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Bioorganic & Medicinal Chemistry Letters 16 (2006) 5567–5571
Bioorganic & Medicinal Chemistry Letters

Novel thienopyrrole glycogen phosphorylase inhibitors: Synthesis, in vitro SAR and crystallographic studies

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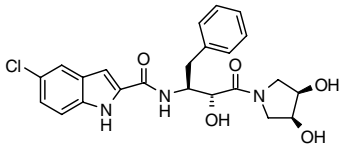
Received 28 February 2006; revised 7 July 2006; accepted 5 August 2006
Available online 30 August 2006

Abstract—Two series of novel thienopyrrole inhibitors of recombinant human liver glycogen phosphorylase a (GPa) which are effective in reducing glucose output from rat hepatocytes are described. Representative compounds have been shown to bind at the dimer interface site of the rabbit muscle enzyme by X-ray crystallography.

Type 2 diabetes is a complex disease caused by defects in both the action and secretion of insulin, leading to fasting hyperglycaemia and vascular complications. It has become the fourth leading cause of death and affects 150 million people worldwide, with its prevalence expected to double by the year 2025.[1] Current therapies do not achieve adequate glycaemic control,[2] hence there is a need for new, effective pharmacological agents.

Glycogen phosphorylase (GP) catalyses the breakdown of glycogen to glucose-1-phosphate. In the liver this is metabolised further to glucose, which is then secreted into the bloodstream. One approach to reducing hyperglycaemia in Type 2 diabetes is to seek to restore normal net hepatic glucose balance by inhibition of hepatic GP.[3] The more active phosphorylated form (GPa) of the enzyme is a homodimer; its activity is modulated by phosphorylation status and by a number of endogenous and synthetic ligands.[4–7] Of particular interest is an inhibitory allosteric binding site[8] at the dimer interface for which a series of synthetic ligands has been identified,[4] including ingliforib (CP368296) which has undergone phase II clinical evaluation. We report here the identification of novel thienopyrrole inhibitors, which we have shown by X-ray crystallography to bind at the allosteric site situated at the dimer interface.



We prepared a series of inhibitors derived from thieno[2,3-*b*]pyrrole and thieno[3,2-*b*]pyrrole carboxamides and compared their activity to the known 5-chloroindole **1** (Table 1). The isolated enzyme and cell assay values found for **1** match well with those from the literature.[4]

Keywords: Glycogen phosphorylase; Diabetes; Thienopyrrole.
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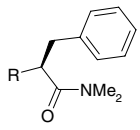
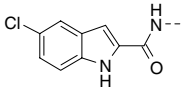
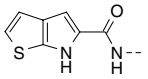
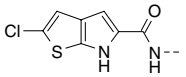
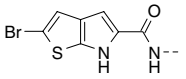
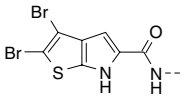
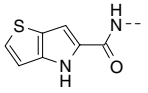
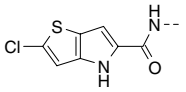
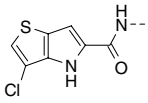
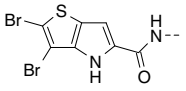
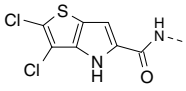
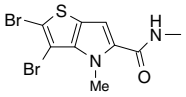
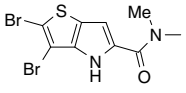
doi:10.1016/j.bmcl.2006.08.047

Table 1. Thienopyrrole SAR



Compound	R	Enzyme inhibition[a] IC_{50}[b] nM	Cell[c] IC_{50}[b] μM
1		51	2.6
2		2310	
3		17	1.4
4		31	1.6
5		20% at 10 μM	
6		35% at 100 μM	
7		577	
8		390	
9		23	
10		5	1.0
11		>10,000	1.1
12		>10,000	

[a] Using recombinant human liver GPa: glucose-1-phosphate production from glycogen monitored by a multienzyme coupled assay.[9]

[b] $n = \geqslant 3$ unless otherwise stated.

