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Enzyme Inhibitors

Discovery of a New Class of Inhibitors of *Mycobacterium tuberculosis* Protein Tyrosine Phosphatase B by Biology-Oriented Synthesis**

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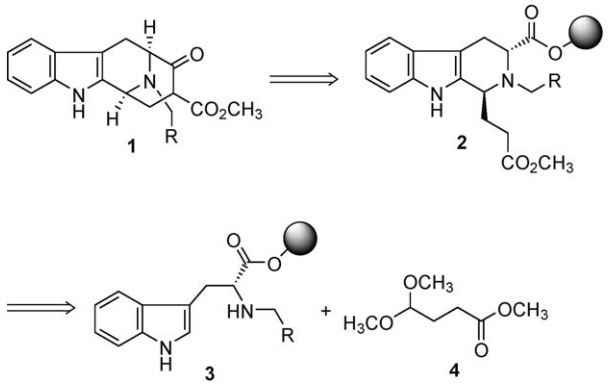
The causative organism of pulmonary tuberculosis, *Mycobacterium tuberculosis*, secretes two eukaryote-like protein tyrosine phosphatases, MptpA and MptpB, which selectively dephosphorylate human host proteins involved in interferon-γ signaling pathways, thereby preventing the initiation of host defense mechanisms.[1] The inhibition of these enzymes might prevent the proliferation of *Mycobacterium tuberculosis* in human host macrophages and therefore represents a promising strategy for the development of selective antibiotics against this severe pathogen.[2] As *Mycobacterium tuberculosis* is a major human pathogen, and as antibiotic-resistant strains spread rapidly, new chemotherapeutic approaches are urgently required.

In the de novo development of structurally new enzyme inhibitors, the relevance of compound classes to nature is of particular importance. Natural products can be regarded as biologically prevalidated starting points for the generation of compound collections, as their underlying scaffolds were selected evolutionarily as suitable core structures for protein binding.[3,4] We recently introduced a treelike structural classification of natural products (SCONP)[5] as a scaffold-generating principle for library synthesis. Furthermore, we established the concept of biology-oriented synthesis (BIOS), which places biological relevance and prevalidation at the focus of synthetic efforts. For example, core structures of natural products are used as scaffolds for compound collections.[6] Thus, BIOS creates focused diversity around a biologically validated starting point in vast chemical-structure space and aims to extend this diversity locally.[7]

The combination of these two concepts has directed our attention to scaffolds derived from indole alkaloids as promising candidates for the development of MptpB inhibitors.[6] We have now applied BIOS to macrolines, a family of more than 100 indole alkaloids with diverse biological activities and a common tetracyclic cycloocta[*b*]indole framework. However, as no comparative analysis of the biological activity of compounds with the tetracyclic cycloocta[*b*]indole motif has yet been reported, this scaffold is a proper test case for exploiting the potential of BIOS.

β-Ketoesters of cycloocta[*b*]indoles **1** were chosen as the target structures for library design because they are available from *trans*-configured diesters **2** by a one-pot regioselective epimerization and Dieckmann cyclization.[8] The intermediates **2** should be accessible by an asymmetric Pictet–Spengler reaction of resin-bound secondary amines, which may be obtained by reductive amination (Scheme 1). This challenging synthetic plan thus includes two asymmetric transformations during library generation.[9]

As the enantiospecific Pictet–Spengler cyclization requires strongly acidic reaction conditions, the base-labile but acid-stable hydroxymethylbenzoic acid (HMBA) linker was chosen for solid-phase synthesis. With this linker, cleavage from the solid support and the Dieckmann cyclization could be carried out in a very practical one-pot reaction.



Scheme 1. Retrosynthetic analysis of the cycloocta[*b*]indole target structure.

