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Obtaining new flavanones exhibiting antifungal activities by methyltrioxorhenium-catalyzed epoxidation–methanolysis of flavones

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ABSTRACT

New 3-hydroxy-2-methoxyflavanones have been obtained through epoxidation–methanolysis of the corresponding flavone with urea–hydrogen peroxide (UHP)/methyltrioxorhenium (CH_3ReO_3, MTO) catalytic system in methanol as nucleophilic solvent. After acetylation of the reaction mixtures, the corresponding *cis*- and *trans*-3-acetoxy-2-methoxyflavanones have been isolated and characterized by spectroscopic analyses. Their antifungal activity has been tested in vitro against three fungal strains of common saprotrophic soil and seed fungi, such as *Trichoderma koningii*, *Fusarium solani* and *Cladosporium herbarum*, potentially pathogenic for humans. Some aspects of the structure–activity relationship of the most active compounds have been evaluated. The mycelial growth of *T. koningii* and *C. herbarum* has been totally inhibited from *cis*-3-acetoxy-2,6-dimethoxyflavanone **7c** and *cis*-3-acetoxy-2,7-dimethoxyflavanone **13c** at the lowest concentration (0.5×10^{-4} M).

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1. Introduction

Flavonoids are polyphenolic secondary metabolites ubiquitous in edible plant foods and beverages. In fact, they naturally occur in fruits, vegetables, nuts, seeds, tea and wine, and are an integral part of the human diet.[1] In the plant kingdom, they are involved in important biological processes such as nitrogen fixation, photosensitization, energy transfer, plant growth, control of respiration and photosynthesis.[2] Figure 1 shows the generic chemical structure of flavonoids (**a**) based on the flavan nucleus and the numbering system used to distinguish the carbon positions around the molecule. The three phenolic rings are referred to as the A, B and C (or pyrane) rings. Among them, flavones and flavanones have an oxo group in C4; flavones have also a double bond in C2–C3 (Fig. 1, **b** and **c**). These compounds as well as their mixtures possess high antifungal potential against different species usually occurring on grains,[3] seeds and pods.[4]

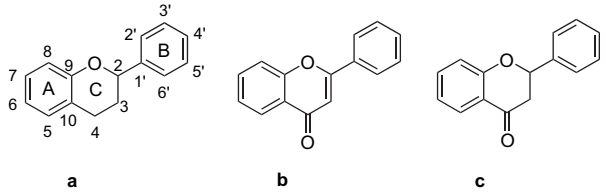

Figure 1. The generic structure of flavonoids.

With the aim of synthesizing new flavanones, we have considered to functionalize the double bond of flavones by an oxidative addition pathway. It is known that the C2–C3 double bond of these compounds is unreactive towards classical oxidants such as H_2O_2/OH^-,[5] $KMnO_4$,[6] NiO_2,[7] SeO_2 [8] and $Tl(OAc)_3$.[9] Only freshly prepared acetone solutions of dimethyldioxirane[10] or methyl(trifluoromethyl)dioxirane[11] are able to convert flavones into the corresponding epoxides. However, dioxiranes are difficult to handle being gaseous, explosive and having a poor solubility. Furthermore, to the best of our knowledge, no general catalytic oxidation procedures for the oxidative attack of the double bond of flavones have been reported. In the last years, methyltrioxorhenium (CH_3ReO_3, MTO)[12] together with hydrogen peroxide or urea–hydrogen peroxide [13] has become an important catalyst for a variety of synthetic transformations such as the epoxidation of alkenes,[14] conjugated dienes,[15] oxidation of alkynes,[16] aromatic derivatives,[17] sulfur compounds,[18] anilines and amines,[19] phosphines,[20] C–H and Si–H bonds [21] and the Bayer–Villiger rearrangement.[22] The reactive intermediates for these oxidations are monoperoxo $[CH_3Re(O)_2O_2]$ and bis-peroxo $[CH_3ReO(O)_2]\eta^2$-rhenium complexes (Scheme 1).[23]

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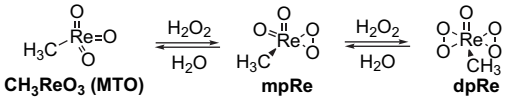


Scheme 1. H_2O_2/CH_3ReO_3 catalytic system.

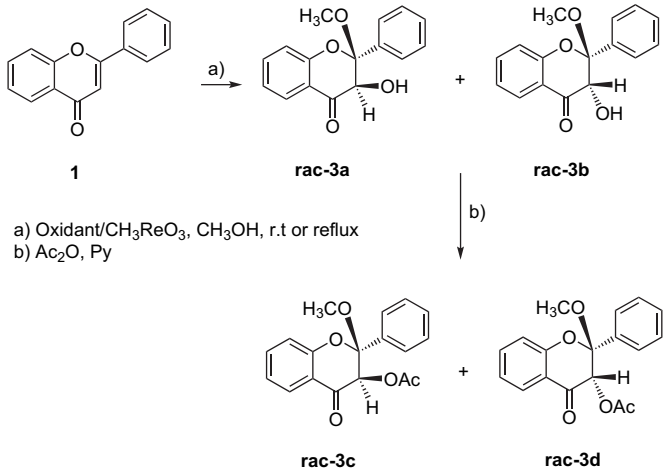
For many years, our research group has been investigating the reactivity of the hydrogen peroxide/methyltrioxorhenium catalytic system on natural organic compounds in order to get new bioactive compounds and fine chemicals.[24] In particular, we first described the Bayer–Villiger oxidation of the flavanone C-ring to prepare new lactones exhibiting apoptotic activity on tumoral cell lines [25] and the oxidation of the A-ring of catechins to obtain new *p*-benzoquinones.[26]

In this paper, we report the epoxidation–methanolysis of the double bond of monosubstituted flavones catalyzed by methyltrioxorhenium and urea–hydrogen peroxide (UHP) as the oxidant in the presence of methanol as nucleophilic solvent. After acetylation of the oxidation products, the stable new *cis*- and *trans*-3-acetoxy-2-methoxyflavanone derivatives were tested as antifungal agents against three fungal strains of common saprotrophic soil and seed fungi,[27] such as *Trichoderma koningii*, *Fusarium solani* and *Cladosporium herbarum*, potentially pathogenic for humans.[28] Some considerations on structure–activity relationships are also reported.

