Bioorganic & Medicinal Chemistry Letters 18 (2008) 3603–3606

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design and synthesis of novel furoquinoline based inhibitors of multiple targets in the PI3K/Akt-mTOR pathway

Manoj V. Lohar [a], Ramswaroop Mundada [a], Mandar Bhonde [b], Amol Padgaonkar [b], Vijaykumar Deore [a], Nilambari Yewalkar [a], Dimple Bhatia [b], Maggie Rathos [b], Kalpana Joshi [b], Ram A. Vishwakarma [a], Sanjay Kumar [a,*]

[a] Department of Medicinal Chemistry, Nicholas Piramal Research Centre, Goregaon (East), Mumbai 400 063, India
[b] Department of Pharmacology, Nicholas Piramal Research Centre, Goregaon (East), Mumbai 400 063, India

ARTICLE INFO

Article history:
Received 16 November 2007
Revised 11 April 2008
Accepted 30 April 2008
Available online 4 May 2008

ABSTRACT

We herein report the design and synthesis of furoquinoline based novel molecules (**16**–**36**) and their in vitro multiple targeted inhibitory potency against PI3K/Akt phosphorylation and mTOR using cell based and cell-free kinase assay. In particular, compound **23** in addition to PI3K-mTOR inhibitory potency, it has shown potent inhibition of hypoxia-induced accumulation of HIF-1α protein in U251-HRE cell line. The inhibitory activities of compound **23** were confirmed by Western blot analysis, using human non-small cell lung carcinoma H-460 cell line and glioblastoma U251 cell lines.

Phosphatidylinositol-3-kinases (PI3Ks) are members of a family of lipid kinases that regulate cellular metabolism and growth by phosphorylation of the 3-position of phosphatidylinositol diphosphate (PIP_2) to phosphatidylinositol triphosphate (PIP_3). The PI3K signalling pathway is negatively regulated by PTEN. Which is most frequently mutated in human cancer, leading to amplification of signalling and as such is a promising target for small molecule inhibition, with potential therapeutic target for anti-cancer drug development.[1–5] Based on the primary structure and mechanism of action PI3Ks are divided into two major classes viz. class I and class II.[6,7] The class I PI3Ks are further divided into class IA enzymes: p110α, p110β and p110δ which are activated by tyrosine kinase receptors whereas the only member of class IB enzyme p110γ, is activated by G-protein-coupled receptor. The class II PI3Ks C2α, C2β and C2γ are characterized by the presence of C2 domain at C-terminus. The recent studies with isoform-specific small-molecule inhibitors helped to elucidate the distinct cellular function of different class I isoforms (p110α, p110β, p110δ and p110γ). It has been reported that inhibition of p110α is essential to affect growth suppression in malignant cell lines.[8] The other class I isoforms have their therapeutic potential in other disease areas viz. inflammation, autoimmune disease (p110δ and p110γ) and thrombosis (p110β).[1,3,9–11]

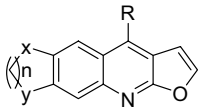
It has been reported that the inhibition of multiple target in the PI3K/Akt pathway (PI3K/Akt, mTOR, HIF-1α, VEGF) may lead to greater therapeutic potential.[12,13] Akt, which is also named as protein kinase B (PKB), is an important downstream effector of growth factor signalling cascade that generate proliferative and antiapoptotic responses. The PI3K/Akt-mTOR pathway is also responsible for the regulation of hypoxia-inducible factor-1. HIF-1 is a heterodimer consisting of α and β subunit. The HIF-1α subunit is degraded rapidly in normoxic conditions and stabilized under hypoxic conditions, while HIF-1β is constitutively expressed. In general, the availability and activity of HIF-1α protein determine the bioactivity of HIF-1.[14] HIF-1α is master regulator of transcriptional response to oxygen deficiency. It is upregulated in response to hypoxia and growth factor stimuli. In addition to HIF-1's association in tumour progression, HIF-1 has been implicated in the regulation of genes involved in angiogenesis, for example, vascular endothelial growth factor (VEGF), inducible nitric oxide synthase and anaerobic metabolism (glycolytic enzymes).[15] Overexpression of HIF-1α has been demonstrated in many common human cancers such as pancreatic carcinoma, lung carcinoma, colorectal carcinoma and glioblastoma, to name a few.[16] Moreover, a link with HIF-1 signalling pathway has been suggested from the studies with various cell types.[17,18] Recently, several inhibitors have been identified to inhibit PI3K including wortmannin and Ly-294002.[19,20] The example of HIF-1α inhibitors includes YC-1, PX-478 and topotecan.[21–23]

