4-Hydroxy-6-methoxyaurones with High-Affinity Binding to Cytosolic Domain of P-Glycoprotein

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A series of 4-hydroxy-6-methoxyaurones and 4,6-dimethoxyaurones has been synthesised and tested for their binding affinity toward the nucleotide-binding domain of P-glycoprotein, an ABC (ATP-Binding Cassette) transporter which mediates the resistance of cancer cells to chemotherapy. These compounds differ from each other by the nature of the substituent on the aurone B-ring. The binding affinity seems to be linked to the nature of the substituent, as well as to the presence or the absence of a hydroxy group at position 4. The most active compounds were 4′-bromo-4-hydroxy-6-methoxyaurone and 4-hydroxy-4′-iodo-6-methoxyaurone.

Key words aurone; binding affinity; P-glycoprotein; multidrug resistance

Multidrug resistance (MDR) of tumor cells to cytotoxic drugs is a major problem in cancer chemotherapy. Cells selected for MDR are characterized by cross-resistance to a wide variety of compounds, either natural or synthetic.[1)] The pharmacological basis for MDR appears to be a decreased accumulation and retention of drugs by these cells. It has been reported that the mechanism by which tumor cells acquire this MDR phenotype is the overexpression of an ATP-dependent membrane glycoprotein called P-glycoprotein (Pgp), that may be capable of binding a drug and expelling it from the cell.[2—5)] The driving force of drug efflux is Pgp-mediated ATP hydrolysis, the liberated energy of which is used for the drug transport.[6)]

During the past decade, a tremendous effort has been made to find compounds which are able to overcome MDR by restoring the intracellular accumulation of antitumor agents in resistant cells.[7,8)] Our group, has investigated the field of flavonoids which interact with the C-terminal nucleotide-binding domain of P-glycoprotein and may constitute potential MDR modulators. We have studied flavones, flavonols, chalcones and other phenolic compounds, and have identified the structural criteria required for a high binding-affinity.[9—13)]

Continuing our efforts in identifying Pgp-mediated MDR modulators, we wish to report here for the first time the binding of aurones, which constitute a naturally-occurring subclass of flavonoids frequently found in plants, where they contribute to the coloration of fruits and flowers (Fig. 1).[14)]

In previous studies we reported that the presence of a 2–3 double bond, a 4-ketone group, a 7-methoxy group, and a 5-hydroxy or methoxy group are favourable for high binding-affinity of flavones toward the C-terminal nucleotide-binding domain (NBD2) of Pgp. Halogenation on the B-ring of flavones (A-ring in chalcones) significantly increases the binding affinity toward NBD2 of P-glycoprotein.[9—12)] Therefore, we decided to identify, synthesise and test aurones where these structural criteria are conserved (Fig. 2).

Chemistry

We earlier reported a general and convenient synthesis of aurones.[15)] It employed (Chart 1) the condensation of phloroglucinol **1** with chloroacetonitrile in the presence of $ZnCl_2$, and the obtained imine was then hydrolysed to give the ketone **2**, which was cyclised in basic medium to provide the benzofurane **3**. Protection of the hydroxyls provided the 4,6-dimethoxybenzofurane **4**, and condensation of the latter with a benzaldehyde derivative gave the aurone **5**. The last step was a selective demethylation utilising BBr_3 to obtain the target aurone **6**. Under the same conditions, deprotection of aurones **5g** and **5h** gave a complex of several compounds which were difficult to purify. Other methods used (Me_3SI; Py·HCl; HBr/AcOH) failed to yield the desired compounds.

