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Optimization of the 4-Aryl Group of 4-Aryl-pyridine Glucagon Antagonists: Development of an Efficient, Alternative Synthesis

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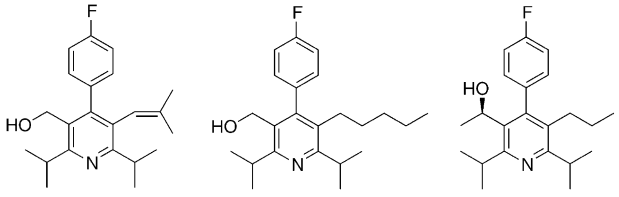
Abstract—A narrow structure–activity relationship was established for the 4-aryl group in 4-aryl-pyridine glucagon antagonists, with only small substituents being well-tolerated, and only at the 3′- and 4′-positions. However, substitution with a 2′-hydroxy group gave a ca. 3-fold increase in activity (e.g., 4′-fluoro-2′-hydroxy analogue **33**, $IC_{50} = 190$ nM). For efficient preparation of 2′-substituted phenylpyridines, a novel synthesis via pyrones and 4-methoxy-pyridines was developed.

As the incidence of Type 2 diabetes increases toward 200 million cases worldwide by 2010,[1] the search for effective therapies continues in earnest along several mechanistic strategies.[2] For example, the peptide hormone glucagon functions to trigger hepatic glucose production, and a strong body of evidence has prompted the pursuit of small-molecule glucagon receptor antagonists for the treatment of Type 2 diabetes.[3] Recently, we reported the discovery of 4-phenyl-pyridines as a novel class of selective glucagon antagonists [e.g., screening hit **1**, human glucagon receptor (hGR) binding affinity $IC_{50} = 7$ μM].[4] Investigation of substituents on the pyridine ring led to significantly more potent analogues such as **2** (hGR binding affinity $IC_{50} = 0.70$ μM; cAMP production $IC_{50} = 0.40$ μM) and **3** (hGR binding affinity $IC_{50} = 0.11$ μM; cAMP production $IC_{50} = 0.065$ μM).[4] In this article, we describe the optimization of the 4-aryl group in this compound series, and the development of an effective, alternative synthetic route to these analogues.



1 **2** **3**

Preparation of analogues of **2** having a variety of 4-aryl groups was achieved via application of the Hantzsch dihydropyridine synthesis.[5] Oxidation to the corresponding pyridine diester,[6] followed by mono-reduction to ester–alcohol, oxidation to ester–aldehyde, Wittig olefination, reduction and hydrogenation provided the desired analogues in generally excellent overall yields (Scheme 1).[4]

Following the Topliss sequential method to guide lead optimization,[7] the parent **4** and 4-chloro analogue **9** were first prepared, and were found to be somewhat less potent as glucagon antagonists than the lead **2** (Table 1).[8] The subsequent analogues prompted by the Topliss paradigm (e.g., **11**, **22**, **12**, or **13**, **10**, **14**, **15**, **27**, **24**) provided no significant gain in potency, relative to the parent (**4**). Pursuing a more extensive variety of analogues, it was found that small substituents (fluoro,

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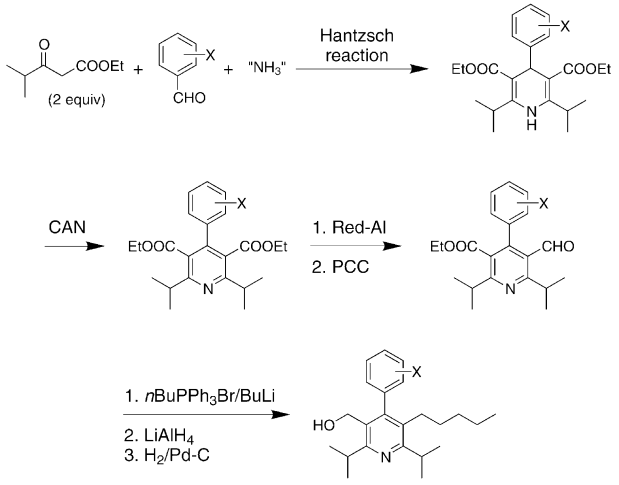
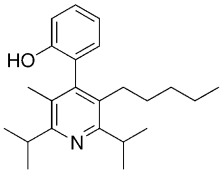


Scheme 1. Synthesis of analogues of **2**.

