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Prenylated Xanthones as Potential P-Glycoprotein Modulators

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Abstract—Dimethylallyl (DMA) derivatives of a naturally occurring xanthone (decussatin **1**) were prepared. Their activity as potential P-glycoprotein inhibitors was monitored by affinity of direct binding and compared to that of corresponding DMA-flavones. Both classes of compounds exhibited the same structure–activity relationships. Decreasing polarity enhanced the binding affinity for the P-glycoprotein C-terminal cytosolic domain since DMA derivatives were more active, but unsubstituted hydroxyl group close to the carbonyl was required for efficient activity.

Introduction

Xanthones are secondary metabolites occurring in restricted higher plants, fungi or lichens families.[1] In our study on barks of *Trema orientalis* (Ulmaceae), we isolated, for the first time in Ulmaceae family, four xanthones, including decussatin (1-hydroxy-3,7,8-trimethoxyxanthone **1**) (to be published). Xanthones and flavonoids have related structures. Thus, in our course to biologically active compounds and regarding the activity of flavones and chalcone derivatives[2,3] in the field of modulation of multidrug resistance, we prepared prenylated derivatives of xanthone **1**. In this report, we briefly describe the semisynthesis of five new products (**2**–**6**), starting from compound **1**, and discuss their activity as potential P-glycoprotein modulators, compared to those of corresponding flavone derivatives (**8**–**11**).

Results and Discussion

Whereas there were many ways to total synthesis of xanthones,[4] semisynthesis of prenylated xanthones was performed by a known reaction previously designed for chalcones,[5] to yield analogues of the naturally occurring decussatin (**1**).[6,7] Thus, isoprenylation of **1** (Scheme 1) with 3,3-dimethylallyl bromide resulted in a mixture of compounds. After purification, the major product (**2**) (69%), identified as a 3′,3′-dimethylallyl ether of **1**, was submitted to a Claisen rearrangement in boiling diethylaniline[8] to give four isomeric products (**3**–**6**). They were identified by means of their spectral data[8] (Table 1).

Formation of **3** (18%) and **5** (8%) was explained by the classical Claisen rearrangement. They were respectively identified as the-2-(1′,1′-DMA) and the-4-(3′,3′-DMA) derivatives of **1**. Compound **4** (2%) was concluded to be 3,7,8-trimethoxy-4′,5′-dihydro-4′,4′,5′-trimethylfurano(2′,3′:1,2)xanthone and resulted from cyclization of **3**. Compound **6** (9%) was characterized as the-2-(3′,3′-DMA) derivative of **1**. Furthermore, and unlike **5**, formic acid cyclization of **6** afforded **7** which was identified by its dihydropyran ring.[8]

The formation of **6** from compound **2** could only be explained by a ring-opening in **6** to give a benzophenone intermediate. The re-cyclization of which could then produce a mixture of **5** and **6**. This is close to the Wessely–Moser mechanism previously proposed for other xanthones.[9,10]

Since flavone and chalcone derivatives have been identified as ligands of P-glycoprotein, and in order to discover other compounds with high binding affinity, the above

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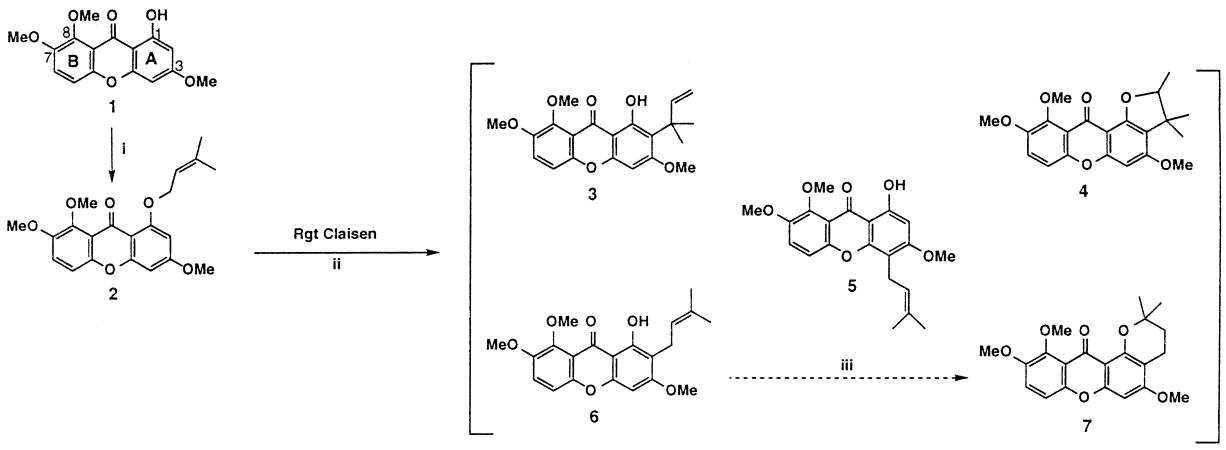

Scheme 1. (i) $(CH_3)_2CO$, K_2CO_3, 3,3-dimethylallyl bromide, reflux, 6 h; (ii) *N,N*-diethylaniline, N_2, reflux, 4 h; (iii) HCO_2H, reflux, 1 h.

xanthones were tested on the purified C-terminal cytosolic domain of P-glycoprotein, except for xanthone **7** which was obtained in too low amount and as an unpurified compound. In addition, binding affinities of the corresponding flavones analogues **8**–**11** (Daskiewicz et al., to be published) have been measured. The direct binding was measured by the quenching of protein intrinsic-fluorescence, as previously described for other classes of flavonoids:[11] dissociation constant, K_d, and maximal fluorescence-quenching, ΔF_{max}, were determined with the Grafit program (Erithacus software).

