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N-Oleoyl-1,2,3,4-Tetrahydroisoquinolines as Conformationally Restricted Inhibitors of Acyl-CoA:Cholesterol Acyl Transferase (ACAT)

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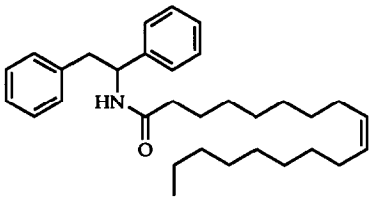
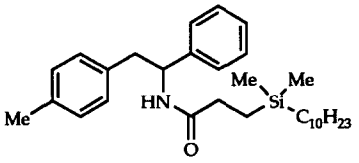
Abstract: Oleoyl-amides of variously substituted 1,2,3,4-tetrahydroisoquinolines were synthesized and assayed for their inhibition of acyl-CoA:cholesterol acyl transferase (ACAT) as conformationally restricted analogs of I and II. These were found to be moderate to good inhibitors of ACAT.

The critical role of acyl-CoA:cholesterol acyl transferase (ACAT) in the transport and storage of cholesterol has been well established. For this reason the therapeutic potential of ACAT inhibitors has been recognized for lipid lowering and anti-atherosclerotic therapies.[2]



I



II

Compounds I[3] and II[4] have been reported as potent ACAT inhibitors. This report addresses the importance of conformations adopted by the diphenylethyl moiety of I and II by evaluating the 3-phenyl- and the 1-benzyl-N-acyl-tetrahydroisoquinolines III and IV as two possible series of restricted analogs of I or II, Figure 1.

Molecular modeling calculations were used to ascertain the conformational bias of the diphenylethyl fragment of the amine in I (for ease of calculations acetyl was used as the acid fragment) and indicated no conformational bias in the acyclic system. The relative energy difference between the staggered conformation **A** or the gauche conformations **B** and **C** conformations was about 1-2 Kcal/mol., making all of these three conformations readily accessible, Figure 2.

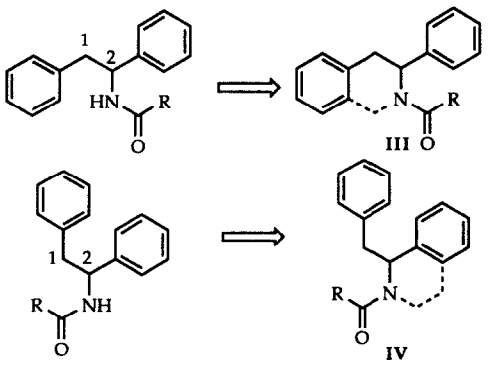
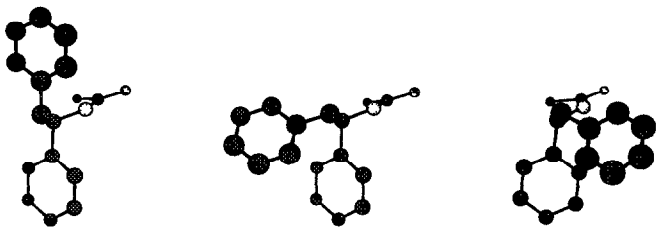


Figure 1

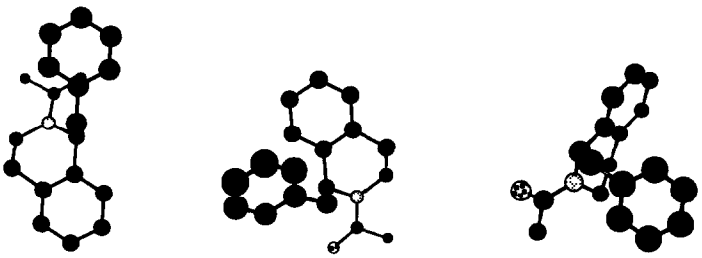


A B C

Conformer	Torsion Angle	Relative Energy (Kcal)
A	182.6	0
B	299.8	1.19
C	60.4	1.84

Figure 2

Molecular modeling calculations on the 3-phenyl- and the 1-benzyl-N-acetyl-tetrahydroiso-quinolines confirmed that the completely rigid 3-phenyl-N-acetyl-tetrahydroisoquinolines were good mimics for conformation **A**. The flexibility around the C1-C2 bond of the 1-benzyl-N-acetyl-tetrahydroisoquinolines allows them to adopt the three conformations **A'**, **B'** and **C'**, Figure 3, corresponding to **A**, **B** and **C**. The conformational flexibility of the 1-benzyl-N-acetyl-tetrahydroiso-quinolines would allow them to be direct mimics of the acyclic compounds and hence, serve to provide a correlation between the acyclic series and the isoquinoline series.



