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Structure-activity relationship of leucyladenylate sulfamate analogues as leucyl-tRNA synthetase (LRS)-targeting inhibitors of Mammalian target of rapamycin complex 1 (mTORC1)

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ABSTRACT

Leucyl-tRNA synthetase (LRS) plays an important role in amino acid-dependent mTORC1 signaling, which is known to be associated with cellular metabolism and proliferation. Therefore, LRS-targeting small molecules that can suppress mTORC1 activation may provide an alternative strategy to current anticancer therapy. In this work, we developed a library of leucyladenylate sulfate analogues by extensively modifying three different pharmacophoric regions comprising adenine, ribose and leucine. Several effective compounds were identified by cell-based mTORC1 activation assays and further tested for anticancer activity. The selected compounds mostly exhibited selective cytotoxicity toward five different cancer cell lines, supporting the hypothesis that the LRS-mediated mTORC1 pathway is a promising alternative target to current therapeutic approaches.

1. Introduction

Mammalian/mechanistic target of rapamycin (mTOR) is a multi-protein complex that functions as a serine-threonine protein kinase. mTOR is known to be involved in various signaling pathways that govern cell growth and immune responses. mTOR consists of two distinct protein domains, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). It has been reported that S6 kinase 1 (S6K1) and the translation regulators eukaryotic translation initiation factor 4E (elF4E)-binding protein 1 (4E-BP1) are the two major substrates for mTORC1 and participate in the signal transduction pathway regulating protein synthesis.[1] Given its crucial role in cell growth and metabolism, it is not surprising that overactivity of mTORC1 has been linked to a wide range of human diseases, such as diabetes, neurodegenerative diseases, and cancers.[2–4] Extracellular signals such as growth hormones and amino acids activate the mTOR pathway. In particular, the phosphorylation of S6K1 and 4E-BP1 by activated mTORC1 triggers downstream pathways involving biosynthesis and proliferation.[5] Because specific inhibition of mTORC1 can suppress cell growth and proliferation, rapamycin and their analogues, also known as rapalogs, have been widely tested for anticancer activity.[6,7] While these compounds are highly specific allosteric inhibitors of mTORC1, rapamycin treatment alone did not appear to generate sufficient anticancer activity.[8,9] Several studies have also suggested that a partial blockade of the mTORC1-related pathway could cause rapamycin-resistance in cancer cells. These results imply that the mTOR pathway constitutes a promising new drug target. It may also be possible to develop alternative, more effective therapeutic strategies in addition to rapalogs.

It has been reported that leucine and arginine are the two major amino acids that regulate the amino acid-dependent activation of the mTORC1 signaling pathway.[10,11] Although it is still unclear how these amino acids activate the mTORC1 pathway, recent studies have proposed that leucyl-tRNA synthetase (LRS) may play a critical role in leucine sensing by activating RagD GTPase, which subsequently

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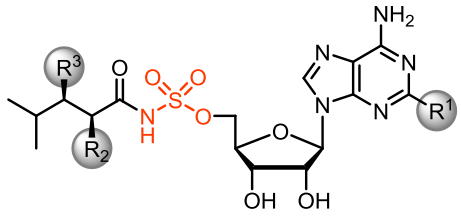


1 R^1 = H R^2 = NH_2 R^3 = H
2 R^1 = H R^2 = OH R^3 = OH
3 R^1 = I R^2 = OH R^3 = H

Figure 1. Representative leucyladenylate sulfamate analogues.

promotes the amino acid-dependent mTORC1 activation.[12,13] LRS, a class I aminoacyl-tRNA synthetase (ARS), catalyzes the ATP-dependent ligation of leucine to its tRNA to form leucyl tRNA. Since LRS acts as a GTPase-activating protein (GAP) for Rag GTPase, molecules that bind to LRS and interfere with the Rag GTPase activity are also likely to block the mTORC1 activation.[12] As a proof of concept, it has been demonstrated that leucinol, a leucine analogue, was able to block the amino acid-mediated activation of mTORC1 without affecting the catalytic function of LRS.[14,15] Considering that this LRS-mediated mTORC1 activation is independent of the mTOR-rapamycin interaction, LRS inhibitors such as leucinol may provide an alternative therapeutic option to suppress the mTORC1 activity and overcome rapamycin resistance.

Based on this idea, we have previously developed the leucyladenylate sulfamate analogues as mTORC1 inhibitors that specifically bind to LRS.[16] Among the three representative compounds (**1–3**, Fig. 1) identified in our previous study, leucyladenylate sulfamate **1** blocked the catalytic activity of LRS but did not affect leucine-induced mTORC1 activation, whereas compound **3** inhibited mTORC1 activation without markedly compromising the catalytic activity of LRS compared to compounds **1** and **2**. More importantly, our mechanistic study indicated that mTORC1 inhibition by compounds **2** and **3** was not due to direct interaction with mTOR but from blocking the leucine-sensing ability of LRS. While both compounds **2** and **3** demonstrated anticancer activity, only compound **3** demonstrated specific activity against colon cancer cells expressing overactive mTORC1.

In this report, we developed a series of novel leucyladenylate sulfate analogues to optimize their anticancer activities. We divided the leucyladenylate sulfamate scaffold into three pharmacophoric regions, the base (adenine), ring (ribose) and side chain (leucyl sulfamate), as described in Fig. 2. We modified each region extensively by adding and deleting functional groups and synthesized a library of compounds that were further tested for *in vitro* activity against leucine-induced mTORC1 activation. Several compounds with high activity were selected to assess their cytotoxicities against various cancer cell lines.

2. Results and discussion

2.1. Chemistry

For the syntheses of leucyladenylate sulfamate and its N^6-methyladenylate derivatives (Scheme 1), the key intermediate 5′-*O*-sulfamoyl-2′,3′-*O*-diacetyl adenosine was prepared from adenosine in 4 steps.[16,17] The sulfamate was coupled with the corresponding acids, and then deacetylation with sodium methoxide provided the leucyladenylate sulfamate derivatives (**5**). The N^6-methyladenylate derivatives (**6**) were synthesized from N^6-methyladenosine, which was prepared from adenosine in 3 steps by following the same method described for the synthesis of **4**.

For the syntheses of 2-chloroadenylate, 2-iodoadenylate and inosine derivatives (Scheme 2), commercially available 2-chloroadenosine was converted to 2-chloroadenylate derivatives (**6**) by following the method of Scheme 1. 2-Iodoadenylate derivatives (**7**) were synthesized from 5′-*O*-sulfamoyl 2-iodoadenosine, which was prepared from guanosine in 4 steps.[16] The synthesis of inosine derivatives (**8**) was accomplished from inosine by following the methods described for the synthesis of **4**.

For the syntheses of 2′-deoxyadenylate and 3′-deoxyadenylate arabinose derivatives (Scheme 3), commercially available 2′-deoxyadenosine and 3′-deoxyadenosine, which was prepared from adenosine in 5 steps, were converted to 2′-deoxyadenylate (**9**) and 3′-deoxyadenylate (**10**) derivatives, respectively, by following the method in Scheme 1. The synthesis of arabinose derivatives (**11**) was carried out from commercially available adenine 9-β-D-arabinofuranoside by following the same route for the corresponding ribose derivatives (**4**).

For the syntheses of 3′-deoxy-3′-aminoadenylate derivatives (Scheme 4), the *N*-benzyl-2′,3′-oxazolone derivative of the key intermediate 3′-amino-3′-deoxyadenosine was prepared from adenosine in 5 steps according to a previous report.[18] The benzyl group of the intermediate was readily cleaved by the mild process of oxidative debenzylation using alkali metal bromide.[19] The removal of the 5′-TBDPS group followed by sulfamoylation provided the 5′-*O*-sulfamoyl 2′,3′-oxazolone intermediate, which was converted to 3′-deoxy-3′-aminoadenylate (**12**) and its oxazolone (**13**) derivatives according to the above method.

2.2 Biological activity

To assess the biological activity of the synthesized derivatives, we first determined the inhibition of leucine-induced phosphorylation of S6 Kinase (S6K) in HEK293 cells by performing Western blot analysis.[16] In this assay, we pretreated HEK293 cells with each compound at a fixed concentration (200 μM) together with compound **2** (200 μM), rapamycin (100 nM) and leucinol (800 μM), followed by the addition of leucine for 10 min to activate mTORC1. We first examined a group of six compounds (**14–19**) that contained a modified functional group in each pharmacophoric region including the base, ring, and side chain of the previously reported compound **2**. The newly introduced or modified moieties are marked in red in Fig. 3. Based on the band intensity of the phosphorylated S6K (pS6K), we found that compounds **16** and **17** showed more potent inhibition of S6K phosphorylation than did compound **2**, while compound **18** demonstrated comparable activity. Compounds **14**, **15**, and **19** did not inhibit S6K phosphorylation at the same concentration. When we further analyzed S6K phosphorylation at four different concentrations (20, 50, 100, and 200 μM) of compounds **16** and **17**, both compounds demonstrated dose-dependent inhibition, while the effect of compound **16** appeared to be more potent than **17**. None of the tested compounds affected the amount of the total Akt or phosphorylated Akt (pAkt), indicating that these effects are only specific toward mTORC1. Overall, it appears that the 2′-deoxyribose group is favorable for inhibition (compound **16**), and the 3′-deoxy side chain and (*S*)-2-hydroxy-4-methylpentanoyl group do not adversely affect inhibition (compound **17**). The stereochemistry of the leucyl side chain and the 3′-deoxyribose does not have any significant effect on inhibition, while the 2-iodoadenine (compound **18**) is likely to be beneficial for its effect.

Next, we tested compounds **20–27** for additional SAR analysis, and the results are described in Fig. 4. In this group, compounds **22**, **26**, and **27** exhibited improved activity compared to compound **2,** while compounds **20** and **25** maintained comparable inhibition. On the other hand, compounds **21**, **23**, and **24** did not show any activity. These results suggest that the modification of the adenine to a hypoxanthine group improved inhibition (compounds **26–27**) while the modification of ribose to arabinose did not affect activity (compounds **21–22**). Incorporation of the 2-chloro group in the adenine also did not appear to enhance inhibition (**23–25**).

