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(-)-SCH 57939: SYNTHESIS AND PHARMACOLOGICAL PROPERTIES OF A POTENT, METABOLICALLY STABLE CHOLESTEROL ABSORPTION INHIBITOR

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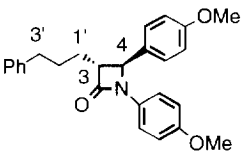
Abstract: Previous SAR studies of C-3 side chain modified analogs of (-)-SCH 48461,[1,3,4] as well as information concerning the metabolic stability this series, enabled us to design a cholesterol absorption inhibitor (i.e., (-) **2a**, SCH 57939) with tenfold higher potency and greatly enhanced metabolic stability. The synthesis and pharmacological profile, including the role of relative stereochemistry at both the C3 and 1' positions in determining the SAR of these compounds, will be discussed.

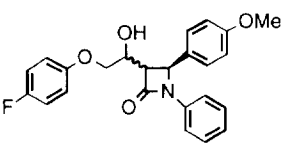
(-)-SCH 48461[1-5] is a potent inhibitor of cholesterol absorption that acts primarily in the intestinal wall and is transported in the enterohepatic circulatory system (ECS), accounting for its exposure to liver metabolism.[2] Increasing potency and metabolic stability were the basic criteria for future generations of these drugs. Modification of the C-3 side-chain of (-)-SCH 48461 has resulted in dramatic changes in the pharmacological profile, particularly in analogs that have been modified separately at the C-1' and C-3' positions.[3,4]

In this paper, we probe the effects of combining changes at the C-1' and C-3' position of the side chain in both the *cis* and *trans* azetidinone series.[3,4,6] In addition, we report that these changes, in combination with appropriate substitution on the aryl rings, result in inhibitors which are both more potent and metabolically stable than the parents. The chosen targets (Figure 1) are compounds **1**: in which the C-3 side-chain is protected

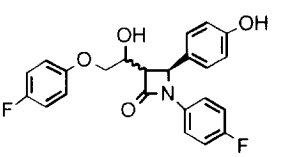
Figure 1 Targets which combine structural modifications at the 1' and 3' positions.



(-)-SCH 48461



1a= (1'S)-3,4-*trans*
c= (1'R)-3,4-*trans*

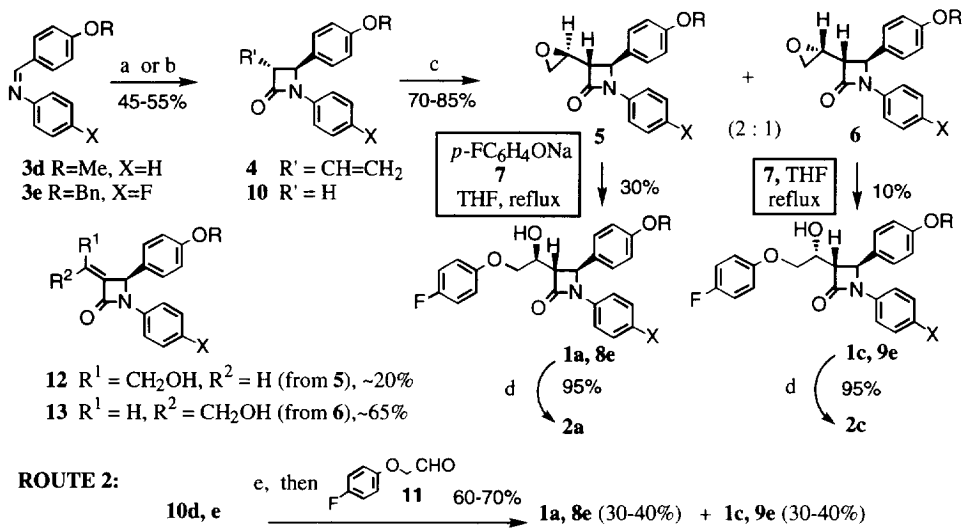


2a= (1'S)-3,4-*trans* ((-)-SCH 57939)
b= (1'S)-3,4-*cis*
c= (1'R)-3,4-*trans*

against metabolism, and **2**: in which the N-1 and C-4 aryl groups respectively are, a) protected against N-aryl hydroxylation by the incorporation of an electron withdrawing fluorine, and, b) "pre-metabolized" by replacing the metabolically labile[3] C-4 ArOMe with the corresponding phenol.

Chemistry: Scheme 1 shows the general routes to the *trans* azetidinone series. Route 1 had the advantage of producing easily separable epoxide intermediates **5** and **6** which could then be reacted with a variety of phenoxides such as **7** or other nucleophiles to form **8** or **9** of known relative stereochemistry. However, lower yields were obtained due to the formation of rearranged by products **12** and **13**. We developed Route 2 to circumvent this problem[7]. The enolate of C-3 unsubstituted azetidinone **10d**, when reacted with aldehyde **11**, produced an 88% yield of a mixture of **1a** and **1c** plus ~10% of the corresponding *cis* azetidinones all of which were separable by HPLC.[8] Route 2 had the added advantage of being adaptable into an asymmetric synthesis[7].

