Bioorganic & Medicinal Chemistry Letters 21 (2011) 5239–5243

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and evaluation of quinazolin-4-ones as hypoxia-inducible factor-1α inhibitors

Wenwei Huang *, Ruili Huang, Matias S. Attene-Ramos, Srilatha Sakamuru, Erika E. Englund, James Inglese, Christopher P. Austin, Menghang Xia

NIH Chemical Genomics Center, National Human Genome Research Institute, NIH, 9800 Medical Center Dr., Rockville, MD 20850, USA

ARTICLE INFO

Article history:
Received 2 June 2011
Revised 8 July 2011
Accepted 11 July 2011
Available online 21 July 2011

Keywords:
Hypoxia-inducible factor-1α
Quinazolin-4-ones
Parallel synthesis

ABSTRACT

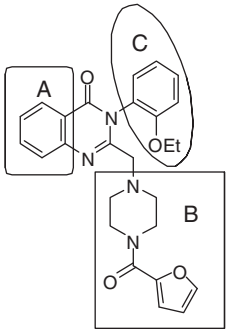
Quinazolin-4-one **1** was identified as an inhibitor of the HIF-1α transcriptional factor from a high-throughput screen. HIF-1α up-regulation is common in many cancer cells. In this Letter, we describe an efficient one-pot sequential reaction for the synthesis of quinazolin-4-one **1** analogues. The structure–activity relationship (SAR) study led to the 5-fold more potent analogue, **16.**

Published by Elsevier Ltd.

Hypoxia-inducible factor (HIF-1) is a dimeric transcription factor consisting of an oxygen regulated α-component and a constitutively expressed β-component. At normal oxygen levels, HIF-1α is degraded via the pVHL-mediated ubiquitin-proteosomal pathway. Under hypoxic conditions, HIF-1α rapidly accumulates and dimerizes with HIF-1β. This heterodimer binds to the DNA hypoxia-response element (HRE) and activates a diverse array of target genes.[1] This pathway is particularly relevant to the cancer field because oxygen levels in tumors are commonly lower than in the surrounding tissues. Hypoxic cells are resistant to radiation damage and their distances from blood vessels reduce the potency of anti-cancer drugs. Hypoxia additionally promotes the up-regulation of genes involved in drug resistance. HIF-1 is directly responsible for the induction of numerous genes that are present at higher levels in cancer cells, in particular VEGF. The overexpression of HIF-1 has been related to the aggressiveness and vascularity of tumors, and mortality rate in patients. Despite the introduced difficulties in treating hypoxic tumors, the hypoxic environment found in tumor cells can be exploited for targeted therapy. One strategy to achieve this involves the identification of HIF-1 inhibitors as potential anti-cancer drugs.[2] We recently reported a high-throughput cell-based HIF-1 mediated β-lactamase reporter gene assay. Upon screening a library of 73,000 compounds (PubChem AID:915 (http://pubchem.ncbi.nlm.nih.gov)), several compounds were identified as novel inhibitors of the HIF-1 signaling pathway.[3] Among these hits, quinazolin-4-one **1** (NCGC00056044) showed good drug-like properties and was selected for further exploration.

Three areas were selected for structure-activity relationship (SAR) studies: (1) substitution in area A; (2) piperazine region B; and (3) phenyl substitution in area C (Fig. 1).

To facilitate our compound synthesis for the SAR study, we modified a reported method[4] to remove the need for intermediate purification. In addition, a microwave reactor was used to accelerate the synthesis. Acylation of anthranilic acid **2** with chloroacetyl chloride gave **3,** which was treated with aniline **4** to afford chloride **5** (Scheme 1). The chloride was reacted with amine **6** to give compounds **1**, **7–36**. All three steps were conducted in one-pot without

