Appl Microbiol Biotechnol (2015) 99:1081–1096
DOI 10.1007/s00253-014-6295-1

MINI-REVIEW

Fungal naphtho-γ-pyrones—secondary metabolites of industrial interest

Elodie Choque · Youssef El Rayess · José Raynal · Florence Mathieu

Received: 8 October 2014 / Revised: 2 December 2014 / Accepted: 4 December 2014 / Published online: 19 December 2014

Abstract Naphtho-γ-pyrones (NGPs) are secondary metabolites mainly produced by filamentous fungi (*Fusarium* sp., *Aspergillus* sp.) that should be considered by industrials. Indeed, these natural biomolecules show various biological activities: anti-oxidant, anti-microbial, anti-cancer, anti-HIV, anti-hyperuricuric, anti-tubercular, or mammalian triacylglycerol synthesis inhibition which could be useful for pharmaceutical, cosmetic, and/or food industries. In this review, we draw an overview on the interest in studying fungal NGPs by presenting their biological activities and their potential values for industrials, their biochemical properties, and what is currently known on their biosynthetic pathway. Finally, we will present what remains to be discovered about NGPs.

Keywords Naphtho-γ-pyrones · Filamentous fungi · Aurasperone · Rubrofusarin · Anti-oxidant

Introduction

Because of their negative perception of chemicals, some consumers presently claimed and were ready to pay significant premiums for natural products (Young 1998; Sebranek and Bacus 2007; Karre et al. 2013; Aneja et al. 2014). In order to satisfy this demand, manufacturers are constantly seeking new natural molecules with helpful properties that could replace or diminish synthetically produced chemicals in their products. In this search for new natural compounds, secondary metabolites produced by plant, fungi, or bacteria are an important potential resource due to their diversity and their various biological activities. For example, toxins such as aflatoxins, ochratoxins, or fumonisins have a detrimental effect on humans and animals (Nielsen et al. 2009; Kew 2013; Wu et al. 2014). On the other hand, other secondary metabolites are beneficial and can be used as food additives, pigments, antibiotics, anti-oxidant, or anti-cancer agents (Archer et al. 2008; Nielsen et al. 2009; Chang et al. 2011; Lee and Pan 2012).

Among the polyketide secondary metabolites prospected, naphtho-γ-pyrones (NGPs) are widespread in nature; hence, they are produced not only by a wide variety of filamentous fungi but also by lichen (Ernst-Russell et al. 2000), higher plants (Li et al. 2001; Graham et al. 2004; Lee et al. 2006), and echinoderms (Bokesch et al. 2010, Chovolou et al. 2011). These natural compounds present a broad range of biological activities such as anti-oxidant, anti-microbial, or anti-tumor (Koyama et al. 1988; Zhang et al. 2007; Barrow and McCulloch 2009; Nielsen et al. 2009; Lu et al. 2014). As an example of application of NGPs in industry, it was demonstrated that an *Aspergillus niger* extract containing NGPs protected lard from being oxidized (Zaika and Smith 1975). Besides, a patent was recently filed for the preparation of a herbal extract of *Cassia tora* leaves for treating anxiety disorders (WO 2010109318 A1). Relying on previous works describing hepatoprotective and anti-mutagenic properties of NGPs extracted from *Cassia tora*, authors of the patent suggest that NGPs extracted from the leaves of *Cassia tora* could be one of the active compounds for treatment against anxiety (Wong et al. 1989; Choi et al. 1997).

There is few data available in the literature regarding the biological role of NGPs in their producing organisms. YWA1, a monomer NGP, is described to be a precursor of the black

Elodie Choque and Youssef El Rayess contributed equally to this work.

E. Choque · J. Raynal · F. Mathieu (✉)
Laboratoire de Génie Chimique, UMR 5503 CNRS/INPT/UPS, Université de Toulouse, INP-ENSAT, 1, avenue de l'Agrobiopôle, 31326 Castanet-Tolosan, France
e-mail: mathieu@ensat.fr

Y. El Rayess
Faculté des Sciences Agronomiques et Alimentaires, Université Saint-Esprit de Kaslik, B.P. 446, Jounieh, Lebanon

pigment dihydroxynaphthalene (DHN)-melanin in *Aspergillus* sp. (Watanabe et al. 1998; Tsai et al. 1998) and of the red pigment aurofusarin in *Fusarium* sp. (Malz et al. 2005; Frandsen et al. 2006). Rubrofusarin, another monomer NGP, was also linked to aurofusarin synthesis in *Fusarium* sp. (Frandsen et al. 2006). Besides, recent results suggest that NGPs could be involved in virulence of producing fungal strains. Indeed, Slesiona and colleagues (2012) showed that YWA1 was sufficient to allow *Aspergillus fumigatus* escape from macrophages by inhibition of phagolysosomes acidification during the first step of infection in invasive bronchopulmonary aspergillosis (Langfelder et al. 1998; Tsai et al. 1998; Thywißen et al. 2011; Slesiona et al. 2012; reviewed in Heinekamp et al. 2012). Going further, Slesiona and colleagues (2012) conditionally expressed *wA* gene, required for YWA1 production, in *Aspergillus terreus*, a strain that does not produced this precursor. This conditional expression led to the production of YWA1 and was sufficient to prevent acidification of phagolysosomes (Slesiona et al. 2012). This result emphasizes the role of NGPs in the interaction of *Aspergillus* sp. with macrophages during the infection process. Some of the remaining questions are for example: are other NGPs linked to fungal pigmentation and/or virulence? Are they involved in other biological processes?

Unlike higher plants, filamentous fungi, such as *A. niger*, are frequently used in the fermentation industry for the production of primary and secondary metabolites like organic acids (Alvarez-Vasquez et al. 2000; Roukas 2000) and enzymes (Maldonado and Strasser de Saad 1998) because they are easy to handle and allow the massive production of interesting compounds. As NGPs represent the most abundant family of secondary metabolites in *A. niger* (Nielsen et al. 2009), it makes this filamentous fungus a good candidate for the production of these molecules. The aim of this review is to provide an overview of the production of NGPs by filamentous fungi. We will present their industrial potential, then their biochemical properties. Even though, several drawbacks, which will be presented in this review, now prevent their massive production. Describing what is known on the biosynthetic pathway of NGPs and what remains to be known will help us in improving the industrial process to produce/extract these potentially interesting molecules.

Biological activities of fungal NGPs and their potential industrial applications

The biological effects or activities of NGPs produced by different filamentous fungi have been partially studied. For example, the biological activities of some NGPs have already been reviewed showing anti-viral, anti-microbial, insecticidal, and anti-estrogenic activities (Barrow and McCulloch 2009; Lu et al. 2014). In the search of natural compounds with attractive biological properties, NGPs produced by filamentous fungi were identified within two opposite strategies: (i) screening for a specific activity and elucidation of responsible compounds (Singh et al. 2003; Song et al. 2004; Shaaban et al. 2012; Kong et al. 2013) and (ii) biochemical discovery of new family members and testing of their putative anti-microbial properties (Ehrlich et al. 1984; Akiyama et al. 2003; Bouras et al. 2005). An exhaustive list of NGPs produced by filamentous fungi is presented in Table 1, including producer organisms and their described biological properties like anti-HIV, anti-tubercular, anti-hyperuricemia, anti-microbial, anti-tumor, and anti-oxidant, as many biological activities could be valued in pharmaceutical, cosmetic, and food industry.

It should be noticed that NGPs were first described as toxical compounds (Ghosal et al. 1979). We would like to discuss this point before going further into the description of beneficial biological activities of NGPs that could be valued by industrials. In late 1970s, Ghosal and collaborators showed that intraperitoneal injection of 50 mg/kg of mixture containing NGPs, extracted from *A. niger* van Teigh cultured on mango pulp, caused albino mice and rats death through central nervous system (CNS) depression (Ghosal et al. 1979). Conversely, another study showed that oral ingestion by rats of 0.1 % of their daily ration, of a methanol extract from *A. niger* ATCC 9029 containing NGPs, during 60 days did not seem to cause an acute toxicity for animals but show a hepatoprotective effect (Rabache and Adrian 1982). The differences between these studies resided in the fungal strain from which the NGPs were extracted, the organic solvents used for the extraction, the quantity, and the way it was administered. Besides, considering the way that NGPs were extracted before administration, it could not be excluded that mycotoxins should have been present in the mixture but biochemically undetectable. Indeed, other experimental studies show that NGP extracts could be toxic when intraperitoneally injected, even though, they do not exclude the putative presence of highly toxic bioactive malformin compounds in the extract (DeLucca et al. 1983; Ehrlich et al. 1984). Through bioactive fractionation, Zhan et al. (2007) show that cytotoxicity of an organic extract of *Aspergillus tubingensis* was due to malformin A1 and not to any of the ten NGPs present in the extract (asperpyrone D, TMC256A1, rubrofusarin B, fonsecin B, fonsecin, fonsecinone A, asperpyrone A, aurasperone A, dianhydro-aurasperone C, aurasperone E) (Zhan et al. 2007). None of these ten NGPs showed toxicity on different types of cancer cell line or normal human fibroblast when tested on cell culture to a concentration of 5 μg/ml (Zhan et al. 2007). It seems that NGPs are not toxic when a small dose is daily orally ingested (Rabache and Adrian 1982). Even though, chosen extracts will require toxicological assessments (dermatological, pharmaceutical, etc.) before their use in pharmaceutical, cosmetic, or food industry.

