Chapitre 4

Les inhibiteurs du cytochrome P450 (CYP) 1B1

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Avant-propos

Le Chapitre 4 de mon mémoire est constitué d'une revue scientifique qui a été soumise au journal *European Journal of Medicinal Chemistry* le 8 mars 2017, acceptée le 13 avril 2017 et publiée en ligne le 18 avril 2017. Il a ensuite été incorporé au volume 135 de 2017 aux pages 296 à 306. Le numéro d'identification de cette revue est 10.1016/j.ejmech.2017.04.042.

Mon directeur de recherche, Donald Poirier, a participé activement à la rédaction de ce manuscrit en me guidant dans l'organisation de sa structure, dans la recherche bibliographique et en m'apportant différentes suggestions et corrections pour bonifier le manuscrit.

J'ai pour ma part réalisé la recherche bibliographique, le plan de la revue, la première version ainsi que la version finale du manuscrit en y ajoutant les dernières découvertes autour de l'inhibition de la CYP1B1.

Résumé

L’enzyme cytochrome P450 1B1 (CYP1B1) humaine est impliquée dans le métabolisme de divers médicaments. Elle catalyse l'hydroxylation des composés aryliques, générant ainsi des métabolites plus polaires qui peuvent être facilement excrétés. L’enzyme CYP1B1 est également connue pour sa capacité à activer de nombreux composés procarcinogènes en métabolites carcinogènes. Par exemple, elle peut hydroxyler la 17β-estradiol (E2) en 4-hydroxy-E2, ce qui peut favoriser la carcinogénèse en raison de la puissante activité estrogénique de ce métabolite. La 4-hydroxy-E2 peut également être transformée en E2-3,4-quinone, un composé pouvant former des sites apuriniques en se liant de façon covalente à l'ADN. Depuis que les niveaux d'expression élevés de CYP1B1 ont été signalés dans divers cancers, mais pas dans les tissus normaux, cette enzyme représente une cible thérapeutique intéressante. Cette revue met l'accent sur les différentes familles d'inhibiteurs de la CYP1B1, en particulier les composés rapportés depuis 2003.

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Inhibitors of cytochrome P450 (CYP) 1B1

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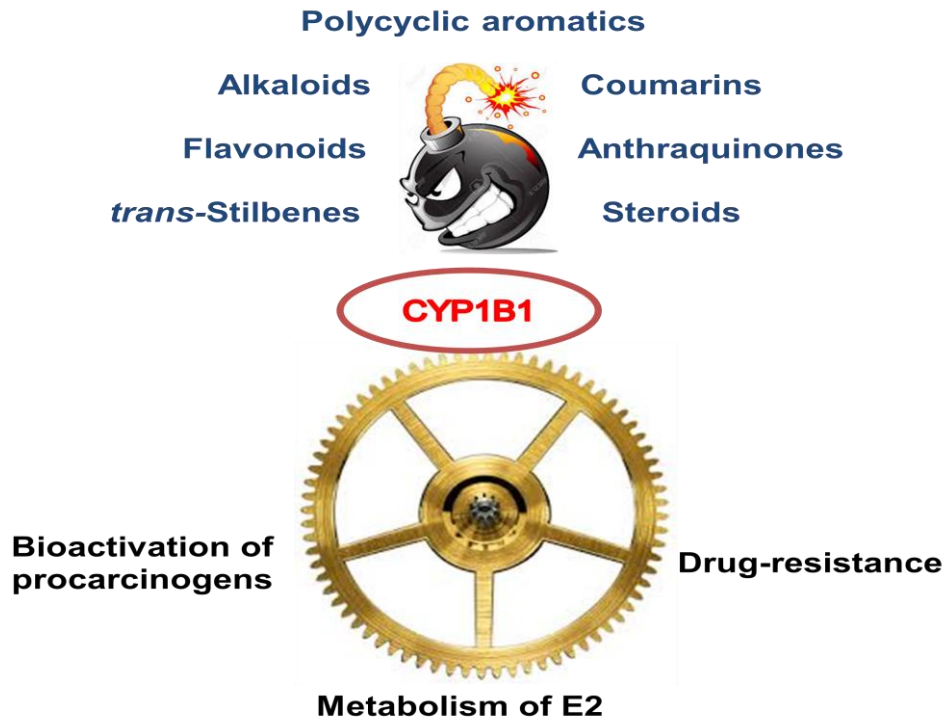
Abstract

Human cytochrome P450 1B1 (CYP1B1) is involved in the metabolism of various drugs. This enzyme catalyzes the hydroxylation of aryl compounds, thus generating more polar metabolites that can be easily excreted. CYP1B1 is also known for its ability to activate procarcinogens into carcinogens. For example, it can hydroxylate 17β-estradiol (E2) into 4-hydroxy-E2, which can promote tumorigenesis as a potent estrogen, or after being transformed into E2-3,4-quinone. Since elevated expression levels of CYP1B1 have been reported in various cancers, but not in normal tissues, this enzyme represents an interesting therapeutic target. This review put emphasis on different families of inhibitors, especially those reported since 2003.



1. Introduction

Cytochromes P450 (CYPs) constitute a large family of hemo- proteins involved in many reduction and oxidation reactions on both endogenic and xenobiotic molecules of various sizes [1,2]. This enzyme family is primarily made up of monooxygenases which are present in several animals, plants, fungi, bacteria, protists, archaea and also in some viruses [3]. More than 21,000 CYPs have been identified to date, and 18 families, including over 50 enzymes are found in humans [1,4]. Cytochrome P450-mediated diseases are associated with anormal steroidogenesis; defects in cholesterol, fatty and bile acid pathways; vitamin D dysregulation and with retinoid dysfunction during fertilization, implantation, embryogenesis, foetogenesis and neonatal development [5].

The CYP1 subfamily contains three members: CYP1A1, CYP1A2 and CYP1B1. Human CYP1B1 share 41 and 40% amino acid sequence homology with human CYP1A1 and CYP1A2, respectively, while the latter two are 72% identical [6]. Three-dimensional structures of human CYP1A1, CYP1A2 and CYP1B1 have been reported and can be consulted in the Protein Data Bank [7-9]. CYP1A1 is expressed in the liver but also in many extrahepatic tissues such as pancreas, thymus, uterus and small intestine, while CYP1A2 is constitutively expressed in the liver [10]. CYP1B1 is mainly expressed in extrahepatic tissues such as breast, prostate and uterus. Furthermore, a low level of CYP1B1 mRNA has been detected in several normal human tissues such as kidney, liver, intestine, eye tissue and brain [6,10,11]. CYP1A1 and CYP1B1 have been widely studied because they are involved in the conversion of a large number of polycyclic aromatic hydrocarbons (PAHs) into carcinogens [12]. Also, it should be emphasized that CYP1 enzymes are involved in the modulation of pro-inflammatory and inflammatory pathways through the metabolism of leukotrienes and eicosanoids [13]. Moreover, it has been shown that some flavonoid-type compounds could act as CYP1 substrates, leading to the formation of more antiproliferative agents within cancer cells [14].

