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IMIDAZOLIDINONES AND PYRAZOLONES AS NOVEL ACAT INHIBITORS: CHEMISTRY AND BIOLOGICAL ACTIVITY[1]

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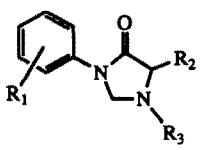
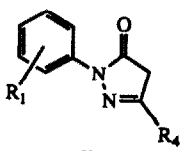
Abstract: Our continued interest in inhibitors of acyl-coenzyme A:cholesterol acyltransferase (ACAT) has led us to study a series of imidazolidinones (I) and a series of pyrazolones (II), two systems structurally distinct from our other reported series. The synthesis and biological activity of I and II are reported.

Acyl-coenzyme A:cholesterol acyltransferase (ACAT, EC 2.3.1.26) is the intracellular enzyme responsible for the esterification of cholesterol in tissues from all mammalian species.[2] There is considerable evidence supporting a role for ACAT in intestinal cholesterol esterification, and hence, in the absorption of dietary and biliary cholesterol.[3] In fact, inhibitors of this enzyme have been shown to prevent the absorption of dietary cholesterol and lower plasma total cholesterol in numerous animal models,[4] and therefore, could be useful in the treatment of hypercholesterolemia. There is also evidence that inhibition of arterial wall ACAT may have a beneficial effect on atherosclerotic lesions.[3,5]

Previous work in our laboratories led to the development of a series of fatty acid anilides which were potent at inhibiting ACAT in vitro and at lowering plasma total cholesterol in vivo.[5] Due to the encouraging biological results with this series of compounds,[5b] we explored modifications of these fatty acid anilides which led to a series of ß-ketoamide ACAT inhibitors which also inhibited ACAT in vitro and reduced plasma total cholesterol in vivo.[6] Because of our continued interest in the area of ACAT inhibition, we studied two series of conformationally constrained analogs, imidazolidinones (I), derived from our amino acid and glycine anilide series,[7,8] and pyrazolones (II), analogs of the ß-ketoamides.[6] We felt that exploration of these rigid systems might expand our understanding of the structural requirements for potent ACAT inhibition both in vitro and in vivo.



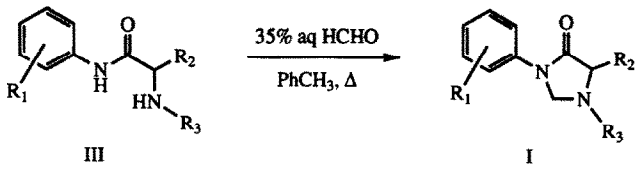
I



II

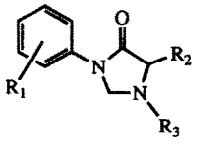
Chemistry

The imidazolidinones (I) listed in Table I were synthesized by the condensation of various amino acid anilides (III) with a twofold excess of 35% aqueous formaldehyde[9] (eq 1).



III I (1)

Table I. In Vitro and In Vivo Activity of Substituted Imidazolidinones (I)



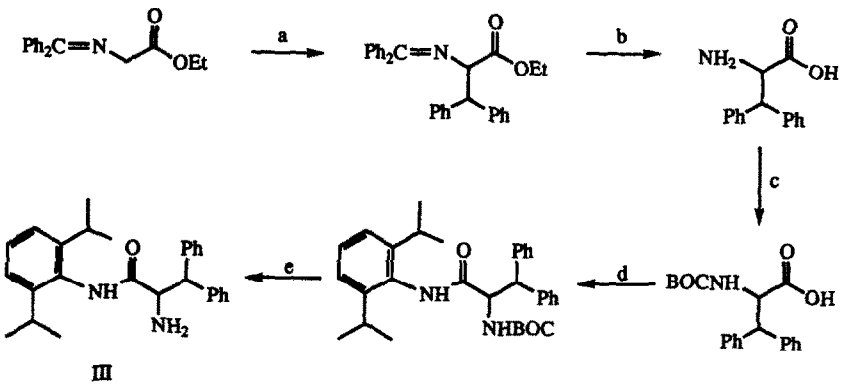
I

EXAMPLE	R_1	R_2	R_3	IAI[a] IC_{50} (μM)	APCC[b] (% Δ in TC)
1[f]	2,6-$(CHMe_2)_2$	$CHPh_2$	H	3.9	-14[c]
2	2,6-$(CHMe_2)_2$	H	$CHPh_2$	1.3	*d*
3[f]	2,6-$(CHMe_2)_2$	CH_2Ph	H	5.5	-9
4	2,6-$(CHMe_2)_2$	H	CH_2CHPh_2	>5[e]	-6

