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Study of Thiazoline Derivatives for the Design of Optimal Fungicidal Compounds Using Multiple Linear Regression (MLR)

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Rice blast is the most serious disease of rice due to its harmfulness and its world wide distribution. *Magnaporthe grisea* is the cause of rice blast disease and destroys rice enough to feed several tens of millions of people each year. Fungicides are commonly used to control rice blast. But *M. grisea* acquires resistance to chemical treatments by genetic mutations. 2-Phenylimino-1,3-thiazolines were proposed as a novel class of fungicides against *M. grisea* in the previous study. To develop compounds with a higher biological activity, a new series of 2-phenylimino-1,3-thiazolines was synthesized and its fungicidal activity was determined against *M. grisea*. The QSAR analysis was carried out on a series of 2-phenylimino-1,3-thiazolines. The QSAR results showed the dependence of fungicidal activity on the structural and physicochemical features of 2-phenylimino-1,3-thiazolines. Our results could be used as guidelines for the study of the mode of action and further design of optimal fungicides.

Key Words : Thiazoline derivatives, *Magnaporthegrisea*, QSAR, Multiple linear regression

Introduction

Magnaporthe grisea, a hemibiotrophic fungal pathogen, causes rice blast disease. This rice blast fungus has been reported to occur in over 85 countries. The rice losses by this fungus are between 10% and 30% of the annual harvest.[1] Many fungicides are used against rice blast disease, including tricyclazole, isoprothiolane and probenazole. However, *M. grisea* can establish resistance to chemical treatments. It is thought that this fungus is able to evolve into drug-resistant strain by genetic mutations.[2]

In our previous study, 2-phenylimino-1,3-thiazolines were proposed as a novel class of fungicides against *M. grisea*. Most of 2-phenylimino-1,3-thiazolines showed 90% inhibition of fungal infection at a concentration of 100 ppm but the targets inhibited are unknown. The quantitative structure-activity relationship (QSAR) analysis was carried out on the 2-phenylimino-1,3-thiazolines using multiple linear regression (MLR) to determine the structural and physicochemical features responsible for their fungicidal activity.[3-5]

In this current study, we synthesized a new series of 2-phenylimino-1,3-thiazolines that have diverse substituents in various positions of thiazolines used in the previous study. The fungicidal activity (pI_{50}; logarithm of a reciprocal concentration for 50% inhibition) of each compound was determined against *M. grisea.* Then, QSAR analysis[6,7] was

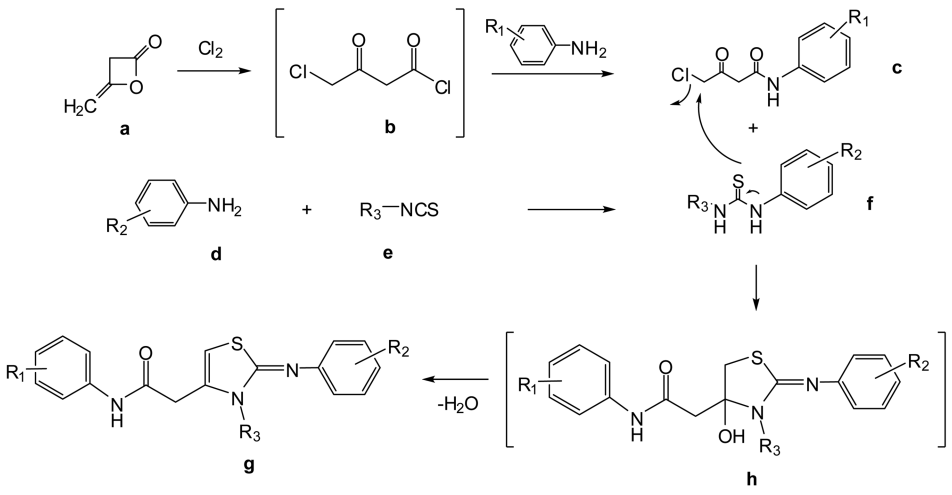


Figure 1. Reaction scheme for synthesis of 2-phenylimino-1,3-thiazoline analogues (**g**).

carried out on a series of 2-phenylimino-1,3-thiazolines in order to explore the dependence of fungicidal activity on their structural and physicochemical features.

