O.27 - p-Hydroxyphenylpyruvate dioxygenase, a herbicide target site for natural β-triketones

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Abstract

Weed control relies primarily on the use of synthetic herbicides. However, concerns over their potential impact on the environment and health necessitate the development of alternative and safer weed management tools. Natural herbicides present themselves as a potential bridge between traditional and organic agriculture (Duke et al. 2000). *p*-Hydroxyphenylpyruvate dioxygenase (HPPD) is a key enzyme in the biosynthesis of prenyl quinones. Inhibition of HPPD reduces plastoquinone levels and has deleterious effects on carotenoid synthesis and the photosynthetic apparatus. This enzyme is the target site of ••-triketone herbicides (e.g., sulcotrione and mesotrione) (Lee et al., 1997). The inhibitory activity of natural ••-triketones (e.g., flavesone, grandiflorone and leptospermone) and several analogues against HPPD was tested. Modeling of the binding of the triketones to HPPD and conformational molecular field analysis (CoMFA) determined that bulky substituents on the ring structure hindered binding to HPPD. The length of the aliphatic tail also modulated the activity of the compounds. Preliminary greenhouse data indicates that while these natural ••-triketones may not have optimal structural features of their synthetic counterparts for *in vivo* herbicidal activity, the activity of the ••-triketone enriched essential oil can be improved with the use of surfactants to a level sufficiently high to be potentially developed as natural tools for weed management.

Weed control relies primarily on the use of synthetic herbicides. However, concerns over their potential impact on the environment and health necessitate the development of alternative and safer weed management tools. Natural herbicides present themselves as a potential bridge between traditional and organic agriculture (Duke et al. 2000).

p-Hydroxyphenylpyruvate dioxygenase (HPPD) is a non-heme, iron II containing, β-keto acid-dependent enzyme that catalyses the formation of homogentisic acid (HGA) via a mechanistically complex reaction involving the oxidative decarboxylation of the 2-oxoacid side chain of 4-hydroxyphenylpyruvate (4-HPP), the subsequent hydroxylation of the aromatic ring, and a 1,2 (ortho) rearrangement of the carboxymethyl group. HGA is a precursor in the biosynthesis of prenyl quinones and tocopherols. Plastoquinone (a prenylquinone) is an essential cofactor for phytoene desaturase (Norris et al., 1995). Phytoene desaturase cannot function in the absence of plastoquinone, which leads to a deleterious reduction in the levels of carotenoids in plants. The reduced pool of carotenoids is unable to quench the excess energy generated by photosynthesis, causing a rapid degradation of chlorophylls (photodynamic bleaching).

HPPD is the target site of β-triketone herbicides (e.g., sulcotrione and mesotrione) (Lee et al., 1997). These herbicides were ostensibly derived from leptospermone, an β-triketone allelochemical from the bottlebrush plant (Callistemon spp.) that was known to cause photobleaching of foliar tissues (Gray et al., 1980). β-triketones (e.g., leptospermone, flavesone, agglomerone, tasmonone and grandiflorone) are also common in many Australasian woody plants (e.g., *Leptospermum*, *Eucalyptus*, and *Corymbia*) (Hellyer, 1968; van Klink et al., 1999; Douglas et al., 2004). Essential oils from these plants have antifungal, antimicrobial, antiviral, insecticidal, molluscicidal and herbicidal activities. For example, the triketone-enriched fraction of the essential oil of manuka (*Leptospermum scoparium* (Forst. & Forst. f.)) consists of 12.1 % leptospermone and smaller amounts of isoleptospermone, flavesone, and grandiflorone. This oil is

phytotoxic and causes bleaching of foliage similar to that caused by synthetic triketone herbicides. Manuka oil and its triketone components cause decreases in both chlorophyll and carotenoids in treated seedlings (Dayan et al. 2007).

