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Improving the solubility of anti-proliferative thieno[2,3-*b*] quinoline-2-carboxamides

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ABSTRACT

Thieno[2,3-*b*]pyridines are a class of compounds known for their potent anti-proliferative activities against a range of human cancer cell lines. In this research, a number of strategies to generate analogues that have improved aqueous solubility whilst retaining the potent anti-proliferative actions, compared to previously-explored compounds in this class, were made. Herein we report the synthesis of 80 novel compounds, comprising two series, all based on the thieno[2,3-*b*]pyridine core structure. Overall, it was found that introducing alternative heterocycles did not notably improve the solubility or retain anti-proliferative activity seen in previously-reported analogues. However, pleasingly it was discovered, that the best strategy for improving the solubility was the alteration of the appended alkyl ring to introduce polar groups such as alcohols, ketones and substituted amine groups. In addition to this finding, we have discovered a thieno[2,3-*b*]pyridine, **15e**, with greater aqueous solubility that has ever been seen for this class of compounds that is also a potent inhibitor of cancer cell growth, with IC_{50}′s in the nanomolar range. This new lead structure will form the basis of future explorations into this class of compounds.

1. Introduction

The notable antiproliferative activity of compounds bearing the thieno[2,3-*b*]pyridine scaffold (Fig. 1) is well-documented against an extensive array of human cancer cell lines.[1] Of particular note is the potency of this class of compounds against triple-negative (i.e. lacking the hallmark progesterone and oestrogen receptors and human epidermal growth factor receptor 2 (HER2)) breast cancers; thienopyridines have been shown to inhibit proliferation with GI_{50} values in the low nanomolar range against triple-negative breast cancer lines MDA-MB-231 and MDA-MB-468.[2]

The mechanisms for the cytotoxic action of thienopyridines are varied, with these compounds exhibiting a range of effects on cellular growth and development. Effects include severe growth restriction, rounding and blebbing of the plasma membrane, decreased copper trafficking of antioxidant 1 copper chaperone (Atox1) and copper chaperone for superoxide dismutase (CCS) proteins, induced cell cycle arrest in M-phase and slowed migration in scratch assays.[2a,2b] Many of these stated effects induced by these compounds bear a resemblance to the behaviours demonstrated in phosphoinositide phospholipase C-δ_1 (PLC-δ_1) and δ_3 isoform knockdown cells for MDA-MB-231, which corroborates with the observation, discovered through virtual high throughput screening (vHTS), that this class of compounds bind with the PLC – γ2 protein.[2a,2b] This enzyme plays a critical role in cell signalling pathways involved in cell proliferation and motility.[2b,3] In saying this, there is recent conjecture that interactions with tyrosyl-DNA phosphodiesterase I (TDP1), A_{2A} Receptor Antagonists (A_{2A}AR), G-Protein coupled receptor (GPCR), copper trafficking protein Atox or tubulin may also account for some of the antiproliferative activity exhibited by this class of compounds.[4]

In addition to exploring the molecular target and specific mechanism by which these compounds impart their activities, the key research focus for this compound class has been the development of a consistent SAR and optimisation of anti-proliferative activity. Points of variation to the core thieno[2,3-*b*]pyridine structure have been identified and explored through the testing of commercially-available compounds and synthesis

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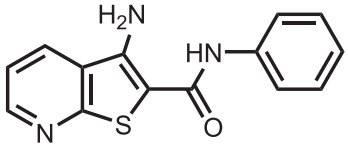


Fig. 1. Core structure of the thieno[2,3-*b*]pyridine class of anti-proliferative compounds.

of a large library of commercially-unattainable derivatives (Fig. 2).[1e–1g,2a,4b,5] It has been uncovered that one of the most critical features of the base thieno[2,3-*b*]pyridine was the free amino group (pink) – all derivatives with this moiety removed or altered had a drastic decrease in biological activity. Replacing the sulfur atom with an oxygen atom (green) did not produce a discernible difference in compound potency, although the resulting oxypyridines were more difficult to synthesise. A clear trend in the substitution pattern on the *N*-phenyl ring was discovered (red), with 2,3-disubstitution being the most favourable pattern. Additionally, an extensive exploration into the adjunction of an alkyl ring fused to the pyridine was investigated (blue) – it was found that this additional ring improves the cytotoxicity of the compounds, with a larger ring and additional keto or alcohol groups being optimal. IC_{50} values were in the low nanomolar (~10 nm) range for the most active of these compounds against various human cancer cell lines.

Despite the demonstrated potent anti-proliferative properties *in vitro* a limiting factor in that these compounds have a poor aqueous solubility profile, which limits the dose at which they can be tested and the efficacy of the compounds.[2b,4b]

Following on from this finding, efforts have been turned to improving the solubility profiles of these compounds while trying to retain biological activity. To date, two main strategies have been attempted. The first of these strategies was modification of the chemical structure from a thieno[2,3-*b*]pyridine to a pyrrolo[2,3-*b*]pyridine.[4a,6] Unfortunately, while there was a marked improvement in aqueous solubility (1000-fold from 1.21 μg/mL for the thienopyridine to 1340 μg/mL for the corresponding pyrrolo[2,3-*b*]pyridine, with a *N*-propyl-morpholine group) the compounds were devoid of biological activity. A second strategy formulating the thienopyridines in a solubilising polymer, cholesteryl-poly(allylamine) (Ch5-PAA) showed some success in delivering the thienopyridine a pancreatic cell line but there is still vast room for improvement.[7]

When considering new compounds the current SAR suggested two possible areas of modification that did not immediately suggest a loss in biological activity (Fig. 3) – that was addition of solubilising groups/motifs to the alkyl ring (blue) or to the *N*-phenyl ring (red). Herein we report the synthesis, activity and solubility of a range of derivatives, designed to explore the potential of modifying the thienopyridine structure in these positions in an effort to level the balance between solubility and activity and find potent anti-proliferative thienopyridines with appropriate aqueous solubility such to have the potential to move forward to the clinic.

2. Results and discussion

2.1. Compound series 1 chemistry; synthesis of thieno[2,3-b]quinoline-2-carboxamide derivatives

Of the two identified areas of modification, the first that was altered was the structure and nature of the alkyl ring appended to the central pyridine. As stated, a range of alkyl ring sizes in this section of the molecule, have been explored, as well as the presence of alcohol and keto substituents on this ring.[1e–1g,4b,5] What has not previously been investigated is the presence of alcohol/keto groups in thienopyridines lacking the alkyl ring. It was thought that these polar groups would better-contribute to the solubility of the compound when not locked into a ring structure.

Synthesis of the desired thieno[2,3-*b*]pyridines was achieved in an efficient sequence starting from diketone **1**, analogous to that reported previously.[2a,5] The first multi-component step involved reaction of **1** with dimethylformamide dimethyl acetal (DMFDMA) to give the corresponding enamine,[8] which was subsequently reacted with cyanothioacetamide, followed by acidification with aqueous acid, which provided carbonitrile **2**. Several chloroanilines **3a-m**[5] were coupled with carbonitrile **2** by heating at reflux in the presence of Na_2CO_3, where the basic conditions also effected concomitant cyclisation to give desired 5-acetyl thieno[2,3-*b*]pyridines **4a-m** in moderate to quantitative yields. Successfully achieving the synthesis of these keto-containing analogues, the alcohols of five of these ketones were generated by reduction of the ketone in **4a-e** providing alcohols **5a-e**. These five analogues have differing substitution on the aromatic ring; no substituents, 2-methyl, 2-methyl-3-chloro, 4-methoxy and 1-naphthyl. These five patterns are those most frequently used in this class of thienopyridine compounds, with a strongly-associated and established general SAR – compounds with 2,3-disubstitution (e.g. 2-methyl-3-chloro and 1-naphthyl) have excellent bioactivity, compounds with no and 2-methyl substitution have relatively moderate activity and compounds with 4-methoxy substitution have relatively poor activity, in a given series (see Scheme 1).

Another strategy to potentially improve compound solubility, was to change the appended alkyl ring, incorporating heteroatoms into this ring. It was thought that integrating a nitrogen/oxygen into the alkyl ring moiety may improve compound solubility as before, but this time with retention of anti-proliferative activity. As such, final compounds were targeted with a secondary and tertiary amine functionalities in the alkyl ring, as well as a series of oxygenated derivatives (Scheme 2).

First focussing on the nitrogen-containing analogues; substituted 4-

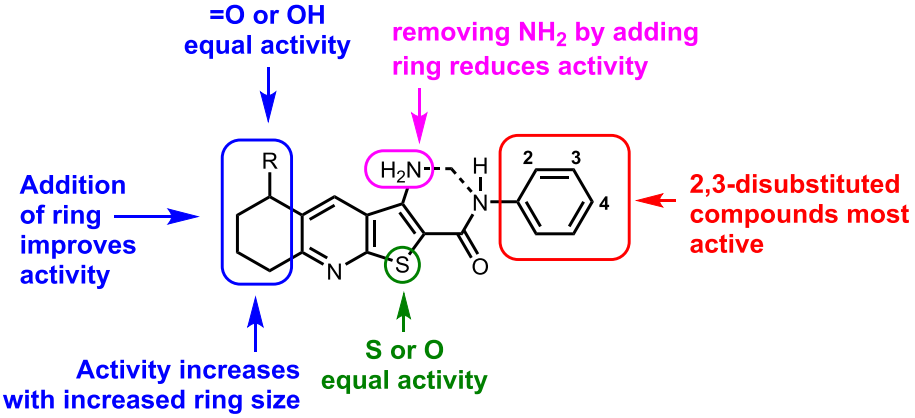


Fig. 2. Established SAR and structural variations of the core thieno[2,3-*b*]pyridine that have previously been investigated.

