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Substituted Imidazoles as Glucagon Receptor Antagonists

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Abstract—A modestly active, nonselective triarylimidazole lead was optimized for binding affinity with the human glucagon receptor. This led to the identification of a 2- and/or 4-alkyl or alkyloxy substituent on the imidazole C4-aryl group as a structural determinant for significant enhancement in binding with the glucagon receptor (e.g., **41**, $IC_{50} = 0.053\ \mu M$) and selectivity ($>1000\times$) over p38 MAP kinase in this class of compounds.

Glucagon is a 29-amino acid peptide hormone produced by the α-cells in the pancreas and is a major counter-regulatory hormone to insulin in the maintenance of glucose homeostasis.[1] Binding of glucagon to its receptor, which signals via G-proteins and which is primarily located in the liver, leads to enhanced levels of intracellular cyclic AMP and Ca^{2+}. This results in increased hepatic gluconeogenesis and glycogenolysis and attenuates the ability of insulin to inhibit these processes.[2] According to a bihormonal hypothesis, in Type II diabetes, elevated levels of circulating glucagon result in increased rates of hepatic glucose synthesis and glycogen metabolism, translating to excessive plasma glucose levels.[3] Therefore, antagonists of the glucagon receptor have the potential to modulate the rate of hepatic glucose output and improve insulin responsiveness in the liver, resulting in a decrease in fasting plasma glucose levels in diabetics.

Peptidyl antagonists of the glucagon receptor have been well-studied and several antagonists (without any partial agonism) have been reported recently,[4a–c] although facile metabolic cleavage remains of concern.[4d–f] Recent years have seen a considerable interest in the pursuit of nonpeptide glucagon antagonists,[5] for example, diaminostyryl-dichloroquinoxaline,[6] substituted benzimidazoles,[7] pyridylphenyls and biphenyls[8] have been described.

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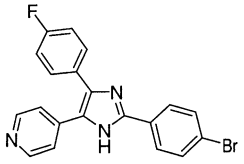
A screening effort to identify small nonpeptide antagonists of the human glucagon receptor (hGlur) led to the discovery of the triarylimidazole **1**. This compound exhibited an IC_{50} of 0.27 μM in the hGlur assay but also registered an IC_{50} of 0.16 μM in a p38 mitogen-activated protein (MAP) kinase assay. Herein, we describe the transformation of this lead into potent and selective triarylimidazole glucagon receptor antagonists.[9]



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Scheme 1 shows one of several routes used to prepare the imidazoles studied. Acylation of the anion of a protected 4-pyridinemethanol with a Weinreb amide provided a 2-(4-pyridyl), 2-siloxyaryl ketone. This compound was heated with an aldehyde in the presence of ammonium acetate and an oxidizing agent to form the triarylimidazole.[10] In some cases, another step is required to reach the target compound.[11] Compounds **1**–**21**, **25**–**27**, **30**–**34**, and **36**–**39** were prepared in this manner.

Compounds **22**–**23** were prepared by treatment of **21** with sodium hydride and iodomethane followed by chromatographic separation. The structural assignments were based on NOE difference spectra between the N-CH_3 and the *ortho*-protons of the aromatic ring.

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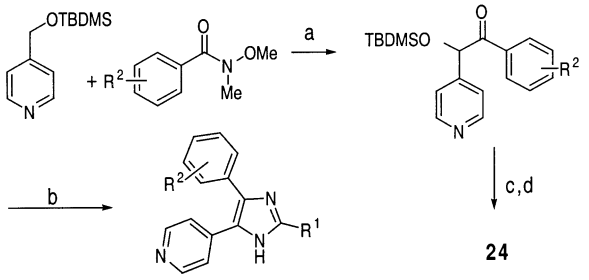


