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Synthesis of isothiazol-3-one derivatives as inhibitors of histone acetyltransferases (HATs)

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ABSTRACT

High-throughput screening led to the identification of isothiazolones **1** and **2** as inhibitors of histone acetyltransferase (HAT) with IC50s of 3 μM and 5 μM, respectively. Analogues of these hit compounds with variations of the *N*-phenyl group, and with variety of substituents at C-4, C-5 of the thiazolone ring, were prepared and assayed for inhibition of the HAT enzyme PCAF. Potency is modestly favoured when the *N*-aryl group is electron deficient (4-pyridyl derivative **10** has IC_{50} = 1.5 μM); alkyl substitution at C-4 has little effect, whilst similar substitution at C-5 causes a significant drop in potency. The ring–fused compound **38** has activity (IC_{50} = 6.1 μM) to encourage further exploration of this bicyclic structure. The foregoing SAR is consistent with an inhibitory mechanism involving cleavage of the S–N bond of the isothiazolone ring by a catalytically important thiol residue.

1. Introduction

Histone acetylation and deacetylation play key roles in modifying chromatin structure and thereby regulating gene transcription.[1–5] Acetylation catalysed by histone acetyltransferases (HATs)[6,7] occurs at specific lysine residues in the flexible N-terminal histone tails that protrude from the nucleosome surface. Each acetylation step removes a positive charge from the histone and multiple reactions induce major conformational changes of the protein, thereby modulating transcriptional activity. These structural changes can be reversed by removal of acetyl groups catalysed by histone deacetylases (HDACs). A balance of acetylation and deacetylation is important for normal cell proliferation, growth, and differentiation; and abnormal histone acetylation states may contribute to a variety of diseases including HIV, COPD[8–10] and cancer.[11–13] Thus a variety of solid and haematological cancers contain HAT genes that have undergone gene amplification, mutation and translocation.[7,14,15] For example, AIB-1 (amplified in breast cancer-1, also known as ACTR) is a nuclear hormone receptor co-activator with HAT activity that is amplified and over-expressed in breast cancer.[16] The in vitro and in vivo proliferation of prostate cancer is associated with elevated levels of the HAT p300, and reduced expression of p300 using siRNA causes decreased prostate cell growth.[17]

There are several families of HATs, grouped according to the number of highly conserved structural motifs. These include the GNAT family (GCN5, PCAF (p300/cyclicAMP-responsive element binding protein associated factor)), the MYST group (Sas2, Tip60, MORF) and the p300/CBP family.[1] Inhibitors of one or more of these HATs would be useful tools to enable the role of HAT enzymes in cancer development to be examined and may represent starting points for the development of anti-cancer agents. The first reported inhibitors of HAT (p300 and PCAF) were analogues of the co-substrate acetyl coenzyme A, and Lys CoA was observed to inhibit p300 with an IC_{50} value of 50 nM.[18] Peptidic analogues of Lysyl-CoA were then prepared,[19,20] culminating in a derivative that is cell permeable.[21] Anacardic acid, a lipophilic naturally occurring derivative of salicylic acid, inhibits p300 with IC_{50} = 5 μM[22] whilst related synthetic lipophilic acids are generally less potent.[23] A second, structurally more complex, natural product garcinol[12] inhibits p300 and PCAF with IC_{50}s of ca. 6 μM whilst LTK-14, prepared by monomethylation of the catechol functionality of garcinol, is a more specific p300 inhibitor[24] that does not inhibit PCAF. Both compounds inhibit multiplication of HIV but only LTK-14 is non toxic to the host T-cells. Alpha-methylene butyrolactones,[25] benzylidene acetones,[26] and alkylidene malonates[27] have also been reported as inhibitors of HAT. All these compounds have the potential to act as Michael acceptors towards biological nucleophiles but it is not clear whether this is relevant to their inhibitory activity.

We have reported the discovery, by high throughput screening, of isothiazolones **1** and **2** as inhibitors of HAT[28] and described initial SAR findings and the biological properties of these compounds.

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Here we report exploratory medicinal chemistry aimed at clarifying the structural requirements for biological activity and the scope for developing this hit series towards a drug for cancer therapy (see Fig. 1).

2. Chemistry

We have shown previously[28] that *N*-phenylisothiazolones with a variety of aryl substituents are potent inhibitors of the HAT enzymes p300 and PCAF, and that *N*-alkylisothiazolones have reduced potency. Here we report an extension of these studies to include *N*-pyridylisothiazolones and to explore the effect of introducing substituents at the 4- and 5-positions of the isothiazolone ring. The isothiazolone **3**, required as a reference compound, was prepared according to the procedure of Beeley et al.,[29] and the same method was used for the synthesis of the *N*-pyridyl-isothiazolones **8–10** (Scheme 1). Starting from 3-(*Z*)-(benzylsulfanyl)propenoic acid (**4**),[29] access was readily gained to the respective 2-, 3-, and 4-pyridyl amide sulfoxides **5–7** using standard amide formation techniques followed by oxidation with 3-chloroperoxybenzoic acid. Ring closure was achieved using trichloroacetic anhydride in dichloromethane (Scheme 1), with no sign of competing Pummerer-related products.[29]

Access to 4-dimethylaminophenyl and 4-morpholinophenyl isothiazolones **13** and **14** (Scheme 2) was accomplished in a similar manner to the pyridyl series but significant problems were encountered during the oxidation step due to competing N-oxidation. Even under acidic conditions using sodium periodate[30] or hydrogen peroxide as oxidants yields were very low. However, the use of hydrogen peroxide in hexafluoroisopropanol proved successful in achieving selective *S*-mono-oxidation. This fluorous solvent has been reported to enhance dramatically the rate of S-oxidation by hydrogen peroxide and a mechanistic rationale has been proposed.[31] Under these conditions the sulfoxides **11** and **12** were formed in 63% and 59% yield, respectively. The required isothiazolones **13** and **14** were then obtained from **11** and **12**, respectively, by treatment with trichloroacetic anhydride in dichloromethane (Scheme 2).

The synthesis of 5-substituted isothiazolones is shown in Scheme 3. Commencing with trityl protected propargyl alcohol, access was gained to the anilide **16** via deprotonation with *n*-BuLi and acetylide anion quenching with phenyl isocyanate. 1,2-Addition of benzyl mercaptan was then accomplished with moderate selectivity (2:1) in favour of the desired (*Z*)-isomer (**17**) over the undesired (*E*) isomer using methanolic alkaline conditions. Selective crystallisation from MeCN allowed the efficient separation of the component isomers. Finally, S-oxidation using 3-chloroperoxybenzoic acid afforded the sulfoxide **18** which was then immediately cyclised to 5-substituted isothiazolone **19** in good yield. The trityl ether derivative **19** was directly converted to 5-acetoxymethylisothiazolone **21** by treatment with acetyl chloride, following a literature procedure.[32] Additionally, removal of the trityl protecting group was achieved using trifluoroacetic acid to afford the hydroxymethyl derivative **20** and this alcohol was derivatised further to afford 5-phenylureidomethyl-isothiazolone **22**, and 5-azidomethyl-isothiazolone **23** as outlined in Scheme 3.