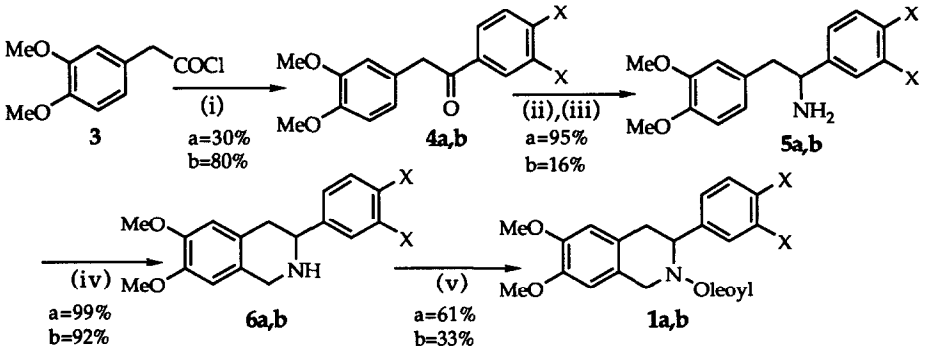
A' B' C'

Conformer	Torsion Angle	Relative Energy (Kcal)
A'	180.5	0
B'	299.7	1.14
C'	69.4	2.03

Figure 3

Synthesis

(i) *3-Phenyl-N-oleoyl-tetrahydroisoquinolines* : The synthesis 3-phenyl-N-oleoyl-1,2,3,4-tetrahydroisoquinolines is outlined in Scheme 1. Acylation of benzene or veratrole with the acid chloride **3** under Friedel-Craft conditions gave the ketones **4a,b** which were converted to the respective oximes, reduction of the oximes yielded the amines **5a,b**. Treatment of the amines



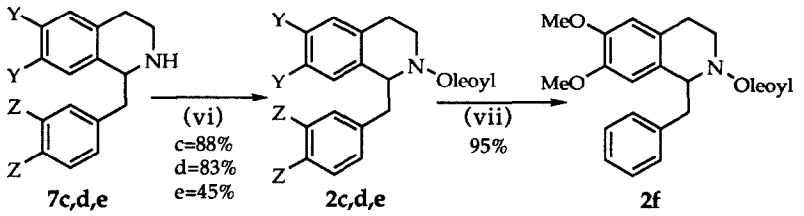
(a) X=H; (b) X=OMe

(i) $AlCl_3$,  ; (ii) $NH_2OH.HCl$, :Py; (iii) 10%Pd/C, 1MHCl in EtOH, H_2(45psi)
(iv) 36% HCHO, 1.5MHCl in MeOH; e) Oleic acid, DCC, DMAP, CH_2Cl_2

Scheme 1

5a,b with 36% formaldehyde and methanolic hydrochloric acid resulted in the formation of the isoquinolines **6a,b**.[5] Acylation of **6a,b** with oleic acid gave the desired N-oleoyl-tetrahydroisoquinolines **1a** and **1b**.

(ii) *1-Benzyl-N-oleoyl-tetrahydroisoquinolines* : Acylation of the isoquinolines **7c,d**[6] and **e**[7] gave the desired amides **2c,d** and **e**, Scheme 2. **2e** was alkylated with dimethyl sulfate to give **2f**.



7c,d,e **2c,d,e** **2f**

(c) Y and Z = H; (d) Yand Z = OMe; (e) Y = OH, Z = H;

(vi) Oleic acid, DCC, DMAP, CH_2Cl_2; (vii) K_2CO_3, Me_2SO_4, Acetone

Scheme 2

Modeling

(i) *3-Phenyl-N-acetyl-tetrahydroisoquinolines* : The starting structure was built in SYBYL Version 5.5[8] using standard fragments and minimized using the MM2* forcefield in Macromodel Batchmin Version 3.5[9]. The SYBYL SEARCH routine was used to search the three acylic bonds of the diphenylethyl moiety as well as the C-N bond. The C1-C2 (ethyl) and C-N bonds were rotated through a full 360 degree arc at 30 degree increments, while the C-phenyl bonds were varied from 0 to 180 degrees at 60 degree increments. The general Van der Waals screening factor was set to 0.90 and the 1-4 Van der Waals screening factor was set to 0.80. The resulting set of 36 conformations was minimized using Batchmin to derivative convergence of .01 Kcal/Ang-mol. Duplicate or near duplicate conformations were removed to give three rotamers about the C1-C2 bond.

