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Synthesis and anticancer activity of novel rapamycin C-28 containing triazole moiety compounds

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Abstract

Rapamycin is an mTOR allosteric inhibitor with multiple functions such as immunosuppressive, anticancer, and lifespan prolonging activities. Its C-43 semi-synthetic derivatives temsirolimus and everolimus have been used as mTOR targeting anticancer drugs in the clinic. Following our previous research on antitumor rapalogs modified on the C-43 position, 13 novel rapamycin triazole hybrids (**6a–g**, **7a–f**) were designed and synthesized on the C-28 position of rapamycin via Huisgen's reaction. Anticancer assays indicated that the targeted derivatives containing phenyl and 4-methylphenyl groups showed an obvious raise in anticancer activity. On the contrary, the compounds with methoxyl, amine, and halogen groups on the benzene ring displayed lower anticancer activity. Compound **6c**, as the most active compound, showed a stronger inhibition effect as compared with rapamycin for almost all of the tested cell lines ($p < 0.01$), except PC-3. Meanwhile, the effect of **6c** on inducing apoptosis and cell cycle arrest in A549 cells was more powerful than that of rapamycin. In addition, **6c** inhibited the phosphorylation of mTOR and its downstream key kinases 4EBP1 and p70S6K1 in A549 cells, indicating that **6c** also effectively inhibits the mTORC1 signaling pathway as rapamycin. On the basis of these findings, **6c** may have the potential to be developed as a new mTOR inhibitor against specific cancers.

KEYWORDS
anticaner activity, rapamycin, semi-synthesis, triazole

1 | INTRODUCTION

The mTOR (mammalian target of rapamycin) inhibitor rapamycin (also known as sirolimus), a nitrogen-containing 36-membered macrolide, was discovered as an antifungal antibiotic produced by *Streptomyces hygroscopics* AY-994 in a soil sample from Easter Island in 1975,[1,2] it combines with FKBP12 (FK506 binding protein 12) to form a rapamycin-FKBP12 complex which inhibits mTORC1 pathway by indirect binding to the FRB domain of mTOR.[3,4] As a potent mTOR inhibitor, rapamycin has been developed as a multifunctional drug with antifungal, immunosuppressive, anti-proliferative, antitumor activities,[1,5–10] and recently, found to improve cardic function,[11,12] slow aging as well as prolong mouse lifespan.[13–16] Correspondingly, FDA approved rapamycin as an immunosuppressant indicated for the prophylaxis of organ rejection in patients receiving renal transplants, as drug-eluting stents coating

to prevent coronary restenosis following balloon angioplasty as well as an mTOR targeted antitumor drug for the treatment of patients with lymphangioleiomyomatosis.

A number of semi-synthetic derivatives of rapamycin have been developed and some of them are *in clinic*. Temsirolimus, made from rapamycin by acylation of the C-43, was approved as the first mTOR target antitumor drug for advanced renal cell carcinoma.[17] Then, everolimus, *O*-alkylation in position C-43 of rapamycin, is used not only as a mTOR target drug to treat advanced renal cell carcinoma, neuroendocrine tumors, renal angiomyolipoma as well as breast cancer, etc. *in clinic*,[18–20] but also as an immunosuppressant and stent coating. Other two rapalogs, biolimus A9 and zotarolimus, ethoxyethyl ether and tetrazole derivatives of rapamycin, respectively, were also approved as stent coating. It is observed that all rapamycin analogs (rapalogs) applied *in clinic* are modified at position C-43 of rapamycin (Figure 1).

In our previous work, rapamycin was chemically modified in the position C-43 and a number of new rapalogs with antitumor activities were obtained. Among these new rapalogs, rapamycin triazole derivative **9e**[21] and thiazole derivative **9b**[22] etc. exhibited stronger antitumor activities compared with rapamycin.[23] To obtain more potent rapalogs and further explore their structure–activity relationship, we tried to modify the structure of rapalogs we had reported before[21] by migrating side chain moiety from the C-43 position to C-28 position as showed in Figure 2. It may open a new structural space if we can develop more potent rapalogs modified at C-28 position. Prompted by the aforementioned findings, in the current study, we hereby report the synthesis and biological evaluation of novel rapalogs incorporating triazole moiety at C-28 position. Besides, the mechanism of action for **6c** was also preliminary elucidated.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The synthesis of the C-28 triazole-based rapalogs was accomplished according to the synthetic protocol showed in Scheme 1. As we know that rapamycin contains two secondary-hydroxyl groups at positions C-28 and C-43, it should be difficult to achieve a selective synthesis of C-28 esters, but Nan et al.[24] had already overcome these difficulties by providing a region-selective synthesis of 28-esters of rapamycin. Therefore, we would like to use the same method to synthesize target rapalogs which were modified with 28-esters linkage. First of all, rapa-28-OTMS (**1**) was obtained by treating rapamycin with trimethylsilyl chloride, imidazole, and H_2SO_4 at 0°C for 12 h. Following, compound **2** was synthesized by mixing **1** with *tert*-butyldimethylsilyl chloride, imidazole at 6°C for 5 h. Secondly, compound **2** was treated with bromoacetyl bromide using pyridine in DCM, which subsequently reacted with 2 N H_2SO_4 in acetone to afford intermediate **4** as white solids. The reaction of bromide **4** with sodium azide resulted in azido-rapamycin (**5**) yields of 40.3%. Finally, the targeted compounds **6a–g**, **7a–f**, were obtained by Huisgen's reaction that **5** underwent cycloaddition with 1-ethynyl-substituted benzene, propargyl alcohol, 1-diethylamino-2-propyne, using CuI as catalyzer. All intermediates and targeted compounds were purified by column chromatography on silica gel and the chemical structures were confirmed by IR, MS, HRMS, ^{1}H NMR and ^{13}C NMR, which would be explained in Section 4.

2.2 | Biological activity

2.2.1 | Novel rapalogs modified in the position C-28 of rapamycin possessed effect against cancer cells proliferation

As shown in Table 1, most of the tested compounds were more potent than rapamycin against A549 cells, and all of targeted compounds showed less potency for PC-3 cell line when compared with rapamycin. Compound **6c**, with a 4-methylphenyl moiety on the triazole ring, as the most active compound, showed stronger inhibition effect as compared with rapamycin on all of tested cell lines ($p < 0.01$), except PC-3. Among the targeted derivatives containing different substituted benzene group on the triazole ring, the compounds **6a** (H), **6b** (3-Me), and **6c** (4-Me) displayed more potent activity than rapamycin against most of tested cell lines. On the contrary, compounds **6d** (2-Cl),

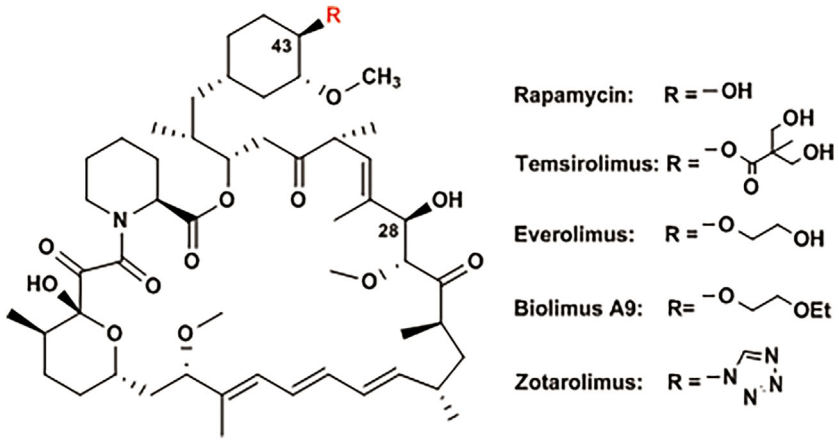


FIGURE 1 Structure of rapamycin and analogs

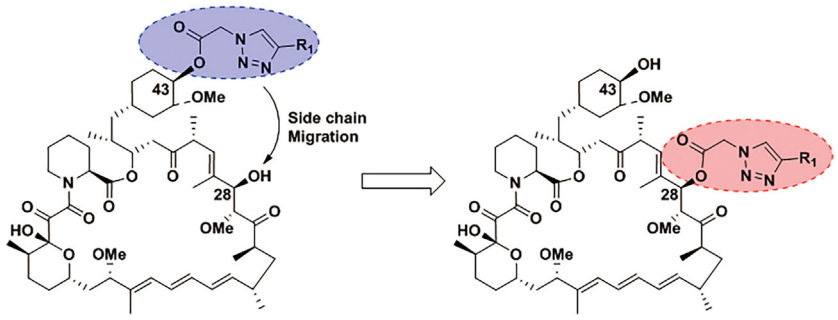
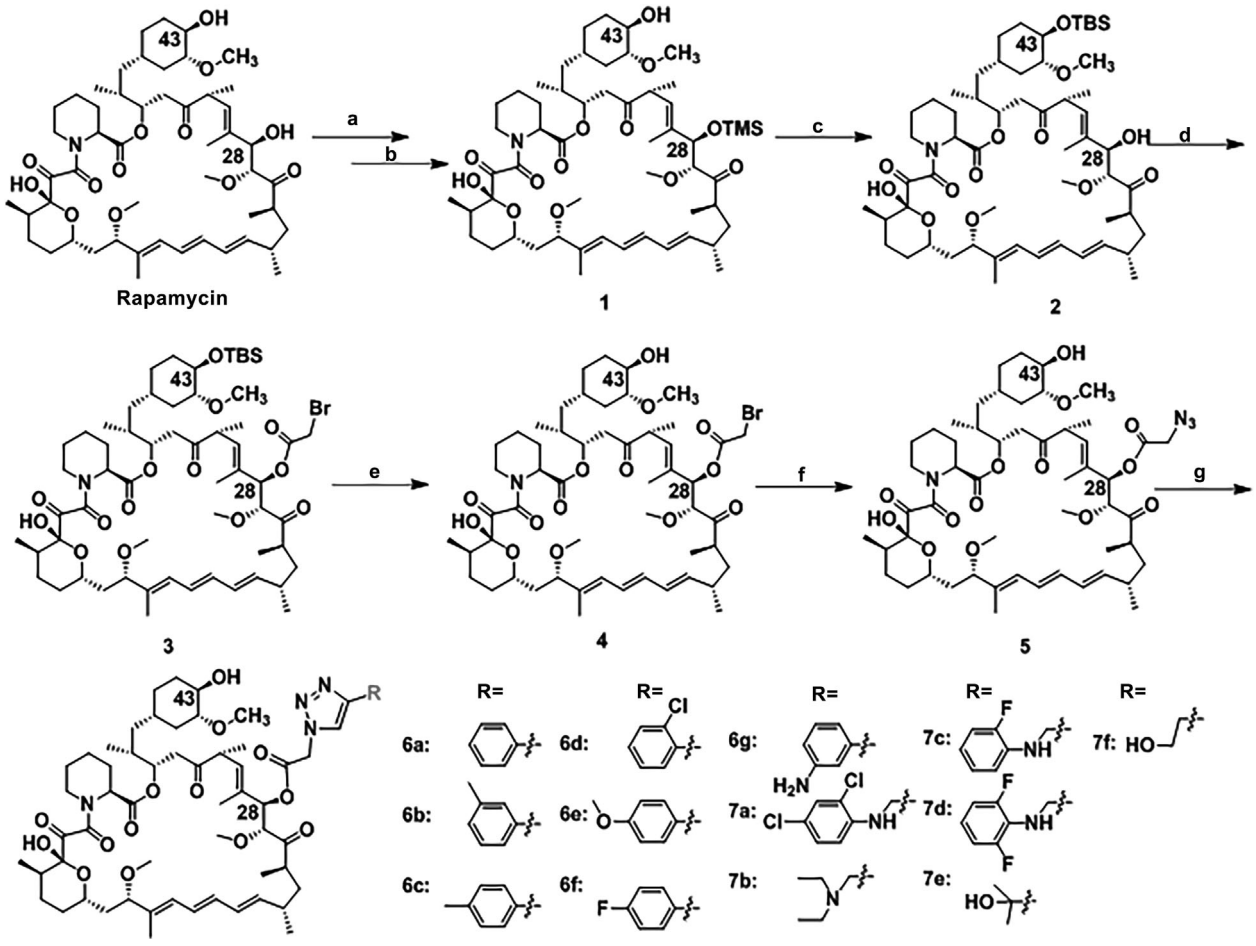


