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# Synthesis and Evaluation of the First Fluorescent Antagonists of the Human P2Y$_2$ Receptor Based on AR-C118925

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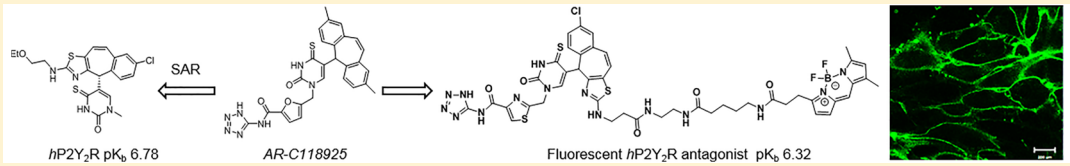
Supporting Information




**ABSTRACT:** The human P2Y$_2$ receptor (*h*P2Y$_2$R) is a G-protein-coupled receptor that shows promise as a therapeutic target for many important conditions, including for antimetastatic cancer and more recently for idiopathic pulmonary fibrosis. As such, there is a need for new *h*P2Y$_2$R antagonists and molecular probes to study this receptor. Herein, we report the development of a new series of non-nucleotide *h*P2Y$_2$R antagonists, based on the known, non-nucleotide *h*P2Y$_2$R antagonist AR-C118925 (**1**), leading to the discovery of a series of fluorescent ligands containing different linkers and fluorophores. One of these conjugates, **98**, displayed micromolar affinity for *h*P2Y$_2$R (p$K_d$ = 6.32 ± 0.10, $n$ = 17) in a bioluminescence-energy-transfer (BRET) assay. Confocal microscopy with this ligand revealed displaceable membrane labeling of astrocytoma cells expressing untagged *h*P2Y$_2$R. These properties make **98** one of the first tools for studying *h*P2Y$_2$R 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 on the basis of their primary signaling through specific G proteins[1] and sequence homology. The first subgroup includes the P2Y$_{1,2,4,6,11}$ receptors, which primarily signal though G$_q$, whereas the second subgroup, signaling through G$_i$, encompasses the P2Y$_{12,13,14}$ receptor subtypes.[2] Notably, the P2Y$_2$ receptor (P2Y$_2$R) 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 P2Y$_2$R is predominately G$_q$-coupled, receptor activation leads to the stimulation of phospholipase C, IP$_3$ release, and the elevation of the intracellular $Ca^{2+}$ concentration, as well as the initiation of protein kinase C and the activation of the mitogen-activated protein-kinase cascade.

Defining the clinical role for P2Y$_2$R antagonism has been hampered by the lack of high-affinity and druglike 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 the migration of tumor cells and hence cancer proliferation. More importantly, P2Y$_2$R was identified as the primary mediator of this effect; a strong reduction of tumor cell metastasis was observed in P2Y$_2$R-deficient mice, revealing a therapeutic potential of P2Y$_2$R antagonists as antimetastatic agents.[5,6] Recently, it has been reported that both inflammation and fibrosis were reduced in P2Y$_2$R-deficient mice compared with those in wild-type animals. In addition, mechanistic studies have demonstrated that the recruitment of neutrophils into the lungs, the proliferation and migration of lung fibroblasts, and IL-6 production are all key P2Y$_2$R-mediated processes. These studies clearly demonstrate the involvement of P2Y$_2$R subtypes in the pathogenesis of fibrotic lung diseases in humans and mice and support the development of selective P2Y$_2$R antagonists for the treatment of idiopathic pulmonary fibrosis (IPF).[7] To date, the only reported high-affinity P2Y$_2$R antagonists were those developed by scientists from AstraZeneca resulting in the non-nucleotide P2Y$_2$R antagonist AR-C118925 (**1**).[8,9]

Several in vivo and ex vivo studies using **1** have been reported that further validate the therapeutic benefits of P2Y$_2$R antagonists. Importantly, it was shown that **1**, which was reported to be inactive at 10 μM against a panel of 37 other

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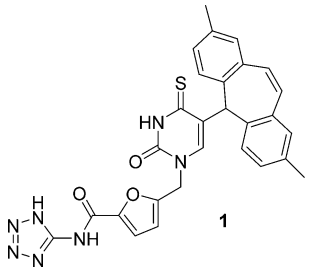


1

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 $P2Y_2R$.[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 scientists 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 the 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 nanoluciferase (NLuc), to quantify ligand−receptor interactions and determine the affinities 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 affinities, it is proposed that the fluorescent ligands designed from these might also have affinities in this range. This may prove problematic for techniques which directly monitor fluorescent-ligand binding, but NanoBRET has been shown to display low, nonspecific binding at high fluorescent-ligand concentrations.[18,19]

## RESULTS AND DISCUSSION

**Synthesis and Evaluation of the *h*$P2Y_2R$ Antagonists.** The medicinal-chemistry strategy involved an initial exploration of the structure−activity relationship (SAR) around **1** in order to enable the design of structural analogues with improved predicted physicochemical properties and to guide our design strategy by highlighting suitable linking sites to attach the fluorophore groups (Figure 1 and Table 1).

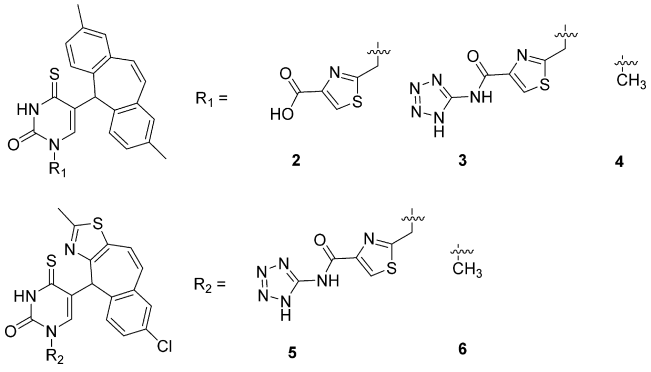



**Figure 1.** SARs for the $P2Y_2R$ antagonists showing the change from the furan in **1** to thiazole (reduced lipophilicity) and the change from 2,8-dimethyl-5*H*-dibenzo[*a*,*d*][7]annulene to the 7-chloro-4*H*-benzo-[5,6]cyclohepta[1,2-*d*]thiazole tricyclic ring system.

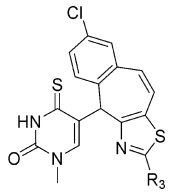
**Table 1. Estimated Affinity Values for $P2Y_2R$ Antagonists 1−6 Obtained from the Calcium-Mobilization Assay**

| example | *h*$P2Y_2$ $pK_b$[a] | example | *h*$P2Y_2$ $pK_b$[a] |
|---|---|---|---|
| **1** | 7.51 ± 0.09 (12) | **4** | IA[b] |
| **2**[c] | 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 using the Gaddum equation from the shift of the UTPγS concentration−response curve brought about by the addition of a single concentration of the antagonist. The data shown are the means ± SEM, and the numbers of separate experiments are given in parentheses. [b]IA = inactive; i.e., less than a 50% inhibition of the response to 0.1 μM UTPγS in the presence of a 10 μM concentration of the compound. UTPγS $EC_{50}$ = 7.9 ± 1.3 nM ($n$ = 25). [c]The literature value is $pA_2$ = 5.7.[8]

**1** has previously been shown to have a 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 $P2Y_2R$. Replacement of the 2,8-dimethyl-5*H*-dibenzo[*a*,*d*][7]annulene with a 7-chloro-4*H*-benzo[5,6]cyclohepta[1,2-*d*]thiazole tricyclic ring gave **5** ($pK_b$ = 6.5, cLogP = 3.4).[21] In addition, a nonparallel SAR with $P2Y_2R$ affinity was observed in the replacement of the *N*-1 thiouracil substituent with a methyl group, which gave compound **6**, whereas the corresponding analogue, **4**, proved inactive. In order to explore this intriguing finding and study this SAR, we synthesized a range of analogues of **6** (Table 2).

**Table 2. Calcium-Mobilization Activities for the $P2Y_2R$ Antagonists 6−19**



| example | $R_3$ | *h*$P2Y_2$ $pK_b$[a] |
|---|---|---|
| **7** | $NH_2$ | 6.56 ± 0.14 (4) |
| **8** | PhNH | IA[b] (3) |
| **9** | $PhCH_2NH$ | IA (3) |
| **10** | $PhCH_2CH_2NH$ | IA (3) |
| **11** | 1-methyl piperazin-4-yl | IA (3) |
| **12** | morpholinyl | IA (3) |
| **13** | piperidinyl | IA (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-isopropoxyethan-1-aminyl | 6.49 ± 0.09 (3) |
| **18** | 2-phenoxyethan-1-aminyl | IA (3) |
| **19** | phenyl | IA (3) |

[a]The estimated affinity value for each antagonist ($pK_b$) was calculated using the Gaddum equation from the shift of the UTPγS concentration−response curve brought about by the addition of a single concentration of the antagonist. The data shown are the means ± SEM, and the numbers of separate experiments are given in parentheses. [b]IA = inactive; i.e., less than a 50% inhibition of the response to 0.1 μM UTPγS in the presence of a 10 μM concentration of the compound ($n$ = 3).

From the SAR study of **6**, it was shown that replacing the thiazole 2-methyl substituent with an amino group increased *h*$P2Y_2R$ affinity (compare **6** with **7**), whereas a sterically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demanding substituent, such as that in **19**, resulted in a complete loss of affinity for $h$P2Y$_2$R. We therefore explored the substitution of the amino group and showed that both compounds with sterically demanding amino groups (**8**, **9**, and **10**) and compounds with cyclic tertiary amines (**11**, **12**, and **13**) were inactive. However, the linear, less sterically demanding alkyl amino groups, such as those in **14**, **15**, **16**, and **17**, increased $h$P2Y$_2$R affinity, although the bulkier 2-phenoxyethan-1-amino substituent in **18** resulted in the compound being inactive.

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

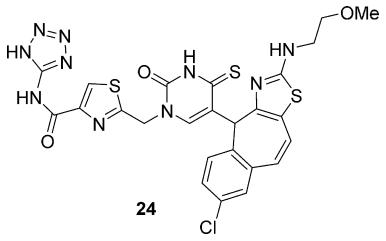
**Table 3. Calcium-Mobilization Activities for the Resolved Enantiomers of 14 and 16**

| example | racemic compound | enantiomeric excess (ee)[a] | $h$P2Y$_2$ p$K_b$[b] |
|---|---|---|---|
| **20** | **14** | 99% | 6.63 ± 0.11 (6) |
| **21** | | 78% | 5.82 ± 0.05 (3) |
| **22** | **16** | 95% | IA[c] (3) |
| **23** | | 96% | 6.78 ± 0.05 (6) |

[a]The compounds were separated using Phenomenex's Lux 5 $\mu$m amylose-2 stationary phase. [b]The estimated affinity value for each antagonist (p$K_b$) was calculated using the Gaddum equation from the shift of the UTP$\gamma$S concentration−response curve brought about by the addition of a single concentration of the antagonist. The data shown are the means ± SEM, and the numbers of separate experiments are given in parentheses. [c]IA = inactive; i.e., less than a 50% inhibition of the response to 0.1 $\mu$M UTP$\gamma$S in the presence of a 10 $\mu$M concentration of the compound ($n$ = 3).

From these results, it is possible to see that the $h$P2Y$_2$R-antagonist affinities observed for the racemic compounds **14** and **16** reside largely in enantiomers **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 their absolute chiralities could not be used for structural determination. However, vibrational circular dichroism was used for **22** and **23**, from which spectra were acquired for both samples and fitted to the calculated spectra.[22−24] The results (Supporting Information) showed that there was a good match between the spectrum of **22** and the calculated spectrum for the (*S*)-enantiomer; therefore, **23** was assigned as the active (*R*)-enantiomer (Figure 2).

