Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 3197–3212

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and Biological Activity of a Novel Class of Pyridazine Analogues as Non-competitive Reversible Inhibitors of Protein Tyrosine Phosphatase 1B (PTP1B)

Charlotta Liljebris,[a,*] Jessica Martinsson,[a] Lars Tedenborg,[a] Meredith Williams,[a] Emma Barker,[b] James E. S. Duffy,[b] Alf Nygren[a] and Stephen James[a]

[a]*Biovitrum AB, Department of Medicinal Chemistry and Biology, SE-75137 Uppsala, Sweden*
[b]*BioFocus, Sittingbourne Research Centre, Sittingbourne, Kent ME9 8AZ, UK*

Received 3 April 2002; accepted 31 May 2002

Abstract—A series of novel pyridazine analogues were prepared and the structure–activity relationship of their behavior as inhibitors of PTP1B was evaluated. Most of the analogues had potencies in the low micromolar range. The in vitro kinetics of this compound series demonstrated that they were reversible non-competitive binders. This indicates that there may exist another site in the enzyme through which enzyme activity can be inhibited, which is not a recognized interaction domain. Some of the analogues exhibited high selectivity for other PTPases, for example, compound **12mp** showed 20-fold selectivity for PTP1B ($IC_{50} = 5.6\,\mu M$) versus both TCPTP and LAR ($>100\,\mu M$, respectively). In contrast to many tyrosine phosphatase mimetic inhibitors, this compound class lacks negative charge and thus showed high permeability across cell membranes. Selective analogues in the series were analyzed in an in vitro cellular assay, which showed increased insulin-stimulated insulin receptor phosphorylation.

Introduction

Insulin resistance in the liver and peripheral tissues, together with pancreatic cell defects, is the common causes of Type 2 diabetes.[1] The biological actions of insulin are initiated when insulin binds to the α-unit of its receptor, resulting in stimulation of intrinsic receptor tyrosine kinase activity of the β-subunit of the receptor and its autophosphorylation, and the subsequent phosphorylation of intracellular substrates.[2–4] In the insulin resistant state in Type 2 diabetes patients, the insulin-signaling cascade is attenuated, due to a defect at a site distal to the binding of insulin to its receptor, which has previously been proposed to be at the level of the insulin receptor itself.[5,6]

Protein tyrosine phosphatases (PTPases) constitute a diverse family of enzymes and are responsible for the selective dephosphorylation of tyrosine residues.[7,8] Several PTPases, including PTP1B, LAR, PTPα and PTPε, are capable of dephosphorylating the insulin receptor, and thereby attenuating the tyrosine kinase activity.[9–12] In addition, a role for certain PTPs, including PTP1B, a cytosolic nonreceptor PTPase, in the insulin resistance associated with diabetes and obesity has been suggested by some clinical studies in which correlations between the levels of PTP1B expression in muscle and adipose tissue and insulin resistant states were found.[13,14] This is supported by several cellular and biochemical studies where PTP1B has been shown to play a major role in the dephosphorylation of the insulin receptor.[15] Recent studies with PTP1B knockout mice have demonstrated that loss of PTP1B activity resulted in an enhancement of the insulin sensitivity and decreased susceptibility to diet-induced obesity.[16] Thus, PTP1B might be an attractive therapeutic target in Type 2 diabetes and obesity.

Interest in the development of specific PTP1B inhibitors has increased over recent years.[8,17,18] Due to the electrostatic properties of the phosphatase active site, it has been difficult to develop reversible competitive PTP1B inhibitors lacking negative charge. Most of the early PTP1B inhibitors are compounds with a

*Corresponding author. Tel.: +46-8-697-3869; fax: +46-8-697-3914; e-mail: charlotta.liljebris@biovitrum.com

0968-0896/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0968-0896(02)00176-1

non-hydrolyzable phosphotyrosine mimetic often incorporated into a peptide-like scaffold.[8] Although much progress has been made, most of the inhibitors developed so far have properties which make them unsuitable as drugs due to lack of cell permeability.[8,17] To date only a few small molecule PTP1B inhibitors have been demonstrated to show in vivo activity.[18]

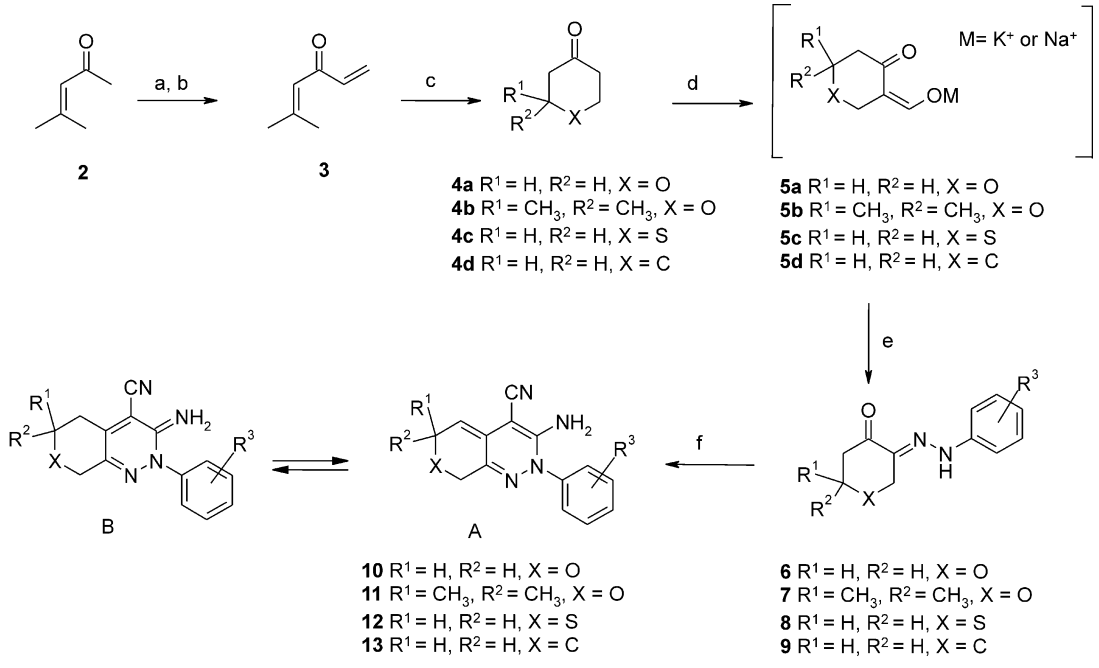
In our high throughput screening program, we have identified compound **11** (3-amino-6,6-dimethyl-2-phenyl-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile, commercially available) as a novel reversible PTP1B inhibitor with an IC_{50} value of 5.7 μM. Interestingly, this compound displayed non-competitive kinetics, and is therefore seemingly not binding within the phosphatase active site pocket. This opens up the exciting possibility to find a new binding site for the PTP1B enzyme. The non-polar properties, and small molecular weight of this compound class, made it an interesting starting point to develop specific orally available PTP1B inhibitors. In this paper, we describe the synthesis and in vitro pharmacology properties of this compound class as PTP1B inhibitors. Furthermore, selected analogues in the series showed increased insulin receptor phosphorylation in a cellular assay, which resulted in enhanced insulin receptor activity.

Results and Discussion

Chemistry

Preparation of the target analogues is shown in Scheme 1. The pyranone analogues **4a** and **4c** were commercially available, whereas the geminal dimethyl pyranone **4b** could be prepared in two steps following literature methods. A Mannich reaction between mesityl oxide **2** and formaldehyde gave the divinyl ketone **3**.[19] The divinyl ketone **3** was oxidatively ringclosed using $HgSO_4$[20] which gave the requisite pyranone **4b**. Condensation with ethyl formate furnished the enolate analogues **5a–c** using either NaH or K^t-BuO as bases. When K^{t}BuO was used, the resulting enolate could be stored as a solid, otherwise the enolate was used directly in the next step. The phenylhydrazones **6–9** where prepared by reacting the enolate with the diazonium salt of the corresponding aniline.[21,22] Cyclization using malonitrile in DMSO furnished the target pyridazine analogues **10**.[23] As previously described,[23] we found that the pyridazine analogues were in equilibrium between the tautomeric forms A and B in solution (Scheme 1). NMR experiments showed that the dimethyl analogues **11a–n** mostly existed in the amine tautomer A, whereas for **10** and **12**, the equilibrium was shifted towards the imine form B. We also discovered that the analogues most prone to be in the imine form, often where chemically unstable under pharmacological conditions (pH 7.4, 37 °C, 24 h).[24] An exception to this finding was the sulphur pyridazine analogue **12** which was found to be stable under pharmacological conditions. Encouraged by the indication that the geminal dimethyl group stabilises the core structure, a series of analogues of **11**, with substituent variations in the phenyl ring **11a–11n** (o, m, p), were prepared according to Scheme 1. All of these analogues showed good pharmacological stability.[24]

The nitrile functionality of **10** could be converted to the corresponding primary amide by heating **10** in concentrated H_2SO_4 to provide compound **14** (Scheme 2). Furthermore, acylation of the amine group of **10** with acetic anhydride gave the diacetylated analogue **15** (Scheme 2).

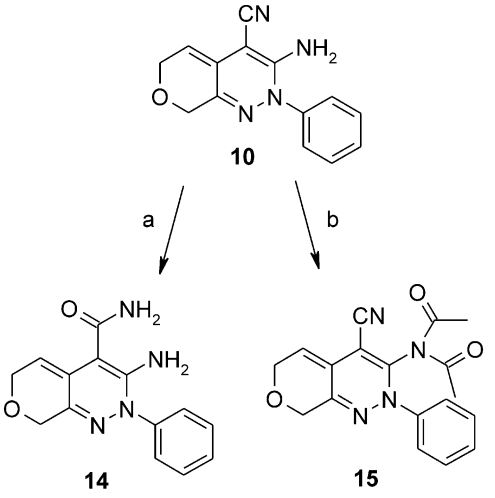


Scheme 1. Reagents: (a) paraformaldehyde, Et_2NH, MsOH, H_2O (b) Ac_2O, DMAP (c) $HgSO_4$, H_2SO_4, H_2O (d) K^{t}BuO/THF or NaH/EtOH, HCO_2Et; (e) $R^3(C_6H_4)N_2^+Cl^-$, −5 °C; (f) malononitrile, morpholine, DMSO.

A route analogous to Scheme 1 was employed to vary the ring size of **13**, as shown in Scheme 3. Starting from the appropriate cyclo ketone analogues the corresponding hydrazones **16** and **18** were prepared. Treatment with malonitrile in DMSO and morpholine as base, afforded the pyridazine analogues **17** and **19**.

In vitro PTP activity

The inhibitory activity of the pyridazine analogues on PTP1B was measured using pNPP as the substrate (Table 1). Compound **10**, lacking the geminal dimethyl groups, showed surprisingly high potency as PTP1B inhibitor ($IC_{50} = 0.35\,\mu M$). However, it was discovered that the chemical stability at physiological conditions[24] of analogues lacking the geminal dimethyl groups was universally bad, and therefore this compound class was not pursued any further. Instead, a series of analogues to **11** with variations in the phenyl ring were prepared **11a**–**11n** (Table 1). The structure–activity relationship for these analogues was found to be quite flat with IC_{50} values ranging from 1 to 17 μM. However, some para substituted analogues **11ap**, **11dp**, **11ep** and **11mp**, were found to be inactive as inhibitors of PTP1B, which could be due to steric hindrance in this position of the binding site. This is supported by the lower activity of the para phenoxy analogue **11gp**. The most active analogues in this series were the *ortho* ethyl and *ortho* butyl analogues **11co** and **11lo** ($IC_{50} = 1.6$ and 1.5 μM, respectively).

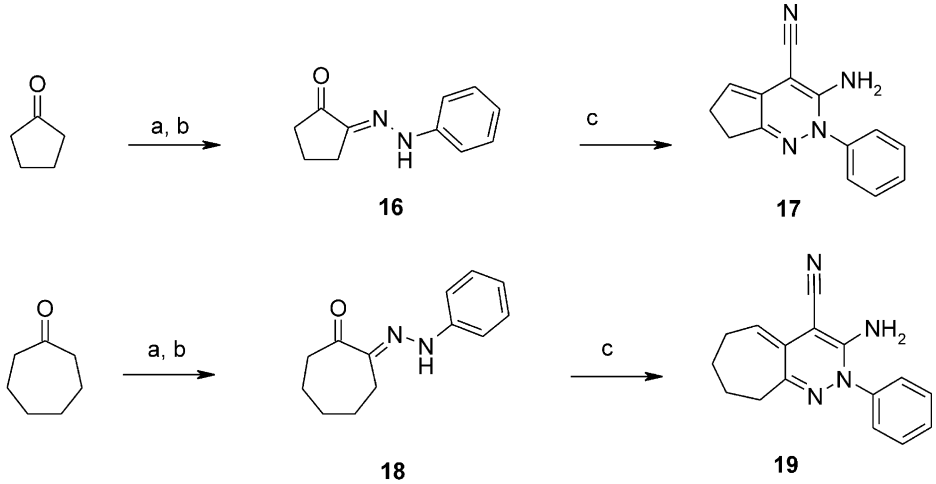


Scheme 2. Reagents: (a) concd H_2SO_4, 80–90 °C; (b) Ac_2O, Hunigs base, CH_2Cl_2.

Replacing the oxygen with sulphur or carbon (**12** and **13**, respectively) was not found to be beneficial for the PTP1B inhibitory activity, with equal or lower activity compared to the parent compound **11**.

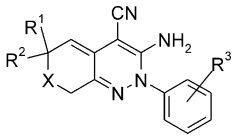
Modification of the ring structure size (cyclopentane analogue **17** and cycloheptane analogue **19**) resulted in total loss of inhibitory activity ($IC_{50} > 100\,\mu M$). The amide analogue **14** and di-acetyl analogue **15** were also found to be inactive as PTP1B inhibitors ($IC_{50} > 100\,\mu M$). This shows that the core ring structure in the parent compound **11** is essential for the activity.

Compounds were also tested for inhibition of other recombinant, purified PTP enzymes (LAR, SHP-2 and TCPTP) as an initial assessment of their specificity. Results are summarized in Table 1. In general, most compounds are non-selective towards these phosphatases with equal inhibitory activities as for PTP1B. However there are some interesting exceptions. The *para* triflouromethyl analogue **11ap** was inactive as an inhibitor of PTP1B and SHP-2, but showed equal activity for LAR and TCPTP with IC_{50} values in the low micromolar range. Furthermore, the *meta* phenoxy analogue **11gm** was found to be equally active for PTP1B, SHP-2 and TCPTP but was inactive towards LAR. Maybe the most exciting finding with regard to selectivity are analogues which showed 6–10-fold selectivity for PTP1B against TCPTP (**11ao**, **11bm**, **11co**, **11 ho**, **11 hp** and **11no**). Also, one compound, **12mp**, showed a 20-fold selectivity for PTP1B against both LAR and TCPTP. Due to the high degree of homology within the catalytic active site cleft of PTP1B and TCPTP, it has proven to be very difficult to gain selectivity with small competitive inhibitory compounds. To gain TCPTP selectivity within the active site cleft, the compounds need to be relatively large with small possibilities to be cell permeable. Analogues in the current series exert their effects outside the catalytic



Scheme 3. Reagents: (a) NaH, HCO_2Et, EtOH; (b) $PhN_2^+Cl^-$, −5 °C; (c) malononitrile, morpholine, DMSO.

