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Inhibition by pesticides of the DJ-1/Park7 protein related to Parkinson disease.

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Parkinson's disease is a severe neurodegenerative disease. Several environmental contaminants such as pesticides have been suspected to favor the appearance of this pathology. The protein DJ-1 (or Park7) protects against the development of Parkinson's disease. Thus, the possible inhibitory effects of about a hundred pesticides on human DJ-1 have been studied. We identified fifteen of them as strong inhibitors of DJ-1 with IC50 values between 0.02 and 30 μ M. Thiocarbamates are particularly good inhibitors, as shown by thiram that acts as an irreversible inhibitor of an esterase activity of DJ-1 with an IC50 value of 0.02 μ M. Thiram was also found as a good inhibitor of the protective activity of DJ-1 against glycation. Such inhibitory effects could be one of the various biological effects of these pesticides that may explain their involvement in the development of Parkinson's disease.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease with complex etiology and variable pathology (Kalia and Lang, 2015). Most cases originate from a combination of factors that are not fully characterized. Nevertheless, several molecular mechanisms were historically identified as common markers of the disease, including α -synuclein misfolding and aggregation, and such processes play a central role in the occurrence of PD (Kazantsev and Kolchinsky, 2008). Regarding its complex etiology, although multifactorial, the causes of PD have mainly two components, a genetic component, and an environmental component. Among various environmental factors, pesticides exposure has been found as a major candidate in PD pathogenesis. Indeed, an increased risk of developing PD has been observed in people exposed for a long period of time to pesticides (Baldi et al. 2021; Brown et al. 2006). Some studies were able to investigate the risks of specific chemicals, such as paraquat, maneb, organochlorines and rotenone

(Vellingiri et al. 2022). A review by Tanner et al. summarized the state of knowledge on this topic (Tanner et al. 2011). The chemicals for which most data link them to an increased PD risk are paraquat and rotenone, with exposure associated with a 2–3-fold higher PD risk over the general population (Yan et al. 2018). More recently a study estimated the consequences of exposure for 157 pesticides in the Netherlands population. Environmental exposure to pesticides was estimated using a spatio-temporal model, based on agricultural crops around the residential address. Four of these 157 pesticides were considered a priori relevant for PD: paraquat, maneb, lindane, and benomyl (Brouwer et al. 2017). Interestingly, two of them are carbamates or thiocarbamates (R-NH-CO-NH-R or R-NH-CS-S-R), that are highly reactive towards biomolecules through modification of their thiol residues.

Thus, the association between exposure to agricultural pesticides and PD has long been a topic of study in the field of environmental health. However, the various molecular mechanisms that are at the origin of the effects of pesticides on the development of PD are not known in most

Abbreviations: AGE, advanced glycation end products; BSA, bovine serum albumin; DTT, dithiothreitol; GMP, guanosine monophosphate; MGO, methylglyoxal; PD, Parkinson's disease; pNPA, para-nitro-phenyl acetate.

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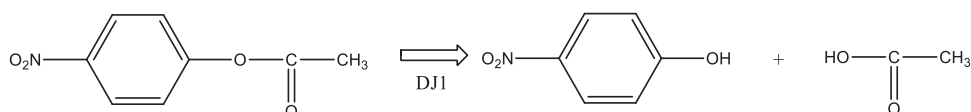
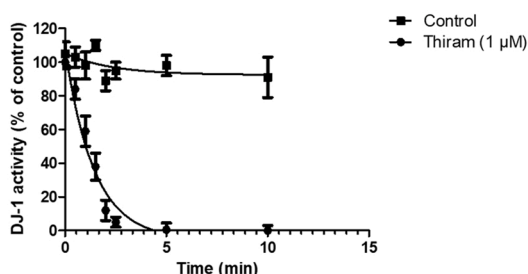


Fig. 1. Esterase activity of DJ-1 towards pNPA as substrate.

a: Inhibition of the DJ-1 esterase activity as a function of the time of preincubation of DJ-1 with thiram. Mean values from three independent experiments.



b: Inhibition of the DJ-1 esterase activity with thiram before and after dialysis. Mean values from three independent experiments.

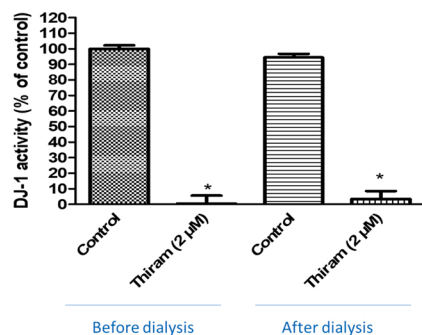


Fig. 2. Kinetic analysis of DJ-1 inhibition by thiram a: Inhibition of the DJ-1 esterase activity as a function of the time of preincubation of DJ-1 with thiram. Mean values from three independent experiments. b: Inhibition of the DJ-1 esterase activity with thiram before and after dialysis. Mean values from three independent experiments.

cases.

A major factor contributing to cellular and organism aging is the accumulation of advanced glycation end products (AGEs) on proteins, lipids and nucleic acids (Chaudhuri et al. 2018; Miranda et al. 2016; Singh et al. 2001). The AGEs comprise a large number of heterogeneous chemical structures and a majority of them have a propensity to cross-link proteins, which alters their structure and function. The glycation process (also called non-enzymatic glycosylation) is initiated by a non-enzymatic reaction between sugar-derived aldehydes and protein or nucleic acid nucleophiles such as amino or thiol groups. This reaction was discovered by the French chemist Louis-Camille Maillard in 1912 and the major glycating agents are reducing sugars (glucose, fructose, ribose, and their phosphorylated derivatives) and glyoxals such as methylglyoxal (MGO, $\text{CH}_3\text{CO-CHO}$) (Maillard, 1912). Glyoxals are formed as by-products of glucose metabolism and are responsible for 65% of cellular glycation events. The condensation reaction begins with the rapid formation of a hemithioacetal with thiols and of amino-carbinols with NH_2 groups of biomolecules. Nucleic acids undergo permanent glycation by MGO and the most susceptible nucleotides are guanosine and deoxyguanosine (Thornalley, 2008). Then, a series of dehydrations, oxidations and rearrangements result in a myriad of products: Amadori's, AGEs and protein and/or DNA crosslinks. Several recent studies implicate glycation as an important process in the pathogenesis of PD (Miranda et al. 2016; Sadowska-Bartosz and Bartosz, 2016). Thus, it has been shown that α -synuclein itself, a protein that

plays a central role in PD, can be glycated in models of this pathology (Guerrero et al. 2013). Recently, Outeiro and colleagues established that the glycation process enhances α -synuclein toxicity in vitro and in vivo, in *Drosophila* and mice.