We next focused on diversifying the leucyl side chain with various heterocycles and inosine analogues. As shown in Fig. 5, compounds

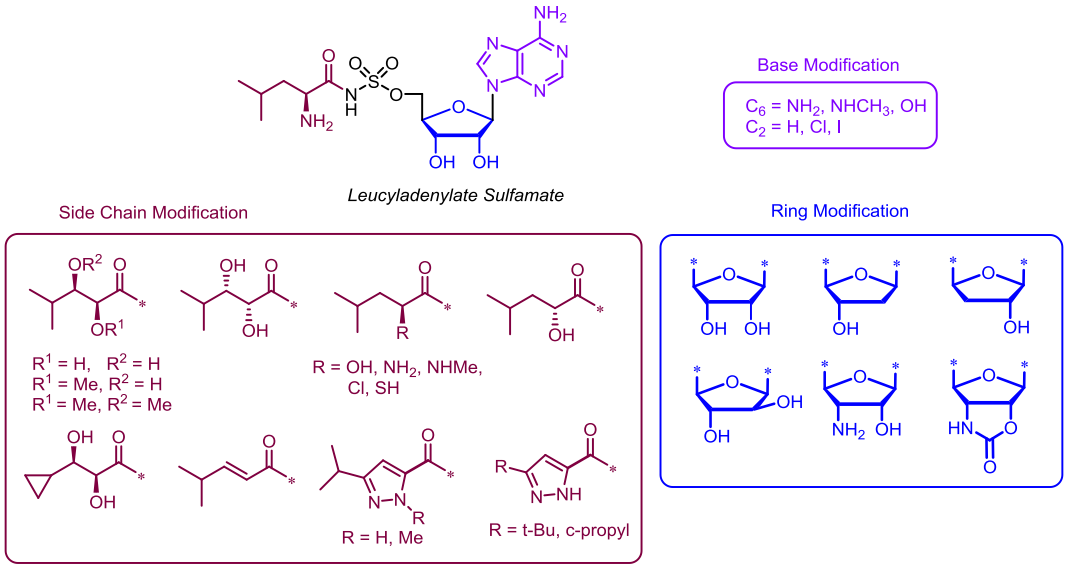
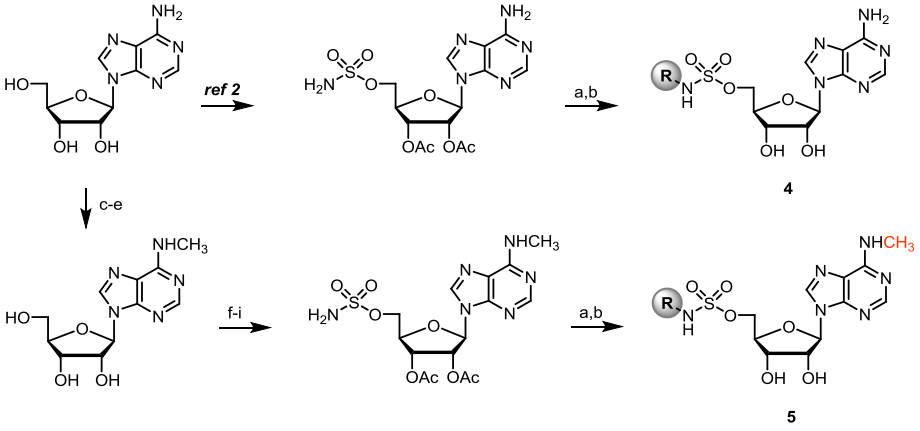
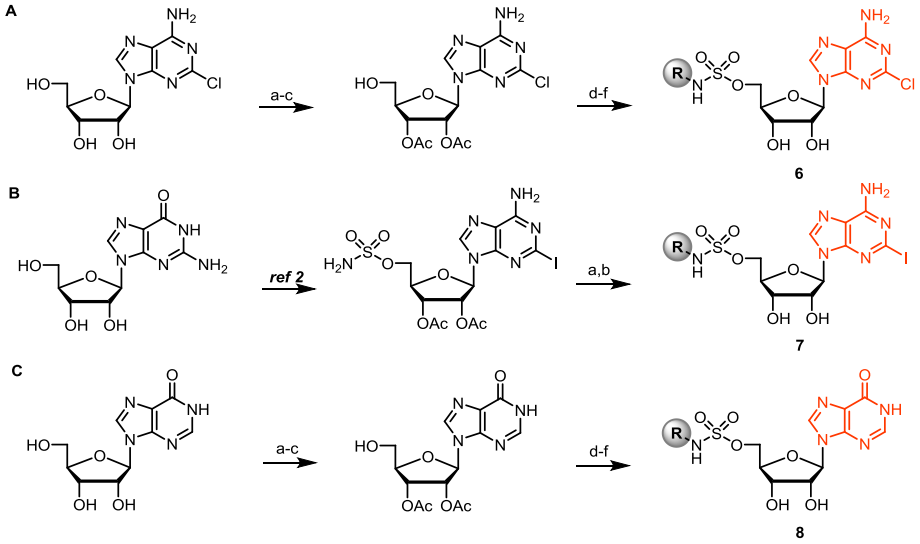


Figure 2. Structure-activity relationship of leucyladenylate sulfamate.

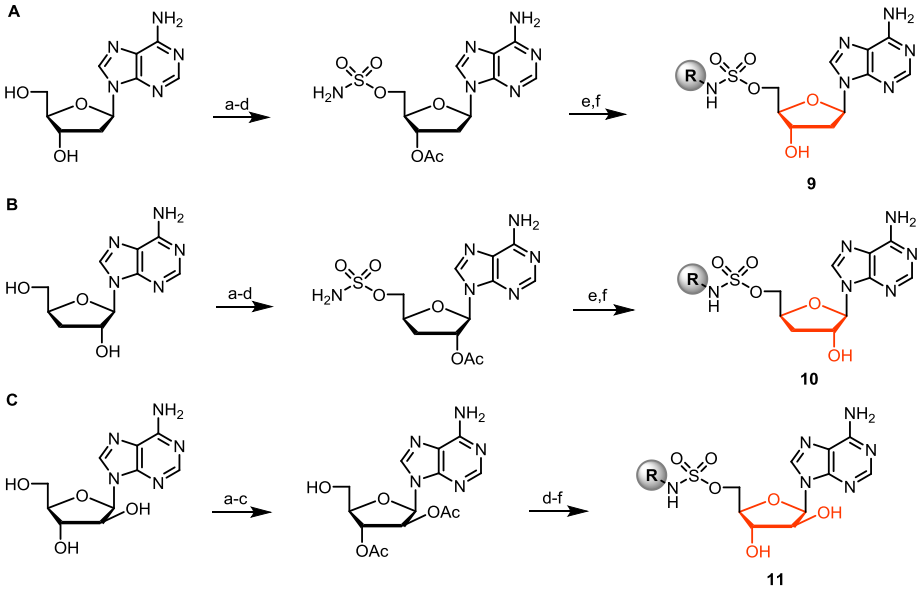


Scheme 1. Synthesis of leucyladenylate sulfamate and its N^6-methyl derivatives. *Reagents and conditions*: (a) acid, DCC, DMAP, anhyd. CH_2Cl_2, r.t., 12 h, 45 ~ 80%; (b) 0.02 M NaOMe in MeOH, r.t., 2 h, 68 ~ 85%; (c) Ac_2O, pyridine, 60 °C, 8 h; (d) CH_3Br, DBU, MeCN, r.t.; (e) 7 M NH_3 in MeOH; (f) MMTCl, anhyd. pyridine, anhyd. DMF, 0 °C to r.t., 24 h; (g) Ac_2O, TEA, DMAP, CH_3CN, 0 °C to r.t., 4 h; (h) 80% aq. AcOH, r.t., 12 h; (i) i) NaH, anhyd·THF, 0 °C, 1 h and ii) NH_2SO_2Cl, anhyd·THF, 0 °C to r.t., 5 h.

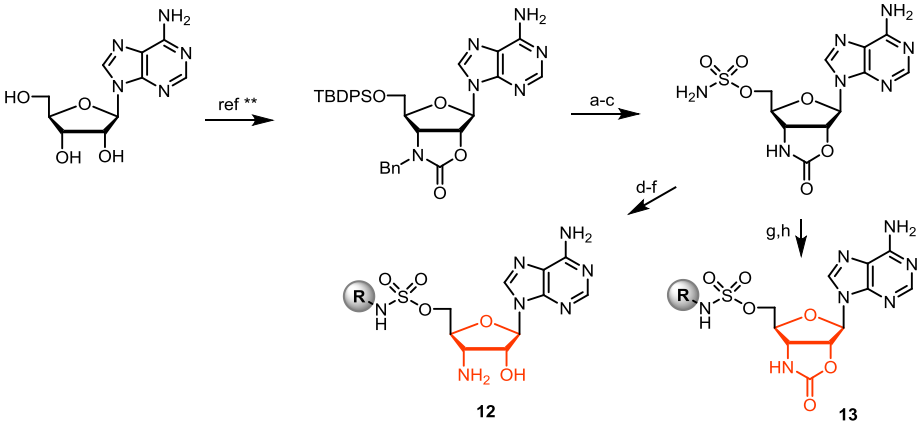
29–30 showed potent activity compared to compound **2** or leucinol. Compounds **28** and **32–34** appeared to demonstrate similar inhibitory effect compared to compound **2**, whereas compounds **31** and **35–37** did not inhibit mTORC1. Overall, SAR analysis of this group of compounds indicated that the addition of a rigid cyclopropyl group at the end of the leucyl side chain (**28**, **29**, **34**, and **37**) demonstrated comparable activity, except for compound **37**. In addition to the cyclopropyl group in the side chain region, the modification of the 2′,3′-dihydroxyl group of ribose into oxazolone (**34**) favorably affected its activity. The replacement of the leucyl group with a bioisosteric 1-methyl pyrazole



Scheme 2. Synthesis of 2-chloroadenylate, 2-iodoadenylate and inosine derivatives. *Reagents and conditions*: **Route A:** (a) MMTCl, anhyd. pyridine, anhyd. DMF, 0 °C to r.t., 24 h; (b) acetic anhydride, TEA, DMAP, CH_3CN, 0 °C to r.t., 4 h; (c) 80% aq. AcOH, r.t., 12 h; (d) i) NaH, anhyd·THF, 0 °C, 1 h and ii) NH_2SO_2Cl, anhyd·THF, 0 °C to r.t., 5 h; (e) acid, DCC, DMAP, anhyd. CH_2Cl_2, r.t., 12 h; (f) 0.02 M NaOMe in MeOH, r.t., 2 h. **Route B:** (a) acid, DCC, DMAP, anhyd. CH_2Cl_2, r.t., 12 h, 45 ~ 80%; (b) 0.02 M NaOMe in MeOH, r.t., 2 h, 68 ~ 85%. **Route C:** (a) MMTCl, anhyd. pyridine, anhyd. DMF, 0 °C to r.t., 24 h; (b) acetic anhydride, TEA, DMAP, CH_3CN, 0 °C to r.t., 4 h; (c) 80% aq. AcOH, r.t., 12 h; (d) i) NaH, anhyd·THF, 0 °C, 1 h and ii) NH_2SO_2Cl, anhyd·THF, 0 °C to r.t., 5 h; (e) acid, DCC, DMAP, anhyd. CH_2Cl_2, r.t., 12 h; (f) 0.02 M NaOMe in MeOH, r.t., 2 h.



