Attaining tissue selectivity for drugs depends in part on receptor heterogeneity. Besides the distinction of A_1 and A_2 adenosine receptors, we have found considerable species difference in drug specificity of A_1 receptors in brain tissue.[83] Thus, the xanthine DPX varies as much as 500-fold in potency at A_1 receptors in different species. If comparable differences in drug specificity for receptors can be identified in different tissues of a single species, tissue-specific drugs could be developed.

In principle, receptor-binding techniques should considerably facilitate the enhancement of drug potency by structure–activity analysis. For the xanthines, blockade of adenosine receptors can be readily augmented by structure–activity analysis. We developed a series of substituted xanthines with greatly enhanced potencies at adenosine receptors.[40] Thus, a 8-phenyl substituent augments the potency of theophylline about 1,000-fold. Replacing the 1,3-dimethyl substituents of theophylline with 1,3-dipropyl substituents produces yet a further 10-fold enhancement of potency. Varying substituents on the 8-phenyl ring also alters potency. A 2-amino-4-chloro substitution on the 8-phenyl group provides a 6-fold augmentation of potency compared to the unsubstituted 8-phenyl group. Combining all these substituents results in 1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine (PACPX), a compound which displays a K_i for adenosine A_1 receptors in bovine brain membranes of 22 pM. It is 4000000 more times potent than xanthine itself and 70000 times more potent than theophylline.

In summary, receptor-binding techniques provide powerful strategies for drug development in numerous therapeutic classes. The examples of benzodiazepine and adenosine receptors reviewed in detail here are only two instances. Comparable accomplishments should be feasible with all the known drug and neurotransmitter receptors.

Acknowledgment. Work in this area was supported by USPHS Grants DA-00266, MH-18501, and NS-16375, RSA Award DA-00074, and grants of the McKnight Foundation and International Life Science Institute.

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Communications to the Editor

Khellin Analogues. 1. General Topological Requirements for Lipid-Altering Activity in Furochromones

Sir:

The distribution of cholesterol among the various lipoproteins in the plasma is recognized as a powerful predictor of risk of cardiovascular disease and, in particular, of atherosclerosis.[1] Of the four classes of plasma lipoproteins, chylomicrons (specifically, chylomicron remnants),[2a] very low density lipoproteins (VLDL),[2b] and low density lipoproteins (LDL)[3-5] are considered atherogenic, whereas high density lipoproteins (HDL) have been reported to be antiatherogenic[6] (i.e., protective against atherosclerosis). The atherogenicity of LDL arises from increasing evidence that suggests that most cellular cholesterol is derived through the internalization and catabolism of LDL cholesterol by the cell.[7a] Since LDL is a product of VLDL catabolism,[7b] this latter lipoprotein is likewise regarded as being atherogenic. HDL cholesterol has been demonstrated to be a good predictor of coronary artery disease.[8] The inverse relationship between plasma levels of HDL and mortality from cardiovascular disease suggests that high levels of HDL may be protective against atherosclerosis,[9] and, in fact, considerable attention has been devoted to the metabolic control of HDL and factors affecting circulating HDL levels.[10] It remains to be seen whether manipulation of HDL levels will in itself alter the development of cardiovascular disease. A great deal of effort has gone into the identification of drugs that lower VLDL and LDL cholesterol and elevate HDL cholesterol, since such drugs should be antiatherosclerotic and, thus, provide a valuable therapy for reducing the risk of cardiovascular disease.[11]

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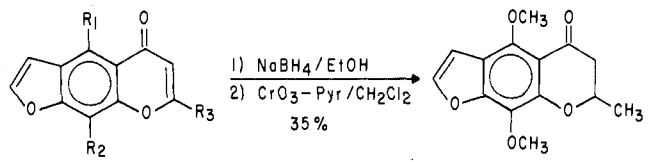
Scheme I



