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Synthesis and antifungal activity of new *N*-(1-phenyl-4-carbetoxypyrazol-5-yl)-, *N*-(indazol-3-yl)- and *N*-(indazol-5-yl)-2-iodobenzamides

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Abstract

N-(1-Phenyl-4-carbetoxypyrazol-5-yl)-, *N*-(indazol-3-yl)- and *N*-(indazol-5-yl)-2-iodobenzamides **6**, with a Benodanil-like structure, were synthesized by refluxing in acetic acid the corresponding benzotriazinones **5** with potassium iodide for 1 h in order to study the role on the antifungal activity of the *N*-substitution with an aromatic heterocyclic system on benzamide moiety. Among the tested iododerivatives, compounds **6d**,**f**,**g**,**h** possess interesting activities toward some phytopathogenic fungal strains.

Keywords: *N*-(1-Phenyl-4-carbetoxypyrazol-5-yl)-2-iodobenzamides; *N*-(Indazol-3-yl)-2-iodobenzamides; *N*-(Indazol-5-yl)-2-iodobenzamides; Antifungal activity

1. Introduction

Benodanil, namely *N*-phenyl-2-iodobenzamide (**I**), active principle of Carilus® (BASF) [1,5], is one of the most significant fungicide anilides structurally connected with the wide class of fungicide carboxamides known as *cis*-crotoanilides (**II**). It exhibits a spectrum of antifungal activities primarily including smuts (*Ustilaginales*), rusts (*Uredinales*), and rots caused by *Rhizoctonia solani* [1–5].

Previously [6], we had investigated the synthesis of *N*-isoxazolyl-2-iodobenzamides **III** and **IV** with a benodanil-like structure, which showed interesting antifungal activity against *Phytophtora citricola* saw., *Botrytis cinerea* Pers., *Rhizoctonia* sp., and *Alternaria* sp. Particularly, *Alternaria* sp., besides being a very common phytotoxic fungi, can also play a considerable role in human pathology, especially in patients with immunological deficiency. Its pathogenic role in humans is mainly expressed by asthma [7], even if cases of dermal alternariosis, occurring during an immunosuppressive therapy, are reported [8].

Among the iododerivative **III** and **IV**, *N*-(3-methylisoxazol-5-yl)-4-chloro-2-iodobenzamide (**IIIa**) and *N*-(5-methylisoxazol-3-yl)-2-iodobenzamide (**IVa**) possess interesting activities against the aforesaid fungal strains in several cases similar to that of Benodanil **I** taken as reference drug [6].

On the basis of such results, we synthesized new *N*-(heteroaryl)-2-iodobenzamides in order to study the role of the *N*-substitution with an aromatic heterocyclic system on benzamide moiety.

In particular, owing to their antimicrobial activity reported in literature [9–11], the pyrazolyl and indazolyl moiety were selected as heterocyclic systems for new *N*-(heteroaryl)-2-iodobenzamides.

2. Chemistry

N-(1-Phenyl-4-carbetoxypyrazol-5-yl)-2-iodobenzamides **6a**–**c**, *N*-(indazol-5-yl)-2-iodobenzamides **6d**–**f** and *N*-(indazol-3-yl)-2-iodobenzamides **6g**,**h** were obtained by a previously described method (Scheme 1) [6].

