Synthesis and Evaluation of the first Fluorescent Antagonists of the Human $P2Y_2$ Receptor based on AR-C118925.

Sean Conroy[1,#], Nicholas D. Kindon[1,#], Jacqueline Glenn[2,3], Leigh A. Stoddart[2,3], Richard J. Lewis[4], Stephen J. Hill[2,3], Barrie Kellam[1,3], Michael J. Stocks[1]*

[1] School of Pharmacy, Centre for Biomolecular Sciences, University Park Nottingham, Nottingham, NG7 2RD, UK.

[2] Division of Physiology, Pharmacology & Neuroscience, Medical School, University of Nottingham, Nottingham, NG7 2UH, UK.

[3]Centre of Membrane Proteins and Receptors, University of Birmingham and University of Nottingham, the Midlands, NG7 2UH, UK.

[4] Medicinal Chemistry, Respiratory, Inflammation and Autoimmunity, IMED Biotech Unit, AstraZeneca, 431 83 Mölndal, Gothenburg, Sweden

[#]These authors contributed equally to this work.

KEYWORDS: $P2Y_2R$, antagonists, fluorescence, BRET, G Protein-Coupled Receptor, GPCR

ABSTRACT

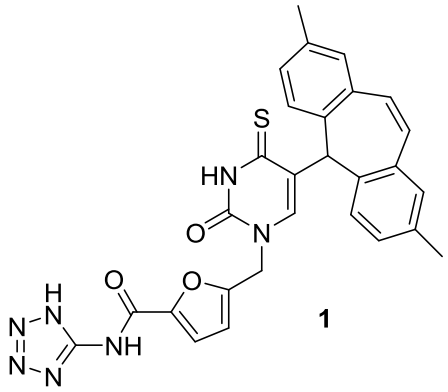
The human $P2Y_2$ receptor (*h*$P2Y_2R$) is a G protein-coupled receptor that shows promise as a therapeutic target for many important conditions including anti-metastatic cancer therapy and more recently for the treatment of idiopathic pulmonary fibrosis. As such, there is a need for new *h*$P2Y_2R$ antagonists and molecular probes to study this receptor. Herein, we report the development of a new series of non-nucleotide *h*$P2Y_2R$ antagonists leading to the discovery of a series of fluorescent ligands containing different linkers and fluorophores based on the known, non-nucleotide *h*$P2Y_2R$ antagonist AR-C118925 (**1**). One of these conjugates **98** displayed micromolar affinity for the *h*$P2Y_2R$ (pK_d = 6.32 ± 0.10; *n*=17) using a bioluminescence energy transfer (BRET) assay. Confocal microscopy with this ligand revealed displaceable membrane labeling of astrocytoma cells expressing un-tagged *h*$P2Y_2R$**.** These properties, make **98** one of the first tools for studying *h*$P2Y_2R$ distribution and organization.

INTRODUCTION

P2Y receptors (P2YRs) are G protein-coupled receptors (GPCRs) that are activated by extracellular nucleotides. The P2Y family is composed of eight members encoded by distinct genes that can be subdivided into two groups based on their primary signaling through specific G proteins[1] and sequence homology. The first sub-group includes the $P2Y_{1,2,4,6,11}$ receptors which primarily signal though G_q, with the second sub-group signaling through G_i encompassing the $P2Y_{12,13,14}$ receptor subtypes.[2] Notably, the $P2Y_2$ receptor ($P2Y_2R$) is activated by the endogenous agonists uridine-5′-triphosphate (UTP *h*$P2Y_2$, EC_{50} = 140 nM) and adenosine-5′-triphosphate (ATP, *h*$P2Y_2$, EC_{50} = 230 nM).[3] As the $P2Y_2R$ is predominately G_q-coupled, receptor activation leads to stimulation of phospholipase C, IP_3 release and elevation of intracellular Ca^{2+}

concentration, as well as the initiation of protein kinase C and activation of the mitogen activated protein kinase cascade.

Defining the clinical role for $P2Y_2R$ antagonism has been hampered by the lack of high affinity and drug-like receptor antagonists.[4] However, it has been reported that ATP released from tumor cell-activated platelets induces the opening of the endothelial barrier, leading to migration of tumor cells and hence cancer proliferation. More importantly, the $P2Y_2R$ was identified as the primary mediator of this effect; a strong reduction of tumor cell metastasis was observed in $P2Y_2R$ deficient mice revealing a therapeutic potential of $P2Y_2R$ antagonists as anti-metastatic agents.[5, 6] Recently, it has been reported that both inflammation and fibrosis were reduced in $P2Y_2R$-deficient mice compared to wild type animals. In addition mechanistic studies have demonstrated that recruitment of neutrophils into the lungs, proliferation and migration of lung fibroblasts and IL-6 production are all key $P2Y_2R$-mediated processes. These studies clearly demonstrate the involvement of $P2Y_2R$ subtypes in the pathogenesis of fibrotic lung diseases in humans and mice and support the development of selective $P2Y_2R$ antagonists for the treatment of idiopathic pulmonary fibrosis (IPF).[7] To date, the only reported high affinity $P2Y_2R$ antagonists were those developed by scientists from AstraZeneca resulting in the non-nucleotide $P2Y_2R$ antagonist AR-C118925 (**1**).[8, 9]



1

Several *in vivo* and *ex vivo* studies using **1** have been reported further validating the therapeutic benefit of $P2Y_2R$ antagonists. Importantly, it has been shown that **1**, which was reported to be inactive at 10 μM against a panel of 37 other receptors, was able to concentration-dependently antagonize ATPγS-induced mucin secretion in an *ex vivo* model of human bronchial epithelial cells.[10] In addition, Müller *et al* recently demonstrated that **1** was a selective, high affinity reversible antagonist of the $P2Y_2R$.[11]

We were drawn to the exciting possibility of using **1** as a chemical template to design new $P2Y_2R$ antagonists[12] and synthesize fluorescently labeled chemical tools to further probe $P2Y_2R$ function.[13] Using fluorescence as a means to study GPCRs allows access to a large range of pharmacological techniques that can capture dynamic processes in living cells.[14] In particular, fluorescently labeled receptor antagonists have been developed to target GPCRs allowing visualization of GPCR function at the cellular level.[15–17] In addition, fluorescent ligands can be used in resonance energy transfer (RET) techniques, in particular those that utilize the luciferase NanoLuc, to quantify ligand-receptor interactions and determine the affinity of unlabeled ligands.[18] This offers advantages for receptors such as $P2Y_2R$ for which there are currently no commercially available radio ligands. In addition, as the reported antagonists for $P2Y_2R$ have mid to high affinity it is proposed that fluorescent ligands designed on these ligands might also have affinity in this range. This may prove problematic for techniques which directly monitor fluorescent ligand binding but NanoBRET has been shown to display low non-specific binding at high fluorescent ligand concentrations.[18, 19]

RESULTS AND DISCUSSION

Synthesis and evaluation of $hP2Y_2R$ antagonists. The medicinal chemistry strategy involved an initial exploration of the structure activity relationship (SAR) around **1**, to enable the design of

structural analogues with improved predicted physicochemical properties and to guide our design strategy through highlighting suitable linking sites to attach the fluorophore groups (Figure 1 and Table 1).

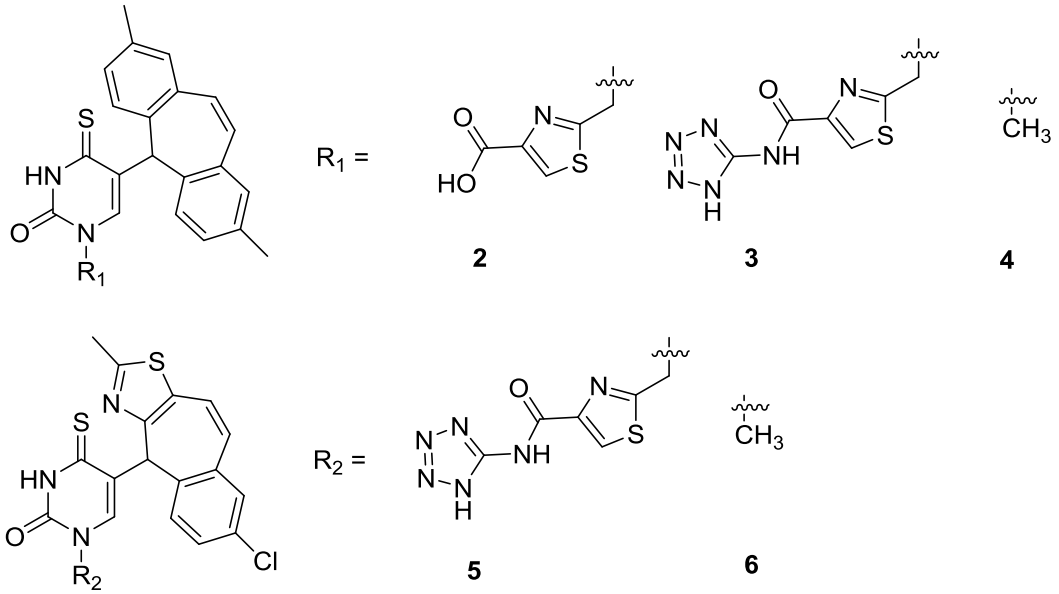


Figure 1. SAR for $P2Y_2R$ antagonists showing changes from the furan in **1**, to thiazole (reduced lipophilicity) and change from 2,8-dimethyl-5H-dibenzo[a,d][7]annulene to 7-chloro-4H-benzo[5,6]cyclohepta[1,2-d]thiazole tricylic ring system.

Table 1 The estimated affinity value for $P2Y_2R$ antagonists **1-6** obtained from the calcium mobilization assay

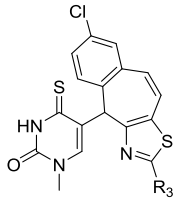
Example	*h*P2Y$_2$ pK$_b$[a]	Example	*h*P2Y$_2$ pK$_b$[a]
1	7.51 ± 0.09 (12)	**4**	i.a.
2[b]	6.43 ± 0.08 (3)	**5**	6.48 ± 0.10 (3)
3	7.11 ± 0.14 (7)	**6**	5.99 ± 0.03 (7)

[a] The estimated affinity value for each antagonist (pK$_b$) was calculated from the shift of UTPγS concentration response curve brought about by addition of a single concentration of antagonist using the Gaddum equation. i.a. = less than 50% inhibition of the response to 0.1μM UTPγS in the

presence of 10 μM compound. UTPγS EC_{50} = 7.9 ± 1.3 nM (*n*=25). [b] literature value pA_2 5.7[8] Data shown is mean ± SEM, number of separate experiments given in parentheses.

1 has previously been shown to have high lipophilicity (cLogP 4.2)[20] and poor physicochemical properties for oral delivery.[8] We therefore synthesized the thiazole analogue **3** (cLogP 3.8), with little loss of affinity for the $P2Y_2R$. Replacement of the 2,8-dimethyl-5H-dibenzo[a,d][7]annulene to 7-chloro-4H-benzo[5,6]cyclohepta[1,2-d]thiazole tricylic ring gave **5** (pK_b 6.5, clogP 3.4). [21] In addition, non-parallel SAR was observed in the replacement of the *N*-1 thiouracil substituent with a methyl group to give a compound **6** with $P2Y_2R$ affinity, whereas the corresponding analogue **4** proved inactive. In order to explore this intriguing finding we synthesized a range of analogues of **6** to study this SAR (Table 2).

Table 2 Calcium mobilization activity for $P2Y_2R$ antagonists **6-19**



Example	R_3	*h*$P2Y_2$ pK_b[a]
7	NH_2	6.56 ± 0.14 (4)
8	PhNH	i.a. (3)
9	$PhCH_2NH$	i.a. (3)
10	$PhCH_2CH_2NH$	i.a. (3)
11	1-methyl piperazin-4-yl	i.a. (3)
12	morpholinyl	i.a. (3)
13	piperidinyl	i.a. (3)
14	2-methoxyethan-1-aminyl	6.60 ± 0.21 (4)
15	2-methoxypropan-1-aminyl	6.56 ± 0.09 (3)
16	2-ethoxyethan-1-aminyl	6.73 ± 0.05 (3)

17	2-*iso*propoxyethan-1-aminyl	6.49 ± 0.09 (3)
18	2-phenoxyethan-1-aminyl	i.a. (3)
19	phenyl	i.a. (3)

[a] The estimated affinity value for each antagonist (pK_b) was calculated from the shift of the UTPγS concentration response curve brought about by addition of a single concentration of antagonist using the Gaddum equation. i.a. = less than 50% inhibition of 0.1μM UTPγS response in the presence of 10 μM compound (n=3). n.b. the corresponding uracil derivatives were inactive at 10 μM compound. Data shown is mean ± SEM, number of separate experiments given in parentheses.

From the SAR study of **6**, it was shown that replacing thiazole 2-methyl substituent with an amino group increased *h*P2Y$_2$R affinity (compare **6** with **7**) whereas a sterically demanding substituent, such as **19** showed complete loss of affinity for the *h*P2Y$_2$R. We therefore explored substitution of the amino group and showed that both sterically demanding amino groups (**8**, **9** and **10**) and cyclic tertiary amines (**11**, **12** and **13**) were inactive. However the linear, less sterically demanding alkyl amino groups (**14**, **15**, **16** and **17**) increased *h*P2Y$_2$R affinity, although the bulkier 2-phenoxyethan-1-amino substituent **18** proved inactive.

Thus far, all the compounds synthesized were tested as racemic mixtures. To try and determine whether the activity resided in one enantiomer, resolution of **14** and **16** was achieved through semi-preparative chiral HPLC and the biology of the resolved enantiomers independently assessed (Table 3).

Table 3 Calcium mobilization activity for resolved enantiomers of **14** and **15**

Example	Racemic compound	Enantiomeric excess (ee)[a]	*h*P2Y$_2$ Pk_b[b]
20	**14**	99%	6.63 ± 0.11 (6)
21		78%	5.82 ± 0.05 (3)
22	**16**	95%	i.a. (3)

23	96%	6.78 ± 0.05 (6)

[a] Compounds separated using Phenomenex's Lux 5 μm amylose-2 stationary phase. [b] The estimated affinity value for each antagonist (pK_b) was calculated from the shift of UTPγS concentration response curve brought about by addition of a single concentration of antagonist using the Gaddum equation. i.a. = less than 50% inhibition of 0.1μM UTPγS response in the presence of 10 μM compound (n=3). Data shown is mean ± SEM, number of separate experiments given in parentheses.

From these results it is possible to see that the *h*P2Y$_2$R antagonist affinity observed for the racemic compounds (**14** and **16**) resides largely in just one enantiomer **20** and **23** respectively. Although some antagonist activity is observed for **21**, this may be attributed to residual active enantiomer **20** which constitutes 11% of the sample. Unfortunately, the resolved enantiomers proved to be amorphous powders and so single crystal X-ray determination of the absolute chirality could not be used for structural determination. However, vibrational circular dichroism of **22** and **23** was used, where spectra were acquired on both samples and fitted to the calculated spectra.[22–24] The results (supplementary information) showed that there was a good match for **22** with the calculated spectrum for the *(S)*- enantiomer and therefore **23** was assigned as the active *(R)*-enantiomer (Figure 2).

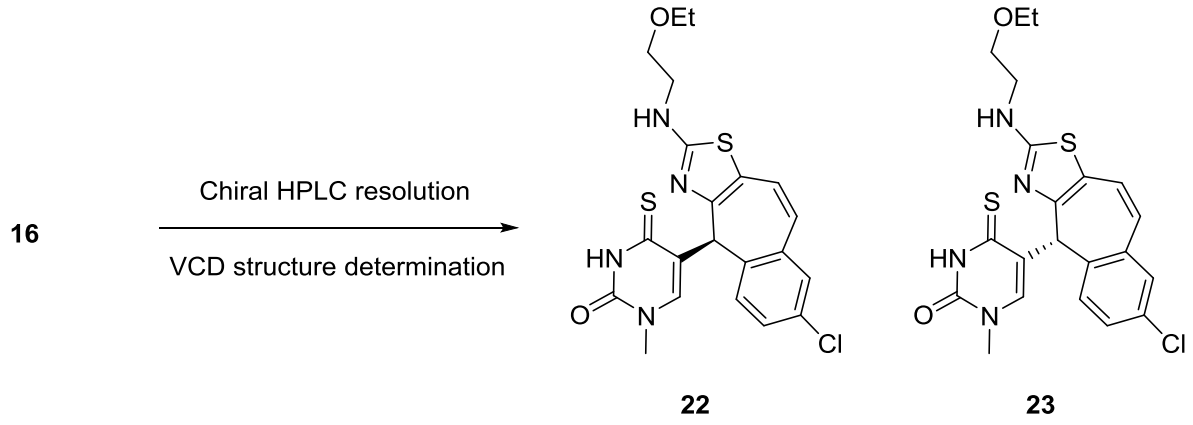
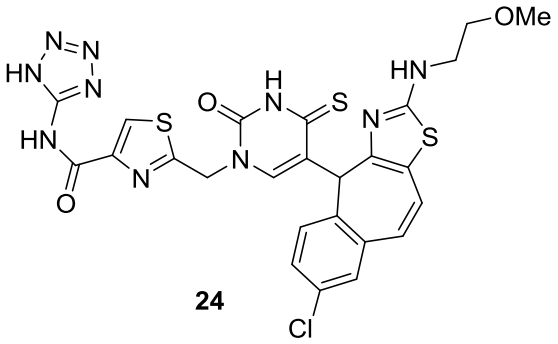


Figure 2. Chiral resolution of **16** and structural assignment made by vibrational circular dichroism.

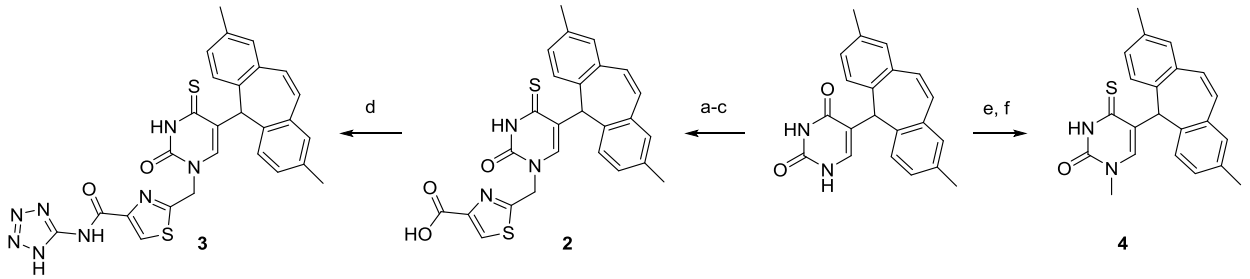
In an attempt to increase affinity within the new series of compounds we incorporated key structural features of **14** and **5** to generate compound **24.**



24

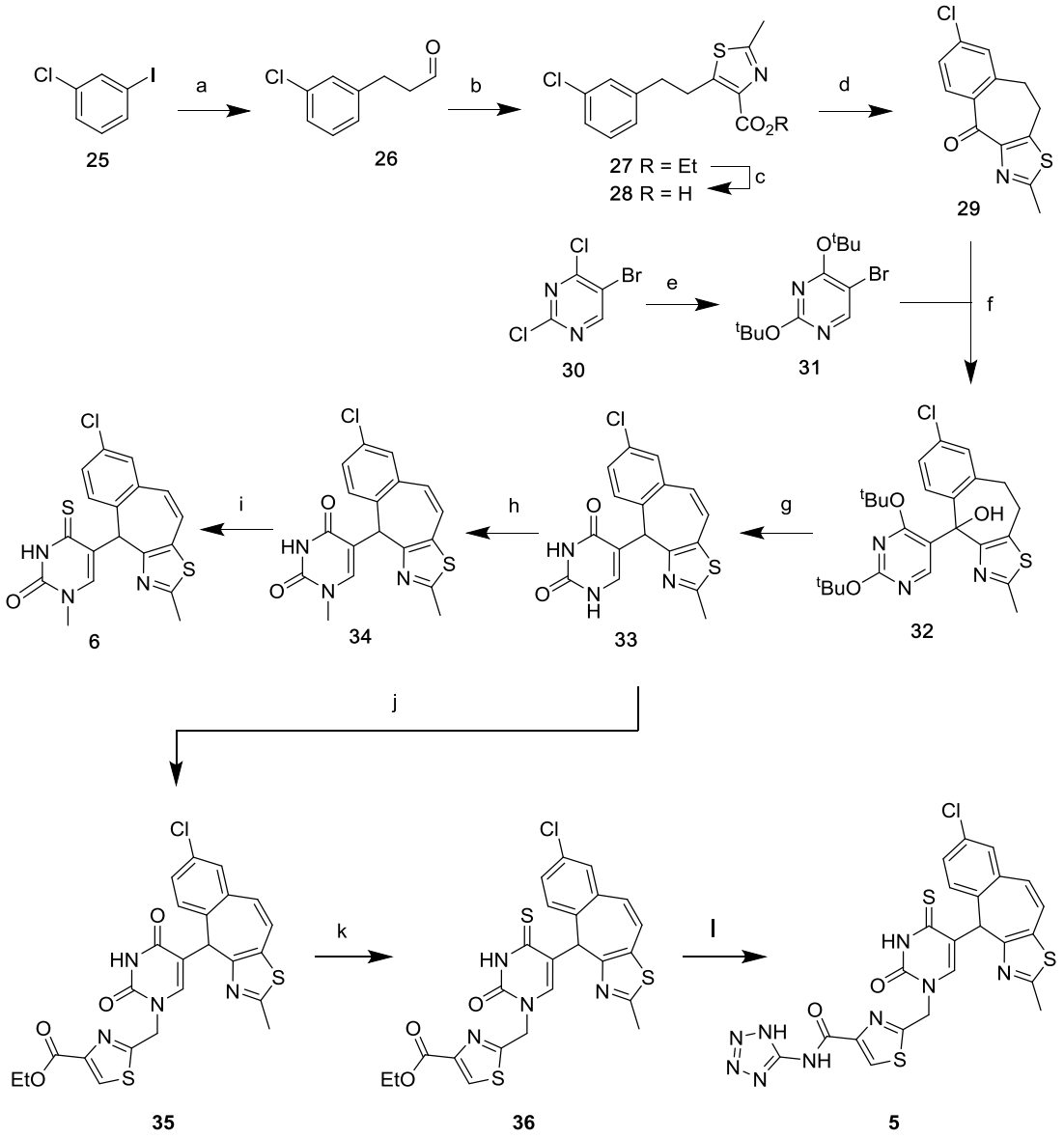
However, **24** did not demonstrate the expected increase in affinity from combining the features of **14** and **5** and showed a similar level of *h*P2Y$_2$R affinity (pK_d 7.02 ± 0.05, n=4) to both **1** and **3**, demonstrating non-additive SAR within the series of compounds.[25]

The synthesis of compounds **2-4** is illustrated in Scheme 1. Alkylation of 5-(2,8-dimethyl-*5H*-dibenzo[a,d][7]annulen-5-yl)pyrimidine-2,4(1H,3H)-dione[8] with ethyl 2-(bromomethyl)thiazole-4-carboxylate followed by treatment with Lawesson's reagent and saponification gave **2**[8] which was reacted with 2-amino tetrazole using benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate activation to afford **3**. In a similar manner, alkylation with methyl iodide followed by conversion to the thiouracil gave **4**, in good over all yield.



Scheme 1. (a) (i) *N,O-bis*(trimethylsilyl)trifluoroacetamide, DCM, reflux, 18 h; (ii) ethyl 2-(bromomethyl)thiazole-4-carboxylate, 50^{o}C, 24 h (55%). (b) Lawesson's reagent, 1, 4-dioxane, 100^{o}C, 18 h (85%). (c) NaOH, methanol/H_2O, reflux, 1 h (91%). (d) 5-aminotetrazole, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate, DMF, DIPEA, rt, 1 h

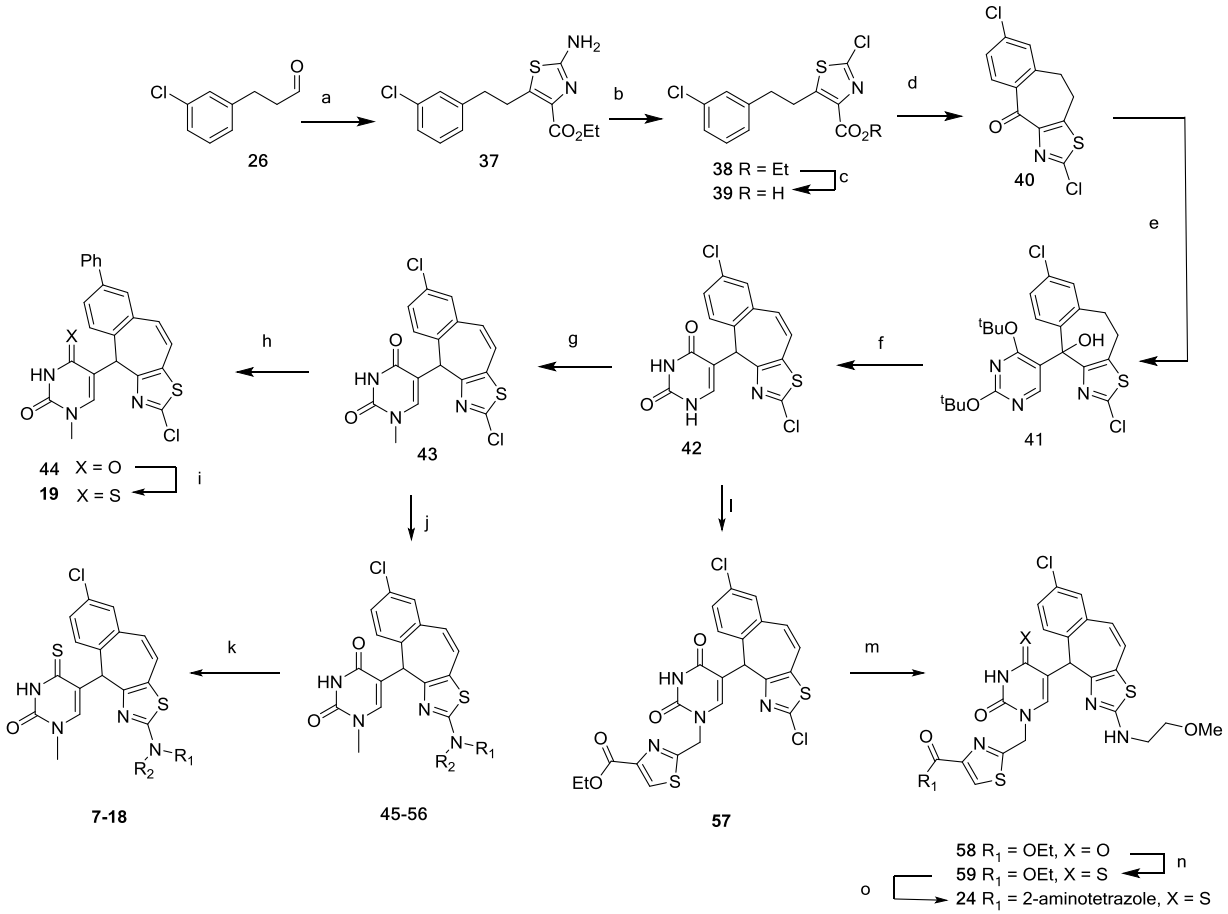
(49%). (e) (i) *N,O-bis*(trimethylsilyl)trifluoroacetamide, DCM, reflux, 18 h; (ii). iodomethane, 50°C, 24 h (61%). (f) Lawesson's reagent, 1,4-dioxane, reflux, 18 h (40%).