The protein DJ-1 (or Park7) has a protective role towards the development of PD since the PARK7 gene is associated with recessive and sporadic forms of PD (Bonifati et al. 2003). Intensive studies have been undertaken to figure out DJ-1 function(s) and its role in the etiology of this neurodegenerative disease. Thus, DJ-1 has been proposed to take part in various physiological pathways related to the promotion of cell survival (Oh and Mouradian, 2017). It also modulates oxidative and electrophilic stresses. DJ-1 activates the Nrf2-mediated antioxidant response (Neves et al. 2022). It catalytically protects and/or repairs various biomolecules against glycation by glyoxals (Matsuda et al. 2017; Richarme et al. 2017; Richarme et al. 2015) or detoxifies reactive compounds produced during glycolysis (Heremans et al. 2022; Lee et al. 2012). Although the nature of its physiological substrates is still a matter of controversy (Andreeva et al. 2019; Galligan et al. 2018; Jun and Kool, 2020; Matsuda et al. 2017; Mazza et al. 2022; Mulikova et al. 2021; Pfaff et al. 2017a, 2017b; Richarme, 2017; Richarme and Dairou, 2017; Richarme et al. 2017; Richarme et al. 2015) DJ-1 is involved in the protection against the glycation processes.

This article reports the effects of about 100 pesticides belonging to the main chemical classes, such as organophosphates, organochlorines, arylureas, carbamates, aryloxyalkanoic acids, triazoles, neonicotinoids and pyrethroids, on human recombinant DJ-1. It shows that some of them are irreversible inhibitors of DJ-1 at the sub-micro molar level, revealing a new mode of action of these molecules. This strong inhibitory effect could be involved in the role of those pesticides in the development of PD.

2. Materials and methods

2.1. Materials

Most chemical and biochemical reactants were purchased from Sigma-Aldrich or Merck (St. Louis, United States). Plasmids for human DJ-1 were obtained from Dr. Sun-Sin Cha (Lee et al. 2003). Proteins were desalted with Micro Bio-Spin 6 columns (Biorad, Hercules, United States). UV-Visible spectra were recorded on a Carry 300 or Biotek PowerWave XS spectrometers. Gels were imaged on a LAS 4000 (GE Healthcare, Chicago, United States). The Liquid Chromatography (LC) system was composed of Shimadzu apparatus (Kyoto, Japan) equipped with a LC30AD pump, a SiL30AC auto-sampler coupled with a photodiode array detector PDA20A and a triple quadrupole mass detector 8060.

2.2. Human DJ-1 production and purification

Human DJ-1 was expressed and purified as previously described (Richarme et al. 2015). Briefly, the DJ-1 expression strain was grown in Luria-Bertani medium to an $\text{A}_{600} = 0.6$ and then induced with 1 mM isopropyl β -D-1-thiogalactopyranoside for 4 h. Bacteria were lysed by ultrasonic disruption, and the 100,000 g supernatant was used for DJ-1 purification. DJ-1 was first purified on a DEAE-Sephacel (Pharmacia, NJ, United States) column equilibrated in 30 mM Tris buffer, pH 8, containing 20 mM NaCl, 0.5 mM EDTA, and 1 mM dithiothreitol at 4 °C. Protein was eluted with a linear gradient of 20–400 mM NaCl in equilibration buffer. DJ-1 was then purified on a hydroxyapatite column

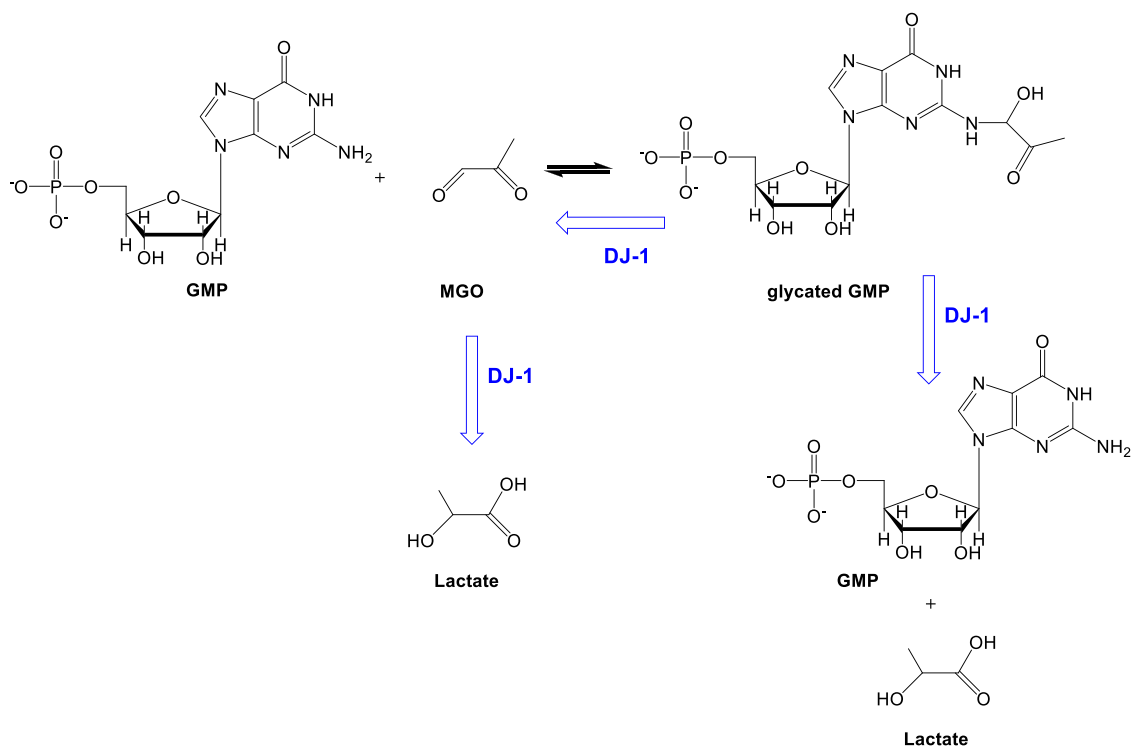


Fig. 3. Protective activity of DJ1 against glycation of GMP by MGO.

