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Glycogen phosphorylase inhibitory effects of 2-oxo-1,2-dihydropyridin-3-yl amide derivatives

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ABSTRACT

Glycogen phosphorylase (GP) plays a crucial role in the conversion of glycogen to glucose-1-phosphate (and in turn glucose) and is a promising target for therapeutic intervention in diabetes. In this study we synthesized new derivatives of 2-oxo-1,2-dihydropyridin-3-yl amides using a facile aminolysis reaction, in which different alkyl and aryl esters and amides are substituted at N-1 and C-3 of the heterocyclic ring. The in vitro inhibitory activity of compounds against glycogen phosphorylase was evaluated. From this series the most potent compound exhibits good GPa inhibition (IC_{50} = 6.3 μM). A preliminary study of these compounds showed that anti-GP activity was decreased by the incorporation of a C3–N carbonyl group and favored by increased lipophilicity.

1. Introduction

Glycogen phosphorylase (GP) is a therapeutic target for treating hyperglycemia,[1–4] as it plays a crucial role in the breakdown of glycogen to glucose-1-phosphate in liver and skeletal muscle. Interest in GP as a therapeutic target has increased since it was validated in diabetic *ob/ob* mice.[5] Recent work has also shown that blocking the interaction of GPa with the C-terminus of G_L-PP-1 increases glycogen synthesis in primary rat hepatocytes.[6] Of the known GP binding sites, the majority of inhibitors target either the catalytic site[3] which binds glucose,[7,8] glycogen and analogues of these;[9] the allosteric (AMP) site which binds adenosine monophosphate (AMP), inosine 5′-monophosphate, adenosine tri-phosphate, and glucose-6-phosphate;[1] the purine nucleoside site (an allosteric site also referred to as 'the inhibitor site'), which is active toward purine derivatives, such as caffeine;[10] and a range of heterocyclic compounds; or the indole site (another allosteric site) at the enzyme dimer interface which primarily binds indoles.[11–14] Recently a possible novel binding site on the surface of the protein located roughly 32 Å from any other binding site has been reported.[15] Most compounds that inhibit GP have been screened in vitro, with some inhibitors also screened in vivo. Comprehensive reviews on a range of GP inhibitors have been reported elsewhere.[1,2,4,5,16,17]

Recent inhibitors of GP are primarily glucose, purine, or indole structures, that target the catalytic site, the AMP site and the indole site, and in some cases appear to have reached a limit in potency. Discovery of new structural diversity is important to identifying new potent inhibitors of GP that are likely to be progressed through the drug discovery process. We have opted for an innovative chemogenomics strategy to identify a new molecular scaffold for inhibitor design. This approach is focused on the C-terminus of the hepatic glycogen-binding subunit G_L (encoded by the gene *PPP1R3B*) of protein phosphatase-1 (PPP1). GPa binds to residues 269–284 at the C-terminus end of the G_L-subunit of PPP-1 (G_L (*PPP1R3B*[18]). This C-terminus sequence is absent from the other glycogen-targeting subunits, G_M (*PPP1R3A*),[19] R5/PTG (*PPP1R3C*),[20,21] and R6 (*PPP1R3D*),[22] (*PPP1R3E*)[23] and (*PPP1R3F*),[24] and (*PPP1R3G*).[24] The binding site does not vary between mammalian species (rat, mouse, human), which suggests that allosteric regulation of GPa activity by G_L-PPP1 is important in mammals[25,26] and that G_L has the highest glycogenic potency.[27] In addition G_L binding occurs in the presence of caffeine and glucose. As caffeine and glucose bind to unique sites that are homologous in muscle and liver phosphorylases, G_L is not binding at the same site. During the course of our work, other studies provided evidence that a GP inhibitor can block the interaction of GPa with the G_L C-terminus[6,28,29] suggesting that this interaction could be targeted in the development of a GPa inhibitor. In the present work, a chemogenomics strategy was implemented based on the reported analysis of the homology of amino acids between rat G_L, rabbit G_M and human G_M (Fig. 1).[18]

Using this comparison (Fig. 1) and in a de novo approach, we identified the amino acid sections that were unique within the human G_L C-terminus binding sequence (Fig. 1). Considering synthetic accessibility and flexibility for generation of a library of

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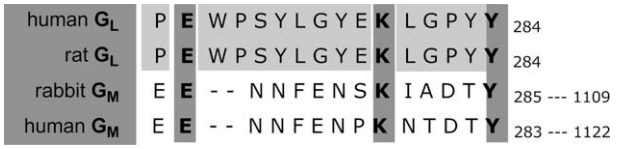


Figure 1. Comparison of the amino acid sequence homology of rat liver G_L and human liver G_L, with the N-terminal regions of G_M from rabbit and human skeletal muscle. Homologous residues are shown in bold. The sequences are taken from Figure 2 in Ref. 18.

compounds we selected as our scaffold a pyridone ring, a mimetic of the Leu-Gly residues contained within the C-terminal residues associated with activity[6,28,29] (Fig. 2). As the scaffold is to be extended to form derivatives it needs to be located along from the end C-terminal residues. Formation of derivatives (Fig. 2) by substitution of the C3 amino group, and aminolysis of the N1-ester side chain was used to generate a library of pyridone derivatives. The inhibition activity of the synthesized compounds was evaluated against GPa. Herein we report on a discovery library, with an excellent hit rate, and identify a 2-oxo-1,2-dihydropyridin-3-yl amide, bearing appropriate side chains, as a new lead compound for in vitro GPa inhibition.

2. Chemistry

The synthesis of 2-pyridone derivatives **6a–c**, **6t**, **7a–d**, **7s–x**, **8a–f**, **8t**, **9a–e**, **9g–w**, and **9y** was achieved following the steps outlined in Scheme 1. Aminopyridone **1**[30,31] was obtained from commercially available 2-hydroxy-3-nitropyridine by treatment with sodium hydride and ethyl bromoacetate, followed by reduction with hydrogen in the presence of 10% Pd/C in EtOAc.[32] Reductive amination of amine **1** with propionaldehyde gave **2** (67%), and with benzaldehyde gave **3** (98%). Alternatively, acylation of amine **1** with acetyl chloride gave **4** (98%), and with benzoyl chloride gave **5** (99%).[32] Esters **2–5** were converted to amide derivatives by using direct aminolysis (e.g., excess of amine (5 μL/mg) at a reaction temperature of 22, 90, or 120 °C, respectively, for 15 min for up to 4 h), which was previously found to be a mild and atom efficient procedure.[32] Our previous work had shown that the aminolysis proceeded readily with primary amines, and some secondary amines. This influenced the choice of amines used in the aminolysis of esters **2–5** and the selection of derivatives based on the pyridone scaffold **1**. Pyridone esters (**2–5**) were reacted with a range of

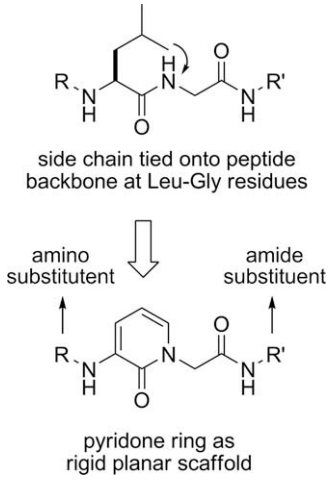


Figure 2. Pyridone mimetic of Leu-Gly residues in the C-terminus (residues 269–284) of human G_L.

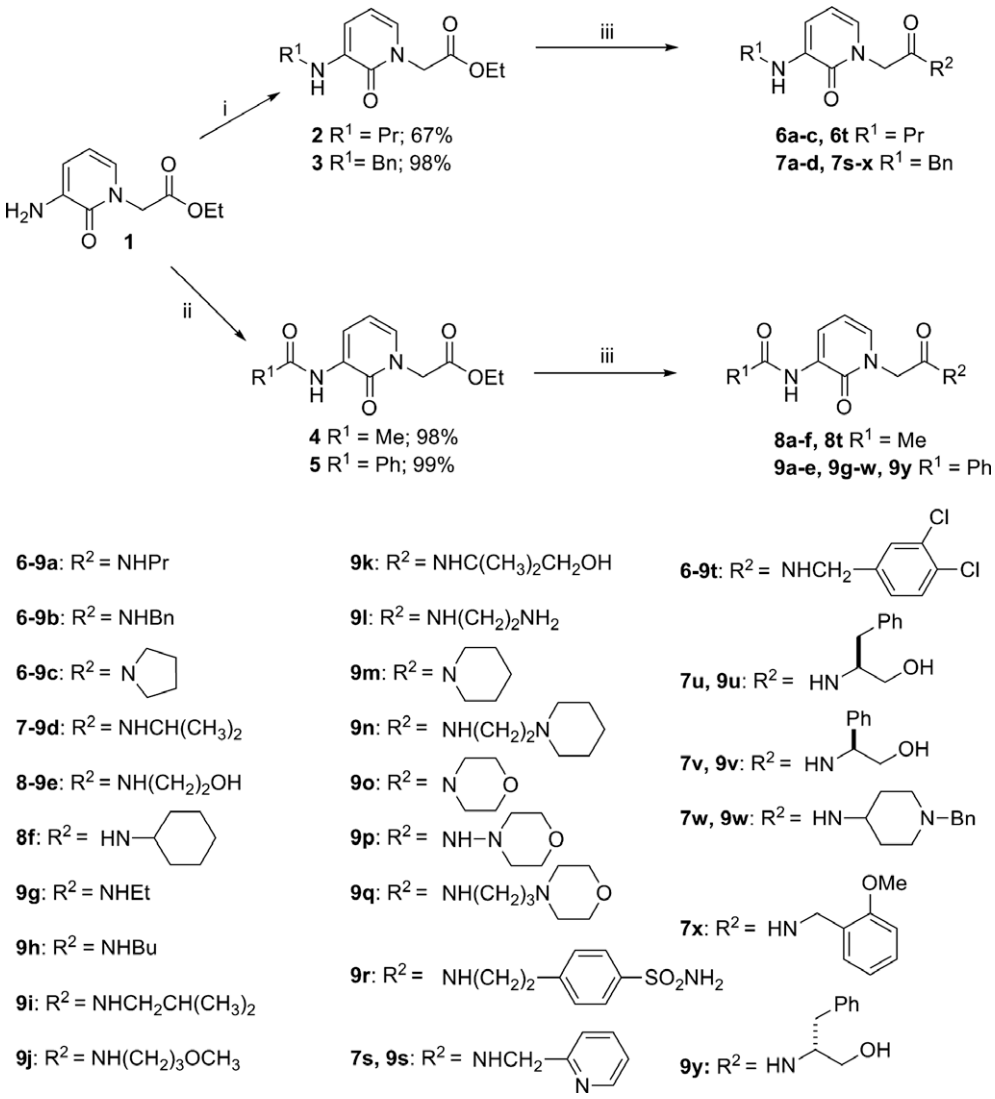
amines with lipophilic groups (e.g., ethyl, propyl, butyl, isopropyl groups; **6–9a**, **7–9d**, **9g–j**, Scheme 1), hydrophilic groups (e.g., hydroxyl group; **7u–v**, **8–9e**, **9k**, **9u–v**, Scheme 1), aromatic groups (e.g., benzyl group; **6–9b**, **6t**, **7s–x**, **9r–w**, **9y**, Scheme 1), a basic amino group (**9l**, Scheme 1), or containing a ring system (e.g., pyrrolidine, cyclohexyl group; **6–9c**, **8f**, Scheme 1) and included privileged drug-like structures,[33] such as piperidine and morpholine (**9m**, **9o**, Scheme 1) that were also attached with extension linkers such as ethylene and propylene (**9n**, **9p**, **9q**, Scheme 1). The majority of right hand modifications were carried out on easily purified and stable pyridone ester **5**. In the aminolysis procedure, amino alcohols reacted only at the amine end to give the pyridone amide (**7u–v**, **8e**, **9e**, **9k**, **9u–v**, Scheme 1). In the case of ethylenediamine (**9l**, Scheme 1), dimer formation was avoided by use of excess ethylenediamine, and mono pyridone amide **9l** was obtained exclusively. These compounds were purified by recrystallization, and characterized (Microanalysis, mass spectrometry, IR, ^{1}H, and ^{13}C NMR spectroscopy). To facilitate discussion, these derivatives will be referred to by the left, then right hand side substituents; for example, benzyl propyl pyridone **7a**.

