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Discovery of Mycobacterium Tuberculosis Protein Tyrosine Phosphatase A (MptpA) Inhibitors Based on Natural Products and a Fragment-Based Approach

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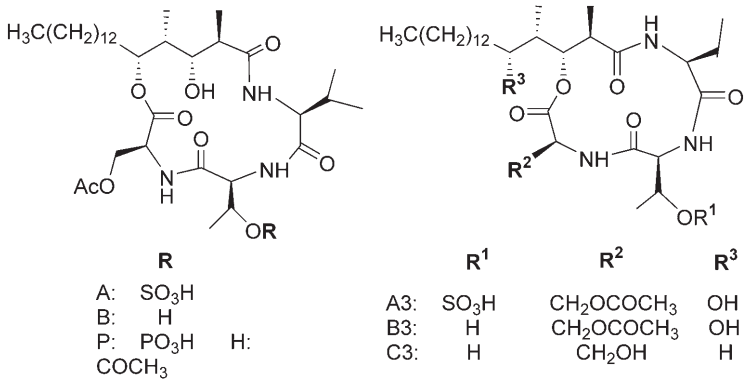
Protein phosphorylation and dephosphorylation reactions are at the heart of innumerable biological processes. Aberrant protein phosphorylation contributes to the development of many human diseases including cancer and diabetes.[1] Due to this biological importance, protein kinases, which catalyse protein phosphorylation, and their antagonists, protein phosphatases (PPs), have moved into the focus of a rapidly growing number of medicinal-chemistry and chemical-biology research programs.[2–5] Several bacterial pathogens produce eukaryotic-like protein phosphatases that have been implicated in virulence. A particularly important case is *Myobacterium tuberculosis*, which is the causative agent of tuberculosis (TB) and a major cause of mortality around the world.[6] *M. tuberculosis* has two functional phosphatases, MptpA and MptpB.[7] These enzymes are secreted by growing mycobacterial cells. They are believed to mediate mycobacterial survival in host cells by dephosphorylating proteins that are involved in interferon-γ signaling pathways.[8,9] About one third of the world's population is infected with *M. tuberculosis*, and there is an increasing spread of drug-resistant mycobacteria. Therefore, there is a growing need for the development of new therapeutic agents for the treatment of tuberculosis. In the light of this urgent demand, the Mptps have been proposed as new potential anti-TB drug targets.[9] However, to date, inhibitors of these enzymes have not been described.

Here we describe the discovery of MptpA inhibitors by two different and complementary approaches for the identification of initial hits in screening collections, namely natural-product-inspired and fragment-based library development.

We have previously forwarded the notion that biologically active natural products should be regarded as evolutionarily selected and biologically prevalidated starting points for inhibitor development.[10] Based on this principle and the fact that MptpA is a tyrosine phosphatase,[7,11] we have investigated whether natural products and their analogues that have already served as guiding structures for the discovery of new classes of phosphatase inhibitors[12,13] could be employed for the identification of the first Mptp inhibitors.

Initially the stevastelins (Scheme 1) were considered as possible starting points for the development of MptpA inhibitors.



Scheme 1. Structures of representative members of the stevastelin family.

These microbial metabolites have been shown to be inhibitors of the *Vaccinia* virus related dual-specificity phosphatase, VHR,[14] which dephosphorylates extracellular regulated kinases 1/2 (Erk1/2) and Jnk1/2.[15]

In terms of structure, the stevastelins can be regarded as cyclodepsipeptides. They are composed of a tripeptide unit that forms a macrolactone with either one of two hydroxy groups present in a diketide fragment, although stevastelin C3 contains only one hydroxy group (Scheme 1). Analogous cyclodepsipeptides **8**–**12** were chosen as model compounds. These incorporate two stereogenic centers in the long-chain hydroxy acid.