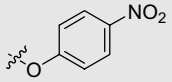
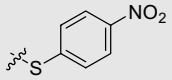
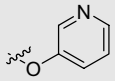
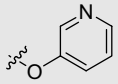
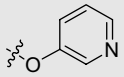
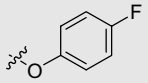
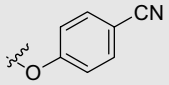
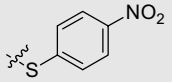
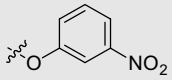
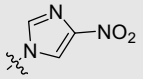
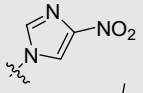
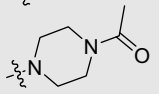
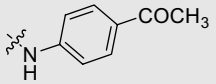
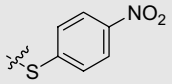
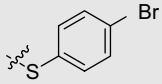
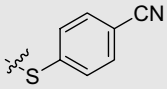
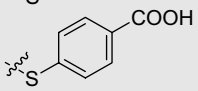
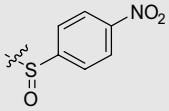
As a part of our ongoing research in the identification of novel multiple target inhibitors for anti-cancer drug development, we herein report the design, synthesis and therapeutic evaluation of natural product based molecules, which can significantly inhibit multiple targets of same pathway (PI3K-Akt-mTOR-HIF) in order to enhance the potency towards various cancer cell types relative to drugs that address only a single target of the particular pathway. The role of PI3K/Akt, mTOR and HIF-1α is well known in inflamma-

* Corresponding author. Tel.: +91 22 30818317; fax: +91 22 30818334.
E-mail address: sanjay.kumar@piramal.com (S. Kumar).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.04.078

Table 1
In vitro inhibition of PI3K/Akt-mTOR activity in cell-based assay for compounds **16–36**[a]



Compound	*x*	*y*	*n*	R	PI3K[b]	mTOR.[b]
16	O	O	1		NI	47
17	O	O	1		NI	44
18	O	O	1		NI	51
19	H	OCH_3	0		67	NI
20	OCH_3	OCH_3	0		43	NI
21	O	O	1		45	NI
22	O	O	1		58	NI
23	H	OCH_3	0		48	49
24	H	OCH_3	0		43	NI
25	H	OCH_3	0		64	NI
26	O	O	1		57	NI
27	OCH_3	OCH_3	0		64	NI
28	OCH_3	OCH_3	0		58	NI
29	H	OCH_3	0		58	NI
30	O	O	1		40	45
31	H	OCH_3	0		NI	53
32	OCH_3	OCH_3	0		NI	NI
33	H	OCH_3	0		NI	NI
34	H	OCH_3	0		NI	NI
35	H	OCH_3	0		NI	NI
36	H	OCH_3	0		NI	NI
Ly-294002					40	45