Experimental

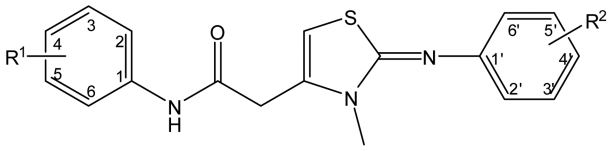
Synthetic Procedures. The 2-phenylimino-1,3-thiazolines were synthesized according to the procedure.[8] 2-Phenylimino-1,3-thiazolines **g** were synthesized from the three building blocks, γ-chloroacetoacetanilides **c**, amines **d** and isothiocyanates **e**. The chemical library of 2-phenylimino-1,3-thiazolines **g** was synthesized by the sequential reaction of amines **d**, isothiocyanates **e** and then γ-chloroacetoacetanilides **g** through thioureas **f**. We prepared the 2-phenylimino-1,3-thiazoline analogues that have various substituents at all positions (*ortho*-, *meta*- and *para*-positions in both R^1 and R^2 aromatic rings).

Biological Assay. The fungicidal activities (pI_{50}) of 2-phenylimino-1,3-thiazolines were listed in Table 1. The inhibitory value of each compound was measured according to the methods of previous study.[5] In the presence of compound, rice (*Oryza sativa* L., cv. Nakdong) plants were inoculated with *M. grisea*. After 5 days the percentage of infected leaf area was recorded and the inhibitory value was determined as shown in the Eq. (1).

$$A = \%\text{control value} = 100[(a-b)/a] \quad (1)$$

In Eq. (1), a is the area of infection (%) of leaves sprayed with Tween 20 solution as a controland b is the area of infection (%) of leaves treated with compound. The molar

Table 1. Structures of 2-phenylimino-1,3-thiazoline analogues, experimental activities (pI_{50}) against *Magnaporthegrisea*, and descriptors used in MLR



