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Discovery of (*S*)-4-isobutyloxazolidin-2-one as a novel leucyl-tRNA synthetase (LRS)-targeted mTORC1 inhibitor

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ABSTRACT

A series of leucinol analogs were investigated as leucyl-tRNA synthetase-targeted mTORC1 inhibitors. Among them, compound **5**, (*S*)-4-isobutyloxazolidin-2-one, showed the most potent inhibition on the mTORC1 pathway in a concentration-dependent manner. Compound **5** inhibited downstream phosphorylation of mTORC1 by blocking leucine-sensing ability of LRS, without affecting the catalytic activity of LRS. In addition, compound **5** exhibited cytotoxicity against rapamycin-resistant colon cancer cells, suggesting that LRS has the potential to serve as a novel therapeutic target.

Mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase, regulating various signaling processes that are crucial for cell survival, such as cell growth, proliferation, metabolism and autophagy.[1] mTOR forms two structurally distinct protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2); mTORC1 controls protein synthesis by sensing and integrating a wide range of extracellular and intracellular signals such as growth factors, nutrients, energy status, and various stressors, whereas mTORC2 regulates the cytoskeleton and metabolism.[2] Interestingly, amino acids, particularly leucine, regulate mTORC1 activity via specific mechanisms independent of other environmental signals.[3,4] Although how amino acids control mTORC1 activity has not been fully elucidated, the Rag family of GTPases appears to regulate mTORC1 in response to amino acid sufficiency.[5,6] More specifically, it has been proposed that leucyl-tRNA synthetase (LRS) activates the Rag GTPase in the presence of leucine, and promotes the lysosomal translocation of mTORC1 for activation.[7,8] It has been also demonstrated that certain leucine analogs, such as leucinol, inhibited leucine-induced mTORC1 activation,[9,10] probably by blocking leucine-sensing ability of LRS,[8] suggesting that LRS-targeted inhibitors can suppress mTORC1 activity.

Overactive mTORC1 is associated with many pathological conditions, including obesity, diabetes, neurodegenerative diseases, and cancers.[11–13] Rapamycin and its analogs, also called rapalogs, have been widely used to study underlying mechanisms of mTORC1 activation in the pathogenesis of these diseases.[14] While rapamycin is considered to be a highly selective allosteric inhibitor of mTORC1, recent studies indicate that it does not completely shut down all the effects of mTORC1, implying the existence of 'rapamycin-resistant' or 'rapamycin-insensitive' pathways.[15] One possible explanation for this phenomenon is that the kinase activity of mTORC1 changes depending on the nutrient and growth factor levels, therefore exhibiting differential sensitivity to rapamycin.[16] mTORC1 inhibitors that do not bind mTOR, but block other regulators such as LRS, would provide a powerful tool to study mTORC1 specific pathways and related disorders. In addition, since rapamycin resistance is regarded as one of the contributing factors to poor efficacy of rapalogs in cancer therapy,[17,18] these inhibitors have the potential to offer a new therapeutic option.

To develop LRS-targeted mTORC1 inhibitors, we first designed and synthesized a series of leucinol analogs. Leucinol **1** and compounds **2**–**12** were prepared by following the pathway described in Scheme 1. Compounds **2** and **6** were prepared by carbonylation or methylation from leucinol respectively, which was prepared from commercially available *N*-Cbz-leucine by 3 steps. Compounds

