Bioorganic & Medicinal Chemistry Letters 21 (2011) 1185–1190

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

Elizabeth Fullam [a], Areej Abuhammad [a], David L. Wilson [b], Matthew C. Anderton [a], Steve G. Davies [b], Angela J. Russell [a,b], Edith Sim [a,*]

[a] Department of Pharmacology, University of Oxford, Mansfield Road, Oxford OX1 3QT, UK
[b] Department of Chemistry, Chemistry Research Laboratory, Mansfield Road, Oxford, OX1 3TA, UK

ARTICLE INFO

*Article history:*
Received 17 November 2010
Revised 14 December 2010
Accepted 18 December 2010
Available online 24 December 2010

*Keywords:*
Tuberculosis
Arylamine *N*-acetyltransferase
β-Amino alcohols
Enzyme inhibition
Drug discovery

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

* Corresponding author. Tel.: +44 1865 271884; fax: +44 1865 271853.
*E-mail address:* edith.sim@pharm.ox.ac.uk (E. Sim).

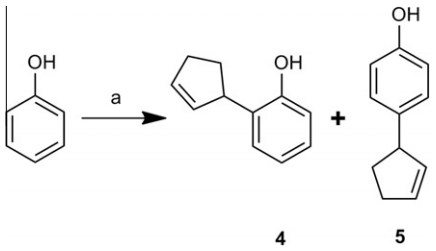
0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.12.099

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.

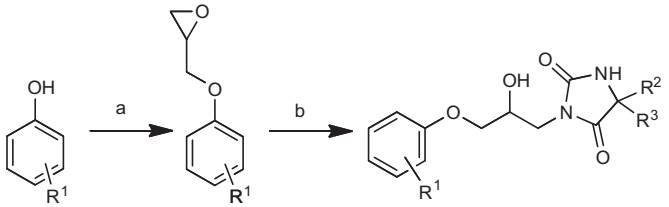
A series of β-amino alcohols were synthesized with derivatisation occurring either on the aryl ring and/or on the hydantoin moiety of the molecule, Schemes 1 and 2.[18,19] In order to resynthesise the hit compound **1**, *ortho*- and *para*-substituted cyclopentenylphenol **4** and **5**, respectively, 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

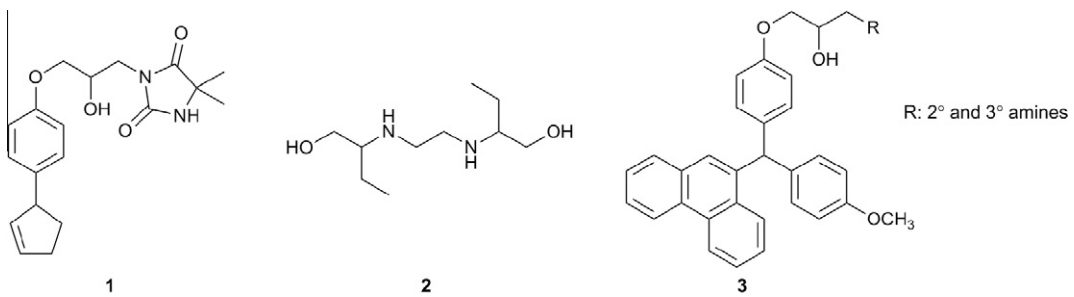


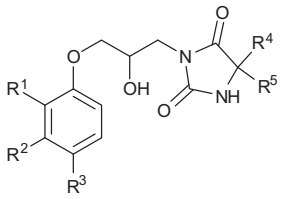

**Figure 1.** β-Amino alcohols. Compound **1** is the hit compound identified from the high-throughput screen, compound **2** is ethambutol currently used as a front-line drug for the treatment of tuberculosis and analogues of compound **3** have been reported to inhibit the growth of *Mycobacterium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