No	R^1	R^2	EXP (pI_{50})	Descriptors					
				$SA_R{}^{2\,a}$	$SV_R{}^2{}_{C4}{}^b$	CLOGP[c]	$\sigma_{pR}{}^{1d}$	$\sigma_{mpR}{}^{2e}$	$SV_R{}^1{}_{C2,3}{}^f$
1*	4-OCH_3	4'-OCF_3	7.00	161.6	36.40	5.62	-0.27	0.35	0.00
2	4-OCH_3	4'-SCH_3	6.07	162.8	39.66	5.15	-0.27	0.00	0.00
3	4-CH_2CN	4'-Cl	5.03	144.6	21.90	4.66	0.18	0.23	0.00
4	4-CH_2CN	4'-CH_2CH_3	5.25	158.0	36.88	4.97	0.18	-0.15	0.00
5	4-CHF_2	3'-Cl,4'-F	4.42	145.3	4.81	5.55	0.32	0.43	0.00
6	4-CH_2CH_3	3'-NO_2	5.52	145.8	0.00	2.26	-0.15	0.71	0.00
7	4-CH_3	2'-F,4'-F	6.03	131.8	4.32	5.31	-0.17	0.06	0.00
8*	4-CHF_2	2'-F	5.03	127.6	0.00	4.86	0.32	0.00	0.00
9	4-OCH_3	2'-Cl	5.10	142.5	0.00	5.31	-0.27	0.00	0.00
10	4-OCH_3	2',4',6'-CH_3	4.79	178.4	17.90	6.09	-0.27	-0.17	0.00
11	4-OCH_3	2',6'-Cl,4'-CF_3	6.63	188.9	38.99	6.90	-0.27	0.54	0.00
12	4-OCH_2CH_3	2'-F,4'-F	6.46	131.8	4.32	5.41	-0.24	0.06	0.00
13	4-OCH_2CH_3	2'-F,4'-Cl,5'-$OCH(CH_3)_2$	4.33	199.8	22.44	5.86	-0.24	0.23	0.00
14	4-CH_2CN	2'-F,4'-Cl	5.34	144.1	21.09	4.80	0.18	0.23	0.00
15	2-F	4'-Br	5.81	137.7	31.94	5.18	0.00	0.23	14.20
16	2-F,4-Cl	3'-F	4.82	129.1	0.00	5.27	0.23	0.34	13.15
17	2-F,4-Cl	4'-OCF_3	4.90	161.6	36.40	6.28	0.23	0.35	13.15
18	2-F,4-Cl	4'-CF_3	5.25	156.2	33.26	6.01	0.23	0.54	13.15
19	2-Cl,4-CH_3	4'-Cl	4.93	144.8	21.90	5.85	-0.17	0.23	21.49
20*	3-Cl,4-CH_3	4'-F	4.54	128.8	5.56	6.13	-0.17	0.06	22.06
21*	3-F,4-OCH_3	4'-Cl	5.24	144.8	21.90	5.45	-0.27	0.23	12.16
22*	3-F,4-OCH_3	4'-CN	6.68	144.9	23.38	4.17	-0.27	0.66	12.26
23	3-Cl,4-OCH_3	4'-CH_3	4.69	144.6	23.36	5.78	-0.27	-0.17	25.19
24	3-Cl,4-OCH_3	4'-CN	5.62	144.9	23.38	4.71	-0.27	0.66	25.19
25*	2-CH_3,4-OCH_3	2'-F	4.56	127.6	0.00	4.07	-0.27	0.00	23.65
26	4-OCH_3	2'-F,5'-F	6.55	128.6	0.00	4.49	-0.27	0.00	0.00
27	4-OCH_3	2'-CH_3,4'-CH_3	4.76	162.6	18.63	5.20	-0.27	-0.17	0.00
28	4-CH_2CH_3	4'-C_6H_5	4.57	209.2	48.03	7.20	-0.15	-0.01	0.00
29	3-Cl,4-CH_3	3'-F	4.97	129.1	0.00	6.13	-0.17	0.34	22.06
30	3-F,4-OCH_3	3'-F	5.02	129.1	0.00	4.43	-0.27	0.34	12.26

[a]$SA_R{}^2$: is the Connolly surface area of R^2 aromatic ring with substituents. [b]$SV_R{}^2{}_{C4}$: is the non-oversubstituent volume at C_4' in R^2 aromatic ring. [c]CLOGP: is the Hydrophobic parameter (Partition Coefficients) of total molecule structure. [d]$s_{pR}{}^1$: is the Hammett sigma constants of *para*-substituents in R^1 aromatic ring. [e]$s_{mpR}{}^2$: is the sum of Hammett sigma constants of meta- and *para*-substituents in R^2 aromatic ring. [f]$SV_R{}^1{}_{C2,3}$: is the non-overlap steric volume between each analogue has substituents at C_2 and C_3 carbons in R^1 aromatic ring and reference molecule (benzene). *Test Set

concentration for 50% inhibition against *M. grisea* (EC_{50}) of each compound was determined by probit analysis.[9] And pI_{50} was calculated from EC_{50}, according to the Eq. (2).[10]

