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Transcription profiling of the *Neurospora crassa* response to a group of synthetic (thio)xanthenes and a natural acetophenone



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ABSTRACT

Xanthenes are a class of heterocyclic compounds characterized by a dibenzo- γ -pyrone nucleus. Analysis of their mode of action in cells, namely uncovering alterations in gene expression, is important because these compounds have potential therapeutic applications. Thus, we studied the transcriptional response of the filamentous fungus *Neurospora crassa* to a group of synthetic (thio)xanthone derivatives with antitumor activity using high throughput RNA sequencing. The induction of ABC transporters in *N. crassa*, particularly *atrB* and *cdr4*, is a common consequence of the treatment with xanthenes. In addition, we found a group of genes repressed by all of the tested (thio)xanthone derivatives, that are evocative of genes downregulated during oxidative stress. The transcriptional response of *N. crassa* treated with an acetophenone isolated from the soil fungus *Neosartorya siamensis* shares some features with the (thio)xanthone-elicited gene expression profiles. Two of the (thio)xanthone derivatives and the *N. siamensis*-derived acetophenone inhibited the growth of *N. crassa*. Our work also provides framework datasets that may orientate future studies on the mechanisms of action of some groups of xanthenes.

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Introduction

Xanthenes are a class of heterocyclic compounds characterized by a dibenzo- γ -pyrone nucleus [1–7]. They are composed by a unique chemical scaffold, the tricyclic aromatic system (C₆–C₃–C₆) named xanthene-9-one [2,5,7]. They are commonly found as secondary metabolites in higher plants, fungi and lichens [8], and can be obtained by synthetic methods [9]. This class of compounds is composed by a rigid heteroaromatic tricyclic platform which may be considered as a ‘privileged structure’ since it can provide different ligands through modification of functional groups, allowing them to interact with a large variety of biological targets [10]. The biological activity of xanthenes and their derivatives is based on their tricyclic scaffold but depends on the nature and position of the different substituents [2]. Since xanthenes may play a role as anti-

proliferative and pro-apoptotic agents [2,11–14], several derivatives, including (thio)xanthone derivatives, have been synthesized and tested in human cancer cell lines [14,15], but not in fungi. It is thus important to add data from different organisms to the current knowledge on the mechanism of action of xanthenes.

XP13 (3,4-dihydro-12-hydroxy-2,2-dimethyl-2H,6H-pyrano [3,2-b]-xanthen-6-one) (Fig. 1) is a prenylated analogue of 3,4-dihydroxyxanth-9-one [11,13]. XP13 was shown to possess very potent anticancer activity in leukemia [11] and breast cancer [13] cells. XP13 was also shown to reduce cell proliferation, arrest the cell cycle at the S phase and induce apoptosis [11]. In addition, XP13 was shown to inhibit the interaction of p53 with MDM2 and mimicked the activity of known p53 activators, leading to the activation of p53-dependent transcriptional activity [16].

In this work, we have also tested the effects of three thioxanthone derivatives on *N. crassa*—TX129 (1-[(3,4-dimethoxybenzyl)amino]-4-propoxy-9H-thioxanthen-9-one), TX34 (1-(piperidin-1-yl)-4-propoxy-9H-thioxanthen-9-one) and TX87 (1-[[2-(1,3-benzodioxol-5-yl)ethyl]amino]-4-propoxy-9H-thioxanthen-9-one) (Fig. 1). These compounds are aminated thioxanthenes and were obtained by synthesis after being designed to inhibit cancer cell proliferation and

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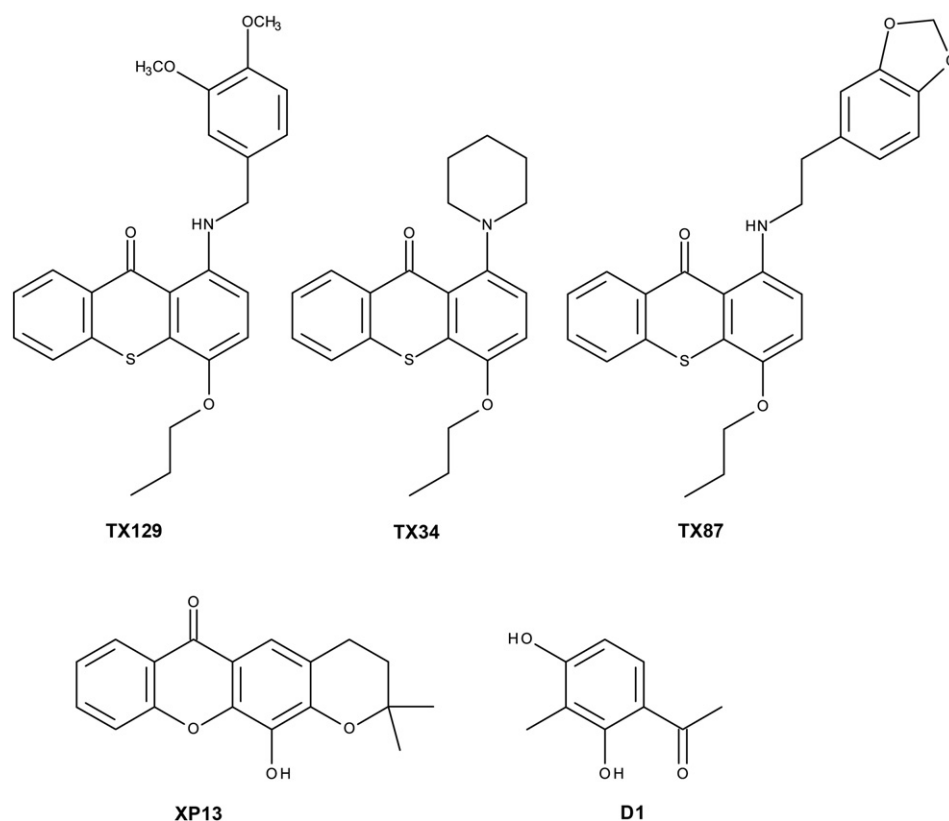


