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Synthesis of GSK3β mimetic inhibitors of Akt featuring a novel extended dipeptide surrogate

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ABSTRACT

Akt is a cardinal nodal point in PI3K signaling pathway and confers resistance to apoptosis through inactivation of regulatory substrates such as GSK3β. Efforts to inhibit the kinase activity of Akt have largely focused on targeting the ATP-binding domain of Akt. Here, we present the design and synthesis of conformationally constrained GSK3β mimics featuring a novel extended dipeptide surrogate core. This effort resulted in the identification of a novel substrate mimetic Akt inhibitor (**11**) with low micromolar activity in vitro (Akt1 IC_{50} = 3.1 μM).

Akt (PKB) is a central protein in the phosphoinositide 3-kinase (PI3K) signaling pathway and a key regulator of cell growth, cell-cycle progression, transcription, and metabolism.[1–3] Akt is known to phosphorylate over 20 substrates, many of which are involved in the induction of apoptosis and in the arrest of cell proliferation.[4,5] Intracellular proteins such as GSK3αβ, Bad, and FoxO are inactivated upon phosphorylation by Akt and thus prevented from carrying out their normal biological functions. Although mutations in the genes encoding Akt are less common, hyperactivation of Akt frequently occurs due to upstream amplification of PI3K or loss of PTEN, its most important negative regulator.[6–11] Enhanced Akt activity is the hallmark of several aggressive malignancies including hormone-refractory prostate cancer, breast cancer, and pancreatic adenocarcinoma.[8,12–15] As a result, Akt has emerged as an attractive target for the development of novel anticancer therapeutics.[6,7,16–21]

Three homologous isoforms of Akt (Akt1, Akt2, and Akt3) sharing >85% sequence homology have been identified in humans.[22–24] Each of these isoforms harbors an N-terminal pleckstrin homology (PH) domain that acts as a binding site for phosphatidylinositol 3,4,5-triphosphate (PIP3). Upon binding PIP3, Akt undergoes a conformational change and localizes to the plasma membrane where it is activated by the kinases PDK1 and mTORC2. Since constitutive recruitment of Akt results in its hyperactivation, a number of existing Akt inhibitors are designed to antagonize the PH domain–PIP3 interaction. Like most kinases, a great deal of effort has also been devoted to inhibition of the ATP-binding pocket of Akt as a direct means to abrogate its activity.[16–21] Given the highly conserved nature of the PH and ATP-binding domains across a wide range of kinases, the development of selective Akt inhibitors remains a challenge.

In contrast to the above strategies, several groups have been interested in developing Akt inhibitors that specifically target the substrate binding groove adjacent to the ATP-binding site.[25–33] While achieving ligand complementarity in the relevant protein–protein interaction (PPI) region is expected to be more topochemically demanding, such inhibitors may also exhibit better kinase selectivity relative to PH and ATP-binding domain antagonists.[24] Early work based on GSK3β revealed the minimal peptide sequence required for Akt1 activity, with several GSK3β-based polypeptides exhibiting IC_{50} values in the low to sub-micromolar range (~10−0.1 μM).[30,31] A subsequent X-ray crystal structure of a ternary Akt1 complex revealed that the GSK3β peptide adopts a highly extended conformation when bound to the PPI domain (Fig. 1).[34] Recent efforts to reduce peptide character while maintaining this extended conformation have led to the identification of peptidomimetic Akt1 inhibitors with activities comparable to or better than the parent peptides.[25–33] Potent bisubstrate inhibitors that covalently link an ATP-site binder to a GSK3β-based polypeptide have also been reported.[35]

Recently, our group reported the synthesis of a suite of constrained bicyclic scaffolds designed to mimic the conformational and electronic properties of highly extended dipeptides.[36]

