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CoA Adducts of 4-Oxo-4-Phenylbut-2-enoates: Inhibitors of MenB from the *M. tuberculosis* Menaquinone Biosynthesis Pathway

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Abstract

A high-throughput screen led to the discovery of 2-amino-4-oxo-4-phenylbutanoate inhibitors of the 1,4-dihydroxy-2-naphthoyl-CoA synthase (MenB) from the menaquinone biosynthesis pathway in *Mycobacterium tuberculosis*. However, these compounds are unstable in solution and eliminate to form the corresponding 4-oxo-4-phenylbut-2-enoates that then react with CoA *in situ* to form nanomolar inhibitors of MenB. The potency of these compounds results from interaction of the CoA adduct carboxylate with the MenB oxyanion hole, a conserved structural motif in the crotonase superfamily. 4-Oxo-4-chlorophenylbutenoyl methyl ester has MICs of 0.6 and 1.5 μg/ml against replicating and nonreplicating *M. tuberculosis*, respectively, and it is proposed that the methyl ester penetrates the cell where it is hydrolyzed and reacts with CoA to generate the active antibacterial. The CoA adducts thus represent an important foundation for the development of novel MenB inhibitors, and suggest a general approach to the development of potent inhibitors of acyl-CoA binding enzymes.

Keywords

Menaquinone; MenB; 1,4-dihydroxy-2-naphthoyl-CoA synthase; CoA; HTS; *o*-succinylbenzoic acid

Tuberculosis is a global health threat and efforts to combat the spread of this disease are hindered by the emergence of drug resistance, coupled with the ability of *Mycobacterium tuberculosis* to survive in a latent, non-replicating state for many years.[1–2] Since latent mycobacteria are thought to consume ATP in order to remain viable,[3] compounds that interfere with bacterial respiration hold the promise of targeting both replicating as well as non-replicating bacterial populations.[4] *M. tuberculosis* utilizes menaquinone (vitamin K2), a polyisoprenylated naphthoquinone, as the lipid soluble redox cofactor in the electron transport chain, and efforts are underway to validate enzymes in the menaquinone biosynthesis pathway as targets for drug discovery.[5–6] The menaquinone biosynthesis pathway in *M. tuberculosis* mirrors that found in *Escherichia coli* (Figure 1),[7–8] and

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Supporting Information Detailed experimental procedures for the synthesis of compounds and biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

transposon site hybridization has identified *menC*, *menD* and *menE* as essential for growth of *M. tuberculosis*.[9] Recently Sassetti and coworkers have shown that *menB* is also essential (Dr. Sassetti, personal communication), while inhibitors of MenA are active against both replicating and non-replicating bacterial populations.[6] In the present study we now report the discovery of a series of 2-CoA-4-oxo-4-phenylbutanoic acids that target MenB, the 1,4-dihydroxy-2-naphthoyl-CoA (DHNA-CoA) synthase which catalyzes an intramolecular Claisen condensation (Dieckmann) reaction leading to the formation of DHNA-CoA from O-succinylbenzoate (OSB) (Figure 1).[8]

The substrate for MenB is unstable and consequently we used a coupled assay[8] to screen 105,091 small molecules (see supplementary methods).[10] Following the primary screen, in which compounds were tested at a single concentration of 12.5 μg/ml, we obtained 455 hits that had at least 30% inhibition relative to control. Within these hits, we identified 7 compounds (Figure 2) that possessed the backbone structure of OSB, the substrate for MenE. We hypothesized that these molecules could inhibit either MenE or MenB, and so compounds were first evaluated for their ability to inhibit MenE directly.[11] Using this assay it was found that 1548L21 (Figure 2) did not inhibit MenE (100 nM) up to a concentration of 300 μM (data not shown).

