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Glucose-6-Phosphatase Catalytic Enzyme Inhibitors: Synthesis and In Vitro Evaluation of Novel 4,5,6,7-Tetrahydrothieno[3,2-*c*]- and -[2,3-*c*]pyridines

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Abstract—The discovery of the first class of potent glucose-6-phosphatase catalytic site inhibitors, substituted 4,5,6,7-tetrahydrothieno[3,2-*c*]- and -[2,3-*c*]pyridines, is described. Optimisation of this series involved solution phase combinatorial synthesis and very potent compounds were prepared with IC_{50} values down to 140 nM. The structure–activity relationship (SAR) of these compounds indicates that: a tetrahydrothieno[3,2-*c*]pyridine core ring system and the isomeric [2,3-*c*] system are equipotent and much better than the corresponding benzo analogue, 1,2,3,4-tetrahydro-isoquinoline. The 4-substituent of the tetrahydrothieno[3,2-*c*]pyridine ring has to be a phenyl group, optionally substituted with a lipophilic 4-substituent, such as trifluoromethoxy or chloro. The 5-substituent of the tetrahydrothieno[3,2-*c*]pyridine ring has to be a substituted benzoyl; anisoyl and (*E*)-3-furan-3-ylacryloyl are the best of the investigated groups. Substitution in the benzoyl *ortho* position seems to be forbidden, whereas substitution in the *meta* position is tolerated only if a methoxy *para* substituent is present. These SAR findings were parallel to those obtained in the 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine system. Enantioselectivity in enzyme recognition was observed and the activity resided in all cases only in one of the enantiomers.

Introduction

Regulation of hepatic glucose production is an attractive way to reduce the increased blood glucose levels seen in type 2 diabetic patients.[1–4] Inhibition of different liver enzymes involved in either glycogenolysis or gluconeogenesis seem therefore to be potential targets for development of new drugs as alternatives to the existing treatments of type 2 diabetes.[5] Glucose-6-phosphatase (G-6-Pase), which is predominantly found in the liver, catalyses the terminal step in both glycogenolysis and gluconeogenesis by converting glucose-6-phosphate (G-6-P) to glucose, and is, therefore, a key enzyme in blood glucose homeostasis.[1] The G-6-Pase is a multicomponent system comprising the G-6-Pase catalytic enzyme with its active site located at the luminal site of the endoplasmic reticulum, a specific transporter T1 which mediates entry of G-6-P into the luminal compartment, and transporters T2 and T3 which mediates export to the cytosol of inorganic phosphate and glucose, respectively.[6,7] It has been shown that the rate of hydrolysis of G-6-P and the hepatic glucose output were increased under diabetic conditions.[8,9] The increased activity could mainly be accounted for by increased G-6-Pase catalytic enzyme protein.[10,11] Recently, peroxyvanadium compounds have been found to be potent inhibitors of the G-6-Pase catalytic enzyme,[12] which makes this site a potential target in control of excess glucose production seen in diabetes.[12]

Very few compounds with activity on the G-6-Pase enzyme complex are known. A series of chlorogenic acid derivatives have been found to inhibit the T1 translocase,[13] 2-hydroxy-5-nitrobenzaldehyde is described[14] as an inhibitor of both T1 and T2, and a report on a carbohydrate (6R-C-methylglucose) as a specific inhibitor of the G-6-Pase enzyme has been published.[15] The K_i of the 6R-C-methylglucose was reported to be in the mM range.

We have screened our compound library for compounds which are able to inhibit the activity of the G-6-Pase catalytic site, and in this publication we report a series of 4,5,6,7-tetrahydrothienopyridines, the first report on

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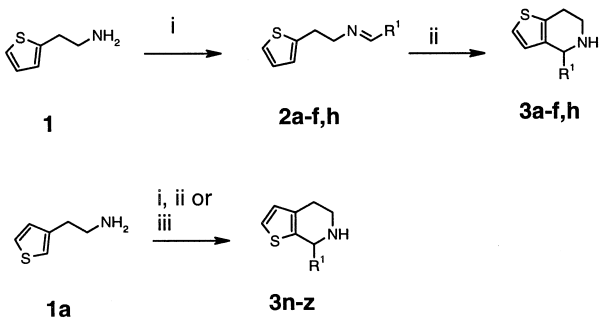
non-carbohydrate compounds with potent activity at the catalytic site only.

Neither 4,5,6,7-tetrahydrothieno[3,2-*c*]pyridines carrying an acyl substituent in the 5-position and a cyclic substituent in the 4-position nor 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridines carrying an acyl substituent in the 6-position and a cyclic substituent in the 7-position are well described in the literature.

The preparation of 4-substituted 4,5,6,7-tetrahydrothieno[3,2-*c*]pyridines have been reported, but no 4-aryl-5-acyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridines were among the described compounds.[16–19] Ohkubo et al.[18] have described a series of 4,5,6,7-tetrahydrothieno[3,2-*c*]pyridines bearing hydrogen, methyl or ethyl on the 5-position with anticonvulsant activities. Russell[20] reported a series of 5-aminoalkyl substituted tetrahydrothienopyridines as antiarrythmic agents and Vecchietti et al.[21] have investigated a series of 4-aminoalkyl-5-arylacetyl derivatives and found that they possess potent activity as kappa opioid analgesics.

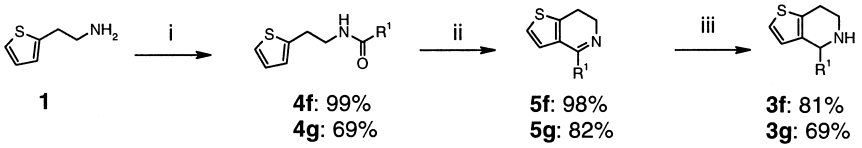


Scheme 1. Preparation of 4,5,6,7-tetrahydrothienopyridines, imine route. (i) R^1CHO, EtOH, TEA; (ii) TFA, rt; (iii) a. R^1CHO, PhH, △, Dean–Stark, b. TFA.

Chemistry

Some 4-substituted 4,5,6,7-tetrahydrothieno[3,2-*c*]thienopyridines (4-chlorophenyl **3j**, 4-methoxyphenyl, **3k**, 4-trifluoromethylphenyl **3l** as well as 4-trifluoromethoxyphenyl **3m**) were commercially available, new compounds were prepared via the imine and amide routes as depicted in Schemes 1 and 2, respectively.

The imine route (Scheme 1 and Table 1, entries **a**–**f**, **h**, **n**–**y**) gave good yields for benzaldehyde derivatives (70–100%) while thiophene, cyclohexyl as well as 1-methylpiperidine-4-carboxaldehydes gave low yields both at room temperature and at elevated temperatures. These compounds were more conveniently prepared as shown in Scheme 2 using the amide route. Commercially available 2-(2-thienyl)ethylamine (**1**) or 2-(3-thienyl) ethylamine (**1a**) were treated with an aldehyde under

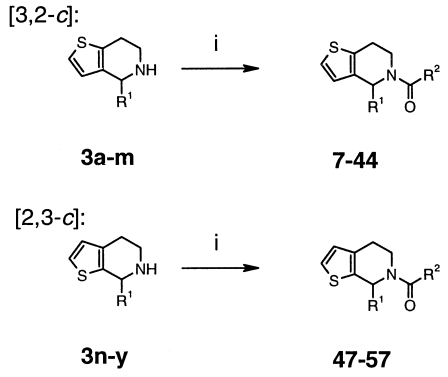


Scheme 2. Preparation of 4,5,6,7-tetrahydrothieno[3,2-*c*] pyridines, amide route. (i) a. R^1COCl, TEA, DCM, or b. R^1CO_2H, HOBt, EDAC, DMF, (ii) $POCl_3/P_2O_5$, △; (iii) $NaBH_4$, MeOH.