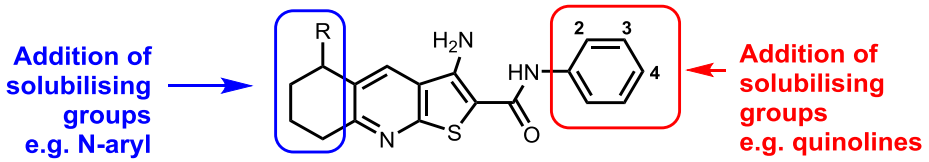
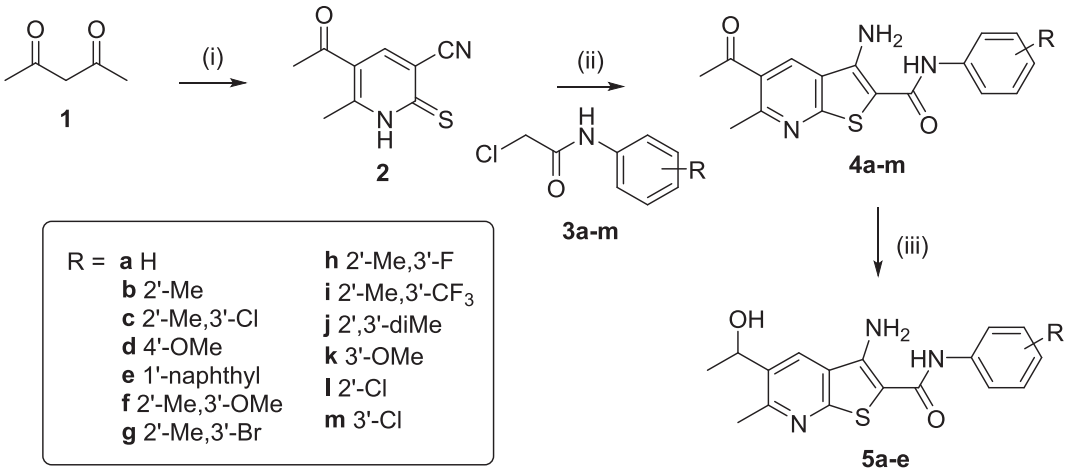
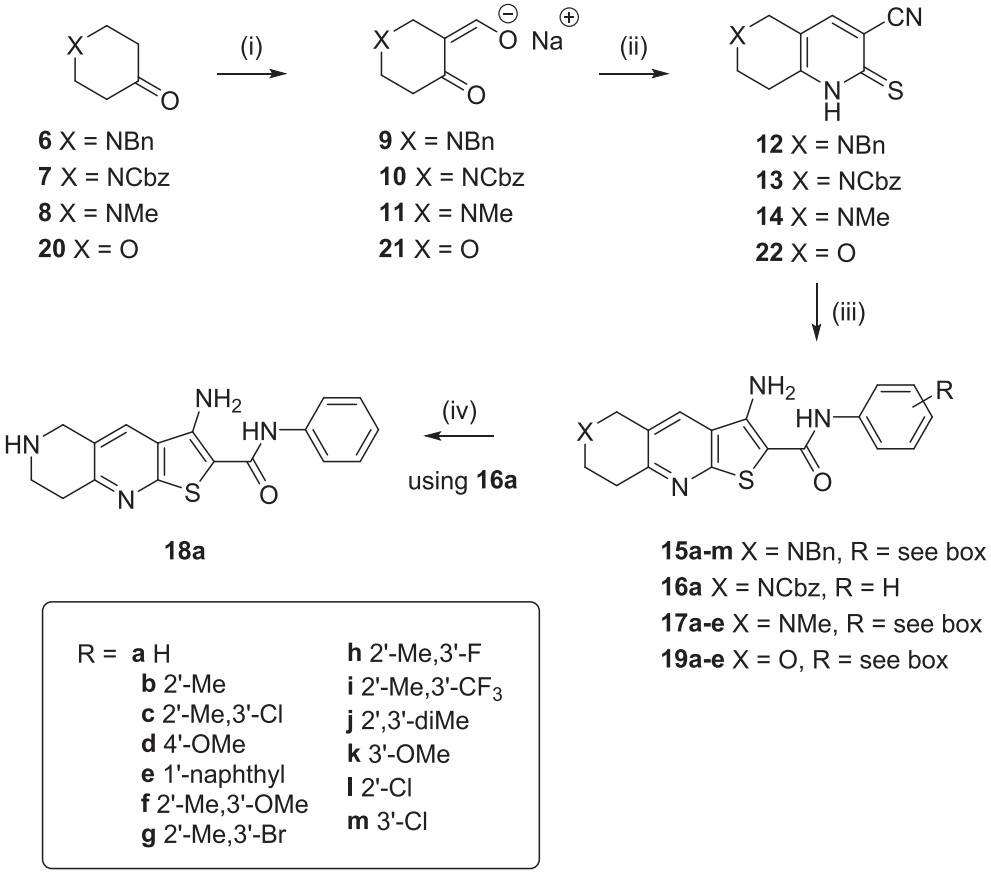


Fig. 3. Strategies explored in this work to improve the aqueous solubility and retain the biological activity of thieno[2,3-*b*]pyridines.



Scheme 1. Synthesis of 5-acetyl thieno[2,3-*b*]pyridines **4a-m** and 5-(1-hydroxyethyl) thieno[2,3-*b*]pyridines **5a-e**. *Reagents and conditions:* (i) DMF.DMA (1 equiv.), dioxane, r.t., 24 h; Na (2 equiv.), cyanothioacetamide (1 equiv.), MeOH, r.t., 1 h; reflux, 4 h; 2 M HCl, 97%. (ii) Acetamides **3** (1 equiv.), Na_2CO_3 (1.06–2 equiv.), EtOH, reflux, 24–48 h, 47%-quant. (iii) $NaBH_4$ (1 equiv.), MeOH, THF, r.t., 2 h; H_2O, 50%-quant.



Scheme 2. Synthesis of thienopyridines **15a-m**, **16a**, **17a-e, 18a** and **19a-e**. *Reagents and conditions:* (i) Ethyl formate (1 equiv.), Na (1 equiv.), cat. EtOH, diethyl ether, r.t., 48 h, 43–84%; (ii) cyanothioacetamide (1 equiv.), piperidinium acetate, H_2O, reflux, 24 h; AcOH, r.t. 24 h, 49–97%; (iii) Acetamides **3** (1 equiv.), Na_2CO_3 (1.06–2 equiv.), EtOH, reflux, 24–48 h, 13–77%; (iv) **16a** only, 12 M HCl, r.t. 24 h, 24%.

piperidones **6**–**8** were converted to salts **9**–**11** through their reaction with freshly-prepared sodium ethoxide and ethyl formate. These salts **9**–**11** were heated at reflux with cyanothioacetamide and piperidinium acetate, followed by acidification with acetic acid, consequently providing carbonitriles **12**–**14**. *N*-Benzyl-substituted carbonitrile **12** was successfully reacted with all available 2-haloacetamides **3a-m**, producing the desired *N*-benzyl-piperidine thienopyridines **15a-m**. In contrast, it was decided to only react *N*-Cbz-substituted carbonitrile **13** with non-substituted *N*–phenyl acetamide **3a** because of the low yield and instability of the resulting product **16a**. For *N*-Me-substituted carbonitrile **14**, haloacetamides **3a-e** with the most-common substitution patterns, representing the full scope of relative activities, were chosen to react, producing *N*-Me-piperidine thienopyridines **17a-e**. Finally, the Cbz group in **16a** was removed in strongly acidic conditions, generating the unsubstituted piperidine analogue **18a**. The oxygenated derivatives **19a-e** were accessed through the corresponding reactions, starting with oxan-4-one **20**, giving salt **21**, followed by carbonitrile **22** which was coupled with haloacetamides **3a-e**, producing the required thienopyridines **19a-e**. The successful synthesis of the oxygenated compounds completed Series 1, those with alteration solely on the alkyl ring appended to the core thienopyridine.

2.2. Anti-proliferative activity and aqueous solubility of Series 1 compounds

Following on from the synthesis of Series 1, both the antiproliferative activity and aqueous solubility of all synthesised compounds were measured (Table 1). The anti-proliferative action was measured against colon cancer HCT116 cell line and breast cancer MDA-MB-231 cell line with all compounds being initially tested at 1 μM concentration and IC_{50} values were determined for the active compounds with less than 10% relative growth when exposed to 1 μM of compounds.

First, looking at the anti-proliferative activity of the compounds, it was apparent that the aforementioned SAR related to the substitution on the aromatic ring generally held true for all analogues, with compounds possessing 2-methyl-3-chloro (**c**) and 1-naphthyl (**e**) substitutions reducing the growth of both cancer cell lines, with the greatest efficacy for thienopyridines **4**, **5** and **15** compared to the other analogues **a**, **b** and **d**. For thienopyridines **4** and **15**, it should be noted that the most active analogues were the **g** and **j** analogues, with 2-methyl-3-bromo and 2,3-dimethyl substitutions, respectively; like 2-methyl-3-chloro (**c**) and 1-naphthyl (**e**), these compounds all have 2,3-disubstitution which has been consistently shown to be most effective. Encouragingly, 5-acetyl thieno[2,3-*b*]pyridines **4** and 5-(1-hydroxyethyl) thieno[2,3-*b*] pyridines **5** along with *N*-benzyl-piperidine thienopyridines **15** all included analogues that exhibited excellent anti-proliferative activity that effectively reduced all cellular growth at 1 μM and boasting IC_{50}′s in the 70–200 nM range, an excellent finding. Disappointingly, the other *N*-Me piperidine and pyrano-thienopyridines **17** and **19** were shown to be generally inactive against the tested cancer cell lines.