The 5-methyl-isothiazolone derivative **28** was prepared as shown in Scheme 4. The synthesis starts with but-2-ynoic acid (**24**) which was converted to isomers of **25** by reacting with benzyl mercaptan in methanol/water. Amide formation was effected via the acid chloride, and oxidation of the sulfide **26** was accomplished using 3-chloroperoxybenzoic acid in dichloromethane (Scheme 4). Cyclisation was achieved utilising trichloroacetic acid in dichloromethane. The 5-phenyl analogue of **3**, that is, compound **29** (Table 2), is a known compound and was prepared by the literature method.[33]

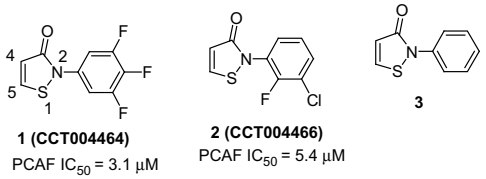
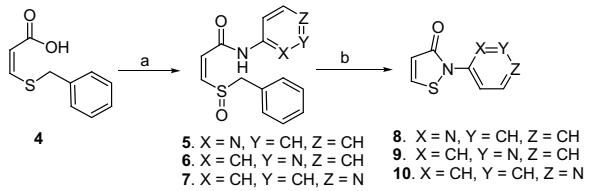
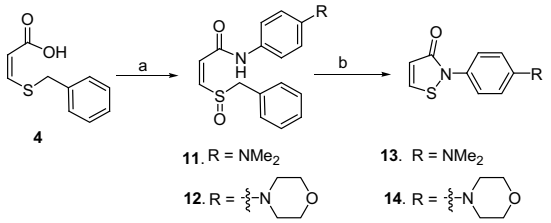


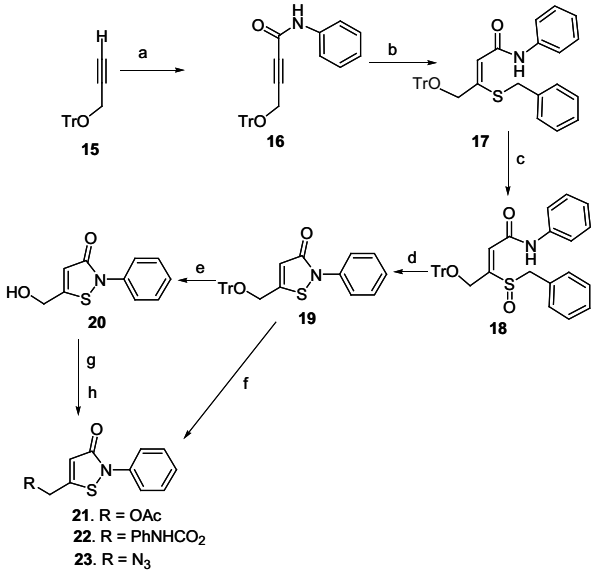
Figure 1.



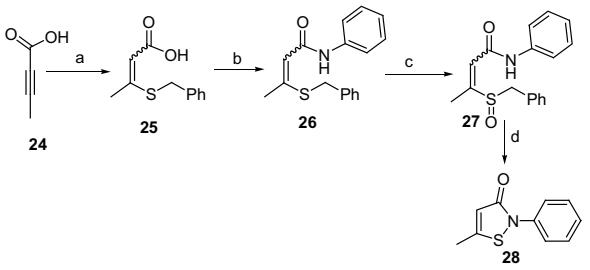
Scheme 1. Reagents and conditions: (a) i—$(COCl)_2$, CH_2Cl_2, DMF, room temp., 30 min, ii—aminopyridine, CH_2Cl_2, −20 °C to room temp., 1 h, iii—3-chloroperoxybenzoic acid, CH_2Cl_2, −10 °C, 10 min; (b) $(Cl_3CCO)_2O$, CH_2Cl_2, 0 °C, 1 h.



Scheme 2. Reagents and conditions: (a) i—substituted aniline, EtN(*i*Pr)$_2$, CH_2Cl_2, 0 °C, 10 min, ii—PyBOP, CH_2Cl_2, 0 °C to room temp., 18 h, iii—H_2O_2, hexafluoroisopropanol, 2 M HCl, room temp., 2 h; (b) $(Cl_3CCO)_2O$, CH_2Cl_2, 0 °C, 1 h.



Scheme 3. Reagents and conditions: (a) i—*n*-BuLi, THF, −78 °C, 45 min, ii—PhNCO (single rapid addition); (b) BnSH, Et_3N, MeCN, room temp., 30 min; (c) 3-chloroperoxybenzoic acid, CH_2Cl_2, −10 °C, 10 min; (d) $(Cl_3CCO)_2O$, CH_2Cl_2, 0 °C, 1 h; (e) 4% TFA in CH_2Cl_2, 5 min; (f) AcCl, CH_2Cl_2, room temp., 36 h; (g) PhNCO, pyridine, CH_2Cl_2, 48 h; (h) i—*p*-toluenesulfonic anhydride, CH_2Cl_2, (*i*Pr)$_2$NEt, DMAP, room temp., 2 h, ii—NaN_3, DMF, 4 h.



Scheme 4. Reagents and conditions: (a) benzyl mercaptan, Na_2CO_3, H_2O/MeOH; (b) i—thionyl chloride, reflux, 3 h, ii—benzene, aniline, 0 °C, 30 min; (c) 3-chloroperoxybenzoic acid, CH_2Cl_2, −10 °C, 10 min; (d) $(Cl_3CCO)_2O$, CH_2Cl_2, 0 °C.

Access to the 4-subsituted series was gained via 4-methyl-isothiazolone **30** and 4-methyl-5-chloroisothiazolone **34** (Schemes 5 and 6). For this study **30** and **34** were prepared by treating *N*,*N*′-bis-phenyl-3,3′-dithiodiisobutyramide with SO_2Cl_2.[34] Bromination at the 4-methyl position of **30** was achieved using *N*-bromosuccinimide (NBS) in the presence of a catalytic quantity of AIBN in boiling carbon tetrachloride, affording the 4-bromomethyl derivative **31**, which was then transformed into 4-methoxymethyl-isothiazolone **32** and 4-azidomethyl-isothiazolone **33** using sodium methoxide and sodium azide, respectively (Scheme 5).

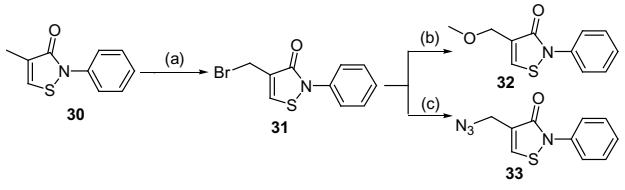
Treatment of 4-methyl-5-chloro-isothiazolone **34** with NaOMe in methanol at reflux effected nucleophilic displacement at the reactive C-5 position affording 4-methyl-5-methoxy-isothiazolone **35** in 24% yield (Scheme 6).

Finally, the pyridyl-fused isothiazolone **38**, a known compound,[35] was prepared in two steps from 2-mercaptonicotinic acid (**36**) by first forming the amide **37** via PyBOP® carboxyl activation followed by cyclisation to **38** using iodine and triethylamine in dichloromethane (Scheme 7).

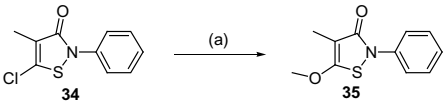
3. Biological evaluation

All compounds were tested as inhibitors of HAT activity using filter binding assays for PCAF and p300 as previously described.[28,36]

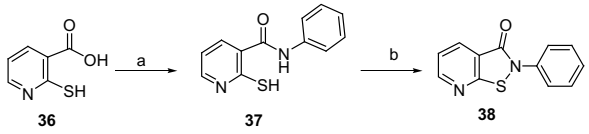
Replacement of the phenyl ring in **3** with pyridyl, 4-morpholinophenyl or 4-dimethylaminophenyl led to an increase in HAT inhibitory activity (Table 1). The 4-pyridyl derivative **10** was the most potent compound in this series inhibiting the HAT activity of PCAF in the low μM range (PCAF IC_{50} = 1.5 μM). Because these compounds are inhibitors of PCAF and p300, with similar potencies versus both HAT enzymes (Table 1) subsequent studies were carried out on PCAF only. The effects on PCAF inhibitory activity, of substitution in the isothiazolone ring of **3**, were strongly dependent on position. (Table 2). Whereas the 4-methyl derivative **30** had a similar PCAF IC_{50} value to that of the parent compound **3**, the 5-methyl derivative **28** had no detectable activity. The introduction of functionalised alkyl groups at C-4 and C-5 resulted in further loss of potency, (compounds, **20–23**, **28**, **29**, **35**, Table 2), with the exception of the 5-Cl compound **34**. All the foregoing results are consistent with the view that the HAT activity of this class



Scheme 5. Reagents and conditions: (a) NBS, CCl_4, AIBN, 24 h, reflux, 36%; (b) NaOMe, MeOH, 25 °C, 24 h; (c) NaN_3, DMF, 1 h.

