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Chemical synthesis, biological evaluation and structure–activity relationship analysis of azaisoindolinones, a novel class of direct enoyl-ACP reductase inhibitors as potential antimycobacterial agents

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ABSTRACT

The synthesis and biological evaluation of azaisoindolinone compounds embedding a lipophilic chain on the framework were performed. These compounds were designed as InhA inhibitors and as anti-*Mycobacterium tuberculosis* agents. Structure–activity relationships concerning the length and the location of the lipophilic chain around the azaisoindolinone framework, the suppression of the phenyl group, the bioisosteric substitution of ether link and alkylating of the tertiary hydroxyl and the hemiamidal nitrogen were also investigated, revealing insightful information and thereby enabling further diversification of the azaisoindolinone scaffold for new antitubercular agents.

1. Introduction

Tuberculosis is one of the world's most important infectious diseases causing over one million deaths annually. The rapid development of strains resistant to existing antitubercular compounds, mainly isoniazid and rifampicine, underscores the urgent need to identify new lead molecules with novel properties and mechanisms of action.

Isoniazid (INH), a frontline antitubercular agent, is a pro-drug that requires activation by the mycobacterial catalase peroxidase enzyme (KatG) to form the active metabolite (INH-NAD adduct, Fig. 1), which exerts its lethal effect on intracellular target.[1–4] The INH-NAD adduct inhibits the mycolic acid biosynthesis in *Mycobacterium tuberculosis* by affecting InhA, an enoyl-ACP reductase enzyme of the type II fatty acid synthesis (FAS-II) system.[5] This enzyme is recognized and validated as an important drug target in *M. tuberculosis* since its homolog in human is absent. It is well established nowadays that resistance to INH largely results from mutations in *M. tuberculosis* KatG that diminish its ability to convert INH into its active form.[6–9] Thus, the development of direct inhibitors of InhA is an attractive strategy.

Our previous work based on the development of INH-NAD truncated analogs as new antitubercular agents inspired from the active metabolite of INH led to derivatives exhibiting InhA inhibitory activity (Fig. 1).[10–12] These compounds embedding a lipophilic fragment upon the phenyl ring (second generation) were planned as direct bisubstrate inhibitors of InhA enzyme. We hypothesized that the presence of this lipophilic chain would show interest in establishing new interactions in the deep hydrophobic substrate cavity and increasing thereby the affinity of the inhibitor for the InhA target. Indeed, the presence of the alkoxy lipophilic chain in compound **2a** proved necessary for the inhibitory activity towards InhA since its absence (compound **1**) abolished the activity (Fig. 1).

Among the previously synthesized INH-NAD truncated adducts (Fig. 1), the dihydropyridine (DHP)-based analogs **4**, despite of their inhibitory activity towards InhA, were too unstable (oxidation of DHP ring and loss of the *N*-substituent (**2**)) for further development. In the pyridinium series, the presence of the positive charge may represent an obstacle to the membrane crossing, discarding them to be validated as hit molecules. In contrast, the stable azaisoindolinone compound **2a** (chain OC_{12}) showed, in addition to its InhA inhibitory activity, a very interesting antimicrobial qualitative profile, as compared to isoniazid. As isoniazid, it was able to inhibit *M. smegmatis* and *M. tuberculosis* growth while it did not inhibit *Corynebacterium glutamicum* growth (a strain which does not have InhA homologs) and *Escherichia coli* growth (a strain which has an homolog of the enoyl-ACP reductase InhA called FabI).[13] All together these results suggested that in the case of **2a** one of the target might be InhA. Thus, encouraged by these results, we selected the azaisoindolinone **2a** as a hit compound for further studies on targeting InhA.

In this Letter, we described the design, the synthesis of the modified azaisoindolinones, their evaluation towards InhA potency

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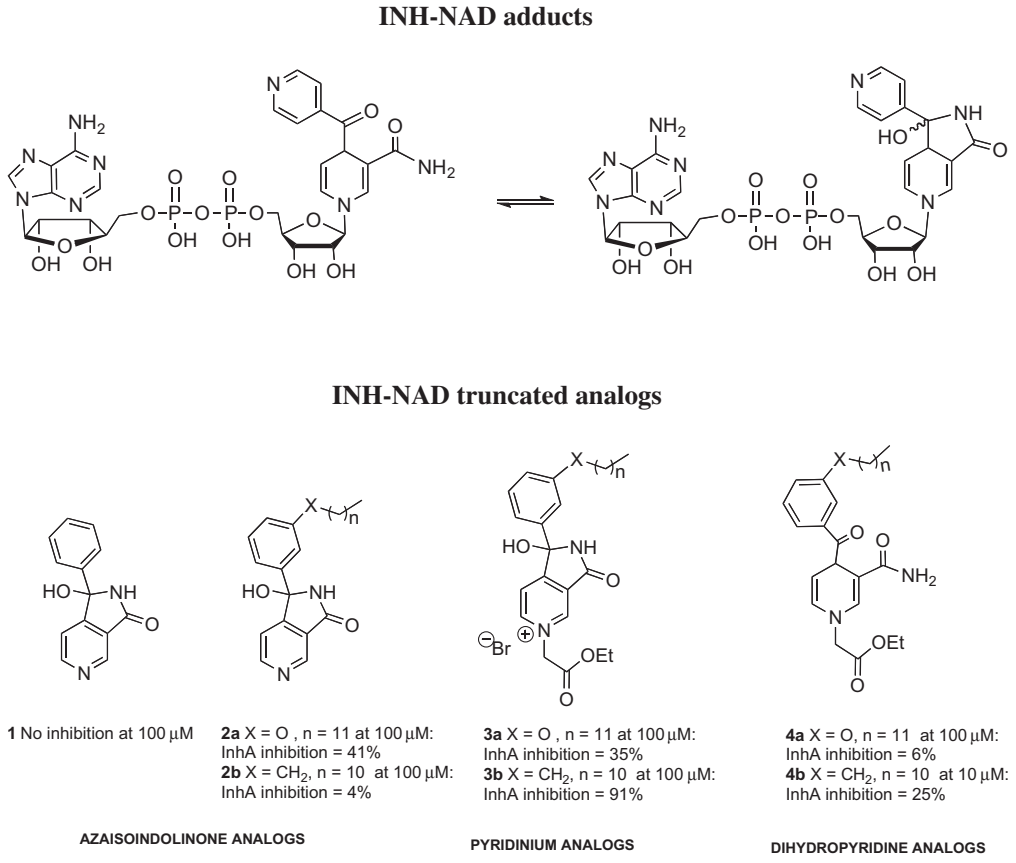


Figure 1. Structures of INH-NAD adducts and truncated analogs.[10]

and *M. tuberculosis* growth and the structure–activity relationship (SAR) analysis. This work aims at optimizing the potentiating effect of the alkoxy lipophilic chain, attached to the azaisoindolinone scaffold, in order to precise the pharmacophore elements necessary to interact with InhA and further develop new and more potent antituberculosis agents.

2. Results and discussion

2.1. Chemistry

The target compounds synthesized in this study were designed for a SAR study. The modifications concerning the length and the location of the lipophilic chain around the azaisoindolinone framework, the suppression of the phenyl group, the bioisosteric substitutions of the ether link and alkylation of the tertiary hydroxyl C-7OH, (R^1) and the hemiamidal N-8H (R^2) were performed (Fig. 2). We were also interested in investigating further structural influence of each enantiomer over InhA activity. To extract pertinent information from the SAR exploration, only one parameter was changed each time.

