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Synthesis and antifungal activity of new *N*-isoxazolyl-2-iodobenzamides

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Abstract

N-Isoxazolyl-2-iodobenzamides **3** and **9**, with a benodanil-like structure, were synthesized by refluxing in acetic acid the corresponding benzotriazinones **2** and **8** with potassium iodide for 1 h with the aim to ascertain if they were active as fungicides against *Phytophthora citricola* Saw., *Botrytis cinerea* Pers., *Rhizoctonia* sp. and *Alternaria* sp. Among the tested iodo derivatives, compounds **3b** and **9a** possess interesting activities against the aforesaid fungal strains in several cases similar to that of benodanil **I** taken as reference drug.

Keywords: *N*-Isoxazolyl-2-iodobenzamides; Antifungal activity

1. Introduction

Since the discovery of systemic antifungal properties of carboxamides [1], several compounds, such as carboxin (Vitavax®) [1,2], oxycarboxin (Plantavax®) [1,3], fenfuram (Panoram®) [1,4], and benodanil (Carilus®) [1,5], were synthesized and introduced as agricultural fungicides [1].

Among these, benodanil, namely *N*-phenyl-2-iodobenzamide **I** (Fig. 1), active principle of Carilus® (BASF), exhibited a spectrum of activities including primarly smuts (*Ustilaginales*), ruts (*Uredinales*), and rots caused by *Rhizoctonia solani* [1,5,6]. It is also known that 2-iodobenzamides bearing a heterocycle nucleus, i.e. **II** (Fig. 1) are useful compounds to control the attack of mildew [7].

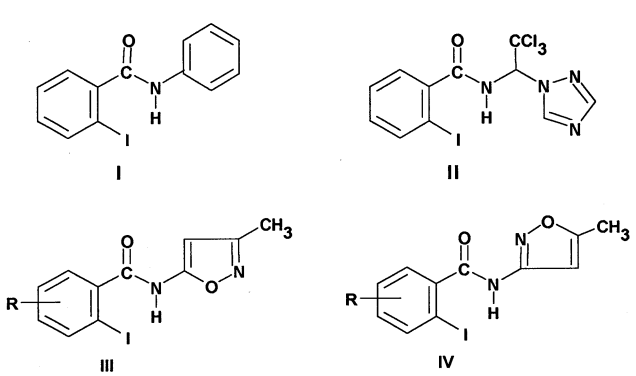

Fig. 1. Structure of the agricultural fungicides **I–IV**.

Owing to facile availability in our hands of the starting material, we synthesized the *N*-isoxazolyl-2-iodobenzamides **III** and **IV** (Fig. 1), with a benodanil-like structure, with the aim to ascertain if they were active as agricultural fungicides.

Compounds **III** and **IV** were therefore tested at 100 μg/ml against four representative genera of phytopathogenic fungi: *Phytophthora citricola* Saw. (*Mastigomycotina*), *Botrytis cinerea* Pers. ex Fr. (*Deuteromycotina*, *Hyphomycetes*), anamorph of *Botryotinia fuckeliana* (De Bary) Whetzel (*Ascomycotina*), *Rhizoctonia* sp. (*Deuteromycotina*, *Micelia sterilia*), anamorph of many *Basidiomycotina* (*Ceratobasidium*, *Thanatephorus*, etc.), and *Alternaria* sp. (*Deuteromycotina*, *Hyphomycetes*) anamorph of many *Ascomycotina*.

