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1 **Synthesis of bleomycin-inspired RNA ligands targeting the biogenesis of oncogenic miRNAs**

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4

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12

1 **Abstract**

2 Non-coding RNAs (ncRNAs) play pivotal roles in the regulation of gene expression and represent a  
3 promising target for the development of new therapeutic approaches. Among these ncRNAs,  
4 microRNAs (miRNAs or miRs) are involved in the regulation of gene expression and their  
5 dysregulation has been linked to several diseases such as cancers. Indeed, oncogenic miRNAs are  
6 overexpressed in cancer cells thus promoting tumorigenesis and maintenance of cancer stem cells that  
7 are resistant to chemotherapy and often responsible for therapeutic failure. Here we describe the  
8 design and synthesis of new small-molecule RNA binders able to inhibit the biogenesis of oncogenic  
9 miRNAs and targeting efficiently cancer stem cells. Through the biochemical study of their interaction  
10 with the target and thanks to intracellular assays, we describe the structure-activity relationships for  
11 this new series of RNA ligands and we identify compounds bearing a very promising antiproliferative  
12 activity against cancer stem cells.

13

14

## 1 **Introduction**

2 The majority of current marketed drugs is directed against protein targets despite the fact that  
3 druggable proteins represent an extremely small percentage of what the human genome encodes.<sup>1,2</sup>  
4 Indeed, the major part of intracellular DNA produces non-coding RNAs (ncRNAs) involved in the  
5 essential regulation of a large number of biological processes. Taking these ncRNAs into account as  
6 potential drug targets would thus greatly widen the landscape of current medicinal chemistry and some  
7 drugs are already on the market for this scope.<sup>3</sup> Among them, various classes of antibiotics, such as  
8 aminoglycosides, tetracyclines or oxazolidinones, impair protein synthesis in bacteria thanks to their  
9 ability to target the prokaryotic ribosomal RNA and have been on the market since many decades.<sup>4</sup>  
10 More recently, Risdiplam entered the market as a modulator of pre-mRNA splicing in Spinal Muscular  
11 Atrophy (SMA) to correct the splicing of SMN protein thus restoring its biological functions.<sup>5</sup> These  
12 examples show the potential of a therapeutic approach based on the targeting of biologically relevant  
13 RNAs and hold the promise for future discoveries.

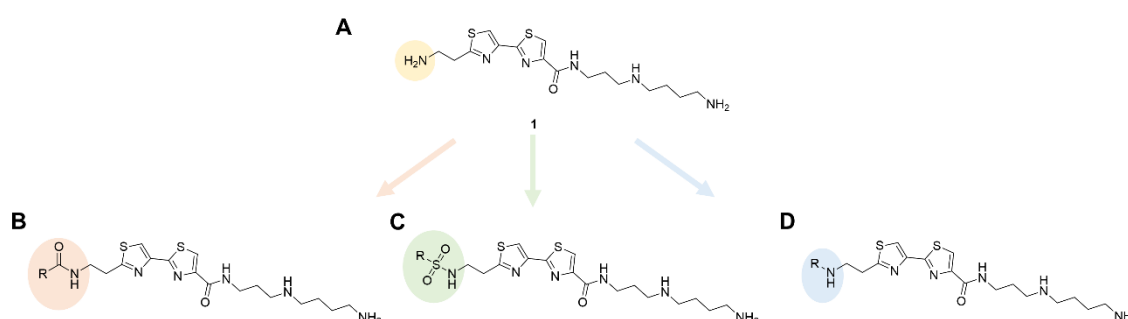
14 Among the most promising ncRNA targets, microRNAs (miRNAs or miR) have been explored since  
15 they are directly involved in the regulation of gene expression.<sup>6</sup> MiRNAs are short ncRNAs of about  
16 22-24 nucleotides able to inhibit mRNA translation upon recognition of various mRNA sequences.  
17 Each miRNA is responsible for the regulation of hundreds of proteins and the miRNAs network is  
18 pivotal for cellular homeostasis. Indeed, deregulations in the expression of miRNAs has been directly  
19 linked to a number of pathologies such as cancer. In this context, it has to be noted that miRNAs also  
20 emerged as key regulators of stemness, pluripotency maintenance, self-renewal control, differentiation  
21 and epithelial-to-mesenchymal transition (EMT) in cancer stem cells (CSCs). The presence of CSCs in  
22 several types of cancers is believed to be the main cause of failure of chemotherapy and radiotherapy  
23 and their targeting upon inhibition of specific miRNAs biogenesis could represent a promising  
24 strategy.<sup>7</sup> Various examples of small-molecule ligands were reported as inhibitors of the biogenesis of  
25 one or more overexpressed miRNAs in cancer.<sup>8</sup> Such inhibitors bind to the stem-loop structured  
26 precursors of the targeted miRNA (pre-miRNA or pri-miRNA) and block their processing thus  
27 inhibiting the production of the corresponding miRNA. Some of these compounds were discovered  
28 upon high-throughput screening, such as functionalized polyamines as inhibitors of miR-372  
29 biogenesis,<sup>9</sup> or by virtual screening such as compound AC1MMYR2 bearing inhibition activity for the  
30 same target.<sup>10,11</sup> The group of Disney and coworkers also developed a methodology to discover  
31 specific RNA ligands called Inforna allowing for the identification of binders specific for a particular  
32 RNA motif such as internal loops or bulges.<sup>12,13</sup> This efficient approach was applied to various  
33 oncogenic miRNAs with great successes in terms of specificity and biological activity in cells and *in*  
34 *vivo*.<sup>14,15,16</sup>

35 We also developed an original approach for the design of oncogenic miRNAs inhibitors based on the  
36 design and synthesis of multimodal binders where different RNA binding domains are conjugated to

1 bring both affinity and selectivity for a pre-miRNA target. In this context, we employed the  
2 aminoglycoside neomycin as a strong but non-selective ligand, artificial nucleobases able to  
3 selectively interact with RNA base pairs and amino acids constituting the natural RNA binders in  
4 peptides.<sup>17,18</sup> The resulting compounds were designed to target a particular oncogenic miRNA, miR-  
5 372, involved in the development of various cancers such as gastric cancers.<sup>19</sup> The biological  
6 evaluation of the synthesized conjugates *in vitro* and in cancer cells showed that some compounds  
7 displayed a specific inhibition of proliferation of cancer cells overexpressing the targeted miRNA,  
8 while cells that do not express miR-372 were not affected. The biological activity was directly linked  
9 to the inhibition of the biogenesis of miR-372 upon binding to its precursor pre-miR-372 and  
10 inhibiting its processing.

11 In the search for new RNA binding domains that could be added to this multimodal design, we then  
12 focused our attention on bleomycins. These anticancer natural compounds are glycopeptides isolated  
13 for the first time by Umezawa et al . from *Streptomyces verticellus* and that are composed of a metal-  
14 binding domain and a C-terminal substituted bithiazole known to be involved in DNA and RNA  
15 binding.<sup>20</sup> The anticancer activity of bleomycins is due to a complex mechanism of action. Indeed,  
16 bleomycins are topoisomerase II inhibitors but they are also able to induce iron-mediated cleavage of  
17 DNA and RNA. Recently the activity of bleomycins in RNA binding and cleavage has been studied  
18 showing that it is possible to take advantage of this kind of compounds to synthesize bioactive RNA  
19 ligands with RNA-cleavage properties.<sup>21</sup>

20 In this work, we decided to take advantage of the RNA binding properties of the C-terminal domain of  
21 bleomycin A5 (compound **1**, Figure 1A) to prepare new RNA binders as inhibitors of the biogenesis of  
22 oncogenic miRNAs. We first observed that **1** was a good RNA binder upon evaluation of its affinity  
23 for the precursor of miR-21 (pre-miR-21), this latter being a recognized oncogenic miRNA in various  
24 cancers.<sup>22</sup> Given that its affinity for the target was particularly promising, we decided to synthesize  
25 new analogs of **1** upon coupling new substituents on one of the free amino groups and using three  
26 different linkages: (i) amide (Figure 1B), (ii) sulfonamide (Figure 1C) and (iii) secondary amine  
27 (Figure 1D).



28  
29 **Figure 1.** Chemical structure of compound **1** constituted by the bithiazole-spermidine side chain of

1 bleomycin A5 (A) and general structures of the new series of analogues synthesized and presented in  
2 this work: amide derivatives (B), sulfonamide derivatives (C) and reductive amination derivatives (D).

3  
4 This led us to the preparation of 24 new compounds whose biological evaluation led to the  
5 identification of conjugates able to bind selectively oncogenic pre-miRNAs and inhibit their  
6 processing into mature miRNA. We also assessed the biological activity in cancer cells as well as the  
7 toxicity on healthy cells to identify two hit compounds showing a promising antiproliferative activity  
8 on glioblastoma cancer cells. The most promising compounds were finally evaluated on patient-  
9 derived cell lines to appreciate their ability to target cancer stem cells. The ability of some of the  
10 newly synthesized compounds to target efficiently cancer stem cells associated with the correlation  
11 between this activity and the inhibition of oncogenic miRNAs maturation make them promising leads  
12 bearing an original mechanism of action for future developments.

13

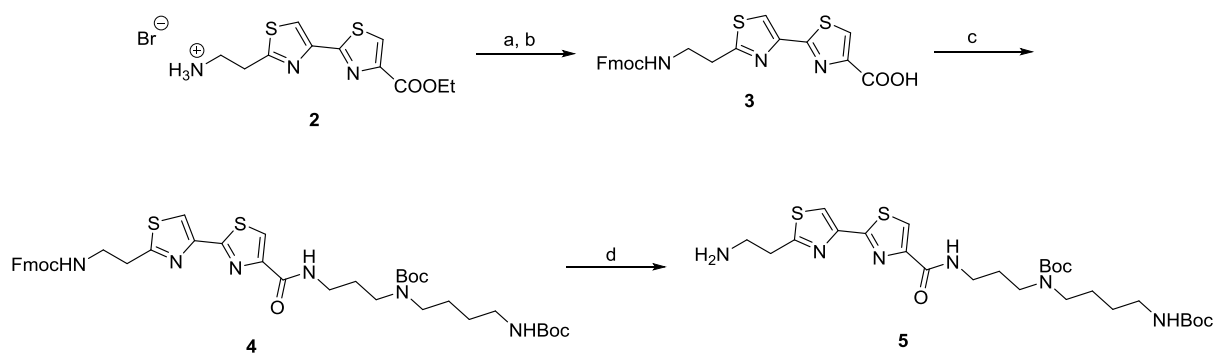
## 14 **Results and Discussion**

### 15 **Design and synthesis of new bithiazole derivatives**

16 The aim of this work is to discover new RNA ligands able to inhibit the biogenesis of oncogenic  
17 miRNAs in a selective manner and induce an inhibition of proliferation of cancer cells. To design new  
18 original and effective RNA binders, we decided to take advantage of the DNA/RNA binding domain  
19 of bleomycin A5. The complex mechanism of action of bleomycins as anticancer agents involves three  
20 levels.<sup>20</sup> First, bleomycins interact with DNA and RNA thanks to the presence of a C-terminal domain  
21 including a bithiazole scaffold bearing a positively charged side chain. Second, bleomycins induce the  
22 cleavage of DNA and RNA thanks to the iron chelating properties of the N-terminal domain including  
23 a pyrimidine, an acetyl chain and a  $\beta$ -aminoalanine. Third, bleomycins target preferentially cancer  
24 cells thanks to the presence of the carbohydrate moiety. In this work, we chose the C-terminal domain  
25 of bleomycin A5 where the lateral chain is a spermidine conjugated to the bithiazole scaffold  
26 (compound **1**, Figure 1A) to measure its affinity for an oncogenic pre-miRNA and in particular for  
27 pre-miR-21. Compound **1** was previously reported as a strong DNA binder lacking the cleavage  
28 potency of bleomycin.<sup>23</sup> The targeted pre-miR-21 is the direct precursor of miR-21 which is an  
29 oncogenic miRNA in a wide variety of cancers and has been widely studied in the literature for its role  
30 in oncogenesis. A preliminary study was first performed on compound **1** to assess if it could represent  
31 a good and selective RNA binder. To this aim, we applied a previously reported fluorescence-based  
32 assay where the targeted RNA sequence is labeled at the 5'-end with a fluorophore. Binding of a small  
33 molecule will induce a change in the environment of the fluorophore and a variation of fluorescence.<sup>24</sup>  
34 The result of this first assay showed that compound **1** bears a very promising affinity for pre-miR-21  
35 of 3.63  $\mu$ M and maintains its affinity when in competition with a large excess of tRNA or DNA  
36 (Figure 2 and Table S1 in Supporting Information) thus suggesting the ability to selectively bind pre-

1 miR-21 with respect to other nucleic acid structures. A FRET-based assay was then used to measure  
2 the ability of **1** to inhibit pre-miR-21 processing by Dicer.<sup>24</sup> In this assay, the pre-miRNA target is  
3 double labeled with a fluorophore at the 5'-end and a quencher at the 3'-end in order to have  
4 appearance of fluorescence when Dicer cleaves the pre-miRNA. In the presence of RNA binders able  
5 to inhibit Dicer processing the increase of fluorescence is inhibited. In this assay, compound **1** did not  
6 show the ability to inhibit pre-miR-21 processing. We thus designed new analogs of this compound to  
7 assess whether it would be possible to obtain compounds displaying improved affinity, selectivity as  
8 well as an inhibition activity for pre-miR-21 processing. Various positions of compound **1** could be  
9 modified and here we decided to substitute the free amine linked to the bithiazole while keeping the  
10 spermidine moiety intact (Figure 1B-D). This allows us to keep the main scaffold of compound **1**  
11 intact to avoid major changes that could reduce affinity. We introduced various aliphatic, aromatic and  
12 heteroaromatic substituents *via* the formation of amide (Figure 1B), sulfonamide (Figure 1C) or  
13 reductive amination products (Figure 1D). These three links allow for the introduction of new binding  
14 domains while maintaining the –NH– group that could be involved in the formation of relevant  
15 hydrogen bonds or electrostatic interactions with the target. The chosen substituents were chosen for  
16 their ability to form selective interactions to preferentially bind the structure of targeted pre-miRNA  
17 over different and unrelated DNA and RNA structures as it will be detailed below.

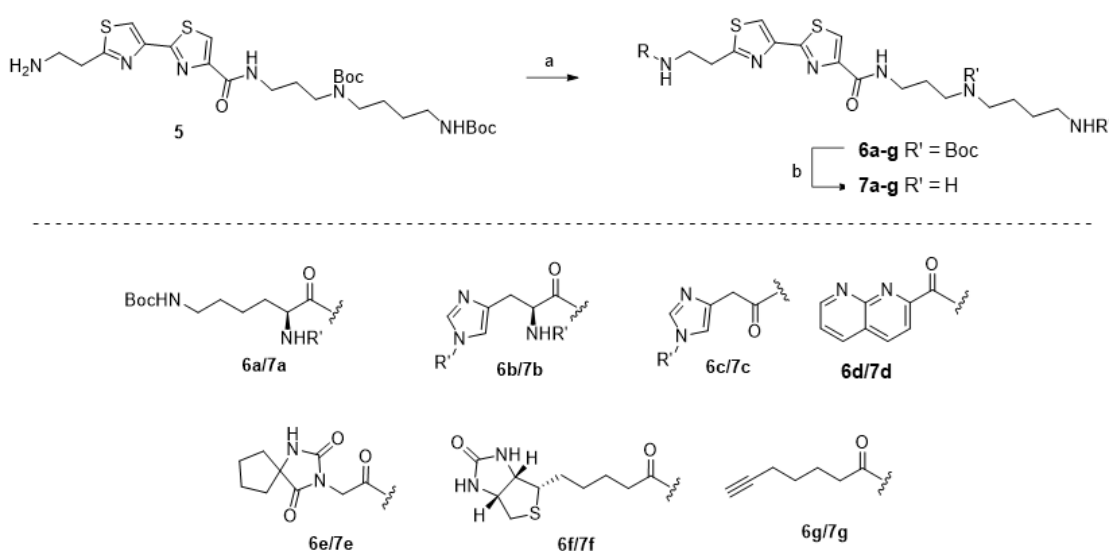
18 To synthesize these analogs, we first needed to prepare a common precursor containing the desired  
19 amino group available for coupling while protecting the others. To this aim, we prepared intermediate  
20 **5** as illustrated in Scheme 1. Indeed, we started with compound **2**, prepared following previously  
21 reported procedures,<sup>23</sup> and performed a saponification of the terminal ester directly followed by  
22 protection of the amine using a Fmoc group that led to the formation of compound **3** in 90% yield over  
23 the two steps. Peptide coupling of **3** with *N4,N8*-di-tert-butoxycarbonylspermidine was then performed  
24 in the presence of EDC and HOSu leading to compound **4** in 83% yield. The deprotection of the  
25 Fmoc-protected amine in the presence of piperidine in DMF led to **5** in 83% yield, allowing for the  
26 following the specific modification of the free amine. Once this key intermediate in hand, we reacted  
27 the primary amine with the appropriate substituents to obtain new original compounds.



28

1 **Scheme 1.** Synthesis of common precursor **5**. Reagents: a) LiOH, H<sub>2</sub>O/ THF, 0°C, r.t.; b) FmocOSu,  
2 H<sub>2</sub>O/Dioxane, r.t.; c) *N*<sup>4</sup>,*N*<sup>8</sup>-di-tert-butoxycarbonylspermidine, EDC, HOSu, DCM, r.t.; d) Piperidine,  
3 DMF, r.t..

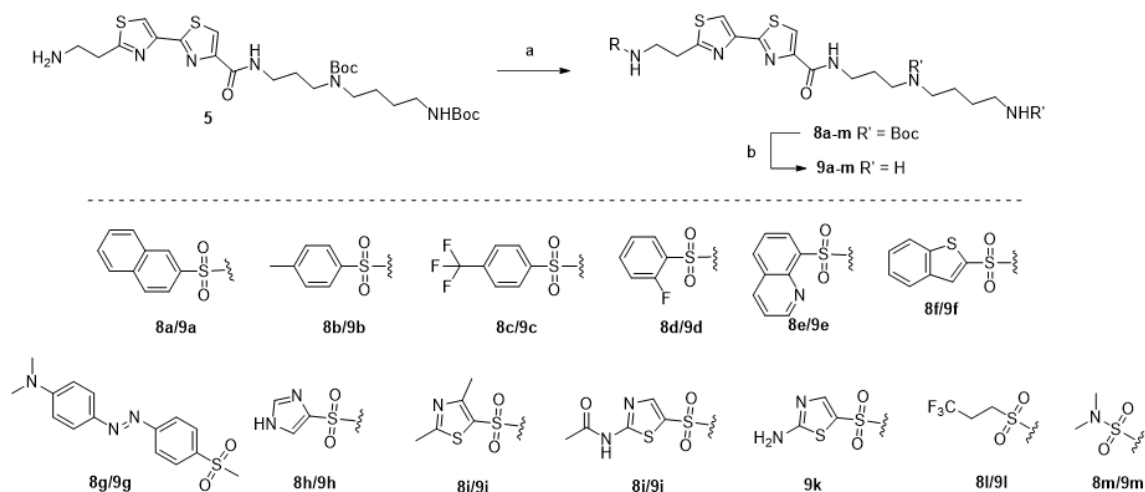
4  
5 As illustrated in Scheme 2, in the context of the amide series, we decided to use a direct amide  
6 formation upon reacting compound **5** with carboxylic acids (see Figure S2 in the Supporting  
7 Information for the structures of the employed carboxylic acids **12a-g**). To improve the RNA binding  
8 properties of compound **1**, we first chose lysine (as in **7a**) and histidine (as in **7b**) as basic amino acids  
9 that previously showed very good binding features when used in other conjugates.<sup>25</sup> We also coupled **5**  
10 with compound **12c** (as in **7c**) to assess the importance of the α-NH<sub>2</sub> of histidine in binding. These  
11 moieties were compared with extended aromatic substituents such as 1,8-naphthyridine (as in **7d**). This  
12 moiety was previously described in other selective RNA ligands.<sup>26</sup> Finally, aliphatic and sp<sup>3</sup>-  
13 containing structures were used such as 2,4-dioxo-1,3-diazaspiro[4,4]nonan (as in **7e**), biotin (as in **7f**)  
14 and pentynyl (as in **7g**) substituents. We chose to perform the peptide coupling in the presence of EDC  
15 and HOSu for the carboxylic acids **12a-f** and in the presence of HBTU and DIPEA for the carboxylic  
16 acid **12g**. The use of an uronium reagent for the coupling of compound **12g** was preferred in order to  
17 increase the carboxyl activation, since a too weak activation was observed in the presence of  
18 carbodiimide, certainly due to the weak reactivity of this carboxylic acid. Compounds **6a-g** were  
19 obtained with yields ranging from 22 to 90%. The lowest yields were observed for the preparation of  
20 compounds **6c** and **6d** and were due to difficulties during purification steps. A final deprotection step  
21 in acidic medium led to desired conjugates **7a-g** in 68-97% yields.



22  
23 **Scheme 2.** Synthesis of the amide series: compounds **7a-g**. Reagents: a) **12a-f** (Figure S2 in  
24 Supporting Information), EDC, HOSu, r.t. / **12g**, HBTU, DIPEA, r.t.; b) TFA, DCM, r.t. or HCl,  
25 MeOH, r.t..



