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Design and synthesis of triazolopyrimidine acylsulfonamides as novel anti-mycobacterial leads acting through inhibition of acetohydroxyacid synthase

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ABSTRACT

Novel triazolopyrimidine acylsulfonamides class of antimycobacterial agents, which are mycobacterial acetohydroxyacid synthase (AHAS) inhibitors were designed by hybridization of known AHAS inhibitors such as sulfonyl urea and triazolopyrimidine sulfonamides. This Letter describes the synthesis and SAR studies of this class of molecules by variation of two parts of the molecule, the phenyl and triazolopyrimidine rings. SAR study describes optimisation of enzyme potency, whole cell potency and evidence of mechanism of action.

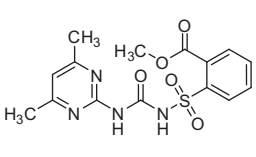
Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (Mtb), is responsible for over 1.4 million deaths annually and 9 million new cases of infection each year. The re-emergence of TB due to HIV co-infection, multi-drug resistant (MDR-TB) and extremely drug resistant (XDR-TB) strains of Mtb has created a global epidemic with serious consequences if left unaddressed.[1] The resolution of the current TB epidemic requires not only prevention of new infections but also new medicines that are safe and effective against drug sensitive and drug resistant Mtb strains.[2]

It is well known in the literature that drugs that act via novel mechanisms of action would be effective against both drug sensitive as well as drug resistant TB and could also potentially help to replace one or more of the current drugs that exhibit severe side effects.

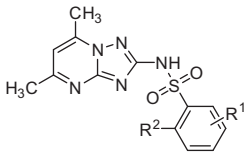
In the recent literature, acetohydroxyacid synthase (AHAS) has been recognized as an attractive enzyme target for discovering novel anti-TB compounds.[3–6] AHAS is the first enzyme in the pathway for the de novo biosynthesis of branched chain amino acids [BCAA] isoleucine, leucine and valine (*ilv*). AHAS catalyses the irreversible decarboxylation of pyruvate and the condensation of the acetaldehyde moiety with a second molecule of pyruvate to give 2-acetolactate, or with a molecule of 2-ketobutyrate to yield 2-aceto-2-hydroxybutyrate, common precursors for the synthesis of all three branched chain amino acids. Since mycobacteria synthesize all the amino acids required for their protein synthesis, the amino-acid biosynthesis pathways are essential for their survival.

Such BCAA pathway are absent in humans.[7] As AHAS is also an essential enzyme in plants it has been successfully targeted for developing novel herbicides. Commercially marketed herbicides such as sulfonylurea sulfometuron methyl (SMM)[8] and triazolopyrimidine sulfonamides[9a] are known to be potent inhibitors of plant AHAS (Fig. 1).

Interestingly, SMM has been shown to inhibit the growth of Mtb and shown to be efficacious in a murine mouse model, albeit at



Sulfometuron Methyl (SMM)



Triazolopyrimidine Sulfonamides
Flumetasulam, wherein R^1= 1-F and R^2 = F

Figure 1. Plant AHAS inhibitors as herbicides.

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higher dose (500 mg/kg, ip), suggesting that AHAS is an essential target for the survival of Mtb.[3]

However, recent studies have shown that inactivation of *ilv*B1 (coding for AHAS large subunit) gene in Mtb leads to BCAA auxotrophy and attenuation of virulence in mice. This may be due to either uptake of BCAA from in vivo environment or *ilv*B1 mutant having ability to synthesize some BCAA using an alternative mechanism, or combination of both.[10]

Based on the above studies, one would assume that inhibiting AHAS in Mtb with a small molecule inhibitor would result in bacteriostatic effect in vivo and not bactericidal, such compounds when given in combination with other anti-tuberculosis drugs may lead to better efficacy. In fact, we have recently demonstrated that rifampicin, a potent RNA polymerase inhibitor and one of the front-line TB drugs, potentiates the killing effects of several of the AHAS inhibitors in vitro.[11] Therefore, the focus of the current study was to identify potent Mtb specific AHAS inhibitors with improved whole cell potency against Mtb.

