Communications to the Editor

Substituted 2-Azaspiro[5.3]nonan-1-ones as Potent Cholesterol Absorption Inhibitors: Defining a Binding Conformation for SCH 48461

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Discussion. During the course of investigations into the design of novel hypolipidemic agents, SCH 48461 was identified as a very potent cholesterol absorption inhibitor.[1] Although the mechanism of action of these compounds is not yet known, there is good evidence to suggest that these compounds affect the function of a specific protein in the cholesterol absorption pathway.[2]

Initial structure–activity relationship (SAR) investigation indicated that both the *cis*- and the *trans*-diastereomers SCH 48461 and SCH 48678 were potent cholesterol absorption inhibitors in our *in vivo* assay, with reductions of liver cholesterol ester (L/CE) levels of 66% and 69%, over control, respectively.[1] The corresponding enantiomers of SCH 48461 and SCH 48678, however, were inactive at a comparable dose. This clearly demonstrated that chirality at the C-4 carbon of the β-lactam ring was crucial but the chirality at the C-3 carbon was not. Also the aryl moiety in the C-3 side chain and the length of the side chain were critical for activity.[3] This suggested that even though the chirality at C-3 was not important, the nature of the substituent was. If SCH 48461 and SCH 48678 were binding to the same site on a protein, then they should have a common orientation for the side chain such as the "*cis*" orientation shown in Figure 2.

If our hypothesis that the "*cis*" orientation of the side chain in the acyclic β-lactams SCH 48461 and SCH 48678 is the bioactive conformation is valid, then the acyclic β-lactams where such an orientation is precluded should be inactive. For C-3-disubstituted compounds **C**, Figure 3, the *gauche*-pentane interaction should prevent the phenylpropyl side chain from attaining the "*cis*" conformation and hence should be inactive. Disubstituted compounds **2** and **3** were indeed found to be inactive. This was evidence in support of our hypothesis that a "*cis*" orientation of the C-3 phenylpropyl chain was probably the bioactive conformation for SCH 48461 and SCH 48678. Furthermore, conformational analyses of the C-3 side chains of C-3 mono- and disubstituted β-lactams **D** and **E**, Figure 4, confirmed the absence of most of the "*cis*" conformations in the disubstituted structure **E** compared to the monosubstituted structure **D**.

This study presents the results of our efforts to determine this common orientation of the side chain in SCH 48461 and SCH 48678 by designing conformationally restricted analogs. If these analogs are active, then they would not only define a binding orientation of the

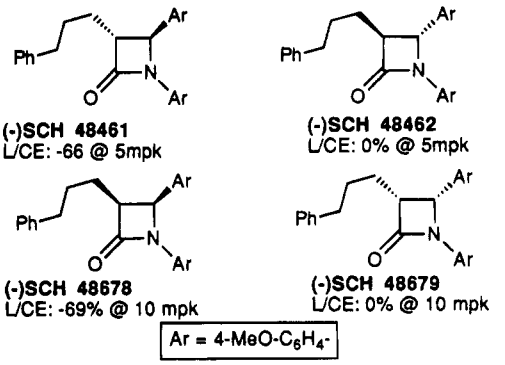

Figure 1.

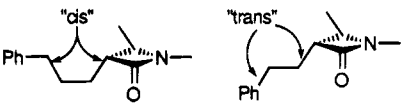

Figure 2.

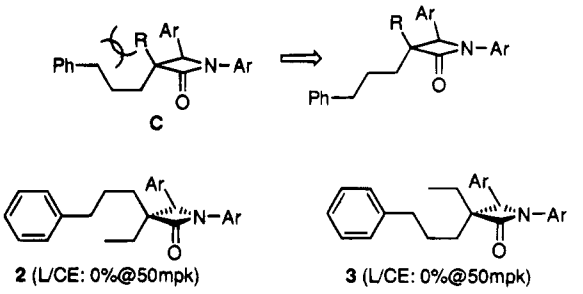

Figure 3.

