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

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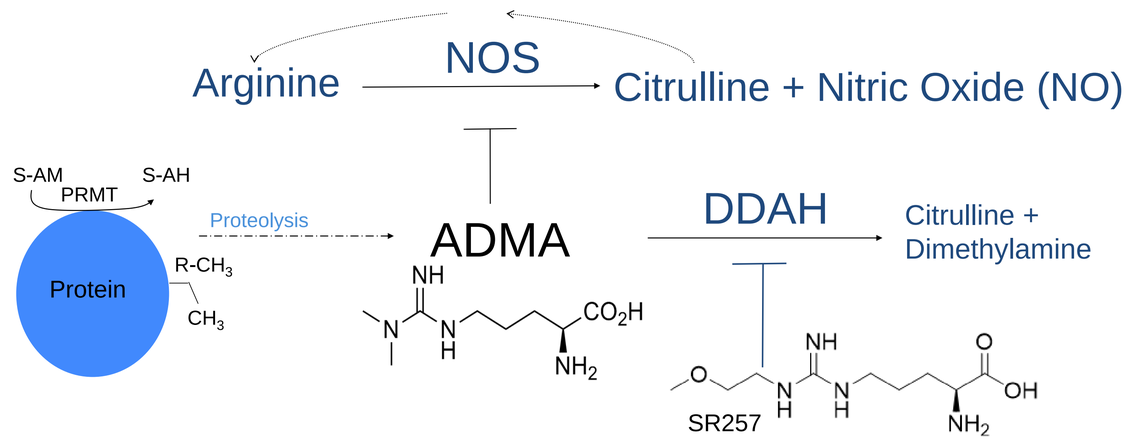
Introduction

Dimethylarginine dimethylaminohydrolases (DDAH) metabolise the endogenous nitric oxide synthase (NOS) inhibitors: asymmetric dimethylarginine (ADMA) and monomethylarginine[1]. In sepsis excessive nitric oxide partially contributes to acute circulatory failure; pharmacological DDAH1 inhibition has been proposed in order to increase circulating methylarginine concentrations and reduce NO levels[2]. The N^G-methoxyethyl arginine substituent, SR257, inhibits DDAH1, with an IC50 22 μM, without directly inhibiting NOSs[1,3].



Methods

Chemical synthesis: Acyclic and cyclic N^G,N^G-disubstituted arginines were made as previously described[4] using Katritzky's synthesis preparing trisubstituted guanidines from di-(benzotriazol-1-yl)methanimine[5].

In silico prediction: Molecular docking was employed to explore interactions of the N^G, N^G-disubstituted arginines (table 1) with human DDAH1 (PDB 2JAJ; hDDAH1 bound to SR257) using Glide (Schrödinger[6]) and Autodock4[7]. The published SR257 ligand was used to define the binding site with both software tools.

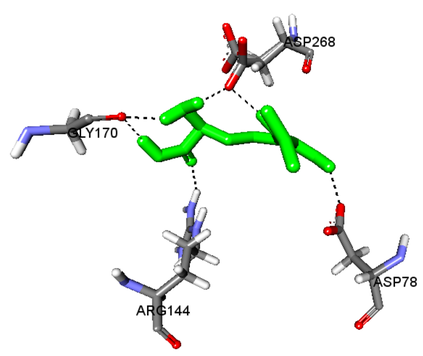


Figure 1: Human DDAH-1 (PDB 2JAJ) predicted active site complex with substrate ADMA.

***In vitro* DDAH1 assay:** Recombinant human DDAH1 activity was measured using colorometric citrulline assay[8] containing ADMA (100 μM), and phosphate buffered saline (pH7.4); 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:** N^G,N^G-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.

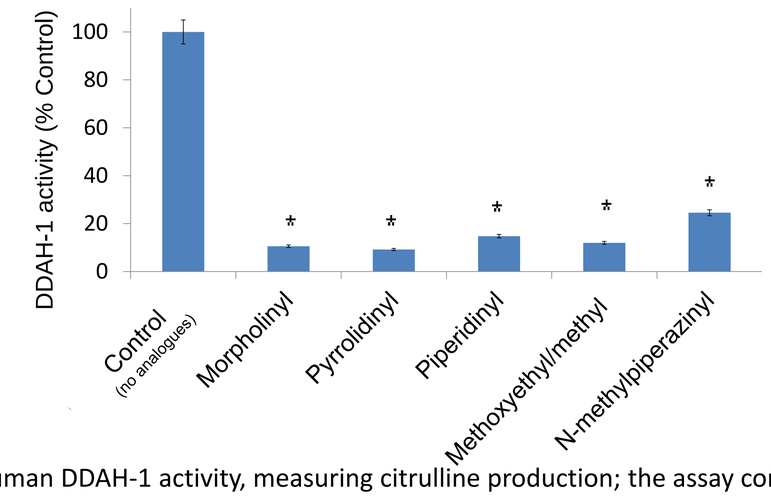


Figure 2: Human DDAH-1 activity, measuring citrulline production; the assay contained ADMA (100 μM) with N^G,N^G-disubstituted arginine analogues (100 μM), n=3, *p<0.05 with ANOVA.

