Accepted Manuscript

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PII: S0223-5234(16)30032-0

DOI: 10.1016/j.ejmech.2016.01.031

Reference: EJMECH 8321

To appear in: *European Journal of Medicinal Chemistry*

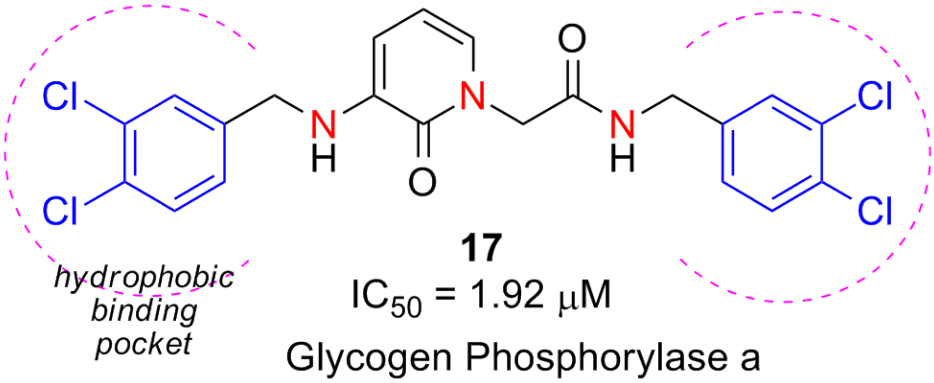
Received Date: 8 December 2015

Revised Date: 18 January 2016

Accepted Date: 18 January 2016

Please cite this article as: 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, *European Journal of Medicinal Chemistry* (2016), doi: 10.1016/j.ejmech.2016.01.031.

Graphical Abstract



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European Journal of Medicinal Chemistry
journal homepage: www.elsevier.com

2-Oxo-1,2-dihydropyridinyl-3-yl amide-based GPa inhibitors: Design, synthesis and structure-activity relationship study

Wendy A. Loughlin[a,*], Ian D. Jenkins[b], N. David Karis[b], Stephanie S. Schweiker[b, o], Peter C. Healy[a,c]

[a]*School of Natural Sciences, Griffith University, Brisbane, Qld, 4111, Australia*
[b]*Eskitis Institute for Drug Discovery, Griffith University, Brisbane, Qld, 4111, Australia*
[c] *Central Analytical Research Facility, Queensland University of Technology, Brisbane, Qld, 4001, Australia*

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Glycogen phosphorylase inhibitors
Pyridone amides
Peptide mimetic
Structure Activity Relationship
Type 2 diabetes

ABSTRACT

Glycogen phosphorylase (GP), which plays a crucial role in the conversion of glycogen to glucose-1-phosphate, is a target for therapeutic intervention in diabetes. In this study, we report the design and synthesis of 29 new derivatives of 2-oxo-1,2-dihydro pyridin-3-yl amides, as potential inhibitors of GP. The hit rate (45%) was high with 13 compounds inhibiting GPa (between 33% at 4.40 mM and an IC_{50} of 1.92 μM). Two lead compounds were identified as compounds exhibiting good GPa inhibition (IC_{50} = 2.1 and 1.92 μM). SAR analysis of these compounds revealed sensitivity of GPa to the length of the 2-oxo-1,2-dihydro pyridin-3-yl amide derivative and a preference for inclusion of a 3,4-dichlorobenzyl moiety

* Corresponding author:

E-mail address: w.loughlin@griffith.edu.au

[o] Present address: Faculty of Health Science and Medicine, Bond University, Gold Coast, Queensland, 4229, Australia

1. Introduction

Current medication for Type 2 diabetes commonly has adverse side effects, which has identified a need for new and better treatments. Control of glycogenolysis (the breakdown of glycogen to glucose) is a promising approach to control of blood glucose levels. Glycogen Phosphorylase (GP) is a key enzyme in the glycogenolysis pathway and plays a crucial role in carbohydrate metabolism, catalysing the phosphorolysis of glycogen to form glucose-1-phosphate which can be utilised to form ATP in muscle cells or in the case of hepatocytes, to form glucose for maintaining blood glucose levels. This is reflected in the distribution of three main isoforms of GP in the body; muscle, liver, brain. Studies have confirmed the efficacy of inhibitors of GP on hepatic glycogen balance and blood glucose control, thus making GP a molecular therapeutic target for the design of compounds that could treat hyperglycaemia associated with Type II Diabetes Mellitus [1-7]. GP is a highly regulated allosteric enzyme with multiple binding sites for inhibitors which include the catalytic site which binds glucose derivatives [8,9], the glycogen storage site [10] and binding sites [11] for allosteric effectors (purine site [12],[1] indole site at the enzyme dimer interface [13,14], allosteric (AMP) site [1,15] and new allosteric site [16]). Glucose derivatives, which bind to the catalytic site of GP, represent the most widely studied group of inhibitory molecules [17-19], However, other structural classes of inhibitors show promise; for example GPa has been validated as a target in diabetic ob/ob mice [20] for indole inhibitors. Comprehensive reviews on a range of GP inhibitors have been reported in the patent literature [21,22] and elsewhere [1-7, 18, 23-31].

Previously, we reported a chemogenomics strategy for GP inhibitor design [32], 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) [33-35]. 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, a 'first generation' library of 2-oxo-1,2-dihydropyridin-3-yl amides was synthesized and screened against GPa. Benzyl 3,4-dichlorobenzyl pyridone **1** (est. IC_{50} = 6.3 μM) was identified as the lead GPa inhibitor, in the context of the other 2-oxo-1,2-dihydropyridin-3-yl amide inhibitors that were identified from the same study [32]; for example: benzyl benzylpyridone **2** (est. IC_{50} = 34.2 μM) and benzoyl dichlorobenzyl pyridone **3** (est. IC_{50} = 162 μM). A preliminary analysis showed that inhibitory activity of a compound appeared to be favoured by aromatic ligands (positions (b) and (c), Figure 1), whereas inclusion of a carbonyl group adjacent to C3-N (position (a), Figure 1) was detrimental to potency [32].

A GP-**1** ligand X-Ray structure has yet to be obtained to establish the binding site of the 2-oxo-1,2-dihydropyridin-3-yl amides to GP. Herein, we report on a second-generation library consisting of a range of derivatives of benzyl 3,4-dichlorobenzyl pyridone **1**. We maintained the use of the pyridone moiety as the primary scaffold, as it provides diversity points through the C3 amino group and the N1 of the pyridone ring. An excellent hit rate was obtained and the inhibitory activity of the library of pyridone derivatives provided useful Structure-Activity Relationships (SAR) for the 2-oxo-1,2-dihydropyridin-3-yl amide inhibitors of GPa.

2. Chemistry

The synthesis of 2-oxo-1,2-dihydropyridin-3-yl amide derivatives with variations at the C3-amino group and the N1 of the pyridone is outlined in Schemes 1 and 2. Based on our previous study [32], the derivatives were designed to include functional groups that had apparent pharmacophoric features (aromatic ring and aromatic hydrophobic moiety (positions b and c, Figure 1); and lack of a hydrogen bond acceptor at C3-N (position a, Figure 1). In particular the derivatives were designed to explore (a) diversity at the C3-amino group through use of (i) readily available aldehydes which explored the optimal position for the chlorine substituent on the aromatic ring (R^1 = a-d, Scheme 1), (ii) readily available heterocyclic aromatic rings (R^1 = e-h, Scheme 1), and (iii) *para*-toluenesulfonyl as another aromatic functional group (R^1 = i, Scheme 1); (b) combinations of chlorobenzyl substituents at both the C3-amino group and the N1 of the pyridine (Scheme 1) and (c) the structural length of the derivatives, and thus placement of the aromatic moiety, through shortening or extending the substituent at the N1 of the pyridone and/or the C3-amino group (Schemes 1 and 2). Design and choice of derivatives was guided further by (i) previous results where benzyl 3,4-dichlorobenzyl pyridone **1** and benzyl benzyl pyridone **2** displayed an order of magnitude of difference in inhibition of GPa, (ii) iterative GPa assay results obtained during the present study and (iii) commercial availability of aldehyde and amine precursors.

Reductive amination of aminopyridone ester **4** [36] (Scheme 1), with a range of readily available aldehydes, gave pyridone esters **5-11** and **13** in good to excellent yields (84-99%). Interestingly, whereas reductive animation of aminopyridone ester **4** with N-methyl pyrrole aldehyde was unsuccessful, introduction of an electron withdrawing group led to reductive amination of **4** with N-tosylpyrrole-2-carbaldehyde [37] and gave tosylpyrrole pyridine ester **12** (78%). With esters **5-13** in hand, conversion to a library of amides was carried out using direct aminolysis, which was previously found to be a mild and atom-efficient procedure [36]. Pyridone esters **5-13** were reacted with 3,4-dichlorobenzylamine to incorporate the apparent favored moiety and derivatives **14-17** and **20-24** were obtained in excellent yields (93-100%). Two further examples were prepared to facilitate SAR comparison at positions (b) and (c) in Figure 1. Aminolysis of 3,4-dichlorobenzyl aminopyridone ester **8** with benzylamine and propylamine gave the derivatives **18** (95%) and **19** (97%), respectively. In addition selected benzoyl derivatives were chosen to confirm the difference in activity between the carbonyl or methylene moiety at position (a) in Figure 1. Acylation of aminopyridone ester **4** with 2-chlorobenzoyl chloride and phenyl isocyanate gave **25** and **26** (89-97%). Esters **25** and **26** were converted to amide derivatives **27**, **28** and **29** (88-100%) (Scheme 1) by aminolysis with 3,4-dichlorobenzylamine, benzylamine, and propylamine, respectively. Attempted formation of the corresponding phenylurea 3,4-dichlorobenzyl pyridone from ester **26** and 3,4-dichlorobenzylamine resulted in decomposition (see supplementary data).

Alteration of the spacing of the aromatic moieties and flexibility of the alkyl linking chains at the C3-amino group and the N1 of the pyridine was explored through insertion or deletion of an ethylene group. This required a different synthetic approach (Scheme 2). 2-Hydroxy-3-nitropyridine **30** was N-alkylated with the corresponding bromoacetophenones **31-36** generated from bromoacetyl bromide and an amine (aniline, 3,4-dichloroaniline, phenylethylamine, 3,4-dichlorobenzylamine, 3,4-dichloro phenylethylamine, 2,4-dichlorophenylethylamine) in moderate to excellent yields (30-93%). Two methods were used for the generation of nitropyridones **37-42**. Addition of bromoacetophenones **31-34** to a mixture of NaH and 2-hydroxy-3-nitropyridine **30** in THF and then irradiation of the solution under microwave conditions (150°C, 60 mins) gave nitropyridones **37-40** in good to excellent yields (68-100%).

Precipitation of the product from solution required the controlled use of 1.1 equivalents of NaH. In the alternative method, NaH and 2-hydroxy-3-nitropyridine **30** were reacted in THF, then the solvent was removed. The resultant solid and bromoacetophenones **35** or **36** were heated at 140^{o}C for 20 hrs to give nitropyridones **41-42** in moderate yield (44-63%)

Reduction of nitropyridones **37-42** by hydrogenation over palladium on carbon at atmospheric pressure gave aminopyridones **43-48**, which were unstable and used directly in the next step. Reductive amination using $NaBH(OAc)_3$, aminopyridones **43-48**, and an aldehyde (benzaldehyde, 3,4-dihlorobenzaldehyde, *ortho*-chlorobenzaldehyde, *meta*-chloro benzaldehyde, *para*-chlorobenzaldehyde, dihydrocinnam aldehyde; whereas cinnamaldehyde remained unreacted) gave the target compounds **50-63** in low to moderate yields (5-32%). This was due to the high instability of the intermediate aminopyridones **43-48.** Again a selected benzoyl derivative was prepared. Benzoylation of aminopyridone **44** with benzoyl chloride in the presence of triethylamine gave **49** (30%).

All compounds were purified by chromatography and recrystallization and characterized (Microanalysis, mass spectrometry, FTIR, ^{1}H, and ^{13}C NMR spectroscopy). *To facilitate discussion, these derivatives will be referred to by the left (LHS), followed by the right hand side (RHS) substituents; for example, 4-chlorobenzyl 3,4-dichlorobenzyl pyridone* **16**.

3. X-ray Structures of 13 and 37

In conjunction with the synthetic work, pyridone ester **13** (not active against GPa), and nitro pyridone **37** formed crystals suitable for single crystal X-ray diffraction studies (see supplementary data). The ORTEP-3 diagram of the molecular structures of pyridone ester **13** and nitro pyridone **37** are shown in Figures 2 and 3.

