J|A|C|S
COMMUNICATIONS

Published on Web 01/08/2003

Novel PI Analogues Selectively Block Activation of the Pro-survival Serine/Threonine Kinase Akt

Alan P. Kozikowski,*,† Haiying Sun,† John Brognard,‡ and Phillip A. Dennis*,‡

Drug Discovery Program, Department of Neurology, Georgetown University, 3900 Reservoir Road, NW, Washington, DC 20007, and Cancer Therapeutics Branch, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland 20889

Received September 11, 2002; E-mail: kozikowa@georgetown.edu

Phosphorylation regulates many cellular processes, and abnormal regulation of kinases or phosphatases that control phosphorylation contributes to human diseases such as cancer. Considerable attention is therefore being directed toward the design of selective kinase inhibitors.[1] The serine/threonine kinase Akt (or PKB) is the prototypic kinase that promotes cellular proliferation, growth, and survival in vitro and tumor formation in vivo. Through inactivation of the regulatory phosphatase PTEN, activation of *ras* or growth factor receptors, or amplification of the upstream kinase, phosphatidylinositol 3-kinase, active Akt has been detected in many types of human cancers (Figure 1).[2] Despite the greater understanding of the role of Akt in cancer biology, however, the study of Akt has been limited by the lack of specific inhibitors directed against Akt. Development of Akt inhibitors would not only facilitate in vitro studies but would also identify lead compounds for potential drug development. Herein we disclose a novel class of modified phosphatidylinositol (PI) analogues that selectively block activation of Akt and downstream substrates without affecting activation of the upstream kinase, PDK-1, or other kinases downstream of *ras* such as MAPK in cancer cells that have high levels of constitutively active Akt. Our studies suggest that these analogues are effective lead compounds for the development of drugs designed to inhibit Akt activity and ultimately treat neoplastic diseases.

Previously, we had reported on the ability of the phosphatidylinositol analogue DPIEL (3-deoxyphosphatidylinositol ether lipid) to inhibit platelet derived growth factor-stimulated activation of Akt and growth factor-stimulated cell growth. Using this as our lead structure, we now sought compounds of improved metabolic stability and of anticancer potential through manipulation of the 2-OH group. As the degradation of inositol phospholipids occurs through the enzymatic action of PI-specific phospholipase C (PI-PLC) with formation of a 1,2-cyclic phosphate, we chose to explore the effect of both alkylation as well as deletion of the 2-OH group to block formation of the cyclic phosphate.[3]

Compound **1** was prepared from quebrachitol according to our previous method[4] and transformed into intermediates **3**−**6** as shown in Scheme 1. These compounds were carried on to the ether lipids **13**−**16** as shown in Scheme 2. From **3** and **6**, the carbonate analogues **20** and **21** were also prepared (Scheme 3).

To test the biological activity of these PI analogues, we chose two lung cancer cell lines that we had previously shown to have high endogenous levels of Akt activity, H157 cells and H1703 cells.[5] H157 cells have increased Akt activity as a result of mutant *K-ras* and mutant PTEN status. H1703 cells have wild-type PTEN, and the cause of their increased Akt activity is unknown. Compounds **13**, **16**, **7**, or **20** were added at 10 μM to both cell lines, and phosphorylation of S473, which is necessary and indicative of activation of Akt, was assessed by immunoblotting with phospho-specific antibodies directed against S473 (Figure 2). Phosphorylation of Akt was decreased by **13** and **16**, but not by **7**, which lacks the inositol headgroup, or by **20**, which contains a carbonate linker. Similar decreases in Akt phosphorylation were observed when **14** or **15** were added to these cells (data not shown). Interestingly, DPIEL was ineffective in these assays, indicating that, while DPIEL previously inhibited PDGF-induced Akt phosphorylation, it could not inhibit endogenous Akt phosphorylation or activity in these cells. Levels of native Akt were not affected by these compounds. To

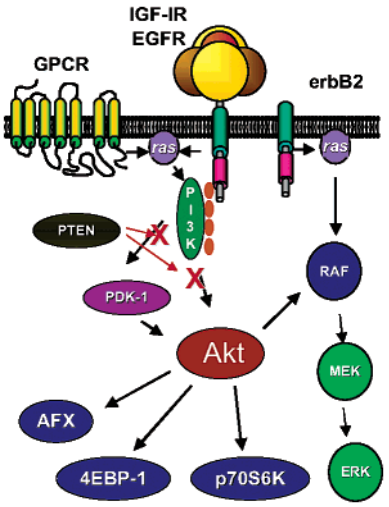
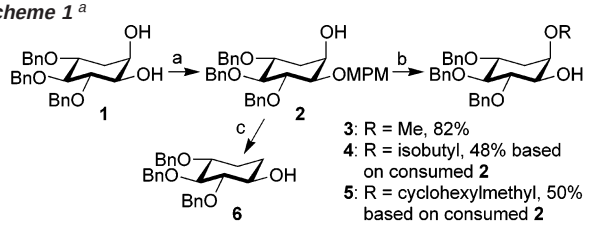


