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Novel 3,4-Dihydroquinolin-2(1*H*)-one Inhibitors of Human Glycogen Phosphorylase *a*

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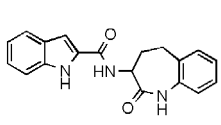
Dedicated to the memory of Chris A. Willoughby

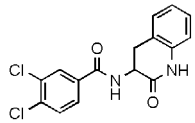
Abstract—The preparation of a series of substituted indoles coupled to six- and seven-membered cyclic lactams is described and their role as human glycogen phosphorylase *a* inhibitors discussed. The SAR of the indole moiety and lactam ring are presented.

A recent World Health Organization report estimates that approximately 130 million people worldwide are afflicted with type 2 diabetes, a number projected to double over the next two decades.[1] Patients suffer from progressive insulin insensitivity that manifests itself in both postprandial and fasting hyperglycemia. Current therapy targets restoration of normal glucose levels with treatment determined by disease progression. One investigational approach involves restricting hepatic glucose output (HGO), a major demonstrated contributor to diabetic hyperglycemia.[2] HGO derives from two sources, gluconeogenesis, and the breakdown of stored glycogen catalyzed by glycogen phosphorylase *a* (GPa). In addition, a portion of the glucose derived from gluconeogenesis, which is elevated in type 2 diabetics relative to normal patients,[3] cycles through glycogen via the glycogenolytic pathway.[4] Previous reports indicated GPa inhibition to be efficacious in lowering blood glucose in diabetic models,[5,6] thereby validating it as an attractive therapeutic target for lowering blood glucose in the treatment of type 2 diabetes.

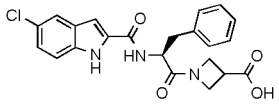
Initial glycogen phosphorylase inhibitor leads **1** and **2** originated from high throughput screening of our compound collection.[7] Both inhibitors possessed comparable potency against both the liver and muscle GPa isoforms (hLGPa and hMGPa, respectively). The following data represents our preliminary findings of a novel, bicyclic lactam scaffold.



1
hLGPa 1.06uM
hMGPa 2.44uM



2
hLGPa 1.37uM
hMGPa 2.31uM



CP-403,700
hLGPa 45nM

Lead structures **1** and **2** resemble an inhibitor class[6] that bind in an allosteric site at the enzyme homodimeric interface. The reported enzyme-inhibitor co-crystal, 1EXV,[8] suggests that both the indole moiety from **1**, and the potential indole replacement 3,4-dichlorophenyl group from **2** bind in a restricted hydrophobic pocket. The overall structural homology shared by compound **1** and CP-403700 suggests common enzyme–inhibitor interactions.

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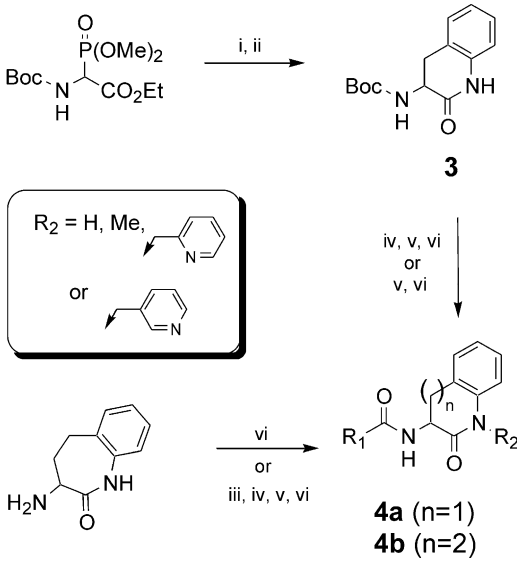
Other potentially significant functionality in **1** and **2** are the carboxamide moiety that links the two ring systems in each inhibitor and the lactam carbonyl. The corresponding functional groups in 1EXV form a hydrogen bonding lattice spanning the binding pocket,[8a] thereby reinforcing the importance of these hydrogen bonding groups. In addition, variation of lactam ring size could alter the lactam phenyl group orientation. Selection between both lead isosteric scaffolds and subsequent optimization provided broad SAR for this reported class of inhibitor.

