Tri-Substituted Imidazoles as *Mycobacterium tuberculosis* Glutamine Synthetase Inhibitors

Johan Gising,†,‡ Mikael T. Nilsson,†,‡ Luke R. Odell,† Samir Yahiaoui,† Martin Lindh,‡ Harini Iyer,‡ Achyut M. Sinha,‡ Bachally R. Srinivasa,‡ Mats Larhed,‡ Sherry L. Mowbray,‡ Anders Karlén*†

†Department of Medicinal Chemistry, Organic Pharmaceutical Chemistry, BMC, Uppsala University, Box 574, SE-751 23 Uppsala, Sweden. ‡Department of Cell and Molecular Biology, Structural Biology, BMC, Uppsala University, Box 596, SE-751 24 Uppsala, Sweden. †AstraZeneca India Private Limited, Bellary Road, Hebbal, Bangalore 560024, India.

Supporting Information Placeholder

ABSTRACT: *Mycobacterium tuberculosis* glutamine synthetase (*MtGS*) is a promising target for anti-tuberculosis drug discovery. In a recent high-throughput screening study we identified several classes of *MtGS* inhibitors targeting the ATP-binding site. We now explore one of these classes, the 2-tert-butyl-4,5-diarylimidazoles, and present the design, synthesis and X-ray crystallographic studies leading to the identification of *MtGS* inhibitors with submicromolar IC₅₀ values and promising anti-tuberculosis MIC values.

INTRODUCTION

*Mycobacterium tuberculosis*, the causative agent of tuberculosis, is one of the world’s most deadly pathogens, leading to almost 1.7 million deaths annually despite more than a hundred years of research.¹ Rapidly emerging drug-resistant and multidrug-resistant strains have provided additional impetus to the search for new therapeutic agents to combat the disease.²⁻³ *M. tuberculosis* glutamine synthetase (*MtGS*; EC 6.3.1.2) catalyzes the conversion of glutamate, ammonia and ATP to glutamine, phosphate and ADP.⁴ *MtGS* plays a key role in controlling the ammonia levels within infected host cells and so contributes to the pathogen’s capacity to inhibit phagosome acidification and phagosome-lysosome fusion.⁵⁻⁶ Furthermore, *MtGS* is believed to be involved in cell wall biosynthesis; it is found extracellularly in large quantities, which is related to a role in the production of the poly-δ-glutamate-glutamine that is a major component of the cell wall in pathogenic mycobacteria.⁷ Treatment of *M. tuberculosis* with antisense oligonucleotides to GS mRNA⁸ or with the GS inhibitor L-methionine-S-sulfoximine (MSO, ¹,Figure 1a)⁹ inhibits biosynthesis of poly-L-glutamate-glutamine as well as the bacterial growth.⁸⁻⁹ Compound ¹ also shows in vivo efficacy in a guinea pig model, suggesting GS as a promising and druggable target in the treatment of *M. tuberculosis*.¹⁰ The structure of *MtGS* incubated with ¹, which is phosphorylated in situ to form the transition-state analogue L-methionine-S-sulfoximine phosphate (MSO-P, ²,Figure 1a), has been reported, showing how the inhibitor interacts in the amino acid-binding site.¹¹ In the present study we explore a new class of *MtGS* inhibitors derived from a recent high throughput screening (HTS) study.¹²⁻¹³ This screen targeted the ATP-binding

![Figure 1](https://example.com/figure1.png)

**Figure 1.** a) Reference GS inhibitor ¹ and phosphorylated transition-state analogue ². These two compounds bind to the amino acid-binding site of MtGS. b) Common scaffold of the HTS hits of cluster ². c, d) The two most potent compounds from cluster ² in the HTS study. Compounds ³ and ⁷ interact with the ATP-binding site of MtGS.¹⁴