Scheme 3. Synthesis of 2′-deoxyadenylate derivatives. *Reagents and conditions*: **Route A:** (a) MMTCl, anhyd. pyridine, anhyd. DMF, 0 °C to r.t., 24 h, 53%; (b) Ac_2O, TEA, DMAP, CH_3CN, 0 °C to r.t., 4 h, 89%; (c) 80% aq. AcOH, r.t., 12 h, 48%; (d) i) NaH, anhyd·THF, 0 °C, 1 h and ii) NH_2SO_2Cl, anhyd·THF, 0 °C to r.t., 5 h, 77%; (e) acid, DCC, DMAP, anhyd. CH_2Cl_2, r.t., 12 h, 25 ~ 45%; (f) 0.02 M NaOMe in MeOH, r.t., 2 h, 30 ~ 50%. **Route B:** (a) TBDPSCl, TEA, DMF, DMAP, r.t., overnight, 73%; (b) Ac_2O, pyridine, 60 °C, 8 h, 93%; (c) TBAF, THF, 0 °C, 1 h, 89%; (d) NH_2SO_2Cl, NaH, anhyd·THF, 0 °C to r.t., 5 h, 75%; (e) acid, DCC, DMAP, anhyd. CH_2Cl_2, r.t., 12 h, 65–69%; (f) 0.02 M NaOMe in MeOH, r.t., 2 h, 30–65%. **Route C:** (a) MMTCl, anhyd. pyridine, anhyd. DMF, 0 °C to r.t., 24 h; (b) Ac_2O, TEA, DMAP, CH_3CN, 0 °C to r.t., 4 h; (c) 80% aq. AcOH, r.t., 12 h; (d) i) NaH, anhyd·THF, 0 °C, 1 h and ii) NH_2SO_2Cl, anhyd·THF, 0 °C to r.t., 5 h; (e) acid, DCC, DMAP, anhyd. CH_2Cl_2, r.t., 12 h; (f) 0.02 M NaOMe in MeOH, r.t., 2 h.



Scheme 4. Synthesis of 3′-deoxy-3′-aminoadenylate derivatives. (a) KBr, oxone, ACN, 70 °C, 24 h, 63%; (b) TBAF, THF, 0 °C to r.t., overnight, 99%; (c) NH_2SO_2Cl, NaH, anhyd·THF, 0 °C to r.t., 5 h, 82%; (d) *N*-Boc-Leu, DCC, DMAP, anhyd·THF, r.t., 12 h, 64%; (e) 1 N NaOH, THF, r.t., 48 h; (f) 1 N HCl in MeOH, r.t., overnight; (g) acid, DCC, DMAP, anhyd·THF:DMF, r.t., 12 h, 44–55%; (h) 0.02 M NaOMe, r.t., 4 h, 87–90%.

surrogate also appeared to help maintain their activity (**32**, **33**). The replacement of the (*S*)-2-hydroxyl group with a chlorine improved mTORC1 inhibition (**30**); however, when the adenine was replaced with hypoxanthine, the inversion of the same stereocenter with (*R*)-2-hydroxyl (**35**) or the replacement of the (*S*)-2-hydroxyl with a sulfhydryl group (**36**) adversely affected inhibition.

We also tested another group of compounds (**38–46**) that contained similarly modified functional scaffolds as described in Fig. 6. In this series, only compounds **39**, **41** and **42** maintained mTORC1 inhibition, while the remaining compounds showed much less activity. Interestingly, we found that compounds **38–40** only differ by one substituent in the 3-position of pyrazole; however, only **39** showed inhibitory effect. It should also be noted that compounds **41** and **42** appeared to show better activity compared to their hypoxanthine-containing counterparts (**35** and **36**), suggesting that the adenine group is important for activity. The replacement of the (*S*)-2-hydroxyl group in compound **42** with an *N*-methyl amino group (**43**) appeared to adversely affect its activity. Neither 3′-deoxy-3′-aminoadenylate (**44**) nor 2′-deoxyadenylate (**45**) inhibited S6K phosphorylation, and 2′-deoxyadenylate with a (2*R*,3*S*)-2,3-dihydroxy side chain (**46**) also showed no activity.

In our final group of compounds, we mostly focused on derivatizing the leucyl side chain with a slight modification of the ribose or adenine. As shown in Fig. 7, compounds in this series did not inhibit the phosphorylation of S6K in general. However, some of the compounds tested, particularly compounds **47–49**, appeared to inhibit the phosphorylation of AKT, which is catalyzed by activated mTORC2 but not mTORC1. Contrary to our observations with compound **16**, when the leucyl side chain of the 2′-deoxyadenylate was modified with a (*S*)-2-hydroxyl side chain (**49**) or a (2*S*,3*R*)-2-methoxy-3-hydroxyl side chain (**51**), compounds **49** and **51** lost their activities, probably due to their significant structural differences with (*R*)-3-hydroxyl or the leucine group. In addition, unlike compound **30**, which contained 2-chloroadenylate with a (*S*)-2-chloro side chain, compound **52** (adenine without chlorine substituent) and compound **53** (N^6-methyladenylate) also showed no activity, indicating that the substituents in the adenine group play a significant role in activity. Compound **56** also lost activity when its ribose was modified to oxazolone. Although we observed favorable activity with replacement of adenine with hypoxanthine in compound **26** and favorable interaction with a cyclopropyl side chain at the end of the leucyl side chain, compound **57** containing both of these groups did not show any activity.

For overall comparison, we repeated the Western blot experiments with our previously published compounds **1–3** and newly synthesized compounds **17–18** in one panel as described in Fig. 8. In this blot, it is clearly shown that the substitution of the (*S*)-2-amine group with a (*S*)-2- hydroxyl group improved the activity (compound **17**) compared to compound **1**, and the additional modification of the 2-iodoadenine further enhanced activity (compound **3**); however, the addition of a (*R*)-3-hydroxyl group to the same compounds (compounds **2** and **18**) did not appear to improve activity.

Based on these SAR analyses, we selected a total of 8 compounds to assess anticancer activity by performing sulforhodamine B (SRB)

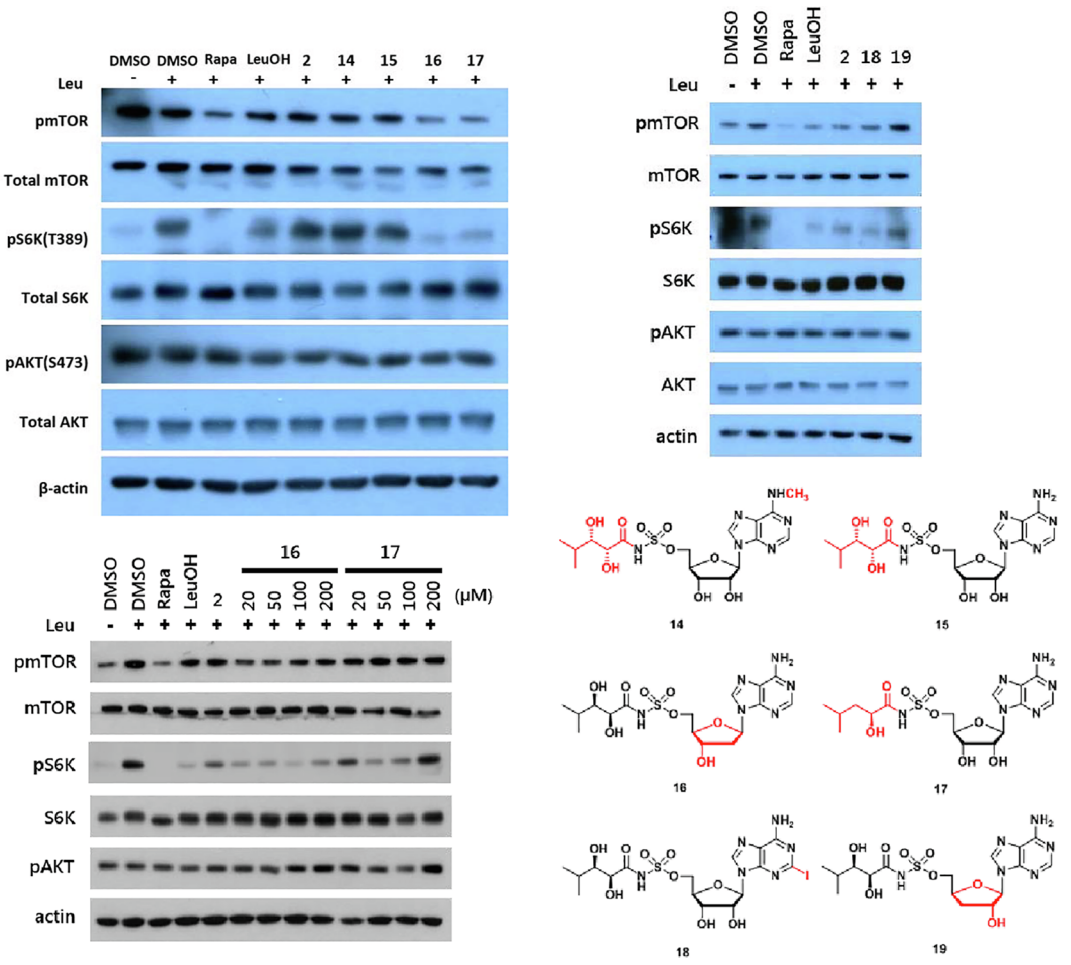


Figure 3. Inhibition of leucine-induced mTORC1 activation in HEK293 cells treated with compounds **14–19.**

