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# Synthesis and biological testing of novel pyridoisothiazolones as histone acetyltransferase inhibitors

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ABSTRACT

We present a combination of database screening, synthesis and in vitro testing to identify novel histone acetyltransferase (HAT) inhibitors. The National Cancer Institute compound collection (NCI) and several commercial databases were filtered by similarity-based virtual screening to find new HAT inhibitors. Employing the recombinant HAT p300/CBP-associated factor (PCAF) and two different histone substrates for screening, pyridoisothiazolones were identified as inhibitors of human PCAF. Due to the limited solubility of the initial hits, we synthesized and tested them on PCAF. The compounds inhibit the proliferation of cancer cells. In summary, valuable chemical tools and potential lead candidates for new anticancer agents directed against HATs as new targets have been identified.



## 1. Introduction

Acetylation and deacetylation of histones have emerged as key mechanisms regulating transcriptional activity. Histone acetyltransferases (HATs) catalyze the transfer of acetyl groups to lysine residues in histones, which results in a more open conformation of nucleosomes and increased accessibility of regulatory proteins to DNA.[1] The acetylation status of several non-histone proteins including p53, ataxia-telangiectasia mutant (ATM), heat shock protein 90, and α-tubulin, is intimately related to their functions. Reversible acetylation of α-tubulin marks stabilized microtubule structures and may contribute to regulating microtubule dynamics.[2] Acetylation of p53 tumor suppressor protein by two HAT subtypes (p300/cAMP-responsive element binding protein (CBP) and p300/CBP-associated factor (PCAF)) was linked to its transactivation potential and ability to regulate cell cycle arrest and apoptosis.[3] Furthermore, several HATs were found to be genetically altered in a variety of cancer types.[4] In view of the increasing evidence that associates HAT function with cancer formation and progression, these enzymes are appealing as drug targets for the development of small-molecule inhibitors.

Whereas an increasing number of HDAC inhibitors is available nowadays, only a few HAT inhibitors have been described so far. They possess various degrees of selectivity and cell permeability. Peptide-CoA conjugates (Lys-CoA and H3-CoA-20)[5] as well as a few natural small molecules such as anacardic acid[6] and analogues,[7] garcinol,[8] and curcumin[9] and the γ-butyrolactone MB-3 were described as potent p300 or PCAF inhibitors.[10] Moreover, the selective inhibition of p300 HAT by semi-synthetic derivatives of garcinol (i.e., LTK-14)[11] was described. Recently, Aherne and co-workers used high throughput screening to identify isothiazolone derivatives as irreversible PCAF inhibitors.[16] The compounds were shown to decrease the cellular acetylation level in a time- and concentration dependent manner and they inhibited the growth of a panel of human tumor cell lines. It was also shown that the preservative agent Kathon CG, which also contains an isothiazolone structure, inhibits HAT and cancer cell growth.[12]

In order to identify new inhibitors of HATs, we applied a strategy that combined computational screening methods with a robust biochemical assay that has been successfully applied to several targets recently.[13–15] Virtual database filtering was used to conduct virtual screening with the National Cancer Institute (NCI) compound collection and several commercial compound libraries. As search query we used the isothiazolone and isothiazolidinone ring system which was derived from recently identified isothiazolone HAT inhibitors. Subsequently, the initial hits were docked into the active site of the HAT PCAF. Thus, 21 compounds were identified as inhibitor candidates and these were then tested in a PCAF in vitro assay. Due to the limited solubility of some of the identified hits and to obtain further structure-activity relationships, a series

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of further analogs were synthesized and biologically tested. We show for the first time that pyridoisothiazolones inhibit PCAF in the submicromolar range and are promising anticancer lead structures with good cellular activity.