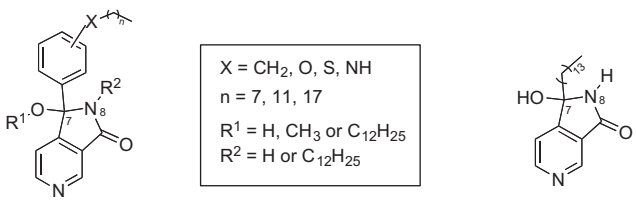


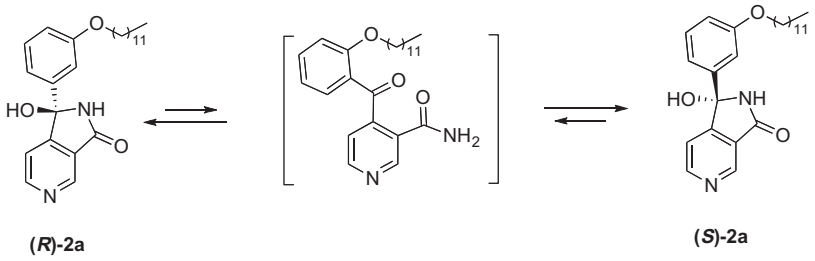
Figure 2. Modifications effected around azaisoindolinone framework. The numbering does not follow the IUPAC nomenclature.

2.1.1. Enantiomers separation by chiral supercritical fluid chromatography (SFC)

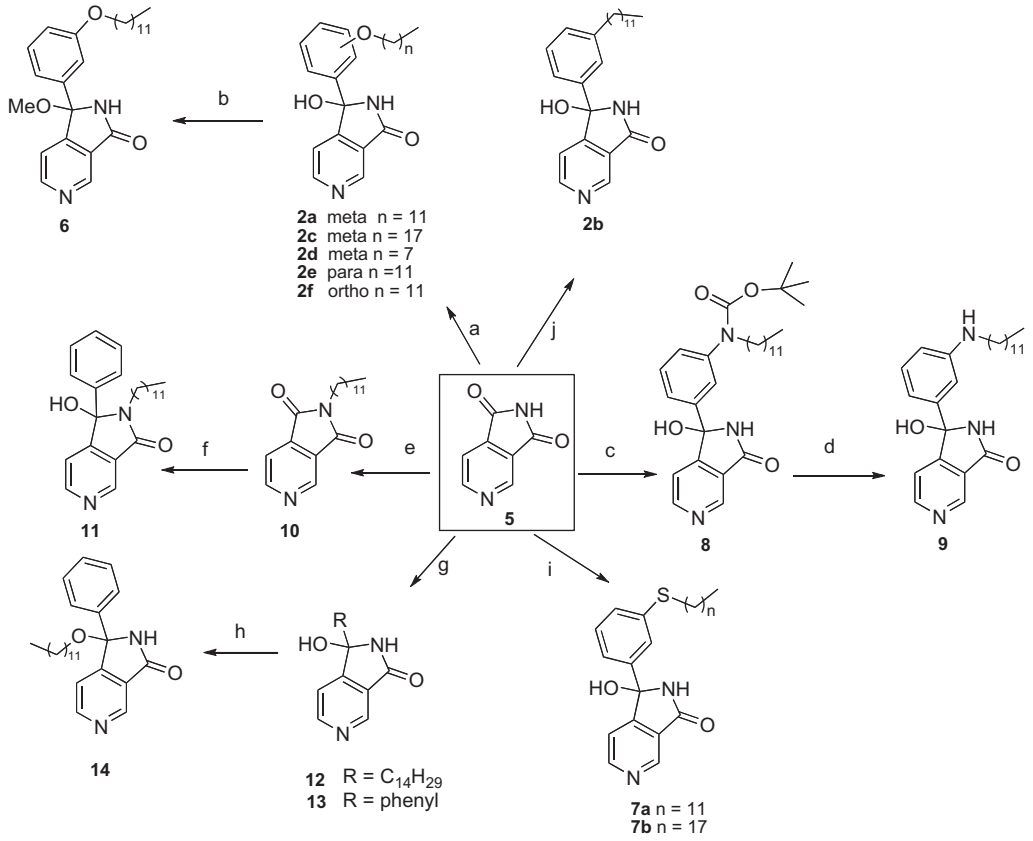
The racemic sample of **2a** was submitted to a chiral chromatography analysis in order to separate the two enantiomers. A solution of (*RS*)-**2a** in methanol was injected in a SFC chain and each enantiomer (R_t = 6.36 and 8.98 min) was collected individually and re-injected after 20 h for control. The re-injection of each isomer showed the apparition of a second peak in a 1:1 ratio (see Supplementary data), which corresponded to the opposite enantiomer. These analytical results clearly indicate that a racemisation step occurs in solution probably through an open intermediate structure (keto-amide, Scheme 1) that cannot be detected by ^{1}H and ^{13}C NMR analysis. Thereby, in this work all compounds were analyzed as a racemate mixture.

2.1.2. Synthesis

The chemical synthetic routes of target compounds are presented in Scheme 2. The new compounds studied in this work contain lipophilic chains with different lengths, positions and linker atoms (**2c**, **2d**, **2e**, **2f**, **7a** and **7b**). They have been synthesized according to our previously described procedure using the commercial 3,4-pyridine-dicarboximide reagent (**5**) as starting material (Scheme 2).[10] The various substituted aryl halide compounds employed in these synthesis were obtained directly by treating corresponding halogenophenols/thiols with halogenoalkanes in the presence of base (see Supplementary data). Compounds **2a** and **2b** have already been reported by us in the literature.[10] For preparation of compound **9**, containing a NH linker, a protection/deprotection step of the amine function with BOC group was required. The alkylation of the tertiary hydroxyl (C-7OH) of **2a** and **13** was performed in the presence of $SOCl_2$ in the appropriate refluxing alcohol to provide **6** and **14**, respectively. Compound **12**



Scheme 1.



Scheme 2. Reagents and conditions: (a) $BrC_6H_4OC_{12}H_{25}$ or $BrC_6H_4OC_{18}H_{37}$ or $BrC_6H_4C_8H_{17}$, *n*-BuLi, THF, −78 °C; (b) $SOCl_2$, CH_3OH, reflux; (c) $BrC_6H_4N(C_{12}H_{25})COOC(CH_3)_3$), *n*-BuLi, −78 °C/1 h; (d) CF_3COOH, CH_2Cl_2, 0 °C; (e) $C_{12}H_{25}I$, K_2CO_3, DMF, 50 °C; (f) PhMgBr, THF, −78 °C; (g) $C_{14}H_{29}MgBr$ or PhLi, THF, −78 °C; h) $SOCl_2$, $C_{12}H_{25}OH$, reflux; (i) $BrC_6H_4SC_{12}H_{25}$ or $BrC_6H_4SC_{18}H_{37}$, *n*-BuLi, −78 °C/1 h; (j) *n*-dodecylboronic acid, $Pd(dppf)Cl_2$, Ag_2O, K_2CO_3, THF, 80 °C.

was obtained by reaction of the corresponding alkyl Grignard reagent upon **5**. Nucleophilic substitution reaction of dicarboximide **5** with iodododecane, carried out in DMF and in the presence of K_2CO_3 at 50 °C afforded **10**, which followed by a reaction with PhMgBr and a purification step gave **11**.