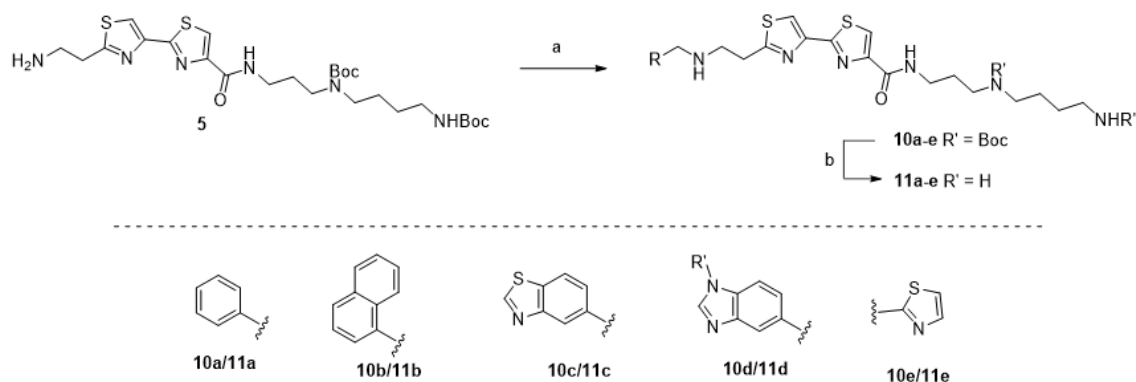
1  
 2 In the context of the sulfonamide series, as illustrated in Scheme 3, compound **5** was coupled with  
 3 commercially available sulfonyl chlorides (see Figure S3 in Supporting Information for the employed  
 4 sulfonyl chloride **13a-m**) leading to the preparation of twelve derivatives (**9a-9m**). Regarding the  
 5 choice of moieties, substituted and unsubstituted phenyls (as in **9a-d**) were first exploited to provide  
 6 additional interactions with RNA such as  $\pi$ -stacking. Thus, the use of naphthalene and different  
 7 phenyls substituted by a methyl or fluorinated group allow us to evaluate the influence and the type of  
 8 additional interactions that these groups could bring. Indeed, this type of compounds can interact with  
 9 RNA by hydrophobic interactions or non-classical hydrogen bonds made by the fluorine atoms. Next,  
 10 different heteroaromatic rings were exploited to evaluate the influence of different heteroatoms. We  
 11 chose the benzothiophene (as in **9f**), quinolone (as in **9e**), imidazole (as in **9h**) and thiazole analogs (as  
 12 in **9i**, **9j** and **9k**) moieties for their RNA ligand properties. Indeed, these moieties can interact with  
 13 nucleic acids either by intercalation or *via* a network of electrostatic and non-electrostatic bonds. A  
 14 diazobenzene moiety, inspired by the structure identified by Deiters et al. in an HTS screen,<sup>27</sup> was also  
 15 employed (as in **9g**) since it is capable of bringing about both  $\pi$ -stacking interactions and new  
 16 hydrogen bonds. Finally, aliphatic chains and methylated amines (as in **9l** and **9m**) were also tested.  
 17 Sulfonyl chlorides **13a-l** were coupled to the free amine of compound **5** under basic conditions in  
 18 DMF to form compounds **8a-8l** in yields ranging from 29 to 81%. The final step of cleavage of the  
 19 Boc groups led to desired compounds **9a-9m** in yields ranging from 50 to 99% over two steps.



20  
 21 **Scheme 3.** Synthesis of the sulfonamide series: compounds **9a-m**. Reagents: a) **13a-m** (Figure S3 in  
 22 Supporting Information), Et<sub>3</sub>N, DMF, r.t.; b) TFA, DCM, r.t. or HCl, MeOH, r.t. or HCl, MeOH,  
 23 65°C.

24  
 25 Finally, we chose to introduce new functional groups on the amine of **5** *via* reductive amination upon  
 26 reaction with aldehydes **14a-d** (see Figure S4 for the structures of these reagents). Concerning the

1 chosen groups, we opted for phenyl (as in **11a**) and naphthalene (as in **11b**) allowing to bring  
 2 additional interactions with RNA such as  $\pi$ -stacking but heteroaromatic motifs were also used with  
 3 benzothiazole (as in **11c**), and benzimidazole (as in **11d**). Benzimidazole is often employed for the  
 4 preparation of RNA ligands<sup>28</sup> and benzothiazole was chosen by analogy to this moiety. These two  
 5 motifs will be compared with phenyl to conclude on the influence of heteroatoms in this type of  
 6 structures. Thus, the reaction between the aldehydes **14a-d** and **5** allowed the formation of compounds  
 7 **10a-d** with yields ranging from 38% to 90% (Scheme 4). Following a deprotection reaction,  
 8 compounds **11a-d** were obtained in yields from 82 to 95%.



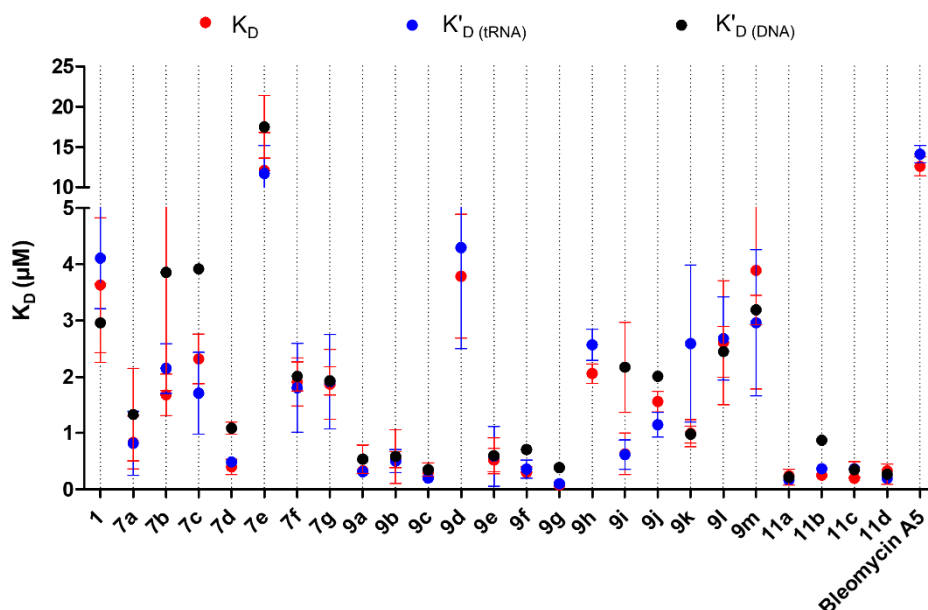
9  
 10 **Scheme 4.** Synthesis of the reductive amination series: compounds **11a-d**. Reagents: a) **14a-d**, AcOH,  
 11 NaBH<sub>3</sub>CN, MeOH, r.t.; b) HCl, MeOH, r.t.

12  
 13 All compounds were fully characterized by NMR, HRMS and HPLC before using them in  
 14 biochemical and biological assays.

15  
 16 ***In vitro* affinity for pre-miR-21**

17 Once the molecules synthesized, the *in vitro* biochemical evaluation was performed on pre-miR-21 as  
 18 previously done for compound **1** in order to assess whether the added modifications had improved the  
 19 affinity for the target. As illustrated in Figure 2 and Table S1 in the Supporting Information, the  
 20 dissociation constants obtained for amides **7a-g** are in the low micromolar range except **7d** which has  
 21 a submicromolar K<sub>D</sub>. More specifically, compound **1** has a K<sub>D</sub> of 3.63  $\mu$ M while compounds **7a** and  
 22 **7b**, bearing the addition of an amino acid, have an increased affinity for the target (K<sub>D</sub> of 0.835 and  
 23 1.68  $\mu$ M, respectively). When the histidine of **7a** was replaced by the imidazole as in **7c**, the affinity  
 24 for pre-miR-21 was only slightly reduced suggesting that the  $\alpha$ -amino group is not involved in major  
 25 interactions with the target. Increasing the aromaticity as in naphthyridine compound **7d** allowed a  
 26 strong increase in the affinity with a K<sub>D</sub> of 0.400  $\mu$ M and this compound is the best ligand of this  
 27 series. The presence of aliphatic substituents as in **7f** and **7g** led to K<sub>D</sub> of 1.91 and 1.87  $\mu$ M,  
 28 respectively, in the same range as the other derivatives. The introduction of new substituents *via* an

1 amide bond thus allowed us to improve binding in particular with compound **7d** as the most promising  
2 bearing an affinity 9 times better than parent compound **1**. Noteworthy, bleomycin A5 bears a  $K_D$  of  
3  $12.6 \pm 1.2 \mu\text{M}$  when tested in the same conditions.



4  
5 **Figure 2.**  $K_D$  values (●) of compounds **1**, **7a-g**, **9a-l** and **11a-d** against pre-miR-21 72-mer fragment  
6 labeled at the 5'-end with Fluoresceine. Selectivity for pre-miR-21 in the presence of 100 eq. of tRNA  
7 (●) and of DNA (●) is reported.  $K_D$  values were determined from duplicates performed over three  
8 independent experiments using the change in fluorescence intensity. Error bars represent the standard  
9 deviation determined from three independent experiments.

10  
11 Regarding the sulfonamide series, most of the newly synthesized compounds showed excellent affinity  
12 for pre-miR-21 and several compounds, such as **9a**, **9b**, **9c**, **9e**, **9f**, **9g** and **9i** have sub-micromolar  
13 affinities. These compounds contain extended aromatic substituents (**9a**, **9e**, and **9f**) or a p- $\text{CF}_3$ -phenyl  
14 substituent (**9c**) and bear  $K_D$  as low as 76 nM for **9g**. All other compounds bear  $K_D$  between 0.330  $\mu\text{M}$   
15 to 3.79  $\mu\text{M}$ . These results suggest that the presence of aromatic and heteroaromatic motifs and of a  
16 sulfonamide linkage are particularly favorable for the affinity. Compound **9g** is the strongest binder of  
17 this series with an affinity more than 50 times higher than parent compound **1**.

18 Finally, compounds **11a-d** obtained upon reductive amination showed excellent submicromolar  
19 dissociation constants suggesting that the introduction of a secondary amine function during  
20 conjugation is also favorable for affinity. In particular, the aromatic substituents of **11a** and **11b**  
21 increased the affinity with  $K_D$  of 0.240  $\mu\text{M}$  and 0.255  $\mu\text{M}$ , respectively. These affinity values were  
22 maintained using the heterocycles benzothiazole as in **11c** and benzimidazole as in **11d**.

23 A first assessment of the selectivity of the newly synthesized compounds was performed upon  
24 measurement of  $K_D$ s in the presence of a large excess (100 eq.) of competitors, such as tRNA and

1 DNA, that are largely present inside cells and could represent potential off targets. Interestingly, all  
 2  $K_{D}$ s were maintained in the presence of tRNA (Figure 2 and Table S1 in the Supporting Information)  
 3 suggesting that these compounds bind preferentially to the pre-miRNA stem-loop instead of more  
 4 structured RNAs. More important differences were observed in the presence of DNA, regardless of the  
 5 sequence, since some compounds lost their affinity of 3 to 6 times. As expected, bleomycin A5 loses  
 6 its affinity for pre-miR-21 when this latter is in competition with DNA.

7

### 8 **Inhibition of pre-miR-21 processing by Dicer**

9 To test if the improved binding affinity of the newly synthesized conjugates would lead to better  
 10 inhibition activity, we measured  $IC_{50}$ s for the inhibition of pre-miR-21 processing by Dicer of all  
 11 conjugates. Surprisingly, only some of them were able to inhibit the Dicer cleavage of pre-miR-21 and  
 12 these compounds are reported in Table 1 with the corresponding  $IC_{50}$  values. Indeed, only compounds  
 13 **7f**, **9a**, **9c**, **9f-i**, **11a** and **11c-d** showed a measurable  $IC_{50}$  value. Compounds **9g** and **11c** showed a very  
 14 good inhibition activity with an  $IC_{50}$  of 1.62  $\mu$ M for **9g** and 3.74  $\mu$ M for **11c** leading to the observation  
 15 that the best ligands are also the best inhibitors. Compounds **9a**, **9c**, **9f**, **9h** and **11d** bearing mainly  
 16 submicromolar  $K_D$  showed  $IC_{50}$  in the range of 10-20  $\mu$ M. Finally, compounds **7f**, **9i** and **11a** showed  
 17  $IC_{50}$ s higher than 20  $\mu$ M.

18

19 **Table 1.**  $IC_{50}$  values ( $\mu$ M) measured for compounds indicated against the processing of pre-miR-21 by  
 20 recombinant enzyme or cell lysates and the corresponding ratio.

<b>ID</b>	<b><math>IC_{50}</math> (recombinant enzyme)</b>	<b><math>IC'_{50}</math> (cell lysates)</b>	<b><math>IC'_{50}/IC_{50}</math></b>
<b>1</b>	no inhibition	-	-
<b>7f</b>	38.3 $\pm$ 3.1	no inhibition	-
<b>9a</b>	17.3 $\pm$ 1.2	148 $\pm$ 11.2	8.5
<b>9c</b>	16.4 $\pm$ 1.3	92.8 $\pm$ 5.8	5.6
<b>9f</b>	11.8 $\pm$ 1.7	55.5 $\pm$ 9.2	4.7
<b>9g</b>	1.62 $\pm$ 0.090	6.93 $\pm$ 2.4	4.3
<b>9h</b>	20.3 $\pm$ 1.8	not tested	-
<b>9i</b>	75.9 $\pm$ 6.6	not tested	-
<b>11a</b>	31.2 $\pm$ 6.7	195 $\pm$ 12.3	6.2
<b>11c</b>	3.74 $\pm$ 0.77	4.68 $\pm$ 0.52	1.2
<b>11d</b>	20.3 $\pm$ 1.9	50.1 $\pm$ 4.3	2.5
<b>Bleomycin A5</b>	no inhibition	-	-

$IC_{50}$  experiments were performed in the presence of 50 nM of pre-miR-21 beacon and 0.5 U of recombinant enzyme or 250  $\mu$ g/mL of MCF7 whole cell lysates in buffer A (20 mM Tris-HCl, pH 7.4, 12 mM NaCl, and 1 mM DTT).

21

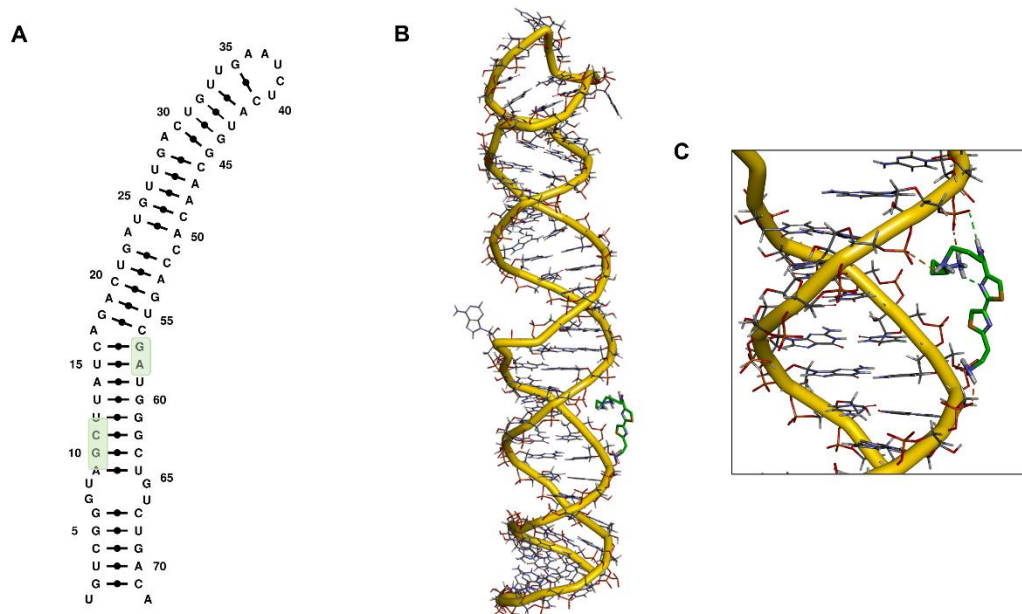
1 As demonstrated by these values, and the fact that all other compounds were not able to inhibit Dicer  
2 processing of pre-miR-21 at the tested concentrations, we could not observe a clear correlation  
3 between  $K_D$  and  $IC_{50}$  because binding itself is not sufficient for efficient inhibition. Binding to a  
4 functional binding site, i.e. the site of cleavage by Dicer, is essential, so compounds having the same  
5 affinity can have different activity if the binding site changes.<sup>29</sup> Noteworthy, bleomycin A5 did not  
6 show any inhibition activity at the tested concentrations (up until 1 mM) further proving that the newly  
7 synthesized compounds show different properties with respect to bleomycin itself. Altogether, the  
8 obtained results suggest that sulfonamides and reductive amination products are the best binders and  
9 inhibitors, with compounds **9g** and **11c** leading to the best results in terms of affinity, selectivity and  
10 inhibition activity.

11

### 12 **Molecular docking**

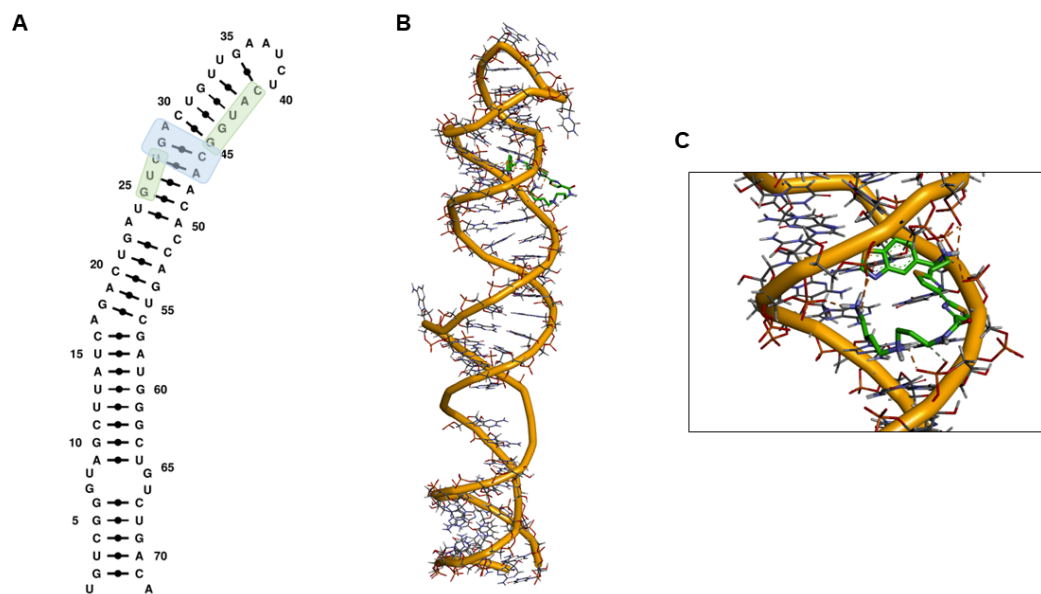
13 As mentioned above, there is for some compounds a lack of correlation between binding affinity and  
14 inhibition activity. This is illustrated by the fact that even if the best inhibitors are among the best  
15 binders, some compounds bearing a very good affinity did not show a good inhibition activity. To gain  
16 further insights into the binding site of these compounds, we chose to perform molecular docking  
17 studies on pre-miR-21 target for parent compound **1**, having a good  $K_D$  but no inhibition, for  
18 compound **9e**, bearing excellent affinity but no inhibition activity as well as the two best binders and  
19 inhibitors **9g** and **11c**. For this purpose, the 3D stem-loop sequence of the studied pre-miRNAs was  
20 generated from the primary and secondary sequences ([www.miRBase.org](http://www.miRBase.org)) and the 3D model was then  
21 constructed using the MC Fold/MC-Sym protocol (<https://major.ircic.ca/MC-Fold/>). Molecular docking  
22 using AutoDock provides the ten conformations with the lowest energy levels and the study of each of  
23 them allows a hypothesis about the interaction site. For the studied compounds, we chose to illustrate  
24 the binding site that most often occurs in the ten obtained poses. This means that other binding sites  
25 are possible and that the obtained results suggest a possible explanation for the correlation between  
26 affinity and inhibition activity.

27 The docking of **1** with pre-miR-21 is illustrated in Figure 3 and suggests a binding site in the major  
28 groove of pre-miR-21, close to nucleotides G10 and G57. The fact that the proposed binding site is not  
29 located in the cleavage site of Dicer enzyme could contribute to the lack of activity of this compound.



1  
 2 **Figure 3.** Pre-miR-21 hairpin loop where the region of the cleavage site of Dicer is indicated in blue  
 3 and the one of binding of compound **1** is indicated in green (A). Docking of **1** with pre-miR-21 (B and  
 4 C) performed by using autodock 4, in which the grid boxes were fixed on the entire RNA sequence.

5  
 6 As illustrated in Figure 4, the docking of **11c** to pre-miR-21 suggest a binding site in the double-  
 7 stranded region close to the catalytic site of Dicer. This could explain not only the better affinity of  
 8 this compound compared to **1**, but also the good inhibition activity, the binding site being more  
 9 favorable for blocking Dicer processing. Similarly, as illustrated in Figure S6 in Supporting  
 10 Information, **9g** could also interacts with residues close to the cleavage site of Dicer enzyme. The  
 11 comparison of these docking results with the one obtained in the presence of compound **9e**, bearing  
 12 good affinity but weak inhibition activity, suggested that compound **9e** could interact in the region of  
 13 nucleotides U15-C16 and C51-C52 and its the binding site is probably not favorable for inhibition as  
 14 observed for **1**.



1  
 2 **Figure 4.** Pre-miR-21 hairpin loop where the region of the cleavage site of Dicer is indicated in blue  
 3 and the one of binding of compound **11c** is indicated in green (A). Docking of **11c** with pre-miR-21 (B  
 4 and C) performed by using autodock 4, in which the grid boxes were fixed on the entire RNA  
 5 sequence.

6  
 7 In conclusion, while docking studies cannot demonstrate which is the exact binding site of the studied  
 8 compounds, differences in the site of interaction of compounds bearing similar affinities but different  
 9 inhibition activities could be explained by differences in the binding site. The binding sites of the two  
 10 active molecules **9g** and **11c** seems to be probably close to the cleavage region of Dicer on pre-miR-  
 11 21. In contrast, compounds **1** and **9e** that are not active, seem to interact in the stem region that does  
 12 not appear to prevent Dicer processing.

### 14 **Selectivity profile**

15 Compounds **9g** and **11c** are the best inhibitors of pre-miR-21 processing since their binding site is  
 16 particularly favorable for the activity. To further appreciate the potential of these compounds, various  
 17 assays to evaluate their selectivity have been performed. Selectivity of RNA ligands is a major  
 18 concern in the field since the RNA sequence and structure offer various binding possibilities especially  
 19 when the ligand is represented by a positively charged molecule as is the case for the compounds  
 20 presented here. As described above, all compounds have been tested for their selectivity against tRNA  
 21 and DNA representing abundant competitors inside cells, and compound **11c** was among the most  
 22 selective (Figure 2). However, given the importance of the selectivity features of this kind of  
 23 compound, we decided to perform further assays.

1 First, to assess the selectivity of inhibition in the presence of intracellular competitors, we measured  
2 the  $IC_{50}$ s of the best inhibitors using cell lysates instead of the recombinant enzyme. The obtained  
3 results, illustrated in Table 1, showed that all compounds, except **11c**, lose activity from 2.5 times  
4 (**11d**) to 8.5 times (**9a**) until compound **7f** that loses entirely its activity of inhibition. **11c** maintains a  
5 low  $IC_{50}$  with lysates (3.74  $\mu$ M in the presence of recombinant enzyme and 4.68  $\mu$ M in the presence of  
6 cell lysates) suggesting an excellent selectivity for the target.