The compounds were dissolved in dimethylsulfoxide and then diluted in aqueous medium, to be assayed for binding in vitro to the P-glycoprotein purified domain (Table 2).

The data clearly showed that prenylation at either position 2 (xanthones **3** and **6**) or position 4 (xanthone **5**) on ring A increased the binding affinity by 50- to 100-fold. In contrast, the gain in affinity was not observed when prenylation concerned the hydroxyl group at position 1, in either a linear way (xanthone **2**) or by cyclization with position 2 (xanthone **4**). Comparable changes in affinity were observed with similarly derivatized flavones: prenylation at either position 6 (flavone **9**) or position 8 (flavone **10**) produced a 80-fold increase in affinity as compared to flavone **8**, whereas prenylation of the hydroxyl group at position 5 (flavone **11**) produced a very limited effect.

The present results show the key role played by the ring A hydroxyl group at either position 1 of xanthones or position 5 of flavones. This might be attributed to mimicking, together with the vicinal carbonyl on ring C, of the adenine moiety of ATP, as demonstrated by co-crystallization of cyclin-dependent kinase 2[12] and Hck tyrosine kinase[13] with flavonoids or derivatives. The high increase in affinity due to hydrophobic substitution of ring A, by prenylation, is expected to strengthen xanthone and flavone interaction at the steroid-interacting

Table 1. ^{13}C NMR (50 MHz, $CDCl_3$) spectral data of **1**–**6**

Compd	1[a]	2	3	5	6
1	164.3 s	161.1 s	162.3 s	162.2 s	159.9 s
2	97.2 d	96.2 d	118.6 s	94.0 d	111.6 s
3	167.1 s	164.2 s	165.7 s	164.0 s	164.1 s
4	92.2 d	92.1 d	89.8 d	107.0 s	88.9 d
4a	157.7 s	159.0 s	155.6 s	153.5 s	155.6 s
4b	151.3 s	150.2 s	150.7 s	151.3 s	151.1 s
5	113.2 d	111.9 d	112.4 d	112.8 d	112.6 d
6	121.2 d	118.7 d	120.4 d	120.6 d	120.4 d
7	150.0 s	149.3 s	149.2 s	149.2 s	149.2 s
8	149.2 s	149.0 s	149.0 s	149.0 s	149.0 s
8a	116.0 s	118.5 s	116.0 s	115.5 s	116.0 s
9a	102.3 s	108.2 s	104.0 s	103.7 s	104.0 s
9	181.6 s	175.0 s	181.4 s	181.6 s	181.1 s
3-OMe	56.1 q	55.6 q	55.3 q	56.0 q	55.9 q
7-OMe	57.1 q	57.1 q	57.2 q	57.3 q	57.3 q
8-OMe	61.5 q	61.6 q	61.7 q	61.7 q	61.6 q
1′		66.5 t	41.1 s	21.5 t	21.4 t
2′		119.9 d	150.7 d	122.2 d	122.2 d
3′		136.7 s	106.6 t	131.5 s	131.8 s
4′		18.4 q	29.0 q	17.8 q	17.8 q
5′		25.7 q	29.0 q	25.7 q	25.8 q

[a]Recorded in $CDCl_3$ and acetone-d_6. The carbons of **1**, **3** and **5** were assigned by ^{13}C-^{1}H long-range COLOC.

Table 2. Differential affinity of binding to P-glycoprotein recombinant domain of prenylated xanthones; comparison to 7-MEM-chrysin derivatives. Xanthones and flavones with similar prenylation on ring A were positioned on the same lines of the table

Xanthone derivative	Apparent K_d (μM)	ΔF_{max} (%)	Flavone derivative	Apparent K_d (μM)	ΔF_{max} (%)
1	28.8±3.4	89.2± 5.7	**8**	8.05± 0.63	73.1± 2.3
3	0.25± 0.05	45.8± 1.3	**9**	0.10± 0.04	42.3± 2.0
6	0.39± 0.22	35.0± 4.0			
5	0.59± 0.05	81.9± 1.9	**10**	0.10± 0.01	48.8± 0.6
2	13.9± 0.88	81.8± 1.9	**11**	6.10± 0.34	81.8± 1.9
4	24.8± 1.4	87.8± 1.8			

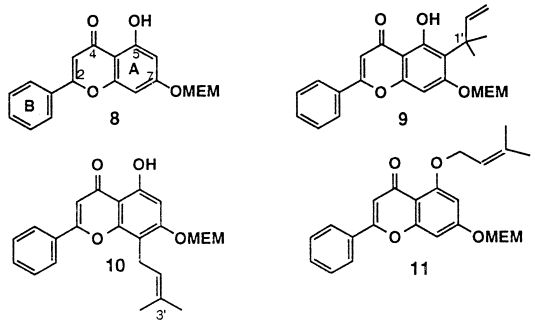

Figure 1.

hydrophobic region previously shown to be close to the ATP-binding site.[11] In contrast, substitution of the critical hydroxyl appears to markedly change the orientation of bound compound.

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