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B-ring Substituted 5,7-Dihydroxyflavonols with High-Affinity Binding to P-Glycoprotein Responsible for Cell Multidrug Resistance

Ahcène Boumendjel,[a,*] Frédéric Bois,[a] Chantal Beney,[a]
Anne-Marie Mariotte,[a] Gwenaëlle Conseil[b] and Attilio Di Pietro[b]

[a] *Département de Pharmacochimie Moléculaire UMR-CNRS 5063, UFR de Pharmacie de Grenoble 38706, La Tronche, France*
[b] *Institut de Biologie et Chimie des Protéines, UMR 5086 CNRS/Université Claude Bernard-Lyon I, 7 Passage du Vercors, 69367 Lyon, France*

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Abstract—Starting from the interaction of galangin (3,5,7-trihydroxyflavone) with a cytosolic nucleotide-binding domain of P-glycoprotein, a series of flavonol derivatives was synthesized and tested for their binding affinity towards the same target. The 5,7-dihydroxy-4′-iodoflavonol and 5,7-dihydroxy-4′-*n*-octylflavonol derivatives displayed much higher binding affinities, with respective increases of 6- and 93-fold as compared to galangin. © 2000 Elsevier Science Ltd. All rights reserved.

There is now general consensus that one of the major mechanisms by which tumor cells become resistant to several of the most efficient anticancer drugs presently available is a decreased intracellular drug bioavailability. Such a multidrug resistance (MDR) phenotype has often been correlated to the increased expression of a 170-kDa plasma membrane glycoprotein (*P*-gp) which acts as a cellular 'pump' by extruding the anticancer compounds and preventing their antitumor effect.[1] Two intracellular ATP-binding sites have been described in P-gp and the active export of cytotoxic compounds requires the energy derived from their ATPase activity.[2] Different ways have been undertaken to restore chemosensitivity of the MDR tumor cells to cytotoxic drugs. Besides attempts to discover anticancer drugs which would not be transported substrates for P-gp through maintaining a sufficient activity towards their molecular target, another pharmacological approach consists of trying to sufficiently inhibit P-gp pump functioning in order to restore cell sensitivity to anticancer drugs.[3]

By using a purified recombinant protein corresponding to a cytosolic nucleotide-binding domain of P-gp, we have previously demonstrated that flavonoids are efficient P-gp inhibitors, by binding to cytosolic sites which partly overlap the ATP-binding site and the modulators interacting region.[4] Studies of structure–activity relationships have concluded that flavones are more efficient than isoflavones, while flavonols and chalcones are even more active.

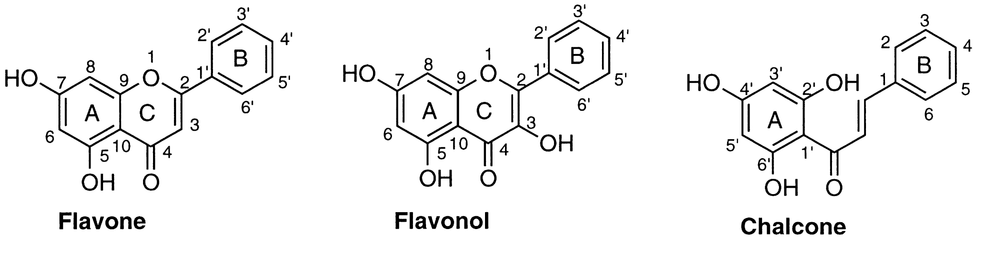

Figure 1.

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*Corresponding author. Tel.: +33-4-7651-8688; fax: +33-4-7663-7165; e-mail: ahcene.boumendjel@ujf-grenoble.fr

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Hydroxyl groups at positions 3 and 5 are essential for high-affinity binding to P-gp. In a recent study conducted with chalcones, we have shown that the presence of a hydrophobic substituent on the B-ring (at 4-position; Fig. 1) considerably enhanced the binding affinity.[5,6] However, the instability of chalcones (due to their cyclization to give inactive flavanones) makes them less desirables for further studies. In order to prepare high-affinity and much more stables flavonols to be used for in vivo studies, we have targeted and synthesized a series of flavonols (3,5,7-trihydroxyflavones) with different hydrophobic substituents at the 4′-position.

Chemistry

Flavonols **4** were synthesized in two steps starting from 2,4,6-trihydroxy-ω-methoxyacetophenone **1** (**1** was prepared from phloroglucinol and methoxyacetonitrile according to Wähälä and Hase).[7] Condensation of **1** with an appropriately substituted benzoyl chloride in the presence of K_2CO_3 in acetone afforded directly the diketone **2** which was not isolated but cyclized by treatment with AcOH to give 5,7-dihydroxy-3-methoxyflavones **3**. In one case (R = F), the expected 5,7-dihydroxy-3-methoxyflavone **3a** was not obtained. The main product was the 7-fluorobenzoyl-5-hydroxy-3-methoxyflavone. The latter was then treated with KOH in MeOH to give **3a** in a quantitative yield. Demethylation of methoxyflavones **3** with BBr_3 in CH_2Cl_2 gave flavonols **4** (Scheme 1).

