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Antimycotic activity of 4-thioisosteres of flavonoids towards yeast and yeast-like microorganisms

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ABSTRACT

Different substituted methoxy- and hydroxy-4-thioisosteres of flavonoids were prepared and their in vitro antimycotic activity towards yeast (*Candida* spp., *Clavispora* spp., *Cryptococcus* spp., *Filobasidiella* spp., *Issatchenkia* spp., *Pichia* spp., *Kluyveromyces* spp., *Saccharomyces* spp. and *Yarrowia* spp.) and yeast-like (*Prototheca* spp.) microorganisms was tested. Further insights in the biological activities of these antioxidant, oestrogenic and antimicrobial biomimetic derivatives were obtained.

Flavonoids are ubiquitous in photosynthesising eukaryotic cells and therefore occur widely in the plant kingdom.[1] They are found in fruit, vegetables, nuts, seeds, stems and flowers as well as tea, wine,[2] propolis and honey,[3] and represent a common constituent of the human diet[4] with a daily dietary intake of mixed flavonoids ranging from hundreds to thousands of mg in occidental countries.[5] In plants they provide colours,[1,6] protect from fungal pathogens and UV-B radiation[3,6] and are involved in photosensitisation, energy transfer, growth regulation, respiration and photosynthesis. Flavonoids are becoming the subject of human medical research. They have been reported to possess many useful properties, including enzyme inhibition, anti-inflammatory, oestrogenic, antimicrobial,[7] antiallergic, antioxidant,[1] vascular and cytotoxic antitumour activities.[5] Owing to the widespread ability of flavonoids to inhibit spore germination of plant pathogens, they have been proposed for use as antimycotic drugs against yeasts and filamentous fungi known as human pathogens, like *Candida albicans*,[8] *Aspergillus flavus*,[9] *Aspergillus tamarii, Cladosporium sphaerospermum, Penicillium digitatum* and *Penicillium italicum*.[10]

During the last years, 4-thiaflavans, the compounds with a sulfur atom replacing the C4 in the C ring of the Flavan skeleton (Fig. 1), have emerged as biologically interesting isosteres for their oestrogenic,[11] antimicrobial[12] and antioxidant[13] activities.

In particular, exploiting our practical and flexible access to hydroxy-4-thiaflavans, based on the inverse electron-demand hetero Diels–Alder reaction (HDAR) of *o*-thioquinones with styrenes (Scheme 1), we were able to prepare properly substituted derivatives which, for their ability as 'catechin-like' and/or 'tocopherol-like' radical scavengers,[13] metal (i.e., Fe^{2+} ions) chelators[13d] as well as hydroperoxides quenchers,[13e] can be considered valuable multi-defence antioxidants.

With the aim to better understand the range of biological activity of these compounds, and considering a possible use as additives against oxidation of tissues or different materials, including foods and rubbers, we were interested in the measurement of their antimycotic activity. In this light, we prepared 4-thiaflavans **1**–**12**, reported in Scheme 1, whose structure was chosen taking into consideration the ability as antioxidants,[13] and the structural substitution patterns of natural flavonoids.

The possibility to assemble, the thiaflavan skeleton fusing two properly pre-equipped moieties through a HDAR, allowed the easy modulation of the substitution pattern on both A and B aromatic

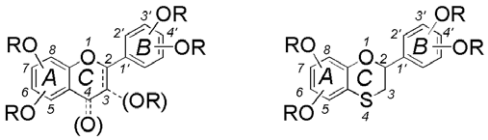

Figure 1. Flavan and 4-thiaflavan skeletons.

