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Design, synthesis, SAR and biological investigation of 3-(carboxymethyl) rhodanine and aminothiazole inhibitors of *Mycobacterium tuberculosis* Zmp1

Mattia Mori [a,b], Davide Deodato [a], Mohan Kasula [a], Davide M. Ferraris [c], Adriana Sanna [d], Alessandro De Logu [e], Menico Rizzi [c], Maurizio Botta [a,f,*]

[a] Department of Biotechnology, Chemistry and Pharmacy, University of Siena, via Aldo Moro 2, 53019 Siena, Italy
[b] Center for Life Nano Science@Sapienza, Istituto Italiano di Tecnologia, viale Regina Elena 291, 00161 Roma, Italy
[c] DiSF-Dipartimento di Scienze del Farmaco, Università del Piemonte Orientale, Via Bovio 6, 28100 Novara, Italy
[d] Department of Public Health, Clinical and Molecular Medicine, University of Cagliari, Via Porcell 4, 09124 Cagliari, Italy
[e] Department of Life and Enviromental Sciences, University of Cagliari, Via Porcell 4, 09124 Cagliari, Italy
[f] Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology, College of Science and Technology, Temple University, BioLife Science Bldg., Suite 333, 1900 N 12th Street, Philadelphia, PA 19122, USA

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ABSTRACT

Sixteen 3-(carboxymethyl)rhodanines, and twelve aminothiazoles as rhodanine-mimetics were designed, synthesized and tested as inhibitors of the Zmp1 enzyme from *Mycobacterium tuberculosis* (Mtb). Almost all rhodanines (**5a–d**, **5f–n**, and **7a–b**) exhibited Zmp1 inhibition with IC_{50} values in the range 1.3–43.9 μM, whereas only aminothiazoles **12b** and **12d** proved active with IC_{50} values of 41.3 and 35.7 μM, respectively. Structure-activity relationships (SAR) were coupled with molecular modeling studies to highlight structural determinants for Zmp1 inhibition. Moreover, rhodanines **5a** and **5c** induced 23.4 and 53.8% of Mtb growth inhibition in THP-1 infected cells, respectively, at the non-toxic concentration of 10 μg/ml. This work represents a step forward in targeting Zmp1 by small molecules.

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb) that has been one of the top ten causes of death worldwide in 2015.[1] To control TB spread, in the '90s the WHO has launched the DOTS (Directly Observed Treatment, Short Course) strategy that proved successful in effectively achieving cure rates over 90% in countries where the health system works well. On the contrary, DOTS proved notably less successful in cases of HIV co-infections or in patients infected by multidrug-resistant (MDR), extensively drug-resistant (XDR) and totally drug-resistant (TDR) Mtb strains.[2–4] Therefore, novel and effective strategies to treat and control TB are still urgently needed, and may be achieved by targeting Mtb validated targets or Mtb proteins that are relevant for its replication and survival into the host.[5–7] In this context, Mtb-secreted extracellular proteins are attracting much interest either as candidate drug targets or biomarkers of active and latent TB, mostly because of their predominant role in virulence, in mediating host-pathogen interaction, and in attenuating host immune response.[8–10] Among them, the extracellular zinc metalloprotease 1 (Zmp1)[11–13] has been reported to play a key role in phagosome maturation and to elicit TB-specific humoral immune response,[14] thus enhancing the overall survival of Mtb in the host. In a guinea pig model of TB infection, Zmp1 deletion has led to increase the protective efficacy of the live vaccine *Mycobacterium bovis* BCG,[12] in agreement with Master et al.,[15] showing that Zmp1 deletion is associated to virulence attenuation. In contrast, Muttucumaru et al. have showed that deletion of the Zmp1 gene leads to bacterial hypervirulence in a murine model.[16] Nevertheless, it is clear from multiple reports that Zmp1 plays a relevant role in host–pathogen interaction, and that the design of specific small molecule inhibitors could be a valuable strategy towards anti-TB therapeutics. Zmp1 inhibitors may also serve as tools to further understanding the pathogenic role of Zmp1.