Scheme 2. Reagents and conditions: (a) Allyl alcohol, tetrabutylammonium chloride, DMF, 3% mol. palladium(II)acetate, $NaHCO_3$, 50°C, 18 h (92%). (b) (i) ethyl dichloroacetate, sodium ethoxide, diethyl ether, 30 min, 0-40°C; (ii) thioacetamide, ethanol, reflux, 3 h (30% over 2 steps). (c) NaOH, H_2O/THF (1:1), rt, 18 h (99%). (d) (i) oxalyl chloride, cat. DMF, DCM, rt, 3 h; (ii) aluminium(III)chloride, DCM, rt, 18 h (39% over two steps). (e) Sodium *tert*-butoxide, THF, 0°C-rt, 18 h (61%) (f) (i) *n*-butyllithium, THF, -78°C, 30 mins; (ii) **29**, -78°C to rt, 1 h (65%). (g) trifluoroacetic acid, reflux, 72 h (36%). (h) (i) *N,O-bis*(trimethylsilyl)trifluoroacetamide, DCM, reflux, 18 h; (ii) iodomethane, 50°C, 24 h (61%). (i) Lawesson's reagent, 1,4-dioxane, reflux, 18

h (40%). (j) (i) *N,O-bis*(trimethylsilyl)trifluoroacetamide, DCM, reflux, 18 h; (ii) ethyl 2-(bromomethyl)thiazole-4-carboxylate, 50°C, 24 h (57%). (k) Lawesson's reagent, 1, 4-dioxane, 100°C, 18 h (89%). (l) (i) NaOH, methanol/H_2O, reflux, 1 h (91%). (ii) 5-aminotetrazole, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate, DMF, DIPEA, rt, 1 h (30%).

Scheme 2 shows the synthetic route to **5** and **6**. The first step involved performing a Heck reaction, coupling 3-chloroiodobeneze **25** to allyl alcohol and this successfully isomerized *in situ* yielding the desired aldehyde **26**. This was reacted with ethyl dichloroacetate in a Darzens condensation [25] to generate a α-chloro epoxide which was reacted directly with thioacetamide to afford desired 2-methylthiazole **27** with moderate yields achieved over two steps. Freshly prepared sodium ethoxide, generated from sodium metal in dried ethanol was found to be the optimal base for the Darzens condensation. Saponification gave carboxylic acid **28** and treatment with oxalyl chloride generated the acyl chloride which was immediately cyclized to give the tricyclic ketone **29** as a single regioisomer. Lithiation of the di *tert*-butyl ether-protected uracil **30**,[25] was readily achieved with *n*-butyllithium and this underwent a 1, 2-addition to ketone **29** to give tertiary alcohol **32**. Concomitant deprotection and dehydration gave uracil **33** through heating in trifluoroacetic acid. Although the yield for this reaction was poor, other acidic conditions were ineffective. Alkylation at the *N*1-position of the uracil was achieved in a one-pot process of silylation with *N,O-bis*(trimethylsilyl)trifluoroacetamide (BSTFA), alkylation with iodomethane and subsequent desilylation to give **34**. Finally, reaction with Lawesson's reagent gave **6**. From the uracil intermediate **33** alkylation with ethyl 2-(bromomethyl) thiazole-4-carboxylate[26] gave **35** which was subsequently reacted with Lawesson's reagent to give **36**. Hydrolysis was followed by reaction of the resulting carboxylic acid with 5-aminotetrazole and benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate to give **5**.



Scheme 3. Reagents and conditions: (a) (i) Ethyl dichloroacetate, sodium ethoxide, diethyl ether, 30 min, 0-40ºC; (ii) thiourea, ethanol, reflux, 2.5 h (46% over 2 steps). (b) copper(II)chloride, *tert*-butyl nitrite, acetonitrile, rt, 2 h (65%) (c) NaOH, H_2O/THF (1:1), rt, 18 h (quant.). (d) (i) Oxalyl chloride, cat. DMF, DCM, rt, 3 h; (ii) $AlCl_3$, DCM, rt, 18 h (83% over two steps). (e) (i). **31**, n-butyllithium, THF, -78ºC, 30 mins; (ii). **82**, -78ºC to rt, 1 h (79%). (f) 1:1 acetic acid/1,4-dioxane 140ºC,10 min (58%). (g) (i) *N,O-bis*(trimethylsilyl)trifluoroacetamide, DCM, reflux, 18 h; (ii). iodomethane, 50ºC, 24 h (61%) (h) Phenylboronic acid, Na_2CO_3, 1% mol. *bis*(triphenylphosphine) palladium(II)chloride, 1,4-dioxane, water, MW, 150^{0}C, 5 min (68%). (i) Lawesson's reagent, 1,4-dioxane, reflux, 18 h (76%). (j) R_1R_2NH, 1, 4-dioxane, MW, 100ºC, 4h (51-92 %). (k) Lawesson's reagent, 1,4-dioxane, reflux, 18 h (44-88%). (l) (a) (i) N,O-*bis*(trimethylsilyl)trifluoroacetamide, 1,2-dichloroethane, reflux, 18 h; (ii) ethyl 2-(bromomethyl)thiazole-4-carboxylate, reflux, 48 h (91%). (m) 2-Methoxyethyl amine, triethylamine, 1, 4-dioxane, reflux, 72 h (59%). (n) Lawesson's reagent, 1, 4-dioxane, reflux, 18 h (81%). (o) (i) NaOH, methanol/H_2O, reflux, 1 h (91%). (ii) 5-Aminotetrazole, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate, DMF, DIPEA, rt, 2 h (34%).

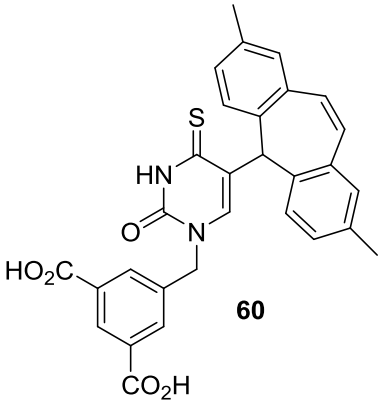
In a similar sequence to Scheme 2, aldehyde **26** was reacted with ethyl dichloroacetate and the resulting crude α-chloro epoxide reacted with thiourea to afford the 2-aminothiazole **37** which was converted to the 2-chlorothiazole **38** (Scheme 3). The ethyl ester was hydrolyzed to afford

carboxylic acid **39,** converted to the acid chloride and cyclized to tricyclic ketone **40**. Lithiation of the *tert*-butyl ether-protected uracil **31** and reaction with **40** gave the tertiary alcohol **41**. After screening a range of milder acidic conditions, heating to 140ºC with MW irradiation in 1:1 acetic acid/1,4-dioxane for 10 minutes was found to be optimal for the formation of the desired uracil intermediate **42,** which was subsequently methylated to give **43**. The chlorine atom in compound **43** was readily displaced through nucleophilic aromatic substitution with a range of primary and secondary amines on heating under basic conditions. These conditions were unsuccessful when using aniline and, in this instance, heating in the microwave with hydrochloric acid (catalytic) proved successful to give **46.** Microwave-based conditions were employed for the substitution with ammonia in the synthesis of **45**. A Suzuki reaction with phenylboronic acid gave **44**. Using Lawesson's reagent, it was then possible to convert these uracil derivatives (**44-54**) to the respective 4-thiouracil derivatives (**6-19**). Employing a route analogous to the synthesis of compound **7**, it was possible to generate the desired tetrazole analogue **24**. Uracil intermediate **42** was alkylated at the N1-position with ethyl 2-(bromomethyl)thiazole-4-carboxylate to give **57**. This was reacted with 2-methoxyethylamine to afford **58**, which was subsequently reacted with Lawesson's reagent to give the 4-thiouracil **59**. Hydrolysis, followed by benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate activation and reaction with 5-aminotetrazole, afforded **24**.

Synthesis of hP2Y$_2$R fluorescent ligands. With a view to developing a series of fluorescent conjugates suitable for both a bioluminescence resonance energy transfer (BRET) ligand binding assay [18,27–29] and imaging through confocal microscopy, we embarked on a strategy to synthesize bodipy conjugates, specifically dyes bodipy A (absorption max 628 nm, emission max 642 nm) or

bodipy B (absorption max 503 nm, emission max 509 nm) as this would allow us the opportunity of ligand choice in future imaging work.

Two positions on to the $P2Y_2R$ antagonist core structure were considered for attachment of the linker and fluorophore (Figure 3). In order to simplify the synthetic chemistry and increase SAR within the series, we examined replacement of the furan ring of **1** and the thiazole ring of **2** with a 1,3,5 tri-substituted phenyl ring. This would allow attachment of the acyl tetrazole group in addition to providing a second free carboxylic acid group to attach the fluorescent conjugates. Fortunately, the 3,5-dicarboxylic acid **60** (*h*$P2Y_2R$ pK_d 6.53 ± 0.04, *n*= 7) was well tolerated with no loss of affinity for the $P2Y_2R$ compared with compound **2**. Therefore, the first series of compounds have the linker-fluorophore attached *via* the phenyl ring of **60**.



60

Having established that alkoxyalkyl amines are tolerated for activity in the 2-position of the thiazole in the 4*H*-benzo[5,6]cyclohepta[1,2-*d*]thiazol-4-yl) tricyclic ring of compounds of the type shown in Figure 2, this position was chosen as the second point of attachment of the linker-fluorophore. Finally, a third generation of fluorescent ligands would be explored containing the optimal second generation fluorescent ligand with incorporation of the acyl tetrazole functional group.

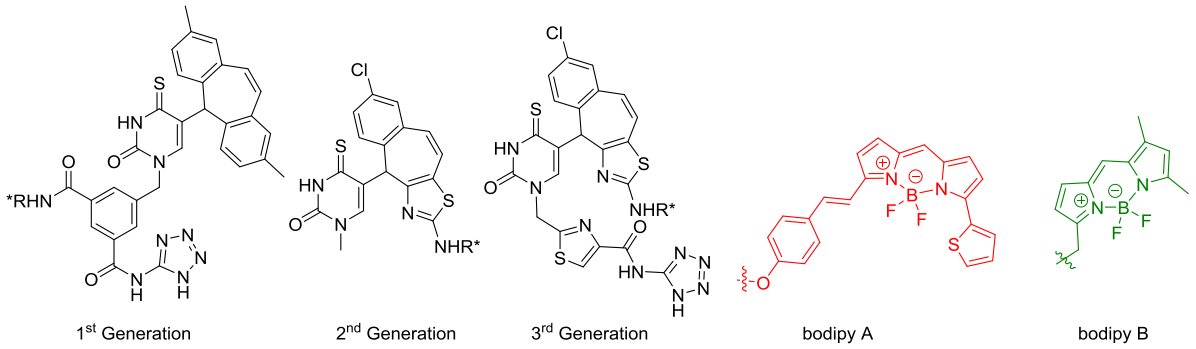
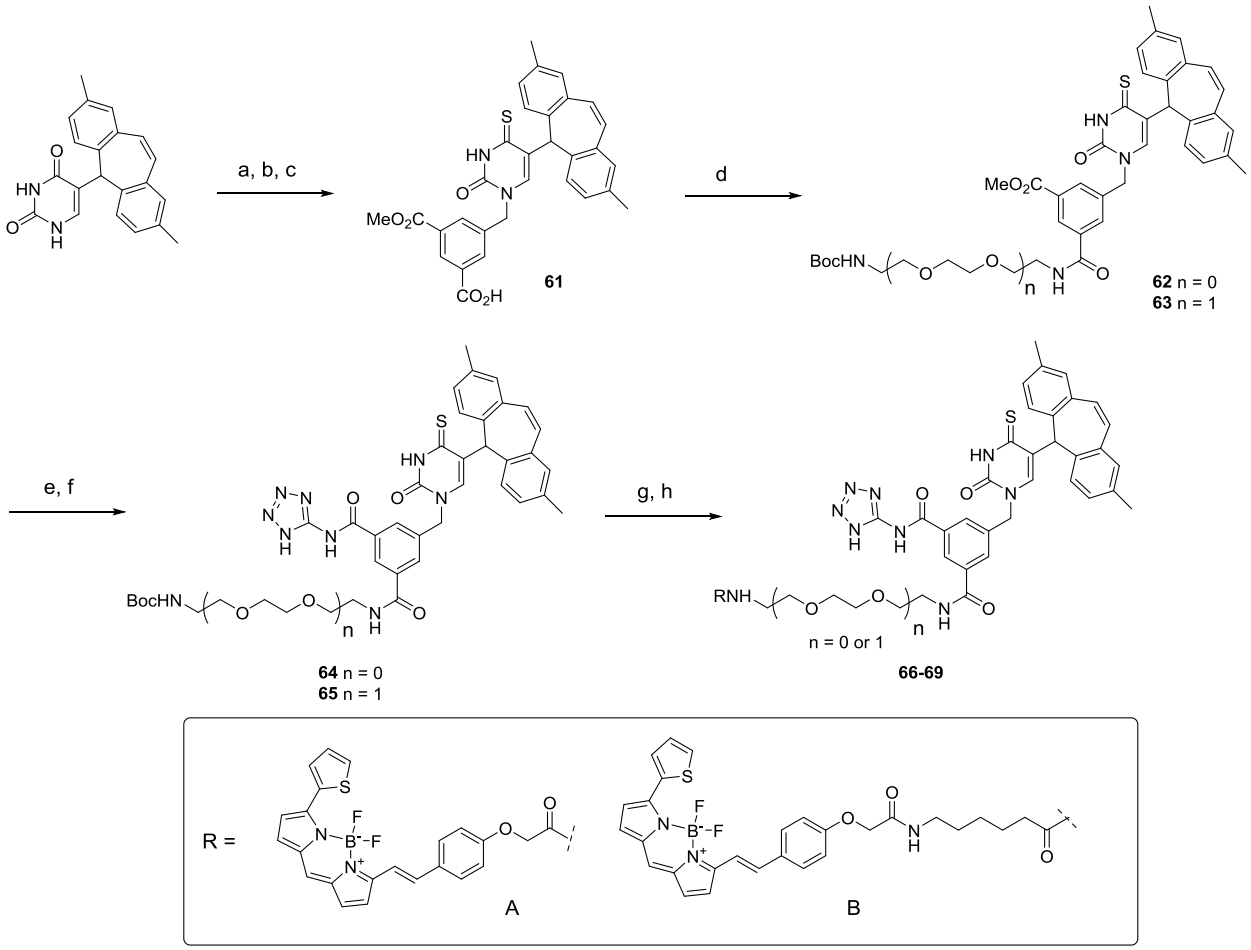


Figure 3. Design of fluorescent $P2Y_2R$ antagonists based from SAR on **1**. The figure shows potential attachment points from which to attach either bodipy A or bodipy B *via* a suitable linking group selected. * Represents attachment points for fluorophore linkage *via* a suitable linking group.

First generation hP2Y₂R fluorescent ligands. The general synthetic route to the first generation $P2Y_2R$ fluorescent antagonists is shown in Scheme 4.



Scheme 4. a) Dimethyl 5-(bromomethyl)*iso*phthalate, BSTFA, 1,2-dichloroethane, reflux, 16 h. b) Lawesson's reagent, 1,4-dioxane, reflux, 16 h. c) 2eq NaOH, MeOH, toluene, H_2O, 4h. 65% (3 steps) d) *tert*-butyl (2-(2-aminoethoxy)ethyl)carbamate (53%) or *tert*-butyl (2-aminoethyl)carbamate (63%), NEt_3, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate, DMF, rt, 0.5 h. e) LiOH, MeOH, H_2O, 16 h, rt. 14-30%. f) 4-aminotetrazole, NEt_3, Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate, DMF, 1h, rt. g) 4M HCl in 1,4-dioxane, 1h. h) bodipy-SE, DIPEA, DMF, rt, 1-4 h.15-45%.

Alkylation of 5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)pyrimidine-2,4(1H,3H)-dione[8] with dimethyl 5-(bromomethyl)isophthalate, followed by treatment with Lawesson's reagent and selective hydrolysis of one of the methyl esters gave **61**. The carboxylic acid **61** was activated using benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate and coupled to form the appropriate amide (**62**-**63**). The second ester was then hydrolyzed and converted to the amidotetrazoles (**64**-**65**). Finally, the Boc protecting group was removed and the resulting amine

coupled to the fluorophore using the appropriate commercially available BODIPY succinimidyl ester to give a small library of 4 fluorescent conjugates (**66-69**).

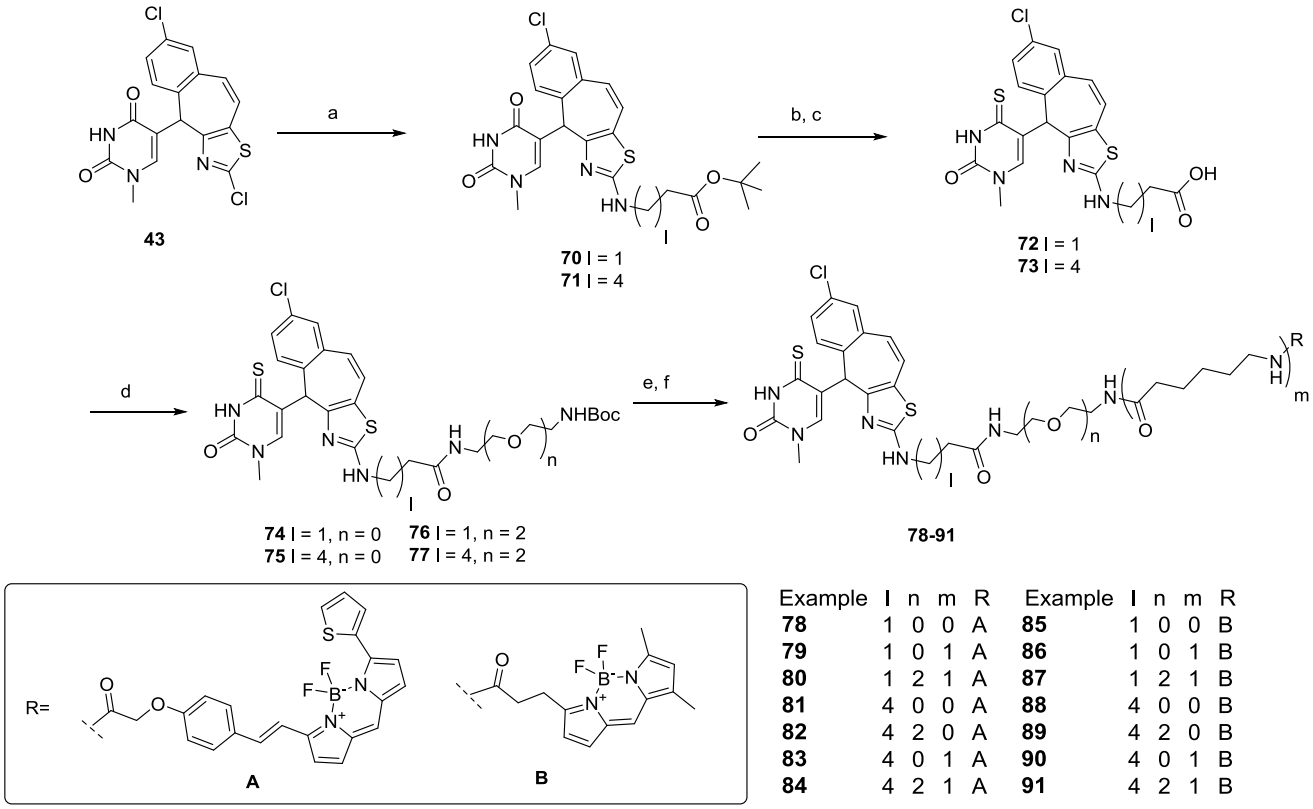
To determine whether any of these conjugates had affinity at the $P2Y_2R$ and consequently if they could be used in NanoBRET binding assays, a 1321N1 astrocytoma cell-line expressing recombinant $P2Y_2R$ tagged on its *N*-terminus with NanoLuc (NanoLuc-$P2Y_2R$) was generated. The NanoLuc-tagged $P2Y_2$ receptors exhibited normal calcium signals (EC_{50} for UTPγS of 91 ± 12 nM; *n=3*). These NanoLuc-$P2Y_2$ cells were treated with increasing concentrations of **66-69** and then treated with the NanoLuc substrate furimazine, before the resulting BRET signal was monitored. All four compounds showed moderate to low affinity for the NanoLuc-$P2Y_2R$ (Table 4), with the conjugates **68** and **69** having the higher affinities. However, this did illustrate the power of using NanoBRET to monitor ligand binding to low affinity receptors.

Table 4: Affinity of **66**, **67**, **68** and **69** determined in 1321N1 astrocytoma cells expressing recombinant NLuc-$P2Y_2R$

Example	n[b]	R[c]	NanoBRET pK_d[a]
66	0	A	5.56 ± 0.1 (3)
67	0	B	5.78 ± 0.18 (3)
68	1	A	6.12 ± 0.07 (3)
69	1	B	6.07 ± 0.15 (3)

[a]pK_d values derived from saturation binding curves. [b, c] n and R previously defined from Scheme 4. Data shown is mean ± SEM, number of separate experiments given in parentheses.

Second generation $hP2Y_2R$ fluorescent ligands. The general synthetic route to the second generation $P2Y_2R$ fluorescent antagonists, in which the linker-fluorophore is attached to the thiazole ring, is illustrated in Scheme 5.



Example	l	n	m	R	Example	l	n	m	R
78	1	0	0	A	**85**	1	0	0	B
79	1	0	1	A	**86**	1	0	1	B
80	1	2	1	A	**87**	1	2	1	B
81	4	0	0	A	**88**	4	0	0	B
82	4	2	0	A	**89**	4	2	0	B
83	4	0	1	A	**90**	4	0	1	B
84	4	2	1	A	**91**	4	2	1	B

Scheme 5. Reagents and conditions: (a) amine, NEt_3, 1,4-dioxane, reflux, 72 h. 96-97% (b) Lawesson's reagent, 1,4-dioxane, 100ºC, 18 h. 39-74% (c) TFA, DCM, rt, 30 min. (d) amine, Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate, DMF, DIPEA, rt, 1 h. 36-58% (2 steps) (e) TFA, DCM, rt, 30 min; f) bodipy-SE, DIPEA, DMF, rt, 1-4 h. 10-54% (2 steps).

Nucleophilic displacement of the 2-chloro substituent of **43** by either *tert*-butyl 3-aminopropanoate or *tert*-butyl 6-aminohexanoate gave the corresponding *tert*-butyl esters (**70** and **71**). Treatment with Lawesson's reagent in 1,4-dioxane at 100ºC resulted in formation of the 4-thiouracils (**72** and **73**). The carboxylic acids were generated from the *tert*-butyl esters using TFA-mediated hydrolysis and subsequently coupled with *tert*-butyl (2-aminoethyl)carbamate or *tert*-butyl (2-(2-aminoethoxy)ethyl)carbamate to generate the corresponding amides (**74**-**77**). The Boc protecting group was removed using TFA and the resulting amines coupled with the appropriate commercially available bodipy-succinimidyl ester (SE), generating a small library of 14 fluorescent conjugates (**78**-**91**). These fluorescent conjugates were initially tested using the

aforementioned NanoBRET binding assay at fixed concentrations of 10 µM in the presence and absence of 10 µM **1** (see figure S1 in the supplementary material). It was found that **80, 85, 86** and **87** generated the largest specific NanoBRET signal, therefore their affinity was determined from saturation binding assays demonstrating the excellent signal to noise ratios observed from the NanoBRET assay even for low affinity conjugates (Figure 4; Table 5).

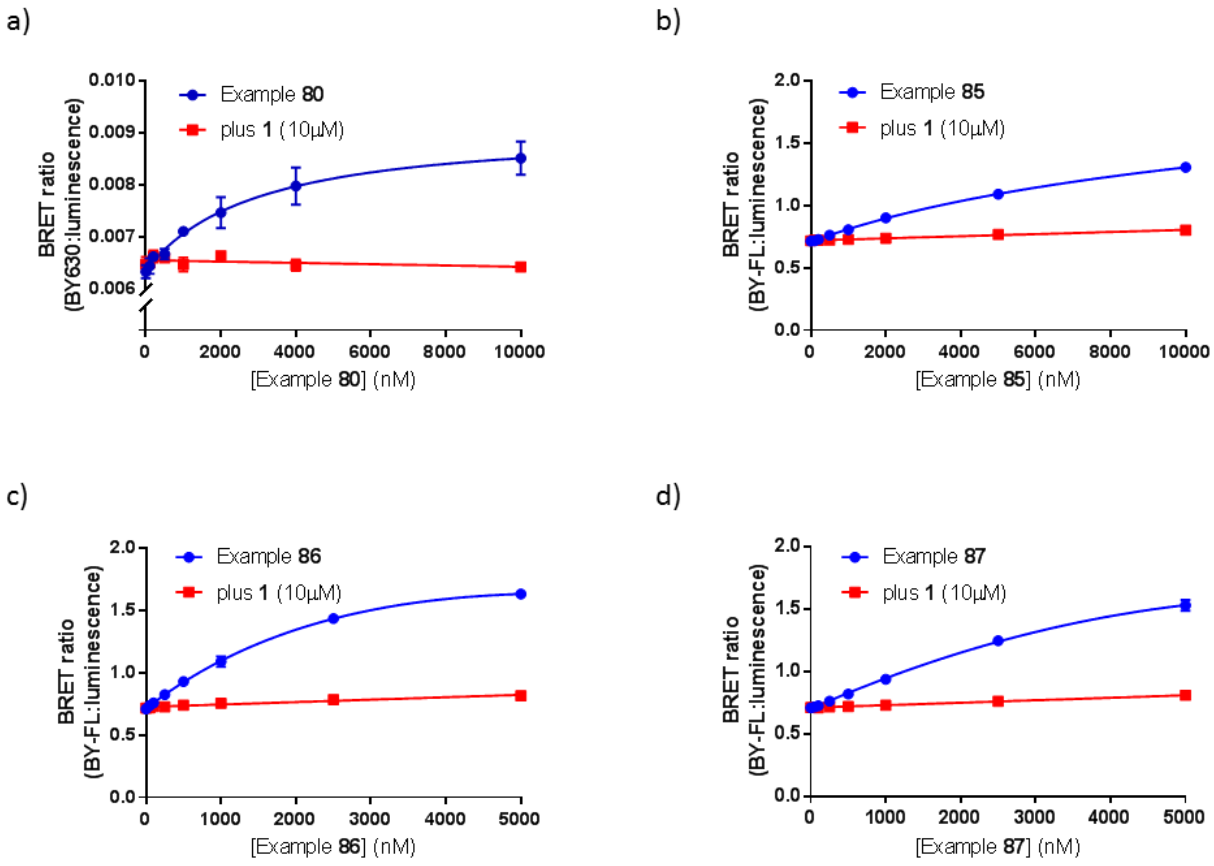


Figure 4. NanoBRET saturation binding isotherm determined in 1321N1 astrocytoma cells expressing recombinant NLuc-P2Y$_2$R. This has been performed for (a) **80**, (b) **85**, (c) **86** and (d) **87** in the absence and presence of 10 µM **1**. Data points are mean values ± SEM (n= 3 or 4).

Table 5. Affinity of **85**, **86**, **87** and **80** determined in 1321N1 astrocytoma cells expressing recombinant NLuc-P2Y$_2$R

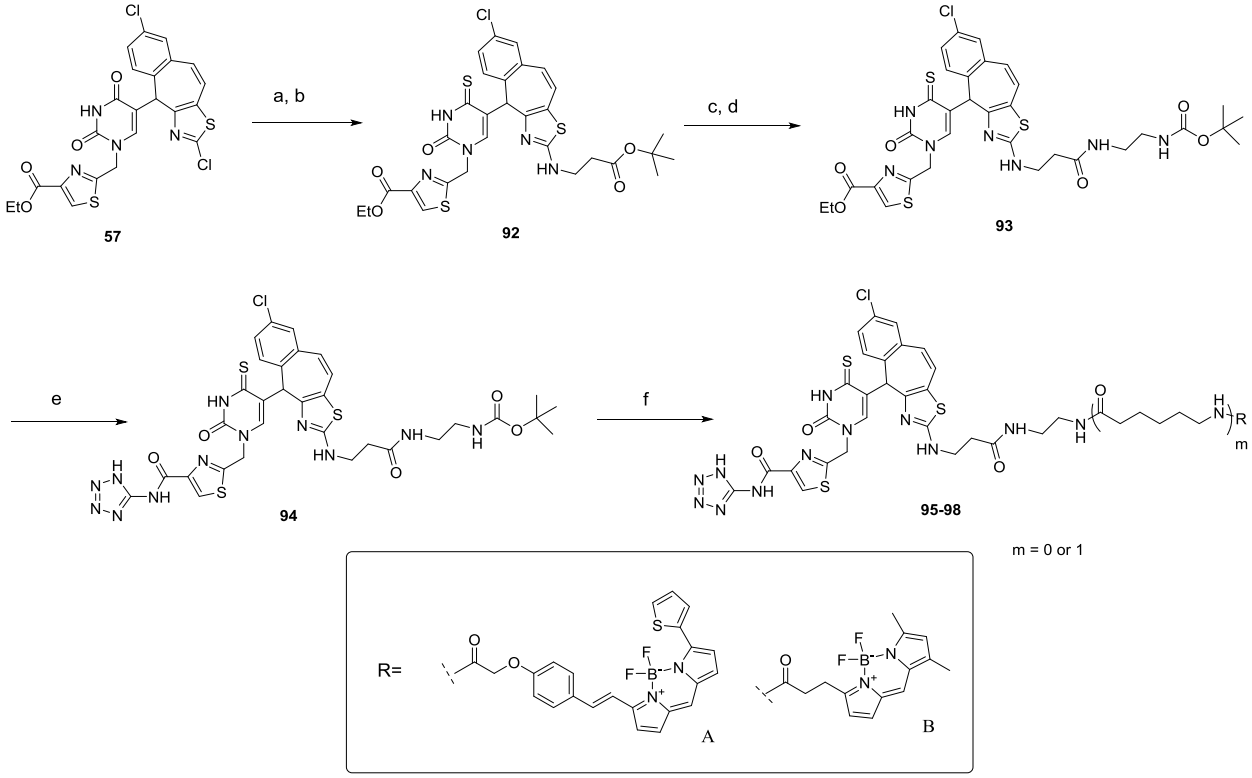
Example	pK_d
80	5.29 ± 0.17 (4)
85	4.91 ± 0.14 (4)
86	5.67 ± 0.10 (4)
87	5.38 ± 0.19 (3)

pK_d values derived from saturation binding curves. Data shown is mean ± SEM, number of separate experiments given in parentheses.

