Enzyme Inhibitors

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Identification of Thiazolidinones Spiro-Fused to Indolin-2-ones as Potent and Selective Inhibitors of the *Mycobacterium tuberculosis* Protein Tyrosine Phosphatase B**

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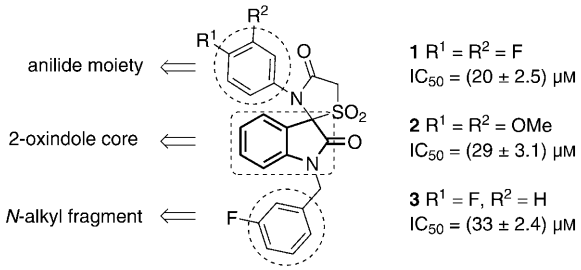
Mycobacterium tuberculosis protein tyrosine phosphatases A (MptpA) and B (MptpB) mediate pathogen survival in macrophages by the dephosphorylation of host proteins that are involved in key pathways of the immune system.[1–3] Since MptpB has no direct human orthologues, inhibitors active against MptpB offer unique opportunities for the development of new therapeutic approaches for the treatment of tuberculosis.[3–5]

As the enzyme is secreted into the host cell by growing mycobacteria, inhibitors would not have to pass the bacterial cell wall. This aspect is particularly relevant, since phosphatase inhibitors typically contain polar acidic groups that may be ionized at physiological pH values and can be expected to display poor cell permeability and low oral bioavailability. New inhibitor classes with good selectivity profiles and improved pharmacological properties are therefore in high demand. Herein we report the identification of indolin-2-on-3-spirothiazolidinones as a new class of potent and selective inhibitors of MptpB. To identify novel MptpB-inhibitor classes, we screened a library of more than 40000 compounds in a 384-well format with *p*-nitrophenyl phosphate (pNPP) as the substrate.[5a] We identified compounds **1**–**3** as primary hits with IC_{50} values in the mid-micromolar range (Scheme 1).



Scheme 1. Structures and IC_{50} values of the primary hits identified by the screening of a compound library for the inhibition of MptpB; positions suitable for structural modification are highlighted.

The identified inhibitors contain a 2-oxindole moiety, which is found in a large number of natural products with a broad spectrum of biological activity.[6] They might therefore be biologically prevalidated starting points for further compound development.[7,8] To the best of our knowledge, spirooxindoles, such as **1**–**3**, have not been reported as phosphatase inhibitors before. To develop potent, selective inhibitors and to delineate a structure–activity relationship (SAR) for the newly identified inhibitor scaffold, we modified the structure of the phenyl substituent on the thiazolidinone moiety and the *N*-alkyl substituent on the 2-indolinone moiety (Scheme 1).

The synthesis of indolin-2-on-3-spirothiazolidinones (Scheme 2) commenced with the alkylation of 1*H*-indole-2,3-diones **4** with different benzyl bromides. Condensation of the products **5** or **4** itself with various anilines resulted in the formation of isatin-3-imines, which underwent efficient cyclization in the presence of mercaptoacetic acid to spirothiazolidinones **6**. The obtained sulfides **6** were readily oxidized with *meta*-chloroperbenzoic acid (mCPBA) to give a focused library of 200 indolin-2-on-3-spirothiazolidinones **7** in greater than 95% purity (as determined by HPLC). Further structural modifications (to produce compounds **8**–**12**) were carried out as depicted in Scheme 2 (see the Supporting Information for details).

Investigation of the MptpB-inhibiting activity of this family of compounds revealed a crucial role of the dihalogenated anilide fragment (Table 1). Compounds containing two fluorine atoms or a fluorine and a chlorine atom in *meta* and *para* positions of the anilide fragment (compounds **7a**–**7k**) were preferred over analogues bearing mono- (compound **7p**) or dialkyl substituents (compound **7q**). The introduction of a nitro group in the 5-position of the 2-