FIGURE 2 Design of the target compounds

6e (4-MeO), **6f** (4-F), and **6g** (3-NH_2) showed lower activity. These results implied that the different substituted groups on the benzene group may be highly related with their activity. Interestingly, it is worth to mention that the introduction of aryl-amino-methyl group on triazole ring, such as **7a**, **7c**, and **7d**, also led to an obvious raise in anticancer activity while hydroxyl-alkyl (**7e** and **7f**) caused anticancer potency to be lowered significantly. More interestingly, we had reported the synthesis and antitumor activities of C-43 triazole-based rapamycin derivatives, in which the side chain of compound **4a**, **4b**, **5e**[21] were almost the same with **6c**, **6f**, **7a**, respectively, but the anticancer activities of **4a**, **4b**, **5e** were much weaker, indicating that the anticancer activities of the rapalogs



SCHEME 1 Syntheses of compounds **6a–f** and **7a–f**. Reagents and conditions: (a, b) TMSCl, 0.5 N H_2SO_4, EA, 0°C; (c) TBSCl, imidazole, DMF, 6°C; (d) bromoacetyl bromide, pyridine, DCM, −2°C; (e) 1 N H_2SO_4, acetone, 0°C; (f) NaN_3, KI, DMF, 55°C; (g) CuI, DIPEA, acetonitrile, 25°C

TABLE 1 Anti-proliferative IC_{50} of rapalogs on human cancer cell lines

	IC_{50}[a] (μM), mean ± SD				
Compound no.	A549[b]	PC-3[b]	CASKI[b]	769-P[b]	ECA-109[b]
6a	14.2 ± 0.7**	15.9 ± 0.6	16.2 ± 0.3	16.7 ± 0.5**	19.2 ± 0.4**
6b	14.6 ± 0.3**	14.7 ± 0.1	14.2 ± 0.4*	15.4 ± 0.2**	18.6 ± 0.2**
6c	12.8 ± 0.4**	13.1 ± 0.4	13.1 ± 0.4**	13.9 ± 0.4**	14.8 ± 0.4**
6d	18.5 ± 0.5	20.6 ± 0.4	21.1 ± 0.4	24.3 ± 0.4	23.4 ± 0.4
6e	17.1 ± 0.4	17.4 ± 0.4	27.1 ± 0.4	28.3 ± 0.4	29.1 ± 0.4
6f	16.1 ± 0.4*	n.d.[c]	n.d.[c]	25.3 ± 0.4	n.d.[c]
6g	17.6 ± 0.2	17.1 ± 0.5	25.2 ± 0.2	25.3 ± 0.7	23.2 ± 0.5*
7a	n.d.[c]	15.1 ± 0.2	20.7 ± 0.3	n.d.[c]	20.4 ± 0.1*
7b	12.4 ± 0.8**	16.4 ± 0.8	22.9 ± 0.8	23.8 ± 0.8	22.3 ± 0.8
7c	12.7 ± 0.8**	15.8 ± 0.8	20.3 ± 0.8	22.4 ± 0.8**	22.9 ± 0.8
7d	12.8 ± 0.5**	16.5 ± 0.7	18.3 ± 0.4*	21.3 ± 0.4**	21.6 ± 0.4
7e	17.3 ± 0.4	18.1 ± 0.8	30.4 ± 0.8	34.4 ± 0.8	31.2 ± 0.8
7f	17.9 ± 0.8	17.8 ± 0.8	36.1 ± 0.8	37.5 ± 0.8	>50
Rapamycin	18.1 ± 0.6	12.3 ± 0.7	15.2 ± 0.4	24.5 ± 0.6	21.8 ± 0.4

[a]IC_{50} was tested by the SRB assay. Experiments were performed in triplicate and the results were presented as average values. *$p < 0.05$, **$p < 0.01$.
[b]A549, human non-small cell lung cancer cell line; PC-3, human prostatic cancer cell line; CASKI, human cervical cancer line; 769-P, human renal carcinoma cell line; and ECA-109, human esophageal cancer cell line, were employed to test the anticancer activities of new rapalogs.
[c]n.d.: not determined.

modified on C-28 position were stronger than that of the rapalogs modified on C-43 position.

2.2.2 | 6c induced apoptosis in A549 cells

To determine whether **6c**-induced cell death was related to induction of apoptosis, A549 was treated with **6c** in different concentration for 48 h and Annexin V staining assay was performed with flow cytometry. As displayed in Figure 3, similar to rapamycin, **6c** showed the action on inducing A549 cells apoptosis in dose-dependent manner. As the concentration of which from 10 to 20 μM, the apoptosis rate of A549 increased from 3.83 to 7.97%. At the same concentration, the apoptosis rate of A549 by **6c** (4.05%) was higher than that by rapamycin (3.54%). It indicated that the effect of **6c** was stronger than of rapamycin in inducing cell apoptosis.

2.2.3 | 6c induced cell cycle arrest in G1 phase in A549

To further investigate whether the growth inhibitory effect of **6c** on A549 was partly due to cell cycle arrest, A549 was exposed to various concentrations of **6c** for 48 h and the DNA content of cells was measured by flow cytometry. As showed in Figure 4 and Table 2, **6c** caused accumulation of A549 in the G1 phase of cell cycle in dose-dependent manner. At the same concentration of 15 μM, **6c** exhibited more effect on arrest of A549 in the G1 phase of cell cycle than that of rapamycin.

2.2.4 | 6c blocked mTOR signaling pathway in A549

mTOR pathway is an important driver of tumor development and progression. Western blot analysis showed that **6c** inhibited the phosphorylation of mTOR at Ser 2448 which is an important phosphorylation site for mTOR activity. Furthermore, the phosphorylation of mTOR major downstream 4E-BP1 and p70S6K1 were significantly decreased by **6c** in dose-dependent manner (Figure 5). These results indicated that **6c** repressed tumor cell growth by blocking mTOR signaling pathway, which was same as rapamycin.

2.2.5 | Binding mode analysis

To further elucidate the binding mode of compounds, docking analysis was performed by means of the MOE software and a proposed binding mode was obtained. As shown in Figure 6, detailed analysis of the interactions revealed the following features. First, three carbonyl oxygen atoms of lactone in **6c** formed three hydrogen-bonding interactions with IleA56, TyrA82, and LysA52, respectively. Meanwhile, one hydrogen bond formed between the hydroxyl group of hemiacetal and AspA37. In addition, methylene (—CH2—) of side chain in **6c** formed hydrogen bond with GlnA53. Second, the triene moiety fitted into the hydrophobic pocket that was formed by LeuB2031, TrpB2101, Phe2108, etc. Notably, the benzene group of side chain was almost out of docking pocket, thus it may explain the similar activity for these series derivatives because varied substituted groups on the benzene ring would not have great positive effect for the binding result.

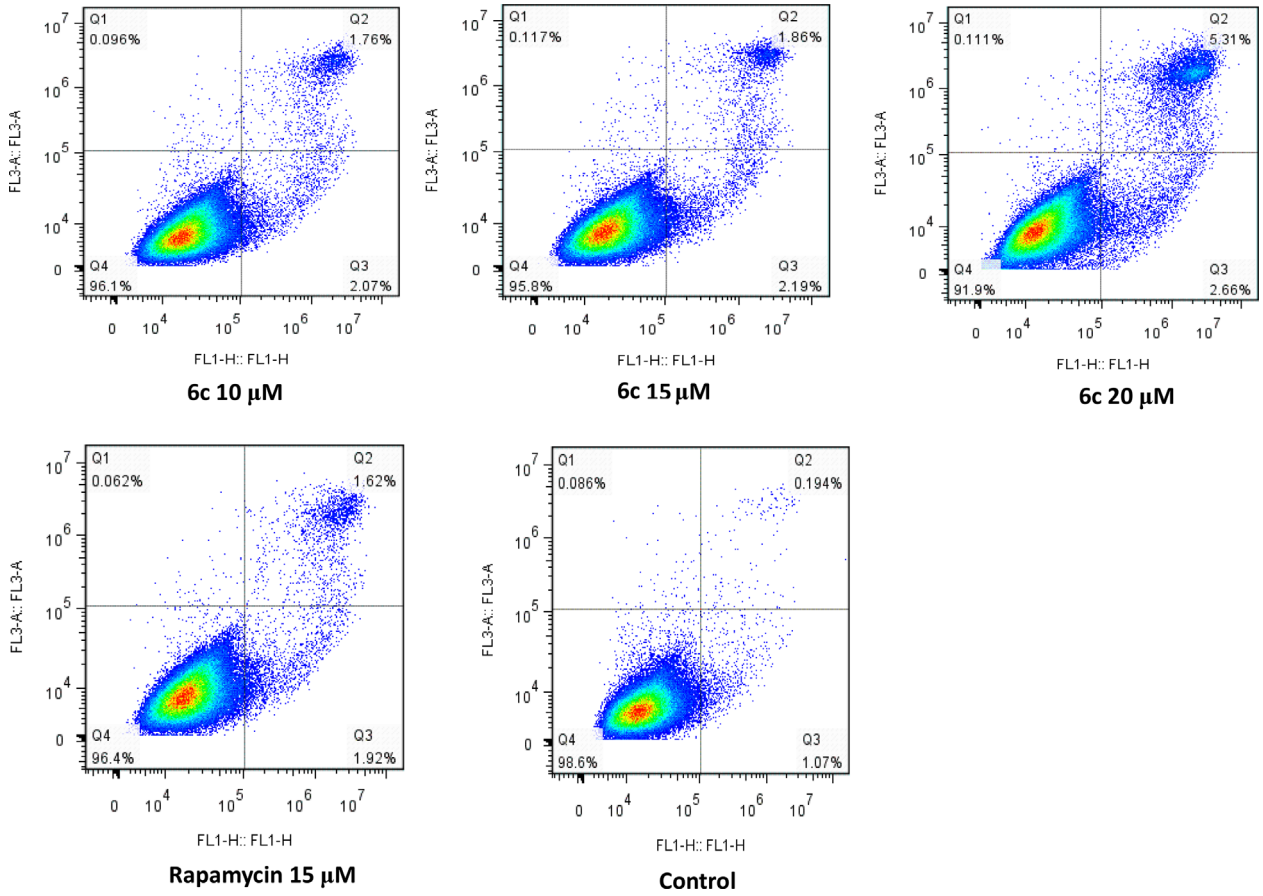


FIGURE 3 Effect of **6c** and rapamycin on apoptosis in A549 cells. A549 was treated with **6c** or rapamycin for 48 h at the indicated concentrations. Cells were stained with Annexin V-FITC and propidium iodide and analyzed by flow cytometry

3 | CONCLUSIONS

This work presents the synthesis of rapamycin–triazole hybrids modified on the position C-28 of rapamycin and their anticancer activity against different cancer cell lines. To our delight, the most promising compound **6c** showed stronger effect than rapamycin not only in anticancer activity against most of tested cancer cell lines but also in inducing cancer cell apoptosis, cell cycle arrest, and inhibition of mTOR pathway. In addition, conclusions regarding the structure–activity relationships could be tentatively drawn as two aspects. (1) Among target compounds (**6a–g**), the result indicated that the introduction of hydrogen or methyl group on the benzene ring made a good contribution to potency, with following rank order: H < Me. Compounds with halogen or methoxyl groups on the benzene ring lost significant potency against 769-P and ECA-109 cell lines. (2) Moreover, the introduction of aryl-amino-methyl group on triazole ring is advantageous to the potency while the introduction of hydroxyl-alkyl (**7e**, **7f**) moiety has negative impact on their anti-cancer activities. In conclusion, based on the experimental data and the binding mode, modification of rapamycin on C-28 position is a promising approach to obtain more potent mTOR inhibitors and **6c** holds the potential to become a new therapeutic mTOR inhibitor against various cancers.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | Reagents and general procedures

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. All melting points were obtained on a Buchi Melting Point M-560 (Buchi Labortechnik, Flawil, Switzerland) and were recorded. Mass spectra (MS) were taken in ESI mode Agilent 1100 LC-MS (Agilent, Palo Alto, CA, USA), $[a]_D$ values were recorded on Autopol I-Rudolph (USA). High resolution mass spectra (HRMS) were recorded on Agilent QTOF 6520 instrument. Nuclear magnetic resonance spectroscopy was performed using Bruker Advance, 600 MHz spectrometers (Bruker, Germany) with TMS as an internal standard. IR spectra (KBr disks) were recorded with a Bruker IFS 55 instrument (Bruker).

The InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

4.1.2 | Preparation of Rapa-28-OTMS (1)

Rapamycin (10.0 g, 10.9 mmol) and imidazole (9.4 g, 137.2 mmol) were dissolved in dry ethyl acetate (115.0 mL) and the solution was stirred at

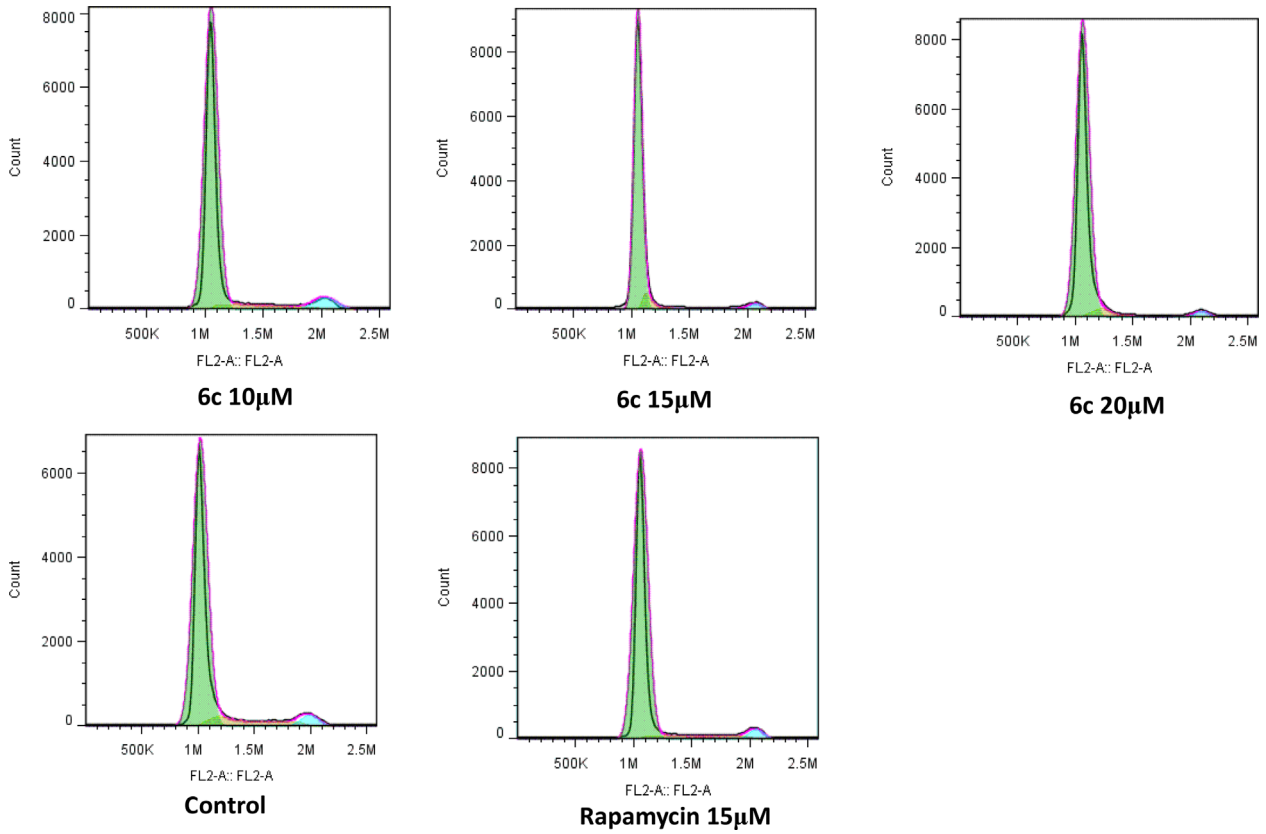


FIGURE 4 Effect of **6c** and rapamycin on cell cycle arrest in A549. A549 cells were treated with **6c** or rapamycin for 48 h at the indicated concentrations. Cells were stained with annexin V-FITC and analyzed by flow cytometry

0°C, then a solution of trimethylsilyl chloride (9.3 g, 85.3 mmol) was added dropwise to the reaction solution within 38 min. After the Rapa was transformed into Rapa-28,43-bis-OTMS, a 66 mL of 0.5 N sulfuric acid was added dropwise for 15 min and the mixture was stirred for 3 h at 0°C. The organic layer was washed with saturated $NaHCO_3$ aqueous solution and brine, dried over Na_2SO_4, filtered, and concentrated to afford **1** (10.3 g) as white solids in 95.6% yield; m.p.: 145.8–147.5°C; MS (ESI) *m/z*: 1008.5000 $(M+Na)^+$; $[a]_D$ −25.7 (*c* = 1.0, MeOH); ^1H NMR (600 MHz, DMSO-d_6) δ 6.47 (s, 1H), 6.41 (dd, *J* = 14.3, 11.5 Hz, 1H), 6.23 (dd, *J* = 24.6, 13.7 Hz, 1H), 6.15 (dd, *J* = 21.1, 10.9 Hz, 1H), 6.10 (d, *J* = 15.1 Hz, 1H), 5.46 (dd, *J* = 14.8, 9.6 Hz, 1H), 5.11 (d, *J* = 10.2 Hz, 1H), 5.01 (d, *J* = 3.6 Hz, 1H), 4.95 (d, *J* = 4.8 Hz, 1H), 4.68–4.62 (m, 1H), 4.02 (t, *J* = 9.3 Hz, 2H), 3.87 (d, *J* = 5.7 Hz, 1H), 3.61 (dd, *J* = 33.1, 18.7 Hz, 1H), 3.45 (d, *J* = 12.6 Hz, 1H), 3.33 (s, 3H), 3.29 (dd, *J* = 19.4, 8.7 Hz, 1H), 3.17 (s, 3H), 3.14 (t, *J* = 5.7 Hz, 2H), 3.08 (d, *J* = 21.0 Hz, 3H), 2.82 (dd, *J* = 15.5, 8.0 Hz, 1H), 2.72 (dd, *J* = 33.3, 11.9 Hz, 1H), 2.50 (d, *J* = 12.8 Hz, 1H), 2.23 (s, 1H), 2.13 (d, *J* = 9.6 Hz, 1H), 2.05–2.01 (m, 1H), 1.85 (d, *J* = 9.3 Hz, 1H), 1.83 (s, 1H), 1.77 (s, 3H), 1.67 (s, 1H), 1.64 (s, 3H), 1.61 (s, 1H), 1.38 (d, *J* = 12.7 Hz, 1H), 1.30 (s, 1H), 1.24 (d, *J* = 11.3 Hz, 1H), 1.20 (d, *J* = 11.1 Hz, 1H), 0.99 (d, *J* = 6.3 Hz, 3H), 0.88 (d, *J* = 6.3 Hz, 3H), 0.83 (d, *J* = 6.3 Hz, 3H), 0.78 (d, *J* = 6.5 Hz, 3H), 0.74 (d, *J* = 6.4 Hz, 3H), 0.03–0.05 (m, 9H).

TABLE 2 Cell cycle distribution of A549 cells after **6c** or rapamycin treatment for 48 h

	Cell cycle distribution (%)		
Compound (μM)	G_1	S	G_2
6c (10)	127.73	12.56	6.03
6c (15)	145.12	7.53	3.24
6c (20)	148.12	6.28	2.79
Rapamycin (15)	137.12	9.13	5.18
Control	122.24	14.16	6.23

4.1.3 | Preparation of Rapa-43-OTBDMS (2)

Rapa-28-OTMS (10.3 g, 10.5 mmol) and imidazole (24.5 g, 360.4 mmol) were added to *N*,*N*-dimethylformamide (DMF, 110.0 mL) at 6°C, a solution of *tert*-butyldimethylsilyl chloride (TBDMSCl) (14.7 g, 138.3 mmol) in DMF (10.0 mL) was added within 20 min. The mixture was stirred at 6°C for 5 h. The reaction mixture was diluted with DMF and washed thoroughly with water, 1 N HCl aqueous solution, $NaHCO_3$ aqueous solution and brine, dried over Na_2SO_4, filtered, and concentrated. Silica gel chromatography (PE/EA 4:1) of the crude mixture afforded **2** (6.0 g, 46.3%); m.p.: 113.4–

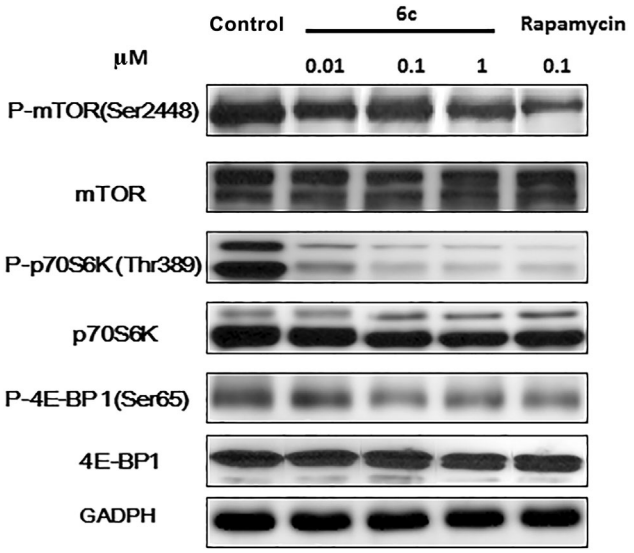


FIGURE 5 Inhibition of **6c** and rapamycin on the phosphorylation of mTOR, p70S6K, 4E-BP1 in A549 cells. Cells were treated with **6c** or rapamycin for 48 h and lysed, proteins were subjected to SDS–PAGE and transferred to nitrocellulose membranes. Bands were visualized by using the enhanced chemiluminescence Western blot detection system

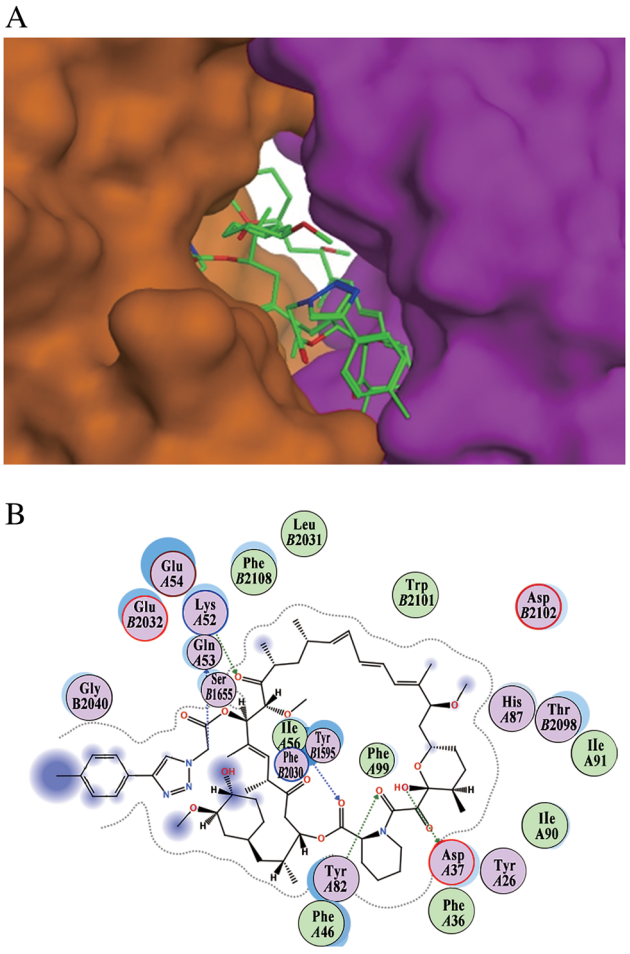


FIGURE 6 The binding model of compound **6c** with FKBP12-FRB. (A) Overview of predicted 3D binding modes of **6c** with the FKPB12-FRB crystal structure (PDB: 1FAP); (B) predicted 2D binding modes of **6c** with the FKPB12-FRB crystal structure

115.6°C; MS (ESI) *m/z*: 1050.6000 $(M+Na)^+$; $[a]_D$ −13.2 (*c* = 1.0, MeOH); ^1H NMR (600 MHz, DMSO-d_6) δ 6.46 (d, *J* = 1.4 Hz, 1H), 6.44–6.37 (m, 1H), 6.23 (dd, *J* = 14.5, 10.6 Hz, 1H), 6.17–6.11 (m, 1H), 6.11 (d, *J* = 4.5 Hz, 1H), 5.47 (dd, *J* = 14.9, 9.6 Hz, 1H), 5.28 (d, *J* = 4.5 Hz, 1H), 5.11 (d, *J* = 10.1 Hz, 1H), 5.01–4.97 (m, 1H), 4.95 (d, *J* = 6.0 Hz, 1H), 4.05–3.99 (m, 2H), 3.95 (d, *J* = 4.6 Hz, 1H), 3.45 (d, *J* = 12.7 Hz, 1H), 3.33 (s, 4H), 3.20–3.17 (m, 1H), 3.16 (s, 3H), 3.13 (s, 1H), 3.06 (s, 3H), 2.76–2.71 (m, 1H), 2.44–2.39 (m, 1H), 2.24–2.18 (m, 1H), 2.14–2.08 (m, 1H), 2.04 (dd, *J* = 15.0, 6.9 Hz, 1H), 1.85–1.80 (m, 1H), 1.74 (s, 4H), 1.68 (s, 1H), 1.68–1.66 (m, 1H), 1.64 (s, 4H), 1.60 (d, *J* = 4.8 Hz, 1H), 1.59–1.56 (m, 2H), 1.53 (s, 2H), 1.38 (d, *J* = 10.5 Hz, 1H), 1.29 (d, *J* = 11.8 Hz, 3H), 1.25 (d, *J* = 11.1 Hz, 2H), 0.98 (d, *J* = 6.5 Hz, 3H), 0.88 (d, *J* = 6.5 Hz, 3H), 0.86 (s, 9H), 0.83 (d, *J* = 6.4 Hz, 4H), 0.78 (d, *J* = 6.7 Hz, 3H), 0.74 (d, *J* = 6.7 Hz, 3H), 0.04 (d, *J* = 1.4 Hz, 6H).