1 $R_1 = R_2 = OCH_3$; $R_3 = CH_3$
2 $R_1 = R_2 = H$; $R_3 = CH_3$
3 $R_1 = OCH_3$; $R_2 = H$; $R_3 = CH_3$
4 $R_1 = H$; $R_2 = OCH_3$; $R_3 = CH_3$

5

Khellin (1),[12] a naturally occurring furochromone isolated from *Ammi visnaga L.*, reduces VLDL and LDL cholesterol and elevates HDL cholesterol in the normocholesterolemic male S.E.A. Japanese quail,[13] as well as in man.[14] Furthermore, khellin significantly reduces deposition of arterial cholesterol in male, S.E.A. Japanese quail fed an atherogenic diet.[13] These results have stimulated us to discern the general topological features of furochromones that are most influential in imparting the observed lipid-altering and antiatherosclerotic activity to this class of compounds. This communication describes some of our initial results in our structure–activity relationship program with regard to the lipid-altering activity of furochromones.

Khellin possesses two noteworthy molecular features that immediately offer a logical starting point for a structure–activity relationship investigation. First, khellin is a linear furochromone bearing methoxyl substituents at carbons 4 and 9 and a methyl substituent at carbon 7. These three substituents are the only elements that disrupt the planar character of khellin. In addition, and equally interesting, is the presence in khellin of five oxygen atoms, which constitute a variety of subfunctional units (furan, benzofuran, chromone, pyrone) and functionalities (ketone, methoxyl). In recognition of these unique features, two questions arise: (1) is planarity, or near planarity, required for the lipid-altering activity of furochromones, and (2) what is the significance of the heteroatom substitution pattern and the presence of the various subfunctional units in khellin.

Chemistry.[15] Furochromone **2** (mp 183–184 °C; lit.[16] 186 °C) was prepared by a slight modification of the procedure of Davies[16] in which the γ-pyrone ring was attached to the required benzofuran by the method of Dann.[17] Visnagin (**3**), also a naturally occurring furochromone isolated from *Ammi visnaga L.*, was prepared from khellol glucoside.[18] Basic hydrolysis ($NaOH/H_2O$/2 h reflux) of the glucoside gave visnagone (mp 106–108 °C, lit.[19] 106–108 °C), which upon Claisen condensation with ethyl acetate (NaH/THF), followed by acid-catalyzed cyclodehydration of the intermediate β-diketone, yielded visnagin (mp 138–140 °C, lit.[20] 139–140 °C). Furochromone **4** (mp 197–199 °C, lit.[17] 196–197 °C) was prepared as described by Dann.[17] The 6,7-dihydrofurochromone **5** was prepared from khellin in either a one- or two-step process. Reduction of **1** with lithium aluminum hydride ($LiAlH_4$/THF/−78 °C) as described by Dann and Volz[21] or sodium borohydride reduction ($NaBH_4$/CH_3CH_2OH) of **1**, followed by oxidation (CrO_3–Pyr/CH_2Cl_2), gave the 5-ketofurochromone **5** (mp 104–106 °C; lit.[21] 105–106 °C) (Scheme I).

Table I. Serum Lipoprotein Cholesterol Alterations with Furochromones in Normocholesterolemic Male S.E.A. Japanese Quail

no.	dose, mg/(kg day)	serum lipoprotein cholesterol (T/C)[a] HDL	VLDL + LDL	total
1	50	1.46**	0.37**	1.21*
2	50	1.05	0.84	1.01
3	50	1.42**	0.51**	1.21*
4	50	1.19*	0.77	1.09
5	50	1.45**	0.55**	1.24*
8	50	1.36**	0.42**	1.15
10	50	1.17	0.74	1.07
11	50	1.40**	0.49**	1.17
12	50	1.63**	0.57**	1.35**
13	50	0.92	0.37**	0.82
14	50	1.68**	0.47**	1.36**
15	50	1.33**	0.43**	1.09

[a] * = treated mean is significantly different from control mean; $p < 0.05$. ** = treated mean is significantly different from control mean; $p < 0.001$.