Table 1. Physicochemical data for **2a**,**b**,**d** and **3a–h, n–y**[c]

Compounds	R^1	Yield **2** (%)	Mp (°C)	Yield[a] **3** (%)	Mp (°C)
[3,2-*c*]:					
a	4-Dimethylaminophenyl	77	76.8–77	95	95–98
b	4-Nitrophenyl	76	83.9–84.4	85	128–129.5
c	4-Pyridyl			71	81.8–83.8
d	2-Thienyl	36	ND[e]	15	96–97.3
e	5-Chloro-2-thienyl			10	Oil
f	Cyclohexyl			9 (81)[b]	Oil
g	1-Methyl-4-piperidinyl			69 [b]	Oil
h	Phenyl			24	83.8–84.2
[2,3-*c*]:					
n	4-Trifluoromethoxyphenyl			16	190–195[c]
o	4-Chlorophenyl			81	93.6–93.8
p	4-Methoxyphenyl			42	Oil
q	2,4-Dichlorophenyl			91 [d]	Oil
r	2,4-Dimethoxyphenyl			95 [d]	Oil
s	3-Methoxyphenyl			100 [d]	Oil
t	3-Fluoro-4-methoxyphenyl			97 [d]	Oil
u	3,4-Methylenedioxyphenyl			100 [d]	Oil
v	2-Methoxyphenyl			100 [d]	Oil
x	3,5-Dichlorophenyl			94 [d]	Oil
y	3-Fluorophenyl			100 [d]	Oil

[a]Yields refer to imine route overall yields.
[b]Amide route, overall yields.
[c]Hydrogen oxalate.
[d]Yields determined by HPLC (ELS-detection).
[e]ND: Not determined.



Scheme 3. Preparation of substituted 4,5,6,7-tetrahydrothienopyridines **7**–**44** and **47**–**57**. (i) R^2COCl, Et_3N, DMF, or R^2CO_2H, HOBt, EDAC, DMF.

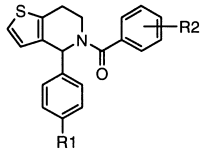
formation of an imine which was isolated either by filtration or by concentration in vacuo. The imines were cyclised immediately without any purification. The crude imines were highly prone towards hydrolysis to amine and aldehyde, as column chromatography resulted in hydrolysis to the starting materials.

Ring closure of the imines were performed in CF_3CO_2H (TFA) either at room temperature or at reflux to afford the desired 4,5,6,7-tetrahydro[3,2-*c*]- and -[2,3-*c*]pyridines (**3a**–**f, h, n**–**y**).

Preparation of compounds **3f**–**g** proceeded in good yields using the amide route depicted in Scheme 2. Amides **4** were formed either from reaction of **1** with the appropriate carboxylic acid chloride/triethylamine or from carboxylic acids using HOBt/EDAC as coupling reagents. Cyclisation of amides **4** with $POCl_3$ or mixtures of P_2O_5/$POCl_3$ gave yields of $>80\%$, subsequent reduction with $NaBH_4$ gave the desired tetrahydrothieno[3,2-*c*]pyridines in yields $>69\%$.

The target substituted 4,5,6,7-tetrahydrothieno[3,2-*c*]- and -[2,3-*c*]pyridines **7**–**44** and **47**–**57** were prepared from the various substituted 4,5,6,7-tetrahydrothienopyridines **3a**–**y** by means of conventional amide formation procedures (Scheme 3). In order to prepare a large number of compounds with a large diversity for exploring the structural requirements of the inhibitors for activity, the procedure was modified to work in a solution phase parallel fashion. This method was conveniently used to prepare >200 analogues of this type. Selected compounds were prepared and characterised fully while all the library compounds were characterised by HPLC-MS. Biological results obtained from the active library compounds did not differ significantly from the corresponding data obtained from pure, characterised compounds (Table 7). (Test solutions were made by weighing out the evaporation residues). Further, selected compounds were resolved into the pure enantiomers using semi-preparative HPLC. Generally, HPLC-resolution of an active library compound gave two optical

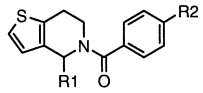
Table 2. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridines. SAR of the 5-benzoyl group

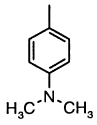
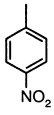
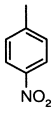
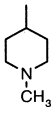


Compound	R1	R2	IC_{50} (μM)	HPLC-MS[a]		
				R_t (min)	M + 1 (*m/z*)	ELS purity (%)
7	Cl	4-OMe	0.62	15.90	384	100
8	OCF_3	4-OMe	0.81	16.43	434	93
9	OMe	4-OMe	0.80	14.73	380	100
10	CF_3	4-OMe	0.51	16.15	418	97
11	CF_3	3-OMe	>100	16.15	418	98
12	OMe	2-Cl	>78	15.10	384	100
13	OCF_3	4-OEt	1.1	17.03	448	88
14	Cl	4-F	1.8	16.03	372	100
15	Cl	4-Cl	3.3	16.83	388	100
16	Cl	4-NH_2	1.4	11.65	369	100
17	Cl	4-OH	1.7	14.10	370	59
18	Cl	4-CH_2OH	0.51	13.73	384	100
19	OMe	4-CH_2OH	>100	12.10	380	100
20	OCF_3	4-Ph	>100	18.00	480	96
21	OCF_3	4-^{t}Bu	>100	18.48	460	96
22	Cl	H	4.9	15.97	354	99
23	OCF_3	3-Cl-4-OMe	1.7	16.78	468	94
24	OCF_3	3,4-di-Cl	>100	17.68	472	92
25	OCF_3	3,4-OCH_2O	2.5	15.98	448	94
26	OCF_3	5-Indolyl[b]	2.7	15.72	443	94
27	OCF_3	5-Benzimidazolyl[c]	4.4	9.68	444	93

[a]HPLC-MS conditions: See Experimental.
[b]Whole 5-substituent is 5-indolylcarbonyl.
[c]Whole 5-substituent is 5-benzimidazolylcarbonyl.

Table 3. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridines. SAR of the 4-phenyl group



Compound	R1	R2	IC_{50} (μM)	HPLC-MS[a]		
				R_t (min)	M + 1 (*m*/*z*)	ELS purity (%)
28		OMe	0.61	14.83	350	92
29		Cl	1.1	15.90	354	77
30		CH_2OH	1.9	12.33	350	82
31		OMe	0.75	9.55	393	85
32		OMe	1.2	14.80	395	66
33		CH_2OH	100	12.44	395	48
34		OMe	6.0	14.58	356	79
35		OMe	6.1	16.38	391	58
36		OMe	> 100	8.50	351	91
37		OMe	> 100	8.75	371	100
38		OMe	> 100	16.18	356	> 98

[a]HPLC-MS conditions: See Experimental.

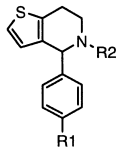
isomers of which one was approximately 2-fold more potent as compared to the library compound, whereas the other isomer was inactive.

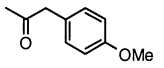
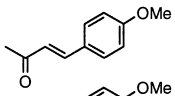
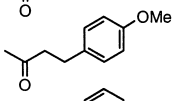
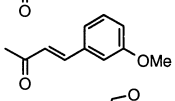
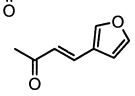
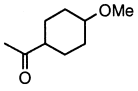
Initially we investigated the SAR of the 5-benzoyl group using the commercially available thieno[3,2-*c*]pyridines **3j–m** as seen in Table 2 where selected compounds are listed. Using the thieno[3,2-*c*]pyridines **3a–h** the SAR of the 4-phenyl group was explored further as seen in Table 3. To investigate compounds with a spacer between the benzene ring and the carbonyl group of the benzamides a series of compounds were prepared, some of which are listed in Table 4. The isomeric 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridines were prepared and tested, along with other substituents/substitution patterns in the 7-aryl group as listed in Table 5. Lastly, selected compounds were resolved into their optically pure enantiomers as listed in Table 6.

Biological results

Initial information on the ability of these compounds to inhibit the glucose-6-phosphatase activity at the catalytic site using TritonX-100 disrupted pig microsomes were obtained using five concentrations (0, 1, 25, 50, 100 μM) of test compounds. Compounds of interest were

Table 4. 4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridines. Further SAR of the 5-benzoyl group



Compound	R1	R2	IC_{50} (μM)	HPLC-MS[a]		
				R_t (min)	M + 1 (*m*/*z*)	ELS purity (%)
39	OCF_3		> 100	16.25	448	93
40	OCF_3		2.3	16.64	460	88
41	CF_3		> 100	16.41	446	59
42	CF_3		> 100	16.54	444	89
43	OMe		1.8	14.50	366	93
44	OCF_3		> 100	16.03	440	96

[a]HPLC-MS conditions: See Experimental.

further evaluated by determination of full dose–response curves to calculate the IC_{50} values. Moreover, in some cases the inhibitory mode of action was investigated. For these compounds, the mode of action was always competitive (data not shown).