4. Biology

GP activity of compounds **5-29**, **49-63** was measured, using the in vitro GP screen reported in other recent studies [34,38], in the direction of glycogen synthesis [39] by the formation of inorganic phosphate from glucose-1-phosphate [40,41]. The results for compounds that displayed levels of inhibition of GP are listed in Tables 1-3 and Table S1 (supplementary data). By comparison, a typical IC_{50} of 229 ± 2, 283 ± 10 or 477 ± 14 μM was obtained for the caffeine standard. In addition to compounds **21** (Table 2), **50** (Table 1) and **61** (Table 3), the compounds not included in Tables 1-3 and Table S1 (compounds **5**, **6**, **9-11**, **13**, and **25-29**), did not inhibit GPa at a maximal concentration used in the assay (i.e., <20% at 222 μM). An apparent IC_{50} inhibitory concentration was recorded at 50% inhibition for assays reaching ≥ 90% inhibition, where compounds with a Hillslope > 5.0 were included in Table S1 (compounds **22**, **49**, **55** and **57**; supplementary data). The high Hillslope values suggest that analogues **22**, **49**, **55** and **57** may have formed precipitates in the assay (not observable by eye) leading to turbidity and creating false positives due to light scattering interference in the colorimetric inhibition assay. Thus these compounds were excluded from Tables 1-3 and only referenced in the SAR analysis.

The compounds with defined activity against GPa and meeting acceptable Hillslope values were analysed for ligand efficiency (LE) [42] and ligand-efficiency-dependent lipophilicity (LELP) [43] to provide further assessment of the quality of the hits obtained from the GPa screening. The calculated values for LE and LELP for compounds **1, 2**, **14-18, 20**, **23**, **56**, **59**, and **63** are shown in Table 4.

5. Calculation of molecular physiochemical properties

Compounds (**5-29**, **49-63**) (Schemes 1 and 2) 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 [44], and Molinspiration [45] are listed for the compounds **1-3**, **7**, **8**, **12**, **14-24**, **49-63** (Tables 1-3 and Table S1). The compounds in Tables 1-3 and Table S1 have a predicted Log P <5 in accordance with Lipinski's rules [46,47], and LogP < 3.5 (compounds **2**, **3**, **7**, **8**, **12**, **18**, **19**, **20**, **21**, **22**, **24**, **49**, **50**) the preferred value for lead–like compounds [48]. All compounds in Tables 1-3 and Table S1 have molecular weights <500 (except for **23**) and a TPSA under 120 Å [49]. The majority of compounds do not violate the 'rule of five' (#OHNH ≤ 5; #ON <10; #RB <8); with **12**, **22**, **23**, **52**, **53**, **56-58**, **62**, and **63** having the number of rotatable bonds (an important predictor of good oral bioavailability [49-51]) ≥8. Compounds could be classified into those with lower calculated solubility from the interval between 4 and 100 mg/L [50] (compounds **1-3**, **7**, **8**, **12**, **18-24**, **49**, **51-54**, **56**, **62** and **63**), and those with lower calculated solubility of < 4 mg/L (compounds **14-17**, **55**, **57-61**). However apparent solubility observed in the bioassay (Table 1) varied from the LogP predictions. It can also be noted that there were no correlations between TPSA and GPa inhibition levels, illustrated by compounds **19** (TPSA 63.01; 67% at 222 μM), **18** (TPSA 63.01; IC_{50} = 10.2μM), and **23** (TPSA 102.2; IC_{50} = 3.4 μM) (Tables 1 and 2).

6. Results and discussion

The preliminary lead compound **1** [32] upon which this study was based suggested that inclusion of a 3,4-dichlorobenzyl group at position (c) (Figure 1) and a methylene group at position (a) (Figure 1) were favored moieties for GPa inhibitory activity. A library of 29 compounds was generated which explored structural variations of compound **1** and were multiply grouped as sub-sets based on:

(i) Creation of analogues of **1** with a 3,4-dichlorobenzyl group at position (c) and explore aryl variation at position (b): Compounds **20-24**.

(ii) Selected benzoyl analogs (**27-29** and **49**) as 'structural controls' for the GPa inhibition assay

(iii) Lengthening the methylene linker at position (b) or position (c): Compounds **52**, **53**, **56-58**, **62** and **63**.

(iv) Shortening the methylene linker at position (b) or position (c): Compounds **50**, **51**, **54**, **55**, **59**, **60** and **61**.

(v) Variation of the aryl chloro substitution at position (b) or (c) or (b) and (c): Compounds **14-24** and **51-61**.

The library of 29 target compounds gave rise to 23 compounds that inhibited GPa (between 33% at 4.40 mM and an IC_{50} of 1.92 μM); an initial hit rate of 79% for the library.

In the previous study, the 3,4-dichlorobenzyl substituent had only been used at position (c) with benzyl at position (b). The 3,4-dichlorobenzyl substituent is present in successful drugs, such as the antidepressant Zoloft (Sertraline), the most prescribed antidepressant in the U.S. in 2013 [52]. In the present study, analogs of **1** with the 3,4-dichlorobenzyl substituent at position (c) and a range of aryl substituents at position (b) (compounds

20-24) (Table 2) were screened against GPa. Inhibition of GPa by these derivatives was variable; for example N-tosyl-pyrrole **23**, displayed a potent activity with an IC_{50} of 3.4 μM, whereas pyridyl **21** was inactive, perhaps as a result of protonation of the pyridyl nitrogen. We noted that the structural controls, benzoyl (**27** and **28**) and urea (**29)** analogs were inactive or gave a possible false positive result (**49**; see section 4 biology). This result confirmed the previous SAR that incorporation of a C3-N carbonyl group generally resulted in a decrease in inhibition of GPa. Similarly, incorporation of other polar groups such as sulfonyl at C3-N also resulted in a significant reduction in inhibition of GPa (cf **1**, IC_{50} 6.3 μM and **24**, 37% @ 222 μM)

It was interesting to note that the apparent length of the pyridone analogs, as a function of shortening or lengthening the methylene linkers between the substituent and N or C3-N of the pyridone ring, had an impact on the potency of GPa inhibition. Approximations for an extended conformation (ChemBio3D Ultra 11.0) were used to aid comparison to benzyl 3,4-dichlorobenzyl pyridone (**1**; est IC_{50} 6.3 μM), which was ~19.5Å in length. Increased length of an analog (eg. **52**; ~20 Å and **62**; ~21.5 Å) gave decreased inhibition levels of GPA values to > 60 μM (**63**; IC_{50} = 68μM) or negligible inhibition of GPa (**52**, **53**, **58** and **62**; 22-93%) (Tables 1 and 2). Similarly, shorter analogs (eg. **51**; ~ 16 Å) decreased GPa inhibition values to >20 μM (**59**), negligible (**51**, **54** and **60**; 19-41%) or not active (**50** and **61**) (Tables 1-3). However, specific non-extended conformations of a compound are likely to further promote favorable interactions with GPa *in situ*, where the length/floppiness of a compound will impact accordingly.

In conjunction with the screening of the final target compounds, ester intermediates **5-12** and **25** (Scheme 1) were also screened. Interestingly, three of the ester intermediates showed marginal inhibitory activity against GPa; **7** (75% @ 222μM), **8** (43% @ 222μM), and **12** (75% @ 222μM; Table S1). The left-hand side functional groups were present in target compounds with GPa inhibition (e.g. compounds **16**, **17** and **23**). The left hand side of esters **7**, **8** and **12** may be favoured for either their lipophilic properties, possible π-stacking with an aromatic ring, or their steric fit with GPa.

Testing the preference for the 3,4-dichlorobenzyl substituent at positions (b) and (c) was illustrated by comparison of 3,4-dichlorobenzyl propyl **19** (67% inhibition @ 222μM) and 3,4-dichlorobenzyl benzyl **18** (IC_{50} = 10.2 μM) to benzyl 3,4-dichlorobenzyl **1** (IC_{50} = 6.3 μM) and benzyl benzyl **2** (IC_{50} 34.2 μM) (Table 1). Incorporation of the 3,4-dichlorobenzyl moiety at the RHS (position c) improved inhibition levels of the compounds against GPa. Further exploration of the chloro substitution at the aryl substituent at the LHS (position b) revealed a preference by GPa for derivatives with *meta* **15**, *para* **16** or *meta and para* **17,** rather than *ortho* chloro substituents (Table 3). The improved activity of the bis-dichloro analogue **17** suggests either the GPa binding pocket is sufficiently large to accommodate two dichlorobenzyl substituents (for a folded conformation of **17**; Figure 4A), or there are two separate hydrophobic binding pockets (for an extended conformation of **17**; Figure 4B). The even larger N-(*p*-tosyl)-pyrrole is also readily accommodated (**23**, IC_{50} = 3.4 μM). Despite the ability of the hydrophobic pocket(s) to accommodate relatively large substituents, the sensitivity of GPa to the length of the analogues indicates an analogue-enzyme interaction that has specific spatial constraints.

The Ligand Efficiency (LE) and Ligand-Efficiency-dependent Lipophilicity (LELP) were calculated for the compounds in Tables 1-3 and compared with the IC_{50} values (Table 4). Elsewhere a lower limit of LE (0.3) and a range of -10 < LELP < 10 for LELP has been discussed [42]. Upon consideration of the values for LE and LELP in combination with the potency observed, as compared to the previous lead **1** (IC_{50} = 6.3 μM), two tiers of hits were proposed for the compounds in Table 4. Compounds **15, 16** and **17** were considered to be Tier One hits (IC_{50} < 6.3 μM and LE ≥ 0.25), with compounds **1**, **14**, **18**, **20** and **23** being Tier Two hits (IC_{50} < 20 μM and LE ≤ 0.25). The remaining compounds in Table 4 had an IC_{50} > 20 μM. Thus the lead compounds with improved potency were narrowed to *para*-chlorobenzyl 3,4-dichlorobenzyl **16** and 3,4-dichlorobenzyl 3,4-dichlorobenzyl **17** (Tier One hits).

7. Conclusion

The design, synthesis and testing of a second generation of 29 new pyridone amide derivatives resulted in 23 compounds that inhibited GPa (between 33% at 4.4 mM and an IC_{50} of 1.92 μM). The hit rate from this second generation library was high with 13 compounds (hit rate 45%) displaying IC_{50} values < 70 μM. Two new lead compounds **16** and **17** (IC_{50}=2.1 and 1.92 μM respectively) from the dichlorobenzyl pyridone class of GPA inhibitors have been identified. Both were more potent that the previously reported **1** (IC_{50}=6.3 μM). The sensitivity of GPa to the length of the analogues suggests that the binding site has limited spatial tolerance. The SAR suggests that GPa has a sufficiently large hydrophobic binding pocket to accommodate two 3,4-dichlorobenzyl substituents, or there are two separate hydrophobic binding pockets, each of which can accommodate a dichlorobenzyl group. The improvement in potency and increased SAR understanding supports further studies on dichlorobenzyl pyridones as a class of GPa inhibitors.

8. Experimental Section

8.1 General

All reagents were purchased from commercial suppliers and used without further purification. 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 valued 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 sample 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).

The synthesis of the following compounds are reported in the Supplementary data: Esters **5-13**, **25** and **26**, Bromoacetamides **31-36**; 3-Nitropyridones **37-42**; 3-Aminopyridones **43-48**.

8.2 Experimental Procedures

8.2.1 General procedure for Aminolysis at 120 °C: Preparation of compounds **14-18**, **20-24**, **27**, and **28**.

Aminolysis was carried out in a conical micro scale reaction vessel by mixing amine (5 µL amine per mg of ester) and ester (0.15-0.6 g). 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.

8.2.1.1 2-[3'-[(2'''-Chlorobenzyl)amino]-2'-oxopyridin-1'(2H)-yl]-N-(3'',4''-dichlorobenzyl)acetamide **14** Colourless solid (0.281 g, 100%). M.p 184-188 °C (dec.). FTIR (KBr) ν: 3399, 3265, 3089, 1659, 1642, 1605, 756, 727 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.31 (d, 2H, *J* = 6.0 Hz, 1-NHCH_2), 4.35 (d, 2H, *J* = 6.0 Hz, 3'-NHCH_2), 4.60 (s, 2H, H2), 5.99-6.06 (m, 3H, H4', H5', NH), 6.86 (dd, 1H, *J* = 6.0, 2.4 Hz, H6'), 7.25-7.31 (m, 4H, H3''', H4''', H5''', H6''), 7.42-7.46 (m, 1H, H6'''), 7.55 (d, 1H, *J* = 2.0 Hz, H2''), 7.57 (d, 1H, *J* = 8.4 Hz, H5''), 8.70 (t, 1H, *J* = 6.0 Hz, 1-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 41.1 (1-NHCH$_2$), 43.9 (3'-NHCH$_2$), 51.5 (C2), 105.7 (C5'), 106.6 (C4'), 125.2 (C6'), 127.2 (C4'''), 127.5 (C6''), 128.5 (C3''' or C5'''), 128.6 (C3''' or C5'''), 129.1 (C2''), 129.2 (C6'''), 129.3 (C4'), 130.4 (C5''), 130.9 (C3'') 132.1 (C1'''), 136.0 (C2'''), 137.6 (C3'), 140.5 (C1''), 157.0 (C2'), 167.3 (C1). ESI-MS *m/z* 456.1 (M+Li$^+$). Anal. Calc. for $C_{21}H_{18}O_2N_3Cl_3$: C, 55.96; H, 4.03; N, 9.32%. Found: C, 56.11; H, 3.92; N, 9.17%.