The synthesis of cyclic stevastelin analogues **8**–**12** was accomplished as shown in Scheme 2. *Anti*-configured isomers **8**, **9**, and **12** were obtained from racemic *anti*-configured β-hydroxy acid thioester (**1**).[16]

After esterification of alcohol **1** with serine derivative **2**, and selective removal of the N-terminal 9-fluorenylmethoxycarbonyl (Fmoc) group, diastereomeric *anti*-isomers **3** and **4** were conveniently separated by flash chromatography. The absolute

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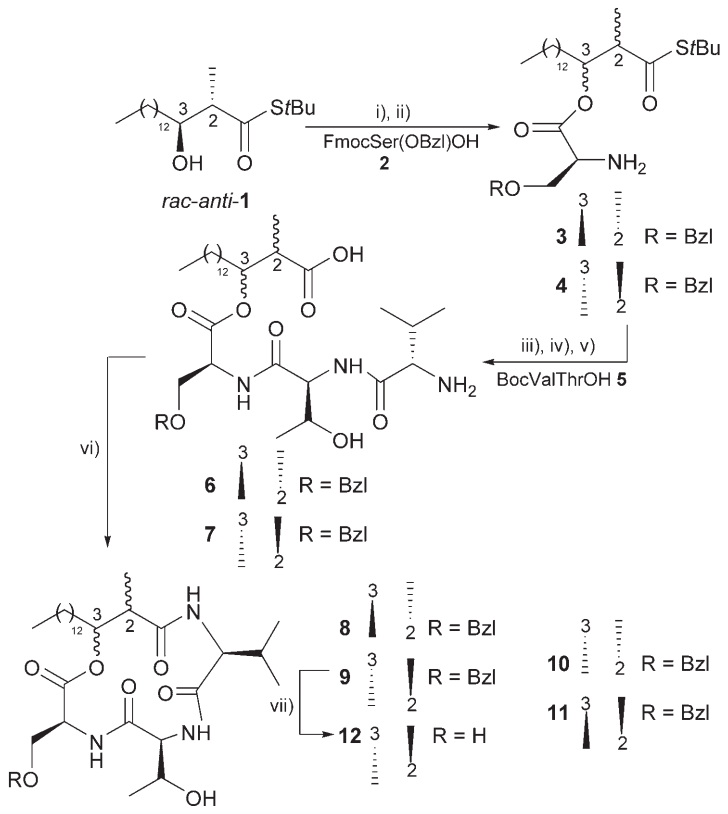


Scheme 2. Synthesis of cyclic stevastelin analogues. i) 1 equiv Fmoc–Ser(OBzl)OH (**2**), 1.1 equiv *i*PrNCN*i*Pr (DIC), 0.1 equiv 4-(*N*,*N*-dimethylamino)pyridine (DMAP), CH_2Cl_2, RT, overnight, 82%; ii) CH_2Cl_2/Et_2NH 4:1, RT, 3 h, (*S*,*S*)-isomer: 43%, (*R*,*R*)-isomer: 42%; iii) 1 equiv **5**, 1.2 equiv DIC, 1.2 equiv 1-hydroxybenzotriazole (HOBt), 1.25 equiv $(iPr)_2NEt$, CH_2Cl_2/DMF 10:1, 0 °C, 5 min, RT, overnight, 70–76%; iv) CH_2Cl_2/TFA 1:1, RT, 30 min, quant; v) 3.0 equiv *N*-bromosuccinimide (NBS), THF/H_2O 4:1, 8 h, 70%; vi) 2.0 equiv HBTU, 4.0 equiv $(iPr)_2NEt$, CH_2Cl_2/DMF 10:1, RT, overnight, 19–42%; vii) Pd-C,H_2, RT, 7 d, 58%.

configuration of the β-hydroxy acid group incorporated into **3** and **4** was ascertained by saponification of ester **4**, isolation of the free β-hydroxy acid, and comparison of its specific rotation with a reference compound. The reference compound was synthesized by means of an *anti*-selective aldol reaction that employed the Evans aldol methodology.[17] Subsequent amino acid chain elongation with dipeptide **5**, and removal of the N- and the C-terminal protecting groups yielded acyclic intermediates **6** and **7**.

Macrolactamization was achieved in moderate yield by using 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluoro phosphate (HBTU) as condensing reagent. The *O*-benzyl protecting group of the serine side chain was removed from *anti*-isomer **9** by hydrogenolysis to yield compound **12**. This cyclic depsipeptide is a direct analogue of stevastelin C3 (Scheme 1), and contains two instead of three stereocenters in the hydroxy acid component.