$$pI_{50} = -\log(EC_{50}/MW) \quad (2)$$

MW: Molecular weight of each compound

Multiple Linear Regression Analysis. The 3D structures of 2-phenylimino-1,3-thiazoline analogues were geometrically optimized by using AM1 Hamiltonian in a MOPAC software package. The descriptors for MLR analysis were derived from the following structural and physicochemical features: (1) molecular weight, (2) non-overlap steric volume between each analogue and reference molecule (benzene), (3) the highest occupied molecular orbital energy, HOMO, (4) the lowest unoccupied molecular orbital energy, LUMO, (5) the Connolly surface area of each ring with substituents, (6) the octanol-water partition coefficient[11] (CLOGP), (7) partial charges of each carbon atom in each ring and (8) the Hammett constants[12] of substituents at various positions in aromatic rings. Molecular mechanics calculations were carried out by Insight II package.[5] MLR analysis was carried out using anin house program.[13] In order to ensure the reliability of our MLR model, the data set was divided into training and test sets.

Results and Discussion

Table 1 illustrates the structuresand their fungicidal activities of 30 thiazoline derivatives. The 2-phenylimino-1,3-thiazoline analogues have two aromatic rings (R^1 and R^2). The R^1 aromatic ring is attached to the carboxanilide group on the left side of the thiazoline ring. The R^2 aromatic ring is attached to the imino atom on the right side of the thiazoline ring; the carbon atoms in each aromatic ring are numbered counter-clockwise. All 2-phenylimino-1,3-thiazolines have a methyl substituent in the thiazoline ring and various substituents in R^1 and R^2 aromatic rings, as shown in Figure 1 and Table 1.

Multiple Linear Regression Analysis. Data set of the 2-phenylimino-1,3-thiazoline analogues was divided into training and test sets, which consist of 24 and 6 derivatives respectively. Values of the Hammett sigma constants, s_{pR}^1 and s_{mpR}^2, in R^1 and R^2 aromatic rings for the compounds in the training set are –0.27 to 0.32, –0.17 to 0.71, respectively which include the ranges of those values, –0.27 to 0.32, 0.00 to 0.66 respectively in the test set. Therefore the analyzed test set lies within the applicability domain of the training set. A leave-one-out cross-validated r^2 (LOO q^2), validation criteria, was used to select MLR model that have high predictability.[14] The final MLR model showing the highest q^2 value (0.634) was as follows:

$$\text{Fungicidal activity} = -0.046(\pm 0.013)\, SA_R^2 + 0.046(\pm 0.013)\, SV_R^2{}_{C4} - 0.069(\pm 0.022)\, SV_R^1{}_{C2C,3} + 0.251(\pm 0.211)\, \text{CLOGP} - 2.605(\pm 0.821)\, s_{pR}^1 + 1.559(\pm 0.686)\, \sigma_{mpR}^2 + 10.053(\pm 1.537)$$

$N = 24$, $r^2 = 0.818$, $s = 0.331$, $F = 12.753$, $p < 0.0001$

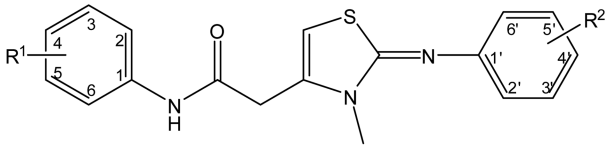
Table 2. Statistical parameters of final MLRmodel

Descriptors	Coefficient	Significance	95% confidence interval	
			Low	Up
Constant	10.053	0.000	8.516	11.590
SA_R^2	-0.046	0.000	-0.060	-0.033
$SV_R^2{}_{C4}$	0.046	0.000	0.030	0.061
CLOGP	0.251	0.023	0.040	0.463
σ_{pR}^1	-2.605	0.000	-3.426	-1.783
σ_{mpR}^2	1.559	0.000	0.872	2.245
$SV_R^1{}_{C2,3}$	-0.069	0.000	-0.091	-0.047

$N = 24$, $r^2 = 0.818$, $s = 0.331$, $F = 12.753$, $p < 0.0001$

This optimum MLR model was built using six descriptors; the Connolly surface area (SA_R^2), the substituent volumes ($SV_R^2{}_{C4}$ and $SV_R^1{}_{C2C3}$), the octanol-water partition coefficient (CLOGP) and the Hammett sigma constants (σ_{pR}^1 and