CYP1B1 is the most interesting target among the three CYP1 mentioned above. Indeed, it should be noted that CYP1B1 is the most efficient enzyme catalyzing the hydroxylation of the potent estrogenic C18-steroid 17β-estradiol (E2) (Fig. 1) [15]. Moreover, it is overexpressed in various types of human cancers (breast, lung, colon, esophagus, skin, testis, lymph node and brain), but not in healthy tissues [16]. This enzyme is also a good marker for the prevention of certain cancers, such as breast cancer. In addition to its involvement in the

activation of many PAHs such as benzo[α]pyrene, CYP1B1 has a distinct selectivity for the 4-hydroxylation of E2, whereas CYP1A1 and CYP1A2 are mainly implicated in the 2-hydroxylation of E2 [17]. Unlike 2-hydroxy-E2 and its oxidation product E2-2,3-quinone, 4-hydroxy-E2 can be oxidized into E2-3,4-quinone, which has a high mutagenic potential by interacting covalently with DNA, thus leading to the formation of depurinating adducts [18]. Finally, CYP1B1 is also involved in the metabolism of some anticancer agents such as docetaxel, leading to drug resistance associated with the overexpression of CYP1B1 [19,20]. Therefore, an inhibitor of CYP1B1 could be useful in certain multitherapies. Clearly, the inhibition of CYP1B1 represents a promising therapeutic strategy because it would permit a therapeutic action at three different levels (**Fig. 1**): (1) by inhibiting the formation of 4-hydroxy-E2, (2) by inhibiting the bioactivation of procarcinogens, and (3) by reducing drugresistance [1,20]. However, it is important to specifically inhibit CYP1B1 because CYP1A1 plays a significant role in the detoxication of environmental procarcinogens, and contributes also to the metabolic activation of dietary compounds with cancer preventive activity [21].

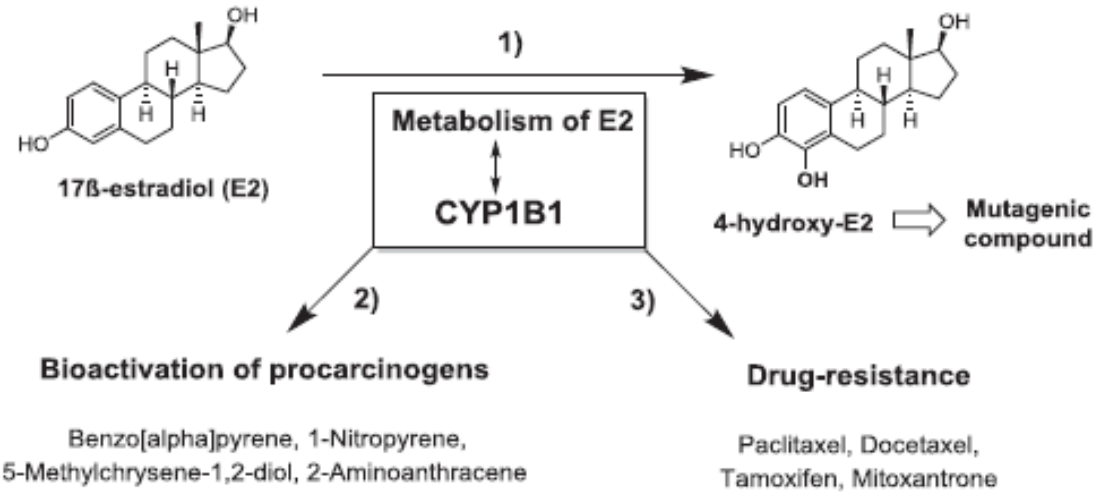


Fig. 1. Involvement of CYP1B1 in the development of cancer.

In a review article published in 2003 [1], Chun and Kim clearly exposed the state of knowledge of CYP1B1 and its inhibitors by grouping them according to several families: synthetic aromatic compounds, naturally occurring coumarins, flavonoids, naturally occurring stilbenes and analogs, anthraquinones and anti-cancer agents. Among these families of compounds, we note that the flavonoids and the stilbenes have been the most studied for the inhibition of CYP1B1. Stilbene family thus provided the best inhibitors in terms of activity and selectivity, particularly with the 2,4,3',5'-tetramethoxystilbene which is one of the most

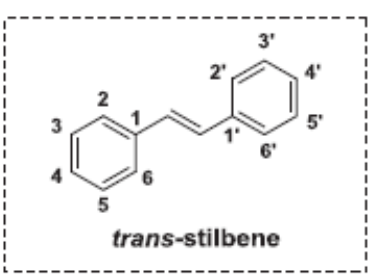
selective inhibitors of CYP1B1 [22]. Moreover, this compound also specifically inhibits the 4-hydroxylation of E2 by CYP1B1. There are also some exotic compounds which have shown some inhibitory activity against CYP1B1. This is the case, for example, of organoselenium compounds, some anticancer agents (e.g. flutamide) and certain alkaloids (e.g. rutaecarpine) [1,23-27]. Moreover, it should be noted that feedback inhibition of CYP1B1 by methoxyestrogens has been reported [28].

Since 2003, improved versions of known inhibitors or novel inhibitors have been the subject of new publications; we therefore deemed it relevant to write a new review article. It should be emphasized that two review articles were recently published. In 2014, Cui and Li wrote a review of different CYP1 inhibitors and reported several CYP1-activated prodrugs [29]. Their exhaustive review presents a complete listing of the chemicals interacting with CYP1 enzymes and provides key information based on structureactivity relationship (SAR) studies. The second review article, published in 2016 by Dong et al. [30], focuses on flavonoids and naphthoflavonoids as CYP1 substrates and inhibitors. In our minireview article, we focused on the CYP1B1 inhibition and we presented, across different families, various new CYP1B1 inhibitors published between 2003 and 2017.

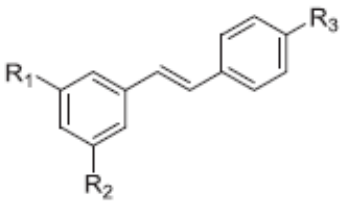
2. Stilbenes

Stilbenes have been studied extensively as CYP1B1 inhibitors and they generally represent the most active and selective inhibitors among the different previously mentioned families [1]. The basic structure of stilbenes (**Fig. 2**) has two aromatic groups on either side of a double bond. For instance, the trihydroxylated transstilbene resveratrol (**1**) is present in red wine and is a chemopreventive agent due to its antioxidant activity [31]. However, resveratrol is not selective for CYP1B1 because it also inhibits CYP1A1 [32,33] and its activity was quite low in comparison with some other stilbenes, which are much more active inhibitors [1]. Subsequently, several derivatives of resveratrol have been developed, including, for example, compounds **2-11** bearing methoxy (CH_3O) or thiomethyl (CH_3S) groups instead of the hydroxy (OH) group generally found in basic scaffold. Among those derivatives without a thiomethyl group, compounds **2**, **3** and **4** appear to be good inhibitors of CYP1A1 (K_i = 0.13, 0.16 and 0.57 μM, respectively), but they are less active against CYP1B1 with K_i values (0.90, 2.06 and 0.91 μM, respectively) similar to that of resveratrol (K_i = 0.8 μM) [34]. Within the stilbene derivatives bearing a thiomethyl group and at less one methoxy group,

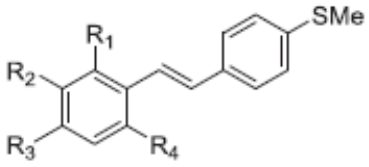
compounds **5** and **6** (K_i = 0.041 and 0.030 μM, respectively) exhibit a better activity against CYP1B1 than resveratrol (IC_{50} = 0.3, 0.5 and 11.2 μM, respectively) [35]. Furthermore, some derivatives bearing up to three methoxy groups and one thiomethyl group have also been studied. In this series, compound **7** was the best CYP1B1 inhibitor (IC_{50} = 0.5 μM), but it was not selective for CYP1A1 (IC_{50} = 0.4 μM) although 13-fold selective for CYP1A2 (IC_{50} = 6.5 μM). The introduction of a chlorine atom instead of the three methoxy groups (compound **8**) kept the CYP1B1 inhibitory activity (IC50 = 0.3 μM), but increased the selectivity for CYP1A2 and CYP1A1 by 48- and 3-fold, respectively [36]. These results highlight the influence of the nature and the position of substituents over the activity and the selectivity of stilbene derivatives toward CYP1 enzymes.



