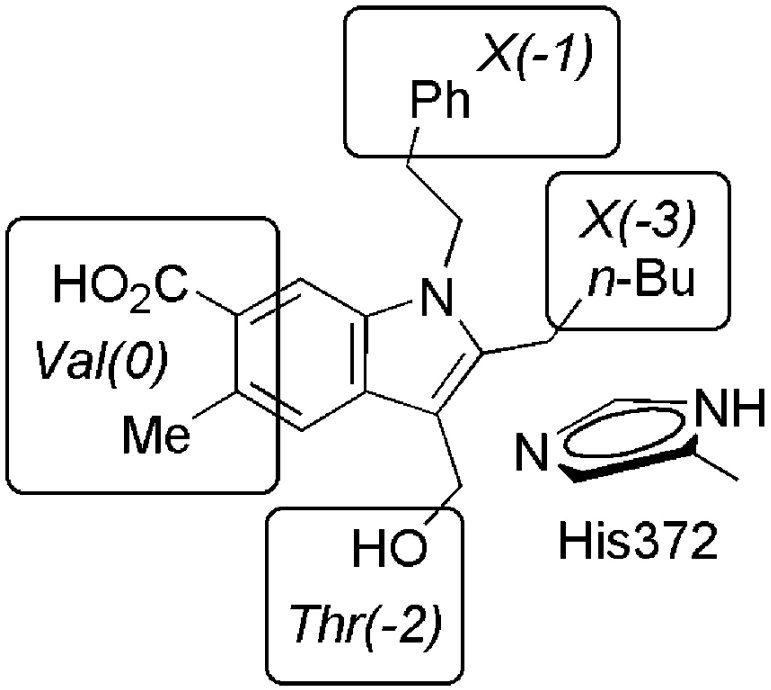
Communication

A Selective Irreversible Inhibitor Targeting a PDZ Protein Interaction Domain

Naoaki Fujii, Jose J. Haresco, Kathleen A. P. Novak, David Stokoe, Irwin D. Kuntz, and R. Kiplin Guy

J. Am. Chem. Soc., **2003**, 125 (40), 12074-12075• DOI: 10.1021/ja035540l • Publication Date (Web): 11 September 2003



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 3 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 09/11/2003

A Selective Irreversible Inhibitor Targeting a PDZ Protein Interaction Domain

Naoaki Fujii,[†] Jose J. Haresco,[§] Kathleen A. P. Novak,[†] David Stokoe,[||] Irwin D. Kuntz,[§] and R. Kiplin Guy*[,†,‡,||]

Department of Pharmaceutical Chemistry, Department of Cellular and Molecular Pharmacology, Laboratory for Molecular Dynamics and Design, and Cancer Research Institute, University of California at San Francisco, Genentech Hall, Mission Bay, 600 16th Street 2280, San Francisco, California 94143-2280

Received April 9, 2003; E-mail: rguy@cgl.ucsf.edu

The MAGIs are a small family of adaptors that are widely expressed in the human body and function to maintain signaling that regulates tissue organization and differentiation. MAGI3 binds to the tumor suppressor PTEN, a lipid/protein phosphatase,[1,2] and colocalizes PTEN with the oncoprotein Akt/PKB, a protein kinase.[3] In this context, PTEN prevents activation of PKB and suppresses PKB signaling, whereas release of PTEN from MAGI-3 increases Akt/PKB signaling. Normally, Akt/PKB signaling ensures cell survival during response to cellular insults by suppressing apoptosis.[4] However, PTEN mutants that cause constitutive Akt/PKB signaling have been associated with human cancers.[5] The exact role of the interaction of PTEN and MAGI in oncogenesis has been controversial. Chemical disruption of this interaction would be a unique and temporally controlled way to investigate the role of Akt/PKB signaling in transformation and cancer. We report here the structure-based design, synthesis, and evaluation of a potent irreversible inhibitor of the interaction of PTEN and MAGI3 − the first specifically targeted irreversible inhibitor of a protein interaction.