Table 1. In vitro activity of pyridazine analogues **10–13**



Compd	R^1	R^2	R^3	X	IC_{50} (μM)			
					PTP1B	LAR	SHP-2	TCPTP
10	H	H	H	O	0.35±0.1[a]	0.6±0.01[a]	ND	2.1±0.1[a]
11	CH_3	CH_3	H	O	5.7±0.6	9.0±0.6	8.2±0.6	1.5[a]
12	H	H	H	S	1.1±0.2[a]	3.1±0.2[a]	ND	4.6±0.2[a]
12ip	H	H	4-OMe	S	1.3±0.05	6.0±0.2	8.0±0.8	1.5±0.1
12ko	H	H	2-F	S	4.4±0.2	ND	ND	ND
12kp	H	H	4-F	S	2.0±0.1	ND	ND	ND
12mo	H	H	2-*i*Propyl	S	2.0±0.2	2.9±0.6	2.4	0.9±0.1
12mp	H	H	4-*i*Propyl	S	5.6±0.7	>100	5.2	>100
13	H	H	H	C	10.4±0.3[a]	26.0±3.0[a]	4.8±0.1[a]	65.0±11[a]
11ao	CH_3	CH_3	2-CF_3	O	3.9±0.5	7.1±2.1	4.7±0.6	25.9±1.8
11am	CH_3	CH_3	3-CF_3	O	2.8±0.02	5.9±1.4	10.5±1.9	6.1±0.5
11ap	CH_3	CH_3	4-CF_3	O	>100	3.7	>100	5.8±0.5
11bo	CH_3	CH_3	2-CH_2OH	O	11.1±1.0	7.9±0.05	6.5±0.5	46.0±6.0
11bm	CH_3	CH_3	3-CH_2OH	O	2.4±0.3	10.2±0.5	3.8±0.5	28.2±0.2
11bp	CH_3	CH_3	4-CH_2OH	O	1.7±0.05	5.7±1.1	14.4±0.6	4.5±0.1
11co	CH_3	CH_3	2-CH_2CH_3	O	1.6±0.1	4.0±0.4	2.8±0.3	16.4±1.4
11cm	CH_3	CH_3	3-CH_2CH_3	O	8.2±1.2	15.4±1.2	6.8±0.2	7.6±0.3
11cp	CH_3	CH_3	4-CH_2CH_3	O	2.8±1.1	21.9±0.1	6.1±0.1	7.5±0.5
11do	CH_3	CH_3	2-NO_2	O	17.5±1.3	11.9±0.1	19.5±0.2	13.8±0.8
11dp	CH_3	CH_3	4-NO_2	O	>100	>100	>100	>100
11em	CH_3	CH_3	3-$COOCH_3$	O	2.1±0.01	7.2±0.7	5.0±1.5	4.8±0.7
11ep	CH_3	CH_3	4-$COOCH_3$	O	>100	>100	>100	>100
11fm	CH_3	CH_3	3-$NHCOCH_3$	O	2.3±0.02	9.3±0.2	6.0±1.2	6.5±0.8
11fp	CH_3	CH_3	4-$NHCOCH_3$	O	6.1±0.4	16.9±1.8	4.6±0.5	12.0±0.6
11go	CH_3	CH_3	2-Oph	O	15.8±0.5	8.0±1.1	10.8±0.1	5.9±0.1
11gm	CH_3	CH_3	3-Oph	O	3.9±0.3	>100	9.4±2.7	5.9±1.1
11gp	CH_3	CH_3	4-Oph	O	22.7±1.6	21.7±0.4	>100	>100
11 ho	CH_3	CH_3	2-OCH_2CH_3	O	3.4±0.6	6.2±0.4	5.7±0.4	26.2±1.5
11 hp	CH_3	CH_3	4-OCH_2CH_3	O	3.2±0.4	5.0±0.2	5.5±0.5	28.2±0.2
11io	CH_3	CH_3	2-OCH_3	O	6.2±3.8	12.1±0.2	9.5±1.2	8.6±2.6
11im	CH_3	CH_3	3-OCH_3	O	3.6±0.6	8.3±1.0	8.8±0.8	7.3±0.2
11ip	CH_3	CH_3	4-OCH_3	O	2.2±0.3	9.8±1.8	8.7±0.1	9.8±5.9
11jp	CH_3	CH_3	4-CH_2CH_2OH	O	2.9±0.6	9.4±1.4	8.2±0.23	9.3±0.1
11ko	CH_3	CH_3	2-F	O	2.4±0.5	5.7±1.4	8.4±0.3	7.9±0.7
11km	CH_3	CH_3	3-F	O	4.5±0.5	11.8±0.3	12.5±0.6	20.3±1.5
11kp	CH_3	CH_3	4-F	O	4.9±0.9	9.6±0.4	5.5±0.5	10.0±1.8
11lo	CH_3	CH_3	2-Butyl	O	1.5±0.05	8.3±0.4	4.7±1.2	4.0±0.3
11lp	CH_3	CH_3	4-Butyl	O	7.0±0.7	14.0±1.5	11.7±3.3	9.7±0.3
11mm	CH_3	CH_3	3-*i*Propyl	O	8.2±0.4	12.1±0.2	12.0±1.8	17.4±2.8
11mp	CH_3	CH_3	4-*i*Propyl	O	>100	>100	>100	>100
11no	CH_3	CH_3	2-Morpholinyl	O	2.9±0.5	3.4±0.1	4.6±0.5	21.0±0.6

[a]Concentration giving 50% inhibition relative to the maximum inhibition observed with sodium vanadate. All other values are the inflexion point of inhibition curves.

binding site, which opens up the possibility to gain TCPTP selectivity with retained bioavailability. It is not obvious, however, to see a structure–activity pattern of the selective compounds. More analogues need to be prepared in this series to elucidate this further.

Kinetics

The characteristics of the inhibition of PTP1B by this compound series were analysed using compound **11** as a representative compound. PTP1B was incubated with increasing concentrations of compound **11** and full velocity dose curves were performed (Fig. 1). Non-linear regression analysis showed that the data best fit non-competitive models of inhibition (reduced χ^2 0.0003). Re-plotting of the data as Lineweaver–Burke transformations (Fig. 1) confirmed this result, showing that this compound series behaves as non-competitive inhibitors of PTP1B. The PTP family comprises over 100 family members, containing both trans-membrane receptor-like enzymes and soluble cytosolic enzymes.[25] There is considerable homology within the active sites of the family members and considerable diversity outside the active site. Certain cytosolic PTPs contain other protein domains, such as src homology 2 (SH2) domains, PEST domains and retinaldehyde binding protein-like domains, which are involved in intracellular targeting strategies and are important for protein regulation.[26] PTP1B is noticeable in the absence of other recognized protein domains outside the active site, apart from a C-terminal endoplasmic reticulum targeting sequence. Data with this compound series therefore indicate that

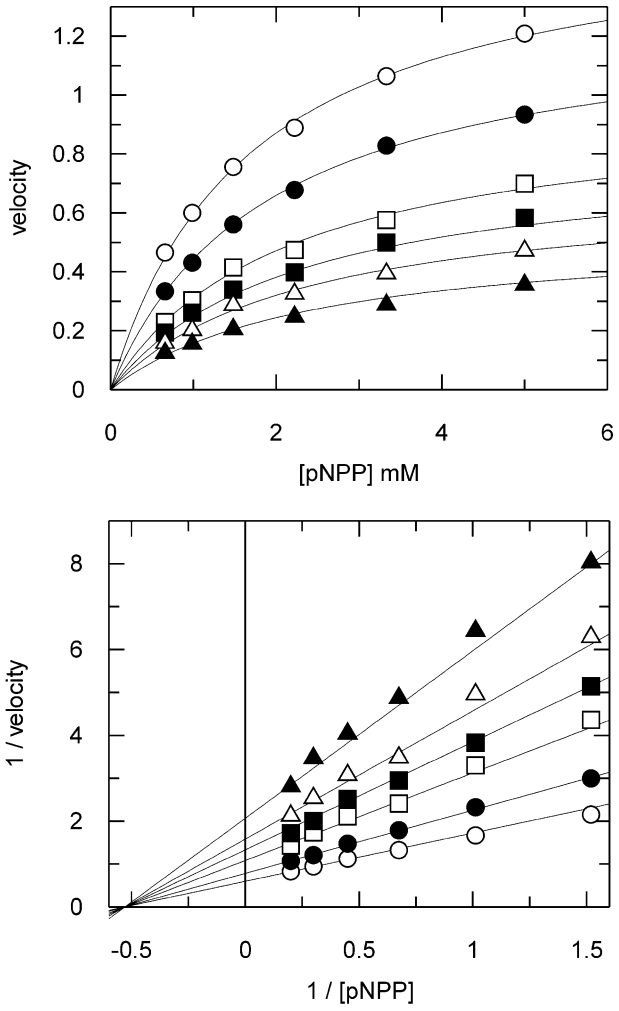


Figure 1. Compound **11** behaves as a non-competitive inhibitor or PTP1B. Top panel: velocity curves performed with 20 ng PTP1B in the presence of increasing concentrations of compound 11 (open circles, 0 μM, filled circles, 1.25 μM, open squares, 3.3 μM, filled squares, 5 μM, open triangles, 6.7 μM, filled triangles, 10 μM). Data are representative of several experiments, each point the average of quadruplicates. Solid lines are fits of the data to the Henri Michaelis Menten equation. Lower panel: Lineweaver–Burke transformations of data from top panel.

there may exist another site in the enzyme through which enzyme activity can be inhibited which is not a recognized interaction domain.

Cellular activity of selected compounds

Cell permeability experiments were performed according to published procedures,[27] which showed that this class of compounds transit the cell membrane readily (data not shown). This is in contrast to reports of other PTP inhibitors where ability to cross the plasma membrane has been limiting. We therefore examined the effects of this compound class in an insulin receptor cellular assay. Incubation of L6 myotubes with compounds **11**, **11co** and **11io** followed by stimulation with sub-maximal concentrations of insulin showed that insulin receptor phosphorylation was increased relative to vehicle-treated cells (Fig. 2). The effects of the compounds were more modest than those of sodium vanadate, in keeping with the reversible inhibition of PTP activity they cause. The compounds alone had no effect on insulin receptor phosphorylation (data not shown). Thus, treatment of cells with specific compounds resulted in enhanced insulin receptor activity.

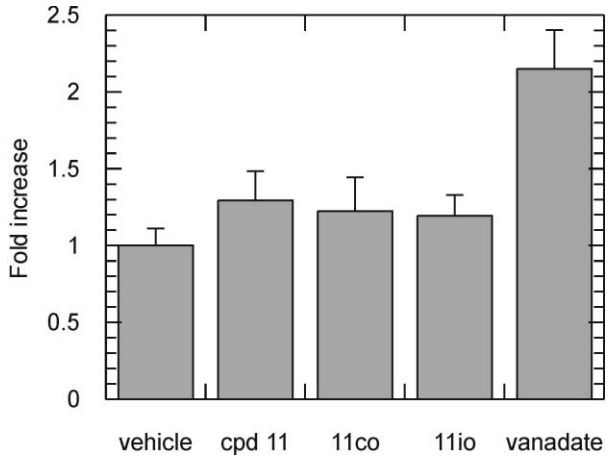


Figure 2. Compound class enhances insulin-stimulated tyrosine phosphorylation of the insulin receptor. L6 myotubes were pre-treated for 30 min in the presence and absence of the indicated compounds. Cells were subsequently stimulated with 25 nM insulin for 5 min and insulin receptor phosphorylation was measured as described in the Methods section. Data are the taken from at least three independent experiments and normalized to insulin-stimulated cell responses in the presence of vehicle alone. All treatments resulted in statistically significant increases in insulin receptor phosphorylation compared with vehicle controls ($p < 0.03$, Students t-test).

Conclusion

A series of novel pyridazine analogues has been prepared, which were shown to be inhibitors of PTP1B with IC_{50} values in the low micromolar range. In vitro kinetics of this compound class demonstrate that they are reversible non-competitive inhibitors, and thus not binding within the active site cleft of PTP1B. To our knowledge, this is the first time non-competitive binders of PTP1B have been reported, and this indicates that there may exist another site in the enzyme through which enzyme activity can be inhibited which is not a recognized interaction domain. The compounds were analyzed for their inhibitory activity of other PTPases, which demonstrated surprisingly high selectivity of some of the analogues. Compound **12mp** was 20-fold more selective for PTP1B against both LAR and TCPTP. TCPTP is the phosphatase, which has highest homology with PTP1B within the active site cleft against which it has thus been difficult to gain selectivity with competitive binders. Furthermore, due to the relatively small molecular weight and non-polar properties of this compound class they were shown to transit cell membranes readily. Selected analogues in the series, **11**, **11co** and **11io**, were analyzed in an in vitro cellular assay which showed that they increased insulin receptor phosphorylation and thereby prolonged the activated state of the insulin receptor. The interesting features of this compound class makes them an attractive starting point to be developed into more potent orally available PTP1B inhibitors and thus therapy for Type 2 diabetes.

Experimental

NMR spectra were recorded on a Varian 400 MHz spectrometer, a Bruker Advance DPX 400 or a Bruker DRX 500 and chemical shifts are given in ppm using tetramethylsilane as an internal standard at 25 °C. HPLC analyses were performed using; System A: Waters Xterra MS C18 column (100 × 4.6 mm, 5 μ) eluting with a gradient of 5% ACN in 95% water to 95% ACN in 5% water (0.2% TFA buffer) over 3.5 min then 95% ACN in 5% water (0.2% TFA buffer) for a further 2.5 min at a flow rate of 3 mL/min on a Waters 600E system with monitoring at 254 nm; System B: Hewlett-Packard 1100 instrument with a Nucleosil C-18 column (250×4.6 mm, 3 μM) thermostated at 25 °C, eluting with water (0.1% TFA)/acetonitrile at a flow rate of 1 mL/min and gradients with a 5-min isocratic run followed by a 10-min gradient, with UV detection at 254-nm. Thin layer chromatography was carried out using pre-coated silica gel F-254 plates (thickness 0.25 mm). IR spectra were recorded on a Perkin-Elmer Spectrum 1000 FTIR spectrometer. Electrospray MS spectra were obtained on a Micromass platform LCMS spectrometer. The Biotage Quad 3 system was used for parallel flash purification. Silica gel column chromatography was performed using YMC gel, silica 120 Å S-50 μm.