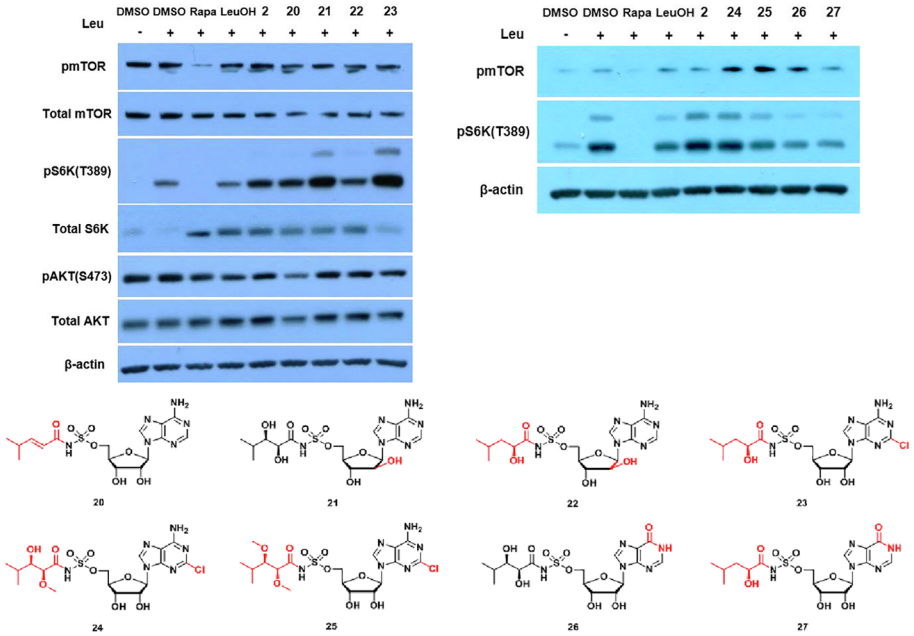


Figure 4. Inhibition of leucine-induced mTORC1 activation in HEK293 cells treated with compounds **20–27**.

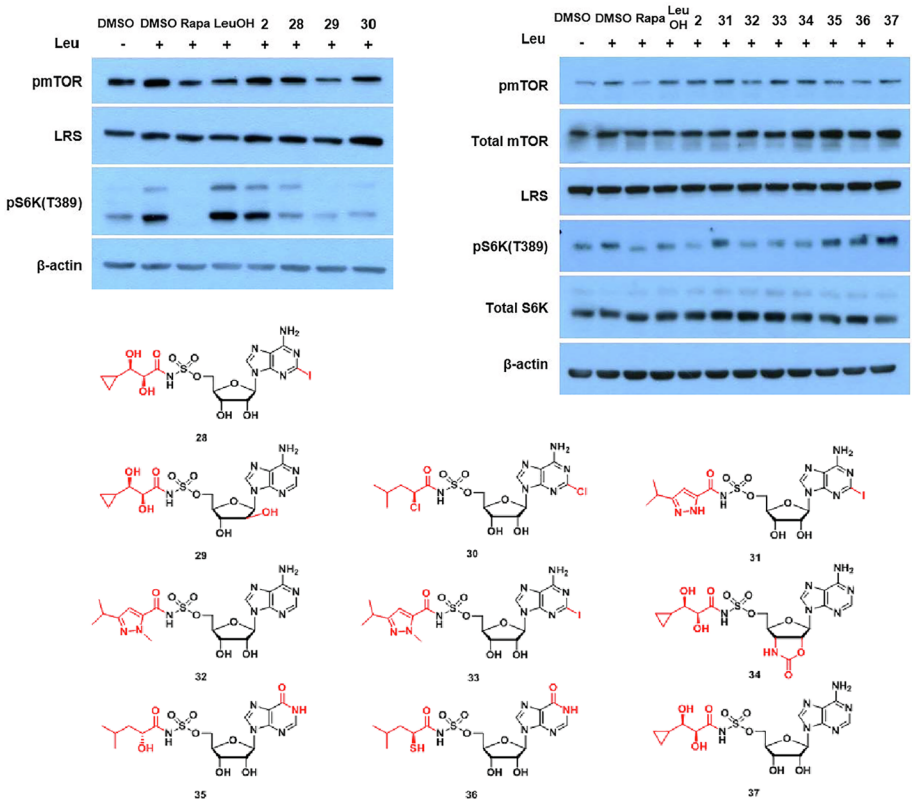


Figure 5. Inhibition of leucine-induced mTORC1 activation in HEK293 cells treated with compounds **28–37.**

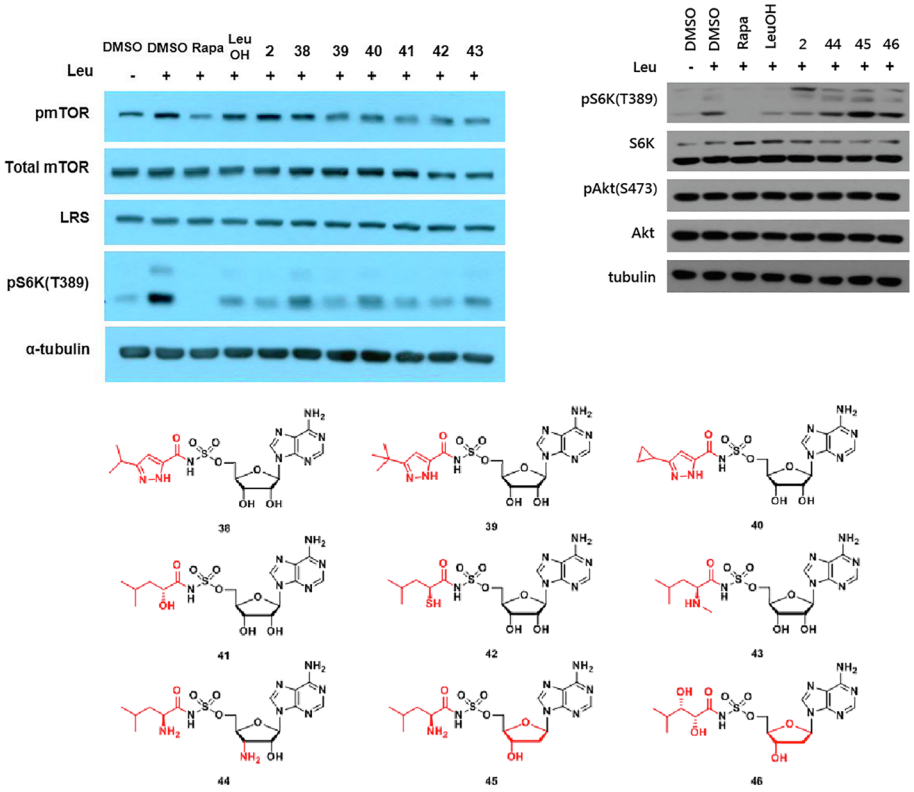


Figure 6. Inhibition of leucine-induced mTORC1 activation in HEK293 cells treated with compounds **38–46.**

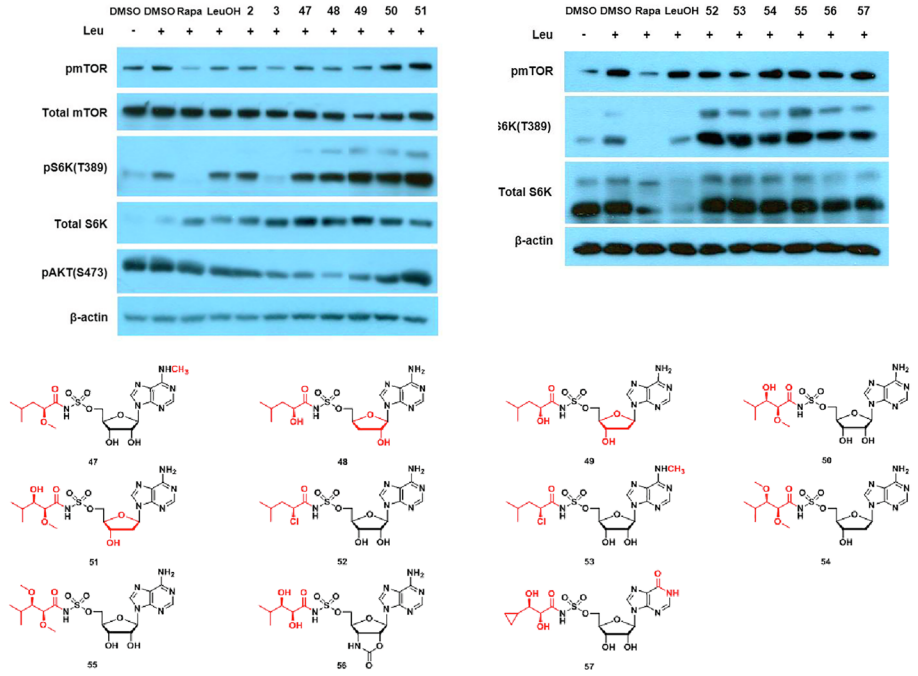


Figure 7. Inhibition of leucine-induced mTORC1 activation in HEK293 cells treated with compounds **47**–**57**.

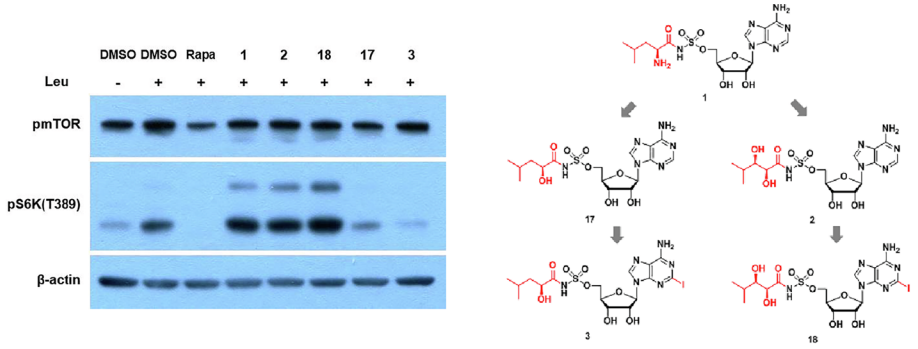


Figure 8. Inhibition of leucine-induced mTORC1 activation in HEK293 cells treated with compounds **1**–**3, 17** and **18**.

