Inhibition of Dimethylarginine Dimethylamino Hydrolase (DDAH) and Arginine Deiminase (ADI) by Pentafluorophenyl (PFP) sulfonates

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A range of pentafluorophenyl (PFP) sulfonate esters derived from the reaction of PFP vinyl sulfonate and various nitrones are shown to have significant inhibitory activity against the bacterial enzymes DDAH and ADI.

Nitric oxide (NO) is an important mediator of intracellular signaling and has attracted interest as a target for therapeutic intervention, as it is widely acknowledged that there are a variety of disease states for which NO is implicated.[1] One of the most significant problems associated with the design of inhibitors, is to target the pathological excess NO production without disrupting essential NO-mediated processes, often by seeking selectivity for a particular NOS isoform. One method for potentially circumventing these problems is the indirect modulation of NO levels by inhibition of the enzyme DDAH which is responsible for controlling levels of N^G-methyl-L-arginine (MMA) and N^G, N^G, dimethyl-L-arginine (ADMA) which are endogeneous inhibitors of NOS.[2,3]

Inhibition of bacterial DDAH[4] is also of interest as it offers opportunities for the development of new anti-bacterial agents. The structurally related enzyme arginine deiminase (ADI) is also a possible antibacterial/antiprotozoal target, as various pathogenic organisms utilize ADI to generate ATP under anaerobic conditions.[5a]

Recently high-resolution structures of a bacterial DDAH[6] and ADI[5] have been disclosed and it has been shown in both enzymes that the active site comprises a catalytic triad containing an acidic residue (Glu / Asp), a basic residue (His) and a cysteine residue (Cys) (Figure 1). Both enzymes are known to catalyze the conversion of the substrate(s) MMA and ADMA to citrulline as shown in figure 1.

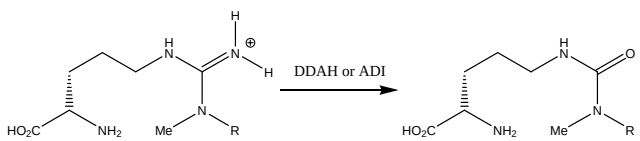


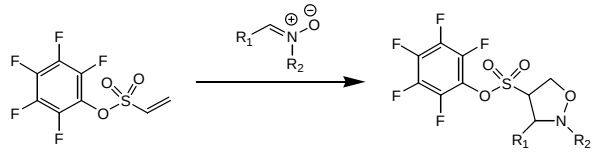
Figure 1*:* Conversion of ADMA (R = Me) and / or MMA (R = H) to citrulline are catalysed by DDAH and ADI.

In a recent communication Knipp *et al.* described the cysteine modification of DDAH by HcyNO and proposed this as a lead for the possible development of covalent inhibitors of DDAH and ADI.[7] In that work it was proposed that it should be possible to rationally design covalent inhibitors of DDAH based on those findings. That work has prompted us to disclose our own studies, which identify novel small molecule inhibitors of DDAH and ADI. Whilst the development of small molecule inhibitors of both DDAH and ADI is appealing, it is notable that there is only one known inhibitor of bacterial DDAH, which has modest affinity and is an arginine homologue,[8] and there are no known inhibitors of ADI. Herein we disclose our preliminary studies on the use of pentafluorophenyl (PFP) sulfonates as an unprecedented new class of enzyme inhibitors. The biological activity of the PFP-sulfonate group is completely unexplored and is highlighted here by the development of inhibitors of DDAH and ADI

As had previously been noted by one of us in the disclosure of the crystal structure of DDAH, the active site resembles that of a cysteine protease with a catalytic triad (Fig 1).[5]

The work of Roush *et al.* on the use of sulfonates and sulfonamides as inhibitors of cysteine proteases,[9] stimulated us to speculate that it may be possible to generate inhibitors of DDAH and ADI based on sulfonates and sulfonamides or closely related structures. This would offer an opportunity to develop molecular scaffolds, which would be markedly different in their structure to arginine mimetics, which may be a more obvious class of potential inhibitor. In order to test this speculative hypothesis we decided to evaluate a diverse collection of heterocyclic PFP-sulfonates as potential inhibitors of DDAH and ADI.

Our previously disclosed synthetic approach to such species was based on the 1,3-dipolar cycloaddition reaction of a PFP-sulfonate with nitrones (Scheme 1).[10]



Scheme 1. Synthetic approach to heterocyclic PFP-sulfonates via 1,3-dipolar cycloaddition

An initial screen of a variety of PFP-sulfonates and related structures at relatively high concentrations (500 µm, data not shown) provided some encouraging inhibition of both pseudomonas enzymes DDAH and ADI. At 50 µm a smaller selection of PFP sulfonates retained significant activity and their molecular structures are shown in Figure 2.