5-Methyl-hexa-1,4-dien-3-one (3). A solution of diethyl amine (73 g, 1.0 mol) and methane sulfonic acid (100 g, 1.04 mol) in 2-propanol (50 mL) was dissolved in ice water (100 g). To the resultant water solution was mesityl oxide (98 g, 1 mol) and paraformaldehyde (60 g, 2 mol) added and the mixture was refluxed for 4 h. The solution was cooled and extracted with ether to remove unreacted mesityloxide. Ether and a concentrated sodium hydroxide solution was carefully added to the ice-cold solution until it was alkaline. The aqueous solution was extracted with ether, and the combined ether extracts were washed with water, dried and evaporated. To the neat material was DMAP (1 g) and acetic anhydride (100 mL, 1 mol) added dropwise, keeping the temperature below 60 °C. The mixture was stirred 2 h at room temperature, then the reaction mixture was diluted with water and hexane. Sodium bicarbonate was added until all gas evolution had ceased. After neutralization, the hexane solution was separated and the water solution was extracted with hexane, all hexane extracts were washed with water to remove the diethylacetamide. The hexane solution was dried and evaporated. The crude product was distilled in vacuum with a 10 cm long NS29 column filled with glass rings. In order to avoid polymerisation, hydroquinone was added to the crude material. The fraction at 57–63 °C/18 mmHg was collected which gave 36.6 g (33%) of the crude divinyl ketone **3**: GC 86%. An analytical sample was prepared by redistillation which gave **3** as an oil. GC 96%; (bp 70 °C at 15 mmHg, lit: bp 60–61 °C at 22 mmHg); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95 (s, 3H); 2.2 (s, 3H); 5.9.25 (m, 4H).

2,2-Dimethyl-tetrahydro-pyran-4-one (4b). A solution of **3** (approximately 0.92 mol), HgSO4 (9.2 g, 0.31) and sulphuric acid (16.6 g, 0.16 mol) in water (1.4 L) was stirred vigorously at 100 °C for 4 h. The reaction mixture was cooled, saturated with NaCl and extracted with EtOAc. The organic phase was dried and evaporated and the crude product was distilled in vacuum without column. The fraction at 75–81 °C/20 mm Hg was collected which gave 79 g (67%) of **4b**; GC 94%. An analytical sample was redistilled to give pure material **4b** as an oil. GC 99%; ^{1}H NMR: (400 MHz, $CDCl_3$) δ 1.27 (s, 6H); 2.36 (s, 2H); 2.39 (t, 2H); 4.01 (t, 2H). Anal. calcd for $C_7H_{12}O_2$: C, 65.60; H, 9.44; found: C, 65.43; H, 9.75.

General methods to prepare the hydrazone intermediates 6–9, 16 and 18:

Method A. Exemplified by the preparation of 3-(phenyl-hydrazono)-tetrahydro-pyran-4-one (6). To dry ethanol (60 mL) at −20 °C was added sodium hydride (slowly) (3.92 g, 0.098 mol). Tetrahydro-4H-pyran-4-one (**4a**) (9.79 g, 0.098 mol) was then added, followed by ethyl formate (11.9 mL, 0.147 mol) and the reaction mixture stirred at room temperature overnight. The solvent was then removed under reduced pressure and the residue dissolved in water (40 mL). The resultant solution was added to a solution of the diazonium salt of aniline, prepared by the gradual addition of a solution of sodium nitrite (6.76 g, 0.098 mol) in water (20 mL) to a solution of aniline (9.11 g, 0.098 mol) in 2 M HCl (98 mL, 0.196 mol) at −5 to 0 °C. The resultant mixture was stirred at this temperature for 1 h. The product was then extracted with DCM, washed with water, dried ($MgSO_4$) and the solvent removed under reduced pressure to give a brown solid (14.5 g, 73%). The product **6** was then recrystallized from ethanol (8.17 g, 41%); HPLC 92%, $R_T = 3.74$ min (System A); ^{1}H NMR ($CDCl_3$) δ 7.39.23 (m, Ph), 4.58 (s, CH_2), 4.01 (t, CH_2), 2.65 (t, CH_2). The hydrazone was used directly in the condensation reaction without further characterization.

2,2-Dimethyl-5-(phenyl-hydrazono)-tetrahydro-pyran-4-one (7). Prepared according to method A using 2,2-dimethyl-tetrahydro-pyran-4-one (**4b**). The hydrazone was used in the condensation reaction without further characterization.

Method B. Exemplified by the preparation of 2,2-dimethyl-5-[(2-trifluoromethyl-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7ao). A solution of 2,2-dimethyl-tetrahydro-pyran-4-one **4b** (3.0 g, 23.4 mmol) and ethyl formate (3.0 mL, 37.2 mmol) in dry THF (30 mL) was placed under nitrogen. To the solution was added K^t-BuO (2.63 g, 23.4 mmol) in small portions during 10 min. A yellow precipitate was formed almost immediately. The mixture was stirred at room temperature for 3 h. The solvent was then evaporated and the crude product **5b** was dried in vacuum. The crude material was dissolved in EtOH (18 mL) and divided into portions and stored in freezer until used.

A solution of the diazoniumsalt of 2-trifluoromethyl-aniline was prepared by the gradual addition of a solution of sodium nitrite (273 mg, 3.95 mmol) in water

(2 mL) to a solution of 2-trifluoromethyl aniline (637 mg, 3.95 mol) in 1.00 M HCl (7.90 mL, 7.90 mmol) at −5 °C. The cold ethanol solution (3 mL) of the potassium salt of 5-(hydroxymethylene)-2,2-dimethyl-tetrahydro-4H-pyran-4-one **5b** (3.90 mmol) was added dropwise and the resulting mixture was stirred vigorously at −5° C for 1 h. The reaction mixture was diluted with DCM and washed with water. The organic phase was dried with $MgSO_4$ and evaporated which gave 0.9755 g of **7ao** as an orange solid. (83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.05 (s, 0.7H), 7.86 (d, 1H), 7.55 (d, 1H), 7.50 (t, 1H), 7.08 (t, 1H), 4.64 (s, 2H), 2.58 (s, 2H), 1.36 (s, 6H). MS (EI) m/z 300.0 $(M+H)^+$.

2,2-Dimethyl-5-[(3-trifluoromethyl-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7am). Prepared according to method B using **4b** and 3-trifluoromethyl-aniline. Yield: 0.948 g (80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.68 (s, 1H), 7.54 (br.s, 1H), 7.42 (m, 1H), 7.35 (m, 1H), 7.27 (m, 1H), 4.63 (s, 2H), 2.56 (s, 2H), 1.36 (s, 6H). MS (ES) m/z 300 $(M+H)^+$.

2,2-Dimethyl-5-[(4-trifluoromethyl-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7ap). Prepared according to method B using **4b** and 4-trifluoromethyl-aniline. Yield: 0.94 g (80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.63 (s, 1H), 7.56 (d, 2H), 7.31 (d, 2H), 4.63 (s, 2H), 2.56 (s, 2H), 1.36 (s, 6H). MS (ES) m/z 300 $(M+H)^+$.

2,2-Dimethyl-5-[(2-hydroxymethyl-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7bo). Prepared according to method B using **4b** and (2-amino-phenyl)-methanol. Yield: 0.45 g (66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (dd, 1H), 7.33 (dt, 1H), 7.22 (dd, 1H), 7.01 (dt, 1H), 4.77 (s, 2H), 4.64 (s, 2H), 2.55 (s, 2H), 1.35 (s, 6H). MS (EI) m/z 262 $(M+H)^+$.

2,2-Dimethyl-5-[(3-hydroxymethyl-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7bm). Prepared according to method B using **4b** and (3-amino-phenyl)-methanol. Yield: 0.55 g (81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (m, 1H), 7.26 (d, 1H), 7.15 (dd, 1H), 7.03 (d, 1H), 4.69 (s, 2H), 4.61 (s, 2H), 2.53 (s, 2H), 1.35 (s, 6H). HMS (EI) m/z 262 $(M+H)^+$.

2,2-Dimethyl-5-[(4-hydroxymethyl-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7bp). Prepared according to method B using **4b** and (4-amino-phenyl)-methanol. Yield: 0.52 g (77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.74 (s, 1H), 7.32 (d, 2H), 7.24 (d, 2H), 4.65 (s, 2H), 4.61 (s, 2H), 2.53 (s, 2H), 1.35 (s, 6H). MS (EI) m/z 262 $(M+H)^+$.

2,2-Dimethyl-5-[(2-ethyl-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7co). Prepared according to method B using **4b** and 2-ethyl-phenylamine. Yield: 0.89 g (66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.05 (s, 1H), 7.67 (dd, 1H), 7.22 (dt, 1H), 7.16 (dd, 1H), 7.01 (dt, 1H), 4.65 (s, 2H), 2.67 (q, 2H), 2.55 (s, 2H), 1.36 (s, 6H), 1.31 (t, 3H). MS (EI) m/z 260 $(M+H)^+$.

2,2-Dimethyl-5-[(3-ethyl-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7cm). Prepared according to method B using **4b** and 3-ethyl-phenylamine. Yield: 0.56 g (84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.75 (s, 1H), 7.22 (dd, 1H), 7.11 (m, 1H), 7.06 (m, 1H), 6.89 (dd, 1H), 4.62 (s, 2H), 2.64 (q, 2H), 2.53 (s, 2H), 1.35 (s, 6H), 1.24 (tr, 3H). MS (EI) m/z 260 $(M+H)^+$.

2,2-Dimethyl-5-[(4-ethyl-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7cp). Prepared according to method B using **4b** and 4-ethyl-phenylamine. Yield: 0.53 g (78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.8 (s, 1H), 7.16 (m, 4H), 4.61 (s, 2H), 2.61 (q, 2H), 2.52 (s, 2H), 1.34 (s, 6H), 1.22 (tr, 3H). MS (EI) m/z 260 $(M+H)^+$.

2,2-Dimethyl-5-[(2-nitro-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7do). Prepared according to method B using **4b** and 2-nitro-phenylamine. Yield: 0.44 g (82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (dd, 1H), 8.22 (dd, 1H), 7.59 (m, 1H), 7.05 (m, 1H), 4.68 (s, 2H), 2.64 (s, 2H), 1.38 (s, 6H). The hydrazone proton is here outside the spectral range. MS (EI) m/z 277 $(M+H)^+$.

2,2-Dimethyl-5-[(4-nitro-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7dp). Prepared according to method B using **4b** and 4-nitro-phenylamine. Yield: 0.66 g (92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.62 (s, 1H), 8.21 (d, 2H), 7.30 (d, 2H), 4.64 (s, 2H), 2.59 (s, 2H), 1.37 (s, 6H). MS (EI) m/z 277 $(M+H)^+$.

2,2-Dimethyl-5-[(3-carboxymethyl-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7em). Prepared according to method B using **4b** and 3-amino-benzoic acid methyl ester. Yield: 0.46 g (82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.71 (s, 1H), 7.90 (m, 1H), 7.70 (dd, 1H), 7.44 (m, 1H), 7.38 (dd, 1H), 4.64 (s, 2H), 3.93 (s, 3H), 2.55 (s, 2H), 1.36 (s, 6H). MS (EI) m/z 290 $(M+H)^+$.

2,2-Dimethyl-5-[(4-carboxymethyl-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7ep). Prepared according to method B using **4b** and 4-amino-benzoic acid methyl ester. Yield: 0.46 g (82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.64 (s, 1H), 8.00 (d, 2H), 7.27 (d, 2H), 4.63 (s, 2H), 3.89 (s, 3H), 2.56 (s, 2H), 1.36 (s, 6H). MS (EI) m/z 290 $(M+H)^+$.

2,2-Dimethyl-5-[(3-acetamido-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7fm). Prepared according to method B using **4b** and *N*-(3-amino-phenyl)-acetamide. Yield: 0.48 g (85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.64 (s, 1H), 7.54 (s, 1H), 7.23 (d, 1H), 7.14 (m, 2H), 6.96 (d, 1H), 4.61 (s, 2H), 2.53 (s, 2H), 2.18 (s, 3H), 1.35 (s, 6H). MS (EI) m/z 289 $(M+H)^+$.

2,2-Dimethyl-5-[(4-acetamido-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7fp). Prepared according to method B using **4b** and *N*-(4-amino-phenyl)-acetamide. Yield: 0.52 g (70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.8 (s, 1H), 7.45 (d, 2H), 7.22 (d, 2H), 7.11 (br.s, 1H), 4.61 (s, 2H), 2.52 (s, 2H), 2.17 (s, 3H), 1.35 (s, 6H). MS (ESI+) m/z 290 $(M+H)^+$.

2,2-Dimethyl-5-[(3-phenoxy-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7gm). Prepared according to method B using **4b** and 3-phenoxy-phenylamine. Yield: 0.58 g

(92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.65 (s, 1H), 7.35 (m, 2H), 7.24 (m, 1H), 7.12 (m, 1H), 7.02 (m, 2H), 6.95 (m, 2H), 6.95 (m, 2H), 6.66 (m, 1H), 4.58 (s, 2H), 2.52 (s, 2H), 1.34 (s, 6H). MS (EI) *m*/*z* 324 $(M+H)^+$.

2,2-Dimethyl-5-[(4-phenoxy-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7gp). Prepared according to method B using **4b** and 4-phenoxy-phenylamine. Yield: 0.49 g (77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.84 (s, 1H), 7.32 (m, 2H), 7.25 (m, 1H), 7.23 (m, 1H), 7.07 (m, 1H), 7.01 (m, 1H), 6.99 (m, 2H), 6.97 (m, 1H), 4.61 (s, 2H), 2.53 (s, 2H), 1.35 (s, 6H). MS (EI) *m*/*z* 324 $(M+H)^+$.

2,2-Dimethyl-5-[(2-ethoxy-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7 ho). Prepared according to method B using **4b** and 2-ethoxy-phenylamine. Yield: 0.49 g (69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.85 (s, 1H), 7.62 (dd, 1H), 6.96 (m, 2H), 6.87 (dd, 1H), 4.64 (s, 2H), 4.13 (q, 2H), 2.54 (s, 2H), 1.53 (t, 3H), 1.35 (s, 6H). MS (EI) *m*/*z* 276 $(M+H)^+$.

2,2-Dimethyl-5-[(4-ethoxy-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7 hp). Prepared according to method B using **4b** and 4-ethoxy-phenylamine. Yield: 0.51 g (72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.92 (s, 1H), 7.19 (d, 2H), 6.87 (d, 2H), 4.60 (s, 2H), 4.01 (q, 2H), 2.51 (s, 2H), 1.40 (t, 3H), 1.34 (s, 6H). MS (EI) *m*/*z* 276 $(M+H)^+$.

2,2-Dimethyl-5-[(4-methoxy-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7ip). Prepared according to method B using **4b** and 4-methoxy-phenylamine. Yield: 0.83 g (80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.90 (s, 1H), 7.18 (d, 2H), 6.87 (d, 2H), 4.59 (s, 2H), 3.79 (s, 3H), 2.50 (s, 2H), 1.33 (s, 6H). MS (EI) *m*/*z* 262 $(M+H)^+$.