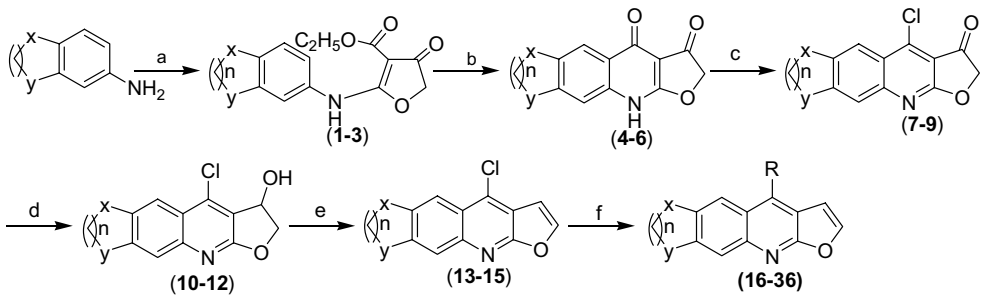
[a] Values are means of three experiments.
[b] % Inhibition at 10 μM in H460 cell line. Ly-294002 was evaluated at 30 μM to get significant inhibition; NI, No inhibition observed up to 30 μM.

tion and cancer. Based on the various isoforms of the PI3K (p110 α, β, γ and δ) and their homology, we thought to take advantage of this information and selected an anti-inflammatory natural product, which on suitable modification could yield novel molecules with anti-cancer properties. A sizable number of natural products are known in the literature with potent anti-inflammatory activity. One of them being Evolitrine, an alkaloid, which has been isolated from several plants sources and has been reported for its antifeedant, anti-inflammatory and anti-cancer activity.[24,25] Though it shows significant anti-inflammatory property its anti-cancer properties have a lot of scope for improvement. Thus we wanted to make modifications on Evolitrine and enhance its anti-cancer properties targeting the PI3K/Akt-mTOR/HIF-1α pathway.

The compounds (**16–36**) described in this Letter (Table 1) are prepared as outlined in Scheme 1.[25] In brief, the condensation of suitably substituted anilines with diethylmalonate was carried out by treating sodium diethylmalonate salt with chloroacetyl chloride to get compounds **1–3**, which on thermal cyclization in diphenyl ether yielded corresponding substituted dihydrofuran quinoline dione (**4–6**). Compounds **4–6** on treatment with Aliquat 336 and $POCl_3$ yielded substituted 4-chlorofuro-quinoline-3-one (**7–9**), which on subsequent reduction with $NaBH_4$ yielded 3-hydroxy compounds **10–12**. The 3-hydroxy compound on dehydration with $KHSO_4$ yielded substituted 4-chlorofuroquinoline **13–15** in quantitative yield. The target compounds **16–36** (Table 1) were prepared by treating substituted 4-chlorofuroquinoline **13–15** with a variety of substituted phenols/thiophenols/amines/*N*-heterocycles using NaH in DMF at 150 °C for 10–15 h in 30–70% yields.

The in vitro inhibitory potency of all newly synthesized target molecules **16–36** are evaluated against the phosphorylation of Akt at Ser 473, which is an universally accepted read-out of PI3K activity and phosphorylation of p70S6 kinase at Thr 389 which is a bonafide read-out of mTOR activity by using human non-small cell lung cancer H-460 cells as per previously described assay protocol.[26] We found several compounds which inhibited either PI3K (**19–30**) or mTOR (**16–18**, **23**, **30–31**) or both PI3K and mTOR (**23**, **30**) activities (Table 1). All the compounds (**16–36**) were tested at 10 μM concentration in PI3K and mTOR assay and inhibitory activity has been reported in % inhibition. Ly-294002 was used as a standard PI3K and mTOR inhibitor in all the experiments, which shows 40% inhibition in PI3K and 45% inhibition in mTOR assay at 30 μM concentration (Table 1).

We have synthesized a series of molecules by keeping furoquinoline backbone constant and doing the modification particularly at positions 4, 6 and 7 of furoquinoline ring. The positions 6 and 7 have been explored by using 7-methoxy or 6,7-dimethoxy or 6,7-methylenedioxo groups. The modification at positions 6 and 7 re-



1, 4, 7, 10, 13: x = H, y = -OCH_3, n = 0; **2, 5, 8, 11, 14**: x = y = -OCH_3, n = 0; **3, 6, 9, 12, 15**: x = y = -O-, n = 1

