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N,*N*-Dibenzyl-*N*′-benzylidenehydrazines: Potent Competitive Glucose-6-phosphatase Catalytic Enzyme Inhibitors

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Abstract—A novel class of *N*,*N*-dibenzyl-*N*′-benzylidenehydrazines as potent and competitive glucose-6-phosphatase catalytic site inhibitors are described. Optimisation of this series identified compounds with IC_{50} values as low as 170 nM.

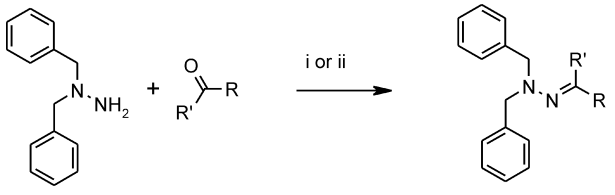
Introduction

Increased hepatic glucose production seems to play a pivotal role for the elevated plasma glucose levels seen in type 2 diabetic patients.[1] Inhibitors of liver enzymes involved in either glycogenolysis or gluconeogenesis are potential targets for development of new drugs as alternatives to the existing treatments of type 2 diabetes.[2–6] Among those targets glucose-6-phosphatase (G-6-Pase) has attracted considerable attention[5–7] since this enzyme is mainly located in the liver and catalyses the final step in both glycogenolysis and gluconeogenesis by converting glucose-6-phosphate (G-6-P) to glucose. According to the substrate transport model,[8] which is the most widely accepted model, G-6-Pase comprises of the G-6-Pase catalytic enzyme with its active site located at the luminal site of the endoplasmic reticulum, a specific transporter T_1 which mediates entry of G-6-P into the luminal compartment, and transporters T_2 and T_3 which mediate export to the cytosol of inorganic phosphate and glucose, respectively.[9,10] It has been shown that the rate of hydrolysis of G-6-P[11] and the hepatic glucose output[1] is increased under diabetic conditions. The increased activity is mainly accounted for by increased G-6-Pase catalytic enzyme protein,[12,13] which justifies the catalytic protein as a potential target in the control of excess glucose production observed in diabetes. Recently, we have shown that a new class of compounds, 4,5,6,7-tetrahydrothieno[3,2-*c*]- and -[2,3-*c*]pyridines, are potent competitive inhibitors of the G-6-Pase catalytic enzyme.[7,14] This suggests that specific inhibitors of the G-6-Pase catalytic protein could be an alternative to the T_1-translocase inhibitors as potential anti-diabetic drugs targeting G-6-Pase.

In this publication, we report a new series of *N*,*N*-dibenzyl-N′-benzylidenehydrazines that are potent competitive inhibitors of the G-6-Pase catalytic protein.

Chemistry

Hydrazones derived from aldehydes were prepared[15] by mixing equimolar amounts of *N*,*N*-dibenzylhydrazine and aldehydes in a 2:1 mixture of DMF and triethyl orthoformate. When the reaction was complete, the solvents were removed in vacuo. Hydrazones of ketones were similarly prepared[16] in DMF with a catalytic amount of HOAc (Scheme 1). Under these reaction



Scheme 1. (i) (aldehydes) DMF/$HC(OEt)_3$ (2:1); (ii) (ketones) DMF, cat. HOAc.

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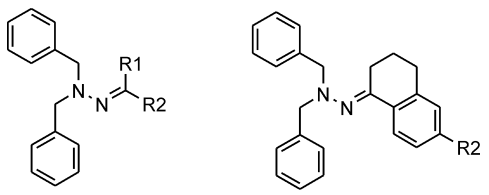
Table 1.



3, 4

Compd	R1	R2	IC_{50}[a] (μM)
1	H	Ph	1.5
2	Me	Ph	25
3		OMe	>50
4		H	25
5	H	Cyclohexyl	2.4
6	H	Bn	>100
7	H	4-OMe-Ph	0.22
8	H	3-OMe-Ph	0.7
9	H	2-OMe-Ph	>100
10	H	4-Cl-Ph	0.24
11	H	4-OH-Ph	2.4
12	H	4-NMe_2-Ph	100
13	H	3,5-Cl_2-Ph	>100
14	H	3,4-Cl_2-Ph	2
15	H	2-Furyl	1.1
16	H	2-Thienyl	1.5
17	H	2-Pyridyl	10
18	H	3-Pyridyl	3.7
19	H	4-Pyridyl	17
20	H	5-Et-Furyl	0.33
21	H	5-Cl-2-Thienyl	0.17
22	H	5-$HOCH_2$-Furyl	16
23	H	5-NO_2-Furyl	>100

[a]Assay described previously[7] using Triton X-100 disrupted pig liver microsomes.

conditions we have never observed by NMR or LC–MS the formation of *E*/*Z*-isomers of the hydrazone bond.

