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# Insecticidal activity of flupyrazofos KH502 against *Plutella xylostella* : a CoMFA study

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**Abstract :** In recent years, the diamondback moth (DBM), *Plutella xylostella* has become one of the most important pests for cruciferous plants in the world. A new type of the thiophosphoryl pyrazole insecticide, called KH502. Its outstanding insecticidal activity could be effective alternative against DBM. We investigated, using the comparative molecular field analysis (CoMFA) method, The structure-activity relationship of various thiophosphorylpyrazole derivatives and structure requirement for insecticidal activity. We found, the key substructures in pyrazole derivatives, the trifluoro-methyl group and the thiophosphoryl group. Both play an important role in insecticidal activity with the binding site. The three dimensional Quantitative Structure Activity Relationship (QSAR) analysis could provide useful information for the structural requirements of pyrazole insecticide as an insecticidal and the design of a new insecticide.(Received October 2, 2004; accepted September 23, 2004)

## Introduction

For over several decades now, the diamondback moth (DBM), *Plutella xylostella*, has become one of the most important pests for cruciferous plants in the world. The species is believed to have originated from southeast Europe, they are widely spread over the world is attributed to its supper migratory habit and the extended cultivation of host its (Chu, 1992). Furthermore, the continuous growth of host plants and favorable climatic conditions result in DBM attaining high populations with overlapping generations year round. If the populations were not managed properly from the early growth stage of the crop, the insects, with their excessive feeding on the leaves at larval stage, could cause a serious yield loss.

For the past 20 years, many insecticide compounds have been developed to control DBM. Recently, we developed a new phosphorothionate insecticide against DBM. It has low mammalian toxicity [$LD_{50}$ (*rat, oral*) 372-605 mg/kg] (Kim *et al.*, 1997; Sung-Bo Chemical Co. Report), called **KH502** (*o,o*-diethyl-*o*-(1-phenyl-3-trifluoromethyl-5-pyrazoyl) thiophosphoric acid ester: Fig. 1)(Hwang *et al.*, 1988, Hwang *et al.*, 1989). As a contact and stomach poison, it inhibits acetylcholinesterase without showing mutagenicity or phytotoxicity (Sung-Bo Chemical Co. Report). Thermo-stability (Cho and Han 1992), photostability (Cho *et al.*, 1993) and *in vitro* metabolism were studied using rat liver microsomes (Lee *et al.*, 1997). It effects on aerobic soil (Kim *et al.*, 1998; Jeong *et al.*, 2001).

However, little work has been done on Quantitative Structure Activity Relationship (QSAR) study. Thus, we tried to study the three-dimensional (3-D) arrangement of the pharmacophoric elements to increase the insecticide's biological activity against DBM. Our study aimed to understand the physicochemical effects of the substituents, $R^1$ on the pyrazole ring, on the insecticidal activity measured with DBM. The analysis has been made quantitatively with

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physicochemical substituents and/or molecular parameters and regression analysis (3D-QSAR).

## Materials and Methods

### Molecular Modeling

All molecular modeling and comparative molecular fieldanalysis (CoMFA) (Cramer *et al.*, 1988) studies described here were performed on Silicon Graphics workstation (Origin R10000, 250 MHz) using the SYBYL (v 6.7)program (Gasteiger and Marsili, 1980; Streitwieser, 1961). A set of 31 pyrazole derivatives with various $R^1$, $R^2$ and $R^3$ were grouped as models, A and B were used for the 3D-QSAR study (Table 1). In our first work, we studied the derivatives of model A where only $R^1$ group was changed, without variation in the $R^2$ and $R^3$ groups. And then, we tried to study about the variation of $R^1$, $R^2$, and $R^3$ groups in model B. All ligand's structures were constructed using the "Sketch Molecule". Their initial geometries for further conformational analysis were optimized by the Tripos force field with the Powell method and a distance-dependent dielectric function until the gradient reached 0.05 kcal/mol/Å. Partial atomic charges required for the calculation of the electrostatic interaction energies were calculated by the Gasteiger-Hückel method (SYBYL Molecular Modeling System). A grid search was performed changing rotable torsional angles by 60° increments from 0 to 360°. The low-energy conformers from the result of the grid search were reoptimized by removing all constraints and clustering manually. The common conformation from each compound was selected and suitably aligned by atom-by-atom fitting (Fig. 2). Compound **1** was

