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Human Glucagon Receptor Antagonists Based on Alkylidene Hydrazides

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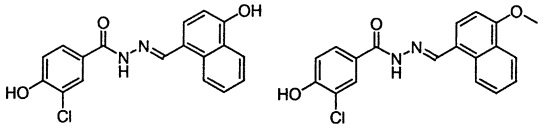
Abstract—A series of alkylidene hydrazide derivatives containing an alkoxyaryl moiety was optimized. The resulting hydrazide-ethers were competitive antagonists at the human glucagon receptor. Pharmacokinetic experiments showed fast clearance of most of the compounds tested. A representative compound [4-hydroxy-3-cyanobenzoic acid (4-isopropylbenzyloxy-3,5-dimethoxy-methylene)hydrazide] with an IC_{50} value of 20 nM was shown to reduce blood glucose levels in fasted rats.

Glucagon stimulates glycogenolysis and gluconeogenesis resulting in increased levels of glucose in the blood.[1,2] In diabetes, the bihormonal hypothesis implicates not only the lack of an insulin effect but also a paradoxically elevated relative level of circulating glucagon. It has been demonstrated that immunoneutralizing both exogenously administered as well as endogenous glucagon in various animal species effectively alleviates glucagon-stimulated hyperglycemia.[3–5] Thus, small molecule glucagon receptor antagonists[6–9] may be useful agents for the treatment of diabetes.

Previously we reported the discovery of a class of human glucagon receptor antagonists exemplified by 3-chloro-4-hydroxybenzoic acid (4-hydroxy-1-naphthylmethylene)hydrazide (**1**) prepared from the condensation of 3-chloro-4-hydroxybenzoylhydrazide and 4-hydroxynaphthaldehyde.[10] Loss of hydrogen-bond donation capability of **1** (IC_{50} = 200 nM) by methylation of the naphthylhydroxy group to compound **2** (IC_{50} = 870 nM) did not result in substantial loss of affinity. This observation prompted us to further elaborate structure **1** through alkylation of the naphthylhydroxy group. In this Letter we report further optimization of a series of alkylidene hydrazides having high affinity for the human glucagon receptor, representative in vitro metabolism, in vivo pharmacokinetic and pharmacodynamic results.



The alkylidene hydrazides were prepared either individually or in parallel as illustrated by the example in Scheme 1. Alkoxyarylaldehydes or ketones, obtained by alkylation of hydroxyarylaldehydes or ketones in the presence of potassium carbonate in acetonitrile, were condensed with hydrazides in DMSO. The starting hydrazides were prepared either by hydrazinolysis of the corresponding esters, or by amide-bond formation with *t*-butyl carbazate followed by deprotection. Indoles **18** and **19** were prepared by alkylation of formylindoles under sodium hydride conditions followed by hydrazone formation. Hydrazide **20** was obtained by reduction of hydrazone **9** with $NaBH_4$/cat. TFA.

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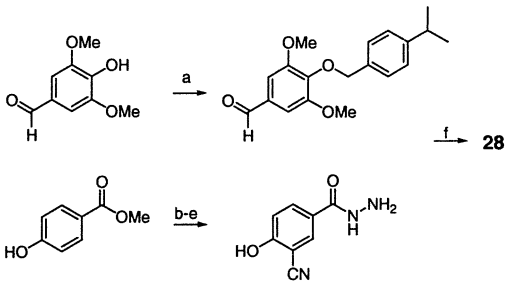
Coupling of alkoxydimethoxyphenethylamine with hydroxychlorobenzoic acid or the corresponding Pinner imidate[11] gave **21** or **22**, respectively. Compound **23** was prepared from the condensation of hydroxychloroaniline with the corresponding alkoxyphenylacrylic acid via HBTU activation. The sulfonylhydrazone **24** was obtained from the condensation of the corresponding sulfonylhydrazide[12,13] with the alkoxybenzaldehyde.

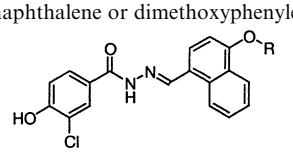
A targeted library consisting of hydrazide-ethers prepared according to Scheme 1 was produced. Screening of the library at 1 μM using ^{127}I-labeled glucagon resulted in identification of compounds with low nanomolar affinity for the human glucagon receptor with preference for the 4-hydroxynaphthyl and the 3,5-dimethoxy-4-hydroxybenzyl groups as the core moiety of the molecules. Among the alkylating agents used for *O*-alkylation of the core aryl moieties, hydrophobic aromatic groups were preferred by the receptor. Structure–activity relationships of representative resynthesized pure compounds are summarized in Table 1. Compounds alkylated with *para*-substituted benzyl groups were among the most potent (**3**–**4** and **9**–**11**), whereas compounds alkylated with hydrophilic groups such as **5** and **12** were less potent. Addition of two ethyl groups to the primary amide **5** resulted in compound **6**, displaying a 10-fold increase in binding affinity, indicating that lipophilic groups were preferred by the receptor. Incorporation of groups containing heteroatoms with hydrogen bond acceptor properties into this region of the molecules also resulted in compounds with modest affinity. For example, the pyranyl **7** and 1,2,4-oxadiazolyl **8** had IC_{50} values of 140 and 160 nM, respectively. The combination of 4-isopropylbenzyl with 3,4-dimethoxy-4-hydroxybenzaldehyde as in compound **9** (IC_{50} = 23 nM) led to a 10-fold improvement in binding affinity compared to **1** (IC_{50} = 200 nM).