[c] Inhibition of glucose output from primary rat hepatocytes following glucagon challenge[9] determined from seven concentration points unless otherwise stated.

Replacement of the chloroindole with the unsubstituted thieno[2,3-*b*]pyrrole system **2** led to a novel inhibitor, albeit with a considerably reduced inhibitory activity–however, this could be restored by introduction of a 2-chloro or 2-bromo (**3** and **4**) substituent. This is consistent with the halothienopyrrole moiety binding in a similar manner to that reported for the chloroindole,[8] that is, binding deeply into a hydrophobic pocket at the interface site bounded by Leu 63, Pro 229 and Trp 67. Interestingly, dibromo substitution (**5**) led to a marked reduction in inhibition, possibly due to a severe steric clash between the additional bromine atom and the protein (possibly Val64, which is 3.92 Å from the chloroindole).

SAR in the isomeric thieno[3,2-*b*]pyrrole series is subtly different, with very little activity seen for the unsubstituted compound (**6**), moderate activity restored by monohalogenation (**7** and **8**), 2,3-dibromo (**9**) now tolerated and optimal activity found with 2,3-dichloro substitution (**10**). The alternative inverted orientation of the thiophene ring in this series appears to result in the second halo atom being directed towards a less sterically demanding region of the protein. Methylation on either the pyrrole nitrogen (**11**) or the carboxamide group (**12**) leads to loss of activity, consistent with the key H-bonds the unsubstituted groups make to the backbone carbonyl groups of Asn 187 and Thr 38, respectively.

Exploration of a variety of amide N-substituents by library synthesis led to the discovery of two novel structural types with promising activity (Table 2). The 3,4-dihydro-2-quinolone moiety led to potent inhibitor compounds in both the thieno[3,2-*b*]pyrrole (**14**) and the thieno[2,3-*b*]pyrrole (**15**) series, against the enzyme and also against glucose output from rat hepatocytes.

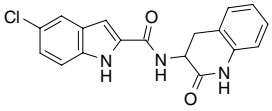
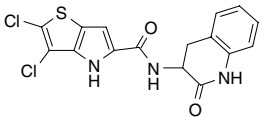
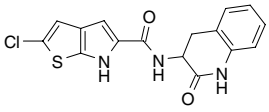
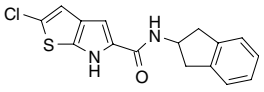
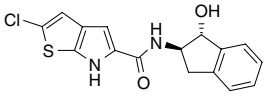
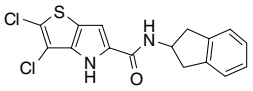
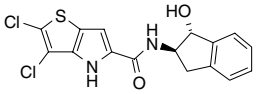
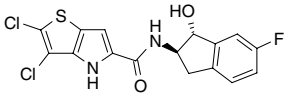
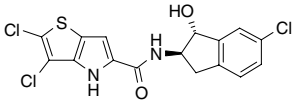
Other workers have reported the identification of the corresponding 5-chloroindole (**13**) derivative,[5] which we found to show enzyme potency comparable to the thienopyrroles, but apparently reduced potency in rat hepatocytes.

Indan-2-yl was also identified as a modestly active amide N-substituent (**16**) in the chlorothieno[2,3-*b*]pyrrole series with a *trans* 1-hydroxy substituent leading to a slightly more active inhibitor (**17**). The dichlorothieno[3,2-*b*]pyrrole analogues **18** and **19** showed an increase in potency more marked than for the previous amide N-substituents. Halo substitution in the indan aromatic ring leads to a general reduction in activity (compounds **20** and **21**), consistent with the restricted space in the aromatic binding pocket.

