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Synthesis and SAR of 1-Hydroxy-1*H*-benzo[*d*]imidazol-2(3*H*)-ones as Inhibitors of D-Amino Acid Oxidase

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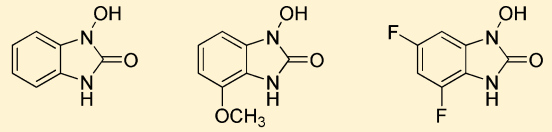
Supporting Information

ABSTRACT: A series of 1-hydroxy-1*H*-benzo[*d*]imidazol-2(3*H*)-ones were synthesized and evaluated for their ability to inhibit human and porcine forms of D-amino acid oxidase (DAAO). The inhibitory potency is largely dependent on the size and position of substituents on the benzene ring with IC_{50} values of the compounds ranging from 70 nM to greater than 100 μM. Structure−activity relationships of this new class of DAAO inhibitors will be presented in detail along with comparisons to previously published SAR data from other classes of DAAO inhibitors. Two of these compounds were given to mice orally together with D-serine to assess their effects on plasma D-serine pharmacokinetics.



KEYWORDS: *D-amino acid oxidase (DAAO), D-serine, NMDA receptors, glucuronidation*

Cumulative evidence suggests that allosteric activation of the *N*-methyl-D-aspartate (NMDA) receptor provides a new therapeutic avenue for the treatment of schizophrenia.[1] NMDA receptor glycine modulatory site agonists (glycine, D-serine, and D-alanine) were reported to improve not only positive symptoms but also negative symptoms and cognitive impairments where existing antipsychotics have failed to show significant efficacy.[2] D-Serine is particularly attractive since it is more permeable than glycine to the blood−brain barrier.[3] Furthermore, because there is no known signal transduction site modulated by the low concentrations of D-serine other than the glycine modulatory site, D-serine can facilitate NMDA receptor function without affecting other central nervous system receptors. Clinical development of D-serine, however, is hampered by the high doses of D-serine (60−120 mg/kg) required for optimal efficacy,[4] which may not be tolerated due to potential nephrotoxic effects.

Two independent studies suggest that the underlying cause of D-serine-induced nephrotoxicity can be attributed to hydrogen peroxide generated by D-amino acid oxidase (DAAO)-mediated metabolism of D-serine in the kidneys.[5,6] DAAO is a flavoenzyme that catalyzes the oxidation of D-amino acids to the corresponding imino acids and hydrogen peroxide and is predominantly responsible for clearance of orally administered D-serine in mice.[7] Coadministration of D-serine and a small molecule DAAO inhibitor may mitigate the problem related to D-serine clinical use by reducing DAAO-mediated metabolism of D-serine, thus enabling a dose reduction as well as prevention of nephrotoxicity.

Indeed, one of the early DAAO inhibitors, 6-chlorobenzo[*d*]isoxazol-3-ol (CBIO) **1** (Figure 1), was found to improve the oral bioavailability of D-serine in rodents.[8] In addition, enhanced brain levels of D-serine were achieved when D-serine was dosed in conjunction with **1**. The potency of D-serine was significantly improved in a preclinical model of schizophrenia when it was coadministered with **1**.[9] Thus, coadministration of D-serine and a DAAO inhibitor may offer a new type of treatment for schizophrenia complementary to existing antipsychotics, which primarily target dopamine receptors.

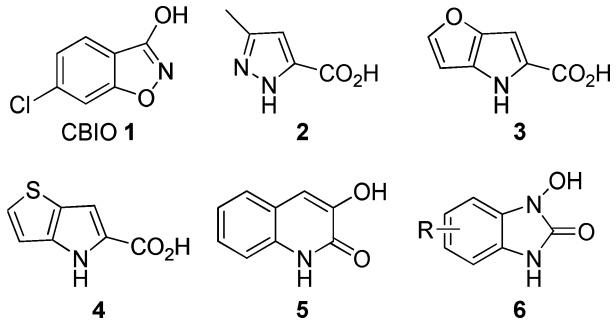


Figure 1. Representative inhibitors of DAAO.

