

# QSARs OF ANTI-FUNGAL ACTIVITY OF FURAN CARBOXANILIDE DERIVATIVES AGAINST WILD AND MUTANT STRAINS OF *USTILAGO MAYDIS*

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## Abstract

The structural requirements for the inhibitor activity of various furan carboxanilide derivatives against succinate dehydrogenase complex (SDC) activity in mitochondria of either wild or mutant strains of *Ustilago maydis* were investigated with the aid of Hansch QSAR analysis. It has been found that the inhibitor activity against both types of enzymes is best related to the  $\Sigma\sigma_P$  or  $\Sigma\sigma_M$  of the substituents on the phenyl ring and to the hydrophobicity of substituents at ortho (with negative slope) and meta position (with positive slope). Similarly, the activity decreases with the increase in the width of substituents at ortho position. However, while the activity decreases with the increase in the width of substituents at para position of phenyl ring for the wild type enzyme, the reverse is true for the mutant type enzyme. Moreover, in spite of wild type enzyme, the activity against mutant type enzyme is related to the presence of a hydrogen acceptor group at meta position of the phenyl ring. These data indicate that the difference between wild and mutant types of SDC enzymes in the *U. maydis* has been brought about by the appearance of an amino acid residue with the ability to form hydrogen binding in the active site of the mutant enzyme.

## Introduction

Carboxanilide derivatives such as carboxin are widely used as anti-fungal agents against corn smut, *Ustilago maydis* [1]. It has been reported that the anti-fungal activity of these compounds is due to the inhibition of electron transport of the mitochondrial succinate dehydrogenase complex (SDC). However, some strains of *U. maydis* are resistant to carboxin and it is believed that this resistance is caused by the mutation

of the SDC enzyme [1].

G.A. White [1] tested several furan carboxamide derivatives against both wild and mutant types of SDC enzymes and showed that some of these compounds are active against mutant enzymes. The aim of the present work is to apply a QSAR (Hansch analysis) method to elucidate the structural requirements for the activity against each type of enzyme and to shed light on the nature of the mutation.

## Methods

QSARs were examined by computerized multiple

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gression analysis [2]. The numerical values of the substituent parameters of various furan carboxamide derivatives (Table I) substituted at various positions of the phenyl ring were correlated with their potency  $-\log(IC_{50})$  against the SDC enzyme of the mitochondrial isolated from spordia of either wild or carboxin resistant mutant strains of *U. maydis* [1]. The following physiochemical parameters were used in this study:

Verloop's steric parameters, various widths (B1, B2, B3), and length (L) of individual substituents [3]; and Aromatic substituent constants of individual substituents at the C<sub>2</sub>-C<sub>5</sub> position of the phenyl ring [4].

The values of substituent constants for each compound are shown in Table II and their intervariable relation matrix is reported in Table III.

The validity of regression was judged by (i) standard deviation of estimate, SD; (ii) T-test value; (iii) F-test value; (iv) level of significance, p; (v) and correlation coefficient, r. The estimated standard error of each coefficient is shown in parentheses following its value.

### Results and Discussion

Hansch analysis detects a quantitative relationship between the pharmacological activity of derivatives and their pharmacological properties. It has been used successfully to gain understanding of the mechanism of drug action on a molecular level and in designing new drugs. This method has been applied here to distinguish the structural requirements for the anti-fungal activity of furan carboxamide derivatives against the wild and mutant types of *U. maydis* to provide some evidence regarding the nature of mutation. It has been found that several equations could be derived to relate the physiochemical parameters of substituents of furan carboxamide derivatives to their biological activity against either wild or mutant types of *U. maydis*. The most significant equations are presented here.

#### Effect of Substitution on the Phenyl Ring on Anti-Fungal Activity Against the Wild Type SDC Enzyme

The potency for anti-fungal activity of furan carboxanilide [expressed as  $-\log(IC_{50})$ ] when various groups are substituted at the ortho (o), meta (m) and para (p) positions of the phenyl ring is correlated best with the  $\pi$ , MR,  $\Sigma\sigma_P$ ,  $\Sigma\sigma_M$  and hydrogen donor ability of the substituents as described by Eqs. (1, 2,

$$-\log(IC_{50}) = 5.5275 (0.189) + 1.1866 (0.361) \pi_m - 0.0623 (0.021) MR_p - 0.951 (0.412) \Sigma\sigma_P \quad (1)$$

$$n=20 \quad r=0.804 \quad SD=0.5966 \quad F_{(3,16)}=9.75 \quad p<0.005$$

Sixty-four and six tenths percent of the variance in the biological activity [ $-\log(IC_{50})$ ] is explained by Eq. (1).