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

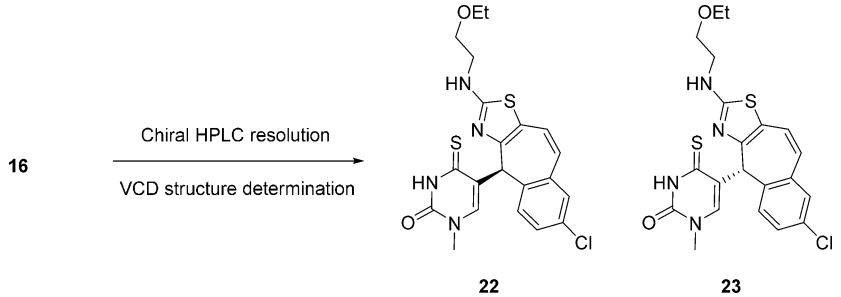



**24**

However, **24** did not demonstrate the expected increase in affinity from the combination of features from **14** and **5** and instead showed a level of $h$P2Y$_2$R affinity ( p$K_d$ = 7.02 ± 0.05, $n$ = 4) similar to those of **1** and **3**, demonstrating that the SARs within the series of compounds were nonadditive.[25]

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

Scheme 2 shows the synthetic route to compounds **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 compound was reacted with ethyl dichloroacetate in a Darzens condensation[25] to generate an $\alpha$-chloro epoxide, which was reacted directly with thioacetamide to afford the 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 the 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 compound underwent a 1,2-addition to the ketone, **29**, to give the tertiary alcohol, **32**. Concomitant deprotection and dehydration resulted in uracil, **33**, via 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, which give **34**. Finally, a 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




**22** **23**

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



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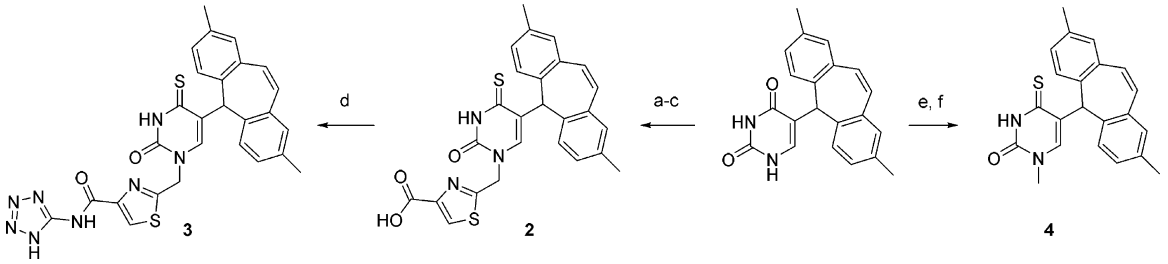
**Scheme 1. Synthesis of Compounds 2−4**[a]



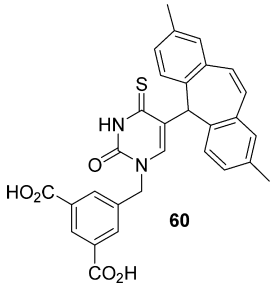

[a]Reagents and conditions: (a) (i) *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide, DCM, reflux, 18 h; (ii) ethyl 2-(bromomethyl)thiazole-4-carboxylate, 50 °C, 24 h (55%). (b) Lawesson's reagent, **1**, 4-dioxane, 100 °C, 18 h (85%). (c) NaOH, methanol/$H_2O$, reflux, 1 h (91%). (d) 5-Aminotetrazole, 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%).

followed by a reaction of the resulting carboxylic acid with 5-aminotetrazole and benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate to give **5**.

In a similar sequence to Scheme 2, aldehyde **26** was reacted with ethyl dichloroacetate, and the resulting crude $\alpha$-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 the carboxylic acid, **39**, which was converted to the acid chloride and cyclized to the tricyclic ketone, **40**. Lithiation of the *tert*-butyl ether-protected uracil, **31**, and a reaction with **40** gave the tertiary alcohol, **41**. After screening a range of milder acidic conditions, heating to 140 °C with microwave irradiation in acetic acid/1,4-dioxane (1:1) for 10 min 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 a nucleophilic aromatic substitution with a range of primary and secondary amines upon heating under basic conditions. These conditions were unsuccessful when aniline was used, and in this instance, heating in the microwave with hydrochloric acid (catalytic) proved successful in giving **46**. Microwave-based conditions were employed for the substitution with ammonia in the synthesis of **45**. A Suzuki reaction with phenylboronic acid gave **44**. Through the use of Lawesson's reagent, it was then possible to convert these uracil derivatives (**44**−**54**) to the respective 4-thiouracil derivatives (**6**−**19**). Through 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 *N*1-position with ethyl 2-(bromomethyl)thiazole-4-carboxylate to give **57**. This compound 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 a reaction with 5-aminotetrazole afforded **24**.

**Synthesis of the *h*P2Y$_2$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 via confocal microscopy, we embarked on a strategy to synthesize BODIPY conjugates, specifically with the dyes BODIPY A (628 nm absorption max, 642 nm emission max) or BODIPY B (503 nm absorption max, 509 nm emission max) as this would allow us the opportunity of ligand choice in future imaging work.

Two positions on the P2Y$_2$R-antagonist core structure were considered for the attachment of the linker and fluorophore (Figure 3). In order to simplify the synthetic chemistry and increase the SARs within the series, we examined the replacement of the furan ring of **1** and the thiazole ring of **2** with 1,3,5-trisubstituted phenyl rings. This would allow the attachment of the acyl tetrazole group in addition to providing a second free carboxylic acid group to which the fluorescent conjugates could be attached. Fortunately, the 3,5-dicarboxylic acid on **60** (*h*P2Y$_2$R p$K_d$ = 6.53 ± 0.04, $n$ = 7) was well tolerated with no loss of affinity for P2Y$_2$R compared with the affinity of compound **2**. Therefore, the first series of compounds had the linker fluorophore attached via the phenyl ring of **60**.




Having established that alkoxyalkyl amines are tolerated in terms of activity in the 2-position of the thiazole in the 4*H*-benzo[5,6]cyclohepta[1,2-*d*]thiazol-4-yl) tricyclic rings 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 were explored that contained the optimal second-generation fluorescent ligand with an incorporated acyl tetrazole functional group.

**First-Generation *h*P2Y$_2$R Fluorescent Ligands.** The general synthetic route for the first-generation P2Y$_2$R fluorescent antagonists is shown in Scheme 4.

Alkylation of 5-(2,8-dimethyl-5*H*-dibenzo[*a*,*d*][7]annulen-5-yl)pyrimidine-2,4(1*H*,3*H*)-dione[8] with dimethyl 5-(bromomethyl)isophthalate followed by a treatment with Lawesson's reagent and the selective hydrolysis of one of the methyl esters gave **61**. This carboxylic acid (**61**) was activated using benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate and coupled to form the appropriate amides (**62** and **63**). The second esters of **62** and **63** were then hydrolyzed and converted to amidotetrazoles (**64** and **65**). Finally, the Boc protecting group was removed, and the resulting amine was coupled to the fluorophore with the appropriate commercially available BODIPY succinimidyl ester to give a small library of four fluorescent conjugates (**66**−**69**).

To determine whether any of these conjugates had affinities for P2Y$_2$R and consequently if they could be used in



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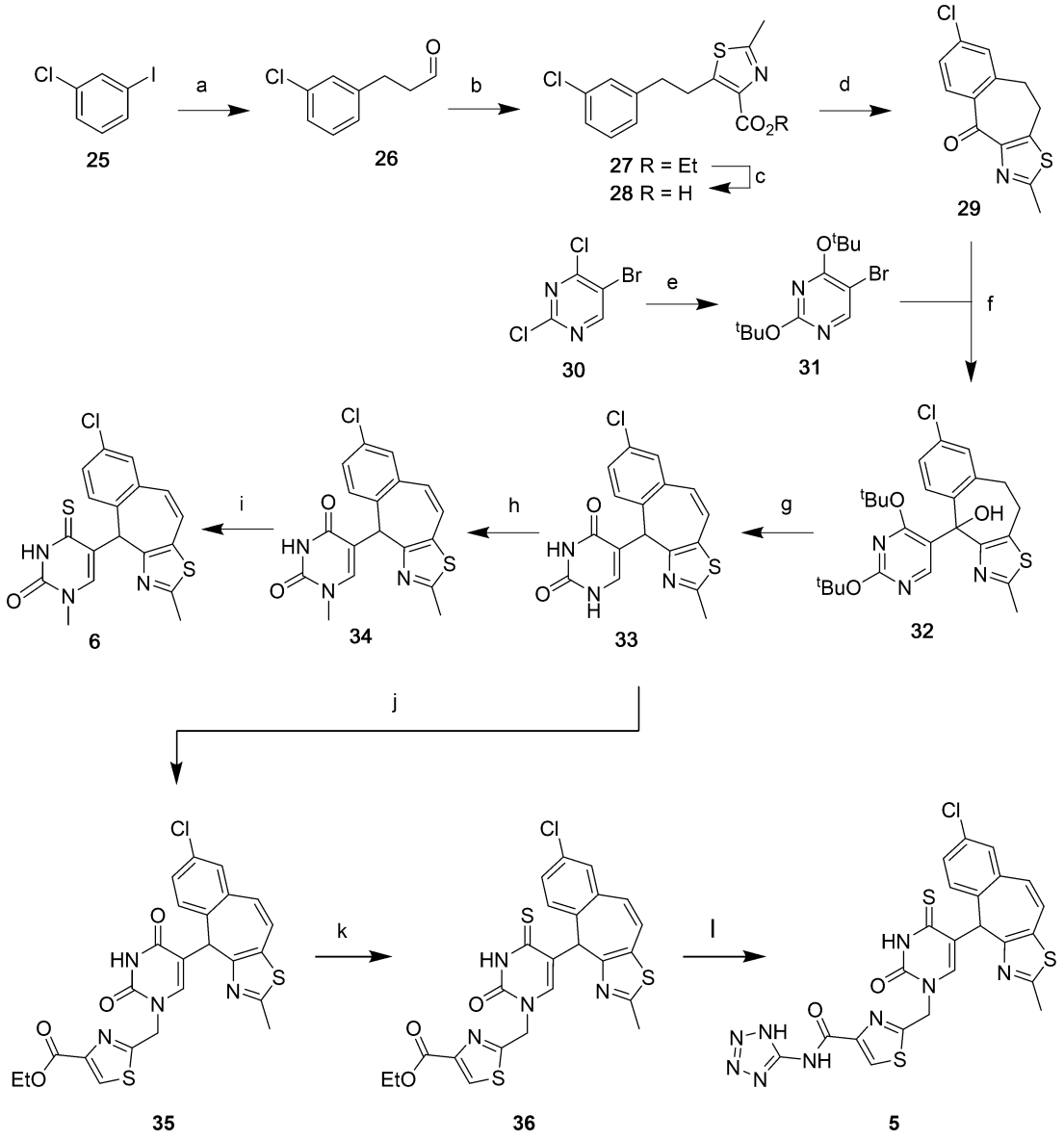
**Scheme 2. Synthesis of Compounds 5 and 6**[a]




[a]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 two steps). (c) NaOH, $H_2O$/THF (1:1), rt, 18 h (99%). (d) (i) Oxalyl chloride, catalytic DMF, DCM, rt, 3 h; (ii) aluminum(III)chloride, DCM, rt, 18 h (39% over two steps). (e) Sodium *tert*-butoxide, THF, 0 °C to rt, 18 h (61%). (f) (i) *n*-Butyllithium, THF, −78 °C, 30 min; (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%).

NanoBRET binding assays, a 1321N1 astrocytoma cell line expressing recombinant $P2Y_2R$ tagged on its N-terminus with NLuc (NLuc-$P2Y_2R$) was generated. The NLuc-tagged $P2Y_2$ receptors exhibited normal calcium signals (UTP$\gamma$S $EC_{50}$ = 91 ± 12 nM, $n$ = 3). These NLuc-$P2Y_2$ cells were treated with increasing concentrations of **66**−**69** and then treated with the NLuc substrate, furimazine, before the resulting BRET signal was monitored. All four compounds showed moderate to low affinities for the NLuc-$P2Y_2R$ (Table 4), with conjugates **68** and **69** having higher affinities. However, this did illustrate the power of using NanoBRET to monitor ligand binding to low-affinity receptors.

**Second-Generation *h*$P2Y_2R$ Fluorescent Ligands.** The general synthetic route for the second-generation $P2Y_2R$ fluorescent antagonists, in which the linker fluorophores are attached to the thiazole rings, is illustrated in Scheme 5.