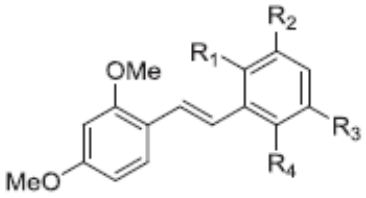
trans-stilbene



1 : $R_1 = R_2 = R_3 = OH$
(resveratrol)
2 : $R_1 = R_3 = OH$; $R_2 = OMe$
(pinostilbene)
3 : $R_1 = R_2 = OH$; $R_3 = OMe$
(desoxyrhapontigenin)
4 : $R_1 = R_2 = OMe$; $R_3 = OH$
(pterostilbene)



5 : $R_1 = OMe$; $R_2 = R_3 = R_4 = H$
(2-methoxy-4'-thiomethyl-*trans*-stilbene)
6 : $R_1 = R_3 = R_4 = H$; $R_2 = OMe$
(3-methoxy-4'-thiomethyl-*trans*-stilbene)
7 : $R_1 = R_3 = R_4 = OMe$; $R_2 = H$
(2,4,6-trimethoxy-4'-thiomethyl-*trans*-stilbene)
8 : $R_1 = Cl$; $R_2 = R_3 = R_4 = H$
(2-chloro-4'-thiomethyl-*trans*-stilbene)



9 : $R_1 = R_4 = H$; $R_2 = R_3 = OMe$
(2,4,3',5'-tetramethoxy-*trans*-stilbene)
10 : $R_1 = R_2 = R_4 = OMe$; $R_3 = H$
(2,2',3',4,6'-pentamethoxy-*trans*-stilbene)
11 : $R_1 = R_4 = OMe$; $R_2 = R_3 = H$
(2,4,2',6'-tetramethoxy-*trans*-stilbene)

Fig. 2. Stilbene derivatives as inhibitors. The basic structure including carbon numbering is represented in the dashed square.

After the identification of 2,4,3',5'-tetramethoxy-trans-stilbene (compound **9**) as a highly potent and selective competitive inhibitor of CYP1B1 with IC_{50} values of 6 nM, 300 nM and 3 μM for CYP1B1, CYP1A1 and CYP1A2, respectively [22], and as part of their ongoing program to develop CYP1B1 inhibitors, Chun and collaborators [37] reported a new series of

13 derivatives of compound **9**. Compound **10**, having five methoxy groups instead of four, was found to be a more potent and selective inhibitor of CYP1B1 than lead compound **9** (IC_{50} = 3.3 and 6 nM, respectively). Among the stilbene derivatives mentioned above, compound **11** (with four methoxy groups) has shown the best inhibitory activity toward CYP1B1 with an IC_{50} value of 1.77 nM. In comparison to compound **9**, its selectivity for CYP1A1 was better, but its selectivity for CYP1A2 was clearly lower [37,38]. In relation to breast cancer, compound **9** was used to modulate ductal growth of the developing murine mammary gland. This first generation CYP1B1 inhibitor blocked the conversion of 17β-estradiol (E2) to both 2-OH-E2 and 4-OH-E2, but did not regulate CYP1B1 at the level of transcription [39].

3. Polycyclic aromatic compounds

Synthetic aromatic compounds have long been recognized as inhibitors of CYP1 enzymes. Typical examples among this class are polycyclic aromatic hydrocarbons (PAHs), such as **12** and **13** (**Fig. 3**), which demonstrated a potent inhibition of CYP1B1 (IC_{50} = 2 and 30 nM) with a selectivity over CYP1A1 (IC_{50} = 41 and 151 nM) [40]. Shimada et al. [41] examined the mechanism of inhibition of a selection of four PAHs (compounds **13-16**) that inhibited CYP1A1, CYP1A2 and CYP1B1. The results suggest different mechanisms of inhibition for CYP1A1, CYP1A2 and CYP1B1 by PAHs and related compounds. However, only K_i (2.3 μM) and kinactivation (0.04 min^{-1}) values for compound **16** have been determined for CYP1B1. Extending their work, Shimada et al. [42] tested 23 PAHs and demonstrated the potential of 15 PAHs to inhibit CYP1B1. Three of them, compounds **17-19**, potently and selectively inhibited CYP1B1 over CYP1A1 and CYP1A2. Tested in the same conditions, dibenz[*a*,*h*]anthracene (**17**) was found to be a more potent and selective inhibitor than α-naphthoflavone (**20**) (IC_{50} = 9.0 and 5.2 nM, respectively). A complex mixture of PAHs extracted from coal tar, the Standard Reference Material (SRM) 1597, competitively inhibited both CYP1B1 and CYP1A1, but the inhibitory effect was stronger on CYP1B1 [43]. Another not well defined mixture of PAHs, the urban dust particulate matter, was also reported to noncompetitively inhibit CYP1B1 and CYP1A1 in a dose-dependent manner [44].

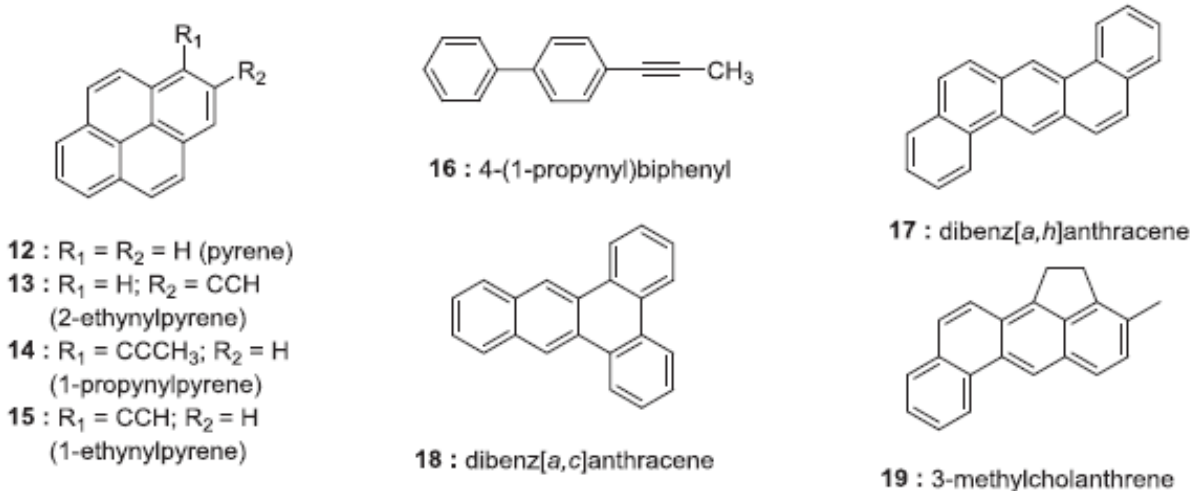


Fig. 3. Polycyclic aromatic compounds as inhibitors.