4.1.4 | Preparation of Rapa-28-*O*-(2-bromoacetyl)-43-OTBDMS (3)

Rapa-43-OTBDMS (6.0 g, 5.8 mmol) and pyridine (17.7 g, 0.2 mol) were added into dichloromethane (DCM, 100.0 mL) at −2°C. A solution of bromoacetyl bromide (9.0 mL, 104.2 mmol) in DCM (15.0 mL) was added into this mixture within 40 min and further stirred for 10 min. The reaction mixture was diluted with DCM and washed thoroughly with water, 1 N HCl aqueous solution, $NaHCO_3$ aqueous solution and brine, dried over Na_2SO_4, filtered, and concentrated. Silica gel chromatography (PE/EA 4:1) of the crude mixture afforded **3** (4.8 g, 70.8%); m.p.: 94.1–96.4°C; MS (ESI) *m/z*: 1170.5000 $(M+Na)^+$; $[a]_D$ −35.5 (*c* = 1.0, MeOH); ^1H NMR (600 MHz, DMSO-d_6) δ 6.42 (d, *J* = 3.8 Hz, 1H), 6.41–6.37 (m, 1H), 6.23 (dd, *J* = 10.3, 4.8 Hz, 1H), 6.15 (dd, *J* = 12.9, 7.1 Hz, 1H), 6.12 (d, *J* = 7.0 Hz, 1H), 5.51–5.47 (m, 1H), 5.05 (d, *J* = 11.3 Hz, 1H), 5.02–4.96 (m, 2H), 4.94 (d, *J* = 5.4 Hz, 5H), 4.57 (s, 1H), 4.08–3.98 (m, 3H), 3.91 (d, *J* = 2.8 Hz, 1H), 3.63 (dd, *J* = 20.6, 9.3 Hz, 1H), 3.45 (d, *J* = 12.6 Hz, 1H), 3.33 (dd, *J* = 2.6, 1.5 Hz, 3H), 3.32–3.27 (m, 1H), 3.22–3.19 (m, 3H), 3.17 (d, *J* = 2.2 Hz, 3H), 3.15–3.13 (m, 2H), 3.05 (d, *J* = 2.8 Hz, 3H), 2.88–2.80 (m, 1H), 2.67 (dd, *J* = 9.2, 6.2 Hz, 1H), 2.39 (dd, *J* = 16.1, 9.2 Hz, 1H), 2.12 (d, *J* = 13.1 Hz, 1H), 2.04–1.99 (m, 1H), 1.90 (d, *J* = 13.1 Hz, 1H), 1.86–1.80 (m, 1H), 1.78–1.73 (m, 1H), 1.67 (s, 1H), 1.63–1.59 (m, 1H), 1.55 (d, *J* = 12.8 Hz, 1H), 1.53 (s, 1H), 1.27 (s, 1H), 1.26–1.22 (m, 1H), 1.21–1.16 (m, 1H), 0.98 (d, *J* = 2.1 Hz, 3H), 0.87 (d, *J* = 1.9 Hz, 3H), 0.85–0.83 (m, 12H), 0.83–0.82 (m, 3H), 0.78 (d, *J* = 7.8 Hz, 3H), 0.74 (dd, *J* = 6.5, 2.4 Hz, 3H), 0.59 (dd, *J* = 23.8, 11.9 Hz, 1H), −0.03 to 0.06 (m, 6H).

4.1.5 | Preparation of Rapa-28-*O*-(2-bromoacetyl) (4)

Rapa-28-O-(2-bromoacetyl)-43-OTBDMS (4.8 g, 4.2 mmol) was dissolved in acetone (70.0 mL) and the solution was stirred at 0°C, and

dilute sulfuric acid (2.0 mol/L, 18.0 mL) was added into the solution and further stirred for 18 h. The reaction mixture was diluted with EtOAc and washed thoroughly with $NaHCO_3$ aqueous solution and brine, dried over Na_2SO_4, filtered, and concentrated. Silica gel chromatography (PE/EA 1:1) of the crude mixture afforded **4** (3.6 g, 79.5%); m.p.: 101.3–104.6°C; MS (ESI) *m/z*: 1056.4000 $(M+Na)^+$; $[a]_D$ −23.8 (*c* = 1.0, MeOH); ^1H NMR (600 MHz, DMSO-d_6) δ 6.42 (d, *J* = 4.5 Hz, 1H), 6.40 (d, *J* = 3.6 Hz, 1H), 6.23 (d, *J* = 4.0 Hz, 1H), 6.19–6.15 (m, 1H), 6.12 (s, 1H), 5.47 (d, *J* = 5.3 Hz, 1H), 5.09 (s, 1H), 4.98 (d, *J* = 3.8 Hz, 1H), 4.94 (s, 1H), 4.65 (s, 1H), 4.02–4.00 (m, 2H), 3.91 (d, *J* = 2.7 Hz, 1H), 3.62 (d, *J* = 6.2 Hz, 1H), 3.44 (d, *J* = 2.1 Hz, 1H), 3.33 (s, 3H), 3.26 (s, 1H), 3.22 (s, 3H), 3.19 (d, *J* = 2.1 Hz, 1H), 3.05 (s, 3H), 2.84–2.82 (m, 1H), 2.73 (dd, *J* = 8.8, 5.2 Hz, 1H), 2.51 (dt, 2H), 2.46–2.42 (m, 1H), 2.10 (d, *J* = 11.8 Hz, 2H), 1.82 (s, 3H), 1.66–1.65 (m, 1H), 1.61 (s, 3H), 1.58 (s, 1H), 1.41 (s, 1H), 1.30 (s, 1H), 0.99 (d, *J* = 6.6 Hz, 3H), 0.88–0.86 (m, 3H), 0.84 (dd, *J* = 4.8, 3.8 Hz, 3H), 0.77 (d, *J* = 6.8 Hz, 3H), 0.74 (d, *J* = 6.6 Hz, 3H).

4.1.6 | Preparation of Rapa-28-*O*-(2-azidopropiomyl) (5)

Rapa-28-*O*-(2-bromoacetyl) (3.6 g, 3.5 mmol) was dissolved in DMF (50.0 mL) and the solution was stirred at 55°C, catalytic quantity KI and NaN_3 (0.9 g, 13.8 mmol) was added into the solution and further stirred for 1 h. The reaction mixture was diluted with EtOAc and washed thoroughly with water, 1 N HCl aqueous solution, $NaHCO_3$ aqueous solution and brine, dried over Na_2SO_4, filtered, and concentrated. Silica gel chromatography (PE/EA 1:1) of the crude mixture afforded **5** (1.4 g, 40.3%); m.p.: 100.3–103.7°C; MS (ESI) *m/z*: 1019.5000 $(M+Na)^+$; $[a]_D$ −13.7 (*c* = 1.0, MeOH); ^1H NMR (600 MHz, DMSO-d_6) δ 6.47 (s, 1H), 6.40 (dd, *J* = 14.6, 11.3 Hz, 1H), 6.24 (dd, *J* = 14.5, 10.7 Hz, 1H), 6.15 (dd, *J* = 15.7, 5.3 Hz, 1H), 6.12 (d, *J* = 4.6 Hz, 1H), 5.47 (dd, *J* = 15.0, 9.7 Hz, 1H), 5.34 (t, *J* = 5.9 Hz, 1H), 5.05 (d, *J* = 10.1 Hz, 1H), 4.98–4.93 (m, 2H), 4.61 (s, 1H), 4.36 (d, *J* = 3.8 Hz, 1H), 4.09–3.97 (m, 2H), 3.95 (d, *J* = 9.8 Hz, 1H), 3.63 (dd, *J* = 17.2, 5.6 Hz, 1H), 3.46 (d, *J* = 13.9 Hz, 1H), 3.33 (s, 3H), 3.30–3.27 (m, 1H), 3.27–3.24 (m, 1H), 3.22 (s, 3H), 3.13–3.08 (m, 1H), 3.05 (s, 3H), 2.86–2.82 (m, 1H), 2.67 (dd, *J* = 17.5, 2.7 Hz, 1H), 2.52–2.49 (m, 1H), 2.44–2.36 (m, 2H), 2.24 (s, 2H), 2.13 (d, *J* = 12.5 Hz, 1H), 1.85 (s, 1H), 1.82 (s, 3H), 1.62 (s, 3H), 1.59–1.55 (m, 4H), 1.28 (s, 1H), 1.00 (d, *J* = 6.5 Hz, 6H), 0.89–0.86 (m, 6H), 0.84–0.82 (m, 1H), 0.77 (d, *J* = 6.7 Hz, 3H), 0.74 (d, *J* = 6.6 Hz, 3H).

4.1.7 | General procedure for the preparation of compounds 6a–g, 7a–f

Mix of azidorapamycin (0.3 g, 0.3 mmol), 1-ethynyl-substituted benzene or other types of alkynes (3.0 equiv), catalytic quantity CuI and ethyldiisopropylamine (0.1 mL) in acetonitrile (10.0 mL). The heterogeneous mixture was stirred vigorously overnight at room temperature. TLC analysis indicated complete consumption of the azidorapamycin within 2–24 h, depending on the substrate and steric hindrance. The reaction mixture was diluted with EtOAc and washed thoroughly with water, 1 N HCl aqueous solution, $NaHCO_3$ aqueous solution and brine, dried over Na_2SO_4, filtered, and concentrated. Silica gel chromatography (PE/EA 1:1) of the crude mixture afforded the targeted compounds.