## 2. Results and discussion

We chose to computationally screen the National Cancer Institute (NCI)'s 3D database since it contains structurally diverse synthetic compounds collected from many laboratories around the world as well as natural products. The recently identified isothiazolone inhibitor **V**,[16] which acts as an irreversible inhibitor by covalently binding to Cys574 at the PCAF active site, was used to derive a search query for the in silico screening. The crystal structure of PCAF complexed with the cofactor Acetyl-CoA (pdb code 1CMO) was used for docking studies. We identified 51 compounds with an isothiazolone or isothiazolidinone substructure that were subsequently docked into the PCAF substrate binding site to test whether they are able to bind at the catalytic site. 32 compounds were identified that contain a reactive S–N bond which was located in close proximity to the active site Cys574 in the model (S–S distance below 4.5 Å). Among these, 15 compounds (Fig. 1 and Table 1) could be obtained from the NCI. Using the same search query five other pyridoisothiazolones were identified and purchased from commercial suppliers (Ambinter, ChemDiverse, Enamine, see Fig. 2).

The 21 selected compounds were tested for in vitro inhibition of histone acetyltransferase activity using recombinant human PCAF. Biotinylated oligopeptide sequences from both histones $H3_{1-21}$ and $H4_{2-24}$ were used as the substrates. The conversion was detected with a primary antibody against acetylated histone H3 and H4 and quantification was achieved by using a secondary Europium labelled antibody with a final measurement of time resolved fluorescence. This assay had been used before for the determination of cellular hypoacetylation caused by isothiazolones.[16] We had previously used the same approach for the determination of histone methyltransferase activity in vitro and in cancer cells[17,18] and also use it here for the characterization of in vitro HAT inhibition. The published isothiazolone **V**[16] was synthesized and used as reference inhibitor.

The most active pyridoisothiazolones showed PCAF inhibition in the submicromolar range. In most cases a higher potency was observed on the H4 substrate. Thus, the binding of the histone substrate seems to influence the structure or reactivity (or both) of the acetyltransferase with regard to inhibitor responsiveness.

The amino-substituted isothiazolone **NSC 145097** (Fig. 1, Table 1) as well as the isothiazolone-thione **NSC 279225** (Fig. 1, Table 1) did not show significant HAT inhibition. Isothiazolones substituted with an aminocarbonylmethyl linker (**A4033-0171899**, **K783-6791**, and **K783-6798** (Fig. 2)) showed only moderate inhibition on PCAF inhibition. The highest potency was observed for the pyridoisothiazolones **NSC 694614** and **NSC 694622** (Fig. 1, Table 1). Generally, the nitro substituted compounds from the NCI repository suffered from severe solubility problems and this made determination of the cellular activity impossible. In some cases, compounds precipitated in the cell culture medium and no growth inhibition could be observed. For that reason and in order to obtain

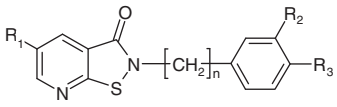



**Figure 1.** Pyridoisothiazolones scaffold for compounds from the NCI compound repository and synthesized compounds **4a-g** and **8a-h**.

**Table 1**
Chemical structures of pyridoisothiazolones from the NCI compound repository and synthesized compounds **4a-g** and **8a-h** according to the scaffold in Figure 1

| Compound | *n* | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|---|
| NSC 694614 | 0 | $CH_3$ | H | Br |
| NSC 694615 | 0 | H | H | Br |
| NSC 694616 | 0 | $NO_2$ | H | Br |
| NSC 694617 | 0 | H | H | $NO_2$ |
| NSC 694618 | 0 | $NO_2$ | H | $NO_2$ |
| NSC 694619 | 0 | $CH_3$ | H | $OC_6H_5$ (OPhe) |
| NSC 694620 | 0 | $NO_2$ | H | $OC_6H_5$ (OPhe) |
| NSC 694621 | 0 | H | H | $OCH_3$ |
| NSC 694622 | 0 | $NO_2$ | H | $OCH_3$ |
| NSC 694623 | 0 | H | H | $C_4H_9$ |
| NSC 698599 | 0 | $CH_3$ | H | $NO_2$ |
| NSC 698600 | 0 | $CH_3$ | H | $OCH_3$ |
| NSC 700864 | 0 | $C_6H_5$ (Phe) | H | $C_4H_9$ |
| **4a** | 0 | H | H | H |
| **4b** | 0 | H | H | Cl |
| **4c** | 0 | H | Cl | Cl |
| **4d** | 0 | H | H | F |
| **4e** | 0 | H | F | F |
| **4f** | 0 | H | H | $CH_3$ |
| **4g** | 0 | H | $OCH_3$ | H |
| **8a** | 1 | H | H | H |
| **8b** | 1 | H | H | Cl |
| **8c** | 1 | H | Cl | Cl |
| **8d** | 1 | H | H | F |
| **8e** | 1 | H | F | F |
| **8f** | 1 | H | H | $CH_3$ |
| **8g** | 1 | H | $OCH_3$ | H |
| **8h** | 1 | H | H | $CF_3$ |

