**In silico and in vitro approaches to develop Dimethylarginine dimethylaminohydrolase-1 inhibitors**

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**Introduction**

Dimethylarginine dimethylaminohydrolases (DDAH) metabolise the endogenous nitric oxide synthase (NOS) inhibitor dimethylarginine (ADMA) and monomethylarginine. In sepsis excessive nitric oxide partially contributes to acute circulatory failure; pharmacological DDAH inhibition has been proposed to increase circulating methylarginine concentrations and reduce NO levels. The N²,N²-disubstituted arginine substitute, SR257, inhibits DDAH1, with an IC₅₀ 22 µM, without directly inhibiting NOS.¹,²

**Methods**

**Chemical synthesis:** Acyclic and cyclic N²,N²-, N⁰,N⁰-, and N⁰ substituted arginines were made as previously described using Katritzky's synthesis preparing trisubstituted guanidines from di-benzobziazol-1-y1)methanimine.³

**In silico prediction:** Molecular docking was employed to explore interactions of the N², N²-disubstituted arginines (table 1) with human DDAH1 (PDB 2JAJ, hDDAH1 bound to SR257) using Glide (Schrödinger)⁴ and Autodock 4. The published SR257 ligand was used to define the binding site with both software tools.

**In vitro DDAH1 assay:** Recombinant human DDAH1 activity was measured using colorometric citrulline assay⁶ containing ADMA (100 µM), and phosphate-buffered saline (pH 7.4), with symmetric dimethylarginine (100 µM), not a substrate for DDAH1, as blank. Experiments were carried out in duplicate, and repeated on at least 3 separate occasions.

**Results**

**In vitro DDAH1 assay:** N⁰,N⁰-disubstituted arginine analogues (100 µM) reduced the activity of human recombinant DDAH1 activity to less than 25% of control in the presence of 100 µM ADMA substrate. The morpholinyl and pyrrolidinyl substituents reduced hDDAH1 activity to less than 10% of control.

**Discussion**

Both Autodock4 and Glide docking predicted higher binding energies for morpholinyl, pyrrolidinyl and piperidinyl than the known SR257 compound. The morpholinyl compound has been described to be a weak inhibitor of hDDAH1. In vitro assays confirmed these N⁰,N⁰-disubstituted arginines reduced DDAH1 activity. There was variation between Glide and Autodock in the docking predictions for methoxymethyl methyl and N-methylpiperazinyl.

**In silico prediction** of DDAH1 ligand interactions may assist in the future design and development of novel N⁰,N⁰-disubstituted arginines.

**References**


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