Derivatives of 3,4-dihydroxyhydrocinnamic acid at the 6-position as mechanistic probes of L-DOPA dioxygenase

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Abstract

Dioxygenase enzymes are essential protein catalysts for the breakdown of catecholic rings, structural components of plant woody tissue. This powerful chemistry is used in nature to make antibiotics and other bioactive materials as well as degrade plant material, but we have a limited understanding of the breadth and depth of substrate space for these powerful enzymes. To this end, we report the syntheses, redox potentials and pKas of 3,4-dihydroxyhydrocinnamic acid (DHHCA) derivatives substituted at the 6-position and their characterization as substrates of L-DOPA dioxygenase from *Streptomyces lincolnensis*. The cleavage of diverse catecholic substrates is an important element of bioremediation. Extradiol dioxygenase cleavage of DHHCA derivatives also promises to yield insight into mechanism and provide synthons for various applications.

Background & Motivation

- The L-DOPA dioxygenase enzyme (Fig. 1) is a catalyst used for the breakdown of catechols as it cleaves the aromatic ring using vicinal oxygen chelation (Fig. 2). [1]

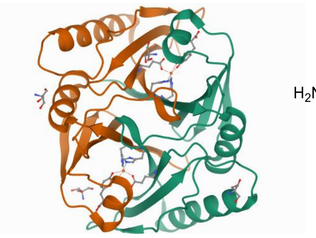

Figure 1: The crystal structure of L-DOPA dioxygenase from *Streptomyces sclerotialus*.

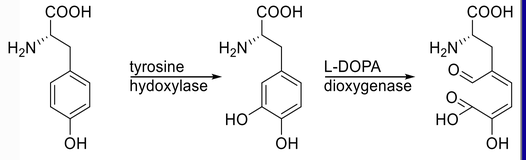

Figure 2: *S. lincolnensis* L-DOPA dioxygenase cleaves the aromatic ring for biosynthesis of lincomycin.

- There is currently a gap in knowledge regarding the specific mechanisms by which this reaction occurs. In order to better understand this mechanism, we seek to explore the substrate space with the creation of various dihydroxyhydrocinnamic acid analogues.
- With a better understanding of the enzyme's mechanism, there is the potential for creating a process to mimic the action of this enzyme in a lab setting.. Additionally, it can provide access to novel therapeutics and bioremediation.
- The following electron withdrawing substituents have a record of good activity with the enzyme and have been synthesized for in-vitro assays in the enzyme (Fig. 3). [2]

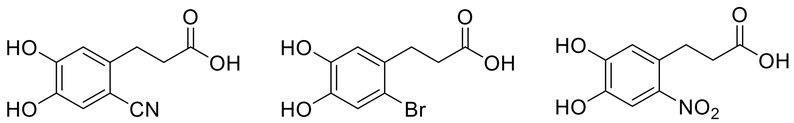

Figure 3: DHHCA and derivatives with electron withdrawing substituents.

Acknowledgements

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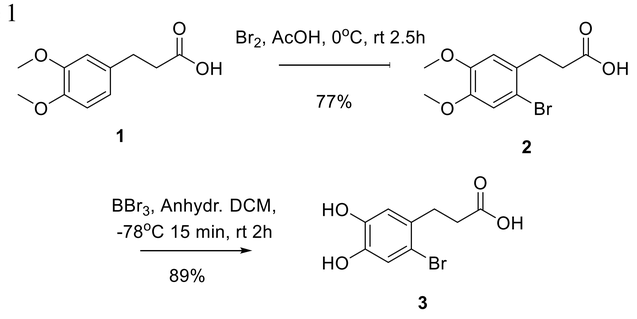
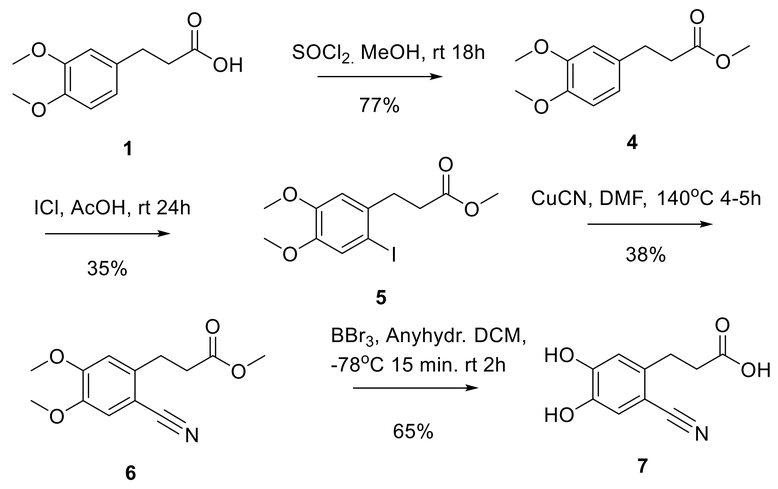
Synthesis of Bromo-DHHCA

