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Development of potent, orally active 1-substituted-3,4-dihydro-2-quinolone glycogen phosphorylase inhibitors

Alan M. Birch,[a,*] Peter W. Kenny,[a] Nikos G. Oikonomakos,[b] Ludovic Otterbein,[a] Paul Schofield,[a] Paul R. O. Whittamore[a] and Dave P. Whalley[a]

[a]*AstraZeneca, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK*
[b]*Institute of Organic and Pharmaceutical Chemistry, The National Hellenic Research Foundation, 48, Vas. Constantinou Ave. 116 35 Athens, Greece*

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Abstract—A series of substituted 3,4-dihydro-2-quinolone glycogen phosphorylase inhibitors, which have potential as antidiabetic agents, is described. Initial members of the series showed good enzyme inhibitory potency but poor physical properties. Optimisation of the 1-substituent led to 2,3-dihydroxypropyl compounds which showed good in vitro potency and improved physical properties, together with good DMPK profiles and acute in vivo efficacy in a rat model. X-ray crystallographic data are presented, showing an unexpected variety of binding orientations at the dimer interface site.

Abnormally high output of glucose from the liver contributes significantly to the elevated plasma glucose levels found in diabetic patients.[1] A key mechanism in this raised hepatic glucose output (HGO) is the action of the enzyme glycogen phosphorylase to liberate glucose from its polymeric storage form glycogen.[2] The active form of the enzyme (GPa) is a homodimer, its activity being inhibited by glucose, glucose-6-phosphate and ATP, but stimulated by AMP and by phosphorylation.[3] Two allosteric inhibitor binding sites have been identified: a purine binding site where inhibitors such as caffeine interact and a novel binding site at the interface between the two dimer subunits,[2,4,5] for which synthetic ligands have been described. Here we report a further series of novel potent glycogen phosphorylase inhibitors, some of which show improved physical and DMPK properties and good activity in a rat model of diabetes.

Previously, we have reported a series of thienopyrrolecarboxamide inhibitors, including the 3,4-dihydro-2-quinolone (DHQ) derivatives **1** and **2**.[6] These compounds showed good potency both in an in vitro GPa assay[7] and in a glucose output assay from rat hepatocytes[7] (Table 1). However, the high plasma protein binding (as determined using the equilibrium dialysis technique at 37 °C, analysing by generic LC/UV/MS) and poor solubility (as measured in 0.1 M phosphate at pH 7.4 following 24 h of agitation) for these compounds limited their potential for further development. Dihydroquinolone GPa inhibitors have been reported previously, but no physical property, DMPK, in vivo or crystallographic data were included.[8]

As an initial attempt to improve properties, substitution at the quinolone 1-position was explored, starting with the 1-methyl compound **3**, which showed similar potency and physical properties (Table 1) to those of its parent compound. Simple functionalised alkyl substituents such as the ether **4**, the ketone **8**, the sulfide **5**, and the sulfone **7** all showed similar enzyme inhibitory potencies but generally without significant improvements in solubility or protein binding. However, the sulfoxide **6** did give an indication that improvements in protein binding could be achieved through incorporation of more polar groups.

The 1-acetic acids **9**–**10** showed similar potencies together with the expected significant increase in aqueous

Keywords: Glycogen phosphorylase; Glycogen phosphorylase inhibitor; 3,4-Dihydro-2-quinolone; Allosteric inhibitor; Dimer interface; X-ray crystallography; Physical properties; DMPK properties; Solubility; Plasma protein binding; Oxidative cyclisation; Thienopyrrole; Chloroindole; Hepatic glucose output; Glucose lowering in vivo.
* Corresponding author. Tel.: +44 1625 514650; e-mail: alan.birch@astrazeneca.com