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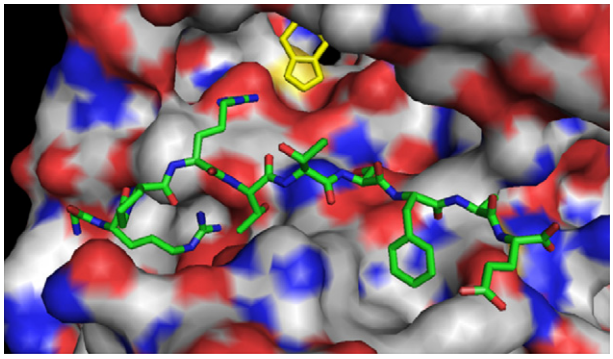
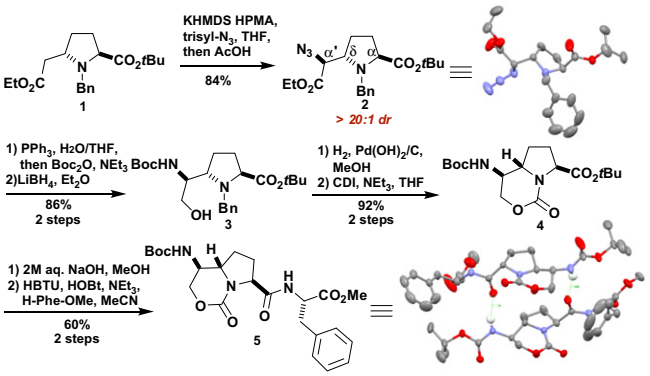


Figure 1. X-ray crystal structure of a GSK3β peptide (green) bound to Akt1 in the presence of an ATP-binding site inhibitor (yellow). PDB code 1O6K.



Scheme 1. Synthesis of scaffold **4**.[36]

Molecular modeling and X-ray crystallography indicated that some of our scaffolds do in fact approximate the conformation of dipeptides found in β-sheets. In this Letter, we report the design, synthesis, and biological evaluation of peptidomimetic Akt inhibitors featuring an extended dipeptide surrogate in place of the central Thr–Thr motif in the Akt-binding domain of GSK3β.

A series of susbtrate mimetic Akt inhibitors was designed based on the parent GSK3β peptide sequence (GRPRTTSFAE) shown in Figure 2.[34] First, we elected to replace the conformationally extended Thr–Thr dipeptide with one of our bicyclic scaffolds in an effort to enforce a sawtooth peptide backbone arrangement. Introduction of the Val-Phe-NHBn motif at the C-terminus was prompted by SAR previously carried out by Hamilton and coworkers on a series of GSK3β mimics.[25,26] We also chose to probe modifications at the N-terminus while retaining the biologically important guanidino group. Finally, we settled on incorporation of a bicyclic carbamate scaffold as our extended dipeptide surrogate due to its conformational properties, ease of synthesis, and the presence of an oxygen atom to more closely mimic the native threonine side chain.

Synthesis of the requisite extended dipeptide surrogate commenced with homoproline derivative **1**, which is readily available from pyroglutamic acid (Scheme 1).[36,37] Regio- and diastereoselective electrophilic azidation under optimized conditions afforded azidoester **2** in high yield. The stereochemical outcome of the reaction was confirmed by X-ray diffraction. Elaboration into the target scaffold proceeded as shown to give **4** in 66% overall yield from **1** (five steps).[36] Although diffraction quality crystals of **4** could not be obtained, converstion to phenylalanyl derivative **5** afforded a crystalline solid suitable for conformational analysis by X-ray. Interestingly, this compound exists as a head-to-tail dimer in the solid state and features two intermolecular H-bonds at either end of the bicyclic scaffold. The dipeptide surrogate motif in compound **5** also exhibits a highly extended conformation with an N–C(O) dipeptide distance very close that found in a typical β-strand dipeptide (5.9 vs 5.8–6.0 Å).

