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Carbazates as potent inhibitors of hormone-sensitive lipase

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Abstract—The central role of adipose tissue hormone-sensitive lipase in regulating fatty acid metabolism makes it a potential pharmacological target for the prevention of peripheral insulin resistance in obese, prediabetic and diabetic individuals. The synthesis of a new series of carbazates is presented. Modification of the phenolic 4-position in a series of 1,2,3,4-tetrahydroisoquinoline and morpholine derived carbazates, yielded inhibitors of the catalytic activity of this enzyme with nanomolar potency.

1. Introduction

In type 2 diabetes, hyperglycemia is accompanied by abnormalities in lipid metabolism. The elevation of circulating free fatty acids (FFA), in particular, has received much attention and is widely considered a possible pathogenetic factor in the disease.[1–3] Increased influx of FFA leads to peripheral insulin resistance seen as decreased glucose uptake in muscle and increased hepatic glucose production. The mechanism for this effect has been attributed to glucolipotoxia.[4] Furthermore, increased FFA flux to the liver results in enhanced triglyceride synthesis and assembly into secreted VLDL, contributing to the abnormal lipoprotein profile seen in diabetics and insulin resistant individuals.[5]

The driving force behind the increased flux of plasma FFA is an overflow, in adipose tissue, of fatty acids derived from increased intracellular lipolysis and from hydrolysis of chylomicron triglycerides of direct dietary origin.

The key rate-limiting enzyme in regulating lipolysis in adipose tissue is hormone-sensitive lipase (HSL) which catalyses the first and second step in the breakdown of triglycerides, releasing two molecules of fatty acid and one molecule of monoacylglycerol, which is then hydrolysed by a separate, specific monoacylglycerol lipase.[6] Cholesterol fatty acid esters can also be readily hydrolysed by HSL. As all lipases HSL has a low activity against water-soluble esters such as *p*-nitrophenyl acetate or butyrate. The activity of HSL is acutely regulated by hormones which influence the levels of cAMP and hence the activity of protein kinase A, which activates HSL by phosphorylation of specific serine residues. The prime players are catecholamines and insulin where cetecholamines via induction of cAMP activates HSL and lipolysis while insulin potently reduces cAMP levels and hence lipolysis.[7] Thus, the central role of HSL regulating the release of fatty acids stored as triglycerides in adipose tissue makes it a potential pharmacological target for the prevention of peripheral insulin resistance in obese, prediabetic and diabetic individuals.

Common structural features of lipases and several esterases are that they adopt the so-called α/β hydrolase fold and perform catalysis using a catalytic triad consisting of a serine, an aspartic or glutamic acid and a histidine residue. Most members of this esterase-lipase family utilize a serine catalytic mechanism, reminiscent of the serine protease mechanism. The mechanism for inhibition of choline esterases by aryl carbamate esters has been studied in detail, involving covalent carbamoylation of the serine-OH and subsequent hydrolysis of the enzyme-inhibitor complex.[8] In this paper the synthesis of some novel carbazates along with in vitro inhibitory effects on HSL activity are presented.

2. Enzyme assay

Hormone-sensitive lipase activity was measured as the release over time of ^{3}H-oleic acid from a

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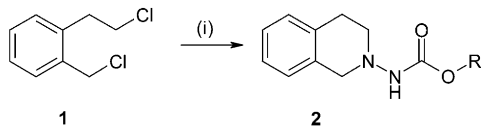


1 2

Scheme 1. Reagents and conditions: (i) H_2NNHCO_2R, Et_3N, DMF, 60 °C, 2–15%.

