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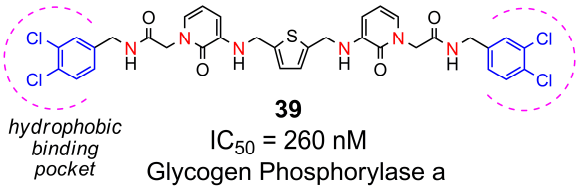
Graphical Abstract



39

IC_{50} = 260 nM

Glycogen Phosphorylase a

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Discovery of new nanomolar inhibitors of GPa: Extension of 2-oxo-1,2-dihydropyridinyl-3-yl amide-based GPa inhibitors

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ABSTRACT

Glycogen Phosphorylase (GP) is a functionally active dimeric enzyme, which is a target for inhibition of the conversion of glycogen to glucose-1-phosphate. In this study we report the design and synthesis of 14 new pyridone derivatives, and seek to extend the SAR analysis of these compounds. The SAR revealed the minor influence of the amide group, importance of the pyridone ring both spatially around the pyridine ring and for possible π-stacking, and confirmed a preference for inclusion of 3,4-dichlorobenzyl moieties, as bookends to the pyridone scaffold. Upon exploring a dimer strategy as part of the SAR analysis, the first extended 2-oxo-dihydropyridinyl-3-yl amide nanomolar based inhibitors of GPa (IC_{50} = 230 and 260 nM) were identified.

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1. Introduction

The non-insulin dependent (type 2) form of diabetes mellitus (T2DM) is a metabolic disorder, which has almost quadrupled since 1980 to 422 million adults in 2014 [1]. The increasing predominance of T2DM has identified a need for new and better treatments for improved glycemic control, in particular from hepatic glucose output [2]. Glycogen Phosphorylase (GP) is a validated molecular therapeutic target for the design of compounds that could treat hyperglycaemia associated with T2DM [3-5]. GP is the rate-limiting enzyme in the glycogenolysis pathway, catalysing the phosphorolysis of 'storage' glycogen to form glucose-1-phosphate which can be used, in the case of hepatocytes, to form glucose. The three main isoforms of GP occur in the muscle, liver and brain. However, it is the liver isoform of GP that is directly involved in control of blood glucose homeostasis [6].

GP is a highly regulated allosteric enzyme with multiple binding sites for inhibitors [3, 7-9] which include the catalytic site which binds glucose derivatives, the glycogen storage site and binding sites for allosteric effectors (purine site, indole site at the enzyme dimer interface, allosteric (AMP) site the new allosteric site) and the quercetin binding site [10]. Glucose derivatives, which bind to the catalytic site of GP, represent the most widely studied group of inhibitory molecules [7, 11, 12], including in vivo studies [13]. However, other structural classes of inhibitors show promise; for example GPa has been validated as a target in diabetic ob/ob mice [14] for indole inhibitors, and in streptozotocin-treated rats for glucopyranosylidene-spirothiohydantoin [15]. Comprehensive reviews on a range of GP inhibitors have been reported in the patent literature [16-18] and elsewhere [3-5, 7, 8, 19-25]. Although inhibitors of GP comprise a broad range of structural diversity, the majority of reports of nanomolar inhibitors have been focused towards glucopyranosyl inhibitors [24, 26, 27], with the best Ki currently being 26 nM [28]; along with isolated cases of bi-aryl anthranilimides (IC_{50} of 3-7 nM) targeting the AMP site [29] and 5-chloro-indole-2-carboximide derivatives incorporating a fluorinated tetrahydro napthalenyl (IC_{50} of 20 nM), with predicted binding at the dimer interface [30].

Previously, we reported a chemogenomics strategy for GP inhibitor design [31], based on studies which showed that a GP inhibitor can block the interaction of the C-terminus of the hepatic glycogen-binding subunit GL (encoded by the gene PPP1R3B) of protein phophatase-1 (PP-1) [32]. A pyridone ring was established as a useful mimetic of the Leu-Gly residues of the C-terminus residues identified with GP activity. Arising from this work benzyl 3,4-dichlorobenzyl pyridone **3** (IC_{50} = 6.3 μM) [31] (Fig. 1) provided the lead for subsequent Structure-Activity Relationships (SAR) studies, in which the pyridone moiety was maintained as the primary scaffold, providing diversity points through the C3 amino group and the N1 of the pyridone ring. Improved potency was observed with two lead compounds identified (Fig. 1); 4-chlorobenzyl 3,4-dichlorobenzyl pyridone **2** (IC_{50} = 2.1 μM) [31] and 3,4-dichlorobenzyl 3,4-dichlorobenzyl pyridone **1** (IC_{50} = 1.9 μM) [33]. A GP-**1**, **2** or **3** ligand X-Ray structure has yet to be obtained to establish the binding site of the 3,4-dichlorobenzyl 2-oxo-1,2-dihydropyridin-3-yl amides to GP. The present report discloses further SAR and in vitro evaluation, where the structural variance of the central pyridone ring through 'scaffold hopping' [34], along with a dimer strategy [35, 36] (in the context that GPa has multiple binding sites) was explored; resulting in the discovery of new nanomolar inhibitors of GPa.

2. Chemistry

In the present study we sought to further explore structural variance of the previously identified lead compounds; 3,4-dichlorobenzyl 3,4-dichlorobenzyl pyridone **1** [33], 4-chlorobenzyl 3,4-dichlorobenzyl pyridone **2** [33] and benzyl 3,4-dichlorobenzyl pyridone **3** [31]. The derivatives presented here explore shortening and lengthening the derivatives with concomitant presence/absence of amide groups, and modification of the pyridone scaffold. Design and choice of derivatives was guided further by (i) previous results where benzyl 3,4-dichlorobenzyl pyridone **3** and 3,4-dichlorobenzyl benzyl pyridone **4** displayed an order of magnitude of difference in inhibition of GPa to the corresponding benzyl benzyl pyridone analog (IC_{50} = 34.2 μM) [33], and (ii) commercial availability of aldehyde and amine precursors. *To facilitate discussion, derivatives will be referred to by the left (LHS), followed by the right hand side (RHS) substituents as drawn herein; for example, benzyl 3,4-chlorobenzyl pyridone* **3**.

The synthesis of 2-oxo-1,2-dihydropyridin-3-yl amide derivatives with a shortened linker at the N1 of the pyridone is outlined in Scheme 1. Based on our previous studies [31, 33], the aromatic functionality at the right hand side was maintained but the amide section of the linker removed; compounds **11** and **12**. The methyl analog **8** was prepared for comparison. 2-Hydroxy-3-nitropyridine **5** was N-alkylated with benzylbromide or methyl iodide in the presence of NaH. Reduction of nitropyridones **6** and **9** by hydrogenation over palladium on carbon at atmospheric pressure gave aminopyridones **7** and **10**, which were unstable and used directly in the next step. Reductive amination using $NaBH(OAc)_3$, aminopyridones **7** and **10**, and benzaldehyde or 3,4-dichlorobenzaldehyde gave the target compounds **8**, **11** and **12** in excellent yields (83-98%) (Scheme 1).

We sought to explore structural tolerance further on the LHS, in an extension of our previous studies [31, 33], by introducing a rigid left hand aromatic substituent and/or increasing the number of carbonyl moieties. The phthalate group was considered, as well as the thiourea group, which is commonly used in drug design [37]. Amino ethyl ester pyridone **13** [38] and phthalic anhydride were heated at reflux in the presence of $MgSO_4$ to give phthalimide ethyl ester pyridone **14** in excellent yield (88%) (Scheme 2). Direct aminolysis was previously found to be a mild and atom-efficient procedure [38]. Attempted aminolysis of **14** with 3,4-dichlorobenzylamine at 120 ^oC for 4 hrs, following the general procedure (section 8.2.5), resulted in formation of a complex mixture. In contrast, aminolysis of **14** with propylamine at room temperature for 15 minutes gave diamide **15** (61%) and amino propyl pyridone **16** (20%). Formation of diamide **15** and amino propyl pyridone **16** confirmed that cleavage of the phthalic group had occurred through nucleophilic attack by propyl amine. A mechanism for the deprotection is proposed in Scheme 2, which involves formation of a N-propylamine intermediate **A**, followed by intramolecular attack by the propyl amide nitrogen atom and cleavage to form intermediates **B** and **C**. Reaction of a second molecule of propyl amine with N-propylphthalimide **B** would generate diamide **15**, in conjunction with aminolysis of the intermediate ester **C** to form amino propyl pyridone **16**. In another synthetic variation, reaction of aminoethyl ester **13** with commercially available benzoyl isothiocyanate at room temperature for 2 hrs gave benzoylthiourea ethyl ester **17** in excellent yield (98%) (Scheme 3). Subsequent aminolysis of **17** with propylamine at room temperature for 15 minutes again resulted in cleavage products, and the formation of amino thiourea **18**, as an unstable oil (87%). No further variations to the previously reported phenylurea derivative [33] were explored.

Identification of the importance of the pyridone scaffold led to a scaffold-hopping [34] exercise, where the pyridone was alkylated at position 4, and the aromaticity removed from the pyridone ring, as observed in a 2-oxopiperidine moiety. Alkylation of 4-methyl nitropyridone **19** with ethyl bromoacetate, followed by reduction of **20** by hydrogenation over palladium on carbon at atmospheric pressure and reductive amination of aminopyridone **21**, using $NaBH(OAc)_3$ in the presence of benzaldehyde, gave benzyl ester **22** in excellent yield (72%) (Scheme 4). Aminolysis of benzyl ester **22** with 3,4-dichlorobenzylamine gave the target compound, benzyl 3,4-dichlorobenzyl methylpyridone **23**, an analog of the previously reported benzyl 3,4-dichlorobenzyl pyridone **1** [31].

Aminolysis of commercially available ethyl 2-oxopiperidine-3-carboxylate **24** with benzylamine or 3,4-dichlorobenzylamine gave amides **25** and **26** respectively, in quantitative yield (100%). Whereas attempted alkylation of **24** with ethyl bromoacetate resulted in complex mixtures, reaction of amides **25** and **26** with benzoyl chloride, and amide **25** with 2-chlorobenzoyl chloride at reflux for 16 hrs, gave the derivatives **27**, **28**, and **29** respectively (Scheme 5). Analogs of **27**, **28**, and **29** represent partial scaffold hopping analogs of **1**, with the benzyl- and 2-chlorobenzyl moieties used in the structures of the 2-oxopiperidine analogs.

We noted from our previous work that some extended derivatives, such 3,4-dichlorobenzyl ethylenephenyl pyridone (IC_{50} = 31.3 μM) [31], displayed inhibitory activity against GPa. In the present study, structural extension of analogs was examined using the pyridone scaffold. Initially, click chemistry [39] was explored as a rapid approach to structural extension, and to trial how robust the pyridone was to the conditions of the 1,3-diplar addition. Reaction of benzyl ethyl ester pyridone **30** with propargylamine at room temperature for 16 hrs gave benzyl propargyl pyridone **31**. A modified procedure to the standard click chemistry protocol [40] was used, due to solubility considerations. Reaction of freshly generated benzyl azide **32** and benzyl propargyl pyridone **31**, using methanol as solvent, in a copper catalyzed 1,3-dipolar addition [41] gave benzyl triazole benzyl pyridone **33** in excellent yield (85%) (Scheme 6).

Next, elongated dimer derivatives were considered. Heating two equivalents of amine ester **35** [38] with one equivalent of ethylenediamine at 120 ^oC using a solid melt approach did not yield the target dimer **36**. Instead, amine ester **34** [31] and ester **35** were heated at 120 ^oC for 4hrs, then cooled to room temperature. Recrystallisation of the resultant solid gave *bis*-benzoyl ethylamine dimer **36** in excellent yield (92%) (Scheme 7). A potentially efficient alternative approach to dimer formation was through outward extension from a central linker, such as thiophene or 1,4-phenyl. This route provides synthetic accessibility to a range of derivatives via the selections used in the central aromatic moiety and the aminolysis step. Three examples demonstrate the synthetic principle (Scheme 7). Firstly, reaction of 2,5-thiophenedicarboxaldehyde **37** with two equivalents of amino ethyl ester pyridine **13**, followed by reduction with $NaBH(OAc)_3$ gave diester thiophene dimer **38** (82%). Aminolysis of diester thiophene dimer **38** with two equivalents of 3,4-dichlorobenzylamine at 120 ^oC gave *bis*-3,4-dichlorobenzylbenzyl thiophene dimer **39** in excellent yield (98%). Next, reaction of 1,4-phenylene diisocyanate **40** with ethyl ester pyridine **13**, gave diester diurea dimer **41** (84%). Aminolysis of **41** with propylamine at room temperature gave bis-propylamine diurea dimer **42** in excellent yield (94%); confirming the stability of the diurea central linker. In the final example, aminolysis of diester diurea dimer **41** with 3,4-dichlorobenzylamine at 120 ^oC gave *bis*-3,4-dichlorobenzyl diurea dimer **43**, in good yield (78%). Multiple recrystallizations of compound **43** provided a sample of sufficient purity (90%) for in vitro testing.

All compounds were purified by chromatography and recrystallization and characterized (Microanalysis, mass spectrometry, FTIR, ^1H, and ^{13}C NMR spectroscopy).

3. X-ray Structures of 8, 11, 19, 20 and 38

In conjunction with the synthetic work, methylpyridone **8**, (low activity against GPa; 34% at 222 μM)), benzyl pyridone **11** (IC_{50} = 33.0 μM), compound **19**, intermediate ester **20** thiophene dimer ester **38** (not active) formed crystals suitable for single crystal X-ray diffraction studies (see supplementary data for representative ORTEP plots). The ORTEP-3 diagram of the molecular structures of methylpyridone **8**, benzyl pyridone **11** and intermediate ester thiophene dimer **38** are shown in Figs. 2, 3 and 5. Intermediate ester thiophene dimer **38** displayed an extended conformation with disorder observed for the central thiophene moiety (Fig 5). A strong intermolecular O-H···O(carbonyl) and O···H-N(amine) hydrogen bonding network was observed between two molecules in the packing diagram for benzyl pyridone **11** (Fig. 4).

4. Biology

GP activity of compounds **8**, **11**, **12**, **23**, **27-29**, **33**, **36**, **38**, **39** and **41-43** was measured, using the reported in vitro GP screen [42, 33], in the direction of glycogen synthesis by the formation of inorganic phosphate from glucose-1-phosphate. An apparent IC_{50} inhibitory concentration was recorded at 50% inhibition for assays reaching ≥ 90% inhibition, with Hillslope values observed in the range 1.3 to 3.3. The results for compounds that displayed levels of inhibition of GP are listed in Table 1. By comparison, a typical IC_{50} of 227 ± 5 or 182 ± 3 μM was obtained for the caffeine standard. Compounds **12**, **27** and **36** were included in Table 1 for structural comparison and did not inhibit GPa at a maximal concentration used in the assay (i.e., <20% at 222 μM).

The compounds with defined activity against GPa and meeting acceptable Hillslope values were analysed for ligand efficiency (LE) [43] and ligand-efficiency-dependent lipophilicity (LELP) [44] to provide further assessment of the quality of the hits obtained from the GPa screening. The calculated values for LE and LELP for compounds **11**, **39**, **41**, **42** and **43** are shown in Table 2.

5. Calculation of molecular physiochemical properties

Compounds **8**, **11**, **12**, **23**, **27-29**, **33**, **36**, **39** and **41-43** (Schemes 1 and 4-7) were converted to their SMILES-codes. Numerical values for lipophilicity (Log P), solubility (Log S), polar surface area (PSA), number of H-bond donors (#OHNH), number of oxygen and nitrogen atoms (#ON), and number of rotatable bonds (#RB) were calculated with ALOGPS 2.1 [45], and Molinspiration [46] are listed for the compounds **8**, **11**, **12**, **23**, **27-29**, **33**, **36**, **39** and **41-43** (Table 1). The compounds in Table 1 have a predicted Log P <5 in accordance with Lipinski's rules [67], and LogP < 3.5 (compounds **8**, **12**, **27**, **29**, **33**, **36**, **41**, **42**) the preferred value for lead–like compounds [48].