The 7-demethylfurochromone **8** was prepared in two steps from khellinone **6** (Scheme II).[12] Treatment of **6** with a slight excess of *N,N*-dimethylformamide dimethyl acetal (1.1 equiv/80 °C/2.5 h) afforded the enaminone **7** in good yield.[22] Treatment of **7** with aqueous acid in tetrahydrofuran gave the desired furochromone **8** (mp 182–184 °C; lit.[23] 182 °C) in 48% yield from **6**. The 2,3-dihydrofurochromone **10** was also prepared from khellinone **6**. Hydrogenation of **6** (H_2/Pd/EtOH) gave **9** (mp 104–105 °C; lit.[24] 103–104 °C) in excellent yield. Construction of the γ-pyrone ring under standard conditions (1. NaH/EtOAc; 2. $HCl/CHCl_3/\Delta$) then afforded **10** (mp 146–148 °C; lit.[25] 150–151 °C) in 54% yield. Furochromones **11–15** were prepared from khellinone (**6**) and the appropriate ester utilizing a Claisen-like condensation–cyclodehydration (Scheme III).[26]

Pharmacology. Adult, male, S.E.A. Japanese quail at 5–7 weeks of age were randomly distributed into 20 groups of 5 birds per group. They were housed individually in 10 cage units and fed a commercial diet.[27] Drugs were dissolved or dispersed in 100 mL of 95% ethanol and mixed into the diet. Three control groups received diet mixed with ethanol alone, one group received a positive standard (50 mg of khellin per kg of body weight), and 16 groups received test compounds.

After 2 weeks on the diet, the birds were bled from the right jugular vein. Individual serum samples were analyzed for HDL and LDL cholesterol levels. The lower density lipoproteins (0.2 mL serum) were precipitated at 4 °C

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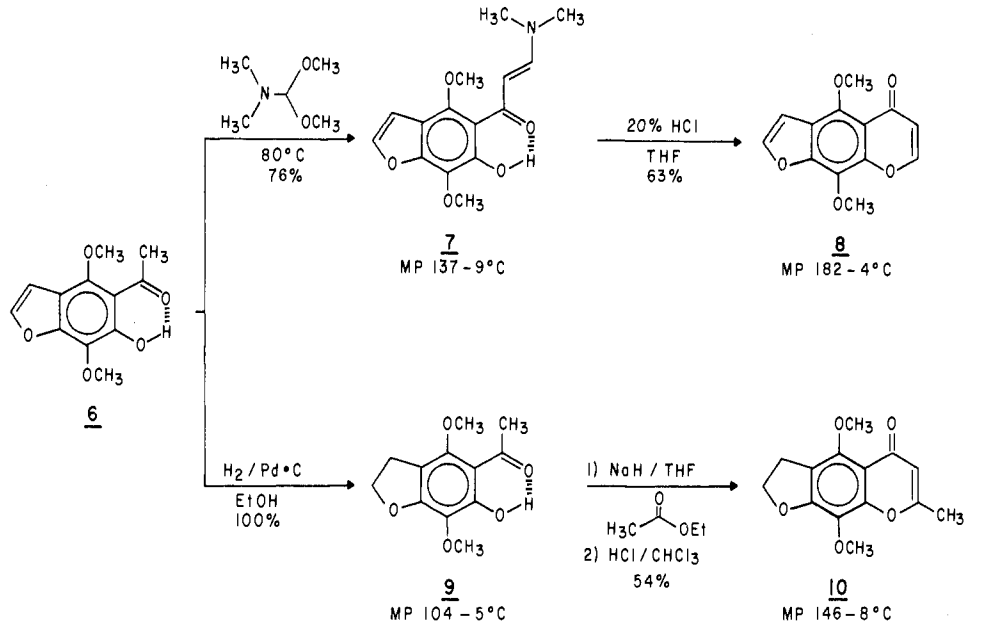
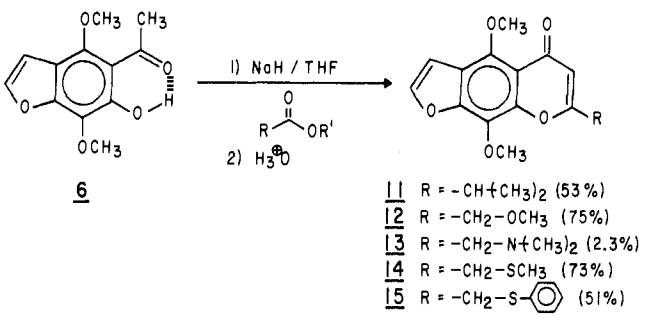
Scheme II