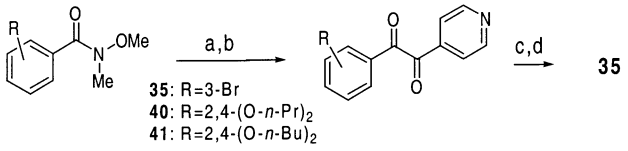
Scheme 1. (a) LDA, THF, −30°→0 °C, 50–75%; (b) R^1CHO, $Cu(OAc)_2$, NH_4OAc, HOAc, 115 °C, 4 h, 22–80%; (c) $(n\text{-}Bu)_4NF$, THF, −60 °C, $(4\text{-MeS})C_6H_4\text{-COCl}$, 30%; (d) NH_4OAc, HOAc, 90 °C, 3 h, 40%.

The oxazole **24** was prepared via the requisite ester as shown in Scheme 1.

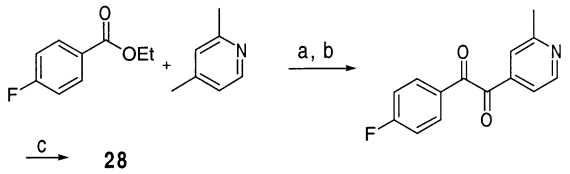
In the dione route (Scheme 2), acylation of the anion of 4-picoline with the requisite Weinreb amide provided the corresponding ketone, which was oxidized to the dione and then transformed to triarylimidazoles **40** and **41**. For the synthesis of **35**, subsequent to imidazole formation, palladium(0)-mediated biaryl coupling reaction between the 3-bromo functionality of the triarylimidazole and the corresponding phenylboronic acid provided the desired analogue.

A modified version of Scheme 2 was used to prepare compounds **28** and **29**. As shown in Scheme 3, the requisite substituted pyridyl moiety was prepared from deprotonation of the corresponding lutidine. Subsequent oxidation and imidazole formation provided the desired compound.

The glucagon receptor binding affinity of the compounds examined was determined by measuring the reduction in binding of ^{125}I-glucagon to hGlur expressed in CHO cell membranes in the presence of the test compound, as previously described.[12] In the presence of physiological concentrations of Mg^{2+} (5 mM), a reduction of 2- to 25-fold in binding affinity was observed for the



Scheme 2. (a) 4-Picoline, LDA, THF, −40°→0°C, 81%; (b) SeO_2, HOAc, 50%; (c) $(4\text{-Cl})C_6H_4CHO$, NH_4OAc, HOAc, 65%; (d) $Pd(PPh_3)_4$, NaOH, $C_6H_5B(OH)_2$, toluene, EtOH, 70%.



Scheme 3. (a) $NaN(TMS)_2$, THF, 0 °C, 20%; (b) SeO_2, Ac_2O, 75 °C, 24%; (c) $(4\text{-Cl})C_6H_4CHO$, NH_4OAc, HOAc, 60%.

compounds reported herein [e.g., for **1**: hGlur $IC_{50} = 0.27\ \mu M\ (-Mg^{2+})$; $IC_{50} = 2.9\ \mu M\ (+Mg^{2+})$].[9,12,13] Assays were run in duplicate and the inter-assay variability was ~50%.

As a counterscreen, the inhibition of human p38α MAP kinase (p38) by the test compounds was assessed as described previously.[14] Assays were run in duplicate and the inter-assay variability was ~3-fold.

Initially, the screening protocol was run in the absence of divalent magnesium ions.[12] Indeed, the discovery of the lead compound (**1**) and the preliminary development of the SAR of this class of glucagon receptor antagonists relied on this version of the assay. Table 1 shows that among C2-aryl substituted imidazoles, a 4-substituent on the aryl ring is preferred (**1**–**4**) The 4-bromo derivative (**1**) was the most active among 4-halo, 4-alkyl, and 4-aryl substitutions studied (**1** and **5**–**10**). Excessive steric bulk was not tolerated (**9** vs **10**). Analogues with a heteroatom directly attached to the 4-position of the imidazole C2-aryl moiety fared poorly (**11**–**13**). Those with a distal heteroatom or polar group also had moderate potency (**14**–**15**). Compounds **1** and **16**–**18** demonstrate the importance of the directionality of the ring substituent and the deleterious effect of a polar heteroatom in the ring (**16** vs **18**). Data from the α-naphthyl (**19**) and the phenethyl (**20**) derivatives show the effect of relaxing the structural constraint of the phenyl ring. Taken together, these data suggest that the binding space of these triaryl imidazoles with the glucagon receptor at the 2-position of the imidazole is largely hydrophobic, best accommodated by a 4-substituted aryl group, rather narrow, and quite directional towards the 4-position of the phenyl ring. As shown in Table 1, no separation from p38 activity was found in this series.