7 Second, to evaluate the ability of the synthesized compounds to bind other pre-miRNA structures, we  
8 measured the dissociation constants of all compounds against three other pre-miRNAs sequences: pre-  
9 miR-18a, pre-miR-148a and pre-miR-373. All these miRNAs are oncogenic in various cancers. Pre-  
10 miR-18a belongs to the miR-17-92 cluster and is highly expressed in glioblastoma, esophageal cancer,  
11 colon cancer, pancreatic cancer and liver cancer tissues.<sup>30</sup> MiR-148a is aberrantly expressed in various  
12 cancers and has been identified as an oncogenic in glioblastoma or tumor suppressor in other cancers  
13 including gastric, colorectal or pancreatic cancer.<sup>31</sup> MiR-373 has been identified as oncogenic in  
14 gastric cancer as well as various other tissues.<sup>32</sup> The comparison of  $K_D$  values measured on the  
15 corresponding pre-miRNAs showed some differences in the binding affinities even if all compounds  
16 maintain a good affinity for these other pre-miRNAs (Table S2 in Supporting Information). More  
17 specifically, compound **1** shows 8-fold improvement in affinity for pre-miR-18a and pre-miR-148a,  
18 while **9g** and **11c** showed similar affinities for the four tested pre-miRNAs.

19 Third, we measured  $IC_{50}$  values for these three pre-miRNAs and their processing by Dicer enzyme. In  
20 accordance with the  $K_D$  values, the tested compounds were able to inhibit Dicer processing of the three  
21 additional miRNAs in a similar way than pre-miR-21 (Table S3 in Supporting Information). However,  
22 some differences have been detected since compound **9c** loses activity when tested on pre-miR-373  
23 while **9a**, **9f** and **11a** are more active on pre-miR-373 than the other three pre-miRNAs. The most  
24 active compounds on pre-miR-21 processing inhibition, compounds **9g** and **11c**, are also good  
25 inhibitors of the processing of the other pre-miRNAs. The obtained result demonstrates that **11c** is not  
26 only a very strong pre-miRNA binder but also the most efficient and selective inhibitor with a clear  
27 selectivity for pre-miRNAs structures.

28 Altogether, these selectivity assays, combined with the previous ones in the presence of tRNA and  
29 DNA, show that even if the synthesized compounds do not discriminate between the four tested  
30 oncogenic pre-miRNAs, differences in affinity and inhibition activities can be observed suggesting  
31 that it is possible to obtain partial selectivity as shown by tRNA and DNA competition experiments  
32 and by inhibition experiments in the presence of cell lysates. This suggests that it could be possible to  
33 reach the desired biological activity without any visible toxicity. This further support the hypothesis  
34 that the molecular mechanism of inhibition relies on RNA binding and not on non-selective Dicer  
35 inhibition. Indeed, we previously demonstrated that targeting a small set of miRNAs could be an  
36 efficient approach to inhibit the proliferation of cancer cells overexpressing these miRNAs and that

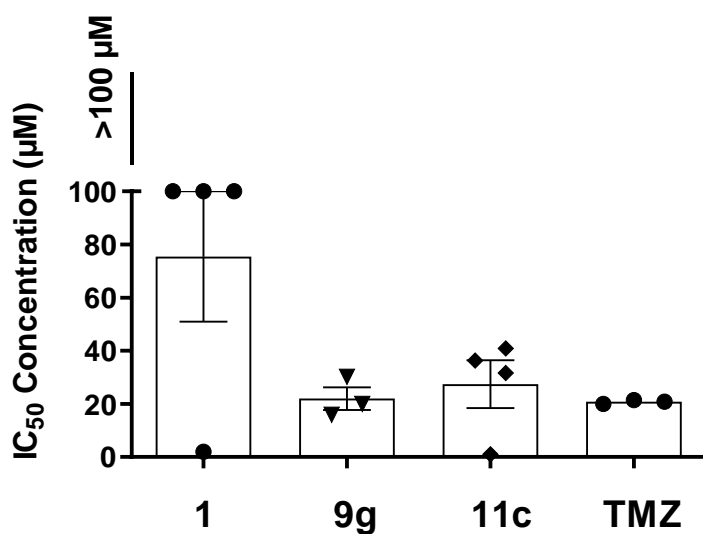


1 selectivity in cells depends upon miRNAs levels of expression.<sup>9</sup> We thus decided to pursue the  
2 evaluation of the most promising compounds in cell cultures.

#### 4 **Study of the intracellular activity**

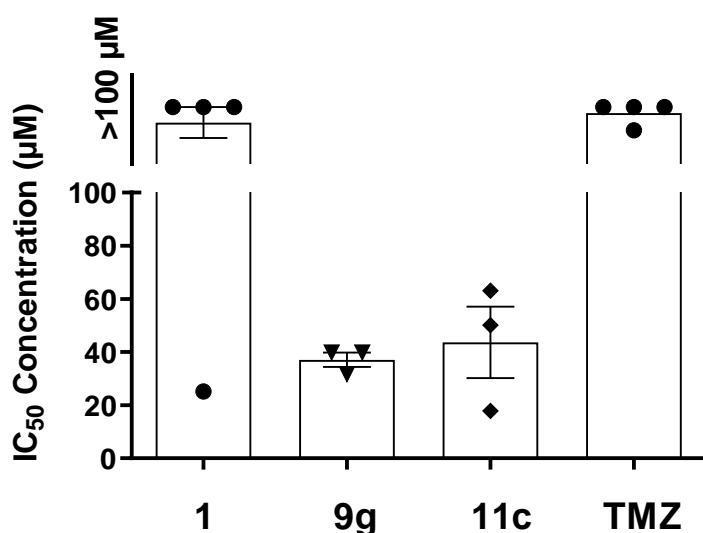
5 The studied pre-miRNAs are overexpressed and oncogenic in various cancers. While it is known that  
6 these pre-miRNA targets are quite flexible and can adapt to different ligands thus being difficult to  
7 target specifically, it is important to note that interfering with a set of oncogenic miRNAs instead of  
8 specifically with one of them, could lead to a more comprehensive and yet specific biological activity.  
9 To test the biological activity of the most promising compounds in cells, we thus chose glioblastoma  
10 U87 cell line. Glioblastoma is an aggressive type of brain tumor with a life expectancy at the moment  
11 of diagnosis included between 12 and 24 months. No cure exists for this cancer since while surgical  
12 resection associated to radiotherapy and chemotherapy increase life expectancy of a few months, these  
13 treatments are unfortunately palliative. The most commonly employed chemotherapeutic agent is  
14 temozolomide (TMZ) that is a cytotoxic alkylating agent. The therapeutic failure in the treatment of  
15 glioblastoma seems to be mainly due to the heterogeneity of this type of cancer and to the presence of  
16 stem-like cells that are tumorigenic and resistant to chemotherapy. We thus decided to assess  
17 antiproliferative activity of the synthesized compounds in cancer cells, measuring the effect of all  
18 compounds on the cell viability of U87 glioblastoma cell line. It has been largely demonstrated in the  
19 literature that this cell line overexpresses miR-21, miR-18a and miR148a.<sup>29,33</sup> Among all synthesized  
20 compounds, only **1**, **9g** and **11c** were able to inhibit the proliferation of U87 cells with IC<sub>50</sub> values of  
21 37.4 μM for **1**, 45.3 μM for **9g** and 16.5 μM for **11c** (Table 2 and Figure S7 in Supporting  
22 Information) while the IC<sub>50</sub> of TMZ is reported to be over 100 μM.<sup>34,35</sup> All compounds were also  
23 tested on MCR5 fibroblasts to assess the potential off-target toxicity of the active compounds on  
24 healthy cells. Compound **1** is also toxic on healthy cells, showing the same IC<sub>50</sub> value on MCR5 cells,  
25 compound **9g** shows a 30% inhibition of proliferation at 50 μM while **11c** is not toxic for MCR5 in the  
26 range of tested concentrations. Compound **11c** that is the one bearing the best results during *in vitro*  
27 studies of affinity and selectivity, is thus also the best inhibitor of the proliferation of U87 cells  
28 without apparent toxicity. This suggests that even if **9g** and **11c** showed the ability to bind other pre-  
29 miRNAs, this does not induce toxicity and could even increase the phenotypic effect since miR-148a  
30 and miR-18a are also overexpressed in U87 cells. Indeed, it has been already suggested that interfering  
31 with a network of oncogenic miRNAs could be a more efficient approach for a comprehensive  
32 antiproliferative activity.

1 A



2

3 B



4

5 **Figure 5.** Cell viability measured using wst-1 assay of two different primary cultures of glioblastoma  
6 exposed to compounds **1**, **9g** and **11c**. A) Cell viability of N13-1300 PDCL and B) N15-0385 PDCL.

7

8 As mentioned above, the main hurdle in the treatment of glioblastoma is the presence of CSCs that are  
9 resistant to chemotherapy. During recent years, a great interest has been put into the search for  
10 compounds able to inhibit the proliferation of CSCs with the identification of some compounds acting  
11 specifically on these cells, such as salinomycin.<sup>36</sup> One of the methodologies employed to study the  
12 effect of small molecules on the proliferation of CSCs is to employ primary cultures or patient-derived  
13 cell lines (PDCLs) that mainly contain CSCs and best represent the heterogeneity of the tumor in  
14 patients. The use of primary cultures for intracellular evaluation of cytotoxic compounds allows for

1 the appreciation of the effect of the molecules on cells that are phenotypically and genetically closer to  
 2 patients' cells and to assess the effect of the inhibitors on the proliferation of CSCs. Thus, to confirm  
 3 the potential of these compounds for the inhibition of proliferation of glioblastoma cancer cells, we  
 4 also performed viability assays on GBM PDCLs. To this aim, we employed two PDCLs (N13-1300  
 5 and N15-0385) that are part of the GloTEx cell line bank (ICM, Paris Brain Institute), cultured under  
 6 neurosphere conditions.<sup>37,38,39</sup> As illustrated in Figure 5, while the impact of **1** on these PDCLs is  
 7 variable and overall does not appear significant, , compounds **9g** and **11c** inhibit PDCLs proliferation  
 8 with an IC<sub>50</sub> in the 2-digit micromolar range (22-45 μM; Table 2), i.e. at least equivalent (for N13-  
 9 1300) or much lower (for N15-0385) than the IC<sub>50</sub>s obtained with temozolomide in these PDCLs.  
 10 Interestingly, the inhibition of PDCLs proliferation is similar in both PDCLs, suggesting a general  
 11 effect on GBM cells.

12

13 **Table 2.** IC<sub>50</sub> values (μM) for the inhibition of proliferation of cancer cell lines U87 and MCR5, based  
 14 on CellTiter Glo (Promega) assay, or GBM PDCLs based on wst-1 assay.

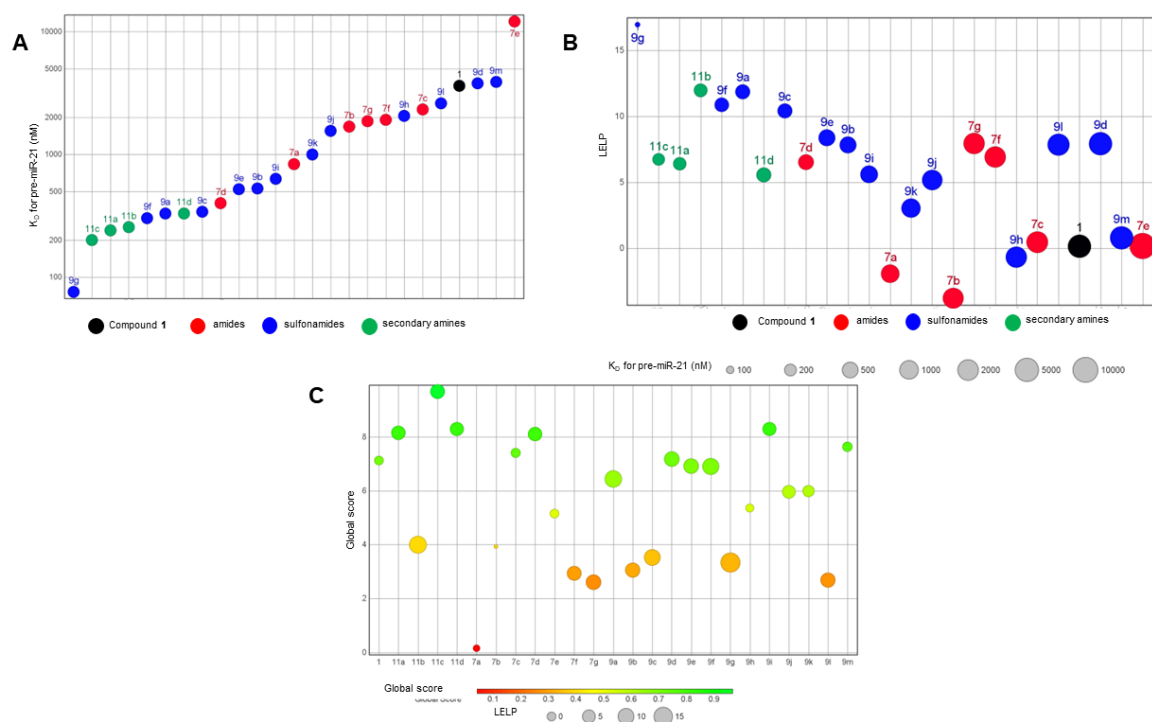
<b>ID</b>	<b>U87</b>	<b>MCR5</b>	<b>PDCL N15-0385</b>	<b>PDCL N13-1300</b>
<b>1</b>	37.4 ± 3.5	16.6 ± 1.5	>100	67.3 ± 32.6
<b>9g</b>	45.3 ± 5.8	> 100	37.1 ± 2.7	22.0 ± 4.3
<b>11c</b>	16.5 ± 0.50	> 100	44.7 ± 13.5	24.5 ± 12.1
<b>TMZ</b>	-	-	> 100	20.8 ± 0.4

15

16

### 17 **Structure-activity relationships**

18 As demonstrated by the obtained results about *in vitro* affinity, selectivity, inhibition activity and  
 19 biological activity in cells, sulfonamide derivative **9g** and amine derivative **11c** are among the most  
 20 promising compounds. To gain further insights into the structure-activity relationships of this series of  
 21 RNA ligands, we performed a chemoinformatic study using Data Warrior (DW) application. First, as  
 22 already mentioned, there is a correlation between the linker employed and the measured K<sub>D</sub> for pre-  
 23 miR-21 with reductive amination products being the best binders, followed by sulfonamide and amide  
 24 derivatives (Figure 6A).



1  
2 **Figure 6.** Structure activity relationships study performed using Data Warrior. A) Influence of the  
3 employed linker on the value of  $K_D$  for pre-miR-21. B) Relationships between LELP and binding  
4 affinity. All molecules are displayed in ascending order of the activity ( $K_D$ ) from left to right and  
5 colored according to their related family (amide, secondary amine and sulfonamide linker). C)  
6 Graphical illustration of the global score as a function of the chemical structure of the compound and  
7 its LELP.

8  
9 A few exceptions can be identified depending on the introduced substituent. For example, **7d**  
10 containing the naphthyridine substituent is a very good ligand even if containing the amide linker.  
11 Similarly, the sulfonamide derivative **9f** (bearing a benzothiazole moiety) is among the very good  
12 binders while its sulfonamide counterpart **9m** (with a dimethylamino substituent) does not bind as  
13 strongly. This shows, as expected, that the affinity results from the cooperative activity of both the  
14 substituent and the employed linker. To have a more precise view of structure-activity relationships  
15 we also evaluated the ligand efficiency lipophilic price (LELP) that is expressed as  $\text{clogP/LE}$  with  
16 ligand efficiency (LE) being a measure for the activity ( $K_D$ ) normalized by the number of heavy atoms  
17 (non H-atoms).<sup>39</sup> Most promising compounds in terms of affinity and druggability should show LELP  
18 values between 0 and 7.5. Indeed, LELP expresses that lipophilicity (which results into a loss of  
19 bioavailability when increased) and molecular weight (which increases activity at LE constant) should  
20 then be compensated by higher activity at the target. As illustrated in Figure 6B, when lipophilicity  
21 and molecular weight are taken into consideration, only four compounds (**11a**, **11c**, **11d** and **7d**)  
22 remain in the desirable ranges of both LELP and  $K_D$  to represent promising hits ( $0 < \text{LELP} < 7.5$  and

1  $K_D < 1000$  nM). Noteworthy, **9g** which is one of the best binders does not bear desirable properties and  
2 show a LELP just below 16.5. Finally, we decided to take into account multiple properties that have a  
3 direct impact on either the drug likeness, the toxicity risk assessment or the desired biological activity.  
4 In particular, we chose to build a score for each compound using six contributions: drug likeness,  
5 Lipinski rule of five, drug score, LELP regarding the affinity for pre-miR-21 and  $IC_{50}$  for pre-miR-21  
6 processing. We attributed a score between 0 (non-desirable) and 1 (desirable) for each individual  
7 property and a global score is the obtained calculating the mean value of each of the six chosen  
8 properties and limits (see Supporting Information protocol for limits choices). The closest the global  
9 score is to 1, the best properties the compound will have. As illustrated in Figure 6C, among the most  
10 promising compounds **11a**, **11c**, **11d** and **7d** only compound **11c** exhibits a very high score (0.97).  
11 Compounds **11a**, **11d** and **7d** show lower but still interesting overall scores ( $> 0.8$ ). Despite having the  
12 best  $K_D$  and  $IC_{50}$  values, compound **9g** is one of the worst compounds when considering the  
13 combination of the selected properties. In conclusion, compound **11c** represents the best compromise  
14 in the synthesized series of compounds in terms of drug likeness, toxicity risk and biological activity  
15 and deserves further development.

16

## 17 **Conclusion**

18 In conclusion, we pursued the multimodal design of new RNA ligands thanks to the conjugation of the  
19 C-terminal lateral chain of bleomycin A5 to various chemical scaffolds known to be able to interact  
20 with RNA residues. This allowed for the design and the synthesis of new RNA ligands able to inhibit  
21 the biogenesis of oncogenic pre-miRNAs. Three series of compounds have been synthesized using  
22 amide, carbamate and reductive amination leading to a total of 24 compounds that have been studied  
23 for their affinity, selectivity and inhibition activity against four oncogenic miRNA: miR-21, miR-18a,  
24 miR-148a and miR-373. The obtained results indicate that the series containing a sulfonamide or a  
25 secondary amine as a linker for the bleomycin A5 C-terminal chain are the strongest binders of pre-  
26 miR-21 and among these compounds, **9g** and **11c** are the strongest binders as well as the best  
27 inhibitors. A molecular docking study suggests that the potential binding site is located in the cleavage  
28 site of Dicer as opposed to other good binders that bind to different regions and are not able to induce  
29 the same inhibition activity. A detailed study of the selectivity of these compounds showed that even if  
30 they are not specific for one single RNA structure, they are selective for pre-miRNAs and have the  
31 potential to inhibit the processing of oncogenic ones. To further confirm this potential, we evaluated  
32 the biological activity on glioblastoma U87 cells that overexpress miR-21, miR-18a and miR-148a and  
33 observed a good antiproliferative activity especially for compounds **9g** and **11c** that are not toxic on  
34 healthy cells. We also demonstrated that these promising compounds maintain their biological activity  
35 on PDCLs thus having the potential to target glioblastoma CSCs that are usually resistant to classical  
36 chemotherapy. Since no therapy exist for this aggressive type of cancer and the only palliative  
37 treatment is based on TMZ used at high doses and toxic, these compounds, despite a moderate

1 activity, could represent promising tools to affect the proliferation of glioblastoma cells lines and  
2 CSCs thus opening the way to the discovery of new anticancer molecules for this aggressive and  
3 incurable type of brain cancer.

#### 4 **Experimental Section**

##### 5 **Chemistry**

6 Reagents and solvents were purchased from Aldrich, Alfa Aesar, Fluorochem, ABCR, Carlo Erba,  
7 Acros and used without further purification. Flash column chromatography was carried out on silica  
8 gel columns (Interchim Puriflash silica HP 15 to 50  $\mu\text{m}$ ) on Puriflash® instrument (Interchim).  
9 Analytical thin-layer chromatography (TLC) was conducted on Sigma Aldrich precoated silica gel and  
10 compounds were visualized by irradiation (254 nm) and/or by staining with ninhydrin or vanilin  
11 stains.

12  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on Bruker AC 200, 400 and 500 MHz spectrometers.  
13 Chemical shifts are reported in parts per million (ppm,  $\delta$ ) referenced to the residual  $^1\text{H}$  resonance of  
14 the solvent ( $\text{D}_2\text{O}$   $\delta$  4.79;  $\text{CDCl}_3$   $\delta$  7.26;  $\text{CD}_3\text{OD}$   $\delta$  3.31) and referenced to the residual  $^{13}\text{C}$  resonance of  
15 the solvent ( $\text{CDCl}_3$   $\delta$  77.2;  $\text{CD}_3\text{OD}$   $\delta$  49.0). Splitting patterns are designed as follows: s (singlet), d  
16 (doublet), t (triplet), m (multiplet), and br (broad). Coupling constant (J) are listed in hertz (Hz).

17 Low resolution mass spectra (MS) were obtained with a Bruker Daltonics Esquire 3000+ electrospray  
18 spectrometer equipped with API ionization source. High-resolution mass spectrometry (HRMS) was  
19 carried out on a LTQ Orbitrap hybrid mass spectrometer with an electrospray ionization probe  
20 (ThermoScientific, San Jose, CA) by direct infusion from a pump syringe, to confirm the correct molar  
21 mass and high purity of the compounds.

22 HPLC analyses were performed using a Water Alliance 2695 pump coupled to a Water 996  
23 photodiode array detector and a two different columns were used: Method A: X-Select CSHTM  
24 fluorophenyl reverse-phase C18 column (50 x 4.6 mm, 5  $\mu\text{m}$ ) for analytical HPLC analysis and  
25 reverse-phase C18 column (250 x 10 mm, 5  $\mu\text{m}$ ) for semipreparative HPLC. Method B: Phenomenex  
26 Synergi™ FusionRP 80A reverse-phase C18 column (250 x 4.6 mm, 5  $\mu\text{m}$ ) for analytical HPLC  
27 analysis and reverse-phase C18 column (250 x 10 mm, 5  $\mu\text{m}$ ) for semipreparative HPLC. All  
28 experiments were run out at room temperature using a gradient of  $\text{CH}_3\text{CN}$  containing 0.1% TFA  
29 (eluent B) in water containing 0.1% TFA (eluent A) at flow rates of 1 and 3.5 mL/min for the  
30 analytical and semipreparative HPLC, respectively. The gradient is 5% to 40% eluent B in eluent A  
31 over 20 min, 40% to 100% eluent B over 5 min, 100% eluent B over 5 min and 100% to 5% eluent B  
32 over 5 min. Method B analytical HPLC was employed to assess the purity of all tested compounds.  
33 Purity was in all cases > 95%.