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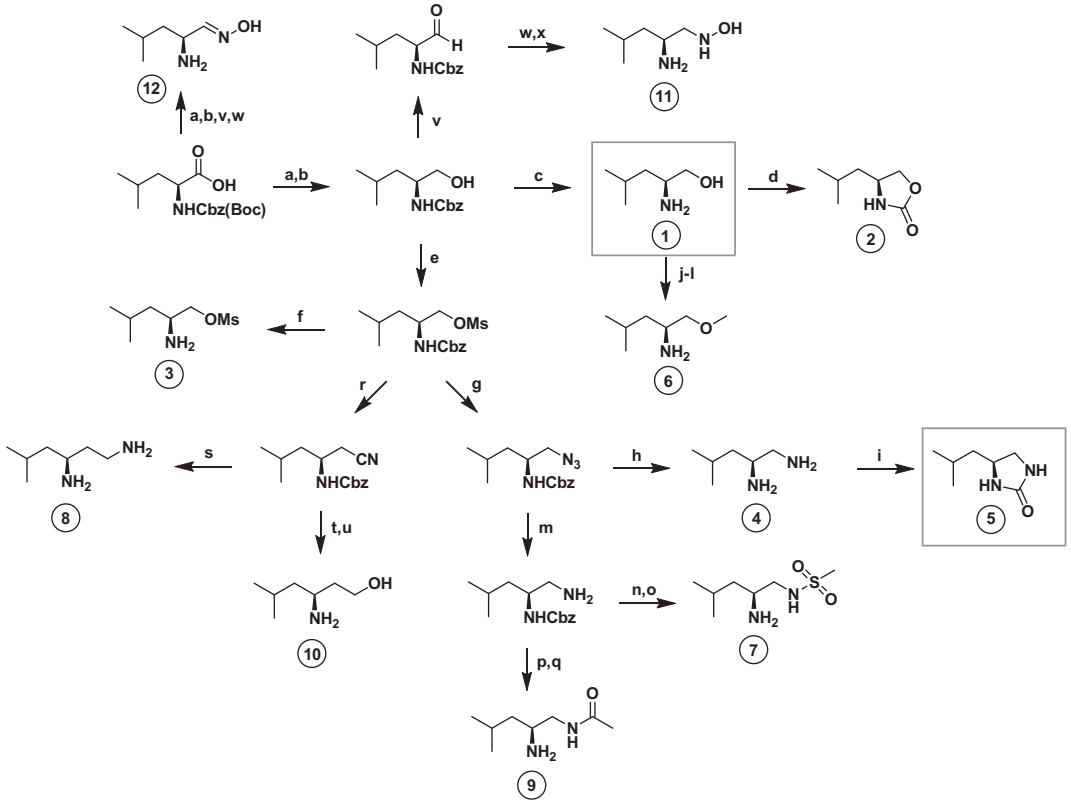
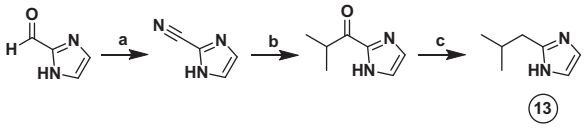


Scheme 1. Synthesis of leucinol analogs. *Reagents and conditions:* (a) cat. concd H_2SO_4, MeOH, reflux, 12 h, 99% for *N*-Cbz, 45% for *N*-Boc; (b) $NaBH_4$, EtOH, 0 °C → rt, 56% for *N*-Cbz, 55% for *N*-Boc; (c) Pd/C, H_2, MeOH, rt, overnight, 89%; (d) 1,1′-carbonyldiimidazole, DMF, rt, 12 h, 39%; (e) MsCl, TEA, MC, 0 °C → rt, 2 h, 90%; (f) 10% Pd/C, H_2, MeOH, rt, 12 h, 89%; (g) NaN_3, DMF, 80 °C, 4 h, 78%; (h) 10% Pd/C, H_2, MeOH, rt, 12 h, 33%; (i) 1,1′-carbonyldiimidazole, MC, rt, 12 h, 54%; (j) NaH, MeI, DMF, 0 °C → rt, 12 h; (k) benzyl chloroformate, K_2CO_3, acetonitrile, 0 °C → rt, 5 h, 65%; (l) 10% Pd/C, H_2, 2 M NH_3 in MeOH, rt, 12 h, 99%; (m) PPh_3, cat. H_2O, THF, rt, 12 h, 83%; (n) MsCl, TEA, MC, rt, 1 h, 89%; (o) 10% Pd/C, H_2, 2 M NH_3 in MeOH, rt, 12 h, 79%; (p) acetic anhydride, 80 °C, 12 h, 99%; (q) 10% Pd/C, H_2, 2 M NH_3 in MeOH, rt, 12 h, 98%; (r) NaCN, 15-crown-5, DMF, 60 °C, 48 h, 73%; (s) 10% Pd/C, H_2, MeOH, rt, 12 h, 70%; (t) 6 N HCl, reflux, 12 h, 99%; (u) BH_3·THF, THF, reflux, overnight, 15%; (v) Dess–Martin periodinane, MC, rt, 2 h, 61% for *N*-Cbz, 80% for *N*-Boc; (w) hydroxylamine hydrochloride, Na_2CO_3, MeOH, rt, 12 h, 66% for *N*-Cbz, 67% for *N*-Boc; (x) 10% Pd/C, H_2, MeOH, rt, 12 h, 86%.