(ii) *1-Benzyl-N-acetyl-tetrahydroisoquinolines* : Modeling was done as above, except that a ring search was included in the SYBYL SEARCH procedure. Both acyclic bonds of the benzyl moiety were searched as well as the N-C bond of the acetamide moiety. The resulting set of 197 conformations was minimized and duplicates were removed as above to give eight unique conformations representing three rotamers about the isoquinoline-benzyl bond: Three for rotamer A, four for rotamer B, and one for rotamer C. Different conformations within a rotamer

family are due to higher energy isoquinoline ring conformations and amide rotamers. Energies are reported for the lowest energy conformation in each family.

ACAT Microsomal Assay

ACAT activity was assessed by measuring the transfer of [^{3}H]-oleic acid from [^{3}H]-oleoyl-CoA to cholesterol to give radiolabeled cholesteryl ester by rat liver microsomes.[10] The microsomes[11] and the [^{3}H]-oleoyl CoA[12] were prepared as described and the assay performed in a total volume of 50 µL. Each incubation contained 0.1 M $KHPO_4$ (pH 7.4), 2 µM dithiothreitol, 12.5 µg microsomal protein, and 300 µg of bovine serum albumin. Test compound was dissolved in DMSO and added into the reactions in a 1 µL volume. Incubations were then pre-incubated for 15 minutes at 37°C. The reactions were initiated by the addition of 3 µL [^{3}H]-oleoyl CoA (1 µCi/incubation, final concentration 10 µM). After incubation for an additional 15 min. at 37°C, the incubations were terminated by application of a 15 µl aliquot to individual lanes on a silica gel TLC plate. The plates were developed with petroleum ether:diethyl ether:acetic acid (90:10:1). Regions corresponding to the migration of authentic cholesteryl oleate were scraped into scintillation vials and the radioactivity was quantified.

Results & Discussion

The results of the ACAT inhibition assay are given in Table 1. The data indicates that of the 3-phenyl- and the 1-benzyl-N-oleoyl-tetrahydroisoquinolines tested there is not much difference in activity in the two series and they are all moderate to good ACAT inhibitors.[13] The inhibition data suggested that compounds **1a, 2c, 2d** and **2f** were equipotent to **I** (87 % Inhib. ACAT @ 25 µM) in the ACAT microsomal assay. However, the IC_{50} values for the more active

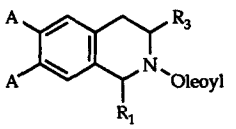


Table 1

#	A	R_1	R_3	IC_{50} (µM)	Inhib(%) @ 25µM
1a	OMe	H	C_6H_5	3.8	86
1b	OMe	H	3,4-$(OMe)_2$-C_6H_5	–	67
2c	H	$C_6H_5CH_2$	H	4.8	81
2d	OMe	3,4-$(OMe)_2$-$C_6H_5CH_2$	H	2.7	91
2e	OH	$C_6H_5CH_2$	H	–	73
2f	OMe	$C_6H_5CH_2$	H	–	63

compounds **1a**, **2c** and **2d** indicated that these were 20 fold less active than I (IC_{50} = 0.15 μM). These results indicate that the N-oleoyl-tetrahydroisoquinolines are only moderate to good inhibitors of ACAT. It also seems from the activity of compound **1a** that the extended conformations of **I** and **II** are ACAT binding conformations. However, our results do not rule out the possibility that gauche or other intermediate confomations are also binding conformations. Both compounds **I** and **II** have been reported as selective and potent inhibitors of ACAT and we had hoped to probe for the important binding conformation of **I**/**II** in the enzyme active site.[14] Our initial data does not provide us with an answer. Investigation of other restricted analogs of **I** and **II** are ongoing in our laboratories and will be reported at a later date.

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References and Notes

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13. Acyclic analogs related to **1a** and **1b** were found to be equipotent to **I** and **II** in our ACAT microsomal assay suggesting that the presence of either the methoxy or hydroxy group does not affect the level of activity for these compounds. Manuscript in preparation.
14. The mechanism of action of ACAT inhibitors is of considerable debate due to the absence of enzyme kinetic data. The absence of such information precludes the conclusion that a particular ACAT inhibitor is bound to the enzyme active site. There is speculation that some of these ACAT inhibitors may be active by binding to an allosteric site or by disrupting the microsomal membrane structure.