The synthesis of the six-membered lactam series was relatively straightforward, beginning with a Horner–Emmons condensation of the *N*-Boc-ethyl-2-(dimethylphosphono)glycinate and 2-nitro-benzaldehyde, followed by nitro group reduction and subsequent cyclization to afford Boc-lactam **3**. Alkylation, deprotection and coupling to the corresponding aryl carboxylate using standard methods afforded the substituted products of general structure, **4a** (Scheme 1). The seven-membered lactam series was synthesized from the commercially available 3-amino-2,3,4,5-tetrahydro-1-benazepin-2-one to which was coupled the corresponding aryl carboxylic acid affording the products of general structure **4b** (Scheme 1). In example **15**, the amino-lactam was Boc-protected, followed by methylation, deprotection, and coupled to the corresponding carboxylic acid (Scheme 1).

Initial SAR comparison of acyl aromatic groups indicated a strong preference for the indole group (**6** and **9**) over the 3,4-dichlorphenyl-substituted compounds (**2** and **5**). One possible explanation stemmed from the hydrogen bonding potential of the indole nitrogen, which contacted a GP backbone carbonyl in the aforementioned X-ray structure.[8] The effect of lactam ring size could be inferred from a comparison of lactams **6** and **12** with **9** and **15**, respectively, which indicates a clear preference for the six-membered lactam rings over their seven-membered counterparts. The specific stereochemistry preferred by the enzyme was also investigated.[9] The data appears to be somewhat ambiguous in that unalkylated (*R*)-enantiomer **7** was more active (2-fold) than the corresponding (*S*)-enantiomer **8**, while both enantiomers are essentially equally potent upon lactam alkylation (**13** vs **14**).

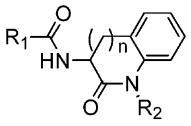
As a result of our findings in Table 1,[10] the indole moiety was investigated further. While literature precedence[6,8] establishes the 5-chloroindole moiety as optimal, we were interested in a more comprehensive SAR study. The synthesis of inhibitors **18**–**36** followed from EDC-mediated coupling of deprotected amino-lactam with each corresponding indole-2-carboxylic acid, as outlined in Scheme 1. Although several of the indole-2-carboxylic acids were commercially available, the majority were prepared from their substituted aniline **16**. Directed iodination *ortho* to the amine group,[11] followed by coupling with pyruvic acid and cyclization at 105 °C afforded the desired indole **17**.[12] The 5-phenylindole-2-carboxylate used for the synthesis of **27** required Suzuki coupling of phenylboronic acid onto 5-bromoindole-2-carboxylate prior to amide bond formation. The related 6-phenyl indole-2-carboxylate leading to **30** was derived from the reduction of 3-nitrobiphenyl to afford the aryl amine input relevant to Scheme 2.[13] Lastly, the 7-aminoindole-2-carboxylate from **33** was generated via reduction of the corresponding 7-nitro-indole-2-carboxylate derivative used for the synthesis of **32**.[12]

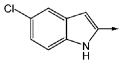
Analysis of the indole SAR confirmed the limited space surrounding the heterocyclic scaffold (Table 2).[10] Although smaller substituents at C5–C7 were tolerated,

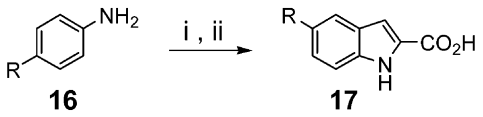


Scheme 1. Reagents and conditions: (i) 2-nitro-benzaldehyde, NaH, THF, 0 °C to rt, 1 h; (ii) Pd/C, MeOH, H_2, 40 psi, 3 h, then EtOAc, reflux, 24 h; (iii) Boc_2O, DCM; (iv) NaO*t*Bu, $\mathbf{R_2}$-X, DMF, rt, 1 h; (v) acetyl chloride, MeOH, 50 °C, 30 min; (vi) $\mathbf{R_1}$-CO_2H, EDC.

