Synthesis and fungistatic activity of aryl-substituted naphthalene- and indene-2-carbonitriles*

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ABSTRACT

4-Aryl-substituted 1-aminonaphthalene-2-carbonitriles and 3-phenyl-1-oxo-1*H*-indene-2-carbonitriles were prepared in a two--step synthesis from appropriate carbonyl compounds and were evaluated *in vitro* for growth-inhibiting activity against some phytopathogenic fungi: *Fusarium culmorum, Penicillium expansum, Botrytis cinerea* and *Alternaria* species. The results were compared with the activity of a commercial fungicide – *captan*. The highest fungistatic activity was revealed by 1-amino-4-phenylnaphthalene-2-carbonitrile. An efficient synthesis of 1-amino-4-arylnaphthalene-2-carbonitriles involves condensation of diarylacetaldehydes or 1,1-diarylacetones with malonodinitrile and cyclization of the obtained aryl-ylidenemalonodinitriles in concentrated sulfuric acid. The benzannulation reaction is accompanied by a *quasi*-aromatic rearrangement. The indenone derivatives were synthesized in the same manner from substituted benzophenones. As the formation of five-membered ring during the cyclization of diarylmethylidenemalonodinitriles was less effective, the rearrangement was not observed.

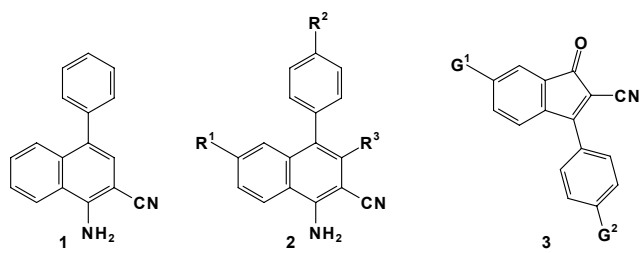
INTRODUCTION

There are few known aromatic compounds imposing an effect on fungi, for instance *chlorothalonil* (2,4,5,6-tetrachloro--1,3-benzenedicarbonitrile), the active ingredient of many commercial fungicides (e.g. Agrothalonil 500 SC, Bravo 500 SC, Clortosip 500 SC, Gwarant 500 SC, Rywal 500 SC). *Chlorothalonil* is effective against a broad range of phytopathogenic fungi attacking many agronomic and vegetable crops. The search for easily biodegradable, especially non-halogenated fungicides is of great interest, being also a challange for organic chemists and plant-protection specialists. In our earlier studies (Kulig et al. 2002; Wilamowski et al. 2001) we found that some alkyl-substituted, carbocyclic, vicinal aminonitriles are highly active against some phytopathogenic fungi, such as *Fusarium culmorum, Alternaria brassicicola, Botrytis cinerea* and *Penicillium expansum*. These fungi are common pathogens of many plants, which may cause seedling's damping-off, root and stem rot and mould of fruits (Borecki 1996). The obtained results encouraged us to determine the influence of a variety of substituents on fungistatic activity of the 1-aminonaphthalene-2-carbonitrile system. Particularly, inserting aryl groups to the core structure became an interesting issue. Furthermore, we extended our studies to 3-phenyl-1-oxo-1*H*-indene--2-carbonitriles derivatives.

MATERIAL AND METHODS

1-Amino-4-phenylnaphthalene-2-carbonitrile (**1**) and its derivatives **2** were obtained from diarylacetaldehydes or 1,1-diarylacetones in a two-step, efficient synthesis described in our earlier report (Scheme 1) (Kozik et al. 2006). 3-Aryl-1-oxoindene--2-carbonitriles (**3**) were prepared from benzophenones according to the procedures described in the literature (Campaigne et al. 1974; Campaigne and Schneller 1976; Davey et al. 1999; Ong and Keoshkerian 1984). Tested phytopathogenic fungi were obtained from the Department of Plant Protection, University of Agriculture in Krakow, Poland.

