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N-Alkoxysulfamide, *N*-Hydroxysulfamide, and Sulfamate Analogues of Methionyl and Isoleucyl Adenylates as Inhibitors of Methionyl-tRNA and Isoleucyl-tRNA Synthetases

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Abstract—A series of sulfamate surrogates of methionyl and isoleucyl adenylate have been investigated as MetRS and IleRS inhibitors by modifications of the sulfamate linker and adenine moieties. The discovery of 2-iodo Ile-NHSO$_2$-AMP (**58**) as a potent *Escherichia coli* IleRS inhibitor revealed that a significant hydrophobic interaction between the 2-substituent of Ile-NHSO$_2$-AMP and the adenine binding site of IleRS provided its high potency to the enzyme.

The aminoacyl-tRNA synthetases (aaRSs) are a family of enzymes that precisely attach amino acids to tRNA molecules containing the corresponding anticodon. The resulting aminoacyl-tRNAs are substrates for translation in protein synthesis and are pivotal in determining how the genetic code is interpreted as amino acids.[1] The enzymes have been regarded as novel bacterial targets, particularly for the treatment of antibiotic-resistant bacterial strains, such as MRSA and VRE.[2–4] Since the activity of aminoacyl-tRNA synthetases is essential in all living organisms, the clinical application of the enzyme inhibitors primarily depends on the selectivity to the pathogen synthetases over the human cell counterparts.

Aminoacyl adenylate (aa-AMP, **I**), an intermediate in the enzymatic reaction, has been used as a starting prototype to discover novel aaRS inhibitors because of its tighter binding affinity than the substrates, amino acid and ATP, which is generally greater by two or three orders of magnitude. Most modifications of aminoacyl adenylates have focused on several important issues as antibiotic candidates, such as chemical stability, tight binding, and pathogen selectivity. Since the mixed anhydride acylphosphate bond of the intermediate is readily susceptible to hydrolysis, its substitution with non-hydrolyzable bioisosteres has been most extensively investigated to find potent aminoacyl adenylate inhibitors. To date, alkylphosphate (**II**),[5–7] ester (**III**, aa-CO$_2$–AMP),[8–10] amide (**IV**, aa-CONH–AMP),[8] hydroxamate (**V-VI**, aa-CON(OH)–AMP),[8–10] sulfamate (**VII**, aa-NHSO$_3$–AMP),[5,6,11–14] and sulfamide (**VIII**, aa-NHSO$_2$NH–AMP)[6] surrogates have been used to replace the labile acylphosphate of the aminoacyl adenylate (Fig. 1). Among them, the sulfamate moiety has exhibited both tight binding affinity and stability to a variety of aaRSs, such as AlaRS,[11] ArgRS,[5] HisRS,[5] IleRS,[12] ProRS,[13] SerRS,[14] ThrRS,[5] and TyrRS.[6] Particularly, an isoleucyl sulfamate analogue bearing the dihydroxytetrahydropyran moiety of pseudomonic acid had exceptionally ultrapotent binding affinity, with a value in the femtomolar range for binding to the *S. aureus* IleRS.[12] As a similar strategy to modify an acylphosphate linkage of an aminoacyl adenylate, we recently reported that ester (**III**), amide (**IV**), and hydroxamate analogues (**V**, **VI**) of methionyl and isoleucyl adenylate, as stable surrogates of aminoacyl adenylate,

