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SYNTHESIS OF POTENT INHIBITORS OF HISTIDINOL DEHYDROGENASE[1]

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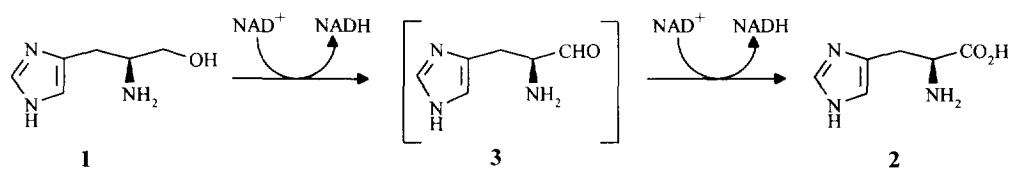
Abstract: Novel inhibitors of histidinol dehydrogenase are described. The most potent inhibitors, compounds **18** (K_i^* = 4.4 nM) and **19** (K_i^* = 2.9 nM) exploit a hitherto unreported lipophilic binding pocket adjoining the active site. Preliminary SAR data for this pocket are detailed. The electrophilic ketone **6** designed to bind to an active site nucleophile was a considerably weaker inhibitor (IC_{50} ~20μM). Copyright © 1996 Elsevier Science Ltd

Inhibition of essential amino acid biosynthesis is a well established mode of herbicidal action,[2] and is attractive from a toxicological viewpoint since the enzymes involved are not present in mammals. Consequently, agrochemical companies have expended considerable effort in devising inhibitors of established and novel target enzymes. Recently, several groups including those at AgrEvo,[3] Ciba-Geigy[4, 5, 6] and Zeneca[7] have become interested in inhibitors of histidine biosynthesis as potential herbicides. In particular, the sixth enzyme in the pathway, imidazole glycerol phosphate dehydratase,[3, 4, 7] and the ninth and final enzyme, histidinol dehydrogenase,[5, 6] have been investigated.

This communication describes the results of some of our investigations into the design and synthesis of inhibitors of histidinol dehydrogenase (HDH) (EC 1.1.1.23). The enzyme converts L-histidinol (**1**) to L-histidine (**2**) in an irreversible two step (four-electron) oxidation *via* a Bi Uni Uni Bi Ping Pong mechanism as shown in Scheme 1.[6] We considered HDH to be particularly attractive as a target for the rational design of active site inhibitors for three reasons. Firstly, it is the only enzyme in the biosynthetic pathway for which the substrate does not bear a phosphate group and hence inhibitors should be relatively non-polar and therefore

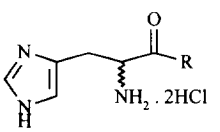
SCHEME 1



1 **3** **2**

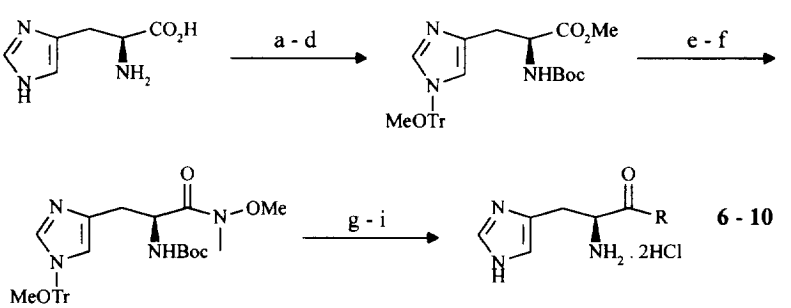
more typically herbicide-like in nature. Secondly, the intermediate L-histidinal (**3**) is bound extremely tightly to the enzyme (K_D = 1.4 x 10^{-11} M),[8] possibly as a serine-hemiacetal,[9] which suggested to us that electrophilic ketones might be good inhibitors.[10] Finally, HDH is mechanistically related to the well studied enzyme hydroxymethyl glutaryl CoA reductase[11] for which potent inhibitors are known.[12] Many of these inhibitors are composed of a substrate analogue with a lipophile attached at the position of hydride transfer. We hoped that a similar strategy might furnish inhibitors of HDH.

