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The Antifungal Activity of Alkyl Benzimidazol-2-ylcarbamates and Related Compounds[a]

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The fungistatic activity against *Penicillium digitatum* and *Diplodia natalensis* decreased slightly in ascending a homologous series of alkyl esters of benzimidazol-2-ylcarbamic acid from the methyl ester (carbendazim) to the pentyl ester; the hexyl and octyl esters were inactive. 2-(Acylamino)benzimidazoles were slightly less active than the analogous alkyl benzimidazol-2-ylcarbamates. Introduction of a methylene bridge between the benzimidazole ring and the 2-methoxycarbonylamino group abolished antifungal activity. Methylation of either the carbamate nitrogen or an imidazole nitrogen of carbendazim produced inactive compounds. Replacement of the benzimidazole ring of carbendazim with various other ring systems was accompanied by a marked reduction in antifungal activity.

1. Introduction

The antifungal activity of 2-acylamino- and 2-alkoxycarbonylamino-derivatives of benzimidazoles, benzothiazoles and benzoxazoles has been described in several patents over the past 20 years.[1–5] The best known of these fungicides, benomyl and carbendazim, have been extensively used to control a number of fungal diseases of growing plants and of plant products after harvest.[6] Numerous 2-substituted benzimidazoles and related compounds have been presented in the patent literature without a clear definition of their antifungal spectrum or any indication of their relative activity. Indeed, some of the compounds listed in the patents exhibit very weak antifungal action. This investigation was undertaken to elucidate the structural requirements for antifungal activity in the 2-substituted benzimidazoles and related compounds.

2. Experimental

2.1. Materials

Most of the alkyl benzimidazol-2-ylcarbamates were prepared by the reaction of *o*-phenylenediamine or a derivative with the appropriate dialkyl 2-methylisothiourea-1,3-dicarboxylate.[7] The silver salt of carbendazim was obtained by adding an equivalent quantity of silver nitrate to an ammoniacal solution of carbendazim in propan-2-ol. Carbendazim–copper complex was precipitated from a dimethylformamide solution of both carbendazim and copper diacetate by the addition of water. The 2-acylaminobenzimidazoles were prepared by reaction of benzimidazol-2-ylamine with an acyl halide. Compound **14** was produced by treatment of 2-methylaminobenzimidazole with excess of methyl chloroformate, followed by removal of the methoxycarbonyl group from the imidazole nitrogen by hydrolysis. Carbendazim was treated with methyl chloroformate to give methyl 1-(methoxycarbonyl)benzimidazol-2-ylcarbamate. The following compounds were provided as follows: benomyl by Dupont de Nemours & Co., Inc.; mecarbinzid by BASF Wynan-

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dotte Corp.; cypendazole by Bayer A.G.; methyl benzothiazol-2-ylcarbamate (**16**) by Pfizer Inc. The purity of the compounds was established by thin-layer chromatography and the structures confirmed by infrared and mass spectra.

2.2. Antifungal assays

The spectrum of antifungal activity was evaluated by observing the inhibitory action of several concentrations of each compound in potato-dextrose agar upon the growth of thirteen species of fungi (Table 1) which varied in their sensitivities to this class of compounds. The test fungi are

Table 1. Antifungal spectrum of 2-substituted-benzimidazoles and benzothiazole compounds

Test fungus	Inhibition (%) by compounds[a] at 1 μg ml^{-1} in agar medium			
	5	**11**	**3**	**16**
Monilinia fructicola (Wint.) Honey	100	100	100	100
Botrytis cinerea Pers. ex Fr.	100	100	100	100
Diplodia natalensis P. Evans	100	73	100	81
Phomopsis citri Fawc.	100	100	100	25
Penicillium digitatum Sacc.	100	100	100	100
Thielaviopsis paradoxa (de Seynes) Höhn	100	100	100	100
Glomerella cingulata (Stonem.) Spauld. & Schrenk	100	100	100	82
Fusarium roseum (Link emend. & Snyd. & Hans.)	0	0	11	11
Aspergillus niger V. Tiegh.	25	16	0	0
Alternaria citri Ell. & Pierce	0	0	0	0
Geotrichum candidum Link ex. Pers.	0	0	0	0
Phytophthora citrophthora (R.E. Sm. & E.H. Sm.) Leonian	0	0	0	0
Rhizopus stolonifer (Ehr. ex Fr.) Vuill.	0	0	0	0