Scheme 1. Synthesis of 3,4-*trans*-2-Azetidinone Targets

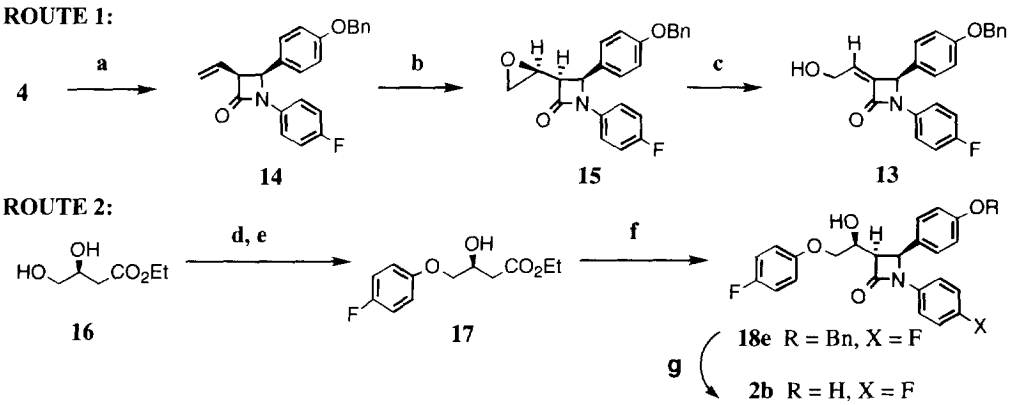


Reagents and conditions: a. (for **4**): MeCH=CHCOCl, Bu_3N, toluene, reflux. b. (for **10**): $BrZnCH_2CO_2Et$, THF. c. mCPBA, CH_2Cl_2, RT. d.(for **8e** and **9e**) H_2, 10% Pd/C, THF. e. LDA, THF, -60° C.

Both a racemic and stereoselective synthesis of the *cis*-azetidinones is shown in (Scheme 2). Although *trans*-**4** could be isomerized to *cis*-**14** by kinetic protonation of the azetidinone enolate, the derived epoxide **15** gave only the rearranged product **13** on reaction with phenoxides. Ultimately, a *cis* isomer was obtained in a highly stereoselective manner (**18e** then **2b**, Route 2) from ethyl-(3S)-3,4-dihydroxybutanoate (**16**)[9] using a diethyl- zinc modified ester enolate-imine condensation.[10]

Biological Results: The cholesterol-lowering activities of these compounds in the cholesterol-fed hamster model[1, 11] are shown in Table 1. Simple incorporation of an (1'S)-hydroxyl in the *p*-fluorophenoxy series (i.e. **(±)-1a** (Table 1) resulted in comparable *in vivo* activity to that of the parent.[3] The minus enantiomer (i.e., **(-)-1a,**) proved to have a ED_{50} value lower by a factor of ten when compared to the parent deshydroxy analog[3] (ED_{50} = 2.2 mpk) in the identical assay. This data encouraged us to prepare the *cis* (1'S)-hydroxy analog (**2b**, Scheme 2),[12] a change in geometry which, in the parent carba series,[4] resulted in increased potency. This same modification when applied to the *p*-fluorophenoxy series was detrimental to potency .[12]

Scheme 2. Synthesis of 3,4-*cis* -Azetidinone Targets



Reagents and Conditions: a. LDA, THF, -70°C; then BHT. b. mCPBA, CH_2Cl_2. c. **7** (Scheme 1), THF. d. TsCl, py., e. *p*-FC_6H_4OMgBr, THF. f. Et_2Zn, THF; then $LiN(TMS)_2$ folowed by imine **3** (Scheme 1, see ref. 10). g. H_2, 10% Pd/C, THF.

Table 1 Cholesterol lowering activity[11] of 1' hydroxy-2'-aryloxy analogs of (-)- SCH 48461.

No.	1'(R or S)* 3,4(C or T)	% L/CE	ED50 (MPK)	No.	1'(R or S)* 3,4(C or T)	% L/CE	ED50 (MPK)
(±)-1a	S, T	-57 @ 1 mpk		**(-)-2a**	S, T	-70 @ 0.3 mpk	0.27
(-)-1a	S, T	-84 @ 1 mpk	0.3	**(±)-2b**	S, C	-0 @ 1 mpk	
(±)-1c	R, T	-48 @ 10 mpk		**(-)-2b**	S, C	-0 @ 1 mpk	
(±)-2a	S, T	-38 @ 1 mpk	0.7	**(±)-2c**	R, T	-39 @ 10 mpk	

*R and S designate relative stereochemistry; C= *cis*, T= *trans* and designate 3,4-azetidinone geometry.