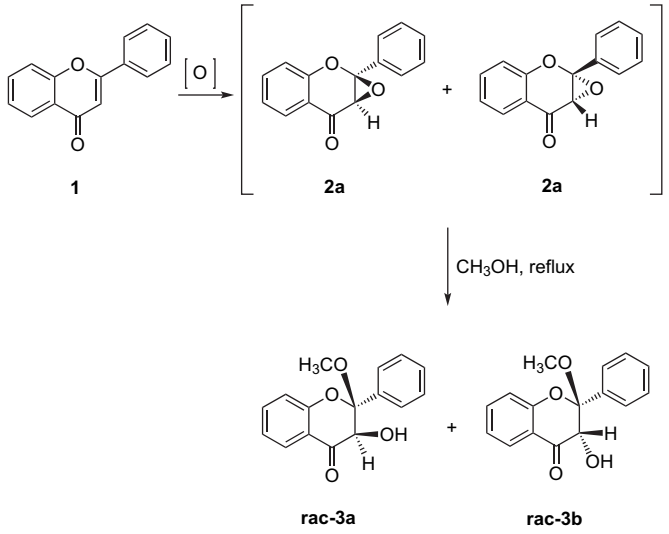
2. Results and discussion

Preliminary experiments on oxidation were carried out using flavone **1** as the substrate model (Scheme 2). Hydrogen peroxide (50% water solution) or urea–hydrogen peroxide (UHP) was used as oxidants in the presence of a catalytic amount of methyltrioxorhenium. In these reaction conditions, the corresponding *cis*- and *trans*-3-hydroxy-2-methoxyflavanones **3a** and **3b** were obtained. The best conversion of the flavone **1** was obtained using UHP/MTO in methanol at reflux (conversion and yield: 62%). Due to their instability during the chromatographic purification, compounds **3a** and **3b** were isolated and characterized as acetates after acetylation of the crude reaction mixture. As expected, the corresponding *cis*- and *trans*-3-acetoxy-2-methoxyflavanones **3c** and **3d** were isolated in a 32/68 ratio (**3c**/**3d**=1.0/2.1) and characterized by spectroscopic analyses.

After double bond epoxidation by the catalytic system UHP/CH_3ReO_3, the obtained epoxides **2a**,**b** undergo a rapid ring opening reaction in the solvolytic conditions to get **3a** and **3b** (Scheme 3), as reported by Adam for the dioxirane reaction.[10] All efforts to isolate the epoxides **2a**,**b** in a non-nucleophilic solvents failed. In fact, in dichloromethane and diethyl ether no appreciable conversion of the flavone **1** occurred. The structural assignation of compounds **3a** and **3b** was confirmed by NOESY experiments on the corresponding acetylated products **3c** and **3d**. An S_N1-like mechanism probably operated in these conditions as suggested by the polarimetric measurements performed on the *cis*- and *trans*-3-hydroxy-2-methoxyflavanones **3c** and **3d**, both of them resulted as a racemic mixture. The **3c**/**3d**=32/68 ratio was explained by molecular calculations as an outcome of the different stability of the cis–trans stereoisomers.



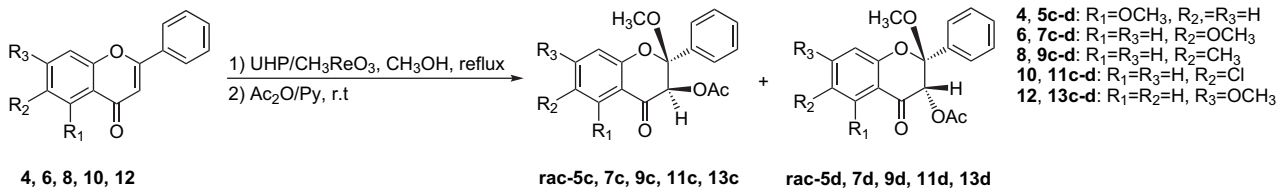
Scheme 2. Oxidation–methanolysis of the flavone **1** with the UHP or H_2O_2/CH_3ReO_3 catalytic system.



Scheme 3. Possible mechanism of oxidation–methanolysis of the flavone **1** with the UHP/CH_3ReO_3 catalytic system.

After these initial investigations, we extended the epoxidation–methanolysis to the monosubstituted flavones **4**, **6**, **8**, **10** and **12**. The successive acetylation of the crude mixtures allowed the isolation of the corresponding new *cis*- and *trans*-flavanones **5c**,**d**, **7c**,**d**, **9c**,**d** and **13c**,**d** (Scheme 4, Table 1). The unreactivity of 6-chloroflavone **10** (Table 1, entry 4) with UHP/MTO was attributed to the deactivating effect of the chlorine substituent on the double bond of the benzopyranic ring.