Analogues of 1548L21 were synthesized (Scheme S1) and evaluated for their ability to inhibit MenB as well as bacterial growth (Table 1, supplementary material). Although several compounds inhibited MenB with IC_{50} values below 10 μM (**4** and **5**), there was generally a poor correlation between enzyme inhibition and antibacterial activity. For example, **1** is at least 10-fold more active in the antibacterial assay than **4** (MIC 6.25 μg/ml compared to 50 μg/ml), but is a significantly poorer inhibitor of MenB (IC_{50} 52 μM). Subsequent studies revealed that the inhibitors undergo *retro*-Michael addition,[12] leading to the regeneration of the (*E*)-benzoylacrylic acid and the amine in solution. The 2-aminobutanoates have half-lives of ~10 min to 12 hr at pH 7, which is significant since the MIC measurements take at least 24–48 hour to complete. In addition, the IC_{50} values were determined using the same protocol as that developed for the HTS in which compounds were pre-incubated in reaction buffer with all components except MenE for 1h prior to determining enzyme activity, suggesting that compound stability was also a factor in the enzyme inhibition studies. Indeed, these compounds were found not to inhibit MenB if the reaction was initiated immediately after addition of inhibitor (Table 1).

To strengthen the connection between compound stability and enzyme inhibition, we synthesized a series of compounds which were unable to undergo *retro*-Michael addition (Figure 1S). This included analogues **1S** and **2S** in which the α protons were replaced with fluorines or methyl groups, **3S** in which the ketone was replaced with a hydroxyl group, and **4S**, **5S**, **6S** in which the amine was replaced with an amide, heterocycle or sulfur, respectively. Compounds **1S** to **6S** were stable in solution and did not inhibit MenB up to a concentration of 50 μM. In addition, we also synthesized the monomethyl and deuterium analogues **7S** and **8S** which were still able to undergo elimination and, as a consequence, also retained the ability to inhibit MenB. Based on these results, we hypothesized that the inhibitory activity of the compounds was related to their stability in solution.

Since the (*E*)-benzoylacrylic acid is a Michael acceptor, we speculated that CoA in the reaction mixture might react with the acid, and it was found that the IC_{50} value for inhibition of MenB by compound **3a** decreased as the concentration of CoA increased (data not shown). This suggested that inhibition of MenB might result from an adduct formed between the (*E*)-benzoylacrylic acid and CoA. Subsequently the (*E*)-benzoylacrylic acid and CoA were incubated together at pH 7.0 for 2 hr and the major product was purified by HPLC. Analytical data confirmed that the CoA thiol had added to the C2 carbon of the acid.

To generate SAR data for MenB inhibition, a series of CoA adducts were synthesized and evaluated (Table 2). In general, addition of a bulky substituent at either the *meta* or *para* position of the phenyl group resulted in significant reduction in enzyme inhibition. Additionally, incorporation of electron-donating substituents at either the *ortho* or *para* positions also decreased inhibitor potency. In contrast, introduction of an electron-withdrawing substituent into the aromatic ring resulted in an increase in enzyme inhibition. Perusal of the data in Table 2 indicates that the most potent compounds (**10** and **16**) have IC_{50} values of ~100 nM which is close to ½[E_o]. To gain further mechanistic insight into the functioning of these compounds, we thus used steady state kinetic methods to study the inhibition of MenB by **7**, **10** and **16**. These compounds were found to be noncompetitive (mixed) inhibitors of MenB. Consistent with their respective IC_{50} values (106 and 470 nM, respectively), the K_i and K_i' values of the 2,4-diCl derivative **16** (49 and 290 nM, respectively) were significantly lower than the corresponding values for the 4-Cl analogue **7** (0.35 and 1.6 μM, respectively). Clearly there is a preference for an electron withdrawing substituent ortho to the succinyl side chain, which of course is the position normally occupied by the OSB carboxyl group. In order to account for noncompetitive inhibition, we are currently exploring the possibility that binding of inhibitor to one subunit in the MenB homohexamer can modulate the activity of adjacent subunits.