3. X-ray structures of 7u, 7v, and 9k

Pyridones **7u**, **7v**, and **9k** formed crystals suitable for single crystal X-ray diffraction studies (see Supplementary data for representative ORTEP plots). The solid state conformation was of interest to see whether N–H groups from one pyridone molecule (in the crystal lattice) establish hydrogen bonds with the C=O groups in another pyridone molecule (in the same crystal lattice), reflective of, for example, a 'beta-strand-like' peptide conformation. The results of this work showed **7u** and **7v** to crystallize as hydrated, isomorphous structures. The water molecules occupy hydrophilic regions within the crystal lattice, forming a strong inter-molecular O–H···$O_{(carbonyl)}$, O–H···$O_{(water)}$, and O···H–$O_{(hydroxyl)}$, O···H–$N_{(amine)}$ hydrogen bonding network as illustrated in Figure 3. The amide proton forms chains of N–H···O hydrogen bonds with the hydroxyl rather than with the amide carbonyl group as usually found for amide systems. The C3–N3 bond lengths of 1.357(7) Å (**7u**) and 1.366(7) Å (**7v**) are similar to those observed in the structures of secondary anilines[34] and reflect conjugation of this bond with the pyridone ring. By comparison, the structure of **9k** crystallized with two independent molecules in the crystal lattice. For both molecules the amino substituent group is approximately co-planar with the pyridone ring. The structure of this fragment is characterized by the presence of intermolecular π···π interactions between the pyridone rings and a relative absence of hydrogen bonding interactions by the carbonyl and amine groups with intermolecular O···H–N bonding observed only for the amide carbonyl in the first molecule and the pyridone carbonyl in the second. In this structure, the change in the nitrogen atom from an amine to an amide results in an increase in the C3–N3 bond lengths to 1.403(3) and 1.398(3) Å. The amide groups within the two molecules of **9k** are oriented at right angles to the pyridone plane and exhibit strong intermolecular hydrogen bonding interactions between the carbonyl, amide and hydroxyl groups.

4. Biology

GP activity of compounds **2–5**, **6a–c**, **6t**, **7a–d**, **7s–x**, **8a–f**, **8t**, **9a–e**, **9g–w**, and **9y** was measured, using the in vitro GP screen reported in other recent studies,[28,35] in the direction of glycogen synthesis[11] by the formation of inorganic phosphate from glucose-1-phosphate.[36,37] The results for compounds that displayed levels of inhibition of GP are listed in Table 1. By comparison, an IC_{50} = 283 ± 10 μM was obtained for the caffeine standard. The



Scheme 1. Reagents and conditions: (i) For **2**, propionaldehyde, $NaBH(OAc)_3$, CH_2Cl_2, rt, 16 h, 67%; for **3**, benzaldehyde, $NaBH(OAc)_3$, CH_2Cl_2, rt, 16 h, 98%; (ii) for **4**, acetyl chloride, Et_3N, CH_2Cl_2, rt, 4 h, 98%; for **5**, benzoyl chloride, Et_3N, CH_2Cl_2, rt, 4 h, 99%; (iii) amine, 22, 90, or 120 °C, 15 min, 2, 4, or 16 h.

compounds not included in Table 1 (compounds **2–5**, **6a**, **6c**, **7a**, **7c–d**, **7s**, **8a–f**, **9a–e**, **9g–s**, **9u–w**, and **9y**), did not inhibit GPa at the maximal concentration used in the assay (i.e., <20% at 222 μM).

5. Calculation of molecular physicochemical properties

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,[38] and are listed for the active compounds **6–7b**, **6–9t**, **7u–w**, and **7x** (Table 1). The compounds in Table 1 have a predicted Log *P* <3.52 in accordance with Lipinski's rules,[39,40] and another study,[41] have molecular weights <500 and a PSA under 120 Å^2,[42] and none violate the 'rule of five' (#OHNH ⩽ 5; #ON <10; #RB <8); with only **7u** having a number of rotatable bonds (an important predictor of good oral bioavailability[42–44]) >8. Compounds could be classified into those with lower calculated solubility from the interval between 4 and 100 mg/L (compounds **6t**, **7b**, **7t**, **7x**, **8t**, **9b**, and **9t**), and those with calculated solubility from the interval between 0.1 and 3.5 g/L sufficient for fast adsorption, (compounds **6b**, **7u–w**, and **8b**). Overall, it appears that incorporation of a C3–N carbonyl group (e.g., vis-à-vis **7t** and **9t**) led to a decrease in the activity against GPa, whereas inhibition of GPa activity was generally favored by increased lipophilicity (Log *P* >1.3, Table 1; e.g., vis-à-vis **7b** and **7t**).

6. Results and discussion

Initially, 34 compounds (**2–5**, **6a–c**, **7a–d**, **8a–f**, **9a–e**, and **9g–r**, Scheme 1) were synthesized and tested for their inhibition activities against GPa. Compounds **2–5**, **6a**, **6c**, **7a**, **7c–d**, **8a–f**, **9a–e**, and **9g–r** did not inhibit GPa at the maximal concentration used in the assay (222 μM). However, propyl benzyl pyridone **6b** and benzyl benzyl pyridone **7b** inhibited GPa with estimated IC_{50} values of 343 and 34.2 μM, respectively. These values compared favorably to the value obtained for the caffeine standard (IC_{50} = 283 ± 10 μM). The estIC_{50} value for **6b** could be the result of non-specific inhibition of GPa, whereas the lower value obtained for **7b** is more likely associated with specific inhibition of GPa. We noted that a carbonyl group at position **a** (Fig. 4) appeared to lead to non-inhibition of GPa (cf. propyl benzyl pyridone **6b** (estIC_{50} = 343 μM) and acetyl benzyl pyridone **8b** (not active); benzyl benzyl pyridone **7b** (estIC_{50} = 34.2 μM) and benzoyl benzyl pyridone **9b** (not active)). Furthermore, when the benzyl and propyl group side chains were swapped (**b**↔**c**, Fig. 4),

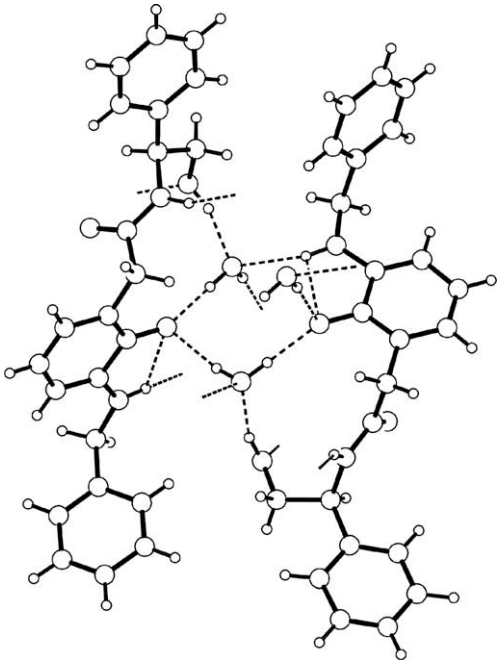


Figure 3. Representation of the H-bonding structure of **7v**.

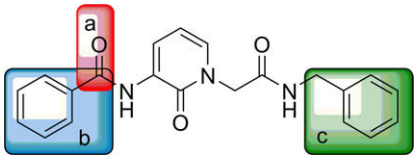


Figure 4. A preliminary analysis for pyridone amide derivatives; position **a** = carbonyl group not favored; position **b** = position **c** = aromatic group favored.

benzyl propyl pyridone **7a** did not inhibit GPa, whereas propyl benzyl pyridone **6b** displayed moderate inhibition of GPa ($estIC_{50}$ = 343 μM). From the initial compound set (compounds **2–5**, **6a–c**, **7a–d**, **8a–f**, **9a–e**, and **9g–r**, Scheme 1), benzyl benzyl pyridone **7b** represented the only compound with aromatic functional groups at both positions **b** and **c** (Fig. 4). The above results suggested that an aromatic functionality in position **c** in combination with an aromatic group at position **b** (such as benzyl) was a favorable structural combination for inhibition of GPa. Thus, we selected benzyl benzyl pyridone **7b** for structural modification of the right-hand amide aromatic substituent (position **c**), whilst maintaining the benzyl group at position **b**.

A further 12 compounds (**7s–x**, **9s–w**, **9y**) were synthesized (Scheme 1). Modification of the amide substituent at position **c** was readily achieved by changing the amine used in the aminolysis reaction, but within the constraints of the requirement for an aromatic substituent at position **c**, availability of the amine and compatibility with the aminolysis procedure (which excludes, e.g., carboxylic acid groups). Included in the second set of compounds were aromatic amines with halogen atoms (to increase lipophilicity; **7t**), with an aliphatic hydroxyl group (to increase hydrophobicity and introduce a hydrogen bonding site; **7u**, **7v**), a methoxy group (to change the electronic distribution; **7x**), a pyridine or amine group (to increase basicity; **7s**, **7w**), and a different linker (to alter the steric/position of aromatic ring; **7u–w**). Benzoyl pyridones, **9s–w**, were synthesized to provide some 'structural controls' for the GPa inhibition assay.

