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POTENT, ORALLY ABSORBED GLUCAGON RECEPTOR ANTAGONISTS

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Abstract: The SAR of 2-pyridyl-3,5-diaryl pyrroles, ligands of the human glucagon receptor and inhibitors of p38 kinase, were investigated. This effort resulted in the identification of 2-(4-pyridyl)-5-(4-chlorophenyl)-3-(5-bromo-2-propyloxyphenyl)pyrrole **49** (L-168,049), a potent (Kb = 25 nM), selective antagonist of glucagon. © 1999 Elsevier Science Ltd. All rights reserved.

Introduction

Glucagon is a major counterregulatory hormone to insulin, stimulating glycogenolysis and gluconeogenesis.[1] Glucagon is a 29 amino acid peptide produced in the α-cells of the pancreas from a precursor peptide preproglucagon which is processed in the pancreas to give glucagon and, in the intestine, to provide glucagon like peptide 1 (GLP-1).[2] The receptors for glucagon are found primarily in the liver. The glucagon receptor is a 7 membrane spanning G-protein linked protein. Occupation of the receptor on hepatocytes by glucagon stimulates adenyl cyclase and increases free Ca^{2+}, resulting in increased glucose output.[3]

Hyperglucagonemia occurs in both insulin dependant diabetes (Type I) and non-insulin dependent diabetes (Type II). In both disease states the glucagon/insulin and glucagon/glucose ratios are elevated. The bihormonal hypothesis proposes that both insulin and glucagon contribute to elevated levels of glucose in diabetics.[4] Therefore, a glucagon receptor antagonist may mediate the formation of hepatic glucose, lower fasting plasma glucose levels and improve glucose tolerance in diabetics.

Considerable progress directed at identifying a peptide antagonist of glucagon has been reported.[5] A nonpeptide antagonist would have pharmacokinetic advantages over a peptidyl drug. CP-99,711 was reported to displace ^{125}I-glucagon from rat liver receptors at low micromolar concentrations and to antagonise glucagon stimulated cyclic adenosine monophosphate (cAMP) production in rat liver homogenates.[6] More recently substituted pyridines and biphenyls have been claimed as glucagon antagonists.[7] We wish to report the discovery of a series of ligands of the human and murine glucagon receptor.[8] Of these ligands, two, **11** and **49**, have been demonstrated to be orally bioavailable antagonists of the human glucagon receptor.

Chemistry

Compounds were prepared by adaptation of methods 1–3 which are illustrated in Scheme 1.[9] Condensation of the silyl acyloin **A** with an acetophenone **B** provided low yields of the pyrroles **C** in a one pot reaction.[10] A higher yielding route was developed from the 4-fluorophenyl acetopyridine **D**, prepared by alkylation of 4-pyridine carboxaldehyde dimethylacetal with 4-fluorobenzyl bromide followed by acid catalyzed hydrolysis.[11] Alkylation of **D** with α-bromo ketones,[12] followed by condensation with ammonia formed *in situ* by heating ammonium carbonate in acetic acid formed pyrroles **F** in excellent yield. Analogs with 3-position variations were predominantly prepared via method 3. Chalcones **G** are commercially available or readily prepared by condensation of 4′-chloro acetophenone **F** with an aldehyde. Application of the Stetter reaction provided the 1,4-diketone **H** in good yield, which in turn were converted to the desired pyrroles **I**.[13] The general synthetic method and chemical conversions amongst analogs are indicated below the Tables 1–3.

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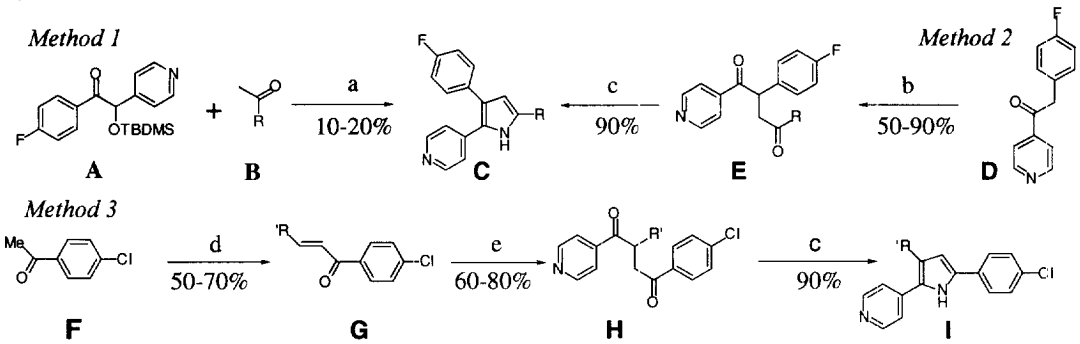
Scheme 1

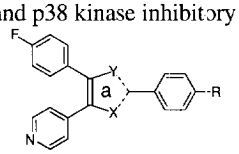


a. $RCOCH_3$, KCN, EtOH, reflux then add excess NH_4OAc reflux. b. $NaN(TMS)_2$, DMSO, $RCOCH_2Br$. c. NH_4OAc, HOAc, 110°C. d. R'CHO, NaOH, EtOH. e. NaCN (cat.), DMF, pyridaldehyde.