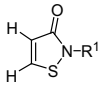


Scheme 6. Reagents and conditions: (a) NaOMe, MeOH, reflux, 5 h.



Scheme 7. Reagents and conditions: (a) i—aniline, PyBOP, EtN(*i*Pr)2, CH_2Cl_2, 0 °C to room temp.; (b) I_2, Et_3N, CH_2Cl_2.

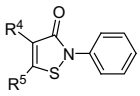
Table 1
Effect of variations on the *N*-aryl ring on inhibition of HAT activity for PCAF and p300



Compound	R^1	PCAF IC_{50} (μM)	p300 IC_{50} (μM)
3	Phenyl	28 ± 5.6	23 ± 4.9
8	2-Pyridyl	19 ± 2.5	28 ± 4.0
9	3-Pyridyl	6.5 ± 2.2	14.4 ± 3.7
10	4-Pyridyl	1.5 ± 0.39	3.0 ± 0.6
13	4-Dimethylaminophenyl	5.4 ± 1.4	ND
14	4-Morpholinophenyl	7.5 ± 2.4	9.9 ± 1.6

Each IC_{50} value is the mean (±SD) for $n > 3$ determinations. ND = not determined.

Table 2
Effect on PCAF inhibition of 4- and 5-substitution in isothiazol-3-ones



Compound	R^4	R^5	PCAF IC_{50}, μM
3	H	H	28 ± 5.6
20	H	CH_2OH	109
21	H	CH_2OCOMe	162
22	H	$CH_2OCONHPh$	>200
23	H	CH_2N_3	58
28	H	Me	>200
29	H	Ph	>200
30	Me	H	32
32	CH_2OMe	H	102
33	CH_2N_3	H	42.7
34	Me	Cl	19.8
35	Me	OMe	>200
38	See Scheme 7	See Scheme 7	6.1 ± 1.9

Each IC_{50} value is the mean of two independent determinations or the mean (±SD) for $n > 2$ determinations.

of compounds is dependent on nucleophilic attack by a biologically important nucleophile (especially thiol) at the sulfur atom of the thiazolone with concomitant ring-opening. For example the presence of a 5-alkyl substituent would sterically hinder nucleophilic attack at ring S, but this effect would be counterbalanced for the 5-Cl substituent by its greater electronegativity.

The activity of the 5-chloro derivative **34** (similar to the parent compound **30**) may also be enhanced by the formation of a highly reactive thioacetyl chloride, a result of tautomerism of the mercaptoacrylamide (Scheme 8). This plausible mechanism was proposed by Collier et al. to account for the increased antimicrobial activity of 5-chloro-*N*-methylisothiazol-3-one.[37]

The pyridylisothiazolone **38** which incorporates C-4 and C-5 substituents as a fused aromatic ring was approximately four times more potent against PCAF compared with **3** (Table 2). This is an encouraging result as it affords new opportunities to vary the structure of this series of HAT inhibitors in attempts to separate enzyme inhibition and unwanted chemical reactivity.

It should be noted that isothiazolone derivatives have been reported to inhibit a variety of biochemical processes including cytokine-induced cartilage destruction,[33] telomerase,[38] and $p56^{lck}$ kinase activity.[39] The inhibitory mechanism for the latter was proposed to involve the formation of a covalent disulfide bond as a result of a reaction of cysteine SH groups within the $p56^{lck}$ catalytic domain with the isothiazolone ring.[39] It has also been reported that some isothiazolones possess biocidal activity[37] or antibacterial activity.[40]

Overall these results suggest that the HAT inhibitory activity of isothiazolones may be dependent on their chemical reactivity. If so, their potential for clinical utility would be limited, as the more potent candidates would be expected to react promiscuously with biological nucleophiles. Future work will be directed towards fused pyridylisothiazolone **38**, aiming to explore in more detail whether chemical reactivity can be minimised without compromising HAT inhibition.

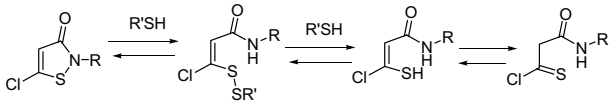
4. Experimental

Anhydrous solvents were obtained from Aldrich and used without further purification unless otherwise stated. All other reagents were obtained from commercial suppliers, and used without further purification.

Thin layer chromatography (TLC) was performed on pre-coated aluminium sheets of silica (60 F_{254}, Merck) and visualised with short-wave UV analysis. Merck Silica 60 (Art 15111) was used in low-pressure column chromatography unless stated otherwise. LCMS analysis was conducted using gradient elution (MeOH/0.1% HCO_2H in H_2O) and a Supelco Discovery C18 HPLC column (5 cm × 0.46 cm, 5 μm). Samples were injected using a Gilson 215 liquid handler. The HPLC system employed a Thermoseparations P4000 quaternary pump and UV 2000 detector operating at 254 nm. HPLC eluent passed directly into an LCQ ion trap mass spectrometer (Finnigan LCQ) operating in electrospray ionisation mode. Fast atom bombardment (FAB) mass spectra were determined with VG ZAB-SE spectrometer. ^{1}H NMR spectra were recorded at 250 MHz using a Bruker AC250 spectrometer with the residual solvent (in DMSO-d_6) or tetramethylsilane (in $CDCl_3$) as standard. Field strengths are expressed in units of δ (ppm) and peak multiplicities are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; br s, broad singlet; m, multiplet. Infrared spectra were obtained using a Perkin–Elmer 1720X FT-IR. Melting points were determined on a Kofler block and are uncorrected. Optical rotations were obtained using a Perkin–Elmer Model 141 Polarimeter with a sodium lamp as radiation source. Elemental analyses were determined by C, H, N. Analysis Limited, Leicester and Warwick Analytical Service, Warwick.

4.1. 3-(*Z*)-(Benzylsulfanyl)propenoic acid (4)

This compound was prepared as described by Beeley et al.[29] To a stirred solution of sodium carbonate (3.3 g, 31.4 mmol) in water (34 mL) was added propynoic acid (2 g, 28.5 mmol) followed by methanol (26 mL). Benzyl mercaptan was added rapidly to the mixture, which was then refluxed for 4 h. After cooling to room temperature the mixture was carefully acidified to pH 1 with 2 M HCl and then extracted into EtOAc. The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated in vacuo. Recrystallisation of the residue (ethyl acetate/hexane) afforded the title compound as colourless needles (5.4 g, 27.8 mmol, 98%).



Scheme 8.

4.2. Synthesis of pyridyl-propenamides 5–7

*Example procedure: 3-(Z)-(Benzylsulfinyl)-N-(3-pyridyl)propenamide (**6**)*: To a stirred slurry of 3-(*Z*)-(benzylsulfanyl)propenoic acid (500 mg, 2.57 mmol) and dimethylformamide (1 drop) in dichloromethane at room temperature under nitrogen was added oxalyl chloride (1.42 mL, 2 M in dichloromethane, 2.83 mmol). The mixture was stirred until effervescence could no longer be detected and then added dropwise to a stirred solution of 3-aminopyridine (266 mg, 2.83 mmol), pyridine (0.42 mL, 5.15 mmol) and dichloromethane (8 mL) at −20 °C. The reaction was allowed to warm to room temperature over 1 h and then quenched by the addition of $NaHCO_3$. The resultant mixture was extracted with dichloromethane (2 × 10 mL), dried over Na_2SO_4 and evaporated in vacuo. This afforded a 2:1 *E/Z* mixture (596 mg, 2.21 mmol, 86%) containing 3-(*Z*)-(benzylsulfanyl)-*N*-(3-pyridyl)propenamide as a light tan solid.

The *E/Z* mixture (596 mg, 2.21 mmol) in dichloromethane (35 mL) at −20 °C was treated with 3-chloroperoxybenzoic acid (725 mg, 2.21 mmol). The mixture was stirred for 10 min and then quenched by the addition of aqueous sodium hydrogen sulfite. The reaction was warmed to room temperature, diluted with satd $NaHCO_3$ (4 mL) and extracted with dichloromethane (3 × 15 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated in vacuo. Flash column chromatography of the residue (5%MeOH/EtOAc) afforded 3-(*Z*)-(benzylsulfinyl)-*N*-(3-pyridyl)propenamide (**6**) as a crystalline solid (140 mg, 0.49 mmol, 22%).