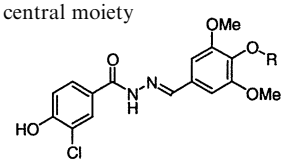


Scheme 1. Reagents and conditions: (a) K_2CO_3, CH_3CN, 4-isopropylbenzyl chloride, reflux 18 h (87%); (b) ICl, AcOH (30%); (c) CuCN, DMF, reflux 2 h (37%); (d) KOH, H_2O/THF, then HCl (87%); (e) *t*-BuOCONHNH$_2$, DIEA, PYBOP, then TFA (56%); (f) DMSO (82%).

Modifications of the naphthalene core moiety in **3** or the 3,5-dimethoxyphenylene moiety in **9** are shown in Table 2. Replacement of both methoxy groups in **9** (IC_{50} = 23 nM) with methyl groups in **13** (IC_{50} = 330 nM) resulted in a 15-fold decrease in affinity. Removal of one or both methoxy groups from **9** resulted in **14** and **15**, respectively, exhibiting 50-fold loss of affinity. Replacement of one methoxy for a carboxy group (**16**) resulted in further loss of affinity. Truncation of the naphthalene in **3** (IC_{50} = 53 nM) for a dimethylphenylene core **17** (IC_{50} = 740 nM) likewise exhibited a loss of affinity. On the other hand, compounds containing a 1,3- or 1,4-disubstituted indole bicyclic system (**18** or **19**) were well tolerated by the receptor, the 1,4-substituted

Table 1. In vitro activities of glucagon antagonists containing a naphthalene or dimethoxyphenylene central moiety





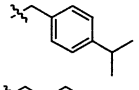
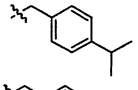
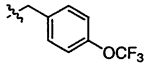
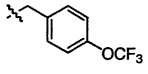
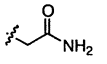
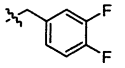
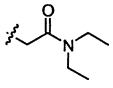
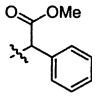
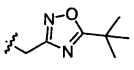
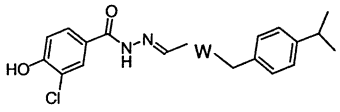
No.		IC_{50} (μM)	No.		IC_{50} (μM)
3		0.053	**9**		0.023
4		0.058	**10**		0.18
5		1.2	**11**		0.15
6		0.13	**12**		0.69
7		0.14			
8		0.16			

Table 2. In vitro activities of glucagon antagonists with modified naphthalene or dimethoxyphenylene moieties



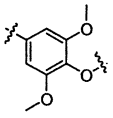
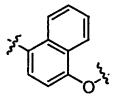
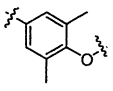
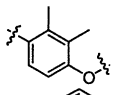
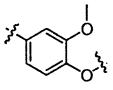
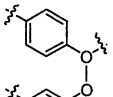
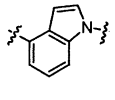
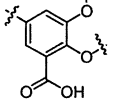
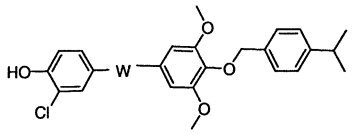
No.	W	IC_{50} (μM)	No.	W	IC_{50} (μM)
9		0.023	**3**		0.053
13		0.33	**17**		0.74
14		1.3	**18**		0.39
15		1.5	**19**		0.084
16		30			

Table 3. Explorations of alkylidene hydrazide isosteres



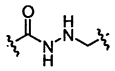
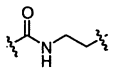
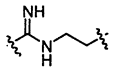
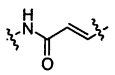
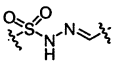
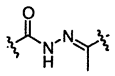
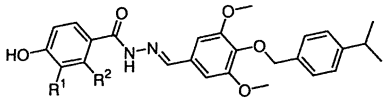
No.	W	IC_{50} (μM)
20		0.88
21		1.90
22		5.20
23		6.40
24		78.30
25		>100

Table 4. Effect of R^1 and R^2 substituents in the phenol ring



No.	R^1	R^2	IC_{50} (μM)
9	Cl	H	0.023
26	F	H	0.11
27	NO_2	H	0.050
28	CN	H	0.020
29	H	H	0.69
30	Cl	Cl	0.031
31	F	F	0.048
32	NH_2	H	0.94
33	OH	H	2.7

indole **19** being more potent (84 nM). These results indicated that the arylene core moiety needs to be hydrophobic and electron-rich in order to give compounds with high affinity towards the human glucagon receptor.