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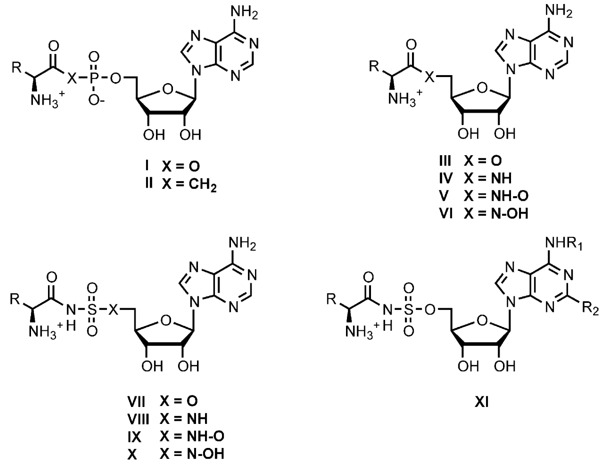
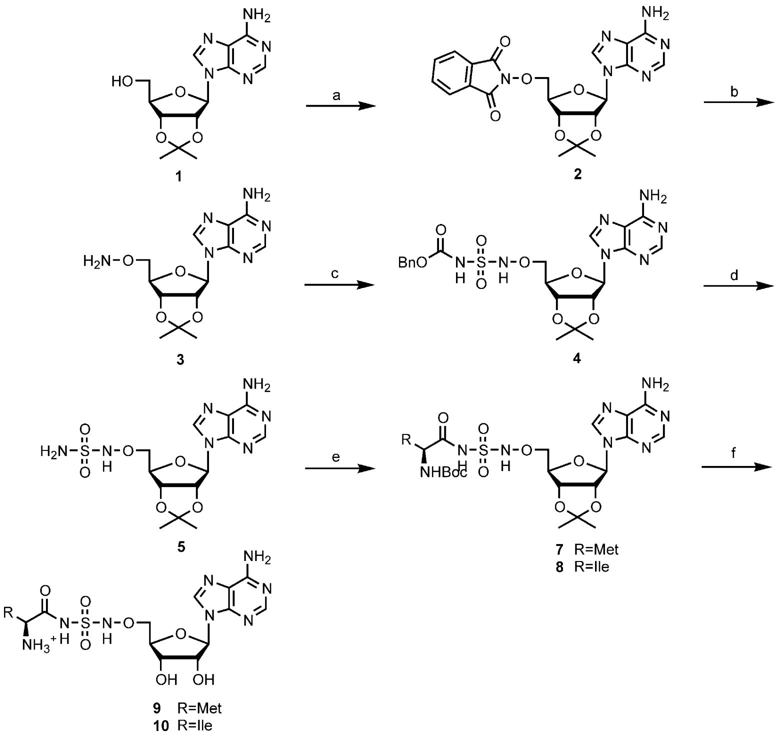


Figure 1.

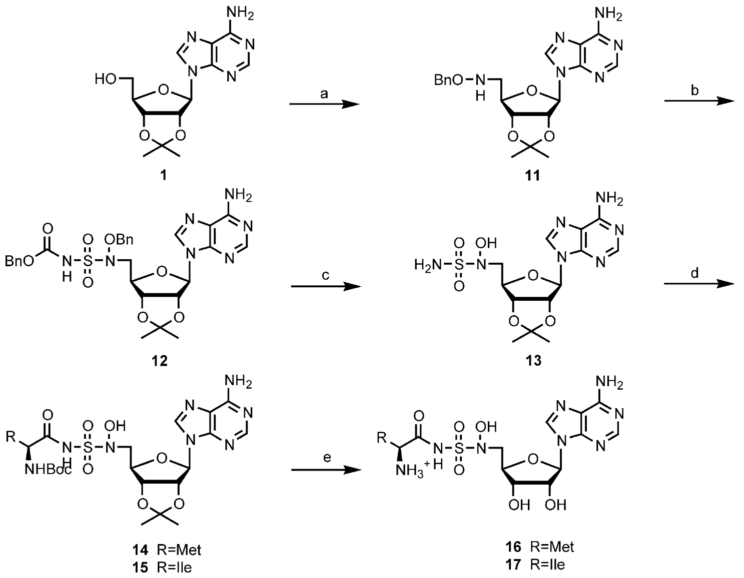
have moderate to potent inhibitory activities,[8–10] which were much lower than those of the corresponding sulfamate analogues (**VII**).

