Bioorganic & Medicinal Chemistry Letters, Vol.3, No.2, pp. 353-356, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

SYNTHESIS OF 4(5)-IMIDAZOLYL ALLYLAMINES AND PROPARGYLAMINES AS INHIBITORS OF DIAMINE OXIDASE

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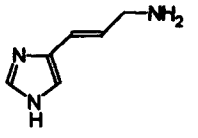
(*Received in USA* 2 *November* 1992)

ABSTRACT: A series of four side-chain unsaturated histamine analogs were synthesized and evaluated as inhibitors of Diamine Oxidase, (DAO). All four target compounds were potent competitive inhibitors of DAO with K_i values ranging from 0.17 to 7.1 μM.

Diamine oxidase, (DAO, EC[1.4.3.6]), is a copper containing amine oxidase whose known physiological substrates include histamine, putrescine and cadaverine.[1] DAO, once thought to be a pyridoxal enzyme, or a PQQ enzyme[2] has recently been shown to contain the tyrosine-derived cofactor topa quinone.[3] Originally named histaminase, DAO was discovered over sixty years ago, but its physiological functions remain unclear and the role of DAO in a variety of systems is under active investigation.[4] Aminoguanidine, a non-competitive reversible inhibitor of DAO,[5] has been employed in many of these studies, but potent, selective, competitive inhibitors could provide additional information concerning the structural binding requirements of the enzyme active site which are not available using the simple compound aminoguanidine. We undertook the synthesis and evaluation of a series of potential mechanism-based irreversible inhibitors of DAO for use in such studies. Consideration of the published substrate specificities of DAO[6] and other amine oxidases[7] led us to synthesize side-chain unsaturated analogs of histamine, compounds 1, 2, 3, and 4 as inhibitors of DAO.



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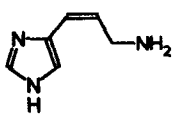

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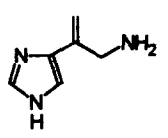

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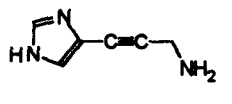

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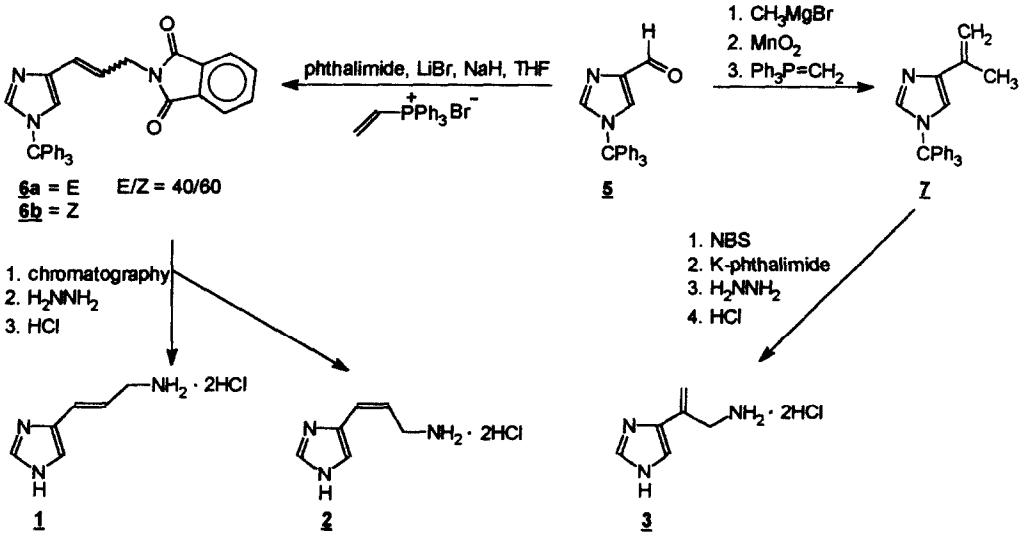
The syntheses of imidazolyl-allylamines 1, 2, and 3 are shown in Scheme I. We prepared compound 5, N-trityl-4-formylimidazole, from 4(5)-

hydroxymethylimidazole[8] according to published procedures.[9] Following Meyers' modification[10] of a general procedure for synthesis of allyl amines, we combined 5 with a mixture of vinyltriphenylphosphonium bromide, sodium hydride, lithium bromide, and phthalimide in THF to afford a 90% yield of a 40/60 mixture of E and Z alkenes 6a and 6b. Without LiBr this reaction affords an E/Z ratio of 8/92. Isomers 6a and 6b were readily separated by silica gel chromatography, and each isomer deprotected with hydrazine followed by 1N HCl to afford target compounds 1 and 2 in 80% yields.[11]

