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Original article

Synthesis and cytotoxicity of thieno[2,3-*b*]pyridine and furo[2,3-*b*]pyridine derivatives

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ABSTRACT

Forty seven thieno[2,3-*b*]pyridines-2-carboxamides, furo[2,3-*b*]pyridines-2-carboxamides and tetrahydrothieno[2,3-*b*]quinolones-2-carboxamides derivatives were synthesized and tested for their antiproliferative activity against the NCI-60 cell lines. The 5-keto-tetrahydrothieno[2,3-*b*]quinolones-2-carboxamides (series **17**) were found to have the greatest activity, with the compound containing a 3-methoxyphenylcarboxamide (compound **17d**) being the most active, with GI_{50} values in the low nanomolar range against a range of cell lines, in particular the melanoma cell line MDA-MD-435 (GI_{50} – 23 nM) and the breast cancer cell line MDA-MB-468 (GI_{50} – 46 nM). Molecular modelling of series **17** against phosphoinositide specific-phospholipase C reveals that the side chains of the amino acids His356, Glu341, Arg549 and Lys438 are involved in hydrogen bonding with the ligands as well as a lipophilic pocket is occupied by the phenyl carboxamide moiety.

1. Introduction

Breast cancer is the most common cancer in women even though early detection and the introduction of new therapies have resulted in decline in mortality [1–4]. Hormone-dependent breast cancer is commonly treated with tamoxifen, whilst trastuzumab is effective against human epidermal growth factor receptor 2 (HER2) positive cancers [5]. Tumours lacking the oestrogen receptor (ER), the progesterone receptor (PR) and HER2 are classified as triple-negative breast cancer [6,7], with the MDA-MB-231/468 cell lines belonging to this type of cancer [8].

Recently, Reynisson et al. [9] identified a series of potential phosphoinositide specific-phospholipase C-γ (PLC-γ) inhibitors using a virtual high throughput screening (vHTS) method and subsequently assessed them for their antiproliferative activity [10]. Some of the most potent compounds contained a thieno[2,3-*b*]pyridine-3-amine scaffold, e.g. **1** and **2**, or 3*H*-pyrimido[5,4-*b*]indol-4(5*H*)-one scaffold, e.g. **3** (Fig. 1). Further analysis of these classes of compounds revealed their activity against a range of cancer cell lines including triple-negative breast cancer cell lines [11,12]. We therefore wished to prepare a series of bicyclic and tricyclic analogues containing the motifs found in these compounds and compare their antiproliferative activity to previously examined compounds in the aim of finding more potent agents.

Previous molecular modelling studies [10–12] indicated that the aryl carboxamide and the 3-amino moiety of compounds **1** and **2** have hydrogen bonding interactions within the PLC active site (Fig. 2). Furthermore, the phenyl ring is embedded in a lipophilic pocket. Modelling has also indicated the keto group of **1** and substituents at the 8-position of **3** also positively contribute to binding. To investigate each of these binding interactions three series of compounds were prepared. In the first compound series, the thieno and furopyridine-2-carboxamides, different substituted phenyl rings were designed to investigate the lipophilic contacts of the binding pocket and also the effect of substitution at C-5 (Fig. 3). In a second compound series, the pyrimidinone and triazinone analogues, the 3-amino functional group is incorporated into a ring system, thus eliminating the hydrogen donor ability to the amino acids GLU341 (Fig. 2), shedding light on the importance of this putative intermolecular interaction. Thirdly, various *N*-aryl-tetrahydrothieno- and furo[2,3-*b*]quinoline-2-carboxamides, a compound series which has antiproliferative activity [10–12], would further investigate the optimal substitution of the phenyl ring and the alteration of the keto group. Comparison of the activity of 5-substituted-thienopyridine-2-carboxamides with *N*-aryl-5-oxo-tetrahydrothieno[2,3-*b*]quinoline-2-carboxamides (or furo

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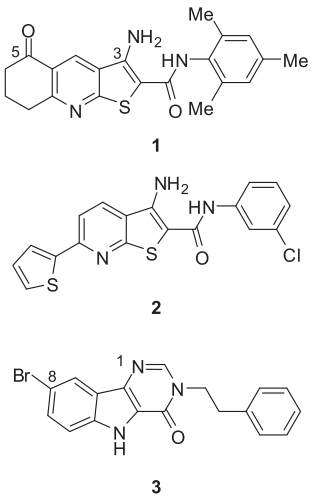


Fig. 1. Previously discovered inhibitors of PLC-γ.

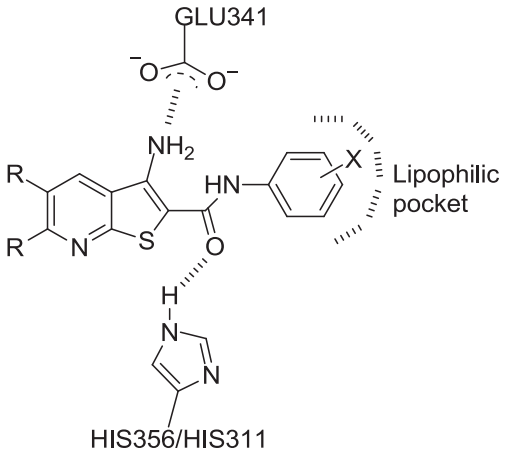


Fig. 2. Predicted interactions of thienopyridine-2-carboxamides in the PLC binding pocket.

derivatives), to investigate the functional group requirement at this position, whilst modifications of the heterocyclic core along with the addition of halogen substituents, has the potential to further refine the requirements for strong inhibitory activity.

2. Results and discussion

2.1. Chemistry

2.1.1. Thienopyridine-2-carboxamide analogues

Thieno[2,3-*b*]pyridines **4a**–**i** were prepared in two steps from thioglycolic acid **5** (Scheme 1). The synthesis began by preparing the mercaptoacetamides **6a**–**c**, with different substituted phenyl moieties, by heating the corresponding anilines and thioglycolic acid **5** without solvent. Whilst thieno[2,3-*b*]pyridine-2-carboxamides such as **4** are often prepared through initial formation of 2-(substituted-thio)pyridines [13,14], it was found that reaction of 2-mercapto-*N*-phenylacetamide **6a** with 2-chloro-3-pyridine carbonitrile **7a**, using sodium carbonate, in refluxing ethanol, gave the desired thieno[2,3-*b*]pyridine **4a** in 73% yield in a single step. Analogues **4b** and **4c** with 4-methoxy or 3-chlorophenyl moieties were prepared similarly from pyridine **7a** in 60% and 90% yields respectively. 5-Halo-substituted thieno[2,3-*b*]pyridines **4d**–**i** were prepared using the same conditions as for **4a**–**c**, reaction of 2,5-dichloronicotinonitrile **7b** or 5-bromo-2-chloro-3-cyano-pyridine **7c** with mercaptoacetamides **6a**–**c** gave 5-halo-thieno[2,3-*b*]pyridines **4d**–**i** in 40–99% yields.

Derivatization of thieno[2,3-*b*]pyridines **4a**–**i** into thienopyrimidinones **8a**–**i** was achieved by heating thienopyridine-2-carboxamides **4a**–**i** in excess triethyl orthoformate (Scheme 1). The addition of acetic acid was found to be essential for successful conversion [15] and gave thienopyrimidinones **8a**–**i** in 41–99% yields.

Another series of analogues, the thienotriazinones **9a**–**i**, were prepared by diazotization of **4a**–**i** using sodium nitrite [15]. These reactions gave the desired thienotriazinones **9a**–**i** in 50–99% yields (Scheme 1). In total 27 analogues with the thieno[2,3-*b*]pyridine core structure were easily prepared with diversity in the aryl carboxamide, at the 5-position and in the chemical nature of the 3-amino position.

2.1.2. Furopyridine-2-carboxamide analogues

Following successful synthesis of the thienopyridine series, it was attempted to prepare furopyridine-2-carboxamides **10a**–**i** using the same method (Scheme 2). 2-Hydroxyacetamides **11a**–**c** were prepared from glycolic acid **12** in the same manner as thioacetamides **6**, giving amides **11a**–**c** in 56–99% yields. Reaction of **11a**–**c** with chloropyridines **7a**–**c**, using sodium carbonate in ethanol, however did not give furopyridine-2-carboxamides **10a**–**i** and gave only cyanopyridines **13a**–**i** in 19–92% yields, with the polyhalogenated derivatives **13f** and **13i** being obtained in the lowest yields. Using cyanopyridine **13a**, a variety of bases were explored to facilitate the furan ring formation, and whilst NaOH, NaOMe and Et_3N were all unsuccessful, giving mixtures of amide-hydrolysed or polymeric material, the use of hindered base KO^tBu successfully gave the desired furopyridine-2-carboxamide **10a** in quantitative yield. Similarly, reaction of **13b** and **13c** gave **10b** and **10c**, though in reduced yields of 75% and 57%, respectively. Reaction of **13d**, which has a non-substituted phenyl amide, gave furopyridine **10d** in 74% yield but reaction of all other halogenated

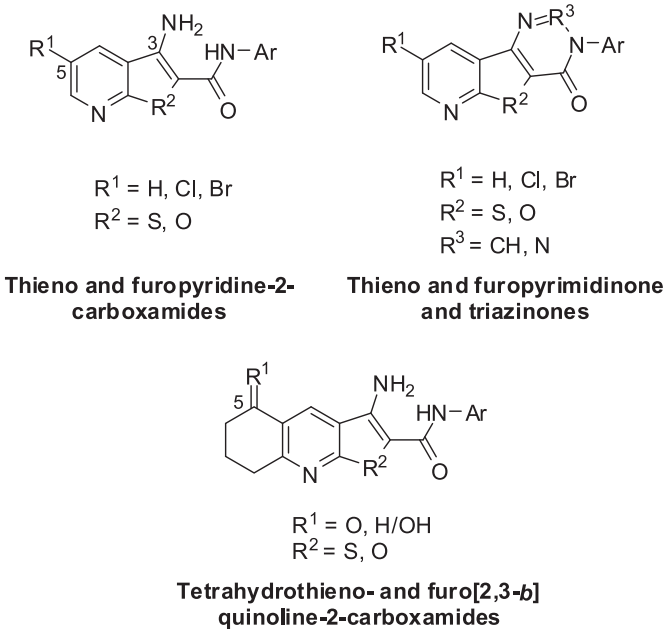
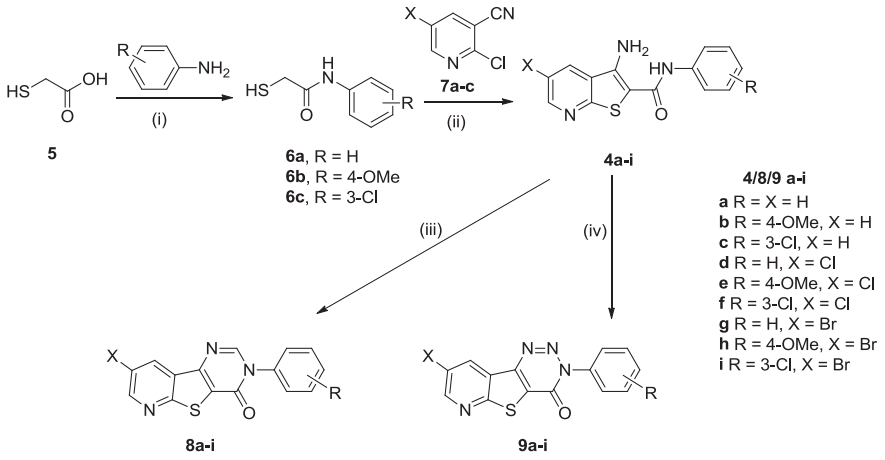
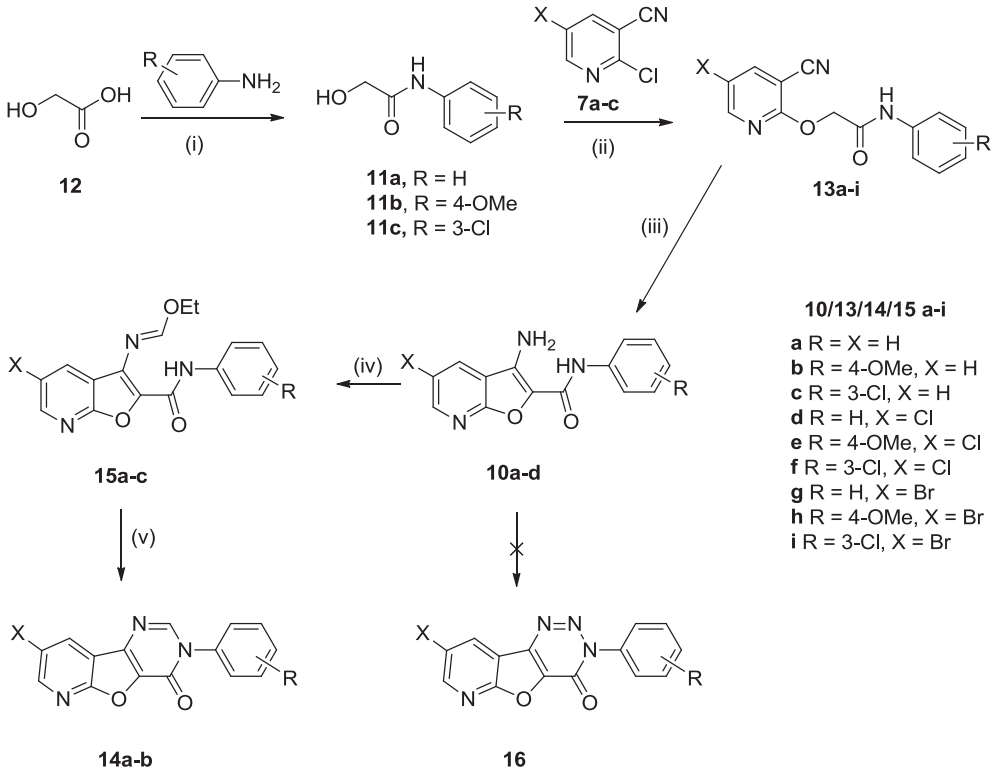


Fig. 3. Proposed heterocyclic inhibitors of the PLC enzyme.



Scheme 1. Reagents and conditions: (i) 1 equiv. substituted aniline, 130 °C, 3–5 h, **6a**–**c** 48–99%; (ii) 1 equiv. **7a**–**c**, 1.05 equiv. **6a**–**c**, Na_2CO_3, EtOH, reflux, 18 h, **4a**–**i** 40–99%; (iii) 10 equiv. $(EtO)_3CH$, CH_3CO_2H, 1–15 h, **8a**–**i** 41–99%; (iv) 20 equiv. $NaNO_2$, CH_3CO_2H, H_2O, 0 °C, 2 h, **9a**–**i** 50–99%.



Scheme 2. Reagents and conditions: (i) 1 equiv. substituted aniline, 130 °C, 3–5 h, **11a**–**c** 56–99%; (ii) 1 equiv. **7a**–**c**, 1.05 equiv. **6a**–**c**, Na_2CO_3, EtOH, reflux, 18 h, **13a**–**i** 19–92%; (iii) 1.2 equiv. KO^{t}Bu, THF, 80 °C, 3 h, **10a**–**d** 57–99%; (iv) 10 equiv. $(EtO)_3CH$, CH_3CO_2H, 1–15 h, **8a**–**i** 41–99%; (v) Na_2CO_3, EtOH, reflux, 15 h, **14a** 12%, **14b** 20%.

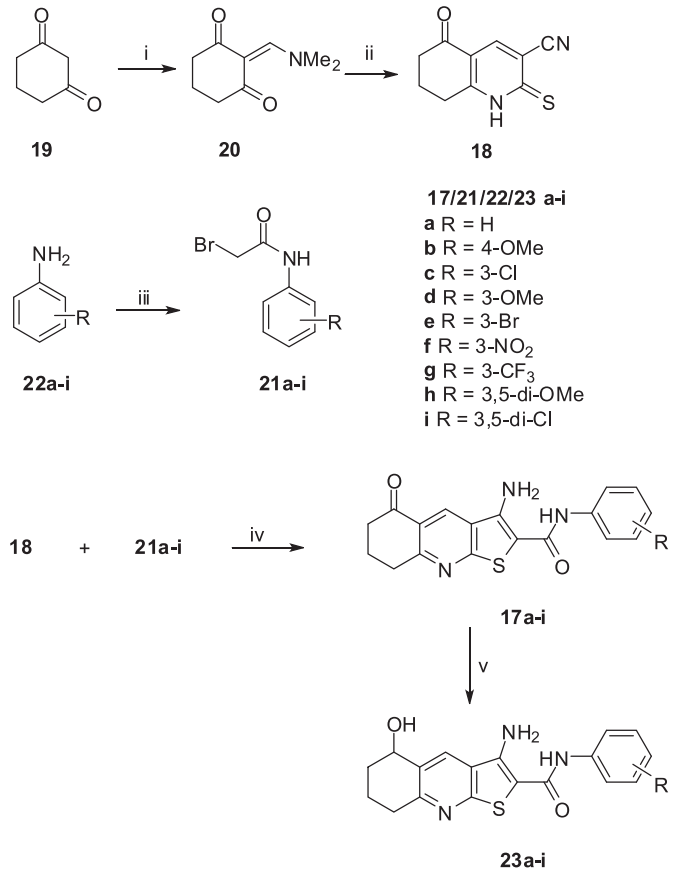
pyridines **13e**–**i**, under the same conditions, led to mixtures of products, even with very short reaction times. Use of the previously attempted bases on **13e**–**i** also led to complex mixtures of products, indicating the reduced stability of the polyhalogenated derivatives of both **10** and **13**. With similar biological results obtained between our initial set of furanopyridines **10** and thienopyridines **4** (see below) it was decided to abandon further optimisation of the synthesis of analogues **10**.

An attempt was made to prepare furopyrimidinones **14a**–**d** using the same procedure as used for the synthesis of **8a**–**i**. However, reaction of furopyridines **10a**–**c** in this case gave formimidates **15a**–**c** in 53–77% yields, whilst the reaction of **10d** gave only a complex mixture of products with no **14d** or **15d** isolated. Base-induced cyclisation of formimidates **15a**–**c**, using Na_2CO_3 in ethanol, gave **14a** and **14b**, albeit in a low yields of 12% and 20%, respectively, whilst the halogenated derivative **14c** was found to be unstable.

Finally, using furopyridines **10a**–**c** it was attempted to prepare a series of furotriazinones **16**, in a similar manner to the synthesis of thienotriazinones **9a**–**i**. Unfortunately, despite a number of different reaction times and reagent concentrations being attempted, none of the desired products were obtained.

2.1.3. Tetrahydrothieno[2,3-b]quinoline-2-carboxamide analogues

After determining the lower stability of oxygen-containing heterocycles **10**, **14** and **16** and coupled with the biological data showing no significant difference in antiproliferative activity between the already prepared oxygen and sulphur heterocycles (see below), it was decided to focus on the synthesis of *N*-aryl-tetrahydrothieno[2,3-*b*]quinoline-2-carboxamides rather than their furan equivalents. Whilst some derivatives of **17** are commercially available [10] there is almost no synthetic data reported for this class of compound [13]. The synthesis of these analogues **17a**–**i** began with the preparation of thiohexahydroquinoline **18** using our modification [11] of a literature method (Scheme 3) [16]. Firstly we performed a multi-component reaction [17–20] where 1,3-cyclohexanedione **19** was reacted with dimethylformamide dimethyl acetal (DMFDMA) [15] to give enamine **20**, which was then immediately reacted with cyanothioacetamide giving thiohexahydroquinoline **18** in 82% yield. Bromoacetamides **21a**–**i** were prepared in 51–99% yields from the acylation, at 0 °C, of various substituted anilines **22a**–**i** with bromoacetyl bromide. It was found that reaction at higher temperatures led to further amination at the α-bromide [21]. Similar to the synthesis of thieno[2,3-*b*]pyridine-2-carboxamides **4a**–**i**, we found that reaction of thiohexahydroquinoline **18** with sodium carbonate and bromoacetamide **21a**–**i**, gave the desired 5-oxo-thieno[2,3-*b*]pyridines **17a**–**i** in 14–99% yields in a single step. Finally reduction of the keto group in **17a**–**i** using sodium borohydride, in a mixture of methanol and THF, gave 5-hydroxy-thieno[2,3-*b*]pyridines **23a**–**i** in 66–99% yields.



Scheme 3. Reagents and conditions: (i) DMFDMA, DMF, 24 h; (ii) cyanothioacetamide, NaH, DMF, 24 h 82% (2 steps); (iii) 1 equiv. **22a**–**i**, 1.1 equiv. bromoacetyl bromide, Et_3N, CH_2Cl_2, 1 h, 51–99%; (iv) Na_2CO_3, EtOH, reflux, 18 h, 14–99%; (v) 1 equiv. $NaBH_4$, MeOH/THF, 2 h, 66–99%.