4. Flavonoids

Flavonoids have been extensively studied to develop CYP1 inhibitors (**Fig. 4**). These polyphenol compounds are secondary plant metabolites, and they are called chromophores because they are responsible for the different colors in fruits and flowers. Major flavonoids are divided into several subgroups: flavones, flavonols, dihydroflavonols, flavanones, flavanols and anthocyanidins. More rarely, plants can contain other types of flavonoids which are denominated isoflavonoids, neoflavonoids and minor flavonoids (e.g. chalcones). Also, it should be emphasized that flavonoids are a major source of antioxidants in our diet [45]. Moreover, flavonoids have shown good anticancer properties, particularly because they interact with CYPs. It is important to underline that dietary flavonoids could act as CYP1 inhibitors, as CYP1 substrates, and as substrates and inhibitors of CYP1 enzymes, depending of the degree of hydroxylation and/or methylation of the A and B rings [14,46].

Within this family, we find for example the α-naphthoflavone (compound **20**) which is recognized as a potent inhibitor of CYP1 enzymes with IC_{50} values of 60, 6 and 5 nM for CYP1A1, CYP1A2 and CYP1B1, respectively [40]. The first 3D-crystal structure of a complex between CYP1B1 and an inhibitor was in fact obtained with compound **20** [9]. The results showed that both CYP1B1 and CYP1A2 exhibit narrow active site cavities, which underlie similarities in their substrate profile, despite significant differences in their amino acid sequences. However, it should be emphasized that compound **20** adopts a distinctly different orientation in each enzyme reflecting its binding capacity. Several derivatives of this compound have therefore been developed in order to improve the selectivity for CYP1B1 *vs* CYP1A1 and CYP1A2. SAR studies have shown that this may be enabled by the introduction

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of methoxy groups within the naphthalene moiety and by adding a fluorine in C30 on the B ring [20]. Thereby, this study has led to the development of compound **21**, which is the most potent CYP1B1 inhibitor known to date with an IC_{50} value of 0.043 nM and the most selective *vs* CYP1A1 (267 fold) and CYP1A2 (853 fold). Using molecular docking based on x-ray structure of CYP1B1-compound **20** complex, Cui et al. [20] demonstrated that compound **21** tightly fit in the active site of CYP1B1. Furthermore, water-soluble derivatives of **21** have been synthesized in order to address the phenomenon of drug-resistance. This has led to the development of compound **22** which has allowed a total elimination of docetaxel-resistance at 10 μM in MCF-7/1B1 cells, but its inhibitory activity for CYP1B1 dropped to 12.3 nM [20].

A series of pyranoflavones, which are flavonoid derivatives that contain a second pyran ring, has been developed in order to investigate the active site cavities of CYP1 enzymes [47]. Among the 14 novel flavone derivatives tested, compound **23** was found to be the most selective CYP1B1 inhibitor (IC_{50} = 0.1 μM) *vs* CYP1A1 and CYP1A2 (IC_{50} = 10 and 20 μM, respectively). Most of these compounds inhibited the CYP1B1 with IC_{50} values ranging from ~5 to ~100 nM, which values are close to the IC_{50} of compound **20**.

As previously mentioned, the introduction of hydroxy and/or methoxy groups in flavones results in a modulation of the inhibitory activity of these compounds for CYP1 enzymes. The tetrahydroxylated flavonoid fisetin (**25**) is thus a stronger CYP1B1 inhibitor (2 fold) than unsubstituted flavone (**24**) [48]. Similarly, compounds **26-29**, containing one or three hydroxy groups in their backbone, increased the inhibitory activity (IC_{50}) for CYP1B1 from 0.6 μM (flavone (**24**), no OH) to 0.09, 0.21, 0.25 and 0.003 μM, respectively [49]. Compounds **30** and **31**, which have been developed by the introduction of one- and two-methoxy groups into 5,7-dihydroxyflavone, respectively, have also shown a good inhibitory activity toward CYP1B1 with respective IC_{50} values of 0.014 and 0.019 μM [49]. Di- and tri-methoxylated flavones **32-35** were found to be potent inhibitors of benzo[α]pyrene-induced CYP1B1 mRNA expression in human oral SCC-9 cells [50]. Notice that the presence and the number of hydroxy groups on the B-ring is a determining factor for the selectivity of flavonoids toward CYP1 enzymes. Indeed, compound **36**, which has no hydroxy functions on the B-ring, preferentially inhibits CYP1A2 (K_i = 0.5 μM) while compound **37**, with two hydroxy groups on the B-ring, mainly inhibits CYP1A1 activity (Ki = 2.4 μM) [51]. However, compounds **36-38**, which are all three 5,7-dihydroxyflavones, have shown similar inhibitory activity against CYP1B1 (IC_{50} = 0.28,

0.36 and 0.36 μM). In contrast, the presence of hydroxy groups on A-ring and of methoxy groups on B-ring plays a key role for the activity and selectivity of flavonoids toward CYP1B1. For example, compound **39**, which is a natural methoxyflavonoid, has shown an inhibitory activity about five times higher for CYP1B1 (IC_{50} = 19.7 nM) in comparison to CYP1A1 (IC_{50} = 94.7 nM) [52]. Moreover, it should be noted that compound **39** prevents the 4-hydroxylation of E2 by CYP1B1 over the 2-hydroxylation of E2 by CYP1A1 [52,53].