[a]ACAT inhibition was determined in vitro (IAI) by incubation of the compounds with ^{14}C-oleoyl-CoA and intestinal microsomes isolated from cholesterol-fed rabbits. Activity is expressed as the micromolar concentration of the compound required to inhibit enzyme activity by 50% (IC_{50}).[10] Each determination was performed in triplicate. [b]Cholesterol-lowering activity was assessed in rats fed a single, high-fat, high-cholesterol meal, in which the plasma total cholesterol was determined by enzymatic methods described by Allain, et al.[11] This acute in vivo data (APCC) is expressed as percent change in total cholesterol in cholic acid (0.5%)-cholesterol (1.5%)-peanut oil (5.5%)-fed rats. All animals were dosed at 30 mg/kg. [c]Significantly different from control, $p < 0.05$, using unpaired two-tailed t test. The average vehicle control value for total cholesterol was 214 mg/dL. [d]Not tested. [e]3% at 5 μM. [f]Racemic amino acid anilide precursors (III) were used as the starting materials.

The anilide precursor for Example 1 was synthesized by alkylating N-(diphenylmethylene) glycine ethyl ester with bromodiphenylmethane under phase transfer conditions[12] (Scheme I). Hydrolysis of the alkylated imine with 6N HCl, protection of the amino acid with BOC-anhydride, formation of the amide with the isobutyryl mixed anhydride of the BOC-protected amino acid and 2,6-diisopropylaniline, followed by removal of the BOC with HCl gas, yielded the desired amino acid anilide. The syntheses of the amino acid anilide precursors for Examples 2 - 4 have been published previously.[7,8]

Scheme I.

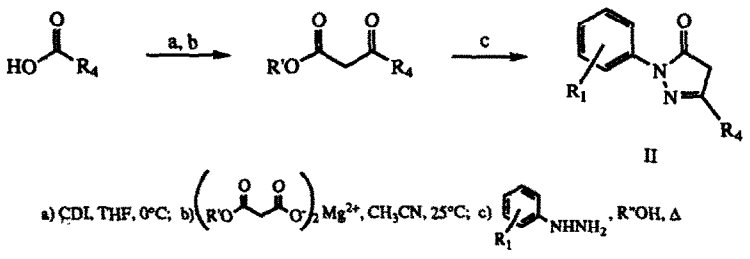


III

a) Ph_2CHBr, n-$Bu_4N^+HSO_4^-$, 10% NaOH, CH_2Cl_2; b) 6N HCl; c) $(t\text{-}BuO_2C)_2O$, NEt_3, H_2O/dioxane; d) $iBuO_2CCl$, Et_3N, 2,6-diisopropylaniline; e) HCl(g), CH_2Cl_2, 0°C

The pyrazolones (II) listed in Table II were prepared by the synthetic route shown in Scheme II. Treatment of the appropriate carboxylic acid with 1,1-carbonyldiimidazole, followed by the addition of the magnesium salt of either the ethyl or *p*-nitrobenzyl malonate, yielded the required ß-ketoester.[6] Condensation of these ß-ketoesters with various phenylhydrazines in refluxing alcohol yielded pyrazolones II.

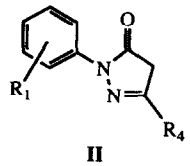
Scheme II.



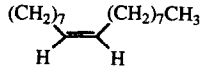
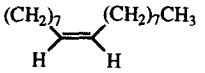
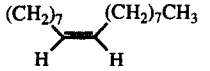
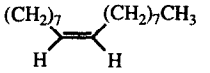
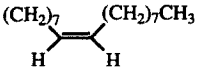
II

a) CDI, THF, 0°C; b) $\left(R'O\right)_2Mg^{2+}$, CH_3CN, 25°C; c) R_1 $NHNH_2$, R"OH, Δ

Table II. In Vitro and In Vivo Activity of Substituted Pyrazolones (II)



II

EXAMPLE	R_1	R_4	IAI[a] IC_{50} (μM)	APCC[b] (% Δ in TC)
5[c]	2,4-Me_2		>5[e]	-26[f]
6[c]	2,4-F_2		>5[g]	*h*
7[c]	2,4-$(NO_2)_2$		>5[i]	-26[f]
8[c]	2,4-Cl_2		9.9	*h*
9[c]	2,6-Cl_2		22.9	-18[f]
10[d]	2,6-Me_2	$(CH_2)_9CH_3$	>5[j]	*h*
11[d]	2,6-Me_2	CH_2CHPh_2	50.8	*h*

[a]ACAT inhibition in vitro. See Footnote *a*, Table I. [b]Denotes percent change in total cholesterol in cholic acid-cholesterol-peanut oil-fed rats. See Footnote *b* Table I. [c]The 2,4- and 2,6-disubstituted phenylhydrazines for Examples 5 through 9 were commercially available. [d]For Examples 10 and 11, the HCl salt of the 2,6-dimethylphenylhydrazine was synthesized in high yield by treating 2,6-dimethylaniline with sodium nitrite, followed by sodium sulfite and hydrochloric acid.[13] [e]35% at 5 μM. [f]Significantly different from control, $p < 0.05$. See Footnote *c*, Table I. [g]18% at 5 μM. [h]Not tested. [i]1% at 5 μM. [j]16% at 5 μM.