2.2. Biology

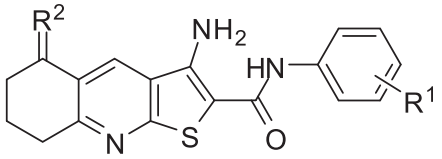
Heterocyclic compounds are frequently shown to inhibit the growth of cancerous cell lines [21–24] with inhibition of selected cell lines most often reported. A total of 47 analogues were selected and tested against the National Cancer Institute's human tumour cell lines (NCI-60), where the growth inhibition of compounds is compared to untreated cells at 100% [25]. The NCI-60 cancer cell panel includes leukaemia's, melanomas, and cancers of renal, ovarian, prostate, colon, lung, central nervous system and breast origin [26]. Analysis of the NCI-60 results of **17a**–**c**, previously published by Feng et al. [10], and 5-oxo-tetrahydrothieno[2,3-*b*]quinoline-2-carboxamides **17d**–**i** showed that unsubstituted analogue **17a** and 3-substituted phenyl derivatives **17c**, **17d** and **17e** showed the greatest activity. The 4-methoxyphenyl derivative **17b** had the lowest inhibition levels, which is in line with previous findings that the *para*-position is not favoured [12]. Analogues with strong electron-withdrawing groups at the 3-position (**17f**, **17g**) or 3,5-disubstitution (**17h**, **17i**) had reduced activity or were inactive. The NCI results of the similar alcohol analogues **23a**–**i** were similar to those for the corresponding keto derivative. Again the 3-substituted phenyl derivatives (**23c**, **23d**, **23e**) were the most active, however in this series the analogues **23f** and **23g** were more active than their keto counterparts. The similarity between the biological results for the keto **17** and alcohol **23** series suggest that they are inhibiting the same enzyme or a class of highly related enzymes (Table 1).

Compounds **17a**, **17c**, **17d**, **17e**, **23a**, **23c**, **23d**, **23e**, **23f** and **23g** were selected for dose response testing based on their bioactivity and the results are given in Table 2. The Growth Inhibition at 50% (GI_{50}) was derived from these measurements [27]. Six tumour cell lines were particularly affected, i.e., MDA-MB-435 (melanoma), MDA-MB-468 (breast), HS 578T (breast), NCI-H522 (non-small cell lung cancer), OVCAR–3 (ovarian) and RXF 393 (renal). Many other cell lines also showed good response, e.g., COLO 205 (colon), SNB–75 (CNS), BT 549 (breast) and MDA-MB-231 (breast). All the biological data is given in the Supplementary Information section.

It can be seen in Table 2 that *meta* methoxy substitution on the phenyl ring causes the most growth inhibition for both the keto **17** and alcohol series **23**. These derivatives **17/23d** show either the best or one of the best GI_{50} values for the cell lines shown. When the averaged GI_{50} values for the *meta* methoxy derivatives (**17/23d**) are correlated an R^2 of 0.969 is obtained, which is a strong indication that they are inhibiting the same enzyme. The other series are less correlated (**17/23a** − 0.497, **17/23c** − 0.325 and **17/23e** − 0.534). The keto methoxy compound **17d** is a better inhibitor than its alcohol **23d** counterpart for the entire cell lines considered in Table 2. Indeed, when the other substitution patterns are considered the trend is that the ketone derivatives are usually more active.

We then compared the activity of the tetrahydrothieno[2,3-*b*]quinoline-2-carboxamides **17** and **23** with the synthetic compounds not containing a cyclohexane ring. Compounds **4a**–**d** and **10a**–**d** were relatively inactive, showing that a furan ring in the core structure did not provide any advantage over compounds with more easily synthesizable thiophenes (Table 3). Similar to compounds **17** and **23**, analogues with a 4-methoxyphenyl carboxamide moiety had the lowest inhibition means with an average of 96.8%, whereas analogues with 3-chlorophenyl carboxamide had improved activities with an average of 84.9%. Also in the more active 3-chlorophenyl carboxamide analogues, activity improved as the size of the substituent at the 5-position increased from H (e.g. **8c**, 104.1%) to Cl (e.g. **8f**, 77.6%) to Br (e.g. **8i**, 69.3%) but did not reach the level of the corresponding 3-chlorophenyl derivatives **17c** (36.3%) and **23c** (27.1%) showing that derivatives containing a cyclohexanone or cyclohexanol ring are much more favourable

Table 1
The NCI Mean percentages (%) of growth at 10 μM as compared to untreated cells (100% growth) for compound series **17** and **23**.



17a-i and 23a-i

Compound	R^1	R^2	NCI-60 mean (%)
17a[a]	H	O	30.2
17b[a]	4-OMe	O	103.0
17c[a]	3-Cl	O	36.3
17d	3-OMe	O	24.9
17e	3-Br	O	31.6
17f	3-NO_2	O	66.0
17g	3-CF_3	O	88.3
17h	3,5-di-OMe	O	84.9
17i	3,5-di-Cl	O	94.8
23a	H	H/OH	30.0
23b	4-OMe	H/OH	95.5
23c	3-Cl	H/OH	27.1
23d	3-OMe	H/OH	24.0
23e	3-Br	H/OH	27.6
23f	3-NO_2	H/OH	50.3
23g	3-CF_3	H/OH	32.8
23h	3,5-di-OMe	H/OH	81.6
23i	3,5-di-Cl	H/OH	96.3

[a] Data from Ref. [10].

than the tested halide derivatives. The antiproliferation testing of the triazinones **9** and pyrimidinones **8** and **14** showed similar results as the thieno **4** and furopyridine-2-carboxamides **10**, although none of the triazinone and pyrimidinone analogues had directly comparable inhibition activities, showing that functionalization of the 3-amino group was detrimental to the bioactivity.

2.3. Molecular modelling study

Protein-ligand docking was undertaken on all 47 synthetic analogues with the GOLD software using four scoring functions (see experimental methods 4.8 for details). As the PLC-γ crystal structure is not available, the known PLC-δ isoform was used as a model, since the highest amino acid similarity between the isoforms occur in their substrate binding site [9]. There are predicted hydrogen bonding interactions between the 3-amino moiety, with GLU341, and amide carbonyl, with HIS356 and the HIS311 in all of the thienopyridine- **4**, furopyridine- **10** and tetrahydrothieno[2,3-*b*]quinoline-2-carboxamide **17** and **23** series. Also predicted is the insertion of the phenyl ring in a lipophilic pocket. Tetrahydrothieno[2,3-*b*]quinolines **17**, with a ketone carbonyl group at the 5-position, are predicted to further interact with both ARG549 and LYS438. The same is found in the *S*-enantiomers, but *not* the *R*-enantiomers, of the alcohol series **23**. It can be hypothesised that the *S*-enantiomers are responsible for the bioactivity of the **23** series, due to superior hydrogen bonding, since a racemic mixture was used in the experiments (Fig. 4). These findings are in line with previous modelling studies on compounds from series **17** [9–12].

Compound **17b** has consistently the worst score for the scoring functions used, except for Gold Score, which correlates with the biological results. Furthermore, the NCI mean and the predicted scores generated by ChemPLP for the **17** series have a reasonable linear correlation (R^2 − 0.443). This is also the trend observed for alcohols **23**. Interestingly, ChemPLP is reported to give the best results of the scoring functions available in GOLD [28].

Similar to the biological activities there were no significant differences found in the binding scores between compounds **4** and **8**, with a thiophene ring, and compounds **10** and **14** with a furan ring. Furthermore, this series of compounds had lower binding scores than the more biologically active tetrahydrothieno[2,3-*b*]quinolines **17** and **23** (as shown in Tables S1 and S2 in the Supplementary Information). Pyrimidinones, **8** and **14**, and triazinone **9** series showed the molecules had rotated by 180° in the binding pocket and showed hydrogen bonding between N-1 and HIS311 rather than interactions with GLU341, and no interactions between the amide carbonyl with either HIS311 or HIS356. These results suggested that the important interaction between GLU341 and the 3-amino group in **4** and **10** is blocked by the formation of the pyrimidinone (in **8** and **14**) and triazinone (in **9**) moieties further explaining the lower bioactivity of these compounds.

When the configurations of all analogues were scored and ranked it was noticed that analogues with a 3-substituent on the aryl carboxamide generally have the highest scores, whereas *para*-methoxy phenyl derivatives of **4/8/9/10/14/17b/23b** usually had the lowest scores, which correlated well with the obtained biological results. The scoring data is given in the Supplementary Information section.

Thienopyridines and furopyridines with 5-chloro (**4d/e/f** and **10d**) or 5-bromo (**4g/h/i**) substitution generally have higher, but not significantly higher, scores versus analogues with no substituent (**4a/b/c** and **10a/b/c**). The binding scores of these compounds when compared with the corresponding tetrahydrothieno[2,3-*b*]quinolines **17** which have the equivalent of a ketone at the 5-position, were significantly lower. The ketone carbonyl group is predicted to interact with both ARG549 and LYS438, whilst the halogens at the 5-position have limited contact with these amino acids.

3. Conclusions

A number of thieno[2,3-*b*]pyridine **4**/**8**/**9**, furo[2,3-*b*]pyridines **10**/**14**/**16** and tetrahydrothieno[2,3-*b*]quinolines **17**/**23** were synthesised and tested for their antiproliferative activity against a range of cancer cell lines. Tetrahydrothieno[2,3-*b*]quinolines **17**/**23** with a non-electron withdrawing substituent at the 3-position were found to be the most active with **17d** having the greatest activity in NCI testing. Thieno[2,3-*b*]pyridine-2-carboxamides **4** and furo[2,3-*b*]pyridines-2-carboxamides **10** and their derivatives **8**/**9**/**14**/**16** were found to were found to have diminished bioactivities in comparison with analogues **17**/**23**. In both of these series compounds with substitution at the 5-position were more active than those without substitution, and in general the larger the substituent the greater the activity. The biological results correlate well with the predicted binding of the ligands in the PLC active site, however further experimental work is required to confirm these observations, as it is a challenging endeavour to demonstrate mechanistic action of small molecule inhibitors in complex biological system.

4. Experimental section

4.1. General experimental

All reactions were carried out under a nitrogen atmosphere in dry, freshly distilled solvents unless otherwise noted. All optical rotation measurements were determined at 20 °C on the sodium D line (λ = 589 nm, 0.1 dm cell). All NMR spectra were recorded on either Bruker Avance DRX 300 MHz or 400 MHz spectrometers at ambient temperatures. Chemical shifts are reported

Table 2
The GI_{50} (50% growth inhibition) in nanomolar (nM) are shown for six tumour cell lines: MDA-MB-435 (melanoma), MDA-MB-468 and HS 578T (breast), NCI-H522 (non-small cell lung cancer), OVCAR-3 (ovarian) and RXF 393 (renal). All derivatives were tested twice and both values are given.

Compound	MDA-MB-435	MDA-MB-468	HS 578T	NCI-H522	OVCAR-3	RXF 393
17a[a]	88/137	173/150	333/825	150/309	243/287	192/189
17c[a]	45/71	45/171	452/342	190/285	305/245	251/913
17d	22/23	44/47	342/73	44/57	58/64	55/127
17e	54/157	247/272	630/566	176/350	316/408	265/522
23a	66/50	186/290	X/362	196/401	274/344	211/349
23c	120/123	267/247	831/3150	237/793	298/325	304/515
23d	25/23	55/142	199/531	73/149	68/164	133/278
23e	53/115	186/303	395/X	369/460	301/340	233/558
23f	416/391	2030/2200	16100/7500	4610/8090	3440/5950	4140/11100
23g	306/338	1790/1600	9040/4300	2860/56700	3720/4890	3150/10100

[a] Data from Ref. [10]; X: data not available.

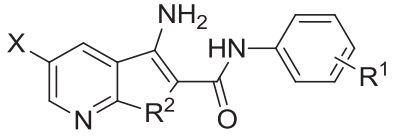
relative to the solvent peak of chloroform (δ 7.26 for ^{1}H and δ 77.0 for ^{13}C) or DMSO (δ 2.50 for ^{1}H and δ 39.5 for ^{13}C). ^{1}H NMR data is reported as position (δ), relative integral, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak; qd, quartet of doublets), coupling constant (J, Hz), and the assignment of the atom. ^{13}C NMR data are reported as position (δ) and assignment of the atom. All NMR assignments were performed using HSQC and HMBC experiments. High-resolution mass spectroscopy (HRMS) was carried out by either chemical ionization (CI) or electrospray ionization (ESI) on a MicroTOF-Q mass spectrometer. Unless noted, chemical reagents were used as purchased.

4.2. Synthesis of 2-mercaptoacetamides **6** and 2-hydroxyacetamides **11**

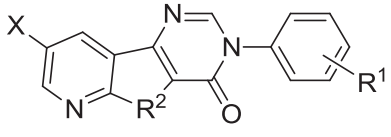
4.2.1. 2-Mercapto-N-phenylacetamide **6a**

Aniline (2.0 g, 21.7 mmol) was added to a solution of thioglycolic acid **5** (2.0 g, 21.7 mmol) in toluene (10.8 mL) and the mixture heated at reflux, under an atmosphere of nitrogen, using a Dean–Stark apparatus. After 5 h the resulting mixture was cooled to room temperature and hexane (11 mL) added. The mixture was cooled on ice, and the resultant precipitate was filtered to give the *title product* **6a** (1.75 g, 48%) as a yellow solid. m.p. 122–125 °C. lit. m.p. 112 °C [29]. ^{1}H NMR (400 MHz; $CDCl_3$) 2.03 (1H, t, J = 9.1 Hz,

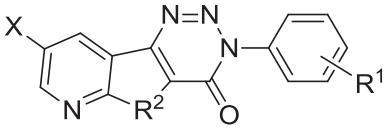
Table 3
The NCI Mean percentages (%) of growth at 10 μM as compared to untreated cells (100% growth) for compounds **4**, **8**, **9**, **10** and **14**.



4 and 10



8 and 14



9

Compound	X	R^1	R^2	NCI-60 mean (%)
4a	H	H	S	97.5
4b	H	4-OMe	S	96.4
4c	H	3-Cl	S	92.8
4d	Cl	H	S	91.8
4e	Cl	4-OMe	S	95.1
4f	Cl	3-Cl	S	73.4
4g	Br	H	S	91.5
4h	Br	4-OMe	S	96.8
4i	Br	3-Cl	S	70.7
8b	H	4-OMe	S	105.3
8c	H	3-Cl	S	104.1
8e	Cl	4-OMe	S	83.8
8f	Cl	3-Cl	S	77.6
8g	Br	H	S	84.3
8h	Br	4-OMe	S	99.6
8i	Br	3-Cl	S	69.3
9a	H	H	S	102.9
9b	H	4-OMe	S	106.5
9c	H	3-Cl	S	98.2
9e	Cl	4-OMe	S	97.9
9f	Cl	3-Cl	S	72.9
9g	Br	H	S	96.3
9h	Br	4-OMe	S	97.0
9i	Br	3-Cl	S	94.4
10a	H	H	O	96.9
10b	H	4-OMe	O	98.4
10c	H	3-Cl	O	96.1
10d	Cl	H	O	95.3
14b	H	4-OMe	O	87.7

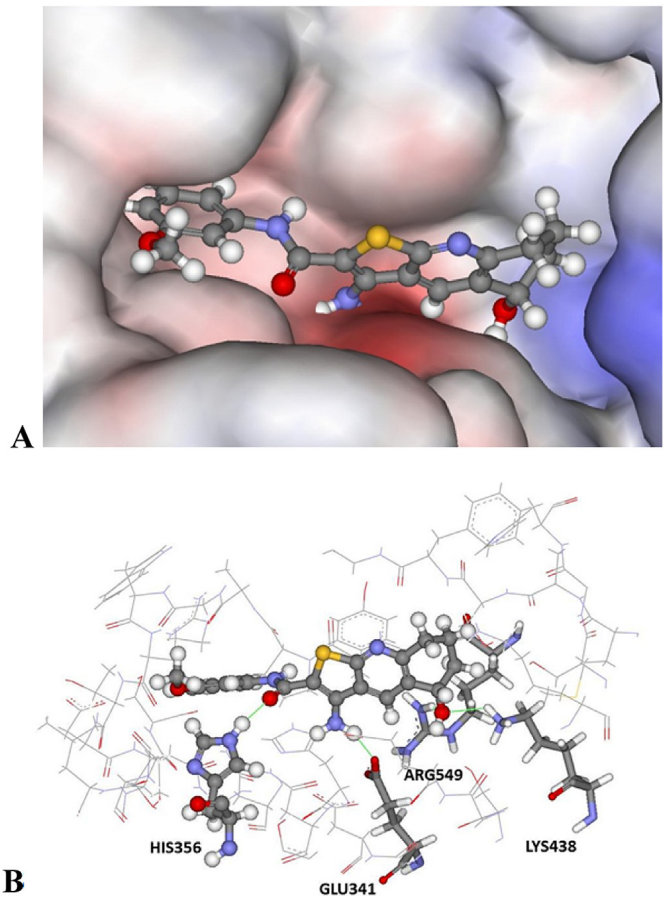


Fig. 4. The docked configuration of *S*-**23d** in the binding site of PLC-δ1 using ChemPLP. (A) The protein surface is rendered and ligand *S*-**23d** is shown. The phenyl group of *S*-**23d** occupies a lipophilic cavity to the left hand side and the methoxy substituent occupies a pocket. Red depicts a positive partial charge on the surface, blue depicts negative partial charge and grey shows neutral/lipophilic areas. (B) Hydrogen bonds are depicted as green lines between ligand *S*-**23d** and the amino acids HIS356, GLU431 ARG549 and LYS438. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

SH), 3.41 (2H, d, $J = 9.1$ Hz, CH_2), 7.13–7.18 (1H, m, Ar–H), 7.35–7.37 (2H, m, Ar–H), 7.54–7.61 (2H, m, Ar–H), 8.58 (1H, s, NH). The ^{1}H NMR data was in agreement with the literature values [29].

4.2.2. 2-Mercapto-N-(4′-methoxyphenyl)acetamide **6b**

A mixture of thioglycolic acid **5** (0.11 mL, 1.62 mmol) and *p*-anisidine (0.20 g, 1.62 mmol) were stirred for 5 h, at 130 °C, under an atmosphere of nitrogen. The mixture was then cooled to room temperature and diluted with DCM (15 mL), washed with 2 M HCl (2 × 15 mL), H_2O (15 mL), and brine (15 mL), dried (Na_2SO_4) and the solvent was removed *in vacuo* to give the *title product* **6b** (0.32 g, 99%) as a light purple solid. m.p. 116–118 °C. lit. m.p.118 °C [30]. ^{1}H NMR (400 MHz; $CDCl_3$) 2.01 (1H, t, $J = 9.3$ Hz, SH), 3.39 (2H, d, $J = 9.3$ Hz, CH_2), 3.80 (3H, s, OCH_3), 6.88 (2H, d, $J = 9.0$ Hz, H-3′ and H-5′), 7.45 (2H, d, $J = 9.0$ Hz, H-2′ and H-6′), 8.40 (1H, s, NH). ^{13}C NMR (400 MHz; $CDCl_3$) (100 MHz; $CDCl_3$) 29.0 (C-2), 55.5 (OCH_3), 114.2 (C-3′ and C-5′), 121.8 (C-2′and C-6′), 130.4 (C-1′), 156.8 (C-1′), 167.0 (C=O).

4.2.3. N-(3′-Chlorophenyl)-2-mercaptoacetamide **6c**

Using the same procedure as for amide **6b**, using 3-chloroaniline (0.23 mL, 2.17 mmol) and thioglycolic acid **5** (0.15 mL, 2.17 mmol), stirring for 3 h at 130 °C. After workup the *title product* **6c** (0.43 g, 97%) was obtained as a white solid. m.p. 85–86 °C. lit. m.p.75 °C [30]. ^{1}H NMR (400 MHz; $CDCl_3$) 2.04 (1H, t, $J = 9.2$ Hz, SH), 3.39 (2H, d, $J = 9.2$ Hz, CH_2), 7.12 (1H, d, $J = 8.0$ Hz, H-4′), 7.23–7.28 (1H, m, H-5′), 7.40 (1H, d, $J = 8.0$ Hz, H-6′), 7.66 (1H, s, H-2′), 8.57 (1H, br s, NH). The ^{1}H and data was in agreement with the literature values [29].

