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Investigation of cholinesterase inhibitory potential of chlorinated phenols

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Abstract

Chlorinated phenols (i.e., totally 19 compounds from mono-chlorophenols to pentachlorophenol) have been synthesized in large amounts and used for diverse purposes within the last century. Their worldwide application in different areas also resulted in their accumulation within the environment. Therefore, it is always a topic of concern to investigate the biological effects of these compounds by various scientific disciplines, including but not limited to, toxicology, ecology, and xenobiotic metabolism. In this study, we aimed to screen the cholinesterase inhibitory potential of chlorinated phenols. The results indicated that chlorinated phenols have low to moderate potential as inhibitors of cholinesterases and the potential depends on the substitution level of phenol.

Keywords

Acetylcholinesterase, butyrylcholinesterase, chlorinated phenols, metabolism, xenobiotics.

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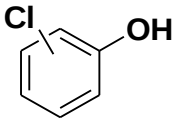
INTRODUCTION

Chlorinated phenols consist of totally 19 compounds, representing mono-, di-, tri-, tetra-, and penta-chlorine substituted phenol analogues (Exon, 1984). Following the first identification of phenol, pentachlorophenol, and some tri-chlorophenol derivatives as fungicides, herbicides, insecticides, and antimicrobial agents, their annual production reached to enormous levels particularly after 1930s (Figure 1) (Ahlborg *et al.,* 1980; Jensen *et al.,* 1973). Beside, tetrachlorophenols were also intensively used as wood preservatives (Puhakka *et al.,* 1992). This, overall, triggered their accumulation in the environment concomitant to the questioning of their toxic effects on living things, including human (Igbinosa *et al.,* 2013). Toxicity has been also questioned related to their synthesis impurities including dibenzodioxines and dibenzofurans (Nilsson *et al.,* 1974).



Three monochloro-, six dichloro-, six trichloro-, three tetrachloro-, and one pentachloro substituted phenol analogues

Figure 1: The general structure of chlorinated phenols.

Today, beside occupational exposure, living things are exposed to these compounds from various sources including water (Lampi *et al.,* 1990). Therefore, the metabolism aspect of these compounds have been investigated in detail. These compounds, in general have diverse tissue distribution including liver and kidney (Wagner *et al.,* 1991). Although majority of the pharmacokinetic studies depend on the results obtained for pentachlorophenol, in general many of these compounds have been shown to be eliminated from the organism in less than 24 hours (Nikkilä *et al.,* 2003). In terms of metabolic pathways, the studies indicated particularly the interaction of these compounds with Cyp450 enzymes, resulting in the production of oxidative dechlorination products. These are either quinone or more hydroxylated phenol derivatives (Mehmood *et al.,* 1996). Beside the metabolism in human, nature also directs the degradation of these compounds in soil through microorganisms ending up with mono-chlorinated derivatives (Murthy *et al.,* 1979). Regarding their importance with respect to exposure from the environment, there has always been interest to investigate the interaction of these compounds with different biological cascades (Gulcan *et al.,* 2008). From this perspective, in this study, we aimed to screen the potential of chlorinated phenols

to inhibit cholinesterase enzymes (i.e., acetylcholinesterase (AChE), and butyrylcholinesterase (BuChE)).

MATERIALS AND METHODS

The chlorinated phenol derivatives were obtained from Sigma Aldrich (CA, USA). Their purities were more than 99 % as stated on their labels. Therefore, no other purification was conducted on the reagents.

Determ*ination of AChE and BChE inhibitory activities*

The modified spectrophotometric method of Ellman was used to determine of AChE and BuChE inhibitory activities of the chlorinated phenols (Gulcan *et al.,* 2014). The enzymes used for cholinesterase activity studies were electric eel AChE (eeAChE) (Sigma) and equine BuChE (Sigma).

Acetylthiocholine iodide and butyrylthiocholine chloride (Sigma, St. Louis, MO, USA) were employed as substrates of the reaction. 5, 5´-Dithio-bis (2-nitrobenzoic) acid (DTNB, Sigma, St. Louis, MO, USA) was used for the measurement of the cholinesterase activity. Briefly, 50 mM Tris HCl buffer (pH 8.0), 6.8 mM DTNB, 2 µl of sample solutions and 10 µl of AChE/BChE solution were added in a 96-well microplate. The reaction was then initiated with the addition of 10 µl of acetylthiocholine iodide/butyrylthiocholine chloride. The hydrolysis of acetylthiocholine iodide/butyrylthiocholine chloride was monitored at a wavelength of 412 nm by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines at 27°C for 5 min, catalyzed by enzymes (Varioskan Flash, Thermo Scientific, USA). The measurements and calculations were evaluated by using SkanIt Software 2.4.5 RE for Varioskan Flash software. Percentage of inhibition of AChE and BuChE was determined by comparing the rates of reaction of samples relative to blank sample (methanol) using the formula (E-S)/E x 100, where E is the activity of enzyme without test sample and S is the activity of enzyme with test sample. The experiments were done in triplicate. Donepezil hydrochloride, and rivastigmine were used as reference compounds. The percent inhibition at 100 and 50 µM was obtained for each test compound with standard compounds.

RESULTS AND DISCUSSION

The activities obtained for the potential of chlorinated phenol derivatives to inhibit cholinesterase enzymes are shown in Table 1. Accordingly, the compounds displayed varying activities depending on the chlorine substitution pattern.