Discussion

When comparing **7**, **8**, **9** and **10** (Table 2), it is evident that the *p*-substituent of the 4-phenyl group has no significant impact on potency.

The substitution pattern of the 5-benzoyl group is much more important for inhibitory activity, the 4-methoxy group being by far the best. Moving this group to the 3-position (**11**) results in loss of all activity. A 4-Cl-substituent is also tolerated, although about 5-fold less potent (**15**). Similarly, moving this group to the 2-position (**12**) all activity is lost. An unsubstituted 5-benzoyl group (**22**) is roughly as potent as a 4-chlorobenzoyl group.

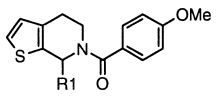
As can be seen in Table 2, many other groups are tolerated in this *para*-position, for example hydroxy (**17**), hydroxymethyl (**18**), amino (**16**), and fluoro (**14**) groups confer good activity. There are, however, steric constraints, as compounds with more bulky aliphatic and aromatic *para* substituents are not active, for example *tert*-butyl (**21**) and phenyl (**20**).

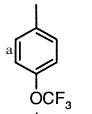
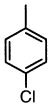
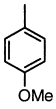
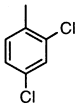
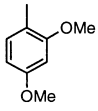
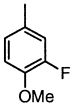
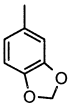
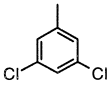
Compounds with 3,4-disubstituted benzoyl groups can be active, provided that the 4-substituent is a heteroatom capable of being a hydrogen bond donor and/or acceptor, compare for example **23** with **24** (Table 2). Activity is lost, however, compared to the 4-methoxy group. Ring systems, as in **27**, **26** and **25** are also allowed with respect to activity.

Many other acyl groups than substituted benzoyl have been tried and the general picture is that positioning of a methoxy group or another hydrogen bond acceptor within narrow limits is crucial for activity. Thus, 4-methoxyphenylacetyl (**39**) and its homologue 3-(4-methoxyphenyl)propionyl (**41**) are inactive, whereas 4-methoxycinnamyl (**40**) is active (Table 4). Moving this methoxy group to the *meta* position gave an inactive compound (**42**). It was not possible with other aromatic substituents to regain activity for any of the three mentioned 'non-benzoyl' systems. Another acyl group that confers good activity is the (*E*)-3-furan-3-ylacryloyl group (**43**). This group presents the oxygen atom of the furan ring roughly in the same space, as does the 4-methoxybenzoyl group. It is remarkable that a fully saturated acyl group (4-methoxycyclohexylcarboxyl, **44**, Table 4) is devoid of any activity, indicating that interactions with the π-electrons of the aromatic ring or those of the 3-furan-3-ylacryloyl group are implicated in the enzyme inhibition.

It is evident that the combination of 'allowed' substituents on the 4-phenyl and 5-benzoyl groups is not additive with respect to inhibitory activity, as exemplified by **18** and **19**. With the 4-methoxyphenyl building block (**3k**) there were generally much fewer library hits than

Table 5. 4,5,6,7-Tetrahydrothieno[2,3-*c*]pyridines. SAR of the 7-aryl group



Compound	R1	IC_{50} (μM)	HPLC-MS[a] R_t (min)	HPLC-MS[a] M + 1 (*m*/*z*)	HPLC-MS[a] ELS purity
47		0.35	16.50	434	93
48		0.26	—[b]	—[b]	—[b]
49		0.80	—[b]	—[b]	—[b]
50		> 100	6.71	417	50%
51		> 100	5.82	409	77%
52		4.9	5.39	380	73%
53		4.6	5.97	397	64%
54		0.78	5.87	394	73%
55		> 100	5.38	380	75%
56		> 100	6.97	418	80%
57		25	5.38	368	67%

[a]HPLC-MS metod B used for compound **50**–**57**. See Experimental.
[b]See Table 7 for data.

obtained with **3j**, **3l** and **3m**, and only compounds with anisoyl and (*E*)-3-furan-3-ylacryloyl as 5-substituents consistently showed up as potent library hits.

We then turned to investigate the SAR of the 4-substituent of the 4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine system (Table 3) originating from the intermediates **3a**–**i**. An unsubstituted phenyl group is as potent as any of the 4-Cl-, 4-CF_3-, and 4-OCF_3-phenyl systems (compare for example **28** with **8**). Even with less favourable substituents on the 5-benzoyl group, such as 4-chloro- (**29**) and hydroxymethyl (**30**), potency is maintained.

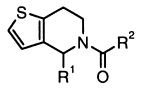
Likewise, a 4-dimethylamino substituent on the 4-phenyl group (**31**) is about as good as the unsubstituted or 4-Cl-, 4-CF_3-, and 4-OCF_3-phenyl systems. In contrast, neither a 4-pyridyl group (**36**), nor a 1-methyl-4-

Table 6. Optically resolved compounds

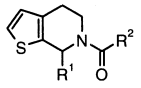
Compound	ee ($[\alpha]_D^{20}$)	IC_{50} (μM)	Column	R_t (min)	Flow (mL/min)
7A	100%	> 100	W[b,g]	44–50[b] *15.5*[a]	12 *1*[a]
7B	98.0% (−170.0)	0.31	W[b]	62–72 *20.8*[a]	12 *1*[a]
8A	> 99.8%	0.31	W[b]	24–30 *12.0*[a]	10 *1*[a]
8B	99.1%	> 100	W[b]	41–47 *15.8*[a]	10 *1*[a]
9A	> 99.9%	0.23	W[e]	27–30 *9.3*[a]	10 *1*[a]
9B	98.8%	> 100	W[e]	39–45 *12.3*[a]	10 *1*[a]
10A	> 99.0%	0.18	W[b]	40–43 *12.6*[a]	10 *1*[a]
10B	99.0%	> 100	W[b]	55–59 *16.4*[a]	10 *1*[a]
15A	100%	0.63	W[b]	28–32 *9.9*[a]	10 *1*[a]
15B	99.5%	> 100	W[b]	51–58 *15.5*[a]	10 *1*[a]
17A	100%	1.0	W[b]	24–29 *8.9*[a]	10 *1*[a]
17B	99.4%	> 100	W[b]	42–50 12.5[a]	10 *1*[a]
27A	100%	> 100	C[c,h]	16–19 *8.4*[a]	6 *0.6*[a]
27B	> 99.0%	8.70	C[c]	27–35 *14.7*[a]	6 *0.6*[a]
43A	99.8%	> 100	C[d]	20–24 *12.9*[a]	6 *0.55*[a]
43B	99.8%	0.91	C[d]	30–36 *19.8*[a]	6 *0.55*[a]
46A	100% (+175.4)	1.6	W[b]	27–32 *9.2*[a]	10 *1*[a]
46B	> 99.5% (−170.6)	> 100	W[b]	62–74 *17.0*[a]	10 *1*[a]
47A	> 99.8%	0.16	W[f]	32–36 *10.5*[a]	10 *1*[a]
47B	> 98.0%	> 100	W[f]	45–51 *14.5*[a]	10 *1*[a]
49A	100%	0.24	W[f]	26–30 *9.1*[a]	10 *1*[a]
49B	99.2%	> 100	W[f]	38–43 *11.9*[a]	10 *1*[a]

[a]Retention times and flow rates in italics refers to data from analytic runs.
[b]Eluted isocratically with a mixture of heptane: 2-propanol (1:1).
[c]Eluted isocratically with a mixture of heptane:ethanol:diethylamine (70:30:0.1).
[d]Eluted isocratically with a mixture of heptane:ethanol (3:2).
[e]Eluted isocratically with a mixture of heptane:ethanol (1:1).
[f]Eluted isocratically with a mixture of heptane:ethanol (4:1).
[g]W: Column: (*R*,*R*)-Whelk-O (Regis). 21.1×250 mm for preparative runs, 4.6 250 mm for analytical runs.
[h]C: Column: Chiralpak AS. 20×250 mm for preparative runs, 4.6×250 mm for analytical runs.