Table 1
Antitumoral activities of the selected leucyladenylate sulfamates on the growth of various cancer cell lines.[a]

IC_{50} (μM)	A549	HCT116	MDA-MB-231	SK-HEP-1	SNU638	MRC5
16	5.04	6.52	6.27	5.74	4.72	> 20
17	1.00	3.47	2.61	1.76	4.50	16.65
25	> 20	> 20	> 20	> 20	> 20	> 20
26	> 20	> 20	> 20	> 20	> 20	> 20
27	> 20	> 20	> 20	> 20	> 20	> 20
28	4.84	7.27	16.72	5.86	4.77	> 20
29	6.93	9.14	17.78	7.26	5.70	> 20
30	6.27	7.51	7.75	5.87	5.50	> 20
Etoposide	0.47	1.22	3.38	0.41	0.34	12.72

[a] A549, lung cancer cells; HCT116, colon cancer cells; MDA-MB-231, breast cancer cells; SK-Hep-1, liver cancer cells; SNU638, stomach cancer cells; MRC5, normal lung epithelial cells.

colorimetric assays.[20] The selected compounds were tested in five different cancer cell lines together with etoposide as a positive control, and the results are summarized in Table 1. Most of the tested compounds generally showed moderate anticancer activity, having IC_{50} values in the low micromolar range, except for compounds **25**–**28**. More importantly, these compounds were active against cancer cells while showing marginal to low cytotoxicity against normal cells (MRC5). We believe that these results again support our hypothesis that the selective inhibition of the mTORC1 pathway by LRS-targeting molecules is a viable strategy for the development of anticancer agents.

3. Conclusions

In this work, we investigated the structure-activity relationship of a series of leucyladenylate sulfamate derivatives as LRS-targeted mTORC1 inhibitors. We modified each of three pharmacophoric regions in the leucyladenylate sulfamate scaffold consisting of adenine, ribose and a leucyl side chain, and evaluated their inhibitory effects by cell-based assays. Our SAR analyses indicated that the structural modifications in any one of these regions, adenine, ribose and leucine, can affect the biological activity significantly likely due to the small binding pocket in the LRS. In particular, the modification of the leucyl side chain region appears to be most critical, although this is not surprising given that these compounds inhibit mTORC1 by affecting leucine-sensing of LRS. When we further tested several selected compounds in a panel of cancer cell lines, compounds **16**, **17**, **28**, **29** and **30** were found to be effective at low micromolar IC_{50} concentrations without significant cytotoxicity toward normal cells. We believe that the leucyladenylate sulfamate analogues possess highly specific activity toward the LRS-mediated mTORC1 inhibition without significantly affecting the total expression of mTOR complex or LRS, which is an

advantageous and promising feature for potential anticancer therapy. Currently, we are optimizing the pharmacophore via ligand-based modeling and cell-based assays to generate LRS-targeting mTORC1 inhibitors with favorable pharmacokinetic properties for future *in vivo* applications.

4. Experimental

4.1. General experimental

All chemical reagents were commercially available. Melting points were determined on a Büchi Melting Point B-540 apparatus and are uncorrected. Silica gel column chromatography was performed on silica gel 60, 230–400 mesh, Merck. ^{1}H NMR spectra were recorded on a JEOL JNM-LA 300 at 300 MHz, Bruker Avance DE/AVANCE Digital 500 at 500 MHz and a JEOL JNM-ECA-600 at 600 MHz. Chemical shifts are reported in ppm units with Me_4Si as a reference standard. Mass spectra were recorded on a VG Trio-2 GC–MS and 6460 Triple Quad LC/MS.

4.1.1. Sulfamoylation

To a solution of starting material (1 eq) in anhydrous THF was added NaH (3 eq) slowly at 0 °C. After stirring for 30 min, freshly prepared sulfamoyl chloride (3 eq) was added. The reaction mixture was gradually warmed to room temperature and stirred for several hours. After the starting material disappeared, the reaction was quenched with MeOH and then, the solvent was evaporated. The crude residue was partitioned between EtOAc and H_2O and the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel (EtOAc:MeOH = 10:1) to give the sulfamoylated product.

4.1.2. Amide coupling

To a solution of 5′-*O*-sulfamoylated intermediate (1 eq) in anhydrous CH_2Cl_2 was added the corresponding acid (1.5 eq) and DMAP (0.05 eq) at room temperature. 1 M DCC in CH_2Cl_2 (1.5 eq) was added dropwise and the reaction mixture was stirred for 12 h at room temperature. The reaction mixture was filtered through celite pad, washed with EtOAc and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel (EtOAc:MeOH = 10:1) to give the desired compound.

4.1.3. Acetyl deprotection

The starting material was dissolved in 0.02 M sodium methoxide solution in MeOH and stirred for 2 h at room temperature. After the reaction was completed, DOWEX 50WX8 hydrogen form resin was added in portions until pH of the reaction mixture reached ca. 6 ~ 7. The reaction mixture was filtered and concentrated under reduced pressure to give the pure final compound.

4.2. Chemical spectra

4.2.1. ((2R,3S,4R,5R)-3,4-Dihydroxy-5-(6-(methylamino)-9H-purin-9-yl) tetrahydrofuran-2-yl)methyl ((2R,3S)-2,3-dihydroxy-4-methylpentanoyl) sulfamate (14)

Yield = 56%, white solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.49 (s, 1H), 8.24 (s, 1H), 6.07 (d, J = 5.50 Hz, 1H), 4.61(t, J = 5.12 Hz, 1H), 4.39–4.26 (m, 3H), 4.08 (s, 1H), 3.49 (d, J = 8.21 Hz, 1H), 3.10 (m, 2H), 1.00 (d, J = 6.62 Hz, 3H), 0.95 (d, J = 6.62 Hz, 3H); HRMS (ESI) m/z calcd for $C_{17}H_{26}N_6O_9S$ $[M + H]^+$490.1482, found: 491.1563.

4.2.2. ((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((2R,3S)-2,3-dihydroxy-4-methylpentanoyl)sulfamate (15)

Yield = 61%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.52 (s, 1H), 8.18 (s, 1H), 6.07 (d, J = 5.51 Hz, 1H), 4.60 (t, J = 5.32 Hz, 1H), 4.38 (t, J = 5.0 Hz, 1H), 4.32 (m, 2H), 4.28 (m, 2H), 4.13 (s, 1H), 3.48 (d, J = 9.21 Hz, 1H), 0.99 (d, J = 6.8 Hz, 3H), 0.93 (d, J = 6.42, 3H); HRMS (ESI) m/z calcd for $C_{16}H_{24}N_6O_9S$ $[M + H]^+$477.1325, found: 277.1380.

4.2.3. ((2R,3S,5R)-5-(6-Amino-9H-purin-9-yl)-3-hydroxytetrahydrofuran-2-yl)methyl ((2S,3R)-2,3-dihydroxy-4-methylpentanoyl)sulfamate (16)

Yield = 63%, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 8.49 (s, 1H), 8.17 (s, 1H), 6.49 (t, J = 6.80 Hz, 1H), 4.63 (t, J = 2.75 Hz, 1H), 4.30–4.24 (m, 2H), 4.19 (q, J = 2.80 Hz, 1H), 4.10 (s, 1H), 3.48 (d, J = 11.60 Hz, 1H), 2.76 (dd, J = 6.30, 0.75 Hz, 1H), 2.47 (dd, J = 6.10, 3.05 Hz, 1H), 1.90–1.83 (m, 1H), 1.01 (d, J = 6.60 Hz, 3H), 0.94 (d, J = 6.75 Hz, 3H); HRMS (ESI) m/z calcd for $C_{16}H_{24}N_6O_8S$ $[M + H]^+$ 461.1454, found: 461.1443.

4.2.4. ((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((S)-2-hydroxy-4-methylpentanoyl) sulfamate (17)

Yield = 88%, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 8.50 (s, 1H), 8.19 (s, 1H), 6.08 (d, J = 5.35 Hz, 1H), 4.64 (t, J = 7.90 Hz, 1H), 4.38 (t, J = 3.80 Hz, 1H), 4.35–4.25 (m, 3H), 4.03 (dd, J = 9.15, 3.25 Hz, 1H), 1.65–1.43 (m, 3H), 0.91 (t, J = 6.75 Hz, 6H); HRMS (ESI) m/z calcd for $C_{16}H_{24}N_6O_8S$ $[M + H]^+$ 461.1454, found: 461.1445.

4.2.5. ((2R,3S,4R,5R)-5-(6-Amino-2-iodo-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((2S,3R)-2,3-dihydroxy-4-methylpentanoyl)sulfamate (18)

Yield = 72%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.41 (s, 1H), 6.01 (d, J = 5.35 Hz, 1H), 4.56 (t, J = 5.12 Hz, 1H), 4.39–4.36 (m, 3H), 4.07 (m, 1H), 3.50 (d, J = 9.00 Hz, 1H), 1.87 (m, 1H), 1.00 (d, J = 6.75 Hz, 3H), 0.94 (d, J = 6.75 Hz, 3H); HRMS (ESI) m/z calcd for $C_{16}H_{23}IN_6O_9S$ $[M + H]^+$ 603.0292, found: 603.0281.

4.2.6. ((2S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-4-hydroxytetrahydrofuran-2-yl)methyl ((2S,3R)-2,3-dihydroxy-4-methylpentanoyl)sulfamate (19)

Yield = 52%, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 8.46 (s, 1H), 8.18 (s, 1H), 5.99 (d, J = 1.55 Hz, 1H), 4.70–4.66 (m, 1H), 4.65–4.64 (m, 1H), 4.43 (dd, J = 11.25, 2.35 Hz, 1H), 4.26 (dd, J = 11.25, 3.25 Hz, 1H), 4.11 (s, 1H), 3.51 (d, J = 8.10 Hz, 1H), 2.40 (dd, J = 9.00, 5.65 Hz, 1H), 2.11 (dd, J = 7.15, 4.45 Hz, $H_{3''}$), 1.00 (d, J = 6.65 Hz, 3H), 0.94 (d, J = 6.75 Hz, 3H); HRMS (ESI) m/z calcd for $C_{16}H_{24}N_6O_8S$ $[M + H]^+$ 461.1454, found: 461.1452.

