averages with 95% confidence intervals shown in parentheses of three independent experiments.

assay measures the ability of a kinase enzyme to phosphorylate a peptide substrate and detects functional inhibitors of kinase activity independent of mechanism of action. Alkyl halides **5**–**7**, α -chloroacetamide **11**, and vinyl amide **14** were all inactive as AKT1 inhibitors, with IC₅₀s of >100 μ M. α -Bromoacetamide **12**, α -iodoacetamide **13**, and maleimides **8** and **16** all inhibited AKT1 in the low micromolar range of concentration. Boc-Phe-vinyl ketone **3**, however, inhibited AKT1 at submicromolar concentration, with an IC₅₀ of 580 nM. Known AKT inhibitors in clinical trials from GlaxoSmithKline (GSK690693) and Merck (MK-2206) were used as controls. The IC₅₀ values determined for these compounds in our assay were consistent with reported values (GSK690693, 2 nM;²⁰ MK-2206, 8 nM²¹).

Having identified Boc-Phe-vinyl ketone **3** as a potential candidate for an AKT-selective covalent inactivator, we wished to confirm that this compound inhibits AKT by a covalent mechanism of action. As our first experiment, we inactivated the electrophile of **3** by reducing the vinyl ketone to the ethyl

ketone **17** (Scheme 4). Testing of **17** for inhibition of AKT1 in the Z'-LYTE assay showed that this compound is inactive, with

Scheme 4. Synthesis of Ethyl Ketone 17^a

^aReagents and conditions: (a) Pd/C, H₂, EtOAc, rt, overnight, quantitative.

an IC₅₀ >100 μ M (Table 1). As a second method to determine whether **3** is a covalent inhibitor, we examined the time-dependence of its inhibition of AKT1. We retested **3** in the Z'-LYTE assay, varying the incubation time of the kinase with **3** prior to the kinase reaction step from 0 to 60 min (Figure 2). The data show a progressive, time-dependent decrease in IC₅₀ values, a characteristic of irreversible inhibitors that interact covalently with their target enzyme.

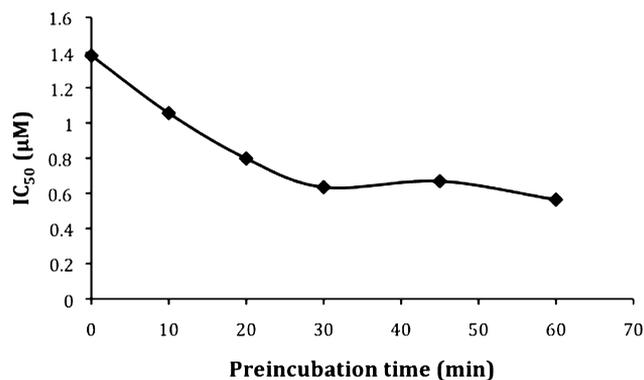


Figure 2. Inhibition of AKT1 by Boc-Phe-vinyl ketone **3** is time-dependent.

To evaluate the selectivity of Boc-Phe-vinyl ketone **3**, we first tested its ability to inhibit c-AMP-dependent protein kinase A (PKA α), also in the Z'-LYTE assay. PKA α , like AKT, is a member of the AGC kinase family and is the most closely related kinase to AKT, sharing 43% sequence identity in the kinase catalytic domain.²² Boc-Phe-vinyl ketone **3** inhibited PKA α with an IC₅₀ of 52.6 μ M and is thus ~90-fold more selective for AKT1 than PKA α . To further evaluate the selectivity of **3**, we tested its inhibitory effects in a panel of 45 kinases using the SelectScreen Profiling Service from Life Technologies (see Table S1 in the Supporting Information for complete data set). This panel of 45 kinases was composed of 22 kinases that contain an activation loop cysteine similar to AKT (see Table S1, Supporting Information, for sequences and alignments) and 23 kinases that do not have an activation loop cysteine. All 45 of the kinases contained at least one cysteine, with 40 of the kinases containing >5 cysteines and six kinases containing >30 cysteines (see Table S1, Supporting Information, for the number of cysteines per kinase). Of the 45 kinases tested, only four were inhibited as well or better than AKT: AMPK α 1, CaMKIV, CHK2, and MELK. As can be seen in Table 2, all four of these kinases contain a cysteine in the same position of their activation loops as in AKT. Most interestingly, these kinases all belong to the CAMK family of kinases. Other