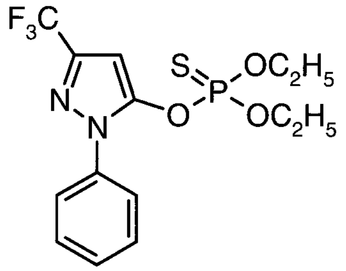
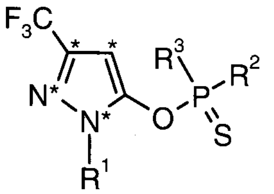


Fig. 1. Structure of KH502

Table 1. Structure and insecticidal activity of models A (left) and B (right)



**Model A**

| # | $R^1$ | $R^2$ | $R^3$ | $-log(LC_{50})$ (*in vivo*) |
|---|---|---|---|---|
| 1 | $C_6H_5$ | | | 5.85 |
| 2 | $CH_2C(Br)=CH_2$ | | | 4.21 |
| 3 | $CH_2C(CH_3)=CH_2$ | | | 4.35 |
| 4 | $CH_2C\equiv CH$ | | | 4.63 |
| 5 | $CH_2CF_3$ | | | 3.69 |
| 6 | $CH_2CH_2Br$ | | | 4.72 |
| 7 | $CH_2CH_2Cl$ | $OC_2H_5$ | $OC_2H_5$ | 4.36 |
| 8 | $CH_2CH_2C\equiv N$ | | | 4.60 |
| 9 | $CH_2CH_2OH$ | | | 4.64 |
| 10 | $C_2H_5$ | | | 4.66 |
| 11 | $CH_2CH=CH_2$ | | | 4.54 |
| 12 | $CH(CH_3)_2$ | | | 4.64 |
| 13 | $CH_3$ | | | 3.89 |
| 14 | pyrimidin-2-yl | | | 4.28 |
| 15 | pyridin-2-yl | | | 5.70 |

**Model B**

| # | $R^1$ | $R^2$ | $R^3$ | $-log(LC_{50})$ (*in vivo*) |
|---|---|---|---|---|
| 16 | $CH_2C\equiv CH$ | $OC_2H_5$ | $C_6H_5$ | 4.18 |
| 17 | $CH_2C\equiv CH$ | $OC_2H_5$ | $C_6H_5$ | 4.63 |
| 18 | $CH_2CF_3$ | $OC_2H_5$ | $OCH_2C\equiv CH$ | 3.20 |
| 19 | $CH_2CF_3$ | $OCH_3$ | $C_6H_5$ | 5.13 |
| 20 | $CH_2CH_2Cl$ | $OC_2H_5$ | $C_6H_5$ | 4.82 |
| 21 | $CH_2CH_2Cl$ | $OCH_3$ | $C_6H_5$ | 4.28 |
| 22 | $C_2H_5$ | $-OCH_2C(CH_3)_2CH_2O-$ | | 3.44 |
| 23 | $C_2H_5$ | $OC_2H_5$ | $C_6H_5$ | 5.39 |
| 24 | $C_2H_5$ | $OC_2H_5$ | $OCH_2C\equiv CH$ | 4.06 |
| 25 | $C_2H_5$ | $OCH_3$ | $C_6H_5$ | 5.24 |
| 26 | $CH_2CH=CH_2$ | $OC_2H_5$ | $C_6H_5$ | 4.52 |
| 27 | $CH(CH_3)_2$ | $-OCH_2C(CH_3)_2CH_2O-$ | | 3.46 |
| 28 | $CH_3$ | $OCH_2C\equiv CH$ | $C_6H_5$ | 4.35 |
| 29 | $CH_3$ | $OC_2H_5$ | $C_6H_5$ | 4.55 |
| 30 | $CH_3$ | $OC_2H_5$ | $OCH_2C\equiv CH$ | 4.03 |
| 31 | $CH_3$ | $OCH_3$ | $C_6H_5$ | 4.63 |

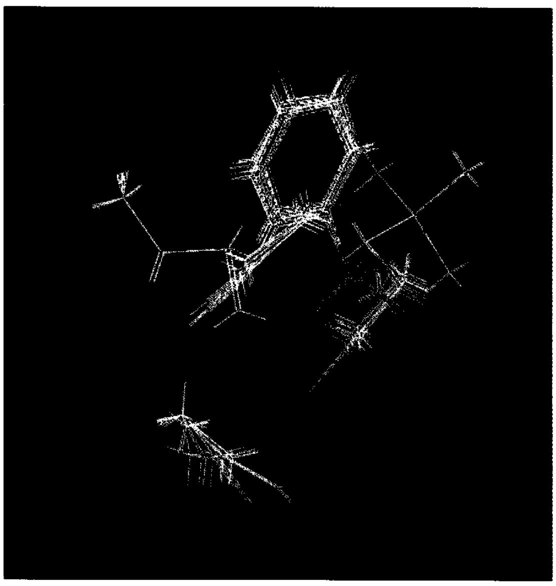


Fig. 2. Superimposition of model A and B.