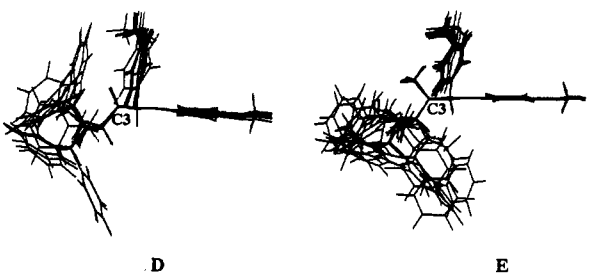

Figure 4. Conformationally accessible orientations of the side chain for C-3 monosubstituted (**D**) and disubstituted (**E**) β-lactams.

C-3 side chain in SCH 48461 and SCH 48678 but should also have enhanced potency.

Modeling. To confirm that there were conformations which placed the side chain phenyl in a position which is equally accessible to both SCH 48461 and SCH 48678, conformational searching was carried out on the minimized structures using the SEARCH command in Sybyl.[4] The four acyclic torsions of the side chain of SCH 48461 were systematically varied at 30° increments to generate a conformational ensemble. During the search, the distances between the centroid of the phenylpropyl side chain phenyl and both the lactam carbonyl and the proton at C-4 were recorded. Subsequently, the search of SCH 48678 was conducted with a constraint on these distances equal to the range also available to SCH 48461. The resulting set of conforma-

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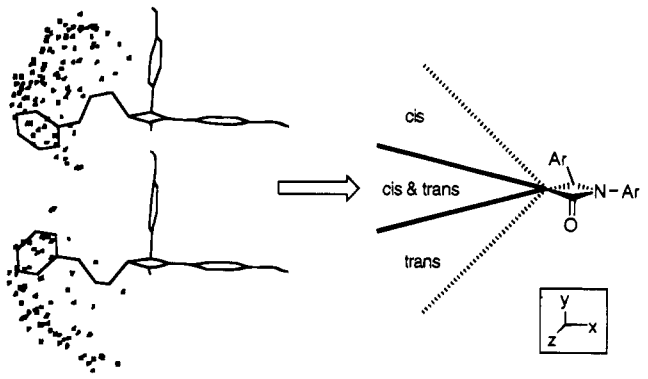


Figure 5. Conformational analysis of *cis*- and *trans*-azetidinone side chains. Symbol (+) indicates location of the centroid of the phenylpropyl side chain phenyl. Actual conformation shown is random (edge view).

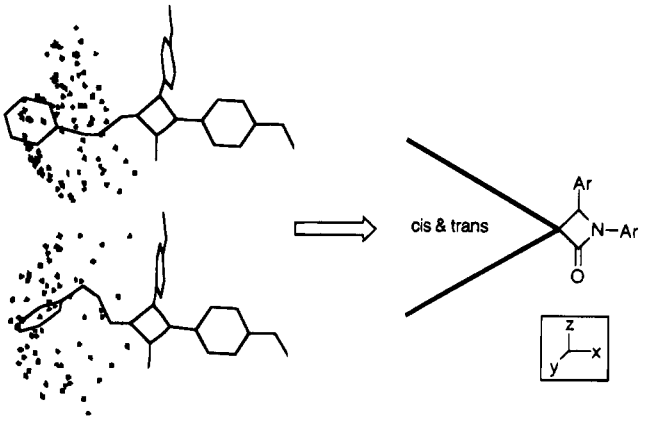


Figure 6. Conformational analysis of *cis*- and *trans*-azetidinone side chains. Symbol (+) indicates location of the centroid of the phenylpropyl side chain phenyl. Actual conformation shown is random (top view).

tions for both SCH 48461 and SCH 48678 is shown in Figures 5 and 6. These sets of conformations show that there is a common region of space for the orientation of the side chain for these two compounds. The common orientation of the side chain, in the $x-y$ plane (β-lactam), is confined to a relatively small area accessible by the "*cis*" conformation of the phenylpropyl side chain, Figure 5. In the β-lactam $x-z$ plane however, there seems to be no bias, and the entire area is accessible to both diastereomers, Figure 6.