Table 2. Kinases Inhibited in SelectScreen Panel

kinase	% inhibition @ 1 μM of 3	activation loop cysteine
AKT1	50	³⁰⁸ TFCGT ³¹²
AMPK α 1	46	¹⁸³ TSCGS ¹⁸⁷
CaMKIV	50	²⁰⁰ TVCGT ²⁰⁴
CHK2	46	³⁸³ TLCGT ³⁸⁷
MELK	62	¹⁶⁷ TCCGS ¹⁷¹

kinases of the AGC family that carry an activation loop cysteine similar to AKT (such as PKA α , PKC α , PKC θ , PKG1, RSK1, MSK1, and p70S6K) were not inhibited by 3. We are currently analyzing the consensus sequences for AMPK α 1, CaMKIV, CHK2, and MELK to generate hypotheses for binding sites and potential use of 3 as an inactivator of these kinases (see Supplemental Discussion in the Supporting Information). Also of note, the other 18 kinases tested that carry the activation loop cysteine similar to AKT and all 23 of the kinases that contained cysteines in the protein but not specifically in the activation loop were not inhibited by 3 (see Table S1 in the Supporting Information). Taken together this data demonstrates that 3 selectively inhibits only a subset of kinases that contain the activation loop cysteine and does not inhibit the function of kinases lacking the activation loop cysteine.

To determine if inhibition of AKT by Boc-Phe-vinyl ketone 3 is effective in a cellular context, we performed growth inhibition assays in HCT116 (colon cancer) and H460 (nonsmall cell lung cancer) cell lines. Both of these cell lines carry genetic mutations that drive AKT signaling by constitutively activating the PI3K/AKT signaling pathway. One mutation occurs in the large subunit of PI3K and one mutation constitutively activates KRas.²³ Selective inhibition of AKT in cancer cell lines has been shown to inhibit growth.^{20,24} Known AKT inhibitors MK-2206 and GSK690693 were again used as control compounds. Boc-Phe-vinyl ketone 3 was moderately potent in the growth inhibition assay (Table 3 and Figure 3) and the IC₅₀ values for

Table 3. Growth Inhibition of HCT116 and H460 Cancer Cell Lines by AKT Inhibitors

compd	IC ₅₀ (μM) ^a	
	HCT116 cells	H460 cells
3	21.5 \pm 4.0	22.9 \pm 6.4
GSK690693	8.2 \pm 0.5	5.4 \pm 2.2 ^b
MK-2206	7.5 \pm 2.3	5.4 \pm 3.1
17	>100	>100

^aSee Supporting Information for details. ^b*n* = 4.

3 were within 2- to 4-fold of the known AKT inhibitors in the HCT116 and H460 cell lines. Interestingly, the ratio of the cellular IC₅₀ to the IC₅₀ with recombinant AKT is much smaller with Boc-Phe-vinyl ketone 3 (ratio of 37 for HCT116 and 39 for H460) than for with GSK690693 (ratio of 4100 for HCT116 and 2700 for H460) or MK-2206 (ratio of 750 for HCT116 and 540 for H460), demonstrating that 3 is more efficient in inhibiting growth in these cell lines. This ability to inhibit cell growth at a moderately potent concentration in cells while only having a moderately potent IC₅₀ against recombinant enzyme could be the result of several factors including the increased biochemical efficiency due to irreversible binding of the covalent mechanism of action, increased cell permeability relative to MK-2206 and GSK690693, or inhibition of off-target cellular enzymes (such

