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Synthesis and biological evaluation of unprecedented classes of spiro-β-lactams and azido-β-lactams as acyl-CoA:cholesterol acyltransferase inhibitors

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Abstract—Unprecedented classes of four- and five-membered hydroxyl-spiro-β-lactams and hydroxyl-azido-β-lactams were prepared via regioselective ring opening of hydroxyl-epoxides. The potential of these particular β-lactams as biologically active compounds has been confirmed by the results obtained in ACAT inhibition assays.

Acyl-CoA cholesterol *O*-acyltransferase (ACAT, EC 2.3.1.26) is an allosteric enzyme responsible for the esterification of cholesterol with acyl-CoA fatty acids to produce cholesteryl esters.[1] This enzyme plays an important role in the adsorption of cholesterol, the secretion of hepatic very low-density lipoprotein (VLDL), and the accumulation of cholesteryl esters in the arterial lesions.[2] Therefore, ACAT is a key enzyme in controlling cholesterol metabolism and represents a promising target for the development of therapeutic agents.[3] In mammals, two ACAT genes encode for two different proteins,[4] ACAT1 and ACAT2, that may function in distinct and complementary manner. While ACAT1 is more ubiquitous, ACAT2 is present exclusively in hepatocytes and intestinal cells.[5] Inhibition of ACAT activity reduces plasma cholesterol levels by suppressing the assembly and secretion of low-density lipoprotein in liver and chylomicron in intestine. Cholesterol metabolism is tightly associated with hypercholesterolemia, the major risk factors associated with coronary heart disease (CHD) and with the neurodegenerative Alzheimer's disease.[6] For these reasons, during the last 30 years there has been intensive work on the development of ACAT inhibitors[7] designed to control hypercholesterolemia, atherosclerosis, and Alzheimer's disease. A number of classes of compounds were reported to inhibit in vitro ACAT-catalyzed cholesterol esterification. Unfortunately, many of them were not successful in vivo or failed in early clinical trials.[8]

New interesting spiro-β-lactam-containing structures have been recently reported due to their structural features and their bioactivity.[9] In particular, spiro-β-lactam-based structures, containing hydroxy and keto functions in the C3 side-chain, were found to be potent cholesterol absorption inhibitors (CAI). Over the last few years, the synthesis of polyfunctionalized azetidin-2-ones, possessing the requirements for cholesterol absorption control, has received great attention.[10]

Although several different targets have been recently identified for cholesterol absorption control,[11] ACAT test could give some indication to develop novel potential inhibitors.

We report herein the synthesis and biological evaluation of ACAT inhibitors of unprecedented classes of four- and five-membered hydroxyl-spiro-β-lactams and hydroxyl-azido-β-lactams, through the regioselective ring opening of hydroxyl-epoxides.

The regio- and stereoselective ring opening of oxiranes provides a convenient way to prepare polyfunctionalized compounds. Moreover, the stereochemical outcome of the epoxidation of the double bond by *m*-chloroperbenzoic acid (MCPBA) is strongly influenced by steric factors.[12] It is well-known that allylic and homoallylic

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alcohols can be stereoselectively epoxidized with peracids, due to the complex formation between the peroxy acid and the unsaturated alcohol. The synthetic route to the spiro-β-lactams described in this paper is summarized in Scheme 1.

Trans epoxide **2a** was obtained in 85% yield and 85:15 diastereomeric ratio by treatment of rac-1-benzyl-3(but-1′enyl)-3(1′′hydroxybenzyl)azetidin-2-one **1a** with meta-chloroperbenzoic acid (MCPBA).[13]

The pure major isomer **2a** could be isolated by flash chromatography on silica gel and was submitted to intramolecular epoxide ring opening in the presence of $BF_3{\cdot}Et_2O$ (1 equiv).[14] Under these conditions the unprecedented oxetane spiro-derivative **3a** was obtained in 90:10 regioisomeric ratio, with respect to the corresponding five membered ring.[15]

The NOESY-1D analysis performed on **3a** showed a strong enhancement of H^3 signal when H^1 was irradiated, thus suggesting a cis-relationship between the two hydrogens.

With the aim to test the bioactivity of these new classes of compounds, the same reaction sequence was repeated on enantiomerically pure epoxides. By treatment of **2b**[13] under the above-reported conditions, the four-membered oxetane spiro-derivative **3b** was obtained as major product with respect to its five-membered regioisomer **4b**.

In order to test the influence of C4 configuration on bioactivity, the intramolecular epoxide ring opening was performed also on compound **2c**,[13] having (*S*) configuration at C4 of the lactam ring. By treatment of **2c** with boron trifluoride, **3c** and **4c** were obtained in good yield and in 34:66 regioisomeric ratio.