^{3}H-trioleoylglycerol emulsion stabilized with phospholipids in the presence of bovine serum albumin as fatty acid acceptor[9] using purified recombinant human hormone-sensitive lipase with a specific activity of 2.6 U/mg protein and triolein as substrate at 37 °C.[10,11]

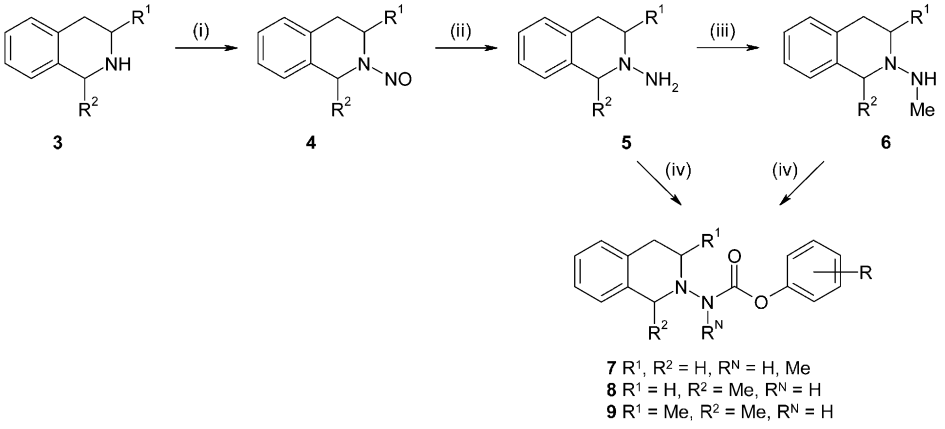
3. Chemistry

The synthesis of the carbazates starts either from 1-(2-chloroethyl)-2-chloromethylbenzene (**1**)[12,13] and a hydrazinecarboxylic acid ester[14,15] (Scheme 1) or more practically via reaction of a chloroformate with a (substituted) 2-amino-3,4-dihydro-1*H*-isoquinoline **5** (Scheme 2). This compound is readily prepared from the corresponding 1,2,3,4-tetrahydroisoquinoline **3** by reaction with isoamyl nitrite[16] or with sodium nitrite and sulphuric acid,[17] followed by reduction of the nitroso compound **4** with lithium aluminium hydride. The *N*-methyl analogue **6** was prepared by reaction of **5** (R^1, $R^2 = H$) with formic acid[18] followed by reduction of the formylhydrazine intermediate with lithium aluminium hydride.[19]

A third method of synthesizing carbazates is by reaction of a carbamoyl chloride with an alcohol or phenol. This method was successfully applied on solid phase supported synthesis (Scheme 3). *N*-Amino-1,2,3,4-tetrahydroisoquinoline was attached to the solid support[20] by reductive amination with sodium cyanoborohydride. Reaction with triphosgene afforded the corresponding hydrazinecarbonyl chloride **11**, which was reacted with a variety of phenols to give the resin bound carbazates **12**. Reaction with 25% trifluoroacetic acid in dichloromethane gave the free carbazates in high purity.