Rapa-28-*O*-(2-(4-(phenyl)-1*H*-1,2,3-trizole-1-yl)acetyl) (6a)

This compound was obtained as white solids in 69.2% yield; m.p.: 116.3–118.3°C; MS (ESI) *m/z*: 1098.6000 $(M+Na)^+$; $[a]_D$ −20.7 (c 1.0, MeOH); IR (KBr) cm^{-1}: 3428.0, 2940.5, 1728.3, 1641.7, 1450.7, 1372.9, 1095.3, 990.3, 766.6. ^1H NMR (600 MHz, DMSO-d_6) δ 8.43 (s, 1H), 7.86 (d, *J* = 1.0 Hz, 1H), 7.84 (s, 1H), 7.46 (d, *J* = 7.4 Hz, 1H), 7.43 (s, 1H), 7.33 (dd, *J* = 13.3, 5.9 Hz, 1H), 6.44 (d, *J* = 1.1 Hz, 1H), 6.37 (dd, *J* = 14.3, 11.4 Hz, 1H), 6.22 (dd, *J* = 17.0, 6.4 Hz, 1H), 6.19–6.11 (m, 1H), 6.10 (d, *J* = 4.1 Hz, 1H), 5.63–5.48 (m, 1H), 5.37 (dd, *J* = 23.2, 10.0 Hz, 2H), 5.09 (d, *J* = 7.3 Hz, 1H), 5.03–4.94 (m, 2H), 4.57–4.52 (m, 1H), 4.35 (d, *J* = 3.6 Hz, 1H), 4.31–4.10 (m, 1H), 4.06–3.96 (m, 1H), 3.65 (dd, *J* = 33.1, 11.7 Hz, 1H), 3.46 (d, *J* = 13.5 Hz, 1H), 3.29 (d, *J* = 6.6 Hz, 3H), 3.27–3.22 (m, 1H), 3.21 (s, 3H), 3.19–3.07 (m, 2H), 3.03 (d, *J* = 16.7 Hz, 3H), 2.85–2.75 (m, 1H), 2.72–2.60 (m, 1H), 2.40 (dd, *J* = 17.5, 8.2 Hz, 1H), 2.35–2.27 (m, 1H), 2.16 (dd, *J* = 27.4, 14.1 Hz, 1H), 2.02 (dd, *J* = 15.4, 7.9 Hz, 1H), 1.90 (d, *J* = 12.0 Hz, 1H), 1.83 (d, *J* = 13.9 Hz, 1H), 1.74–1.64 (m, 3H), 1.62–1.49 (m, 6H), 1.41 (dd, *J* = 27.3, 13.6 Hz, 2H), 1.24 (dd, *J* = 13.6, 7.8 Hz, 2H), 1.20–1.10 (m, 3H), 1.10–1.00 (m, 2H), 0.98 (t, *J* = 6.4 Hz, 3H), 0.91–0.79 (m, 6H), 0.79–0.69 (m, 6H), 0.56 (dd, *J* = 23.7, 11.9 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 208.7, 206.8, 198.6, 169.2, 166.8, 165.5, 146.3, 139.0, 137.9, 132.3, 131.7, 130.5, 128.7, 127.8, 126.9, 125.6, 125.1, 122.6, 98.9, 83.7, 82.1, 81.7, 77.8, 73.9, 73.1, 66.1, 57.1, 56.6, 55.4, 50.9, 50.3, 45.2, 43.6, 40.8, 40.6, 40.0, 39.8, 39.6, 39.5, 39.3, 39.1, 39.0, 37.9, 35.1, 35.0, 34.6, 33.7, 32.8, 32.5, 31.3, 29.7, 28.6, 26.1, 24.4, 22.4, 21.4, 20.4, 19.4, 18.9, 15.5, 15.2, 14.9, 14.1, 13.0, 11.1, 10.4; HRMS (ESI): calcd. for $C_{61}H_{86}N_4NaO_{14}$ $[M+Na]^+$ = 1098.6115. Found = 1098.6141.

Rapa-28-*O*-(2-(4-(3-methylphenyl)-1*H*-1,2,3-trizole-1-yl)acetyl) (6b)

This compound was obtained as white solids in 38.2% yield; m.p.: 121.7–123.9°C; MS (ESI) *m/z*: 1112.6000 $(M+Na)^+$; $[a]_D$ −32.2 (c 1.0, MeOH); IR (KBr) cm^{-1}: 3449.0, 2936.4, 1725.0, 1646.0, 1450.2, 1375.8, 1249.8, 1091.4, 989.2, 784.3. ^1H NMR (600 MHz, DMSO-d_6) δ 8.40 (d, *J* = 6.2 Hz, 1H), 7.67 (s, 1H), 7.63 (d, *J* = 7.6 Hz, 1H), 7.33 (dd, *J* = 14.0, 6.5 Hz, 1H), 7.15 (d, *J* = 7.2 Hz, 1H), 5.45 (dd, *J* = 22.4, 12.7 Hz, 1H), 5.38–5.31 (m, 2H), 5.15–5.05 (m, 1H), 5.03–4.93 (m, 2H), 4.55 (t, *J* = 6.2 Hz, 1H), 4.35 (d, *J* = 3.5 Hz, 1H), 4.27 (d, *J* = 3.9 Hz, 1H), 4.06–3.95 (m, 1H), 3.65 (dd, *J* = 32.2, 11.9 Hz, 1H), 3.46 (d, *J* = 13.5 Hz, 1H), 3.29 (d, *J* = 7.7 Hz, 3H), 3.27–3.23 (m, 1H), 3.19 (d, *J* = 13.9 Hz, 3H), 3.12 (dd, *J* = 15.7, 10.7 Hz, 2H), 3.03 (d, *J* = 16.4 Hz, 3H), 2.84–2.76 (m, 1H), 2.66 (d, *J* = 15.3 Hz, 1H), 2.43–2.37 (m, 1H), 2.36 (s, 3H), 2.33–2.29 (m, 1H), 2.19–2.11 (m, 1H), 2.02 (dd, *J* = 15.0, 7.6 Hz, 1H), 1.86 (dd, *J* = 30.5, 14.0 Hz, 3H), 1.74–1.66 (m, 3H), 1.54 (d, *J* = 14.8 Hz, 5H), 1.45–1.36 (m, 2H), 1.34–1.27 (m, 2H), 1.22 (d, *J* = 13.9 Hz, 2H), 1.20–1.12 (m, 3H), 1.04 (dd, *J* = 18.7, 6.3 Hz, 3H), 0.97 (d, *J* = 6.3 Hz, 3H), 0.87 (t, *J* = 9.6 Hz, 4H), 0.77–0.70 (m, 8H), 0.55 (dd, *J* = 23.8, 11.9 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 208.7, 206.8, 198.7, 169.2,

166.8, 165.6, 146.4, 139.0, 137.8, 132.3, 131.8, 130.4, 128.6, 128.5, 126.9, 125.7, 122.5, 122.3, 110.0, 98.9, 83.7, 82.1, 81.7, 77.8, 73.9, 73.1, 66.1, 57.1, 56.6, 55.4, 50.9, 50.3, 45.2, 43.6, 40.9, 40.6, 40.0, 39.8, 39.6, 39.5, 39.3, 39.1, 39.0, 37.8, 35.1, 34.9, 34.6, 33.8, 32.8, 32.5, 31.3, 29.7, 26.1, 24.4, 21.4, 20.9, 20.4, 15.5, 15.2, 14.9, 14.1, 13.0, 10.4; HRMS (ESI): calcd. for $C_{62}H_{88}N_4NaO_{14}$ $[M+Na]^+$ = 1112.6279. Found = 1112.6297.

Rapa-28-*O*-(2-(4-(4-methylphenyl)-1*H*-1,2,3-trizole-1-yl)acetyl) (6c)

This compound was obtained as white solids in 62.5% yield; m.p.: 124.1–127.3°C; MS (ESI) *m/z*: 1112.6000 $(M+Na)^+$; $[a]_D$ −40.3 (c 1.0, MeOH); IR (KBr) cm^{-1}: 3459.5, 2930.5, 1721.2, 1638.8, 1454.5, 1247.7, 1091.4, 999.5. ^1H NMR (600 MHz, DMSO-d_6) δ 8.36 (s, 1H), 7.73 (d, *J* = 7.0 Hz, 2H), 7.27 (t, *J* = 17.0 Hz, 2H), 6.43 (s, 1H), 6.41–6.30 (m, 1H), 6.30–6.15 (m, 1H), 6.15–6.03 (m, 2H), 5.54–5.41 (m, 1H), 5.36 (d, *J* = 23.1 Hz, 2H), 5.05 (d, *J* = 39.3 Hz, 1H), 4.97 (d, *J* = 11.1 Hz, 2H), 4.30 (d, *J* = 41.9 Hz, 2H), 4.01 (s, 1H), 3.62 (d, *J* = 11.0 Hz, 1H), 3.46 (d, *J* = 11.5 Hz, 1H), 3.28 (s, 3H), 3.20 (s, 3H), 3.18–3.10 (m, 2H), 3.04 (s, 3H), 2.79 (s, 1H), 2.66 (d, *J* = 16.8 Hz, 1H), 2.44–2.38 (m, 1H), 2.33 (s, 3H), 2.15 (d, *J* = 13.3 Hz, 1H), 2.01 (d, *J* = 6.6 Hz, 1H), 1.86 (dd, *J* = 33.4, 12.8 Hz, 3H), 1.78 (s, 1H), 1.67 (d, *J* = 8.8 Hz, 3H), 1.50 (d, *J* = 27.5 Hz, 5H), 1.46–1.35 (m, 2H), 1.23 (s, 2H), 1.20–1.10 (m, 3H), 1.05 (d, *J* = 7.4 Hz, 3H), 0.97 (d, *J* = 5.4 Hz, 3H), 0.83 (dd, *J* = 17.8, 5.8 Hz, 6H), 0.74 (s, 6H), 0.60–0.53 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 208.3, 206.6, 198.6, 169.2, 166.8, 165.6, 146.4, 139.0, 137.9, 137.1, 132.3, 131.7, 130.4, 129.3, 127.7, 126.9, 125.6, 125.1, 122.2, 108.2, 98.9, 83.7, 82.1, 81.7, 77.8, 73.9, 73.1, 66.1, 57.1, 56.6, 55.4, 50.9, 50.3, 45.2, 40.8, 40.6, 40.0, 39.8, 39.6, 39.5, 39.3, 39.1, 39.0, 37.9, 35.1, 35.0, 34.6, 33.7, 32.8, 32.5, 31.3, 29.7, 26.1, 24.4, 21.4, 20.7, 20.4, 15.5, 15.2, 14.9, 14.1, 13.0, 10.4. HR-MS(ESI): calcd. for $C_{62}H_{88}N_4NaO_{14}$ $[M+Na]^+$ = 1112.6269. Found = 1112.6297.

Rapa-28-*O*-(2-(4-(2-chlorophenyl)-1*H*-1,2,3-trizole-1-yl)acetyl) (6d)

This compound was obtained as yellow solids in 36.4% yield; m.p.: 111.2–114.3°C; MS (ESI) *m/z*: 1155.5000 $(M+Na)^+$; $[a]_D$ −12.8 (c 1.0, MeOH); IR (KBr) cm^{-1}: 3452.0, 2939.1, 1722.0, 1640.6, 1454.2, 1195.3, 1100.2, 991.2. ^1H NMR (600 MHz, DMSO-d_6) δ 8.63 (d, *J* = 13.4 Hz, 1H), 8.12 (t, *J* = 8.6 Hz, 1H), 7.60 (dd, *J* = 31.5, 7.7 Hz, 1H), 7.55–7.44 (m, 1H), 7.40 (t, *J* = 7.1 Hz, 1H), 6.47 (s, 1H), 6.39 (dd, *J* = 26.1, 14.6 Hz, 1H), 6.28 (dd, *J* = 36.5, 27.6 Hz, 1H), 6.18 (dd, *J* = 21.6, 10.2 Hz, 1H), 6.11 (d, *J* = 10.9 Hz, 1H), 5.47–5.40 (m, 1H), 5.37 (d, *J* = 14.6 Hz, 1H), 5.03 (d, *J* = 9.9 Hz, 1H), 4.96 (d, *J* = 18.6 Hz, 1H), 4.58 (dd, *J* = 15.6, 11.9 Hz, 1H), 4.30 (dd, *J* = 30.2, 11.6 Hz, 1H), 4.23–4.10 (m, 1H), 4.00 (t, *J* = 19.6 Hz, 1H), 3.65 (dd, *J* = 37.1, 12.7 Hz, 1H), 3.46 (d, *J* = 11.3 Hz, 2H), 3.29 (s, 3H), 3.25 (dd, *J* = 12.4, 4.7 Hz, 1H), 3.20 (s, 1H), 3.13 (dd, *J* = 27.6, 13.4 Hz, 3H), 3.05 (s, 3H), 2.81 (dd, *J* = 15.3, 8.2 Hz, 1H), 2.68 (t, *J* = 16.1 Hz, 1H), 2.40 (dd, *J* = 17.3, 8.1 Hz, 1H), 2.19 (s, 1H), 2.12 (t, *J* = 14.2 Hz, 1H), 2.02 (d, *J* = 7.2 Hz, 1H), 1.89–1.81 (m, 3H), 1.75–1.65 (m, 3H), 1.59–1.50 (m, 5H), 1.45–1.33 (m, 2H), 1.25 (dd, *J* = 40.0, 14.1 Hz, 2H), 1.16 (dd, *J* = 21.8, 7.9 Hz, 2H), 1.04 (dd, *J* = 18.6, 6.3 Hz, 2H), 1.00–0.95 (m, 3H), 0.94 (s, 3H), 0.88–0.81 (m, 6H), 0.76–0.70 (m, 3H), 0.56 (dd, *J* = 23.9, 12.0 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 208.8, 207.1, 199.1, 185.0, 182.3, 169.6, 167.4, 142.9, 139.6, 139.2, 138.3, 138.1, 132.8, 132.2, 130.7, 130.7, 129.8, 129.4, 127.9, 127.4, 126.1, 99.4, 84.2, 82.5, 78.2, 74.3, 73.6, 71.8, 66.5, 57.5, 57.1, 55.9, 51.4, 50.6, 45.5, 44.0, 40.4, 40.2, 40.1, 39.9, 39.8, 39.7, 39.5, 38.4, 35.6, 33.3, 33.0, 31.8, 30.0, 28.6, 26.8, 24.9, 22.0, 21.4, 19.7, 16.0, 15.7, 15.4, 14.4, 13.6, 10.9, 9.9. HRMS (ESI): calcd. for $C_{61}H_{85}ClN_4NaO_{14}$ $[M+Na]^+$ = 1155.5643. Found = 1155.5622.