2,2-Dimethyl-5-[(4-(2-hydroxyethyl)phenyl)-hydrazono]-tetrahydro-pyran-4-one (7jp). Prepared according to method B using **4b** and 2-(4-amino-phenyl)-ethanol. Yield: 0.83 g (76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.76 (s, 1H), 7.20 (m, 4H), 4.61 (s, 2H), 3.84 (tr, 2H), 2.84 (tr, 2H), 2.53 (s, 2H), 1.35 (s, 6H). MS (EI) *m*/*z* 276$(M+H)^+$.

2,2-Dimethyl-5-[(2-fluoro-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7ko). Prepared according to method B using **4b** and 2-fluoro-phenylamine. Yield: 0.78 g (79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.68 (s, 1H), 7.65 (m, 1H), 7.10 (m, 2H), 6.96 (m, 1H), 4.63 (s, 2H), 2.56 (s, 2H), 1.36 (s, 6H). MS (EI) *m*/*z* 250 $(M+H)^+$.

2,2-Dimethyl-5-[(2-butyl-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7lo). Prepared according to method B using **4b** and 2-butyl-phenylamine. Yield: 0.48 g (85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.02 (s, 1H), 7.67 (dd, 1H), 7.21 (m, 1H), 7.13 (m, 1H), 6.99 (m, 1H), 4.65 (s, 2H), 2.64 (t, 2H), 2.55 (s, 2H), 1.64 (m, 2H), 1.46 (m, 2H), 1.36 (s, 6H), 0.97 (t, 3H). MS (EI) *m*/*z* 288 $(M+H)^+$.

2,2-Dimethyl-5-[(4-butyl-phenyl)-hydrazono]-tetrahydro-pyran-4-one (7lp). Prepared according to method B using **4b** and 4-butyl-phenylamine. Yield: 0.40 g (72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.8 (s, 1H), 7.17 (d, 2H), 7.12 (d, 2H), 4.61 (s, 2H), 2.57 (t, 2H), 2.52 (s, 2H), 1.57 (m, 4H), 1.34 (s, 6H), 0.92 (t, 3H). MS (EI) *m*/*z* 288 $(M+H)^+$.

2,2-Dimethyl-5-[(4-(isopropyl)phenyl)-hydrazono]-tetrahydro-pyran-4-one (7mp). Prepared according to method B using **4b** and 4-isopropyl-phenylamine. Yield: 0.195 g (60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.80 (s, 1H), 7.26 (s, 2H), 7.18 (s, 2H), 4.61 (s, 2H), 2.87 (m, 1H), 2.52 (s, 2H), 1.34 (s, 6H), 1.23 (d, 6H). MS (ES) *m*/*z* 274 $(M+H)^+$.

2,2-Dimethyl-5-[(2-(4-morpholinyl)phenyl)-hydrazono]-tetrahydro-pyran-4-one (7no). Prepared according to method B using **4b** and 2-(4-morpholinyl)phenylamine. Yield: 0.43 g (52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.85 (s, 1H), 7.65 (d, 1H), 7.13 (d, 2H), 7.02 (m, 1H), 4.64 (s, 2H), 3.99 (t, 4H), 2.92 (t, 4H), 2.55 (s, 2H), 1.36 (s, 6H). MS (ESI+) *m*/*z* 318.24 $(M+H)^+$.

3-(Phenyl-hydrazono)-tetrahydro-thiopyran-4-one (8). Prepared according to method A using tetrahydro-thiopyran-4-one.

2-(Phenyl-hydrazono)-cyclohexanone (9). Prepared according to method A using cyclohexanone (**4d**). HPLC 98%, R_T: 2.41 min (System A). ^{1}H NMR ($CDCl_3$) δ 7.33–7.22 (m, Ph), 2.73–2.68 (m, CH_2), 2.54–2.49 (m, CH_2), 1.88–1.82 (m, CH_2CH_2). MS (ESI+) *m*/*z* 225.1 ($[M+Na]^+$). Anal. calcd for $C_{12}H_{14}N_2O$: C, 71.26; H, 6.98; N, 13.85. Found: C, 71.11; H, 6.52; N, 13.76.

2-(Phenyl-hydrazono)-cyclopentanone (16). Prepared according to method A using cyclopentanone. HPLC 96%, R_T: 1.97 min (System A). ^{1}H NMR ($CDCl_3$) δ 7.34–7.19 (m, Ph), 2.80–2.75 (m, CH_2), 2.51–2.45 (m, CH_2), 2.19–2.06 (m, CH_2). MS (ESI+) *m*/*z* 225.1 ($[M+H]^+$). Anal. calcd for $C_{11}H_{12}N_2O{\cdot}1/2\ H_2O$: C, 66.98; H, 6.64; N, 14.20; found: C, 66.64; H, 6.78; N, 14.13.

2-(Phenyl-hydrazono)-cycloheptanone (18). Prepared according to method A using cycloheptanone. Used directly in condensation reaction without further characterization.

General methods to prepare the pyridazine analogues 10–13

Method C. exemplified by the preparation of 3-amino-2-phenyl-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile (10). To the hydrazone **6** (1.0 g, 4.9 mmol) in DMSO (3 mL) was added malononitrile (0.32 g, 4.9 mmol) and morpholine (0.43 mL, 4.9 mmol) and the reaction mixture was stirred at 80 °C for 15 min. The mixture was cooled and the product was purified by column chromatography over silica using a gradient of hexane/ethyl acetate (5:1 to 1:1), which gave 1.12 g (91%) of the pyridazine **10** as a brown solid. The HCl salt was then prepared and the compound freeze-dried;

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.69–7.25 (m, Ph), 6.14 (s, NH_2), 5.05–5.01 (m, CH), 4.27–4.25 (m, CH_2), 4.18 (s, CH_2). HPLC 95%, R_T: 3.73 min (System A). MS (ESI+) m/z 253.1 $(M+H)^+$. HRMS (EI) for $C_{14}H_{12}N_4O$: calcd 252.1008; found 252.1011.

Method D. Exemplified by the preparation of 3-amino-6,6-dimethyl-2-[2-(trifluoromethyl)phenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11ao). A solution of hydrazone **7ao** (0.97 g, 3.23 mmol), malononitrile (261 mg, 3.95 mmol) and morpholine (345 μL, 3.95 mmol) in DMSO (2 mL) was heated at 80° C for 30 min. The reaction mixture was diluted with $CHCl_3$, washed with water, dried ($MgSO_4$) and evaporated. The crude product was purified by column chromatography on silica gel with a gradient of hexane/ethyl acetate (4:1 to 1:1). The purified material was dissolved in EtOAc and HCl in EtOAc was added. The product was filtered, washed with EtOAc and dried in vacuum. Gave 0.667 g (54%) of the title compound **11ao**. The two tautomers imin/enamine are present in a 60:40 ratio. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imin) 9.43 (br s, 2H), 8.09 (d, 1H), 8.04 (m, 1H), 7.95 (m, 1H), 7.88 (d, 1H), 4.71 (s, 2H), 3.20 (q, 2H), 1.34 (d, 6H). (enamine) (54%) 7.85 (d, 1H), 7.80 (m, 1H), 7.70 (m, 1H), 7.57 (d, 1H), 6.34 (s, 2H), 4.98 (s, 1H), 4.18 (q, 2H), 1.27 (s, 6H). MS (ESI+) m/z 349.29 $(M+H)^+$. HPLC 99%, R_T: 12.46 min (System B. 30–60% acetonitrile over 10 min). HRMS for $C_{17}H_{15}F_3N_4O$: calcd 348.1198; found 348.1199; Anal calcd for $C_{17}H_{15}F_3N_4O{\cdot}Cl$ H: C, 53.06; H, 4.19; N, 14.56; found: C, 52.84; H, 4.38; N, 15.36.

3-Amino-6,6-dimethyl-2-[3-(trifluoromethyl)phenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11am). The hydrazone 7am (0.948 g, 3.16 mmol) was dissolved in DMSO (2 mL). Malononitrile (263 mg, 3.98 mmol) and morpholine (345 μL, 3.96 mmol) was added. The reaction conditions and workup procedure followed method D which gave 0.514 g (42%) of the title compound **11am**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 9.43 (br.s, 2H), 8.06 (m, 2H), 7.93 (m, 2H), 4.73 (s, 2H), 3.16 (s, 2H), 1.33 (s, 6H). (enamine) 7.74 (m, 2H), 7.68 (m, 2H), 6.38 (s, 2H), 5.00 (s, 1H), 4.23 (s, 2H), 1.26 (s, 6H). The ratio of imine/enamine is 1:0.3. MS (ESI+) m/z 349.31 $(M+H)^+$. HPLC 99%, R_T: 13.75 min (System B; 30–60% acetonitrile over 10 min). HRMS (EI) for $C_{17}H_{15}F_3N_4O$: calcd 348.1198; found 348.1197.

3-Amino-6,6-dimethyl-2-[4-(trifluoromethyl)phenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11ap). The hydrazone 7ap (0.940 g, 3.13 mmol) was dissolved in DMSO (2 mL). Malononitrile (269 mg, 4.07 mmol) and morpholine (345 μL, 3.96 mmol) was added. The reaction conditions and workup procedure followed method D which gave 0.657 g (52%) of the title compound **11ap**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imin) 9.41 (br.s, 2H), 8.10 (d, 2H), 7.87 (d, 2H), 4.74 (s, 2H), 3.16 (s, 2H), 1.35 (s, 6H). (enamine) 7.82 (d, 2H), 7.59 (d, 2H), 6.42 (br. s, 2H), 5.03 (s, 1H), 4.24 (s, 2H), 1.27 (s, 6H). The ratio of the two tautomers is 1:0.3. MS (ESI+) m/z 349.29 $(M+H)^+$. HPLC 99%, R_T: 13.84 min (System B; 30–60% acetonitrile over 10 min). HRMS (EI) for $C_{17}H_{15}F_3N_4O$: calcd 348.1198; found 348.1192.

3-Amino-6,6-dimethyl-2-[2-(hydroxymethyl)phenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11bo). The hydrazone **7bo** (0.445 g, 1.70 mmol) was dissolved in DMSO (10 mL). Malononitrile (273 mg, 4.13 mmol) and piperazinomethyl polystyrene resin (1.70 g, 1.08 mmol/g, 1.1 equiv) was added. The reaction mixture was heated at 80 °C for 12 h and then diluted with CH_2Cl_2 and the resin was filtered and washed with CH_2Cl_2. The organic phase was washed with water and extracted with 1 M HCl (aq) × 3. The aqueous phase was neutralized and the free amine was back-extracted with EtOAc, dried ($MgSO_4$) and evaporated. The pure product was dissolved in Et_2O and HCl in Et_2O was added dropwise. The product was filtered, washed with Et_2O and dried in vacuum which gave 0.319 g (54%) of the title compound **11bo**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 9.23 (br.s, 2H), 7.70 (d, 1H), 7.65 (dt, 1H), 7.56 (dt, 1H), 7.50 (dd, 1H), 4.72 (q, 2H), 4.40 (q, 2H), 3.12 (s, 2H), 1.37 (s, 3H), 1.34 (s, 3H). (enamine) 7.61 (m, 1H), 7.46 (m, 1H), 7.37 (m, 1H), 7.26 (d, 1H), 6.01 (br.s, 2H), 4.95 (s, 1H), 4.20 (s, 2H), 1.27 (s, 3H), 1.26 (s, 3H). The ratio between imine/enamine is 1:0.15. MS (ESI+) m/z 311.27 $(M+H)^+$. HPLC 90%, R_T: 12.87 min (System B; 30–60% acetonitrile over 10 min). HRMS (EI) for $C_{17}H_{18}N_4O_2$: calcd 310.1430; found 310.1438.

3-Amino-6,6-dimethyl-2-[3-(hydroxymethyl)phenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11bm). The hydrazone **7bm** (0.546 g, 2.08 mmol) was dissolved in DMSO (2 mL). Malononitrile (170 mg, 2.57 mmol) and piperidine (255 μL, 2.58 mmol) was added. The reaction conditions and workup procedure followed method D which gave 0.538 g (75%) of the title compound **11bm**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 9.24 (br.s, 2H), 7.56–7.63 (m, 3H), 7.44 (m, 1H), 4.72 (s, 2H), 4.62 (s, 2H), 3.10 (s, 2H), 1.34 (s, 6H). (enamine) 7.43 (m, 1H), 7.35 (d, 1H), 7.26 (m, 1H), 7.21 (d, 1H), 6.10 (br. s, 2H), 4.96 (s, 1H), 4.53 (s, 2H), 4.22 (s, 2H), 1.27 (s, 6H). The ratio between imine/enamine is 1:0.1. MS (ESI+) m/z 311.26 $(M+H)^+$. HPLC 94%, R_T: 5.35 min (System B; 30–60% acetonitrile over 10 min). HRMS (EI) for $C_{17}H_{18}N_4O_2$: calcd 310.1430; found 310.1440.

3-Amino-6,6-dimethyl-2-[4-(hydroxymethyl)phenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11bp). The hydrazone **7bp** (0.518 g, 1.97 mmol) was dissolved in DMSO (2 mL). Malononitrile (184 mg, 2.57 mmol) and piperidine (255 μL, 2.58 mmol) was added. The reaction conditions and workup procedure followed method D which gave 0.324 g (47%) of the title compound **11bp**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 9.24 (br.s, 2H), 7.62 (d, 2H), 7.53 (d, 2H), 4.72 (s, 2H), 4.63 (s, 2H), 3.13 (s, 2H), 1.34 (s, 6H). (enamine) 7.41 (d, 2H), 7.30 (d, 2H), 6.88 (br.s, 2H), 4.95 (s, 1H), 4.54 (s, 2H), 4.21 (s, 2H), 1.26 (s, 6H). The ratio of imine/enamine is 1:0.14. MS (ESI+) m/z 311 $(M+H)^+$. HPLC 95%, R_T: 5.30 min (System B; 30–60% acetonitrile

over 10 min). HRMS (EI) for $C_{17}H_{18}N_4O_2$: calcd 310.1430; found 310.1434.