[a] Compounds: **5**, carbendazim;
11, pentyl benzimidazol-2-ylcarbamate;
3, *N*-(benzimidazol-2-yl)propionamide;
16, methyl benzothiazol-2-ylcarbamate.

responsible for post-harvest diseases of citrus and other fruits; the benzimidazole fungicides are extensively utilised for the control of these diseases. The concentration (ED_{50}) of each compound, required to reduce radial growth of *Penicillium digitatum* and *Diplodia natalensis* on agar medium to one-half of that in the absence of the compound, was determined. Samples of the test compounds were dissolved in dimethyl sulphoxide (DMSO) containing the surfactant, polysorbate 20 (Tween 20; 6 mg ml^{-1}), and these solutions were diluted with water to give stock solutions or suspensions of the test compound. A maximum of 5 ml of stock solution was added to melted (55°C) potato-dextrose agar (Difco; 55 ml) and sterile water was added to give a final volume of 60 ml. Aliquots (15 ml) of the agar medium were pipetted into each of two Petri plates. The agar was allowed to solidify and one 8-mm diameter disc of the same agar medium containing 30-h hyphae of *P. digitatum* or *D. natalensis* was placed in the centre of each Petri plate on the surface of the medium. The diameter of the fungal colony on medium containing the test compound was measured after incubation for 5 days at 25°C and compared with that of the control containing only DMSO and polysorbate 20. The inhibition of colony growth at each concentration of the test compound was plotted on a probit scale *vs* the log(concentration) and the ED_{50} value was estimated graphically. The ED_{50} value was determined in this manner on at least two separate occasions. The antifungal spectrum of each compound was determined in a similar fashion, except that the culture medium was seeded with spores or mycelial fragments of the fungi (Table 1), rather than hyphae of an exact age as in the dose–response tests.

3. Results

Although benzimidazol-2-ylamine exhibited little inhibitory action against any of the thirteen species of fungi tested, a number of derivatives of this compound in which a single amino hydrogen was substituted with an acyl or alkoxycarbonyl group were highly active against fungi which were sensitive to this class of compound. The typical antifungal spectrum of all active compounds is shown in Table 1. *Alternaria citri*, *Geotrichum candidum*, *Phytophthora citrophthora* and *Rhizopus stolonifer* were not strongly inhibited by these compounds at 100 μg ml^{-1}, the highest concentration tested. *Fusarium roseum* and *Aspergillus niger* were characteristically more tolerant of this class of fungicides than the first seven species listed in Table 1.

The concentration of each compound estimated to give 50% inhibition (ED_{50}) of the radial growth of *P. digitatum* and *D. natalensis* is given in Table 2. Both of these fungi were inhibited to a

Table 2. Inhibition of mycelial growth of *Penicillium digitatum* and *Diplodia natalensis* by 2-substituted benzimidazoles