The affinity of **16** is noteworthy as, in our experience, substrate or product analogues of CoA-dependent enzymes do not generally bind with high affinity to their respective enzymes. Indeed, potent inhibitors of CoA-binding enzymes are largely unknown except in a few specific cases such as 2-octynoyl-CoA which is a mechanism based inhibitor of acyl-CoA dehydrogenase.[13] However, analysis of the MenB reaction suggests an explanation for the potent inhibition of MenB by the CoA adducts. A mechanism for the MenB catalyzed reaction has been proposed in which an intramolecular proton abstraction by the OSB carboxylate leads to the formation of a resonance stabilized carbanion.[8] Model building suggests that the CoA adducts adopt a bound structure that resembles the intermediate required for α-proton abstraction (Figure 3), which may account for the high affinity of these compounds for MenB. Of key importance is the location of the free carboxylate of the adduct, which is bound in the oxyanion hole formed by Gly-105 and Gly-161.[8,14] The oxyanion hole is a conserved structural feature within the crotonase superfamily and plays a central role in catalysis by stabilizing the carbanion/enolate formed during reactions catalyzed by this superfamily.[15] Indeed, many other CoA binding enzymes outside the crotonase superfamily also function to increase either thioester electrophilicity or α-proton acidity through interactions with the acyl-CoA thioester carbonyl, and thus our data suggest a general structural feature that may facilitate the development of inhibitors of CoA-dependent drug targets.

To investigate the importance of the proposed interaction between the oxyanion hole and the adduct carboxylate, we synthesized two additional compounds **17** and **18** (Scheme S1, supplementary material) which have similar structures to the CoA adducts but lack a free carboxylate group. Compared with the CoA adducts, the CoA thioester (**17**) has a significantly lower affinity for MenB (Table 2) with an IC_{50} value of 2.2 μM compared to 100 nM for the corresponding adduct (**16**). In addition, compound **18** displayed no significant inhibition up to a concentration of 400 μM. These two compounds thus support the importance of the free carboxylate for CoA adduct binding.

The CoA adducts were found to have limited antibacterial activity (data not shown), presumably due to poor uptake by the bacteria. We speculated that CoA addition could occur once the benzoylacrylic acid had penetrated the cell and, while the benzoylacrylic acid **3a** had limited antibacterial activity, the corresponding benzoylacrylic acid methyl ester **3b**, as well as the fluoro analogue **2b**, displayed potent antibacterial activity (Table 3, MIC 0.64

μg/ml). Indeed **3b** also had potent activity in a low oxygen recovery assay (LORA) against nonreplicating *M. tuberculosis* (NRP-MTB) (Table 3, MIC 1.5 μg/ml), which is promising given that apart from rifampicin (MIC 0.4 μg/ml) many current drugs are inactive against NRP-MTB.[16] Interestingly, it has been reported that the *menB* gene is upregulated when H37Rv is grown under oxygen-limiting conditions,[17] supporting the possibility that this enzyme is an intracellular target for the CoA adducts. Based on these results, we propose that protection of the acid aids entry of the compounds into the cell where they then undergo reaction(s), such as a Michael addition by CoA that results in formation of the antibacterial species. Although we cannot rule out the possibility that the compounds react with other nucleophiles in the cell, we observed that the addition of 1,4-dihydroxy-2-naphthoic acid (DHNA; 100 μg/ml) to the media was able to rescue the growth of *M. tuberculosis* H37Rv that had been treated with **2b** or **3b** at 2xMIC. DHNA is a downstream product of the MenB reaction that has previously been used in complementation experiments to elucidate the order and identity of enzymes that comprise the menaquinone biosynthesis pathway.[18] Thus the ability of DHNA to rescue growth supports our current hypothesis that the Michael acceptors inhibit menaquinone biosynthesis in *M. tuberculosis*.