Fig. 1. Chemical structures of the compounds used in this study. TX129: 1-[(3,4-dimethoxybenzyl)amino]-4-propoxy-9H-thioxanthen-9-one; TX34: 1-(piperidin-1-yl)-4-propoxy-9H-thioxanthen-9-one; TX87: 1-[[2-(1,3-benzodioxol-5-yl)ethyl]amino]-4-propoxy-9H-thioxanthen-9-one; XP13: 3,4-dihydro-12-hydroxy-2,2-dimethyl-2H,6H-pyranopyrano [3,2-b]-xanthen-6-one; D1: 2,4-dihydroxy-3-methylacetophenone.

to block the human P-glycoprotein [15,17]. These compounds were obtained by merging the structural skeleton of thioxanthenes with moieties important for P-glycoprotein modulation [17]. TX34 has been shown to behave as a non-competitive inhibitor of the P-glycoprotein and to block the growth of cancer cells *in vitro* [15,17].

Here we used the filamentous fungus *N. crassa* as a model to study the transcriptional response to a group of synthetic (thio)xanthone derivatives using high-throughput RNA sequencing (RNA-seq) to assess alterations in gene expression. Our data suggest that, in *N. crassa*, some ABC transporter-encoding genes and the transcription factor *czt-1* are induced by different (thio)xanthone derivatives and that a group of genes – the majority of which encode currently uncharacterized proteins – are repressed by all of the tested compounds. XP13 (3,4-dihydro-12-hydroxy-2,2-dimethyl-2H,6H-pyranopyrano [3,2-b]-xanthen-6-one) led to the strongest transcriptional response and growth impairment of *N. crassa*. The present study is the first to describe the effects of (thio)xanthenes in fungi and will be important to understand their mechanism of action.

Material and methods

Strains, culture media and compounds

Standard procedures for the handling of wild type *N. crassa* cells (FGSC 2489) were employed [18]. Cells were grown in Vogel's MM supplemented with 1.5% (w/v) sucrose [19]. Agar at 1.5% (w/v) concentration was added to obtain solid medium. Conidia were harvested by the addition of sterile dH_2O , agitation and filtration through cheesecloth in 7-day cultures on Vogel's MM with agar at 26 °C.

The xanthone XP13 and the thioxanthenes TX34, TX87, and TX129 were synthesized according to previously described procedures [11, 17]. The purity of each compound was determined by HPLC–DAD analysis using an isocratic elution of MeOH:H₂O basified with TEA (1%) or

acidified with CH₃COOH (1%) at a constant flow rate of 1.0 ml/min. All tested compounds possessed a purity of at least 95%. (Thio)xanthone derivatives and 2,4-dihydroxy-3-methylacetophenone (D1) were dissolved in DMSO (Sigma-Aldrich).

RNA sequencing (RNA-seq)