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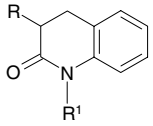
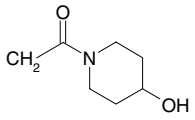
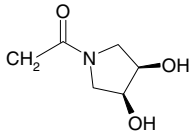
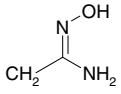
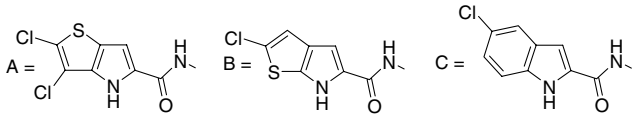
Table 1. In vitro activity and physical property data for 1-substituted DHQs



Compound	R	R^1	Enzyme inhibition[b] IC_{50}[c] (μM)	Cell[d] IC_{50} (μM)	Plasma protein binding % free	Sol. (μM)
1	A[a]	H	0.132	0.56	0.03	0.72
2	B[a]	H	0.041	0.69	0.14	0.33
3	B	Me	0.223 ($n = 1$)	1.4	0.04	0.38
4	B	CH_2CH_2OMe	0.05	1.7	0.18	1.9
5	B	CH_2CH_2SMe	0.163 ($n = 1$)	5.0[e]	0.03	0.29
6	B	$CH_2CH_2S(O)Me$	0.048	6.0[e]	1.11	1.2
7	B	$CH_2CH_2S(O)_2Me$	0.063	4.0[e]	0.50	0.50
8	B	$CH_2C(O)Et$	0.084	7.0[e]	0.15	0.27
9	A	CH_2CO_2H	0.063	2.7	0.12	595
10	C[a]	CH_2CO_2H	0.237	20[e]	ND	2100
11	B	CH_2CONH_2	0.135	1.2	0.38	5.3
12	B	$CH_2CONHMe$	0.079	1.3	0.45	5.4
13	B	CH_2CONMe_2	0.161	IA at 30	0.62	0.74
14	B	$CH_2CH_2CONHMe$	0.150	9.0	0.51	16
15	B	$CH_2CONHOH$	0.103	8.0[e]	0.04	15
16	B	$CH_2CONH(CH_2)_2OH$	0.121	15	0.97	14
17	B		0.709	ND	1.83	0.13
18	B	$CH_2CONHCH(CH_2\ OH)_2$	0.104	ND	2.63	17
19	B		0.140	IA at 30	2.35	2.2
20	B	$CH_2CONHSO_2Me$	0.399	IA at 30	0.37	1100
21	B		0.075	9.8	0.6	6.9
22	B	CH_2CO_2Me	0.084	1.9	ND	0.44
23	B	CH_2CN	0.028	3[e]	0.09	0.58

[a] 

[b] Using recombinant human liver GPa: glucose-1-phosphate production from glycogen monitored by a multienzyme coupled assay (Ref. 7).
[c] $n \geqslant 3$ unless otherwise stated.
[d] Inhibition of glucose output from primary rat hepatocytes following glucagon challenge (Ref. 7), determined from 7 concentration points unless otherwise stated.
[e] Determined from 3 concentration points IA $\leqslant$ 50% inhibition at stated dose.

solubilities. However, plasma protein binding remained high and activity in whole hepatocytes dropped. Consistent with this, compound **9** showed poor intrinsic permeability and high efflux potential in a Caco-2 assay (Papp A–B 0.3, B–A 11 cm $s^{-1} \times 10^{-6}$). Amide derivatives of these acids showed generally similar potencies against the enzyme, although activity dropped with tertiary or bulky substitution.

Incorporation of hydroxy groups in compounds **15**–**19** led to some modest increases in solubility and decreases in plasma protein binding. The acidic *N*-sulfonylamide **20** showed the expected improvement in solubility but was without activity in hepatocytes, presumably as a result of poor cellular permeability. The hydroxamidine, ester and nitrile **21**–**23** all showed no significant improvement in physical properties.