The oxidation performed on 5,7-dimethoxyflavone **14** in the same experimental conditions led to the formation of a complex mixture of products. However, by monitoring the oxidation at room temperature, despite lower conversion of the substrate (35%), we obtained a mixture of two compounds, inseparable by column



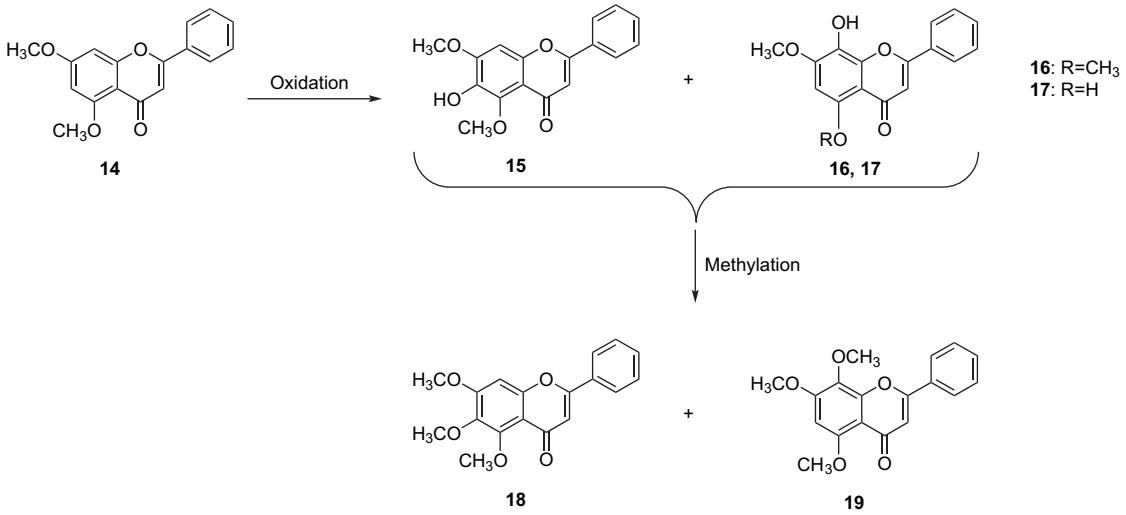
Scheme 4. Oxidation of the flavones **4**, **6**, **8**, **10** and **12** with the UHP/CH_3ReO_3 catalytic system in methanol and successive acetylation.

Table 1
Oxidation of the flavones **4**, **6**, **8**, **10** and **12**, and successive acetylation of the products as depicted in Scheme 4

Entry	Substrate	Reaction time (h)[a]	Conv. (%)	Yields (%)[b,c]	Ratio
1	**4**	24	62	**5c**: 25, **5d**: 75	**5c**/**5d**=1.0/3.0
2	**6**	24	50	**7c**: 29, **7d**: 71	**7c**/**7d**=1.0/2.4
3	**8**	24	66	**9c**: 26, **9d**: 74	**9c**/**9d**=1.0/2.8
4	**10**	48	<5	—	—
5	**12**	24	52	**13c**: 22, **13d**:78	**13c**/**13d**=1.0/3.5

[a] UHP (6 equiv), CH_3ReO_3 (0.05 mmol), CH_3OH, reflux.
[b] Calculated after chromatographic purification.
[c] Calculated on the converted substrate.

chromatography on silica gel, assigned as 6-hydroxy-5,7-dimethoxyflavone **15** (yield: 10%) and 8-hydroxy-5,7-dimethoxyflavone **16** (yield: 10%, Scheme 5). A third compound with a lower R_f was eluted having the structure of 5,8-dihydroxy-7-methoxyflavone **17**, probably derived from oxidative demethylation of **16** (yield: 15%). Therefore, in the presence of two C-5 and C-7 methoxy groups on the aromatic A ring, the catalytic oxidant system did not attack the C2–C3 double bond, the oxyfunctionalization of the activated A ring being favoured, as already reported by us for the methylated catechins.[26] After methylation of mixture of compounds **15** and **16**, the corresponding products **18** and **19** were easily separated by chromatographic column and characterized by spectroscopic analysis. As expected, the methylation of **16** and **17** gave the same compound **19**.



Oxidation: H_2O_2 (50% water solution, 2 eq.); CH_3ReO_3 (0.05 mmol), CH_3OH, 25 °C, 2h
Methylation: DMF, CH_3I (10 eq.), 25 °, 24 h

Scheme 5. Oxidation of the flavones **4**, **6**, **8** and **10** with the UHP/CH_3ReO_3 catalytic system in methanol and successive acetylation.

