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New chemotypes for wALADin1-like inhibitors of delta-aminolevulinic acid dehydratase from *Wolbachia* endobacteria

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ABSTRACT

Substituted benzimidazoles of the wALADin1-family have recently been identified as a new class of species-selective inhibitors of delta-aminolevulinic acid dehydratase (ALAD) from *Wolbachia* endobacteria of parasitic filarial worms. Due to its *Wolbachia*-dependent antifilarial activity, wALADin1 is a starting point for the development of new drugs against filarial nematodes. We now present several other chemotypes of ALAD inhibitors that have been identified based upon their molecular similarity to wALADin1. A tricyclic quinoline derivative (wALADin2) with a different inhibitory mechanism and improved inhibitory potency and selectivity may represent an improved drug lead candidate.

The enzyme δ-aminolevulinic acid dehydratase (ALAD, also known as porphobilinogen synthase, E.C. 4.2.1.24) catalyzes the condensation of two molecules of 5-aminolevulinic acid (5-ALA) to porphobilinogen as the first common step of heme synthesis.[1] This enzyme has been proposed as a drug target in a variety of human pathogens including the endosymbiotic *Wolbachia* α-proteobacteria of filarial nematodes.[2] These parasitic worms are the causative agents of lymphatic filariasis and onchocerciasis and infect more than 150 million people in the tropics and subtropics.[3–5] As these worms are unable to synthesize tetrapyrroles de novo the endobacteria, which have conserved a functional heme biosynthetic pathway, are expected to supply their hosts with this essential biomolecule.[2,6–8] Experimental evidence suggests that the worms are indeed dependent upon *Wolbachia* for heme, as blocking of the tetrapyrrole biosynthesis enzymes ALAD or ferrochelatase results in worm death in vitro.[2] The differences in metal cofactor usage between the Mg^{2+}-regulated *Wolbachia* ALAD enzyme and the Zn^{2+}-dependent human ortholog and the corresponding differences in protein structure, in principle, enable species-selective inhibition of this pathway.[2,9,10]

We have recently reported the discovery of wALADin1 as the most potent member of a new class of trisubstituted benzimidazole carboxylic acid compounds with species-selective inhibitory activity against ALAD from *Wolbachia* endobacteria of the filarial worm *Brugia malayi* (wALAD) but not of the human ortholog (hALAD).[11] Since wALADin1 elicited pronounced antifilarial activity in an ex vivo worm culture assay it holds promise as a lead compound in the development of novel antifilarial drugs. However, the aim of the present study was to identify other specific ALAD inhibitors based on different chemical scaffolds. These alternative chemotypes might offer new possibilities in the design of inhibitors with improved potency, specificity and other drug-activity related properties.

Based on the structure of wALADin1 (Fig. 1A) and the related benzimidazole compound 24C7 (Fig. 1B), also identified in the original high-throughput wALAD activity screen but with weaker inhibitory potency, a fingerprint-based similarity search was conducted based on three conceptually different molecular fingerprints: a fragment-based fingerprint, MACCS;[12] an atom-environment fingerprint, ECFP4;[13] and a pharmacophore-based fingerprint, GpiDAPH3.[14] Chemical similarity was quantitatively measured as the fingerprint overlap between reference and ~3.75 million vendor catalog molecules using the Tanimoto coefficient.[15] Compounds were ranked according to their similarity val-

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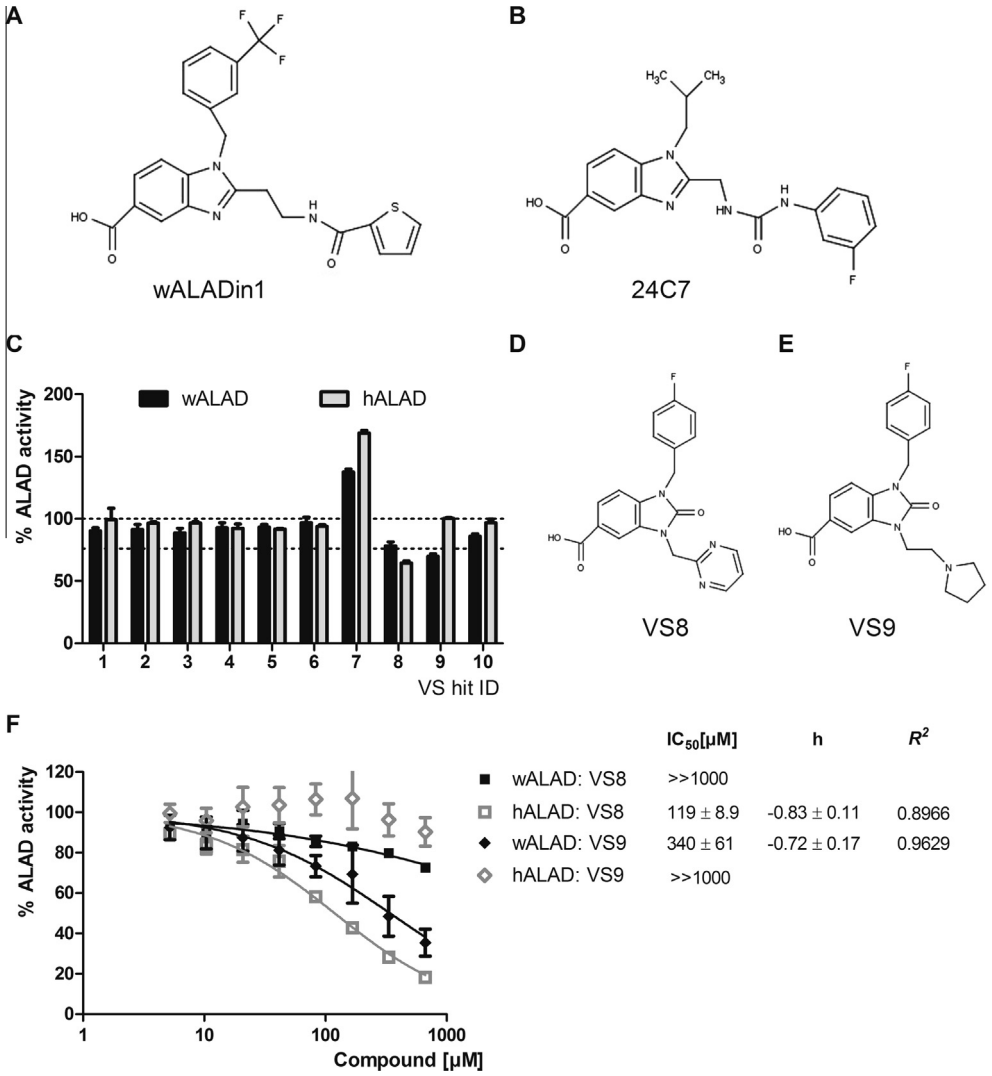

