# Molecular Interactions Between Human Cytochrome P450 1A2 and Flavone Derivatives

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**Abstract:** Activation by human cytochrome P450 1A2 (hCYP1A2) of heterocyclic amines is assumed to trigger of a number of carcinogenic processes. In this work, a group of natural inhibitors of human cytochrome P450 1A2 reported in literature has been theoretically analysed. These consist of flavone hydroxylated derivatives, natural compounds that exist in plants and associated products. Different theoretical/computational tools were used to describe the specific molecular interactions between these compounds and hCYP1A2. Based on this analysis, a method is proposed for helping the selection of specific molecular features that enhance protein-inhibitor interaction.

**Key Words:** Flavonoids, cytochrome P450, CYP1A2, stabilization energy.

## INTRODUCTION

CYPs are ubiquitous enzymes that undertake an important role in detoxification of the organism, by oxidizing xenobiotic substances such as pesticides and food additives to excretable products. They are also involved in the biosynthetic pathways of endogenous compounds such as fatty acids, retinoic acid and steroids. One negative side effect of their oxidative action is the activation of carcinogenic compounds to reactive mutagens [1-3]. For that reason, the understanding of the inhibition of this type of CYPs is a necessary step towards lowering the possibilities of such carcinogenic process to occur.

Different flavonoids have already been shown to lower the carcinogenic activity of human CYP1A2 (hCYP1A2) [4]. Flavonoids are plant secondary metabolites responsible for odor, taste and coloration. They are ubiquitous in various constituents of the human diet such as vegetables, fruit, tea and red wine. Their high antioxidant activity has been associated with prevention against diseases caused by oxidative damage and their pharmacological relevance includes also anti-inflammatory and antiviral action [4-7]. In humans, flavonoids interact with homologous enzymes in particularly with CYP1A1/1A2 isoforms [5-9]. This gives rise to yet another beneficial role attributed to these phytochemicals – inhibition of CYP1A1/1A2 activation of promutagens.

In this work, the hCYP1A2 inhibitory potency variation of a series of six flavonoids has been studied (see Fig. (**1**)). The experimental data we will be using concerns the inhibitory strength of different flavone derivatives scaled according to the correspondent IC50 values concerning the inhibition of methoxyresorufin demethylase (MROD) activity in microsomes containing c-DNA expressed hCYP1A2 [8].

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The use of a set of ligands with the same basic structure and a high rigidity allows for their inhibitory strength to be correlated with a simple substitution pattern, such as the number of hydroxyl groups and their position in the conjugated rings.

The six flavonoids were shown to be competitive inhibitors towards hCYP1A2 [8]. Competitive inhibition takes place when both substrate and inhibitor compete for binding into the same active site. Geometrical characteristics, such as shape, volume and surface, involved in enzyme-ligand complementarities, have an important role in this process. The flavones used in this study are very similar from a geometrical point of view and other differential characteristics should be selected to discriminate the inhibitory potency within this group. The analysis of the electrostatic potential pattern of the ligands together with both the inhibitor-enzyme complex geometry and energy for the series of flavones offers a number of clues on the physical properties that best contribute to their inhibitory potency over hCYP1A2.

## METHODS

The initial structures of the flavone derivatives studied in this work have been modelled in InsightII [10] using the crystallographic structure of 3,5,7-trihydroxyflavone taken from Cambridge Database [11]. All these structures have been subsequently optimized using the Gaussian98 package [12], at the B3LYP/6-31G* level.

Molecular electrostatic potentials (MEPs) of the ligands were generated at the B3LYP/6-31G* level. We used MOLEKEL [13] to map the electrostatic potential onto an electron density surface of 0.002 electrons/bohr$^3$ (generally used, corresponding to about 95% of the electronic charge) and to draw three-dimensional electrostatic potential isosurfaces. The latter are used to predict long-range interactions [14-16].

