Bioorganic & Medicinal Chemistry Letters 26 (2016) 5272–5276

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Targeting cytochrome P450 (CYP) 1B1 with steroid derivatives

Donald Poirier [a,b,*], Jenny Roy [a,b], Francisco Cortés-Benítez [a,c], Raphaël Dutour [a,b]

[a] Laboratory of Medicinal Chemistry, Endocrinology and Nephrology Unit, CHU de Québec – Research Center (CHUL, T4-42), 2705 Laurier Boulevard, Québec (Québec) G1V 4G2, Canada
[b] Department of Molecular Medicine, Faculty of Medicine, Université Laval, Québec (Québec), Canada
[c] Department of Pharmacy, Faculty of Chemistry, National Autonomous University of Mexico, Mexico City, Mexico

ARTICLE INFO

Article history:
Received 12 July 2016
Revised 15 September 2016
Accepted 16 September 2016
Available online 17 September 2016

Keywords:
CYP1B1
Enzyme
Inhibitor
Screening
Steroid

ABSTRACT

Inhibition of cytochrome P450 1B1 (CYP1B1) represents a promising therapeutic strategy, because it would enable action at three different levels: (1) by inhibiting the formation of mutagenic 4-hydroxy-estradiol, (2) by inhibiting the bioactivation of procarcinogens, and (3) by reducing drug-resistance. Surprisingly, few steroids were reported as inhibitors of CYP1B1. From a screening performed with 90 steroid derivatives, we identified thioestrone (**B19**) as an inhibitor (IC_{50} = 3.4 μM) of CYP1B1. Molecular modeling studies showed that the 3-SH group of **B19** is closer (3.36 Å) to the iron atom of the heme system than the 3-OH group of enzyme substrates estrone and estradiol (4.26 Å and 3.58 Å, respectively). **B19** also produced a better docking GOLD score that correlated with the inhibitory results obtained. The estrane derivative **B19** represents an interesting lead compound that can be easily modified to extend the structure–activity relationship study and to provide a next generation of more powerful CYP1B1 inhibitors.

Cytochromes P450 (CYPs) constitute a large family of hemoproteins involved in many reduction and oxidation reactions on both endogenic and xenobiotic molecules of various sizes.[1,2] The CYP1 subfamily is comprised of 3 members: CYP1A1, CYP1A2 and CYP1B1. The later is predominantly found in extrahepatic mesodermal cells, including steroidogenic tissue (ovaries, testes and adrenal glands) and in steroid-responsive tissue (breast, uterus, and prostate).[3] These CYP1 members have been widely studied because they are involved in the conversion of a large number of polycyclic aromatic hydrocarbons (PAHs), such as benzo[α]pyrene, into carcinogens.[4,5] Certain CYP1 members would also be involved in the modulation of pro-inflammatory and inflammatory pathways via the metabolism of leukotrienes and eicosanoids[6], whereas others are known as tumor suppressor enzymes via the metabolism of flavonoid-type compounds.[7–10] CYP1 members can also catalyze the hydroxylation of 17β-estradiol (E2), a C18-steroid known to be a powerful natural estrogenic hormone, but CYP1B1 showed a distinct selectivity for the 4-hydroxylation of E2 (Fig. 1) whereas CYP1A1 and CYP1A2 are mainly involved in the 2-hydroxylation of E2.[11,12] Unlike 2-hydroxy-E2 and its oxidation product, E2-2,3-quinone, 4-hydroxy-E2 can be oxidized into E2-3,4-quinone, a product having a high mutagenic potential by covalent interaction with DNA.[13]

CYP1B1 is overexpressed in various types of human cancers (breast, lung, colon, esophagus, skin, testis, lymph node and brain), but not in healthy tissue.[14] This enzyme is also a good marker for the prevention of certain cancers such as breast cancer. CYP1B1 is also involved in the metabolism of some anticancer agents, such as docetaxel, leading to drug-resistance associated with the overexpression of CYP1B1.[15–17] Clearly, inhibition of CYP1B1 represents a promising strategy because it would enable therapeutic action at 3 different levels (Fig. 1): (1) by inhibiting the formation of mutagenic 4-hydroxy-E2, (2) by inhibiting the bioactivation of procarcinogens, and (3) by reducing drug-resistance.[1]