4.2.4. 2-Hydroxy-N-phenylacetamide ***11a***

A mixture of aniline (0.36 mL, 3.94 mmol) and glycolic acid **12** (0.30 g, 3.94 mmol) were stirred at 130 °C for 5 h. The mixture was then cooled to room temperature and left to stand for 1 h. Diethyl ether (20 mL) was added and the resultant solid was filtered to give the *title product* **11a** (0.31 g, 56%) as a white solid. m.p. 90–92 °C. lit. m.p. 92–94 °C [31]. ^{1}H NMR (400 MHz; d_4-MeOH) 4.13 (2H, s, CH_2), 7.11–7.15 (1H, m, Ar–H), 7.33 (2H, d, $J = 8.0$ Hz, Ar–H), 7.61 (2H, d, $J = 8.0$ Hz, Ar–H). The ^{1}H NMR data was in agreement with the literature values [31].

4.2.5. 2-Hydroxy-N-(4′-methoxyphenyl)acetamide ***11b***

A mixture of glycolic acid **12** (0.25 g, 3.25 mmol) and *p*-anisidine (0.40 g, 3.25 mmol) were stirred at 130 °C for 3 h. The mixture was then cooled to room temperature and left to stand for 1 h. The resultant solid was filtered to give the *title product* **11b** (0.45 g, 76%) as a dark purple solid. m.p. 87–89 °C. lit. m.p. 95–96 °C [32]. ^{1}H NMR (400 MHz; $CDCl_3$) 3.77 (3H, s, OCH_3), 4.16 (2H, s, CH_2), 6.85 (2H, d, $J = 9.0$ Hz, H-3′ and H-5′), 7.42 (2H, d, $J = 9.0$ Hz, H-2′ and H-6′), 8.38 (1H, s, NH). The ^{1}H NMR data was in agreement with the literature values [33].

4.2.6. N-(3′-Chlorophenyl)-2-hydroxyacetamide ***11c***

Using the same procedure as for amide **11b**, glycolic acid **12** (0.20 g, 2.63 mmol) and 3-chloroaniline (0.28 mL g, 2.63 mmol) stirring at 130 °C for 5 h. After workup the *title product* **11c** (0.49 g, 99%) was obtained as a light orange solid. m.p. 92–95 °C. ^{1}H NMR (400 MHz; d_6-acteone) 3.65 (1H, s, OH), 4.14 (2H, s, CH_2), 7.12 (1H, d, $J = 8.0$ Hz, H-4′), 7.31–7.35 (1H, m, H-5′), 7.64 (1H, d, $J = 8.0$ Hz, H-6′), 7.99 (1H, s, H-2′), 9.34 (1H, br s, NH). ^{1}H NMR (100 MHz; d_6-acteone) 62.9 (CH_2), 117.0 (C-6′), 120.2 (C-2′), 124.3 (C-4′), 131.0 (C-5′), 134.7 (C-3′), 140.8 (C-1′), 171.8 (C=O). IR: υ_{max} (film)/cm^{-1}: 3374, 3280, 3188, 3068, 2924, 1650, 1594, 1551, 1070, 968. *m*/*z* (ESI+): 210 (^{37}Cl MNa$^+$, 31%), 208 (^{35}Cl MNa$^+$, 100); HRMS (ESI$^+$) found (^{37}Cl MNa$^+$): 210.0106, $C_8H_8NO_2^{37}ClNa$ requires 210.0107. Found (^{35}Cl MNa$^+$): 208.0136, $C_8H_8NO_2^{35}ClNa$ requires 208.0136.

4.3. Synthesis of thienopyridine-2-carboxamides ***4*** and furopyridine-2-carboxamides ***10***

4.3.1. General procedure A: synthesis of the thienopyridine-2-carboxamides ***4*** and cyanopyridines ***13*** from pyridines ***7***

A mixture of 2-mercaptoacetamides **6a**–**c** or 2-hydroxyacetamides **11a**–**c** (1.05 mmol), pyridine **7a**–**c** (1 mmol) and anhydrous sodium carbonate (1.05 mmol) in absolute ethanol (1 mL) was stirred at reflux for 15 h. The mixture was then cooled to room temperature and the solvent removed *in vacuo*. The resultant crude solid was washed with small amounts of water and the remaining solid was recrystallized, using methanol to give the thienopyridine-2-carboxamide **4** or cyanopyridine **13**.

4.3.1.1. 3-Amino-N-phenylthieno[2,3-b]pyridine-2-carboxamide ***4a***. The reaction was carried out according to general procedure A using acetamide **6a** (1.0 g, 5.98 mmol) and pyridine **7a** (0.79 g, 5.70 mmol) to give the *title product* **4a** (1.17 g, 73%) as a yellow solid. m.p. 247–249 °C; ^{1}H NMR (400 MHz; d_6-DMSO) 7.07–7.11 (1H, m, Ar–H), 7.33 (2H, t, $J = 7.9$ Hz, Ar–H), 7.39 (2H, s, NH_2), 7.48 (1H, dd, $J = 4.5$, 8.0 Hz, H-5), 7.71 (2H, d, $J = 7.9$ Hz, Ar–H), 8.51 (1H, dd,

J = 1.5, 8.0 Hz, H-4), 8.68 (1H, dd, J = 1.5, 4.5 Hz, H-6), 9.45 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 96.6 (C-2), 119.4 (C-5), 121.1 (Ar–CH), 123.4 (Ar–CH), 126.1 (C-3a), 128.4 (Ar–CH), 130.9 (C-4), 138.9 (Ar–C), 146.8 (C-3), 150.2 (C-6), 158.7 (C-7a), 163.9 (C=O); IR: υ_{max} (film)/cm^{-1}: 3428, 3318, 3227, 3061, 1642, 1588, 1488, 1239; m/z (ESI+): 292 (MNa$^+$, 100%). HRMS (ESI$^+$) found (MNa$^+$): 292.0521, $C_{14}H_{11}N_3OSNa$ requires 292.0515.

*4.3.1.2. 3-Amino-N-(4′-methoxyphenyl)thieno[2,3-b]pyridine-2-carboxamide **4b**.* The reaction was carried out according to general procedure A using acetamide **6b** (1.20 g, 6.08 mmol) and pyridine **7a** (0.80 g, 5.79 mmol) to give the *title product* **4b** (1.09 g, 60%) as a yellow solid. m.p. 199–201 °C; ^{1}H NMR (400 MHz; d_6-DMSO) 3.75 (3H, s, OCH_3), 6.91 (2H, d, J = 9.0 Hz, H-3′ and H-5′), 7.33 (2H, s, NH_2), 7.47 (1H, dd, J = 4.5, 8.0 Hz, H-5), 7.58 (2H, d, J = 9.0 Hz, H-2′ and H-6′), 8.49 (1H, dd, J = 1.5, 8.0 Hz, H-4), 8.67 (1H, dd, J = 1.5, 4.5 Hz, H-6), 9.36 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 55.1 (OCH_3), 96.8 (C-2), 113.6 (C-3′ and C-5′), 119.4 (C-5), 122.9 (C-2′ and C-6′), 126.1 (C-3a), 130.8 (C-4), 131.8 (C-1′), 146.4 (C-3), 150.1 (C-6), 155.5 (C-4′), 158.6 (C-7a), 163.6 (C=O); IR: υ_{max} (film)/cm^{-1}: 3515, 3446, 3335, 3051, 1631, 1595, 1502, 1324, 1235, 1177, 1026; m/z (ESI+): 322 (MNa$^+$, 100%). HRMS (ESI$^+$) found (MNa$^+$): 322.0629, $C_{15}H_{13}N_3O_2SNa$ requires 322.0621.

*4.3.1.3. 3-Amino-N-(3′-chlorophenyl)thieno[2,3-b]pyridine-2-carboxamide **4c**.* The reaction was carried out according to general procedure A using acetamide **6c** (0.30 g, 1.49 mmol) and pyridine **7a** (0.20 g, 1.42 mmol) to give the *title product* **4c** (0.39 g, 90%) as a yellow solid. m.p. 240–241 °C; ^{1}H NMR (400 MHz; d_6-DMSO) 7.12 (1H, d, J = 8.0 Hz, H-4′), 7.33–7.37 (1H, m, H-5′), 7.48 (3H, dd, J = 4.5, 8.0 Hz, NH_2, H-5), 7.67 (1H, d, J = 8.0 Hz, H-6′), 7.94 (1H, s, H-2′), 8.53 (1H, dd, J = 1.5, 8.0 Hz, H-4), 8.69 (1H, dd, J = 1.5, 4.5 Hz, H-6), 9.61 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 96.1 (C-2), 119.1 (C-6′), 119.5 (C-5), 120.2 (C-2′), 122.9 (C-4′), 126.0 (C-3a), 130.0 (C-5′), 131.0 (C-4), 132.7 (C-3′), 140.6 (C-1′), 147.3 (C-3), 150.4 (C-6), 158.8 (C-7a), 164.0 (C=O); IR: υ_{max} (film)/cm^{-1}: 3455, 3323, 3068, 1643, 1586, 1475, 1303, 1232; m/z (ESI+): 328 (^{37}Cl MNa$^+$, 35%), 326 (^{35}Cl MNa$^+$, 100); HRMS (ESI$^+$) found (^{37}Cl MNa$^+$): 328.0103, $C_{14}H_{10}N_3OS^{37}ClNa$ requires 328.0096. Found (^{35}Cl MNa$^+$): 326.0127, $C_{14}H_{10}N_3OS^{35}Cl$ Na requires 326.0125.

*4.3.1.4. 3-Amino-5-chloro-N-phenylthieno[2,3-b]pyridine-2-carboxamide **4d**.* The reaction was carried out according to general procedure A using acetamide **6a** (0.10 g, 0.61 mmol) and pyridine **7b** (0.10 g, 0.58 mmol) to give the *title product* **4d** (0.07 g, 40%) as a yellow solid. m.p. 214–216 °C; ^{1}H NMR (400 MHz; d_6-DMSO) 7.09–7.13 (1H, m, Ar–H), 7.34 (2H, d, J = 8.0 Hz, Ar–H), 7.36 (2H, s, NH_2), 7.69 (2H, d, J = 8.0 Hz, Ar–H), 8.71 (2H, m, H-4 and H-6), 9.54 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 98.7 (C-2), 121.2 (Ar–CH), 123.6 (Ar–CH), 127.0 (C-3a and C-5), 128.4 (Ar–CH), 130.2 (C-4), 138.7 (Ar–C), 145.7 (C-3), 148.5 (C-6), 156.6 (C-7a), 163.5 (C=O); IR: υ_{max} (film)/cm^{-1}: 3449, 3419, 3326, 1688, 1591, 1525, 1490, 1237, 1112; m/z (ESI+): 328 (^{37}ClMNa$^+$, 35%), 326 (^{35}ClMNa$^+$, 100%); HRMS (ESI$^+$) found (^{37}ClMNa$^+$): 328.0095, $C_{14}H_{10}N_3OS^{37}ClNa$ requires 328.0096. Found (^{35}Cl MNa$^+$): 326.0128, $C_{14}H_{10}N_3OS^{35}ClNa$ requires 326.0125.

*4.3.1.5. 3-Amino-5-chloro-N-(4′-methoxyphenyl)thieno[2,3-b]pyridine-2-carboxamide **4e**.* The reaction was carried out according to general procedure A using acetamide **6b** (0.12 g, 0.61 mmol) and pyridine **7b** (0.10 g, 0.58 mmol) to give the *title product* **4e** (0.14 g, 74%) as a yellow solid. m.p. 214–216 °C; ^{1}H NMR (400 MHz; d_6-DMSO) 3.74 (3H, s, OCH_3), 6.91 (2H, d, J = 9.0 Hz, H-3′ and H-5′), 7.30 (2H, s, NH_2), 7.57 (2H, d, J = 9.0 Hz, H-2′ and H-6′), 8.69 (2H, m, H-4 and H-6), 9.45 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 55.1 (OCH_3), 99.0 (C-2), 113.6 (C-3′ and C-5′), 123.0 (C-2′ and C-6′), 127.0 (C-3a), 127.1 (C-5), 130.2 (C-4), 131.7 (C-1′), 145.2 (C-3), 148.3 (C-6), 155.6 (C-4′), 156.5 (C-7a), 163.3 (C=O); IR: υ_{max} (film)/cm^{-1}: 3424, 3311, 3016, 2927, 1638, 1594, 1502, 1342, 1109; m/z: (ESI+): 358 (^{37}Cl MNa$^+$, 36%), 356 (^{35}Cl MNa$^+$, 100); HRMS (ESI$^+$) found (^{37}Cl MNa$^+$): 358.0194, $C_{15}H_{12}N_3O_2S^{37}Cl$ Na requires 358.0202. Found (^{35}Cl MNa$^+$): 356.0222, $C_{15}H_{12}N_3O_2S^{35}ClNa$ requires 356.0231.

*4.3.1.6. 3-Amino-5-chloro-N-(3′-chlorophenyl)thieno[2,3-b]pyridine-2-carboxamide **4f**.* The reaction was carried out according to general procedure A using acetamide **6c** (0.12 g, 0.61 mmol) and pyridine **7b** (0.10 g, 0.58 mmol) to give the *title product* **4f** (0.20 g, 99%) as a yellow solid. m.p. 258–260 °C; ^{1}H NMR (400 MHz; d_6-DMSO) 7.13 (1H, d, J = 8.1 Hz, H-4′), 7.33–7.37 (1H, m, H-5′), 7.44 (2H, s, NH_2), 7.64 (1H, d, J = 8.1 Hz, H-6′), 7.91 (1H, s, H-2′), 8.71 (2H, s, H-4 and H-6), 9.68 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 98.3 (C-2), 119.2 (C-6′), 120.3 (C-2′), 123.0 (C-4′), 126.9 (C-3a), 127.0 (C-5), 130.0 (C-5′), 130.4 (C-4), 132.7 (C-3′), 140.6 (C-1′), 146.1 (C-3), 148.7 (C-6), 156.6 (C-7a), 163.6 (C=O); IR: υ_{max} (film)/cm^{-1}: 3460, 3334, 3055, 1657, 1590, 1522, 1420, 1346, 1231, 1113; m/z (ESI+): 363 ($^{37}Cl_2$MNa$^+$, 14%), 361 ($^{35}Cl^{37}$ClMNa$^+$, 78%), 359 ($^{35}Cl_2$MNa$^+$, 100%); HRMS (ESI$^+$) found ($^{37}Cl_2$ MNa$^+$): 363.9670, $C_{14}H_9N_3OS^{37}Cl_2Na$ requires 363.9677. Found ($^{35}Cl^{37}$Cl MNa$^+$): 361.9704, $C_{14}H_9N_3OS^{35}Cl^{37}ClNa$ requires 361.9706. Found ($^{35}Cl_2$ MNa$^+$): 359.9737, $C_{14}H_9N_3OS^{35}Cl_2Na$ requires 359.9736.

*4.3.1.7. 3-Amino-5-bromo-N-phenylthieno[2,3-b]pyridine-2-carboxamide **4g**.* The reaction was carried out according to general procedure A using acetamide **6a** (0.08 g, 0.49 mmol) and pyridine **7c** (0.10 g, 0.46 mmol) to give the *title product* **4g** (0.07 g, 42%) as a yellow solid. m.p. 224–226 °C; ^{1}H NMR (400 MHz; d_6-DMSO) 7.07–7.11 (1H, m, Ar–H), 7.33 (2H, d, J = 7.9 Hz, Ar–H), 7.37 (2H, s, NH_2), 7.69 (2H, d, J = 7.9 Hz, Ar–H), 8.78 (1H, d, J = 2.0 Hz, H-6), 8.84 (1H, d, J = 2.0 Hz, H-4), 9.54 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 98.4 (C-2), 115.5 (C-5), 121.2 (Ar–CH), 123.6 (Ar–CH), 127.6 (C-3a), 128.4 (Ar–CH), 133.2 (C-4), 138.8 (Ar–C), 145.6 (C-3), 150.4 (C-6), 156.9 (C-7a), 163.5 (C=O); IR: υ_{max} (film)/cm^{-1}: 3446, 3412, 3321, 1632, 1588, 1345, 1311, 1103; m/z (ESI+): 371 (^{81}BrMNa$^+$, 100%), 369 (^{79}BrMNa$^+$, 87); HRMS (ESI$^+$) found: (^{81}Br MNa$^+$) 371.9599, $C_{14}H_{10}N_3OS^{81}BrNa$ requires 371.9600. Found (^{79}Br MNa$^+$): 369.9621, $C_{14}H_{10}N_3OS^{79}BrNa$ requires 369.9620.

*4.3.1.8. 3-Amino-5-bromo-N-(4′-methoxyphenyl)thieno[2,3-b]pyridine-2-carboxamide **4h**.* The reaction was carried out according to general procedure A using acetamide **6b** (0.10 g, 0.49 mmol) and pyridine **7c** (0.10 g, 0.46 mmol) to give the *title product* **4h** (0.12 g, 71%) as a yellow solid. m.p. 242–243 °C; ^{1}H NMR (400 MHz; d_6-DMSO) 3.75 (3H, s, OCH_3), 6.91 (2H, d, J = 9.0 Hz, H-3′ and H-5′), 7.31 (2H, s, NH_2), 7.57 (2H, d, J = 9.0 Hz, H-2′ and H-6′), 8.76 (1H, d, J = 2.0 Hz, H-6), 8.82 (1H, d, J = 2.0 Hz, H-4), 9.44 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 55.1 (OCH_3), 98.7 (C-2), 113.6 (C-3′ and C-5′), 115.5 (C-5), 123.0 (C-2′ and C-6′), 127.7 (C-3a), 131.7 (C-4), 133.1 (C-1′), 145.2 (C-3), 150.3 (C-6), 155.6 (C-4′), 156.8 (C-7a), 163.2 (C=O); IR: υ_{max} (film)/cm^{-1}: 3401, 3281, 3201, 3132, 1649, 1595, 1501, 1346, 1107; m/z (ESI+): 401 (^{81}Br MNa$^+$, 100%), 399 (^{79}Br MNa$^+$, 98%); HRMS (ESI$^+$) found (^{81}Br MNa$^+$): 401.9698, $C_{15}H_{12}N_3O_2S^{81}BrNa$ requires 401.9706. Found (^{79}Br MNa$^+$): 399.9723, $C_{15}H_{12}N_3O_2S^{79}BrNa$ requires 399.9726.

*4.3.1.9. 3-Amino-5-bromo-N-(3′-chlorophenyl)thieno[2,3-b]pyridine-2-carboxamide **4i**.* The reaction was carried out according to general procedure A using acetamide **3c** (0.10 g, 0.49 mmol) and pyridine **6c** (0.10 g, 0.46 mmol) to give the *title product* **4i** (0.12 g, 67%) as a yellow solid. m.p. 272–275 °C; ^{1}H NMR (400 MHz; d_6-DMSO) 7.11 (1H, d, J = 8.0 Hz, H-4′), 7.32–7.36 (1H, m, H-5′), 7.43

(2H, s, NH_2), 7.62 (1H, d, J = 8.0 Hz, H-6′), 7.91 (1H, s, Hz, H-2′), 8.77 (1H, d, J = 2.0 Hz, H-6), 8.83 (1H, d, J = 2.0 Hz, H-4), 9.69 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 98.7 (C-2), 115.5 (C-5), 119.4 (C-6′), 120.4 (C-2′), 122.8 (C-4′), 127.6 (C-3a), 130.0 (C-5′), 132.7 (C-4), 133.2 (C-3′), 141.2 (C-1′), 145.7 (C-3), 150.4 (C-6), 157.0 (C-7a), 163.8 (C=O); IR: υ_{max} (film)/cm^{-1}: 3458, 3331, 3053, 1657, 1588, 1420, 1343, 1102; m/z (ESI+): 385 (^{81}Br^{37}Cl MH$^+$, 25%), 383 (^{81}Br^{35}Cl MH$^+$ and ^{79}Br^{37}Cl MH$^+$, 100%), 381 (^{79}Br^{35}Cl MH$^+$, 75%); HRMS (ESI$^+$) found (^{81}Br^{37}Cl MH$^+$): 385.9356, $C_{14}H_{10}N_3OS^{81}Br^{37}Cl$ requires 385.9361. Found (^{79}Br^{35}Cl MH$^+$): 381.9406, $C_{14}H_{10}N_3OS^{79}Br^{35}Cl$ requires 381.9411.