Results and Discussion

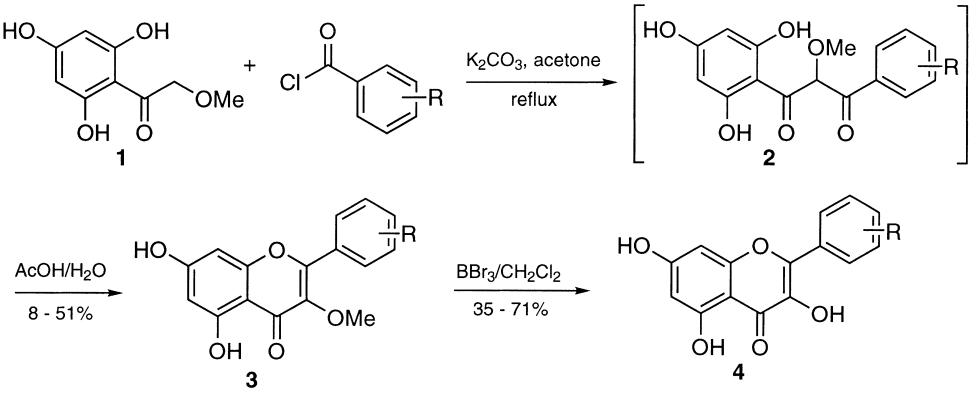
The binding of flavonols to the purified C-terminal nucleotide-binding domain (NBD2) of P-gp was directly measured by the quenching of protein intrinsic fluorescence due to a single tryptophan residue, as previously described for other classes of flavonoids.[4] The results of this study are listed in Table 1.

Our lead compound was galangin (3,5,7-trihydroxyflavone) which was used as a reference and interacted with NBD2 with a K_D of 5.9 ± 0.2 μM. Addition of a hydroxyl group at position 4′ of the B-ring produced a slightly negative effect when comparing kaempferol (6.7 ± 0.3 μM) to galangin. On the contrary, the presence of a hydrophobic methoxy group (in kaempferide) slightly increased the binding affinity as monitored by quenching ($K_D = 5.0 \pm 0.2$ μM).

As seen from Table 1, no significant effect was produced by introducing a fluorine in **4a** ($K_D = 6.8 \pm 0.28$ μM), whereas two chlorine atoms at positions 2′ and 4′ in **4b** produced a positive effect ($K_D = 4.0 \pm 0.23$ μM). A much higher increase in affinity was observed by introducing an iodine in **4d** ($K_D = 1.06 \pm 0.08$ μM), which was probably due to its hydrophobic character as concluded previously with chalcone derivatives.[5] Interestingly, an intermediate 3-fold increase in affinity was observed by replacing the complete B-ring with a diphenylmethyl substituent in **4c**. Finally, the most striking increase in binding affinity was observed by an octyl group which produced a 93-fold higher affinity in **4e** as compared to galangin. The choice of this substituent relies on our previous study conducted on chalcones which shows that an octyl group is the most optimal substituent, and further increase in the chain length began to alter the binding. The fact that the maximal quenching of fluorescence was significantly reduced (62.1 ± 3.3%) as compared to all other compounds (≥84.6 ± 2.0%) might indicate a differently orientated binding that would differentially modify tryptophan environment.

Table 1. Role of flavonol B-ring substituents on the binding affinity for NBD2

Compound	R	K_D (μM)	ΔF_{max} (%)
Galangin	H	5.9 ± 0.2	95.0 ± 1.6
Kaempferol	4′-OH	6.7 ± 0.3	97.6 ± 1.0
Kaempferide	4′-OCH_3	5.0 ± 0.2	96.7 ± 0.6
4a	4′-F	6.8 ± 0.28	91.6 ± 1.2
4b	2′, 4′-Cl	4.0 ± 0.23	85.3 ± 1.3
4c	PhR = $-CH(Ph)_2$	2.0 ± 0.22	84.6 ± 2.0
4d	4′-I	1.06 ± 0.08	95.5 ± 2.0
4e	4′-*n*-C_8H_{17}	0.057 ± 0.02	62.1 ± 3.3



Scheme 1.

In summary, the binding affinity of eight flavonols to P-glycoprotein was evaluated by using a purified domain (NBD2). The potency was dependent on the hydrophobic substituent on the B-ring. The two most potent derivatives, 5,7-dihydroxy-4′-iodoflavonol and 5,7-dihydroxy-4′-octylflavonol are being evaluated for their ability to modulate the MDR.

Acknowledgements

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