Compounds **7s**, **9s**, **9–w**, and **9y** did not inhibit GPa at <222 μM. Benzyl pyridones **7t–x** and benzoyl pyridone **9t** inhibited GPa as reported in Table 1. The hit rate from the initial discovery library (46 compounds) was high with six compounds (hit rate 13%) displaying $estIC_{50}$ values <350 μM. In addition, an acceptable hit was identified (compound **7t**, $estIC_{50}$ = 6.3 μM), indicating that the initial design approach shows promise. The activity observed for benzyl dichlorobenzyl pyridone **7t** appeared to support the preference for aromatic groups at positions **b** and **c** (Fig. 4). Where a direct structural comparison could be made between a benzyl or benzoyl group at position **b** (Fig. 4) inhibitory activity was lost (vis-à-vis **7u** and **9u**; **7v** and **9v**; **7w** and **9w**) or significantly reduced (vis-à-vis **7t** and **9t**) in the presence of the benzoyl group. This observation appeared to further support the earlier observation that a carbonyl group at position **a** (Fig. 4) was detrimental to the inhibitory activity of these pyridone derivatives against GPa. However, it was intriguing that one benzoyl analogue, compound **9t** (IC_{50} = 162 μM), displayed inhibitory activity toward GPa. It was also notable that the direct benzyl analog of **9t**, dichloropyridone **7t** ($estIC_{50}$ = 6.3 μM), was the most potent compound, and also contained the lipophilic dichlorobenzyl group at position **c** (Fig. 4). Two final compounds were synthesized (propylamine dichlorobenzyl pyridone **6t** and acetyl dichlorobenzyl pyridone **8t**; Scheme 1), in which the dichlorobenzyl ligand at position **c** was combined with position **b** ligands (Fig. 4) used previously in this study. Compounds **6t** and **8t** inhibited GPa (Table 1) at poorer levels than for **7t** and **9t**. Benzyl dichlorobenzyl pyridone **7t** was in the order of 25-, 60- and 300-fold more potent than **9t**, **8t**, and **6t**, respectively (Table 1). Preliminary analysis show that inclusion of a carbonyl group at position **c** (Fig. 4) was detrimental to inhibitory activity. The inhibitory activity of pyridone amide derivatives against GPa appears to be favored by aromatic ligands at positions **a** and **b** (Fig. 4).

Table 1
Calculated physical (ALOGPS 2.1) and GP inhibition data for **6**–**7b**, **6**–**9t**, **7u**–**w**, and **7x**.

#	R^1	R^2	MW	GPa % inhibition[a] or $estIC_{50}$[b] (μM)	Log *P*	Log *S*[c] (g/L)	#RB	#ON	#OHNH	TPSA
6b	Pr	NHBn	299	343 (80% at 222)	1.63 ± 0.44	0.824	7	5	2	63.1
6t	Pr	$NHCH_2(3,4\text{-}diClC_6H_4)$	368	1770 (40% at 222)	2.85 ± 0.43	0.051	7	5	2	63.1
7b	Bn	NHBn	347	34.2 (96% at 111)	2.25 ± 0.51	0.096	7	5	2	63.1
7t	Bn	$NHCH_2(3,4\text{-}diClC_6H_4)$	416	6.3 (94% at 222)	3.52 ± 0.49	0.004	7	5	2	63.1
7u	Bn	$NHCH(Bn)CH_2OH$	391	94% at 222	1.98 ± 0.50	0.167	9	6	3	83.3
7v	Bn	$NHCH(Ph)CH_2OH$	377	120.4 (40% at 222)	1.71 ± 0.50	0.273	8	6	3	83.4
7w	Bn	$NHCH(-CH_2CH_2-)_2NBn$	430	813 (45% at 222)	2.78 ± 0.68	0.201	8	6	2	66.4
7x	Bn	$CHCH_2(2\text{-}OCH_3C_6H_4)$	377	308 (38% at 222)	2.34 ± 0.60	0.067	8	6	2	72.4
8t	Me	$NHCH_2(3,4\text{-}diClC_6H_4)$	368	372 (45% at 222)	1.33 ± 0.86	0.054	5	6	2	80.2
9t	Ph	$NHCH_2(3,4\text{-}diClC_6H_4)$	430	162 (45% at 222)	3.00 ± 0.71	0.005	6	6	2	80.2

[a] Average values from 4 determinations (duplicates on two separate occasions) with a Hill slope between 0.5 and 3.0 and *Z′* values of ~0.8.
[b] Estimated IC_{50} reported with % GPa inhibition observed at maximal concentration.
[c] Calculated from Log *S* value determined from ALOGPS 2.1.

7. Conclusion

The design, synthesis and testing of a range of pyridone amides led to the identification of selected oxo-1,2-dihydropyridin-3-yl amide derivatives as a new 'type' of GPa inhibitor. The X-ray structure determination of three representative examples (**7u**, **7v**, **9k**) confirmed strong intermolecular H-bonding patterns were possible in such pyridone derivatives. The hit rate from the initial discovery library (46 compounds) was high with six compounds (hit rate 13%) displaying estIC$_{50}$ values <350 μM. In addition, an acceptable hit was identified (compound **7t**, estIC$_{50}$ = 6.3 μM) which was considerably more active than caffeine (283 μM), the commonly used standard, indicating that the initial design approach shows promise. Inhibitory activity of a compound appeared to be favored by aromatic ligands, whereas inclusion of a C3–N carbonyl group was detrimental to the potency of a compound. In the future, a more detailed SAR is required to shed insight on the positioning of specific functional groups within a defined pyridone amide. We view benzyl dichlorobenzyl pyridone **7t** as a promising hit compound for a new class of GPa inhibitors, deserving of further studies.

8. Experimental

8.1. Chemistry general

In general, reagents and solvents were purchased and used without further purification. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in the deuterated solvents indicated. Chemical shifts are reported in parts per million (δ units, relative to the reported solvent as an internal standard (e.g., $CDCl_3$ ^{13}C, 77.0 ppm). ^{1}H NMR and ^{13}C NMR spectral assignments are supported by gCOSY, gHSQC, and gHMBC analysis. Mass spectral data, MS were obtained by electrospray ionization (ESI). For preparative scale chromatography, silica gel (60–200 mesh) was used. Melting points are uncorrected. Compounds **1**, **4**, **5**, **8a–f**, **9a–e**, **9k**, **9m**, **9o**, and **9u** were prepared as previously reported.[32]

8.2. General procedure for the preparation of compounds 6a–c, 6t, 7a–d, 7s, 7t, 7w, 7x, 8t, 9g–j, 9l, 9n, 9p–q, 9s–t, 9w

Method A: Aminolysis was carried out in a conical micro scale reaction vessel by mixing amine (5 μL amine per mg of ester **4** or **5**) and ester **4** or **5** (0.02–1.0 g). The reaction mixture was heated for 2, 4, 16, or 48 h at 90 or 120 °C; *Method B:* Aminolysis was carried out according to method A with the reaction mixture stirred at room temperature for 15 min or 16 h. *Workup*: 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 h). 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, washed with diethyl ether and dried to give the required compounds **6a–c**, **6t**, **7a–d**, **7s**, **7t**, **7w**, **7x**, **8t**, **9g–j**, **9l**, **9n**, **9p–q**, **9s–t**, **9w** as colorless solids, in 95–99% purity, as determined by ^{1}H NMR spectroscopy.

8.2.1. 2-[2′-Oxo-3′-(propylamino)pyridin-1′(*2H*)-yl]-*N*-propylacetamide (6a)

Compound **2** (0.300 g, 1.26 mmol), and neat propylamine (1.5 mL) were treated at room temperature for 15 min using method B. Compound **6a** was obtained (0.307 g, 97%). Mp 135–136 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (3H, t, J = 7.2 Hz, 1-NHCH$_2$CH$_2$C*H*$_3$), 0.99 (3H, t, J = 7.2 Hz, 3′-NHCH$_2$CH$_2$C*H*$_3$), 1.47 (2H, tq, J = 7.2, 7.2 Hz, 1-NHCH$_2$C*H*$_2$), 1.67 (2H, tq, J = 7.2, 7.2 Hz, 3′-NHCH$_2$C*H*$_2$), 3.03 (2H, t, J = 7.2 Hz, 3′-NHC*H*$_2$), 3.15 (2H, dt, J = 7.0, 6.8 Hz, 1-NHC*H*$_2$), 4.56 (2H, s, H2), 6.20 (1H, dd, J = 6.6, 6.6 Hz, H5′), 6.24 (1H, m, H4′), 6.71 (1H, dd, J = 6.6, 1.8 Hz, H6′), 6.94 (1H, br s, Wh$_{½}$ ~ 16 Hz, 1-NH), NH not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.2 (1-NHCH$_2$CH$_2$*C*H$_3$), 11.6 (3′-NHCH$_2$CH$_2$*C*H$_3$), 21.9 (3′-NHCH$_2$*C*H$_2$), 22.5 (1-NHCH$_2$*C*H$_2$), 41.2 (1-NH*C*H$_2$), 45.2 (3′-NH*C*H$_2$), 54.0 (C2), 107.4 (C4′), 108.3 (C5′), 123.2 (C6′), 138.5 (C3′), 157.8 (C2′), 167.4 (C1); MS (ESI) m/z (%): 274.2 ([M+Na]$^+$, 100); 258.2 ([M+Li]$^+$, 100). Anal. Calcd for $C_{13}H_{21}O_2N_3$: C, 62.13; H, 8.42; N, 16.72. Found: C, 61.80; H, 8.34; N, 16.57.

8.2.2. *N*-Benzyl-2-[2′-oxo-3′-(propylamino)pyridin-1′(*2H*)-yl]acetamide (6b)

Compound **2** (0.300 g, 1.26 mmol), and neat benzylamine (1.5 mL) were treated at 120 °C for 2 h using method A. Compound **6b** was obtained (0.347 g, 92%). Mp 153–155 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.90 (3H, t, J = 7.2 Hz, CH_3), 1.56 (2H, tq, J = 7.2, 7.2 Hz, CH_3C*H*$_2$), 2.97 (2H, dt, J = 6.8, 6.8 Hz, 3′-NHC*H*$_2$), 4.30 (2H, d, J = 6.0 Hz, CH_2), 4.57 (2H, s, H2), 5.24 (1H, t, J = 6.0 Hz, NH), 6.09 (1H, dd, J = 6.8, 6.8 Hz, H5′), 6.17–6.19 (1H, m, H4′), 6.82 (1H, dd, J = 6.8, 1.2 Hz, H6′), 7.22–7.33 (5H, m, Ph), 8.59 (1H, t, J = 6.0 Hz, 1-NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 11.5 (CH_3), 21.3 (CH_3C*H*$_2$), 42.1 (CH_2), 44.2 (3′-NH*C*H$_2$), 51.4 (C2), 105.5 (C4′), 105.9 (C5′), 124.4 (C6′), 126.8 (*p*-Ph), 127.2 (*o*-Ph), 128.2 (*m*-Ph), 138.2 (C3′), 139.2 (*i*-Ph), 157.0 (C2′), 167.0 (C1); MS (ESI) m/z (%): 322.2 ([M+Na]$^+$, 100), 300.2 ([M+H]$^+$, 20); 306.3 ([M+Li]$^+$, 100). Anal. Calcd for $C_{17}H_{21}N_3O_2$: C, 68.21; H, 7.07; N, 14.04. Found: C, 68.14; H, 6.88; N, 13.78.