RESULTS AND DISCUSSION

We first sought a synthetic route to the two most active HTS hits in order to re-synthesize them, confirm their activity and initiate X-ray crystallographic studies. This proved to be problematic; despite exploration of various synthetic routes, we were never able to re-synthesize compound ³. However, the tri-substituted imidazole ⁷ could be readily synthesized, and was therefore used in the hit expansion of this cluster. The synthesis started from 2-bromo-6-methoxynaphthalene ⁴ with two consecutive Sonogashira couplings followed by an oxidation
and cyclization to form the imidazole ring (see Scheme 1). The ethyne was introduced by a fast microwave assisted Sonogashira method using ethynyltrimethylsilane, dichlorobis(triphenylphosphine)-palladium, copper iodide and acetonitrile:diethylamine (1:1) as solvent. In situ deprotection with saturated K₂CO₃ in methanol gave the 2-ethynyl-6-methoxynaphthalene (5) in 85% yield. Employing the same protocol, the 4-pyridyl moiety was incorporated in moderate yield (6a, 47%). Oxidation of the ethyne 6a to the di-ketone intermediate with potassium permanganate in aqueous acetone proved to be a very sensitive reaction, and often led to oxidative cleavage of the triple bond. Changing the standard buffer system NaHCO₃/MgSO₄ to NaH₂PO₄/Na₂HPO₄, and thus lowering the pH from 7.5 to 5, provided a more robust protocol to furnish the diketone. In our attempts to prepare 3, the identical oxidation conditions with the corresponding quinoline intermediate only gave over-oxidation, and no conditions were identified that suppressed oxidative cleavage. Finally the imidazole ring was synthesized by in situ trapping of the diketone with various aldehydes and ammonium acetate allowing straight-forward variation of the tert-butyl group position (R₂). The re-synthesized HTS hit 7a had an IC₅₀ of 3.1 µM, which is 30-fold higher than the IC₅₀ obtained in the HTS study (0.1 µM), due to our more stringent assay conditions. Unfortunately, neither 3 (from the AstraZeneca library) nor the resynthesized 7a was active in the mycobacterial growth assay (MICs > 32 µg/mL).

Scheme 1. Synthetic Route to Compounds 7a–m.

Reagents and conditions: (a) ethynyltrimethylsilane, Pd(PPh₃)₄Cl₂, CuI, MeCN, diethylamine, MW 120 °C, 15 min, then K₂CO₃, MeOH, rt, 2h, 85%; (b) bromoaryl/heteroaryl, Pd(PPh₃)₄Cl₂, CuI, MeCN, diethylamine, MW 80–120 °C, 15 min, 22–63%; (c) KMnO₄, phosphate buffer; (d) aldehyde, ammonium acetate, n-butanol, 50–65 °C, 0.5–5 h, 10–63%.

Since numerous 4-substituted aryl imidazoles of 7a (Figure 1) had already been identified and evaluated in the HTS study, we focused instead on optimizing positions 2 (the tert-butyl group) and 5 (the 4-pyridyl group). The importance of the position of the nitrogen in the 4-pyridyl ring was first examined by synthesizing the phenyl and pyrimidine analogues (Table 1, 7b–d). The same synthetic strategy as described above was applied, replacing the 4-pyridyl with aryl/heteroaryl groups introduced in the second Sonogashira reaction. Cyclization with pivalaldehyde gave the target compounds 7b–d in which the nitrogen was either removed (7b) or its position altered (7c–d). The results clearly indicate the importance of having a nitrogen in the 4-position of the pyridine ring; all compounds lacking this feature were inactive (IC₅₀ > 25 µM).

To investigate the importance of steric bulk in position 2 of the imidazole ring, compounds with smaller alkyl chains were synthesized. Cyclizing the di-ketone with propionaldehyde, acetaldehyde and formaldehyde gave ethyl, methyl and hydrogen, respectively, at position 2 of the imidazole ring (7e–g in Table 1). Removal of two methyl groups generated a slightly more active compound (7e, 2.2 µM vs 7a, 3.1 µM). Removal of yet another methyl group yielded 7f, which was inactive. Likewise, complete removal of the tert-butyl group gave the inactive compound 7g. Clearly, there is a preference for relatively bulky substituents in this position.

