μM, respectively. The lower activity of 3 was consistent with less efficient polyglutamylation (Table I), but obviously could reflect other factors such as transport and binding to a putative biochemical target. We are unaware of any folic acid analogues that possess this level of in vitro antitumor activity when ring A is opened.

An experiment was performed to determine whether inhibition of cell growth by 1 could be prevented with thymidine (TdR) and/or hypoxanthine (Hx). Incubation of L1210 cells for 72 h in the presence of 1 (5 μM) and TdR (5.6 μM) alone, Hx (100 μM) alone, or a combination of TdR (5.6 μM) and Hx (100 μM), afforded partial protection, suggesting that 1 possesses antifolate activity. However, the fact that *complete* protection was not observed at these normally protective concentrations of TdR and Hx indicates that growth inhibition by 1 may arise in part from interference with metabolic processes other than de novo thymidylate or purine nucleotide biosynthesis. At this time the specific enzyme or enzymes inhibited by polyglutamates of 1 are unknown.

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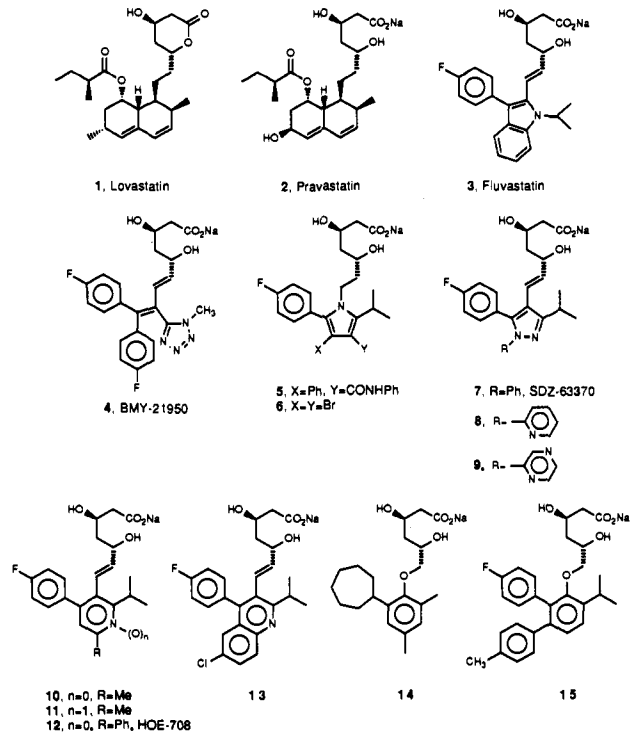
Relationship between Tissue Selectivity and Lipophilicity for Inhibitors of HMG-CoA Reductase

It is now well-established that inhibition of the enzyme HMG-CoA reductase (HMGR) is an effective means for lowering plasma total and LDL-cholesterol in hypercholesterolemic patients.[1] However, the long-term safety of these agents is still unproven. Although recent clinical experience with lovastatin (1) has indicated that it is well-tolerated in man,[2] some adverse reactions have been noted; particularly, elevated liver enzymes,[3] sleep disturbances,[4] and myositis.[5] Recently, there has been considerable controversy in the literature regarding both the nature and existence of tissue (liver) selectivity for various HMGR inhibitors, and whether confining their action to the liver would reduce the incidence of adverse reactions. The initial report[6] describing tissue selectivity for pravastatin (2) suggested that pravastatin and lovastatin were equipotent at inhibiting cholesterol biosynthesis in cultured rat hepatocytes, but pravastatin was 100 times less potent than lovastatin at inhibiting biosynthesis in human skin fibroblasts. This selectivity was further supported by ex vivo rat studies, which demonstrated that pravastatin inhibited cholesterol biosynthesis only in lipoprotein-producing organs (liver and intestine), whereas lovastatin and mevastatin (compactin) also inhibited cholesterol biosynthesis significantly in kidney, lung, spleen, prostate, and testis. The assertion that pravastatin is more tissue selective than lovastatin has been disputed, however, on the basis of measurements of peripheral drug distribution employing a bioassay[7] as well as the uptake and tissue distribution of radiolabeled drug.[8] More recently, other HMGR inhibitors have been reported to display liver selectivity.[9] It has been proposed that tissue selectivity is influenced primarily by the relative lipophilicity of the drugs, with the relatively more hydrophilic compounds showing higher liver selectivity.[10]

Since we had prepared HMGR inhibitors possessing considerable variation in structure and lipophilicity during the course of our program in this area, we decided to test this hypothesis directly. Thus, we compared a selection of potent inhibitors possessing a broad range of calculated lipophilicities (CLOGP) for their abilities to inhibit sterol synthesis in tissue cubes derived from rat liver, spleen, and testis. The results of these studies are the subject of this report.