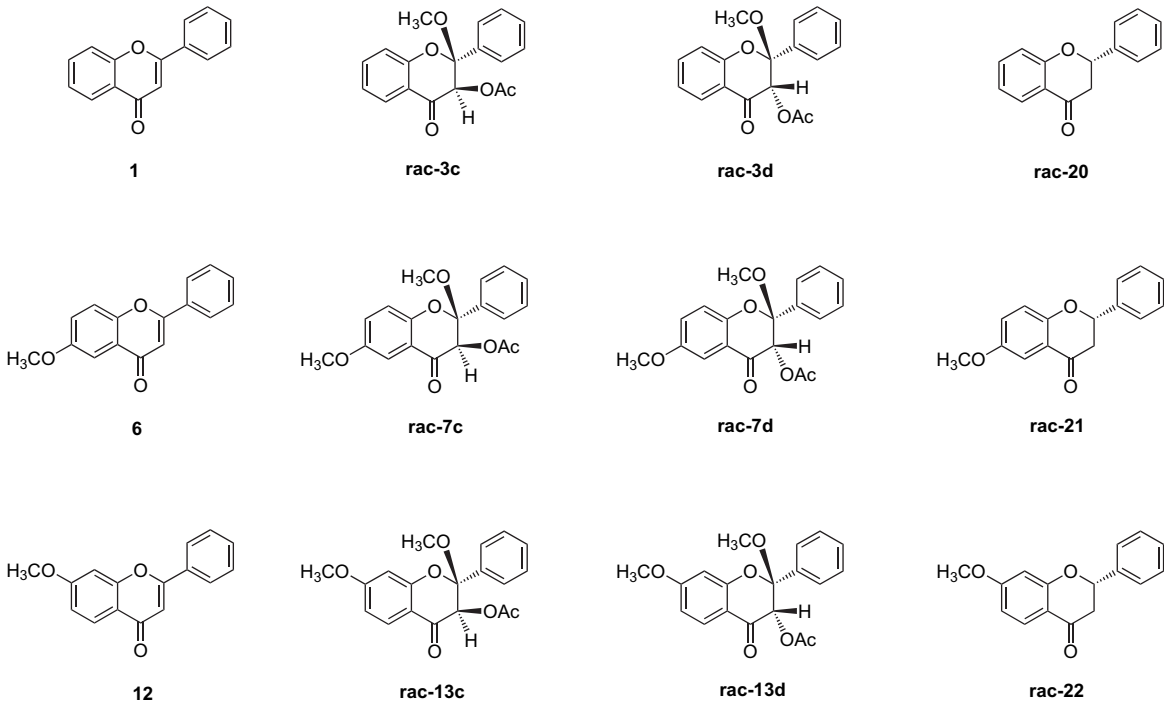


Figure 2. Compounds tested on the antifungal activity.

Table 2
Inhibition (%) of linear mycelial growth of *T. koningii*, *F. solani* and *C. herbarum*[a,b]

Entry	Compound	*T. koningii*			*F. solani*			*C. herbarum*		
		8×10^{-4} M	2×10^{-4} M	0.5×10^{-4} M	8×10^{-4} M	2×10^{-4} M	0.5×10^{-4} M	8×10^{-4} M	2×10^{-4} M	0.5×10^{-4} M
1	**1**	79.3	85.1	26.4	63.7	58.2	5.80	27.1	28.0	13.3
2	**3c**	78.3	75.0	55.6	55.0	44.7	23.1	72.7	66.0	41.1
3	**3d**	53.0	43.5	32.3	16.7	7.40	3.02	48.8	32.3	26.1
4	**5c**	40.0	10.9	4.01	9.22	3.21	2.20	28.8	7.30	—
5	**5d**	55.8	38.7	32.0	14.2	—	—	31.2	9.80	—
6	**6**	35.2	35.2	32.5	—	—	—	11.0	13.3	14.5
7	**7c**	74.2	85.2	98.9	13.8	4.10	3.20	67.0	73.5	100
8	**7d**	—	—	—	—	—	2.50	—	—	—
9	**9c**	49.2	48.3	53.5	15.6	9.60	9.70	55.3	44.5	39.1
10	**9d**	48.4	31.8	23.9	5.50	—	—	23.0	9.70	6.40
11	**12**	62.0	62.7	52.2	24.3	23.9	12.4	59.7	53.8	48.6
12	**13c**	87.8	98.0	100	42.9	46.0	50.0	59.1	100	100
13	**13d**	15.6	14.7	5.30	2.01	3.80	4.81	7.20	—	—
14	**20**	68.4	66.6	62.0	15.8	11.7	13.2	8.90	11.4	—
15	**21**	31.0	30.5	33.2	—	—	—	3.50	4.60	—
16	**22**	65.0	60.0	53.0	24.0	14.5	2.80	45.8	43.2	42.4

[a] Data were evaluated by analysis of the variance; the probability of single differences was calculated at 5% level; all data were statistically significant at this level.
[b] Non-reported data indicated as "—" correspond to no significant growth inhibition.

The obtained products are the methylated derivatives of natural bioactive compounds. In fact, 5,6,7-trimethoxyflavone **17** is the trimethyl ether of baicalein, the main component of a traditional Chinese herbal medicine *Scutellaria baicalensis* having multiple biological activities including antiallergic, anticarcinogenic and anti-HIV properties.[29] Recent studies report that this compound has an important role in the cure of Parkinson's disease inhibiting the fibril formation of α-synuclein or disaggregating its existing fibrils;[30] 5,7,8-trimethoxyflavone **18** is the 5,7-dimethyl ether of wogonin,[31] a component of pharmaceutical preparations for treatment of gastrointestinal diseases, regeneration of nerve and neural stem cells and antiinflammatory agents.[32]