2.2. Biology

2.2.1. InhA activity inhibition

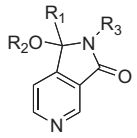
The target compounds **2a**, **2b**, **2c**, **2d**, **6**, **7a**, **7b**, **9**, **11**, **12** and **14** were initially screened in vitro for their ability to inhibit InhA activity. The InhA inhibition percentage was determined according to the method previously described[14] measuring the decrease of the absorbance at 340 nm. The molecules were tested at 100 and/or 30 μM in case of solubility problems. Triclosan was tested as positive control. The data are summarized in Table 1.

Among the various evaluated azaisoindolinones, **2c** and **7a** displayed better inhibitory potencies towards InhA than the parent molecule **2a** at 100 μM. At 30 μM, **7b** and **14** also exhibited superior InhA inhibitory activity when compared to **2a**.

2.2.2. *M.* tuberculosis growth inhibition

Compounds **2a**, **2b**, **2c**, **2d**, **6**, **7a**, **7b, 9**, **11**, **12** and **14** were evaluated in vitro with regards to bacterial growth inhibition of pathogenic mycobacterial species *M. tuberculosis.* The minimum inhibitory concentrations (MIC) of the newly and previously synthesized compounds were determined against *M. tuberculosis* by using twofold serial dilutions technique and using a colorimetric test involving 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) reduction measured at 570 nm. These compounds were compared to isoniazid and ciprofloxacine as standards antituberculosis drugs. The MICs were defined as the concentration of the compounds required to give complete inhibition of mycobacterial growth. The IC_{50} were only determined for the best compounds (with MIC inferior to 100 μM). The results are presented in Table 1. These data reported in Table 1 showed

Table 1
Effects of modifications upon the azaisoindolinone framework on enzyme-inhibitory and antibacterial activities



Entry	Compound	R1	R2	R3	InhA inhibition (%)		MIC (μM) MTB	IC_{50} (μM) MTB
					100 μM	30 μM		
1	**2a**	Ph-*m*OC_{12}	H	H	27	12	156	–
2	**2b**	Ph-*m*C_{12}	H	H	4	–	9.8	7
3	**2c**	Ph-*m*OC_{18}	H	H	67	28	25	12
4	**2d**	Ph-*m*OC_8	H	H	12	–	250	–
5	**2e**	Ph-*p*OC_{12}	H	H	16	1	>100	–
6	**2f**	Ph-*o*OC_{12}	H	H	0	0	>100	–
7	**6**	Ph-*m*OC_{12}	Me	H	0	–	25	10
8	**7a**	Ph-*m*SC_{12}	H	H	66	15	12.5	6.5
9	**7b**	Ph-*m*SC_{18}	H	H	ND*	26	>100	–
10	**9**	Ph-*m*NHC_{12}	H	H	9 InhA activation (%)	1	>100	–
11	**11**	Ph	H	C_{12}	13	0	12.5	4.5
12	**12**	C_{14}	H	H	10 InhA activation (%)	–	>100	–
13	**14**	Ph	C_{12}	H	ND*	20	25	9.5
14		Triclosan[a]				37[a]	–	43.1[b]
15		Isoniazid			–	–	0.6	–
16		Ciprofloxacin			–	–	2.5	–

* ND not determined due to solubility problems.
[a] Tested at 10 μM.
[b] Ref. 10.

that some new synthesized compounds exhibited significant antibacterial activity against *M. tuberculosis* with MIC values inferior to that of **2a**. In addition, some of them were also inhibitors of the InhA activity. Among the assayed compounds, **2b**, **7a** and **11** were found to be the three most active derivatives with IC_{50} values in the range 4.5–7.0 μM.

2.3. Structure–activity relationships analysis

First, it was investigated if the aryl fragment bearing the lipophilic chain was essential for the enzymatic inhibitory activity or if the lipophilic moiety could be directly attached to the hemiamidal core. When an aliphatic chain (C_{14}) (entry 12) was connected directly on the C-7 position with suppression of the phenyl ring, no InhA inhibitory activity and no inhibition of *M. tuberculosis* growth were observed. This shows the critical requirement of the aryl ring in maintaining the inhibitory activity towards InhA and *M. tuberculosis* growth. We concluded that the aryl group, as a spacer between hemiamidal cycle and the lipophilic chain, is fundamental for the observed inhibitory enzymatic activity. Probably the phenyl ring imposes a more favorable rigid conformation allowing the hydrocarbon chain to insert into the hydrophobic substrate cavity of InhA.

By retaining the phenyl ring intact, we tried to ascertain the optimal effect of the chain around the azaisoindolinone scaffold. Firstly, the substitution pattern of the phenyl ring was examined. The synthesized *ortho*, *meta* and *para* derivatives exhibited different behaviors towards InhA activity. It was found that when the dodecyloxyl substituent was moved to the *para* position (**2e**), the InhA inhibitory activity decreased, when compared to the hit molecule **2a** (entries 1 and 5). Shifting the chain to the *ortho* position (**2f**) completely abolished the activity (entries 1 and 6).

When compounds **2e** and **2f** were evaluated against *M. tuberculosis* growth, no significant improvement of antibacterial activity was observed with respect to **2a**. Their antimycobacterial MIC values were superior to 100 μM (entries 1, 5 and 6).

From the enzymatic data, the best place for the lipophilic aliphatic chain is the *meta* position of the phenyl group. This result suggests that the substituent located at this position could help orienting the chain towards better interaction within the substrate site.

In the second time, the possibility of introducing the lipophilic chain on the hemiamidal region (C-7OH and N-8H) was also investigated. When the lipophilic chain (C_{12}) was attached at the hydroxyl group of the C-7 position of the hemiamidal ring, the compound could not be tested at 100 μM due to its poor solubility in the enzymatic reaction medium (entry 13). However, the enzymatic percentage of inhibition obtained for **14** (C-7OC_{12}) at 30 μM is slightly better than the hit molecule **2a** at the same concentration (entries 1 and 13). Furthermore, this analog is also a more efficient inhibitor of *M. tuberculosis* growth as compared with **2a**.

Compound **11**, holding a C_{12} chain at N-8 position of the hemiamidal cycle, showed a poor InhA inhibitory activity. However, it exhibited one of the best activities against *M. tuberculosis* strains indicating that **11** has probably an other target than InhA. Compared with the hit molecule **2a**, the inhibitory activity of **11** upon *M. tuberculosis* growth was remarkably improved by a factor 12 (entries 1 and 11).

The strategy of bioisosteric replacement is a powerful and highly productive tool in analog design. On the basis of this concept, the oxygen atom link of the chain in **2a** was changed to nitrogen (**9**), sulfur (**7a**) or suppressed (**2b**, C_{12} chain) to ascertain whether the oxygen link atom is implicated in the binding interaction with the target. One can observe that the replacement of the oxygen linker by a NH group completely abolished the activity (entries 1 and 10) whereas the replacement of the *O*-atom by *S*-atom improved the inhibitory activity towards InhA (entries 1 and 8). On the other hand, the C_{12} analog **2b** is not an efficient inhibitor of InhA.[10] These results allow us to think that the presence of an electronegative heteroatom (*O* or *S*) is essential for binding affinity.