Biology

Glucagon receptor binding affinity was determined by radioligand binding assay by measurement of the reduction in binding of ^{125}I-glucagon to the human glucagon receptor (hGLUR) expressed on CHO cells.[14] The affinity of synthetic analogs for the receptor was reduced five to tenfold in the presence of physiological concentrations of Mg^{2+} (5 mM). Therefore, binding affinity was also determined for the more potent analogs in the presence of Mg^{2+} (5 mM). The importance of murine models of diabetes necessitated the determination of mGLUR affinity. p38 inhibition was determined as described previously.[15] Functional activity was determined by measurement of the inhibition of glucagon stimulated cAMP synthesis in CHO cells expressing the hGLUR.[13]

Table 1. Glucagon receptor binding affinity and p38 kinase inhibitory potency of initial screening leads.



#	X	Y	a	R	Synthetic Method	IC_{50} p38 μM n = ( )	IC_{50} hGLUR $-Mg^{2+}$ μM n = ( )
SB203580	N	NH	imidazole	S(O)Me	ref 16	0.037 (69)	18% @ 2 μM (1)
1	N	NH	imidazole	SMe	ref 17	0.117 (1)	0.49 (1)
2	CH	NH	pyrrole	S(O)Me	a	0.0053 (29)	31% @ 2 μM (2)
3	NH	CH	pyrrole	S(O)Me	a	0.67 (2)	1.1 (8)
4	CH	NH	pyrrole	SMe	3	0.044 (1)	0% @ 2 μM (2)
5	NH	CH	pyrrole	SMe	1	1.23 (1)	0.29 (1)

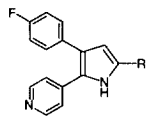
a: **4** or **5** $K_2S_2O_8$, HOAc, H_2O

Results

Screening of the Merck sample collection for compounds with affinity for the cloned human glucagon receptor (hGLUR) identified **1**, the precursor to SB203580, an inhibitor of p38 kinase,[17] as a weak hGLUR ligand.[18] We had prepared the regioisomeric pyrrole analogs of SB203580 and its precursor **1** in an effort to identify p38 inhibitors with advantages over SB203580.[19] Of the two pyrrole regioisomeric analogs of **1**, **2** and **3** the proximal analog **3** had substantially reduced kinase potency (126-fold) and was a ligand for the hGLUR. The synthetic precursor to **3**, **5**, was fivefold more potent than the sulfoxide **3** as a hGLUR ligand with reduced p38 inhibitory potency. Therefore, the SAR of 2-pyridyl pyrroles was explored further.

The effect of modification of the 5-position of 2-(4-pyridyl)-3-(4-fluorophenyl)pyrroles is illustrated in Table 2. The absence of a 5-substituent (**6**) or presence of a small alkyl group (**7**) disrupted hGLUR affinity while maintaining p38 potency. The N-methyl-4-piperidinyl analog (**8**) provided a highly potent and selective p38 inhibitor. hGLUR binding was restored by introduction of cyclohexyl (**9**) or phenyl analog **10**. Halogen substitution improved glucagon binding while reducing p38 potency (**11–14**). Glucagon receptor affinity and p38 potency were both reduced by alkoxy or alkyl substitution (**15–18**). Incorporation of acidic, basic or electron withdrawing substituents containing pi bonds such as ester, nitro or cyano reduced hGLUR binding while increasing p38 potency (**18–23**). The data suggested that the optimal pyrrole 5-substituent for hGLUR was 4-chlorophenyl (**11**).

Table 2. hGLUR binding affinity and p38 inhibitory potency of pyrrole 5 position analogs.