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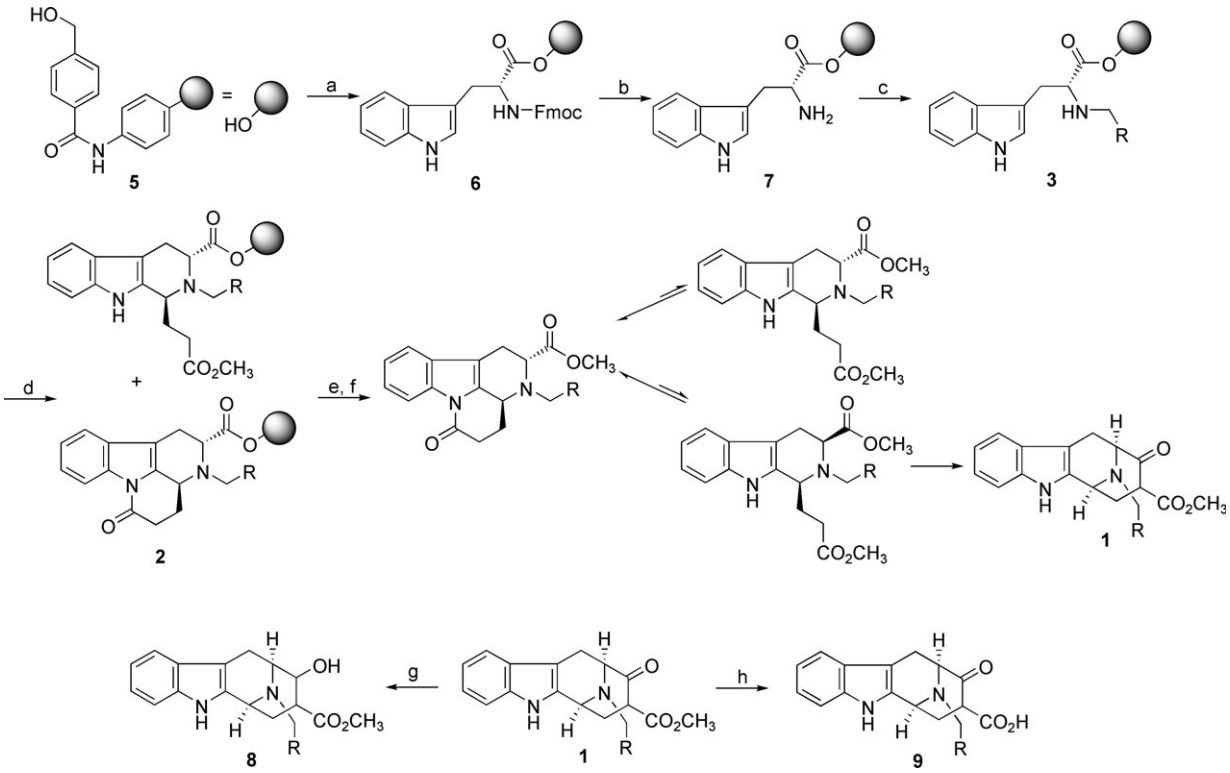
The synthesis of cycloocta[*b*]indoles **1** is exemplified in Scheme 2 for derivatives based on D-tryptophan. The HMBA resin **5** loaded with D- or L-Fmoc-tryptophan was deprotected and subjected to a two-step reductive amination to yield secondary amines **3**. A Pictet–Spengler reaction under acidic conditions with methyl-4,4-dimethoxybutyrate (**4**) resulted in the formation of the desired 1,3-t*rans* isomers **2** either as β-carbolines or as the corresponding δ-lactams. Release from the solid support, regioselective epimerization via a lactam intermediate, and subsequent Dieckmann cyclization upon the addition of sodium methoxide led to the desired *cis* β-ketoesters **1**. The analogous reactions in solution were studied in detail with a benzyl derivative as a model compound by Yu et al., who proposed a spiroindolenine intermediate and an intramolecular rearrangement to explain the observed stereoselectivity in the Pictet–Spengler reaction.[8b] Therefore, we verified the absolute configuration of the final products on the basis of that of compound **1a** (Table 1) by comparison of its specific rotation with literature values ($[\alpha]_D^{22} = -177°$ ($c = 1\ g\,dL^{-1}$, $CHCl_3$); lit.:[8b] $[\alpha]_D^{22} = -177.4°$) and NMR spectroscopic characterization.[8]

The six-step synthetic sequence led to the desired β-ketoesters **1** in 12–75% overall yield after HPLC purification (for selected examples, see Table 1 and the Supporting Information). The use of D- and L-tryptophan in combination with differently substituted aromatic, heteroaromatic, and allylic aldehydes gave rise to more than 100 isomerically pure tetracyclic alkaloid analogues in greater than 90% purity (as determined by HPLC). Moreover, the library could be enlarged further by the solution-phase reduction of **1** to β-hydroxyesters **8**. Additionally, we converted several esters **1** into β-ketoacids **9** to obtain more-water-soluble derivatives (for selected examples, see Table 1). Importantly, these β-ketoacids were sufficiently stable for use in enzyme screening and NMR spectroscopic experiments.