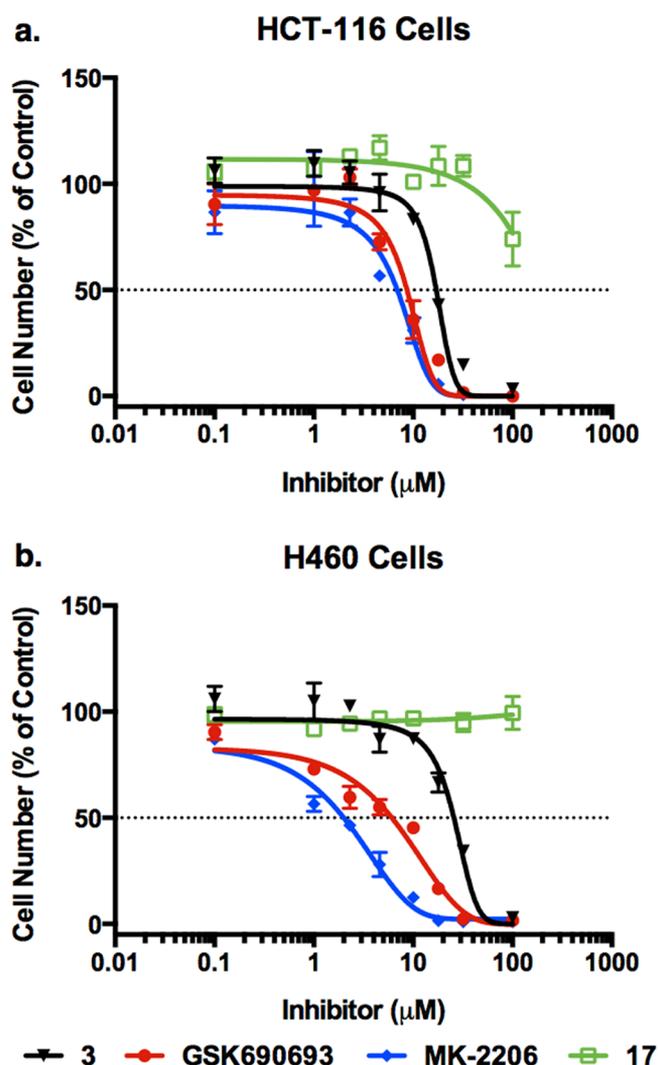


Figure 3. Growth inhibition of HCT116 colon cancer cells (a) and H460 nonsmall cell lung cancer cells (b) by Boc-Phe-vinyl ketone 3, MK-2206, GSK690693, and Boc-Phe-ethyl ketone 17.

as those in Table 2) or enzymes that have not yet been identified. In addition, the cellular data provide evidence that Boc-Phe-vinyl ketone 3 is cell permeable. Our hypothesis is that the compound is actively transported by amino acid transporters, which might account for this increase in inhibition efficiency. Finally, as expected, the Boc-Phe-ethyl ketone 17 was inactive in both cell lines (IC₅₀ > 100 μM ; Table 3 and Figure 3). These data are in agreement with the inhibition data with recombinant AKT1 and demonstrates that the vinyl ketone Michael acceptor is required for inhibition.

Lastly, we determined the amino acid of AKT1 that is covalently modified by Boc-Phe-vinyl ketone 3 using mass spectrometry analysis. For this experiment, we used 18, a fluorescein-labeled analogue of 3 (Figure 4), in order to confirm protein labeling prior to mass spectrometry analysis. Incubation of AKT1 with 18 was followed by gel electrophoresis, confirmation of labeling by in-gel fluorescence scanning (see Figure S1 in the Supporting Information), excision of the labeled protein band, and trypsin digest. Using a LTQ Orbitrap Velos mass spectrometer, an average of 93% coverage was obtained. Phe-vinyl ketone 18 (788.26 amu) was shown to modify the AKT fragment containing Cys310 (Table 4).

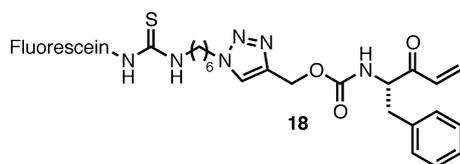


Figure 4. Fluorescein-labeled Phe-vinyl ketone **18** used for mass spectrometry experiment (see Supporting Information).

Modifications of other amino acids in AKT, including Cys296, were not observed.

