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SYNTHETIC MODELS RELATED TO FURANOCOUMARIN-CYP 3A4 INTERACTIONS. SYNTHESIS OF FURANOCOUMARIN DERIVATIVES AS POTENT INHIBITORS OF CYP 3A4

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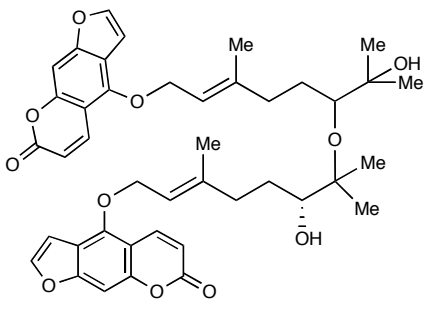
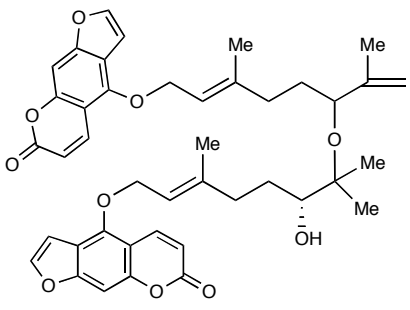
Abstract – We prepared a series of furanocoumarin derivatives that have inhibitory effects on the activity of human cytochrome P450 (CYP) 3A4. The reported furanocoumarin dimers paradisins A and B from grapefruit juice showed potent CYP 3A4 inhibition with an IC_{50} value of 0.07 μM. Synthetic furanocoumarin derivatives, which are more stable and accessible than paradisins, exhibited comparable activity against CYP 3A4.

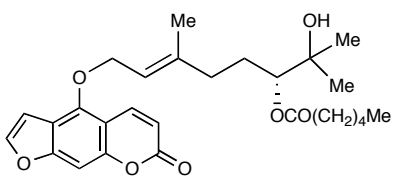
More than a decade has passed since it was unintentionally discovered that grapefruit juice interacts with certain drugs.[1] The coadministration of these drugs with grapefruit juice can markedly elevate drug bioavailability and can alter pharmacokinetic and pharmacodynamic parameters of drugs.[2] Although these drugs have no structural similarities, furanocoumarin dimers, paradisin A (**1**) and B (**2**), present in grapefruit juice have been found to be extremely potent inhibitors of CYP 3A4 activity in the intestine (Fig. 1).[3] The broad biological properties of furanocoumarin derivatives and their low concentrations in