Reassuringly, the affinities determined for the non-fluorescent $P2Y_2R$ antagonist **1**, with either **86** (pK_i 7.45 ± 0.13, *n* = 4) or **87** (pK_i 7.32 ± 0.13, *n* = 4) where consistent with the affinity determined using the $P2Y_2R$ functional assay (pK_b 7.51 ± 0.09); demonstrating that the $P2Y_2R$ fluorescent ligands could be used in a NanoBRET assay for determining the affinity of non-fluorescent $P2Y_2R$ antagonists. The clear demonstration of saturable specific binding with these low affinity fluorescent ligands confirmed the utility of the NanoBRET binding format and the ability to exploit the good signal to noise ratio of this proximity-based assay. To explore the opportunity to develop higher affinity fluorescent conjugates, we embarked on a synthetic strategy to incorporate the affinity-enhancing acyl-tetrazole functional group into the fluorescent compounds.

Third generation $P2Y_2R$ fluorescent ligands. The general synthetic route to the third generation $P2Y_2R$ fluorescent antagonists is illustrated in Scheme 6. Displacement of the chlorine atom in compound **57** with *tert*-butyl 3-aminopropanoate gave *tert*-butyl ester **92**. Treatment with TFA afforded conversion of the *tert*-butyl ester to the corresponding acid which was immediately activated and coupled to *tert*-butyl (2-aminoethyl)carbamate **93** using HATU. Hydrolysis of the ethyl ester, activation of the carboxylic acid using benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate and coupling with 5-aminotetrazole gave the

amidotetrazole **94**. The Boc protecting group was removed and the resulting amine attached to the fluorophore using the appropriate bodipy succinimidyl ester (Scheme 6).



Scheme 6. Reagents and conditions: (a) amine, NEt_3, 1,4-dioxane, reflux, 72 h. (89%). (b) Lawesson's reagent, 1,4-dioxane, reflux, 18 h (48%). (c) TFA, DCM, rt, 30 min (55%). (d) *tert*-butyl (2-aminoethyl)carbamate, HATU, DCM, DIPEA, rt, 24 h (63%). (e) 1. NaOH, MeOH, reflux, 4 h; 2. 5-aminotetrazole, Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate, DIPEA, DMF, rt 4 h (28% over 2 steps). (f) 1. TFA, DCM, rt, 30 min; 2. bodipy-SE, DIPEA, DMF, rt, 1-4 h. (11-18%, 2 steps). The structures of compounds **95-98** are assigned in table 6.

In contrast to non-fluorophore compounds, **14** and **24** where there was little change in affinity in changing the uracil *N*1-substituent, in three of the four compounds (**95-98**) there was a significant increase in affinity relative to the non-amidotetrazole series - compare **98** & **86**, **96** & **85** and **97** & **80** (Table 5 and Table 6).

Table 6. Affinity of **95**, **96**, **97** and **98** determined in 1321N1 astrocytoma cells expressing recombinant NLuc-P2Y$_2$R

Example	m[b]	R[c]	NanoBRET pK_d[a]
95	0	A	6.05 ± 0.12 (3)
96	0	B	7.05 ± 0.05 (3)
97	1	A	6.89 ± 0.06 (7)
98	1	B	6.32 ± 0.10 (17)

[a]pK_d values derived from saturation binding curves [b, c] m and R previously defined from Scheme 6. Data shown is mean ± SEM, number of separate experiments given in parentheses.

Pharmacological evaluation of third generation P2Y$_2$R fluorescent ligands. To further evaluate the utility of fluorescent ligands to study the pharmacology of the P2Y$_2$R, one bodipy A (**97**) and one bodipy B (**98**) linked fluorescent ligand was chosen for further studies. Initially, we confirmed that **97** and **98** still retained the ability to functionally antagonize the P2Y$_2$R (Figure 5). In the Ca^{2+} mobilization assay, a modest rightwards shift of the agonist dose-response curve was observed for both 10μM **97** (pK_b 5.69 ± 0.05, *n*=8) and 10μM **98** (pK_b 5.87 ± 0.05, *n*=7).

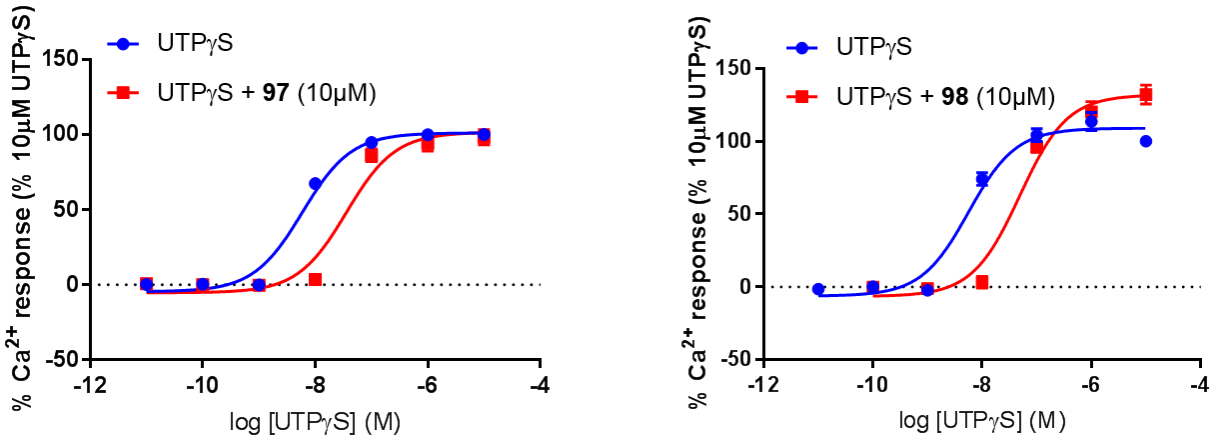


Figure 5. Pharmacological evaluation of: **97** and **98** showing the effect on Ca^{2+} mobilization in *h*P2Y$_2$R-1321N1 cells induced by 0.1 μM UTPγS (*n*=7 *or* 8).

Compounds **97** and **98** bought about a clear concentration-dependent increase in the BRET ratio in the NLUC-tagged *h*P2Y$_2$R assay (Figure 6). This was antagonized by **1** (Figures 6 c & d) yielding pKi values of 7.66 ± 0.11 (*n* = 9) and 7.38 ± 0.04 (*n* = 6) for antagonism of **97** and **98** binding respectively.

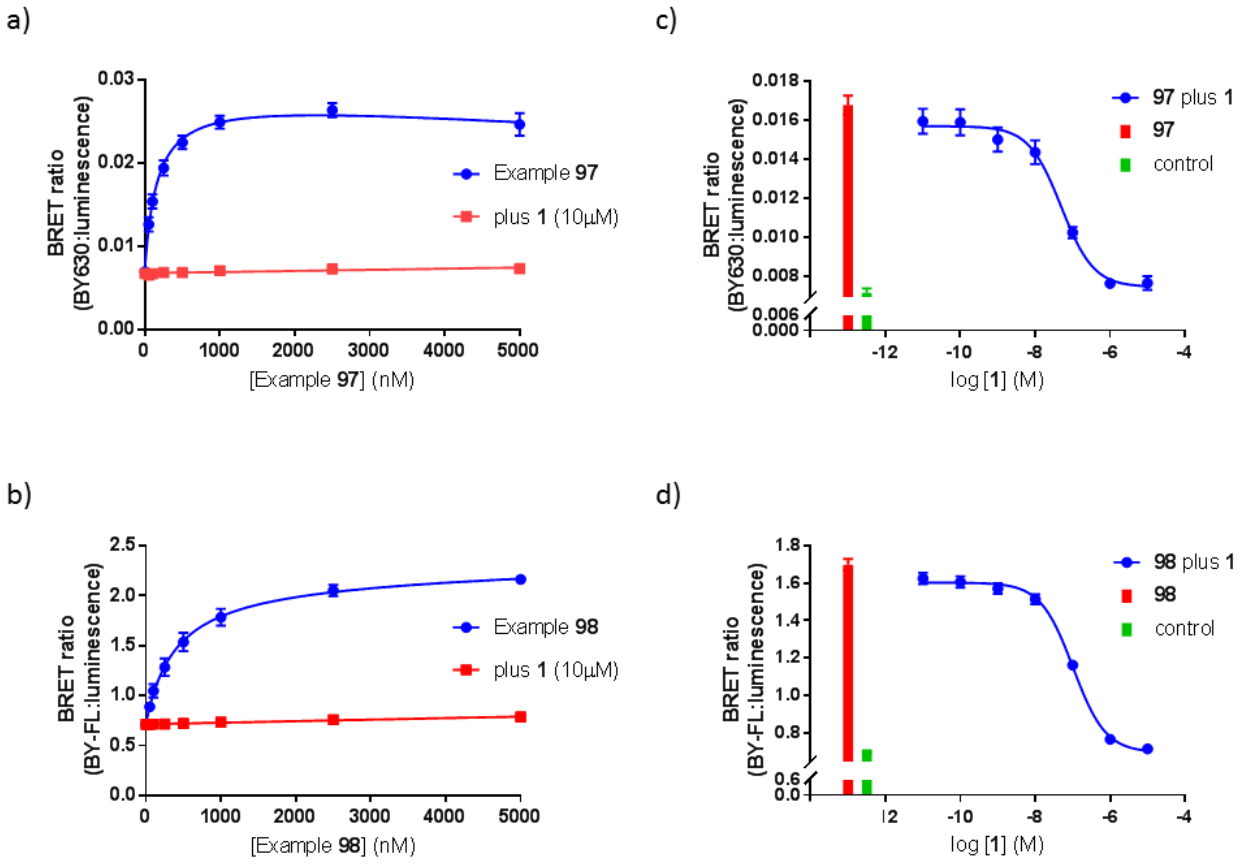
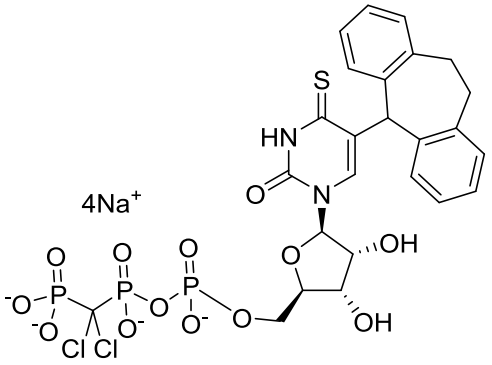


Figure 6. Pharmacological evaluation of: a) **97** and b) **98** showing BRET saturation binding (*n*=7); c) and d) Displacement of **97** (c) (100nM, *n=9*) and **98** (d) (1μM, *n=6*) binding in NLuc-P2Y$_2$ 1321N1 cells by **1.** Values show Mean ± SEM.

To further evaluate the utility of the fluorescent conjugate **98** in the NanoBRET-ligand binding assay the affinity of a selection of P2Y$_2$R antagonists (**1**, **3**, **6**, **22**, **23**, **60**, **86**) and the previously

reported stabilized triphosphate P2Y$_2$R antagonist **99**[8] over a range of P2Y$_2$R affinity were determined in competition binding experiments.



(99)

All eight compounds induced a concentration dependent decrease in specific binding of **98** that enabled their affinity to be determined. There was a good correlation in the values obtained in the NanoBRET assay to those determined in the Ca^{2+} mobilization assay (Table 7). In addition to antagonists, the NanoBRET assay was also used to estimate the affinity of UTPγS. As there has been no reports of radioligands for the P2Y$_2$R, this measurement has not previously been possible.

Table 7. Comparison of affinity estimates obtained in Ca^{2+} mobilization and NanoBRET assays

Example	pK_b Ca^{2+} mobilization[a]	pK_i NanoBRET (**98**)[b]
1	7.51 ± 0.09 (12)	7.38 ± 0.04 (6)
3	7.11± 0.14 (7)	6.89 ± 0.05 (6)
6	5.99 ± 0.03 (7)	5.81 ± 0.10 (6)
22	<5.0 (6)	5.28 ± 0.05 (3)
23	6.74 ± 0.10 (6)	6.56 ± 0.16 (3)
60	6.53 ± 0.04 (7)	6.84 ± 0.02 (6)
86	6.30 ± 0.05 (6)	6.40 ± 0.01 (6)
99	6.59 ± 0.12 (3)[c]	7.08 ± 0.03 (3)
UTPγS	ND	5.46 ± 0.04 (6)

[a] The estimated affinity value for each antagonist (pK_b) was calculated from the shift of the agonist dose response curve brought about by addition of a single concentration of antagonist using the Gaddum equation. [b] Measured in competition binding experiment using fluorescent ligand **98**. [c] reported pA_2 8.0[8]. ND = not determined due to agonist activity. Data shown is mean ± SEM, number of separate experiments given in parentheses.

The availability of both a green (**98**) and a red (**97**) fluorescent $P2Y_2R$ ligand with reasonable affinity for the *h*$P2Y_2R$ suggested that they may both have utility for imaging of the receptor in living cells. Confocal microscope images of fluorescent ligands **97** and **98** with astrocytoma 1321N1 cells expressing *h*$P2Y_2R$ (Figures 7a and c) showed localized membrane fluorescence and very little intracellular fluorescence. When cells were pretreated with **1**, the specific-membrane fluorescence of **97** and **98** was substantially reduced (Figures 7b and d), indicating that the majority of the membrane fluorescence observed was specific labeling of the $P2Y_2R$.

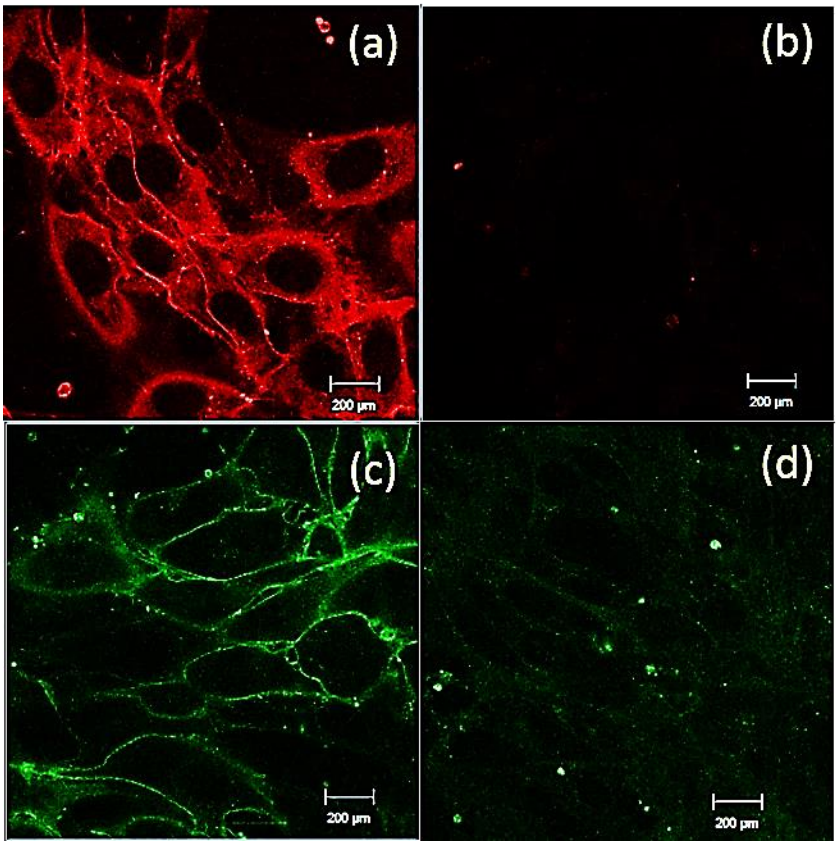


Figure 7. Visualization of the binding of **97** (a) and **98** (c) on astrocytoma cells expressing *h*P2Y$_2$R. In each case, the images in the left hand column shows a confocal image with 100 nM of the fluorescent ligand, while the right hand column shows the images (b) and (d) with the addition of 10 μM of **1.** In all conditions, cells were incubated for 30 min at 37°C in the presence or absence of 10 μM of **1**. Single equatorial confocal images were then obtained in the continued presence of the fluorescent ligand (**97** or **98**) and/or unlabeled antagonist. Images shown are from a single experiment representative of 4 performed.

CONCLUSION

We have described the synthesis and evaluation of new examples of acidic *h*P2Y$_2$R antagonists based on the known *h*P2Y$_2$R antagonist **1**. In addition, we have shown the discovery of a new series of neutral *h*P2Y$_2$R antagonists and demonstrated SAR leading to the identification of potent *h*P2Y$_2$R antagonists (such as **20** and **23**). In addition, we have shown a stereochemical preference for biological activity within this series as typified by the resolved examples **20** & **21** and **22** and **23**. Vibrational circular dichroism has suggested that, in the case of **16** all *h*P2Y$_2$R biological activity resides in the *(R)*-enantiomer **23** – although single crystal X-ray work will be required to confirm this initial stereochemical assignment. The SAR studies led to the identification of suitable linking sites for attachment of the fluorescent ligand to generate three distinct series of fluorescently-labelled *h*P2Y$_2$R antagonists. From this extensive synthetic work, two examples (**97** and **98**) were identified demonstrating both functional antagonist activity (Ca^{2+} mobilization assay) and sufficient affinity for the *h*P2Y$_2$R through a new bioluminescence resonance energy transfer (BRET) assay. In addition, confocal microscopy revealed clear, displaceable membrane labeling of astrocytoma cells expressing the *h*P2Y$_2$R**.** The excellent imaging properties, make **97** and **98** ideal tools for studying *h*P2Y$_2$R distribution and organization. Finally, the discovery of the new

*h*P2Y$_2$R antagonist fluorescent ligands (**97** and **98**) became realized as a result of an extensive program of synthetic chemistry where it proved essential to explore the parallel changes of linker attachment points, fluorophores and linking group.[30] From this study, only a very few fluorescent conjugates were shown to possess sufficient affinity to enable the establishment of a new NanoBRET-based fluorescent assay for the identification of new *h*P2Y$_2$R fragments and ligands.

EXPERIMENTAL SECTION

CHEMISTRY - GENERAL METHODS

Chemicals and solvents were provided by Fisher Scientific UK, Acros Organics, Sigma-Aldrich, Merck Millipore or Fluorochem. BODIPY®FL-X-NHS (D6102) and BODIPY®630/650-X-NHS (D10000) were purchased from Molecular Probes (Invitrogen, UK). All reactions were monitored by TLC using Merck Silica Gel 60Å F254 TLC plates or by LC-MS. Unless otherwise stated, all compounds were dried under high vacuum either at rt or within an oven at 40ºC. LC-MS data was collected on a Shimadzu UFLCXR HPLC system coupled to an Applied Biosystems API 2000 LC/MS/MS electrospray ionization (ESI). The column used was a Phenomenex Gemini-NX 3 µm-110Â C18, 50x2mm at 40ºC. The flow rate was 0.5 mL/min, the UV detection was at 220 nm and 254 nm. Method 1 for the LC-MS ran for 1 min at 5% B; 5 to 98% B over 2 min, 98% B for 2 min, 98 to 5% B over 0.5 min and then 5% for 1 min. Method 2 for the LC-MS ran for 1.5 min at 10% B; 10 to 98% B over 8 min; 98% B for 2 min; 98 to 10% B over 0.5 min and then 10% B for 1 min. Where solvent A: 0.1% formic acid in water; solvent B: acetonitrile. Unless otherwise stated compounds reported had a purity >95% at the wavelength and method quoted. HRMS data was collected on a Bruker microTOF II mass spectrometer using electrospray ionization (ESI-TOF). Adducts within error of ±10 ppm are reported. Preparative RP-HPLC was performed on a Waters 2767 sample manager coupled to Waters 2525 binary gradient module and a Waters 2457 dual

wavelength absorbance detector. The column used was a Phenomenex Gemini® 5 μm NX C18 110 Å, 150x21 mm at ambient temperature. The flow rate was 40 mL/min, the UV detection was at 254 nm. Method 3 for the preparative RP-HPLC ran for 1 min at 10% B; then 10 to 35% B over 4 min; then 35 to 40% B over 20 min; then 40 to 90% B over 2 min; then 90 to 10% B over 2 min and then for 1 min at 10% B. Method 4 for the preparative RP-HPLC ran for 1 min at 10% B; 10 to 45% B over 4 min; 45 to 50% B over 20 min; 50 to 90% B over 2 min; 90 to 10% B over 2 min and then for 1 min at 10% B. Where solvent A was 0.1% trifluoroacetic acid in water and solvent B was 0.1% trifluoroacetic acid in acetonitrile. Chiral-HPLC was performed on a Dionex ICS-3000 SP single pump coupled to a Rheodyne 9725i PEEKinjector coupled to a Dionex UltiMate 3000 Variable Wavelength Detector. The columns used were Phenomenex Lux® 5 μm Amylose-2, 250 x 4.6 mm and 250 x 10 mm; for analytical and semi-preparative runs respectively. These operated at ambient temperature and, unless otherwise stated, the mobile phase was 25% ethanol/hexane with a flow rate of 1 mL/min for analytical runs and 5 mL/min for semi-preparative runs. The UV detection was at 254 nm. NMR spectroscopy was performed using a Bruker AV(III) HD 400 NMR spectrometer equipped with a 5 mm BBFO+ probe, recording ^{1}H and ^{13}C NMR at 400.25 MHz and 100.66 MHz respectively; or a Bruker AV(III) 500 NMR spectrometer equipped with a 5 mm dual $^{1}H/^{13}C$ helium-cooled cryoprobe, recording ^{1}H and ^{13}C NMR at 500.13 MHz and 125.77 MHz respectively. NMR data was processed using iNMR (version 5.5.7) referencing spectra to residual solvents. Chemical shifts are quoted as δ: values in ppm; coupling constants J are given in Hz and multiplicities are described as follows: s - singlet, d - doublet, t - triplet, q - quartet, qi - quintet, sep – septet, m – multiplet, app – apparent, br - broad.

Non standard abbreviations used in experimental: BODIPY® (boron-dipyrromethene); Calcd. (calculated); ESI (electrospray ionization); FC (flash chromatography); HPLC (high performance

liquid chromatography); HRMS (high resolution mass spectrometry); LC-MS (liquid chromatography mass spectrometry); MW (microwave); PREP (preparative); RM (reaction mixture); RP (reverse phase); TLC (thin layer chromatography).

All compounds submitted for biological screening had a purity >95%.

General Procedure 1 – Substitution of 2-chlorothiazole to generate 2-aminothiazoles

A stirred solution of 43 (0.15-0.50 mmol) dissolved in anhydrous 1,4-dioxane (0.1 M, 1.5-5.0 mL) was treated with a chosen amine (4 eq., 0.45-2.00 mmol) and triethyl amine (10 eq. 1.50-5.00 mmol) under N_2 and heated either to reflux for 36-72 h until completion was observed by TLC or LC-MS or heated in a microwave reactor at 100ºC for 4 h. The reaction mixture was concentrated *in vacuo* directly onto silica gel and purified by chromatography on silica gel (2-5% MeOH/DCM w.0.1% 880 NH_3) to afford the desired 2-aminothiazoles **45-56**.

General Procedure 2 – Thionation of uracils to 4-thiouracils using Lawesson's reagent.

A stirred suspension/solution of the uracil (0.05-0.25 mmol) and Lawesson's reagent (2 stoichiometric eq./1 molar eq., 0.05-0.25 mmol) in anhydrous 1,4-dioxane (0.05 M, 1-5 mL) were heated to reflux (120^0C) under N_2 for 18 h generating deep yellow/orange solutions. These were allowed to cool to room temperature and the crude reaction mixture concentrated *in vacuo* onto silica gel and, unless otherwise stated, the title compound purified by chromatography on silica gel eluting with (0-5% MeOH/DCM) to afford the desired 4-thiouracil.

General Procedure 3 - Synthesis of BODIPY® labelled, fluorescent conjugates.

A solution of the Boc–protected amine (2.00 μmol) dissolved in DCM (0.50 mL) was treated with TFA (0.25 mL) and stirred at RT for 30 min. This was then diluted with toluene (10 mL) and

concentrated *in vacuo* to 1/5th volume; this was repeated 3 times before concentrating to dryness to afford the TFA salt of the conjugate. This was dissolved in DMF (500 μL), treated with DIPEA (25 μL), followed by a solution of either BODIPY®FL-X-NHS (1.00 mg, 2.00 μmol) in DMF (150 μL); BODIPY®630/650-X-NHS (1.32 mg, 2.00 μmol) in DMF (150 μL); or a solution of BODIPY®FL-NHS or BODIPY®630/650-NHS; generated *in situ* by mixing BODIPY®FL-CO_2H X (0.78mg, 2.00 μmol) or BODIPY®630/650-CO_2H (0.90mg, 2.00 μmol) with HATU (0.76 mg, 2.00 μmol), NHS (0.23 mg, 2.00 μmol) and DIPEA (25 μL) in DMF (150 μL) for 30 min before addition. These were then allowed to react at room temperature in the absence of light for 1-4 h until, completion observed by LC-MS, and then purified directly from the reaction mixture by preparative reverse phase HPLC to afford the desired fluorescent conjugate after lyophilization.

2-((5-(2,8-Dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-N-(1H-tetrazol-5-yl)thiazole-4-carboxamide (**3**). PyBrop® (0.046 g, 0.1 mmol) was added to a stirred solution of 2-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)thiazole-4-carboxylic acid (**2**) (0.04 g, 0.082 mmol), 5-aminotetrazole monohydrate (0.018 g, 0.18 mmol) and triethylamine (0.036 g, 0.36 mmol) in DMF (1 mL). After 10 minutes the reaction mixture was partitioned between 1M hydrochloric acid and ethyl acetate. The ethyl acetate solution was washed with water and brine and evaporated to dryness. Purification was by silica gel chromatography eluting with ethyl acetate:methanol:acetic acid, 93:5:2. Yield 0.025 g, 0.049 mmol, 49% as a yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 12.73 (s, 1H), 12.17 (s, 1H), 11.96 (s, 1H), 8.74 (s, 1H), 7.46 (d, J = 7.8 Hz, 2H), 7.14 (ddd, J = 7.7, 1.9, 0.8 Hz, 2H), 7.10 (s, 2H), 7.08 (s, 1H), 6.75 (s, 2H), 5.75 (s, 1H), 5.25 (s, 2H), 2.23 (s, 6H). Rt 3.02 (254nm); (m/z): 555.0 (M+1).

