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Analysis of β-amino alcohols as inhibitors of the potential anti-tubercular target *N*-acetyltransferase

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ABSTRACT

The synthesis and inhibitory potencies of a novel series of β-amino alcohols, based on the hit-compound 3-[3′-(4″-cyclopent-2‴-en-1‴-ylphenoxy)-2′-hydroxypropyl]-5,5 dimethylimidazolidine-2,4-dione as specific inhibitors of mycobacterial *N*-acetyltransferase (NAT) enzymes are reported. Effects of synthesised compounds on growth of *Mycobacterium tuberculosis* have been determined.

Tuberculosis (TB) remains one of the leading causes of death from a single infectious disease worldwide each year. In 2009 the World Health Organisation (WHO) reported 1.7 million people died from TB, equal to 4700 deaths a day, (http://www.who.int/tb/publications/global_report/2010/) and also reported 9.4 million new cases of TB in the same year. The increase in prevalence of HIV and the emergence of multi-drug resistant (MDR) and extreme drug resistant strains (XDR) means that new drugs are urgently required to prevent a potential pandemic.[1,2]

The arylamine *N*-acetyltransferase (NAT) enzyme has been identified in a number of eukaryotic and prokaryotic species including *Mycobacterium tuberculosis*, the causative agent of TB and has been identified as a potential new target for the treatment of tuberculosis. When the gene was deleted from *Mycobacterium bovis* BCG (Δ*nat*) the resulting knockout organism was found to have low levels of mycolic acids and an alteration in the cell wall architecture.[3] The Δ*nat* mutant strain was found to have increased sensitivity to the antibiotics hygromycin and gentamycin that have previously been shown to have little effect on wild-type *M. bovis* BCG and *M. tuberculosis*. Importantly these Δ*nat* mutant strains were found to be more susceptible to intracellular killing within mouse macrophages.[3]

NAT enzymes utilise the donor cofactor acetyl coenzyme A to acetylate a broad range of substrates including arylamines, *N*-arylhydroxyamines and aryl hydrazines[4] and acyl hydrazides including isoniazid,[5] which is one of the front-line treatments for tuberculosis. The crystal structures of NAT enzymes from a number of species have been solved, including *Salmonella typhimurium*,[6] *Pseudomonas aeruginosa*,[7] *Nocardia farcinica*,[8] human NAT2[9] and the mycobacterial species *Mycobacterium smegmatis*[10] and *Mycobacterium marinum*.[11] All NAT enzymes are found to have very similar 3-dimensional structures and consist of three distinct domains with the active site containing a catalytic triad formed from a cysteine, a histidine and an aspartate residue. Recently the cofactor binding site has been identified in human NAT2[9] and *M. marinum*[11] and found to differ between mammalian and bacterial species.[11]

In order to further understand and investigate the role of NAT as an essential target within mycobacteria, small molecule inhibitors of NAT enzymes are required so that a chemical genomic approach can be undertaken to complement the genetic studies which had been previously carried out on Δ*nat* strains of *M. bovis* BCG[3] and *M. smegmatis*.[12] Therefore, an in-house library of 5000 selected commercial compounds were screened for in vitro enzymic inhibition against a panel of prokaryotic and eukaryotic NAT enzymes.[13,14] A smaller manual screen had been previously carried out and been successful in identifying inhibitors of prokaryotic NAT enzymes.[15] From the larger, automated high-throughput screen, six compounds were identified as specific inhibitors of the prokaryotic NAT enzymes with these compounds displaying no inhibitory effect on the eukaryotic enzymes also screened.[13] One of the compounds identified from the more extensive screen

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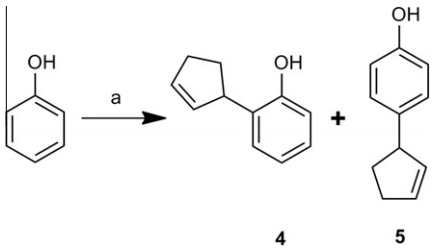
was the β-amino alcohol 3-[3′-(4″-cyclopent-2‴-en-1‴-ylphenoxy)-2′-hydroxypropyl]-5,5-dimethylimidazolidine-2,4-dione **1**. The novel class of compound, that inhibited prokaryotic NATs with greater than 80% inhibition at a concentration of 30 μM, had not previously been reported to have antibacterial activity. However, it was interesting to note that a β-amino-alcohol motif has been incorporated into ethambutol **2**, Figure 1,[16] a front-line drug currently used for the treatment of tuberculosis. Also, phenanthracene derivatives **3**, Figure 1, have been reported to have anti-tubercular activity at a minimum inhibitor concentration (MIC) as low as 3.12 μg/mL,[17] Figure 1. We report here on the synthesis of a series of β-amino alcohols which have diversification around the aryl and hydantoin moieties of the purported hit compound **1**, the in vitro evaluation of this series of compounds as inhibitors of NAT enzymes and growth of mycobacteria species and the identification of preliminary structure–activity relationships.