Our approach towards designing AHAS inhibitors with improved whole cell potency was to utilize the structures **1** and **2** as starting points. We hypothesized that hybridization of structural features of sulfonylureas and triazolopyrimidine sulfonamides would result in compounds such as triazolopyrimidines acylsulfonamide (**3**) that may maintain overall structural features required to bind to the bacterial enzyme through the critical residues (Fig. 2). The triazolopyrimidine acylsulfonamides **3** are also already known in literature as herbicides *or* plant growth regulants.[12,13]

It is interesting to note here that the genesis of triazolopyrimidine sulfonamides itself has its origin in the conformational restriction of sulfonylureas (Fig. 3).[9a] Overlay of SMM (crystal bound conformation as in 1YI0),[9b] triazolopyrimidine sulfonamide (Flumetasulam) and triazolopyrimidine acylsulfonamide **3d** is depicted in Fig.4.

As evident from the overlay, the common feature present in both sulfonylureas and triazolopyrimidine sulfonamides is the presence of SO_2NH group. The carbonyl group of **3d** occupies the same region as that of carbonyl group present in SMM and allows the pyrimidine part of triazolopyrimidine ring (**3d**) to come closer to same position as that of pyrimidine ring of SMM.

To validate our hypothesis, we evaluated the binding mode of SMM and compound **3d** through docking using Mtb AHAS homology model built over plant AHAS (1YI0) as a template. Docking studies (Fig. 4) suggest that the binding mode and the observed interactions of SMM and **3d** were quite similar to sulfonylureas class of compounds, reported in the crystal structure of plant and yeast AHAS.[9b,c] The carbonyl of **3d** and one of the nitrogen atom of triazolopyrimidine ring forms crucial hydrogen bond (HB) interactions with terminal nitrogen's of conserved Arg377 mimicking carbonyl and pyrimidine ring nitrogen of SMM. The sulfonyl oxygen of **3d** forms HB interaction with Lys197 whereas the

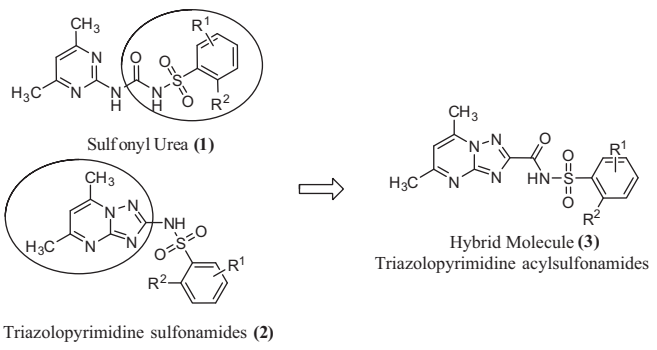


Figure 2. Hybridization approach to get triazolopyrimidines acylsulfonamide.

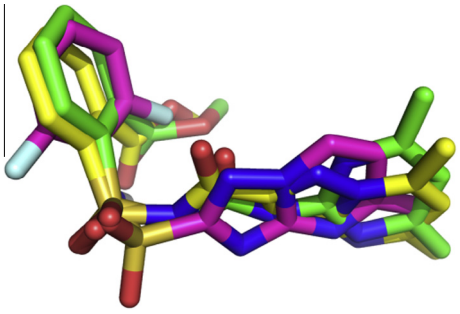


Figure 3. Overlay of Flumetasulam (magenta) and compound **3d** (yellow) onto crystal structure (1YI0) bound conformation of sulfometuron (green).