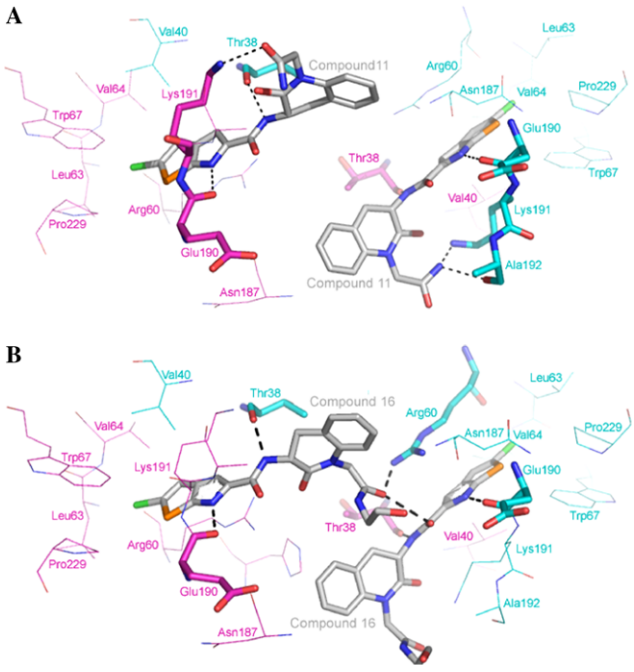
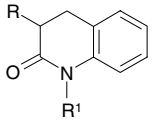


Figure 1. Inhibitor environment in the crystal structure of rabbit muscle glycogen phosphorylase (A) complexed with compound **11** and (B) with compound **16**. Residues from one monomer are coloured pink; residues from the other blue. The figures were prepared using PyMol (DeLano Scientific).

Crystallographic study[9] of the complex between **11** and rabbit muscle glycogen phosphorylase (Fig. 1A) revealed two inhibitor molecules at the dimer interface, consistent with earlier reports.[2,4,5] Although crystallographic experiments were carried out with compounds racemic at the DHQ 3-position, we have generally found that the two bound molecules have the same stereochemistry, the *S*-isomer in the case of **11.** The thienopyrrole system binds into a hydrophobic pocket defined by the residues Val40 (from the one monomer) and Val64, Arg60, Leu63, Trp67, Lys191 and Pro229 (from the other monomer). The more hydrophobic dichlorothieno[3,2-*b*]pyrrole group is the most potent of the three alternative systems described, but has the poorest solubility and the highest protein binding. In the refined structure, the pendant primary amides of the two ligands adopt different orientations. In one of the ligands, the amide NH_2 is close to the cationic nitrogen of Lys191 and also forms a hydrogen bond with the backbone carbonyl of Ala192. The other inhibitor molecule chelates Lys191 between the carbonyl groups of the primary amide and dihydroquinolone. Crystallographic study[9] on the *N*-(hydroxyethyl) analogue **16** (Fig. 1B) reveals a hydrogen bond interaction between the two inhibitor molecules, with one adopting a new binding mode using the opposite enantiomer. The carbonyl oxygen of the *R* enantiomer only interacts with Arg60 that lies against the iso-TP ring of the other ligand. The structure of 16 shows the spatial resolution of a racemic mixture into the two binding pockets. For each inhibitor molecule, attempting to fit the opposite enantiomer into electron density results in a higher strain and less probable 'syn' arrangement of the inferred positions of the amide hydrogen with the nearest carbon hydrogen (see Supplementary material for conformational analyses through examination of structures in CCDD and through calculation). In both complexes, the crystal space group change on ligand soaking[9] permits small changes between the two now crystallographically independent binding pockets in the dimer interface to stabilise different binding modes. The hydrogen-bonding contact between the two bound enantiomers of **16** hints at cooperativity. These observations illustrate the difficulty of both interpreting SAR and predicting binding modes for this system. These structures suggest that the hydrophobic interactions with the thienopyrrole system are the most important for inhibitor binding, with a

Table 2. In vitro activity and physical property data for 1-(di)hydroxyalkyl DHQs