4.3.1.10. 2-(3″-Cyanopyridin-2″-yloxy)-N-phenylacetamide ***13a***. The reaction was carried out according to general procedure A using acetamide **11a** (0.15 g, 0.99 mmol) and pyridine **7a** (0.13 g, 0.95 mmol) to give the *title product* **13a** (0.12 g, 49%) as a white solid. m.p. 157–159 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 5.11 (2H, s, CH_2), 7.04–7.08 (1H, m, H-5″), 7.20–7.23 (1H, m, H-4′), 7.31 (2H, d, J = 7.8 Hz, H-3′ and H-5′), 7.57 (2H, d, J = 7.8 Hz, H-2′ and H-6′), 8.32 (1H, d, J = 7.2 Hz, H-4″), 8.42 (1H, d, J = 7.2 Hz, H-6″), 10.20 (1H, br s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 64.7 (C-2), 95.7 (C-3″), 115.2 (CN), 117.8 (C-5″), 119.2 (C-2′ and C-6′), 123.5 (C-4′), 128.8 (C-3′ and C-5′), 138.5 (C-1′), 144.1 (C-4″), 151.5 (C-6″), 162.5 (C-2″), 165.7 (C-1); IR: υ_{max} (film)/cm^{-1}: 3350, 3067, 2954, 2849, 2235, 1668, 1576, 1534, 1444, 1246, 1037; m/z (ESI+): 276 (MNa$^+$, 100%). High Resolution (ESI$^+$) found (MNa$^+$): 276.0743, $C_{14}H_{11}N_3O_2Na$ requires 276.0743.

4.3.1.11. 2-(3″-Cyanopyridin-2″-yloxy)-N-(4′-methoxyphenyl)acetamide ***13b***. The reaction was carried out according to general procedure A using acetamide **11b** (0.15 g, 0.83 mmol) and pyridine **7a** (0.11 g, 0.79 mmol) to give the *title product* **13b** (0.13 g, 57%) as a purple solid. m.p. 174–175 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 3.80 (3H, s, OCH_3), 5.03 (2H, s, CH_2), 6.89 (2H, d, J = 9.0 Hz, H-3′ and H-5′), 7.11–7.14 (1H, m, H-5″), 7.52 (2H, d, J = 9.0 Hz, H-2′ and H-6′), 7.98 (1H, dd, J = 2.0, 7.5 Hz, H-4″), 8.15 (1H, br s, NH), 8.42 (1H, dd, J = 2.0, 7.5 Hz, H-6″); ^{13}C NMR (100 MHz; $CDCl_3$) 55.5 (OCH_3), 65.7 (C-2), 97.2 (C-3″), 114.3 (C-3′ and C-5′), 114.7 (CN), 118.1 (C-5″), 121.8 (C-2′ and C-6′), 130.0 (C-1′), 143.0 (C-4″), 151.6 (C-6″), 156.9 (C-4′), 162.0 (C-2″), 164.8 (C-1); IR: υ_{max} (film)/cm^{-1}: 3246, 3044, 2939, 2843, 2230, 1655, 1581, 1539, 1444, 1235, 1031; m/z (ESI+): 306 (MNa$^+$, 100%). HRMS (ESI$^+$) found (MNa$^+$): 306.0854, $C_{15}H_{13}N_3O_3Na$ requires 306.0849.

4.3.1.12. N-(3′-Chlorophenyl)-2-(3″-cyanopyridin-2″-yloxy)acetamide ***13c***. The reaction was carried out according to general procedure A using acetamide **11c** (0.10 g, 0.54 mmol) and pyridine **7a** (0.07 g, 0.51 mmol) to give the *title product* **13c** (0.06 g, 39%) as a white solid. m.p. 170–171 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 5.12 (2H, s, CH_2), 7.12 (1H, d, J = 8.0 Hz, H-4′), 7.20–7.23 (1H, m, H-5″), 7.32–7.36 (1H, m, H-5′), 7.44 (1H, d, J = 8.0 Hz, H-6′), 7.77 (1H, s, H-2′), 8.13 (1H, d, J = 7.5 Hz, H-4″), 8.42 (1H, d, J = 7.5 Hz, H-6″); ^{13}C NMR (100 MHz; $CDCl_3$) 64.7 (C-2), 95.7 (C-3″), 115.1 (CN), 117.6 (C-6′), 117.8 (C-5″), 118.7 (C-2′), 123.2 (C-4′), 130.5 (C-5′), 133.1 (C-3′), 139.9 (C-1′), 144.1 (C-4″), 151.4 (C-6″), 162.4 (C-2″), 166.2 (C-1); IR: υ_{max} (film)/cm^{-1}: 3294, 3069, 2949, 2841, 2229, 1672, 1578, 1540, 1421, 1257; m/z (ESI+): 312 (^{37}Cl MNa$^+$, 31%), 310 (^{35}Cl MNa$^+$, 100%); HRMS (ESI$^+$) found (^{37}Cl MNa$^+$): 312.0332, $C_{14}H_{10}N_3O_2^{37}ClNa$ requires 312.0325. Found (^{35}Cl MNa$^+$): 310.0358, $C_{14}H_{10}N_3O_2^{35}ClNa$ requires 310.0354.

4.3.1.13. 2-(5″-Chloro-3″-cyanopyridin-2″-yloxy)-N-phenylacetamide ***13d***. The reaction was carried out according to general procedure A using acetamide **11a** (0.09 g, 0.61 mmol) and pyridine **7b** (0.10 g, 0.58 mmol) to give the *title product* **13d** (0.15 g, 92%) as an orange solid. m.p. 149–152 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 5.02 (2H, s, CH_2), 7.14–7.18 (1H, m, H-4′), 7.36 (2H, d, J = 8.0 Hz, H-3′ and H-5′), 7.60 (2H, d, J = 8.0 Hz, H-2′ and H-6′), 7.98 (1H, d, J = 2.5 Hz, H-4″), 8.16 (1H, s, NH), 8.56 (1H, d, J = 2.5 Hz, H-6″); ^{13}C NMR (100 MHz; $CDCl_3$) 66.1 (C-2), 98.2 (C-3″), 113.4 (CN), 120.0 (C-2′ and C-6′), 125.0 (C-4′), 125.7 (C-5″), 129.2 (C-3′ and C-5′), 136.7 (C-1′), 142.2 (C-4″), 150.2 (C-6″), 160.4 (C-2″), 164.6 (C-1); IR: υ_{max} (film)/cm^{-1}: 3274, 3207, 3147, 3108, 3070, 2235, 1677, 1556, 1449, 1301, 1253; m/z: (ESI+): 312 (^{37}Cl MNa$^+$, 38%), 310 (^{35}Cl MNa$^+$, 100%); HRMS (ESI$^+$) found (^{37}Cl MNa$^+$): 312.0321, $C_{14}H_{10}N_3O_2^{37}ClNa$ requires 312.0325. Found (^{35}Cl MNa$^+$): 310.0354, $C_{14}H_{10}N_3O_2^{35}ClNa$ requires 310.0354.

4.3.1.14. 2-(5″-Chloro-3″-cyanopyridin-2″-yloxy)-N-(4′-methoxyphenyl)acetamide ***13e***. The reaction was carried out according to general procedure A using acetamide **11b** (0.11 g, 0.61 mmol) and pyridine **7b** (0.10 g, 0.58 mmol) to give the *title product* **13e** (0.10 g, 55%) as a purple solid. m.p. 160–162 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 3.80 (3H, s, OCH_3), 5.00 (2H, s, CH_2), 6.88 (2H, d, J = 9.0 Hz, H-3′ and H-5′), 7.50 (2H, d, J = 9.0 Hz, H-2′ and H-6′), 7.94 (1H, d, J = 2.5 Hz, H-4″), 8.06 (1H, s, NH), 8.36 (1H, d, J = 2.5 Hz, H-6″); ^{13}C NMR (100 MHz; $CDCl_3$) 55.5 (OCH_3), 66.1 (C-2), 98.1 (C-3″), 113.4 (CN), 114.3 (C-3′ and C-5′), 121.8 (C-2′ and C-6′), 125.6 (C-5″), 129.8 (C-1′), 142.2 (C-4″), 150.2 (C-6″), 156.9 (C-4′), 160.4 (C-2″), 164.4 (C-1); IR: υ_{max} (film)/cm^{-1}: 3330, 3073, 2929, 2838, 2232, 1664, 1550, 1507, 1453, 1426, 1241, 1037; m/z: (ESI+): 342 (^{37}Cl MNa$^+$, 39%), 340 (^{35}ClMNa$^+$, 100%); HRMS (ESI$^+$) found: (^{37}Cl MNa$^+$) 342.0435, $C_{15}H_{12}N_3O_3^{37}ClNa$ requires 342.0431. Found (^{35}Cl MNa$^+$): 340.0461, $C_{15}H_{12}N_3O_3^{35}ClNa$ requires 340.0459.

4.3.1.15. 2-(5″-Chloro-3″-cyanopyridin-2″-yloxy)-N-(3′-chlorophenyl)acetamide ***13f***. The reaction was carried out according to general procedure A using acetamide **11c** (0.11 g, 0.61 mmol) and pyridine **7b** (0.10 g, 0.58 mmol) to give the *title product* **8f** (0.03 g, 19%) as a white solid. m.p. Decomp. >350 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 5.01 (2H, s, CH_2), 7.14 (1H, d, J = 8.0 Hz, H-4′), 7.26–7.30 (1H, m, H-5′), 7.40 (1H, d, J = 8.0 Hz, H-6′), 7.77 (1H, s, H-2′), 7.96 (1H, d, J = 2.5 Hz, H-4″), 8.17 (1H, s, NH), 8.36 (1H, d, J = 2.5 Hz, H-6″); ^{13}C NMR (100 MHz; $CDCl_3$) 66.0 (C-2), 98.2 (C-3″), 113.4 (CN), 118.0 (C-6′), 120.1 (C-2′), 125.1 (C-4′), 125.8 (C-5″), 130.1 (C-5′), 134.9 (C-3′), 137.8 (C-1′), 142.2 (C-4″), 150.2 (C-6″), 160.2 (C-2″), 164.7 (C-1); IR: υ_{max} (film)/cm^{-1}: 3460, 3315, 2231, 1677, 1592, 1452, 1297, 1160, 1035; m/z (ESI+): 347 (^{37}Cl$_2$ MNa$^+$, 10%), 345 (^{35}Cl^{37}Cl MNa$^+$, 59), 343 (^{35}Cl$_2$ MNa$^+$, 100). HRMS (ESI$^+$) found (^{37}Cl$_2$ MNa$^+$): 347.9908, $C_{14}H_9N_3O_2^{37}Cl_2Na$ requires 347.9905.

4.3.1.16. 2-(5″-Bromo-3″-cyanopyridin-2″-yloxy)-N-phenylacetamide ***13g***. The reaction was carried out according to general procedure A using acetamide **11a** (0.07 g, 0.49 mmol) and pyridine **7c** (0.10 g, 0.46 mmol) to give the *title product* **13g** (0.10 g, 65%) as a white solid. m.p. 169–172 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 5.01 (2H, s, CH_2), 7.14–7.18 (1H, m, Ar–H), 7.36 (2H, d, J = 7.8 Hz, Ar–H), 7.60 (2H, d, J = 7.8 Hz, Ar–H), 8.07 (1H, d, J = 2.5 Hz, H-4″), 8.15 (1H, s, NH), 8.45 (1H, d, J = 2.5 Hz, H-6″); ^{13}C NMR (100 MHz; $CDCl_3$) 66.0 (C-2), 98.7 (C-3″), 112.7 (C-5″), 113.3 (CN), 120.0 (Ar–CH), 125.1 (Ar–CH), 129.2 (Ar–CH), 136.7 (Ar–C), 144.8 (C-4″), 152.5 (C-6″), 160.8 (C-2″), 164.5 (C-1); IR: υ_{max} (film)/cm^{-1}: 3274, 3146, 3108, 3065, 2235, 1679, 1554, 1448, 1152, 1056; m/z (ESI+): 355 (^{81}Br MNa$^+$, 96%), 353 (^{79}BrMNa$^+$, 100%); HRMS (ESI$^+$) found (^{81}Br MNa$^+$): 355.9825, $C_{14}H_{10}N_3O_2^{81}BrNa$ requires 355.9829. Found (^{79}Br MNa$^+$): 353.9842, $C_{14}H_{10}N_3O_2^{79}BrNa$ requires 353.9849.

4.3.1.17. 2-(5″-Bromo-3″-cyanopyridin-2″-yloxy)-N-(4′-methoxyphenyl)acetamide ***13h***. The reaction was carried out according to general procedure A using acetamide **11b** (0.09 g, 0.49 mmol) and

pyridine **7c** (0.10 g, 0.46 mmol) to give the *title product* **13h** (0.08 g, 47%) as a purple solid. m.p. 150–153 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 3.80 (3H, s, OCH_3), 5.00 (2H, s, CH_2), 6.89 (2H, d, J = 9.0 Hz, H-3′ and H-5′), 7.50 (2H, d, J = 9.0 Hz, H-2′ and H-6′), 8.06–8.10 (2H, m, H-4″ and NH), 8.45 (1H, d, J = 2.4 Hz, H-6″); ^{13}C NMR (100 MHz; $CDCl_3$) 55.5 (OCH_3), 66.0 (C-2), 98.7 (C-3″), 112.6 (C-5″), 113.4 (CN), 114.3 (C-3′ and C-5′), 121.9 (C-2′ and C-6′), 129.8 (C-1′), 144.8 (C-4″), 152.5 (C-6″), 156.9 (C-4′), 160.8 (C-2″), 164.4 (C-1); IR: υ_{max} (film)/cm^{-1}: 3332, 3062, 2995, 2936, 2230, 1668, 1553, 1453, 1304, 1041; *m/z* (ESI+): 385 (^{81}Br MNa$^+$, 100%), 383 (^{79}Br MNa$^+$, 96); HRMS (ESI$^+$) found: (^{81}Br MNa$^+$) 385.9931, $C_{15}H_{12}N_3O_3^{81}BrNa$ requires 385.9934. Found (^{79}BrMNa$^+$): 383.9949, $C_{15}H_{12}N_3O_3^{79}BrNa$ requires 383.9954.

*4.3.1.18. 2-(5″-Bromo-3″-cyanopyridin-2″-yloxy)-N-(3′-chlorophenyl)acetamide **13i**.* The reaction was carried out according to general procedure A using acetamide **11c** (0.09 g, 0.49 mmol) and pyridine **7c** (0.10 g, 0.46 mmol) to give the *title product* **8i** (0.07 g, 39%) as a white solid. m.p. 146–148 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 5.01 (2H, s, CH_2), 7.14 (1H, d, J = 8.0 Hz, H-4′), 7.25–7.29 (1H, m, H-5′), 7.40 (1H, d, J = 8.0 Hz, H-6′), 7.76 (1H, s, H-2′), 8.08 (1H, d, J = 2.4 Hz, H-4″), 8.18 (1H, s, NH), 8.45 (1H, d, J = 2.4 Hz, H-6″); ^{13}C NMR (100 MHz; $CDCl_3$) 66.0 (C-2), 98.7 (C-3″), 112.8 (C-5″), 113.3 (CN), 118.0 (C-6′), 120.1 (C-2′), 125.1 (C-4′), 130.1 (C-5′), 134.9 (C-3′), 137.8 (C-1′), 144.9 (C-4″), 152.5 (C-6″), 160.7 (C-2″), 164.7 (C-1); IR: υ_{max} (film)/cm^{-1}: 3313, 3066, 2937, 2229, 1675, 1592, 1452, 1423, 1297, 1150; *m/z* (ESI+): 391 (^{81}Br^{37}ClMNa$^+$, 24%), 389 (^{81}Br^{35}ClMNa$^+$ and ^{79}Br^{37}ClMNa$^+$, 100%), 387 (^{79}Br^{35}ClMNa$^+$, 69%); HRMS (ESI$^+$) found (^{81}Br^{37}Cl MNa$^+$): 391.9400, $C_{14}H_9N_3O_2^{81}Br^{37}ClNa$ requires 391.9409. Found (^{79}Br^{35}ClMNa): 387.9458, $C_{14}H_9N_3O_2^{79}Br^{35}ClNa^+$ requires 387.9459.

*4.3.2. General procedure B: synthesis of the furopyridine-2-carboxamides **10** from cyanopyridines **13***

A mixture of cyanopyridine **13** (1.0 mmol) and potassium tert-butoxide (1.2 mmol) in THF (10 mL) was heated at 80 °C for 3 h, under an atmosphere of nitrogen. The mixture was cooled and the solvent removed *in vacuo*. The crude solid was washed with small amounts of water and dried under vacuum to give the furopyridine-2-carboxamide **10**.

*4.3.2.1. 3-Amino-N-phenylfuro[2,3-b]pyridine-2-carboxamide **10a**.* The reaction was carried out according to general procedure B using 2-(3-cyanopyridin-2-yloxy)-*N*-phenylacetamide **13a** (0.1 g, 0.40 mmol) to give the *title product* **10a** (0.10 g, 99%) as a yellow solid. m.p. 197–200 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 6.43 (2H, s, NH_2) 7.05–7.09 (1H, m, Ar–H), 7.32 (2H, d, J = 7.6 Hz, Ar–H), 7.39 (1H, dd, J = 4.7, 7.8 Hz, H-5), 7.84 (2H, d, J = 7.6 Hz, Ar–H), 8.39 (1H, dd, J = 1.7, 7.8 Hz, H-4), 8.47 (1H, dd, J = 1.7, 4.7 Hz, H-6), 9.97 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 114.7 (C-3a) 118.9 (C-5), 120.2 (Ar–CH), 123.2 (Ar–CH), 124.1 (C-2), 128.5 (Ar–CH), 131.2 (C-4), 136.3 (C-3), 138.8 (Ar–C), 147.9 (C-6), 158.8 (C-7a), 159.5 (C=O); IR: υ_{max} (film)/cm^{-1}: 3427, 3310, 2918, 2849, 1640, 1596, 1561, 1403, 1348, 1240; *m/z* (ESI+): 276 (MNa$^+$, 100%). HRMS (ESI$^+$) found (MNa$^+$): 276.0749, $C_{14}H_{11}N_3O_2Na$ requires 276.0743.

*4.3.2.2. 3-Amino-N-(4′-methoxyphenyl)furo[2,3-b]pyridine-2-carboxamide **10b**.* The reaction was carried out according to general procedure B using 2-(3-cyanopyridin-2-yloxy)-*N*-(4-methoxyphenyl)acetamide **13b** (0.10 g, 0.35 mmol) to give the *title product* **10b** (0.08 g, 75%) as a green solid. m.p. 145–148 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 3.75 (3H, s, OCH_3), 6.37 (2H, s, NH_2) 6.90 (2H, d, J = 8.9 Hz, H-3′ and H-5′), 7.39 (1H, dd, J = 4.8, 7.5 Hz, H-5), 7.74 (2H, d, J = 8.9 Hz, H-2′ and H-6′), 8.38 (1H, d, J = 1.7, 7.5 Hz, H-4), 8.46 (1H, d, J = 1.7, 4.8 Hz, H-6), 9.88 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 55.1 (OCH_3), 113.6 (C-3′ and C-5′), 114.8 (C-3a) 118.9 (C-5), 121.8 (C-2′ and C-6′), 124.3 (C-2), 131.1 (C-4), 131.9 (C-1′), 135.8 (C-3), 147.7 (C-6), 155.3 (C-4′), 158.8 (C-7a), 159.2 (C=O); IR: υ_{max} (film)/cm^{-1}: 3450, 3217, 3054, 2923, 2839, 1634, 1595, 1406, 1349, 1234; *m/z* (ESI+): 306 (MNa$^+$, 100%). HRMS (ESI$^+$) found (MNa$^+$): 306.0850, $C_{15}H_{13}N_3O_3Na$ requires 306.0849.

*4.3.2.3. 3-Amino-N-(3′-chlorophenyl)furo[2,3-b]pyridine-2-carboxamide **10c**.* The reaction was carried out according to general procedure B using *N*-(3-chlorophenyl)-2-(3-cyanopyridin-2-yloxy) acetamide **13c** (0.10 g, 0.35 mmol) to give the *title product* **10c** (0.06 g, 57%) as a yellow solid. m.p. 230–232 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 6.53 (2H, s, NH_2), 7.11 (1H, d, J = 8.0 Hz, H-4′), 7.32–7.36 (1H, m, H-5′), 7.40 (1H, dd, J = 4.8, 7.8 Hz, H-5), 7.79 (1H, d, J = 8.0 Hz, H-6′), 8.08 (1H, s, H-2′), 8.41 (1H, dd, J = 1.6, 7.8 Hz, H-4), 8.48 (1H, dd, J = 1.6, 4.8 Hz, H-6), 10.19 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 114.6 (C-3a), 118.4 (C-6′), 119.0 (C-5), 119.5 (C-2′), 122.7 (C-4′), 123.7 (C-2), 130.1 (C-5′), 131.4 (C-4), 132.8 (C-3′), 136.9 (C-3), 140.5 (C-1′), 148.2 (C-6), 158.9 (C-7a), 159.6 (C=O); IR: υ_{max} (film)/cm^{-1}: 3440, 3276, 3173, 3069, 1671, 1590, 1535, 1408, 1346; *m/z* (ESI+): 312 (^{37}ClMNa$^+$, 32%), 310 (^{35}ClMNa$^+$, 100%); HRMS (ESI$^+$) found (^{37}Cl MNa$^+$): 312.0326, $C_{14}H_{10}N_3O_2^{37}ClNa$ requires 312.0322. Found (^{35}Cl MNa$^+$): 310.0353, $C_{14}H_{10}N_3O_2^{35}ClNa$ requires 310.0354.

