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Functionalized 3-amino-imidazo[1,2-*a*]pyridines: A novel class of drug-like *Mycobacterium tuberculosis* glutamine synthetase inhibitors

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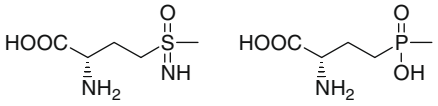
ABSTRACT

3-Amino-imidazo[1,2-*a*]pyridines have been identified as a novel class of *Mycobacterium tuberculosis* glutamine synthetase inhibitors. Moreover, these compounds represent the first drug-like inhibitors of this enzyme. A series of compounds exploring structural diversity in the pyridine and phenyl rings have been synthesized and biologically evaluated. Compound **4n** was found to be the most potent inhibitor (IC_{50} = 0.38 ± 0.02 μM). This compound was significantly more potent than the known inhibitors, L-methionine-*SR*-sulfoximine and phosphinothricin.

Tuberculosis (TB) is one of the world's major public health problems and has been declared a global health emergency by the World Health Organization.[1] In 2006, there were approximately 9 million new cases of TB, resulting in an estimated 1.7 million deaths.[2] In addition, the emergence of multi-drug resistant *Mycobacterium tuberculosis* strains (causing MDR-TB), the growing rate of TB incidence, the lethal combination represented by HIV co-infection and the lack of any new antituberculosis agent in the last 40 years, all indicate an urgent need for the development of novel TB therapies. In particular, new lead structures are required with novel modes of action.[3,4] In the last decade *M. tuberculosis* glutamine synthetase (*Mt*GS), a key enzyme required for nitrogen metabolism and mycobacterial cell-wall biosynthesis, has emerged as a potential target for antibiotics against TB.[1,4–6] Exposure of *M. tuberculosis* to the known GS inhibitor L-methionine-*SR*-sulfoximine **1** (MSO) (Fig. 1) has been shown to inhibit both cell wall formation and mycobacterial growth.[1,6] Thus, the development of new *Mt*GS inhibitors could be useful in developing an effective treatment for MDR-TB. The majority of known GS inhibitors are simple glutamate analogues and of these, MSO and phosphinothricin **2** (PPT) are the most widely investigated.[7] These inhibitors have been used as lead compounds in several studies,[5,7–12] albeit they are polar, flexible, non-selective[13] and not particularly drug-like. Accordingly, we were interested in the identification and development of more drug-like non-amino acid derived *Mt*GS inhibitors.



1 **2**

Figure 1. Structure of MSO (**1**) and PPT (**2**).

During the course of a high-throughput compound screen and a subsequent lead validation process, 3-amino-imidazo[1,2-*a*]pyridines were identified as a novel class of *Mt*GS inhibitors (Fig. 2). It was quickly recognized that this would provide a scaffold amenable to the rapid exploration of structure–activity relationships. Indeed, a diverse range of analogues can be readily obtained, in one step, via an Ugi-type cyclization.[14–19] Herein, we describe the high-speed synthesis and *Mt*GS inhibitory activity of two libraries of differentially substituted 3-amino-imidazo[1,2-*a*]pyridines, **3** and **4**.

We decided to focus our initial investigations on evaluating the effect of altering the pyridine ring substituent. Accordingly, 3-amino-imidazo[1,2-*a*]pyridines (**3a**–**m**) were prepared by a microwave-assisted multicomponent reaction (MCR) between cyclopentylisonitrile, 3-hydroxy-4-methoxy benzaldehyde and an appropriately substituted 2-aminopyridine.[19] All 2-aminopyridines were commercially available with the exception of 5-methoxy-2-aminopyridine, which was prepared by the treatment of 5-iodo-2-aminopyridine with MeOH and CuI under Buchwald's