Figure 1. The Akt pathway. Akt can be activated in a PI3K-dependent manner by stimulation of G protein-coupled receptors (GPCR), growth factor receptors such as IGF-IR or EGFR or ras. PI3K generates the phospholipids, phosphatidylinositol 3,4-bisphosphate, and phosphatidylinositol 3,4,5-trisphosphate. PIP_2 and PIP_3 bind to PDK-1 and Akt and induce translocation to the plasma membrane where phosphorylation of Akt occurs. Levels of PIP_2 and PIP_3 are regulated by the tumor suppressor phosphatase, PTEN. Activation of Akt subsequently increases phosphorylation of downstream substrates such as the kinase c-Raf, the transcription factors AFX, 4EBP-1, or p70S6K, which control the initiation phase of protein translation.

Scheme 1 [a]

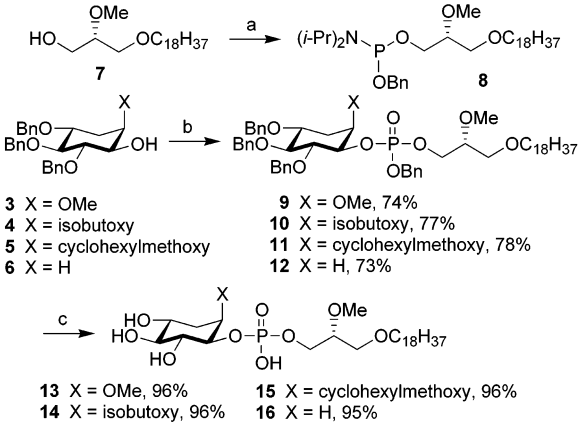


[a] Reagents and conditions: (a) Bu_2SnO, toluene, reflux; *p*-$MeOC_6H_4CH_2Cl$, CsF, DMF, rt, 90%; (b) (i) NaH, MeI, or isobutyl bromide or cyclohexylmethyl bromide, 0 °C, DMF; (ii) CAN, CH_3CN-H_2O 4:1 (v/v), 0 °C to rt; (c) (i) NaH, CS_2, then MeI, 0 °C, DMF; (ii) Bu_3SnH, AIBN, toluene, reflux; (iii) CAN, CH_3CN-H_2O 4:1 (v/v), 0 °C to rt, 81% over three steps.

† Georgetown University.
‡ National Cancer Institute.

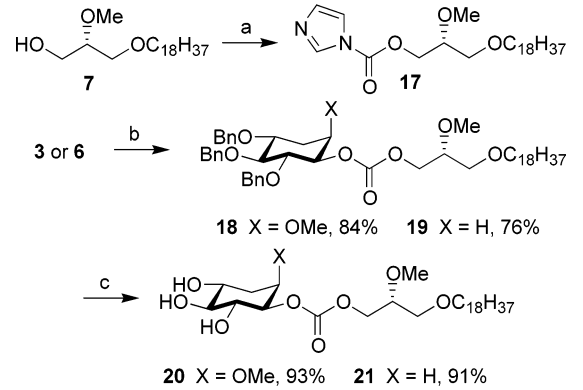
10.1021/ja0285159 CCC: $25.00

Scheme 2 [a]



[a] Reagents and conditions: (a) $BnOP[N(i\text{-}Pr)_2]_2$, diisopropylammonium tetrazolide, CH_2Cl_2, rt, 98%; (b) (i) **8**, 1*H*-tetrazole, rt, CH_2Cl_2; (ii) *m*-CPBA, 0 °C to rt, CH_2Cl_2; (c) H_2, 20% $Pd(OH)_2$−C, *t*-BuOH, rt, 1 atm.

Scheme 3 [a]



[a] Reagents and conditions: (a) $(imd)_2CO$, toluene, reflux; (b) **17**, DBU, toluene, reflux; (c) H_2, 20% $Pd(OH)_2$−C, *t*-BuOH, rt, 1 atm.

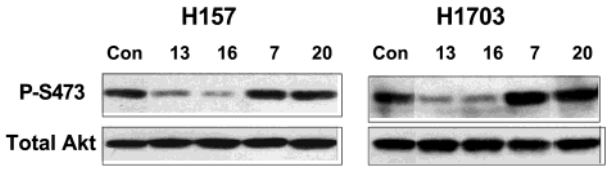


Figure 2. Effects of novel PI analogues on Akt phosphorylation. H157 and H1703 cells were treated with 10 μM **13**, **16**, **7**, or **20** for 2 h (Con - untreated cells). Cells were lysed, and extracts were run on 10% SDS polyacrylamide gels. Proteins were transferred to nitrocellulose, and immunoblotting was performed with phospho-specific S473 antibodies or antibodies against native Akt. Only **13** or **16** decreased Akt phosphorylation without affecting total levels of Akt protein.