We have previously measured the aqueous solubility of compounds in this class, where the best-reported solubility was that of a pyrrolo[2,3-*b*]pyridine (1340 μg/mL).[4a] Unfortunately, this compound was both unstable and biologically-inactive. The corresponding thienopyridine demonstrated better bioactivity, but a poor solubility profile (1.21 μg/mL). In that study, the best solubility of a thienopyridine was 3.53 μg/mL. Pleasingly, it was found that several of the compounds in Series 1 (i.e. **5a** 3.51 μg/mL, **5b** 29.54 μg/mL, **15e** 3.97 μg/mL, **17b** 5.61 μg/mL, **18a** 40.47 μg/mL, **19b** 7.52 μg/mL, **19e** 4.00 μg/mL) of this study showed equivalent and improved solubility compared to this previous-best by a thienopyridine. This showed that our strategy of introducing non-ring fused alcohol/keto groups and heteroatoms into the alkyl ring, as successful in improving the aqueous solubility in many instances, although there was no specific approach that produced several, consistently more soluble, analogues than the others. Furthermore, unfortunately it can be seen that increased solubility is generally coupled with greatly-reduced anti-proliferative activity; the only compound to have aqueous solubility better than that previously-reported and any notable anti-proliferative activity was **15e** (solubility: 3.51 μg/mL; IC_{50} 640 nM and 644 nM against HCT-116 and MDA-MB-231, respectively).

Three of the aforementioned analogues with increased thermodynamic solubility (**5b**, **17b** and **19b**) have 2-methyl substitution on the *N*-phenyl ring. Though unfortunately these compounds had negligible effect on cancer cell growth, it is of interest to note that this *N*-phenyl substitution pattern also results in analogues that have decreased melting points compared to that generally seen for thienopyridines, the majority of which melt at very high temperatures, though it should be noted that in this study the melting points were determined from non-recrystalised samples. The trend in melting points however is likely due to their extended planar shape resulting in a tightly packed crystal structure, held together by intermolecular forces that are difficult to disrupt. The relationship between melting point and solubility is well known,[9] and relates to the principle that the intermolecular forces holding the crystal structure together must be overcome for the compound to melt or dissolve, although this is a complicated relationship

Table 1
Anti-proliferative activity (at 1 μM) and IC_{50} value (nM) of Series 1 compounds against HCT-116 and MDA-MB-231 cancer cell lines. Thermodynamic aqueous solubility (μM/mL) of Series 1 compounds. * denotes sample degradation during solubility testing.

	Mean Relative Growth of 1μM in Cancer Cell Line (%)		IC_{50} nM		Thermodynamic Solubility (μg/mL)
	HCT-116	MDA-MB-231	HCT-116	MDA-MB-231	
4a	109.4	119.6			3.00
4b	109.5	97.1			1.49
4c	10.0	13.7	573	636	1.63
4d	82.9	84.6			0.13
4e	7.8	13.6	591	633	1.08
4f	96.8	99.7			1.52
4g	4.0	11.0	183	219	1.15
4h	102.6	113.8			0.35
4i	105.7	67.7			1.17
4j	2.1	8.9	422	506	0.68
4k	96.5	96.0			1.10
4l	87.3	99.6			0.13
4m	101.2	101.6			1.52
5a	95.3	107.4			3.51
5b	99.9	97.9			29.54
5c	2.2	11.7	72	76	2.41
5d	97.5	114.4			1.79
5e	1.7	9.9	171	81	2.21
15b	96.4	109.1			0.26
15c	2.3	4.0	193	150	0.77
15d	87.4	94.1			0.197
15e	4.7	19.3	640	644	3.97
15f	100.6	106.1			1.89
15g	1.0	3.5	151	122	0.61
15h	98.1	97.3			0.84
15i	69.7	61.5	>1000	>1000	1.31
15j	1.5	4.0	201	162	1.72
15k	85.2	90.7			0.30
15l	99.4	99.8			1.06
15m	89.9	80.5			0.213
16a	3.1	17.5			1.93*
17a	100.2	91.2			–
17b	101.0	95.3			5.61
17c	101.0	95.3			0.44*
17d	98.9	90.3			2.17
17e	93.5	89.0			3.06
18a	100.4	89.7			40.47
19a	91.5	98.9			1.03
19b	100.6	103.3			7.52
19c	78.1	50.9			1.15*
19d	80.1	83.9			3.19
19e	100.3	78.3			4.00

depending on many factors, including the polarity of the solvent. Based off this observation, it was thought that this information could be used in future analogue studies, where a lower melting point could be used as an indicator of increased thermodynamic solubility potential.

This increased solubility of compounds **5b**, **17b**, and **19b** was not predicted by calculated LogP values performed by ALOGPS 2.1 software[10], which predicted values very similar to many other thienopyridines in this series (Tables 2 and S1). The most soluble analogue, **18a**, was reflected in the predicted LogP, which was 2.21, the lowest predicted LogP in Series 1.

Compounds **4h**, **4l**, and **15b**, which had some of the lowest thermodynamic solubilities, did have a notable increase in calculated LogP values, reflecting their increased lipophilic nature. However, while **15b** has the highest calculated LogP value in this group, many other **15** analogues had higher LogP values despite having better aqueous solubility.

It was then decided to investigate an additional series, Series 2, carrying through the alkyl ring variations with the best activity (i.e. 5-acetyl thieno[2,3-*b*]pyridines **4** and 5-(1-hydroxyethyl) thieno[2,3-*b*] pyridines **5** along with *N*-benzyl-substituted thienopyridines **15**) and alter the phenyl aromatic moiety, replacing it with alternative heterocyclic systems. It was also decided that this approach would be used for the keto-containing cyclohexyl thienopyridines **23** that have been investigated previously, and to which the formerly-most soluble thienopyridine, **23a**, belongs.

2.3. Compound series 2 chemistry; synthesis of thieno[2,3-b]quinoline-2-carboxamide derivatives

Synthesis of the Series 2 compounds was straightforward, following the aforementioned synthetic route, albeit with novel acetamides **3n-w** to facilitate the introduction of these new heterocyclic motifs. Heterocycles chosen to investigate were a range of quinolines and isoquinolines, benzodioxanes and pyridines. Acetamides **3n-w** were generated in moderate to excellent yields through the reaction of the target aminoheterocycles **24n-w**, with chloroacetyl chloride in basic conditions (Scheme 3). These acetamides where then coupled with carbonitriles **2**, **12** and **25**, providing the desired thienopyridines **4n-w**, **15o-u** and **23n-w**, respectively. To access the final set of compounds in the series, the acetyl functionality in **4n-w** was reduced to give alcohols **5n-w**.

2.4. Anti-proliferative activity and aqueous solubility of Series 2 compounds

As for Series 1, the anti-proliferative activity and solubility of all synthesised compounds in Series 2 was analysed (Table 3). Surprisingly, it was found that very few of the analogues produced any activity of note and no compounds prevented the growth of cancer cells with efficacy close to that matching the first series. These results indicated that replacing a substituted phenyl motif for a more-polar heterocyclic alternative was extremely detrimental to the compound bioactivity.

Concerning the solubility of the Series 2 compounds, there was no observable, clear, improvement in solubility compared to Series 1 compounds, although there were compounds (**5o** and several of the *N*-benzyl-substituted thienopyridines **15**) that were shown to be more soluble than the previously-reported best thienopyridine **20a**. Unfortunately, none of these derivatives were effective as **15e**, from Series 1, in inhibiting the growth of cancer cells.

Table 2
Selected LogP and LogS values as calculated by ALOGPS 2.1[10].

	ALOGPS 2.1	
	LogP	LogS
5b	2.72	−4.66
17b	3.02	−4.49
19b	2.61	−4.63
4h	3.24	−5.08
4l	3.44	−5.02
15b	4.22	−5.34
15e	5.10	−5.77
18a	2.21	−4.54

The calculated LogP values for Series 2 indicated that the compounds containing the pyridine moiety on the *N*-phenyl ring (compounds **4**, **5** and **20v** and **w**) had the lowest values out of all compounds in Series 1 and 2, indicating they should have a higher affinity for the aqueous phase (Tables 4 and S1). Out of these, **5v** seemed to reflect this the most, with a thermodynamic solubility of 5.43 μg/mL.

The two standouts in terms of their thermodynamic solubility in Series 2 were **5o** and **5r** which gave the respective solubilities of 18.54 and 10.06 μg/mL. These compounds contain a secondary alcohol, a group that was introduced to increase hydrogen bonding. However, the calculated LogP and LogS values did not show any significant difference compared to other quinolines.

While these compounds in Series 2 did not produce results that would warrant further investigation, the synthesis and investigation of this series has allowed for us to rule out this as a potential avenue for modification in the future.

2.5. Chemical space

The calculated molecular descriptors MW (molecular weight), log *P* (water-octanol partition coefficient), HD (hydrogen bond donors), HA (hydrogen bond acceptors), PSA (polar surface area) and RB (rotatable bonds)) are given in Table S2. The MW of the ligands lies in the range of 324.4 and 507.4 g mol^{-1} and falls into Drug-like chemical space except for derivative **15g** reaching into Known Drug Space (KDS), **15g** has a bromine moiety explaining its high value. The Log *P* values are from 1.2 to 5.1 spanning the three defined volumes of chemical space; lead-like, drug-like and KDS. The other four descriptors all lie within drug-like chemical space except HD, which reaches into lead-like chemical space (for the definition of lead-like, drug-like and KDS regions (see Ref. 11 and Table S3).

The Known Drug Indexes (KDIs) for the ligands were calculated to gauge the balance of the molecular descriptors (MW, log P, HD, HA, PSA and RB). This method is based on the analysis of drugs in clinical use; the statistical distribution of each descriptor is fitted to a Gaussian function and normalized to 1 resulting in a weighted index.[12] Both the summation of the indexes (KDI_{2a}) and multiplication (KDI_{2b}) methods were used as shown for KDI_{2a} in Equation **1** and for KDI_{2b} in Equation **2**; the numerical results are given in Table S2 in the Supplementary section.