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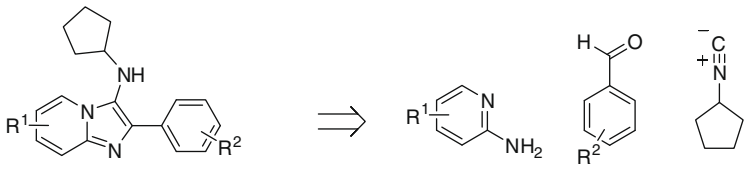
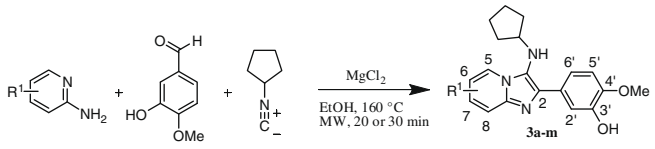


3 and **4**

Figure 2. 3-Amino-imidazo[1,2-*a*]pyridines and retrosynthetic analysis showing assembly via a one-pot three-component condensation reaction.

Table 1
Synthesis and biological evaluation of 3-amino-imidazo[1,2-*a*]pyridines (**3a–m**)



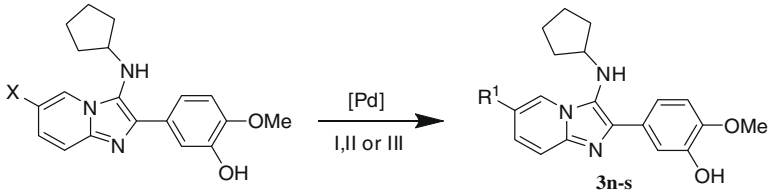
Entry	Product[a]	R^1	IC_{50}[b] (μM)
1	**3a**	H	>50
2	**3b**	6-Me	>50
3	**3c**	6-OMe	31.3 ± 0.7
4	**3d**	6-F	>50
5	**3e**	6-Cl	11.2 ± 1.5
6	**3f**	6-Br	8.8 ± 0.4
7	**3g**	6-I	4.8 ± 0.5
8	**3h**	6-CF_3	>50
9	**3i**	6-CN	>50
10	**3j**	5-Br	>50
11	**3k**	8-Br	21.3 ± 1.6
12	**3l**	6-Br, 8-Br	>50
13	**3m**	6-Br, 8-Me	12.7 ± 0.7
MSO	—	—	51 ± 6[c]
PPT	—	—	1.9 ± 0.4[c]

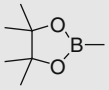
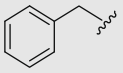
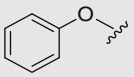
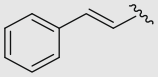
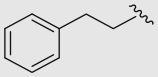
[a] Purity >95% by HPLC or ^{1}H NMR.
[b] Values are means of three experiments ± standard error.
[c] See Ref. 10.

modified Ullman reaction conditions (1,10-phenanthroline, Cs_2CO_3, 110 °C)[20] providing **3c**. The MCR reactions were typically irradiated, in a sealed vessel, for 20–30 min at 160 °C in the presence of $MgCl_2$ and the products were isolated either by simple filtration, recrystallization or flash chromatography.[21] The potency of the compounds **3a–m** from this first series against *Mt*GS was evaluated[22] and these results are reported in Table 1.

From Table 1 it is clear that a pyridine ring substituent is beneficial for *Mt*GS inhibition and that a number of synthesized analogues were significantly more active than MSO (i.e., **3c**, **3e–g**, **3k**, **3m**). The most active compounds contained large halogen atoms (Cl, Br or I) in the 6-position (i.e., **3f**, IC_{50} = 8.8 ± 0.4 μM and **3g** IC_{50} = 4.8 ± 0.5 μM). Interestingly, the 6-fluorine, 6-trifluoromethyl and 6-nitrile containing compounds were essentially inactive (**3d**, **3h** and **3i** IC_{50} >50 μM), suggesting that the potency of **3e**, **3f** and **3g** is not related purely to their electron withdrawing nature. The corresponding 5- and 8-bromine substituted analogues displayed considerably weaker inhibitory activity (**3j**, IC_{50} >50 μM and **3k**, IC_{50} = 21.3 ± 1.6 μM) compared to **3f**, indicating that there may be some specific hydrophobic or van der Waals interactions accessible only by a substituent in the 6-position. The introduction of an additional 8-bromine or 8-methyl substituent did not improve compound potency (**3l**, IC_{50} >50 μM and **3m**, IC_{50} = 12.7 ± 0.7 μM, respectively).