Discussion

The importance of the hydrazones (Table 1) being derived from aldehydes was evident (**1** vs **2**). Many other ketone-derived hydrazones were prepared (e.g., **3** and **4**) but none of them proved to have any significant inhibitory action. Saturation of the aromatic ring of the benzylidene group (**5**) gave a slight decrease in G-6-Pase activity, whereas chain elongation of the benzylidene group of **1** to the corresponding phenetylidene group resulted in an inactive compound (**6**). Substitution of the benzylidene ring with a 4-methoxy group resulted in a marked increase in potency (**7**). While the 4-methoxy proved to be better than the 3-substituted analogue (**8**), the *ortho*-isomer (**9**) was totally inactive. Other 4-substituents were also found to result in active compounds, for example 4-chloro (**10**), equipotent with (**7**), and 4-hydroxy (**11**). Other substituents resulted in inactive compounds, for example 4-dimethylamino (**12**) and 3,5-dichloro (**13**). With 3,4-dichloro (**14**), inhibition was partially restored. Compounds derived from heteroaromatic aldehydes were indeed also found to be active inhibitors, furyl (**15**) and thienyl (**16**) being equipotent

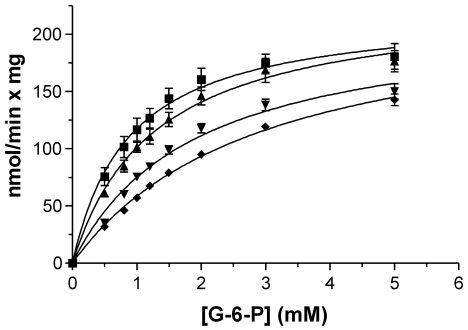


Figure 1. Michaelis–Menten curves for glucose-6-phosphatase activity from disrupted pig microsomes in the presence of 0 (■), 0.5 (▲), 1.0 (▼) and 2.5 (◆) μM (**15**). The V_{max} values (nmol/mg protein×min) were calculated to be 221.9±9.1; 230.9±1.7; 216.9±11.9 and 230.9±12.6 for 0, 0.5, 1.0 and 2.5 μM, respectively, and the corresponding K_m values (mM) were calculated to be 0.9±0.1; 1.3±0.1**; 2.0±0.1*** and 2.9±0.2***. Data are averages from 3 individual determinations ±SEM. **$P<0.01$; ***$P<0.001$ compared to control value (Student's *t*-test). Using the Michaelis–Menten equation for competitive inhibition, the K_i could be calculated to be 1.0±0.1 μM.

with phenyl (**1**) and better than pyridyl (**17**, **18** and **19**). Appropriate 5-substituents on the furyl and thienyl groups gave highly potent glucose-6-phosphatase catalytic site inhibitors [e.g., 5-ethyl-2-furyl (**20**) and 5-chloro-2-thienyl (**21**)]. The interaction of the R2-moiety with the enzyme is probably an unspecific lipophilic interaction with optimal spatial contacts giving more potent compounds. The increase in polarity and resulting loss of inhibitory activity in going from **20** to **22** or **23** could result from unfavourable polar to lipophilic interactions. Further, too much bulk was not well tolerated (e.g., **2**, **3**, **4** and **13**). Selected compounds (e.g., (**15**), Fig. 1) were further tested to determine the inhibitory mode of action. For these compounds, the mode of action was always competitive, as V_{max} was unchanged in the presence of inhibitor whereas the K_m was reduced (Fig. 1). Assuming that all of the compounds were competitive inhibitors, K_i can be calculated from $K_i = IC_{50}/(1+([G\text{-}6\text{-}P]/K_m))$. Since [G-6-P] is 0.5 mM and K_m is 0.9 mM (Fig. 1), K_i can be calculated as $K_i = 0.64 \times IC_{50}$. Selected compounds were assayed against other phosphatases, alkaline phosphatase (AP) and fructose-1,6-bisphosphatase (FB) to investigate if there was a selectivity issue associated with these compounds. No significant inhibition of either AP with, for example **15**, **16** and **19**, or of FB with, for example, **11** and **19** was observed (data not shown), suggesting that the compounds were selective for G-6-Pase.

Conclusion

In conclusion, *N*,*N*-dibenzyl-*N*′-benzylidenehydrazines represent a novel class of potent competitive glucose-6-phosphatase catalytic site inhibitors.

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15. Preparation of hydrazones derived from aldehydes, general procedure: *N*,*N*-dibenzylhydrazine (5 g, 23.6 mmol) was dissolved in DMF (20 mL), and 4-chlorobenzaldehyde (3.3 g, 23.6 mmol) was added followed by $HC(OEt)_3$ (10 mL) and the reaction mixture was stirred at rt for 16 h. Concentration in vacuo furnished 7.39 g (94%) of *N*,*N*-dibenzyl-N′-(4-chlorobenzylidene)hydrazine (**10**), mp 118–120 °C. ^1H NMR ($CDCl_3$): δ 4.52 (4H, s), 7.08 (1H, s), 7.2–7.3 (12H, m), 7.40 (2H, d). Anal. calcd for $C_{21}H_{19}ClN_2$: C, 75.33%; H, 5.72%; N, 8.37%. Found: C, 75.07%; H, 5.80%, N, 8.34%.
16. Preparation of hydrazones derived from ketones, general procedure: *N*,*N*-dibenzylhydrazine (32 mg, 0.15 mmol) and 6-methoxytetralone (26 mg, 0.15 mmol) were dissolved in DMF (1.5 mL), and HOAc (9 μL) was added. The reaction mixture was stirred at rt for 16 h. Concentration in vacuo furnished *N*,*N*-dibenzyl-N′-(6-methoxy-1,2,3,4-tetrahydronaphthalen-1-ylidene)hydrazine (**3**). HPLC–MS: $m/z = 371$ (M + 1); ELS purity 90%.