Table 2. Results of CoMFA

| | | Model A | Model B |
|---|---|---|---|
| Cross-validated $R^2$ | | 0.69 | 0.75 |
| Optimum # of components | | 3 | 4 |
| Standard error of estimate | | 0.15 | 0.15 |
| Conventional $R^2$ | | 0.95 | 0.95 |
| F-value | | 64.57 | 64.53 |
| Relative | Electron static | 45 | 40 |
| Contributions (%) | Steric | 55 | 60 |

used as a template owing to its high activity. The atoms used for the 3D-alignment were two nitrogens and two carbons in the pyrazole ringthat were represented by stars (Table 1).

For a probe atom in CoMFA field, an $sp^3$ hybridized carbon atom with +1 charge was used. The grid points were regularly spaced per 2 Å to form a grid box (18X20X22 Å), following the automatic procedure in CoMFA program. After constructing the CoMFA lattice, the interaction energy of steric and electrostatic fields was calculated for each molecule at every the grid point surrounding the aligned molecules in 3D-space. To correlate these fieldenergy terms with their DBM insecticidal activity, a partial least square (PLS) analysis was used with cross validation to measure the predictive power of the model and to get the optimum number of components for the next step. The final PLS analysis was performed by no-cross validation with an optimum number of components reported from the cross validation result. The steric and electrostatic fields were

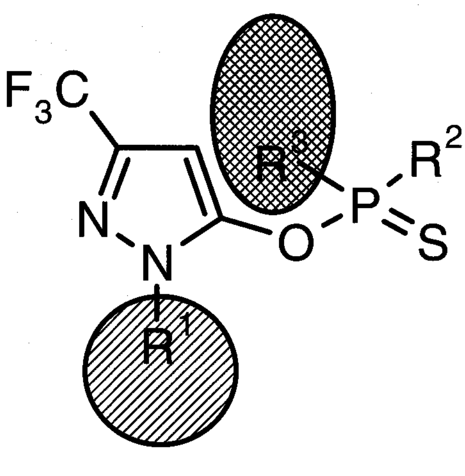


Fig. 3. The analysis of 3D-QSAR.
( $R^1$ : more negative charge-favorable region ,
$R^3$ : more bulky group-favorable region )

scaled according to CoMFA standard deviation in order to give them the same potential weights on the resulting QSAR. The constructed 3D-QSAR PLS model was then used to predict the insecticidal activity of new compounds, replacing X group with 221 new substituents, as suggested by the CoMFA module (Table 4).

## Results and Discussion

From the structure-activity relationships in the *in vivo* experiments, trifluoro-methyl and thiophosphoryl groups at the pyrazole ring were known as the very important groups to show insecticidal activity. Two groups were located in the same position of the 3D-space from the conformational search (Fig. 3). In order to compare the structure-activity relationship for $R^1$ and $R^{2,3}$, we divided the compounds into two groups, models A and B. The derivatives of model A were changed only in the $R^1$group, without alteration in the $R^2$ and $R^3$ groups. However, the derivatives of model B were changed into different substituents of $R^1$, $R^2$, and $R^3$ groups. We tried 3D-QSAR analysis using the CoMFA program (Table 2). The steric and electrostatic fields were displayed as a contour map in Fig. 4.

In model A, the CoMFA analysis showed a good correlation with cross-validated $R^2$ and conventional $R^2$ to be 0.690 and 0.946, respectively. Model B also was shown a good correlation with cross-validated $R^2$ and conventional $R^2$ to be 0.745 and 0.949, respectively. The relative contributions by steric and electrostatic factor were 55% and 45% for model A, and 60% and 40% for model B

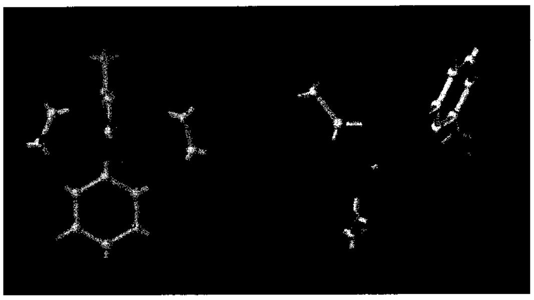


Fig. 4. CoMFA maps of models A (left) and B (right).
(Blue region : more positive charge-favorable region, Red region : more negative charge-favorable region, Green region : more bulky group-favorable region, Yellow region : less bulky group-favorable region)