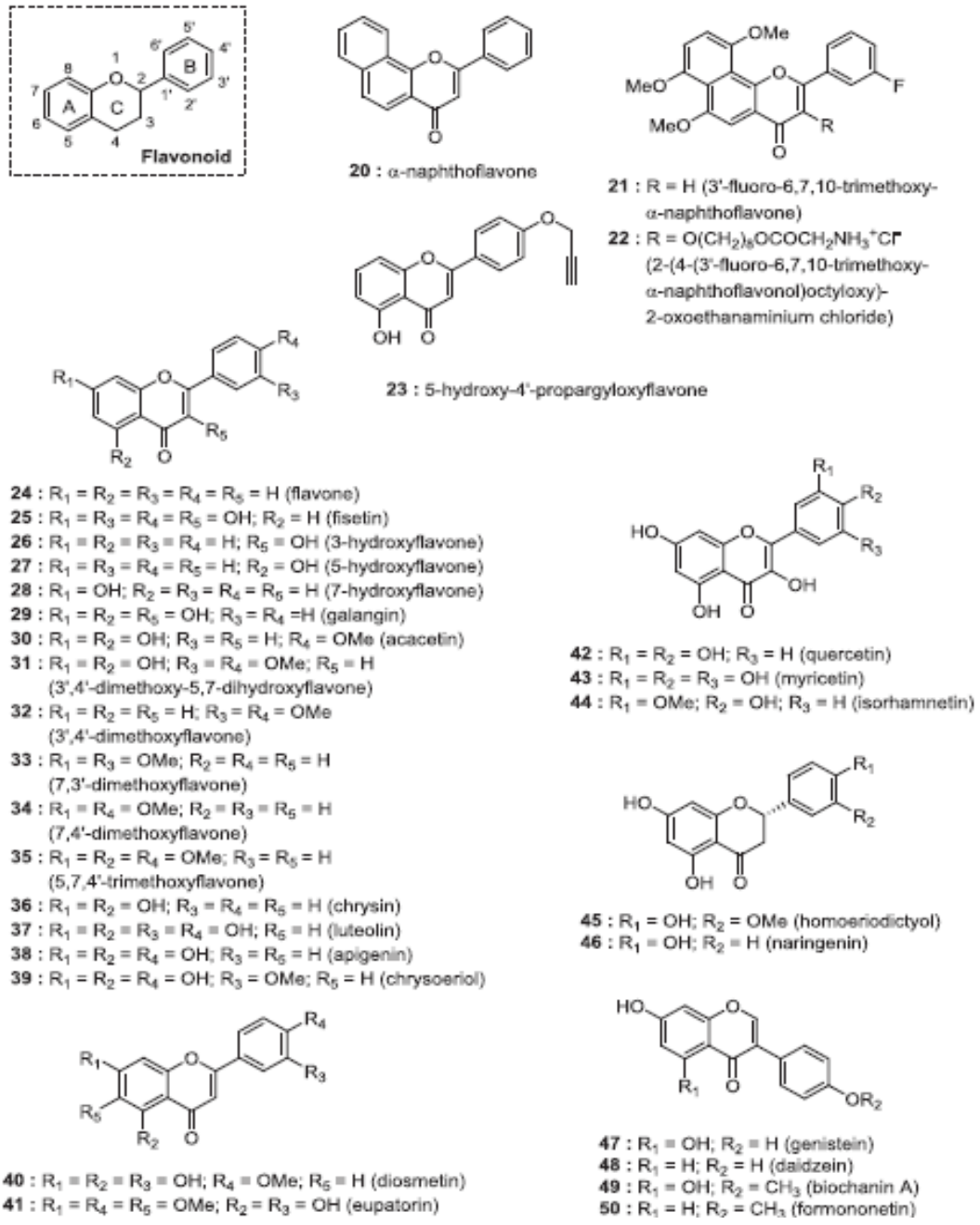


Fig. 4. Flavone derivatives as inhibitors. The basic structure including carbon numbering is represented in a dashed square.

Since the chemopreventive action of dietary constituents appears to be related to the inhibition of CYP1A1 and CYP1B1 by flavonoids, Androutsopoulos et al. [46] tested a series of 14 different flavonoids. The most potent CYP1B1 inhibitors were the methoxylated flavones acacetin (**30**), diosmetin (**40**), eupatorin (**41**), the di-hydroxylated flavone chrysin (**36**) and the polyhydroxylated flavonols quercetin (**42**) and myricetin (**43**) (K_i = 7, 16, 35, 16, 23 and 27 nM, respectively). The same group [14,54] has also observed that the conversion of compound **40** to compound **37** by CYP1 enzymes contributes to the enhancement of their anticancer activity, since compound **37** is a weak CDK4 and EGFR inhibitor. Another interesting point is that flavones (e.g. **39**) and flavonols (e.g. **44**), both with a double bond between C2 and C3 on the C-ring, show a greater inhibitory activity against CYP1 enzymes than flavanones (e.g. **45**), which do not have this double bond [55]. Otherwise, compound **46**, a flavanone present in lemon and having two hydroxy groups on the A-ring and one on the B-ring, inhibits CYP1B1 at above 5 μM but not CYP1A1 activity [56]. Although less potent inhibitors, some flavanone derivatives, such as compound **45**, selectively inhibit CYP1B1 activity (IC_{50} = 0.24 μM) compared to CYP1A1 and CYP1A2 (IC_{50} = 19.1 and 37.8 μM, respectively) [57]. Finally, it must be noted that the binding affinity depends on the interactions between the methoxy groups of these compounds and the specific residues of CYP1 enzymes. In fact, compounds **39** and **44** fit well to the active site of CYP1B1 but not with that of CYP1A1 and CYP1A2 because of the steric effect between the methoxy group of these flavonoids and Ser-122 in CYP1A1 and Thr-124 in CYP1A2 [55]. Isorhamnetin (**44**) was the most potent CYP1B1 inhibitor (K_i = 3 nM) of a series of three flavonol aglycones isolated from *Ginkgo biloba* extracts [58]. In another study, genistein (**47**) and daidzein (**48**), the O-demethylated metabolites of the predominant isoflavones in red clover, biochanin A (**49**) andformononetin (**50**), inhibited the CYP1B1 with K_i values of 1.9 and 3.7 μM, respectively [59].

5. Coumarins

Coumarins constitute a class of phenolic compounds that are derived from coumarin, also known as *2H*-1-chromen-2-one (**Fig. 5**). These naturally occurring chemicals have potential as chemopreventive agents and some are known to interact with CYP1 enzymes. In an extension of their work published in 2002 [60], DiGiovanni's group identified compounds **51-54** as competitive inhibitors of CYP1B1 with K_i values of 587, 11, 6 and 1 μM, respectively

[61]. Another group has also studied the inhibitory activity against CYP1B1 of three furocoumarins contained in grapefruit juice. IC_{50} values of 7.17, 3.56 and 8.89 μM were thus obtained for compounds **51**, **55** and **56**, respectively [62]. It should be noted that the inhibitory activity for CYP1B1 varies depending on the enzyme substrate. Indeed, the inhibitory effects of these three compounds were weaker when BROD (benzyloxyresorufin *O*-dealkylase) was the enzyme activity measured instead of EROD (ethoxyresorufin *O*-dealkylase) [62].

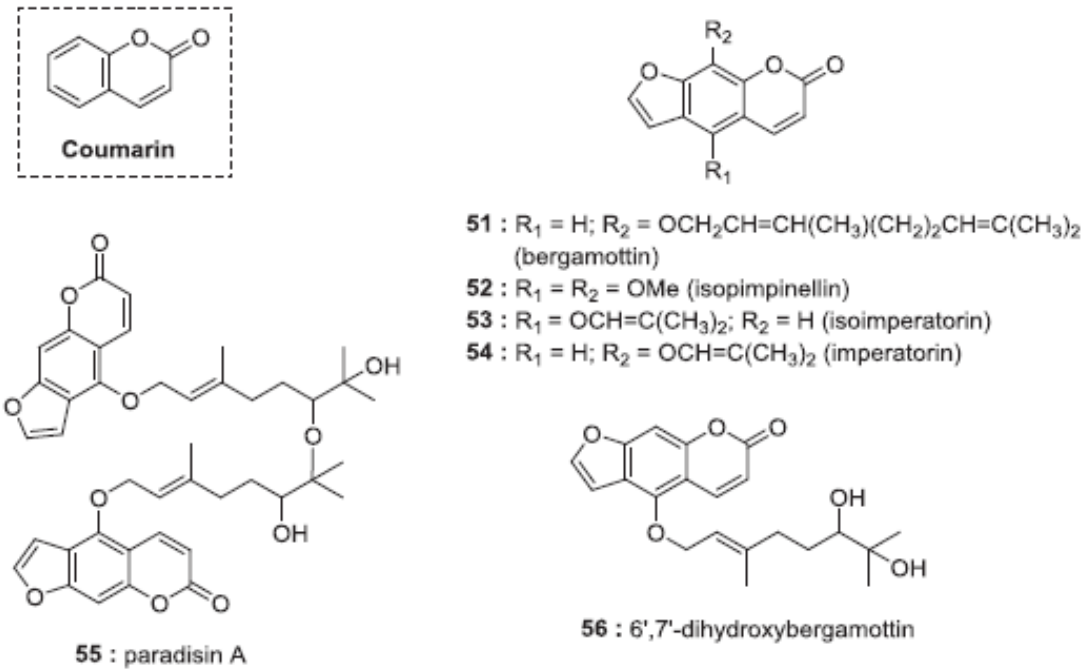


Fig. 5. Coumarin derivatives as inhibitors. The basic structure is represented in a dashed square.