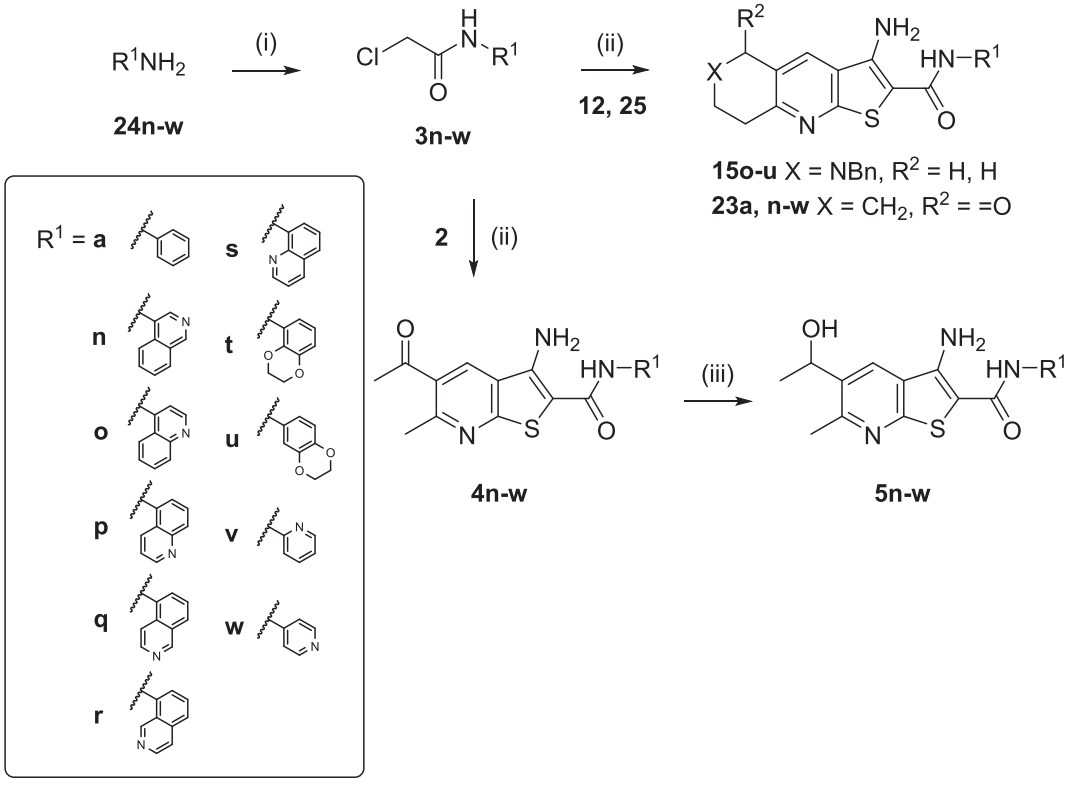
$$KDI_{2a} = I_{MW} + I_{\log P} + I_{HD} + I_{HA} + I_{RB} + I_{PSA} \quad (1)$$

$$KDI_{2b} = I_{MW} \times I_{\log P} \times I_{HD} \times I_{HA} \times I_{RB} \times I_{PSA} \quad (2)$$

The KDI_{2a} values for the ligands range from 5.49 to 5.98 with a theoretical maximum of 6 and the average of 4.08 for known drugs. KDI_{2b} range from 0.56 to 0.98, with a theoretical maximum of 1 and with KDS average of 0.18. For both indexes very good values are obtained for the ligands showing their excellent balance of physicochemical properties for biocompatibility. This is reflected in very active derivatives, e.g. **15c**, albeit better water solubility is desired. The conclusion can be drawn that all of the derivatives permeate through the cell membranes and only those inhibiting enzymes are active whereas the rest does not have a biological effect.

2.6. Summary

In this research, a number of strategies to improve the aqueous solubility whilst retaining potent anti-proliferative actions against cancer cell lines were investigated. This study led to the synthesis of 80 novel compounds, comprising two series, all based on the previously-reported thieno[2,3-*b*]pyridine core structure, modifying the structure of the appended alkyl ring (Series 1) and further amending the structure



Scheme 3. Synthesis of thienopyridines **4n-w**, **5n-w** **15o-u** and **23n-w**. *Reagents and conditions:* (i) chloroacetyl chloride (1 equiv.), Et_3N (1.2 equiv.), CH_2Cl_2, 1 h, 0 °C; 2 h, r.t., 18–97% (ii) Acetamides **3** (1 equiv.), Na_2CO_3 (2 equiv.), EtOH, reflux, 48 h, 29%-quant. (iii) $NaBH_4$ (1 equiv.), MeOH, THF, r.t., 2 h; H_2O, 68%-quant.

through the replacement of the phenyl ring with alternative heterocycles (Series 2). Overall, it was found that introducing the alternative heterocycles did not notably improve the solubility and in fact generally led to a large loss in anti-proliferative activity. It was discovered, however, that the best strategy for improving the solubility was the alteration of the appended alkyl ring to introduce polar groups such as alcohols, ketones and substituted amine groups. While this trend was not consistent, in the course of this study, we have discovered a thieno[2,3-*b*]pyridine, **15e**, with greater aqueous solubility that has ever been seen for this class of compounds that is also a potent inhibitor of cancer cell growth, with IC_{50}'s in the nanomolar range.

3. Experimental details and methodology

3.1. Synthesis of compounds

General details: All reactions were carried out under a nitrogen atmosphere in dry, freshly distilled solvents unless otherwise noted. All NMR spectra were recorded on either Bruker Avance DRX 300 MHz or 400 MHz spectrometers at ambient temperatures. Chemical shifts are reported relative to the solvent peak of chloroform (δ 7.26 for ^{1}H and δ 77.0 for ^{13}C), DMSO (δ 2.50 for ^{1}H and δ 39.5 for ^{13}C) or acetone (δ 2.05 for 1H and δ 29.8 for ^{13}C). ^{1}H NMR data is reported as position (δ), relative integral, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of triplets; dd, doublet of doublets; tt, triplet of triplets; 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.

General procedures:

*General procedure A: Synthesis of thieno[2,3-b]pyridine-2-carboxamide derivatives **4a-w, 15a-u, 16a, 17a-e, 19a-e** and **23a,n-w***

A mixture of carbonitriles **2**, **12**–**14, 20** and **25** (1 equiv.), acetamides **3a-w** (1 equiv.), and anhydrous sodium carbonate (1.06–2 equiv.) in absolute ethanol (4.00 mL per mmol acetamide) was stirred at reflux for 24–48 h. The ethanol was then removed *in vacuo* and the remaining residue recrystallised from methanol to give the thieno[2,3-*b*] pyridine-2-carboxamide derivatives **4a-w**, **15a-u**, **16a**, **17a-e**, **19a-e** and **23a**,***n*-w**.

*General procedure B: Synthesis of thieno[2,3-b]pyridine-2-carboxamide alcohols **5a-e** and **5n-w***

To a solution of ketones **4a-e** and **4n-w** (1 equiv.) in dry THF was added a solution of sodium borohydride (1 equiv.) in methanol, dropwise, over 15 min. The mixture was stirred at r.t. for 2 h, water added and stirred for a further 5 min before the volatiles were removed *in vacuo*. The residue was diluted with water, extracted with ethyl acetate, washed with H_2O and dried with $MgSO_4$ to give the thieno[2,3-*b*]pyridine-2-carboxamide alcohols **5a-e** and **5n-w**.

*General procedure C: Synthesis of carbonitriles **13, 14** and **20***

To a mix of sodium metal (1 equiv.) in dry ether (1 mL/mmol ketone) under an atmosphere of N_2, was added dropwise a solution of ketones **7**, **8** and **16** (1 equiv.), and ethyl formate (1 equiv.) in ether. Absolute ethanol (cat.) was then added as an initiator and the mixture stirred at r. t. for 24–72 h. The resulting solid was filtered, washed with ether and dried *in vacuo* to give the sodium enolate salts **10**, **11** and **18**, which were used without further purification. A mixture of sodium enolate salts **10**, **11** and **18** (1 equiv.), cyanothioacetamide (1 equiv.), and piperidinium acetate solution [20% acetic acid, 45% water, 35% piperidine] in water was heated at reflux for 24 h before being acidified with acetic acid while hot. The reaction mixture was allowed to cool to r.t. and stirred for a further 12 h before the residue was filtered off, washed with ice water and collected to give the carbonitriles **13**, **14** and **20**.

*General procedure D: Synthesis of acetamides **3n-w***

To a solution of a substituted aniline **21n-w** (1 equiv.) and triethylamine (1.2 equiv.) in CH_2Cl_2 at 0 °C was added chloroacetyl chloride

Table 3
Anti-proliferative activity (at 1 μM) and IC_{50} (nM) of Series 2 compounds against HCT-116 and MDA-MB-231 cancer cell lines. Thermodynamic aqueous solubility (μM/mL) of Series 2 compounds. * denotes sample degradation during solubility testing.

	Mean Relative Growth of 1μM in Cancer Cell Line (%)		Thermodynamic Solubility (μg/mL)
	HCT-116	MDA-MB-231	
4n	96.2	105.0	0.09
4o	101.7	91.6	0.26*
4p	93.0	98.7	1.30
4q	111.0	99.8	–
4r	108.3	100.6	1.06
4s	97.0	86.7	3.00
4t	72.5	103.8	0.26*
4u	103.9	102.7	0.19
4v	91.7	92.9	0.45
4w	103.4	97.2	0.45
5n	108.9	106.1	–
5o	93.6	107.1	18.54
5p	92.0	112.2	0.89
5q	110.3	101.9	7.55
5r	83.1	94.6	10.06
5s	109.5	101.9	–
5t	58.4	62.3	3.06
5u	94.9	104.7	0.27
5v	96.5	93.8	5.43
5w	110.9	112.8	2.85
15o	82.8	104.1	3.83
15p	103.9	91.3	0.62
15q	109.9	108.1	1.12
15r	102.8	107.6	2.79
15s	98.2	103.7	1.17
15t	88.2	89.3	2.10
15u	83.2	94.9	0.70
23n	97.8	110.6	0.71
23o	100.7	107.2	0.64
23p	96.3	96.9	0.41
23q	102.7	107.0	1.67
23r	98.1	115.0	1.94
23s	100.6	99.2	0.44
23t	18.6	17.8	0.12
23u	76.2	101.9	0.32
23v	10.2	27.0	0.64
23w	110.1	107.8	1.54

Table 4
Selected LogP and LogS values as calculated by ALOGPS 2.1[n].