Alternaria sp., besides being very common phytotoxic fungi, can also play a considerable role in human pathology, especially in patients with immunological deficiency. Its pathogenic role in human pathology is mainly expressed by asthma [8], even if cases of der-

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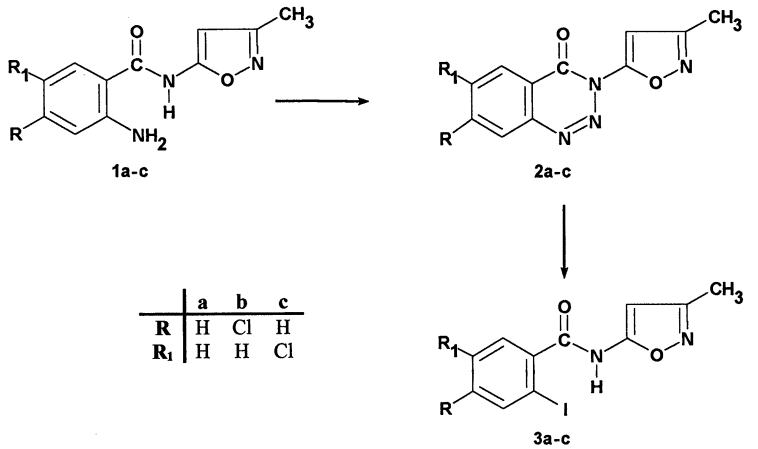


Scheme 1.

maly alternariosis, occurring during an immunosuppressive therapy, are reported too [9].

2. Chemistry

N-(Isoxazol-5-yl)-2-iodo-4-R-5-R_1-benzamides **3a–c** and *N*-(isoxazol-3-yl)-2-iodo-4-R-5-R_1-benzamides **9a–c** were synthesized, as shown in Schemes 1 and 2. The starting *N*-(isoxazol-3-yl)-2-nitro-4-R-5-R_1-benzamides **6a–c**, were prepared by condensing the proper 2-nitroaroyl chloride **4a–c** with 5-methyl-3-aminoisoxazole **5**, in chloroform solution. When compounds **6** were treated with stannous chloride in concentrated hydrochloric acid the *N*-(isoxazol-3-yl)-2-amino-4-R-5-R_1-benzamides **7a–c** were obtained. Treatment of compounds **1** [10] and **7** with potassium nitrite in acetic acid afforded the corresponding benzotriazinones **2a** [12], **b,c** and **8a–c**. Finally, the 2-iodo-*N*-isoxazolylbenzamides **3** and **9** were obtained by refluxing compounds **2** and **8** in acetic acid with potassium iodide for 1 h.

Proof of structure of all new products was achieved on the basis of microanalyses and spectroscopic evidences (see Section 4).

3. Biological results and discussion

Compounds **3a–c** and **9a–c** were screened for antifungal activity at 100 μg/ml against the following isolates of phytopathogenic fungi: *P. citricola*, *B. cinerea*, *Rhizoctonia* sp. and *Alternaria* sp. Results are reported in Tables 1 and 2.

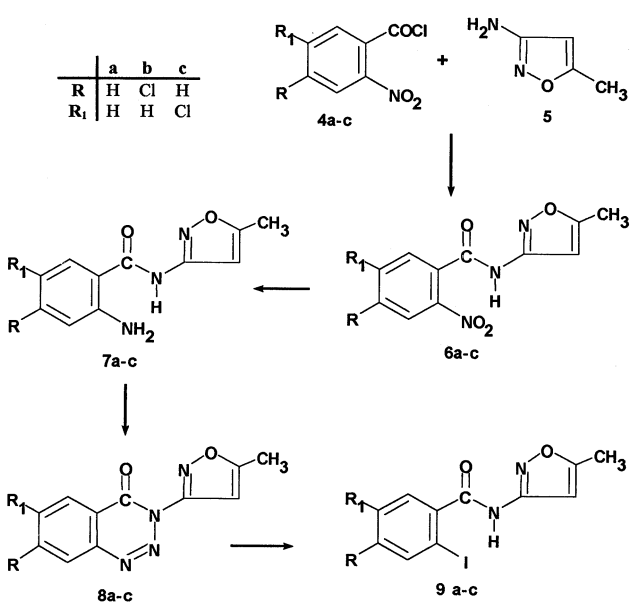
Compounds **3b** and **9a** which resulted the most active compounds in the above preliminary screening (Table 1) were tested again at lower concentrations (50, 25, 12.5, 6.2 μg/ml). The reference drug benodanil was included in all tests as positive control (Tables 1 and 2).

