HETEROCYCLES, Vol. 65, No. 8, 2005, pp. 1817 - 1820
Received, 19th May, 2005, Accepted, 6th July, 2005, Published online, 7th July, 2005

SYNTHETIC MODELS RELATED TO FURANOCOUMARIN – CYP3A4 INTERACTIONS. SYNTHESIS OF FURANOCOUMARIN DIMERS THAT HAVE INHIBITORY EFFECTS ON ACYIVITY OF HUMAN CYP3A4

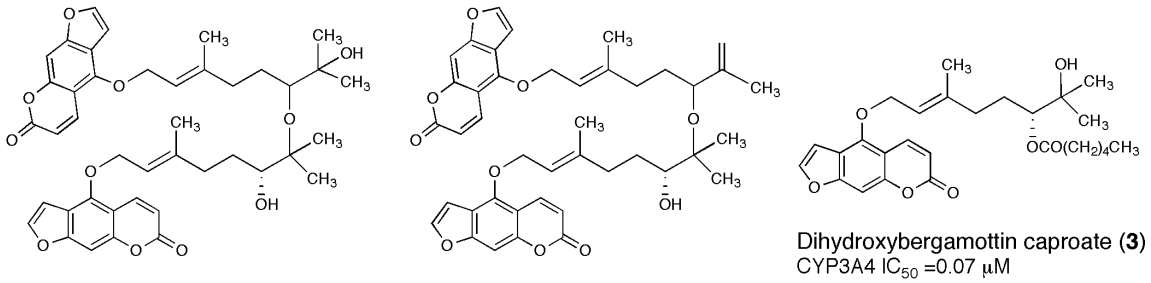
Kazuaki Oda,* Hitomi Kasai, Yuki Yamaguchi, Teruki Yoshimura, Keiji Wada, Minoru Machida, and Naozumi Nishizono*

Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido 061-0293, Japan.
e-mail: k-oda@hoku-iryo-u. ac. jp

Abstract □ Synthesis of a series of furanocoumarin dimers that have inhibitory effects on the activity of human cytochrome P450 (CYP) 3A4 is described. The reported furanocoumarin dimers paradisins A and B from grapefruit juice showed potent CYP3A4 inhibition with an IC_{50} value of 0.07 □M. Synthetic furanocoumarin dimer (**10**), which is more stable and accessible than paradisins, exhibited comparable activity against CYP 3A4 (IC_{50} = 0.02 □M).

In 1991, Bailey *et al*. reported that concomitant oral administration of grapefruit juice increased the bioavailability of dihydropyridine-type calcium channel blockers, felodipine and nifedipine.[1] Similar phenomena were subsequently reported for clinically important drugs.[2] Although these drugs have no structural similarities, it was shown that compounds in grapefruit juice affect the drug metabolism in humans by inhibition of cytochrome P450 (CYP) 3A4. Recently, Ohta *et al*. have reported the isolation of furanocoumarin dimers, paradisin A (**1**) and paradisin B (**2**) from grapefruit juice, as specific CYP3A4 inhibitors with an IC_{50} value of 0.07 □M.[3] They also