**Table 1**
Comparison of compound **1** and **6** as inhibitors of NAT enzymes

| Enzyme | | PANAT | | MSNAT | | MMNAT | | MMNAT |
|---|---|---|---|---|---|---|---|---|
| Substrate | | INH | 5AS | INH | 5AS | INH | 5AS | HLZ |
| $IC_{50}$ (μM) | 1 *para* | >50 | >50 | >50 | >50 | >50 | >50 | ND |
| $IC_{50}$ (μM) | 6 *ortho* | 9.4 ± 1.4 | 17.7 ± 1.5 | 37.0 ± 1.2 | 16.2 ± 0.9 | 37.0 ± 1.8 | 33.1 ± 1.5 | 1.5 ± 0.09 |

The $IC_{50}$ (μM) values for compounds **1** and **6**. The NAT activity assay[14] was used to determine the specific activities of the pure recombinant MMNAT, MSNAT or PANAT protein in the presence of five concentrations of compound **1** and **6** with the substrates isoniazid (INH) or 5AS (500 μM). Hydralazine (HLZ) was used at a concentration of 150 μM. Each inhibitor concentration was carried out in triplicate. The concentration at which there was 50% inhibition of activity was determined graphically. ND is not determined.

**Table 2**
Effect of analogues of **1** on MMNAT enzymic activity




| Compound | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | % inhibition |
|---|---|---|---|---|---|---|
| **6** | Cyclopentenyl | H | H | Me | Me | 58 |
| **1** | H | H | Cyclopentenyl | Me | Me | 0 |
| **7** | F | H | H | Me | Me | 0 |
| **8** | Br | H | H | Me | Me | 24 |
| **9** | Me | H | H | Me | Me | 0.5 |
| **10** | $NO_2$ | H | H | Me | Me | 4 |
| **11** | Ph | H | H | Me | Me | 24 |
| **12** | H | Ph | H | Me | Me | 6 |
| **13** | H | H | Ph | Me | Me | 8 |
| **14** | Naphthyl | Naphthyl | H | Me | Me | 28 |
| **15** | H | H | H | Me | Me | 0 |
| **16** | H | H | H | Ph | H | 0 |
| **17** | H | H | H | H | H | 0 |

The substitution of the aryl ring $R^1$–$R^3$ and substituted hydantoin $R^4$–$R^5$ are shown. The percentage inhibition of MMNAT enzymic activity at 30 μM compound is calculated by comparison to a 5% DMSO control. The NAT activity assay[14] was used to determine the activity with isoniazid as substrate. Each measurement was carried out in triplicate.

an effort to rationalise the preliminary structure–activity relationships for this series of β-amino alcohols. Compounds were docked into the MMNAT structure (PDB code 2VFB) using the GOLD suite of programs.[22] Ten docking solutions for each ligand were outputted by the program and the docking solution with the best score, was selected and analysed. The docking solutions indicated that both the *ortho*- and *para*-compounds **6** and **1** are able to enter a cleft of MMNAT that leads up to the active-site cysteine, although remote from the cofactor acetyl-CoA binding site, Figure 2.[11] A number of hydrophobic binding interactions are predicted for each. Both compounds (**1** and **6**) are positioned in a similar location in the protein with the main difference between the two predicted docking solutions of compounds **1** and **6** being the orientation of the cyclopentenyl group. In the *ortho*-isomer **6** the cyclopentenyl group is orientated towards the Sγ of active site cysteine. In the case of the *para*-isomer **1** the cyclopentenyl group is orientated away from the active site cysteine. This is consistent with the experimental data that *ortho*-compound **6** is an inhibitor of prokaryotic NAT enzymes, whereas the *para*-compound **1** is a poor inhibitor of NAT enzymic activity.