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 100 °C resulted in the formation of the 4-thiouracils (**72** and **73**). The carboxylic acids were generated from the *tert*-butyl esters via TFA-mediated hydrolysis and subsequently coupled with *tert*-butyl (2-aminoethyl)carbamate or *tert*-butyl



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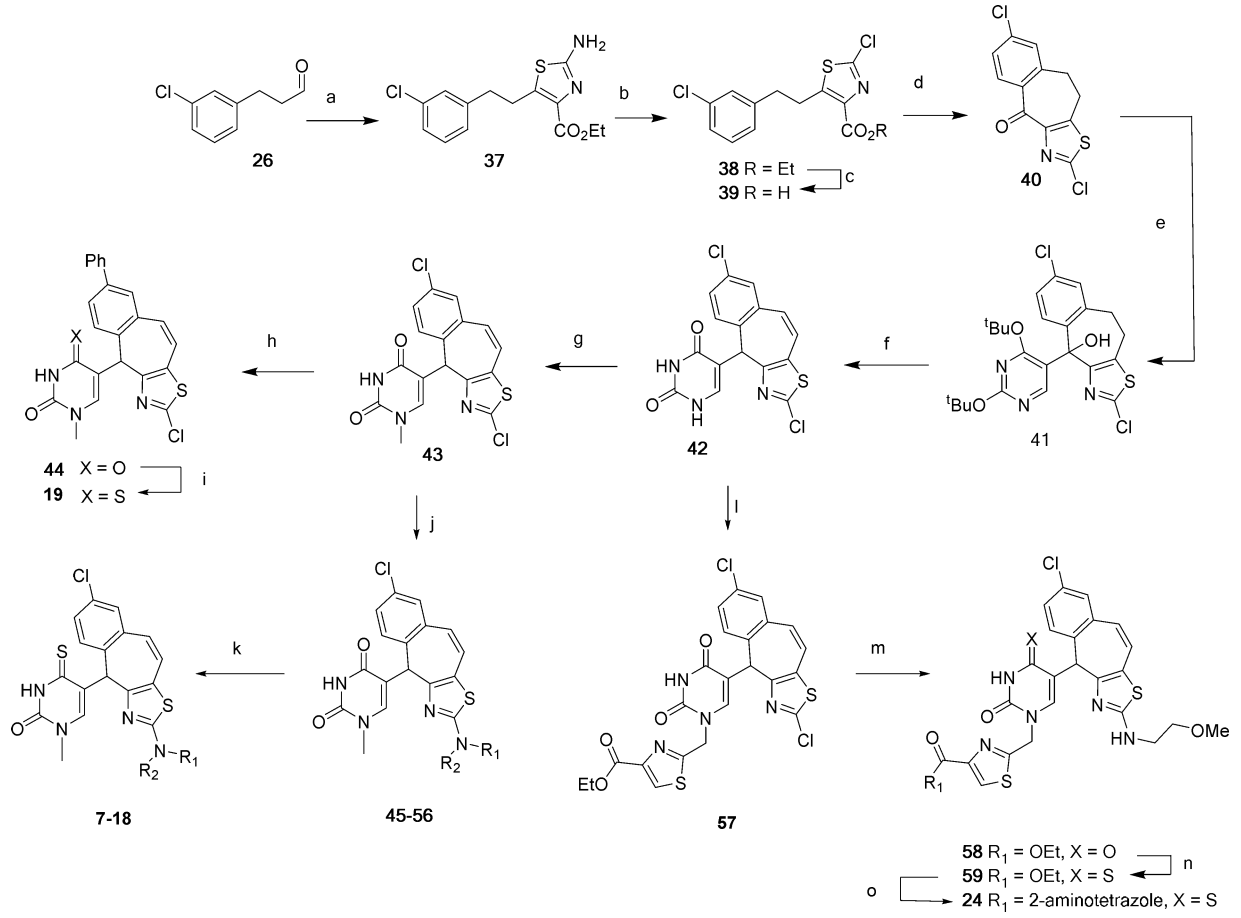
**Scheme 3. Synthesis of Compound 24**[a]




[a]Reagents and conditions: (a) (i) Ethyl dichloroacetate, sodium ethoxide, diethyl ether, 30 min, 0−40 °C; (ii) thiourea, ethanol, reflux, 2.5 h (46% over two 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, catalytic DMF, DCM, rt, 3 h; (ii) $AlCl_3$, DCM, rt, 18 h (83% over two steps). (e) (i) **31**, *n*-butyllithium, THF, −78 °C, 30 min; (ii) **82**, −78 °C to rt, 1 h (79%). (f) Acetic acid/1,4-dioxane (1:1), 140 °C, 10 min (58%). (g) (i) *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide, DCM, reflux, 18 h; (ii) iodomethane, 50 °C, 24 h (61%). (h) Phenylboronic acid, $Na_2CO_3$, 1 mol % bis(triphenylphosphine) palladium(II)chloride, 1,4-dioxane, water, microwave irradiation, 150 °C, 5 min (68%). (i) Lawesson's reagent, 1,4-dioxane, reflux, 18 h (76%). (j) $R_1R_2NH$, 1,4-dioxane, microwave irradiation, 100 °C, 4 h (51−92%). (k) Lawesson's reagent, 1,4-dioxane, reflux, 18 h (44−88%). (l) (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%).

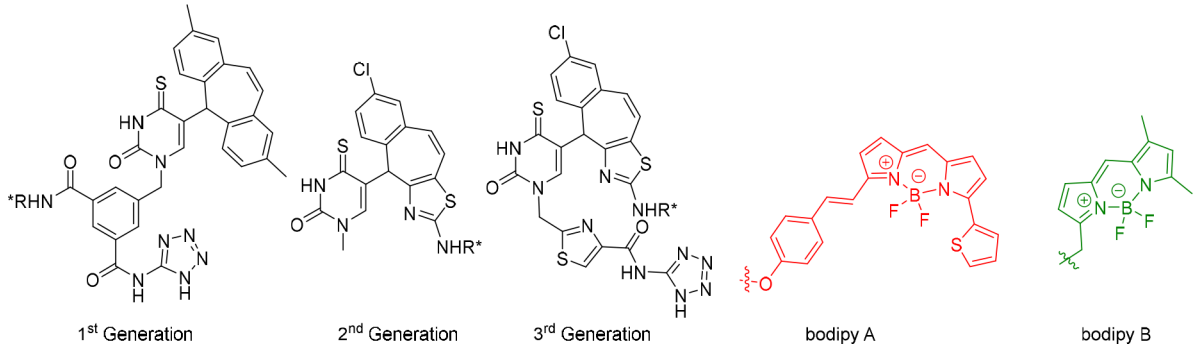



**Figure 3.** Design of the fluorescent $P2Y_2R$ antagonists based on the SARs of **1**. The figure shows the potential attachment points to which either BODIPY A or BODIPY B could be attached via a suitable linking group. The asterisks (*) represent the attachment points for the fluorophore linkage via a suitable linking group.

(2-(2-aminoethoxy)ethyl)carbamate to generate the corresponding amides (**74**−**77**). The Boc protecting groups were removed using TFA, and the resulting amines were 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 $\mu$M in the presence or absence of 10 $\mu$M **1**



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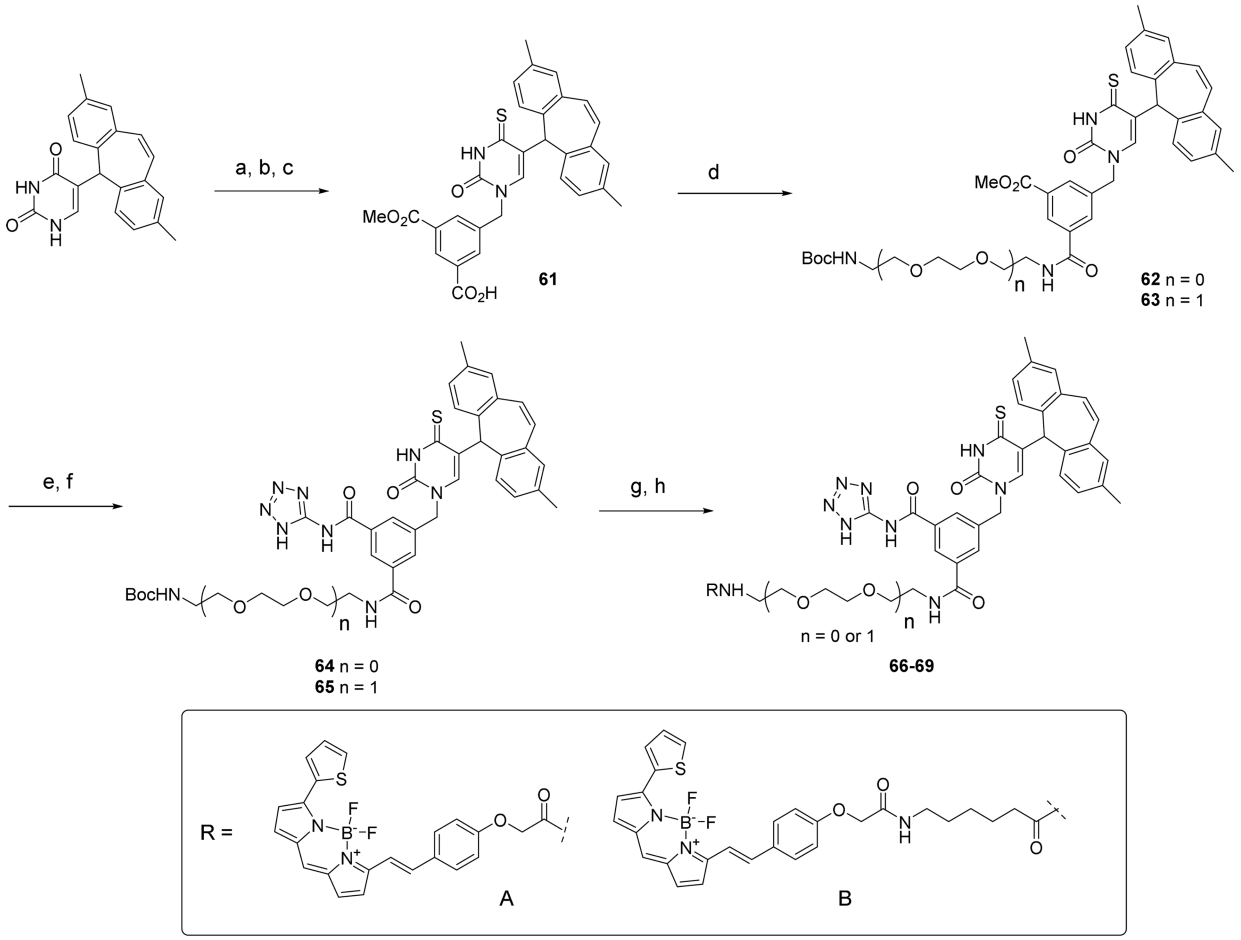
**Scheme 4. General Synthetic Route for the First-Generation $P2Y_2R$ Fluorescent Antagonists**[a]




[a]Reagents and conditions: (a) Dimethyl 5-(bromomethyl)isophthalate, BSTFA, 1,2-dichloroethane, reflux, 16 h. (b) Lawesson's reagent, 1,4-dioxane, reflux, 16 h. (c) NaOH (2 equiv), MeOH, toluene, $H_2O$, 4 h, 65% (three 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, 1 h, rt. (g) HCl (4 M) in 1,4-dioxane, 1 h. (h) BODIPY-SE, DIPEA, DMF, rt, 1−4 h, 15−45%.

**Table 4. Affinities of 66, 67, 68, and 69 Determined in 1321N1 Astrocytoma Cells Expressing Recombinant NLuc-$P2Y_2R$**

| example | $n$[a] | R[b] | NanoBRET $pK_d$[c] |
|---|---|---|---|
| **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]See Scheme 4. [b]A and B correspond to the R groups in Scheme 4. [c]The $pK_d$ values were derived from saturation binding curves. The data shown are the means ± SEM, and the numbers of separate experiments are given in parentheses.

(see Figure S1 in the Supporting Information). It was found that **80**, **85**, **86**, and **87** generated the largest specific NanoBRET signals; therefore, their affinities were determined from saturation binding assays, demonstrating the excellent signal-to-noise ratios observed from NanoBRET assays even for low-affinity conjugates (Figure 4 and Table 5).

Reassuringly, the affinities determined for the nonfluorescent $P2Y_2R$ antagonist, **1**, with either **86** ($pK_i$ = 7.45 ± 0.13, $n$ = 4) or **87** ($pK_i$ = 7.32 ± 0.13, $n$ = 4) were consistent with the affinities 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 affinities of nonfluorescent $P2Y_2R$ antagonists. The clear demonstration of the saturable specific binding of 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 affinity-enhancing acyl-tetrazole functional groups into the fluorescent compounds.