A. thaliana HPPD was heterologously expressed and purified as described by Meazza et al. (2002). The inhibitory activity of natural β-triketones and several analogues against HPPD was tested. The structure-activity relationship derived from the dose-response curves of 19 natural β-triketones analogues against the enzyme activity of HPPD (Figure 1) provided a wide range of I_{50} values that were used as the basis for a conformational molecular field analysis (CoMFA) and docking study.

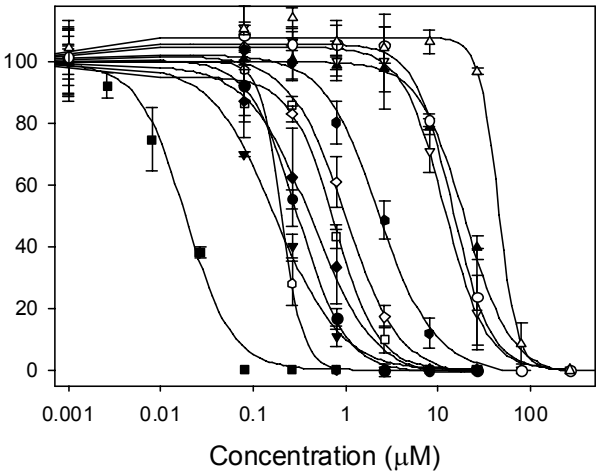

Figure 1. Inhibition of HPPD by the triketones samples set used in this study. Symbols are M32 (△), M33 (▽), M34 (○), M41 (□), M47 (◇), M62 (), M64 (▲), M80 (▼), M81 (●), M82 (■), M228 (◆), and M665 (⬣). Each data point represents the mean of three independent experiment ± 1 SD. Compounds M35, M79, M83, M85, M86, M497 and M498 are not displayed because their I_{50} was greater than 50 μM.

Computer modeling illustrated that leptospermone and other triketones may bind to HPPD in a manner similar to that of sulcotrione. The most active compounds fit within the substrate binding domain (Figure 2), whereas the inactive triketones (e.g., M83, M85, M86) had bulky side chains that exceeded the dimensions of the cavity. A Fe^{2+} metal known to play a key role in the catalytic reaction interacts with the 1,3-diketone moiety of the inhibitors coordinated via an octahedral complex with three strictly conserved active site residues (Glu373, His287 and His205) and a critical binding site H_2O molecule, providing a strong ligand orientation and binding force. These interactions are consistent with those established with several classes of potent 1,3-diketone-type HPPD inhibitors.

Analysis of the catalytic domain of HPPD reveals that a lipophilic region near the Fe^{2+} favors the binding of ligands with lipophilic moieties. For this reason, triketones with longer side chains tend to have higher inhibitory activity than those with shorter side chains (e.g., M82>M47>M41>M33>M32). However, compounds with chains longer than M82 (e.g., M62 and M81 have lower activity because they extend beyond the lipophilic domain within the catalytic site.

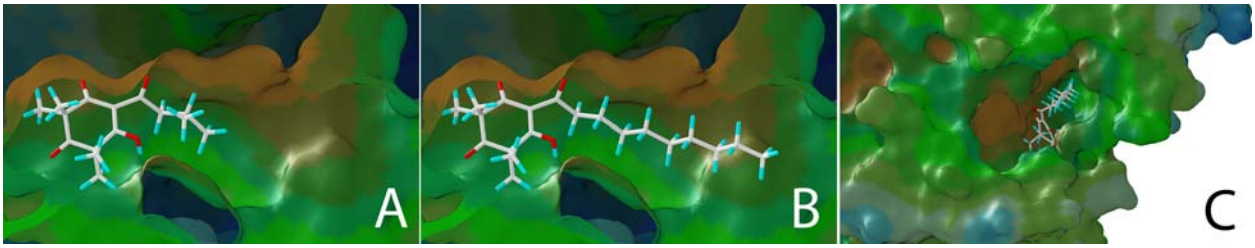

Figure 2. Binding mode of A) leptospermone (M33) and B) M82 within the substrate binding domain of HPPD. C) View of M82 within the channel leading to the binding domain

CoMFA illustrated that bulky substituents on the ring structure hindered binding to HPPD. The pattern is a reflection of the volume limitation near the Fe^{2+} atom. The length of the aliphatic tail also modulated the activity of the compounds (Figure 3).