A family of five N-terminally modified peptidomimetics were synthesized as shown in Scheme 2. Although selective acidolysis of scaffold **4** met with difficulties, we found that the *t*-butyl ester could be efficiently hydrolyzed under basic conditions. Condensation with H-Val-Phe-NHBn and treatment with HCl/dioxane afforded intermediate **6** in high yield. Compounds **7** and **8** were obtained by coupling to the corresponding amino acids, followed by Boc acidolysis, amine guanidinylation with Goodman's reagent,

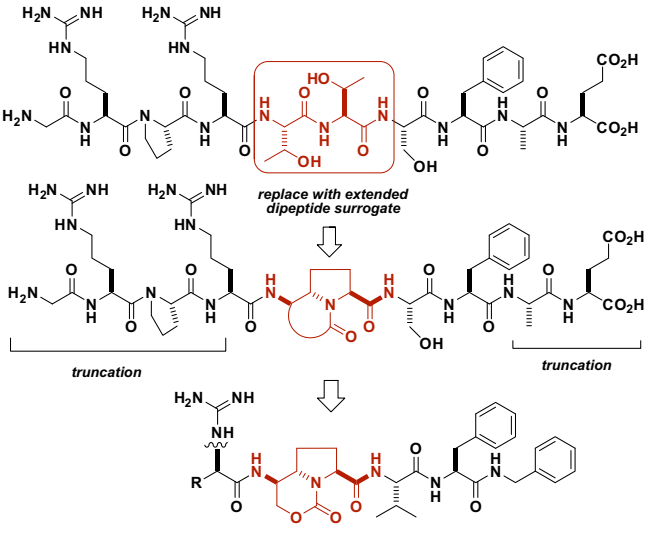
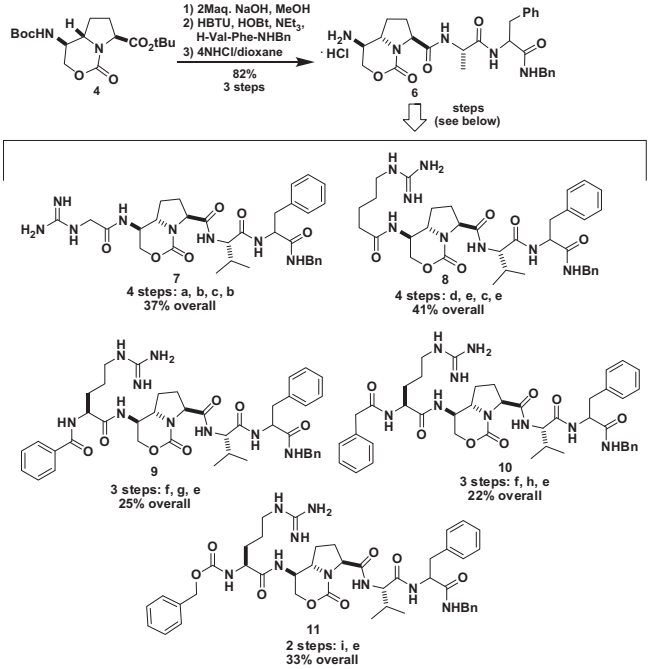


Figure 2. Design of GSK3β mimics.



Scheme 2. Reagents and conditions: (a) HBTU, HOBt, NEt_3, Boc-Gly-OH, DMF; (b) 4 N HCl/dioxane; (c) Goodman's reagent, NEt_3, DCM; (d) HBTU, HOBt, NEt_3, Boc-$NH(CH_2)_4CO_2H$, DMF; (e) TFA/DCM; (f) HBTU, HOBt, NEt_3, Fmoc Arg(Boc)$_2$-OH, DMF; (g) BzCl, NEt_3, DCM; (h) BnCOCl, NEt_3, DCM; (i) HBTU, HOBt, NEt_3, Cbz-Arg(Boc)$_2$-OH, DMF.