3. Antifungal activities

The new *cis*- and *trans*-3-acetoxy-2-methoxyflavanones **3c**–**3d**, **5c**–**5d**, **7c**–**7d**, **9c**–**9d** and **13c**–**13d** were screened in vitro for their antifungal activity against three fungal strains of common saprotrophic soil and seed fungi, [27] such as *T. koningii*, *F. solani* and *C. herbarum*, potentially pathogenic for humans.[28] The antifungal activity of the most active compounds **3c**, **7c** and **13c** was compared with those of the corresponding starting materials (flavones **1**, **6** and **12**) and of the structurally analogous flavanones **20**–**22** (Fig. 2). All new compounds were tested at three different concentrations (0.5, 2.0 and 8.0×10^{-4} M). As reported in Table 2, a significant antifungal activity against the three fungal strains has been observed for all tested compounds. The only exception is flavanone **7d**. According to Weidenborner et al. and Silva et al.,[4] no linear relationship between the concentration and the antifungal activity has been observed for some tested compounds. As shown, all new cis derivatives are more active than the corresponding trans derivatives (compare entry 2 with entry 1; entry 6 with entry 5; entry 10 with entry 9; entry 14 with entry 13; entry 16 with entry 15). Between them, the cis derivatives **3c**, **7c** and **13c** are the most active compounds against the three fungal strains tested. In particular, also at low concentration (0.5×10^{-4} M) compounds **7c** and **13c** totally inhibited the growth of *T. koningii* and *C. herbarum* (see entries 6 and 10). Their activity was compared with those of the corresponding starting materials such as flavones **1**, **6** and **12** and of the structurally analogous flavanones **20**–**22** (Fig. 2). As reported in Table 2, the antifungal activities of flavones **1**, **6** and **12** against *T. koningii, F. solani* and *C. herbarum* were similar to flavanones **20**–**22** (compare entry 1 with entry 14; entry 6 with entry 15; entry 12 with entry 22). These activities appeared to increase in the new cis-synthesized flavanones **3c**, **7c** and **13c**.

4. Conclusions

New *cis*- and *trans*-3-hydroxy-2-methoxyflavanones have been obtained by the oxidation of the flavone double bond with urea–hydrogen peroxide (UHP)/methyltrioxorhenium (CH_3ReO_3, MTO) catalytic system in methanol as nucleophilic solvent. After acetylation of the reaction products and chromatographic purification, the corresponding *cis*- and *trans*-3-acetoxy-2-methoxyflavanones were isolated. These compounds have been tested as antifungal agents against three fungal strains of common saprotrophic soil and seed fungi, such as *T. koningii*, *F. solani* and *C. herbarum*, potentially pathogenic for humans. A structure–activity relationship showed that the introduced chemical modification influences the antifungal activity. In fact, the reaction products were generally more active than the corresponding starting materials (commercial flavones) and the structural analogues (commercial flavanones). Moreover, the relative stereochemistry of the 2-methoxy and 3-acetoxy groups was an important factor. In fact, the cis isomers showed higher activity than the corresponding trans isomers. In particular, the cis derivatives **3c**, **7c** and **13c** possessed the highest activity against all three fungal strains tested, even at low concentration (0.5×10^{-4} M).

5. Experimental

5.1. General

All reagents were of the highest grade available and were used as such (Sigma–Aldrich–Fluka). Chromatographic purifications were performed on columns packed with Merck silica gel 60, 230–400 mesh. Thin layer chromatography was carried out using Merck platen Kieselgel 60 F_{254}. ^1H and ^{13}C NMR spectra were recorded on a Bruker 200 MHz. Mass spectra were recorded with a GC Shimadzu GC-17A equipped with an electron beam of 70 eV, an SPB column (25 m×0.30 mm and 0.25 mm film thickness) and a mass-selective detector QP 6000. The injector temperature was 280 °C. An isothermal temperature profile of 60 °C for 5 min, followed by a 10 °C/min temperature gradient to 250 °C for 10 min was used. Chromatographic grade helium was used as the carrier gas.

5.2. Oxidation of flavones: general procedure

A 10 ml reaction flask was charged with methyltrioxorhenium (0.05 mmol) and urea–hydrogen peroxide (2.0 mmol) in methanol (5 ml). The stirred solution became yellow due to the formation of peroxo complexes and after 5 min the flavone derivative (0.5 mmol)

was added. The mixture was stirred at reflux for 8–24 h. The oxidation was monitored by TLC and GC–MS analysis. Depending on the substrate, successive equivalents of urea–hydrogen peroxide were added. At the end of the reaction, the solvent was removed under reduced pressure; the residue was extracted with dichloromethane (3×10 ml), dried over Na_2SO_4 and concentrated to afford a yellow oil. The reaction mixture was dissolved in dry pyridine (1 ml) and then a large excess of acetic anhydride (20 mmol) was added dropwise. The mixture was stirred at room temperature overnight. At the end of the reaction, the solution was neutralized with a solution of 1 M HCl and then extracted with ethyl acetate (3×10 ml). After washing with a saturated solution of NaCl, the crude was dried over Na_2SO_4. The solvent was removed under reduced pressure to afford a yellow oil. The residue was purified by preparative TLC or by flash chromatography on silica gel using dichloromethane/methanol or ethyl acetate/hexane mixtures as eluents. Products were identified by ^{1}H NMR, ^{13}C NMR, GC–MS and elemental analyses.

Spectroscopic and analytical data of these compounds are given below.

*5.2.1. cis-3-Acetoxy-2-methoxyflavanone (**3c**)*

Yellow oil. ^{1}H NMR, δ (ppm): 2.10 (s, 3H, $OCOCH_3$), 3.11 (s, 3H, OCH_3), 5.77 (s, 1H, H-3), 7.13–7.18 (m, 2H, PhH), 7.42–7.45 (m, 3H, PhH), 7.59–7.70 (m, 3H, PhH), 7.89 (m, 1H, PhH). ^{13}C NMR, δ (ppm): 20.4 (CH_3), 51.2 (OCH_3), 77.4 (CH), 106.2 (C), 118.0 (CH), 120.7 (C), 122.6 (CH), 126.8 (CH), 128.3 (CH), 128.7 (CH), 129.5 (CH), 134.7 (C), 136.4 (CH), 156.4 (C), 169.2 (C), 187.2 (C=O). *m*/*z* (EI) 270 (M^+-42). Anal. Calcd for $C_{18}H_{16}O_5$ (312.32): C, 69.22; H, 5.17; O, 25.62. Found: C, 69.24; H, 5.16; O, 25.60.

