Communications to the Editor

Selective Inhibition of Mammalian Lanosterol 14α-Demethylase: A Possible Strategy for Cholesterol Lowering[1,†]

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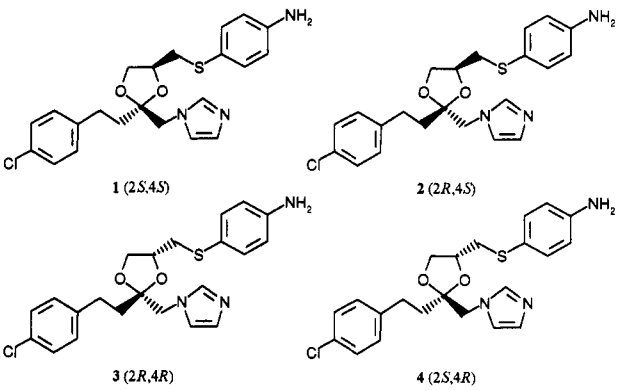
Reduction of serum cholesterol in man, particularly that carried in low-density lipoproteins, has been shown to reduce the incidence of coronary heart disease.[2] Lanosterol 14α-demethylase (P-450$_{14DM}$) catalyses the first step in the conversion of lanosterol to cholesterol in mammals[3] and, as such, provides a possible intervention point for inhibiting cholesterol biosynthesis.

Although there is relatively little work on the inhibition of mammalian lanosterol demethylase (14-DM),[4] the related enzyme in fungi has been extensively studied following the discovery that the antifungal activity of 1-substituted azoles[5] is a consequence of blocking this enzyme.[6] Inhibition of this cytochrome P-450 results from coordination of the azole nitrogen to the heme iron, with the lipophilic ligand attached to the azole occupying the binding site for lanosterol. These type II inhibitors thus prevent both binding of the substrate and oxygen activation. Although fungi synthesize ergosterol as their primary sterol, the 14-demethylation of lanosterol is common to both pathways of cholesterol and ergosterol synthesis. Indeed, the orally active antifungal agent ketoconazole has been shown to inhibit rat 14-DM[7] and to lower serum cholesterol in man.[8,9] However, ketoconazole is relatively nonselective; it inhibits cytochromes P-450 involved in steroid biosynthesis, cholesterol metabolism, and xenobiotic metabolism and lowers testosterone and corticosteroid levels in man.[10] Further modifications of ketoconazole have led to structures exquisitely selective for *fungal* lanosterol demethylase relative to mammalian P-450 enzymes, including 14-DM.[6] Taking work on antifungals as a starting point, while cognizant that different structure–activity relationships would be encountered, we undertook a search for azole inhibitors of *mammalian* 14-DM having minimal effects on other important P-450 enzymes. We report here the preparation and biological activity of the first non-steroidal *selective inhibitor of mammalian lanosterol 14α-demethylase*, compound 1 (RS-21607), as a potential strategy for cholesterol lowering in man.

The preparation of 1 (Scheme I) commenced with Swern oxidation[11] of alcohol **5**[12] to give ketone **6**. Transketalization of **6** with (*S*)-solketal tosylate (from (*R*)-solketal) required catalysis by *n*-BuOH (*p*-TsOH) for a satisfactory rate, giving the separable *cis*-(2*S*,4*S*) and *trans*-(2*R*,4*S*) ketal tosylates **7** and **8**. Further reaction with 4-aminobenzenethiol (acetone, K_2CO_3, reflux) gave the *cis*-(2*S*,4*S*) and *trans*-(2*R*,4*S*) thioethers **1** and **2**. Use of (*R*)-solketal tosylate gave the corresponding *cis*-(2*R*,4*R*) and *trans*-(2*S*,4*R*) enantiomers **3** and **4**. The optical purities were determined to be >99.8% by HPLC on an Ultron ES-OVM column.



Compound **1** competitively inhibited lanosterol 14α-demethylation in hepatic microsomes[13] from rat, hamster, and human with apparent K_i values of 2.5 ± 1.5, 1.4, and 0.79 ± 0.35 nM, respectively. Comparative values for the enantiomer **3** and ketoconazole were as follows: (**3**) 37 ± 11, 40.4, and 17.7 nM, and (ketoconazole) 65, 24.5, and 63.5 nM, respectively. Apparent K_i values for the *trans* isomers **2** and **4** against rat lanosterol demethylase were 117 and 11 nM, respectively. As is found for related antifungal agents, the 2*S*-*cis* stereoisomer is most potent. Selectivity relative to other cytochrome P-450 enzymes involved in steroid biosynthesis and steroid and drug metabolism was determined using progesterone 17α,20-lyase, cholesterol 7α-hydroxylase, aromatase, corticoid 11β-hydroxylase, and progesterone hepatic 6β-hydroxylase as described previously,[13] giving apparent K_i values of 447, 1625 ± 784, 7.6 ± 1.3, 35, and 28 nM, respectively, for **1**, and 54, 109 ± 47, 13, 15, and 33 nM, respectively, for the enantiomer **3**. The IC_{50} values for cholesterol 20,22-lyase were 18 400 and 15 200 nM, respectively. Thus the 2*S*,4*S* isomer **1** shows good selectivity for the demethylase relative to other P-450 enzymes, and a favorable separation of activity from its enantiomer **3**. Most remarkable is the separation of activity against cholesterol 7α-hydroxylase, the rate-limiting enzyme in the catabolism of cholesterol, where most of the activity of the racemate is found in the 2*R*,4*R* isomer. Inhibition of this enzyme would presumably tend to increase circulating cholesterol levels. Compounds were evaluated for inhibition of cellular cholesterol biosynthesis from [2-^{14}C]acetate using human fibroblasts in tissue culture. Compound **1** was approximately 2 orders of magnitude more effective than **3** and 400 times more