(Bio-Gel HTP from Bio-Rad) equilibrated in 20 mM phosphate buffer, pH 8, containing 20 mM NaCl, 0.5 mM EDTA and 1 mM dithiothreitol at 4 °C. Protein was eluted with a linear gradient of 20–250 mM sodium phosphate buffer, pH 8. Then, DJ-1 was dialyzed overnight against 50 mM phosphate buffer (Na₂HPO₄), pH 8, 20 mM NaCl, 0.5 mM EDTA and 1 mM dithiothreitol at 4 °C. Purified protein was quantitated with Bradford reagent (Bradford, 1976). Purity was assessed by SDS-PAGE and/or western blot with anti-DJ-1 as antibody. Proteins were kept at – 80 °C until use.

2.3. Enzyme assay—detection of DJ-1 esterase and deglycase activity

Prior to any experiment, DJ-1 enzyme was reduced by 10 mM dithiothreitol (DTT) for 30 min and then dialyzed overnight at 4 °C against 50 mM phosphate buffer. Protein concentration was determined using a Bradford assay with BSA (Bovine serum albumin) as standard (Bio-rad). DJ-1 esterase activity was performed as described previously (Vazquez-Mayorga et al. 2016) in a total volume of 200 μL: ten μL of DJ-1 solution (0.2 μM final) were added to 190 μL of a 2.8 mM solution of p-nitrophenylacetate (pNPA), prepared in PBS from a 200 mM stock solution in dimethylsulfoxide. DJ-1 esterase activity was determined by monitoring the slope of the absorbance at 405 nm vs time at 37 °C.

The activity of DJ-1 concerning the protection against guanosine monophosphate glycation by methylglyoxal was determined by LC as previously reported (Richarme et al. 2015). Briefly guanosine monophosphate (GMP) 100 μM was incubated at 37 °C in N₂-gassed 50 mM phosphate buffer pH 7.0, with 400 μM MGO, in the absence or presence of 4 μM DJ-1. The samples were analyzed by LC on a C18 Reverse phase LC Kinetex® - Phenomenex column equilibrated in water with formic acid 0.1% at 45 °C, and eluted with a linear gradient with acetonitrile with 0.1% formic acid. The kinetics of GMP protection by DJ-1 was followed by measuring the area under the peak of the glycated GMP peak of the LC chromatogram (absorbance at 254 nm and Mass Spectrometry detection).

2.4. Effects of pesticides on DJ-1 activity

The inhibitory effects of pesticides were determined by running the enzymatic assay as described above, in the presence of pesticide (5 μM). This activity was compared to the activity of a similar DJ-1 solution without pesticide (100%). pNPA hydrolysis by pesticides without DJ-1 was also used as control and showed no significant esterase activity unless otherwise stated. For pesticides that show DJ-1 inhibition greater than 25% under the previous conditions, IC₅₀ determinations were made.

To test whether the reaction of DJ-1 with pesticide was irreversible, purified enzyme (1 μM final) was first incubated in presence or absence of pesticide for 30 min at 37 °C. The samples were then dialyzed overnight at 4 °C against phosphate buffer prior to enzyme assay. Controls were carried out with non-dialyzed samples.

Kinetic data with thiram were obtained at 37 °C as follows: to 1 mL of a 50 nM solution of DJ-1 was added 1 μL of a 1 mM solution of thiram (final concentrations: 1 μM). The esterase activity was recorded at various time intervals by monitoring of pNPA hydrolysis. The bimolecular rate constant k_{inac} was determined by Kitz & Wilson methodology proposed in 1962, the time-course of an irreversible inhibition assay can be described by a rising exponential curve by fitting (Kitz and Wilson, 1962).

2.5. Effects of pesticides on the oxidation state of DJ-1

Western blotting was performed as previously described (Bahmed et al. 2016). We used antibodies against native and Cys106-oxidized DJ-1 (Sigma) and the blots were then developed using an enhanced chemiluminescence western blotting kit according to the manufacturer's instructions (Amersham Pharmacia Biotech, Piscataway, NJ). Images were quantitated using ImageJ software.

2.6. Statistical analyses

Each experiment was performed independently at least three times.

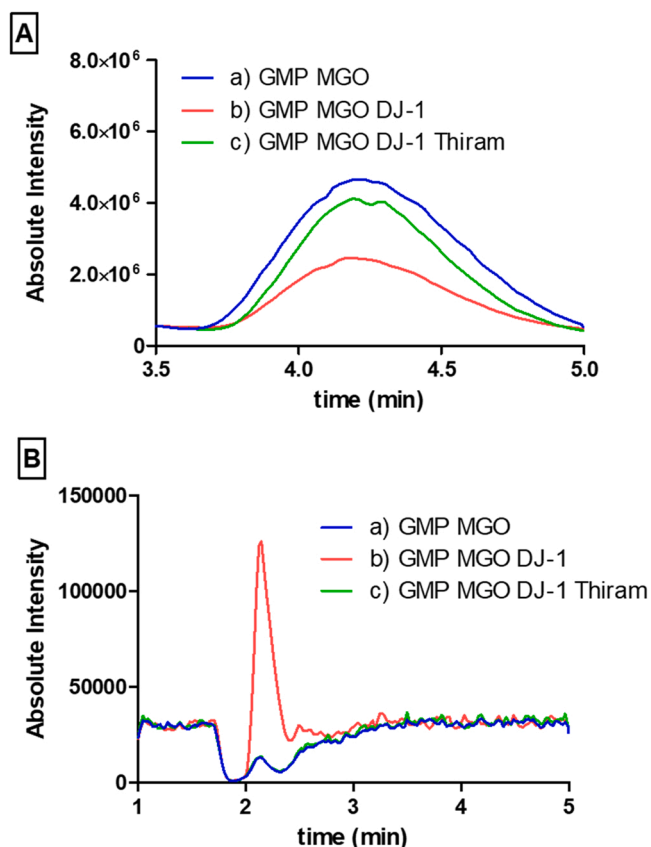


Fig. 4. Inhibition by thiram of the protective effect of DJ-1 against GMP glycation. LCMS profiles of glycated GMP ($m/z = 436.22$) under the following conditions: (a) GMP (100 μM) incubated with MGO (400 μM), (b) GMP (100 μM) incubated with MGO (400 μM) and DJ-1 (4 μM), (c) GMP (100 μM) incubated with MGO (400 μM), DJ-1 (4 μM), and Thiram (10 μM). LCMS profiles of lactate ($m/z = 89.00$) under the following conditions: (a) GMP (100 μM) incubated with MGO (400 μM), (b) GMP (100 μM) incubated with MGO (400 μM) and DJ-1 (4 μM), (c) GMP (100 μM) incubated with MGO (400 μM), DJ-1 (4 μM), and Thiram (10 μM).