Conidial suspensions at 1×10^6 cells/ml were incubated in minimal medium for 6 h (26 °C, 140 rpm, constant light), followed by the addition of the indicated compounds (or the DMSO control solvent) for 1 more hour. Cells were harvested using 0.45 μ m filters and immediately frozen in liquid nitrogen. Total RNA was isolated by the Trizol–Phenol–Chloroform method, three times for control samples and once for the treatments with (thio)xanthenes and the acetophenone. After digestion of 25 μ g RNA with TURBO DNase (Life Technologies), mRNA was purified using Dynabeads oligo(dT) magnetic beads (Life Technologies). The mRNA was chemically fragmented using the Ambion RNA fragmentation kit (Life Technologies). First and second strand cDNA synthesis was achieved using appropriate kits (Life Technologies). The Illumina TruSeq kit was employed to generate the cDNA libraries with indexing adapters essentially following the manufacturer's protocol. After purification of the libraries with AMPure XP beads (Roche), the quality of the libraries was analyzed in an Agilent 2100 Bioanalyzer. The cDNA libraries were sequenced in an Illumina HiSeq2000 and single reads of 50 bp were obtained. Sequencing data was handled essentially with Tophat, Cufflinks and Cuffdiff [20]. Expression levels are presented as Fragments/Reads Per Kilobase of transcript per Million mapped reads (FPKM/RPKM). The resulting dataset is available at the NCBI GEO database (<http://www.ncbi.nlm.nih.gov/geo/>; series record: GSE53040). Functional enrichment of sets of genes was assessed with FunCat [21] and Venn diagrams were obtained using Venny [22].

Evaluation of growth in microplates

For growth assessment in liquid Vogel's MM, 1×10^4 conidia/ml were incubated at 26 °C, 100 rpm, under constant light in 96-well plates (200 μ l/well) and absorbance at 620 nm [23] was followed (Thermo Electron Corporation Multiskan EX) during 24 h. At least three independent experiments were performed in each case. The Mann–Whitney statistical test was used to make comparisons between conditions.

Results and discussion

Transcriptional profiling and functional enrichment analyses of the *N. crassa* response to (thio)xanthone derivatives

N. crassa cells were grown for 6 h in liquid Vogel's medium, treated for 1 h with 20 μ M of each of the (thio)xanthone derivatives TX129, TX34, TX87 and XP13 (Fig. 1), and processed for RNA-seq. Treatment with the different compounds led to an alteration of the expression

levels of a significantly different fraction of genes (Supplemental File 1). XP13 was the xanthone derivative eliciting the most active transcriptional response, altering the expression of 15.7% of the genes (Fig. 2A, i–iv, left panels). TX34, TX87 and TX129 modified the expression of 3.5, 1.1 and 0.6% of the genes, respectively. As a comparison, a 1-hour treatment with 20 μ M staurosporine, a protein kinase inhibitor that induces cell death in *N. crassa*, causes the alteration of approximately 20% of the genes [24]. Interestingly, all the (thio)xanthone derivatives caused a large downregulation of gene expression, particularly TX87 and XP13, with 94.5% and 88.4% of repressed genes among those with altered expression (Fig. 1A, i–iv, right panels). These data indicate that treatment with (thio)xanthone derivatives mainly leads to the decrease in expression levels of *N. crassa* genes, which is considerably different from the fungal response to staurosporine, which causes a similar percentage of gene induction and repression [24].

FunCat [21] was employed to evaluate if there were enriched functional categories among datasets of genes which showed increased or reduced expression levels for each compound (Supplemental File 2