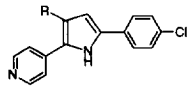
#	R	Synthetic Method	IC_{50} hGLUR-Mg^{2+} μM n = ( )	IC_{50} hGLUR+Mg^{2+} μM n = ( )	IC_{50} mGLUR-Mg^{2+} μM n = ( )	IC_{50} mGLUR+Mg^{2+} μM n = ( )	IC_{50} p38 μM	IC_{50} hGlUR +Mg^{2+}/IC_{50} p38
6	H	a	>3 (1)	>3 (1)	-	-	0.16	>18
7	Me	2	>3 (1)	-	-	-	0.32	>9
8	N-Me-piperidinyl	b	7% @ 2 (1)	-	-	-	0.02	>130
9	c-hex	2	0.09 (2)	0.49	>1	>1	0.18	2.7
10	Ph	1	0.39 (6)	-	2.7 (1)	-	0.8	-
11	4-Cl-Ph	1	0.08 (7)	0.8 (1)	0.2 (1)	43% @ 1 (2)	1.4	0.57
12	3-Cl-Ph	2	0.04 (2)	0.99 (2)	0.46 (1)	28% @ 1 (1)	0.42	2.3
13	2,4-(Cl)-Ph	2	0.04 (2)	0.95 (2)	0.34 (1)	13% @ 1 (2)	0.42	2.2
14	4-F-Ph	1	0.09 (5)	0.35 (1)	0.43 (2)	7% @ 1 (1)	0.34	1.0
15	4-OMe-Ph	1	0.59 (5)	-	-	-	2.8	
16	4-Me-Ph	1	0.25 (3)	2.2 (1)	-	-	1.69	1.3
17	4-Et-Ph	1	0.37 (1)	-	-	-	1.36	-
18	4-tBu-Ph	1	0.85 (1)	-	-	-	>10 (2)	-
19	4-(NO_2)-Ph	2	0.36 (2)	-	-	-	0.05	-
20	4-(CO_2Et)-Ph	2	0.85 (2)	-	-	-	0.26	-
21	4-(CN)-Ph	2	0.14 (3)	4.9 (1)	0.23 (3)	42% @ 3 (2)	0.05	102
22	4-(NH_2)-Ph	c	1.4 (2)	-	-	-	0.19	-
23	4-(COOH)-Ph	d	>3 (2)	-	-	-	0.09	-

a: i. Method 2 with allyl bromide, ii. O_3, Me_2S, iii. NH_4OAc, HOAc. b: 2 with N-(Cbz)-4-($BrCH_2CO$)-piperidinyl followed by LAH, THF reflux. c: **19**, $CoCl_2$, $NaBH_4$. d: **20**, KOH, MeOH.

mGLUR binding was determined for the more active hGLUR ligands. Affinity was less for the murine than that for the human receptor. The reduction in affinity varied from twofold (**11**) to tenfold (**12**).

The functional efficacy and species selectivity of **11** was determined. Compound **11** inhibited glucagon (100 pM) stimulated cAMP synthesis in CHO cells expressing the hGLUR (IC_{50} = 2 μM). Compound **11** does not inhibit glucagon binding to rat, guinea pig and rabbit liver membranes, but did inhibit binding to dog and mouse liver membranes (IC_{50} = 80 and 200 nM (-Mg^{2+}), respectively). Compound **11** was orally bioavailable as established by dosing at 3.0 mg/kg po in rats and measuring systemic levels of **11** (as determined by HPLC) of 230 (+/– 105) and 553 (+/– 107) ng/mL at 0.33 and 2.0 h, respectively.

Table 3. hGLUR binding affinity and p38 inhibitory potency of pyrrole 3-position analogs.