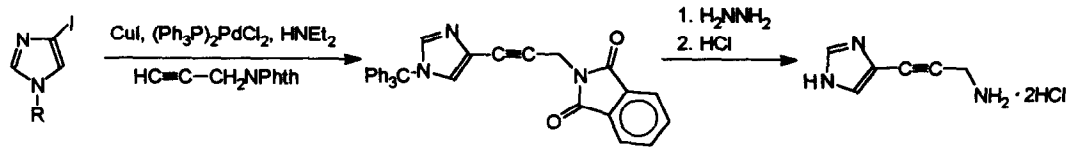
Reaction of 5 with methyl magnesium bromide to form a 2° alcohol, (80%), followed by oxidation with MnO_2, (90%), and reaction of the ketone thus formed[12] with triphenylphosphonium methylid afforded propene derivative 7, (50%). Allylic bromination of 7 with NBS,[13] (50%), followed by displacement of the allylic bromide with potassium phthalimide, (60%), and removal of protecting groups with hydrazine and HCl, (80%), afforded target compound 3.



Scheme I.

Our synthesis of imidazolyl-propargylamine, target compound 4, is shown in Scheme II. 4(5)-Iodoimidazole, (8a), was synthesized from imidazole by published procedures[14] and converted to N-trityl-4-iodoimidazole, (8b), by reaction with triphenylmethylchloride and Et_3N in DMF, (98%). Coupling of 8b with N-propargylphthalimide (prepared from propargylamine and phthalic anhydride) in a reaction catalyzed by CuI and $(Ph_3P)_2PdCl_2$[15] afforded a 57%

yield of compound 9. Hydrazine and HCl were used to remove the protecting groups from 9 to give compound 4 in 60% yield.



8a R = H
8b R = CPh_3

9

4

Scheme II.

Target compounds 1, 2, 3, and 4, were assayed for their ability to inhibit hog kidney DAO in a spectrometric assay developed by Bardsley et al. using p-dimethylaminomethylbenzylamine as substrate.[16] The mode of inhibition was determined from Hanes plots of [S]/v vs. [S]. All four compounds had a pattern of parallel lines indicating competitive inhibition.[17] The K_i values were obtained from replots of the intercepts vd inhibitor concentration and the results are shown in Table I. All of the target compounds were very good inhibitors of DAO with K_i values in the low- to sub-micromolar range. Both 1 and 2, with K_i values of 0.20 and 0.17 μM, respectively, compare well with aminoguanidine (K_i= 0.14 μM,[5]), the standard inhibitor of DAO employed in most studies.

Table I. Unsaturated Histamine Analogs As DAO Inhibitors

Compound	Mode of Inhibition	K_i, μM
1	Competitive	0.20
2	Competitive	0.17
3	Competitive	7.1
4	Competitive	1.7

One of the criteria for a mechanism-based irreversible inhibitor or affinity label is time dependent inactivation of the target enzyme.[18] We

could readily observe such inactivation of DAO within one minute with 1,4-diaminobutyne, a known (but non-selective) mechanism-based inhibitor of DAO.[18] However, none of the target compounds exhibited any evidence of time dependent inactivation. All of the inhibitors demonstrated very strong inhibition which nevertheless remained constant for twenty minutes, at which time the experiments were halted. Therefore these compounds are not mechanism-based irreversible inhibitors of DAO, but represent a new class of potent reversible competitive inhibitors. These compounds provide valuable information on steric constraints to binding at the active site of DAO.

Acknowledgement: This work was supported in part by N.I.H. Grant AI31193.

References and Notes

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