Compounds **8**, **11**, **12**, **23**, **27-29**, **33** in Table 1 have molecular weights <500, a TPSA under 120 Å [49], and do not violate the 'rule of five' (#OHNH ≤ 5; #ON <10; #RB <8), with **32** having number of rotatable bonds ≥8 (an important predictor of good oral bioavailability [49-51]). Not surprisingly, the extended derivatives, compounds **36**, **39**, **41-43** in Table 1 have molecular weights >500, a TPSA over 120 Å, have 1-3 violations of the

'rule of five' (#OHNH ≤ 5; #ON <10; #RB <8), in conjunction with the number of rotatable bonds being ≥8.

The calculated solubility of compounds in Table1 varied from a moderate calculated solubility of > 100mg/L (compounds **12**, **27**, **33**, **36**), to a lower calculated solubility between 4 and 100 mg/L [51] (compounds **8**, **11**, **23**, **28**, **29**, **41**, **42**), or those with very low calculated solubility of < 4 mg/L (compounds **39** and **43**). Absorption of a drug is usually very low if the calculated solubility is <0.00001 mg/L, as shown in a study of the rate-limiting steps of human oral absorption of 238 drugs [52]. However all compounds were observed to display solubility in the bioassay (Table 1).

6. Results and discussion

The previous study had established that GPa was sensitive to the length of the linkers and presence of 3,4-dichlorobenzyl groups at positions (a) and/or (d) on the central pyridone scaffold (Fig. 1). In the present study, targeted analogs (14 compounds) were generated to explore some remaining SAR considerations. Exploration of the structural tolerance on the LHS was considered with targets containing increased hydrogen bonding carbonyl groups, as represented in the phthalate and thiourea groups. Synthetic studies did not yield the desired analogs, due to the high reactivity of the phthalate and thiourea groups to the amine nucleophile. However, three other SAR considerations were resolved, which derived from sub-sets based on:

(i) Creation of analogs of **4** that removed the hydrogen bond donor/acceptor moiety, the amide group, at position (c) (Fig. 1): Compounds **8**, **11** and **12** (Scheme 1).

(ii) Alteration of the pyridone scaffold, through two variations of 'scaffold hopping'; alkylation at the 4-position on the pyridone ring and removal of the rigidity of the pyridone scaffold, to create a partial derivative with increased scaffold flexibility: Compounds **23**, and **27-29** (Schemes 4 and 5).

(iii) Significant extension of the pyridone, to explore a 'dimer' strategy. The synthetic approach to dimer formation included a trial using click chemistry (compound **33;** Scheme 6) and the successful use of a central dimer unit, such as ethylene, thiophene and benzene: Compounds **36**, **39**, **42** and **43** (Scheme 7).

The group of 14 target compounds gave rise to 11 compounds that inhibited GPa (between 29% at 222 μM and an IC_{50}= 230 or 260 nM) and uncovered two new extended pyridone amides as a scaffold for nanomolar inhibition of GPa.

In the previous study of derivatives with a central pyridone scaffold and amide at position (c), there was preference for the 3,4-dichlorobenzyl substituent at positions (a) and (d) (Fig. 1). The 3,4-dichlorobenzyl substituent is present in successful drugs, such as the antidepressant Zoloft (Sertraline) [53]. In the present study, compounds **8**, **11** and **12** (Table 1) were screened against GPa. Whereas benzyl benzyl compound **12** was inactive, the 3,4-dichlorobenzyl benzyl compound **11** displayed increased inhibition of GPa (**11**, IC_{50} = 33.0 μM) and compound **8** displayed minimal inhibition of GPa (**8**, 34% @ 222 μM). Notably, comparison of compound **11** (IC_{50} = 33.0 μM) and compound **4** (IC_{50} = 10.2 μM) [33] (Fig. 1), where the amide group is absent or present respectively, indicates that the amide moiety at position (c) has an influence on improved inhibition of GPa. These results also affirm the preference for a LHS 3,4-dichlorobenzyl substituent on the pyridone scaffold.

A popular term in drug discovery is 'scaffold hopping', where identified and active substituents to the left and right hand side of the central scaffold is changed [34]. Substitution of the pyridone ring with a more flexible oxopiperidine ring scaffold was synthetically problematic. As a compromise partial oxopiperidine derivatives, albeit with increased hydrogen bond acceptors, such as compounds **27-29**, were generated by a synthetically accessible route. Compounds **27-29** displayed negligible or no inhibition of GPa (Table 1). In contrast, inhibition of GPa by a substituted pyridone scaffold derivative, compound **23**, was noteworthy. Benzyl, 3,4-dichlorobenzyl pyridone **3** displayed potent activity (IC_{50} = 6.3 μM), whereas inclusion of a 4-methyl group on the scaffold, as in benzyl, 3,4-dichlorobenzyl 4-methylpyridone **23** resulted in negligible inhibition of GPa (38% at 222 μM). The results for **23** vis-à-vis **3** indicates an analogue-enzyme interaction that has specific spatial constraints around the pyridone ring.

During the course of the project "click chemistry" was considered as a trial for rapid extension of pyridone derivatives. The trial compound **33** inhibited GPa at a negligible level (60% at 222 μM), which could be attributable to both the incorporation of the polar group triazole (cf. the benzyl benzyl analog IC_{50} = 34 μM) [33]) and increased length of the derivative as previously observed [33]. In the last study we had suggested that specific non-extended conformations of a compound were likely to further promote favorable interactions with GPa *in situ*, where the length/floppiness of a compound will impact accordingly. In consideration of this notion and that GPa is a dimeric enzyme, significant extension of the pyridone using a 'dimer' strategy was explored. The use of dimers is not common, but is a known strategy within drug design to simultaneously target two spatially separated sites [35, 36]. Three central moieties, ethylene, thiophene and benzene, were used to gain synthetic access to dimer derivatives. Compounds **36**, **39**, **42** and **43**, along with ester intermediates **38** and **41** were screened against GPa. In the context of the previous work [31], it was not surprising that the *bis*-benzoyl ethylamine dimer **36** was inactive; continuing to confirm the previous SAR that incorporation of a C3-N carbonyl group generally resulted in a decrease in inhibition of GPa [31, 33].

However, *bis*-isopropyl 'benzene' dimer **42** displayed a good activity with an IC_{50} of 1.6 μM and surprisingly *bis*-3,4-dichlorobenzyl thiophene dimer **39**, and *bis*-3,4-dichlorobenzyl 'benzene' dimer **43**, both displayed potent activity with IC_{50} values of 260 and 230 nM respectively; the first pyridone nanomolar inhibitors of GPa. We noted that the ester intermediates **38** and **41** displayed minimal inhibition of GPa (**38**, 29% @ 222 μM) or good inhibition of GPa (**41**, IC_{50} = 3.10 μM). Although **41** was present in trace amounts of the sample of **43** screened against GPa, its inhibitory activity was an order of magnitude less potent than *bis*-3,4-dichlorobenzyl 'benzene' dimer **43**.

Testing the SAR consideration for the amide group at position (c) indicated the amide moiety (compound **11**; IC_{50} = 33 μM c.f. compound **4**, IC_{50} = 10.2 μM) had a minor influence on inhibition of GPa. By contrast, disruption of the pyridone scaffold at position (b) by either 4-methyl substitution (compound **23,** 38% at 222 μM c.f. compound **3**, IC_{50} = 6.3 μM) or as in the partial derivatives (compounds **27-29**; typically 39% at 222 μM), significantly decreased inhibition of GPa and affirmed the influence of the spatial constraints of the central pyridone scaffold, and possible π-stacking. The previous lead, 3,4-dichlorobenzyl, 3,4-dichlorobenzyl pyridone **1** remains the lead for pyridone analogs with LHS and RHS substituents and an

amide at position (c). Further exploration of extended dimers revealed the first nanomolar pyridone derivatives; compound **39**, (IC_{50} = 230 nM) and compound **43** (IC_{50} = 260 nM).

It was interesting to note the apparent length of the dimer pyridone analogs. Approximations for an extended conformation (ChemBio3D Ultra 11.0) were used to aid comparison to benzyl 3,4-dichlorobenzyl pyridone (**3**; IC_{50} = 6.3 μM), which was ~19.5 Å in length. Compounds **38** (~33 Å), **41** (~30 Å) and **43** (~32 Å), were notably longer than any of the derivatives previously reported [33], yet displayed improved potency. It is interesting to note that GPa is a functionally active dimeric enzyme [54], in which the two subunits are mutually interdependent. In addition, potent 5-chloroindole GPa inhibitors at the 'new allosteric site' located at the dimer interface [9], have displayed a variety of binding orientations including occupation by two singular molecules [30, 55, 56]. In light of this, the improved activities of the extended *bis*-dichloro dimers **39** and **43**, suggests binding could be occurring across two spatially separated sites, potentially across the two subunits of GPa, for either extended or various folded conformations of **39** and **43**.

The Ligand Efficiency (LE) and Ligand-Efficiency-dependent Lipophilicity (LELP) were calculated for the compounds in Table 1 and compared with the IC_{50} values (Table 2). Elsewhere a lower limit of LE (0.3) and a range of -10 < LELP < 10 for LELP has been discussed [43]. Nanomolar pyridone inhibitors **39** and **43**, showed good enzyme potency but poorer physical properties, whereas the better micromolar inhibitors **41** and **42** (IC_{50} = 3.1 and 1.6 μM) displayed LELPs in the desired range. Thus the lead nanomolar inhibitors, **39** and **43**, would require further optimization of the physical properties if developed further.

7. Conclusion

The design, synthesis and testing of new pyridone amide derivatives resulted in increased SAR knowledge and 11 new compounds that inhibited GPa (between 29% at 222 μM and IC_{50} = 230 nM). The amide moiety at position (c) (Fig. 1) had a minor influence on improved potency. Scaffold hopping established that the pyridone scaffold at position (b) (Fig. 1) without substitutions, such as a 4-methyl group, had influence on GPa inhibition both spatially around the pyridone ring and for possible π-stacking. The 3,4-dichloro substituent remain favoured at positions (a) and (d) (Fig. 1) for improved potency of 2-oxo-1,2-dihydropyridinyl-3-yl amide derivatives. The first extended 2-oxo-1,2-dihydropyridinyl-3-yl amides and nanomolar inhibitors of GPa were identified as compounds **39** and **43**, however both these compounds had physical properties that require further optimization. Nonetheless, the improved GPa inhibition observed for **39** and **43**, combined with the flexible length of these compounds, suggested dimer binding could be occurring across two spatially separated sites. Worthwhile future work would include dimer inhibitor-GP X-ray studies.

8. Experimental Section

8.1 General

All reagents, and compounds **5**, **19**, **24**, **37** and **40** were purchased from commercial suppliers and used without further purification. The procedures for the preparation of compounds **13**,[38] **30**,[31] **34**,[31] **35**[38] are reported elsewhere. Column chromatography was performed using silica gel 60 Å (0.040-0.063 mm). Analytical thin layer chromatography (TLC) was performed using aluminium plates coated with silica gel 69 F254 (0.2 mm), and visualized by means of ultra-violet irradiation (254 nm) or vanillin dip. Melting points were measured on a variable temperature apparatus by the capillary method and are uncorrected. Temperatures are reported in ^{o}C. High resolution mass spectroscopy (HRMS) was performed on a Fourier Transform Mass Spectrometer equipped with an electrospray source (ESI-FTMS). Mass spectra were recorded using electrospray as the ionization technique. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 at 300 or 400 MHz. Coupling constants J are in Hertz (Hz). Chemical shifts are reported in parts per million, using the appropriate signal for solvent as a reference. IR spectra were recorded on a FT-IR Spectrometer as KBr discs for solids or as neat samples for oils. Absorption maxima are reported in wavenumbers (cm^{-1}). Where given, systematic compound names are those generated by ChemDraw Ultra 11.0 following IUPAC conventions (Supplementary Data; Note on compound nomenclature).

8.2 Experimental Procedures

8.2.1 General procedure for alkylation: Preparation of compounds **6**, **9** and **20**.

Sodium hydride (95%, 1.5 or 2.0 equivalents) was added in portions to 2-hydroxy-3-nitro pyridine **5** (0.200 g or 2.00 g) or 4-methyl-3-nitropyridin-2(1*H*)-one **19** (0.663 g) in dry THF (20 or 50 mL). The resulting suspension was stirred for 30 minutes. Methyl iodide (1.1 equivalents), benzyl bromide (2.0 equivalents) or ethyl bromoacetate (1.1 equivalents) was added dropwise. The resulting yellow suspension was heated to 55 °C under nitrogen for 16 hours (**6**, **9**) or 36 hours (**20**). The red reaction mixture was filtered and the solid thoroughly washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The crude product was purified by silica column chromatography (as indicated below; **6**, **9**, and **20**)

8.2.1.1 1-Methyl-3-nitropyridin-2(1H)-one **6**. Chromatography (0-20% ethyl acetate /dichloromethane gradient, with 0.5% Et_3N). Yellow solid (0.216 g, 98%): M.p 172-176 °C (dec.) (Lit.[57]176 ^{o}C). FTIR (KBr) ν: 3432, 3085, 1683, 1593, 1540, 1340, 1279, 1111, 915, 768 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.65 (s, 3H, CH_3), 6.27 (dd, 1H, *J*=7.6, 6.8 Hz, H5), 7.70 (dd, 1H, *J* = 6.4, 2.0 Hz, H6), 8.28 (dd, 1H, *J* = 7.6, 2.0 Hz, H4). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 38.8 (CH_3), 103.2 (C5), 138.6 (C3, C4), 144.7 (C6), 154.8 (C2). ESI-MS m/z 176.8 ($M+Na^+$), 160.8 ($M+Li^+$).

8.2.1.2 1-Benzyl-3-nitropyridin-2(1H)-one **9**. Chromatography (0-20% ethyl acetate /dichloromethane gradient, with 0.5% Et_3N). Yellow solid (3.291 g, 100%): M.p 87-91 °C. FTIR (KBr) ν: 3072, 1671, 1536, 1279, 772, 698 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.17 (s, 2H, CH_2), 6.26 (dd, 1H, *J* = 7.6, 6.8 Hz, H5), 7.26-7.30 (m, 5H, C_6H_5), 7.75 (dd, 1H, *J* = 6.4, 2.0 Hz, H4), 8.23 (dd, 1H, *J* = 7.6, 2.0 Hz, H6). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 53.5 (CH_2), 103.7 (C5), 129.0 (*o*-C_6H_5)), 129.1 (*p*-C_6H_5), 129.5 (*m*-C_6H_5), 135.0 (*i*-C_6H_5), 138.8 (C6), 139.3 (C3), 143.8 (C4), 154.8 (C2). ESI-MS m/z 253.0 ($M+Na^+$). HRMS m/z: 253.0586; calc. for $C_{12}H_{10}O_3N_2Na$: 253.0584 ($M+Na^+$).

8.2.1.3 Ethyl 2-(4'-methyl-3'-nitro-2'-oxopyridin-1'(2H)-yl)acetate **20**. Chromatography (0-40% ethyl acetate /dichloromethane gradient, with 0.5% Et_3N). Orange crystals (5.49 g, 99%); M.p 78-80 °C. FTIR (KBr) ν: 3412, 2995, 1757, 1667, 1610, 1532, 1205, 780 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.16 (t, 3H, *J* = 7.2 Hz, CH_3), 2.16 (s, 3H, 4'-CH_3), 4.11 (q, 2H, *J* = 7.2 Hz, CH_2), 4.59 (s, 2H, H2), 6.10 (d, 1H, *J* = 7.2 Hz, H5'), 7.34 (d, 1H, *J* = 7.2 Hz, H6'). ^{13}C NMR (100 MHz, $CDCl_3$) δ: δ 14.2 (CH_3), 18.0 (4'-CH_3), 50.7 (C2), 62.3 (CH_2), 107.9 (C5'), 130.9 (C6'), 141.5 (C3'), 145.5 (C4'), 154.9 (C2'), 167.1 (C1). ESI-MS m/z 263.1 ($M+Na^+$). Anal. Calc. for

$C_{10}H_{12}O_5N_2$: C, 50.00; H, 5.04; N, 11.66%. Found: C, 50.04; H, 5.01; N, 11.34%.