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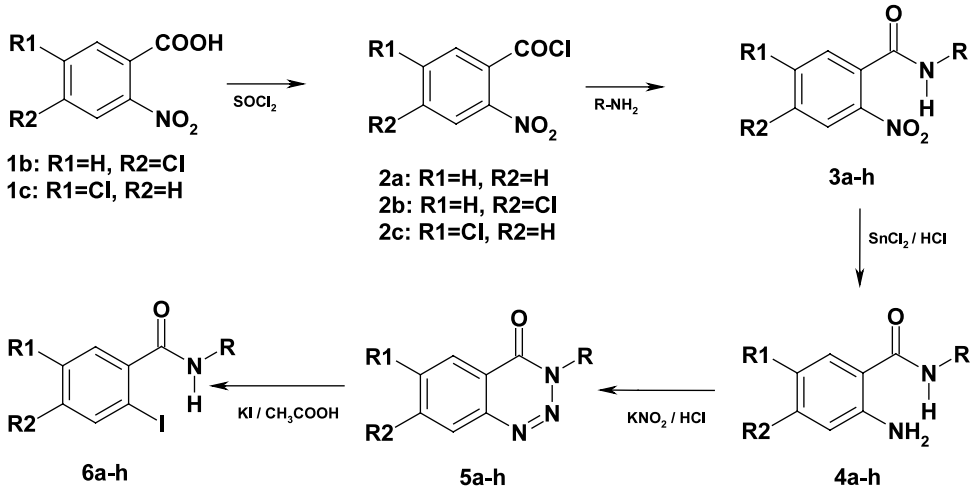

Scheme 1.

All new products were characterized on the basis of elemental analysis, infrared (IR) and nuclear magnetic resonance (^{1}H NMR) spectra.

3. Biological results and discussion

2-Iodobenzamides **IIIa**, **IVa**, **6a**–**h** and Benodanil **I**, included in all tests as positive control, were screened for antifungal activity at 100 μg/ml against the following isolates of phytopathogenic fungi: *P. citrophthora*, *B. cinerea*, *R. solani* and *Alternaria* sp. (Table 1); compounds **6d**, **6f**–**h** which showed the best activities in the above preliminary screening, were tested again at lower concentrations of 50, 25, 12.5, and 6.2 μg/ml (Table 2).

In particular, against *P. citrophthora* compounds **6d**,**f**–**h** were more active than Benodanil **I** at 50 μg/ml concentration, and, except for compound **6d**, they exhibited good activities at lower concentrations in which Benodanil **I** resulted inactive.

2-Iododerivatives **6** did not show noteworthy antifungal activity against all the other isolates of phytopathogenic fungi, having activities lower than the reference drug Benodanil **I**.

Moreover they were also evaluated for their antifungal activity against human pathogenic yeasts, like *Candida albicans* ATCC10231 and *Candida tropicalis* ATCC 13803, but no compounds resulted active at the screening concentration of 100 μg/ml.

Data reported in Tables 1 and 2 showed that iodobenzamides **6**, bearing in the position 3 the indazolyl moiety, possess interesting activities towards some phytopathogenic fungal strains, especially against *P. citrophthora*; no significant activities were shown if the 3 position was substituted with the pyrazolyl moiety.

A comparison of the antifungal activities against *P. citrophthora* between compounds **6**, Benodanil **I** and the previously synthesized compounds **IIIa** and **IVa** [6] emphasize the positive role of the introduction in the 3 position of the indazolyl moiety with respect to the references.

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 Nujol mull supported on NaCl disk; ^{1}H NMR spectra were obtained in $CDCl_3$ or DMSO-d_6 using a Brüker AC-E 250 MHz spectrometer (TMS as the internal standard). Elemental analyses (C, H, N), performed by

Table 1
Inhibitory effects of compounds **IIIa**, **IVa**, **6a**–**h** and Benodanil **I** at 100 μg/ml on radial growth of some phytopathogenic fungal strains

Comp.	% Inhibition			
	P. citrophthora	*B. cinerea*	*R. solani*	*Alternaria* sp.
IIIa	30	58.5	52	43
IVa	52.5	44.3	27.8	17.7
6a	ns [a]	ns	14.7	10.5
6b	ns	12.8	12.6	28.2
6c	10.2	ns	ns	11.3
6d	44.8	54	ns	35.8
6e	ns	13.7	14.2	ns
6f	53.8	49	19	36.8
6g	42.1	50.4	17.3	38.3
6h	45.6	19.5	11.7	36.2
Benodanil	16.5	64.5	79.4	56.9