4.2.7. ((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((E)-4-methylpent-2-enoyl) sulfamate (20)

Yield = 90%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.36 (s, 1H), 8.20 (s, 1H), 6.91(dd, J = 15.4 Hz, 6.4 Hz, 1H), 6.04 (m, 1H), 5.81 (d, J = 15.4 Hz, 1H), 4.65 (m, 1H), 4.51 (m, 2H), 4.37 (m, 1H), 4.29 (m, 1H), 2.43 (m, 1H), 1.04 (d, J = 2.02 Hz, 3H), 1.01(d, J = 2.02 Hz, 3H); HRMS (ESI) m/z calcd for $C_{16}H_{22}N_6O_7S$ $[M + H]^+$ 443.1271, found: 443.1342.

4.2.8. ((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((2S,3R)-2,3-dihydroxy-4-methylpentanoyl)sulfamate (21)

Yield = 77%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.04 (s, 1H), 7.83 (s, 1H), 6.07 (d, J = 4.45 Hz, 1H), 4.08–3.92 (m, 4H), 3.79 (m, 1H), 3.70 (d, J = 2.00 Hz, 1H), 2.40 (dd, J = 9.00, 5.65 Hz, 1H), 2.11 (dd, J = 7.15, 4.45 Hz, 1H), 1.00 (d, J = 6.65 Hz, 3H), 0.94 (d, J = 6.75 Hz, 3H); HRMS (ESI) m/z calcd for $C_{16}H_{24}N_6O_9S$ $[M + H]^+$ 477.1325, found: 477.1311.

4.2.9. ((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((S)-2-hydroxy-4-methylpentanoyl) sulfamate (22)

Yield = 87%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.04 (s, 1H), 7.83 (s, 1H), 6.07 (d, J = 4.45 Hz, 1H), 4.48 (m, 1H), 4.43 (m, 2H), 4.36 (t, 1H), 4.28 (q, 1H), 4.02 (dd, 1H), 1.81 (m, 1H), 1.48 (m, 2H), 0.92 (s, 3H), 0.90 (s, 3H); HRMS (ESI) *m/z* calcd for $C_{16}H_{24}N_6O_8S$ $[M + H]^+$ 461.1376, found: 461.1365.

4.2.10. ((2R,3S,4R,5R)-5-(6-Amino-2-chloro-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((S)-2-hydroxy-4-methylpentanoyl) sulfamate (23)

Yield = 68%, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 8.37 (s, 1H), 5.99 (d, J = 5.35 Hz, 1H), 4.58 (t, J = 3.80 Hz, 1H), 4.43 (m, 2H), 4.36 (m, 1H), 4.28 (q, 1H), 4.02 (dd, J = 9.15, 3.25 Hz, 1H), 1.81 (m, 1H), 1.48 (m, 2H), 0.92 (s, 3H), 0.90 (s, 3H); HRMS (ESI) *m/z* calcd for $C_{16}H_{23}ClN_6O_8S$ $[M + H]^+$ 495.0987, found: 495.1068.

4.2.11. ((2R,3S,4R,5R)-5-(6-Amino-2-chloro-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((2S,3R)-3-hydroxy-2-methoxy-4-methylpentanoyl)sulfamate (24)

Yield = 99%, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 8.48 (s, 1H), 6.01 (d, J = 5.35 Hz, 1H), 4.59 (t, J = 5.10 Hz, 1H), 4.39 (t, J = 3.90 Hz, 1H), 4.32 (m, 2H), 4.28 (m, 1H), 3.66 (d, J = 3.55 Hz, 1H), 3.47 (m, 1H), 3.36 (s, 3H), 1.85 (m, 1H), 0.97 (d, J = 6.65 Hz, 3H), 0.90 (d, J = 6.75 Hz, 3H); HRMS (ESI) *m/z* calcd for $C_{17}H_{25}ClN_6O_9S$ $[M + H]^+$ 525.1165, found: 525.1147.

4.2.12. ((2R,3S,4R,5R)-5-(6-Amino-2-chloro-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((2S,3R)-2,3-dimethoxy-4-methylpentanoyl)sulfamate (25)

Yield = 43%, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 8.49 (s, 1H), 6.01 (d, 1H), 4.61 (t, 1H), 4.38 (m, 1H), 4.34 (m, 2H), 4.28 (m, 1H), 3.67 (d, 1H), 3.40 (s, 3H), 3.33 (s, 3H), 3.21 (m, 1H), 1.91 (m, 1H), 0.97 (d, 3H), 0.89 (s, 3H); HRMS (ESI) *m/z* calcd for $C_{18}H_{27}ClN_6O_9S$ $[M + H]^+$ 539.1322, found 539.1287.

4.2.13. ((2R,3S,4R,5R)-3,4-Dihydroxy-5-(6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-2-yl)methyl ((2S,3R)-2,3-dihydroxy-4-methylpentanoyl)sulfamate (26)

Yield = 95%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.33 (s, 1H), 7.95 (s, 1H), 5.95 (d, J = 5.31 Hz, 1H), 4.51 (t, J = 5.31 Hz, 1H), 4.29 (t, J = 3.48 Hz, 1H), 4.18 (m, 2H), 4.28 (m, 1H), 3.97 (s, 1H), 3.40 (d, J = 8.79 Hz, 1H), 1.85 (m, 1H), 0.92 (d, J = 6.57 Hz, 3H), 0.85 (d, J = 6.78 Hz, 3H); HRMS (ESI) *m/z* calcd for $C_{16}H_{23}N_5O_9S$ $[M + H]^+$ 478.1238, found 478.1230.

4.2.14. ((2R,3S,4R,5R)-3,4-Dihydroxy-5-(6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-2-yl)methyl ((S)-2-hydroxy-4-methylpentanoyl) sulfamate (27)

Yield = 92%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.44 (s, 1H), 8.05 (s, 1H), 6.06 (d, J = 5.67 Hz, 1H), 4.65 (t, J = 5.31 Hz, 1H), 4.37 (m, 1H), 4.28 (m, 4H), 3.98 (dd, J = 3.66 Hz, 1H), 1.82 (m, 1H), 1.54 (m, 2H), 0.91 (t, J = 6.42 Hz, 6H); HRMS (ESI) *m/z* calcd for $C_{16}H_{23}N_5O_9S$ $[M + H]^+$ 462.1289, found 462.1266.

4.2.15. ((2R,3S,4R,5R)-5-(6-Amino-2-iodo-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((2S,3R)-3-cyclopropyl-2,3-dihydroxypropanoyl)sulfamate (28)

Yield = 75%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.06 (s, 1H), 5.65 (d, J = 5.50 Hz, 1H), 4.21 (t, J = 5.10 Hz, 1H), 4.02–3.91 (m, 4H), 3.66 (d, J = 2.21 Hz, 1H), 2.77 (d, J = 9.11 Hz, 1H), 0.82 (m, 1H), 0.91 (m, 4H); HRMS (ESI) *m/z* calcd for $C_{16}H_{21}IN_6O_9S$ $[M + H]^+$ 601.0292, found 601.0147.

4.2.16. ((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((2S,3R)-3-cyclopropyl-2,3-dihydroxypropanoyl)sulfamate (29)

Yield = 65%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.04 (s, 1H), 7.83 (s, 1H), 6.07 (d, J = 4.42 Hz, 1H), 4.08–3.92 (m, 4H), 3.79 (m, 1H), 3.70 (d, J = 2.01 Hz, 1H), 2.78 (m, 1H), 0.84 (m, 1H), 0.20 (m, 4H); HRMS (ESI) *m/z* calcd for $C_{16}H_{21}IN_6O_9S$ $[M + H]^+$ 601.0135, found 601.0121.

4.2.17. ((2R,3S,4R,5R)-5-(6-Amino-2-chloro-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((S)-2-chloro-4-methylpentanoyl) sulfamate (30)

Yield = 50%, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 8.46 (s, 1H), 6.01 (d, J = 5.95 Hz, 1H), 4.62 (t, J = 5.25 Hz, 1H), 4.36 (dd, J = 4.7, 3.15 Hz), 4.30 (m, 2H), 4.28 (m, 1H), 4.22 (dd, J = 8.3, 6.05 Hz), 1.78 (m, 2H), 1.71 (m, 1H), 0.91 (dd, J = 23.55, 6.3 Hz); HRMS (ESI) *m/z* calcd for $C_{16}H_{22}Cl_2N_6O_7S$ $[M + H]^+$ 513.0804, found 513.0705.

4.2.18. ((2R,3S,4R,5R)-5-(6-Amino-2-iodo-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl (3-isopropyl-1H-pyrazole-5-carbonyl)sulfamate (31)

Yield = 69%, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 8.49 (s, 1H), 6.55 (s, 1H), 6.01 (d, 1H), 4.61 (t, 1H), 4.38 (m, 1H), 4.32 (m, 2H), 4.18 (m, 1H), 3.67 (d, 1H), 3.40 (m, 4H), 0.89 (m, 6H); HRMS (ESI) *m/z* calcd for $C_{17}H_{21}IN_8O_7S$ $[M + H]^+$ 609.0455, found 609.0435.

4.2.19. ((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl (3-isopropyl-1-methyl-1H-pyrazole-5-carbonyl)sulfamate (32)

Yield = 69%, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 8.49 (s, 1H), 6.01 (d, 1H), 4.61 (t, 1H), 4.38 (m, 1H), 4.34 (m, 1H), 4.28 (m, 1H), 3.67 (d, 1H), 3.40 (s, 2H), 3.33 (s, 3H), 3.21 (m, 1H), 1.91 (m, 1H), 0.89 (s, 6H); HRMS (ESI) *m/z* calcd for $C_{18}H_{24}N_8O_7S$ $[M + H]^+$ 497.1322, found 497.1287.

4.2.20. ((2R,3S,4R,5R)-5-(6-Amino-2-iodo-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl (3-isopropyl-1-methyl-1H-pyrazole-5-carbonyl)sulfamate (33)

Yield = 72%, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 8.49 (s, 1H), 6.01 (d, 1H), 4.38 (m, 1H), 4.34 (m, 2H), 4.28 (m, 1H), 3.67 (d, 1H), 3.40 (s, 3H), 3.33 (s, 3H), 3.21 (m, 1H), 1.91 (m, 1H), 0.97 (d, 3H), 0.89 (s, 3H); HRMS (ESI) *m/z* calcd for $C_{18}H_{23}IN_8O_7S$ $[M + H]^+$ 623.0455, found 623.0441.