3-Amino-6,6-dimethyl-2-[2-ethylphenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11co). The hydrazone **7co** (0.884 g, 3.40 mmol) was dissolved in DMSO (10 mL). Malononitrile (410 mg, 6.20 mmol) and piperazinomethyl polystyrene resin (3.40 g, 1.08 mmol/g, 1 equiv) was added. The reaction mixture was heated at 80 °C for 12 h and then diluted with CH_2Cl_2 and the resin was filtered and washed with CH_2Cl_2. The organic phase was washed with water and extracted with 1 M HCl (aq) × 3. The aqueous phase was neutralized and the free amine was back-extracted with EtOAc, dried ($MgSO_4$) and evaporated. The pure product was dissolved in Et_2O and HCl in Et_2O was added dropwise. The product was filtered, washed with Et_2O and dried in vacuum which gave 0.550 g (47%) of the title compound **11co**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 7.63 (m, 1H), 7.57 (d, 1H), 7.48 (m, 2H), 4.71 (s, 2H), 3.13 (s, 2H), 2.44 (q, 2H), 1.37 (s, 3H), 1.34 (s, 3H), 1.11 (t, 3H). (enamine) 7.41 (m, 2H), 7.31 (t, 1H), 7.25 (d, 1H), 6.03 (br.s, 2H), 4.94 (s, 1H), 4.20 (s, 2H), 2.44 (q, 2H), 1.27 (s, 6H), 1.11 (t, 3H). The ratio of imin/enamine is 1:0.1.MS (ESI+) m/z 309.29 $(M+H)^+$. HPLC 99%, R_T: 12.69 min (System B; 30–60% acetonitrile over 10 min). HRMS (EI) for $C_{18}H_{20}N_4O$: calcd 308.1637; found 308.1634.

3-Amino-6,6-dimethyl-2-[3-ethylphenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11cm). The hydrazone **7cm** (0.564 g, 2.20 mmol) was dissolved in DMSO (2 mL). Malononitrile (170 mg, 2.57 mmol) and piperidine (255 μL, 2.58 mmol) was added. The resulting mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with $CHCl_3$, washed with water, dried ($MgSO_4$) and evaporated. The crude product was dissolved in EtOAc/Et_2O and HCl in EtOAc was added dropwise. The product was filtered, washed with EtOAc and Et_2O and dried in vacuum which gave 0.336 g (44%) of the title compound **11cm**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.23 (t, 3H), 1.34 (s, 6H), 2.72 (q, 2H), 3.10 (s, 2H), 4.72 (s, 2H), 7.40 (m, 1H), 7.43 (m, 1H), 7.54 (m, 1H), 7.60 (m, 1H), 9.24 (br. s, 2H). (imine); ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 9.24 (br. s, 2H), 7.60 (m, 1H), 7.54 (m, 1H), 7.43 (m, 1H), 7.40 (m, 1H), 4.72 (s, 2H), 3.10 (s, 2H), 2.72 (q, 2H), 1.34 (s, 6H), 1.23 (t, 3H). (enamine) 7.28 (m, 1H), 7.19 (m, 3H), 6.08 (s, 2H), 4.96 (s, 1H), 4.22 (s, 2H), 2.69 (q, 2H), 1.26 (s, 6H), 1.21 (t, 3H). The ratio of imine/enamine is 1:0.06. MS (ESI+) m/z 309 $(M+H)^+$. HPLC 99%, R_T: 13.10 min (System B; 30–60% acetonitrile over 10 min). HRMS (EI) for $C_{18}H_{20}N_4O$: calcd 308.1637; found 308.1642.

3-Amino-6,6-dimethyl-2-[4-ethylphenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11cp). The hydrazone **7cp** (0.526 g, 2.02 mmol) was dissolved in DMSO (2 mL). Malononitrile (170 mg, 2.57 mmol) and piperidine (255 μL, 2.58 mmol) was added. The resulting mixture was heated at 80 °C for 30 min. The reaction mixture was diluted with $CHCl_3$, washed with water, dried ($MgSO_4$) and evaporated. The crude product was dissolved in EtOAc/Et_2O and HCl in EtOAc was added dropwise. The product was filtered, washed with EtOAc and Et_2O and dried in vacuum which gave 0.488 g (70%) of the title product **11cp**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 9.24 (br.s, 2H), 7.51 (m, 4H), 4.72 (s, 2H), 3.13 (s, 2H), 2.73 (q, 2H), 1.34 (s, 6H), 1.25 (t, 3H). (enamine) 7.28 (m, 4H), 6.07 (br.s, 2H), 4.95 (s, 1H), 4.21 (s, 2H), 1.26 (s, 6H), 1.20 (t, 3H). The ratio of imine/enamine is 1:0.13. MS (ESI+) m/z 309 $(M+H)^+$. HPLC 99%, R_T: 13.46 min (System B; 30–60% acetonitrile over 10 min). HRMS (EI) for $C_{18}H_{20}N_4O$: calcd, 308.1637, found 308.1628.

3-Amino-6,6-dimethyl-2-[2-nitrophenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11do). The hydrazone **7do** (0.443 g, 1.59 mmol) was dissolved in DMSO (2 mL). Malononitrile (145 mg, 2.19 mmol) and morpholine (170 μL, 1.95 mmol) was added. The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with EtOAc and washed with water, then extracted with 1 M HCl (aq) × 3. The aqueous phase was neutralized and the free amine was back-extracted to EtOAc. The organic phase was dried ($MgSO_4$) and evaporated. The pure amine was redissolved in EtOAc/Et_2O and HCl (Et_2O) was added. The product was filtered, washed with Et_2O and dried in vacuum which gave 0.348 g (61%) of the title compound **11do**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 9.89 (br.s, 2H), 8.48 (m, 1H), 8.14 (m, 1H), 8.02 (m, 1H), 7.69 (m, 1H), 4.72 (d, 2H), 3.19 (d, 2H), 1.34 (s, 6H). (enamine) 8.14 (m, 1H), 7.96 (m, 1H), 7.84 (m, 1H), 7.68 (m, 1H), 6.50 (br.s, 2H), 5.04 (s, 1H), 4.17 (s, 2H), 1.27 (s, 6H). The ratio of imine/enamine is 1:1. MS (ESI+) m/z 326.23 $(M+H)^+$. HPLC 97%, R_T: 9.58 min (System B; 30–60% acetonitrile over 10 min). HRMS for $C_{16}H_{15}N_5O_3$: calcd 325.1175; found 325.1173.

3-Amino-6,6-dimethyl-2-[4-nitrophenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile (11dp). The hydrazone 7dp (0.657 g, 2.37 mmol) was dissolved in DMSO (2 mL). Malononitrile (170 mg, 2.57 mmol) and piperidine (255 μL, 2.58 mmol) was added. The resulting mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with $CHCl_3$ and the product spontaneously crystallized. The product was filtered, washed with MeOH and dried in vacuum which gave 0.410 g (53%) of the title product **11dp**; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (d, 2H), 7.64 (d, 2H), 6.54 (s, 2H), 5.09 (s, 1H), 4.27 (s, 2H), 1.28 (s, 6H). The product was isolated as free amine and the only tautomer present was the amine. MS (ESI+) m/z 326 $(M+H)^+$. HPLC 99%, R_T: 10.04 min (System B; 30–60% acetonitrile over 10 min). HRMS for $C_{16}H_{15}N_5O_3$: calcd 325.1175; found 325.1175.

3-Amino-6,6-dimethyl-2-[3-carboxymethylphenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11em). The hydrazone **7em** (0.461 g, 1.59 mmol) was dissolved in DMSO (2 mL). Malononitrile (126 mg, 1.91 mmol) and morpholine (170 μL, 1.95 mmol) was added. The reaction mixture was stirred

at room temperature for 1 h. The reaction mixture was diluted with EtOAc and washed with water, then extracted with 1 M HCl (aq) × 3. The aqueous phase was neutralized and the free amine was back-extracted to EtOAc. The organic phase was dried ($MgSO_4$) and evaporated. The pure amine was redissolved in EtOAc/Et_2O and HCl (Et_2O) was added. The product was filtered, washed with Et_2O and dried in vacuum which gave 0.281 g (47%) of the title compound 11em. ^1H NMR (400 MHz, DMSO-d_6) δ (imine) 9.39 (s, 2H), 8.20 (m, 2H), 7.86.89 (m, 2H), 4.74 (s, 2H), 3.91 (s, 3H), 3.16 (s, 2H), 1.34 (s, 6H). (enamine) 7.95 (m, 1H), 7.86 (m, 1H), 7.64 (m, 2H), 6.33 (br. s, 2H), 5.00 (s, 1H), 4.24 (s, 2H), 3.87 (s, 3H), 1.27 (s, 6H). The ratio of imine/enamine is 1:0.2. MS (ESI+) m/z 339.23 $(M+H)^+$. HPLC 96%, R_T: 8.36 min (System B; 30–60% acetonitrile over 10 min). HRMS for $C_{18}H_{18}N_4O_3$: calcd 338.1379; found 338.1384.

3-Amino-6,6-dimethyl-2-[4-carboxymethylphenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11ep). The hydrazone **7ep** (0.617 g, 2.12 mmol) was dissolved in DMSO (2 mL). Malononitrile (170 mg, 2.57 mmol) and piperidine (255 μL, 2.58 mmol) was added. The reaction conditions and workup procedure followed method D which gave 0.458 g (58%) of the title compound **11ep**. ^1H NMR (400 MHz, DMSO-d_6) δ (imine) 9.40 (br.s, 2H), 8.25 (d, 2H), 7.73 (d, 2H), 4.74 (s, 2H), 3.93 (s, 3H), 3.15 (s, 2H), 1.35 (s, 6H). (enamine) 8.01 (d, 2H), 7.52 (d, 2H), 5.03 (s, 1H), 4.25 (s, 2H), 3.88 (s, 3H), 1.27 (s, 6H). The ratio of imine/enamine is 1:0.3. MS (ESI+) m/z 339.25 $(M+H)^+$. HPLC 99%, R_T: 10.24 min (System B; 30–60% acetonitrile over 10 min). HRMS for $C_{18}H_{18}N_4O_3$: calcd 338.1379; found 338.1390.

3-Amino-6,6-dimethyl-2-[3-acetamidophenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11fm). The hydrazone **7fm** (0.481 g, 1.66 mmol) was dissolved in DMSO (2 mL). Malononitrile (133 mg, 2.01 mmol) and morpholine (170 μL, 1.95 mmol) was added. The reaction mixture was stirred at room temperature for 1 h and then diluted with EtOAc, washed with water, and extracted with 1 M HCl (aq) × 3. The aqueous phase was neutralized and the free amine was back-extracted to EtOAc. The organic phase was dried ($MgSO_4$) and evaporated. The pure amine was redissolved in EtOAc/Et_2O and HCl (Et_2O) was added. The product was filtered, washed with Et_2O and dried in vacuum, which gave 0.337 g (54%) of the title compound **11fm**. ^1H NMR (400 MHz, DMSO-d_6) δ 1.34 (s, 6H), 2.10 (s, 3H), 3.11 (s, 2H), 4.73 (s, 2H), 7.21 (d, 1H), 7.58 (t, 1H), 7.68 (d, 1H), 8.12 (s, 1H), 8.98 (br.s, 2H), 10.69 (s, 1H). (imine); ^1H NMR (400 MHz, DMSO-d_6) δ (imine) 10.69 (s, 1H), 8.98 (br.s, 2H), 8.12 (s, 1H), 7.68 (d, 1H), 7.58 (t, 1H), 7.21 (d, 1H), 4.73 (s, 2H), 3.11 (s, 2H), 2.10 (s, 3H), 1.34 (s, 6H). (enamine) 10.3 (s, 1H), 8.12 (s, 1H), 7.68 (d, 1H), 7.37 (t, 1H), 7.01 (d, 1H), 6.17 (br.s, 2H), 4.96 (s, 1H), 4.22 (s, 2H), 2.06 (s, 3H), 1.27 (s, 6H). The ratio of imine/enamine is 1:0.15. MS (ESI+) m/z 338.26 $(M+H)^+$. HPLC 97%, R_T: 6.13 min (System B; 30–60% acetonitrile over 10 min). HRMS for $C_{18}H_{19}N_5O_2$: calcd 337.1539; found 337.1543.

3-Amino-6,6-dimethyl-2-[4-acetamidophenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11fp). The hydrazone **7fp** (0.520 g, 1.79 mmol) was dissolved in DMSO (2 mL). Malononitrile (170 mg, 2.57 mmol) and piperidine (255 μL, 2.58 mmol) was added. The resulting mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with $CHCl_3$, washed with water, dried ($MgSO_4$) and evaporated. The crude product was dissolved in EtOAc/Et_2O and HCl in EtOAc was added dropwise. The product was filtered, washed with EtOAc and Et_2O and dried in vacuum, which gave 0.510 g (76%) of the title product **11fp**. ^1H NMR (400 MHz, DMSO-d_6) δ (imine) 10.77 (s, 1H), 9.1 (br. s, 2H), 7.93 (d, 2H), 7.49 (d, 2H), 4.72 (s, 2H), 3.11 (s, 2H), 2.91 (s, 3H), 1.33 (s, 6H). (enamine) 10.38 (s, 1H), 7.70 (d, 2H), 7.26 (d, 2H), 4.93 (s, 1H), 4.21 (s, 2H), 2.07 (s, 3H), 1.26 (s, 6H). The ratio of enamine/imin is 1: 0.1. MS (ESI+) m/z 338.30 $(M+H)^+$. HPLC 99%, R_T: 5.88 min (System B; 30–60% acetonitrile over 10 min). HRMS for $C_{18}H_{19}N_5O_2$: calcd 337.1539; found 337.1535.

3-Amino-6,6-dimethyl-2-[2-phenoxyphenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11go). The hydrazone **7go** (0.197 g, 0.61 mmol) was dissolved in DMSO (0.5 mL). Malononitrile (40 mg, 0.61 mmol) and morpholine (50 μL, 0.61 mmol) was added . The reaction conditions and workup procedure followed method C, which gave 0.141 g (62%) of the pyridazine **11go**; ^1H NMR (400 MHz, DMSO-d_6) δ 1.38 (s, 6H), 3.18 (s, 2H), 4.76 (s, 2H), 7.11.7 (m, 9H) (imine); ^1H NMR (400 MHz, DMSO-d_6) δ (imine) 7.11–7.7 (m, 9H), 4.76 (s, 2H), 3.18 (s, 2H), 1.38 (s, 6H). (enamine) 7.11–7.7 (m, 9H), 6.33 (br.s, 2H), 4.95 (s, 1H), 4.22 (s, 2H), 1.38 (s, 6H). The ration of imine/enamine is 1:0.3. HPLC 98%, R_T: 3.41 min (System A). MS (ESI+) m/z 373.17 $(M+H)^+$. HRMS for $C_{22}H_{20}N_4O_2$: calcd 372.1586; found 372.1579.