As can be seen from Table 1 the majority of these compounds were found to have activity against both DDAH and ADI at 50 µm concentration. These preliminary data indicate that there is greater inhibition of DDAH compared with ADI. IC_{50} values were determined for a small selection of the most active species and it can be seen that compounds **2**, **3**, **4**, **6** and **9** exhibit

† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See http://dx.doi.org/10.1039/b000000x/

significant activity against DDAH and compounds **3** and **6** also show significant activity against ADI.

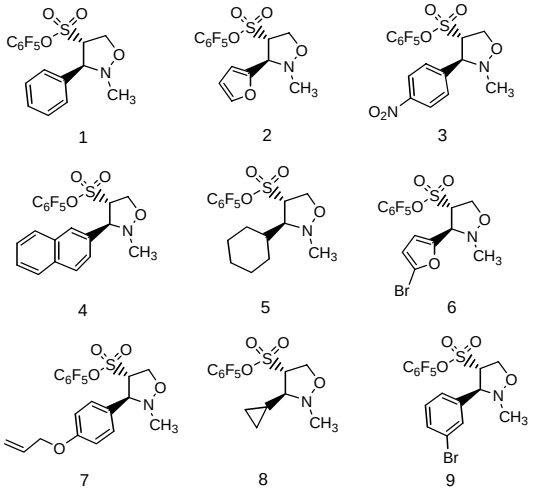


Figure 2: Molecular structures of PFP sulfonates exhibiting significant inhibition of PaDDAH and PaADI

These data suggest that there is potential for considerable optimization to give a new series of inhibitors based on the PFP-isoxazolidine structural motif.

Table 1. Activity of PFP-sulfonates against PaDDAH and PaADI (1mM substrate)

Entry	DDAH Inhibition at 50 μM	IC_{50} / DDAH, μM	ADI Inhibition at 50 μM	IC_{50} / ADI, μM
1	30%	-	14%	-
2	63%	34	27%	246
3	76%	21	35%	74
4	56%	32	33%	167
5	40%	-	26%	-
6	65%	16	38%	103
7	44%	-	14%	-
8	41%	-	15%	-
9	58%	58	27%	-

In order to assess the nature of the inhibition we carried out experiments on reversibility and time-dependence (Figure 3).

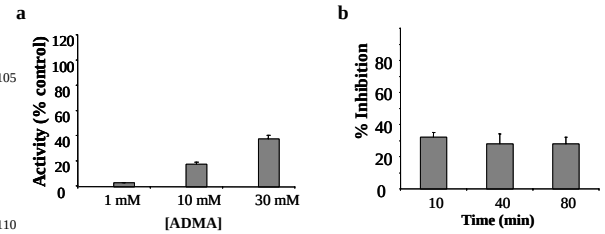


Figure 3. (a) Effect of increasing substrate concentration on inhibition of PaDDAH by compound **6**, 75μM. (b) Time-dependence of PaDDAH inhibition by compound **3**, 10μM.

Inhibition by compound **6** can be at least partially reversed by addition of increased amounts of substrate (Figure 3a), suggesting competitive inhibition. The large excess of substrate required to reverse inhibition may indicate that the inhibitor is more tightly bound than the substrate. In a separate experiment, compound **3** exhibited a constant level of DDAH inhibition over an 80-minute period (Figure 3b). Covalent inhibitors would show a time-dependent increase in inhibition as the enzyme becomes progressively irreversibly bound, therefore the evidence suggests that our inhibitors are not acting by a covalent mechanism. This is consistent with the observed reversibility of inhibition.[11]

In summary we have described new inhibitors of the enzymes DDAH and ADI. From these experiments it would appear that these PFP-sulfonates may be reversible inhibitors of DDAH. Irrespective of the detailed mechanism underlying the inhibition, this is the first time that non-substrate-like inhibitors for DDAH have been identified and these are the most potent inhibitors of bacterial DDAH currently known. Moreover these results identify the first small molecules to inhibit the enzyme ADI. The present study has also demonstrated that the PFP-sulfonate motif may play an important role in future studies directed toward identification of small molecule enzyme inhibitors and / or ligands for proteins. The simplicity with which diverse arrays of PFP-derivatives can be prepared may facilitate further small-molecule discovery activities. The further development of this work to identify details of the molecular interaction of these PFP-sulfonate derivatives with DDAH and ADI are underway.

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