As part of our continuing effort to find potent and pathogen-selective inhibitors of MetRS and IleRS as potential antibacterial candidates, we herein describe the *N*-alkoxysulfamide (**IX**, aa-$NHSO_2NHO$–AMP) and *N*-hydroxysulfamide (**X**, aa-$NHSO_2N(OH)$–AMP) analogues of methionyl and isoleucyl adenylates, as sulfamate surrogates, and a SAR investigation of the adenine moiety of sulfamate (**XI**, aa-$NHSO_3$–AMP) analogues, which provides crucial information regarding the interaction of the isoleucyl adenylate with IleRS.

The synthesis of *N*-alkoxy sulfamide (**9**, **10**) analogues, as sulfamate surrogates, is outlined in Scheme 1. *O*-Adenosylhydroxylamine (**3**), as an intermediate for the synthesis of *N*-adenosyloxy sulfamide, was prepared from the commercially available 2′,3′-isopropylideneadenosine (**1**) by the Mitsunobu reaction with *N*-hydroxyphthalimide, followed by the hydrazine reaction, as reported previously.[8] Treatment of **3** with *N*-(carbobenzyloxy)sulfamoyl chloride[15] and subsequent catalytic hydrogenation provided the sulfamate intermediate **5**. The condensation of **5** with *N*-Boc methionine and *N*-Boc isoleucine by DCC afforded the *N*-alkoxysulfamate analogues (**7**, **8**), whose protecting groups were hydrolyzed under acidic conditions to produce the final *N*-methionyl(or isoleucyl)-*N*′-adenosyloxy sulfamide compounds, **9** and **10**, respectively. For the synthesis of the *N*-hydroxy sulfamide (**16**, **17**) analogues shown in Scheme 2, the Mitsunobu reaction of 2′,3′-isopropylideneadenosine (**1**) with *O*-benzylhydroxylamine produced the *O*-benzyl-*N*-adenosylhydroxylamine (**11**), which was converted to the final *N*-methionyl (or isoleucyl)-*N*′-adenosyl-*N*′-hydroxy sulfamides (**16**, **17**) by following a sequence similar to that described in Scheme 1. In order to investigate the structure–activity relationship toward the adenine moiety in the sulfamate analogues of methionyl and isoleucyl adenylate, we synthesized several 2-substituted analogues, as shown in Schemes 3 and 4. 2-Chloro adenosine is commercially available, and 2-iodo-6-methylamino adenosine was prepared according to the literature.[16] 2-Iodo, 2-ethynyl, and 2-hexynyl substituted analogues of adenosine were synthesized by the modified method of Matsuda et al.[17] The key iodo-intermediate **21** was prepared from 2-amino-6-chloropurine in 3 steps. The



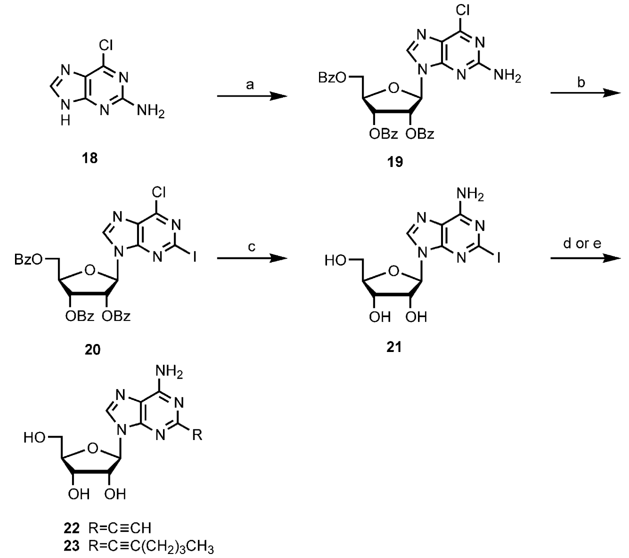
Scheme 1. Reagents and conditions: (a) *N*-hydroxyphthalimide, DEAD, PPh_3, THF, 83%; (b) N_2H_4, EtOH, reflux, 96%; (c) $BnO_2CNHSOCl$, NEt_3, THF–CH_2Cl_2, 81%; (d) H_2, Pd–C, MeOH, 97%; (e) $BocNCH(R)CO_2H$, DCC, DMAP, CH_2Cl_2, 99% for Met, 96% for Ile; (f) CF_3CO_2H, anisole, CH_2Cl_2, 82% for Met, 98% for Ile.