#	R	Syn. Method	IC_{50} hGLUR $-Mg^{2+}$ μM n = ( )	IC_{50} hGLUR $+Mg^{2+}$ μM n = ( )	IC_{50} mGLUR $-Mg^{2+}$ μM n = ( )	IC_{50} mGLUR $+Mg^{2+}$ μM n = ( )	IC_{50} μM p38	IC_{50} hGLUR $+Mg^{2+}$/ IC_{50} p38
24	H	a	0.14 (2)	>3 (2)	0.7 (1)	>3 (1)	>10	-
25	iPr	3	0.032 (2)	0.11 (1)	0.06 (1)	>3 (1)	>10	-
26	c-hex	3	0.1 (2)	>3 (1)	0.06 (1)	>3 (1)	>10	-
27	CO_2Et	2	0.09 (2)	0.4 (1)	0.5 (2)	>3 (2)	>1	-
28	COOH	b	>3 (1)	ND	ND	ND	>1	-
29	CO_2Bn	c	0.06 (3)	1.3 (1)	0.8 (1)	8% @1 (2)	69% @ 40	-
30	CONHBn	d	>3 (1)	ND	ND	ND	0% @ 1	-
31	Ph	3	0.06 (2)	0.38 (2)	ND	ND	0.21	1.8
11	4-F-Ph	1	0.08 (7)	0.8 (1)	0.19 (1)	43% @1 (2)	1.4	0.57
32	4-Cl-Ph	2	0.19 (4)	0.81 (1)	0.22 (5)	42% @3 (3)	0.16	5.0
33	4-Br-Ph	2	0.14 (3)	2.3 (2)	ND	ND	0.3	7.6
34	3-Br-Ph	2	0.15 (4)	1.7 (1)	0.23 (1)	48% @1 (2)	0.08	21.2
35	2-Br-Ph	2	0.026 (2)	2.3 (2)	ND	ND	1.0	2.3
36	4-(OMe)-Ph	2	0.1 (4)	1.6 (1)	0.24 (2)	16% @1 (2)	1.15	1.4
37	3-(OMe)-Ph	3	0.06 (2)	0.45 (2)	ND	ND	0.42	1.1
38	2-(OMe)-Ph	3	0.07 (2)	0.5 (1)	0.4 (1)	6% @1 (2)	2.65	0.18
39	2-(OEt)-Ph	3	0.04 (2)	0.5 (2)	0.34 (1)	22% @1 (1)	14.2	0.03
40	2-(OPr)-Ph	3	0.008 (3)	0.14 (1)	0.03 (4)	0.7 (1)	2.84	0.05
41	2-(Oallyl)-Ph	3	0.017 (2)	0.26 (2)	0.27 (1)	50% @3 (1)	4.74	0.05
42	2-(OBu)-Ph	3	0.01 (2)	0.18 (2)	0.08 (1)	1.2 (1)	16% @ 1	-
43	2-(OHx)-Ph	3	0.04 (2)	0.5 (2)	0.34 (1)	22% @1 (1)	2.68	0.18
44	2-(OiPr)-Ph	3	0.03 (2)	0.91 (2)	0.7 (1)	>1 (1)	5.54	0.16
45	2-(OiBu)-Ph	3	0.003 (2)	0.2 (2)	0.15 (2)	17% @1 (1)	3.76	0.05
46	2-(NHPr)-Ph	e	0.02 (2)	0.27 (1)	0.44 (1)	>3 (1)	52% @ 5	-
47	2,6-(OPr)-Ph	3	0.04 (2)	0.29 (2)	0.67 (1)	>3 (1)	5% @ 10	-
48	2-(OPr)-4-Br-Ph	3	0.004 (2)	0.13 (1)	0.049 (2)	0.83 (2)	-	-
49	2-(OPr)-5-Br-Ph	3	0.007 (4)	0.17 (4)	0.037 (2)	0.25 (2)	1.44	0.12
50	2-(OPr)-5-Cl-Ph	3	0.006 (2)	0.17 (2)	0.05 (1)	1.0 (1)	31% @10	-

a: **27** LiOH, H_2O, reflux. b: **27**, LiOH, H_2O. c: **28**, EDC, DMAP,. BnOH, DMF. d: **28**, EDC, HOBt, NMM, $BnNH_2$. e: i. Method 3 with nitrochalcone. ii. H_2, Pd/C, MeOH. iii. EtCHO, NaOAc, $NaCNBH_3$, MeOH

Further structural variation was focussed on the pyrrole 3-position with 4-chlorophenyl at the 5-position. Removal of the pyrrole 3-position substituent (**24**) gave a compound almost equipotent with **11**. Addition of alkyl substituents (**25**, **26**), a phenyl ring (**31**), or carboxy alkyl group (**27**, **29**) provided analogs with good glucagon receptor affinity. p38 inhibitory potency was of consequence only with the phenyl analog **31**. The carboxylic acid and amide analogs **28** and **30** lost all glucagon affinity. Replacement of fluoro in **11** with chloro (**32**) had little effect on GLUR binding but increased p38 potency tenfold. Comparison of the 4, 3 and 2 bromophenyl isomers **33–35** suggested that a 2-subsituent could potentially improve GLUR binding whilst reducing p38 potency (**35** hGLUR IC_{50} = 26 nM and p38 IC_{50} = 1.0 μM). The 4, 3 and 2 methoxy analogs **36–38** were approximately equipotent. Increasing the size of the 2 substituent from methoxy to ethoxy **39** and propoxy **40** improved hGLUR binding affinity and p38 selectivity. No improvement was elicited by alkoxy analogs **41–44**. The isobutoxy analog **45** was a more potent hGLUR ligand than propoxy analog **40** in the absence of magnesium. However, in the presence of magnesium the binding affinity was approximately the same. Replacement of the oxygen linking atom in **40** by nitrogen in **46** had little effect on either hGLUR or p38 potency.

