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**Novel 2-Oxoglutarate Analogues Modulate the Epigenetic Activity of the Cancer-related Human Enzyme Aspartate/Asparagine- $\beta$ -Hydroxylase**

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The human oxygenase aspartate/asparagine- $\beta$ -hydroxylase (AspH) catalyzes posttranslational  $\beta$ -hydroxylations of specific Asp/Asn-residues in epidermal growth factor-like domains (EGFDs) by using 2-oxoglutarate (2OG) as a cofactor.<sup>[1]</sup> AspH is overexpressed in certain cancer cells and translocates from the endoplasmic reticulum to the cell surface membrane, a process which correlates to enhanced tumor invasiveness and poor clinical prognosis.<sup>[2]</sup> We recently reported the production of a truncated human AspH-construct in *E. coli* that was catalytically active<sup>[3]</sup> and used for the development of a high-throughput mass spectrometry-based assay to monitor AspH-activity *in vitro*. Herein, we report a novel synthesis of non-natural 2OG analogues which were either alternative AspH-cofactors or small-molecule AspH-inhibitors.

Commercial starting materials were converted in three synthetic steps to previously inaccessible 2OG analogues bearing alkyl-, aryl-, or heteroaryl-substituents. This novel synthesis compares favorably to literature-reported syntheses as it features a broad substrate scope, high reaction yields, and simple reaction protocols. A diverse library of 2OG analogues (>35) was obtained and evaluated in the AspH-assay. Potent AspH-inhibitors were identified and ranked according to their (IC<sub>50</sub>)-values, constituting the first detailed structure activity relationship study on AspH using small-molecules with a known mode of inhibition (replacing 2OG in the active site). Several 2OG analogues could substitute 2OG as cofactor and thus promote enzymatic turnover. Michaelis constants (K<sub>M</sub>) were determined and used to compare the efficiency of the 2OG analogues with 2OG. The selectivity of the novel AspH-inhibitors and -cofactors was evaluated using structurally and functionally related human 2OG-dependent oxygenases (i.e. PHD2, FIH, JMJD5). Finally, crystallographic experiments were performed to obtain further structural information on the substrate requirements of the AspH active site; thus enabling future studies to design selective AspH-inhibitors as potential anticancer therapeutics.

In conclusion, a novel efficient synthesis of 2OG analogues was developed providing access to important chemical tools for mechanistic and inhibition studies of 2OG-dependent oxygenases. The relevance of this compound class was highlighted by the identification of novel potent and selective small-molecule inhibitors of the cancer-related human enzyme AspH.

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