Table 1. In vitro phosphorylase inhibitory activity: six versus seven-membered lactams[10]

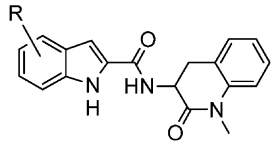


Compd	*n*	R_1	R_2	HLGPa IC_{50} (nM)	HMGPa IC_{50} (nM)
2-rac.	1		H	1400	2300
5-rac.	2			6400	21,700
6-rac.	1		H	44	38
7-(*R*)	1			25	14
8-(*S*)	1			47	39
9-rac.	2			105	55
10-(*R*)	2			99	83
11-(*S*)	2			240	240
12-rac.	1		Me	25	32
13-(*R*)	1			61	58
14-(*S*)	1			57	57
15-rac.	2			305	105

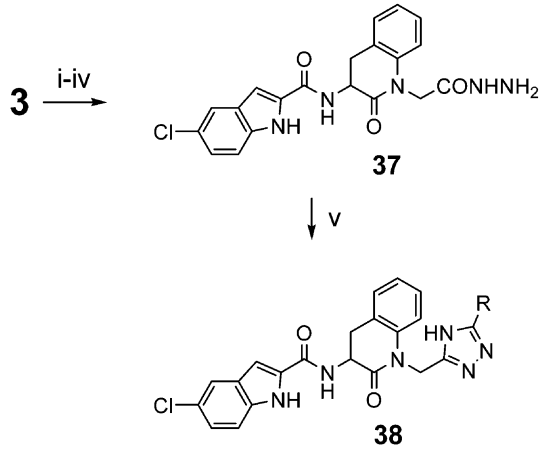


Scheme 2. Reagents and conditions: (i) Ag_2SO_4, I_2, EtOH, rt, 16 h; (ii) pyruvic acid, DABCO, $Pd(OAc)_2$, DMF, 105 °C, 4 h.

Table 2. In vitro phosphorylase inhibitory activity of indole analogues[10]



Compd	R	HLGPa IC_{50} (nM)	HMGPa IC_{50} (nM)
18	H	160	56
19	4-Cl	1600	2100
20	5-Br	30	73
12	5-Cl	25	32
21	5-F	120	89
22	5-Me	120	98
23	5-Et	2500	850
24	5-OMe	1700	1700
25	5-CN	180	110
26	5-CF_3	1500	550
27	5-Ph	>20,000	>20,000
28	5-CO_2Me	5300	2700
29	6-Cl	99	69
30	6-Ph	>20,000	>20,000
31	7-Cl	88	88
32	7-NO_2	300	120
33	7-NH_2	1200	6800
34	4-CO_2Me, 5-Cl	>20,000	>20,000
35	5-Cl, 6-CO_2Me	>20,000	>20,000
36	5-Cl, 7-F	83	55

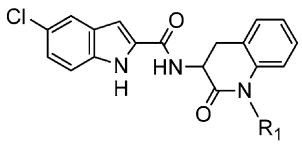


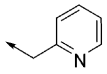
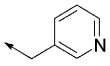
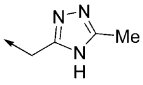
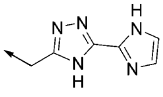
Scheme 3. (i) NaOtBu, methyl bromoacetate; (ii) HCl; (iii) 5-chloroindole-2-carboxylic acid, EDC; (iv) NH_2–NH_2, reflux; (v) R-$CSNH_2$, (4:1) pyr: *n*BuOH, reflux.

the 5-chloroindole analogue (**12**) proved to be the most active.

