Synthetic Models Related to Furanocoumarin–CYP 3A4 Interactions. Comparison of Furanocoumarin, Coumarin, and Benzofuran Dimers as Potent Inhibitors of CYP3A4 Activity

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Furanocoumarin derivatives (dimers and monomers) present in commercially available grapefruit juice have the capacity to inhibit the activity of human CYP3A4. Such interactions are believed to result from the mechanism-based inhibition of CYP3A4 activity in the intestine. The aim of this work was to synthesize and test a series of dimers with a view to determining the relationship between structure and inhibitory activity and determining whether they might make suitable probes of CYP3A4 activity. We prepared a series of furanocoumarin, coumarin, and benzofuran derivatives that have inhibitory effects on the activity of human CYP3A4. A synthetic benzofuran dimer, which is more accessible than furanocoumarin dimers, exhibited activity against CYP3A4 comparable to that of furanocoumarin dimers.

Key words grapefruit juice; furanocoumarin dimer; inhibitor; CYP3A4; benzofuran dimer

Grapefruit juice, when consumed with certain orally administered drugs, has been shown to increase the bioavailability of the drugs.[1] This interaction was first discovered when grapefruit juice was administered with the calcium antagonist felodipine. Many studies on the effects of grapefruit juice on drugs of therapeutic importance have since been performed.[2] Although these drugs have no structural similarities, furanocoumarin derivatives, present in grapefruit juice have been found to be extremely potent inhibitors of CYP3A4 activity in the intestine.[3] However, there is no clear evidence that any one compound is primarily responsible, and it is possible that the interaction arises from the accumulative effect of a number of constituents present in grapefruit juice. Of the various compounds isolated from grapefruit juice, furanocoumarin dimers, paradisin A and B, were found to be the most potent inhibitors of CYP3A4 activity (Fig. 1). The broad biological properties of furanocoumarin derivatives and their low concentrations in grapefruit juice prompted us to investigate the synthesis of furanocoumarin derivatives. Previously, we[4] and another group[5] individually proposed the synthesis of novel furanocoumarin dimers as potent inhibitors and candidates for suitable probes of CYP3A4 activity. The results indicated that furanocoumarin dimers having suitable chain length and rigidity (containing a carbon–carbon double bond) in the linker part between the two furanocoumarin rings had strong inhibitory activity. Herein we report the synthesis of furanocoumarin, coumarin, and benzofuran dimers having the same linker part and the results of assays of their inhibitory effects on CYP3A4 activity with a view to determining the relationship between the ring part structure (ring systems and suitable location of the linking chain) and inhibitory effect on CYP 3A4 activity (Fig. 2). In this study, in order to compare inhibitory effects on CYP3A4 activity in a simpler system, we selected dimers having a flexible five-methylene chain as a linker, accepting the possibility that such dimers might have slightly weaker inhibitory effects on CYP3A4 activity.

A series of furanocoumarin dimers (**3a**—**c**) were prepared by the reactions reported in a previous paper.[4] 4-Hydroxyfuranocoumarin (bergaptol)[6] and 9-hydroxyfuranocoumarine (xanthotoxol) were used as starting materials (Chart 1). Furanocoumarin dimers (**3a**: 4–4 dimers) in which two furanocoumarin rings are linked by five methylenic chains at the 4 position of the furanocoumarin ring were synthesized from 1,5-dibromopentane, 4-hydroxyfuranocoumarin, and Cs_2CO_3 in CH_3CN. Similarly, furanocoumarin dimers (**3b**: 9–9 dimers) linked at the 9 position of the furanocoumarin ring were synthesized from 1,5-dibromopentane, 9-hydroxyfuranocoumarin, and K_2CO_3 in DMF at 80 °C. An unsymmetrical furanocoumarin dimer (**3c**: 4–9 dimer) was prepared by reaction in DMF of 4-hydroxyfuranocoumarin with 9-(5-bromopentyloxy)-7*H*-furo[3,2-*g*]chromen-7-one previously obtained by the reaction of 1,5-dibromopentane with 9-hydroxyfuranocoumarine. Next, in order to determine the furan por-