*4.3.2.4. 3-Amino-5-chloro-N-phenylfuro[2,3-b]pyridine-2-carboxamide **10d**.* The reaction was carried out according to general procedure B using 2-(5-chloro-3-cyanopyridin-2-yloxy)-*N*-phenylacetamide **13d** (0.10 g, 0.35 mmol) to give the *title product* **10d** (0.074 g, 74%) as a yellow solid. m.p. 229–230 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 6.43 (2H, s, NH_2), 7.06–7.09 (1H, m, Ar–H), 7.33 (2H, t, J = 7.4 Hz, Ar–H), 7.82 (2H, d, J = 7.4 Hz, Ar–H), 8.51–8.54 (2H, m, H-4 and H-6), 10.04 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 115.9 (C-3a), 120.3 (Ar–CH), 123.4 (Ar–CH), 125.5 (C-2), 125.6 (C-5), 128.5 (Ar–CH), 130.4 (C-4), 135.4 (C-3), 138.6 (Ar–C), 146.0 (C-6), 157.0 (C-7a), 159.2 (C=O); IR: υ_{max} (film)/cm^{-1}: 3430, 3184, 3060, 2849, 1640, 1594, 1532, 1443, 1269; *m/z* (ESI+): 312 (^{37}Cl MNa$^+$, 29%), 310 (^{35}Cl MNa$^+$, 100); HRMS (ESI$^+$) found (^{37}Cl MNa$^+$): 312.0319, $C_{14}H_{10}N_3O_2^{37}ClNa$ requires 312.0325. Found (^{35}Cl MNa$^+$): 310.0348, $C_{14}H_{10}N_3O_2^{35}ClNa$ requires 310.0354.

*4.4. Synthesis of thieneopyrimidinones **8** and furopyrimidinones **14***

*4.4.1. General procedure C: synthesis of the thieneopyrimidinones **8** and formimidates **15** from amines **4** and **10***

A mixture of thieno- **4** or furo-pyridine **10** (1 mmol) and triethyl orthoformate (10 mmol) were heated at the stated temperature. Acetic acid (9 mmol) was then added dropwise over 5 min. The mixture was then cooled to room temperature and the crude solid product collected by filtration. Recrystallization from methanol gave thieneopyrimidinones **8** or formimidates **15**.

*4.4.1.1. 3-Phenylpyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one **8a**.* The reaction was carried out according to general procedure C using carboxamide **4a** (0.20 g, 0.74 mmol) with heating at 130 °C to give the *title product* **15a** (0.14 g, 65%) as a white solid. m.p. 245–246 °C. ^{1}H NMR (400 MHz; $CDCl_3$) 7.46–7.61 (6H, m, 5 × Ar–H and H-8), 8.29 (1H, s, H-2), 8.59 (1H, dd, J = 1.6, 8.1 Hz, H-9), 8.82 (1H, dd, J = 1.6, 4.6 Hz, H-7); ^{13}C NMR (100 MHz; $CDCl_3$) 120.5 (C-8), 124.1(C-4a), 127.0 (Ar–CH), 128.4 (C-9a), 129.6 (Ar–CH), 129.8 (Ar–CH), 131.7 (C-9), 136.8 (Ar–C), 148.2 (C-2), 150.2 (C-9b), 151.2 (C-7), 157.1 (C-4), 163.1 (C-5a). The ^{1}H NMR data was in agreement with the literature values [33].

*4.4.1.2. 3-(4′-Methoxyphenyl)pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one **8b**.* The reaction was carried out according to

general procedure C using carboxamide **4b** (0.20 g, 0.67 mmol) with heating at 130 °C for 1 h to give the *title product* **8b** (0.08 g, 40%) as a white solid. m.p. 238–239 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 3.89 (3H, s, OMe) 7.07 (2H, d, J = 8.8 Hz, H-3′ and H-5′), 7.38 (2H, d, J = 8.8 Hz, H-2′ and H-6′), 7.50–7.53 (1H, dd, J = 4.3, 8.0 Hz, H-8), 8.27 (1H, s, H-2), 8.59 (1H, dd, J = 1.3, 8.0 Hz, H-9), 8.82 (1H, dd, J = 1.3, 4.3 Hz, H-7); ^{13}C NMR (100 MHz; $CDCl_3$) 55.7 (OCH_3), 115.0 (C-3′ and C-5′), 120.5 (C-8), 124.0 (C-4a), 128.2 (C-2′ and C-6′), 128.4 (C-9a), 129.5 (C-1′), 131.7 (C-9), 148.5 (C-2), 150.1 (C-9b), 151.1 (C-7), 157.4 (C-4), 160.3 (C-4′), 163.1 (C-5a); IR: υ_{max} (film)/cm^{-1}: 3051, 2985, 2848, 1680, 1585, 1514, 1387, 1239, 1015; m/z (ESI+): 332 (MNa^+, 100%). HRMS (ESI^+) found (MNa^+): 332.0467, $C_{16}H_{11}N_3O_2SNa^+$ requires 332.0464.

*4.4.1.3. 3-(3′-Chlorophenyl)pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one **8c**.* The reaction was carried out according to general procedure C using carboxamide **4c** (0.10 g, 0.33 mmol) with heating at 150 °C for 2 h to give the *title product* **8c** (0.07 g, 72%) as a white solid. m.p. 228–229 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 7.37–7.41 (1H, m, ArH) 7.50–7.55 (4H, m, ArH and H-8), 8.25 (1H, s, H-2), 8.60 (1H, dd, J = 1.6, 8.0 Hz, H-9) 8.83 (1H, dd, J = 1.6, 5.0 Hz, H-7); ^{13}C NMR (100 MHz; $CDCl_3$) 120.6 (C-8), 124.0 (C-4a), 125.3 (C-4′), 127.5 (C-5′), 128.3 (C-9a), 129.9 (C-2′), 130.7 (C-6′), 131.8 (C-9), 135.4 (C-3′), 137.8 (C-1′), 147.7 (C-2), 150.2 (C-9b), 151.3 (C-7), 156.8 (C-4), 163.1 (C-5a); IR: υ_{max} (film)/cm^{-1}: 3060, 1688, 1582, 1541, 1471, 1218, 1380, 1068; m/z (ESI+): 337 (^{37}Cl MNa^+, 32%), 335 (^{35}Cl MNa^+, 100); HRMS (ESI^+) found (^{37}Cl MNa^+): 337.9941, $C_{15}H_8N_3OS^{37}ClNa$ requires 337.9940. Found (^{35}Cl MNa^+): 335.9972, $C_{15}H_8N_3OS^{35}ClNa$ requires 335.9969.

*4.4.1.4. 8-Chloro-3-phenylpyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one **8d**.* The reaction was carried out according to general procedure C using carboxamide **4d** (0.02 g, 0.07 mmol) with heating at 130 °C for 6 h to give the *title product* **8d** (0.01 g, 24%) as a white solid. m.p. 255–258 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 7.46–7.48 (2H, m, H-3′ and H-5′), 7.55–7.62 (3H, m, H-4′, H-2′ and H-6′), 8.28 (1H, s, H-2), 8.57 (1H, d, J = 2.5 Hz, H-9), 8.76 (1H, d, J = 2.5 Hz, H-7); IR: υ_{max} (film)/cm^{-1}: 3059, 3015, 1668, 1560, 1526, 1488, 1453, 1233, 1214, 1096; m/z (ESI+): 337 (^{37}Cl MNa^+, 33%), 335 (^{35}Cl MNa^+, 100); HRMS (ESI^+) found (^{37}Cl MNa^+): 337.9947, $C_{15}H_8N_3OS^{37}ClNa$ requires 337.9940. Found (^{35}Cl MNa^+): 335.9978, $C_{15}H_8N_3OS^{35}ClNa$ requires 335.9969.

*4.4.1.5. 8-Chloro-3-(4′-methoxyphenyl)pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one **8e**.* The reaction was carried out according to general procedure C using carboxamide **4e** (0.10 g, 0.30 mmol) with heating at 130 °C for 4 h to give the *title product* 8**e** (0.10 g, 97%) as a white solid. m.p. 287–288 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 3.89 (3H, s, OMe), 7.07 (2H, d, J = 8.9 Hz, H-3′ and H-5′), 7.37 (2H, d, J = 8.9 Hz, H-2′ and H-6′), 8.27 (1H, s, H-2), 8.56 (1H, d, J = 2.4 Hz, H-9), 8.75 (1H, d, J = 2.4 Hz, H-7); IR: υ_{max} (film)/cm^{-1}: 3051, 3017, 2923, 2837, 1671, 1571, 1532, 1507, 1377, 1243, 1102; m/z (ESI+): 368 (^{37}Cl MNa^+, 36%), 366 (^{35}Cl MNa^+, 100%); HRMS (ESI^+) found (^{37}Cl MNa^+): 368.0056, $C_{16}H_{10}N_3O_2S^{37}ClNa$ requires 368.0046. Found (^{35}Cl MNa^+): 366.0083, $C_{16}H_{10}N_3O_2S^{35}ClNa$ requires 366.0074.

*4.4.1.6. 8-Chloro-3-(3′-chlorophenyl)pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one **8f**.* The reaction was carried out according to general procedure C using carboxamide **4f** (0.10 g, 0.30 mmol) with heating at 130 °C for 4 h to give the *title product* **8f** (0.10 g, 99%) as a white solid. m.p. 268–270 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 7.37–7.39 (1H, m, ArH), 7.51–7.54 (3H, m, ArH), 8.25 (1H, s, H-2), 8.57 (1H, d, J = 2.4 Hz, H-9), 8.77 (1H, d, J = 2.4 Hz, H-7); IR: υ_{max} (film)/cm^{-1}: 3673, 3077, 2978, 2901, 1669, 1561, 1525, 1212, 1096, 1055; m/z (ESI+): 373 ($^{37}Cl_2$ MNa^+, 13%), 371 (^{35}Cl^{37}Cl MNa^+, 67%), 369 ($^{35}Cl_2$ MNa^+, 100%); HRMS (ESI^+) found ($^{37}Cl_2$ MNa^+): 373.9513, $C_{15}H_7N_3OS^{37}Cl_2Na$ requires 373.9520. Found (^{35}Cl^{37}ClMNa^+): 371.9542, $C_{15}H_7N_3OS^{35}Cl^{37}ClNa$ requires 371.9550. Found ($^{35}Cl_2$ MNa^+): 369.9572, $C_{15}H_7N_3OS^{35}Cl_2Na$ requires 369.9579.

*4.4.1.7. 8-Bromo-3-phenylpyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one **8g**.* The reaction was carried out according to general procedure C using carboxamide **4g** (0.15 g, 0.43 mmol) with heating at 70 °C for 15 h to give the *title product* **8g** (0.03 g, 20%) as a white solid. m.p. 259–260 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 7.46–7.48 (1H, m, H-4′), 7.55–7.7.62 (4H, m, H-2′, H-6′, H-3′ and H-5′), 8.28 (1H, s, H-2), 8.72 (1H, d, J = 2.3 Hz, H-9), 8.85 (1H, d, J = 2.3 Hz, H-7); IR: υ_{max} (film)/cm^{-1}: 3049, 1685, 1568, 1526, 1490, 1455, 1382, 1234; m/z (ESI+): 381 (^{81}Br MNa^+, 93%), 379 (^{79}Br MNa^+, 100%); HRMS (ESI^+) found (^{81}Br MNa^+): 381.9437, $C_{15}H_8N_3OS^{81}BrNa$ requires 381.9443. Found (^{79}Br MNa^+): 379.9459, $C_{15}H_8N_3OS^{79}BrNa$ requires 379.9464.

*4.4.1.8. 8-Bromo-3-(4′-methoxyphenyl)pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one **8h**.* The reaction was carried out according to general procedure C using carboxamide **4h** (0.15 g, 0.40 mmol) with heating at 70 °C for 15 h to give the *title product* **8h** (0.07 g, 43%) as a white solid. m.p. 287–290 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 3.89 (3H, s, OMe), 7.07 (2H, d, J = 9.0 Hz, H-3′ and H-5′), 7.37 (2H, d, J = 9.0 Hz, H-2′ and H-6′), 8.26 (1H, s, H-2), 8.71 (1H, d, J = 2.4 Hz, H-9), 8.84 (1H, d, J = 2.4 Hz, H-7); IR: υ_{max} (film)/cm^{-1}: 3047, 2929, 2836, 1672, 1571, 1507, 1239, 1051; m/z (ESI+): 411 (^{81}Br MNa^+, 92%), 409 (^{79}Br MNa^+, 100%), HRMS (ESI^+) found (^{81}Br MNa^+): 411.9554, $C_{16}H_{10}N_3O_2S^{81}BrNa$ requires 411.9549. Found (^{79}Br MNa^+): 409.9571, $C_{16}H_{10}N_3O_2S^{79}BrNa$ requires 409.9569.

*4.4.1.9. 8-Bromo-3-(3′-chlorophenyl)pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one **8i**.* The reaction was carried out according to general procedure C using carboxamide **4i** (0.15 g, 0.39 mmol) with heating at 70 °C for 15 h to give the *title product* **15i** (0.13 g, 82%) as a white solid. m.p. 287–290 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 7.36–7.39 (1H, m, ArH), 7.50–7.54 (3H, m, ArH), 8.25 (1H, s, H-2), 8.72 (1H, d, J = 2.2 Hz, H-9), 8.85 (1H, d, J = 2.2 Hz, H-7); IR: υ_{max} (film)/cm^{-1}: 3095, 3064, 1683, 1563, 1528, 1423, 1369, 1218; m/z (ESI+): 417 (^{81}Br^{37}Cl MNa^+, 28%), 415 (^{81}Br^{35}Cl MNa^+ and ^{79}Br^{37}Cl MNa^+, 100%), 413 (^{79}Br^{35}Cl MNa^+, 65); HRMS (ESI^+) found (^{81}Br^{37}Cl MNa^+): 417.9023, $C_{15}H_7N_3OS^{81}Br^{37}ClNa$ requires 417.9024. Found (^{79}Br^{35}Cl MNa^+): 413.9074, $C_{15}H_7N_3OS^{79}Br^{35}ClNa$ requires 413.9074.

*4.4.1.10. Ethyl N-(2-(phenylcarbamoyl)furo[2,3-b]pyridin-3-yl)formimidate **15a**.* The reaction was carried out according to general procedure C using carboxamide **10a** (0.10 g, 0.40 mmol) with stirring at room temperature for 1 h to give the *title product* **15a** (0.09 g, 77%) as a white solid. m.p. 238–240 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 1.50 (3H, t, J = 7.1 Hz, CH_3), 4.53 (2H, q, J = 7.1 Hz, OCH_2), 7.13–7.17 (1H, m, Ar–H), 7.34 (1H, dd, J = 4.7, 7.7 Hz, H-5), 7.35–7.39 (2H, m, Ar–H), 7.66–7.68 (2H, m, Ar–H), 8.06 (1H, dd, J = 1.6, 7.7 Hz, H-4), 8.47 (1H, s, NCH), 8.51 (1H, dd, J = 1.6, 4.7 Hz, H-6), 9.28 (1H, s, NH); IR: υ_{max} (film)/cm^{-1}: 3247, 3100, 3069, 2978, 1674, 1619, 1595, 1541, 1443, 1277, 1245, 1149; m/z (ESI+): 332 (MNa^+, 100%); HRMS (ESI^+) found (MNa^+): 332.1002, $C_{17}H_{15}N_3O_3Na^+$ requires 332.1006.

*4.4.1.11. Ethyl N-(2-((4′-methoxyphenyl)carbamoyl)furo[2,3-b]pyridin-3-yl)formimidate **15b**.* The reaction was carried out according to general procedure C using carboxamide **10b** (0.10 g, 0.35 mmol) with stirring at room temperature for 1 h to give the *title product* **15b** (0.63 g, 53%) as a green solid. m.p. 255–257 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 1.49 (3H, t, J = 7.1 Hz, CH_3), 3.81 (3H, s, OMe), 4.50 (2H, q, J = 7.1 Hz, CH_2), 6.90 (2H, d, J = 8.8 Hz, H-3′ and H-5′), 7.31

(1H, dd, J = 4.8, 7.8 Hz, H-5), 7.56 (2H, d, J = 8.8 Hz, H-2′ and H-6′), 8.04 (1H, dd, J = 1.4, 7.8 Hz, H-4), 8.46 (1H, s, NCH′), 8.47 (1H, dd, J = 1.4, 4.8 Hz, H-6), 9.19 (1H, s, NH); IR: υ_{max} (film)/cm^{-1}: 3249, 3071, 2980, 2937, 2838, 1666, 1623, 1545, 1410, 1238, 1148; m/z (ESI+): 362 (MNa^+, 100%). HRMS (ESI^+) found (MNa^+): 362.1106, $C_{18}H_{17}N_3O_4Na$ requires 362.1111.

*4.4.1.12. Ethyl N-(2-((3′-chlorophenyl)carbamoyl)furo[2,3-b]pyridin-3-yl)formimidate **15c**.* The reaction was carried out according to general procedure C using carboxamide **9c** (0.10 g, 0.35 mmol) with heating at 80 °C for 1 h to give the *title product* **16c** (0.07 g, 58%) as a white solid. m.p. 239–241 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 1.52 (3H, t, J = 7.2 Hz, CH_3), 4.54 (2H, q, J = 7.2 Hz, OCH_2), 7.12 (1H, d, J = 8.0 Hz, H-4′), 7.27–7.31 (1H, m, H-5′), 7.34 (1H, dd, J = 4.8, 8.0 Hz, H-5), 7.49 (1H, d, J = 8.0 Hz, H-6′), 7.80 (1H, s, H-2′), 8.07 (1H, dd, J = 1.8, 8.0 Hz, H-4), 8.47 (1H, s, NCH), 8.52 (1H, dd, J = 1.8, 4.8 Hz, H-6), 9.32 (1H, s, NH); IR: υ_{max} (film)/cm^{-1}: 3098, 3070, 2977, 1681, 1622, 1593, 1542, 1478, 1410, 1276, 1242, 1150; m/z (ESI+): 368 ($^{37}ClMNa^+$, 31%), 366 ($^{35}ClMNa^+$, 100%). HRMS (ESI^+) found (^{37}Cl MNa^+): 368.0583, $C_{17}H_{14}N_3O_3^{37}ClNa$ requires 368.0588. Found (^{35}Cl MNa^+): 366.0614, $C_{17}H_{14}N_3O_3^{35}ClNa$ requires 366.0616.

*4.4.2. General procedure D: synthesis of furopyrimidinones **14** from formimidates **15***

A mixture of formimidates **15** (1.0 mmol) and anhydrous sodium carbonate (1.05 mmol) in absolute ethanol (3 mL) was stirred at reflux for 18 h. The mixture was then cooled to room temperature and the solvent removed *in vacuo*. The resultant crude solid was washed with small amounts of water and the remaining solid was recrystallized, using methanol to give the furopyrimidinone **14**.

*4.4.2.1. 3-Phenylpyrido[3′,2′:4,5]furo[3,2-d]pyrimidin-4(3H)-one **14a**.* The reaction was carried out according to general procedure D using formimidate **15a** (0.10 g, 0.32 mmol) to give the *title product* **14a** (0.01 g, 12%) as a white solid. m.p. 240–242 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 7.46–7.62 (6H, m, Ar–H and H-8), 8.25 (1H, s, H-2), 8.48 (1H, dd, J = 1.7, 7.7 Hz, H-4), 8.67 (1H, dd, J = 1.7, 4.7 Hz, H-6); IR: υ_{max} (film)/cm^{-1}: 3060, 2988, 2919, 1700, 1564, 1527, 1490, 1397, 1347, 1185, 1112; m/z (ESI+): 286 (MNa^+, 100%); HRMS (ESI^+) found (MNa^+): 286.0584, $C_{15}H_9N_3O_2Na$ requires 286.0587.

*4.4.2.2. 3-(4-Methoxyphenyl)pyrido[3′,2′:4,5]furo[3,2-d]pyrimidin-4(3H)-one **14b**.* The reaction was carried out according to general procedure D using formimidate **15b** (0.10 g, 0.30 mmol) to give the *title product* **18b** (0.02 g, 20%) as a green solid. m.p. 260–263 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 3.89 (3H, s, OMe), 7.07 (2H, d, J = 8.8 Hz, H-3′ and H-5′), 7.37 (2H, d, J = 8.8 Hz, H-2′ and H-6′), 7.50 (1H, dd, J = 4.9, 7.7 Hz, H-8), 8.24 (1H, s, H-2), 8.47 (1H, dd, J = 1.6, 7.7 Hz, H-9), 8.67 (1H, dd, J = 1.6, 4.9 Hz, H-7); IR: υ_{max} (film)/cm^{-1}: 3064, 3008, 2919, 2847, 1703, 1606, 1564, 1507, 1398, 1302, 1234, 1110; m/z (ESI+): 316 (MNa^+, 100%); HRMS (ESI^+) found (MNa^+): 316.0687, $C_{16}H_{11}N_3O_3Na$ requires 316.0693.