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Scheme I

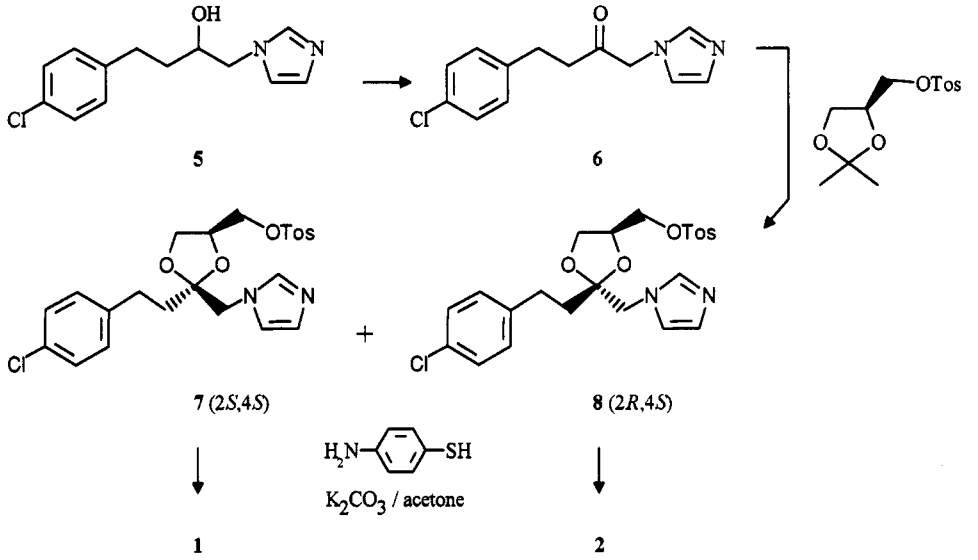

Table I. Effects on Total Serum Cholesterol (TC) in Hamsters[a]

compd	dose (mg/kg)	TC (mg/dL)[b]	% lowering
1	100[c]	41.8 ± 3.3*	62
	30[c]	71.0 ± 2.6*	36
	10[c]	85.1 ± 5.2*	23
3	100[c]	86.0 ± 5.0*	22
	30[c]	96.4 ± 2.7*	13
	10[c]	105.7 ± 2.7*	5
vehicle		110.9 ± 2.6	
2	60[d]	120.4 ± 10.8*	15
4	60[d]	85.1 ± 3.4*	40
vehicle		141.7 ± 3.3	

[a] Test compounds were administered orally for 3 days, and blood was drawn 18 h following the final dose. [b] Mean ± SE of eight animals. [c] Dosed once daily (4 pm). [d] Dosed b.i.d. *Significant difference from vehicle (propylene glycol) controls ($p < 0.1$).

effective than ketoconazole in inhibiting synthesis in these cells (IC_{50} values:[14] 0.09, 8, and 37 nM, respectively). Lanosterol (and/or dihydrolanosterol) was the principal radiolabeled intermediate formed. Compounds 1–4 were further evaluated orally in male Syrian hamsters for effects on serum cholesterol. In keeping with the *in vitro* results, 1 was more effective than its stereoisomers in reducing serum cholesterol *in vivo* (Table I). In further studies (Tables II and III), 1 and its dihydrochloride salt (1·2HCl) were superior to lovastatin and ketoconazole after once daily administration for 14 days. Analysis of the lipoprotein profiles at doses causing similar reductions in cholesterol levels showed a statistically significant increase in the HDL/total cholesterol ratio for 1·2HCl (but not lovastatin), with most of the decrease in cholesterol occurring in the LDL fraction. [1·2HCl (50 mg/kg): TC (30%↓*), HDL 50.0 ± 2.5 mg/dL (17%↓*), LDL 24.0 ± 2.1 mg/dL (52%↓*), HDL/TC 56.7 ± 1.5% (19%↑*), Apo A-1 124.1 ± 6.3 mg/dL, Apo B 19.5 ± 1.4* mg/dL. Lovastatin (100 mg/kg): TC (26%↓*), HDL 48.9 ± 5.1 mg/dL (19%↓*), LDL 34.2 ± 3.5 mg/dL (32%↓*), HDL/TC 51.0 ± 1.4% (7%↑), Apo A-1 99.0 ± 6.8* mg/dL, Apo B 21.2 ± 1.3* mg/dL. Vehicle control: HDL 60.4 ± 2.1 mg/dL, LDL 50.6 ± 3.6 mg/dL, HDL/TC 47.5 ± 0.9%, Apo A-1 129.3 ± 3.7 mg/dL, Apo B 30.7 ± 0.6 mg/dL. (Significant difference from control: (*) $p < 0.05$ using Dunnett's t test.)] The effect on triglycerides was variable.