Scheme 1. Reagent and conditions: (a) NaH, chloroacetyl chloride, TEA, diethyl malonate, THF, 24 h, 45–65%; (b) diphenyl ether, 250 °C, 30 min, 70–80%; (c) Aliquat 336, $POCl_3$, rt, 48 h, 65–70%; (d) $NaBH_4$, MeOH, 1 h, 90–92%; (e) $KHSO_4$, dioxane, reflux, 2–3 h, 65–75%; (f) substituted phenol/thiophenol/amine/*N*-heterocyles, NaH, DMF, 150 °C, 10–15 h, 30–70%.

veal that 7-methoxy was more suitable modification for getting an active entity. We explored position 4 by substituting with variety of R groups (Table 1) viz. phenoxy, thiophenoxy, *N*-phenyl, pyridoxy, imidazo, piperazine, etc. While evaluating the activity in PI3K and mTOR several molecules (**19**–**30**) were found active in PI3k assay. Upon screening this subset of molecules for PI3K and mTOR dual activity only two molecules (**23** and **30**) exhibited significant activity. Then these two molecules were subjected for HIF-1α assay and compound **23** was found active thus demonstrating multiple target inhibitor profile. To perform a SAR on compound **23** the para nitro group was substituted with a electron donating group like amine (data not shown), electronically neutral group (halogen) but neither of these molecules exhibited any significant activity. Thus other EWG (**34**, **35**, and **36**) were tried at this position but as shown in Table 1 none of them demonstrate any activity. Compound **23** turns out to be the most active multi targeted (PI3K/Akt-mTOR-HIF-1α) inhibitor of this furoquinoline series. Upon screening Evolitrine for similar assay it exhibited no activity in PI3K, mTOR or HIF-1α up to 30 μM concentration.

To address question whether the inhibition of Akt phosphorylation was a result of targeting the PI3 kinase inhibition or due to direct inhibition of Akt phosphorylation, we performed cell-free kinase assay with PI3K inhibitors (**19**–**30**) using recombinant PI3Kα (p110α). The read-out of this assay was the formation of PI3P (phosphatidylinositol-3-phosphate) from PI (phosphatidylinositol) in presence of PI3Kα as per published procedure.[8] Surprisingly, we found that only compound **23** showed significant inhibition in PI3 kinase (p110α) assay with IC_{50} 4 μM (data not shown). The compounds, which inhibited Akt phosphorylation in cell-based assay, but showed no inhibition of PI3 kinase activity, might be directly inhibiting Akt without affecting the PI3 kinase (p110α). The PI3K-mTOR inhibitory potency of compound **23** was further confirmed by Western blot analysis using H-460 cell line for phosphorylation of Akt (PI3K) and 4E-BP1 (mTOR) at concentrations of 3 and 10 μM. We observed a concentration dependent inhibition of phosphorylation of PI3K and mTOR targets (Fig. 1a). The dual (PI3K and mTOR) inhibitory potency of compound **23** encouraged us to see the effect of these inhibitors in downstream signalling pathway.

For the same we decided to evaluate the inhibitory potency of the target molecules **16**–**36** against hypoxia-induced HIF-1 activation in U251-HRE glioblastoma cell line. The inhibitory potencies of HIF-1 activation were obtained using a HRE mediated cell based reporter gene assay under hypoxic conditions (1% O_2, 94% N_2 and 5% CO_2) as well as DFX induced hypoxic condition similar to published protocol.[15] In this assay out of all reported compounds **16**–**36**, only compound **23** has showed significant inhibition of hypoxia-induced HIF-1 activation, with IC_{50} 3 μM (data not shown). YC-1 a known HIF-1 inhibitor used as standard (IC_{50} 14.8 μM).