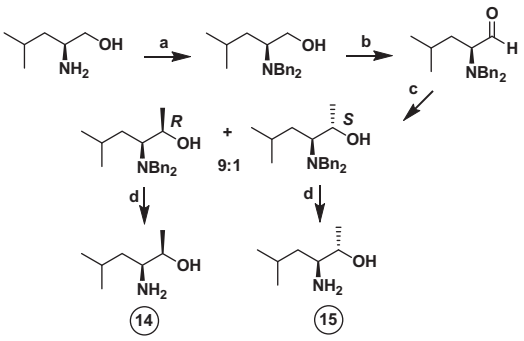
3, **8**, and **10** were synthesized from the mesylated product of *N*-Cbz-leucinol; compound **3** was obtained via deprotection of the *N*-Cbz group, and compounds **8** and **10** were prepared by displacing the mesylate with a nitrile group followed by reduction or reductive hydrolysis respectively. Displacement of the mesylate with sodium azide yielded an azido intermediate, which was then used to prepare compounds **4**, **5**, **7**, and **9**. Hydrogenation in the presence of 10% palladium afforded diamine compound **4**, and carbonylation of compound **4** yielded a cyclic analog, compound **5**; selective reduction of the azide produced the *N*-Cbz protected monoamine intermediate, and subsequent methanesulfonylation or acylation, followed by deprotection produced compound **7** and **9** respectively. To prepare compound **11**, *N*-Cbz protected leucinol was oxidized to aldehyde by Dess–Martin periodinane, and subsequent condensation with hydroxylamine, followed by deprotection yielded compound **11**. Compound **12** was prepared from *N*-Boc protected leucine by applying the same procedure carried out for compound **11.**

Compound **13** was synthesized from commercially available imidazole-2-carboxaldehyde (Scheme 2); the aldehyde group was converted to a nitrile group which was then reacted with isopropyl magnesium chloride to produce an imidazole intermediate containing an isobutyl ketone group. The ketone group was completely reduced by using Ni–Al catalyst to generate compound **13**.

Leucinol analogs with a chiral α-methyl group were synthesized by following the pathway described in Scheme 3. *N*-Dibenzyl protected leucinol was oxidized to aldehyde for subsequent Grignard reaction, providing a racemic alcohol mixture of *R*- and *S*-isomers



Scheme 2. Synthesis of imidazole analog of leucinol. *Reagents and conditions:* (a) i) hydroxylamine hydrochloride, pyridine, rt, 2 h, ii) acetic anhydride, 80 °C → rt, 5 h, 80%; (b) isopropylmagnesium chloride, THF, 0 °C → rt, 3 h, 77%; (c) Ni–Al alloy, H_2O, reflux, 48 h, 50%.



Scheme 3. Synthesis of α-methyl analogs of leucinol. *Reagents and conditions:* (a) benzyl bromide, K_2CO_3, aq MeOH, 65 °C, 2 h, 41%; (b) oxalyl chloride, DMSO, TEA, MC, −78 °C, 2 h; (c) CH_3MgI, ether, 0 °C → rt, 3 h, 60% (*R*:*S* = 9:1); (d) 10% Pd/C, H_2, MeOH. rt, overnight, 62% for **14**, 47% for **15**.

with a ratio of 9:1. Final debenzylation of purified enantiomers yielded compounds **14** (*R*-isomer) and **15** (*S*-isomer). The stereochemistry of each compound was assigned based on the previous report.[19]

As a primary screening, we first assessed the effect of each compound on leucine-induced phosphorylation of S6 Kinase (S6K) by immunoblotting. S6K is one of the mTORC1 substrates, and pretreatment with leucinol appears to block phosphorylation of S6K by inhibiting LRS.[8] We treated HEK293 cells with each compound at 1 mM concentration, along with rapamycin as a control at 100 nM; and then activated mTORC1 by treating cells with leucine for 10 min. As demonstrated in Figure 1, leucine treatment induces phosphorylation of S6 K, whereas pretreatment with rapamycin or deprivation of leucine blocked the phosphorylation. Pretreatment of leucinol also inhibited the phosphorylation to some extent, while compounds, **2**, **5**, **11**, and **13** appeared to inhibit the phosphorylation more effectively than leucinol at the same concentrations. It is interesting to note that all four compounds have multiple heteroatoms that can participate in additional hydrogen bond interactions compared to leucinol. It is also noticeable that compounds **2**, **5**, and **13** are all cyclic analogs. Introduction of an additional methylene group or chiral α-methyl group did not generate any desirable activity. Addition of a relatively bulky functional group such as methanesulfonyl, mesyl, and acetyl group at the alcohol position also do not produce inhibitory effects, suggesting that leucine binding site is highly specific and only afford a small sized residue.