chloro, and methyl) were well tolerated at the 4′-position (**2**, **9**, and **13**), but gave rise to significant losses in activity when bonded at the 3′- or 2′-positions. A steep reduction in activity was seen as substituent size increased at the 4′-position, from methyl to ethyl and phenyl (**13**, **16**, and **18**). The substituent size limitations were also found to apply to electron-withdrawing and -donating substituents (e.g., **22**–**27**). With these results defining a rather tight binding pocket, it was gratifying to find that substitution by hydroxy at the 2′-position (**30**) afforded a ca. 3-fold improvement over the parent compound (**4**). Substitution at this position with amino, representing another H-bond donor/acceptor, was also quite well tolerated (**31**). A larger H-bond donor group at this position, however, was not (**32**). Finally, the beneficial effects of the 4′-F and 2′-OH substituents were found to be additive, with analogue **33** exhibiting a further increase in potency: hGR binding affinity $IC_{50}=190$ nM; cAMP production $IC_{50}=120$ nM. Although the factors responsible for the favorable 2′-OH substitution are not fully understood, consideration of molecular models indicates that this group may engage in hydrogen-bonding with the hydroxymethyl moeity on the pyridyl ring. It is noteworthy that slight displacement of the hydroxy group position gives rise to considerably less potent compounds (**34** and **35**). Also, the 2′-hydroxy moiety can not act as a surrogate for the 3-hydroxymethyl group, as **36** is relatively inactive (11% inhibition at 20 μM). Finally, it should be noted that the 2′-substituted analogues (e.g., **30** and **33**) are chiral (racemates), by virtue of atropisomerism[9] owing to restricted rotation about the phenyl–pyridine bond.



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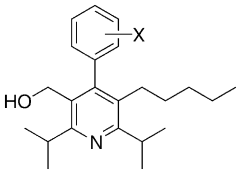
Identification of the favorable 2′-hydroxy substituent presented a new challenge, however. While the Hantzsch reaction afforded high yields in most cases, synthesis of the diester **37** (a precursor to **33**)[4b] was

Table 1. Glucagon antagonist activities[8] of analogues of **2**

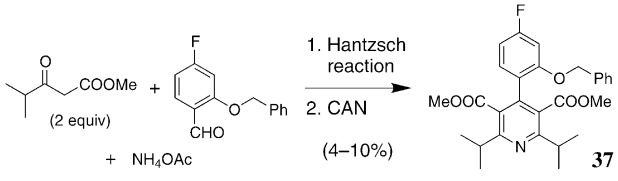


Compd	X	% inhibition (20 μM)	IC_{50} (μM)
2	4-F		0.70
4	H		1.4
5	3-F		3.0
6	2-F		9.0
7	3,4-F_2		1.1
8	2,4-F_2		11
9	4-Cl		0.8
10	3-Cl	49	
11	3,4-Cl_2	39[a]	
12	2,4-Cl_2	8	
13	4-CH_3		1.0
14	3-CH_3	46	
15	2-CH_3	33	
16	4-Et	42	
17	4-*i*Pr	34	
18	4-Ph	2	
19	2,3-$(CH)_4$	6	
20	3,4-$(CH)_4$	16	
21	3,4-$(CH_2)_4$	37	
22	4-CF_3	35	
23	3-CF_3	25	
24	4-SO_2CH_3	14[a]	
25	4-OCH_3	37	
26	3-OCH_3	48	
27	2-OCH_3	27	
28	4-OH	24	
29	3-OH		6.0
30	2-OH		0.50
31	2-NH_2		2.3
32	2-$NHCOCH_3$	13	
33	4-F-2-OH		0.19
34	2-CH_2OH	44	
35	4-F-3-CH_2OH		19[a]

[a]The *E*-pent-1-enyl rather than pentyl derivative was tested.[8]

quite problematic (Scheme 2). Indeed, the literature is replete with examples of low-yielding Hantzsch syntheses utilizing 2-substituted benzaldehydes.[10] We therefore embarked upon an alternative route to diester **37**, in which a pyrone would be converted to a 4-halopyridine, and then undergo a nucleophilic displacement by a carbon nucleophile such as an aryl Grignard reagent.

At the outset of this work, a survey of the literature revealed very few examples of pyrones having ester groups at the 3,5-positions and any carbon substituents at the 2,6-positions. Half of these examples had perfluoroalkyl groups at the 2- and/or 6-positions, and none had the required 2,6-diisopropyl pattern. Synthetic



