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# In vitro and in silico investigations of the binding interactions between chlorophenols and trypsin

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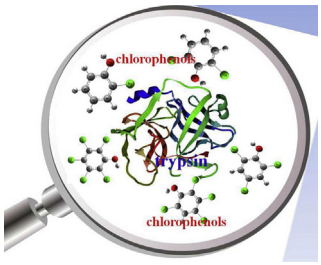
## HIGHLIGHTS

- Binding interactions of five chlorophenols with trypsin were investigated.
- The number of chlorine atoms of chlorophenols partly affected the binding ability of them to trypsin.
- Noncovalent interactions stabilized the trypsin–chlorophenols complexes.
- There was the one main binding site of trypsin for chlorophenols.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Being the first-degree toxic pollutants, chlorophenols (CP) have potential carcinogenic and mutagenic activity and toxicity. Since there still lacks studies on molecular interactions of chlorophenols with trypsin, one major binding target of many exogenous environmental pollutants, the binding interactions between five chlorophenols, 2-CP, 2,6-DCP, 2,4,6-TCP, 2,4,6-TCP, 2,3,4,6-TCP and PCP and trypsin were characterized by the combination of multispectroscopic techniques and molecular modeling. The chlorophenols bind at the one main site of trypsin and the binding induces the changes of microenvironment and global conformations of trypsin. Different number of chloride atoms significantly affects the binding and the binding constants $K_A$ ranks as $K_A$ (2-CP) < $K_A$ (2,6-DCP) ≈ $K_A$ (2,4,6-TCP) < $K_A$ (2,3,4,6-TCP) < $K_A$ (PCP). These chlorophenols interacts with trypsin mainly through hydrophobic interactions and via hydrogen bonding interactions and aromatic–aromatic π–π stacking interaction. Our results offer insights into the binding mechanism of chlorophenols with trypsin and provide important information for possible toxicity risk of chlorophenols to human health.



## 1. Introduction

Chlorophenols were classified as first-degree toxic pollutants by US Environmental Protection Agency [1] and they caused increasing public concern due to their toxicity and widespread applications [2]. The pollutions of chlorophenols are contributed often from industrial and agricultural sources such as industrial wastewater,

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wood preservative degradation products of chlorinated pesticides and herbicides [3,4]. Chlorophenols can be easily transported through the cell membrane and bio-accumulate in aquatic organisms because of their lipophilicity. Exposure of chlorophenols may result in potential toxicity to human health [5], thus it is necessary to evaluate their hazards on nontarget organisms [6].

Chlorophenols are recalcitrant to biodegradation and are therefore persistent in the environment. The numbers of chlorine atoms in chlorophenols affect their biodegradability and toxicity of chlorophenols [7]. Chattaraj et al. firstly have used the best quantitative structure–activity relationship (QSAR) models in ecotoxicology to analyze the toxicity of chlorophenols against *Daphnia magna* [6]. Kishino et al. have studied the relation between the acute toxicity of chlorophenols in fish and the numbers of chlorine atoms in chlorophenols [8]. The interactions of some chlorophenols with serum albumin have also been studied *in vitro* [9–11]. Up to now, the molecular interactions of chlorophenols with digestive proteases have been rarely studied and the effect of chlorophenols on the activity of proteases needs to be further investigated at the atomic level [12].

As the most abundant proteases in nature, trypsin (EC 3.4.21.4) plays an essential role in digestion and deconstruction of food proteins and other physiological processes including apoptosis, signal transduction, and immune response [13]. Trypsin is a well-known target that many exogenous environmental pollutants can bind with it and affect the conformation as well as the activity of trypsin [14–16]. In present study, the molecular interactions of 2-chlorophenol (2-CP), 2,6-dichlorophenol (2,6-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), 2,3,4,6-tetrachlorophenol (2,3,4,6-TTP), and pentachlorophenol (PCP) (Fig. 1) with trypsin was investigated by *in vitro* spectroscopic techniques and *in silico* modeling. The applied multiple spectroscopic techniques probed the conformational changes of trypsin upon the binding of chlorophenols. The underlying binding mechanism was further elucidated by molecular modeling. The obtained information is helpful for the understanding of the distribution of chlorophenols and can provide essential information for the risk assessment of chlorophenols.

