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In vitro SAR of (5-(2*H*)-isoxazolonyl) ureas, potent inhibitors of hormone-sensitive lipase

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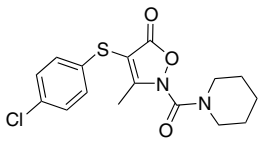
Abstract—A series of (5-(2*H*)-isoxazolonyl) ureas were developed as nanomolar inhibitors of hormone-sensitive lipase, an enzyme of potential importance in the treatment of diabetes.

Hormone-sensitive lipase[1] (HSL) is unique among lipases in having a catalytic domain whose activity is modulated by phosphorylation. In adipose tissue, it catalyzes the rate-limiting step in intracellular lipolysis, acting mainly on diacylglycerols. The enzyme has a broad substrate specificity, however, and provides almost all the neutral cholesterol ester hydrolase activity in steroidogenic tissues such as the adrenals[2] and significant amounts in macrophages.[3]

HSL is a component of the metabolic switch between the use of glucose or free fatty acids (FFA) as energy sources. Adipose HSL activity is normally inhibited by insulin, and is thus elevated in hypoinsulinemic states such as fasting. HSL remains active in type II diabetes, however, despite elevated insulin levels, presumably through loss of insulin's inhibitory effect. The resulting fatty acid flux stimulates inappropriate hepatic gluconeogenesis. High FFA levels are further suspected to play a role in the (still unclear) mechanisms of insulin resistance itself. Inhibition of lipolysis thus has therapeutic implications for type II diabetes, with the potential to treat hyperglycemia, dyslipidemia, and possibly their underlying metabolic defects.

We have previously described the in vivo effects of an isoxazolone inhibitor of HSL, compound **59**.[4] Pyrrolopyrazinediones and carbamoyl triazoles have been reported as HSL inhibitors.[5] Otherwise, the only small-molecule HSL inhibitors that have been described outside the patent literature[6] are natural products.[7] A number of reports of cholesterol esterase inhibitors[8–12] may also represent HSL inhibitors.

High-throughput screening for HSL inhibitors identified compound **1**, with an IC_{50} of 6 nM against the recombinant human enzyme. This inhibition was (slowly) reversible, in agreement with a mechanism proposed for such heterocyclic HSL inhibitors.[5a] Isoxazolone ureas are not well-precedented in pharmaceutical structures,[13] but no heterocyclic modifications were tolerated. Accordingly, medicinal chemistry efforts sought to vary the substituents on the urea nitrogen and on the isoxazolone itself.



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The compounds were assayed[14] against recombinant human HSL at pH 7.2, using tricaproin as substrate with

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colorimetric detection of the resulting liberated fatty acid (NEFA Detection Kit, Wako Bioproducts). Activity in a cellular system was assayed by inhibition of forskolin-stimulated lipolysis in differentiated 3T3-L1 cells, using the same colorimetric readout.

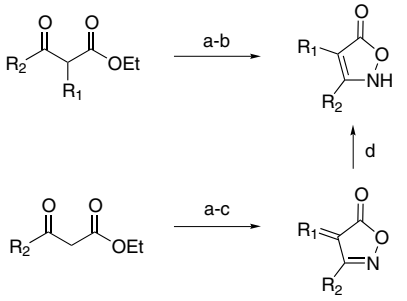
Synthetic routes to these compounds were relatively straightforward (Scheme 1). Substituted β-keto esters (commercially available, or prepared by standard alkylations of ethyl acetoacetate) were reacted with hydroxylamine and sodium hydroxide at room temperature. Subsequent addition of aqueous HCl followed by heating ensured the formation of the isoxazol-5-one product in 50–80% yield, as opposed to the isomeric 3-one.[15] Alternatively, the unsubstituted isoxazolone (from reaction of ethyl acetoacetate) could be condensed with a ketone[16] to provide the alkene, and reduced directly[17] with borohydride to provide isoxazol-5-ones in similar yields.

N-acylation of these heterocycles was typically carried out (Scheme 2) by treatment with excess phosgene, followed by evaporation and reaction with the desired amine. On a larger scale, the CDI-based urea synthesis of Batey et al.[18] was more convenient. Both methods reliably gave N-acylated products, while alternatives could produce mixtures of N- and O-acylation.[19]

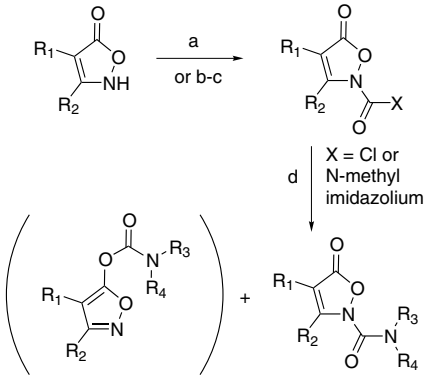
Small alkyl groups were generally preferred at the R_2 position, and this substituent was standardized as a methyl group. Replacement of the thioaryl R_1 group was well-tolerated as long as the substituent remained reasonably bulky (Table 1). Branched alkyl groups and ether-containing chains showed particular potency. These trends large carried over into the cellular assay, with some notable exceptions (vide infra.). IC_{50} values in the 3T3-L1 assay, while very reproducible, showed wider SAR variations here and in other structural series (see the ether compounds **16** and **17**, e.g.).