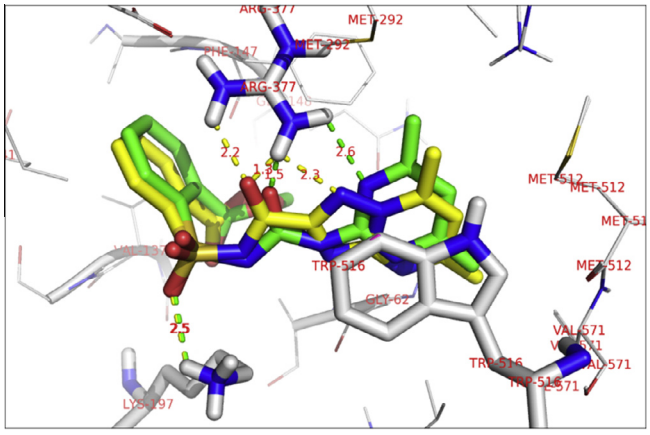


Figure 4. Comparison of Hypothetical binding modes of SMM (stick model, green) and compound **3d** (stick mode, yellow) in Mtb AHAS homology model (grey, important residues are shown in stick model). Dash line indicates hydrogen bond interactions.

triazolopyrimidine ring of **3d** forms a very strong π–π stacking interaction with indolyl group of Trp516. Thus, the newly designed triazolopyrimidine acylsulfonamide class of compounds may act as novel Mtb AHAS inhibitors.

Coincidentally acylsulfonamides, such as **4** and **5**, have been reported to be inhibitors of plant AHAS[14] thus giving credence to our thinking that bioisosteric replacement of sulfonamide with acylsulfonamides may be an valid strategy to identify new inhibitors of Mtb AHAS (Fig. 5).

We have designed and synthesized differently substituted triazolopyrimidine acylsulfonamide class of compounds with variations in the phenyl sulfonamide part and tested. Here, we present AHAS enzyme inhibition and MIC data for triazolopyrimidine acylsulfonamides in Table 1.

The title series of triazolopyrimidine acylsulfonamides were synthesized in a convergent fashion by coupling sulfonamides **11** and triazolopyrimidine carboxylic acid **9** using EDCI as coupling reagent (Scheme1). **9** was obtained by condensing ethyl 5-amino-4*H*-1,2,4-triazole-3-carboxylate (**6**) with acetylacetone (**7**) as per the literature procedure.[12] The sulfonamides used were either commercially available or were conveniently synthesized by

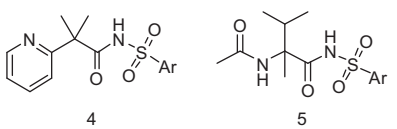
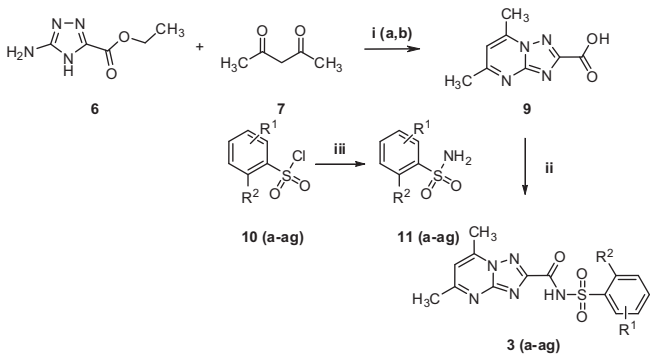


Figure 5. Acylsulfonamides as AHAS inhibitors.