In order to understand the effects of *ortho*-, *meta*- and *para*-substitution the appropriate biphenyl compounds *ortho*-biphenyl **11**, *meta*-biphenyl **12** and *para*-biphenyl **13** were synthesised and tested for activity on the MMNAT enzyme and the results are shown in Table 2. The *ortho*-biphenyl derivative **11** inhibits MMNAT enzymic activity by 24% whilst the *para*-biphenyl analogue **13** shows only 8% inhibition and the meta-biphenyl compound **12** inhibits MMNAT activity by just 6%. These results for the biphenyl series of compounds **11-13** show the same trend as for the cyclopentenyl derivatives **1** and **6** in which the *ortho*-substituted compounds are the most potent inhibitors of MMNAT enzymic activity. It should be noted that the *ortho*-biphenyl substituted derivate **11** (24% inhibition) has less inhibitory potency than the *ortho*-cyclopentenyl counterpart **6** (58% inhibition).

To explain these experimental results in structural terms, docking studies were carried out (Fig. 3). The docking results suggest that each inhibitor binds within the same position of the MMNAT protein with each ligand being within a distance of 4 Å from the active-site Cys70. These docking results suggest that the *ortho*-phenyl substituent **11** is orientated away from the active site cysteine, in a manner that stabilises the binding interaction between the protein and ligand through π-stacking interactions between the phenyl-substituent group of the ligand and with residue Phe130 (Fig. 3D). This may explain the experimental result in which compound **11** is a less potent inhibitor than compound **6**. Furthermore, compound **6** may result in greater inhibition of MMNAT enzymic activity than compound **11** as a result of *ortho*-cyclopentenyl compound **6** having fewer conformational restrictions than the *ortho*-biphenyl compound **11**. The cyclopentenyl-ring of compound **6** may adopt an envelope con-

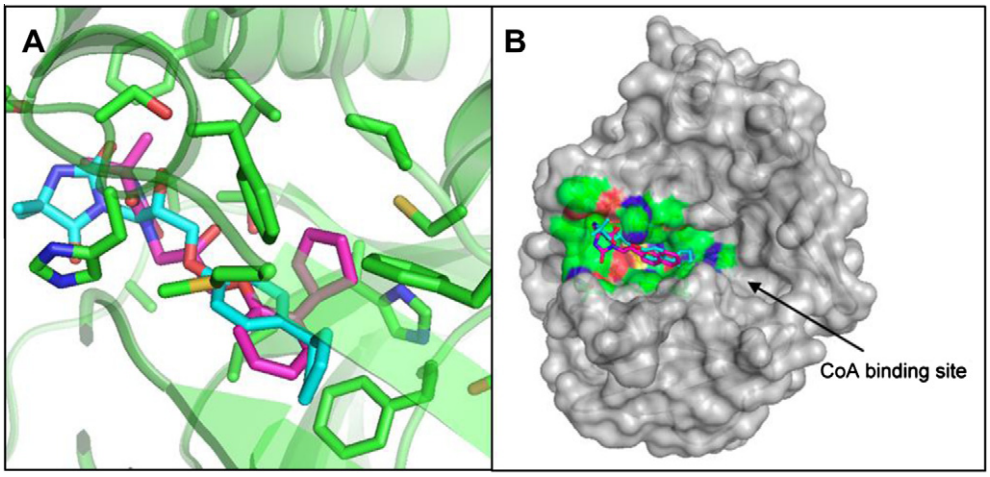



**Figure 2.** Comparison of the *ortho*- and *para*-cyclopentenyl substitution on interaction with MMNAT. (A) Compounds **1** and **6** were docked into the GOLD docking program. The residues which are predicted to interact with the ligands are shown in stick format. The *ortho* isomer **6** carbon atom's are shown in magenta and the *para* isomer **1** carbon atoms are coloured blue. (B) A surface representation of MMNAT (PDB code 2VFB), indicating the predicted cleft for binding of the compounds **1** and **6**. The CoA binding site is indicated.

