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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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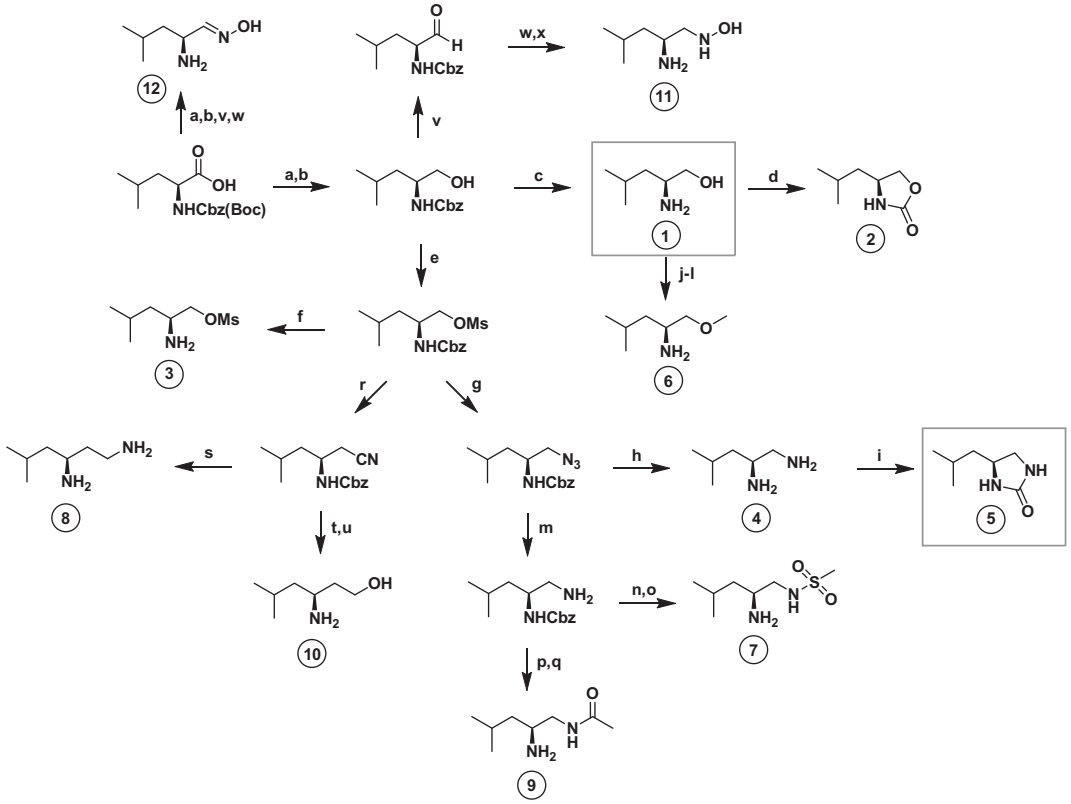
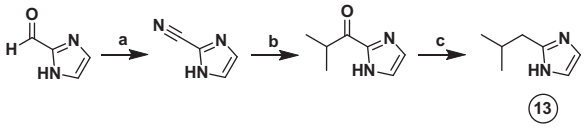


Scheme 1. Synthesis of leucinol analogs. *Reagents and conditions:* (a) cat. concd H_2SO_4, MeOH, reflux, 12 h, 99% for *N*-Cbz, 45% for *N*-Boc; (b) $NaBH_4$, EtOH, 0 °C → rt, 56% for *N*-Cbz, 55% for *N*-Boc; (c) Pd/C, H_2, MeOH, rt, overnight, 89%; (d) 1,1′-carbonyldiimidazole, DMF, rt, 12 h, 39%; (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%.

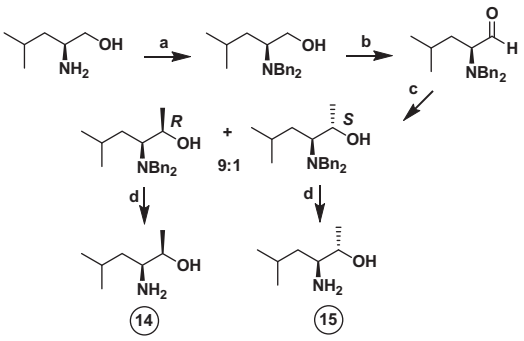
3, **8**, and **10** were synthesized from the mesylated product of *N*-Cbz-leucinol; compound **3** was obtained via deprotection of the *N*-Cbz group, and compounds **8** and **10** were prepared by displacing the mesylate with a nitrile group followed by reduction or reductive hydrolysis respectively. 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**.