Bioorganic & Medicinal Chemistry Letters 18 (2008) 4186–4190

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of potent and cell-active allosteric dual Akt 1 and 2 inhibitors

Tony Siu [a,*], Jun Liang [a], Jeannie Arruda [a], Yiwei Li [b], Raymond E. Jones [b], Deborah Defeo-Jones [b], Stanley F. Barnett [b], Ronald G. Robinson [b]

[a] Department of Medicinal Chemistry, Merck Research Laboratories, Merck & Co., 3535 General Atomics Court, San Diego, CA 92129, USA
[b] Department of Cancer Research, Merck Research Laboratories, Merck & Co., PO Box 4, West Point, PA 19486, USA

ARTICLE INFO

Article history:
Received 3 March 2008
Revised 18 May 2008
Accepted 19 May 2008
Available online 24 May 2008

Keywords:
Akt
Kinase
Allosteric
Cell active

ABSTRACT

This paper describes the improvement of cell potency in a class of allosteric Akt 1 and 2 inhibitors. Key discoveries include identifying the solvent exposed region of the molecule and appending basic amines to enhance the physiochemical properties of the molecules. Findings from the structure–activity relationships are discussed.

The PI3K/Akt pathway has attracted much attention in the cancer research community due to its involvement in multiple cell survival, growth, energy metabolism, and proliferation pathways.[1] Akt, also known as Protein Kinase B (PKB), is a serine/threonine kinase that plays a crucial role in the regulation of the PI3K/Akt pathway. Akt exists as three isoforms: 1, 2, and 3, all of which contain a pleckstrin homology (PH) domain in addition to the kinase catalytic domain. Interaction of phosphatidyinositol-3,4,5-triphosphate with the PH domain recruits Akt to the membrane from the cytoplasm. Upon translocation to the membrane, Akt undergoes a conformational change and is phosphorylated by phosphoinositide-dependent kinase 1 (PDK1) at threonine 308 and by phosphoinositide-dependent kinase 2 (PDK2) at serine 473 for full activation.

Support for the use of Akt kinase as a molecular target for cancer is provided by human cancers with mutations that upregulate the PI3K/Akt pathway strongly. Most notable is the loss of function of PTEN, a phosphatase and negative regulator of Akt, that is deleted or mutated in >50% of human cancers.[2] Several therapeutic strategies can be envisioned due to the many roles Akt plays in neoplastic transformation. Because Akt is a crucial component of the survival pathway, inhibition of the enzyme through small molecule perturbation in synergy with chemotherapy could potentially sensitize cancer cells to undergo apoptosis.[3] In addition, inhibition of Akt kinase could have the benefit of reversing chemotherapeutic and radiation therapy resistance. This mechanism of action would have a substantial impact on a broad spectrum of cancers. Furthermore, small molecule inhibition of Akt kinase as a monotherapy could inhibit tumorigenesis through deregulation of the cell growth and proliferation pathways that Akt controls.[1b]

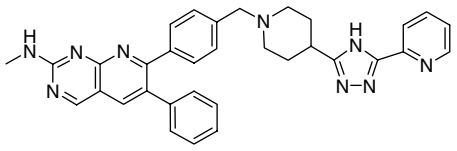
In previous letters, Merck Research Laboratories have reported a strategy for targeting Akt kinase 1 and 2 using selective allosteric inhibitors.[4] This strategy involves targeting the PH domain as opposed to the catalytic ATP-binding domain of Akt. By designing molecules that bind allosterically, these inhibitors have the advantage of achieving kinase specificity. As a result of this selectivity, the likelihood of encountering toxic adverse effects due to inhibiting multiple kinases should be minimized.

Merck Research Laboratories have recently published a series of bisphenyl analogs represented by **1** and **2** (Fig. 1) as allosteric inhibitors of Akt.[4e] Most notable is pyridopyrimidine **1**, which possesses potent intrinsic and cellular activity against Akt 1. However, **1** suffers from modest cellular Akt 2 activity. Our focus was to expand on these initial findings to obtain potent cell-based active dual Akt 1 and 2 inhibitors.[4e] Continuing our strategy to obtain selective kinase activity by targeting the allosteric site of Akt, we report in this letter a detailed structure activity relationship based on a novel scaffold of Akt inhibitors with potent enzymatic and cell-based activities against Akt 1 and 2.