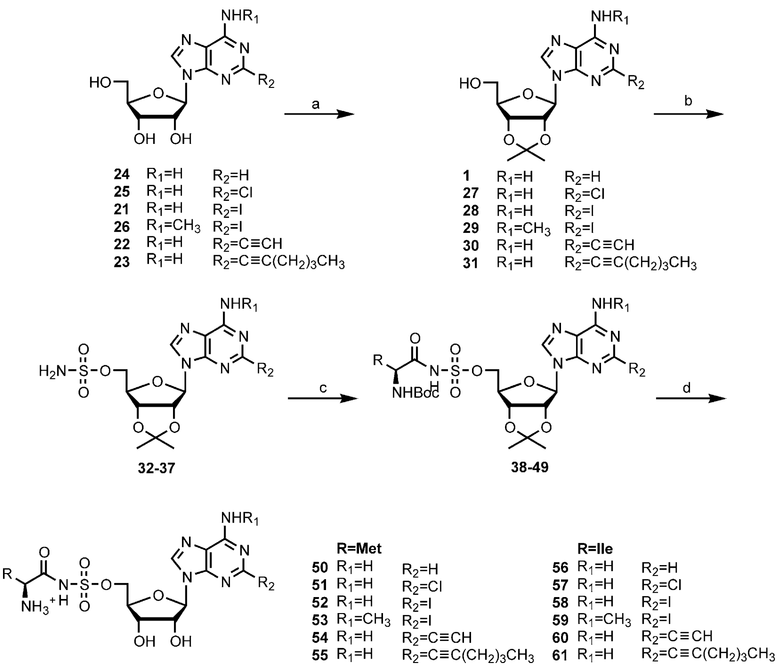
Scheme 2. Reagents and conditions: (a) BnONH–HCl, DEAD, PPh$_3$, THF, 98%; (b) BnO$_2$CNHSOCl, NEt$_3$, THF–CH$_2$Cl$_2$, 77%; (c) H$_2$, Pd–C, MeOH, 93%; (d) BocNCH(R)CO$_2$H, DCC, DMAP, CH$_2$Cl$_2$, 68% for Met, 96% for Ile; (e) CF$_3$CO$_2$H, anisole, CH$_2$Cl$_2$, 74% for Met, 75% for Ile.

palladium-catalyzed cross-coupling reaction of **21** with alkynes provided the 2-alkyne substituted adenosines, **22** and **23**. The adenosine analogues, **21**–**26**, were acetonided to the 2′,3′-*O*-isopropylidenes **27**–**31**, and the 5-hydroxyls were reacted with sodium hydride and sulfamoyl chloride as previously reported[13,18,19] to furnish the 5-*O*-sulfamoylated adenosines **32**–**37**. Finally, the aminoacylation of **32**–**37** with methionine or isoleucine followed by acidic hydrolysis afforded the methionyl (**50**–**55**) and isoleucyl (**56**–**61**) sulfamate analogues, respectively.