3-Amino-6,6-dimethyl-2-[3-phenoxyphenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11gm). The hydrazone **7gm** (0.579 g, 1.78 mmol) was dissolved in DMSO (2 mL). Malononitrile (132 mg, 2.00 mmol) and morpholine (170 μL, 1.95 mmol) was added and the mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with EtOAc and washed with water, then extracted with 1 M HCl (aq) × 3. The aqueous phase was neutralized and the free amine was back-extracted to EtOAc. The organic phase was dried ($MgSO_4$) and evaporated. The pure amine was redissolved in EtOAc/Et_2O and HCl (Et_2O) was added. The product was filtered, washed with Et_2O and dried in vacuum, which gave 0.367 g (50%) of the title compound **11gm**. ^1H NMR (400 MHz, DMSO-d_6) δ 1.32 (s, 6H), 3.11 (s, 2H), 4.71 (s, 2H), 7.19 (m, 2H), 7.22 (m, 2H), 7.34 (m, 2H), 7.45 (m, 2H), 7.70 (t, 1H), 9.40 (br.s, 2H). (imine); ^1H NMR (400 MHz, DMSO-d_6) δ (imine) 9.40 (br.s, 2H), 7.70 (t, 1H), 7.45 (m, 2H), 7.34 (m, 2H), 7.22 (m, 2H), 7.19 (m, 2H), 4.71 (s, 2H), 3.11 (s, 2H), 1.32 (s, 6H). (enamine) 7.18–7.47 (m, 5H), 7.11 (m, 2H), 7.02 (m, 1H), 6.97 (m, 1H), 6.28 (br.s, 2H), 4.96 (s, 1H), 4.21 (s, 2H), 1.25 (s, 6H). The ration of imine/enamine is 1:0.12. MS (ESI+) m/z 373.23

$(M+H)^+$. HPLC 94%, R_T: 4.99 min (System B; 60–90% acetonitrile over 10 min). HRMS for $C_{22}H_{20}N_4O_2$: calcd 372.1586; found 372.1592.

3-Amino-6,6-dimethyl-2-[4-phenoxyphenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11gp). The hydrazone **7gp** (0.488 g, 1.50 mmol) was dissolved in DMSO (2 mL). Malononitrile (140 mg, 2.00 mmol) and morpholine (170 μL, 1.95 mmol) was added. The reaction mixture was diluted with EtOAc and washed with water, then extracted with 1 M HCl (aq) × 3. The aqueous phase was neutralized and the free amine was back-extracted to EtOAc. The organic phase was dried ($MgSO_4$) and evaporated. The pure amine was redissolved in EtOAc/Et_2O and HCl (Et_2O) was added. The product was filtered, washed with Et_2O and dried in vacuum, which gave 0.391 g (64%) of the title compound **11gp**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 9.29 (br.s, 2H), 7.60 (m, 2H), 7.47 (m, 2H), 7.26 (d, 2H), 7.24 (m, 1H), 7.16 (dd, 2H), 4.73 (s, 2H), 3.13 (s, 2H), 1.34 (s, 6H). (enamine) 7.40 (m, 3H), 7.36 (m, 2H), 7.08 (m, 4H), 6.26 (br.s, 2H), 4.95 (s, 1H), 4.22 (s, 2H), 1.26 (s, 6H). The ratio of imine/enamine is 1:0.1. MS (ESI+) m/z 373.23 $(M+H)^+$. HPLC 95%, R_T: 5.02 min (System B; 60–90% acetonitrile over 10 min). HRMS for $C_{22}H_{20}N_4O_2$: calcd 372.1586; found 372.1588.

3-Amino-6,6-dimethyl-2-[2-ethoxyphenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11ho). The hydrazone **7ho** (0.491 g, 1.78 mmol) was dissolved in DMSO (10 mL) and malononitrile (289 mg, 4.37 mmol) and piperazinomethyl polystyrene resin (1.67 g, 1.08 mmol/g, 1 equiv) was added. The reaction mixture was heated at 80 °C for 12 h. The reaction mixture was diluted with CH_2Cl_2 and the resin was filtered and washed with CH_2Cl_2. The organic phase was washed with water and extracted with 1 M HCl (aq) × 3. The aqueous phase was neutralized and the free amine was back-extracted with EtOAc, dried ($MgSO_4$) and evaporated. The pure product was dissolved in Et_2O and HCl in Et_2O was added dropwise. The product was filtered, washed with Et_2O and dried in vacuum, which gave 0.349 g (54%) of the title compound **11ho**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 9.14 (br.s, 2H), 7.63 (dt, 1H), 7.50 (dd, 1H), 7.36 (d, 1H), 7.19 (dt, 1H), 4.73 (q, 2H), 3.16 (q, 2H), 1.35 (s, 3H), 1.33 (s, 3H), 1.24 (t, 3H). (enamine) 7.41 (t, 1H), 7.27 (d, 1H), 7.18 (m, 1H), 7.01 (m, 1H), 4.91 (s, 1H), 4.10 (s, 2H), 4.17 (q, 2H), 1.29 (s, 6H), 1.24 (t, 3H). The ratio of imine/enamine is 1:0.1. MS (ESI+) m/z 325.29 $(M+H)^+$. HPLC 98%, R_T: 12.23 min (System B; 30–60% acetonitrile over 10 min). HRMS for $C_{18}H_{20}N_4O_2$: calcd 324.1586; found 324.1586.

3-Amino-6,6-dimethyl-2-[4-ethoxyphenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11hp). The hydrazone **7hp** (0.508 g, 1.83 mmol) was dissolved in DMSO (10 mL) and malononitrile (290 mg, 4.38 mmol) and piperazinomethyl polystyrene resin (2.16 g, 1.08 mmol/g, 1.3 equiv) was added. The reaction mixture was heated at 80 °C for 12 h. The reaction mixture was diluted with CH_2Cl_2 and the resin was filtered and washed with CH_2Cl_2. The organic phase was washed with water and extracted with 1 M HCl (aq) × 3. The aqueous phase was neutralized and the free amine was back-extracted with EtOAc, dried ($MgSO_4$) and evaporated. The pure product was dissolved in Et_2O and HCl in Et_2O was added dropwise. The product was filtered, washed with Et_2O and dried in vacuum, which gave 0.393 g (59%) of the title compound **11hp**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 9.17 (br.s, 2H), 7.50 (d, 2H), 7.19 (d, 2H), 4.72 (s, 2H), 4.13 (q, 2H), 3.12 (s, 2H), 1.37 (t, 3H), 1.34 (s, 6H). (enamine) 7.25 (d, 2H), 6.99 (d, 2H), 4.93 (s, 1H), 4.20 (s, 2H), 4.06 (q, 2H), 1.37 (t, 3H), 1.26 (s, 6H). The ratio of imine/enamine is 1:0.1; MS (ESI+) m/z 325.27 $(M+H)^+$; 98%, R_T: 12.87 min (System B; 30–60% acetonitrile over 10 min); HRMS for $C_{18}H_{20}N_4O_2$: calcd 324.1586; found 324.1577.

3-Amino-6,6-dimethyl-2-[2-methoxyphenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11io). The hydrazone **7io** (0.36 g, 1.37 mmol) was dissolved in DMSO (0.5 mL). Malononitrile (91 mg, 1.37 mmol) and morpholine (120 μL, 1.37 mmol) was added. The reaction conditions and workup procedure followed method C, which gave 0.384 g (90%) of the pyridazine **11io** as an orange solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 9.51 (br.s, 2H), 7.68–7.65 (m, 1H), 7.50–7.48 (m, 1H), 7.38–7.36 (m, 1H), 7.23–7.20 (m, 1H), 4.75 (d, J = 15.0 Hz, 1H), 4.70 (d, J = 15.0 Hz, 1H), 3.84 (s, 3H), 3.18 (d, J = 15 Hz, 1H), 3.14 (d, J = 15 Hz, 1H), 1.35 (s, 3H), 1.33 (s, 3H). (enamine) 7.46.43 (m, 1H), 7.30.28 (m, 1H), 7.18.16 (m, 1H), 7.04.01 (m, 1H), 4.91 (s, 2H), 4.18 (s, 2H), 3.80 (s, 3H), 1.26 (s, 6H). The ratio of imine/enamine is 7:3. HPLC 99%, R_T: 3.01 min (System A). MS (ESI+) m/z 311.18 $(M+H)^+$. HRMS for $C_{18}H_{20}N_4O_2$: calcd 310.1430; found 310.1421.

3-Amino-6,6-dimethyl-2-[3-methoxyphenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11im). The hydrazone **7im** (0.47 g, 1.80 mmol) was dissolved in DMSO (0.5 mL). Malononitrile (119 mg, 1.80 mmol) and morpholine (160 μL, 1.80 mmol) was added . The reaction conditions and workup procedure followed method C, which gave 0.481 g (86%) of the pyridazine **11im** as an orange solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 8.16 (tr, J = 8.16 Hz, 1H), 7.33 (dd, J = 8.48, 2.51 Hz, 1H), 7.28–7.26 (m, 1H), 7.22–7.20 (m, 1H), 4.81 (s, 2H), 3.90 (s, 3H), 3.22 (s, 2H), 1.42 (s, 6H). (enamine) 7.46 (tr, J = 8.17 Hz, 1H), 7.07–7.05 (m, 1H), 7.01–7.99 (m 2H), 6.21 (s, 1H, N–H), 5.04 (s, 1), 4.30 (s, 2), 3.85 (s, 3H), 1.34 (s, 6H). The ratio of imine/enamine is 8:2. HPLC 99%. MS (ESI+) m/z 311.18 $(M+H)^+$. HRMS for $C_{17}H_{18}N_4O_2$: calcd 310.1430; found 310.1424.

3-Amino-6,6-dimethyl-2-[4-methoxyphenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11ip). The hydrazone (0.832 g, 3.17 mmol) was dissolved in DMSO (2 mL). Malononitrile (265 mg, 4.01 mmol) and morpholine (345 μL, 3.96 mmol) was added. The reaction conditions and workup procedure followed method D, which gave 0.651 g (59%) of the title compound **11ip**. ^{1}H NMR (400 MHz, DMSO-d_6) δ

(imin) 9.20 (br.s, 2H), 7.51 (s, 2H), 7.22 (d, 2H), 4.72 (s, 2H), 3.86 (s, 2H), 3.12 (s, 2H), 1.35 (s, 6H). (enamine) 7.27 (d, 2H), 7.01 (d, 2H), 6.04 (br.s, 2H), 4.90 (s, 1H), 4.20 (s, 2H), 3.79 (s, 2H), 1.26 (s, 6H). The ratio of imin/enamine is 1:0.1. MS (ESI+) m/z 311.31 $(M+H)^+$. HPLC 98%, R_T: 9.74 min (System B; 30–60% acetonitrile over 10 min). HRMS for $C_{17}H_{18}N_4O_2$: calcd 310.1430; found 310.1405.

3-Amino-6,6-dimethyl-2-[4-(2-hydroxyethyl)phenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11jp). The hydrazone **7jp** (0.832 g, 3.01 mmol) was dissolved in DMSO (2 mL). Malononitrile (266 mg, 4.03 mmol) and morpholine (345 μL, 3.96 mmol) was added. The reaction conditions and workup procedure followed method D, which gave 0.690 g (64%) of the title compound **11jp**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 9.25 (br.s, 2H), 7.51 (m, 4H), 4.71 (s, 2H), 3.68 (t, 2H), 3.12 (s, 2H), 2.84 (t, 2H), 1.34 (s, 6H). (enamine) 7.27 (m, 4H), 6.05 (br.s, 2H), 4.95 (s, 1H), 4.21 (s, 2H), 3.62 (t, 2H), 2.76 (t, 2H), 1.26 (s, 6H). The ratio of imin/enamine is 1:0.15. MS (ESI+) m/z 325.33 $(M+H)^+$. HPLC 97%, R_T: 5.88 min (System B; 30–60% acetonitrile over 10 min). HRMS for $C_{18}H_{20}N_4O_2$: calcd 324.1586; found 324.1581.

3-Amino-6,6-dimethyl-2-[2-fluorophenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11ko). The hydrazone **7ko** (0.778 g, 3.16 mmol) was dissolved in DMSO (2 mL). Malononitrile (268 mg, 4.06 mmol) and morpholine (345 μL, 3.96 mmol) was added. The reaction conditions and workup procedure followed method D, which gave 0.625 g (60%) of the title compound **11ko**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 9.59 (br.s, 2H), 7.48–7.77 (m, 4H), 4.73 (d, 2H), 3.14 (s, 2H), 1.34 (d, 6H). (enamine) 7.26–7.75 (m, 4H), 6.57 (br.s, 2H), 4.99 (s, 1H), 4.21 (s, 2H), 1.27 (s, 6H). Ratio of imin/enamine is 1:0.5. MS (ESI+) m/z 299.30 $(M+H)^+$. HPLC 99%, R_T: 8 45 min (System B; 30–60% acetonitrile over 10 min). HRMS for $C_{16}H_{15}FN_4O$: calcd 298.1230; found 298.1235.

3-Amino-6,6-dimethyl-2-[3-fluorophenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile (11km). The hydrazone **7km** (0.57 g, 2.28 mmol) was dissolved in DMSO (1 mL). Malononitrile (151 mg, 2.28 mmol) and morpholine (200 μL, 2.28 mmol) was added. The reaction conditions and workup procedure followed method C. The product was purified by preparative HPLC and then freeze-dried to give the TFA salt of the pyridazine **11km** as a yellow solid (0.273 g, 40%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 7.79–7.74 (m, 1H), 7.60–7.45 (m, 2H), 7.29–7.21 (m, 1H), 4.23 (s, 2H), 3.16 (s, 2H), 1.34 (s, 3H), 1.27 (s, 3H). (enamine) 7.79–7.74 (m, 1H), 7.60–7.45 (m, 2H), 7.29–7.21 (m, 1H), 6.31 (br.s, 1H, N–H), 4.99 (s, 1H), 4.77 (s, 2H), 1.34 (s, 3H), 1.27 (s, 3H). Ratio of imin/enamine is 45:55. HPLC 99%, R_T: 1.65 min (System A). MS (ESI+) m/z 299.1 $(M+H)^+$ HRMS for $C_{16}H_{15}FN_4O$: calcd 298.1230; found 298.1235.

3-Amino-6,6-dimethyl-2-[4-fluorophenyl]-2,8-dihydro-6H-pyrano[3,4-c]pyridazine-4-carbonitrile (11kp). The hydrazone **7kp** (0.19 g, 0.76 mmol) was dissolved in DMSO (1 mL). Malononitrile (50 mg, 0.76 mmol) and morpholine (70 μL, 0.76 mmol) was added. The reaction conditions and workup procedure followed method C which gave 0.18 g (79%) of **11kp** as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 7.68–7.65 (m, 2H), 7.56–7.53 (m, 2H), 4.96 (s, 1H), 4.21 (s, 2H), 1.34 (s, 6H). (enamine) 7.43–7.40 (m, 2H), 7.31–7.28 (m, 2H), 6.20 (br.s, 2H, N–H), 4.73 (s, 2H), 3.15 (s, 2H), 1.26 (s, 6H). Ratio of imin/enamine 80:20. HPLC 96%, R_T: 1.61 min (System A). MS (ESI+) m/z 299.1 $(M+H)^+$. HRMS for $C_{16}H_{15}FN_4O$: calcd 298.1230; found 298.1236.

