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Structural and enzyme activity studies demonstrate that aryl substituted 2,3-butadienamine analogs inactivate *Arthrobacter globiformis* amine oxidase (AGAO) by chemical derivatization of the 2,4,5-trihydroxyphenylalanine quinone (TPQ) cofactor ☆

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ABSTRACT

Copper amine oxidases (CAOs) are a family of redox active enzymes containing a 2,4,5-trihydroxyphenylalanine quinone (TPQ) cofactor generated from post translational modification of an active site tyrosine residue. The *Arthrobacter globiformis* amine oxidase (AGAO) has been widely used as a model to guide the design and development of selective inhibitors of CAOs. In this study, two aryl 2,3-butadienamine analogs, racemic 5-phenoxy-2,3-pentadienylamine (POPDA) and racemic 6-phenyl-2,3-hexadienylamine (PHDA), were synthesized and evaluated as mechanism-based inactivators of AGAO. Crystal structures show that both compounds form a covalent adduct with the amino group of the substrate-reduced TPQ, and that the chemical structures of the rac-PHDA and rac-POPDA modified TPQ differ by the allenic carbon that is attached to the cofactor. A chemical mechanism accounting for the formation of the respective TPQ derivative is proposed. Under steady-state conditions, no recovery of enzyme activity is detected when AGAO pre-treated with rac-PHDA or rac-POPDA is diluted with excess amount of the benzylamine substrate (100-fold K_m). Comparing the IC_{50} values further reveals that the phenoxy substituent in POPDA offers an approximately 4-fold increase in inhibition potency, which can be attributed to a favourable binding interaction between the oxygen atom in the phenoxy group and the active site of AGAO as revealed by crystallographic studies. This hypothesis is corroborated by the observed >3-fold higher partition ratio of PHDA compared to POPDA. Taken together, the results presented in this study reveal the mechanism by which aryl 2,3-butadienamines act as mechanism-based inhibitors of AGAO, and the potency of enzyme inactivation could be fine-tuned by optimizing binding interaction between the aryl substituent and the enzyme active site.

1. Introduction

Copper amine oxidases (CAOs) are a family of redox active enzymes that catalyze the oxidative deamination of primary amines to aldehydes, with concomitant reduction of O_2 to H_2O_2 (Scheme 1 [1–3]). CAOs are nearly ubiquitous in nature, as they are found in plants, most yeasts, microorganisms, and mammals but somewhat surprisingly not in archaea. Most CAOs contain a 2,4,5-trihydroxyphenylalanine quinone (TPQ) cofactor, which is derived from the post-translational modification of an active site tyrosine residue [4–5]. During enzyme catalysis, the primary amine substrate condenses with TPQ to form a substrate Schiff base adduct (SSB). The SSB then tautomerizes to a product Schiff base (PSB), which upon hydrolysis affords a reduced aminated TPQ (TPQ_{amr})

Abbreviations: CAO, copper-containing amine oxidase; AGAO, *Arthrobacter globiformis* amine oxidase; PHDA, racemic 6-phenyl-2,3-hexadienylamine; POPDA, racemic 5-phenoxy-2,3-pentadienylamine; PSB, product Schiff base; SSB, substrate Schiff base; TPQ, trihydroxyphenylalanine quinone

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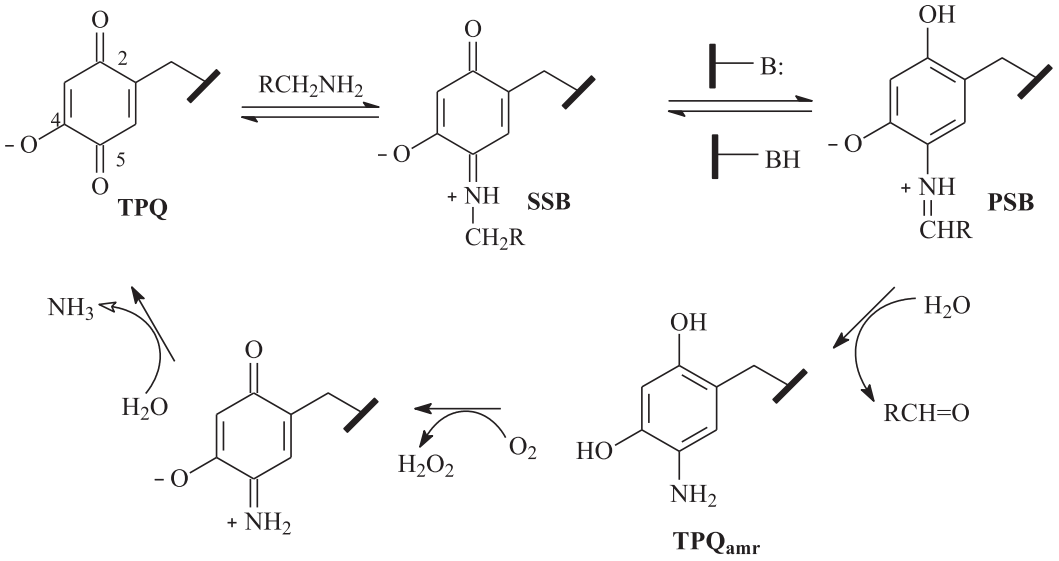

Scheme 1. Proposed mechanism for the TPQ-catalyzed oxidation of primary amine in copper amine oxidases.

and an aldehyde. These events are collectively known as the reductive half-reaction. In the oxidative half-reaction, the TPQ_{amr} is oxidized by O_2 to form quinonimine, which then undergoes hydrolysis to release ammonia to regenerate the TPQ cofactor.