*4.5. Synthesis of the triazinones **9***

*4.5.1. General procedure E: synthesis of the triazinones **9** from thienopyridine-2-carboxamides **4***

To a solution of thienopyridine-2-carboxamides **4** (1.0 mmol) in acetic acid (2 mL) at 0 °C, was added a solution of sodium nitrite (10 mmol) in water (3 mL) dropwise over 15 min. After 2 h the resultant solid was collected by filtration and recrystallized from ethanol to give triazinones **9**.

*4.5.1.1. 3-Phenylpyrido[3′,2′:4,5]thieno[3,2-d][1,2,3]triazin-4(3H)-one **9a**.* The reaction was carried out according to general procedure E using carboxamide **4a** (0.10 g, 0.37 mmol) to give the *title product* **19a** (0.08 g, 78%) as a white solid. m.p. 210–212 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 7.52–7.71 (6H, m, Ar–H and H-8), 8.84 (1H, d, J = 8.0 Hz, H-9), 8.90 (1H, d, J = 8.0 Hz, H-7); ^{13}C NMR (100 MHz; $CDCl_3$) 121.6 (C-8), 126.1 (Ar–CH), 126.9 (C-4a), 128.8 (C-9a) 129.2 (Ar–CH), 129.6 (Ar–CH), 132.2 (C-9), 138.3 (Ar–C), 146.3 (C-9b), 151.9 (C-7), 153.1 (C-5a), 162.8 (C-4); IR: υ_{max} (film)/cm^{-1}: 3052, 1680, 1585, 1512, 1368, 1269, 1041; m/z (ESI+): 303 (MNa^+, 100%); HRMS (ESI^+) found (MNa^+): 303.0309, $C_{14}H_8N_4OSNa$ requires 303.0311.

*4.5.1.2. 3-(4′-Methoxyphenyl)pyrido[3′,2′:4,5]thieno[3,2-d][1,2,3]triazin-4(3H)-one **9b**.* The reaction was carried out according to general procedure E using carboxamide **4b** (0.10 g, 0.33 mmol) to give the *title product* **19b** (0.10 g, 99%) as a white solid. m.p. 238–240 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 3.90 (3H, s, OMe) 7.08 (2H, d, J = 8.9 Hz, H-3′ and H-5′), 7.61 (2H, d, J = 8.9 Hz, H-2′ and H-6′), 7.65 (1H, dd, J = 4.7, 8.0 Hz, H-8), 8.84 (1H, dd, J = 1.6, 8.0 Hz, H-9), 8.89 (1H, dd, J = 1.6, 4.7 Hz, H-7); IR: υ_{max} (film)/cm^{-1}: 3075, 3009, 2969, 2940, 2833, 1673, 1588, 1503, 1365, 1241, 1174; m/z (ESI+): 333 (MNa^+, 100%); HRMS (ESI^+) found (MNa^+): 333.0416, $C_{15}H_{10}N_4O_2SNa$ requires 333.0417.

*4.5.1.3. 3-(3′-Chlorophenyl)pyrido[3′,2′:4,5]thieno[3,2-d][1,2,3]triazin-4(3H)-one **9c**.* The reaction was carried out according to general procedure E using carboxamide **4c** (0.10 g, 0.33 mmol) to give the *title product* **9c** (0.10 g, 98%) as a white solid; m.p. 273–274 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 7.52–7.53 (2H, m, H-4′ and H-5′), 7.64–7.68 (2H, m, H-5 and H-6′), 7.75 (1H, s, H-2′), 8.85 (1H, d, J = 7.5 Hz, H-9), 8.92 (1H, d, J = 7.5 Hz, H-7); IR: υ_{max} (film)/cm^{-1}: 3089, 3055, 1687, 1587, 1511, 1472, 1370, 1044; m/z (ESI+): 338 (^{37}Cl MNa^+, 33%), 336 (^{35}Cl MNa^+, 100); HRMS (ESI^+): Found (^{37}Cl MNa^+) 338.9894, $C_{14}H_9N_4OS^{37}ClNa^+$ requires 338.9892. Found (^{35}Cl MNa^+) 336.9915, $C_{14}H_9N_4OS^{35}ClNa$ requires 336.9921.

*4.5.1.4. 8-Chloro-3-phenylpyrido[3′,2′:4,5]thieno[3,2-d][1,2,3]triazin-4(3H)-one **9d**.* The reaction was carried out according to general procedure E using carboxamide **4d** (0.02 g, 0.07 mmol) to give the *title product* **9d** (0.01 g, 43%) as a white solid; m.p. 219–222 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 7.53–7.62 (3H, m, H-3′, H-4′ and H-5′), 7.68–7.70 (2H, m, H-2′ and H-6′), 8.83–8.86 (2H, m, H-7 and H-9); IR: υ_{max} (film)/cm^{-1}: 3671, 3333, 2987, 2903, 1684, 1406, 1393, 1252, 1056, 864; m/z (ESI+): 338 (^{37}Cl MNa^+, 33%), 336 (^{35}Cl MNa^+, 100%); HRMS (ESI^+) found (^{37}Cl MNa^+): 338.9891, $C_{14}H_7N_4OS^{37}ClNa$ requires 338.9892. Found (^{35}Cl MNa^+): 336.9921, $C_{14}H_7N_4OS^{35}ClNa$ requires 336.9921.

*4.5.1.5. 8-Chloro-3-(4′-methoxyphenyl)pyrido[3′,2′:4,5]thieno[3,2-d][1,2,3]triazin-4(3H)-one **9e**.* The reaction was carried out according to general procedure E using carboxamide **4e** (0.15 g, 0.45 mmol) to give the *title product* **9e** (0.16 g, 99%) as a green solid. m.p. 289–290 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 3.90 (3H, s, OMe) 7.09 (2H, d, J = 8.9 Hz, H-3′ and H-5′), 7.61 (2H, d, J = 8.9 Hz, H-2′ and H-6′), 8.80 (1H, d, J = 2.4 Hz, H-9), 8.83 (1H, d, J = 2.4 Hz, H-7); IR: υ_{max} (film)/cm^{-1}: 3396, 3072, 2991, 2837, 1682, 1606, 1505, 1252, 1176, 1108, 1033; m/z (ESI+): 369 ($^{37}ClMNa^+$, 37%), 367 ($^{35}ClMNa^+$, 100); HRMS (ESI^+) found (^{37}Cl MNa^+): 369.0000, $C_{15}H_9N_4O_2S^{37}ClNa$ requires 368.9998. Found (^{35}Cl MNa^+): 367.0026, $C_{15}H_9N_4O_2S^{35}ClNa^+$ requires 367.0027.

*4.5.1.6. 8-Chloro-3-(3′-chlorophenyl)pyrido[3′,2′:4,5]thieno[3,2-d][1,2,3]triazin-4(3H)-one **9f**.* The reaction was carried out according to general procedure E using carboxamide **4f** (0.10 g, 0.29 mmol) to give the *title product* **9f** (0.10 g, 99%) as a white solid. m.p. 258–260 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 7.52–7.54 (2H, m, ArH),

7.61–7.64 (1H, m, ArH), 7.74–7.75 (1H, m, ArH), 8.81 (1H, d, $J = 2.4$ Hz, H-9), 8.85 (1H, d, $J = 2.4$ Hz, H-7); IR: υ_{max} (film)/cm^{-1}: 3335, 3053, 1689, 1582, 1505, 1331, 1253, 1107, 1044; *m/z* (ESI+): 374 ($^{37}Cl_2$ MNa^+, 18%), 372 ($^{35}Cl^{37}Cl$ MNa^+, 65%), 370 ($^{35}Cl_2$ MNa^+, 100%); HRMS (ESI$^+$) found ($^{37}Cl_2$ MNa^+): 374.9446, $C_{14}H_6N_4OS^{37}Cl_2Na$ requires 374.9473. Found ($^{35}Cl_2$ MNa^+): 370.9522, $C_{14}H_6N_4OS^{35}Cl_2Na$ requires 370.9532.

4.5.1.7. 8-Bromo-3-phenylpyrido[3′,2′:4,5]thieno[3,2-d][1,2,3]triazin-4(3H)-one ***9g***. The reaction was carried out according to general procedure E using carboxamide **4g** (0.06 g, 0.17 mmol) to give the *title product* **9g** (0.03 g, 50%) as a white solid. m.p. 218–220 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 7.53–7.62 (3H, m, Ar–H), 7.67–7.70 (2H, m, Ar–H), 8.93 (1H, d, $J = 2.2$ Hz, H-9), 8.97 (1H, d, $J = 2.2$ Hz, H-7); IR: υ_{max} (film)/cm^{-1}: 3048, 2029, 2850, 1679, 1502, 1486, 1391, 1327, 1250, 1097, 1040; *m/z* (ESI+): 382 (^{81}Br MNa^+, 96%), 380 ($^{79}BrMNa^+$, 100%); HRMS (ESI$^+$) found (^{81}Br MNa^+) 382.9389, $C_{14}H_7N_4OS^{81}BrNa$ requires 382.9396. Found (^{79}Br MNa^+): 380.9410, $C_{14}H_7N_4OS^{79}BrNa$ requires 380.9416.

4.5.1.8. 8-Bromo-3-(4′-methoxyphenyl)pyrido[3′,2′:4,5]thieno[3,2-d][1,2,3]triazin-4(3H)-one ***9h***. The reaction was carried out according to general procedure E using carboxamide **4h** (0.10 g, 0.26 mmol), acetic acid (0.52 mL, 9.10 mmol) and sodium nitrite (0.50 g, 7.25 mmol) in water (2 mL) to give the *title product* **9h** (0.10 g, 99%) as a green solid. m.p. 281–282 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 3.90 (3H, s, OMe), 7.09 (2H, d, $J = 7.8$ Hz, H-3′ and H-5′), 7.61 (2H, d, $J = 7.8$ Hz, H-2′ and H-6′), 8.92 (1H, d, $J = 2.4$ Hz, H-9), 8.96 (1H, d, $J = 2.4$ Hz, H-7); IR: υ_{max} (film)/cm^{-1}: 3670, 3073, 2979, 2906, 2835, 1682, 1606, 1505, 1254, 1177, 1035; *m/z* (ESI+): 412 (^{81}Br MNa^+, 93%), 410 (^{79}Br MNa^+, 100%); HRMS (ESI$^+$) found (^{81}Br MNa^+): 412.9500, $C_{15}H_9N_4O_2S^{81}BrNa^+$ requires 412.9502. Found (^{79}Br MNa^+): 410.9515, $C_{15}H_9N_4O_2S^{79}BrNa$ requires 410.9522.

4.5.1.9. 8-Bromo-3-(3′-chlorophenyl)pyrido[3′,2′:4,5]thieno[3,2-d][1,2,3]triazin-4(3H)-one ***9i***. The reaction was carried out according to general procedure E using carboxamide **4i** (0.10 g, 0.26 mmol), acetic acid (0.52 mL, 9.14 mmol) and sodium nitrite (0.50 g, 7.25 mmol) in water (2 mL) to give the *title product* **9i** (0.08 g, 82%) as a white solid. m.p. 292–293 °C; ^{1}H NMR (400 MHz; $CDCl_3$) 7.53–7.63 (3H, m, H-4′, H-5′ and H-6′), 7.74 (1H, s, H-2′), 8.94 (1H, d, $J = 2.2$ Hz, H-9), 8.97 (1H, d, $J = 2.2$ Hz, H-7); IR: υ_{max} (film)/cm^{-1}: 3671, 3099, 3054, 2978, 2902, 1689, 1579, 1503, 1412, 1252, 1097, 1048; *m/z* (ESI+): 418 ($^{81}Br^{37}Cl$ MNa^+, 29%), 416 ($^{81}Br^{35}Cl$ MNa^+ and $^{79}Br^{37}ClMNa^+$, 100%), 414 ($^{79}Br^{35}Cl$ MNa^+, 78); HRMS (ESI$^+$) found ($^{81}Br^{37}Cl$ MNa^+): 418.8983, $C_{14}H_6N_4OS^{81}Br^{37}ClNa$ requires 418.8988. Found ($^{79}Br^{35}Cl$ MNa^+): 414.9037, $C_{14}H_6N_4OS^{79}Br^{35}ClNa$ requires 414.9037.

4.6. Synthesis of the tetrahydrothieno[2,3-b]quinoline-2-carboxamides ***17*** *and* ***23***

4.6.1. General procedure F: formation of the N-aryl bromoacetamides ***21a–i***

To a solution of substituted aniline **22a–i** (0.81 mmol) and triethylamine (0.89 mmol) in DCM (2 mL) at 0 °C was added bromoacetyl bromide (0.81 mmol) dropwise over 15 min, and stirring was continued for an additional 1 h at 0 °C. The mixture was diluted with DCM (10 mL), washed with HCl (2 × 10 mL), H_2O (1 × 10 mL), sat. aqueous $NaHCO_3$ (1 × 10 mL), brine (1 × 10 mL), dried (Na_2SO_4), and the solvent removed *in vacuo* to give bromoacetamides **21a–i**.

4.6.1.1. 2-Bromo-N-phenylacetamide ***21a***. The reaction was carried out in accordance with general procedure F using aniline **22a** (0.10 mL, 1.07 mmol) to give the *title product* **21a** (0.12 g, 51%) as a white solid. m.p. 135–137 °C. lit. m.p. 130–131 [34]. ^{1}H NMR (400 MHz; $CDCl_3$) 4.03 (2H, s, H-2), 7.15–7.19 (1H, m, Ar–H), 7.36 (2H, d, $J = 8.0$ Hz, Ar–H), 7.53 (2H, d, $J = 8.0$ Hz, Ar–H), 8.10 (1H, s, NH). The ^{1}H NMR data was in agreement with the literature values [35].

4.6.1.2. 2-Bromo-N-(4′-methoxyphenyl)acetamide ***21b***. The reaction was carried out in accordance with general procedure F using *p*-anisidine **22b** (0.09 mL, 0.81 mmol) with the brown crude product being further purified by trituration (DCM) to give the *title product* **21b** (0.12 g, 52%) as a white solid. m.p. 129–132 °C. lit. m.p. 131–132 °C [36], ^{1}H NMR (400 MHz; $CDCl_3$) 3.80 (3H, s, OCH_3), 4.02 (2H, s, H-2), 6.89 (2H, d, $J = 9.0$ Hz, H-3′ and H-5′), 7.42 (2H, d, $J = 9.0$ Hz, H-2′ and H-6′), 8.05 (1H, s, NH). The ^{1}H NMR data was in agreement with the literature values [36].

4.6.1.3. 2-Bromo-N-(3′-chlorophenyl)acetamide ***21c***. The reaction was carried out in accordance with general procedure F using 3-chloroaniline **22c** (0.10 g, 0.78 mmol) to give the *title product* **21c** (0.20 g, 100%) as a light orange solid. m.p. 80–83 °C. lit. m.p. 80–81 °C [36]. ^{1}H NMR (400 MHz; $CDCl_3$) 4.02 (2H, s, H-2), 7.15 (1H, d, $J = 8.0$ Hz, H-4′), 7.26–7.30 (1H, m, H-5′), 7.39 (1H, d, $J = 8.0$ Hz, H-6′), 7.65 (1H, s, H-2′), 8.12 (1H, s, NH). The ^{1}H NMR data was in agreement with the literature values [36].

4.6.1.4. 2-Bromo-N-(3′-methoxyphenyl)acetamide ***21d***. The reaction was carried out in accordance with general procedure F using 3-methoxyaniline **22d** (0.09 mL) to give the *title product* **21d** (0.15 g, 78%) as a brown solid. m.p. 87–89 °C. lit. m.p. 98.5–99.5 °C [37]. ^{1}H NMR (400 MHz; $CDCl_3$) 3.81 (3H, s, OCH_3), 4.01 (2H, s, H-2), 6.72 (1H, d, $J = 8.1$ Hz, H-4′), 7.01 (1H, d, $J = 8.1$ Hz, H-6′), 7.22–7.26 (2H, m, H-2′ and H-5′), 8.12 (1H, s, NH). ^{13}C NMR (100 MHz; $CDCl_3$) 29.5 (C-2), 55.4 (OCH_3), 105.8 (C-2′), 111.0 (C-4′), 112.2 (C-6′), 129.8 (C-5′), 138.1 (C-1′), 160.2 (C-3′), 163.4 (C=O). The ^{1}H NMR data was in agreement with the literature values [37].

4.6.1.5. 2-Bromo-N-(3′-bromophenyl)acetamide ***21e***. The reaction was carried out in accordance with general procedure F using 3-bromoaniline **4e** (0.40 g, 2.33 mmol) to give the *title product* **21e** (0.68 g, 99%) as a yellow solid. m.p. 97–100 °C. ^{1}H NMR (400 MHz; $CDCl_3$) 4.02 (2H, s, H-2), 7.22 (1H, d, $J = 8.0$ Hz, H-4′), 7.29–7.31 (1H, m, H-5′), 7.45 (1H, d, $J = 8.0$ Hz, H-6′), 7.78 (1H, s, H-2′), 8.13 (1H, s, NH); ^{13}C NMR (100 MHz; $CDCl_3$) 29.3 (C-2), 118.5 (C-6′), 122.8 (C-3′), 123.0 (C-2′), 128.3 (C-4′), 130.4 (C-5′), 138.2 (C-1′), 163.4 (C-1); IR: υ_{max} (film)/cm^{-1}: 3249, 3182, 3107, 3070, 1649, 1606, 1589, 1537, 1471, 1421, 1235; *m/z* (ESI+): 317 ($^{81}Br_2$ MNa^+, 50%), 315 ($^{79}Br^{81}Br$ MNa^+, 100%), 313 ($^{79}Br_2$ MNa^+, 55%); HRMS (ESI$^+$) found ($^{81}Br_2$ MNa^+): 317.8751, $C_8H_7NO^{81}Br_2Na$ requires 317.8746. Found ($^{79}Br_2$ MNa^+) 313.8794, $C_8H_7NO^{79}Br_2Na$ requires 313.8787.

4.6.1.6. 2-Bromo-N-(3′-nitrophenyl)acetamide ***21f***. The reaction was carried out in accordance with general procedure F using 3-nitroaniline **4f** (0.10 g, 0.72 mmol) to give the *title product* **21f** (0.18 g, 93%) as a brown solid. m.p. 112–114 °C. lit. m.p. 110–112 [38]. ^{1}H NMR (400 MHz; $(CD_3)_2CO$) 4.11 (2H, s, H-2), 7.62–7.67 (1H, m, H-5′), 7.98–8.01 (2H, m, H-4′ and H-6′), 8.72 (1H, s, H-2′), 10.02 (1H, s, NH). The ^{1}H NMR data was in agreement with the literature values [38].

4.6.1.7. 2-Bromo-N-(3′-(trifluoromethyl)phenyl)acetamide ***21g***. The reaction was carried out in accordance with general procedure F using 3-(trifluoromethyl)aniline **4g** (0.40 g, 2.48 mmol) to give the *title product* **21g** (0.70 g, 100%) as a brown solid. m.p. 78–81 °C. lit. m.p. 82 [39]. ^{1}H NMR (400 MHz; $CDCl_3$) 4.04 (2H, s, H-2), 7.41–7.50 (2H, m, H-4′ and H-5′), 7.75 (1H, d, $J = 8.0$ Hz, H-6′), 7.83 (1H, s, H-

2′), 8.28 (1H, s, NH). The ^{1}H data was in agreement with the literature values [40].

*4.6.1.8. 2-Bromo-N-(3′,5′-dimethoxyphenyl)acetamide **21h**.* The reaction was carried out in accordance with general procedure F using 3,5-dimeoxyaniline **4h** (0.40 g, 2.61 mmol) to give the *title product* **21h** (0.70 g, 100%) as an orange solid. m.p. 90–93 °C. ^{1}H NMR (400 MHz; $CDCl_3$) 3.78 (6H, s, OCH_3), 4.00 (2H, s, H-2), 6.28 (1H, t, J = 2.2 Hz, H-4′), 6.76 (2H, d, J = 2.2 Hz, H-2′, and H-6′), 8.08 (1H, s, NH); ^{13}C NMR (100 MHz; $CDCl_3$) 29.5 (C-2), 55.4 (OCH_3), 97.5 (C-4′), 98.4 (C-2′ and C-6′), 138.7 (C-1′), 161.1 (C-3′ and C-5′), 163.7 (C-1); IR: υ_{max} (film)/cm^{-1}: 3267, 3222, 3161, 3099, 2957, 2840, 1670, 1598, 1555, 1418, 1393, 1299, 1200, 1151; *m/z* (ESI+): 297 (^{81}Br MNa$^+$, 91%), 295 (^{79}Br MNa$^+$, 100%); HRMS (ESI$^+$) found (^{81}BrMNa$^+$): 297.9883, $C_{10}H_{12}NO_3^{81}BrNa$ requires 297.9876. Found (^{79}Br MNa$^+$) 295.9900, $C_{10}H_{12}NO_3^{79}BrNa^+$ requires 295.9893.