Scheme 1



R 1, R 2 = H, CH_3, Cl
R 3 = H, CH_3

G 1, G 2 = H, CH_3, Cl

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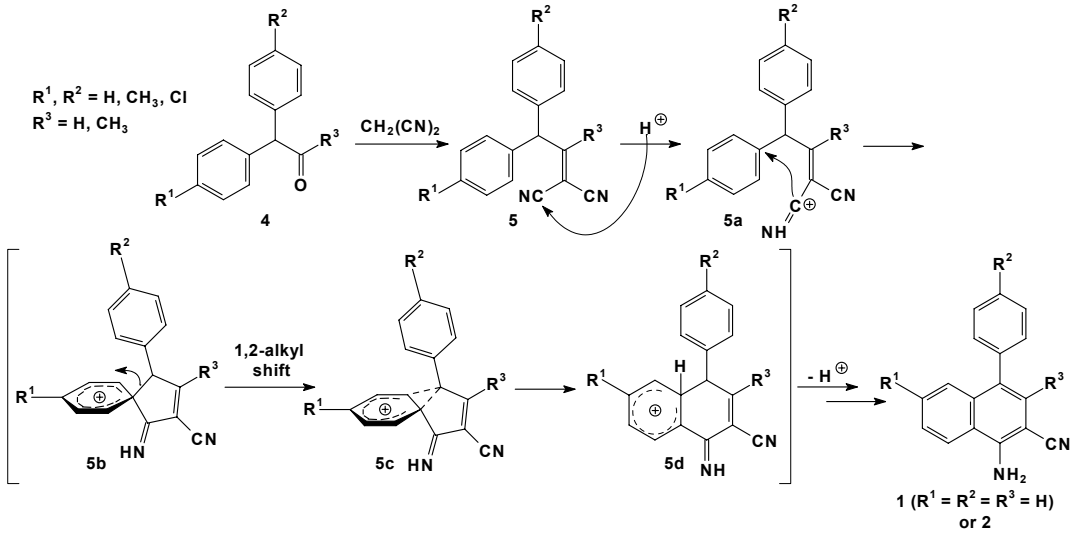
Scheme 2



BIOLOGICAL ASSAYS

The fungistatic activity of aminonitrile **1** was investigated against phytopathogenic fungi *Fusarium culmorum* (W. G. Sm.) Sacc., *Alternaria brassicicola* (Schw.) Wiltsh., *Botrytis cinerea* Pers. and *Penicillium expansum* Thom. (Borecki 1996). The nitriles **2** and **3** were tested on *F. culmorum* and *A. brassicicola* or *Alternaria alternata* (Fr.) Keissler, alternatively. The experiments were conducted according to the standard method used in laboratory investigations of fungicides (Borecki 1984). Sterile agarized Czapek-Dox (Kiraly et al. 1977) media were prepared at 45°C with an addition of acetone solutions at concentrations 40, 4 and 0.4 mg/dm^3 of the nitriles **2** and **3** and, respectively, 20, 2 and 0.2 mg/dm^3 of the aminonitrile **1**. The media were poured into 8 cm Petri dishes (10 mL per dish), left for two days to evaporate acetone and then they were inoculated with PDA discs (Ø5 mm) of overgrown mycelium. In case of *P. expansum* the medium was inoculated by pricking with a laboratory needle covered with fungus spores. Fungi were incubated in the thermostat at 20 (±2)°C and four days after inoculation the diameter of the cultures was measured. The percentage of inhibition of fungi growth was calculated in accordance with Abbott's formula (Abbott 1925). These values were defined by comparing the growth of fungi colonies on media containing examined compounds to the control colony on the medium with one equivalent of acetone only. Tests were carried out in five replicates and results were statistically analyzed using analysis of variance. The significance of the differences between combinations was estimated using Duncan's test ($\alpha = 0.05$). For the nitriles which exhibited the highest level of fungistatic activity, concentrations that would give 50% of fungi growth inhibition (*effective concentration* – EC_{50}) were calculated by a probit analysis using program "Regres", written in the Institute of Industrial Organic Chemistry Branch Pszczyna. The EC_{50} values that exceeded the range of tested concentrations were estimated by extrapolation.

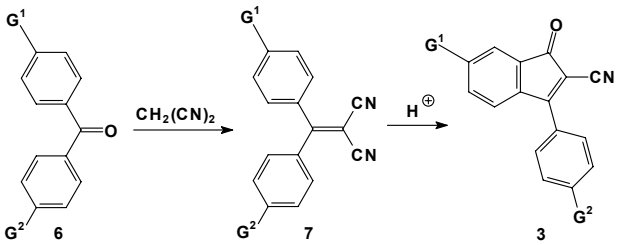
RESULTS AND DISCUSSION

Synthesis of naphthalene- and indene-2-carbonitriles

The two-step synthesis of 1-amino-4-phenylnaphthalene--2-carbonitrile (**1**) and its derivatives **2** involves condensation of the carbonyl compounds **4** with malonodinitrile and cyclization of obtained aryl-ylidenemalonodinitriles **5** in concentrated sulfuric acid (Scheme 2) (Kozik et al. 2006). The benzannulation reaction is accompanied with a *quasi*-aromatic rearrangement. The pathway appears to involve the *ipso* electrophilic attack of the activated nitrile group **5a** and the formation of spiroarenium cation **5b**. The crucial rearrangement of the cation **5b** leads to stable, vicinal aminonitriles **1** and **2**.