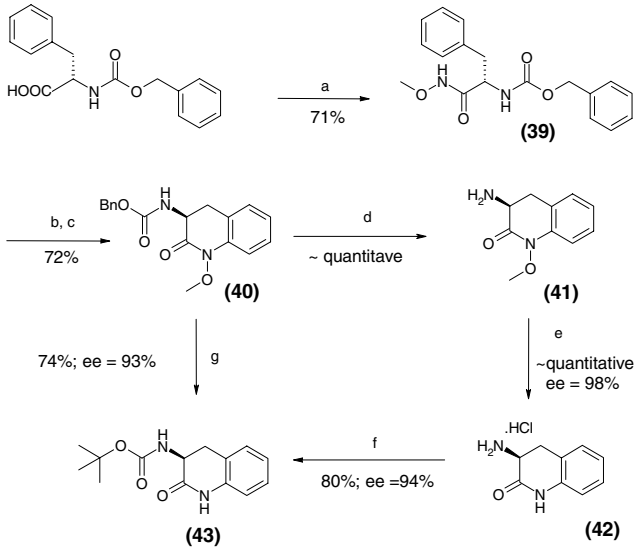
Compound	R	DHQ 3-config.	R^1	Enzyme inhibition[b] IC_{50}[c] (μM)	Cell IC_{50}[d] (μM)	Plasma protein binding % free	Sol. (μM)
24	A[a]	*R,S*-	CH_2CH_2OH	0.007 (n = 1)	0.6[e]	0.03	1.1
25	B[a]	*R,S*-	CH_2CH_2OH	0.026	0.79	0.25	14
26	C[a]	*R,S*-	CH_2CH_2OH	0.017	4.0[e]	0.06	2.0
27	A	*R,S*-	$CH_2CH(CH_2OH)_2$	0.038	0.83	0.23	1.2
28	B	*R,S*-	$CH_2CH(CH_2OH)_2$	0.060	2.7	0.51	4.0
29	C	*R,S*-	$CH_2CH(CH_2OH)_2$	0.066	3.0	0.21	2.7
30	B	*R,S*-	$CH_2CH_2CH_2OH$	0.021	1.1	0.22	4.3
31	B	*R,S*-	(*R,S*)-$CH_2CH(OH)Et$	0.044	2.0	0.11	4.9
32	A	*R,S*-	(*R*)-$CH_2CH(OH)CH_2OH$	0.002	0.26	0.08	3.1
33	B	*R,S*-	(*R*)-$CH_2CH(OH)CH_2OH$	0.029	1.1	0.36	190
34	C	*R,S*-	(*R*)-$CH_2CH(OH)CH_2OH$	0.044	1.3	0.20	45
35	B	*R*-	(*R*)-$CH_2CH(OH)CH_2OH$	0.040	ND	ND	11
36	B	*S*-	(*R*)-$CH_2CH(OH)CH_2OH$	0.027	ND	ND	35
37	B	*R*-	(*S*)-$CH_2CH(OH)CH_2OH$	0.021	ND	ND	12
38	B	*S*-	(*S*)-$CH_2CH(OH)CH_2OH$	0.012	ND	ND	16

Footnotes as for Table 1.

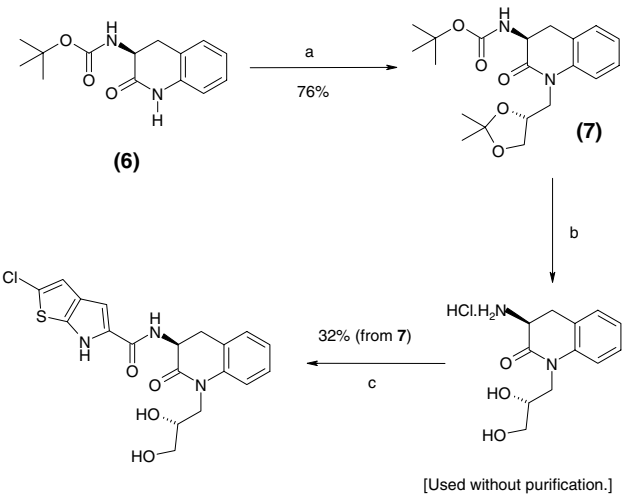
variety of options acceptable for the remaining parts of the molecules, depending on size and flexibility.