##### 34 **Synthetic procedures**

##### 35 **General procedures**

##### 36 **General procedure A - Boc deprotection with TFA**

1 To a solution of Boc-product (1 eq.) in CH<sub>2</sub>Cl<sub>2</sub> was added trifluoroacetic acid (12 to 48 eq.) and the  
2 reaction mixture was stirred 16 h at room temperature. Solvent was removed under reduced pressure  
3 and the residual TFA co-evaporated with toluene. If possible, the product was precipitated in Et<sub>2</sub>O or  
4 cyclohexane and lyophilized to give the desired product.

#### 5 **General procedure B - Boc deprotection with HCl**

6 To a solution of Boc-product (1 eq.) in MeOH was added a solution of hydrochloric acid (15 to 60 eq.)  
7 in MeOH and the reaction mixture was stirred 16 h at room temperature. Solvent was removed under  
8 reduced. If possible, the product was precipitated in Et<sub>2</sub>O or cyclohexane and lyophilized to give the  
9 desired product.

#### 10 **General procedure C - sulfonamide formation**

11 To a solution of tert-butyl (3-(2'-(2-aminoethyl)-[2,4'-bithiazole]-4-carboxamido)propyl)(4-((tert-  
12 butoxycarbonyl)amino)butyl)carbamate 2.20 (named Bithiazole Boc-spermidine) (1 eq.) in DMF was  
13 added the sulfonyl chloride (1.2 eq.) and triethylamine (1.6 eq.) and the reaction mixture was stirred  
14 16 h at room temperature. The solution was concentrated under reduced pressure and the crude  
15 product was purified by flash chromatography (FC) on a silica gel column using a mixture CH<sub>2</sub>Cl<sub>2</sub>/  
16 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10) as the eluent.

#### 17 **General procedure D - reductive amination**

18 To a solution of Bithiazole Boc-spermidine 2.20 (1 eq.) in MeOH was added the aldehyde (1.1 eq.)  
19 and the mixture was stirred 0.5 h. The pH was adjusted at 5 with acetic acid if needed. Sodium  
20 cyanoborohydride (2.1 eq.) was then added and the reaction mixture was stirred 16 h at room  
21 temperature. The solvent was concentrated under reduced pressure and the crude product was  
22 dissolved in CH<sub>2</sub>Cl<sub>2</sub> and the organic phase was washed with NaHCO<sub>3</sub>, then H<sub>2</sub>O before being dried  
23 over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduce pressure. The crude product was purified by FC on a  
24 silica gel column using a mixture CH<sub>2</sub>Cl<sub>2</sub>/(CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90:10) as the eluent.

#### 25 **Ethyl 2'-[2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino]ethyl-2,4'-bithiazole-4-carboxylate (3).**

26 To a solution of 2-(2-aminoethyl)thiazole-4-carbothioamide **2** (1.81 g, 4.98 mmol) in H<sub>2</sub>O (40 mL) at  
27 0°C, was added dropwise a solution of LiOH (0.30 g, 12.5 mmol, 2.5 eq.) in H<sub>2</sub>O (10mL). The mixture  
28 was stirred at room temperature until complete disappearance of the ethyl ester. The solvent was then  
29 removed under reduced pressure, the crude product was dissolved in a 3/2 mixture of H<sub>2</sub>O/dioxane (50  
30 mL) and 9-fluorenylmethyl *N*-succinimidyl carbonate (1.86 g, 5.50 mmol, 1.1 eq.) was added. The  
31 reaction mixture was stirred at room temperature overnight. H<sub>2</sub>O (15 mL) was added to the mixture,  
32 and it was acidified with HCl (1M) until pH 2, before being extracted with EtOAc. The organic phase  
33 was washed with acidic H<sub>2</sub>O (15 mL x 3) and brine (15 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated  
34 under reduced pressure. The crude product was purified by FC on a silica gel column using a mixture  
35 CH<sub>2</sub>Cl<sub>2</sub>/MeOH from 100/0 to 80/20 as the eluent to give compound **3** as a white solid (2.14 g, 90%).  
36  $R_f = 0.40$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 80/20); <sup>1</sup>H NMR (200 MHz, CD<sub>3</sub>OD) δ 8.22 (s, 1H), 8.14 (s, 1H), 7.73 (d, J

1 = 7.0 Hz, 2H), 7.58 (d, J = 7.6 Hz, 2H), 7.42 – 7.19 (m, 4H), 4.35 (d, J = 6.7 Hz, 2H), 4.17 (t, J = 6.7  
2 Hz, 1H), 3.61 – 3.51 (m, 2H), 3.23 (t, J = 6.6 Hz, 2H); <sup>13</sup>C NMR (100MHz, CD<sub>3</sub>OD) δ 170.7, 164.6,  
3 164.4, 150.5, 149.2, 145.3, 142.6, 128.9, 128.8, 128.1, 126.2, 120.9, 118.8, 67.7, 47.5, 41.4, 34.1; MS  
4 (ESI) *m/z* 477.93 (M+H)<sup>+</sup> (Theoretical 478.08).

5 **2'-(((9H-Fluoren-9-yl)methoxy)carbonyl)-aminoethyl-2,4'-bithiazole-4-[3-(4-N-**  
6 **tertbutoxycarbonyl-aminobutyl)-N-tertbutoxycarbonyl-aminopropyl]carboxamide (4).** To a  
7 solution of Fmoc-bithiazole acid **3** (0.20 g, 0.42 mmol) in CH<sub>2</sub>Cl<sub>2</sub>/DMF (16 mL/0.4 mL) was added  
8 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.11 g, 0.71 mmol, 1.7 eq.) and  
9 *N*-hydroxysuccinimide (0.08 g, 0.70 mmol, 1.7 eq.) and the mixture was stirred 4 h at room  
10 temperature. *N4,N8*-di-tert-butoxycarbonyl-spermidine (0.21 g, 0.61 mmol, 1.4 eq.) was added and  
11 the reaction mixture was stirred 4 h at room temperature. The organic mixture was washed with H<sub>2</sub>O  
12 (8 mL x 3) then brine (8 mL) before being dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced  
13 pressure. The crude product was purified by FC on a silica gel column using a mixture CH<sub>2</sub>Cl<sub>2</sub>/MeOH  
14 from 100/0 to 96/4 as the eluent to give desired product **4** as a white foam (0.28 g, 83%). *R<sub>f</sub>* = 0.71  
15 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10); <sup>1</sup>H NMR (400MHz, CD<sub>3</sub>OD) δ 8.12 (s, 2H), 7.77 (d, J = 7.5 Hz, 2H),  
16 7.61 (d, J = 7.4 Hz, 2H), 7.37 (t, J = 7.5 Hz, 2H), 7.27 (t, J = 7.4 Hz, 2H), 4.33 (d, J = 6.8 Hz, 2H),  
17 4.18 (t, J = 6.8 Hz, 1H), 3.56 (t, J = 6.5 Hz, 2H), 3.42 (t, J = 6.7 Hz, 2H), 3.31 (m, 2H), 3.27 – 3.22 (m,  
18 4H), 3.04 (t, J = 6.8 Hz, 2H), 1.86 (br, 2H), 1.56 (br, 2H), 1.48 – 1.38 (m, 20H); <sup>13</sup>C NMR (101MHz,  
19 CDCl<sub>3</sub>) δ 161.4, 156.5, 156.2, 144.1, 141.6, 127.9, 127.2, 125.2, 123.3, 120.2, 116.6, 79.8, 66.9, 47.5,  
20 46.9, 40.4, 40.2, 33.3, 31.1, 28.7, 28.6, 27.7; MS (ESI) *m/z* 825.93 (M+Na)<sup>+</sup> (Theoretical 803.33).

21 **2'-Aminoethyl-2,4'-bithiazole-4-[3-(4-N-tertbutoxycarbonyl-aminobutyl)-N-tertbutoxycarbonyl-**  
22 **aminopropyl]carboxamide (5).** To a solution of Fmoc-bithiazole Boc-spermidine **4** (0.31 g, 0.39  
23 mmol) in DMF (6 mL) was added piperidine (1.50 mL, 15.2 mmol, 40 eq.). The reaction mixture was  
24 stirred at room temperature for 5 h, the solvent was removed under reduced pressure and the crude  
25 product was purified by FC on a silica gel column using a mixture CH<sub>2</sub>Cl<sub>2</sub>/MeOH from 100/0 to 80/20  
26 as the eluent leading to compound **5** as a yellow oil (0.19 g, 83%). *R<sub>f</sub>* = 0.19 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 96/4); <sup>1</sup>H  
27 NMR (400 MHz, CD<sub>3</sub>OD) δ 8.17 (s, 1H), 8.16 (s, 1H), 3.43 (t, J = 6.7 Hz, 2H), 3.32 – 3.29 (m, 2H),  
28 3.26 – 3.21 (m, 4H), 3.15 (t, J = 6.2 Hz, 2H), 3.04 (t, J = 6.7 Hz, 2H), 1.87 (br, 2H), 1.57 (br, 2H), 1.48  
29 -1.40 (m, 20H); <sup>13</sup>C NMR (101MHz, CD<sub>3</sub>OD) as a mixtures of 2 isomers δ 169.9, 162.6, 162.0, 157.2,  
30 150.3, 148.2, 123.5, 116.9, 79.6, 78.4, 46.7, 46.5, 44.9, 44.1, 40.6, 39.6, 36.9, 36.5, 35.0, 28.5, 27.9,  
31 27.4, 27.3, 27.0, 25.6, 25.1; MS (ESI) *m/z* 583.07 (M+H)<sup>+</sup> (Theoretical 583.27).

32 **2'-Aminoethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)aminopropyl]carboxamide (1).** Deprotection of  
33 bithiazole-spermidine **5** (122 mg, 0.18 mmol) was performed following general procedure B and and  
34 hydrochloric acid (0.45 mL, 5.36 mmol, 30 eq.) in MeOH (3 mL). The product was precipitated in  
35 Et<sub>2</sub>O leading to compound **1** as a yellow oil (80 mg, 88%). HPLC *R<sub>t</sub>* = 5.3 min, analytical HPLC  
36 method A; <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O) δ 8.23 (s, 1H), 8.14 (s, 1H), 3.59 – 3.45 (m, 6H), 3.18 – 3.06 (m,  
37 4H), 3.03 (t, J = 7.0 Hz, 2H), 2.09 – 1.98 (m, 2H), 1.83 – 1.69 (m, 4H); <sup>13</sup>C NMR (101MHz, D<sub>2</sub>O) δ



1 168.1, 163.7, 163.0, 148.7, 147.3, 125.2, 119.2, 46.9, 45.1, 38.7, 38.5, 36.2, 29.6, 25.8, 23.9, 22.7;  
2 HRMS (ESI)  $m/z$  383.16870 (M+H)<sup>+</sup> (C<sub>16</sub>H<sub>27</sub>N<sub>6</sub>OS<sub>2</sub> requires 383.16823).

3 **2'-(Na,Nw-Ditertbutoxycarbonyl-L-lysynamido)ethyl-2,4'-bithiazole-4-[3-(4-N-**  
4 **tertbutoxycarbonyl-aminobutyl)-N-tertbutoxycarbonyl-aminopropyl]carboxamide (6a).** To a  
5 solution of Boc-L-Lys(Boc)-OH **12a** (31.8 mg, 0.09 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (4 mL) was added 1-ethyl-3-  
6 (3-dimethylaminopropyl)carbodiimide (22.8 mg, 0.15 mmol, 1.7 eq.) and *N*-Hydroxysuccinimide  
7 (17.6 mg, 0.16 mmol, 1.8 eq.) and the mixture was stirred 4 h at room temperature. Bithiazole Boc-  
8 spermidine **5** (58.2 mg, 0.10 mmol, 1.1eq.) was then added and the reaction mixture was stirred 4 h at  
9 room temperature. The organic mixture was washed with H<sub>2</sub>O (1 mL x 3) then brine (1 mL) before  
10 being dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The crude product was purified by  
11 FC on a silica gel column using a mixture CH<sub>2</sub>Cl<sub>2</sub>/MeOH from 100/0 to 95/5 as the eluent leading to  
12 compound **6a** as a brown oil (45.6 mg, 54%). R<sub>f</sub> = 0.51 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 96/4); HPLC Rt = 22.2 min,  
13 analytical HPLC method A; <sup>1</sup>H NMR (400MHz, CD<sub>3</sub>OD) δ 8.17 (s, 1H), 8.15 (s, 1H), 3.98 – 3.92 (m,  
14 1H) 3.77 – 3.68 (m, 1H), 3.67 – 3.55 (m, 1H), 3.43 (t, J = 6.6 Hz, 2H), 3.34 – 3.30 (m, J = 3.2 Hz, 2H),  
15 3.30 – 3.18 (m, 4H), 3.04 (t, J = 6.7 Hz, 2H), 2.98 (t, J = 6.7 Hz, 2H), 1.87 (br, 2H), 1.68 (br, 1H), 1.56  
16 (br, 3H), 1.49 – 1.36 (m, 42H, H-15); <sup>13</sup>C NMR (101MHz, CD<sub>3</sub>OD) as a mixture of 2 isomers δ 175.5,  
17 170.9, 164.1, 163.5, 158.6, 157.9, 151.8, 149.7, 125.0, 118.6, 81.1, 80.7, 79.9, 56.3, 48.0, 47.9, 46.3,  
18 45.5, 41.0, 41.0, 39.9, 38.2, 37.8, 33.7, 33.1, 30.8, 30.7, 30.0, 29.3, 29.0, 28.9, 28.5, 27.0, 26.5, 24.1;  
19 HRMS (ESI)  $m/z$  911.47394 (M+H)<sup>+</sup> (C<sub>42</sub>H<sub>71</sub>N<sub>8</sub>O<sub>10</sub>S<sub>2</sub> requires 911.47291).

20 **2'-(L-Lysynamido)ethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-aminopropyl]carboxamide (7a).**  
21 Deprotection of compound **6a** (42.0 mg, 0.05 mmol) was performed following general procedure A  
22 and trifluoroacetic acid (0.12 mL, 1.57 mmol, 34 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL). The product was precipitated  
23 in Et<sub>2</sub>O leading to compound **7a** as a brown oil (41.2 mg, 93%). HPLC Rt = 2.5 min, analytical HPLC  
24 method A; <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O) δ 8.24 (s, 1H), 8.14 (s, 1H), 3.92 (t, J = 6.5 Hz, 1H), 3.89 – 3.80  
25 (m, 1H), 3.69 – 3.58 (m, 1H), 3.54 (t, J = 6.5 Hz, 2H), 3.43 – 3.26 (m, 2H), 3.18 – 3.07 (m, 4H), 3.04  
26 (t, J = 6.7 Hz, 2H), 2.88 (t, J = 7.5 Hz, 2H), 2.07 – 2.00 (m, 2H), 1.83 – 1.72 (m, 6H), 1.63 – 1.52 (m,  
27 2H), 1.35 – 1.22 (m, 2H); <sup>13</sup>C NMR (101MHz, D<sub>2</sub>O) δ 170.8, 169.5, 163.6, 162.9, 148.8, 147.0, 125.1,  
28 119.0, 53.0, 46.9, 45.1, 39.1, 38.8, 38.7, 36.2, 31.9, 30.3, 26.3, 25.8, 23.9, 22.7, 21.2; HRMS (ESI)  $m/z$   
29 511.26349 (M+H)<sup>+</sup> (C<sub>22</sub>H<sub>39</sub>N<sub>8</sub>O<sub>2</sub>S<sub>2</sub> requires 511.26319).

30 **2'-(Na,Nim-Ditertbutoxycarbonyl-L-histidinamido)ethyl-2,4'-bithiazole-4-[3-(4-N-**  
31 **tertbutoxycarbonyl-aminobutyl)-N-tertbutoxycarbonyl-aminopropyl]carboxamide (6b).** To a  
32 solution of Boc-His(Boc)-OH DCHA **12b** (76.6 mg, 0.14 mmol, 1.8 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (4 mL) was added  
33 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (27.6 mg, 0.18 mmol, 2.3 eq.) and  
34 *N*-hydroxysuccinimide (21.0 mg, 0.18 mmol, 2.3 eq.) and the mixture was stirred 2 h. Bithiazole  
35 Boc-spermidine **5** (47.7 mg, 0.08 mmol) was then added and the reaction mixture was stirred 4 h at  
36 room temperature. The organic mixture was washed with H<sub>2</sub>O (2 mL x 3) then brine (2 mL) before  
37 being dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The crude product was purified by

1 FC on a silica gel column using a mixture CH<sub>2</sub>Cl<sub>2</sub>/(CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10) from 100/0 to 60/40 as the  
2 eluent leading to compound **6b** as a white solid (66.4 mg, 90%). R<sub>f</sub> = 0.34 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 96/4); <sup>1</sup>H  
3 NMR (400MHz, CD<sub>3</sub>OD) δ 8.16 (s, 1H), 8.14 (s, 1H), 8.08 (s, 1H), 7.28 (s, 1H), 4.34 – 4.28 (m, 1H),  
4 3.75 – 3.65 (m, 1H), 3.65 – 3.55 (m, 1H), 3.42 (t, J = 6.4 Hz, 2H), 3.33 – 3.29 (m, 2H), 3.26 – 3.20 (m,  
5 4H), 3.04 (t, J = 6.0 Hz, 2H), 3.00 - 2.94 (m, 1H), 2.87 – 2.75 (m, 1H), 1.86 (s, 2H), 1.62 – 1.54  
6 (m, 11H), 1.49 – 1.33 (m, 29H); <sup>13</sup>C NMR (101MHz, CD<sub>3</sub>OD) as a mixture of 2 isomers δ 174.3,  
7 170.8, 164.1, 163.5, 158.6, 157.6, 151.9, 149.7, 148.2, 138.4, 125.0, 118.6, 116.4, 81.1, 80.8, 79.9,  
8 55.8, 48.2, 48.0, 46.4, 45.7, 41.1, 40.0, 38.4, 38.0, 33.8, 31.9, 30.8, 30.7, 30.0, 29.4, 28.9, 28.8, 28.2,  
9 27.1, 26.6; HRMS (ESI) *m/z* 920.43823 (M+H)<sup>+</sup> (C<sub>42</sub>H<sub>66</sub>N<sub>9</sub>O<sub>10</sub>S<sub>2</sub> requires 920.43686).

10 **2'-(L-Histidinamido)ethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-aminopropyl]carboxamide (7b).**

11 Preparation of compound **7b** was performed following general procedure A starting from Boc-  
12 protected histidine-bithiazole-spermidine **6b** (62.0 mg, 0.07 mmol) and trifluoroacetic acid (0.13 mL,  
13 1.70 mmol, 24 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (3 mL). The product was precipitated in Et<sub>2</sub>O to give compound **7b** as a  
14 brown powder (57.0 mg, 87%). HPLC Rt = 2.6 min, analytical HPLC method A; <sup>1</sup>H NMR (400MHz,  
15 D<sub>2</sub>O) as a mixture of 2 isomers δ 8.68 (d, J = 1.1 Hz, 0,2H), 8.62 (d, J = 1.1 Hz, 0,8H), 8.22 (s, 1H),  
16 8.11 (s, 1H), 7.40 (s, 0,2H), 7.32 (s, 0,8H), 4.24 (t, J = 7.1 Hz, 0,2H), 4.09 (t, J = 7.1 Hz, 0,8H), 3.79  
17 (dt, J = 6.6 Hz, 13.6 Hz, 1H), 3.65 – 3.57 (m, 1H), 3.54 (t, J = 6.7 Hz, 2H), 3.39 – 3.24 (m, 4H), 3.18 –  
18 3.07 (m, 4H), 3.04 (t, J = 6.8 Hz, 2H), 2.09 – 1.99 (m, 2H), 1.84 – 1.70 (m, 4H); <sup>13</sup>C NMR (101MHz,  
19 D<sub>2</sub>O) as a mixture of 2 isomers δ 170.6, 167.9, 163.6, 162.8, 148.8, 147.0, 134.2, 134.0, 125.8, 125.0,  
20 119.0, 118.1, 117.8, 53.2, 51.9, 46.9, 45.1, 39.1, 38.7, 36.2, 31.7, 26.2, 25.8, 25.6, 23.9, 22.7; HRMS  
21 (ESI) *m/z* 520.22729 (M+H)<sup>+</sup> (C<sub>22</sub>H<sub>34</sub>N<sub>9</sub>O<sub>2</sub>S<sub>2</sub> requires 520.22714).

22 **2'-[N-Im-tertButoxycarbonyl-imidazole-4-acetamido]ethyl-2,4'-bithiazole-4-[3-(4-N-**  
23 **tertbutoxycarbonyl-aminobutyl)-N-tertbutoxycarbonyl-aminopropyl]carboxamide (6c).** To a

24 solution of imidazole acetic acid **12c** (28.2 mg, 0.12 mmol, 1.3 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (4 mL) 1-ethyl-3-(3-  
25 dimethylaminopropyl)carbodiimide (24.9 mg, 0.16 mmol, 1.8 eq.) and *N*-hydroxysuccinimide  
26 (18.4 mg, 0.16 mmol, 1.8 eq.) were added and the reaction mixture was stirred 4 h at room  
27 temperature. Bithiazole Boc-spermidine **5** (55.0 mg, 0.09 mmol) was then added and the reaction  
28 mixture was stirred 4 h at room temperature. The organic mixture was washed with H<sub>2</sub>O (2 mL x 3)  
29 then brine (2 mL) before being dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The crude  
30 product was purified by FC on a silica gel column using a mixture CH<sub>2</sub>Cl<sub>2</sub>/(CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10)  
31 from 100/0 to 80/20 as the eluent and leading to compound **6c** as a yellow oil (16.2 mg, 22%). R<sub>f</sub> =  
32 0.68 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10); <sup>1</sup>H NMR (400MHz, CD<sub>3</sub>OD) δ 8.16 (s, 1H), 8.14 (s, 1H), 8.09 (s, 1H),  
33 7.36 (s, 1H), 3.66 (t, J = 6.7 Hz, 2H), 3.49 (s, 2H), 3.43 (t, J = 6.7 Hz, 2H), 3.34 – 3.30 (m, 2H), 3.30 –  
34 3.21 (m, 4H, H-2), 3.04 (t, J = 6.7 Hz, 2H), 1.87 (br, 2H), 1.64 - 1.52 (m, 11H), 1.51 – 1.37 (m, 20H);  
35 <sup>13</sup>C NMR (101MHz, CD<sub>3</sub>OD) as a mixture of 2 isomers δ 172.7, 170.9, 164.2, 163.6, 158.7, 151.9,  
36 149.7, 148.2, 138.5, 138.0, 125.0, 118.6, 116.9, 87.3, 81.2, 80.0, 48.2, 48.0, 45.9, 45.7, 41.1, 40.3,

1 38.4, 38.0, 36.2, 33.7, 30.1, 29.4, 29.0, 28.9, 28.5, 28.2, 27.2, 26.8; HRMS (ESI)  $m/z$  791.35889  
2 (M+H)<sup>+</sup> (C<sub>36</sub>H<sub>55</sub>N<sub>8</sub>O<sub>8</sub>S<sub>2</sub> requires 791.35788).