The synthesized methionyl and isoleucyl adenylate analogues were evaluated as inhibitors of *E. coli* MetRS and IleRS, respectively (Table 1). The inhibitory activities were determined by measuring the decrease of the aminoacylation product, the [^{35}S]methionyl *E. coli*-tRNA$^{\mathrm{Met}}$ or [^{3}H]isoleucyl *E. coli*-tRNA$^{\mathrm{Ile}}$, in the presence of different chemical concentrations as previously reported.[20] Sulfamate analogues of methionyl adenylate (Met-NHSO$_3$–AMP) (**50**) and isoleucyl adenylate (Ile-NHSO$_3$–AMP) (**56**) were evaluated as references in our assay protocol, and showed values of IC$_{50}$ = 0.007 and 0.098 μM, respectively. The replacement of the sulfamate oxygen with –NH-*O*- provided the *N*-alkoxysulfamide analogues (**9**, **10**), which exhibited much lower inhibitory activities than the corresponding sulfamates (**50**, **56**), but similar activities as the ester and hydroxamate surrogates reported previously.[9] The *N*-hydroxysulfamides (**16**, **17**) resulting from the replacement of the sulfamate oxygen with –N(OH)– attenuated the inhibition of MetRS and IleRS as compared to the *N*-alkoxysulfamides. Since the sulfamate analogues of methionyl and isoleucyl adenylate had shown the most potent inhibitory activities, as compared to the other isosteres studied thus far, we wanted to investigate the SAR of their adenine region by creating aminoacyl adenylates with different adenine derivatives. Four functional groups, including chloro, iodo, ethynyl, and hexynyl groups were introduced at the 2-position of the sulfamate analogues of the methionyl and isoleucyl adenylates. In the methionyl adenylate analogues, the 2-substituted analogues (**51**, **52**, **54**, **55**) showed moderate inhibitory activities, with a range of IC$_{50}$ = 1.1–7.4 μM, but were less potent than the unsubstituted parent compound (**50**). Among the isoleucyl adenylate analogues, the 2-chloro (**57**) analogue exhibited moderate inhibition, while the 2-iodo (**58**) and 2-ethynyl (**60**) analogues showed significantly improved inhibitory activity to IleRS, with values of IC$_{50}$ = 0.014 and 0.039 μM, which were 7- and 2.5-fold more potent than the parent compound (**56**), respectively. However, the substitution with a more extended hexynyl to afford **61** led



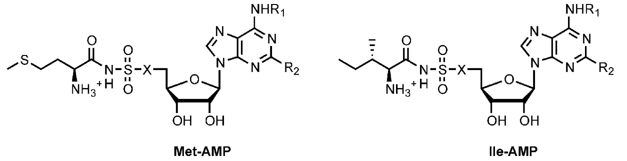
Scheme 3. Reagents and conditions: (a) (i) HMDS, (NH$_4$)$_2$SO$_4$, reflux; (ii) *O*-tribenzoylribosyl-1-acetate, TMSOTf, ClCH$_2$CH$_2$Cl, 83%; (b) I$_2$, CH$_2$I$_2$, isoamyl nitrite, CuI, reflux, 85%; (c) NH$_3$, MeOH, 83%; (d) 23: (i) HC≡CTMS, (PPh$_3$)$_2$PdCl2, CuI, Et$_3$N, DMF, 80 °C, 84%; (ii) NH$_3$, MeOH, 87%; (e) 24: (i) HC≡C(CH$_2$)$_3$CH$_3$, (PPh$_3$)$_2$PdCl2, CuI, Et$_3$N, DMF, 80 °C, 89%.



Scheme 4. Reagents and conditions: (a) camphorsulfonic acid, acetone, 65–75%; (b) NH_2SO_2Cl, NaH, DME, 60–80%; (c) $BocNCH(R)CO_2H$, DCC, DMAP, CH_2Cl_2, 85–95%; (d) CF_3CO_2H, CH_2Cl_2-H_2O, 45–65%.

to a dramatic loss in the inhibitory activity. 6-Methylation of the 2-iodo analogues (**52**, **58**) to furnish **53** and **59** was ineffective for the enzyme inhibition, as they showed 160 and 1600-fold reductions, respectively. This finding suggested that the 6-amino group is crucial to interact with the IleRS active site, where the two amino hydrogens form hydrogen bonds with two carbonyl oxygens in the enzyme, as previously examined in the complex between Ile-AMS and the X-ray structure of the *Thermus thermophilus* IleRS.[21]