Compounds **3b** and **9a** showed percentages of growth inhibition against *P. citricola* comparable to the control at 50, 25, 12.5 μg/ml, respectively. At 6.2 μg/ml only benodanil **I** showed a poor inhibition.

Against *B. cinerea* compound **3b** exhibited good activity only at 100 μg/ml and moderate or poor activity at 50 and 25 μg/ml, respectively; compound **9a** showed a moderate activity at 100 μg/ml and a very poor inhibition at 50 μg/ml; percentages of growth inhibition of the reference drug were much better than those of the tested compounds at all tested concentrations except 6.2 μg/ml.

For that concerning *Rhizoctonia* sp., compounds **3b** showed a good activity at 100 μg/ml and a weak activity at 50 μg/ml, while compound **9a** exhibited a poor activity only at 100 μg/ml. Benodanil showed good activities at 100 and 50 μg/ml but not a significant percentage of growth inhibition at lower concentrations.

Compound **3b** was more active than the reference drug against *Alternaria* sp. at all tested concentrations



Scheme 2.

Table 1
Inhibitory effects of compounds **3a–c**, **9a–c** and benodanil at 100 μg/ml on radial growth of some phytopathogenic fungal strains

Compound	% Inhibition			
	Phytophthora citricola	*Botrytis cinerea*	*Rhizoctonia* sp.	*Alternaria* sp.
3a	16.6	ns[a]	ns	ns
3b	38.0	58.5	52.0	43.0
3c	ns	11.1	ns	ns
9a	36.3	44.3	27.8	17.7
9b	ns	11.1	ns	ns
9c	ns	12.5	ns	ns
Benodanil	36.0	67.1	63.7	31.5

[a] ns, not significant i.e. below 10% of inhibition; values are the mean of at least three determinations.

Table 2
Inhibitory effects of compounds **3b**, **9a** and benodanil on radial growth of selected fungal strains

Compound	Concentration (μg/ml)	% Inhibition			
		Phytophthora citricola	*Botrytis cinerea*	*Rhizoctonia* sp.	*Alternaria* sp.
3b	50	30.5	39.5	19.2	31.2
	25	25.2	16.9	ns	29.4
	12.5	24.7	ns	ns	11.2
	6.2	ns[a]	ns	ns	ns
9a	50	23.1	11.8	ns	ns
	25	20.6	ns	ns	ns
	12.5	18.9	ns	ns	ns
	6.2	ns	ns	ns	ns
Benodanil	50	34.1	55.2	53.8	ns
	25	22.3	45.7	ns	ns
	12.5	18.0	24.8	ns	ns
	6.2	18.0	ns	ns	ns

[a] ns, not significant i.e. below 10% of inhibition; values are the mean of at least three determinations.

except 6.2 μg/ml, while compound **9a** showed a weak activity only at 100 μg/ml.

Iodo derivatives **3a–c** and **9a–c** were also evaluated for their in vitro growth inhibiting activity against the yeasts *C. albicans* ATCC 10231 and *C. tropicalis* ATCC 13803, but no compound showed activity at the highest tested concentration (200 μg/ml).

To summarize, compounds **3b** and **9a** possess interesting activities against some fungal strains of agricultural interest, in several cases similar to the reference drug. Further investigations, like in vivo research and toxicity, are in progress.

4. Experimental

4.1. Chemistry

Melting points were determined on a Büchi tottoli apparatus and are uncorrected; IR spectra were recorded with a Jasco IR-810 spectrophotometer as a Nujol mull supported on a NaCl disk; ^{1}H NMR spectra were obtained in $CDCl_3$ or DMSO-d_6 using a Brüker AC-E 250 MHz spectrometer (using TMS as the internal standard). Elemental analyses (C, H, N) performed by the Dipartimento di Scienze Farmaceutiche, Università di Catania, were within $\pm$ 0.4% of the theoretical values.

4.1.1. 2-Nitrobenzoylchloride ***4a–c***

Compound **4a** is commercially available. Substituted 2-nitrobenzoylchlorides **4b,c** were obtained by refluxing the proper 2-nitrobenzoic acid derivative (0.04 mol) with thionyl chloride (28.9 ml) for 5 h [11].