No.	Compound and structure[a]		$ED_{50} \times 10^7$ (M)	
			P. digitatum	*D. natalensis*
1	Benzimidazol-2-ylamine	X—NH_2	4500	7500
2	*N*-(Benzimidazol-2-yl)acetamide	X—NH—CO—CH_3	57	57
3	*N*-(Benzimidazol-2-yl)propionamide	X—NH—CO—C_2H_5	10	19
4	*N*-(Benzimidazol-2-yl)butyramide	X—NH—CO—C_3H_7	21	33
5	Methyl benzimidazol-2-ylcarbamate[b]	X—NH—CO—O—CH_3	1.8	1.8
6	Ethyl benzimidazol-2-ylcarbamate	X—NH—CO—O—C_2H_5	2.1	3.6
7	Propyl benzimidazol-2-ylcarbamate	X—NH—CO—O—C_3H_7	3.6	11.4
8	Allyl benzimidazol-2-ylcarbamate	X—NH—CO—O—CH_2—$CH{=}CH_2$	4.4	10.1
9	Butyl benzimidazol-2-ylcarbamate	X—NH—CO—O—C_4H_9	4.3	16.3
10	Isobutyl benzimidazol-2-ylcarbamate	X—NH—CO—O—CH_2—$CH(CH_3)_2$	8.1	23.2
11	Pentyl benzimidazol-2-ylcarbamate	X—NH—CO—O—C_5H_{11}	3.4	14.0
12	Hexyl benzimidazol-2-ylcarbamate	X—NH—CO—O—C_6H_{13}	>3800	>3800
13	Octyl benzimidazol-2-ylcarbamate	X—NH—CO—O—C_8H_{17}	>3500	>3500
14	Methyl *N*-(benzimidazol-2-yl)-*N*-methylcarbamate	X—$N(CH_3)$—CO—O—CH_3	>4900	>4900
15	Methyl *N*-(benzimidazol-2-ylmethyl)-carbamate	X—CH_2—NH—CO—O—CH_3	>4900	>4900

[a] X = Benzimidazol-2-yl.
[b] Carbendazim.

similar degree by active compounds, although *P. digitatum* was somewhat more sensitive to certain compounds. *N*-(Benzimidazol-2-yl)propionamide (**3**) was more inhibitory to both fungi than the acetamide homologue (**2**) and slightly more active than *N*-(benzimidazol-2-yl)butyramide (**4**). Activity of the corresponding carbamates (—CH_2— replaced by —O—) was somewhat higher (compare **3** with **5** and **4** with **6**). Fungistatic activity appeared to decline slightly with increase in length of the carbon chain of the alkoxy group up to the 5-carbon member of this series. Branching and unsaturation at the beta-carbon of the alkoxy group did not have a great influence upon the activity of the 3–4 carbon esters (**7–10**). The pentyl ester (**11**) was only slightly less active than the methyl ester (**5**) and both compounds exhibited the same range of antifungal activity (Table 1). Activity was lost by increasing the size of the alkoxy group beyond five carbon atoms. The hexyl (**12**) and the octyl esters (**13**) at 100 μg ml^{-1} had very little effect upon the growth of any of the test fungi listed in Table 1.

Insertion of a methylene carbon between the imidazole ring and the carbamate nitrogen completely abolished antifungal activity (compare **5** with **15**). Replacement of the hydrogen on the carbamate nitrogen with a methyl group also resulted in an inactive compound (compare **5** with **14**).

Methylation of the imidazole nitrogen of carbendazim completely abolished activity ($ED_{50} > 4900 \times 10^{-7}$ M). Compounds with more labile substituents on the imidazole nitrogen, such as benomyl, mecarbinzid, cypendazole, and methyl 1-methoxycarbonylbenzimidazol-2-ylcarbamate showed activity very similar to carbendazim. The carbendazim–copper complex also showed similar activity. Apparently these compounds decomposed to carbendazim under the conditions oi the antifungal assay. The silver complex was slightly less active than carbendazim ($ED_{50} \approx 8 \times 10^{-7}$ M).

Table 3. Inhibition of mycelial growth of *Penicillium digitatum* and *Diplodia natalensis* by *N*-substituted analogues of carbendazim

No.	Compound and structure	$ED_{50} \times 10^7$(M)	
		P. digitatum	*D. natalensis*
16	Methyl benzothiazol-2-ylcarbamate	33	26
17	Methyl imidazol[4,5-*b*]pyridin-2--ylcarbamate	99	100
18	Methyl imidazol[4,5-*c*]pyridin-2--ylcarbamate	2300	>5200
19	Methyl naphth[2,3-*d*]imidazol--2-ylcarbamate	>4100	>4100
20	Methyl naphth[1,2-*d*]imidazol--2-ylcarbamate	68	2000