With the knowledge that a compound's potency was unlikely to be negatively affected by any moderately sized 1-substituent, further variations containing functionality likely to improve physical properties were explored. A series of 1-(hydroxyalkyl) compounds showed most promise (Table 2). The 1-(2-hydroxyethyl) compounds showed good enzyme and cell potencies for the isomeric thienopyrroles **24** and **25**, with the corresponding 5-chloroindole **26** somewhat less active. Plasma protein binding remained high and solubilities low for these compounds, with the thieno[2,3-*b*]pyrrole system showing the best balance of properties. Incorporation of dihydroxyalkyl substituents generally maintained good potency whilst improving physical properties. Compound **33** (racemic at the dihydroquinolone 3-position) in particular showed much improved solubility, as measured on amorphous material. The four individual diastereoisomers of this system **35**–**38** all showed similar enzyme potencies and solubilities, the latter being lower than for the mixed diastereoisomers, as expected. The synthesis of these individual diastereomers is detailed below; exemplified by the 3-(*S*)-2′-(*R*)-form.

(*S*)-3-Amino-3,4-dihydro-1*H*-quinolin-2-one **42** was prepared, in high enantiomeric excess,[10] via oxidative cyclisation[11] of an *N*-methoxycarboxamide of (*S*)-ZPheOH **39** and **40** (Scheme 1). The subsequent two-step hydrogenolysis, effecting removal of the *Z*-group, then removal of the methoxy group under acidic conditions, to give **42**,[11,12] was followed by BOC-protection to afford **43**. Alternatively, a one-pot hydrogenolysis may



Scheme 1. Reagents and conditions: (a) CH_3ONH_2·HCl, Et_3N (2 equiv), EDAC, HOBt, DMF, rt; (b) DCM, argon, 0 °C, TFA (2.7 equiv), $(CF_3CO_2)IPh$ (1.05 equiv), 1.5 h; (c) excess 10% aq Na_2CO_3; (d) H_2, 10% Pd/C, 1 bar, EtOAc, 12 h; (e) EtOH/water (1:1), 2 M HCl to pH 3, H_2, 10% Pd/C, 3 bar, 25 °C, 33 h; (f) DCM, Et_3N, $(Boc)_2O$, rt; (g) $(Boc)_2O$ (1.1 equiv), Et_3N (1.1 equiv), 10% Pd/C, 15 bar, 50 °C.



Scheme 2. Reagents and conditions: (a) DMF, argon, 0 °C, NaH, 20 min, then ROTf[13] 45 min to rt; (b) 1,4-dioxane/HCl, rt, 8 h, then H_2O, 45 min; (c) 2-chloro-6*H*-thieno[2,3-*b*]pyrrole-5-carboxylic acid, HOBt, EDAC, Et_3N to pH 8, DMF, rt, 3.5 h.

be employed to remove the methoxy group with concomitant replacement of benzylcarbamate by *tert*-butylcarbamate, proceeding from **40** to **43**.

Amine **42** was protected as the *tert*-butyl carbamate, before subjecting the cyclic amide to basic conditions for subsequent alkylations, in order to maintain the chiral integrity of the 3-amino position (Scheme 2). In like manner, all four diasteroisomers **35**–**38** were synthesised from the respective homo-chiral precursors and recrystallised from *i*PrOH. Recrystallisation in all four cases improved the enantiomeric purity at the 3-position, but reduced it at the 2′-position. The final composition of the four diastereomers is reported in Table 3.

Variation of the thieno[2,3-*b*]pyrrole group gave **32** and **34** which had comparable in vitro profiles, although **33** displayed the best balance of properties. Comparison of rat pharmacokinetic profiles for compounds from the various sub-series (Table 4) again showed **33** to have the best profile with complete bioavailability, high permeability with low efflux potential, low clearance, moderate volume of distribution and a 11 h half-life.

