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Amides of Piperidine, Morpholine and Piperazine Substituted 1-Phenylethylamines: Inhibitors of AcylCoA:cholesterol Acyltransferase (ACAT) Activity *in vitro* and *in vivo*

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Abstract—Amides of some substituted 1,2-diarylethylamines have been shown to exhibit potent acylCoA:cholesterol acyltransferase (ACAT, EC 2.3.1.26) inhibitory activity *in vitro* in microsomal ACAT assays but show poor *in vivo* activity in a cholesterol-fed hamster model. In an effort to design ACAT inhibitors that are potent in both our *in vitro* and *in vivo* assays a series of amides of piperidine, morpholine and piperazine substituted 1-phenylethylamines were synthesized. Compounds of this series were found to be very potent inhibitors of ACAT in a microsomal ACAT assay and also exhibited potent activity in a cholesterol-fed hamster model.

Introduction

AcylCoA:cholesterol acyltransferase (ACAT, EC 2.3.1.26) is a microsomal enzyme responsible for conversion of free cholesterol into fatty acid cholesteryl esters.[1] Prior to packaging into chylomicrons and secretion into the lymph, intestinally derived free cholesterol is esterified via ACAT. Hence, inhibition of intestinal ACAT activity may significantly inhibit intestinal cholesterol absorption and help reduce serum lipid levels in humans. This has been demonstrated in animal models of hypercholesterolemia. Inhibition of hepatic ACAT activity may also reduce the secretion of cholesteryl esters in lipoproteins by the liver. Furthermore, since ACAT-derived cholesterol esters have been implicated in the formation of atherosclerotic plaques, ACAT inhibition may arrest progression and promote regression of plaques. This would then address a possible solution to a major determinant of risk in the occurrence of coronary artery disease (CAD).[2]

Various classes of compounds have been shown to inhibit ACAT activity.[3] However most of these compounds, including amides **1** and **2** (Fig. 1)[3f,g] are lipophilic and have poor bioavailability which translates into poor *in vivo* activity. It has been reported that compounds with low systemic bioavailability will lack the desired effect on hepatic and macrophage ACAT activity and not have antiatherosclerotic activity.[4]

Others have struggled with this issue as well and have come to the conclusion that the lipophilicity plays an important part in ACAT inhibitory activity and have attempted to correlate bioavailability and lipophilicity.[5] As part of our efforts directed at designing potent ACAT inhibitors, both *in vitro* and *in vivo*, we have previously investigated the structure–activity relationships in derivatives of **2** and a related series of diphenylacetamides **3** using both a rat intestinal microsomal ACAT assay and a cholesterol-fed hamster model. It was observed that in general, introduction of a polar group on the phenyl ring of the diphenylethylamine moiety attenuated the *in vitro*, rat intestinal microsomal, assay ACAT activity, with the extent of this attenuation roughly following the polarity of the group.[6] However, this attenuation of *in vitro* activity due to the introduction of the polar group was associated with a concomitant improvement in the *in vivo* activity of these compounds.[6] This effect of a polar moiety in increasing the *in vivo* activity has been reported recently.[7]

Previous SAR studies of the 1,2-diphenylethyl amides had indicated that replacement of either of the phenyl rings in

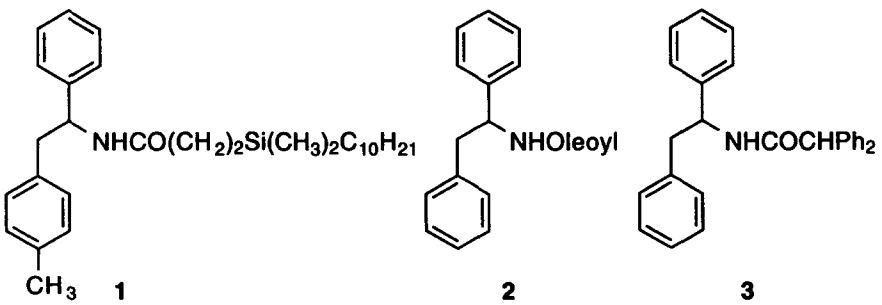
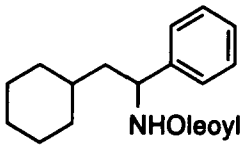


Figure 1.

2 with a cyclohexyl ring yielded compounds such as **4** and **5** (Fig. 2) that were still very potent inhibitors of ACAT *in vitro* but compound **5** had no *in vivo* activity. With this information in hand we decided to investigate the activity of a series of ethyl amides of type **6** (Fig. 3) that had one of the phenyl groups replaced by a 'polar cyclohexyl' moiety such as piperidines, piperazines and morpholines. The results of this study are reported here.



