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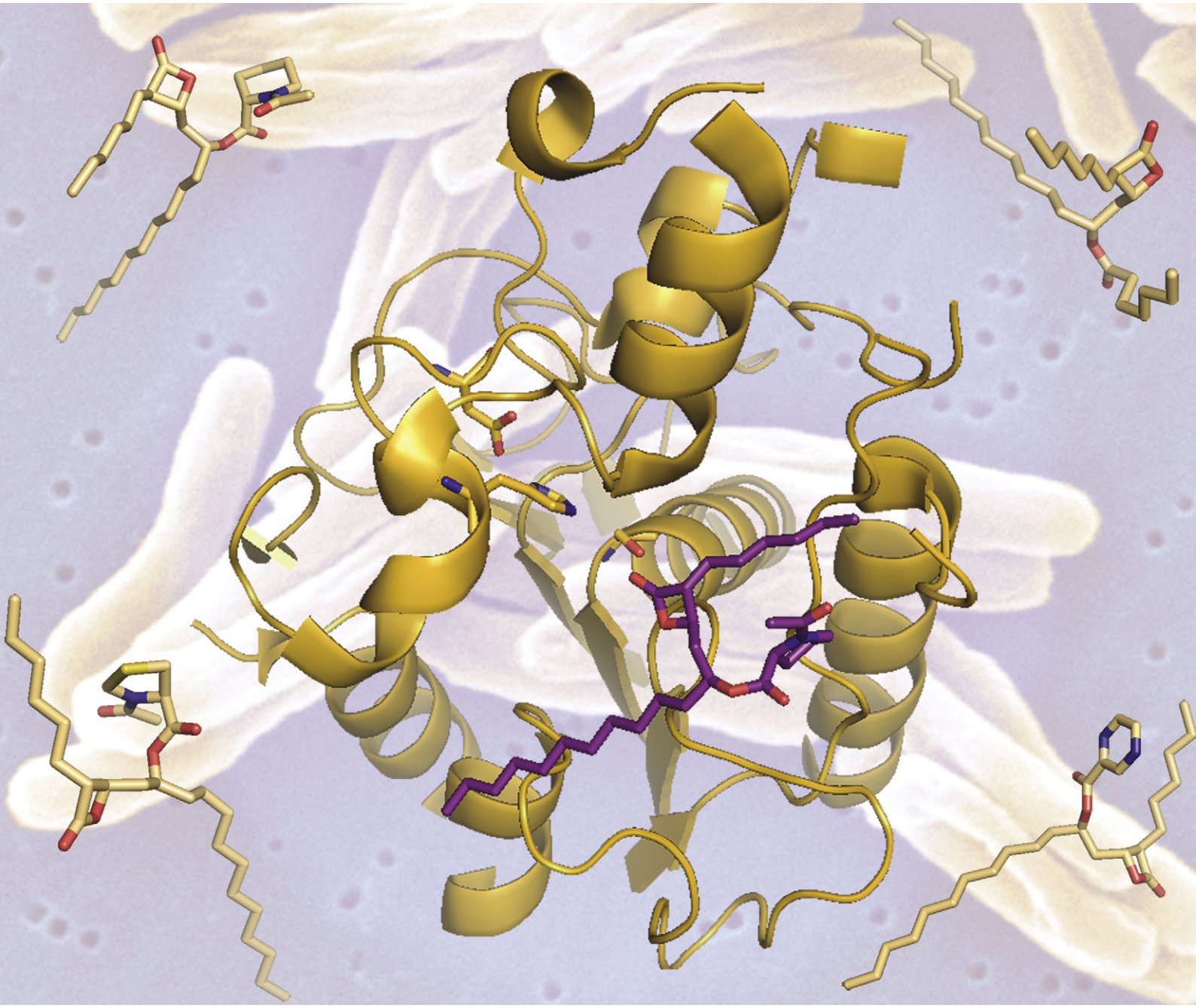

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COMMUNICATION

Inhibitors of an essential mycobacterial cell wall lipase (Rv3802c) as tuberculosis drug leads†

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The first targeted inhibitors of an essential *M. tuberculosis* cell wall lipase, Rv3802c, are described. Lead compounds exhibited nanomolar inhibition of the enzyme, and encouraging antibacterial activity against *M. tuberculosis in vitro*, supporting Rv3802c as a novel TB drug target.