3 **2'-[Imidazole-4-acetamido]ethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-aminopropyl]carboxamide**  
4 **(7c)**. Preparation of compound **7c** was performed following general procedure A starting from Boc-  
5 protected histidine-bithiazole-spermidine **6c** (15.3 mg, 0.02 mmol) and trifluoroacetic acid (0.06 mL,  
6 0.78 mmol, 39 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL). The product was precipitated in Et<sub>2</sub>O to give compound **7c** as a  
7 yellow oil (13.6 mg, 84%). HPLC Rt = 2.6 min, analytical HPLC method A; <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O)  
8 δ 8.58 (s, 1H), 8.21 (s, 1H), 8.05 (s, 1H), 7.25 (s, 1H), 3.73 (s, 2H), 3.67 (t, J = 6.1 Hz, 2H), 3.55 (t, J =  
9 6.5 Hz, 2H), 3.28 (t, J = 6.1 Hz, 2H), 3.18 – 3.08 (m, 4H), 3.05 (t, J = 6.4 Hz, 2H), 2.10 – 2.00 (m,  
10 2H), 1.78 (s, 4H); <sup>13</sup>C NMR (101MHz, D<sub>2</sub>O) δ 170.7, 170.4, 163.6, 162.8, 148.7, 146.8, 133.4, 126.7,  
11 125.0, 118.9, 117.2, 46.9, 45.1, 39.1, 38.7, 36.2, 32.0, 31.1, 25.7, 23.9, 22.7; HRMS (ESI)  $m/z$   
12 491.20117 (M+H)<sup>+</sup> (C<sub>21</sub>H<sub>31</sub>N<sub>8</sub>O<sub>2</sub>S<sub>2</sub> requires 491.20059).

13 **2'-[1,8-Naphthyridine-2-carboxamido]ethyl-2,4'-bithiazole-4-[3-(4-N-tertbutoxycarbonyl-**  
14 **aminobutyl)-N-tertbutoxycarbonyl-aminopropyl]carboxamide (6d)**. To a solution of 1,8-  
15 naphthyridine-2-carboxylic acid **12d** (19.2 mg, 0.11 mmol, 1.4 eq.) in CH<sub>2</sub>Cl<sub>2</sub>/DMF (3 mL/ 0.2mL)  
16 was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (20.5 mg, 0.13 mmol, 1.6 eq.) and  
17 N-hydroxysuccinimide (15.8 mg, 0.14 mmol, 1.4 eq.) and the mixture was stirred 4 h at room  
18 temperature. Bithiazole Boc-spermidine **5** (43.6 mg, 0.08 mmol) was then added and the reaction  
19 mixture was stirred 4 h at room temperature. The organic mixture was washed with H<sub>2</sub>O (2 mL x 3)  
20 then brine (2 mL) before being dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The crude  
21 product was purified by FC on a silica gel column using a mixture Cyclohexane/EtOAc from 80/20 to  
22 5/95 as the eluent leading to compound **6d** as a brown oil (31.9 mg, 39%). R<sub>f</sub> = 0.24  
23 (Cyclo/EtOAc30/70); <sup>1</sup>H NMR (400MHz, CD<sub>3</sub>OD) δ 9.14 (dd, J = 1.9 Hz, 4.3 Hz, 1H), 8.59 (d, J =  
24 8.4 Hz, 1H), 8.53 (dd, J = 1.9 Hz, 8.2 Hz, 1H), 8.31 (d, J = 8.4 Hz, 1H), 8.10 (s, 2H), 7.72 (dd, J = 4.3  
25 Hz, 8.2 Hz, 1H), 3.96 (t, J = 6.8 Hz, 2H), 3.46 (t, J = 6.8 Hz, 2H), 3.41 (t, J = 6.8 Hz, 2H), 3.31 (m,  
26 2H), 3.22 (t, J = 7.2 Hz, 2H), 3.03 (t, J = 6.7 Hz, 2H), 1.84 (br, 2H), 1.54 (br, 2H), 1.47 – 1.37 (m, ,  
27 20H); <sup>13</sup>C NMR (101MHz, CD<sub>3</sub>OD) as a mixture of two isomers δ 175.2, 170.5, 166.2, 164.0, 163.4,  
28 158.5, 155.8, 155.1, 154.0, 151.6, 149.6, 140.9, 139.7, 125.9, 124.9, 121.1, 118.5, 81.0, 79.8, 48.1,  
29 47.9, 46.3, 45.5, 41.0, 40.4, 38.2, 37.8, 33.7, 30.7, 29.9, 29.3, 28.8, 28.7, 27.0, 26.5; MS (ESI)  $m/z$   
30 771.27 (M+MeOH+H)<sup>+</sup> (Theoretical 771.97).

31 **2'-[1,8-Naphthyridine-2-carboxamido]ethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-**  
32 **aminopropyl]carboxamide (7d)**. The unprotected product **7d** was synthesised following the general  
33 procedure B starting from Boc-protected naphthyridine-bithiazole-spermidine **6d** (25.8 mg, 0.03  
34 mmol) and hydrochloric acid (0.13 mL, 1.56 mmol, 52 eq.) in MeOH (1.5 mL). The product was  
35 crystallized in Et<sub>2</sub>O to give **7d** as a brown solid (10.1 mg, 82%). HPLC Rt = 11.9 min, analytical  
36 HPLC method B; <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O) δ 9.35 (d, J = 4.6 Hz, 1H), 9.15 (dd, J = 1.2 Hz, 8.3 Hz,  
37 1H), 8.82 (d, J = 8.5 Hz, 1H), 8.35 (d, J = 8.5 Hz, 1H), 8.16 (dd, J = 4.6 Hz, 8.3 Hz, 1H), 8.02 (s, 1H),

1 8.00 (s, 1H), 3.89 (t, J = 6.5 Hz, 2H), 3.48 (t, J = 6.7 Hz, 2H), 3.41 (t, J = 6.5 Hz, 2H), 3.16 -3.08 (m,  
2 4H), 3.04 (t, J = 6.8 Hz, 2H), 2.08 - 1.95 (m, 2H), 1.84 – 1.70 (m, 4H); <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O) δ  
3 170.4, 166.1, 163.3, 162.6, 154.7, 153.0, 151.7, 148.3, 146.8, 140.3, 138.8, 124.8, 124.5, 123.9, 119.8,  
4 119.2, 46.9, 45.1, 39.5, 38.8, 36.2, 32.1, 25.7, 23.9, 22.7; HRMS (ESI) *m/z* 539.20099 (M+H)<sup>+</sup>  
5 (C<sub>25</sub>H<sub>31</sub>N<sub>8</sub>O<sub>2</sub>S<sub>2</sub> requires 539.20059).

6 **2'-[2-(2,4-Dioxo-1,3-diazaspiro[4.4]nonan-3-yl)acetamido]ethyl-2,4'-bithiazole-4-[3-(4-N-**  
7 **tertbutoxycarbonyl-aminobutyl)-N-tertbutoxycarbonyl-aminopropyl]carboxamide (6e).** To a  
8 solution of dioxo-diazaspiro acetic acid **12e** (20.2 mg, 0.09 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (4 mL) 1-ethyl-3-(3-  
9 dimethylaminopropyl)carbodiimide (25.0 mg, 0.16 mmol, 1.8 eq.) and *N*-hydroxysuccinimide (17.8  
10 mg, 0.16 mmol, 1.8 eq.) were added and the reaction mixture was stirred 4 h at room temperature.  
11 Bithiazole Boc-spermidine **5** (57.6 mg, 0.10 mmol, 1.1 eq.) was then added and the reaction mixture  
12 was stirred 5 h at room temperature. The organic mixture was washed with H<sub>2</sub>O (2 mL x 3) then brine  
13 (2 mL) before being dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The crude product  
14 was purified by FC on a silica gel column using a mixture CH<sub>2</sub>Cl<sub>2</sub>/(CH<sub>2</sub>Cl<sub>2</sub>/MeOH) from 100/0 to  
15 50/50 as the eluent to give compound **6e** as a white solid (25.0 mg, 34%). R<sub>f</sub> = 0.63 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH  
16 90/10); HPLC Rt = 20.7 min, analytical HPLC method A; <sup>1</sup>H NMR (400MHz, CD<sub>3</sub>OD) δ 8.17 (s, 1H),  
17 8.14 (s, 1H), 4.12 (s, 2H), 3.67 (t, J = 6.8 Hz, 2H), 3.43 (t, J = 6.7 Hz, 2H), 3.34 – 3.30 (m, 2H), 3.29 –  
18 3.20 (m, 4H), 3.04 (t, J = 6.8 Hz, 2H), 2.13 – 2.07 (m, 2H), 1.87 – 1.81 (m, 8H), 1.56 (br, 2H), 1.49 –  
19 1.38 (m, 20H); <sup>13</sup>C NMR (101 MHz, CD<sub>3</sub>OD) as a mixture of two isomers δ 180.0, 170.7, 169.2,  
20 164.2, 163.6, 158.7, 157.9, 151.8, 149.7, 125.1, 118.7, 81.1, 80.0, 48.2, 48.0, 46.5, 45.7, 41.5, 41.1,  
21 40.3, 38.7, 38.3, 38.0, 33.8, 30.1, 29.5, 29.0, 28.9, 27.2, 26.8, 26.3; MS (ESI) *m/z* 777.00 (M+H)<sup>+</sup>  
22 (Theoretical 777.33).

23 **2'-[2-(2,4-Dioxo-1,3-diazaspiro[4.4]nonan-3-yl)acetamido]ethyl-2,4'-bithiazole-4-[3-(4-**  
24 **aminobutyl)-aminopropyl]carboxamide (7e).** Preparation of compound **7e** was performed following  
25 general procedure A starting from Boc-protected dioxodiazaspiro-bithiazole-spermidine **6e** (20.0 mg,  
26 0.03 mmol) and trifluoroacetic acid (0.06 mL, 0.78 mmol, 26 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL). The product was  
27 precipitated in Et<sub>2</sub>O to give compound **7e** as a colourless oil (16.6 mg, 79%). HPLC Rt = 7.0 min,  
28 analytical HPLC method A; <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O) δ 8.23 (s, 1H), 8.08 (s, 1H), 4.14 (s, 2H),  
29 3.69 (t, J = 5.9 Hz), 3.55 (t, J = 6.6 Hz, 2H), 3.29 (t, J = 5.9 Hz 2H), 3.19 – 3.08 (m, 4H), 3.04 (t, J =  
30 7.1 Hz, 2H), 2.10 – 2.00 (m, 2H), 1.99 – 1.91 (m, 2H), 1.85 – 1.70 (m, 10H); <sup>13</sup>C NMR (101MHz,  
31 D<sub>2</sub>O) δ 180.3, 170.6, 168.6, 163.2, 162.8, 156.9, 148.7, 147.0, 125.0, 119.0, 68.6, 46.9, 45.1, 40.6,  
32 39.0, 38.7, 37.8, 37.1, 36.2, 32.0, 25.8, 24.9, 23.9, 22.7; HRMS (ESI) *m/z* 577.23822 (M+H)<sup>+</sup>  
33 (C<sub>25</sub>H<sub>37</sub>N<sub>8</sub>O<sub>4</sub>S<sub>2</sub> requires 577.23737).

34 **2'-[Biotinamido]ethyl-2,4'-bithiazole-4-[3-(4-N-tertbutoxycarbonyl-aminobutyl)-N-**  
35 **tertbutoxycarbonyl-aminopropyl]carboxamide (6f).** To a solution of biotine **12f** (29.4 mg,  
36 0.12 mmol, 1.4 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (3 mL), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (22.1 mg, 0.14  
37 mmol, 1.6 eq.) and *N*-hydroxysuccinimide (16.2 mg, 0.14 mmol, 1.6 eq.) were added and the reaction

1 mixture was stirred 4 h at room temperature. Bithiazole Boc-spermidine **5** (50.4 mg, 0.09 mmol) was  
2 added, and the reaction mixture was stirred 5 h at room temperature. The organic mixture was washed  
3 with H<sub>2</sub>O (2 mL x 3) then brine (2 mL) before being dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under  
4 reduced pressure. The crude product was purified by FC on a silica gel column using a mixture  
5 CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10) from 60/40 to 10/90 as the eluent to give compound **6f** as a yellow solid (33.7  
6 mg, 49%). R<sub>f</sub> = 0.64 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10); <sup>1</sup>H NMR (400MHz, CD<sub>3</sub>OD) δ 8.18 (s, 1H), 8.16 (s, 1H),  
7 4.48 – 4.42 (m, 1H), 4.26 – 4.21 (m, 1H), 3.70 – 3.57 (m, 2H), 3.46 – 3.40 (m, 2H), 3.34 – 3.30 (m,  
8 2H), 3.30 – 3.21 (m, 4H), 3.14 – 3.08 (m, 1H), 3.07 – 3.01 (m, 2H), 2.88 (dd, J = 4.8 Hz, 12.7 Hz, 1H),  
9 2.68 (d, J = 12.7 Hz, 1H), 2.19 (t, J = 7.3 Hz, 2H), 1.87 (br, 2H), 1.73 – 1.30 (m, 28H); <sup>13</sup>C NMR  
10 (101MHz, CD<sub>3</sub>OD) as a mixture of 2 isomers δ 176.4, 171.0, 166.2, 164.2, 163.6, 158.7, 151.8, 149.7,  
11 125.1, 118.7, 81.2, 80.0, 63.4, 61.7, 57.1, 48.2, 48.1, 46.4, 45.7, 41.2, 41.1, 40.2, 38.4, 38.1, 36.9,  
12 33.9, 30.9, 30.8, 29.8, 29.6, 29.4, 29.0, 28.9, 27.0, 27.1, 26.6; MS (ESI) *m/z* 809.07 (M+H)<sup>+</sup>  
13 (Theoretical 809.34).

14 **2'-[Biotinamido]ethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-aminopropyl]carboxamide (7f).**

15 Preparation of compound **7f** was performed following general procedure A starting from Boc-  
16 protected biotine-bithiazole-spermidine **6f** (27.2 mg, 0.03 mmol) and trifluoroacetic acid (0.08 mL,  
17 1.04 mmol, 35 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL). The product was precipitated in Et<sub>2</sub>O to give compound **7f** as a  
18 yellow oil (16.4 mg, 68%). HPLC Rt = 7.2min, analytical HPLC method A; <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O)  
19 δ 8.20 (s, 1H), 8.08 (s, 1H), 4.44 (dd, J = 4.8 Hz, 7.8 Hz, 1H), 4.16 (dd, J = 4.4 Hz, 7.9 Hz, 1H), 3.72 –  
20 3.56 (m, 2H), 3.52 (t, J = 6.6 Hz, 2H), 3.27 (t, J = 5.5 Hz, 2H), 3.17 – 3.06 (m, 4H), 3.03 (t, J = 7.0 Hz,  
21 2H), 2.98 – 2.91 (m, 1H), 2.81 (dd, J = 4.9 Hz, 13.0 Hz, 1H), 2.63 (d, J = 13.0 Hz, 1H), 2.15 (t, J = 7.0  
22 Hz, 2H), 2.08 – 1.97 (m, 2H), 1.84 – 1.68 (m, 4H), 1.46 – 1.34 (m, 3H), 1.35 – 1.22 (m, 1H), 1.10 –  
23 0.98 (m, 2H); <sup>13</sup>C NMR (101MHz, D<sub>2</sub>O) δ 176.6, 171.2, 165.1, 163.4, 163.1, 162.8, 125.1, 119.1, 61.8,  
24 60.1, 55.1, 47.0, 45.2, 39.7, 38.7, 38.7, 36, 35.4, 32.1, 27.5, 27.4, 25.8, 25.3, 23.9, 22.7; HRMS (ESI)  
25 *m/z* 609.24640 (M+H)<sup>+</sup> (C<sub>26</sub>H<sub>41</sub>N<sub>8</sub>O<sub>3</sub>S<sub>3</sub> requires 609.24583).

26 **2'-[Heptinoic-7-carboxamido]ethyl-2,4'-bithiazole-4-[3-(4-*N*-tertbutoxycarbonyl-aminobutyl)-*N*-**  
27 **tertbutoxycarbonyl-aminopropyl]carboxamide (6g).** To a solution of heptynoic acid **12g** (16.0 μL,

28 0.13 mmol, 1.4 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) was added *N,N,N',N'*-tetramethyl-*O*-(1*H*-benzotriazol-1-  
29 yl)uronium hexafluorophosphate (83.7 mg, 0.22 mmol, 2.4 eq.) and *N,N*-diisopropylethylamine (75.0  
30 μL, 0.46 mmol, 5.1 eq.) and the reaction mixture was stirred 5 min at room temperature. Bithiazole  
31 Boc-spermidine **5** (49.9 mg, 0.09 mmol) was then added, and the reaction mixture was stirred 2 h at  
32 room temperature. The organic mixture was washed with H<sub>2</sub>O (2 mL x 3) then brine (2 mL) before  
33 being dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The crude product was purified by  
34 FC on a silica gel column using a mixture CH<sub>2</sub>Cl<sub>2</sub>/(CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10) from 100/0 to 50/50 as the  
35 eluent leading to compound **6g** as a white solid (34.8 mg, 59%). R<sub>f</sub> = 0.81 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 96/4); <sup>1</sup>H  
36 NMR (400MHz, CD<sub>3</sub>OD) δ 8.17 (s, 1H), 8.15 (s, 1H), 3.63 (t, J = 6.6 Hz, 2H), 3.43 (t, J = 6.6 Hz,  
37 2H), 3.33 – 3.29 (m, 2H), 3.29 – 3.20 (m, 4H), 3.04 (t, J = 6.7 Hz, 2H), 2.23 – 2.10 (m, 5H), 1.87 (br,

1 2H), 1.73 – 1.65 (m, 2H), 1.56 (br, 2H), 1.52 – 1.36 (m, 22H); <sup>13</sup>C NMR (101MHz, CD<sub>3</sub>OD) as a  
2 mixture of 2 isomers δ 176.2, 170.9, 164.2, 163.6, 158.7, 151.8, 149.7, 125.0, 118.7, 84.7, 81.2, 80.0,  
3 70.0, 48.2, 46.4, 45.9, 41.1, 40.2, 38.4, 38.0, 36.6, 33.9, 30.8, 29.3, 29.0, 28.9, 28.5, 27.2, 26.7, 26.2,  
4 18.9; MS (ESI) *m/z* 690.87 (M+H)<sup>+</sup> (Theoretical 691.32).

5 **2'-[Heptinoic-7-carboxamido]ethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-**  
6 **aminopropyl]carboxamide (7g)**. Preparation of compound **7g** was performed following general  
7 procedure A starting from Boc-protected heptyn-bithiazole-spermidine **6g** (34.8 mg, 0.05 mmol) and  
8 trifluoroacetic acid (0.10 mL, 1.31 mmol, 26 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (3 mL). The product was precipitated in  
9 Et<sub>2</sub>O to give compound **7g** as a yellow oil (35.1 mg, 97%). <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O) δ 8.18 (s, 1H),  
10 8.04 (s, 1H), 3.62 (t, J = 5.8 Hz, 2H), 3.52 (t, J = 6.6 Hz, 2H), 3.25 (t, J = 5.8 Hz, 2H), 3.16 – 3.06 (m,  
11 4H), 3.02 (t, J = 6.7 Hz, 2H), 2.21 (t, J = 2.5 Hz, 1H), 2.16 (t, J = 7.3 Hz, 2H), 2.08 – 1.96 (m, 4H),  
12 1.83 – 1.69 (m, 4H), 1.55 – 1.44 (m, 2H), 1.31 – 1.21 (m, 2H); <sup>13</sup>C NMR (101MHz, D<sub>2</sub>O) δ 176.5,  
13 170.9, 163.5, 159.9, 148.7, 146.9, 125.0, 119.1, 85.5, 69.2, 46.9, 45.1, 38.8, 36.2, 35.3, 32.1, 27.0,  
14 25.8, 24.6, 23.9, 22.7, 17.2; HRMS (ESI) *m/z* 491.22629 (M+H)<sup>+</sup> (C<sub>23</sub>H<sub>35</sub>N<sub>6</sub>O<sub>2</sub>S<sub>2</sub> requires 491.22574);  
15 HPLC Rt = 8.1 min, analytical HPLC method A.

16 **2'-[Naphthalene-2-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-*N*-tertbutoxycarbonyl-aminobutyl)-**  
17 ***N*-tertbutoxycarbonyl-aminopropyl]carboxamide (8a)**. Compound **8a** was prepared following  
18 general procedure C starting from Bithiazole Boc-spermidine **5** (45.0 mg, 0.08 mmol), naphthalene-2-  
19 sulfonyl chloride **13a** (22.1 mg, 0.10 mmol, 1.2 eq.) and triethylamine (20.0 μL, 0.12 mmol, 1.6 eq.) in  
20 DMF (1 mL). The crude product was purified by FC on a silica gel column using a mixture  
21 CH<sub>2</sub>Cl<sub>2</sub>/(CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10) from 100/0 to 70/30 as the eluent leading to compound **8a** as a dark  
22 yellow solid (32.0 mg, 54%). R<sub>f</sub> = 0.7 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 96/4); <sup>1</sup>H NMR (400MHz, CD<sub>3</sub>OD) as a  
23 mixture of 2 isomers δ 8.27 (s, 1H), 8.03 (s, 0.5H), 7.96 (s, 1H), 7.87 (s, 0.5H), 7.86 – 7.81 (m, 1H),  
24 7.80 – 7.72 (m, 1H), 7.66 (t, J = 4.1 Hz, 1H), 7.54 – 7.44 (m, 2H, 2H), 3.41 – 3.29 (m, 4H), 3.23 (m,  
25 2H), 3.19 – 3.04 (m, 4H), 2.96 (t, J = 5.9 Hz, 2H), 1.78 (br, 2H), 1.48 (br, 2H), 1.42 – 1.26 (m, 20H);  
26 <sup>13</sup>C NMR (101MHz, CD<sub>3</sub>OD) as a mixture of two isomers δ 169.7, 168.6, 162.6, 162.0, 157.1, 150.20,  
27 148.0, 137.0, 134.7, 132.1, 129.3, 128.9, 128.5, 127.8, 127.6, 127.3, 123.5, 123.4, 121.9, 117.30,  
28 117.15, 79.8, 46.63, 45.0, 44.2, 42.2, 39.7, 37.0, 36.5, 32.9, 32.8, 29.7, 29.4, 27.7, 27.0, 25.7, 25.2; MS  
29 (ESI) *m/z* 772.93 (M+H)<sup>+</sup> (Theoretical 773.27).