4.1.2. 3-(3-Methylisoxazol-5-yl)-1,2,3-benzotriazin-4(3H)-ones ***2b,c***

Compounds **2b,c** were prepared by general methods previously described [12]. The physical and spectroscopic data are reported in Table 3.

4.1.3. N-(3-Methylisoxazol-5-yl)-2-iodobenzamides ***3a–c***

A solution of 0.01 mol of the proper benzotriazinones **2a** [12]**, b,c** in glacial acetic acid (200 ml) was refluxed for 1 h with 0.02 mol of potassium iodide.

Table 3
Physical and spectroscopic data for compounds **2b**,**c**, **3a–c**, **6a–c**, **7a–c**, **8a–c** and **9a–c**

Compound	M.p. (°)[a]	Formula	Yield (%)	IR (Nujol) (cm^{-1})	^1H NMR (δ)[b]
2b	187–188	$C_{11}H_7N_4O_2Cl$	85	1710 (CO)	2.44 (s, 3H, CH_3); 6.67 (s, 1H, isoxazole H-4); 7.78–8.41 (a set of signals, 3H, aromatic protons).
2c	225–226	$C_{11}H_7N_4O_2Cl$	88	1700 (CO)	2.42 (s, 3H, CH_3); 6.65 (s, 1H, isoxazole H-4); 7.95–8.38 (a set of signals, 3H, aromatic protons).
3a	146-149	$C_{11}H_9N_2O_2I$	33	3320–3040 (NH); 1680 (CO)	2.14 (s, 3H, CH_3); 6.37 (s, 1H, isoxazole H-4); 7.16–7.91 (a set of signals, 4H, aromatic protons); 9.51 (s, 1H, exchangeable NH).
3b	157–159	$C_{11}H_8N_2O_2ClI$	20	3310–3100 (NH); 1680 (CO)	2.19 (s, 3H, CH_3); 6.37 (s, 1H, isoxazole H-4); 7.38–7.88 (a set of signals, 4H, aromatic protons); 9.54 (s, 1H, exchangeable NH).
3c	181–183	$C_{11}H_8N_2O_2ClI$	39	3320–3100 (NH); 1680 (CO)	2.21 (s, 3H, CH_3); 6.38 (s, 1H, isoxazole H-4); 7.12–7.83 (a set of signals, 3H, aromatic protons); 9.38 (s, 1H, exchangeable NH).
6a	177–179	$C_{11}H_9N_3O_4$	43	3300–3020 (NH); 1695 (CO)	2.42 (s, 3H, CH_3); 6.74 (s, 1H, isoxazole H-4); 7.77–8.18 (a set of signals, 4H, aromatic protons); 11.66 (s, 1H, exchangeable NH).
6b	214–216	$C_{11}H_8N_3O_4Cl$	53	3400–3010 (NH); 1680–1670 (CO)	2.42 (s, 3H, CH_3); 6.74 (s, 1H, isoxazole H-4); 7.80–8.26 (a set of signals, 3H, aromatic protons); 11.73 (s, 1H, exchangeable NH).
6c	162–163	$C_{11}H_8N_3O_4Cl$	70	3300–3000 (NH); 1695 (CO)	2.43 (s, 3H, CH_3); 6.74 (s, 1H, isoxazole H-4); 7.82–8.22 (a set of signals, 3H, aromatic protons); 11.73 (s, 1H, exchangeable NH).
7a	188–189	$C_{11}H_{11}N_3O_2$	90	3480–3040 (NH and NH_2); 1665 (CO)	2.40 (s, 3H, CH_3); 6.55–7.77 (a set of signal, 7H, aromatic protons, isoxazole H-4, exchangeable NH_2); 10.92 (s, 1H, exchangeable NH).
7b	202–203	$C_{11}H_{10}N_3O_2Cl$	90	3500–3020 (NH and NH_2); 1680 (CO)	2.40 (s, 3H, CH_3); 6.53–7.75 (a set of signal, 6H, aromatic protons, isoxazole H-4, exchangeable NH_2); 10.99 (s, 1H, exchangeable NH).
7c	198–200	$C_{11}H_{10}N_3O_2Cl$	60	3500–3020 (NH and NH_2); 1680 (CO)	2.40 (s, 3H, CH_3); 6.68–7.82 (a set of signal, 6H, aromatic protons, isoxazole H-4, exchangeable NH_2); 11.07 (s, 1H, exchangeable NH).
8a	173–174	$C_{11}H_8N_4O_2$	70	1720–1700 (CO)	2.56 (s, 3H, CH_3); 6.63 (s, 1H, isoxazole H-4); 7.86–8.45 (a set of signals, 4H, aromatic protons).
8b	202–203	$C_{11}H_7N_4O_2Cl$	65	1710 (CO)	2.56 (s, 3H, CH_3); 6.60 (s, 1H, isoxazole H-4); 7.81–8.40 (a set of signals, 3H, aromatic protons).
8c	208–209	$C_{11}H_7N_4O_2Cl$	44	1700 (CO)	2.56 (s, 3H, CH_3); 6.08 (s, 1H, isoxazole H-4); 7.96–8.65 (a set of signals, 3H, aromatic protons).
9a	147–148	$C_{11}H_9N_2O_2I$	43	3220–3100 (NH); 1685–1675 (CO)	2.35 (s, 3H, CH_3); 6.88 (s, 1H, isoxazole H-4); 7.16–7.93 (a set of signals, 4H, aromatic protons); 10.17 (s, 1H, exchangeable NH).
9b	170–172	$C_{11}H_7N_4O_2ClI$	50	3300–3100 (NH); 1705 (CO)	2.37 (s, 3H, CH_3); 6.86 (s, 1H, isoxazole H-4); 7.43–7.93 (a set of signals, 3H, aromatic protons); 10.33 (s, 1H, exchangeable NH).
9c	178–179	$C_{11}H_7N_4O_2ClI$	61	3300–3100 (NH); 1705 (CO)	2.38 (s, 3H, CH_3); 6.89 (s, 1H, isoxazole H-4); 7.15–7.86 (a set of signals, 3H, aromatic protons); 10.90 (s, 1H, exchangeable NH).