8.2.1.2 2-[3'-[(3'''-Chlorobenzyl)amino]-2'-oxopyridin-1'(2H)-yl]-N-(3'',4''-dichlorobenzyl)acetamide **15** Colourless solid (0.402 g, 95%). M.p 175-178 °C (dec.). FTIR (KBr) ν: 3322, 3252, 3052, 1642, 1585, 780, 727, 682 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.29-4.31 (m, 4H, 1-NHCH_2, 3'-NHCH_2), 4.60 (s, 2H, H2), 6.00-6.07 (m, 2H, H5', H4'), 6.14 (t, 1H, *J* = 6.4 Hz, 3'-NH), 6.83-6.85 (m, 1H, H6'), 7.25-7.34 (m, 5H, H6'', H2''', H4''', H5''', H6'''), 7.54 (d, 1H, *J* = 1.0 Hz, H2''), 7.57 (d, 1H, *J* = 8.4 Hz, H5''), 8.70 (t, 1H, *J* = 6.0 Hz, 1-NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 41.1 (1-NHCH$_2$), 45.4 (3'-NHCH$_2$), 51.5 (C2), 105.7 (C5'), 106.6 (C4'), 125.0 (C6'), 125.7 (C2''' or C6'''), 126.6 (C4''' or C5'''), 126.7 (C2''' or C6'''), 127.5 (C6''), 129.1 (C2''), 129.3 (C4''), 130.1 (C4''' or C5'''), 130.3 (C5''), 130.9 (C3''), 133.1 (C3''') 137.6 (C3'), 140.5 (C1''), 142.3 (C1'''), 157.0 (C2'), 167.3 (C1). ESI-MS *m/z* 456.1 (M+Li$^+$). Anal. Calc. for $C_{21}H_{18}O_2N_3Cl_3$: C, 55.96; H, 4.03; N, 9.32%. Found: C, 55.78; H, 3.92; N, 9.26%.

8.2.1.3 2-[3'-[(4'''-Chlorobenzyl)amino]-2'-oxopyridin-1'(2H)-yl]-N-(3'',4''-dichloro benzyl)acetamide **16** Colourless solid (0.277 g, 99%). M.p 213-218 °C (dec.). FTIR (KBr) ν: 3334, 3260, 1650, 1597, 829, 702 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.27 (d, 2H, *J* = 6.4 Hz, 3'-NHCH_2), 4.30 (d, 2H, *J* = 5.6 Hz, 1-NHCH_2), 4.58 (s, 2H, H2), 5.98-6.09 (m, 3H, H4', H5', 3'-NH), 6.85 (dd, 1H, *J* = 6.4, 2.0 Hz, H6'), 7.28 (dd, 1H, *J* = 8.4, 2.0 Hz, H6''), 7.31-7.37 (m, 4H, H2''', H3''', H5''', H6'''), 7.54 (d, 1H, *J* = 2.0 Hz, H2''), 7.57 (d, 1H, *J* = 8.4 Hz, H5''), 8.68 (brt, 1H, *J* = 5.6 Hz, 1-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 41.1 (1-NHCH$_2$), 45.3 (3'-NHCH$_2$), 51.5 (C2), 105.7 (C5'), 106.6 (C4'), 125.0 (C6'), 127.5 (C6''), 128.2 (C2''', C6'''), 128.8 (C3''', C5'''), 129.1 (C2''), 129.2 (C4''), 130.4 (C5''), 130.9 (C3''), 131.2 (C4'''), 137.6 (C3'), 138.5 (C1''') 140.6 (C1''), 157.0 (C2'), 167.3 (C1); ESI-MS m/z 458.2 (M+Li$^+$). Anal. Calc. for $C_{21}H_{18}O_2N_3Cl_3$: C, 55.96; H, 4.03; N, 9.32%. Found: C, 55.96; H, 3.93; N, 9.22%.

8.2.1.4 N-(3'',4''-Dichlorobenzyl)-2-[3'-[(3''',4'''-dichlorobenzyl) amino]-2'-oxopyridin-1'(2H)-yl]acetamide **17** Colourless solid (0.410 g, 100%). M.p 186-190 °C. FTIR (KBr) ν: 3354, 3256, 3072, 1650, 1601, 1556, 719 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.29 (t, 4H, *J* = 6.2 Hz, 1-NHCH_2, 3'-NHCH_2), 4.59 (s, 2H, H2), 6.01 (dd, 1H, *J* = 6.8, 6.8 Hz, H5'), 6.06 (dd, 1H, *J* = 7.2, 1.6 Hz, H4'), 6.20 (t, 1H, *J* = 6.2 Hz, 3'-NH), 6.84 (dd, 1H, *J* = 6.8, 1.6 Hz, H6'), 7.26-7.32 (m, 2H, H6'', H6'''), 7.53-7.58 (m, 4H, H2'', H2''' and H5'', H5'''), 8.69 (t, 1H, *J* = 6.2 Hz, 1-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 41.1 (1-NHCH$_2$), 44.8 (3'-NHCH$_2$), 51.5 (C2), 105.7 (C5'), 106.7 (C4'), 125.2 (C6'), 127.3 (C6'' or C6'''), 127.5 (C6'' or C6'''), 128.9 (C2'' or C2'''), 129.0 (C3'' or C3''' or C4'' or C4'''), 129.1 (C2'' or C2'''), 129.3 (C3'' or C3''' or C4'' or C4'''), 130.3 (C5'' or C5'''), 130.4 (C5'' or C5'''), 130.88 (C3'' or C3''' or C4'' or C4'''), 130.9 (C3'' or C3''' or C4'' or C4''') 137.4 (C3'), 140.5 (C1'''), 141.1 (C1''), 157.0 (C2'), 167.3 (C1). ESI-MS *m/z* 492.2 (M+Li$^+$). Anal. Calc. for $C_{21}H_{17}O_2N_3Cl_4$: C, 51.99; H, 3.53; N, 8.66%. Found: C, 51.97; H, 3.34; N, 8.60%.

8.2.1.5 N-Benzyl-2-[3'-[(3'',4''-dichlorobenzyl)amino]-2'-oxo pyridin-1'(2H)-yl]acetamide **18** Colourless solid (0.334 g, 95%). M.p 214-216 °C (dec.). FTIR (KBr) ν: 3334, 3269, 1663, 1642, 1597, 731, 694 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.28-4.31 (m, 4H, 3'-NHCH_2, 1-NHCH_2), 4.58 (s, 2H, H2), 6.00 (dd, 1H, *J* = 6.8, 6.8 Hz, H5'), 6.05 (dd, 1H, *J* = 7.8, 1.6 Hz, H4'), 6.23 (brt, 1H, *J* = 6.0 Hz, NH), 6.84 (dd, 1H, *J* = 6.8, 1.6 Hz, H6'), 7.21-7.34 (m, 6H, Ph, H6''), 7.54-7.57 (m, 2H, H2'', H5''), 8.61 (brt, 1H, *J* = 6.0 Hz, 1-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 42.1 (3'-NHCH$_2$), 44.8 (1-NHCH$_2$), 51.4 (C2), 105.6 (C5'), 106.7 (C4'), 125.2 (C6'), 126.8 (*p*-Ph), 127.2 (*o*-Ph), 127.3 (C6''), 128.2 (*m*-Ph), 128.9 (C2''), 129.1 (C4''), 130.4 (C5''), 130.9 (C3''), 137.4 (C3'), 139.1 (*i*-Ph), 141.1 (C1''), 157.0 (C2'), 167.0 (C1). ESI-MS *m/z* 438.1 (M+Na$^+$), 416.2 (M+H$^+$), 422.2 (M+Li$^+$). Anal. Calc. for $C_{21}H_{19}O_2N_3Cl_2$: C, 60.59; H, 4.60; N, 10.09%. Found: C, 60.63; H, 4.66; N, 10.08%.

8.2.1.6 N-(3'',4''-Dichlorobenzyl)-2-[2'-oxo-3'-[(2'''-thienyl methyl)amino]pyridin-1'(2H)-yl]acetamide **20** Colourless solid (0.273 g, 96%). M.p 190-191 °C (dec.). FTIR (KBr) ν: 3314, 3265, 1646, 1585, 756, 698 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.29 (d, 2H, *J* = 6.0 Hz, 1-NHCH_2), 4.46 (d, 2H, *J* = 6.0 Hz, 3'-NHCH_2), 4.58 (s, 2H, H2), 5.91 (t, 1H, *J* = 6.0 Hz, 3'-NH), 6.06 (dd, 1H, *J* = 6.8, 6.8 Hz, H5'), 6.25 (d, 1H, *J* = 6.8 Hz, H4'), 6.86 (d, 2H, *J* = 6.0 Hz, H6'), 6.95 (dd, 1H, *J* = 5.0, 3.6 Hz, H4'''), 7.03 (d, 1H, *J* = 3.6 Hz, H3'''), 7.27 (dd, 1H, *J* = 8.0, 1.2 Hz, H6''), 7.36 (d, 1H, *J* = 5.0 Hz, H5'''), 7.54 (d, 1H, *J* = 1.2 Hz, H2'') 7.57 (d, 1H, *J* = 8.0 Hz, H5''), 8.70 (t, 1H, *J* = 6.0 Hz, 1-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 41.0 (1-NHCH$_2$), 41.5 (3'-NHCH$_2$), 51.5 (C2), 105.6 (C5'), 107.0 (C4'), 124.6 (C5'''), 125.0 (C3'''), 125.3 (C6'), 126.7 (C4'''), 127.5 (C6''), 129.1 (C2''), 129.2 (C4''), 130.4 (C5''), 130.9 (C3''), 137.5 (C3'), 140.5 (C1'') 143.3 (C2'''), 157.0 (C2'), 167.3 (C1). ESI-MS m/z 428.2 (M+Li$^+$). Anal. Calc. for $C_{19}H_{17}O_2N_3SCl_2$: C, 54.04; H, 4.06; N, 9.95%. Found: C, 53.72; H, 4.17; N, 9.86%.

8.2.1.7 N-(3'',4''-Dichlorobenzyl)-2-[2'-oxo-3'-[(pyridin-2'''-yl methyl)amino]pyridin-1'(2H)-yl]acetamide **21** Colourless solid (0.271 g, 93%). M.p 165-169 °C (dec.). FTIR (KBr) ν: 3412, 3248, 3072, 1675, 1589, 760 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.30 (d, 2H, *J* = 6.0 Hz, 1-NHCH_2), 4.35 (d, 2H, *J* = 6.0 Hz, 3'-NHCH_2), 4.60 (s, 2H, H2), 6.05 (dd, 1H, *J* = 7.0, 7.0 Hz, H5'), 6.12 (dd, 1H, *J* = 7.2, 1.6 Hz, H4'), 6.20 (t, 1H, *J* = 6.0 Hz, 3'-NH), 6.86 (dd, 1H, *J* = 6.8, 1.6 Hz, H6'), 7.24-7.27 (m, 1H, H6''), 7.29 (d, 1H, *J* = 2.0 Hz, H5'''), 7.32 (d, 1H, *J* = 7.6 Hz, H3'''), 7.55 (d, 1H, *J* = 1.6 Hz, H2''), 7.57 (d, 1H, *J* = 8.4 Hz, H5''), 7.73 (ddd, 1H, *J* = 7.6, 7.6, 2.0 Hz, H4'''), 8.52-8.54 (m, 1H, H6'''), 8.70 (t, 1H, *J* = 6.0 Hz, 1-NH). ^{13}C NMR (100 MHz,

DMSO-d_6) δ: 41.1 (1-NH*C*H$_2$), 47.9 (3'-NH*C*H$_2$), 51.5 (C2), 105.8 (C5'), 106.7 (C4'), 121.2 (C3'"), 122.2 (C5'"), 125.1 (C6'), 127.5 (C6"), 129.1 (C2"), 129.2 (C3"), 130.4 (C5"), 130.9 (C4"), 136.7 (C4'"), 137.8 (C3'), 140.6 (C1") 148.8 (C6'"), 157.0 (C2'), 158.2 (C2'"), 167.3 (C1). ESI-MS m/z 417.2 (M+Na$^+$), 242.3 (M-NHCH$_2$C$_6$H$_3$Cl$_2^+$). HRMS m/z: 417.0878; calc. for $C_{20}H_{19}O_2N_4Cl_2$: 417.0880 (M+H$^+$).

8.2.1.8 N-(3",4"-Dichlorobenzyl)-2-[3'-[(4'"-nitrobenzyl)amino]-2'-oxopyridin-1'(2H)-yl]acetamide **22** Yellow solid (0.197 g, 94%). M.p 191-193 °C (dec.). FTIR (KBr) ν: 3326, 3265, 3072, 2929, 1663, 1646, 1597, 1520, 1344, 1250, 723 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.30 (d, 2H, *J* = 6.0 Hz, 1-NHC*H*$_2$), 4.43 (d, 2H, *J* = 6.4 Hz, 3'-NHC*H*$_2$), 4.59 (s, 2H, H2), 5.97-6.02 (m, 2H, H4', H5'), 6.28 (t, 1H, *J* = 6.4 Hz, 3'-NH), 6.84 (dd, 1H, *J* = 6.0, 2.8 Hz, H6'), 7.28 (dd, 1H, *J* = 8.0, 2.0 Hz, H6"), 7.54 (d, 1H, *J* = 2.0 Hz, H2"), 7.57 (d, 2H, *J* = 8.8 Hz, H2'", H6'"), 7.58 (d, 1H, *J* = 8.0 Hz, H5"), 8.17 (d, 2H, *J* = 8.8 Hz, H3'", H5'"), 8.69 (t, 1H, *J* = 6.0 Hz, 1-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 41.1 (1-NH*C*H$_2$), 45.5 (3'-NHCH$_2$), 51.5 (C2), 105.6 (C5'), 106.7 (C4'), 123.5 (C3'", C5'"), 125.2 (C6'), 127.5 (C6"), 128.0 (C2'", C6'"), 129.1 (C2"), 129.2 (C4"), 130.4 (C5"), 130.9 (C3"), 137.5 (C3'), 140.6 (C1"), 146.4 (C4'"), 148.1 (C1'"), 157.0 (C2'), 167.3 (C1). ESI-MS m/z 483.1 (M+Na$^+$), 461.1 (M+H$^+$), 467.1 (M+Li$^+$). HRMS m/z: 461.0794; calc. for $C_{21}H_{19}O_4N_4Cl_2$: 461.0778 (M+H$^+$).