Table 2
Synthesis and biological evaluation of 6-substituted 3-amino-imidazo[1,2-*a*]pyridines (**3n–s**)



Entry	Starting material (X)	Method	Product[a]	R_1	IC_{50}[b] (μM)
1	Br–	I	**3n**		>50
2	Br–	I	**3o**		>50
3		II	**3p**		>50
4	—	As per **3c**	**3q**		>50
5	Br–	I	**3r**		>50
6	**3r**	III	**3s**		>50

[a] Purity >95% by HPLC or ^{1}H NMR.
[b] Values are means of three experiments. I; Boronic acid (3 equiv), $Pd(PPh_3)_4$ (7%), Cs_2CO_3 (3.5 equiv), DMF, MW, 120 °C, 20 min. II; benzyl bromide (0.8 equiv), $Pd(dppf)Cl_2$ (10%), K_2CO_3 (3 equiv), EtOH, H_2O, MW, 130 °C, 30 min. III; **3r** 10% Pd/C (10%), H_2 (1 atm.), DMF, 25 °C, 18 h.

To further explore the effect of large hydrophobic groups in the 6-position, we decided to prepare a series of compounds where the halogen was exchanged for various aryl moieties. The target compounds were designed to explore the available chemical space and, in the case of **3o** increase solubility and H-bond potential. These molecules were smoothly prepared via microwave-assisted Suzuki cross-coupling reactions[23–25] utilizing either aryl bromide **3f** or the corresponding 6-boronic acid pinacol ester derivative, which was prepared from 2-aminopyridine-5-boronic acid pinacol ester according to Table 1. Compound **3q** was synthesized as per **3c**, however MeOH was replaced by phenol in the Ullman reaction. In addition, treatment of **3r** under catalytic hydrogenation conditions (Pd/C, H_2) afforded the saturated phenylethylene derivative **3s**. These compounds were then assessed for their ability to inhibit *Mt*GS and the results are reported in Table 2.

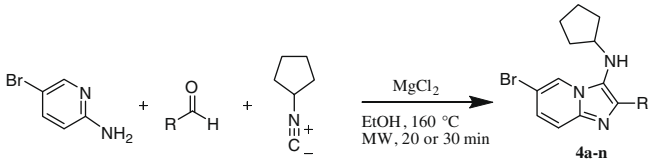
Unfortunately, the compounds in this series (**3n**–**s**) failed to show any significant *Mt*GS inhibitory activity, highlighting a lack of tolerance towards the introduction of large aryl substituents in the 6-position of the 3-amino-imidazo[1,2-*a*]pyridines.

Finally, utilizing compound **3f** as the lead structure, we decided to investigate the effect of altering the C-2 aryl substituent (R) on *Mt*GS inhibition. Thus, a series of compounds were synthesized from cyclopentylisonitrile, 5-bromo-2-aminopyridine and an appropriately substituted aldehyde as per Table 1. Compounds **4a**–**n** were evaluated for their *Mt*GS inhibitory activity and the results are presented in Table 3.