8.2.3. 1-[2′-Oxo-2′-(pyrrolidin-1″-yl)ethyl]-3-(propylamino)pyridin-2(*1H*)-one (6c)

Compound **2** (0.300 g, 1.26 mmol), and neat pyrrolidine (1.5 mL) were treated at 90 °C for 4 h using method A. Compound **6c** was obtained (0.311 g, 94%). Mp 113–114 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 (3H, t, J = 7.2 Hz, CH_3), 1.66 (2H, tq, J = 7.2, 6.6 Hz, CH_3C*H*$_2$), 1.86 (2H, tt, J = 6.8, 6.8 Hz, H3″ or H4″), 2.00 (2H, tt, J = 6.8, 6.8 Hz, H3″ or H4″), 3.02 (2H, t, J = 7.2 Hz, 3-NHC*H*$_2$), 3.50 (2H, t, J = 6.8 Hz, H2″ or H5″), 3.59 (2H, t, J = 6.8 Hz, H2″ or H5″), 4.66 (2H, s, H1′), 6.16 (1H, dd, J = 7.0, 7.0 Hz, H5), 6.23 (1H, m, H4), 6.68 (1H, m, H6), NH not observed; ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9 (CH_3), 22.2 (CH_3C*H*$_2$), 24.3 (C3″ or C4″), 26.4 (C3″ or C4″), 45.5 (CH_2NH), 46.3 (C2″ or C5″), 46.4 (C2″ or C5″), 50.9 (C1′), 107.5 (C4, C5), 124.2 (C6), 138.6 (C3), 157.9 (C2), 165.2 (C2′); MS (ESI) m/z (%): 286.3 ([M+Na]$^+$, 45); 270.3 ([M+Li]$^+$, 100). Anal. Calcd for $C_{14}H_{21}N_3O_2$: C, 63.85; H, 8.04; N, 15.96. Found: C, 63.73; H, 8.24; N, 15.68.

8.2.4. *N*-(3′,4′-Dichlorobenzyl)-2-[2″-oxo-3″-(propylamino)pyridin-1″(*2H*)-yl]acetamide (6t)

Compound **2** (0.300 g, 1.26 mmol), and neat 3,4-dichlorobenzylamine (1.5 mL) were treated at 120 °C 4 h using method A. Compound **6t** was obtained (0.423 g, 91%). Mp 174–176 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90 (3H, t, J = 7.4 Hz, CH_3), 1.56 (2H, tq, J = 7.2, 7.2 Hz, CH_3C*H*$_2$), 2.96 (2H, dt, J = 6.8, 6.8 Hz, 3′-NHC*H*$_2$), 4.29 (2H, d, J = 6.0 Hz, 1-NHC*H*$_2$), 4.57 (2H, s, H2), 5.21 (1H, br t, J = 6 Hz, 3′-NH), 6.09 (1H, dd, J = 7.0, 7.0 Hz, H5′), 6.18 (1H, dd, J = 7.2, 1.6 Hz, H4′), 6.83 (1H, dd, J = 6.8, 1.6 Hz, H6′), 7.27 (1H, dd, J = 8.4, 2.0 Hz, H6″), 7.54 (1H, d, J = 2.0 Hz, H2″), 7.57 (1H, d, J = 8.0 Hz, H5″), 8.66 (1H, br t, J = 6.0 Hz, 1-NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.2 (CH_3), 22.1 (CH_3C*H*$_2$), 41.7 (1-NH*C*H$_2$), 44.9 (3′-NH*C*H$_2$), 52.3 (C2), 106.3 (C5′), 106.7 (C4′), 125.1 (C6′), 128.2 (C6″), 129.8 (C2″), 129.9 (C3″), 131.1 (C5″), 131.6 (C4″),

138.9 (C3′), 141.3 (C1″), 157.7 (C2′), 168.0 (C1); MS (ESI) *m/z* (%):374.2 ([M+Li]$^+$, 100). Anal. Calcd for $C_{17}H_{19}Cl_2N_3O_2$: C, 55.45; H, 5.20; N, 11.41. Found: C, 55.43; H, 5.03; N, 11.17.

8.2.5. 2-[3′-(Benzylamino)-2′-oxopyridin-1′(*2H*)-yl]-*N*-propylacetamide (7a)

Compound **3** (0.200 g, 0.698 mmol), and neat propylamine (1.0 mL) were treated at room temperature for 15 min using method B. Compound **7a** was obtained (0.201 g, 96%). Mp 162–165 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.85 (3H, t, J = 7.4 Hz, CH_3), 1.41 (2H, tq, J = 7.4, 7.2 Hz, CH_3CH_2), 3.02 (2H, dt, J = 6.6, 6.0 Hz, 1-NHCH_2), 4.27 (2H, d, J = 4.0 Hz, PhCH_2), 4.49 (2H, s, H2), 5.94–6.00 (2H, m, H5′, NH), 6.05 (1H, dd, J = 7.0, 1.8 Hz, H4′), 6.77 (1H, dd, J = 7.0, 1.8 Hz, H6′), 7.19–7.24 (1H, m, Ph), 7.28–7.32 (4H, m, Ph), 8.07 (1H, br t, J = 6.0 Hz, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 11.5 (CH_3), 22.8 (CH_3CH_2), 41.4 (1-NHCH$_2$), 47.8 (PhCH$_2$), 54.2 (C2), 108.3 (C5′), 108.7 (C4′), 124.1 (C6′), 127.5 (*o*-Ph), 127.6 (*p*-Ph), 128.9 (*m*-Ph), 138.1 (C3′), 138.5 (*i*-Ph), 158.0 (C2′), 167.5 (C1); MS (ESI) *m/z* (%): 322.1 ([M+Na]$^+$, 55); 306.2 ([M+Li]$^+$, 100). Anal. Calcd for $C_{17}H_{21}N_3O_2$: C, 68.21; H, 7.07; N, 14.04. Found: C, 68.21; H, 7.14; N, 13.82.

8.2.6. *N*-Benzyl-2-[3′-(benzylamino)-2′-oxopyridin-1′(*2H*)-yl]acetamide (7b)

Compound **3** (0.210 g, 0.733 mmol), and neat benzylamine (1.1 mL) were treated at 120 °C for 4 h using method A. Compound **7b** was obtained (0.243 g, 95%). Mp 184–185 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.33 (2H, s, 3′-NHCH_2), 4.45 (2H, d, J = 5.6 Hz, 1-NHCH_2), 4.64 (2H, s, H2), 5.45 (1H, br s, $Wh_{½} \sim 21$ Hz, 3′-NH), 6.19 (1H, dd, J = 7.0, 7.0 Hz, H5′), 6.28 (1H, dd, J = 7.0, 1.6 Hz, H4′), 6.79 (1H, dd, J = 7.0, 1.6 Hz, H6′), 7.24–7.36 (11H, m, 3′-NHCH$_2$*Ph*, 1-NHCH$_2$*Ph*, 1-NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 43.7 (1-NHCH$_2$), 48.0 (3′-NHCH$_2$), 54.3 (C2), 108.5 (C5′), 109.0 (C4′), 124.5 (C6′), 127.6, 127.7, 128.9 (*o*, *m*, *p*-3′-NHCH$_2$*Ph*, *o*, *m*, *p*-1-NHCH$_2$*Ph*), 137.8 (*i*-3′-NHCH$_2$*Ph*, *i*-1-NHCH$_2$*Ph*), 138.1 (C3′), 158.1 (C2′), 167.5 (C1); MS (ESI) *m/z* (%): 370.2 ([M+Na]$^+$, 100), 348.2 ([M+H]$^+$, 85); 354.2 ([M+Li]$^+$, 100). Anal. Calcd for $C_{21}H_{21}N_3O_2$: C, 72.60; H, 6.09; N, 12.09. Found: C, 72.72; H, 6.17; N, 12.09.

8.2.7. 1-[2′-Oxo-2′-(pyrrolidin-1″-yl)ethyl]-3-(benzylamino)pyridin-2(*1H*)-one (7c)

Compound **3** (0.300 g, 1.05 mmol) and neat pyrrolidine (1.5 mL) were treated at 90 °C for 4 h using method A. Compound **7c** was obtained (0.323 g, 99%). Mp 137–139 °C (dec); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.89 (3H, tt, J = 7.0, 6.8 Hz, H3″ or H4″), 2.02 (2H, tt, J = 7.0, 6.8 Hz, H3″ or H4″), 3.52 (2H, t, J = 7.0 Hz, H2″ or H5″), 3.66 (2H, t, J = 7.0 Hz, H2″ or H5″), 4.32 (2H, d, J = 5.6 Hz, CH_2NH), 4.68 (2H, s, H1′), 5.36–5.42 (1H, m, NH), 6.12 (1H, dd, J = 7.0, 7.0 Hz, H5), 6.17 (1H, dd, J = 7.4, 1.8 Hz, H4), 6.69 (1H, dd, J = 6.6, 1.8 Hz, H6), 7.24–7.28 (1H, m, 1H, Ph), 7.31–7.38 (4H, m, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.3 (C3″ or C4″), 26.4 (C3″ or C4″), 46.3 (C2″ or C5″), 46.4 (C2″ or C5″), 48.0 (CH_2) 50.9 (C1′), 107.4 (C5), 108.6 (C4), 124.8 (C6), 127.4 (*p*-Ph), 127.5 (*o*-Ph), 128.8 (*m*-Ph), 138.2 (*i*-Ph), 138.4 (C3), 157.9 (C2), 165.2 (C2′); MS (ESI) *m/z* (%): 334.0 ([M+Na]$^+$, 45); 318.0 ([M+Li]$^+$, 100). HRMS: calcd for [M+Na]$^+$ $C_{18}H_{21}N_3O_2Na$ 334.1526, found 334.1542.

8.2.8. 2-[3′-(Benzylamino)-2′-oxopyridin-1′(*2H*)-yl]-*N*-isopropylacetamide (7d)

Compound **3** (0.300 g, 1.05 mmol) and neat isopropylamine (1.5 mL) were treated at room temperature for 16 h using method B. Compound **7d** was obtained (0.285 g, 91%). Mp 173–174 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.06 (6H, d, J = 7.2 Hz, CH_3), 3.81 (1H, dsept {8 lines}, J = 7.0, 6.8 Hz, CH), 4.27 (2H, d, J = 6.4 Hz, CH_2), 4.46 (2H, s, H2), 5.98 (2H, m, H5′, CH_2NH), 6.05 (1H, dd, J = 7.2, 1.6 Hz, H4′), 6.77 (1H, dd, J = 6.8, 1.6 Hz, H6′), 7.19–7.24 (1H, m, Ph), 7.28–7.32 4H, (m, Ph), 8.00 (1H, br d, J = 7.6 Hz, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.1 (2 × CH_3), 41.3 (CH), 46.8 (CH_2NH), 51.8 (C2), 106.2 (C5′), 107.1 (C4′), 125.6 (C6′), 127.4 (*p*-Ph), 127.7 (*o*-Ph), 129.0 (*m*-Ph), 138.5 (C3′), 140.1 (*i*-Ph), 157.6 (C2′), 166.5 (C1); MS (ESI) *m/z* (%): 322.2 ([M+Na]$^+$, 100), 300.2 ([M+H]$^+$, 35); 306.3 ([M+Li]$^+$, 100). Anal. Calcd for $C_{17}H_{21}N_3O_2$: C, 68.21; H, 7.07; N, 14.04. Found: C, 68.17; H, 7.01; N, 13.82.