In order to understand the binding mode of these new inhibitors, they were soaked into crystals of rabbit muscle GPb (non-phosphorylated form). The complexes gave well-defined high-resolution structures.[10]

The structure of **15** (Fig. 1A) showed similarities to structures previously reported for 5-chloroindole derivatives, with two molecules of inhibitor per molecule of GPb dimer, and the thieno[2,3-*b*]pyrrole group binding

Table 2. Amide N-substituents

Compound	Structure	Enzyme inhibition[a] IC_{50}[b] nM	Cell[c] IC_{50}[b] μM
13	 (+/-)	86	7[d]
14	 (+/-)	132	0.56
15	 (+/-)	41	0.69
16		806	
17	 (+/-)	319	3.6
18		197	
19	 (+/-)	9	1.5
20	 (+/-)	49	1.3
21	 (+/-)	90	

[a] Using recombinant human liver GPa: glucose-1-phosphate production from glycogen monitored by a multienzyme coupled assay.[9]
[b] $n = \geqslant 3$ unless otherwise stated.
[c] Inhibition of glucose output from primary rat hepatocytes following glucagon challenge[9] determined from 7 concentration points unless otherwise stated.
[d] Determined from three concentration points.

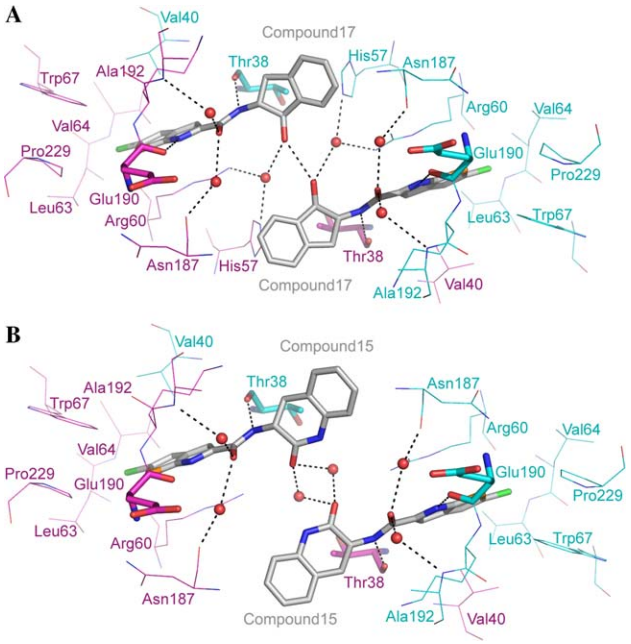
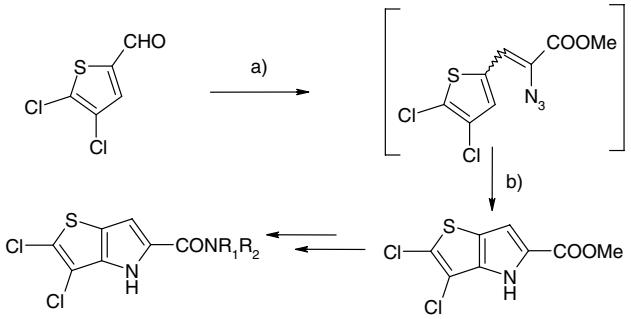


Figure 1. Inhibitor environment in the crystal structure of rabbit muscle glycogen phosphorylase complexed with compound **15** (A) or with compound **17** (B). Residues from one monomer are coloured pink; residues from the other blue. The figures were prepared using PyMol (DeLano Scientific).

in the same hydrophobic binding pocket as the indole. This pocket is formed from Val40 (from the one monomer) and Val64, Arg60, Leu63, Trp67 and Pro229 (from the other monomer). In addition, the guanidinium group of Arg60 makes a favourable electrostatic interaction with the indole π-system. The amide carbonyl group forms a water-bridged H-bond to Asn187 and the amide NH interacts with the backbone carbonyl of Thr38, from the second protein subunit.