Our chemistry allowed a full exploration of what proved to be the most sensitive area, the urea nitrogen. Acyclic ureas showed a wide range of activity (Table 2). Monosubstitution usually lowered potency against the enzyme, and invariably led to inactivity in the cellular assay (**20**–**23**). Meanwhile, the enzyme activity seemed intolerant of steric bulk at this position (see **39**–**40**). These trends led us to monomethyl disubstituted ureas. Wide variations in potency were still the rule, but benzyl and heteroarylmethyl compounds (**25**–**27** and **30**–**31**) showed very good activity. Note, however, the inactivity of the substituted benzyl compounds **28** and **29**. These SAR trends could be reliably extrapolated to the other 4-substituents in the previous table.

Among cyclic amines (Table 3), six-membered rings remained the most attractive. Larger rings (**55**–**56**) showed similar steric liabilities as in the acyclics. Additional heteroatoms (**46**–**47**, **53**–**54**) generally lowered enzymatic or cellular potency, with the exception of N-benzyl and N-arylpiperazines (**48**–**52**).

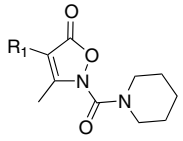


Scheme 1. Reagents and conditions: (a) NH_2OH, aq NaOH; (b) aq HCl; (c) ketone, aq HCl; (d) $NaBH_4$, MeOH.



Scheme 2. Reagents and conditions: (a) phosgene, CH_2Cl_2; (b) N,N′-carbonyldiimidazole; (c) CH_3I; (d) R_3R_4NH.

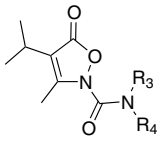
Table 1. IC_{50} values (in nM) for 4-substituted 5-(2*H*)-isoxazolones



Compound	R_1	IC_{50} (HSL) (nM)[a]	IC_{50} (3T3-L1) (nM)[a]
1	S4-Chlorophenyl	6	135
2	SO_2(4-chloro)phenyl	35	1270
3	S Phenyl	6	190
4	Benzyl	5	90
5	CH_3	13	655
6	Ethyl	18	325
7	*n*-Butyl	7	61
8	Isopropyl	2	75
9	Isobutyl	5	32
10	*tert*-Butyl	14	90
11	Cyclopentyl	6	22
12	Cyclohexyl	11	27
13	4-Tetrahydropyranyl	71	200
14	4-Tetrahydrothiopyranyl	11	98
15	4-Tetrahydrothiopyranyl dioxide	55	380
16	$CH_2CH_2OCH_3$	10	180
17	CH_2CH_2OPh	4	2
18	CH_2(N-CH_3)-2-indolyl	17	177
19	Phenyl	14	150

[a] Values are means of three experiments.

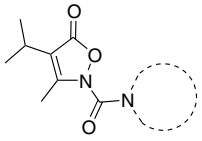
Table 2. IC_{50} values (in nM) for acyclic ureas



Compound	R_3	R_4	IC_{50} (HSL) (nM)[a]	IC_{50} (3T3-L1) (nM)[a]
20	H	*tert*-Butyl	>1000	Na
21	H	Phenyl	>1000	Na
22	H	8-Quinolinyl	Na	Na
23	H	CH_2cyclohexyl	72	Na
24	CH_3	Cyclohexyl	9	>1000
25	CH_3	Benzyl	5	67
26	CH_3	(2-Fluoro)benzyl	4	10
27	CH_3	(3-Fluoro)benzyl	5	12
28	CH_3	(4-Fluoro)benzyl	111	1000
29	CH_3	(4-Methyl)benzyl	408	>1000
30	CH_3	CH_2(2-furanyl)	4	9
31	CH_3	CH_2(2-thienyl)	2	3
32	CH_3	CH_2CH_2CN	>1000	Na
33	CH_3	CH_2CH_2Ph	180	300
34	CH_3	CH_2CH_2 (2-indolyl)	Na	Na
35	CH_3	CH_2CONH_2	>1000	Na
36	CH_3	Phenyl	7	190
37	CH_3	4-Chlorophenyl	17	>1000
38	CH_3	2-Pyridyl	240	Na
39	Ethyl	Ethyl	1400	600
40	Isopropyl	Isopropyl	Na	Na

[a] Values are means of three experiments (Na = not active).