4.2.21. ((3aR,4S,6R,6aR)-6-(6-Amino-9H-purin-9-yl)-2-iminohexahydrofuro[3,4-d]oxazol-4-yl)methyl ((2S,3R)-3-cyclopropyl-2,3-dihydroxypropanoyl)sulfamate (34)

Yield = 68%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.68 (s, 1H), 8.58 (s, 1H), 6.34 (s, 1H), 4.48 (t, J = 3.50 Hz, 1H), 4.43 (d, J = 2.32 Hz, 1H), 4.26 (m, 2H), 4.20 (dd, J = 11.15, 3.75 Hz, 1H), 4.00 (m, 1H), 3.09 (d, J = 8.80 Hz, 1H), 2.71 (dd, J = 8.97, 2.55 Hz, 1H), 1.14 (m, 1H), 0.57 (m, 1H), 0.49 (m, 1H), 0.38 (m, 1H), 0.30 (m, 1H); HRMS (ESI) *m/z* calcd for $C_{17}H_{21}N_7O_9S$ $[M + H]^+$ 500.1278, found 500.1266.

4.2.22. ((2R,3S,4R,5R)-3,4-Dihydroxy-5-(6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-2-yl)methyl ((R)-2-hydroxy-4-methylpentanoyl) sulfamate (35)

Yield = 81%, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 8.31 (s, 1H), 8.05 (s, 1H), 6.27 (s, 1H), 4.80 (s, 1H), 4.71 (t, J = 1.61 Hz, 1H), 4.56 (dd, J = 13.90, 2.35 Hz, 1H), 4.36 (m, 2H), 4.05 (d, J = 5.45 Hz, 1H), 1.85 (m, 1H), 1.52 (m, 2H), 0.93 (dd, J = 11.95, 5.65 Hz, 6H); HRMS (ESI) *m/z* calcd for $C_{16}H_{23}N_5O_9S$ $[M + H]^+$ 462.1373, found 462.1305.

4.2.23. ((2R,3S,4R,5R)-3,4-Dihydroxy-5-(6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-2-yl)methyl ((R)-2-mercapto-4-methylpentanoyl) sulfamate (36)

Yield = 99%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.36 (s, 1H), 8.06 (s, 1H), 6.06 (s, 1H), 4.43 (m, 1H), 4.40 (m, 1H), 4.33 (m, 2H), 4.29 (m, 1H), 3.63 (m, 1H), 1.78 (m, 1H), 1.68 (m, 1H), 1.49 (m, 1H), 0.90 (m, 6H); HRMS (ESI) *m/z* calcd for $C_{16}H_{23}N_5O_8S_2$ $[M + H]^+$ 478.0988, found 478.1056.

4.2.24. ((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((2S,3R)-3-cyclopropyl-2,3-dihydroxypropanoyl)sulfamate (37)

Yield = 82%, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 8.51 (s, 1H), 8.18 (s, 1H), 6.08 (d, J = 5.60 Hz, 1H), 4.64 (t, J = 5.30 Hz, 1H), 4.38 (dd, J = 8.20, 3.50 Hz, 1H), 4.35–4.27 (m, 3H), 4.00 (d, J = 2.10 Hz, 1H), 3.11 (dd, J = 9.10, 2.00 Hz, 1H), 1.17–1.11 (m, 1H), 0.56–0.29 (m, 4H); HRMS (ESI) *m/z* calcd for $C_{16}H_{22}N_5O_9S$ $[M + H]^+$ 475.1247, found: 475.1245.

4.2.25. ((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl (3-isopropyl-1H-pyrazole-5-carbonyl)sulfamate (38)

Yield = 72%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.51 (s, 1H), 8.18 (s, 1H), 6.55 (s, 1H), 6.08 (d, J = 5.60 Hz, 1H), 4.64 (t, J = 5.30 Hz, 1H), 4.38 (dd, J = 8.20, 3.50 Hz, 1H), 4.35–4.27 (m, 1H), 4.00 (d, J = 2.10 Hz, 1H), 3.11 (dd, J = 9.10, 2.00 Hz, 1H),0.89 (m, 6H); HRMS (ESI) *m/z* calcd for $C_{17}H_{22}N_8O_7S$ $[M + H]^+$ 483.1332, found: 483.1320.

4.2.26. ((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl (3-(tert-butyl)-1H-pyrazole-5-carbonyl)sulfamate (39)

Yield = 72%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.50 (s, 1H), 8.17 (s, 1H), 6.54 (s, 1H), 6.08 (d, J = 5.60 Hz, 1H), 4.64 (t, J = 5.30 Hz, 1H), 4.38 (dd, J = 8.20, 3.50 Hz, 1H), 4.35–4.27 (m, 1H), 3.99 (m, 1H), 3.11 (m, 1H),0.89 (m, 9H); HRMS (ESI) *m/z* calcd for $C_{18}H_{24}N_8O_7S$ $[M + H]^+$ 497.1489, found: 487.1478.

4.2.27. ((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl (3-cyclopropyl-1H-pyrazole-5-carbonyl)sulfamate (40)

Yield = 65%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.50 (s, 1H), 8.17 (s, 1H), 6.54 (s, 1H), 6.08 (d, J = 5.60 Hz, 1H), 4.64 (t, J = 5.30 Hz, 1H), 4.38 (dd, J = 8.20, 3.50 Hz, 1H), 4.35–4.27 (m, 2H), 3.99 (m, 1H), 3.11 (m, 1H), 0.56–0.29 (m, 4H); HRMS (ESI) *m/z* calcd for $C_{17}H_{20}N_8O_7S$ $[M + H]^+$ 481.1176, found: 481.1165.

4.2.28. ((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((R)-2-hydroxy-4-methylpentanoyl) sulfamate (41)

Yield = 72%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.56 (s, 1H) 8.31 (s, 1H), 6.37 (s, 1H), 5.02 (d, J = 13.6, 1H), 4.75 (m, 2H), 4.38 (t, J = 4.8, 1H), 4.11 (d, J = 5.41 Hz, 1H), 4.01 (m, 1H), 1.84 (m, 1H), 1.51 (m, 1H), 0.90 (t, J = 5.60 Hz, 6H); HRMS (ESI) *m/z* calcd for $C_{16}H_{24}N_6O_8S$ $[M + H]^+$ 461.1533, found 461.1520.

4.2.29. ((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((S)-2-mercapto-4-methylpentanoyl)sulfamate (42)

Yield = 77%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.44 (s, 1H), 8.18 (s, 1H), 6.09 (d, 1H), 4.67 (m, 1H), 4.49 (s, 1H), 4.49 (s, 1H), 4.41 (s, 2H), 4.33 (m, 3H), 3.63 (s, 1H), 0.88–0.94 (m, 6H); HRMS (ESI) *m/z* calcd for $C_{16}H_{26}N_6O_7S_2$ $[M + H]^+$ 477.1221, found 477.1209.

4.2.30. ((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl (methyl-L-leucyl)sulfamate (43)

Yield = 99%, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 8.51 (s, 1H, Ar, 1H), 8.19 (s, 1H, Ar, 1H), 6.08 (d, J = 5.70, 1H), 4.65 (t, J = 5.32 Hz, 1H), 4.39 (t, J = 5.30 Hz, 1H), 4.35 (d, J = 3.75 Hz,m 1H), 4.33 (d, J = 3.75 Hz, 1H), 4.28 (m, 2H), 3.12 (t, J = 7.71 Hz, 1H), 2.39 (s, 3H), 1.73 (m, 1H), 1.56 (m, 1H), 1.38 (m, 1H), 0.93 (d, J = 6.50 Hz, 3H), 0.86 (d, J = 6.60 Hz, 3H); HRMS (ESI) *m/z* calcd for $C_{17}H_{27}N_7O_7S$ $[M + H]^+$ 474.1349, found 474.1289.

4.2.31. ((2S,3S,4R,5R)-3-Amino-5-(6-amino-9H-purin-9-yl)-4-hydroxytetrahydrofuran-2-yl)methyl (L-leucyl)sulfamate (44)

Yield = 78%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.64 (s, 1H), 8.59 (s, 1H), 6.35 (s, 1H), 4.50 (t, J = 3.51 Hz, 1H), 4.45 (d, J = 2.62 Hz, 1H), 4.27 (d, J = 2.40 Hz, 1H), 4.22 (m, 2H), 3.39 (m, 1H), 1.73 (m, 1H), 1.62 (m, 1H), 1.41 (m, 1H), 0.92 (m, 6H); HRMS (ESI) *m/z* calcd for $C_{17}H_{24}N_8O_7S$ $[M + H]^+$ 485.1645, found 485.1633.

4.2.32. ((2R,3S,5R)-5-(6-Amino-9H-purin-9-yl)-3-hydroxytetrahydrofuran-2-yl)methyl (L-leucyl)sulfamate (45)

Yield = 60%, white solid; ^{1}H NMR (300 MHz, D_2O) δ 7.75 (s, 1H), 7.69 (s, 1H), 3.46 (m, 1H), 3.15 (s, 2H), 3.05 (s, 2H), 1.06 (m, 6H), 0.27 (m, 6H); HRMS (ESI) *m/z* calcd for $C_{16}H_{25}N_7O_6S$ $[M + H]^+$ 444.1645, found 444.1633.

4.2.33. ((2R,3S,5R)-5-(6-Amino-9H-purin-9-yl)-3-hydroxytetrahydrofuran-2-yl)methyl ((2R,3S)-2,3-dihydroxy-4-methylpentanoyl)sulfamate (46)

Yield = 71%, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 8.51 (s, 1H), 8.18 (s, 1H), 6.49 (t, J = 6.85 Hz, 1H), 4.63–4.62 (m, 1H), 4.30–4.24 (m, 2H), 4.19 (d, J = 3.70 Hz, 1H), 4.06 (m, 1H), 3.5 (dd, J = 8.85, 1.25 Hz, 1H), 2.75 (qd, J = 7.25, 3.60 Hz, 1H), 2.46 (qd, J = 6.05, 3.10 Hz, 1H), 1.88–1.81 (m, H), 0.99 (d, J = 6.70 Hz, 3H), 0.94 (d, J = 6.70 Hz, 3H); HRMS (ESI) *m/z* calcd for $C_{16}H_{24}N_6O_8S$ $[M + H]^+$ 461.1454, found: 461.1408.