8.2.2 General procedure for hydrogenation: Preparation of compounds **7**, **10** and **21**.

1-Methyl-3-nitropyridin-2(1*H*)-one **6** (0.620 g), 1-Benzyl-3-nitropyridin-2(1*H*)-one **9** (2.0 g) or Ethyl 2-(4'-methyl-3'-nitro-2'-oxopyridin-1'(2*H*)-yl)acetate **20** (2.00 g) and 10% Pd/C (11% w/w) was flushed with nitrogen then degassed (sonication) ethanol (60 or 200 mL) was added under nitrogen. The resulting suspension was then flushed with H_2 and hydrogenated for 16 hours under balloon pressure. The reaction was quenched by flushing with nitrogen and addition of Celite®. The resulting suspension was filtered through a pad of Celite® and activated charcoal, using ethyl acetate to wash the plug. The solvent was removed under reduced pressure to give crude compound as an unstable oil, which was used directly in the next step.

8.2.2.1 3-Amino-1-methylpyridin-2(1H)-one **7**. Unstable pale yellow/green oil, (0.498 g, 100%). R_f 0.45 (isopropyl alcohol:dichloromethane 1:9 with 0.5% triethylamine).

8.2.2.2 3-Amino-1-benzylpyridin-2(1*H*)-one **10**. Unstable pale blue/green oil, (1.74 g, 100%). R_f 0.44 (ethyl acetate:dichloromethane 1:4 with 0.5% triethylamine).

8.2.2.3 Ethyl (3-amino-4-methyl-2-oxopyridin-1(2H)-yl)acetate **21**.Unstable brown oil (1.75 g, 100%). R_f 0.33 (ethyl acetate:dichloromethane 2:3 with 0.5% triethylamine).

8.2.3 General procedure for reductive amination: Preparation of compounds **8**, **11, 12, 22** and **38**.

The amine (1.0 equiv) was dissolved in DCE (anhydrous) and the aldehyde (1.5 equiv) was added under an atmosphere of nitrogen. The solution was stirred at rt/reflux for 16 hrs then $NaBH(OAc)_3$ (2 equiv) was added. The resulting suspension was stirred at rt for 16 hrs. The reaction was quenched with K_2CO_3 (1 M) and stirred vigorously for 30 mins before the phases were separated. The aqueous phase was extracted with DCM. The combined organic layers were washed (brine), dried (anhydrous $MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica column chromatography (as indicated below; (**8**, **11**, **12**, **22**) or recrystallized from acetone/diethyl ether (**38**).

*8.2.3.1 3-[(3',4'-Dichlorobenzyl)amino]-1-methylpyridin-2(1*H*)-one* **8**. 3-Amino-1-methylpyridin-2(1*H*)-one **7** (, 0.500 g, 4.03 mmol), 3,4-dichlorobenzaldehyde (1.06 g, 6.04 mmol) and sodium triacetoxyborohydride (1.71 g, 8.06 mmol) were reacted at room temperature. Chromatography (0-40% ethyl acetate /dichloromethane gradient, with 0.5% Et_3N). Compound **8** Blue crystals (0.947 g, 83%): M.p 122-126 °C (dec.). FTIR (KBr) ν: 3326, 3048, 2909, 1634, 1605, 1569, 1487, 719 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 3.54 (s, 3H, CH_3), 4.26 (d, 2H, *J* = 6.0 Hz, 3-NHCH_2), 5.52 (brs, 1H, $Wh_{½}$ ~ 15 Hz, NH), 5.98-6.03 (m, 1H, H4, H5), 6.61 (dd, 1H, *J* = 5.2, 3.2 Hz, H6), 7.12 (dd, 1H, *J* = 8.0, 2.0 Hz, H6'), 7.35 (d, 1H, *J* = 8.0 Hz, H5'), 7.38 (d, 1H, *J* = 2.0 Hz, H2'). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 37.6 (CH_3), 46.6 (3-$NHCH_2$), 107.3 (C4, C5), 124.5 (C6), 126.5 (C6'), 129.1 (C2'), 130.8 (C5'), 131.3 (C4'), 133.9 (C3'), 138.3 (C3), 139.2 (C1'), 158.2 (C2). ESI-MS m/z 289.1 ($M+Li^+$). HRMS m/z: 283.0394; calc. for $C_{13}H_{13}ON_2Cl_2$: 283.0399 ($M+H^+$).

*8.2.3.2 1-Benzyl-3-[(3',4'-dichlorobenzyl)amino]pyridin-2(1*H*)-one* **11**. 3-Amino-1-benzylpyridin-2(1*H*)-one **10** (0.870 g, 4.34 mmol), 3,4-dichlorobenzaldehyde (1.14 g, 6.52 mmol) and sodium triacetoxyborohydride (1.84 g, 8.69 mmol) were reacted at room temperature. Chromatography (0-10% ethyl acetate /dichloromethane gradient, with 0.5% Et_3N). Compound **11** Blue crystals (1.264 g, 81%): M.p 117-120 °C. FTIR (KBr) ν: 3457, 3318, 1634, 1593, 735 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 4.26 (s, 2H, 3-$NHCH_2$), 5.15 (s, 2H, 1-CH_2), 5.55 (brs, 1H, $Wh_{½}$ ~ 15 Hz, NH), 5.99-6.05 (m, 2H, H4, H5), 6.64 (dd, 1H, *J* = 6.0, 2.4 Hz, H6), 7.14 (dd, 1H, *J* = 8.0, 2.0 Hz, H6'), 7.23-7.33 (m, 5H, C_6H_5), 7.36 (d, 1H, *J* = 8.0 Hz, H5'), 7.40 (d, 1H, *J* = 2.0 Hz, H2'). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 46.8 (3-$NHCH_2$), 52.6 (1-CH_2), 107.4 (C5), 107.5 (C4), 123.6 (C6), 126.6 (C6'), 128.2 (*p*-C_6H_5), 128.3 (*o*-C_6H_5), 129.1 (*m*-C_6H_5), 129.3 (C2'), 130.9 (C5'), 131.4 (C4'), 133.0 (C3'), 136.8 (*i*-C_6H_5), 138.7 (C3), 139.2 (C1'), 158.0 (C2). ESI-MS m/z 381.2 ($M+Na^+$). HRMS m/z: 359.0710; calc. for $C_{19}H_{17}ON_2Cl_2Na$: 359.0712 ($M+Na^+$).

*8.2.3.3 1-Benzyl-3-(benzylamino)pyridin-2(1*H*)-one* **12**. 3-Amino-1-benzylpyridin-2(1*H*)-one **10** (0.330 g, 1.65 mmol), benzaldehyde (0.252 mL, 2.47 mmol) and sodium triacetoxyborohydride (0.699 g, 3.30 mmol) were reacted at room temperature. Chromatography (0-10% ethyl acetate /dichloromethane gradient, with 0.5% Et_3N). Compound **12** Pale blue crystals (0.467 g, 98%): M.p 91-95 °C. FTIR (KBr) ν: 3330, 3023, 1634, 1589, 735, 690 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 4.38 (d, 2H, *J* = 5.6 Hz, 3-NHCH_2), 5.23 (s, 2H, 1-CH_2), 5.64 (brt, 1H, *J* = 5.6 Hz, NH), 6.13 (dd, 1H, *J* = 7.0, 7.0 Hz, H5), 6.16 (dd, 1H, *J* = 7.2, 1.6 Hz, H4), 6.71 (dd, 1H, *J* = 6.8, 1.6 Hz, H6), 7.32-7.45 (m, 10H, C_6H_5). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 47.9 (3-$NHCH_2$), 52.6 (1-CH_2), 106.9 (C4), 107.7 (C5), 123.0 (C6), 127.5 (1-CH_2-*m*-C_6H_5 or *m*-C_6H_5), 127.6 (*o*-C_6H_5), 128.1 (1-CH_2-*p*-C_6H_5 or *p*-C_6H_5), 128.3 (1-CH_2-*p*-C_6H_5 or *p*-C_6H_5), 128.9 (1-CH_2-*o*-C_6H_5), 129.1 (1-CH_2-*m*-C_6H_5 or *m*-C_6H_5), 137.0 (1-CH_2-*i*-C_6H_5), 138.7 (*i*-C_6H_5), 139.2 (C3), 158.1 (C2). ESI-MS m/z 313.2 ($M+Na^+$). Anal. Calc. for $C_{19}H_{18}ON_2$: C, 78.59; H, 6.25; N, 9.65%. Found: C, 78.92; H, 6.12; N, 9.60%.

*8.2.3.4 Ethyl 2-[3'-(benzylamino)-4'-methyl-2'-oxopyridin-1'(2*H*)-yl]acetate* **22**. Ethyl 2-(3'-amino-4'-methyl-2'-oxopyridin-1'(2*H*)-yl)acetate **21** (0.875 g, 4.16 mmol), benzaldehyde (0.425 mL, 4.16 mmol) and anhydrous sodium sulfate (4 equivalents, 2.37 g, 16.6 mmol) were heated at reflux, then reacted with sodium triacetoxyborohydride (1.76 g, 8.32 mmol). Chromatography (ethyl acetate/dichloromethane using a gradient from 0 to 20% ethyl acetate, with 0.5% Et_3N). Compound **22** Brown oil (0.906 g, 72%): FTIR (KBr) ν: 3330, 2974, 1744, 1650, 1597, 1205, 731, 702 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.24 (t, 3H, *J* = 7.2 Hz, CH_2CH_3), 2.17 (s, 3H, CH_3), 4.19 (q, 2H, *J* = 7.2 Hz, CH_2CH_3), 4.31 (s, 2H, 3'-NHCH_2), 4.58 (s, 2H, H2), 5.93 (d, 1H, *J* = 7.2 Hz, H5'), 6.65 (d, 1H, *J* = 7.2 Hz, H6'), 7.18-7.31 (m, 5H, C_6H_5), NH not observed. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.2 (CH_2CH_3), 19.1 (CH_3), 51.1 (C2), 51.3 (3'-$NHCH_2$), 62.0 (CH_2CH_3), 111.4 (C5'), 126.9 (C6'), 127.4 (*p*-C_6H_5), 127.8 (*m*-C_6H_5), 128.7 (*o*-C_6H_5), 129.6 (C4'), 136.7 (C3'), 140.1 (*i*-C_6H_5), 159.6 (C2'), 168.1 (C1). ESI-MS m/z 323.1 ($M+Na^+$). HRMS m/z: 301.1535; calc. for $C_{17}H_{21}O_3N_2$: 301.1547 ($M+H^+$).

*8.2.3.5 [3-({5'-[(1''-Ethoxycarbonylmethyl-2''-oxo-1',2''-dihydro-pyridin-3''-ylamino)-methyl]-thiophen-2'-ylmethyl}-amino)-2-oxo-pyridin-1(2*H*)-yl]-acetic acid ethyl ester* **38**. Ethyl (3-amino-2-oxopyridin-1(*2H*)-yl)acetate **13** [56] (1.27 g, 6.47 mmol), 2,5-thiophenedicarboxaldehyde **37** (0.5 equivalents, 0.454 g, 3.24 mmol) and anhydrous sodium sulfate (2 equivalents, 1.84 g, 12.9 mmol) were heated at reflux then reacted with sodium triacetoxyborohydride (2.74 g, 12.9 mmol). Chromatography (ethyl acetate/dichloromethane using a gradient from 0 to 80% ethyl acetate, with 0.5% Et_3N) Compound **38** Blue crystals (1.32 g, 81%): M.p 166-169 °C. FTIR (KBr) ν: 3334, 1740, 1646, 1597, 1201, 747 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.19 (t, 6H, *J* = 7.2 Hz, 2xCH_3), 4.12 (q, 4H, *J* = 7.2 Hz,

2xCH$_2$), 4.37 (d, 4H, *J* = 6.0 Hz, 3-NHC*H*$_2$, 3"-NHC*H*$_2$), 4.66 (s, 4H, 1-CH$_2$, 1"-CH$_2$), 5.96 (t, 2H, *J* = 6.0 Hz, 2xNH), 6.08 (dd, 2H, *J* = 7.0, 7.0 Hz, H5, H5"), 6.25 (dd, 2H, *J* = 7.2, 1.6 Hz, H4, H4"), 6.83 (s, 2H, H3', H4'), 6.87 (dd, 2H, *J* = 6.8, 1.6 Hz, H6, H6"). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.0 (2xCH$_3$), 41.5 (3-NHCH$_2$, 3"-NHCH$_2$), 50.3 (1-CH$_2$, 1"-CH$_2$), 60.8 (2xCH$_2$), 106.1 (C5, C5"), 106.9 (C4, C4"), 124.4 (C6, C6"), 124.5 (C3', C4'), 137.5 (C3, C3"), 141.9 (C2', C5'), 156.9 (C2, C2"), 168.1 (2xCO). ESI-MS m/z 523.2 (M+Na$^+$). HRMS m/z: 523.1623; calc. for $C_{24}H_{28}O_6N_4SNa$: 523.1622 (M+Na$^+$).

8.2.4 General procedure for Aminolysis at room temperature: Preparation of compounds **18**, **31** and **42**.

Aminolysis was carried out in a conical micro scale reaction vessel by mixing amine (5 μL amine per mg of ester) and ester. The reaction mixture was stirred for 1 hour (**17**, **41**) or 48 hours (**30**) at room temperature. Excess amine was removed under vacuum when possible, or the reaction mixture was transferred to a conical flask and diluted with diethyl ether. The diethyl ether mixture was cooled in a freezer overnight (16 hours). Excess amine was separated from the crude solid product or oil by filtration and washing with additional diethyl ether. Crude solid was recrystallized by dissolving in hot acetone and then adding small amounts of diethyl ether, the resulting solution was cooled in a freezer overnight forming a white solid precipitate or oil. The white precipitate was filtered and washed with diethyl ether. The solution was decanted and the oil washed with ether.

8.2.4.1 2-[3-[(Aminocarbonothioyl)amino]-2-oxopyridin-1(2H)-yl]-N-propylacetamide **18**. Ethyl 2-(3'-{[(benzoylamino)carbonothioyl]amino}-2'-oxo pyridine-1'(2*H*)-yl]acetate **17** (0.040 g, 0.111 mmol) and neat propylamine (0.200 mL) were reacted. Byproduct **18** Brown unstable oil (0.026 g, 87%). ^{1}H NMR (400 MHz, CDCl$_3$) δ: 0.84 (t, 3H, *J* = 7.4 Hz, 1-NHCH$_2$CH$_2$C*H*$_3$), 1.41 (tq, 2H, *J* = 7.6, 7.4 Hz, 1-NHCH$_2$C*H*$_2$), 3.03 (dt, 2H, *J* = 6.8, 6.0 Hz, 1-NHC*H*$_2$), 4.56 (s, 2H, H2), 6.23 (dd, 1H, *J* = 7.0, 7.0 Hz, H5'), 7.30 (dd, 1H, *J* = 6.8, 1.6 Hz, H6'), 7.91 (brs, 2H, Wh$_{½}$ ~ 18 Hz, 3'-NHCSN*H*$_2$), 8.14 (brt, 1H, *J* = 5.6 Hz, 1-NH), 8.84 (dd, 1H, *J* = 7.4, 1.6 Hz, H4'), 9.27 (brs, 1H, Wh$_{½}$ ~ 41 Hz, 3'-NH). ESI-MS m/z 291.2 (M+Na$^+$).