Rapa-28-*O*-(2-(4-(4-methoxylphenyl)-1*H*-1,2,3-trizole-1-yl)-acetyl) (6e)

This compound was obtained as yellow solids in 15.4% yield; m.p.: 113.9–115.8°C; MS (ESI) *m/z*: 1128.6000 $(M+Na)^+$; $[a]_D$ −33.5 (c 1.0, MeOH); IR (KBr) cm^{-1}: 3455.0, 2931.3, 1723.8, 1639.4, 1449.6, 1246.9, 1095.1, 993.7. ^1H NMR (600 MHz, DMSO-d_6) δ 8.32 (d, *J* = 5.5 Hz, 1H), 7.85–7.74 (m, 2H), 7.12–6.99 (m, 2H), 6.47 (d, *J* = 8.0 Hz, 1H), 6.38 (dd, *J* = 14.5, 11.4 Hz, 1H), 6.21 (dd, *J* = 14.4, 10.9 Hz, 1H), 6.11 (dd, *J* = 18.5, 10.6 Hz, 2H), 5.43 (dd, *J* = 15.0, 9.8 Hz, 1H), 5.39–5.32 (m, 2H), 5.13–5.05 (m, 1H), 5.02–4.92 (m, 2H), 4.59 (d, *J* = 4.0 Hz, 1H), 4.37 (d, *J* = 3.2 Hz, 1H), 4.31–4.11 (m, 1H), 4.03 (d, *J* = 10.9 Hz, 1H), 3.79 (s, 3H), 3.63 (dd, *J* = 32.8, 21.3 Hz, 1H), 3.47 (d, *J* = 12.9 Hz, 1H), 3.29 (s, 3H), 3.21 (s, 3H), 3.17–3.13 (m, 2H), 3.05 (s, 3H), 2.80 (dd, *J* = 15.6, 8.3 Hz, 1H), 2.66 (dd, *J* = 31.8, 16.8 Hz, 1H), 2.40 (dd, *J* = 17.3, 8.2 Hz, 2H), 2.24–2.13 (m, 1H), 2.10 (d, *J* = 23.6 Hz, 1H), 2.05–1.97 (m, 1H), 1.87 (dd, *J* = 38.0, 13.4 Hz, 3H), 1.75–1.65 (m, 3H), 1.55–1.40 (m, 5H), 1.33 (d, *J* = 34.9 Hz, 2H), 1.25 (d, *J* = 14.3 Hz, 2H), 1.20–1.12 (m, 3H), 1.06 (ddd, 3H), 0.98 (d, *J* = 6.3 Hz, 3H), 0.90–0.80 (m, 6H), 0.76–0.70 (m, 6H), 0.58–0.52 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 207.8, 200.4, 198.4, 185.3, 181.7, 180.7, 174.1, 169.7, 166.3, 159.5, 152.8, 147.9, 135.8, 126.8, 114.8, 99.6, 84.0, 73.5, 64.5, 56.8, 55.6, 46.1, 40.4, 40.2, 40.1, 39.9, 39.8, 39.7, 39.5, 33.1, 21.7, 16.0, 15.5, 15.4, 14.0, 11.2, 10.5; HRMS (ESI): calcd. for $C_{62}H_{88}N_4NaO_{15}$ $[M+Na]^+$ = 1128.6238. Found = 1128.6246.

Rapa-28-*O*-(2-(4-(4-fluorinphenyl)-1*H*-1,2,3-trizole-1-yl)acetyl) (6f)

This compound was obtained as white solids in 30.3% yield; m.p.: 118.7–120.3°C. MS (ESI) *m/z*: 1116.6000 $(M+Na)^+$; $[a]_D$ −15.6 (c 1.0, MeOH); IR (KBr) cm^{-1}: 3456.2, 2941.4, 1717.0, 1638.4, 1453.1, 1374.0, 1193.2, 1096.0. ^1H NMR (600 MHz, DMSO-d_6) δ 8.42 (d, *J* = 4.9 Hz, 1H), 7.88 (dd, *J* = 8.5, 5.5 Hz, 2H), 7.29 (t, *J* = 8.8 Hz, 2H), 6.44 (s, 1H), 6.37 (dd, *J* = 14.4, 11.3 Hz, 1H), 6.24–6.16 (m, 1H), 6.12 (t, *J* = 12.6 Hz, 1H), 6.08 (d, *J* = 10.7 Hz, 1H), 5.46–5.41 (m, 1H), 5.33 (s, 2H), 5.07 (d, *J* = 22.9 Hz, 1H), 4.98 (d, *J* = 10.9 Hz, 2H), 4.59–4.52 (m, 1H), 4.35 (d, *J* = 3.3 Hz, 1H), 4.28 (s, 1H), 4.05–3.97 (m, 1H), 3.62 (d, *J* = 12.3 Hz, 1H), 3.46 (d, *J* = 13.5 Hz, 1H), 3.28 (s, 3H), 3.26–3.22 (m, 1H), 3.20 (s, 3H), 3.17 (d, *J* = 6.3 Hz, 2H), 3.04 (s, 3H), 2.80 (dd, *J* = 15.6, 8.3 Hz, 1H), 2.66 (d, *J* = 14.9 Hz, 1H), 2.39 (dd, *J* = 17.4, 8.2 Hz, 1H), 2.15 (d, *J* = 13.5 Hz, 1H), 2.03–1.98 (m, 1H), 1.86 (dd, *J* = 31.1, 14.9 Hz, 3H), 1.74–1.65 (m, 3H), 1.54 (d, *J* = 15.5 Hz, 5H), 1.43 (dd, *J* = 24.6, 12.6 Hz, 2H), 1.22 (d, *J* = 15.2 Hz, 2H), 1.19–1.13 (m, 3H), 1.04 (dd, *J* = 14.9, 9.0 Hz, 3H), 0.97 (d, *J* = 6.4 Hz, 3H), 0.85 (d, *J* = 6.4 Hz, 6H),

0.75–0.70 (m, 6H), 0.55 (d, *J* = 11.7 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 208.7, 206.5, 198.7, 169.2, 166.9, 165.5, 160.7, 145.4, 139.0, 137.9, 132.3, 131.7, 130.4, 127.2, 127.1, 122.5, 115.8, 115.6, 98.9, 83.7, 82.1, 81.7, 77.8, 73.9, 73.1, 66.1, 57.0, 56.6, 55.4, 50.9, 50.3, 45.2, 43.6, 40.8, 40.5, 40.0, 39.8, 39.6, 39.5, 39.3, 39.1, 39.0, 37.9, 35.1, 35.0, 34.6, 33.7, 32.8, 32.5, 31.3, 26.2, 24.4, 21.4, 20.4, 15.5, 15.2, 14.9, 14.1, 12.9, 10.4; HRMS(ESI): calcd. for $C_{61}H_{85}FN_4NaO_{14}$ $[M+Na]^+$ = 1116.6030. Found = 1116.6046.

Rapa-28-*O*-(2-(4-((3-aminophenyl)aminomethylene)-1*H*-1,2,3-trizole-1-yl)acetyl) (6g)

This compound was obtained as white solids in 79.1% yield; m.p.: 132.3–135.8°C; MS (ESI) *m/z*: 1136.6000 $(M+Na)^+$; $[a]_D$ −17.6 (c 1.0, MeOH); IR (KBr) cm^{-1}: 3377.9, 2933.9, 1728.3, 1642.2, 1447.2, 1198.1, 1093.8, 993.8. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.27 (s, 1H), 7.12 (s, 1H), 7.09–7.04 (m, 1H), 6.95 (t, *J* = 7.2 Hz, 1H), 6.54 (dd, *J* = 7.9, 1.5 Hz, 1H), 6.47 (s, 1H), 6.39 (dd, *J* = 14.6, 11.4 Hz, 1H), 6.21 (dd, *J* = 14.5, 10.8 Hz, 1H), 6.11 (dd, *J* = 14.5, 11.0 Hz, 2H), 5.43 (dd, *J* = 14.9, 9.7 Hz, 1H), 5.35 (dd, *J* = 10.3, 7.4 Hz, 2H), 5.16 (s, 2H), 5.10 (d, *J* = 9.1 Hz, 1H), 5.01–4.95 (m, 2H), 4.61–4.57 (m, 1H), 4.34 (d, *J* = 3.7 Hz, 1H), 4.31–4.25 (m, 1H), 4.02 (t, *J* = 8.1 Hz, 1H), 3.63 (d, *J* = 12.9 Hz, 1H), 3.47 (d, *J* = 12.8 Hz, 1H), 3.30 (s, 3H), 3.27 (s, 1H), 3.21 (s, 3H), 3.05 (d, *J* = 3.9 Hz, 3H), 2.84–2.79 (m, 1H), 2.70–2.65 (m, 1H), 2.42 (dd, *J* = 17.5, 8.2 Hz, 1H), 2.20 (s, 1H), 2.16 (d, *J* = 13.0 Hz, 1H), 2.02 (dd, *J* = 14.7, 7.2 Hz, 1H), 1.88–1.81 (m, 3H), 1.79 (s, 3H), 1.68 (d, *J* = 11.2 Hz, 3H), 1.61 (d, *J* = 6.4 Hz, 3H), 1.60–1.50 (m, 5H), 1.46–1.36 (m, 2H), 1.31–1.23 (m, 2H), 1.22–1.13 (m, 3H), 1.10–1.01 (m, 3H), 0.98 (d, *J* = 6.5 Hz, 3H), 0.86 (t, *J* = 5.7 Hz, 3H), 0.77 (d, *J* = 6.5 Hz, 3H), 0.74 (t, *J* = 6.9 Hz, 6H), 0.57 (d, *J* = 11.8 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 209.3, 207.4, 199.3, 169.7, 167.4, 166.1, 149.4, 147.5, 139.5, 138.4, 132.8, 132.2, 131.4, 130.9, 129.7, 127.5, 122.7, 114.0, 113.5, 110.9, 99.4, 84.2, 82.6, 78.2, 74.4, 73.6, 66.6, 57.6, 57.1, 55.9, 55.3, 51.4, 50.7, 45.7, 44.1, 41.1, 40.7, 40.4, 40.2, 40.1, 39.9, 39.8, 39.7, 39.5, 38.4, 35.6, 35.5, 35.2, 34.2, 33.3, 33.0, 31.8, 30.2, 30.1, 26.8, 24.9, 22.0, 20.9, 16.0, 15.8, 15.4, 14.5, 13.6, 10.9; HRMS (ESI): calcd. for $C_{61}H_{87}N_5NaO_{14}$ $[M+Na]^+$ = 1136.6142. Found = 1136.6132.