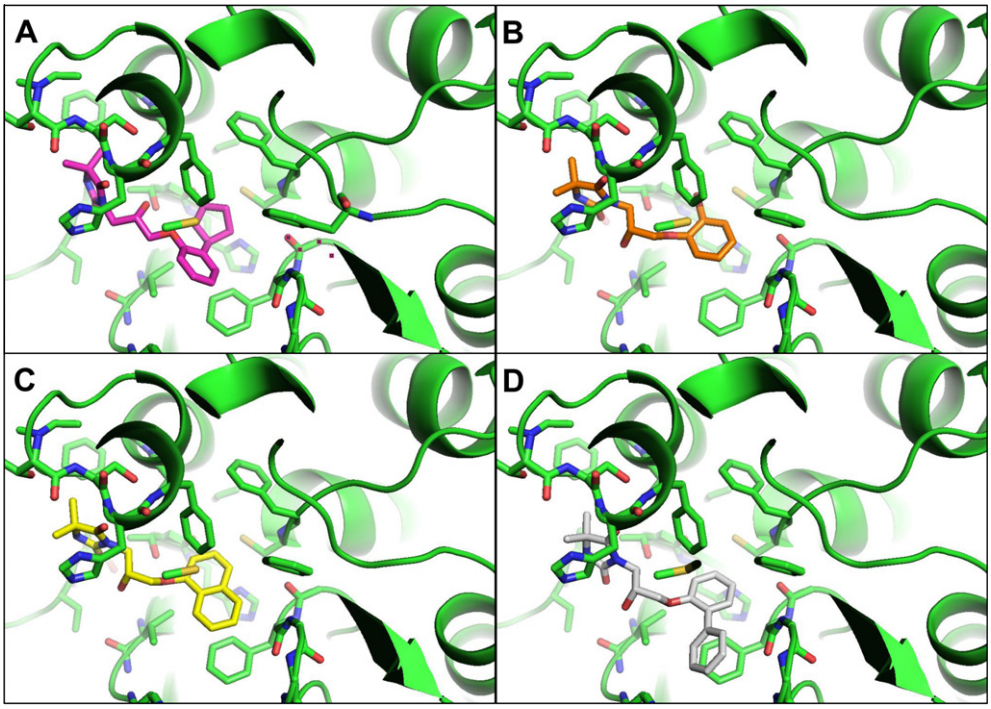



**Figure 3.** Comparison of the *ortho*-substitution of the β-amino alcohol inhibitors interaction with MMNAT. Compounds **6** (magenta, A), **8** (orange, B), **14** (yellow, C), **11** (grey, D) were docked into the MMNAT protein (PDB code 2VFB) using the GOLD docking program. The residues which are predicted to interact with the ligands are shown in stick format.

formation thus enabling close contacts between the inhibitor and the active-site of the cleft of MMNAT to occur. The rigid aromatic biphenyl compound **11** is locked in its orientation, is less flexible, and therefore may not fit the MMNAT pocket as well as compound **6** resulting in less inhibitory potency of the *ortho*-biphenyl compound **11**. *ortho*-Naphthyl **14** also contains rigid aromatic rings and has similar potency to **11**. Docking studies of the naphthyl analogue **14** show that this compound is also predicted to bind in the same orientation as the cyclopentenyl substituted compound **6**, with the naphthyl group orientated towards the active-site cysteine (Fig. 3C). The bromo-compound **8** is also predicted to bind in a similar position in the MMNAT protein, Figure 3B. Bromo-compound **8** is likely to be a better inhibitor than fluoro-compound **7**, which does not have any inhibitory effect upon the activity of MMNAT, as a result of the increased size of the bromine atom, compared to fluorine.

Recently the structure of MMNAT has been solved with the substrate hydralazine (HLZ) (PDB code 3LTW,[23]). When the docking results of the *ortho*-cyclopentenyl compound **6** are overlaid upon the solved HDZ-MMNAT structure it is observed that the *ortho*-cyclopentenyl moiety of compound **6** is in the same position of the HDZ substrate, Figure 4. Therefore it is proposed that the mechanism of inhibition of these *ortho*-β-amino alcohol compounds is through their ability to prevent substrates of the MMNAT protein being able to access and undergo acetylation at the active-site Cys70.

