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Effect of Structural Modification on the Inhibitory Selectivity of Rutaecarpine Derivatives on Human CYP1A1, CYP1A2, and CYP1B1

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Abstract—Derivatives of a CYP1A2 inhibitor rutaecarpine were synthesized to have potent and selective inhibition of human CYP1 members. Structural modelling shows a good fitting of rutaecarpine with the putative active site of human CYP1A2. Among the derivatives, 10- and 11-methoxyrutaecarpine are the most selective CYP1B1 inhibitors. 1-Methoxyrutaecarpine and 1,2-dimethoxyrutaecarpine are the most selective CYP1A2 inhibitors.

Cytochrome P450 (P450, CYP) 1 family plays an important role in the detoxication and activation of various xenobiotics and endogenous compounds, such as estradiol.[1,2] Up to date, three members, CYP1A1, CYP1A2, and CYP1B1, are identified in human. CYP1A1 and CYP1A2 have 80% identity in amino acid sequence and are about 40% identical with CYP1B1.[3] CYP1 enzymes have overlapped substrate specificity but show differential tissue distribution, substrate preference, and stereo-specific oxidations.[3,4]

Lewis et al.[5] reported that several hydrogen bond donor/ acceptor side chains of the putative active site of CYP1A enzymes become essentially hydrophobic residues in CYP1B1. These differences could change ligand orientation in the active site and possibly cause the interactions of ligands with CYP1A enzymes different from CYP1B1. Selective inhibitors of CYP1 enzymes are important agents for toxicological and pharmacological application including the identification of CYP1 members involved in the activation and detoxication of xenobiotics and the development of chemoprotective agents.

Many types of compounds have been tested for their inhibitory effects on CYP1 members.[6–8] However, the inhibitory potency and selectivity of modified derivatives were not well investigated together with the molecular modelling of their core-structure. Our previous report demonstrated that an alkaloid rutaecarpine (**1**, Table 1) was a selective and potent inhibitor of CYP1A2 in mouse and human liver microsomes.[9] Rutaecarpine may provide a good core structure for investigation of the inhibitory selectivity. Kim et al.[8] reported that 2,3′,4,5′-tetramethoxy stilbene selectively inhibited CYP1B1. The positions of methoxy substituents are important in determining the inhibitory potency and selectivity. To examine the effect of structural modification on inhibitory selectivity, we have synthesized a series of methoxylated and halogenated derivatives (Table 1). Molecular modelling of the interaction of rutaecarpine and CYP1A2 was performed and the inhibitory effects of rutaecarpine derivatives on human CYP1A1, CYP1A2, and CYP1B1 were studied.

Rutaecarpine (**1**), 10-methoxyrutaecarpine (**2**), and 11-methoxyrutaecarpine (**3**) were synthesized from tryptamine, 5-methoxytryptamine, and 6-methoxytryptamine, respectively.[10,11] 7,8-Dehydrorutaecarpine (**4**) was obtained by dehydrogenation of rutaecarpine with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in

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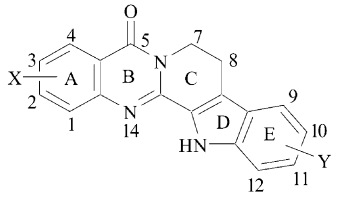
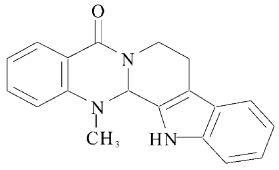
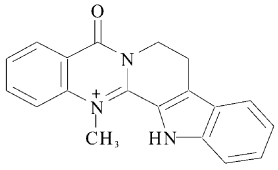
Table 1. IC_{50} values of rutaecarpine and its derivatives for CYP1A1, CYP1A2, and CYP1B1-catalyzed 7-ethoxyresorufin *O*-deethylation activities in bacterial membrane expressing human P450s



1-14



15, evodiamine



16, dehydroevodiamine

Compd	IC_{50} (nM)			IC_{50} ratio	
	CYP1A1	CYP1A2	CYP1B1	1A1/1A2	1B1/1A2
1, Rutaecarpine, X = Y = H	260 ± 30	22 ± 3	55 ± 11	13	3
2, X = H, Y = 10-OMe	1780 ± 380	2920 ± 690	84 ± 17	0.6	0.03
3, X = H, Y = 11-OMe	2000 ± 300	1980 ± 180	110 ± 20	1	0.06
4, X = Y = H, 7,8 = Δ	32 ± 3	30 ± 4	69 ± 22	1	2
5, X = 1-OMe, Y = H	450 ± 90	11 ± 0	71 ± 10	45	7
6, X = 2-OMe, Y = H	850 ± 50	76 ± 8	170 ± 8	11	2
7, X = 3-OMe, Y = H	1280 ± 100	280 ± 40	140 ± 7	5	0.5
8, X = 1,2-di-OMe, Y = H	970 ± 30	29 ± 3	240 ± 30	33	8
9, X = 1,3-di-OMe, Y = H	420 ± 8	41 ± 5	80 ± 9	11	2
10, X = 2,3-di-OMe, Y = H	1580 ± 170	> 500,000[a]	160 ± 20	< 0.003	< 0.0003
11, X = 3,4-di-OMe, Y = H	870 ± 120	1730 ± 290	140 ± 10	0.5	0.08
12, X = 2-Cl, Y = H	1200 ± 70	39 ± 4	37 ± 11	30	1
13, X = 3-Cl, Y = H	1920 ± 140	29 ± 5	90 ± 35	64	3
14, X = 3-Br, Y = H	2470 ± 320	150 ± 44	120 ± 20	16	0.8
15, Evodiamine	24,560 ± 1890	16,300 ± 810	690 ± 130	2	0.04
16, Dehydroevodiamine	18,050 ± 660	5630 ± 680	330 ± 10	3	0.06