$$-\log(IC_{50}) = 6.298 (0.182) - 0.7199 (0.204) \pi_o - 2.0142 (0.549) H-Don_m - 0.1065 (0.019) MR_p - 0.9619 (0.352) \Sigma\sigma_P \quad (2)$$

$$n=20 \quad r=0.870 \quad SD=0.5118 \quad F_{(4,15)}=11.62 \quad p<0.005$$

Seventy-five and six tenths percent of the variance in the biological activity [ $-\log(IC_{50})$ ] is explained by Eq. (2).

$$-\log(IC_{50}) = 6.7331 (0.217) - 0.7961 (0.204) \pi_o - 1.836 (0.530) H-Don_m - 0.1095 (0.019) MR_p - 2.1160 (0.733) \Sigma\sigma_M \quad (3)$$

$$n=20 \quad r=0.875 \quad SD=0.5022 \quad F_{(4,15)}=12.22 \quad p<0.005$$

Seventy-six and five tenths percent of the variance in the biological activity [ $-\log(IC_{50})$ ] is explained by Eq. (3).

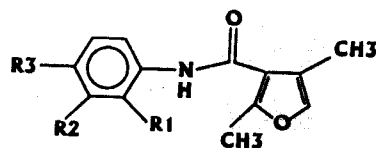
From these equations it can be deduced that for obtaining the best anti-fungal activity against wild type SDC enzyme the following properties at phenyl ring are required:

- The substituent at ortho position must be a hydrophilic one.
- The substituent at meta position must be hydrophobe with low hydrogen donor ability.
- The substituent at para position must be a small group (low MR).

In addition, the electronic properties of the substituent will affect the biological activity and usually substituents with negative  $\sigma_P$  or  $\sigma_M$  are preferred. Therefore, the activity of compounds such as 15 & 16 (with high activity) and compound 13 (with low activity) agree with the above mentioned conclusion. Similarly, the low activity of compounds 2, 18 and 19 refer to high electron induction ability of compound 2 and electronic resonance and size of substituents of compounds 18 and 19.

#### Effects of Substitution on the Phenyl Ring on the Anti-Fungal Activity Against the Mutant Type of SDC Enzyme

The anti-fungal activity of furan carboxamides against the mutant type of SDC enzyme when various groups are substituted at o, m and p positions of the phenyl ring is correlated best with the  $\pi$ , B1, B2,  $\Sigma\sigma_P$  and hydrogen acceptor ability of the substituents as described by Eqs. (4, 5, 6).



**Table I.** The structure and biological activity of furan carboxamide derivatives used in this analysis\*

Compound	R <sub>1</sub> (o)	R <sub>2</sub> (m)	R <sub>3</sub> (p)	-log(IC <sub>50</sub> ) <sub>W</sub>	-log(IC <sub>50</sub> ) <sub>M</sub>
1	H	H	H	6.55	4.60
2	CF <sub>3</sub>	H	H	4.77	3.74
3	C <sub>2</sub> H <sub>5</sub>	H	H	5.72	4.12
4	O-n-Pr	H	H	5.46	4.85
5	O-i-Pr	H	H	5.92	4.30
6	O-n-Bu	H	H	5.28	4.69
7	C <sub>6</sub> H <sub>5</sub>	H	H	5.49	4.13
8	OCH <sub>3</sub>	H	CH <sub>3</sub>	6.64	5.05
9	O-n-Pr	5-CH <sub>3</sub>	H	5.15	4.29
10	H	CH <sub>3</sub>	H	6.00	5.64
11	H	CF <sub>3</sub>	H	5.85	4.77
12	H	Cl	H	6.02	4.42
13	H	OH	H	4.53	5.00
14	H	OCH <sub>3</sub>	H	6.07	5.68
15	H	O-n-Pr	H	7.60	6.03
16	H	O-i-Pr	H	6.60	5.60
17	H	H	O-i-Pr	4.60	5.77
18	H	H	COCH <sub>3</sub>	4.10	4.31
19	H	H	C <sub>6</sub> H <sub>5</sub>	4.00	4.57
20	H	H	Br	4.94	5.35

\*The [-log(IC<sub>50</sub>)] median anti-fungal concentration against wild and mutant strains of *U. maydis* is taken from Ref. 1.

