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Synthesis and biological evaluation of a series of tangeretin-derived chalcones

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ABSTRACT

A series of chalcones polyoxygenated on the ring A (with pentamethoxy or 2′-hydroxy-3′,4′,5′,6′-tetramethoxy substitution patterns) was synthesized from tangeretin, a natural *Citrus* flavonoid. These chalcones were evaluated for their antiproliferative, activation of apoptosis, inhibition of tubulin assembly and antileishmanial activities. Comparison with the reference analogous 3′,4′,5′-trimethoxylated chalcones showed that such peroxygenated substitution patterns on the ring A were less beneficial to these activities.

Chalcones are natural or synthetic compounds bearing the 1,3-diphenylprop-2-en-1-one **1** framework, that have displayed a wide pharmacological spectrum including, among others, cytotoxic, antitumour, antiviral, and antiprotozoal activities.[1] Many studies of chalcones related to cancer have demonstrated the positive influence of a polymethoxylated ring A on cytotoxicity, though the optimal substitution pattern remains to be defined.[2,3] For instance, a 3′,4′,5′-trimethoxyphenyl ring A is present in the strongly cytotoxic chalcones **2, 3**, and **5**, that interfere with the mitotic phase of the cell cycle.[4–7] The biological profile of **2, 3**, and **5** can be easily related to the structural analogy of these chalcones with combretastatin A4 **6** and its amino analog **7**, two powerful inhibitors of tubulin assembly now under clinical investigation as their respective prodrugs **8** and **9**.[8]

More recently, it was shown that dimethoxylation or trimethoxylation at 2′,4′,6′-carbons were highly beneficial to cell cycle arrest at G_2/M, while hydroxylation at 2′ was generally detrimental[9] (this last observation was in contrast with previous results showing high cytotoxicity of 2′-hydroxylated chalcones against Jurkat and U 937 cancer cells[10]). So a clear correlation between the polymethoxylation pattern of chalcones on the ring A and their cytotoxicity seems difficult to establish. Starting from tangeretin **10** we used this natural polymethoxylated flavone isolated from *Citrus* for a straightforward access to chalcones peroxygenated on the ring A through the easily available acetophenones **11a** and **11b**. Until now, only two of such chalcones, pedicin **12**, and isodidymocarpin **13**, have been studied with regard to cytotoxicity that proved to be weak [(**12**: IC_{50} (KB cells) 21.2 μM; IC_{50} (Inhibition of tubulin assembly) 300 μM.[11] Compound **13**: IC_{50} (P-388 cells) 11.1 μ M[12]]. As chalcones **12** and **13** are unsubstituted on the ring B, we undertook synthesis and cytotoxicity evaluation of chalcones **14a** and **14b,** and **16a** and **16b** bearing, respectively, on the ring B the substitution pattern of reference antimitotic chalcones **2** and **5**.

Chemistry: Synthesis of these new chalcones was achieved from tangeretin **10** in two, three, or four steps according to scheme 1: (a) basic degradation of **10** into acetophenone **11a**;[13] (b) methylation of **11a** to the pentamethoxyacetophenone **11b**; (c) Claisen–Schmidt condensation of **11a** or **11b** with isovanillin in a mixture MeOH–aqueous KOH giving **14a** and **14b** respectively; (d) same Claisen–Schmidt condensation of **11a** or **11b** but with 3-nitro-4-methoxybenzaldehyde leading to **15a** and **15b**, respectively;[14] (e) reduction of nitrochalcones **15a** or **15b** with $SnCl_2$ providing the aminochalcones **16a** or **16b**. Reference 3′,4′,5′-trimethoxylated chalcones **2, 4**, and **5** were prepared in the same way from 3′,4′,5′-trimethoxyacetophenone. Lastly, a Claisen–Schmidt reaction between 3-nitrobenzaldehyde and 3′,4′,5′-trimethoxyacetophenone or acetophenone **11a** led to compounds **17**[3] or **18**, the 4-demethoxylated analogs of 3-nitrochalcones **4** and **15a** (vide infra Biology).