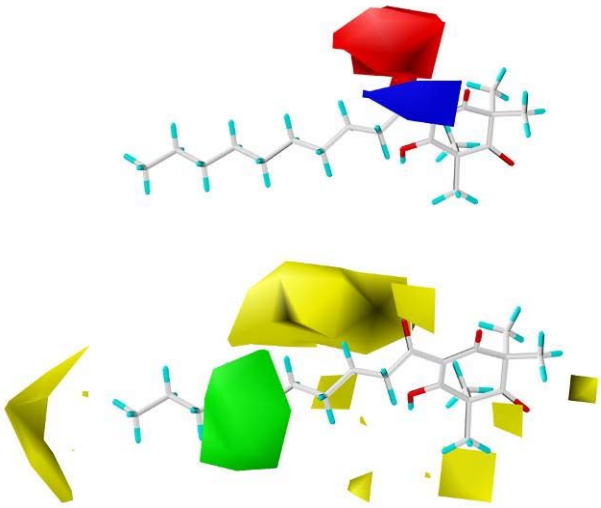

Figure 3. CoMFA maps of the β-triketones. Green areas mean increase in steric bulk is favoured and yellow areas mean increase in steric bulk is not favoured. Red areas mean negative charge and H-bond acceptor are favoured and positive charge and H-bond donors are not favoured. Blue areas mean positive charge and H-bond donors are favoured and negative charge and H-bond acceptors are not favoured

The models provided good prediction of the activity (Figure 4). The CoMFA has a r^2 value of 0.961, which indicates that this model represents the relationship between the structures and their inhibitory activity fairly well. CoMFA also provides a q^2 value (cross-validated r^2) of 0.629, which represents how well the model can predict the activity of compounds structurally related to the dataset used to develop the model. Experimentally, any model with a q^2 greater than 0.5 has a strong and statistically valid predictive power.

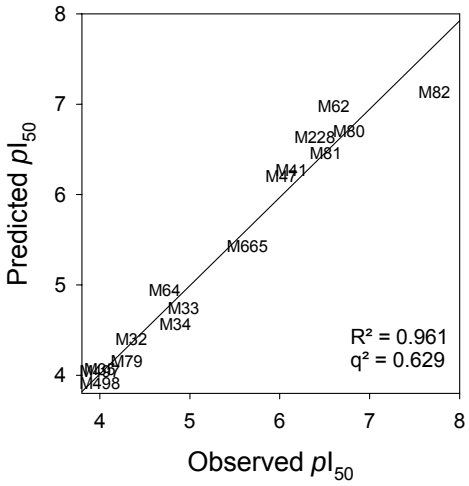

Figure 4. Leave-one-out validation model showing the relationship between the measured activity and the predicted activity calculated by the CoMFA model.

Greenhouse experiment.

The potency of the triketone-rich essential oil of manuka was tested on several monocotyledonous (*Sorghum halepense* (L.) Pers., *Echinochloa crus-galli* (L.) Beauv.,*Lolium rigidum*, Gaudin), dicotyledonous (*Abutilon theophrasti*, Medic., *Amaranthus retroflexus* (L.), and *Ipomoea hederacea* (L.) Jacq.), and sedge (*Cyperus rotundus*, L.) weeds. The effect of various surfactants (nonionic, silicon-based and crop oil concentrate) was also tested to determine whether these adjuvants can be used to increase this herbicidal activity of the essential oil. The experiments are ongoing and data will be presented at the conference. However, preliminary data indicates that while these natural β-triketones may not have optimal structural features of their synthetic counterparts for *in vivo* herbicidal activity, the activity of the β-triketone enriched essential oil can be improved with the use of surfactants to a level sufficiently high to be potentially developed as natural tools for weed management.

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