2-Propoxy group analogs were prepared. 2,6-Dipropoxy analog **47** was less potent than the mono-propoxy analog **40**. Substitution with bromo at the 4-position (**48**) gave an analog equipotent with **40**. Interestingly, the 5-bromo-2-propoxy analog **49** was equipotent with **40** at the hGLUR but demonstrated a threefold improvement in mGLUR binding in the presence of Mg^{2+}. The importance of the 5-substituent is illustrated by the four fold reduction in mGLUR binding in the presence of Mg^{2+} elicited by the chloro analog **50**. Compound **49** was chosen for further pharmacological and pharmacokinetic studies.

Analog **49** inhibited glucagon (100 pM) stimulated cAMP synthesis in CHO cells expressing the hGLUR (IC_{50} = 41 nM). Schild regression analysis derived a Kb = 25 nM with a slope of 0.6, supporting a non-competive mechanism of action. Murine liver membranes produce cAMP in response to glucagon (C max = 2.0 pmoles/mg membrane in response to 10^{-7} M glucagon) stimulation. No cAMP was formed on treatment of murine liver membranes with 10^{-5}M **49**, demonstrating that **49** is not a glucagon receptor agonist. Compound **49** blocked cAMP formation stimulated by glucagon in murine liver membranes. Compound **49** does not bind to the GLP-1 receptor or other targets currently under investigation in these laboratories. Oral bioavailability was established in rats and mice as illustrated in Table 4.

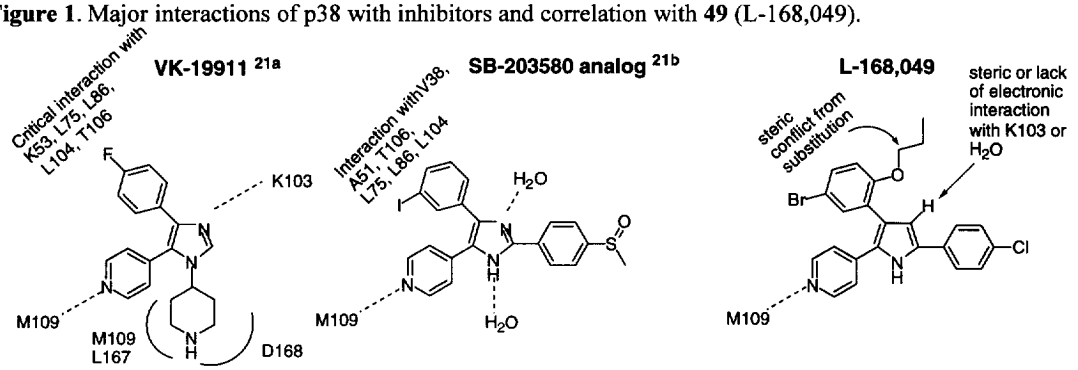
Table 4. Concentration (μM) of **49** in plasma following oral dosing in rats and mice.[20]

Species/dose po	Plasma source	0.25 h	1.0 h	2.0 h	4.0 h	6.0 h	24 h
Rat/3.0 mg/kg	Portal	0.025	1.24	1.40			
	Systemic	0.009	0.59	0.84			
Mouse/50 mg/kg	Systemic	1.7	15.2	11.7	16.5	13.1	0.7

Conclusions

We have shown that 2-pyridyl-3,5-diaryl pyrroles may be developed into orally active, potent, antagonists of glucagon at the human glucagon receptor. Appropriate substitution of the 3-aryl group in conjunction with positioning of the pyridyl ring proximally to the pyrrole NH provided selectivity against p38 kinase inhibition. Recently X-ray crystal structures of p38 complexed with inhibitors[21a,b] have been published and discussed[21c] as well as inhibitor binding studies with single amino acid mutants.[21d] This data supports a model for inhibitor/p38 binding which correlates with the p38 SAR data accumulated herein. Figure 1 illustrates how p38 inhibition in this series of compounds may be reduced by introduction of a 2-propoxy group on the pyrrole 3-position aryl ring (thought to be deeply embeded within the enzyme on binding) and the removal of the nitrogen atom of the imidazole 3-position. The glucagon inhibitory potency and pharmacokinetic properties of **49** suggest that **49**, and analogs derived therefrom, may be useful tools to determine the therapeutic relevance of glucagon inhibition in animal models of diabetes.

Figure 1. Major interactions of p38 with inhibitors and correlation with **49** (L-168,049).



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