30 **2'-[Naphthalene-2-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-**  
31 **aminopropyl]carboxamide (9a)**. Deprotection was performed following general procedure B starting  
32 from Boc-protected naphthalene sulphonamide **8a** (26.0 mg, 0.03 mmol) and of hydrochloric acid (0.09  
33 mL, 1.08 mmol, 36 eq.) in MeOH (1.5 mL). The product was precipitated in Et<sub>2</sub>O to give compound  
34 **9a** as a slightly yellow solid (32.0 mg, 97%). HPLC Rt = 15.1 min, analytical HPLC method A; <sup>1</sup>H  
35 NMR (400MHz, D<sub>2</sub>O) δ as a mixture of isomers 8.20 (s, 0.3H), 8.17 (s, 0.3H), 8.03 (s, 0.8H), 8.20 (s,  
36 0.3H), 7.99 (m, 0.3H), 7.95 (s, 0.7H), 7.88 (m, 0.3H), 7.69 – 7.60 (m, 2H), 7.54 – 7.52 (m, 1H), 7.52 –  
37 7.32 (m, 3H), 3.61 – 3.50 (m, 4H), 3.22 – 3.10 (m, 6H), 3.09 – 3.04 (m, 2H), 2.12 – 2.04 (m, 2H),

1 1.86 – 1.74 (m, 4H); <sup>13</sup>C NMR (101 MHz, D<sub>2</sub>O) as a mixture of isomers δ 171.1, 169.6, 163.4, 161.8,  
2 148.1, 146.6, 142.9, 135.5, 134.1, 131.2, 129.6, 128.8, 128.5, 127.6, 127.5, 127.1, 124.9, 120.9,  
3 119.2, 118.6, 47.01, 45.24, 42.35, 38.80, 36.30, 31.73, 25.80, 23.93, 22.79, 22.21, 21.72; HRMS (ESI)  
4 *m/z* 573.17865 (M+H)<sup>+</sup> (C<sub>26</sub>H<sub>32</sub>N<sub>6</sub>O<sub>3</sub>S<sub>3</sub> requires 573.7870).

5 **2'-[4-Methylphenyl-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-*N*-tertbutoxycarbonyl-**  
6 **aminobutyl)-*N*-tertbutoxycarbonyl-aminopropyl]carboxamide (8b).** Compound **8b** was prepared  
7 following general procedure C starting from Bithiazole Boc-spermidine **5** (44.4 mg, 0.08 mmol), *p*-  
8 toluene sulfonyl chloride **13b** (19.2 mg, 0.10 mmol, 1.3 eq.) and triethylamine (20.0 μL, 0.12 mmol,  
9 1.6 eq.) in DMF (1 mL). The crude product was purified by FC on a silica gel column using a mixture  
10 CH<sub>2</sub>Cl<sub>2</sub>/ MeOH from 100/0 to 95/05 as the eluent leading to compound **8b** as a white solid (44.0 mg,  
11 78%). *R<sub>f</sub>* = 0.35 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 96/4); HPLC *R<sub>t</sub>* = 20.9 min, analytical HPLC method B; <sup>1</sup>H NMR  
12 (400MHz, CD<sub>3</sub>OD) δ 8.15 (s, 1H), 8.08 (s, 1H), 7.68 (d, *J* = 8.2 Hz, 2H), 7.30 (d, *J* = 8.2 Hz, 2H),  
13 3.42 (t, *J* = 6.7 Hz, 2H), 3.34 – 3.29 (m, 4H), 3.26 – 3.15 (m, 4H), 3.04 (t, *J* = 6.6 Hz, 2H), 2.36 (s,  
14 3H), 1.86 (br, 2H), 1.56 (br, 2H), 1.50- 1.37 (m, 20H); <sup>13</sup>C NMR (101MHz, CD<sub>3</sub>OD) as a mixture of 2  
15 isomers δ 170.2, 163.9, 163.4, 158.5, 151.6, 149.4, 144.7, 138.7, 130.7, 128.0, 124.8, 118.5, 81.0,  
16 79.8, 48.0, 47.9, 46.3, 45.5, 43.4, 41.0, 38.2, 37.8, 34.2, 29.9, 29.2, 28.8, 28.7, 28.3, 27.0, 26.5, 21.5;  
17 MS (ESI) *m/z* 736.80 (M+H)<sup>+</sup> (Theoretical 735.30).

18 **2'-[4-Methylphenyl)sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-**  
19 **aminopropyl]carboxamide (9b).** Deprotection was performed following general procedure B from  
20 Boc-protected *p*-toluene sulphonamide **8b** (39.0 mg, 0.05 mmol) and of hydrochloric acid (0.18 mL,  
21 2.16 mmol, 43 eq.) in MeOH (1 mL). The product was precipitated in Et<sub>2</sub>O to give compound **9b** as a  
22 white solid (29.6 mg, 92%). HPLC *R<sub>t</sub>* = 12.2 min, analytical HPLC method B; <sup>1</sup>H NMR (400MHz,  
23 D<sub>2</sub>O) δ 8.02 (s, 1H), 7.73 (s, 1H), 7.39 (d, *J* = 7.4 Hz, 2H), 7.00 (d, *J* = 7.4 Hz, 2H), 3.50 – 3.43 (m,  
24 2H), 3.30 – 3.22 (m, 2H), 3.15 – 3.06 (m, 4H), 3.06 – 2.96 (m, 4H), 2.08 – 1.96 (m, 5H), 1.83 – 1.70  
25 (m, 4H); <sup>13</sup>C NMR (101MHz, D<sub>2</sub>O) δ 169.5, 163.1, 162.6, 148.6, 146.8, 144.2, 135.3, 129.7, 126.3,  
26 124.9, 118.7, 47.0, 45.2, 42.2, 38.8, 36.3, 32.1, 25.8, 23.9, 22.8, 20.6; HRMS (ESI) *m/z* 537.17743  
27 (M+H)<sup>+</sup> (C<sub>23</sub>H<sub>33</sub>N<sub>6</sub>O<sub>3</sub>S<sub>3</sub> requires 537.17708).

28 **2'-[4-Trifluoromethylphenyl)sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-*N*-tertbutoxycarbonyl-**  
29 **aminobutyl)-*N*-tertbutoxycarbonyl-aminopropyl]carboxamide (8c).** Compound **8c** was prepared  
30 following general procedure C starting from Bithiazole Boc-spermidine **5** (45.7 mg, 0.08 mmol), 4-  
31 (trifluoromethyl)benzenesulfonyl chloride **13c** (23.9 mg, 0.10 mmol, 1.2 eq.) and triethylamine (20.0  
32 μL, 0.12 mmol, 1.6 eq.) in DMF (1 mL). The crude product was purified by FC on a silica gel column  
33 using a mixture CH<sub>2</sub>Cl<sub>2</sub>/ (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10) from 100/0 to 70/30 gave **8c** as a slightly yellow oil  
34 (44.3 mg, 70%). *R<sub>f</sub>* = 0.35 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 96/4); <sup>1</sup>H NMR (400MHz, CD<sub>3</sub>OD) δ 8.15 (s, 1H), 8.08 (s,  
35 1H), 8.00 (d, *J* = 8.2 Hz, 2H), 7.81 (d, *J* = 8.3 Hz, 2H), 3.45 – 3.38 (m, 4H), 3.34 – 3.29 (m, 2H), 3.26  
36 – 3.19 (m, 4H), 3.04 (t, *J* = 6.7 Hz, 2H), 1.91 – 1.817 (br, 2H), 1.62 – 1.51 (m, 2H), 1.49 – 1.37 (m,  
37 20H); <sup>13</sup>C NMR (101MHz, CD<sub>3</sub>OD) as a mixture of 2 isomers δ 170.1, 163.8, 163.4, 158.5, 157.7,

1 151.7, 149.5, 145.9, 135.3, 135.0, 134.7, 134.4, 128.7, 127.4, 127-3, 124.8, 123.5, 120.8, 118.6, 81.0,  
2 79.8, 48.0, 47.9, 46.3, 45.5, 43.5, 41.0, 38.2, 37.8, 34.3, 30.0, 29.27, 28.8, 28.7, 28.3, 27.0, 26.5. MS  
3 (ESI)  $m/z$  789.27 (M+H)<sup>+</sup> (Theoretical 790.30).

4 **2'-[4-Trifluoromethylphenyl)sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-**  
5 **aminopropyl]carboxamide (9c)**. Deprotection was performed following general procedure B starting  
6 from Boc-protected 4-(trifluoromethyl)benzene sulfonamide **8c** (58.9 mg, 0.07 mmol) and of  
7 hydrochloric acid (0.19 mL, 2.22 mmol, 32 eq.) in MeOH (1 mL). The product was precipitated in  
8 Et<sub>2</sub>O to give compound **9c** as a brown solid (24.5 mg, 50%). HPLC Rt = 15.1 min, analytical HPLC  
9 method A; <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O)  $\delta$  7.89 (s, 1H), 7.75 – 7.67 (m, 3H), 7.40 (d, J = 7.7 Hz, 2H), 3.51  
10 – 3.42 (m, 2H), 3.41 – 3.32 (m, 2H), 3.16 – 3.08 (m, 4H), 3.08 – 2.99 (m, 4H), 2.09 – 1.97 (m, 2H),  
11 1.85 – 1.71 (m, 4H, H-14). <sup>13</sup>C NMR (101MHz, D<sub>2</sub>O)  $\delta$  169.4, 163.0, 162.1, 148.7, 146.7, 142.8,  
12 133.4, 133.2, 127.0, 126.2, 124.7, 124.4, 121.7, 118.5, 47.1, 45.2, 42.3, 38.8, 36.3, 32.2, 25.8, 23.9,  
13 22.8; HRMS (ESI)  $m/z$  591.15021 (M+H)<sup>+</sup> (C<sub>23</sub>H<sub>32</sub>N<sub>6</sub>O<sub>3</sub>S<sub>3</sub> requires 591.14881).

14 **2'-[2-Fluorophenyl-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-N-tertbutoxycarbonyl-aminobutyl)-**  
15 **N-tertbutoxycarbonyl-aminopropyl]carboxamide (8d)**. Compound **8d** was prepared following  
16 general procedure C starting from Bithiazole Boc-spermidine **5** (44.5 mg, 0.08 mmol), 2-  
17 fluorobenzenesulfonyl chloride **13d** (12.0  $\mu$ L, 0.09 mmol, 1.2 eq.) and triethylamine (20.0  $\mu$ L, 0.12  
18 mmol, 1.6 eq.) in DMF (1 mL). The crude product was purified by FC on a silica gel column using a  
19 mixture CH<sub>2</sub>Cl<sub>2</sub>/ (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10) from 100/0 to 70/30 as the eluent leading to compound **8d** as  
20 a white solid (33.0 mg, 58%). R<sub>f</sub> = 0.28 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 96/4); HPLC Rt = 20.4 min, analytical HPLC  
21 method B; <sup>1</sup>H NMR (400MHz, CD<sub>3</sub>OD)  $\delta$  8.16 (s, 1H), 8.08 (s, 1H), 7.84 (t, J = 7.5 Hz, 1H), 7.62 –  
22 7.54 (m, 1H), 7.31 – 7.18 (m, 2H), 3.49 – 3.37 (m, 4H), 3.36 – 3.27 (m, 2H), 3.26 – 3.18 (m, 4H), 3.04  
23 (t, J = 6.5 Hz, 2H), 1.89 (br, 2H), 1.56 (br, 2H), 1.50 – 1.35 (m, 20H); <sup>13</sup>C NMR (101MHz, CD<sub>3</sub>OD) as  
24 a mixture of 2 isomers  $\delta$  170.3, 164.1, 163.6, 161.6, 159.0, 158.7, 157.8, 157.5, 151.8, 149.6, 136.4,  
25 136.3, 131.5, 130.0, 129.9, 125.8, 125.8, 125.0, 118.7, 118.4, 118.2, 81.1, 80.0, 48.2, 48.0, 46.5, 45.7,  
26 43.5, 41.1, 38.4, 38.0, 34.4, 30.6, 30.1, 29.4, 29.0, 28.9, 27.1, 26.7; MS (ESI)  $m/z$  740.93 (M+H)<sup>+</sup>  
27 (Theoretical 741.25).

28 **2'-[2-Fluorophenyl-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-**  
29 **aminopropyl]carboxamide (9d)**. Deprotection was performed following general procedure A from  
30 Boc-protected fluorophenyl sulphonamide **8d** (28.5 mg, 0.04 mmol) and trifluoroacetic acid (0.12 mL,  
31 1.57 mmol, 39 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL). The product was precipitated in Et<sub>2</sub>O to give compound **9d** as a  
32 slightly yellow solid (32.0 mg, 97%). HPLC Rt = 11.8 min, analytical HPLC method B; <sup>1</sup>H NMR  
33 (400MHz, D<sub>2</sub>O)  $\delta$  8.04 (s, 1H), 7.74 (s, 1H), 7.57 (t, J = 7.0 Hz, 1H), 7.37 (dd, J = 6.5 Hz, 12.8 Hz,  
34 1H), 7.06 (t, J = 6.7, 1H), 7.04 – 6.97 (m, 1H), 3.48 (dd, J = 8.9 Hz, 15.5 Hz, 2H), 3.40 (d, J = 5.8 Hz,  
35 2H), 3.17 – 3.04 (m, 6H), 3.01 (t, J = 10.9 Hz, 2H), 2.07 – 1.95 (m, 2H), 1.82 – 1.67 (m, 4H); <sup>13</sup>C  
36 NMR (101MHz, D<sub>2</sub>O)  $\delta$  169.7, 163.2, 163.0, 159.4, 156.9, 148.5, 146.7, 135.7, 135.6, 129.3, 126.4,



1 126.3, 124.7, 124.6, 118.8, 117.19, 117.0, 46.9, 45.2, 42.2, 38.7, 36.3, 32.2, 25.7, 23.9, 22.7; HRMS  
2 (ESI),  $m/z$  541.15234 (M+H)<sup>+</sup> (C<sub>22</sub>H<sub>30</sub>FN<sub>6</sub>O<sub>3</sub>S<sub>3</sub> requires 541.15201).

3 **2'-[Quinoline-8-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-*N*-tertbutoxycarbonyl-aminobutyl)-*N*-**  
4 **tertbutoxycarbonyl-aminopropyl]carboxamide (8e).** Compound **8e** was prepared following general  
5 procedure C starting from Bithiazole Boc-spermidine **5** (44.8 mg, 0.08 mmol), quinoline-8-sulfonyl  
6 chloride **13e** (21.4 mg, 0.09 mmol, 1.1 eq.) and triethylamine (20.0  $\mu$ L, 0.12 mmol, 1.6 eq.) in DMF (1  
7 mL). The crude product was purified by FC on a silica gel column using a mixture  
8 CH<sub>2</sub>Cl<sub>2</sub>/ (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10) from 100/0 to 0/100 as the eluent leading to compound **8e** as a slightly  
9 yellow solid (48.2 mg, 81%).  $R_f$  = 0.52 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 96/4); HPLC  $R_t$  = 20.6 min, analytical HPLC  
10 method B; <sup>1</sup>H NMR (400MHz, CD<sub>3</sub>OD)  $\delta$  8.74 (br, 1H), 8.35 (d,  $J$  = 7.0 Hz, 1H), 8.29 (d,  $J$  = 8.1 Hz,  
11 1H), 8.15 (s, 1H), 8.11 (d,  $J$  = 8.0 Hz, 1H), 7.90 (s, 1H), 7.65 (t,  $J$  = 8.0 Hz, 1H), 7.45 (dd,  $J$  = 4.3 Hz,  
12 8.3 Hz, 1H), 3.48 – 3.40 (m, 4H), 3.34 – 3.29 (m, 2H), 3.23 (t,  $J$  = 7.2 Hz, 2H), 3.12 (t,  $J$  = 6.3 Hz,  
13 2H), 3.04 (t,  $J$  = 6.7 Hz, 2H), 1.87 (br, 2H), 1.56 (br, 2H), 1.51 – 1.36 (m, 20H); <sup>13</sup>C NMR (101MHz,  
14 CD<sub>3</sub>OD) as a mixture of 2 isomers  $\delta$  170.0, 164.8, 163.4, 158.5, 157.7, 152.3, 151.6, 149.2, 144.2,  
15 138.1, 137.5, 134.9, 131.8, 130.3, 126.8, 124.9, 123.5, 118.5, 81.0, 79.8, 48.0, 47.8, 46.3, 45.5, 43.6,  
16 41.0, 38.3, 37.8, 33.6, 30.7, 29.9, 28.8, 28.3, 27.0, 26.5; MS (ESI)  $m/z$  774.00 (M+H)<sup>+</sup> (Theoretical  
17 774.27).

18 **2'-[Quinoline-8-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-**  
19 **aminopropyl]carboxamide (9e).** Deprotection was performed following general procedure B starting  
20 from Boc-protected quinoline sulphonamide **8e** (47.1 mg, 0.06 mmol) and of hydrochloric acid (0.15  
21 mL, 1.80 mmol, 30 eq.) in MeOH (1 mL). The product was precipitated in Et<sub>2</sub>O to give compound **9e**  
22 as a slightly yellow solid (36.3 mg, 92%). HPLC  $R_t$  = 13.8 min, analytical HPLC method B; <sup>1</sup>H NMR  
23 (400MHz, D<sub>2</sub>O)  $\delta$  8.99 – 8.89 (m, 1H), 8.68 – 8.62 (m, 1H), 8.42 – 8.31 (m, 1H), 8.10 – 7.98 (m, 2H),  
24 7.75 – 7.65 (m, 1H), 7.70 (m, 1H), 7.38 – 7.35 (m, 1H), 3.58 – 3.50 (m, 4H), 3.22 – 3.08 (m, 4H, H-  
25 12), 3.09 – 2.97 (m, 4H), 2.13 – 2.01 (m, 2H), 1.87 – 1.71 (m, 4H); <sup>13</sup>C NMR (101MHz, D<sub>2</sub>O)  $\delta$  169.8,  
26 163.2, 161.5, 148.5, 147.7, 146.1, 146.0, 135.8, 135.1, 134.0, 129.7, 129.3, 128.5, 124.9, 122.6, 118.7,  
27 47.0, 45.3, 42.7, 38.8, 36.4, 31.5, 25.8, 23.9, 22.8; HRMS (ESI)  $m/z$  574.17389 (M+H)<sup>+</sup>  
28 (C<sub>25</sub>H<sub>32</sub>N<sub>7</sub>O<sub>3</sub>S<sub>3</sub> requires 574.17233).

29 **2'-[Benzo[b]thiophene-2-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-*N*-tertbutoxycarbonyl-**  
30 **aminobutyl)-*N*-tertbutoxycarbonyl-aminopropyl]carboxamide (8f).** Compound **8f** was prepared  
31 following general procedure C starting from Bithiazole Boc-spermidine **5** (44.6 mg, 0.08 mmol),  
32 benzo[b]thiophene-2-sulfonyl chloride **13f** (20.9 mg, 0.10 mmol, 1.2 eq.) and triethylamine (20.0  $\mu$ L,  
33 0.12 mmol, 1.6 eq.) in DMF (1 mL). Purification by FC (CH<sub>2</sub>Cl<sub>2</sub>/ (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10) from 100/0  
34 to 0/100) gave **8f** as a slightly yellow solid (24.6mg, 39%).  $R_f$  = 0.18 (Cyclohexane/EtOAc 4/6); <sup>1</sup>H  
35 NMR (400MHz, CD<sub>3</sub>OD)  $\delta$  8.11 (s, 1H), 8.02 (s, 1H), 7.89 – 7.81 (m, 3H), 7.46 – 7.37 (m, 2H), 3.52  
36 (t,  $J$  = 6.5 Hz, 1H), 3.43 (t,  $J$  = 6.7 Hz, 2H), 3.34 – 3.29 (m, 2H), 3.28 – 3.19 (m, 4H), 3.04 (t,  $J$  = 6.7  
37 Hz, 2H), 1.87 (br, 2H), 1.62 – 1.51 (m, 2H), 1.50 – 1.35 (m, 20H); <sup>13</sup>C NMR (101MHz, CD<sub>3</sub>OD) as a

1 mixture of two isomers  $\delta$  170.3, 164.0, 163.6, 158.7, 151.7, 149.6, 143.1, 143.0, 139.3, 130.1, 128.4,  
2 126.8, 126.6, 125.0, 123.8, 118.7, 81.2, 80.0, , 48.2, 48.1, 46.4, 45.7, 43.9, 41.1, 38.4, 38.0, 34.2, 30.1,  
3 29.5, 29.0, 28.9, 28.7, 28.5, 27.2, 26.7; MS (ESI)  $m/z$  778.93 (M+H)<sup>+</sup> (Theoretical 779.23).

4 **2'-[Benzo[b]thiophene-2-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-**  
5 **aminopropyl]carboxamide (9f)**. Deprotection was performed following general procedure B starting  
6 from Boc-protected benzothiophène sulphonamide **8f** (19.6 mg, 0.03 mmol) and of hydrochloric acid  
7 (0.13 mL, 1.56 mmol, 52 eq.) in MeOH (1.5 mL). The product was precipitated in Et<sub>2</sub>O to give  
8 compound **9f** as a yellow foam (13.1 mg, 80%). HPLC Rt = 13.7 min, analytical HPLC method B; <sup>1</sup>H  
9 NMR (400MHz, D<sub>2</sub>O)  $\delta$  7.91 (s, 1H), 7.54 (s, 1H), 7.51 – 7.46 (m, 2H), 7.44 – 7.38 (m, 1H), 7.15 –  
10 7.07 (m, 2H), 3.48 (m, 4H), 3.08 (m, 8H), 2.10 – 1.98 (m, 2H), 1.84 – 1.70 (m, 4H); <sup>13</sup>C NMR  
11 (101MHz, D<sub>2</sub>O)  $\delta$  169.5, 163.2, 161.8, 148.2, 146.7, 141.0, 139.5, 137.0, 129.4, 127.0, 125.5, 125.1,  
12 122.3, 118.5, 47.0, 45.2, 42.6, 38.8, 36.3, 31.8, 25.8, 23.9, 22.8; HRMS (ESI)  $m/z$  579.13409 (M+H)<sup>+</sup>  
13 (C<sub>24</sub>H<sub>31</sub>N<sub>6</sub>O<sub>3</sub>S<sub>4</sub> requires 579.13350).