Table 4. Mass Spectrometry Data for AKT1 Fragment Modified by 18^a

	³⁰⁸ TFCGTPEYLAPVLEDNDYGR ³²⁸	
	mass found	mass calculated
unmodified	2389.04	2389.06
modified with 18 ^b	3177.33	3177.32

^aExperimental details for the mass spectrometry experiments can be found in the Supporting Information. Two new ions (+3 and +4) were found from the modified peptide with an unambiguous MS/MS sequence. ^bExact mass of Boc-Phe-vinyl ketone **18** is 788.26 amu (calculated).

Strategies to develop substrate-competitive kinase inhibitors that disrupt protein–protein interactions are highly sought because they offer the potential of selectivity for a specific kinase. This selectivity is derived from utilizing the same structural elements as Nature for binding. One method to improve the poor potency of substrate-competitive kinase inhibitors is to develop ones that act by a covalent mechanism of action. As a first step in this process, we have designed and synthesized a covalent inactivator of AKT, Boc-Phe-vinyl ketone **3**, and shown that it is potent (against recombinant enzyme and in a cell growth inhibition assay), selective (for AKT and a subset of kinases that contain an activation loop cysteine), and selectively labels Cys199 as designed.

In comparison to known noncovalent substrate-competitive inhibitors of AKT, Boc-Phe-vinyl ketone **3** is more potent than the small-molecule inhibitor reported by Hamilton ($IC_{50} = 12 \mu M$;²⁵ Figure 5) but less potent than the heptapeptide reported by Livnah ($IC_{50} = 0.2 \mu M$;⁹ Figure 5) (see Table S3 in the Supporting Information for a comparison of the assay

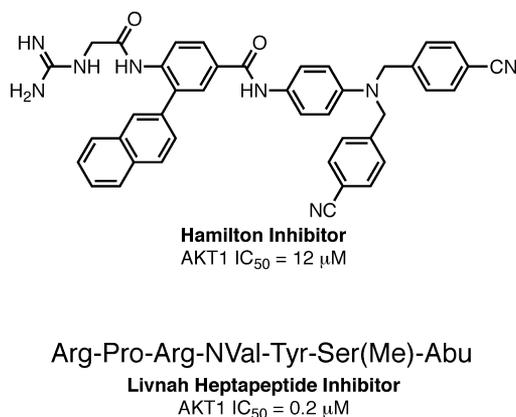


Figure 5. Known noncovalent substrate-competitive inhibitors of AKT.

conditions for these three inhibitors). Boc-Phe-vinyl ketone **3**, however, is quite a small compound (MW = 276), and its selectivity and potency can likely be improved by adding additional structural elements from the GSK3 β substrate peptide or small molecule fragments that bind in a similar fashion. This work is currently underway in our laboratory.

The activation-loop cysteine targeted by the design strategy in this work is conserved in kinases in the AGC family (including PKA, PKC, MSK, RSK, S6K, and SGK)²⁶ and CAMK family (AMPK α 1, CaMKIV, CHK2, and MELK). We are currently working to develop covalent inactivators and substrate-competitive covalent inhibitors of these enzymes using this conserved cysteine and their unique substrate consensus sequences.

■ ASSOCIATED CONTENT

Supporting Information

Experimental data for the synthesis and characterization of all compounds, SelectScreen kinase panel data and supplementary discussion, assay protocols for inhibition of AKT and PKA, cell growth inhibition, time-dependence experiments, mass spectrometry experiments, and comparison of assay conditions for noncovalent substrate-competitive inhibitors of AKT. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Fischer, P. M. The design of drug candidate molecules as selective inhibitors of therapeutically relevant protein kinases. *Curr. Med. Chem.* **2004**, *11*, 1563–1583.
- (2) Cohen, P. The development and therapeutic potential of protein kinase inhibitors. *Curr. Opin. Chem. Biol.* **1999**, *3*, 459–465.
- (3) Denessiouk, K. A.; Johnson, M. S. When fold is not important: A common structural framework for adenine and AMP binding in 12 unrelated protein families. *Proteins: Struct., Funct., Bioinf.* **2000**, *38*, 310–326.
- (4) Traut, T. W. Physiological concentrations of purines and pyrimidines. *Mol. Cell. Biochem.* **1994**, *140*, 1–22.