Several studies aiming to better understand the carcinogenic PAHs and the chemopreventive role of certain natural compounds such as flavonoids, alkaloids, coumarins, anthraquinones and *trans*-stilbene derivatives, such as resveratrol, have identified different families of CYP1 inhibitors, including those of CYP1B1.[1] Surprisingly, only the 16α-fluoro-5-androsten-17-one and three methoxyestradiols were reported as steroidal inhibitors of CYP1B1.[18,19] However, C18-steroid E2 and its oxidized form at position 17, estrone (E1) are both targets of several CYPs including CYP1B1.[20]

Our research group developed expertise in the use of steroid nuclei (C18-, C19- and C21-steroids) as a basis for the development of enzyme inhibitors of the steroidogenesis.[21–29] We therefore used our collection of steroids and steroid derivatives to perform a screen on the CYP1B1. A standard test[30] using the resorufin ethyl

* Corresponding author. Tel.: +1 418 654 2296; fax: +1 418 654 2298.
E-mail address: donald.poirier@crchul.ulaval.ca (D. Poirier).

http://dx.doi.org/10.1016/j.bmcl.2016.09.046
0960-894X/

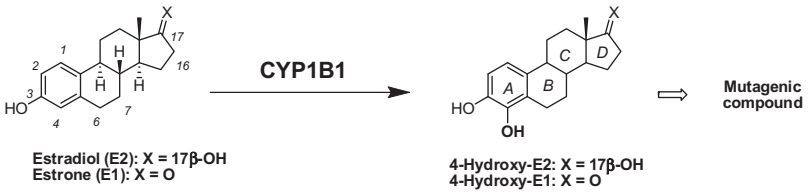

Figure 1. The conversion of estrogenic C18-steroid hormones by CYP1B1. Partial numbering of carbons (left structure) and steroid (A–D) ring identification (right structure).

ether as substrate and a preparation of human CYP1B1 was used to evaluate the inhibitory activity of several steroidal compounds. This assay was validated and found reproducible using the known CYP1B1 inhibitor α-naphthoflavone[31], which inhibited 89.2 ± 9.4% of the CYP1B1 activity at 3 μM. A first series of steroid hormones was next tested (Fig. 2) and the results oriented the screening toward the C18-steroid family. Indeed, the steroid estrone (E1) produced the better inhibition (40%) at 3 μM.

A second series of 26 C18-steroid derivatives (**B1**–**B26**) representing different kinds of A-ring modifications was also tested as CYP1B1 inhibitors (Table 1; *Series B*). Only one compound inhibited significantly the enzyme better than E1, namely **B19**, with 61.6% of inhibition at 3 μM. **B19** is an E1 derivative where the 3-OH was replaced by a thiol (SH) group. Other modifications such as methylation of the 3-OH, its replacement by a small group, or adding a group at position 2 were found to be detrimental to inhibitory activity. This was also the case for all kinds of A-ring modifications of E2. B-ring and D-ring modifications were also addressed in a third series of 19 C18-steroid derivatives (**C1**–**C19**) (Table 1; *Series C*), but the chemical group or the side-chain introduced on E1 or E2 nucleus did not provide CYP1B1 inhibition to the level of thioestrone (**B19**) or E1 (**A6**). Similarly, a series of C19-steroids, mainly androsterone and 5α-androstane-3α,17β-diol derivatives, were tested as inhibitors but they did not generate interesting results (data not shown).

The concentration inhibiting 50% of CYP1B1 activity (IC_{50} values) was determined for 3-thioestrone (**B19**) and the reference inhibitor α-naphthoflavone, but cannot be determined for the weak inhibitors E1 and E2 (Fig. 3). The curves generated using different concentrations of each compound demonstrated a concentration-dependent inhibition of CYP1B1 and provided IC_{50} values of 0.3 and 3.4 μM for α-naphthoflavone and **B19**, respectively. The steroid inhibitor **B19** is thus 10-times less potent than the non-steroidal inhibitor used as reference, but the former represents an interesting lead compound that can be easily modified to extend the structure–activity relationship study reported in Table 1.