The structure of compound **17** (Fig. 1b; *R*,*R*-diastereoisomer) bound to the enzyme shows many similar features to those described above, the binding mode of the thieno[2,3-*b*]pyrrole-5-carboxamide system being essentially identical. The aromatic ring of the indan system binds in the hydrophobic pocket in a similar way to that described above for the dihydroquinolone. Again, H-bonding interaction is seen between the two inhibitor molecules, but in this case it is direct rather



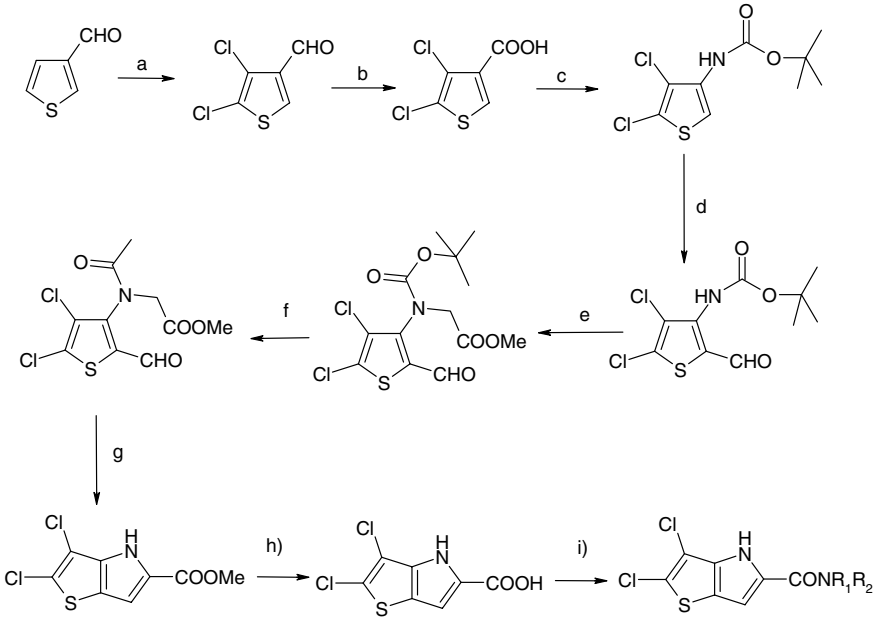
Scheme 1. Reagents and conditions: (a) NaOMe, $MeO_2CCH_2N_3$, MeOH; (b) reflux toluene.

than water mediated, presumably with each hydroxy group acting both as donor and acceptor. The hydroxy groups are seen to make a further, water-bridged H-bond to His57.

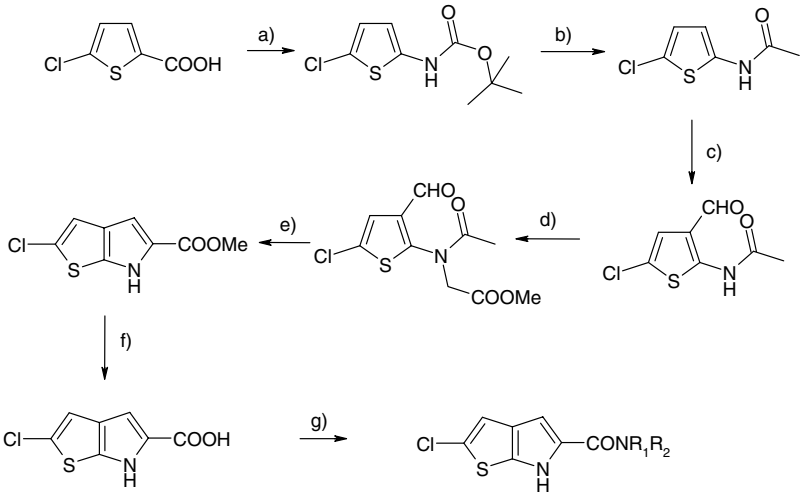
The general synthetic routes to thieno[2,3-*b*]pyrrole and thieno[3,2-*b*]pyrrole carboxamide inhibitors are shown in Schemes 1–3.