**Table II.** Substituent constants for C<sub>2</sub>-(o), C<sub>3</sub>-(m), C<sub>4</sub>-(p) substituted furan carboxamides against wild and mutant strains of *U. maydis*\*

Comp.	π <sub>o</sub>	π <sub>m</sub>	σP <sub>m</sub>	H-Acpt <sub>m</sub>	H-Dont <sub>m</sub>	σP <sub>p</sub>	MR <sub>p</sub>	ΣσM	ΣσP	B1 <sub>o</sub>	B2 <sub>p</sub>
1	0.00	0.00	0.00	0.00	0.00	0.00	1.03	0.00	0.00	1.00	1.00
2	0.88	0.00	0.00	0.00	0.00	0.00	1.03	0.43	0.54	1.98	1.00
3	1.02	0.00	0.00	0.00	0.00	0.00	1.03	-0.07	-0.15	1.52	1.00
4	1.05	0.00	0.00	0.00	0.00	0.00	1.03	0.10	-0.25	1.35	1.00
5	0.36	0.00	0.00	0.00	0.00	0.00	1.03	0.10	-0.45	1.35	1.00
6	1.55	0.00	0.00	0.00	0.00	0.00	1.03	0.10	-0.32	1.35	1.00
7	1.92	0.00	0.00	0.00	0.00	0.00	1.03	0.06	-0.01	1.70	1.00
8	-0.02	0.00	0.00	0.00	0.00	-0.17	5.65	0.05	-0.44	1.35	1.00
9	1.05	0.56	-0.17	0.00	0.00	0.00	1.03	0.03	-0.42	1.35	1.00
10	0.00	0.56	-0.17	0.00	0.00	0.00	1.03	-0.07	-0.17	1.00	1.00
11	0.00	0.88	0.54	0.00	0.00	0.00	1.03	0.43	0.54	1.00	1.00
12	0.00	0.71	0.23	0.00	0.00	0.00	1.03	0.37	0.23	1.00	1.00
13	0.00	-0.67	-0.37	1.00	1.00	0.00	1.03	0.12	-0.37	1.00	1.00
14	0.00	-0.02	-0.27	1.00	0.00	0.00	1.03	0.12	-0.27	1.00	1.00
15	0.00	1.05	-0.25	1.00	0.00	0.00	1.03	0.10	-0.25	1.00	1.00
16	0.00	0.36	-0.45	1.00	0.00	0.00	1.03	0.10	-0.45	1.00	1.00
17	0.00	0.00	0.00	0.00	0.00	-0.45	17.06	0.10	-0.45	1.00	3.13
18	0.00	0.00	0.00	0.00	0.00	0.50	11.18	0.38	0.50	1.00	1.90
19	0.00	0.00	0.00	0.00	0.00	-0.01	25.36	0.06	-0.01	1.00	1.70
20	0.00	0.00	0.00	0.00	0.00	0.23	8.88	0.39	0.23	1.00	1.95

\*The values of constants are taken from Ref. 3 and 4. For the structure and biological activity see Table I.

III. Intervariable correlation matrix for the substituent constants shown in Table II

	$\pi_o$	$\pi_m$	$\sigma P_m$	H-Acpt <sub>m</sub>	H-Dont <sub>m</sub>	$\sigma P_p$	MR <sub>p</sub>	$\Sigma\sigma M$	$\Sigma\sigma P$	B1 <sub>o</sub>	B2 <sub>p</sub>
	1.000										
	-0.162	1.000									
	0.072	0.273	1.000								
cpt <sub>m</sub>	-0.325	0.011	-0.708	1.000							
on <sub>m</sub>	-0.149	-0.499	-0.364	0.459	1.000						
	-0.018	-0.014	0.007	-0.015	-0.007	1.000					
	-0.317	-0.216	0.108	-0.243	-0.112	-0.079	1.000				
1	-0.204	0.148	0.482	-0.112	-0.037	0.412	0.053	1.000			
	-0.069	0.184	0.611	-0.356	-0.187	0.543	0.084	0.774	1.000		
	0.775	-0.223	0.106	-0.354	-0.163	-0.087	-0.300	-0.015	0.095	1.000	
	-0.286	-0.195	0.098	-0.220	-0.101	-0.185	0.772	0.173	0.046	-0.312	1.000

$$\log(IC_{50}) = 6.4766 (0.447) - 1.4168 (0.361) B1_o - 0.7016 (0.302) \Sigma\sigma P \quad (4)$$

$$20 \quad r = 0.757 \quad SD = 0.4477 \quad F_{(2,17)} = 11.40 \quad p < 0.005$$