- (5) Han, K.-C.; Yeon Kim, S.; Gyeong Yang, E. Recent advances in designing substrate-competitive protein kinase inhibitors. *Curr. Pharm. Des.* **2012**, *18*, 2875–2882.
- (6) Pinna, L. A.; Ruzzene, M. How do protein kinases recognize their substrates? *Biochim. Biophys. Acta, Mol. Cell Res.* **1996**, *1314*, 191–225.
- (7) Obata, T.; Yaffe, M. B.; Leparo, G. G.; Piro, E. T.; Maegawa, H.; Kashiwagi, A.; Kikkawa, R.; Cantley, L. C. Peptide and protein library screening defines optimal substrate motifs for AKT/PKB. *J. Biol. Chem.* **2000**, *275*, 36108–36115.
- (8) Luo, Y.; Smith, R. A.; Guan, R.; Liu, X.; Klinghofer, V.; Shen, J.; Hutchins, C.; Richardson, P.; Holzman, T.; Rosenberg, S. H.; Giranda, V. L. Pseudosubstrate peptides inhibit Akt and induce cell growth inhibition. *Biochemistry* **2004**, *43*, 1254–1263.
- (9) Litman, P.; Ohne, O.; Ben-Yaakov, S.; Shemesh-Darvish, L.; Yechezkel, T.; Salitra, Y.; Rubnov, S.; Cohen, I.; Senderowitz, H.; Kidron, D.; Livnah, O.; Levitzki, A.; Livnah, N. A novel substrate mimetic inhibitor of PKB/Akt inhibits prostate cancer tumor growth in mice by blocking the PKB pathway. *Biochemistry* **2007**, *46*, 4716–4724.
- (10) Parang, K.; Cole, P. A. Designing bisubstrate analog inhibitors for protein kinases. *Pharmacol. Ther.* **2002**, *93*, 145–157.
- (11) Lavogina, D.; Enkvist, E.; Uri, A. Bisubstrate inhibitors of protein kinases: from principle to practical applications. *ChemMedChem* **2010**, *5*, 23–34.
- (12) Johnson, D. S.; Weerapana, E.; Cravatt, B. F. Strategies for discovering and derisking covalent, irreversible enzyme inhibitors. *Future Med. Chem.* **2010**, *2*, 949–964.
- (13) Barf, T.; Kaptein, A. Irreversible protein kinase inhibitors: balancing the benefits and risks. *J. Med. Chem.* **2012**, *55*, 6243–6262.
- (14) Yang, J.; Cron, P.; Good, V. M.; Thompson, V.; Hemmings, B. A.; Barford, D. Crystal structure of an activated Akt/protein kinase B ternary complex with GSK3-peptide and AMP-PNP. *Nat. Struct. Mol. Biol.* **2002**, *9*, 940–944.
- (15) Korwar, S.; Nguyen, T.; Ellis, K. C. Preparation and evaluation of deconstruction analogues of 7-deoxykalafungin as AKT kinase inhibitors. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 271–274.
- (16) Toral-Barza, L.; Zhang, W.-G.; Huang, X.; McDonald, L. A.; Salaski, E. J.; Barbieri, L. R.; Ding, W.-D.; Krishnamurthy, G.; Hu, Y. B.; Lucas, J.; Bernan, V. S.; Cai, P.; Levin, J. I.; Mansour, T. S.; Gibbons, J. J.; Abraham, R. T.; Yu, K. Discovery of lactoquinomycin and related pyranonaphthoquinones as potent and allosteric inhibitors of AKT/PKB: mechanistic involvement of AKT catalytic activation loop cysteines. *Mol. Cancer Ther.* **2007**, *6*, 3028–3038.