For the synthesis of *syn*-configured stevastelin analogues **10** and **11**, the two corresponding *syn*-configured long chain β-hydroxy acids were synthesized in entiomerically pure form by using the Evans *syn*-aldol protocol.[18] Each acid was then converted into the corresponding benzyl thioester by following the strategy delineated above for the *anti*-isomers (except for the separation of diastereomers). The two cyclic depsipeptides **10** and **11** were then synthesized from the benzyl thioesters.

Cyclic stevastelin analogues **8**–**12** were then investigated as possible inhibitors of several different phosphatases. The screen included Cdc25A and VHR as two typical dual specificity phosphatases, MptpA (for cloning, expression and purification see Supporting Information), and PTP1B as a prototypical tyrosine phosphatase. VHR is a physiological regulator of ERKs, which belong to the mitogen-activated protein (MAP) kinase family;[15,19] the Cdc25 dual-specific protein phosphatase family is critical for cell-cycle control;[20] and PTP1B is a key negative regulator of insulin-receptor activity. PTP1B inhibitors are expected to enhance insulin sensitivity and act as effective therapeutics for the treatment of type 2 diabetes, insulin resistance, and obesity.[3] The tyrosine phosphatase CD45, which is a positive regulator of signal transduction in B- and T-cells,[21] and the prototypical serine/threonine phosphatase PP1, which regulates numerous intracellular processes[22] were also included in the screen.

The results of the screen are displayed in Table 1. The data demonstrate that variation of the absolute configuration and structure in the cyclic stevastelin analogues led to pronounced selectivity for individual phosphatases. Thus, *anti*-configured compounds **8** and **9** selectively inhibited VHR. Compound **12**, which was obtained from **9** by *O*-debenzylation, proved to be a selective inhibitor of Cdc25A. Gratifyingly, *syn*-configured stevastelin analogue **10** inhibited MptpA with appreciable potency and displayed similar activity towards VHR and PTP1B. Stereoisomer **11** was not active, that is, it did not inhibit any of the phosphatases with IC_{50} values < 30 μM. Phosphatases CD45 and PP1 were not inhibited by any of the cyclic stevastelin analogues.

These results demonstrate that by varying the configuration in the β-hydroxy acid part of the stevastelin analogues and

Table 1. IC_{50} values for inhibition of different phosphatases by stevastelin analogues **8**–**12**.[a]

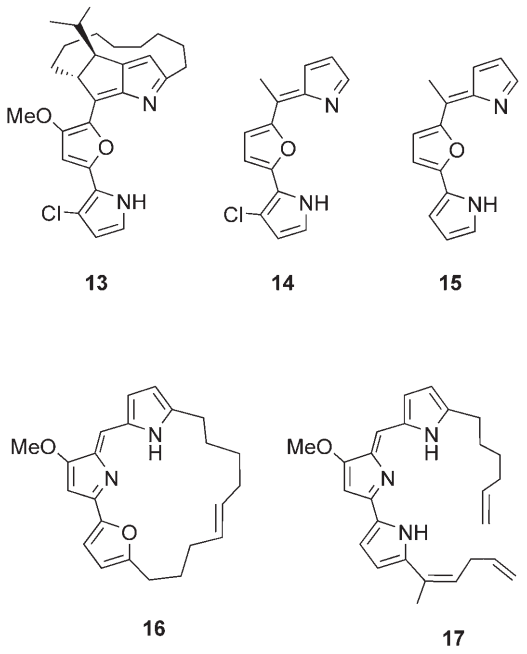
Compound	VHR IC_{50} [μM]	Cdc25A IC_{50} [μM]	PTP1B IC_{50} [μM]	MptpA IC_{50} [μM]	CD45 IC_{50} [μM]	PP1 IC_{50} [μM]
8	25.0 ± 5.1	n.a.	n.a.	n.a.	n.a.	n.a.
9	12.2 ± 0.6	n.a.	n.a.	n.a.	n.a.	n.a.
10	6.5 ± 1.2	n.a.	11.3 ± 2.9	8.8 ± 5.9	n.a.	n.a.
11	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
12	n.a.	15.5 ± 3.9	n.a.	n.a.	n.a.	n.a.