8.2.1.9 N-(3",4"-Dichlorobenzyl)-2-[2'-oxo-3'-(1-{[(para-methyl phenyl)sulfonyl]-1H-pyrrol-2'"-ylmethyl}amino)pyridine-1'(2H)-yl]acetamide **23** Colourless solid (0.781 g, 100%). M.p 129-133 °C (dec.). FTIR (KBr) ν: 3334, 3220, 3056, 1679, 1581, 1360, 1168, 735, 700 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.35 (s, 3H, CH$_3$), 4.30 (d, 2H, *J* = 6.0 Hz, 1-NHC*H*$_2$), 4.34 (d, 2H, *J* = 6.4 Hz, 3'-NHC*H*$_2$), 4.57 (s, 2H, H2), 5.70 (t, 1H, *J* = 6.4 Hz, 3'-NH), 5.90 (dd, 1H, *J* = 7.2, 1.6 Hz, H4'), 5.95 (dd, 1H, *J* = 7.0, 7.0 Hz, H5'), 6.16-6.17 (m, 1H, H4'"), 6.23 (dd, 1H, *J* = 3.2, 3.2 Hz, H3'"), 6.83 (dd, 1H, *J* = 6.8, 1.6 Hz, H6'), 7.27 (dd, 1H, *J* = 8.0, 2.0 Hz, H6"), 7.34 (dd, 1H, *J* = 3.6, 2.0 Hz, H5'"), 7.40 (d, 2H, *J* = 8.4 Hz, *m*-Ph), 7.54 (d, 1H, *J* = 2.0 Hz, H2"), 7.56 (d, 1H, *J* = 8.0 Hz, H5"), 7.79 (d, 2H, *J* = 8.4 Hz, *o*-Ph), 8.69 (t, 1H, *J* = 6.0 Hz, 1-NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.0 (CH$_3$), 41.0 (3'-NHCH$_2$), 41.0 (1-NH-*C*H$_2$), 51.5 (C2), 105.5 (C5'), 106.8 (C4'), 111.9 (C4'"), 114.0 (C3'"), 123.1 (C5'"), 125.3 (C6'), 126.7 (*o*-Ph), 127.5 (C6"), 129.1 (C2"), 129.2 (C4"), 130.3 (*m*-Ph), 130.4 (C5"), 130.9 (C3"), 131.7 (C2'"), 135.2 (*i*-Ph), 137.1 (C3'), 140.5 (C1"), 145.4 (*p*-Ph), 156.9 (C2'), 167.3 (C1). ESI-MS m/z 581.1 (M+Na$^+$), 559.1 (M+H$^+$); 565.1 (M+Li$^+$). HRMS m/z: 559.0979; calc. for $C_{26}H_{25}O_4N_4SCl_2$: 559.0968 (M+Na$^+$).

8.2.1.10 N-(3'",4'"-Dichlorobenzyl)-2-[3'-{[(4"-methylphenyl) sulfonyl]amino}-2'-oxopyridin-1'(2H)-yl]acetamide **24** Colourless solid (0.411 g, 100%). M.p 248-250 °C (dec.). FTIR (KBr) ν: 3403, 3126, 1691, 1646, 1581, 1152, 882, 821, 751 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.32 (s, 3H, CH$_3$), 4.27 (d, 2H, *J* = 6.0 Hz, NHC*H*$_2$), 4.56 (s, 2H, H2), 6.16 (dd, 1H, *J* = 7.2, 7.2 Hz, H5'), 7.25 (dd, 1H, *J* = 8.0, 2.0 Hz, H6'"), 7.30-7.34 (m, 4H, H4', H6', H3", H5"), 7.50 (d, 1H, *J* = 2.0 Hz, H2'"), 7.56 (d, 1H, *J* = 8.4 Hz, H5'"), 7.75 (d, 2H, *J* = 8.0 Hz, H2", H6"), 8.73 (t, 1H, *J* = 6.0 Hz, 3'-NH), 9.00 (brs, 1H, Wh$_{½}$ ~ 16 Hz, 1-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.0 (CH$_3$), 41.1 (NHCH$_2$), 51.6 (C2), 104.3 (C5'), 124.1 (C4'), 126.8 (C2", C6"), 127.3 (C3'), 127.5 (C6'"), 129.1 (C2'"), 129.3 (C4'"), 129.6 (C3", C5"), 130.4 (C5'"), 130.9 (C3'"), 134.5 (C6'), 137.0 (C1"), 140.4 (C1'") 143.4 (C4"), 156.9 (C2'), 166.7 (C1). ESI-MS m/z 502.0 (M+Na$^+$), 480.1 (M+H$^+$). HRMS m/z: 480.0523; calc. for $C_{21}H_{20}O_4N_3Cl_2S$: 480.0546 (M+H$^+$).

8.2.1.11 2-Chloro-N-(1'-{2"-[(3'",4'"-dichlorobenzyl)amino]-2"-oxoethyl}-2'-oxo-1',2'-dihydropyridin-3-yl)benzamide **27** Colourless solid (0.407 g, 98%). M.p 232-234 °C. FTIR (KBr) ν: 3326, 3273, 3056, 2912, 1638, 1532, 768, 743, 657 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.31 (d, 2H, *J* = 5.6 Hz, 2"-NHC*H*$_2$), 4.69 (s, 2H, H1"), 6.34 (dd, 1H, *J* = 6.8, 6.8 Hz, H5'), 7.26 (d, 1H, *J* = 8.4 Hz, H6'"), 7.42-7.57 (m, 6H, H3, H4, H5, H6', H2'", H5'"), 7.63 (d, 1H, *J* = 7.2 Hz, H6), 8.32 (d, 1H, *J* = 6.8 Hz, H4'), 8.78 (brt, 1H, *J* = 5.6 Hz, 2"-NH), 9.45 (s, 1H, 3'-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 41.1 (2"-NHCH$_2$), 51.9 (C1"), 104.8 (C5'), 124.0 (C4'), 127.3 (C3), 127.5 (C6'"), 128.0 (C3'), 129.1 (C2'"), 129.3 (C4'"), 129.5 (C6), 129.7 (C2), 129.9 (C5'"), 130.3 (C5), 130.9 (C3'"), 131.7 (C4), 134.0 (C6') 135.4 (C1), 140.4 (C1'"), 156.9 (C2'), 164.7 (3'-NHCO), 166.8 (C2"). ESI-MS m/z 470.1 (M+Li$^+$). Anal. Calc. for $C_{21}H_{16}O_3N_3Cl_3$: C, 54.27; H, 3.47; N, 9.04%. Found: C, 54.34; H, 3.33; N, 8.95%.

8.2.1.12 N-{1'-[2"-(Benzylamino)-2"-oxoethyl]-2'-oxo-1',2'-dihydro pyridin-3'-yl}-2-chlorobenzamide **28** Colourless solid (0.311 g, 88%). M.p 196-198 °C. FTIR (KBr) ν: 3330, 3273, 3074, 1646, 1589, 1524, 743, 694, cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.21 (d, 2H, *J* = 5.6 Hz, CH$_2$), 4.69 (s, 2H, H1"), 6.33 (dd, 1H, *J* = 7.2, 7.2 Hz, H5'), 7.23-7.33 (m, 5H, Ph), 7.42-7.45 (m, 2H, H6', H4'), 7.48-7.55 (m, 2H, H3, H5), 7.63 (dd, 1H, *J* = 7.6, 1.6 Hz, H6), 8.32 (d, 1H, *J* = 6.4 Hz, H4'), 8.70 (brt, 1H, *J* = 5.6 Hz, 2"-NH), 9.48 (s, 1H, 3'-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 42.2 (CH$_2$), 51.7 (C1"), 104.7 (C5'), 124.0 (C4'), 126.8 (*p*-Ph), 127.2 (*o*-Ph), 127.3 (C3), 128.0 (C3'), 128.3 (*m*-Ph), 129.5 (C6), 129.7 (C2), 129.8 (C5), 131.6 (C4), 134.0 (C6') 135.5 (C1), 139.0 (*i*-Ph), 156.9 (C2'), 164.8 (3'-NHCO), 166.5 (C2"). ESI-MS m/z 418.1 (M+Na$^+$), 396.1 (M+H$^+$); 402.3 (M+Li$^+$). Anal. Calc. for $C_{21}H_{18}O_3N_2Cl$: C, 63.72; H, 4.58; N, 10.62%. Found: C, 63.76; H, 4.50; N, 10.51%.

8.2.2 General procedure for Aminolysis at room temperature: Preparation of compounds **19** and **29.**

Aminolysis was carried out in a conical micro scale reaction vessel by mixing amine (5 μL amine per mg of ester) and ester (0.2 or 1.18g). The reaction mixture was stirred for 1 hour 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 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.

8.2.2.1 2-[3'-[(3",4"-Dichlorobenzyl)amino]-2'-oxopyridin-1'(2H)-yl]-N-propylacetamide **19** Colourless solid (0.201 g, 97%). M.p 193-195 °C (dec.). FTIR (KBr) ν: 3322, 3256, 3081, 2968, 1646, 1581, 1483, 756 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.85 (t, 3H, *J* = 7.4 Hz, 1-NHCH$_2$CH$_2$C*H*$_3$), 1.41 (tq, 2H, *J* = 7.2, 7.2 Hz, 1-NHCH$_2$C*H*$_2$), 3.02 (dt, 2H, *J* = 6.8, 6.0 Hz, 1-NHCH$_2$), 4.28 (d, 2H, *J* = 6.4 Hz, 3'-NHC*H*$_2$), 4.49 (s, 2H, H2), 5.98 (dd, 1H, *J* = 6.8, 6.8 Hz, H5'), 6.04 (dd, 1H, *J* = 7.2, 1.6 Hz, H4'), 6.19 (t, 1H, *J* = 6.4 Hz, 3'-NH), 6.79 (dd, 1H, *J* = 6.8, 1.6 Hz, H6'), 7.30 (dd, 1H, *J* = 8.0, 1.6 Hz, H6"), 7.55 (d, 2H, *J* = 8.0 Hz, H5"), 7.56 (d, 1H, *J* = 1.6 Hz, H2"), 8.07 (t, 1H, *J* = 5.4 Hz, 1-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 11.4 (1-NHCH$_2$CH$_2$*C*H$_3$), 22.3 (1-NHCH$_2$*C*H$_2$), 40.4 (1-NHCH$_2$), 44.8 (3'-NHCH$_2$), 51.2 (C2), 105.5 (C5'), 106.6 (C4'), 125.2 (C6'), 127.3 (C6"), 128.9 (C2"), 129.1 (C4") 130.4 (C5"), 130.9 (C3"), 137.4 (C3'), 141.1 (C1"), 156.9 (C2'), 166.7 (C1). ESI-MS m/z

374.2 (M+Li^+). HRMS m/z: 368.0918; calc. for $C_{17}H_{20}O_2N_3Cl_2$: 368.0927 (M+H^+).

8.2.2.2 2-[3'-[(Anilinocarbonyl)amino]-2'-oxopyridin-1'(2H)-yl] -N-propylacetamide **29** Colourless solid (1.228 g, 100%). M.p 193-196 °C (dec.). FTIR (KBr) ν: 3326, 3293, 1646, 1581, 1548, 1499, 751, 694 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.85 (t, 3H, *J* = 7.4 Hz, CH_3), 1.42 (tq, 2H, *J* = 7.2, 7.2 Hz, 1-NHCH$_2$C*H*$_2$), 3.04 (dt, 2H, *J* = 6.8, 6.0 Hz, NHC*H*$_2$), 4.58 (s, 2H, H2), 6.23 (dd, 1H, *J* = 7.2, 7.2 Hz, H5'), 6.94-6.97 (m, 1H, *p*-Ph), 7.20 (dd, 1H, *J* = 6.8, 1.6 Hz, H6'), 7.26 (t, 2H, *J* = 7.8 Hz, *m*-Ph), 7.43 (d, 2H, *J* = 8.0 Hz, *o*-Ph), 8.07 (dd, 1H, *J* = 7.6, 1.6 Hz, H4'), 8.14 (brt, 1H, *J* = 6.0 Hz, 1-NH), 8.55 (s, 1H, 3'-NH), 9.51 (s, 1H, 3'-NHCON*H*). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 11.3 (CH_3), 22.3 (1-NHCH$_2$*C*H$_2$), 40.4 (1-NH*C*H$_2$), 51.6 (C2), 105.1 (C5'), 117.9 (*o*-Ph), 119.1 (C4'), 121.9 (*p*-Ph), 128.8 (*m*-Ph), 129.7 (C3'), 130.8 (C6'), 139.6 (*i*-Ph), 152.4 (CO), 156.7 (C2'), 166.4 (C1). ESI-MS m/z 351.3 (M+Na^+); 329.3 (M+H^+); 335.3 (M+Li^+). Anal. Calc. for $C_{17}H_{20}O_3N_4$: C, 62.18; H, 6.14; N, 17.06%. Found: C, 62.27; H, 6.18; N, 17.16%.