Table 7. Physicochemical and biological data for fully characterised 4,5,6,7-tetrahydrothieno[3,2-*c*]- and-[2,3-*c*]pyridines



8, 9, 10, 17, 27 and 43



48-49

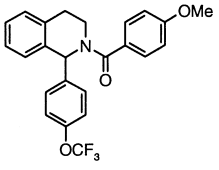
Compound	R^1	R^2	Mp (°C)	Yield (%)	Anal.	IC_{50} (μM)
8	4-Trifluoromethoxyphenyl	4-Methoxyphenyl	68–70	98	CHN	0.27
9	4-Methoxyphenyl	4-Methoxyphenyl	126.5–127.3	84	CHN	0.80
10	4-Trifluoromethylphenyl	4-Methoxyphenyl	86.1–88.8	88	CHN	0.34
17	4-Chlorophenyl	4-Hydroxyphenyl	207–212	99	CHN	1.7
27	4-Trifluoromethoxyphenyl	5-Benzimidazolyl	187–>200	87	CHN	4.4
43	4-Methoxyphenyl	2-(3-Furanyl)ethenyl	75–77	20	CHN	1.2
48	4-Chlorophenyl	4-Methoxyphenyl	129.5–130.0	91	CHN	0.26
49	4-Methoxyphenyl	4-Methoxyphenyl	Oil	81	CHN	0.80

piperidyl group (**37**) are tolerated at all, indicating unfavourable contacts with the basic nitrogens in these systems. In the case of **37**, the inactivity can also be attributed to the absence of aromatic π-electrons (compare with **38**). Similarly as seen with 4-(4-methoxyphenyl) group, a 4-(4-nitrophenyl) group is only active when paired with the 4-methoxybenzoyl group in the 5-position confers activity (**32**), and other 'allowed' substituents on the 5-benzoyl group are not active at all (e.g. 4-hydroxymethyl **33**).

Saturated systems replacing the 4-phenyl group are not tolerated at all, since the cyclohexyl system only give inactive compounds (e.g., **38**), indicating that also this aromatic ring system is involved in recognition and inhibition of the enzyme.

A 2-thienyl (**34**) group as well as a 5-chloro-2-thienyl (**35**) group as the 4-substituent of the 4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine system is clearly less tolerated (by about 10-fold) as compared to the parent phenyl or 4-(4-chlorophenyl) groups.

Further, we have prepared a series of analogues wherein the central tetrahydrothieno[3,2-*c*]pyridine ring was replaced by a 1,2,3,4-tetrahydro-isoquinoline system, as seen in **45**. These analogues were all virtually inactive, indicating that it is also in the core ring system that benzene is not a good thiophene bioisoster for this target.



45

We then investigated the influence of changing the 4,5,6,7-tetrahydro[3,2-*c*]pyridine core ring system to the isomeric [2,3-*c*] ring system. The SAR of the 5-acyl group of the [3,2-*c*] series was nicely paralleled with that of the 6-acyl group of the [2,3-*c*] series, the 4-methoxybenzoyl group being the best followed by 2-(3-furanyl)acryloyl > 5-chlorothiophen-2-ylcarbonyl > 4-chlorobenzoyl (data not shown).

The substituents of the 7-phenyl group of the [2,3-*c*] series have a large impact on the inhibitory activity (Table 5). The 4-trifluoromethoxy (**47**) and 4-chloro (**48**) groups are equipotent and marginally better than the 4-methoxy group (**49**). An *ortho* substituent is not tolerated at all (**50**, **51** and **55**) while *meta* substituted phenyl groups are active, although less active than the *para* substituted ones. Compare for example **49** with **52** which is about 6-fold less active. A 3,5-dichlorophenyl group (**56**) is not tolerated at all while a 3-fluorophenyl group is almost inactive (**57**). A 3,4-disubstituted phenyl group is also tolerated. When comparing the 3-fluoro-4-methoxyphenyl group (**53**) with the 4-methoxyphenyl

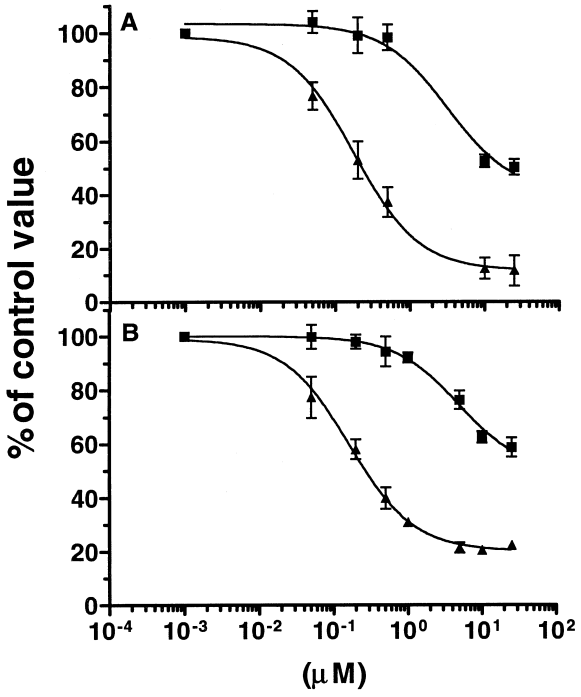


Figure 1. Dose–response curves for inhibition of pig G-6-Pase catalytic enzyme activity by 10A, 10B, 47A and 47B. Dose-response curves for inhibition of pig glucose-6-phosphatase activities using disrupted microsomes by **10a**(▲) and **10b** (■) (A) and **47a** (▲) and **47b** (■) (B). Glucose-6-phosphatase activities were measured using 0.5 mM glucose-6-phosphatase as the substrate. Data are from 3–4 individual experiments with SEM indicated by vertical bars if they extend beyond the symbols. IC_{50} values (nM) were calculated to be 183±36 and 159±26 for compound **10a** and **47a** respectively.

group (**49**) it is evident, however, that only some activity is lost. A 3,4-methylenedioxyphenyl group (**54**) is equipotent with the 4-methoxyphenyl group.

As these compounds are so potent G-6-Pase catalytic enzyme inhibitors as racemates, we wanted to study the G-6-Pase inhibition properties of the individual enantiomers. Generally the compounds were efficiently resolved with baseline separations for minutes as seen in Table 6. The first eluting enantiomers are denoted 'A' and the second eluting enantiomers are denoted 'B'. In all cases the activity of the racemate could be attributed to the activity of one of the enantiomers, as the other enantiomer was inactive. Also, as could be expected, the activities of the active enantiomers were about twice as much as the activity of the racemates. Representative dose-response curves are given in Figure 1.

Further, we have obtained information on absolute configuration (X-ray) of **46A** and **10A**. These compounds were both found to be of (*S*) configuration.

Conclusion

In summary, we have described the first class of potent non-carbohydrate glucose-6-phosphatase catalytic enzyme inhibitors, 4-aryl-5-benzoyl-4,5,6,7-tetrahydrothieno[3, 2-*c*]pyridines and 7-aryl-6-benzoyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridines.

The structure–activity relationship of these compounds indicates that: (1) Tetrahydrothieno[3,2-*c*]- and -[2,3-*c*]pyridine core ring systems are much better than the corresponding benzo analogue, 1,2,3,4-tetrahydro-isoquinoline. (2) The 4-substituent of the tetrahydrothieno[3,2-*c*]pyridine ring system (or the 7-substituent of the [2,3-*c*] system) has to be a phenyl group, optionally substituted with a lipophilic 4-substituent, such as trifluoromethoxy or chloro. A thienyl group is less favoured. Saturated ring systems are not allowed. (3) The 5-substituent of the tetrahydrothieno[3,2-*c*]pyridine ring system (or the 6-substituent of the [2,3-*c*] system) has to be a 4-substituted benzoyl such as anisoyl, although compounds with a 5-(*E*)-3-furan-3-ylacryloyl group are roughly equipotent. Substitution in the benzoyl *meta* position is less tolerated, whereas substitution in the benzoyl *ortho* position seems to be forbidden (at least in the [3,2-*c*] series). Saturated ring systems are not allowed. (4) The observed high enantioselectivity in enzyme recognition and inhibition is in good accordance with the high potency of the inhibitors.