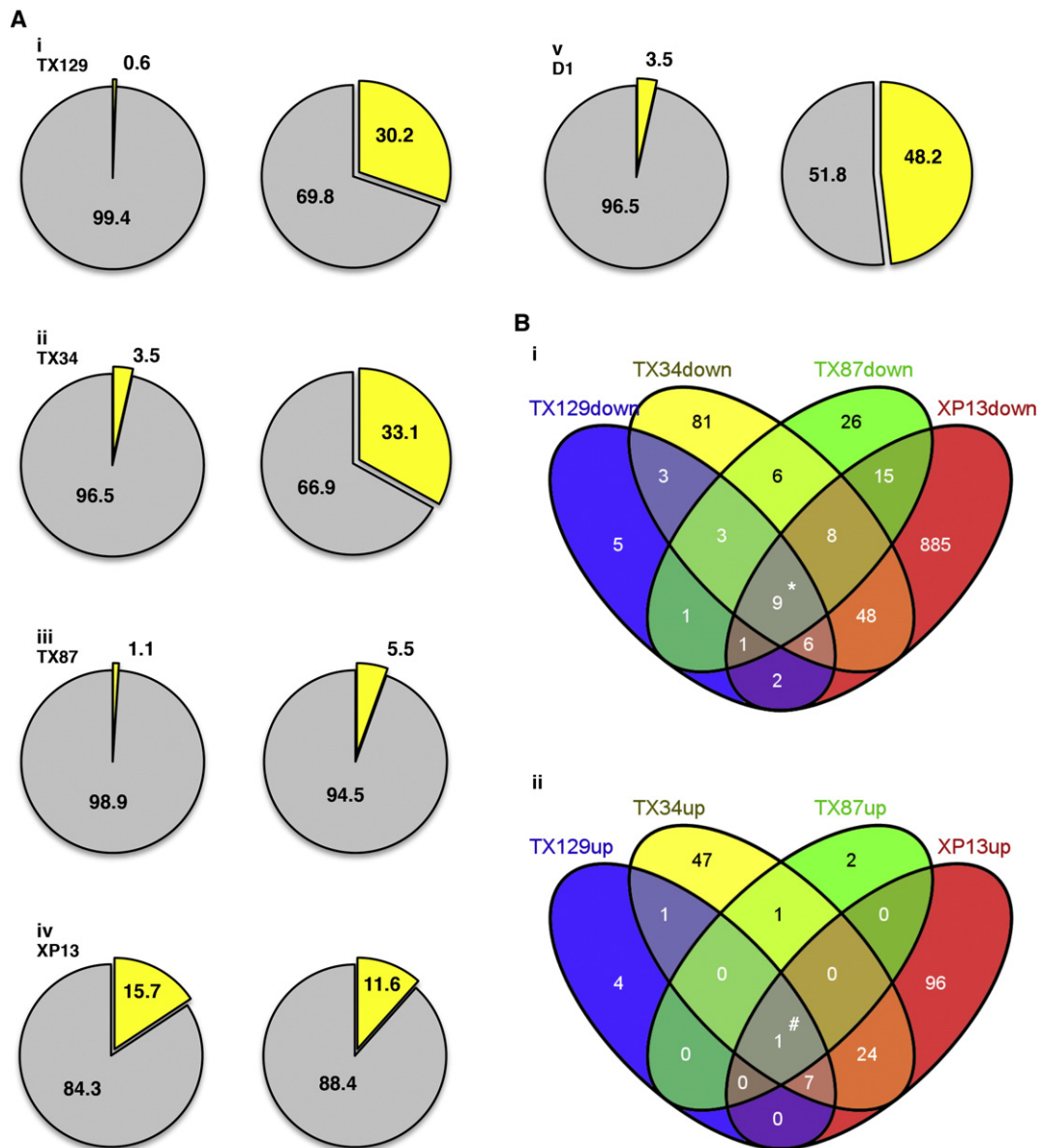


Fig. 2. Overview of the *N. crassa* transcriptional response to (thio)xanthone derivatives and a secondary metabolite from *N. siamensis*. (A) The percentage of genes with altered expression upon treatment with each of the indicated compounds (left panel; yellow: altered genes; grey: unaltered genes) and the fraction of induced and repressed genes (right panel; yellow: induced genes; grey: repressed genes) was calculated for cells treated with TX129 (i), TX34 (ii), TX87 (iii), XP13 (iv) and D1 (v). (B) Venn diagrams were used to assess the amount of (thio)xanthone-specific repressed (i) and induced (ii) genes. * and # indicate genes that are either repressed or induced by all of the tested (thio)xanthone derivatives, respectively.

Table 1Functional enrichment analysis of genes whose expression was altered by the (thio)xanthone derivatives (*P*-value <0.0001).

FunCat ID	Category	<i>P</i> -value
<i>TX129 (induced genes)</i>		
32.01.01	Oxidative stress response	1.99E – 05
32.05.01	Resistance proteins	4.82E – 05
32.07	Detoxification	1.28E – 05
<i>TX34 (induced genes)</i>		
1	Metabolism	2.21E – 14
01.05	C-compound and carbohydrate metabolism	2.84E – 05
01.06	Lipid, fatty acid and isoprenoid metabolism	2.01E – 08
20.01.01	Ion transport	8.37E – 05
20.01.01.01	Cation transport (H ⁺ , Na ⁺ , K ⁺ , Ca ²⁺ , NH ⁴⁺ , etc.)	2.28E – 05
20.03.22	Transport ATPases	2.48E – 07
20.09.16.01	Type I protein secretion system (ABC-type transport systems)	4.96E – 06
32.05.01	Resistance proteins	1.51E – 05
34.01	Homeostasis	5.09E – 05
34.01.01	Homeostasis of cations	2.33E – 05
34.01.01.01	Homeostasis of metal ions (Na, K, Ca etc.)	1.96E – 06
<i>XP13 (induced genes)</i>		
1	Metabolism	1.29E – 07
01.05	C-compound and carbohydrate metabolism	6.58E – 06
2	Energy	1.42E – 06
20.01.01	Ion transport	6.58E – 06
20.01.27	Drug/toxin transport	1.07E – 05
20.03.22	Transport ATPases	4.32E – 07
20.03.25	ABC transporters	7.67E – 05
20.09.16.01	Type I protein secretion system (ABC-type transport systems)	3.16E – 05
32	Cell rescue, defense and virulence	3.78E – 09
32.01.01	Oxidative stress response	2.01E – 07
32.07	Detoxification	1.92E – 13
32.07.05	Detoxification by export	5.63E – 06
32.07.07	Oxygen and radical detoxification	2.07E – 05
<i>XP13 (repressed genes)</i>		
10	Cell cycle and DNA processing	2.54E – 07
10.03	Cell cycle	1.57E – 05
10.03.01	Mitotic cell cycle and cell cycle control	8.94E – 06
11	Transcription	4.17E – 13
11.02	RNA synthesis	9.80E – 08
11.02.03	mRNA synthesis	4.04E – 06
11.02.03.04	Transcriptional control	4.06E – 07
11.04	RNA processing	1.47E – 05
11.04.01	rRNA processing	1.43E – 07
16.03	Nucleic acid binding	3.42E – 05
18.02.05	Regulator of G-protein signaling	9.64E – 05
20.09	Transport routes	6.46E – 05

