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Activity, toxicity, molecular docking, and environmental effects of three imidazolinone herbicides enantiomers

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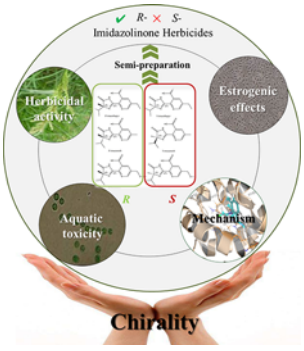
HIGHLIGHTS

- *R*- IMI herbicides are more efficient and less harmful than *S*-IMI herbicides and their racemates.
- Molecular structure contributes to the enantioselective bioactivity of IMI herbicides.
- Molecular docking can be used to explain the mechanism.

GRAPHICAL ABSTRACT



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ABSTRACT

All imidazolinone (IMI) herbicides are chiral consisting of two enantiomers; however, studies on the enantioselectivities of their interactions are limited. This study is a systematic assessment of the enantiomers and racemates of IMI herbicides, including semi-preparation and determination of absolute configurations, stereoselective bioactivity toward target organisms (*Echinochloa crus-galli* and *Microcystis aeruginosa*), and toxicity toward Michigan Cancer Foundation-7 (MCF-7) cells. *R*-imidazolinones were found to be more active than *S*-IMIs in the inhibition of target organisms, and neither enantiomer had estrogenic activity. Moreover, the relationship between the molecular structures and the efficiency of target growth inhibition by the IMI herbicides was investigated. Molecular modeling provided the rational structural basis for the enantioselectivity of the acetohydroxyacid synthase (AHAS) activity of the IMIs. These findings encourage the application of enantiopure *R*-IMI herbicides to capitalize on their advantages over the racemates.

1. Introduction

Up to 40% of the members of several classes of pesticides used in China are chiral (Ye et al., 2009), and herbicides constitute the largest portion of those pesticides. The racemic mixtures of chiral herbicides comprise two or more enantiomers (Zhang et al., 2012a, 2012b) that often show significantly different degradation rates and toxicities in the environment. Often one enantiomer is target-active, while the others are inactive or less active but still increase the pollution load on the environment. However, such herbicides are mostly produced and applied as their racemates (Garrec and Jordan, 2004) and enter the

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environment as 1:1 mixtures of their enantiomers (Qian et al., 2009). In addition, the present studies on the ecotoxic risks and environmental fates of chiral herbicides often focus on racemates with little information available on the enantioselectivities of chiral herbicides and their activities and toxicities in animals (Liu et al., 2009). The available information is often non-specific and incomplete (Ye et al., 2009). To enhance their herbicidal efficiency and reduce the environmental load and public health risks, studying these compounds and beginning to use them in their enantiopure forms is necessary.

Imazethapyr along with imazaquin, imazameth, imazamox, imazapic, and imazapyr compose the unique class of synthetic compounds known as the imidazolinone (IMI) herbicides. They are widely utilized throughout the world for their high herbicidal activity and weed control ability and are still commercially available in China. As they have water solubilities of 20–100 mg L^{-1} and their half-live in soil are weeks long, they are categorized as moderately persistent herbicides (Vogue, 2013). Imidazolinone herbicides all have an asymmetrically substituted carbon atom, and therefore consist of two enantiomers. Differences in the reactivities of the two enantiomers of IMI herbicides with acetolactate synthase (ALS) enzyme in plants have been reported with the *R*-enantiomers being 8–10 times more potent in their inhibition than the *S*-enantiomers (Los, 1984). *R*-imazethapyr (*R*-IM) was found to display a stronger inhibitory effect than *S*-imazethapyr (*S*-IM) in maize and rice. In addition, the inhibition ability of the racemate-IM (*Rac*-IM) fell between that of *R*-IM and that of *S*-IM (Zhou et al., 2009). However, based on accumulated evidence, the enantioselectivity of IMIs in plants has received relatively little attention. Systematic research on their effects on target and non-target biota is scarce.

Arabidopsis thaliana is a model organism to test herbicidal activity. However, the *Echinochloa crus-galli* (*E. crus-galli*) is one of the real target-active weeds. Weeds such as *E. crus-galli* can greatly reduce the yield of agricultural crops by competing for soil nutrients. Imidazolinone herbicides can be applied to eliminate them either pre- or post- emergence. In natural bodies of water including oceans, water blooms occur frequently (Otten et al., 2012). The blooms can have the appearance of blue-green paint or scum and comprises cyanobacteria (Ye et al., 2013). Such blooms may attenuate light in the water column, causing water anoxia, discoloration of the water, and alterations to the structure of the food web (Lewis et al., 2011). Cyanobacteria have been a growing concern for drinking water utilities that use lakes or rivers as their source water. Thus, water blooms can have adverse effects on public health (Ross et al., 2006; Yang et al., 2012). Imidazolinone herbicides enter the water and inevitably impact the growth and life cycle of cyanobacteria. *Microcystis aeruginosa* (*M.aeruginosa*) is one of typical cyanobacteria. Apart from differences in biological activities toward the target organisms, herbicides also have enantioselective toxicities (Liu et al., 2009; Konwick et al., 2005; Zhang et al., 2012a, 2012b). Because a wide variety of the synthetic chemicals that have entered the ecosystem interfere with hormone-regulated physiological processes, there is also a need to examine their adverse effects. Estrogenic effects and thyroid hormone disrupting effects are the most frequently tested. Michigan Cancer Foundation-7 (MCF-7) cells are always used to test the estrogenic effects of the herbicide.

