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# Structure based mimicking of Phthalic acid esters (PAEs) and inhibition of hACMSD, an important enzyme of the tryptophan kynurenine metabolism pathway

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ABSTRACT

Human α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (hACMSD) is a zinc containing amidohydrolase which is a vital enzyme of the kynurenine pathway in tryptophan metabolism. It prevents the accumulation of quinolinic acid (QA) and helps in the maintenance of basal Trp-niacin ratio. To assess the structure based inhibitory action of PAEs such as DMP, DEP, DBP, DIBP, DEHP and their metabolites, these were docked into the active site cavity of hACMSD. Docking results show that the binding affinities of PAEs lie in the comparable range (−4.9 kca/mol–7.48 kcal/mol) with Dipicolinic acid (−6.21 kcal/mol), a substrate analogue of hACMSD. PAEs interact with the key residues such as Arg47 and Trp191 and lie within the 4 Å vicinity of zinc metal at the active site of hACMSD. Dynamics and stability of the PAEs-hACMSD complexes were determined by performing molecular dynamics simulations using GROMACS 5.14. Binding free energy calculations of the PAEs-hACMSD complexes were estimated by using MMPBSA method. The results emphasize that PAEs can structurally mimic the binding pattern of tryptophan metabolites to hACMSD, which further leads to inhibition of its activity and accumulation of the quinolate in the kynurenine pathway of tryptophan metabolism.



## 1. Introduction

In the brain, elevated levels of quinolinic acid (QA) are often associated with the pathogenesis of different neurodegenerative disorders including Alzheimer's and Huntington's disease [1–3]. In kynurenine pathway of tryptophan metabolism, α-amino-β-carboxymuconate-ε-semialdehyde (ACMS) is metabolized to α-amino-β-muconate-ε-semialdehyde (AMS) via action of important enzyme ACMS decarboxylase, further AMS converted to acetyl CoA as shown in Fig. 1 [4,5]. To maintain the basal Trp-niacin ratio, ACMS is non-enzymatically metabolized to quinolate (QA) which further leads to the NAD formation [6]. Thus, the presence of key enzyme ACMSD prevents the accumulation of quinolate [7]. Various studies related to ACMSD show that enzyme is zinc-dependent amidohydrolase maintaining quinolinic acid (QA) and NAD homeostasis [8]. Disturbance in the basal levels of QA is associated with many physiological and pathological conditions related to the central nervous system (CNS) [9]. Thus, ACMSD act as a checkpoint and regulates the balance between the relative QA levels.

Several studies have reported that ACMSD is an critical enzyme for tryptophan metabolism [10,11]. Phthalic acid esters (PAEs) are commonly used for the industrial manufacturing of lubricants, various adhesives, pest repellents, and plastics [12,13]. Several PAEs are long-established environmental endocrine disrupters, peroxisome proliferators and induce reproductive and developmental toxicities [14–18]. It has been reported that PAEs can imbalance the Trp-niacin basal ratio in the tryptophan metabolism pathway. In rats, it has been reported that the conversion ratio of tryptophan to niacin has increased with increase in the dietary concentration of di-(2-ethylhexyl) phthalate (DEHP) [19]. This study shows that the increase in the amount of quinolinic acid with an increase in DEHP concentration is associated with the inhibition of enzymatic activity of ACMSD. Similarly, di-*n*-butyl phthalate (DnBP) is reported to be linked with alteration in trp to niacin ratio in the weaning rats, which were fed with niacin-free and tryptophan limited diet [20].

It has been shown that DEHP degrades to Phthalic acid via an intermediate mono-(2-ethylhexyl) phthalate (MEHP) [21]. In another report, it has been shown that DEHP and its metabolite MEHP increased QA production in the rats [22]. This study reveals that the structural similarity of DEHP and MEHP to tryptophan metabolites is responsible for the noticeable changes in normal

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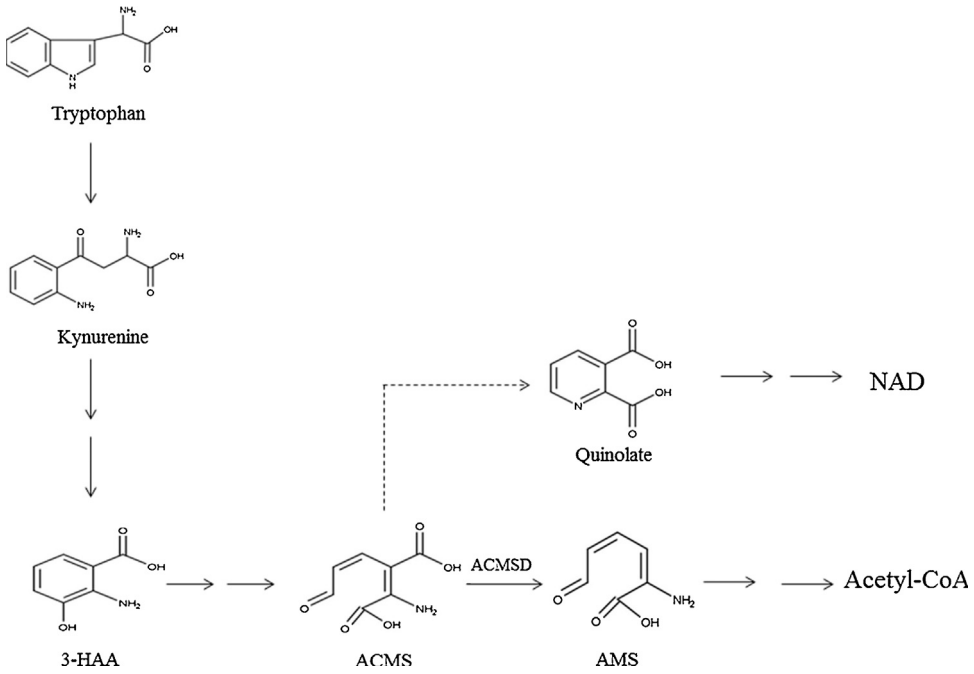


