J. Pesticide Sci. **11**, 579–584 (1986)

Original Article

Structure-activity Relationships of *N*-Methyl-2′-methoxybenzanilides against Rice Blast*

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(Received April 3, 1986)

The thirty-four *N*-methyl-2′-methoxy(substituted)benzanilides were synthesized and their preventive activities against rice blast were studied by the pot test. The substitution at the para-position of benzoyl moiety varied the activity to various extents. The inhibitory activity of *N*-methyl-2′-methoxy-4-substituted benzanilides on the penetration of infection pegs from appressoria of *Pyricularia oryzae* were also measured by using cellophane film as a model of plant cell walls and the structure-activity relationships were analyzed using the physicochemical parameters and regression analysis. The inhibitory activities were related to the bulkiness and electronic effect of the substituents and parabolically to the hydrophobicity of the molecules.

INTRODUCTION

N-Methyl-4-*tert*-butylbenzanilides have been found to possess potent fungicidal activity against rice blast caused by *Pyricularia oryzae*.[1] *N*-Methyl-4-*tert*-butyl-2′-methoxybenzanilide was practically inactive on the spore germination, appressorium formation, mycerial growth and spore formation of rice blast fungus, but highly active on the penetration of infection pegs from appressoria (appressorial penetration) into cellophane film[1] as a model of plant cell walls.[2,3] Such findings suggested that the main action of this compound is the inhibition of the appressorial penetration into epidermal cells of rice plant.[1] In the previous paper,[4] we reported the relationship between the structure of amine moiety of 4-*tert*-butylbenzamides or anilides and the preventive activity against rice blast. The oxygen atom at the ortho-position of aniline ring was found to play a significant role for increasing the activity. In this experiment, we synthesized 34 *N*-methyl-2′-methoxy-(substituted)benzanilides and studied the preventive activity by the pot test. We have also determined the inhibitory activity of *N*-methyl-2′-methoxy-4-substituted benzanilides on the appressorial penetration into cellophane film and studied the relationship with the preventive activity by the pot test. Furthermore, in order to clarify the substituent effect of the acid moiety, we analyzed the relationships between the chemical structure and the inhibitory activity by means of physicochemical parameters and regression analysis.

MATERIALS AND METHODS

1. *Synthesis of Compounds*

All compounds were prepared by the reaction of *N*-methyl ortho-anisidine with respec-

* Fungicidal Activity of *N*-Methylbenzanilides (Part 2). For Part 1, see Ref. 4).

tive benzoyl chlorides as previously reported.[4] 2- (or 3-) *tert*-Butylbenzoic acid was prepared by Grignard reaction from respective bromo *tert*-butylbenzene.[5] 4-Isopropyl-, *n*-butyl- and *tert*-butylbenzoic acid were obtained commercially. The other benzoic acids were prepared by Friedel-Crafts reaction from alkylbenzene and oxalyl chloride.[6] These acids were converted into benzoyl chlorides with thionyl chloride. The structure of the compounds were comfirmed by IR and NMR spectroscopy and for some compounds by the elementary analysis for C, H and N. All melting and boiling points were uncorrected. A typical procedure is shown below.

N-Methyl-2'-methoxybenzanilide (**1**): To a mixture of *N*-methyl ortho-anisidine (2.7 g, 0.02 mol) and sodium bicarbonate (1.7 g, 0.02 mol) in 70 ml of acetone was added benzoyl chloride (2.8 g, 0.02 mol) with stirring at room temperature. After 2 hr, the reaction mixture was poured into water and extracted with 200 ml of toluene. The organic layer was washed with water and diluted HCl, dried over anhydrous sodium sulfate and concentrated. The residue was recrystallized from ligroin to give 4.3 g (89%) of **1**, mp 115–116°C. NMR ($CDCl_3$) δ: 3.33 (3H, s), 3.67 (3H, s), 6.60–7.73 (9H, m). Anal. Found: C, 74.48; H, 6.43; N, 5.61, Calcd. for $C_{15}H_{15}NO_2$: C, 74.67; H, 6.27; N, 5.80%.