From a spectral point of view, a striking difference between series **a** (2′-OH) and **b** (2′-OMe) could be noted in the ^{1}H NMR spectra: in the series **a**, signals of both olefinic protons were deshielded with chemical shifts for Hα and Hβ very near [2 doublets (J = 16 Hz) at δ 7.75 and 7.85 ppm in **15a**; 2 doublets (J = 16 Hz) at δ 7.77 and 7.78 ppm in **16a**], or identical in **14a** (one singlet of

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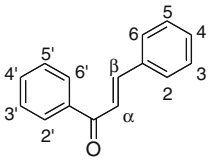

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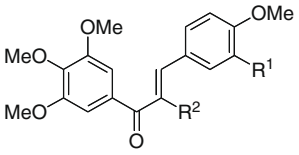
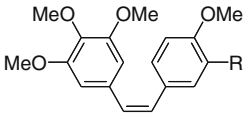
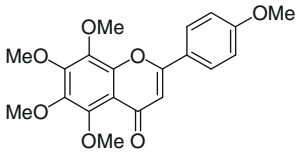


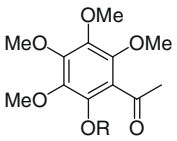
R^1	R^2	
OH	H	**2**
OH	Me	**3**
NO_2	H	**4**
NH_2	H	**5**



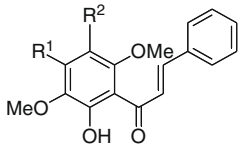
R	
OH	**6**
NH_2	**7**
OPO_3Na_2	**8**
NHserine, HCl	**9**



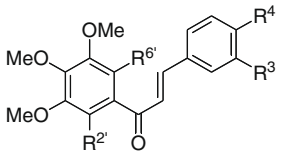
10



R	
H	**11a**
Me	**11b**



R^1	R^2	
OMe	OH	**12**
OH	OMe	**13**

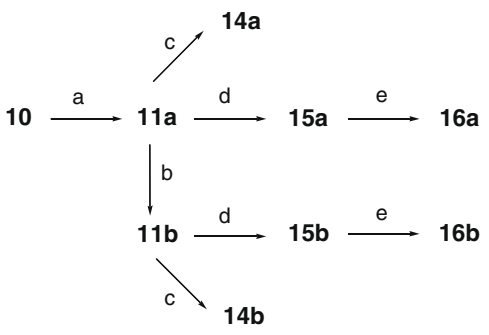


$R^{2'}$	$R^{6'}$	R^3	R^4	
OH	OMe	OH	OMe	**14a**
OMe	OMe	OH	OMe	**14b**
OH	OMe	NO_2	OMe	**15a**
OMe	OMe	NO_2	OMe	**15b**
OH	OMe	NH_2	OMe	**16a**
OMe	OMe	NH_2	OMe	**16b**
H	H	NO_2	H	**17**
OH	OMe	NO_2	H	**18**

two protons at δ 7.79 ppm) ; in the series **b**, the difference between Hα and Hβ chemical shifts was much more marked (about 0.4 ppm) as in compounds **2, 4**, and **5** [two doublets (J = 16 Hz) for Hα and Hβ, respectively, at 6.93 and 7.30 ppm in **15b**, 6.87 and 7.26 ppm in **16b** and 6.85 and 7.26 ppm in **14b**]. The coupling constant of 16 Hz observed in all the spectra (except **14a**) indicates the formation of only the expected *E* isomers. The strong variation of the olefinic signals between series **a** and **b** correlates certainly with a marked difference of conformations, that is related to the presence in the series **a** of an intramolecular hydrogen bonding between the 2′-hydroxyl proton and carbonyl oxygen.

Biology: The antiproliferative effect of chalcones was asseyed on KB human buccal carcinoma cells and the activation of apoptosis with DEVD-AMC as substrate in HL60 human leukemia cells. Inhibition of tubulin assembly (ITA) was determined according to Zavala and Guenard's method.[15] Compounds were tested at 0.1 mg/ml ($\approx 2 \times 10^{-4}$ M) and estimated inactive when they decreased by less than 30% the maximum assembly rate of tubulin in the absence of drug. The IC_{50} was calculated only for the most active compounds and expressed in relation to deoxypodophyllotoxin (DPPT) in terms of the $IC_{50}/IC_{50\ DPPT}$ ratio. As depicted in Table 1, all the chalcones polyoxygenated on the ring A displayed a weak capacity to inhibit cell proliferation (still weaker for compounds **14b–16b** of the series **b**). This was in contrast to the strong activity observed with the 3′,4′,5′-trimethoxylated chalcones **2** and **5**. The measured ITA of the four most active chalcones **2**, **5** and **14a**, **16a** was in good correlation with the cell growth inhibition and matched the activation of apoptosis: **2** and **5** were potent activators (100 nM) compared to **14a** and **16a** (10 μM) while **15a** was inactive. Lastly, it was noticed that compounds having the intramolecular bond between the 2′-hydroxyl and carbonyl function (**14a and 16a**) exhibit significantly higher ITA that those which do not have this bond (**14b and 16b**).