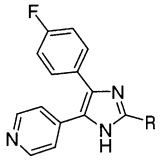
Table 2 shows the crucial role of the basic imidazole hydrogen. Comparing the data from **21** versus **22**–**24**, it is clear that the NH is required for reasonable glucagon binding.

The significance of the 4-pyridyl group on the 5-position of the imidazole was studied. Data from compounds **25**–**27** (Table 3) show that this 4-pyridyl nitrogen cannot be substituted by a carbon or an exocyclic oxygen atom (e.g., the OH of phenol in **27**). Alkyl substitutions around the pyridyl ring were also studied (**28**–**29**). These all gave compounds with inferior binding to the glucagon receptor compared to the unsubstituted 4-pyridyl derivative **5**.

For the compounds presented above, the glucagon receptor affinity, where they are significant, are invariably accompanied by submicromolar activities in the MAP kinase assay. A breakthrough in the separation of this kinase activity from glucagon receptor binding came when the SAR of the 4-position of the imidazole was examined.

Studies on the 4-halo substituted 4-aryl moiety (**5** and **30**–**31**) suggested that larger substituents might increase glucagon receptor binding and could decrease p38

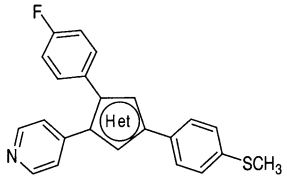
Table 1. In vitro binding potencies of triarylimidazoles for the human glucagon receptor and p38 MAP kinase: imidazole-C2 variations



Compound	R	hGlur IC_{50} (−Mg, μM)	p38 IC_{50} (μM)	Compound	R	hGlur IC_{50} (−Mg, μM)	p38 IC_{50} (μM)
1	(4-Br)Ph	0.27	0.16	**11**	(4-NH_2)Ph	2.0	0.07
2	C_6H_5	>20	0.13	**12**	(4-OH)Ph	na[a]	0.08
3	(2-Br)Ph	na[1]	0.09	**13**	(4-OMe)Ph	13	0.10
4	(3-Br)Ph	1.4	0.15	**14**	(4-CN)Ph	8.0	0.21
5	(4-Cl)Ph	0.40	0.08	**15**	(4CO_2Me)Ph	8.7	0.30
6	(4-F)Ph	2.0	0.10	**16**	(5-Br)-2-thienyl	2.2	0.11
7	(4-I)Ph	0.51	0.10	**17**	(4-Br)-2-thienyl	2.8	0.10
8	(4-Me)Ph	1.3	0.09	**18**	(5-Br)-2-furyl	na[a]	0.19
9	(4-*i*Pr)Ph	0.70	0.28	**19**	α-naphthyl	1.5	0.34
10	(4-Ph)Ph	10	0.30	**20**	phenethyl	na[a]	0.13

[a]na = <20% inhibition at 2 μM under the assay conditions.