Two billion people are currently infected with *Mycobacterium tuberculosis*, the etiological agent of tuberculosis (TB). In 2008, there were estimated to be 9.4 million new *M. tuberculosis* infections and 1.8 million deaths as a result of TB, the most by any single infectious agent.[1] The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *M. tuberculosis* highlights the need for new therapies, which operate *via* novel modes of action.[2–4] One of the key features contributing to the successful pathogenesis of *M. tuberculosis* is the unique nature of the complex lipid-rich cell wall.[5,6] The enzymes involved in the biosynthesis of mycolic acid, a key component of the mycobacterial cell wall,[7] are considered promising targets for TB drug development, as they are often essential for the survival and virulence of *M. tuberculosis*.[8–10] Recently, the enzyme Rv3802c (found within the mycolic acid gene cluster) was reported to possess significant lipase, phospholipase A, and thioesterase activities, with catalysis occurring *via* the action of a serine containing catalytic triad.[11] The essential nature of Rv3802c for the survival of *M. tuberculosis*[12] and other mycobacteria,[13] coupled with its retention within the cell wall,[14] makes it an ideal candidate for TB drug discovery as potential inhibitors would not need to traverse the waxy cell coat. The pancreatic lipase inhibitor tetrahydrolipstatin (THL (**1**), Scheme 1) used for the treatment of obesity (marketed as Xenical®) has recently been shown to inhibit the essential *M. tuberculosis* lipase Rv3802c,[7,13] and has also displayed modest anti-mycobacterial activity.[14] Given this unexplored activity of THL we were interested in using this privileged scaffold as a starting point for the development of the first targeted Rv3802c inhibitors for the discovery of new TB drug leads with a novel mode of action.

In the absence of structural information for Rv3802c, a first generation inhibitor library was designed based on the THL pharmacophore. To this end, THL (**1**) was isolated by Soxhlet extraction from the contents of Xenical® capsules and, after 4 synthetic steps, selectively protected diol **2** was furnished in high yield (see Supplementary Information).[15] Treatment of **2** with benzenesulfonyl chloride and pyridine at −18 °C provided the desired β-lactone in good yield. Subsequent deprotection of the C-5 silyl ether was achieved using a buffered solution of tetrabutylammonium fluoride (TBAF) and acetic acid[16] to afford the corresponding alcohol **3** in 63% yield over the two steps (Scheme 1).

With alcohol **3** in hand, we next embarked on the divergent synthesis of a library of Rv3802c inhibitors. Compounds were prepared by condensing **3** with the desired carboxylic acid using *N*,*N*′-diisopropylcarbodiimide (DIC) and *N*,*N*-dimethylaminopyridine (DMAP),[17] directly affording the corresponding inhibitors **4–14** and, after subsequent manipulation, **15–20** in moderate to high yields. A diverse series of side chains were incorporated in order to interrogate the specificity of Rv3802c for the side chain moiety. In particular, lipophilic side chains were incorporated in **4–7** to probe for hydrophobic regions within the active site. Incorporation of aromatic and heteroaromatic rings in **8–11** allowed hydrogen bonding and π–π and cation–π interactions between side chain residues and backbone functionalities to be assessed. Furthermore, a number of flexible non-aromatic heterocyclic side chains were also incorporated in inhibitors **12–20**.

With the desired inhibitor library (**4–20**) prepared, these compounds were screened for their inhibition of Rv3802c in a polyoxyethylene sorbitan lipase assay (see Supplementary Information).[11] Gratifyingly, many of the compounds proved to be significantly more potent inhibitors of the target enzyme compared with THL (Table 1). Clear structure–activity trends can be delineated from the inhibition results based on the nature of the appended ester side chain. In particular, compounds bearing smaller lipophilic groups, *i.e.* **6** (cyclopropyl) and **7** (methyl), displayed moderate activity with IC_{50} values of 23 μM and 11 μM respectively. Conversely the incorporation