SAR exploration was also carried out by modifying the length of the lipophilic aliphatic chain (OC_8 and OC_{18}) in order to explore the presumed hydrophobic binding cavity. Thus, compounds **2c** (OC_{18}) and **2d** (OC_8) were prepared to establish the optimal length. The rank order of InhA inhibitory potency was **2c** (OC_{18}) > **2a**

(OC_{12}) > **2d** (OC_8). Increasing the length of the alkyl chain resulted in significant improvement of the activity (entries 1, 3 and 4). For the sulfur derivative, the C_{12} chain was also replaced by a C_{18} chain. In this case, the benefic effect of a more lipophilic chain towards InhA inhibition was also significant (entries 8 and 9). However, for **7b** this positive effect was not reflected in inhibition of *M. tuberculosis* growth.

Next, methylation of the tertiary alcohol at C-7 (**2a**) dramatically decreased the InhA inhibitory activity (entries 1 and 7). On the one hand, it may suggest that the hydroxyl group is important to establish binding interactions with InhA target. On the other hand, this free OH group may play a fundamental role in hemiamidal opening process (as evidenced by us by the chiral HPLC experiments) towards a keto-amide form, which may be the key species for interaction with InhA.[15] Surprisingly, this protection resulted in an improved activity of **6** against *M. tuberculosis* growth.

2.4. Cytotoxicity evaluation on eukaryotic cells

The most efficient anti *M. tuberculosis* compounds (**2a**, **2b**, **2c**, **6**, **7a**, **11** and **14**) were submitted to in vitro cytotoxicity assay. Their cytotoxicity was determined over THP-1 cells and expressed by MIC values. The MIC values of these compounds towards THP-1 cell cytotoxicity (see Supplementary data) were higher than $MIC_{M.tuberculosis}$ values but the difference could not satisfy the drug-like properties.

3. Conclusion

In the present study, it was demonstrated, by the first time, that aryl azaisoindolinone framework undergoes epimerization at the C-7 carbon probably via a cycle opening pathway. A series of azaisoindolinones embedding a lipophilic chain was synthesized and the structure–activity relationship analysis, carried out around the parent azaisoindolinone scaffold **2a**, permitted to precise some pharmacophore elements. The best molecular interaction with the InhA enzyme was achieved by the following modifications: (i) the lipophilic chain must be attached through the phenyl ring to the azaisoindolinone scaffold, (ii) 12 or 18 carbons long chain bound to oxygen or sulfur atom to the *meta* position of the phenyl ring and (iii) a free tertiary hydroxyl at C-7 position.

The improvements in the InhA inhibitory activity brought by these structural modifications were also correlated with an increase of the inhibition of the growth of *M. tuberculosis*. Compounds **2c** and **7a** showed superior InhA inhibitory property and better activity against *M. tuberculosis* growth than the parent molecule **2a**.

In contrast, yet interesting, some structural modifications led to a loss of InhA inhibitory activity (compounds **2b**, **6** and **11**) still associated to a capacity to inhibit *M. tuberculosis* growth. The mechanism of action of these compounds does not involve InhA as a target.

Although the initial strategy of targeting the hydrophobic substrate cavity of InhA enzyme with azaindolinones bearing long hydrocarbon chain led to compounds with better affinity for InhA and better activity against *M. tuberculosis*, their cytotoxicity towards eukaryote cells precludes their development. With the contribution of molecular computational modeling we plan to modify the chemical structure in order to gain more in InhA selectivity and thereby compensate the toxicity.

4. Experimental part

4.1. General

The melting points were determined on an Electrothermal 9300 capillary melting point apparatus and are not corrected. Infra-red spectra were recorded on a Perkin-Elmer spectrometer GX 2000. ^{1}H NMR spectra were recorded on a Bruker spectrometer at 250, 300, 400 and 500 MHz using $CDCl_3$, DMSO-d_6 or MeOD-d_4 as the solvent. For ^{1}H NMR the residual proton signal of the deuterated solvent was used as an internal reference: $CDCl_3$ δ = 7.29 ppm, DMSO = 2.50 ppm and MeOD = 3.31 ppm. ^{13}C NMR spectra were recorded on a Bruker spectrometer at 63, 75, 100 and 126 MHz. Mass spectra (EI) were obtained on an MS-Nermag R10-10 spectrometer. For the MS–ESI a Perkin-Elmer SCIEX API 365 spectrometer was used and for the MS–CI a Thermo electron TQS 7000 was employed. Column chromatography was performed on silica gel (Merck 0.063–0.200 mm). The purity of compounds was assessed by microanalysis and/or by thin layer chromatography coupled to ^{13}C NMR. All reagents were obtained from commercial suppliers and were used without further purification. Anhydrous solvents were freshly distilled before use. THF and ether were dried with sodium in the presence of benzophenone. Dimethylformamide was dried on calcium hydride.

The syntheses of intermediates are described in the Supplementary data. Compounds **2a** and **2b** are described in the literature.[10] Compounds **2c**, **2d**, **2e**, **2f**, **7a**, **7b** and **8** were synthesized according to the representative protocol described below for **2c** and the spectral characterization is described below.

Compound **11** was prepared as previously described[16] using **10** as starting material. Compounds **12** and **13** were synthesized adapting this same protocol.

The protocols for the preparation of the new compounds **6**, **9**, **10** and **14** are described below.

4.1.1. Chiral chromatography

Supercritical fluid chromatography (SFC) was performed with an OD–H 10 mm × 250 mm column eluted with 15% MeOH at 12 mL/min (P 120 bar, oven temperature = 40 °C) for purification. An OD–H 4.6 mm × 250 mm column eluted with 15% MeOH at 4 mL/min (P 100 bar, oven temperature = 35 °C) was used for analytical chromatography.

4.2. (*RS*)-1-hydroxy-1-[3-(octadecyloxy)-phenyl]-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridin-3-one (2c)

To a solution of the suitable bromoether (1.00 g; 0.6 mmol) in dry tetrahydrofurane (18 mL), under argon atmosphere and at −40 °C was added *n*-butyllithium 2.5 M (1.7 mL; 2.0 mmol). After addition, the temperature was maintained at −50 °C for 30 min. Then the temperature was decreased at −78 °C and this solution was added upon a solution of 3,4-pyridinedicarboximide (175 mg, 1.2 mmol) in dry tetrahydrofurane (15 mL). The reaction mixture was stirred for 1 h at −50 °C. Then the reaction was quenched with a saturated ammonium chloride solution (30 mL) and the mixture was extracted with ethyl acetate (3 × 20 mL). The organic phases were combined, dried over sodium sulfate and concentrated under vaccum. The residue (860 mg) was purified by column chromatography (SiO_2) (eluent: dichloromethane/methanol: 100/0 to 93/7) to furnish **2c** as a mixture of *para*/*meta* compounds. The *para* regioisomer was isolated by a recrystallization in acetone/methanol (3 days).