With the possibility of a common conformation substantiated, we wanted to design a conformationally restricted analog that best incorporated this information. To achieve this the conformational sets for SCH 48461 and SCH 48678 were minimized in Batchmin[4] to give a set of unique conformations for SCH 48461 and SCH 48678. These two sets were spatially aligned so that the azetidinone nuclei were coincident, and the sets were visually inspected to identify conformations which placed the side chain phenyl group in a similar position. The side chain conformations of SCH 48461 and SCH 48678 which gave the greatest overlap for the side chain phenyl were then extracted and merged. This was minimized in Batchmin resulting in the initial spirocyclic azetidinone structure **1**, Figure 7.

The spirocyclic compound **1** is a composite of two diastereomers: a *syn*- diastereomer, where the phenyl group and the β-lactam carbonyl moiety are disposed *cis*- on the cyclohexyl ring, and an *anti*-diastereomer,

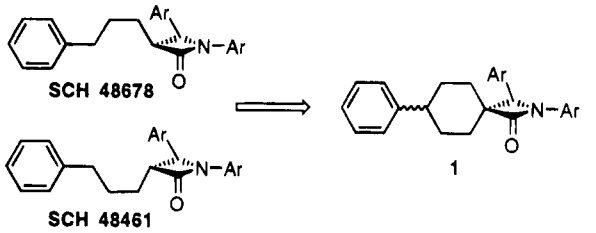


Figure 7.

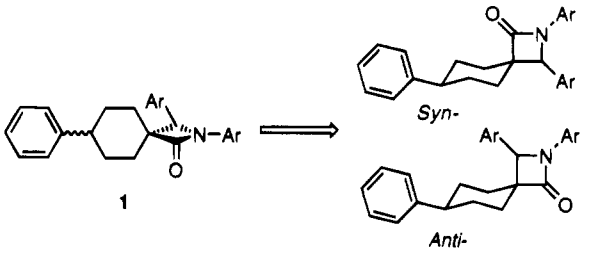


Figure 8.

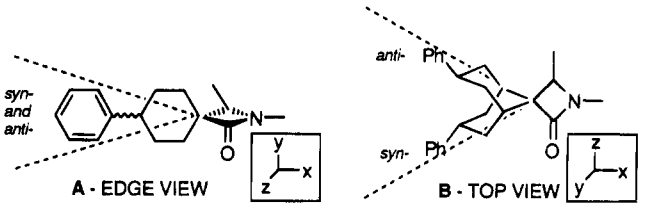
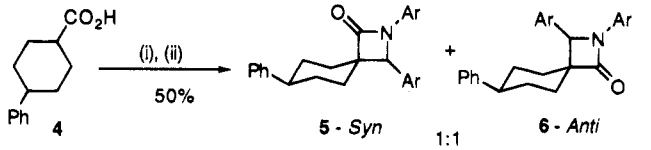


Figure 9. Overlay of the *syn*- and *anti*-spirocyclic azetidinones and the area occupied by the C-3 side chain in both the acyclic diastereomers.

Scheme 1[a]



[a] (i) $(COCl)_2$, CH_2Cl_2; (ii) 4-MeO-C_6H_4CH=NC_6H_4-OMe-4, Et_3N, CH_2Cl_2.

where they are *trans* to each other, Figure 8. When overlaid on to the common region of space occupied by the C-3 side chain in both SCH 48461 and SCH 48678, the *syn*- and *anti*-diastereomers of the spirocyclic β-lactams, Figure 9A, confirm a good orientation fit in the β-lactam $x-y$ plane. In the $x-z$ plane the *syn*- and *anti*-diastereomers span two extreme ends of the accessible area; hence, any difference in activity between the two diastereomers would help define the binding and nonbinding regions in this plane, Figure 9B.