Grubmeyer *et al.* have reported on a number of HDH inhibitors, the most potent of which was the methyl ketone **4** (K_i = 5 μM).[13] We began our studies by resynthesising **4**, and using similar methodology,[13] the ethyl ketone **5**, which we found to be a less potent inhibitor than **4** (Table 1). The perfluroethyl ketone **6**, which we hoped might bind as a hemiacetal, was made, in 36% overall yield, as outlined in Scheme 2. The key step of this synthesis involves the condensation of perfluoroethyllithium[14] with a Weinreb amide derived from histidine. This compound was a disappointingly poor inhibitor even though it was more tightly bound than the non-fluorinated analogue **5** (Table 1).[15]



4: R = Me
5: R = Et

SCHEME 2



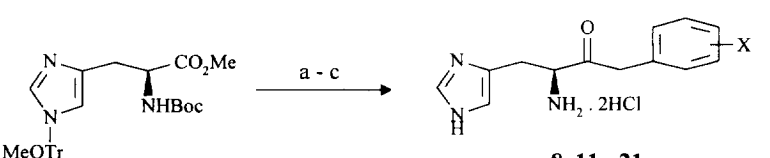
(a) MeOH, HCl, Δ, 16h; (b) 2.3eq. $(Boc)_2O$, 2eq. Et_3N, MeOH, r.t., 16h; (c) 0.1eq. K_2CO_3, MeOH, Δ, 16h;
(d) 1.1eq. MeOTrCl, 1.5eq. Et_3N, DMF, r.t., 16h; (e) 2eq. KOH, aq. MeOH, r.t., 2h;
(f) 2.3eq. CDI, DCM, r.t., 1h. Purge with N_2 then add 5.7eq. MeNHOMe.HCl, 27eq. Et_3N, r.t., 16h;
(g) 3eq. RM, THF, -78°C to 0°C, 4h; (h) 60% aq. TFA, r.t., 1h;[17] (i) aq. 1M HCl

TABLE 1

Structure	R	IC_{50} (μM) (*E. coli*)[16]
4	Me	10
5	Et	> 100
6	C_2F_5	20
7	Ph	> 100
8	CH_2Ph	1
9	CH_2CH_2Ph	100
10	$CH_2CH_2CH_2Ph$	80

Our attention next turned to the search for a lipophilic pocket adjacent to the active site, as found in hydroxymethyl glutaryl CoA reductase.[12] We elected to probe for the presence of this pocket with a phenyl ring attached *via* a chain of variable length as seen in structures **7 - 10**. These compounds were synthesised as shown in Scheme 2 in 42 - 54% overall yields from L-histidine. As can be seen from Table 1, the enzyme showed a very marked preference for the benzyl ketone **8**. The fact that this compound was a stronger inhibitor than the methyl ketone **4** implied that we had indeed discovered a lipophilic binding site adjoining the active site of HDH. In as much as the nature or even existance of such a lipophilic pocket might be organism dependent we decided to switch from using the *E. coli* enzyme to a recombinant plant enzyme from cabbage.[5, 16] When tested, the benzyl ketone **8** was found to also be a good inhibitor of the cabbage enzyme and in fact was bound ten times more tightly than to the *E. coli* enzyme. We now set out to explore the SAR of the lipophilic pocket by preparing a range of substituted benzylic ketones **11 - 21** (Table 2), using a modified synthesis strategy involving the condensation of the dianion of various substituted phenylacetic acids with a protected histidine methyl ester (Scheme 3). In the case of compounds **13** and **16**, the aromatic amino group was protected as an ethyl carbamate during the synthesis, and compound **21** was made by the bromination (Br_2, AcOH) of the fully protected analogue of **16**. Based on the results shown in Table 2, it was clear that substitution in the 4-position of the phenyl group could lead to increased levels of inhibition.

SCHEME 3



8, 11 - 21

(a) 3-4eq. $ArCH_2CO_2H$, 6-8eq. LHMDS, THF, -78°C to r.t., 16h; (b) 60% aq. TFA, r.t., 1h;[17] (c) aq. 1M HCl

Structures **18** (4 - Br)and **19** (4 - Phenyl) are the most potent inhibitors of this enzyme identified to date and showed competitive slow binding kinetics with K_i^* values of 4.4 and 2.9 nM, respectively. It was interesting to note that despite the fact that a 3-amino substituent (Structure **13**) was well tolerated, it was incompatible with further lipophilic substitution in the 4-position (Structure **21**). Since it was clear that the lipophilic pocket could effectively bind a 3,4-disubstituted phenyl group (Structure **20**), we inferred that the 3-amino group may have caused the phenyl group to bind in an alternative more sterically demanding orientation.