Table 3. Test sets and their predicted activities

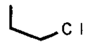
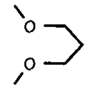
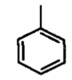
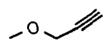
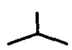
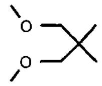
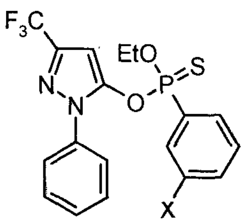
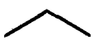
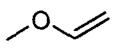
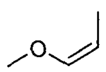
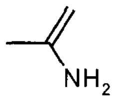
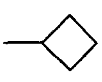
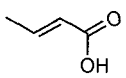
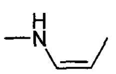
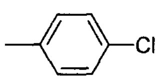
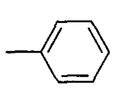
| # | $R^1$ | $R^2$ | $R^3$ | *Predicted -logLC$_{50}$(M)* | *Actual insecticidal activity -logLC$_{50}$(M) (in vivo)* |
|---|---|---|---|---|---|
| 1 |  | |  | 3.55 | 3.34 |
| 2 |  |  |  | 4.17 | 4.28 |
| 3 |  | |  | 3.28 | 3.46 |

Table 4. Results of 3D-CoMFA optimization. The best active compound's logLC$_{50}$ is 6.01.



| # | X | Predicted -logLC$_{50}$(*M*) |
|---|---|---|
| 1 |  | 6.01 |
| 2 |  | 5.82 |
| 3 |  | 5.77 |
| 4 |  | 5.73 |
| 5 |  | 5.69 |
| 6 |  | 5.67 |
| 7 |  | 5.67 |
| 8 |  | 5.63 |
| 9 |  | 5.62 |
| 10 |  | 5.54 |

respectively.

In the CoMFA map of model A, the positively charged favorable regions at $R^1$ were shown because negatively charged halogen atoms in inactive compound **5** highly decreased the insecticidal activity. The Negatively charged favorable regions at $R^1$ came from the existence of pyridine ring of compound **15**with high insecticidal activity. More bulky group favorable regions were created from the best active compound **1** with the phenyl ring. In the CoMFA map of model B, negatively charged favorable region at $R^1$ was from the inactive compounds (24, 26) that had methyl proton. A more bulky group favorable region was generated because of the active compounds with methoxy and ethoxy group at $R^3$ position.

In order to test the predictability of the CoMFA model S, we calculated, by using model B through a regression equation from CoMFA (Table 3), the insecticidal activities of three compound whose actual activities were known in the test sets. The calculation results showed that the calculated log(LC$_{50}$) was well predicted by the model B.

Because model B had a good predictive power for $R^3$, we predicted improved insecticidal activities of new compounds with modified at $R^3$ position. The QSAR- optimizedinterface in the advanced CoMFA model was used to build a series of analogues and to estimate expected activities chosen from 221 distinct substituents provided by the SYBYL program. After reviewing 221 substituents for the $R^3$ group, the bulky groups like the ethyl group has the possibility to increase their insecticidal activity against DBM, among all the best-known compounds' activity (Table 4).

We have carried out the CoMFA analysis on the 3-trifluoromethyl-5-hydroxypyrazole derivatives of the new insecticide for DBM. The CoMFA result showed good structure-activity relationships. These results will provide useful information about understanding the pharmacophore of pyrazoyl organophosphates and designing new insecticides.

## Acknowledgements

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**배추좀나방에대한flupyrazofosKH502의살충활성CoMFA**

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**요약 :** 십자화과 식물을 가해하는 세계적으로 중요한 해충의 하나인 배추좀나방(diamondback moth, *Plutella xylostella*)을 방제를 하기위하여 개발된 새로운 형태의 thiophosphoryl pyrazole계 살충제 KH502 유도체들의 구조와 살충활성을 CoMFA 방법으로 조사한 결과 효소의 결합부위에서 가장 중요한 역할을 하는 작용기는 trifluoro-methyl group과 thiophosphoryl group으로 밝혀졌다. 이와 같은 3D-QSAR 분석결과는 새로운 배추좀나방 방제용 살충제를 설계하는데 유용한 정보로 이용할 수 있을 것이다.

색인어 : 배추좀나방, CoMFA(Comparative Molecular Field Analysis), 피라졸계 살충제

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