The synthesized cycloocta[*b*]indoles were investigated for their inhibitory potential in enzymatic-activity assays with the tyrosine phosphatases MptpA, MptpB, VE-PTP, PTP1B, and TC-PTPN2, as well as the dual-specificity phosphatase Cdc25A (Table 2 and Table in the Supporting Information). Compounds with an IC_{50} value of less than 10 μM were considered as hits. The screen yielded potent inhibitors of the tyrosine phosphatase MptpB with IC_{50} values in the low micromolar range (Table 2). Compounds **1i** and **1k** inhibited MptpB selectively with IC_{50} values of 7.04 ± 0.99 and 9.64 ± 0.93 μM, respectively, without inhibiting other phosphatases up to a concentration of 100 μM (Table 2, entries 4 and 6). Compound **1j** was almost as selective and inhibited MptpB with an IC_{50} value of 8.26 ± 4.57 μM (Table 2, entry 5). The macroline derivative **1f** was found to be the most potent MptpB inhibitor of the library, with an IC_{50} value of 4.71 ± 1.14 μM and an at least 15-fold selectivity for MptpB versus



Scheme 2. Solid-phase synthesis of cycloocta[*b*]indoles: a) 2,6-dichlorobenzoyl chloride (8 equiv), pyridine (12 equiv), L- or D-Fmoc-tryptophan (4 equiv); b) 20% piperidine in DMF, room temperature, 2×10 min; c) 1. RCHO (5 equiv), $HC(OMe)_3/CH_2Cl_2$ (1:1), room temperature, 12 h; 2. $Na(CN)BH_3$ (5 equiv), 20% HOAc in THF, 0°C, overnight; d) 1. 15% TFA in CH_2Cl_2, room temperature, 2×5 min; 2. methyl-4,4-dimethoxybutanoate (**4**, 10 equiv), 15% TFA in CH_2Cl_2, room temperature, 3 days; e) 1. 15% Et_3N in CH_2Cl_2, room temperature, 2×5 min; 2. 1 M NaOMe in MeOH/dioxane (1:1), 50°C, overnight; f) NaOMe (35 equiv), MeOH/toluene (1:9), reflux, 3 days, 12–75%; g) $NaBH_4$ (2 equiv) in EtOH, −60°C→RT, overnight, 71–92%; h) 15% TFA in H_2O/acetonitrile (1:1), room temperature, overnight, 75–81%. DMF = *N*,*N*-dimethylformamide, Fmoc = 9-fluorenylmethoxycarbonyl, TFA = trifluoroacetic acid.

Table 1: Synthesis of selected cycloocta[*b*]indoles.

Entry	Compound	Yield[a] [%]
1	**1a**	56
2	**1b**	61
3	**1c**	54
4	**1d**	55
5	**1e**	55
6	**8a**	23
7	**9a**	34
8	**9b**	37

[a] Yield of the cycloocta[*b*]indole after solid-phase synthesis and subsequent solution-phase reactions. Note: The formula numbers differ from the numbers used in the Supporting Information.

PTP1B and VE-PTP. MptpA, TC-PTPN2, and Cdc25A were not inhibited by **1f** up to a concentration of 100 μM (Table 2, entry 1). Remarkably, the compound collection also contained in **1m** and **1h** two inhibitors of PTP1B, with IC_{50} values of 8.90 ± 0.80 and 11.9 ± 1.9 μM, respectively (Table 2, entries 8 and 3). These compounds are novel examples of the pharmaceutically important class of non-acidic PTP1B inhibitors.[10] Furthermore, the inhibition profiles of all macroline derivatives clearly indicate that the *S* configuration at positions C6 and C10 is crucial for inhibitory activity, as the *R* enantiomers were entirely inactive. Moreover, the β-ketoester moiety is essential for MptpB inhibition, as reduction of the β-keto group abolished biochemical activity completely. The influence of the *N*-benzyl moiety on MptpB inhibition is more difficult to describe. However, it seems that an aromatic moiety that contains an oxygen atom either within the aromatic ring system or as a phenol substituent is favored (Table 2, entries 1–4 and 6). Alternatively, benzyl moieties that are *meta*,*para*-disubstituted with electron-withdrawing groups contribute to potent inhibition (Table 2, entries 5 and 7).

To gain deeper insight into the mode of MptpB inhibition by these compounds, **1i** was subjected to Lineweaver–Burk analysis. The kinetic data suggest a mixed-type inhibition mode involving competitive and noncompetitive binding (Figure 1). The K_I value of 1.34 ± 0.32 μM calculated for **1i** on the basis of this inhibition mode proves the strong inhibitory potency of this substance class.