and Table 1). The dataset of TX129-induced genes was enriched in molecules involved in the response to oxidative stress and in resistance and detoxification. In turn, TX34 induced genes involved in metabolism (carbon, carbohydrate and lipid metabolism) and in ion transport and homeostasis. The FunCat analysis was particularly useful in the case of XP13-altered genes. The XP13-induced dataset was highly enriched in genes involved in cell rescue, defense and virulence (namely genes related with detoxification by members of the ABC transporter family

and genes implicated in the response to oxidative stress) and in genes implicated in metabolism and energy. On the other hand, XP13-repressed genes seem to be related to cell cycle and RNA synthesis and processing. The latter feature is shared with staurosporine [24], suggesting that some pathways may be disturbed by both compounds.

Venn diagrams were built in order to evaluate whether repressed or induced genes were shared between the (thio)xanthone derivatives. For most of the genes, the alteration in expression levels elicited by

Table 2

Genes that are repressed (*) and induced (#) by all of the tested (thio)xanthone derivatives and the respective FPKM.

Shared genes	FPKM					
	DMSO	TX129	TX34	TX87	XP13	D1
NCU04907 *	56.17	22.52 (0.40)	13.86 (0.25)	23.56 (0.42)	14.85 (0.26)	16.02 (0.29)
NCU00999 *	65.75	25.78 (0.39)	13.65 (0.21)	24.39 (0.37)	25.60 (0.39)	50.77 (0.77, ns)
NCU07235 *	14.72	4.89 (0.33)	1.36 (0.09)	5.06 (0.34)	1.22 (0.08)	4.57 (0.31)
NCU11307 *	65.40	29.14 (0.45)	20.47 (0.31)	15.30 (0.23)	20.00 (0.31)	26.42 (0.40)
NCU09020 *	11.89	4.13 (0.35)	3.96 (0.33)	4.06 (0.34)	0.93 (0.08)	6.41 (0.54, ns)
NCU09428 *	5.67	0.89 (0.16)	1.16 (0.20)	1.50 (0.26)	0.54 (0.10)	1.02 (0.18)
NCU04910 *	85.13	25.19 (0.30)	26.93 (0.32)	26.09 (0.31)	34.94 (0.41)	31.90 (0.37)
NCU11922 *	35.84	8.82 (0.25)	4.41 (0.12)	14.70 (0.41)	5.61 (0.16)	10.08 (0.28)
NCU02098 *	48.30	17.72 (0.37)	9.38 (0.19)	22.13 (0.46)	6.04 (0.13)	37.14 (0.77, ns)
NCU09830 #	2.31	342.31 (148.19)	427.86 (185.22)	99.49 (43.07)	357.96 (154.96)	11.46 (4.96)

Fold change versus control (DMSO) is indicated between parentheses. ns: not significant.

Table 3
Annotation details of genes that are repressed (*) and induced (#) by all of the tested (thio)xanthone derivatives.

Gene	Annotation ^a	Conserved domains ^a	Observations ^a	STS [24] ^b	PHS [25] ^c	MENA [26] ^d
NCU04907	Hypothetical protein	Methyltransferase domain	Oxidative stress	Down	Down	Down
NCU00999	Hypothetical protein					
NCU07235	Hypothetical protein			Down		
NCU11307	CCC1	VIT family	Iron homeostasis; vacuolar iron transporter; oxidative stress	Down		Up
NCU09020	Hypothetical protein	C4-dicarboxylate transporter/malic acid transport protein		Down		
NCU09428	Hypothetical protein		Oxidative stress	Down		Down
NCU04910	Hypothetical protein	Methyltransferase domain	Oxidative stress	Down	Down	Down
NCU11922	Hypothetical protein		Also annotated as NCU16555	Down		Down
NCU02098	G/U mismatch-specific DNA glycosylase	Uracil DNA glycosylase superfamily; Mismatch-specific thymine-DNA glycosylase	Base-excision repair; oxidative stress			Down
NCU09830	ABC multidrug transporter	ABC transporter	Oxidative stress			Up ^e

STS: staurosporine, PHS: phytosphingosine, MENA: menadione.

