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Synthesis of kojic acid derivatives as secondary binding site probes of D-amino acid oxidase

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ABSTRACT

A series of kojic acid (5-hydroxy-2-hydroxymethyl-4*H*-pyran-4-one) derivatives were synthesized and tested for their ability to inhibit D-amino acid oxidase (DAAO). Various substituents were incorporated into kojic acid at its 2-hydroxymethyl group. These analogs serve as useful molecular probes to explore the secondary binding site, which can be exploited in designing more potent DAAO inhibitors.

D-Amino acid oxidase (DAAO, EC 1.4.3.3) is a flavoenzyme that catalyzes the oxidative deamination of neutral D-amino acids and produces the corresponding α-keto acids, ammonia, and hydrogen peroxide.[1,2] In humans, DAAO is predominantly expressed in the liver, kidneys, and some regions of the brain and is primarily responsible for the metabolism of neutral D-amino acids including D-serine, an endogenous agonist at the NMDA receptor glycine modulatory site. DAAO has gained substantial interest as a therapeutic target for disorders associated with NMDA receptor hypofunction such as schizophrenia[3,4] as well as neuropathic pain in which hydrogen peroxide is believed to act as a key contributor.[5] Thus, substantial efforts have been made recently to identify potent and selective DAAO inhibitors as novel therapeutic agents.

Benzoic acid[6] is one of the early DAAO inhibitors and has served as a useful probe to study the mechanism and physiological role of the enzyme. Indeed, the first crystal structure of human DAAO was solved as a complex with benzoic acid,[7] providing critical insights into the structural details of the DAAO active site (Fig. 1A). Benzoic acid binds parallel to the flavin ring on the re face of the cofactor while it interacts with the side chain of Tyr224, which stacks against the face of the benzene ring opposite to the cofactor. The carboxylate group is coplanar with the benzene ring and forms key hydrogen bonds with Arg283 and Tyr228. Although Gly313 appears to play a minor role in the binding of benzoic acid to the DAAO active site, the carbonyl group of Gly313 participates in a critical hydrogen bond with the nitrogen group of imino-DOPA generated in situ by oxidation of D-DOPA (Fig. 1B).[8]

In addition to benzoic acid, a variety of structurally diverse DAAO inhibitors have been discovered to date (Fig. 2).[4] Consistent with the crystal structure of DAAO in complex with benzoic acid, the majority of DAAO inhibitors share common structural features, namely an aromatic ring system with a carboxylic acid or its bioisostere. For instance, 6-chlorobenzo[*d*]isoxazol-3-ol **1** (IC_{50} = 188 nM),[9] 3-hydroxyquinolin-2(1*H*)-one **2a** (IC_{50} = 4 nM),[10] and 3-hydroxychromen-2-one **2b** (IC_{50} = 440 nM)[11] are considered benzoic acid derivatives in which the carboxylic acid was replaced by an isoxazol-3-ol, an α-hydroxylactam, and an α-hydroxylactone, respectively. 4,6-Difluoro-1-hydroxy-1*H*-benzo[*d*]imidazol-2(3*H*)-one **3** (IC_{50} = 80 nM) represents a newly discovered pharmacophore based on the cyclic *N*-hydroxyurea scaffold.[12] The relatively higher potency of compound **2a** can be attributed to its ability to form a hydrogen bond with the carbonyl group of Gly313 as shown by its co-crystal structure with human DAAO.[10] In these series, SAR studies indicated that sterically hindered substituents are not well tolerated on the benzene ring, which is consistent with the limited space available at the active site of DAAO.

Fused pyrrole carboxylic acids **4a** (IC_{50} = 141 nM) and **4b** (IC_{50} = 245 nM),[13] pyrrole-2-carboxylic acid **5a** (IC_{50} <10 μM),[14] and 1*H*-pyrazole-3-carboxylic acid **5b** (IC_{50} <100 μM)[14] represent additional classes of DAAO inhibitors for which extensive SAR studies have been conducted. It is conceivable that these compounds bind to the DAAO active site in a manner similar to that of benzoic acid with an additional interaction between the NH

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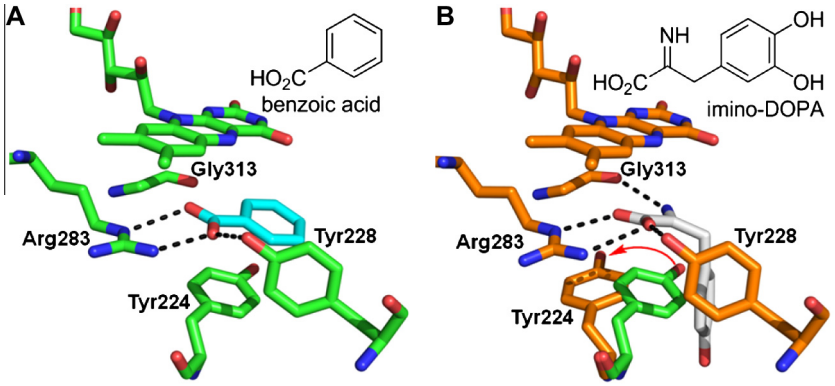


Figure 1. (A) Benzoic acid (cyan) bound to the active site of DAAO (2DU8). Key residues and FAD (flavin adenine dinucleotide) are shown in green. (B) Imino-DOPA (white) bound to the active site of DAAO (2E82). Key residues and FAD are shown in orange. Tyr224 residue of 2DU8 (green) is superimposed to highlight the repositioning (red arrow) of this residue upon binding of imino-DOPA.