8.2.3 Preparation of N-{1-[2'-(3'',4''-Dichlorophenylamino)-2'-oxoethyl]-2-oxo-1,2-dihydropyridin-3-yl}benzamide **49**

Benzoyl chloride (0.14 mL, 1.5 equiv) was added to a solution of compound **44** (250 mg, 1.0 equiv) and Et_3N (0.17 mL, 1.5 equiv) in DCM (anhydrous) under nitrogen. The resulting solution was stirred 16 hrs at rt, and then concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (DCM/Et_3N, 99.5:0.5).

8.2.3.1 N-{1-[2'-(3'',4''-Dichlorophenylamino)-2'-oxoethyl]-2-oxo-1,2-dihydropyridin-3-yl}benzamide **49** Pale yellow gum (100 mg, 30 %). FTIR (KBr) ν: 3249, 3070, 1640, 1528, 764, 690, 623 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.85 (s, 2H, 1'CH$_2$), 6.40 (t, 1H, *J* = 5.5 Hz, H4), 7.40-7.65 (m, 6H, *p*-C_6H_5, *m*-C_6H_5, H5, H6, H6''), 7.80-8.00 (m, 2H, *o*-C_6H_5, H''), 8.30 (dd, 1H, *J* = 0.6, 7.5 Hz, H2'') 9.30 (s, 1H, 3-NH), 10.75 (s, 1H, 1''-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 52.5 (C2'), 105.1 (C4), 119.0 (C5), 120.2 (C6''), 124.9 (C2''), 127.1 (C6), 128.0 (C4'') 128.4 (*p*-$CH_2C_6H_5$), 128.7 (*o*-$CH_2C_6H_5$), 129.2 (*m*-$CH_2C_6H_5$), 130.8 (C5''), 132.6 (C3''), 133.6 (C3), 138.7 (C1''), 157.1 (*i*-$CH_2C_6H_5$), 164.8 (C2), 165.9 (3-NHC=O), 167.3 (C1'). ESI-MS m/z 437.81 (M+Na^+), 415.82 (M+H^+). HRMS m/z: 438.0367; calc. for $C_{20}H_{15}Cl_2N_3O_3$: 438.0383 (M+Na^+).

8.2.4 General procedure for reductive amination: Preparation of compounds **50-63**

The amine (1.0 equiv) was dissolved in DCE (anhydrous) and the aldehyde (1.1 equiv) was added under an atmosphere of nitrogen. The solution was stirred at rt for 16 hrs then $NaBH(OAc)_3$ (2 equiv) was added. The resulting suspension was stirred 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; **50**, **60**, **61**, **63**) or recrystallized from dichloromethane (**52**, **53**, **56**, **57**, **58**, **62**), or dichloromethane/hexane (**51**, **54**, **55**, **59**).

8.2.4.1 2-(3'-(Benzylamino)-2'-oxopyridin-1' (2''H)-yl)-N-phenyl acetamide **50** Chromatography (ethyl acetate/hexane: 80/20). Pale blue solid (100 mg, 32 %). M.p. 193-197 °C. FTIR (KBr) ν: 3318, 3070, 1659, 1600, 760, 698 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.30 (d, *J* = 5.0 Hz, 2H, 3'-NHC*H*$_2$), 4.75 (s, 2H, H2), 5.90-6.10 (m, 3H, H4', H5', 3'-NH), 6.84 (d, 1H, *J* = 5.5 Hz, H6'), 7.20 (t, 1H, *J* = 5.5 Hz, *p*-C_6H_5), 7.20-7.25 (m, 1H, *p*-$CH_2C_6H_5$), 7.30-7.40 (m, 5H, *m*-C_6H_5, *m*-$CH_2C_6H_5$, *o*-$CH_2C_6H_5$) 7.45-7.62 (m, 3H, *o*-$CH_2C_6H_5$, *o*-C_6H_5) 10.30 (s, 1H, CONH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 46.0 (3'-NHCH$_2$), 51.9 (C2), 105.7 (C4'), 106.6 (C5'), 118.9 (*o*-C_6H_5), 123.3 (*p*-C_6H_5), 124.9 (C6'), 126.7 (*p*-$CH_2C_6H_5$), 127.0 (*o*-$CH_2C_6H_5$) 128.3 (*m*-C_6H_5), 128.8 (*m*-$CH_2C_6H_5$), 137.8 (C3'), 138.8 (*i*-C_6H_5), 139.4 (i-$CH_2C_6H_5$), 157.0 (C2'), 165.7 (C1). ESI-MS m/z 355.9 (M+Na^+). HRMS m/z: 356.1352; calc. for $C_{20}H_{19}N_3O_2$: 356.1369 (M+Na^+).

8.2.4.2 2-(3'-(Benzylamino)-2'-oxopyridin-1' (2''H)-yl)-N-(3',4'-dichlorophenyl)acetamide **51** Pale blue solid (80 mg, 18 %). M.p. 195-196 °C. FTIR (KBr) ν: 3290, 3100, 1659, 1524, 760, 698, 623 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.30 (d, *J* = 5.0 Hz, 2H, 3'-NHC*H*$_2$), 4.75 (s, 2H, H2), 6.00-6.10 (m, 3H, H4', H5', 3'-NH), 6.83 (d, 1H, *J* = 5.0 Hz, H6'), 7.20-7.25 (m, 1H, *p*-$CH_2C_6H_5$), 7.30-7.40 (m, 4H, *o*-$CH_2C_6H_5$, *m*-$CH_2C_6H_5$), 7.45-7.60 (m, 2H, H5'', H6'') 7.95-8.99 (m, 1H, H2''), 10.63 (s, 1H, 1''-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 46.0 (3'-NHCH$_2$), 52.0 (C2), 105.8 (C4'), 106.6 (C5'), 118.9 (C''), 120.1 (C''), 124.7 (C''), 125.1 (C6'), 126.7 (*p*-$CH_2C_6H_5$), 126.9 (*o*- $CH_2C_6H_5$), 128.3 (*m*-$CH_2C_6H_5$), 130.8 (C''), 131.0 (C''), 137.8 (C3'), 138.8 (C''), 139.4 (*i*-$CH_2C_6H_5$), 157.0 (C2''), 166.3 (C1). ESI-MS m/z 123.83, 125.84 (M+Na^+). HRMS m/z: 402.0766; calc. for $C_{20}H_{18}Cl_2N_3O_2$: 402.0771 (M+H^+).

8.2.4.3 N-(3',4'-dichlorophenethyl)-2-(3''-(benzylamino)-2''-oxo pyridin-1'' (2H)-yl)acetamide **52** Pale green solid (90 mg, 20 %). M.p. 140-146 °C. FTIR (KBr) ν: 3246, 3392, 3077, 1642, 1529, 764, 692, 624 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.60 (s, 1H, 3''-NH), 2.72 (t, 2H, *J* = 6.8 Hz, 1-NHCH$_2$C*H*$_2$), 3.25-3.30 (m, 2H, 1-NHC*H*$_2$), 4.30 (d, 2H, *J* = 4.3 Hz, 3''-NHC*H*$_2$), 4.50 (s, 2H, H2), 5.97-6.11 (m, 2H, H4'', H5''), 6.75 (d, 1H, *J* = 4.0 Hz, H6'), 7.18-7.29 (m, 2H, H2', H5'), 7.30-7.40 (m, 5H, C_6H_5), 7.53 (d, 1H, *J* = 6.0 Hz, H6''), 8.30-8.40 (m, 1H, 1-NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 33.9 (1-NHCH$_2$**C**H$_2$), 38.8 (1-NHCH$_2$), 46.0 (3''-NHCH$_2$) 51.1 (C2), 105.6 (C4''), 106.3 (C5''), 124.7 (C6'), 126.7 (C2'), 126.9 (*o*-C_6H_5), 127.3 (*p*-C_6H_5), 128.3 (*m*-C_6H_5), 128.7 (C5'), 129.3 (C4'), 130.3 (C3'), 130.7 (C6''), 137.8 (C1'), 139.3 (C3''), 140.7 (*i*-C_6H_5), 156.9 (C2''), 166.9 (C1). ESI-MS m/z 452.05, 454.05 (M+Na^+). HRMS m/z: 468.0658; calc. for $C_{22}H_{21}Cl_2N_3O_2$: 468.0642 (M+Na^+).

8.2.4.4 N-(2',4'-Dichlorophenethyl)-2-(3''-(benzylamino)-2''-oxo pyridin-1'' (2H)-yl)acetamide **53** Pale blue solid (99 mg, 21 %). M.p. 166-170 °C. FTIR (KBr) ν: 3246, 3392, 3077, 1642, 1529, 764, 692, 624 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.76-2.92 (m, 2H, 1-NHCH$_2$C*H*$_2$), 3.23-3.40 (m, 2H, 1-NHC*H*$_2$), 4.28 (d, 2H, *J* = 6.6 Hz, 3''-NHC*H*$_2$), 4.50 (s, 2H, H2), 5.75-6.15 (m, 3H, H4'', H5'', H6'), 6.76 (d, 1H, *J* = 7.3 Hz, H5'), 7.18-7.44 (m, 6H, H3', C_6H_5), 7.50-7.64 (m, 1H, H6''), 8.20-8.30 (m, 1H, 1-NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 32.2 (1-NHCH$_2$*C*H$_2$), 38.2 (1-NHCH$_2$), 46.0 (3''-NHCH$_2$) 51.2 (C2), 105.6 (C4''), 106.3 (C5''), 124.7 (C5'), 126.6 (C3'), 126.9 (*o*-C_6H_5), 127.3 (*p*-C_6H_5), 128.2 (*m*-C_6H_5), 128.5 (C6'), 131.7 (C4'), 132.5 (C2'), 133.9 (C6''), 135.7 (C3''), 137.8 (C1'), 139.3 (*i*-C_6H_5), 156.9 (C2''), 166.9 (C1). ESI-MS m/z 452.05, 454.05 (M+Na^+). HRMS m/z: 430.1079; calc. for $C_{24}H_{25}Cl_2N_3O_2$: 430.1084 (M+H^+).

8.2.4.5 2-(3'-(3'',4''-Dichlorobenzylamino)-2'-oxopyridin-1' (2''H) -yl)-N-phenylacetamide **54** Pale blue solid (122 mg, 22 %). M.p. 212-214 °C. FTIR (KBr) ν: 3300, 3070, 1650, 1599, 760, 698, 623 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.24 (d, *J* = 5.0 Hz, 2H, 3'-NHC*H*$_2$), 4.76 (s, 2H, H2), 6.00-6.10 (m, 2H, H4', H5'), 6.20 (t, 1H, *J* = 5.5 Hz, 3'-NH), 6.83 (d, 1H, *J* = 5.5 Hz, H6'), 7.18 (t, 1H, *J* = 5.5 Hz, *p*-C_6H_5), 7.30-7.38 (m, 1H, 3H, H6'', *m*-

C_6H_5), 7.50-7.64 (m, 4H, H5", H2", *o*-C_6H_5) 10.30 (s, 1H, 2H, 1-NH, H5"), 7.52-7.60 (m, 2H, H3', H4"), 8.15-8.26 (m, 1H, H6"). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 32.2 (1-NHCH$_2$*C*H$_2$), 38.2 (1-NHCH$_2$), 44.7 (3"-NHCH$_2$) 51.2 (C2), 105.5 (C4"), 110.2 (C5"), 125.6 (C5'), 127.1 (C6'"), 127.3 (C3'), 128.5 (C5'"), 128.9 (C2'"), 129.0 (C6'), 130.4 (C4'"), 131.4 (C4'), 132.5 (C2'), 133.9 (C6"), 135.8 (C3"), 137.4 (C3'"), 138.1 (C1'), 141.0 (C1'"), 157.1 (C2"), 167.0 (C1). ESI-MS m/z 519.96, 521.95, 523.95 (M+Na$^+$). HRMS m/z: 520.0107; calc. for $C_{22}H_{19}Cl_4N_3O_2$: 520.0124 (M+Na$^+$).

CONH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 44.7 (3'-NHCH$_2$), 51.9 (C2), 105.6 (C4'), 108.8 (C5'), 118.8 (*o*-C_6H_5), 123.2 (*p*-C_6H_5), 125.3 (C6'), 127.3 (C"), 128.7 (*m*-C_6H_5), 128.8 (C"), 128.9 (C"), 130.4 (C"), 130.9 (C"), 137.4 (C3'), 138.8 (*i*-C_6H_5), 141.0 (C"), 156.9 (C2'), 165.7 (C1). ESI-MS m/z 423.94, 425.89 (M+Na$^+$). HRMS m/z: 402.0779; calc. for $C_{20}H_{18}Cl_2N_3O_2$: 402.0771 (M+H$^+$).

