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Synthesis and biological evaluation of pyrrole-based chalcones as CYP1 enzyme inhibitors, for possible prevention of cancer and overcoming cisplatin resistance

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ABSTRACT

Inhibitors of CYP1 enzymes may play vital roles in the prevention of cancer and overcoming chemo-resistance to anticancer drugs. In this letter, we report synthesis of twenty-three pyrrole based heterocyclic chalcones which were screened for inhibition of CYP1 isoforms. Compound **3n** potently inhibited CYP1B1 with an IC_{50} of ~0.2 μM in Sacchrosomes™ and CYP1B1-expressing live human cells. However, compound **3j** which inhibited both CYP1A1 and CYP1B1 with an IC_{50} of ~0.9 μM, using the same systems, also potently antagonized B[*a*]P-mediated induction of AhR signaling in yeast (IC_{50}, 1.5 μM), fully protected human cells from B[*a*]P toxicity and completely reversed cisplatin resistance in human cells that overexpress CYP1B1 by restoring cisplatin's cytotoxicity. Molecular modeling studies were performed to rationalize the observed potency and selectivity of enzyme inhibition by compounds **3j** and **3n**.

Cytochrome P450 (CYP) enzymes are heme containing monooxygenases. Around 15 of them are responsible for phase I metabolism acting mainly to hydroxylate pharmaceuticals and exogenous substances, some of which may have the potential to be carcinogenic.[1] The CYP1 sub-family of enzymes consists of 1A1, 1B1 and 1A2 isoforms. The CYP1A1 isoform catalyzes hydroxylation of a large number of pro-carcinogens, such as polyaromatic hydrocarbons (PAHs), oxides, amines, and estrogens (E) converting them to cytotoxic, mutagenic and carcinogenic chemicals.[2–5] Overexpressed CYP1A1 isoform can metabolize the PAH benzo[*a*]pyrene [BaP] into benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide which subsequently can form a quinone intermediate which can covalently react with DNA and lead to DNA damage.[6–15] Similarly, CYP1B1 overexpression is responsible for the increased metabolism of anticancer drugs such as paclitaxel, docetaxel, doxorubicin, mitoxantrone, tamoxifen, and cisplatin in various cancer cells.[16] As a result, the cellular efficacy of cytotoxic drugs is reduced and eventually cancer cells, which overexpress CYP1B1, become resistant to a variety of chemotherapeutic agents. Recent studies have demonstrated that CYP1B1 inhibitors can overcome docetaxel resistance,[16,17] as well as cisplatin resistance in CYP1B1-overexpressing cells.[18]

Various biochemical and cellular studies suggest that basal expression of CYP1 enzymes in healthy tissue is very low, in contrast to tissues exposed to pro-carcinogens or drugs. This selective behavior offers an opportunity to a medicinal chemist and biologist for prevention of CYP1A1-mediated lung carcinogenesis caused by B[*a*]P in individuals who smoke.

Several natural and synthetic compounds have been reported as potent inhibitors of CYP1 enzymes e.g. resveratrol, quercetin, and rosmarinic acid.[19–21] In continuation of our efforts in this area,[18,22,23] herein we report design and synthesis of 2-pyrrole based chalcones, to combat CYP1B1-mediated cisplatin resistance and CYP1A1-mediated B[*a*]P toxicity. A total 23 chalcones were synthesized as potential CYP1 family inhibitors using the classical Claisen-Schimdt condensation.[24–26] This reaction offers coupling of

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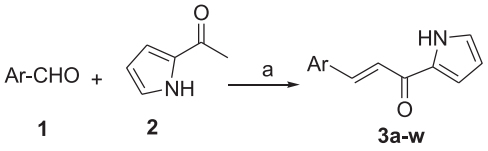
equimolar amounts of aromatic aldehydes and acetophenones in either acidic or basic conditions or vice versa. 2-Pyrrole chalcones synthesized using solution phase or solid grinding method gives moderate to good yields (24–73%). The synthetic scheme is provided in Scheme 1. All compounds were characterized by NMR, IR and HRMS analysis.

All synthesized 2-pyrrole chalcones were screened for inhibition of CYP1, CYP2 and CYP3 family enzymes using Sacchrosomes™ which are endoplasmic reticulum-bound CYP-reductase complexes isolated from recombinant baker's yeast, *Saccharomyces cerevisiae*. Results of CYP inhibition are depicted in Table 1. *In-vitro* CYP inhibition data suggest that all chalcones selectively inhibit CYP1 enzymes. The 2-pyrrole chalcone **3j** inhibits all three isoforms of CYP1 with almost equal potency. However, another 2-pyrrolyl chalcone derivative **3n** selectively inhibits CYP1B1 with an IC_{50} value of 210 nM. Based on *in vitro* data, few key structure-activity relationship (SAR) features have been drawn for these heterocyclic chalcones. These include (a) 2-pyrrole substituted class of CYP1 inhibitors can be selective for CYP1B1 isoform (b) halogen or methoxy substitution on non-heterocyclic ring (e.g. 2-chloro substituted compound **3n**) is essential for activity (c) alkoxy substitution on the non-heterocyclic ring is critical for potency of CYP1 inhibition.