Mp 174–176 °C; ν_{max} (film)/cm^{-1}: 1672s (CO), 1612s; δH (250 MHz, $CDCl_3$, TMS) 4.20 (1H, d, *J* 12.7, CH_2), 4.45 (1H, d, *J* 12.7, CH_2), 6.67 (1H, d, J_{cis} 10.0, H-2), 6.72 (1H, d, J_{cis} 10.0, H-3), 7.34–7.43 (5H, m, Ph), 7.43–7.45 (1H, m, 5′-H), 8.22 (1H, ddd, *J* 8.37, 2.53, 1.42, 6′-H), 8.30 (1H, dd, *J* 4.9, 1.42, 4′-H), 8.8 (1H, dd, *J* 2.53, 0.6, 2′-H); δC (63 MHz, $CDCl_3$) 60.0, 123.7, 127.1, 127.2, 128.3, 128.4, 130.2, 130.4, 135.1, 140.9, 144.8, 152.8, 162.3; FAB-HRMS: measured 309.0663; calcd for $C_{15}H_{14}N_2O_2SNa$ $(M+Na)^+$: 309.0674.

Compounds **5** and **7** were prepared by a procedure analogous to that described for the 3-pyridyl derivative **6**.

4.2.1. 3-(*Z*)-(Benzylsulfinyl)-*N*-(2-pyridyl)propenamide (5)

Mp 155 °C (dec); (found: C, 62.82; H, 4.95; N, 9.65; $C_{15}H_{14}N_2O_2S$ requires C, 62.82; H, 4.93, 9.78); ν_{max} (film)/cm^{-1}: 3400–3200w (NH), 1669s, (CO); δH (250 MHz, $CDCl_3$, TMS) 4.25 (1H, d, *J* 12.6, CH_2), 4.37 (1H, d, *J* 12.6, CH_2), 6.5 (1H, d, J_{cis} 10.1, CH), 6.78 (1H, d, J_{cis} 10.1, CH), 7.13–7.19 (1H, m, 4′-H), 7.30–7.41 (5H, m, Ph), 7.80–7.87 (1H, m, 6′-H), 8.29–8.35 (2H, m, 3′-H and 5′-H); δC (63 MHz, $CDCl_3$) 60.0, 114.8, 120.5, 127.2, 128.3, 128.5, 130.5, 130.7, 138.7, 147.7, 151.2, 155.9, 162.2; FAB-HRMS: measured 287.0864; calcd for $C_{15}H_{15}N_2O_2S$ $(M+H)^+$: 287.0854.

4.2.2. 3-(*Z*)-(Benzylsulfinyl)-*N*-(4-pyridyl)propenamide (7)

Mp 161 °C (dec.); ν_{max} (film)/cm^{-1}: 3600–3300br (NH), 1684s (CO); δH (250 MHz, $CDCl_3$, TMS) 4.21 (1H, d, *J* 12.6, CH2), 4.47 (1H, d, *J* 12.6, CH2), 6.48 (2H, s, 2 × CH), 7.30–7.42 (5H, m, Ph), 7.53 (2H, d, *J* 6.3, 2′-H), 8.45 (2H, d, *J* 6.3, 3′-H), 10.9 (1H, br s, NH); δC (63 MHz, $CDCl_3$) 60.1, 113.9, 128.3, 128.6, 128.7, 130.2, 130.6, 145.3, 150.2, 153.9, 162.8; *m/z* (LC–MS) 287.0 $(M+H)^+$.

4.3. Synthesis of pyridyl-isothiazolo-3-ones 8–10

*Example procedure: 2-(3-Pyridyl)-isothiazol-3(2H)-one (**9**)*:

To a stirred slurry of 3-(*Z*)-(benzylsulfinyl)-*N*-(3-pyridyl)propenamide (120 mg, 0.42 mmol) in dry dichloromethane (3 mL) at 0 °C under nitrogen was added trichloroacetic anhydride (0.092 mL, 0.50 mmol). After 30 min the reaction mixture was diluted with dichloromethane (10 mL) and quenched by the addition of satd $NaHCO_3$ (5 mL). The aqueous phase was extracted with dichloromethane (2 × 5 mL), dried over Na_2SO_4 and concentrated in vacuo. Column chromatography (20% MeOH/EtOAc) of the residue afforded the title compound as a powdery white solid (34 mg, 48%).

Mp 118–119 °C; (found: C, 54.12; H, 3.40; N, 15.47; $C_8H_6N_2OS$ requires C, 53.92; H, 3.39, 15.72); ν_{max} (film)/cm^{-1}: 1662s (CO); δH (250 MHz, $CDCl_3$, TMS) 6.35 (1H, d, *J* 6.6, 4-H), 7.39 (1H, ddd, *J* 8.5, 4.7, 0.58, 5′-H), 8.02–8.07 (1H, m, 6′-H), 8.25 (1H, d, *J* 6.6, 5-H), 8.55 (1H, dd, *J* 4.7, 1.43, 4′-H), 8.81 (1H, d, *J* 2.5, 2′-H); δC (62 MHz, $CDCl_3$) 114.6, 123.7, 131.7, 133.6, 140.2, 145.0, 148.0, 167.5; FAB-HRMS: measured 179.0276; calcd for $C_8H_7N_2OS$ (M+H)$^+$: 179.0279.

Compounds **8** and **10** were prepared by a procedure analogous to that described for the 3-pyridyl derivative **9**.

4.3.1. 2-(2-Pyridyl)-isothiazol-3(2*H*)-one (8)

Mp 100–105 °C; ν_{max} (film)/cm^{-1}: 1657s (CO); δH (250 MHz, $CDCl_3$, TMS) 6.29 (1H, d, $J_{4,5}$ 6.4, 4-H), 7.15 (1H, ddd, *J* 7.3, 4.9, 0.9, 5′-H), 7.79 (1H, ddd, *J* 8.4, 7.3, 1.8, 4′-H), 8.14 (1H, d, *J* 6.4, 5-H), 8.34–8.38 (1H, m, 6′-H), 8.63 (1H, dd, *J* 8.4, 0.9, 3′-H); δC (83 MHz, $CDCl_3$) 113.6, 116.5, 120.9, 138.5, 141.6, 147.5, 150.0, 167.1; FAB-HRMS: measured 179.0276; calcd for $C_8H_7N_2OS$ (M+H)$^+$: 179.0276.

4.3.2. 2-(4-Pyridyl)-isothiazol-3(2*H*)-one (10)

Mp 102 °C (dec); (found: C, 53.19; H, 3.39; N, 15.13; $C_8H_6N_2OS$ requires C, 53.92; H, 3.39, 15.72); ν_{max} (film)/cm^{-1}: 1663s (CO); δH (250 MHz, $CDCl_3$, TMS) 6.27 (1H, d, *J* 6.4, 4-H), 7.67 (2H, d, *J* 4.7, 2′-H), 8.18 (1H, d, *J* 6.4, 5-H), 8.56 (2H, d, *J* 4.7, 3′-H); δC (83 MHz, $CDCl_3$) 115.8, 117.5, 145.1, 146.8, 151.3, 170.0; FAB-HRMS: measured 179.0276; calcd for $C_8H_7N_2OS$ (M+H)$^+$: 179.0279.

4.4. Synthesis of anilino-propenamides 11 and 12

*Example preparation: 3-(Z)-(Benzylsulfinyl)-2-N-(4-Morpholinoanilino)-propenamide (**12**)*: PyBOP® (1.07 g, 2.06 mmol) was added portionwise to a stirred solution of 3-(Z)-(benzylsulfanyl)propenoic acid (400 mg, 2.06 mmol), diisopropylethylamine (1.07 mL, 6.2 mmol) and 4-morpholinoaniline (349 mg, 1.96 mmol) in DCM (15 mL) at 0 °C. The mixture was stirred for 30 min at 0 °C and then warmed to room temperature for 18 h. The reaction mixture was diluted with 1 M NaOH (40 mL) and then extracted into $CHCl_3$ (2 × 20 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated to 5 mL. With vigorous stirring, hexane (40–50 mL) was added until crystallisation occurred. The resultant pink solid was filtered, rinsed with cold diethyl ether (2 × 5 mL) and then dried under high vacuum to afford 3-(*Z*)-(benzylsulfanyl)-2-*N*-(4-morpholinoanilino)-propenamide (520 mg, 1.46 mmol, 71%).