In other respects, a recent article reporting some highly selective antileishmanial 3-nitrochalcones led us to evaluate antileish-



Scheme 1. Reagents and conditions: (a) EtOH–40% aq KOH, reflux, 5 h, 56%; (b) iodomethane, K_2CO_3, DMF, rt, 1 h, quantitative yield; (c) MeOH–50% aq KOH 1:1, isovanillin, rt, 15 h, 31% (**14a**), 64% (**14b**); (d) MeOH–50% aq KOH 10:1, 3-nitro-4-methoxybenzaldehyde, rt, 15 h, 60% (**15a**), 50% (**15b**); (e) $SnCl_2$, MeOH, 60 °C, 2 h, 74% (**16a**), 34% (**16b**).

Table 1
Antiproliferative, proapoptotic, antitubulin, and antileishmanial activities of synthesized chalcones

Compound	Cytotoxicity on KB cells[a] IC_{50} (μM)	Activation of apoptosis in HL60[b]	ITA activity IC_{50} (μM)	Antileishmanial activity on *L. donovani* promastigotes[f] IC_{50} (μM)	Antileishmanial activity on *L. amazonensis* amastigotes[g] IC_{50} (μM)
2	100% IC_{50} = 0.017	100 nM: 3.6×	IC_{50} = 4.3 1.3[c]	nd	nd
4	44%		nd[d]	IC_{50} = 2.8	Inactive[h]
5	100% IC_{50} = 0.031	100 nM: 3.6×	IC_{50} = 3.7 1.0[c]	nd	nd
14a	49%	10 μM: 3.2×	IC_{50} = 13 3.8[c]	IC_{50} = 25	Inactive[h]
14b	22%		Inactive[e]	IC_{50} = 20	nd
15a	43%	10 μM: 1.2×	Inactive[e]	IC_{50} = 5.3	Inactive[h]
15b	19%		Inactive[e]	nd	nd
16a	40%	10 μM: 2.9×	IC_{50} = 14 4.1[c]	IC_{50} = 44	Inactive[h]
16b	29%		Inactive[e]	nd	nd
17	29%		nd	IC_{50} = 0.4	IC_{50} = 26 IS[i] = 2.8
18	31%		nd	IC_{50} = 4.4	IC_{50} = 26 IS = 2.5

[a] As measured by the MTS assay after 72 h incubation of cells with drug; results are expressed as the percentage of inhibition of cell growth with 10 to 6 M chalcone concentration; IC_{50} was calculated only for the two most active compounds.
[b] Activation of caspases 3/7 activity: optimal concentration of compound and fold activation.
[c] IC_{50} chalcone/IC_{50} deoxypodophyllotoxin.
[d] Not determined.
[e] Estimated inactive when decreasing by less than 30% the maximum assembly rate of tubulin without drug.
[f] As measured by the MTT assay after 72 h incubation of parasite with the drug.
[g] As measured after 30 h incubation of infected macrophages with the drug.
[h] Estimated inactive when amastigotes are still lodged within parasitophore vacuole, inside macrophages, as compared to DMSO control.
[i] Index of selectivity defined by the ratio $IC_{50\ \text{murine macrophages}}/IC_{50\ \text{amastigotes}}$.

manial activity of the intermediate nitrochalcones **4** and **15a**.[16] As the same letter displayed also the detrimental influence of almost all substitutions at C-4 on this activity, we decided to compare **4** and **15a** to their 4-unsubstituted analogs **17** and **18**. The antileishmanial activity (Table 1) was determined in vitro against promastigotes of *Leishmania donovani* strain LV9 (MHOM/ET/67/HU3) clone which were grown as described previously,[17] and against intracellular amastigotes of *Leishmania amazonensis* strain LV9 (MPROB/BR/1972/M1841) which were isolated from lesions and purified as described earlier.[18] Cytotoxicity against murine macrophages allowed evaluation of the compound selectivity (Index of selectivity $IS = IC_{50\ \text{macrophages}}/IC_{50\ \text{amastigotes}}$). The evaluation on promastigotes of compounds **14a–16a** confirmed the positive effect of a nitro group at C-3 since **15a** is much more active than **14a** and **16a**, but comparison **15a** vs **4** was slightly in favor of **4**. Removing the methoxyl group at C-4 increased the antipromastigotes activity within the 3′,4′,5′-trimethoxylated series (**17** vs **4**), but not within the series **a** (**18** vs **15a**). In compounds **17** and **18** the lack of substitution at C-4 had a significant impact on efficacy against amastigotes. Only these two compounds lacking substitution at C-4 showed any efficacy in this regard.

In conclusion, our study showed that the peroxygenated substitution patterns of the ring A present in the series **a** and **b** were less beneficial to antiproliferative, activation of apoptosis, antimitotic, and antileishmanial activities than the more classical and available 3′,4′,5′-trimethoxy substitution. However, this SAR was deduced from only few examples of chalcones, and needs to be generalized to a larger series bearing other substitution patterns on the ring B.

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