8.2.4.6 2-(3'-(3'', 4''-Dichlorobenzylamino)-2'-oxopyridin-1(2''H)-yl)-N-(3',4'-dichlorophenyl)acetamide **55** Blue solid (40 mg, 8 %). M.p. 120-125 °C. FTIR (KBr) ν: 3285, 1653, 1524, 760, 698, 623 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.26 (d, *J* = 5.0 Hz, 2H, 3'-NHC*H*$_2$), 4.72 (s, 2H, H2), 6.00-6.10 (m, 2H, H4', H5'), 6.22 (t, 1H, *J* = 5.9 Hz, 3'-NH), 6.87 (d, *J* = 5.9 Hz, 1H, H6'), 7.28 (d, *J* = 8.0 Hz, 1H, H6'"), 7.43 (dd, 1H, *J* = 2.0, 5.0 Hz, 1H, H6"), 7.50-7.60 (m, 3H, H5", H5'", H2'") 7.96 (d, *J* = 2.0 Hz, 1H, H2"), 10.60 (s, 1H, 1"-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 44.7 (3'-NHCH$_2$), 52.0 (C2), 105.7 (C4'), 106.8 (C5'), 118.9 (C6"), 120.1 (C2"), 124.7 (C4"), 125.1 (C6'), 127.3 (C6'"), 128.9 (C5"), 129.0 (C3'"), 130.4 (C5'"), 130.7 (C4'"), 130.9 (C2'"), 131.0 (C3"), 137.4 (C3'), 138.8 (C1"), 141.0 (C1'"), 156.9 (C2'), 166.3 (C1). ESI-MS m/z 493.75 (M+Na$^+$). HRMS m/z: 491.9787; calc. for $C_{20}H_{15}Cl_4N_3O_2$: 491.9811 (M+Na$^+$).

8.2.4.7 2-(3'-(3'',4''-Dichlorobenzylamino)-2'-oxopyridin-1' (2H)-yl)-N-phenethylacetamide **56** Pale blue solid (254 mg, 32 %). M.p. 183-184 °C. FTIR (KBr) ν: 3249, 3300, 3070, 1640, 1528, 764, 690, 623 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.72 (t, 2H, *J* = 7.7 Hz, NHCH$_2$C*H*$_2$), 3.24-3.32 (m, 2H, NHC*H*$_2$CH$_2$), 4.28 (d, 2H, *J* = 6.5 Hz, 3'-NHC*H*$_2$), 4.50 (s, 2H, H2), 5.96-6.07 (m, 2H, H5', H4'), 6.22 (t, 1H, *J* = 6.5 Hz, 3'-NH), 6.78 (dd, 1H, *J* = 3.0, 6.5 Hz, H6'), 7.15-7.34 (m, 6H, C_6H_5, H6"), 7.52-7.60 (m, 2H, H2", H5"), 8.20 (t, 1H, *J* = 4.8 Hz, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 35.0 (NHCH$_2$*C*H$_2$), 40.3 (NH*C*H$_2$CH$_2$), 44.7 (3'-NHCH$_2$) 51.2 (C2), 105.5 (C4'), 106.6 (C5'), 125.1 (C6'), 126.0 (C4"), 127.3 (*p*-C_6H_5), 128.3 (*o*-C_6H_5), 128.6 (*m*-C_6H_5), 128.9 (C6"), 129.0 (C2"), 130.4 (C5"), 130.9 (C3"), 137.4 (C3'), 139.3 (C1"), 141.0 (*i*-C_6H_5), 156.9 (C2'), 166.8 (C1). ESI-MS m/z 429.93, 431.91 (M+H$^+$). HRMS m/z: 452.0883; calc. for $C_{22}H_{21}Cl_2N_3O_2$: 452.0903 (M+Na$^+$).

8.2.4.8 2-(3'-(3'',4''-Dichlorobenzylamino)-2'-oxopyridin-1' (2H)-yl)-N-(3''',4'''-dichlorophenethyl)acetamide **57** Pale green solid (164 mg, 23 %). M.p. 163-165 °C. FTIR (KBr) ν: 3283, 1653, 1523, 761, 690, 622 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.66-2.78 (m, 2H, 1-NHCH$_2$C*H*$_2$), 3.23-3.30 (m, 2H, 1-NHC*H*$_2$), 4.28 (d, 2H, *J* = 6.5 Hz, 3'-NHC*H*$_2$), 4.45 (s, 2H, H2), 5.95-6.04 (m, 2H, H4', H5'), 6.72-6.280 (m, 1H, H6", H6'"), 7.15-7.40 (m, 3H, H2'", H2", H5"), 7.50-7.60 (m, 2H, H5'", H6'), 8.15-8.23 (m, 1H, 1-NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 33.9 (1-NHCH$_2$**C**H$_2$), 35.0 (1-NHCH$_2$), 44.7 (3'-NHCH$_2$) 51.2 (C2), 105.5 (C4'), 106.6 (C5'), 125.0 (C6"), 126.8 (C6'"), 127.3 (C5'"), 128.2 (C2'"), 128.6 (C5"), 129.3 (C2"), 130.3 (C4'"), 130.6 (C3'"), 130.7 (C4"), 130.9 (C3"), 137.4 (C6'), 139.4 (C3'), 140.0 (C1'"), 141.0 (C1"), 156.9 (C2'), 166.9 (C1). ESI-MS m/z 452.05, 454.05 (M+Na$^+$). HRMS m/z: 520.0117; calc. for $C_{22}H_{19}Cl_4N_3O_2$: 520.0124 (M+Na$^+$).

8.2.4.9 N-(2',4'-Dichlorophenethyl)-2-(3''-(3''',4'''-dichlorobenzyl amino)-2''-oxopyridin-1''(2H)-yl)acetamide **58** Pale blue solid (86 mg, 16 %). M.p. 119-123 °C. FTIR (KBr) ν: 3283, 1653, 1523, 761, 690, 622 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.75-2.86 (m, 2H, 1-NHCH$_2$C*H*$_2$), 3.25-3.30 (m, 2H, 1-NHC*H*$_2$), 4.30 (d, 2H, *J* = 4.0 Hz, 3"-NH), 4.49-4.51 (m, 2H, 3"-NHC*H*$_2$), 5.00 (s, 2H, H2), 5.96-6.24 (m, 2H, H6', H5"), 6.40-6.46 (m, 2H, H5', H6'"), 6.76-6.82 (m, 1H, H2'"), 7.20-7.40 (m,

8.2.4.10 2-(3'-(2'-Chlorobenzylamino)-2'-oxopyridin-1' (2''H)-yl)-N-(3',4'-dichlorophenyl)acetamide **59** Pale blue solid (100 mg, 21 %). M.p. 130-134 °C. FTIR (KBr) ν: 3299, 1653, 1528, 765, 696, 626 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.36 (d, 2H, *J* = 6.5 Hz, 3'-NHC*H*$_2$), 4.75 (s, 2H, H2), 6.00-6.10 (m, 3H, H4', H5', 3'-NH), 6.90 (d, 1H, *J* = 7.1 Hz, H6'), 7.21-7.30 (m, 3H, H6'", H5'", H4'"), 7.40-7.50 (m, 2H, H6", H3'"), 7.50 (d, 1H, *J* = 7.1 Hz, H5'") 7.96 (d, *J* = 1.4 Hz, 1H, H2"), 10.65 (s, 1H, 1"-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 43.8 (3'-NHCH$_2$), 52.0 (C2), 105.8 (C4'), 106.7 (C5'), 118.9 (C6"), 120.1 (C6"), 124.7 (C4"), 125.1 (C6'), 127.1 (C5'"), 128.4 (C5'"), 128.5 (C4'"), 129.2 (C3'"), 130.7 (C5"), 131.0 (C3"), 132.0 (C2"), 135.9 (C3'), 137.5 (C1"), 138.8 (C1'"), 156.9 (C2'), 166.3 (C1). ESI-MS m/z 157.78, 159.80 (M+Na$^+$). HRMS m/z: 458.0191; calc. for $C_{20}H_{16}Cl_3N_3O_2$: 458.0200 (M+Na$^+$).

8.2.4.11 2-(3'-(3''-Chlorobenzylamino)-2'-oxopyridin-1' (2''H)-yl)-N-(3',4'-dichlorophenyl)acetamide **60** Chromatography (DCM/Et$_3$N, 9:1) and recrystallisation (DCM/Hexane). Pale blue solid (100 mg, 18 %). M.p. 175-178 °C. FTIR (KBr) ν: 3249, 1649, 1523, 768, 690, 623 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.38 (d, 2H, *J* = 5.6 Hz, 3'-NHC*H*$_2$), 4.72 (s, 2H, H2), 6.00-6.10 (m, 2H, H4', H5'), 6.22 (t, 1H, *J* = 7.4 Hz, 3'-NH), 6.89 (dd, 1H, *J* = 0.8, 7.4 Hz, H6'), 7.20-7.35 (m, 4H, H6'", H5'", H4'", H2'"), 7.49 (dd, 1H, *J* = 1.6, 8.1 Hz, H6"), 7.55 (d, 1H, *J* = 8.1 Hz, H5") 7.92 (d, 1H, *J* = 1.6 Hz, H2"), 10.60 (s, 1H, 1"-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 45.3 (3'-NHCH$_2$), 52.0 (C2), 105.8 (C4'), 106.7 (C5'), 118.9 (C6"), 120.1 (C2"), 124.7 (C4"), 124.9 (C6'), 125.6 (C6'"), 126.6 (C4'"), 126.7 (C2'"), 130.1 (C5'"), 130.8 (C5"), 131.0 (C3"), 133.0 (C3'"), 137.5 (C3'), 138.8 (C1"), 142.3 (C1"'), 156.9 (C2'), 166.3 (C1). ESI-MS m/z 457.78, 459.80 (M+Na$^+$). HRMS m/z: 458.0193; calc. for $C_{20}H_{16}Cl_3N_3O_2$: 458.0200 (M+Na$^+$).

8.2.4.12 2-(3'-(4''-Chlorobenzylamino)-2'-oxopyridin-1' (2''H)-yl)-N-(3',4'-dichlorophenyl)acetamide **61** Chromatography (DCM/Et$_3$N, 99:1) and recrystallisation (DCM/Hexane). Pale blue solid (100 mg, 18 %). M.p. > 300 °C (dec.). FTIR (KBr) ν: 3253, 1644, 1524, 760, 698, 623 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.33 (d, 2H, *J* = 5.6 Hz, 3'-NHC*H*$_2$), 4.65 (s, 2H, H2), 6.00-6.10 (m, 2H, H4', H5'), 6.22 (t, 1H, *J* = 7.0 Hz, 3'-NH), 6.85 (dd, 1H, *J* = 0.8, 7.4 Hz, H6'), 7.25-7.40 (m, 4H, H6'", H5'", H2'", H3'"), 7.45-7.60 (m, 2H, H6", H5"), 7.90 (d, 1H, *J* = 1.6 Hz, H2"), 10.69 (s, 1H, 1"-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 45.2 (3'-NHCH$_2$), 52.0 (C2), 105.8 (C4'), 106.7 (C5'), 118.9 (C6"), 120.1 (C2"), 124.7 (C4"), 124.9 (C6'), 127.9 (C2'"), 128.2 (C6'"), 128.8 (C5'"), 129.2 (C3'"), 130.7 (C5"), 131.0 (C3"), 131.1 (C4'"), 137.6 (C3'), 138.5 (C1"), 138.9 (C1'"), 156.9 (C2'), 166.4 (C1). ESI-MS m/z 457.78, 459.78 (M+Na$^+$). HRMS m/z: 458.0212; calc. for $C_{20}H_{16}Cl_3N_3O_2$: 458.0200 (M+Na$^+$).

8.2.4.13 N-(3',4'-Dichlorobenzyl)-2-(3''-(3'''-phenylpropylamino)-2''-oxopyridin-1'' (2H)-yl)acetamide **62** Pale green solid (82 mg, 24 %). M.p. 148-149 °C. FTIR (KBr) ν: 3247, 3390, 3074, 1640, 1528, 764, 690, 623 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.69-1.97 (m, 2H, H2'"), 2.63 (t, 2H, *J* = 8.4 Hz, H3'"), 2.93-3.17 (m, 2H, H1'"), 4.25 (d, 2H, *J* = 6.0 Hz, 3"-NHC*H*$_2$),

4.60 (s, 2H, H2), 5.33 (t, 1H, *J* = 6.0 Hz, 3"-NH), 6.12-6.20 (m, 2H, H4", H5"), 6.82 (d, 1H, *J* = 8.4 Hz, H6"), 7.10-7.38 (m, 6H, H6', C_6H_5) 7.50-7.62 (m, 2H, H5', H2'), 8.70 (t, 1H, *J* = 6.0 Hz, 1-NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 29.6 (C2'"), 32.6 (C3'"), 41.0 (C1'") 41.9 (1-$NHCH_2$), 51.5 (C2), 105.6 (C4"), 105.9 (C5"), 124.4 (C6"), 125.7 (C4'), 126.1 (C6'), 127.4 (*p*-C_6H_5), 128.2 (*m*-C_6H_5), 129.1 (*o*-C_6H_5), 129.2 (C2'), 130.3 (C5'), 130.8 (C3'), 138.1 (C3"), 140.5 (C1'), 141.9 (*i*-C_6H_5), 156.9 (C2"), 167.3 (C1). ESI-MS m/z 444.11, 446.12 ($M+H^+$). HRMS m/z: 466.1044; calc. for $C_{23}H_{23}Cl_2N_3O_2$: 466.1059 ($M+Na^+$).