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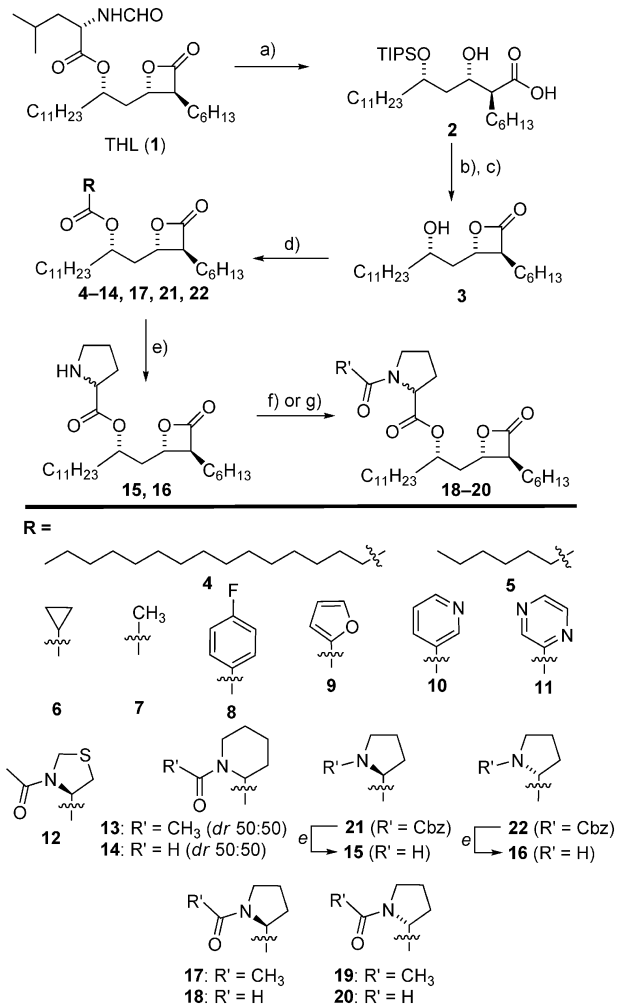

Scheme 1 (a) 47% over 4 steps;[16] (b) benzenesulfonyl chloride, pyridine, −18 °C, 48 h, 74%; (c) *n*-Bu_4NF, AcOH, THF, −20 °C, 16 h, 63%; (d) RCO_2H, DIC, DMAP, DCM/DMF, 0 °C to 25 °C, 15–72 h, 28%–quant; (e) for **21** and **22**; H_2, Pd/C, MeOH, 25 °C, 4 h, 62–68%; (f) for **16**; AcCl, Et_3N, DCM, 25 °C, 30 min, 85%; (g) for **15** and **16**; AcOCHO, DCM, 25 °C, 15 min, 55–72%.

of larger lipophilic hexyl and palmityl ester side chains in **4** and **5**, respectively, invoked a drop in potency.

Compounds **8** and **9** bearing *p*-fluorophenyl and 2-furanyl ester side chains were also inactive against the enzyme up to concentrations of 25 μM. Interestingly, the introduction of nitrogen-containing aromatic side chains (**10** and **11**) led to a significant increase in potency, with IC_{50} values of 6.0 μM and 1.2 μM, respectively. The most potent compounds tested were **12**, bearing an L-thiazolidyl ester side chain, and **17–20** containing L- and D-prolyl ester side chains, with IC_{50} values ranging from 200–800 nM. In particular, compounds **17** and **18** bearing *N*-acetyl-L-prolyl and *N*-formyl-L-prolyl ester side chains were the most active, both exhibiting IC_{50} values of 200 nM against the enzyme. Notably, these inhibitors are over an order of magnitude more potent than the original lead compound, THL.

The superior activity displayed by inhibitors **17** and **18** when compared with the corresponding free amines **15** and **16** (IC_{50} = 1.8 μM and 2.0 μM) highlights the importance of the *N*-acyl moiety for inhibition. Expansion from a five-membered

Table 1 *In vitro* inhibition of *M. tuberculosis* cell wall lipase Rv3802c and *M. tuberculosis* by THL and inhibitors **4–20**