^a *N. crassa* database at the Broad Institute (www.broadinstitute.org/annotation/genome/neurospora/MultiHome.html).

^b The dataset was obtained by RNA-seq.

^c The dataset was obtained by microarray analysis.

^d The dataset was obtained by digital gene expression profiling.

^e NCU09830 was the gene with the highest induction by menadione.

the (thio)xanthone derivatives was drug-specific (Fig. 2A–B). However, nine genes (Fig. 2B, i, marked with *) were repressed by all of the (thio)xanthone derivatives – NCU04907, NCU00999, NCU07235, NCU11307, NCU09020, NCU09428, NCU04910, NCU11922, and NCU02098 – and one gene (Fig. 2B, ii, marked with #) was induced by all of them—NCU09830. Table 2 presents the FPKMs for these genes in control- as well as in (thio)xanthone-treated cells and Table 3 describes their annotation. The function of the majority of these proteins is still unknown and they are all annotated as 'hypothetical proteins'. However, based on previous transcription profiling work, we noticed a clear trend that indicates that some of these genes are involved in oxidative stress because, as in the case of the (thio)xanthone derivatives, they were either repressed – NCU04907, NCU09428, NCU04910 and NCU02098 – or induced – NCU09830 – by different oxidative stress inducers, namely staurosporine, phytosphingosine and menadione [24–27]. Thus, the results suggest that xanthone derivatives affect the cellular machinery that is implicated in the response to oxidative stress. In addition, we observed that among the nine genes repressed by all of the tested (thio)xanthone derivatives, two encode proteins that harbor a methyltransferase domain, suggesting that proteins that possess this functional domain may be involved in the mechanism of action of this type of compounds.

We have recently characterized CZT-1, a transcription factor encoded by NCU09974 that is involved in staurosporine-induced cell

death in *N. crassa* [24]. Treatment with TX34 and XP13 also led to a strong induction of *czt-1* (Fig. 3A), further emphasizing its role in the response to cellular stress. In addition, NCU07235, NCU11307, NCU09020 and NCU11922, genes whose expression is repressed by all of the (thio)xanthone derivatives, are strongly repressed in a $\Delta czt-1$ deletion mutant [24], suggesting that these genes are under the regulatory control of the CZT-1 transcription factor.

Analysis of ABC transporter-encoding genes in the (thio)xanthone-treated gene expression datasets

We compiled a list of described and predicted ABC transporter-encoding genes in *N. crassa* and analyzed their expression levels upon treatment with the (thio)xanthone derivatives. Interestingly, we found that some genes encoding ABC transporters increased in expression level upon treatment with different (thio)xanthone derivatives. Besides NCU09830 (induced by all of the tested (thio)xanthone derivatives), we also observed that NCU05591 (induced by TX129, TX34 and XP13), NCU07546 and NCU09975 (induced by TX34 and XP13) and NCU04161 (induced by XP13) were induced by more than one of the compounds (Fig. 3B). Thus, our data indicate that NCU09830, NCU05591 and other ABC transporter-encoding genes appear to be an important target for xanthenes in *N. crassa*.

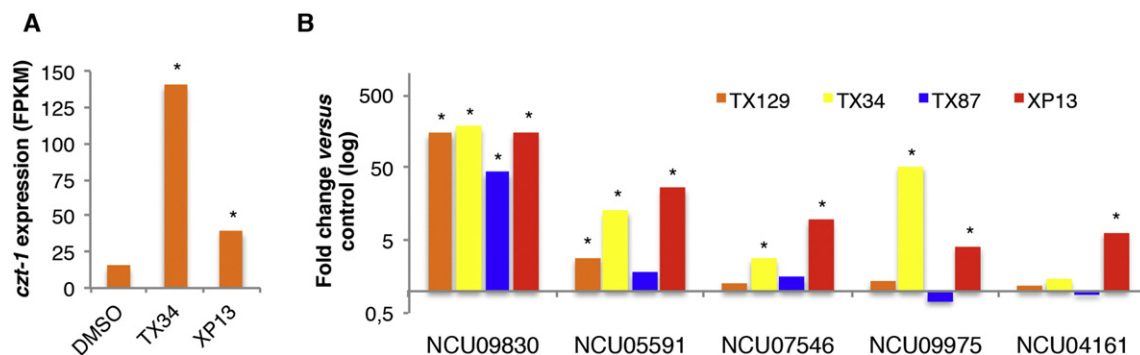


Fig. 3. (Thio)xanthone derivatives induce the expression of *czt-1* and of different members of the ABC transporter family. (A) The levels of expression (in FPKMs) of NCU09974 (*czt-1*) in *N. crassa* cells treated with TX34, XP13 (or DMSO) are presented. (B) The levels of expression (in FPKMs) of NCU09830 (*atr*), NCU05591 (*cdr4*), NCU07546, NCU09975 (*abc-3*) and NCU04161 in *N. crassa* cells treated with the (thio)xanthone derivatives were obtained and the fold change versus the control (DMSO) was calculated. *, *P*-value < 0.05.

