Electronic Supplementary Material (ESI) for MedChemComm.

Supplementary Information

Isolation, expression and biochemical characterization of recombinant hyoscyamine-6β-hydroxylase from *Brugmansia sanguinea* – tuning the scopolamine production

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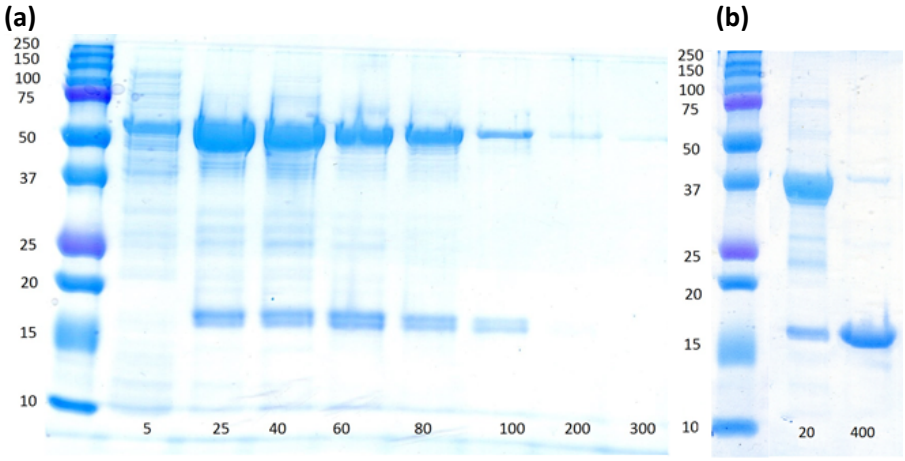

Figure S1. (**a**) Purification of SUMO-BsH6H analyzed by comassie blue stained SDS-page. Amounts of imidazole used to elute the protein are indicated under each line (mM). (**b**) After cleavage of the SUMO-tag, the native BsH6H remains in the flowthrough (first lane).