Table 3 shows that a C-2 phenyl ring with a hydrogen bond donor in the 3′-position (i.e., **4e**, OH or **4l**, NH_2) is a clear requisite for enzyme inhibition. Placement of the –OH in the 2′- or 4′-positions (**4f** and **4b**, respectively) results in complete loss in activity. Removal of the hydrogen bond donor capacity through the introduction of a methoxy group rendered the inactive compound **4g**. Interestingly, **4e** displays a twofold increase in activity compared to **3f** suggesting that the 4′-methoxy group is not required for inhibition. The introduction of electron withdrawing substituents (**4i** and **4j**) was also detrimental to activity. However, these substituents may also disrupt the orientation of the phenyl ring (**4i**) or intramolecularly bind the 3′-OH (**4j**) giving rise to the loss in potency. The corresponding 3′-aniline (**4l**) and 3-methylalcohol (**4m**) compounds were twofold less potent than **4e** (IC_{50} = 10.1 ± 1.1 μM and 13.2 ± 1.5 μM vs 3.3 ± 0.6 μM, respectively) demonstrating a preference for a phenol group in the 3′-position. Rewardingly, the introduction of a carboxylic acid rendered the nanomolar potent inhibitor, **4n** (IC_{50} = 0.38 ± 0.02 μM).

In summary, three small series of trisubstituted 3-amino-imidazo[1,2-*a*]pyridines have been investigated as *Mt*GS inhibitors. The compounds represent the first non-amino acid derived inhibitors of this enzyme. The most effective compounds possessed low micromolar (**3f**, IC_{50} = 8.8 ± 0.4 μM, **3g**, IC_{50} = 4.8 ± 0.5 μM, **4e**, IC_{50} = 3.3 ± 0.6 μM) or nanomolar potency (**4n** IC_{50} = 0.38 ± 0.02 μM). Compound **4n** was significantly more active than both MSO (IC_{50} = 51 ± 6 μM) and PPT (IC_{50} = 1.9 ± 0.4 μM) the most potent known *Mt*GS inhibitors. Given their drug-like nature, we anticipate they will serve as important lead compounds in the search for new anti-tuberculosis agents. The chemistry established can easily be used to smoothly produce additional inhibitors. Work is currently underway within our laboratory utilizing these structures to expand the SAR developed herein.

Table 3
Synthesis and biological evaluation of 3-amino-imidazo[1,2-*a*]pyridines (**4a**–**n**)



4a-n

Entry	Product[a]	R	IC_{50}[b] (μM)
1	**4a**	H	>50
2	**4b**	3′-OMe,4′-OHC_6H_3	>50
3	**4c**	C_6H_5	>50
4	**4d**	4′-OHC_6H_4	nd[c]
5	**4e**	3′-OHC_6H_4	3.3 ± 0.6
6	**4f**	2′-OHC_6H_4	>50
7	**4g**	3′-$OMeC_6H_4$	>50
8	**4h**	2′,3′-$DiOMeC_6H_3$	>50
9	**4i**	2′-Cl,3′-OHC_6H_3	>50
10	**4j**	3′-OH,4′-$NO_2C_6H_3$	>50
11	**4k**	3′-$NO_2C_6H_4$	>50
12	**4l**	3′-$NH_2C_6H_4$	10.1 ± 1.1
13	**4m**	3′-$(CH_2OH)C_6H_4$	13.2 ± 1.5
14	**4n**	3′-$(COOH)C_6H_4$	0.38 ± 0.02

[a] Purity >95% by HPLC or ^{1}H NMR.
[b] Values are means of three experiments ± standard error.
[c] IC_{50} could not be determined due to poor solubility.