Proteins in the MAGI family contain six PDZ protein interaction domains.[6−11] MAGI3 binds to PTEN using its second PDZ domain (MAGI3-PDZ2). Class I PDZ domains, such as MAGI3-PDZ2, recognize the carboxy terminus of their binding partner (ligand) through the consensus sequence X-S/T-X-V and include a conserved histidine in the PDZ domain that interacts with the ligand's −2 residue.[12] The −1 and −3 residues of the ligand help determine specificity for individual PDZ domains within the class. The high structural homology between PDZ domains, with or without bound ligand, suggested the feasibility of designing compounds targeted to MAGI3 PDZ2 domain based on the crystal structure of liganded PSD-95 PDZ3[13] using a combination of visual inspection and DOCK.

The distances and angles between the α carbons of residues (0) and (−1) of the PSD95-PDZ3 ligand CRIPT (amino acid sequence Y-K-Q-T-S-V), bound to PSD95, were measured using Weblab and matched by inspection to those of simple rigid cores. Potential scaffolds were used to generate a virtual library with diversity positions designed to mimic the crucial side chains, and the overall predicted fit of these virtual compounds to the PDZ domain was evaluated using DOCK.[14,15] Initial studies indicated that positioning an ionizable group near the conserved histidine in the PDZ domain might produce an irreversible inhibitor. DOCKing experiments with 3-hydroxymethylindole **1** indicated that it could both place critical functional groups for binding in proximity to their orientation in the bound ligand and properly orient the hydroxymethyl group to trap the histidine (Figure 1).

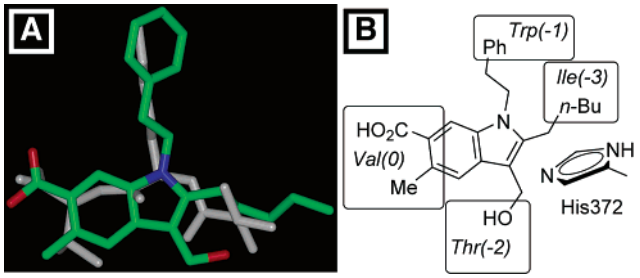


Figure 1. The design of **1**. Panel A shows an overlay of the peptide ligand for PSD95-PDZ3 (gray) and the designed small molecule inhibitor of ligand binding **1** (green). Panel B shows the structure of **1** and the rationalization of the placement of the ionizable hydroxyl group to capture the conserved histidine in the binding site.

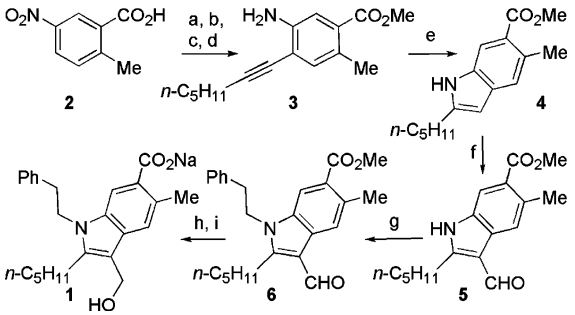


Figure 2. Synthesis of **1**: (a) MeI (2 equiv), K_2CO_3 (3 equiv), DMF, 40 °C, 1 h. (b) H_2, 10% Pd(C), MeOH, room temperature, 2 h, two steps quant. (c) ICl (1.6 equiv), $CaCO_3$ (3 equiv), MeOH, H_2O, room temperature, 1 h, 34%. (d) 1-heptyne (5 equiv), $PdCl_2(PPh_3)_2$ (0.15 equiv), CuI (0.3 equiv), Et_2NH (large excess), DMF, room temperature, 2 h. (e) $PdCl_2(PhCN)_2$ (0.2 equiv), DMF, 80 °C, 40 min, two steps overall 87%. (f) $POCl_3$ (1.3 equiv), DMF, 5 °C, quant. (g) $BrCH_2CH_2Ph$ (10 equiv), Cs_2CO_3 (5 equiv), DMF, room temperature, 19 h, 44%. (h) $NaBH_4$ (excess), MeOH, 5 °C. (i) NaOH, H_2O, MeOH, 65 °C, 12 h.

The synthesis of **1** proceeded smoothly (Figure 2). Sequential esterification of 2-methyl-5-nitrobenzoic acid, reduction of the nitro group, and ortho iodination gave methyl 5-amino-4-iodo-2-methyl benzoate. Crosscoupling with 1-heptyne gave alkynylaniline **3**, which afforded indole **4** after heating with palladium. Formylation of **4** gave **5**. Alkylation of **5** with phenylethyl bromide gave **6**. Sequential reduction of the aldehyde and hydrolysis of the ester then produced **1**. While compound **1** is unstable under acidic conditions, it is stable in a neutral environment. Treatment of **1** with a large excess of imidazole (pH 7, PBS) afforded an imidazole adduct, as expected (data not shown).