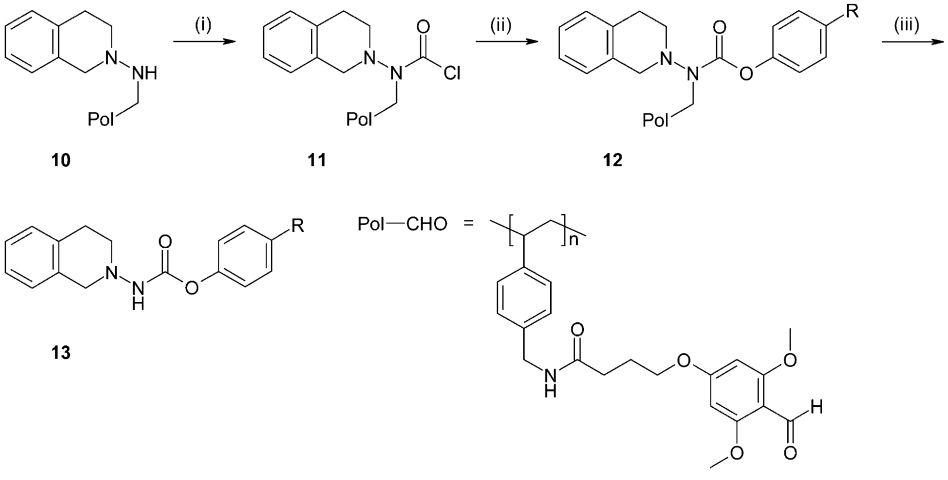
4. Results

From our initial results with compounds **14**–**17** (Table 1), which were prepared according to Scheme 1, it became clear that simple aliphatic carbazates showed very low potency in inhibiting HSL. Only with the aro-



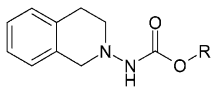
3 4 5 6

Scheme 2. Reagents and conditions: (i) isoamyl nitrite or $NaNO_2$, H_2SO_4; (ii) $LiAlH_4$, Et_2O, reflux, 2 h, 54–56% over 2 steps; (iii) for R^1, $R^2 = H$ (a) HCO_2H, reflux, 1 h; (b) $LiAlH_4$, Et_2O, 19%; (iv) phenyl chloroformate, iPr_2NEt or Et_3N, dichloromethane, 2–63%.



Scheme 3. Reagents and conditions: (i) triphosgene, iPr_2NEt, dichloromethane; (ii) DABCO, phenol, DMF; (iii) 25% TFA in dichloromethane, 24–31% overall yield.

Table 1. Inhibition of HSL by initially synthesised (3,4-dihydro-1*H*-isoquinolin-2-yl)-carbamic acid esters



Compd[a]	R	% Inhibition	IC_{50} (nM)
14	*t*Bu	8	NT[b]
15	Et	9	NT
16	CH_2Ph	4	NT
17	Ph	NT	205

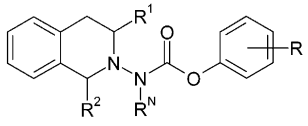
[a] All new compounds gave satisfactory analytic and spectral data.
[b] Not tested.

matic carbazate **17** a strong inhibition was observed. This prompted us to synthesize a number of derivatives of this compound and to developing a SAR around this class of compounds.

From the results obtained with the compounds **18**–**29** (Table 2) it became clear that steric as well as electronic effects play an important role in the inhibition of HSL. *Ortho*-substituents have a dramatic influence on the activity of the carbazates as is clearly demonstrated by the much lower activity of compound **18** compared to compound **19**. The two *ortho*-substituents in compound **20** are protecting the carbazate functionality completely from nucleophilic attack by the serine in the active site, making it no longer a pseudo-substrate for the enzyme. Also a small substituent on the nitrogen atom of the carbazate changes the molecule from a highly active inhibitor (**21**) into a completely inactive compound (**24**). On the other hand, small substituents in the tetrahydroisoquinoline part of the molecule (compounds **22** and **23**) hardly have any influence on the activity.

The importance of polar effects on the activity of the carbazates as inhibitors is clearly demonstrated with

Table 2. Inhibition of HSL activity by a series of (3,4-dihydro-1*H*-isoquinolin-2-yl)-carbamic acid phenyl esters



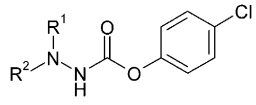
Compd[a]	R	R^1	R^2	R^N	% Inhibition	IC_{50} (nM)
18	2-MeO	H	H	H	76	6400
19	4-MeO	H	H	H	97	360
20	2,6-di-Cl	H	H	H	3	NT[b]
21	4-Cl	H	H	H	98	19
22	4-Cl	Me	H	H	99	35
23	4-Cl	H	Me	H	91	32
24	4-Cl	H	H	Me	2	NT[b]
25	4-COPh	H	H	H	21	NT[b]
26	4-CH_2Ph	H	H	H	97	97
27	4-CO_2Me	H	H	H	57	9952
28	4-NHCOMe	H	H	H	7	NT[b]
29	4-$NHCO_2$-*t*Bu	H	H	H	98	3