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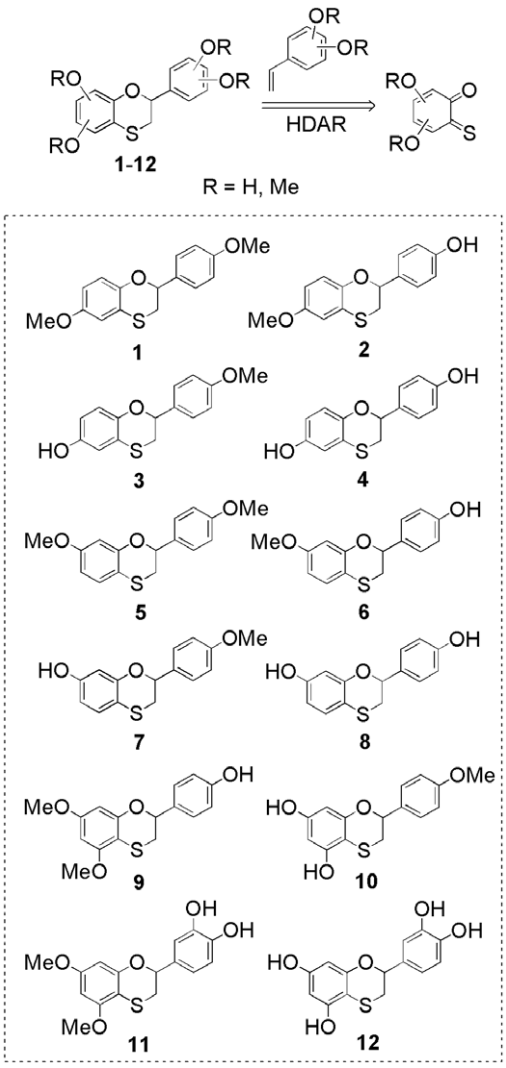

Scheme 1. Retrosynthetic approach and structures of 4-thiaflavans **1**–**12** tested in this study.

rings: a difficult task using natural flavonoids as starting materials. Accordingly, yeast (*Candida* spp., *Clavispora* spp., *Cryptococcus* spp., *Filobasidiella* spp., *Issatchenkia* spp., *Pichia* spp., *Kluyveromyces* spp., *Saccharomyces* spp. and *Yarrowia* spp.) and yeast-like (*Prototheca*spp.) microorganisms belonging to species considered as opportunistic pathogens for humans and animals were selected as target microorganisms.[14]

Data obtained are reported in Tables 1 and 2. Preliminarily, the activity of derivatives **1**–**12** was checked towards a wide range of strains and is reported as diameter of growth inhibition halo (mm) in Table 1.[15] On the basis of the antimycotic spectrum of derivatives **1**–**12** (Table 1) and the characteristics and availability of target strains, seven thiaflavans (**3**, **4**, **6–8**, **10** and **11**) and 10 yeasts were selected for the determination of the minimal inhibitory concentrations (MICs, expressed as μg/mL), reported in Table 2.[16]

On the whole, we can observe a certain activity for seven out of twelve of the thiaflavans tested. Due to the lack of a rationale elucidating the observed antimycotic activity, only qualitative, yet worthy of note, considerations can be done.

The lack of hydroxy groups on A and B rings, like in **1** and **5**, prevents antimycotic activity. This is in perfect agreement with data that emerged in a preliminary evaluation, and convinced us not to further investigate fully methoxylated derivatives and sulfur oxidized thiaflavans, which have demonstrated to be inactive as well.[12] Apart from this evidence, our results showed, at least for the 12 compounds tested in this study, that no direct relationship exists between the number of OH groups and the whole anti-yeast activity, as it could be expected in consideration of the similarity with natural flavonoids (vide infra).

The ineffectiveness of derivative **12**, bearing four hydroxy groups, the better antiradical derivative amongst those tested,[13] is probably the more explicit demonstration that no relationship exists between the antioxidant activity and the antimycotic activity of these hydroxy-4-thiaflavans. This is corroborated by the comparison of derivative **6** with **7** or **9** with **10** where the increasing antiradical ability corresponds to a decreasing antimycotic activity.

Data reported in Tables 1 and 2 clearly indicate that the 4′,7-substitution pattern, with at least a free OH group, as in compounds **6**–**8**, represents the better situation to achieve a high de-

Table 1
Diameters of growth inhibition halos (standard error ⩽ 0.5 mm) of thiaflavans **1**–**12** towards yeast and yeast-like microorganisms

Species[a]	DBVPG[b]	1	2	3	4	5	6	7	8	9	10	11	12
Candida albicans	6133[T]						17.5		16.2		12.4		
Candida albicans	6157						16.5	14.1	18.3			12.1	
Candida glabrata	7212								13.1				
Pichia guilliermondii	6140[T]								17.2				
Candida tropicalis	3982[T]						20.2		15.5				
Candida zeylanoides	6163						19.4		16.4				
Clavispora lusitaniae	6142[T]				12.9		15.3		12.4			13.9	
Issatchenkia orientalis	6782[T]						17.6		14.0				
Kluyveromyces marxianus	6141[T]				13.3		24.3		20.2		14.2	14.9	
Saccharomyces cerevisiae	6173[T]				15.2		24.1		15.6		15.1	14.6	
Saccharomyces cerevisiae	6500						18.3	20.6	19.2		14.7	14.9	
Yarrowia lipolitica	6053[T]				14.3		15.8	15.6	16.5				
Cryptococcus laurentii	3883				13.8		18.2		22.6			13.1	
Cryptococcus laurentii	4272			22.0	16.4		30.2	26.4	28.5		17.5	19.3	
Cryptococcus laurentii	6265[T]				16.9		20.6	16.8	17.8		13.3	2.6	
Filobasidiella neoformans	3428						20.0	14.6	17.4				
Filobasidiella neoformans	6010[T]			13.6	15.5		35.1	18.1	15.5				
Filobasidiella neoformans	6225				11.2		18.4	14.9	17.0				
Filobasidiella neoformans	6981						25.1	14.2	15.6				
Filobasidiella neoformans	6982							12.9					
Prototheca wickerhamii	8879						20.7		17.8			14.5	