Table 1. Inhibitory activities of the synthesized compounds toward *E. coli* MetRS and IleRS



Number	X	R_1	R_2	IC_{50}
Met-AMP				
9	NHO	H	H	19.7
16	N(OH)	H	H	106
50	O	H	H	0.007
51	O	H	Cl	7.4
52	O	H	I	1.2
53	O	CH_3	I	191
54	O	H	C≡CH	1.1
55	O	H	$C{\equiv}C(CH_2)_3CH_3$	1.9
Ile-AMP				
10	NHO	H	H	12.8
17	N(OH)	H	H	132
56	O	H	H	0.098
57	O	H	Cl	5.5
58	O	H	I	0.014
59	O	CH_3	I	22.2
60	O	H	C≡CH	0.039
61	O	H	$C{\equiv}C(CH_2)_3CH_3$	1.1

The high potency of the 2-iodo (**58**) and 2-ethynyl (**60**) analogues of Ile-$NHSO_3$–AMP suggested that IleRS may contain a cavity to afford such bulky groups. This finding prompted us to search for the presence of such a pocket in IleRS. Recently, the structural basis for the recognition of an isoleucyl-adenylate by IleRS was identified by the crystal structure of the IleRS from *Thermus thermophilus* in complex with the sulfamide, Ile-AMS (Ile-$NHSO_2NH$–AMP). Careful examination of the adenine binding site of Ile-AMS with the enzyme revealed a small hole (circle with arrows) around the 2-position of adenine, as shown in Figure 2a. The docking study with the 2-iodo sulfamate analogue (**58**) was performed on the X-ray model above, and the modeled results are shown in Figure 2b (ball and stick) and c (spacefill).[22] The modeling study revealed that the 2-iodo atom (violet color) of **58** was accomodated by the cavity shown in Figure 2a very well, in which the Gly56, Gln59, Gly551, His581, Gly582, Leu583, Ile584, and Arg628 amino acids were present within a 5 Å range from the iodo moiety. This docking result confirmed that the enhanced potency of **58** resulted from its better binding to the active site of IleRS by accomodating the 2-iodo moiety into the hole. Also, the dimensions of the hole were not sufficient to hold a bulkier group, like the hexynyl group of **61**, which explains its lower inhibitory activity. Nevertheless, the reduced inhibitory activity of the 2-chloro analogue (**57**) cannot be explained by this model, and its investigation is still under way.

In summary, the potent MetRS and IleRS inhibitions by the sulfamate analogues of Met-AMP and Ile-AMP were optimized by modifications of the sulfamate linker