test whether the PI analogues affected other components in the Akt pathway, we assessed the activation state of multiple components in the Akt pathway using phospho-specific antibodies in immunoblotting experiments (Figure 3). We chose to test the activation state of PDK-1, a kinase upstream of Akt that activates Akt,[6] and four proteins whose phosphorylation is increased in response to Akt activation, 4EBP-1,[7] p70S6K,[8] AFX,[9] and c-Raf.[10] We also tested two members of the MAPK family, ERK, which is activated downstream of ras, and p38. When compound **13**, **16**, or **7** was added to H157 cells, only inhibition of Akt and downstream components was observed. Phosphorylation of PDK-1 was not affected. Akt phosphorylation was decreased by **13** or **16**, but not **7**. Levels of total Akt were unaffected. Of the downstream substrates tested, phosphorylation of 4EBP-1 was attenuated most by **13** or **16**. Phosphorylation of AFX or p70S6K was attenuated less. c-Raf phosphorylation was also inhibited by **13** or **16**. Interestingly, this correlated with increased phosphorylation of ERK1/2, which is consistent with prior observations that phosphorylation of c-Raf by Akt can inhibit the MEK/ERK pathway.[10,11] Phosphorylation of p38, a MAPK kinase activated by cellular stress, was also increased. Similar inhibition of Akt and downstream substrates was observed when **14** or **15** was tested (data not shown). Together, our data indicate that 2-modified, 3-deoxy PI analogues are capable of decreasing activation of Akt. PI analogues with phosphate linkers were more effective than those with carbonate linkers (compare **13** or **16** with **20**). Most importantly, **13** and **16** inhibited activation of Akt and select downstream substrates without decreasing phosphorylation of PDK-1, or other kinases downstream of ras such as MAPK. On the basis of the increased phosphorylation of p38, a kinase whose activity is increased under conditions of cellular stress, we have begun experiments evaluating these compounds for toxicity in these cell lines. Compounds **13** and **16** are potent inducers of apoptosis and selectively kill a variety of cancer cell lines that contain high levels of active Akt and depend on Akt for survival (data not shown). Thus, this report is the first to identify PI analogues as effective Akt inhibitors that decrease Akt activity and cause apoptosis in cancer cells. These compounds have potential for use as therapeutic agents in cancer therapy. Future studies will characterize the mechanism of inhibition of Akt and suitability of these analogues for in vivo use.[12]

Acknowledgment. We are indebted to the National Institutes of Health (CA61015-07) and to the intramural program of the NCI for their support of these investigations.

Supporting Information Available: Figure 3 and experimental procedures and characterization data for new compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Cohen, P. *Nat. Rev. Drug Discov.* **2002**, *1*, 309−315.
(2) Vivanco, I.; Sawyers, C. L. *Nat. Rev. Cancer* **2002**, *2*, 489−501.
(3) Hondal, R. J.; Zhao, Z.; Kravchuk, A. V.; Liao, H.; Riddle, S. R.; Yue, X.; Bruzik, K. S.; Tsai, M. D. *Biochemistry* **1998**, *37*, 4568−4580.
(4) Kozikowski, A. P.; Qiao, L.; Tückmantel, W.; Powis, G. *Tetrahedron* **1997**, *53*, 14903−14914.
(5) Brognard, J.; Clark, A. S.; Ni, Y.; Dennis, P. A. *Cancer Res.* **2001**, *61*, 3986−3997.
(6) Stephens, L.; Anderson, K.; Stokoe, D.; Erdjument-Bromage, H.; Painter, G. F.; Holmes, A. B.; Gaffney, P. R.; Reese, C. B.; McCormick, F.; Tempst, P.; Coadwell, J.; Hawkins, P. T. *Science* **1998**, *279*, 710−714.
(7) Gingras, A. C.; Kennedy, S. G.; O'Leary, M. A.; Sonenberg, N.; Hay, N. *Genes Dev.* **1998**, *12*, 502−513.
(8) Burgering, B.; Coffer, P. *Nature* **1995**, *376*, 599−602.
(9) Kops, G. J.; de Ruiter, N. D.; De Vries-Smits, A. M.; Powell, D. R.; Bos, J. L.; Burgering, B. M. *Nature* **1999**, *398*, 630−634.
(10) Zimmermann, S.; Moelling, K. *Science* **1999**, *286*, 1741−1744.
(11) Rommel, C.; Clarke, B. A.; Zimmermann, S.; Nuñez, L.; Rossman, R.; Reid, K.; Moelling, K.; Yancopoulos, G. D.; Glass, D. J. *Science* **1999**, *286*, 1738−1741.
(12) For a review on antitumor ether lipids, see: Arthur, G.; Bittman, R. *Biochim. Biophys. Acta* **1998**, *1390*, 85−102.

JA0285159