Yield 32% (187 mg – mixture of *para*/*meta* 2:1 regioisomers). White solid, mp 113 °C. TLC R_f: 0.35 (dichloromethane/methanol 95:5, v/v). IR (cm^{-1}): 3141, 3059, 2920, 2851, 1708, 1607, 1578, 1286, 1258, 1142, 1096, 1082, 1045, 1030, 841, 700. ^{1}H NMR (500 MHz, MeOD) δ (ppm): 8.93 (s, 1H); 8.73 (d, *J* = 5.4 Hz, 1H); 7.46 (dd, *J* = 1.0 and 5.1 Hz, 1H); 7.27 (t, *J* = 8.0 Hz, 1H); 7.17 (m, 1H); 7.07 (dd, *J* = 1.0 and 8.8 Hz, 1H); 6.91 (dd, *J* = 8.3 Hz and *J* no mesurable, 1H); 4.00–3.97 (m, 2H); 1.81–1.75 (m, 2H); 1.48–1.31 (m, 30H); 0.92 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ (ppm): 168.5 (C); 159.6 (C); 159.4 (C); 152.6 (CH); 144.5 (CH);

141.1 (C); 129.4 (CH); 126.4 (C); 117.9 (CH); 117.4 (CH); 114.5 (CH); 111.8 (CH); 67.9 (CH_2); 31.6 (CH_2); 29.3–29.2 (10 × CH_2); 28.98 (CH_2); 28.94 (CH_2); 28.91 (CH_2); 25.7 (CH_2); 22.2 (CH_2); 12.9 (CH_3), 1C missing. MS (ESI) *m/z*: 495.5 [M+H$^+$]. HRMS (ESI): for $C_{31}H_{47}N_2O_3$ calcd: 495.3587; found: 495.3618. Anal. Calcd for $C_{31}H_{46}N_2O_3 \cdot CH_3OH \cdot 0.2\ CH_3COCH_3$: C, 72.73; H, 9.59; N, 5.20. Found: C, 72.32; H, 9.53; N, 4.92.

4.3. (*RS*)-1-hydroxy-1-[3-(octyloxy)phenyl]-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridin-3-one (2d)

Yield 16% (100 mg – mixture of *para*/*meta* isomers). The *para* regioisomer was obtained after a column chromatography (dichloromethane to dichloromethane/methanol 95: 5, v/v). White solid, mp 97 °C. TLC R_f: 0.35 (dichloromethane/methanol 95:5, v/v). IR (cm^{-1}): 3350, 3060, 2918, 2849, 1714, 1613, 1465, 1340, 1207, 1067, 727. ^{1}H NMR (500 MHz, MeOD) δ (ppm): 8.93 (s, 1H); 8.73 (d, J = 5.0 Hz, 1H); 7.48 (dd, J = 0.8 Hz and 5.1 Hz, 1H); 7.29 (t, J = 8.0 Hz, 1H); 7.18 (d, J = 2.0 Hz, 1H); 7.07 (d, J = 8.0 Hz, 1H); 6.91 (dd, J = 1.8 and 7.3 Hz, 1H); 4.00–3.96 (m, 2H); 1.77 (q, J = 6.5 Hz, 2H); 1.49–1.45 (m, 2H); 1.41–1.33 (m, 8H); 0.93 (t, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ (ppm): 168.4 (C); 159.5 (C); 159.4 (C); 152.6 (CH); 144.4 (CH); 141.0 (C); 129.4 (CH); 126.4 (C); 118.0 (CH); 117.3 (CH); 114.3 (CH); 111.7 (CH); 87.8 (C); 67.7 (CH_2); 31.6 (CH_2); 29.1 (CH_2); 29.0 (CH_2); 28.9 (CH_2); 25.8 (CH_2); 22.3 (CH_2); 12.9 (CH_3). MS (ESI) *m/z*: 355.4 [M+H$^+$]. HRMS (ESI): for $C_{21}H_{27}N_2O_3$ calcd: 355.2022; found: 355.2050. Anal. Calcd for $C_{21}H_{26}N_2O_3 \cdot 0.5\ CH_3OH$: C, 69.73; H, 7.56; N, 7.56. Found: C, 69.40; H, 7.24; N, 7.41.

4.4. (*RS*)-1-[4-(dodecyloxy)phenyl]-1-hydroxy-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridin-3-one (2e)

Yield 21% (46.5 mg – mixture of *para*/*meta* 2:1 regioisomers). The *para* regioisomer was isolated by recrystallization in acetone. White solid, mp 118–119 °C. TLC R_f: 0.84 (dichloromethane/methanol 92.5:7.5, v/v). IR (cm^{-1}): 3302, 3060, 2919, 2852, 1688, 1605, 1510, 1475, 1242, 1174, 1081, 1035, 931, 826, 716. ^{1}H NMR (500 MHz, DMSO) δ (ppm): 9.42 (s, 1H,); 8.85 (s, 1H); 8.72 (d, J = 5.0 Hz, 1H); 7.39 (d, J = 10.0 Hz, 2H); 7.38 (d, J = 5.0 Hz, 1H); 7.07 (s, 1H); 6.90 (d, J = 10.0 Hz, 2H); 3.93 (t, J = 7.5 Hz, 2H); 1.70–1.67 (m, 2H); 1.40–1.37 (m, 2H); 1.27–1.20 (m, 16H); 0.86 (t, J = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ (ppm): 167.5 (C); 159.2 (C); 159.1 (C); 153.4 (CH); 145.0 (CH); 132.8 (C); 127.3 (2 × CH); 126.5 (C); 118.2 (CH); 114.7 (2 × CH); 87.6 (C); 67.9 (CH_2); 31.8 (CH_2); 29.5–29.1 (7 × CH_2); 25.9 (CH_2); 22.5 (CH_2); 14.4 (CH_3). MS (ESI) *m/z*: 433 [M+Na$^+$], 411 [M+H$^+$]. HRMS (DCI-CH_4): for $C_{25}H_{35}N_2O_3$ calcd: 411.2648; found: 411.2634.

4.5. (*RS*)-1-[2-(dodecyloxy)phenyl]-1-hydroxy-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridin-3-one (2f)

Yield 39% (195 mg – mixture of *para*/*meta* regioisomers). The *para* regioisomer was isolated by recrystallization in acetone/methanol. White solid, mp 132 °C. TLC R_f: 0.71 (dichloromethane/methanol 95:5, v/v). IR (cm^{-1}): 3199, 3061, 2920, 2851, 1708, 1619, 1470, 1284, 1243, 1073, 1045, 1022, 847, 798, 748. ^{1}H NMR (500 MHz, MeOD) δ (ppm): 8.90 (d, J = 1.1 Hz, 1H); 8.69 (d, J = 5.3 Hz, 1H); 8.07 (dd, J = 1.8 and 7.8 Hz, 1H); 7.37 (td, J = 1.8 and 7.5 Hz, 1H); 7.32 (dd, J = 1.4 and 5.1 Hz, 1H); 7.05 (dt, J = 1.0 and 7.7 Hz, 1H); 6.88 (dd, J = 0.7 and 8.3 Hz, 1H); 3.74 (dt, J = 6.3 and 9.2 Hz, 1H); 3.56 (dt, J = 6.3 and 9.1 Hz, 1H); 1.35–1.00 (m, 20H); 0.92 (t, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ (ppm): 169.1 (C); 160.5 (C); 155.9 (C); 152.1 (CH); 143.8 (CH); 130.2 (CH); 128.5 (C); 127.8 (CH); 125.8 (C); 119.8 (CH); 117.3 (CH); 111.6 (CH); 85.6 (C); 67.8 (CH_2); 31.7 (CH_2); 29.4 (3 × CH_2); 29.21 (CH_2); 29.16 (CH_2); 29.12 (CH_2); 28.7 (CH_2); 25.7 (CH_2); 22.4 (CH_2); 13.1 (CH_3). MS (ESI) *m/z*: 433.5 [M+Na$^+$]; 411.5 [M+H$^+$]; 393.4 [M+H$^+$−H_2O]. HRMS (ESI) for $C_{25}H_{35}N_2O_3$: calcd: 411.2648; found: 411.2681. Anal. Calcd for $C_{25}H_{34}N_2O_3 \cdot 0.5\ CH_3OH$: C, 71,80; H, 8.51; N, 6.57. Found: C, 71.59; H, 8.26; H, 6.64.