8.2.4.14 N-(3',4'-Dichlorophenethyl)-2-(3''-(3-phenylpropyl amino)-2''-oxopyridin-1''(2H)-yl)acetamide **63** Chromatography (ethyl acetate/hexane/Et_3N, 80:19.5:0.5). Pale blue/green gum (30 mg, 5 %). FTIR (KBr) ν: 3243, 3391, 3074, 1641, 1520, 762, 690, 623 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.40 (s, 1H, 3"-NH), 1.83-2.01 (m, 2H, 3"-$NHCH_2CH_2$), 2.65-2.82 (m, 4H, 3"-$NHCH_2CH_2CH_2$, 3"-$NHCH_2$), 3.10 (t, 2H, *J* = 5.4 Hz, 1-$NHCH_2CH_2$), 3.62 (t, 2H, *J* = 5.4 Hz, 1-$NHCH_2$), 4.60 (s, 2H, 2x H2), 6.20-6.31 (m, 2H, H4", H5"), 6.74 (dd, 1H, *J* = 1.9, 5.7 Hz, H6'), 7.12-7.28 (m, 6H, H3', C_6H_5), 7.36-7.44 (m, 2H, H5', H6"), 1-NH not observed. ^{13}C NMR (75 MHz, DMSO-d_6) δ: 31.6 (3"-$NHCH_2CH_2CH_2$), 34.2 (3"-$NHCH_2CH_2$), 35.4 (1-$NHCH_2CH_2$), 41.5 (1-$NHCH_2$), 43.3 (3"-$NHCH_2$), 53.3 (C2), 108.8 (C4"), 109.3 (C5"), 125.0 (C6'), 126.9 (C2'), 129.4 (*p*-C_6H_5), 129.4 (*o*-C_6H_5), 129.5 (*m*-C_6H_5), 129.9 (C5'), 131.1 (C4'), 131.5 (C3'), 131.9 (C6"), 139.9 (C1'), 141.3 (C3"), 143.0 (*i*-C_6H_5), 159.8 (C2"), 169.6 (C1). ESI-MS m/z 480.08, 482.09 ($M+Na^+$). HRMS m/z: 480.1198; calc. for $C_{24}H_{25}Cl_2N_3O_2$: 480.1216 ($M+Na^+$).

9. X-ray structure determination

A full cif deposition resides with the Cambridge Crystallographic Data Centre, CCDC for **13**, and **37**; 1439368, and 1439367, 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 lg/mL) activity was measured as described [13,38] in the direction of glycogen synthesis by the formation of inorganic phosphate from glucose-1-phosphate [40] 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 [41]. 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^+% (^{1}H NMR purity; compounds **5-13, 19, 21-26** and **50-63**) or 100% purity (microanalytical purity; compounds **14-18, 20, 27-29** and **49**)

11. Computational Studies

11.1 Ligand Efficiency (LE). Ligand Efficiency [42] 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 units kcal/mol per non-hydrogen atom. Following the practice of substitution of pKd with pIC50, LE can be expressed as LE = (1.37/HA) x pIC50 [42]. pIC50 values were calculated from the IC50 values [53]. 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 [43] was calculated for compounds using the definition of LELP = the ratio of log P and ligand efficiency (LE).

Conflict of interest

None.

Acknowledgments

This research was supported by the Eskitis Institute, Griffith University and partially from a grant from the Diabetes Australia Research Trust. N. D. Karis and S. S. Schweiker were both in receipt of an Australian Postgraduate Award.

Supplementary Data

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

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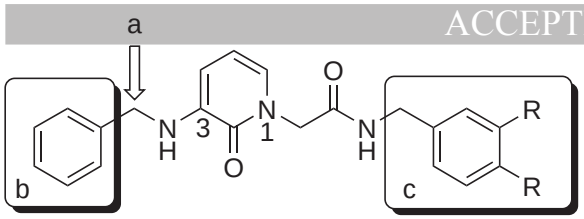
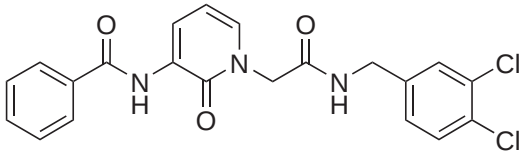


1 R = Cl; GPa IC_{50} = 6.3 µM

2 R = H; GPa IC_{50} = 34.2 µM



3 GPa IC_{50} = 162 µM

Fig. 1. A preliminary analysis for pyridone amide derivatives; position **a** - carbonyl group not favored; positions **b, c** - aromatic group favored.

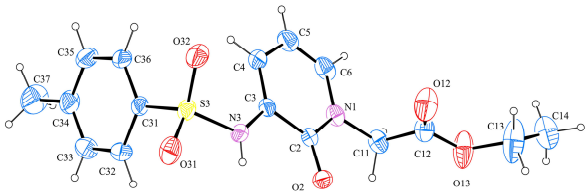


Fig. 2. ORTEP-3 diagram of the molecular structure pyridone ester **13** Displacement ellipsoids for non-hydrogen atoms are drawn with 30% probability.

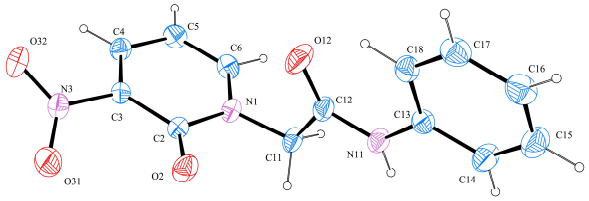


Fig. 3. ORTEP-3 diagram of the molecular structure nitro pyridone **37** Displacement ellipsoids for non-hydrogen atoms are drawn with 30% probability.

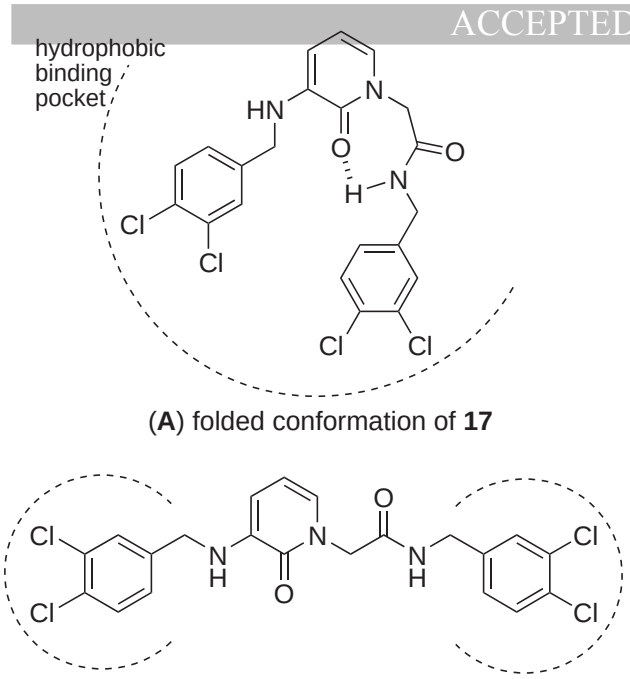
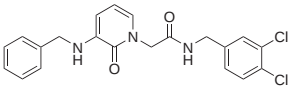
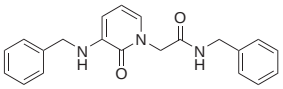
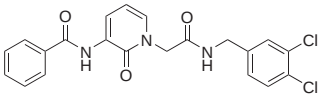
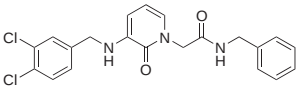
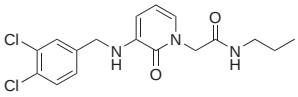
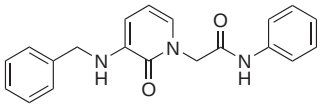
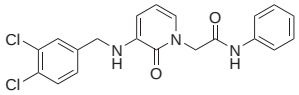
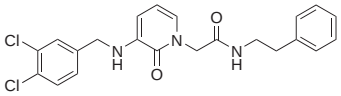


Fig. 4. Proposed (**A**) folded and (**B**) extended conformations of benzyl 3,4-dichlorobenzyl 3,4-dichlorobenzyl pyridone **17** and interactions with a hydrophobic binding pocket

Table 1. GP inhibition data and calculated physical data values[a] for RHS derivatives **18**, **19**, **50**, **54** and **56** produced via Schemes 1 and 2 and compounds **1-3** [32].

#	Structure	MW	GPa inhibition (% at μM) or IC_{50} (μM)[b,d]	Log *P*	Log S (g/L)	#RB	#ON	#OHNH	TPSA
1		416	6.3[b]	3.52±0.49	0.004	7	5	2	63.1
2		347	34.2[b]	2.25±0.51	0.096	7	5	2	63.1
3		430	162[c]	3.00±0.71	0.005	6	6	2	86.2
18		416	10.2[b]	3.48±0.60	0.012	7	5	2	63.1
19		368	67%@222	2.82±0.52	0.059	7	5	2	63.1
50		333	not active	2.32±0.54	0.180	6	5	2	63.1
54		402	19%@440	3.56±0.59	0.007	6	5	2	63.1
56		430	31.3[b]	3.80±0.60	0.009	8	5	2	63.1

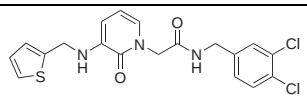
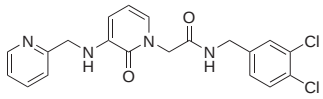
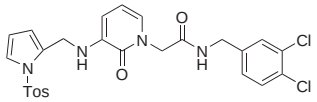
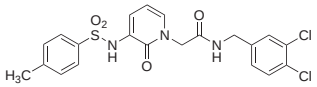
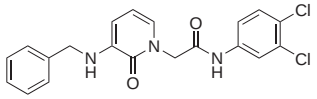
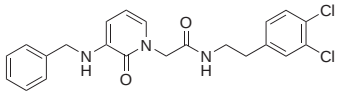
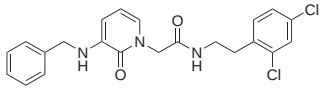
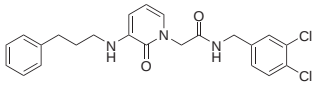
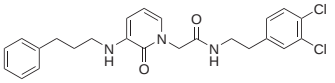
[a] Calculated with ALOPS 2.1 and Molinspiration

[b] IC_{50} = Inhibition concentration at 50% inhibition for assays reaching ≥ 90% inhibition.

[c] est $IC5_0$ reported with % GPa inhibition observed at maximal concentration; 45% at 222μM.

[d] The caffeine standard IC_{50} was 283 ± 10 μM for **1-3**; 229 ± 2 μM for **18-19** and 490 ± 14 μM for **54** and **56**.

Table 2. GP inhibition data and calculated physical data values[a] for RHS derivatives **20**, **21**, **23**, **24**, **51-53**, **62** and **63** produced via Schemes 1 and 2

#	Structure	MW	GPa inhibition (% at μM) or IC_{50} (μM)[b,c] or appIC_{50} (μM)[d]	Log *P*	Log S (g/L)	#RB	#ON	#OHNH	TPSA
20		422	19.5[c]	3.22±0.48	0.012	7	5	2	63.1
21		417	not active	2.52±0.57	0.070	7	6	2	76.0
23		559	3.4[b]	3.63±0.79	0.020	9	8	2	102.2
24		480	37%@222	3.24±0.62	0.013	7	7	2	97.3
51		402	41%@90	3.56±0.59	0.007	6	5	2	63.1
52		430	80%@444	3.81±0.60	0.009	8	5	2	63.1
53		430	93%@44	3.80±0.60	0.009	8	5	2	63.1
62		444	33%@4.4[e]	4.11±0.57	0.005	9	5	3	63.1
63		458	68[b]	4.55±0.62	0.004	10	5	2	63.1

[a] Calculated with ALOPS 2.1 and Molinspiration

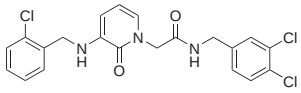
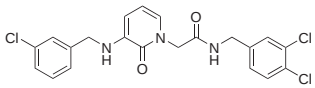
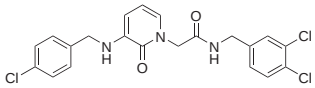
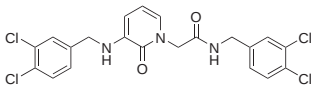
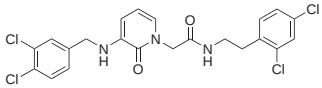
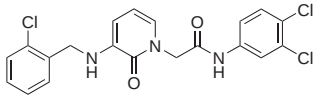
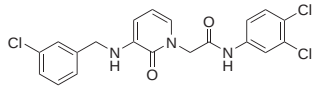
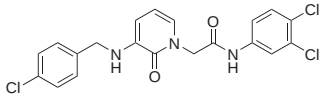
[b] IC_{50} = Inhibition concentration at 50% inhibition for assays reaching ≥ 90% inhibition.

[c] The caffeine standard IC_{50} was 229 ± 2 μM for **20-24** and 490 ± 14 μM for **51-53** and **62-63**.

