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

Smith, C.L., Zloh, M. and Rossiter, S.

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

Authors: C.L. Smith, M. Zloh* and S. Rossiter*
Department of Life Sciences, University of Westminster, London
*Department of Pharmacy, University of Hertfordshire, Hatfield

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, and pharmacological DDAH1 inhibition has been proposed in order to increase methylarginines and reduce NO levels [2]. The SR257 arginine analogue, with N^G-methoxyethyl substituent, inhibits DDAH1 with an IC50 22 µM without directly inhibiting NOSs[1,3].

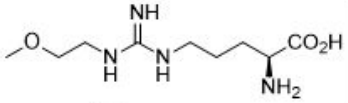
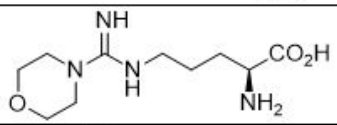
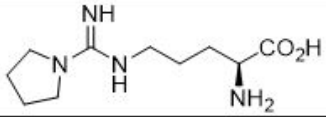
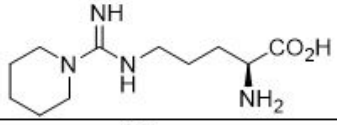
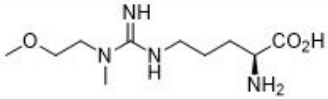
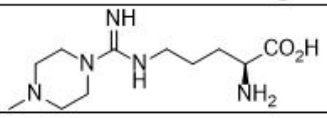
Methods: Acyclic and cyclic N^G,N^G-disubstituted arginines were made as previously described[4] using Katritzky's synthesis preparing trisubstituted guanidines from di-(benzotriazol-1-yl)methanimine[5].

Molecular docking was employed to explore interactions of these N^G,N^G-disubstituted arginines with human DDAH1 (PDB 2JAJ) using Glide (Schroedinger[6]) and Autodock4[7]. The published SR257 ligand was used to define the binding site with both software tools.

Recombinant human DDAH1 activity was measured using colorometric citrulline assay[8] containing ADMA (100 µM), sodium phosphate (10 mM 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: Recombinant DDAH1 activity was reduced to less than 25% of control (ADMA substrate, 100 µM) in the presence of 100 µM piperidinyl, methoxyethyl/methyl, *N*-methylpiperazinyl, with morpholinyl and pyrrolidinyl substituents reducing activity to less than 10% of control.

The *in silico* Glide docking score and predicted Autodock4 binding energy for human DDAH1 (PDB, 2JAJ) for the known SR257 DDAH1 inhibitor and N^G,N^G-disubstituted arginines are shown in the table:

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

Conclusion: Both Autodock4 and Glide docking predicted higher binding energies for morpholinyl, pyrrolidinyl and piperinyl than the known SR257 compound. *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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