6. Anthraquinones

The family of anthraquinones refers to a group of molecules derived from anthraquinone, which is itself a derivative of anthracene (**Fig. 6**). Anthraquinone is a polycyclic aromatic hydrocarbon found in some animals and plants. Two inhibitors of CYP1B1 reported among this class of compounds are purpurin (**57**) and alizarin (**58**) with K_i values of 0.7 and 0.5 μM, respectively, but these compounds are not very selective *vs* CYP1A1 and CYP1A2 [63]. Only one study [64] has been conducted on this subfamily of inhibitors after 2003. However, this study focused on the inhibition of CYP1A1, CYP1A2, CYP2B1 and/or CYP2A6 by derivatives of compound **59** (emodin), but not on the inhibition of CYP1B1. Emodin (**59**) is a natural anthraquinone isolated from *Rheum emodi* which has shown an inhibitory activity toward CYP1A1 and CYP1A2 with respective IC_{50} values of 12.25 and 3.73 μM [64]. Among

the 12 analogs that have been tested, compound **60** was the best inhibitor of CYP1A1 and CYP1A2 with IC_{50} values of 0.40 and 0.53 μM, respectively. Docking studies using 3D structure of the enzymes were also carried out and revealed the structural characteristics responsible for their potency and selectivity [64].

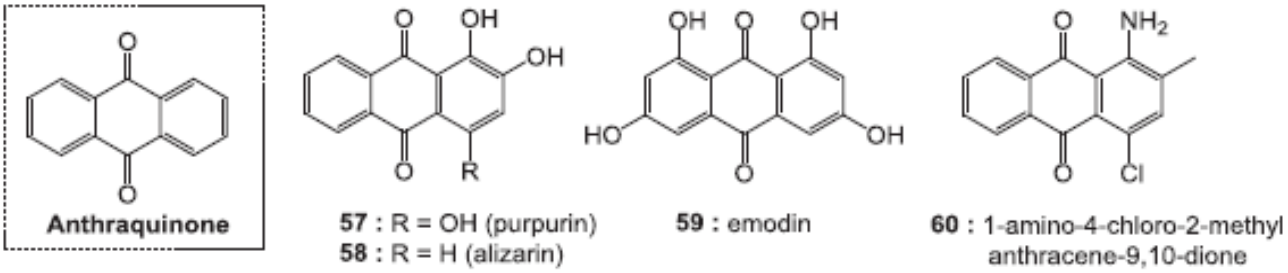
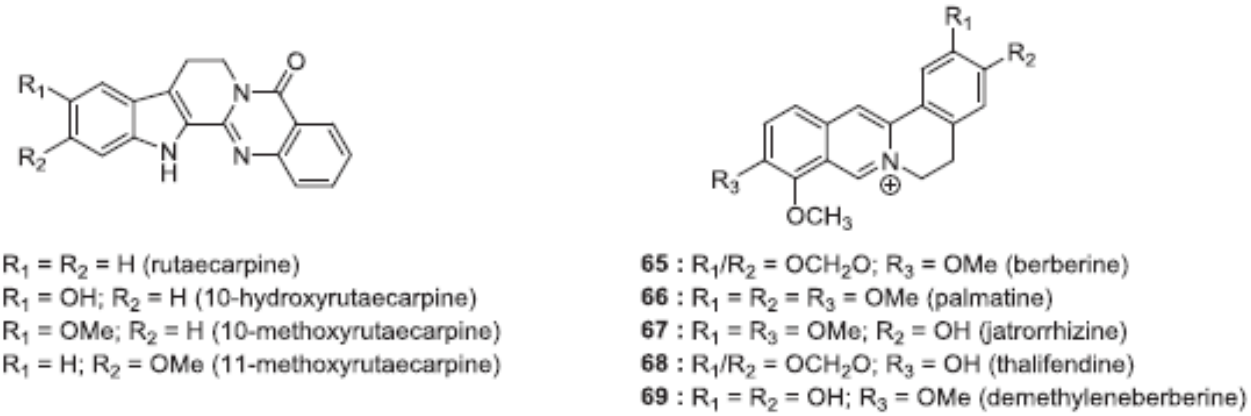


Fig. 6. Anthraquinone derivatives as inhibitors. The basic structure is represented in a dashed square.

7. Natural alkaloids

Alkaloids constitute a family of various nitrogen compounds, usually heterocyclics, and they are mainly found in plants. Rutaecarpine (**61**) (**Fig. 7**), a natural quinazolinocarboline alkaloid isolated from *Evodia rutaecarpa*, has been used to inhibit CYP1 enzymes and results show that it seems to preferentially inhibit CYP1A2 [65]. In order to obtain CYP1 inhibitors, several derivatives of rutaecarpine were then developed, notably by addition of hydroxy and methoxy functions on the basic core. Among these derivatives, 10-hydroxyrutaecarpine (**62**) inhibits the activity of CYP1A1, CYP1A2 and CYP1B1 with IC_{50} values of 2.56, 2.57 and 0.09 μM, respectively [26]. Methoxylated rutaecarpines **63** and **64** were however less potent CYP1B1 inhibitors than compound **61** (IC_{50} = 84, 110 and 55 nM, respectively), but they are much more selective inhibitors of CYP1B1 over CYP1A1 (21.2 and 18.2 times for **63** and **64**) and CYP1A2 (34.8 and 18.0 times for **63** and **64**) [27]. Within this family of inhibitors, the inhibitory activity of some protoberberines against CYP1 enzymes has also been studied [66]. This is the case of compounds **65-67**, which inhibit the activity of CYP1A1 and CYP1B1, but have little effect toward CYP1A2 activity. Compound **65**, a pharmacologically active alkaloid that is found for example in *Coptis chinensis* medicinal herb,was the most active and selective inhibitor of CYP1B1 with a K_i value of 44 nM [67]. It also preferentially inhibits the 4-hydroxylation of E2 over the 2- hydroxylation [67,68]. Compounds **68** and **69** (IC_{50} = 4.1 and

9.9 μM, respectively), which are structurally closely related to berberine (**65**), also inhibit the 4-hydroxylation of E2 by CYP1B1, but they are weaker inhibitors than **65** (IC_{50} = 0.33 μM).



61 : $R_1 = R_2$ = H (rutaecarpine)
62 : R_1 = OH; R_2 = H (10-hydroxyrutaecarpine)
63 : R_1 = OMe; R_2 = H (10-methoxyrutaecarpine)
64 : R_1 = H; R_2 = OMe (11-methoxyrutaecarpine)

65 : $R_1/R_2 = OCH_2O$; R_3 = OMe (berberine)
66 : $R_1 = R_2 = R_3$ = OMe (palmatine)
67 : $R_1 = R_3$ = OMe; R_2 = OH (jatrorrhizine)
68 : $R_1/R_2 = OCH_2O$; R_3 = OH (thalifendine)
69 : $R_1 = R_2$ = OH; R_3 = OMe (demethyleneberberine)

Fig. 7. Natural alkaloids as inhibitors.