The alkylidene hydrazide region proved to be sensitive to modification. Several alkylidene hydrazide equivalents were explored (Table 3). The isosteres consisted of a four atom system possessing the ability to exist in an iminol-like tautomeric form and containing at least one sp^2 carbon center. The isosteres investigated were at least one order of magnitude less active than the hydrazone **9**. Reduction of the sp^2 hydrazone carbon in **9** to the sp^3 hydrazide **20**, replacement of the planar amide moiety with the tetrahedral sulfonamide **24**, and

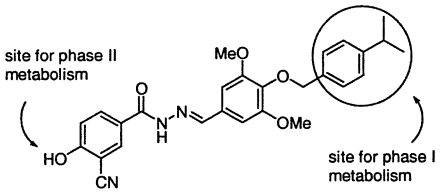


Figure 1. Sites of metabolism for **28**.

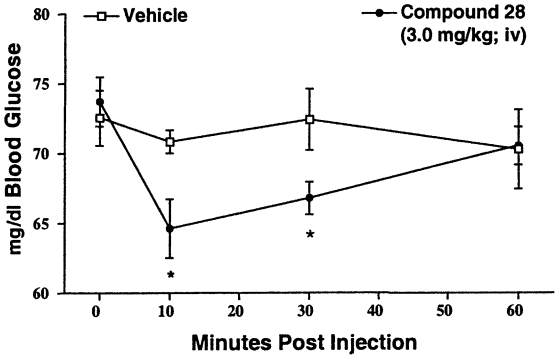


Figure 2. Compound **28** significantly reduced blood glucose at 10 and 30 min post injection. At 60 min post injection blood glucose was not different from control. $*p<0.05$ versus vehicle.

methylation of the hydrazone carbon to **25** led to compounds with decreased activities. It appeared that the position of the carbonyl and the nitrogen functionalities, and overall geometry of this region, were crucial to the overall recognition of the molecule by the receptor.

In vitro metabolism studies in rat liver microsomes indicated that the hydroxyl group in the benzoyl ring in **9** underwent rapid glucuronidation. To better understand the effect of this site on receptor affinity and the metabolic stability of this class of compounds, analogues containing various R^1 and R^2 substituents in the phenolic ring were prepared (Table 4). Previously we suggested[10] that the presence of an electron-withdrawing substituent lowered the pK_a of the hydroxy functionality, thus enhancing its ability to hydrogen-bond with the receptor. As expected, electron-withdrawing substituents such as chloro **9**, fluoro **26**, nitro **27**, and cyano **28** at the R^1 position resulted in compounds with much improved affinity compared to the unsubstituted phenol **29**. The chloro **9** and the cyano **28** were equipotent. Compound **28** displayed a mean IC_{50} value of 20 nM in a competition-binding assay using ^{127}I-radiolabeled glucagon and membranes from cells transfected with the human glucagon receptor. Compound **28** had a mean IC_{50} value of 1.0 nM for the rat receptor. Addition of a second halogen atom at R^2 (**30** and **31**) did not significantly affect the potency of the compounds. Electron-donating substituents at R^1 such as the amino group in **32** or the hydroxy group in **33** resulted in compounds with significantly less affinity for the receptor. In vitro metabolism experiments indicated a significantly diminished glucuronidation rate for **28** (27 pmol/min/mg protein) compared to **9**

(169 pmol/min/mg protein). In vivo pharmacokinetic experiments indicated that **28** had a significantly longer half-life than **9** ($t_{1/2} = 60$ min vs 18 min).

Compound **28** was investigated further with respect to the major metabolites formed in vitro and it was found that the compound was metabolized in both human and rat liver microsomes via both phase I (oxidation) and phase II (glucuronidation) pathways (Fig. 1). The major metabolic pathways in rat liver microsomes were identified to be hydroxylation, further oxidation to a carbonyl, or elimination of the alcohol to an alkene in the isopropyl group. Glucuronidation of the phenol moiety was found to be minor. Replacement of the chlorine atom by a cyano group therefore resulted in a metabolic swap from phase II to phase I metabolism within this series of compounds.

The nonpeptide glucagon antagonist **28** significantly lowered blood glucose in fasted rats (Fig. 2). Under the experimental conditions, maintenance of blood glucose levels in fasted rats is largely dependent on glucagon. Compound **28** did not affect resting glucose levels in fed rats (data not shown).

In summary, a series of alkylidene hydrazides was optimized to low nanomolar affinities at the human glucagon receptor. Compound **28** had an IC_{50} value of 20 nM and significantly lowered blood glucose in fasted rats. Replacement of the chlorine substituent with a cyano group in the C3 position of the benzoyl ring led to a metabolism swap from largely glucuronidation of the compound at the hydroxyl group to rapid phase I metabolism of the isopropyl group in compound **28**. Further in vitro metabolism and PK optimization of this series will be reported in the near future.

Acknowledgements

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