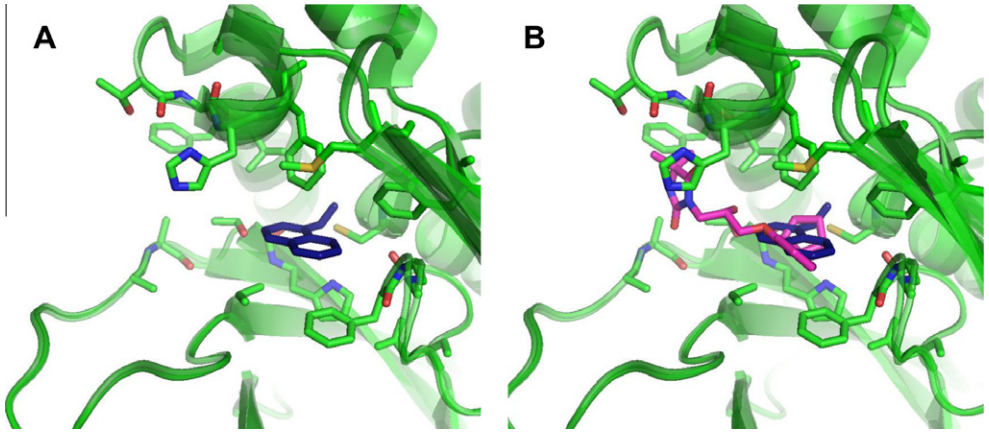



**Figure 4.** Comparison of docked *ortho*-isomer **6** with the binding site of hydralazine in MMNAT. (A) The binding site of hydralazine (dark blue) in the active-site of MMNAT is shown (PDB code 3LTW). (B) The docking result of the *ortho*-isomer **6** is overlaid upon the hydralazine-MMNAT structure. The *ortho*-isomer **6** carbon atoms are shown in magenta.

The predicted conformations of the inhibitors of MMNAT indicate that they each bind in a similar orientation and position in the MMNAT protein, Figure 5A. However, the *ortho*-cyclopentenyl compound **6** docking result predicts that the hydroxyl moiety of the β-amino alcohol functionality points in an opposing direction with compounds **8**, **11** and **14**. The orientation of this hydroxyl-moiety of **6** is positioned ideally to form a hydrogen bond with Thr109 and therefore this extra ligand-protein interaction formed may go some way to explaining why compound **6** is a better inhibitor than **8**, **11** and **14**, Figure 5. Previous studies of compounds that have been identified as substrates and inhibitors of NAT proteins have suggested that more lipophilic compounds are preferred.[15] In this study the orientation, conformation, size and steric factors also appear to play an important role in the inhibitory effect of this series of compounds studied.

Substituted hydantoin compounds **15**, **16** and **17**, had no effect on the activity of the protein, suggesting that this moiety of the potential inhibitor has little impact on enzymic activity.

Having shown that the *ortho*-compound **6** is able to inhibit the activity of MMNAT, the effects of this compound and also the 'hit' *para*-compound **1** on the growth of *M. marinum* were investigated.[24] Compound **6** displayed moderate inhibition of growth