Experimental

General

Melting points were determined on a Büchi 535 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained on Bruker Avance DRX 300 and DPX 200 systems (300 MHz and 200 MHz, respectively) operating at room temperature. Optical rotation data were obtained using a Perkin–Elmer Polarimeter Model 241. Microanalyses were performed by Novo Nordisk Analytical Department.

The HPLC-MS analyses were performed on a PE Sciex API 100 LC/MS System using a Waters^{TM} 3×150 mm 3.5 μ C-18 Symmetry column and positive ionspray with a flow rate at 20 μL/min. The column was eluted with a linear gradient of 5–90% A, 85–0% B and 10% C in 15 min at a flow rate of 1 mL/min (solvent A = acetonitrile, solvent B = water and solvent C = 0.1% trifluoroacetic acid in water).

The HPLC-MS method B used for analysing compounds **50**–**57** is identical to that described above with the following settings: Column: YMC ODS-A 120Å s-5μ 3×50 mm, gradient 5–90% acetonitrile in 0.05% TFA linearly during 7.5 min at 1.5 mL/min.

1-(4-Trifluoromethoxyphenyl)-1,2,3,4-tetrahydroisoquinoline (**45**) was prepared using the 'imine route' (similarly as previously described[22]).

[4-(4-Methoxyphenyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]-pyridin-5-yl]-(4-trifluoromethylphenyl)-methanone (**46**) is commercially available (Maybridge).

Dimethyl-{4-[(2-thiophen-2-ylethylimino)methyl]phenyl} amine, (2a). 2-(2-Thienyl)-ethylamine (5 g, 39.4 mmol), 4-dimethylaminobenzaldehyde (5.9 g, 94 mmol), triethylamine (6 mL) and ethanol (150 mL) were mixed at room temperature. The reaction mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated to 75 mL by evaporation in vacuo and the solid formed was filtered and dried to afford 6.82 g (67%) dimethyl-{4-[(2-thiophen-2-ylethylimino)methyl]-phenyl}amine, (**2a**). Mp: 76.8–77 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.0 (s, 6H), 3.15 (t, 2H), 3.8 (t, 2H), 6.65 (d, 2H), 6.8 (d, 1H), 6.95 (dd; 1H), 7.1 (dd; 1H), 7.6 (d, 2H), 8.1 (s, 1H), ^{13}C NMR ($CDCl_3$) δ 32.3, 40.7, 63.3, 112.1, 124.0, 124.7, 125.6, 127.2, 130.1, 143.1, 152.5, 162.26. Calcd for $C_{15}H_{18}N_2S$: C, 69.73; H, 7.02; N, 10.84. Found: C, 69.84; H, 7.13; N, 10.84.

Dimethyl-[4-(4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-4-yl) phenyl]amine (3a). Dimethyl-{4-[(2-thiophen-2-yl-ethylimino)methyl]phenyl}amine (1 g, 3.9 mmol) was added to TFA (20 mL) at once (exothermic reaction). The reaction mixture was stirred at room temperature for 72 h, and then evaporated in vacuo. The crude oil was suspended in dichloromethane (75 mL) and extracted with 1N hydrochloric acid (50 mL). The aqueous phase was added to 2N sodium hydroxide to pH 10, then extracted with dichloromethane (3×125 mL). The organic phase was dried with $MgSO_4$, filtered, evaporated in vacuo to afford 0.96 g (95%) dimethyl-[4-(4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-4-yl)phenyl]amine (**3a**). Mp: 95–98 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.75 (broad s, 1H), 2.6–3.3 (m, 4H), 2.75 (s, 6H), 4.7 (s, 1H), 6.45 (d, 1H), 6.5 (d, 2H), 6.9 (d, 1H), 7.1 (d, 2H); ^{13}C NMR ($CDCl_3$) δ 26.7, 41.2, 43.1, 60.0, 113.0, 121.9, 127.1, 129.6, 132.3, 135.2, 150.5. Calcd for $C_{15}H_{18}N_2S$: C, 69.73; H, 7.02; N, 10.84. Found: C, 69.20; H, 7.06; N, 10.80.

(4-Nitrobenzylidene)-(2-thiophen-2-ylethyl)amine (2b). 2-(2-Thienyl)ethylamine (5 g, 39.4 mmol), 4-nitrobenzaldehyde (6 g, 39.7 mmol) and triethylamine (10 mL) were dissolved in ethanol (100 mL). The reaction mixture was stirred at room temperature for 48 h. The solid formed was filtered and dried to afford 7.75 g (76%) (4-nitrobenzylidene)-(2-thiophen-2-ylethyl)amine (**2b**). Mp: 83.9–84.4 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.2 (t, 2H), 3.95 (t, 2H), 6.3 (dd, 1H), 6.4 (dd, 1H), 7.4 (d, 2H), 8.3 (d, 2H), 8.25 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 31.6, 63.3, 124.2, 124.3, 125.8, 126.2, 127.2, 129.2, 142.0, 142.3, 160.0.

4-(4-Nitrophenyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine trifluoroacetate (3b). (4-Nitrobenzylidene)-(2-thiophen-2-ylethyl)amine (1 g, 3.8 mmol) was added to trifluoroacetic acid (100 mL) at once (exothermic reaction). The reaction mixture was stirred at room temperature for 72 h, then evaporated in vacuo. Crystallization from a mixture of diethyl ether and dichloromethane afforded 1.2 g (85%) 4-(4-nitrophenyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine trifluoroacetate (**3b**). Mp: 128–129.5 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.3 (m, 2H), 3.6 (m, 2H), 4.6 (broad s, 1H), 5.3 (d, 1H), 7.3 (d, 1H), 7.6 (d, 2H), 8.3 (d, 2H); ^{13}C NMR ($CDCl_3$) δ 26.1, 45.1, 62.0, 128.5, 129.5, 129.8, 133.7, 135.1, 138.0, 146.2. Calcd for $C_{15}H_{13}N_2SF_3O_4$: C, 48.13; H, 3.48; N, 7.49. Found C, 47.96; H, 3.57; N, 7.32.

4-Pyridin-4-yl-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (3c). 2-(2-Thienyl)ethylamine (2 g, 15.7 mmol), pyridine-4-carboxaldehyde (1.68 g, 15.7 mmol), triethylamine (1 mL) and ethanol (15 mL) were mixed and the reaction mixture was stirred at room temperature for 15 h, then evaporated in vacuo. The crude oil was added to trifluoroacetic acid (75 m) at once (exothermic reaction). The reaction mixture was stirred at room temperature for 0.5 h, then evaporated in vacuo. The residue was dissolved in dichloromethane (150 mL) and washed with 2N sodium hydroxide (100 mL). The aqueous phase was extracted with dichloromethane (3×50 mL). The combined organic phases were dried with $MgSO_4$, filtered and evaporated in vacuo to give an oil (3.21 g) which was crystallised from a mixture of dichloromethane and hexane to afford 2.4 g (71%) 4-pyridin-4-yl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (**3c**). Mp: 81.8–83.8 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.15 (broad s, 2H), 2.7–3.4 (m, 4H), 5.0 (s, 1H), 6.45 (d, 1H), 7.15 (d, 1H), 7.25 (dd; 2H), 8.05 (dd; 2H); ^{13}C NMR ($CDCl_3$) δ 26.3, 42.6, 59.3, 122.8, 123.8, 126.2, 135.3, 136.0, 150.4, 152.8. Calcd for $C_{12}H_{12}N_2S{\cdot}1H_2O$: C, 61.51; H, 6.02; N, 11.96. Found C, 61.64; H, 5.98; N, 11.79.

(2-Thiophen-2-ylethyl)thiophen-2-ylmethyleneamine (2d). 2-(2-Thienyl)ethylamine (2 g, 15.7 mmol), 2-thienyl-carboxaldehyde (1.76 g, 15.7 mmol), triethylamine (1 mL) and ethanol (15 mL) were mixed, and the reaction mixture was stirred at room temperature for 96 h. The mixture was evaporated in vacuo, and the residue was crystallised from hexane, to afford 1.26 g (36%) (2-thiophen-2-ylethyl)thiophen-2-ylmethyleneamine (**2d**). ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.2 (t, 2H), 3.8 (t, 2H), 6.85 (d, 1H), 6.9 (dd; 1H), 7.05 (dd, 1H), 7.1 (d, 1H), 7.25 (d, 1H), 7.45 (d, 1H), 8.25 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 31.8, 63.0, 124.1, 125.7, 127.2, 127.8, 129.4, 131.0, 142.6, 142.8, 155.6.