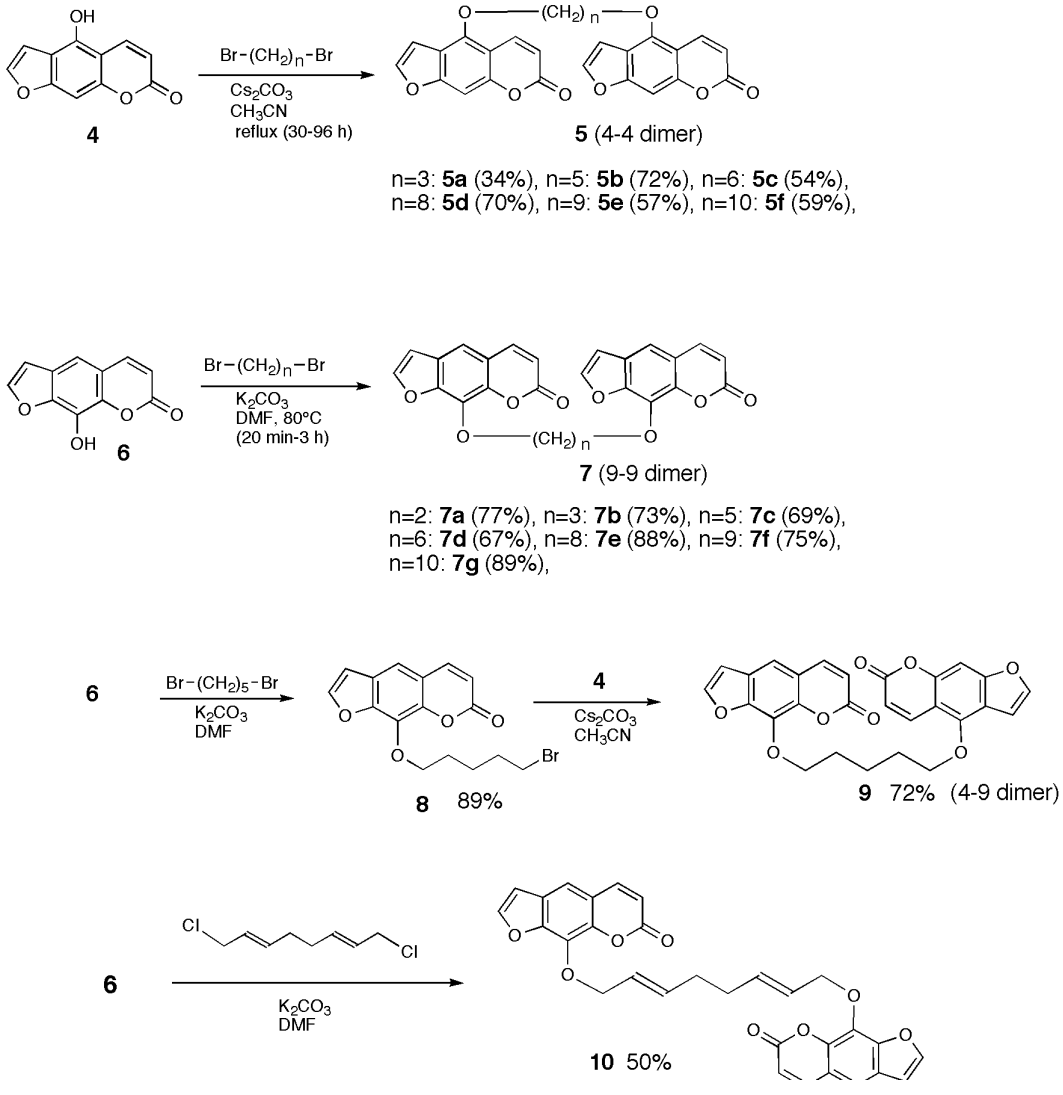
Scheme 1



proposed a semi-synthetic dihydroxybergamottin carproate (**3**), derived from bergamottin in grapefruit juice, as a potent and stable inhibitor of CYP3A4 activity.[4] The relevant biological properties of furanocoumarin dimers and their low concentration in grapefruit juice prompted us to investigate the synthesis of furanocoumarin dimers. Herein we report the synthesis of more stable and accessible furanocoumarin dimers having strong inhibitory effects on CYP 3A4 activity.

The synthetic route to afford various furanocoumarin dimers (**5**, **7**, **9**, and 1**0**) is outlined in Scheme 2. 4-Hydroxyfuranocoumarin (**4**: bergaptol) and 9-hydroxyfurano-coumarine (**6:** xanthotoxol) were used as starting materials. Furanocoumarin dimers (**5**: 4-4 dimers) in which two furanocoumarin rings are linked by flexible polymethylenic chains of various lengths at the 4 position of the furanocoumarin ring were synthesized from commercially available ω,ω-dibromoalkanes, 4-hydroxyfuranocoumarin (**4**), and $CsCO_3$ in CH_3CN. Similarly, furanocoumarin dimers (**7**: 9-9 dimers) linked at the 9 position of the furanocoumarin ring were synthesized from commercially available ω,ω-dibromoalkanes, 9-hydroxyfuranocoumarin (**6**), and K_2CO_3 in DMF at

Scheme 2



80°C. An unsymmetrical furanocoumarin dimer (**9**: 4-9 dimer) was prepared by reaction in DMF of **4** with 9-(5-bromopentyloxy)-7*H*-furo[3,2-*g*]chromen-7-one (**8**) previously obtained by the action of 1,5-dibromopentane on **6**. Next, in order to determine the rigidity of the functional group responsible for CYP3A4 inhibition, we prepared furanocoumarin dimer (**10**)[5] that contained a carbon-carbon double bond in the linker part from (*2E,6E*)-1,8-dichloroocta-2,6-diene and **6** according to the procedure used for **7**.

Assay of inhibition of CYP3A4 activity by these furanocoumarin dimers was based on the microsomal testosterone 6□-hydroxylation.[6] The results are shown in Table 1. A comparison of 4-

Table 1. Inhibition of CYP3A4 activity by furanocoumarin dimers (0.5 μM).[7]

Compound	Residual Percentage Activity (Mean ± S.D., n=3)	Compound	Residual Percentage Activity (Mean ± S.D., n=3)
5b	19.4 ± 4.2	**7d**	7.9 ± 0.4
5d	65.4 ± 2.7	**7e**	8.2 ± 1.8
5f	87.3 ± 6.5	**7f**	15.3 ± 1.6
7a	26.3 ± 0.4	**7g**	26.1 ± 3.6
7b	18.8 ± 1.5	**9**	15.7 ± 0.8
7c	15.1 ± 2.1	**10**	4.8 ± 0.4