Table 4

Functional enrichment analysis of genes whose expression was altered by D1 (P -value <0.0001).

FunCat ID	Category	P -value
<i>D1 (induced genes)</i>		
1	Metabolism	1.41E – 05
01.05	C-compound and carbohydrate metabolism	1.70E – 05
02.16	Fermentation	3.89E – 05
32.01.01	Oxidative stress response	2.48E – 05
32.07	Detoxification	9.99E – 08
<i>D1 (repressed genes)</i>		
1	Metabolism	2.89E – 07
01.01	Amino acid metabolism	1.27E – 07
01.01.06	Metabolism of the aspartate family	2.69E – 06
01.02	Nitrogen, sulfur and selenium metabolism	3.97E – 05
01.03.01	Purine nucleotide/nucleoside/nucleobase metabolism	4.14E – 05
01.05.13	Transfer of activated C-groups	6.39E – 05
01.05.13.03	Tetrahydrofolate-dependent C-transfer	7.71E – 06

NCU09830 and NCU05591 encode ATRB and CDR4, respectively, and share high similarity with ABC transporters of the pleiotropic drug resistance (PDR) subfamily [28]. In *N. crassa*, ATRB was found in a group of menadione-induced genes [27] while CDR4 is an important mediator of resistance to azole drugs [28]. NCU09975 and NCU07546 are ABC transporters that belong to the multidrug resistance (MDR) subfamily. NCU09975 encodes ABC-3, an ABC transporter that performs the efflux of staurosporine in *N. crassa* [29,30]. NCU04161 is an ABC transporter

that belongs to the multidrug resistance-associated protein (MRP) subfamily. Interestingly, the transcription factor CZT-1 regulates the expression of NCU09975, NCU07546 and NCU05591 [24], indicating that it may have an important role during the fungal response to (thio)xanthones.

The fungal secondary metabolite 2,4-dihydroxy-3-methylacetophenone elicits a defined transcriptional response in N. crassa

We also evaluated the transcriptional response of *N. crassa* cells to 2,4-dihydroxy-3-methylacetophenone (termed 'D1' from now on) (Fig. 1), a secondary metabolite from the culture of *Neosartorya siamensis* KUFC 6349 collected from soil in Thailand [31]. A 1-hour treatment of *N. crassa* cells with 20 μ M D1 resulted in the alteration of the expression levels of 3.5% of the genes (Fig. 2A, v, left panel), which is comparable with the TX34 profile. D1 caused a similar fraction of gene induction and repression (Fig. 2A, v, right panel). Functional enrichment among the genes that were either induced or repressed by D1 was evaluated using FunCat (Supplemental File 2 and Table 4). D1 induced genes involved in carbon compound and carbohydrate metabolism, fermentation, oxidative stress response and detoxification (Table 4), indicating an active response of the fungus in the presence of this compound. In fact, analysis of the genes included in the 'detoxification' category revealed that D1 led to the upregulation of genes within the ABC transporter family, namely NCU05591 (*cdr4*), NCU07546 and NCU09830 (*atr*), which is a shared feature with some of the aforementioned xanthone

