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The discovery of bioisoster compound for plumbagin using the knowledge-based rational method

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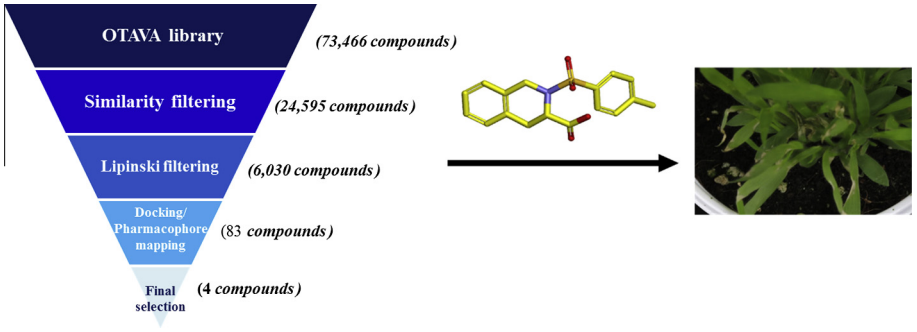
HIGHLIGHTS

- We endeavored to discovery plumbagin bioisostere as herbicide targeting to *At*KAPAS.
- The metabolic site of plumbagin was identified.
- Compound library was filtered by performing a similarity-based virtual screeening.

GRAPHICAL ABSTRACT

The novel tetrahydro-isoquinoline-3-carboxylic acid derivative, **compound 2**, was identified as the new herbicide from a commercial compound library using a knowledge-based computational method.



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ABSTRACT

Arabidopsis thaliana 7-Keto-8-AminoPelargonic Acid Synthase (*At*KAPAS) is a crucial herbicide target, and *At*KAPAS inhibitors are widely available in the agrochemical market. The herbicide plumbagin is known as a potent inhibitor for *At*KAPAS but it is extremely toxic. In this study, we identified the metabolic site of plumbagin and also performed a similarity-based library analysis using 2D fingerprints and a docking study. Four compounds as virtual hits were derived from plumbagin. Treatment of *Digitaria ciliaris* with **compound 2**, one of four hit compounds, stunted the growth of leaves and the leaf tissue was desiccated or burned within three days. Thus, we expect that **compound 2** will be developed as a new herbicide and additionally our strategy will provide helpful information for optimizing lead compounds.

Introduction

7-Keto-8-Aminopelargonic acid synthase from the *Arabidopsis thaliana* plant (*At*KAPAS) introduced in our previous research [1] is a novel potent herbicide target that is involved in the early steps of the creation of the biotin (vitamin H) biosynthesis pathway. Since biosynthetic steps of biotin are found only in plants, we expect that inhibition of the potent target *At*KAPAS will not affect the human metabolic system [1]. It is thus an ideal target for designing and developing commercial herbicides in the agrochemical market. We recently studied the 3D-structure *At*KAPAS with a theoretical method, homology modeling, because the 3D-structure of *At*KAPAS was not known [2]. In addition, we also found that

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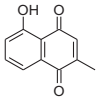
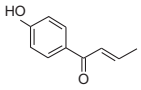
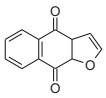
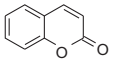
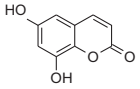
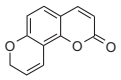
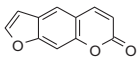
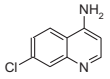
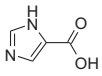
Table 1
Structural and diverse indications derived from plumbagin.

No.	Structure	Indication	Similarity[a]	Refs.
1		Activating Nrf2/ARE pathway	1.00	[4]
2		SDH inhibitor (antimalarial agent)	0.81	[11]
3		Antibacterial agent	0.78	[5]
4		Activating Nrf2/ARE pathway	0.76	[4]
5		Natural product extraction	0.76	[9]
6		Natural product extraction	0.68	[9]
7		Breast cancer cell inhibitor	0.64	[10]
8		Mycothiol biosynthesis pathway inhibitor	0.63	[7]
9		Anti-HIV activity agents	0.63	[12]
10		Antibacterial agent	0.63	[5]
11		Breast cancer cell inhibitor	0.61	[10]
12		Antibacterial agent	0.53	[5]
13		Glutathione reductase inhibitor	0.43	[8]
14		Pin1 inhibitor	0.27	[6]
15		Pin1 inhibitor	0.26	[6]

[a] Calculated to the plumbagin (No. 1) as reference ligand by Tanomoto coefficient (FCFC4).