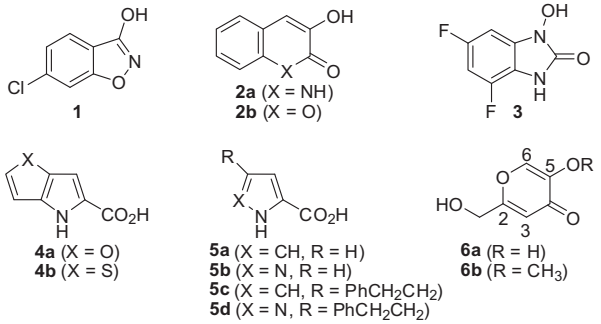


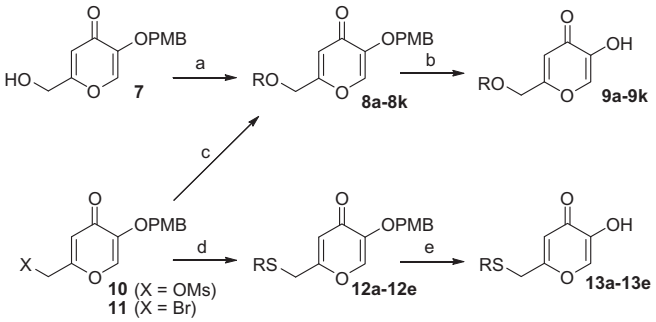
Figure 2. Representative inhibitors of DAAO.

(of the pyrrole or pyrazole ring) and Gly313. One unique aspect of the pyrrole and pyrazole carboxylates lies in their ability to gain affinity to DAAO by incorporating a side chain. For instance, when the 4-position of **5a** was substituted by a phenethyl group, the IC_{50} value is in the submicromolar range as exemplified by **5c** (IC_{50} <1 μM). Similarly, substituted compound **5d** also exhibited improved potency (IC_{50} <1 μM) over the parent compound **5b**.[14]

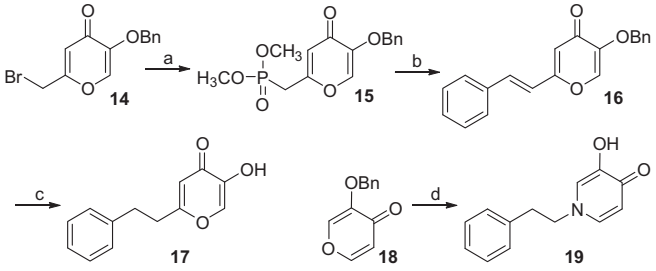
Given the limited space available at the active site on the re face of the flavin ring, the side chains of **5c** and **5d** likely stretch into another hydrophobic pocket adjacent to the active site in order to gain additional binding affinity. On a related note, human DAAO in complex with imino-DOPA (2E82) shows its catechol moiety taking a position nearly perpendicular to the flavin ring (Fig. 1B).[8] This mode of binding was enabled by the repositioning of Tyr224, which swings away from the active site. The Tyr224 shift results in the widening of the substrate entry path of DAAO by 2–3 Å compared to the 2DU8 structure and creates space for the catechol moiety of imino-DOPA.[15] We hypothesized that the side chains of compounds **5c** and **5d** stretched into the same hydrophobic cavity occupied by the catechol moiety of imino-DOPA. This hypothesis prompted us to design new DAAO inhibitors exploiting this secondary binding site.

Kojic acid **6a**, 5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one, represents another class of DAAO inhibitors with a distinct pharmacophore. Kojic acid **6a** was originally identified as an inhibitor of porcine DAAO with a K_i value of 21 μM.[6] Given its structural similarity to compound **2a**, we speculate that the 5-hydroxy-4*H*-pyran-4-one moiety acts as a carboxylic acid surrogate and binds to the active site of DAAO. Indeed, as described later, 5-methoxy derivative **6b** was found to be completely devoid of any inhibitory activity. However, we speculate that the 2-hydroxymethyl group of kojic acid plays an insignificant role in binding of kojic acid to DAAO and serves as an attachment point for various substituents that may allow us to perform systematic SAR studies exploring the secondary binding site. Herein, we report the synthesis and SAR of kojic acid derivatives as molecular probes for the secondary binding site of DAAO. These new DAAO inhibitors should provide new insights into the molecular features enabling the additional interaction at the secondary binding site.

As shown in Scheme 1, 2-(hydroxymethyl)-5-((4-methoxybenzyl)oxy)-4*H*-pyran-4-one **7** and its derivatives **10** and **11** served as starting materials[16] for the synthesis of the *O*-alkylated derivatives of kojic acid. Reactive organic halides can be directly coupled with compound **7** through its 2-hydroxymethyl group to give compounds **8**. Subsequently, the *p*-methoxybenzyl group of **8** can be removed by TFA to give the desired products **9**. Either mesylate



Scheme 1. Reagents and conditions: (a) NaH, DMF, RI or RBr, 43–75%; (b) TFA, dichloromethane, rt, 4 h, 36–96%; (c) ROH, NaH, DMF, 0 °C to rt, 4 h, 26–93%; (d) RSH, NaH, DMF, 0 °C to rt, 4 h, 16–56%; (e) TFA, dichloromethane, rt, 4 h, 30–78%.



Scheme 2. Reagents and conditions: (a) $P(OMe)_3$, benzene, reflux, 24h, 90%; (b) NaH, THF, 0 °C, then benzaldehyde, 12 h, 0 °C to rt, 66%; (c) 6 N HCl/HOAc, TFA, reflux, 48 h then H_2 (1 atm), Pd/C (5%), rt, 1 h, 50%; (d) MeOH, phenethylamine, 72 h, rt then H_2 (1 atm), 5% Pd/C (5%), EtOAc, 1 h, 50%.