The binding mode of macroline derivatives to MptpB was characterized by NMR spectroscopy. For this purpose, saturation transfer difference (STD) NMR experiments[11] were performed with the more-water-soluble β-keto acids. For example, we studied the interaction of MptpB with **9b**, which also proved to be a potent MptpB inhibitor ($IC_{50} = 6.91 \pm 2.39$ μM; Figure 2a). As the STD screening of compounds often identifies numerous hits, we carried out subsequent ^{15}N TROSY NMR experiments (TROSY = transverse relaxation-optimized spectroscopy), which enable the identification of ligand binding through chemical-shift perturbations (CSP) of an ^{15}N-labeled protein backbone. These experiments revealed small CSPs indicative of binding upon the addition of **9b** (Figure 2b).

The substrate analogue Fmoc-phosphonomethylphenylalanine (Fmoc-Pmp-OH) was used to map the binding site of the inhibitor. Comparison of the CSPs induced by the substrate analogue in the high-resolution ^{15}N TROSY spectrum of the phosphatase with the CSPs observed upon the addition of **9b** (Figure 2b) shows clearly that the two compounds bind simultaneously to two different sites of MptpB. An additional competition experiment between Fmoc-Pmp-OH and **9b** (Figure 2c) confirmed this unexpected result. To gain further insight into the binding mode, the interaction of 9b with the Phe(161, 222) residues located at the active site of the phosphatase was investigated. Therefore, MptpB was selectively labelled with [^{15}N]phenylalanine (Phe). Interestingly, the addition of **9b** induced only weak CSPs, which indicates that neither a

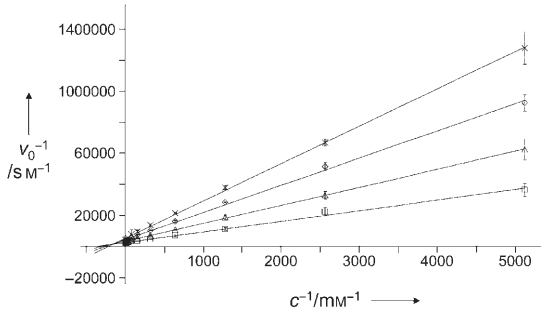
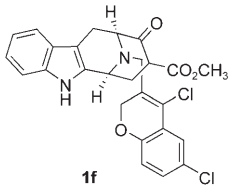
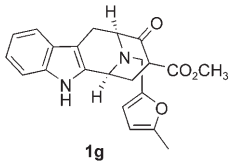
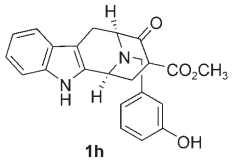
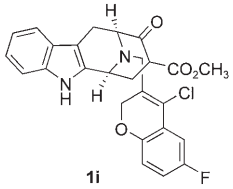
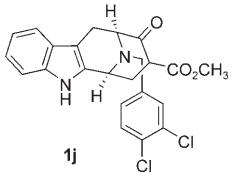
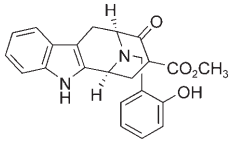
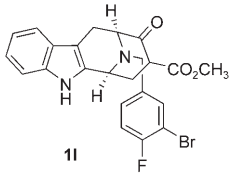
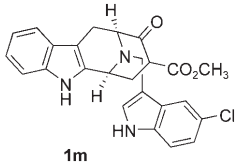


Figure 1. Lineweaver–Burk plot of **1i** at four concentrations: □ no inhibitor, △ 16.2 μM, ◇ 32.4 μM, × 64.8 μM.

Table 2: MptpB inhibitors identified by biochemical screening.[a]

Entry	Compound	IC_{50} [μM]					
		MptpA	MptpB	PTP1B	TC-PTPN2	VE-PTP	Cdc25A
1	 **1f**	n.a.	4.71 ± 1.14	84.2 ± 21.0	n.a.	73.2 ± 6.0	n.a.
2	 **1g**	n.a.	5.95 ± 1.43	34.2 ± 3.1	n.a.	68.3 ± 4.6	n.a.
3	 **1h**	n.a.	6.10 ± 1.17	11.9 ± 1.9	9.88 ± 2.14	31.8 ± 2.4	85.4 ± 3.1
4	 **1i**	n.a.	7.04 ± 0.99	n.a.	n.a.	n.a.	n.a.
5	 **1j**	n.a.	8.26 ± 4.57	n.a.	n.a.	n.a.	97.2 ± 7.1
6	 **1k**	n.a.	9.64 ± 0.93	n.a.	n.a.	n.a.	n.a.
7	 **1l**	n.a.	10.7 ± 7.2	63.2 ± 28.3	n.a.	32.0 ± 4.2	n.a.
8	 **1m**	n.a.	11.6 ± 7.4	8.90 ± 0.80	11.3 ± 2.3	30.2 ± 4.6	n.a.