*5.2.2. trans-3-Acetoxy-2-methoxyflavanone (**3d**)*

Colourless oil. ^{1}H NMR, δ (ppm): 1.74 (s, 3H, $OCOCH_3$), 3.08 (s, 3H, OCH_3), 5.48 (s, 1H, H-3), 7.09–7.20 (m, 2H, PhH), 7.39–7.43 (m, 3H, PhH), 7.60–7.66 (m, 3H, PhH), 7.90 (m, 1H, PhH). ^{13}C NMR, δ (ppm): 20.1 (CH_3), 50.3 (OCH_3), 72.1 (CH), 104.9 (C), 118.1 (CH), 119.8 (C), 122.4 (CH), 127.0 (CH), 127.4 (CH), 128.4 (CH), 129.4 (CH), 134.8 (C), 136.6 (CH), 156.8 (C), 167.9 (C), 187.0 (C=O). *m*/*z* (EI) 270 (M^+-42). Anal. Calcd for $C_{18}H_{16}O_5$ (312.32): C, 69.22; H, 5.17; O, 25.62. Found: C, 69.18; H, 5.15; O, 25.67.

*5.2.3. cis-3-Acetoxy-2,5-dimethoxyflavanone (**5c**)*

Colourless oil. ^{1}H NMR, δ (ppm): 2.10 (s, 3H, CH_3), 3.11 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 5.62 (s, 1H, H-3), 6.68 (dd, 2H, PhH), 7.35–7.50 (m, 4H, PhH), 7.62–7.68 (m, 2H, PhH). ^{13}C NMR, δ (ppm): 20.1 (CH_3), 51.2 (OCH_3), 56.2 (OCH_3), 77.5 (CH), 105.3 (CH), 105.2 (C), 109.9 (CH), 115.9 (C), 127.0 (CH), 128.4 (CH), 129.5 (CH), 134.8 (C), 136.6 (CH), 158.4 (C), 161.2 (C), 169.4 (C), 184.7 (C=O). *m*/*z* (EI) 300 (M^+-42). Anal. Calcd for $C_{19}H_{18}O_6$ (342.35): C, 66.66; H, 5.30; O, 28.04. Found: C, 66.56; H, 5.30; O, 28.14.

*5.2.4. trans-3-Acetoxy-2,5-dimethoxyflavanone (**5d**)*

Colourless oil. ^{1}H NMR, δ (ppm): 1.74 (s, 3H, CH_3), 3.08 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 5.41 (s, 1H, H-3), 6.77 (dd, 2H, PhH), 7.35–7.56 (m, 4H, PhH), 7.58–7.68 (m, 2H, PhH). ^{13}C NMR, δ (ppm): 20.1 (CH_3), 50.4 (OCH_3), 56.1 (OCH_3), 73.4 (CH), 104.4 (C), 105.0 (CH), 110.0 (CH), 113.3 (C), 127.0 (CH), 128.3 (CH), 129.3 (CH), 134.9 (C), 136.4 (CH), 158.5 (C), 161.0 (C), 167.9 (C), 185.6 (C=O). *m*/*z* (EI) 300 (M^+-42). Anal. Calcd for $C_{19}H_{18}O_6$ (342.35): C, 66.66; H, 5.30; O, 28.04. Found: C, 66.60; H, 5.32; O, 28.08.

*5.2.5. cis-3-Acetoxy-2,6-dimethoxyflavanone (**7c**)*

Colourless oil. ^{1}H NMR, δ (ppm): 2.09 (s, 3H, CH_3), 3.09 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 5.75 (m, 1H, C-3), 7.04–7.19 (m, 2H, PhH), 7.32–7.45 (m, 4H, PhH), 7.64–7.69 (m, 2H, PhH). ^{13}C NMR, δ (ppm): 20.1 (CH_3), 51.1 (OCH_3), 55.8 (OCH_3), 77.5 (CH), 106.1 (C), 107.9 (CH), 119.4 (CH), 120.7 (C), 124.8 (C), 127.1 (CH), 128.3 (CH), 129.4 (CH), 134.8 (C), 150.6 (C), 154.8 (C), 167.9 (C), 187.2 (C=O). *m*/*z* (EI) 342 (M^+). Anal. Calcd for $C_{19}H_{18}O_6$ (342.35): C, 66.66; H, 5.30; O, 28.04. Found: C, 66.62; H, 5.28; O, 28.10.

*5.2.6. trans-3-Acetoxy-2,6-dimethoxyflavanone (**7d**)*

Colourless oil. ^{1}H NMR, δ (ppm): 1.74 (s, 3H, CH_3), 3.06 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 5.46 (m, 1H, H-3), 7.07–7.18 (m, 2H, PhH), 7.35–7.42 (m, 4H, PhH), 7.60–7.64 (m, 2H, PhH). ^{13}C NMR, δ (ppm): 20.1 (CH_3), 50.2 (OCH_3), 55.8 (OCH_3), 71.9 (CH), 104.7 (C), 108.0 (CH), 119.4 (CH), 119.7 (C), 125.5 (CH), 127.1 (CH), 128.3 (CH), 129.4 (CH), 134.9 (C), 151.2 (C), 154.8 (C), 167.9 (C), 187.2 (C=O). *m*/*z* (EI) 342 (M^+). Anal. Calcd for $C_{19}H_{18}O_6$ (342.35): C, 66.66; H, 5.30; O, 28.04. Found: C, 66.60; H, 5.24; O, 28.16.