## 2. Materials and methods

### 2.1. Materials

Trypsin (from porcine pancreas) and *N*-benzoyl-D, L-arginine-*p*-nitroanilide (BApNA) were purchased from Sigma-Aldrich Company and used without further purification. 2-CP, 2,6-DCP, 2,4,6-TCP, 2,3,4,6-TTP and PCP were obtained from Aladdin Industrial Corporation. The other reagents were all of analytical purity. The five chlorophenol solutions ($0.0125\,mol\,L^{-1}$) were prepared in pH 7.40 Tris–HCl buffer ($0.05\,mol\,L^{-1}$ Tris, $0.1\,mol\,L^{-1}$ NaCl) containing 20% methanol, respectively. Trypsin solution was also prepared in Tris–HCl buffer. BApNA ($3.0 \times 10^{-3}\,mol\,L^{-1}$) was dissolved in DMSO and was stored at 0–4 °C. For the CD experiments, a phosphate buffer ($0.01\,mol\,L^{-1}$) of pH 7.40 was exclusively prepared in ultrapure water purified with a Milli-Q purification system.

### 2.2. Molecular modeling

The geometries of chlorophenols, tyrosyle (Tyr) and tryptophan (Trp) were optimized using DFT B97-D [17] and the 6-31++G (d) by Gaussian 09 [18] and employed the ultrafine pruned numerical integration grid with 99 radial shells and 590 angular point per shell. The optimized geometries were characterized as minima through calculation of harmonic vibration frequencies. The requested convergences on RMS density matrix and on MAX density matrix were 1.00D-08 and 1.00D-06, respectively.

Chlorophenols were automatically docked into the binding site of trypsin (PDB ID 2ZQ1, 1.68 Å) [19] By Autodock 4.2.3 program [20]. The grid box size of trypsin–chlorophenols systems and Trp–chlorophenols (or Tyr–chlorophenols systems) were

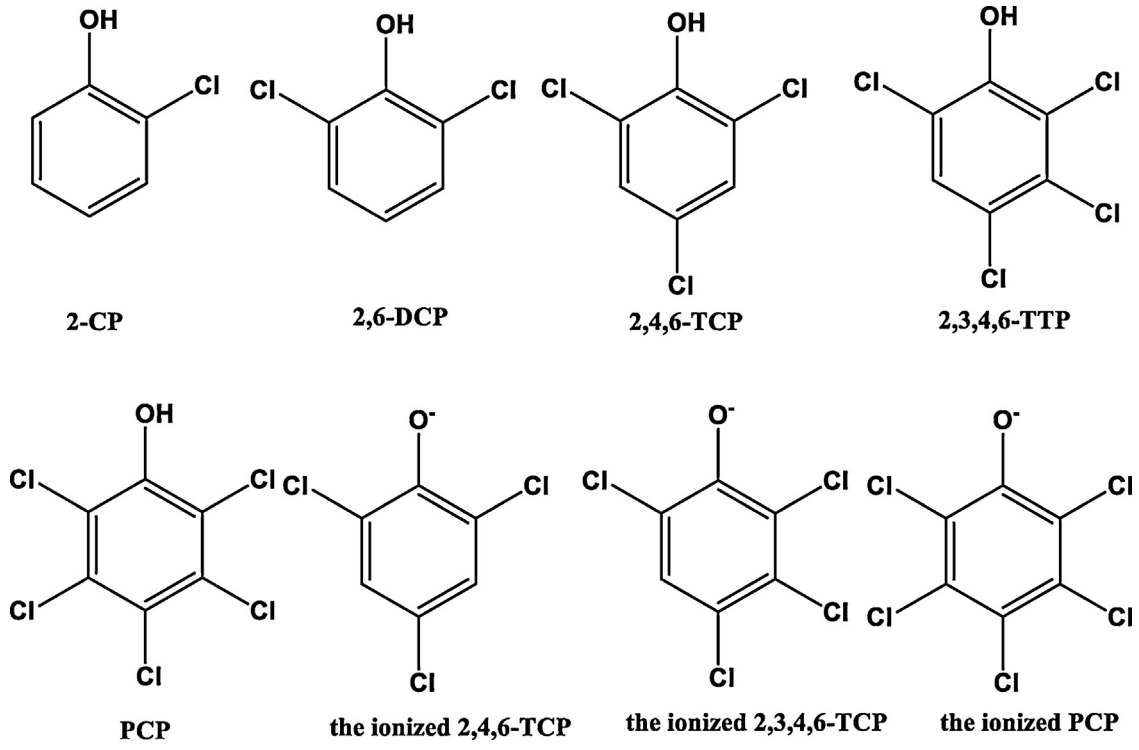


**Fig. 1.** The molecular structure of chlorophenols.