2. *Biological Tests*

2.1 *Preventive activity*

The preventive values against rice blast caused by *Pyricularia oryzae* were obtained by the method previously reported.[4]

2.2 *Inhibitory activity on the appressorial penetration into cellophane film*

The cellophane film 22.9 μ thickness and 15 mm square (22/32 Type, Visking Co., Ltd.) was washed with running water for 2 hr, two times with distilled water and dried. A piece of filter paper, 9 cm in diameter, was sufficiently impregnated with a test compound in fresh rice leaf homogenate diluted 100 times (w/v) with 1/100 M phosphate buffer (pH 5.6) and placed in a petri dish of 9 cm diameter. Three pieces of cellophane films were put on the filter paper. Then the cellophane film was sprayed with spore suspension and placed in a moist chamber at 27°C. After incubation for 48 hr, the film was put on a slide glass and stained with a few drops of zinc chloride reagent (ZnCl 50 g, KI 20 g, I_2 0.5 g and water 100 ml) and the number of penetration by infection pegs emerging from appressoria into the film was counted under microscope. The inhibitory activity was expressed as the percentage of inhibition of the penetration. The molar I_{50} values were determined from the plot of the inhibitory activity against the concentration on the logarithmic probability scale.

2.3 *Physical parameter*

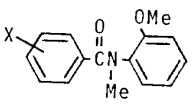
The structure-activity correlation has been analyzed by the method developed by Hansch and Fujita[7] using such physicochemical substituent parameters as hydrophobic π,[8] electronic σ,[9,10] Taft-Kutter-Hansch steric constant E_s[11,12] and STERIMOL L.[13] As the π values, those derived from partition coefficients of substituted acetophenones in the 1-octanol/water system were used.[14] The STERIMOL parameter L, which was developed by Verloop, expresses the length of substituents in Å along the axis connecting the α-atom of substituents with the rest of the molecule. For the sake of simplicity, the reference points for E_s and L are shifted to make H=0. The squared correlation matrix of variables is shown in Table 3.

RESULTS AND DISCUSSION

1. *Preventive Activity of N-Methyl-2'-methoxy-(substituted)benzanilides*

As shown in Table 1, *N*-methyl-2'-methoxybenzanilide (**1**) exhibited moderate preventive activity. The substitution at the ortho-position of benzoyl moiety of a methyl (**2**), chloro (**3**), methoxy (**4**) or nitro (**5**) group reduced the activity in whole. At the meta-position, the substitution of a methyl (**7**) or nitro (**10**) group maintained the activity and a chlorine atom (**8**) slightly increased. The substitution at the ortho or meta-position of a *tert*-butyl group (**6** and **11**) that showed the high activity at the para-position (**19**) stopped the activity. Moreover, the substitution at the para-position varied the activity to various extents. These findings suggest that the preventive activity of this series of compounds is highly position specific. The substitution at the para-position

Table 1 Physical property and preventive activity of *N*-methyl-2′-methoxybenzanilides.



No.	X	mp (°C) bp (°C/mmHg)	Evaluation of activity[a]	
			500 ppm	100 ppm
1	H	115–116	B	D
2	2-Me	79–81	D	
3	2-Cl	101–103	D	
4	2-MeO	64–67	D	
5	2-NO_2	118–119	D	
6	2-*t*-Bu	155–156/0.06	D	
7	3-Me	90–91	B	D
8	3-Cl	59–61	B	B
9	3-MeO	83–85	D	
10	3-NO_2	116–118	B	D
11	3-*t*-Bu	175/0.2	D	
12	4-Me	59–60	A	B
13	4-Et	70–71	A	B
14	4-*n*-Pr	32–33	A	B
15	4-*i*-Pr	74–76	A	A
16	4-*n*-Bu	157–162/0.03	A	C
17	4-*i*-Bu	151–157/0.02	A	B
18	4-*s*-Bu	69–71	A	B
19	4-*t*-Bu	117–119	A	A
20	4-*n*-Pent	169–176/0.04	A	C
21	4-Pr(Me)CH	153–158/0.03	A	B
22	4-*t*-Pent	83–85	A	A
23	4-*n*-Hex	182–185/0.04	A	D
24	4-*c*-Hex	90–92	A	D
25	4-Cl	67–69	B	D
26	4-Br	95–96	A	C
27	4-I	108–109	A	D
28	4-MeO	84–86	B	D
29	4-EtO	98–100	B	D
30	4-*i*-PrO	85–86	B	B
31	4-*i*-BuO	143–153/0.02	B	B
32	4-CF_3	36–37	B	D
33	4-NO_2	138–140	D	
34	4-NH_2	186–187	D	