Parallel studies at this point produced the structure of MtGS in complex with 2, 3 (obtained from AstraZeneca’s in-house compound library), phosphate and magnesium at 2.15 Å resolution (crystallographic R-factor 22.5%, Figure 2a). An overlay of this structure on that of the complex with 2, ADP and magnesium (PDB entry 2BVC) is shown in Figure 2b; an rmsd of 0.2 Å is obtained when the Cos of residues 4–478 in the A chains are compared. Compound 2 occupies exactly the same position in the amino acid-binding site in each case; all three of the metal ions are also in equivalent locations. The 4-pyridyl moiety of 3 is at essentially the same place as the 6-membered pyrimidine ring of ADP, with the inhibitor’s ring nitrogen providing the only hydrogen bond to protein (via the side chain of Ser280), in an interaction equivalent to that of N1 of ADP’s adenine ring. The interaction with this serine explains why a nitrogen at the 4-position of the pyridine ring is so important for binding affinity (vide supra). The imidazole ring and tert-butyl moieties of 3 occupy roughly the same space as the ribose of the nucleotide. Thus the R₂ group is found at the end of the ribose proximal to its linkage with the phosphates. A phosphate ion assumes the position of the β-phosphate of ADP. The quinoline moiety extends out toward the solvent, making van der Waals interactions with the protein only near Ala362 (not shown). Correspondingly, the electron density of this group is poorer.

Figure 2. a) MtGS (yellow carbons) in complex with 2 (MSO-P), 3 (gray carbons), phosphate and magnesium. Hydrogen bond between 3 and Ser280 is shown as a black dashed line. Nearby magnesium ions are green spheres. b) Superposition of the MtGS structure with 3 on that with bound ADP (PDB entry 2BVC) allows comparison to nucleotide (pink carbons) binding. Protein/ADP hydrogen bonds are pink dashed lines.
Reagents and conditions: (a) EtOH, Pd(OAc)$_2$, Xantphos, DBU, Mo(CO)$_6$, MW 120 °C, 30 min, 93%; (b) 2-fluoro-4-methylpyridine, NaHMDS, THF, 0 °C, 2 h, 64%; (c) HBr, DMSO, 70 °C, 2 h then pivalaldehyde, ammonium acetate, n-butanol, 50 °C, 2 h, 71%; (d) 11a: (i) diphenylmethanamine, dioxane, MW 200 °C, 10 h, (ii) Pd/C, NH$_2$OAc, MeOH, MW 120–140 °C, 2 h, 28%; 11b: methylamine (2.0 M in THF), MW 150 °C, 17 h, 18%; 11c: ammonium hydroxide, DMF, MW 150 °C, 12 h, 85%; 11d: AcOH, H$_2$O, MW 190 °C, 2 h, 91%.

Table 1. Activities of Tri-Substituted Imidazoles

<table>
<thead>
<tr>
<th>Compd</th>
<th>R$_1$</th>
<th>R$_2$</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td></td>
<td></td>
<td>0.049 ± 0.006 2</td>
</tr>
<tr>
<td>7b</td>
<td></td>
<td></td>
<td>1.2 ± 0.1 64</td>
</tr>
<tr>
<td>7c</td>
<td></td>
<td></td>
<td>&gt;25</td>
</tr>
<tr>
<td>7d</td>
<td></td>
<td></td>
<td>3.1 ± 0.1 &gt;256</td>
</tr>
<tr>
<td>7e</td>
<td></td>
<td></td>
<td>&gt;25</td>
</tr>
<tr>
<td>7f</td>
<td></td>
<td></td>
<td>&gt;25</td>
</tr>
<tr>
<td>7g</td>
<td></td>
<td></td>
<td>&gt;25</td>
</tr>
<tr>
<td>7h</td>
<td></td>
<td></td>
<td>&gt;25</td>
</tr>
<tr>
<td>7i</td>
<td></td>
<td></td>
<td>&gt;25</td>
</tr>
<tr>
<td>7j</td>
<td></td>
<td></td>
<td>&gt;25</td>
</tr>
<tr>
<td>7k</td>
<td></td>
<td></td>
<td>&gt;25</td>
</tr>
<tr>
<td>7l</td>
<td></td>
<td></td>
<td>&gt;25</td>
</tr>
<tr>
<td>7m</td>
<td></td>
<td></td>
<td>&gt;25</td>
</tr>
</tbody>
</table>