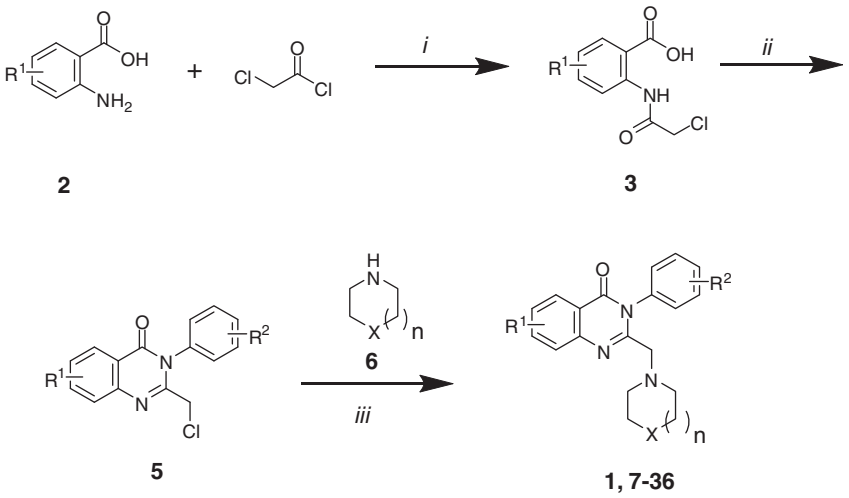


1

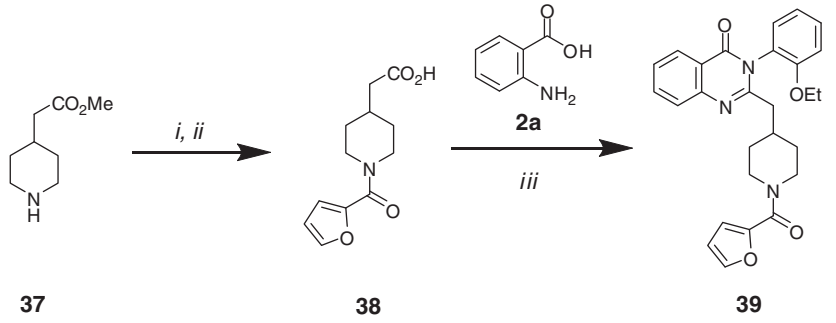
Figure 1. Optimization plan for NCGC00056044 (**1**).

* Corresponding author. Tel.: +1 301 217 5740; fax: +1 301 217 5736.
E-mail address: huangwe@mail.nih.gov (W. Huang).

0960-894X/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2011.07.043



Scheme 1. Reagents and conditions: (i) *i*PrNEt$_2$, ACN, rt; (ii) ArNH$_2$ (**4**), POCl$_3$, MW 150 °C, 15 min; (iii) K$_2$CO$_3$, EtOH, MW 150 °C, 5 min; then amine **6**, MW 150 °C, 10 min.



Scheme 2. Reagents and conditions: (i) *i*PrNEt$_2$, DCM, 2-furoyl chloride; (ii) LiOH; (iii) 2-ethoxyaniline, **2a**, pyridine, MW 230 °C, 10 min.

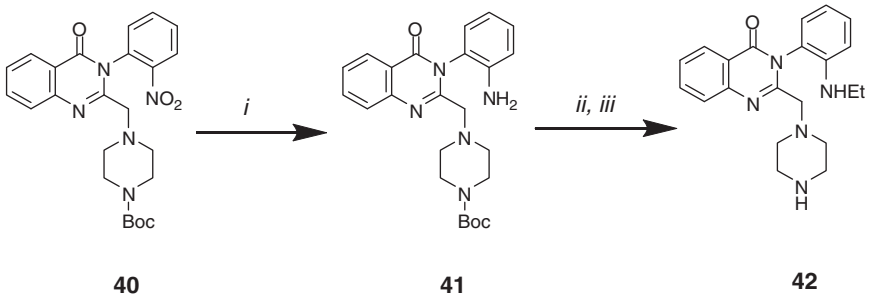
the need for intermediate isolation. This protocol was carried out in a parallel fashion to prepare the analogues which were purified via HPLC.[5]

Compound **39** was prepared as described in Scheme 2. Reaction of **37** with 2-furoyl chloride, followed by a hydrolysis reaction yielded acid **38**. The desired **39** was obtained via a microwave assisted one-pot three-component reaction of **38**, acid **2a**, and 2-ethoxyaniline.[6]

Scheme 3 describes the synthesis of the area C analogue **42.** Nitro-reduction of **40** gave **41**. Alkylation of the aniline nitrogen in **41** using ethyl iodide followed by a Boc-deprotection gave **42**.

