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Combinatorial Synthesis of SSAO Inhibitors Using Sonogashira Coupling: SAR of Aryl Propargylic Amines

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Abstract—The structure–activity relationships for semicarbazide-sensitive amine oxidase (SSAO) inhibitors based on arylpropynylamines was investigated using solution-phase combinatorial Sonogashira coupling. The results suggest that binding to the active site occurs by coordination of the amine to the proximal copper(II) and formation of a π-complex between topaquinone and the electron-rich aryl group of the inhibitor.

Semicarbazide-sensitive amine oxidases (SSAOs) are a widespread group of enzymes found in plants and animals, possessing a copper atom and an oxidised tyrosine residue (topaquinone) in their active site.[1,2] While they appear to be involved in the oxidative deamination of endogenous amines, their precise function has yet to be fully elucidated. However in humans, SSAOs have been implicated in the physiopathology of diabetes. Elevated levels of SSAO have been reported in diabetics and it is believed that vascular damage may be due to the toxicity of formaldehyde and hydrogen peroxide, formed by the action of SSAO on endogenous methylamine.[3] Drugs that inhibit SSAOs may thus prove useful as prophylactics preventing long-term retinal damage associated with diabetes mellitus.[4]

In the course of other work, we found that the arylpropenylamine **2**, acted as a potent inhibitor of bovine plasma SSAO (EC 1.4.3.6). Compound **2** was synthesised in a straightforward manner as shown in Scheme 1.

Treatment of methyl 3,4-methylenedioxycinnamate with diisobutylaluminum hydride in THF followed by reaction of the alcohol with thionyl chloride afforded the arylpropenyl chloride **1** in 72% yield. Heating this chloride with methylamine in THF in a sealed tube gave a mixture of both **2** and the disubstituted tertiary amine.

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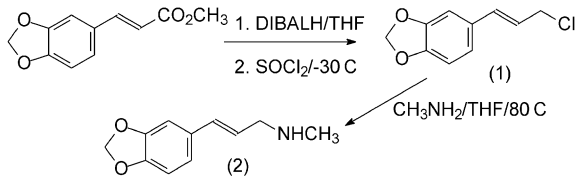
Enzymatic assay using the oxidation of benzylamine by bovine plasma SSAO was carried out and showed that amine **2** was a potent inhibitor ($K_i = 2.7\,\mu M$). Kinetic analysis using Leonora indicated that **2** acted as a competitive inhibitor.[5]

We initially suspected that **2** may inhibit the enzyme by reversible addition to topaquinone forming a quinone iminium species, which is unable to undergo conversion to an aryl imine (Scheme 2). This view was supported by reports that, aside from one exception,[6] only primary amines are substrates for SSAOs. With their therapeutic potential in mind, we sought a simple procedure for rapidly evaluating structure–activity relationships of this class of compounds.

The Sonogashira reaction is amenable to combinatorial synthesis on solid support[8] and a recent report describes the solid-phase synthesis of closely related tertiary arylpropynylamines using Sonogashira coupling, followed by a Mannich condensation (Scheme 3).[9] However, we sought a procedure which would be amenable to the synthesis of primary, secondary and tertiary amines in



Scheme 1. Synthesis of arylpropenylamine inhibitor.

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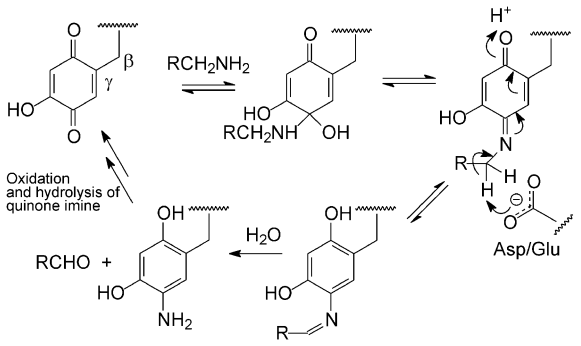
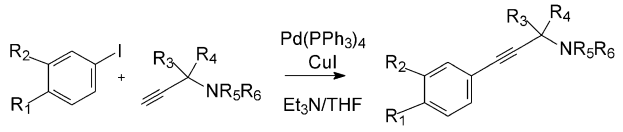


Scheme 2. Proposed mechanism of SSAO oxidative deamination.[7]



Scheme 3. Sonogashira coupling of iodoarenes with propargyl amines.

solution. We were also encouraged to adopt a Sonogashira reaction as the key step in our synthesis by reports of the generation of libraries in solution-phase organometallic couplings.[10] In addition to this, while the coupling of aryl halides with propargyl amines had not been reported, many other examples of the Sonogashira reaction suggested that there would be no need for protecting the amine.[11] This synthetic plan would enable us to examine the SAR for both the aryl and the amine portions of the substrate. While this would afford alkynes and not alkenes, it was envisioned that this route would provide both arylpropynyl and arylpropenyl amines, after partial reduction of the triple bond. Both these groups of compounds were of interest as potential SSAO inhibitors. This communication reports the inhibition of bovine SSAO by a small indexed library of arylpropynylamines.