Inhibitor	IC_{50} Rv3802c (μM)[a]	IC_{50} *M. tuberculosis* (μM)[b]
THL **1**	3.8 ± 1.2	15 ± 5.6
4	>25	>25
5	>25	>25
6	23 ± 1.9	11 ± 4.0
7	11 ± 2.5	1.3 ± 0.2
8	>25	>25
9	>25	6.0 ± 4.0
10	6.0 ± 2.1	2.5 ± 0.5
11	1.2 ± 0.3	2.9 ± 1.2
12	0.7 ± 0.1	1.4 ± 0.1
13	1.6 ± 0.3	1.9 ± 0.2
14	1.4 ± 0.1	1.3 ± 0.2
15	1.8 ± 0.1	4.9 ± 0.4
16	2.0 ± 0.1	3.7 ± 0.6
17	0.2 ± 0.01	1.5 ± 0.2
18	0.2 ± 0.01	1.3 ± 0.2
19	0.8 ± 0.1	4.0 ± 0.6
20	0.6 ± 0.1	1.4 ± 0.1

[a] Kinetic parameters for Rv3802c: K_M = 4.52 mM, k_{cat} = 0.14 s^{-1}. [b] *M. tuberculosis* = H37Rv strain.

to a six-membered ring in pipecolic acid derivatives **13** and **14** led to a drop in potency when compared to **17** and **18** (IC_{50} = 1.6 μM and 1.4 μM) indicating that the larger six-membered ring is not as well accommodated in the active site. Modifying the chirality of the prolyl ester side chain in inhibitors **19** and **20**, bearing *N*-formyl-D-prolyl and *N*-acetyl-D-prolyl ester side chains, also led to a slight decrease in potency against Rv3802c (IC_{50} = 800 nM and 600 nM).

We were next interested in examining the mechanism of inhibition of Rv3802c by compound **17**. To this end, 5 μM of **17** was first incubated with Rv3802c for 30 min before the addition of 1, 5 and 10 mM concentrations of the substrate (see Supplementary Information). In this study **17** acted as a covalent irreversible inhibitor of Rv3802c at 1 mM and 5 mM substrate concentrations. It should be noted that at a substrate concentration of 10 mM, and after monitoring substrate hydrolysis for 1 h, up to 20% of enzyme activity was restored, suggesting that the inhibitors are slowly reversible, albeit after long incubation times and in the presence of a 2000-fold molar excess of the substrate.

Having elucidated a number of potent inhibitors of Rv3802c, we next focussed on assessing the *in vitro* antibacterial activity. Compounds **4–20** were screened against *M. tuberculosis* H37Rv, a virulent human strain, using a microplate-based assay with Alamar blue readout (MABA) (see Supplementary Information).[18–20] Overall there was good correlation between the activity displayed against Rv3802c and *M. tuberculosis*, with active inhibitors showing antibacterial effects (Table 1). The most potent inhibitors of *M. tuberculosis* growth were **12**, **17**, **18**, and **20**, bearing *N*-acetyl-L-thiazolidyl, *N*-acetyl-L-prolyl, *N*-formyl-L-prolyl, and *N*-formyl-D-prolyl ester side chains, which displayed IC_{50} values between 1.3 and 1.5 μM; an order of magnitude more active than THL (IC_{50} = 15 μM). These four compounds all prevented the growth of *M. tuberculosis* with minimum inhibition concentrations (MICs) of *ca.* 3 μM. It is important to note that compounds **12**, **17**, **18**, and **20** were also the most potent inhibitors of Rv3802c, thus providing further compelling evidence for the essential nature of this

enzyme and its potential as a new TB drug target. Toxicity studies were also performed with these lead compounds against the human embryonic kidney cell line HEK293 (see Supplementary Information). Gratifyingly, these analogues displayed similarly low cytotoxicity when compared to THL, a drug approved for use in humans. Specifically, analogues displayed no significant effect on cell activity up to concentrations of 50 μM (over 10 times the concentration required for complete inhibition of *M. tuberculosis* growth *in vitro*).

In summary, we have reported the first potent inhibitors of the *M. tuberculosis* lipase, Rv3802c. Located in the mycobacterial cell wall, this enzyme is essential for viability and, as such, represents an exciting new TB drug target. Several of the analogues synthesised in this study exhibited sub-micromolar inhibition of the enzyme. In general, compounds that displayed potent activity against the enzyme also possessed encouraging antibacterial activity against *M. tuberculosis in vitro* and now serve as novel TB drug leads for further investigation. Future studies will involve screening the lead compounds (**12**, **17**, **18** and **20**) in *M. tuberculosis*-infected mice models to gauge the efficacy of the inhibitors as anti-tubercular agents *in vivo*. These compounds also represent leads for the design and synthesis of second-generation inhibitors, work towards which is currently underway in our laboratories.

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