Table 3. Experimental pI_{50} versus calculated pI_{50} in MLR



No	R1	R2	Exp (pI_{50})	MLR	
				Cal	Cal-Exp
1*	4-OCH_3	4'-OCF_3	7.00	6.88	-0.12
2	4-OCH_3	4'-SCH_3	6.07	6.31	0.24
3	4-CH_2CN	4'-Cl	5.03	5.41	0.38
4	4-CH_2CN	4'-CH_2CH_3	5.25	4.95	-0.30
5	4-CHF_2	3'-Cl,4'-F	4.42	4.76	0.34
6	4-CH_2CH_3	3'-NO_2	5.52	5.35	-0.17
7	4-CH_3	2'-F,4'-F	6.03	6.01	-0.02
8*	4-CHF_2	2'-F	5.03	4.52	-0.51
9	4-OCH_3	2'-Cl	5.10	5.48	0.38
10	4-OCH_3	2',4',6'-CH_3	4.79	4.56	-0.23
11	4-OCH_3	2',6'-Cl,4'-CF_3	6.63	6.35	-0.28
12	4-OCH_2CH_3	2'-F,4'-F	6.46	6.21	-0.25
13	4-OCH_2CH_3	2'-F,4'-Cl,5'-$OCH(CH_3)_2$	4.33	4.26	-0.07
14	4-CH_2CN	2'-F,4'-Cl	5.34	5.43	0.09
15	2-F	4'-Br	5.81	5.80	-0.01
16	2-F,4-Cl	3'-F	4.82	4.41	-0.41
17	2-F,4-Cl	4'-OCF_3	4.90	4.83	-0.07
18	2-F,4-Cl	4'-CF_3	5.25	5.17	-0.08
19	2-Cl,4-CH_3	4'-Cl	4.93	5.12	0.19
20*	3-Cl,4-CH_3	4'-F	4.54	4.88	0.36
21*	3-F,4-OCH_3	4'-Cl	5.24	5.92	0.68
22*	3-F,4-OCH_3	4'-CN	6.68	6.33	-0.35
23	3-Cl,4-OCH_3	4'-CH_3	4.69	4.56	-0.13
24	3-Cl,4-OCH_3	4'-CN	5.62	5.57	-0.05
25*	2-CH_3,4-OCH_3	2'-F	4.56	4.22	-0.34
26	4-OCH_3	2'-F,5'-F	6.55	5.92	-0.63
27	4-OCH_3	2'-CH_3,4'-CH_3	4.76	5.11	0.35
28	4-CH_2CH_3	4'-C_6H_5	4.57	4.73	0.16
29	3-Cl,4-CH_3	3'-F	4.97	5.05	0.08
30	3-F,4-OCH_3	3'-F	5.02	5.56	0.54

*Test Set : $r^2 = 0.806$, $s = 0.427$

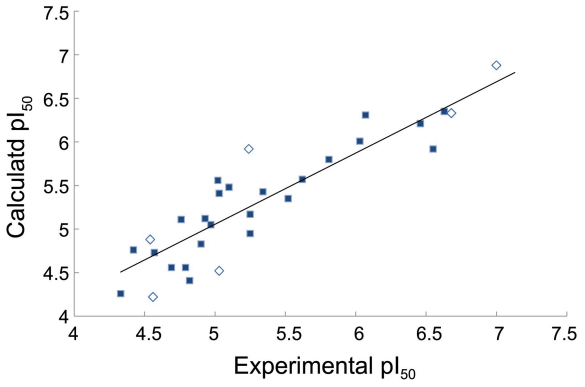


Figure 2. Plot of experimental versus calculated pI_{50} for training set (■) and test set (◇) in MLR model.