Based on the available X-ray structure of Zmp1/ligand complex,[17] we recently identified the 3-(carboxymethyl)rhodanine as privileged scaffold of Zmp1 inhibitors by disclosing **ZTB23(R)**, **ZTB28(R)** and **ZTB29(R)** (Fig. 1) as confirmed hits.[18] Subsequent

* Corresponding author at: Department of Biotechnology, Chemistry and Pharmacy, University of Siena, via Aldo Moro 2, 53100 Siena, Italy.
E-mail address: botta@unisi.it (M. Botta).

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to our work, two reports on rhodanine and quinolidene-rhodanine Zmp1 inhibitors have been published.[19,20]

Here, to further understanding the structure–activity relationships (SAR) of the 3-(carboxymethyl)rhodanine scaffold and to investigate its effect on Mtb growth, we designed, synthesized and tested *in silico* and *in vitro* sixteen rhodanines and twelve aminothiazoles as possible rhodanine-mimetics (Fig. 1).

Particularly, we modified *i*) the amino acid moiety of rhodanines responsible for zinc coordination,[18] *ii*) the nature of the aromatic ring linked to the rhodanine core, and *iii*) the position and type of substituents to the phenyl ring (Fig. 1). Moreover, to understand the relevance of the rhodanine core, it was replaced by the aminothiazole moiety (Fig. 1). The preparation of the rhodanine derivatives followed the synthetic route already described previously (see also Supporting Information for chemistry directions and compounds characterization).[18] Briefly, D-amino acids **1a**–**c** were reacted with carbon disulfide in water to give compounds **2a**–**c**, which were treated *in situ* with sodium chloroacetate and lastly with HCl, affording cyclized compounds **3a**–**c**. Knoevenagel condensation with the opportune benzaldehydes **4a**–**i**, using β-alanine as base (Scheme 1), furnished the final Z-rhodanines **5a**–**n** listed in Table 1. ^{1}H NMR analysis of compounds **5a**–**n** confirmed the presence of a single peak for the olefin proton (CH=) in the range of 7.70–7.50 ppm, at lower field values than expected for the *E*-isomer. This observation suggests the *Z* configuration of the double bond due to the higher termodynamic stability of this isomer.[21,22] Heteroaromatic derivatives **7a**–**b** bearing a pyridine and a thiophene substituent were similarly obtained by using the corresponding heteroaromatic aldehydes **6a**–**b** (Scheme 2).

Aminothiazole derivatives were obtained as outlined in Scheme 3. Starting material was D-phenylalanine **1a**, which was converted to the corresponding methyl ester **8** by treatment with thionyl chloride in methanol. Compound **8** was then reacted with methoxycarbonyl isothiocyanate, using DIPEA as the base, to give the *N*-methoxycarbonylthiourea **9** that was hydrolyzed with sodium hydroxide to furnish the thiourea **10**, bearing a free carboxylic acid function. The aminothiazole products were synthesized according to the Hantzch thiazole synthesis,[23] in which