The structure for human CYP1A2 used for docking the flavone derivatives is a homology model built as described in

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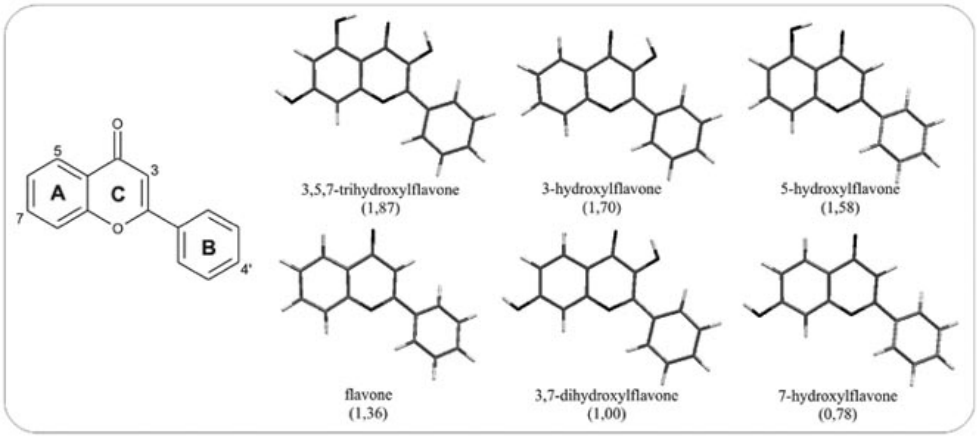


**Fig. (1).** The flavone derivatives studied by Zhai *et al*. - in parenthesis are the correspondent values for –log[IC50(M)].

Fonseca *et al.* [17]. The final geometry was used for automated docking of the flavone derivatives with GOLD [18], using the standard default settings. The best 5 results of 50 docking runs for each ligand were analyzed. A distance constraint of 6 Å between the most likely carbon atom to be oxidized, C4', and the iron atom [9, 19, 20] was used. Subsequently we refined the docking results. First, the docking position was geometry optimized using the complete solvated model, keeping the protein fixed, with programme CHARMM and corresponding force field [21]. Then, an energy minimization was performed allowing the side-chains to move together with the ligands. The united atom force-field parameters and 12 Å non-bonded cut-off distance were used. Solvent was treated explicitly by using an 8 Å layer of TIP3P water. Harmonic constrains were applied to the water molecules located more than 5 Å away from the enzyme. Minimizations used 500 steps of steepest descent followed by conjugate gradient.

After determining the most stable docking configurations, the affinity of a flavonoid to an enzyme active site can be evaluated by the correspondent stabilization energy. This has been calculated as follows:

$$\Delta E^{Stab}(cyp:flv) = E(cyp:flv) - \left[E(cyp) + E(flv)\right] \qquad \textbf{(1)}$$

where, *E(cyp : flv)*, *E(cyp)* and *E(flv)* are the total energies of the hCYP1A2:ligand complex, the enzyme and the flavonoid, respectively. The stabilization energy will be presented as normalized with respect to the weakest ligand:

$$\Delta\Delta E^{Stab}(cyp:flv) = E(cyp:flv) - E(cyp:flv\_weakest) - \left[E(flv) - E(flv\_weakest)\right] \qquad \textbf{(2)}$$

The interaction energies in the optimized complexes were determined with the INTER utility available in CHARMM [21]. This quantity, generally named $\Delta E^{inter}$, is one of the components of the stabilization energy:

$$\Delta E^{Stab}(cyp:flv) = \Delta E^{Inter}(cyp,flv) + \Delta E^{rearr}(cyp) + \Delta E^{rearr}(flv) \qquad \textbf{(3)}$$

In equation [3], $\Delta E^{rearr}$ *(cyp)* and $\Delta E^{rearr}$ *(flv)* are the conformational rearrangement energies for the hCYP1A2 enzyme and the flavonoid, respectively. Energy of this type is associated with the transition from the optimized geometry of the correspondent fragment to the characteristic geometry assumed by this species in the rearranged complex *CYP:flv*. In the same equation, $\Delta E^{inter}$ *(cyp,flv)* is the interaction energy between the enzyme and the flavonoid within the same rearranged complex. This quantity can be calculated for any given pair of fragments of the complex (enzyme/ligand, heme/ligand, etc). We will present this quantity normalized with respect to the weakest ligand, $\Delta\Delta E^{inter}$.