Compound **33** showed no activity against a panel of cytochrome P450 isoforms (1A2, 2C9, 2C19, 2D6, 3A4; <50% inhibition at 10 μM) and no inhibition of the hERG encoded potassium channel.

Compound **33** was next tested in a glucagon challenge model of diabetes in Zucker rats.[14] In this model admin-

Table 3. Purity profiles of stereoisomers of compound **33**

Diastereoisomer	Composition
3-(*S*)-2′-(*R*)-	<1% 3-(*R*)-2′-(*R*)-form; <2% 3-(*S*)-2′-(*S*)-form
3-(*S*)-2′-(*S*)-	<1% 3-(*R*)-2′-(*S*)-form; <2% 3-(*S*)-2′-(*R*)-form
3-(*R*)-2′-(*R*)-	<1% 3-(*S*)-2′-(*R*)-form; 7% 3-(*R*)-2′-(*S*)-form
3-(*R*)-2′-(*S*)-	<1% 3-(*S*)-2′-(*S*)-form; 6% 3-(*R*)-2′-(*R*)-form

Table 4. Pharmacokinetic data for compounds

Compound	Rat clearance (mL/min/Kg)	Rat Vdss (L/Kg)	Rat $t_{1/2}$ h	Bioavail. (%)	Caco-2 A–B cm/s × 10^{-6}	Caco-2 B–A cm/s × 10^{-6}
11	1.2	1.4	16	35	12	7
16	5.1	1.4	5.4	12	4	14
28	2.5	2.2	13	41–94	17	10
33	2.4	1.9	11	>100	29	29

Compounds dosed at 2.1–4.0 mg/kg (iv) and 8.1–8.9 mg/kg (po) to male AP Wistar rats.

istration of glucagon increases hepatic glucose output through cAMP mediated activation of GPa.

Compounds were dosed po to between four and six animals in each group (basal blood glucose was around 5–6 mM) and did not affect pre-glucagon challenge blood glucose at doses which inhibited the glucagon response. When dosed at 12.5 μM/kg (dose volume 5 mL/kg) 45 min prior to the glucagon, compound **33** caused a 46% lowering of the induced glucose increase, measured 90 min after the compound dose.

In summary, optimisation of a series of 1-substituted 3,4-dihydro-2-quinolone GPa inhibitors resulted in compound **33**, which exhibits good potency in vitro and in vivo, a good DMPK profile and improved physical properties.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.10.037.

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9. Protein crystals kindly provided by N.G.O were grown using published procedures.[15] On soaking the inhibitors (2.5 mM and 5 mM for compounds **11** and **16**, respectively, for 30 min), these crystals underwent a space group change from tetragonal to orthorhombic symmetry, such that the dimer dyad is no longer crystallographic. Crystals could be flash-cooled to 100 K directly from the stabilization buffer which contained 10 mM BES, pH 6.7, 0.1 mM EDTA and 35% DMSO. Diffraction data were collected at SRS, Daresbury, at 100 K. The crystals of the complex with **11** have space group $P2_12_12_1$, unit cell 114.1, 125.2, 128.4 Å. 144,829 unique reflections from 492,418 observations to 1.9 Å give 81.3% completeness with R_{merge} = 9.3%. The final R-factor is 22.8% (R_{free} using 5% of data is 26.9%). The mean temperature factor is 38.2 Å^2 for protein atoms and 39.1 Å^2 for the ligand. Crystals of the complex with **16** have space group $P2_12_12_1$ and unit cell 113.9, 124.4, 128.3 Å. 143,632 unique reflections from 330,353 observations to 1.9 Å give 82.5% completeness with R_{merge} = 10.2%. The final model has an R-factor of 24.3% (R_{free} using 5% of data is 28.5%). Mean atomic temperature factor for the protein is 23.6 and for the inhibitor is 37.2 Å^2. Data analysis and structure solution used programs from the CCP4 suite.[16] The inhibitors were modelled into electron density using Quanta2000 (Accelrys). The model was refined using CNX (Accelrys) and Refmac5[16]. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 2IEG and 2IEI. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].
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