Rapa-28-*O*-(2-(4-((2,4-dichlorophenyl)aminomethylene)-1*H*-1,2,3-trizole-1-yl)acetyl) (7a)

This compound was obtained as white solids in 42.9% yield; m.p.: 110.2–113.8°C; MS (ESI) *m/z*: 1195.5000 $(M-H)^-$; $[a]_D$ −16.9 (c 1.0, MeOH); IR (KBr) cm^{-1}: 3425.6, 2933.6, 1722.3, 1641.0, 1425.3, 1096.6, 989.3. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.91–7.87 (m, 1H), 7.36 (t, *J* = 6.7 Hz, 1H), 7.14 (dt, *J* = 7.0, 3.5 Hz, 1H), 6.81 (d, *J* = 8.9 Hz, 1H), 6.47 (s, 1H), 6.39 (dd, *J* = 14.3, 11.5 Hz, 1H), 6.20 (dd, *J* = 23.1, 8.5 Hz, 1H), 6.16–6.08 (m, 2H), 6.06 (d, *J* = 5.9 Hz, 1H), 5.49 (dd, *J* = 21.0, 10.7 Hz, 1H), 5.36–5.25 (m, 2H), 5.08 (d, *J* = 7.4 Hz, 1H), 5.00 (d, *J* = 9.9 Hz, 1H), 4.96 (d, *J* = 4.1 Hz, 1H), 4.61 (d, *J* = 4.0 Hz, 1H), 4.44 (d, *J* = 5.9 Hz, 2H), 4.29 (d, *J* = 3.5 Hz, 1H), 4.05–3.94 (m, 2H), 3.93 (d, *J* = 7.1 Hz, 1H), 3.63 (d, *J* = 12.0 Hz, 1H), 3.46 (d, *J* = 12.8 Hz, 1H), 3.34 (s, 3H), 3.29–3.20 (m, 2H), 3.19 (d, *J* = 5.5 Hz, 3H), 3.04 (d, *J* = 18.5 Hz, 3H), 2.85–2.79 (m, 1H), 2.72–2.60 (m, 1H), 2.44–2.31 (m, 2H), 2.24 (d, *J* = 28.6 Hz, 1H), 2.17–2.11 (m, 1H), 2.05–1.97 (m, 1H), 1.89 (dt, *J* = 25.7, 14.6 Hz, 3H), 1.68 (s, 3H), 1.59–1.47 (m, 5H), 1.45–1.35 (m, 2H), 1.27 (dd, *J* = 25.5, 13.6 Hz, 2H), 1.22–1.13 (m, 3H), 1.08–1.03 (m, 2H), 0.98 (d, *J* = 6.3 Hz, 3H), 0.94 (d, *J* = 7.0 Hz, 1H), 0.86 (d, *J* = 6.4 Hz, 3H), 0.85–0.82 (m, 1H), 0.80 (d, *J* = 6.3 Hz, 3H), 0.74 (t, *J* = 6.4 Hz, 6H), 0.57 (dd, *J* = 23.7, 11.9 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 209.3, 207.2, 199.2, 169.7, 167.4, 166.1, 145.4, 143.2, 139.5, 138.4, 132.9, 132.2, 130.9, 128.6, 128.1, 127.4, 126.5, 124.7, 119.7, 118.8, 112.9, 107.4, 99.4, 84.2, 82.6, 82.2, 78.1, 74.2, 73.7, 71.8, 66.6, 57.6, 57.2, 55.9, 51.4, 50.6, 45.6, 44.1, 41.0, 40.4, 40.2, 40.1, 39.9, 39.8, 39.7, 39.5, 38.7, 35.6, 35.2, 34.1, 33.3, 33.0, 31.8, 30.0, 26.5, 25.1, 22.0, 20.9, 19.5, 16.0, 15.7, 15.4, 14.4, 13.7, 10.9 HRMS(ESI): calcd. for $C_{62}H_{86}Cl_2N_5O_{14}$ $[M-H]^-$ = 1195.5608. Found = 1195.5627.

Rapa-28-*O*-(2-(4-(methene-(*N*,*N*-diethyl))-1*H*-1,2,3-trizole-1-yl)-acetyl) (7b)

This compound was obtained as white solids in 67.6% yield; m.p.: 105.5–108.3°C; MS (ESI) *m/z*: 1108.6000 $(M+H)^+$; $[a]_D$ −34.2 (c 1.0, MeOH); IR (KBr) cm^{-1}: 3442.5, 2943.6, 1726.6, 1643.0, 1456.6, 1209.4, 1106.6, 999.2. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.91 (s, 1H), 6.45 (d, *J* = 22.6 Hz, 1H), 6.39 (dd, *J* = 14.3, 11.5 Hz, 1H), 6.21 (dd, *J* = 24.1, 9.5 Hz, 1H), 6.16–6.12 (m, 1H), 6.11 (d, *J* = 9.9 Hz, 1H), 5.47–5.41 (m, 1H), 5.32 (s, 2H), 5.00 (d, *J* = 9.9 Hz, 1H), 4.95 (d, *J* = 3.7 Hz, 2H), 4.59 (d, *J* = 27.7 Hz, 1H), 4.34 (d, *J* = 3.4 Hz, 1H), 4.27 (d, *J* = 18.1 Hz, 1H), 4.00 (dd, *J* = 12.6, 9.0 Hz, 1H), 3.77 (s, 2H), 3.63 (d, *J* = 11.6 Hz, 1H), 3.46 (d, *J* = 12.8 Hz, 1H), 3.30 (d, *J* = 7.7 Hz, 3H), 3.28 (d, *J* = 8.1 Hz, 1H), 3.20 (s, 3H), 3.16 (d, *J* = 12.6 Hz, 2H), 3.05 (s, 3H), 2.84–2.78 (m, 1H), 2.65 (d, *J* = 15.7 Hz, 1H), 2.53–2.46 (m, 4H), 2.39 (dd, *J* = 17.5, 8.3 Hz, 1H), 2.23 (s, 1H), 2.14 (d, *J* = 18.0 Hz, 1H), 2.04–1.99 (m, 1H), 1.85 (t, *J* = 11.8 Hz, 3H), 1.79 (s, 3H), 1.68 (dd, *J* = 18.4, 9.8 Hz, 3H), 1.62 (s, 3H), 1.56 (dd, *J* = 18.9, 12.3 Hz, 5H), 1.41 (dd, *J* = 21.6, 12.6 Hz, 2H), 1.31–1.24 (m, 2H), 1.17 (dd, *J* = 18.5, 11.2 Hz, 3H), 1.04 (t, *J* = 6.9 Hz, 9H), 0.99 (d, *J* = 6.4 Hz, 3H), 0.87 (d, *J* = 6.3 Hz, 3H), 0.82 (d, *J* = 6.4 Hz, 3H), 0.74 (dd, *J* = 5.9, 3.5 Hz, 6H), 0.56 (d, *J* = 11.8 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 207.4, 199.1, 191.7, 169.6, 167.0, 166.0, 144.9, 138.1, 132.2, 127.5, 123.8, 99.4, 84.2, 82.2, 77.9, 74.2, 73.2, 66.5, 60.8, 57.2, 55.9, 51.4, 50.2, 45.6, 40.4, 40.2, 40.1, 39.9, 39.8, 39.7, 39.5, 35.4, 32.9, 31.7, 29.8, 29.5, 22.0, 16.0, 15.7, 15.4, 14.3, 13.4, 10.8; HRMS (ESI): calcd. for $C_{60}H_{94}N_5O_{14}$ $[M+H]^+$ = 1108.6792. Found = 1108.6787.

Rapa-28-*O*-(2-(4-((2-fluorinphenyl)aminomethylene)-1*H*-1,2,3-trizole-1-yl)acetyl) (7c)

This compound was obtained as white solids in 69.7% yield; m.p.: 82.5–85.5°C; MS (ESI) *m/z*: 1168.6000 $(M+Na)^+$; $[a]_D$ −22.7 (c 1.0, MeOH); IR (KBr) cm^{-1}: 3422.6, 2933.7, 1723.4, 1649.1, 1444.3, 1197.6, 1100.1, 988.3. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.89 (s, 1H), 7.20–7.12 (m, 1H), 7.02–6.98 (m, 1H), 6.93 (d, *J* = 8.0 Hz, 1H), 6.82–6.77 (m, 1H), 6.57–6.52 (m, 1H), 6.47 (s, 1H), 6.39 (dd, *J* = 14.7, 11.3 Hz, 1H), 6.25–6.19 (m, 1H), 6.17–6.12 (m, 1H), 6.12–6.09 (m, 1H), 5.43 (dd, *J* = 14.8, 9.8 Hz, 1H), 5.31 (d, *J* = 5.4 Hz, 2H), 5.09 (d, *J* = 4.1 Hz, 1H), 5.00 (d, *J* = 10.0 Hz, 1H), 4.96 (d, *J* = 5.0 Hz, 1H), 4.61 (dd, *J* = 6.4, 4.4 Hz, 1H), 4.39 (d, *J* = 5.8 Hz, 2H), 4.01 (dd, *J* = 19.4, 9.2 Hz, 1H), 3.95

(dd, *J* = 6.3, 1.1 Hz, 1H), 3.63 (d, *J* = 12.8 Hz, 1H), 3.45 (t, *J* = 14.0 Hz, 1H), 3.31 (s, 3H), 3.29 (d, *J* = 3.9 Hz, 1H), 3.19 (t, *J* = 5.6 Hz, 3H), 3.05 (s, 3H), 2.85–2.80 (m, 1H), 2.66 (d, *J* = 15.6 Hz, 1H), 2.43–2.37 (m, 2H), 2.24–2.20 (m, 1H), 2.16–2.12 (m, 1H), 2.02 (dd, *J* = 14.6, 7.1 Hz, 1H), 1.85 (t, *J* = 13.1 Hz, 3H), 1.70–1.64 (m, 3H), 1.59–1.50 (m, 5H), 1.40 (dd, *J* = 27.2, 13.9 Hz, 2H), 1.27 (dd, *J* = 14.2, 9.2 Hz, 2H), 1.22–1.16 (m, 3H), 1.05 (ddd, *J* = 16.3, 10.9, 9.1 Hz, 3H), 0.99 (d, *J* = 6.4 Hz, 3H), 0.87 (d, *J* = 6.4 Hz, 3H), 0.82 (d, *J* = 6.5 Hz, 3H), 0.74 (dd, *J* = 6.4, 3.1 Hz, 6H), 0.58 (d, *J* = 11.8 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 209.1, 207.4, 199.2, 169.6, 167.4, 166.2, 152.2, 150.2, 146.0, 143.6, 139.6, 138.4, 136.6, 132.9, 132.2, 130.9, 127.4, 125.1, 121.8, 118.5, 116.2, 114.7, 112.6, 99.4, 84.2, 82.6, 79.5, 78.2, 76.3, 74.3, 74.0, 73.7, 71.8, 66.6, 57.6, 57.2, 55.9, 51.4, 50.4, 45.6, 44.1, 40.4, 40.2, 40.1, 39.9, 39.8, 39.7, 39.5, 38.5, 35.6, 35.2, 33.3, 33.0, 31.8, 30.0, 28.6, 26.6, 22.0, 21.4, 20.9, 19.7, 16.0, 15.7, 15.4, 14.4, 13.7, 10.9, 10.0; HRMS (ESI): calcd. for $C_{62}H_{88}FN_5NaO_{14}$ $[M+Na]^+$ = 1168.6204. Found = 1168.6184.