*5.2.7. cis-3-Acetoxy-2-methoxy-6-methylflavanone (**9c**)*

Yellow oil. ^{1}H NMR, δ (ppm): 2.09 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 3.10 (s, 3H, OCH_3), 5.74 (m, 1H, H-3), 7.04 (d, 1H, *J*=8.3 Hz, PhH), 7.37–7.44 (m, 4H, PhH), 7.64–7.69 (m, 3H, PhH). ^{13}C NMR, δ (ppm): 20.1 (CH_3), 20.3 (CH_3), 51.1 (OCH_3), 77.6 (CH), 106.0 (C), 114.0 (C), 117.7 (CH), 120.3 (C), 126.5 (CH), 127.1 (CH), 128.3 (CH), 129.4 (CH), 132.0 (C), 137.5 (CH), 154.3 (C), 169.3 (C), 187.4 (C=O). *m*/*z* (EI) 326 (M^+). Anal. Calcd for $C_{19}H_{18}O_5$ (326.35): C, 69.93; H, 5.56; O, 24.51. Found: C, 69.99; H, 5.50; O, 24.51.

*5.2.8. trans-3-Acetoxy-2-methoxy-6-methylflavanone (**9d**)*

Colourless oil. ^{1}H NMR, δ (ppm): 1.73 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 3.06 (s, 3H, OCH_3), 5.46 (s, 1H, H-3), 7.07 (d, 1H, *J*=8.4 Hz, PhH), 7.38–7.43 (m, 4H, PhH), 7.60–7.65 (m, 2H, PhH), 7.73 (s, 1H, PhH). ^{13}C NMR, δ (ppm): 20.1 (CH_3), 20.4 (CH_3), 50.2 (OCH_3), 72.1 (CH), 104.7 (C), 117.9 (CH), 119.4 (C), 127.0 (CH), 128.3 (CH), 129.4 (CH), 132.0 (C), 134.9 (C), 137.6 (CH), 154.8 (C), 167.9 (C), 187.3 (C=O). *m*/*z* (EI) 326 (M^+). Anal. Calcd for $C_{19}H_{18}O_5$ (326.35): C, 69.93; H, 5.56; O, 24.51. Found: C, 69.90; H, 5.50; O, 24.60.

*5.2.9. cis-3-Acetoxy-2,7-dimethoxyflavanone (**13c**)*

Yellow oil. ^{1}H NMR ($CDCl_3/CD_3OD$), δ (ppm): 2.09 (s, 3H, CH_3), 3.13 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 5.71 (s, 1H, H-3), 6.60–6.71 (m, 2H, PhH), 7.38–7.44 (m, 3H, PhH), 7.63–7.68 (m, 2H, PhH), 7.83 (d, 1H, *J*=8.6 Hz, PhH). ^{13}C NMR ($CDCl_3/CD_3OD$), δ (ppm): 20.1 (CH_3), 51.2 (OCH_3), 55.8 (OCH_3), 72.1 (CH), 101.8 (CH), 106.2 (C), 110.1 (CH), 114.3 (C), 127.1 (CH), 128.3 (CH), 129.2 (CH), 129.4 (CH), 134.9 (C), 158.4 (C), 166.4 (C), 169.3 (C), 185.7 (C=O). *m*/*z* (EI) 342 (M^+). Anal. Calcd for $C_{19}H_{18}O_6$ (342.35): C, 66.66; H, 5.30; O, 28.04. Found: C, 66.62; H, 5.34; O, 28.04.

*5.2.10. trans-3-Acetoxy-2,7-dimethoxyflavanone (**13d**)*

Colourless oil. ^{1}H NMR, δ (ppm): 1.72 (s, 3H, CH_3), 3.09 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 5.45 (m, 1H, H-3), 6.62–6.70 (m, 2H, PhH), 7.36–7.46 (m, 3H, PhH), 7.57–7.63 (m, 2H, PhH), 7.86 (d, 1H, *J*=8.6 Hz, PhH). ^{13}C NMR, δ (ppm): 20.1 (CH_3), 50.4 (OCH_3), 55.7 (OCH_3), 72.1 (CH), 101.7 (CH), 105.2 (C), 110.3 (CH), 113.5 (C), 127.1 (CH), 128.3 (CH), 129.2 (CH), 129.4 (CH), 134.9 (C), 158.9 (C), 166.6 (C), 167.9 (C), 185.7 (C=O). *m*/*z* (EI) 342 (M^+). Anal. Calcd for $C_{19}H_{18}O_6$ (342.35): C, 66.66; H, 5.30; O, 28.04. Found: C, 66.60; H, 5.26; O, 28.14.