Table 1 Listing of fungal NGPs, their producing strains, and their described biological properties

NGPs	Strains		Activities	References
Asperpyrone	Asperpyrone A	*A. KJ-9*; *A. niger*; *A. tubingensis*		Akiyama et al. (2003); Zhang et al. (2007); Zhan et al. (2007); Li et al. (2013); Xiao et al. (2014)
	Asperpyrone B	*A. niger*	Anti-microbial	Akiyama et al. (2003); Song et al. (2004)
	Asperpyrone C	*A. niger*	Anti-fungal	Akiyama et al. (2003); Zhang et al. (2007)
	Asperpyrone D	*A. tubingensis*; *A. niger*		Zhan et al. (2007); Li et al. (2013)
	Asperpyrone E	*A. niger*		Li et al. (2013)
Aurasperone	Aurasperone A	*A. alternata*; *A. niger*; *A. tubingensis*; *A. fonsecaeus*; *Aspergillus* sp. *FKI-3451*; *A. awamori*; *A. aculeatus*	Anti-microbial; xanthine oxidase inhibition	Tanaka et al. (1966); Wang and Tanaka (1966); Ghosal et al. (1979); Priestap (1984); Ehrlich et al. (1984); Akiyama et al. (2003); Song et al. (2004); Campos et al. (2005); Zhang et al. (2007); Zhan et al. (2007); Sakai et al. (2008); Shaaban et al. (2012)
	Isoaurasperone A	*A. KJ-9*; *A. niger*		Ghosal et al. (1979); Ehrlich et al. (1984); Li et al. (2013); Xiao et al. (2014)
	Aurasperone B	*A. alternata*; *A. niger*; *A. fonsecaeus*; *A. awamori*; *A. vadensis*	Anti-fungal; anti-oxidant	Tanaka et al. (1966); Tanaka et al. (1972); Priestap (1984); Ehrlich et al. (1984); Bouras et al. (2005); De Vries et al. (2005); Zhang et al. (2007); Shaaban et al. (2012)
	Aurasperone C	*A. alternata*; *A niger*; *A. awamori*		Tanaka et al. (1966); Tanaka et al. (1972); Ehrlich et al. (1984); Bouras et al. (2005); Shaaban et al. (2012)
	Dianhydro-aurasperone C	*A. KJ-9*; *A. niger*; *A. M39*; *A. tubingensis*	Reverse multidrug resistance on human KB cells	Ikeda et al. (1990); Zhang et al. (2007); Zhan et al. (2007); Li et al. (2013); Xiao et al. (2014);
	Aurasperone D	*A. niger*		Ghosal et al. (1979); Ehrlich et al. (1984); Bouras et al. (2005); Li et al. (2013)
	Aurasperone E	*A. niger*; *A. tubingensis*		Ehrlich et al. (1984); Bouras et al. (2005); Zhan et al. (2007)
	Aurasperone F	*A. alternata*; *A. niger*		Ghosal et al. (1979); Bouras et al. (2005); Shaaban et al. (2012)
	Isoaurasperone F	*A. niger*		Li et al. (2013)
	Aurasperone G	*A. niger*		Bouras et al. (2007)
Cephalochromin	Cephalochromin	*C. vilior*; *F508*; *Cephalosporium* sp.; *SCF-125*; *Nectria* sp.; *Pseudoanguillospora* sp.; *Verticillium* sp. *K-113*	Anti-proliferative effect cancer cell; anti-bacterial; nitric oxide production inhibition; calmodulin-sensitive phosphodiesterase inhibition;	Haskins and Knapp (1969); Carey and Nair (1975); Matsumoto et al. (1975); Koyama et al. (1988); Hegde et al. (1993); Ishii et al. (2005); Zheng et al. (2007); Kock et al.

Table 1 (continued)

NGPs	Strains		Activities	References
			botulinum neurotoxin serotype A inhibitor	(2009); Cardellina et al. (2012); Hsiao et al. (2014)
	Cephalochromin 5-methyl ether	*Cephalosporium* sp.		Ishii et al. (2005)
	Cephalochromin A 5,5′-dimethyl ether	*Cephalosporium* sp.		Ishii et al. (2005)
Chaetochromin	Chaetochromin A	*Fusarium* sp.; *Chaetomium* sp.	Nitric oxide production inhibition; botulinum neurotoxin serotype A inhibitor; anti-diabetes	Sekita et al. (1980); Koyama et al. (1988); Singh et al. (2003); Ishii et al. (2005); Paranagama et al. (2007); Cardellina et al. (2012); Xu et al. (2014); Qiang et al. (2014)
	Chaetochromin A 5,5′-dimethyl ether	*Chaetomium* sp.		Ishii et al. (2005)
	Isochaetochromin A_1	*Penicillium* sp. *FKI-4942*	Inhibition of triacylglycerol synthesis in mammalian cells	Ugaki et al. (2012)
	Isochaetochromin A_2	*Chaetomium microcephalum*		Xu et al. (2014)
	Chaetochromin B	*Chaetomium* sp.; *Fusarium* sp.		Koyama et al. (1988); Singh et al. (2003); Xu et al. (2014)
	Isochaetochromin B	*Fusarium* sp.	HIV-1 integrase inhibition	Singh et al. (2003)
	Isochaetochromin B_1	*Penicillium* sp. *FKI-4942*; *Fusarium* sp.	Inhibition of triacylglycerol synthesis in mammalian cells HIV-1 integrase inhibition	Singh et al. (2003); Ugaki et al. (2012)
	Isochaetochromin B_2	*Penicillium* sp. *FKI-4942*; *M. anisopliae*; *Fusarium* sp.	Inhibition of triacylglycerol synthesis in mammalian cells; anti-tubercular; HIV-1 integrase inhibition	Singh et al. (2003); Ugaki et al. (2012); Kong et al. (2013)
	Chaetochromin C	*Fusarium* sp.		Koyama et al. (1988)
	Chaetochromin D	*Fusarium* sp.		Koyama et al. (1988); Singh et al. (2003)
	Isochaetochromin D_1	*Fusarium* sp.	HIV-1 integrase inhibitory activity	Singh et al. (2003)
Flavasperone	Flavasperone	*A. niger* sp.; *A. carbonarius*; *A. fonsecaeus*		Lund et al. (1953); Bycroft et al. (1962); Ghosal et al. (1979); Ehrlich et al. (1984); Zhang et al. (2008)
Fonsecin	Fonsecin	*A. alternata*; *A. carbonarius*; *A. niger*; *A. tubingensis*; *A. fonsecaeus*	Anti-mycobacterial	Galmarini et al. (1962); Ehrlich et al. (1984); Bouras et al. (2005); Zhan et al. (2007); Zhang et al. (2008); Shaaban et al. (2012)
	Fonsecin B	*A. alternata*; *A. tubingensis*		Zhan et al. (2007); Lee et al. (2010); Shaaban et al. (2012)
	10,10′-Bifonsecin B	*A. carbonarius*		Zhang et al. (2008)
	Fonsecin monomethyl ether	*A. niger*		Ehrlich et al. (1984)
Fonsecinone	Fonsecinone A	*A. KJ-9*; *A. niger*; *A. tubingensis*; *A. fonsecaeus*; *Aspergillus aculeatus*;	Anti-microbial	Priestap (1984); Akiyama et al. (2003); Song et al. (2004); Campos et al.

Table 1 (continued)

NGPs	Strains		Activities	References
		Cladosporium herbarum		(2005);Ye et al. (2005); Zhang et al. (2007); Zhan et al. (2007); Chiang et al. (2011);Xiao et al. (2014)
	Fonsecinone B	*A. niger*; *A. fonsecaeus*	Anti-oxidant	Priestap (1984); Zhang et al. (2007)
	Fonsecinone C	*A. niger*; *A. fonsecaeus*		Priestap (1984); Zhang et al. (2007)
	Fonsecinone D	*A. niger*; *A. fonsecaeus*	Anti-oxidant	Priestap (1984; Zhang et al. (2007)
Hypochromin	Hypochromin A	*H. vinosa*	Inhibitory effects on tyrosinase, proliferation, migration, and tubule formation	Ohkawa et al. (2010)
	Hypochromin B	*H. vinosa*	Inhibitory effects on tyrosinase, proliferation, migration, and tubule formation	Ohkawa et al. (2010)
	SC2051	*H. vinosa*	Inhibitory effects on tyrosinase, proliferation, migration, and tubule formation	Ohkawa et al. (2010)
Indigotides	Indigotide B	*C. indigotica*; *M. anisopliae*		Asai et al. (2012); Kong et al. (2013)
	Indigotide G	*M. anisopliae*		Kong et al. (2013)
	Indigotide H	*M. anisopliae*		Kong et al. (2013)
Nigerasperone	Nigerasperone A	*A. niger*		Zhang et al. (2007)
	Nigerasperone B	*A. niger*		Zhang et al. (2007)
	Nigerasperone C	*A. niger*	Anti-fungal; anti-oxidant	Zhang et al. (2007)
Nigerone	Nigerone	*A. niger*; *A. carbonarius*		Ehrlich et al. (1984); Divirgilio et al. (2007); Zhang et al. (2008)
	Nigerone methyl ether	*A. niger*		Ehrlich et al. (1984)
	Isonigerone	*A. carbonarius*		Zhang et al. (2008)
	6′-*O*-demethylnigerone	*A. carbonarius*		Zhang et al. (2008)
	8′-*O*-demethylisonigerone	*A. carbonarius*	Anti-microbial	Zhang et al. (2008); Lee et al. (2013)
	8′-*O*-demethylnigerone	*A. carbonarius*	Anti-microbial	Zhang et al. (2008); Lee et al. (2013)
Rubasperone	Rubasperone A	*A. tubingensis*		Huang et al. (2010)
	Rubasperone B	*A. tubingensis*		Huang et al. (2010)
	Rubasperone C	*A. tubingensis*	α-Glucosidase inhibition	Huang et al. (2010)
	Rubasperone D	*A. tubingensis*		Huang et al. (2011)
	Rubasperone E	*A. tubingensis*		Huang et al. (2011)
	Rubasperone F	*A. tubingensis*		Huang et al. (2011)
Rubrofusarin	Rubrofusarin	*A. niger*; *A. tubingensis*; *Fusarium* sp.	Tyrosinase inhibition	Ashley et al. (1937); Tanaka and Tamura (1961); Ghosal et al. (1979); Ehrlich

Table 1 (continued)

NGPs	Strains		Activities	References
				et al. (1984); Huang et al. (2010)
	Rubrofusarin B	*A. KJ-9*; *A. alternata*; *A. M39*; *A. niger*; *A. tubingensis*; *A. carbonarius*; *Cladosporium herbarum*	Reverse multidrug resistance on human KB cells; anti-microbial; anti-tumoral	Ikeda et al. (1990); Song et al. (2004); Ye et al. (2005); Zhan et al. (2007); Zhang et al. (2008); Huang et al. (2010); Shaaban et al. (2012); Xiao et al. (2014)
	Rubrofusarin-6-*O*-α-D-ribofuranoside	*A. niger*		Li et al. (2013)
	Rubrofusarin-6-*O*-β-(4-*O*-methylglucopyranoside)	*T. lutearostata*		Isaka et al. (2007)
TMC 256	TMC-256A1	*A. carbonarius*; *A. tubingensis*; *A. niger*	IL-4 signal transduction inhibition	Sakurai et al. (2002); Zhan et al. (2007); Zhang et al. (2008); Li et al. (2013)
	TMC-256B1	*A. niger*	IL-4 signal transduction inhibition	Sakurai et al. (2002)
	TMC-256C1	*A. niger*	IL-4 signal transduction inhibition	Sakurai et al. (2002)
Ustilaginoidin	Ustilaginoidin A	*Villosiclava virens*		Shibata et al. (1963); Koyama et al. (1988); Koyama and Natori (1988)
	Isoustilaginoidin A	*Verticillium* sp. *K-113*		Matsumoto et al. (1975)
	Dihydroisoustilaginoidin A	*A. butyri*; *Verticillium* sp. *K-113*	Nitric oxide production inhibitory activity	Matsumoto et al. (1975); Ishii et al. (2005)
	Ustilaginoidin B	*Villosiclava virens*		Shibata and Ogihara (1963)
	Ustilaginoidin C	*Villosiclava virens*		Shibata and Ogihara (1963)
	Ustilaginoidin D	*M. anisopliae*; *Fusarium* sp.	Anti-tubercular	Koyama et al. (1988); Singh et al. (2003); Kong et al. (2013)
	Ustilaginoidin E	*Fusarium* sp.		Koyama et al. (1988)
	Ustilaginoidin F	*Villosiclava virens*		Koyama et al. (1988)
	Ustilaginoidin G	*Villosiclava virens*		Koyama et al. (1988)
	Ustilaginoidin H	*Villosiclava virens*		Koyama et al. (1988)
	Ustilaginoidin I	*Villosiclava virens*		Koyama et al. (1988)
	Ustilaginoidin J	*Villosiclava virens*		Koyama et al. (1988)
YWA1	YWA1	*Aspergillus* sp.; *Fusarium* sp.		Fujii et al. (2001); Frandsen et al. (2011)