and final deprotection. Compounds **9** and **10** were prepared via condensation with Fmoc-Arg(Boc)$_2$-OH, acylation, and Boc deprotection. Synthesis of **11** employed Cbz-Arg(Boc)$_2$-OH in place of the Fmoc derivative. All compounds were purified by RP-HPLC prior to biological evaluation. Curiously, we noted that each of the five compounds was an inseparable mixture of diastereomers, indicating racemization of one of the chiral centers. We later identified the Phe residue to be the site of stereochemical erosion. This event occurs during synthesis of H-Val-Phe-NHBn as confirmed by careful HPLC analysis. Similar epimerization has been observed previously in a related series of compounds.[25]

We next evaluated the ability of compounds **7–11** to inhibit Akt1 in vitro. Compounds were tested in a single dose duplicate model at a concentration of 50 μM using staurosporine as a control. Inhibition was measured as a function of crosstide (GRPRTSSFAEG) phosphorylation by His-tagged Akt1 in the presence of ^{33}P-labeled 10 μM ATP. While compound **7** exhibited almost no activity, compounds **8–11** inhibited Akt1 significantly at 50 μM concentration.

Dose-response curves were then generated for the more active compounds (**8–11**). As shown in Figure 3, each of the GSK3β mimics inhibited the activity of Akt1 in dose-dependent fashion with IC_{50} values in the low micromolar range. Based on the structures of **7–11**, the absence of an N-terminal hydrophobic group has a negative effect on activity, as does a shortening of the guanidine tether (see Table 1, **7 vs 8**). The most potent inhibitor in the series, Cbz-Arg-[5,6 carbmate scaffold]-Val-Phe-NHBn (**11**), exhibited an IC_{50} of 3.1 μM. The enhanced potency of **11** relative to **9** and **10** may also be due to the increased length of the tether bearing the aromatic moiety group (Cbz vs benzyl or phenacyl). Compound **11** compares favorably with previously reported inhibitors of Akt in terms of in vitro activity, despite only harboring one canonical peptide bond (between Val and Phe).

In summary, we have synthesized a set of GSK3β mimics featuring a novel extended dipeptide surrogate. Compounds **7–11** were designed to demonstrate the ability of our constrained scaffold to act as a useful conformational probe. Evaluation of these compounds for activity against Akt1 led to the discovery of **11** as a low micromolar inhibitor. Given the potential advantages of a substrate mimetic strategy for targeting Akt, compound **11** represents an attractive lead compound for drug design. We are currently evaluating the whole cell activity and protease stability of **11** to enable further development. Structure–activity relationship studies are also underway and will be reported in due course.

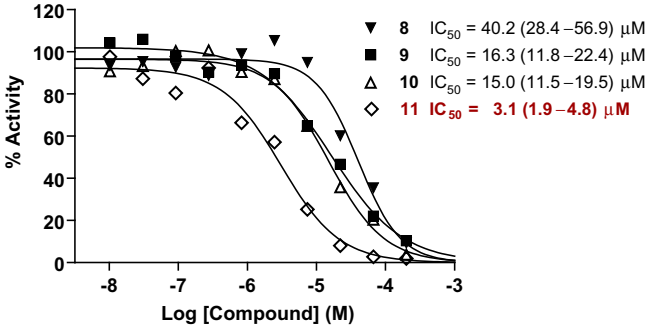


Figure 3. Akt1 dose–response curves for **8–11** (95% confidence interval).

Table 1
Effect of **7–11** on Akt1 activity at 50 μM inhibitor concentration

Compound	% Akt1 activity at 50 μM	
	Run 1	Run 2
7	89.4	92.1
8	23.9	21.1
9	14.6	16.0
10	21.6	16.8
11	2.2	2.4

Acknowledgments

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Supplementary data

Supplementary data (crystallographic data (excluding structure factors) for compounds **2** and **5** have been deposited with the Cambridge Crystallographic Data Centre as supplementary CCDC publications numbers 813013 and 813016. Experimental procedures and spectral data for all new compounds, and copies of NMR spectra for inhibitors **7–11**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.09.079.

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