Scheme III



overnight with a mixture of 1.3 mL of 0.02 M Tris buffer, 0.2 mL of 0.2% heparin, and 0.2 mL of 0.4 M $CaCl_2$ at pH 7.4. After centrifugation at 4 °C, the supernate containing HDL cholesterol was poured into centrifuge tubes, 1.6 mL of absolute methanol added, the solution was mixed, let stand at 4 °C overnight, and centrifuged, and the supernate was discarded. The VLDL plus LDL precipitate was resuspended in 0.1 mL of 1 N NaOH and then extracted with 1.9 mL of 2-propanol for cholesterol determination. The HDL precipitate was extracted with 3.8 mL of 2-propanol for cholesterol determination.

Data from each study were statistically analyzed as a one-way classification[28] with values transformed to logarithms to achieve more homogeneous within-group variances. The mean response for each test compound was compared with the control mean with the LSD test.[29] Significant differences from the control mean are indicated by one asterisk for $p \leq 0.05$ and two asterisks for $p \leq 0.01$. Results are presented as treated mean/control mean ratios.

Results and Discussion

Removal of both the C-4 and C-9 methoxyl groups, compound **2** (see Table I), resulted in complete loss of lipid-altering activity. The 9-methoxyfurochromone **4** (C-4 methoxyl absent) exhibited marginal activity, while visnagin (**3**) was found to be equal in potency to khellin. The 5,6-dihydrofurochromone **5** was quite active, while the 2,3-dihydrofurochromone **10** was devoid of activity at the screening dose. Removal or replacement of the C-7 methyl group in khellin with alkyl, heteroalkyl, or heteroalkylaryl groups was not detrimental to the lipid-altering activity of the molecule.

These results indicate that within this series, the C-4 methoxyl group is required for lipid-altering activity, while the C-9 methoxyl is neither required nor significantly contributes to the observed lipidemic activity in this test system. Interestingly, the planar furan ring is critical to lipid-altering activity, while absence of planarity in the C-ring, as well as a C-7 substituent, is well tolerated. It is important to point out that the mechanism by which these furochromones affect lipid levels is unknown. Our data have provided insight into the initial questions raised concerning the influence of planarity and various subfunctional units and functionalities on activity. However, the lack of activity of the 2,3-dihydro analogue, which represents a change in both planarity and subfunctional units, raises an interesting question regarding the relationships between molecular modifications of the furochromone nucleus, metabolic biotransformations, absorption, distribution, clearance, and the observed lipid-altering activity.

Registry No. 1, 82-02-0; **2**, 7674-96-6; **3**, 82-57-5; **4**, 87249-41-0; **5**, 3380-63-0; **8**, 49572-91-0; **10**, 26239-04-3; 11, 76301-21-8; **12**, 76301-18-3; **13**, 76301-38-7; **14**, 76301-19-4; **15**, 76301-20-7.

Supplementary Material Available: Physical and analytical properties of compounds **5**, **7**, and **11-15** (3 pages). Ordering information is given on any current masthead page.

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Structure-Activity Relationships for and Potentiation of the Antimitogenic Activity of 2-5A Core Derived from 2-5A, a Mediator of Interferon Action

Sir:

One mechanism by which interferon exerts its antiviral effect is through the 2-5A system.[1] Double-stranded RNA,

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