Compound **1** irreversibly blocks ligand binding to the MAGI3-PDZ2 domain (Figure 3). Pretreatment of MAGI3-PDZ2 with **1** blocked ligand binding in a dose-dependent manner (Figure 3A). While efficacy of peptide ligand binding dropped with increasing concentrations of **1**, ligand affinity did not change − consistent

[†] Department of Pharmaceutical Chemistry.
[‡] Department of Cellular and Molecular Pharmacology.
[§] Laboratory for Molecular Dynamics and Design.
[||] Cancer Research Institute.

10.1021/ja035540l CCC: $25.00

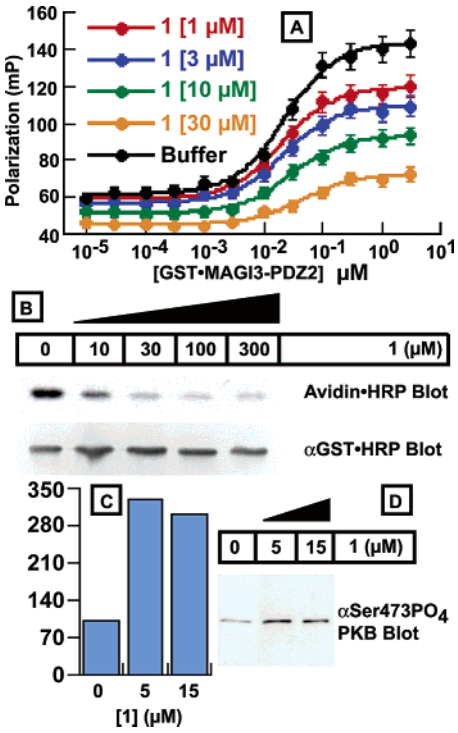


Figure 3. The activity of **1**. Panel A shows that pretreating a GST·MAGI3-PDZ2 fusion protein with increasing concentrations of **1** prior to allowing binding of a fluorescently labeled PTEN carboxy terminal peptide lowers the amount of peptide ligand binding as monitored by fluorescence polarization. Panel B shows that treating a GST·MAGI3-PDZ2 fusion protein with the biotin conjugate of **1** gives a covalently attached biotin conjugate of the protein, as demonstrated by SDS-PAGE purification of the adduct and avidin·HRP blotting, and that this conjugation is inhibited by pretreatment with **1**. The GST control immunoblot shows there is no change in overall protein present in each sample. Panel C shows that treatment of HCT116 cells with **1** causes a 3-fold increase in endogenous PKB activity, as measured by substrate turnover after immunopurification of the enzyme from the cells. Panel D shows that the same treatment also leads to phosphorylation of the endogenous PKB, as measured by blotting of the cellular lysate with an antibody specific to phospho-PKB.

with an irreversible inhibitor. Additionally, treatment of MAGI3-PDZ2 with a biotin conjugate of **1** afforded a covalent adduct whose formation was disrupted by pretreatment of MAGI3-PDZ2 with **1** (Figure 3B). This result is consistent with covalent modification of a specific site on the protein by **1**. Mass spectrometry of MAGI3-PDZ2 treated with **1** revealed a single modified species with a mass consistent with the addition of a single equivalent of **1** (see Supporting Information). Mutation of the targeted conserved histidine in the PDZ domain to alanine gave a stable protein that was unreactive with reagent **1**, indicating that the single site of modification is most probably the targeted histidine (see Supporting Information). As is consistent with the weak ionization potential of the indole alcohol, complete reaction between MAGI3-PDZ2 and **1** required either high excess of **1** (as shown) or extended reaction times (see Supporting Information).