8.2.4.2 2-[3'-(Benzylamino)-2'-oxopyridin-1'(2H)-yl]-N-prop-2''-yn-1''-ylacetamide **31**. Ethyl 2-(2'-oxo-3'-(benzylamino)pyridinyl-1'(*2H*)-yl acetate **30** [47](1.00 g, 3.49 mmol), and neat propargylamine (2.5 mL) were reacted. Compound **31** Colourless solid (0.897 g, 87%): M.p 171-175 °C (dec.). FTIR (KBr) ν: 3330, 3301, 3277, 1659, 1634, 1597, 723 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.12-3.13 (m, 1H, H3"), 3.88-3.90 (m, 2H, H1"), 4.27 (d, 2H, *J* = 6.0 Hz, 3'-NHC*H*$_2$), 4.53 (s, 2H, H2), 5.96-6.02 (m, 2H, H5', NH), 6.05-6.07 (m, 1H, H4'), 6.78-6.80 (m, 1H, H6'), 7.19-7.23 (m, 1H, *p*-C$_6$H$_5$), 7.28-7.31 (m, 4H, *o*, *m*-C$_6$H$_5$), 8.58 (brt, 1H, *J* = 5.2 Hz, 1-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 28.0 (C1"), 46.1 (3'-NHCH$_2$), 51.0 (C2), 73.2 (C2"), 80.9 (C3"), 105.7 (C5'), 106.5 (C4'), 124.8 (C6'), 126.7 (*p*-C$_6$H$_5$), 127.0 (*m*-C$_6$H$_5$), 128.3 (*o*-C$_6$H$_5$), 137.8 (C3'), 139.4 (*i*-C$_6$H$_5$), 157.0 (C2'), 166.8 (C1). ESI-MS m/z 318.3 (M+Na$^+$). HRMS m/z: 296.1387; calc. for $C_{17}H_{18}O_2N_3$: 296.1394 (M+H$^+$).

8.2.4.3 2-[2^a-oxo-3^a-(3^b-{4^c-[3^d-(2^e-oxo-1^e-propyl carbamoyl methyl-1^e, 2e-dihydropyridin-3^e-yl)ureido] phenyl}ureido) pyredin-1^a(2H)-yl]-N-propylacetamide **42**. 2-[3a-(3b-{4c-[3d-(1e-Ethoxycarbonylmethyl-2e-oxo-1e,2e-dihydropyridin-3e-yl)ureido]phenyl}ureido)-2a-oxo-dihydropyridin-1a(2*H*)-yl] acetic acid ethyl ester **41** (0.300 g, 0.543 mmol), and propylamine (1.5 mL) were reacted. Compound **42** Colourless solid (0.294 g, 94%): M.p > 300 °C (dec.). FTIR (KBr) ν: 3293, 3089, 2958, 1650, 1552, 1507, 1189, 735, 674 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.85 (t, 6H, *J* = 7.4 Hz, 1-NHCH$_2$CH$_2$C*H*$_3$, H3^f), 1.43 (dq, 4H, *J* = 7.2, 6.8 Hz, 1-NHCH$_2$C*H*$_2$, H2^f), 3.04 (dt, 4H, *J* = 6.8, 6.0 Hz, 1-NHC*H*$_2$, H1^f), 4.57 (s, 4H, H2, 1^e-CH$_2$), 6.22 (dd, 1H, *J* = 7.2, 7.2 Hz, H5^a, H5^e), 7.19 (dd, 1H, *J* = 7.2, 2.4 Hz, H6^a, H6^e), 7.34 (s, 4H, H2^c, H3^c, H5^c, H6^c), 8.06 (d, 2H, *J* = 7.2 Hz, H4^a, H4^e), 8.13 (t, 2H, *J* = 5.4 Hz, 1-NH, 1^e-CH$_2$CON*H*), 8.49 (s, 2H, H1^b, H3^d), 9.39 (s, 2H, H3^b, H1^d). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 11.4 (1-NHCH$_2$CH$_2$*C*H$_3$, C3^f), 22.3 (1-NHCH$_2$*C*H$_2$, C2^f), 40.4 (1-NHCH$_2$, C1^f), 51.6 (C2, 1^e-CH$_2$), 105.1 (C5^a, C5^e), 118.7 (C2^c, C3^c, C5^c, C6^c), 118.9 (C4^a, C4^e), 129.8 (C3^a, c3^e), 130.6 (C6^a, C6^e), 134.0 (C1^c, C4^c), 152.4 (C2^b, C2^d), 156.7 (C2^a, C2^e), 166.4 (C2, 1^e-CH$_2$*C*O). ESI-MS m/z 601.3 (M+Na$^+$). HRMS m/z: 601.2477; calc. for $C_{28}H_{34}O_6N_8Na$: 601.2494 (M+Na$^+$).

8.2.5 General procedure for Aminolysis at 120 °C: Preparation of compounds **23**, **25**, **26**, **39**, *and* **43**.

Aminolysis was carried out in a conical micro scale reaction vessel by mixing amine (5 μL amine per mg of ester) and ester. The reaction mixture was heated for 4 hours at 120 °C. Excess amine was removed under vacuum when possible, or the reaction mixture was transferred while still warm to a conical flask and diluted with diethyl ether. The diethyl ether mixture was cooled in a freezer overnight (16 hours). Excess amine was separated from the crude solid product by filtration and washing with additional diethyl ether. The crude solid was recrystallized by dissolving in hot acetone and then adding small amounts of diethyl ether. The resulting solution was cooled in a freezer overnight forming a white solid precipitate. The white precipitate was filtered and washed with diethyl ether.

8.2.5.1 2-(3-Amino-2-oxopyridin-1(2H)-yl)-N-propylacet amide **15** and N,N'-*Dipropylphthalamide* **16** Ethyl 2-[3'-(1",3"-dioxo-1",3"-dihydro-2"*H*-isoindol-2"-yl)-2'-oxopyridin-1'(2*H*)-yl]acetate **14** (0.378 g, 1.16 mmol), and neat 3,4-dichlorobenzylamine (1.9 mL) propylamine were reacted for 15 minutes, according to the general procedure. The crude product was isolated by column chromatography (0 to 40% ethyl acetate:dichloromethane with 0.5% triethylamine.

By-product **15** Unstable blue oil (0.047 g, 20%): LRMS (ESI) *m/z* (%): 232.1 ([M+Na]$^+$, 100); 216.1 ([M+Li]$^+$, 100). R$_f$ 0.32 (ethyl acetate:dichloromethane 2:3 with 0.5% triethylamine).

Byproduct **16** [58] White solid (0.239 g, 61%): M.p 133-135 °C (Lit. [81] 134-135 °C); ^{1}H NMR (400 MHz, CDCl$_3$) δ: 0.88 (t, 6H, *J* = 7.6 Hz, 2xCH$_3$), 1.50 (tq, 4H, *J* = 7.2, 7.2 Hz, 2xNHCH$_2$C*H*$_2$), 3.16 (dt, 4H, *J* = 6.8, 6.8 Hz, 2xNHC*H*$_2$), 7.23-7.29 (m, 4H, C$_6$H$_5$), 7.36-7.39 (m, 2H, 2xNH). ESI-MS m/z 271.2 (M+Na$^+$).

8.2.5.2 2-[3'-(Benzylamino)-4'-methyl-2'-oxopyridin-1'(2H)-yl]-N-(3'',4''-dichlorobenzyl) acetamide **23**. Ethyl 2-[3'-(benzylamino)-4'-methyl-2'-oxopyridin-1'(2*H*)-yl]acetate **22** (0.400 g, 1.33 mmol), and neat 3,4-dichlorobenzylamine (2.0 mL) were reacted. Compound **23** Colourless solid (0.572 g, 100%): M.p 166-168 °C (dec.). FTIR (KBr) ν: 3436, 3269, 2970, 1659, 1605, 740, 678 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.10 (s, 3H, CH$_3$), 4.29 (d, 2H, *J* = 6.0 Hz, 1-NHC*H*$_2$), 4.32 (d, 2H, *J* = 6.8 Hz, 3'-NHC*H*$_2$), 4.55 (s, 2H, H2), 5.07 (brt, 1H, *J* = 6.8 Hz, 3'-NH), 5.94 (d, 1H, *J* = 7.0 Hz, H5'), 6.99 (d, 1H, *J* = 7.0 Hz, H6'), 7.17-7.29 (m, 6H, *o*, *m*, *p*-C$_6$H$_5$, H6"), 7.55 (m, 2H, H2", H5"), 8.67 (brt, 1H, *J* = 6.0 Hz, 1-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 18.3 (CH$_3$), 41.0 (1-NHCH$_2$), 49.6 (3'-NHCH$_2$), 51.8 (C2), 109.5 (C5'), 126.7 (*p*-C$_6$H$_5$), 127.1 (*o*-C$_6$H$_5$), 127.2 (C4'), 127.5 (C6"), 128.1 (C6'), 128.2 (*m*-C$_6$H$_5$), 129.1

(C5''), 129.2 (C4''), 130.3 (C2''), 131.1 (C3''), 135.7 (C3'), 140.6 (C1''), 141.1 (*i*-C_6H_5), 158.4 (C2'), 167.4 (C1). ESI-MS m/z 452.1 ($M+Na^+$). HRMS m/z: 430.1073; calc. for $C_{22}H_{22}O_2N_3Cl_2$: 430.1084 ($M+H^+$).

8.2.5.3 N-Benzyl-2-oxopiperidine-3-carboxamide **25**. Ethyl 2-oxopiperidine-3-carboxylate **24** (2.00 g, 11.7 mmol), and neat benzylamine (5.0 mL) were reacted. Compound **25** Colourless solid (2.71 g, 100%): M.p 121-124 °C. FTIR (KBr) ν: 3256, 3081, 2950, 1683, 1634, 1556, 743, 694 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.55-1.62 (m, 1H, H5), 1.77-1.98 (m, 3H, H4, H5, H6), 3.13-3.18 (m, 3H, H3, H4, H6), 4.23-4.35 (m, 2H, 3-CONHCH_2), 7.19-7.32 (m, 5H, C_6H_5), 7.67 (brs, 1H, $Wh_{½}$ ~ 7 Hz, H1) 8.45 (brt, 1H, J = 5.6 Hz, 3-CON*H*). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.6 (C5), 24.6 (C4), 41.3 (C6), 42.1 (3-CONH*C*H_2), 48.1 (C3), 126.6 (*p*-C_6H_5), 127.0 (*o*-C_6H_5), 128.2 (*m*-C_6H_5), 139.4 (*i*-C_6H_5), 168.1 (C2), 170.0 (3-CO). ESI-MS m/z 255.0 ($M+Na^+$). Anal. Calc. for $C_{13}H_{16}O_2N_2$: C, 67.22; H, 6.94; N, 12.06%. Found: C, 67.51; H, 6.96; N, 12.11%.

8.2.5.4 N-(3',4'-Dichlorobenzyl)-2-oxopiperidine-3-carbox amide **26.** Ethyl 2-oxopiperidine-3-carboxylate **24** (2.00 g, 11.7 mmol), and neat 3,4-dichlorobenzylamine (5.0 mL) were reacted Compound **26** Colourless solid (3.52 g, 100%): M.p 171-174 °C (dec.). FTIR (KBr) ν: 3363, 3179, 3036, 2938, 1675, 1655, 1528, 817 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.57-1.63 (m, 1H, H5), 1.76-1.97 (m, 3H, H4, H5, H6), 3.13-3.17 (m, 3H, H3, H4, H6), 4.20 (dd, 1H, J = 8.0, 4.0 Hz, CH_2NH), 4.36 (dd, 1H, J = 8.0, 4.0 Hz, CH_2NH), 7.28 (dd, 1H, J = 8.4, 1.6 Hz, H6'), 7.55 (d, 1H, J = 8.4 Hz, H5'), 7.59 (d, 1H, J = 2.0 Hz, H2'), 7.70 (brs, 1H, $Wh_{½}$ ~ 5 Hz, H1) 8.54 (brt, 1H, J = 6.4 Hz, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.6 (C5), 24.3 (C4), 41.0 (CH_2), 41.3 (C6), 48.2 (C3), 127.3 (C6'), 128.9 (C2'), 129.0 (C4'), 130.3 (C5'), 130.8 (C3'), 140.8 (C1'), 168.0 (C2), 170.2 (3-CO). ESI-MS m/z 301.1 ($M+H^+$). Anal. Calc. for $C_{13}H_{14}O_2N_2Cl_2$: C, 51.85; H, 4.69; N, 9.30%. Found: C, 51.93; H, 4.85; N, 9.21%.

8.2.5.5 *N-(3^b,4^b-Dichlorobenzyl)-2-(3^a-{[5^c-({1^d-[(3^e,4^e-di chlorobenzylcarbamoyl) methyl] -2^d-oxo-1^d,2^d-dihydropyridin-3^d-ylamino}methyl)thiophen-2^c-ylmethyl]amino}-2^a-oxo-pyridin-1^a(2H)-yl)acetamide* **39**. [3-({5'-[(1''-Ethoxycarbonylmethyl-2''-oxo-1',2''-dihydro-pyridin-3''-ylamino)-methyl]-thiophen-2'-ylmethyl}-amino)-2-oxo-pyridin-1(2H)-yl]-acetic acid ethyl ester **38** (0.300 g, 0.599 mmol), and neat 3,4-dichlorobenzyl amine (1.5 mL) were reacted. Compound **39** Colourless solid (0.418 g, 92%): M.p 233-236 °C (dec.). FTIR (KBr) ν: 3395, 3269, 3068, 2925, 1679, 1650, 1605, 1577, 1556, 1475, 1262, 870, 805, 727 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ: 4.29 (d, 4H, J = 6.0 Hz, 2xCONHCH_2), 4.36-4.38 (m, 4H, 3^a-NHCH_2, 3^d-NHCH_2), 4.57 (s, 4H, 1^a-CH_2, 1^d-CH_2), 5.87 (t, 2H, J = 6.4 Hz, 3^a-NH, 3^d-NH), 6.05 (dd, 2H, J = 7.0, 7.0 Hz, H5^a, H5^d), 6.25 (dd, 2H, J = 7.4, 1.6 Hz, H4^a, H4^d), 6.84-6.86 (m, 4H, H6^a, H6^d, H3^c, H4^c), 7.27 (dd, 2H, J = 8.4, 2.0 Hz, H6^b, H6^e), 7.53 (d, 2H, J = 2.0 Hz, H2^b, H2^e), 7.57 (d, 2H, J = 8.0 Hz, H5^b, H5^e), 8.66 (t, 2H, J = 6.4 Hz, 2xCONH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 41.1 (2xCONHCH_2), 41.6 (3^a-NHCH_2, 3^d-NHCH_2), 51.5 (1^a-CH_2, 1^d-CH_2), 105.7 (C5^a, C5^d), 106.9 (C4^a, C4^d), 124.5 (C3^c, C4^c), 125.2 (C6^a, C6^d), 127.5 (C6^b, C6^e), 129.1 (C2^b, C2^e), 129.2 (C4^b, C4^e), 130.4 (C5^b, C5^e), 130.9 (C3^b, C3^e), 137.5 (C3^a, C3^d), 140.5 (C1^b, C1^e), 141.9 (C2^c, C5^c), 157.0 (C2^a, C2^d) 167.3 (2xCO). ESI-MS m/z 783.1 ($M+Na^+$). HRMS m/z: 759.0846; calc. for $C_{34}H_{31}O_4N_6Cl_4S$: 759.0876 ($M+H^+$).

