Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 4125–4128

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A New Class of Glycogen Phosphorylase Inhibitors

Zhijian Lu,[a,*] Joann Bohn,[a] Raynald Bergeron,[b] Qiaolin Deng,[a] Kenneth P. Ellsworth,[b] Wayne M. Geissler,[b] Georgianna Harris,[b] Peggy E. McCann,[b] Brian McKeever,[a] Robert W. Myers,[b] Richard Saperstein,[b] Christopher A. Willoughby,[a] Jun Yao[b] and Kevin Chapman[a]

[a]*Department of Basic Chemistry, Merck Research Laboratories, Merck & Co., Inc., PO Box 2000, Rahway, NJ 07065, USA*
[b]*Departments of Biochemistry and Metabolic Disorders, Merck Research Laboratories, Merck & Co., Inc., PO Box 2000, Rahway, NJ 07065, USA*

Received 21 February 2003; accepted 6 August 2003

Abstract—A new class of diacid analogues that binds at the AMP site not only are very potent but have ~10-fold selectivity in liver versus muscle glycogen phosphorylase (GP) in the in vitro assay. The synthesis, structure, and in vitro and in vivo biological evaluation of these liver selective glycogen phosphorylase inhibitors are discussed.
© 2003 Elsevier Ltd. All rights reserved.

It is known that excessive hepatic glucose production (HGP) is a significant factor contributing to the diabetic hyperglycemia. The liver is the major regulator of plasma glucose levels in the post-absorptive state, and in type 2 diabetics HGP is significantly higher than non-diabetics.[1,2] The liver produces glucose by gluconeogenesis and glycogenolysis. Type 2 diabetics have been reported to display elevated gluconeogenic rates.[2,3] Efforts to control HGP with gluconeogenesis inhibitors have not been very successful except for metformin. On the other hand, the reduction of glycogenolysis to reduce HGP via inhibition of glycogen phosphorylase has generated a lot of interest in recent years.[4] In humans, glucose is stored as glycogen in high concentrations in skeletal muscle and liver tissues, where glycogen degradation is regulated by the control of the activity of glycogen phosphorylase.

Our research efforts have been focused on searching for liver selective human glycogen phosphorylase inhibitors to treat type 2 diabetic patients. We wanted to avoid inhibiting the muscle glycogen phosphorylase due to concern about the effect of McArdle's disease.

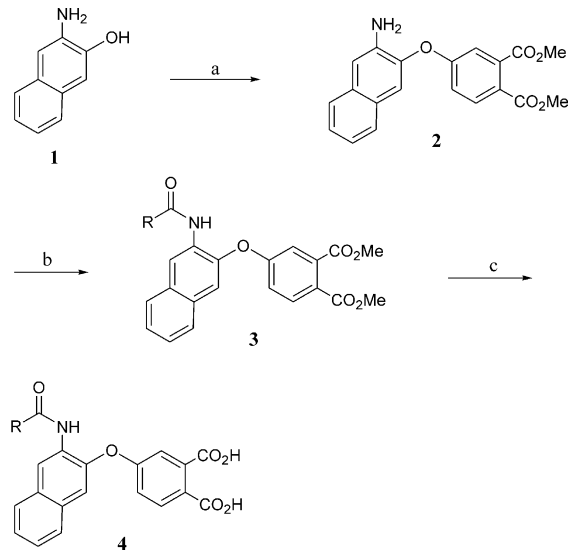
McArdle's disease is a defect in glycogen metabolism confined to muscle where the muscle phosphorylase activity is absent. The patient is otherwise normal and well developed except exhibits limited capability to perform strenuous exercise. Published data[4] and our internal research[5] have shown that the dimer interface inhibitors display only marginal (2- to 3-fold) or no selectivity between liver and muscle isozymes. On the other hand, our research has found that a class of diacid analogues not only are very potent but have about 10-fold selectivity liver versus muscle in the in vitro assay. One of the best compounds in the series, compound **11h**,[6] was evaluated in the glucagon challenge PD model and it lowered glucose more than 50% at 30 mpk at 60 min after oral administration. X-ray crystallography studies indicate that this class of compounds binds at the AMP site. In this letter we discuss the synthesis, structure, as well as in vitro and in vivo biological evaluation of these glycogen phosphorylase inhibitors.