[d] app. IC_{50} = apparent IC_{50} inhibition concentration at 50% inhibition for assays reaching < 90% inhibition: **20** (73% at 222μM).

[e] mM

Table 3. GP inhibition data and calculated physical data values[a] for compounds **14-17**, **58-60** and **61** produced via Schemes 1 and 2

#	Structure	MW	GPa inhibition (% at μM) or IC_{50} (μM)[b,c] or appIC_{50} (μM)[d]	Log *P*	Log S (g/L)	#RB	#ON	#OHNH	TPSA
14		451	18.5[c]	3.94±0.58	0.002	7	5	2	63.1
15		451	5.66[b]	3.94±0.59	0.002	7	5	2	63.1
16		451	2.1[b]	4.08±0.64	0.002	7	5	2	63.1
17		485	1.92[b]	4.49±0.64	0.0005	7	5	2	63.1
58		499	22%@44	4.97±0.73	0.0004	8	5	2	63.1
59		437	57[b]	4.15±0.66	0.002	6	5	2	63.1
60		437	33%@20	4.15±0.66	0.002	6	5	2	63.1
61		437	not active	4.17±0.65	0.003	6	5	2	63.1

[a] Calculated with ALOPS 2.1 and Molinspiration

[b] IC_{50} = Inhibition concentration at 50% inhibition for assays reaching ≥ 90% inhibition.

[c] The caffeine standard IC_{50} was 229 ± 2 μM for **14-17** and 490 ± 14 μM for **58-61**.

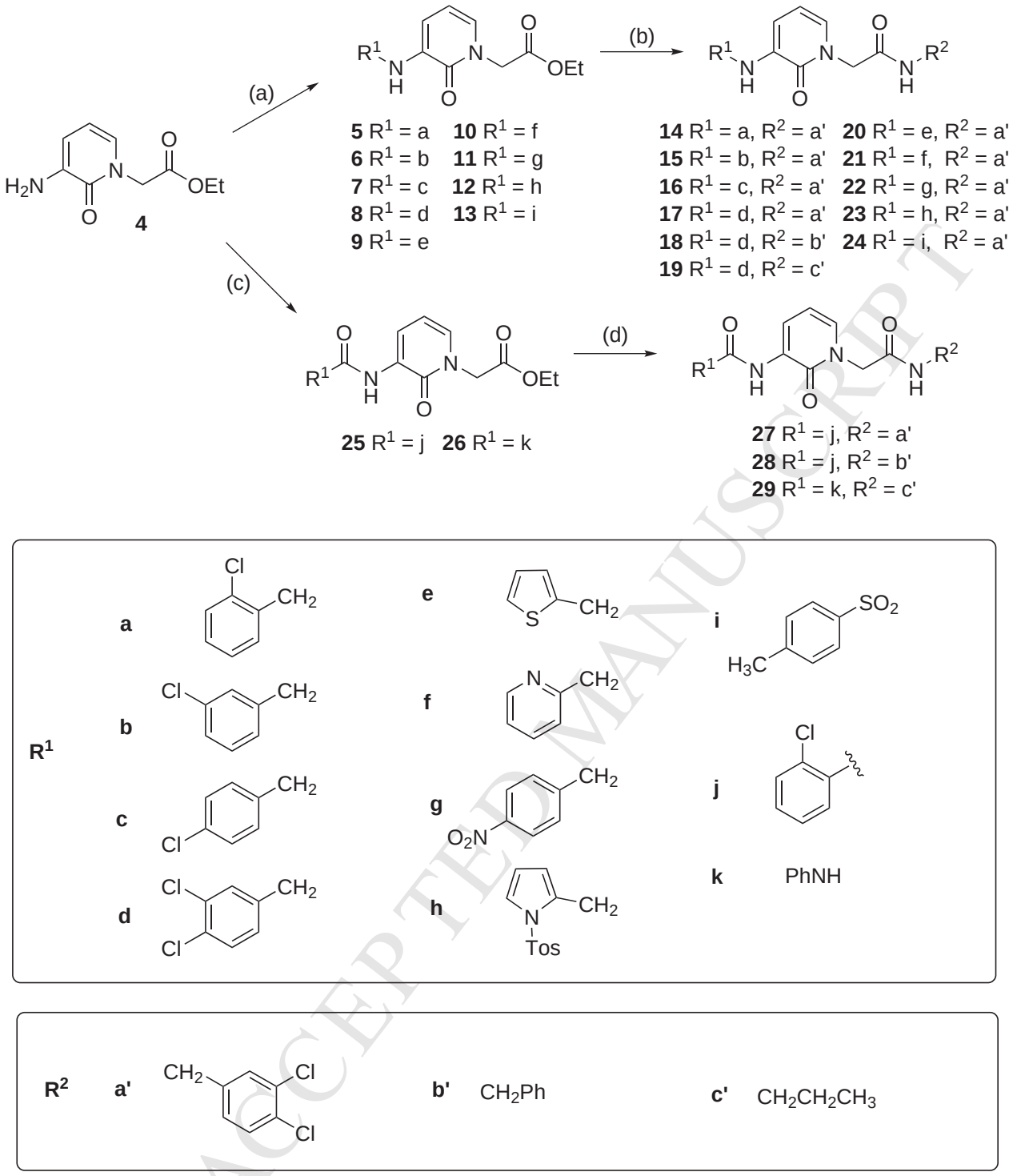
[d] app. IC_{50} = apparent IC_{50} inhibition concentration at 50% inhibition for assays reaching < 90% inhibition: **14** (77% at 222μM).

Table 4. Calculated Ligand Efficiency (LE) and Ligand-Efficiency-dependent Lipophilicity (LELP) of compounds **1**, **2**, **14-18**, **20**, **23**, **56**, **59** and **63**

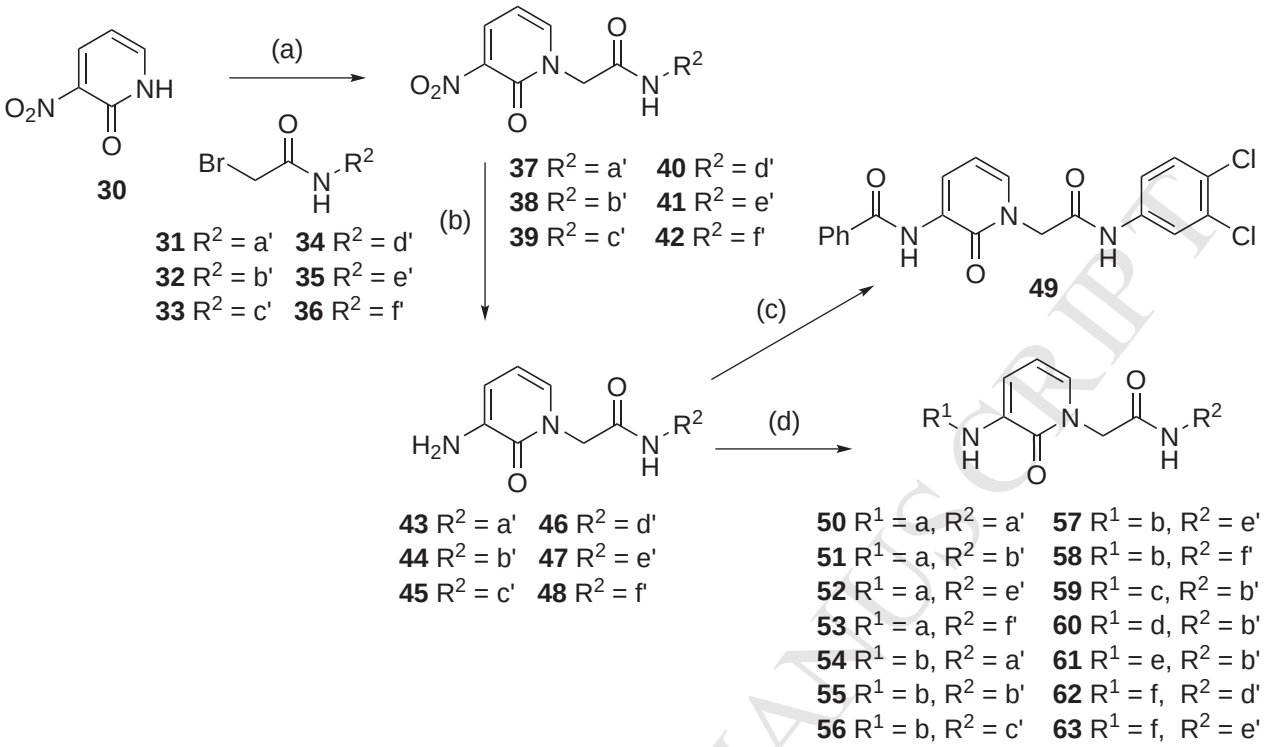
Compound	IC_{50} (μM)[a]	LE	cLogP[b]	LELP
1	6.3	0.24	3.52	14.75
2	34.2	0.24	2.25	9.55
14	18.5	0.22	3.94	17.60
15	5.66	0.25	3.94	15.87
16	2.1	0.27	4.08	15.19
17	1.92	0.26	4.49	17.18
18	10.2	0.24	3.48	14.23
20	19.5	0.25	3.22	12.96
23	3.4	0.24	3.63	15.00
56	31.3	0.21	3.80	17.83
59	57	0.21	4.15	19.96
63	68	0.18	4.55	24.67

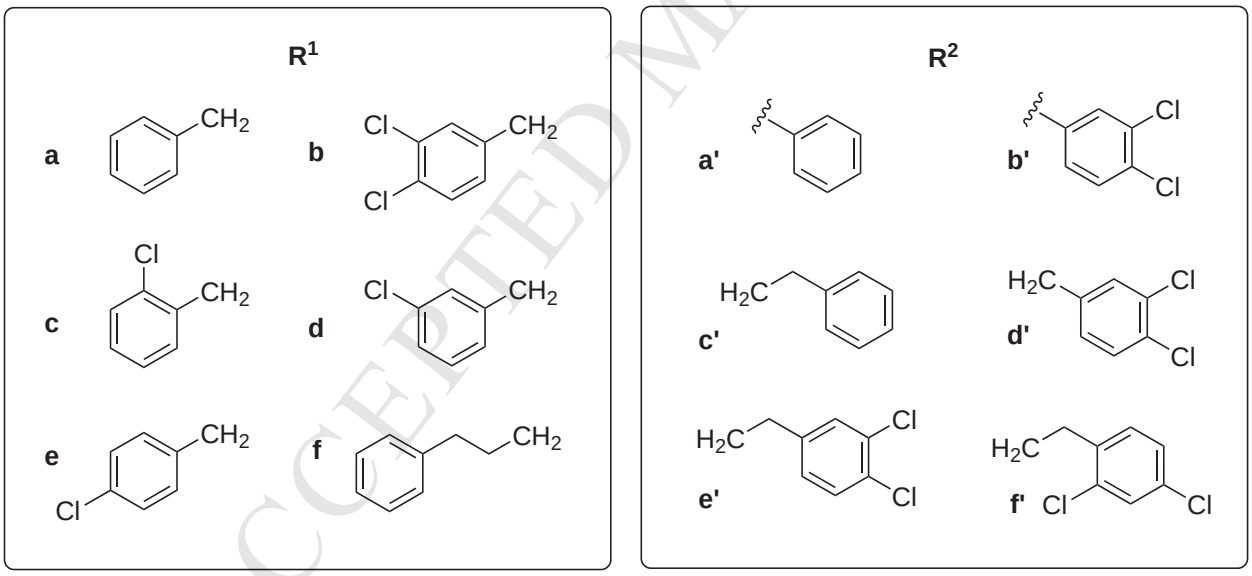
[a] Values as reported in Tables 1-3

[b]Calculated with ALOGPS 2.1



Scheme 1. (a) aldehyde or *p*-toluenesulfonyl chloride, 2hrs, rt then $NaBH(OAc)_3$, 16 hrs, rt. (b) amine, 4 hrs, 120 °C; (c) 2-chlorobenzoyl chloride, Et_3N, 4 hrs, rt (compound **25**) or phenyl isocyanate, 4hrs, rt (compound **26**); (d) amine, 4 hrs, 120 °C (compounds **27**,**28**) or 1hr, rt (compound **29**).





Scheme 2. (a) NaH, THF, microwave 150 °C, 60 min or NaH, 140 °C, 20 hrs; (b) H_2, Pd/C. CH_3OH, 6-48 hrs, rt; (c) benzoyl chloride, Et_3N, 16 hrs, rt; (d) $NaBH(OAc)_3$, 16 hrs, rt.

2-Oxo-1,2-dihydropyridinyl-3-yl amide-based Glycogen Phosphorylase inhibitors: Design, synthesis and structure-activity relationship study

Wendy A. Loughlin, Ian D. Jenkins, N, David Karis, Stephanie S. Schweiker, Peter C. Healy

Highlights

- Design and synthesis of 29 new pyridone amide inhibitors of Glycogen Phosphorylase
- Second generation library hit rate was 45%, with 13 compounds inhibiting GPa
- Two lead compounds **16** and **17** with improved GPa inhibition (IC_{50} = 2.1 and 1.92 μM)
- SAR analysis revealed sensitivity of GPa to the length of the pyridone amide
- SAR analysis revealed a preference for inclusion of a 3,4-dichlorobenzyl moiety