Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 173–176

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

3-Deoxy-3-substituted-D-*myo*-inositol Imidazolyl Ether Lipid Phosphates and Carbonate as Inhibitors of the Phosphatidylinositol 3-Kinase Pathway and Cancer Cell Growth

Youhong Hu,[a] Emmanuelle J. Meuillet,[b] Margareta Berggren,[b] Garth Powis[b] and Alan P. Kozikowski[a,*]

[a]*Drug Discovery Program, Department of Neurology, Georgetown University Medical Center, 3900 Reservoir Road NW, Washington, DC 20007, USA*

[b]*Arizona Cancer Center, 1515 North Campbell Avenue, Tucson, AZ 85724, USA*

Received 11 September 2000; accepted 31 October 2000

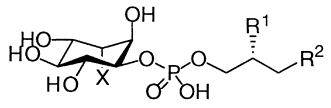
Abstract—3-Modified D-*myo*-inositol imidazolyl ether lipid phosphates and a carbonate were synthesized and evaluated as inhibitors of PI3-K and Akt. These data are presented along with IC_{50} values for the inhibition of the growth of three cancer cell lines.

Phosphatidylinositol is known to undergo phosphorylation at multiple sites to generate diverse phosphoinositides that in turn regulate several signal transduction pathways.[1] In one of these pathways, PI(4,5)P_2 can be hydrolyzed by PI-PLC to give the water-soluble inositol trisphosphate I(1,4,5)P_3 together with the lipophilic component diacylglycerol (DAG). DAG is an endogenous activator of protein kinase C (PKC), while I(1,4,5)P_3 interacts with a receptor on the endoplasmic reticulum[2] to release intracellular Ca^{2+}. In a second pathway, PI 3-kinase (PI3-K) phosphorylates the 3-position of phosphatidylinositols to give a class of 3-phosphorylated PIPs that have the unique ability to bind to the pleckstrin homology (PH) domains of a number of signaling proteins such as Akt. Akt becomes fully activated upon phosphorylation by phosphatidylinositol dependent kinases (PDKs), and it regulates cell survival and proliferation by phosphorylating a number of downstream targets such as Bad, an inhibitor of apoptosis.[3] Three mammalian isoforms of Akt have been identified, Akt1, Akt2, and Akt3. Akt1 has been found to be overexpressed in gastric adenocarcinomas while Akt2 is overexpressed in breast, ovarian, and pancreatic cancer.[4] Another important counterpart to PI3-K is the tumor suppressor PTEN. Mutations in the PTEN tumor suppressor gene appear to be a common occurrence in a number of human cancers.[5] Thus, chemical modulation of the PI3-K dependent phospholipid signaling cascade may offer a basis for the selective control of cancer cell growth while minimizing effects on normal cells.

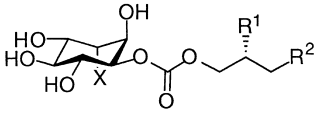
In pursuit of this particular strategy for anticancer drug development, we have reported on the chemistry and biology of a number of phosphatidylinositol analogues embodying modifications to the 3-position of the inositol ring, as well as to the lipid portion and the phosphate group. Specifically, subsequent to our finding that 1D-3-deoxyphosphatidyl-*myo*-inositol (**1**) was able to block the growth of HT-29 human colon carcinoma cells ($IC_{50} = 35.0\,\mu M$),[6] the DAG portion of this molecule was replaced by a more stable ether lipid moiety; certain ether lipids are in fact known to possess antitumor activity of their own. This strategy delivered the PI analogue **2**, which was found to be a reasonably active inhibitor of both PI3-K ($IC_{50} = 14.8 \pm 5.6\,\mu M$) and Akt ($IC_{50} = 1.5 \pm 0.3\,\mu M$), and to block the growth of HT-29 colon cancer cells ($IC_{50} = 2.1\,\mu M$).[7] Additionally, we have recently reported that the PI analogues **3** and **6** bearing an axially oriented 3-hydroxymethyl group are also reasonably good inhibitors of Akt, with compound **6** being more selective than DPIEL (**2**) for inhibiting Akt over PI3-K.[8] In general, in studies of some of these PI analogues in vivo, we have been plagued by problems relating to the poor solubility of these lipids, and by

*Corresponding author. Tel.: +1-202-687-0686; fax: +1-202-687-5065; e-mail: kozikowa@giccs.georgetown.edu

0960-894X/01/$ - see front matter
PII: S0960-894X(00)00640-5



1 X = H, $R^1 = R^2$ = O-palmitoyl
2 X = H, R^1 = OMe, $R^2 = OC_{18}H_{37}$-*n*
3 X = CH_2OH, R^1 = OMe, $R^2 = OC_{18}H_{37}$-*n*
4 X = CH_2OH, R^1 = 1-imidazolyl, $R^2 = OC_{18}H_{37}$-*n*
5 X = H, R^1 = 1-imidazolyl, $R^2 = OC_{18}H_{37}$-*n*



6 X = CH_2OH, R^1 = OMe, $R^2 = OC_{18}H_{37}$-*n*
7 X = CH_2OH, R^1 = imidazolyl, $R^2 = OC_{18}H_{37}$-*n*

Figure 1. Structures of 3-deoxy-3-substituted PI analogues.