Based on the primary screening results, we decided to focus on compounds **2**, **5**, **11**, and **13**, and further determined the inhibitory effects on leucine-induced mTORC1 activation at the various concentrations by immunoblotting. As shown in Figure 2, all four compounds inhibited S6K phosphorylation in a dose-dependent manner while their apparent potency decreases in the order of **5** > **13** > **11** > **2**. The cyclic analogs, compounds **5** and **13** appear to be more potent than leucinol, however, another cyclic derivative

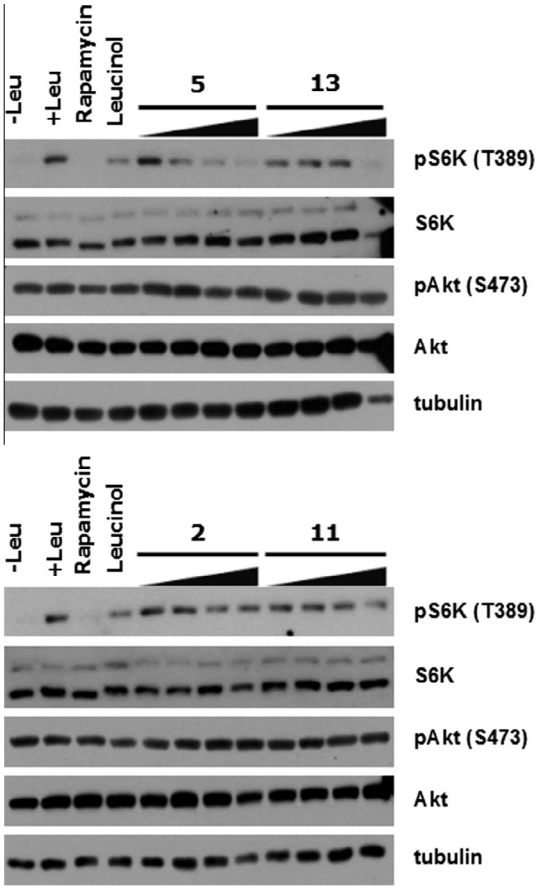


Figure 2. Dose-dependent inhibition of LRS-mediated mTORC1 activation of selected leucinol derivatives on HEK293 cells. Rapamycin was treated at 100 nM, leucinol was at 800 μM, and compounds **2**, **5**, **11**, and **13** were treated at 100, 200, 400, and 800 μM.

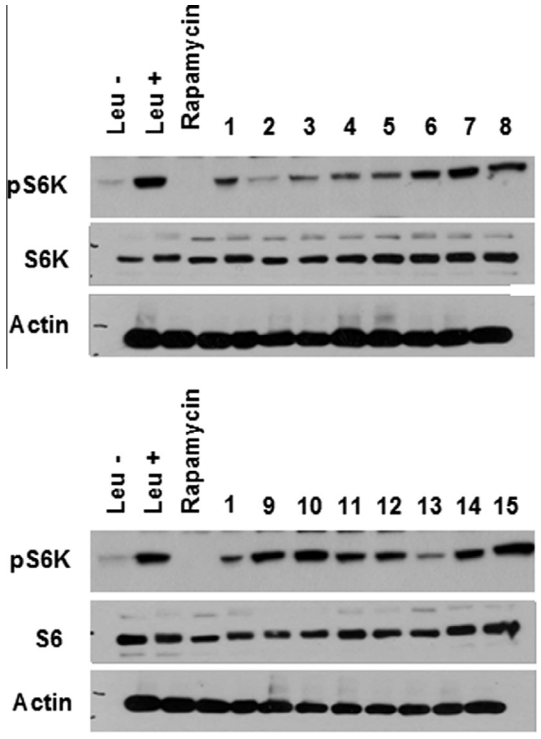


Figure 1. Inhibition of LRS-mediated mTORC1 activation of synthesized leucinol analogs on HEK293 cells.

compound **2** only differs from compound **5** by one atom, and yet exhibits significantly lower activity. Additionally, we examined the effect of each compound on mTORC2 activation by using Akt as the marker.[1] All tested compounds including rapamycin and leucinol do not affect the cellular levels of Akt or phosphorylated Akt (pAkt) indicating that biological effects of these compounds are indeed mTORC1 specific.