First of all, the inhibition potential of each compound was found to be concentration dependent. In other words, the potential of the compounds to inhibit both cholinesterase enzymes were measured higher with the increasing concentration. On the other hand, the compounds were found more active on AChE, and their potential on the inhibition of BuChE was found lower for almost each compound analyzed.

Small variances were observed within the group of compounds possessing the same number of chlorine substitutions. The average inhibition percentage of mono-chlorinated phenols was found lower in comparison to the rest of the compounds. However, the increase in the potential to inhibit the cholinesterase enzymes corresponding to the number of chlorine substitutions was too different from di-chlorine substituted derivatives to the tetra-substituted analogues, although the average percent inhibition depending on the group of compounds possessing the same of number of chlorine substitution was found to be almost the same.

The highest activity was observed for pentachlorophenol for the inhibition of AChE, while its BuChE inhibition was found almost identical for each of other molecules analyzed.

There is limited data on the investigation of chlorinated phenols with respect to their interaction with cholinesterases (Klemmer *et al.*, 1980; Igisu *et al.*, 1993). From this perspective, the study presented here, for the first time, have indicated the potential of chlorinated phenols to act as cholinesterase inhibitors. Overall, the activities of phenol compounds were found quite low to be further analyzed with respect to the metabolism and toxicological perspectives. Indeed, the concentrations employed within this study are relatively high for acute exposure conditions. However, the potential of the compounds obtained might be evaluated from the occupational or environmental perspectives leading to the chronic exposure to one of these compounds.

Table 1: The potential the title compounds to inhibit AChE and BuChE enzymes.

Chlorinated phenols	% Inhibition (AChE) 50µM	% Inhibition (AChE) 100µM	% Inhibition (BuChE) 50µM	% Inhibition (BuChE) 100µM
2-chlorophenol	9.6 ± 0.3	19.0 ± 0.1	5.2 ± 0.2	9.2 ± 0.7
3-chlorophenol	5.1 ± 0.4	22.1 ± 0.3	4.3 ± 0.9	7.0 ± 0.5
4-chlorophenol	4.7 ± 0.3	18.7 ± 0.2	4.6 ± 0.1	7.3 ± 0.5
2,3-dichlorophenol	14.5 ± 0.1	21.5 ± 0.4	8.2 ± 0.5	11.3 ± 0.4
2,4-dichlorophenol	11.0 ± 0.1	28.3 ± 0.7	6.1 ± 0.2	9.3 ± 0.2
2,5-dichlorophenol	15.0 ± 0.37	27.1 ± 0.9	5.0 ± 0.5	8.3 ± 0.2
2,6-dichlorophenol	12.2 ± 0.7	30.2 ± 0.1	7.3 ± 0.4	10.1 ± 0.1
3,4-dichlorophenol	10.1 ± 0.5	31.1 ± 0.3	7.2 ± 0.1	9.8 ± 0.3
3,5-dichlorophenol	18.2 ± 0.4	25.2 ± 0.2	8.4 ± 0.2	11.3 ± 0.1
2,3,4-trichlorophenol	9.5 ± 0.3	18.3 ± 0.2	7.5 ± 0.2	8.1 ± 0.2
2,3,5-trichlorophenol	16.4 ± 0.5	27.0 ± 0.9	5.4 ± 0.1	8.0 ± 0.1
2,3,6-trichlorophenol	17.7 ± 0.4	25.2 ± 0.6	7.0 ± 0.6	10.3 ± 0.8
2,4,5-trichlorophenol	10.7 ± 0.3	30.3 ± 0.1	5.5 ± 0.1	10.7 ± 0.9
2,4,6-trichlorophenol	8.4 ± 0.1	20.0 ± 0.5	4.5 ± 0.5	11.9 ± 0.1
3,4,5-trichlorophenol	9.3 ± 0.5	19.7 ± 0.8	3.9 ± 0.2	10.3 ± 0.4
2,3,4,5-tetrachlorophenol	14.2 ± 0.6	35.2 ± 0.5	4.1 ± 0.5	14.2 ± 0.6
2,3,4,6-tetrachlorophenol	14.0 ± 0.3	37.3 ± 0.4	4.5 ± 0.6	9.0 ± 0.3
2,3,5,6-tetrachlorophenol	17.0 ± 0. 7	33.3 ± 0.2	6.1 ± 0.9	13.8 ± 0.7
Pentachlorophenol	19.0 ± 0.1	38.1 ± 0.4	7.0 ± 0.4	13.9 ± 0.2
Donepezil	89.0 ± 0.8	92.3 ± 0.9	83.2 ± 0.5	87.2 ± 0.9
Rivastigmine	69.9 ± 0.2	79.1 ± 0.3	78.8 ± 1.1	81.3 ± 0.5

CONCLUSION

It is apparent that the research on the investigation of environmentally exposured compounds and their activities inside the organisms has always been a topic of concern. In this study, the potential of chlorinated phenols to act as cholinesterase inhibitors was investigated and it was concluded that these molecules have negligible significance in terms of a side or toxic effect with respect to cholinesterase inhibition. However, the low potential must be at the same time evaluated depending on the acute/chronic exposure concomitant to the presence of other molecules.

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