4.6. (*RS*)-1-[3-(dodecyloxy)phenyl)]-1-methoxy-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridin-3-one (6)

To a solution of **2a** (100 mg; 0.24 mmol) in methanol (6 mL) was added 0.5 mL of thionyl chloride. The mixture was heated at reflux under argon for 16 h. Then the reaction was treated with a saturated solution of $NaHCO_3$ (5 mL) and extracted with ethyl acetate (3 × 8 mL). The combined organic layers were dried over anhydrous sodium sulfate and, after filtration, concentrated under reduced pressure to furnish 93 mg of a yellow oil. The residue was purified by column chromatography (dichloromethane/methanol 95:5).

Yield 31% (32 mg). White solid. TLC R_f: 0.60 (dichloromethane/methanol 95:5, v/v). IR (cm^{-1}): 2922, 2852, 1726, 1603, 1438, 1287, 1051, 698. ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm): 9.12 (bd s, 1H); 8.80 (bd s, 1H); 7.31–7.25 (m, 2H); 7.13 (s, 1H); 7.09 (t, J = 7.0 Hz, 1H); 6.91–6.87 (m, 2H); 3.97 (t, J = 6.4 Hz, 2H); 3.19 (s, 3H); 1.79 (t, J = 5.5 Hz, 2H); 1.45–1.28 (m, 18H); 0.91 (t, J = 5.9 Hz, 3H); ^{13}C NMR (63 MHz, $CDCl_3$) δ (ppm): 168.1 (C); 159.6 (C); 154.9 (C); 153.2 (CH); 146.0 (CH); 139.7 (C); 130.0 (CH); 118.0 (CH); 117.5 (CH); 114.9 (CH); 112.1 (CH); 91.9 (C); 68.2 (CH_2); 50.9 (CH_3); 31.9 (CH_2); 29.7–29.3 (7 × CH_2); 26.0 (CH_2); 22.7 (CH_2); 14.1 (CH_3); 1Cq missing. MS (ESI) *m/z*: 425.6 [M+H$^+$]. HRMS (ESI) for $C_{26}H_{37}N_2O_3$: calcd: 425.2804; found: 425.2847.

4.7. (*RS*)-1-[3-(dodecylsulfanyl)phenyl]-1-hydroxy-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridin-3-one (7a)

Yield 42%, (200 mg – mixture of *para*/*meta* regioisomers). The *para* regioisomer was isolated by recrystallization in acetone.[17] White solid, mp 86 °C. TLC R_f: 0.31 (dichloromethane/methanol 95:5, v/v). IR (cm^{-1}): 3159, 2917, 2849, 1698, 1614, 1587, 1464, 1347, 1205, 1067, 964, 784, 698. ^{1}H NMR (250 MHz, MeOD) δ (ppm): 8.94 (s, 1H); 8.74 (d, J = 5.0 Hz, 1H), 7.56 (s, 1H); 7,46 (d, J = 4.6 Hz, 1H); 7.35–7.30 (m, 3H); 2.96 (t, J = 7.0 Hz, 2H); 1.65–1.56 (m, 2H); 1.53–1.25 (m, 18H); 0.94 (t, J = 6.0 Hz, 3H). ^{13}C NMR (63 MHz, MeOD) δ (ppm): 168.3 (C); 159.0 (C); 152.7 (CH); 150.0 (C); 144.5 (CH); 140.0 (C); 138.0 (C); 128.9 (CH); 128.4 (CH); 125.2 (CH); 122.5 (CH); 118.0 (CH); 87.6 (C); 70.1 (CH_2); 32.6 (CH_2); 31.7 (CH_2); 29.4–28.8 (6 × CH_2); 28.4 (CH_2); 22.2 (CH_2); 13.0 (CH_3). MS (ESI) *m/z*: 449.2 [M+Na$^+$]; 427.3 [M+H$^+$]. HRMS (ESI) for $C_{25}H_{35}N_2O_2S$: calcd: 427.2419; found: 427.2459. Anal. Calcd for $C_{25}H_{34}N_2O_2S \cdot 0.6\ CH_3COCH_3$: C, 69.75; H, 8.21; N, 6.07. Found: C, 69.43; H, 7.99; H, 6.33.

4.8. (*RS*)-1-hydroxy-1-[3-(octadecylsulfanyl)phenyl]-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridin-3-one (7b)

Yield 39%, (104 mg – mixture of *para*/*meta* regioisomers). The *para* regioisomer was isolated by column chromatography (SiO_2/eluent CH_2Cl_2/MeOH 99:1 to 96:4). White solid, mp: 92 °C. TLC R_f: 0.42 (dichloromethane/methanol 95:5, v/v). IR (cm^{-1}): 3066, 2916, 2851, 1722, 1612, 1591, 1472, 1341, 1200, 1140, 1060, 954, 885, 838, 777, 727, 715, 693. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.64 (s, 1H); 8.49 (d, J = 4.8 Hz, 1H); 8.24 (s, 1H); 7,56 (s, 1H); 7,31–7.24 (m, 4H); 2.91 (t, J = 7.2 Hz, 2H); 1.69–1.59 (m, 2H); 1.29–1.20 (m, 30H); 0.90 (t, J = 5.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 168.4 (C); 158.6 (C); 152.7 (CH); 144.9

(C); 139.3 (CH); 138.6 (C); 129.3 (C); 128.4 (CH); 125.5 (CH); 125.2 (CH); 122.5 (CH); 118.0 (CH); 88.1 (C); 33.2 (CH_2); 31.9 (CH_2); 29.7 (8 × CH_2); 29.6 (CH_2); 29.5 (CH); 29.4 (CH_2); 29.2 (CH_2); 29.0 (CH_2); 28.9 (CH_2); 22.7 (CH_2); 14.1 (CH_3). HRMS (ESI) for $C_{31}H_{47}N_2O_2S$: calcd: 511.3358; found: 511.3355.