In addition to **1**, several structurally distinct DAAO inhibitors have been indentified to date.[10] These include 5-methylpyrazole-3-carboxylic acid **2**,[11] 4*H*-furo[3,2-*b*]pyrrole-5-carboxylic acid **3**,[12] 4*H*-thieno[3,2-*b*]pyrrole-5-carboxylic acid **4**,[13] and 3-hydroxyquinolin-2(1*H*)-one **5** (Figure 1).[14] The discovery of **5** is of particular interest from the viewpoint of exploring new pharmacophores for DAAO inhibitors as it presents possibilities for other chemical moieties to serve as effective carboxylic acid surrogates. The crystal structure of **5** bound to human DAAO

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highlights some key hydrogen-bonding interactions (Figure 2A).[14] The 3-hydroxyl group is involved in two hydrogen

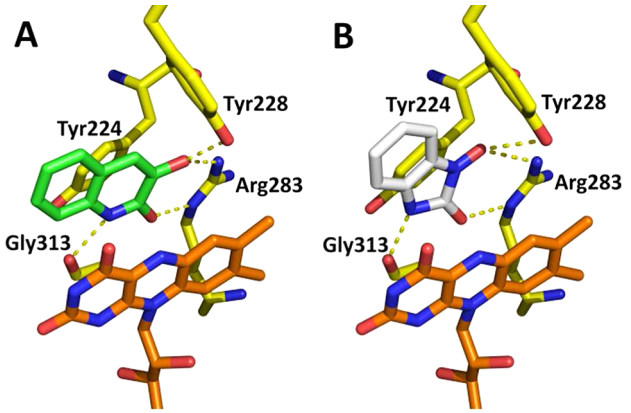


Figure 2. (A) Compound **5** (green) bound to the active site of DAAO (3G3E). (B) Proposed binding mode of **6a** (white) to the active site of DAAO. Hydrogen-bonding interactions are shown as yellow dashed lines.

bonds, one with Tyr228 and the other with Arg283. Additional interactions with the Arg283 residue are made by the 2-carbonyl group, while the lactam NH donates a hydrogen bond to the backbone carbonyl of Gly313. We hypothesized that the lactam component of **5** can be replaced by other moieties as long as these key interactions are preserved. Along this line, we explored a variety of functional groups as alternatives to this component and found that a cyclic *N*-hydroxyurea was an effective ring system to constitute potent DAAO inhibitory activity. Here, we disclose the synthesis and structure−activity relationship studies of 1-hydroxy-1*H*-benzo[*d*]imidazol-2(3*H*)-ones **6** as novel small molecule DAAO inhibitors (Figure 1).

As shown in Figure 3, 1-hydroxy-1*H*-benzo[*d*]imidazol-2(3*H*)-one **6a** inhibited a human form of DAAO with an IC_{50} value of 0.6 μM. To identify structural features that

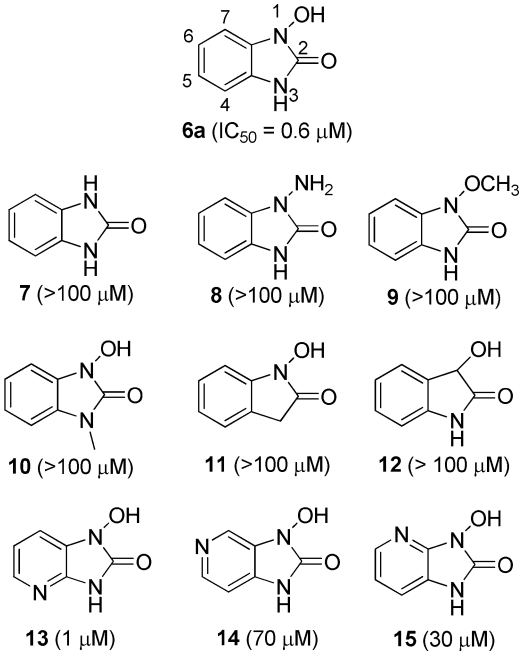


Figure 3. Preliminary SAR studies of analogues of **6a**.

contribute to the high DAAO inhibitory potency of **6a**, we systematically modified particular functional groups of this molecule and synthesized compounds **7**−**15** (Figure 3).

Displacement of the 1-hydroxyl group with a hydrogen atom (compound **7**) or amino group (compound **8**) led to a complete loss of activity. Methylation of the 1-hydroxyl group (compound **9**) also resulted in a complete loss of activity. This is most likely caused by loss of hydrogen-bonding interactions with Arg283 and Tyr228. These residues represent a key component of the DAAO active site by interacting with a carboxylate group of D-amino acid substrates. All known competitive DAAO inhibitors take advantage of hydrogen-bonding interactions with these two residues.