The results shown in figures are, if not otherwise stated, mean values \pm S.E.M of 3–6 independent experiments. Statistical analysis was performed using analysis of ANOVA test using Prism (GraphPad Software).

3. Results and discussion

About hundred pesticides belonging to the main pesticide chemical classes were tested as possible inhibitors of DJ-1. First, they were tested as possible inhibitors of the esterase activity of human recombinant DJ-1 using pNPA as substrate (Fig. 1) (Maksimovic et al. 2021; Vazquez-Mayorga et al. 2016). Table 1 shows the percentage of inhibition observed after 5 min of incubation of 0.2 μM DJ-1 in the presence of 2.8 mM pNPA and 5 μM pesticide. Under these conditions, only a little number of pesticides led to a significant inhibition of DJ-1. This was the case of three carbamates (benomyl, formetanate and oxamyl), five organochlorines (captan, DDE, dienochlore, folpet and lindane), one pyrethroid (resmethrin), two organophosphates, (diazinon and pirimiphos methyl) and two unclassified pesticides (paraquat and dithianon) with various potencies (from 10% to 85% of inhibition). However, one family of pesticides emerges as major inhibitors of DJ-1, the thiocarbamate series (from 70% to 100% of inhibition). Interestingly, a thiocarbamate used as a drug, disulfiram, was recently reported as a good inhibitor of the esterase activity of DJ-1 (Wu et al. 2022).

Table 2 indicates the IC_{50} values found for those pesticides having led to a significant inhibition of DJ-1 (higher than 25%). Almost all the

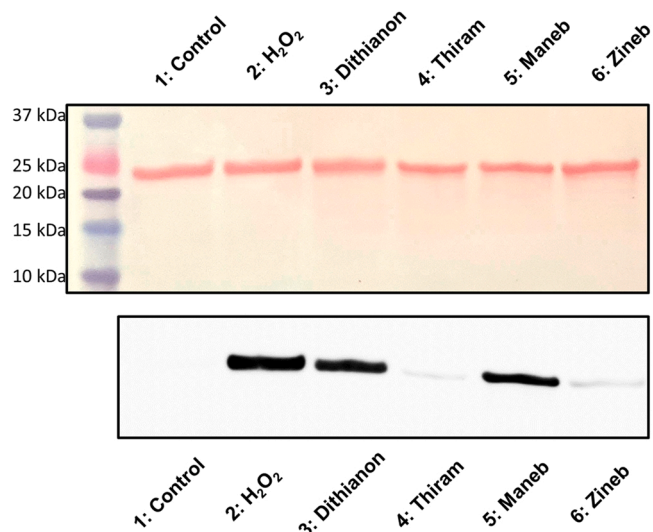


Fig. 5. Effects of pesticides on the oxidation state of DJ-1. Ponceau-stained full blot (above) and the immunostained DJ-1 band (below). Western blotting was performed with an antibody against Cys106-oxidized DJ-1 under the following conditions: 1. DJ-1 (10 μM), 2. DJ-1 (10 μM) with 100 μM H₂O₂, 3. DJ-1 (10 μM) with 100 μM dithianon, 4. DJ-1 (10 μM) with 100 μM thiram, 5. DJ-1 (10 μM) with 100 μM maneb and 6. DJ-1 (10 μM) with 100 μM zineb.

tested thiocarbamates and dithianon led to remarkably low IC_{50} values, equal or lower than 1 μM . Thiram was found to be the best inhibitor under these conditions, with an IC_{50} value of about 0.02 μM . It is important to compare these IC_{50} values with the concentrations found after human exposure to those pesticides. Thus, for example, several recent studies have revealed that humans are exposed through their consumption of fruit and vegetables and their drinking water to concentrations of maneb up to 450 $\mu\text{g/L}$ (ie 1.7 μM) (Asghar et al. 2022; Carrasco Cabrera and Medina Pastor, 2021; Onwona-Kwakye et al. 2020; Ozhan and Alpertunga, 2008). We found an IC_{50} for maneb of 0.06 μM that is much lower than the concentration reported upon maneb exposure.

Experiments based on preincubation of DJ-1 in the presence of the inhibitors were then performed to determine the type of inhibition of those thiocarbamates. Fig. 2a shows that the inhibition level observed with 1 μM thiram clearly increased with the time of preincubation of DJ-1 in the presence of this inhibitor. A complete inhibition of DJ-1 was observed after 5 min preincubation of DJ-1 with 1 μM thiram, indicating that this pesticide acts as an irreversible inhibitor of DJ-1. Determination of the k_{inact} led to a value of $3.5 \pm 0.5 \cdot 10^4 \text{ M}^{-1} \cdot \text{s}^{-1}$. Dialysis at equilibrium experiments of the incubates of DJ-1 in the presence of thiram confirmed that DJ-1 remained inactive under these conditions and that thiram acted as an irreversible inhibitor (Fig. 2b).

Some of the pesticides having led to a strong inhibitory effect on the esterase activity of DJ-1 were tested on a previously described assay measuring the protecting activity of DJ-1 towards glycated GMP formation upon reaction of GMP with MGO (Fig. 3), resulting in lactate formation, (Richarme et al. 2017). Fig. 4 A shows the LCMS chromatograms of glycated GMP ($m/z = 436.22$), alone (a) or after incubation with DJ-1 (b), or after incubation with 4 μM DJ-1 previously treated during 30 min at room temperature with 10 μM thiram (c). Fig. 4B shows the formation of lactate ($m/z = 89.00$) under identical conditions. Those results show that thiram is also an inhibitor of this protective activity of DJ-1 against glycated GMP formation. Similar results were obtained with maneb and dithianon.

As a first attempt to determine the mechanism of the inhibition of DJ-1 by the pesticides indicated in Table 2, experiments have been done to know whether some of them could lead to an irreversible oxidation of cysteine 106, a crucial residue of the DJ-1 active site (Blackinton et al.

Table 1
Inhibition of human DJ-1 by pesticides.