4-(Thiophen-2-yl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (3d). The imine **2d** (1.26 g, 5.7 mmol) was added to trifluoroacetic acid (25 mL) and the reaction mixture was stirred at 60 °C for 12 h. The reaction mixture was evaporated in vacuo. The residue was dissolved in dichloromethane (30 mL) and washed with 2N sodium hydroxide (30 mL). The aqueous phase was extracted with dichloromethane (3×25 mL). The combined organic phases were dried with $MgSO_4$, filtered and evaporated in vacuo. The residue (1.15 g) was purified by column chromatography on silica gel eluting with a mixture of dichloromethane:methanol (19:1) to afford 0.18 g (5%) 4-(thiophen-2-yl)-4,5,6,7-tetrahydrothieno [3,2-*c*]pyridine (**3d**). Mp: 96–97.3 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.0 (s, 1H), 2.85 (dt; 2H), 3.1–3.35 (m, 2H), 5.3 (s, 1H), 6.15 (d, 1H), 6.9 (s, 1H), 6.95 (d, 1H), 7.05 (d, 1H), 7.2 (dd; 1H); ^{13}C NMR ($CDCl_3$) δ: 26.3, 42.1, 55.1, 122.3, 125.3, 125.7, 126.7, 126.8, 135.3, 136.7, 148.5.

4-(5-Chlorothiophen-2-yl)-4,5,6,7-tetrahydrothieno[3,2-c] pyridine (3e). 2-(2-Thienyl)-ethylamine (1 g, 7.9 mmol) and 5-chlorothiohene-2-carboxaldehyde (1.15 g, 7.9 mmol) were mixed (exothermic reaction). The separated water was decanted and the remaining oil was heated until reflux. The mixture was allowed to cool to room temperature. Then TFA (10 mL) was added under stirring, and then refluxed for 16 h. The reaction mixture was evaporated in vacuo, dissolved in dichloromethane (50 mL) washed with 1N NaOH (50 mL). The aqueous phase was extracted with dichloromethane (50 mL). The combined organic phases were dried with $MgSO_4$, filtered, and evaporated in vacuo. The residue (1.2 g) was purified on a silica gel column eluting first with dichloromethane and then with a mixture of dichloromethane:methanol (19:1) giving 0.2 g (9.9%) 4-(5-chlorothiophen-2-yl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (**3e**) as an oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.15 (broad s, 1H), 2.7 (m, 2H), 3.0–3.3 (m, 2H), 5.2 (s, 1H), 6.65 (m, 2.5H), 7.0 (d, 1.5H); ^{13}C NMR ($CDCl_3$) δ 41.9, 55.3, 122.6, 124.8, 125.8, 126.4, 129.7, 135.3, 135.6, 147.6. Calcd for $C_{11}H_{10}NS_2Cl$: C, 51.65; H, 3.94; N, 5.48. Found C, 51.75; H, 3.80; N, 5.44.

4-Cyclohexyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (3f, imine route). 2-(2-Thienyl)ethylamine (2 g, 15.7 mmol) and cyclohexylcarboxaldehyde (1.76 g, 15.7 mmol) were mixed in ethanol (15 mL). The reaction mixture was stirred for 5 days. The solvent was removed in vacuo. The residual brown oil was added to trifluoroacetic acid (40 mL) and stirred for 72 h at room temperature. The reaction mixture was evaporated in vacuo. The remaining oil was added to dichloromethane (50 mL) washed with 1N NaOH (100 mL). The aqueous phase was extracted with dichloromethane (30 mL). The combined dichloromethane phases were added to activated carbon, dried with $MgSO_4$, filtered and evaporated in vacuo. The residue (2.91 g) was purified by column chromatography on

silica gel eluting with a mixture of dichloromethane: methanol (9:1) to afford 0.33g (9%) 4-cyclohexyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (**3f**). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.0–1.9 (m, 10H), 2.6 (m, 1H), 2.75 (m, 1H), 2.9 (m, 1.5H), 3.25–3.6 (m, 1.5H), 3.9 (dd; 1H), 6.75 (d, 1H), 7.0 (d, 1H).

Cyclohexanecarboxylic acid (2-thiophen-2-ylethyl)amide (4f). 2-(2-Thienyl)ethylamine (3 g, 23.6 mmol) was added to a solution of cyclohexanecarboxylic acid (3.02 g, 23.6 mmol) and HOBt (3.61 g, 23.6 mmol) in DMF (150 mL), EDAC (6.78 g, 35.4 mmol) was added and the resulting mixture was stirred for 15 h at room temperature. The mixture was evaporated in vacuo. The remaining oil was added to ethyl acetate (200 mL) and washed with brine (100 mL), 0.5 N HCl (100 mL) and 1N NaOH (100 mL). The organic phase was dried with $MgSO_4$, filtered and evaporated in vacuo giving 5.51g (99%) cyclohexanecarboxylic acid (2-thiophen-2-ylethyl)amide (**4f**) as white crystals. Mp 86.7–87.2 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.1–1.5 (m, 5H), 1.6–1.9 (m, 5H), 2.05 (tt, 1H), 3.0 (t, 2H), 3.5 (dt, 2H), 5.6 (broad s, 1H), 6.8 (dd, 1H), 6.95 (dd, 1H), 7.1 (dd, 1H); ^{13}C NMR ($CDCl_3$) δ: 26.1, 30.1, 30.4, 41.0, 45.9, 45.95, 124.3, 125.7, 127.5, 141.9, 176,5.

[1-Methylpiperidin-4-carboxylic acid]-(2-thiophen-2-yl) ethyl amide (4g). 2-Thiophen-2-ylethylamine (3 g, 23.6 mmol) in DMF (150 mL) was added to a premixed (2 min.) solution containing HOBt (3.61 g (23.6 mmol), diisopropylethylamine (8.23 mL, 47.2 mmol) and *N*-methyl-piperidin-4-yl-carboxylic acid, hydrochloride in DMF (50 mL), then 6.78g (0.0354 mol) EDAC was added. The reaction mixture was stirred at room temperature for 48 h. The solvent was evaporated in vacuo. The remaining oil was added to H_2O (100 mL), 1N NaOH (50 mL) and dichloromethane (100 mL). The aqueous phase was extracted with dichloromethane (3×75 mL). The combined organic phases were dried with $MgSO_4$, filtered and evaporated in vacuo giving 7.2 g. Recrystallisation from a mixture of ethyl acetate and pentane afforded 4.1g (69%) [1-methylpiperidin-4-carboxylic acid]-(2-thiophen-2-yl)ethyl amide (**4g**) as white crystals. Mp: 114.2–114.7 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.6–2.1 (broad multiplet, 6H), 2.25 (s, 3H), 2.9 (d, 2, 5H), 3.1 (t, 2H), 3.15 (p, 0.5H), 3.5 (q, 2H), 5.95 (broad s, 1H), 6.8 (d, 1H), 7.9 (dd, 1H), 7.13 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 29.3, 30.2, 41.1, 43.1, 46.7, 55.6, 124.3, 125.7, 127.4, 141.7, 175.

4-(1-Methylpiperidin-4-yl)-6,7-dihydrothieno[3,2-*c*]pyridine (5g). [1-Methylpiperidin-4-carboxylic acid]-(2-thiophen-2-yl)ethyl amide (1 g, 40 mmol) was dissolved in toluene (25 mL) and added to $POCl_3$ (1.1 mL, 12 mmol). The resulting mixture was heated at 80 °C for 19 h. Toluene was decanted from a yellow precipitate. The yellow crystals were dissolved in dichloromethane (150 mL), ice (50 mL) and 2N NaOH (50 mL). The aqueous phase was extracted with dichloromethane (2×75 mL) the combined organic phase and was dried with $MgSO_4$, filtered, and evaporated in vacuo to give 0.77 g (82%) of 4-(1-methylpiperidin-4-yl)-6,7-dihydro-thieno[3,2-*c*]pyridine (**5g**) as an oil. ^{1}H NMR ($CDCl_3$) δ 1.8 (m, 4H), 2.05 (dt, 2H), 2.25 (s, 3H), 1.6 (h, 1H), 2.7 (t, 2H), 2.95 (broad d, 2H), 3.75 (t, 2H), 7.05 (d, 1H), 7.15 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 22.6, 30.3, 42.8, 46.9, 48.1, 56.4, 122.2, 124.0, 128.5, 129.4, 131.2, 143.7, 166.4.