Table 1
Mtb AHAS pIC_{50}, Mtb MIC and Mtb MIC in presence of *ilv*

Compound	R^1	R^2	Mtb AHAS [a] pIC_{50} [b]	Mtb MIC [c] (μg/ml)	Mtb MIC+200 (μg/ml) *ilv* [d]
SMM	—	—	6.12	16	>32
4	—	—	<4.52	>32	ND [e]
3a	*o*-CF_3	H	6.98	⩽0.13	>32
3b	*o*-Cl	H	6.82	2	>32
3c	*o*-Br	H	7.04	0.25	>32
3d	*o*-COOMe	H	7.15	0.25	>32
3e	*o*-OCF_3	H	7.60	0.03	>32
3f	*o*-F	H	5.24	>32	>32
3g	*o*-CH_3	H	6.29	1	>32
3h	*o*-OCH_3	H	5.81	0.5	>32
3i	*o*-$OCHF_2$	H	>7.2	<0.25	>32
3j	*o*-CH_2CN	H	6.59	32	ND
3k	*m*-Br	H	5.31	32	ND
3l	*m*-CF_3	H	5.36	16	ND
3m	*m*-OCF_3	H	5.60	16	ND
3n	*p*-OCF_3	H	<4.52	>32	ND
3o	*p*-CF_3	H	<4.52	>32	ND
3ab	*o*-Cl	Me	6.70	0.25	>32
3ae	*o*-OCF_3	Me	7.75	⩽0.03	>32
3ag	*o*-CH_3	Me	6.54	0.5	>32

[a] Mtb AHAS: *Mycobacterium tuberculosis* Acetohydroxyacid synthase.
[b] pIC_{50}: –log IC_{50} (Mtb AHAS inhibition).
[c] MIC: Minimum Inhibitory Concentration (unit as given).
[d] *ilv* : Isoleucine, Leucine and Valine.
[e] ND: Not determined.



Scheme 1. Reagents and conditions: (i) (a) Piperidine/EtOH, 100 °C, reflux, overnight (b) 2.5 N NaOH/EtOH (ii) CH_3CN/EDCI, DMAP, 50 °C 3 h (iii) aq NH_3/CH_3CN, 0 °C to rt, 2 h.

converting respective sulfonyl chlorides (**10**) to sulfonamides using aqueous ammonia.

In addition to these molecules, SMM was also tested to establish the baseline. The acylsulfonamide **4** was found to be inactive against Mtb AHAS but the newly designed acylsulfonamides (**3a**–**3ai**) showed potent activity in the primary enzyme assay as shown in Table 1.

As shown in Table 1, the ortho substituted analogs (**3a**–**3j**) showed potent AHAS inhibition (pIC_{50} from **5.24** to >**7.2**), whereas, the *meta*-substituted analogs (**3k**–**3m**) were moderately active with pIC_{50} in the range of (5.3–5.6) and the para substituted analogs (**3n** and **3o**) were inactive. Although electron withdrawing groups in the ortho position of phenyl ring seems to be favoured, this is not always the case as seen with compounds **3g** and **3h**, which have electron donating groups. Analysis of SAR reveals that the inhibition could be ascribed to electron withdrawing nature as well as size of ortho substitution of phenyl ring. The moderate activity of the methoxy and methyl groups (**3g**, **3h**) reinforces the fact that both electronics as well as steric factor plays an important role in bringing AHAS inhibition.

The presence of another ortho substituent in the phenyl portion of triazolopyrimidine acylsulfonamide, such as a methyl group may lead to twisting of the phenyl ring with respect to the rest of the molecule. Based on this data, we synthesized a few 2,6 disubstituted sulfonamides (**3ab**, **3ae**, **3ag**) with methyl group at R^2 position. This result suggested that twisting of the aryl ring may not be an important determinant for activity of this class of molecules and in-fact it had potentiated the AHAS inhibition for 2,6 disubstituted compounds.