8.2.5.6 *N-(3^b, 4^b-Dichlorobenzyl)-2-(3^a-{3^c-[4^d-(3^e-{1^f-[(3^g, 4^g-dichlorobenzylcarbamoyl) methyl]-2^f-oxo-1^f, 2^f-dihydropyridin-3^f-yl}ureido)phenyl]ureido}-2^a-oxopyridin-1^a (2H)-yl)acetamide* **43**. 2-[3a-(3b-{4c-[3d-(1e-Ethoxy carbonyl methyl-2e-oxo-1e,2e-dihydropyridin-3e-yl)ureido]phenyl}ureido)-2a-oxo-dihydro pyridin-1a(2H)-yl]acetic acid ethyl ester **41** (0.300 g, 0.543 mmol), and neat 3,4-dichlorobenzyl amine (1.5 mL) were reacted. Compound **43** Colourless solid (0.318 g, 72%) with a minor presence of **41** (10%): M.p > 320 °C (dec.). FTIR (KBr) ν: 3326, 3085, 1650, 1585, 1544, 1507, 1193, 747 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ: 4.31 (d, 4H, J = 6.0 Hz, 1-NHCH_2, 1^f-CH_2CONHCH_2), 4.67 (s, 4H, H2, 1^f-CH_2), 6.25 (dd, 2H, J = 7.0, 7.0 Hz, H5^a, H5^f), 7.23 (dd, 2H, J = 6.8, 1.6 Hz, H6^a, H6^f), 7.27-7.29 (m, 2H, H6^b, H6^g), 7.34 (s, 4H, H2^d, H3^d, H5^d, H6^d), 7.53 (d, 2H, J = 2.0 Hz, H2^b, H2^g), 7.58 (d, 2H, J = 8.4 Hz, H5^b, H5^g), 8.07 (dd, 2H, J=7.2, 1.6 Hz, H4^a, H4^f), 8.49 (s, 2H, H1^c, H3^e), 8.73 (t, 2H, J = 6.0 Hz, 1-NH, 1^f-CH_2CONH), 9.41 (2, 2H, H3^c, H1^e). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 41.8 (1-NHCH_2, 1^f-CH_2CONHCH_2), 52.4 (C2, 1^f-CH_2), 106.0 (C5^a, C5^f), 119.3-119.7 (C4^a, C4^f, C2^d, C3^d, C5^d, C6^d), 128.2 (C6^b, C6^g), 129.9 (C2^b, C2^g), 130.0 (C4^b, C4^g), 130.6 (C6^a, C6^f), 131.1 (C5^b, C5^g), 131.3 (C3^a, C3^f), 131.6 (C3^b, C3^g), 134.7 (C1^d, C4^d), 141.2 (C1^b, C1^g), 153.1 (C2^c, C2^e), 157.5 (C2^a, C2^f), 167.7 (C1, 1^f-CH_2CO). ESI-MS m/z 835.0 ($M+Na^+$). HRMS m/z: 833.0929; calc. for $C_{36}H_{30}N_8O_6Cl_4$: 833.0935 ($M+H^+$).

8.2.6 General procedure for benzoylation: Preparation of compounds **27-29**.

Benzoyl chloride (2.0 equiv) or 2-chlorobenzoyl chloride (2.0 equiv) and carboxamide (1.0 equiv) were refluxed in dry toluene for 16 hours. The toluene was removed under reduced pressure. The crude product was purified by silica gel column chromatography (ethyl acetate/dichloromethane 5% with 0.5% Et_3N).

8.2.6.1 1-Benzoyl-N-*benzyl-2-oxopiperidine-3-carboxamide* **27**. Colorless solid (0.597 g, 82%): M.p 145-147.5 °C (dec.); FTIR (KBr) ν: 3265, 3089, 2942, 1693, 1683, 1283, 731, 702 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.81-1.92 (m, 1H, H5), 2.02-2.12 (m, 3H, 2xH4, H5), 3.53 (t, 1H, *J* = 6.8 Hz, H3), 3.66-3.78 (m, 2H, H6), 4.25-4.35 (m, 2H, CH_2), 7.21-7.31 (m, 5H, $CH_2C_6H_5$), 7.36-7.39 (m, 2H, *m*-C_6H_5), 7.48-7.52 (m, 1H, *p*-C_6H_5), 7.64-7.66 (m, 2H, *o*-C_6H_5), 8.60 (brt, 1H, *J* = 6.0 Hz, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.3 (C5), 25.6 (C4), 42.2 (CH_2), 46.0 (C6), 50.9 (C3), 126.8 (*p*-$CH_2C_6H_5$), 127.1 (*o*-$CH_2C_6H_5$), 128.0 (*m*-C_6H_5), 128.1 (*o*-C_6H_5), 128.3 (*m*-$CH_2C_6H_5$), 131.5 (*p*-C_6H_5), 135.7 (*i*-C_6H_5), 139.1 (*i*-$CH_2C_6H_5$), 169.5 (3-CO), 171.1 (C2), 174.2 (1-CO). ESI-MS m/z 359.2 ($M+Na^+$). Anal. Calc. for $C_{20}H_{20}O_3N_2$: C, 71.41; H, 5.99; N, 8.33%. Found: C, 71.61; H, 5.90; N, 8.34%.

8.2.6.2 1-Benzoyl-N-*(3',4'-dichlorobenzyl)-2-oxopiperidine-3-carboxamide* **28**. Colourless solid (0.599 g, 89%): M.p 144-146 °C (dec.). FTIR (KBr) ν: 3269, 3072, 1687, 1655, 1287, 719 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.83-1.90 (m, 1H, H5), 2.01-2.12 (m, 3H, H5, 2xH4), 3.54 (t, 1H, *J* = 6.8 Hz, H3), 3.64-3.77 (m, 2H, H6), 4.24-4.35 (m, 2H, CH_2), 7.23 (dd, 1H, *J* = 8.4, 2.0 Hz, H6'), 7.35-7.38 (m, 2H, *m*-C_6H_5), 7.48-7.51 (m, 2H, *p*-C_6H_5, H2'), 7.54 (d, 1H, *J* = 8.4 Hz, H5'), 7.62-7.64 (m, 2H, *o*-C_6H_5), 8.67 (t, 1H, *J* = 6.0 Hz, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.3 (C5), 25.4 (C4), 41.1 (CH_2), 46.0 (C6), 50.9 (C3), 127.4 (C6'), 127.9 (*m*-C_6H_5), 128.0 (*o*-C_6H_5), 129.1 (C2'), 129.2 (C4'), 130.4 (C5'), 130.8 (C3'), 131.5 (*p*-C_6H_5), 135.7 (*i*-C_6H_5), 140.4 (C1'), 169.6 (3-*C*O), 171.0 (C2), 174.2 (1-*C*O). ESI-MS m/z 427.1 ($M+Na^+$). HRMS m/z: 405.0777; calc. for $C_{20}H_{19}O_3N_2Cl_2$: 405.0767 ($M+H^+$).

8.2.6.3 N-*Benzyl-1-(2'-chlorobenzoyl)-2-oxopiperidine-3-carboxamide* **29**. Colorless solid (0.597 g, 75%): M.p 102-105 °C. FTIR (KBr) ν: 3314, 3072, 2946, 1704, 1687, 1642, 1160, 751, 719 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.81-1.88 (m, 1H, H5), 2.02-2.09 (m, 3H, H4, H5, H6), 3.53 (t, 1H, *J* = 7.2 Hz, H3), 3.73-3.88 (m, 2H, H4, H6), 4.25 (d, 2H, *J* = 6.0 Hz, 3-

CONHC*H*$_2$), 7.19-7.46 (m, 9H, C_6H_5, H3', H4', H5', H6'), 8.50 (t, 1H, *J* = 5.8 Hz, 3-CON*H*). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.3 (C5), 24.7 (C4), 42.2 (3-CONH*C*H$_2$), 44.7 (C6), 50.9 (C3), 126.8 (*p*-C_6H_5), 126.9 (C3' or C4' or C5' or C6'), 127.1 (*o*-C_6H_5), 127.5 (C3' or C4' or C5' or C6'), 128.2 (*m*-C_6H_5), 128.8 (C2'), 129.2 (C3' or C4' or C5' or C6'), 130.3 (C3' or C4' or C5' or C6'), 137.6 (C1'), 139.0 (*i*-C_6H_5), 168.8 (3-*C*O), 169.4 (1-CO), 170.3 (C2). ESI-MS m/z 393.2 (M+Na$^+$). HRMS m/z: 371.1152; calc. for $C_{20}H_{20}O_3N_2Cl$: 371.1157 (M+H$^+$).

8.2.7 Preparation of Ethyl 2-[3'-(1'',3''-dioxo-1'',3''-dihydro-2''H-isoindol-2''-yl)-2'-oxopyridin-1'(2H)-yl]acetate **14**.

Ethyl (3-amino-2-oxopyridin-1(*2H*)-yl)acetate **13** [56] (1 equivalent, 0.950 g, 4.84 mmol) was dissolved in dry toluene (25 mL), phthalic anhydride (2.0 equivalents, 1.43 g, 9.68 mmol) and anhydrous $MgSO_4$ (10.0 equivalents, 5.82 g, 48.4 mmol) was added, and the resulting mixture heated at reflux for 16 hours. Excess toluene was removed under reduced pressure and the resulting mixture dissolved in ethyl acetate (50 mL) and washed with aqueous hydrochloric acid (2M, 1 x 25 mL), potassium carbonate (1M, 1 x 25 mL), brine (2 x 25 mL), and dried (anhydrous $MgSO_4$). The crude mixture was concentrated under reduced pressure, and resulting oil purified by silica column chromatography (ethyl acetate/dichloromethane 0-20% with 0.5% Et_3N). Compound **14** Colourless oil (1.28 g, 81%). FTIR (KBr) ν: 3477, 2978, 1728, 1663, 1389, 1201, 719 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.23 (t, 3H, *J* = 7.2 Hz, CH_3), 4.17 (q, 2H, *J* = 7.2 Hz, CH_2), 4.71 (s, 2H, H2), 6.33 (dd, 1H, *J* = 7.0, 7.0 Hz, H5'), 7.50-7.54 (m, 2H, H4', H6'), 7.75-7.78 (m, 2H, H4'', H7''), 7.84-7.87 (m, 2H, H5'', H6''). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.8 (CH_3), 50.8 (C2), 61.6 (CH_2), 104.8 (C5'), 122.8 (C3'), 123.5 (C4'', C7''), 131.7 (C3a'', C7a''), 134.3 (C5'', C6''), 139.5 (C6'), 140.3 (C4'), 158.5 (C2'), 166.5 (C1'', C3''), 167.1 (C1). ESI-MS m/z 349.1 (M+Na$^+$). HRMS m/z: 349.0801; calc. for $C_{17}H_{14}O_5N_2Na$: 349.0795 (M+Na$^+$).

8.2.8 Preparation of Ethyl 2-(3'-{[(benzoylamino)carbonothioyl]amino}-2'-oxopyridin-1'(2H)-yl] acetate **17.**

Benzoyl isothiocyanate (1.1 equivalents, 0.149 mL, 1.11 mmol) was added dropwise under nitrogen to a solution of ethyl (3-amino-2-oxopyridin-1(*2H*)-yl)acetate **13** [56] (0.197 g, 1.01 mmol) in dry tetrahydrofuran (5 mL). The resulting mixture was stirred 2 hours at room temperature, then concentrated under reduced pressure and the resulting suspension was diluted with diethyl ether and cooled in a freezer over night. The crude solid was filtered, washed with diethyl ether and then recrystallized (acetone/diethylether). Compound **17** Colourless solid (0.354 g, 98%): M.p 180-181 °C (dec.). FTIR (KBr) ν: 3420, 2974, 2361, 1740, 1540, 1520, 1225, 756, 702 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.22 (t, 3H, *J* = 7.2 Hz, CH_3), 4.16 (q, 2H, *J* = 7.2 Hz, CH_2), 4.82 (s, 2H, H2), 6.41 (dd, 1H, *J* = 7.2, 7.2 Hz, H5'), 7.51-7.58 (m, 3H, H6', *m*-C_6H_5), 7.63-7.67 (m, 1H, *p*-C_6H_5), 7.94-7.97 (m, 2H, *o*-C_6H_5), 9.30 (dd, 1H, *J* = 7.2, 1.6 Hz, H4'), 11.57 (s, 1H, 3'-NHCSN*H*), 3'-NH not observed. ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.0 (CH_3), 50.8 (C2), 61.2 (CH_2), 104.6 (C5'), 125.0 (C4'), 128.4 (*m*-C_6H_5), 128.6 (C3'), 128.7 (*p*-C_6H_5), 132.0 (*i*-C_6H_5), 133.1 (*p*-C_6H_5), 134.3 (C6'), 157.3 (C2'), 167.7 (C1), 167.8 (3'-NHCSNH*C*O), 176.8 (3'-NH*C*S). ESI-MS m/z 382.2 (M+Na$^+$). HRMS m/z: 382.0850; calc. for $C_{17}H_{17}O_4N_3SNa$: 382.0832 (M+Na$^+$).

8.2.9 Preparation of Benzyl azide **32**.

Benzyl bromide (1.0 equivalent, 0.695 mL, 5.85 mmol) was added dropwise to a solution of sodium azide (2 equivalents, 0.760 g, 11.7 mmol) in (3:1 acetone/water, 24 mL) and the resulting mixture stirred at room temperature for 1 hour. The reaction was diluted with water (20 mL) and extracted with ethylacetate (3 x 30 mL). The combined layers were washed with brine (2 x 20 mL), dried (anhydrous $MgSO_4$) and concentrated under reduced pressure to give compound **32** as a crude colourless oil (0.793 g, 100%). Due to instability **32** was diluted with dry methanol (100 mL) to give a 0.117 M stock solution that was kept under nitrogen in a Schlenk tube and used in the next step.

8.2.10 Preparation of 2-[3'-(Benzylamino)-2'-oxopyridin-1'(2H)-yl]-N-(1''-benzyl-1H-1'',2'',3''-triazol-4''-yl)acetamide **33**.

Ethyl 2-(2'-oxo-3'-(benzylamino)pyridinyl-1'(*2H*)-yl acetate (187) (0.100 g, 0.339 mmol), was stirred with ascorbic acid (40 mol%, 0.0239 g, 0.135 mmol) and $CuSO_4 \cdot 5H_2O$ (20 mol%, 0.0169 g, 0.0677 mmol) in a solution of methanol and water (9:1). Benzyl azide **32** was added dropwise and the resulting mixture stirred at room temperature for 2 hours to give a colourless precipitate. The precipitate was filtered from the reaction solution and washed with cold methanol, resulting solid was recrystalised (methanol/acetone/diethyl ether). Compound **33** Colourless solid (0.124 g, 86%): M.p 192-194 °C (dec.). FTIR (KBr) ν: 3281, 3085, 1687, 1642, 1589, 1479, 1266, 731, 698 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.27 (d, 2H, *J* = 6.4 Hz, 3'-NHC*H*$_2$), 4.31 (d, 2H, *J* = 5.6 Hz, 1-NHC*H*$_2$), 4.52 (s, 2H, H2), 5.56 (s, 2H, 1''-CH_2), 5.93 (t, 1H, *J* = 6.4 Hz, 3'-NH), 5.99 (dd, 1H, *J* = 7.0, 7.0 Hz, H5'), 6.07 (dd, 1H, *J* = 7.2, 1.6 Hz, H4'), 6.78 (dd, 1H, *J* = 6.8, 1.6 Hz, H6'), 7.19-7.25 (m, 1H, 3'-NHCH$_2$-*p*-C_6H_5 or 1''-CH_2-*p*-C_6H_5), 7.28-7.38 (m, 9H, C_6H_5), 7.99 (s, 1H, H5''), 8.61 (t, 1H, *J* = 5.6 Hz, 1-NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 34.3 (1-NH*C*H$_2$), 46.1 (3'-NHCH$_2$), 51.2 (C2), 52.7 (1''-CH_2), 105.7 (C5'), 106.5 (C4'), 123.1 (C5''), 124.8 (C6'), 126.7 (3'-NHCH$_2$-*p*-C_6H_5 or 1''-CH_2-*p*-C_6H_5), 127.0 (3'-NHCH$_2$-*o*-C_6H_5), 128.0 (1''-CH_2-*o*-C_6H_5), 128.1 (3'-NHCH$_2$-*p*-C_6H_5 or 1''-CH_2-*p*-C_6H_5), 128.3 (3'-NHCH$_2$-*m*-C_6H_5 or 1''-CH_2-*m*-C_6H_5), 128.7 (3'-NHCH$_2$-*m*-C_6H_5 or 1''-CH_2-*m*-C_6H_5), 136.0 (1''-CH_2-*i*-C_6H_5), 137.8 (C3'), 139.4 (3'-NHCH$_2$-*i*-C_6H_5), 144.9 (C4''), 157.0 (C2'), 167.0 (C1). ESI-MS m/z 451.2 (M+Na$^+$). HRMS m/z: 451.1831; calc. for $C_{24}H_{24}O_2N_6Na$: 451.1853 (M+Na$^+$).