## RESULTS AND DISCUSSION

### Electrostatic Potentials

Molecular recognition processes are usually involved in the approach of the ligands to the active site entrance and its subsequent binding. In this context, the electrostatic pattern recognition has been demonstrated to have a crucial role [14, 22-24]. By observing the molecular electrostatic pattern it is possible to detect the potential sites for H-bonding and other noncovalent interactions formation, which could be very important for a correct orientation of the inhibitor inside the enzyme [14-16, 24]. The local minima in the potential surface corresponds to areas, which are susceptible of electrophilic attack while the regions predisposed to nucleophilic interactions are only recognizable when displayed at a certain distance from the nucleus (the highest positive peak of electrostatic potential in the molecule), which is why we have displayed the MEP mapped onto a molecular surface.

Fig. (**2**) shows the electrostatic potential mapped onto an electron density isosurface (0.002 electrons/bohr$^3$) of the flavone hydroxylated derivatives studied experimentally by Zhai *et al.*, those of the new ligands drawn in this study, and of one aminoflavone substrate. The regions of lowest electrostatic potential are in red and the peaks of positive electrostatic potential are in blue. The regions of lower electrostatic potential seem to carry the features which will be determinant for molecular recognition and are represented separately in the same figure. The aminoflavone derivative is presented as a model for the features that are important for enzyme-ligand complementarity, as it is a molecule that has a high specificity for hCYP1A2 [20].

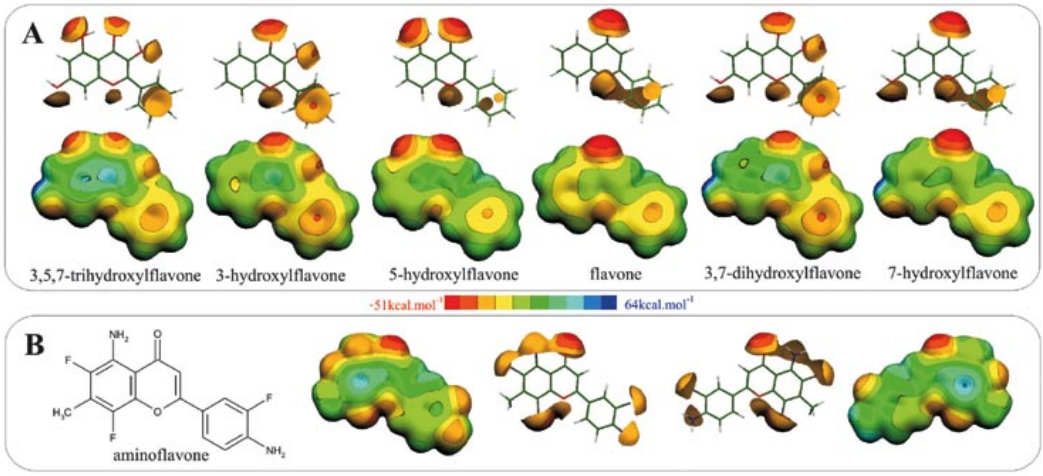


**Fig. (2).** Electrostatic potential mapped onto an electron density isosurface (0.002 electrons/bohr3). The regions of lowest electrostatic potential are represented separately in the second row for each particular set of compounds. (a) Compounds studied experimentally by Zhai *et al*. (b) Specific aminoflavone substrate for hCYP1A2.

The fact that the B ring from these flavones has no electronegative substituent and that phenyl rings have a very low reactivity will definitely contribute to their inhibitory properties. The common feature between the best inhibitor of the set and the aminoflavone is the spreading of the lowest potential peak between the oxo group and the hydroxyl groups on carbons C3 and C5 in the flavones and also for the fluoride bound to carbon C6 in the aminoflavone. This delocalization seems to be a molecular recognition feature related to an increase in inhibitory power by means of specific interaction with the active site of hCYP1A2. If we compare 3-hydroxylflavone and 5-hydroxylflavone with flavone, and 3,5,7-trihydroxylflavone with 5-hydroxylflavone/3-hydroxylflavone/3,7-dihydroxylflavone, this seems obvious. If this negative potential area is wide, meaning there are hydroxyl substituents on both C3 and C5, then another negative potential spot subsequent to C5 will contribute to a higher inhibitory power. Actually, in the aminoflavone, there is a methyl group in C7, correspondent to a positive potential area, followed by a fluoride group, which corresponds to negative potential. This combination seems to favor a good fit in the active site, and should be related to this compound's specificity towards hCYP1A2.