Chemical Class	Compounds	% of Inhibition	
Aryloxy alkanolic acids	Cyhalofop-butyl	< 5%	
	Diclofop-methyl	< 5%	
	Fenoxaprop-P	< 5%	
	Fluazifop-P-butyl	< 5%	
	MCPA	< 5%	
	MCPB	< 5%	
	Mecoprop-P	< 5%	
	Quizalofop-P-ethyl	< 5%	
Aryl ureas	Chlortoluron	< 5%	
	Foramsulfuron	< 5%	
	Linuron	< 5%	
	Metobromuron	< 5%	
	Metsulfuron-methyl	< 5%	
	Nicosulfuron	< 5%	
	Rimsulfuron	< 5%	
	Tribenuron-methyl	< 5%	
	Tritosulfuron	< 5%	
	Carbamates	Asulam	< 5%
Benomyl		85 ± 6%	
Carbaryl		< 5%	
Carbendazim		< 5%	
Chlorpropham		< 5%	
Diethofencarb		< 5%	
Formetanate		56 ± 8%	
Iprovalicarb		< 5%	
Methomyl		< 5%	
Oxamyl		22 ± 7%	
Phenmedipham		< 5%	
Pirimicarb		< 5%	
Propamocarb		< 5%	
Prosulfocarb		< 5%	
Thiabendazole		< 5%	
Neonicotinoids		Imidacloprid	< 5%
		Thiacloprid	< 5%
		Thiamethoxam	< 5%
Organochlorines	Boscalid	< 5%	
	Bixafen	< 5%	
	captan	57 ± 9%	
	Chlordecon	< 5%	
	DDE	17 ± 4%	
	DDT	< 5%	
	Dieldrin	< 5%	
	Dienochlore	35 ± 5%	
	Endosulfan	< 5%	
	Folpet	25 ± 4%	
	Lindane	12 ± 4%	
	Metolachlore	< 5%	
	Organophosphates	Acephate	< 5%
		Diazinon	10 ± 4%
Dichlorvos		< 5%	
Dimethoate		< 5%	
Ethion		< 5%	
Fenitrothion		< 5%	
Glyphosate		< 5%	
Isocarbophos		< 5%	
Malathion		< 5%	
Methyl parathion		< 5%	
Monocrotophos		< 5%	
Parathion (ethyl)		< 5%	
Phosmet		< 5%	
pirimiphos methyl		14 ± 3%	
Profenofos		< 5%	
Tolclofos-methyl		< 5%	
Triazophos		< 5%	
Pyrethroids		Allethrin	< 5%
	Bifenthrin	< 5%	
	Cyfluthrin	< 5%	
	Cypermethrin	< 5%	
	Deltamethrin	< 5%	
	Esfenvalerate	< 5%	
	Fenvalerate	< 5%	
	Fluvalinate	< 5%	
	Permethrin	< 5%	
	Phenothrin	< 5%	

Table 1 (continued)

Chemical Class	Compounds	% of Inhibition
Thiocarbamates	Resmethrin	26 ± 4%
	Tetramethrin	< 5%
	Tralomehrin	< 5%
	Mancozeb	100 ± 5%
	Maneb	100 ± 6%
	Propineb	100 ± 3%
	Thiram	100 ± 1%
	Triallat	70 ± 12%
Triazoles	Zineb	100 ± 7%
	Ziram	100 ± 6%
	Difenoconazole	< 5%
	Hexaconazole	< 5%
	Metconazole	< 5%
	Myclobutanil	< 5%
Others	Penconazole	< 5%
	Tebuconazole	< 5%
	Triticonazole	< 5%
	Atrazine	< 5%
	Cyprodinil	< 5%
	Dinitramin	< 5%
	Dithianon	100 ± 4%
	Iprodione	< 5%
	Mepanipyrim	< 5%
	Mesotrione	< 5%
	Paraquat	20 ± 4%
Rotenone	< 5%	
Sedaxane	< 5%	
Tembotrione	< 5%	

% inhibition of the DJ1 esterase activity (using pNPA as substrate) upon incubation of DJ-1 in the presence of 2.8 mM pNPA and 5 µM pesticide; mean values +/- S.E.M. from 6 to 9 experiments.

2009; Canet-Aviles et al. 2004; Kinumi et al. 2004). For that purpose, western-blot analyses of DJ-1 incubation mixtures were done by using a specific antibody against an oxidized form of DJ-1, specifically at the level of Cys106. Fig. 5 compares the analysis of untreated DJ-1 with DJ-1 pretreated with 100 µM H₂O₂ and confirmed that the antibody specifically recognizes S-oxidized DJ-1 (line 1 vs 2). Interestingly, analysis of DJ-1 pretreated with maneb (line 5) showed the presence of S-oxidized DJ-1 whereas this was not the case for DJ-1 pretreated with thiram or zineb. The oxidation of Cys106 observed upon treatment of DJ-1 with maneb, that is a Mn(II) complex, could be due to the binding and reaction of dioxygen (O₂) with its Mn(II) center with formation of superoxide. Accordingly, incubation of DJ-1 with thiram or zineb that did not contain such a Mn(II) redox active center did not lead to Cys106 oxidation (Fig. 5).

Similar experiments performed on DJ-1 pretreated with dithianon (Fig. 5) showed the formation of S-oxidized DJ-1 whereas this was not the case for DJ-1 pretreated with folpet, formetanate, lindane, resmethrine, oxamyl or triallate (data not shown). This oxidation of Cys106 by dithianon could be due to its particular activated quinone structure able to receive an electron from Cys106 with formation of a stable radical anion.

From those data, it seems that oxidation of Cys106 would only be involved in the inhibition of DJ-1 by a few pesticides, such as maneb, mancozeb (data not shown) or dithianon. The mechanisms of inhibition of DJ-1 by the other pesticides mentioned in Table 2 remain to be determined.

4. Conclusion

Our study of the possible inhibitory effects of about hundred pesticides on human DJ-1 has shown that some twenty of them are inhibitors of the esterase activity of DJ-1 (Table 1). Fifteen of them are good inhibitors with IC₅₀ values between 0.02 and 30 µM (Table 2). The pesticides of the thiocarbamate series are particularly good inhibitors. Thus, thiram was found as an irreversible DJ-1 inhibitor with an IC₅₀ value of 0.02 µM. All those fifteen pesticides have been linked to the

Table 2
IC₅₀ values obtained for the inhibition of DJ1 (esterase activity) by pesticides.