8.2.11 Preparation of N-[1-({2'-[2''-(3'''-Benzoylamino-2'''-oxo-pyridin-1'''(2H)-yl)acetylamino] ethylcarbamoyl}methyl)-2-oxo-1,2-dihydropyridin-3-yl]benzamide **36**.

N-{1-[2'-(2''-Aminoethylamino)-2'-oxoethyl]-2-oxo-1,2-dihydro pyridin-3-yl} benzamide **34** [47] (0.050 g, 0.159 mmol), and ethyl [3-(benzoylamino)-2-oxopyridin-1(*2H*)-yl]acetate **35** [56] (2 equivalents, 0.096 g, 0.318 mmol) were mixed together and the resulting powder transferred to a conical micro-scale reaction vessel. The solid mixture was reacted at 120 ^{o}C for 4 hrs. Heating was carried out without stirring until a solution had formed, and then with stirring until all of the eliminated ethanol was evaporated and a solid was re-formed. The crude reaction mixture was transferred and re-dissolved in warm acetone with small amounts of methanol if needed. Small amounts of diethyl ether were then added to the resulting solution before it was cooled in a freezer overnight, forming a white solid precipitate. The white precipitate was filtered and washed with diethyl ether. Compound **36** Colourless solid (0.083 g, 92%): M.p 282-285 °C (dec.). FTIR (KBr) ν: 3379, 3285, 3089, 1650, 1593, 1520, 1368, 760, 711 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.18 (s, 4H, H1', H2') 4.64 (s, 4H, CH_2, H2''), 6.34 (dd, 2H, *J* = 7.2, 7.2 Hz, H5, H5'''), 7.42 (dd, 2H, *J* = 6.8, 1.6 Hz, H6, H6'''), 7.49-7.53 (m, 4H, *m*-C_6H_5, *m*-C_6H_5'''), 7.57-7.61 (m, 2H, *p*-C_6H_5, *p*-C_6H_5'''), 7.89 (d, 2H, *J*=7.2 Hz, *o*-C_6H_5, *o*-C_6H_5'''), 8.25 (s, 2H, 2xNH), 8.28 (dd, 2H, *J* = 7.6, 1.6 Hz, H4, H4'''), 9.28 (s, 2H, 3-NH, 3'''-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 38.3 (C1', C2'), 51.7

(CH_2, C2''), 105.0 (C5, C5'''), 123.8 (C4, C4'''), 127.1 (*o*-C_6H_5, *o*-C_6H_5'''), 128.0 (C3, C3'''), 128.8 (*m*-C_6H_5, *m*-C_6H_5'''), 132.0 (*p*-C_6H_5, *p*-C_6H_5'''), 133.7 (C6, C6'''), 133.9 (*i*-C_6H_5, *i*-C_6H_5'''), 157.1 (C2, C2'''), 164.8 (3-NH*C*O, 3'''-NH*C*O), 166.6 (CO, C1'''). ESI-MS m/z 591.1 ($M+Na^+$). HRMS m/z: 591.1965; calc. for $C_{30}H_{28}O_6N_6Na$: 591.1963 ($M+Na^+$).

8.2.12 Preparation of 2-[3^a-(3^b-{4^c-[3^d-(1^e-Ethoxycarbonylmethyl-2^e-oxo-1^e,2^e-dihydropyridin-3^e-yl) ureido]phenyl}ureido)-2^a-oxo-dihydropyridin-1^a(2H)-yl]acetic acid ethyl ester **41**.

Ethyl (3-amino-2-oxopyridin-1(*2H*)-yl)acetate (61) (1 equivalent, 1.27 g, 6.48 mmol), and 1,4-phenylene diisocyanate **40** (0.5 equivalents, 0.519 g, 3.24 mmol) were reacted at room temperature in dry acetonitrile for 16 hours, forming a pink precipitate. The reaction mixture was filtered and the filtrate was recrystallized (methanol/acetone/diethyl ether). Compound **41** Colourless solid. (1.50 g, 84%): M.p > 300 °C (dec.). FTIR (KBr) ν: 3326, 3297, 3085, 1740, 1642, 1597, 1552, 1532, 1512, 1189, 743, 707 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.21 (t, 6H, *J* = 7.2 Hz, 2xCH_3), 4.15 (q, 4H, *J* = 7.2 Hz, 2xCH_2), 4.77 (s, 4H, 3^e-CH_2, 3^a-CH_2), 6.28 (dd, 2H, *J* = 7.2, 7.2 Hz, H5^a, H5^e), 7.27 (dd, 2H, *J* = 6.8, 1.2 Hz, H6^a, H6^e), 7.34 (s, 4H, H2^c, H3^c, H5^c, H6^c), 8.08-8.10 (m, 2H, H4^a, H4^e), 8.53 (s, 2H, H1^b, H3^d), 9.39 (s, 2H, H3^b, H1^d). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.0 (2xCH_3), 50.7 (1^a-CH_2, 1^e-CH_2), 61.0 (2xCH_2), 105.7 (C5^a, C5^e), 118.7 (C2^c, C3^c, C5^c, C6^c), 119 (C4^a, C4^e), 129.9 (C3^a, c3^e, C6^a, C6^e), 134.0 (C1^c, C4^c), 152.4 (C2^b, C2^d), 156.8 (C2^a, C2^e), 167.9 (C1, 1^e-CH_2*C*O). ESI-MS m/z 575.2 ($M+Na^+$). HRMS m/z: 575.1861; calc. for $C_{26}H_{28}O_8N_6Na$: 575.1880 ($M+Na^+$).

9. X-ray structure determination

A full cif deposition resides with the Cambridge Crystallographic Data Centre, CCDC for **8**, **11**, **19**, **20** and **38**; 1477196, 1477197, 1477198, 1477199, and 1477200 respectively. Crystal data for **8**, **11**, **19**, **20** and **38**, and ORTEP plots for **19** and **20** and crystal packing diagram for **8** are described in the Supplementary data.

10. GPa inhibition assay

RMGPa (Rabbit Muscle Glycogen Phosphorylase a from Sigma) (0.475 μg/mL) activity was measured as described [33] in the direction of glycogen synthesis by the formation of inorganic phosphate from glucose-1-phosphate using a 384 well plate at 22 ^oC in 45 μL of buffer containing 50 mM Hepes (pH 7.2), 100 mM KCl, 2.5 mM EGTA, 2.5 mM $MgCl_2$, 0.25 mM glucose-1-phosphate, and 1 mg/mL glycogen with a 30 min incubation time. Phosphate was measured at 620 nm, 5 min after the addition of 150 μL of 1 M HCl containing 10 mg/mL ammonium molybdate and 0.38 mg/mL malachite green. Test compounds were added to the assay in 5 μL of 14% DMSO. Compounds were tested against a caffeine standard in 11 point concentration–response curve in duplicate on two separate occasions. Data was analyzed using GraphPad Prism v.4.03. A nonlinear regression (curve fit) analysis with a sigmoidal dose–response equation (variable slope) was applied to generate IC_{50} and Hill slope values. The reported IC_{50} had a Hill slope between 0.7 and 3.0 and a Z' value of ~0.8. Compounds were screened with maximal concentrations of 222 μM (unless indicated otherwise). The assay was carefully monitored for signs of compound insolubility. The results are presented as mean values from 4 determinations. Samples used in screening were of 98$^+$% purity (^1H NMR purity; compounds **8**, **11**, **23**, **28**, **29, 33**, **36**, **38, 39**, **41** and **42**) or 100% purity (microanalytical purity; compounds **12** and **27**) or 90+% purity (^1H NMR purity; compound **43**).

11. Computational Studies

11.1 Ligand Efficiency (LE). Ligand Efficiency [43] was calculated for a temperature of 300K using the following equation.

$LE = \Delta g = (\Delta G)/N$

where $\Delta G = -RT\ln Kd$ and N is the number of non-hydrogen atoms. The units used for LE were kcal/mol per non-hydrogen atom. Following the practice of substitution of pKd with pIC_{50}, LE can be expressed as LE = (1.37/HA) x pIC_{50} [43]. pIC_{50} values were calculated from the IC_{50} values [59]. The LE values were comparable as the assay conditions were the same for all tested compounds.

11.2 Ligand-Efficiency-dependent Lipophilicity (LELP). Ligand-Efficiency-dependent Lipophilicity [45] was calculated for compounds using the definition of LELP = the ratio of log P and ligand efficiency (LE).

Conflict of interest

None.

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Supplementary Data

Supplementary data associated with this article can be found in the online version, at……

References and Notes

[1] WHO. Global report on diabetes. Geneva: World Health Organization, 2016.
[2] M. Roden, E. Bernroider, Hepatic glucose metabolism in humans—its role in health and disease. Best Pract. Res. Cl. En. Metab.17 (2003) 365-383.
[3] N.G. Oikonomakos, L. Somsak, Advances in glycogen phosphorylase inhibitor design, Curr. Opin. Investig. Drugs 9 (2008) 379-395.
[4] L. Somsák, K. Czifrak, M. Tóth, E. Bokor, E.D. Chrysina, K.-M. Alexacou, J.M. Hayes, C. Tiraidis, E. Lazoura, D.D. Leonidas, S.E. Zographos, N.G. Oikonomakos, New inhibitors of glycogen phosphorylase as potential antidiabetic agents, Curr. Med. Chem. 15 (2008) 2933-2983.
[5] L. Agius, Physiological Control of Liver Glycogen Metabolism: Lessons from Novel Glycogen Phosphorylase Inhibitors, Mini-Rev. Med. Chem. 12 (2010) 1175-1187.
[6] L. Agius, New hepatic targets for glycaemic control in diabetes. Best Pract. Res. Cl. En. Metab. 21 (2007) 587-605.
[7] L. Somsák, Glucose derived inhibitors of glycogen phosphorylase, Comptes Rendus Chim. 14 (2011) 211-223.
[8] J. M. Hayes, A. L. Kantsadi, D. D. Leonidas, Natural products and their derivatives as inhibitors of glycogen phosphorylase: potential treatment for type 2 diabetes. Phytochem. Rev., 13(2), (2014) 471-498.
[9] N.G. Oikonomakos, V.T. Skamnaki, K.E. Tsitsanou, N.G. Gavalas, L.N. Johnson, A new allosteric site in glycogen phosphorylase b as a target for drug interactions, Structure 8 (2000) 575-584.
[10] A.L. Kantsadi, Apostolou, A. Theofanous, S. Stravodimos, G.A. Kyriakis, E. Gorgogietas, V.A. Chatzileontiadou, D.S.M. Pegiou, K. Skamnaki, V.T. Stagos, D. Kouretas, D. Psarra, A.-M.G. Haroutounian, S.A. Leonidas, D.D. Biochemical and biological assessment of the inhibitory potency of extracts from vinification