Rapa-28-*O*-(2-(4-((2,6-difluorinphenyl)aminomethylene)-1*H*-1,2,3-trizole-1-yl)acetyl) (7d)

This compound was obtained as white solids in 61.9% yield; m.p.: 85.7–88.4°C; MS (ESI) *m/z*: 1186.6000 $(M+Na)^+$; $[a]_D$ −28.9 (c 1.0, MeOH); IR (KBr) cm^{-1}: 3383.1, 2932.4, 1723.6, 1647.0, 1459.9, 1196.3, 1090.5, 999.3. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.84 (s, 1H), 7.07–6.94 (m, 2H), 6.91 (d, *J* = 7.7 Hz, 1H), 6.66 (s, 1H), 6.47 (s, 1H), 6.43–6.35 (m, 1H), 6.24–6.17 (m, 1H), 6.12 (d, *J* = 8.1 Hz, 2H), 5.47–5.38 (m, 1H), 5.28 (d, *J* = 24.9 Hz, 2H), 5.12 (d, *J* = 31.5 Hz, 1H), 5.03–4.92 (m, 2H), 4.59 (t, *J* = 16.4 Hz, 1H), 4.48 (d, *J* = 6.0 Hz, 2H), 4.42–4.25 (m, 1H), 3.96 (dd, *J* = 30.0, 24.2 Hz, 1H), 3.65 (t, *J* = 20.7 Hz, 1H), 3.46 (d, *J* = 11.9 Hz, 1H), 3.31 (s, 3H), 3.29 (s, 1H), 3.19 (s, 3H), 3.15–3.09 (m, 2H), 3.05 (s, 3H), 2.82 (s, 1H), 2.65 (d, *J* = 16.2 Hz, 1H), 2.39 (dd, *J* = 17.5, 8.8 Hz, 1H), 2.22 (s, 1H), 2.13 (s, 1H), 2.04–1.97 (m, 1H), 1.85 (s, 3H), 1.77 (s, 3H), 1.68 (s, 3H), 1.62 (s, 3H), 1.59–1.50 (m, 5H), 1.42 (d, *J* = 10.4 Hz, 2H), 1.27 (s, 2H), 1.17 (dd, *J* = 22.2, 8.5 Hz, 3H), 1.05 (d, *J* = 12.6 Hz, 3H), 0.99 (d, *J* = 5.1 Hz, 3H), 0.89–0.84 (m, 3H), 0.82 (s, 3H), 0.74 (d, *J* = 5.2 Hz, 6H), 0.57 (d, *J* = 11.3 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 209.1, 207.4, 199.4, 169.3, 166.2, 153.6, 152.2, 146.5, 139.6, 138.4, 132.8, 132.1, 130.9, 127.4, 125.6, 124.3, 117.5, 112.8, 112.2, 102.1, 99.4, 84.3, 82.6, 82.2, 78.0, 74.2, 73.7, 71.8, 66.6, 57.6, 57.2, 55.9, 51.3, 50.4, 45.6, 41.0, 40.4, 40.2, 40.1, 40.0, 39.8, 39.7, 39.5, 35.5, 35.1, 33.0, 31.8, 30.3, 28.6, 26.6, 24.9, 22.0, 19.6, 16.0, 15.7, 15.4, 14.4, 13.8, 10.9, 10.0; HRMS (ESI): calcd. for $C_{62}H_{87}F_2N_5NaO_{14}$ $[M+Na]^+$ = 1186.6110. Found = 1186.6088.

Rapa-28-*O*-(2-(4-(2-hydroxylisopropyl)-1*H*-1,2,3-trizole-1-yl)-acetyl) (7e)

This compound was obtained as white solids in 37.7% yield; m.p.: 94.7–96.8°C; MS (ESI) *m/z*: 1080.6000 $(M+Na)^+$; $[a]_D$ −12.8 (c 1.0, MeOH); IR (KBr) cm^{-1}: 3452.6, 2939.7, 2355.6, 1722.6, 1642.2, 1454.8, 1374.9, 1196.2, 1089.6, 991.3. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.81 (s, 1H), 6.47 (d, *J* = 1.1 Hz, 1H), 6.40 (dd, *J* = 14.5, 11.3 Hz, 1H), 6.22 (dd, *J* = 14.7, 10.7 Hz, 1H), 6.14 (dd, *J* = 26.7, 12.2 Hz, 2H), 5.42 (dd, *J* = 17.3, 7.7 Hz, 1H), 5.34 (d, *J* = 3.0 Hz, 1H), 5.12 (d, *J* = 6.4 Hz, 1H), 5.07 (d, *J* = 3.3 Hz, 1H), 4.99–4.93 (m, 2H), 4.61 (d, *J* = 4.3 Hz, 1H), 4.31 (d, *J* = 3.9 Hz, 1H), 4.30–4.24 (m, 1H), 4.04–3.97 (m, 1H), 3.63 (d, *J* = 13.3 Hz, 1H), 3.46 (d, *J* = 12.5 Hz, 1H), 3.31 (s, 3H), 3.28 (d, *J* = 4.4 Hz, 1H), 3.20 (s, 3H), 3.15 (d, *J* = 4.5 Hz, 2H), 3.05 (s, 3H), 2.84–2.80 (m, 1H), 2.67 (dd, *J* = 17.7, 2.8 Hz, 1H), 2.43 (dd, *J* = 17.7, 8.3 Hz, 2H), 2.21 (d, *J* = 8.6 Hz, 1H), 2.12 (s, 1H), 2.04–2.01 (m, 1H), 1.88–1.81 (m, 3H), 1.77 (s, 3H), 1.69–1.65 (m, 3H), 1.62 (s, 3H), 1.60–1.50 (m, 5H), 1.47 (s, 6H), 1.44–1.35 (m, 2H), 1.22–1.14 (m, 3H), 1.10–1.01 (m, 3H), 0.99 (d, *J* = 6.5 Hz, 3H), 0.88 (d, *J* = 6.5 Hz, 3H), 0.84 (d, *J* = 6.5 Hz, 3H), 0.74 (t, *J* = 6.0 Hz, 6H), 0.58 (d, *J* = 11.9 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 209.5, 207.4, 199.1, 169.3, 167.3, 166.2, 156.2, 138.2, 132.1, 127.5, 122.3, 99.3, 84.2, 73.6, 67.4, 57.7, 57.2, 55.9, 51.1, 50.5, 45.5, 40.4, 40.2, 40.1, 40.0, 39.8, 39.7, 39.5, 35.6, 32.8, 31.0, 29.9, 26.5, 22.0, 20.9, 16.0, 15.8, 13.9, 10.8; HRMS (ESI): calcd. for $C_{58}H_{88}N_4NaO_{15}$ $[M+Na]^+$ = 1080.6228. Found = 1080.6246.

Rapa-28-*O*-(2-(4-(hydroxyethyl)-1*H*-1,2,3-trizole-1-yl)acetyl) (7f)

This compound was obtained as white solids in 39.7% yield; m.p.: 81.6–83°C; MS (ESI) *m/z*: 1066.6000 $(M+Na)^+$; $[a]_D$ −39.8 (c 1.0, MeOH); IR (KBr) cm^{-1}: 3423.9, 2933.7, 1728.8, 1637.3, 1449.1, 1192.8, 1096.3, 986.8. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.77 (s, 1H), 6.47 (d, *J* = 1.4 Hz, 1H), 6.39 (dd, *J* = 14.7, 11.3 Hz, 1H), 6.25–6.19 (m, 1H), 6.15–6.12 (m, 1H), 6.11 (d, *J* = 10.5 Hz, 1H), 5.50–5.43 (m, 1H), 5.31 (t, *J* = 4.7 Hz, 2H), 5.27 (s, 1H), 5.11–5.06 (m, 1H), 5.00–4.93 (m, 2H), 4.71–4.67 (m, 2H), 4.62–4.57 (m, 1H), 4.34 (d, *J* = 3.7 Hz, 1H), 4.26 (dd, *J* = 18.8, 3.8 Hz, 1H), 4.01 (t, *J* = 9.9 Hz, 1H), 3.66–3.60 (m, 1H), 3.49–3.43 (m, 1H), 3.32–3.29 (m, 3H), 3.29 (d, *J* = 4.4 Hz, 1H), 3.20 (s, 3H), 3.16 (dd, *J* = 6.6, 3.9 Hz, 2H), 3.06 (d, *J* = 4.7 Hz, 3H), 2.84–2.80 (m, 1H), 2.77 (dd, *J* = 12.7, 5.6 Hz, 2H), 2.66 (dd, *J* = 17.6, 2.6 Hz, 1H), 2.40 (dd, *J* = 17.6, 8.4 Hz, 1H), 2.21 (d, *J* = 8.8 Hz, 1H), 2.17–2.12 (m, 2H), 2.02 (dd, *J* = 15.4, 7.4 Hz, 1H), 1.85 (t, *J* = 12.3 Hz, 3H), 1.79 (s, 3H), 1.71–1.65 (m, 3H), 1.62 (s, 3H), 1.60–1.50 (m, 5H), 1.42 (dd, *J* = 26.9, 14.2 Hz, 2H), 1.29–1.23 (m, 2H), 1.22–1.13 (m, 3H), 1.10–1.01 (m, 3H), 0.99 (d, *J* = 6.5 Hz, 3H), 0.86 (d, *J* = 6.5 Hz, 3H), 0.81 (d, *J* = 6.5 Hz, 3H), 0.74 (d, *J* = 6.6 Hz, 6H), 0.57 (dd, *J* = 23.8, 12.0 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 207.4, 199.1, 191.7, 169.6, 167.0, 166.0, 144.9, 138.1, 132.2, 127.5, 123.8, 99.4, 84.2, 82.2, 77.9, 74.2, 73.2, 66.5, 60.8, 57.2, 55.9, 51.4, 50.2, 45.6, 40.4, 40.2, 40.1, 39.9, 39.8, 39.7, 39.5, 35.4, 32.9, 31.7, 29.8, 29.5, 22.0, 16.0, 15.7, 15.4, 14.3, 13.4, 10.8; HRMS (ESI): calcd. for $C_{57}H_{86}N_4NaO_{15}$ $[M+Na]^+$ = 1066.6058. Found = 1066.6090.

4.2 | Biological assays

4.2.1 | Reagents

Rapamycin (Fujian Kerui Pharmaceutical Co. Ltd.) and new rapalogs were dissolved in dimethyl sulfoxide (DMSO) to prepare a 10.0 mg/mL stock solution and stored at −20°C. Sulforhodamine B (SRB, Sigma) was dissolved in 1% glacial acetic acid to prepare a 0.4% solution. Antibodies included those against S6K1, 4E-BP1, mTOR, phospho-S6K1 (Thr389), phospho-4E-

BP1 (Thr70), phospho-mTOR (Ser2448) were purchased from Cell Signaling, USA.

4.2.2 | Cell culture

All cancer cells were purchased from Cell Bank of Chinese Academy of Sciences, Shanghai Institute of Cell Biology (Shanghai, China). Renal cell adenocarcinoma 769-P and esophageal squamous carcinoma ECA-109 were maintained in RPMI-1640 medium (Gibco, USA). Lung carcinoma A549 was maintained in F12 medium (Gibco). Cervical cancer cell line CASKI cells were maintained in RPMI medium (Gibco, USA), prostate adenocarcinoma PC-3 was maintained in F12 medium (Gibco). All media were supplemented with 10% fetal bovine serum (Gibco) and gentamycin (80.0 U/mL), and all cells were grown in a humid incubator (37°C, 5% CO_2).

4.2.3 | Anticancer activities

The anticancer activities of the compounds were evaluated against A549, PC-3, CASKI, 769-P, ECA-109 cancer cell lines by sulforhodamine B (SRB, Sigma) assay *in vitro*, as described previously.[21]

Results were expressed as mean ± standard deviation (SD) of independent experiments, and statistical analysis was performed using Student's *t*-test. All statistical analyses were performed using SPSS 20 statistical analysis software. Values with $p < 0.05$ were considered to indicate statistical significance.

4.2.4 | Apoptotic cell staining

Annexin V and propidium iodide (PI) staining analysis was performed following the manufacturer's instruction (Vazyme Biotech, China). Briefly, A549 cells were seeded at a density of 10^5 cells/well on 6-well plates at 37°C for 24 h, then treated with compounds and incubated for another 48 h, following washed in cold phosphate-buffered saline (PBS, Gibco). Cells (1×10^6 cells/mL) were stained with a mixture containing 500.0 µL of 1 × binding buffer, 5.0 mL of Annexin V (Annexin-V-FITC), and 5.0 µL of PI, incubated at room temperature in the dark for 5 min, and cells apoptosis rate was conducted using a BD ACCURIC6 flow cytometer (BD Biosciences).

4.2.5 | Cell cycle analysis

Cell cycle analysis was also performed following the manufacturer's instruction (Vazyme Biotech, China). Briefly, after treated with **6c** or rapamycin for 48 h, A549 cells were washed with cold PBS and fixed in 70% ethanol at 4°C for 12 h. Then cells were washed again and suspended in 200 µL of cold PBS. Twenty microliter of RNase solution were added to the cell suspension and maintained at 37 °C for 30 min and then the cell suspension was stained in 400 µL of PI staining solution at 4°C for 30 min in the dark. The measurements were performed using a BD ACCURIC6 flow cytometer.

4.2.6 | Western blotting analysis

Western blotting analysis was performed as described previously.[17] Briefly, cells were lysed in 2% SDS. Equal amounts of lysate protein were subjected to SDS–PAGE and transferred onto nitrocellulose membranes (Bio-Rad Laboratories, USA). The target protein carrying membranes were blocked with 5% milk and incubated with the primary antibody at 4°C overnight. The membranes were then washed in TBST buffer three times and incubated with the secondary antibody for 1 h. Wash the membrane as previous procedure. Bands were visualized by using the enhanced chemiluminescence Western blotting detection system (GE, USA).

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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