Results and Discussion

Aurones appear attractive from the perspective of drug design. Besides their contribution to the flowers of some popular ornamental plants, such as snapdragon and cosmos,[14)] they possess a wide range of biological activities. They have been described as antifungal agents and as phytoalexins.[16)] The naturally-occurring aureusidin (4,6,3′,4′-tetrahydroxyaurone) was found to be a potent inhibitor of iodothyronine-deiodinase.[17)] Recently, Kayser *et al.* reported the drug-potential of aurones in *Leishmania* infections.[18)] The advantage

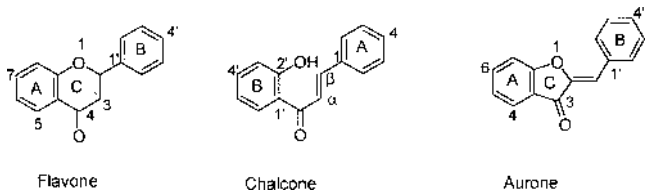

Fig. 1

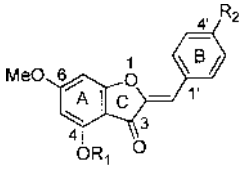

Fig. 2. Structure of Aurones Targeted in This Study

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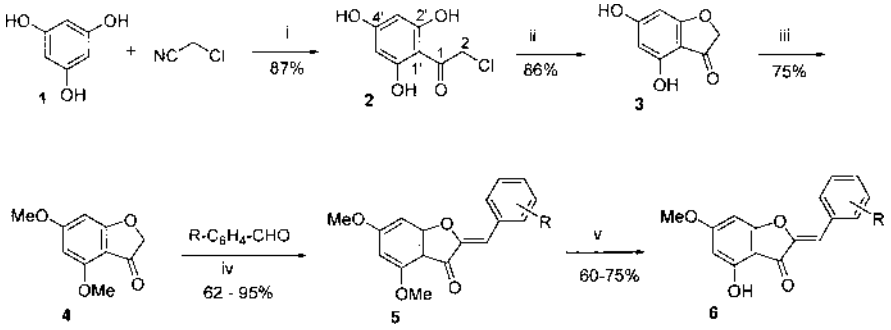

(i) a. $ZnCl_2$, HCl, Et_2O, 0 °C ; b. 1 N HCl, 100 °C. (ii) MeONa, MeOH, reflux, 2 h. (iii) MeI, K_2CO_3, DMF, 80 °C, 1 h. (iv) KOH, MeOH/H_2O, rt, 1 h. (v) BBr_3/CH_2Cl_2, 24 h.

Chart 1

Table 1. Role of Aurone Substituents on the Affinity for NBD2 of Pgp

Aurone	Substituent at position 4′	K_D (μM)	ΔF_{max} (%)
5a	H	7.0 ± 1.1	99.9 ± 5.8
5b	F	2.9 ± 0.7	88.6 ± 0.9
5c	Cl	0.99 ± 0.2	88.5 ± 6.3
5d	Br	0.82 ± 0.08	84.1 ± 2.5
5e	I	0.54 ± 0.04	85 ± 2.1
5f	CN	20 ± 4.6	100 ± 8
5g	$-N(CH_3)_2$	2.6 ± 0.4	72 ± 3.5
5h	3′,4′,6′-OCH_3	92 ± 43	87.6 ± 28
6a	H	1.32 ± 0.33	63.4 ± 6.3
6b	F	2.7 ± 0.8	85.1 ± 10.5
6c	Cl	0.46 ± 0.08	74.5 ± 3.4
6d	Br	0.15 ± 0.07	80 ± 7.9
6e	I	0.26 ± 0.03	78.1 ± 2.6
6f	CN	2.9 ± 1	46.2 ± 7.6

of such molecules is their high stability, as compared to chalcones which can easily undergo a cyclisation to yield inactive flavanones.

The binding of aurones to the purified C-terminal cytosolic domain of Pgp was measured by the quenching of protein intrinsic fluorescence, as previously described.[9] The dissociation constant (K_D) and the maximal fluorescence quenching (ΔF_{max}) were determined using the Grafit program.[19] As shown in Table 1, 4,6-dimethoxyaurones are less active than their corresponding 4-hydroxy-6-methoxyaurones, and halogenated aurones are the most active. The binding affinity of the latter increases with the size (hydrophobicity) of the halogen. Substitution of the 4-hydroxyaurone hydrogen at position 4′ with a bromo atom increases the binding affinity by nearly 8.8-fold. Overall, the data clearly show that the binding affinity is most likely correlated to the lipophilicity of the halogen rather than to hydrogen-bond capacity. The size of the halogen may also contribute to the binding affinity. The presence of heteroatoms other than halogens is unfavourable for high-binding affinity as can be deduced from K_D of **5g** and **5h**.