The syntheses of these diacid analogues are summarized in Schemes 1 and 2. Commercially available 3-amino-2-naphthol was mixed with dimethyl 4-nitrophthalate and potassium carbonate in DMF at 100 °C for 12 h to afford **2**. Compound **2** reacted with the corresponding acid chlorides to give amides **3**. Amides **3** were then either treated with phenyltrimethylsilane and iodine at 110 °C or with aqueous NaOH to afford final diacids **4**.

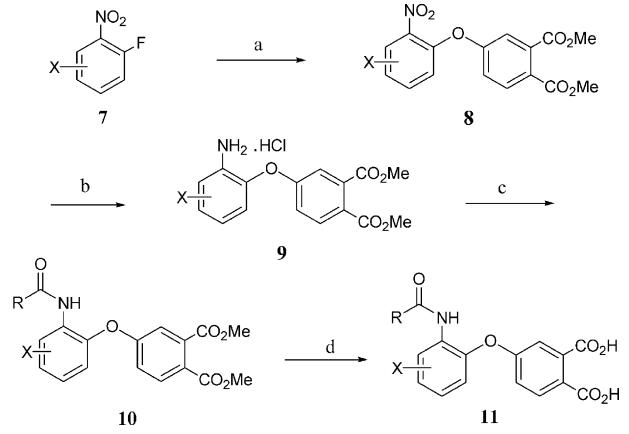
In a similar fashion, the substituted phenyl analogues were prepared. Commercially available 4-hydroxy

*Corresponding author. Tel.: +1-732-594-4392; fax: +1-732-594-9473; e-mail: zhijian_lu@merck.com

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.08.046



Scheme 1. Synthesis of naphthyl diacids: (a) dimethyl 4-nitrophthalate, K_2CO_3, DMF, 100 °C; (b) RCOCl, CH_2Cl_2, DIEA or RCO_2H, EDC, DIEA, CH_2Cl_2, 49–75%; (c) PhTMS, I_2, 110 °C or NaOH, $MeOH/H_2O$, 12–68%.

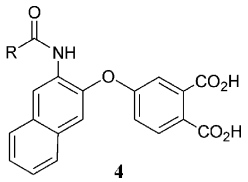


Scheme 2. Synthesis of phenyl diacids: (a) K_2CO_3, DMF, 100 °C, **6**; 66%; (b) H_2, Pd/C, EtOH, 40 psi; HCl; 100%; (c) RCOCl, DIEA, CH_2Cl_2 or RCO_2H, EDC, DIEA, CH_2Cl_2; 18–43%; (d) PhTMS, I_2, 110 °C, 28–70%.

phthalic acid (**5**) was treated with thionyl chloride in methanol under refluxing conditions to provide dimethyl 4-hydroxyphthalate (**6**) in good yield. 1-Fluoro 2-nitrobenzenes (**7**) were treated with **6** and potassium carbonate in DMF at 100 °C to yield ethers **8**. Hydrogenation of **8** with Pd/C at 40 psi gave amines which were converted into hydrochloride salts **9**. These amides were coupled with the corresponding acids or acid chlorides to afford amides **10**. Final deprotection of **10** using phenyltrimethylsilane and iodine at 110 °C gave the desired products **11**.

Compounds **4a–i** and **11a–k** were evaluated in the enzyme inhibition assay against human liver and muscle glycogen phosphorylase and the cell based assay.[7] The results are outlined in Tables 1 and 2. Initially, we investigated the SAR of **4** around the aromatic amides. Phenyl amide **4a** is a weak GP inhibitor to both liver and muscle with a moderate selectivity (4.3-fold). The 3-nitrophenyl analogue **4b** is about 100-fold more potent than **4a** for the liver with a selectivity ratio of 6. Introducing a nitrogen atom at the α-position of the phenyl carboxylic amide improves liver selectivity by 17-fold (**4c** vs **4a**). Combining these features, the pyridine analogue **4d**,[8] is the most potent compound ($IC_{50liver}$ = 1 nM) in the series with a slight drop of selectivity (3-fold). Compared with **4d**, the chloropyridyl and the methoxypyridyl analogues are similarly potent with improved selectivity. The methyl and the trifluoromethyl analogues are 10-fold less potent than **4d**, while the ethyl analogue loses only 4-fold of potency against the liver enzyme. All three of the alkyl analogues display better selectivity than **4d** (Table 1).