Evaluation based on the Glide score as well as visual inspection. Despite their promising docking poses, compounds 7j–m all lacked inhibitory activity at 25 µM.

Going back to the superimposed X-ray structures, we decided to substitute the 4-pyridyl group of 7a with a 2-aminopyridine-4-yl, which would possibly allow the formation of a hydrogen bond to the backbone carbonyl oxygen of Lys361, similar to the interaction seen for N6 of ADP (Figure 2b). Another synthetic approach was employed in the synthesis of the aminopyridines 11a–d, avoiding the sensitive oxidation of the triple bond. Ethyl 6-methoxy-2-naphthoate (8) was synthesized in 93% yield by a palladium-catalyzed carbonylation of 2-bromop-4-methylpyridine with NaHMDS via nucleophilic substitution of 8. As an alternative to creating the imidazole ring from the diketone, an α-bromination was performed on 9 followed by cyclization with ammonium acetate and pivalaldehyde. Attempts to re-synthesize the HTS hit 3 by this route again failed because we were never able to substitute the ethoxy of ethyl quinoline-3-carboxylate with 4-methylpyridine. At this point, it was encouraging to see that the fluorne in compound 10 contributed to an approximate 2.5-fold increase of affinity compared to the hit compound 7a (IC$_{50}$ 1.2 µM vs 3.1 µM, Table 1). The amino-pyridine 11a was synthesized via microwave-assisted nucleophilic substitution of the fluorne of 10 with diphenylmethanamine followed by deprotection through catalytic hydrogenation. Remarkably, compound 11a was over 60 times better than the reference compound 7a (0.049 µM vs 3.1 µM, Table 1), suggesting an important new interaction with the enzyme. For comparison, the methylamino and the dimethylamino groups were incorporated in a similar way. Compound 11b, having one methyl group, showed a 100-fold loss in activity compared to 11a, making it even less potent than 7a. Substituting the fluorne with a dimethylamino group.

Scheme 2. Synthetic Route to Compounds 11a–d.
At this time, we succeeded in obtaining a crystal structure of MtGS to support a hydrogen bond between the 2pamino bonds as cyan dashed lines. b) Superposition of the protein structures are essentially identical. The compounds had promising MIC values; the most potent enzyme inhibition and antibacterial activity are attained.

This moderate level of cytotoxicity is not unusual among compounds in the early stages of drug development, and the situation is expected to improve as more effective enzyme inhibition and antibacterial activity are attained.

CONCLUSIONS

A recent HTS study targeting the ATP-binding site of MtGS produced several active classes of compounds. One of these, the 2-tert-butyl-4,5-diaryl imidazoles, contained several hits, and a complex with one of the inhibitors with MtGS could be obtained by X-ray crystallography. Based on this structure our initial SAR explorations could be rationalized as well as the importance of having a nitrogen, acting as a hydrogen-bond acceptor, in the 4-position of the pyridine ring. Building out from the tert-butyl group, a series of compounds was designed to reach into the ribose-binding site of the ATP pocket. However, these compounds lacked inhibitory activity at 25 µM. An alternate synthetic strategy in which a 2-amino group was introduced into the 4-pyridyl ring was then pursued. This gave us our best inhibitor (11a) with an IC50 of 0.049 µM on MtGS and an MIC of 2 µg/mL against M. tuberculosis. We were also able to obtain an X-ray structure of this compound bound to MtGS, which showed that instead of forming a hydrogen bond to the backbone carbonyl oxygen of Lys361 as predicted from docking studies, the 2-amino group formed an additional interaction with the hydroxyl oxygen of Ser280 in its primary binding mode.