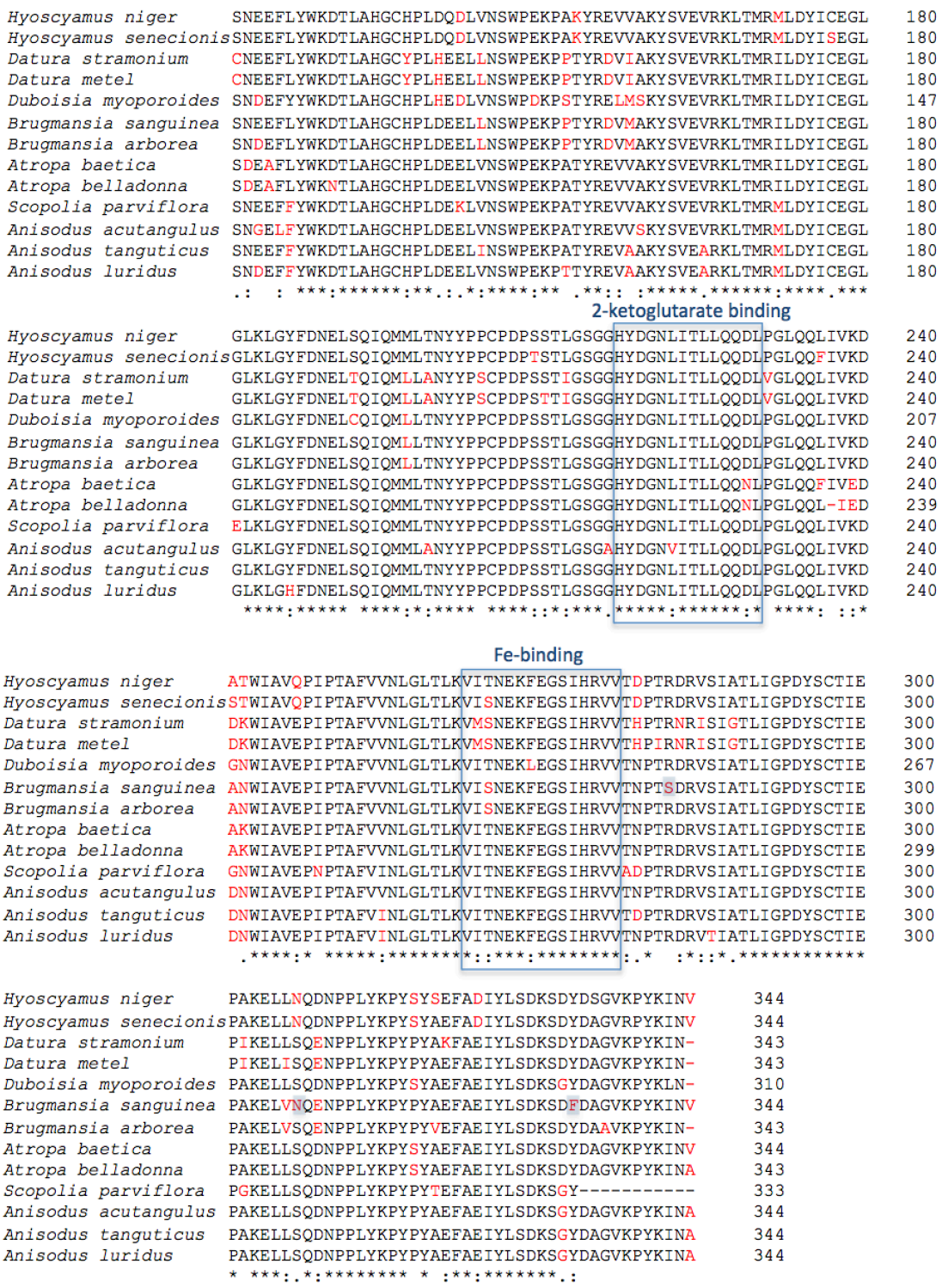

Figure S2. Multiple sequence alignments for comparison of BsH6H with other reported H6H homologues. Two iron binding and one co-factor region are boxed. Red letters indicate different residues with regard to the consensus. Blue highlighted letters depict significantly different residues in *Brugmansia sanguinea*-H6H compared to other H6H homologues. Accession numbers: ABG89397.1 (*H. niger*); AFP99876.1 (*H. senecionis*); ALD59774.1 (*D. stramonium*); AAQ04302.1 (*D. metel*);

AQU12714.1 (*D. myoporoides*); ALD59773.1 (*B. arborea*); ABR15749.1 (*A. baetica*); AEM91979.1 (*A. belladonna*); AAY53932.1 (*S. parviflora*); ABM74185.1 (*A. acutangulus*); AAQ75700.1 (*A. tanguticus*); AGL76991.1 (*A. luridus*).

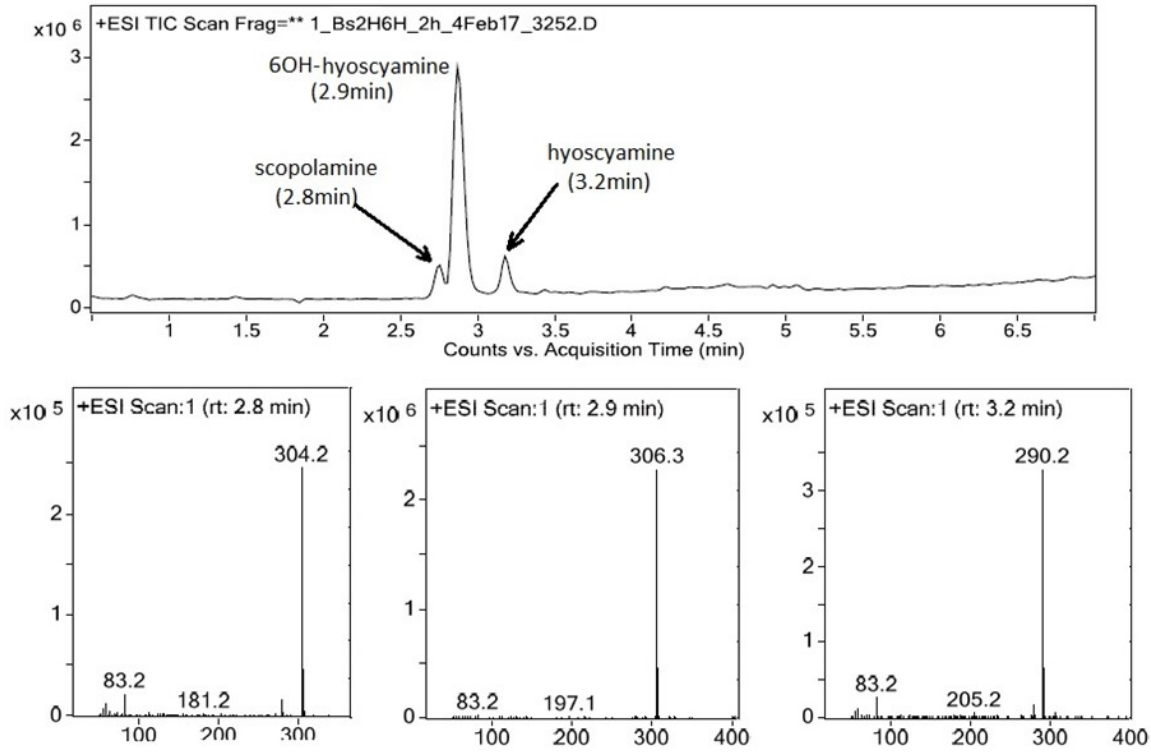

Figure S3. LCMS analysis of the transformation of hyoscyamine **1** (3.2 min) to intermediate **2** (2.9 min) and scopolamine **3** (2.8 min) by BsH6H and MS identification of products.