Chemistry. The C-3 spirocyclic β-lactams were synthesized *via* the ketene–imine protocol[5] starting with 4-phenylcyclohexanecarboxylic acid (**4**), Scheme 1. The resulting *syn*- and *anti*-diastereomers **5** and **6** were separated by silica gel chromatography. The structures of **5** and **6** were determined by NMR based on the coupling patterns of each proton resonance and the COSY spectra. (The methine proton H-6_a in both **5** and **6**, Figure 10, is a triplet of triplet (J = 12, 12, 4.5, 4.5 Hz), consistent with an axial proton coupling to two equitorial protons. The axial protons H-5_a and H-7_a appear individually as a doublet of quartet (J = 12, 12, 12, 4.5 Hz), consistent with coupling to a geminal, two axial and one equitorial proton. The axial protons H-4_a and H-8_a appear individually as a doublet of triplet (J = 12, 12, 4.5 Hz), consistent with couplings to a geminal,

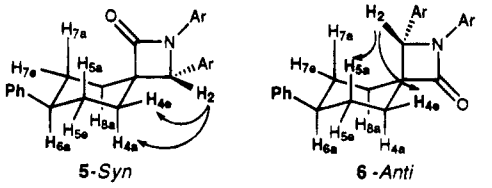


Figure 10. Cyclohexyl ring protons within NOE distance of H-2.

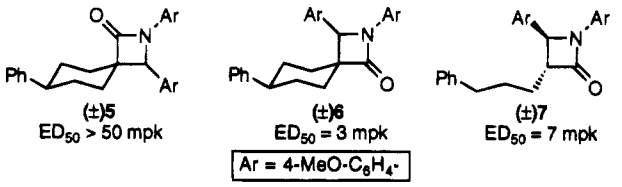


Figure 11.

one axial, and one equitorial proton. All equatorial protons appear as doublets with a large geminal coupling constant of 12 Hz and smaller couplings of 4.5 Hz and less. Couplings obtained from COSY spectra clearly indicate that the four protons at C-4 and C-5 are coupled to each other as are the four protons at C-7 and C-8. With confirmation of the spirocyclic structure of the azetidinones and assignment of every proton signal by both the coupling pattern and COSY spectra, the diastereomers were identified from the difference NOE spectra obtained by irradiating the methine proton H-2. In **5**, the methine proton H-2 was found to be close to both the axial and equitorial protons H-4_a and H-4_e. On the other hand, in **6**, the same proton was close to the equitorial proton H-4_e and the axial proton H-5_a and not H-4_a. The proximity of these protons was also independently established by molecular modeling.)

Results and Discussion. The compounds **5** and **6** were tested in our cholesterol-fed hamster model,[6] and much to our satisfaction **6** was very active (L/CE reduction of 87% at 50 mpk; ED_{50} = 3 mpk), while **5** was inactive (L/CE reduction of 17% at 50 mpk; ED_{50} > 50 mpk), Figure 11. The activity of **6** provides evidence for our hypothesis of the orientation of the side chain in the binding conformation. Also the difference in activity between **5** and **6** clearly defines the allowed and disallowed domains for the orientation of the side chains of SCH 48461 and SCH 48678, Figure 8, and supports the idea that the target for these compounds is a specific protein involved in cholesterol absorption. Also, as we had hoped, the conformationally restricted **6** is more potent compared to **7** (racemate of SCH 48461).

The conformational rigidity of **5** and **6** very narrowly defines the binding and nonbinding domains, Figure 12, and this leaves regions around the narrow binding

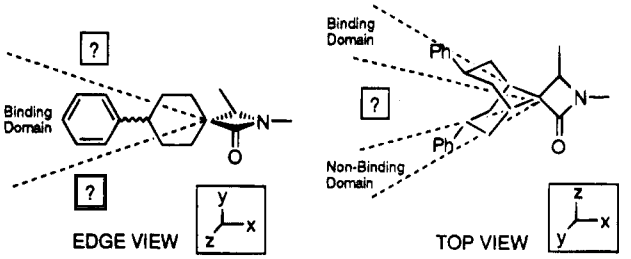


Figure 12.

domain that are conformationally accessible to the acyclic side chain. An investigation of various regioisomers and ring sizes of the spirocyclic β-lactams will help fully define the boundaries of the binding and nonbinding domains.

The identification of this new class of compounds that are potent cholesterol absorption inhibitors has led us to continue our investigation of the SAR for these compounds. The results from this investigation will be reported in a future publication.

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Supporting Information Available: Experimental procedures, including ^{1}H-NMR data and C,H,N analyses (3 pages). Ordering information is given on any current masthead page.

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