Mp 205 °C; (found: C, 67.33; H, 6.23; N, 7.72; $C_{20}H_{22}N_2O_2S$ requires C, 67.77; H, 6.26, 7.90); ν_{max} (film)/cm^{-1}: 3400–3300w (NH), 1633s (CO); δH (250 MHz, $CDCl_3$, TMS) 3.09 (4H, t, *J* 4.8, CH_2N), 3.84 (4H, t, *J* 4.8, CH_2O), 3.94 (2H, s, CH_2S), 5.84 (1H, d, *J* 9.9, 2-H), 6.80–6.86 (2H, m, 2 × CH), 6.92 (1H, d, *J* 9.9, 3-H), 7.27–7.34 (5H, m, Ph), 7.45–7.48 (2H, m, 2 × CH); FAB-HRMS: measured 354.1412; calcd for $C_{20}H_{22}N_2O_2S$ (M)$^+$: 354.1402.

2 M HCl (0.5 mL, 1.00 mmol) was added dropwise to a stirred solution of 3-(*Z*)-(benzylsulfanyl)-2-*N*-(4-morpholinoanilino)-propenamide (226 mg, 0.64 mmol) in hexafluoroisopropanol (3.3 mL). With vigorous stirring, H_2O_2 (30% solution, 0.20 mL) was added over a 2 h period. Immediately after the complete addition, the reaction was quenched by the addition of aq sodium hydrogen sulfite (3 mL) and $NaHCO_3$ (1 mL). The organic phase was separated, dried with Na_2SO_4 and evaporated to dryness. The residue was recrystallised from methanol to afford 3-(*Z*)-(benzylsulfinyl)-2-*N*-(4-morpholinoanilino)-propenamide (**12**) as a yellow powder (139 mg, 0.375 mmol, 59%).

Mp 235–238 °C (dec.); ν_{max} (film)/cm^{-1}: 1656s (CO); δH (250 MHz, $CDCl_3$, TMS) 3.07–3.11 (4H, m, 2 × CH_2N), 3.80–3.84 (4H, m, 2 × CH_2O), 4.15 (1H, d, *J* 12.6, CH_2S), 4.36 (1H, d, *J* 12.6, CH_2S), 6.36 (1H, d, *J* 9.9, 2-H), 6.48 (1H, d, *J* 9.9, 3-H), 6.86 (2H, d, *J* 9.0, 2 × CH), 7.28–7.35 (5H, m, Ph), 7.49 (2H, d, *J* 9.0, 2 × CH); δC (62 MHz, $CDCl_3$) 50.5, 60.0, 66.3, 117.0, 121.2, 121.3, 128.3, 128.4, 128.6, 130.6, 132.3, 146.7, 152.4, 161.5; *m*/*z* (LC–MS) 371.0 (M+H)$^+$.

4.4.1. 3-(*Z*)-(Benzylsulfinyl)-2-*N*-(4-dimethylaminoanilino)-propenamide (11)

Compound **11** was prepared by a procedure analogous to that described for the morpholinoanilino derivative **12**, that is, 3-(*Z*)-(benzylsulfanyl)-2-*N*-(4-dimethylaminoanilino)-propenamide was first prepared and then was oxidised to the corresponding sulfoxide **11**.

3-(Z)-(Benzylsulfanyl)-2-N-(4-dimethylaminoanilino)-propenamide: Mp 163–166 °C; (found: C, 69.14; H, 6.32; N, 9.07; $C_{18}H_{20}N_2OS$ requires C, 69.2; H, 6.45, 8.97) ν_{max} (film)/cm^{-1}: 3400–3200w (NH), 1637s (CO); δH (250 MHz, $CDCl_3$, TMS) 2.90 (6H, s, 2 × NMe), 3.93 (2H, s, CH_2S), 5.83 (1H, d, *J* 9.9, 2-H), 6.66 (2H, d, *J* 8.9, 2 × CH), 6.89 (1H, d, *J* 9.9, 3-H), 7.25–7.36 (5H, m, Ph), 7.42 (2H, d, *J* 8.9, 2 × CH); FAB-HRMS: measured 312.1284; calcd for $C_{18}H_{20}N_2OS$ (M)$^+$: 312.1296.

*3-(Z)-(Benzylsulfinyl)-2-N-(4-dimethylaminoanilino)-propenamide (**11**): Mp* 200–204 °C; (found: C, 64.94; H, 6.11; N, 8.21; $C_{18}H_{20}N_2O_2S$. 0.25 H_2O requires C, 64.94; H, 6.21, 8.41) ν_{max} (Nujol)/cm^{-1}: 1661s (CO); δH (250 MHz, $CDCl_3$, TMS) 2.86 (6H, s, 2 × Me), 4.08 (1H, d, *J* 12.5, CH_2S), 4.30 (1H, d, *J* 12.5, CH_2S), 6.56 (1H, d, $J_{2,3}$ 10.2, 2-H), 6.61 (1H, d, $J_{3,2}$ 10.2, 3-H), 6.71 (2H, d, *J* 9.0, 2 × CH), 7.31–7.42 (5H, m, Ph), 7.48 (2H, d, *J* 9.0, 2 × CH), 10.4 (1H, br s, NH); δC (62 MHz, $CDCl_3$) 40.3, 59.8, 112.5, 120.6, 127.7, 127.9, 128.2, 128.3, 130.3, 131.9, 147.5, 152.8, 161.0; FAB-HRMS: measured 328.1250; calcd for $C_{18}H_{20}N_2O_2S$ (M)$^+$: 328.1245).

4.5. Synthesis of the aniline-isothiazol-3(2*H*)-one derivatives 13 and 14

*Example preparation: 2-(4-Morpholinoaniline)-isothiazol-3(2H)-one (**14**)*: To a stirred slurry of 3-(*Z*)-(benzylsulfinyl)-*N*-(4-morpholinoanilino)-propenamide (95 mg, 0.257 mmol) in dry dichloromethane (9 mL) at 0 °C under nitrogen was added trichloroacetic anhydride (0.056 mL, 0.308 mmol). After 30 min the reaction was diluted with dichloromethane (10 mL) and quenched by the addition of satd $NaHCO_3$ (3 mL). The aqueous phase was extracted with $CHCl_3$ (3 × 10 mL), dried over Na_2SO_4 and evaporated in vacuo. Column chromatography (100% EtOAc) of the residue afforded 2-(4-morpholinoaniline)-isothiazol-3(2*H*)-one (**14**) as a tan coloured solid (6.5 mg, 0.025 mmol, 10%).

Mp 197–201 °C; ν_{max} (film)/cm^{-1}: 1645s (CO); δH (250 MHz, $CDCl_3$, TMS) 3.21 (4H, t, *J* 4.9, CH_2N), 3.90 (4H, t, *J* 4.9, CH_2O), 6.35 (1H, d, *J* 6.3, 4-H), 7.00 (2H, d, *J* 8.9, 2 × CH), 7.45 (2H, d, *J* 8.9, 2 × CH), 8.16 (1H, d, *J* 6.3, 5-H); δC (62 MHz, $CDCl_3$) 49.1,

66.6, 114.7, 116.0, 122.3, 126.5, 139.2, 151.4, 167.8; *m*/*z* (LC-MS) 263.1 $(M+H)^+$.

4.5.1. 2-(4-Dimethylaminoaniline)-isothiazol-3(2*H*)-one (13)

Compound **13** was prepared by a procedure analogous to that described for the morpholinoanilino derivative **14**.