further structure–activity relationships a series of analogs was synthesized on the basis of the derived initial hits.

*N*-Substituted pyridoisothiazolones were synthesized by two various routes. Starting out from 2-mercaptonicotinic acid, *N*-phenyl and *N*-benzyl derivatives (Fig. 1, Table 1) were prepared in order to determine structure–activity relationships. After protection of the reactive thiol group of the starting material, amide formation with aniline derivatives and following oxidative cyclisation provided *N*-aryl substituted derivatives (Scheme 1, compounds **4a**–**g**).[19] *N*-Benzyl-derivatives (compounds **8a–h**) were obtained by nucleophilic substitution using the parent pyridoisothiazolone structure (compound **7**, Scheme 2) and benzyl bromides.[20–22]

For the most potent compound from the class of *N*-benzyl pyridoisothiazolones (**8c**), a two-step sulphur oxidation was carried out in order to investigate the influence of the oxidation state on the inhibitory activity. Treatment with $KHSO_5$ leads to the racemic sulphoxide **9** and the subsequent phase-transfer oxidation with hypochloride provides the sulphone **10** (Scheme 3).[23] For the oxidized analogues the reduced inhibitory properties in vitro against PCAF/$H3_{1-21}$ (Table 2) can be correlated with the increasing sulphur oxidation state. With each oxidation step a huge reduction of the $IC_{50}$ value was observed. Mono- and dioxidized pyridoisothiazolone derivatives are probably much less reactive. The electron withdrawal effect of the oxygen on the sulphur hampers the S–N bond cleavage.[24] Due to the sterically more demanding sulphoxide and sulphone group it cannot be excluded that the low activity is resulting from unfavourable steric effects.[25]

Molecular modelling studies were carried out to analyze the binding mode of the identified pyridoisothiazolones. The PCAF crystal structure complexed with the cosubstrate CoA was used to dock the inhibitors into the substrate and cosubstrate binding pocket. The docking studies (e.g., for compound **4d**, Fig. 3) showed a preferred binding mode in which the isothiazolone S–N bond is located nearby the active site Cys574. A T-shaped aromatic pi–pi stacking interaction is observed between the pyridine ring of the pyridoisothiazolones and Tyr616 (distance pyridine-N/aromatic plane 3.8 Å). The binding of the compounds is further stabilized