1,4-naphthoquinone compounds including plumbagin could be employed as a good chemical lead for an S. angulatus herbicide working for *At*KAPAS [3]. In that paper, we reported that plumbagin was the most potent compound and its herbicidal activity had an IC_{50} value of 2.1 μM by *in vitro* assay. Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone), a natural compound, from the leaves of *Plumbago auriculata*, has long been known as a folk remedy. On this basis, it has been comprehensively studied as a chemotherapeutic agent by many researchers. Plumbagin and its analogs have been used as diverse agents, providing antioxidant, anti-inflammatory, anti-cancer, anti-bacterial, anti-fungal and anti-HIV activities, as shown in Table 1 [4–12]. However, concerns have been raised about its safety, perhaps due to reports on its vesicant and abortifacient properties. These have been adequately dealt with in a report by the National Toxicology Program, part of the National Institute of Health (http://ntp.niehs.nih.gov/) [13]. Plumbagin gives rise to crucial cytotoxic action toward human keratinocytes (HaCaT) as a transformed epidermal human cell line [14,15]. It is a strong inducer of reactive oxygen species (ROS) and a depleting agent of cellular glutathione [15]. More specifically, plumbagin is involved in two different mechanisms, namely, redox cycling and reaction with glutathione (GSH). Plumbagin transformed the corresponding semiquinone radicals by redox cycling and stoichiometrically converted GSH to GSSG in a metabolic system. Moreover, it was reported that plumbagin induces hepatic toxicity [9].

Thus, to resolve this dilemma and to obtain a novel plumbagin bioisostere that can be available as a potential herbicide inhibitor, we carried out a molecular similarity analysis and a docking study using a commercial library as a knowledge-based computational method in this research. We described the metabolic site that may induce the toxicity of plumbagin as mentioned, expeditiously discovered four alternative compounds *via* these computational methods and finally obtained one active compound among these four compounds through *in vivo* study.

Materials and methods

Toxicity prediction

Computational toxicology experiments utilizing The Toxicity Prediction protocol (TOPKAT) in Discovery Studio 3.5 software can play a major role in decreasing time to market, in reducing animal experiments, in assessing human health risks, and in planning a strategy for the pharmaceutical and chemical development process [16]. TOPKAT computes and validates the toxicity and the environmental effects of chemicals from their structure. This method is useful for rapidly assessing a broad range of toxicity of compounds solely from their 2D molecular structure. Thus, we simulated *in silico* prediction to explore intermediates, metabolites or pollutants of plumbagin and to assess agricultural chemical products for potential safety concerns. The two different tests of plumbagin were defined as simulation 1, for the Ames mutagenicity, and simulation 2, for the rodent carcinogenicity by NTP. The computed probability value is between 0.0 and 1.0. For Ames mutagenicity, a chemical is tested against five strains of *Salmonella typhimurium*. The developed biological model for toxicity prediction includes TA100, TA1535, and TA98, using a Histidine Reversion Assay [17]. We thereby obtained a response that the query chemical will be a mutagen or non-mutagen. We used computational models for carcinogenicity prediction created by Helma Kramer [18]. with the data set of the National Toxicology Program (NTP). The probability of rodent carcinogenicity was represented as either 0 or 1, denoting either "non-carcinogen" or "carcinogen". Thus, we explored the novel bioisostere of plumbagin based on the results of toxicological calculation.

Compound sources and filters

All compounds used in this study were obtained from OTAVA Ltd. (http://www.otavachemicals.com). Predicted physicochemical properties of the compounds were calculated using Accelrys, Pipeline Pilot 8.5 software and Advanced Chemistry Development, Inc. (ACD/Labs), Percepta 2012 Build, 2203. The database included the molecular weight, the number of rotatable bonds, the calculated octanol–water partition coefficient (calculated logP or ClogP), the number of hydrogen-bond donors, the number of hydrogen-bond acceptors, the number of chiral centers, the number of chiral double bonds (E/Z isomerism), the polar surface area, the net charge,