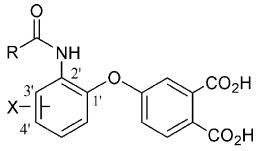
Table 1. SAR of naphthyl diacid analogues[7]



Compd	R	HLGP (nM)	HMGP (nM)	Ratio	GRAPH (μM)
4a		2880	12,300	4	>20
4b		23	140	6	7.5
4c		167	844	5	>20
4d		1	3	3	0.3
4e		2	12	6	1.5
4f		2	12	6	3.1
4g		10	57	6	3.5
4h		4	29	7	2.8
4i		12	80	7	2.2

In a different SAR study, we attempted to determine the effect of the substitutions on the central phenyl ring. For a better comparison, we used the best amides from the previous study. In the non-substituted phenyl series **11**(**a–e**), we observed a similar trend: $NO_2 > Cl \approx OMe$. The nitropyridyl analogue **11a** is very potent (3 nM) and selective (8-fold). Other phenyl analogues (**11b–c**) are about 10-fold less potent than the corresponding naphthyl analogues (**4e–f**). Interestingly the selectivity ratios for the non-substituted phenyl analogues (**11a–e**) are 8- to 12-fold better than the naphthyl analogues. But in the cell based assay, only **11a** and **11e** show low μM inhibitory activity. In the substituted phenyl series (**11f–k**), we investigated the effect of the fluorine substitution at

Table 2. SAR of phenyl diacid analogues[7]



11

Compd	R	X	HLGP (nM)	HMGP (nM)	Ratio	GRAPH (μM)
11a		H	3	25	8	1
11b		H	17	181	10	8
11c		H	20	200	10	13
11d		H	39	468	12	15
11e		H	16	167	10	3
11f		3′-F	250	1860	7	3
11g		3′-F	1	9	9	0.3
11h		3′-F	10	60	6	0.3
11i		3′-F	11	111	10	1
11j		4′-F	13	109	8	1.8
11k		4′-F	78	647	8	11

various positions. As shown in Table 2, 3′-fluoro phenyl analogue **11f** has moderate liver activity, but it displays a low μM activity in the cell based assay. If this trend holds, the substituted pyridine analogues should be significantly better. It turns out that these analogues display better cell activity (**11g**,**h**,**i**) while maintaining enzymatic inhibitions. For example, compound **11g** is 3 times more active both in the inhibition assay and in the cell assay than **11a**. In fact, compound **11g** is another excellent GP inhibitor ($IC_{50} = 1$ nM) along with **4d** as the results of our SAR studies have shown. The cell based assay shows that these 3′-fluorinated phenyl analogues are 9- to 29-fold more active than the corresponding non-fluorinated analogues but the 4′-fluorinated analogues do not improve the cell based activity.

In the light of these studies, we further investigated how these acids would act on the mouse and rat liver enzymes. Not surprisingly, all of the selected compounds display similar activity among the human, mouse and rat liver enzymes. The result might give us a

Table 3. A comparison among human, mouse and rat liver enzymes[7]

Compd	HLGP (nM)	Mouse liver GP (nM)	Rat liver GP (nM)
11b	17.4	14	16
11c	20	18	24
11e	16	14	19
11f	249	207	302
11h	10	<9	<9