Going through medical consideration, some NGPs have been described for their anti-tubercular, anti-tumor, anti-HIV, and anti-hyperuricosuric effects (Singh et al. 2003; Song et al. 2004; Shaaban et al. 2012) (Table 1). For example, aurasperone A and rubrofusarin B, extracted from *A. niger* IFB-E003, show a xanthine oxidase inhibitory effect equivalent to the one of allopurinol, a commonly used anti-hyperuricosuric. These NGPs also show anti-tumor properties against a colon cancer cell line (Song et al. 2004). In the search of new and more powerful drug against tuberculosis, Shaaban et al. (2012) show that rubrofusarin B and aurasperone A, extracted from *Alternaria alternata* D2006, have high anti-microbial properties and show really weak toxicity at a 10 μg/ml concentration (Shaaban et al. 2012). Anti-tumor and anti-microbial activities of NGPs were recently detailed by Lu and collaborators (2014). Finally, considering anti-HIV drugs, four NGPs of the chaetochromin family, extracted from *Fusarium* sp., show good inhibitory properties against HIV-1 integrase and should be deeply studied for their therapeutic use (Singh et al. 2003).

Thanks to literature and personal unpublished data, it is possible to think that one of the most attractive properties of NGPs was their anti-oxidant capacity. First described by Rabache and colleagues (1982), the anti-oxidant activity of NGPs seems to have both a beneficial effect on rats growing and a hepatoprotective activity, probably due to a better intestinal absorption of retinol and tocopherol (Rabache et al. 1982). Xie et al. (2012) have recently shown that a previous animal treatment with Cassia seed ethanol extract (CSE) protected mice against CCl4-induced liver injury via an enhancement of the anti-oxidant capacity (Xie et al. 2012). It was previously demonstrated that CSE contained NGPs (Kitanaka et al. 1998; Li et al. 2001). Anti-oxidant properties of NGPs were also described for nigerasperone C, aurasperone B, fonsecinone B, and fonsecinone D, extracted from *A. niger* EN-13, which show an anti-oxidant capacity two times lower than the one of BHT (Zhang et al. 2007). Besides, experiments using 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) test on *A. niger* C-433, which did not produce ochratoxin A (Bouras et al. 2005; Bouras et al. 2007), show that the anti-oxidant power of an ethanol extract containing NGPs is equivalent to vitamin C (unpublished personal data). Unlike natural anti-oxidants currently used (vit. C, vit. D, vit. E, etc.) (Scotter and Castle 2004; Burke 2007; Stamford 2012), the anti-oxidant capacity of this ethanol extract is also stable over the time and resists to temperatures up to 60 °C (unpublished personal data). Study of the anti-oxidant properties of NGPs will allow having significant impacts in a variety of areas. As described above, the anti-oxidant activity of NGPs prevents lard oxidation (Zaika and Smith 1975), so NGPs could be a good substitute to actual conservators such as sulfur or synthetic anti-oxidants. In health and cosmetics industry, NGPs could be an ideal dietary supplement or formulate in cream as anti-skin-aging compounds (Ye et al. 2014).

Chemistry of fungal NGPs

Naphtho-γ-pyrones (NGPs) belong to the family of naphthopyrones. They are C13 (C6-C4-C3) basic skeleton molecules consisting of a naphthalene core and a pyrone core. Greek letters (α, β, γ) indicate the position of the oxygen atom relative to the carbonyl group on the pyrone core (Barrow and McCulloch 2009) (Fig. 1a). The naphthopyrone group comprises 18 isomeric forms. Only three can exist as NGPs. As shown in Fig. 1a, one isomeric form has the basic skeleton organized in a linear form where the remaining two forms have angular organization. Up to now, the NGP family (Table 1) is composed of various monomers, fonsecin, flavasperone, rubrofusarin, etc. (Fig. 2a), and dimers, asperpyrones, aurasperones, fonsecinones, nigerones, etc, whose diversity is presented in Fig. 2b. Dimers result from the association of two monomers, depending on their structure (linear and angular), the position of the chemical bond between monomers, and the decoration present on both naphthalene and pyrone cores (Ehrlich et al. 1984; Chiang et al. 2011). As mentioned in Fig. 2, some NGPs share the same structure, but they are conformational isomers. Conformation of these NGPs was recently reviewed by Lu et al. (2014).

The relationship between the structure of NGPs and their biological activities is poorly described in the literature. Concerning the anti-oxidant activity, it is possible to make a comparison with what it is described on naphtho-α-pyrones by Leitão et al. (2002). They suggest that the anti-oxidant capacity of naphthopyrones produced by *Paepalanthus* sp. is due to presence and number of free hydroxyl groups (Leitão et al. 2002). Unpublished personal data show that aurasperone C has a higher anti-oxidant capacity than aurasperone B and F (unpublished personal data). As shown in Fig. 2b, aurasperone C has five free hydroxyl groups while aurasperone B and F have only four hydroxyl groups. Besides, aurasperone F has a higher anti-oxidant activity than aurasperone B, suggesting that the position of the hydroxyl groups is important too.

Elucidation of NGP structures so far was mostly done using semi-preparative reverse-phase HPLC purification and mass spectrometry analysis (Campos et al. 2005; Bouras et al. 2005). It was shown that NGPs contain a fully conjugated system, giving rise to very characteristic UV/vis spectra, strongly absorbing between 200 and 450 nm (Fig. 1b). The absorption spectra mainly depend on the isomeric form and the polymerization degree (Nielsen et al. 2009) (Fig. 2b). As example, flavasperone (monomeric, angular) presents two peaks with equal maximum

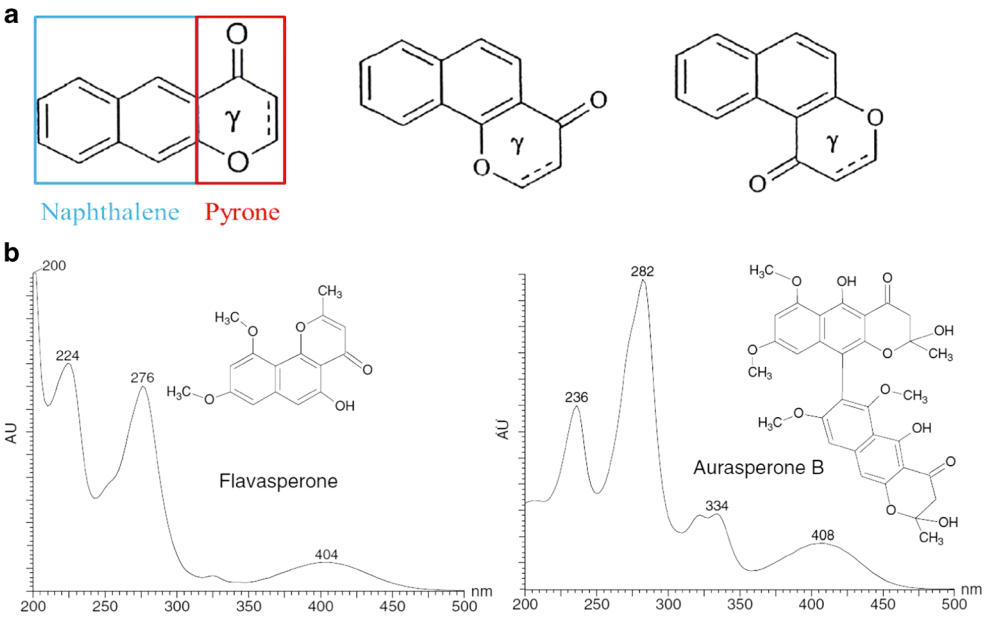


Fig. 1 Biochemical properties of NGPs. **a** Linear and angular forms of NGPs. Both present a naphthalene core and a pyrone core. **b** UV/vis spectra of flavasperone (monomer) and aurasperone B (dimer) adapted from Nielsen et al. (2009)

absorbance (224 nm, 276 nm) whereas aurasperone B (dimeric, linear) shows a maximum absorbance peak (282 nm) and three little peaks (236, 334, 408 nm) (Nielsen et al. 2009; for UV/vis spectra references, please refer to Table 1) (Fig. 1b). Unfortunately, despite of the possible discrimination of different NGPs using their specific absorption spectra, reliable standards are missing to precisely estimate the NGP concentration of purified extracts. Indeed, the only standard presently available is rubrofusarin, a NGP monomer linear, isolated from *Fusarium graminearum* (Adipogen, CAS 3567-00-8).

Their specific structure confers NGP hydrophobic characters allowing their extraction by several organic solvents, such as ethyl acetate (Xiao et al. 2014; Li et al. 2013; Zhang et al. 2007), acetone (Zhang et al. 2007), methanol (Akiyama et al. 2003; Bouras et al. 2005; Bouras et al. 2007), chloroform/methanol mix (Song et al. 2004), or methylene chloride (Ehrlich et al. 1984). Thanks to this hydrophobic property, it is also possible to consider their extraction by a nontoxic organic solvent as ethanol, as it is already done in higher plants (Kitanaka et al. 1998; Li et al. 2001; Xie et al. 2012). Even though, the issue remains the purity of the extract because one-step extraction with any organic solvent leads to a crude extract containing all hydrophobic compounds present in matrices. Right now, purification process for isolation and structure elucidation of NGPs is composed of numerous steps of drying, elution, and chromatography (Akiyama et al. 2003; Li et al. 2013; Xiao et al. 2014). However, purity of these molecules is not a prerequisite for their potential industrialization, as ethanol extracts are already used (Xie et al. 2012, WO 2010109318 A1). Considering their attractive biological properties, it is interesting to better understand their biosynthetic pathway in order to optimize their production.

The poorly known biosynthetic pathway of NGPs

In the purpose of improving NGP production/extraction process, a strong knowledge of their biosynthetic pathway is a relevant fact. Unfortunately, only few data are available in the literature. The first step of NGP biosynthetic pathway elucidation was done by Watanabe and colleagues (1998) (Watanabe et al. 1998; Watanabe et al. 1999). Studying conidial pigmentation in *Aspergillus nidulans* (*A. nidulans*), they showed that *wA* gene encodes a polyketide synthase responsible for the formation of the linear monomer NGP, YWA1, required for the formation of the dark green conidial pigment (Watanabe et al. 1998). Indeed, *A. nidulans* strain lacking *wA* gene produces white conidia. It was lately shown that the C-terminal part of *wA* was required for the naphthopyrone synthesis (Fujii et al. 2001). Indeed, *A. nidulans* strain expressing a C-term-modified *wA* gene produces a heptaketide isocoumarin instead of YWA1. Authors assume that the C-term part of *wA*, and particularly a Claisen cyclase domain, is involved in the cyclization of the second aromatic ring of YWA1 (Fujii et al. 2001).