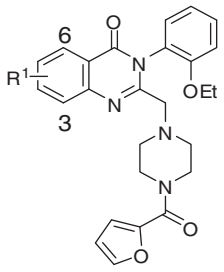
All analogues were evaluated in a cell-based HIF-1 mediated β-lactamase reporter gene assay under hypoxic conditions.[7] Area A showed little tolerance for substitution (Table 1). The C-6 methoxy (**7**), C-5 iodo (**9**), and C-4 and C-5 dimethoxy (**10**) substitutions were inactive. Compound **8** with a methyl group at C-6 was active, but it was 3-fold less potent than the original hit (**1**). Considering these results, our efforts focused on the optimization of areas B and C (Fig. 1).

Modification of the piperazine region B is shown in Table 2. Acetylation of N-4 (**11**) resulted in similar activity to the hit compound (**1**), but capping the piperazine nitrogen with a benzamide (**12**) or ethyl carbamate (**13**) resulted in a loss of activity. N-4 methylation (**14**) or benzylation (**15**) resulted in a 2-fold and 64-fold loss of activity respectively. Ultimately, the most active compound was the unsubstituted N-4 analogue (**16**), which was about 5-fold more potent than **1**. N-4 was critical for activity because when it was replaced with either a carbon (**19**) or oxygen (**18**), activity was lost.



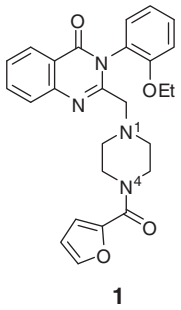
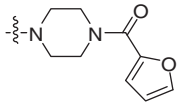
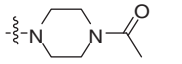
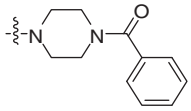
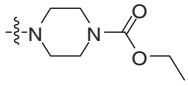
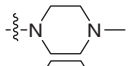
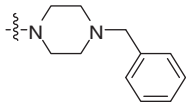
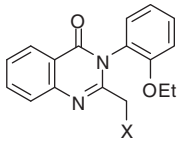
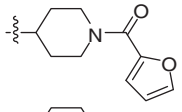
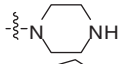
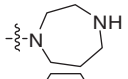
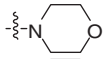
Scheme 3. Reagents and conditions: (i) $Na_2S_2O_4$; MW 100 °C, 10 min (ii) EtI, DMF, *i*Pr$_2$NEt, K$_2$CO$_3$, MW 150 °C, 15 min; (iii) DCM, TFA, rt 2 h.

Table 1
Modification at the R^1 position*

Structure	Entry	Compd	R^1	1% O_2 IC_{50} (μM)
	1	**1**	H	0.43
	2	**7**	6-Methoxy	Inactive
	3	**8**	6-Methyl	1.2
	4	**9**	5-Iodo	Inactive
	5	**10**	4,5-Dimethoxy	Inactive

* Values of IC_{50} are the mean of three independent experiments.

Table 2
SAR study for the piperazine region*

	Entry	Compd	X	1% O_2 IC_{50} (μM)
 **1**	1	**1**		0.43
	2	**11**		0.47
	3	**12**		1.7
	4	**13**		9.4
	5	**14**		0.81
	6	**15**		27.5
	7	**39**		27.6
	8	**16**		0.09
	9	**17**		0.16
	10	**18**		27.6
	11	**19**		Inactive

* Values of IC_{50} are the mean of three independent experiments.

In fact, both piperazine nitrogens were important because replacement of N-1 with a carbon (**39**) also resulted in a 40-fold decrease in activity. Finally, the piperazine ring was expanded to homopiperazine (**17**) and there was a slight loss in activity relative to **16**, but this analogue was still more potent than **1**.

The modification of area C was explored in Table 3. The first set of compounds was based on piperazine scaffold A (Table 3, entries 1–12) and there was almost no tolerance for substitution. The only moderately successful analogue was 2-OMe (**29**), but even this was 8-fold less active than **1**. Scaffold B presented a greater opportunity for SAR analysis (entries 13–20). Large alkoxy groups, such as benzyloxy (**33**), or iso-butyloxy (**34**) at C-2 resulted in significant loss of activity in comparison with ethoxy (**16**). Moving the methoxy group from the 2- to 4- position resulted in a complete loss of activity (**29** vs. **36**). A dramatic substitution effect was observed at the 5-position. Replacement of the nitro group (**32**) with a CF_3 (**31**) resulted in more than a 20-fold improvement in potency. Finally, by comparing **35**, **16**, and **42**, the ethoxy group appeared to be better than ethoxythio or ethylamine at the C-2 position.