Table 1 shows data for a wide range of pyridopyrimidine amines, which were tested against Akt 1 and 2. Included are the Akt IC_{50} values which are derived from biochemical inhibition of synthetic peptide phosphorylation, as well as Akt cell IC_{50} values which represent inhibition of phosphorylation of Akt isozymes in C33a cancer cells.[5] Our strategy centered on preserving the pyrido-

* Corresponding author at present address: Department of Drug Optimization, Merck Research Laboratories, Merck & Co., 33 Avenue Louis Pasteur, Boston, MA 02115, USA.
E-mail address: tony_siu@merck.com (T. Siu).

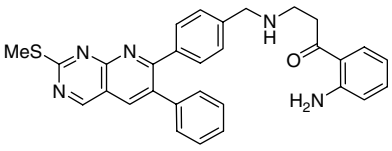
0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.05.085



1

Akt 1/2 IC_{50} = 6.0/94 nM

Cell Akt 1/2 IC_{50} = 20/899 nM



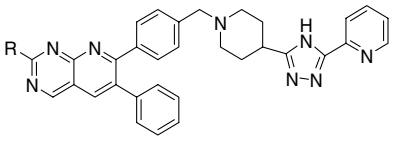
2

Akt 1/2 IC_{50} = 15/90 nM

Cell Akt 1/2 IC_{50} = 78/388 nM

Figure 1. Merck Akt allosteric inhibitors.

Table 1
SAR on pyridopyrimidine analogs



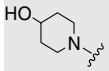
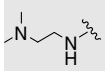
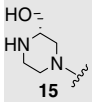
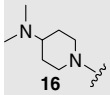
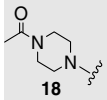
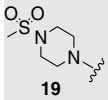
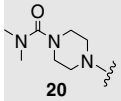
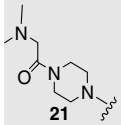
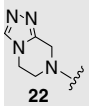
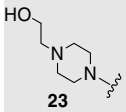
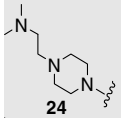
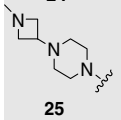
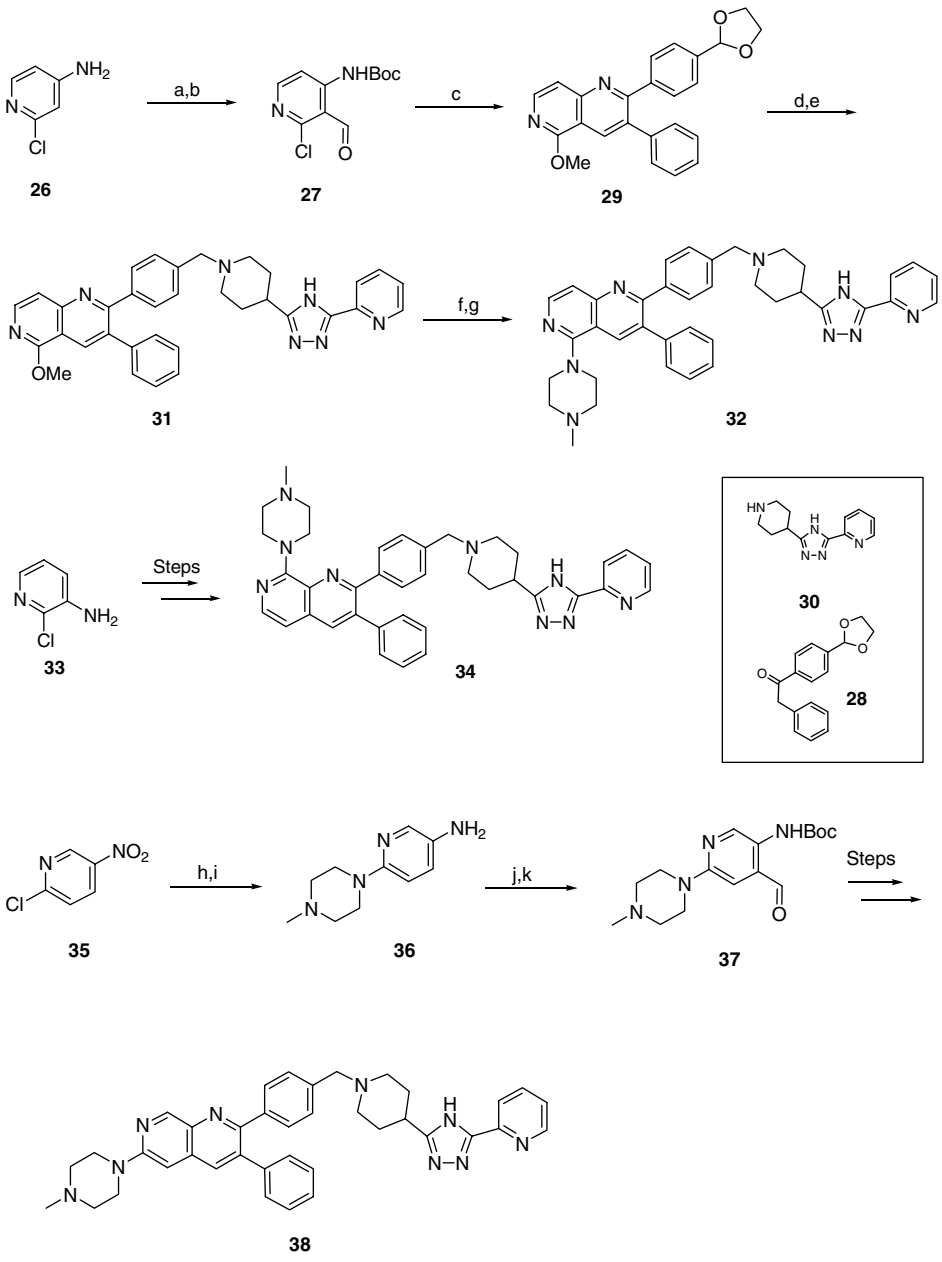
	Enzyme IC_{50} (nM)		Cell IC_{50} (nM)	
	Akt1	Akt2	Akt1	Akt2
 **3**	20	252	75	462
 **4**	18	93	36	229
 **5**	5	43	19	243
 **6**	7	45	17	168
 **7**	28	102	42	184
 **8**	38	297	NA	
 **9**	45	77	46	101
 **10**	8	25	36	44
 **11**	17	47	75	125
 **12**	9	27	36	52
 **13**	43	29	194	199