EXPERIMENTAL PROCEDURES

General methods. Microwave-assisted reactions were performed in sealed vials dedicated for microwave processing, using a Smith Synthesizer. NMR spectra were recorded on a Varian Mercury plus for 1H at 399.9 MHz and for 13C NMR at 100.5 MHz. Analytical HPLC-UV/MS analysis of pure products were performed on a Gilson HPLC system equipped with a Finnigan AQA quadrupole mass spectrometer using a 4 mL/min CH3CN/H2O gradient (0.05% HCOOH) and detection by UV (DAD) and MS (ESI+). All compounds were determined to be >95% pure by HPLC-UV at 254 nm.

4-(2-tert-butyl-4-(6-methoxynaphthalen-2-yl)-1H-imidazol-5-yl)pyridin-2-amine (11a). To a microwave vial (2.5 mL) was added 10 (50 mg, 0.13 mmol), diphenylmethanamine (1.5 mL) and dioxane (1.5 mL). The vial was then sealed under air and heated at 200 °C by microwave irradiation for 10 h. After cooling, the mixture was filtered through a plug of silica, eluted with EtOAc:hexane (1:1) and the filtrate concentrated in vacuo. The crude mixture was then taken up in MeOH (2.0 mL) and transferred to a microwave vial (2–5 mL) loaded with Pd/C (10%, 5 mg) and ammonium acetate (100 mg, 1.3 mmol). The vial was then sealed under air and heated at 120 °C by microwave irradiation for 2 h. After cooling, Pd/C (10%, 10 mg) and the combined organic phases were concentrated in vacuo.

For 11a, an IC50 of 24.7 µM in this test, which is almost 5-fold greater than the MIC of 5.4 µM (2 µg/mL). This moderate level of cytotoxicity is not unusual among compounds in the early stages of drug development, and the situation is expected to improve as more effective enzyme inhibition and antibacterial activity are attained.
ASSOCIATED CONTENT
Supporting Information. Additional experimental details concerning: synthesis of all compounds, protein expression, purification and activity/inhibition studies, spectroscopic data as well as structural studies. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes
PDB codes for the MtGS-3 and MtGS-11a structures are 3ZXR and 3ZXX.

AUTHOR INFORMATION

Corresponding Author
* Tel: +46-18-4714293; Fax: +46-18-4714474; E-mail: anders.karlen@orgfarm.uu.se

Author Contribution
* J. Gising and M. T. Nilsson contributed equally to this work.

ACKNOWLEDGMENT
We thank Johan Gustavsson for preparative work, as well as Dr Aleh Yahorau, Department of Pharmaceutical Biosciences, Uppsala University, for conducting HRMS analyses. The Swedish Foundation for Strategic Research (SSF), the Swedish Research Council (VR) and the EU Sixth Framework Program NM4TB (CT:018 923) provided financial support.

ABBREVIATIONS
MtGS, glutamine synthetase from Mycobacterium tuberculosis; MIC, minimum inhibitory concentration; PDB, Protein Data Bank; WHO, World Health Organization; HTS, high throughput screening; MSO, L-Methionine-S-sulfoximine; MSO-P, L-Methionine-S-sulfoximine phosphate; MW, microwaves.

REFERENCES
(1) www.who.org.
(19) Legion; Tripos;St Louis, MO, 1998.
TABLE OF CONTENTS GRAPHICS
1.  R = H
2.  R = PO₃H₂

Common scaffold of cluster 2

IC₅₀ = 0.02 μM
MIC > 32 μg/mL

IC₅₀ = 0.10 μM
MIC > 32 μg/mL
56x39mm (300 x 300 DPI)
IC₅₀ = 49 nM
MIC = 2 µg/mL