*4.6.1.9. 2-Bromo-N-(3′,5′-dichlorophenyl)acetamide **21i**.* The reaction was carried out in accordance with general procedure F using 3,5-dichloroaniline **4i** (0.50 g, 3.09 mmol) to give the *title product* **21i** (0.70 g, 100%) as a brown solid. m.p. 90–95 °C. ^{1}H NMR (400 MHz; $CDCl_3$) 3.78 (6H, s, OCH_3), 4.00 (2H, s, H-2), 6.28 (1H, t, J = 2.2 Hz, H-4′), 6.76 (2H, d, J = 2.2 Hz, H-2′, and H-6′), 8.08 (1H, s, NH). ^{13}C NMR (100 MHz; $CDCl_3$) 29.1 (C-2), 118.2 (C-2′ and C-6′), 125.2 (C-4′), 135.4 (C-3′ and C-5′), 138.7 (C-1′), 163.6 (C-1); IR: υ_{max} (film)/cm^{-1}: 3297, 3260, 3183, 3121, 3081, 1666, 1586, 1536, 1437, 1409, 1313, 1113; *m/z* (ESI+): 309 ($^{37}Cl_2^{81}$Br MNa$^+$, 5%), 307 (^{35}Cl^{37}Cl^{81}Br MNa$^+$ and $^{37}Cl_2^{79}$Br MNa$^+$, 42%), 305 ($^{35}Cl_2^{81}$Br MNa$^+$ and ^{35}Cl^{37}Cl^{79}Br MNa$^+$, 100%), 303 ($^{35}Cl_2^{79}$Br MNa$^+$, 62%); HRMS (ESI$^+$) found ($^{37}Cl_2^{81}$Br MNa$^+$): 309.8831, $C_8H_6^{37}Cl_2^{81}BrNONa$ requires 309.8823. Found ($^{35}Cl_2^{79}$Br MNa$^+$): 303.8910, $C_8H_6^{35}Cl_2^{79}BrNONa$ requires 303.8902.

*4.6.1.10. 5-Oxo-2-thioxo-1,2,5,6,7,8-hexahydroquinoline-3-carbonitrile **18**.* A mixture of 1,3-cyclohexanedione **19** (0.50 g, 4.46 mmol) and dimethyl formamide dimethyl acetyl (0.59 mL, 4.46 mmol) in DMF (15 mL) was stirred, under an atmosphere of nitrogen, for 24 h at room temperature to form enamine **20**. Separately, a solution of sodium hydride (0.21 g, 8.92 mmol) and cyanothioacetamide (0.45 g, 4.46 mmol) in DMF (15 mL) was stirred for 10 min under an atmosphere of nitrogen, at room temperature, and then transferred into the enamine **20** mixture and stirred for a further 24 h. The mixture was then acidified to pH 4 using conc. HCl and stirred further for 24 h. The resultant solid was filtered and recrystallized from ethanol to give the *title product* **18** (0.73 g, 82%) as a brown solid. m.p. >350 °C. [lit. [15] m.p. >300 °C]; ^{1}H NMR (400 MHz; d_6-DMSO) 2.02–2.08 (2H, m, H-7), 2.51–2.54 (2H, m, H-6), 2.99–3.02 (2H, m, H-8), 8.24 (1H, s, H-4), 14.4 (1H, s, NH); ^{13}C NMR (100 MHz; DMSO-d_6) 19.9 (C-8), 26.7 (C-9), 36.5 (C-7), 114.8 (CN) 116.2 and 118.2 (C-5 and C-3), 140.1 (C-4), 161.4 (C-2), 180.6 (C-10), 192.5 (C=O). The ^{1}H NMR data was in agreement with the literature values [15].

*4.6.2. General procedure G: synthesis of 5-oxo-tetrahydrothieno [2,3-b]quinoline-2-carboxamides analogues **17a–i***

A mixture of 2-bromoacetamides **21a–i** (0.49 mmol), 5-oxo-2-thioxo-1,2,5,6,7,8-hexahydroquinoline-3-carbonitrile **18** (0.49 mmol) and anhydrous sodium carbonate (0.52 mmol) in absolute ethanol (2 mL) was stirred at reflux for 18 h. The mixture was cooled to room temperature, and the solvent was removed *in vacuo*. The crude product was washed using small amounts of water and the remaining solid, filtered and recrystallized from methanol to give the 5-oxo-tetrahydrothieno[2,3-*b*]quinolines **17a–i**.

*4.6.2.1. 3-Amino-5-oxo-N-phenyl-5,6,7,8-tetrahydrothieno[2,3-b] quinoline-2-carboxamide **17a**.* The reaction was carried out in accordance with general procedure G using 2-bromo-*N*-phenylacetamide **21a** (0.11 g, 0.49 mmol) to give the *title product* **17a** (0.07 g, 41%) as a yellow solid. m.p. 280–282 °C. lit. m.p. 291–292 °C [13]. ^{1}H NMR (400 MHz; d_6-DMSO) 2.13–2.19 (2H, m, H-7), 2.73 (2H, t, J = 6.2 Hz, H-6), 3.20 (2H, t, J = 6.2 Hz, H-8), 7.07–7.12 (1H, m, H-4′), 7.34 (2H, dd, J = 1.0, 8.3 Hz, H-3′ and H-5′), 7.59 (2H, s, NH_2), 7.69 (2H, dd, J = 1.0, 8.3 Hz, H-2′ and H-6′), 9.05 (1H, s, H-4), 9.50 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 21.2 (C-7), 32.3 (C-8), 38.1 (C-6), 96.1 (C-2) 121.2 (C-2′ and C-6′), 123.5 (C-4′), 124.4 (C-4a), 125.2 (C-3a), 128.4 (C-3′ and C-5′), 130.0 (C-4), 138.8 (C-1′), 147.4 (C-3), 162.1 (C-9a), 163.7 (NC=O), 164.0 (C-8a), 197.0 (C-5); IR: υ_{max} (film)/cm^{-1}: 3408, 3348, 3292, 3048, 2954, 2884, 1676, 1627, 1584, 1528, 1433, 1315, 1232; *m/z* (ESI+): 360 (MNa$^+$, 100%); HRMS (ESI$^+$) found (MNa$^+$) 360.0777, $C_{18}H_{15}N_3O_2SNa$ requires 360.0777.

*4.6.2.2. 3-Amino-N-(4′-methoxyphenyl)-5-oxo-5,6,7,8-tetrahydrothieno[2,3-b]quinoline-2-carboxamide **17b**.* The reaction was carried out in accordance with general procedure G using 2-bromo-*N*-(4-methoxyphenyl)acetamide **21b** (0.12 g, 0.49 mmol) to give the *title product* **17b** (0.03 g, 14%) as a yellow solid. m.p. 321–324 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 2.12–2.19 (2H, m, H-7), 2.73 (2H, t, J = 6.4 Hz, H-6), 3.20 (2H, t, J = 6.4 Hz, H-8), 3.75 (3H, s, OCH_3), 6.91 (2H, d, J = 9.0 Hz, H-3′ and H-5′), 7.51 (2H, s, NH_2), 7.56 (2H, d, J = 9.0 Hz, H-2′ and H-6′), 9.02 (1H, s, H-4), 9.41 (1H, s, NH); ^{13}C NMR (100 MHz; DMSO-*d6*) 21.2 (C-7), 32.3 (C-8), 38.1 (C-6), 55.1 (OCH_3) 96.3 (C-2) 113.6 (C-3′ and C-5′), 123.0 (C-2′ and C-6′), 124.4 (C-4a), 125.2 (C-3a), 129.9 (C-4) 131.6 (C-1′), 147.1 (C-3), 155.6 (C-4′) 162.1 (C-9a) 163.5 (NC=O), 164.0 (C-8a), 197.1 (C-5); IR: υ_{max} (film)/cm^{-1}: 3428, 3332, 2952, 1674, 1593, 1507, 1462, 1408, 1231, 1033; *m/z* (ESI+): 390 (MNa$^+$, 100%); HRMS (ESI$^+$) found (MNa$^+$): 390.0874, $C_{19}H_{17}N_3O_3SNa^+$ requires 390.0883.

*4.6.2.3. 3-Amino-N-(3′-chlorophenyl)-5-oxo-5,6,7,8-tetrahydrothieno[2,3-b]quinoline-2-carboxamide **17c**.* The reaction was carried out in accordance with general procedure G using 2-bromo-*N*-(3-chlorophenyl)acetamide **21c** (0.12 g, 0.49 mmol) to give the *title product* **17c** (0.14 g, 77%) as a yellow solid. m.p. 276–278 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 2.12–2.19 (2H, m, H-7), 2.73 (2H, t, J = 6.2 Hz, H-6), 3.20 (2H, t, J = 6.2 Hz, H-8), 7.13 (1H, d, J = 8.0 Hz, H-4′), 7.33–7.37 (1H, m, H-5′), 7.64 (3H, d, J = 8.0 Hz, H6′ and NH_2), 7.90 (1H, s, H-2′), 9.04 (1H, s, H-4), 9.65 (1H, s, NH). The ^{1}H NMR data was in agreement with the literature values [11].

*4.6.2.4. 3-Amino-N-(3′-methoxyphenyl)-5-oxo-5,6,7,8-tetrahydrothieno[2,3-b]quinoline-2-carboxamide **17d**.* The reaction was carried out in accordance with general procedure G using 2-bromo-*N*-(3-methoxyphenyl)acetamide **21d** (0.12 g, 0.49 mmol) to give the *title product* **17d** (0.14 g, 77%) as a yellow solid. m.p. 248–250 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 2.13–2.19 (2H, m, H-7), 2.73 (2H, t, J = 6.4 Hz, H-6), 3.20 (2H, t, J = 6.4 Hz, H-8), 3.76 (3H, s, OCH_3), 6.67 (1H, d, J = 8.1 Hz, H-4′), 7.21–7.25 (1H, m, H-5′), 7.32 (1H, d, J = 8.1 Hz, H-6′), 7.38 (1H, s, H-2′), 7.59 (2H, s, NH_2), 9.04 (1H, s, H-4), 9.46 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 21.2 (C-7), 32.4 (C-8), 38.1 (C-6), 55.0 (OCH_3) 96.0 (C-2), 106.6 (C-2′), 109.1 (C-4′), 113.2 (C-6′), 124.4 (C-4a), 125.2 (C-3a), 129.1 (C-5′), 130.0 (C-4), 140.1 (C-1′), 147.6 (C-3), 159.3 (C-3′) 162.1 (C-9a), 163.7 (NC=O), 164.1 (C-8a), 197.1 (C-5); IR: υ_{max} (film)/cm^{-1}: 3416, 3325, 3046, 2945, 2842, 1673, 1631, 1582, 1519, 1428, 1243, 1158, 1044; *m/z* (ESI+): 390 (MNa$^+$, 100%); HRMS (ESI$^+$) found (MNa$^+$): 390.0881, $C_{19}H_{17}N_3O_3SNa$ requires 390.0883.

*4.6.2.5. 3-Amino-N-(3′-bromophenyl)-5-oxo-5,6,7,8-tetrahydrothieno[2,3-b]quinoline-2-carboxamide **17e**.* The reaction

was carried out in accordance with general procedure G using 2-bromo-*N*-(3-bromophenyl)acetamide **21e** (0.14 g, 0.49 mmol) to give the *title product* **17e** (0.18 g, 87%) as a yellow solid. m.p. 301–303 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 2.11–2.18 (2H, m, H-7), 2.72 (2H, t, J = 6.2 Hz, H-6), 3.19 (2H, t, J = 6.2 Hz, H-8), 7.23 (1H, d, J = 8.0 Hz, H-4′), 7.26–7.30 (1H, m, H-5′), 7.63 (2H, s, NH_2), 7.67 (1H, d, J = 8.0 Hz, H-6′), 8.04 (1H, s, H-2′), 9.03 (1H, s, H-4), 9.61 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 21.2 (C-7), 32.4 (C-8), 38.1 (C-6), 96.4 (C-2) 119.7 (C-6′), 121.2 (C-3′), 123.2 (C-2′), 124.4 (C-4a) 125.2 (C-3a) 125.6 (C-4′), 130.1 (C-4), 130.3 (C-5′), 141.3 (C-1′), 147.6 (C-3), 162.2 (C-9a) 163.9 (NC═O), 164.1 (C-8a), 197.0 (C-5); IR: υ_{max} (film)/cm^{-1}: 3432, 3352, 3312, 1677, 1634, 1582, 1521, 1417, 1245, 1054; *m*/*z* (ESI+): 440 (^{81}Br MNa$^+$, 46%), 438 (^{79}Br MNa$^+$, 45%), 360 ([MNa-Br]$^+$, 100%). HRMS (ESI$^+$) found (^{81}Br MNa$^+$): 439.9865, $C_{18}H_{14}N_3O_2S^{81}BrNa$ requires 439.9862. Found (^{79}Br MNa$^+$) 437.9883, $C_{18}H_{14}N_3O_2S^{79}BrNa$ requires 437.9882.

4.6.2.6. 3-Amino-N-(3′-nitrophenyl)-5-oxo-5,6,7,8-tetrahydrothieno [2,3-b]quinoline-2-carboxamide ***17f***. The reaction was carried out in accordance with general procedure G using 2-bromo-*N*-(3-nitrophenyl)acetamide **21f** (0.13 g, 0.49 mmol) to give the *title product* **17f** (0.19 g, 100%) as a yellow solid. m.p. 290–292 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 2.13–2.19 (2H, m, H-7), 2.74 (2H, t, J = 6.2 Hz, H-6), 3.21 (2H, t, J = 6.2 Hz, H-8), 7.61–7.65 (1H, m, H-5′), 7.74 (2H, s, NH_2), 7.94 (1H, d, J = 8.0 Hz, H-4′), 8.15 (1H, d, J = 8.0 Hz, H-6′), 8.77 (1H, s, H-2′), 9.08 (1H, s, H-4), 9.95 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 21.2 (C-7), 32.4 (C-8), 38.1 (C-6), 95.1 (C-2) 114.8 (C-2′), 117.7 (C-4′), 124.5 (C-4a), 125.0 (C-3a) 126.6 (C-6′) 129.8 (C-5′), 130.3 (C-4), 140.3 (C-1′), 147.8 (C-3), 148.5 (C-3′), 162.3 (C-9a) 164.0 (NC═O), 164.5 (C-8a), 197.0 (C-5); IR: υ_{max} (film)/cm^{-1}: 3444, 3322, 2960, 2871, 1672, 1643, 1583, 1525, 1320, 1296, 1238, 1096; *m*/*z* (ESI+): 405 (MNa$^+$, 49%), 360 ([MNa-NO_2]$^+$, 100%). HRMS (ESI$^+$) found (MNa$^+$): 405.0632, $C_{18}H_{14}N_4O_4SNa$ requires 405.0628.

4.6.2.7. 3-Amino-5-oxo-N-(3′-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydrothieno[2,3-b]quinoline-2-carboxamide ***17g***. The reaction was carried out in accordance with general procedure G using 2-bromo-*N*-(3-(trifluoromethyl)phenyl)acetamide **21g** (0.14 g, 0.49 mmol) to give the *title product* **17g** (0.16 g, 88%) as a yellow solid. m.p. 289–292 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 2.12–2.18 (2H, m, H-7), 2.72 (2H, t, J = 6.3 Hz, H-6), 3.19 (2H, t, J = 6.3 Hz, H-8), 7.39 (1H, d, J = 8.0 Hz, H-4′), 7.53–7.57 (1H, m, H-5′), 7.66 (2H, s, NH_2), 7.97 (1H, d, J = 8.0 Hz, H-6′), 8.18 (1H, s, H-2′), 9.04 (1H, s, H-4), 9.77 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 21.2 (C-7), 32.4 (C-8), 38.1 (C-6), 96.2 (C-2) 117.0 (C-2′), 119.2 (C-4′), 122.9 (C-3′), 124.4 (C-4a and C-6′), 125.2 (C-3a), 128.8 (CF_3), 129.5 (C-5′), 130.1 (C-4), 140.5 (C-1′), 147.7 (C-3), 162.2 (C-9a), 164.1 (NC═O), 164.1 (C-8a), 197.0 (C-5); IR: υ_{max} (film)/cm^{-1}: 3427, 3353, 3311, 3074, 2955, 2876, 1675, 1631, 1581, 1525, 1475, 1402, 1301, 1231, 1094; IR: υ_{max} (film)/cm^{-1}: 3429, 3362, 3313, 2953, 1677, 1642, 1585, 1538, 1439, 1318, 1251, 1228, 1107; *m*/*z* (ESI+): 428 (MNa$^+$, 75%), 360 ([MNa-CF_3]$^+$, 100%). HRMS (ESI$^+$) found (MNa$^+$) 428.0657, $C_{19}H_{14}F_3N_3O_2SNa$ requires 428.0651.

4.6.2.8. 3-Amino-N-(3′,5′-dimethoxyphenyl)-5-oxo-5,6,7,8-tetrahydrothieno[2,3-b]quinoline-2-carboxamide ***17h***. The reaction was carried out in accordance with general procedure G using 2-bromo-*N*-(3,5-dimethoxyphenyl)acetamide **21h** (0.40 g, 1.46 mmol) to give the *title product* **17h** (0.17 g, 29%) as a yellow solid. m.p. 279–280 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 2.13–2.16 (2H, m, H-7), 2.72 (2H, t, J = 5.6 Hz, H-6), 3.19 (2H, t, J = 5.6 Hz, H-8), 3.74 (6H, s, OCH_3), 6.23 (1H, s, H-4′), 7.03 (2H, s, H-2′ and H-6′), 7.60 (2H, s, NH_2), 9.03 (1H, s, H-4), 9.38 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 21.2 (C-7), 32.3 (C-8), 38.1 (C-6), 55.1 (OCH_3), 95.6 and 96.0 (C-2 and C-4′), 99.0 (C-2′ and C-6′), 124.4 (C-4a), 125.1 (C-3a), 130.1 (C-4), 140.7 (C-1′), 147.6 (C-3), 160.2 (C-3′ and C-5′), 162.1 (C-9a), 163.7 (NC═O), 164.1 (C-8a), 197.0 (C-5); IR: υ_{max} (film)/cm^{-1}: 3467, 3348, 3279, 2941, 2838, 1686, 1633, 1580, 1540, 1476, 1450, 1424, 1267, 1156, 1066; *m*/*z* (ESI+): 420 (MNa$^+$, 100%); HRMS (ESI$^+$) found (MNa$^+$): 420.0997, $C_{20}H_{19}N_3O_4SNa$ requires 420.0988.

4.6.2.9. 3-Amino-N-(3′,5′-dichlorophenyl)-5-oxo-5,6,7,8-tetrahydrothieno[2,3-b]quinoline-2-carboxamide ***17i***. The reaction was carried out in accordance with general procedure G using 2-bromo-*N*-(3,5-dichlorophenyl)acetamide **22i** (0.50 g, 1.78 mmol) to give the *title product* **17i** (0.25 g, 35%) as a yellow solid. m.p. 269–270 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 2.12–2.15 (2H, m, H-7), 2.70 (2H, t, J = 5.5 Hz, H-6), 3.17 (2H, t, J = 5.5 Hz, H-8), 7.20 (1H, s, H-4′), 7.65 (2H, s, NH_2), 7.82 (2H, s, H-2′ and H-6′), 9.00 (1H, s, H-4); ^{13}C NMR (100 MHz; d_6-DMSO) 21.2 (C-7), 32.3 (C-8), 38.1 (C-6), 96.3 (C-2) 118.8 (C-2′ and C-6′), 121.8 (C-4′), 124.4 (C-4a), 125.1 (C-3a), 130.1 (C-4), 133.6 (C-3′ and C-5′), 142.4 (C-1′), 147.9 (C-3), 162.3 (C-9a), 164.1 (NC═O), 164.2 (C-8a), 197.0 (C-5); IR: υ_{max} (film)/cm^{-1}: 3433, 3327, 2941, 1675, 1648, 1578, 1527, 1439, 1399, 1265, 1230; *m*/*z* (ESI+): 431 ($^{37}Cl_2$ MNa$^+$, 16%), 429 ($^{35}Cl^{37}Cl$ MNa$^+$ 68%), 427 ($^{35}Cl_2$ MNa$^+$, 100%); HRMS (ESI$^+$) found ($^{37}Cl_2$ MNa$^+$): 431.9936, $C_{18}H_{13}N_3O_2S^{37}Cl_2Na$ requires 431.9939. Found ($^{35}Cl_2$MNa$^+$): 427.9998, $C_{18}H_{13}N_3O_2S^{35}Cl_2Na$ requires 427.9998.