Table 1 (*continued*)

	Enzyme IC_{50} (nM)		Cell IC_{50} (nM)	
	Akt1	Akt2	Akt1	Akt2
 **14**	79	154	208	223
 **15**	25	67	61	421
 **16**	10	22	23	70
 **17**	5	73	35	684
 **18**	5	16	15	145
 **19**	7	23	20	196
 **20**	18	40	13	177
 **21**	6	6	3	19
 **22**	19	138	125	735
 **23**	9	31	17	41
 **24**	4	4	10	39
 **25**	12	9	6	27



Scheme 1. Synthesis of regioisomers. Reagents and conditions: (a) $(BOC)_2O$, DMAP, Et_3N, CH_2Cl_2; (b) i— *t*-BuLi, THF, −78 °C; ii—DMF; (c) **28**, 25% sodium methoxide in MeOH, 100 °C; (d) 1 N HCl; (e) **30**, $NaBH(OAc)_3$, DMF; (f) i—4 N HCl/THF; ii—$POCl_3$, MeCN, reflux; (g) methyl-piperazine 100 °C, dioxane; (h) methyl-piperazine, 100 °C, dioxane; (i) H_2, 5% Pd/C, EtOAc; (j) $(BOC)_2O$, DMAP, Et_3N, CH_2Cl_2; (k) i— *t*-BuLi, THF, −78 °C; ii—DMF.

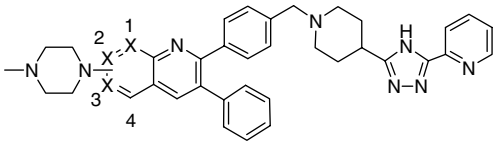
pyrimidine biphenyl core and introducing a variety of amines in the northwest region of the molecule.