It has been shown that recombinant cytosolic domains of P-glycoprotein contain a steroid-interacting hydrophobic region in close proximity to the ATP-binding site.[20] The key roles played by the A-ring and α,β-unsaturated ketone system of flavones in mimicking the adenine moiety of ATP have been reported.[21,22] Based on these data, we can postulate that aurones overlap the two binding sites of NBD2 where the B-ring substituted by the hydrophobic halogen would bind to the steroid-interacting region and the A/C rings would interact with the ATP-binding site. Efforts are under way to synthesise aurones possessing more hydrophobic (alkyl chains) on the B-ring in order to obtain derivatives with high binding affinity.

Experimental

General Melting points were measured on a Fisher micromelting point apparatus and are uncorrected. MS spectra were determined on a JEOL HX-110 spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured on Bruker AC-200 spectrometer and were referenced to internal standards, tetramethylsilane (δ_H=0.00, δ_C=0.0); $CDCl_3$ (δ_H=7.27, δ_C=77.0). Electron-impact mass spectra were obtained at 70 eV using a Trio 1000 instrument. Elemental analyses were performed by the analytical department of CNRS, Vernaison, France. Thin-layer chromatography (TLC) was carried out using Merck silica gel F-254 plates (0.25 mm thick). Flash chromatography was carried out using Merck silica gel 60, 200—400 mesh. All solvents were distilled prior to use. The spectral and analytical data of 2′,4′,6′-trihydroxy-2-chloroacetophenone (**2**); 4,6-dihydroxybenzofuran-3(2*H*)-one (**3**); 4,6-dimethoxybenzofuran-3(2*H*)-one (**4**) and aurones **5a**, **5b**, **5c**, **5d**, **5g**, **5h** have been reported (ref. 15).

(*Z*)-4,6-Dimethoxy-(4′-iodoobenzylidene)benzofuran-3(2*H*)-one (**5e**): mp 165—167 °C (EtOAc). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 3.91 (3H, s), 3.95 (3H, s), 6.14 (1H, d, J=1.8 Hz), 6.38 (1H, d, J=1.8 Hz), 6.65 (1H, s), 7.55 (2H, d, J=8.4 Hz), 7.75 (2H, d, J=8.6 Hz). MS *m/z*: 409 $(M+1)^+$. *Anal*. Calcd for $C_{17}H_{13}IO_4$: C, 50.02; H, 3.21; I, 15.68. Found: C, 49.98; H, 3.17; I, 15.64.

(*Z*)-(4′-Cyanobenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (**5f**): mp 195—198 °C (EtOAc). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 3.96 (3H, s), 4.18 (3H, s), 6.53 (1H, d, J=1.4 Hz), 6.69 (1H, d, J=1.4 Hz), 7.20 (1H, s), 7.69 (2H, d, J=8.5 Hz), 7.94 (2H, d, J=8.5 Hz). MS *m/z*: 307 (M^+). *Anal*. Calcd for $C_{18}H_{13}NO_4$: C, 70.35; H, 4.26; N, 4.56. Found: C, 70.31; H, 4.23; N, 4.53.

4-Hydroxy-6-methoxyaurones (6a—f), General Procedure A solution of aurone (**5**) in CH_2Cl_2 was treated with BBr_3 (1 M in CH_2Cl_2, 2 eq) at room temperature for 24 h, after which, the solution was poured into ice water and extracted with CH_2Cl_2. The CH_2Cl_2 was dried over Na_2SO_4 and concentrated and the crude was purified by column chromatography eluted with CH_2Cl_2 to yield pure aurone (**6**). Under the same conditions, aurones (**5g**) and (**5h**) gave a complex mixture of several compounds.