4.9. *tert*-Butyl *N*-dodecyl-3-[(*RS*)-1-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrrolo [3,4-*c*]pyridin-1-yl)]phenylcarbamate (8)

Yield 37% (255 mg – mixture of *para*/*meta* 2:1 regioisomers). The *para* regioisomer was isolated by recrystallization in acetone. TLC R_f: 0.64 (dichloromethane/methanol 92.5:7.5, v/v). IR (cm^{-1}): 3228, 2924, 2854, 1698, 1603, 1454, 1366, 1149, 1071, 1041, 733, 702. ^1H NMR (250 MHz, MeOD) δ (ppm): 8.94 (s, 1H); 8.73 (d, J = 5.1 Hz, 1H); 7.46–7.36 (m, 4H); 7.22–7.19 (m, 1H); 3.62 (t, J = 6.9 Hz, 2H); 1.49–1.44 (m, 2H); 1.38–1.25 (m, 27 H); 0.90 (t, J = 6.2 Hz, 3H). ^{13}C NMR (75 MHz, MeOD) δ (ppm): 167.5 (C); 159.7 (C); 153.4 (C); 152.6 (CH); 144.5 (CH); 142.6 (C); 140.5 (C); 128.9 (CH); 127.0 (CH); 126.2 (C); 124.5 (CH); 123.3 (CH); 118.0 (CH); 87.0 (C); 80.3 (C); 49.6 (CH_2); 31.7 (CH_2); 29.4–29.1 (5 × CH_2); 28.9 (CH_2); 28.0 (CH_2); 27.2 (3 × CH_3); 26.3 (CH_2); 22.3 (CH_2); 13.1 (CH_3). MS (DCI/NH_3) m/z: 527.3 [M+NH_4^+]; 510.3 [M+H^+]. HRMS (DCI–CH_4): for $C_{30}H_{44}N_3O_4$ calcd: 510.3332, found: 510.3323.

4.10. (*RS*)-1-[3-(dodecylamino)phenyl]-1-hydroxy-1,2-dihydro-3*H*-pyrrolo[3,4-*c*] pyridin-3-one (9)

To a solution of **8** (32 mg, 62.7 mmol) in CH_2Cl_2 was added 0.11 mL of trifluoroacetic acid under argon and at 0 °C. The reaction was stirred at 0 °C temperature for 16 h and then quenched with a saturated solution of $NaHCO_3$ (pH 7–8). The organic phase was separated, washed by an aqueous $NaHCO_3$ solution, dried over anhydrous Na_2SO_4 and then concentrated under vaccum. The residue obtained was purified by a column chromatography of silica (eluent: dichloromethane/methanol 4%) to give **9**.

Yield 66% (17 mg). TLC R_f: 0.83 (dichloromethane/methanol 95:5, v/v). IR (cm^{-1}): 3393, 3194, 2921, 2852, 1704, 1674, 1603, 1466,1433, 1352, 1143, 1098, 1038, 773, 695. ^1H NMR (500 MHz, MeOD) δ (ppm): 8.91 (s, 1H); 8.70 (d, J = 5.1 Hz, 1H); 7.47 (dd, J = 1.0 and 6.3 Hz, 1H); 7.12 (t, J = 8.1, 1H); 6.87 (dd, J = 2.0 and 2.0 Hz, 1H), 6.78 (dd, J = 1.6 and 7.8 Hz, 1H), 6.60 (dd, J = 1.6 and 8.1 Hz, 1H); 3.06 (t, J = 6.9 Hz, 2H); 1.63–1.52 (m, 2H); 1.39–1.20 (m, 18H); 0.92 (t, J = 6.6 Hz, 3H). ^{13}C NMR (75 MHz, MeOD) δ (ppm): 168.5 (C); 159.8 (C); 152.4 (CH); 149.5 (C); 144.3 (CH); 140.0 (C); 129.0 (CH); 126.4 (C); 118.0 (CH); 113.2 (CH); 112.6 (CH); 109.6 (CH); 89.0 (C); 43.5 (CH_2); 31.7 (CH_2); 29.4–29.3 (4 × CH_2); 29.2 (CH_2); 29.06 (CH_2); 28.9 (CH_2); 26.9 (CH_2); 22.3 (CH_2); 13.0 (CH_3). MS (DCI) m/z: 438 [M^++C_2H_5], 410 [M+H^+]. HRMS (DCI): for $C_{25}H_{36}N_3O_2$ calcd: 410.2808, found: 410.2810.

4.11. 2-Dodecyl-1*H*-pyrrolo[3,4-*c*]pyridine-1,3-dione (10)

To a solution of 3,4-pyridinedicarboximide (**5**) (200 mg; 1.35 mmol) in DMF (13.5 mL), 1-iododododecane (400 mg; 1.35 mmol) and potassium carbonate (931.5 mg; 6.75 mmol) were added. The mixture was stirred for 12 h at 50 °C under nitrogen. Then water (15 mL) was added and the reaction mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic phases were dried over anhydrous sodium sulfate, filtrated and concentred in vaccum to give **10**.

Yield 57% (245 mg). TLC R_f: 0.77 (dichloromethane/methanol 95:5, v/v). Beige solid, mp 56 °C. IR (cm^{-1}): 2915, 2847, 1705, 1400, 1065, 1058, 865. ^1H NMR (250 MHz, $CDCl_3$) δ (ppm): 9.17 (s, 1H); 9.09 (d, J = 4.8 Hz, 1H); 7.78 (d, J = 4.0 Hz, 1H); 3.75 (t, J = 7.2 Hz, 2H); 1.70–1.64 (m, 2H); 1.34–1.27 (m, 18H); 0.92 (t, J = 5.8 Hz, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ (ppm): 167.2 (C); 166.8 (C); 155.5 (CH); 144.6 (CH); 139.6 (C); 127.2 (C); 116.7 (CH); 38.5 (CH_2); 31.9 (CH_2); 29.6–29.1 (6 × CH_2); 28.4 (CH_2); 26.8 (CH_2); 22.7 (CH_2); 14.1 (CH_3). MS (ESI) m/z: 317 [M+H]. HRMS (ESI): for $C_{19}H_{29}N_2O_2$: calcd: 317.2229; found: 317.2271.

4.12. (*RS*)-2-(dodecyl)-1-hydroxy-1-phenyl-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridin-3-one (11)

In this case PhMgBr was used in the place of PhLi. However the reaction can be performed with both reagents.

Yield 68% (42 mg – mixture of *para*/*meta* 1.5:1 regioisomers). The *para* regioisomer was isolated by recrystallization in acetone. White solid, mp 80 °C. TLC R_f: 0.66 (dichloromethane/methanol 95:5, v/v). IR (cm^{-1}): 3387, 2918, 2850, 1683, 1662, 1449, 1429, 1398, 1371, 1060, 702. ^1H NMR (500 MHz, MeOD) δ (ppm): 8.97 (s, 1H), 8.71 (d, J = 5.1 Hz, 1H); 7.43–7.36 (m, 6H); 3.51–3.45 (m, 1H); 3.12–3.06 (m, 1H); 1.65–1.23 (m, 20H); 0.91 (t, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ (ppm): 166.3 (C); 158.2 (C); 152.5 (CH); 144.0 (CH); 137.9 (C); 128.6 (CH); 128.4 (2 × CH); 126.8 (C); 125.9 (2 × CH); 117.9 (CH); 91.2 (C); 39.4 (CH_2); 31.7 (CH_2); 29.4–29.1 (5 × CH_2); 28.8 (CH_2); 28.2 (CH_2); 26.8 (CH_2); 22.3 (CH_2); 13.0 (CH_3). MS (ESI) m/z: 417.3 [M+Na^+], 395.4 [M+H^+]. HRMS (ESI) for $C_{25}H_{35}N_2O_2$, calcd: 395.2699; found: 395.2708. Anal. Calcd for $C_{25}H_{34}N_2O_2$: C, 76.10; H, 8.69; N, 7.10. Found: C, 76.09; H, 8.52; N, 6.91.