4 dimer series (**5b**, **5d**, and **5f**)[8] showed that the values of residual percentage activity of CYP3A4 were very sensitive to chain length. **5b**, having five methylenes as a linking chain, showed the most potent inhibition, and the inhibitory activity of dimers (**5d** and **5f**) decreased with increase in chain length. The influence of the location of the linking chain on the furanocoumarin ring was studied. A comparison of three types of dimers (**5b:** 4-4 dimer, **7c:** 9-9 dimer, and **9:** 4-9 dimer) having five methylenes as a linking chain showed that **7c** had the strongest inhibitory effects. To determine the most appropriate length of linking chain, the number of methylenes was varied from 2 to 10 in the series of 9-9 dimers (**7**). **7d** (n=6) and **7e** (n=8) showed strong inhibitory effects on CYP3A4 activity. Finally, we tested furanocoumarin dimer (**10**) that contained a carbon-carbon double bond (total 8 carbons) in the linker part. The inhibitory effect of **10** on CYP3A4 activity was significantly greater than that of **7e** having eight methylenes as a flexible linking chain. Interestingly, **10** showed the most potent inhibition with an IC_{50} value of 0.02 □M. [9]

The results indicated that furanocoumarin dimers having suitable chain length and rigidity in the linker part had strong the inhibitory activity. We prepared a furanocoumarin dimer (**10**) that was more stable and accessible than paradisins and exhibited comparable activity against CYP3A4. Through their inhibitory effects on the activity of the drug-metabolizing enzyme CYP3A4,

administration of furanocoumarin derivatives might enable doses of drugs to be reduced and thus reduce side effects. Elucidation of the CYP3A4-inhibitor interaction may be an important subject in the pharmacokinetics of clinically used drugs.

ACKNOWLEDGEMENTS

This study was partially supported by the "Academic Frontier" Project of the Ministry of Education, Culture, Sports, Science, and Technology, Japan. We are grateful to the staff of the Center for Instrumental Analysis, Hokkaido University, for the elemental analysis and HRMS spectra measurements.

REFERENCES AND NOTES

1. D. G. Bailey, J. D. Spence, C. Munoz, and J. M. Arnold, *Lancet*, 1991, **337**, 268.
2. L.-Q. Guo, K. Fukuda, T. Ohta, and Y. Yamazoe, *Drug. Metab. Dispos.*, 2000, **28**, 766 and references cited therein.
3. M. P. Ducharme, L. H. Warbasse, and D. J. Edwards, *Clin. Pharmacol. Ther.,* 1995, **57**, 485; G. C. Yee, D. L. Stanley, L. J. Pessa, C. T. Dalla, S. E. Beltz, and J. Ruiz, *Lancet*, 1995, **345**, 995; H. H. Kupferschmidt, H. R. Ha, W. H. Ziegler, P. J. Meier, and S. Krahenbuhl, *Clin. Pharmacol. Ther.*, 1995, **58**, 20.
4. T. Ohta, M. Nagahashi, S. Hosoi, and S. Tsukamoto, *Bioorg. Med. Chem.,* 2002, **10**, 969.
5. Furanocoumarin dimer (**10**), mp 156-157 °C (ethanol), ^{1}H-NMR (500 MHz, $CDCl_3$) □ 1.99-2.00 (4H, m), 4.88 (4H, d, *J*= 5.7 Hz), 5.67 (2H, dt, *J* =5.7, 15.5 Hz), 5.72 (2H, dt, *J*= 5.7, 15.5 Hz), 6.36 (2H, d, *J*=9.7 Hz), 6.81 (2H, d, *J*= 2.3 Hz), 7.37 (2H, s), 7.69 (2H, d, *J*= 2.3 Hz), 7.77 (2H, d, *J*=9.7 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) □31.6, 74.2, 106.9, 113.4, 114.7, 116.5, 125.7, 126.0, 131.3, 135.9, 143.7, 144.5, 146.8, 148.5, 160.6. MS *m/e* 510 (M^+), *Anal*. Calcd for $C_{30}H_{22}O_8$: C, 70.58; H, 4.34. Found: C, 70.66; H, 4.32.
6. W. Tassaneeyakul, L.-Q. Guo, K. Fukuda, T. Ohta, and Y. Yamazoe, *Arch. Biochem. Biophys.,* 2000, **58**, 1667.
7. Inhibitory effect on testosterone 6□-hydroxylation was expressed as a percentage of the residual activity compared with the control.
8. Owing to the poor solubility of some 4-4 dimers in aqueous solution, selected data are listed.
9. Furanocoumarin dimer (**10**) showed dose-dependent inhibitory effects on human microsomal testosterone 6□-hydroxylation in the range from 0.005 to 0.3 □M (6 points). The value of IC_{50}, the concentration required for 50% inhibition of CYP3A4 activity, was calculated from the data of duplicate measurements.