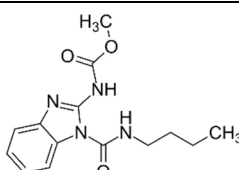
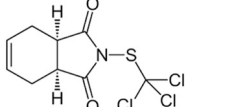
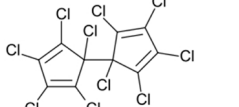
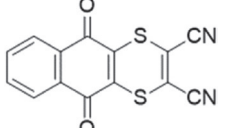
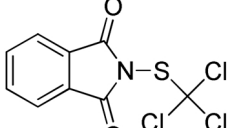
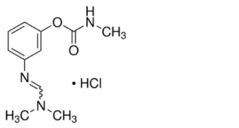
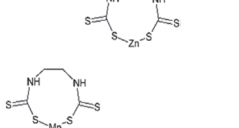
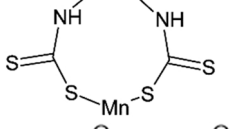
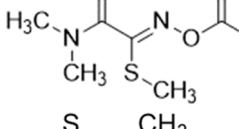
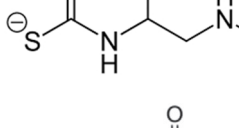
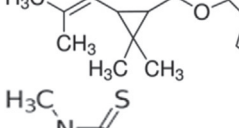
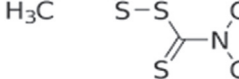
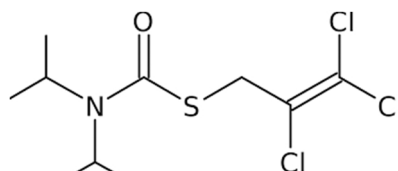
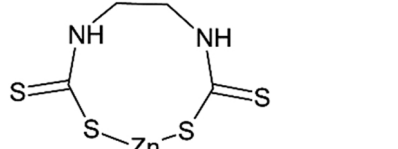
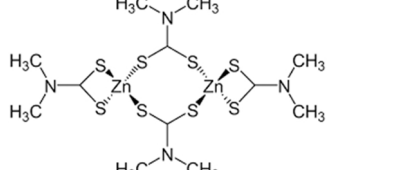
Compounds	Structure	IC ₅₀ (μM)
Benomyl		0.92 ± 0.1
Captan		6.1 ± 0.3
Dienochlore		19.2 ± 0.2
Dithianon		1.1 ± 0.2
Folpet		15.1 ± 2.3
Formetanate		3.2 ± 0.3
Mancozeb		0.09 ± 0.01
Maneb		0.06 ± 0.01
Oxamyl		24.8 ± 0.3
Propineb		0.17 ± 0.02
Resmethrine		18.1 ± 0.8
Thiram		0.018 ± 0.002

Table 2 (continued)

Compounds	Structure	IC ₅₀ (μM)
Triallat		0.99 ± 0.11
Zineb		0.17 ± 0.02
Ziram		0.15 ± 0.02

development of PD (Brouwer et al. 2017; Brown et al. 2006; Tanner et al. 2011). Interestingly, a recent study of the consequences of the exposure for 157 pesticides in the Netherland population has shown that four of those pesticides, paraquat, maneb, lindane and benomyl, were relevant for the development of PD (Brouwer et al. 2017). Interestingly, those four pesticides were found to act as inhibitors of DJ-1 (Tables 1 and 2).

Several biological effects of some pesticides have been reported to contribute to the development of PD. Thus, for instance, rotenone act as inhibitor of mitochondrial complex1 (Betarbet et al. 2006; Betarbet et al. 2000; Schiller and Zickermann, 2022), Paraquat increases the formation of free radicals and oxidative stress (Ranjbar et al. 2018) and benomyl and some dithiocarbamates act as inhibitors of the aldehyde dehydrogenase that is responsible for the detoxication of a dopamine metabolite, 3,4-dihydroxyphenyl acetaldehyde (Burke et al. 2014; Fitzmaurice et al. 2014; Fitzmaurice et al. 2013). Our results show that some of the hundred pesticides tested in our study are strong inhibitors of DJ-1. However, the esterase activity of DJ-1 has so far no identified physiological role and further experiments using *in cellulo* and *in vivo* models are necessary to show that this inhibition of DJ-1 could be one of the various biological effects of those pesticides involved in the development of PD.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Julien DAIROU reports financial support was provided by French national Health Safety Agency and FRM.

Data availability

Data will be made available on request.