At the same time, Tsai and colleagues (1998), working on conidial morphology and virulence of *A. fumigatus*, characterize *alb1* gene, *A. nidulans wA* ortholog, putatively encoding the pentakide tetrahydroxynaphthalene (THN) (Tsai et al. 1998). THN is a precursor in the DHN-melanin, a well-known virulence factor of both plant and human (Langfelder et al. 2003; Heinekamp et al. 2012). *alb1* was then overexpressed in *Aspergillus oryzae*, which does not possess an orthologous gene (Watanabe et al. 2000). This overexpression leads to the production of the heptaketide naphthopyrone YWA1 instead of THN (Watanabe et al. 2000). These results suggest that *alb1* encodes a naphthopyrone synthase instead

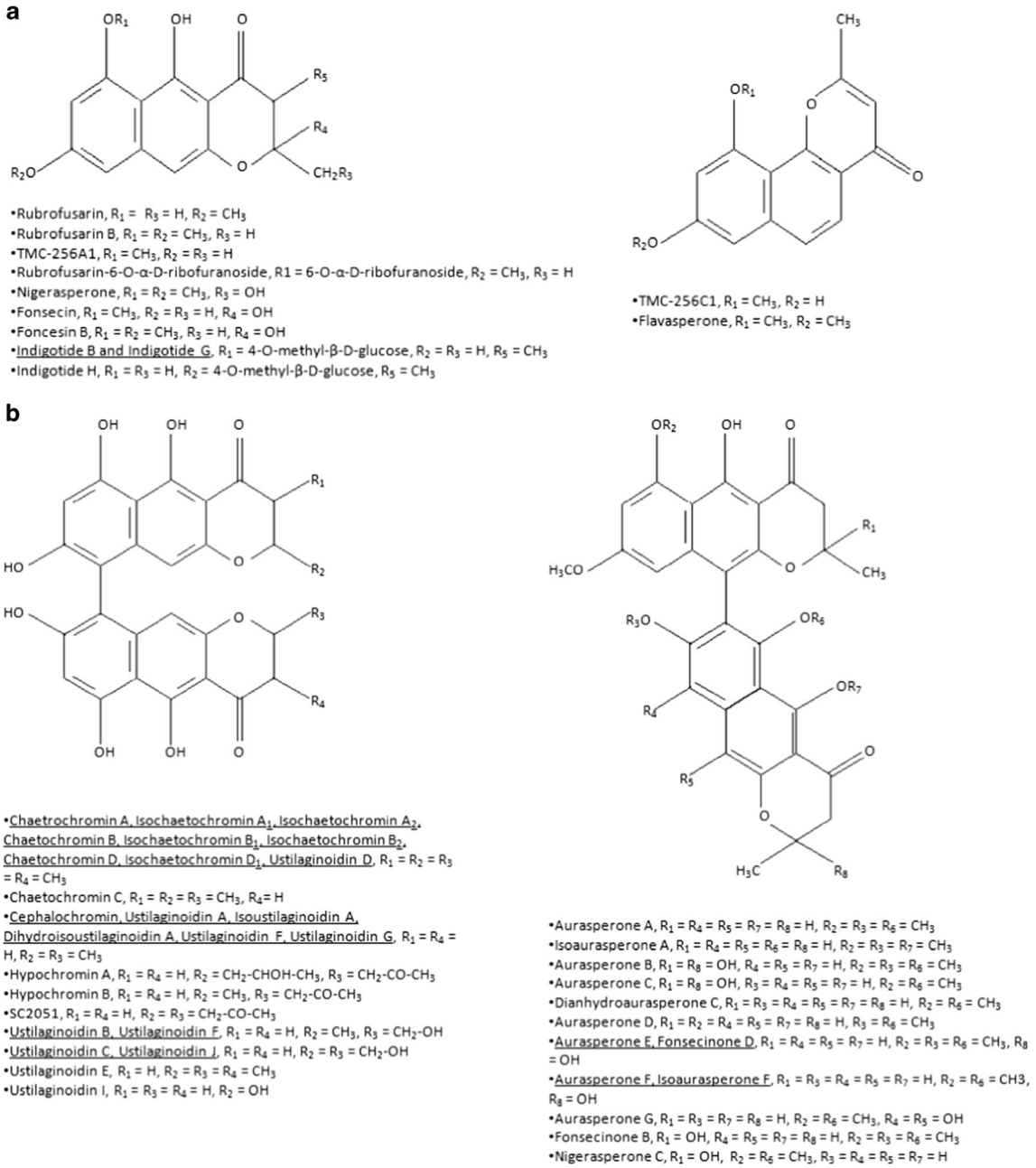
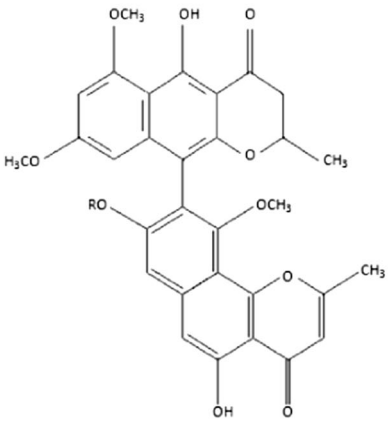


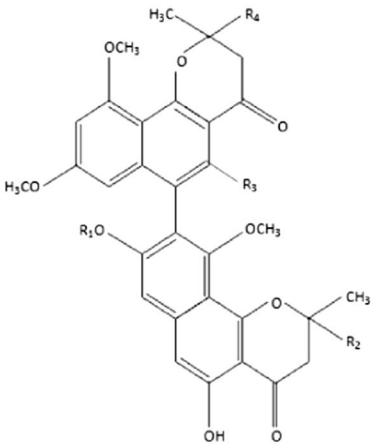
Fig. 2 Structural formula of the presented naphtho-γ-pyrones. **a** NGP monomers. **b** NGP dimers. *Underlined NGPs names* with the same structural formula represent stereoisomers

of a THN synthase. Besides, it was then described that the esterase-like enzyme Ayg1 allows the formation of THN from YWA1, in *A. fumigatus* (Fujii et al. 2004). Interestingly, in *Colletotrichum lagenarium*, it was shown that synthesis of the THN pentaketide only required the presence of the polyketide synthase PKS1 (Watanabe and Ebizuka 2004). Unlike *Aspergillus* sp., NGP production is not described in *Colletotrichum* sp. So, in *Aspergillus* sp., the presence of this additional first step, leading to the formation of YWA1, suggests that this monomer NGP could be a common precursor for both DHN-melanin and NGPs.

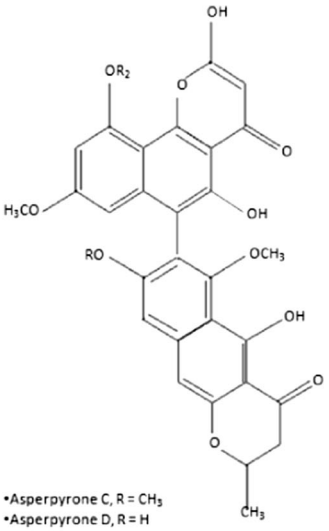
Considering NGP biosynthesis in *Aspergillus* sp., two simultaneous studies show that *albA*, *alb1* ortholog in *A. niger*, was responsible of YWA1 synthesis which seems to be a precursor of both DHN-melanin and NGPs produced by this filamentous fungus (Jørgensen et al. 2011b; Chiang et al. 2011) (Fig. 3). Jørgensen et al. (2011b) tried to elucidate black pigmentation biosynthetic pathway in *A. niger* N402, using UV mutagenesis to select spore color mutants. Thus, they described three different spore color mutants: fawn, olive, and brown. Using a complementation method, they showed that a specific gene required for DHN-melanin biosynthesis



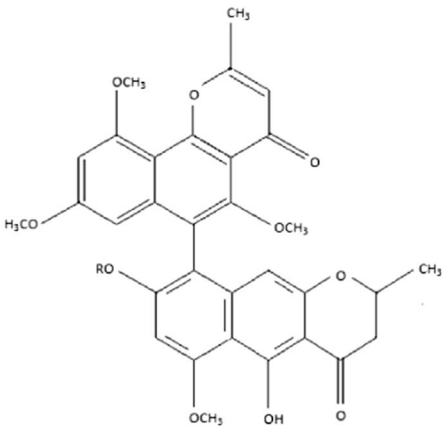
•Asperpyrone A, R = H
•Fonsecinone A, R = CH_3



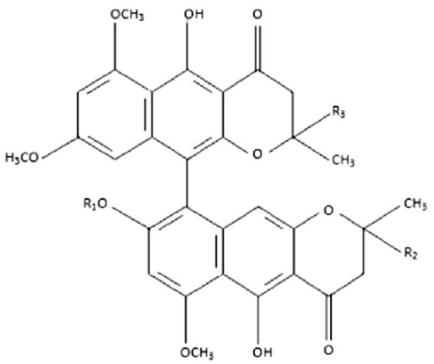
•Asperpyrone B, R_1 = CH_3, R_2 = R_4 = H, R_3 = OH
•Fonsecinone A, R_1 = R_2 = R_4 = H, R_3 = OH
•Nigerasperone B, R_1 = CH_3, R_2 = R_3 = R_4 = OH



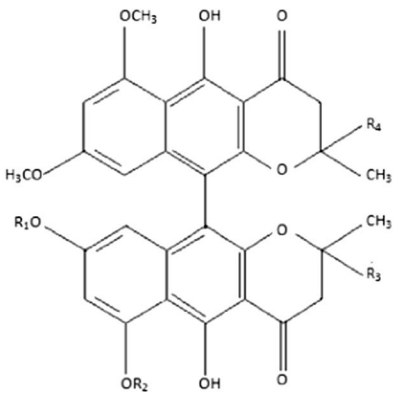
•Asperpyrone C, R = CH_3
•Asperpyrone D, R = H



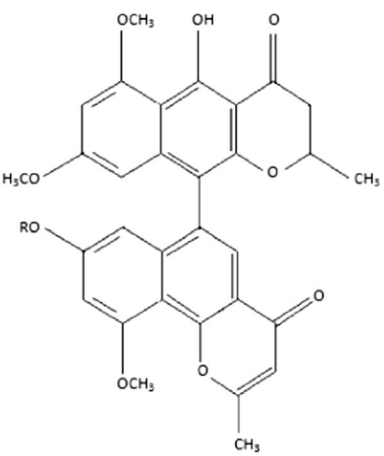
•Rubasperone C, R = CH_3
•Rubasperone D, R = H



•Rubasperone A, R_1 = R_2 = R_3 = H
•Rubasperone B, R_1 = CH_3, R_2 = R_3 = H
•Rubasperone E, R_1 = CH_3, R_2 = H, R_3 = OH
•Rubasperone F, R_1 = CH_3, R_2 = OH, R_3 = H