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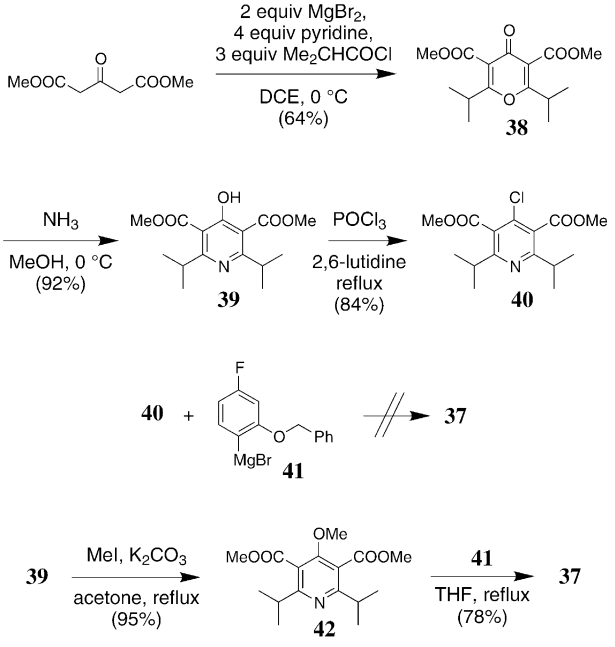
Scheme 2. Hantzsch route to intermediate **37**.

procedures reported for this class include a process via the anhydrous magnesium complex of diethyl acetone-1,3-dicarboxylate[11] or the condensation of copper ethyl acetoacetate with phosgene.[12] After investigation of a variety of reaction conditions, we found that the putative magnesium complex of dimethyl-1,3-acetonedicarboxylate could be formed in situ and then converted to the 2,6-diisopropyl pyrone **38**, by an efficient, one-pot process in 64% yield[13] (Scheme 3). In related examples, the 2,6-dimethyl and 2,6-dicyclohexyl analogues were prepared in similar yields, whereas the 2,6-di-*tert*-butyl and 2,6-diphenyl analogues were not obtained in significant yield through this procedure. Treatment of pyrone **38** with ammonia (gaseous or aqueous) readily gave the 4-hydroxy-pyridine **39**,[14] from which could be prepared the 4-chloro-pyridine **40**.[15] Unfortunately, however, treatment of **40** with aryl Grignard reagents such as **41** did not provide the desired coupled products.

We reasoned that an alternative leaving group on the pyridine diester might facilitate the coupling reaction. Noting that Meyers and colleagues have made extensive use of the methoxy group as nucleofuge in their elegant biaryl syntheses,[16] we speculated that the methoxy group might serve as an efficient leaving group in our own system, and were encouraged by the fact that it could be readily accessed from **39**. Methylation of **39** proved straightforward,[17] and, gratifyingly, warming diester **42** with Grignard reagent **41** afforded phenylpyridine **37** in 78% yield.[18] Moreover, purification of compound **37** involved only a simple trituration from hexane to afford analytically pure product. Curiously, we observed no addition of the Grignard reagent to the methyl ester groups in **42**, a consideration that had caught our attention at the outset.



Scheme 3. Alternative synthesis of intermediate **37**.

In summary, variation of the 4-aryl group in 4-aryl pyridine glucagon antagonists was investigated, and a wide variety of substituents was found to give reduced activity, as compared to the 4′-fluorophenyl lead **2**. Substitution by a 2′-hydroxy moiety was surprisingly well tolerated (**30**), and the most potent analogue identified in this study was a 4′-fluoro-2′-hydroxy-phenyl analogue (**33**, $IC_{50} = 190$ nM). To permit the efficient preparation of such 2′-substituted phenyl-pyridines, a novel synthesis via pyrones and 4-methoxy-pyridines was developed, that proceeds in high yield from readily available starting materials. In a future article, we will describe the effect of combining the optimized substitutions on the 4-aryl group (such as in **33**) and the pyridine ring (such as in **3**).