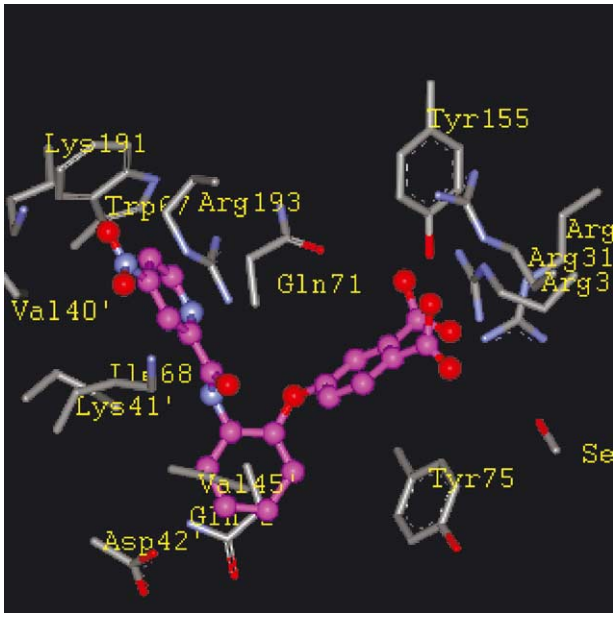


Figure 1. X-ray crystallographic representation of the complex of compound **11a** with neighboring residues of rabbit muscle glycogen phosphorylase.

useful guide to correlate the animal study with the potential human studies. Table 3 summarizes these data.

To understand where and how these acids are bound to the enzyme, an X-ray crystallographic analysis has been performed. Given the similarity between the liver and muscle enzymes and among the animal species, we chose the readily available rabbit muscle enzyme. Compound **11a** was co-crystallized with the rabbit muscle enzyme (details to be published elsewhere).[10] The crystal structure of the complex, solved to 2 Å resolution, shows that **11a** binds at the AMP site of the enzyme and the molecule bends to a V-shape (Fig. 1). The diacid forms a number of stabilizing interactions with Arg 81, Arg 309 and Arg 310 and Tyr 155. The central aromatic ring inserts into a lipophilic pocket containing Val 45′, Gln 72 and Tyr 75. The pyridine ring of **11a** fits into another pocket formed by Trp 67 and Val 40′, Ile 68 and Gln 71. The nitro group appears to stack with the guanidinium group of Arg 193. It is still not fully understood why the pyridyl nitrogen is so important for the binding. It is hypothesized that the nitrogen atom in the ring may change the electron distribution of the aromatic ring facilitating the beneficial interactions in the pocket. Figure 1 shows the picture of the binding site.

Compound **11h** was chosen for further in vivo studies based on its excellent cell inhibitory activity and reason-

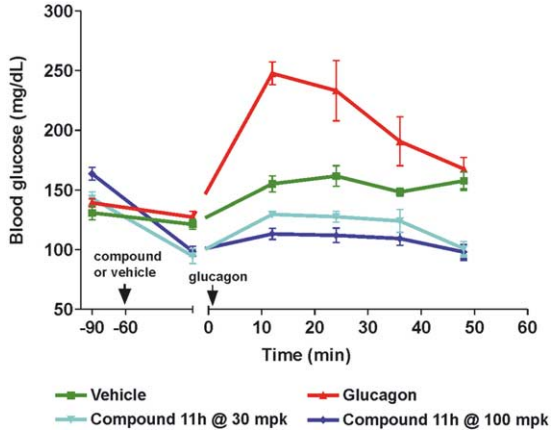


Figure 2. Effect of **11h** on glucagon-induced glucose levels.

able pharmacokinetics.[9] It was carried out in glucagon challenge model. In the study, **11h** was dosed in the animals at 30 and 100 mpk. The plasma glucose levels of the treated and untreated C57BI/6j mice were then monitored. Sixty minutes later, glucagon was introduced. As shown in Figure 2, in the treated group, the glucose levels were reduced initially at both 30 and 100 mpk. Once dosed with glucagon, the glucose level in the untreated animal was significantly higher than the vehicle; on the other hand, the glucose levels of the treated group increased only slightly. The glucose levels were lower than that of the vehicle as well (Fig. 2). This indicates that even dosed with glucagon compound **11h** is able to lower the glucose level significantly.

The in vivo selectivity study of compound **11h** will be the subject of another publication.[11] In summary, we have discovered a new class of glycogen phosphorylase inhibitors that bind at the AMP site. One of the best of these inhibitors has shown an ability to reduce plasma glucose in vivo.