14 **2'-[4-(Dimethylamino)azobenzene-4'-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-*N*-**  
15 **tertbutoxycarbonyl-aminobutyl)-*N*-tertbutoxycarbonyl-aminopropyl]carboxamide (8g)**. The title  
16 compound **8g** was prepared following the general procedure C starting from Bithiazole Boc-  
17 spermidine **5** (56.8 mg, 0.10 mmol), (E)-4-((4-(dimethylamino)phenyl)diazenyl)benzenesulfonyl  
18 chloride **13g** (32.0 mg, 0.11 mmol, 1.2 eq.) and triethylamine (0.02 mL, 0.15 mmol, 1.6 eq.) in DMF  
19 (1.5 mL). Purification by FC (CH<sub>2</sub>Cl<sub>2</sub>/ (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10) from 100/0 to 90/10) gave **8g** as a red  
20 solid (54.2 mg, 66%). HPLC Rt = 21.7 min, analytical HPLC method B; R<sub>f</sub> = 0.34 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH  
21 96/4); <sup>1</sup>H NMR (400MHz, CD<sub>3</sub>OD)  $\delta$  8.06 (s, 1H), 8.00 (s, 1H), 7.84 (d, J = 8.6 Hz, 2H), 7.80 – 7.73  
22 (m, 4H), 6.74 (d, J = 9.0 Hz, 2H), 3.39 (t, J = 6.2 Hz, 2H), 3.36 – 3.32 (m, 2H), 3.29 – 3.21 (m, 2H),  
23 3.21 – 3.14 (m, 4H), 3.05 (s, 6H), 3.01 (t, J = 6.5 Hz, 2H), 1.79 (br, 2H), 1.52 (br, 2H), 1.47 –  
24 1.34 (m, 20H); <sup>13</sup>C NMR (101MHz, CD<sub>3</sub>OD) as a mixture of 2 isomers  $\delta$  170.2, 163.9, 163.4, 158.6,  
25 156.7, 154.9, 151.7, 149.6, 144.8, 141.4, 129.1, 126.9, 124.9, 123.6, 118.7, 112.7, 81.1, 79.9, 48.2,  
26 48.0, 46.4, 45.7, 43.6, 41.1, 40.6, 38.4, 38.0, 34.3, 30.0, 29.4, 29.0, 28.9, 27.1, 26.6; MS (ESI)  $m/z$   
27 870.00 (M+H)<sup>+</sup> (Theoretical 870.34).

28 **2'-[4-(Dimethylamino)azobenzene-4'-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-**  
29 **aminopropyl]carboxamide (9g)**. Deprotection was performed following general procedure A starting  
30 from Boc-protected diazobenzene-bithiazole-spermidine **8g** (48.9 mg, 0.056 mmol) and trifluoroacetic  
31 acid (0.18 mL, 2.35 mmol, 42 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL). The product was precipitated in Et<sub>2</sub>O to give  
32 compound **9g** as an orange solid (49.8 mg, 99%). HPLC Rt = 14.6 min, analytical HPLC method A;  
33 <sup>1</sup>H NMR (400MHz, CD<sub>3</sub>OD)  $\delta$  8.15 (s, 1H), 8.04 (s, 1H), 7.87 (d, J = 9.0 Hz, 2H), 7.81 (d, J = 9.0 Hz,  
34 4H), 6.81 (d, J = 9.2 Hz, 2H), 3.49 (t, J = 6.3 Hz, 2H), 3.41 (t, J = 6.5 Hz, 2H), 3.20 (t, J = 6.5 Hz, 2H),  
35 3.10 (s, 6H), 3.08 – 3.02 (m, 4H), 2.98 (t, J = 6.9 Hz, 2H), 2.06 – 1.95 (m, 2H), 1.86 – 1.70 (m, 4H);  
36 <sup>13</sup>C NMR (101MHz, CD<sub>3</sub>OD)  $\delta$  170.4, 164.5, 164.2, 156.8, 155.0, 151.2, 149.5, 144.8, 141.5, 129.1,

1 126.9, 125.5, 123.6, 118.8, 112.8, 48.3, 46.6, 43.6, 40.5, 40.1, 37.2, 34.3, 27.9, 25.7, 24.4; HRMS  
2 (ESI)  $m/z$  670.24115 (M+H)<sup>+</sup> (C<sub>30</sub>H<sub>40</sub>N<sub>9</sub>O<sub>3</sub>S<sub>3</sub> requires 670.24107).

3 **2'-[1H-Imidazole-4-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-N-tertbutoxycarbonyl-**  
4 **aminobutyl)-N-tertbutoxycarbonyl-aminopropyl]carboxamide (8h)**. Compound **8h** was prepared  
5 following general procedure C starting from Bithiazole Boc-spermidine **5** (45.2 mg, 0.08 mmol), 1H-  
6 imidazole-4-sulfonyl chloride **13h** (15.7 mg, 0.09 mmol, 1.1 eq.) and triethylamine (20.0  $\mu$ L,  
7 0.12 mmol, 1.6 eq.) in DMF (1 mL). Purification by FC (CH<sub>2</sub>Cl<sub>2</sub>/ (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10) from 100/0  
8 to 0/100) gave **8h** as a colourless oil (28.5 mg, 51%).  $R_f$  = 0.43 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 94/6); <sup>1</sup>H NMR  
9 (400MHz, CD<sub>3</sub>OD)  $\delta$  8.16 (s, 1H), 8.14 (s, 1H), 7.82 (d, J = 0.9 Hz, 1H), 7.69 (d, J = 0.9 Hz, 1H), 3.47  
10 – 3.40 (m, 4H), 3.32 – 3.28 (m, 4H), 3.25 (t, J = 7.0 Hz, 2H), 3.06 (t, J = 7.0 Hz, 2H), 1.88 (br, 2H),  
11 1.58 (br, 2H), 1.49 – 1.38 (m, 20H); <sup>13</sup>C NMR (101MHz, CD<sub>3</sub>OD) as a mixture of 2 isomers  $\delta$  170.4,  
12 164.0, 163.4, 158.5, 157.6, 151.7, 149.5, 138.7, 124.9, 121.9, 118.5, 81.0, 79.8, 48.0, 47.9, 46.3, 45.6,  
13 43.5, 41.0, 38.3, 37.9, 34.4, 29.8, 29.3, 28.8, 28.7, 28.3, 26.8, 26.4; MS (ESI)  $m/z$  713.00 (M+H)<sup>+</sup>  
14 (Theoretical 713.91).

15 **2'-[1H-Imidazole-4-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-**  
16 **aminopropyl]carboxamide (9h)**. Deprotection was performed following general procedure B starting  
17 from Boc-protected *p*-toluene sulphonamide **8h** (24.4 mg, 0.03 mmol) and of hydrochloric acid (0.09  
18 mL, 1.08 mmol, 36 eq.) in MeOH (1 mL). The product was precipitated in Et<sub>2</sub>O to give compound **9h**  
19 as yellow oil (14.7 mg, 79%). HPLC  $R_t$  = 9.9 min, analytical HPLC method A; <sup>1</sup>H NMR (500MHz,  
20 D<sub>2</sub>O)  $\delta$  8.28 (s, 1H), 8.10 (s, 1H), 7.90 (s, 1H), 7.74 (s, 1H), 3.62 – 3.53 (m, 4H), 3.2.7 (t, J = 6.2 Hz,  
21 2H), 3.22 – 3.17 (m, 2H), 3.16 – 3.12 (m, 2H), 3.07 (t, J = 7.0 Hz, 2H), 2.12 – 2.05 (m, 2H), 1.80 –  
22 1.64 (m, 4H); <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O) as a mixture of two isomers  $\delta$  170.5, 163.7, 163.2, 162.9,  
23 148.7, 146.9, 138.0, 136.4, 125.1, 121.9, 119.0, 117.5, 115.2, 46.9, 45.1, 42.2, 38.8, 36.2, 32.3, 25.8,  
24 23.9, 22.73; HRMS (ESI)  $m/z$  513.15320 (M+H)<sup>+</sup> (C<sub>19</sub>H<sub>29</sub>N<sub>8</sub>O<sub>3</sub>S<sub>3</sub> requires 513.15193).

25 **2'-[2,4-Dimethylthiazole-5-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-N-tertbutoxycarbonyl-**  
26 **aminobutyl)-N-tertbutoxycarbonyl-aminopropyl]carboxamide (8i)**. Compound **8i** was prepared  
27 following general procedure C starting from Bithiazole Boc-spermidine **5** (44.8 mg, 0.08 mmol), 2,4-  
28 dimethylthiazole-5-sulfonyl chloride **13i** (19.7 mg, 0.10 mmol, 1.2 eq.) and triethylamine (20.0  $\mu$ L,  
29 0.12 mmol, 1.6 eq.) in DMF (1 mL). Purification by FC (CH<sub>2</sub>Cl<sub>2</sub>/ (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10) from 100/0  
30 to 0/100) gave **8i** as a yellow oil (23.6mg, 40%).  $R_f$  = 0.10 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 92/8); HPLC  $R_t$  = 21.3  
31 min, analytical HPLC method B; <sup>1</sup>H NMR (400MHz, CD<sub>3</sub>OD)  $\delta$  8.17 (s, 1H), 8.11 (s, 1H), 3.49 (t, J =  
32 6.5 Hz, 2H), 3.43 (t, J = 6.7 Hz, 2H), 3.36 – 3.29 (m, 2H), 3.27 – 3.21 (m, 4H), 3.06 (t, J = 6.7 Hz,  
33 2H), 2.59 (s, 3H), 2.51 (s, 3H), 1.89 (br, 2H), 1.59 (br, 2H), 1.50 – 1.36 (m, 20H); <sup>13</sup>C NMR  
34 (101MHz, CD<sub>3</sub>OD) as a mixture of 2 isomers  $\delta$  170.7, 170.3, 164.1, 163.6, 158.7, 156.1, 151.8, 149.7,  
35 132.1, 125.1, 118.7, 81.2, 80.0, 48.1, 46.4, 45.7, 43.7, 41.1, 38.4, 38.0, 34.2, 29.4, 29.0, 28.9, 28.5,  
36 27.2, 26.7, 19.1, 16.3; MS (ESI)  $m/z$  757.87 (M+H)<sup>+</sup> (Theoretical 756.26).

1 **2'-[2,4-Dimethylthiazole-5-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-**  
2 **aminopropyl]carboxamide (9i).** Deprotection was performed following general procedure B starting  
3 from Boc-protected dimethylthiazole sulphonamide **8i** (18.6 mg, 0.03 mmol) and of hydrochloric acid  
4 (0.13 mL, 1.56 mmol, 52 eq.) in MeOH (1.5 mL). The product was precipitated in Et<sub>2</sub>O to give  
5 compound **9i** as a yellow solid (14.0 mg, 89%). HPLC Rt = 11.3 min, analytical HPLC method B; <sup>1</sup>H  
6 NMR (400MHz, D<sub>2</sub>O) δ 8.22 (s, 1H), 7.97 (s, 1H), 3.64 – 3.57 (m, 2H), 3.54 (t, J = 6.6 Hz, 2H), 3.23 –  
7 3.08 (m, 6H), 3.04 (t, J = 6.8 Hz, 2H), 2.32 (s, 3H), 2.28 (s, 3H), 2.10 – 2.00 (m, 2H), 1.83 – 1.71 (m,  
8 4H); <sup>13</sup>C NMR (101MHz, D<sub>2</sub>O) δ 171.0, 170.3, 154.2, 129.3, 118.8, 47.0, 45.2, 42.5, 38.8, 36.3, 31.9,  
9 25.8, 23.9, 22.8, 17.6, 14.9; HRMS (ESI) *m/z* 558.14386 (M+H)<sup>+</sup> (C<sub>21</sub>H<sub>32</sub>N<sub>7</sub>O<sub>3</sub>S<sub>4</sub> requires 558.14440).

10 **2'-[2-Acetamidothiazole-5-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-*N*-tertbutoxycarbonyl-**  
11 **aminobutyl)-*N*-tertbutoxycarbonyl-aminopropyl]carboxamide (8j).** Compound **8j** was prepared  
12 following general procedure C starting from Bithiazole Boc-spermidine **5** (90.6 mg, 0.16 mmol), 2-  
13 acetamidothiazole-5-sulfonyl chloride **13j** (40 mg, 0.17 mmol, 1.1 eq.) and triethylamine (40.0 μL,  
14 0.24 mmol, 1.6 eq.) in DMF (2 mL). The crude product was purified by FC on a silica gel column  
15 using a mixture CH<sub>2</sub>Cl<sub>2</sub>/ (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10) from 100/0 to 0/100 as the eluent leading to  
16 compound **8j** as a beige solid (35.5 mg, 29%). R<sub>f</sub> = 0.14 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 96/4); <sup>1</sup>H NMR (400MHz,  
17 CDCl<sub>3</sub>) δ 11.52 (br, 1H), 8.08 (s, 1H), 7.89 (s, 1H), 7.84 (s, 1H), 7.53 (br, 1H), 6.16 (br, 1H), 4.66 (br,  
18 1H), 3.62 – 3.55 (m, 2H), 3.51– 3.38 (m, 2H), 3.38 – 3.30 (m, 2H), 3.30 – 3.24 (m, 2H), 3.24 – 3.16  
19 (m, 2H), 3.16 – 3.06 (m, 2H), 2.28 (s, 3H), 1.83 (br, 2H), 1.67 – 1.50 (m, 2H), 1.50 – 1.37 (m, 20); <sup>1</sup>H  
20 NMR (100MHz, CDCl<sub>3</sub>) as a mixture of 2 isomers δ 168.8, 168.1, 161.4, 156.3, 156.2, 156.1, 150.9,  
21 147.9, 130.0, 123.6, 117.0, 79.9, 77.4, 46.9, 46.8, 45.7, 44.1, 42.4, 41.5, 40.4, 40.3, 34.3, 32.929.9,  
22 29.8, 29.5, 27.7, 26.0; MS (ESI) *m/z* 786.87 (M+H)<sup>+</sup> (Theoretical 785.25).

23 **2'-[2-Acetamidothiazole-5-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-**  
24 **aminopropyl]carboxamide (9j).** Deprotection was performed following general procedure B starting  
25 from Boc-protected *N*-(thiazol-2-yl)acetamide sulphonamide **8j** (15.0 mg, 0.02 mmol) and of  
26 hydrochloric acid (0.03 mL, 0.38 mmol, 20 eq.) in MeOH (1.5 mL). The product was precipitated in  
27 Et<sub>2</sub>O to give compound **9j** as a brown solid (8.9 mg, 71%). <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O) as a mixture of  
28 two isomers δ 8.21 (s, 1H), 8.08 (s, 0.15H), 7.91 (s, 0.85H), 7.69 (s, 0.85H), 7.68 (s, 0.15H), 3.63 –  
29 3.50 (m, 4H), 3.28 – 3.07 (m, 6H), 3.04 (t, J = 6.9 Hz, 2H), 2.10 – 2.00 (m, 5H), 1.84 – 1.70 (m, 4H);  
30 <sup>13</sup>C NMR (101MHz, D<sub>2</sub>O) δ 171.1, 170.3, 163.3, 162.6, 162.4, 146.9, 142.9, 128.8, 125.1, 119.3, 47.0,  
31 45.2, 42.5, 38.8, 36.3, 31.53, 25.8, 23.9, 22.8, 21.7; HRMS (ESI) *m/z* 587.13568 (M+H)<sup>+</sup>  
32 (C<sub>21</sub>H<sub>31</sub>N<sub>8</sub>O<sub>4</sub>S<sub>4</sub> requires 587.13456).

33 **2'-[2-Aminothiazol-5-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-**  
34 **aminopropyl]carboxamide (9k).** Deprotection was performed from Boc-protected *N*-(thiazol-2-  
35 yl)acetamide sulphonamide **8j** (10.3 mg, 0.01 mmol) and of hydrochloric acid (22 μL, 0.26 mmol, 20  
36 eq.) in MeOH (1 mL). The reaction mixture was stirred at 65°C for 16h. The product was precipitated  
37 in Et<sub>2</sub>O to give compound **9k** as a brown solid (11.1mg, quantitative). <sup>1</sup>H NMR (500MHz, D<sub>2</sub>O) δ 8.26

1 (s, 1H), 8.11 (s, 1H), 7.50 (s, 1H), 3.61 – 3.52 (m, 4H), 3.28 – 3.22 (m, 2H), 3.19 – 3.10 (m, 4H), 3.06  
2 (t, J = 6.8 Hz, 2H), 2.10 – 1.98 (m, 2H), 1.82 – 1.69 (m, 4H); <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O) δ 170.5,  
3 163.7, 148.8, 147.2, 145.8, 141.5, 125.2, 125.1, 121.4, 119.1, 47.0, 45.2, 42.5, 38.8, 36.3, 31.9, 25.8,  
4 23.9, 22.8; HRMS (ESI) *m/z* 545.12561 (M+H)<sup>+</sup> (C<sub>19</sub>H<sub>29</sub>N<sub>8</sub>O<sub>3</sub>S<sub>4</sub> requires 545.12400).

5 **2'-[3,3,3-Trifluoropropane-1-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-*N*-tertbutoxycarbonyl-**  
6 **aminobutyl)-*N*-tertbutoxycarbonyl-aminopropyl]carboxamide (8l).** Compound **8l** was prepared  
7 following general procedure C starting from Bithiazole Boc-spermidine **5** (45.3 mg, 0.08 mmol),  
8 3,3,3-trifluoropropane-1-sulfonyl chloride **13l** (12.0 μL, 0.09 mmol, 1.2 eq.) and triethylamine (20.0  
9 μL, 0.12 mmol, 1.6 eq.) in DMF (1 mL). The crude product was purified by FC on a silica gel column  
10 using a mixture CH<sub>2</sub>Cl<sub>2</sub>/ (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10) from 100/0 to 70/30 gave **8l** as a white solid (33.0 mg,  
11 58%). *R<sub>f</sub>* = 0.40 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 96/4); HPLC *R<sub>t</sub>* = 19.9 min, analytical HPLC method B; <sup>1</sup>H NMR  
12 (400MHz, CD<sub>3</sub>OD) δ 8.19 – 8.16 (m, 2H), 3.58 (t, J = 6.7 Hz, 2H), 3.43 (t, J = 6.7 Hz, 2H), 3.34 –  
13 3.29 (m, 4H), 3.28 – 3.20 (m, 4H), 3.04 (t, J = 6.8 Hz, 2H), 2.69 – 2.55 (m, 2H), 1.87 (br, 2H), 1.57 (br,  
14 2H), 1.50 – 1.37 (m, 20H); <sup>13</sup>C NMR (101MHz, CD<sub>3</sub>OD) as a mixtures of 2 isomers δ 170.3, 164.0,  
15 163.4, 158.5, 151.7, 149.6, 131.5, 128.8, 126.0, 123.3, 124.9, 118.6, 81.0, 79.8, 47.9, 46.2, 46.1, 45.6,  
16 43.3, 41.0, 38.2, 37.9, 34.9, 30.3, 30.0, 29.7, 29.4, 29.3, 29.2, 28.8, 28.7, 28.3, 27.0, 26.5; MS (ESI)  
17 *m/z* 742.93 (M+H)<sup>+</sup> (Theoretical 743.25).

18 **2'-[3,3,3-Trifluoropropane-1-sulfonamido]ethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-**  
19 **aminopropyl]carboxamide (9l).** Deprotection was performed following general procedure B starting  
20 from Boc-protected trifluoropropyl sulphonamide **8l** (19.2 mg, 0.03 mmol) and hydrochloric acid  
21 (0.12 mL, 1.57 mmol, 52 eq.) in MeOH (1.5 mL). The product was precipitated in Et<sub>2</sub>O to give  
22 compound **9l** as a white foam (13.9 mg, 87%). HPLC *R<sub>t</sub>* = 10.8 min, analytical HPLC method B; <sup>1</sup>H  
23 NMR (400MHz, D<sub>2</sub>O) δ 8.10 (s, 1H), 7.99 (s, 1H), 3.51 (t, J = 6.0 Hz, 2H), 3.45 (t, J = 6.6 Hz, 2H),  
24 3.35 – 3.28 (m, 2H), 3.21 (t, J = 6.0 Hz, 2H), 3.10 – 2.99 (m, 4H), 2.96 (t, J = 6.8 Hz, 2H), 2.61 – 2.45  
25 (m, 2H), 2.01 – 1.91 (m, 2H), 1.76 – 1.62 (m, 4H); <sup>13</sup>C NMR (101MHz, D<sub>2</sub>O) δ 170.4, 163.7, 162.8,  
26 148.7, 147.0, 129.8, 127.0, 124.3, 121.6, 125.1, 119.1, 47.0, 45.2, 45.0, 44.9, 42.2, 38.8, 36.2, 33.0,  
27 28.5, 28.2, 27.9, 27.6, 25.8, 23.9, 22.8; HRMS (ESI) *m/z* 543.14948 (M+H)<sup>+</sup> (C<sub>19</sub>H<sub>30</sub>F<sub>3</sub>N<sub>6</sub>O<sub>3</sub>S<sub>3</sub> requires  
28 543.14881).