Further studies are required to confirm that CoA addition and MenB inhibition occurs within the cell and to fully elucidate the mechanism of action of these compounds. In addition, it will be important to further improve the affinity of the adducts for MenB and also to replace the CoA portion of the adducts by more drug-like groups, and in this regard we note the studies by Pereira *et al* in which inhibitors of *E. coli* acetyltransferase GlmU were identified that occupy the CoA binding site in this enzyme.[19] Since the benzoylacrylic acid and CoA bind to adjacent sites on MenB, we are also exploring the possibility that MenB catalyzes adduct formation in an analogous fashion to that observed, for example, in the assembly of inhibitors via *in situ* click chemistry.[20] It is interesting to note that the IC_{50} values for enzyme inhibition by the parent 2-aminobutanoates (Table 1) correlate with their stability and hence reactivity of the butenoate that is formed. There is also a correlation between IC_{50} and K_i values for inhibition of MenB by the resulting adducts, indicating that the more electrophilic butenoates bind more tightly to the enzyme, presumably due to favorable interactions between the enzyme and halogens at the 2 and 4 positions. A current goal of our research program is to develop butenoates with increased affinity for the enzyme but reduced electrophilicity, thus improving the selectivity of MenB inhibition by increasing the likelihood that adduct formation would occur on the enzyme. In this regard, we note the recent discussions concerning the utility of covalent enzyme inhibitors that contain appropriately tuned electrophilic groups.[21]

Finally, in addition to providing a promising starting point for the development of MenB inhibitors, the work described here also serves to reinforce issues that can arise with leads identified through HTS.[22] Although the present leads are stable in DMSO, their reactivity under aqueous conditions reinforces the necessity to consider compound stability under the assay conditions employed, which is of particular importance for measurements that require prolonged incubation such as antibacterial assays. In the present work the use of a coupled assay that included free CoA resulted in the discovery of CoA adducts that bind with high affinity to MenB and that represent a promising foundation for the development of novel antibacterial agents that target menaquinone biosynthesis in *M. tuberculosis* as well as other pathogenic bacteria that have this pathway. Importantly, our data also support the proposal that MenB may be an appropriate target in non-replicating populations of *M. tuberculosis*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Bloom BR, Murray CJ. Tuberculosis: commentary on a reemergent killer. Science. 1992; 257:1055–64. [PubMed: 1509256]
2. Boshoff HI, Barry CE 3rd. Tuberculosis - metabolism and respiration in the absence of growth. Nat Rev Microbiol. 2005; 3:70–80. [PubMed: 15608701]
3. Gengenbacher M, Rao SP, Pethe K, Dick T. Nutrient-starved, non-replicating Mycobacterium tuberculosis requires respiration, ATP synthase and isocitrate lyase for maintenance of ATP homeostasis and viability. Microbiology. 2010; 156:81–7. [PubMed: 19797356]
4. Andries K, Verhasselt P, Guillemont J, Gohlmann HW, Neefs JM, Winkler H, Van Gestel J, Timmerman P, Zhu M, Lee E, Williams P, de Chaffoy D, Huitric E, Hoffner S, Cambau E, Truffot-Pernot C, Lounis N, Jarlier V. A diarylquinoline drug active on the ATP synthase of Mycobacterium tuberculosis. Science. 2005; 307:223–7. [PubMed: 15591164]
5. Lu X, Zhang H, Tonge PJ, Tan DS. Mechanism-based inhibitors of MenE, an acyl-CoA synthetase involved in bacterial menaquinone biosynthesis. Bioorg Med Chem Lett. 2008
6. Dhiman RK, Mahapatra S, Slayden RA, Boyne ME, Lenaerts A, Hinshaw JC, Angala SK, Chatterjee D, Biswas K, Narayanasamy P, Kurosu M, Crick DC. Menaquinone synthesis is critical for maintaining mycobacterial viability during exponential growth and recovery from non-replicating persistence. Mol Microbiol. 2009; 72:85–97. [PubMed: 19220750]
7. Meganathan R. Biosynthesis of menaquinone (vitamin K2) and ubiquinone (coenzyme Q): a perspective on enzymatic mechanisms. Vitam Horm. 2001; 61:173–218. [PubMed: 11153266]
8. Truglio JJ, Theis K, Feng Y, Gajda R, Machutta C, Tonge PJ, Kisker C. Crystal Structure of Mycobacterium tuberculosis MenB, a Key Enzyme in Vitamin K2 Biosynthesis. J Biol Chem. 2003; 278:42352–60. [PubMed: 12909628]
9. Sassetti CM, Boyd DH, Rubin EJ. Genes required for mycobacterial growth defined by high density mutagenesis. Mol Microbiol. 2003; 48:77–84. [PubMed: 12657046]
10. Li X, Liu N, Zhang H, Knudson SE, Slayden RA, Tonge PJ. Synthesis and SAR studies of 1,4-benzoxazine MenB inhibitors: novel antibacterial agents against Mycobacterium tuberculosis. Bioorg Med Chem Lett. 2010; 20:6306–9. [PubMed: 20850304]
11. Obrien WE. Continuous spectrophotometric assay for argininosuccinate synthetase based on pyrophosphate formation. Anal Biochem. 1976; 76:423–430. [PubMed: 11707]
12. Jenner G. Comparative study of physical and chemical activation modes. The case of the synthesis of beta-amino derivatives. Tetrahedron. 1996; 52:13557–13568.
13. Powell PJ, Thorpe C. 2-Octynoyl coenzyme A is a mechanism-based inhibitor of pig kidney medium-chain acyl coenzyme A dehydrogenase: isolation of the target peptide. Biochemistry. 1988; 27:8022–8028. [PubMed: 3233192]
14. Li HJ, Li X, Liu N, Zhang H, Truglio JJ, Mishra S, Kisker CF, Garcia-Diaz M, Tonge PJ. Mechanism of the Intramolecular Claisen Condensation Reaction Catalyzed by MenB, a Crotonase Superfamily Member. Biochemistry. 2011
15. Zhang, H.; Machutta, CA.; Tonge, PJ. Fatty Acid Biosynthesis and Oxidation. In: Mander, L.; Lui, H-W., editors. Comprehensive Natural Products Chemistry II Chemistry and Biology. Vol. 8. 2010. p. 231-275.
16. Hurdle JG, Lee RB, Budha NR, Carson EI, Qi J, Scherman MS, Cho SH, McNeil MR, Lenaerts AJ, Franzblau SG, Meibohm B, Lee RE. A microbiological assessment of novel nitrofuranylamides as anti-tuberculosis agents. J Antimicrob Chemother. 2008; 62:1037–45. [PubMed: 18693235]
17. Ramchandra P, Sturm AW. Expression of the naphthoate synthase gene in *Mycobacterium tuberculosis* in a self-generated oxygen depleted liquid culture system. Anaerobe. 2010; 16:610–613. [PubMed: 21094263]