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References

- Andreeva, A., Bekkhozhin, Z., Omertassova, N., Baizhumanov, T., Yeltay, G., Akhmetali, M., Toibazar, D., Utepbergenov, D., 2019. The apparent deglycase activity of DJ-1 results from the conversion of free methylglyoxal present in fast equilibrium with hemithioacetals and hemiaminals. *J. Biol. Chem.* 294, 18863–18872.
- Asghar, M., Yaqoob, M., Munawar, N., Nabi, A., 2022. Determination of Mancozeb and Maneb using flow injection chemiluminescence detection. *Int. J. Environ. Ch.* 102, 2586–2606.
- Bahmed, K., Messier, E.M., Zhou, W., Tudor, R.M., Freed, C.R., Chu, H.W., Kelsen, S.G., Bowler, R.P., Mason, R.J., Kosmider, B., 2016. DJ-1 modulates nuclear erythroid 2-related factor-2-mediated protection in human primary alveolar type II cells in smokers. *Am. J. Respir. Cell Mol. Biol.* 55, 439–449.
- Baldi, I., Botton, J., Chevri er, C., Coumoul, X., Elbaz, A., Goujon, S., Jouzel, S., Monnereau, A., Multigner, L., Salles, B., Siroux, V. and Spinosi, J., 2021, Pesticides et effets sur la sant e. In:  E.E. Sciences (Ed), EXPERTISE COLLECTIVE.
- Betarbet, R., Canet-Aviles, R.M., Sherer, T.B., Mastroberardino, P.G., McLendon, C., Kim, J.H., Lund, S., Na, H.M., Taylor, G., Bence, N.F., Kopito, R., Seo, B.B., Yagi, T., Yagi, A., Klinefelter, G., Cookson, M.R., Greenamyre, J.T., 2006. Intersecting pathways to neurodegeneration in Parkinson's disease: effects of the pesticide rotenone on DJ-1, alpha-synuclein, and the ubiquitin-proteasome system. *Neurobiol. Dis.* 22, 404–420.
- Betarbet, R., Sherer, T.B., MacKenzie, G., Garcia-Osuna, M., Panov, A.V., Greenamyre, J. T., 2000. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat. Neurosci.* 3, 1301–1306.
- Blackinton, J., Lakshminarasimhan, M., Thomas, K.J., Ahmad, R., Greggio, E., Raza, A.S., Cookson, M.R., Wilson, M.A., 2009. Formation of a stabilized cysteine sulfinic acid is critical for the mitochondrial function of the parkinsonism protein DJ-1. *J. Biol. Chem.* 284, 6476–6485.
- Bonifati, V., Rizzu, P., van Baren, M.J., Schaap, O., Breedveld, G.J., Krieger, E., Dekker, M.C., Squitieri, F., Ibanez, P., Joosse, M., van Dongen, J.W., Vanacore, N., van Swieten, J.C., Brice, A., Meco, G., van Duijn, C.M., Oostra, B.A., Heutink, P., 2003. Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. *Science* 299, 256–259.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254.
- Brouwer, M., Huss, A., van der Mark, M., Nijssen, P.C.G., Mulleners, W.M., Sas, A.M.G., van Laar, T., de Snoo, G.R., Kromhout, H., Vermeulen, R.C.H., 2017. Environmental exposure to pesticides and the risk of Parkinson's disease in the Netherlands. *Environ. Int.* 107, 100–110.
- Brown, T.P., Rumsby, P.C., Capleton, A.C., Rushton, L., Levy, L.S., 2006. Pesticides and Parkinson's disease—is there a link? *Environ. Health Perspect.* 114, 156–164.
- Burke, W.J., Bronstein, J.M., Fitzmaurice, A., 2014. Aldehyde dehydrogenase variation enhances effect of pesticides associated with Parkinson disease. *Neurology* 83, 1879–1880.
- Canet-Aviles, R.M., Wilson, M.A., Miller, D.W., Ahmad, R., McLendon, C., Bandyopadhyay, S., Baptista, M.J., Ringe, D., Petsko, G.A., Cookson, M.R., 2004. The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization. *Proc. Natl. Acad. Sci. USA* 101, 9103–9108.
- Carrasco Cabrera, L., Medina Pastor, P., 2021. The 2019 European Union report on pesticide residues in food. *EFSA J.* 19, e06491.
- Chaudhuri, J., Bains, Y., Guha, S., Kahn, A., Hall, D., Bose, N., Gugliucci, A., Kapahi, P., 2018. The role of advanced glycation end products in aging and metabolic diseases: bridging association and causality. *Cell Metab.* 28, 337–352.
- Fitzmaurice, A.G., Rhodes, S.L., Cockburn, M., Ritz, B., Bronstein, J.M., 2014. Aldehyde dehydrogenase variation enhances effect of pesticides associated with Parkinson disease. *Neurology* 82, 419–426.
- Fitzmaurice, A.G., Rhodes, S.L., Lulla, A., Murphy, N.P., Lam, H.A., O'Donnell, K.C., Barnhill, L., Casida, J.E., Cockburn, M., Sagasti, A., Stahl, M.C., Maidment, N.T., Ritz, B., Bronstein, J.M., 2013. Aldehyde dehydrogenase inhibition as a pathogenic mechanism in Parkinson disease. *Proc. Natl. Acad. Sci. USA* 110, 636–641.
- Galligan, J.J., Wepy, J.A., Streeter, M.D., Kingsley, P.J., Mitchener, M.M., Wauchope, O. R., Beavers, W.N., Rose, K.L., Wang, T., Spiegel, D.A., Marnett, L.J., 2018. Methylglyoxal-derived posttranslational arginine modifications are abundant histone marks. *Proc. Natl. Acad. Sci. USA* 115, 9228–9233.
- Guerrero, E., Vasudevaraju, P., Hegde, M.L., Britton, G.B., Rao, K.S., 2013. Recent advances in alpha-synuclein functions, advanced glycation, and toxicity: implications for Parkinson's disease. *Mol. Neurobiol.* 47, 525–536.
- Heremans, I.P., Caligiore, F., Gerin, I., Bury, M., Lutz, M., Graff, J., Stroobant, V., Vertommen, D., Teleman, A.A., Van Schaftingen, E., Bommer, G.T., 2022. Parkinson's disease protein PARK7 prevents metabolite and protein damage caused by a glycolytic metabolite. *Proc. Natl. Acad. Sci. USA* 119.
- Jun, Y.W., Kool, E.T., 2020. Small substrate or large? Debate over the mechanism of glycation adduct repair by DJ-1. *Cell Chem. Biol.* 27, 1117–1123.
- Kalia, L.V., Lang, A.E., 2015. Parkinson's disease. *Lancet* 386, 896–912.
- Kazantsev, A.G., Kolchinsky, A.M., 2008. Central role of alpha-synuclein oligomers in neurodegeneration in Parkinson disease. *Arch. Neurol.* 65, 1577–1581.
- Kinumi, T., Kimata, J., Taira, T., Ariga, H., Niki, E., 2004. Cysteine-106 of DJ-1 is the most sensitive cysteine residue to hydrogen peroxide-mediated oxidation in vivo in human umbilical vein endothelial cells. *Biochem. Biophys. Res. Commun.* 317, 722–728.