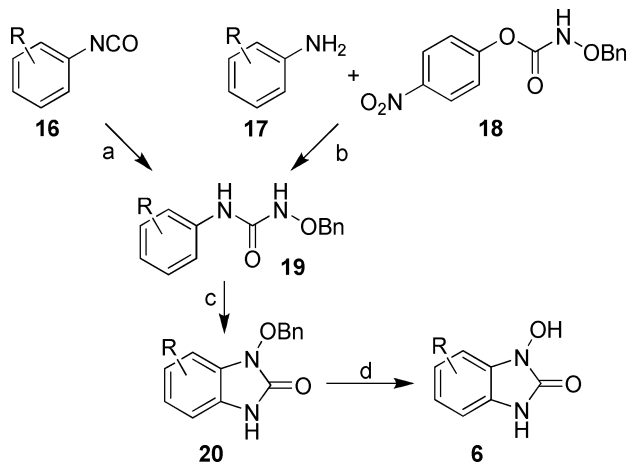
Methylation of the 3-position nitrogen (compound **10**) as well as displacement with a methylene group (compound **11**) caused a complete loss of potency. The 3-position nitrogen group of **6a** presumably forms a hydrogen bond with the backbone carbonyl of Gly313 in a similar manner as the amino group of **5** (Figure 2B). This explains the lack of potency in **10** and **11**, which do not have the ability to form hydrogen-bonding interactions with Gly313.

We also synthesized three pyridine derivatives (compounds **13**−**15**) to assess the effect of a nitrogen atom in the benzene ring on the binding affinity to DAAO. One of these molecules, compound **13**, was slightly less potent than **6a**, while the other two derivatives were much weaker DAAO inhibitors.

Preliminary SAR indicates that the cyclic *N*-hydroxyurea is an essential element for potent inhibition of DAAO. Furthermore, the pyridinyl nitrogen appears to play only a small role in enhancing potency. Hence, subsequent SAR studies were directed toward a wide variety of 1-hydroxy-1*H*-benzo[*d*]-imidazol-2(3*H*)-ones in which various substituents were introduced onto the benzene ring at the 4-, 5-, 6-, and/or 7-positions.

The general synthetic route to 1-hydroxy-1*H*-benzo[*d*]-imidazol-2(3*H*)-ones is illustrated in Scheme 1. A key intermediate **19** was prepared either from phenylisocyanate **16** or aniline **17**.[15] Oxidative ring closure of **19** was carried out with lead tetraacetate,[16] providing the bicyclic core structure **20**.

Scheme 1. Synthesis of 1-Hydroxy-1*H*-benzo[*d*]imidazol-2(3*H*)-ones[a]



[a]Reagents and conditions: (a) $BnONH_2$, DCM, rt, 80−100%. (b) Et_3N, DCM, rt, 64−84%. (c) $Pb(OAc)_4$, $CHCl_3$, rt, 9−83%. (d) H_2, 10% Pd/C, EtOH, 13−90% or HBr, AcOH, reflux, 28%.

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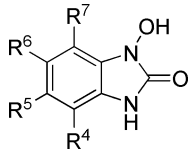
Subsequent removal of the benzyl protecting group by catalytic hydrogenation afforded the desired 1-hydroxy-1*H*-benzo[*d*]-imidazol-2(3*H*)-ones **6**.

Inhibitory potencies of the 1-hydroxy-1*H*-benzo[*d*]imidazol-2(3*H*)-ones were measured in both human and porcine forms of DAAO using previously reported assay methods.[8] Using this assay, compound **5** was found to inhibit human and porcine DAAO with IC_{50} values of 0.02 and 0.08 μM, respectively. Table 1 summarizes the in vitro DAAO inhibitory data of the 1-hydroxy-1*H*-benzo[*d*]imidazol-2(3*H*)-ones **6**.