4.13. (*RS*)-1-hydroxy-1-tetradecyl -1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridin-3-one (12)

Yield 54% (505 mg – mixture of *para*/*meta* 2:1 regioisomers). The *para* isomer was isolated by preparative HPLC (column Sunfire C18 150 × 4.6 mm; eluent: TFA 0.1% /CH_3CN 40:60; RT_{para} = 12.4 - min and RT_{meta} = 13.2 min). TLC R_f: 0.83 (dichloromethane/methanol 95:5, v/v). White solid, mp 137 °C. IR (cm^{-1}): 3313, 3054, 2916, 2850, 1697, 1613, 1595, 1470, 1359, 1154, 1021, 830, 795, 719. ^1H NMR (250 MHz, MeOD) δ (ppm): 8.90 (s, 1H); 8.81 (d, J = 5.1 Hz, 1H); 7.68 (d, J = 5.0 Hz 1H); 2.13–2.04 (m, 2H); 1.30–1.27 (m, 24H); 0.94 (t, J = 5.9 Hz, 3H). ^{13}C NMR (63 MHz, MeOD) δ (ppm): 167.8 (C); 158.2 (C); 152.4 (CH); 144.2 (CH); 127.5 (C); 117.6 (CH); 88.0 (C); 37.7 (CH_2), 31.7 (CH_2); 29.4 -29.0 (9 × CH_2); 23.3 (CH_2); 22.3 (CH_2); 13.0 (CH_3). MS (ESI) m/z: 369.4 [M+Na^+], 347.5 [M+H^+]. HRMS for $C_{21}H_{35}N_2O_2$, calcd: 347.2699; found: 347.2694.

4.14. (*RS*)-1-dodecyloxy-1-phenyl-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridin-3-one (14)

To a solution of hemiamidal **13**[16] (200 mg; 0.87 mmol) in 1-dodecanol (34 mL) was added 2 mL of thionyl chloride. The mixture was heated at reflux and under inert atmosphere for 3 h. The reaction was monitored by TLC. After complete transformation of the starting material, the reaction was stopped. The reaction was treated with saturated solution of $NaHCO_3$ (30 mL) and extracted with ethyl acetate (3 × 25 mL). The combinated organic layers were dried over anhydrous sodium sulfate and concentrated under vaccum. The residue obtained as a yellow oil was purified by column chromatography (eluent: gradient dichloromethane/methanol: 100/0 to 96/4) to obtain **14**.

Yield 30% (105 mg). White solid, mp 46 °C. TLC R_f: 0.39 (dichloromethane/methanol 96:4, v/v). IR (cm^{-1}): 2922, 1714, 1606, 1449, 1051, 747, 696. ^1H NMR (250 MHz, $CDCl_3$) δ (ppm): 9.10 (bd s, 1H); 8.79 (bd s, 1H); 7.58–7.51 (m, 2H); 7.43–7.35 (m, 3H); 7.29–7.25 (m, 1H); 6.96 (s, 1H); 3.51 (pseudo q, J = 6.5 Hz, 1H); 3.04 (pseudo q, J = 6.3 Hz, 1H); 1.65–1.57 (m, 2H); 1.50–1.20 (m, 18H); 0.90 (t, J = 6.0 Hz, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ (ppm): 168.5 (C); 155.6 (C); 153.2 (CH); 146.0 (CH); 138.7 (C); 129.0 (CH); 128.8

(2 × CH); 126.5 (C); 125.5 (2 × CH); 117.9 (C); 91.6 (C); 63.4 (CH_2); 31.9 (CH_2); 29.6–29.3 (7 × CH_2); 26.1 (CH_2); 22.7 (CH_2); 14.1 (CH_3). MS (ESI) *m/z*: 395.4 [M+H$^+$]. HRMS: for $C_{25}H_{35}N_2O_2$: calcd: 395.2699; found: 395.2693. Anal. Calcd for $C_{25}H_{34}N_2O_2$ 0.75 CH_3OH: C, 73.89; H, 8.91; N, 6.69. Found: C, 74.33; H, 8.71; N, 6.24.

4.15. Enzymatic inhibition experiments

M. tuberculosis InhA was overexpressed in *E. coli* and purified as previously described.[18] Isoniazid and NADH were obtained from Sigma-Aldrich. The substrate 2-*trans*-decenoyl-CoA was synthesized from 2-*trans*-decenoic acid using the mixed anhydride method and purified according to the procedure described by Goldman and Vagelos.[19] The concentration of the substrate was determined on the basis of ε_{260} = 22 600 M^{-1} cm^{-1}.

For the enzymatic inhibition assays, pre-incubation reactions were performed in 90 μL (total volume) of 30 mM PIPES buffer solution, 150 mM NaCl, pH 6.8 at 25 °C containing 70 nM InhA, 200 μM cofactor (NADH) and the tested compound (at 100 or 30 μM). DMSO was used as co-solvent and its final concentration was 0.5%. After 5 min of pre-incubation, the addition of 35 μM substrate (*trans*-2-decenoyl-CoA) initiated the reaction which was followed at 340 nm (oxidation of NADH) and at 25 °C using a thermostated Uvikon 923 spectrophotometer (Bio-Tek Kontron Instruments). Control reactions were carried out under the same conditions as those described above but without ligands, and triclosan (10 μM) was used as a positive control. The initial rates of the reactions were calculated. The inhibitory activity of each compound tested was expressed as the percentage inhibition of InhA activity (initial velocity of the reaction) with respect to the control experiments. All activity assays were performed in triplicate.

4.16. Bacterial growth inhibition

The susceptibility of *M. tuberculosis* H_{37}Rv to all synthesized compounds was evaluated by using a colorimetric microassay based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma–Aldrich) to formazan by metabolically active cells.[20,21] Briefly, serial two-fold dilutions of the inhibitor solubilized in DMSO were prepared in 100 μL Middlebrook 7H9 broth base (Difco) and dispensed into 96-well microtiter plates, to each well of which were added 100 μL of *M. tuberculosis* (diluted in 7H9 broth to an OD_{600} of 0.02). Plates were incubated for six days at 37 °C, and MTT was added (50 μL of a 1 mg/mL solution). Plates were incubated for a further 24 h and solubilization buffer [DMF/SDS 20% (w/v), 1:2] was added to each well. Absorbance was measured at 570 nm. The MIC was determined to be the lowest concentration of compound that inhibited visible growth of the bacterial culture (the absorbance value obtained for untreated bacilli was taken as a growth control). The IC_{50}, corresponding to the concentration that inhibits 50% of the bacterial growth, was determined by fitting the data by non-linear regression using the program GraphPad Prism 5.0. Isoniazid- and ciprofloxacin-treated cultures were used as positive controls. Each mother solution was prepared in DMSO at concentrations 100 times greater than the highest test concentration.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.09.017.

References and notes

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