5-(2,8-Dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-1-methyl-4-thioxo-3,4-dihydropyrimidin-2(1H)-one (**4**) was prepared using the procedures for **34** and **6**, using 5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)pyrimidine-2,4(1H,3H)-dione (0.14 g, 0.42 mmol). Yield 0.08 g, 0.2 mmol, as a yellow solid, 48% over the two steps. ^{1}H NMR (400 MHz, DMSO) δ 12.53 (s, 1H), 7.47 (d, *J* = 7.8 Hz, 2H), 7.20 (s, 2H), 7.16 (ddd, *J* = 7.8, 1.9, 0.8 Hz, 2H), 7.01 (s, 1H), 6.95 (s, 2H), 5.82 (s, 1H), 3.17 (s, 3H), 2.29 (s, 6H). Rt 2.87 (254nm); (m/z): 361.4 (M+1)

2-((5-(7-Chloro-2-methyl-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-N-(1H-tetrazol-5-yl)thiazole-4-carboxamide (5). A stirred solution of **36** (65 mg, 0.120 mmol) in MeOH (10 mL) was treated with 2M NaOH (0.18 mL, 0.36 mmol) and heated to reflux for 30 min under N_2. Completion was observed by TLC and the RM concentrated to 1/3rd volume, diluted with ethyl acetate (20 mL), washed with 1M HCl (10 mL), brine (10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the corresponding acid as an orange solid (60 mg). A stirred solution of the acid (30 mg, 0.058 mmol) in DMF (3 mL) was treated with DIPEA (40µL) and 5-aminotetrazole monohydrate (12 mg, 0.117 mmol) followed by PyBroP® (41 mg, 0.087 mmol) and stirred at rt for 2 h. until completion was observed by LC-MS. The RM was quenched with water (c.a. 2 drops), diluted with ethyl acetate (20 mL), partitioned with 1M HCl (10 mL) and the organics extracted with ethyl acetate (2 x 10 mL), combined washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* azeotroping DMF with toluene (3 x 50 mL) to afford an orange oil. This was purified by washing with 10% MeOH/DCM before eluting off the desired compound with 10% MeOH/DCM w. 1% acetic acid affording the title compound **5**, an orange solid (10 mg, 0.017 mmol, 30%). LC-MS (ESI+) Rt: 2.79 min (254 nm, Method 1); (m/z): 581.9 $[M(^{35}Cl)+H]^+$ H.MS-TOF (ESI-) (*m/z*): $[M-H]^-$ calcd. for $C_{23}H_{15}ClN_9O_2S_3$, 580.0205; found, 580.0186. ^{1}H NMR (500 MHz, DMSO-d_6) δ:

12.90 (s, 1H), 8.50 (s, 1H), (d, *J* = 8.4 Hz, 1H), 7.66 (s, 1H), 7.50 (d, *J* = 2.3 Hz, 1H), 7.42 (dd, *J* = 8.4, 2.3 Hz, 1H), 7.06 (s, 2H), 6.53 (s, 1H), 5.36 (d, *J* = 15.7 Hz, 1H), 5.30 (d, *J* = 15.7 Hz, 1H), 2.59 (s, 3H) (N.B, -CONH & Tetrazole-NH not observed).

5-(7-Chloro-2-methyl-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methyl-4-thioxo-3,4-dihydropyrimidin-2(1H)-one (6). Following General Procedure 2, **34** (60 mg, 0.161 mmol) was converted to the title compound **6** which was isolated at 1% MeOH/DCM, a yellow solid (25 mg, 0.065 mmol, 40%). LC-MS (ESI+) Rt: 2.85 min (254 nm, Method 1); (m/z): 388.1 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ: 9.39 (s, 1H), 7.70 (d, *J* = 8.3 Hz, 1H), 7.38 (dd, *J* = 8.3, 2.2 Hz, 1H), 7.35 (d, *J* = 2.1 Hz, 1H), 7.19 (s, 1H), 6.95 (d, *J* = 11.6 Hz, 1H), 6.91 (d, *J* = 11.6 Hz, 1H), 6.22 (s, 1H), 3.33 (s, 3H), 2.72 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 188.4, 166.9, 151.1, 147.9, 140.4, 135.7, 134.0, 132.7, 132.5, 130.7, 129.9, 129.3, 129.1, 120.3, 119.2, 45.9, 37.0, 19.4.

5-(2-Amino-7-chloro-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methyl-4-thioxo-3,4-dihydropyrimidin-2(1H)-one (7). Following General Procedure 2, **45** (45 mg, 0.121 mmol) was converted to the title compound **7** and isolated at 2% MeOH/DCM as a yellow solid (4.8 mg, 0.012 mmol, 10%). LC-MS (ESI+) Rt: 2.51 min (254 nm, Method 1); (m/z): 389.0 $[M(^{35}Cl)+H]^+$

5-(7-Chloro-2-(phenylamino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methyl-4-thioxo-3,4-dihydropyrimidin-2(1H)-one (8). Following General Procedure 2, **46** (45 mg, 0.100 mmol) was converted to the title compound **8** which was isolated at 2% MeOH/DCM, a yellow solid (39 mg, 0.084 mmol, 84%). LC-MS (ESI+) Rt: 3.06 min (254 nm, Method 1); (m/z): 465.2 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 12.73 (s, 1H), 10.38 (s, 1H), 7.94 (s, 1H), 7.59 (dd, *J* = 8.7, 1.1 Hz, 2H), 7.55 (d, *J* = 8.7 Hz, 1H), 7.51 (d, *J* = 2.3 Hz, 1H), 7.39 (dd, *J* = 8.4, 2.3 Hz, 1H), 7.35 (dd, *J* = 8.6, 7.4 Hz, 2H), 7.03 (d, *J* = 11.7 Hz, 1H), 7.00 (d, *J* = 11.7 Hz, 1H), 7.02-

6.98 (m, 1H), 5.74 (s, 1H), 3.39 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 189.4, 164.0, 155.2, 148.5, 148.4, 144.5, 141.0, 137.6, 134.7, 130.9, 129.5, 128.9, 128.5, 128.2, 122.4, 122.3, 118.2, 117.8, 117.4, 44.7, 37.3.

5-(2-(Benzylamino)-7-chloro-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methyl-4-thioxo-3,4-dihydropyrimidin-2(1H)-one (9). Following General Procedure 2, **47** (30 mg, 0.065 mmol) was converted to the title compound **95** which was isolated at 2% MeOH/DCM, a yellow solid (25 mg, 0.053 mmol, 81%). LC-MS (ESI+) Rt: 3.01 min (254 nm, Method 1); (m/z): 478.9 [M(^{35}Cl)+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.65 (s, 1H), 8.42 (t, J = 5.8 Hz, 1H), 7.63 (s, 1H), 7.54 (d, J = 8.5 Hz, 1H), 7.44 (d, J = 2.3 Hz, 1H), 7.34-7.30 (m, 5H), 7.27-7.24 (m, 1H), 6.86 (s, 2H), 5.69 (s, 1H), 4.48 (dd, J = 15.3, 5.9 Hz, 1H), 4.41 (dd, J = 15.3, 5.6 Hz, 1H), 3.26 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 189.2, 169.5, 148.8, 148.5, 143.5, 139.0, 137.6, 134.2, 131.8, 130.8, 129.01, 128.84, 127.93, 127.90, 127.6, 126.9, 122.7, 117.5, 117.0, 48.1, 45.1, 37.2.

5-(7-Chloro-2-(phenethylamino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methyl-4-thioxo-3,4-dihydropyrimidin-2(1H)-one (10). Following General Procedure 2, **48** (35 mg, 0.071 mmol) was converted to the title compound **10** at 2% MeOH/DCM as a yellow solid (21 mg, 0.043 mmol, 60%). LC-MS (ESI+) Rt: 3.03 min (254 nm, Method 1); (m/z): 492.8 [M(^{35}Cl)+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.67 (s, 1H), 8.04 (t, J = 5.4 Hz, 1H), 7.64 (s, 1H), 7.57 (d, J = 8.6 Hz, 1H), 7.45 (d, J = 2.3 Hz, 1H), 7.35 (dd, J = 8.4, 2.3 Hz, 1H), 7.32-7.24 (m, 4H), 7.22-7.18 (m, 1H), 6.87 (s, 2H), 5.72 (s, 1H), 3.52-3.45 (m, 2H), 3.27 (s, 3H), 2.87 (t, J = 7.3 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 189.2, 169.3, 148.5, 143.5, 142.4, 139.7, 137.6, 134.1, 131.9, 130.8, 129.14, 129.01, 128.8, 127.9, 126.73, 126.61, 122.7, 117.6, 116.6, 46.1, 45.2, 37.2, 35.1.

5-(7-Chloro-2-(4-methylpiperazin-1-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methyl-4-thioxo-3,4-dihydropyrimidin-2(1H)-one (11). Following General Procedure 2, **49** (47 mg, 0.103 mmol) was converted to the title compound **11** which was isolated at 5% MeOH/DCM w.0.1% 880 NH_3, as a yellow solid (4.5 mg, 0.0095 mmol, 9%). LC-MS (ESI+) Rt: 2.28 min (254 nm, Method 1); (m/z): 472.1 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 12.67 (s, 1H), 7.61 (d, J = 8.5 Hz, 1H), 7.54 (s, 1H), 7.47 (d, J = 2.3 Hz, 1H), 7.36 (dd, J = 8.4, 2.4 Hz, 1H), 6.94 (d, J = 11.6 Hz, 1H), 6.91 (d, J = 11.7 Hz, 1H), 5.79 (s, 1H), 3.46-3.41 (m, 4H), 3.28 (s, 3H), 2.47-2.42 (m, 4H), 2.27 (s, 3H).

5-(7-Chloro-2-morpholino-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methyl-4-thioxo-3,4-dihydropyrimidin-2(1H)-one (12). Following General Procedure 2, **50** (44 mg, 0.100 mmol) was converted to the title compound **12** which was isolated at 2% MeOH/DCM, a yellow solid (20 mg, 0.044 mmol, 44%). LC-MS (ESI+) Rt: 2.94 min (254 nm, Method 1); (m/z): 459.2 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 12.66 (s, 1H), 7.59 (d, J = 8.5 Hz, 1H), 7.56 (s, 1H), 7.46 (d, J = 2.3 Hz, 1H), 7.35 (dd, J = 8.4, 2.3 Hz, 1H), 6.95 (d, J = 11.6 Hz, 1H), 6.91 (d, J = 11.7 Hz, 1H), 5.77 (s, 1H), 3.69 (t, J = 4.9 Hz, 4H), 3.39 (t, J = 4.8 Hz, 4H), 3.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 189.2, 171.3, 149.2, 148.5, 143.3, 137.4, 134.1, 132.2, 131.0, 129.2, 128.0, 127.6, 122.4, 118.5, 117.3, 65.8, 48.3, 45.4, 37.2.

5-(7-Chloro-2-(piperidin-1-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methyl-4-thioxo-3,4-dihydropyrimidin-2(1H)-one (13). Following General Procedure 2, **51** (44 mg, 0.100 mmol) was converted to the title compound **13** which was isolated at 2% MeOH/DCM as a yellow solid (34 mg, 0.074 mmol, 74%). LC-MS (ESI+) Rt: 3.14 min (254 nm, Method 1); (m/z): 456.8 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 12.65 (s, 1H), 7.60 (d, J = 8.5 Hz, 1H), 7.51 (s, 1H), 7.44 (d, J = 2.3 Hz, 1H), 7.34 (dd, J = 8.4, 2.3 Hz, 1H), 6.90 (d, J = 11.6 Hz, 1H), 6.87 (d, J

= 11.7 Hz, 1H), 5.76 (s, 1H), 3.40 (s, 4H), 3.27 (s, 3H), 1.57 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 189.2, 171.0, 149.5, 148.5, 143.1, 137.4, 133.9, 132.4, 130.9, 129.2, 127.9, 127.0, 122.4, 117.8, 117.3, 49.2, 45.5, 37.2, 25.1, 23.9.

5-(7-Chloro-2-((2-methoxyethyl)amino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methyl-4-thioxo-3,4-dihydropyrimidin-2(1H)-one (14). Following General Procedure 2, **52** (40 mg, 0.093 mmol) was converted to the title compound **14** at 2% MeOH/DCM a yellow solid (14 mg, 0.031 mmol, 34%). LC-MS (ESI+) Rt: 2.74 min (254 nm, Method 1); (m/z): 446.9 [M(^{35}Cl)+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.65 (s, 1H), 8.00 (t, J = 5.4 Hz, 1H), 7.63 (s, 1H), 7.55 (d, J = 8.5 Hz, 1H), 7.44 (d, J = 2.3 Hz, 1H), 7.33 (dd, J = 8.4, 2.3 Hz, 1H), 6.86 (s, 2H,), 5.68 (s, 1H), 3.49-3.46 (m, 2H), 3.43-3.39 (m, 2H), 3.28 (s, 3H), 3.26 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 189.2, 169.4, 148.8, 148.5, 143.5, 137.6, 134.1, 130.8, 129.0, 127.8, 126.8, 122.7, 117.4, 116.7, 70.6, 58.4, 45.2, 44.2, 37.2.

The two enantiomers were isolated by Chiral-HPLC: **20** (Rt: 34.46 min; 99% ee) and **21** (Rt: 43.43 min; 78% ee).

5-(7-Chloro-2-((3-methoxypropyl)amino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methyl-4-thioxo-3,4-dihydropyrimidin-2(1H)-one (15). Following General Procedure 2, **53** (36 mg, 0.080 mmol) was converted to the title compound **15** which was isolated at 2% MeOH/DCM, a yellow solid (35 mg, 0.076 mmol, 95%). LC-MS (ESI+) Rt: 2.75 min (254 nm, Method 1); (m/z): 461.0 [M(^{35}Cl)+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.66 (s, 1H), 7.94 (t, J = 5.5 Hz, 1H), 7.65 (s, 1H), 7.57 (d, J = 8.5 Hz, 1H), 7.45 (d, J = 2.3 Hz, 1H), 7.35 (dd, J = 8.4, 2.3 Hz, 1H), 6.87 (s, 2H), 5.70 (s, 1H), 3.38 (t, J = 6.2 Hz, 2H), 3.30 (s, 3H), 3.28-3.24 (m, 2H), 3.23 (s, 3H), 1.78 (app.qi, J = 6.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 189.2, 169.5, 149.0, 148.5,

143.4, 137.6, 134.1, 130.8, 129.0, 127.8, 126.7, 122.7, 117.5, 116.5, 110.0, 69.8, 58.4, 45.2, 41.9, 37.2, 29.2.

5-(7-Chloro-2-((2-ethoxyethyl)amino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methyl-4-thioxo-3,4-dihydropyrimidin-2(1H)-one (16). Following General Procedure 2, **54** (36 mg, 0.080 mmol) was converted to the title compound **16** which was isolated at 2% MeOH/DCM, a yellow solid (34 mg, 0.074 mmol, 92%). LC-MS (ESI+) Rt: 2.81 min (254 nm, Method 1); (m/z): 461.0 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 12.66 (s, 1H), 8.00 (t, J = 5.5 Hz, 1H), 7.64 (s, 1H), 7.57 (d, J = 8.5 Hz, 1H), 7.45 (d, J = 2.3 Hz, 1H), 7.35 (dd, J = 8.4, 2.3 Hz, 1H), 6.87 (s, 2H), 5.70 (s, 1H), 3.53-3.50 (m, 2H), 3.45 (q, J = 7.0 Hz, 2H), 3.43-3.38 (m, 2H), 3.30 (s, 3H), 1.11 (t, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 189.3, 169.4, 148.8, 148.5, 143.5, 137.6, 134.1, 132.0, 130.8, 129.0, 127.8, 126.7, 122.7, 117.5, 116.7, 68.5, 65.9, 45.2, 44.5, 37.2, 15.6.

The two enantiomers were isolated by Chiral-HPLC: **22** (Rt: 28.32 min; 95% ee) and **23** (Rt: 36.68 min; 96% ee).

5-(7-Chloro-2-((2-isopropoxyethyl)amino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methyl-4-thioxo-3,4-dihydropyrimidin-2(1H)-one (17). Following General Procedure 2, **55** (37 mg, 0.080 mmol) was converted to the title compound **17** which was isolated at 2% MeOH/DCM, a yellow solid (30 mg, 0.063 mmol, 79%). LC-MS (ESI+) Rt: 2.88 min (254 nm, Method 1); (m/z): 475.1 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 12.66 (s, 1H), 7.97 (t, J = 5.4 Hz, 1H), 7.63 (s, 1H), 7.57 (d, J = 8.5 Hz, 1H), 7.45 (d, J = 2.3 Hz, 1H), 7.35 (dd, J = 8.4, 2.3 Hz, 1H), 6.87 (s, 2H), 5.70 (s, 1H), 3.57 (sep., J = 6.1 Hz, 1H), 3.52-3.49 (m, 2H), 3.39-3.37 (m, 2H), 3.30 (s, 3H), 1.08 (d, J = 6.1 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 189.2, 169.4, 148.5, 143.5,

137.6, 134.1, 132.0, 130.8, 129.0, 127.9, 126.7, 122.7, 117.5, 116.7, 71.3, 66.0, 45.2, 44.9, 37.2, 22.51, 22.48.

5-(7-Chloro-2-((2-phenoxyethyl)amino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methyl-4-thioxo-3,4-dihydropyrimidin-2(1H)-one (18). Following General Procedure 2, **56** (39 mg, 0.080 mmol) was converted to the title compound **18**. This was isolated at 2% MeOH/DCM and further purification was achieved by preparative RP-HPLC (Method 3) isolating the title compound 18 at Rt: 18.57 min which was freeze dried to an orange solid (8 mg, 0.016 mmol, 20%). LC-MS (ESI+) Rt: 3.02 min (254 nm, Method 1); (m/z): 509.0 $[M(^{35}Cl)+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.66 (s, 1H), 8.20 (t, *J* = 5.3 Hz, 1H), 7.63 (s, 1H), 7.57 (d, *J* = 8.5 Hz, 1H), 7.45 (d, *J* = 2.2 Hz, 1H), 7.35 (dd, *J* = 8.4, 2.3 Hz, 1H), 7.29 (app.t, *J* = 8.0 Hz, 2H), 6.97-6.92 (m, 3H), 6.88 (s, 2H), 5.72 (s, 1H), 4.13 (t, *J* = 5.4 Hz, 2H), 3.64 (app.q, *J* = 5.4 Hz, 2H), 3.27 (s, 3H).

5-(7-Chloro-2-phenyl-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methyl-4-thioxo-3,4-dihydropyrimidin-2(1H)-one (19). Following General Procedure 2, **44** (15 mg, 0.035 mmol) was converted to the title compound **19** which was purified by FC (20-50% ethyl acetate/petroleum ether) and isolated at 50% ethyl acetate/petroleum ether, a yellow solid (12 mg, 0.027 mmol, 76%). LC-MS (ESI+) Rt: 3.20 min (254 nm, Method 1); (m/z): 450.1 $[M(^{35}Cl)+H]^+$. LC-MS (ESI+) Rt: 6.99 min (254 nm, Method 2); (m/z): 450.0 $[M(^{35}Cl)+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.76 (s, 1H), 7.96-7.94 (m, 2H), 7.88 (s, 1H), 7.67 (d, *J* = 8.4 Hz, 1H), 7.58 (d, *J* = 2.1 Hz, 1H), 7.53-7.49 (m, 3H), 7.45 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.27 (d, *J* = 11.6 Hz, 1H), 7.22 (d, *J* = 11.8 Hz, 1H), 6.04 (s, 1H), 3.37 (s, 3H).. ^{13}C NMR (101 MHz, DMSO-d_6) δ: 189.3, 167.2, 152.5, 148.5, 144.1, 137.0, 135.4, 133.2, 131.60, 131.50, 131.36, 131.1, 129.90, 129.79, 129.61, 129.0, 126.5, 122.0, 117.2, 45.0, 37.2.

2-((5-(7-Chloro-2-((2-methoxyethyl)amino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-N-(1H-tetrazol-5-yl)thiazole-4-carboxamide (**24)**. A stirred solution of **59** (140mg, 0.23 mmol) in MeOH (10 mL) was treated with 2M NaOH (0.35 mL, 0.69 mmol) and heated to reflux for 1 h. under N_2. Completion was observed by TLC and the crude evaporated directly onto silica and purified by FC, washing with 5% MeOH/DCM before eluting off the desired compound with 20% MeOH/DCM w. 1% acetic acid to afford a yellow solid. This was dissolved in EA (20 mL), washed with 1M HCl (10 mL), brine (10mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the corresponding carboxylic acid as an orange solid (120 mg, 0.20 mmol, 91%). LC-MS (ESI+) Rt: 2.68 min (254 nm, Method 1); (m/z): 573.9 $[M(^{35}Cl)+H]^+$. The acid (20 mg, 0.035 mmol) in DMF (1.5 mL) was treated with DIPEA (50 µL) and 5-aminotetrazole monohydrate (22 mg, 0.203 mmol) followed by PyBroP (24 mg, 0.051 mmol) and stirred at RT for 2 h. until completion was observed by LC-MS. The RM was quenched with water (c.a. 2 drops), diluted with EA (20 mL), partitioned with 1M HCl (10mL) and the organics extracted with EA (2 x 10 mL), combined washed with brine (10mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* azeotroping DMF with toluene (3 x 50 mL) to afford an orange oil. This was purified by washing with 10% MeOH/DCM before eluting off the desired compound with 10% MeOH/DCM w. 1% acetic acid affording the title compound. Further purification was achieved by preparative RP-HPLC (Method 3) isolating the title compound **24** at Rt: 7.37 min which was freeze dried to an orange solid (8 mg, 0.016 mmol, 34%).LC-MS (ESI+) Rt: 2.71 min (254 nm, Method 1); (m/z): 640.9 $[M(^{35}Cl)+H]^+$. LC-MS (ESI+) Rt: 5.12 min (254 nm, Method 2); (m/z): 640.9 $[M(^{35}Cl)+H]^+$. H.MS-TOF (ESI-) (*m/z*): $[M-H]^-$ calcd. for $C_{25}H_{20}ClN_{10}O_3S_3$, 639.0576; found, 639.0569. ^1H NMR (400 MHz, DMSO-d_6) δ: 12.89 (s, 1H), 12.30-12.23 (m, 1H), 8.74 (s, 1H), 7.98 (t, J = 4.9 Hz, 1H), 7.76 (s, 1H), 7.57 (d, J = 8.5

Hz, 1H), 7.40 (d, *J* = 2.2 Hz, 1H), 7.34 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.76 (s, 2H), 5.67 (s, 1H), 5.45 (d, *J* = 16.0 Hz, 1H), 5.30 (d, *J* = 16.0 Hz, 1H), 3.42-3.33 (m, 4H), 3.22 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 189.8, 176.9, 169.4, 165.9, 159.4, 148.0, 141.9, 137.5, 133.7, 131.2, 130.8, 129.44, 129.35, 128.9, 128.7, 127.8, 126.7, 122.6, 117.7, 116.7, 70.6, 58.3, 49.4, 46.3, 44.1.

3-(3-Chlorophenyl)propanal (26). A stirred solution of 3-chloroiodobenzene (50.00 g, 210 mmol), allyl alcohol (21.4 mL, 18.27 g, 314 mmol), tetrabutylammonium chloride (58.26 g, 210 mmol) and $NaHCO_3$ (17.61 g, 210 mmol) dissolved in anhydrous DMF (150 mL) cooled in an ice bath under N_2 was treated portion-wise with $Pd(OAc)_2$ (1.40 g, 6.29 mmol) over 30 min. This was then heated to 50ºC for 18 h. and consumption of the 3-chloroiodobenzene was observed by TLC (15% ethyl acetate/petroleum ether). The reaction was concentrated *in vacuo*, azeotroping the DMF with toluene (3 x 200 mL). The resulting black gum was dissolved in Et_2O (300 mL) and water (300 mL), filtered, the organics were extracted with Et_2O (2 x 300 mL), combined, washed with brine (100 mL), dried over $MgSO_4$ and concentrated *in vacuo* to afford a black oil. Further purification was achieved by FC (10-40% Et_2O/PE) affording the title compound **27** (32.30 g, 192 mmol, 92%). LC-MS (ESI+) Rt: 2.72 min (254 nm, Method 1); (m/z): not observed. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.81 (t, *J* = 1.2 Hz, 1H), 7.22-7.17 (m, 3H), 7.07 (dt, *J* = 7.1, 1.6 Hz, 1H), 2.93 (t, *J* = 7.4 Hz, 2H), 2.80-2.76 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 200.9, 142.5, 134.3, 129.9, 128.5, 126.6, 126.51, 44.9, 27.7.

Ethyl 5-(3-chlorophenethyl)-2-methylthiazole-4-carboxylate (27). A stirred solution of **26** (24.00 g, 143 mmol) and ethyl dichloroacetate (22.42 g, 143 mmol) dissolved in anhydrous diethyl ether (120 mL) cooled to below -10ºC in an ice/salt bath, under N_2, was treated with a freshly prepared solution of sodium ethoxide (2.2 M, 50 mL, 110 mmol) over a 15 min period ensuring the temperature did not raise above 0ºC; generating a pale orange suspension. This was stirred for

45 min and then warmed to 40ºC over 30 min. This was then quenched with water (250 mL) and the organics extracted with diethyl ether (3 x 150 mL). These were combined, washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford to afford an orange oil. This was dissolved in ethanol (80 mL) and added dropwise to a warm solution of thioacetamide (16.09 g, 214 mmol) in ethanol (80 mL) heated to 65ºC over a 10 min period. This was heated to reflux for 3 h. generating a red solution with a white precipitate which was allowed to cool to rt and concentrated *in vacuo*. The residue was then diluted with diethyl ether (200 mL), partitioned with $NaHCO_3$ (100 mL) and water (100 mL). The organics where extracted with diethyl ether (3 x 200 mL), combined, washed with brine (100 mL) dried over $MgSO_4$, filtered and concentrated *in* vacuo to afford a brown oil. This was purified by FC (10-30% ethyl acetate/petroleum ether) to afford the title compound **27** at 15% ethyl acetate/petroleum ether, a yellow solid (13.21 g, 42.7 mmol, 30%). LC-MS (ESI+) Rt: 3.04 min (254 nm, Method 1); (m/z): 310.0 $[M(^{35}Cl)+H]^+$ ^1H NMR (400 MHz, $CDCl_3$) δ: 7.21-7.19 (m, 3H), 7.08-7.06 (m, 1H), 4.42 (q, *J* = 7.1 Hz, 2H), 3.48 (t, *J* = 7.8 Hz, 2H), 2.95 (t, *J* = 7.8 Hz, 2H), 2.67 (s, 3H), 1.41 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 162.5, 162.2, 148.4, 142.3, 140.6, 134.2, 129.7, 128.6, 126.73, 126.59, 61.2, 37.0, 28.8, 19.3, 14.5.

5-(3-Chlorophenethyl)-2-methylthiazole-4-carboxylic acid (28). A stirred solution of **27** (13.00 g, 42.0 mmol) in THF (60 mL) was treated with a solution of NaOH (2.52g, 63.0 mmol) in water (60 mL) this was stirred for 24 h. at rt. This was treated with 2M HCl (100 mL) and the organics extracted with ethyl acetate (3 x100 mL), combined, washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the title compound **28**, a pale yellow solid (11.68 g, 41.4 mmol, 99%). ^1H NMR (400 MHz, $CDCl_3$) δ: 9.38-9.07 (br.s, 1H), 7.25-7.19 (m, 3H), 7.10 (dt, *J* = 6.5, 1.9 Hz, 1H), 3.52 (t, *J* = 7.8 Hz, 2H), 2.98 (t, *J* = 7.8 Hz, 2H), 2.69 (s,

3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 163.2, 149.6, 142.0, 139.6, 134.3, 129.8, 128.7, 126.8, 126.7, 36.8, 28.8, 18.9.

7-Chloro-2-methyl-9,10-dihydro-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-one (29). A stirred solution of **28** (11.27 g, 40 mmol) in DCM (120 mL) under N_2 was treated with oxalyl chloride (6.00 mL, 70 mmol) and catalytic DMF (c.a. 2 drops) and stirred for 3 h. at rt. This was then concentrated *in vacuo* azeotroping residual oxalyl chloride with toluene (3 x 50 mL) to afford an orange solid. This was dissolved in DCM (175 mL), cooled in an ice bath under N_2 and treated portion-wise with aluminium(III)chloride (21.33 g, 160 mmol) generating a black solution. This was allowed to warm to rt and stirred for 18 h. This was gradually added to a stirred slurry of ice and 2M HCl (400 mL) and allowed to warm to rt. The organics were extracted with ethyl acetate (3 x 400 mL), combined, washed with brine (300 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a crude solid. This was purified by FC (20-100% ethyl acetate/petroleum ether) to afford the title compound **29** at 50% ethyl acetate/petroleum ether, a beige solid (4.16 g, 15.6 mmol, 39%). LC-MS (ESI+) Rt: 2.70 min (254 nm, Method 1); (m/z): 264.1 $[M(^{35}Cl)+H]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.77 (d, J = 8.4 Hz, 1H), 7.54 (d, J = 2.1 Hz, 1H), 7.48 (dd, J = 8.4, 2.2 Hz, 1H), 3.28-3.25 (m, 2H), 3.21-3.18 (m, 2H), 2.63 (s, 3H).