The electrostatic potential isosurfaces at 7 kcal $mol^{-1}$ (white) and -7 kcal $mol^{-1}$ (grey) for the flavone hydroxylated derivatives are represented in Fig. (**3**). Also in these maps we can see that the spreading of the negative density towards the A ring correlates with higher inhibition power. Here it is more evident that the best inhibitors have, on the A ring, a small positive density spot in between two negative regions. In the aminoflavone substrate, the same spot corresponds to a methyl substituent between the two fluoride substituents on positions 6 and 8 and in 3,5,7-trihydroxylflavone it is related to the hydrogen atom on C6.

In the following discussion it will be shown how besides playing a role in molecular recognition these features are involved in complex stabilization.

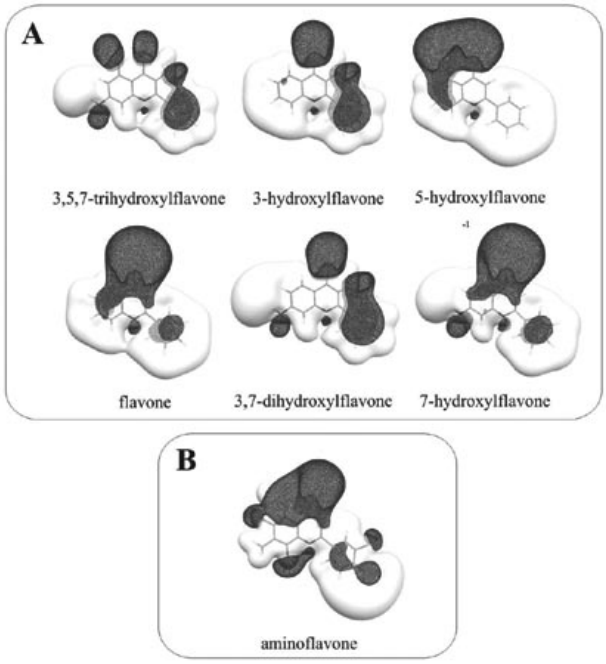


**Fig. (3).** Electrostatic potential isosurfaces at 7 kcal $mol^{-1}$ (white/transparent) and -7 kcal $mol^{-1}$ (grey/chickenwire) for the flavone hydroxylated derivatives. (a) Compounds studied experimentally by Zhai *et al*. (b) Specific aminoflavone substrate for hCYP1A2.

## Docking

The results of docking optimization are shown schematically in Fig. (**4**) and (**5**).

The main points for electrostatic interaction between ligand and enzyme are the hydrogen bonds between the side-chains of $Thr_{498}$, $Tyr_{112}$ and $Asn_{234}$ and both the hydroxyl substituents of the flavone derivatives and the oxo group on