To investigate the mechanisms of leucine analogs in LRS-mediated mTORC1 activation, we examined the effects of compounds **5** and **13** on the catalytic activity of LRS. More specifically, since LRS catalyzes leucylation in the presence of tRNA, we carried out leucylation assays on these compounds. As shown in Figure 3, both compounds **5** and **13** are poor inhibitors of aminoleucylation, having IC_{50} values in the high millimolar range. Given that both compounds inhibited phosphorylation of S6K at micromolar concentrations, our result indicates that these leucine analogs do not affect catalytic activity of LRS, which is in agreement with the previously reported observation that LRS activates mTORC1 signaling via leucine recognition without involving tRNA charging.[8]

Since mTORC1 is found to be overactive in many cancers, we evaluated cytotoxicity of compound **5** in cancer cells. In particular, mTORC1 is reported to be hyperactive in human colorectal cancer,[20] however, rapamycin appears to be mostly ineffective.[21] We selected a human colon cancer cell line (SW620) which is known to be rapamycin resistant,[22] to see whether blocking LRS can solve the resistance issue. After treating cells with each compound, we performed cytotoxicity assays by using CellTox Green fluorescent dye which stains the DNA from dead cells (Fig. 4). As

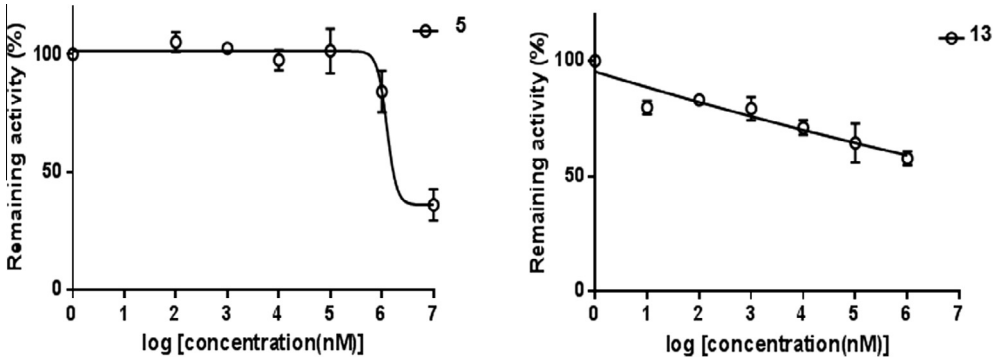


Figure 3. Aminoacylation activity of compounds **5** and **13**.

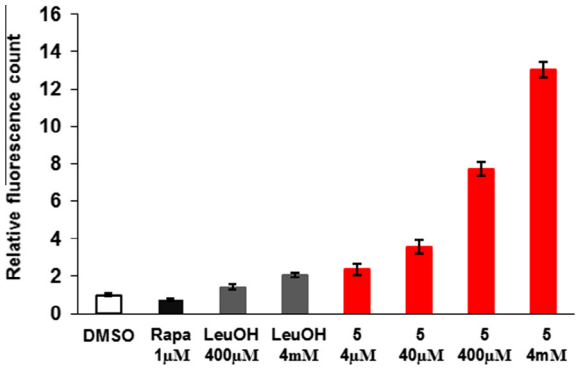


Figure 4. Cytotoxicity of compound **5** in rapamycin-resistant colon cancer cell (SW620).

we expected, rapamycin did not exhibit significant cytotoxicity while leucinol seemed to be marginally cytotoxic even at the high concentrations; however, compound **5** demonstrated a dose-dependent cytotoxicity, exerting a much greater magnitude of cytotoxic effects at the same concentrations compared to leucinol. Considering that compound **5** was more potent inhibitor of mTORC1 than leucinol, cytotoxicity of compound **5** could be attributed to mTORC1 inhibition.