5-Bromo-2,4-di-tert-butoxypyrimidine (31). To a stirred solution of 5-bromo-2,4-dichloropyrimidine (5.00 g, 22.00 mmol) in anhydrous THF (70 mL) cooled in an ice bath under N_2, a suspension of sodium *tert*-butoxide (6.35 g, 66.0 mmol) in anhydrous THF (50 mL) was added dropwise via a dropping funnel over a 30 min period. This was allowed to warm to rt and stirred for a further 18 h. generating a dark brown solution. The RM was then quenched with aq. NH_4Cl (10 mL), diluted with water (100 mL), the organics extracted with ethyl acetate (3 x 75 mL), combined, washed with brine (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*

to afford a black oil. Further purification was achieved by FC (2.5% diethyl ether/petroleum ether w. 0.1% Et_3N) to afford the title compound **31** a clear oil that crystallized to a white solid on standing (4.08 g, 13.50 mmol, 61%). LC-MS (ESI+) Rt: 3.32 min (254 nm, Method 1); (m/z): 305.2 $[M(^{81}Br)+H]^+$, 303.2 $[M(^{79}Br)+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.24 (s, 1H), 1.64 (s, 9H), 1.59 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 165.7, 163.0, 159.0, 99.6, 83.3, 80.8, 28.3, 28.3.

7-Chloro-4-(2,4-di-tert-butoxypyrimidin-5-yl)-2-methyl-9,10-dihydro-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-ol (32). A stirred solution of **31** (3.03 g, 10.00 mmol) in unstabilized, anhydrous THF (80 mL) under N_2 was cooled to -78ºC and treated dropwise with a solution of *n*-butyllithium (2.46 M in hexane, 4.1 mL, 10.50 mmol) and stirred for 30 min generating a dark orange solution. This was then treated dropwise with a solution of **29** (2.64 g, 10.00 mmol) dissolved in unstabilized, anhydrous THF (20 mL) and stirred at -78ºC for 15 min, allowed to warm to rt and stirred for 1 h. The RM was then quenched with NH_4Cl (50 mL), diluted with water (50 mL) and the organics extracted with ethyl acetate (3 x 100 mL). These were combined, washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford an orange oil. Further purification was achieved by FC (5-20% ethyl acetate/petroleum ether) affording the title compound **32** at 15% ethyl acetate/petroleum ether, a clear oil that foamed and crystallized to a white solid under high vacuum (3.04 g, 6.23 mmol, 65%). LC-MS (ESI+) Rt: 3.36 min (254 nm, Method 1); (m/z): 488.2 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 7.88 (s, 1H), 7.38 (d, J = 8.5 Hz, 1H), 7.33 (d, J = 2.3 Hz, 1H), 7.24 (dd, J = 8.5, 2.4 Hz, 1H), 6.13 (s, 1H), 3.25-3.18 (m, 1H), 2.97-2.94 (m, 2H), 2.87-2.80 (m, 1H), 2.53 (s, 3H), 1.56 (s, 9H), 1.18 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: - 167.0, 163.2, 160.2, 156.1, 151.7, 143.3, 141.4, 131.84, 131.67, 129.37, 129.24, 125.8, 122.1, 81.2, 80.0, 75.3, 31.8, 28.6, 28.1, 26.9, 19.0.

5-(7-Chloro-2-methyl-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)pyrimidine-2,4(1H,3H)-dione (33). A stirred solution of 32 (1.54 mmol, 0.750 g) in trifluoroacetic acid (20 mL) was heated to reflux under N_2 for 72 h. generating a black solution. This concentrated *in vacuo* to give dark red gum which was trituration with Et_2O (3 x 10 mL) to give orange solid (454 mg) which was purified by FC (2-10% MeOH/DCM w. 0.1% 880 NH_3) affording the title compound **33** at 4% MeOH/DCM w. 0.1% 880 NH_3, a pink solid (200 mg, 0.56 mmol, 36%). LC-MS (ESI+) Rt: 2.80 min (254 nm, Method 1); (m/z): 358.1 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 11.07 (s, 1H), 10.57 (d, J = 6.1 Hz, 1H), 7.53 (d, J = 8.3 Hz, 1H), 7.53 (d, J = 2.2 Hz, 1H), 7.45 (dd, J = 8.3, 2.3 Hz, 1H), 7.08 (d, J = 11.7 Hz, 1H), 7.01 (d, J = 11.7 Hz, 1H), 6.66 (d, J = 5.5 Hz, 1H), 5.50 (s, 1H), 2.64 (s, 3H).

5-(7-Chloro-2-methyl-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methylpyrimidine-2,4(1H,3H)-dione (34). A stirred suspension of **33** (100 mg, 0.28 mmol) in DCM (15 mL) under N_2 was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.3 mL, 287 mg, 1.18 mmol) and heated to reflux for 18 h. This was cooled, treated with iodomethane (1.5 mL) and heated to 50°C for 24 h. until completion was observed by LC-MS. This was concentrated *in vacuo* affording an oil which was dissolved in MeOH and re-concentrated *in vacuo* to afford as a solid. This was purified by FC (1-4% MeOH/DCM w. 0.1% 880 NH_3) to afford the title compound **34** at 2% MeOH/DCM w. 0.1% 880 NH_3 (64 mg, 0.17 mmol, 61%). LC-MS (ESI+) Rt: 2.64 min (254 nm, Method 1); (m/z): 372.0 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 11.26 (s, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.51 (d, J = 2.2 Hz, 1H), 7.44 (dd, J = 8.3, 2.3 Hz, 1H), 7.07 (d, J = 11.7 Hz, 1H), 7.01 (d, J = 11.7 Hz, 1H), 6.90 (s, 1H), 5.52 (s, 1H), 3.15 (s, 3H), 2.65 (s, 3H).

Ethyl 2-((5-(7-chloro-2-methyl-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)thiazole-4-carboxylate (35). A stirred suspension of **33**

(160 mg, 0.28 mmol) in 1,2-dichloroethane (10 mL) was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.3 mL, 1.18 mmol) and heated to reflux for 18 h. under N_2. This was then treated with ethyl 5-(bromomethyl)thiophene-3-carboxylate (104 mg, 0.42mmol) dissolved in 1,2-dichloroethane (5 mL) and refluxed for 48 h. This was concentrated *in vacuo* to a gum, treated with MeOH (20 mL) and then re-concentrated *in vacuo* to a solid. This was purified by chromatography on silica gel (1-5% MeOH/DCM) affording the title compound **35** at 3% MeOH/DCM (84 mg, 0.16 mmol, 57%). LC-MS (ESI+) Rt: 2.82 min (254 nm, Method 1); (m/z): 526.9 $[M(^{35}Cl)+H]^+$ ^1H NMR (400 MHz, $CDCl_3$) δ: 8.28-8.25 (br.s, 1H), 8.22 (s, 1H), 7.56 (d, *J* = 8.3 Hz, 1H), 7.38 (dd, *J* = 8.3, 2.2 Hz, 1H), 7.29 (d, *J* = 2.3 Hz, 1H), 6.87 (s, 1H), 6.84 (d, *J* = 11.6 Hz, 1H), 6.75 (d, *J* = 11.7 Hz, 1H), 5.79 (s, 1H), 5.21 (d, *J* = 15.2 Hz, 1H), 4.98 (d, *J* = 15.2 Hz, 1H), 4.51 (q, *J* = 7.1 Hz, 2H), 2.71 (s, 3H), 1.48 (t, *J* = 7.1 Hz, 3H).

Ethyl 2-((5-(7-chloro-2-methyl-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)thiazole-4-carboxylate (36). Following General Procedure 2, **35** (80 mg, 0.151 mmol) was converted to the title compound **36** at 2% MeOH/DCM, a yellow solid (68 mg, 0.125 mmol, 89%). LC-MS (ESI+) Rt: 3.01 min (254 nm, Method 1); (m/z): 542.8 $[M(^{35}Cl)+H]^+$ ^1H NMR (400 MHz, $CDCl_3$) δ: 9.63 (s, 1H), 8.24 (s, 1H), 7.69 (d, *J* = 8.3 Hz, 1H), 7.36 (dd, *J* = 8.3, 2.1 Hz), 7.29 (d, *J* = 2.2 Hz, 1H), 7.25 (s, 1H), 6.91 (d, *J* = 11.6 Hz, 1H), 6.84 (d, *J* = 11.5 Hz, 1H), 6.20 (s, 1H), 5.26 (d, *J* = 15.1 Hz, 1H), 5.03 (d, *J* = 15.0 Hz, 1H), 4.52 (q, *J* = 7.1 Hz, 2H), 2.71 (s, 3H), 1.49 (t, *J* = 7.1 Hz, 3H).

Ethyl 2-amino-5-(3-chlorophenethyl)thiazole-4-carboxylate (37). A stirred solution of **26** (18.50 g, 110 mmol) and ethyl dichloroacetate (17.29 g, 110 mmol), dissolved in anhydrous diethyl ether (90 mL) cooled to below -10°C in an ice/salt bath under N_2, was treated with a freshly prepared solution of sodium ethoxide (2.2M, 50 mL, 110 mmol) over a 15 min period ensuring the

temperature did not raise above 0ºC generating a pale orange suspension. This was stirred for 45 min and warmed to 40ºC over 30 min. This was then quenched with water (250 mL) and the organics extracted with diethyl ether (3 x 150 mL). These were combined, washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford to afford an orange oil. This was dissolved in ethanol (60 mL) and added dropwise to a warm solution of thiourea (12.56 g, 165 mmol) in ethanol (60 mL) heated to 65ºC over a 10 min period. This was heated to reflux for 2.5 h. generating a purple/red solution and white precipitate which was then allowed to cool to rt, and concentrated *in vacuo*. The residue was then diluted with diethyl ether (200 mL), partitioned with $NaHCO_3$ (100 mL) and water (100 mL). The organics where extracted with diethyl ether (3 x 200 mL), combined, washed with brine (100 mL) dried over $MgSO_4$, filtered and concentrated *in* vacuo to afford a brown oil. This was taken up in a diethyl ether (100 mL) and filtered th.ough a plug of silica washing with diethyl ether (3 x 200 mL). The filtrate was concentrated to afford an orange solid which was triturated with diethyl ether/hexane to give the title compound **37** a yellow solid (15.70 g, 50.5 mmol, 46%). LC-MS (ESI+) Rt: 2.81 min (254 nm, Method 1); (m/z): 311.3 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.21-7.19 (m, 3H), 7.09-7.07 (m, 1H), 5.09 (s, 2H), 4.36 (q, *J* = 7.1 Hz, 2H), 3.37 (t, *J* = 7.8 Hz, 2H), 2.91 (t, *J* = 7.8 Hz, 2H), 1.39 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 163.4, 162.3, 142.6, 139.5, 136.9, 134.3, 129.8, 128.8, 126.9, 126.6, 61.1, 37.1, 28.8, 14.6.

Ethyl 2-chloro-5-(3-chlorophenethyl)thiazole-4-carboxylate (38). A stirred solution of **37** (15.00 g, 48.26 mmol) in degassed, anhydrous acetonitrile (240 mL) under N_2 was treated with copper(II)chloride (12.92 g, 96.25 mmol) and then treated dropwise, *via* a syringe pump, with *tert*-butyl nitrite (11.50 mL, 96.25 mmol) over a 15 min period at rt ensuring the temperature did not rise above 25ºC. This was stirred for a further 2 h. at rt, quenched with 2M HCl (250 mL), stirred

for 10 min and then the organics extracted with ethyl acetate (3 x 250mL). These were combined, washed with brine (200 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford an orange oil. Further purification was achieved by FC (10% ethyl acetate/petroleum ether) to afford the title compound **38**, an orange oil (10.41 g. 31.53 mmol, 65%). LC-MS (ESI+) Rt: 3.18 min (254 nm, Method 1); (m/z): 330.0 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.21-7.18 (m, 3H), 7.05 (dt, *J* = 6.0, 2.1 Hz, 1H), 4.40 (q, *J* = 7.1 Hz, 2H), 3.48 (t, *J* = 7.7 Hz, 2H), 2.95 (t, *J* = 7.7 Hz, 2H), 1.40 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 161.2, 150.5, 148.3, 141.7, 139.8, 134.4, 129.9, 128.6, 126.9, 126.7, 61.6, 36.6, 28.9, 14.4.

2-Chloro-5-(3-chlorophenethyl)thiazole-4-carboxylic acid (39). A stirred solution of **38** (10.00 g, 30.3 mmol) in THF (40 mL) was treated with a solution of NaOH (1.817 g, 45.4 mmol) in water (40 mL) this was stirred for 24 h. at rt. This was treated with 2M HCl (100 mL) and the organics extracted with ethyl acetate (3 x 100 mL), combined, washed with brine (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the title compound **39** as a pale yellow solid (9.15 g, 30.3 mmol, 100%.). LC-MS (ESI+) Rt: 2.88 min (254 nm, Method 1); (m/z): 301.9 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.24-7.20 (m, 3H), 7.09-7.06 (m, 1H), 3.53 (t, *J* = 7.7 Hz, 2H), 2.98 (t, *J* = 7.7 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 162.4, 151.9, 148.6, 141.4, 138.8, 134.5, 130.0, 128.7, 127.0, 126.7, 36.4, 28.9.

2,7-Dichloro-9,10-dihydro-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-one (40). A stirred solution of **39** (7.00 g, 23.17 mmol) in DCM (70 mL) under N_2 was treated with oxalyl chloride (3.47 mL, 40.54 mmol) and catalytic DMF (c.a. 2 drops) and stirred for 3 h. at rt. This was then concentrated *in vacuo* azeotroping residual oxalyl chloride with toluene (3 x 50 mL) to afford an orange solid. The solid was dissolved in DCM (100 mL), cooled in an ice bath under N_2 and treated portionwise with aluminium(III)chloride (12.36 g, 92.68 mmol) generating a black solution. This

was allowed to warm to rt and stirred for 18 h. This was gradually added to a stirred slurry of ice and 2M HCl (250 mL) and allowed to warm to rt. The organics were extracted with ethyl acetate (3 x 200 mL), combined, washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford and orange solid. This was triturated with diethyl ether/hexane to afford the title compound **40**, a beige solid (5.48 g, 19.28 mmol, 83%). LC-MS (ESI+) Rt: 2.88 min (254 nm, Method 1); (m/z): 284.0 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 7.78 (d, J = 8.4 Hz, 1H), 7.55 (d, J = 2.1 Hz, 1H), 7.48 (dd, J = 8.4, 2.2 Hz, 1H), 3.29-3.26 (m, 2H), 3.24-3.21 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 183.5, 153.2, 148.6, 147.9, 142.1, 137.7, 137.0, 132.6, 129.9, 127.7, 33.5, 26.7.

2,7-Dichloro-4-(2,4-di-tert-butoxypyrimidin-5-yl)-9,10-dihydro-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-ol (41). A stirred solution of **31** (1.516 g, 5.00 mmol) in unstabilized, anhydrous THF (40 mL) under N_2 was cooled to -78ºC and treated dropwise with a solution of *n*-butyllithium (2.25M in hexane, 2.33 mL, 5.25 mmol) and stirred for 30 min generating a dark orange solution. This was then treated dropwise with a solution of **82** (1.421 g, 5.00 mmol) dissolved in unstabilized, anhydrous THF (10 mL) and stirred at -78ºC for 15 min, allowed to warm to rt and stirred for 1 h. The RM was then quenched with aq. NH_4Cl (20 mL), diluted with water (20 mL) and the organics extracted with ethyl acetate (3 x 50 mL). These were combined, washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford an orange oil. Further purification was achieved by FC (5-20% ethyl acetate/petroleum ether) affording the title compound **41** at 15% ethyl acetate/petroleum ether a clear oil that foamed and crystallized to a white solid under high vacuum (2.00 g, 3.93 mmol, 79%). LC-MS (ESI+) Rt: 3.46 min (254 nm, Method 1); (m/z): 508.3 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.02 (d, J = 8.5 Hz, 1H), 7.67 (s, 1H), 7.31 (dd, J = 8.5, 2.3 Hz), 7.16 (d, J = 2.2 Hz, 1H), 5.16 (s, 1H),

3.12-3.04 (m, 2H), 2.87-2.77 (m, 1H), 2.72-2.66 (m, 1H), 1.61 (s, 9H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.1, 163.5, 156.9, 150.8, 147.1, 142.6, 137.9, 133.11, 133.07, 129.9, 128.2, 126.5, 120.1, 82.5, 80.4, 74.4, 31.2, 28.42, 28.40, 26.7.

5-(2,7-Dichloro-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)pyrimidine-2,4(1H,3H)-dione (42). A solution of **41** (1.990 g, 3.91 mmol) in 1,4-dioxane (19.5 mL) was treated with acetic acid (19.5 mL) and heated to 140^{O}C with stirring in the MW for 10 min. This concentrated *in vacuo* azeotroping residual acetic acid with toluene (3 x 50 mL) to afford a brown solid. Purification was achieved by FC (5% MeOH/DCM) to afford the title compound **42**, as an orange solid (851 mg, 2.25 mmol, 58%). LC-MS (ESI+) Rt: 2.71 min (254 nm, Method 1); (m/z): 378.1 [M(^{35}Cl)+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.08 (s, 1H), 10.60 (s, 1H), 7.58 (d, *J* = 2.1 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.49 (dd, *J* = 8.3, 2.2 Hz, 1H), 7.12 (d, *J* = 11.9 Hz, 1H) 7.07 (d, *J* = 11.7 Hz, 1H), 6.63 (s, 1H), 5.48 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 163.4, 151.2, 150.6, 149.0, 138.6, 136.2, 134.9, 133.1, 131.8, 131.5, 130.1, 129.8, 120.5, 108.3, 43.7.

5-(2,7-Dichloro-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methylpyrimidine-2,4(1H,3H)-dione (43). A stirred suspension of **42** (1.430 g, 3.80 mmol) in 1,2-dichloroethane (40 mL) under N_2 was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (4.1 mL, 3.912g, 15.20 mmol) and heated to reflux for 18 h. generating an orange solution. This was cooled, treated with iodomethane (4.7 mL, 10.737 g, 76.0 mmol) and heated to 50^{0}C for 6 h. until completion was observed by LC-MS. This was concentrated *in vacuo* affording an orange oil which was dissolved in MeOH and re-concentrated *in vacuo* to afford a yellow solid. This was triturated with diethyl ether (3 x 30 mL) to afford the title compound **43** an orange solid (1.466 g, 3.73 mmol, 98%). LC-MS (ESI+) Rt: 2.83 min (254 nm, Method 1); (m/z): 392.0 [M(^{35}Cl)+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.27 (s, 1H), 7.56 (d, *J* = 2.3 Hz, 1H), 7.55 (d, *J* = 8.3 Hz, 1H), 7.48 (dd, *J* = 8.3,

2.2 Hz, 1H), 7.11 (d, *J* = 11.8 Hz, 1H), 7.05 (d, *J* = 11.7 Hz, 1H), 6.87 (d, *J* = 0.9 Hz, 1H), 5.50 (s, 1H), 3.14 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 163.1, 151.1, 150.6, 148.8, 143.2, 136.2, 134.8, 133.2, 132.1, 131.8, 131.5, 130.2, 129.7, 120.6, 108.4, 43.9, 36.0.

5-(7-Chloro-2-phenyl-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methylpyrimidine-2,4(1H,3H)-dione (44). A suspension of **43** (39 mg, 0.099 mmol), phenylboronic acid (15 mg, 0.12 mmol), Na_2CO_3 (32 mg, 0.30 mmol), bis(triphenylphosphine) palladium(II)chloride (7 mg, 0.01 mmol) in 1,4-dioxane (0.8 mL) and water (0.2 mL) were heated in a MW to 150^{O}C for 5 min generating a black solution. This was concentrated directly onto silica a purified by FC (20-50% ethyl acetate/toluene) affording the title compound **44** at 30% ethyl acetate/toluene (29 mg, 0.067 mmol, 68%). LC-MS (ESI+) Rt: 3.01 min (254 nm, Method 1); (m/z): 433.8 [M(^{35}Cl)+H]$^+$. LC-MS (ESI+) Rt: 6.22 min (254 nm, Method 2); (m/z): 434.1 [M(^{35}Cl)+H]$^+$ (N.B. 85% purity at 254nm; 95% purity at 220nm). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.30 (s, 1H), 7.96-7.94 (m, 2H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.56 (d, *J* = 2.2 Hz, 1H), 7.53-7.49 (m, 3H), 7.48 (dd, *J* = 8.3, 2.3 Hz, 1H), 7.19 (d, *J* = 11.7 Hz, 1H), 7.11 (d, *J* = 11.8 Hz, 1H), 7.06 (s, 1H), 5.62 (s, 1H), 3.18 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 167.4, 163.2, 151.7, 151.1, 143.3, 136.4, 135.1, 133.2, 131.6, 131.1, 130.8, 130.0, 129.8, 129.3, 126.47, 126.44, 121.4, 110.0, 108.8, 43.9, 36.1.

5-(2-Amino-7-chloro-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methylpyrimidine-2,4(1H,3H)-dione (45). A stirred solution of **43** (140 mg, 0.357 mmol) in 1,4-dioxane (1 mL) was treated with 880 NH_3 (1 mL) and heated in a MW to 100^{o}C with stirring for 4 h. The RM was concentrated directly onto silica and purified by FC (5-20% MeOH DCM w.0.1% 880 NH_3) affording the title compound **45** at 20% MeOH DCM w.0.1% 880 NH_3 (68 mg, 0.182 mmol, 51%). LC-MS (ESI+) Rt: 2.37 min (254 nm, Method 1); (m/z): 372.8 [M(^{35}Cl)+H]$^+$ ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.23 (s, 1H), 7.44 (d, *J* = 8.7 Hz, 1H), 7.43 (d, *J* = 2.4 Hz, 1H), 7.37 (dd, *J* = 8.2,

2.3 Hz), 7.36-7.32 (br.s, 2H), 6.93 (s, 1H), 6.79 (d, *J* = 11.6 Hz, 1H), 6.76 (d, *J* = 11.8 Hz, 1H), 5.22 (s, 1H), 3.16 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 169.9, 163.2, 151.1, 142.8, 137.0, 134.0, 133.2, 131.7, 131.0, 129.4, 128.2, 126.0, 122.2, 117.3, 108.9, 43.9, 36.1

5-(7-Chloro-2-(phenylamino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methylpyrimidine-2,4(1H,3H)-dione (46). A stirred solution of **43** (75 mg, 0.191 mmol) and aniline (0.07 mL, 0.76 mmol) dissolved in 1,4-dioxane (2 mL) were treated with conc. HCl (ca. 2 drops) and heated with stirring in the MW at 140^{0}C for 1h. This was evaporated directly onto silica and purified by FC (2-5% MeOH/DCM w. 0.1% 880 NH_3) affording the title compound **46** at 3% MeOH/DCM w. 0.1% 880 NH_3, a pale yellow solid (63 mg, 0.14 mmol, 74%). LC-MS (ESI+) Rt: 2.92 min (254 nm, Method 1); (m/z): 448.9 [M(^{35}Cl)+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.46-11.03 (br.s, 1H), 10.73-10.19 (br.s, 1H), 7.61 (d, *J* = 7.7 Hz, 2H), 7.50 (d, *J* = 8.5 Hz, 1H), 7.47 (d, *J* = 2.1 Hz, 1H), 7.40 (dd, *J* = 8.3, 2.2 Hz, 1H), 7.33 (app.t, *J* = 7.9 Hz, 2H), 7.13 (s, 1H), 6.98 (t, *J* = 7.4 Hz, 1H), 6.94-6.88 (m, 2H), 5.29 (s, 1H), 3.19 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 164.3, 151.3, 147.6, 143.52, 141.1, 137.0, 134.4, 132.7, 131.1, 129.5, 129.4, 128.6, 127.75, 127.73, 122.2, 121.9, 118.5, 117.8, 108.8, 43.6, 36.2.

5-(2-(benzylamino)-7-chloro-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methylpyrimidine-2,4(1H,3H)-dione (47). Following General Procedure 1, **43** (75 mg, 0.191 mmol) was reacted with benzylamine (0.08 mL, 0.76 mmol) to afford the title compound **47** at 4% MeOH/DCM w. 0.1% 880 NH_3 a pale yellow solid (45 mg, 0.097 mmol, 51%). LC-MS (ESI+) Rt: 2.81 min (254 nm, Method 1); (m/z): 462.9 [M(^{35}Cl)+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.25 (s, 1H, s, 1H), 8.41 (t, *J* = 5.8 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 1H), 7.43 (d, *J* = 2.3 Hz, 1H), 7.38-7.32 (m, 5H), 7.29-7.24 (m, 1H), 7.03 (s, 1H), 6.82 (d, *J* = 11.6 Hz, 1H), 6.78 (d, *J* = 11.7 Hz, 1H), 5.22 (s, 1H), 4.50 (dd, *J* = 15.2, 5.8 Hz, 1H), 4.42 (dd, *J* = 15.3, 5.8 Hz, 1H), 3.16 (s,

3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 169.6, 163.3, 151.1, 147.8, 143.1, 139.0, 137.0, 134.2, 132.9, 131.0, 129.3, 128.8, 128.3, 128.0, 127.6, 126.2, 122.2, 117.2, 108.9, 48.1, 43.8, 36.2.

5-(7-Chloro-2-(phenethylamino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methylpyrimidine-2,4(1H,3H)-dione (48). Following General Procedure 1, **43** (75 mg, 0.191 mmol) was reacted with phenethylamine (0.10 mL, 0.76 mmol) to afford the title compound **48** at 3% MeOH/DCM w. 0.1% 880 NH_3 a pale yellow solid (51 mg, 0.10 mmol, 54%). LC-MS (ESI+) Rt: 2.82 min (254 nm, Method 1); (m/z): 477.0 $[M(^{35}Cl)+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.23 (s, 1H), 8.00 (t, J = 5.4 Hz, 1H), 7.44 (d, J = 8.5 Hz, 1H), 7.42 (d, J = 2.2 Hz, 1H), 7.36 (dd, J = 8.3, 2.3 Hz, 1H), 7.31-7.23 (m, 4H), 7.19 (tt, J = 6.9, 2.0 Hz, 1H), 6.98 (s, 1H), 6.80 (d, J = 11.6 Hz, 1H), 6.76 (d, J = 11.8 Hz, 1H), 5.22 (s, 1H), 3.49-3.41 (m, 2H), 3.15 (s, 3H), 2.86 (t, J = 7.3 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 169.5, 163.3, 151.1, 148.0, 143.0, 139.7, 137.1, 134.1, 133.1, 131.0, 129.32, 129.16, 128.8, 128.2, 126.6, 126.1, 122.2, 116.8, 108.9, 46.2, 43.9, 36.2, 35.1.

5-(7-Chloro-2-(4-methylpiperazin-1-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methylpyrimidine-2,4(1H,3H)-dione (49). Following General Procedure 1, **43** (75 mg, 0.191 mmol) was reacted with *N*-methylpiperazine (0.08 mL, 0.76 mmol) to afford the title compound **49** at 6% MeOH/DCM w.0.1% 880 NH_3 a pale yellow solid (64 mg, 0.14 mmol, 71%). LC-MS (ESI+) Rt: 2.13 min (254 nm, Method 1); (m/z): 456.0 $[M(^{35}Cl)+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.25 (s, 1H), 7.47 (d, J = 8.6 Hz, 1H), 7.45 (d, J = 2.2 Hz, 1H), 7.38 (dd, J = 8.3, 2.3 Hz, 1H), 6.96 (s, 1H), 6.88 (d, J = 11.6 Hz, 1H), 6.82 (d, J = 11.7 Hz, 1H), 5.29 (s, 1H), 3.46-3.42 (m, 4H), 3.16 (s, 3H), 2.52-2.47 (m, 4H), 2.29-2.25 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 171.0, 163.2, 151.1, 148.3, 142.9, 136.8, 134.2, 133.2, 131.1, 129.5, 128.4, 126.8, 121.9, 118.7, 108.7, 53.9, 47.8, 45.8, 44.1, 36.2.