Five *N*-substituted analogues of carbendazim were compared with the parent compound (Table 3). Methyl benzothiazol-2-ylcarbamate **(16)**, which is formally derived by substituting sulphur for the imino-nitrogen of the imidazole ring of carbendazim, exhibited only one-twentieth the activity of carbendazim. The aza analogues **(17, 18)** of carbendazim had greatly reduced activity. The analogous methyl naphth[2,3-*d*]imidazol-2-ylcarbamate **(19)** and methyl naphth[1,2-*d*]-imidazol-2-ylcarbamate **(20)** were essentially devoid of activity.

4. Discussion

The activity spectrum of each fungistatic 2-substituted benzimidazole examined in this investigation was qualitatively similar to those described for thiabendazole,[8] benomyl,[9] and thiophanate-methyl.[10] The spontaneous transformation of thiophanate-methyl at low concentrations to carbendazim in a physiological environment has been well documented.[11] The antifungal ranges shown in Table 1 appear to be characteristic of the antifungal compounds of this type, all of which probably exert their activity by binding to the protein tubulin in the cell.[12] Furthermore, isolates of *P. digitatum* that were resistant to carbendazim were resistant also to other active compounds in this investigation.

Investigations on the relationship of structure to herbicidal activity in the phenylcarbamates suggest that compounds of this type are bound to the site of activity in plant cells by hydrogen

bonds or a charge-transfer complex arising through the amido group R^1—NH—CO—R^2 of these herbicides.[13, 14] R^1 is thought to influence penetration into the plant cell and hydrophobic bonding at the active site, as well as having an electronic influence on hydrogen bonding of the amido group. The principal role of R^2 is to provide the optimal electron density at the carbonyl group. The present investigation of the alkyl benzimidazol-2-ylcarbamates and related compounds appears to implicate the involvement of the analogous moieties in these molecules in the determination of antifungal activity.

Substitution of the imino hydrogen of phenylcarbamates is reported to prevent intermolecular hydrogen bonding by these compounds,[15] and in the present investigation, such modification of carbendazim destroyed antifungal activity. Interference with resonance between the imidazole ring and imino group by insertion of a —CH_2— group between these two moieties should weaken hydrogen bonds formed through the imino group. Modification of carbendazim in this fashion eliminated antifungal activity. The relatively high fungistatic activity of *N*-(benzimidazol-2-yl)-propionamide suggests that the ester function is not essential to activity, but rather that is provides the optimum release of electrons to the imino group for hydrogen bonding within the cell of the sensitive fungus. The benzimidazole nucleus not only provides a desirable lipophilic balance to this class of fungicides, but should be important in binding the molecule at the site of action. The opportunity for hydrogen bonding through nitrogen atoms of the imidazole ring[16] and the planarity of the benzimidazole nucleus are both factors which should lead to a strong affinity for a complementary site. Hydrogen bonding through the imidazole ring should be weakened in the aza analogues (**17** and **18**). Inactivity of the naphtho analogues could result from excessive hydrophobic character or steric hindrance at the active site. The participation of hydrogen bonding through the imidazole nitrogen is indicated by the inferior fungistatic activity of compounds in which the potential for hydrogen bonding at this site is impaired or abolished, as in the silver salt of carbendazim, methyl 1-methylbenzimidazol-2-ylcarbamate, and methyl benzothiazol-2-ylcarbamate. Methylation of the imidazole nitrogen of fenbendazole (General Medical Council approved name for methyl 5-(phenylthio)benzimidazol-2-ylcarbamate) is reported to reduce anthelmintic activity.[17] It is to be expected that the other 1-substituted compounds (benomyl, mecarbinzid, cypendazole, and methyl 1-methoxycarbonylbenzimidazol-2-ylcarbamate) would be degraded spontaneously and rapidly to carbendazim in the dilute solutions involved in the assay for antifungal activity.[18, 19]

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