The enantiomerically pure spiroderivatives **3b**, **4b**, **3c**, and **4c** were tested as ACAT inhibitors, using Lovastatin as reference standard (IC_{50} = 12 μM from the literature data, IC_{50} = 16.8 μM when concurrently tested).[16] The results obtained from the enzymatic assays, performed following esterification of [^{14}C]-cholesterol with palmitoyl-CoA in the presence of the spiro-lactam (10 μm), are reported in Table 1.

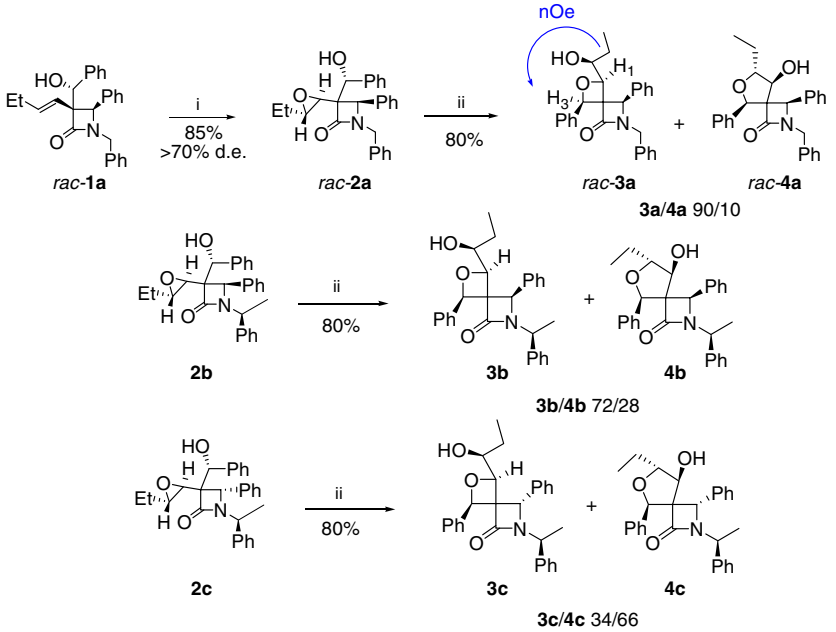
The biological evaluation displayed modest results (entries 1–4), nevertheless interesting information may be drawn. Comparison of the biological activities of oxetane-spiroderivatives **3b** and **3c** (entries 1 and 3) with the corresponding furane-derivatives **4b** and **4c** (entries 2 and 4) showed that four-membered rings were more active than five-membered ones.

We ascribed this major inhibitory effect to the major accessibility of the hydroxyl group, that in the furane-derivatives it is directly linked to the spirocyclic rigid structure, while in the oxetanes it is linked to a linear side-chain.

Furthermore, the comparison between the inhibitory activity of (4*S*)- and (4*R*)-spiro-derivatives showed an influence of the C4 stereochemistry of the lactam ring on bioactivity.

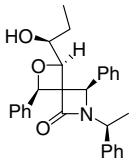
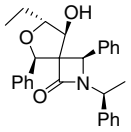
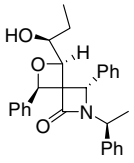
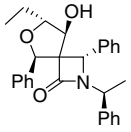
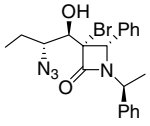
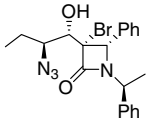
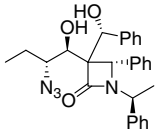
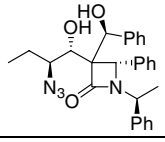
In fact, while (4*S*)-**4c** (entry 4, 27%) was slightly more active than (4*R*)-**4b** (entry 2, 23%), a major increase in inhibition could be observed comparing (4*S*)-**3c** (entry 3, 66%) with the corresponding (4*R*)-**3b** (entry 1, 45%).

Although the reason why **3c** exhibited such higher activity in respect to **3b** is still unclear, this is a useful information for the improvement of efficacy for these and other related classes of compounds.



Scheme 1. Reagents and conditions: (i) MCPBA (1.5 equiv), CH_2Cl_2, rt, overnight; (ii) $BF_3{\cdot}Et_2O$ (1 equiv), CH_2Cl_2, rt, 1 h.

Table 1. ACAT inhibition for spiroderivative and azido-lactams

Entry	Compound	Structure	% Inhibition[a,b] (10 μM)
1	**3b**		45
2	**4b**		23
3	**3c**		66
4	**4c**		27
5	**5c**[c]		65
6	**5d**[c]		60
7	**6c**		79
8	**6d**		87

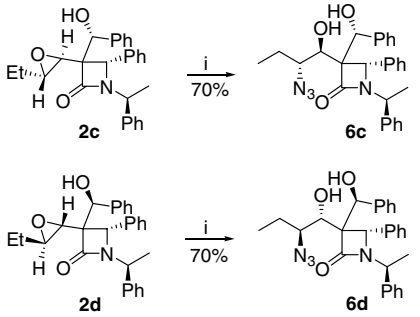
[a] Measured by quantitation of [^{14}C]Cholesterol ester by column chromatography.
[b] Lovastatin as reference standard (IC_{50} = 12 μM from the literature data, IC_{50} = 16.8 μM when concurrently tested).
[c] See Ref. 17.