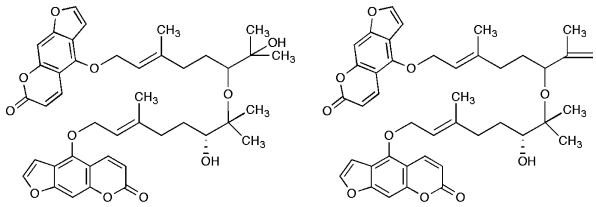

Fig. 1. Furanocoumarin Dimers in Grapefruit Juice

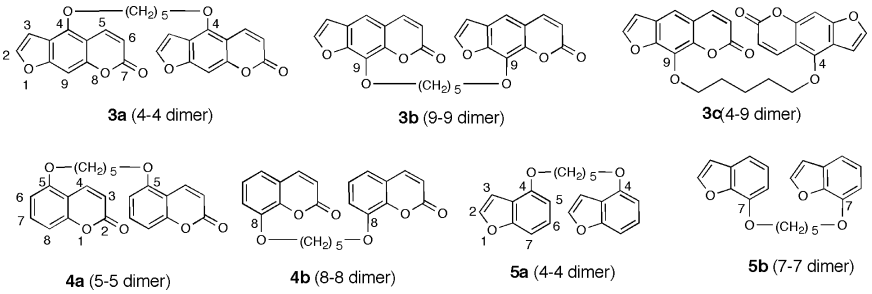

Fig. 2. Structures of Synthetic Dimers

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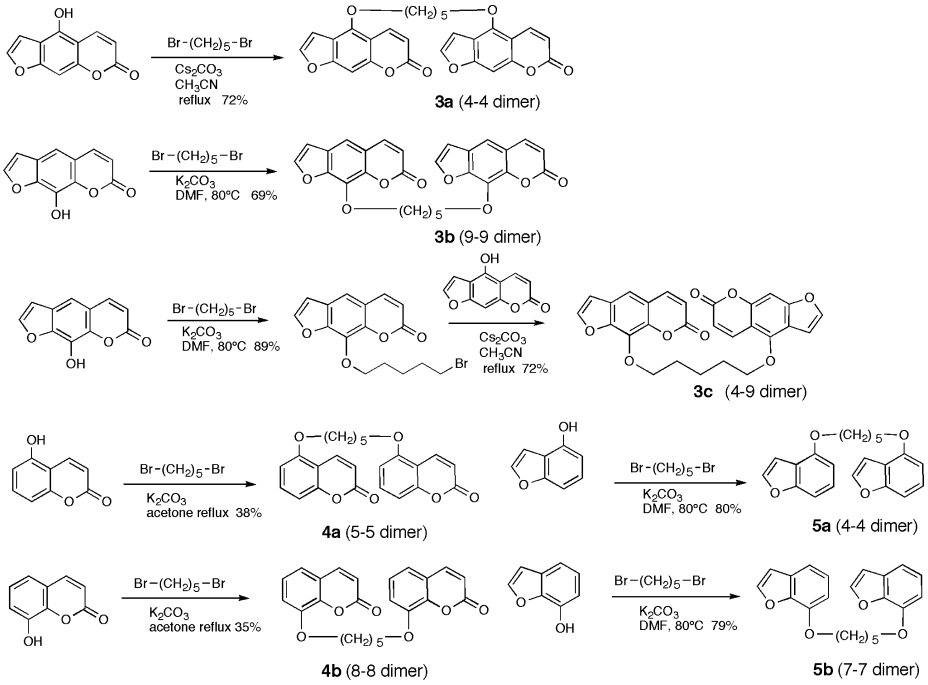

Chart 1. Synthesis of Various Dimers

Table 1. IC_{50} Values of Various Dimers Based on 6β-Hydroxylation of Testosterone

Compound	IC_{50} values (μM)	Compound	IC_{50} values (μM)
3a	0.13	**4b**	>10
3b	0.02	**5a**	0.10
3c	0.03	**5b**	1.02
4a	>10		

tion of the tricyclic ring system responsible for CYP3A4 activity inhibition, we prepared coumarin dimers (**4a**, **b**) in which two coumarin rings are linked by five methylenic chains at the 5 or 8 position of the coumarin from appropriate 5- or 8-hydroxycoumarin[7] and 1,5-dibromopentane, according to the procedure used for **3b**. Furthermore, to determine the 2-pyrone portion of the tricyclic ring system responsible for CYP3A4 activity inhibition, benzofuran dimers (**5a**, **b**) were prepared from appropriate 4-[8] or 7-hydroxybenzofuran[9] and 1,5-dibromopentane, according to the procedure used for **3b**.

Assays for inhibition of CYP3A4 activity (GENTEST Co. Human CYP3A4+cytochrome b5+P450 reductase (Baculovirus)) by these dimers were carried out based on microsomal testosterone 6β-hydroxylation.[10] The results are shown in Table 1.[11] First, the influence of the location of the linking chain on the furanocoumarin ring was studied. A comparison of three types of dimers (**3a**: 4–4 dimer, **3b**: 9–9 dimer, and **3c**: 4–9 dimer) having five-methylene chain as a linker showed that **3b** and **3c** had the strongest inhibitory effects. The two coumarin dimers (**4a**, **b**) having the linking chain at either the 5- or 8-position were found to be weak inhibitors of CYP3A4 activity. IC_{50} values were greater than 10 μM, the highest inhibitor concentration used. Next, we tested benzofuran dimers (**5a**, **b**) having the linking chain at either the 4- or 7-position. Interestingly, 4–4 dimer **5a** showed a 10-fold higher inhibition potency than that of 7–7 dimer **5b**, indicating the importance of the location of the linking chain. The inhibitory effect of **5a** on CYP3A4 activity (IC_{50}: 0.10 μM) was comparable with that of ketoconazole (IC_{50}: 0.11 μM), a known inhibitor of CYP3A4 activity. Finally, we prepared[12] and tested the inhibitory activity of a benzofuran monomer (**6**: 4-pentyloxybenzofuran) having a five-carbon chain at the 4-position to compare its inhibitory activity with that of dimers. IC_{50} value of **6** was greater than 10 μM, the highest inhibitor concentration used.