4.2.34. ((2R,3S,4R,5R)-3,4-Dihydroxy-5-(6-(methylamino)-9H-purin-9-yl)tetrahydrofuran-2-yl)methyl ((S)-2-methoxy-4-methylpentanoyl) sulfamate (47)

Yield = 80%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.45 (s, 1H), 8.18 (s, 1H), 5.99 (d, J = 1.83 Hz, 1H), 4.71 (m, 2H), 4.42 (dd, J = 11.34, 2.55 Hz, 1H), 4.23 (dd, J = 11.19, 3.30 Hz, 1H), 4.08 (dd, J = 9.15, 3.66 Hz, 1H), 2.40 (d, J = 2.49 Hz, 1H), 2.11 (qd, J = 6.03, 2.88 Hz, 1H), 1.82–1.80 (m, 1H), 0.91 (d, J = 2.58 Hz, 3H), 0.89 (d, J = 2.73 Hz, 3H); HRMS (ESI) *m/z* calcd for $C_{17}H_{26}N_6O_8S$ $[M + H]^+$ 475.1689, found: 475.1672.

4.2.35. ((2S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-4-hydroxytetrahydrofuran-2-yl)methyl ((S)-2-hydroxy-4-methylpentanoyl) sulfamate (48)

Yield = 83%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.45 (s, 1H), 8.18 (s, 1H), 5.99 (d, J = 1.83 Hz, 1H), 4.71–4.65 (m, 2H), 4.44 (dd, J = 11.34, 2.55 Hz, 1H), 4.27 (dd, J = 11.19, 3.30 Hz, 1H), 4.08 (dd, J = 9.15, 3.66 Hz, 1H), 2.41 (qd, J = 8.97, 2.49 Hz, 1H), 2.11 (qd, J = 6.03, 2.88 Hz, 1H), 1.82–1.80 (m, 1H), 1.60–1.42 (m, 2H), 0.91 (d, J = 2.58 Hz, 3H), 0.89 (d, J = 2.73 Hz, 3H); HRMS (ESI) *m/z* calcd for $C_{16}H_{24}N_6O_7S$ $[M + H]^+$ 445.1584, found: 445.1571.

4.2.36. ((2R,3S,5R)-5-(6-Amino-9H-purin-9-yl)-3-hydroxytetrahydrofuran-2-yl)methyl ((S)-2-hydroxy-4-methylpentanoyl) sulfamate (49)

Yield = 76%, white solid; ^{1}H NMR (600 MHz, CD_3OD) δ 8.47 (s, 1H), 8.18 (s, 1H), 6.49 (dd, J = 7.32, 5.94 Hz, 1H), 4.62 (q, J = 2.76 Hz, 1H), 4.28–4.23 (m, 2H), 4.20 (q, J = 3.66 Hz, 1H), 3.99 (dd, J = 9.60, 3.18 Hz, 1H), 2.77 (dd, J = 7.80, 6.00 Hz, 1H), 2.46 (dd, J = 5.94, 0.60 Hz, 1H), 1.86–1.80 (m, 1H), 1.57 (dd, J = 9.18, 3.66 Hz,

1H), 1.45 (dd, J = 9.60, 5.04 Hz, 1H), 0.92 (d, J = 6.36 Hz, 3H), 0.90 (d, J = 6.90 Hz, 3H); HRMS (ESI) m/z calcd for $C_{16}H_{24}N_6O_7S$ $[M + H]^+$ 445.1505, found: 445.1473.

4.2.37. ((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((2S,3R)-3-hydroxy-2-methoxy-4-methylpentanoyl)sulfamate (50)

Yield = 79%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.46 (s, 1H), 8.19 (s, 1H), 6.08 (d, J = 5.31 Hz, 1H), 4.66 (t, J = 5.49 Hz, 1H), 4.40–4.28 (m, 4H), 3.70 (d, J = 3.48 Hz, 1H), 3.42–3.41 (m, 1H), 3.33 (s, 3H), 1.88–1.81 (m, 1H), 0.98 (d, J = 6.78 Hz, 3H), 0.91 (d, J = 6.78 Hz, 3H); HRMS (ESI) m/z calcd for $C_{17}H_{26}N_6O_9S$ $[M + H]^+$ 491.1638, found: 491.1622.

4.2.38. ((2R,3S,5R)-5-(6-Amino-9H-purin-9-yl)-3-hydroxytetrahydrofuran-2-yl)methyl ((2S,3R)-3-hydroxy-2-methoxy-4-methylpentanoyl)sulfamate (51)

Yield = 68%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.55 (s, 1H), 8.22 (s, 1H), 6.66 (t, J = 6.24 Hz, 1H), 6.50 (s, 1H), 4.56–4.68 (m, 4H), 4.20–4.29 (m, 3H), 3.43 (s, 3H), 2.43 (m, 1H) 2.03 (m, 1H), 0.95(m, 6H); HRMS (ESI) m/z calcd for $C_{17}H_{26}N_6O_8S$ $[M + H]^+$ 475.1689, found: 475.1678.

4.2.39. ((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((S)-2-chloro-4-methylpentanoyl) sulfamate (52)

Yield = 70%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.51 (s, 1H), 8,20 (s, 1H), 6.09 (d, J = 6.21 Hz, 1H), 4.69 (t, J = 5.82 Hz, 1H), 4.38–4.20 (m, 5H), 1.78 (m, 3H), 0.96–0.87 (m, 6H); HRMS (ESI) m/z calcd for $C_{16}H_{23}ClN_6O_7S$ $[M + H]^+$ 479.1134, found: 479.1123.

4.2.40. ((2R,3S,4R,5R)-3,4-Dihydroxy-5-(6-(methylamino)-9H-purin-9-yl)tetrahydrofuran-2-yl)methyl ((S)-2-chloro-4-methylpentanoyl)sulfamate (53)

Yield = 81%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.63 (s, 1H), 8.27 (s, 1H), 6.36 (s, 1H), 6.08 (d, J = 5.12 Hz, 1H), 4.64 (t, J = 5.31 Hz, 1H), 4.49–4.30 (m, 3H), 3.23 (s, 3H), 3.13 (m, 1H), 1.78 (m, 2H), 1.30 (t, J = 7.31 Hz, 1H), 0.97–0.85 (m, 6H); HRMS (ESI) m/z calcd for $C_{17}H_{25}ClN_6O_7S$ $[M + H]^+$ 493.1301, found 493.1291.

4.2.41. ((2R,3S,5R)-5-(6-Amino-9H-purin-9-yl)-3-hydroxytetrahydrofuran-2-yl)methyl ((2S,3R)-2,3-dimethoxy-4-methylpentanoyl)sulfamate (54)

Yield = 75%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.36 (s, 1H), 8.14 (s, 1H), 6.39 (t, J = 6.60 Hz, 1H), 4.55 (q, J = 2.94 Hz, 1H), 4.39–4.28 (m, 2H), 4.13 (q, J = 3.27 Hz, 1H), 3.63 (d, J = 3.12 Hz, 1H), 3.28 (s, 3H), 3.21 (s, 3H), 3.10 (dd, J = 7.68, 3.87 Hz, 1H), 2.70 (q, J = 7.14 Hz, 1H), 2.43–2.32 (m, 1H), 1.89–1.77 (m, 1H), 0.87 (d, J = 6.60 Hz, 3H), 0.80 (d, J = 6.78 Hz, 3H); HRMS (ESI) m/z calcd for $C_{18}H_{27}N_6O_8S$ $[M + H]^+$ 489.1846, found 489.1852.

4.2.42. ((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((2S,3R)-2,3-dimethoxy-4-methylpentanoyl)sulfamate (55)

Yield = 82%, white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 8.42 (s, 1H), 8.28 (s, 1H), 6.07 (d, J = 4.95 Hz, 1H), 4.67 (t, J = 4.68 Hz, 1H), 4.61–4.50 (m, 2H), 4.41 (t, J = 5.85 Hz, 1H), 4.31 (t, J = 3.48 Hz, 1H), 3.71 (d, J = 3.66 Hz, 1H), 3.36 (s, 3H), 3.33 (s, 3H), 3.17–3.11 (m, 1H), 2.01 (m, 1H), 0.99 (d, J = 6.60 Hz, 3H), 0.89 (d, J = 6.51 Hz, 3H); HRMS (ESI) m/z calcd for $C_{18}H_{28}N_6O_9S$ $[M + H]^+$ 505.1795, found 505.1782.

4.2.43. ((3aR,4S,6R,6aR)-6-(6-Amino-9H-purin-9-yl)-2-iminohexahydrofuro[3,4-d]oxazol-4-yl)methyl ((2S,3R)-2,3-dihydroxy-4-methylpentanoyl)sulfamate (56)

Yield = 75%, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 8.71 (s, 1H), 8.58 (s, 1H), 6.34 (s, 1H), 4.47 (t, J = 5.21 Hz, 1H), 4.43 (d, J = 4.25 Hz, 1H), 4.28 (d, J = 4.25 Hz, 1H), 4.23 (m, 1H), 4.07 (s, 1H), 3.48 (s, 1H), 1.85 (m, 1H), 0.98 (m, 6H); HRMS (ESI) m/z calcd for $C_{17}H_{23}N_7O_9S$ $[M + H]^+$ 502.1434, found 502.1442.

4.2.44. ((2R,3S,4R,5R)-3,4-Dihydroxy-5-(6-oxo-1,6-dihydro-9H-purin-9-yl)tetrahydrofuran-2-yl)methyl ((2S,3R)-3-cyclopropyl-2,3-dihydroxypropanoyl)sulfamate (57)

Yield = 80%, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 8.41 (s, 1H), 8.04 (s, 1H), (6.04 (d, J = 5.40 Hz, 1H), 4.58 (t, J = 5.21 Hz, 1H), 4.36 (t, J = 4.62 Hz, 1H), 4.15–4.32 (m, 3H), 4.00 (d, J = 2.00 Hz), 3.11 (dd, J = 2.00, 9.11 Hz, 1H), 1.11–1.18 (m, 1H), 0.51 (m, 1H), 0.44 (m, 1H). 0.29–0.39 (m, 2H); HRMS (ESI) m/z calcd for $C_{16}H_{21}N_5O_{10}S$ $[M + H]^+$ 476.1166, found 476.1152.

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