σ_{mpR}^2). Predictability of the model is shown in the results of test set (r^2 value of 0.806 in Table 3 and Figure 2). The statistical results of the model are summarized in Table 2.

σ_{pR}^1 is the Hammett sigma constants of *para*-substituents in R^1 aromatic ring. The Hammett sigma constants describe the electronic effect of substituents on the reaction or interaction center. The 2-phenylimino-1,3-thiazoline analogues, which have electronic donating substituent (4-OCH_3) at the *para*-position in the R^1 aromatic ring showed high fungicidal activities. In contrast, the analogues containing the electronic withdrawing *para*-substituents (4-Cl, 4-Br, 4-CH_2CN, and 4-CHF_2) showed relatively low fungicidal activities. These results show correlation between the fungicidal activities and electronic effects of *para*-substituents in the R^1 aromatic ring of the 2-phenylimino-1,3-thiazolines on corresponding interaction. Therefore, we suggest that the electronic donating capabilities of *para*-substituents in the R^1 aromatic ring contribute positively to the interaction between the 2-phenylimino-1,3-thiazolines and *M. grisea*.

σ_{mpR}^2 is the sum of the Hammett sigma constants for electronic effects of *meta*- and *para*-substituents in the R^2 aromatic ring. The 2-phenylimino-1,3-thiazolines containing electronegative substituents at these positions showed high fungicidal activities. The resultsimply that the electronic withdrawing capability of *meta*- and *para*-substituents in the R^2 aromatic ring contributes positively to the interaction between the 2-phenylimino-1,3-thiazolines and target fungus.

CLOGP, coefficient of octanol-water partition, is utilized to explain the influences of cellular uptake, distribution and bio-availability of the compounds. CLOGP was selected as an effective factor of fungicidal activities. It is proposed that these bio-availabilities of the 2-phenylimino-1,3-thiazoline analogues are correlated with the fungicidal activity.

SA_R^2, Connolly surface area of R^2 aromatic ring, is related to the molecular shape of substituents in R^2 aromatic ring. The 2-phenylimino-1,3-thiazolines that have bulky substituents in R^2 aromatic ring showed low fungicidal activities. We carefully suggest that the low fungicidal activities are generated by the steric hindrance between substituents in the R^2 aromatic ring and the interaction site of target fungus.

$SV_R{}^2{}_{C4}$ is the volume of *para*-substituents in R^2 aromatic ring. QSAR results reveal that there is a positive correlation between the volume of *para*-substituents in R^2 aromatic ring and the fungicidal activity.

$SV_R{}^1{}_{C2C3}$ is the sum of volumes of the substituents at the *ortho*- and *meta*-positions in the R^1 aromatic ring. $SV_R{}^1{}_{C2C3}$ had negative contribution for fungicidal activity. It seems that *ortho*- and *meta*-substituents interfere with interaction between 2-phenylimino-1,3-thiazolines and target fungus.

Conclusion

The quantitative structural-activity relationship (QSAR) analysis was carried out on a series of 2-phenylimino-1,3-thiazolines in order to determine the structural and physicochemical features responsible for their fungicidal activity. The QSAR results showed the dependence of the fungicidal activity on the structural and physicochemical features of 2-phenylimino-1,3-thiazolines. Firstly, the electronic properties at the *para*-position of both R^1 and R^2 aromatic rings are important for fungicidal activity against *M. grisea*; electron-donating substituents contributed positively to fungicidal activity at the *para*-position in R^1 aromatic ring, whereas electron-withdrawing substituents contributed positively to the activity at the *para*-position in R^2 aromatic ring. Secondly, the large substituents at the *ortho*- and *meta*-positions of both R^1 and R^2 aromatic ring interfere the interaction between 2-phenylimino-1,3-thiazolines and *M. grisea*. Lastly, the bio-availability of each 2-phenylimino-1,3-thiazoline analogueis correlated with its fungicidal activity. These results could be used as guidelines for the study of the mode of action and further design of optimal fungicides.

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