To confirm HIF-1α inhibition, compounds **18** and **16** were evaluated in a Western blot analysis.[8] At 1 μM, **16** completely suppressed HIF-1α accumulation while **18** had no effect on the protein accumulation (Fig. 2). This result is in agreement with the compounds' activities observed in the cell-based assay.

Table 3
SAR study for R^2 position*

Scaffold	Entry	Compd	Scaffold	R^2	1% O_2 IC_{50} (μM)
	1	**20**	A	4-F	Inactive
	2	**21**	A	2-tBu	Inactive
	3	**22**	A	2-NO2	Inactive
	4	**1**	A	2-EtO	0.43
	5	**23**	A	H	Inactive
	6	**24**	A	2-Cl	Inactive
	7	**25**	A	2-Me	Inactive
	8	**26**	A	2-Benzyloxy	Inactive
	9	**27**	A	2-F	Inactive
	10	**28**	A	2-PhO	Inactive
Scaffold A					
	11	**29**	A	2-MeO	3.3
	12	**30**	A	2-MeO, 5-CF3	Inactive
	13	**31**	B	2-MeO, 5-NO2	0.2
	14	**32**	B	2-EtNH	4.2
	15	**42**	B	2-Benzyloxy	4.1
	16	**33**	B	2-Benzyloxy	22.9
	17	**34**	B	2-isobutoxy	6.2
Scaffold B					
	18	**35**	B	2-EtS	3.5
	19	**36**	B	4-MeO	Inactive
	20	**16**	B	2-EtO	0.09

* Values of IC_{50} are the mean of three independent experiments.

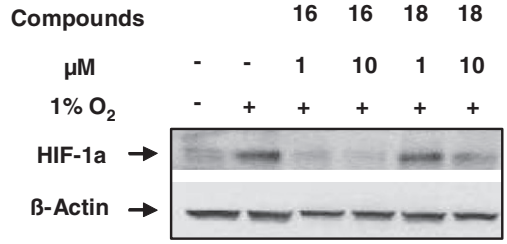


Figure 2. Effect of compounds **16** and **18** on the accumulation of the HIF-1α protein under hypoxia conditions.

However, compound **18** at 10 μM also inhibited HIF-1α protein accumulation. Stockwell and co-workers reported that these quinazolin-4-ones caused rapid death of human tumor cells (BJ-TERT/LT/ST/RAS^{V12} cells) via RAS-RAF-MEK dependent signaling.[9] Because Ras, a well known oncogene, has been shown to stimulate HIF-1α expression via the Raf/Mek/ERK pathway,[10] it is possible that the activity of these quinazolin-4-ones against HIF-1α accumulation is via the RAS signaling pathway.

In conclusion, we have identified a series of novel quinazolin-4-one HIF-1α inhibitors. A library synthesis and SAR studies revealed analogue **16** as the new lead, which was almost 5-fold more potent than the hit from the primary screen (**1**). The inhibition of HIF-1α was further confirmed in Western blot analyses. Detailed mechanistic studies and evaluation of these compounds as anti-cancer agents in rare types of cancer are currently under way and will be reported in due course.

Acknowledgments

We thank Paul Shinn and Danielle Van Leer for compound management, William Leister and Jeremy Smith for analytical chemistry. This research was supported by the Molecular Libraries Initiative of the National Institutes of Health Roadmap for Medical Research, National Institutes of Health.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.043.