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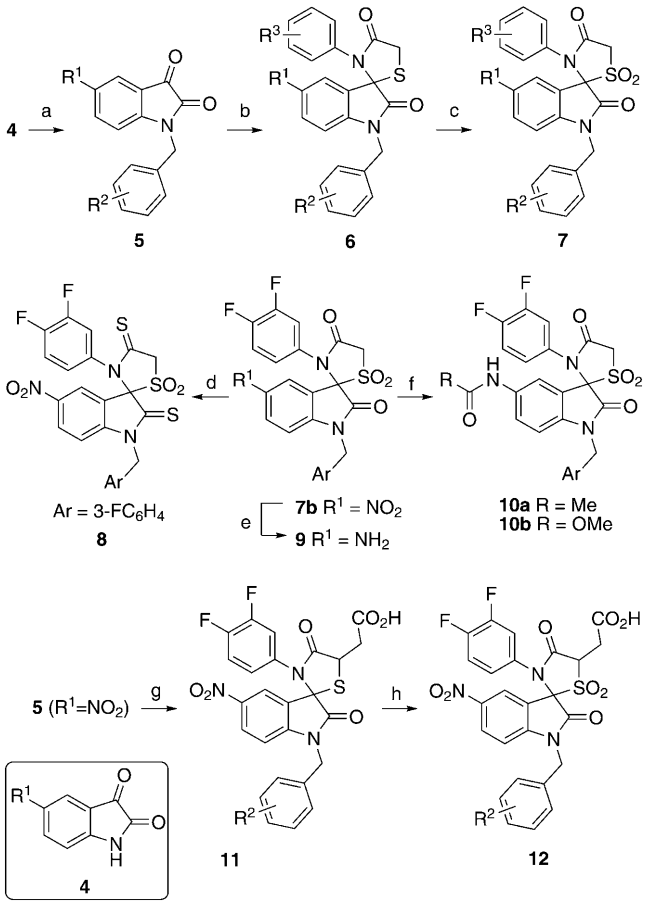

Scheme 2. Synthesis of indolin-2-on-3-spirothiazolidinones: a) NaH, DMF, 0 °C, then $ArCH_2Br$, 6 h, 80–95 %; b) 1) $Ar'NH_2$, EtOH, reflux, 6 h; 2) mercaptoacetic acid, toluene, reflux, 16 h, 45–82 %; c) mCPBA (5 equiv), $CHCl_3$, room temperature, 48 h, 78–92 %; d) Lawesson reagent, toluene, reflux, 2 h, 75 %; e) HCO_2NH_4, Pd/C, EtOH, reflux, 3 h, 79 %; f) RCOCl, pyridine, room temperature, 12 h, 89–92 %; g) 1) 3,4-difluoroaniline, EtOH, reflux, 6 h; 2) mercaptosuccinic acid, toluene, reflux, 16 h, 69–77 %; h) Oxone (5 equiv), $MeOH/H_2O$ (1:1), room temperature, 24 h, 65–74 %. DMF = *N*,*N*-dimethylformamide.

Table 1: IC_{50} values for the inhibition of MptpB by indolin-2-on-3-spirothiazolidinones.[a]

Compound	R^1	R^2	R^3	IC_{50} [μM]
1	H	3-F	3,4-di-F	20 ± 2.5
7a	NO_2	4-Br	3,4-di-F	1.1 ± 0.3
7b	NO_2	3-F	3,4-di-F	1.2 ± 0.2
7c	NO_2	4-Cl	3,4-di-F	1.8 ± 0.4
7d	NO_2	3-Cl	3,4-di-F	2.1 ± 0.6
7e	NO_2	3-Cl	3-Cl-4-F	2.7 ± 0.4
7f	NO_2	4-Cl	3-Cl-4-F	2.7 ± 0.7
7g	NO_2	4-CN	3,4-di-F	3.1 ± 0.4
7h	NO_2	$3\text{-}CF_3$	3,4-di-F	3.2 ± 0.5
7i	NO_2	3,4-di-F	3-Cl-4-F	3.3 ± 0.3
7j	NO_2	4-F	3,4-di-F	3.6 ± 0.8
7k	NO_2	$3\text{-}CF_3$	3-Cl-4-F	3.9 ± 0.8
7l	SO_2NH_2	4-F	3,4-di-F	16.4 ± 1.9
7m	CO_2Me	4-F	3,4-di-F	18.1 ± 2.1
7n	OCF_3	4-F	3,4-di-F	22.3 ± 2.4
7o	OMe	4-F	3,4-di-F	27.2 ± 2.2
7p	NO_2	4-F	4-Me	18.4 ± 2.1
7q	NO_2	4-F	3,4-di-Me	n.a.[b]
7r	NO_2	4-OH	3,4-di-F	8.2 ± 1.9
7s	NO_2	4-OMe	3,4-di-F	22.1 ± 2.1
7t	NO_2	–	3,4-di-F	n.a.[b]
8[c]	NO_2	3-F	3,4-di-F	9.6 ± 1.4
9	NH_2	3-F	3,4-di-F	31.4 ± 2.5
10a	$NHCO_2Me$	3-F	3,4-di-F	34.7 ± 2.8