byproducts of Vitis vinifera extracts against glycogen phosphorylase. Food Chem. Toxicol. 67(2014) 35-43.
[11] M. Tóth, B. Szőcs, T. Kaszás, T. Docsa, P. Gergely, L. Somsák, Synthesis of 2-(β-D-glucopyranosylamino)-5-substituted-1,3,4-oxadiazoles for inhibition of glycogen phosphorylase, Carbohyd. Res. 381 (2013) 196-204.
[12] E. Bokor, Z. Széles, T. Docsa, P. Gergely, L. Somsák, C-Glucopyranosyl-1, 2, 4-triazol-5-ones: synthesis and inhibition of glycogen phosphorylase. Carbohyd. Res. 429 (2016) 128-134.
[13] D. Goyard, B. Kónya, A.S. Chajistamatiou, E.D. Chrysina, J. Leroy, S. Balzarin, M. Tournier, D. Tousch, P. Petit, C. Duret, P. Maurel, Glucose-derived spiro-isoxazolines are anti-hyperglycemic agents against type 2 diabetes through glycogen phosphorylase inhibition. Eur. J. Med. Chem. 108 (2016) 444-454.
[14] D.J. Hoover, S. Lefkowitz-Snow, J.L. Burgess-Henry, W.H. Martin, S.J. Armento, I.A. Stock, R.K. McPherson, P.E. Genereux, E.M. Gibbs, J.L. Treadway, Indole-2-carboxamide inhibitors of human liver glycogen phosphorylase, J. Med. Chem. 41 (1998) 2934-2938.
[15] T. Docsa, B. Marics, J. Nemeth, C. Huse, L. Somsák, P. Gergely B. Peitl, B. Insulin sensitivity is modified by a glycogen phosphorylase inhibitor: glucopyranosylidene-spiro-thiohydantoin in streptozotocin-induced diabetic rats. Curr. Top. Med. Chem. 15 (2015) 2390-2394.
[16] D.J. Baker, P.L. Greenhaff, J.A. Timmons, Glycogen phosphorylase inhibition as a therapeutic target: a review of the recent patent literature, Expert Opin. Ther. Pat. 16 (2006) 459-466.
[17] N. Gaboriaud-Kolar, A-L. Skaltsounis, Glycogen phosphorylase inhibitors: a patent review (2008 - 2012), Expert Opin. Ther. Pat. 23 (2013) 1017-1032.
[18] M. Donnier-Maréchal, S. Vidal, Glycogen phosphorylase inhibitors: a patent review (2013-2015). Expert Opin. Ther. Pat. 26 (2016) 199-212.
[19] T. Barf, Intervention of hepatic glucose production. Small molecule regulators of potential targets for type 2 diabetes therapy, Mini-Rev. Med. Chem. 4 (2004) 897-908.
[20] E.D. Chrysina, The prototype of glycogen phosphorylase, Mini-Rev. Med. Chem. 10 (2010) 1093-1101.
[21] W.A. Loughlin, Recent advances in the allosteric inhibition of glycogen phosphorylase, Mini-Rev. Med. Chem. 10 (2010) 1139-1155.
[22] B.R. Henke, Inhibition of glycogen phosphorylase as a strategy for the treatment of type 2 diabetes, RSC Drug Discov. Series 27 (2012) 324-365.
[23] J.P. Praly, S. Vidal, Inhibition of glycogen phosphorylase in the context of type 2 diabetes, with focus on recent inhibitors bound at the active site, Mini-Rev. Med. Chem. 10 (2010) 1102-1126.
[24] S. Kun, É. Bokor, G. Varga, B. Szőcs, A. Páhi, K. Czifrák, M. Tóth, L. Juhász, T. Docsa, P. Gergely, L. Somsák, New synthesis of 3-(β-D-glucopyranosyl)-5-substituted-1, 2, 4-triazoles, nanomolar inhibitors of glycogen phosphorylase. Eur. J. Med. Chem., 76 (2014) 567-579.
[25] A.A. Spasov, N.I., Chepljaeva, E.S.Vorob'ev, Glycogen phosphorylase inhibitors in the regulation of carbohydrate metabolism in type 2 diabetes. Russ. J. Bioorg. Chem. 42 (2016) 133-142.
[26] L. Somsák, V. Nagy, S. Vidal, K. Czifrák, E. Berzsényi, J.P. Praly, Novel design principle validated: Glucopyranosylidene-spiro-oxathiazole as new nanomolar inhibitor of glycogen phosphorylase, potential antidiabetic agent. Bioorg. Med. Chem. Lett. 18 (2008) 5680-5683.
[27] E. Bokor, S. Kun, T. Docsa, P. Gergely, L. Somsák, 4(5)-Aryl-2-C-glucopyranosyl-imidazoles as New Nanomolar Glucose Analogue Inhibitors of Glycogen Phosphorylase. Med. Chem. Lett. 6 (2015) 1215-1219.
[28] A.L. Kantsadi, E. Bokor, S. Kun, G.A. Stravodimos, D.S. Chatzileontiadou, D.D. Leonidas, E. Juhász-Tóth, A. Szakács, G. Batta, T. Docsa, P. Gergely, Synthetic, enzyme kinetic, and protein crystallographic studies of C-β-D-glucopyranosyl pyrroles and imidazoles reveal and explain low nanomolar inhibition of human liver glycogen phosphorylase. Eur. J. Med. Chem. 123 (2016) 737-745.
[29] S.A. Thomson, P. Banker, D.M. Bickett, J.A. Boucheron, H.L. Carter, D.C. Clancy, J. P. Cooper, S.H. Dickerson, D.M. Garrido, R.T. Nolte, A.J. Peat, Anthranilimide based glycogen phosphorylase inhibitors for the treatment of type 2 diabetes. Part 3: X-ray crystallographic characterization, core and urea optimization and in vivo efficacy. Bioorg. Med. Chem. Lett. 19 (2009) 1177-1182.
[30] K. Onda, R. Shiraki, T. Ogiyama, K. Yokoyama, K. Momose, N. Katayama, M. Orita, T. Yamaguchi, M. Furutani, N. Hamada, M. Takeuchi, Design, synthesis, and pharmacological evaluation of N-bicyclo-5-chloro-1H-indole-2-carboxamide derivatives as potent glycogen phosphorylase inhibitors. Bioorg. Med. Chem. 16 (2008)10001-10012.
[31] N.D. Karis, W.A. Loughlin, I.D. Jenkins, P.C. Healy, Glycogen phosphorylase inhibitory effects of 2-oxo-1,2-dihydropyridin-3-yl amide derivatives, Bioorg. Med. Chem. 17 (2009) 4724-4733.
[32] A. Pautsch, N. Stadler, O. Wissdorf, E. Langkopf, W. Moreth, R. Streicher, Molecular recognition of the protein phosphatase 1 glycogen targeting subunit by glycogen phosphorylase, J. Biol. Chem. 283 (2008) 8913-8918.
[33] W.A. Loughlin, I.D. Jenkins, N.D. Karis, S.S. Schweiker, P.C. Healy, 2-Oxo-1, 2-dihydropyridinyl-3-yl amide-based GPa inhibitors: Design, synthesis and structure-activity relationship study. Eur. J. Med. Chem., 111 (2016) 1-14.
[34] H. Zhao, Scaffold selection and scaffold hopping in lead generation: a medicinal chemistry perspective. Drug Discov. Today. 12 (2007) 149-155.
[35] P. Sauerberg, J.P. Mogensen, L. Jeppesen, L.A. Svensson, J. Fleckner, J. Nehlin, E.M. Wulff, I. Pettersson, Structure–activity relationships of dimeric PPAR agonists. Bioorg. Med. Chem. Lett. 15 (2005)1497-1500.
[36] P. R. Carlier, D.M. Du, Y.F. Han, J. Liu, E. Perola, I.D. Williams, Y.P. Pang, Dimerization of an inactive fragment of huperzine A produces a drug with twice the potency of the natural product. Angew. Chem. Int. Ed. 39 (2000) 1775-1777.
[37] N.A. Meanwell, Synopsis of some recent tactical application of bioisosteres in drug design. J. Med. Chem. 54 (2001) 2529-2591.
[38] N.D. Karis, W.A. Loughlin, I.D. Jenkins, A facile and efficient method for the synthesis of novel pyridone analogues by aminolysis of an ester under solvent-free conditions, Tetrahedron 63 (2007)12303-12309.
[39] H. C. Kolb, M.G., Finn, K.B. Sharpless, Click chemistry: diverse chemical function from a few good reactions. Angew. Chem. Int. Ed. 40 (2001) 2004-2021.
[40] V.V. Rostovtsev, L.G. Green, V.V. Fokin, K.B. Sharpless, A stepwise huisgen cycloaddition process: copper (I)-catalyzed regioselective "ligation" of azides and terminal alkynes. Angew. Chem. Int. Ed. 114 (2002) 2596-2599.
[41] M. Meldal, C.W. Tornøe, Cu-catalyzed azide-alkyne cycloaddition. Chem. Rev. 108 (2008) 2952-3015.
[42] X. Wen, J. Xia, K. Cheng, L. Zhang, P. Zhang, J. Liu, L. Zhang, P. Ni, H. Sun, Pentacyclic triterpenes. Part 5: Synthesis and SAR study of corosolic acid derivatives as inhibitors of glycogen phosphorylases, Bioorg. Med. Chem. Lett. 17 (2007) 5777-5782.
[43] A.L. Hopkins, G.M. Keserü, P.D. Leeson, D.C. Rees, C.H. Reynolds, The role of ligand efficiency metrics in drug discovery, Nature Rev. Drug Discov. 13 (2014) 105-121.
[44] G. M. Keserü, G. M. Makara, The influence of lead discovery strategies on the properties of drug candidates. Nat. Rev. Drug Discov. 8 (2009) 203-212.
[45] I.V. Tetko, V.Y. Tanchuk, Application of associative neural networks for prediction of lipophilicity in ALOGPS 2.1 program, J. Chem. Inf. Comp. Sci. 42 (2002) 1136-1145.
[46] Molinspiration Cheminformatics (2015). Accessed July –December 2015, <www.molinspiration.com>.
[47] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliver. Rev. 46 (2001) 3-26.
[48] S.J. Teague, A.M. Davis, P.D. Leeson, T. Oprea, The design of leadlike combinatorial libraries, Angew. Chem. Int. Ed. 38 (1999) 3743-3748.
[49] F. Darvas, G. Keseru, A. Papp, A.; Dorman, G.; L. Urge, P. Krajcsi. In silico and ex silico ADME approaches for drug discovery, Curr. Top. Med. Chem. 2 (2002) 1287-1304.
[50] H.F.F. Refsgaard, B.F. Jensen, P.B. Brockhoff, S.B. Padkjær, M. Guldbrandt, M.S. Christensen, In Silico Prediction of Membrane Permeability from Calculated Molecular Parameters, J. Med. Chem. 48 (2005) 805-811.
[51] D.F. Veber, S.R. Johnson, H-Y. Cheng, B.R. Smith, K.W. Ward, K.D. Kopple. Molecular Properties That Influence the Oral Bioavailability of Drug Candidates, J. Med. Chem. 45 (2002) 2615-2623.
[52] Y.H. Zhao, M.H. Abraham, J. Lee, A. Hersey, C.N. Luscombe, G. Beck, B. Sherborne, I. Cooper, Rate-limited steps of human oral absorption and QSAR studies, Pharm. Res. 19 (2002) 1446-1447.

[53] J.M. Grohol (2014). "Top 25 Psychiatric Medication Prescriptions for 2013". Psych Central. Retrieved 4 December 2015.< http://psychcentral.com/lib/top-25-psychiatric-medication-prescriptions-for-2013/>.
[54] D. Barford, L.N. Johnson. The allosteric transition of glycogen phosphorylase. Nature 340 (1989) 609-616.
[55] P.R. Whittamore, M.S. Addie, S.N. Bennett, A.M. Birch, M. Butters, L. Godfrey, P.W. Kenny, A.D. Morley, P.M. Murray, N.G. Oikonomakos, L.R. Otterbein, Novel thienopyrrole glycogen phosphorylase inhibitors: synthesis, in vitro SAR and crystallographic studies. Bioorg. Med. Chem. Lett. 16 (2006) 5567-5571.
[56] A.M. Birch, P.W. Kenny, N.G. Oikonomakos, L. Otterbein, P. Schofield, P.R. Whittamore, D.P. Whalley, Development of potent, orally active 1-substituted-3, 4-dihydro-2-quinolone glycogen phosphorylase inhibitors. Bioorg. Med. Chem. Lett. 16 (2007).394-399.
[57] A.E. Tschitschibabin, R.A. Konovalova, Über nitro-derivate von methylierten formen des α-amino-pyridins, Ber. Deut. Chem. Gesell. (B Series) 55 (1922) 1712-1717.
[58] W. Endres, Synthesis of phthalic acid diamides, Arch. Pharm. 305 (1972) 571-579.
[59] C. Selvaraj, S.K. Tripathi, K. K. Reddy, S.K. Singh, Tool development for Prediction of pIC_{50} values from the IC_{50} values - A pIC_{50} value calculator. Curr. Trends Biotech. Pharm. 5 (2011) 1104-1109.

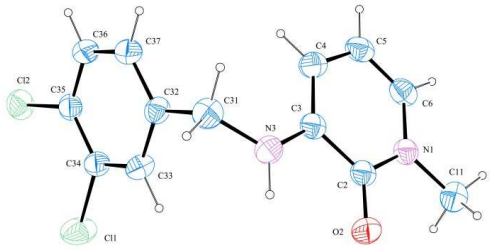

Fig. 2. ORTEP-3 diagram of the molecular structure **8**. Displacement ellipsoids for non-hydrogen atoms are drawn with 30% probability.

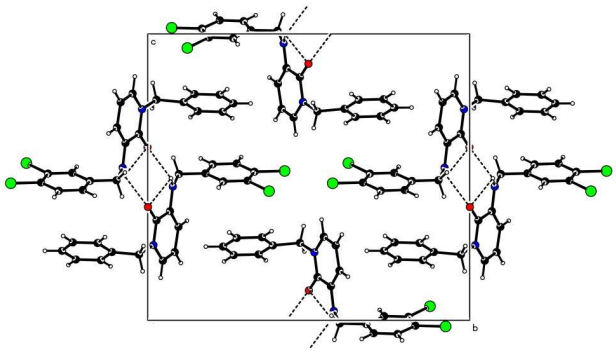

Fig. 3. ORTEP-3 diagram of the molecular structure **11**. Displacement ellipsoids for non-hydrogen atoms are drawn with 30% probability

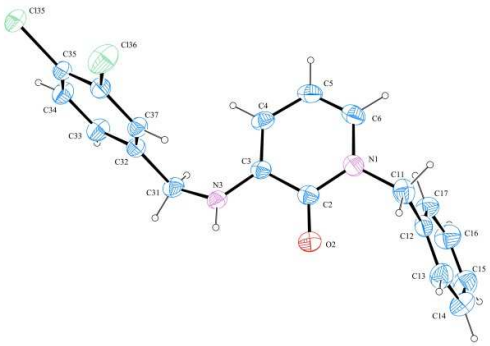

Fig. 4. Packing diagram of the molecular structure of benzyl pyridone **11** (IC_{50} = 33.0 μM).

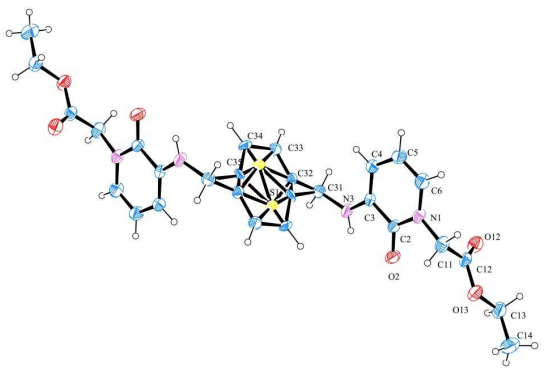

Fig. 5. ORTEP-3 diagram of the molecular structure pyridone ester **38** Displacement ellipsoids for non-hydrogen atoms are drawn with 30% probability.

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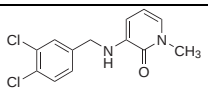
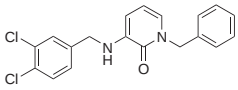
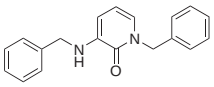
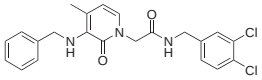
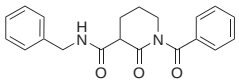
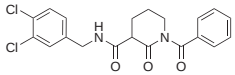
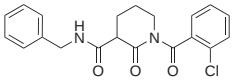
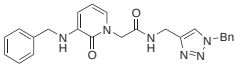
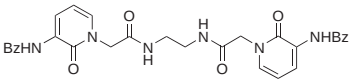
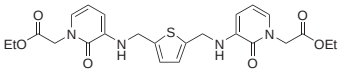
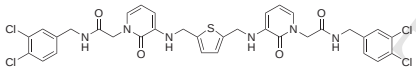
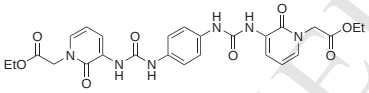
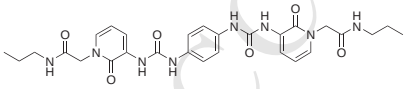
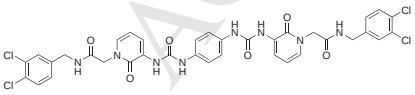
Table 1.
GP inhibition data and calculated physical data values[a] for **8, 11, 12, 23, 27-29, 33, 36, 39,** and **41-43** (Schemes 1 and 4-7).

#	Structure	MW	GPa inhibition (% at μM) or IC_{50} (μM)[b,c,d]	Log *P*	Log S (g/L)	#RB	#ON	#OHNH	TPSA
8		283	34% @ 222	2.75±0.50	0.098	3	3	1	34.0
11		359	33.0[b]	4.32±0.56	0.006	5	3	1	34.0
12		290	not active	3.10±0.58	0.140	5	3	1	34.0
23		430	38% @ 222	3.56±0.34	0.009	7	5	2	63.1
27		336	not active	2.28±0.61	0.240	4	5	1	66.5
28		405	39% @ 222	3.55±0.56	0.010	4	5	1	66.5
29		370	38% @ 222	2.91±0.56	0.049	4	5	1	66.5
33		429	60% @ 222	1.87±0.68	0.970	9	8	2	93.9
36		568	not active	0.78±1.12	0.240	11	12	4	160.4
38		500	29% @ 222	1.41±1.00	0.650	14	10	2	120.7
39		760	0.26[b]	4.61±1.20	0.00002	14	10	4	126.3
41		552	3.10[b]	0.74±1.09	0.049	12	14	4	178.9
42		578	1.60[b]	0.47±1.06	0.028	12	14	6	184.5
43		812	0.23[b]	3.88±1.19	0.000001	12	14	6	184.5

[a] Calculated with ALOPS 2.1 and Molinspiration [b] IC_{50} = Inhibition concentration at 50% inhibition for assays reaching ≥ 85% inhibition @ 2.2μM (compounds **39** and **43**), 22.2μM (compound **42**) or 222μM (compounds **11** and **41**). [c] The caffeine standard IC_{50} was 227 ± 5 μM for **11**, **12**, **23**, and **42**; and 182 ± 3 μM for **8**, **27-29**, **33**, **36**, **38**, **39**, **41** and **43**.