[a] The dephosphorylation of pNPP was measured by its absorption change at 405 nm. The concentration (μM) of inhibitors for which the enzyme activity is reduced to 50% is shown. The values were determined from at least three independent experiments; n.a.: not active ($IC_{50} > 30$ μM).

combining different substitution patterns in the amino acid side chains (compare **9** and **12**), selective phosphatase inhibitors can be developed. In particular, it appears possible that potent MptpA inhibitors can be obtained by employing the stevastelin structure as a guiding unit. A strategy for the discovery of such inhibitors could, for instance, consist of the synthesis of cyclic tripeptide libraries that are analogous to **8**–**12** and incorporate differently configured β-hydroxy acids.

Encouraged by these findings we investigated whether MptpA inhibitors could also be identified from the roseophilin and prodigiosin alkaloid classes.[23] These natural products and their analogues were recently found to define a new and non-obvious core motif for the development of tyrosine phosphatase inhibitors.[13]

To this end, roseophilin (**13**), its enantiomer, and analogues **14** and **15**, which lack the macrocyclic aliphatic ring, were investigated as potential MptpA inhibitors (Scheme 3 and Table 2). Gratifyingly, **15** proved to be a potent inhibitor of the phosphatase with an IC_{50} value of 9.4 μM.



Scheme 3. Roseophilin and prodigiosin analogues that were investigated as inhibitors of MptpA.

Similarly, we investigated a series of nonylprodigiosin analogues; this class of compounds has also delivered unprecedented phosphatase inhibitors.[13] Like the roseophilin analogues, these compounds contain an azafulvene unit but differ from roseophilin in the positioning of this structural element and in the type of aromatic rings that form the backbone.

A total of ten cyclic and acyclic prodigiosin analogues were investigated. They included compound **16** which differs from nonylprodigiosin by a furan instead of pyrrole ring and by an additional double bond in the aliphatic bridge. Although, this cyclic analogue of the natural product was not an inhibitor,

Table 2. IC_{50} [μM] values for inhibition of different phosphatases by roseophilin and nonylprodigiosin analogues **13**–**17**.[a]

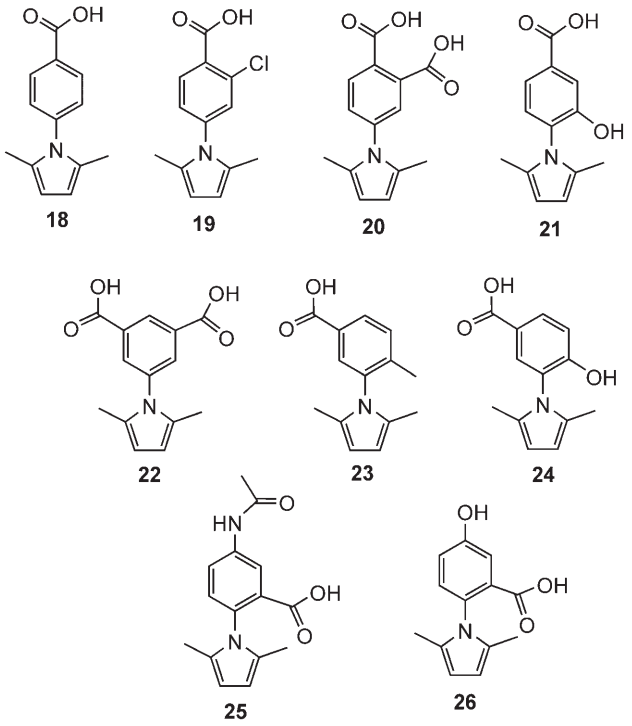
Compound	VHR IC_{50} [μM]	Cdc25A IC_{50} [μM]	PTP1B IC_{50} [μM]	MptpA IC_{50} [μM]
13	n.a.	28 ± 3	n.a.	n.a.
14	9 ± 7	n.a.	n.a.	n.a.
15	4.9 ± 3	n.a.	3.3 ± 1	9.4 ± 2.1
16	28 ± 13	n.a.	23 ± 11	n.a.
17	9 ± 7	n.a.	12 ± 2	28.7 ± 9.7

[a] The dephosphorylation of pNPP was measured by its absorption change at 405 nm. The concentration (μM) of inhibitors for which the enzyme activity is reduced to 50% is shown. The values were determined from at least three independent experiments; n.a.: not active (IC_{50} > 30 μM).

the related open-chain derivative, **17**, turned out to inhibit the enzymatic activity of MptpA with an IC_{50} value of 28.7 ± 9.7 μM (Table 2). These results demonstrate that prodigiosin- and roseophilin-type alkaloids can also serve as guiding structures for the development of MptpA inhibitors.