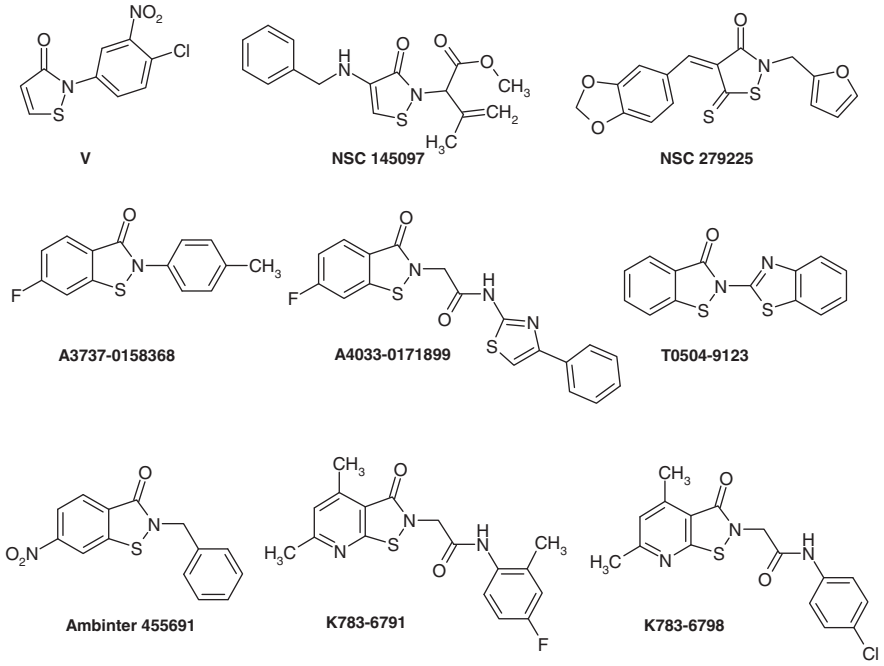
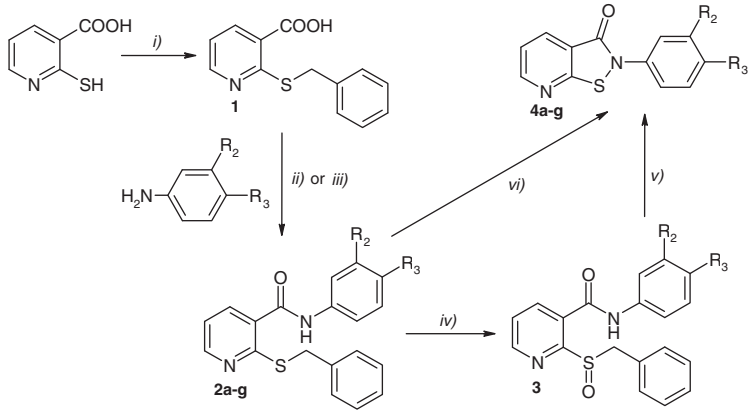



**Figure 2.** Chemical structures of further isothiazolones.



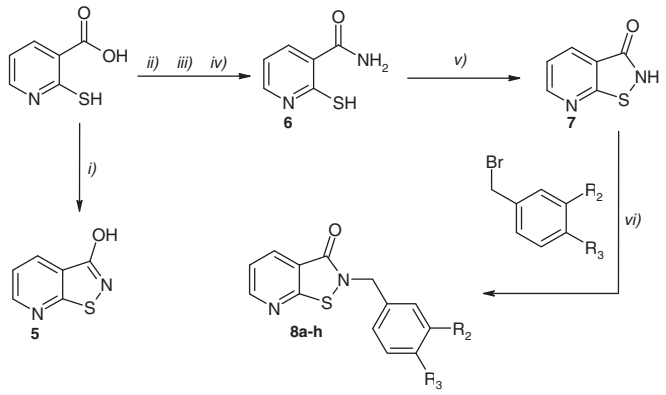

**Scheme 1.** Synthesis routes for compounds with *N*-phenylpyridoisothiazolones scaffold (compounds **4a–g**). Reagents and conditions: (i) BnCl, KOH, isopropyl alcohol, reflux, 30 min; (ii) BOP–Cl, DIPEA, $CH_2Cl_2$, rt, overnight; (iii) IBCF, $Et_3N$, $CH_2Cl_2$, rt, overnight; (iv) *m*-CPBA, $CH_2Cl_2$, 0 °C, 15 min rt, overnight; (v) TCAA, $CH_2Cl_2$, 0–25 °C, 4 h; (vi) $SO_2Cl_2$, $Cl(CH_2)_2Cl$, reflux, 45 min; (rt: room temperature).

by van der Waals interactions with aliphatic and aromatic residues of the pocket (Leu526, Met 529, Ala613 and Phe617). The observed binding mode of the potent inhibitors **NSC 694614-NSC 694623** (Fig. 1, Table 1) as well as the novel compounds **4a–g** (Fig. 1, Table 1) is very similar and no correlation between the docking scores (Goldscore, Chemscore) and the PCAF inhibition data could be derived. Introducing a methylene group between the aromatic substituent and the isothiazole core (e.g., compound **8h**) results in increasing the distance Cys574/S–N bond and is therefore unfavourable.