In this work, the enantioselective effects of three of the IMI herbicides on the target organisms *E. crus-galli* and *M. aeruginosa* and non-target MCF-7 cells were investigated. The relationship between their molecular structures and their bioactivities was explored. In addition, molecular docking studies were performed to explain the experimental enantioselectivity observations. The objectives of the present study were to determine which enantiomers of IMI herbicides are the most efficient and least harmful. This work can also be helpful for ascertaining the relationships between their molecular structures and their herbicidal activities.

2. Materials and methods

2.1. Preparation of IMI herbicide enantiomers

Imazethapyr, imazameth and imazamox with chemical purities ≥97.0% were kindly donated by Xinyi Yongcheng Chemical Industrial Co., Ltd. (Jiangsu, China). The stereochemistries of the IMI herbicides are shown in Fig. 1. Chiral separations were performed on a Waters 2535 semi-preparative HPLC system (Waters Corp., Milford, USA). The enantioseparation conditions followed those of Qian et al. (2009)) with some modifications. The enantiomers were separated on an OJ-H column (5 μm, 250 mm × 10 mm i.d., Daicel), and the mobile phase was *n*-hexane/ethanol/acetic acid (50/50/0.1) at a flow rate of 5 mL min^{-1}. The herbicides were dissolved in ethanol and the injection volume was 50 μL. The purity was tested by injecting one enantiomer into the instrument, then calculating the area ratio peak1/(peak1 + peak2) and peak 2 (peak1 + peak2). The enantiopure compounds were then dissolved in ethanol and stored at 4 °C.

2.2. Stereochemical characterization of IMI herbicide enantiomers

Electronic circular dichroism (ECD) spectra of the IMI enantiomers were obtained using a Jasco J-1500 CD spectrometer (Tokyo, Japan) at room temperature. *n*-Hexane was used as the solvent. Spectra were collected over 185–400 nm, with a 200 nm min^{-1} scan speed. A 1 mm path length quartz cuvette was used.

The calculated ECD were acquired using time-dependent density functional theory (TDDFT) methods with Gaussian 09 (Frisch et al., 2009). The structures of *R*-and *S*-IMI herbicides were obtained from The PubChem Project, and ComputeVOA was used to search the configurations. Then, the geometries of the enantiomers were further optimized at the level of the B3LYP/6-31G(d,p) basis set (Becke, 1993; Hehre et al., 1986). Then, the calculated Boltzmann population-weighted spectra for each configuration were obtained (Xie et al., 2016).

2.3. Stability of IMI herbicide enantiomers

We have previously explored the configuration transformation potential (*R*-enantiomers transformed to *S*-enantiomers or *S*-transformed to *R*-enantiomers) of the herbicide enantiomers at different temperatures (4 °C and 30 °C) and in diverse organic solvents and water (Xie et al., 2016). In this study, we also test the solvent and thermal stability of IMI herbicide enantiomers. Blowing nitrogen was used to evaporate the solvents from the enantiomers, then ethanol, isopropanol, acetone, ethyl acetate, *n*-hexane were added. Aliquots of the stock solutions of the enantiomers (0.8 mL) were dried, dissolved in 0.8 mL of acetone, and diluted with ultrapure water to 40 mL. The composition of the enantiomers were analyzed after being dried and dissolved in 0.8 mL of hexane by normal-phase high performance liquid chromatography (HPLC) after 2, 5, 10, and 15 days of storage at 4 and 30 °C.

2.4. Target enantioselective bioactivity

A typical weed, *E. crus-galli*, was selected as the target vegetation. Its seedlings were obtained from Zhejiang Research Institute of Chemical Industry, China. They were surface sterilized by sequential washing with 4% sodium hypochlorite, 75% ethanol, and sterilized water. The seeds were germinated (30 °C, 48 h) before they were utilized as the model weed in this work. Healthy, actively growing and uniformly germinated seedlings were selected for herbicidal activity experiments. Ten seedlings were used in each dish (9 cm), and the treatments and the controls were repeated in triplicate. The enantiomers and racemates were dissolved in acetone, and the solutions were added to media to give final acetone concentrations of 0.1% (v/v) for each treatment and control solution. Imazethapyr and imazameth were applied to the