The data in Table 1 shows that compounds **3a**–**3ag** with varied enzyme inhibition potencies showed moderate to potent Mtb MIC. In general, the cellular potency (MIC) fairly correlates with enzyme potency (pIC_{50}) for this series. A closer look at the data suggested that the MICs did not correlate well with enzyme inhibition (pIC_{50}) for few compounds (e.g. **3b** and **3h**). This could be due to differential bacterial permeability or efflux property of the compounds resulting in moderate correlation between pIC_{50} and MIC for these compounds. It is interesting to note that additional methyl substitution at R^2 position not only potentiated the enzyme potency but also improved MIC as compared to its mono substituted counterpart (e.g. **3b** vs **3ab**). This pattern is structurally consistent with all 2,6 di-substituted compounds (**3ab**, **3ab**, and **3ag**). Furthermore, when compounds were tested in the presence of *ilv*, the MICs were elevated suggesting that growth inhibition is due to the amino-acids starvation and not by any other mechanism and that the target is auxotrophic in nature.

In conclusion, we have demonstrated triazolopyrimidine acylsulfonamides as new leads with potent anti-TB activity acting through the inhibition of Mtb AHAS enzyme. The molecules exhibited potent Mtb AHAS inhibition as well as Mtb MIC via the expected mechanism of action and were more potent than sulfonylureas and triazolopyrimidine sulfonamides reported earlier.

These molecules are uniquely placed to serve as leads, as there are sufficient structural handles that can be explored within the triazolopyrimidine ring for further optimization to identify a candidate drug with the potential to synergize with a RNA polymerase inhibitor such as Rifampicin.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.02.054.

References and notes

1. World Health Organization. *Global TB control Report* 2012. http://www.who.int/tb/publications/global_report/en/index.html.
2. Koul, A.; Amoult, E.; Lounis, N.; Guillemont, J.; Andries, K. *Nature* **2011**, *469*, 483.
3. Grandoni, J. A.; Marta, P. T.; Schloss, J. V. *J. Antimicrob. Chemother.* **1998**, *42*, 475.
4. Sohn, H.; Lee, K.; Ko, Y.; Ryu, J.; Woo, J.; Koo, D.; Shin, S.; Ahn, S.; Shin, A.; Song, C.; Jo, E.; Park, J.; Kim, H. *Int. J. Antimicrob. Ag.* **2008**, *31*, 567.
5. Zohar, Y.; Einav, M.; Chipman, D. M.; Barak, Z. *BBA-Proteins Proteom.* **2003**, *1649*, 97.
6. Wang, D.; Zhu, X.; Cui, C.; Dong, M.; Jiang, H.; Li, Z.; Liu, Z.; Zhu, W.; Wang, J. *J. Chem. Inf. Model.* **2013**, *53*, 343.
7. Chipman, D.; Barak, Z.; Schloss, J. V. *BBA-Protein Struct. Mol. Enzymol.* **1998**, *1385*, 401.
8. LaRossa, R. A.; Schloss, J. V. *J. Biol. Chem.* **1984**, *259*, 8753.
9. (a) Kleschick, W. A.; Costales, M. J.; Dunbar, J. E.; Meikle, R. W.; Monte, W. T.; Pearson, N. R.; Snider, S. W.; Vinogradoff, A. P. *Pestic. Sci.* **1990**, *29*, 341; (b) McCourt, J. A.; Pang, S. S.; King-Scott, J.; Guddat, L. W.; Duggleby, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 569; (c) McCourt, J. A.; Pang, S. S.; Guddat, L. W.; Duggleby, R. G. *Biochemistry* **2005**, *44*, 2330.
10. Awasthy, D.; Gaonkar, S.; Shandil, R. K.; Yadav, R.; Bharath, S.; Marcel, N.; Subbulakshmi, V.; Sharma, U. *Microbiology* **2009**, *155*, 2978.
11. Balganesh, M.; Nandan, S. WO2007132189.
12. Tseng, C. EP 0244166.
13. Tseng, C. US 4838925.
14. Cloudsdale, I. S.; Anderson, R. J.; Chinn, H. R.; Craig, G. W.; Deng, P. N.; Herberich-Patton, L.; Pomes, J. C. *ACS Symp. Ser. (Synthesis and Chemistry of Agrochemicals IV)* **1995**, *584*, 37.