Table 1. Inhibition of DAAO by 1-Hydroxy-1*H*-benzo[*d*]imidazol-2(3*H*)-ones[a]



					IC_{50} (μM)[a]	
cmpd	R^4	R^5	R^6	R^7	human	porcine
6a	H	H	H	H	0.6	1
6b	F	H	H	H	0.1	0.5
6c	CH_3	H	H	H	0.2	0.4
6d	CH_2CH_3	H	H	H	80	20
6e	OCH_3	H	H	H	0.1	0.4
6f	CH_2Ph	H	H	H	10	50
6g	OCH_2CH_3	H	H	H	50	50
6h	*i*-Pr	H	H	H	20	10
6i	*n*-Pr	H	H	H	20	30
6j	*sec*-butyl	H	H	H	>100	40
6k	CH_3	CH_3	H	H	20	1
6l	CH_3	F	H	H	0.07	0.7
6m	OCH_2-CH_2		H	H	0.8	0.2
6n	CH_3	H	CH_3	H	20	4
6o	F	H	F	H	0.08	0.4
6p	H	F	H	H	0.9	1
6q	H	CH_3	H	H	4	4
6r	H	F	F	H	70	30
6s	H	CH_3	F	H	2	1
6t	H	CH_2-CH_2		H	30	10
6u	H	H	F	H	0.4	0.6
6v	H	H	Cl	H	9	2
6w	H	H	CH_3	H	30	7
6x	H	H	F	F	8	20
6y	H	H	CH_2-CH_2		>100	>100
6z	H	H	F	CH_3	>100	>100
6aa	H	H	H	F	3	20
6ab	H	H	H	CH_3	40	>100

[a]Assay methods are described in the Supporting Information.

When each of the four aromatic hydrogen atoms was substituted by a fluorine atom, the resulting compounds (**6b**, **6p**, **6u**, or **6aa**) exhibited no significant loss of potency except for compound **6aa**. When each of the four aromatic hydrogen atoms was substituted by a methyl group, it was only tolerated at the 4-position (**6c**), and the rest of the compounds (**6q**, **6w**, and **6ab**) exhibited significantly higher IC_{50} values. In general, smaller substituents are more preferred at all four positions, although the 4-position appears more tolerant of slightly larger substituents. Indeed, fluorine-, methyl-, and methoxy-substituted analogues (**6b**, **6c**, and **6e**) exhibited inhibitory potency superior to **6**. In contrast, bulkier substitutions (**6f**–**j**) resulted in significant loss of inhibitory potency. While some of the 5- or 6-substituted analogues were found to be potent DAAO inhibitors, all of the 7-substituted compounds exhibited weaker inhibitory potency. Disubstitution at the 4-, 5-, and/or 6-positions was well tolerated as exemplified by compounds **6l** and **6o**. Among those tested, four compounds (**6b**, **6e**, **6l**, and **6o**) exhibited potent DAAO inhibitory activity with IC_{50} values equal to or less than 100 nM.

The overall SAR trends are largely consistent with those of analogues derived from **1** and **5**. In general, bulky substituents on the aryl ring of **6** are not tolerated at the active site of DAAO. This is consistent with the findings from the crystallographic studies indicating the limited space available within the substrate binding site, which is parallel to the flavin ring. A more thorough SAR analysis revealed that substitution patterns preferred for 1-hydroxy-1*H*-benzo[*d*]imidazol-2(3*H*)-ones[14] better correlate with those of 3-hydroxyquinolin-2(1*H*)-ones than those of benzo[*d*]isoxazol-3-ols.[8] For example, substitution at the corresponding 4-position of the benzo[*d*]-isoxazol-3-ol series by a fluorine or methyl group resulted in a substantial decline in the inhibitory potency as opposed to compounds **6b** and **6c**, displaying improved potency over **6a**. Although speculative, it is conceivable that a similar binding mode is in effect for 1-hydroxy-1*H*-benzo[*d*]imidazol-2(3*H*)-ones and 3-hydroxyquinolin-2(1*H*)-ones, which share the common α-hydroxycarbonyl moiety. However, none of the hydroxy-1*H*-benzo[*d*]imidazol-2(3*H*)-ones tested was as potent as compound **5**. This could be due to the optimal interaction of compound **5** with the carboxylate binding site of DAAO, which cannot be reproduced by the *N*-hydroxylurea moiety of **6**.

It was previously reported that some DAAO inhibitors are more potent against the human form of DAAO than the rat form.[14] In contrast, as shown in Table 1, there was little difference in potency when 1-hydroxy-1*H*-benzo[*d*]imidazol-2(3*H*)-ones were tested against human and porcine forms of the enzyme. It should be noted that weaker binding to the rat enzyme might be attributed to the conformational changes of rat DAAO due to the deletion of Leu27.[14] Interestingly, the porcine enzyme possesses the Leu27 residue, and this may be the reason for similar potency seen in both human and porcine forms of DAAO.