•10,10′-Bifonsecin B, R_1 = R_2 = CH_3, R_3 = R_4 = OH
•Nigerone, R_1 = R_2 = CH_3, R_3 = R_4 = H
•6′-O-Demethylnigerone, R_1 = CH_3, R_2 = R_3 = R_4 = H
•8′-O-Demethylnigerone, R_2 = CH_3, R_1 = R_3 = R_4 = H



•8′-O-Demethylisonigerone, R = H
•Isonigerone, R = CH_3

Fig. 2 (continued)

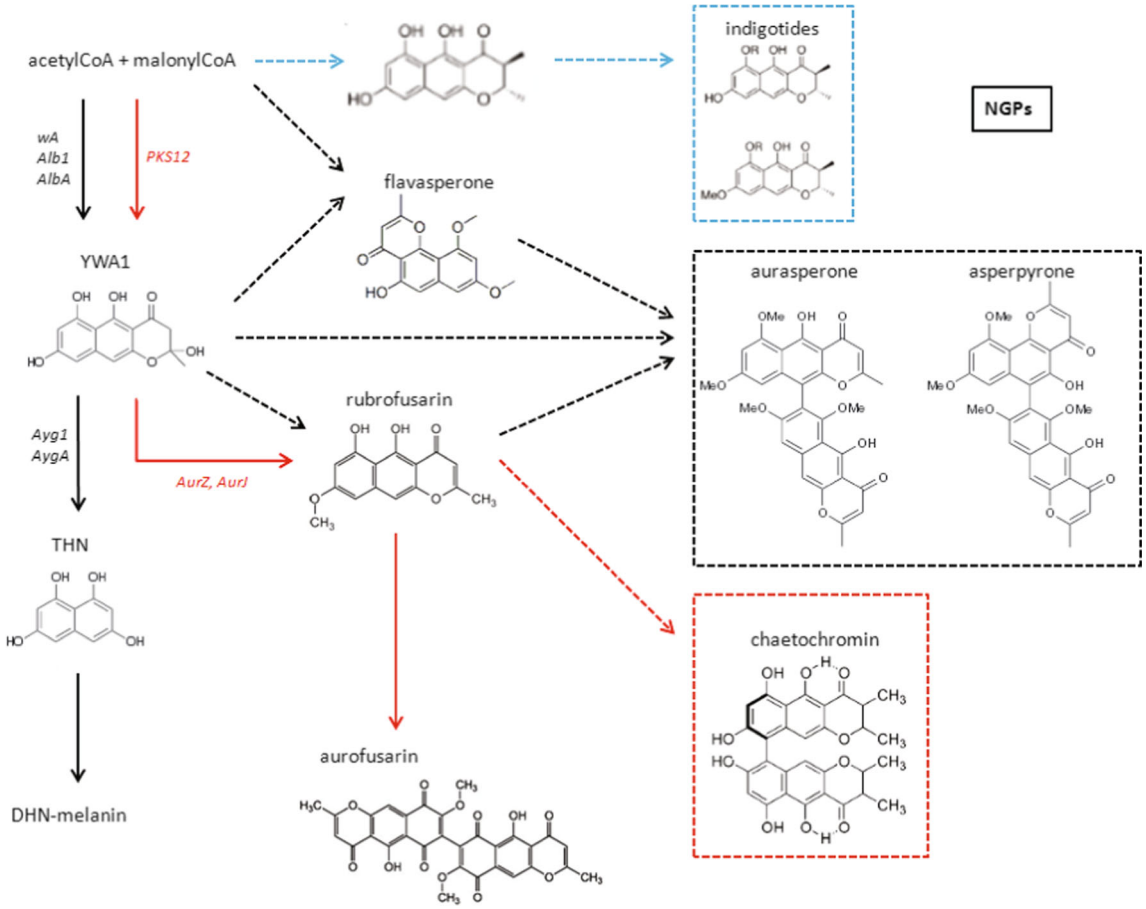


Fig. 3 Biosynthesis pathways of NGPs. *Black arrows* represent common biosynthesis pathways of DHN-melanin and NGPs in *Aspergillus* sp., *red arrows* represent putative common biosynthesis pathways of aurofusarin and NGPs in *Fusarium* sp., and *blue arrows* represent NGP biosynthetic pathway in *Cordyceps indigotica*. When *arrows are represented with a full line*, the biosynthetic pathway is known whereas when *arrows are represented with dotted line*, the biosynthetic pathway has to be discovered

was mutated in each color phenotype. Mutation on *albA* was responsible for the "fawn" phenotype while mutations on *aygA* and *abrA* (ortholog of *A. fumigatus ayg1* and *abr1*) gave olive and brown phenotypes, respectively. Interestingly, they also showed that in addition to a light color indicating a defect in pigment synthesis, fawn mutants were not able to produce NGPs, suggesting that AlbA is also involved in the biosynthetic pathway of NGPs (Jørgensen et al. 2011b). Olive and brown mutants were still able to produce some NGPs, suggesting that AygA and AbrA are not involved in NGP biosynthetic pathway (Jørgensen et al. 2011b). Those results were confirmed by Chiang and collaborators (2011) who deleted *albA* and *aygA* in *A. niger* ATCC1015 in order to determinate cryptic secondary metabolites by suppressing NGP production (Chiang et al. 2011). However, compared to the work done by Jørgensen et al. (2011a, b), fungal phenotypes of mutants are different. Various hypotheses can be made to explain those differences: genetic background of each strain, culture medium and growth conditions, hazardous mutation versus deletion, or the major point of DNA repair mechanism disruption used by Chiang et al. (2011) to facilitate *A. niger* transformation and which can lead to huge genetic variation. Indeed, it may be seriously considered that disruption of DNA repair machinery could let appeared spontaneous mutations emphasizing the colorless phenotype and the lack of NGPs.

The precursor YWA1 was also described in the biosynthetic pathway of the homodimeric naphthoquinone aurofusarin, a red color pigment produced by *Fusarium* sp. (Fig. 3) (Malz et al. 2005; Frandsen et al. 2006; Frandsen et al. 2011). Identification of the gene cluster responsible for the synthesis of aurofusarin indicates that nor-rubrofusarin and rubrofusarin, two NGP monomers, were precursors of this red pigment (Frandsen et al. 2006). It was first admitted that *F. graminearum* PKS12 was the polyketide synthase responsible of nor-rubrofusarin production as an intermediate to aurofusarin (Malz et al. 2005). Determination of AurZ and AurJ functions allows demonstrating that rubrofusarin synthesis passes through the production of YWA1 by PKS12 (Frandsen et al. 2011) (Fig. 3). As described above for *wA* gene in *A. nidulans*, it was shown that a Claisen cyclase domain in the C-terminal part of PKS12 was required for the formation of YWA1 (Sørensen et al. 2012). Disruption of this

PKS12 domain leads to the formation of a lactone, citreoisocoumarin, instead of YWA1. This result suggests the presence of a conserved regulatory mechanism in the formation of YWA1. Then, AurZ is responsible for the production of nor-rubrofusarin from YWA1, and AurJ was required next for the production of rubrofusarin (Frandsen et al. 2011). Rugbjerg et al. (2013) showed that PKS12, AurZ, and AurJ were necessary and sufficient for the production of rubrofusarin. Indeed, heterologous expression of these three proteins in *Saccharomyces cerevisiae* allows weak production of rubrofusarin by the yeast (Rugbjerg et al. 2013). Interestingly, some NGP dimers, such as chaetochromin or ustilaginoidin, are also produced by *Fusarium* sp. (Singh et al. 2003). However, any study describes a link between aurofusarin biosynthetic pathway and the one of chaetochromin or ustilaginoidin. Questions remaining are does YWA1 and/or rubrofusarin are precursors for NGP biosynthesis in *Fusarium* sp. and is rubrofusarin the second key step, after YWA1, in the general biosynthetic pathway of NGPs (Fig. 3). Besides, it has to be noticed that AurZ is the first representative of a novel class of dehydratases that act on hydroxylated γ-pyrones (Frandsen et al. 2011). This information will be significant for bioinformatic selection of putative candidates involved in the biosynthetic pathways of NGPs in fungal organisms.

Obviously, NGPs and fungal pigments are clearly biosynthetically linked in both *Aspergillus* sp. (DHN-melanin or *A. nidulans* dark green pigment) and *Fusarium* sp. (aurofusarin) (Malz et al. 2005; Chiang et al. 2011; Jørgensen et al. 2011b). It was even proposed that a second type of polymeric subunit made upstream from YWA1 could be incorporated into the final melanin polymer in *A. fumigatus* (Tsai et al. 1999; Fujii et al. 2004; Wheeler et al. 2008). The role of this particular biosynthetic link between NGPs and pigment is not yet elucidated and introduces important questions on the fungal role of these molecules.

Does NGP biosynthesis could depend of more than one pathway? Some studies suggest that it could be the case. Indeed, still working on conidia coloration of *A. niger* N402, Jørgensen et al. (2011a, b) show that flavasperone and aurasperone B, two NGPs, were associated to conidia formation (Jørgensen et al. 2011a). Besides, they showed that flavasperone production did not show the same dependence on AlbA depending on the culture conditions, submerged against subaerial (Jørgensen et al. 2011a, b). This difference could be explained by induction of a complementary PKS or an efficient metabolism of flavasperone in subaerial settings. In the same study, they determined that low level of aurasperone B was found in a mutant derived from *ΔalbA* background suggesting partial redundancy to synthesize NGPs (Fig. 3) (Jørgensen et al. 2011a). The question here is does *A. niger* genome contain a candidate polyketide synthase for this complementary biosynthetic pathway hypothesis? It was shown that more than 70 % of fungal PKS-encoding gene clusters were transcriptionally suppressed under various standard laboratory culture conditions (Scherlach and Hertweck 2009; Zerikly and Challis 2009; Fisch et al. 2009). That is why, treatment of *A. niger* ATCC 1015 with suberoylanilide hydroxamic acid (SAHA), an inhibitor of histone deacetylase (HDAC) used for epigenetic studies, led to transcription upregulation of many secondary metabolites encoding biosynthetic gene clusters (Fisch et al. 2009). Comparing the conserved domain composition of the polyketide synthases present in *A. niger*, they showed that only one PKS, An03g05440, possesses the same conserved domains as AlbA (Fisch et al. 2009). Besides, genes encoding those PKS show a strong homology. Interestingly, *An03g05440* was strongly upregulated by SAHA whereas *albA* was not. This specific property of *An03g05440* can lead to the use of SAHA for a better understanding of NGP biosynthetic pathway in *A. niger*. Supporting those results, the use of an HDAC inhibitor on *Cordyceps indigotica* (*C. indigotica*) culture allows the purification of indigotides, characterized as NGPs (Asai et al. 2012) (Fig. 3). Trying to understand the way that indigotides are synthesized by *C. indigotica*, Asai and collaborators (2012) established the profile of a NGP precursor synthesized from malonyl CoA, different from YWA1. This putative precursor supports the hypothesis of multiple biosynthetic pathways for the production of NGPs.