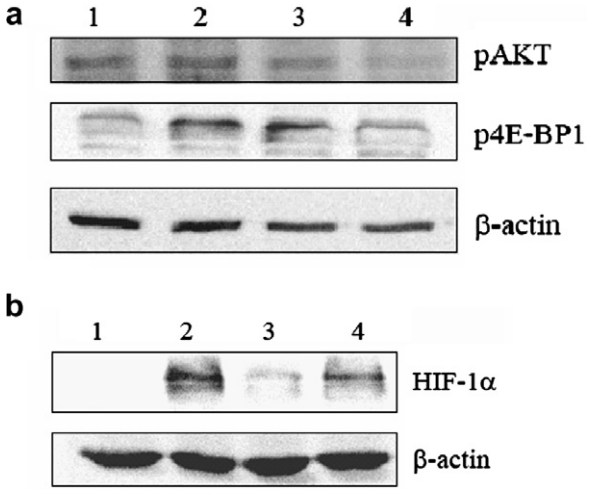


Figure 1. (a) H460 cells were either left untreated (lane 1), or were treated with 20 % FCS (lane 2) in the presence of 3 μM compound **23** (lane 3) or 10 μM compound **23** (lane 4). (b) U251-HRE-Luc cells were either left untreated (lane 1), or were treated for 6 h with DFX (lane 2), in the presence of 0.5 μM Topotecan (lane 3) or 10 μM compound **23** (lane 4).

To confirm the HIF-1 inhibitory activity, compound **23** was evaluated by Western blot analysis for the expression of HIF-1α protein in U251-HRE cell line (Fig. 1b).

These results suggest that compound **30** is a dual inhibitor of PI3K and mTOR activities, and compound **23** is a multiple inhibitor of PI3K, mTOR and HIF-1α. However, further studies will unravel the mechanism of action of these inhibitors in the context of PI3K isoforms (p110α, p110β, p110γ, p110δ) as well as antiproliferative and in vivo efficacy.

In conclusion, we have designed and synthesized variety of furoquinoline based compounds. The primary screening shows that 7-methoxy-4-(4-nitrophenylthio)furo[2,3-*b*]quinoline (**23**) is a multi-target inhibitor of PI3K/Akt-mTOR-HIF-1α and identified as most potent inhibitor in this series. Further investigations are warranted to establish more detailed mechanism of these molecules.

Acknowledgments

Authors are grateful to Dr. Giovanni Melillo, Development Therapeutics Program-Tumor Hypoxia Laboratory, National Cancer Institute at Frederick, Maryland USA. We are also thankful to department of analytical chemistry for providing nmr, mass, microanalysis and hplc for characterization of all novel molecules

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.04.078.