A number of heterocyclic substituted six-membered lactam inhibitors were prepared in order to examine the SAR proximal to the N1-position. Qualitative analysis

Table 3. In vitro phosphorylase inhibitory activity of *N*1-substituted lactams[10,14]



Compd	R_1	HLGPa IC_{50} (nM)	Hepatocyte EC_{50} (μM)
7-(*R*)	H	25	1.8
8-(*S*)		47	7.8
13-(*R*)	Me	61	13.2
14-(*S*)		57	8.4
39-rac.		110	20.7
40-(*R*)		120	14.5
41-(*S*)		140	11.6
42-rac.		130	16.6
43-rac.		140	1.2
44-rac.		160	3.7

of 1EXV suggested that stabilizing van der Waals interactions in such a polar pocket might enhance potency. An overlay of compound **12** with CP-403700 (data not shown), reveals the predictably similar orientation of the dihydroquinolone carbonyl and phenylalanine carbonyl, lending support to the idea of the *N*-substituents projecting into the water filled cavity of the homodimer interface.

The incorporation of hydrophilic groups may however alter the physical properties of the inhibitor to positively impact cell potency. Inhibitors **39**–**42** were prepared following the methodology outlined in Scheme 1, while triazolylmethyl-substituted lactams (**38**) were generated from cyclization of hydrazide **37** with various thioamides (Scheme 3).

Data analysis indicated no clear SAR for the lactam N1-substituent in both the enzyme and cell-based assays (Table 3),[14] except for the general trend toward a slight loss of potency against HGPa observed for the arylmethyl substituents. However, the magnitude of the potency shifts from enzyme to cell-based GP inhibition tracked with the relative hydrophilicity of the N1-side chain.[15] Specifically, the polar triazoylmethyl lactams (**43**–**44**) possessed 9- and 23-fold shifts in IC_{50}, while the remaining IC_{50} values were, at best, 72-fold shifted (**7**) for the same comparison. A plausible explanation for observed effect was improved cell penetration due to greater amphiphilic character of inhibitors possessing the more polar lactam sidechains.

In summary, the (*R*)-six-membered unsubstituted cyclic lactam coupled to the 5-chloroindole-2-carboxylic acid (**7**) was the most potent in vitro GP inhibitor (25 nM).

Substitution of the triazolylmethyl group at N1 (**43**) led to an improvement in cell-based GP inhibition (1.2 μM) that revealed an apparent correlation between lactam substituent polarity and improved cell activity.

Acknowledgements

A special thanks to Anthony Ogawa for his time during many useful discussions and mentorship.

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9. Separation of racemic Boc-lactam **3** by chiral NP-HPLC [chiral AD column; isocratic (92:8) heptane/*i*PrOH] afforded individual enantiomers that were carried on as shown in Scheme 1. A chiral synthesis of this peptidomimetic will be reported separately.
10. Phosphorolysis of glycogen using recombinant human liver or muscle enzyme. Glucose-1-phosphate production was monitored via an enzymatic assay involving phosphoglucomutase/glucose-6-phosphate dehydrogenase-mediated NADH production (*ex*. 340 nm, *em*. 465 nm). The standard error for liver and muscle enzyme activity is 0.17/0.14 ($n > 8$), respectively, based on an internal standard.
Phosphorylase *a* activity was measured at the sub-saturating substrate levels in the physiologic glycogenolytic direction by a modification (manuscript in preparation reference) of the method in which glucose-1-phosphate production is coupled to NAD reduction using phosphoglucomutase dehydrogenase. For further details, see: Maddailh, V. T.; Madsen, N. B. *J. Biol. Chem.* **1966**, *241*, 3873.
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13. Not shown.
14. Glucagon-stimulated glycogenolysis in primary rat hepatocytes. Inhibition of glucose production determined by digesting remaining glycogen and quantifying by monitoring glucose dehydrogenase-mediated NADPH production (*ex*. 340 nm, *em*. 465 nm). The standard error for hepatocyte activity is 0.15.
15. Due to incomplete solubility data, global logD calculations were performed on the compounds in Table 3 that showed no sensitivity toward lactam N1-side-chain variation, presumably due to the dominant effect of the 5-chloroindole carboxamide moiety. Subsequent logD determination for the truncated N1-substituted-3-acetamido-tetrahydroquinoline yielded the noted correlative trend.