Mp 154 °C (dec.); (found: C, 59.14; H, 5.47; N, 11.63; $C_{11}H_{12}N_2OS$. 0.25 H_2O requires C, 58.77; H, 5.60, N, 12.46); ν_{max} (film)/cm^{-1}: 1634s (CO); δH (250 MHz, $CDCl_3$, TMS) 2.96 (6H, s, 2 × Me), 6.29 (1H, d, *J* 6.4, 4-H), 6.73 (2H, d, *J* 8.8, 2 × CH), 7.32 (2H, d, *J* 8.8, 2 × CH), 8.09 (1H, d, *J* 6.4, 5-H); δC (62 MHz, $CDCl_3$) 40.8, 113.0, 114.6, 125.3, 126.8, 139.1, 149.6, 168.0; FAB-HRMS: measured 220.0665; calcd for $C_{11}H_{12}N_2OS$ $(M)^+$: 220.0670).

4.6. 4-Trityloxy-but-2-ynoic acid phenylamide (16)

n-BuLi (0.69 mL, 1.11 mmol) was added to a stirred solution of 1-trityloxy-prop-2-yne[41] (300 mg, 1.0 mmol) in dry THF (2 mL) at −78 °C. After 45 min and the observance of a white precipitate, phenylisocyanate was added rapidly and stirred for a further 10 min. The reaction was quenched by the addition of satd NH_4Cl (5 mL) and the resultant mixture extracted into diethyl ether (3 × 10 mL). The combined extracts were dried over Na_2SO_4 and evaporated. Flash column chromatography of the residue (5:1 hexane/EtOAc as eluant) afforded 4-trityloxy-but-2-ynoic acid phenylamide as fine needles (266 mg, 0.64 mmol, 64%). Mp 141 °C; ν_{max} (film)/cm^{-1}: 3400–3300 m (NH), 1646s (CO); δH (250 MHz, $CDCl_3$, TMS) 3.91 (2H, s, CH_2O), 6.99–7.44 (20H, m, 4 × Ph); δC (62 MHz, $CDCl_3$) 52.6, 79.9, 83.2, 87.9, 119.8, 124.8, 127.4, 128.0, 128.6, 129.0, 137.1, 143.0, 151.3.

4.7. 3-*Z*-Benzylsulfanyl-4-trityloxy-but-2-enoic acid phenylamide (17)

Benzyl mercaptan (94 mg, 0.75 mmol) was added dropwise to a stirred solution of 4-trityloxy-but-2-ynoic acid phenylamide (300 mg, 0.72 mmol) and Et_3N (76 mg, 0.75 mmol) in methanol (9 mL) at room temperature. The reaction mixture was stirred at room temperature for 2 h, then concentrated in vacuo. The residue was dissolved in warm acetonitrile and the solution was stirred for 30 min to afford a white precipitate. The product was collected by filtration, washed with cold acetonitrile (1 mL) and hexane (10 mL) and then subjected to high vacuum until dry. This procedure afforded spectroscopically pure 3-*Z*-benzylsulfanyl-4-trityloxy-but-2-enoic acid phenylamide as a white powder (199 mg, 0.37 mmol, 51%). Mp 177–178 °C; (found: C, 79.49; H, 5.77; N, 2.56; $C_{36}H_{31}NO_2S$ requires C, 79.82; H, 5.77, 2.59); ν_{max} (film)/cm^{-1}: 3400–3300w (NH), 1646s (CO); δH (250 MHz, $CDCl_3$, TMS) 3.78 (2H, s, CH_2O), 3.91 (2H, s, CH_2S), 6.39 (1H, s, CH), 7.04–7.67 (25H, m, 5 × Ph); δC (62 MHz, $CDCl_3$) 35.1, 65.9, 87.7, 117.2, 119.5, 123.9, 127.2, 127.3, 128.0, 128.5, 128.7, 128.9, 136.8, 128.1, 143.3, 150.6; FAB-HRMS: measured 542.2142; calcd for $C_{36}H_{32}NO_2S$ $(M+H)^+$: 542.2154.

4.8. 3-*Z*-Benzylsulfinyl-4-trityloxy-but-2-enoic acid phenylamide (18)

3-*Z*-Benzylsulfanyl-4-trityloxy-but-2-enoic acid phenylamide (180 mg, 0.33 mmol) in dichloromethane (10 mL) at −10 °C was treated with 3-chloroperoxybenzoic acid (109 mg, 52% pure, 0.33 mmol). The mixture was stirred for 20 min and then quenched by the addition of aqueous sodium hydrogen sulfite (2 mL). The reaction was warmed to room temperature, diluted with satd $NaHCO_3$ (4 mL) and extracted with chloroform (3 × 8 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated in vacuo. This afforded crude 3-*Z*-benzylsulfinyl-4-trityloxy-but-2-enoic acid phenylamide as an amorphous white solid (120 mg, 0.22 mmol, 65%). δH (250 MHz, $CDCl_3$, TMS) 3.80 (1H, m, CH_2O), 4.05 (1H, d, *J* 12.5, CH_2S), 4.2 (1H, m, CH_2O), 4.27 (1H, d, *J* 12.5, CH_2S), 6.66 (1H, s, CH), 7.10–7.63 (25H, m, 5 × Ph), 7.85 (1H, br, NH).

4.9. 2-Phenyl-5-(trityloxymethyl)isothiazol-3(2*H*)-one (19)

To a stirred slurry of 3-*Z*-benzylsulfinyl-4-trityloxy-but-2-enoic acid phenylamide (120 mg, 0.22 mmol) in dry dichloromethane (10 mL) at 0 °C under nitrogen was added trichloroacetic anhydride (0.047 mL, 0.28 mmol). After 20 min the reaction was quenched by the addition of satd $NaHCO_3$ (4 mL) and extracted with chloroform (3 × 8 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated in vacuo. Column chromatography (30% EtOAc/hexane) of the residue afforded 2-phenyl-5-trityloxymethyl-isothiazol-3-one as a powdery white solid (44 mg, 45%). Mp <40 °C; δH (250 MHz, $CDCl_3$, TMS) 4.35 (2H, s, CH_2O), 5.97 (1H, s, CH), 7.12–7.56 (20H, m, 4 × Ph); FAB-HRMS: measured 450.1545; calcd for $C_{29}H_{24}NO_2S$ $(M+H)^+$: 450.1528.

4.10. 5-Hydroxymethyl-2-phenylisothiazol-3(2*H*)-one (20)

Trifluoroacetic acid (0.16 mL, 2.07 mmol) was added dropwise to a stirred solution of 2-phenyl-5-trityloxymethyl-isothiazol-3-one (40 mg, 0.089 mmol) in dichloromethane (5 mL). After 5 min, methanol (2 mL) and Et_3N (0.5 mL) were added successively and the mixture evaporated to dryness. Flash column chromatography of the residue afforded 5-hydroxymethyl-2-phenyl-isothiazol-3-one as a colourless crystalline solid (11 mg, 0.053 mmol, 59%). Mp 134–135 °C; (found: C, 57.81; H, 4.39; N, 6.59; $C_{10}H_9NO_2S$ requires C, 57.95; H, 4.38, N, 6.76); δH (250 MHz, $CDCl_3$, TMS) 3.4–3.75 (1H, br, OH), 4.82 (2H, s, CH_2OH), 6.12 (1H, s, CH), 7.29–7.56 (5H, m, Ph); δC (62.9 MHz, $CDCl_3$) 58.6, 108.7, 124.7, 127.4, 129.2, 136.5, 163.6, 168.2; FAB-HRMS: measured 208.0444; calcd for $C_{10}H_{10}NO_2S$ $(M+H)^+$: 208.0432.