The results indicated that furanocoumarin dimers had the most potent inhibitory activity. The weak inhibition observed with coumarin dimer (**4a**) and the strong inhibition observed with benzofuran dimer (**5a**) suggested that the furan portion in the ring system is essential for the activity. The results suggested that the interaction of CYP3A4 with furanocoumarin occurs at the unsaturated furan ring as in the case of interaction of CYP2A6 with furanocoumarins.[13] There is initial formation of an epoxide followed by opening of the furan ring, which is able to covalently bind to the apoprotein of the enzyme, thus irreversibly inactivating it. Interestingly, the benzofuran monomer showed a poor inhibitory effect on CYP3A4 activity. Further study is needed to determine the reason for the large difference between the inhibitory effects of a benzofuran dimer and a benzofuran monomer on CYP3A4 activity.

We prepared various dimers that were more stable and accessible than paradisins (natural furanocoumarin dimers), and some dimers exhibited comparable activity against CYP3A4. Although synthetic furanocoumarin dimers showed the most potent inhibition, it is noteworthy that owing to the difficulty of ring system construction,[6] furanocoumarin dimers are disadvantageous as probes for determining the contribution of the enzyme to drug metabolism, whereas benzofuran dimer having a more simpler ring system seem to be good candidates for suitable probes. Further structural modification of benzofuran dimers with superior activity

against CYP3A4 to that of furanocoumarin dimers is under way. Through their inhibitory effects on the activity of the drug-metabolizing enzyme CYP3A4, administration of certain dimers might enable doses of drugs to be reduced and thus reduce side effects.

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References and Notes

1) Beiley D. G., Spence J. D., Munoz C., Arnold J. M., *Lancet*, **337**, 268—269 (1991).
2) Dahan A., Altman H., *Eur. J. Clin. Nutr.*, **58**, 1—9 (2004) and references cited therein.
3) Guo L.-Q., Fukuda K., Ohta T., Yamazoe Y., *Drug Metab. Dispos.*, **28**, 766—771 (2000) and references cited therein.
4) Oda K., Kasai H., Yamaguchi Y., Yoshimura T., Wada K., Machida M., Nishizono N., *Heterocycles*, **65**, 1817—1820 (2005).
5) Row E., Brown S. A., Stachulski A. V., Lennard M. S., *Drug Metab. Dispos.*, **34**, 324—330 (2006).
6) Oda K., Nishizono N., Tamai Y., Yamaguchi Y., Yoshimura T., Wada K., Machida M., *Heterocycles*, **65**, 1985—1988 (2005).
7) Harayama T., Katsuno K., Nishioka H., Fujii M., Nishita Y., Ishii H., Kaneko Y., *Heterocycles*, **39**, 613—622 (1994).
8) Kneen G., Maddocks P. J., *Synth. Commun.*, **16**, 1635—1640 (1986).
9) Musser J. M., Chakraborty U., Bailey K., Sciortino S., Whyzmuzis C., Amin D., Sutherland C. A., *J. Med. Chem.*, **30**, 62—67 (1987).
10) Tassaneeyakul W., Guo L.-Q., Fukuda K., Ohta T., Yamazoe Y., *Arch. Biochem. Biophys.*, **378**, 356—363 (2000).
11) All dimers except coumarins showed dose-dependent inhibitory effects on human microsomal testosterone 6β-hydroxylation. Dose-dependency data of selected compounds were listed. Compound; residual activity (concentration). **3b**; 79.5% (0.01 μM), 56.1% (0.02 μM), 26.0% (0.05 μM), 18.4% (0.08 μM), 16.7% (0.1 μM). **5b**; 66.6% (0.05 μM), 51.9% (0.1 μM), 38.8% (0.15 μM), 32.8% (0.2 μM), 24.9% (0.3 μM). The value of IC_{50}, the concentration required for 50% inhibition of CYP3A4 activity, was calculated from the data of duplicate measurements.
12) 4-Pentyloxybenzofuran (**6**) was synthesized from 1-bromopentane, 4-hydroxybenzofuran, and K_2CO_3 in DMF at 80 °C.
13) Koenigs L. L., Tager W. F., *Biochemistry*, **37**, 10047—10061 (1988).