References and notes

1. Weidemann, A.; Johnson, R. S. *Cell Death Differ.* **2008**, *15*, 621.
2. (a) Yewalkar, N.; Deore, V.; Padgaonkar, A.; Manohar, S.; Sahu, B.; Kumar, P.; Jalota-Badhwar, A.; Joshi, K.; Sharma, S.; Kumar, S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6426; (b) Park, S.-Y.; Jang, W.-J.; Yi, E.-Y.; Jang, J.-Y.; Jung, Y.; Jeong, J.-W.; Kim, Y.-J. *J. Pineal Res.* **2010**, *48*, 178; (c) Shimizu, K.; Maruyama, M.; Yasui, Y.; Minegishi, H.; Ban, H. S.; Nakamura, H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1453; (d) Narita, T.; Yin, S.; Gelin, C. F.; Moreno, C. S.; Yepes, M.; Nicolaou, K. C.; Van Meir, E. G. *Clin. Cancer Res.* **2009**, *15*, 6128; (e) Kasper, A. C.; Moon, E. J.; Hu, X.; Park, Y.; Wooten, C. M.; Kim, H.; Yang, W.; Dewhirst, M. W.; Hong, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3783; (f) Won, M.-S.; Im, N.; Park, S.; Boovanahalli, S. K.; Jin, Y.; Jin, X.; Chung, K.-S.; Kang, M.; Lee, K.; Park, S.-K.; Kim, H. M.; Kwon, B. M.; Lee, J. J.; Lee, K. *Biochem. Biophys. Res. Commun.* **2009**, *385*, 16; (g) Uno, M.; Ban, H. S.; Nakamura, H. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3166; (h) Diaz-Gonzalez, J. A.; Russell, J.; Rouzaut, A.; Gil-Bazo, I.; Montuenga, L. *Cancer Biol. Ther.* **2005**, *4*, 1055; (i) Harada, H.; Hiraoka, M. *Curr. Signal Transduction Ther.* **2010**, *5*, 188; (j) Mooring, S. R.; Wang, B. H. *Sci. China:Chem.* **2011**, 24; (k) Li, Y.; Ye, D. *Curr. Cancer Drug Tar.* **2010**, *10*, 782; (l) Semenza, G. *Drug Discovery Today* **2007**, 19; (m) DeBerardinis, R. J.; Lum, J. J.; Hatzivassiliou, G.; Thompson, C. B. *Cell Metab.* **2008**, 11; (n) Wang, X.-H.; Cavell, B. E.; Alwi, S. S.; Packham, G. *Biochem. Pharmacol.* **2009**, *78*, 261; Garcia-Maceira, P.; Mateo, J. *Oncogene* **2009**, *28*, 313; (o) Li, S. H.; Shin, D. H.; Chun, Y.-S.; Lee, M. K.; Kim, M.-S.; Park, J.-W. *Mol. Cancer Ther.* **2008**, *7*, 3729; (p) Greenberger, L. M.; Horak, I. D.; Filpula, D.; Sapra, P.; Westergaard, M.; Frydenlund, H. F.; Albaek, C.; Schroder, H.; Orum, H. *Mol. Cancer Ther.* **2008**, *7*, 3598; (q) Ban, H. S.; Yoshikazu, U.; Nakamura, H. *Expert Opin. Ther. Patents* **2011**, 131.
3. Xia, M.; Bi, K.; Huang, R.; Cho, M.-H.; Sakamuru, S.; Miller, S. C.; Li, H.; Sun, Y.; Printen, J.; Austin, C. P.; Inglese, J. *Mol. Cancer* **2009**, *8*, 117.
4. Becklin, R. R.; Chepanoske, C. L.; Pelter, J. M.; Qi, L.; Robbins, P. B.; Sahasrabudhe, S. R.; Selliah, R.; Simmons, K.; Stockwell, B. R.; Venkat, R. G.; Von Rechenberg, M.; Zhen, E. PCT Int. Appl., WO2006081331, 2006.