3-Amino-6,6-dimethyl-2-[2-butylphenyl]-2,8-dihydro-6H-pyrano[3,4-c]pyridazine-4-carbonitrile hydrochloride (11lo). The hydrazone **7lo** (0.477 g, 1.65 mmol) was dissolved in DMSO (2 mL). Malononitrile (131 mg, 1.98 mmol) and morpholine (170 μL, 1.95 mmol) was added. The reaction mixture was stirred at room temperature for 1 h, and was then diluted with EtOAc, washed with water, and extracted with 1 M HCl (aq) × 3. The aqueous phase was neutralized and the free amine was back-extracted to EtOAc. The organic phase was dried ($MgSO_4$) and evaporated. The pure amine was redissolved in EtOAc/Et_2O and HCl (Et_2O) was added. The product was filtered, washed with Et_2O and dried in vacuum, which gave 0.339 g (55%) of the title compound **11lo**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 9.03 (br.s, 2H), 7.61 (m, 1H), 7.56 (m, 2H), 7.49 (m, 2H), 4.71 (s, 2H), 3.13 (s, 2H), 2.39 (m, 2H), 1.47 (m, 2H), 1.36 (s, 3H), 1.34 (s, 3H), 1.26 (m, 2H), 0.83 (t, 3H). (enamine) 7.39 (m, 2H), 7.31 (m, 1H), 7.25 (m, 1H), 6.02 (br.s, 2H), 4.94 (s, 1H), 4.20 (q, 2H), 2.43 (m, 2H), 1.49 (m, 2H), 1.26 (s, 6H), 1.22 (m, 2H), 0.86 (t, 3H). The ratio of imine/enamine is 1:0.2. MS (ESI+) m/z 337.29 $(M+H)^+$. HPLC 94%, R_T: 5.39 min (System B; 60–90% acetonitrile over 10 min). HRMS for $C_{20}H_{24}N_4O$: calcd 338.1379; found 338,1384.

3-Amino-6,6-dimethyl-2-[4-butylphenyl]-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carbonitrile hydrochloride (11lp). The hydrazone **7lp** (0.403 g, 1.39 mmol) was dissolved in DMSO (2 mL). Malononitrile (140 mg, 2.00 mmol) and morpholine (170 μL, 1.95 mmol) was added. The reaction mixture was stirred at room temperature for 1 h, and was then diluted with EtOAc, washed with water, and extracted with 1 M HCl (aq) × 3. The aqueous phase was neutralized and the free amine was back-extracted to EtOAc. The organic phase was dried ($MgSO_4$) and evaporated. The pure amine was redissolved in EtOAc/Et_2O and HCl (Et_2O) was added. The product was filtered, washed with Et_2O and dried in vacuum, which gave 0.301 g (58%) of the title compound **11lp**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 9.25 (br.s, 2H), 7.49 (s, 4H), 4.72 (s, 2H), 3.13 (s, 2H), 2.96 (t, 2H), 1.62 (m, 2H), 1.36 (m, 2H), 1.34 (s, 6H), 0.92 (t, 3H). (enamine) 7.26 (m, 4H), 6.07 (br.s, 2H), 4.95 (s, 1H), 4.21 (s, 2H), 2.61 (m, 2H), 1.60 (m, 2H), 1.36 (m, 2H), 1.26 (s, 6H), 0.92 (t, $J=3$ Hz, 3H). The ratio between imin/enamine is 1:0.15. MS (ESI+) m/z 337.30 $(M+H)^+$. HPLC 95%, R_T: 5.43 min (System B; 60–90% acetonitrile over 10 min). HRMS for $C_{20}H_{24}N_4O$: calcd 336.1950; found 336.1940.

3-Amino-6,6-dimethyl-2-[3-(isopropyl)phenyl]-2,8-dihydro-6H-pyrano[3,4-c]pyridazine-4-carbonitrile hydrochloride (11mm). The hydrazone **7mm** (0.39 g, 1.40 mmol) was dissolved in DMSO (1 mL). Malononitrile (94 mg, 1.40 mmol) and morpholine (120 μL, 1.40 mmol) was added. The reaction conditions and workup procedure followed method C which gave 0.226 g (50%) of **11mm** as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 7.61–7.58 (m, 1H), 7.44 (s, 1H), 7.40–7.38 (m, 1H), 7.21–7.20 (m, 1H), 4.73 (s, 2H), 4.22 (s, 2H), 1.24 (tr, J = 7.22 Hz, 1H), 1.22 (d, J = 7.22 Hz, 6H). (enamine) 7.61–7.58 (m, 1H), 7.44 (s, 1H), 7.32–7.30 (m, 1H), 7.17–7.14 (m, 1H), 6.06 (br.s, 1H, N–H), 4.96 (s, 1H), 4.22 (s, 2H), 1.25 (tr, J = 7.22 Hz, 1H), 1.22 (d, J = 7.22 Hz, 6H). The ratio of imin/enamine is 1:1. HPLC 97%, R_T: 3.39 min (System A). MS (ESI+) m/z 323.21 $(M+H)^+$. HRMS for $C_{19}H_{22}N_4O$: calcd 322.1794; found 322.1791.

3-Amino-6,6-dimethyl-2-[4-(isopropyl)phenyl]-2,8-dihydro-6H-pyrano[3,4-c]pyridazine-4-carbonitrile hydrochloride (11mp). The hydrazone **7mp** (0.192 g, 0.71 mmol) was dissolved in DMSO (2 mL). Malononitrile (47 mg, 0.71 mmol) and morpholine (61 μL, 0.70 mmol) was added. The reaction conditions and workup procedure followed method D, which gave 0.169 g (67%) of the title compound **11mp**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 9.24 (br.s, 2H), 7.55 (d, 2H), 7.49 (d, 2H), 4.71 (s, 2H), 3.13 (s, 2H), 3.02 (sept, 1H), 1.34 (s, 6H), 1.26 (d, 6H). (enamine) 7.34 (d, 2H), 7.26 (d, 2H), 6.07 (br. s, 2H), 4.95 (s, 1H), 4.21 (s, 2H), 2.94 (sept, 1H), 1.26 (s, 6H), 1.22 (d, 6H). The ratio of imine/enamine is 1:0.15. MS (ESI+) m/z 323.35 $(M+H)^+$. HPLC 99%, R_T: 15.33 min (System B; 30–60% acetonitrile over 10 min). HRMS for $C_{19}H_{22}N_4O$: calcd 322.1794; found 322.1788.

3-Amino-6,6-dimethyl-2-[2-(4-morpholinyl)phenyl]-2,8-dihydro-6H-pyrano[3,4-c]pyridazine-4-carbonitrile hydrochloride (11no). The hydrazone **7no** (0.428 g, 1.35 mmol) was dissolved in DMSO (10 mL). Malononitrile (187 mg, 2.83 mmol) and piperazinomethyl polystyrene resin (1.30 g, 1.08 mmol/g, 1 equiv) was added. The reaction mixture was heated at 80 °C for 8 h, then diluted with CH_2Cl_2 and the solid phase reagent was filtered and washed with CH_2Cl_2. The organic phase was washed with water, dried ($MgSO_4$) and evaporated. The crude product was purified by column chromatography on silica gel with petroleum ether/EtOAc 4:1. The purified material was dissolved in EtOAc/Et_2O and HCl in Et_2O was added dropwise. The product was filtered, washed with Et_2O and dried in vacuum, which gave 0.302 g (51%) of the title compound **11no**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (imine) 8.92 (br.s, 2H), 7.65 (dt, 1H), 7.49 (dd, 1H), 7.44 (dd, 1H), 7.37 (dt, 1H), 4.75 (q, 2H), 3.51 (m, 4H), 3.14 (q, 2H), 2.77 (m, 4H), 1.34 (s, 6H). The enamine is present in approximately 5%. MS (ESI+) m/z 366.28 $(M+H)^+$. HPLC 98%, R_T: 10.58 min (System B; 30–60% acetonitrile over 10 min). HRMS for $C_{20}H_{23}N_5O_2$: calcd 365.1852; found 365.1853.

3-Amino-2-phenyl-2,8-dihydro-6*H*-thiopyrano[3,4-*c*]pyridazine-4-carbonitrile (12). Prepared according to method C from the corresponding crude hydrazone **8** which was itself prepared according to method A. Yield: (21%). HPLC 99%, R_T: 3.61 min (System B; 10–95% acetonitrile over 2 min). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.53–7.35 (m, 5H), 5.50 (t, J = 5.0 Hz, 1H), 4.37 (br. s, 2H), 3.42 (s, 1H), 3.35 (d, J = 5.0 Hz, 2H). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 149.68, 145.04, 139.36, 130.09, 129.15, 126.66, 121.74, 117.52, 106.60, 99.89, 31.08, 26.49. MS (ES) m/z 269 (MH^+). Anal. calcd for $C_{14}H_{12}N_4S_1$: C, 62.66; H, 4.51; N, 20.88; S, 11.95; found C, 62.35; H, 4.63; N, 20.76; S, 11.93.

3-Amino-2-(4-methoxyphenyl)-2,8-dihydro-6*H*-thiopyrano[3,4-*c*]pyridazine-4-carbonitrile (12ip). Prepared according to method C from the corresponding crude hydrazone which was itself prepared according to method A. Yield: (58%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.28 (d, J = 8.79 Hz, 2H), 7.02 (d, J = 8.79 Hz, 2H), 5.99 (s, 2H), 5.23 (t, J = 5.02 Hz, 1H), 3.79 (s, 3H), 3.40 (s, 2H), 3.33 (d, J = 5.02 Hz, 2H). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 160.0, 150.09, 144.84, 131.95, 128.32, 121.84, 117.62, 115.24, 106.20, 77.20, 55.65, 31.13, 26.52. MS (ES) m/z 299 (MH^+). HPLC 99%, R_T: 8.32 min (System B; 10–95% acetonitrile over 2 min). HRMS (ESI+) for $C_{15}H_{14}N_4OS$: calcd, 298.0888; found, 298.0892.

3-Amino-2-(2-fluorophenyl)-2,8-dihydro-6*H*-thiopyrano[3,4-*c*]pyridazine-4-carbonitrile (12ko). Prepared according to method C from the corresponding crude hydrazone which was itself prepared according to method A. Yield: (68%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.54–7.58 (m, 2H), 7.39 (t, J = 8.95 Hz, 1H), 7.31 (t, J = 7.69 Hz, 1H), 6.31 (s, 2H), 5.30 (t, J = 5.02 Hz, 1H), 3.41 (s, 2H), 3.34 (d, J = 5.02 Hz, 2H). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 149.98, 145.46, 131.61, 129.83, 125.50, 121.52, 117.66, 117.37, 107.50, 98.96, 77.20, 66.11, 31.00, 26.45. MS (ES) m/z 287 (MH^+). HPLC 99%, R_T: 3.55 min (System B; 10–95% acetonitrile over 2 min). HRMS (ESI+) for $C_{14}H_{11}FN_4S$: calcd 286.0688; found 286.0686.

3-Amino-2-(4-flourophenyl)-2,8-dihydro-6*H*-thiopyrano[3,4-*c*]pyridazine-4-carbonitrile (12kp). Prepared according to method C from the corresponding crude hydrazone which was itself prepared according to method A. Yield: (57%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.42 (m, 2H), 7.30 (m, 2H), 6.16 (s, 2H), 5.26 (t, J = 5.02 Hz, 1H), 3.41 (s, 2H), 3.33 (d, J = 5.02 Hz, 2H). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 149.68, 145.24, 128.89, 128.76, 121.60, 117.25, 116.92, 107.08, 99.90, 77.21, 31.03, 26.48. MS (ES) m/z 287 (MH^+). HPLC 99%, R_T: 1.87 min (System B; 20–80% acetonitrile over 3 min). HRMS (ESI+) for $C_{14}H_{11}FN_4S$: calcd 286.0688; found 286.0692.

3-Amino-2-(2-isopropylphenyl)-2,8-dihydro-6*H*-thiopyrano[3,4-*c*]pyridazine-4-carbonitrile (12mo). Prepared according to method C from the corresponding crude hydrazone which was itself prepared according to method A. Yield: (64%). Predominantly in the imine form. ^{1}H NMR (270 MHz, methanol-d_4) δ 7.70–7.68 (m, J = 4.49 Hz, 2H), 7.52–7.41 (m, 2H), 3.90 (s, 2H), 3.53 (t, J = 6.20 Hz, 2H), 3.12 (t, J = 5.67 Hz, 2H), 2.66 (m, 1H), 1.28 (d, J = 6.86 Hz, 3H), 1.17 (d, J = 6.86 Hz, 3H).

HPLC 90%, R_T: 2.45 min (System A). MS (ES) m/z 311.39 $(M+H)^+$. HRMS (ESI+) for $C_{17}H_{18}N_4S$: calcd 310.1252; found 310.1297.

3-Amino-2-(4-isopropylphenyl)-2,8-dihydro-6*H*-thiopyrano [3,4-*c*]pyridazine-4-carbonitrile hydrochloride (12mp). Prepared according to method C from the corresponding hydrazone and isolated as an HCl salt from ether. The hydrazone precursor was prepared using method A and used in its crude form. Compound **12mp** was isolated as a mixture of tautomers in a ratio of approximately 3:1 imine/enamine. ^{1}H NMR (500 MHz, DMSO-d_6) δ (imine) 7.35 (d, $J=8.48$ Hz, 2H), 7.27 (d, $J=8.16$ Hz, 2H), 5.75 (s, 2H), 5.25 (m, 1H), 3.33 (m, 2H), 2.94 (m, 2H), 1.22 (d, $J=6.91$ Hz, 6H). (enamine) 7.35 (d, $J=8.48$ Hz, 2H), 7.27 (d, $J=8.16$ Hz, 2H), 5.75 (s, 2H), 5.25 (m, 1H), 3.33 (m, 2H), 2.94 (m, 2H), 1.22 (d, $J=6.91$ Hz, 6H). MS (ES) m/z 311 $(M+H^+)$. HPLC 99%, R_T: 3.96 min (System B; 10%–95% acetonitrile over 2 min). HRMS (ESI+) for $C_{17}H_{18}N_4S$: calcd 310.1252; found 310.1252.

3-Amino-2-phenyl-2,6,7,8-tetrahydro-cinnoline-4-carbonitrile (13). Morpholine (64 μL, 0.70 mmol) and malononitrile (0.05 g, 0.70 mmol) was added to hydrazone **9** (0.15 g, 0.7 mmol) dissolved in DMSO (3 mL). The reaction mixture was stirred at 80 °C for 15 min. The mixture was cooled and the solution poured into cold water (20 mL). The resultant precipitate was collected by filtration, recrystallized from ethanol, then freeze-dried. This gave **13** as a brown solid (0.107 g, 58%). HPLC 91%, R_T: 4.04 min (System A). MS (ES) m/z 251.2 $[M+H]^+$). ^{1}H NMR ($CDCl_3$) δ 7.49.24 (m, Ph), 5.28 (t, CH), 4.43 (s, NH_2), 2.38–2.42 (m, CH_2), 2.22–2.25 (m, CH_2), 1.77.80 (m, CH_2).