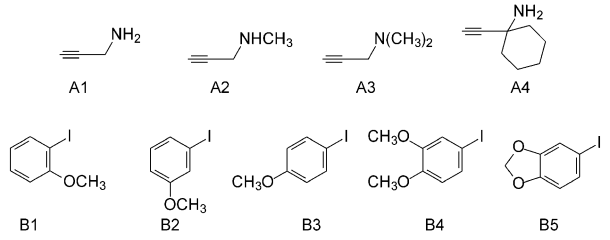


Figure 1. Combinatorial library components.

Table 1. Product matrix showing % inhibition of SSAO[a]

	B1	B2	B3	B4	B5	%I
A1	610	2013	3721	4270	4331	61
A2	450	1485	2745	3150	3195	45
A3	650	2145	3965	**4550**	**4615**	65
A4	280	924	1708	1960	1988	28
%I	10	33	61	70	71	

[a]Values represent products of %I. Values in bold indicate the most potent inhibitors.

For this initial study, commercially available propargyl amines, A1–A4 (Fig. 1), were coupled with a series of alkoxyiodoarenes, B1–B5, selected to explore the importance of both the position of the methoxy group and the presence of the methylenedioxy group. The dioxygenated iodoarenes, B4 and B5, were prepared by iodonation of catechol dimethylether and benzene-1,3-dioxole, respectively, with silver sulfate/iodine.[12]

Coupling of the alkynes with the iodoarenes was carried out in THF/triethylamine at room temperature with appropriate molar proportions of each reactant (Scheme 3).[13] GC–MS was used to confirm consumption of the starting materials, and as expected, in all cases the reactions went to completion with equimolar amounts of the arylpropynylamines formed. The reaction mixtures were purified simply by removal of the solvent, followed by extraction with dilute hydrochloric acid, then basification and back extraction. This afforded mixtures of arylpropynylamines that were suitable for combinatorial enzymatic assay.[14] The results of these assays are shown in Table 1.

While indexed combinatorial assays[15] are fraught with a number of possible complications, a number of trends are apparent from the assays. Firstly, inhibition appears maximised by methoxy substitution in the 4-position as opposed to the 2- or 3-positions; secondly dioxygenation appears to enhance inhibition, and thirdly, substitution on the amine carbon with bulky substituents dramatically reduces inhibition. These results suggested that the arylpropynylamine formed by coupling A3 with B5, and A3 and B4 were the most potent bovine plasma SSAO inhibitors of this group. In order to confirm these results, both these compounds were synthesized in 80 and 81% yield, respectively, by Sonogashira coupling.[16] Enzymatic assay[17] confirmed that the arylpropynylamine derived from A3 and B4 was a marginally more potent inhibitor of bovine plasma SSAO ($K_i = 2.9$ μM), than that from A3 and B5 ($K_i = 3.1$ μM).

The results indicate that increasing electron density of the aryl substituent increases inhibition, possibly due to the formation of a π-complex at the binding site. This led us to closely examine the active sites of the family of enzymes which have been studied quite extensively by crystallographic studies. While the X-ray structure of bovine plasma SSAO has not been reported, the enzymes in this family that have been studied have common conserved regions which correspond to the active site. The topaquinone moiety is involved in oxidative deamination, while the nearby copper(II) is believed to be involved in reoxidation of the reduced amino topaquinone (Scheme 2), thus regenerating the active enzyme.[19] X-ray studies on pea seedling amine oxidase, also a topaquinone/copper-dependant amine oxidase, show that the distance between the copper and the oxygen at the topaquinone 2-position is about 6 Å.[20] This same study also showed considerable structural homology between the enzyme from pea seedlings and *Escherichia coli* amine oxidase. The orientation of the topaquinone unit varies, with considerable flexibility in rotation about the β–γ bond (Scheme 2), having been

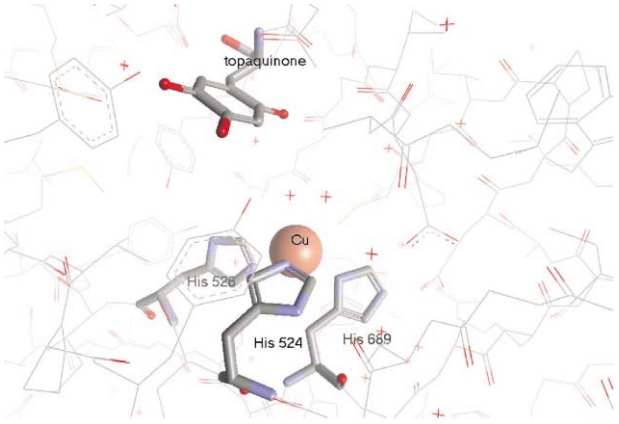


Figure 2. Active site of *E. coli* amine oxidase.[18]