29 **2'-[Dimethylsulfamido]ethyl-2,4'-bithiazole-4-[3-(4-*N*-tertbutoxycarbonyl-aminobutyl)-*N*-**  
30 **tertbutoxycarbonyl-aminopropyl]carboxamide (8m).** Compound **8m** was prepared following  
31 general procedure C starting from Bithiazole Boc-spermidine **5** (45.8 mg, 0.08 mmol),  
32 dimethylsulfamoyl chloride **13m** (0.1 mL, 0.09 mmol, 1.1 eq.) and triethylamine (20.0 μL, 0.12 mmol,  
33 1.6 eq.) in DMF (1 mL). The crude product was purified by FC on a silica gel column using a mixture  
34 CH<sub>2</sub>Cl<sub>2</sub>/ (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10) from 100/0 to 0/100 gave **8m** as a white powder (38.1 mg, 69%). *R<sub>f</sub>* =  
35 0.43 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 96/4); <sup>1</sup>H NMR (400MHz, CD<sub>3</sub>OD) δ 8.18 (s, 1H), 8.17 (s, 1H), 3.50 (t, J = 6.7  
36 Hz, 2H), 3.44 (t, J = 6.7 Hz, 2H), 3.32 – 3.28 (m, 4H, H-2), 3.25 (t, J = 7.0 Hz, 2H), 3.06 (t, J = 6.7  
37 Hz, 2H), 2.76 (s, 6H), 1.88 (br, 2H), 1.58 (br, 2H), 1.51 – 1.39 (m, 20H); <sup>13</sup>C NMR (101MHz,

1 CD<sub>3</sub>OD) as a mixture of 2 isomers  $\delta$  170.6, 164.0, 163.4, 158.5, 157.6, 151.7, 149.5, 124.9, 118.5,  
2 81.0, 79.8, 48.0, 46.3, 45.6, 43.8, 41.0, 38.3, 37.8, 36.9, 34.4, 30.7, 29.9, 28.8, 28.3, 27.0, 26.5; MS  
3 (ESI)  $m/z$  689.8 (M+H)<sup>+</sup> (Theoretical 690,28); HPLC Rt = 19.4 min, analytical HPLC method B.

4 **2'-[Dimethylsulfamido]ethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-aminopropyl]carboxamide**  
5 **(9m)**. Deprotection was performed following general procedure B starting from Boc-protected  
6 dimethylsulfamoyl sulphonamide **8m** (33.1 mg, 0.05 mmol) and of hydrochloric acid (0.12 mL, 1.44  
7 mmol, 30 eq.) in MeOH (1 mL). The product was precipitated in Et<sub>2</sub>O to give compound **9m** as a  
8 slightly yellow foam (27.9 mg, 99%). <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O)  $\delta$  8.03 (s, 1H, H-7), 7.90 (s, 1H, H-4),  
9 3.52 – 3.39 (m, 4H, H-1, H-10), 3.22 – 3.06 (m, 6H, H-2, H-12, H-13), 3.03 (t,  $J$  = 6.4 Hz, 2H, H-16),  
10 2.70 (s, 6H, H-18, H-19), 2.07 – 1.97 (m, 2H, H-11), 1.80 – 1.70 (m, 4H, H-14, H-15); <sup>13</sup>C NMR  
11 (101MHz, D<sub>2</sub>O)  $\delta$  170.4, 163.1, 162.1, 148.5, 146.5, 124.9, 119.0, 47.0, 45.2, 42.4, 38.8, 37.4, 36.3,  
12 32.6, 25.8, 23.9, 22.8; HRMS (ESI)  $m/z$  490.17282 (M+H)<sup>+</sup> (C<sub>18</sub>H<sub>32</sub>N<sub>7</sub>O<sub>3</sub>S<sub>3</sub> requires 490.17233);  
13 HPLC Rt = 11.8 min, analytical HPLC method B.

14 **2'-Phenylaminoethyl-2,4'-bithiazole-4-[3-(4-N-tertbutoxycarbonyl-aminobutyl)-N-**  
15 **tertbutoxycarbonyl-aminopropyl]carboxamide (10a)**. The title compound **10a** was prepared  
16 following the general procedure D starting from Bithiazole Boc-spermidine **5** (44.6 mg, 0.08 mmol),  
17 benzaldehyde **14a** (9.0  $\mu$ L, 0.09 mmol, 1.2 eq.) and sodium cyanoborohydride (11.7 mg, 0.19 mmol,  
18 2.2 eq.) in MeOH (5 mL). Purification by FC (CH<sub>2</sub>Cl<sub>2</sub>/ (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10) from 100/0 to 20/80)  
19 gave **10a** as a yellow oil (19.8 mg, 38%).  $R_f$  = 0.56 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10); <sup>1</sup>H NMR (400MHz,  
20 CD<sub>3</sub>OD)  $\delta$  8.17 (s, 1H), 8.14 (s, 1H), 7.39 – 7.30 (m, 4H), 7.30 – 7.23 (m, 1H), 3.86 (s, 2H), 3.43 (t,  $J$   
21 = 6.7 Hz, 2H), 3.36 – 3.26 (m, 4H), 3.23 (t,  $J$  = 7.2 Hz, 2H), 3.10 (t,  $J$  = 6.9 Hz, 2H), 3.04 (t,  
22  $J$  = 6.7 Hz, 2H), 1.86 (br, 2H), 1.56 (br, 2H), 1.49 – 1.38(m, 20H); <sup>13</sup>C NMR (101MHz, CD<sub>3</sub>OD) as a  
23 mixture of 2 isomers  $\delta$  171.5, 164.0, 163.4, 158.5, 151.7, 149.4, 139.9, 129.6, 129.6, 128.5, 124.8,  
24 118.4, 81.0, 79.8, 54.1, 48.4, 48.1, 47.8, 46.3, 45.6, 41.0, 38.2, 37.9, 33.4, 30.8, 29.9, 28.8, 28.7, 28.4,  
25 27.0, 26.5; MS (ESI)  $m/z$  673.07 (M+H)<sup>+</sup> (Theoretical 673.31); HPLC Rt = 18.7 min, analytical HPLC  
26 method B.

27 **2'-Phenylaminoethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-N-tertbutoxycarbonyl-**  
28 **aminopropyl]carboxamide (11a)**. Preparation of compound **11a** was performed following the  
29 general procedure B starting from Boc-protected benzyl-bithiazole-spermidine **10a** (15.0 mg, 0.02  
30 mmol) and hydrochloric acid (55.0  $\mu$ L, 0.66 mmol, 30 eq.) in MeOH (1 mL). The product was  
31 precipitated in Et<sub>2</sub>O to give **11a** as a brown solid (11.5 mg, 89%). <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O)  $\delta$  8.20 (s,  
32 1H), 8.12 (s, 1H), 7.52 – 7.47 (m, 5H), 4.35 (s, 2H), 3.58 (t,  $J$  = 6.4 Hz, 2H), 3.55 – 3.49 (m, 4H), 3.18  
33 – 3.07 (m, 4H), 3.04 (t,  $J$  = 6.7 Hz, 2H), 2.09 – 1.99 (m, 2H, H), 1.84 – 1.70 (m, 4H); <sup>13</sup>C NMR  
34 (101MHz, D<sub>2</sub>O)  $\delta$  167.8, 163.5, 162.8, 148.7, 147.2, 130.4, 129.8, 129.3, 125.2, 119.2, 51.0, 47.0,  
35 45.4, 45.2, 38.8, 36.2, 28.4, 25.8, 23.9, 22.8; HRMS (ESI)  $m/z$  473.21594 (M+H)<sup>+</sup> (C<sub>23</sub>H<sub>33</sub>N<sub>6</sub>OS<sub>2</sub>  
36 requires 473.21518); HPLC Rt = 3.7 min, analytical HPLC method A.

1 **2'-[1-Naphthyl]aminoethyl-2,4'-bithiazole-4-[3-(4-N-tertbutoxycarbonyl-aminobutyl)-N-**  
2 **tertbutoxycarbonyl-aminopropyl]carboxamide (10b).** The title compound **10b** was prepared  
3 following the general procedure D starting from Bithiazole Boc-spermidine **5** (44.9 mg, 0.08 mmol),  
4 naphthaldehyde **14b** (12.0  $\mu$ L, 0.09 mmol, 1.2 eq.) and sodium cyanoborohydride (11.8 mg,  
5 0.19 mmol, 2.2 eq.) in MeOH (5 mL). Purification by FC ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  90/10) from 100/0  
6 to 20/80) gave **10b** as a yellow solid (52.1 mg, 90%).  $R_f = 0.21$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  96/4);  $^1\text{H}$  NMR  
7 (400MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.12 (s, 1H), 8.09 (s, 1H), 8.07 – 8.01 (m, 1H), 7.86 – 7.80 (m, 1H), 7.76 (d,  $J =$   
8 8.2 Hz, 1H), 7.49 (d,  $J = 6.5$  Hz, 1H), 7.44 – 7.38 (m, 3H), 4.24 (s, 2H), 3.42 (t,  $J = 6.7$  Hz, 2H), 3.27  
9 (t,  $J = 6.6$  Hz, 4H), 3.22 (t,  $J = 7.2$  Hz, 2H), 3.15 (t,  $J = 6.5$  Hz, 2H), 3.03 (t,  $J = 6.7$  Hz, 2H), 1.85 (br,  
10 2H), 1.55 (br, 2H), 1.48 – 1.37 (m,  $J = 16.0$  Hz, 20H);  $^{13}\text{C}$  NMR (101MHz,  $\text{CD}_3\text{OD}$ ) as a mixture of 2  
11 isomers  $\delta$  180.3, 163.7, 163.1, 158.1, 151.3, 148.9, 135.8, 135.0, 132.6, 129.7, 129.5, 128.9, 127.3,  
12 127.1, 126.5, 126.0, 124.2, 118.1, 80.8, 79.7, 51.5, 47.8, 47.7, 46.0, 45.2, 40.7, 38.0, 37.5, 33.5, 30.5,  
13 28.8, 28.7, 28.0, 26.7, 26.2; MS (ESI)  $m/z$  723.13 ( $\text{M}+\text{H}^+$ ) (Theoretical 723.33).

14 **2'-[1-Naphthyl]aminoethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-aminopropyl]carboxamide (11b).**  
15 Preparation of compound **11b** was performed following the general procedure B, starting from Boc-  
16 protected naphthalene-bithiazole-spermidine **10b** (48.0 mg, 0.02 mmol) and hydrochloric acid (55.0  $\mu$ L,  
17 0.66 mmol, 33 eq.) in MeOH (1 mL). The product was precipitated in  $\text{Et}_2\text{O}$  to give **11b** as a pale  
18 orange solid (39.9 mg, 95%);  $^1\text{H}$  NMR (400MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.86 – 7.78 (m, 3H), 7.75 (d,  $J = 8.2$  Hz,  
19 1H), 7.73 – 7.68 (m, 1H), 7.57 (d,  $J = 6.9$  Hz, 1H), 7.40 (t,  $J = 7.7$  Hz, 1H), 7.37 – 7.30 (m, 2H), 4.65  
20 (s, 2H), 3.55 (t,  $J = 6.0$  Hz, 2H), 3.49 – 3.38 (m, 4H), 3.13 – 3.06 (m, 6H), 2.08 – 1.98 (m, 2H), 1.87 –  
21 1.74 (m, 4H);  $^{13}\text{C}$  NMR (101MHz,  $\text{D}_2\text{O}$ )  $\delta$  167.5, 162.9, 162.0, 148.4, 146.4, 133.5, 130.7, 130.6,  
22 129.7, 129.0, 127.5, 126.5, 126.1, 125.6, 124.7, 122.2, 119.1, 47.8, 47.0, 45.6, 45.3, 38.9, 36.4, 28.0,  
23 25.7, 24.0, 22.8; HRMS (ESI)  $m/z$  523.23114 ( $\text{M}+\text{H}^+$ ) ( $\text{C}_{27}\text{H}_{35}\text{N}_6\text{OS}_2$  requires 523.23083).

24 **2'-[5-Benzothiazole]aminoethyl-2,4'-bithiazole-4-[3-(4-N-tertbutoxycarbonyl-aminobutyl)-N-**  
25 **tertbutoxycarbonyl-aminopropyl]carboxamide (10c).** Compound **10c** was prepared following  
26 general procedure D starting from bithiazole Boc-spermidine **5** (45.1 mg, 0.08 mmol), benzothiazole-  
27 2-carbaldehyde **14c** (15.2 mg, 0.09 mmol, 1.1 eq.) and sodium cyanoborohydride (11.4 mg, 0.17  
28 mmol, 2.1 eq.) in MeOH (3 mL). Purification by FC on a silica gel column using a mixture  $\text{CH}_2\text{Cl}_2/$   
29 ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  90/10) from 100/0 to 0/100 as the eluent leading to compound **10c** as a brown oil  
30 (19.0 mg, 65%).  $R_f = 0.50$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  90/10);  $^1\text{H}$  NMR (400MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  9.24 (s, 1H), 8.15  
31 (s, 1H), 8.13 (s, 1H), 8.10 (s, 1H), 8.04 (d,  $J = 8.3$  Hz, 1H), 7.54 (d,  $J = 8.3$  Hz, 1H), 4.06 (s, 2H),  
32 3.42 (t,  $J = 6.7$  Hz, 2H), 3.34 – 3.27 (m, 4H), 3.23 (t,  $J = 7.2$  Hz, 2H), 3.20 – 3.12 (m, 2H), 3.03 (t,  $J =$   
33 6.7 Hz, 2H), 1.89 (br, 2H), 1.56 (br, 2H), 1.43 (m, 20H);  $^{13}\text{C}$  NMR (101MHz,  $\text{CD}_3\text{OD}$ ) as a mixtures  
34 of 2 isomers  $\delta$  171.7, 164.0, 163.4, 158.5, 157.4, 154.4, 151.7, 149.3, 139.5, 133.8, 127.7, 124.8,  
35 123.6, 123.2, 118.4, 81.0, 79.8, 53.9, 48.9, 48.0, 47.9, 46.2, 45.6, 41.0, 38.2, 37.9, 33.6, 30.8, 29.9,  
36 29.2, 28.8, 28.7, 27.0, 26.5; MS (ESI)  $m/z$  730.07 ( $\text{M}+\text{H}^+$ ) (Theoretical requires 730.28); HPLC  $R_t =$   
37 18.7 min, analytical HPLC method B.

1 **2'-[5-Benzothiazole]aminoethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-aminopropyl]carboxamide**  
2 (**11c**). The unprotected product **11c** was synthesised following the general procedure B starting from  
3 Boc-protected benzothiazole-bithiazole-spermidine **10c** (19.2 mg, 0.03 mmol) and hydrochloric acid  
4 (0.13 mL, 1.56 mmol, 52 eq.) in MeOH (1.5 mL). The product was crystallized in Et<sub>2</sub>O to give **11c** as  
5 a brown oil (10.1 mg, 82%). <sup>1</sup>H NMR (400MHz, D<sub>2</sub>O) δ 9.29 (s, 1H), 8.11 (d, J = 10.5 Hz, 2H), 8.05  
6 (s, 1H), 7.57 (d, J = 8.3 Hz, 1H), 4.50 (s, 2H), 3.66 (t, J = 6.2 Hz, 2H), 3.54 – 3.48 (m, 4H), 3.19 –  
7 3.07 (m, 4H), 3.04 (d, J = 7.2 Hz, 2H), 2.09 – 1.96 (m, 2H), 1.84 – 1.68 (m, 4H); <sup>13</sup>C NMR (101MHz,  
8 D<sub>2</sub>O) δ 167.7, 163.5, 147.0, 129.1, 127.1, 123.7, 123.4, 119.1, 50.7, 47.0, 45.3, 45.2, 38.8, 36.3, 28.4,  
9 25.8, 23.9, 22.8; HRMS (ESI) *m/z* 530.18335 (M+H)<sup>+</sup> (C<sub>24</sub>H<sub>32</sub>N<sub>7</sub>OS<sub>3</sub> requires 530.18250); HPLC Rt =  
10 10.1 min, analytical HPLC method B.

11 **N(Im)-tertButoxycarbonyl-1H-benzimidazole-6-carboxaldehyde (14d)**. This compound was  
12 prepared from (1H-benzo[d]imidazol-5-yl)methanol which was synthesised as described in the  
13 literature.<sup>40</sup> To a solution of (1H-benzo[d]imidazol-5-yl)methanol (0.10 g; 0.67mmol) in DMSO  
14 (4 mL) was added a solution of IBX (0.50g; 0.80 mmol; 1.2 eq.) in DMSO (4 mL) and the mixture  
15 was stirred at room temperature for 5 h. The mixture was diluted with EtOAc and the organic phase  
16 was washed with NaHCO<sub>3</sub> (4 mL x 3) then brine (4 mL) and dried over Na<sub>2</sub>SO<sub>4</sub> before being  
17 concentrated under reduce pressure. To a solution of this crude material in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) at 0°C was  
18 added a solution of Boc<sub>2</sub>O (0.18 g; 0.80 mmol; 1.2 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL) and Et<sub>3</sub>N (0.20 mL; 1.34  
19 mmol, 2 eq.) and the mixture was stirred at room temperature for 4 h. The organic phase was washed  
20 with H<sub>2</sub>O (3 mL x 3) then brine (3 mL) and dried over Na<sub>2</sub>SO<sub>4</sub> before being concentrated under  
21 reduced pressure to give compound **14d** as a brown solid (0.08 mg, 48%). R<sub>f</sub> = 0.40  
22 (Cyclohexane/EtOAc 60/40); <sup>1</sup>H NMR (200MHz, CDCl<sub>3</sub>) as a mixtures of 2 isomers δ 10.11 (d, J =  
23 0.6 Hz, 1H), 8.61 (s, 0.5H), 8.58 – 8.52 (m, 1H), 8.30 (d, J = 0.9 Hz, 0.5H), 7.97 (dd, J = 8.5, 1.4 Hz,  
24 1H), 7.92 (s, 1H), 1.72 (s, 9H); <sup>13</sup>C NMR (50MHz, CDCl<sub>3</sub>) δ 191.7, 147.6, 145.2, 143.9, 134.0, 133.5,  
25 126.1, 121.3, 86.9, 28.2.

26 **2'-[6-N(Im)-tertButoxycarbonyl-1H-benzimidazole]aminoethyl-2,4'-bithiazole-4-[3-(4-N-**  
27 **tertbutoxycarbonyl-aminobutyl)-N-tertbutoxycarbonyl-aminopropyl]carboxamide (10d)**.  
28 Compound **10d** was prepared following general procedure D starting from bithiazole Boc-spermidine  
29 **5** (53.0 mg, 0.09 mmol, 1.1 eq), benzothiazole-2-carbaldehyde **14d** (19.4 mg, 0.08 mmol.) and sodium  
30 cyanoborohydride (10.1 mg, 0.16 mmol, 2.0 eq.) in MeOH (2 mL). Purification by FC on a silica gel  
31 column using a mixture CH<sub>2</sub>Cl<sub>2</sub>/ (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90/10) from 100/0 to 0/100 as the eluent led to  
32 compound **10d** as a brown oil (32.0 mg, 49%). R<sub>f</sub> = 0.23 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 94/6); <sup>1</sup>H NMR (400MHz,  
33 CD<sub>3</sub>OD) δ 8.67 (s, 1H), 8.28 (s, 1H), 8.22 (s, 1H), 8.16 (s, 1H), 7.81 (d, J = 7.9 Hz, 1H), 7.56 (d, J =  
34 7.9 Hz, 1H), 4.44 (s, 2H), 3.61 – 3.54 (m, 2H), 3.51 (t, J = 6.3 Hz, 2H), 3.43 (t, J = 6.6 Hz, 2H), 3.36 –  
35 3.26 (m, 2H), 3.23 (dd, J = 7.0 Hz, 13.8 Hz, 2H), 3.04 (t, J = 6.7 Hz, 2H), 1.85 (br, 2H), 1.73 - 1.67  
36 (m, 9H), 1.56 (br, 2H), 1.51 - 1.35 (m, 20H); <sup>13</sup>C NMR (101 MHz, CD<sub>3</sub>OD) δ 175.5, 167.1, 165.8,  
37 163.3, 158.5, 151.8, 148.9, 135.6, 130.6, 127.3, 124.9, 121.6, 119.0, 117.3, 87.7, 81.0, 79.8, 79.6,



1 79.3, 78.9, 68.7, 56.7, 54.9, 47.2, 41.0, 40.3, 31.7, 30.7, 30.5, 30.1, 28.8, 28.7, 28.2, 25.1, 24.0; MS  
2 (ESI)  $m/z$  814.05 (M+H)<sup>+</sup> (Theoretical 813.33).

### 3 **2'-[6-1H-benzimidazole]aminoethyl-2,4'-bithiazole-4-[3-(4-aminobutyl)-**

4 **aminopropyl]carboxamide (11d)**. The unprotected product **11d** was synthesised following the  
5 general procedure B starting from Boc-protected benzimidazole-bithiazole-spermidine **10d** (11.5 mg,  
6 0.01 mmol) and hydrochloric acid (35  $\mu$ L, 0.42 mmol, 42 eq.) in MeOH (1 mL). The product was  
7 crystallized in Et<sub>2</sub>O to give **11d** as a brown oil (9.8 mg, 91%). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O)  $\delta$  9.14 (s,  
8 1H), 8.17 (s, 1H), 8.08 (s, 1H), 7.92 (s, 1H), 7.86 (d,  $J$  = 8.6 Hz, 1H), 7.65 (dd,  $J$  = 8.6, 1.5 Hz, 1H),  
9 4.50 (s, 2H), 3.62 (t,  $J$  = 6.9 Hz, 2H), 3.49 (t,  $J$  = 7.0 Hz, 4H), 3.13 – 3.02 (m, 4H), 2.98 (t,  $J$  = 7.1 Hz,  
10 2H), 2.03 – 1.95 (m, 2H), 1.77 – 1.66 (m, 4H); <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O)  $\delta$  167.8, 163.7, 163.1,  
11 148.9, 147.2, 140.5, 131.2, 129.0, 127.9, 125.2, 119.1, 117.5, 116.3, 115.4, 50.8, 47.0, 45.6, 45.2,  
12 38.8, 36.3, 28.5, 25.8, 23.9, 22.7; HRMS (ESI)  $m/z$  513.22144 (M+H)<sup>+</sup> (C<sub>24</sub>H<sub>33</sub>N<sub>8</sub>OS<sub>2</sub> requires  
13 513.22133).

### 14 **Associated content**

#### 15 **Supporting Information**

16 The supporting information is available free of charge.

17 Molecular formula string (CSV)

18 Supplementary figures and procedures (PDF)

#### 19 **Notes**

20 The authors declare no competing financial interest.

#### 21 **Abbreviations used**

22 CSCs, cancer stem cells; DW, Data Warrior; DIPEA, diisopropylethylamine; EMT, epithelial-to-  
23 mesenchymal transition; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; GBM, glioblastoma;  
24 HOSu, *N*-hydroxysuccinimide; HBTU, (2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium  
25 hexafluorophosphate; K<sub>D</sub>, dissociation constant; LELP, ligand efficiency lipophilic price; miRs,  
26 microRNAs, miRNAs, microRNAs; ncRNAs, non-coding RNAs; PDCLs, patient-derived cell lines;  
27 SMA, Spinal Muscular Atrophy; TMZ, temozolomide.

28

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#### 36 **Abbreviations**



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