(*Z*)-Benzylidene-4-hydroxy-6-methoxybenzofuran-3(2*H*)-one (**6a**): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 3.92 (3H, s), 6.17 (1H, d, J=1.8 Hz), 6.43 (1H, d, J=1.8 Hz), 6.78 (1H, s), 7.38—7.39 (3H, m), 7.83 (2H, dd, J_1=1.8 Hz, J_2=8.1 Hz). MS *m/z*: 268 (M^+). *Anal*. Calcd for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51. Found: C, 71.59; H, 4.48.

(*Z*)-(4′-Fluorobenzylidene)-4-hydroxy-6-methoxybenzofuran-3(2*H*)-one (**6b**): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 3.89 (3H, s), 6.16 (1H, d, J=1.8 Hz), 6.33 (1H, d, J=1.8 Hz), 6.71 (1H, s), 7.13 (2H, dd, J_1=J_2=8.7 Hz), 7.86 (2H, dd, J_1=5.4 Hz, J_2=8.7 Hz). MS *m/z*: 286 (M^+). *Anal*. Calcd for $C_{16}H_{11}FO_4$: C, 67.13; H, 3.87; F, 6.64. Found: C, 67.09; H, 3.83; F, 6.52.

(*Z*)-(4′-Chlorobenzylidene)-4-hydroxy-6-methoxybenzofuran-3(2*H*)-one (**6c**): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 3.90 (3H, s), 6.16 (1H, d, J=1.8 Hz), 6.34 (1H, d, J=1.8 Hz), 6.70 (1H, s), 7.41 (2H, d, J=8.5 Hz), 7.81 (2H, d,

J=8.5 Hz). MS m/z: 302 (M^+). *Anal*. Calcd for $C_{16}H_{11}ClO_4$: C, 63.48; H, 3.66; Cl, 11.71. Found: C, 63.44; H, 3.57; Cl, 11.63.

(Z)-(4′-Bromobenzylidene)-4-hydroxy-6-methoxybenzofuran-3(2H)-one (**6d**): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 3.89 (3H, s), 6.17 (1H, d, J=1.8 Hz), 6.34 (1H, d, J=1.8 Hz), 6.67 (1H, s), 7.57 (2H, d, J=8.5 Hz), 7.73 (2H, d, J=8.5 Hz). MS m/z: 347 (M^+). *Anal*. Calcd for $C_{16}H_{11}BrO_4$: C, 55.36; H, 3.19; Br, 23.02. Found: C, 55.33; H, 3.14; Br, 22.93.

(Z)-(4′-Iodoobenzylidene)-4-hydroxy-6-methoxybenzofuran-3(2H)-one (**6e**): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 3.83 (3H, s), 6.13 (1H, d, J=1.8 Hz), 6.51 (1H, d, J=1.8 Hz), 6.61 (1H, s), 7.67 (2H, d, J=8.5 Hz), 7.84 (2H, d, J=8.5 Hz). MS m/z: 395 (M^+). *Anal*. Calcd for $C_{16}H_{11}IO_4$: C, 48.75; H, 2.81; I, 32.20. Found: C, 48.71; H, 2.77; I, 32.09.

(Z)-(4′-Cyanobenzylidene)-4-hydroxy-6-methoxybenzofuran-3(2H)-one (**6f**): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 3.94 (3H, s), 6.38 (1H, d, J=1.6 Hz), 6.64 (1H, d, J=1.6 Hz), 7.24 (1H, s), 7.70 (2H, d, J=8.5 Hz), 7.92 (2H, d, J=8.5 Hz). MS m/z: 293 (M^+). *Anal*. Calcd for $C_{17}H_{11}NO_4$: C, 69.62; H, 3.78; N, 4.78. Found: C, 69.57; H, 3.73; N, 4.75.

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