Functional inhibitors of the PTEN·MAGI3-PDZ2 interaction should prevent PTEN recruitment to the plasma membrane and allow full activation of PKB. Treatment of HCT116 cells expressing endogenous PTEN, MAGI, and PKB with **1** results in activation of PKB activity by roughly 3-fold, consistent with in vivo changes (Figure 3C). Normal activation of PKB involves phosphorylation of serine 473.[16] Increased levels of phospho-Ser473-PKB were detected in HCT116 cells treated with **1** by immunoblot using an anti-phospho-Ser473 antibody (Figure 3D), thus indicating that the effects on PKB signaling seem likely to be induced by blockade of PTEN activity. At the concentrations that induce these effects, there were no observable effects upon cell viability or morphology not associated with PKB activity. These studies suggest that inhibitor **1** is capable of physiologically inducing PKB signaling through the normal cellular mechanism and that the reagent should prove useful in studies of PKB mediated transformation.

The widespread occurrence of PDZ domains as organizers of signaling pathways makes them an important subject for biological studies. Compound **1** is the first cell permeable specific inhibitor of PDZ domain function. The route used to produce **1** allows the production of highly diverse libraries of analogues targeted to discovering class- and domain-selective inhibitors. These materials should have use both as functional probes of the activity of PDZ domain containing proteins and as proteomics reagents[17] for isolation of active PDZ domains in cells. Studies toward these ends are currently underway and will be reported in due course.

Acknowledgment. We acknowledge The Stewart Trust (R.K.G., K.A.P.N., N.F.), NIH (I.D.K., J.J.H., GM31497& GM56531), and Fujisawa Pharmaceuticals (N.F.) for funding.

Supporting Information Available: Synthetic procedures, biochemical protocols, and cellular protocols and activity data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Wu, X.; Hepner, K.; Castelino-Prabhu, S.; Do, D.; Kaye, M. B.; Yuan, X. J.; Wood, J.; Ross, C.; Sawyers, C. L.; Whang, Y. E. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 4233−4238.
(2) Wu, Y.; Dowbenko, D.; Spencer, S.; Laura, R.; Lee, J.; Gu, Q.; Lasky, L. A. *J. Biol. Chem.* **2000**, *275*, 21477−21485.
(3) Mayo, L. D.; Donner, D. B. *Trends Biochem. Sci.* **2002**, *27*, 462−467.
(4) Brazil, D. P.; Hemmings, B. A. *Trends Biochem. Sci.* **2001**, *26*, 657−664.
(5) Maehama, T.; Dixon, J. E. *Trends Cell Biol.* **1999**, *9*, 125−128.
(6) Mino, A.; Ohtsuka, T.; Inoue, E.; Takai, Y. *Genes Cells* **2000**, *5*, 1009−1016.
(7) Strochlic, L.; Cartaud, A.; Labas, V.; Hoch, W.; Rossier, J.; Cartaud, J. *J. Cell Biol.* **2001**, *153*, 1127−1132.
(8) Dobrosotskaya, I.; Guy, R. K.; James, G. L. *J. Biol. Chem.* **1997**, *272*, 31589−31597.
(9) Aarts, M.; Liu, Y.; Liu, L.; Besshoh, S.; Arundine, M.; Gurd, J. W.; Wang, Y. T.; Salter, M. W.; Tymianski, M. *Science* **2002**, *298*, 846−850.
(10) Sheng, M.; Sala, C. *Annu. Rev. Neurosci.* **2001**, *24*, 1−29.
(11) Fanning, A. S.; Anderson, J. M. *J. Clin. Invest.* **1999**, *103*, 767−772.
(12) Harris, B. Z.; Hillier, B. J.; Lim, W. A. *Biochemistry* **2001**, *40*, 5921−5930.
(13) Doyle, D. A.; Lee, A.; Lewis, J.; Kim, E.; Sheng, M.; MacKinnon, R. *Cell* **1996**, *85*, 1067−1076.
(14) Brooijmans, N.; Kuntz, I. D. *Annu. Rev. Biophys. Biomol. Stuct.* **2003**, *32*, 335−373.
(15) Zou, X.; Sun, Y.; Kuntz, I. D. *J. Am. Chem. Soc.* **1999**, *121*, 8033−8043.
(16) Stokoe, D.; Stephens, L. R.; Copeland, T.; Gaffney, P. R.; Reese, C. B.; Painter, G. F.; Holmes, A. B.; McCormick, F.; Hawkins, P. T. *Science* **1997**, *277*, 567−570.
(17) Greenbaum, D.; Baruch, A.; Hayrapetian, L.; Darula, Z.; Burlingame, A.; Medzihradszky, K. F.; Bogyo, M. *Mol. Cell Proteomics* **2002**, *1*, 60−68.

JA035540L