Blocking the remaining major metabolic sites in the *trans*-2-azetidinone series by replacing the C-4 arylmethylether with the corresponding phenol and introducing an N-p-fluorophenyl as in **(-)-2a** (i.e., SCH 57939, Table 1) maintained potency and, we could now study its pharmacological profile in greater detail.

At a dose of 512 mpk[13] for eight consecutive days, (-)-SCH 57939 showed negligible hepatic enzyme induction in rats.[14] Blood levels in rats were also negligible. In one 3 week Rhesus monkey study (n = 5-6 animals/group), no significant increase in plasma cholesterol was seen at a dietary dose of 0.1 mg/day of (-)-SCH 57939 coadministered with 375 mg cholesterol[2] while the total plasma cholesterol of the untreated cholesterol-fed controls roughly doubled from a baseline of 154 mg/dl ± 11 to 306 mg/dl ± 15. This was typical of the rhesus monkey control results at 3 weeks on the high fat/cholesterol diet.

In summary, using SAR data[3,4,6] as well as data obtained from detailed pharmacological studies on (-)-SCH 48461,[2] we were able to design and synthesize a highly potent cholesterol absorption inhibitor which met our criteria of enhanced potency and metabolic stability .

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References and Notes

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7. For additional details of the asymmetric synthesis see: Shankar, B. B.; Kirkup, M. P.; McCombie, S. W.; Clader, J. W. and Ganguly, A. K., *Tetrahedron Lett.*, in press.
8. The enantiomers were separated (> 99% ee) on a Chiracel AS HPLC column (80% hexane/20% IPA). Absolute stereochemistry of **(-)-2a** ($[a]^{25}_D$ -39.0°, EtOH; mp = 140-141°C), determined by single crystal x-ray analysis of 1'-(4-bromobenzoate) of **8d** (Scheme 1). X-Ray coordinates submitted as supplementary material and deposited in the Cambridge data bank (Optical rotation of **(-)-1a**: $[a]^{25}_D$ -41.4°, mp = 171-172°C).
9. Saito, S.; Ishikawa, T.; Kuroda, A.; Koga, K., and Moriwake, T., *Tetrahedron*, **1992**, *48*, 4067.
10. For a general procedure for reacting ethylzincoxylithium enolates with imines see Oguni, N.; and Ohkawa, Y., *J. Chem. Soc., Chem. Commun.*, **1988**, 1376.
11. Measured as percent reduction in liver cholesterol ester levels (L/CE) verses controls in the 7-day cholesterol-fed hamster model. Drug was administered by oral gavage as a corn oil suspension (See ref 2 for details). All compounds with indicated percent reductions were statistically different from the cholesterol-fed control group. The liver cholesterylester level for the 0.5% cholesterol fed controls was 22.14 mg ± 1.53 mg CE/g wet liver. The typical chow fed control is 1.0 mg CE/g wet liver. The compounds were evaluated in a series of separate seven day cholesterol-fed hamster studies, hence, direct comparisons among compounds were not performed.
12. a. Absolute stereochemistry of **(-) 2b** dictated by chiral starting material and confirmed by comparison of the optical rotation ($[\alpha]^{25}_D$ -83°, EtOH; mp = 136-138°C) with the corresponding alcohol in the carba series.[4] A minus rotation is diagnostic of 4S absolute stereochemistry. 3,4-*Cis* relative stereochemistry was determined by ^{1}H NMR ($CDCl_3$) coupling constants for C4-H (δ 5.8, J=6 Hz) and inferred by literature precedent (see ref. 9).
b. The *cis*-(1'R) alcohol was not prepared in the *p*-fluorophenoxy series as SAR data for the corresponding alcohols in the carba series indicated that 1'R stereochemistry results in reduced activity .
13. A dose equimolar to 500 mpk of (-) SCH 48461 suspended in corn oil, was administered orally to female rats (n = 4) over 8 days. Plasma and enzyme induction analyses were conducted 2 h following the last dose. Blood samples were also collected at 2, 6 and 24 h to assess compound absorption/exposure. Plasma was analyzed using a validated HPLC assay, but drug levels were below the lower limit of quantitation (30-ng/ml).
14. A small but statistically significant increase in microsomal protein content per gram (17.8%, $p < 0.01$) and per total liver (20.8%, $p < 0.05$) relative to control animals was observed. All other enzyme induction parameters (i.e., cytochrome P-450 content, benzphetamine N-demethylase, 7-pentoxyresorufin O-dealkylase and 7-ethoxyresorufinase O- deethylase activity) were unaltered.

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