[a] Crystallization solvent: ethanol.
[b] $CDCl_3$ for compounds **2b,c**, **3a–c**, **8a–c**, and **9a–c**. DMSO-d_6 for compounds **6a–c** and **7 a–c**.

After this time, 500 ml of water were added and the precipitate which separated out was collected, air driedand crystallized from ethanol to give **3**. Compounds **3a–c** are listed in Table 3.

*4.1.4. N-(5-Methylisoxazol-3-yl)-2-nitrobenzamides **6a–c** and N-(5-methylisoxazol-3-yl)-2-aminobenzamides **7a–c***

Compounds **6** and **7** were obtained by preparative methods previously reported [13] (see Table 3).

*4.1.5. 3-(5-Methylisoxazol-3-yl)-1,2,3-benzotriazin-4(3H)-ones **8a–c** and N-(5-methylisoxazol-3-yl)-2-iodobenzamides **9a–c***

All these compounds were synthesized by the same procedure previously employed for **2b,c** and **3a–c**, respectively (see Table 3).

4.2. Biology

The in vitro antifungal activity against isolates of plant pathogenic fungi was evaluated by an agar dilu-

tion method using potato dextrose agar (Oxoid) [14]. A suitable volume of each substance (in a solution of DMSO) was added to 20 ml of molten agar (at 50°C) and the resulting mixture was poured onto plates and allowed to solidify. The plates were inoculated by applying 7 mm diameter mycelium disks, from 10 days fungal cultures, to the center of the agar surface. Plates were incubated at 21 ± 1°C for 3 days and then radial growth was recorded. Percentages of growth inhibition were calculated by comparing mean value of diameters of the mycelia in test plates with that of untreated control plates (with DMSO). Each determination was done in triplicate, percentages of growth inhibition are mean value of at least three independent experiments, the variation was $< 10\%$.