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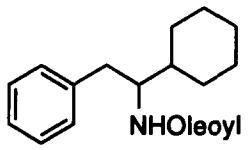
IC_{50} = 0.18mM



5

IC_{50} = 0.17mM

Figure 2.

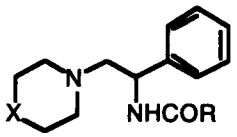


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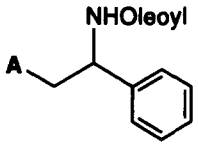
Figure 3.

Chemistry

Various amides of 1,2-disubstituted ethylamines, listed in Table 1, were synthesized following the general procedure shown in Scheme 1.

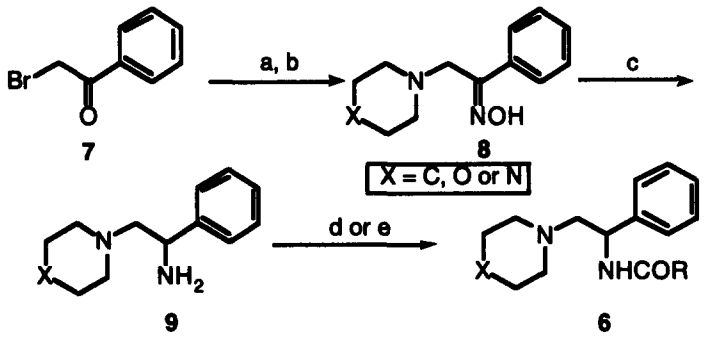
The amines for compounds **10–13** and **15** are commercially available. Compound **14** was obtained by deprotection of the carbamate group in **13** under standard

Table 1. Oleic acid amides of piperidine, morpholine and piperazine substituted phenylethylamines



#	A	IC_{50}(μM)[a]	%Δ L/CE[b]	mp (°C)	MS	Anal.[c]
10		1.2	−12	wax	Calcd: 468 Found: 469	CHN
11		0.3	−21	oil	Calcd: 470 Found: 471	CHN
12		0.3	−55	wax	Calcd: 483 Found: 484	CHN
13		0.06	−22	oil	Calcd: 569 Found: 570	CHN
14		64%@10μM	−54	oil	Calcd: 469 Found: 470	CHN
15		0.01	−35	wax	Calcd: 526 Found: 527	CHN
16		0.18	ND	oil	Calcd: 482 Found: 483	CHN
17		0.03	−21	oil	Calcd: 497 Found: 498	CHN
18		0.34	−25	51–53	Calcd: 484 Found: 485	CHN
19		0.15	−24	oil	Calcd: 498 Found: 499	CHN
20		0.8	0	wax	Calcd: 579 Found: 580	CHN
21		0.5	−18	oil	Calcd: 528 Found: 529	CHN
22		59%@10μM	0	oil	Calcd: 522 Found: 523	CHN

[a]Rat intestinal microsomal assay.
[b]Hepatic cholesterol ester in cholesterol-fed hamster.
[c]All elements shown were within ±0.4% of the calculated percentage.



Reagents: (a) , THF, 0°C; (b) $NH_2OH.HCl$, pyridine;
(c) EtOH, 10%Pd/C, H_2; (d) RCO_2H, DMAP, DCC, CH_2Cl_2;
(e) RCOCl, Et_3N, THF, 0°C

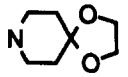
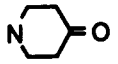
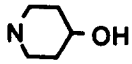
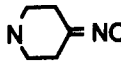
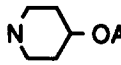
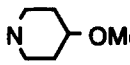
Scheme 1.

conditions. Compounds **16–18** were synthesized by functional group transformations starting with compound **15**. The ketal in **15** proved to be unusually stable to standard deketalization conditions. The hydrolysis was accomplished by using 3 N $HClO_4$ in tetrahydrofuran at 70 °C. The heterocyclic amines for compounds **19–22** were readily synthesized following known literature procedures. The melting points, mass spectral and elemental analyses for all of the analogs are summarized in Tables 1–3.

Discussion

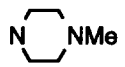
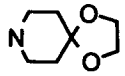
Compounds were evaluated for *in vitro* ACAT inhibitory activity using a rat liver microsomal ACAT assay and for *in vivo* activity using a 7-day cholesterol-fed hamster.