3-Aryl-1-oxoindene-2-carbonitriles (**3**) were prepared according to the procedures described in the literature. The synthesis involves condensation of benzophenones **6** with malonodinitrile catalyzed with titanium(IV) chloride and pyridine (Davey et al. 1999; Ong and Keoshkerian 1984) followed by cyclization of the obtained diarylmethylidenemalonodinitriles **7** in concentrated sulfuric acid (Scheme 3) (Campaigne et al. 1974; Campaigne and Schneller 1976). The cyclization reaction of dinitriles **7** proceeds much slower than it does in the case of dinitriles **5**. Less effective ring closure of **7** is probably caused by the formation of the smaller, five-membered ring. Some of the compounds **7** were cyclized in trifluoromethanesulfonic acid. No rearranged indenones **3** were isolated. Physical and spectroscopic data of all new compounds will be published elsewhere (Kozik, unpublished data).

Scheme 3



Fungistatic activity of naphthalene- and indene-2-carbonitriles

Preliminary tests revealed relatively high fungistatic activity of 1-amino-4-phenylnaphthalene-2-carbonitrile (**1**), in some cases even stronger than the activity of the commercial fungicide – Kaptan 50 WP (a. i. *captan* 50%; Table 1). In case of three out of four studied fungi, EC_{50} values calculated for aminonitrile **1** were lower than for *captan*, especially in case of *F. culmorum* where difference was very significant. On the other hand, phenyl-substituted naphthalene derivative **1** turned out to be less effective than for example its methyl analogue – 1-amino-4-methylnaphthalene-2-carbonitrile (Wilamowski et al. 2001, Table 1). According to those earlier studies, 1-aminonaphthalene--2-carbonitriles similar to the **1**, but having short alkyl group (e. g. methyl, ethyl or *n*-propyl) instead of the phenyl substituent, gave much better results than aminonitrile **1**.

These findings prompted us to synthesize other aryl-substituted 1-aminonaphthalene-2-carbonitriles **2** using our synthetic route to this class of compounds (Kozik et al. 2006) and also extend studies to 3-phenyl-1-oxo-1*H*-indene-2-carbonitriles derivatives **3**. Detailed biological data for the nitriles **2** and **3** were summarized in Table 2. The results were expressed as a percentage of inhibition or stimulation of fungi linear growth.

Fungistatic effect depended on the structure and concentration of the tested compounds **2** and **3** as well as on the species of phytopathogenic fungi. Among those compounds, the nitriles **2e** and **3b** revealed the highest fungistatic activity at all examined concentrations for both *F. culmorum* and *A. alternata*. Table 3 includes EC_{50} values and appropriate correlation coefficients for **2e** and **3b**. The EC_{50} calculated for *F. culmorum* allow to classify both nitriles as a fungicides of moderate activity (class III, EC_{50} = 10-100 mg/dm^3) (Borecki 1984). In case of *A. alternata* the obtained results considerably exceeded the level of 40 mg/dm^3. This fact implies relatively lower effect of **2e** and **3b** on that fungus. To draw better conclusions it would be necessary to make further tests at higher range of concentrations. A few derivatives (**2a**, **2g**, and **3a**) appeared to be only weak fungicides, and the remaining derivatives caused no effect or stimulated fungi growth in a whole range of tested concentrations. None of the investigated compounds altered the morphology of the examined fungi.

Table 1. A comparision of the fungistatic activity of: a) 1-amino-4-phenylnaphthalene-2-carbonitrile (1); b) 1-amino-4-methylnaphthalene-2-carbonitrile; c) the commercial fungicide – Kaptan 50 WP.

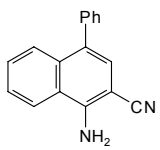
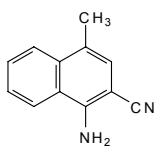
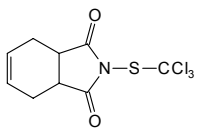
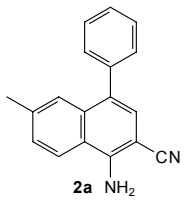
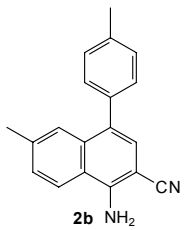
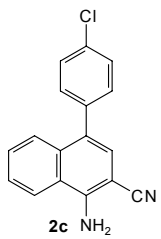
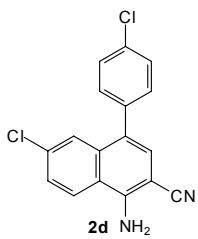
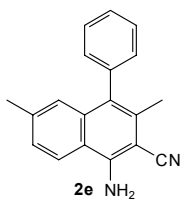
		EC_{50} [mg/dm^3]			
		Fusarium culmorum	***Alternaria brassicicola***	***Botrytis cinerea***	***Penicillium expansum***
a)		93	30	11	20
b)		3.4	25	1.6	8.6
c)		743	11	18	45

Table 2. The percentage inhibition or stimulation of fungi linear growth caused by the nitriles 2 and 3.