Therefore further transformations were carried out on epoxides (4*S*)-**2b** and (4*S*)-**2c**.

As a part of a program directed to the synthesis of β-lactam containing ACAT inhibitors, we have previously reported the stereoselective synthesis of azido-hydroxyl-β-lactams **5c** and **5d**,[17] that displayed encouraging inhibitory effects in enzymatic assays (Table 1, entries 5 and 6). These two structures share as a common feature the presence of 2′-hydroxyl and 3′-azido substituents in anti-relationship, linked to the linear chain at C3 position, and a cis-relationship between a bulky bromine atom linked to C3 and C4-phenyl substituent. Both azides have (*S*) configuration at C4 of the lactam ring.

Therefore, on the basis of the good bioactivity of azides **5c** and **5d**, and following the indications obtained from spiro-derivative analysis, we synthesized azido-derivatives **6c** and **6d**, having the hindering hydroxyphenyl group and a polyfunctionalized flexible chain linked to C3 lactam position and (*S*) configuration of the lactam C4-stereocenter (Scheme 2).

The azido-derivatives **6c** and **6d** were obtained starting from the corresponding epoxides **2c** and **2d** by treatment with Me_2AlN_3 at low temperature in toluene.[18] The ring opening occurred exclusively on the less hindered C2′-position, affording **6c** and **6d** in 70% yield (Scheme 2). No traces of spiro-compounds were detected in the crude reaction mixture.



Scheme 2. Reagents and conditions: (i) NaN_3 (1 equiv), Me_2AlCl (1 equiv), toluene, −78 °C to rt, overnight.

As expected, both **6c** and **6d**, matching all the requirements deduced from the previous experimental observations, gave excellent results in the enzymatic assays, being their inhibitory effect on ACAT noticeably higher than those of the other examples (79% and 87%, respectively, Table 1, entries 7 and 8).

Although the azido function is quite uncommon in naturally occurring species, it is stable in the biological environment and, lacking of toxicity, it has been introduced in a variety of drugs.[19] Furthermore, it could have a lipophilic function and its incorporation into drug molecule represents a strategy for the modulation of pharmacokinetic properties.

In summary, we have synthesized, via intramolecular hydroxyl-epoxides' ring opening, novel classes of spiro-β-lactams: among them compound **3c** showed an encouraging inhibitory activity. The (*S*) configuration at C4 of the lactam seemed to be fundamental for enzymatic inhibition. Finally, ring opening of epoxides **2c** and **2d** with Me_2AlN_3 afforded through a regio- and stereoselective process compounds **6c**–**2d**. The potential of these particular β-lactams as biologically active azetidinones has been confirmed by the results obtained in ACAT inhibition assays that showed improved inhibitory effect. All these compounds retain main features of cholesterol absorption inhibitors, having aromatic groups correctly oriented for a good interaction with the enzyme[20] and hydroxyl moieties[21] that are fundamental for bioactivity.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.01.027.

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14. *General procedure for the intramolecular ring opening of epoxides*. To a solution of epoxide (1 mmol) in CH_2Cl_2 (5 ml), $BF_3{\cdot}Et_2O$ (1.1 mmol, 1.1 equiv, 156 mg, 0.139 ml) was added in one portion. The reaction mixture was stirred at rt for 1 h and then was diluted with CH_2Cl_2 (5 ml). After washing twice with water, the organic layer was separated, dried over Na_2SO_4, and solvent was removed under reduced pressure. The products were purified by flash chromatography on silica gel (cyclohexane/EtOAc, 90:10–50:50). Compund **3c**: $[\alpha] = -72.4$ ($c = 0.5$, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.93 (d, 3H, $J = 7.0$ Hz), 1.06 (t, 3H, $J = 7.3$ Hz), 2.1–2.4 (m, 2H), 2.90 (m, 1H), 4.01 (d, 1H, $J = 11.4$ Hz), 4.25 (s, 1H), 4.68 (q, 1H, $J = 7.0$ Hz,), 5.64 (s, 1H), 6.48 (d, 2H, $J = 7.1$ Hz),

6.68 (d, 1H, $J = 7.8$ Hz), 7.0–7.6 (m, 12H).; ^{13}C NMR δ 9.4, 17.5, 18.6, 42.9, 50.4, 55.8, 65.9, 70.9, 65.7, 70.9(2C), 126.3, 126.5, 126.9, 127.2, 127.4, 128.0, 128.4, 128.5, 128.9, 130.1, 133.3, 128.0, 139.1, 140.0, 168.7; IR (neat): 3406, 3044, 3024, 2962, 2922, 1716, 1490, 1450, 1062 cm^{-1} ; LC-ESI-MS rt 13.3 min, *m/z* 428 (M+1), 450 (M+Na).

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