Acknowledgements

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8. All data reported herein reflect purified and characterized (^{1}H NMR, MS) samples. The human glucagon receptor binding assay was carried out as decribed previously.[4a] Selected compounds were also tested in a functional cell assay,[4a] and were determined to be antagonists. For many of the analogues listed in Table 1, the 3-(1-*E*-pent-1-enyl)-pyridyl derivatives were also tested, and these were found to have glucagon binding activities comparable to or very slightly less than those of the corresponding 3-(1-pentyl)-pyridyl derivatives.
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13. **Dimethyl 2,6-diisopropyl-4-oxo-4*H*-pyran-3,5-dicarboxylate (38)**. To a mixture of dimethyl 1,3-acetone-dicarboxylate (28.7 mL, 0.2 mol) in 200 mL of DCE (dichloroethane) was added magnesium bromide (37.6 g, 0.4 mol), with stirring at room temperature. The mixture was cooled to 0 °C, anhydrous pyridine (65 mL, 0.8 mol) was gradually added, and the mixture was stirred for 30 min. Isobutyryl chloride (90 mL, 0.6 mol) was then added dropwise at 0 °C, and then the mixture was allowed to warm to room temperature as it was stirred overnight. The mixture was again cooled to 0 °C, 100 mL of 1 N HCl was gradually added, and the mixture was stirred for 30 min. The aqueous phase was separated and extracted with DCE (2×). The combined organic phases were washed with brine (2×), dried ($MgSO_4$), and rotary evaporated to provide 43.3 g (73%) of crude product, which was recrystallized from EtOAc–hexane to provide pure **38** as white needles (37.6 g, 64%): mp 100–102 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.9 (s, 6H), 3.1 (sept, 2H), 1.3 (d, 12H); GC/EIMS *m*/*z* 296 (M^+).
14. **Dimethyl 2,6-diisopropyl-4-hydroxy-3,5-pyridine-dicarboxylate (39)**. A solution of **38** (9.10 g, 30.7 mmol) in methanol (100 mL) was cooled to 0 °C, and then anhydrous ammonia gas was bubbled into the solution for 5–10 min. The solution was then stirred at 0 °C for 3–6 h, during which time the pyrone was converted to the less polar pyridone, as monitored by TLC. After complete conversion, the volatiles were removed by rotary evaporation to provide **39** as a white powder (13.5 g, 92%): mp 157–158 °C (EtOAc); ^{1}NMR ($CDCl_3$ 300 MHz) δ 11.85 (bs, 1H), 4.0 (s, 6H), 3.4 (sept, 2H, J = 6.6 Hz), 1.2 (d, 12H, J = 6.6 Hz); GC/EIMS *m*/*z* 295 (M^+).
15. **Dimethyl 2,6-diisopropyl-4-chloro-3,5-pyridinedicarboxylate (40)**. To a flask containing phosphorus oxychloride (30 mL) was gradually added **39** (8.00 g, 27 mmol) with stirring, followed by the dropwise addition of 2,6-lutidine (3.53 mL, 30 mmol). The solution was stirred at reflux ($POCl_3$ bp 106 °C) overnight, and then the mixture was cooled to room temperature. The mixture was slowly and cautiously poured into a well-stirred ice-water mixture (200 mL), and was then stirred for an additional 30 min. Dilute aqueous HCl (10%, 125 mL) was then slowly added, and the mixture stirred for 10 min. The mixture was extracted with diethyl ether (3×), and the combined organic phases were washed with brine, dried ($MgSO_4$), and rotary evaporated to provide the product as crystals (7.12 g, 84%): mp 110–111 °C (EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.0 (s, 6H), 3.0 (sept, 2H), 1.3 (d, 12H); GC/EIMS *m*/*z* 313 (M^+).
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17. **Dimethyl 2,6-diisopropyl-4-methoxy-3,5-pyridine-dicarboxylate (42)**. To a mixture containing **39** (10.0 g, 33.9 mmol) in acetone (100 mL) was added potassium carbonate (7.0 g, 50.8 mmol) at room temperature. The resultant mixture was stirred as iodomethane (5.29 g, 2.3 mL, 37.3 mmol) was added. The mixture was heated at reflux overnight and was then cooled to rt. Most of the solvent was then removed under reduced pressure. The resultant residue was diluted with EtOAc and then washed with water. The organic phase was washed sequentially with 10% hydrochloric acid, saturated aqueous sodium bicarbonate, and brine, dried (Na_2SO_4), and concentrated under reduced pressure to provide **42** as a white solid (10.0 g, 95%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.9 (s, 6H), 3.8 (s, 3H), 2.9 (sept, 2H), 1.2 (d, 12H); GC/EIMS *m*/*z* 309 (M^+).
18. **Dimethyl 2,6-diisopropyl-4-(2′-benzyloxy-4′-fluoro-phenyl)-3,5-pyridinedicarboxylate (37)**. A solution containing dimethyl 2,6-diisopropyl-4-methoxy-3,5-pyridinedicarboxylate (**42**) (52.0 g, 0.168 mol, 1 equiv) in THF (125 mL) was added to a 1.5 M solution of 2-benzyloxy-4-fluorophenylmagnesium bromide (150 mL, 0.269 mol, 1.6 equiv) at reflux. The reaction was heated at reflux for 1 h. The mixture was then cooled to 0 °C and quenched by dropwise addition of saturated aqueous ammonium chloride. The mixture was then extracted with EtOAc and the combined organic layer was washed sequentially with water, saturated aqueous ammonium chloride, and brine. The organic layer was then dried (Na_2SO_4), and rotary evaporated to provide a yellow solid. The solid was triturated from hexane to provide pure **37** as a beige solid (62.5 g, 78%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.20–7.40 (m, 5H), 7.08–7.18 (m, 1H), 6.59–6.78 (m, 2H), 5.02 (s, 2H), 3.51 (s, 6H), 3.17 (sept, 2H, J = 6.6 Hz), 1.24–1.38 (m, 12H); FAB/LSIMS *m*/*z* 480 (MH^+).