Table 2. Diphenylacetic acid amides of piperidine, morpholine and piperazine substituted phenylethylamines

#	A	IC_{50}[a] (mM)	%Δ L/CE[b]	mp (°C)	MS	Anal.[c]
23		64%@10μM	0	134–137	Calcd: 398 Found: 399	CHN
24		3.0	–15	165–169	Calcd: 400 Found: 401	HRMS[d]
25		4.5	–39	142–143	Calcd: 413 Found: 414	CHN
26		0.3	–26	131–133	Calcd: 456 Found: 457	CHN
27		0.5	0	130–131	Calcd: 412 Found: 413	CHN
28		3.0	–25	94–97	Calcd: 414 Found: 415	CHN
29		0.4	0	ND	Calcd: 427 Found: 428	CHN
30		59%@10μM	–25	113–115	Calcd: 456 Found: 457	CHN
31		1.0	–30	120–122	Calcd: 428 Found: 429	CHN

[a]Rat intestinal microsomal assay.
[b]Hepatic cholesterol ester in cholesterol-fed hamster.
[c]All elements shown were within ±0.4% of the calculated percentages.
[d]Calcd [$C_{26}H_{29}N_2O_2$(M + 1)]: 401.2229; found: 401.2224.
ND = Not determined.

Table 3. Amides of various long chain fatty acids

#	A	R	IC_{50}[a] (μM)	%Δ L/CE[b]	mp (°C)	MS	Anal.[c]
32		$C_{17}H_{35}$	0.5	–25	77–78	Calcd: 485 Found: 486	CHN
33		$C_{15}H_{31}$	1.2	–60	74–75	Calcd: 457 Found: 458	CHN
34		$C_{13}H_{27}$	7.0	0	wax	Calcd: 429 Found: 430	CHN
35		$C_{11}H_{23}$	58%@10μM	0	64–67	Calcd: 401 Found: 402	HRMS[d]
36		$C(Me)_2C_{14}H_{29}$	1.1	–40	oil	Calcd: 485 Found: 486	CHN
37		$C(Me)_2C_{12}H_{25}$	0.29	–44	oil	Calcd: 457 Found: 458	CHN
38		$C(Me)_2C_{10}H_{21}$	0.39	0	oil	Calcd: 429 Found: 430	CHN
39		palmitoleoyl	0.16	–37	oil	Calcd: 455 Found: 456	CHN
40		$C_{15}H_{31}$	0.16	–19	wax	Calcd: 500 Found: 501	CHN

[a]Rat intestinal microsomal assay.
[b]Hepatic cholesterol ester in cholesterol-fed hamster.
[c]All elements shown were within ±0.4% of the calculated percentages.
[d]Calcd [$C_{25}H_{44}N_3O(M+1)$]: 402.3484; Found: 402.3475.

Details of both models are given in the Experimental. As reported previously,[8] the change in hepatic cholesteryl ester content proved to be the most sensitive and reproducible measure of *in vivo* efficacy, and hence changes in serum cholesterol were not used to ascertain activity/potency. This endpoint has also been shown to correlate well with the degree of inhibition of cholesterol absorption in this model.

Contrary to our previous results,[6] where we observed an attenuation in *in vitro* activity by the introduction of a polar moiety, comparison of the activity of **4** with **13**, **15**, or **17** indicates for these compounds there is a substantial increase of *in vitro* activity due to presence of the polar amine moieties. As is evident from Table 1 most of these compounds are potent inhibitors of ACAT *in vitro* with compounds **13**, **15** and **17** being the most potent ACAT inhibitors of all of the diphenylethyl amide series that we have investigated. Besides the increased *in vitro* activity, most of these compounds were also active in our *in vivo* assay as we had hoped based on our previous results.[6] The activity of oleic acid amides **10–15** indicates that this activity is retained with a varying degree with variety of heterocyclic rings. However, there seems to be no direct correlation between *in vitro* and *in vivo* activity. For example, compounds **13**, **15** and **17**, the most active *in vitro* are not the most active *in vivo* and compounds **12** and **14**, clearly not the most active *in vitro*, exhibit the best *in vivo* activity. Although the overall *in vivo* activity for the oleamides was not high, we were encouraged by the observation that the introduction of 'polar cyclohexyl' moiety such as piperidines, piperazines and morpholines did induce *in vivo* activity. We then hoped, based on our previous studies on substituted 1,2-diarylethylamines such as **2** and **3**,[6] that replacing the oleic acid moiety with diphenylacetic acid moiety may result in even better *in vivo* activity. Unfortunately, we did not observe the expected increase in *in vivo* potency, as is indicated from the activity of compounds **23–26**, Table 2. For these amides of piperidine, morpholine and piperazine substituted 1-phenylethylamines, though the oleic acid amides are more active *in vitro* than the diphenylacetamides, there does not seem to be much difference in their *in vivo* activity.