5-(7-Chloro-2-morpholino-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methylpyrimidine-2,4(1H,3H)-dione (50). Following General Procedure 1, **43** (75 mg, 0.191 mmol) was reacted with morpholine (0.07 mL, 0.76 mmol) to afford the title compound **50** at 3% MeOH/DCM w.0.1% 880 NH_3 as a pale yellow solid (65 mg, 0.147 mmol, 77%). LC-MS (ESI+) Rt: 2.70 min (254 nm, Method 1); (m/z): 443.1 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 11.25 (s, 1H), 7.47 (d, J = 8.5 Hz, 1H), 7.45 (d, J = 2.3 Hz, 1H), 7.39 (dd, J = 8.3, 2.3 Hz, 1H), 6.97 (s, 1H), 6.89 (d, J = 11.6 Hz, 1H), 6.83 (d, J = 11.7 Hz, 1H), 5.29 (s, 1H), 3.70 (t, J = 4.9 Hz, 4H), 3.40 (t, J = 4.8 Hz, 4H), 3.16 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 171.4, 163.3, 151.1, 148.2, 142.9, 136.8, 134.2, 133.2, 131.1, 129.5, 128.4, 126.9, 121.9, 118.7, 108.7, 65.8, 49.1, 48.2, 36.2.

5-(7-Chloro-2-(piperidin-1-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methylpyrimidine-2,4(1H,3H)-dione (51). Following General Procedure 1, **43** (75 mg, 0.191 mmol) was reacted with piperidine (0.08 mL, 0.76 mmol) to afford the title compound **51** at 3% MeOH/DCM w.0.1% 880 NH_3 as a pale yellow solid (60 mg, 0.136 mmol, 71%). LC-MS (ESI+) Rt: 2.88 min (254 nm, Method 1); (m/z): 441.2 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 11.23 (s, 1H), 7.46 (d, J = 8.5 Hz, 1H), 7.43 (d, J = 2.2 Hz, 1H), 7.36 (dd, J = 8.3, 2.3 Hz, 1H), 6.94 (s, 1H), 6.84 (d, J = 11.6 Hz, 1H), 6.78 (d, J = 11.7 Hz, 1H), 5.26 (s, 1H), 3.43-3.40 (m, 4H), 3.14 (s, 3H), 1.58 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 171.1, 163.3, 151.1, 148.5, 142.8, 136.9, 134.1, 133.3, 131.1, 129.4, 128.3, 126.3, 122.0, 118.0, 108.7, 49.2, 44.1, 36.2, 25.1, 23.9.

5-(7-Chloro-2-((2-methoxyethyl)amino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methylpyrimidine-2,4(1H,3H)-dione (52). Following General Procedure 1, **43** (75 mg, 0.191 mmol) was reacted with 2-methoxyethylamine (0.07 mL, 0.76 mmol) to afford the title compound **52** at 4% MeOH/DCM w.0.1% 880 NH_3 as a pale yellow solid (55mg, 0.128 mmol, 67%). LC-

MS (ESI+) Rt: 2.51 min (254 nm, Method 1); (m/z): 430.9 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 11.23 (s, 1H), 7.98 (t, J = 5.3 Hz, 1H), 7.44 (d, J = 8.6 Hz, 1H), 7.42 (d, J = 2.3 Hz, 1H), 7.36 (dd, J = 8.3, 2.3 Hz, 1H), 6.99 (s, 1H), 6.79 (d, J = 11.6 Hz, 1H), 6.76 (d, J = 11.8 Hz, 1H), 5.21 (s, 1H), 3.48-3.45 (m, 2H), 3.26 (s, 3H), 3.24-3.23 (m, 2H), 3.15 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 169.6, 163.3, 161.6, 151.1, 147.8, 143.0, 137.0, 134.1, 131.0, 129.3, 128.2, 126.1, 122.3, 116.9, 108.8, 70.6, 58.4, 44.2, 43.9, 36.1.

5-(7-Chloro-2-((3-methoxypropyl)amino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methylpyrimidine-2,4(1H,3H)-dione (53). Following General Procedure 1, **43** (50 mg, 0.127 mmol) was reacted with 3-methoxypropylamine (0.05 mL, 0.51 mmol) to afford the title compound **53** which was isolated at 5% MeOH/DCM, a pale yellow solid (52 mg, 0.117 mmol, 92%). LC-MS (ESI+) Rt: 2.54 min (254 nm, Method 1); (m/z): 445.1 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 11.23 (s, 1H), 7.90 (t, J = 5.5 Hz, 1H), 7.44 (d, J = 8.4 Hz, 1H), 7.42 (d, J = 2.2 Hz, 1H), 7.35 (dd, J = 8.3, 2.3 Hz, 1H), 7.00 (s, 1H), 6.80 (d, J = 11.6 Hz, 1H), 6.75 (d, J = 11.7 Hz, 1H), 5.21 (s, 1H), 3.37 (t, J = 6.2 Hz, 2H), 3.28-3.23 (m, 2H), 3.22 (s, 3H), 3.15 (s, 3H), 1.76 (app.qi, J = 6.5 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 169.7, 163.3, 151.1, 148.0, 143.0, 137.0, 134.1, 133.0, 131.0, 129.3, 128.2, 126.0, 122.3, 116.7, 108.9, 69.8, 58.4, 43.9, 41.9, 36.1, 29.2.

5-(7-Chloro-2-((2-ethoxyethyl)amino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methylpyrimidine-2,4(1H,3H)-dione (54). Following General Procedure 1, **43** (50 mg, 0.127 mmol) was reacted with 2-ethoxyethylamine (0.05 mL, 0.51 mmol) to afford the title compound **54** at 5% MeOH/DCM, a pale yellow solid (51 mg, 0.115 mmol, 90%). LC-MS (ESI+) Rt: 2.60 min (254 nm, Method 1); (m/z): 445.1 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 11.24 (s, 1H), 7.98 (t, J = 5.5 Hz, 1H), 7.45 (d, J = 8.5 Hz, 1H), 7.43 (d, J = 2.3 Hz, 1H), 7.37 (dd, J = 8.3,

2.3 Hz, 1H), 7.01 (s, 1H), 6.81 (d, *J* = 11.6 Hz, 1H), 6.77 (d, *J* = 11.7 Hz, 1H), 5.23 (s, 1H), 3.51 (t, *J* = 5.7 Hz, 2H), 3.45 (q, *J* = 7.0 Hz, 2H), 3.43-3.37 (m, 2H), 3.17 (s, 3H), 1.11 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 169.6, 163.3, 151.1, 147.8, 143.0, 137.0, 134.1, 133.1, 131.0, 129.3, 128.2, 126.1, 122.3, 116.9, 108.9, 68.5, 65.9, 44.5, 43.9, 36.1, 15.6.

5-(7-Chloro-2-((2-isopropoxyethyl)amino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methylpyrimidine-2,4(1H,3H)-dione (55). Following General Procedure 1, **43** (50 mg, 0.127 mmol) was reacted with 2-isopropoxyethylamine (0.06 mL, 0.51 mmol) to afford the title compound **55** which was isolated at 5% MeOH/DCM, a pale yellow solid (49 mg, 0.107 mmol, 84%). LC-MS (ESI+) Rt: 2.68 min (254 nm, Method 1); (m/z): 459.2 [M(^{35}Cl)+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.24 (s, 1H), 7.94 (t, *J* = 5.5 Hz, 1H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.43 (d, *J* = 2.1 Hz, 1H), 7.37 (dd, *J* = 8.3, 2.2 Hz, 1H), 7.00 (s, 1H), 6.81 (d, *J* = 11.6 Hz, 1H), 6.77 (d, *J* = 11.7 Hz, 1H), 5.23 (s, 1H), 3.57 (sep., *J* = 6.1 Hz, 1H), 3.52-3.49 (m, 2H), 3.42-3.36 (m, 2H), 3.16 (s, 3H), 1.09 (d, *J* = 6.1 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 169.6, 163.3, 151.1, 147.8, 143.0, 137.0, 134.1, 133.1, 131.0, 129.3, 128.2, 126.0, 122.3, 116.9, 108.9, 71.3, 66.1, 44.9, 43.9, 36.1, 22.51, 22.49.

5-(7-Chloro-2-((2-phenoxyethyl)amino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-1-methylpyrimidine-2,4(1H,3H)-dione (56). Following General Procedure 1, **43** (50 mg, 0.127 mmol) was reacted with 2-phenoxyethylamine (0.07 mL, 0.51 mmol) to afford the title compound **56** at 4% MeOH/DCM, a pale yellow solid (53 mg, 0.108 mmol, 85%). LC-MS (ESI+) Rt: 2.85 min (254 nm, Method 1); (m/z): 483.0 [M(^{35}Cl)+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.24 (s, 1H), 8.17 (t, *J* = 5.5 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.44 (d, *J* = 2.2 Hz, 1H), 7.38 (dd, *J* = 8.3, 2.2 Hz, 1H), 7.29 (dd, *J* = 8.5, 7.5 Hz, 2H), 7.00 (s, 1H), 7.01-6.92 (m, 3H), 6.82 (d, *J* = 11.6 Hz, 1H), 6.79 (d, *J* = 11.7 Hz, 1H), 5.26 (s, 1H), 4.13 (t, *J* = 5.4 Hz, 2H), 3.64 (app.q, *J* = 5.1 Hz, 2H),

3.16 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 169.4, 163.3, 158.8, 151.1, 148.3, 147.7, 143.0, 137.0, 134.1, 131.0, 130.0, 129.4, 128.3, 126.2, 122.2, 117.2, 114.9, 108.8, 66.2, 43.9, 36.1.

Ethyl 2-((5-(2,7-dichloro-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)thiazole-4-carboxylate (57). A stirred suspension of **42** (851 mg, 2.25 mmol) in 1,2-dichloroethane (20 mL) was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (1.8 mL, 6.75 mmol) and heated to reflux for 18 h. under N_2, generating an orange solution. This was then treated with ethyl 2-(bromomethyl)thiazole-4-carboxylate (591 mg, 2.36 mmol) dissolved in 1,2-dichloroethane (3 mL) and refluxed for 72 h. This was concentrated *in vacuo* to a gum, treated with MeOH (20 mL) and then re-concentrated *in vacuo* to a brown solid. This was triturated with diethyl ether (3 x 50 mL) to give the title compound **57** a brown solid (1.125 g, 2.06 mmol, 91%). LC-MS (ESI+) Rt: 2.95 min (254 nm, Method 1); (m/z): 546.9 $[M(^{35}Cl)+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.49 (s, 1H), 8.50 (s, 1H), 7.59 (d, J = 8.5 Hz, 1H), 7.54 (d, J = 2.2 Hz, 1H), 7.51 (dd, J = 8.2, 2.3 Hz, 1H), 7.03 (d, J = 11.9 Hz, 1H), 7.03 (d, J = 1.1 Hz, 1H), 6.96 (d, J = 11.7 Hz, 1H), 5.55 (d, J = 0.9 Hz, 1H), 5.20 (d, J = 15.8 Hz, 1H), 5.16 (d, J = 15.9 Hz, 1H), 4.34 (q, J = 7.1 Hz, 2H), 1.33 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 166.6, 162.7, 161.0, 150.67, 150.55, 148.6, 146.4, 142.0, 136.2, 134.5, 133.3, 132.00, 131.81, 131.5, 130.5, 130.1, 129.7, 120.6, 109.0, 61.4, 48.4, 44.1, 14.7.

Ethyl 2-((5-(7-chloro-2-((2-methoxyethyl)amino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)thiazole-4-carboxylate (58). Following General Procedure 1, **57** (400 mg, 0.73 mmol) was reacted with 2-methoxyethylamine (0.25 mL, 2.92 mmol) to afford the title compound **58** at 3% MeOH/DCM, a pale yellow solid (250 mg, 0.43 mmol, 59%). LC-MS (ESI+) Rt: 2.69 min (254 nm, Method 1); (m/z): 585.8

[M(^{35}Cl)+H]$^{+}$. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.46 (s, 1H), 8.51 (s, 1H), 7.97 (t, *J* = 5.4 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.39 (d, *J* = 2.1 Hz, 1H), 7.37 (dd, *J* = 8.1, 2.3 Hz, 1H), 7.18 (s, 1H), 6.68 (d, *J* = 11.6 Hz, 1H), 6.65 (d, *J* = 11.7 Hz, 1H), 5.27 (d, *J* = 15.9 Hz, 1H), 5.22 (s, 1H), 5.15 (d, *J* = 15.9 Hz, 1H), 4.34 (q, *J* = 7.0 Hz, 2H), 3.46-3.44 (m, 2H), 3.39 (app.q, *J* = 5.6 Hz, 2H), 3.25 (s, 3H), 1.33 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 169.5, 166.5, 163.0, 161.0, 150.7, 147.4, 146.3, 141.8, 137.0, 133.8, 133.0, 131.0, 130.6, 129.2, 128.2, 126.0, 122.3, 116.9, 109.3, 70.6, 61.3, 58.4, 48.5, 44.16, 43.99, 14.7.

Ethyl 2-((5-(7-chloro-2-((2-methoxyethyl)amino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)thiazole-4-carboxylate (59). Following General Procedure 2, **58** (240 mg, 0.41 mmol) was converted to the title compound **59** which was isolated at 2% MeOH/DCM, a yellow solid (200 mg, 0.33 mmol, 81%). LC-MS (ESI+) Rt: 2.87 min (254 nm, Method 1); (m/z): 601.9 [M(^{35}Cl)+H]$^{+}$. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.87 (s, 1H), 8.55 (s, 1H), 8.02-7.98 (br.s, 1H), 7.74 (s, 1H), 7.56 (d, *J* = 8.5 Hz, 1H), 7.40 (d, *J* = 2.3 Hz, 1H), 7.36 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.75 (s, 2H), 5.65 (s, 1H), 5.39 (d, *J* = 15.8 Hz, 1H), 5.26 (d, *J* = 15.8 Hz, 1H), 4.35 (q, *J* = 7.0 Hz, 2H), 3.42-3.40 (m, 2H), 3.37-3.35 (m, 2H), 3.23 (s, 3H), 1.34 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 189.8, 169.3, 165.5, 161.0, 147.9, 146.4, 141.9, 137.5, 133.7, 132.1, 130.93, 130.81, 128.9, 127.8, 126.7, 122.7, 117.6, 116.7, 70.6, 61.4, 58.4, 49.2, 45.3, 44.1, 14.7.

3-((5-(2,8-Dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-5-(methoxycarbonyl)benzoic acid (61) and 5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)isophthalic acid (60). Step a) N,O-*bis*(trimethylsilyl)trifluoroacetamide (0.76 g, 2.96 mmol) was added to a stirred suspension of 5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-

yl)pyrimidine-2,4(1H,3H)-dione (patent: US6107297) (0.49 g, 1.48 mmol) in 1,2-dichloroethane (6 mL) and the reaction mixture heated under reflux, under nitrogen. After 1.5 h, dimethyl 5-(bromomethyl)isophthalate (0.43 g, 1.48 mmol) was added. The reaction mixture was heated under reflux for a further 16 h. After cooling the volatiles were removed under vacuum. Purification was by silica gel chromatography eluting with ethyl acetate:petroleum ether, 50:50 to give dimethyl 5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)isophthalate Yield 0.7 g, 1.3 mmol, 87%. LC-MS (ESI+) Rt 3.10 (254nm, method 1); (m/z): 537.2 (M+1). Step b) Lawesson's reagent (0.8 g, 2 mmol) and the product from step a) (0.7 g, 1.3 mmol) in 1,4-dioxane (8 mmol) was heated under reflux, under nitrogen. After 16h, the volatiles were evaporated under vacuum. Purification was by silica gel chromatography eluting with ethyl acetate:toluene, 30:70 to give dimethyl 5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)isophthalate Yield 0.4 g, 0.72 mmol, 55% as a yellow solid. LC-MS (ESI+) Rt 3.19 (254 nm, method 1); (m/z): 553.7 (M+1). Step c) Sodium hydroxide solution (0.4 mmol, 0.4 mL of 1M solution in water) was added to a mixture of the product from step b) (0.11 g, 0.2 mmol) in methanol (1 mL), toluene (0.5 mL) and water (0.5 mL). After 4h, reaction mixture was partitioned between 2M Hydrochloric acid and ethyl acetate. The ethyl acetate layer was washed with brine, dried (magnesium sulphate), filtered and evaporated *in vacuo*. Purification was by silica gel chromatography eluting with 2% acetic acid in ethyl acetate:toluene, 25:75 then 2% acetic acid in ethyl acetate:toluene, 80:20. Yield of (**61**) 0.07 g, 0.13 mmol, 65% as a yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 13.47 (s, 1H), 12.62 (s, 1H), 8.49 (tt, J = 3.9, 1.7 Hz, 1H), 8.12 (td, J = 13.7, 1.6 Hz, 2H), 7.45 (d, J = 7.8 Hz, 2H), 7.15 (dd, J = 7.9, 1.9 Hz, 2H), 7.09 (td, J = 5.1, 1.9 Hz, 2H), 7.02 (s, 1H), 6.70 (s, 2H), 5.72 (s, 1H), 4.99 (t, J = 4.0 Hz, 2H), 3.97 (s, 3H), 2.27 (s, 6H). LC-MS

(ESI+) Rt 3.05 (254nm); (m/z): 539.6 (M+1). Yield of (**60**) 0.02 g, 0.038 mmol, 19% as a yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 13.47 (s, 2H), 12.62 (s, 1H), 8.49 (q, J = 2.0 Hz, 1H), 8.09 (d, J = 1.6 Hz, 2H), 7.45 (d, J = 7.9 Hz, 2H), 7.15 (dd, J = 7.9, 1.9 Hz, 2H), 7.07 (d, J = 1.9 Hz, 2H), 7.02 (s, 1H), 6.70 (s, 2H), 5.72 (s, 1H), 4.98 (s, 2H), 2.27 (s, 6H). LC-MS (ESI+) Rt 2.81 (254nm, method 1); (m/z): 525.2 (M+1).

Methyl 3-((2-((tert-butoxycarbonyl)amino)ethyl)carbamoyl)-5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)benzoate (**62**). PyBroP® (0.19 g, 0.4 mmol) was added to a stirred solution of 3-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-5-(methoxycarbonyl)benzoic acid (0.15 g, 0.28 mmol), *tert*-butyl (2-aminoethyl)carbamate (0.058 g, 0.36 mmol) and triethylamine (0.15 g, 1.5 mmol) in *DMF* (2 ml). After 0.5 h, the solution was diluted with ethyl acetate (15 mL) and washed with water (2x4 mL) and brine (1x4 mL). The solution was evaporate to dryness under reduced pressure. Purification was by silica gel chromatography eluting with ethyl acetate: 40-60 petroleum ether, 70:30. Yield 0.1 g, 0.15 mmol, 53% as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.47 (s, 1H), 8.50 (s, 1H), 8.04 (dt, J = 7.1, 1.8 Hz, 2H), 7.63 – 7.58 (m, 1H), 7.50 (d, J = 7.8 Hz, 2H), 7.16 (dd, J = 7.9, 1.5 Hz, 1H), 7.07 (s, 1H), 6.90 (s, 1H), 6.68 (s, 2H), 5.74 (s, 1H), 5.33 (s, 1H), 5.10 – 5.05 (m, 1H), 4.79 (s, 2H), 4.02 (s, 3H), 3.68 – 3.60 (m, 2H), 3.50 – 3.44 (m, 2H), 2.32 (s, 6H), 1.44 (s, 8H). LC-MS (ESI+) Rt 3.14 (254nm, method 1); (m/z): 681.2 (M+1)

Methyl 3-((2,2-dimethyl-4-oxo-3,8,11-trioxa-5-azatridecan-13-yl)carbamoyl)-5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)benzoate (**63**). PyBroP® (0.11 g, 0.24 mmol) was added to a stirred solution of 3-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-

yl)methyl)-5-(methoxycarbonyl)benzoic acid (0.1 g, 0.186 mmol), tert-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (0.05 g , 0.2 mmol) and triethylamine (0.1 g, 1 mmol) in DMF (2 mL). After 0.5 h, the solution was diluted with ethyl acetate (15 ml) and washed with water (2x4 mL) and brine (1x4 mL). The solution was evaporate to dryness under reduced pressure. Purification was by silica gel chromatography eluting with ethyl acetate. Yield 0.09 g, 0.12 mmol, 63% as a yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 12.61 (s, 1H), 8.89 (t, J = 5.5 Hz, 1H), 8.48 (t, J = 1.6 Hz, 1H), 8.05 (t, J = 1.7 Hz, 1H), 8.00 (t, J = 1.6 Hz, 1H), 7.44 (d, J = 7.9 Hz, 2H), 7.14 (ddd, J = 7.8, 1.9, 0.8 Hz, 2H), 7.05 (dd, J = 1.9, 0.8 Hz, 2H), 6.94 (s, 1H), 6.76 (t, J = 5.6 Hz, 1H), 6.62 (s, 2H), 5.71 (s, 1H), 4.95 (s, 2H), 3.97 (s, 3H), 3.65 – 3.56 (m, 4H), 3.56 – 3.47 (m, 4H), 3.39 (t, J = 12.3 Hz, 1H), 3.33 – 3.30 (m, 1H), 3.07 (q, J = 6.0 Hz, 2H), 2.27 (s, 6H), 1.37 (s, 9H). LC-MS (ESI+) Rt 3.19 (254nm, method 1); (m/z): 769.6 (M+1)

tert-Butyl (2-(3-((2H-tetrazol-5-yl)carbamoyl)-5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)benzamido)ethyl)carbamate (**64**).

Lithium hydroxide (0.04 g, 1.6 mmol) in water (0.5 mL) was added to a stirred solution of methyl 3-((2-((tert-butoxycarbonyl)amino)ethyl)carbamoyl)-5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)benzoate (0.1 g, 0.15 mmol) in methanol (1.5 mL). After 16h, the mixture was partitioned between ethyl acetate and 2M hydrochloric acid solution. The ethyl acetate solution was washed with brine, dried (magnesium sulphate), filtered and evaporated in vacuo to give 3-((2-((*tert*-butoxycarbonyl)amino)ethyl)carbamoyl)-5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)benzoic acid. Yield 0.08 g, as a yellow solid. LC-MS (ESI+) Rt 2.99 (254nm, method 1); (m/z): 667.5 (M+1)

PyBroP® (0.16 g, 0.34 mmol) was added to a stirred solution of 3-((2-((*tert*-butoxycarbonyl)amino)ethyl)carbamoyl)-5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)benzoic acid (0.09 g, 0.135 mmol), 5-aminotetrazole monohydrate (0.055 g , 0.54 mmol) and triethylamine (0.13 g, 1.3 mmol) in *DMF* (2 ml). After 0.5 h, the solution was diluted with ethyl acetate (15 mL) and washed with 2M hydrochloric acid (1x4 mL), water (2x4 mL) and brine (1x4 mL). The solution was evaporate to dryness under reduced pressure. Purification was by silica gel chromatography eluting with methanol:dichloromethane, 5:95 and then 2% acetic acid in methanol:dichloromethane, 5:95 . Yield 0.03 g, 0.04 mmol, 30% as a yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 12.66 – 12.59 (m, 1H), 12.45 – 11.87 (m, 1H), 8.83 – 8.67 (m, 1H), 8.57 – 8.43 (m, 1H), 8.09 – 7.94 (m, 2H), 7.49 – 7.41 (m, 2H), 7.31 – 7.23 (m, 1H), 7.22 – 7.17 (m, 2H), 7.14 – 7.10 (m, 1H), 7.04 – 7.00 (m, 1H), 6.99 – 6.89 (m, 1H), 6.67 – 6.48 (m, 2H), 5.75 – 5.69 (m, 1H), 5.03 – 4.88 (m, 2H), 3.44 – 3.33 (m, 2H), 3.24 – 3.09 (m, 2H), 2.30 – 2.20 (m, 6H), 1.38 (s, 9H).LC-MS (ESI+) Rt 2.98 (254nm, method 1); (m/z): 734.3 (M+1)

tert-Butyl (2-(2-(2-(3-((2H-tetrazol-5-yl)carbamoyl)-5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)benzamido)ethoxy)ethoxy)ethyl)carbamate (**65**).

Lithium hydroxide (0.04 g, 1.6 mmol) in water (0.5 mL) was added to a stirred solution of methyl 3-((2,2-dimethyl-4-oxo-3,8,11-trioxa-5-azatridecan-13-yl)carbamoyl)-5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)benzoate (0.035 g, 0.046 mmol) in methanol (1.5 mL). After 16h, the mixture was partitioned between ethyl acetate and 2M hydrochloric acid solution. The ethyl acetate solution was washed with brine, dried (magnesium sulphate), filtered and evaporated *in vacuo* to give 3-((2,2-dimethyl-4-oxo-3,8,11-

trioxa-5-azatridecan-13-yl)carbamoyl)-5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)benzoic acid. Yield 0.032 g, as a yellow solid. ^{1}H NMR LC-MS (ESI+) Rt 3.04 (254nm, method 1); (m/z): 755.6 (M+1). PyBroP® (0.042 g, 0.09 mmol) was added to a stirred solution of 3-((2,2-dimethyl-4-oxo-3,8,11-trioxa-5-azatridecan-13-yl)carbamoyl)-5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)benzoic acid (0.05 g, 0.067 mmol), 5-aminotetrazole monohydrate (0.02 g , 0.2 mmol) and triethylamine (0.03 g, 0.3 mmol) in *DMF* (1 mL). After 0.5 h, the solution was diluted with ethyl acetate (15 mL) and washed with 2m hydrochloric acid (1x4 mL), water (2x4 mL) and brine (1x4 mL). The solution was evaporate to dryness under reduced pressure. Purification was by silica gel chromatography eluting with methanol:dichloromethane, 5:95 and then 2% acetic acid in methanol:dichloromethane, 5:95 . Yield 0.009 g, 0.01 mmol, 14% as a yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 12.64 (s, 1H), 12.51 (s, 1H), 8.76 (t, J = 5.6 Hz, 1H), 8.57 (s, 1H), 8.03 (d, 1H), 7.44 (d, J = 7.9 Hz, 2H), 7.12 (dd, J = 8.1, 1.9 Hz, 2H), 7.00 (d, J = 1.9 Hz, 2H), 6.90 (s, 1H), 6.79 – 6.73 (m, 1H), 6.54 (s, 2H), 5.73 (s, 1H), 4.97 (s, 2H), 3.71 – 3.57 (m, 4H), 3.54 (dd, J = 6.1, 4.1 Hz, 4H), 3.40 (d, J = 6.7 Hz, 2H), 3.07 (q, J = 6.0 Hz, 2H), 2.22 (s, 6H), 1.37 (s, 9H). LC-MS (ESI+) Rt 3.02 (254nm, method 1); (m/z): 822.3 (M+1)

(E)-N1-(2-(2-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5H-4λ^{4},5λ^{4}-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)vinyl)phenoxy)acetamido)ethyl)-5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-N3-(1H-tetrazol-5-yl)isophthalamide (**66**). Following General Procedure 3 with *tert*-butyl (2-(3-((2H-tetrazol-5-yl)carbamoyl)-5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)benzamido)ethyl)carbamate (3.2 mg, 4.3x10-3

mmol) and 2,5-dioxopyrrolidin-1-yl *(E)*-2-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)vinyl)phenoxy)acetate (2.3 mg, 4.2x10^{-3} mmol). Yield 0.67 mg, 0.627x10-3 mmol, 15%. LC-MS (ESI+) Rt 3.19 (254nm); (m/z): 1066.4 (M+1).

(E)-N1-(2-(6-(2-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)vinyl)phenoxy)acetamido)hexanamido)ethyl)-5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-N3-(1H-tetrazol-5-yl)isophthalamide (**67**). Following General Procedure 3 with *tert*-butyl (2-(3-((2H-tetrazol-5-yl)carbamoyl)-5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)benzamido)ethyl)carbamate (2.2 mg, 3x10^{-3} mmol) and 2,5-dioxopyrrolidin-1-yl *(E)*-6-(2-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)vinyl)phenoxy)acetamido)hexanoate (2.0 mg, 3x10-3 mmol). Yield 1.2 mg, 1x10-3 mmol, 45%. LC-MS (ESI+) Rt 7.35 (254nm); (m/z): 1179.4 (M+1).