Compound	Concentration [mg/dm³]	Linear growth inhibition (–) or stimulation (+) [%] a)					
		Fusarium culmorum		*Alternaria alternata*		*Alternaria brassicicola*	
 **2a**	40	– 14.2	ef	– 5.8	e	—	—
	4	– 18.0	e	– 4.4	e	—	—
	0.4	– 10.0	f	+ 2.6	f	—	—
 **2b**	40	+ 44.4	n	—	—	+ 33.4	jk
	4	+ 42.6	mn	—	—	+28.8	j
	0.4	+ 32.2	jk	—	—	+ 28.4	j
 **2c**	40	+ 24.0	i	—	—	+ 22.4	hi
	4	+ 34.4	kl	—	—	+ 21.6	hi
	0.4	+ 44.6	n	—	—	+ 31.6	jk
 **2d**	40	+ 54.6	o	—	—	+ 30.4	j
	4	+ 53.2	o	—	—	+ 29.6	j
	0.4	+ 51.2	o	—	—	+ 40.2	l
 **2e**	40	– 56.6	a	– 37.6	b	—	—
	4	– 51.4	b	– 35.8	bc	—	—
	0.4	– 12.2	f	– 8.0	de	—	—

a) In each column mean values marked by the same letter do not differ significantly according to Duncan's test ($\alpha = 0.05$).

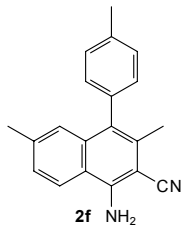
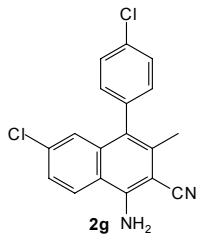
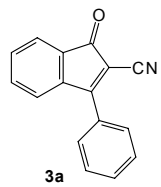
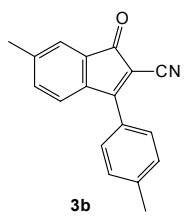
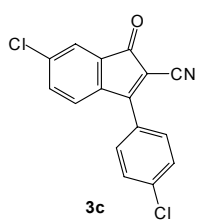
 **2f**	40	+ 3.8	g	—	—	– 13.1	d
	4	+ 28.8	ij	—	—	+ 22.8	i
	0.4	+ 15.8	h	—	—	+ 30.6	j
 **2g**	40	– 18.8	e	—	—	– 26.9	b
	4	– 14.4	ef	—	—	– 9.1	de
	0.4	+ 61.4	p	—	—	+ 36.8	kl
 **3a**	40	– 35.1	d	—	—	– 40.7	a
	4	+ 7.2	g	—	—	– 19.2	c
	0.4	+ 38.0	lm	—	—	+ 11.4	g
 **3b**	40	– 48.4	b	– 43.8	a	—	—
	4	– 42.6	c	– 30.4	c	—	—
	0.4	– 9.6	f	– 12.2	d	—	—
 **3c**	40	+ 4.8	g	—	—	– 5.0	e
	4	+ 4.6	g	—	—	+ 4.4	f
	0.4	+ 42.0	mn	—	—	+ 16.8	gh
SD comp. (A)		1.513		1.544		1.589	
SD conc. (B)		0.828		1.544		1.040	
SD (AxB)		2.620		2.675		2.753	

Table 3. Effective concentration (EC_{50}) values calculated for nitriles 2e and 3b.

Compound	EC_{50} [mg/dm^3]	
	Fusarium culmorum	*Alternaria alternata*
2e	**12.2 (R = 0.907)**	**75.6 (R = 0.885)**
3b	**25.7 (R = 0.914)**	**65.1 (R = 0.986)**

The presence of phenyl group or both nitrile and amino (or carbonyl) groups in the neighbouring positions of naphthalene or indene systems seemed to have the biggest influence on the activity of tested carbonitriles. Inserting of chlorine or methyl substituents diversify the fungistatic properties, but similarly to our earlier report (Wilamowski et al. 2001), no explicit relation between the structure of the investigated compounds and their biological activity were found. Even a small change in substitution pattern of 1-amino-4-phenylnaphthalene-2-carbonitrile reversed the biological effect, as in the case of **2e** and **2f**. These observations fully justify the present preliminary studies and further investigation of other analogues of 1-amino-4-phenylnaphthalene-2-carbonitrile **1** and 3-phenyl-1-oxoindene-2-carbonitrile **3a**. In case of the most active compounds, also studies on greater variety of fungi seem to be on purpose.

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