18. Taber HW, Dellers EA, Lombardo LR. Menaquinone biosynthesis in *Bacillus subtilis* - Isolation of *men* mutants and evidence for clustering of *men* genes. J Bacteriol. 1981; 145:321–327. [PubMed: 6780514]

19. Pereira MP, Blanchard JE, Murphy C, Roderick SL, Brown ED. High-throughput screening identifies novel inhibitors of the acetyltransferase activity of *Escherichia coli* GlmU. Antimicrob Agents Chemother. 2009; 53:2306–2311. [PubMed: 19349513]

20. Lewis WG, Green LG, Grynszpan F, Radic Z, Carlier PR, Taylor P, Finn MG, Sharpless KB. Click chemistry in situ: acetylcholinesterase as a reaction vessel for the selective assembly of a femtomolar inhibitor from an array of building blocks. Angew Chem Int Ed Engl. 2002; 41:1053–7. [PubMed: 12491310]

21. Singh J, Petter RC, Baillie TA, Whitty A. The resurgence of covalent drugs. Nat Rev Drug Discov. 2011; 10:307–317. [PubMed: 21455239]

22. Baell JB, Holloway GA. New substructure filters for removal of Pan Assay Interference Compounds (PAINS) from screening libraries and for their exclusion in bioassays. J Med Chem. 2010; 53:2719–2740. [PubMed: 20131845]