(E)-N1-(2-(2-(2-(2-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)vinyl)phenoxy)acetamido)ethoxy)ethoxy)ethyl)-5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-N3-(1H-tetrazol-5-yl)isophthalamide (**68**). Following General Procedure 3, with tert-butyl (2-(2-(2-(3-((2H-tetrazol-5-yl)carbamoyl)-5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)benzamido)ethoxy)ethoxy)ethyl)carbamate (3.4 mg, 4.14x10-3 mmol) and 2,5-dioxopyrrolidin-1-yl (E)-2-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)vinyl)phenoxy)acetate (2.26 mg, 4.14x10-3 mmol). Yield 0.7 mg, 0.67x10-3 mmol, 17%. LC-MS (ESI+) Rt 7.46 (254nm); (m/z): 1154.6 (M+1).

(E)-N1-(18-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)vinyl)phenoxy)-10,17-dioxo-3,6-dioxa-9,16-diazaoctadecyl)-5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-N3-(1H-tetrazol-5-yl)isophthalamide (**69**). 4M HCl in 1,4-dioxane (1 mL) was added to *tert*-butyl (2-(2-(2-(3-((2H-tetrazol-5-yl)carbamoyl)-5-((5-(2,8-dimethyl-5H-dibenzo[a,d][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)benzamido)ethoxy)ethoxy)ethyl)carbamate (1.5 mg, 1.82x10^{-3} mmol). The solution was stirred for 2h and then evaporated to dryness *in vacuo*. DMF (0.5 mL) was added and to this solution was added di-*iso*propylethylamine (10 mg) and then 2,5-dioxopyrrolidin-1-yl *(E)*-6-(2-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)vinyl)phenoxy)acetamido)hexanoate (1.2 mg, 1.8x10^{-3} mmol). The reaction mixture was stirred in the dark and under nitrogen overnight. Purification was by reverse phase preparative HPLC eluting with a gradient of 50 to 60 % MeCN in water (0.1% formic acid) over 20 min. The fraction containing the product was lyophilised. Yield 1.002mg, 0.79x10-3 mmol, 41%. LC-MS (ESI+) Rt 7.41 (254nm); (m/z): 1267.0 (M+1).

tert-Butyl 3-((7-chloro-4-(1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)propanoate (70). Following General Procedure 1, **43** (200 mg, 0.51 mmol) was reacted with β-alanine-*tert*-butyl ester hydrochloride (370 mg, 2.04 mmol) to afford the title compound **70** which was isolated at 5% MeOH/DCM, a pale yellow solid (248 mg, 0.494 mmol, 96%). LC-MS (ESI+) Rt: 2.79 min (254 nm, Method 1); (m/z): 500.9 [M(^{35}Cl)+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.20 (s, 1H), 7.97 (t, *J* = 5.5 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.44 (d, *J* = 2.2 Hz, 1H), 7.38 (dd, *J* = 8.3, 2.3 Hz, 1H), 7.03 (s, 1H), 6.82 (d, *J* = 11.6 Hz, 1H), 6.79 (d, *J* = 11.7 Hz, 1H), 5.22 (s, 1H), 3.48-3.42 (m, 2H), 3.17 (s, 3H), 1.39 (s,

9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 171.0, 169.3, 163.3, 151.1, 147.8, 143.1, 137.0, 134.2, 133.14, 133.00, 132.96, 131.0, 126.2, 122.2, 117.0, 108.9, 80.5, 43.8, 40.5, 36.2, 35.2, 28.2.

tert-Butyl 6-((7-chloro-4-(1-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)hexanoate (71). Following General Procedure 1, **43** (200 mg, 0.51 mmol) was reacted with *tert*-butyl 6-aminohexanoate (382 mg, 2.04 mmol) to afford the title compound **71** at 5% MeOH/DCM, a pale yellow solid (248 mg, 0.494 mmol, 97%). LC-MS (ESI+) Rt: 2.91 min (254 nm, Method 1); (m/z): 543.0 $[M(^{35}Cl)+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.24 (s, 1H), 7.92 (t, J = 5.4 Hz, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.43 (d, J = 2.2 Hz, 1H), 7.37 (dd, J = 8.3, 2.2 Hz, 1H), 7.01 (s, 1H), 6.81 (d, J = 11.6 Hz, 1H), 6.76 (d, J = 11.7 Hz, 1H), 5.23 (s, 1H), 3.22-3.15 (m, 2H), 3.17 (s, 3H), 2.18 (t, J = 7.3 Hz, 2H), 1.56-1.49 (m, 4H), 1.38 (s, 9H), 1.34-1.30 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 172.7, 169.8, 163.3, 151.1, 148.0, 142.9, 137.1, 134.1, 133.1, 131.0, 129.3, 128.2, 125.9, 122.3, 116.6, 108.9, 79.8, 44.6, 43.9, 36.2, 35.2, 28.7, 28.2, 26.3, 24.8.

3-((7-Chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)propanoic acid (72). Following General Procedure 2, **70** (200 mg, 0.40 mmol) was converted to the thiouracil which was isolated at 2% MeOH/DCM, a yellow solid (124 mg). The intermediate product (100 mg, 0.193 mmol) was dissolved in DCM (12 mL) and was treated with trifluoroacetic acid (6 mL) and stirred at RT for 30 min until completion was observed by TLC. The RM was diluted with toluene (25 mL) and concentrated *in vacuo* to 1/5th volume; this was repeated 3 times before concentrating to dryness to afford a yellow solid. This was purified by FC, washing with 5% MeOH/DCM before eluting the title compound off with 20% MeOH/DCM w. 1% acetic acid, affording **72**, a yellow solid (34 mg, 0.074 mmol, 39%). LC-MS (ESI+) Rt: 2.61 min (254 nm, Method 1); (m/z): 460.9

[M(^{35}Cl)+H]$^{+}$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.66 (s, 1H), 12.45-12.07 (br.s, 1H), 7.99 (t, *J* = 5.2 Hz, 1H), 7.71 (s, 1H), 7.55 (d, *J* = 8.5 Hz, 1H), 7.45 (d, *J* = 2.3 Hz, 1H), 7.35 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.88 (s, 2H), 5.68 (s, 1H), 3.44 (app.q, *J* = 6.1 Hz, 2H), 3.31 (s, 3H).

6-((7-Chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)hexanoic acid (73). Following General Procedure 2, **71** (200 mg, 0.40 mmol) was converted to the thiouracil which was isolated at 2% MeOH/DCM, a yellow solid (124 mg), which was dissolved in DCM (14 mL) was treated with trifluoroacetic acid (7 mL) and stirred at RT for 30 min until completion was observed by TLC. The RM was diluted with toluene (25 mL) and concentrated *in vacuo* to 1/5th volume; this was repeated 3 times before concentrating to dryness to afford a yellow solid. This was purified by FC washing with 5% MeOH/DCM before eluting the title compound off with 20% MeOH/DCM w. 1% acetic acid, affording **73**, a yellow solid (80 mg, 0.159 mmol, 74%). LC-MS (ESI+) Rt: 2.67 min (254 nm, Method 1); (m/z): 502.9 [M(^{35}Cl)+H]$^{+}$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.80-12.53 (br.s, 1H), 12.16-11.93 (br.s, 1H), 7.94 (t, *J* = 5.3 Hz, 1H), 7.62 (s, 1H), 7.58 (d, *J* = 8.5 Hz, 1H), 7.45 (d, *J* = 2.3 Hz, 1H), 7.34 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.86 (s, 2H), 5.71 (s, 1H), 3.29 (s, 3H), 3.23-3.18 (m, 2H), 2.20 (t, *J* = 7.3 Hz, 2H), 1.56-1.48 (m, 4H), 1.36-1.29 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 189.2, 174.9, 169.6, 149.1, 148.5, 143.3, 137.6, 134.0, 132.0, 130.8, 129.0, 127.8, 126.58, 126.56, 122.7, 117.5, 116.4, 45.3, 44.7, 37.2, 34.1, 26.5, 24.7.

tert-Butyl (2-(3-((7-chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)propanamido)ethyl)carbamate (74). A stirred solution **72** (10 mg, 0.022 mmol) in DMF (1 mL) was treated with DIPEA (50 µL) and *N*-Boc-ethylenediamine (21 mg, 0.132 mmol) followed by PyBroP® (15 mg, 0.033 mmol) and stirred at RT for 1h. until completion was observed by LC-MS. The RM was quenched with water (c.a. 2

drops) and concentrated *in vacuo* azeotroping with toluene (2 x 25 mL) to afford an orange oil. This was purified by FC (1-5% MeOH/DCM) affording the title compound **74** at 4% MeOH/DCM, a yellow solid (5.2 mg, 0.0086 mmol, 39%). LC-MS (ESI+) Rt: 2.75 min (254 nm, Method 1); (m/z): 603.1 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 12.67 (s, 1H), 7.95 (t, J = 5.5 Hz, 1H), 7.91 (t, J = 5.4 Hz, 1H), 7.74 (s, 1H), 7.55 (d, J = 8.6 Hz, 1H), 7.45 (d, J = 2.3 Hz, 1H), 7.35 (dd, J = 8.4, 2.3 Hz, 1H), 6.88 (s, 2H), 6.78 (t, J = 5.3 Hz, 1H), 5.66 (s, 1H), 3.45 (app.q, J = 6.5 Hz, 2H), 3.32 (s, 3H), 3.07-3.03 (m, 2H), 2.96 (app.q, J = 6.1 Hz, 2H), 2.38 (t, J = 6.8 Hz, 2H), 1.38 (s, 9H).

tert-Butyl (2-(6-((7-chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)hexanamido)ethyl)carbamate (75). A stirred solution **73** (30 mg, 0.060 mmol) in DMF (3 mL) was treated with DIPEA (150 μL) and *N*-Boc-ethylenediamine (58 mg, 0.362 mmol) followed by PyBroP® (42 mg, 0.090 mmol) and stirred at RT for 1 h. until completion was observed by LC-MS. The RM was quenched with water (c.a. 2 drops) and concentrated *in vacuo* azeotroping with toluene (2 x 25 mL) to afford an orange oil. This was purified by FC (1-5% MeOH/DCM) affording the tile compound **75** at 4% MeOH/DCM (15 mg, 0.023 mmol, 39%). LC-MS (ESI+) Rt: 2.80 min (254 nm, Method 1); (m/z): 645.1 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 12.66 (s, 1H), 7.93 (t, J = 5.4 Hz, 1H), 7.78-7.77 (m, 1H), 7.62 (s, 1H), 7.57 (d, J = 8.5 Hz, 1H), 7.45 (d, J = 2.3 Hz, 1H), 7.34 (dd, J = 8.4, 2.3 Hz, 1H), 6.86 (s, 2H), 6.77 (t, J = 5.5 Hz, 1H), 5.71 (s, 1H), 3.29 (s, 3H), 3.22-3.17 (m, 2H), 3.05 (app.q, J = 5.8 Hz, 2H), 2.98-2.94 (m, 2H), 2.04 (t, J = 7.3 Hz, 2H), 1.55-1.47 (m, 4H), 1.37 (s, 9H), 1.31 (t, J = 7.6 Hz, 2H).

tert-Butyl (2-(2-(2-(3-((7-chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-

yl)amino)propanamido)ethoxy)ethoxy)ethyl)carbamate (76). A stirred solution **72** (10 mg, 0.022 mmol) in DMF (1 mL) was treated with DIPEA (50 μL) and *N*-Boc-2,2′-(ethylenedioxy)diethylamine (33 mg, 0.132 mmol) followed by PyBroP® (15 mg, 0.033 mmol) and stirred at RT for 1h. until completion was observed by LC-MS. The RM was quenched with water (c.a. 2 drops) and concentrated *in vacuo* azeotroping with toluene (2 x 25 mL) to afford an orange oil. This was purified by FC (1-5% MeOH/DCM) affording the tile compound **76** at 4% MeOH/DCM, a yellow solid (8.8 mg, 0.0127 mmol, 58%). LC-MS (ESI+) Rt: 2.76 min (254 nm, Method 1); (m/z): 691.1 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 12.67 (s, 1H), 7.95 (t, J = 5.5 Hz, 2H), 7.79 (s, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.45 (d, J = 2.3 Hz, 1H), 7.34 (dd, J = 8.4, 2.3 Hz, 1H), 6.88 (s, 2H), 6.76 (t, J = 5.4 Hz, 1H), 5.64 (s, 1H).

tert-Butyl (2-(2-(2-(6-((7-chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)hexanamido)ethoxy)ethoxy)ethyl)carbamate (77). A stirred solution **73** (30 mg, 0.060 mmol) in DMF (3 mL) was treated with DIPEA (150 μL) and *N*-Boc-ethylenediamine (58 mg, 0.362 mmol) followed by PyBroP® (42 mg, 0.090 mmol) and stirred at RT for 1 h. until completion was observed by LC-MS. The RM was quenched with water (c.a. 2 drops) and concentrated *in vacuo* azeotroping with toluene (2 x 25 mL) to afford an orange oil. This was purified by FC (1-5% MeOH/DCM) affording the tile compound **77** at 4% MeOH/DCM (16 mg, 0.022 mmol, 36%). LC-MS (ESI+) Rt: 2.81 min (254 nm, Method 1); (m/z): 733.2 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: - 12.66 (s, 1H), 7.93 (t, J = 5.4 Hz, 1H), 7.81 (t, J = 5.6 Hz, 1H), 7.62 (s, 1H), 7.57 (d, J = 8.5 Hz, 1H), 7.44 (d, J = 2.3 Hz, 1H), 7.34 (dd, J = 8.4, 2.3 Hz, 1H), 6.86 (s, 2H), 6.76 (t, J = 5.0 Hz, 1H), 5.71 (s, 1H), 3.51-3.49 (m, 2H), 3.38 (q, J = 5.3 Hz,

6H), 3.29 (s, 3H), 3.22-3.16 (m, 4H), 3.06 (q, *J* = 6.0 Hz, 2H), 2.06 (t, *J* = 7.4 Hz, 2H), 1.57-1.52 (m, 4H), 1.37 (s, 9H), 1.33-1.27 (m, 2H).

(E)-3-((7-Chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)-N-(2-(2-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)vinyl)phenoxy)acetamido)ethyl)propanamide (78). Following General Procedure 3, **74** was converted to BODIPY®630/650 conjugate **78**. This was purified using preparative RP-HPLC (Method 4) isolating **78** at Rt: 13.45 min which was freeze dried to a blue iridescent solid (0.52 mg, 0.54 μmol, 27%). LC-MS (ESI+) Rt: 3.04 min (254 nm, Method 1); (m/z): 935.1 [M(^{35}Cl)+H]$^+$. H.MS-TOF (ESI+) (*m/z*): [M+H]$^+$ calcd. for $C_{45}H_{39}BClF_2N_8O_4S_3$, 935.2001; found, 935.2002. [M+Na]$^+$ calcd. for $C_{45}H_{38}BClF_2N_8NaO_4S_3$, 957.1820; found, 957.1838.

(E)-N-(2-(3-((7-chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)propanamido)ethyl)-6-(2-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)vinyl)phenoxy)acetamido)hexanamide (79). Following General Procedure 3, **74** was converted to BODIPY®630/650-X conjugate **79**. This was purified using preparative RP-HPLC (Method 4) isolating 173 at Rt: 13.37 min which was freeze dried to a blue iridescent solid (0.85 mg, 0.81 μmol, 41%). LC-MS (ESI+) Rt: 3.01 min (254 nm, Method 1); (m/z): 1048.2 [M(^{35}Cl)+H]$^+$. H.MS-TOF (ESI-) (*m/z*): [M-H]$^-$ calcd. for $C_{51}H_{48}BClF_2N_9O_5S_3$, 1046.2694; found, 1046.2657.

(E)-N-(2-(2-(2-(3-((7-Chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)propanamido)ethoxy)ethoxy)ethyl)-

6-(2-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)vinyl)phenoxy)acetamido)hexanamide (80). Following General Procedure 3, **76** was converted to BODIPY®630/650-X conjugate **80**. This was purified using preparative RP-HPLC (Method 4) isolating **80** at Rt: 13.03 min which was freeze dried to a blue iridescent solid (0.57 mg, 0.50 μmol, 25%). LC-MS (ESI+) Rt: 3.01 min (254 nm, Method 1); (m/z): 1136.2 [M(^{35}Cl)+H]$^+$. H.MS-TOF (ESI-) (*m/z*): [M-H]$^-$ calcd. for $C_{55}H_{56}BClF_2N_9O_7S_3$, 1134.3220; found, 1134.3160.

(E)-6-((7-Chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)-N-(2-(2-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5H-5λ^4,6λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)vinyl)phenoxy)acetamido)ethyl)hexanamide (81). Following General Procedure 3, **75** was converted to BODIPY®630/650 conjugate **81**. This was purified using preparative RP-HPLC (Method 4) isolating **81** at Rt: 13.05 min which was freeze dried to a blue iridescent solid (0.41 mg, 0.42 μmol, 21%). LC-MS (ESI+) Rt: 3.04 min (254 nm, Method 1); (m/z): 977.2 [M(^{35}Cl)+H]$^+$. H.MS-TOF (ESI+) (*m/z*): [M+H]$^+$ calcd. for $C_{48}H_{45}BClF_2N_8O_4S_3$, 977.2470; found, 977.2558. [M+Na]$^+$ calcd. for $C_{48}H_{44}BClF_2N_8NaO_4S_3$, 999.2289; found, 999.2380. H.MS-TOF (ESI-) (*m/z*): [M-H]$^-$ calcd. for $C_{48}H_{43}BClF_2N_8O_4S_3$, 975.2325; found, 975.2272.

(E)-6-((7-Chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)-N-(2-(2-(2-(2-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)vinyl)phenoxy)acetamido)ethoxy)ethoxy)ethyl)hexanamide (82). Following General Procedure 3, **77** was converted to BODIPY®630/650 conjugate **82**. This was purified using preparative RP-HPLC (Method 4) isolating **82** at Rt:13.69 min which was freeze dried to a blue

iridescent solid (0.22 mg, 0.21 μmol, 10%). LC-MS (ESI+) Rt: 3.05 min (254 nm, Method 1); (m/z): 1065.2 $[M(^{35}Cl)+H]^+$. H.MS-TOF (ESI-) (*m/z*): $[M-H]^-$ calcd. for $C_{52}H_{51}BClF_2N_8O_6S_3$, 1063.2849; found, 1063.2803.

(E)-6-((7-Chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)-N-(2-(6-(2-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)vinyl)phenoxy)acetamido)hexanamido)ethyl)hexanamide (83). Following General Procedure 3, **75** was converted to BODIPY®630/650-X conjugate **83**. This was purified using preparative RP-HPLC (Method 4) isolating **83** at Rt: 13.27 min which was freeze dried to a blue iridescent solid (0.39 mg, 0.36 μmol, 18%). LC-MS (ESI+) Rt: 3.02 min (254 nm, Method 1); (m/z): 1090.2 $[M(^{35}Cl)+H]^+$. H.MS-TOF (ESI+) (*m/z*): $[M+H]^+$ calcd. for $C_{54}H_{56}BClF_2N_9O_5S_3$, 1090.3311; found, 1090.3293. $[M+Na]^+$ calcd. for $C_{54}H_{55}BClF_2N_9NaO_5S_3$, 1112.3130; found, 1112.3129.

(E)-6-((7-Chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)-N-(18-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)vinyl)phenoxy)-10,17-dioxo-3,6-dioxa-9,16-diazaoctadecyl)hexanamide (84). Following General Procedure 3, **77** was converted to BODIPY®630/650-X conjugate **84**. This was purified using preparative RP-HPLC (Method 4) isolating **84** at Rt: 13.28which was freeze dried to a blue iridescent solid (0.47 mg, 0.40 μmol, 20%). LC-MS (ESI+) Rt: 3.02 min (254 nm, Method 1); (m/z): 1178.2 $[M(^{35}Cl)+H]^+$. H.MS-TOF (ESI+) (*m/z*): $[M+H]^+$ calcd. for $C_{58}H_{64}BClF_2N_9O_7S_3$, 1178.3835; found, 1178.3933. $[M+Na]^+$ calcd. for $C_{58}H_{63}BClF_2N_9NaO_7S_3$, 1200.3654; found, 1200.3772. H.MS-TOF (ESI-) (*m/z*): $[M-H]^-$ calcd. for $C_{58}H_{62}BClF_2N_9O_7S_3$, 1176.3689; found, 1176.3647.

3-((7-Chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)-N-(2-(3-(5,5-difluoro-7,9-dimethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)propanamido)ethyl)propanamide (85). Following General Procedure 3, **74** was converted to BODIPY®FL conjugate **85**. This was purified using preparative RP-HPLC (Method 3) isolating 85 at Rt: 13.82 min which was freeze dried to a red/green iridescent solid (0.84 mg, 1.08 μmol, 54%). LC-MS (ESI+) Rt: 2.80 min (254 nm, Method 1); (m/z): 777.1 $[M(^{35}Cl)+H]^+$. H.MS-TOF (ESI+) (*m/z*): $[M+H]^+$ calcd. for $C_{36}H_{37}BClF_2N_8O_3S_2$, 777.2174; found, 777.2106. $[M+Na]^+$ calcd. for $C_{36}H_{36}BClF_2N_8NaO_3S_2$, 799.1994; found, 799.1960. H.MS-TOF (ESI-) (*m/z*): $[M-H]^-$ calcd. for $C_{36}H_{35}BClF_2N_8O_3S_2$, 775.2029; found, 775.2007.

N-(2-(3-((7-Chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)propanamido)ethyl)-6-(3-(5,5-difluoro-7,9-dimethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)propanamido)hexanamide (86). Following General Procedure 3, **74** was converted to BODIPY®FL-X conjugate **86**. This was purified using preparative RP-HPLC (Method 3) isolating **86** at Rt: 14.37 min which was freeze dried to a red/green iridescent solid (0.54 mg, 0.61 μmol, 30%). LC-MS (ESI+) Rt: 2.81 min (254 nm, Method 1); (m/z): 890.1 $[M(^{35}Cl)+H]^+$. H.MS-TOF (ESI+) (*m/z*): $[M+H]^+$ calcd. for $C_{42}H_{48}BClF_2N_9O_4S_2$, 890.3015; found, 890.3014. $[M+Na]^+$ calcd. for $C_{42}H_{47}BClF_2N_9NaO_4S_2$, 912.2834; found, 912.2862. H.MS-TOF (ESI-) (*m/z*): $[M-H]^-$ calcd. for $C_{42}H_{46}BClF_2N_9O_4S_2$, 888.2869; found, 888.2853.

N-(2-(2-(2-(3-((7-Chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)propanamido)ethoxy)ethoxy)ethyl)-6-(3-(5,5-difluoro-7,9-dimethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-

yl)propanamido)hexanamide (87). Following General Procedure 3, **76** was converted to BODIPY®FL-X conjugate **87**. This was purified using preparative RP-HPLC (Method 3) isolating 87 at Rt: 14.47 min which was freeze dried to a red/green iridescent solid (0.46 mg, 0.47 μmol, 24%). LC-MS (ESI+) Rt: 2.78 min (254 nm, Method 1); (m/z): 978.3 $[M(^{35}Cl)+H]^+$. H.MS-TOF (ESI+) (*m/z*): $[M+Na]^+$ calcd. for $C_{46}H_{55}BClF_2N_9NaO_6S_2$, 1000.3359; found, 1000.3457. H.MS-TOF (ESI-) (*m/z*): $[M-H]^-$ calcd. for $C_{46}H_{54}BClF_2N_9O_6S_2$, 976.3394; found, 976.3355.

6-((7-Chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)-N-(2-(3-(5,5-difluoro-7,9-dimethyl-5H-5λ⁴,6λ⁴-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)propanamido)ethyl)hexanamide (88). Following General Procedure 3, **75** was converted to BODIPY®FL conjugate **88**. This was purified using preparative RP-HPLC (Method 3) isolating 88 at Rt: 15.19 min which was freeze dried to a red/green iridescent solid (0.71 mg, 0.87 μmol, 43%). LC-MS (ESI+) Rt: 2.82 min (254 nm, Method 1); (m/z): 819.2 $[M(^{35}Cl)+H]^+$. H.MS-TOF (ESI+) (*m/z*): $[M+H]^+$ calcd. for $C_{39}H_{43}BClF_2N_8O_3S_2$, 819.2644; found, 819.2598. $[M+Na]^+$ calcd. for $C_{39}H_{42}BClF_2N_8NaO_3S_2$, 841.2463; found, 841.2419.

6-((7-Chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)-N-(2-(2-(2-(3-(5,5-difluoro-7,9-dimethyl-5H-5λ⁴,6λ⁴-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)propanamido)ethoxy)ethoxy)ethyl)hexanamide (89). Following General Procedure 3, **77** was converted to BODIPY®FL conjugate **89**. This was purified using preparative RP-HPLC (Method 3) isolating **89** at Rt:15.97 min which was freeze dried to a red/green iridescent solid (0.41 mg, 0.45 μmol, 23%). LC-MS (ESI+) Rt: 2.83 min (254 nm, Method 1); (m/z): 907.2

[M(^{35}Cl)+H]$^+$. H.MS-TOF (ESI+) (*m/z*): [M+H]$^+$ calcd. for $C_{43}H_{51}BClF_2N_8O_5S_2$, 907.3168; found, 907.3160. [M+Na]$^+$ calcd. for $C_{43}H_{50}BClF_2N_8NaO_5S_2$, 929.2987; found, 929.2989.

6-((7-Chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)-N-(2-(6-(3-(5,5-difluoro-7,9-dimethyl-5H-5λ^4,6λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)propanamido)hexanamido)ethyl)hexanamide (90). Following General Procedure 3, **75** was converted to BODIPY®FL-X conjugate **90**. This was purified using preparative RP-HPLC (Method 3) isolating **90** at Rt: 15.59 min which was freeze dried to a red/green iridescent solid (0.43 mg, 0.46 μmol, 23%). LC-MS (ESI+) Rt: 2.79 min (254 nm, Method 1); (m/z): 932.3 [M(^{35}Cl)+H]$^+$. H.MS-TOF (ESI+) (*m/z*): [M+H]$^+$ calcd. for $C_{45}H_{54}BClF_2N_9O_4S_2$, 932.3484; found, 932.3534. [M+Na]$^+$ calcd. for $C_{45}H_{53}BClF_2N_9NaO_4S_2$, 954.3304; found, 954.3387.

6-((7-Chloro-4-(1-methyl-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)-N-(19-(5,5-difluoro-7,9-dimethyl-5H-5λ^4,6λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)-10,17-dioxo-3,6-dioxa-9,16-diazanonadecyl)hexanamide (91). Following General Procedure 3, **77** was converted to BODIPY®FL-X conjugate **91**. This was purified using preparative RP-HPLC (Method 3) isolating **91** at Rt: 15.92 min which was freeze dried to a red/green iridescent solid (0.45 mg, 0.44 μmol, 22%). LC-MS (ESI+) Rt: 2.80 min (254 nm, Method 1); (m/z): 1020.4 [M(^{35}Cl)+H]$^+$. H.MS-TOF (ESI+) (*m/z*): [M+H]$^+$ calcd. for $C_{49}H_{62}BClF_2N_9O_6S_2$, 1020.4009; found, 1020.4011. [M+Na]$^+$ calcd. for $C_{49}H_{61}BClF_2N_9NaO_6S_{22}$, 1042.3828; found, 1042.3837.

Ethyl 2-((5-(2-((3-(tert-butoxy)-3-oxopropyl)amino)-7-chloro-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-

yl)methyl)thiazole-4-carboxylate (92). Following General Procedure 1, **57** (547 mg, 1.00 mmol) was reacted with β-alanine-*tert*-butyl ester hydrochloride (727 mg, 4.00 mmol) to afford the title compound **92** at 3% MeOH/DCM, a pale yellow solid (585 mg, 0.89 mmol, 89%). LC-MS (ESI+) Rt: 2.97 min (254 nm, Method 1); (m/z): 656.0 $[M(^{35}Cl)+H]^+$. The product was reacted following General Procedure 2, the intermediate uracil (492 mg, 0.75 mmol) was converted to the title compound 92 and isolated at 1.5% MeOH/DCM, a yellow solid (240 mg, 0.36 mmol, 48%). LC-MS (ESI+) Rt: 3.13 min (254 nm, Method 1); (m/z): 672.0 $[M(^{35}Cl)+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.88 (s, 1H), 8.56 (s, 1H), 7.97 (t, *J* = 5.5 Hz, 1H), 7.79 (s, 1H), 7.57 (d, *J* = 8.5 Hz, 1H), 7.41 (d, *J* = 2.3 Hz, 1H), 7.36 (dd, *J* = 8.3, 2.3 Hz, 1H), 6.77 (s, 2H), 5.67 (s, 1H), 5.40 (d, *J* = 15.8 Hz, 1H), 5.27 (d, *J* = 15.8 Hz, 1H), 4.35 (q, *J* = 6.9 Hz, 2H), 3.42 (app.q, *J* = 6.2 Hz, 2H), 2.46 (t, *J* = 6.4 Hz, 2H), 1.35 (s, 9H), 1.34 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 189.8, 171.1, 169.1, 165.4, 161.0, 148.0, 146.4, 137.5, 133.8, 130.99, 130.97, 130.83, 128.9, 127.9, 126.8, 122.7, 117.7, 116.8, 110.0, 80.4, 61.4, 49.3, 45.3, 35.2, 28.2, 22.5, 14.4.