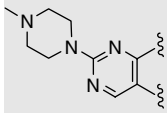
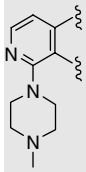
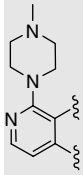
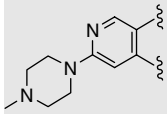
To address the binding environment, we first probed the region with lipophilic substituents such as difluoro pyrrolidine **3** and piperidine **4**. These analogs provided modest intrinsic and cell based potencies. In contrast, the incorporation of a polar heteroatom in the piperidine in the form of morpholine **5** led to slight gains in intrinsic potency (Akt 1/2 IC_{50} = 5/43 nM) with a 2-fold improvement in cellular activity in Akt 2 (cell Akt 1/2 IC_{50} = 19/243 nM) compared to **1**. Several other oxygen-based analogs such as **6** and **7** were explored; however, the cell-based potencies for Akt 2 for both of them were similar to **5**.

Although the morpholine analogs were not at the desired potency level, these results provided an impetus to explore this region of the molecule with more hydrophilic substituents. Further investigation of a set of piperazines (**9**-**13**) led to improved cell-based potency versus Akt 2 with modestly improved intrinsic potency. Notably, we were able to obtain compounds with cellular activity less than 100 nM for Akt 1 and 2 as exemplified by compounds **10** (cell Akt 1/2 IC_{50} = 36/44 nM) and **12** (cell Akt 1/2 IC_{50} = 36/52 nM). The 2,5-dimethyl-substituted piperazine **13**, designed to rotate the piperazine out plane, displayed a 2- to 4-fold loss in cellular potency compared to the methyl piperazine **10**. This result suggests that the conformation of the piperazine ring does not contribute significantly to potency. The flexible acyclic dimethyl amino analog **14** suffered a loss in both intrinsic and cellular potency compared to the piperazines **9**–**13**, signaling the importance of the conformational constraint of the basic nitrogen.

To investigate whether the basic nitrogen of the piperazine substituent is important to the activity of our inhibitors, we examined

Table 2
SAR of methyl piperazine regioisomers



	Compound	Enzyme Akt 1/2 (nM)	Cell Akt 1/2 (nM)
	**12**	9/27	36/52
	**32**	285/539	NA
	**34**	720/3570	NA
	**38**	740/59	924/133

several analogs modulating its basicity. Increasing the basicity by moving the nitrogen out of the ring (**16**) maintained the overall intrinsic and cellular potency. Alternatively, reduction of the basic nitrogen of the piperazine by capping the basic nitrogen as an amide (**18**), sulfonamide (**19**), or urea (**20**) had minimal effects on the intrinsic potency. However, the cell-based potency of the compounds was diminished by 3- to 4-fold compared to **12**, indicating that the basicity of the second nitrogen is crucial to cellular penetration properties. A notable exception is compound **21**. Although the basicity of the nitrogen piperazine is reduced due to the amide moiety, the extended dimethylamino group seems to help maintain the cellular activity.

Encouraged by the increased activity of methyl piperazine (**12**), we proposed appending more aqueous solubilizing groups that extend further out such as compounds **23–25**. In general, introduction of polar functionalities on the piperazine moiety was well tolerated and conferred better overall intrinsic potency. Most notable was **24** with single digit intrinsic potency against Akt 1 and 2 (Akt 1/2 IC_{50} = 4/4 nM) and good overall cellular potency (cell Akt 1/2 IC_{50} = 10/39 nM). These results suggest that the northwest region of our inhibitors is exposed to an aqueous environment as opposed to a hydrophobic pocket. The enhanced intrinsic potency from appending polar functional groups arises possibly from the favorable charge interactions at the solvent interface. In addition, the overall better solubility of the inhibitors might contribute to their enhanced intrinsic and cell-based potencies. This rationale is consistent with properties of other kinase inhibitors with water-solubilizing groups.[6]

