Substrate recognition by P-glycoprotein, drug efflux pump
-Structure-ATPase activity relationship of diverse compounds-

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1. Introduction

Humans are daily exposed to many chemicals and agrochemicals as residues in food and water, or in occupational use. Metabolism and efflux systems have been developed to protect humans from xenobiotics absorbed inside the bodies. P-glycoprotein (P-gp) is a member of the family of proteins labeled ATP-binding cassette (ABC) transporters. P-gp actively transports a wide variety of drugs and xenobiotics out of cells and functions as an efflux pump). Since P-gp recognizes compounds having diverse structures as substrates, it plays an important role for multidrug resistance in treatment of cancers. However, the mechanism of P-gp substrate recognition is complicated and still poorly understood.

In this study, we screened diverse chemicals (steroids etc.) and agrochemicals by measuring ATPase activity of P-gp. It was found that several groups of chemicals including dibenzoylhydrazine (DBH) insecticides could be substrates of P-gp. Three-dimensional QSAR, CoMFA was then performed for the activity of DBH derivatives.

2. Methods

P-glycoprotein expressed by Sf9 insect cells was purified and reconstituted in lecithin liposomes. Reconstituted protein and test compounds were reacted at 37°C for 30 min. The concentration of ADP was then measured with HPLC and ATPase activity was evaluated in terms of relative activity (%) to that of a good P-gp substrate, verapamil 2).

All computations were done with SYBYL ver. 7.3. The X-ray crystallographic structure of a DBH-type insecticide, tebufenozide (Fig. 1) was energy-minimized by Tripos force fields and used as a template. The structure of other derivatives was constructed by modifying the structure of tebufenozide. The electrostatic potential charges of MNDO were calculated for the energy-minimized structure of compounds. Compounds were automatically aligned with the common skeletal chain using SYBYL module, Align Database. The CoMFA for logit-transformed activity (logit A) were performed using default parameters.

3. Results and Discussion

A part of screening results was presented in Fig. 2. As a result of screening, DBH-type insecticides such as tebufenozide and methoxyfenozide, and organophosphates such as iprobenfos showed relatively high ATPase activity. The activity of neonicotinoids, pyrethroids, triazines, and thiocarbamates were not so high. Progesterone among tested steroids showed moderate activity.

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However other steroids such as sex hormones (testosterone and estradiol) and an insect hormone (ecdysone) had no or very low activity. Estradiol, progesterone, and testosterone have been reported to be not substrates of P-gp in previous reports\(^3,4\). Since it was also reported that progesterone significantly stimulated P-gp ATPase activity\(^3\), more investigation is necessary.

Next, the ATPase activity of the A-ring 3,5-dimethyl and 2-Cl derivatives and non-substituted B-ring derivatives of DBHs was evaluated and analyzed with CoMFA. Good CoMFA equations were obtained for 3,5-dimethyl and 2-Cl derivatives, and combined set of the compounds. For non-substituted B-ring derivatives, no significant equation was obtained probably because of low activity of the set of compounds. Introduction of log P did not improve the equations. Based on qualitative SAR and CoMFA results, 3,5-dimethyl groups on the A-ring were more favorable than 2-Cl groups. Regarding B-ring, electropositive 2-substituents and bulkier 3-substituents were favorable while smaller 4-substituents were better for the activity.

Recently the x-ray structures of the complex of cyclic peptide inhibitors (S- and R-isomers) with mouse P-gp were reported\(^5\). The complex structures revealed that each inhibitor binds to a distinct but overlapped site. We are attempting docking of DBHs based on the complex structures even if it is not easy because of the large binding cavity of P-gp. The QSAR results can be useful for determining the P-gp binding site of DBHs and other compounds.

References


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![Fig. 1 Structure of tebufenozide](image)

**Fig. 1**  Structure of tebufenozide

![Fig. 2 ATPase activity (relative activity % to that of verapamil) of agrochemicals (250 μM, n=3)](image)

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