Scheme 1



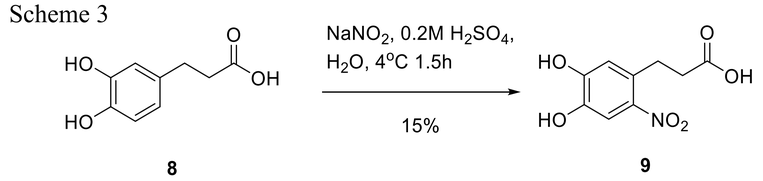
Synthesis of Cyano-DHHCA

Scheme 2



Synthesis of Nitro-DHHCA

Scheme 3



Experimentally-Determined pKa values

Table 1: pKa values of DHHCA and analogues

	DHHCA	Bromo-DHHCA	Cyano-DHHCA	Nitro-DHHCA
First pKa	8.95 ± 0.06	8.20 ± 0.16	7.57 ± 0.08	7.00 ± 0.03
Second pKa	> 10.50	>12.5	> 11	> 11

In Silico Docking of Nitro-DHHCA

Using UCSF Chimera and AutoDock Vina, DHHCA analogues were docked into the active site of L-DOPA dioxygenase (PDB: 6ON3) (Fig. 4). Of the three analogues and control, Nitro-DHHCA had the lowest docking score.

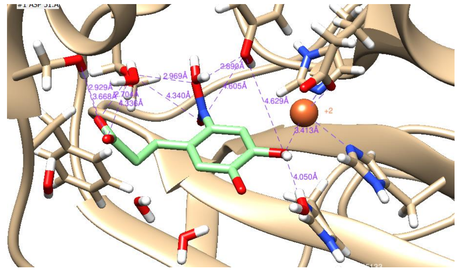


Figure 4: Nitro-DHHCA docked in the L-DOPA dioxygenase active site. Water molecules included. Docking score of -8.0. The control docking score for DHHCA is -6.9.

Electrochemical Studies

- Catechols, under aerobic conditions, can undergo auto-oxidation or enzymatic-catalyzed oxidation to produce highly reactive quinones. In order to characterize the potential for redox cycling, an electrochemical study was performed in order to evaluate the redox behavior of the various analogues.
- This study used cyclic voltammetry; the primary working electrode was a glassy carbon electrode polished on an aluminum plate and the auxiliary (counter) electrode was platinum wire.

Table 2: $E_{1/2}$ values for the DHHCA and analogues

	Dopamine	DHHCA	Bromo-DHHCA	Cyano-DHHCA	Nitro-DHHCA
$E_{1/2}$ (V vs. SCE)	0.1245	0.1555	0.177*	0.2445	0.183*

* Indicated the oxidation was not reversible and only the Ep is shown

Conclusion

- All intermediates have been confirmed by ^{1}H and ^{13}C NMR, and compounds **3**, **7**, and **9** have been additionally confirmed by MS and HPLC.
- The electronic properties of DHHCA and analogues were examined by electrochemistry and pKa. The more electron withdrawing the substituent, the less easily it is oxidized and the lower the pKa value.
- Nitro-DHHCA docked the best in the active site of L-DOPA dioxygenase as determined by computation.
- Preliminary testing of the substrates did not agree with the computational data, showing Nitro-DHHCA to be the least effective of the DHHCA analogues. This could be due to electron withdrawing nature of the nitro group, making it too difficult to properly oxidize.
- Synthesis and testing of each of these compounds continues. These experiments are currently underway.

References

1. Wang Y, Shin I, Fu Y, Colabroy K, Liu A. *Biochem* . 2019, 58(52), 5339-5350.
2. Rote et al, *Synthetic Communications.* (2017) 47, 435-441.