Having identified the favorable properties of piperazine **12**, we were interested in exploring the optimal position for the hydrophilic piperazine groups. Toward that end, several compounds related to **12** were prepared (Scheme 1). Compound **32** was synthesized starting with 2-chloro-4 amino pyridine. Boc protection followed by lithiation and quenching with DMF afforded **27**. Friedlander cyclization with sodium methoxide in methanol and **28** produced **29** with methoxy replacement at the 2-position of the pyridine. Hydrolysis of the acetal with acidic conditions liberated the aldehyde and reductive amination with **30** installed the piperidine triazole subunit to afford **31**. Hydrolysis of the 2-methoxy pyridine under strong acid followed by chlorination with $POCl_3$ and displacement with methyl piperazine provided **32**. Regioisomer **34** was synthesized using the same sequence of steps, but instead starting with 2-chloro-4-amino pyridine **33**. Regioisomer **38** was synthesized starting with 2-chloro-5-nitro-pyridine **35** by displacement of the chloride with methyl piperazine and reduction of the nitro group with palladium on carbon under a balloon atmosphere of hydrogen to afford amine **36**.[7] The amine was protected with Boc anhydride followed by lithiation and trapping with DMF to afford aldehyde **37**. This intermediate was carried on to **38** using the same synthetic sequence as for compound **32**.

Orientation of the methyl piperazine in any position other than position 2 (**12**) dramatically reduced the overall intrinsic potency of these inhibitors (Table 2). For example, positioning the methyl piperazine at positions 1 (**34**) or 4 (**32**) led to a 30- to 80-fold loss in intrinsic potency against Akt 1 and a 20- to 130-fold lost in potency against Akt 2. Interestingly, although orienting the methyl piperazine at position 3 (**38**) diminished Akt 1 activity; a 13-fold selectivity for Akt 2 activity over Akt 1 was observed. It is worth noting that the southwest region of the molecule could potentially be exploited for Akt 2-selective inhibitors. Maximal contact with the solvent exposed region is likely obtained by orienting the hydrophilic piperazine at the 2 position of the A ring.

In summary, potent dual inhibitors of Akt 1 and 2 such as **12**, **23**, and **24** were achieved by introducing modifications to the pyridopyrimidine core. Basic amines attached to the core were key to achieving cellular activity while maintaining the intrinsic potency. Optimal activity was restricted to a regiospecific northwest region of the molecule which presumably projects toward solvent after binding to the enzyme.

Acknowledgments

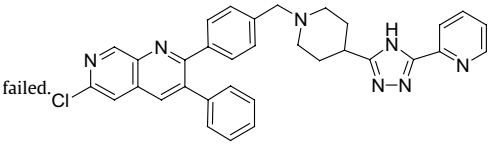
The authors thank Drs. Christopher Cox, Christopher Dinsmore, and Philip Sanderson, for careful reading of this manuscript.

References and notes

1. (a) Luo, J.; Manning, B. D.; Cantley, L. C. *Cancer Cell* **2003**, *4*, 257; (b) Vivanco, I.; Sawyers, C. L. *Nat. Rev. Cancer* **2002**, *2*, 489; (c) Hennessy, B. T.; Smith, D. L.; Ram, R. T.; Yiling, L.; Milla, G. B. *Nat. Rev. Drug Discov.* **2005**, *4*, 989; (d) Cully, M.; You, H.; Levine, A. J.; Mak, T. W. *Nat. Rev. Cancer* **2006**, *6*, 184.
2. (a) Cantley, L. C.; Neel, B. G. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4240; (b) Hsu, J. H.; Shi, Y.; Hu, L. P.; Fischer, M.; Franke, T. F.; Lichenstein, A. *Oncogene* **2002**, *21*, 1391; (c) Page, C.; Lin, H.; Jin, Y.; Castle, V. P.; Nunez, G.; Huang, M.; H.Lin *J. Anticancer Res.* **2000**, *20*, 407.
3. (a) Defeo-Jones, D.; Barnett, S. F.; Fu, S.; Hancock, P. J.; Haskell, K. M.; Leander, K. R.; McAvory, E. M.; Robinson, R. G.; Duggan, M. E.; Lindsley, C. W.; Zhao, Z.; Huber, H. E.; Jones, R. E. *Mol. Cancer Ther.* **2005**, *4*, 271; (b) Brognard, J.; Clark, A. S.; Ni, Y.; Dennis, P. A. *Cancer Res.* **2001**, *61*, 3986.
4. (a) Lindsley, C. W.; Zhao, Z.; Leister, W. H.; Robinson, R. G.; Barnett, S. F.; Defeo-Jones, D.; Jones, R. E.; Hartman, G. D.; Huff, J. R.; Huber, H. E.; Duggan, M. E. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 761; (b) Zhao, Z.; Leister, W. H.; Robinson, R. G.; Barnett, S. F.; Defeo-Jones, D.; Jones, R. E.; Hartman, G. D.; Huff, J. R.; Huber, H. E.; Duggan, M. E.; Lindsley, C. W. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 905; (c) Barnett, S. F.; Bilodeau, M. T.; Lindsley, C. W. *Curr. Top. Med. Chem.* **2005**, *5*, 109; (d) Zhao, Z.; Robinson, R. G.; Barnett, S. F.; Defeo-Jones, D.; Jones, R. E.; Hartman, G. D.; Huber, H. E.; Hartman, G. D.; Huber, H. E.; Duggan, M. E.; Lindsley, C. W. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 49; (e) Wu, Z.; Hartnett, J. C.; Neilson, L.; Robinson, R. G.; Fu, S.; Barnett, S. F.; Defeo-Jones, D.; Jones, R. E.; Kral, A. M.; Huber, H.; Hartman, G. D.; Bilodeau, M. T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1274; (f) Wu, Z.; Robinson, R. G.; Fu, S.; Barnett, S. F.; Defeo-Jones, D.; Jones, R. E.; Kral, A. M.; Huber, H. E.; Kohl, N. E.; Hartman, G. D.; Bilodeau, M. T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2211; (g) Hartnett, J. C.; Barnett, S. F.; Bilodeau, M. T.; Defeo-Jones, D.; Hartman, G. D.;