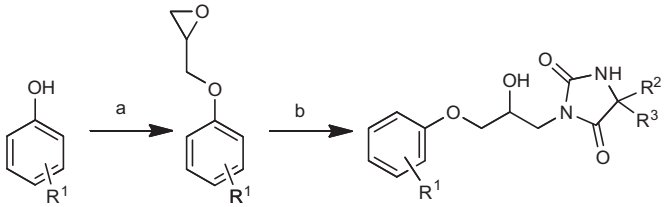
A series of β-amino alcohols were synthesized with derivatisation occurring either on the aryl ring and/or on the hydantoin moiety of the molecule, Schemes 1 and 2.[18,19] In order to resynthesise the hit compound **1**, *ortho*- and *para*-substituted cyclopentenylphenol **4** and **5**, respectively, were prepared via the reaction of freshly prepared cyclopentadiene with phenol, Scheme 1.[18] The reaction proceeded in a non-regioselective manner. Subsequently the product mixture containing *ortho*-**4** and *para*-**5** isomers was separated using column chromatography purification. Other substituted phenols at the *ortho*-, *meta*- and *para*-positions were commercially available and purchased. The corresponding β-amino alcohols were synthesized in two steps starting from the respective substituted phenols, via an epoxide intermediate that was then reacted with commercially available hydantoins,[19] affording resynthesised hit compound **1**, the *ortho*-analogue of the hit compound **6**[20] and analogues **7**–**17**, Scheme 2. The hit compound **1** contained approximately 30% impurity, as determined by ^{1}H NMR. This impurity is believed to be another *para*-substituted compound with isomerisation occurring of the double bond on the cyclopentenyl ring. We were unable to separate this impurity from compound **1** and therefore the results obtained from compound **1** described in this study are from this mixture of *para*-cyclopentenyl compounds. This product showed poor inhibition, Table 1.

The series of β-amino alcohols were tested for their in vitro activity against three bacterial NAT enzymes:[15] NAT from *M. smegmatis* (MSNAT) and NAT from *P. aeruginosa* (PANAT) which were two of the prokaryotic enzymes used in the high-throughput screen[13] and the NAT from *M. marinum* (MMNAT) which is the closest recombinant homologue of the NAT enzyme from *M. tuberculosis* which was available at the time.[11]

Upon testing of the resynthesised hit compound **1** using DTNB to measure the rate of hydrolysis of acetyl CoA with both isoniazid (INH) and 5-aminosalicylate (5AS) as substrates, no inhibitory effect upon the activity of PANAT, MSNAT or MMNAT at a concentration of 50 μM was found. However, interestingly it was found that



Scheme 1. Synthesis of *ortho*- and *para*-cyclopentenylphenol. Reagents and conditions: (a) H_3PO_4, toluene, rt, 2 h then cyclopentadiene, toluene, rt, 2 h.



Scheme 2. Synthesis of β-amino alcohols. Reagents and conditions: (a) epichlorohydrin, K_2CO_3, acetone, reflux 12 h; (b) requisite hydantoin, cat. pyridine, ethanol, reflux, 4 h.

the *ortho*-analogue **6** of the hit compound **1** had inhibitory activity against each of these prokaryotic NAT enzymes, with greatest potency shown against the PANAT version of the NAT enzymes. The IC_{50} values for compound **6** with PANAT were 9 μM when isoniazid (INH) was used as a substrate and 17 μM when 5-aminosalicylate (5AS) was used as a substrate, Table 1. For MMNAT IC_{50} values for compound **6** of 37 and 33 μM were observed for INH and 5AS, respectively, Table 1. When lower concentrations of hydralazine (HLZ) are used as a substrate[21] the IC_{50} values are even lower for compound **6**, Table 1.

The sub-library of synthesized derivatives of compound **1** were tested for their inhibitory effects on MMNAT enzymic activity and the results displaying the percentage inhibition are shown in Table 2. The compounds were all tested at 30 μM and the most potent compound was the *ortho*-cyclopentenyl analogue **6** (58% inhibition) followed by the 1-naphthyl derivative **14** (28% inhibition) and then the *ortho*-phenyl **11** substituted compound (24% inhibition) and *ortho*-bromo substitution **8** (24% inhibition). Compounds that had no substitution of the phenyl ring (**15**–**17**) were found to have no inhibitory effect upon the activity of MMNAT. The replacement of the *ortho*-hydrogen by a fluorine atom **7** also resulted in no increase in inhibitory potency of the compound. When the halogen at the *ortho*-position is a bromine atom **8** rather than fluorine **7**, then an increase in the percentage inhibition of MMNAT from 0% to 24% is observed.

As the crystal structure of MMNAT is available[11] in silico docking studies were carried out for the compounds **1**, **6**, **11** and **14** in

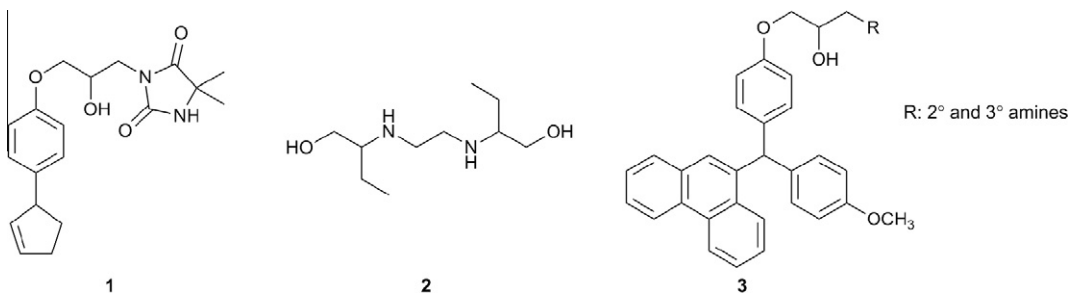


Figure 1. β-Amino alcohols. Compound **1** is the hit compound identified from the high-throughput screen, compound **2** is ethambutol currently used as a front-line drug for the treatment of tuberculosis and analogues of compound **3** have been reported to inhibit the growth of *Mycobacterium tuberculosis*.[16,17,24]