Figure 1. wALAD and hALAD inhibitory activity of hits from in silico screening (A) 10 selected in silico hits were screened at 67 μM for wALAD or hALAD inhibitory activity. Only compounds VS 8 and VS 9 showed >20% inhibition of either ortholog. (B, C) Chemical structures of the substituted benzimidazole-2-one VS8 and VS9, respectively. (D) VS8 specifically inhibited hALAD, while VS9 weakly inhibited wALAD. Graph shows means ± SD of two independent experiments. Curves were fit by non-linear regression using the 'log(inhibitor) versus normalized response–variable slope' algorithm of Prism 5.0. Predictions for IC_{50} and Hill coefficient *h*, as well as the coefficient of determination R^2 are indicated in the graph. (The chemical structures of VS1-7 and VS10 are shown in Supplementary Figure 1.)

ues and reduced to their Bemis and Murcko (BM) scaffolds.[16] The 10 top ranking compounds comprising ten different BM scaffolds from each of the six individual rankings (MACCS, ECFP4 and GpiDAPH2 against wALADin1 alone or wALADin1plus 24C7) were selected. In addition the 40 scaffolds with highest enrichment compared to the entire database in the union of the top 5000 compounds from all 6 database rankings were chosen. Further, the selected 100 compounds were visually inspected for features that had been identified for inhibitory activity of wALADin1 by structure–activity relationship studies previously done, whereby it was determined that the carboxyl function of the inhibitor is essential.[11] Thus, we restricted our experimental analysis to those scaffolds from the top 100 list for which compounds were readily available from commercial sources (ChemBridge, San Diego, CA, USA or Enamine, Kiev, Ukraine) and that featured a carboxyl or another group with (at least partial) a negative charge. 10 compounds (termed VS1–10) met these criteria and were selected for experimental testing in a wALAD activity assay under conditions identical to those previously reported.[11] In brief, in the presence of 67 μM compound, the enzymatic reaction of recombinantly expressed wALAD (500 nM) was carried out in 100 mM Tris buffer (pH 8.0), 5 mM DTT, 1 mM $MgCl_2$ and 200 μM 5-ALA. After 20 min incubation at room temperature, the reaction was stopped with modified Ehrlich's Reagent (1 g 4-dimethylamino benzaldehyde in 42 mL acetic acid, 12 mL perchloric acid, 7.3 mL 12% trichloroacetic acid) and the OD (555 nm) determined. In parallel, the inhibitory activity of the compounds was assessed for the Zn^{2+}-dependent human ortholog (at 250 nM hALAD), for which the assay buffer was 100 mM Tris, pH 7.5, 5 mM DTT, 10 μM $ZnCl_2$. Two compounds that inhibited either ortholog by >25% were the substituted 1,3-dihydro-2H-benzimidazole-2-one structures VS8 and VS9 (Fig. 1C–E). Although both compounds varied only in the identity of the side chain attached to the N_3-atom of the benzimidazole-2-one core, VS8 selectively inhibited hALAD (IC_{50} = 119 ± 9 - μM), while VS9 showed selectivity for wALAD (IC_{50} = 340 ± 61 μM) (Fig. 1F). Thus, this side chain governs specificity of the inhibitor with respect to the different orthologs. This substituent may thus be functionally equivalent to the substituent attached to the C_2 atom of the benzimidazole wALADin1, which we had previously shown controls species-selectivity of the inhibitor. The chemical structures of inactive compounds VS1 -7 and VS10 are shown in Supplementary Figure 1.