In summary, we have developed a series of leucinol analogs that can inhibit mTORC1 signaling pathway by blocking leucine-sensing ability of LRS. These analogs act as a competitive inhibitor of leucine in LRS-mediated activation of mTORC1, thereby inhibiting downstream phosphorylation of S6K. In vitro mTORC1 inhibition study showed that compound **5**, which contains an imidazolidin-2-one side chain, most effectively inhibited leucine-mediated mTORC1 activation among them; however, compound **5** did not suppress mTORC2 activation, nor did it affect catalytic activity of LRS. Furthermore, compound **5** exhibited much higher cytotoxicity than leucinol against rapamycin-resistant colon cancer cells, implying that effective inhibition of leucine-sensing in the mTORC1 pathway may play an important role in conferring cytotoxicity. Taken together, we believe that the blockade of leucine-sensing ability of LRS in the mTORC1 pathway has the potential to serve as a novel therapeutic target.

Acknowledgment

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References and notes

1. Laplante, M.; Sabatini, D. M. *Cell* **2012**, *149*, 274.
2. Wullschleger, S.; Loewith, R.; Hall, M. N. *Cell* **2006**, *124*, 471.
3. Hara, K.; Yonezawa, K.; Weng, Q. P.; Kozlowski, M. T.; Belham, C.; Avruch, J. *J. Biol. Chem.* **1998**, *273*, 14484.
4. Shimobayashi, M.; Hall, M. N. *Cell Res.* **2016**, *26*, 7.
5. Kim, E.; Goraksha-Hicks, P.; Li, L.; Neufeld, T. P.; Guan, K. L. *Nat. Cell Biol.* **2008**, *10*, 935.
6. Sancak, Y.; Peterson, T. R.; Shaul, Y. D.; Lindquist, R. A.; Thoreen, C. C.; Bar-Peled, L.; Sabatini, D. M. *Science* **2008**, *320*, 1496.
7. Sancak, Y.; Bar-Peled, L.; Zoncu, R.; Markhard, A. L.; Nada, S.; Sabatini, D. M. *Cell* **2010**, *141*, 290.
8. Han, J. M.; Jeong, S. J.; Park, M. C.; Kim, G.; Kwon, N. H.; Kim, H. K.; Ha, S. H.; Ryu, S. H.; Kim, S. *Cell* **2012**, *149*, 410.
9. Lynch, C. J.; Fox, H. L.; Vary, T. C.; Jefferson, L. S.; Kimball, S. R. *J. Cell. Biochem.* **2000**, *77*, 234.
10. Wang, X.; Fonseca, B. D.; Tang, H.; Liu, R.; Elia, A.; Clemens, M. J.; Bommer, U. A.; Proud, C. G. *J Biol Chem* **2008**, *283*, 30482.
11. Bove, J.; Martinez-Vicente, M.; Vila, M. *Nat. Rev. Neurosci.* **2011**, *12*, 437.
12. Chiang, G. G.; Abraham, R. T. *Trends Mol. Med.* **2007**, *13*, 433.
13. Zoncu, R.; Efeyan, A.; Sabatini, D. M. *Nat. Rev. Mol. Cell Biol.* **2011**, *12*, 21.
14. Chiarini, F.; Evangelisti, C.; McCubrey, J. A.; Martelli, A. M. *Trends Pharmacol. Sci.* **2015**, *36*, 124.
15. Thoreen, C. C.; Sabatini, D. M. *Autophagy* **2009**, *5*, 725.
16. Kang, S. A.; Pacold, M. E.; Cervantes, C. L.; Lim, D.; Lou, H. J.; Ottina, K.; Gray, N. S.; Turk, B. E.; Yaffe, M. B.; Sabatini, D. M. *Science* **2013**, *341*, 1236566.
17. Guertin, D. A.; Sabatini, D. M. *Cancer Cell* **2007**, *12*, 9.
18. Sabatini, D. M. *Nat. Rev. Cancer* **2006**, *6*, 729.
19. Reetz, M. T.; Drewes, M. W.; Schmitz, A. *Angew. Chem.* **1987**, *99*, 1186.
20. Zhang, Y.-J.; Dai, Q.; Sun, D.-F.; Xiong, H.; Tian, X.-Q.; Gao, F.-H.; Xu, M.-H.; Chen, G.-Q.; Han, Z.-G.; Fang, J.-Y. *Ann. Surg. Oncol.* **2009**, *16*, 2617.
21. Francipane, M. G.; Lagasse, E. *Oncotarget* **2014**, *5*, 49.
22. Zhang, Y.; Zheng, X. S. *Cell Cycle* **2012**, *11*, 594.