Further perspectives

Manufacturers are always seeking for new molecules with beneficial biological properties. Numerous natural extracts are studied for their putative biological properties at a laboratory scale (Akiyama et al. 2003; Shaaban et al. 2012; Xie et al. 2012; Kong et al. 2013). Fungal NGPs show biological properties such as anti-oxidant, anti-tumoral, and antimicrobial activities that could find potential applications in food, health, and cosmetics areas. They are natural molecules produced in large amount by industrial fungi, such as *A. niger* (Nielsen et al. 2009). Besides, they can be co-extracted, with other hydrophobic compounds, in a single step with ethanol allowing accreditation for "non-organic" label, attractive for industry (Kitanaka et al. 1998; Li et al. 2001; Xie et al. 2012). Personal experimental data suggest that ethanol extracts containing NGPs highly resist to temperature up to 60 °C conserving their biological properties, particularly their anti-oxidant activity, and seem to be strongly stable over the time. The dark color of some extracts issued from *A. niger* may be incompatible for their industrial use from consumer's perceptions. However, these crude extracts could still be commercialized as dietary supplements. Besides, the black color of the extracts could still be removed by addition of melanin

inhibitors in culture media or using genetically modified strains unable to produce melanin as described by Jørgensen et al. (2011a, b) and Chiang et al. (2011).

Optimization of the production/extraction process of fungal NGPs has to be done. The first step will be the improvement of culture conditions such as culture media, temperature, pH, incubation time, and the fungal strain chosen. Then, a particular attention should be made at the purification process. As hydrophobic molecules, NGPs are currently extracted with different organic solvents resulting to the simultaneous extraction of other hydrophobic compounds and thus requiring various purification steps, predominantly chromatography (Bouras et al. 2005; Campos et al. 2005). Depending on the potential industrial applications, purification should not be always necessary; the use a fungal bioactive extract containing NGPs could be possible, as it is already the case for ethanol crude extracts produced by plants (WO 2010109318 A1; Xie et al. 2012).

In addition, determination of NGP biosynthetic pathway is absolutely required for the improvement of the production/extraction of fungal NGPs. This knowledge will help guide the production of a specific NGP directly in the culture according to the chosen biological property. Besides, a complete knowledge of this pathway should allow the heterologous synthesis of targeted NGPs as it was done by Rugbjerg and colleagues (2013) for rubrofusarin (Rugbjerg et al. 2013). This specific production will greatly facilitate the current extraction process.

Conflict of interest The authors declare that they have no conflict of interest.

References

Akiyama K, Teraguchi S, Hamasaki Y, Mori M, Tatsumi K, Ohnishi K, Hayashi H (2003) New dimeric naphthopyrones from *Aspergillus niger*. J Nat Prod 66:136–139. doi:10.1021/np020174p

Alvarez-Vasquez F, González-Alcón C, Torres NV (2000) Metabolism of citric acid production by *Aspergillus niger*: model definition, steady-state analysis and constrained optimization of citric acid production rate. Biotechnol Bioeng 70:82–108

Aneja KR, Dhiman R, Aggarwal NK, Aneja A (2014) Emerging preservation techniques for controlling spoilage and pathogenic microorganisms in fruit juices. Int J Microbiol. doi:10.1155/2014/758942

Archer DB, Connerton IF, MacKenzie DA (2008) Filamentous fungi for production of food additives and processing aids. Adv Biochem Eng Biotechnol 111:99–147. doi:10.1007/10_2007_094

Asai T, Yamamoto T, Oshima Y (2012) Aromatic polyketide production in *Cordyceps indigotica*, an entomopathogenic fungus, induced by exposure to a histone deacetylase inhibitor. Org Lett 14:2006–2009. doi:10.1021/ol3005062

Ashley JN, Hobbs BC, Raistrick H (1937) Studies in the biochemistry of micro-organisms: the crystalline colouring matters of *Fusarium culmorum* (W. G. Smith) Sacc. and related forms. Biochem J 31: 385–397

Barrow RA, McCulloch MWB (2009) Linear naphtho-gamma-pyrones: a naturally occurring scaffold of biological importance. Mini-Rev Med Chem 9:273–292

Bokesch HR, Cartner LK, Fuller RW, Wilson JA, Henrich CJ, Kelley JA, Gustafson KR, McMahon JB, McKee TC (2010) Inhibition of ABCG2-mediated drug efflux by naphthopyrones from marine crinoids. Bioorg Med Chem Lett 20:3848–3850. doi:10.1016/j.bmcl.2010.05.057

Bouras N, Mathieu F, Coppel Y, Lebrihi A (2005) Aurasperone F—a new member of the naphtho-gamma-pyrone class isolated from a cultured microfungus, *Aspergillus niger* C-433. Nat Prod Res 19:653–659. doi:10.1080/14786410412331286955

Bouras N, Mathieu F, Coppel Y, Strelkov SE, Lebrihi A (2007) Occurrence of naphtho-gamma-pyrones- and ochratoxin A-producing fungi in French grapes and characterization of new naphtho-gamma-pyrone polyketide (aurasperone G) isolated from *Aspergillus niger* C-433. J Agric Food Chem 55:8920–8927. doi:10.1021/jf071406z

Burke KE (2007) Interaction of vitamins C and E as better cosmeceuticals. Dermatol Ther 20:314–321. doi:10.1111/j.1529-8019.2007.00145.x

Bycroft BW, Dobson TA, Robert JC (1962) Studies in mycological chemistry. Part VIII. The structure of flavasperone ("asperxanthone"), a metabolite of *Aspergillus niger*. J Chem Soc. doi:10.1039/JR9620004179

Campos FR, Barison A, Daolio C, Ferreira AG, Rodrigues-Fo E (2005) Complete 1H and 13C NMR assignments of aurasperone A and fonsecinone A, two bis-naphthopyrones produced by *Aspergillus aculeatus*. Magn Reson Chem 43:962–965. doi:10.1002/mrc.1654

Cardellina JH, Roxas-Duncan VI, Montgomery V, Eccard V, Campbell Y, Hu X, Khavrutskii I, Tawa GJ, Wallqvist A, Gloer JB, Phatak NL, Höller U, Soman AG, Joshi BK, Hein SM, Wicklow DT, Smith LA (2012) Fungal bis-naphthopyrones as inhibitors of botulinum neurotoxin serotype A. ACS Med Chem Lett 3:387–391. doi:10.1021/ml200312s

Carey ST, Nair MS (1975) Metabolites of *Pyrenomycetes* V. Identification of an antibiotic from two species of *Nectria*, as cephalochromin. Lloydia 38:448–449

Chang C-C, Chen W-C, Ho T-F, Wu H-S, Wei Y-H (2011) Development of natural anti-tumor drugs by microorganisms. J Biosci Bioeng 111: 501–511. doi:10.1016/j.jbiosc.2010.12.026

Chiang Y-M, Meyer KM, Praseuth M, Baker SE, Bruno KS, Wang CCC (2011) Characterization of a polyketide synthase in *Aspergillus niger* whose product is a precursor for both dihydroxynaphthalene (DHN) melanin and naphtho-γ-pyrone. Fungal Genet Biol 48:430–437. doi:10.1016/j.fgb.2010.12.001

Choi J, Hj L, Ky P, Jo H, Ss K (1997) In vitro antimutagenic effects of anthraquinone aglycones and naphthopyrone glycosides from *Cassia tora*. Planta Med 63:11–14

Chovolou Y, Ebada SS, Wätjen W, Proksch P (2011) Identification of angular naphthopyrones from the Philippine echinoderm *Comanthus* species as inhibitors of the NF-κB signaling pathway. Eur J Pharmacol 657:26–34. doi:10.1016/j.ejphar.2011.01.039

Delucca AJ, Ehrlich KC, Ciegler A (1983) Toxicity of extracts of *Aspergillus niger* isolated from stored cottonseed. J Food Saf 5: 95–101. doi:10.1111/j.1745-4565.1983.tb00460.x

De Vries RP, Frisvad JC, van de Vondervoort PJI, Burgers K, Kuijpers AFA, Samson RA, Visser J (2005) *Aspergillus vadensis*, a new species of the group of black *Aspergilli*. Antonie Van Leeuwenhoek 87:195–203. doi:10.1007/s10482-004-3194-y

Divirgilio ES, Dugan EC, Mulrooney CA, Kozlowski MC (2007) Asymmetric total synthesis of nigerone. Org Lett 9:385–388. doi:10.1021/ol062468y

Ehrlich KC, DeLucca AJ 2nd, Ciegler A (1984) Naphtho-gamma-pyrone production by *Aspergillus niger* isolated from stored cottonseed. Appl Environ Microbiol 48:1–4

Ernst-Russell MA, Chai CL, Wardlaw JH, Elix JA (2000) Euplectin and coneuplectin, new naphthopyrones from the lichen *Flavoparmelia euplecta*. J Nat Prod 63:129–131

Fisch KM, Gillaspy AF, Gipson M, Henrikson JC, Hoover AR, Jackson L, Najar FZ, Wägele H, Cichewicz RH (2009) Chemical induction of silent biosynthetic pathway transcription in *Aspergillus niger*. J Ind Microbiol Biotechnol 36:1199–1213. doi:10.1007/s10295-009-0601-4

Frandsen RJN, Nielsen NJ, Maolanon N, Sørensen JC, Olsson S, Nielsen J, Giese H (2006) The biosynthetic pathway for aurofusarin in *Fusarium graminearum* reveals a close link between the naphthoquinones and naphthopyrones. Mol Microbiol 61:1069–1080. doi:10.1111/j.1365-2958.2006.05295.x

Frandsen RJN, Schütt C, Lund BW, Staerk D, Nielsen J, Olsson S, Giese H (2011) Two novel classes of enzymes are required for the biosynthesis of aurofusarin in *Fusarium graminearum*. J Biol Chem 286:10419–10428. doi:10.1074/jbc.M110.179853

Fujii I, Watanabe A, Sankawa U, Ebizuka Y (2001) Identification of Claisen cyclase domain in fungal polyketide synthase WA, a naphthopyrone synthase of *Aspergillus nidulans*. Chem Biol 8:189–197

Fujii I, Yasuoka Y, Tsai H-F, Chang YC, Kwon-Chung KJ, Ebizuka Y (2004) Hydrolytic polyketide shortening by ayg1p, a novel enzyme involved in fungal melanin biosynthesis. J Biol Chem 279:44613–44620. doi:10.1074/jbc.M406758200

Galmarini OL, Stodola FH, Raper KB, Fennell DI (1962) Fonsecin, a naphthopyrone pigment from a mutant of *Aspergillus fonsecaeus*. Nature 195:502–503. doi:10.1038/195502a0

Ghosal S, Biswas K, Chakrabarti DK (1979) Toxic naphtho-gamma-pyrones from *Aspergillus niger*. J Agric Food Chem 27:1347–1351