Chart I



Chemistry

All of the inhibitors employed in this study (Chart I) have been reported previously.[11] Representative com-

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Table I

compd	CLOGP[a]	IC_{50}, nM				IC_{50} ratios	
		HMGR[b]	liver[c]	spleen[c]	testis[c]	spleen/liver	testis/liver
11[d]	0.04	9.4	18	266	718	14.8	39.9
2[e]	0.51	40.0	141	228	329	1.6	2.3
4[f]	0.52	13.0	108	1579	638	14.6	5.9
9[g]	0.73	6.6	10	20	64	2.0	6.4
8[g]	1.86	10.0	17	29	82	1.7	4.8
10[h]	2.32	7.7	71	9	17	0.13	0.24
1[i]	3.11	13.0	28	5	4	0.17	0.14
3[j]	3.24	32.0	142	292	4	2.10	0.03
14[g]	3.69	141.0	5170	108	63	0.02	0.01
7[k]	3.70	7.2	34	12	8	0.35	0.24
12[h]	3.92	2.0	8070	77	16	0.01	0.002
13[l]	3.94	480.0	6280	92	184	0.02	0.03
6[l]	4.02	26.0	2726	140	24	0.05	0.009
5[l]	4.06	7.5	39	29	36	0.74	0.9
15[g]	4.82	100.0	6037	375	511	0.06	0.08

[a] Calculated log P of dihydroxy acid (Med Chem Ver 3.54). [b] Microsomal preparation of rat liver HMG-CoA reductase. See ref 14. [c] Values represent the mean of at least two determinations performed with concentrations between 1 and 10000 nM with each concentration run in duplicate. [d] Prepared from 10 by m-CPBA oxidation. [e] Reference 6. We would like to thank Dr. William A. Scott for kindly supplying a sample of compound 2. [f] Reference 8. [g] Manuscript in preparation. [h] Beck, G.; Kesseler, K.; Baader, E.; Bartmann, W.; Bergmann, A.; Granzer, E.; Jendralla, H.; Kerekjarto, B. V.; Krause, R.; Paulus, E.; Schubert, W.; Wess, G. *J. Med. Chem.* **1990**, *33*, 52–60. [i] Extracted from commercial Mevacor. [j] International patent application PCT/WO84/02131, **1984**. [k] U.S. patent 4,613,610, **1986**. [l] Manuscript in press.

pounds were chosen from several distinct chemical series where, in addition to possessing a broad range of lipophilicities, structural features for intrinsic potency had been optimized and compounds of comparably high potency could be chosen. Only compounds **13–15** were chosen from a series with lower intrinsic potency. Relative lipophilicities were estimated for the dihydroxy acids by calculation using the CLOGP program.[12] The degree of ionization was expected to be similar for all of these compounds, since the environment around each carboxyl group is very similar and the variable portion of the molecule is well removed from this site. Thus, no correction was attempted for the ionization which is likely to exist for these acids under the conditions of the experiments. The range of calculated values was broad enough (0.04–4.82) such that it was felt that any significant trends would be detectable.