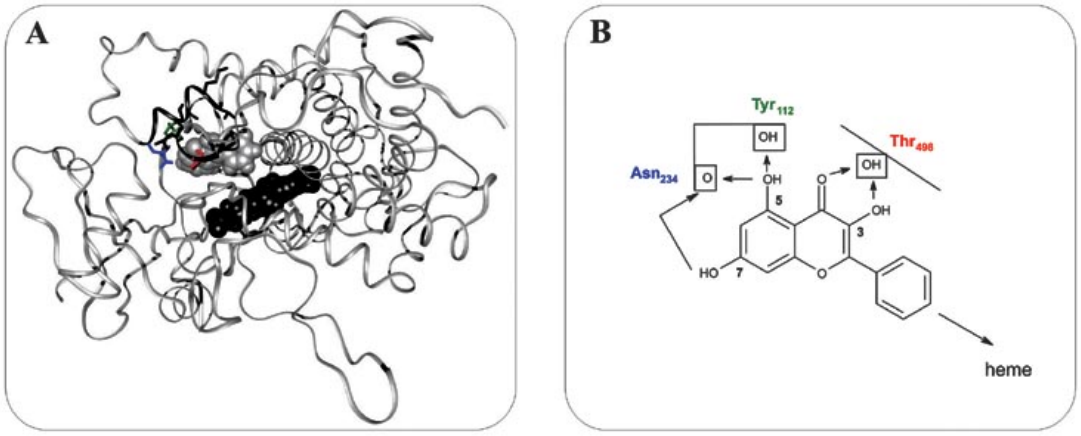


**Fig. (4). (a)** C-alpha trace of the model of hCYP1A2 with 3-hydroxylflavone docked in the active site. The trace in black corresponds to the entrance of the active site area and the side-chains of the corresponding residues are shown in stick. The side-chains of the amino acid residues that make H-bonds with the different flavone derivatives are shown in grey ($Tyr_{112}$, $Asn_{234}$ and $Thr_{498}$). The side-chains of $Val_{227}$, $Glu_{228}$, $Ala_{230}$, $Ser_{231}$, $Gly_{233}$, $Leu_{497}$, and $Met_{499}$ are in black. The heme is represented in black/CPK and 3-hydroxylflavone is represented in white/CPK. The hydrogen bonds formed between the different flavone derivatives and the enzyme are shown schematically in **(b)**.

the carbon atom C4 (see Fig. (**4**)). The interaction between the oxo group on C4 and $Thr_{498}$ is a common feature in all the flavone derivatives complexes. This substituent represents the most electronegative area on the ligands MEP and should be a major spot for molecular recognition. The fact that $Thr_{498}$ is located close to the surface of the active site (see Fig. (**4**)) supports this idea.

Also $Asn_{234}$ and $Tyr_{112}$ are located on the top of the active site, and together with $Thr_{498}$ should be one of the first residues of the active site to make contact with the ligand. The spreading of the negative electrostatic potential of the oxo group on C4 towards the hydroxyl groups in ring A, which seems to be a common feature among the best inhibitors, is also related to a higher stabilization of the ligands by hydrogen bonding of the latter and the neighbouring aminoacid sidechains of $Asn_{234}$ and $Tyr_{112}$ as can be seen in Fig. (**5**). The water molecules, which are located close to the entrance of the active site are also shown. It is noteworthy that the ligands, which have an hydroxyl group on C7 and none on C5 present a less buried docked conformation with the substituent on C7 leaning towards the solvent molecules.

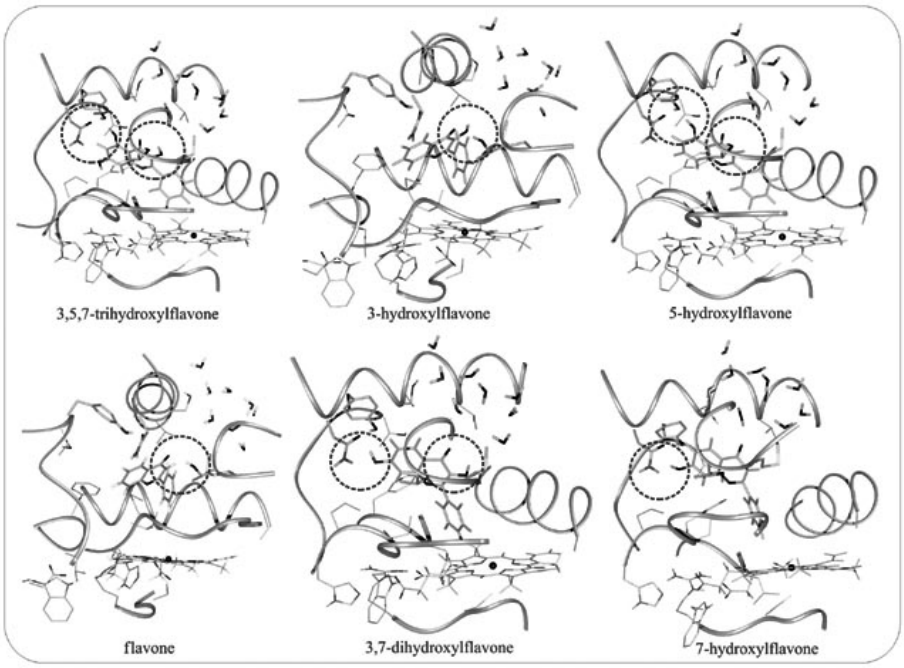


**Fig. (5).** Flavone hydroxylated derivatives docked in the active site of hCYP1A2. The circles show the H-bonds schematically represented in Fig. (**4b**).