To determine the effects of the *N*-hydroxyureas on D-serine plasma levels, mice (n = 3 per time point) were dosed with compounds **6e** or **6o** (30 mg/kg, po) along with D-serine (30 mg/kg, po) with 10% DMSO/90% saline as a vehicle. As shown in Figure 4, compound **6e** showed no effect on plasma D-serine pharmacokinetics. Indeed, subsequent studies revealed that compound **6e** has negligible oral bioavailability in mice (data not shown). When D-serine was coadministered with compound **6o**, plasma levels of D-serine were higher as compared to those of treated with D-serine alone ($^*p < 0.05$ at 30 min after administration). The pharmaco-enhancing effects of **6o**, however, only lasted for a short period of time (<2 h), presumably due to its poor oral bioavailability.

In an attempt to elucidate the mechanism behind the poor oral bioavailability of **6e** and **6o**, their metabolic stability was measured in mouse plasma and liver microsomes (Table 2). Both compounds showed high stability in mouse plasma. In liver microsomes, compound **6e** was substantially metabolized in the presence of NADPH. In contrast, compound **6o** appeared to be resistant to NADPH-dependent CYP450-

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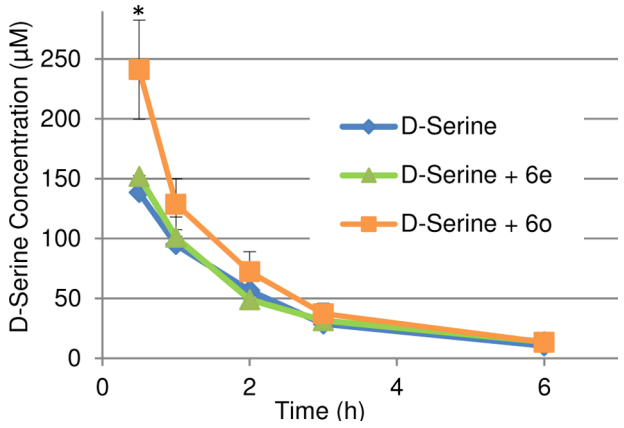

Figure 4. Effects of DAAO inhibitors on plasma D-serine levels following oral coadministration.

Table 2. Metabolic Stability of Compounds 6e and 6o

compd	matrix	cofactor	% remaining (after 1 h)
6e	plasma	none	90
	microsomes	none	~100
	microsomes	NADPH	60
	microsomes	UDPGA	ND[a]
6o	plasma	none	~100
	microsomes	none	~100
	microsomes	NADPH	~100
	microsomes	UDPGA	0.6

[a]Not detected.

mediated oxidation, presumably due to the electron-withdrawing effect of the two fluorine atoms. This may explain the ability of compound **6o** to slightly enhance D-serine levels in vivo. Interestingly, both compounds were completely consumed in the presence of UDPGA. The results clearly show that both compounds undergo phase II glucuronidation in the liver. The most likely site for glucuronidation in these compounds is the 1-hydroxyl group. Unfortunately, as evident from the loss of potency in **7** and **9**, this hydroxyl group is essential for high affinity binding to the DAAO active site. Further structural optimization of the 1-hydroxy-1*H*-benzo[*d*]-imidazol-2(3*H*)-one scaffold will require fine-tuning of both the steric and the electronic environment surrounding the 1-hydroxyl group in a way that does not compromise the inhibitory potency while minimizing the degree of glucuronidation.

In conclusion, we have identified a new series of DAAO inhibitors based on 1-hydroxy-1*H*-benzo[*d*]imidazol-2(3*H*)-one scaffold. Through systematic SAR studies, we identified potent DAAO inhibitors including compounds **6e** and **6o**. While **6o** showed marginal effects on plasma D-serine concentrations, no effects were observed with **6e**. Subsequent studies revealed that these compounds were subjected to phase I and II metabolism. The crystal structure of DAAO in complex with imino-DOPA revealed that its catechol moiety takes a position nearly perpendicular to the flavin ring.[17] This hydrophobic cavity, distinct from the active site, has not been utilized by most DAAO inhibitors and could serve as an additional binding pocket to enhance the affinity to DAAO. Further structural optimization, however, should be attentively directed toward improving metabolic stability while enhancing potency.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis, characterization, and methods of DAAO assay, D-serine pharmacokinetics, and metabolic stability studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

DAAO, D-amino acid oxidase; NMDA, *N*-methyl-D-aspartate; CBIO, 6-chlorobenzo[*d*]isoxazol-3-ol

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