Concentrations of 7-ethoxyresorufin and P450 were 2 μM and 20 nM in the assays, respectively. Rutaecarpine and its derivatives were dissolved in DMSO and added to the incubation mixture of bacterial membranes. The same volume of DMSO was added to the control and the final concentration of DMSO was < 0.5%.

[a]Due to the solubility and DMSO concentration in assay, the maximal concentrations of **10** used for inhibition study were 500 μM. The inhibition by **10**, up to the maximal concentration added, was not sufficient for IC_{50} estimation.

dioxane.[10] Compounds **5**–**14** were synthesized from 1,2,3,4-tetrahydro-β-carboline-1-one[12] and appropriate anthranilic acids in the presence of $POCl_3$.[13,14] 3,5-Dimethoxyanthranilic acid, 3,4-dimethoxyanthranilic acid, and 5,6-dimethoxyanthranilic acid were prepared from 3,5-dimethoxybenzoic acid, 3,4-dimethoxy-2-nitrobenzaldehyde, and 5,6-dimethoxy-2-nitrobenzaldehyde following the method of Grosso et al.,[15] respectively. The other anthranilic acids were commercial available. The structures of compounds **1**–**14** were confirmed by NMR analysis.[10,11,14,16] The syntheses of compounds **8**, **9**, and **11** are first reported. Evodiamine and dehydroevodiamine were isolated from *Evodia rutaecarpa*.[17] Bicistronic human CYP constructs were generously provided by Dr. F. Peter Guengerich (Vanderbilt University, TN, USA). Bacterial membrane fractions of *Escherichia coli* expressing bicistronic human CYP1A1, CYP1A2, and CYP1B1 were prepared following the method of Parikh et al.[18] P450 content and 7-ethoxyresorufin *O*-deethylation activity were determined using the methods of Omura and Sato[19] and Pohl and Fouts,[20] respectively. The concentrations of compounds required for 50% inhibition of catalytic activities (IC_{50}) were calculated by curve fitting (Grafit, Erithacus Software Ltd., Staines, UK).

Rutaecarpine has a planar polycyclic structure, which is a key criterion in defining CYP1 specificity.[5] Rutaecarpine preferentially inhibited CYP1A2 (Table 1) activity in a mixed type of noncompetitive and uncompetitive inhibition (result not shown). The present study used a model of human CYP1A2 generated using the crystal structure of rabbit CYP2C5 as a template based on protein sequence homology.[21] Rutaecarpine was fitted within the CYP1A2 putative active site from interactive docking. Molecular modelling procedures were carried out using the Sybyl software package (Tripos Associates, St. Louis, MO) implemented on a Silicon Graphics Indigo 10000 graphics workstation operating under UNIX. Our modelling result showed a good fitting of rutaecarpine and the putative active site of human CYP1A2 (Fig. 1). Two hydrogen bonds can be formed between the keto- and N14-groups of rutaecarpine and the Thr^{208} and Thr^{473} residues of CYP1A2, respectively. The C-ring moiety of rutaecarpine forms π–π stacking interaction with the aromatic ring of Phe^{205} residue.

The best fit of our modelling results allowed the E-ring moiety of rutaecarpine to approach the heme moiety of CYP1A2 active site pocket (Fig. 1). Introduction of a methoxy group at C10 (**2**) or C11 (**3**) of the E-ring highly increased the IC_{50} value for CYP1A2 with relatively less change of the IC_{50} value for CYP1B1 (Table 1). Compounds **2** and **3** had IC_{50} values for CYP1A2 146- and 99-fold higher than rutaecarpine, respectively.