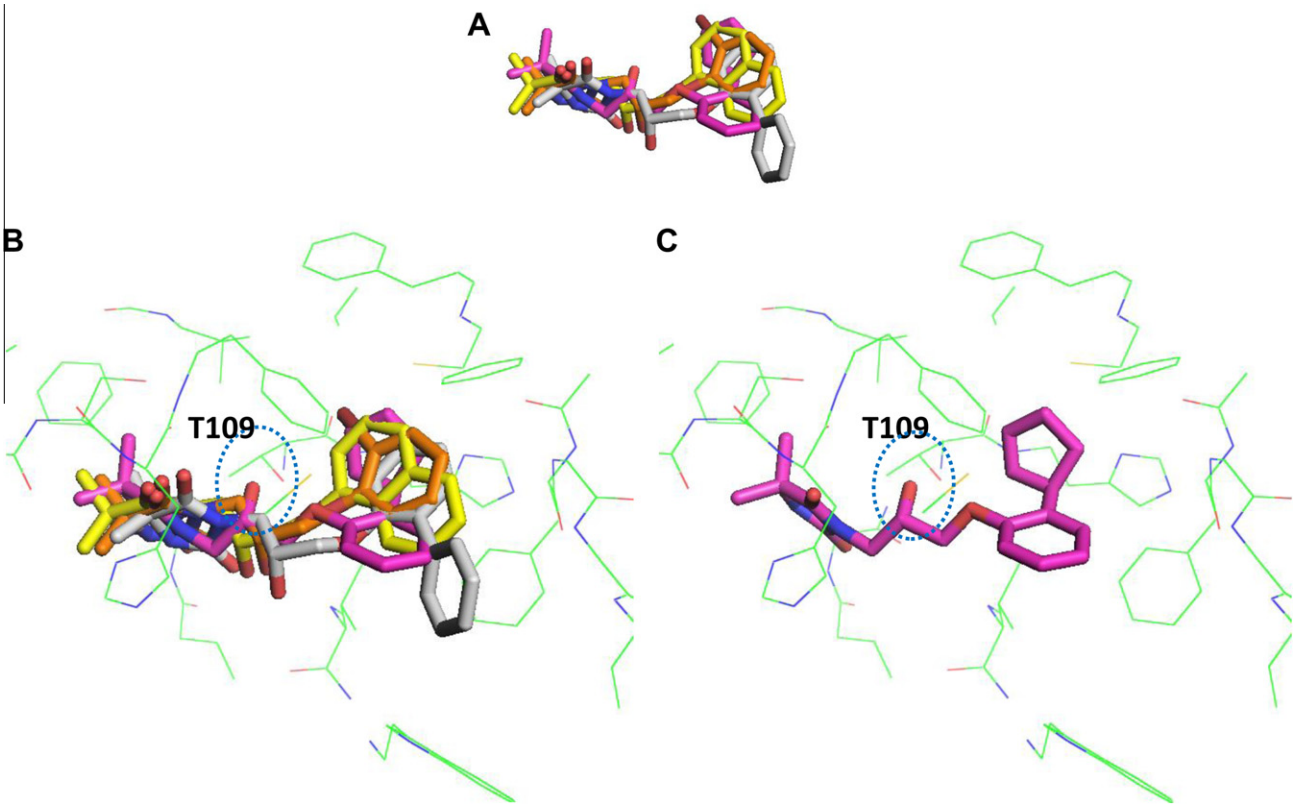



**Figure 5.** The predicted orientation of the docked *ortho*-substituted inhibitors in MMNAT. (A) An overlay of docked solutions of the *ortho*-cyclopentenyl compound **6** (magenta), the bromo-compound **8** (orange), the naphthyl-compound **14** (yellow) and the *ortho*-biphenyl compound **11** (grey). (B) The docked solutions in the active site of MMNAT (PDB code 2VFB). (C) The *ortho*-cyclopentenyl compound **6** (magenta) is shown indicating the H-bond with Thr109. The Oxygen atoms are indicated in red.

against *M. marinum* and at a concentration of 80 μg/mL no growth of *M. marinum* could be observed. The compound in the library purported to be the *para*-analogue **1** had no inhibitory effect on the growth of *M. marinum* at concentrations as high as 100 μg/mL. Compound **6** moderately inhibited the growth of *M. tuberculosis* at 50 μg/mL by 20% compared to the DMSO only control. The remaining synthesised analogues of **1** were not tested for their effect on the inhibition of mycobacterial growth given that they were poor inhibitors of MMNAT at 30 μM and only weak anti-mycobacterial activity was observed for the *ortho*-isomer **6**, which was found to be the most potent MMNAT inhibitor of this series of compound.