Table 2. In vitro binding potencies of various compounds for the human glucagon receptor and p38 MAP kinase



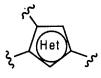
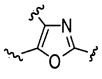
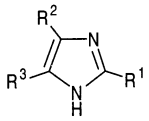
Compound	**21**	**22**	**23**	**24**
				
hGlur (−Mg) IC_{50} (μM or % inh. @ 2μM)	0.49	10%	38%	−8%
p38 IC_{50} (μM)	0.12	0.52	0.86	0.93

Table 3. In vitro binding potencies of triarylimidazoles for the human glucagon receptor and p38 MAP kinase: imidazole-C5 derivatives



Compound	R^1	R^2	R^3	hGlur IC_{50} (−Mg, μM)	p38 IC_{50} (μM)
25	(4-Br)Ph	C_6H_5	4-pyridyl	0.782	0.04
26	(4-Br)Ph	C_6H_5	C_6H_5	37% inh.[a]	2.2
27	(4-Br)Ph	C_6H_5	(4-OH)Ph	24% inh.[a]	0.10
28	(4-Cl)Ph	(4-F)Ph	3-Me(4-pyridyl)	1.1	0.02
29	(4-Cl)Ph	(4-F)Ph	2-Me(4-pyridyl)	38% inh.[a]	0.05

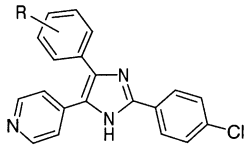
[a]% inhibition at 2 μM.

activity. Several 4-aryl and 4-alkyl derivatives were prepared. As seen on Table 4, this approach indeed allowed the identification of more selective glucagon receptor antagonists (**32**–**34**), the most active being the 4-*n*-butyl derivative (**34**).

A glucagon receptor binding assay incorporating physiological concentrations (5 mM) of Mg^{2+} ions was developed. When tested in this version, the activity of our glucagon-selective compounds decreased by 2- to 25-fold, as shown in Table 4. Compound **34** again stood out in its submicromolar glucagon binding affinity in the $+Mg^{2+}$ assay and selectivity over p38 kinase.

A number of analogues at all positions of the aryl ring were prepared in attempts to identify a selective compound possessing higher binding affinity with the glucagon receptor in the presence of Mg^{2+} (IC_{50} < 100 nM) while maintaining selectivity over p38 kinase. Compounds **35**–**41** are representative of this effort. As

Table 4. In vitro binding potencies of triarylimidazoles for the human glucagon receptor and p38 MAP kinase: imidazole-C4 analogues



Compound	R	hGlur IC_{50} (−Mg, μM)	p38 IC_{50} (μM)	hGlur IC_{50} (+Mg, μM)
5	4-F	0.40	0.080	3.2
30	4-Cl	0.19	0.023	2.2
31	4-I	0.13	0.14	0.75
32	4-Ph	0.14	3.3	3.8
33	4-t-Bu	0.13	>10	3.4
34	4-n-Bu	0.074	>10	0.15
35	3-Ph	0.061	0.59	>3
36	2-OPh	0.0065	0.15	0.29
37	3-OPh	0.013	0.22	0.15
38	4-OPh	0.027	0.25	0.11
39	2-*O*-*n*-Bu	0.0085	>1	0.11
40	2,4(O-*n*-Pr)$_2$	0.013	2.4	0.12
41	2,4(O-*n*-Bu)$_2$	0.0065	20% inh. @ 40 μM	0.053

shown in Table 4, the two phenyl substituted derivatives (**32** and **35**) show that they induced very high Mg^{2+}-shifts in the binding assay to result in relatively inactive compounds. The phenoxy-substituted analogues (**36**–**38**) showed less Mg^{2+}-shifts (**35** vs **37**, **32** vs **38**) to provide **38**, a reasonably potent compound. Unfortunately, **38** and its positional isomers **36** and **37** had considerable p38 MAP kinase activity. The 2-*n*-butoxy derivative[9] **39** was prepared and found to be more selective than the corresponding aryloxy compound **36**. The 2,4-bisalkoxy analogues **40** and **41** were also synthesized and compound **41** indeed met our criteria as a potent and selective triarylimidazole derivative for the glucagon receptor.