[a] All IC_{50} values were determined with *p*-nitrophenyl phosphate (pNPP) as the substrate; the release of *p*-nitrophenol was observed at 405 nm. Data were derived from at least three independent measurements. [b] n.a. = not active (no inhibition up to a concentration of 100 μM). [c] Bisthioamide derivative.

oxindole core (in **7b**) significantly enhanced potency (17-fold) against MptpB over that of our primary hit **1**. Replacement of the nitro group with a sulfonamide (in **7l**), methyl ester (in **7m**), trifluoromethoxy (in **7n**), or methoxy group (in **7o**) led to loss of inhibitory activity. In the same way, analogues with amine (in **9**) or carbamate groups (in **10a**) were essentially inactive.

To investigate the role of the substituted *N*-benzyl fragment for activity against MptpB, we synthesized 13 analogues of **7b** with different substituents on the phenyl ring. Replacement of the fluorine substituent with chlorine (in **7d**) or a trifluoromethyl group (in **7h**), or relocation of the fluorine substituent to the *para* position of the phenyl ring (in **7j**) did not improve potency in comparison to that of **7b**. Replacement of the *para* fluorine substituent with a cyano group (in **7g**) was well-tolerated, whereas the introduction of a *para* hydroxy (in **7r**) or methoxy groups (in **7s**) resulted in a 6- to 18-fold decrease in activity. Furthermore, compounds that either lacked a substituent (in **7t**) or contained alkyl substituents at the 2-oxindole nitrogen atom were inactive (see the Supporting Information). The moderate activity of bisthioamide **8** highlights the importance of the carbonyl groups in **7b**. These groups may serve as hydrogen-bond acceptors when the inhibitor is bound to the target phosphatase. Physicochemical studies, including parallel artificial membrane permeability assays (PAMPA),[9] revealed that the spirothiazolidinones **7** already displayed very appreciable solubility. The additional introduction of a carboxylic acid group (see **12**, Scheme 2) led to a further significant increase in compound solubility and very promising cell permeability, whereby the high inhibitory activity was retained (Table 2).

All indolin-2-on-3-spirothiazolidinones discussed above were initially synthesized and assayed as racemates. To shed light on the relevance of the configuration of the spiro center for inhibitory activity against MptpB, we separated the 10 most potent sulfones into their pure enantiomers by preparative HPLC on a chiral phase (see the Supporting Information for details) and determined their individual IC_{50} values (Table 3). In all cases, the (−) enantiomer (F2) was 7–20 times as potent as the corresponding (+) enantiomer (F1). Notably, the most active compounds displayed IC_{50} values in the nanomolar range (Table 3).

For determination of the absolute configuration of the spiro center, various crystallization procedures were explored but failed to deliver crystals suitable for X-ray diffraction

Table 2: IC_{50} values, solubility, and cell permeability of indolin-2-on-3-spirothiazolidinones **7** and **12**.[a]

	R^2	IC_{50} [μM]	Sol. [μM][b]		R^2	IC_{50} [μM]	Sol. [μM][b]	flux [%][c]
7a	4-Br	1.1 ± 0.3	250	**12a**	4-Br	2.6 ± 0.2	376	20
7c	4-Cl	1.8 ± 0.6	225	**12b**	4-Cl	2.9 ± 0.4	412	20
7d	3-Cl	2.1 ± 0.6	240	**12c**	3-Cl	2.3 ± 0.2	425	22
7h	3-CF_3	3.2 ± 0.4	139	**12d**	3-CF_3	2.4 ± 0.3	421	21
7j	4-F	3.6 ± 0.8	205	**12e**	4-F	4.8 ± 0.6	430	13

[a] All IC_{50} values were determined from at least three independent measurements. [b] Kinetic solubility, as determined by a direct UV assay.[10] [c] Cell permeability, as determined by a parallel artificial membrane permeability assay (PAMPA). Flux [%] denotes concentration (test well)/concentration (control well) × 100.