[a]Grey stripes indicate the strains selected for minimal inhibitory concentration (MIC) determination (see Table 2).
[b]DBVPG accession number.
[T]Type strain of the given species.

Table 2
Minimal inhibitory concentrations (MICs) (μg/mL) of thiaflavans **3**, **4**, **6**–**8**, **10** and **11** towards the selected yeasts

Species	DBVPG[a]	Other collections[b]	**3**	**4**	**6**	**7**	**8**	**10**	**11**
Candida albicans	6133[T]	CBS 562			32		512	512	
Pichia guilliermondii	6140[T]	CBS 566					512		
Candida tropicalis	3982[T]	CBS 94			16		512		
Clavispora lusitaniae	6142[T]	CBS 4413		256	64		512		512
Kluyveromyces marxianus	6141[T]	CBS 834		128	16		128	128	128
Saccharomyces cerevisiae	6173[T]	CBS 1171		256	16		512	512	128
Yarrowia lipolitica	6053[T]	CBS 6124		256	128	256	256		
Cryptococcus laurentii	4272		32	128	8	32	256	256	128
Cryptococcus laurentii	6265[T]	CBS 139		256	16	128	256	128	256
Filobasidiella neoformans	6010[T]	CBS 132	256	512	8	32	256		

[a] DBVPG accession number.
[b] Correspondence with strains collected in other worldwide collections.
[T] Type strain of the given species.

gree of antimycotic activity. Compound **6** (4′-hydroxy-7-methoxy-4-thiaflavan) was in fact the most effective in terms of number of inhibited strains and MICs values. Natural flavans bearing the 4′,7-substitution pattern are known since very long time.[17] Recently some of them have been synthesised and tested against a few microorganisms, including *C. albicans*, showing an activity smaller than the corresponding 4-thioisosteres **6**, **7** and **8**.[18] On the other hand, no activity has been observed for many commercially available flavonones and flavonols, bearing a substitution pattern similar to that of compounds **9**–**12**, when they have been tested against the yeast and yeast-like strains used in this study.[19]

Current literature reports that the ability of hydroxy groups occurring in polyphenol structures to complex proteins (through the formation of either hydrogen or covalent bonds) and carbohydrates can be considered responsible for their bioactivity.[20] Despite we cannot ruling out that the activity of 4-thiaflavans could be ascribed to a similar mechanism, the above considerations, based on data reported in Tables 1 and 2, seem to imply for these compounds the presence of additional mechanism(s) where the sulfur atom in position 4 of C ring might be possibly involved.

In conclusion, we have reported a further achievement in the evaluation of the biological activities of 4-thiaflavans with the identification of the better substitution pattern for the expression of antimycotic activity. Further studies on biological abilities and potential utilisations of these valuable synthetic isosteres of flavonoids are ongoing.

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15. Yeast and yeast-like target strains are conserved in the DBVPG Collection of Industrial Yeasts of the University of Perugia, Italy (www.agr.unipg.it/dbvpg). Working cultures were maintained on YEPG (yeast extract 1%, peptone 1%, glucose 2%, agar 1.5%) slants at 4 °C until use. Preliminary tests on antimycotic activity of 4-thioisoesteres of flavonoids were carried out by the agar diffusion disk bioassay (ADDB).
16. MIC determination was carried out in 96-well microplates (Corning Inc., USA) in agreement with the CLSI (Clinical and Laboratory Standard Institute) recommendations. In Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Approved Standard, 2nd ed.; Document M27-A2, Wayne, PA, USA, 2002. All seven 4-thiaflavans used for the MIC determination were tested in duplicate. No discrepant results were observed in repeated experiments.
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