Panda et al. have tested a library of phenanthracene compounds, Figure 1, that resemble compound **6** and these compounds displayed greater inhibition of growth against *M. tuberculosis* than the *ortho*-compound **6** reported in this study.[17,25] Panda et al. suggested that more hydrophobic, lipophilic compounds are required for inhibiting the growth of the organism.[17] The series of phenanthracene compounds synthesized by Panda et al. are more hydrophobic than compound **6** and therefore may be able to penetrate the 'waxy' cell wall of mycobacteria more easily. Although NAT may represent a potential target for these phenanthracene derivatives it is not possible to discern the molecular target from the studies of Panda et al.[25]

In conclusion, a series of β-amino alcohols were synthesized and we have determined some preliminary structure–activity relationships that show that the *ortho*-substituent of the phenyl ring in this series of compounds plays an essential role in the inhibitory properties of this class of compound in NAT enzymes. Designing future compounds which have a substituent at the *ortho*-position of the phenyl-ring that is primed to interact with the Sγ of Cys70 of MMNAT, may result in a compound with enhanced inhibitory properties. The relative potencies of the *ortho*-**6** and *para*-**1** compounds as NAT inhibitors is also reflected in their relative potencies as inhibitors of growth of *M. marinum* and *M. tuberculosis* although they are each very weak inhibitors of mycobacterial growth.

Furthermore, we have also demonstrated the absolute importance of verifying a hit compound that has been identified as a result of undertaking a high-throughput screen through both resynthesis and biological testing of the compound. These studies have shown that β-amino alcohol compounds represent an interesting class of compound that are worth further investigation in order for novel anti-tubercular drugs to be identified.

## Acknowledgements

The authors wish to thank Dr. Isaac Westwood, Dr. Akane Kawamura and Dr. Adam Le Gresley for helpful discussions.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.099.