**Third-Generation $P2Y_2R$ Fluorescent Ligands.** The general synthetic route for the third-generation $P2Y_2R$ fluorescent antagonists is illustrated in Scheme 6. The displacement of the chlorine atom in compound **57** with *tert*-butyl 3-aminopropanoate gave the *tert*-butyl ester, **92**. Treatment with TFA afforded the conversion of the *tert*-butyl ester to the corresponding acid, which was immediately activated and coupled to *tert*-butyl (2-aminoethyl)carbamate, **93**, with HATU. Hydrolysis of the ethyl ester, activation of the carboxylic acid by benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate, and coupling with 5-aminotetrazole gave the amidotetrazole, **94**. The Boc protecting group was removed,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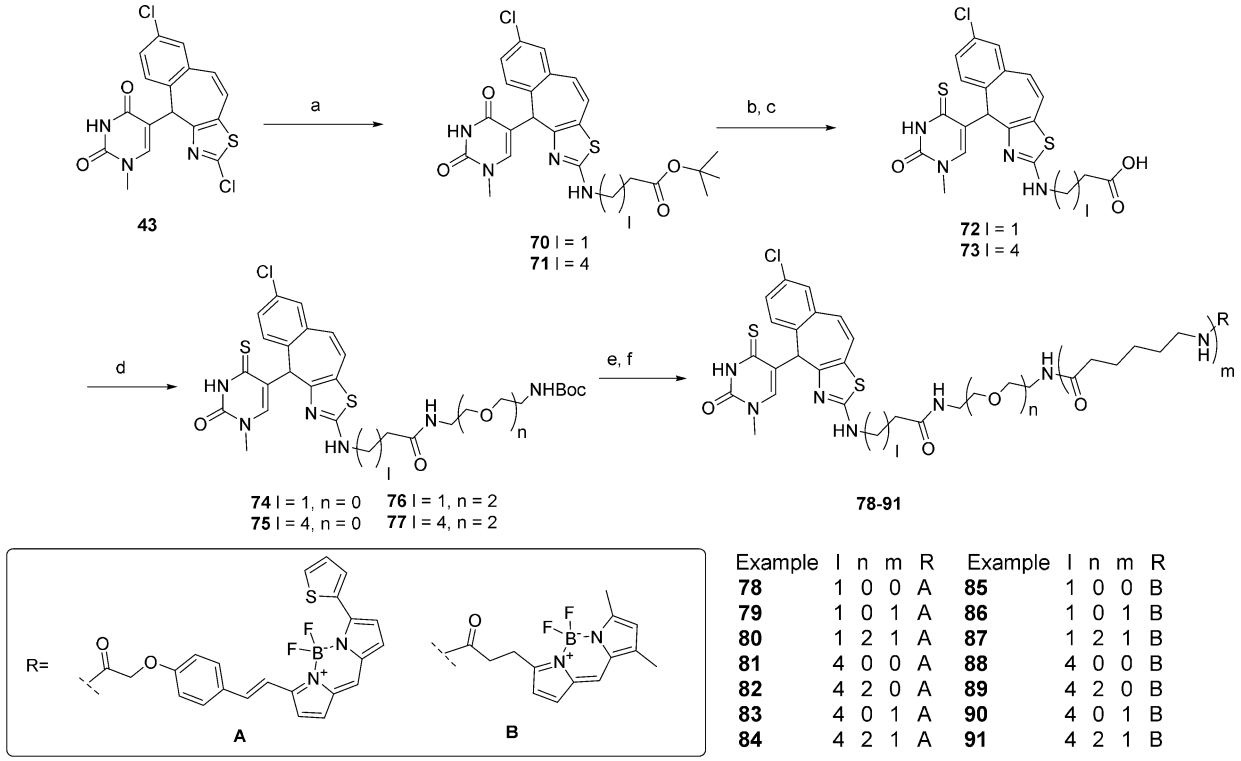
**Scheme 5. General Synthetic Route for the Second-Generation $P2Y_2R$ Fluorescent Antagonists**[a]




| 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 |

[a]Reagents and conditions: (a) Amine, $NEt_3$, 1,4-dioxane, reflux, 72 h, 96–97%. (b) Lawesson's reagent, 1,4-dioxane, 100 °C, 18 h, 39–74%. (c) TFA, DCM, rt, 30 min. (d) Amine, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate, DMF, DIPEA, rt, 1 h, 36–58% (two steps). (e) TFA, DCM, rt, 30 min. (f) BODIPY-SE, DIPEA, DMF, rt, 1–4 h, 10–54% (two steps).

and the resulting amine was attached to the fluorophore with the appropriate BODIPY succinimidyl ester (Scheme 6).

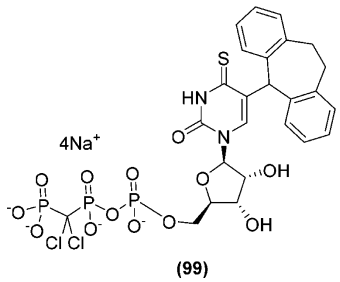
In contrast to the nonfluorophore compounds **14** and **24**, whose affinities hardly changed when their uracil *N*1-substituents were changed, in three of the four compounds (**95**–**98**), there were significant increases in their affinities relative to those of the nonamidotetrazole series (compare **98** with **86**, **96** with **85**, and **97** with **80**; Tables 5 and 6).

**Pharmacological Evaluation of Third-Generation $P2Y_2R$ Fluorescent Ligands.** To further evaluate the utility of the fluorescent ligands in studying the pharmacology of $P2Y_2R$, one BODIPY A and one BODIPY B linked fluorescent ligand (**97** and **98**, respectively) were chosen for further studies. Initially, we confirmed that **97** and **98** still retained the ability to functionally antagonize $P2Y_2R$ (Figure 5). In a $Ca^{2+}$-mobilization assay, modest rightwards shifts of the agonist-dose–response curves were observed for both 10 $\mu$M **97** ($pK_b$ = 5.69 ± 0.05, $n$ = 8) and 10 $\mu$M **98** ($pK_b$ = 5.87 ± 0.05, $n$ = 7).

Compounds **97** and **98** bought about clear concentration-dependent increases in BRET ratios in NLuc-tagged-*h*$P2Y_2R$ assays (Figure 6). These increases were antagonized by **1** (Figure 6c,d), yielding p*K*i values of 7.66 ± 0.11 ($n$ = 9) and 7.38 ± 0.04 ($n$ = 6) for the antagonism of **97** and **98** binding, respectively.

To further evaluate the utility of fluorescent conjugate **98** in the NanoBRET ligand-binding assay, the affinities of a selection of $P2Y_2R$ antagonists (**1**, **3**, **6**, **22**, **23**, **60**, and **86**) and the previously reported stabilized-triphosphate $P2Y_2R$ antagonist, **99**,[8] over a range of $P2Y_2R$ affinities, were determined in competition binding experiments.

All eight compounds induced concentration-dependent decreases in the specific binding of **98**, which enabled their affinities to be determined. There was a good correlation between the values obtained in the NanoBRET assay and those determined in the $Ca^{2+}$-mobilization assay (Table 7). In addition to those of the antagonists, the NanoBRET assay was also used to estimate the affinity of UTP$\gamma$S. As there have been no reports of radio ligands for $P2Y_2R$, this measurement has not previously been possible.




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



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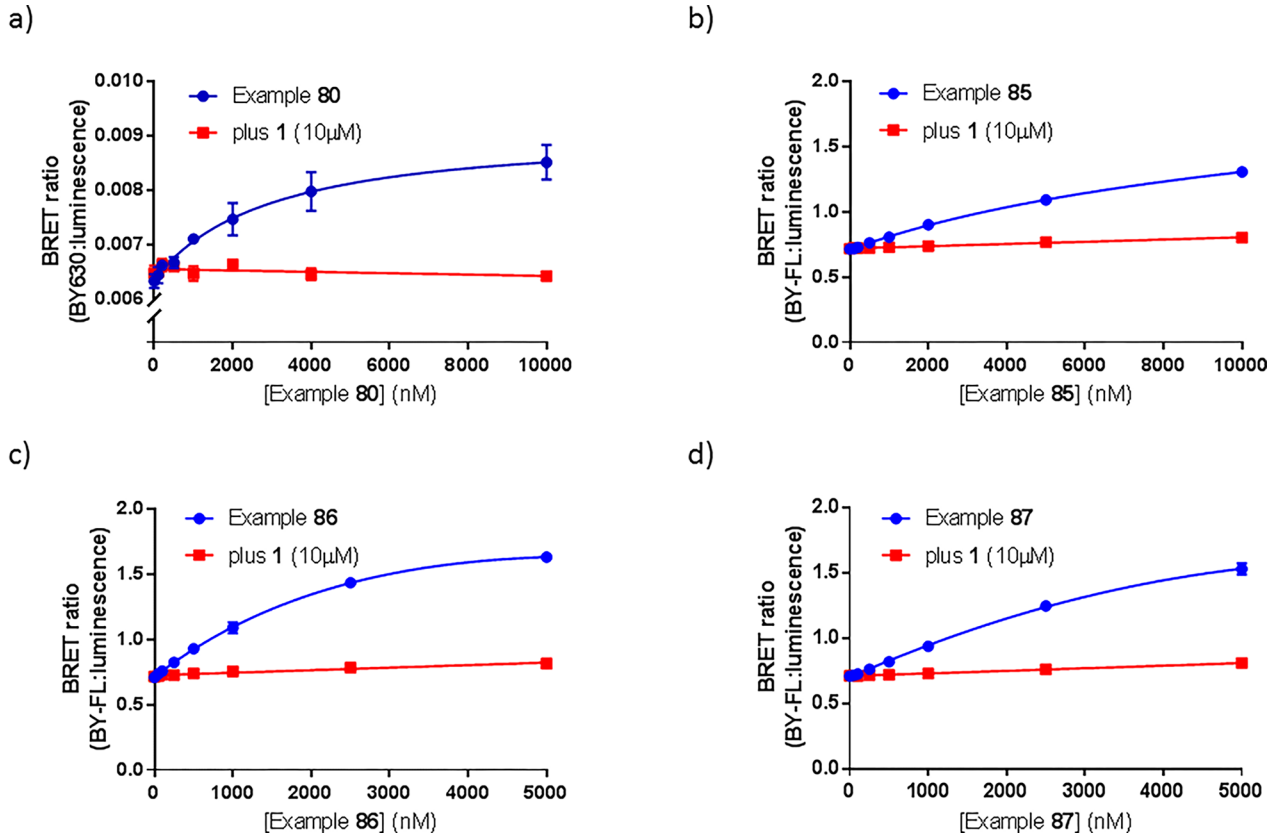


**Figure 4.** NanoBRET saturation binding isotherms, determined in 1321N1 astrocytoma cells expressing recombinant NLuc-P2Y$_2$R, for (a) **80**, (b) **85**, (c) **86**, and (d) **87** in the absence and presence of 10 $\mu$M **1**. The data points are the mean values ± SEM ($n$ = 3 or 4).

**Table 5. Affinities of 85, 86, 87, and 80 Determined in 1321N1 Astrocytoma Cells Expressing Recombinant NLuc-P2Y$_2$R**

| example | p$K_d$[a] |
|---|---|
| **80** | 5.29 ± 0.17 (4) |
| **85** | 4.91 ± 0.14 (4) |
| **86** | 5.67 ± 0.10 (4) |
| **87** | 5.38 ± 0.19 (3) |

[a]The p$K_d$ values were derived from saturation binding curves. The data shown are the means ± SEM, and the numbers of separate experiments are given in parentheses.

## 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 of **20** versus **21** and **22** versus **23**. Vibrational circular dichroism has suggested that in the case of **16**, all of the $h$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 the attachment of the fluorescent ligands, thus generating three distinct series of fluorescently labeled $h$P2Y$_2$R antagonists. From this extensive synthetic work, two examples (**97** and **98**) were identified as demonstrating both functional antagonist activities (in $Ca^{2+}$-mobilization assays) and sufficient affinities for $h$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 $h$P2Y$_2$R. These 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, in which it proved essential to explore the parallel changes of linker-attachment points, fluorophores, and linking groups.[30] From this study, only a few fluorescent conjugates were shown to possess sufficient affinities 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. BODIPYFL-X-NHS (D6102) and BODIPY630/650-X-NHS (D10000) were purchased from Molecular Probes (Invitrogen). All reactions were monitored by TLC using Merck Silica Gel 60 Å F254 TLC plates or by LC-MS. Unless otherwise stated, all compounds were dried under high vacuum either at rt or in an oven at 40 °C. LC-MS data was collected on a Shimadzu UFLCXR HPLC system coupled to an Applied Biosystems API 2000 electrospray-ionization (ESI) LC-MS/MS. The column used was a Phenomenex Gemini-NX (3 $\mu$m, 110 Å, C18, 50 × 2 mm), and it was operated at 40 °C. The flow rate was 0.5 mL/min, and UV detection was at 220 and 254 nm. LC-MS method 1 was 5% B for 1 min, 5−98% B over 2 min, 98% B for 2 min, 98−5% B over 0.5 min, and then 5% B for 1 min. LC-MS method 2 was 10% B for 1.5 min, 10−98% B over