Results

***In silico* DDAH1 predicted binding**: The human DDAH1 (PDB, 2JAJ) structure was used *in silico* to generate both Glide docking scores and predicted Autodock4 binding energy for the known SR257 DDAH1 inhibitor[1] and N^G,N^G-disubstituted arginines (Table 1).

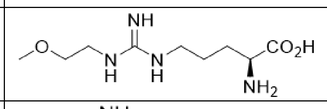
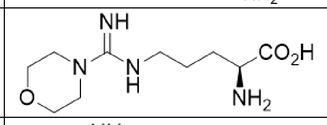
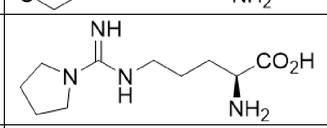
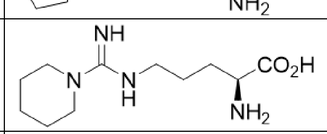
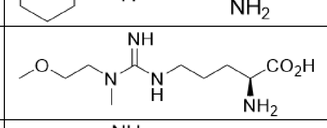
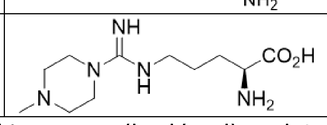
Compound	Structure	Glide docking score (kcal/mol)	Autodock4 binding energy (kcal/mol)
SR257		-5.657	-7.48
Morpholinyl		-9.007	-8.94
Pyrrolidinyl		-8.482	-9.01
Piperidinyl		-9.041	-9.85
Methoxyethyl/methyl		-4.943	-7.82
N-methylpiperazinyl		-4.763	-10.70

Table 1: Predicted Glide docking score (kcal/mol) and Autodock4 binding energy (kcal/mol) for the hDDAH1 (PDB 2JAJ).

SR257 (PDB 2JAJ[1]) forms hydrogen bonds with hDDAH1 Leu29, Asp72, Asp78, Val267, and Asp268 and hydrophobic interactions with Phe75 and Cys273 (Figure 3). Autodock4 predicted all N^G,N^G-disubstituted arginines to interact with Asp78, Arg144 and Asp268; Morpholinyl and Pyrrolidinyl were predicted to interact with Ser31. Methoxyethyl/methyl was the only N^G,N^G-disubstituted arginine predicted to interact with Cys273 (Figure 3).

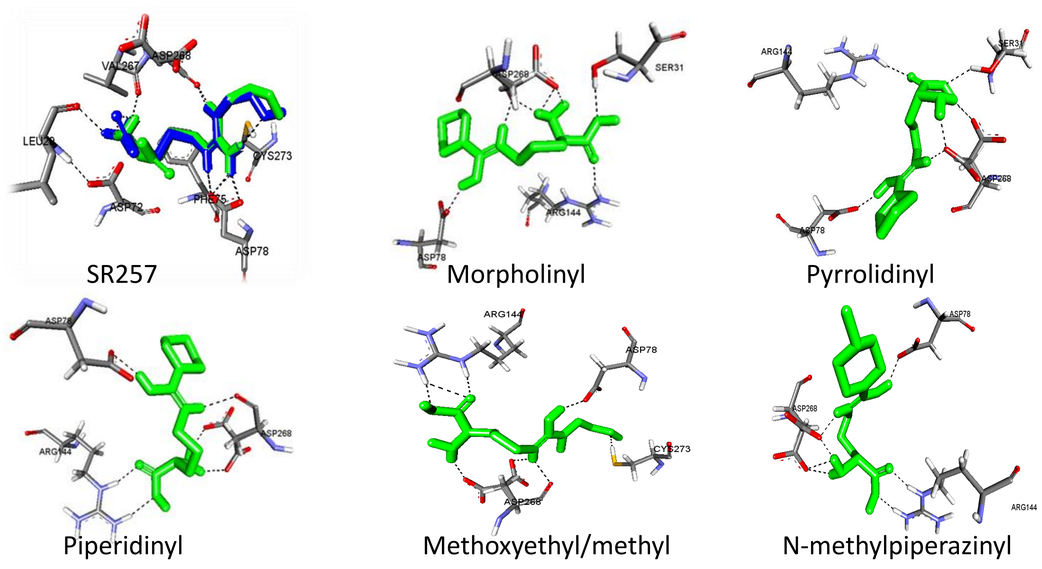


Figure 3: Predicted Human DDAH-1 interactions with N^G,N^G-disubstituted arginines.

Discussion

Both Autodock4 and Glide docking predicted higher binding energies for morpholinyl, pyrrolidinyl and piperinyl than the known SR257 compound. The morpholinyl compound has been described to be a weak inhibitor of hDDAH1[9]. *In vitro* assays confirmed these N^G,N^G-disubstituted arginines reduced DDAH1 activity. There was variation between Glide and Autodock4 in the docking predictions for methoxyethyl/methyl and N-methylpiperazinyl.

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

References

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Acknowledgements

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