Table 2. Calculated Ligand Efficiency (LE) and Ligand-Efficiency-dependent Lipophilicity (LELP) of compounds **11**, **39**, **41**, **42** and **43**

Compound	IC_{50} (μM)[a]	LE	cLogP[b]	LELP
11	33.0	0.26	4.32	16.87
39	0.26	0.18	4.61	25.01
41	3.10	0.19	0.74	3.92
42	1.60	0.19	0.47	2.48
43	0.23	0.17	3.88	23.01

[a] Values as reported in Table 1

[b]Calculated with ALOGPS 2.1

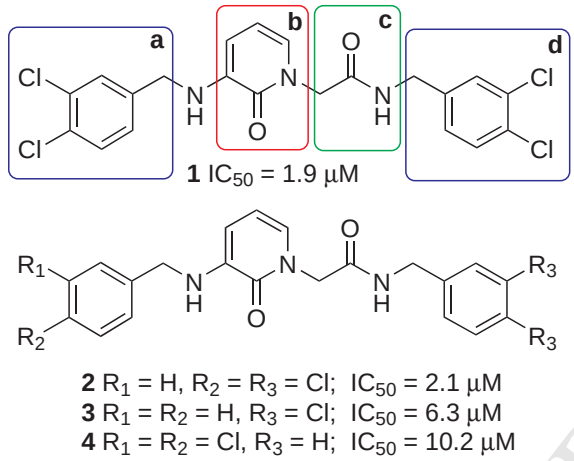
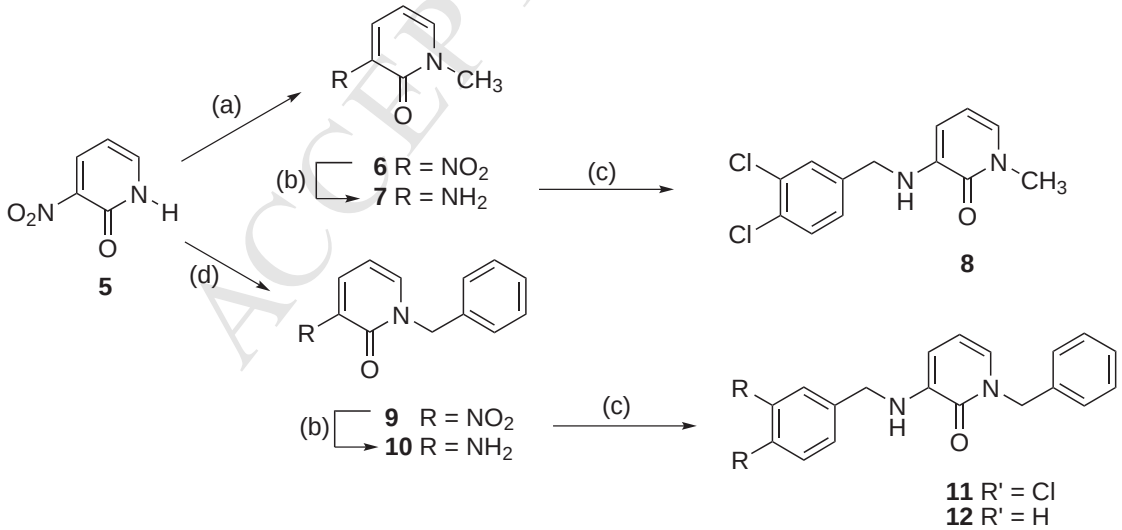
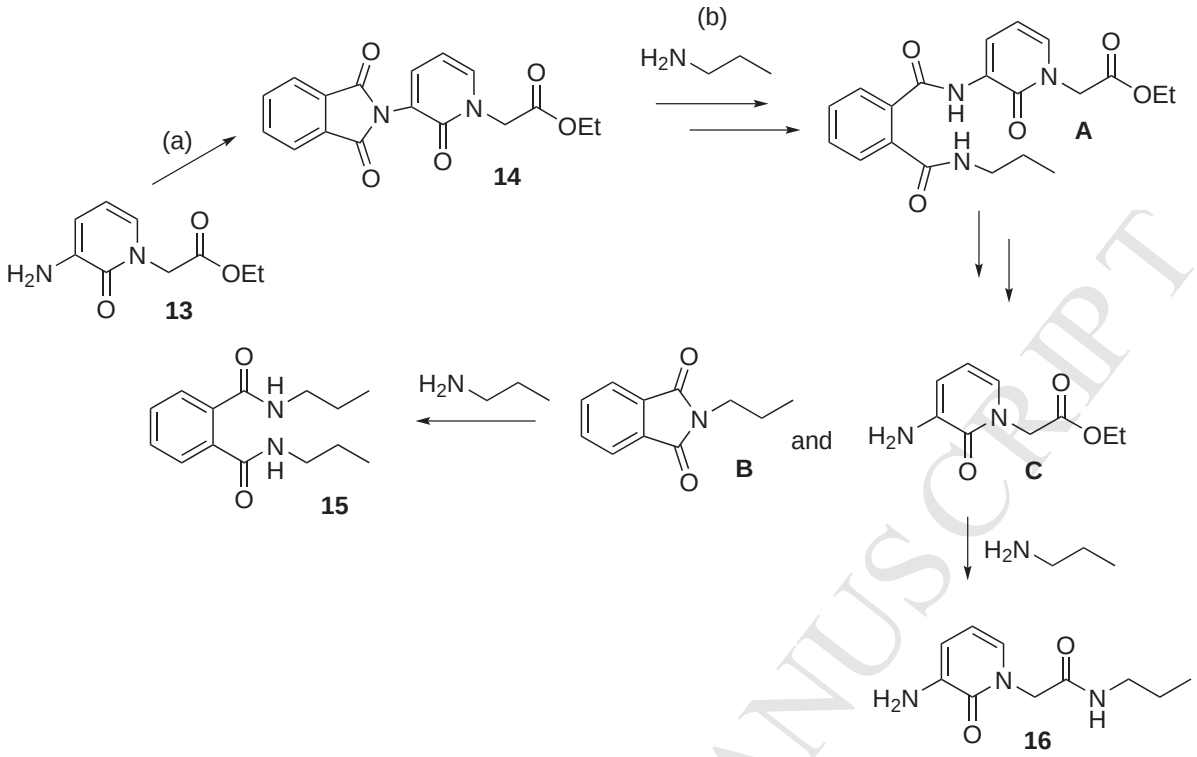


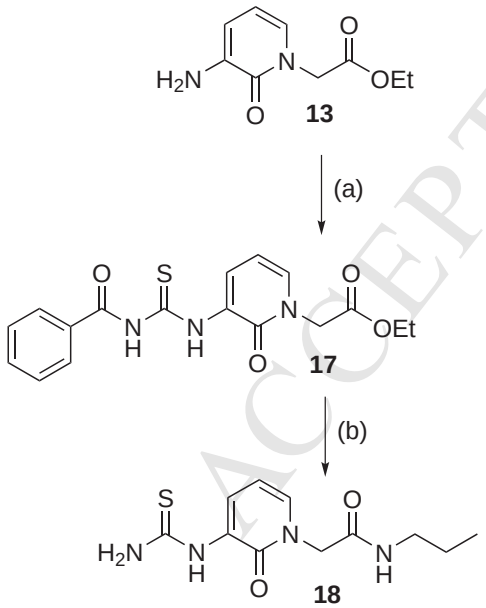
Fig. 1. Lead substituted benzyl benzyl pyridone GPa inhibitors from previous studies.



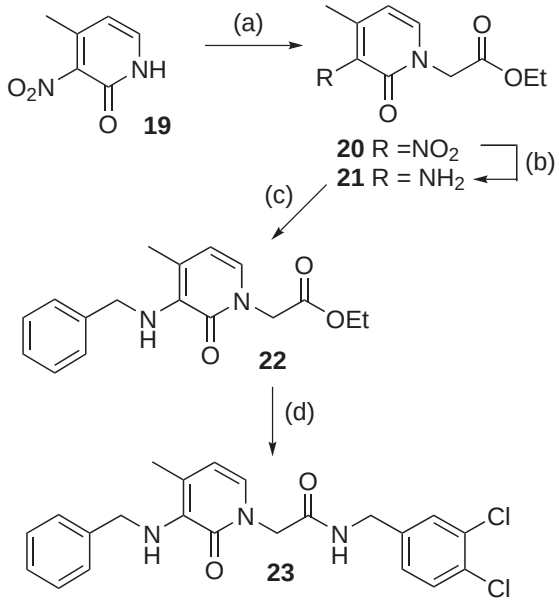
Scheme 1. (a) NaH, methyliodide, THF, 55 °C, 16 hrs; (b) H_2, Pd/C. EtOAc, 16 hrs, rt; (c) benzaldehyde or 3,4-dichlorobenzaldehyde, $NaBH(OAc)_3$, CH_2Cl_2, 16 hrs, rt; (d) NaH, benzylbromide, THF, 55 °C, 16 hrs.



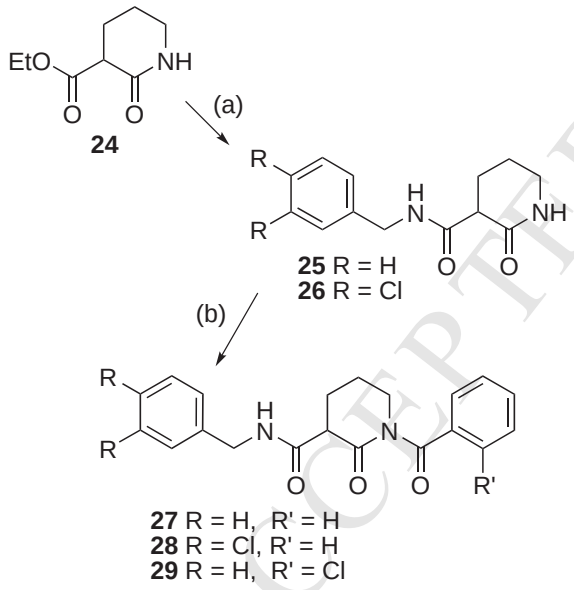
Scheme 2. (a) phthalic anhydride, toluene, reflux, 16hrs; (b) propylamine, 15 mins, rt.



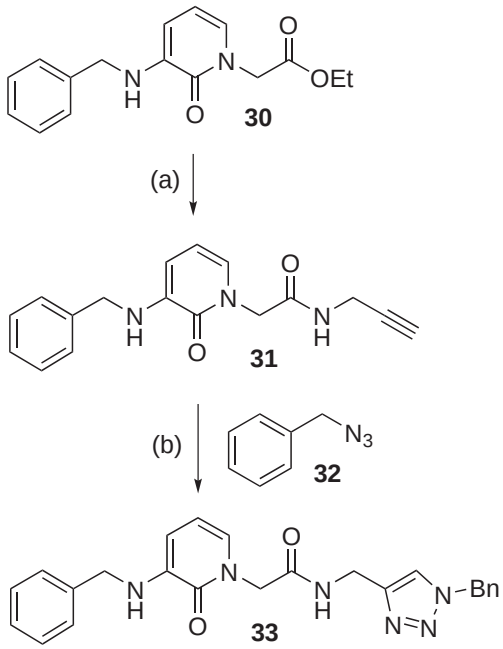
Scheme 3. (a) benzoyl isothiocyanate, THF, rt, 2hrs; (b) propylamine, 15 mins, rt.



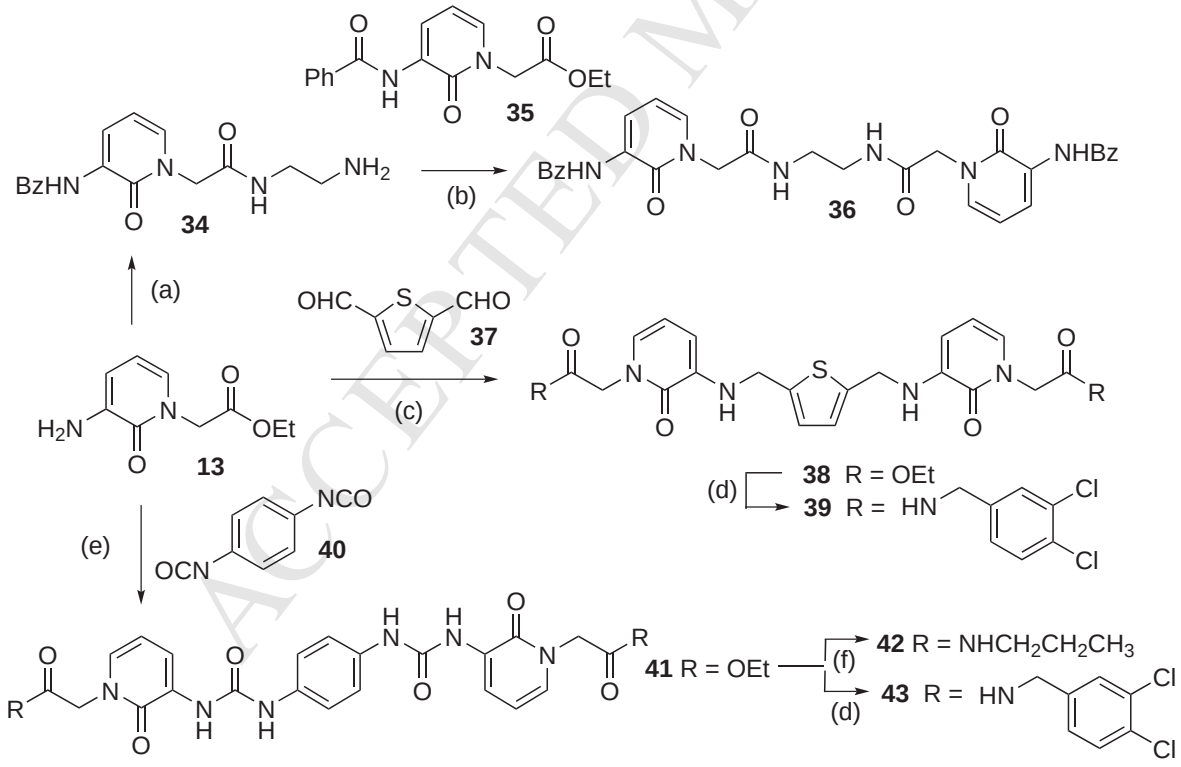
Scheme 4. (a) ethyl bromoacetate, NaH, THF, 55 °C, 36 hrs; (b) H_2, Pd/C. EtOAc, rt, 16 hrs; (c) benzaldehyde, CH_2Cl_2, reflux then $NaBH(OAc)_3$, rt, 16 hrs; (d) 3,4-dichlorobenzylamine, 120 °C, 4 hrs.



Scheme 5. (a) benzylamine or 3,4-dichlorobenzylamine, 120 °C, 4 hrs; (b) benzoyl chloride or 2-chlorobenzoyl chloride, toluene, reflux, 16hrs.



Scheme 6. (a) propargylamine, rt, 16 hrs; (b) **32**, ascorbic acid, $CuSO_4.5H_2O$, $MeOH/H_2O$, rt, 2hrs.



Scheme 7. (a) **13**, benzoyl chloride, Et_3N, CH_2Cl_2, rt, 4 hrs; then ethylenediamine, rt, 15 min; (b) solid melt 1:1, 120 ^oC, 4 hrs; (c) **37**, CH_2Cl_2, reflux, 16 hrs, then $NaBH(OAc)_3$, CH_2Cl_2, rt, 16 hrs; (d) 3,4-dichlorobenzylamine, 120 ^oC, 4 hrs; (e) **40**, CH_3CN, rt, 16hrs; (f) propylamine, rt, 1 hr.

Discovery of new nanomolar inhibitors of GPa: Extension of 2-oxo-1,2-dihydropyridinyl-3-yl amide-based GPa inhibitors

Wendy A. Loughlin, Ian D. Jenkins, N, David Karis, and Peter C. Healy

Highlights

- Design and synthesis of 14 new pyridone amide inhibitors of Glycogen Phosphorylase
- Third generation library hit rate was 36%, with 5 compounds inhibiting GPa with an IC_{50} value
- Dimers **39** and **43** were identified as new nanomolar inhibitors of GPa (IC_{50} = 230 and 260 nM)
- SAR analysis revealed sensitivity of GPa to substitution of the pyridone ring
- SAR analysis revealed some sensitivity of GPa to the presence of the amide group