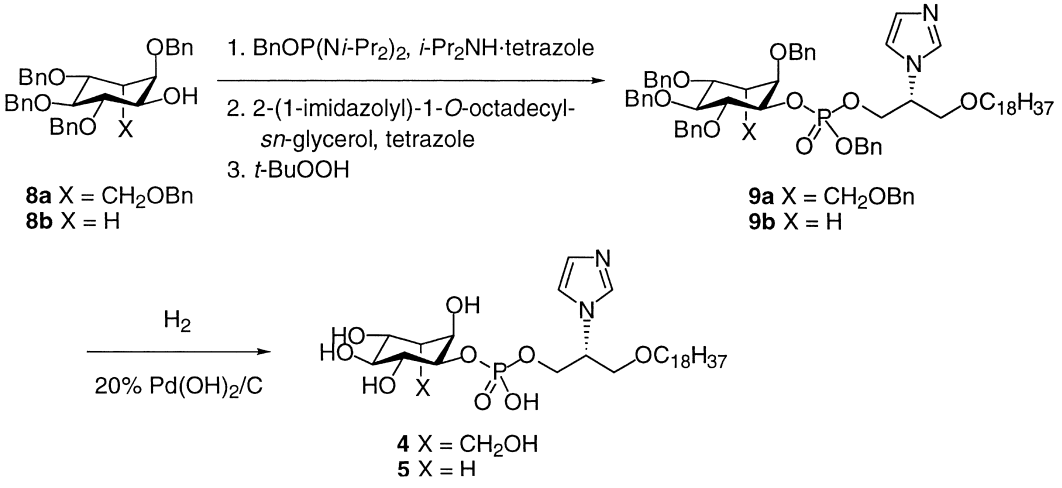
their tendency to form aggregates. Consequently, we have investigated the synthesis and activity of PI analogues in which R^1 of the ether lipid portion has been changed from methoxy to the more basic imidazolyl group (Fig. 1, note that the calculated log *P* for compound **2** is 4.44, while for **4** it is 3.22). The examination of the effect of chemical modification of the lipid portion was of further interest to explore as it may play a role in Akt inhibition as a consequence of alterations in cellular distribution into cytosol and plasma membrane. Interestingly, in this connection we note that the structurally related molecule CPR 1006 belongs to the class of lipid ether drugs and acts as a PKC inhibitor. It blocks the proliferation of HT 29 human colon cancer cells with an IC_{50} of 2 μM.[9] In this communication, the preparation of the two phosphate containing PI analogues **4** and **5** and the related carbonate **7** are described, together with a biological study of their inhibition of Akt, PI3-K, and cancer cell growth.

The imidazolyl bearing ether lipid component was prepared from (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol by a known method,[10] while the inositol components **8a** and **8b** were obtained as reported previously.[7,8] Components **8a** and **8b** were transformed into the corresponding protected phosphatidylinositols **9a** and **9b** using a standard phosphitylation protocol followed by adjustment of the oxidation state of the phosphorus atom.[7,8] Finally, hydrogenolysis of the benzyl groups of the intermediates **9a** and **9b** with 20% $Pd(OH)_2/C$ in *tert*-butanol gave the desired phosphates **4** and **5** (Scheme 1).[11]

The carbonate **7** was prepared using a protocol similar to that employed in the synthesis of **6**.[8] Thus, **8a** was refluxed in toluene with 1,1′-carbonyldiimidazole to furnish a carbamate. This carbamate was used directly without further purification in the coupling reaction with the imidazolyl ether lipid. The two components were refluxed in toluene in the presence of DBU to give compound **10**. Finally, hydrogenolysis of **10** delivered the desired carbonate **7**[12] in good purity (Scheme 2).