By systematic structure–activity studies on all of the 5 positions of the central imidazole ring, a nonselective triarylimidazole glucagon receptor antagonist lead, **1** (hGlur $IC_{50}=2.9\,\mu M$, p38 $IC_{50}=0.16\ \mu M$) was transformed into a selective glucagon receptor antagonist **41** (hGlur $IC_{50}=0.053\,\mu M$, p38 $IC_{50}=20\%$ inh. @ $40\,\mu M$). The key discovery was the identification of a glucagon receptor determinant, an alkyl or alkyloxy group on the phenyl ring of imidazole-C4, which separated the glucagon receptor affinity from the kinase activity and provided the needed enhancement in glucagon receptor binding. This work should serve towards the identification of compounds suitable for studies that provide insights into the role of glucagon receptor antagonism in the management of glucose homeostasis.

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References and Notes

1. (a) Mojsov, S.; Heinrich, G.; Wilson, I. B.; Ravazzola, M.; Orci, L.; Habener, J. *J. Biol. Chem.* **1987**, *261*, 11880. (b) Orsov, C.; Holst, J. J.; Knuhtson, S.; Baldissera, F. G. A.; Poulson, S. S.; Nielsen, O. V. *Endocrinology* **1986**, *119*, 1467.
2. Burcelin, R.; Katz, E. B.; Charron, J. J. *Diabetes Metabolism* **1996**, *22*, 373.
3. Unger, R. H. *Metabolism* **1978**, *27*, 1691.
4. (a) Ahn, J.-M.; Medeiros, M.; Trivedi, D.; Hruby, V. J. *J. Med. Chem.* **2001**, *44*, 1372. (b) Azizeh, B. Y.; Van Tine, B. A.; Trivedi, D.; Hruby, V. J. *Peptides* **1997**, *18*, 633. (c) Van Tine, B. A.; Azizeh, B. Y.; Trivedi, D.; Phelps, J. R.; Houslay, M. D.; Johnson, D. G.; Hruby, V. J. *Endocrinology* **1996**, *137*, 3316. (d) Azizeh, B. Y.; Van Tine, B. A.; Sturn, N. S.; Hutzler, A. M.; Davic, C.; Trivedi, D.; Hruby, F. J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1849. (e) Unson, C. G.; Andreu, D.; Gurzenda, E. M.; Merrifield, R. B. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 4083. (f) Gysin, B.; Johson, D. G.; Trivedi, D.; Hruby, V. J. *J. Med. Chem.* **1987**, *30*, 1409.
5. (a) Livingston, J. N.; Schoen, W. R. *Annu. Rep. Med. Chem.* **1999**, *34*, 189. (b) Nuss, J. M.; Wagman, A. S. *Annu. Rep. Med. Chem.* **2000**, *35*, 211.
6. Collins, J. L.; Dambek, P. J.; Goldstein, S. W.; Faraci, W. L. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 915.
7. Madsen, P.; Knudsen, L. G.; Wiber, F. C.; Carr, R. D. *J. Med. Chem.* **1998**, *41*, 5150.
8. (a) Schmidt, G.; Angerbauer, R.; Brandes, A.; Muller-Gliemann, M.; Bischoff, H.; Schmidt, D.; Wohlfeil, S.; Schoen, W. R.; Ladouceur, G. H.; Cook, J. H.; Lease, T. G.; Wolanin, D. J.; Kramss, R. H.; Hertzog, D. L.; Osterhout, M. H. WO98/04528, 1998; *Chem. Abstr.* **1998**, *128*, 167354s. (b) Cook, J. H.; Doherty, E. N.; Ladouceur, G.; Livingston, J. N.; MacDougall, L. *Abstract of Papers*, Part 2, 216th National Meeting of the American Chemical Society, Boston, MA, Aug. 23–27, American Chemical Society: Washington, DC, 1998; MEDI 285. (c) Ladouceur, G.; Cook, J.; Doherty, E.; Jones, H.; Hertzog, D.; Hundertmark, T.; Korpusik, M.; Lease, T.; Livingston, J.; MacDougall, M.; Osterhout, M.; Phelan, K.; Romero, R.; Shao, C.; Shoen, W. *Abstract of Papers*, Part 1, 218th National Meeting of the American Chemical Society, New Orleans, LA, Aug. 22–26, American Chemical Society: Washington, DC, 1998; MEDI 184.
9. (a) Related work from these laboratories: de Laszlo, S. E.; Hacker, C.; Li, B.; Kim, D.; MacCoss, M.; Mantlo, N.; Pivnichny, J. V.; Colwell, L.; Koch, G. E.; Cascieri, M. A.; Hagmann, W. K. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 641. (b) Kim, D.; Leung, K.-P.; MacCoss, M.; Koch, G. E.; Cascieri, M. A.; Hagmann, W. K. Abstract of Papers, Part 2, 216th National