4-(1-Methylpiperidin-4-yl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (3g). 4-(1-Methylpiperidin-4-yl)-6,7-dihydrothieno[3,2-*c*]pyridine (**5g**) (2.95 g, 12.6 mmol) was dissolved in methanol (75 mL) and 0.71 g (0.0189 mol) sodium borohydride was added in portions. Stirring was continued for 2 h at room temperature. Evaporation in vacuo gave an oil, which was dissolved in dichloromethane (100 mL) and washed with H_2O (100 mL). The aqueous phase was extracted with dichloromethane (2×100 mL). The combined organic phases were dried with $MgSO_4$, filtered and evaporated in vacuo to give 2.98g (100%) 4-(1-methylpiperidin-4-yl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (**3g**). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.5 (dd; 2H), 1.6–2.0 (m, 6H), 2.2 (t, 3H), 2.75 (m, 2H), 2.8–3.0 (m, 3H), 3.35 (dt; 1H), 3.8 (broad s, 1H), 6.8 (d, 1H), 7.05 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 26.6, 27.1, 29.3, 30.0, 40.8, 42.7, 46.8, 56.6, 56.7, 59.1, 121.8, 125.5, 135.4, 137.2. Calcd for $C_{13}H_{20}N_2S$·1.75 H_2O: C, 58.28; H, 8.84; N, 10.46. Found C, 58.52; H, 8.62; N, 10.62.

4-Cyclohexyl-6,7-dihydrothieno[3,2-*c*]pyridine (5f). Compound **4f** (5.5g, 23.2 mmol) was added to a solution of xylene (75 mL) P_2O_5 (9.9 g, 69.9 mmol) and $POCl_3$ (6.6 mL, 3 equiv) at 85 °C. The reaction mixture was heated at 85 °C for 2 h. After cooling, the mixture was added 1N NaOH (100 mL) and solid NaOH until pH 10. The mixture was extracted with toluene (2×50 mL). The combined organic phases were dried with $MgSO_4$, filtered and evaporated in vacuo to give 4.96g (98%) 4-cyclohexyl-6,7-dihydro-thieno[3,2-*c*]pyridine (**5f**) as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.15–1.5 (m, 5H), 1.65 (m, 5H), 2.55–2.7 (m, 1H), 2.75 (dt, 2H), 3.75 (dt, 2H), 7.0 (d, 1H), 7.05 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 22.7, 26.6, 26.9, 31.2, 44.8, 48.1, 122.1, 124.2, 131.7, 143.6, 168.0. Calcd for $C_{13}H_{17}NS$: C, 71.18; H, 7.81; N, 6.39. Found C, 69.60; H, 7.64; N, 6.54.

4-Cyclohexyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (3f, amide route). Compound **5f** (4.25g, 19.4 mmol) was dissolved in methanol (75 mL) and $NaBH_4$ (1.1 g, 29 mmol) was added in portions. The mixture was stirred for 1 h at room temperature. The mixture was then evaporated in vacuo and the remaining oil was dissolved in H_2O (50 mL) and extracted with dichloromethane (3×75 mL). The organic phase was dried with $MgSO_4$, filtered and evaporated in vacuo to afford 4.25g (98%) 4-cyclohexyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (**3f**) as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.0–1.9 (m, 11H), 2.7 (m, 2H), 2.9 (m, 1H), 3.25 (m, 1H), 3.75 (dd, 1H), 6.35 (d, 1H), 7.0 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 26.7, 27.0, 27.1, 27.2, 27.4, 31.0, 43.2, 43.5, 60.3, 121.8, 125.4, 135.2, 137.7. Calcd for $C_{13}H_{19}NS$: C, 70.45; H, 8.65; N, 6.39. Found C, 70.35; H, 8.75; N, 6.66.

4-Phenyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (3h). 2-(2-Thienyl)ethylamine (2 g, 15.7 mmol) and benzaldehyde (1.67 g, 15.7 mmol) were dissolved in toluene (50

mL) and the reaction mixture was heated at reflux until 20 mL of toluene and water was distilled of in a Dean–Stark trap. The remaining mixture was evaporated in vacuo to give the crude imine (3.44 g). The crude imine was added to trifluoroacetic acid (50 mL) at once (exothermic reaction). The reaction mixture was stirred at room temperature for 72 h, and then evaporated in vacuo. The residue was dissolved in dichloromethane (50 mL) and washed with 2N sodium hydroxide (30 mL). The aqueous phase was extracted with dichloromethane (3×30 mL). The combined organic phases were dried with $MgSO_4$, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel eluting with a mixture of dichloromethane and methanol (19:1). This afforded 0.823 g (24%) 4-phenyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (**3h**) as white crystals. Mp: 79.8–80.7 °C ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.95 (broad s, 1H), 2.7–3.3 (m, 4H), 5.0 (s, 1H), 6.45 (d, 1H), 6.95 (d, 1H), 7.25 (m, 5H); ^{13}C NMR ($CDCl_3$) δ: 25.5, 43.0, 60.6, 122.2, 126.8, 128.0, 128.9, 135.4, 137.3, 144.2. Calcd for $C_{13}H_{13}NS$: C, 72.52; H, 6.09; N, 6.51. Found C, 72.18; H, 6.10; N, 6.33.

4-(4-Trifluoromethoxyphenyl)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine (3n). A solution of 2-(3-thienyl)ethylamine[23] (14.7 g, 0.115 mol) and 4-trifluormethoxybenzaldehyde (15.0 g, 0.08 mol) in benzene (200 mL) was refluxed (Dean–Stark trap, H_2O removed) for 4 h. Trifluoroacetic acid (2 mL) was added and the mixture was refluxed for 8 h. After cooling it was made alkaline with NH_4OH and washed with water. The organic phase was dried (K_2CO_3) and evaporated in vacuo to give a residue, which was purified by chromatography on silica-gel (200 g). A by-product was removed by elution with benzene, R_f 0.68 (SiO_2; $CHCl_3$:EtOH:NH_4OH, 200:10:1), probably Schiff base (it was decomposed by an attempt to prepare hydrogen oxalate). Crude **3n** (3.9 g) was obtained by elution with chloroform. R_f 0.47 (SiO_2; $CHCl_3$:EtOH:NH_4OH, 200:10:1). The hydrogen oxalate was prepared by neutralisation of the solution of the above base in diethyl ether with a solution of oxalic acid dihydrated in acetone. Hydrogen oxalate was contaminated with hydrogen oxalate of 2-(3-thienyl)ethylamine. A suspension of the mixture was repeatedly boiled with water and filtrated. This afforded, after drying, pure (**3n**), hydrogen oxalate 2.5 g (8%), mp 190–195 °C. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.22 (s, 3H), 7.59 (d, J=8.6 Hz, 2H), 7.47 (d, J=4.9 Hz, 1H), 7.42 (d, J=8.6 Hz, 2H), 6.97 (d, J=4.9 Hz,1H), 5.73 (s, 1H), 3.35 (bm, 2H), 2.95 (bm, 2H). Calcd for $C_{14}H_{12}F_3NOS$, $C_2H_2O_4$, 1/4 H_2O: C, 48.79; H, 3.71; N, 3.56; F, 14.47; S, 8.14; Found C, 48.69; H, 3.60; N, 3.42; F, 14.83; S, 8.38.