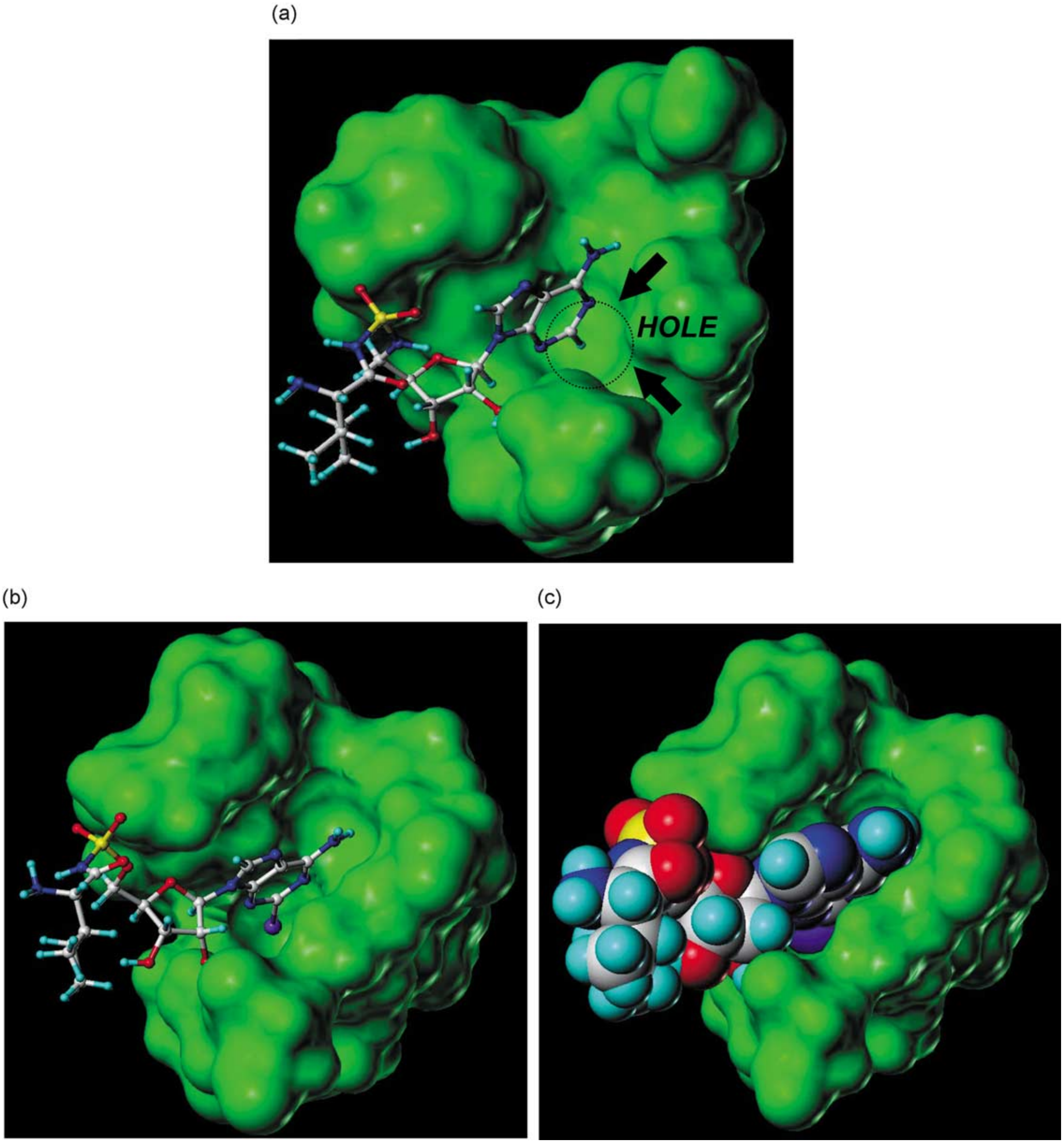


Figure 2. Docking model of (a) Ile-NHSO$_2$NH–AMP[21] and (b, c) 2-iodo analogue of Ile-NHSO$_3$–AMP (**58**) with *T. thermophilus* IleRS.

and the adenine moiety. Replacement of the sulfamate oxygen with N–O groups, to furnish *N*-hydroxy and *N*-alkoxy sulfamide, caused marked reductions in the inhibitory potency to *E. coli* MetRS and IleRS, respectively. However, the modification of the adenine group in the sulfamate surrogates led to a highly potent IleRS inhibitor, the 2-iodo analogue (**58**). A docking study of **58** into the X-ray structure of the *T. thermophilus* IleRS indicated that the potent inhibition by **58** resulted from its snug fit within a hydrophobic cavity around the 2-position of adenine in Ile-NHSO$_3$–AMP.

Acknowledgements

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22. The 2-iodo compound (**58**) was modeled in the *T. thermophilus* IleRS active site, using the X-ray structure of the Ile-AMS (Ile-NHSO$_2$NH–AMP) complex in ref 21, with the Ile-AMS molecule as a guide for the docking. The complex was energy-minimized with SYBYL 6.5, using the Tripos force field. All computational work was done on a Silicon Graphics O$_2$ R10000 workstation.