23. Delano, WL. The PyMOL Molecular Graphics System. 2002. http://www.pymol.org

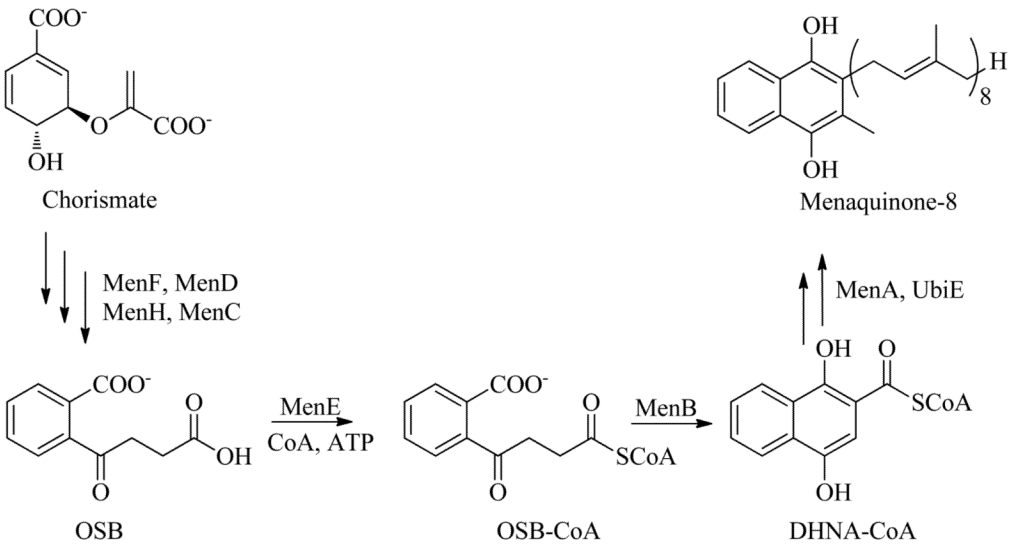


Figure 1. The menaquinone biosynthesis pathway

This pathway converts chorismate to menaquinone in prokaryotes such as *E. coli*, *S. aureus*, *B. subtilis*, and *M. tuberculosis*. Shown in detail are the reactions catalyzed by the OSB-CoA synthase MenE and the 1,4,-dihydroxy-2-naphthoyl-CoA synthase MenB.

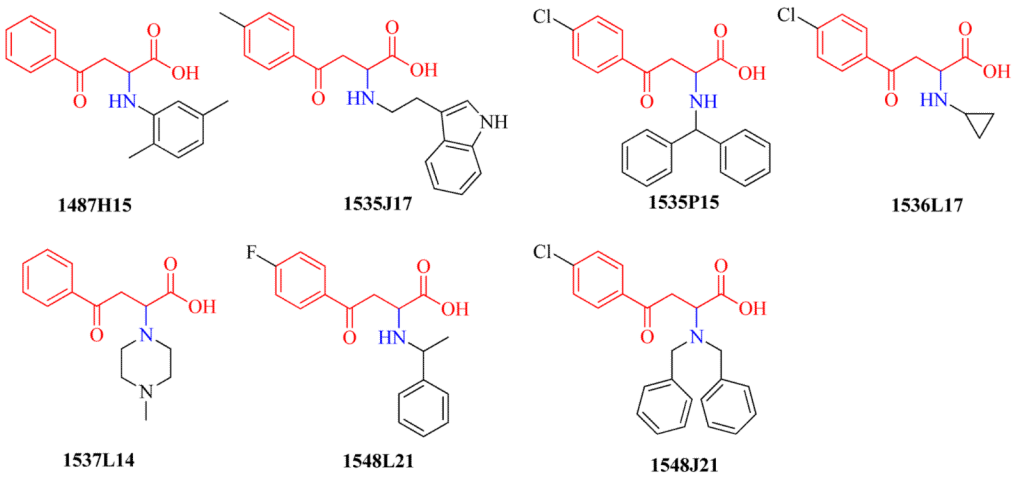
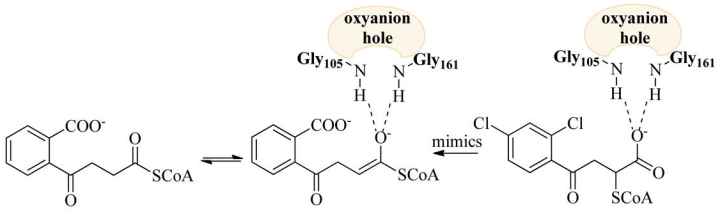


Figure 2. HTS hits containing the OSB core

These compounds were identified in the initial HTS screen conducted using the MenE/MenB coupled assay.