Biological Results

Since there is considerable evidence that the ring-opened dihydroxy acids of lovastatin, pravastatin, and other HMG-CoA reductase inhibitors are the major active moieties circulating in plasma,[13] all compounds were tested in this form. As a measure of intrinsic potency, each compound was first tested for its ability to inhibit microsomal HMGR in vitro.[14] Then, as a measure of hepatic versus peripheral effects, the effects of the compounds on the incorporation of [^{14}C]acetate into sterols were measured in tissue cubes derived from liver, spleen, and testis.[15] Examination of the results of these studies suggests that significant differences exist between compounds and that lipophilicity is an important factor (Table I, compounds arranged by increasing lipophilicity). Thus, compounds with CLOGP < 2 (compounds **11**, **2**, **4**, **9**, and **8**) all appear to possess a moderate degree of tissue selectivity as evidenced by tissue/liver ratios > 1. In general, compounds with CLOGP > 2 are more potent in peripheral tissue than liver. The two exceptions are compound **5**, which is equipotent in hepatic and peripheral tissues, and compound **3**, which potently inhibits sterol synthesis in testis, but not spleen.

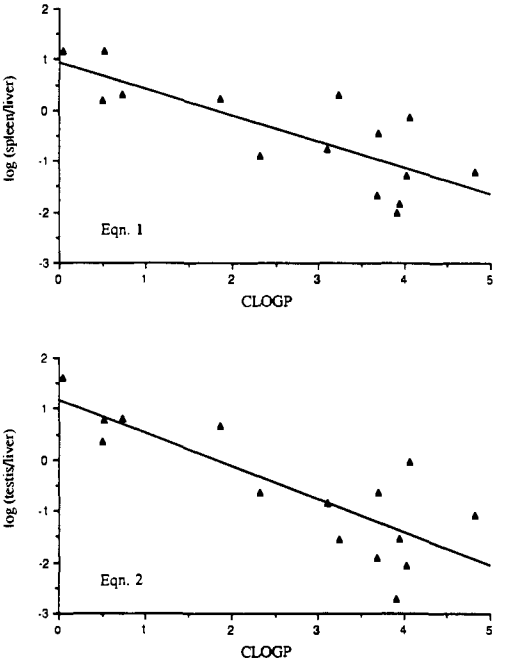

Figure 1. Selectivity vs CLOGP.

Table II. Correlation Coefficients among Biological Activities (N = 15)

	HMGR	liver	spleen	testis
HMGR	1			
liver	0.55	1		
spleen	0.36	0.39	1	
testis	0.33	0.12	0.64	1

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Table III. Coefficients and Statistics of Correlation Equations Relating Activity to Lipophilicity (N = 15)

eq	activity	a	b	c	c'	log β^a	CLOGP^b	r^2	s	F
3a	HMGR	7.79	0.26	−0.08			−	0.14	0.50	0.9
3b	HMGR	7.82	0.13		−0.52	2.41	−	0.13	0.62	0.9
4a	liver	7.27	0.49	−0.20			1.22	0.49	0.83	5.9^d
4b	liver	7.33	0.20		−1.36	2.44	1.66	0.51	0.82	6.2^e
5a	spleen	6.21	1.21^d	−0.24^d			2.48	0.40	0.58	4.0^c
5b	spleen	6.28	0.89^e		−1.61^d	2.30	2.39	0.41	0.57	4.2^c
6a	testis	5.79	1.66^f	−0.31^f			2.65	0.69	0.46	13.5^f
6b	testis	6.09	0.80^f		−2.49^f	3.34	3.02	0.76	0.40	19.1^f

^{a} Equation a = Hansch equation: log (1/act.) = a + b(CLOGP) + c(CLOGP)^2. Equation b = Kubinyi equation: log (1/act) = a + b(CLOGP) + c log (βP + 1). ^{b} Optimal CLOGP. $^c p < 0.05$. $^d p < 0.02$. $^e p < 0.01$. $^f p < 0.001$.

When tissue/liver ratios are plotted against CLOGP (Figure 1),[16] the linear relationships described by eq 1 and 2 are observed. These relationships suggest that selectivity

$$\log (\text{spleen/liver}) = 0.93 - 0.52\text{CLOGP} \quad (1)$$

$$n = 14,\ R^2 = 0.62,\ s = 0.65,\ F = 21.6\ (p < 0.001)$$

$$\log (\text{testis/liver}) = 1.17 - 0.65\text{CLOGP} \quad (2)$$

$$n = 15,\ R^2 = 0.67,\ s = 0.75,\ F = 26.2\ (p < 0.001)$$

is, in fact, directly dependent on lipophilicity. As noted above, the "crossover" point where selectivity is equal between liver and the other tissues is at CLOGP ≅ 2. Below this, compounds are selective for liver; above this, they are selective for the peripheral tissue.