	ALOGPS 2.1	
	LogP	LogS
4v	2.22	−4.38
4w	1.94	−4.39
5o	2.84	−5.00
5r	2.63	−4.99
5t	2.33	−4.29
5v	2.03	−4.20
5w	1.85	−4.23
23v	2.27	−4.35
23w	1.82	−4.36

(1.2 equiv.) over 15 min and the reaction stirred for 1 h at 0 °C, followed by 2 h at r.t. The salt was filtered off and washed with CH_2Cl_2 and EtOAc. This was dissolved in 1:1 MeOH/H_2O, basified with sat. $NaHCO_3$ and extracted with CH_2Cl_2 followed by drying with Na_2SO_4. The solvent was removed *in vacuo* to give the acetamides **3n-w**.

5-Acetyl-6-methyl-2-thioxo-1,2-dihydropyridine-3-carbonitrile ***2***. A mixture of acetylacetone **1** (1.00 mL, 10.0 mmol) and *N,N*-dimethylformamide dimethyl acetal (1.33 mL, 10.0 mmol) in dry dioxane (10.0 mL) was stirred under an atmosphere of nitrogen at r.t. overnight. A second mixture of sodium methoxide was prepared by adding sodium (0.46 g, 20.0 mmol) to dry methanol (10.5 mL, 0.260 mol). Cyanothioacetamide (1.00 g, 10.0 mmol) was added to the sodium methoxide and stirred for 10 min, after which the thioacetamide mixture was added to the acetone mixture and stirred for 1 h at r.t. This was followed by heating at reflux for 4 h. After cooling, the mixture was acidified with ice cold 2 M HCl and water (10 mL) added. The resulting solid was collected by filtration to give the *title product* **2** (1.86 g, 97%) as a light brown solid. m.p. 200–202 °C. Lit. 230–232 °C.[1] ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 2.51 (3H, s, $COCH_3$), 2.64 (3H, s, 6-CH_3), 8.55 (1H, s, H-4), 14.26 (1H, br s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) 19.5 (6-CH_3), 29.0 ($CO\underline{C}H_3$), 113.3 (C-3), 116.6 (CN), 121.1 (C-5), 145.0 (C-4), 157.4 (C-6), 179.2 (C-2), 195.5 ($\underline{C}OCH_3$); IR: ν_{max} (film)/cm^{-1}: 3191, 3021, 2228, 1662, 1589, 1494, 1267; *m/z* (ESI$^+$): 215 (MNa^+, 25%), 163 (55%), 101 (100%); HRMS (ESI$^+$) found (MNa^+): 215.0243 $C_9H_8N_2NaOS$ requires 215.0250.

6-Benzyl-2-thioxo-1,2,5,6,7,8-hexahydro-1,6-naphthyridine-3-carbonitrile ***12***. A solution of substituted 1-benzylpiperidin-4-one **6** (0.470 mL, 2.64 mmol), and ethyl formate (0.210 mL, 2.64 mmol), was added dropwise over 30 min to a solution of sodium methoxide which was prepared by adding sodium metal (60.0 mg, 2.64 mmol) to dry methanol (0.600 mL, 14.0 mmol). The mixture was stirred at r.t. overnight, then diluted with ether and filtered to give the enolate salt **9** (0.33 g, 51%) as a yellow solid which was used immediately without further purification. To enolate salt **9** (0.20 g, 0.836 mmol) was added cyanothioacetamide (84.0 mg, 0.84 mmol) in water (4.13 mL) followed by piperidinium acetate solution (0.79 mL, 20% acetic acid, 45% water, 35% piperidine), which was heated at reflux for 24 h before being acidified with acetic acid while hot. The reaction mixture was allowed to cool to r.t. and stirred for a further 12 h before the residue was filtered off, washed with ice water and collected to give the *title compound* **12** (0.12 g, 49%) as a brown solid. m.p. 179–181 °C. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 2.76–2.82 (4H, m, H-7 and H-8), 3.40 (2H, s, H-5), 3.72 (2H, s, H-6′′), 7.30–7.46 (5H, m, Ar-CH), 7.90 (1H, s, H-4), 14.10 (1H, br s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) 27.0 (C-8), 47.7 (C-7), 51.5 (C-5), 60.7 (C-6′′), 113.6 (C-3), 117.1 (CN), 119.7 (C-4a), 127.3 (C-4′), 128.4 (C-3′ and C-5′), 128.8 (C-2′ and C-6′), 137.8 (C-1′), 143.6 (C-4), 150.9 (C-8a), 176.0 (C-2); ν_{max} (film)/cm^{-1} 2826, 2221, 1597, 1496, 1203, 1174; *m/z* (ESI$^+$): 282 (MH^+, 100%), 227 (10%); HRMS (ESI$^+$) found (MH^+): 282.1059 $C_{16}H_{16}N_3S$ requires 282.1059.

*3-Amino-N-phenyl-5,6,7,8-tetrahydrothieno[2,3-*b*][1,6]naphthyridine-2-carboxamide* ***18a***. A solution of carbamate **16a** (0.50 g, 1.09 mmol) in 12 M HCl (150 mL) was stirred for 96 h followed by basifying with 4 M NaOH solution. The mixture was extracted with EtOAc (200 mL), washed with H_2O (200 mL), brine (200 mL), dried (Na_2SO_4) and the solvent removed *in vacuo* to give the *title compound* **18a** (0.074 g, 24%) as a yellow solid. m.p. 217–219 °C. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 2.93 (2H, t, $J = 5.9$ Hz, H-8), 3.10 (2H, t, $J = 5.9$ Hz, H-7), 4.01 (2H, s, H-5), 7.06 (1H, t, $J = 7.5$ Hz, H-4′), 7.29–7.33 (4H, m, H-3′, H-5′ and NH_2), 7.68 (2H, d, $J = 7.5$ Hz, H-2′ and H-6′), 8.15 (1H, s, H-4), 9.36 (1H, br s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) 32.4 (C-8), 43.0 (C-7), 47.1 (C-5), 95.9 (C-2), 121.1 (C-2′ and C-6′), 123.4 (C-4′), 124.3 (C-3a), 127.8 (C-4a), 128.2 (C-4), 128.4 (C-3′ and C-5′), 139.0 (C-1′), 146.9 (C-3), 156.3 (C-9a), 157.4 (C-8a), 164.1 (2-CONH); IR: ν_{max} (film)/cm^{-1} 3445, 3292, 2924, 2854, 1589, 1522, 1439, 1255, 1064; *m/z* (ESI$^+$): 325 (MH^+, 100%), 232 (10%); HRMS (ESI$^+$) found (MH^+): 325.1110 $C_{17}H_{17}N_4OS$ requires 325.1118.

5-Acetyl-3-amino-6-methyl-N-phenylthieno[2,3-b]pyridine-2-carboxamide ***4a***. The reaction was carried out following general procedure A using carbonitrile **2** (100 mg, 0.52 mmol), acetamide **3a** (110 mg, 0.52 mmol) and anhydrous sodium carbonate (60.0 mg, 0.55 mmol) in absolute ethanol (4.00 mL) to give the *title compound* **4a** (116 mg, 69%) as a light brown solid. m.p. 228–230 °C. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 2.66 (3H, s, 5-$COCH_3$), 2.75 (3H, s, 6-CH_3), 7.07 (1H, t, $J = 7.8$ Hz, H-4′), 7.32 (2H, t, $J = 7.8$ Hz, H-3′ and H-5′), 7.50 (2H, br s, NH_2), 7.68 (2H, d, $J = 7.8$ Hz, H-2′ and H-6′), 9.05 (1H, s, H-4), 9.40 (1H, br s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) 25.1 (6-CH_3), 29.2 (5-$CO\underline{C}H_3$), 96.5 (C-2), 121.1 (C-2′ and C-6′), 123.4 (C-4′), 123.8 (C-3a), 128.37 (C-3′ and C-5′),

128.42 (C-5), 132.9 (C-4), 139.0 (C-1′), 147.0 (C-3), 158.5 (C-6), 160.0 (C-7a), 163.7 (2-CONH), 199.7 (5-COCH$_3$); IR: ν_{max} (film)/cm^{-1} 3396, 3300, 2981, 1682, 1590, 1444, 1255, 1100; *m/z* (ESI$^+$): 348 (MNa$^+$, 80%), 302 (50%), 261 (25%), 227 (100%), 217 (22%); HRMS (ESI$^+$) found (MNa$^+$): 348.0774 $C_{17}H_{15}N_3NaO_2S$ requires 348.0777.

5-Acetyl-3-amino-N-*(isoquinolin-4′-yl)-6-methylthieno[2,3-b]pyridine-2-carboxamide* ***4n***. The reaction was carried out following general procedure A using carbonitrile **2** (44.0 mg, 0.23 mmol), acetamide **3n** (50.0 mg, 0.23 mmol) and anhydrous sodium carbonate (36.0 mg, 0.34 mmol) in absolute ethanol (3.00 mL) for 48 h to give the *title compound* **4n** (58.0 mg, 68%) as a mustard yellow solid. m.p. > 230 °C. ^{1}H NMR (400 MHz, (CD$_3$)$_2$SO) 2.67 (3H, s, 5-COCH$_3$), 2.77 (3H, s, 6-CH$_3$), 7.45 (2H, br s, NH$_2$), 7.69 (1H, t, *J* = 7.3 Hz, H-7′), 7.79 (1H, t, *J* = 7.3 Hz, H-6′), 8.00 (1H, d, *J* = 7.3 Hz, H-5′), 8.15 (1H, d, *J* = 7.3 Hz, H-8′), 8.61 (1H, s, H-3′), 9.04 (1H, s, H-4), 9.17 (1H, s, H-1′), 9.83 (1H, br s, NH); ^{13}C NMR (100 MHz, (CD$_3$)$_2$SO) 25.1 (6-CH$_3$), 29.2 (5-COCH$_3$), 122.9 (C-5′), 124.0 (C-3a), 127.2 (C-7′), 127.4 (C-8′), 128.3 (C-5), 128.5 (C-6′), 129.8 (C-4′ and C-8′a), 132.2 (C-4′a), 132.8 (C-4), 140.0 (C-3′), 146.2 (C-3), 148.9 (C-1′), 157.1 (C-6), 160.2 (C-7a), 165.0 (2-CONH), 199.7 (5-CO). C-2 not observed; IR: ν_{max} (film)/cm^{-1} 3426, 3264, 2981, 2886, 1683, 1586, 1541, 1484, 1248, 1103; *m/z* (ESI$^+$): 399 (MNa$^+$, 100%); HRMS (ESI$^+$) found (MNa$^+$): 399.0891 $C_{20}H_{16}N_4NaO_2S$ requires 399.0886.