Huber, H. E.; Jones, R. E.; Kral, A. M.; Robinson, R. G.; Wu, Z. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2194; (h) Bilodeau, M. T.; Balitza, A. E.; Hoffman, J. M.; Manley, P. J.; Barnett, S. F.; Defeo-Jones, D.; Jones, R. E.; Robinson, R. G.; Smith, A. M.; Huber, H. E.; Hartman, G. D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3178.

5. Detection was performed by homogenous time-resolved fluorescence (HTRF) using a europium chelate. IC_{50} values are reported as the averages of at least two independent determinations; standard deviations are within ±25–50% of IC_{50} values. For Akt enzyme details, see: Barnett, S. F.; Defeo-Jones, D.; Fu, S.; Hancock, P. J.; Haskell, K. M.; Jones, R. E.; Kahana, J. A.; Kral, A.; Leander, K.; Lee, L. L.; Malinowski, J.; McAvoy, E. M.; Nhas, D. D.; Robinson, R.; Huber, H. E. *Biochem. J.* **2005**, *385*, 399. For details on the Akt IPKA assay also see Ref. 3. Values are reported as single determinations ± standard deviations..
6. (a) Chen, P.; Doweyko, A. M.; Norris, D.; Gu, H. H.; Spergel, S. H.; Das, J.; Moquin, R. V.; Lin, J.; Wityak, J.; Iwanowicz, E. J.; McIntyre, K. W.; Shuster, D. J.; Behnia, K.; Chong, S.; de Fex, H.; Pang, S.; Pitt, S.; Shen, D. R.; Thrall, S.; Stanley, P.; Kocy, O. R.; Witmer, M. R.; Kanner, S. B.; Schieven, G. L.; Barrish, J. C. *J. Med. Chem.* **2004**, *47*, 4517; (b) Palmer, B. D.; Trumpp-Kallmeyer, S.; Fry, D. W.; Nelson, J. M.; Showalter, H. H.; Denny, W. A. *J. Med. Chem.* **1997**, *40*, 1519; (c) Hennequin, L. F.; Stokes, E. S.; Thomas, A. P.; Johnstone, C.; Ple, P. A.; Ogilvie, D. J.; Dukes, M.; Wedge, S. R.; Kendrew, J.; Curwen, J. O. *J. Med. Chem.* **2002**, *45*, 1300; (d) Bilodeau, M. T.; Rodman, L. D.; McGaughey, G. B.; Coll, K. E.; Koester, T. J.; Hoffman, W. F.; Hungate, R. W.; Kendall, R. L.; McFall, R. C.; Rickert, K. W.; Rutledge, R. Z.; Thomas, K. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2941.
7. The introduction of the piperazine by displacement with the activating nitro-group was necessary because all efforts to displace the chloride of compound **39** failed.



39