- (17) Salaski, E. J.; Krishnamurthy, G.; Ding, W.-D.; Yu, K.; Insaf, S. S.; Eid, C.; Shim, J.; Levin, J. I.; Tabei, K.; Toral-Barza, L.; Zhang, W.-G.; McDonald, L. A.; Honores, E.; Hanna, C.; Yamashita, A.; Johnson, B.; Li, Z.; Laakso, L.; Powell, D.; Mansour, T. S. Pyranonaphthoquinone lactones: a new class of AKT selective kinase inhibitors alkylate a regulatory loop cysteine. *J. Med. Chem.* **2009**, *52*, 2181–2184.
- (18) Shearn, C. T.; Fritz, K. S.; Reigan, P.; Petersen, D. R. Modification of Akt2 by 4-hydroxynonenal inhibits insulin-dependent Akt signaling in HepG2 cells. *Biochemistry* **2011**, *50*, 3984–3996.
- (19) Yellaturu, C. R.; Bhanoori, M.; Neeli, I.; Rao, G. N. N-Ethylmaleimide inhibits platelet-derived growth factor BB-stimulated Akt phosphorylation via activation of protein phosphatase 2A. *J. Biol. Chem.* **2002**, *277*, 40148–40155.
- (20) Rhodes, N.; Heering, D. A.; Duckett, D. R.; Eberwein, D. J.; Knick, V. B.; Lansing, T. J.; McConnell, R. T.; Gilmer, T. M.; Zhang, S.-Y.; Robell, K.; Kahana, J. A.; Geske, R. S.; Klymenova, E. V.; Choudhry, A. E.; Lai, Z.; Leber, J. D.; Minthorn, E. A.; Strum, S. L.; Wood, E. R.; Huang, P. S.; Copeland, R. A.; Kumar, R. Characterization of an Akt kinase inhibitor with potent pharmacodynamic and antitumor activity. *Cancer Res.* **2008**, *68*, 2366–2374.
- (21) Lindsley, C. W. The Akt/PKB family of protein kinases: a review of small molecule inhibitors and progress towards target validation: a 2009 update. *Curr. Top. Med. Chem.* **2010**, *10*, 458–477.
- (22) Huang, X.; Begley, M.; Morgenstern, K. A.; Gu, Y.; Rose, P.; Zhao, H.; Zhu, X. Crystal structure of an inactive Akt2 kinase domain. *Structure* **2003**, *11*, 21–30.
- (23) Ikediobi, O. N.; Davies, H.; Bignell, G.; Edkins, S.; Stevens, C.; O'Meara, S.; Santarius, T.; Avis, T.; Barthorpe, S.; Brackenbury, L.; Buck, G.; Butler, A.; Clements, J.; Cole, J.; Dicks, E.; Forbes, S.; Gray, K.; Halliday, K.; Harrison, R.; Hills, K.; Hinton, J.; Hunter, C.; Jenkinson, A.; Jones, D.; Kosmidou, V.; Lugg, R.; Menzies, A.; Mironenko, T.; Parker, A.; Perry, J.; Raine, K.; Richardson, D.; Shepherd, R.; Small, A.; Smith, R.; Solomon, H.; Stephens, P.; Teague, J.; Tofts, C.; Varian, J.; Webb, T.; West, S.; Widaa, S.; Yates, A.; Reinhold, W.; Weinstein, J. N.; Stratton, M. R.; Futreal, P. A.; Wooster, R. Mutation analysis of 24 known cancer genes in the NCI-60 cell line set. *Mol. Cancer Ther.* **2006**, *5*, 2606–2612.
- (24) Qing-Bai, S.; Chandralapaty, S.; Qing, Y.; Lobo, J.; Haskell, K. M.; Leander, K. R.; DeFeo-Jones, D.; Huber, H. E.; Rosen, N. Breast tumor cells with PI3K mutation or HER2 amplification are selectively addicted to Akt signaling. *PLoS One* **2008**, *3*, 1–10.
- (25) Kayser-Bricker, K. J.; Glenn, M. P.; Lee, S. H.; Sebt, S. M.; Cheng, J. Q.; Hamilton, A. D. Non-peptidic substrate-mimetic inhibitors of Akt as potential anti-cancer agents. *Bioorg. Med. Chem.* **2009**, *17*, 1764–1771.
- (26) Pearce, L. R.; Komander, D.; Alessi, D. R. The nuts and bolts of AGC protein kinases. *Nat. Rev. Mol. Cell. Biol.* **2010**, *11*, 9–22.