5-Acetyl-3-amino-N-*(2′,3′-dihydrobenzo[b][1′,4′]dioxin-5′-yl)-6-methylthieno[2,3-b]pyridine-2-carboxamide* ***4t***. The reaction was carried out following general procedure A using carbonitrile **2** (68.0 mg, 0.35 mmol), acetamide **3t** (80.0 mg, 0.35 mmol) and anhydrous sodium carbonate (74.0 mg, 0.70 mmol) in absolute ethanol (3.00 mL) for 48 h to give the *title compound* **4t** (106.0 mg, 82%) as a light brown solid. m.p. > 230 °C. ^{1}H NMR (400 MHz, (CD$_3$)$_2$SO) 2.66 (3H, s, 5-COCH$_3$), 2.75 (3H, s, 6-CH$_3$), 4.25–4.27 (2H, m, H-2′), 4.31–4.33 (2H, m, H-3′), 6.70 (1H, dd, *J* = 8.2, 1.5 Hz, H-8′), 6.81 (1H, t, *J* = 8.2 Hz, H-7′), 7.33 (1H, dd, *J* = 8.2, 1.5 Hz, H-6′), 7.42 (2H, br s, NH$_2$), 8.56 (1H, br s, NH), 9.07 (1H, s, H-4); ^{13}C NMR (100 MHz, (CD$_3$)$_2$SO) 25.1 (6-CH$_3$), 29.2 (5-COCH$_3$), 63.9 (C-2′), 64.4 (C-3′), 96.8 (C-2), 113.2 (C-8′), 116.1 (C-6′), 120.0 (C-7′), 124.0 (C-3a), 127.0 (C-5′), 128.5 (C-5), 133.1 (C-4), 136.0 (C-4′a), 143.5 (C-8′a), 146.6 (C-3), 158.5 (C-6), 159.6 (C-7a), 163.1 (2-CONH), 199.7 (5-CO); IR: ν_{max} (film)/cm^{-1} 3414, 3298, 2893, 1687, 1611, 1586, 1446, 1286, 1077, 1088; *m/z* (ESI$^+$): 406 (MNa$^+$, 100%); HRMS (ESI$^+$) found (MNa$^+$): 406.0837 $C_{19}H_{17}N_3NaO_4S$ requires 406.0832.

5-Acetyl-3-amino-6-methyl-N-*(pyridin-2′-yl)thieno[2,3-b]pyridine-2-carboxamide* ***4v***. The reaction was carried out following general procedure A using acetamide **3v** (89.0 mg, 0.52 mmol), carbonitrile **2** (0.10 g, 0.52 mmol) and anhydrous sodium carbonate (0.11 g, 1.0 mmol) in absolute ethanol (2.0 mL) to give the *title compound* **4v** (0.133 g, 78%) as a beige solid. m.p. > 230 °C. ^{1}H NMR (400 MHz, (CD$_3$)$_2$SO) 2.66 (3H, s, COCH$_3$), 2.75 (3H, s, 6-CH$_3$), 7.13 (1H, dt, *J* = 5.0, 1.4 Hz, H-5′), 7.57 (2H, br s, NH$_2$), 7.81 (1H, dt, *J* = 8.6, 1.4 Hz, H-4′), 8.05 (1H, d, *J* = 8.4 Hz, H-3′), 8.36 (1H, dd, *J* = 5.0, 1.4 Hz, H-6′), 9.08 (1H, s, H-4), 9.77 (1H, br s, NH); ^{13}C NMR (100 MHz, (CD$_3$)$_2$SO) 25.2 (6-CH$_3$), 29.2 (COCH$_3$), 96.4 (C-2), 114.8 (C-3′), 119.5 (C-5′), 123.7 (C-3a), 128.4 (C-5), 133.2 (C-4), 137.9 (C-4′), 147.7 and 147.9 (C-3 and C-6′), 152.0 (C-2′), 158.8 (C-6), 160.3 (C-7a), 163.8 (C═O), 199.6 (*C*OCH$_3$); IR: ν_{max}(film)/cm^{-1} 3364, 3180, 2926, 1683, 1619, 1596, 1573, 1456, 1428, 994, 779; *m/z* (ESI): 349 (MNa$^+$, 94%), 327 (MH$^+$, 47%), 233 ($C_{11}H_9N_2O_2S^+$, 57%), 207 ($C_{10}H_{11}N_2OS^+$, 100%), 121 ($C_6H_5N_2O^+$, 82%), 95 ($C_5H_7N_2^+$, 7%); HRMS (ESI$^+$) found (MH$^+$): 327.0908, $C_{16}H_{15}N_4O_2S$ requires 327.0910.

*3-Amino-6-benzyl-N-phenyl-5,6,7,8-tetrahydrothieno[2,3-*b*][1,6]naphthyridine-2-carboxamide* ***15a***. The reaction was carried out following general procedure A using carbonitrile **12** (50.0 mg, 0.178 mmol), acetamide **3a** (38.0 mg, 0.178 mmol) and anhydrous sodium carbonate (20.0 mg, 0.188 mmol) in absolute ethanol (2.00 mL) to give the *title compound* **15a** (25.0 mg, 34%) as a dark brown solid. m.p. > 230 °C. ^{1}H NMR (400 MHz, (CD$_3$)$_2$SO) 2.85 (2H, t, *J* = 5.3 Hz, H-7), 3.03 (2H, t, *J* = 5.3 Hz, H-8), 3.67 (2H, s, H-5), 3.72 (2H, s, H-6′′′), 7.06 (1H, t, *J* = 7.5 Hz, H-4′), 7.26 (2H, br s, NH$_2$), 7.28 (2H, m, H-3′ and H-5′), 7.30–7.37 (5H, m, 5 × Ar-CH), 7.67 (2H, d, *J* = 7.5 Hz, H-2′ and H-6′), 8.14 (1H, s, H-4), 9.36 (1H, br s, NH); ^{13}C NMR (100 MHz, (CD$_3$)$_2$SO) 32.2 (C-8), 49.9 (C-7), 54.5 (C-5), 61.3 (C-6′′′), 96.0 (C-2), 121.1 (C-2′ and C-6′), 123.3 (C-4′), 124.2 (C-3a), 126.3 (C-4a), 127.1 (C-4′′), 128.29 (C-3′, C-5′ and 2 × Ar-C), 128.34 (2 × Ar-C), 128.8 (C-4), 138.1 (C-1′′), 139.0 (C-1′), 146.8 (C-3), 156.6 (C-9a), 156.7 (C–8a), 164.0 (2-CONH); IR: ν_{max} (film)/cm^{-1} 3441, 3332, 3027, 2814, 1606, 1587, 1254, 1106; *m/z* (ESI$^+$): 415 (MH$^+$, 100%), 227 (10%); HRMS (ESI$^+$) found (MH$^+$): 415.1576 $C_{24}H_{23}N_4OS$ requires 415.1587.

*Benzyl 3-amino-2-(phenylcarbamoyl)-7,8-dihydrothieno[2,3-*b*][1,6]naphthyridine-6(5H)-carboxylate* ***16a***. The reaction was carried out following general procedure A using carbonitrile **13** (0.20 g, 0.62 mmol), acetamide **3a** (0.13 g, 0.62 mmol) and anhydrous sodium carbonate (0.13 g, 1.23 mmol) in absolute ethanol (2.50 mL) to give the *title compound* **16a** (0.09 g, 33%) as a light grey solid. m.p. > 230 °C. ^{1}H NMR (400 MHz, (CD$_3$)$_2$SO) 3.06 (2H, t, *J* = 5.9 Hz, H-8), 3.80 (2H, br s, H-7), 4.74 (2H, br s, H-5), 5.15 (2H, s, OCH$_2$), 7.06 (1H, t, *J* = 7.5 Hz, H-4′), 7.28–7.41 (9H, m, H-3′, H-5′, H-2′′, H-3′′, H-4′′, H-5′′, H-6′′ and NH$_2$), 7.67 (2H, d, *J* = 7.5 Hz, H-2′ and H-6′), 8.30 (1H, s, H-4), 9.40 (1H, br s, NH); ^{13}C NMR (100 MHz, (CD$_3$)$_2$SO) 31.8 (C-8), 44.8 (C-7), 53.2 (C-5), 66.5 (OCH$_2$), 121.1 (C-2′, C-6′ and C-3a), 123.3 (C-4′), 124.6 (C-4a), 127.6 (C-2′′ and C-6′′), 127.9 (C-4′′), 128.3 and 128.4 (C-3′, C-5′, C-3′′ and C-5′′), 128.6 (C-4), 136.8 (C-1′′), 139.0 (C-1′), 146.6 (C-3), 154.7 (6-C═O), 156.3 (C-8a), 156.7 (C-9a), 163.9 (2-CONH). C-2 not observed; IR: ν_{max} (film)/cm^{-1} 3497, 3231, 3030, 2938, 1687, 1590, 1256, 1209, 1113; *m/z* (ESI$^+$): 481 (MNa$^+$, 100%), 201 (40%); HRMS (ESI$^+$) found (MNa$^+$): 481.1296 $C_{25}H_{22}N_4NaO_3S$ requires 481.1305.