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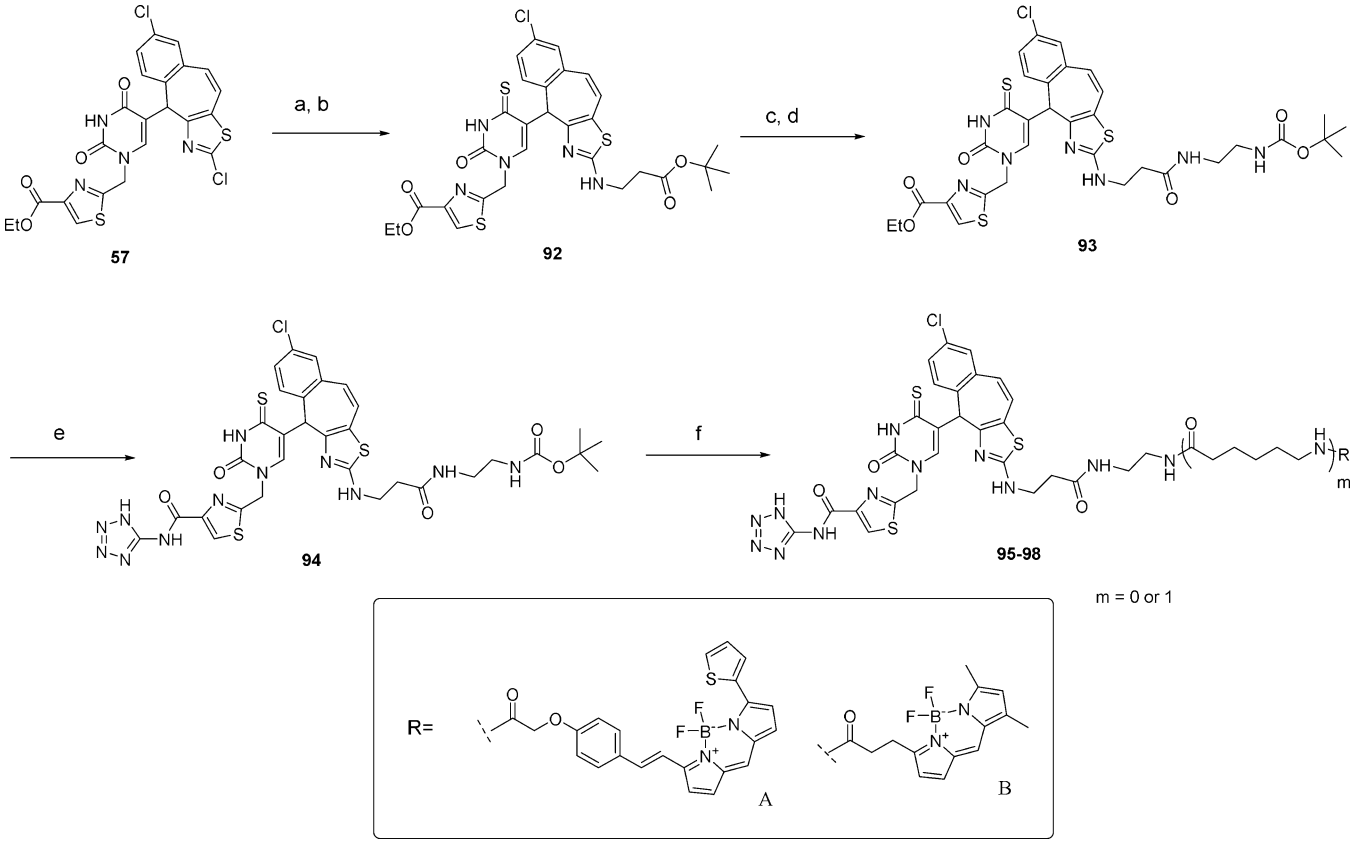
**Scheme 6. General Synthetic Route for the Third-Generation $P2Y_2R$ Fluorescent Antagonists**[a]




[a]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) (i) NaOH, MeOH, reflux, 4 h; (ii) 5-aminotetrazole, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate, DIPEA, DMF, rt, 4 h (28% over two steps). (f) (i) TFA, DCM, rt, 30 min; (ii) BODIPY-SE, DIPEA, DMF, rt, 1−4 h (11−18% over two steps). The structures of compounds **95−98** are assigned in Table 6.

**Table 6. Affinities of 95, 96, 97, and 98 Determined in 1321N1 Astrocytoma Cells Expressing Recombinant NLuc-$P2Y_2R$**

| example | $m$[a] | R[b] | NanoBRET $pK_d$[c] |
|---|---|---|---|
| **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]See Scheme 6. [b]A and B correspond to the R groups in Scheme 6. [c]The $pK_d$ values were derived from saturation binding curves. The data shown are the means ± SEM, and the numbers of separate experiments are given in parentheses.

8 min, 98% B for 2 min, 98−10% B over 0.5 min, and then 10% B for 1 min. Solvent A was 0.1% formic acid in water, and solvent B was acetonitrile. Unless otherwise stated, the compounds reported had purities >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 errors of ±10 ppm were 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-NX (5 μm, 110 Å, C18, 150 × 21 mm) at ambient temperature. The flow rate was 40 mL/min, and UV detection was at 254 nm. Preparative-RP-HPLC method 3 was 10% B for 1 min, 10−35% B over 4 min, 35−40% B over 20 min, 40−90% B over 2 min, 90−10% B over 2 min, and then 10% B for 1 min.

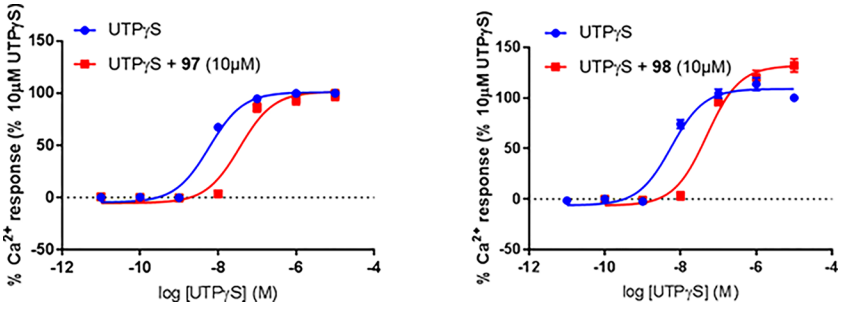



**Figure 5.** Pharmacological evaluations of **97** and **98** showing the effects on $Ca^{2+}$ mobilization in $hP2Y_2R$-1321N1 cells induced by 0.1 μM UTPγS ($n$ = 7 or 8).



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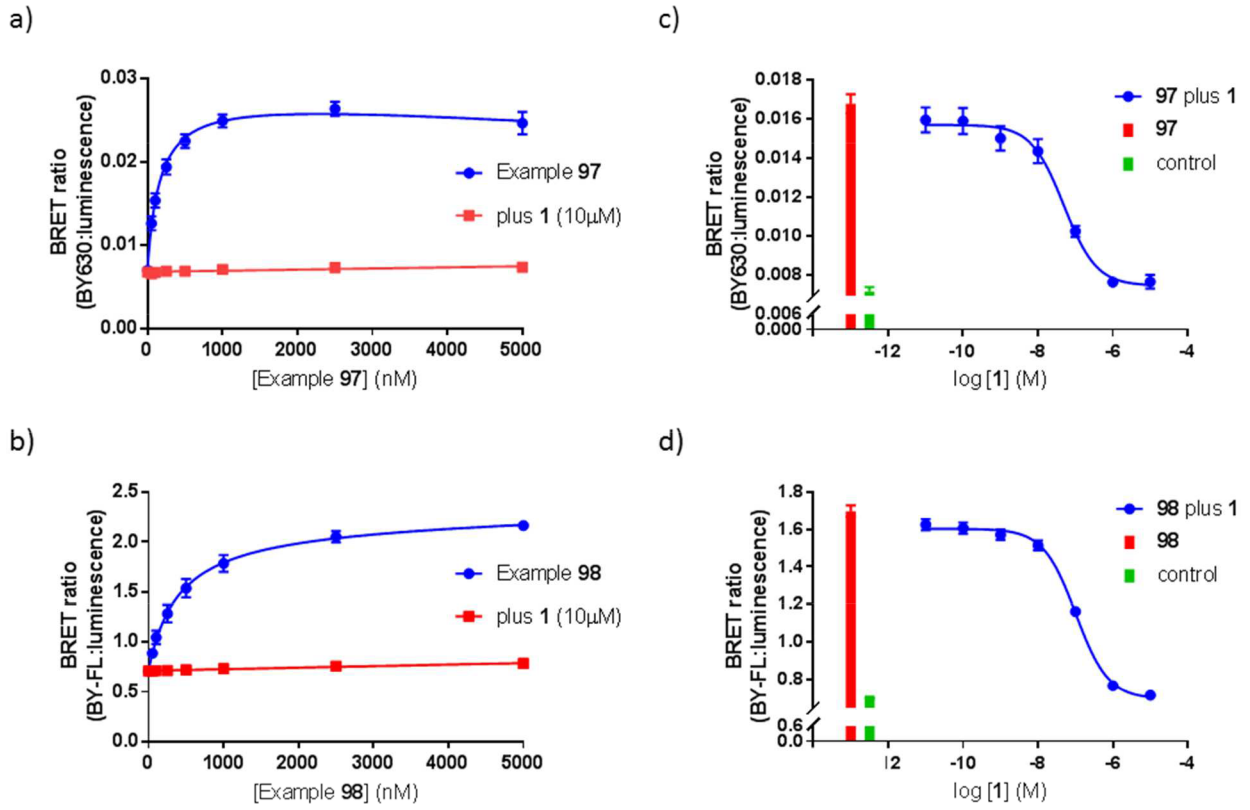


**Figure 6.** Pharmacological evaluations of (a) **97** and (b) **98** showing BRET saturation binding ($n$ = 7). Displacement of (c) **97** (100 nM, $n$ = 9) and (d) **98** (1 $\mu$M, $n$ = 6) binding in NLuc-P2Y$_2$ 1321N1 cells by **1**. The values shown are the means ± SEM.

**Table 7. Comparison of the Affinity Estimates Obtained in the $Ca^{2+}$-Mobilization and NanoBRET Assays**[a]

| example | p$K_b$ $Ca^{2+}$ mobilization[b] | p$K_i$ NanoBRET (**98**)[c] |
|---|---|---|
| **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)[d] | 7.08 ± 0.03 (3) |
| UTPγS | ND[e] | 5.46 ± 0.04 (6) |

[a]The data shown are the means ± SEM, and the numbers of separate experiments are given in parentheses. [b]The estimated affinity value for each antagonist (p$K_b$) was calculated using the Gaddum equation from the shift of the agonist-dose−response curve brought about by the addition of a single concentration of the antagonist. [c]Measured in competition binding experiments with fluorescent ligand **98**. [d]Reported p$A_2$ = 8.0.[8] [e]ND = not determined due to agonist activity.

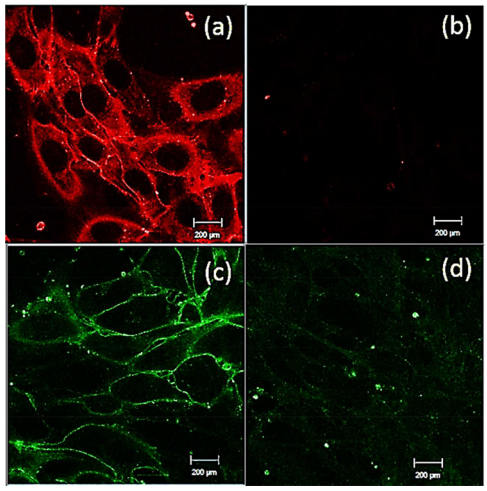


**Figure 7.** Visualization of the binding of (a,b) **97** and (c,d) **98** on astrocytoma cells expressing $h$P2Y$_2$R. The images in the left-hand column (a,c) are confocal image with 100 nM concentrations of the fluorescent ligands, whereas the right-hand column (b,d) shows images of the cells with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

Preparative-RP-HPLC method 4 was 10% B for 1 min, 10−45% B over 4 min, 45−50% B over 20 min, 50−90% B over 2 min, 90−10% B over 2 min, and then 10% B for 1 min. 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 with a Dionex UltiMate 3000 Variable Wavelength Detector. The columns used for the analytical and semipreparative runs were 250 × 4.6 mm and 250 × 10 mm Phenomenex Luxes (5 $\mu$m, Amylose-2), 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 the analytical runs and 5 mL/min for the semipreparative runs. UV detection was at 254 nm. NMR spectroscopy was performed with a Bruker AV(III) HD 400 NMR spectrometer equipped with a 5 mm BBFO+ probe, which recorded the $^1$H and $^{13}$C NMR at 400.25 and 100.66 MHz, respectively, or with a Bruker AV(III) 500 NMR spectrometer equipped with a 5 mm dual $^1$H/$^{13}$C helium-cooled cryoprobe, which recorded the $^1$H and $^{13}$C NMR at 500.13 and 125.77 MHz,


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respectively. The NMR data was processed using iNMR (version 5.5.7), which referenced the spectra to those of the residual solvents. Chemical shifts ($\delta$) are quoted as values in parts per million, and coupling constants ($J$) are given in hertz. Multiplicities are described using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; qi, quintet; sep, septet; m, multiplet; app, apparent; and br, broad.