Meeting of the American Chemical Society, Boston, MA, Aug. 23–27, American Chemical Society: Washington, DC, 1998; MEDI 67.
10. (a) Adams, J. L.; Gallagher, T. F.; Lee, J. C.; White, J. R. WO 93/14081. *Chem. Abstr.* **1993**, *1994*, 120 107005d. (b) Gallagher, T. F.; Seibel, G. L.; Kassis, S.; Laydon, J. T.; Blumenthal, M. J.; Lee, J. C.; Lee, D.; Boehm, J. C.; Fier-Thompson, S. M.; Abt, J. W.; Soreson, M. E.; Smietana, J. M.; Hall, R. F.; Garigipati, R. S.; Bender, P. E.; Erhard, K. F.; Krog, A. J.; Hofmann, G. A.; Sheldrake, P. L.; McDonnell, P. C.; Kuar, S.; Young, P. R.; Adams, J. L. *Bioorg. Med. Chem.* **1997**, *5*, 49. (c) Liverton, N. J.; Butcher, J. W.; Claiborne, F.; Claremon, D. A.; Libby, B. E.; Nguyen, K. T.; Pitzenberger, S. M.; Selnick, H. G.; Smith, G. R.; Tebben, A.; Vacca, J. P.; Varga, S. L.; Agarwal, L.; Dancheck, K.; Forsyth, A. J.; Fletcher, D. S.; Frantz, B.; Hanlon, W. A.; Harper, C. F.; Hofsess, S. J.; Kostura, M.; Lin, J.; Luell, S.; O'Neill, E. A.; Orevillo, C. J.; Pang, M.; Parsons, J.; Rolando, A.; Sahly, Y.; Visco, D. M.; O'Keefe, S. J. *J. Med. Chem.* **1999**, *42*, 2180.
11. All compounds prepared were characterized by mass spectrum (FAB), and 300 MHz, 400 MHz, or 500 MHz ^{1}HNMR
12. Cascieri, M. A.; Koch, G. E.; Ber, E.; Sadowski, S. J.; Louizides, D.; deLaszlo, S. E.; Hacker, C.; Hagmann, W. K.; MacCoss, M.; Chicchi, G. G.; Vicario, P. P. *J. Biol. Chem.* **1999**, *274*, 8694.
13. Divalent cations have been shown to allosterically enhance the binding of glucagon to hGlur (Chicchi, G. G.; Graziano, M. P.; Koch, G.; Hey, P.; Sullivan, K.; Vicario, P. P.; Cascieri, M.A *J. Biol. Chem.* **1997**, *272*, 7765) but their effects on antagonist binding affinity to hGlur are less well understood (see ref 12).
14. (a) LoGrasso, P. V.; Frantz, B.; Rolando, A. M.; O'Keefe, S. J.; Hermes, J. D.; O'Neill, E. A. *Biochemistry* **1997**, *36*, 10422. (b) Selnick, H. G.; Claremon, D. A.; Liverton, N. J. WO 97/12876, 1997; *Chem. Abstr.* **1997**, *126,* 317379d.