References and Notes

1. Consoli, A. *Diabetes Care* **1992**, *15*, 430.
2. Gerich, J. E. *Horm. Metab. Res.* **1992**, *26*, 18.
3. Landau, B. R.; Wahren, J.; Chadramouli, V.; Schumann, W. C.; Ekberg, K.; Kalhan, S. C. *J. Clin. Invest.* **1996**, *98*, 378.
4. (a) Martin, W. H.; Hoover, D. J.; Armento, S. A.; Stock, I. A.; McPherson, R. K.; Danley, D. E.; Stevenson, R. W.; Barrett, E. J.; Treadway, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1776**, *98*, 95. (b) Hoover, D. J.; Lefkowitz-Snow, S.; Burgess-Henry, J. L.; Martin, W. H.; Armento, S. J.; Stock, I. A.; McPherson, R. K.; Genereux, P. E.; Gibbs, E. M.; Treadway, J. L. *J. Med. Chem.* **1998**, *41*, 2934. (c) Rath, V. L.; Ammirati, M.; Danley, D. E.; Ekstrom, J. L.; Gibbs, E. M.; Hynes, T. R.; Mathiowetz, A. M.; McPherson, R. K.; Olson, T. V.; Treadway, J. M. L.; Hoover, D. J. *Chem. Biol.* **2000**, *7*, 677. (d) Oikonomakos, N. G.; Schnier, J. B.; Zographos, S. E.; Skamnaki, V. T.; Tsitsanou, K. E.; Johnson, L. N. *J. Biol. Chem.* **2000**, *275*, 34566. (e) Bergans, N.; Stalmans, W.; Goldmann, S.; Vanstapel, F. *Diabetes* **2000**, *49*, 1419.
5. (a) Chen, L.; Bergeron, R.; Deng, Q.; Ellsworth, K. P.; Geissler, W. G.; Harris, G.; Myers, R. W.; Willoughby, C. A.; Yao, J.; Chapman, K., in preparation. (b) Roseuer, K.; Bergeron, R.; Deng, Q.; Ellsworth, K. P.; Geissler, W. G.; Harris, G.; Myers, R. W.; Willoughby, C. A.; Yao, J.; Chapman, K., in preparation.
6. Analytical data for **11h**: ^{1}H NMR (CD_3OD, 500 MHz) δ 8.55 (d, J = 5.5 Hz, 1H), 8.06 (d, J = 1.5 Hz, 1H), 7.15 (d, J = 8.5 Hz, 1H), 7.61 (dd, J = 5.5, 1.5 Hz, 1H), 7.42 (dd, J = 15, 8.5 Hz, 1H), 7.21 (d, J = 2 Hz, 1H), 7.15 (t, J = 8.5 Hz, 1H), 7.09 (dd, J = 8.5, 2.5 Hz, 1H), 6.99 (d, J = 8.5 Hz, 1H). LC–MS (ES) 431 (M + 1).
7. (a) Phosphorolysis of glycogen using recombinant human liver or muscle enzyme. Glucose production was monitored via enzyme linked assay involving phosphoglucomutase/glucose dehydrogenase-mediated NADH production (ex. 340 nM, em. 465 nM). (b) Glucagon-stimulated phosphorolysis using isolated rat hepatocytes.
8. Analytical data for **4d**: ^{1}H NMR (CD_3OD, 500 MHz) δ 9.00 (s, 1H), 8.91 (d, J = 6 Hz, 1H), 8.75 (d, J = 5.5 Hz, 1H), 8.24 (dd, J = 6, 3 Hz, 1H), 7.94 (d, J = 7.5 Hz, 1H), 7.86 (d, J = 6.5 Hz, 1H), 7.70 (d, J = 6.5 Hz, 1H), 7.59 (m, 1H), 7.56 (d, J = 2.5 Hz, 1H), 7.45 (s, 1H), 7.42 (m, 1H), 7.29 (dd, J = 10, 3 Hz, 1H). LC–MS (ES) 496 (M + Na).
9. Pharmacokinetics data for **11h**: Male *C57BL/6J* mice were dosed iv at 1 mg/kg and orally at 2 mg/kg by gavage (N = 3 for both iv and po). C_{max} = 44 nM; $t_{1/2}$ = 60 min; %F = 4.1.
10. McKeever, B., in preparation.
11. Myers, R. W.; Bergeron, R.; Lu, Z.; Bohn, J.; Deng, Q.; Ellsworth, K. P.; Geissler, W. G.; Harris, G.; Myers, R. W.; Willoughby, C. A.; Yao, J.; Chapman, K., in preparation.