To show that the pyridoisothiazolones bind irreversibly to PCAF, potentially through a reactive S–N bond, we analyzed the role of thiol interactions in the inhibitory activity of the compounds toward PCAF. We determined the effect of a thiol reagent on the PCAF inhibition by **NSC 694622** (Fig. 1, Table 1). Without DTT, an $IC_{50}$ value of 3.42 ± 0.35 μM was determined. In the presence of 1 mmol/L DTT, which alone had no significant effect on enzyme activity, HAT-inhibitory activity was significantly reduced (Fig. 4).

It was reported by Gorsuch et al.[26] that the observed in vitro HAT inhibitory results for isothiazolones are consistent with an attack of a nucleophile (e.g., cysteine-SH) at the sulfur atom of the heterocycle. This proceeds with concomitant ring-opening. The authors showed for example that the presence of a 5-Cl substituent at the isothiazolone ring which is increasing the reactivity of the S–N bond by the higher electronegativity is also increasing the HAT activity. Therefore, we calculated the electronic properties of the tested isothiazolones on the basis of semi-empirical AM1 calculations (AM1 bcc1 partial charge on the S and N atom of the isothiazolone, HOMO and LUMO energies) and tried to correlate them with the observed PCAF inhibition data. No significant correlation between any of the calculated descriptors and the HAT in vitro inhibition was observed indicating that the reactivity of the S–N bond alone is not able to explain the biological activity of the tested compounds. Additionally, the appropriate interaction of the isothiazolone system with Cys574 might be sterically influenced by unfavourable substituents (e.g., **NSC 700864**).

The new pyridoisothiazolones **4d** and **8h**, as well as **NSC 694616** (Fig. 1, Table 1) were then tested for their growth inhibitory properties using SK-N-SH neuroblastoma and MCF7 breast cancer cells (Table 3). The more soluble fluorophenyl isothiazolone



**Scheme 2.** Synthesis routes for compounds with *N*-benzylpyridoisothiazolones scaffold (compounds **8a**–**h**). Reagents and conditions: (i) DPPA, $Et_3N$, pyridine, rt, 1 h; (ii) $SOCl_2$, BOP–Cl, toluene, reflux, 3 h; (iii) $NH_4OH$, $NH_4Cl$, rt, 18 h; (iv) $NaBH_4$, rt, 1 h; (v) conc. $H_2SO_4$, reflux, 3 h; (vi) DIPEA, methanol, rt, overnight; (rt: room temperature).

showed good inhibition especially on the neuroblastoma cell line. Surprisingly, the cellular activity of the benzyl congener was higher, particularly on the SK-N-SH cells, and it was also higher than its own in vitro inhibition. This indicates the involvement of additional mechanisms which indeed have been described for isothiazolones in the literature, for example, inhibition of farnesyltransferases.[26] For compound **4d**, clear time dependence was shown for the antiproliferative action (Fig. 5).

## 3. Conclusions

Isothiazolones had been presented as new lead structures for HAT inhibition in the literature but suffer from general high reactivity. We hoped that compounds with decreased reactivity, for example, obtained by annellation, would still retain HAT inhibition but might be more selective towards that target and could be useful for cellular and eventual further preclinical evaluation as potential anticancer agents. A covalent attachment to thiol groups does not necessarily rule out clinical application as can be shown, for example, for the antiulcer drug omeprazole. Indeed, pyridothiazolones could be identified via virtual screening and in vitro enzyme testing as new PCAF inhibitors. Initial chemical variations of the lead structures led to improved cellular activity as compared to the hits from the in-silico screening. During the course of this work, the group of Aherne just recently published similar compounds and presented them as lead structures with increased potency[27] but no cellular activity was disclosed. We applied a combination of virtual and biological screening approach to potent PCAF inhibitors and show that these pyridoisothiazolones show promising anticancer activity in cellular models. Other mechanisms of action cannot be ruled out at the moment but these initial results merit further structure–activity relationships in this class of compounds.

## 4. Experimental section

### 4.1. Computational methods

All calculations were performed on a Pentium IV 2.2 GHz based Linux cluster (20 CPUs). The GOLD software (Cambridge Crystallographic Data Centre) was used for docking, whereas the calculation of all molecular descriptors and the analysis of the docking results were carried out in MOE2008.10[28] (Chemical Computing Group).