Fifty-seven and three tenths percent of the variance the biological activity [-log(IC<sub>50</sub>)] is explained by (4).

$$\log(IC_{50}) = 3.7655 (0.289) + 0.5530 (0.258) \pi_m + 1.1043 (0.252) H-Acp_m + 0.6081 (0.189) B2_p \quad (5)$$

$$20 \quad r = 0.787 \quad SD = 0.4349 \quad F_{(3,16)} = 8.70 \quad p < 0.005$$

Sixty-two percent of the variance in the biological activity [-log(IC<sub>50</sub>)] is explained by Eq. (5).

$$\log(IC_{50}) = 6.5777 (0.398) - 1.4824 (0.322) B1_o - 1.0926 (0.437) \sigma P_m - 1.334 (0.546) \sigma P_p \quad (6)$$

$$20 \quad r = 0.827 \quad SD = 0.3972 \quad F_{(3,16)} = 11.51 \quad p < 0.005$$

Sixty-eight and three percent of the variance in the biological activity [-log(IC<sub>50</sub>)] is explained by Eq. (6). These equations suggest that the highest activity will be obtained when substituents have the following properties:

The substituent at ortho position must be a small hydrophilic group.

The substituent at meta position must be a lipophile up with low  $\sigma P$  value and the capability to form a hydrogen bond.

The substituent at para position must be a wide up with low electronic resonance potency.

According to these criteria, compound 2 with a

wide and electrically active group (CF<sub>3</sub>) at ortho position has the lowest biological activity, but compounds 14, 15 and 16 with respectively OCH<sub>3</sub>, O-n-Pr and O-i-Pr at ortho position have high biological activities. By comparing the potency of compound 19 (with p-phenyl) and 20 (with p-Br), it can be deduced that the electronic inductive effect (which is high for Br) is more effective than substituents width B2 (which is higher for phenyl). This is in agreement with magnitude of the slopes of these parameters in Eqs. (4, 5, 6).

It could be said that electronically active groups such as Br would influence the distribution of the electronic charges in the molecule especially around the carbonyl group (which is essential for biological activity) and thereby facilitate the binding to the enzyme.

#### Differences between Wild and Mutant Types of SDC Enzyme in *U. maydis*

John J. Maguire and coworkers [5] have reported that there are some differences in the primary structure and the conformation of SDC enzyme in the wild and mutant types. Similarly, the present study shows that structural requirements for optimal inhibitory activity differ in mutant and wild types of the SDC enzyme especially in the mutant type where mutation causes a preference for compounds with H-acceptor groups at meta position of the phenyl ring (Table I).

Some studies on the SDC in various species indicated that this enzyme has a high level of similarity and important parts of the enzyme which may be important in enzyme action are completely preserved [5, 6, 7, 8]. Also, studies on the SDC in *Bacillus subtilis* indicate that at least one amino acid is altered in the mutant type enzyme [5]. This mutation occurred by substitution of Glycin in wild type with Aspartate. This mutation not only exposes a hydrogen donor

group in the active site but also changes the folding of the enzyme. This may also be true for the *U. maydis* and the present QSAR study suggests that the fungal enzyme may be mutated similar to *Bacillus subtilis*. In this case Glycin, or any other similar amino acid, may be exchanged by Aspartate, or any other similar amino acid, so that a hydrogen bond may be formed between hydrogen donor groups of mutated amino acids and hydrogen acceptor groups of various inhibitors. For this reason, the furan carboxamide derivatives, which have a hydrogen acceptor group at meta position of the phenyl ring, are more effective against mutant type enzyme (compare the potency of compound 13 against wild and mutant enzymes). However, due to the steric effect of side chain of mutated amino acid the total volume of active site has been decreased and bulky substituents are no longer tolerated.

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