Acknowledgments

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21. *Example synthesis: Compound* **3f**. To a microwave transparent vial (2–5 mL) with a teflon coated stirring bar was added cyclopentylisonitrile (0.192 g, 2 mmol), 3-hydroxy-4-methoxybenzaldehyde (0.304 g, 2 mmol), 2-amino-5-bromopyridine (0.346 g, 2 mmol), $MgCl_2$ (0.019 g, 0.1 mmol) and EtOH (2 mL). The vial was then sealed under air and heated at 160 °C by microwave irradiation for 20 min using a fixed hold time. After cooling, the mixture was diluted with ethyl acetate and brine (20 mL each) and the two layers separated. The aqueous layer was washed twice with ethyl acetate (20 mL) and the combined organic phases were concentrated in vacuo. The crude product was thereafter purified by recrystallization from ethyl acetate. Yield: 0.530 g, 66%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.39–1.48 (m, 2H), 1.53–1.58 (m, 2H), 1.67–1.77 (m, 4H), 3.05 (d, *J* = 4.4 Hz, 1H), 3.61–3.66 (m, 1H), 3.92 (s, 3H), 6.02 (br s, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 7.15 (dd, *J* = 2.0, 9.2 Hz, 1H), 7.40 (d, *J* = 9.2 Hz, 1H), 7.50 (dd, *J* = 2.0, 8.4 Hz, 1H), 7.60 (d, *J* = 2.0 Hz, 1H), 8.22 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.8, 33.7, 56.2, 59.3, 106.6, 111.0, 113.8, 118.2, 119.6,

122.9, 125.5, 127.3, 127.7, 137.9, 140.0, 146.0, 146.6. LC–ESI-MS: *m/z* 402 (M+1). Calcd for $C_{19}H_{20}BrN_3O_2$: C, 56.73; H, 5.01; N, 10.45. Found: C, 56.44; H, 5.01; N, 10.27.

22. The assay was performed essentially as previously described.[10] However, 25 mM of $MgCl_2$, 1 mM of ATP and 30 mM of monosodium L-glutamate were used and stock solutions of the compounds (10 mM) were prepared in DMSO. A concentration of 50 μM was chosen as the upper limit for IC_{50} determinations due to solubility problems encountered with some compounds at higher concentrations.
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25. *Example synthesis: Compound* **3r**. To a microwave transparent vial (2–5 mL) with a teflon coated stirring bar was added **3f** (0.080 g, 0.2 mmol), *trans*-2-phenylvinylboronic acid (0.088 g, 0.6 mmol), Cs_2CO_3 (0.234 g, 0.7 mmol), $Pd(PPh_3)_4$ (0.016 g, 0.013 mmol) and DMF (2 mL). The vial was then sealed under air and heated at 120 °C by microwave irradiation for 30 min using a fixed hold time. After cooling, the mixture was diluted with ethyl acetate and brine (20 mL each) and the two layers separated. The aqueous layer was washed twice with ethyl acetate (20 mL) and the combined organic phases were concentrated in vacuo. The crude product was thereafter purified by flash chromatography eluting with ethyl acetate/hexane (3:2). Yield: 0.064 g, 65%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.45–1.50 (m, 4H), 1.58–1.62 (m, 2H), 1.70–1.74 (m, 2H), 3.55–3.58 (m, 1H), 3.80 (s, 3H), 4.26 (d, J = 4.6 Hz, 1H), 6.85 (d, J = 8.5 Hz, 1H), 7.04 (d, J = 16.4 Hz, 1H), 7.18 (d, J = 16.4 Hz, 1H), 7.19–7.22 (m, 1H), 7.28–7.37 (m, 3H), 7.45 (dd, J = 1.7, 9.5 Hz, 1H) 7.51 (d, J = 7.5, 2H) 7.55 (dd, J = 2.2, 8.2 Hz, 1H), 7.65 (d, J = 2.4 Hz, 1H), 8.25 (br s, 1H), 8.69 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.0, 33.5, 56.2, 59.0, 112.7, 114.9, 117.0, 118.6, 121.5, 122.3, 122.3, 125.7, 126.3, 126.8, 128.0, 128.2, 128.4, 129.1, 136.5, 137.5, 140.5, 146.7, 147.4. LC–ESI-MS: *m/z* 426 (M+1) Calcd for $C_{27}H_{27}N_3O_2$: C, 76.21; H, 6.40; N, 9.87. Found: C, 76.02; H, 6.39; N, 9.79.