[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 **IIIa**, **IVa**, **6d**, **6f**, **6g**, **6h** and Benodanil **I** at 50, 25, 12.5, 6.2 μg/ml on radial growth of selected phytopathogenic fungal strains

Comp.	Concentration (μg/ml)	%Inhibition		
		P. citrophthora	*B. cinerea*	*Alternaria* sp.
IIIa	50	25	39.5	31.2
	25	12.5	16.9	29.4
	12.5	ns [a]	ns	11.2
	6.2	ns	ns	ns
IVa	50	30	11.8	ns
	25	ns	ns	ns
	12.5	ns	ns	ns
	6.2	ns	ns	ns
6d	50	18.4	34	ns
	25	ns	ns	ns
	12.5	ns	ns	ns
	6.2	ns	ns	ns
6f	50	40	45.2	29.5
	25	40	25.8	ns
	12.5	23	ns	ns
	6.2	ns	ns	ns
6g	50	25.1	34.8	40.5
	25	11.6	21.3	34.2
	12.5	ns	ns	ns
	6.2	ns	ns	ns
6h	50	31.9	nt [b]	34.2
	25	22.8	nt	ns
	12.5	ns	nt	ns
	6.2	ns	nt	ns
Benodanil	50	11.6	56.8	39.5
	25	ns	42.2	15.8
	12.5	ns	27.2	ns
	6.2	ns	ns	ns

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

Dipartimento di Scienze Farmaceutiche-Università di Catania, were within ± 0.4% of theoretical values.

5-Aminoindazole and compound **2a** are commercially available.

3-Aminoindazole [12], 5-amino-4-carbetoxy-1-phenylpyrazole [13] and compounds **5d** [14], **5g** [15], and **5h** [15] were obtained by methods previously described.

*4.1.1. 2-Nitrobenzoylchlorides **2b–c***

Substituted 2-nitrobenzoylchlorides **2b**,**c** were obtained by refluxing for 5 h the opportune 2-nitrobenzoic acid derivatives **1b**,**c** (0.04 mol) with thionyl chloride (28.9 ml) [16].

*4.1.2. N-(Indazol-5-yl)-5-R_1-4-R_2-2-nitrobenzamides **3e–f***

To a cold (ice bath 0–5 °C) magnetic stirred solution of 5-aminoindazole(0.05 mol, 6.65 g) in pyridine (16 ml), 0.05 mol of the proper 2-nitrobenzoyl chloride **2a–c** was added dropwise.

The reaction mixture was left under magnetic stirrer overnight then poured in crushed ice. The solid which separated was filtered off and crystallized to give **3**.

The physical and spectroscopical data of compounds **3** are reported in Table 3.

*4.1.3. N-(1-Phenyl-4carbetoxypyrazol-5-yl)- and N-(indazol-5-yl)-5-R1-4-R2-2-aminobenzamides **4,b,c,e,f***

4.8 mmol of the proper 2-nitrobenzamide **3** were added to a cold (bath ice, 0–5 °C) magnetically stirred suspension of stannous chloride dihydrate (1.8 mmol, 3.6 g) in concentrated HCl (25 ml) at such a rate so that the temperature was maintained below 10 °C (∼1 h). After the complete addition, the mixture was left under magnetic stirrer for 24 h then diluted with cold water (500 ml). Aqueous sodium hydroxyde (40%) was added till the solution became alkaline and the solid which separated was collected and crystallized to give **4**.

The physical and spectroscopical data of compounds **4** are reported in Table 3.

*4.1.4. N-(1-Phenyl-4-carbetoxypyrazol-5-yl)- and N-(indazol-5-yl)-5-R1-4-R2-1,2,3-benzotriazin-4(3H)-ones **5a–c,e,f***

To a cold (ice bath 0–5 °C) magnetically stirred solution of 2-aminobenzamides **4** in 2 M HCl (30 ml), 5.6 mmol of potassium nitrite in a little amount of water was added dropwise.