[a] A: Preventive value above 98%,
B: Preventive value 80–98%,
C: Preventive value 50–80%,
D: Preventive value below 50%.

of alkyl groups (**12**–**24**) generally increased the activity. Especially, the substitution of the highly bulky groups such as isopropyl (**15**), *tert*-butyl (**19**) and *tert*-pentyl (**22**) showed excellent activity. The nitro (**33**) or amino (**34**) substitution stopped the activity. Thus, the physicochemical properties of the substituents at the para-position gave significant effect on the activity.

2. *Inhibitory Activity and QSAR of N-Methyl-2′-methoxy-4-substituted Benzanilides on the Appressorial Penetration*

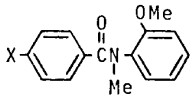
As shown in Table 2, we determined the pI_{50} value of inhibitory activity of *N*-methyl-2′-methoxy-4-substituted benzanilides on the appressorial penetration of rice blast except the nitro and amino analogs that did not show any activity in the pot test. The alkyl groups (**12**–**24**) generally showed high activity. Among the alkyl groups, the activity was the highest in the bulky *tert*-butyl (**19**) and *tert*-pentyl (**22**) groups and was somewhat low in the *n*-butyl (**16**) and *n*-pentyl (**20**) groups. Similar results were obtained by the pot test. Furthermore, the groups (**1**, **23**, **25**, **27**, **28**, **29** and **32**) whose evaluation was D class by the pot test showed lower activity except the cyclohexyl group (**24**). Though the activities of methyl (**12**), isopropyl (**15**) and bromo (**26**) analogs tended to be somewhat lower than expected from the evaluations by the pot test, the tendency of the inhibitory activity well agrees with the preventive activity by the pot test on the whole. This also suggests that the main action of the *N*-methyl-2′-methoxybenzanilides is the inhibition of appressorial penetration of rice blast. In order to clarify the substituent effect of the acid moiety, the quantitative structure-activity relationship between the chemical structure and the inhibitory activity was analyzed.

We examined various combination of substituent parameters. Equation (1) with hydrophobic parameter π was obtained with a relatively good correlation.

$$pI_{50} = -0.693\pi^2 + 2.513\pi + 3.425 \quad (1)$$
$$(\pm 0.574)\,(\pm 1.696)\,(\pm 1.081)$$
$$n = 22,\ s = 0.526,\ r = 0.777,\ F = 14.5$$

In this and following equations, n is the number of compounds, s the standard deviation, r the correlation coefficient, F the F-value of the correlation and figures in parentheses the 99.5% confidence intervals of the cor-

Table 2 Inhibitory activity of *N*-methyl-2′-methoxy-4-substituted benzanilides on appressorial penetration into cellophane film.