3-Amino-2-phenyl-2,8-dihydro-6H-pyrano[3,4-*c*]pyridazine-4-carboxylic acid amide (14). To compound **10** (0.1 g, 0.4 mmol) was added concd H_2SO_4 (0.8 mL) and the reaction mixture was heated at 80–90 °C for 1 h. The mixture was cooled, diluted with water then neutralized with potassium carbonate. The product was extracted with DCM, washed with water, dried ($MgSO_4$) and the solvent removed under reduced pressure which furnished 86 mg (80%) of compound **14** as a pale yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.72–7.69 (m, 3H), 7.58–7.57 (m, 2H), 4.74 (s, 2H), 4.04 (tr, $J=5.96$ Hz, 2H), 3.12 (tr, $J=5.96$ Hz, 1H). HPLC 99%, R_T: 3.63 min (System A). MS (ES) m/z 270.9 $[M+H]^+.]^-$. HRMS (ESI+) for $C_{14}H_{14}N_4O_2$, calcd 270.1117; found 270.1121.

***N*-Acetyl-*N*-(4-cyano-2-phenyl-2,8-dihydro-6H-pyrano [3,4-*c*]pyridazin-3-yl)-acetamide (15).** To compound **10** (0.1 g, 0.4 mmol) in DCM (3 mL) was added hunigs base (0.07 mL, 0.4 mol) and acetic anhydride (0.94 mL, 9.9 mmol) and the reaction mixture stirred at room temperature overnight. Saturated sodium bicarbonate solution was added and the mixture stirred at room temperature for 30 min. The product was then extracted with DCM, washed with water, dried ($MgSO_4$) and the solvent removed under reduced pressure. The product was purified by column chromatography over silica using a gradient of hexane/ethyl acetate (4:1 to 2:1), then freeze-dried. This gave **15** as a dark yellow oil (0.09 g, 77%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.46–7.40 (m, 3H), 7.29–7.25 (m, 2H), 5.54 (tr, $J=5.56$ Hz, 1H), 4.36 (d, $J=5.56$ Hz, 2H), 4.31 (s, 2H), 2.29 (s, 6H). HPLC 95%, R_T: 4.98 min (System A). MS (ES) m/z 293.0 $[M-H]^-$. HRMS (ESI+) for $C_{18}H_{16}N_4O_3$, calcd 336.1222; found 336.1227.

3-Amino-2-phenyl-6,7-dihydro-2H-cyclopenta[*c*]pyridazine-4-carbonitrile (17). Malononitrile (0.18 g, 2.7 mmol) and morpholine (0.23 mL, 2.7 mmol) was added to hydrazone **16** (0.5 g, 2.7 mmol) dissolved in DMSO (1 mL). The reaction mixture was stirred at 80 °C for 15 min. The mixture was cooled and the product was purified by column chromatography over silica using a gradient of hexane/ethyl acetate (5:1 to 1:1), then freeze-dried. This gave **17** as a green solid (0.33 g, 53%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.55–7.36 (m, 5H), 5.13–5.11 (m, 1H), 2.63–2.56 (m, 4H). HPLC 93%, R_T: 3.79 min (System A). MS (ES) m/z 237.0 $[M+H]^+$. HRMS for $C_{14}H_{12}N_4$: calcd 236.1062; found 236.1064.

3-Amino-2-phenyl-6,7,8,9-tetrahydro-2H-cyclohepta[*c*]-pyridazine-4-carbonitrile (19). To hydrazone **18** (0.1 g, 0.46 mmol) in DMSO (1 mL) was added malononitrile (0.031 g, 0.46 mmol) and morpholine (0.04 mL, 0.46 mmol) and the reaction mixture stirred at 80 °C for 15 min. The mixture was cooled and the product was purified by column chromatography over silica using a gradient of hexane/ethyl acetate (3:1 to 1:1), which gave 0.057 g (47%) of compound **19** as a beige solid. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.72–7.69 (m, 3H), 7.60–7.57 (m, 2H), 3.25–3.20 (m, 2H), 3.06–3.02 (m, 2H), 1.97–1.90 (m, 2H), 1.86–1.77 (m, 2H). HPLC 96%, R_T: 4.88 min (System A). MS (ES) m/z 265.3 $[M+H]^+$. HRMS for $C_{16}H_{16}N_4$: calcd 264.1375; found 264.1382.

Measurement of PTP activity

Protein tyrosine phosphatase activity was measured using *p*NPP as the substrate. Assays were performed in 200 μL volumes in buffers comprising 50 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.1% (w/v) BSA and 50 mM Hepes pH 7.2 (for PTP1B, LAR and SHP-2) or 25 mM Tris–HCl/0.01% (v/v) Brij 35 (for TCPTP). *p*NPP was used at concentrations corresponding to the K_m values for the individual enzymes (0.6 mM for TCPTP, 1.25 mM for PTP1B and 6.25 mM for LAR and SHP-2). Assays were performed for different times for different enzymes (45–90 min, ensuring linearity was maintained) and were terminated by addition of 100 μL 0.1 N NaOH. The OD^{405} was measured after 10 min and the extent of reaction was calculated using a molar extinction coefficient of 18,000 M cm^{-1}[17a]

Determination of insulin receptor phosphorylation

L6 myocytes were maintained in minimum essential medium-alpha (α-MEM) supplemented with 10% foetal bovine serum (FBS) and 100 IU/mL penicillin–streptomycin at 37 °C in 5% CO_2. Cells were seeded into 24-well plates and the medium was replaced with αMEM

containing 2% FCS to induce differentiation into myotubes. The medium was changed every other day and cytidine (0.24 mg/mL medium) was added to the cultures at day 7–9 to suspend cycling cells. The cells were used in experiments after over night serum starvation at day 11. They were treated with or without 25 μM compound for 30 min followed by 5 min insulin (25 nM) stimulation. After freezing with liquid N_2 the cells were lysed with a Tris–HCl buffer, pH 7.4, containing 1% Nonidet-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM sodiumorthovanadate, 10 mM β-glycerophosphate, 5 mM sodium pyrophosphate and complete protease inhibitor cocktail from Boehringer. The cell extracts were centrifuged at 14,000 *g* for 10 min and the supernatants were used in the Delfia assay.

A lanthanide-based fluorescent assay (Delfia) was used to detect phosphorylated tyrosines (P-tyr) on the insulin receptor (IR). IR in the cell lysates were captured on a FluoroNunc 96-well plate pre-coated with goat antibody to rabbit IgG (from Cappel) and coated with rabbit antibody to IR (sc-711 from Santa Cruz Biotechnology). P-tyr was detected with a biotinylated monoclonal antibody to P-tyr (PY-99B from Santa Cruz Biotechnology) followed by europium-labelled streptavidin (from Perkin-Elmer). By addition of enhancement solution a highly fluorescent chelate was formed with a long excited halftime which permitted emission measurement after a delay to avoid background interference.

References and Notes

1. (a) DeFronzo, R. A.; Bonadonna, R. C.; Ferrannini, E. *Diabetes Care* **1992**, *15*, 318. (b) Reaven, G. M. *Diabetes/Metabolism Reviews* **1993**, *9* (Suppl. 1), 5 S.
2. White, M. F.; Kahn, C. R. *J. Biol. Chem.* **1994**, *269*, 1.
3. Quon, M. J.; Butte, A. J.; Taylor, S. I. *Trends Endocrin. Metab.* **1994**, *5*, 369.
4. (a) Saltiel, A. R. *Am. J. Physiol.* **1996**, *270*, E375.
5. Häring, H. U. *Diabetalogia* **1991**, *34*, 848.
6. Kahn, C. R. *Diabetes* **1994**, *43*, 1066.
7. Evans, J. L.; Jallal, B. *Exp. Opin. Invest. Drugs* **1999**, *8*, 139.
8. For a review see: Burke, T. R.; Zhang, Z.-Y., Jr. *Biopolymers (Pept. Sci.)* **1998**, *47*, 225.
9. Brandyopadhyay, D.; Kusari, A.; Kenner, K. A.; Liu, F.; Chernoff, J.; Gustafson, T. A.; Kusari, J. *J. Biol. Chem.* **1997**, *272*, 1639.
10. Wang, X. Y.; Bergdahl, K.; Heijbel, A.; Liljebris, C.; Bleasdale, J. E. *Mol. Cell. Endocrinol.* **2001**, *173*, 109.
11. Jacob, K. K.; Sap, J.; Stanley, F. M. *J. Biol. Chem.* **1998**, *273*, 4800.
12. Goldstein, B. J. *J. Cell. Biochem.* **1992**, *48*, 33.
13. Ahmad, F.; Azevedo, J. L.; Cortright, R.; Dohm, G. L.; Goldstein, B. J. *J. Clin. Invest.* **1997**, *100*, 449.
14. Kusari, J.; Kenner, K. A.; Suh, K. I.; Hill, D. E.; Henry, R. R. *J. Clin. Invest.* **1994**, *93*, 1156.
15. (a) Byon, J. C. H.; Kusari, A. B.; Kusari, J. *Mol. Cell. Biochem.* **1998**, *182*, 101. (b) Goldstein, B. J.; Bittner-Kowalczyk, A.; White, M. F.; Harbeck, M. *J. Biol. Chem.* **2000**, *275*, 4283. (c) Egawa, K.; Maegawa, H.; Shimizu, S.; Katsutaro, M.; Yoshihiko, N.; Bryer-Ash, M.; Cheung, A. T.; Kolss, J. K.; Kikkawa, R.; Kashiwagi, A. *J. Biol. Chem.* **2001**, *276*, 10207.
16. (a) Elchebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; Loy, A. L.; Normandin, D.; Cheng, A.; Himms-Hagen, J.; Chan, C. C.; Ramachandran, C.; Gresser, M. J.; Tremblay, M. L.; Kennedy, B. P. *Science* **1999**, *283*, 1544. (b) Klaman, L. D.; Boss, O.; Peroni, O. D.; Kim, J. K.; Martino, J. L.; Zabolotny; Moghal, N.; Lubkin, M.; Kim, Y.-B.; Sharpe, A. H.; Stricker-Krongrad, A.; Shulman, G. I.; Neel, B. G.; Kahn, B. B. *Mol. Cell Biol.* **2000**, *20*, 5479.
17. (a) Taing, M.; Keng, Y. F.; Shen, K.; Wu, L.; Lawrence, D. S.; Zhang, Z.-Y. *Biochemistry* **1999**, *38*, 3793. (b) Bleasdale, J. E.; Ogg, D.; Palazuk, B. J.; Jacob, C. S.; Swanson, M. L.; Wang, W.-Y.; Thompson, D. P.; Conradi, R. A.; Mathews, W. R.; Laborde, A. L.; Stuchly, C. W.; Heijbel, A.; Bergdahl, K.; Bannow, C. A.; Smith, C. W.; Liljebris, C.; Schostarez, H. J.; May, P. D.; Stevens, F. C.; Larsen, S. D. *Biochemistry* **2001**, *40*, 5642. (c) Gao, Y.; Wu, L.; Luo, J. H.; Guo, R.; Yang, D.; Zhang, Z.-Y.; Burke, T. R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 923. (d) Taylor, S. D.; Kotoris, C. C.; Dinaut, A. N.; Wang, Q.; Ramachandran, C.; Huang, Z. *Bioorg. Med. Chem.* **1998**, *6*, 1457. (e) Iverson, L. F.; Andersen, H. S.; Branner, S.; Mortensen, S. B.; Peters, G. H.; Norris, K.; Olsen, O. H.; Jeppesen, C. B.; Lundt, B. F.; Ripka, W.; Moller, K. B.; Moller, N. P. H. *J. Biol. Chem.* **2000**, *275*, 10300. (f) Yokomatsu, T.; Murano, T.; Umesue, I.; Soeda, S.; Shimeno, H.; Shibuya, S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 529. (g) Kotoris, C. C.; Wen, W.; Lough, A.; Taylor, S. D. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1271. (h) Ibrahimi, O. A.; Wu, L.; Zhao, K.; Zhang, Z.-Y. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 457. (i) Larsen, S. D.; Barf, T.; Liljebris, C.; May, P. D.; Ogg, D.; O'Sullivan, T. J.; Palazuk, B. J.; Schostarez, H. J.; Stevens, F. C.; Bleasdale, J. E. *J. Med. Chem.* **2001**, *45*, 598.
18. (a) Malamas, M. S.; Sredy, J.; Moxham, C.; Katz, A.; Xu, W.; McDevitt, R.; Adebayo, F. O.; Sawicki, D. R.; Seestaller, D. S.; Taylor, J. R. *J. Med. Chem.* **2000**, *43*, 1293. (b) Malamas, M. S.; Sredy, J.; Gunawan, I.; Mihan, B.; Sawicki, D. R.; Seestaller, L.; Sullivan, D.; Flam, B. R. *J. Med. Chem.* **2000**, *10*, 995. (c) Wrobel, J.; Sredy, J.; Moxham, C.; Dietrich, A.; Li, Z.; Sawicki, D. R.; Seestaller, L.; Wu, L.; Katz, A.; Sullivan, D.; Tio, C.; Zhang, Z.-Y. *J. Med. Chem.* **1999**, *42*, 3199.
19. (a) Mironov, G. S.; Farberov, M. I.; Orlova, I. M. *J. Gen. Chem. USSR (Engl. Trans.) EN* **1963**, *33*, 1476. (b) Kjeldsen, G.; Knudsen, J. S.; Ravn-Petersen, L. S.; Torssell, B. G. *Tetrahedron* **1983**, *39*, 2237.
20. (a) Nazarov, I. N.; Kuvarzina, M. V. *J. Gen. Chem.* **1948**, *18*, 1328. (b) Johnson, P. Y.; Berchtold, G. A. *J. Org. Chem.* **1970**, *35*, 584.
21. Barbero, M.; Crisma, M.; Degani, I.; Fochi, R.; Perracino, P. *Synthesis* **1998**, 1171.
22. (a) Noravyan, A. S.; Paronikyan, E. G.; Vartanyan, S. A. *Chem. Heterocycl. Compd.* **1983**, *19*, 1157. (b) Pilosyan, S. G.; Dabaeva, V. V.; Mambreyan, Sh.P. *Noravyan, Arm. Khim. Zh.* **1985**, *38*, 747.
23. Paronikyan, E. G.; Noravyan, A. S.; Engoyan, A. P. *Chem. Heterocycl. Compd.* **1996**, *32*, 1213.
24. Compounds were dissolved in phosphate buffer pH 7.4 and kept at 37 °C for 24 h. Chemical stability was analysed by HPLC; if less than 90% of the compound peak remained, the compound was termed unstable.
25. Zhang, Z.-Y. *Curr. Opin. Chem. Biol.* **2001**, *5*, 416.
26. For a recent reviewed see: Tonks, N. K.; Neel, B. *Curr. Opin. Cell Biol.* **2001**, *13*, 182.
27. Garberg, P.; Eriksson, P.; Schipper, N.; Sjostrom, B. *Pharm. Res.* **1999**, *16*, 441.