The solution was left 1 h under magnetic stirrer then poured in cold water. The solid which separated was collected and crystallized to give **5**.

The physical and spectroscopical data of compounds **5** are reported in Table 3.

*4.1.5. N-(1-Phenyl-4-carbetoxypyrazol-5-yl)-, N-(indazol-3-yl)- and N-(indazol-5-yl)-5-R1-4-R2-2-iodobenzamides **6a–h***

A solution of 3.3 mmol of the proper benzotriazinones **5a–c**,**d** [14], **e**,**f**,**g** [15], **h** [15] in glacial acetic acid (54 ml) was refluxed for 1 h with 6.6 mmol of potassium iodide.

After this time, 500 ml of water were added and the precipitate which separated out was collected and crystallized to give **6**.

The physical and spectroscopical data of compounds **6** are reported in Table 3.

4.2. Biology

The in vitro antifungal activity against isolates of planta pathogenic fungi was evaluated by an agar dilution method using potato dextrose agar (Oxoid) [17]. A suitable volume of solution of the test compounds

Table 3
Physical and spectroscopic data for compounds **3e**,**f**, **4b**,**c**,**e**,**f**, **5a**–**c**,**e**,**f** and **6a**–**h**

Comp.	m.p. (°) (ethanol)	Formula	Yields (%)	IR (nujol) (cm^{-1})	^1H NMR [a] (δ)
3e	289–290	$C_{14}H_9N_4O_3Cl$	74	3360–3040 (NH), 1640 (CO).	7.40–8.21 (a set of signals, 7H, aromatic protons); 10.64 (s, 1H, exchangeable NH); 12.97 (s, 1H, exchangeable NH).
3f	298–299	$C_{14}H_9N_4O_3Cl$	79	3320–3060 (NH), 1650 (CO).	7.52–8.23 (a set of signals, 7H, aromatic protons); 10.74 (s, 1H, exchangeable NH); 13.08 (s, 1H, exchangeable NH).
4b	108–110	$C_{19}H_{17}N_4O_3Cl$	54	3480–3100 (NH and NH_2),1720 (CO), 1660 (CO).	1.16 (t, 3H, CH_3); 4.17 (q, 2H, CH_2); 6.56–7.73 (a set of signals, 10H, aromatic protons and exchangeable NH_2); 8.14 (s, 1H, pyrazole H-3); 10.32 (s, 1H, exchangeable NH).
4c	118–120	$C_{19}H_{17}N_4O_3Cl$	64	3520–3220 (NH and NH_2), 1705 (CO), 1650 (CO).	1.72 (t, 3H, CH_3); 4.18 (q, 2H, CH_2); 6.77–7.71 (a set of signals, 10H, aromatic protons and exchangeable NH_2); 8.14 (s, 1H, pyrazole H-3); 10.37 (s, 1H, exchangeable NH).
4e	284–286	$C_{14}H_{11}N_4OCl$	28	3520–3240 (NH and NH_2), 1640 (CO).	6.59 (s, 2H, exchangeable NH_2); 6.81–8.14 (a set of signals, 7H, aromatic protons); 10.10 (s, 1H, exchangeable NH); 12.99 (s, 1H, exchangeable NH).
4f	220–222	$C_{14}H_{11}N_4OCl$	46	3500–3140 (NH and NH_2), 1635 (CO).	6.48 (s, 2H, exchangeable NH_2); 6.77–8.16 (a set of signals, 7H, aromatic protons); 10.13 (s, 1H, exchangeable NH); 13.01 (s, 1H, exchangeable NH).
5a	190–191	$C_{19}H_{15}N_5O_3$	87	1730–1700 (CO).	1.04 (t, 3H, CH_3); 4.15 (q, 2H, CH_2); 7.35–8.30 (a set of signals, 10H, aromatic protons).