All compounds submitted for biological screening had purities >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 equiv, 0.45−2.00 mmol) and triethyl amine (10 equiv, 1.50−5.00 mmol) under $N_2$ and either heated to reflux for 36−72 h until completion was observed by TLC or LC-MS or heated in a microwave reactor at 100 °C for 4 h. The reaction mixture was concentrated in vacuo directly onto a silica gel and purified by chromatography on a silica gel (2−5% MeOH/DCM with 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.** Stirred suspensions or solutions of the uracils (0.05−0.25 mmol) and Lawesson's reagent (2 stoichiometric equiv/1 molar equiv, 0.05−0.25 mmol) in anhydrous 1,4-dioxane (0.05 M, 1−5 mL) were heated to reflux (120 °C) under $N_2$ for 18 h, generating deep-yellow-orange solutions. These were allowed to cool to room temperature, and the crude reaction mixtures were concentrated in vacuo onto silica gels, and unless otherwise stated, the title compounds were purified by chromatography on silica gels and eluted with 0−5% MeOH/DCM to afford the desired 4-thiouracils.

**General Procedure 3: Synthesis of BODIPY-Labeled Fluorescent Conjugates.** A solution of a Boc-protected amine (2.00 $\mu$mol) 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 one-fifth the volume; this was repeated three times before the solution was concentrated to dryness, affording the TFA salt of the conjugate. This was dissolved in DMF (500 $\mu$L), treated with DIPEA (25 $\mu$L) and then with a solution of BODIPYFL-X-NHS (1.00 mg, 2.00 $\mu$mol) in DMF (150 $\mu$L), BODIPY630/650-X-NHS (1.32 mg, 2.00 $\mu$mol) in DMF (150 $\mu$L), BODIPYFL-NHS, or BODIPY630/650-NHS; the latter two were generated in situ by mixing BODIPYFL-$CO_2H$ X (0.78 mg, 2.00 $\mu$mol) or BODIPY630/650-$CO_2H$ (0.90 mg, 2.00 $\mu$mol) with HATU (0.76 mg, 2.00 $\mu$mol), NHS (0.23 mg, 2.00 $\mu$mol), and DIPEA (25 $\mu$L) in DMF (150 $\mu$L) for 30 min before they were added. The solution was then allowed to react at room temperature in the absence of light for 1−4 h until completion was observed by LC-MS. The desired fluorescent conjugate was then purified directly from the reaction mixture by preparative reverse-phase HPLC and 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-5*H*-dibenzo[*a*,*d*][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2*H*)-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 min, the reaction mixture was partitioned between 1 M 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 eluted with ethyl acetate/methanol/acetic acid (93:5:2). Yield: 0.025 g, 0.049 mmol, 49% (yellow solid). $^1$H NMR (400 MHz, DMSO) $\delta$: 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 (254 nm); ($m/z$): 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**).* Compound **4** was prepared using the procedures for **34** and **6** with 5-(2,8-dimethyl-5*H*-dibenzo[*a*,*d*][7]annulen-5-yl)pyrimidine-2,4(1*H*,3*H*)-dione (0.14 g, 0.42 mmol). Yield: 0.08 g, 0.2 mmol, 48% over the two steps (yellow solid). $^1$H NMR (400 MHz, DMSO) $\delta$: 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 (254 nm); ($m/z$): 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 2 M 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 was concentrated to one-third the volume, diluted with ethyl acetate (20 mL), washed with 1 M HCl (10 mL) and 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 (40 $\mu$L) 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 (ca. two drops), diluted with ethyl acetate (20 mL), and partitioned with 1 M HCl (10 mL); the organics were extracted with ethyl acetate (2 × 10 mL), combined, washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The DMF was azeotroped with toluene (3 × 50 mL) to afford an orange oil. This was purified by being washed with 10% MeOH/DCM before the desired compound was eluted with 10% MeOH/DCM and 1% acetic acid to afford the title compound, **5**, as 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-$d_6$) $\delta$: 12.90 (s, 1H), 8.50 (s, 1H), 7.67 (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 and tetrazole-NH were 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 as 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]$^+$. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 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, $CDCl_3$) $\delta$: 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 as 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]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 as 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-$d_6$) $\delta$: 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),



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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-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 with 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]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 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 as 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]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 as 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-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 as 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-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 as 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]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 as 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]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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), which isolated the title compound, **18**, at a retention time of 18.57 min. This compound 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-$d_6$) $\delta$: 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 as 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-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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*



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*(24)*. A stirred solution of **59** (140 mg, 0.23 mmol) in MeOH (10 mL) was treated with 2 M 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 was evaporated directly onto silica, purified by FC, and washed with 5% MeOH/DCM before the desired compound was eluted with 20% MeOH/DCM with 1% acetic acid to afford a yellow solid. This was dissolved in EA (20 mL), washed with 1 M HCl (10 mL) and brine (10 mL), 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 $\mu$L) 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 (ca. two drops), diluted with EA (20 mL), and partitioned with 1 M HCl (10 mL), and the organics were extracted with EA (2 × 10 mL), combined, washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The DMF was azeotroped with toluene (3 × 50 mL) to afford an orange oil. This was purified by being washed with 10% MeOH/DCM before the desired compound was eluted with 10% MeOH/DCM with 1% acetic acid to afford the title compound. Further purification was achieved by preparative RP-HPLC (Method 3), isolating the title compound, **24**, at a retention time of 7.37 min. This compound 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. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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) and cooled in an ice bath under $N_2$ was treated portionwise with $Pd(OAc)_2$ (1.40 g, 6.29 mmol) over 30 min. This was then heated to 50 °C for 18 h, and the consumption of 3-chloroiodobenzene was observed by TLC (15% ethyl acetate/petroleum ether). The reaction was concentrated in vacuo, and the DMF was azeotroped with toluene (3 × 200 mL). The resulting black gum was dissolved in $Et_2O$ (300 mL) and water (300 mL) and filtered, and the organics were extracted with $Et_2O$ (2 × 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), to afford 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, $CDCl_3$) $\delta$: 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, $CDCl_3$) $\delta$: 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) and cooled to below −10 °C 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, and we ensured that the temperature did not rise above 0 °C. This generated a pale-orange suspension. This was stirred for 45 min and then warmed to 40 °C over 30 min. This was then quenched with water (250 mL), and the organics were extracted with diethyl ether (3 × 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) that had been heated to 65 °C 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) and partitioned with $NaHCO_3$ (100 mL) and water (100 mL). The organics where extracted with diethyl ether (3 × 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 as 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 $[M(^{35}Cl)+H]^+$ $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 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, $CDCl_3$) $\delta$: 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 and treated with 2 M HCl (100 mL), and the organics were extracted with ethyl acetate (3 × 100 mL), combined, washed with brine (100 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to afford the title compound, **28**, as a pale-yellow solid (11.68 g, 41.4 mmol, 99%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 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, $CDCl_3$) $\delta$: 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 (ca. two drops) and stirred for 3 h at rt. This was then concentrated in vacuo. The residual oxalyl chloride was azeotroped with toluene (3 × 50 mL) to afford an orange solid. This was dissolved in DCM (175 mL), cooled in an ice bath under $N_2$, and treated portionwise with aluminum(III)chloride (21.33 g, 160 mmol), generating a black solution. This was allowed to warm to rt and was stirred for 18 h. This was gradually added to a stirred slurry of ice and 2 M HCl (400 mL) and allowed to warm to rt. The organics were extracted with ethyl acetate (3 × 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 as 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, $CDCl_3$) $\delta$: 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 was stirred for a further 18 h, generating a dark-brown solution. The RM was then quenched with $NH_4Cl$(aq) (10 mL) and diluted with water (100 mL), and the organics were extracted with ethyl acetate (3 × 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 with 0.1% $Et_3N$) to afford the title compound, **31**, as 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 $[M(^{79}Br)+H]^+$. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.24 (s, 1H), 1.64 (s, 9H), 1.59 (s, 9H). $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$: 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


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THF (80 mL) under $N_2$ was cooled to −78 °C, 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) that had been dissolved in unstabilized, anhydrous THF (20 mL); stirred at −78 °C for 15 min; allowed to warm to rt; and stirred again for 1 h. The RM was then quenched with $NH_4Cl$ (50 mL) and diluted with water (50 mL), and the organics were extracted with ethyl acetate (3 × 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 as 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-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 was concentrated in vacuo to give a dark-red gum, which was triturated with $Et_2O$ (3 × 10 mL) to give an orange solid (454 mg). This was purified by FC (2−10% MeOH/DCM with 0.1% 880 $NH_3$) to afford the title compound, **33**, at 4% MeOH/DCM with 0.1% 880 $NH_3$ as 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-$d_6$) $\delta$: 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 to afford an oil, which was dissolved in MeOH and reconcentrated in vacuo to afford a solid. This was purified by FC (1−4% MeOH/DCM with 0.1% 880 $NH_3$) to afford the title compound, **34**, at 2% MeOH/DCM with 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-$d_6$) $\delta$: 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, 0.42 mmol), 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 reconcentrated in vacuo to a solid. This was purified by chromatography on a silica gel (1−5% MeOH/DCM) to afford 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, $CDCl_3$) $\delta$: 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 as 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, $CDCl_3$) $\delta$: 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) and 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, and we ensured that the temperature did not rise above 0 °C. This generated a pale-orange suspension, which was stirred for 45 min and warmed to 40 °C over 30 min. This was then quenched with water (250 mL), and the organics were extracted with diethyl ether (3 × 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) that had been heated to 65 °C over a 10 min period. This was heated to reflux for 2.5 h, generating a purple-red solution and a white precipitate, which was then allowed to cool to rt and concentrated in vacuo. The residue was then diluted with diethyl ether (200 mL) and partitioned with $NaHCO_3$ (100 mL) and water (100 mL). The organics were extracted with diethyl ether (3 × 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), filtered through a plug of silica, and washed with diethyl ether (3 × 200 mL). The filtrate was concentrated to afford an orange solid, which was triturated with diethyl ether/hexane to give the title compound, **37**, as 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, $CDCl_3$) $\delta$: 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, $CDCl_3$) $\delta$: 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. We ensured the temperature did not rise above 25 °C. This was stirred for a further 2 h at rt, quenched with 2 M HCl (250 mL), and stirred for 10 min, and then the organics were extracted with ethyl acetate (3 × 250 mL). 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**, as 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, $CDCl_3$) $\delta$: 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, $CDCl_3$) $\delta$: 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 and treated with 2 M HCl (100 mL). The organics were extracted with ethyl acetate (3 × 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



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(400 MHz, $CDCl_3$) $\delta$: 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, $CDCl_3$) $\delta$: 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 (ca. two drops) and stirred for 3 h at rt. This was then concentrated in vacuo. The residual oxalyl chloride was azeotroped with toluene (3 × 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 aluminum(III)chloride (12.36 g, 92.68 mmol), generating a black solution. This was allowed to warm to rt and was stirred for 18 h. This was gradually added to a stirred slurry of ice and 2 M HCl (250 mL) and allowed to warm to rt. The organics were extracted with ethyl acetate (3 × 200 mL), combined, washed with brine (100 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to afford an orange solid. This was triturated with diethyl ether/hexane to afford the title compound, **40**, as 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 $[M(^{35}Cl)+H]^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 −78 °C, treated dropwise with a solution of *n*-butyllithium (2.25 M 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) that had been dissolved in unstabilized, anhydrous THF (10 mL); stirred at −78 °C for 15 min; allowed to warm to rt; and stirred for 1 h. The RM was then quenched with $NH_4Cl$(aq) (20 mL) and diluted with water (20 mL), and the organics were extracted with ethyl acetate (3 × 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) to afford the title compound, **41**, at 15% ethyl acetate/petroleum ether as 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 $[M(^{35}Cl)+H]^+$. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 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, $CDCl_3$) $\delta$: 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 °C with stirring in a microwave (MW) for 10 min. This was concentrated in vacuo, and the residual acetic acid was azeotroped with toluene (3 × 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-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 °C for 6 h until completion was observed by LC-MS. This was concentrated in vacuo to afford an orange oil, which was dissolved in MeOH and reconcentrated in vacuo to afford a yellow solid. This was triturated with diethyl ether (3 × 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-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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) was heated in a MW to 150 °C for 5 min, generating a black solution. This was concentrated directly onto silica and purified by FC (20−50% ethyl acetate/toluene) to afford 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 254 nm; 95% purity at 220 nm). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 °C with stirring for 4 h. The RM was concentrated directly onto silica and purified by FC (5−20% MeOH DCM with 0.1% 880 $NH_3$) to afford the title compound, **45**, at 20% MeOH DCM with 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-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 concd HCl (ca. two drops) and heated with stirring in a MW at 140 °C for 1 h. This was evaporated directly onto silica and purified by FC (2−5% MeOH/DCM with 0.1% 880 $NH_3$) to afford the title compound, **46**, at 3% MeOH/DCM with 0.1% 880 $NH_3$ as 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-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 with 0.1% 880 $NH_3$ as 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-$d_6$) $\delta$: 11.25 (s, 1H, s, 1H), 8.41 (t, $J$ = 5.8 Hz, 1H), 7.45 (d, $J$ = 8.5 Hz, 1H),