4.11. 5-Acetoxymethyl-2-phenylisothiazol-3(2*H*)-one (21)

Acetyl chloride (80 μL, 1.1 mmol) was added to a strired solution of 2-phenyl-5-trityloxymethyl-isothiazol-3(2*H*)-one (50 mg, 0.11 mmol) in dichloromethane (1 mL) at room temperature. After 36 h the mixture was diluted with $NaHCO_3$ (satd) (5 mL) and extracted with dichloromethane (3 × 10 mL). The organic extracts were dried over Na_2SO_4 and evaporated in vacuo. Flash column chromatography of the residue (50/50 EtOAc/hexane) afforded the title compound as a crystalline colourless solid (16 mg, 0.064 mmol, 58%). Mp 94–95 °C; ν_{max} (film)/cm^{-1}: 1738s (CO), 1641s (CO); δH (250 MHz, $CDCl_3$, TMS) 2.15 (3H, s, CH_3), 5.21 (2H, s, CH_2), 6.29 (1H, s, 4-H), 7.27–7.58 (5H, m, Ph); δC (62 MHz, $CDCl_3$) 20.4, 59.8, 114.3, 124.6, 127.3, 129.3, 136.4, 152.4, 166.7, 170.8; FAB-HRMS: measured 250.0555; calcd for $C_{12}H_{12}NO_3S$ $(M+H)^+$: 250.0538.

4.12. 5-Phenylureidomethyl-2-phenylisothiazol-3(2*H*)-one (22)

Phenyl isocyanate (5.7 mg, 0.048 mmol) was added to a stirred solution of 5-hydroxymethyl-2-phenyl-isothiazol-3(2*H*)-one (10 mg, 0.048 mmol) and pyridine (0.048 mmol) in dichloromethane (1 mL). After 48 h the precipitate was filtered, washed with dichloromethane (1 mL) and diethyl ether (2 mL). The resultant white powder was re-crystallised from EtOAc to afford the title compound as colourless needles (13 mg, 0.039 mmol). Mp 201–202 °C; (found: C, 62.03; H, 4.34; N, 8.27; $C_{17}H_{14}N_2O_3S$ requires C, 62.56; H, 4.32, N, 8.58); ν_{max} (film)/cm^{-1}: 1723s (CO), 1636s (CO); δH (250 MHz, $CDCl_3$, TMS) 5.32 (2H, s, CH_2), 6.36 (1H, s, 4-

H), 7.05–7.50 (10H, m, 2 × Ph); δC (62 MHz, $CDCl_3$) 60.0, 113.4, 118.7, 123.4, 124.9, 127.6, 128.6, 129.0, 135.6, 137.6, 153.1, 154.7, 167.4; FAB-HRMS: 327.0820; calcd for $C_{17}H_{15}N_2O_3S$ $(M+H)^+$: 327.0803.

4.13. 5-Azidomethyl-2-phenylisothiazol-3(2*H*)-one (23)

To a stirred solution of 5-hydroxymethyl-2-phenyl-isothiazol-3(2*H*)-one (100 mg, 0.483 mmol) in dichloromethane (10 mL) at 0 °C was added diisopropylethylamine (117 μL, 0.67 mmol), 4-dimethylaminopyridine (6 mg, 0.048 mmol) and tosic anhydride (188 mg, 0.58 mmol). After 4 h the reaction was quenched by the addition of $NaHCO_3$ and the resultant mixture extracted into EtOAc (3 × 10 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated in vacuo. Flash column chromatography of the residue (50/50 EtOAc/Hexane as eluant) afforded 5-(*p*-toluenesulfonyloxy)methyl-2-phenyl-isothiazol-3-one as colourless needles (125 mg, 72%). Mp 118 °C; (found: C, 56.24; H, 4.12; N, 3.76; $C_{17}H_{15}NO_4S_2$ requires C, 56.49; H, 4.18, N, 3.88); δH (250 MHz, $CDCl_3$, TMS) 2.46 (3H, s, CH_3), 5.20 (2H, s, CH_2), 6.20 (1H, s, 4-H), 7.3–8.1 (9H, m, 2 × Ar); FAB-HRMS: measured 362.0540; calcd for $C_{17}H_{16}NO_4S_2$ $(M+H)^+$ 362.0521.

A DMF solution (5 mL) of 5-(*p*-toluenesulfonyloxy)methyl-2-phenyl-isothiazol-3(2*H*)-one (100 mg, 0.277 mmol) and NaN_3 (36 mg, 0.55 mmol) were stirred at room temperature for 2 h. After this period the reaction was evaporated to dryness, re-dissolved in EtOAc (10 mL) and washed with $NaHCO_3$ (5 mL). The organic extract was dried over Na_2SO_4 and evaporated. Flash column chromatography of the residue (75% EtOAc/hexane as eluant) afforded the title compound as a colourless oil (55 mg, 0.237 mmol, 85%). Mp 60–64 °C; δH (250 MHz, $CDCl_3$, TMS) 4.60 (2H, s, CH_2), 6.26 (1H, s, 4-H), 7.32–7.59 (5H, m, Ph); δC (62 MHz, $CDCl_3$) 49.1, 113.2, 125.1, 127.9, 129.8, 136.8, 153.8, 167.4; FAB-HRMS: measured 233.0505; calcd for $C_{10}H_9N_4OS$ $(M+H)^+$: 233.0497.

4.14. 5-Methyl-2-*N*-Phenylisothiazol-3(2*H*)-one (28)

Benzyl mercaptan (1.47 g, 11.9 mmol) was added dropwise to a vigorously stirred solution of 2-butynoic acid (1.0 g, 11.9 mmol), Na_2CO_3 (1.38 g, 13.08 mmol), MeOH (8.6 mL) and water (12 mL) at room temperature. The mixture was stirred at reflux for 4 h and then 72 h at room temperature. The mixture was diluted with NaOH (10 mL, 0.5 M), washed with dichloromethane (2 × 15 mL) and the aqueous phase acidified to pH 1 with 2 M HCl. The precipitate thus formed was filtered, washed with 2 M HCl, water and dried under vacuum. This afforded a 2.2:1 *E/Z* mixture (2.01 g, 9.66 mmol, 81%) containing (Z)-3-(methyl)-3-(benzylsulfanyl)propenoic acid (**25**) as a light cream solid. δH (250 MHz, $CDCl_3$, TMS) 2.39 (3H, d, *J* 0.84, Me), 4.02 (2H, s, CH_2), 5.6 (1H, d, CH), 7.28–7.4 (5H, m, Ph).

The *E/Z* mixture **25** (1 g, 4.81 mmol) in dry benzene (2.5 mL) at room temperature was treated with thionyl chloride (0.53 mL, 7.2 mmol) and then refluxed for 3 h. The resultant red solution was evaporated in vacuo, dissolved in dry benzene (5 mL) and then cooled to 0 °C. Aniline (0.87 mL, 9.61 mmol) was added and the mixture stirred for 30 min at 0 °C before being warmed to room temperature. After a further 30 min the reaction mixture was poured onto ice water (20 mL) and diluted with EtOAc (20 mL). The organic phase was successively washed with 1 M HCl (10 mL), satd $NaHCO_3$ (10 mL) and brine (5 mL) and then dried over Na_2SO_4 prior to evaporation in vacuo. Flash column chromatography (40% diethyl ether/hexane) of the crude residue afforded an *E/Z* mixture (1.08 g, 3.81 mmol, 79%) containing (*Z*)-3-(methyl)-3-(benzylsulfanyl)-*N*-phenylpropenamide (**26**) as a white solid. δH (250 MHz, $CDCl_3$, TMS) 2.23 (3H, s, Me), 4.10 (2H, s, CH_2), 5.88 (1H, d, CH), 7.07–7.64 (10H, m, 2 × Ph).

A solution of the *E/Z* vinyl sulfide **26** (100 mg, 0.35 mmol) in dichloromethane (2 mL) at −10 °C under nitrogen was treated portionwise with 3-chloroperoxybenzoic acid (116 mg, 52% pure, 0.35 mmol) and stirred for 10 min. Aqueous $Na_2S_2O_3$ (2 mL) was added and stirred for 10 min whilst warming to room temperature. The reaction mixture was diluted with $NaHCO_3$ (2 mL) and extracted into dichloromethane (3 × 4 mL). The organic extracts were dried over Na_2SO_4 and evaporated in vacuo. This afforded an *E/Z* mixture (100 mg, 0.33 mmol, 95%) containing (*Z*)-3-(methyl)-3-(benzylsulfinyl)-*N*-phenylpropenamide (**27**) as a tan solid.