4.6.3. General procedure H: synthesis of 5-hydroxy-tetrahydrothieno[2,3-b]quinoline-2-carboxamides ***23a–i***

A solution of sodium borohydride (0.30 mmol) in methanol (1 mL) was added dropwise to a solution of 5-oxo-tetrahydrothieno [2,3-*b*]quinoline-2-carboxamide **17** (0.30 mmol) in THF (15 mL) over 15 min. The mixture was stirred for 2 h at room temperature, before H_2O (10 mL) was added and the stirring was continued for further 5 min. The volatile solvents were removed *in vacuo*, and the remaining mixture diluted with H_2O (90 mL). The aqueous mixture was extracted with ethyl acetate (3 × 100 mL), the organic layers were combined and further washed with H_2O (3 × 100 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give 5-hydroxy-tetrahydrothieno[2,3-*b*]quinolines **23a–i**.

4.6.3.1. 3-Amino-5-hydroxy-N-phenyl-5,6,7,8-tetrahydrothieno[2,3-b]quinoline-2-carboxamide ***23a***. The reaction was carried out in accordance with general procedure H using 5-oxo-tetrahydrothieno[2,3-*b*]quinoline **17a** (0.10 g, 0.30 mmol) to give the *title product* **23a** (0.07 g, 66%) as a yellow solid. m.p. 225–228 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 1.71–1.83 (2H, m, H-7), 1.99–2.03 (2H, m, H-6), 2.91–2.97 (2H, m, H-8), 4.72–4.76 (1H, m, H-5), 5.40 (1H, d, J = 6.1 Hz, OH), 7.05–7.09 (1H, m, Ar–H), 7.32 (2H, d, J = 8.4 Hz, Ar–H), 7.39 (2H, s, NH_2), 7.69 (2H, d, J = 8.4 Hz, Ar–H), 8.52 (1H, s, H-4), 9.35 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 18.4 (C-7), 32.0 (C-8), 32.3 (C-6), 66.5 (C-5) 95.6 (C-2) 121.1 (Ar–CH), 123.3 (Ar–CH), 124.5 (C-3a), 128.3 (Ar–CH), 130.7 (C-4), 132.2 (C-4a) 139.0 (Ar–C), 147.2 (C-3), 157.0 (C-9a) 158.8 (C-8a), 164.1 (C═O); IR: υ_{max} (film)/cm^{-1}: 3442, 3299, 3055, 2932, 1590, 1521, 1488, 1436, 1317, 1244, 1068; *m*/*z* (ESI+): 362 (MNa$^+$, 100%), 340 (MH$^+$, 99%). HRMS (ESI$^+$) found (MNa$^+$) 362.0935, $C_{18}H_{17}N_3O_2SNa$ requires 362.0934.

4.6.3.2. 3-Amino-5-hydroxy-N-(4′-methoxyphenyl)-5,6,7,8-tetrahydrothieno[2,3-b]quinoline-2-carboxamide ***23b***. The reaction was carried out in accordance with general procedure H using 5-oxo-tetrahydrothieno[2,3-*b*]quinoline **17b** (0.10 g, 0.27 mmol) to give the *title product* **23b** (0.10 g, 99%) as a yellow solid. m.p. 250–253 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 1.74–1.81 (2H, m, H-7), 2.00–2.03 (2H, m, H-6), 2.86–3.00 (2H, m, H-8), 3.75 (3H, s, OCH_3) 4.72–4.76 (1H, m, H-5), 5.40 (1H, d, J = 6.3 Hz, OH), 6.90 (2H, d, J = 8.0 Hz, H-3′ and H-5′), 7.33 (2H, s, NH_2), 7.56 (2H, d, J = 8.0 Hz, H-

2′ and H-6′), 8.49 (1H, s, H-4), 9.25 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 18.4 (C-7), 32.0 (C-8), 32.3 (C-6), 55.1 (OCH_3), 66.5 (C-5), 95.8 (C-2), 133.5 (C-3′ and C-5′), 122.9 (C-2′ and C-6′), 124.6 (C-3a), 130.6 (C-4), 131.9 (C-1′), 132.2 (C-4a), 146.8 (C-3), 155.5 (C-4′) 156.9 (C-9a) 158.6 (C-8a), 163.9 (C=O); IR: υ_{max} (film)/cm^{-1}: 3672, 3420, 3295, 2924, 1590, 1503, 1298, 1228, 1172, 1032; *m*/*z* (ESI+): 392 (MNa^+, 100%). HRMS (ESI^+): found (MNa^+) 392.1038, $C_{19}H_{19}N_3O_3SNa$ requires 392.1039.

4.6.3.3. 3-Amino-N-(3′-chlorophenyl)-5-hydroxy-5,6,7,8-tetrahydrothieno[2,3-b]quinoline-2-carboxamide ***23c***. The reaction was carried out in accordance with general procedure H using 5-oxo-tetrahydrothieno[2,3-*b*]quinoline **17c** (0.10 g, 0.27 mmol) to give the *title product* **23c** (0.10 g, 98%) as a yellow solid. m.p. 255–257 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 1.71–1.85 (2H, m, H-7), 1.99–2.03 (2H, m, H-6), 2.87–3.00 (2H, m, H-8), 4.72–4.76 (1H, m, H-5), 5.41 (1H, d, J = 6.2 Hz, OH), 7.11 (1H, d, J = 8.0 Hz, H-4′), 7.32–7.36 (1H, m, H-5′), 7.47 (2H, s, NH_2), 7.70 (1H, d, J = 8.0 Hz, H-6′), 7.91 (1H, s, H-2′), 8.53 (1H,s H-4), 9.51 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 18.4 (C-7), 31.9 (C-8), 32.3 (C-6), 66.5 (C-5), 95.0 (C-2) 119.1 (C-6′), 120.2 (C-2′), 122.8 (C-4′), 124.4 (C-3a), 130.0 (C-5′), 130.8 (C-4), 132.3 (C-3′), 132.7 (C-4a), 140.6 (C-1′), 147.8 (C-3), 157.0 (C-9a), 159.0 (C-8a), 164.2 (C=O); IR: υ_{max} (film)/cm^{-1}: 3469, 3381, 3344, 3287, 2933, 2863, 1583, 1516, 1414, 1311, 1240, 1044; *m*/*z* (ESI+): 398 ($^{37}ClMNa^+$, 18%), 396 ($^{35}ClMNa^+$, 48%), 360 (MNa^+-Cl, 100%). HRMS (ESI^+): found ($^{37}ClMNa^+$) 398.0513, $C_{18}H_{16}N_3O_2S^{37}ClNa$ requires 398.0515. Found ($^{35}ClMNa^+$) 396.0541, $C_{18}H_{16}N_3O_2S^{35}ClNa$ requires 396.0544.

4.6.3.4. 3-Amino-5-hydroxy-N-(3′-methoxyphenyl)-5,6,7,8-tetrahydrothieno[2,3-b]quinoline-2-carboxamide ***23d***. The reaction was carried out in accordance with general procedure H using 5-oxo-tetrahydrothieno[2,3-*b*]quinoline **17d** (0.10 g, 0.27 mmol) to give the *title product* **23d** (0.10 g, 98%) as a yellow solid. m.p. 236–239 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 1.79–1.87 (2H, m, H-7), 2.04–2.08 (2H, m, H-6), 2.96–3.02 (2H, m, H-8), 3.80 (3H, s, OCH_3), 4.77–4.81 (1H, m, H-5), 5.45 (1H, d, J = 6.1 Hz, OH), 6.70 (1H, d, J = 8.0 Hz, H-4′), 7.24–7.28 (1H, m, H-5′), 7.37 (1H, d, J = 8.0 Hz, H-6′), 7.43 (1H, s, H-2′), 7.46 (2H, s, NH_2), 8.57 (1H, s, H-4), 9.36 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 18.4 (C-7), 32.0 (C-8), 32.3 (C-6), 55.0 (OCH_3), 66.5 (C-5), 95.5 (C-2), 106.6 (C-2′), 108.9 (C-4′), 113.2 (C-6′), 124.5 (C-3a), 129.1 (C-5′), 130.7 (C-4), 132.3 (C-4a), 140.2 (C-1′), 147.3 (C-3), 157.0 (C-9a) 158.8 (C-8a), 159.3 (C-3′) 164.1 (C=O); IR: υ_{max} (film)/cm^{-1}: 3447, 3338, 3271, 2932, 2833, 1586, 1521, 1448, 1430, 1243, 1161, 1037; *m*/*z* (ESI+): 392 (MNa^+, 39%), 360 (MNa^+-OCH_3, 100%); HRMS (ESI^+): found (MNa^+) 392.1035, $C_{19}H_{19}N_3O_3SNa$ requires 392.1039.

4.6.3.5. 3-Amino-N-(3′-bromophenyl)-5-hydroxy-5,6,7,8-tetrahydrothieno[2,3-b]quinoline-2-carboxamide ***23e***. The reaction was carried out in accordance with general procedure H using 5-oxo-tetrahydrothieno[2,3-*b*]quinoline **17e** (0.10 g, 0.24 mmol) to give the *title product* **23e** (0.10 g, 97%) as a yellow solid. m.p. 240–241 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 1.74–1.83 (2H, m, H-7), 1.97–2.05 (2H, m, H-6), 2.91–2.97 (2H, m, H-8), 4.72–4.76 (1H, m, H-5), 5.40 (1H, d, J = 6.2 Hz, OH), 7.24 (1H, d, J = 8.0 Hz, H-4′), 7.26–7.30 (1H, m, H-5′) 7.48 (2H, s, NH_2), 7.70 (1H, d, J = 8.0 Hz, H-6′), 8.05 (1H, s, H-2′), 8.54 (1H, s, H-4), 9.49 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 18.4 (C-7), 32.0 (C-8), 32.3 (C-6), 66.5 (C-5), 95.0 (C-2), 119.5 (C-6′), 121.2 (C-3′), 123.1 (C-2′), 124.4 (C-3a), 125.7 (C-4′), 130.3 (C-5′), 131.9 (C-4), 132.3 (C-4a), 140.8 (C-1′), 147.8 (C-3), 157.1 (C-9a), 159.0 (C-8a), 164.2 (C=O); IR: υ_{max} (film)/cm^{-1}: 3446, 3322, 2929, 2852, 1580, 1524, 1474, 1400, 1305, 1243, 1056; *m*/*z* (ESI+): 442 ($^{81}BrMNa^+$, 61%), 440 ($^{79}BrMNa^+$, 57%), 360 (MNa^+-Br, 100%). HRMS (ESI^+): found ($^{81}BrMNa^+$) 442.0012, $C_{18}H_{16}N_3O_2S^{81}BrNa$ requires 442.0019. Found ($^{79}BrMNa$) 440.0033, $C_{18}H_{16}N_3O_2S^{79}BrNa$ requires 440.0039.

4.6.3.6. 3-Amino-5-hydroxy-N-(3′-nitrophenyl)-5,6,7,8-tetrahydrothieno[2,3-b]quinoline-2-carboxamide ***23f***. The reaction was carried out in accordance with general procedure H using 5-oxo-tetrahydrothieno[2,3-*b*]quinoline **17f** (0.10 g, 0.26 mmol) to give the *title product* **23f** (0.10 g, 98%) as a yellow solid. m.p. 259–260 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 1.71–1.84 (2H, m, H-7), 1.99–2.03 (2H, m, H-6), 2.92–2.98 (2H, m, H-8), 4.72–4.77 (1H, m, H-5), 5.41 (1H, d, J = 6.3 Hz, OH), 7.56 (2H, s, NH_2), 7.59–7.63 (1H, m, H-5′), 7.92 (1H, d, J = 8.3 Hz, H-4′), 8.15 (1H, d, J = 8.3 Hz, H-6′), 8.56 (1H, s, H-4), 8.77 (1H, s, H-2′), 9.83 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 18.4 (C-7), 31.9 (C-8), 32.3 (C-6), 66.5 (C-5), 95.6 (C-2), 114.8 (C-2′), 117.5 (C-4′), 124.3 (C-3a), 126.6 (C-6′) 129.7 (C-5′), 131.0 (C-4), 132.4 (C-4a), 140.4 (C-1′), 147.8 (C-3), 148.2 (C-3′), 157.1 (C-9a), 159.2 (C-8a), 164.4(C=O); IR: υ_{max} (film)/cm^{-1}: 3422, 3304, 2948, 2922, 2852, 1585, 1527, 1320, 1297, 1243, 1053; *m*/*z* (ESI+): 407 (MNa^+, 86%), 360 (MNa^+-NO_2, 100%). HRMS (ESI^+): found (MNa^+) 407.0780, $C_{18}H_{16}N_4O_4SNa$ requires 407.0784.

4.6.3.7. 3-Amino-5-hydroxy-N-(3′-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydrothieno[2,3-b]quinoline-2-carboxamide ***23g***. The reaction was carried out in accordance with general procedure H using 5-oxo-tetrahydrothieno[2,3-*b*]quinoline **17g** (0.10 g, 0.25 mmol) to give the *title product* **23g** (0.10 g, 99%) as a yellow solid. m.p. 258–260 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 1.71–1.85 (2H, m, H-7), 1.99–2.03 (2H, m, H-6), 2.87–3.00 (2H, m, H-8), 4.72–4.76 (1H, m, H-5), 5.41 (1H, d, J = 6.0 Hz, OH), 7.40 (1H, d, J = 8.0 Hz, H-4′), 7.51 (2H, s, NH_2), 7.56 (1H, t, J = 8.0 Hz, H-5′), 8.00 (1H, d, J = 8.0 Hz, H-6′), 8.20 (1H, s, H-2′), 8.55 (1H, s, H-4), 9.66 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 18.4 (C-7), 31.9 (C-8), 32.3 (C-6), 66.5 (C-5), 94.8 (C-2), 116.8 (C-2′), 119.4 (C-4′), 122.9 (C-3′), 124.2 and 124.4 (C-6′ and C-3a), 129.2 (CF_3), 129.5 (C-5′) 130.9 (C-4), 132.3 (C-4a), 139.9 (C-1′), 147.9 (C-3), 157.1 (C-9a), 159.1 (C-8a), 164.3 (C=O); IR: υ_{max} (film)/cm^{-1}: 3423, 3308, 2922, 2853, 1593, 1549, 1438, 1319, 1115, 1057; *m*/*z* (ESI+): 430 (CF_3MNa^+, 100%); HRMS (ESI^+): found (MNa^+) 430.0809, $C_{19}H_{16}F_3N_3O_2SNa$ requires 430.0808.

4.6.3.8. 3-Amino-N-(3′,5′-dimethoxyphenyl)-5-hydroxy-5,6,7,8-tetrahydrothieno[2,3-b]quinoline-2 carboxamide ***23h***. The reaction was carried out in accordance with general procedure H using 5-oxo-tetrahydrothieno[2,3-*b*]quinoline **17h** (0.10 g, 0.25 mmol) to give the *title product* **23h** (0.73 g, 73%) as a yellow solid. m.p. 199–201 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 1.71–1.85 (2H, m, H-7), 1.99–2.03 (2H, m, H-6), 2.87–3.00 (2H, m, H-8), 3.73 (6H, s, OCH_3) 4.72–4.76 (1H, m, H-5), 5.39 (1H, d, J = 6.2 Hz, OH), 6.22 (1H, t, J = 2.1 Hz, H-4′), 7.04 (2H, d, J = 2.1 Hz, H-2′ and H-6′) 7.42 (2H, s, NH_2), 8.53 (1H,s H-4), 9.25 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 18.4 (C-7), 32.0 (C-8), 32.3 (C-6), 55.1 (OCH_3), 66.5 (C-5), 95.4 (C-2 and C-4′), 98.9 (C-2′ and C-6′) 124.5 (C-3a), 130.8 (C-4), 132.3 (C-4a), 140.8 (C-1′), 147.4 (C-3), 156.9 (C-9a), 158.8 (C-8a), 160.2 (C-3′ and C-5′), 164.1 (C=O); IR: υ_{max} (film)/cm^{-1}: 3671, 3411, 3297, 2928, 2851, 1594, 1529, 1475, 1452, 1416, 1258, 1237, 1155, 1050; *m*/*z* (ESI+): 422 (MNa^+, 100%); HRMS (ESI^+): found (MNa^+) 422.1143, $C_{20}H_{21}N_3O_4SNa$ requires 422.1145.

4.6.3.9. 3-Amino-N-(3′,5′-dichlorophenyl)-5-hydroxy-5,6,7,8-tetrahydrothieno[2,3-b]quinoline-2-carboxamide ***23i***. The reaction was carried out in accordance with general procedure H using 5-oxo-tetrahydrothieno[2,3-*b*]quinoline **17i** (0.10 g, 0.25 mmol) to give the *title product* **23i** (0.10 g, 100%) as a yellow solid. m.p. 267–269 °C. ^{1}H NMR (400 MHz; d_6-DMSO) 1.71–1.83 (2H, m, H-7), 1.99–2.03 (2H, m, H-6), 2.87–3.01 (2H, m, H-8), 4.73–4.76 (1H, m, H-5), 5.42 (1H, d, J = 6.1 Hz, OH), 7.26 (1H, t, J = 2.0 Hz, H-4′), 7.55

(2H, s, NH_2), 7.88 (2H, d, $J = 2.0$ Hz, H-2′ and H-6′), 8.56 (1H, s, H-4), 9.63 (1H, s, NH); ^{13}C NMR (100 MHz; d_6-DMSO) 18.3 (C-7), 31.9 (C-8), 32.3 (C-6), 66.5 (C-5), 94.5 (C-2), 118.6 (C-2′ and C-6′), 122.1 (C-4′), 124.3 (C-3a), 131.0 (C-4), 132.4 (C-4a), 133.7 (C-3′ and C-5′), 141.6 (C-1′), 148.3 (C-3), 157.1 (C-9a), 159.3 (C-8a), 164.2 (C=O); IR: υ_{max} (film)/cm^{-1}: 3432, 3320, 2942, 2922, 2854, 1580, 1528, 1492, 1443, 1404, 1311, 1264, 1056; *m/z* (ESI+): 434 ($^{37}Cl_2MNa^+$, 15%), 432 ($^{35}Cl^{37}ClMNa^+$, 70%), 430 ($^{35}Cl_2MNa^+$, 100%); HRMS (ESI$^+$): found ($^{37}Cl_2MNa$) 434.0102, $C_{18}H_{15}N_3O_2S^{37}Cl_2Na$ requires 434.0096. Found ($^{35}Cl^{37}ClMNa$) 432.0130, $C_{18}H_{15}N_3O_2S^{35}Cl^{37}ClNa$ requires 432.0125. Found ($^{35}Cl_2lMNa$) 430.0159, $C_{18}H_{15}N_3O_2S^{35}Cl_2Na$ requires 430.0154.

4.7. Biological testing

The compounds obtained were submitted to the National Cancer Institute's Developmental Therapeutic Program (DTP) where they were screened against a panel of sixty human tumour cell lines (NCI-60, for further information see Refs. [23,41,42] and references therein). Furthermore, the protocol is given in the Supplementary Information.

4.8. Molecular modelling

The compounds were docked to the crystal structure of PLC-δ1 (PDB ID: 1DJX, resolution 2.3 Å), which was obtained from the Protein Data Bank (PDB) [43,44]. The Scigress Ultra version 7.7.0.47 program [45] was used to prepare the crystal structure for docking, i.e., hydrogen atoms were added, the co-crystallised ligand (D-Myo-Inositol-1,4,5-Triphosphate, IP_3) was removed as well as crystallographic water molecules. The Scigress software suite was also used to build the inhibitors and the MM2 [46] force field was used to optimise the structures. The centre of the binding pocket was defined as the position of the Ca^{2+} ion ($x = 126.257$, $y = 38.394$, $z = 22.370$) with 10 Å radius. Fifty docking runs were allowed for each ligand with default search efficiency (100%). The basic amino acids lysine and arginine were defined as protonated. Furthermore, aspartic and glutamic acids were assumed to be deprotonated. The GoldScore (GS) [47] ChemScore (CS) [48,49], ChemPLP [50] and ASP [51] scoring functions were implemented to validate the predicted binding modes and relative energies of the ligands using the GOLD v5.2 software suite.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.09.001.

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