5b	115–117	$C_{19}H_{14}N_5O_3Cl$	96	1710 (CO).	1.11 (t, 3H, CH_3); 4.18 (q, 2H, CH_2); 7.38–8.33 (a set of signals, 9H, aromatic protons).
5c	100–101	$C_{19}H_{14}N_5O_3Cl$	62	1730–1770 (CO).	1.10 (t, 3H, CH_3); 4.17 (q, 2H, CH_2); 7.36–8.32 (a set of signals, 9H, aromatic protons).
5e	258–260	$C_{14}H_8N_5OCl$	86	1700 (CO).	7.56–8.41 (a set of signals, 7H, aromatic protons); 13.40 (s, 1H, exchangeable NH).
5f	234–236	$C_{14}H_8N_5OCl$	64	3210–3010 (NH), 1700 (CO).	7.55–8.34 (a set of signals, 7H, aromatic protons); 13.35 (s, 1H, exchangeable NH).
6a	180–182	$C_{19}H_{16}N_3O_3I$	94	3260–3080 (NH), 1720 (CO), 1675 (CO).	1.36 (t, 3H, CH_3); 4.32 (q, 2H, CH_2); 7.10–8.02 (a set of signals, 10H, aromatic protons); 8.63 (s, 1H, exchangeable NH).
6b	189–190	$C_{19}H_{15}N_3O_3ClI$	75	3260–3080 (NH), 1720 (CO), 1675 (CO).	1.37 (t, 3H, CH_3); 4.32 (q, 2H, CH_2); 7.34–8.01 (a set of signals, 9H, aromatic protons); 8.66 (s, 1H, exchangeable NH).
6c	208–209	$C_{19}H_{15}N_3O_3ClI$	80	3290–3100 (NH), 1720 (CO), 1675 (CO).	1.37 (t, 3H, CH_3); 4.33 (q, 2H, CH_2); 7.11–8.04 (a set of signals, 9H, aromatic protons); 8.59 (s, 1H, exchangeable NH).
6d	298–299	$C_{14}H_{10}N_3OI$	68	3320–3080 (NH); 1655 (CO).	6.66–7.79 (a set of signals, 8H, aromatic protons); 9.69 (s, 1H, exchangeable NH); 12.16 (s, 1H, exchangeable NH).
6e	301–302	$C_{14}H_9N_3OClI$	81	3300–3100 (NH); 1645 (CO).	7.52–8.25 (a set of signals, 7H, aromatic protons); 10.46 (s, 1H, exchangeable NH); 13.05 (s, 1H, exchangeable NH).
6f	209–210	$C_{14}H_9N_3OClI$	79	3300–3080 (NH); 1650 (CO).	7.29–8.24 (a set of signals, 7H, aromatic protons); 10.49 (s, 1H, exchangeable NH); 13.04 (s, 1H, exchangeable NH).
6g	215–217	$C_{14}H_9N_3OClI$	85	3400–3100 (NH); 1660 (CO).	7.08–8.05 (a set of signals, 7H, aromatic protons); 10.89 (s, 1H, exchangeable NH); 12.80 (s, 1H, exchangeable NH).
6h	230–231	$C_{14}H_9N_3OClI$	92	3380–3220 (NH); 1660 (CO).	7.11–7.98 (a set of signals, 7H, aromatic protons); 10.94 (s, 1H, exchangeable NH); 12.81 (s, 1H, exchangeable NH).

[a] $CDCl_3$ for compounds **3f**, **5a**–**c**,and **6a**–**c**. DMSO-d_6 for compounds **3e**, **4b**–**f**, **5e**,**f** and **6d**–**h**.

(DMSO) was added to 20 ml of molten agar (at 50 °C) to obtain the required concentration 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 the radial growth was recorded. Percentages of growth inhibition were determined 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.

Antifungal activity against the yeasts *C. albicans* ATCC 10231 and *C. tropicalis* ATCC 13803 was carried out by an agar dilution method as described previously [18].

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