Ethyl 2-((5-(2-((3-((2-((tert-butoxycarbonyl)amino)ethyl)amino)-3-oxopropyl)amino)-7-chloro-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)thiazole-4-carboxylate (93). A stirred solution of **92** (200 mg, 0.30 mmol) in DCM (12 mL) was treated with trifluoroacetic acid (6 mL) and stirred at RT for 30 min until completion was observed by TLC. The RM was diluted with toluene (25 mL) and concentrated *in vacuo* to 1/5th volume; this was repeated 3 times before concentrating to dryness to afford a yellow solid. LC-MS (ESI+) Rt: 2.77 min (254 nm, Method 1); (m/z): 616.2 $[M(^{35}Cl)+H]^+$. The intermediate (45 mg, 0.073 mmol) in DCM (3 mL) was treated with Et_3N (60 µL) and *N*-Boc-ethylenediamine (70 mg, 0.438 mmol) followed by HATU (56 mg, 0.146 mmol) and stirred at rt for 24 h. until completion was observed by LC-MS. The RM was quenched with MeOH (1mL)

and concentrated directly onto silica. This was purified by FC (1-5% MeOH/DCM) affording the tile compound **93** at 5% MeOH/DCM, a yellow solid (35 mg, 0.046 mmol, 63%). LC-MS (ESI+) Rt: 2.88 min (254 nm, Method 1); (m/z): 758.2 $[M(^{35}Cl)+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 12.86 (s, 1H), 8.55 (s, 1H), 7.91 (t, *J* = 5.7 Hz, 1H), 7.88 (t, *J* = 6.3 Hz, 1H), 7.75 (s, 1H), 7.58 (d, *J* = 8.5 Hz, 1H), 7.40 (d, *J* = 2.2 Hz, 1H), 7.36 (dd, *J* = 8.3, 2.3 Hz, 1H), 6.78-6.75 (m, 1H), 6.75 (s, 2H), 5.68 (s, 1H), 5.41 (d, *J* = 15.8 Hz, 1H), 5.26 (d, *J* = 15.8 Hz, 1H), 4.35 (q, *J* = 7.0 Hz, 2H), 3.42 (app.q, *J* = 6.2 Hz, 2H), 3.04 (app.q, *J* = 6.0 Hz, 2H), 2.97-2.93 (m, 2H), 2.34 (t, *J* = 6.7 Hz, 3H), 1.39-1.36 (m, 9H), 1.34 (t, *J* = 7.1 Hz, 3H).

tert-Butyl (2-(3-((4-(1-((4-((1H-tetrazol-5-yl)carbamoyl)thiazol-2-yl)methyl)-2-oxo-4-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-7-chloro-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-2-yl)amino)propanamido)ethyl)carbamate (94). A stirred solution of **93** (30 mg, 0.040 mmol) in MeOH (4 mL) was treated with 1M NaOH (120 µL, 0.120 mmol) and heated to reflux for 4 h. under N_2 until completions was observed by LC-MS. This was cooled, treated with 1M HCl (120 µL, 0.120 mmol), diluted with toluene (25 mL) and concentrated *in vacuo* to 1/5th volume; this was repeated 3 times before concentrating to dryness to afford a yellow solid. This was dissolved in DMF (2 mL) treated with DIPEA (35 µL) and 5-aminotetrazole monohydrate (25 mg, 0.240 mmol) followed by PyBroP® (28 mg, 0.60 mmol) and stirred at rt for 4 h. until completion was observed by LC-MS. The RM was quenched with water (ca. 2 drops) and concentrated *in vacuo* azeotroping DMF with toluene (3 x 50 mL) to afford an orange oil. This was purified by FC washing with 10% MeOH/DCM before eluting off the desired compound with 10% MeOH/DCM w. 1% acetic acid affording the title compound **94** (9 mg, 0.011 mmol, 28%). LC-MS (ESI+) Rt: 2.68 min (254 nm, Method 1); (m/z): 797.4 $[M(^{35}Cl)+H]^+$. H.MS-TOF (ESI-) (*m/z*): $[M-H]^-$ calcd. for $C_{32}H_{32}ClN_{12}O_5S_3$, 795.1475; found, 795.1437. ^1H NMR (500 MHz, DMSO-d_6) δ: 12.92-12.78

(br.s, 1H), 8.53-8.43 (br.s, 1H), 7.99-7.89 (s, 2H), 7.74-7.68 (br.s, 1H), 7.58 (d, *J* = 8.4 Hz, 1H), 7.40 (d, *J* = 2.2 Hz, 1H), 7.35 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.78-6.76 (m, 2H), 5.68 (s, 1H), 5.40 (d, *J* = 15.9 Hz, 1H), 5.29 (d, *J* = 15.9 Hz, 1H), 4.12-4.09 (m, 2H), 3.41 (app.q, *J* = 6.5 Hz, 2H), 3.06-3.03 (m, 2H), 2.99-2.94 (m, 2H), 1.36-1.21 (m, 9H).

(E)-2-((5-(7-Chloro-2-((3-((2-(2-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)vinyl)phenoxy)acetamido)ethyl)amino)-3-oxopropyl)amino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-N-(1H-tetrazol-5-yl)thiazole-4-carboxamide (95). Following General Procedure 3, **94** was converted to BODIPY®630/650 conjugate **95**. This was purified using preparative RP-HPLC (Method 4) isolating **95** at Rt: 10.80 min which was freeze dried to a blue iridescent solid (0.35 mg, 0.31 μmol, 16%). LC-MS (ESI+) Rt: 3.00 min (254 nm, Method 1); (m/z): 1129.5 $[M(^{35}Cl)+H]^+$. H.MS-TOF (ESI+) (*m/z*): $[M+H]^+$ calcd. for $C_{50}H_{41}BClF_2N_{14}O_5S_4$, 1129.2011; found, 1129.2000. $[M+Na]^+$ calcd. for $C_{50}H_{40}BClF_2N_{14}NaO_5S_4$, 1151.1831; found, 1151.1823. H.MS-TOF (ESI-) (*m/z*): $[M-H]^-$ calcd. for $C_{50}H_{39}BClF_2N_{14}O_5S_4$, 1127.1866; found, 1127.1818.

(E)-2-((5-(7-Chloro-2-((3-((2-(3-(5,5-difluoro-7,9-dimethyl-5H-5λ^4,6λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)propanamido)ethyl)amino)-3-oxopropyl)amino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-N-(1H-tetrazol-5-yl)thiazole-4-carboxamide (96). Following General Procedure 3, **94** was converted to BODIPY®FL conjugate **96**. This was purified using preparative RP-HPLC (Method 3) isolating **96** at Rt: 12.05 min which was freeze dried to a red/green iridescent solid (0.21 mg, 0.22 μmol, 11%). LC-MS (ESI+) Rt: 2.76 min (254 nm, Method 1); (m/z): 971.4

[M(^{35}Cl)+H]$^+$. H.MS-TOF (ESI+) (*m/z*): [M+H]$^+$ calcd. for $C_{41}H_{39}BClF_2N_{14}O_4S_3$, 971.2185; found, 971.2189. [M+Na]$^+$ calcd. for $C_{41}H_{38}BClF_2N_{14}NaO_4S_3$, 993.2004; found, 993.2002.

(E)-2-((5-(7-Chloro-2-((3-((2-(6-(2-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)vinyl)phenoxy)acetamido)hexanamido)ethyl)amino)-3-oxopropyl)amino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-N-(1H-tetrazol-5-yl)thiazole-4-carboxamide (97). Following General Procedure 3, **94** was converted to BODIPY®630/650-X conjugate **97**. This was purified using preparative RP-HPLC (Method 4) isolating **97** at Rt: 11.28 min which was freeze dried to a blue iridescent solid (0.44 mg, 0.35 μmol, 18%). LC-MS (ESI+) Rt: 2.99 min (254 nm, Method 1); (m/z): 1242.4 [M(^{35}Cl)+H]$^+$. H.MS-TOF (ESI+) (*m/z*): [M+H]$^+$ calcd. for $C_{56}H_{52}BClF_2N_{15}O_6S_4$, 1242.2852; found, 1242.2858. [M+Na]$^+$ calcd. for $C_{50}H_{40}BClF_2N_{14}NaO_5S_4$, 1264.2671; found, 1264.2624.

(E)-2-((5-(7-Chloro-2-((3-((2-(6-(3-(5,5-difluoro-7,9-dimethyl-5H-5λ^4,6λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)propanamido)hexanamido)ethyl)amino)-3-oxopropyl)amino)-4H-benzo[5,6]cyclohepta[1,2-d]thiazol-4-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-N-(1H-tetrazol-5-yl)thiazole-4-carboxamide (98). Following General Procedure 3, **94** was converted to BODIPY®FL-X conjugate **98**. This was purified using preparative RP-HPLC (Method 4) isolating **94** at Rt: 12.69 min which was freeze dried to a red/green iridescent solid (0.35 mg, 0.31 μmol, 18%). LC-MS (ESI+) Rt: 2.75 min (254 nm, Method 1); (m/z): 1084.6 [M(^{35}Cl)+H]$^+$. H.MS-TOF (ESI+) (*m/z*): [M+H]$^+$ calcd. for $C_{47}H_{50}BClF_2N_{15}O_5S_3$, 1084.3026; found, 1085.3014. [M+Na]$^+$ calcd. for $C_{47}H_{49}BClF_2N_{15}O_5S_3$, 1106.2845; found, 1128.2825. H.MS-TOF (ESI-) (*m/z*): [M-H]$^-$ calcd. for $C_{47}H_{48}BClF_2N_{15}O_5S_3$, 1082.2880; found, 1082.2839.

General Pharmacology Methods:

cDNA constructs

To create Nluc-P2Y$_2$R constructs, human P2Y$_2$R DNA (obtained from the Missouri S&T cDNA Resource Centre, Bloomsberg, PA), was amplified by PCR to remove the methionine start signal and cloned into pCR®2.1 (linearized vector; Invitrogen). The P2Y$_2$R was then sub-cloned in-frame with the membrane signal sequence of the 5HT$_{3A}$ receptor and the full length sequence of Nluc luciferase. The NanoLuc-tagged receptors expressed in 1321N1 cells exhibited normal calcium signals (EC_{50} for UTPγS of 91 ± 12 nM; *n=3*).

Cell culture and cell line generation

1321N1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS H.I.) and 2 mM *L*-glutamine at 37°C, 5% CO_2. Mixed-population Nluc-P2Y2R-1321N1 cell lines were generated by transfecting Nluc–P2Y$_2$R construct using Lipofectamine 2000 (Life Technologies) according to the manufacturer's instructions and then subjecting the cells to selective pressure (1 mg/mL G418) for 2–3 weeks. The mixed cell population was then dilution-cloned to obtain cell lines originating from a single cell. Screening for active clones was initially performed using the calcium mobilization assay, followed by detection of luminescence on addition of the Nluc substrate furimazine. On this basis, a single active cloned cell line was selected for use in all NanoBRET assays. 1321N1 cells expressing wild-type P2Y$_2$R (P2Y$_2$R-1321N1) were gifted by Dr Elizabeth Rosethorne, University of Nottingham.

Calcium mobilization assay

$P2Y_2$-1321N1 cells seeded into black-sided 96-well view plates were incubated at 37°C (no CO2) for 45 min in a total volume of 100 μl of HEPES buffered saline solution (HBSS; 10 mM HEPES, 10 mM glucose, 145 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 2 mM sodium pyruvate, and 1.3 mM $CaCl_2$) containing 2.5 mM probenecid, 2.3 μM Fluo 4AM, 0.023% pluronic acid, 0.5mM Brilliant Black, 1U/ml apyrase and the ligand under investigation or vehicle. Plates were then loaded onto a plate reader (FLEXstation; Molecular Devices, Sunnyvale, CA) and fluorescence measured (excitation, 485 nm; emission, 525 nm) every 1.52 s for up to 200 s with the addition of UTPγS at 15 s. For investigation of BODIPY®FL-labelled ligands, the Fluo 4AM was replaced with 2.0 μM X-Rhod-1 AM (excitation, 584 nm; emission, 612 nm).

NanoBRET assay

Saturation and competition binding assays were performed according to the methodology of Stoddart.[18] Briefly, assays were performed on stably transfected Nluc$P2Y_2$-1321N1 cells that were seeded 24 h prior to experimentation in white Thermo Scientific Matrix 96-well microplates. Medium from each well was removed and replaced with HBSS containing apyrase (1U/mL) and the required concentration of fluorescent ligand with or without competing ligand. Upon the addition of fluorescent ligand, cells were incubated for 1 h at 37°C (no CO_2). Nluc substrate furimazine (Promega) was then added to a final concentration of 10 μM and the plate incubated for a further 5 min 37°C (no CO_2). Luminescence and resulting BRET were measured using the PHERAstar FS plate reader (BMG Labtech) at room temperature. Sequential measurements of filtered light emissions were made at 460 nm (80-nm bandpass) and >610 nm (longpass) and the raw BRET ratio calculated by dividing the >610-nm emission by the 460-nm emission (**97**). For assays involving **98**, measurements were made at 475 nm (30-nm bandpass) and 535 nm (30-nm

bandpass) and the raw BRET ratio calculated by dividing the 535-nm emission by the 475-nm emission.

Confocal microscopy

$P2Y_2R$-1321N1 cells were grown to approximately 80% confluency on 8-well Labtek chambered cover glasses (Nunc Nalgene) in normal growth medium. Growth medium was removed and replaced with HBSS containing apyrase (1U/ml) and either **1** (10μM) or vehicle and the cells incubated for 30 min at 37°C (no CO_2). Cells were then incubated with **97** or **98** at the required concentration for 10 min at room temperature, prior to collection of single equatorial confocal images. Images were obtained on a Zeiss LSM710 confocal microscope using a 40× c-Apochromat 1.2NA water-immersion objective. For **97**, images were collected using 633 nm excitation, a 488/561/633 dichroic and emission collected through a 650LP filter. For **98**, 488 nm excitation was used with the same dichroic, with emission collected using a LP575 filter. In each case, a pinhole diameter of 1 Airy Unit was used, and laser power, gain and offset were kept the same for samples within each experiment. In both cases, images are presented as representative of an individual experiment with matched conditions. Linear adjustments to image brightness and contrast have been applied equally across all comparative images using Zen software to prepare images for presentation.

Data analysis

All data were analyzed using GraphPad Prism 6.

For the calcium mobilization experiments as none of the compounds synthesized as part of this study showed any partial agonist action, estimated affinity values (pK_B) were calculated from the

shift of the agonist concentration response curves in the presence of the fluorescent antagonists using equation 1:

$$DR = 1 + \frac{[B]}{K_B} \quad (1)$$

Where DR (dose ratio) is the ratio of the agonist concentration required to stimulate an identical response in the presence and absence of antagonist, [B]. pK_B is –log K_B.

Total and non-specific saturation binding curves were fitted simultaneously using equation 2:

$$BRET\ Ratio = \frac{B_{max} \times [B]}{[B] + K_d} + \left((M \times [B]) + C\right) \quad (2)$$

where B_{max} is the maximal specific BRET signal, [B] is the concentration of fluorescent ligand in nM, K_d is the equilibrium dissociation constant in nM, M is the slope of the non-specific binding component and C is the intercept with the Y-axis. pK_d is – logK_d.

The competition binding curves were fitted using equation 3:

$$\boldsymbol{K_i = \frac{IC_{50}}{1 + \frac{[L]}{K_d}}} \quad (3)$$

where [L] is the concentration of **98** in nM and K_d is the equilibrium dissociation constant of **98** in nM. The IC_{50} is calculated as in equation 4:

$$\%\ inhibition\ of\ specific\ binding = \frac{100 \times [A]}{[A] + IC_{50}} \quad (4)$$

where [A] is the concentration of unlabelled competing drug and IC_{50} is the molar concentration of this competing ligand required to inhibit 50% of the specific binding of the concentration [L] of the labelled ligand.

ASSOCIATED CONTENT

The following files are available free of charge. Molecular Formula Strings (CSV)

Description of VCD structure determination and figure showing binding of BODIPY-630/650-labelled (**78-84**) and BODIPY-FL-labelled (**85-91**) ligands in NLuc-$P2Y_2$-1321N1 cells using the NanoBRET assay (10μM test compound) (pdf)

AUTHOR INFORMATION

Corresponding Author

*Corresponding Author Information: michael.stocks@nottingham.ac.uk. Tel +44 (0)115 951 5151

Author Contributions:

Conceived the study: Hill, Kellam and Stocks.

Managed the project: Hill, Kellam and Stocks.

Chemical synthesis: Conroy, Kindon and Stocks.

VCD structural determination: Lewis

Participated in research design: Hill, Stoddart, Conroy, Kindon, Kellam and Stocks.

Conducted pharmacology experiments: Glenn.

Performed pharmacology data analysis: Glenn, Stoddart and Hill.

Wrote or contributed to the writing of the manuscript: All authors.

Competing Financial Interests

The authors declare no competing financial interests.

Funding Sources

This work was supported by the UK Medical Research Council [grant numbers MR/L016389/1 and MR/N020081/1] and a MRC-funded research studentship Sean Conroy (MRC-funded PhD studentship ref. number 1365529).

ACKNOWLEDGMENT

The authors thank Dr Elizabeth Rosethorne for supply of the $P2Y_2$-1321N1 astrocytoma cells, Mr Nickolaj Groenewoud for assistance with molecular biology and Miss Seema Rajani for assistance with the confocal microscopy work.

ABBREVIATIONS

ATP (adenosine 5'-triphosphate); bodipy (boron-dipyrromethene); BRET (bioluminescence resonance energy transfer); GPCR (G protein-coupled receptor); K_d (dissociation constant of a labelled ligand-receptor complex); K_i (dissociation constant of a ligand-receptor complex determined through a binding assay); NanoBRET (Nano luciferase bioluminescence resonance

energy transfer); NLuc (Nano luciferase); SAR (structure activity relationship); UTPγS (uridine-5'-(g-thio)-triphosphate); VCD (vibrational circular dichroism).

REFERENCES

(1) Abbracchio, M. P. International Union of Pharmacology LVIII: Update on the P2Y G Protein-Coupled Nucleotide Receptors: From Molecular Mechanisms and Pathophysiology to Therapy. *Pharmacol. Rev.* **2006**, *58* (3), 281–341.

(2) Burnstock, G.; Kennedy, C. Is There a Basis for Distinguishing Two Types of P2-Purinoceptor? *Gen. Pharmacol.* **1985**, *16* (5), 433–440.

(3) Lazarowski, E. R.; Watt, W. C.; Stutts, M. J.; Boucher, R. C.; Harden, T. K. Pharmacological Selectivity of the Cloned Human P2U-Purinoceptor: Potent Activation by Diadenosine Tetraphosphate. *Br. J. Pharmacol.* **1995**, *116* (1), 1619–1627.

(4) Conroy, S.; Kindon, N.; Kellam, B.; Stocks, M. J. Drug-like Antagonists of P2Y Receptors-From Lead Identification to Drug Development. *J. Med. Chem.* **2016**, *59* (22), 9981–10005.

(5) Schumacher, D.; Strilic, B.; Sivaraj, K. K.; Wettschureck, N.; Offermanns, S. Platelet-Derived Nucleotides Promote Tumor-Cell Transendothelial Migration and Metastasis *via* $P2Y_2$ Receptor. *Cancer Cell* **2013**, *24* (1), 130–137.

(6) Di Virgilio, F.; Falzoni, S.; Giuliani, A. L.; Adinolfi, E. P2 Receptors in Cancer Progression and Metastatic Spreading. *Curr. Opin. Pharmacol.* **2016**, *29*, 17–25.

(7) Müller, T.; Fay, S.; Vieira, R. P.; Karmouty-Quintana, H.; Cicko, S.; Ayata, K.; Zissel, G.; Goldmann, T.; Lungarella, G.; Ferrari, D.; Virgilio, F. Di; Robaye, B.; Boeynaems, J.-M.;

Blackburn, M. R.; Idzko, M. The Purinergic Receptor Subtype $P2Y_2$ Mediates Chemotaxis of Neutrophils and Fibroblasts in Fibrotic Lung Disease. *Oncotarget* **2017**, *8* (22), 35962–35972.

(8) Kindon, N.; Davis, A.; Dougall, I.; Dixon, J.; Johnson, T.; Walters, I.; Thom, S.; McKechnie, K.; Meghani, P.; Stocks, M. J. From UTP to AR-C118925, the Discovery of a Potent Non Nucleotide Antagonist of the $P2Y_2$ Receptor. *Bioorg. Med. Chem. Lett.* **2017**, *27* (21), 4849–4853.

(9) Kindon, N. D.; Meghani, P.; Thom, S. Preparation of 2-oxo-4-thioxopyrimidin-1-ylmethylheterocyclylcarboxylates as P2-purinoceptor 7-transmembrane G-protein Coupled Receptor Antagonists. WO9854180, **1998**.

(10) Kemp, P. A.; Sugar, R. A.; Jackson, A. D. Nucleotide-Mediated Mucin Secretion from Differentiated Human Bronchial Epithelial Cells. *Am. J. Respir. Cell Mol. Biol.* **2004**, *31* (4), 446–455.

(11) Rafehi, M.; Burbiel, J. C.; Attah, I. Y.; Abdelrahman, A.; Müller, C. E. Synthesis, Characterization, and in Vitro Evaluation of the Selective $P2Y_2$ Receptor Antagonist AR-C118925. *Purinergic Signal.* **2017**, *13* (1), 89–103.

(12) Jacobson, K. A.; Ivanov, A. A.; de Castro, S.; Harden, T. K.; Ko, H. Development of Selective Agonists and Antagonists of P2Y Receptors. *Purinergic Signal.* **2009**, *5* (1), 75–89.

(13) Jayasekara, P. S.; Barrett, M. O.; Ball, C. B.; Brown, K. A.; Hammes, E.; Balasubramanian, R.; Harden, T. K.; Jacobson, K. A. 4-Alkyloxyimino Derivatives of Uridine-5′-

Triphosphate: Distal Modification of Potent Agonists as a Strategy for Molecular Probes of $P2Y_2$, $P2Y_4$, and $P2Y_6$ Receptors. *J. Med. Chem.* **2014**, *57* (9), 3874–3883.

(14) Böhme, I.; Beck-Sickinger, A. G. Illuminating the Life of GPCRs. *Cell Commun. Signal.* **2009**, *7* (1), 16.

(15) Ma, Z.; Du, L.; Li, M. Toward Fluorescent Probes for G-Protein-Coupled Receptors (GPCRs). *J. Med. Chem.* **2014**, *57* (20), 8187–8203.

(16) Sridharan, R.; Zuber, J.; Connelly, S. M.; Mathew, E.; Dumont, M. E. Fluorescent Approaches for Understanding Interactions of Ligands with G Protein Coupled Receptors. *Biochim. Biophys. Acta - Biomembr.* **2014**, *1838* (1), 15–33.

(17) Cottet, M.; Faklaris, O.; Zwier, J. M.; Trinquet, E.; Pin, J.-P.; Durroux, T. Original Fluorescent Ligand-Based Assays Open New Perspectives in G-Protein Coupled Receptor Drug Screening. *Pharmaceuticals* **2011**, *4* (12), 202–214.

(18) Stoddart, L. A.; Johnstone, E. K. M.; Wheal, A. J.; Goulding, J.; Robers, M. B.; Machleidt, T.; Wood, K. V; Hill, S. J.; Pfleger, K. D. G. Application of BRET to Monitor Ligand Binding to GPCRs. *Nat. Methods* **2015**, *12* (7), 661–663.

(19) Hansen, A. H.; Sergeev, E.; Pandey, S. K.; Hudson, B. D.; Christiansen, E.; Milligan, G.; Ulven, T. Development and Characterization of a Fluorescent Tracer for the Free Fatty Acid Receptor 2 (FFA2/GPR43). *J. Med. Chem.* **2017**, *60* (13), 5638–5645.

(20) Instant JChem Was Used for Structure Database Management, Search and Prediction, Instant JChem 16.2.15.0 2016, ChemAxon Http://www.chemaxon.com (Accessed

November 08 2017).

(21) Kindon, N. D.; Meghani, P.; Thom, S. Preparation of Pyrimidinediones and Thioxopyrimidinones as P2-purinoceptor 7-transmembrane (TM) G-protein Coupled Receptor Antagonists. WO9926944, **1999**.

(22) He, Y.; Bo, W.; Dukor, R. K.; Nafie, L. A. Determination of Absolute Configuration of Chiral Molecules Using Vibrational Optical Activity: A Review. *Appl. Spectrosc.* **2011**, *65* (7), 699–723.

(23) Yaguchi, Y.; Nakahashi, A.; Miura, N.; Taniguchi, T.; Sugimoto, D.; Emura, M.; Zaizen, K.; Kusano, Y.; Monde, K. Vibrational CD (VCD) Spectroscopy as a Powerful Tool for Chiral Analysis of Flavor Compounds. ACS Symposium Series Volume1212 *Importance of Chirality to Flavor Compounds*. January 1, **2015** , 35-56. DOI:10.1021/bk-2015-1212.ch003.

(24) Izumi, H.; Ogata, A.; Nafie, L. A.; Dukor, R. K. Structural Determination of Molecular Stereochemistry Using VCD Spectroscopy and a Conformational Code: Absolute Configuration and Solution Conformation of a Chiral Liquid Pesticide, (R)-(+)-Malathion. *Chirality* **2009**, *21* (1E), E172–E180.

(25) Patel, Y.; Gillet, V. J.; Howe, T.; Pastor, J.; Oyarzabal, J.; Willett, P. Assessment of Additive/Nonadditive Effects in Structure−Activity Relationships: Implications for Iterative Drug Design. *J. Med. Chem.* **2008**, *51* (23), 7552–7562.

(26) Khatuya, H.; Hutchings, R. H.; Kuo, G.-H.; Pulito, V. L.; Jolliffe, L. K.; Li, X.; Murray, W. V. Arylpiperazine Substituted Heterocycles as Selective α1a Adrenergic Antagonists.

Bioorg. Med. Chem. Lett. **2002**, *12* (17), 2443–2446.

(27) Machleidt, T.; Woodroofe, C. C.; Schwinn, M. K.; Méndez, J.; Robers, M. B.; Zimmerman, K.; Otto, P.; Daniels, D. L.; Kirkland, T. A.; Wood, K. V. NanoBRET - A Novel BRET Platform for the Analysis of Protein-Protein Interactions. *ACS Chem. Biol.* **2015**, *10* (8), 1797–1804.

(28) Christiansen, E.; Hudson, B. D.; Hansen, A. H.; Milligan, G.; Ulven, T. Development and Characterization of a Potent Free Fatty Acid Receptor 1 (FFA1) Fluorescent Tracer. *J. Med. Chem.* **2016**, *59* (10), 4849–4858.

(29) Lohse, M. J.; Nuber, S.; Hoffmann, C. Fluorescence/Bioluminescence Resonance Energy Transfer Techniques to Study G-Protein-Coupled Receptor Activation and Signaling. *Pharmacol. Rev.* **2012**, *64* (2), 299–336.

(30) Vernall, A. J.; Stoddart, L. A.; Briddon, S. J.; Ng, H. W.; Laughton, C. A.; Doughty, S. W.; Hill, S. J.; Kellam, B. Conversion of a Non-Selective Adenosine Receptor Antagonist into A3-Selective High Affinity Fluorescent Probes Using Peptide-Based Linkers. *Org. Biomol. Chem.* **2013**, *11* (34), 5673.

Table of contents graphic