7-(4-Chlorophenyl)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine (3o). 2-(3-Thienyl)ethylamine (3.15 g, 24.8 mmol) and 4-chlorobenzaldehyde (3.5 g, 2.48 mmol) were mixed without solvent resulting in dissolution of the crystals of the amine followed by precipitation of slightly yellow crystals. The mixture was left at room temperature for 4 h. Trifluoroacetic acid (20 mL) was added and the mixture stirred overnight at room temperature. The solvent was evaporated and the resulting mixture extracted between NaOH (4 M) and dichloromethane, the organic phase was separated, dried with $MgSO_4$ and evaporated resulting in an oil which was purified on silica-gel using dichloromethane:methanol (9:1) as eluent. Yield of (**3o**) 50%, mp 93.6–93.8 C. ^{1}H NMR ($CDCl_3$) δ 7.33–7.1 (m, 4H), 7.13 (d, 1H), 6.82 (d, 1H), 5.12 (s, H), 3.32–2.95 (m, 2H), 2.9–2.6 (m, 2H), 1.95 (broad, 1H NH); ^{13}C NMR ($CDCl_3$): δ 142.7, 137.7, 135.7, 134.0, 129.9, 129.1, 127.7, 123.9, 59.4, 42.7, 27.0. Calcd for $C_{13}H_{12}$ ClNS: C, 62.52%; H, 4.87%; N, 5.61%; Found C, 62.72%; H, 4.87%; N, 5.59.

7-(4-Methoxyphenyl)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine (3p). This compound was prepared from 2-(3-thienyl)ethylamine (1.21 g, 9.5 mmol) and 4-methoxybenzaldehyde (1.30 g, 9.5 mmol) in dry ethanol (8 mL), giving 100% yield of the crude imine which was reacted further with trifluoroacetic acid without further purification as described for (**3o**) resulting in a yield of 42% of (**3p**) isolated as an oil. ^{1}H NMR ($CDCl_3$) δ 7.24–7.21 (m, 2H), 7.1 (d, 1H), 5.09 (s broad, 1H), 3.78 (s, 3H), 3.37–3.21 (m, 1H), 3.12–2.97 (m, 1H), 2.90–2.58 (m, 2H), 1.86 (broad, 1H NH); ^{13}C NMR ($CDCl_3$): δ 159.6, 138.9, 136.6, 135.4, 129.6, 127.6, 123.6, 114.2, 59.6, 55.7, 43.0, 27.1. Calcd for $C_{14}H_{15}NS$: C, 68.54%; H, 6.16%; N, 5.71%; Found C, 68.40%; H, 6.19%; N, 5.72.

7-Substituted 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridines (3q–3y). The 7-substituted 4,5,6,7-tetrahydrothieno[2,3-*c*]-pyridines were prepared from equimolar amounts of appropriate substituted benzaldehyde (0.0078 mol) and 2-(3-thienyl)ethylamine (0.0078 mol) in dry ethanol (8 mL) by shaking for 3 days at room temperature. The mixture was subsequently evaporated to dryness and the resulting oil treated with trifluoroacetic acid (20 mL) by stirring for 24 h followed by addition of NaOH (2M, 10 mL). Extraction with dichloromethane (10 mL) followed by evaporation afforded the desired 7-substituted 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridines as oils. Identity and yield were estimated from the HPLC/MS spectra, data are presented in Table 1.

Preparation of amides: general procedure (Table 7)

(4-Methoxyphenyl)-[4-(4-trifluoromethoxyphenyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-5-yl]methanone (8). 4-Methoxybenzoic acid (0.64 g, 4.2 mmol) was dissolved in DMF (25 mL) and HOBt (0.71 g, 5.0 mmol) was added followed by EDAC (0.96 g, 5.0 mmol). The resulting mixture was stirred at room temperature for 30 min. 4-(4-Trifluoromethoxyphenyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (**3l**, 1.5 g, 5.0 mmol) and DIPEA (1.4 mL, 8.4 mmol) were added and the mixture was stirred at room temperature for 16 h. Water (100 mL) was added and the mixture was extracted with Et_2O (3×20 mL). the combined organic phases were washed with satd NH_4Cl (20 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with a mixture of EtOAc:heptane (1:2). This afforded, after crystallisation from a mixture of heptane and methyl *tert*-butyl ether, 2.13 g (98%) of (4-methoxyphenyl)-[4-(4-trifluoro methoxyphenyl)-4,5,6,7-tetrahydro-thieno[3,2-*c*] pyridin-5-yl]methanone (**8**), mp 68-70 °C. ^{1}H NMR (300 MHz,

$CDCl_3$) δ 2.84 (1H, m), 3.05 (1H, m), 3.28 (1H, m), 3.84 (3H, s), 3.90 (1H, m), 6.75 (1H, d), 6.92 (3H, m), 7.17 (2H, d), 7.21 (1H, d), 7.37 (4H, m). Anal. ($C_{22}H_{18}F_3NO_3S.1/4H_2O$): CHN.

Preparation of amides 7–45 and 47–57: general library procedure

The carboxylic acid (0.15 mmol) was dissolved in DMF (0.25 mL) and a solution of 1-hydroxybenzotriazole (HOBt, 0.15 mmol) in DMF (0.25 mL) was added. A suspension of *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDAC) (0.225 mmol) in DMF (0.25 mL) was added followed by addition of the substituted 4,5,6,7-tetrahydrothienopyridine derivative (0.15 mmol) in DMF (0.25 mL). The reaction mixture was shaken vigorously at room temperature for 24 h. The reaction mixture was added a saturated solution of sodium chloride (2 mL) and ethyl acetate (1 mL) and shaken vigorously for 1 h. The organic phase was removed by pipetting and evaporated in a Savant vacuum centrifuge to afford the product. The resulting oil or crystals were analysed by HPLC-MS.

Optical resolution of amides: general procedure

(+)- and (−)-[4-(4-Chlorophenyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-5-yl]-(4-methoxyphenyl)methanone, (7A and 7B, respectively). Compound **7** (30 mg) was dissolved in a 1:1 mixture of *n*-heptane and 2-propanol (5 mL) and fractionated by HPLC (2 runs) using a 21.1×250 mm (*R*,*R*)-Whelk-O column (Regis). The column was eluted isocratically with a 1:1 mixture of *n*-heptane:2-propanol at a flow rate of 12 mL/min and fractions collected corresponding to 0.8 min/fraction. The eluting enantiomers were detected spectroscopically by measuring absorbance at a wavelength of 225 nm. Two eluting peaks were detected, one corresponding to T_R 44–50 min and one corresponding to T_R 62–72 min. Fractions from the two runs corresponding to T_R 44–50 min were separately pooled and evaporated to yield 12.1 mg of the corresponding (+)-isomer.

100% ee (Determined by HPLC using a 4.6×250 mm (*R,R*)-Whelk-O column eluted with a 1:1 mixture of *n*-heptane and 2-propanol, the flow rate was 1 mL/min, eluting sample was monitored spectroscopically at 225 and 245 nm, T_R 15.5 min).

Fractions from the two runs above corresponding to T_R 62–72 min were separately pooled and evaporated to yield 12.5 mg of the corresponding (−)-isomer.

98% ee (Conditions as described above, T_R 20.8 min). $[\alpha]_D^{20}$ −170.0° (*c* 0.25, ethyl acetate).

Enzyme assays

Materials

Liver from pigs (24 h fasted) were obtained from the animal quarters at Novo Nordisk A/S. Phosphorus Reagent Concentrate (cat no 360-3C) was obtained from Sigma Chemical Company, St. Louis, Mo (USA).

Methods

Glucose-6-phosphatase activity. Pig liver microsomes were prepared essentially as described previously.[24] Microsomes were kept at −80 °C until use. Prior to measurement of G-6-Pase activity, microsomes were treated with 0.5% v/v Triton X-100[25] (the final concentration of Triton X-100 in the assay was 0.04%), and these are referred to as disrupted microsomes. Glucose-6-phosphatase activity in disrupted microsomes (0.05 mg protein) was measured by incubation for 6 min at 30 °C with 0.5 mM glucose-6-phosphate, 30 mM MES (2-(*N*-morpholino)ethanesulfonic acid) pH 6.5, and various compounds dissolved in 100% DMSO in a total volume of 325 μL. The final concentration of DMSO in the assay did not exceed 5%. The reaction was terminated by addition of 100 μL Sigma phosphorus reagent. This mixture was allowed to stand for 2 min and, subsequently, the absorbance was measured at 340 nm. All values were corrected for blank. In some cases, microsomal protein contents were measured using the Bio-Rad DC Protein Assay and human serum albumin as the standard. The IC_{50} values were calculated using GRAPHPAD PRISM (GraphPad software Inc. San Diego, USA).

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