The three new PI imidazolyl ether lipid phosphates and carbonate were tested for their ability to inhibit p100/p85 PI3-K, Akt, and the cell growth of three cell lines using the methods described previously.[8] Data are presented in Table 1 along with comparison data obtained for PI analogues **1**–**3** and **6**. It is interesting to note that imidazolyl ether lipid compounds **4** and **5** containing a phosphate linker both exhibit reasonably good potency for the inhibition of PI3-K, while the carbonate **7** appears less potent. On the other hand, the carbonate **7** is more potent in inhibiting Akt than the phosphates **4** and **5**, although DPIEL **2** still retains the best inhibitory potency for Akt. The imidazolyl ether lipid analogue **7** exhibits an IC_{50} of 15 μM for inhibition of Akt, and it is about 3-fold less potent than the carbonate **6** that bears a C-2 methoxy group in the glycerol portion. Both carbonates **6** and **7** are relatively poor inhibitors of PI3-K.

In terms of the inhibition of cell growth, of the three cell lines tested, the imidazole bearing compounds **4** and **5** exhibit the best potency for inhibition of the MCF-7

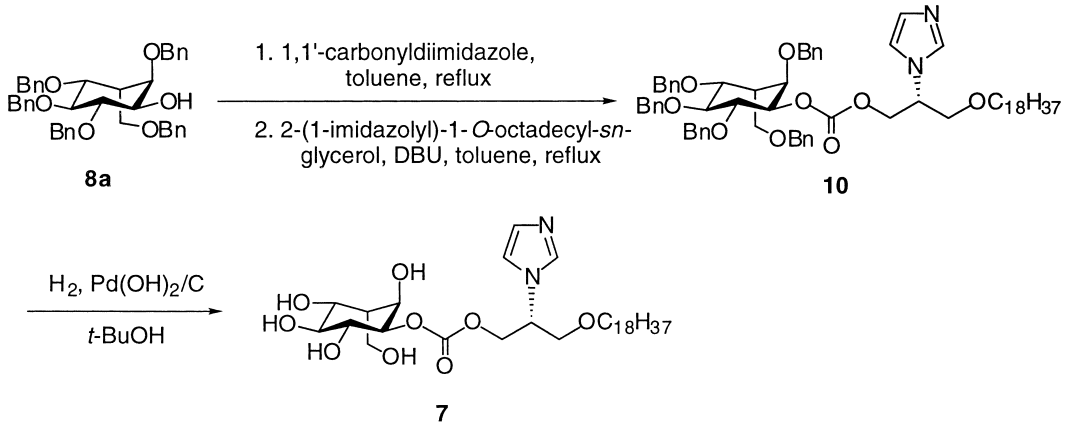


8a X = CH_2OBn
8b X = H

9a X = CH_2OBn
9b X = H

4 X = CH_2OH
5 X = H

Scheme 1. Synthesis of phosphates **4** and **5**.



Scheme 2. Synthesis of carbonate **7**.

Table 1. IC_{50} values for inhibition of Akt, PI3-K, and growth of cancer cell lines by compounds **1**–**7**

Compounds	Akt (IC_{50}, μM)	PI3-K (IC_{50}, μM)	NIH3T3 (IC_{50}, μM)	HT-29 (IC_{50}, μM)	MCF-7 (IC_{50}, μM)
1	27.4±4.4	38.4±4.1	14.0	35.0	10.0
2	1.5±0.3	14.8±5.6	4.5	2.1	7.2
3	7.8±0.8	31.0±7.0	2.0	4.5	5.0
4	20.5±1.8	7.9±3.4	8.2	6.5	2.0
5	37.5±12	14.5±3.2	6.5	15.0	6.0
6	5.0±1.9	83.0±21.0	2.0	10.0	1.25
7	15.0±2.5	49.5±4.6	11.5	15.0	20.0

(breast) cancer cell line; IC_{50} values of 2.0 and 6.0 μM were measured, respectively. However, the ether lipid carbonate **6** is still slightly more potent than either of these two analogues.

In conclusion, the present work details the synthesis of novel 3-substituted-D-*myo*-inositol imidazolyl containing ether lipid phosphates and a carbonate. The ability of these compounds to inhibit both Akt and PI3-K were evaluated, together with the effects of these PI analogues to block cell growth in NIH3T3, HT-29 and MCF-7 cells. Compound **4** was particularly effective in blocking the growth of the MCF-7 cell line.

Acknowledgements

This work was supported by the National Institutes of Health (Grant No. CA 61015) and the Department of Defense (DAMD 17-93-V-3018).