paradisin A (**1**)
CYP3A4 IC_{50} =0.07-0.086 μM



paradisin B (**2**)
CYP3A4 IC_{50} =0.07-0.15 μM

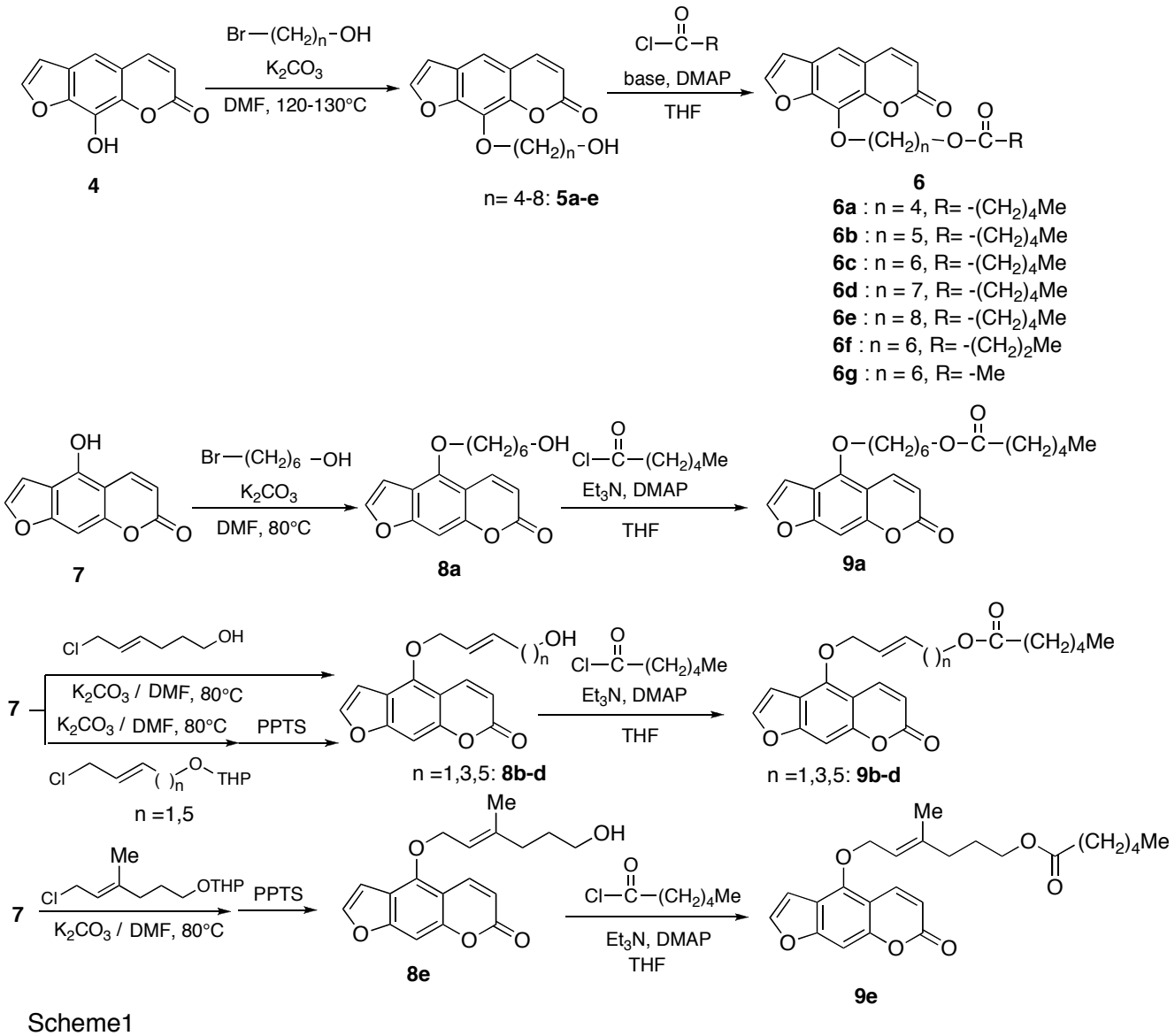


dihydrobergamottin caproate (**3**)
CYP3A4 IC_{50} =0.07 μM

Figure. 1

and another group[5] individually proposed the synthesis of novel furanocoumarin dimers as potent inhibitors and candidates for suitable probes of CYP 3A4 activity. Furthermore, Ohta et al. reported a semi-synthetic dihydroxybergamottin caproate (**3**),[6] derived from bergamottin in grapefruit juice, which is more stable than furanocoumarin dimers and exhibited comparable activity against CYP 3A4 (Fig. 1). Herein we report the synthesis of furanocoumarin derivatives and results of assays of their inhibitory effects on CYP 3A4 activity with a view to defining the relationship between structures and inhibitory effects on CYP 3A4 activity.

A series of furanocoumarin derivatives (**6** and **9**) were prepared by the reactions shown in Scheme 1. 4-Hydroxyfuranocoumarin (**4**: bergaptol)[7] and 9-hydroxyfuranocoumarine (**7**: xanthotoxol) were used as starting materials. Furanocoumarin derivatives (**6**) having flexible polymethylenic chains of various lengths and an ester group in their side chain at the 9 position of the furanocoumarin ring were synthesized from appropriate 9-(ω-hydroxyalkyloxy)-7*H*-furo[3,2-g]chromen-7-one (**5**) and acid chloride



Scheme1

in THF. Similarly, a furanocoumarin derivative (**9a**) having a side chain at the 4 position of the furanocoumarin ring was synthesized from 4-(6-hydroxyhexyloxy)-7*H*-furo[3,2-*g*]chromen-7-one (**8a**) and caproyl chloride in THF. Next, in order to determine the rigidity of the functional group responsible for CYP 3A4 inhibition, we prepared furanocoumarin derivatives (**9b**, **9c**, and **9d**) that contained a carbon-carbon double bond in the side chain from appropriate 9-(ω-hydroxyalkenyloxy)-7*H*-furo[3,2-*g*]chromen-7-one and caproyl chloride according to the procedure used for **9a**. Furthermore, to investigate the effect of a methyl substituent at the alkenyl carbon, a furanocoumarin derivative (**9e)** was prepared.

Assays of inhibition of CYP 3A4 activity (GENTEST Co. Human CYP 3A4 + Cytochrome b5 + P450 reductase (Baculovirus)) by these furanocoumarin derivatives were based on microsomal testosterone 6β-hydroxylation.[8,9] The results are shown in Table 1. A comparison of furanocoumarin derivatives

Table 1. IC_{50} values of furanocoumarin derivatives based on 6β-hydroxylation of testosterone.

Compound	IC_{50} values (μM)	Compound	IC_{50} values (μM)
6a	0.17	**9a**	0.08
6b	0.14	**9b**	0.07
6c	0.12	**9c**	0.07
6d	0.12	**9d**	0.07
6e	0.16	**9e**	0.08
6f	0.20		
6g	0.13		

(**6a**-**e**) having side chains of various lengths and a caproyl ester group in their side chain at the 9 position of the furanocoumarin ring showed that the IC_{50} values of them based on 6β-hydroxylation of testosterone were very sensitive to chain length. **6c**, having six methylenes in the side chain, showed the most potent inhibition, and the inhibitory activity of furanocoumarin derivatives (**6d** and **6e**) decreased with increase in chain length. A comparison of three esters (**6c**: caproyl ester, **6f**: butanoyl ester, and **6g**: acetyl ester) showed that **6c** had the strongest inhibitory effects. The influence of the location of the side chain on the furanocoumarin ring was studied. **9a**, having the same side chain as that of **6c** at the 4 position of the furanocoumarin ring, showed a stronger inhibitory effect on CYP 3A4 activity than that of **6c**. Next, we tested furanocoumarin derivatives **(9b**, **9c**, and **9d)** that contained a carbon-carbon double bond in the side chain. The inhibitory effects on CYP 3A4 activity of these compounds were similar to that of **9a**. Finally, we tested a furanocoumarin derivative (**9e)** that contained a methyl substituent at the alkenyl carbon in the side chain. The inhibitory effect of **9e** on CYP 3A4 activity was not notably different from that of **9c**. Within this series, **9a-e** were found to be the most potent analogues.

The results indicated that furanocoumarin derivatives having an ester moiety and suitable chain length in the side chain and a suitable location of the side chain had strong inhibitory activity. We prepared furanocoumarin derivatives (furanocoumarin monomers) that were more stable and accessible than paradisins (natural fuarnocoumarin dimers) and exhibited comparable activity against CYP 3A4. Through their inhibitory effects on the activity of the drug-metabolizing enzyme CYP 3A4, administration of furanocoumarin derivatives might enable doses of drugs to be reduced and thus reduce side effects. Elucidation of the CYP 3A4-inhibitor interaction may be an important subject in the pharmacokinetics of clinically used drugs.

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