No.	X	π[a]	σ[b]	E_s[b]	L[c]	pI_{50}	
						Obsrd.	Calcd.[d]
1	H	0.00	0.00	0.00	0.00	3.78	3.27
12	Me	0.55	−0.17	−1.24	0.81	4.75	5.05
13	Et	0.95	−0.15	−1.31	2.05	5.83	5.30
14	*n*-Pr	1.43	−0.13	−1.60	2.86	5.45	5.54
15	*i*-Pr	1.41	−0.15	−1.71	2.05	5.85	5.66
16	*n*-Bu	1.93[e]	−0.16	−1.63	4.11	5.24	5.44
17	*i*-Bu	1.86[f]	−0.12	−2.17	2.86	5.77	5.82
18	*s*-Bu	1.86	−0.12	−2.37	2.86	6.12	5.96
19	*t*-Bu	1.81	−0.20	−2.78	2.05	6.70	6.43
20	*n*-Pent	2.43[e]	−0.15	−1.54	4.91	5.02	4.94
21	*n*-Pr(Me)CH	2.36[e]	−0.12[h]	−2.26[l]	4.11	5.54	5.50
22	*t*-Pent	2.31	−0.20[i]	−3.52[l]	2.86	6.54	6.62
23	*n*-Hex	2.93	−0.15[j]	−1.68	6.16	4.26	4.40
24	*c*-Hex	2.29[g]	−0.22	−2.03	4.11	5.59	5.57
25	Cl	0.74	0.23	−0.97	1.46	4.24	4.30
26	Br	0.87	0.23	−1.16	1.76	4.40	4.50
27	I	1.10	0.18	−1.40	2.17	4.87	4.84
28	MeO	0.16	−0.27	−0.55	1.92	4.13	4.34
29	EtO	0.47	−0.24	−0.55	2.74	4.50	4.59
30	*i*-PrO	0.74	−0.45	−0.55	2.74	4.79	5.15
31	*i*-BuO	1.24[e]	−0.32[k]	−0.55	3.99	5.13	5.09
32	CF_3	0.93	0.54	−2.40	1.24	4.68	4.92

a) Unless noted, taken from Ref. 14) as the π values for the substituents of acetophenones or calculated from the following equation which was formulated from experimentally determined partition coefficients in a 1-octanol/water system of acetophenones.[14]

$$\pi_x/\text{Ph COMe}=0.904\pi_x/\text{Ph}+0.156\sigma_x^0+0.376\rho_x+0.05$$

b) Unless noted, taken from Ref. 15).

c) The values were informed by T. Fujita as those taken from the compilation made by A. Verloop, W. Hoogenstraaten and J. Tipker, November 1, 1984.

d) Calculated by Eq. (2).

e) Values estimated from related substituents according to the additivity principle.[16] The value for methyl (or methylene) group is 0.5.[17]

f) Taken as that of *s*-Bu.

g) Calculated by using the σ^0 value of *i*-Pr.

h) Taken as that of *s*-Bu.

i) Taken as that of *t*-Bu.

j) Taken as that of *n*-Pent.

k) Taken as that of *n*-BuO.

l) Taken E_s′ values defined by MacPhee *et al.*[18]

responding constants. Equation (1) indicates the parabolic relationship between the activity and hydrophobic parameter π. The activities were plotted against π values in Fig. 1. The plot for the branched alkyl substituents is always higher than the parabola. Furthermore, in alkyl substituents with the same number of carbon atoms, the relative orders of the activity are isopropyl>*n*-propyl, *tert*-butyl >*sec*-butyl>isobutyl>*n*-butyl, *tert*-pentyl>1-

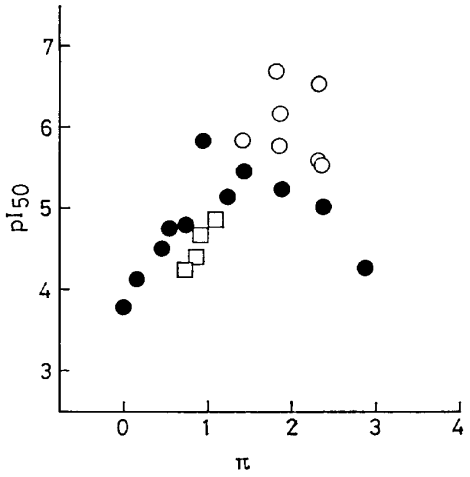


Fig. 1 Relationship between the π values of *N*-methyl-2′-methoxy-4-substituted benzanilides and inhibitory activity on the appressorial penetration of *P. oryzae*.