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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-$d_6$) $\delta$: 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 with 0.1% 880 $NH_3$ as 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-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 with 0.1% 880 $NH_3$ as 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-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 with 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]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 with 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]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 with 0.1% 880 $NH_3$ as a pale-yellow solid (55 mg, 0.128 mmol, 67%). LC-MS (ESI+) Rt: 2.51 min (254 nm, Method 1); ($m/z$): 430.9 [M($^{35}$Cl)+H]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 as 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]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 as 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]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 as 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-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 as 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-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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)


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that had been 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 reconcentrated in vacuo to a brown solid. This was triturated with diethyl ether (3 × 50 mL) to give the title compound, **57**, as 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 $[M(^{35}Cl)+H]^+$. $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 as 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 $[M(^{35}Cl)+H]^+$. $^1H$ NMR (500 MHz, DMSO-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 as a yellow solid (200 mg, 0.33 mmol, 81%). LC-MS (ESI+) Rt: 2.87 min (254 nm, Method 1); (*m*/*z*): 601.9 $[M(^{35}Cl)+H]^+$. $^1H$ NMR (500 MHz, DMSO-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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-5*H*-dibenzo[*a*,*d*][7]annulen-5-yl)pyrimidine-2,4(1*H*,3*H*)-dione[31] (0.49 g, 1.48 mmol) in 1,2-dichloroethane (6 mL), and the reaction mixture was 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 the reaction mixture cooled, the volatiles were removed under vacuum. Purification was by silica-gel chromatography, and the eluent was ethyl acetate/petroleum ether, 50:50, which gave dimethyl 5-((5-(2,8-dimethyl-5*H*-dibenzo[*a*,*d*][7]-annulen-5-yl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl)-isophthalate (0.7 g, 1.3 mmol, 87%). LC-MS (ESI+) Rt: 3.10 (254 nm, 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) were heated under reflux under nitrogen. After 16 h, the volatiles were evaporated under vacuum. Purification was by silica-gel chromatography, and the eluent was ethyl acetate/toluene, 30:70, which gave dimethyl 5-((5-(2,8-dimethyl-5*H*-dibenzo[*a*,*d*][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl)-isophthalate (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: A sodium hydroxide solution (0.4 mmol, 0.4 mL of a 1 M 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 4 h, the reaction mixture was partitioned between 2 M hydrochloric acid and ethyl acetate. The ethyl acetate layer was washed with brine, dried (magnesium sulfate), filtered, and evaporated in vacuo. Purification was by silica-gel chromatography, and the eluents were 2% acetic acid in ethyl acetate/toluene (25:75 and then 80:20). Yield of **61**: 0.07 g, 0.13 mmol, 65% (yellow solid). $^1H$ NMR (400 MHz, DMSO) $\delta$: 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 (254 nm); (*m*/*z*): 539.6 (M+1). Yield of **60**: 0.02 g, 0.038 mmol, 19% (yellow solid). $^1H$ NMR (400 MHz, DMSO) $\delta$: 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 (254 nm, 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-5*H*-dibenzo[*a*,*d*][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2*H*)-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 (2 × 4 mL) and brine (1 × 4 mL). The solution was evaporated to dryness under reduced pressure. Purification was by silica-gel chromatography, and the eluent was 70:30 ethyl acetate/petroleum ether (40−60). Yield: 0.1 g, 0.15 mmol, 53% (yellow solid). $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 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 (254 nm, 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-5*H*-dibenzo[*a*,*d*][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2*H*)-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 (2 × 4 mL) and brine (1 × 4 mL). The solution was evaporate to dryness under reduced pressure. Purification was by silica-gel chromatography, and the eluent was ethyl acetate. Yield: 0.09 g, 0.12 mmol, 63% (yellow solid). $^1H$ NMR (400 MHz, DMSO) $\delta$: 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 (254 nm, 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-5*H*-dibenzo[*a*,*d*][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl)benzoate (0.1 g, 0.15 mmol) in methanol (1.5 mL). After 16 h, the mixture was partitioned between ethyl acetate and a 2 M hydrochloric acid solution. The ethyl acetate solution was washed with brine, dried


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(magnesium sulfate), filtered, and evaporated in vacuo to give 3-((2-((*tert*-butoxycarbonyl)amino)ethyl)carbamoyl)-5-((5-(2,8-dimethyl-5*H*-dibenzo[*a*,*d*][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl)benzoic acid (0.08 g) as a yellow solid. LC-MS (ESI+) Rt: 2.99 (254 nm, 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-5*H*-dibenzo[*a*,*d*][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2*H*)-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 2 M hydrochloric acid (1 × 4 mL), water (2 × 4 mL), and brine (1 × 4 mL). The solution was evaporated to dryness under reduced pressure. Purification was by silica-gel chromatography, and the eluent was methanol/dichloromethane, 5:95, and then 2% acetic acid in methanol/dichloromethane, 5:95. Yield: 0.03 g, 0.04 mmol, 30% (yellow solid). $^1$H NMR (400 MHz, DMSO) $\delta$: 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 (254 nm, 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-5*H*-dibenzo[*a*,*d*][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl)benzoate (0.035 g, 0.046 mmol) in methanol (1.5 mL). After 16 h, the mixture was partitioned between ethyl acetate and a 2 M hydrochloric acid solution. The ethyl acetate solution was washed with brine, dried (magnesium sulfate), 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-5*H*-dibenzo[*a*,*d*][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl)benzoic acid. Yield: 0.032 g (yellow solid). $^1$H NMR LC-MS (ESI+) Rt: 3.04 (254 nm, 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-5*H*-dibenzo[*a*,*d*][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2*H*)-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 2 M hydrochloric acid (1 × 4 mL), water (2 × 4 mL), and brine (1 × 4 mL). The solution was evaporate to dryness under reduced pressure. Purification was by silica-gel chromatography, and the eluent was methanol/dichloromethane, 5:95, and then 2% acetic acid in methanol/dichloromethane, 5:95. Yield: 0.009 g, 0.01 mmol, 14% (yellow solid). $^1$H NMR (400 MHz, DMSO) $\delta$: 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 (254 nm, Method 1); (*m*/*z*): 822.3 (M+1)

*(E)-N1-(2-(2-(4-(2-(5,5-Difluoro-7-(thiophen-2-yl)-5H-4λ⁴,5λ⁴-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-((2*H*-tetrazol-5-yl)carbamoyl)-5-((5-(2,8-dimethyl-5*H*-dibenzo[*a*,*d*][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl)benzamido)ethyl)carbamate (3.2 mg, 4.3 × $10^{-3}$ mmol) and 2,5-dioxopyrrolidin-1-yl (*E*)-2-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5*H*-4$\lambda^4$,5$\lambda^4$-dipyrrolo[1,2-*c*:2′,1′-*f*][1,3,2]-diazaborinin-3-yl)vinyl)phenoxy)acetate (2.3 mg, 4.2 × $10^{-3}$ mmol), the title compound was generated. Yield: 0.67 mg, 0.627 × $10^{-3}$ mmol, 15%. LC-MS (ESI+) Rt: 3.19 (254 nm); (*m*/*z*): 1066.4 (M+1).

*(E)-N1-(2-(6-(2-(4-(2-(5,5-Difluoro-7-(thiophen-2-yl)-5H-4λ⁴,5λ⁴-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-((2*H*-tetrazol-5-yl)carbamoyl)-5-((5-(2,8-dimethyl-5*H*-dibenzo[*a*,*d*][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl)benzamido)ethyl)carbamate (2.2 mg, 3 × $10^{-3}$ mmol) and 2,5-dioxopyrrolidin-1-yl (*E*)-6-(2-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5*H*-4$\lambda^4$,5$\lambda^4$-dipyrrolo[1,2-*c*:2′,1′-*f*]-[1,3,2]diazaborinin-3-yl)vinyl)phenoxy)acetamido)hexanoate (2.0 mg, 3 × $10^{-3}$ mmol), the title compound was generated. Yield: 1.2 mg, 1 × $10^{-3}$ mmol, 45%. LC-MS (ESI+) Rt: 7.35 (254 nm); (*m*/*z*): 1179.4 (M+1).

*(E)-N1-(2-(2-(2-(2-(4-(2-(5,5-Difluoro-7-(thiophen-2-yl)-5H-4λ⁴,5λ⁴-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-((2*H*-tetrazol-5-yl)carbamoyl)-5-((5-(2,8-dimethyl-5*H*-dibenzo[*a*,*d*][7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl)benzamido)-ethoxy)ethoxy)ethyl)carbamate (3.4 mg, 4.14 × $10^{-3}$ mmol) and 2,5-dioxopyrrolidin-1-yl (*E*)-2-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5*H*-4$\lambda^4$,5$\lambda^4$-dipyrrolo[1,2-*c*:2′,1′-*f*][1,3,2]diazaborinin-3-yl)vinyl)-phenoxy)acetate (2.26 mg, 4.14 × $10^{-3}$ mmol), the title compound was generated. Yield: 0.7 mg, 0.67 × $10^{-3}$ mmol, 17%. LC-MS (ESI+) Rt: 7.46 (254 nm); (*m*/*z*): 1154.6 (M+1).

*(E)-N1-(18-(4-(2-(5,5-Difluoro-7-(thiophen-2-yl)-5H-4λ⁴,5λ⁴-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**).* HCl (4 M) in 1,4-dioxane (1 mL) was added to *tert*-butyl (2-(2-(2-(3-((2*H*-tetrazol-5-yl)carbamoyl)-5-((5-(2,8-dimethyl-5*H*-dibenzo[*a*,*d*]-[7]annulen-5-yl)-2-oxo-4-thioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-methyl)benzamido)ethoxy)ethoxy)ethyl)carbamate (1.5 mg, 1.82 × $10^{-3}$ mmol). The solution was stirred for 2 h and then evaporated to dryness in vacuo. DMF (0.5 mL), then di-isopropylethylamine (10 mg), and then 2,5-dioxopyrrolidin-1-yl (*E*)-6-(2-(4-(2-(5,5-difluoro-7-(thiophen-2-yl)-5*H*-4$\lambda^4$,5$\lambda^4$-dipyrrolo[1,2-*c*:2′,1′-*f*][1,3,2]diazaborinin-3-yl)vinyl)phenoxy)acetamido)hexanoate (1.2 mg, 1.8 × $10^{-3}$ mmol) were added to the solution. The reaction mixture was stirred in the dark and under nitrogen overnight. Purification was by reverse-phase preparative HPLC eluted with a gradient of 50−60% MeCN in water (0.1% formic acid) over 20 min. The fraction containing the product was lyophilized. Yield: 1.002 mg, 0.79 × $10^{-3}$ mmol, 41%. LC-MS (ESI+) Rt: 7.41 (254 nm); (*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 as 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-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 as 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


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(400 MHz, DMSO-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 as a yellow solid (124 mg). The intermediate product (100 mg, 0.193 mmol) was dissolved in DCM (12 mL), 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 one-fifth the volume; this was repeated three times before the product was concentrated to dryness to afford a yellow solid. This was purified by FC and washed with 5% MeOH/DCM, and the title compound, **72**, was eluted with 20% MeOH/DCM with 1% acetic acid as 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-$d_6$) $\delta$: 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 as a yellow solid (124 mg). This was dissolved in DCM (14 mL), 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 one-fifth the volume; this was repeated three times before the product was concentrated to dryness to afford a yellow solid. This was purified by FC and washed with 5% MeOH/DCM, and the title compound, **73**, was eluted with 20% MeOH/DCM with 1% acetic acid as 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-$d_6$) $\delta$: 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-$d_6$) $\delta$: 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 of **72** (10 mg, 0.022 mmol) in DMF (1 mL) was treated with DIPEA (50 $\mu$L) and *N*-Boc-ethylenediamine (21 mg, 0.132 mmol) followed by PyBroP (15 mg, 0.033 mmol) and stirred at rt for 1 h until completion was observed by LC-MS. The RM was quenched with water (ca. two drops), concentrated in vacuo, and azeotroped with toluene (2 × 25 mL) to afford an orange oil. This was purified by FC (1−5% MeOH/DCM) to afford the title compound, **74**, at 4% MeOH/DCM as 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]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 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 of **73** (30 mg, 0.060 mmol) in DMF (3 mL) was treated with DIPEA (150 $\mu$L) 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 (ca. two drops), concentrated in vacuo, and azeotroped with toluene (2 × 25 mL) to afford an orange oil. This was purified by FC (1−5% MeOH/DCM) to afford the title 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]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 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 of **72** (10 mg, 0.022 mmol) in DMF (1 mL) was treated with DIPEA (50 $\mu$L) 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 1 h until completion was observed by LC-MS. The RM was quenched with water (ca. two drops), concentrated in vacuo, and azeotroped with toluene (2 × 25 mL) to afford an orange oil. This was purified by FC (1−5% MeOH/DCM) to afford the title compound, **76**, at 4% MeOH/DCM as 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]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 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 of **73** (30 mg, 0.060 mmol) in DMF (3 mL) was treated with DIPEA (150 $\mu$L) 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 (ca. two drops), concentrated in vacuo, and azeotroped with toluene (2 × 25 mL) to afford an orange oil. This was purified by FC (1−5% MeOH/DCM) to afford the title 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]$^+$. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 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-4$\lambda^4$,5$\lambda^4$-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 the BODIPY630/650 conjugate, **78**. This was purified by preparative RP-HPLC (Method 4), which isolated **78** at a retention time of 13.45 min. This compound was freeze-dried to a blue, iridescent solid (0.52 mg, 0.54 $\mu$mol, 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-4$\lambda^4$,5$\lambda^4$-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 the BODIPY630/650-X conjugate, **79**. This was purified by preparative RP-HPLC (Method 4), which isolated **79** at a retention time of 13.37 min. This compound was freeze-dried to a blue, iridescent solid (0.85 mg, 0.81 $\mu$mol, 41%). LC-MS (ESI+)