References and Notes

1. Potter, B. V. L.; Lampe, D. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1933.
2. (a) Berridge, M. J.; Irvine, R. F. *Nature (London)* **1989**, *341*, 197. (b) Berridge, M. J. *Nature (London)* **1993**, *361*, 315. (c) Glazer, R. I. *Curr. Pharm. Des.* **1998**, *4*, 277. (d) Streb, H.; Irvine, R. F.; Berridge, M. J.; Schulz, I. *Nature (London)* **1983**, *306*, 67.
3. (a) Carpenter, C. L.; Cantley, L. C. *Curr. Opin. Cell Biol.* **1996**, *8*, 153. (b) Shepherd, P. R.; Reaves, B. J.; Davidson, H. W. *Trends Cell Biol.* **1996**, *6*, 92. (c) Lemmon, M. A.; Ferguson, K. M.; Schlessinger, J. *Cell* **1996**, *85*, 621. (d) French, M.; Andjelkovich, M.; Ingley, E.; Reddy, K. K.; Falck, J. R.; Hemmings, B. A. *J. Biol. Chem.* **1997**, *272*, 8474. (e) Stokoe, D.; Stephens, L. R.; Copeland, T.; Gaffney, P. R. J.; Resses, C. B.; Painter, G. F.; Holmes, A. B.; McCormick, F.; Hawkins, P. T. *Science* **1997**, *277*, 567. (f) Franke, T. F.; Kaplan, D.; Cantley, L.; Toker, A. *Science* **1997**, *275*, 665. (g) Bellacosa, A.; Chan, T. O.; Ahmed, N. N.; Datta, K.; Malstrom, S.; Stokoe, D.; McCormick, F.; Feng, J.; Tsichlis, P. *Oncogene* **1998**, *17*, 313. (h) Grishin, N. V. *J. Mol. Biol.* **1999**, *291*, 239. (i) Cross, D. A. E.; Alessi, D. R.; Cohen, P.; Andjelkovich, M.; Hemmings, B. A. *Nature* **1995**, *378*, 785. (j) Kauffmann-Zeh, A.; Rodriguez-Viciana, P.; Ulrich, E.; Gilbert, C.; Coffer, P.; Downward, J.; Evan, G. *Nature* **1997**, *385*, 544. (k) Kulik, G.; Klippel, A.; Weber, M. *J. Mol. Cell. Biol.* **1997**, *17*, 1595. (l) Paradis, S.; Ailion, M.; Toker, A.; Thomas, J. H.; Ruvkun, G. *Gen. Dev.* **1999**, *13*, 1438.
4. (a) Coffer, P. J.; Jin, J.; Woodgett, J. R. *Biochem. J.* **1998**, *335*, 1. (b) Staal, S. P. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5034. (c) Bellacosa, A.; Feo, D. D.; Godwin, A. K.; Bell, D. W.; Cheng, J. Q.; Altomare, D. A.; Wan, M.; Dubeau, L.; Scambia, G.; Masciullu, V.; Ferrandina, G.; Panici, P. B.; Mancuso, S.; Neri, G.; Testa, J. R. *Int. J. Cancer* **1995**, *64*, 280. (d) Thompson, F. H.; Nelson, M. A.; Trent, J. M.; Guan, X. Y.; Liu, Y.; Yang, J. M.; Emerson, J.; Adair, L.; Wymer, J.; Balfour, C.; Massey, K.; Weinstein, R.; Alberts, D. S.; Taetle, R. *Cancer Genet. Cytogenet.* **1996**, *87*, 55. (e) Cheng, J. Q.; Ruggeri, B.; Klein, W. M.; Sonoda, G.; Altomare, D. A.; Watson, D. K.; Testa, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 3636.
5. (a) Cantley, L. C.; Neel, B. G. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4240. (b) Lee, J.; Yang, H.; Georgescu, M.; Cristofano, A. D.; Maehama, T.; Shi, Y.; Dixon, J. E.; Pandolfi, P.; Pavletich, N. P. *Cell* **1999**, *99*, 323.
6. Kozikowski, A. P.; Kiddle, J. J.; Frew, T.; Berggren, M.; Powis, G. *J. Med. Chem.* **1995**, *38*, 1053.