Graham JG, Zhang H, Pendland SL, Santarsiero BD, Mesecar AD, Cabieses F, Farnsworth NR (2004) Antimycobacterial naphthopyrones from *Senna obliqua*. J Nat Prod 67:225–227. doi:10.1021/np030348i

Haskins RH, Knapp C (1969) *Cephalosporium* sp. (PRL 2070) and the production of cephalochromin. Can J Microbiol 15:435–437

Hegde VR, Miller JR, Patel MG, King AH, Puar MS, Horan A, Hart R, Yarborough R, Gullo V (1993) SCH 45752—an inhibitor of calmodulin-sensitive cyclic nucleotide phosphodiesterase activity. J Antibiot 46:207–213

Heinekamp T, Thywißen A, Macheleidt J, Keller S, Valiante V, Brakhage AA (2012) *Aspergillus fumigatus* melanins: interference with the host endocytosis pathway and impact on virulence. Front Microbiol 3:440. doi:10.3389/fmicb.2012.00440

Hsiao C-J, Hsiao G, Chen W-L, Wang S-W, Chiang C-P, Liu L-Y, Guh J-H, Lee T-H, Chung C-L (2014) Cephalochromin induces g0/g1 cell cycle arrest and apoptosis in a549 human non-small-cell lung cancer cells by inflicting mitochondrial disruption. J Nat Prod 77:758–765. doi:10.1021/np400517g

Huang H-B, Feng X-J, Liu L, Chen B, Lu Y-J, Ma L, She Z-G, Lin Y-C (2010) Three dimeric naphtho-γ-pyrones from the mangrove endophytic fungus *Aspergillus tubingensis* isolated from *Pongamia pinnata*. Planta Med 76:1888–1891. doi:10.1055/s-0030-1249955

Huang H-B, Xiao Z-E, Feng X-J, Huang C-H, Zhu X, Ju J-H, Li M-F, Lin Y-C, Liu L, She Z-G (2011) Cytotoxic naphtho-γ-pyrones from the mangrove endophytic fungus *Aspergillus tubingensis* (GX1-5E). HCA 94:1732–1740. doi:10.1002/hlca.201100050

Ikeda S, Sugita M, Yoshimura A, Sumizawa T, Douzono H, Nagata Y, Akiyama S (1990) *Aspergillus* species strain M39 produces two naphtho-gamma-pyrones that reverse drug resistance in human KB cells. Int J Cancer 45:508–513

Isaka M, Palasarn S, Kocharin K, Hywel-Jones NL (2007) Comparison of the bioactive secondary metabolites from the scale insect pathogens, anamorph *Paecilomyces cinnamomeus*, and teleomorph *Torrubiella luteorostrata*. J Antibiot 60:577–581. doi:10.1038/ja.2007.73

Ishii R, Horie M, Koyama K, Ishikawa Y, Kitanaka S (2005) Inhibitory effects of fungal bis(naphtho-gamma-pyrone) derivatives on nitric oxide production by a murine macrophage-like cell line, RAW 264.7, activated by lipopolysaccharide and interferon-gamma. Biol Pharm Bull 28:786–790

Jørgensen TR, Nielsen KF, Arentshorst M, Park J, van den Hondel CA, Frisvad JC, Ram AF (2011a) Submerged conidiation and product formation by *Aspergillus niger* at low specific growth rates are affected in aerial developmental mutants. Appl Environ Microbiol 77:5270–5277. doi:10.1128/AEM. 00118-11

Jørgensen TR, Park J, Arentshorst M, van Welzen AM, Lamers G, Vankuyk PA, Damveld RA, van den Hondel CAM, Nielsen KF, Frisvad JC, Ram AFJ (2011b) The molecular and genetic basis of conidial pigmentation in *Aspergillus niger*. Fungal Genet Biol 48:544–553. doi:10.1016/j.fgb.2011.01.005

Karre L, Lopez K, Getty KJK (2013) Natural antioxidants in meat and poultry products. Meat Sci 94:220–227. doi:10.1016/j.meatsci.2013.01.007

Kew MC (2013) Aflatoxins as a cause of hepatocellular carcinoma. J Gastrointest Liver Dis 22:305–310

Kitanaka S, Nakayama T, Shibano T, Ohkoshi E, Takido M (1998) Antiallergic agent from natural sources. Structures and inhibitory effect of histamine release of naphthopyrone glycosides from seeds of *Cassia obtusifolia* L. Chem Pharm Bull 46:1650–1652

Kock I, Draeger S, Schulz B, Elsässer B, Kurtán T, Kenéz Á, Antus S, Pescitelli G, Salvadori P, Speakman J-B, Rheinheimer J, Krohn K (2009) Pseudoanguillosporin A and B: two new isochromans isolated from the endophytic fungus *Pseudoanguillospora* sp. Eur J Org Chem 2009:1427–1434. doi:10.1002/ejoc.200801083

Kong X, Ma X, Xie Y, Cai S, Zhu T, Gu Q, Li D (2013) Aromatic polyketides from a sponge-derived fungus *Metarhizium anisopliae* mxh-99 and their antitubercular activities. Arch Pharm Res 36:739–744. doi:10.1007/s12272-013-0077-7

Koyama K, Natori S (1988) Further characterization of seven bis(naphtho-γ-pyrone) congeners of ustilaginoidins, coloring matters of *Claviceps virens* (*Ustilaginoidea virens*). Chem Pharm Bull 36:146–152

Koyama K, Ominato K, Natori S, Tashiro T, Tsuruo T (1988) Cytotoxicity and antitumor activities of fungal bis(naphtho-gamma-pyrone) derivatives. J Pharmacobio-dyn 11:630–635

Langfelder K, Jahn B, Gehringer H, Schmidt A, Wanner G, Brakhage AA (1998) Identification of a polyketide synthase gene (pksP) of *Aspergillus fumigatus* involved in conidial pigment biosynthesis and virulence. Med Microbiol Immunol 187:79–89

Langfelder K, Streibel M, Jahn B, Haase G, Brakhage AA (2003) Biosynthesis of fungal melanins and their importance for human pathogenic fungi. Fungal Genet Biol 38:143–158

Lee B-H, Pan T-M (2012) Benefit of *Monascus*-fermented products for hypertension prevention: a review. Appl Microbiol Biotechnol 94:1151–1161. doi:10.1007/s00253-012-4076-2

Lee GY, Jang DS, Lee YM, Kim JM, Kim JS (2006) Naphthopyrone glucosides from the seeds of *Cassia tora* with inhibitory activity on advanced glycation end products (AGEs) formation. Arch Pharm Res 29:587–590

Lee H-M, Chan DS-H, Yang F, Lam H-Y, Yan S-C, Che C-M, Ma D-L, Leung C-H (2010) Identification of natural product fonsecin B as a stabilizing ligand of c-myc G-quadruplex DNA by high-throughput virtual screening. Chem Commun (Camb) 46:4680–4682. doi:10.1039/b926359d

Lee YM, Kim MJ, Li H, Zhang P, Bao B, Lee KJ, Jung JH (2013) Marine-derived *Aspergillus* species as a source of bioactive secondary metabolites. Mar Biotechnol 15:499–519. doi:10.1007/s10126-013-9506-3

Leitão GG, Leitão SG, Vilegas W (2002) Quick preparative separation of natural naphthopyranones with antioxidant activity by high-speed counter-current chromatography. Z Naturforsch C J Biosci 57:1051–1055

Li X-B, Xie F, Liu S-S, Li Y, Zhou J-C, Liu Y-Q, Yuan H-Q, Lou H-X (2013) Naphtho-γ-pyrones from endophyte *Aspergillus niger* occurring in the liverwort *Heteroscyphus tener* (Steph.) Schiffn. Chem Biodivers 10:1193–1201. doi:10.1002/cbdv.201300042

Li X-C, Dunbar DC, ElSohly HN, Jacob MR, Nimrod AC, Walker LA, Clark AM (2001) A new naphthopyrone derivative from *Cassia quinquangulata* and structural revision of quinquangulin and its glycosides. J Nat Prod 64:1153–1156. doi:10.1021/np010173h

Lu S, Yian J, Sun W, Meng J, Wang X, Fu X, Wang A, Lai D, Liu Y, Zhou L (2014) Bis-naphtho-gammapyrones from fungi and their bioactivities. Molecules 19(6):7169–7188

Lund NA, Robertson A, Whalley WB (1953) 494. The chemistry of fungi. Part XXI. Asperxanthone and a preliminary examination of aspergillin. J. Chem. Soc. 2434–2439. doi:10.1039/JR9530002434

Maldonado MC, Strasser de Saad AM (1998) Production of pectinesterase and polygalacturonase by *Aspergillus niger* in submerged and solid state systems. J Ind Microbiol Biotechnol 20:34–38

Malz S, Grell MN, Thrane C, Maier FJ, Rosager P, Felk A, Albertsen KS, Salomon S, Bohn L, Schäfer W, Giese H (2005) Identification of a gene cluster responsible for the biosynthesis of aurofusarin in the *Fusarium graminearum* species complex. Fungal Genet Biol 42: 420–433. doi:10.1016/j.fgb.2005.01.010

Matsumoto M, Minato H, Kondo E, Mitsugi T, Katagiri K (1975) Cephalochromin, dihydroisoustilaginoidin A, and isoustilaginoidin A from *Verticillium* sp. K-113. J Antibiot 28:602–604

Nielsen KF, Mogensen JM, Johansen M, Larsen TO, Frisvad JC (2009) Review of secondary metabolites and mycotoxins from the *Aspergillus niger* group. Anal Bioanal Chem 395:1225–1242. doi: 10.1007/s00216-009-3081-5

Ohkawa Y, Miki K, Suzuki T, Nishio K, Sugita T, Kinoshita K, Takahashi K, Koyama K (2010) Antiangiogenic metabolites from a marine-derived fungus, *Hypocrea vinosa*. J Nat Prod 73:579–582. doi:10.1021/np900698p

Paranagama PA, Wijeratne EMK, Gunatilaka AAL (2007) Uncovering biosynthetic potential of plant-associated fungi: effect of culture conditions on metabolite production by *Paraphaeosphaeria quadriseptata* and *Chaetomium chiversii*. J Nat Prod 70:1939–1945. doi:10.1021/np070504b

Priestap HA (1984) New naphthopyrones from *Aspergillus fonsecaeus*. Tetrahedron 40:3617–3624. doi:10.1016/S0040-4020(01)88792-5

Qiang G, Xue S, Yang JJ, Du G, Pang X, Li X, Goswami D, Griffin PR, Ortlund EA, Chan CB, Ye K (2014) Identification of a small molecular insulin receptor agonist with potent antidiabetes activity. Diabetes 63:1394–1409. doi:10.2337/db13-0334

Rabache M, Adrian J (1982) Physiologic effects of the *Aspergillus niger* pigments. 2- Antioxygen property of the naphtho-gamma-pyrones estimated in the rat. Science des Aliments