Antifungal activity against yeasts *C. albicans* ATCC 10231 and *C. tropicalis* ATCC 13803 was carried out by an agar dilution method as described previously [15].

References

[1] P.J. Kuhn, Mode of action of carboxamides, Symp. Br. Mycol. Soc. 9 (1989) 155–183.

[2] R.W. Smiley, D.E. Wilkins, E.L. Klepper, Impact of fungicide seed treatments on *Rhizoctonia* root rot, take-all, eyespot, and growth of winter wheat, Plant Dis. 74 (10) (1990) 782–787.

[3] J.R. Hardison, Chemiotherapeutic control of stipe smut (*Ustilago striiforms*) in grasses by two derivatives of 1,4-oxathin, Phytopathology 57 (1967) 242–245.

[4] P. Ten Hakenn, C.L. Dunn, Structure–activity relationships in group of carboxanilides systematically active against broad bean rust (*Uromyces fabae*) and wheat rust (*Puccinia recondita*), Proc. 6th Br. Insectic. Fungic. Conf. 2 (1971) 453–462.

[5] E.H. Pommer, W. Zwick, Efficacy of systemic fungicides against Basidiomycetes, Indian Phytopathol. 27 (1974) 53–58.

[6] H.R. Kataria, P.R. Verma, G. Racow, Fungicidal control of damping-off and seedling root rot in Brassica species caused by *Rhizoctonia solani* in the growth chamber, Ann. Appl. Biol. 123 (2) (1993) 247–256.

[7] Ger. Offen. DE 3, 215, 771 (Cl. CO7D249/08) 1985; Chem. Abs. 99, 53760y (1983).

[8] J. Salvaggio, L. Aukrust, Mold-induced asthma, J. Allergy Clin. Immunol. 68 (1981) 327–346.

[9] M.C. Machet, E. Stephanon, E. Estève, F. De Closets, A. Barrabès, M. Thèrizol-Ferly, G. Lebret, M.C. Grangeponte, L. Vaillant, Alternariose cutanée survenant au cours de l'évolution d'un pemphigus traité, Ann. Phatol. 14 (3) (1994) 186–191.

[10] S. Plescia, G. Daidone, D. Raffa, M.L. Bajardi, A. Caruso, V. Cutuli, M. Amico-Roxas, Synthesis and pharmacological study of some 3-(isoxazol-5-yl)-quinazolin-4(3*H*)-ones, Farmaco 47 (4) (1992) 465–475.

[11] M.H. Palmer, G.J. Mc Vie, Alkylation and acylation reaction. Part II. The interaction of aryl–oxyacetyl chlorides with aluminium chloride, J. Chem. Soc. B (1968) 745–751.

[12] G. Daidone, D. Raffa, S. Plescia, M.L. Bajardi, Potentially biologically active agents. Note II. Synthesis and pharmacological evaluation of some new 3-isoxazolyl-substituted-1,2,3-benzotriazin-4(3*H*)-ones, Boll. Chim. Farm. 124 (1985) 201–206.

[13] S. Plescia, M.L. Bajardi, D. Raffa, G. Daidone, M. Matera, A. Caruso, M. Amico-Roxas, Synthesis and pharmacological study of some 3-(pyrazol-5-yl)-quinazolin-4(3*H*)-ones, Eur. J. Med. Chem. Chim. Ther. 21 (1986) 291–295.

[14] R. Fioravanti, M. Biava, G.C. Porretta, C. Landolfi, N. Simonetti, A. Villa, E. Conte, A. Porta-Puglia, Research on antibacterial and antifungal agents. XI. Synthesis and antimicrobial activity of *N*-heteroaryl benzylamines and their Schiff bases, Eur. J. Med. Chem. 30 (1995) 123–132.

[15] L.H. Lennette, E.H. Spaulding, P. Truant, in: Manual of Clinical Microbiology, 2nd ed., American Society for Microbiology, Washington, DC, 1974, Ch. 42.