To a stirred slurry of *E/Z* sulfoxide **27** (100 mg, 0.33 mmol) in dry dichloromethane (1.6 mL) at 0 °C under nitrogen was added trichloroacetic anhydride (0.073 mL, 0.40 mmol). After 30 min the reaction was diluted with dichloromethane (10 mL) and quenched by the addition of 1 M NaOH (5 mL). The aqueous phase was extracted with dichloromethane (2 × 5 mL), dried over Na_2SO_4 and evaporated in vacuo. Column chromatography (100% diethyl ether) of the residue afforded 5-methyl-2-phenyl-isothiazol-3-one (**28**) as a colourless crystalline solid (14 mg, 22%). Mp 76–78 °C; ν_{max} (film)/cm^{-1}: 1648s (CO), 1591s (C=C); δH (250 MHz, $CDCl_3$, TMS) 2.44 (3H, s, Me), 6.10 (1H, s, CH), 7.26–7.32 (1H, m, Ph-H_4), 7.4–7.46 (2H, m, Ph-H_3), 7.55–7.58 (2H, m, Ph-H_2); δC (62 MHz, $CDCl_3$) 14.6, 113.4, 124.3, 126.2, 126.9, 136.7, 153.6, 167.7; FAB-HRMS: measured 192.0483; calcd for $C_{10}H_{10}NOS$ $(M+H)^+$: 192.0483.

4.15. 4-Bromomethyl-2-phenylisothiazolo-3(2*H*)-one (31)

A suspension of 4-methyl-2-phenyl-isothiazol-3(2*H*)-one (150 mg, 0.785 mmol), *N*-bromosuccinimide (147 mg, 0.825 mmol) and AIBN (5 mg, 0.03 mmol) in CCl_4 (6 mL) was heated at reflux for 24 h. The reaction was cooled, diluted with dichloromethane (10 mL) and washed successively with 1 M NaOH (5 mL) and brine (5 mL). The organic extracts were dried over Na_2SO_4 and evaporated. Flash column chromatography of the residue (dichloromethane as eluant) afforded the title compound as a gold reflective solid (77 mg, 0.285 mmol, 36%); mp 165 °C (dec.); (found: C, 44.34; H, 2.90; N, 5.16; $C_{10}H_8NOSBr$ requires C, 44.46; H, 2.98, 5.18); δH (250 MHz, $CDCl_3$, TMS) 4.37 (2H, s, CH_2), 7.28–7.65 (5H, m, Ph), 8.36 (1H, s, 5-H); *m/z* FAB-HRMS: measured 269.9600; calcd for $C_{10}H_9BrNOS$ $(M)^+$: 269.9588.

4.16. 4-Methoxymethyl-2-phenyl-isothiazol-3(2*H*)-one (32)

A slurry of 4-bromomethyl-2-phenyl-isothiazolo-3(2*H*)-one (25 mg, 0.092 mmol) and NaOMe (12 mg, 0.22 mmol) were stirred in MeOH (3 mL) at 30 °C for 7 h. After this period the reaction mixture was concentrated to dryness, re-dissolved in dichloromethane (10 mL) and washed with NH_4Cl (5 mL). The organic extract was dried over Na_2SO_4 and evaporated. Flash column chromatography of the residue (40% EtOAc/hexane as eluant) afforded the title compound as a white powder (13 mg, 0.058 mmol, 63%). Mp low melting solid; ν_{max} (film)/cm^{-1}: 1645s (CO); δH (250 MHz, $CDCl_3$, TMS) 3.45 (3H, s, CH_3), 4.34 (2H, s, CH_2), 7.26–7.62 (5H, m, Ph), 8.09 (1H, s, 5-H); δC (62 MHz, $CDCl_3$); 59.3, 69.4, 124.8, 125.1, 127.7, 129.7, 135.2, 137.2, 166.1; FAB-HRMS: measured 222.0575; calcd for $C_{11}H_{12}NO_2S$ $(M+H)^+$: 222.0589.

4.17. 4-Azidomethyl-2-phenyl-isothiazol-3(2*H*)-one (33)

A DMF solution (5 mL) of 4-bromomethyl-2-phenyl-isothiazolo-3(2*H*)-one (100 mg, 0.37 mmol) and NaN_3 (48 mg, 0.74 mmol) were stirred at room temperature for 1 h. After this period the reaction was evaporated to dryness, re-dissolved in EtOAc (25 mL) and washed successively with H_2O (10 mL) and brine (5 mL). The organic extract was dried over Na_2SO_4 and evaporated. Recrystallisation of the residue (EtOAc) afforded the title

compound as an off white solid powder (60 mg, 0.26 mmol, 70%). Mp 99–102 °C; (found: C, 51.39; H, 3.44; N, 23.91; $C_{10}H_8N_4OS$ requires C, 51.71; H, 3.47, N, 24.12); δH (250 MHz, $CDCl_3$, TMS) 4.29 (2H, s, CH_2), 7.33–7.61 (5H, m, Ph), 8.12 (1H, s, 5-H); δC (62 MHz, $CDCl_3$); 48.1, 122.7, 124.8, 127.9, 129.8, 136.0, 136.9, 166.0; FAB-HRMS: measured 233.0505; calcd for $C_{10}H_9N_4OS$ $(M+H)^+$: 233.0497.

4.18. 4-Methyl-5-methoxy-2-phenyl-isothiazol-3(2*H*)-one (35)

A solution of 4-methyl-5-chloro-isothiazol-3(2*H*)-one (50 mg, 0.22 mmol) and NaOMe (24 mg, 0.44 mmol) in MeOH (7.5 mL) were heated at reflux for 5 h. After this period the resultant mixture was evaporated to afford a yellow oil. Purification by flash column chromatography (30% EtOAc/hexane) afforded the title compound as a colourless oil (12 mg, 0.054 mmol, 24%). δH (250 MHz, $CDCl_3$, TMS) 1.92 (3H, s, CH_3), 3.97 (3H, s, CH_3O), 7.22–7.58 (5H, m, Ph); δC (62 MHz, $CDCl_3$) 9.1, 59.7, 101.5, 120.3, 124.9, 126.9, 129.6, 137.5, 168.0; FAB-HRMS: measured 222.0575; calcd for $C_{11}H_{12}NO_2S$ $(M+H)^+$: 222.0589.

4.19. 2-(Phenyl)-isothiazolo[5,4-*b*]pyridin-3(2*H*)-one (38)

PyBOP® (1.68 g, 3.22 mmol) was added portionwise to a stirred solution of 3-mercaptonicotinic acid (500 mg, 3.22 mmol), diisopropylethylamine (168 mL, 9.7 mmol) and aniline (285 mg, 3.06 mmol) in dichloromethane (20 mL) at 0 °C. The mixture was stirred for 30 min and then warmed to room temperature for 1 h. The reaction mixture was diluted satd $NaHCO_3$ (10 mL) and extracted with dichloromethane (2 × 10 mL). The combined organic extracts were then further extracted with 1 M NaOH (2 × 20 mL). The combined alkaline extracts were cooled to 0 °C and carefully acidified to pH 1 using 2 M HCl. The precipitate thus obtained was filtered, rinsed with H_2O (10 mL) and then recrystallised from methanol to afford *N*-(phenyl)-1,2-dihydro-2-thioxo-3-pyridecarboxamide **37** as a highly crystalline yellow solid (0.52 g, 2.26 mmol, 70%).

Triethylamine (0.12 mL, 0.87 mmol) was added dropwise to a stirred slurry of *N*-(phenyl)-1,2-dihydro-2-thioxo-3-pyridecarboxamide (100 mg, 0.43 mmol) and iodine (115 mg, 0.456 mmol) in dichloromethane (10 mL) at room temperature. After 60 min the reaction mixture was washed with satd $Na_2S_2O_3$ and extracted into dichloromethane (3 × 5 mL). The combined extracts were dried over Na_2SO_4 and evaporated in vacuo. Flash column chromatography of the residue (50% EtOAc/Hexane) afforded 2-(phenyl)-isothiazolo[5,4-*b*]pyridin-3-one (**38**) as a silvery crystalline solid (84 mg, 0.37 mmol, 85%). ^{1}H NMR data for this compound was in agreement with the literature.[35]

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