[a] The dephosphorylation of *p*-nitrophenyl phosphate (pNPP) was measured by its absorption change at 405 nm. The inhibitor concentration (μM) at which the enzyme activity is reduced to 50% (IC_{50}) is given. The values were determined from at least three independent experiments; n.a.: not active (no inhibition at an inhibitor concentration of 100 μM).

protein backbone reorientation, which is usually observed during active-site binding, nor a significant change in the protein dynamics occurred (Figure 2d). This finding illustrates again the unusual binding mode of this compound class.

In conclusion, a stereoselective solid-phase synthesis of macroline analogues yielded 120 natural product analogues. Enzymatic-inhibition studies and extensive NMR spectroscopic studies revealed that these macrolines form a new class of potent and selective MptpB inhibitors with a different binding mode from that of the substrate. Consequently, the cycloocta[*b*]indole framework may be a promising scaffold

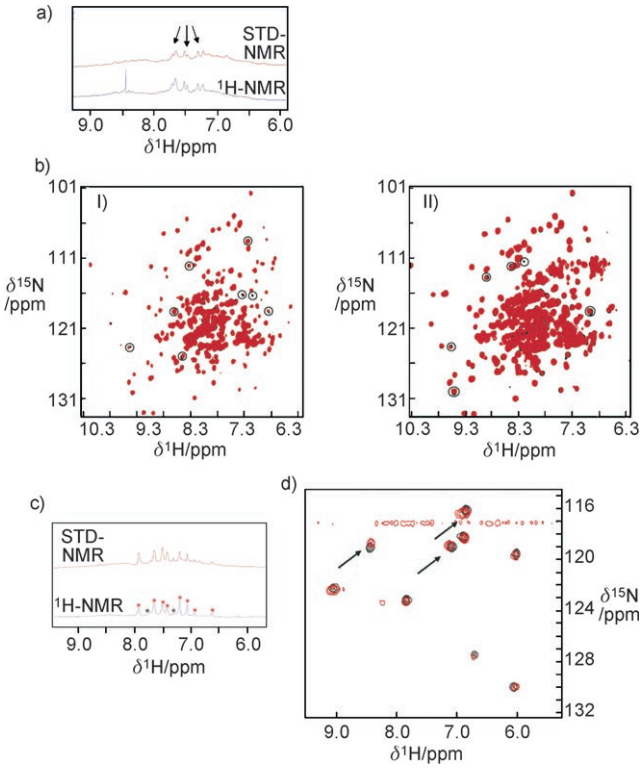


Figure 2. a) STD NMR and ^{1}H NMR spectra of **9b** in the presence of MptpB; arrows indicate characteristic signals: MptpB (0.2 mM; blue) + **9b** (1 mM; red), Na_2HPO_4/NaH_2PO_4 (50 mM), NaCl (150 mM), DTT (10 mM), EDTA (2 mM), 10% DMSO, pH 6.0; b) ^{15}N TROSY spectra of MptpB, and effect of I) **9b** and II) Fmoc-Pmp-OH on MptpB; circles indicate shifted signals. MptpB (0.2 mM; black) + I) **9b** (1 mM; red) and II) Fmoc-Pmp-OH (1 mM; red), Na_2HPO_4/NaH_2PO_4 (50 mM), NaCl (150 mM), DTT (10 mM), EDTA (2 mM), 10% DMSO, pH 6.0; c) STD NMR and ^{1}H NMR spectra from the competition experiment between Fmoc-Pmp-OH (red stars) and **9b** (black stars) in the presence of MptpB: MptpB (0.2 mM), **9b** (1 mM), Fmoc-Pmp-OH (1 mM), Na_2HPO_4/NaH_2PO_4 (50 mM), NaCl (150 mM), DTT (10 mM), EDTA (2 mM), 10% DMSO, pH 6.0; d) ^{15}N TROSY spectrum of [^{15}N]Phe-labeled MptpB, and effect of **9b**; black arrows indicate shifted phenylalanine signals: [^{15}N]Phe-labeled MptpB (0.2 mM; black) + **9b** (1 mM, red), Na_2HPO_4/NaH_2PO_4 (50 mM), NaCl (150 mM), DTT (10 mM), EDTA (2 mM), 10% DMSO, pH 6.0. DMSO = dimethyl sulfoxide, DTT = 1,4-dithiothreitol, EDTA = ethylenediaminetetraacetic acid.

for the development of novel antibiotic agents with activity against *Mycobacterium tuberculosis.*

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