*5.2.11. Mixture (1/1) of 6-hydroxy-5,7-dimethoxyflavone (**15**) and 8-hydroxy-5,7-dimethoxyflavone (**16**)*

Yellow oil. ^{1}H NMR ($CDCl_3/CD_3OD$), δ (ppm): 3.85 (s, 6H, $2OCH_3$), 3.93 (s, 6H, $2OCH_3$), 6.39 (s, 1H, H-6, compound **16**), 6.58 (s, 1H, H-3, compound **16**), 6.62 (s, 1H, H-8, compound **15**), 6.78 (s, 1H, H-3, compound **15**), 7.38–7.46 (m, 6H, PhH), 7.79–7.90 (m, 4H, PhH). ^{13}C NMR ($CDCl_3/CD_3OD$), δ (ppm): 56.2 (OCH_3), 56.4 (OCH_3), 62.1 (OCH_3), 92.5 (CH), 96.1 (CH), 107.7 (CH), 108.6 (C), 112.1 (C), 125.9 (CH), 126.1 (CH), 128.0 (C), 128.9 (CH), 131.2 (CH), 137.4 (C), 144.0 (C), 146.5 (C), 150.7 (C), 151.8 (C), 152.9 (C), 153.2 (C), 161.1 (C), 161.7 (C), 177.7 (C=O), 178.5 (C=O).

*5.2.12. 5,8-Dihydroxy-7-methoxyflavone (**17**)*

Yellow oil. ^{1}H NMR ($CDCl_3/CD_3OD$), δ (ppm): 3.90 (s, 3H, OCH_3), 6.39 (s, 1H, H-6), 6.58 (s, 1H, H-3), 7.36–7.47 (m, 3H, PhH), 7.88–7.93 (m, 2H, PhH). ^{13}C NMR, δ (ppm): 56.0 (CH_3), 93.3 (CH), 101.9 (C), 105.1 (CH), 126.3 (CH), 129.1 (CH), 130.0 (C), 131.9 (CH), 151.4 (C), 156.7 (C), 158.2 (C), 162.9 (C), 181.7 (C=O). *m*/*z* (EI) 284 (M+). Anal. Calcd for $C_{16}H_{12}O_5$ (284.27): C, 67.60; H, 4.26; O, 28.14. Found: C, 67.50; H, 4.28; O, 28.22.

*5.2.13. 5,6,7-Trimethoxyflavone (Baicalein trimethyl ether **18**)*

Yellow oil. ^{1}H NMR ($CDCl_3$), δ (ppm): 3.89 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 6.64 (s, 1H, H-8), 6.79 (s, 1H, H-3), 7.45–7.52 (m, 3H, PhH), 7.82–7.87 (m, 2H, PhH). ^{13}C NMR ($CDCl_3$), δ (ppm): 56.2 (OCH_3 in C-7), 61.2 (OCH_3 in C-6), 61.4 (OCH_3 in C-5), 96.2 (CH), 108.3 (CH), 112.9 (C), 125.9 (CH), 128.9 (CH), 131.2 (CH), 131.5 (C), 140.3 (C), 152.5 (C), 154.5 (C), 157.7 (C), 161.0 (C), 177.1 (C=O). *m*/*z* (EI) 312 (M^+). Anal. Calcd for $C_{18}H_{16}O_5$ (312.32): C, 69.22; H, 5.16; O, 25.62. Found: C, 69.18; H, 5.14; O, 25.68.

*5.2.14. 5,7,8-Trimethoxyflavone (Wogonin dimethyl ether **19**)*

Yellow oil. ^{1}H NMR ($CDCl_3$), δ (ppm): 3.93 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 6.42 (s, 1H, H-6), 6.67 (s, 1H, H-3), 7.45–7.52 (m, 3H, PhH), 7.88–7.93 (m, 2H, PhH). ^{13}C NMR ($CDCl_3$), δ (ppm): 56.2 (OCH_3 in C-5), 56.5 (OCH_3 in C-7), 61.5 (OCH_3 in C-8), 92.6 (CH), 108.3 (CH), 109.1 (C), 125.9 (CH), 128.9 (CH), 130.8 (C), 131.2 (CH), 152.0 (C), 156.3 (C), 156.6 (C), 160.6 (C), 177.8 (C=O); *m*/*z* (EI) 312 (M^+). Anal. Calcd for $C_{18}H_{16}O_5$ (312.32): C, 69.22; H, 5.16; O, 25.62. Found: C, 69.25; H, 5.12; O, 25.63.

5.3. Assay of antifungal activity of flavanones derivatives

Before testing, compounds were dissolved in 100% acetone so that the final concentration of solvent in the test medium did not exceed 1% of the total solution composition. All compounds were used in three different concentrations: 0.5, 2.0 and 8.0×10^{-4} M. All solutions were prepared immediately before testing. *T. koningii*, *F. solani* and *C. herbarum* was used for the antifungal screening test. Prior to testing, each isolate was subcultured on MEA (DIFCO) to ensure optimal growth characteristics and purity. The isolates had been grown for 4–14 days, on MEA at 25 °C. Conidia suspensions were prepared in sterile water supplemented with 0.01% of Tween 80. Each suspension was diluted to obtain the final inoculum, which ranged from 0.5×10^4 to 1.0×10^4 CFU/ml. The inoculum size was determined microscopically using Bürker's chamber and verified by plating 100 μL of serial dilutions of each inoculum onto an MEA plate and incubation until growth became visible. Each Petri dishes (90 mm) containing 12 ml of the medium including the products in required concentrations (added to the agar at temperature below 50 °C) was inoculated with 2 μl of the inoculum suspensions; 5–6 replications for each concentration and fungus were made. The Petri dishes were incubated at 25 °C in the dark to a clearly visible growth on drug-free control. Evaluation of linear growth was conducted by measuring mycelial diameters of each inoculated plate at broadest, medium and smallest diameter, and compared with the corresponding control. The inhibition (%) of linear mycelial mean growth of *T. koningi*, *C. herbarum* and *F. solani* was calculated after incubation for 3, 5 and 6 days, respectively. The data were evaluated by analysis of variance and probability of single differences was calculated at the 5% level.

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