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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-4λ⁴,5λ⁴-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 the BODIPY630/650-X conjugate, **80**. This was purified by preparative RP-HPLC (Method 4), which isolated **80** at a retention time of 13.03 min. This compound 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-5λ⁴,6λ⁴-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 the BODIPY630/650 conjugate, **81**. This was purified by preparative RP-HPLC (Method 4), which isolated **81** at a retention time of 13.05 min. This compound 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-4λ⁴,5λ⁴-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 the BODIPY630/650 conjugate, **82**. This was purified by preparative RP-HPLC (Method 4), which isolated **82** at a retention time of 13.69 min. This compound 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-4λ⁴,5λ⁴-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 the BODIPY630/650-X conjugate, **83**. This was purified by preparative RP-HPLC (Method 4), which isolated **83** at a retention time of 13.27 min. This compound 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-4λ⁴,5λ⁴-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 the BODIPY630/650-X conjugate, **84**. This was purified by preparative RP-HPLC (Method 4), which isolated **84** at a retention time of 13.28. This compound 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-4λ⁴,5λ⁴-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 the BODIPYFL conjugate, **85**. This was purified by preparative RP-HPLC (Method 3), which isolated **85** at a retention time of 13.82 min. This compound 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-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2′,1′-f][1,3,2]diazaborinin-3-yl)propanamido)hexanamide (**86**).* Following general procedure 3, **74** was converted to the BODIPYFL-X conjugate, **86**. This was purified by preparative RP-HPLC (Method 3), which isolated **86** at a retention time of 14.37 min. This compound 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-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2′,1′-f][1,3,2]diazaborinin-3-yl)propanamido)hexanamide (**87**).* Following general procedure 3, **76** was converted to the BODIPYFL-X conjugate, **87**. This was purified by preparative RP-HPLC (Method 3), which isolated **87** at a retention time of 14.47 min. This compound 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 the BODIPYFL conjugate, **88**. This was purified by preparative RP-HPLC (Method 3), which isolated **88** at a retention time of 15.19 min. This compound 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 the BODIPYFL conjugate, **89**. This was purified by preparative RP-HPLC (Method 3), which isolated **89** at a retention time of 15.97 min. This compound 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-5λ⁴,6λ⁴-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 the BODIPYFL-X conjugate, **90**. This was purified by preparative RP-HPLC (Method 3), which isolated **90** at a retention time of 15.59 min. This compound 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.



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[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-5$\lambda^4$,6$\lambda^4$-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 the BODIPYFL-X conjugate, **91**. This was purified by preparative RP-HPLC (Method 3), which isolated **91** at a retention time of 15.92 min. This compound 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 as 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, and the intermediate uracil (492 mg, 0.75 mmol) was converted to the title compound, **92**, which was isolated at 1.5% MeOH/DCM as 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 one-fifth the volume; this was repeated three times before the product was concentrated 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 (1 mL) and concentrated directly onto silica. This was purified by FC (1−5% MeOH/DCM) to afford the title compound, **93**, at 5% MeOH/DCM as 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]$^+$. $^1$H 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 1 M NaOH (120 μL, 0.120 mmol) and heated to reflux for 4 h under $N_2$ until completion was observed by LC-MS. This was cooled, treated with 1 M HCl (120 μL, 0.120 mmol), diluted with toluene (25 mL), and concentrated in vacuo to one-fifth the volume; this was repeated three times before the product was concentrated 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. two drops) and concentrated in vacuo. The DMF was azeotroped with toluene (3 × 50 mL) to afford an orange oil. This was purified by FC and washed with 10% MeOH/DCM before the title compound, **94**, was eluted with 10% MeOH/DCM with 1% acetic acid (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. $^1$H 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-4$\lambda^4$,5$\lambda^4$-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 the BODIPY630/650 conjugate, **95**. This was purified by preparative RP-HPLC (Method 4), which isolated **95** at a retention time of 10.80 min. This compound 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-5$\lambda^4$,6$\lambda^4$-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 the BODIPYFL conjugate, **96**. This was purified by preparative RP-HPLC (Method 3), which isolated **96** at a retention time of 12.05 min. This compound 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-4$\lambda^4$,5$\lambda^4$-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 the BODIPY630/650-X conjugate, **97**. This was purified by preparative RP-HPLC (Method 4), which isolated **97** at a retention time of 11.28 min. This compound 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-5$\lambda^4$,6$\lambda^4$-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 the BODIPYFL-X conjugate, **98**. This was purified by preparative RP-HPLC (Method 4), which isolated **94** at a retention time of 12.69 min. This compound 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,


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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 the NLuc-P2Y$_2$R constructs, human P2Y$_2$R DNA (obtained from the Missouri S&T cDNA Resource Center) was amplified by PCR to remove the methionine start signal and cloned into pCR2.1 (linearized vector, Invitrogen). P2Y$_2$R was then subcloned in-frame with the membrane-signal sequence of the 5HT$_{3A}$ receptor and the full-length sequence of nanoluciferase. The NLuc-tagged receptors expressed in 1321N1 cells exhibited normal calcium signals ($EC_{50}$ for UTP$\gamma$S of 91 ± 12 nM, $n$ = 3).

**Cell Culture and Cell-Line Generation.** The 1321N1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS HI) and 2 mM L-glutamine at 37 °C with 5% $CO_2$. Mixed-population NLuc-P2Y2R-1321N1 cell lines were generated by transfecting the 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 the active clones was initially performed with the calcium-mobilization assay and was followed by the detection of luminescence upon the addition of the NLuc substrate, furimazine. On this basis, a single, active, cloned cell line was selected for use in all of the NanoBRET assays. The 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 without $CO_2$ for 45 min in a total volume of 100 $\mu$L of a 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 $\mu$M Fluo 4AM, 0.023% pluronic acid, 0.5 mM Brilliant Black, 1 U/mL apyrase, and the ligand under investigation or the vehicle. The plates were then loaded onto a plate reader (FLEXstation, Molecular Devices), and the fluorescence was measured (excitation: 485 nm, emission: 525 nm) every 1.52 s for up to 200 s after the addition of UTP$\gamma$S at 15 s. For the investigation of the BODIPYFL-labeled ligands, Fluo 4AM was replaced with 2.0 $\mu$M X-Rhod-1 AM (excitation: 584 nm, emission: 612 nm).

**NanoBRET Assay.** The saturation and competition binding assays were performed according to the methodology of Stoddart.[18] Briefly, the assays were performed on stably transfected NLucP2Y$_2$-1321N1 cells that had been seeded 24 h prior to the experiment in white Thermo Scientific Matrix 96-well microplates. The medium in each well was removed and replaced with HBSS containing apyrase (1 U/mL) and the required concentration of the fluorescent ligand with or without the competing ligand. Upon the addition of the fluorescent ligand, the cells were incubated for 1 h at 37 °C without $CO_2$. The NLuc substrate, furimazine (Promega), was then added to a final concentration of 10 $\mu$M, and the plate was incubated for a further 5 min at 37 °C without $CO_2$. The luminescence and resulting BRET were measured using a PHERAstar FS plate reader (BMG Labtech) at room temperature. For the assays involving **97**, sequential measurements of the filtered light emissions were made at 460 nm (80 nm bandpass) and >610 nm (long-pass), and the raw BRET ratios were calculated by dividing the >610 nm emissions by the 460 nm emissions. For the assays involving **98**, the measurements were made at 475 nm (30 nm bandpass) and 535 nm (30 nm bandpass), and the raw BRET ratios were calculated by dividing the 535 nm emissions by the 475 nm emissions.

**Confocal Microscopy.** The P2Y$_2$R-1321N1 cells were grown to approximately 80% confluency on eight-well Labtek chambered cover glasses (Nunc Nalgene) in normal growth medium. The growth medium was removed and replaced with HBSS containing apyrase (1 U/mL) and either **1** (10 $\mu$M) or the vehicle, and the cells incubated for 30 min at 37 °C without $CO_2$. The cells were then incubated with **97** or **98** at the required concentration for 10 min at room temperature prior to the collection of the single-equatorial confocal images. The images were obtained on a Zeiss LSM710 confocal microscope using a 40× c-Apochromat 1.2NA water-immersion objective. For **97**, the images were collected using a 633 nm excitation wavelength and a 488/561/633 dichroic, and the emissions were collected through a 650LP filter. For **98**, a 488 nm excitation wavelength was used with the same dichroic, and the emissions were collected using an LP575 filter. In each case, a pinhole diameter of 1 airy unit was used, and the laser power, gain, and offset were kept the same for all of the samples within each experiment. For both **97** and **98**, the images presented are as representative of an individual experiment with matched conditions. Linear adjustments to the image brightness and contrast have been applied equally across all of the comparative images using Zen software in order to prepare the images for presentation.

**Data Analysis.** All of the 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 actions, the estimated affinity values (p$K_b$) were calculated from the shifts of the agonist-concentration−response curves in the presence of the fluorescent antagonists using eq 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 the antagonist, b. The p$K_b$ is −log $K_b$.

The total and nonspecific saturation binding curves were fitted simultaneously using eq 2:

$$\text{BRET ratio} = \frac{B_{max} \times [b]}{[b] + K_d} + ((M \times [b]) + C) \quad (2)$$

where $B_{max}$ is the maximum specific BRET signal, [b] is the nanomolar concentration of the fluorescent ligand, $K_d$ is the equilibrium dissociation constant in nanomolar, $M$ is the slope of the nonspecific-binding component, and $C$ is the intercept with the $Y$-axis. The p$K_d$ is −log$K_d$.

The competition binding curves were fitted using eq 3:

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

where [L] is the nanomolar concentration of **98**, and $K_d$ is the equilibrium dissociation constant of **98** in nanomolar. The $IC_{50}$ was calculated as in eq 4:

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

where [A] is the concentration of the unlabeled competing drug, and the $IC_{50}$ is the molar concentration of the competing ligand required to inhibit 50% of the specific binding of the labeled ligand at a given concentration ([L]).

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.8b00139.

Molecular-formula strings (CSV)

Description of the VCD structure determination and figure showing the binding of the BODIPY630/650-labeled (**78**−**84**) and BODIPYFL-labeled (**85**−**91**) ligands in NLuc-P2Y$_2$-1321N1 cells using the NanoBRET assay (PDF)

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### Author Contributions

[⊥]S.C. and N.D.K. contributed equally to this work. S.J.H., B.K., and M.J.S. conceived the study and managed the project. S.C., N.D.K., and M.J.S. performed the chemical syntheses. R.J.L. performed the VCD structural determination. S.J.H., L.A.S., S.C., N.D.K., B.K., and M.J.S. participated in the research design. J.G. conducted the pharmacology experiments. J.G., L.A.S., and S.J.H. performed the pharmacology-data analysis. All of the authors wrote or contributed to the writing of the manuscript.


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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS USED

ATP,adenosine 5′-triphosphate; BODIPY,boron-dipyrromethene; BODIPY,boron-dipyrromethene; BRET,bioluminescence-resonance-energy transfer; ESI,electrospray ionization; FC,flash chromatography; GPCR,G-protein-coupled receptor; HPLC,high-performance liquid chromatography; HRMS,high-resolution mass spectrometry; $K_d$,dissociation constant of a labeled ligand−receptor complex; $K_i$,dissociation constant of a ligand−receptor complex determined through a binding assay; LC-MS,liquid chromatography−mass spectrometry; MW, microwave; NanoBRET,nanoluciferase-bioluminescence-resonance-energy transfer; NLuc,nanoluciferase; PREP,preparative; RM,reaction mixture; RP,reverse phase; SAR,structure−activity relationship; TLC,thin-layer chromatography; UTPγS,uridine-5′-(γ-thio)-triphosphate; VCD,vibrational circular dichroism

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