In an alternative approach MptpA inhibitors were identified from the rationally assorted fragment-based FMP (Forschungsinstitut für Molekulare Pharmakologie) library ($M_{average}$ = 250 Da). The 20000 compounds of this library were selected due to their diverse representation of reportedly bioactive scaffold elements (fragments) and compliance with physicochemical boundaries, which include the Lipinski rules.[24] As a result, hits from this library can be considered as privileged starting points for further structure extension and refinement. The library is accessible to the academic public from the ChemBioNet.[25]

The library was screened in a 384-well format. In order to determine K_i values, primary hits with IC_{50} < 50 μM were validated at different *p*-nitrophenyl phosphate (pNPP) substrate concentrations (1–10 mM). Inhibitor concentrations ranging from 250 nM to 50 μM were used. The screen provided several novel classes of low-molecular-weight inhibitors of phosphatases. The identified inhibitors were typically constructed by the combination of relatively simple chemical fragments. 2,5-Dimethylpyrrol-1-yl benzoic acids (**18**–**26**) were selected as one representative class of compounds with potential for further structure refinement. Preliminary structure–activity investigations were conducted and are summarized in Scheme 4 and Table 3. Hydroxy-substituted pyrrol-1-yl benzoic acids **21**, **24**, and **26** proved to be the superior inhibitors of this class. The 5-hydroxy-2-(pyrrol-1-yl) derivative **26** (K_i = 1.6 μM) was the most active representative. K_i values were also determined for PTP1B. Again the hyxdroxypyrrol-1-yl benzoic acids proved to be the most potent compounds, with K_i values of 1.1 μM (**24**), 1.9 μM (**21**), and 3.0 μM (**26**). Similar structures have previously been reported to modulate the activity of Pin-1.[26] This peptide is a prolyl isomerase that binds the phosphorylated p53 tumor suppressor and is thus pivotal in cancer development.[27] Supposedly, the carboxylic acid residue is recognized in both cases as a mimic of the protein phosphate.



Scheme 4. Representative examples of dimethylpyrrol-1-yl benzoic acids discovered as a novel and powerful hit class for low molecular weight phosphatase inhibitors. The compound class was identified from a fragment-based FMP library that contains 20000 low-molecular-weight compounds.

Table 3. Inhibition of MptpA und PTP1B by (2,5-dimethylpyrrol-1-yl)-benzoic acid inhibitors as identified by screening the fragment-based FMP library.

Compound	MptpA	PTP1B
K_i [μM][a]	K_i [μM][a]	
18	8.0 (±1.3)	3.0 (±0.4)
19	11 (±4.6)	3.6 (±1.2)
20	11 (±6.0)	4.0 (±2.0)
21	18 (±5)	1.9 (±0.7)
22	34 (±13)	5.0 (±2.0)
23	28 (±4.0)	92 (±26)
24	1.9 (±0.2)	1.1 ((±0.2)
25	25 (±8.0)	11 (±2.1)
26	1.6 (±0.4)	3.0 (±1.0)

[a] K_i values were determined by measuring the hydrolysis of pNPP at 405 nm. Primary hits with IC_{50} values below 50 μM were validated at five different substrate concentrations (1.25, 2.5, 5, 7.5, and 10 mmol). At each substrate concentration the absorption was monitored for seven different inhibitor concentrations between 250 nM and 50 μM over 1000 s. K_i values were determined from the resulting Lineweaver–Burke plots. Inhibition was competitive in all cases.

In conclusion, we report the first inhibitors of the *M. tuberculosis* phosphatase, MptpA, and have established the chemistry for the synthesis of these compound classes. Further application of medicinal chemistry methodologies should allow for the development of more potent inhibitors for subsequent biological investigations in iterative cycles.

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