In order to better understand the origin of the observed selectivity, activity was plotted against CLOGP in each tissue examined (Figure 2). Table II shows the correlation matrix of activities. It can be seen that the in vitro potencies are most closely correlated to the activity in liver. Spleen and testis activities have considerable commonality with each other, but not with liver activity. Correlations using either the Hansch parabolic equation[17] or the Kubinyi bilinear model[18] gave similar results (Table III). Thus, the correlations, while not significant for the HMGR activity, do reach statistical significance (95% or better) for each of the tissue activities. For spleen and testis, a CLOGP optimum around 2.5–3.0 is observed with either model. The difference between these tissues and liver is seen most clearly by comparing the b coefficients of the bilinear equations shown in Table III. These estimate the slope of the ascending portion of the curves. For liver, b is not significantly different from 0, indicating an essentially flat relation, while for the two peripheral tissues, significant positive slopes approaching 1 are obtained. All tissues show similar negative slopes at high CLOGP. Thus, the origin of liver selectivity at low CLOGP is found to lie in the relative insensitivity of liver activity to CLOGP as the latter decreases, which is in marked contrast to the decrease in activity observed at low CLOGP in the two peripheral tissues studied. These correlations are not precise, and factors related to other differences between the chemical series are undoubtedly involved, but the general conclusion that liver is remarkably less sensitive to lipophilicity changes than spleen and testis is statistically substantiated. These observations, combined with the lack of correlation of CLOGP with intrinsic potency as measured by HMGR inhibition, point to differential transport properties of liver vs peripheral cells as the source of tissue selectivity.

Discussion

The results of this study support the hypothesis that tissue selectivity is determined primarily by lipophilicity

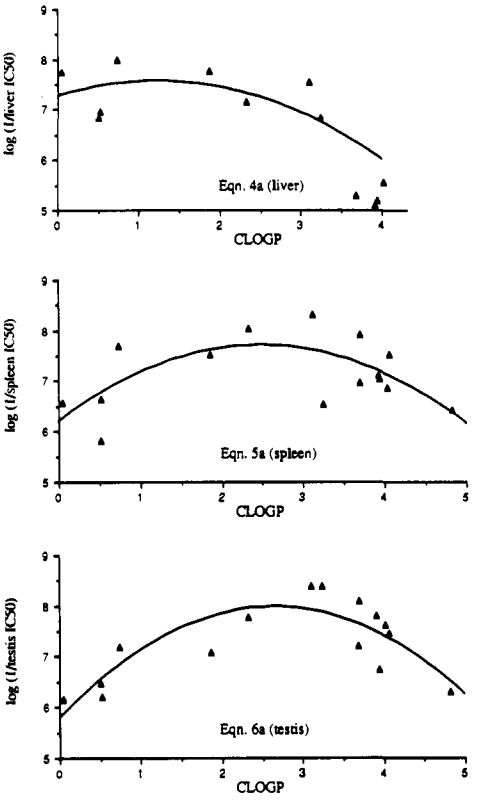

Figure 2. Tissue activity vs CLOGP.

and that an optimal CLOGP range (CLOGP 2–4) exists for inhibition of cholesterol biosynthesis in peripheral tissues, whereas liver does not discriminate compounds on the basis of lipophilicity below this optimum. The dependence of potency on CLOGP in spleen and testis is not unlike the parabolic relationship reported between partition coefficients of steroids and their permeability across excised rabbit cornea.[19] Since the compounds were originally chosen to minimize intrinsic potency differences between the different chemical series represented, the data from the present study suggest that differences in cell membranes between liver and peripheral tissues lead to differential drug penetration, with the liver being much more permeable to compounds with low CLOGPs. This is not meant to imply that potency is not important, but that for a series of diverse but highly potent inhibitors, other factors, e.g., transport properties, are responsible for imparting tissue selectivity. It is noteworthy that the ring-opened dihydroxy acid form of lovastatin, which is close to the optimal CLOGP for penetration into peripheral tissues, is 10 times more potent at inhibiting cholesterol biosynthesis in spleen and testis than in liver. This may explain, in part, the peripheral side effects found with this drug when high plasma drug concentrations occur in