7. (a) Yeates, L. C.; Gallegos, A.; Kozikowski, A. P.; Powis, G. *Anticancer Res.* **1999**, *19*, 4171. (b) Qiao, L.; Nan, F.; Kunkel, M.; Gallegos, A.; Powis, G.; Kozikowski, A. P. *J. Med. Chem.* **1998**, *41*, 3303.
8. Hu, Y.; Qiao, L.; Wang, S.; Rong, S.; Meuillet, E. J.; Berggren, M.; Gallegos, A.; Powis, G.; Kozikowski, A. P. *J. Med. Chem.* **2000**, *43*, 3045.
9. Goekjian, P. G.; Jirousek, M. R. *Curr. Med. Chem.* **1999**, *6*, 877.
10. (a) Nair, H. K.; Peterson, A. C.; Yazdi, P. T.; Franzmair, R. *Bioorg. Med. Chem. Lett.* **1997**, *18*, 2379. (b) Hirth, G.; Barner, R. *Helv. Chim. Acta* **1982**, *65*, 1059.
11. Compound **4**: $[\alpha]_D$ −8.6° (*c* 0.52, $CHCl_3$/MeOH 1:1); ^1H NMR (300 MHz, $CDCl_3/CD_3OD$ 1:1, TMS) δ 9.00 (s, 1H), 7.69 (s, 1H), 7.45 (s, 1H), 4.84 (m, 1H), 4.34 (t, 2H, $J=5.7$ Hz), 4.20 (m, 1H), 4.07 (m, 1H), 3.97 (dd, 1H, $J=9.3$, 6.0 Hz), 3.87 (m, 3H), 3.73 (t, 1H, $J=8.7$ Hz), 3.57 (dd, 1H, $J=11.1$, 8.4 Hz), 3.45 (m, 3H), 2.32 (m, 1H), 1.54 (m, 2H), 1.35–1.24 (m, 30H), 0.89 (t, 3H, $J=6.3$ Hz); ^{13}C NMR (75 MHz, $CDCl_3/CD_3OD$ 1:1) δ 136.08, 122.43, 120.08, 78.26 (d, $J=6.0$ Hz), 75.29, 73.17 (d, $J=5.0$ Hz), 72.55, 70.79, 70.05, 69.65, 64.95 (d, $J=5.0$ Hz). 61.94 (d, $J=7.0$ Hz), 59.25, 47.73, 32.69, 31.12, 30.43, 30.40, 30.36, 30.17, 30.13, 30.11, 26.77, 23.40, 19.30, 14.48; ^{31}P (121 MHz, $CDCl_3/CD_3OD$ 1:1, 85% H_3PO_4) δ 0.58. Compound **5**: $[\alpha]_D$ −23.7° (*c* 0.81, $CHCl_3$/MeOH 1:1); ^1H NMR ($CDCl_3/CD_3OD$ 1:1, TMS) δ 9.02 (s, 1H), 7.72 (s, 1H), 7.48 (s, 1H), 4.34 (t, 2H, $J=5.7$ Hz), 4.15 (m, 1H), 3.87 (m, 4H), 3.69 (t, 1H, $J=9.3$ Hz), 3.46 (m, 2H), 3.33 (m, 1H), 3.18 (t, 1H, $J=9.3$ Hz), 2.09 (dt, 1H, $J=13.5$, 3.9 Hz), 1.54 (m, 3H), 1.27 (m, 30H), 0.89 (t, 3H, $J=6.0$ Hz); ^{13}C NMR ($CDCl_3/CD_3OD$ 1:1) δ 136.10, 122.38, 120.03, 80.38 (d, $J=5.5$ Hz), 78.95, 72.55 (d, $J=5.0$ Hz), 72.39, 69.55, 68.88, 68.35, 64.83 (d, $J=4.6$ Hz), 61.87 (d, $J=6.6$ Hz), 35.91, 32.61, 30.35, 30.03, 26.70, 23.32, 14.32; ^{31}P (121 MHz, $CDCl_3/CD_3OD$ 1:1, 85% H_3PO_4) δ 0.68. Anal. calcd for $C_{30}H_{57}N_2O_9P{\cdot}1.4H_2O$: C, 55.78; H, 9.33. Found: C, 55.77; H, 9.35.
12. Compound **7**: $[\alpha]_D$ −23.8° (*c* 0.49, $CHCl_3$/MeOH 1:1); ^1H NMR ($CDCl_3/CD_3OD$ 1:1, TMS) δ 9.08 (s, 1H), 7.79 (s, 1H), 7.60 (s, 1H), 4.97 (m, 1H), 4.74 (dd, 1H, $J=9.9$, 3.0 Hz), 4.61 (m, 1H), 4.23 (m, 1H), 3.89 (m, 3H), 3.75 (t, 1H, $J=9.3$ Hz), 3.48 (m, 4H), 3.29 (m, 2H), 2.28 (m, 1H), 1.67 (m, 1H), 1.53 (m, 2H), 1.26 (m, 29H), 0.88 (t, 3H, $J=6.3$ Hz); ^{13}C NMR ($CDCl_3/CD_3OD$ 1:1, TMS) δ 155.77, 136.95, 122.84, 121.24, 80.67, 75.91, 72.84, 72.69, 71.16, 69.74, 68.99, 67.30, 61.04, 59.55, 33.21, 30.92, 30.66, 30.62, 27.32, 23.87, 14.59.