TABLE 2

Structure	X	IC_{50} (μM) (Cabbage)[16]	K_i^* (nM) (Cabbage)[16]
8	H	0.1	7.6±0.8
11	2-Br	1	-
12	3-OH	0.3	-
13	3-NH_2	0.05	7.4±3.0
14	3-Br	0.1	-
15	4-OMe	1	-
16	4-NH_2	0.5	-
17	4-Cl	0.3	-
18	4-Br	0.04	4.4±0.15
19	4-Ph	0.04	2.9±0.4
20	3-Br, 4-Br	0.05	-
21	3-NH_2, 4-Br	8	-

Despite having discovered potent inhibitors of the plant enzyme, even the best compounds **18** and **19** were not herbicidal. Nevertheless, we were encouraged to observe that compounds **13** and **18** inhibited the root growth of germinating rice seedlings grown in agar containing Murashige and Skoog basal salts. In the case of compound **13** this biological activity could be reversed specifically by the addition of histidine. These results suggest that there may be an uptake problem, perhaps due to the chemical instability of the free amines or the very low log p of the hydrochlorides.

In conclusion, we have demonstrated that potent inhibitors of HDH can be prepared by exploiting a hitherto unreported lipophilic binding pocket which adjoins the active site. The best compounds (S)-1-(4-bromophenyl)-4-(4-imidazolyl)-3-amino-2-butanone (**18**) and (S)-1-(4-biphenyl)-4-(4-imidazolyl)-3-amino-2-butanone (**19**) bind over 1000 times more tightly than the substrate histidinol or the previously best known inhibitor **4**.

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References and Notes

1 This work represents an ongoing interest within AgrEvo in using biochemical reasoning to generate leads for the discovery of new agrochemicals. For other recent examples see reference 3 and references therein.

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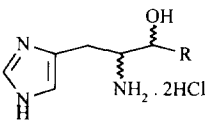
15 We have also prepared several compounds which could potentially trap an enzymic nucleophile upon oxidation at the active site of HDH, for example structures **22** - **24**. These compounds were very poor inhibitors of HDH. However, as far as we were able to determine they were not enzyme substrates and so were not transformed into the active species (i.e. the ketones).



22: R = $CH{=}CH_2$
23: R = $C{\equiv}CH$
24: R = CF_3

16 Enzyme activity was assayed spectrophotometrically essentially as described in Loper and Adams (Loper, J.C.; Adams, E.J. *J. Biol. Chem.*, **1965**, *240*, 788). For IC_{50} determination the assay mixture, containing 150 mM glycine, NaOH (pH 9.2) and 5 mM NAD^+, was preincubated with inhibitor for 10 minutes at 25°C before the reaction was started by the addition of histidinol (40 μM final concentration). *E. coli* HDH ($K_{m\ app}$ histidinol = 12 μM) was cloned from the histidine operon using mutagenic PCR primers. The cabbage enzyme ($K_{m\ app}$ histidinol = 8 μM) was cloned from cDNA by PCR using primers designed to the mature form of the protein predicted by Nagai *et al.* (Nagai, A.; Ward, E.; Beck, J.; Tada, S.; Chang, J-Y.; Scheidegger, A.; Ryals, J. *Proc. Natl. Acad. Sci. USA*, **1991**, *88*, 4133). Although $MnCl_2$ provided a small stimulation of the enzyme, it was not included in the assay because it was found to reduce enzyme stability. For K_i determination the concentration of histidinol was 1 mM and NAD^+ 2 mM. Each value represents the mean of five individual assays with varying inhibitor concentrations.

17 We have found that the TFA is significantly more efficient than HCl at removing the trityl group. However, the resultant TFA salts are usually gums so they were converted into the more easily handled HCl salts which were amorphous powders. In the case of compound **6** the protecting groups were removed by treating with anhydrous HCl in EtOAc for 4.5 hours at room temperature.

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