**Table 1. $IC_{50}$ Values for the Inhibitors Used in this Work Relative to Their Activity Over the *O*-demethylation of Methoxyresorufin by hCYP1A2 Determined by Zhai *et al.*, and the Stabilization Energies of Their Complexes with Human CYP1A2. Quantity $\Delta\Delta E^{Inter}$, Which is the Part of the Total Energy that Shows the Interaction Between Individual Fragments of the Complex, is Presented for the Interactions Between the Flavone Derivatives and hCYP1A2 ($\Delta\Delta E^{Stab}(cyp:flv)$), $Thr_{498}$ ($\Delta\Delta E^{Inter}(Thr_{498}, flv)$), and for the Group of Residues Located on the Entrance of the Active Site Shown in Fig. (4) ($\Delta\Delta E^{Inter}(actsite\_top, flv)$). All Values are Normalized with Respect to the Weakest Inhibitor, 7-hydroxylflavone.**

| Flavonoid (-log[$IC_{50}$(M)]) | $\Delta\Delta E^{Stab}(cyp:flv)$ (kcal $mol^{-1}$) | $\Delta\Delta E^{Inter}(Thr_{498}, flv)$ (kcal $mol^{-1}$) | $\Delta\Delta E^{Inter}(actsite\_top, flv)$ (kcal $mol^{-1}$) |
|---|---|---|---|
| 3,5,7-triOHflavone (1.87) | -43.5 | -8.0 | -6.4 |
| 3-OHflavone (1.70) | -40.6 | -5.9 | -5.4 |
| 5-OHflavone (1.58) | -27.4 | -5.9 | -3.5 |
| Flavone (1.36) | -13.5 | 1.4 | 3.1 |
| 3,7-diOHflavone (1.00) | -0.9 | 2.3 | 1.2 |
| 7-OHflavone (0.78) | 0.0 | 0.0 | 0.0 |

### Stabilization in the Active Site

The stabilization energy of the hCYP1A2/flavone derivatives complexes after geometry optimization is shown in Table **1**, together with its more relevant components and the corresponding $IC_{50}$ values for the inhibitors. It can be seen that the trend observed for the $IC_{50}$ values is maintained for the stabilization energy.

This is also true for some of the components of the stabilization energy, such as the part correspondent to the interaction energy between the flavones and the aminoacid residues at the top of the active site (see values for $\Delta\Delta E^{Inter}$ *(cyp, flv)* in Table **1** and Fig. (**4**)). A stabilizing interaction in this area of the active site favours the best inhibitors (which confirms the importance of the oxo group on the C4 atom), particularly the interaction with $Thr_{498}$. Notice that for the two weaker inhibitors, the previously described less buried docking position result in a lower enzyme-ligand energetic complementarity with respect to the residues located on the top of the active site.

Using this approach, we have also calculated the stabilization energy for the molecule 5,7-dihydroxylflavone, which has been shown to be a more potent inhibitor than flavone [7]. We obtained a value of -33.2 kcal $mol^{-1}$, confirming the correlation between the inhibitory potency of flavonoid inhibitors and the stabilization energy.

## CONCLUSIONS

In this work, a set of theoretical tools for analysing enzyme/inhibitor association were presented. The hCYP1A2 inhibition by flavone hydroxylated derivatives has been studied using several approaches. The MEPs study showed that the negative potential located around the oxo group in C4 is important for enzyme-ligand complementarity, and it becomes more evident when it is spread over the substituents in ring A. The study of specific interactions between the enzyme and the ligands related this molecular recognition feature with a stabilizing interaction resulting from hydrogen bonding between the substituents in the A and C rings of the ligands and the aminoacids located at the top of the active site. The hydroxyl substituents on C3 and C5, which surround the oxo group on C4, have an important role in the fitting of the ligand in the active site. This involves a stabilizing interaction with $Thr_{498}$, which is strong in the best inhibitors of the group. The existence of a negative potential area from C5 to C7 improved the stabilization of the complexes in case there was an hydroxyl substituent in C3. As far as molecular complementarity is concerned, a positive potential between these two negative potential areas increases specificity. Different aspects of the molecular relationship between hCYP1A2 and flavone derivatives were covered in this way. This type of approach should help in the refinement of the binding properties of specific classes of inhibitors.

## ACKNOWLEDGMENTS

We thank the FCT (Fundação para a Ciência e Tecnologia) for a doctoral scholarship for R. F and the NFCR (National Foundation for Cancer Research) Centre for Drug Discovery, University of Oxford, U.K., for financial support.

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**Received: 27 October, 2005 Revised: 28 November, 2005 Accepted: 29 November, 2005**