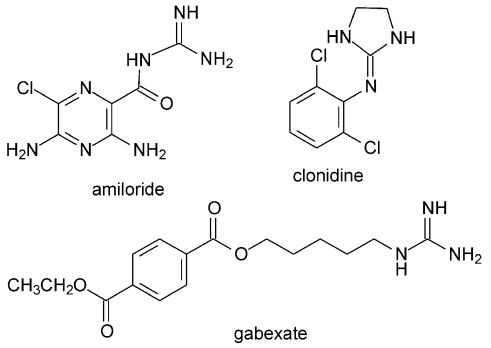


Figure 3. Competitive inhibitors of porcine kidney amine oxidase.[23]

suggested.[21] We speculate that these observations can be explained by the formation of a π-complex between the aromatic ring of the arylpropynylamines and topaquinone with additional binding of the amine to the nearby copper atom (Fig. 2). It is reasonable to assume that ligation of the amine to the copper involves displacement of one of the two water molecules, which along with the histidine residues H442, H444 and H603, form a distorted square-pyramidal geometry.[22]

This proposed binding is supported by the reported competitive inhibition of porcine kidney amine oxidase, a closely related SSAO, by amiloride, gabexate and clonidine (Fig. 3) (K_i 10, 27 and 900 μM, respectively).[23] All of these drugs possess a copper ligand (guanidine functional group) attached to an aromatic ring separated by approximately 6 Å in amiloride, 13 Å gabexate and 4 Å clonidine. Their relative efficacy as inhibitors appears to be related to both the aryl–Cu ligand distance and the electron density of the aryl substituent and thus is consistent with the inhibition mechanism proposed here.

We are now in the process of synthesising a series of new inhibitors, based on these findings which are designed to test this hypothesis. These results will be reported in due course.

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16. Synthesis of 3-benzo[1,3]dioxol-5-yl-prop-2-ynyl)dimethylamine (A3B5). A mixture of 1-dimethylamino-2-propyne (0.310 mL), 3,4-methylenedioxyiodobenzene (120 mg), $Pd(PPh_3)_4$ (28 mg), and CuI (9.3 mg) in dry THF (2.5 mL) and triethylamine (2.5 mL) was stirred under nitrogen for 20 h. The THF and triethylamine were removed under reduced pressure, and the residue was dissolved in Et_2O (15 mL) and extracted with 5% HCl (3×15 mL). The combined aqueous extracts were then neutralised with 5% NaOH and extracted with CH_2Cl_2 (3×100 mL) the combined organic extracts were washed with water and dried over anhydrous Na_2SO_4. Concentration under reduced pressure gave a brown oil which was purified by flash chromatography on silica (CH_2Cl_2/EtOH, 9:1). This afforded the arylpropynylamine (A3B5) as a colourless oil (70 mg, 80%). MS (EI 70 eV): *m/z* 203 (55%), 202 (85), 172 (16), 160 (20), 159 (100), 144 (17), 130 (13), 129 (14), 103 (12), 102 (23), 101 (30), 82 (36), 77 (17), 75 (36), 74 (16), 63 (12), 51 (23), 42 (62). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.32, 6H, s, $N(CH_3)_2$; 3.40, 2H, s, CH_2; 5.92, 2H, s, OCH_2O; 6.71, 1H, d (J=8.1 Hz), H7; 6.86, 1H, d (J=1.5 Hz), H4; 6.93, 1H, dd (J=1.6, 8.0 Hz), H6. ^{13}C NMR (75.5 MHz $CDCl_3$): δ 44.2, 48.6, 82.9, 85.0, 101.2, 108.3, 111.7, 116.5, 126.1, 147.3, 147.6. HR-MS (EI 70 eV): found 203.09508, calcd for $C_{12}H_{13}NO_2$ 203.09464. 3-(3,4-Dimethoxyphenyl)-prop-2-ynyl]dimethylamine (A3B4) was synthesised by the same procedure described for A3B5 in 81% yield. MS (EI 70eV): *m/z* 219 (60%), 218 (100), 204 (14), 203 (14), 202 (17), 188 (29), 187 (16), 176 (21), 175 (100), 161 (12), 160 (11), 132 (10), 131 (44), 130 (13), 115 (10), 103 (13), 102 (19), 89 (21), 82 (50), 63 (28), 58 (15), 57 (10), 42 (60). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.34, 6H, s, $N(CH_3)_2$; 3.41, 2H, s, CH_2; 3.83, 3.85, each 3H, OCH_3; 6.76, 1H, d (J=8.3 Hz), H5; 6.92, 1H, d (J=1.8 Hz), H2; 7.01, 1H, dd (J=1.9, 8.3 Hz), H6. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 44.3, 48.6, 55.9, 83.1, 85.1, 111.1, 114.6, 115.5, 124.9, 148.6, 149.3. HR-MS (EI 70 eV): found 219.12737, calcd for $C_{13}H_{17}NO_2$ 219.12593.
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photometric monitoring of the benzylamine oxidation by Bovine SAO (Sigma) as described by Tabor, C. W.; Tabor, H.; Rosenthal, S. M. *J. Biol. Chem.* **1954**, *208*, 645.
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