[a] All new compounds gave satisfactory analytic and spectral data.
[b] Not tested.

compound **25** versus **26**. After attack of serine on the carbazate the substituted phenol acts as a leaving group. Clearly a carbonyl substituent in the 4-position stabilizes the negative charge of the phenolate, and thereby makes it a better leaving group. The same is true for compound **27** with an ester functionality in 4-position. Also in this case the negative charge is stabilized by the substituent. Based on the stabilizing effect of the substituents, compound **25** should be the best inhibitor but actually the opposite is found, indicating that the polarity of the compounds plays a major role in the inhibition of HSL. Although the result obtained with acetamide **28** was very disappointing and seemed to indicate that an amine functionality in the *para*-position of the phenolic part of the carbazate was not advantageous, a more lipophilic group like the Boc-protected amine in **29** proved to give a compound with very high potency against HSL. However, with these lipophilic substituents the solubility of our compounds might become a severe problem. Therefore, several analogues of compound **21** were prepared, in which the tetrahydroisoquinoline group was replaced by a smaller and less lipophilic heterocycle (Table 3). Although some of the activity was lost changing from a tetrahydroisoquinoline to a morpholine (19 versus 184 nM) as in compound **31**, we were convinced that the activity could be improved by changing the lipophilic substituent in the aromatic part of the molecule.

Based on the promising results obtained with the compounds presented in Tables 2 and 3 we decided to concentrate our efforts on the synthesis of carbazates

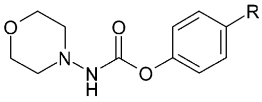
Table 3. Inhibition of HSL by carbamic acid 4-chlorophenyl esters



Compd[a]	R^1, R^2	% Inhibition	IC_{50} (nM)
30	$(CH_2)_5$	99	50
31	$(CH_2)_2O(CH_2)_2$	99	184
32	$(CH_2)_2NMe(CH_2)_2$	98	515
33	$(CH_2)_4$	100	24

[a] All new compounds gave satisfactory analytic and spectral data.

Table 4. Inhibition of HSL by morpholin-4-yl-carbamic acid phenyl esters



Compd[a]	R	% Inhibition	IC_{50} (nM)
34	CH_2CO_2Me	95	716
35	CH_2CO_2Et	99	138
36	$CH_2CO_2CH_2Ph$	100	5
37	NHCOMe	20	NT[b]
38	NHCO-*n*Pr	84	3200
39	$NHCOCH_2$-*t*Bu	99	47
40	NHCOcyclohexyl	99	10
41	NHCO(4-*t*Bu-cyclohexyl)	100	1

[a] All new compounds gave satisfactory analytic and spectral data.
[b] Not tested.

derived from *N*-aminomorpholine and phenols having a lipophilic substituent in 4-position of the aromatic part of the molecule (Table 4).

Changing the size of the ester from a methyl into an ethyl and benzyl (**34**, **35** and **36**) increased the binding affinity from 716 nM to 5 nM. The same trend was found with the amides **37**–**41**. Changing the substituent from an acetamide into a butyramide (**37** vs **38**) or even a more bulky 3,3-dimethylbutyramide as in **35**, increased the inhibition of HSL from only 20% to almost complete inhibition. Increasing the size of the substituent even further resulted in complete inhibition of HSL with an $IC_{50} = 1$ nM for compound **41**.

5. Summary

In conclusion we have identified carbazates derived from *N*-amino-1,2,3,4-tetrahydroisoquinoline and *N*-aminomorpholine as a new class of highly potent inhibitors of human HSL. Polar as well as steric effects play a major role in the activity of these compounds. The binding site of the enzyme tolerates a large number of different substituents in the 4-position of the phenyl ester. Most importantly, highly lipophilic substituents in this position lead to a very strong inhibition of the lipase.

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