Table 3. IC_{50} values (in nM) for cyclic amine-derived ureas



Compound	Cyclic amine	IC_{50} (HSL) (nM)[a]	IC_{50} (3T3-L1) (nM)[a]
8	Piperidine	2	75
41	Pyrrolidine	15	28
42	2,5-Dimethylpyrrolidine	Na	Na
43	3-Pyrroline	72	120
44	Indoline	>1000	Na
45	Tetrahydroquinoline	60	>1000
46	Piperazine	>1000	Na
47	N-Methylpiperazine	314	>1000
48	N-Benzylpiperazine	65	56
49	N-Phenylpiperazine	6	27
50	N-(2-Chlorophenyl)piperazine	30	31
51	N-(3-Methoxyphenyl)piperazine	1	13
52	N-(2-Pyrimidinyl)piperazine	67	51
53	Morpholine	52	>1000
54	Thiomorpholine	8	290
55	Homopiperidine	62	215
56	Azacyclooctane	900	Na

[a] Values are means of three experiments (Na = not active).

In vivo experiments in fasted mice[4,14] (data not shown) indicated that the piperidine-derived compounds were generally more efficacious at lowering plasma free fatty acids than other similarly potent in vitro compounds. SAR studies in this series are shown in Table 4.

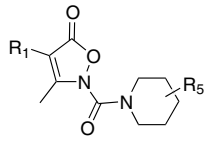
Again, any increase in steric bulk (**57**, **88**) near the proximal nitrogen was disfavored. Substitution at the 3- and 4- positions was generally well tolerated, although many polar groups (**60**–**61**, **80**, **82**–**84**, **91**–**92**) did not fare well. Exceptions were the esters **62** and **81**, but ester isosteres met with mixed success (**85**–**86**).

4-Methyl (**63**–**68**) and 4-phenyl (**70**–**76**) derivatives showed reliably strong enzyme and cellular potency. Note, however, the decreasing activity of the progressively more bulky compounds **77**–**79**.

This class of compounds was very clean in broad activity screens against various receptors and other classes of enzymes, but a counterscreen against a known acyl peptide hydrolase[13b] narrowed the field. Compound **43**, for example, has an IC_{50} against this enzyme of 9 nM, while **8** is >1 μM and **59** is >10 μM. None of the compounds showed appreciable potency against acetylcholinesterase,[5b,20] hepatic lipase, lipoprotein lipase, or pancreatic lipase.

Pharmacokinetic and toxicological findings, and the previously mentioned tests of in vivo efficacy, which will be detailed in separate publications,[14] led to the selection of the 3-methylpiperidine compounds. These showed superior blood levels and ability to suppress lipolysis, with fewer of the side effects seen in compounds of equal or greater potency. Resolution of the amine[21] before coupling showed that the *S*-enantiomer **59** was preferred

Table 4. IC_{50} values (in nM) for piperidine derivatives



Compound	R_1	R_5	IC_{50} (HSL) (nM)[a]	IC_{50} (3T3-L1) (nM)[a]
8	Isopropyl	H	2	75
57	Isopropyl	2-Methyl	93	400
58	Isopropyl	3-(*R*)-Methyl	20	187
59	Isopropyl	3-(*S*)-Methyl	3	28
60	Isopropyl	3-Hydroxymethyl	180	800
61	Isopropyl	3-$CON(Et)_2$	>1000	Na
62	Isopropyl	3-CO_2Et	28	170
63	Isopropyl	4-Methyl	6	40
64	*n*-Butyl	4-Methyl	1	10
65	Isobutyl	4-Methyl	3	18
66	Cyclohexyl	4-Methyl	4	20
67	CH_2CH_2OEt	4-Methyl	3	20
68	4-Tetrahydrothiopyranyl	4-Methyl	6	24
69	Isopropyl	4-Benzyl	102	>1000
70	Isopropyl	4-Phenyl	12	33
71	Isobutyl	4-Phenyl	10	4
72	Cyclopentyl	4-Phenyl	24	8
73	$CH_2CH_2OCH_3$	4-Phenyl	11	5
74	Isopropyl	4-(3-Fluorophenyl)	12	4
75	$CH_2CH_2OCH_3$	4-(3-Fluorophenyl)	20	0.2
76	$CH_2CH_2OCH_3$	4-(3-Methylphenyl)	23	4
77	Isopropyl	4-(4-Methylphenyl)	37	246
78	Isopropyl	4-(4-CF_3-phenyl)	160	—
79	Isopropyl	4-(4-*t*-Bu-phenyl)	480	—
80	Isopropyl	4-COphenyl	250	>1000
81	Isopropyl	4-CO_2Et	6	49
82	Isopropyl	4-Hydroxy	166	450
83	Isopropyl	4-Keto	200	150
84	Isopropyl	4-$CON(CH_3)_2$	>1000	Na
85	Isopropyl	4-(4-CH_3-2-isoxazoyl)	28	73
86	Isopropyl	4-(5-CH_3-2-oxadiazolyl)	>1000	Na
87	Isopropyl	4-N-Piperidinyl	Na	Na
88	Isopropyl	2,5-Dimethyl	460	>1000
89	Isopropyl	3,3-Dimethyl	7	48
90	Isopropyl	3,5-Dimethyl	326	140
91	Isopropyl	4-CN-4-Phenyl	>1000	Na
92	Isopropyl	4-OH-4-Phenyl	>1000	>1000

[a] Values are means of three experiments (Na = not active).

(see Table 4), and this compound was chosen for further evaluation.

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