The initial literature route we employed is illustrated for the dichlorothieno[3,2-*b*]pyrrole.[11] This involves the thermal decomposition of an intermediate vinyl azide (NOT isolated) to a postulated nitrene which subsequently inserts into the thiophene CH bond to afford the desired ring system. As we became more interested in the dichlorothieno[3,2-*b*]pyrrole and chlorothieno[2,3-*b*]pyrrole systems as the basis for a lead optimization programme, we chose to develop novel alternative routes[12,13] that did not involve the potential explosive hazards associated with vinyl azides in general. These are shown in Schemes 2 and 3, and briefly comprise key steps of Curtius rearrangement, metallation/formylation and then aldol cyclisation.

In summary, we have identified series of novel inhibitors of glycogen phosphorylase and have described SAR,



Scheme 2. Reagents and conditions: (a) $AlCl_3$, Cl_2, DCM 20 °C; (b) NaOH, $KMnO_4$, 50 °C; (c) DPPA, Et_3N, *t*-BuOH reflux 12 h; (d) *n*-BuLi, THF, DMF, −78 °C to rt; (e) $KHCO_3$, methyl bromoacetate, DMF, 60 °C 3.5 h; (f) AcOH, Ac_2O, 120 °C 21 h; (g) K_2CO_3, DMF, 60 °C 3 h; (h) MeOH, LiOH, H_2O; (i) standard amide coupling conditions.



Scheme 3. Reagents and conditions: (a) DPPA, Et_3N, *t*-BuOH, reflux 12 h; (b) Ac_2O, AcOH, 120 °C 4 h; (c) DMF, POCl3, DCM, rt 24 h; (d) K_2CO_3, methyl bromoacetate, NMP, *t*-BuOMe, 40 °C 24 h; (e) DMF, K_2CO_3, 60 °C 90 min; (f) NaOH, MeOH, H_2O, reflux; (g) standard amide coupling conditions.

hepatocyte activity, synthetic routes and demonstrated binding at the dimer interface of the rabbit muscle enzyme. Lead optimization in these series and pharmacological profiling of leading compounds will be reported in due course.

Acknowledgments

Thanks to Sue Freeman, Simon Poucher, Gemma Convey, Julie Bartlett and Jenny Thomas for the bioscience testing. Richard Pauptit is thanked for establishing the crystallography collaboration with the Oikonomakos group, who have kindly provided protein and crystals. Thanks to Claire Minshull for the soaking experiments and to Jason Breed, Richard Pauptit, Siân Rowsell and Julie Tucker for synchrotron data collection and analysis.

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9. For in vitro assay experimental procedures, see: Bartlett, J. B.; Freeman, S.; Kenny, P. W.; Morley, A. D.; Whittamore, P. R. O. PCT Intl Application WO200220530, 2002; *Chem. Abstr*. **2002**, *136*, 247485.
10. Protein crystals kindly provided by N.G.O. were grown using published procedures.[14] Diffraction data were collected at SRS, Daresbury, at 100 K for compound **17** and at ESRF, Grenoble, at 100 K for compound **15**. The crystals of the complex with **15** have space group $P4_32_12$, unit cell 126.8, 126.8, 115.1 Å. 39468 unique reflections to 2.3 Å give 97.7% completeness. The final *R*-factor is 18.3% (Rfree using 5% of data is 24.5%). The mean temperature factor is 31.5 $Å^2$ for protein atoms and 47.8 $Å^2$ for the ligand. Crystals of the complex with **17** have space group $P4_32_12$ and unit cell 128.1, 128.1, 116.3 Å. 98,023 unique reflections to 1.6 Å give 74.5% completeness. The final model has an *R*-factor of 18.7% (Rfree using 5% of data is 22.6%). Mean atomic temperature factor for the protein is 18.4 and for the inhibitor is 13.7 $Å^2$. Data analysis and structure solution used programs from the CCP4 suite[15]. The inhibitors were modelled into electron density using Quanta2000 (Accelrys). The model was refined using CNX(Accelrys) and Refmac5.[15] Protein Data Bank deposition codes for the refined structures **15** and **17** are 2GM9 and 2GJ4, respectively.
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