ADHP

Cat. No.: HY-101880

CAS No.: 119171-73-2

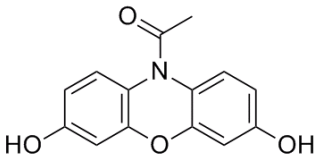
Molecular Formula: $C_{14}H_{11}NO_4$

Molecular Weight: 257.24

Target: Others

Pathway: Others

Storage: Please store the product under the recommended conditions in the Certificate of Analysis.



BIOLOGICAL ACTIVITY

Description	ADHP is a fluorogenic peroxidase substrate (λ_{ex}=530 nm, λ_{em}=590 nm).
In Vitro	To obtain the parameters K_m and k_{cat} for Compound I, two independent methods are used. Initially, the oxidation of ADHP using the injector functionality built-in to the fluorescence plate reader is studied. The auto-injector dispenses the H_2O_2 to initiate the reaction, as a means of generating a set of progress curves. Analysis for MPO-mediated oxidation of ADHP gives a K_m of 31±4 μM and the k_{cat} of 186± 6 s^{-1}.The k_{obs} also increases over the experimental range of ADHP concentrations from 1 to 80 μM and for the converse experiment holding substrate constant over 3 to 45 nM MPO. The apparent second order rate constant obtain from the slope of k_{obs} against ADHP concentration K^{app}_{on} is 2.1±0.2 mM/s[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	ADHP, 4-ABAH, 2-ABAH, 4-BAH, 4-FBAH, 4-NBAH, 4-TFMBAH, 3-DMABAH, NaN_3 and isoniazid are dissolved in DMSO and subsequently diluted into assay buffer. The final concentration of DMSO in the reaction is less than 0.5 % (v/v), which does not affect fluorescence of the oxidized ADHP product 7-hydroxyl-3H-phenoxazin-3-one (resorufin). Reactions of ADHP (20 μ M) are incubated with MPO (2.8 nm) in assay buffer and initiated by the addition of 1/10th volume H_2O_2 from a serial dilution basin. To determine the effect that the simplest benzoic acid hydrazide inhibitor or its analog 4-TFMBAH has on the heme catalytic ability of MPO, MPO (1.2 μM) is incubated for 10 min with different concentrations of BAH inhibitor (0, 0.025, 0.25, 2.5, 12.5 and 25 mM) with ADHP (40 μM) and timing of the reaction is measured following addition of H_2O_2 (20 μM) ADHP. All reactions are measured in assay buffer at room temperature. Samples of 20 μL are added to non-reducing sample loading buffers, and then loaded without prior heating and resolved by 4-15% gradient SDS-polyacrylamide gel electrophoresis[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Jiansheng Huang, et al. Ordered Cleavage of Myeloperoxidase Ester Bonds Releases Active site Heme Leading to Inactivation of Myeloperoxidase by Benzoic Acid Hydrazide Analogs. Arch Biochem Biophys. 2014 Apr 15; 548: 74–85.

Caution: Product has not been fully validated for medical applications. For research use only.

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