3a: Ar = Ph (3-OMe)
3b: Ar = Ph (2,6-di-OMe)
3c: Ar = Ph (2-Cl,5-NO_2)
3d: Ar = Ph (2,3-di-OMe)
3e: Ar = Ph (3,4-Methylene-dioxy)
3f: Ar = Ph (2,4-di-OMe)
3g: Ar = Cinnamyl
3h: Ar = Ph (3,5-di-OMe)
3i: Ar = Ph (2-F)
3j: Ar = Ph (2-OMe)
3k: Ar = Cinnamyl (4-OMe)
3l: Ar = Ph (4-N(CH_3)$_2$)
3m: Ar = Ph (4-OMe)
3n: Ar = Ph (2-Cl)
3o: Ar = Ph (3-Br)
3p: Ar = Ph (3-Br, 4-OMe)
3q: Ar = Furan-2-yl (5-Et)
3r: Ar = Ph (3,4,5-tri-MeO)
3s: Ar = Ph (4-OCF_3)
3t: Ar = Ph (4-COOH)
3u: Ar = Anthracen-9-yl
3v: Ar = Ph (3-Cl)
3w: Ar = Ph (4-Br)

Scheme 1. Reagents and conditions: (a) 5 equivalents NaOH/KOH with solid state grinding using mortar and pestle.

The failure of various CYP inhibitors in drug development is due to the lack of drug-like features and cellular efficacy. In order to overcome this problem, we established CYP-expressing live human cell based assays, for CYP inhibition studies.[18,22,23] The CYP1 inhibitors identified using Sacchrosomes™ were tested for inhibition of CYP1, CYP2 and CYP3 family of enzymes in live human kidney HEK293 cells (results are shown in Table 2).[27,28] The compound **3s**, which was inactive in Sacchrosomes, was also tested in live cells, as a negative control.

The compounds **3j** and **3n** bearing methoxy or chloro substitutions at 2-position inhibit all three CYP1 family enzymes in varying degrees, as it was seen with Sacchrosomes™. It was observed that the 2-pyrrole chalcone **3j** is not CYP1B1 specific since it also inhibits the 1A1 isoform with equal efficacy (IC_{50} = 1.2 μM). However, **3n** is specific to CYP1B1 inhibitor, IC_{50} = 0.25 μM compared to the CYP1A1, IC_{50} = >20 μM. Nonetheless, both **3j** and **3n** display excellent specificity (>20-fold in Sacchrosomes™ and live human cells) for CYP1 family enzymes with respect to CYP2 and CYP3 family isoforms. Inhibition of CYP2/CYP3 enzymes can be the cause of deleterious drug-drug interactions which often thwart further drug development. Sacchrosomes™ and recombinant live human cell assays indicate that the 2-pyrrole chalcones **3j** and **3n** can avoid such harmful interactions.

In order to gain further insight into the experimental CYP inhibition efficacy and selectivity, molecular modeling studies were performed with the chalcones using the 3D structures of isoforms of the CYP1, CYP2 and CYP3 sub-families. Analysis of ANF-bound CYP1A1 and CYP1B1 X-ray derived structures indicate that they

Table 1
CYP inhibition activity of pyrrole chalcones **3a–w** in Sacchrosomes™.[a]

Entry	IC_{50} values for CYP inhibition (μM)						
	1A1	1B1	1A2	2D6	2C9	2C19	3A4
3a	6.2 ± 0.2	5.2 ± 0.1	1.5 ± 0.1	11 ± 0.2	>20	>20	>20
3b	10.8 ± 0.3	>20	10.0 ± 0.5	18 ± 0.4	>20	>20	>20
3c	>20	>20	10.9 ± 0.4	12 ± 0.2	>20	>20	>20
3d	5.2 ± 0.2	>20	9.2 ± 0.4	16 ± 0.3	>20	>20	>20
3e	5.8 ± 0.2	>20	9.7 ± 0.5	14 ± 0.2	>20	>20	>20
3f	11.8 ± 0.2	>20	10.0 ± 02	11 ± 0.1	>20	>20	>20
3g	>20	>20	10.5 ± 0.3	12 ± 0.2	>20	>20	>20
3h	6.1 ± 0.4	>20	9.0 ± 0.2	15 ± 0.4	>20	>20	>20
3i	>20	2.7 ± 0.1	2.2 ± 0.08	16 ± 0.4	>20	>20	>20
3j	0.9 ± 0.07	0.9 ± 0.1	1.1 ± 0.04	20 ± 0.6	>20	>20	>20
3k	>20	>20	13 ± 0.2	18 ± 0.4	>20	>20	>20
3l	>20	>20	12.5 ± 0.1	13 ± 0.2	>20	>20	>20
3m	>20	>20	11.8 ± 0.2	16 ± 0.3	>20	>20	>20
3n	9.1 ± 0.4	0.2 ± 0.04	0.7 ± 0.1	12 ± 0.16	>20	>20	>20
3o	>20	13 ± 0.4	>20	14 ± 0.25	>20	>20	>20
3p	>20	>20	17 ± 0.5	13 ± 0.3	>20	>20	>20
3q	>20	9.2 ± 0.2	2.5 ± 0.08	17 ± 0.4	>20	>20	>20
3r	>20	>20	15 ± 0.4	14 ± 0.2	>20	>20	>20
3s	>20	>20	>20	16 ± 0.3	>20	>20	>20
3t	>20	>20	>20	18 ± 0.4	>20	>20	>20
3u	>20	>20	>20	12 ± 0.15	>20	>20	>20
3v	16 ± 0.5	1.5 ± 0.1	12 ± 0.3	14 ± 0.2	>20	>20	>20
3w	>20	8.5 ± 0.2	13.2 ± 0.2	18 ± 0.4	>20	>20	>20
ANF	0.01 ± 0.002	0.05 ± 0.01	0.03 ± 0.01	>20	>20	>20	>20

[a] α-naphthoflavone (ANF) was used as a control in these studies; The IC_{50} values represent mean and standard deviations from three independent experiments.