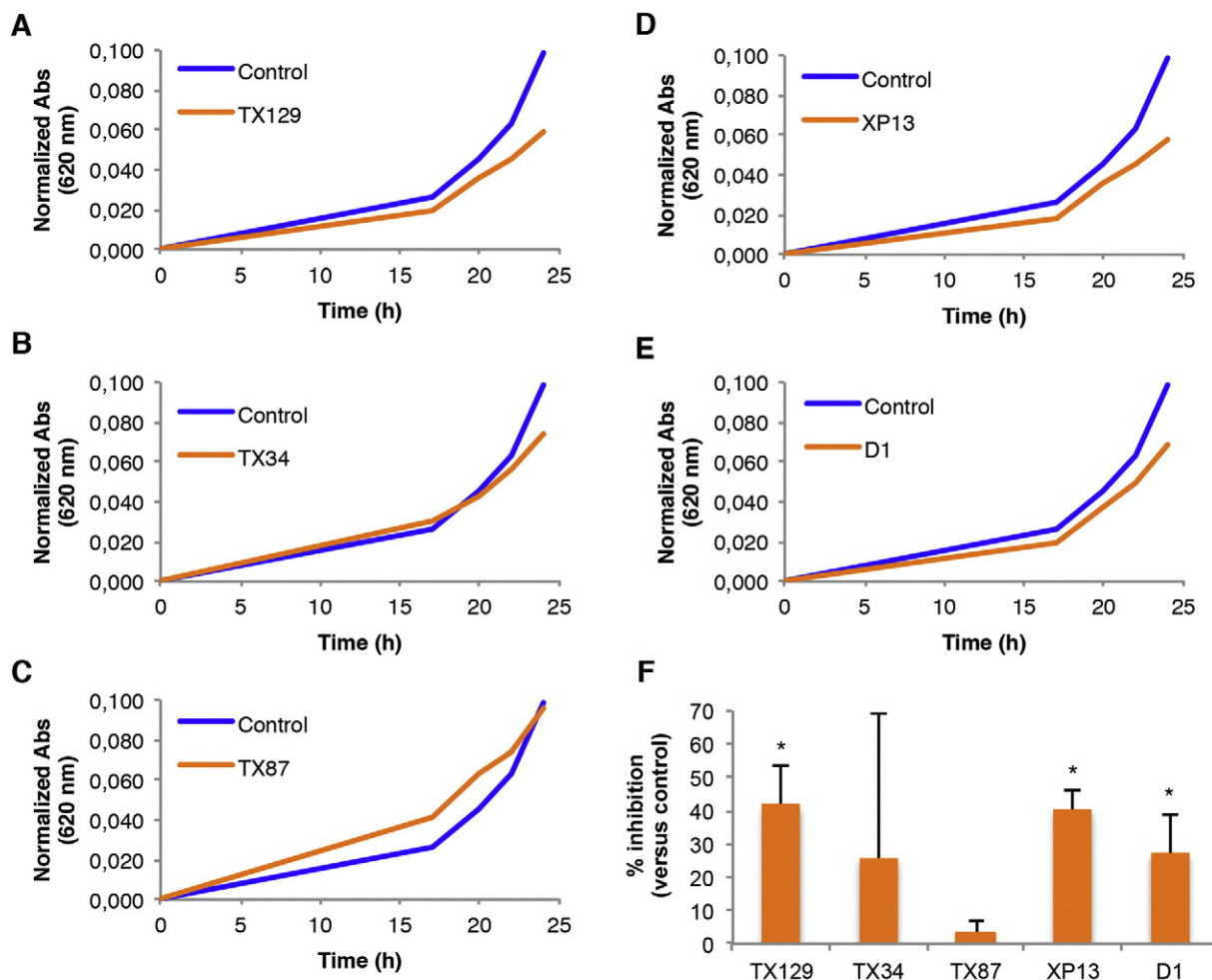


Fig. 4. Growth of *N. crassa* cells in the presence of the (thio)xanthone derivatives and the *N. siamensis*-derived compound D1. (A-E) Growth was evaluated by following the absorbance at 620 nm of *N. crassa* cells in culture medium supplemented with TX129 (A), TX34 (B), TX87 (C), XP13 (D) and D1 (E). (F) The percentage of inhibition caused by each of the compounds at the 24 hour time point is presented. *, P -value <0.05 .

derivatives. Among the repressed genes, there was an enrichment of genes involved in several categories related with cell metabolism, including metabolism of aspartate, nitrogen, sulfur, selenium, purines and tetrahydrofolate (Table 4). Interestingly, also like the tested (thio)xanthone derivatives, D1 led to the repression of NCU04907, NCU07235, NCU11307, NCU09428, NCU04910 and NCU11922 (Table 2), suggesting that D1 and (thio)xanthones activate common intracellular pathways and further emphasizes that the downregulation of these genes is related to a fungal stress response.

(Thio)xanthone derivatives and D1 inhibit the growth of *N. crassa*

Previous reports have shown that xanthone derivatives possess anti-proliferative and pro-apoptotic activity [2,11–14]. Thus, we evaluated the growth of *N. crassa* cells in the presence of the (thio)xanthone derivatives and the acetophenone isolated from *N. siamensis*. TX129, XP13 and D1 markedly inhibited the growth of *N. crassa* while TX34 and TX87 did not have a significant impact on growth (see curves in Fig. 4A–E and quantification of the data for the 24 hour time point in Fig. 4F). Importantly, XP13 ranked first as the compound causing the most dynamic transcriptional response, altering the expression of approximately 16% of the *N. crassa* genes (Fig. 2A, iv). Thus, the aforementioned transcriptional alterations caused by the (thio)xanthone derivatives TX129 and XP13 and the acetophenone D1 are associated with an deleterious effect on fungal growth.

Conclusions

The availability of high-throughput methodologies to manipulate simple models like *N. crassa* constitutes a major advantage to study intracellular pathways that are targeted by exogenous compounds. Our results pointed out that ABC transporters and a group of mostly uncharacterized genes are targets of xanthone and acetophenone derivatives, suggesting that they correspond to common fungal stress response elements. In addition, our work provides several datasets that may orientate future studies on the mechanisms underlying the inhibitory effects on cell growth caused by xanthones.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.gdata.2015.02.001>.

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