Roukas T (2000) Citric and gluconic acid production from fig by *Aspergillus niger* using solid-state fermentation. J Ind Microbiol Biotechnol 25:298–304. doi:10.1038/sj/jim/7000101

Rugbjerg P, Naesby M, Mortensen UH, Frandsen RJ (2013) Reconstruction of the biosynthetic pathway for the core fungal polyketide scaffold rubrofusarin in *Saccharomyces cerevisiae*. Microb Cell Fact 12:31. doi:10.1186/1475-2859-12-31

Sakai K, Ohte S, Ohshiro T, Matsuda D, Masuma R, Rudel LL, Tomoda H (2008) Selective inhibition of acyl-CoA:cholesterol acyltransferase 2 isozyme by flavasperone and sterigmatocystin from *Aspergillus* species. J Antibiot 61:568–572. doi:10.1038/ja.2008.76

Sakurai M, Kohno J, Yamamoto K, Okuda T, Nishio M, Kawano K, Ohnuki T (2002) TMC-256A1 and C1, new inhibitors of IL-4 signal transduction produced by *Aspergillus niger* var niger TC 1629. J Antibiot 55:685–692

Scherlach K, Hertweck C (2009) Triggering cryptic natural product biosynthesis in microorganisms. Org Biomol Chem 7:1753–1760. doi:10.1039/b821578b

Scotter MJ, Castle L (2004) Chemical interactions between additives in foodstuffs: a review. Food Addit Contam 21:93–124. doi:10.1080/02652030310001636912

Sebranek JG, Bacus JN (2007) Cured meat products without direct addition of nitrate or nitrite: what are the issues? Meat Sci 77:136–147. doi:10.1016/j.meatsci.2007.03.025

Sekita S, Yoshihira K, Natori S (1980) Chaetochromin, a bis(naphthodihydropyran-4-one) mycotoxin from *Chaetomium thielavioideum* : application of 13C-1H long-rang coupling to the structure elucidation. Chem Pharm Bull 8:2428–2435

Shaaban M, Shaaban KA, Abdel-Aziz MS (2012) Seven naphtho-γ-pyrones from the marine-derived fungus *Alternaria alternata*: structure elucidation and biological properties. Org Med Chem Lett 2:6. doi:10.1186/2191-2858-2-6

Shibata S, Ogihara Y (1963) Metabolic products of fungi. XXIII. On ustilaginoidins. (3). The structures of ustilaginoidins B and C. Chem Pharm Bull 11:1576–1578

Shibata S, Ogihara Y, Ohta A (1963) Metabolic products of fungi. XXII. On ustilaginoidins. (2). The structures of ustilaginoidin A. Chem Pharm Bull 11:1179–1182

Singh SB, Zink DL, Bills GF, Teran A, Silverman KC, Lingham RB, Felock P, Hazuda DJ (2003) Four novel bis-(naphtho-gamma-pyrones) isolated from *Fusarium* species as inhibitors of HIV-1 integrase. Bioorg Med Chem Lett 13:713–717

Slesiona S, Gressler M, Mihlan M, Zaehle C, Schaller M, Barz D, Hube B, Jacobsen ID, Brock M (2012) Persistence versus escape: *Aspergillus terreus* and *Aspergillus fumigatus* employ different strategies during interactions with macrophages. PLoS ONE 7:e31223. doi:10.1371/journal.pone.0031223

Song YC, Li H, Ye YH, Shan CY, Yang YM, Tan RX (2004) Endophytic naphthopyrone metabolites are co-inhibitors of xanthine oxidase, SW1116 cell and some microbial growths. FEMS Microbiol Lett 241:67–72. doi:10.1016/j.femsle.2004.10.005

Sørensen JL, Nielsen KF, Sondergaard TE (2012) Redirection of pigment biosynthesis to isocoumarins in *Fusarium*. Fungal Genet Biol 49: 613–618. doi:10.1016/j.fgb.2012.06.004

Stamford NPJ (2012) Stability, transdermal penetration, and cutaneous effects of ascorbic acid and its derivatives. J Cosmet Dermatol 11: 310–317. doi:10.1111/jocd.12006

Tanaka H, Tamura T (1961) The chemical constitution of rubrofusarin. Tetrahedron Lett 2:151–155. doi:10.1016/S0040-4039(01)99228-7

Tanaka H, Wang PL, Namiki M (1972) Structure of aurasperone C. Agri Biol Chem 36(13):2511-2517

Tanaka H, Wang P-L, Yamada O (1966) Yellow Pigments of *Aspergillus niger* and *Asp. awamori* Part I. Agric Biol Chem 30:107–113. doi: 10.1271/bbb1961.30.107

Thywißen A, Heinekamp T, Dahse H-M, Schmaler-Ripcke J, Nietzsche S, Zipfel PF, Brakhage AA (2011) Conidial dihydroxynaphthalene melanin of the human pathogenic fungus *Aspergillus fumigatus* interferes with the host endocytosis pathway. Front Microbiol 2: 96. doi:10.3389/fmicb.2011.00096

Tsai HF, Chang YC, Washburn RG, Wheeler MH, Kwon-Chung KJ (1998) The developmentally regulated alb1 gene of *Aspergillus fumigatus*: its role in modulation of conidial morphology and virulence. J Bacteriol 180:3031–3038

Tsai HF, Wheeler MH, Chang YC, Kwon-Chung KJ (1999) A developmentally regulated gene cluster involved in conidial pigment biosynthesis in *Aspergillus fumigatus*. J Bacteriol 181:6469–6477

Ugaki N, Matsuda D, Yamazaki H, Nonaka K, Masuma R, Omura S, Tomoda H (2012) New isochaetochromin, an inhibitor of triacylglycerol synthesis in mammalian cells, produced by *Penicillium* sp. FKI-4942: I. Taxonomy, fermentation, isolation and biological properties. J Antibiot 65:15–19. doi:10.1038/ja.2011.105

Wang P-L, Tanaka H (1966) Yellow pigments of *Aspergillus niger* and *Aspergillus awamori*. Agric Biol Chem 30:683–687. doi:10.1271/bbb1961.30.683

Watanabe A, Ebizuka Y (2004) Unprecedented mechanism of chain length determination in fungal aromatic polyketide synthases. Chem Biol 11:1101–1106. doi:10.1016/j.chembiol.2004.05.015

Watanabe A, Fujii I, Sankawa U, Mayorga ME, Timberlake WE, Ebizuka Y (1999) Re-identification of *Aspergillus nidulans wA* gene to code for a polyketide synthase of naphthopyrone. Tetrahedron Lett 40: 91–94. doi:10.1016/S0040-4039(98)80027-0

Watanabe A, Fujii I, Tsai H, Chang YC, Kwon-Chung KJ, Ebizuka Y (2000) *Aspergillus fumigatus* alb1 encodes naphthopyrone synthase when expressed in *Aspergillus oryzae*. FEMS Microbiol Lett 192: 39–44

Watanabe A, Ono Y, Fujii I, Sankawa U, Mayorga ME, Timberlake WE, Ebizuka Y (1998) Product identification of polyketide synthase coded by *Aspergillus nidulans wA* gene. Tetrahedron Lett 39: 7733–7736. doi:10.1016/S0040-4039(98)01685-2

Wheeler MH, Abramczyk D, Puckhaber LS, Naruse M, Ebizuka Y, Fujii I, Szaniszlo PJ (2008) New biosynthetic step in the melanin pathway of *Wangiella* (*Exophiala*) *dermatitidis*: evidence for 2-acetyl-1,3,6, 8-tetrahydroxynaphthalene as a novel precursor. Eukaryot Cell 7: 1699–1711. doi:10.1128/EC.00179-08

Wong SM, Wong MM, Seligmann O, Wagner H (1989) New antihepatotoxic naphtho-pyrone glycosides from the seeds of *Cassia tora*. Planta Med 55:276–280. doi:10.1055/s-2006-962003

Wu F, Groopman JD, Pestka JJ (2014) Public health impacts of foodborne mycotoxins. Annu Rev Food Sci Technol 5:351–372. doi:10.1146/annurev-food-030713-092431

Xiao J, Zhang Q, Gao Y-Q, Shi X-W, Gao J-M (2014) Antifungal and antibacterial metabolites from an endophytic *Aspergillus* sp. associated with *Melia azedarach*. Nat Prod Res. doi:10.1080/14786419.2014.904308

Xie Q, Guo F-F, Zhou W (2012) Protective effects of cassia seed ethanol extract against carbon tetrachloride-induced liver injury in mice. Acta Biochim Pol 59:265–270

Xu G-B, Yang T, Bao J-K, Fang D-M, Li G-Y (2014) Isochaetomium A2, a new bis(naphthodihydropyran-4-one) with antimicrobial and immunological activities from fungus *Chaetomium microcephalum*. Arch Pharm Res 37:575–579. doi:10.1007/s12272-013-0206-3

Ye YH, Zhu HL, Song YC, Liu JY, Tan RX (2005) Structural revision of aspernigrin A, reisolated from *Cladosporium herbarum* IFB-E002. J Nat Prod 68:1106–1108. doi:10.1021/np050059p

Ye Y, Jia R-R, Tang L, Chen F (2014) In vivo antioxidant and anti-skin-aging activities of ethyl acetate extraction from *Idesia polycarpa* defatted fruit residue in aging mice induced by D-galactose. Evid Based Complement Alternat Med 2014:185716. doi:10.1155/2014/185716

Young J (1998) European market developments in prebiotic- and probiotic-containing foodstuffs. Br J Nutr 80:S231–S233

Zaika LL, Smith JL (1975) Antioxidants and pigments of *Aspergillus niger*. J Sci Food Agric 26:1357–1369. doi:10.1002/jsfa.2740260915

Zerikly M, Challis GL (2009) Strategies for the discovery of new natural products by genome mining. Chembiochem 10:625–633. doi:10.1002/cbic.200800389

Zhang Y, Ling S, Fang Y, Zhu T, Gu Q, Zhu W-M (2008) Isolation, structure elucidation, and antimycobacterial properties of dimeric naphtho-gamma-pyrones from the marine-derived fungus *Aspergillus carbonarius*. Chem Biodivers 5:93–100. doi:10.1002/cbdv.200890017

Zhang Y, Li X-M, Wang B-G (2007) Nigerasperones A approximately C, new monomeric and dimeric naphtho-gamma-pyrones from a marine alga-derived endophytic fungus *Aspergillus niger* EN-13. J Antibiot 60:204–210. doi:10.1038/ja.2007.24

Zhan J, Gunaherath GMKB, Wijeratne EMK, Gunatilaka AAL (2007) Asperpyrone D and other metabolites of the plant-associated fungal strain *Aspergillus tubingensis*. Phytochemistry 68:368–372. doi:10.1016/j.phytochem.2006.09.038

Zheng CJ, Sohn M-J, Lee S, Hong Y-S, Kwak J-H, Kim W-G (2007) Cephalochromin, a FabI-directed antibacterial of microbial origin. Biochem Biophys Res Commun 362:1107–1112. doi:10.1016/j.bbrc.2007.08.144