We next decided to investigate the activity of long chain fatty acid amides, with varying chain lengths, with the intent to optimize on the activity of the oleic acid amides. For this study we chose the *N*-methylpiperazine as our 'polar cyclohexyl moiety' as it was one of the preferred amines for the oleic acid amide. Once again the structure–activity relationship (Table 3) shows that we do not have a

correlation between *in vivo* and *in vitro* activity with respect to chain length, the presence or absence of unsaturation and substitution in the acid chain. The *in vitro* activity decreases with decreasing chain length however, the *in vivo* activity is maximized with the hexadecanamide **33**. The presence of the double bond in the side-chain of these long chain amides also does not seem to have a consistent effect on activity, for although the octadecanamide **32** and the oleic acid amide **12** are equipotent *in vitro*, **32** is less active than **12** *in vivo*. In compounds **33** and **39**, the presence of the double bond in the acid moiety of **39** now results in improved *in vitro* and somewhat diminished *in vivo* activity. The presence of α,α-dimethyl substitution does not have any effect in the *in vitro* or *in vivo* activity for the longer chain hexadecanamides **33** and **36**, but increases both the *in vitro* and *in vivo* activity of **34** (*c.f.* **37**). Comparing the dodecanamides **35** and **38** one finds that the influence is on the *in vitro* activity with both compounds inactive *in vivo*.

In synthesizing these compounds we have generated a novel class of very potent ACAT inhibitors *in vitro* that *also* exhibit moderate to good *in vivo* potency. In our previous study we had found that the introduction of polar substituents was influencing *in vitro* and *in vivo* activity in opposing ways and we needed to address this divergence.[6] In this series of amides we have addressed this issue and it seems that the introduction of 'polar groups' does help the *in vivo* profile of this class of compounds as it has for other investigators.[7] However, in our case the extent of this potentiation of *in vivo* activity seems to be limited and there is a lack of correlation between the *in vivo* and *in vitro* activity.

As we, and others, have endeavored to design potent ACAT inhibitors the major issue has always been their *in vivo* activity. The introduction of 'polar cyclohexyl' moieties has a positive effect on the *in vivo* activity, however the extent of this potentiation and the lack of a correlation in *in vivo* and *in vitro* activity suggests that the factors that influence the *in vivo* activity of these compounds is probably not just due to physicochemical effects such as solubility or lipophilicity. For this series of amides further insight into issues such as their mechanism of delivery to the site of ACAT activity and metabolism will be required to design a more potent ACAT inhibitor *in vivo*.

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We wish to thank Dr D. Burnett, Dr J. W. Clader, Dr T. Fevig, Ms L. Hoos, Mr D. McGregor, Ms A. Smith, Dr W. Vaccaro and Mr B. Weig for their helpful discussions and assistance during this study and Dr P. Das for the high resolution mass spectral data.

Experimental

NMR were recorded on a Varian VXR 200 or Varian Gemini 300 MHz spectrometer. The chemical shifts are reported in ppm down field from an internal tetramethylsilane standard. Melting points were determined on a Unimelt capillary melting point apparatus from Thomas Scientific and are reported uncorrected. Mass spectral data were collected on either a VG-ZAB-SE (Fisons Instruments) double focusing mass spectrometer or HP 5989A MS Engine and Extrel ELQ-400-1 mass spectrometers. Chromatography was performed over Universal Scientific or Selecto Scientific flash silica gel 32-63 mesh. When available chemicals were obtained from commercial sources.

General procedure for the alkylation of α-bromoacetophenone

α-Bromoacetophenone (20 g) was slowly added to a solution of piperidine (25.6 g) in tetrahydrofuran (300 mL) at 0 °C and the reaction mixture stirred overnight as it warmed to ambient temperature. Most of the solvent was removed under vacuum and the crude product mixture diluted with half saturated sodium bicarbonate solution and extracted with EtOAc. The organic layer was washed with brine, dried and concentrated to give 1-phenyl-2-piperidinylethan-1-one (19.55 g).