8. Miscellaneous compounds

In addition to the sub-families of CYP1 inhibitors reported above, additional and varied chemicals were reported inhibiting CYP1B1 (**Fig. 8**). This is the case of steroid derivatives, not covered in the first review article [1], such as the male and female hormones testosterone and 17β-estradiol (E2) (K_i = 411.8 and 1.9 μM) [25]. One of these studies highlighted a feedback inhibition of CYP1A1 and CYP1B1 by methoxyestrogens [28]. In fact, E2 derivatives **70** and **71** have shown an inhibitory activity against the conversion of E2 to 2-hydroxy-E2 by CYP1A1 with K_i values of 88 and 69 μM, respectively. Also, compounds **70-72** inhibited the 4-hydroxylation of E2 by CYP1B1 with K_i values of 36, 28 and 149 μM, respectively [28]. Compound **73** was recently reported as the best CYP1B1 steroidal inhibitor (IC_{50} = 3.4 μM) identified from a screening performed with 90 steroid derivatives [69]. Molecular modeling studies showed that the 3-SH group of compound **73** is close to the iron atom of the heme system of CYP1B1. Another group has studied the effect of compound **74** (fluasterone) on the activity and expression of CYP1A1 and CYP1B1 in breast cancer MCF-7 cells. The results indicate that compound **74** inhibited CYP1A1 activity but had no effect against CYP1B1 [70].

Compounds **75** and **76**, two prenylated bromohydroquinones isolated from the marine algae *Cymopolia barbata*, were identified as potent inhibitors of CYP1A1 with IC_{50} values of 0.39 and 0.93 μM, respectively, whereas compound **76** potently inhibited CYP1B1 with an IC_{50} value of 0.14 μM [71]. The inhibitory effect against the catalytic activity of CYP1 enzymes

by the major components of *Cannabis sativa*, compounds **77-79**, has also been studied [72]. Among these three competitive inhibitors, compound **77** is the less active and the less selective inhibitor of CYP1 enzymes (K_i = 2.47-7.54 μM). Compound **78** is the most powerful inhibitor of CYP1A1 among these three compounds with an apparent K_i value of 0.15 μM. Finally, compound **79** is a potent inhibitor of CYP1A2 and CYP1B1 with respective K_i values of 0.079 and 0.148 μM, but has a lower activity toward CYP1A1 with a K_i value of 0.541 μM [72].

The hydroxylated derivative of limonene, compound **80**, is a dietary monoterpene found in foods such as mints, cranberries and cherries with potential applications in chemoprevention and chemotherapy. It inhibits CYP1B1 activity (K_i of ~2 μM) but not CYP1A1 activity [73]. This compound also inhibited the enzymatic activity induced by dimethylbenz[α]anthracene (DMBA) in MCF-7 cells. Another interesting example is compound **81**, a chemical compound which inhibits p53-mediated gene (tumor suppressor) activation and apoptosis. Indeed, it has been observed that this compound directly affected the catalytic activity of CYP1 enzymes and that compound **81** is a very potent inhibitor of CYP1B1 with an apparent K_i value of 4.38 nM [74].

The effect on CYP1A1 and CYP1B1 of carnosol (**82**), a constituent of polyherbal preparation Zyflamend and a naturally occurring phytopolyphenol found in rosemary that functions as antioxidant and anticarcinogen, was tested by Mohebati et al. [75]. This compound did not inhibit these CYP1 enzymes by direct interaction, but has shown an action toward Hsp90 ATPase leading to reduced levels of AhR (aryl hydrocarbon receptor), blocking of benzo[α]pyrene induction of CYP1A1 and CYP1B1, and inhibition of mutagenesis. Metformin (**83**), the first-line medication for the treatment of type 2 diabetes, decreased CYP1A1 and CYP1B1 expression in breast cancer cells under constitutive and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced conditions [76]. Melatonin (**84**), a drug used in the treatment of some sleep disorders, is a mixed inhibitor of CYP1 enzymes with apparent K_i values of 59, 12 and 14 μM for CYP1A1, CYP1A2 and CYP1B1, respectively [77].

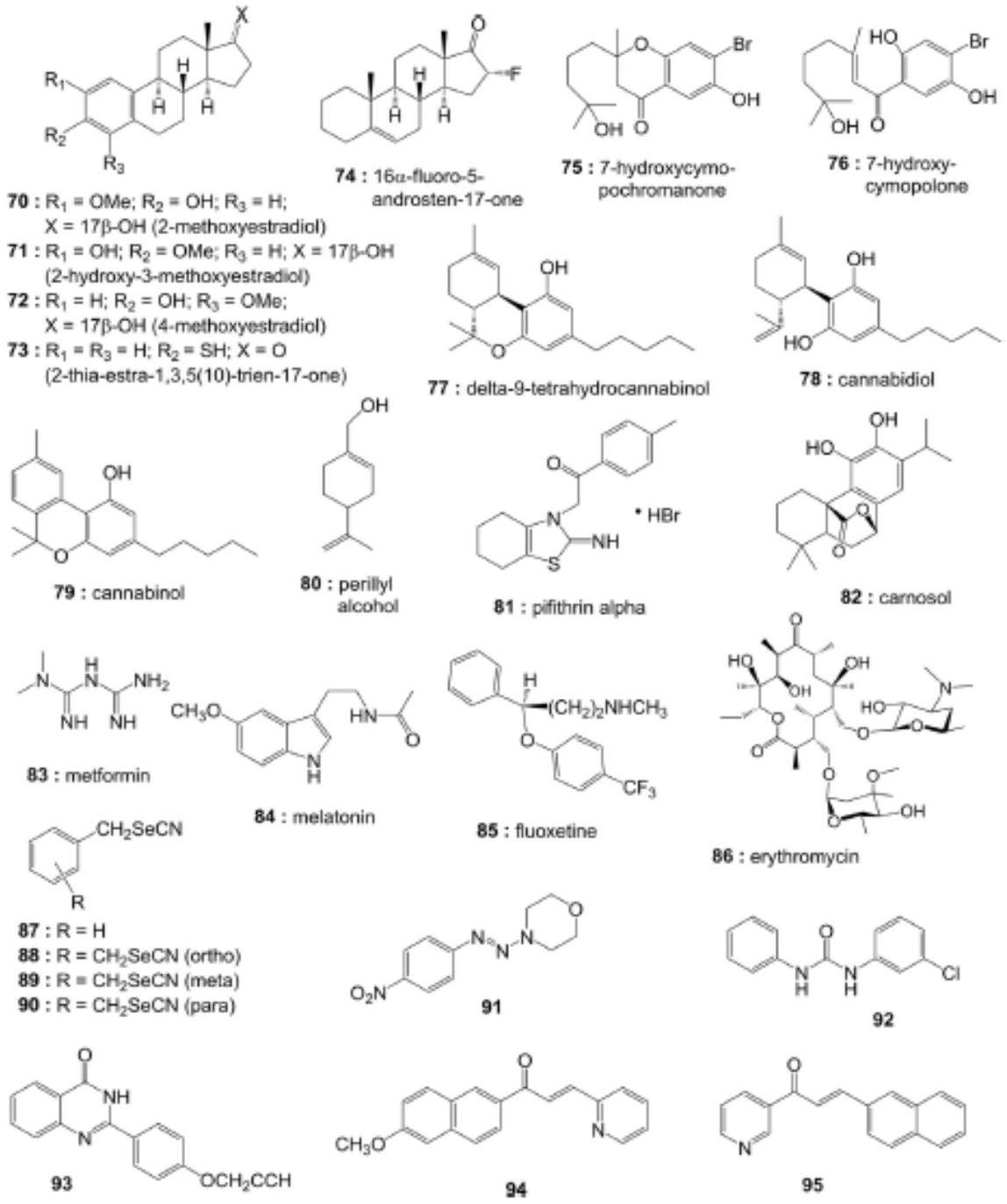


Fig. 8. Miscellaneous compounds as inhibitors.