5. *General procedure for synthesis of compounds* **1**, **7–36**. To a solution of anthranilic acid **2** (0.40 mmol) and Hünig's base (0.60 mmol) in anhydrous acetonitrile (1.0 mL) was added a solution of chloroacetyl chloride (0.44 mmol) in acetonitrile (0.5 mL) at 4 °C. After stirring at rt for 2 h, $POCl_3$ (112 μL, 1.2 mmol, 3.0 equiv) was added followed by a solution of aniline (0.60 mmol) in acetonitrile (0.50 mL). The resulting mixture was heated in a microwave reactor at 150 °C for 15 min, cooled to rt and treated with K_2CO_3 (300 mg) and EtOH (1.0 mL). The mixture was heated at 150 °C for 5 min. To this mixture, a solution of amine (0.44 mmol) in EtOH (0.5 mL) was added. The mixture was heated in microwave for 10 min at 150 °C and the solid was filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative HPLC. Example: 3-(2-Ethoxyphenyl)-2-(piperazin-1-ylmethyl)quinazolin-4(3H)-one (**16**) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, J = 6.9 Hz, 3H), 2.40–2.53 (m, 2H), 2.60–2.72 (m, 2H), 2.91–3.09 (m, 4H), 3.27 (d, J = 14.3 Hz, 1H), 3.34 (d, J = 14.3 Hz, 1H), 3.96–4.17 (m, 2H), 6.99–7.15 (m, 2H), 7.22 (dd, J = 7.6, 1.4 Hz, 1H), 7.38–7.55 (m, 2H), 7.70–7.83 (m, 2H), 8.30 (d, J = 7.6 Hz, 1H); LCMS: R_t = 4.5 min, 98%; HRMS (ESI): m/z calcd for $C_{21}H_{24}N_4O_2$ $[M+1]^+$ 365.1983, found 365.1982.
6. Liu, J. F.; Lee, J. Y.; Dalton, A. M.; Bi, G.; Yu, L. B.; Baldino, C. M.; McElory, E.; Brown, E. *Tetrahedron Lett.* **2005**, 1241.
7. HRE β-lactamase reporter gene assay: HRE-bla ME-180 cells were dispensed at 2500 cells/6 μl/well in 1536-well black wall/clear bottom plates (Greiner Bio-One North America, Monroe, NC) using a Flying Reagent Dispenser (Aurora Discovery, CA). 23 nL of the compound were transferred to the assay plate using a pin tool (Kalypsys, San Diego, CA) resulting in a 261-fold dilution. The assay plates were incubated at 37 °C in hypoxia chamber (1% oxygen) for 17 h. After 1 μL of LiveBLAzer™ B/G FRET substrate (Invitrogen, Carlsbad, CA) mixture was added into the plates, the assay plates were incubated at room temperature for 2.5 h, and the fluorescence intensity (405 nm excitation, and 460 and 530 nm emissions) was measured by an Envision plate reader (Perkin Elmer, Shelton CT).
8. For western blot analysis, 1×10^6 HRE-bla ME-180 cells per well were cultured in a 6-well plate. After 6 h, the cells were treated for 17 h with DMSO in a normal atmosphere (negative control) and 1% oxygen (positive control), or with 1 or 10 μM solutions of **16** or **18** in DMSO at 1% oxygen. After the treatment, the cells were lysed in lysis buffer (Invitrogen) containing 1% of protease inhibitor (Sigma) for 10 min on ice. After the samples were centrifuged for 10 min at 14000 rpm and 4 °C, supernatants were collected and subjected to SDS–PAGE analysis on a 10% Tris–Glycine gel (Invitrogen). Same amount of protein (25 μg) from each sample was loaded into the gel. Proteins were transferred to a PVDF membrane (Invitrogen) and the membrane was blotted with primary antibodies against HIF-1α at a 1:250 dilution (Santa Cruz Biotechnologies, Santa Cruz, CA) or β-Actin at 1:5000 dilution (Sigma) as a loading control. HRP conjugated secondary antibody (1:1000 dilution; anti-goat anti-rabbit or anti-mouse, Santa Cruz, CA) was used together with the Western Blot Luminol reagent kit (Santa Cruz Biotechnology, CA) to develop the membrane that was read with the ChemiDoc XRS system (Bio-Rad, Hercules, CA).
9. (a) Yagoda, N.; von Rechenberg, M.; Zaganjor, E.; Bauer, A. J.; Yang, W. S.; Fridman, A. J.; Smukste, I.; Peltier, J. M.; Boniface, J. J.; Smith, R.; Lessnick, S. L.; Sahasrabudhe, S.; Stockwell, B. R. *Nature* **2007**, *447*, 864; (b) Yang, W. S.; Stockwell, B. R. *Chem. Biol.* **2008**, 234.
10. (a) Lim, J.; Lee, E.; You, H.; Lee, J. W.; Park, J.; Chun, J. *Oncogene* **2004**, 9427; (b) Blum, R.; Jacob-Hirsch, J.; Amariglio, N.; Rechavi, G.; Kloog, Y. *Cancer Res.* **2005**, 999.