## References and notes

1. Dye, C. *Lancet* **2006**, *367*, 938.
2. Das, P.; Horton, R. *Lancet* **2010**, *375*, 1755.
3. Bhakta, S.; Besra, G. S.; Upton, A. M.; Parish, T.; Sholto-Douglas-Vernon, C.; Gibson, K. J.; Knutton, S.; Gordon, S.; DaSilva, R. P.; Anderton, M. C.; Sim, E. *J. Exp. Med.* **2004**, *199*, 1191.
4. Sim, E.; Westwood, I.; Fullam, E. *Expert Opin. Drug Metab. Toxicol.* **2007**, *3*, 169.
5. Upton, A. M.; Mushtaq, A.; Victor, T. C.; Sampson, S. L.; Sandy, J.; Smith, D. M.; van Helden, P. V.; Sim, E. *Mol. Microbiol.* **2001**, *42*, 309.
6. Sinclair, J. C.; Sandy, J.; Delgoda, R.; Sim, E.; Noble, M. E. *Nat. Struct. Biol.* **2000**, *7*, 560.
7. Westwood, I. M.; Holton, S. J.; Rodrigues-Lima, F.; Dupret, J. M.; Bhakta, S.; Noble, M. E.; Sim, E. *Biochem. J.* **2005**, *385*, 605.
8. Martins, M.; Pluvinage, B.; de la Sierra-Gallay, I. L.; Barbault, F.; Dairou, J.; Dupret, J. M.; Rodrigues-Lima, F. *J. Mol. Biol.* **2008**, *383*, 549.
9. Wu, H.; Dombrovsky, L.; Tempel, W.; Martin, F.; Loppnau, P.; Goodfellow, G. H.; Grant, D. M.; Plotnikov, A. N. *J. Biol. Chem.* **2007**, *282*, 30189.
10. Sandy, J.; Mushtaq, A.; Kawamura, A.; Sinclair, J.; Sim, E.; Noble, M. *J. Mol. Biol.* **2002**, *318*, 1071.
11. Fullam, E.; Westwood, I. M.; Anderton, M. C.; Lowe, E. D.; Sim, E.; Noble, M. E. *J. Mol. Biol.* **2008**, *375*, 178.
12. Payton, M.; Gifford, C.; Schartau, P.; Hagemeier, C.; Mushtaq, A.; Lucas, S.; Pinter, K.; Sim, E. *Microbiology* **2001**, *147*, 3295.
13. Westwood, I. M.; Bhakta, S.; Russell, A. J.; Fullam, E.; Anderton, M. C.; Kawamura, A.; Mulvaney, A. W.; Vickers, R. J.; Bhowruth, V.; Besra, G. S.; Lalvani, A.; Davies, S. G.; Sim, E. *Protein Cell* **2010**, *1*, 82.
14. Westwood, I. M.; Kawamura, A.; Russell, A. J.; Sandy, J.; Davies, S. G.; Sim, E. *Comb. Chem. High Throughput screen.* **2011**, *14*, in press. http://www.unboundmedicine.com/medline/ebm/record/21118080/.
15. Brooke, E. W.; Davies, S. G.; Mulvaney, A. W.; Pompeo, F.; Sim, E.; Vickers, R. J. *Bioorg. Med. Chem.* **2003**, *11*, 1227. The compounds were tested using a NAT activity assay which measures the rate of hydrolysis of acetyl CoA by detection with 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) at an absorbance of 405 nm.
16. Thomas, J. P.; Baughn, C. O.; Wilkinson, R. G.; Shepherd, R. G. *Am. Rev. Respir. Dis.* **1961**, *83*, 891.
17. Panda, G.; Shagufta; Mishra, J. K.; Chaturvedi, V.; Srivastava, A. K.; Srivastava, R.; Srivastava, B. S. *Bioorg. Med. Chem.* **2004**, *12*, 5269.
18. Bader, A. R. *J. Am. Chem. Soc.* **1953**, *75*, 5967.
19. Erhardt, P. W.; Woo, C. M.; Gorczynski, R. J.; Anderson, W. G. *J. Med. Chem.* **1982**, *25*, 1402.
20. *Spectroscopic data for* **6**: $^1$H NMR ($CDCl_3$) 400 MHz $\delta$, p. H., s), 1.41 (3H, s), 1.62–1.68 (1H, m), 2.12 (1H, s), 2.32-2.52 (4H, m), 3.73–3.81 (2H, m), 3.93–4.01 (2H, m), 4.25 (1H, br s), 5.78–5.82 (1H, m), 5.91–5.95 (1H, m), 6.78–8.82 (1H, m), 6.88–7.92 (1H, m), 7.11–7.19 (2H, m).
21. Fullam, E.; Kawamura, A.; Wilkinson, H.; Abuhammad, A.; Westwood, I.; Sim, E. *Protein J.* **2009**, *28*, 281.
22. Verdonk, M. L.; Cole, J. C.; Hartshorn, M. J.; Murray, C. W.; Taylor, R. D. *Proteins* **2003**, *52*, 609.
23. Abuhammad, A. M.; Lowe, E. D.; Fullam, E.; Noble, M.; Garman, E. F.; Sim, E. *Protein Cell* **2010**, *1*, 384.
24. Evangelopoulos, D.; Bhakta, S. *Methods Mol. Biol.* **2010**, *642*, 193. Bacteria were grown on solid agar in multiwell plates as spot cultures containing increasing concentrations of inhibitors in individual wells up to 100 μg/ml. Growth was determined by measuring the area of the spot culture over a period of up to 28 days.
25. Panda, G.; Shagufta; Srivastava, A. K.; Sinha, S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5222.