Four drugs found in aquatic environments (fluoxetine, ciprofloxacin, gemfibrozil and erythromycin) were tested for their ability to inhibit CYPs in a panel of fluorescent CYP assays using different substrates [78]. Fluoxetine (**85**) was the best inhibitor of CYP1 activity in rainbow trout microsomes, whereas erythromycin (**86**) was the best in zebrafish expressing CYPs. For CYP1B1 and EROD as substrate, IC_{50} values were 475 and 40 μM for fluoxetine (**85**) and erythromycin (**86**), respectively. Organoselenium compounds **87-90**, which are known to prevent different cancers caused by PAHs, were found to reversibly inhibit

CYP1B1 (IC_{50} = 0.13-0.27 μM) as well as other CYPs, including CYP1A1 (IC_{50} = 0.10-0.45 μM) and CYP1A2 (IC_{50} = 0.20-1.3 μM) [24]. Molecular docking studies with compound **88** provide interesting information regarding the positioning of the inhibitor, and support the view that one of the selenium moieties is more closely located to the heme (Fe-atom) of CYP1B1 than in the cases of CYP1A1 and CYP1A2. The authors also suggest that one of the mechanisms underlying the prevention of cancers caused by PAHs, and tobaccorelated carcinogens, would be the result of the inhibition of CYP1B1 and CYP2A13 by organoselenium compounds [24].

A short series of aryl morpholino triazenes were prepared and the best inhibitor, compound **91**, selectively inhibited CYP1B1 (IC_{50} = 2 μM) over CYP1A1 (IC_{50} = 115 μM) [79]. In this EROD assay, resveratrol (**1**) inhibited both CYP1B1 and CYP1A1 (IC_{50} = 55 and 34 μM, respectively). Chaudhuri's group synthesized three families of new CYP1B1 inhibitors [80-82]. The first family was developed by incorporating two hydrophobic moieties that came from potent CYP1B1 inhibitors, stilbene derivative **9** and flavonoids **20** and **42**, in order to generate biphenyl urea derivatives [80]. From this new scaffold emerged compound **92** as the most potent (IC_{50} = 5 nM) and selective CYP1B1 inhibitor over CYP1A1, CYP1A2, CYP2D6 and CYP3A4 (IC50 > 10 μM). This compound was more potent (10-fold) than α-naphthoflavone (**20**), which is not selective for CYP1A1 and CYP1A2. The second family of CYP1B1 inhibitors is built around a quinazoline scaffold, which is found in the alkaloid rutaecarpine (**61**). From the 20 quinazoline derivatives synthesized, compound **93** was clearly the most efficient inhibitor of CYP1B1 (IC_{50} = 2 nM, 25-fold better than **20**) and the most selective over other CYPs [81]. The third family of inhibitors is based on a pyridylchalcone scaffold. When compared to **20**, representative compounds **94** and **95** produced a better CYP1B1 inhibition (IC_{50} = 50, 9 and 10 nM, respectively), aqueous solubility and selectivity *vs* other CYPs (1A1, 1A2, 2D6 and 3A4) [82]. Both compounds also overcome cisplatinresistance in ovarian cancer cells A2780cis, which is known to overexpress CYP1B1. This result highlights the potential of using a CYP1B1 inhibitor in cancer treatment.

9. Conclusion

CYP1B1 represents a promising biomarker and therapeutic target because it is overexpressed in several types of cancer, is involved in drug-resistance, activates a number of procarcinogenic compounds and catalyzes the conversion of E2 to 4-hydroxy-E2, a mutagenic compound. Several studies have been performed to develop potent and selective inhibitors of CYP1B1, but most of these researches focused on two families of compounds that are already known for their inhibitory activity against CYP1 enzymes: stilbenes and flavonoids. In fact, few studies have been carried out on the other inhibitor families reported by Chun and Kim [1], which are polycyclic aromatics, coumarins, anthraquinones and anticancer agents. However, studies related to the inhibition of CYP1 enzymes by a wide variety of compounds were conducted, extending the nature of chemicals reported as inhibitors.

Table 1. Representative CYP1B1 inhibitors from each family of chemicals.

Family	Name (Number)	CYP1B1 Inhibition	CYP1A1 Inhibition	Selectivity Index	Substrate (Concentration)	Ref.
Stilbenes (Fig. 2)	2,4,2',6'-tetra-methoxystilbene (**11**)	IC_{50} = 1.77 nM	IC_{50} = 350 nM	198	EROD (2 µM)	37
Polycyclic aromatics (Fig. 3)	pyrene (**12**)	IC_{50} = 2 nM	IC_{50} = 41 nM	20.5	EROD (10 µM)	40
Flavonoids (Fig. 4)	3'-fluoro-6,7,10-trimethoxy-α-naphthoflavone (**21**)	IC_{50} = 0.043 nM	IC_{50} = 11.5 nM	267	EROD (0.15 µM)	20
Coumarins (Fig. 5)	imperatorin (**54**)	K_i = 1 µM	N/D	N/D	B[a]P-7,8-diol [a]	61
Anthraquinones (Fig. 6)	purpurin (**57**)	IC_{50} = 2.2 µM K_i = 0.7 µM	IC_{50} = 5.5 µM	2.5	EROD (20 µM; CYP1B1) (40 µM; CYP1A1)	63
Natural alkaloids (Fig. 7)	10-methoxy-rutaecarpine (**63**)	IC_{50} = 84 nM	IC_{50} = 1780 nM	21	EROD (2 µM)	27
Micellaneous (Fig. 8)	pifithrin α (**81**)	IC_{50} = 20.6 nM K_i = 4.38 nM	IC_{50} = 1.5 µM	73	EROD (2.5-20 µM)	74
	Compound **93**	IC_{50} = 2 nM	IC_{50} = 405 µM	202.5	EROD (10 µM)	81

[a] Oxidation of (-) benzo[a]pyrene-7R-trans-7,8-dihydrodiol

In **Table 1**, we reported the best potent and/or selective CYP1B1 inhibitors, their inhibitory activity and their selectivity over CYP1A1, the enzyme involved in the formation of 2-hydroxy-E2 which should not be inhibited in the context of an estrogendependent cancer treatment. It is however not possible to compare several kinds of structurally different inhibitors tested from various research groups while most of the time using different experimental conditions. This is why we also reported some data obtained by Shimada's group. In their interesting study [83], this research group tested the ability of a series of chemicals (16 flavonoids, three stilbenes, six pyrenes, seven naphthalenes, seven phenanthrenes, 10 biphenyls and two steroids) to inhibit the EROD activity catalyzed by

CYP1B1. They also determined that most of these compounds (49/51) induced reverse type I binding spectra with CYP1B1 and provided valuable dissociation constant (K_s) values, which were apparently related to the inhibition potencies (IC_{50} values).

In addition to the improvement of known CYP1B1 inhibitors and the development of new inhibitors, the report of a crystal structure of CYP1B1 complexed with α-naphthoflavone [9] was a major breakthrough in the last decade. Molecular modeling studies using these structural data could thus be a useful tool to guide the synthesis of potent and selective CYP1B1 inhibitors. Another fact that must be emphasized is that very few studies have been conducted on the development of CYP1B1 inhibitors based on a steroidal scaffold. This is surprising given that C18-steroids are substrates of CYP1B1 and considering the role of this enzyme in the biosynthesis of 4-hydroxy-E2, a mutagenic agent.

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