8.2.9. 2-[3′-(Benzylamino)-2′-oxopyridin-1′(2*H*)-yl]-*N*-(pyridin-2″-ylmethyl)acetamide (7s)

Compound **3** (0.300 g, 1.05 mmol), and neat 2-(aminomethyl)pyridine (1.5 mL) were treated at 120 °C for 4 h using method A. Compound **7s** was obtained (0.321 g, 88%). Mp 158–161 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.28 (2H, d, J = 6.0 Hz, 3′-NHCH_2), 4.39 (2H, 1d, J = 6.0 Hz, –NHCH_2), 4.62 (2H, s, H2), 5.98–6.03 (2H, m, H5′, 3′-NH), 6.07 (1H, dd, J = 7.2, 1.8 Hz, H4′), 6.83 (1H, dd, J = 6.8, 1.8 Hz, H6′) 7.20–7.36 (7H, m, *o*, *m*, *p*-Ph, H4″, H6″), 7.75 (1H, ddd, J = 7.7, 7.7, 1.9 Hz, H5″), 8.48–8.50 (1H, m, H3″), 8.71 (1H, br t, J = 5.8 Hz, 1-NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 44.2 (1-NHCH$_2$), 46.1 (3′-NHCH$_2$), 51.5 (C2), 105.7 (C5′), 106.5 (C4′), 120.9 (C6″), 122.1 (C4″), 124.8 (C6′), 126.7 (*p*-Ph), 127.0 (*o*-Ph), 128.3 (*m*-Ph), 136.7 (C5″), 137.8 (C3′), 139.4 (*i*-Ph), 148.8 (C3″), 157.1 (C2′), 158.3 (C1″), 167.3 (C1); MS (ESI) *m/z* (%): 371.2 ([M+Na]$^+$, 30), 349.2 ([M+H]$^+$, 20), 241.0 ([M−NHCH$_2$C$_5$H$_4$N]$^+$, 100); 355.2 ([M+Li]$^+$, 80), 241.0 ([M−NHCH$_2$C$_5$H$_4$N]$^+$, 100). HRMS: calcd for [M+Na]$^+$ $C_{20}H_{20}N_4O_2Na$ 371.1478, found 371.1472.

8.2.10. 2-[3′-(Benzylamino)-2′-oxopyridin-1′(2*H*)-yl]-*N*-(3″,4″-dichlorobenzyl)acetamide (7t)

Compound **3** (0.100 g, 0.349 mmol), and neat 3,4-dichlorobenzylamine (0.5 mL) were treated at 120 °C for 4 h using method A. Compound **7t** was obtained (0.135 g, 93%). Mp 182–183 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.28 (2H, d, J = 6.4 Hz, PhCH_2NH), 4.30 (2H, d, J = 6.0 Hz, 1-NHCH_2), 4.58 (2H, s, H2), 5.96 (1H, br t, J = 6.4 Hz, 3′-NH), 6.01 (1H, dd, J = 7.0, 7.0 Hz, H5′), 6.07 (1H, dd, J = 7.2, 1.6 Hz, H4′), 6.82 (1H, dd, J = 6.8, 1.6 Hz, H6′), 7.19–7.33 (6H, m, *o*, *m*, *p*-Ph, H6″), 7.54 (1H, d, J = 2.4 Hz, H2″), 7.57 (1H, d, J = 8.0 Hz, H5″), 8.68 (1H, br t, J = 6.0 Hz, 1-NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 41.1 (1-NHCH$_2$), 46.1 (PhCH$_2$NH), 51.5 (C2), 105.7 (C5′), 106.5 (C4′), 124.8 (C6′), 126.7 (C6″), 127.0 (*o*-Ph), 127.5 (*p*-Ph), 128.3 (*m*-Ph), 129.1 (C5″), 129.2 (C4″), 130.4 (C2″), 130.9 (C3″), 137.8 (C3′), 139.4 (C1″), 140.5 (*i*-Ph), 157.0 (C2′), 167.3 (C1); MS (ESI) *m/z* (%): 422.2 ([M+Li]$^+$, 100). Anal. Calcd for $C_{21}H_{19}Cl_2N_3O_2$: C, 60.59; H, 4.60; N, 10.09. Found: C, 60.42; H, 4.54; N, 10.27.

8.2.11. 2-[3′-(Benzylamino)-2′-oxopyridin-1′(2*H*)-yl]-*N*-(1″-benzylpiperidin-4″-yl)acetamide (7w)

Compound **3** (0.300 g, 1.05 mmol), and neat 4-amino-1-benzylpiperidine (1.5 mL) were treated at 120 °C for 4 h using method A. Compound **7w** was obtained (0.366 g, 85%). Mp 176–178 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.36–1.45 (2H, m, H2″, H6″), 1.70–1.73 (2H, m, H2″, H6″), 1.99 (2H, br t, J = 10.4 Hz, H3″, H5″), 2.71–2.74 (2H, m, H3″, H5″), 3.43 (2H, s, 4″-CH$_2$), 3.49–3.56 (1H, m, H1″), 4.26 (2H, d, J = 6.4 Hz, CH$_2$), 4.48 (2H, s, H2), 5.95–5.99 (2H, m, H5′, NH), 6.04 (1H, dd, J = 7.2, 1.6 Hz, H4′), 6.76 (1H, dd, J = 6.8, 1.6 Hz, H6′), 7.18–7.33 (10H, m, Ph, 4″-CH$_2$*Ph*), 8.07 (1H, d, J = 7.6 Hz, 1-NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 31.6 (C2″ and C6″), 46.1 (PhCH$_2$), 46.2 (C1″), 51.2 (C2), 51.8 (C3″ and C5″), 62.1 (4″-CH$_2$), 105.5 (C5′), 106.4 (C4′), 124.9 (C6′), 126.7 (*p*-Ph or *p*-4″-CH$_2$Ph), 126.8 (*p*-Ph or *p*-4″-CH$_2$Ph), 127.0 (*m*-Ph or *m*-4″-CH$_2$Ph), 128.1 (*o*-Ph), 128.3 (*m*-Ph or *m*-4″-CH$_2$Ph), 128.7 (*o*-4″-CH$_2$Ph), 137.8 (C3′), 138.6 (*i*-4″-CH$_2$Ph), 139.4 (*i*-Ph), 156.9 (C2′),

166.0 (C1); MS (ESI) *m/z* (%): 431.3 ([M+H]$^+$, 100); 437.3 ([M+Li]$^+$, 45), 431.3 ([M+H]$^+$, 100). Anal. Calcd for $C_{26}H_{30}N_4O_2$: C, 72.53; H, 7.02; N, 13.01. Found: C, 72.63; H, 6.95; N, 12.96.

8.2.12. 2-[3′-(Benzylamino)-2′-oxopyridin-1′(2*H*)-yl]-*N*-(2″-methoxybenzyl)acetamide (7x)

Compound **3** (0.300 g, 1.05 mmol), and neat 2-methoxybenzylamine (1.5 mL) were treated at 120 °C 4 h using method A. Compound **7x** was obtained (0.358 g, 90%). Mp 158–160 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.70 (3H, s, CH_3), 4.25 (2H, d, J = 5.6 Hz, 1-NHC*H*$_2$), 4.27 (2H, d, J = 5.6 Hz, PhC*H*$_2$), 4.59 (2H, s, H2), 5.98–6.02 (2H, m, H5′, NH), 6.06 (1H, dd, J = 7.2, 1.6 Hz, H4′), 6.85 (1H, dd, J = 6.8, 1.6 Hz, H6′), 6.90 (1H, ddd, J = 7.4, 7.4, 1.6 Hz, H5″), 6.95–6.98 (1H, m, H6″), 7.22–7.23 (7H, m, Ph, 3″, 4″), 8.42 (1H, t, J = 5.6 Hz, 1-NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 37.3 (1-NHC*H*$_2$), 46.1 (PhC*H*$_2$), 51.4 (C2), 55.3 (CH_3), 105.7 (C5′), 106.4 (C4′), 110.4 (C3″), 120.1 (C5″), 124.9 (C6′), 126.4 (C1″), 126.7 (C4″), 127.0 (*o*-Ph), 127.8 (C6″), 128.1 (*p*-Ph), 128.3 (*m*-Ph), 137.8 (C3′), 139.4 (*i*-Ph), 156.6 (C2″), 157.1 (C2′), 167.0 (C1); MS (ESI) *m/z* (%): 400.2 ([M+Na]$^+$, 100), 378.2 ([M+H]$^+$, 15); 384.2 ([M+Li]$^+$, 100). Anal. Calcd for $C_{22}H_{23}N_3O_3$: C, 70.01; H, 6.14; N, 11.13. Found: C, 70.24; H, 5.78; N, 11.16.

8.2.13. 2-(3′-Acetamido-2′-oxopyridin-1′(2*H*)-yl)-*N*-(3″,4″-dichlorobenzyl)acetamide (8t)

Compound **4** (0.100 g, 0.420 mmol), and neat 3,4-dichlorobenzylamine (0.5 mL) were treated at 120 °C for 4 h using method B. Compound **8t** was obtained (0.145 g, 94%). Mp 220–225 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.10 (3H, s, CH_3), 4.30 (2H, d, J = 6.0 Hz, CH_2), 4.65 (2H, s, H2), 6.23 (1H, dd, J = 7.2, 7.2 Hz, H5′), 7.27 (1H, dd, J = 8.4, 2.0 Hz, H6″), 7.35 (1H, dd, J = 7.2, 1.6 Hz, H6′), 7.53 (1H, d, J = 2.0 Hz, H2″), 7.58 (1H, d, J = 8.4 Hz, H5″), 8.18–8.20 (1H, m, H4′), 8.74 (1H, br t, J = 6.0 Hz, 1-NH), 9.19 (1H, s, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 24.0 (CH_3), 41.1 (CH_2), 51.8 (C2), 104.8 (C5′), 122.8 (C4′), 127.6 (C6″), 128.7 (C3′), 129.2 (C5″), 129.3 (C4″), 130.4 (C2″), 130.9 (C3″), 132.8 (C6′), 140.5 (C1″), 156.8 (C2′), 164.8 (C1), 166.2 (CO); MS (ESI) *m/z* (%): 390.1 ([M+Na]$^+$, 100), 368.1 ([M+H]$^+$, 90); 374.1 ([M+Li]$^+$, 100). Anal. Calcd for $C_{16}H_{15}Cl_2N_3O_3$: C, 52.19; H, 4.11; N, 11.41. Found: C, 52.19; H, 4.08; N, 11.16.

8.2.14. *N*-{1-[2′-(Ethylamino)-2′-oxoethyl]-2-oxo-1,2-dihydropyridin-3-yl}benzamide (9g)

Compound **5** (0.300 g, 0.999 mmol), and neat ethylamine (5.0 mL, 70% w/w in water) were treated at room temperature for 15 min using method B. The water was removed by freeze-drying prior to recrystallization. Compound **9g** was obtained (0.296 g, 100%). Mp 214–217 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.03 (3H, t, J = 7.2 Hz, CH_3), 3.10 (2H, dq, J = 7.2, 5.6 Hz, CH_2), 4.60 (2H, s, H1′), 6.33 (1H, dd, J = 7.2, 7.2 Hz, H5), 7.40 (1H, dd, J = 7.2, 1.8 Hz, H6), 7.52–7.56 (2H, m, *m*-Ph), 7.59–7.63 (1H, m, *p*-Ph), 7.89–7.91 (2H, m, *o*-Ph), 8.20 (1H, br t, J = 5.2 Hz, 2′-NH), 8.28 (1H, dd, J = 7.2, 1.8 Hz, H4), 9.28 (1H, s, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.5 (CH_3), 36.5 (CH_2), 54.5 (C1′), 107.7 (C5), 126.5 (C4), 130.0 (*o*-Ph), 130.9 (C3), 131.7 (*m*-Ph), 134.9 (*p*-Ph), 136.6 (C6), 136.8 (*i*-Ph), 160.0 (C2), 167.7 (CO), 168.9 (C2′); MS (ESI) *m/z* (%): 322.2 ([M+Na]$^+$, 100); 306.2 ([M+Li]$^+$, 100). HRMS: calcd for [M+H]$^+$ $C_{16}H_{18}N_3O_3$ 300.1343, found 300.1344.