In designing selective inhibitors against CAOs, a mechanism-based strategy targeting the reductive half-reaction has been exploited [6]. In particular, the approach of incorporating an electrophile adjacent to the substrate amino group in primary amines containing an aromatic group led to the synthesis of 4-aryloxy 2-butynamines as selective mechanism-based inhibitors [7]. Previous crystallographic studies of AGAO treated with two 4-aryloxy-2-butynamine analogs revealed a mechanism of enzyme inactivation involving covalent attachment of the α,β-unsaturated aldehyde turnover product generated from amine substrate oxidation to the amino group of TPQ_{amr} derived from the first half-reaction.

To further investigate the kinds of electrophile that can be used to design mechanism-based inhibitors of CAOs, two aryl 2,3-butadienamine analogs, racemic 5-phenoxy-2,3-pentadienylamine (POPDA) and racemic 6-phenyl-2,3-hexadienylamine, (PHDA, Fig. 1) were synthesized. These two compounds contain the 1-amino-2,3-butadiene moiety that has been shown by Qiao and coworkers to inactivate bovine plasma amine oxidase (BPAO) with higher potency compared to 1-amino-3-butyne amine [8]. As aromatic amines containing the 1-amino-3-butyne amine moiety are mechanism-based inactivators of AGAO, it is plausible that rac-PHDA and rac-POPDA will inhibit AGAO. To test this hypothesis, the crystal structures of AGAO treated with rac-PHDA and rac-POPDA, as well as the enzyme inhibition profiles were determined. The findings presented in this study provide a structural framework to guide the design of enzyme selective mechanism-based allenic amine inhibitors for the respective enzyme that may benefit pharmaceutical developments.

2. Materials and methods

2.1. Synthesis of racemic 6-phenyl-2,3-hexadienylamine hydrochloride (PHDA)

A dioxane solution of the t-boc protected propargylamine was refluxed with 3-phenylpropanal, diisopropylamine and freshly prepared CuBr under Argon for 12 h using the method reported in by Casara et al [9]. The reaction was then quenched with 1 N acetic acid followed by extraction with diethyl ether. The organic layer was dried with anhydrous Na_2SO_4, prior to rotary evaporation under reduced pressure. The resulting crude reaction mixture was purified by silica gel flash chromatography to produce the t-boc protected 6-phenyl-hexa-2,3-dienylamine. The t-boc group was deprotected with 3 N HCl at room temperature. Removal of the solvent in excess HCl under vacuum afforded 1-amino-2,3-hexadiene hydrochloride with an overall 20% yield. ^{1}H NMR spectra were obtained using aVarian 400 MHz spectrometers with the chemical shifts being referenced to TMS or the solvent peak. ^{1}H NMR (CD_3OD) δ 7.24 (5H, m), 5.46 (1H, m), 5.29 (1H, m), 3.39 (2H, broad multiplet), 2.75 (2H, m), 2.38 (2H, m); ^{13}C NMR ($CDCl_3$) 206.25, 142.75, 129.74, 129.49, 127.14, 95.22, 86.06, 39.69, 36.20, 31.24; HRFABMS MH^+ m/z observed 174.12804, $C_{12}H_{16}N$ calculated 174.12827.

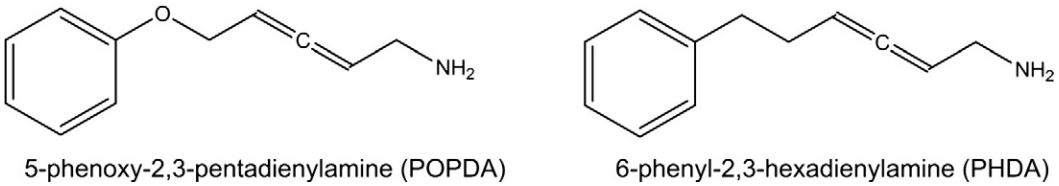

Fig. 1. Chemical structure of 5-phenoxy-2,3-pentadienylamine (POPDA) and 6-phenyl-2,3-hexadienylamine (PHDA). These two compounds were synthesized as described in Materials and methods as a racemic mixture. Both compounds first act as substrates to reduce the TPQ cofactor in AGAO and then chemically modify the reduced TPQ cofactor to inactivate enzyme turnover.