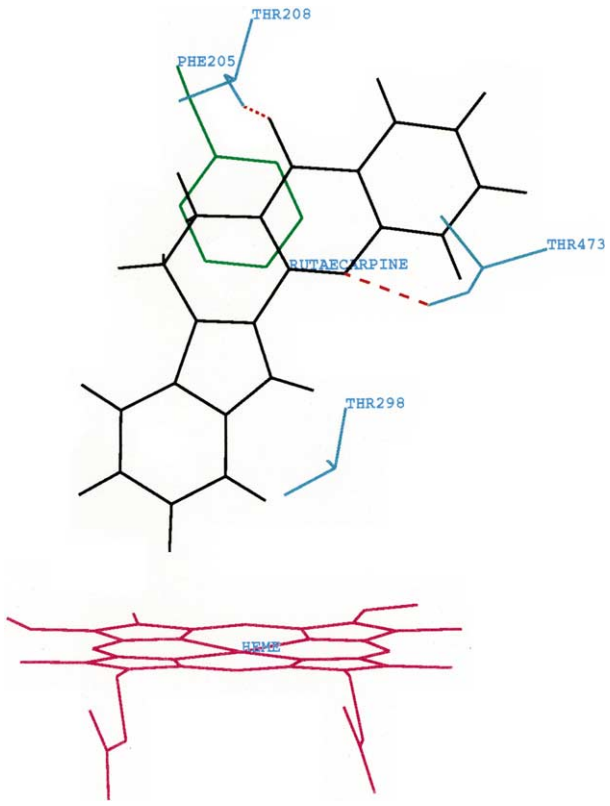


Figure 1. The selective inhibitor, rutaecarpine (black), is shown docked within putative human CYP1A2 active site. The heme (red), rutaecarpine, and several amino acids (blue) are labeled and hydrogen bonds are shown as dashed lines. The numbering of amino acids is from rabbit CYP2C5. The phenyl group of Phe^{205} is shown with green color. $Threonine^{298}$ is the conserved distal threonine in the I-helix.

Their IC_{50} values for CYP1B1 were less than 6% of that for CYP1A1 and CYP1A2. Thus, compounds **2** and **3** were selective inhibitors of CYP1B1.

In comparison with rutaecarpine, compound **4** with the C7–C8 double bond of C-ring provided better π–π stacking interaction with Phe^{205} and resulted in the dramatic decrease of IC_{50} value for CYP1A1. Among all derivatives, compound **4** was the most potent inhibitor of CYP1A1 with an IC_{50} value of 32 ± 3 nM, which was similar to its IC_{50} value for CYP1A2 and about half of that for CYP1B1. The CYP1A2 selectivity of rutaecarpine was lost by the introduction of C7–C8 double bond.

In A-ring modification, compounds 1- (**5**) and 2-methoxyrutaecarpine (**6**) and 1,2- (**8**) and 1,3- (**9**) dimethoxyrutaecarpine preferentially inhibited CYP1A2. Among all derivatives, compounds **5** and **8** had the best CYP1A2 selectivity with IC_{50} ratios for both CYP1A1/CYP1A2 and CYP1B1/CYP1A2 higher than the respective ratios of rutaecarpine. In contrast, 3-methoxyrutaecarpine (**7**) and 2,3- (**10**) and 3,4- (**11**) dimethoxyrutaecarpine preferentially inhibited CYP1B1 with IC_{50} ratios for CYP1B1/CYP1A2 less than 0.5. These results suggested that a methoxy substituent at C1 was important for selective and potent CYP1A2 inhibition. Surprisingly, compound **10** at concentration as high as 500 μM had no inhibitory effect on CYP1A2. It might be good for the use in discriminating CYP1A2 from CYP1A1 and CYP1B1. In comparison with rutaecarpine, the introduction of the electron-withdrawing chlorine at C2 (**12**) and C3 (**13**) appeared to highly increase the IC_{50} value for CYP1A1 with much smaller changes for CYP1A2 and CYP1B1 activities. 3-Bromorutaecarpine (**14**) also had IC_{50} ratio for CYP1A1/CYP1A2 slightly higher than rutaecarpine. However, the discrimination between CYP1A2 and CYP1B1 was not improved. These halogenated derivatives became inhibitors for both CYP1A2 and CYP1B1.

Our modelling result reveals that N14 is important for CYP1A2 binding through hydrogen bond formation (Fig. 1). Therefore, natural alkaloids evodiamine and dehydroevodiamine with an N14-methyl substituent were studied to address the importance of this position. Our results showed that the IC_{50} values of these two alkaloids for all CYP1 enzymes were highly increased, especially CYP1A enzymes (Table 1). These two alkaloids inhibited CYP1A1 and CYP1A2 activity with IC_{50} values 69- to 741-fold greater than the respective IC_{50} values of rutaecarpine (Table 1). Our previous report also showed that evodiamine and dehydroevodiamine had IC_{50} values higher than rutaecarpine in mouse liver microsomes.[9] The N14-methyl substituent might prevent the hydrogen-bond formation and result in high IC_{50} values for both CYP1A1- and CYP1A2-catalyzed oxidations.

In summary, our results of modelling and inhibition studies support the importance of hydrogen bond and π–π stacking interactions. Compared to rutaecarpine, the introduction of methoxy and halogen groups, as indicated in this report, all increased the IC_{50} values for human CYP1A1. For human CYP1 family, 10-methoxyrutaecarpine and 11-methoxyrutaecarpine selectively inhibited CYP1B1 and 1-methoxy-rutaecarpine and 1,2-dimethoxyrutaecarpine selectively inhibited CYP1A2. These rutaecarpine derivatives could be used as selective inhibitors for human CYP1A2 and CYP1B1.

Acknowledgements

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