8.2.15. *N*-{1-[2′-(Butylamino)-2′-oxoethyl]-2-oxo-1,2-dihydropyridin-3-yl}benzamide (9h)

Compound **5** (0.200 g, 0.666 mmol), and neat butylamine (3.0 mL) were treated at room temperature for 16 h using method B. Compound **9h** was obtained (0.192 g, 88%). Mp 201–205 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.87 (3H, t, J = 7.0 Hz, CH_3), 1.26–1.32 (2H, m, C*H*$_2$CH$_3$), 1.36–1.43 (2H, m, NHCH$_2$C*H*$_2$), 3.07 (2H, dt, J = 6.8, 5.6 Hz, NHC*H*$_2$), 4.61 (2H, s, H1′), 6.33 (1H, dd, J = 7.2, 7.2 Hz, H5), 7.40 (1H, dd, J = 7.2, 2.4 Hz, H6), 7.52–7.56 (2H, m, *m*-Ph), 7.59–7.63 (1H, m, *p*-Ph), 7.89–7.91 (2H, m, *o*-Ph), 8.17 (1H, br t, J = 5.6 Hz, 2′-NH), 8.27 (1H, dd, J = 7.2, 2.4 Hz, H4), 9.28 (1H, br s, Wh$_{½}$ ~ 5 Hz, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.6 (CH_3), 19.5 (*C*H$_2$CH$_3$), 31.1 (NHCH$_2$*C*H$_2$), 38.3 (NHCH$_2$), 51.6 (C1′), 104.8 (C5), 123.6 (C4), 127.1 (*o*-Ph), 128.0 (C3), 128.8 (*m*-Ph), 132.0 (*p*-Ph), 133.7 (C6), 133.9 (*i*-Ph), 157.1 (C2), 164.8 (CO), 166.1 (C2′); MS (ESI) *m/z* (%): 334.3 ([M+Li]$^+$, 100). HRMS: calcd for [M+Na]$^+$ $C_{18}H_{21}N_3O_3$Na 350.1475, found 350.1451.

8.2.16. *N*-{1-[2′-(Isobutylamino)-2′-oxoethyl]-2-oxo-1,2-dihydropyridin-3-yl}benzamide (9i)

Compound **5** (0.500 g, 1.66 mmol), and neat isobutylamine (2.5 mL) were treated at room temperature for 16 h using method B. Compound **9i** was obtained (0.499 g, 92%). Mp 200–201.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.85 (6H, d, J = 6.4 Hz, CH_3), 1.69 (1H, m, CH), 2.91 (2H, dd, J = 6.4, 6.4 Hz, NHC*H*$_2$), 4.64 (2H, s, H1′), 6.33 (1H, dd, J = 7.0, 7.0 Hz, H5), 7.40 (1H, dd, J = 7.0, 1.6 Hz, H6), 7.52–7.56 (2H, m, *m*-Ph), 7.59–7.63 (1H, m, *p*-Ph), 7.89–7.91 (2H, m, *o*-Ph), 8.18 (1H, br t, J = 5.6 Hz, 2′-NH), 8.28 (1H, dd, J = 7.2, 1.6 Hz, H4), 9.27 (1H, s, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.1 (2 × CH_3), 28.1 (CH), 46.2 (NHCH$_2$), 51.6 (C1′), 104.8 (C5), 123.6 (C4), 127.1 (*o*-Ph), 128.0 (C3), 128.8 (*m*-Ph), 132.0 (*p*-Ph), 133.8 (*i*-Ph), 133.9 (C6), 157.1 (C2), 164.8 (CO), 166.3 (C2′); MS (ESI) *m/z* (%): 350.3 ([M+Na]$^+$, 100), 328.3 ([M+H]$^+$, 10); 334.3 ([M+Li]$^+$, 100). Anal. Calcd for $C_{18}H_{21}N_3O_3$: C, 66.04; H, 6.47; N, 12.84. Found: C, 65.87; H, 6.54; N, 12.82.

8.2.17. *N*-{1-[2′-(3″-Methoxypropylamino)-2′-oxoethyl]-2-oxo-1,2-dihydropyridin-3-yl}benzamide (9j)

Compound **5** (0.500 g, 1.66 mmol), and neat 3-methoxypropan-1-amine (2.5 mL) were treated at room temperature for 16 h using method B. Compound **9j** was obtained (0.537 g, 94%). Mp 169–171 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.64 (2H, tt, J = 6.8, 6.6 Hz, H2″), 3.12 (2H, dt, J = 6.8, 6.4 Hz, H1″), 3.21 (3H, s, CH_3), 3.33 (t, J = 6.4 Hz, 2H, 3″), 4.61 (2H, s, H1′), 6.33 (1H, dd, J = 7.2, 7.2 Hz, H5), 7.40 (1H, dd, J = 7.2, 1.8 Hz, H6), 7.52–7.56 (2H, m, *m*-Ph), 7.59–7.63 (1H, m, *p*-Ph), 7.89–7.91 (2H, m, *o*-Ph), 8.19 (1H, br t, J = 5.6 Hz, 2′-NH), 8.28 (1H, dd, J = 7.2, 1.8 Hz, H4), 9.28 (1H, s, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 29.1 (C2″), 36.0 (C1″), 51.6 (C1′), 57.9 (CH_3), 69.4 (C3″), 104.9 (C5), 123.6 (C4), 127.1 (*o*-Ph), 128.0 (C3), 128.8 (*m*-Ph), 132.0 (*p*-Ph), 133.7 (C6), 133.9 (*i*-Ph), 157.1 (C2), 164.8 (CO), 166.3 (C2′); MS (ESI) *m/z* (%): 366.3 ([M+Na]$^+$, 100), 344.3 ([M+H]$^+$, 15); 350.3 ([M+Li]$^+$, 100). Anal. Calcd for $C_{18}H_{21}N_3O_4$: C, 62.96; H, 6.16; N, 12.24. Found: C, 62.91; H, 6.15; N, 12.08.

8.2.18. *N*-{1-[2′-(2″-Aminoethylamino)-2′-oxoethyl]-2-oxo-1,2-dihydropyridin-3-yl}benzamide (9l)

Compound **5** (0.300 g, 0.999 mmol), and neat ethylenediamine (1.5 mL) were treated at room temperature for 15 min using method B. Compound **9l** was obtained (0.273 g, 87%). Mp 190–192 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.60 (2H, br s, Wh$_{½}$ ~ 45 Hz, NH$_2$) 2.57 (2H, t, J = 6.4 Hz, H2″), 3.07 (2H, dt, J = 6.4, 6.0 Hz, H1″), 4.63 (2H, s, H1′), 6.33 (1H, dd, J = 7.0, 7.0 Hz, H5), 7.40 (1H, dd, J = 6.8, 1.8 Hz, H6), 7.52–7.56 (2H, m, *m*-Ph), 7.59–7.63 (1H, m, *p*-Ph), 7.89–7.91 (2H, m, *o*-Ph), 8.18 (1H, br t, J = 5.6 Hz, 2′-NH), 8.28 (1H, dd, J = 7.2, 1.8 Hz, H4), 9.28 (1H, br s, W$_{½}$ ~ 17 Hz, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 41.3 (C2″), 42.5 (C1″), 51.7 (C1′), 104.8 (C5), 123.6 (C4), 127.1 (*o*-Ph), 128.0 (C3), 128.8 (*m*-Ph), 132.0 (*p*-Ph), 133.7 (C6), 133.9 (*i*-Ph), 157.1 (C2), 164.8 (CO), 166.4 (C2′); MS (ESI) *m/z* (%): 315.3 ([M+H]$^+$, 100); 321.3 ([M+Li]$^+$, 100). Anal. Calcd for $C_{16}H_{18}N_4O_3$: C, 61.14; H, 5.77; N, 17.82. Found: C, 60.91; H, 5.90; N, 18.02.

8.2.19. *N*-(2-Oxo-1-{2′-oxo-2′-[2″-(piperidin-1‴-yl)ethylamino]ethyl}-1,2-dihydropyridin-3-yl) benzamide (9n)

Compound **5** (0.300 g, 0.999 mmol), and neat 1-(2-aminoethyl)piperidine (1.5 mL) were treated at room temperature for 16 h using method B. Compound **9n** was obtained (0.620 g, 95%). Mp 199–202 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.30–1.37 (2H, m, H4‴), 1.44–1.50 (4H, m, H3‴, H5‴), 2.28–2.32 (6H, m, H2″, H2‴, H6‴), 3.17 (2H, dt, J = 6.8, 6.0 Hz, H1″), 4.63 (2H, s, H1′), 6.33 (1H, dd, J = 7.2, 7.2 Hz, H5), 7.40 (1H, dd, J = 7.0, 1.6 Hz, H6), 7.52–7.56 (2H, m, *m*-Ph), 7.59–7.63 (1H, m, *p*-Ph), 7.89–7.91 (2H, m, *o*-Ph), 8.09 (1H, br t, J = 5.4 Hz, 2′-NH), 8.28 (1H, dd, J = 7.2, 1.6 Hz, H4), 9.28 (1H, s, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 24.0 (C4‴), 25.5 (C3‴, C5‴), 36.4 (C1″), 51.6 (C1′), 54.0 (C2‴, C6‴), 57.6 (C2″), 104.9 (C5), 123.6 (C4), 127.1 (*o*-Ph), 128.0 (C3), 128.8 (*m*-Ph), 132.0 (*p*-Ph), 133.6 (C6), 133.9 (*i*-Ph), 157.1 (C2), 164.8 (CO), 166.2 (C2′); MS (ESI) *m/z* (%): 405.3 ([M+Na]$^+$, 30), 383.3 ([M+H]$^+$, 100); 389.3 ([M+Li]$^+$, 20), 383.3 ([M+H]$^+$, 100). HRMS: calcd for [M+H]$^+$ $C_{21}H_{27}N_4O_3$ 383.2078, found 383.2063.