References and notes

1. Cantley, L. C. *Science* **2002**, *296*, 1655.
2. Hayakawa, M.; Kawaguchi, K.; Kaizawa, H.; Koizumi, T.; Ohishi, T.; Yamano, M.; Okada, M.; Ohta, M.; Tsukamoto, S.; Raynaud, F. I.; Parker, P.; Workman, P.; Waterfield, M. D. *Bioorg. Med. Chem.* **2007**, *15*, 5837.
3. Ward, S.; Sotsios, Y.; Dowden, J.; Bruce, I.; Finan, P. *Chem. Biol.* **2003**, *10*, 207.
4. Ward, S. G.; Finan, P. *Curr. Opin. Pharmacol.* **2003**, *3*, 426.
5. Workman, P. *Biochem. Soc. Trans.* **2004**, *32*, 393.
6. Vanhaesebroeck, B.; Leevers, S. J.; Ahmadi, K.; Timms, J.; Katso, R.; Driscoll, P. C.; Woscholski, R.; Parker, P. J.; Waterfield, M. D. *Annu. Rev. Biochem.* **2001**, *70*, 535.
7. Domin, J.; Waterfield, M. D. *FEBS Lett.* **1997**, *410*, 91.
8. Knight, Z. A.; Gonzalez, B.; Feldman, M. E.; Zunder, E. R.; Goldenberg, D. D.; Williams, O.; Loewith, R.; Stokoe, D.; Balla, A.; Toth, B.; Balla, T.; Weiss, W. A.; Williams, R. L.; Shokat, K. M. *Cell* **2006**, *125*, 733.
9. Engelman, J. A.; Luo, J.; Cantley, L. C. *Nat. Rev. Genet.* **2006**, *7*, 606.
10. Powis, G.; Ihle, N.; Kirkpatrick, D. L. *Clin. Cancer Res.* **2006**, *12*, 2964.
11. Ruckle, T.; Schwarz, M. K.; Rommel, C. *Nat. Rev. Drug Discov.* **2006**, *5*, 903.
12. Xu, Q.; Briggs, J.; Park, S.; Niu, G.; Kortylewski, M.; Zhang, S.; Gritsko, T.; Turkson, J.; Kay, H.; Semenza, G. L.; Cheng, J. Q.; Jove, R.; Yu, H. *Oncogene* **2005**, *24*, 5552.
13. Fan, Q. W.; Knight, Z. A.; Goldenberg, D. D.; Yu, W.; Mostov, K. E.; Stokoe, D.; Shokat, K. M.; Weiss, W. A. *Cancer Cell* **2006**, *9*, 341.
14. Nagle, D. G.; Zhou, Y. D. *Curr. Drug Targets* **2006**, *7*, 355.
15. Rapisarda, A.; Uranchimeg, B.; Scudiero, D. A.; Selby, M.; Sausville, E. A.; Shoemaker, R. H.; Melillo, G. *Cancer Res.* **2002**, *62*, 4316.
16. Zhong, H.; De Marzo, A. M.; Laughner, E.; Lim, M.; Hilton, D. A.; Zagzag, D.; Buechler, P.; Isaacs, W. B.; Semenza, G. L.; Simons, J. W. *Cancer Res.* **1999**, *59*, 5830.
17. Jiang, B. H.; Jiang, G.; Zheng, J. Z.; Lu, Z.; Hunter, T.; Vogt, P. K. *Cell Growth Differ.* **2001**, *12*, 363.
18. Zhong, H.; Chiles, K.; Feldser, D.; Laughner, E.; Hanrahan, C.; Georgescu, M. M.; Simons, J. W.; Semenza, G. L. *Cancer Res.* **2000**, *60*, 1541.
19. Zhu, T.; Gu, J.; Yu, K.; Lucas, J.; Cai, P.; Tsao, R.; Gong, Y.; Li, F.; Chaudhary, I.; Desai, P.; Ruppen, M.; Fawzi, M.; Gibbons, J.; Ayral-Kaloustian, S.; Skotnicki, J.; Mansour, T.; Zask, A. *J. Med. Chem.* **2006**, *49*, 1373.
20. Walker, E. H.; Pacold, M. E.; Perisic, O.; Stephens, L.; Hawkins, P. T.; Wymann, M. P.; Williams, R. L. *Mol. Cell* **2000**, *6*, 909.
21. Yeo, E. J.; Chun, Y. S.; Cho, Y. S.; Kim, J.; Lee, J. C.; Kim, M. S.; Park, J. W. *J. Natl. Cancer Inst.* **2003**, *95*, 516.
22. Welsh, S.; Williams, R.; Kirkpatrick, L.; Paine-Murrieta, G.; Powis, G. *Mol. Cancer Ther.* **2004**, *3*, 233.
23. Rapisarda, A.; Zalek, J.; Hollingshead, M.; Braunschweig, T.; Uranchimeg, B.; Bonomi, C. A.; Borgel, S. D.; Carter, J. P.; Hewitt, S. M.; Shoemaker, R. H.; Melillo, G. *Cancer Res.* **2004**, *64*, 6845.
24. I.-Li. Chen, Y.-L. C., Cherng-Chyi Tzeng, Ih-Sheng Chen. *Helvetica Chemica Acta* **2002**, 85, 2214.
25. Bansi Lal, B. N. B.; Gidwani, R. M.; Lakdawal, A. D.; Joshi, K.; Patwardhan, S. *ARKIVOC* **2005**, *ii*, 77.
26. Versteeg, H. H.; Nijhuis, E.; van den Brink, G. R.; Evertzen, M.; Pynaert, G. N.; van Deventer, S. J.; Coffer, P. J.; Peppelenbosch, M. P. *Biochem. J.* **2000**, *350*, 717.