General procedure for the synthesis of 1,2-disubstituted ethylamines

Hydroxyl amine hydrochloride (10 g) was added to a solution of 1-phenyl-2-piperidinylethan-1-one (19.55 g) in pyridine (70 mL) at 0 °C. The reaction mixture was stirred overnight as it warmed to ambient temperature. It was then poured into excess water and thoroughly extracted with ethyl acetate. The combined organic layers were washed with brine, dried and concentrated. The crude product mixture was purified on a silica gel column eluting with EtOAc:hexane (7:3) to give the corresponding oxime (12.56 g). A solution of the oxime (10 g) in EtOH (200 mL) was reduced over 10% Pd/C (1 g) under H_2 (47 psi) overnight. The catalyst was filtered and the filtrate concentrated to give 1-phenyl-2-piperidinylethylamine (8.74 g).

General procedure for the synthesis of 1,2-disubstituted ethylamides

Method A. Oleoyl chloride (810 mg) was slowly added to a solution of 1-phenyl-2-piperidinylethylamine (500 mg) and triethylamine (545 mg) in tetrahydrofuran (20 mL) at 0 °C. After 2 h the ice-water bath was removed and the reaction mixture stirred for an additional 1 h. It was then poured into half saturated sodium bicarbonate solution and the product extracted with EtOAc. The combined extracts were washed with brine, dried and concentrated. The crude product mixture was purified on a silica gel column eluting with EtOAc:hexane (7:3) containing 1% v/v triethylamine to give *N*-[1-phenyl-2-(1-piperidinyl)ethyl]-9-*z*-octadecenamide (960 mg).

Method B. Diphenylacetic acid (162 mg) was added to a mixture of 1-hydroxybenzotriazole (113 mg), *N*-methylmorpholine (85 mg) and 1-ethyl-3-[3-(dimethylamino)-propyl]-carbodiimide hydrochloride (160 mg) in dimethylformamide (3 mL). To this mixture was then added 1-

phenyl-2-piperidinylethylamine (155 mg) and the reaction mixture stirred for 22 h at ambient temperature. The reaction mixture was poured into water and the product extracted with EtOAc. The organic layer was separated, washed with brine, dried and concentrated. The crude product mixture was purified on a silica gel column eluting with EtOAc:hexane (3:2) containing 1% triethylamine to give a-phenyl-*N*-[1-phenyl-2-(1-piperidinyl)ethyl]benzeneacetamide (146 mg).

N-[2-(4-Oxo-1-piperidinyl)-1-phenylethyl]-9-z-octadecenamide

Perchloric acid (6 mL of a 3 N solution) was added to a solution of *N*-[2-(1,4-dioxa-8-azaspiro-[4.5]dec-8-yl)-1-phenylethyl]-9-*z*-octadecenamide (500 mg) in tetrahydrofuran (6 mL) and the reaction mixture was heated at 70 °C for 5.5 h. The reaction mixture was poured into half saturated sodium bicarbonate solution and product extracted with EtOAc. The organic layer was washed with brine, dried and concentrated. The crude product mixture was purified on a silica gel column eluting with CH_2Cl_2:hexane (98:2) to give *N*-[2-(4-oxo-1-piperidinyl)-1-phenylethyl]-9-*z*-octadecenamide (350 mg).

Rat intestinal microsomal ACAT assay

Assays for acyl CoA:cholesterol acyltransferase (ACAT; EC 2.3.1.26) activity were performed using the incubation conditions described previously.[9]

Cholesterol-fed hamsters

Unless indicated, all animals used in these studies were allowed access to food (chow pellets) and water *ad libitum*. All animals were housed, treated and cared for according to NIH guidelines for humane treatment of laboratory animals and the Animal Welfare Act in a program accredited by the American Association for Accreditation of Laboratory Animal Care. Male Golden Syrian hamsters (Charles River Labs, Wilmington, MA), weighing between 100 and 125 g, were fed rodent chow and provided water *ad libitum*. Treatment protocols consisted of feeding chow which had been supplemented with 0.5% cholesterol for 7 days. During this period the animals were gavaged once daily with test compounds (@ 50 mpk) dissolved in 0.2 mL corn oil. On the last day the animals were killed and liver samples taken for lipid analyses. Samples of liver were extracted for neutral lipid analysis by the method of Folch *et al.*[10] Hepatic neutral lipid composition was determined subsequently using a HPLC method which has been described previously. Data are reported as per cent change in hepatic cholesterol ester content versus control animals receiving the high cholesterol diet (oral gavaged in 0.2 mL corn oil day^{-1}) without drug.

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