8.2.20. *N*-{1-[2′-(Morpholinoamino)-2′-oxoethyl]-2-oxo-1,2-dihydropyridin-3-yl}benzamide (9p)

Compound **5** (0.300 g, 0.999 mmol), and neat 4-aminomorpholine (1.5 mL) were treated at room temperature for 16 h using method B. Compound **9p** was obtained (0.316 g, 89%). Mp 258–260 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.60–3.00 (4H, m, 2 × NCH_2), 3.40–3.90 (4H, m, 2 × CH_2O), 5.74 (2H, s, H1′), 6.33 (1H, dd, J = 7.0, 7.0 Hz, H5), 7.40 (1H, dd, J = 6.6, 1.8 Hz, H6), 7.52–7.55 (2H, m, *m*-Ph), 7.59–7.63 (1H, m, *p*-Ph), 7.89–7.91 (2H, m, *o*-Ph), 8.27–8.30 (1H, m, H4), 8.98 (1H, s, 2′-NH), 9.28 (1H, s, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 50.1 (C1′), 54.8 (NCH_2), 55.7 (NCH_2), 65.8 (2 × CH_2O), 104.8 (C5), 123.4 (C4), 127.1 (*o*-Ph), 128.0 (C3), 128.8 (*m*-Ph), 132.1 (*p*-Ph), 133.7 (C6), 133.8 (*i*-Ph), 157.1 (C2), 164.8 (CO), 168.5 (C2′); MS (ESI) *m/z* (%): 379.2 ([M+Na]$^+$, 100), 357.2 ([M+H]$^+$, 10); 363.2 ([M+Li]$^+$, 100). Anal. Calcd for $C_{18}H_{20}N_4O_4$: C, 60.67; H, 5.66; N, 15.72. Found: C, 60.68; H, 5.70; N, 15.71.

8.2.21. *N*-{1-[2′-(3″-Morpholinopropylamino)-2′-oxoethyl]-2-oxo-1,2-dihydropyridin-3-yl}benz amide (9q)

Compound **5** (0.300 g, 0.999 mmol), and neat 3-morpholinopropylamine (1.5 mL) were treated at room temperature for 16 h using method B. Compound **9q** was obtained (0.356 g, 89%). Mp 165–169 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.56 (2H, tt, J = 7.2, 7.2 Hz, H2″), 2.26–2.32 (6H, m, H3″, 2 × NCH_2), 3.10 (2H, dt, J = 6.8, 5.6 Hz, H1″), 3.53–3.56 (4H, m, 2 × CH_2O), 4.61 (2H, s, H1′), 6.33 (1H, dd, J = 7.0, 7.0 Hz, H5), 7.40 (1H, dd, J = 6.8, 1.6 Hz, H6), 7.54–7.56 (2H, m, *m*-Ph), 7.59–7.63 (1H, m, *p*-Ph), 7.87–7.91 (2H, m, *o*-Ph), 8.19 (1H, br t, J = 5.4 Hz, 2′-NH), 8.28 (1H, dd, J = 7.6, 2.0 Hz, H4), 9.28 (1H, s, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.8 (C2″), 36.9 (C1″), 51.6 (C1′), 53.2 (2 × NCH_2), 55.5 (C3″), 66.1 (2 × CH_2O), 104.7 (C5), 123.5 (C4), 127.0 (*o*-Ph), 127.9 (C3), 128.7 (*m*-Ph), 132.0 (*p*-Ph), 133.6 (C6), 133.8 (*i*-Ph), 157.0 (C2), 164.7 (CO), 166.1 (C2′); MS (ESI) *m/z* (%): 421.3 ([M+Na]$^+$, 10), 399.3 ([M+H]$^+$, 100); 405.3 ([M+Li]$^+$, 25), 399.3 ([M+H]$^+$, 100). Anal. Calcd for $C_{21}H_{26}N_4O_4$: C, 63.30; H, 6.58; N, 14.06. Found: C, 63.16; H, 6.69; N, 14.09.

8.2.22. *N*-{2-Oxo-1-[2′-oxo-2′-(pyridin-2″-ylmethylamino)ethyl]-1,2-dihydropyridin-3-yl}benz amide (9s)

Compound **5** (0.300 g, 0.999 mmol), and neat 2-(aminomethyl)pyridine (1.5 mL) were treated at 120 °C for 4 h using method A. Compound **9s** was obtained (0.336 g, 93%). Mp 206–208 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.41 (2H, d, J = 6.0 Hz, CH_2), 4.76 (2H, s, H1′), 6.35 (1H, dd, J = 7.0, 7.0 Hz, H5), 7.25–7.28 (1H, m, H4″), 7.35 (1H, d, J = 7.6 Hz, H6″), 7.45 (1H, dd, J = 6.8, 1.6 Hz, H6), 7.52–7.56 (2H, m, *m*-Ph), 7.59–7.63 (1H, m, *p*-Ph), 7.77 (1H, ddd, J = 7.6, 7.6, 1.8 Hz, H5″), 7.90–7.92 (2H, m, *o*-Ph), 8.29 (1H, dd, J = 7.2, 1.6 Hz, H4), 8.49–8.51 (1H, m, H3″), 8.83 (1H, br t, J = 6.0 Hz, 2′-NH), 9.30 (1H, s, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 44.3 (CH_2), 51.8 (C1′), 105.0 (C5), 121.1 (C6″), 122.2 (C4″), 123.8 (C4), 127.1 (*o*-Ph), 128.1 (C3), 128.8 (*m*-Ph), 132.1 (*p*-Ph), 133.7 (C6), 133.9 (*i*-Ph), 136.8 (C5″), 148.9 (C3″), 157.2 (C2), 158.2 (C1″), 164.9 (CO), 166.8 (C2′); MS (ESI) *m/z* (%): 369.2 ([M+Li]$^+$, 100). Anal. Calcd for $C_{20}H_{18}N_4O_3$: C, 66.29; H, 5.01; N, 15.46. Found: C, 66.44; H, 4.99; N, 15.40.

8.2.23. *N*-{1-[2′-(3″,4″-Dichlorobenzylamino)-2′-oxoethyl]-2-oxo-1,2-dihydropyridin-3-yl}benz amide (9t)

Compound **5** (0.100 g, 0.333 mmol), and neat 3,4-dichlorobenzylamine (0.5 mL) were treated at 120 °C 4 h using method A. Compound **9t** was obtained (0.143 g, 100%). Mp 261–262 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.32 (2H, d, J = 5.6 Hz, CH_2), 4.71 (2H, s, H1′), 6.34 (1H, dd, J = 7.0, 7.0 Hz, H5), 7.27 (1H, dd, J = 8.4, 2.0 Hz, H6″), 7.45 (1H, dd, J = 7.0, 1.8 Hz, H6), 7.54–7.64 (5H, m, *m*, *p*-Ph, H2″, H5″), 7.89–7.92 (2H, m, *o*-Ph), 8.30 (1H, dd, J = 7.4, 1.8 Hz, H4), 8.80 (1H, br t, J = 5.6 Hz, 2′-NH), 9.30 (1H, s, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 41.1 (CH_2), 52.0 (C1′), 105.1 (C5), 123.6 (C4), 127.0 (*o*-Ph), 127.5 (C6″), 128.0 (C3), 128.8 (*m*-Ph), 129.1 (C2″), 129.3 (C3″ or C4″), 130.4 (C5″), 130.9 (C3″ or C4″), 132.2 (*p*-Ph), 133.6 (C6), 133.9 (*i*-Ph), 140.4 (C1″), 157.1 (C2), 164.8 (CO), 166.8 (C2′); MS (ESI) *m/z* (%): 452.1 ([M+Na]$^+$, 100); 436.1 ([M+Li]$^+$, 100). Anal. Calcd for $C_{21}H_{17}Cl_2N_3O_3$: C, 58.62; H, 3.98; N, 9.77. Found: C, 58.34; H, 3.66; N, 9.59.

8.2.24. *N*-{1-[2′-(1″-Benzylpiperidin-4″-ylamino)-2′-oxoethyl]-2-oxo-1,2-dihydropyridin-3-yl}benz amide (9w)

Compound **5** (0.300 g, 0.999 mmol), and neat 4-amino-1-benzylpiperidine (1.5 mL) were treated at 120 °C for 4 h using method A. Compound **9w** was obtained (0.383 g, 86%). Mp 191–194 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.36–1.46 (2H, m, H2″, H6″), 1.70–1.73 (2H, m, H2″, H6″), 1.97–2.02 (2H, m, H3″, H5″), 2.72–2.75 (2H, m, H3″, H5″), 3.43 (2H, s, 4″-CH_2), 3.49–3.58 (1H, m, H1″), 4.62 (2H, s, H1′), 6.32 (1H, dd, J = 7.0, 7.0 Hz, H5), 7.21–7.32 (5H, m, CH_2*Ph*), 7.39 (1H, dd, J = 6.8, 1.6 Hz, H6), 7.51–7.55 (2H, m, *m*-Ph), 7.58–7.62 (1H, m, *p*-Ph), 7.89–7.91 (2H, m, *o*-Ph), 8.19 (1H, d, J = 7.6 Hz, 2′-NH), 8.27 (1H, dd, J = 7.4, 1.6 Hz, H4), 9.28 (1H, s, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 31.6 (C2″, C6″), 46.3 (C1″), 51.5 (C1′), 51.8 (C3″, C5″), 62.1 (4″-CH_2), 104.7 (C5), 123.6 (C4), 126.8 (*p*-Bn), 127.1 (*o*-Ph), 128.0 (C3), 128.1 (*o*-Bn), 128.7 (*m*-Bn), 128.8 (*m*-Ph), 132.0 (*p*-Ph), 133.8 (C6), 133.9 (*i*-Ph), 138.6 (*i*-Bn), 157.1 (C2), 164.8 (CO), 165.5 (C2′); MS (ESI) *m/z* (%): 445.3 ([M+H]$^+$, 100); 451.3 ([M+Li]$^+$, 30), 445.3 ([M+H]$^+$, 100). Anal. Calcd for $C_{26}H_{28}N_4O_3$: C, 70.25; H, 6.35; N, 12.60. Found: C, 70.21; H, 6.35; N, 12.62.

8.3. General procedure for the preparation of compounds 7u–v, 9r, 9v, and 9y

Method C: A 1:1 equimolar mixture of ester **4** and solid amine was melted together and transferred to a conical micro-scale reaction vessel. The resulting solid mixture was placed in a pre-heated oil bath at 120 °C. Heating was carried out without stirring until a solution was formed and then with stirring until all eliminated ethanol was evaporated and a solid formed, or 4 h if no solid was formed. The crude reaction mixture was transferred to a conical flask and re-dissolved in warm acetone. Small amounts of diethyl ether were added and the resulting solution cooled in a freezer overnight forming a white solid. The required compounds **7u–v**, **8t**, **9r**, **9v**, and **9y** were obtained as colorless solids.