●: H, linear alkyl and alkoxy groups, ○: branched alkyl groups, □: electron withdrawing groups.

methylbutyl>*n*-pentyl and cyclohexyl>*n*-hexyl. On the other hand, the plot for the electron-withdrawing substituents was lower. Thus, the positive steric and negative electronic effect of substituents on the activity was expected. The addition of electronic parameter σ and steric parameter E_s or L terms to Eq. (1) showed better correlation as shown in Eqs. (2) and (3).

$$\begin{aligned} pI_{50} = &-0.491\pi^2 + 1.302\pi - 1.717\sigma \\ &(\pm 0.312)\ (\pm 1.046)\ (\pm 0.910) \\ &-0.745E_s + 3.271 \qquad (2) \\ &(\pm 0.351)\ (\pm 0.544) \\ n=22,\ s=0.257,\ r=0.957,\ F=46.0 \end{aligned}$$

$$\begin{aligned} pI_{50} = &-0.610\pi^2 + 2.906\pi - 1.874\sigma \\ &(\pm 0.317)\ (\pm 0.917)\ (\pm 1.008) \\ &-0.512L + 3.884 \qquad (3) \\ &(\pm 0.269)\ (\pm 0.649) \\ n=22,\ s=0.275,\ r=0.950,\ F=39.7 \end{aligned}$$

Substantially, there is no difference between the standard deviation, the correlation coefficient and *F*-value of Eq. (2) and those of Eq. (3). However, the collinearity between L and π and π^2 is relatively high. Thus, Eq. (2) is better than Eq. (3) for adoption. The use of other steric parameters such as the STERIMOL parameter B_1 or B_4,[13] molar refractivity MR[9,10] and van der Waals volume V_w[19] instead of E_s did not afford any significant improvement. Thus, Eq. (2) is considered the best to represent the relationship between the structure and the inhibitory activity on the appressorial penetration against rice blast.

Table 3 The squared correlation matrix of variables used in Eqs. (2) and (3).

	π	σ	E_s	L
π	0.929	0.045	0.363	0.685
π^2		0.033	0.496	0.664
σ			0.017	0.159
E_s				0.059

Equation (2) indicates the importance of the steric and electronic role of para-substituents as well as their hydrophobic character for the high activity. The activity is related parabolically to hydrophobic parameter π, which suggests that the penetration of the compound through a number of lipordal-aqueous interfaces to reach a critical receptor site plays a significant role for the activity.[20] The negative coefficient of E_s value means that the bulkier the substituent, the greater the activity within this range of substituents. The negative sign of the σ term shows that the electron-donating effect of the substituents on the carbonyl group is favorable to the activity, suggestive that an increase in electron density of the carbonyl group enhances the activity. Hydrogen bond formation at carbonyl oxygen atom with a certain acidic group at the receptor site may be critical for enhancing the activity.

ACKNOWLEDGMENTS

We wish to express our thanks to Prof. Toshio Fujita of Kyoto University and Mr. Seigo Kawada for their invaluable suggestions. We also thank Mr. Fumitaka Yoshida for his advice. Thanks are also due to Mr. Mitsuo Tagawa for his assistance in synthesizing necessary materials.

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要　　約

N-メチル-2′-メトキシベンズアニリド類のイネいもち病に対する構造活性相関*

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嶋崎　功，須田欣孝

34 の *N*-メチル-2′-メトキシ(置換)ベンズアニリド類を合成し，それらのイネいもち病に対する予防効果を測定した．安息香酸部分のパラ位への置換は，活性をかなりの範囲で変化させた．さらに植物の細胞壁のモデルとしてセロファン膜を用い，付着器からの穿入系の侵入に対する *N*-メチル-2′-メトキシ-4-置換ベンズアニリド類の阻害活性を測定し，置換基の物理化学的パラメータと回帰分析により構造と活性の関係を解析した．その結果，阻害活性は，置換基の嵩高さと電子的な性質に関係し，分子の疎水性とパラボリックな関係にあることを認めた．

* *N*-メチルベンズアニリド類の殺菌活性（第2報）