Table 3: IC_{50} values of enantiomerically pure compounds **7a–k** for the inhibition of MptpB.[a]

	IC_{50} [μM]			IC_{50} [μM]	
	F1 (+)[b]	F2 (−)[b]		F1 (+)[b]	F2 (−)[b]
7a	2.9 ± 0.5	0.28 ± 0.06	**7g**	8.8 ± 0.6	1.3 ± 0.1
7b	3.9 ± 0.6	0.32 ± 0.05	**7h**	7.9 ± 0.4	0.38 ± 0.06
7d	9.6 ± 1.4	0.94 ± 0.12	**7i**	11 ± 1.2	1.3 ± 0.2
7e	8.9 ± 0.8	0.51 ± 0.08	**7j**	5.8 ± 1.1	0.52 ± 0.08
7f	9.5 ± 0.5	1.24 ± 0.15	**7k**	9.1 ± 0.4	1.4 ± 0.2

[a] All IC_{50} values were determined from at least three independent measurements. [b] F1 (+) and F2 (−) were assigned the *S* and *R* configuration, respectively.

experiments. We therefore decided to use circular dichroism (CD) spectroscopy in combination with time-dependent density functional theory (TDDFT) calculations to assign absolute configuration.[11] The combination of experimental CD investigations with quantum-chemical CD calculations has proven to be an efficient and reliable method for the assignment of the absolute configuration of various chiral organic molecules.[12] The CD spectra of both enantiomers of **7e** were recorded in acetonitrile and compared with spectra obtained from quantum-chemical calculations at the TD-B2PLYP and TD-PBE0 levels for a model compound in which two of the phenyl rings were replaced with methyl groups to reduce conformational complexity and computing time (see the Supporting Information for details).[13] The almost quantitative agreement between the experimental and simulated spectra revealed that (−)-**7e** (F2) has the *R* configuration (Figure 1).

To characterize the inhibition mode of the spirothiazolidinones, we performed detailed kinetic analysis, including Lineweaver–Burk analysis (see the Supporting Information for details). The indolin-2-on-3-spirothiazolidinones (*R*)-(−)-**7a**, (*R*)-(−)-**7b**, (*R*)-(−)-**7h**, and (*R*)-(−)-**7j** were thus found to be reversible substrate-competitive inhibitors of MptpB with K_i values of (260 ± 30), (250 ± 10), (750 ± 180), and (200 ± 50) nM, respectively.

Finally, we addressed the selectivity of inhibition for different phosphatases. To this end, we performed phosphatase profiling for the 15 most potent inhibitors against a panel

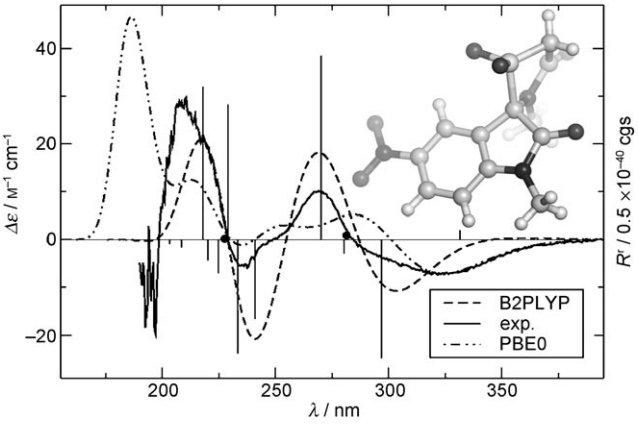

Figure 1. Comparison of the theoretical CD spectra of a model compound (COSMO-PBE0/TZVP′ structure shown) with the CD spectrum of (−)-**7e**. The theoretical spectra were obtained with B2PLYP and PBE0 by using the def2-TZVPP basis set. Bars indicate the positions and rotational strengths of electronic transitions calculated with B2PLYP. Dots mark transitions with small rotational strengths.

of six different phosphatases, including the mycobacterial phosphatase MptpA and the mammalian phosphatases PTP1B, SHP-2, PTPN2, h-PTPβ, and VHR (see the Supporting Information for details), which are involved in a variety of important biological processes in mammalian cells and the establishment of different diseases.[4] All tested compounds showed excellent selectivity in favor of MptpB; the other phosphatases were not inhibited significantly by the compounds at a concentration of 50 μM.

In conclusion, indolin-2-on-3-spirothiazolidinones were identified as a novel class of potent and selective substrate-competitive inhibitors of MptpB and could serve as promising starting points for the development of antibiotics directed against *Mycobacterium tuberculosis*.

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