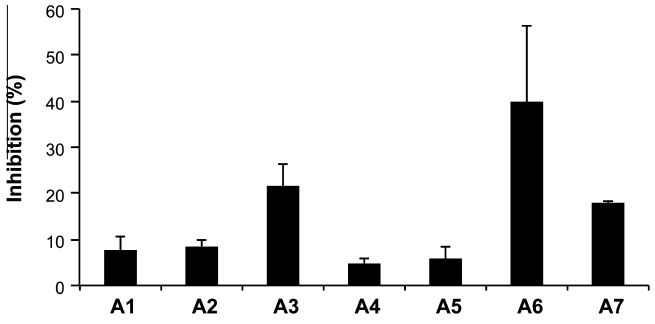

Figure 2. Inhibition of CYP1B1 activity by a series of steroid hormones (**A1**–**A7**) at 3 μM. Pregnenolone (**A1**), dehydroepiandrosterone (**A2**), testosterone (**A3**), dihydrotestosterone (**A4**), androsterone (**A5**), estrone (**A6**) and estradiol (**A7**).

Docking studies were performed using the 3D-structure of CYP1B1 (PDB ID: 3PM0) and GOLD-5.4 software.[32] The chemical structure of E1, E2 and the co-crystalized inhibitor α-naphthoflavone[33] were retrieved from the ZINC database,[34] whereas **B19** was built from the systematic modification of E1. The 4 ligands were energy-minimized by the semi-empirical PM6 method using Gaussian 09 software.[35] Docking simulations were carried out within a 10 Å radius of the co-crystalized molecule using the following parameters: 100 GA runs per molecule and 125,000 operations. GOLD score was chosen as scoring function within the goldscore_p450_csd parameter file. The dockings were ranked according to the value of the GOLD score fitness function; only the best ranked solution for each ligand was included in further analysis.

The best docked conformation for the α-naphthoflavone providing a GOLD score of 74.4 (data not shown) and its predicted binding mode is similar to the co-crystal structure conformation, and superimposes with rmsd (root mean square deviation) of 2.0 Å. As expected, natural enzyme substrates E1 and E2 fitted in the catalytic site with a docking GOLD score of 53.0 and 50.4, respectively (Fig. 4A and B). These scores correlate with the inhibitory results obtained in the screening study (Fig. 2). Similarly, as observed for an aryl ring of α-naphthoflavone[33], the aryl A-ring of E1 and E2 points toward the heme moiety of the enzyme. For steroids E1 and E2, which are natural CYP1B1 ligands, the 3-OH points toward the iron atom and the 4-CH is close and positioned to be hydroxylated by the enzyme. Interestingly, the 3-SH group of **B19** is closer (3.36 Å) to the iron atom than the 3-OH group of E1 and E2 (4.26 Å and 3.58 Å, respectively) (Fig. 4C and D). The thio derivative **B19** also produced a better docking GOLD score (60.3) than E1 and E2. These docking scores also correlate with the inhibitory results we obtained. Thus, the thiophenol ring probably provides a better interaction with the iron than the phenol ring of E1 and E2. In fact, it is well known that the iron atom of P450 enzymes is involved in the formation of a chelate (an octahedral complex with 6 bonds coming from 6 ligands). Since one of these 6 ligands is the oxygen needed in the enzymatic reaction (oxidation or hydroxylation), the presence of another ligand such as the SH group of **B19** will promote the formation of a new complex more stable and without oxygen, thus resulting in an inhibition of the CYP1B1 transformation.

On the other hand, while the co-crystalized α-naphthoflavone produced hydrophobic interactions with Phe-268, Gly-329, Tyr-334, Ala-330 and Leu-509, as well as a *pi*-stacking between the B-ring and the side chain of Phe-231,[33] the docked E1, E2 and **B19** interact with the hydrophobic residues Phe-67, Phe-164, Phe-201 and Ala-259. However, **B19** has additional interactions with amino acids Leu-197 and Thr-263, whereas E1 interacts only with Thr-263, indicating that the best inhibitory activity of **B19** is also promoted by these extra interactions. Therefore, the less polar character of **B19** along with the formation of a chelate between 3-SH and the heme iron are favorable to fit into the hydrophobic pocket of CYP1B1.