- Kitz, R., Wilson, I.B., 1962. Esters of methanesulfonic acid as irreversible inhibitors of acetylcholinesterase. *J. Biol. Chem.* 237, 3245–3249.
- Lee, J.Y., Song, J., Kwon, K., Jang, S., Kim, C., Baek, K., Kim, J., Park, C., 2012. Human DJ-1 and its homologs are novel glyoxalases. *Hum. Mol. Genet.* 21, 3215–3225.
- Lee, S.J., Kim, S.J., Kim, I.K., Ko, J., Jeong, C.S., Kim, G.H., Park, C., Kang, S.O., Suh, P. G., Lee, H.S., Cha, S.S., 2003. Crystal structures of human DJ-1 and Escherichia coli Hsp31, which share an evolutionarily conserved domain. *J. Biol. Chem.* 278, 44552–44559.
- Maillard, L.C., 1912. Action des acides amin es sur les sucres: formation des melanoidines par voie m ethodique. *C. R. Acad. Sci. Ser. 2*, 66–68.
- Maksimovic, I., Finkin-Groner, E., Fukase, Y., Zheng, Q., Sun, S., Michino, M., Huggins, D.J., Myers, R.W., David, Y., 2021. Deglycase-activity oriented screening to identify DJ-1 inhibitors. In: *RSC Med Chem*, 12, pp. 1232–1238.
- Matsuda, N., Kimura, M., Queliconi, B.B., Kojima, W., Mishima, M., Takagi, K., Koyano, F., Yamano, K., Mizushima, T., Ito, Y., Tanaka, K., 2017. Parkinson's disease-related DJ-1 functions in thiol quality control against aldehyde attack in vitro. *Sci. Rep.* 7, 12816.
- Mazza, M.C., Shuck, S.C., Lin, J., Moxley, M.A., Termini, J., Cookson, M.R., Wilson, M.A., 2022. DJ-1 is not a deglycase and makes a modest contribution to cellular defense against methylglyoxal damage in neurons. *J. Neurochem.* 162, 245–261.
- Miranda, V.H., El-Agnaf, O.M., Outeiro, T.F., 2016. Glycation in Parkinson's disease and Alzheimer's disease. *Mov. Disord.* 31, 782–790.
- Mulikova, T., Bekkhozhin, Z., Abdirasil, A., Utepbergenov, D., 2021. A continuous spectrophotometric assay for glutathione-independent glyoxalases. *Anal. Biochem.* 630, 114317.
- Neves, M., Graos, M., Anjo, S.I., Manadas, B., 2022. Modulation of signaling pathways by DJ-1: An updated overview. *Redox Biol.* 51, 102283.
- Oh, S.E., Mouradian, M.M., 2017. Regulation of Signal Transduction by DJ-1. *Adv. Exp. Med Biol.* 1037, 97–131.
- Onwona-Kwakyie, M., Hogarh, J.N., Van den Brink, P.J., 2020. Environmental risk assessment of pesticides currently applied in Ghana. *Chemosphere* 254, 126845.
- Ozhan, G., Alpertunga, B., 2008. Liquid chromatographic analysis of manebe and its main degradation product, ethylenethiouera, in fruit juice. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.* 25, 961–970.
- Pfaff, D.H., Fleming, T., Nawroth, P., Teleman, A.A., 2017a. Evidence Against a Role for the Parkinsonism-associated Protein DJ-1 in Methylglyoxal Detoxification. *J. Biol. Chem.* 292, 685–690.
- Pfaff, D.H., Fleming, T., Nawroth, P., Teleman, A.A., 2017b. Reply to Richarme: Evidence against a role of DJ-1 in methylglyoxal detoxification. *J. Biol. Chem.* 292, 12784–12785.
- Ranjbar, A., Asl, S.S., Firozian, F., Dartoti, H.H., Seyedabadi, S., Azandariani, M.T., Ganji, M., 2018. Role of Cerium Oxide Nanoparticles in a Paraquat-Induced Model of Oxidative Stress: Emergence of Neuroprotective Results in the Brain. *J. Mol. Neurosci.* 66, 420–427.
- Richarme, G., 2017. Response to manuscript by Pfaff et al.: Evidence against a role of DJ-1 in methylglyoxal detoxification. *J. Biol. Chem.* 292, 12783.
- Richarme, G., Dairou, J., 2017. Parkinsonism-associated protein DJ-1 is a bona fide deglycase. *Biochem. Biophys. Res. Commun.* 483, 387–391.
- Richarme, G., Liu, C., Mihoub, M., Abdallah, J., Leger, T., Joly, N., Liebart, J.C., Jurkunas, U.V., Nadal, M., Bouloc, P., Dairou, J., Lamouri, A., 2017. Guanine glycation repair by DJ-1/Park7 and its bacterial homologs. *Science* 357, 208–211.
- Richarme, G., Mihoub, M., Dairou, J., Bui, L.C., Leger, T., Lamouri, A., 2015. Parkinsonism-associated protein DJ-1/Park7 is a major protein deglycase that repairs methylglyoxal- and glyoxal-glycated cysteine, arginine, and lysine residues. *J. Biol. Chem.* 290, 1885–1897.
- Sadowska-Bartosz, I., Bartosz, G., 2016. Effect of glycation inhibitors on aging and age-related diseases. *Mech. Ageing Dev.* 160, 1–18.
- Schiller, J., Zickermann, V., 2022. Binding of natural inhibitors to respiratory complex I. *Pharm. (Basel)* 15.
- Singh, R., Barden, A., Mori, T., Beilin, L., 2001. Advanced glycation end-products: a review. *Diabetologia* 44, 129–146.
- Tanner, C.M., Kamel, F., Ross, G.W., Hoppin, J.A., Goldman, S.M., Korell, M., Marras, C., Bhudhikanok, G.S., Kasten, M., Chade, A.R., Comyns, K., Richards, M.B., Meng, C., Priestley, B., Fernandez, H.H., Cambi, F., Umbach, D.M., Blair, A., Sandler, D.P., Langston, J.W., 2011. Rotenone, paraquat, and Parkinson's disease. *Environ. Health Perspect.* 119, 866–872.
- Thornalley, P.J., 2008. Protein and nucleotide damage by glyoxal and methylglyoxal in physiological systems—role in ageing and disease. *Drug Metab. Drug Inter.* 23, 125–150.
- Vazquez-Mayorga, E., Diaz-Sanchez, A.G., Dagda, R.K., Dominguez-Solis, C.A., Dagda, R. Y., Coronado-Ramirez, C.K., Martinez-Martinez, A., 2016. Novel Redox-Dependent Esterase Activity (EC 3.1.1.2) for DJ-1: Implications for Parkinson's Disease. *Int. J. Mol. Sci.* 17.
- Vellingiri, B., Chandrasekhar, M., Sri Sabari, S., Gopalakrishnan, A.V., Narayanasamy, A., Venkatesan, D., Iyer, M., Kesari, K., Dey, A., 2022. Neurotoxicity of pesticides - A link to neurodegeneration. *Ecotoxicol. Environ. Saf.* 243, 113972.
- Wu, Q., Zhang, M., Wen, Y., He, P., He, Q., Yang, B., Jiang, L., Yuan, M., Cao, J., 2022. Identifying chronic alcoholism drug disulfiram as a potent DJ-1 inhibitor for cancer therapeutics. *Eur. J. Pharm.* 926, 175035.
- Yan, D., Zhang, Y., Liu, L., Shi, N., Yan, H., 2018. Pesticide exposure and risk of Parkinson's disease: Dose-response meta-analysis of observational studies. *Regul. Toxicol. Pharm.* 96, 57–63.