*3-Amino-6-methyl-N-phenyl-5,6,7,8-tetrahydrothieno[2,3-*b*][1,6]naphthyridine-2-carboxamide* ***17a***. The reaction was carried out following general procedure A using carbonitrile **14** (0.20 g, 0.97 mmol), acetamide **3a** (0.21 g, 0.97 mmol) and anhydrous sodium carbonate (0.21 g, 1.95 mmol) in absolute ethanol (4.00 mL) to give the *title compound* **17a** (0.14 g, 44%) as a brown solid. m.p. > 230 °C. ^{1}H NMR (400 MHz, (CD$_3$)$_2$SO) 2.40 (3H, s, NCH$_3$), 2.75 (2H, t, *J* = 5.8 Hz, H-7), 3.04 (2H, t, *J* = 5.8 Hz, H-8), 3.63 (2H, s, H-5), 7.05 (1H, t, *J* = 7.5 Hz, H-4′), 7.30 (4H, m, H-3′, H-5′ and NH$_2$), 7.67 (2H, d, *J* = 7.5 Hz, H-2′ and H-6′), 8.16 (1H, s, H-4), 9.38 (1H, br s, NH); ^{13}C NMR (100 MHz, (CD$_3$)$_2$SO) 32.3 (C-8), 45.4 (NCH$_3$), 52.1 (C-7), 56.6 (C-5), 121.1 (C-2′ and C-6′), 123.2 (C-4′), 124.2 (C-3a), 126.4 (C-4a), 128.3 (C-3′ and C-5′), 128.4 (C-4), 139.1 (C-1′), 146.7 (C-3), 156.6 (C-8a and C-9a), 164.0 (2-CONH). C-2 not observed; IR: ν_{max} (film)/cm^{-1} 3142, 3310, 2939, 1589, 1520, 1437, 1254, 1116; *m/z* (ESI$^+$): 361 (MNa$^+$, 100%); HRMS (ESI$^+$) found (MNa$^+$): 361.1082 $C_{18}H_{18}N_4NaOS$ requires 361.1094.

3-Amino-N-phenyl-7,8-dihydro-5H-pyrano[4,3-b]thieno[3,2-e]pyridine-2-carboxamide ***19a***. The reaction was carried out following general procedure A using carbonitrile **20** (0.10 g, 0.520 mmol), acetamide **3a** (0.11 g, 0.520 mmol) and anhydrous sodium carbonate (0.11 g, 1.04 mmol) in absolute ethanol (2.00 mL) to give the *title compound* **19a** (0.131 g, 77%) as a brown solid. m.p. > 230 °C. ^{1}H NMR (400 MHz, (CD$_3$)$_2$SO) 3.04 (2H, t, *J* = 5.8 Hz, H-8), 4.04 (2H, t, *J* = 5.8 Hz, H-7), 4.85 (2H, s, H-5), 7.07 (1H, t, *J* = 7.5 Hz, H-4′), 7.29–7.33 (4H, m, H-3′, H-5′ and NH$_2$), 7.68 (2H, d, *J* = 7.5 Hz, H-2′ and H-6′), 8.18 (1H, s, H-4), 9.40 (1H, br s, NH); ^{13}C NMR (100 MHz, (CD$_3$)$_2$SO) 31.7 (C-8), 64.7 (C-7), 66.6 (C-5), 96.2 (C-2), 121.1 (C-2′ and C-6′), 123.3 (C-4′), 124.4 (C-3a), 126.6 (C-4a), 126.9 (C-4), 128.4 (C-3′ and C-5′), 139.0 (C-1′), 146.8 (C-3), 155.3 (C-8a), 156.8 (C-9a), 164.0 (2-CONH); IR: ν_{max} (film)/cm^{-1} 3430, 3323, 2938, 1593, 1436, 1237, 1107; *m/z* (ESI$^+$): 348 (MNa$^+$, 100%), 227 (40%), 159 (20%), 101 (18%); HRMS (ESI$^+$) found (MNa$^+$): 348.0774 $C_{17}H_{15}N_3NaO_2S$ requires 348.0777.

3-Amino-5-(1-hydroxyethyl)-6-methyl-N-phenylthieno[2,3-b]pyridine-2-carboxamide ***5a***. The reaction was carried out following general procedure B using ketone **4a** (0.05 g, 0.15 mmol), $NaBH_4$ (6.00 mg, 0.15 mmol) and methanol (0.51 mL) in THF (7.70 mL) to give the *title compound* **5a** (32.0 mg, 64%) as a yellow solid. m.p. > 230 °C. ^{1}H NMR (400 MHz, (CD$_3$)$_2$SO) 1.40 (3H, d, *J* = 6.4 Hz, CHCH$_3$), 2.61 (3H, s, 6-CH$_3$),

5.02 (1H, dq, $J = 6.4$, 3.6 Hz, CHOH), 5.34 (1H, d, $J = 3.6$ Hz, CHOH), 7.06 (1H, t, $J = 7.6$ Hz, H-4′), 7.31 (2H, d, $J = 7.6$ Hz, H-3′ and H-5′), 7.41 (2H, br s, NH_2), 7.68 (2H, d, $J = 7.6$ Hz, H-2′ and H-6′), 8.54 (1H, s, H-4), 9.34 (1H, br s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) 22.2 (6-CH_3), 24.2 (CHCH_3), 64.5 (CHOH), 95.6 (C-2), 121.1 (C-2′ and C-6′), 123.3 (C-4′), 124.5 (C-3a), 127.7 (C-4), 128.3 (C-3′ and C-5′), 136.9 (C-5), 139.0 (C-1′), 147.3 (C-3), 156.2 (C-6), 156.8 (C-7a), 164.0 (2-CONH); IR: ν_{max} (ATR)/cm^{-1} 3308, 3161, 2925, 1589, 1495, 1260, 1092; *m/z* (ESI$^+$): 350 (MNa^+, 100%); HRMS (ESI$^+$) found (MNa^+): 350.0926 $C_{17}H_{17}N_3NaO_2S$ requires 350.0934.

*3-Amino-5-(1-hydroxyethyl)-*N*-(isoquinolin-4′-yl)-6-methylthieno[2,3-b]pyridine-2-carboxamide* ***5n***. The reaction was carried out following general procedure B using ketone **4n** (25.0 mg, 0.07 mmol), $NaBH_4$ (3.80 mg, 0.10 mmol) and methanol (0.33 mL) in THF (3.3 mL) to give the *title compound* **5n** (21.0 mg, 80%) as a yellow semi-solid. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 1.41 (3H, d, $J = 6.4$ Hz, 5-CHCH_3), 2.63 (3H, s, 6-CH_3), 5.05 (1H, dq, $J = 6.4$, 3.8 Hz, CHOH), 5.38 (1H, d, $J = 3.8$ Hz, CHOH), 7.39 (2H, br s, NH_2), 7.71 (1H, tt, $J = 7.0$, 1.4 Hz, H-7′), 7.82 (1H, tt, $J = 7.0$, 1.4 Hz, H-6′), 7.92 (1H, d, $J = 7.0$ Hz, H-5′), 8.18 (1H, d, $J = 7.0$ Hz, H-8′), 8.52 (1H, s, H-3′), 8.57 (1H, s, H-4), 9.23 (1H, s, H-1′), 9.72 (1H, br s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) 22.3 (6-CH_3), 24.2 (CHCH_3), 64.5 (CHOH), 95.3 (C-2), 122.8 (C-5′), 124.5 (C-3a), 127.4 (C-7′), 127.6 (C-8′), 127.8 (C-4), 128.5 (C-8′a), 129.1 (C-4′), 130.3 (C-6′), 132.3 (C-4′a), 137.0 (C-5), 140.8 (C-3′), 147.5 (C-3), 150.2 (C-1′), 156.4 (C-7a), 156.9 (C-6), 165.1 (2-CONH); IR; ν_{max} (film)/cm^{-1} 3401, 3292, 2958, 2852, 1604, 1500, 1410, 1258, 1095, 1023; *m/z* (ESI$^+$): 401 (MNa^+, 100%); HRMS (ESI$^+$) found (MNa^+): 401.1054 $C_{20}H_{18}N_4NaO_2S$ requires 401.1043.

*3-Amino-*N*-(2′,3′-dihydrobenzo[*b*][1′,4′]dioxin-5′-yl)-5-(1-hydroxyethyl)-6-methylthieno[2,3-b]pyridine-2-carboxamide* ***5t***. The reaction was carried out following general procedure B using ketone **4t** (40.0 mg, 0.10 mmol), $NaBH_4$ (6.00 mg, 0.16 mmol) and methanol (0.51 mL) in THF (5.2 mL) to give the *title compound* **5t** (30.0 mg, 75%) as a yellow solid. m.p. > 230 °C. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 1.40 (3H, d, $J = 6.3$ Hz, 5-CHCH_3), 2.61 (3H, s, 6-CH_3), 4.26–4.28 (2H, m, H-2′), 4.32–4.34 (2H, m, H-3′), 5.02 (1H, dq, $J = 6.3$, 3.6 Hz, CHOH), 5.36 (1H, d, $J = 3.6$ Hz, CHOH), 6.68 (1H, dd, $J = 8.0$, 1.6 Hz, H-8′), 6.80 (1H, t, $J = 8.0$ Hz, H-7′), 7.34 (2H, br s, NH_2), 7.40 (1H, dd, $J = 8.0$, 1.6 Hz, H-6′), 8.35 (1H, br s, NH), 8.55 (1H, s, H-4); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) 22.2 (6-CH_3), 24.2 (CHCH_3), 63.9 (C-2′), 64.4 (C-3′ and CHOH), 95.9 (C-2), 112.8 (C-8′), 115.4 (C-6′), 120.0 (C-7′), 124.7 (C-3a), 127.2 (C-5′), 127.9 (C-4), 135.4 (C-4′a), 137.1 (C-5), 143.4 (C-8′a), 146.9 (C-3), 155.8 (C-7a), 156.9 (C-6), 163.4 (2-CONH); IR: ν_{max} (film)/cm^{-1} 3406, 3395, 3152, 2922, 2853, 1613, 1536, 1442, 1249, 1099, 1074; *m/z* (ESI$^+$): 408 (MNa^+, 100%); HRMS (ESI$^+$) found (MNa^+): 408.0985 $C_{19}H_{19}N_3NaO_4S$ requires 408.0988.