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man.[20] As previously reported,[6,8,14b] we also found that the more hydrophilic inhibitors pravastatin (2) and BMY-21950 (4) were more tissue selective than lovastatin in vitro. It might also be concluded from this data that tissue selectivity is not related to a particular structural feature or series of inhibitors, since 2, 4, 8, 9, and 11, which are from four very different chemical series of inhibitors, possess comparable degrees of selectivity. Also of note is the fact that conversion of pyridine 10 to *N*-oxide 11 results in 100- and 35-fold decreases in potency in spleen and testis, respectively, while increasing potency in liver 3-fold. Similar, though less dramatic changes are seen in the pyrazole series (compounds 7–9) on replacement of the *N*-phenyl ring by 2-pyridyl or 2-pyrazinyl.

Of the compounds possessing CLOGP > 2, compound 5 is unusual, in that unlike the other inhibitors, which are more potent in peripheral tissues than in liver, it is equipotent in all tissues. This result suggests that the tissue selectivity reported previously for this compound in cell culture[21] and in vivo[22] is not due to differential tissue potencies, but to some other factor, such as first-pass metabolism.

In summary, with isolated tissue cubes from rat liver, spleen, and testis, it has been shown that the tissue selectivity of a diverse group of potent inhibitors of HMG-CoA reductase was not related to a particular structural feature but was highly dependent on the ability of peripheral tissues to discriminate between compounds on the basis of lipophilicity. This conclusion was supported by a quantitative structure–activity relationship analysis, which not only demonstrated that liver potency was insensitive to changes in lipophilicity at low CLOGP and that a parabolic dependence of potency on lipophilicity (CLOGP) existed in the two peripheral tissues examined but also revealed a linear relationship between lipophilicity and tissue selectivity (tissue IC_{50}/liver IC_{50}). Although the relevance of these observations to the clinical situation is uncertain, these studies suggest that liver selectivity is based on differential membrane sensitivity to lipophilicity, with low CLOGP compounds showing significant selectivity for liver over peripheral tissues. Studies relating the lipophilicity of HMGR inhibitors to tissue selectivity ex vivo and in vivo will be the subject of future reports from these laboratories.

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8-(Dicyclopropylmethyl)-1,3-dipropylxanthine: A Potent and Selective Adenosine A_1 Antagonist with Renal Protective and Diuretic Activities

Adenosine elicits a wide variety of physiological responses[1] via interactions with two major subtypes of extracellular receptors, designated as A_1 and A_2. Considerable efforts to search for selective antagonists have been invested in order to elucidate the physiological role of adenosine.[2] Theophylline (1; Figure 1) and caffeine (2) exert pharmacological effects primarily through blockade of adenosine receptors. However, they are virtually nonselective antagonists and have weak affinity for A_1 and A_2 receptors. Studies of structure–activity relationships of xanthines[3–8] revealed that alkyl substitution at the 1- and 3-positions markedly increased affinity at both A_1 and A_2 receptors. On the other hand, 8-aryl or 8-cycloalkyl substitution resulted in selective and potent A_1 antagonists. Further studies suggest that the sp^3 carbons containing cycloalkyl ring has more favorable interactions with a hydrophobic pocket of the A_1 receptor than the sp^2 carbons in an aryl ring.[5,9] Thus 8-cyclopentyl-1,3-dipropylxanthine (4)[5,10] has been known as the most potent A_1 antagonist.

With the aim of characterizing the hydrophobic interactions between the 8-substituent in xanthine and the A_1-receptor site, we designed xanthines with several substituted methyl group (6) on the basis of compound (4) and A_1-selective antagonist 8-(2-methylcyclopropyl)-1,3-dipropylxanthine (5).[11] The present study describes a new xanthine derivative that is a selective and potent A_1 antagonist and exhibits interesting pharmacological activities.

Synthetic methods are outlined in Scheme I. Acylation of the appropriate 5,6-diaminouracil (7)[12] with a carboxylic acid or its acid chloride, followed by treatment with aqueous sodium hydroxide or phosphorus oxychloride under reflux, gave the corresponding xanthines (6).[13]

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