**Fig. 1.** Kynurenine pathway of tryptophan metabolism. Tryptophan is metabolized to kynurenine and further to 3-Hydroxy anthranilic acid (3-HAA). Then, ACMSD participates in a reaction (marked with a solid arrow) and directs the conversion of α-amino-β-carboxymuconate-ε-semialdehyde (ACMS) to α-amino-β-muconate-ε-semialdehyde (AMS), which is further metabolized to Acetyl-CoA. ACMS is also non-enzymatically converted to quinolate (marked with a dotted arrow), which further leads to nicotinamide adenine dinucleotide (NAD) biosynthesis.

tryptophan metabolism and caused the inhibition of ACMSD activity.

Although, numerous studies have shown that phthalates are involved in disturbing the basal trp to niacin ratio, but the elucidation of the binding mode and important interactions of PAEs with hACMSD have not been reported yet. This study highlights the important interactions of phthalates with human α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (hACMSD) which eventually inhibit the hACMSD activity and leads to the accumulation of quinolate. The crystal structure of the hACMSD along with substrate analogue Dipicolinic acid, PDC (PDB ID: 4IH3) is available [8]. PDC binds in the zinc containing active site of hACMSD and shows the interaction with Arg47 and Trp191. In this study, five commonly used PAEs and their corresponding monophthalates used for the docking studies with hACMSD are: dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DnBP), di-isobutyl phthalate (DIBP), di-(2-ethylhexyl) phthalate (DEHP), monomethyl phthalate (MMP), monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MBP), mono-iso butyl phthalate (MIBP), and mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxyhexyl) phthalate (MEOHP). Molecular docking and simulation studies were used to investigate the binding mode and stability of these PAEs to human ACMSD. The results conclude that these phthalates can efficiently bind and can inhibit the activity of hACMSD. Hence, the binding of PAEs with hACMSD affect the basal trp to niacin ratio which further accumulates the quinolate in the tryptophan metabolism pathway.

## 2. Material and methods

### 2.1. Protein and ligand preparation

Computational docking and simulation studies were performed in order to assess the interaction of the phthalates with hACMSD. The coordinates of hACMSD were retrieved from the crystal structure of hACMSD bound to a substrate analogue Dipicolinic acid (PDC) (PDB ID: 4IH3) from the RCSB database [8]. Geometric optimization of the protein was done by using the Clean Geometry module of Discovery Studio (DS) 4.0 suite by Accelerys (San Diego, CA, USA). Side-chain torsion angles varying more than 30° from the ideal values were corrected. The conformational quality was checked using the Minimize and refine protein module of DS suite, in which water molecules were eliminated and the CHARMM27 force field was used for the protein receptor [23]. The structure was minimized for 1000 steps by utilizing the smart-minimizer algorithm with 0.1 RMS gradient cut-off to remove the steric overlaps.

### 2.2. Molecular docking of ligands

AutoDock 4.2.6 was used to perform the docking of PAEs with hACMSD [24]. Autodock utilizes a semiempirical free energy force field to calculate the binding free energy of a small molecule to a macromolecule. Receptor molecule was prepared by adding explicit hydrogen molecules and associated Kollman charges (16.0) by utilizing the AutoDock Tools 1.5.6 and saved in.pdbqt file format. Five commonly used diphthalates and their corresponding monophthalates used for the docking studies with hACMSD are: dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DnBP), di-isobutyl phthalate (DIBP), di-(2-ethylhexyl) phthalate (DEHP), monomethyl phthalate (MMP), monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MBP), mono-iso butyl phthalate (MIBP), mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono-(2-ethyl-5-oxyhexyl) phthalate (MEOHP). As a positive control, substrate analogue of hACMSD, dipicolinic acid (PDC) was docked and compared with binding affinity scores of PAEs. The 3D structures of all the phthalates were drawn using Marvin suite 17.13 [http://www. chemaxon.com/marvin/sketch/index.jsp] and minimized using DS suite. The properties of phthalate compounds and their structures are shown in Table 1. The ligands were pre-