*3-Amino-5-(1-hydroxyethyl)-6-methyl-*N*-(pyridin-2′-yl)thieno[2,3-b]pyridine-2-carboxamide* ***5v***. The reaction was carried out following general procedure B using ketone **4v** (50.0 g, 0.15 mmol), $NaBH_4$ (6.0 mg, 0.15 mmol), methanol (0.50 mL) in THF (8.0 mL) to give the *title compound* **5v** (63.0 mg, quant.) as a bright yellow solid. m.p. > 230 °C. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 1.41 (3H, s, CHCH_3), 2.61 (3H, s, 6-CH_3), 5.02 (1H, q, $J = 3.6$ Hz, 5-CH), 5.36 (1H, d, $J = 3.6$ Hz, OH), 7.12 (1H, dt, $J = 5.0$, 2.0 Hz, H-5′), 7.48 (2H, br s, NH_2), 7.80 (1H, dt, $J = 8.0$, 2.0 Hz, H-4′), 8.05 (1H, d, $J = 8.0$ Hz, H-3′), 8.34 (1H, dd, $J = 5.0$, 0.4 Hz, H-6′), 8.57 (1H, s, H-4), 9.46 (1H, br s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) 22.3 (6-CH_3), 24.2 (CHCH_3), 64.4 (CHOH), 95.7 (C-2), 114.7 (C-3′), 119.3 (C-5′), 124.4 (C-3a), 127.9 (C-4), 137.0 (C-5), 137.9 (C-4′), 147.9 and 148.0 (C-3 and C-6′), 152.0 (C-2′), 156.4 (C-6), 157.1 (C-7a), 164.1 (C═O); IR: ν_{max}(film)/cm^{-1} 3437, 3323, 2923, 1640, 1576, 1513, 1426, 1301, 1090, 765; *m/z* (ESI): 351 (MNa^+, 54%), 329 (MH^+, 31%), 235 ($C_{11}H_{11}N_2O_2S^+$, 70%), 209 ($C_{10}H_{13}N_2OS^+$, 100%), 121 ($C_6H_5N_2O^+$, 43%); HRMS (ESI$^+$) found (MH^+): 329.1062, $C_{16}H_{17}N_4O_2S$ requires 329.1067.

6-Benzyl-2-thioxo-1,2,5,6,7,8-hexahydro-1,6-naphthyridine-3-carbonitrile ***13***. The reaction was carried out following general procedure C using benzyl 4-oxopiperidine-1-carboxylate **7** (4.00 g, 17.0 mmol), ethyl formate (1.39 mL, 17.0 mmol), sodium metal (400 mg, 17.0 mmol), dry ether (94.0 mL) and absolute ethanol (0.1 mL) to give enolate salt **10** (2.90 g, 59%) as a pink solid which was used immediately without further purification. Enolate salt **10** (2.80 g, 9.88 mmol), cyanothioacetamide (1.00 g, 9.88 mmol), water (9.70 mL), piperidinium acetate solution (0.870 mL) and glacial acetic acid (1.34 mL) were used to give the *title compound* **13** (3.13 g, 97%) as a brown solid. m.p. 135–137 °C. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 2.83 (2H, m, H-8), 4.09 (2H, br s, H-7), 4.50 (2H, s, H-5), 5.12 (2H, s, OCH_2), 7.28–7.39 (5H, m, Ar-H), 8.01 (1H, s, H-4), 8.59 (1H, br s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) 26.3 (C-8), 37.7 (C-7), 42.8 (C-5), 66.6 (OCH_2), 114.0 (C-3), 117.0 (CN and C-4a), 127.4 (2 × Ar-C), 127.6 (C-4′), 127.8 (2 × Ar-C), 128.7 (C-4), 136.6 (C-1′), 150.4 (C-8a), 154.4 (C═O), 176.1 (C-2); ν_{max} (film)/cm^{-1} 3355, 2989, 2209, 1657, 1593, 1474, 1235, 1208, 1112; *m/z* (ESI$^+$): 348 (MNa^+, 100%), 201 (55%); HRMS (ESI$^+$) found (MNa^+): 348.0759 $C_{17}H_{15}N_3NaO_2S$ requires 348.0777.

*2-Chloro-*N*-(isoquinolin-4-yl)acetamide* ***3n***. The reaction was carried out following general procedure D using 4-isoquinolinamine **24n** (0.20 g, 1.39 mmol), chloroacetyl chloride (0.13 mL, 1.67 mmol) and triethylamine (0.23 mL, 1.67 mmol) in CH_2Cl_2 (6.9 mL) to give the *title compound* **3n** (100 mg, 32%) as an off-white solid. m.p. > 230 °C. ^{1}H NMR (400 MHz, $CDCl_3$) 4.36 (2H, s, CH_2), 7.69 (1H, t, $J = 6.8$ Hz, H-7), 7.80 (1H, t, $J = 6.8$ Hz, H-6), 7.86 (1H, d, $J = 6.8$ Hz, H-5), 8.04 (1H, d, $J = 6.8$ Hz, H-8), 8.67 (1H, br s, NH), 8.96 (1H, s, H-3), 9.17 (1H, s, H-1); ^{13}C NMR (100 MHz, $CDCl_3$) 43.3 (CH_2), 120.1 (C-5), 126.8 (C-4), 127.8 (C-7), 128.5 (C-8a), 128.9 (C-4), 130.3 (C-4a), 131.2 (C-6), 138.6 (C-3), 151.0 (C-1), 164.8 (C═O); IR: ν_{max} (film)/cm^{-1} 3248, 3105, 2973, 1667, 1544, 1499, 1210, 782; *m/z* (ESI$^+$): 245 (^{37}ClMNa$^+$, 40%), 243 (^{35}ClMNa$^+$, 100%), 199 (10%); HRMS (ESI$^+$) found (^{37}ClMNa$^+$): 245.0268 $C_{11}H_9^{37}ClN_2NaO$ requires 245.0268. Found (^{35}ClMNa$^+$): 243.0289 $C_{11}H_9^{35}ClN_2NaO$ requires 243.0296.

*2-Chloro-*N*-(2,3-dihydrobenzo[*b*][1,4]dioxin-5-yl)acetamide* ***3t***. The reaction was carried out following general procedure D using 2,3-dihydrobenzo[*b*][1,4]dioxin-5-amine **24t** (0.15 mL, 1.32 mmol), chloroacetyl chloride (0.13 mL, 1.58 mmol) and triethylamine (0.22 mL, 1.58 mmol) in CH_2Cl_2 (6.60 mL) to give the *title compound* **3t** (300 mg, quant.) as brown crystals. m.p. 104–106 °C. ^{1}H NMR (400 MHz, $CDCl_3$) 4.27 (2H, s, CH_2), 4.27–4.30 (2H, m, H-2), 4.34–4.36 (2H, m, H-3), 6.68 (1H, dd, $J = 8.2$, 1.5 Hz, H-8), 6.84 (1H, t, $J = 8.2$ Hz, H-7), 7.87 (1H, dd, $J = 8.2$, 1.5 Hz, H-6), 8.73 (1H, br s, NH); ^{13}C NMR (100 MHz, $CDCl_3$) 43.2 (Cl-CH_2), 64.3 (C-2), 64.9 (C-3), 112.6 (C-6), 113.3 (C-8), 121.1 (C-7), 126.6 (C-5), 133.3 (C-4a), 143.4 (C-8a), 163.8 (C═O); IR: ν_{max} (film)/cm^{-1} 3282, 3154, 2946, 1676, 1555, 1506, 1063, 809; *m/z* (ESI$^+$): 252 (^{37}ClMNa$^+$, 40%), 250 (^{35}ClMNa$^+$, 100%); HRMS (ESI$^+$) found (^{37}ClMNa$^+$): 252.0221 $C_{10}H_{10}^{37}ClNNaO_3$ requires 252.0215. Found (^{35}ClMNa$^+$): 250.0248 $C_{10}H_{10}^{35}ClNNaO_3$ requires 250.0241.

3.2. Cell proliferation assay

As described in detail previously,[13] cell proliferation was measured using a ^{3}H-thymidine incorporation assay by seeding 3000 cells in each well using 96 well plates with varying concentrations of inhibitors for three days. Experiments were performed in triplicate with a minimum of two experimental repeats. Briefly, 0.04 μCi of ^{3}H-thymidine was added to each well five hours prior to harvest, after which the cells were harvested onto glass fibre filters using an automated TomTec harvester. The filters were incubated with Betaplate Scint and thymidine incorporation determined with a Trilux/Betaplate counter. Effects of inhibitors on the incorporation of ^{3}H-thymidine into DNA will be determined relative to the control samples. The human breast cancer cell line MDA-MB-231 and the colon cancer cell line HCT116 were purchased from the American Type Culture Collection (ATCC).

3.3. Drug solubility assay

Intrinsic aqueous drug solubility was determined by forming a supersaturated solution of drug compound in water. The suspension was sonicated for 15 min and centrifuged at 13,000 rpm for 6 min. After 15 min the supernatant was decanted and total drug content solubilised was analysed using reverse phase HPLC. The solution was analysed using an Agilent 1260 Infinity/DAD (100–900 nm) with a Zorbax eclipse XDB (8.5 μm, 4.6 mm × 150 mm) column. The mobile phase comprised of 80:20 acetonitrile: H_2O containing 45 mM ammonium formate buffered to pH 3.5 with formic acid. Flow rate was 1 mL/min with 5 μL injection volume.

3.4. Cheminformatics

The Scigress version FJ 2.6 program (Scigress Ultra V. F.J 2.6. **2016**) was also used to build the ligands and the MM2[14] force field was used to identify the global minimum using the CONFLEX method[15] followed by structural optimization. The QikProp v6.4 (QikProp v6.2: Schrödinger, LLC, New York NY (2020) software package was used to calculate the molecular descriptors. The reliability of QikProp is established for the descriptors.[16] The Known Drug Indexes (KDI) were calculated from the molecular descriptors as described by Eurtivong and Reynisson.[12]

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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