(a)



(b)

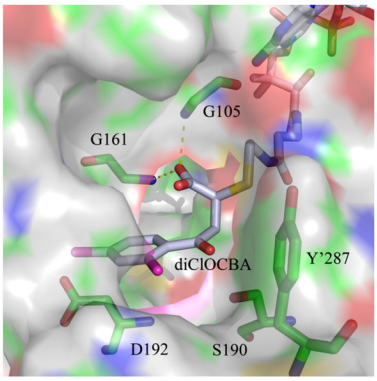


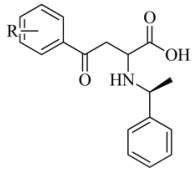
Figure 3. Proposed structure of a CoA adduct bound to MenB

(a) Proposed structure of the CoA adduct bound to MenB. The CoA adduct adopts a structure that mimics the resonance-stabilized carbanion formed during the MenB catalyzed reaction.

(b) The 2,4-dichloro CoA adduct (**16**) modeled into the active site of MenB. This model was built using the structure of acetoacetyl-CoA bound to MenB (1Q51.pdb),[8] and shows the proposed interaction between the adduct carboxylate and the MenB oxyanion hole (G105 and G161). Also shown are three conserved MenB residues (S190, D192 and Y'287). The figure was made using pymol.[23]

Table 1

Activity of the 2-amino-4-oxo-4-phenylbutanoic acids

Compound	Structure	R	IC_{50} (μM)[a] (w/o incubation)	IC_{50} (μM)[a] (w incubation)	MIC (μg/mL)[b]	$t_{1/2}$ (hr)[c]
1		H	>120	52.7±6.1	6.25	11.6
2		4-F	>120	14.4±2.3	12.5	12.2
3		4-Cl	>120	11.7±1.8	50	6.8
4		2-Cl	>120	8.4±0.8	50	0.4
5		2-CF_3	>120	3.2±0.5	25	0.2
6		2-OMe	>120	>120	12.5	11.6

[a] IC_{50} values for MenB inhibition were determined at a MenB concentration of 150 nM. Assays were initiated by addition of MenE either immediately after adding inhibitor to the assay (w/o incubation) or after a 1 h incubation (w incubation).

[b] Antibacterial activity against *M. tuberculosis* H37Rv.

[c] Stability at pH 7.4 and 25°C.

Table 2

Inhibition of MenB by the 2-CoA-4-oxo-4-phenylbutanoic acids

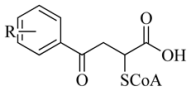
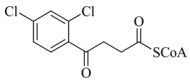
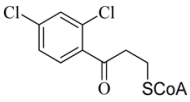
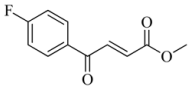
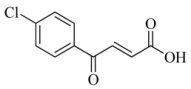
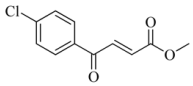
Compound	Structure	R	IC_{50} (nM)[a]	K_i (nM) and K_i' (nM)
7		4-Cl	468±62	350±50 and 1630±280
8		4-OMe	33500±2600	
9		2-F	204±37	
10		2-Cl	103±23	97±17 and 792±63
11		2-Br	135±22	
12		2-I	421±57	
13		2-NO_2	154±24	
14		2-OMe	12100±1000	
15		3-Cl	14100±1500	
16		2,4-diCl	106±26	49±6 and 286±7
17			2200±200	
18			>400000	

Table 3

Antibacterial activity of the 4-oxo-4-phenylbut-2-enoates against H37Rv

Compound	Structure	MIC (μg/ml)[a]	MIC (μg/ml)[b]
2b[c]		0.64	ND
3a		25	ND
3b[c]		0.64	1.5

[a] MIC determined in the microplate dilution assay under aerobic conditions.

[b] MIC determined in the in low oxygen recovery assay against NRP-MTB. ND, not determined.

[c] Cytoxicity assays indicated that the selectivity index of **2b** and **3b** is great than 20.