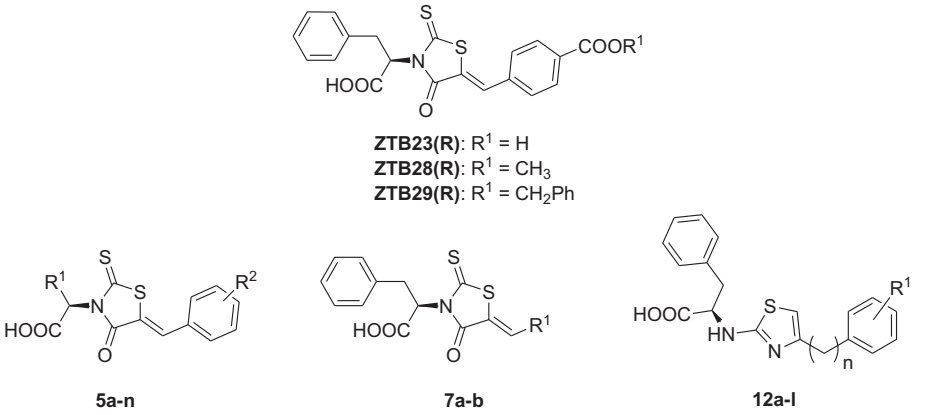
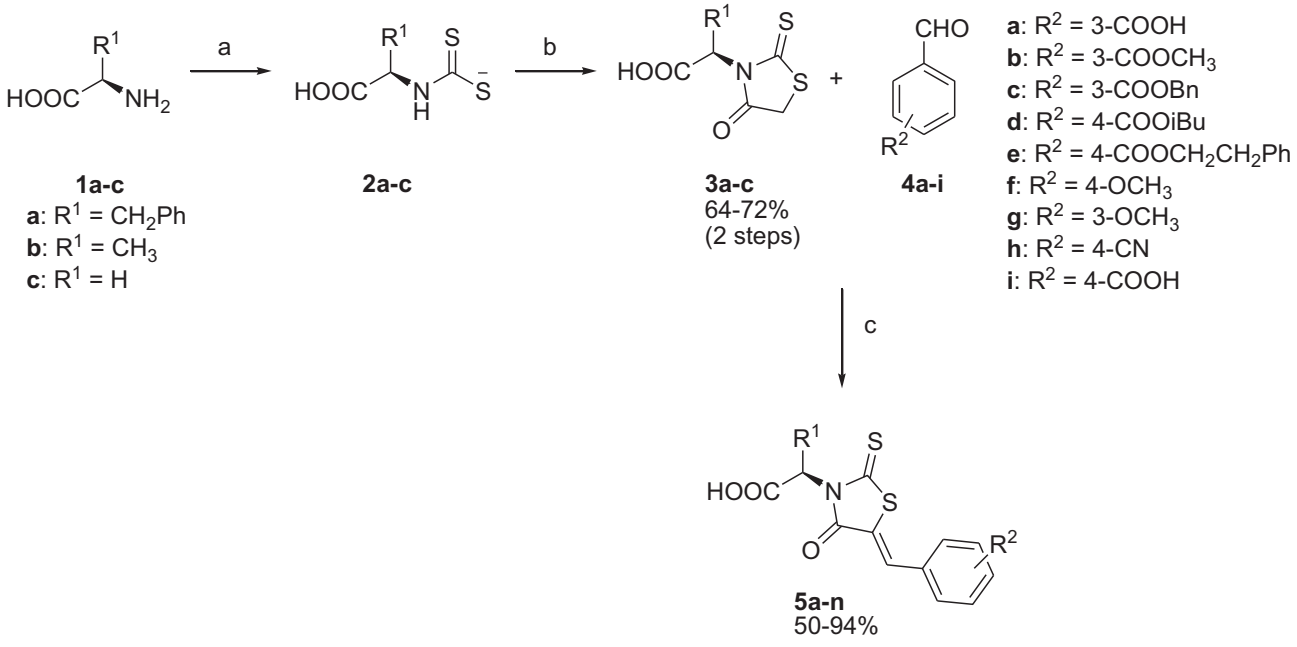


Fig. 1. Chemical structure of Zmp1 inhibitors **ZTB23(R)**, **ZTB28(R)** and **ZTB29(R)** identified previously (top),[18] and rational design of novel rhodanine and aminothiazole derivatives investigated in this work as Zmp1 inhibitors (bottom).



Scheme 1. [a]*Reagents and conditions*: (a) carbon disulfide, NaOH, H_2O, r.t. 12 h; (b) *i.* sodium chloroacetate solution, r.t. 2 h; *ii.* 6 N HCl, cat. $POCl_3$, 75 °C, 4 h; (c) β-alanine, AcOH, ref, 6 h.