Results and Discussion

We previously determined[14] that 2,6-disubstitution on the phenyl ring was important for potent ACAT inhibition in vitro. It was also found that by increasing the size of the 2,6-alkyl substituents[5,14] (i.e., from methyl to isopropyl) on the phenyl ring, potency was improved significantly. This strong dependence of activity (or in vitro potency) on the size of the ortho substituents is probably best interpreted as a requirement to assure a perpendicular orientation of the required amide carbonyl function with the aromatic ring. Because of this requirement, the aryl substituents utilized in this study were 2,6-diisopropyl for the imidazolidinone series. However, for the pyrazolones, 2,6-diisopropyl substitution was not possible due to the difficulty in synthesizing

2,6-diisopropylphenylhydrazine, and our inability to cyclize the adduct of the ß-ketoester intermediate (Scheme II) with 2-isopropyl-6-methylhydrazine. With smaller 2,6-substituents (Cl, Me), the cyclization was successful, although yields were low.

The various side chains (i.e., R_2, R_3, R_4) in both series of compounds were abstracted from potent ACAT series studied previously in our laboratories. Examples 5 - 10 (Table II) incorporate the oleoyl and C-10 alkyl chain exemplified in the fatty acid anilide and ß-ketoamide series, respectively.[5,6] The oleoyl analogs (Examples 5 - 9) had no comparable activity to the similar open-chain fatty acid anilides previously reported.[5] Also, nearly complete inactivity resulted for Example 10, a derivative of the corresponding ß-ketoamide.[6] Examples 1 - 4 (Table I) and Example 11 (Table II) incorporate phenyl group(s) at various distances from the carbonyl. It had been determined that a phenyl group three atoms away from the urea carbonyl seemed to be the optimal spatial arrangement for potent ACAT inhibition.[15] In this study, only Example 2 (Table I) contains a phenyl group three atoms away from the carbonyl group. This compound is the most potent of all five analogs, supporting the evidence of this previous reported observation. Examples 1 - 4 can also be compared to the amino acid and glycine anilide series.[7,8] Examples 1 and 3 had similar activity to their amino acid anilide analogs. As previously reported,[7] substitution in the α-position of the amino acid anilides results in the loss of in vitro activity. However, Examples 2 and 4 showed a marked decrease in activity when compared to their open chain glycine anilide derivatives.[8] Apparently, the amide NH, present in the glycine anilides, is important for ACAT inhibition.[6] When the corresponding glycine anilides are condensed with formaldehyde to give Examples 2 and 4, this NH is not present, and a loss of activity is observed.

In general, the imidazolidinones (I) (e.g., Examples 1 - 3, IC_{50} = 3.9, 1.3, and 5.5 μM, respectively) were at least 2-fold more potent than the pyrazolones (II) at inhibiting ACAT in vitro (e.g., Examples 8, 9, and 11, IC_{50} = 9.9, 22.9, and 50.8 μM, respectively). However, the reduction in plasma total cholesterol was quite similar (-14%, Example 1, vs -18%, Example 9). Perhaps more surprising were the reductions in plasma total cholesterol observed with Examples 5 and 7, which were very weak ACAT inhibitors in vitro (IC_{50} = 35% at 5 μM and 1% at 5 μM, respectively), yet produced reductions in plasma total cholesterol (-26%) which were significantly different from control animals. Whether these changes in plasma total cholesterol are due to ACAT inhibition is unclear.

In conclusion, two structurally different systems, imidazolidinones (I) and pyrazolones (II), have been synthesized and studied as potential ACAT inhibitors in vitro and hypocholesterolemics in vivo. From our observations, this type of modification caused a reduction in both ACAT inhibition in vitro and cholesterol lowering in vivo. Apparently, either the enzyme site cannot tolerate such restricted molecules or the requirement for a hydrogen bond donor seen previously is such as to render these compounds inactive. However, despite the weak ACAT inhibition displayed by these compounds in vitro, when dosed orally at 30 mg/kg, some examples produced statistically significant reductions in plasma total cholesterol.

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