8.3.1. (*S*)-2-[3′-(Benzylamino)-2′-oxopyridin-1′(2*H*)-yl]-*N*-(1″-hydroxy-3″-phenylpropan-2″-yl) acetamide (7u)

Compound **3** (0.300 g, 1.05 mmol), and neat (*S*)-2-amino-3-phenyl-1-propanol (0.158 g, 1.05 mmol) were treated at 120 °C for 1 h using method C. Compound **7u** was obtained (0.344 g, 84%). Mp 156–158 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.64 (1H, dd, J = 7.8, 13.4 Hz, H3″), 2.83 (1H, dd, J = 5.8, 13.8 Hz, H3″), 3.28–3.39 (2H, m, H1″), 3.83–3.91 (1H, m, H2″), 4.27 (2H, d, J = 6.4 Hz, CH_2), 4.48 (2H, ABq, J = 15.2 Hz, H2), 4.77 (1H, t, J = 5.4 Hz, OH), 5.95–6.03 (2H, m, H5′, 3′-NH), 6.04 (1H, dd, J = 7.2, 1.6 Hz, H4′), 6.65 (1H, dd, J = 6.8, 1.6 Hz, H6′), 7.15–7.31 (10H, m, Ph, 3″-Ph), 8.04 (1H, d, J = 8.4 Hz, 1-NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 36.5 (C3″), 46.1 (CH_2), 51.1 (C2), 52.7 (C2″), 62.2 (C1″), 105.6 (C5′), 106.3 (C4′), 124.6 (C6′), 125.9 (*p*-Ph or *p*-3″-Ph), 126.7 (*p*-Ph or *p*-3″-Ph), 127.0 (*o*-Ph), 128.1 (*m*-Ph), 128.3 (*m*-3″-Ph), 129.1 (*o*-3″-Ph), 137.8 (C3′), 137.8 (*i*-3″-Ph), 139.4 (*i*-Ph), 156.9 (C2′), 166.5 (C1); MS (ESI) *m*/*z* (%): 414.2 ([M+Na]$^+$, 100), 392.2 ([M+H]$^+$, 10); 398.2 ([M+Li]$^+$, 100). Anal. Calcd for $C_{23}H_{25}N_3O_3$: C, 70.57; H, 6.44; N, 10.73. Found: C, 70.58; H, 6.36; N, 10.81.

8.3.2. (*S*)-2-[3′-(Benzylamino)-2′-oxopyridin-1′(2*H*)-yl]-*N*-(2″-hydroxy-1″-phenylethyl)acetamide (7v)

Compound **3** (0.300 g, 1.05 mmol), and neat (*S*)-2-amino-2-phenylethanol (0.144 g, 1.05 mmol) were treated at 120 °C for 4 h using method C. Compound **7v** was obtained (0.336 g, 85%). Mp 181–181.5 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.58 (2H, dd, J = 5.8, 5.8 Hz, H2″), 4.26 (2H, d, J = 6.4 Hz, CH_2), 4.61 (2H, ABq, J = 15.4 Hz, H2), 4.81–4.88 (2H, m, H1″, OH), 5.94–5.96 (2H, m, H5′, NH), 6.04 (1H, dd, J = 7.2, 1.6 Hz, H4′), 6.77 (1H, dd, J = 6.8, 1.6 Hz, H6′), 7.18–7.34 (10H, m, Ph, 1″-Ph), 8.55 (1H, d, J = 8.4 Hz, 1-NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 46.0 (CH_2), 51.2 (C2), 55.1 (C1″), 64.6 (C2″), 105.6 (C5′), 106.4 (C4′), 124.8 (C6′), 126.7 (*p*-Ph, or *p*-1″-Ph), 126.8 (*p*-Ph, or *p*-1″-Ph), 126.9 (*o*-Ph), 127.0 (*o*-1″-Ph), 128.0 (*m*-Ph), 128.3 (*m*-1″-Ph), 137.8 (C3′), 139.4 (*i*-Ph), 140.9 (*i*-1″-Ph), 157.0 (C2′), 166.5 (C1); MS (ESI) *m*/*z* (%): 400.2 ([M+Na]$^+$, 100), 378.2 ([M+H]$^+$, 10); 384.2 ([M+Li]$^+$, 100). Anal. Calcd for $C_{22}H_{23}N_3O_3$: C, 70.01; H, 6.14; N, 11.13. Found: C, 69.75; H, 6.37; N, 11.03.

8.3.3. *N*-[1-(2′-{2′-[4‴-(Aminosulfonyl)phenyl]ethylamino}-2′-oxoethyl)-2-oxo-1,2-dihydro pyridine-3-yl]benzamide (9r)

Compound **5** (0.200 g, 0.666 mmol), and neat 4-(2-aminoethyl)benzenesulfonamide (0.133 g, 0.666 mmol) were treated at 120 °C for 1 h using method C. Compound **9r** was obtained (0.302 g, 100%). Mp 258–260 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.81 (2H, t, J = 7.0 Hz, H2″), 3.32–3.36 (2H, m, H1″ and H_2O), 4.62 (2H, s, H1′), 6.34 (1H, dd, J = 7.0, 7.0 Hz, H5), 7.30 (2H, s, NH_2), 7.38–7.43 (3H, m, H6, H2‴, H6‴), 7.52–7.56 (2H,m, *m*-Ph), 7.59–7.63 (1H, m, *p*-Ph), 7.76 (2H, d, J = 8.4 Hz, H3‴, H5‴), 7.91 (2H, d, J = 7.2 Hz, *o*-Ph), 8.29 (1H, m, H4), 8.35 (1H, t, J = 5.4 Hz, 2′-NH), 9.29 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 34.7 (C2″), 40.0 (C1″), 51.6 (C1′), 104.9 (C5), 123.7 (C4), 125.7 (C3‴, C5‴), 127.1 (o-Ph), 128.0 (C3), 128.8 (*m*-Ph), 129.2 (C2‴, C6‴), 132.1 (*p*-Ph), 133.7 (C6), 133.9 (*i*-Ph), 142.1 (C4‴), 143.5 (C1‴), 157.1 (C2), 164.9 (CO), 166.5 (C2′); MS (ESI) *m*/*z* (%): 477.2 ([M+Na]$^+$, 100), 455.2 ([M+H]$^+$, 35); 461.3 ([M+Li]$^+$, 100). HRMS: calcd for [M+Na]$^+$ $C_{22}H_{22}O_5N_4SNa$ 477.1203, found 477.1192.

8.3.4. (*S*)-*N*-(1-{2′-[(2″-Hydroxy-1″-phenylethyl)amino]-2′-oxoethyl}-2-oxo-1,2-dihydropyridin-3-yl) benzamide (9v)

Compound **5** (0.300 g, 0.999 mmol), and neat (*S*)-2-amino-2-phenylethanol (0.137 g, 0.999 mmol) were treated at 120 °C for 1 h using method C. Compound **9v** was obtained (0.356 g, 91%). Mp 207–210 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.58 (2H, dd, J = 5.6, 5.6 Hz, H2″), 4.74 (2H, s, H1′), 4.84 (1H, dt, J = 8.0, 6.2 Hz, H1″), 4.91 (1H, t, J = 5.6 Hz, OH), 6.31 (1H, dd, J = 7.2, 7.2 Hz, H5), 7.21–7.33 (5H, m, 1″-*Ph*), 7.39 (1H, dd, J = 6.8, 1.6 Hz, H6), 7.51–7.55 (2H, m, *m*-Ph), 7.59–7.62 (1H, m, *p*-Ph), 7.88–7.90 (2H, m, *o*-Ph), 8.26 (1H, dd, J = 7.2, 1.6 Hz, H4), 8.69 (1H, d, J = 8.4 Hz, 2′-NH), 9.27 (1H, s, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 51.5 (C1′), 55.2 (C1″), 64.6 (C2″), 104.8 (C5), 123.6 (C4), 126.8 (*p*′-Ph), 127.0 (*o*′-Ph), 127.1 (*o*-Ph), 128.0 (C3), 128.1 (*m*′-Ph), 128.8 (*m*-Ph), 132.0 (*p*-Ph), 133.8 (*i*-Ph), 133.9 (C6), 140.9 (*i*′-Ph), 157.1 (C2), 164.8 (CO), 166.1 (C2′); MS (ESI) *m*/*z* (%): 414.3 ([M+Na]$^+$, 100), 392.3 ([M+H]$^+$, 15); 398.3 ([M+Li]$^+$, 100). Anal. Calcd for $C_{22}H_{21}N_3O_4$: C, 67.51; H, 5.41; N, 10.74. Found: C, 67.54; H, 5.20; N, 10.74.

8.3.5. (*R*)-*N*-(1-{2′-[(1″-Benzyl-2″-hydroxyethyl)amino]-2′-oxoethyl}-2-oxo-1,2-dihydropyridin-3-yl)benzamide (9y)

Compound **5** (0.300 g, 0.999 mmol), and neat (*R*)-2-amino-3-phenyl-1-propanol (0.151 g, 0.999 mmol) were treated at 120 °C for 1 hour using method C. Compound **9y** was obtained (0.364 g, 90%). Mp 207–209 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.65 (1H, dd, J = 13.6, 7.6 Hz, 1″-C*H*$_2$), 2.85 (1H, dd, J = 13.6, 6.0 Hz, 1″-C*H*$_2$), 3.29–3.40 (2H, m, H2″), 3.83–3.91 (1H, m, H1″), 4.61 (2H, ABq, J = 15.6 Hz, H1′), 4.80 (1H, t, J = 5.2 Hz, OH), 6.31 (1H, dd, J = 7.2, 7.2 Hz, H5), 7.16–7.31 (H6, m, 6H, CH_2*Ph*), 7.52–7.56 (2H, m, *m*-Ph), 7.59–7.63 (1H, m, *p*-Ph), 7.89–7.91 (2H, m, *o*-Ph), 8.17 (1H, d, J = 8.0 Hz, 2′-NH), 8.26 (1H, dd, J = 7.2, 1.6 Hz, H4), 9.28 (1H, s, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 37.2 (CH_2Ph), 52.1 (C1′), 53.6 (C1″), 62.8 (C2″), 105.5 (C5), 124.3 (C4), 126.7 (*p*-CH_2Ph), 127.8 (*o*-Ph), 128.7 (C3), 128.9 (*m*-CH_2Ph), 129.5 (*m*-Ph), 129.9 (*o*-CH_2Ph), 132.7 (*p*-Ph), 134.3 (C6), 134.6 (*i*-Ph), 139.6 (*i*-CH_2Ph), 157.8 (C2), 165.5 (CO), 166.7 (C2′); MS (ESI) *m*/*z* (%): 428.3 ([M+Na]$^+$, 100), 406.3 ([M+H]$^+$, 15); 412.3 ([M+Li]$^+$, 100). Anal. Calcd for $C_{23}H_{23}N_3O_4$: C, 68.13; H, 5.72; N, 10.36. Found: C, 68.07; H, 5.61; N, 10.30.

9. X-ray structure determination

A full cif deposition resides with the Cambridge Crystallographic Data Centre, CCDC for **9k**, **7u**, and **7v**; 698940, 698941, 698942, respectively. Crystal data, ORTEP plots 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[11,35] in the direction of glycogen synthesis by the formation of inorganic phosphate from glucose-1-phosphate[36] using a 384 well plate at 22 °C 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.[37] 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 1.2 and a Z' value of ~0.8. Compounds were screened with maximal concentrations of 222 μM. 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+% (^{1}H NMR purity; compounds **2**, **4**, **5**, **7c**, **7s**, **8f**, **9g–h**, **9m–n**, **9r**) or 100% (micro-analytical purity; compounds **3**, **6a–c**, **6t**, **7a–b**, **7d**, **7t–x**, **7g–j**, **8a–e**, **8t**, **9a–e**, **9i–l**, **9o–q**, **9s–w**, **9y**) purity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.04.049.

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