In a separate experiment, male hamsters were treated with 1·2HCl (50 mg/kg, po, q.d.) for 14 days then fasted before a final dose 17 h later. After 1 h, [2-^{14}C]mevalonate was administered ip, and blood and tissues were collected 1.5 h later. The synthesis of labeled cholesterol was inhibited by 98% ($p < 0.05$) in the liver, whereas isotopically-labeled methylsterol levels were increased 5.1-fold ($p < 0.05$) in the liver, but only 2.3-fold in the serum, relative to controls. Total serum cholesterol was reduced by 48% ($p < 0.05$). GC and GC/MS analysis of the total (labeled and unlabeled) methylsterol fractions showed that over the course of the experiment, dihydrolanosterol increased approximately 100-fold in the liver and 30-fold

Table II. Effects of 14-Day Dosing on Total Serum Cholesterol (TC) in Hamsters[a]

compd	dose (mg/kg)	TC (mg/dL)[b]	% lowering
1	50	74.7 ± 4.3*	37
	25	82.9 ± 4.4*	30
	10	104.1 ± 4.6*	12
	5	115.5 ± 5.1	2
2	100	113.3 ± 5.7	4
	50	112.4 ± 4.6	5
	25	125.8 ± 5.3	6↑[d]
	10	125.2 ± 2.3	6↑[d]
lovastatin	50	99.4 ± 1.9*	16
	25	111.2 ± 3.7	6
	10	102.8 ± 4.2*	13
	5	112.2 ± 3.2	5
ketoconazole	200	83.1 ± 6.3*	30
	100	105.0 ± 5.2*	11
	50	111.4 ± 2.2	6
	25	125.2 ± 2.0	6↑[d]
control[c]		118.3 ± 3.5	

[a] Test compounds were administered orally once a day (4 pm) for 14 days and blood was drawn 18 h following the final dose. [b] Mean ± SE of 7–8 animals. [c] $n = 16$. *Significant difference from vehicle (propylene glycol) controls ($p < 0.1$). [d] Increase.

Table III. Effects of 14-Day Dosing on Total Serum Cholesterol (TC) in Hamsters[a]

compd	dose (mg/kg)	TC (mg/dL)[b]	% lowering
1·2HCl	100	65.6 ± 5.6*	49
	75	76.0 ± 4.0*	40
	50	88.9 ± 5.7*	30
	25	117.4 ± 6.9	8
lovastatin	200	79.5 ± 4.9*	38
	150	80.3 ± 8.2*	37
	100	94.9 ± 7.8*	26
	50	102.8 ± 6.1*	19
	25	114.2 ± 3.8	10
vehicle[c]		127.4 ± 4.8	

[a,b] See Table II. [c] $n = 8$.

in serum, while lanosterol levels were increased in liver but not serum, relative to controls. Although methylsterols are significantly elevated over naturally occurring levels, the actual amounts in serum (0.67 mg/dL) are minimal relative to serum cholesterol levels (53.2 mg/dL for the treated group). 14-Methylsterols are known to be preferentially excreted in bile, and levels in excess of these have been observed in man without apparent adverse effects.[9] These data confirm that inhibition of lanosterol demethylase is the primary mechanism of cholesterol lowering in hamsters and, further, show that the 24,25-reductase pathway in lanosterol metabolism is not inhibited. Inhibition of cholesterol synthesis at a late stage with minimal build up of precursors offers a potential advantage over HMGCoA-reductase inhibitors of preserving other products derived from mevalonate (e.g., dolichol, ubiquinone). On the basis of the potency, selectivity, and favorable effect on lipoproteins, compound 1·2HCl (RS-21607-197) is currently undergoing clinical development as a hypocholesterolemic agent.

Supplementary Material Available: Experimental procedures, including analytical and spectral data, for the preparation of 1–4 and 6–10 (4 pages). Ordering information is given on any current masthead page.

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(14) Determined graphically from six concentrations run in triplicate.