### 4.2. Ligand docking

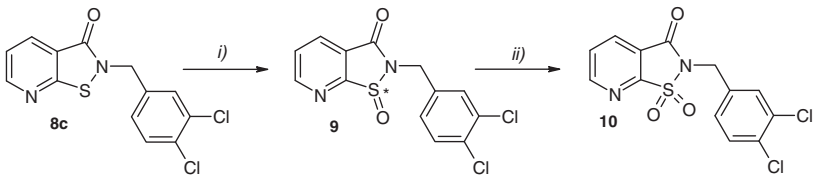
The crystal structure of human PCAF in complex with Acetyl-CoA (pdb code 1CM0, chain B) resolved at 2.30 Å was taken from the Protein Data Bank. The cofactor and the water molecules were removed, hydrogen atoms were added and the protein was minimized using the AMBER force field[29] and the conjugate gradient minimization (MOE 2008.10) until the gradient of 0.1 kcal/mol was reached. Docking of the ligands was carried out using the GOLD 4.0 program with default settings. A sphere of 14 Å around the S-atom of Cys574 was defined for ligand docking. Details about the GOLD program can be found elsewhere.[30] To test the applicability of the docking tool, a control docking was carried out with the cofactor acetyl-CoA. Using GoldScore as scoring function an RMSD value of 1.45 was derived for the top-ranked conformation of Acetyl-CoA (data not shown). For all compounds under study the GoldScore as well as the ChemScore was calculated and analyzed.

### 4.3. Database screening

The 260,000 3D structures of the National Cancer Institute (NCI) database, generated with the program CORINA, were obtained from the NCI homepage (http://dtp.nci.nih.gov/docs/3d_database/dis3d.html) and were imported as Mol2 files into the MOE program. The 3D structures of the Ambinter, ChemDiverse, Chemical Block and Enamine were retrieved from the ZINC database (http://zinc.docking.org). Using an isothiazolone/isothiazolidinone ring as search query we identified 51 compounds from the NCI database that were subsequently docked into the PCAF protein structure using the GOLD program as described above. 32 molecules were successfully docked into the CoA binding pocket showing a distance <5 Å between the S–N bond and the thiol group of Cys574 (distance S to S). From the 32 compounds selected, 15 could be obtained from the NCI. By using the same isothiazolone search query six further compounds were identified and purchased from Ambinter, ChemDiverse, Chemical Block, and Enamine.

### 4.4. Calculation of electronic properties

Geometrey optimization and calculation of partial charges was carried out at the semi-empirical level using the AM1 paramterization (Dewar et al., 1985). ESP partial charges were calculated on the basis of the AM1 optimized geometries. Energies for the highest occupiet molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) were calculated.



**Scheme 3.** Synthesis routes for sulphur oxidation of compound **8c**. Reagents and conditions: (i) $KHSO_5$ (oxone®), methanol, rt, 1 h; (ii) NaClO, TBAB, ethyl acetate, rt, 1 h; (rt: room temperature). *Racemic.

**Table 2**
Inhibition of PCAF histone acetyltransferase activity for histone substrates H3 (amino acid residues 1–21) and H4 (amino acid residues 2–24): $IC_{50}$ value [μM] ± standard error [μM] or enzyme inhibition [%] at the specified concentration

| Compound | PCAF, H3 (1–21) | PCAF, H4 (2–24) |
|---|---|---|
| NSC 694614 | 2.99 ± 0.27 | n.t. |
| NSC 694615 | 4.43 ± 0.20 | n.t. |
| NSC 694616 | 4.91 ± 0.39 | 0.86 ± 0.08 |
| NSC 694617 | 4.10 ± 0.60 | 2.99 ± 0.32 |
| NSC 694618 | 11.8 ± 1.83 | 15.8 ± 5.76 |
| NSC 694619 | 12.9 ± 0.95 | n.t. |
| NSC 694620 | 48% @ 10 μM | n.t. |
| NSC 694621 | 5.71 ± 0.30 | n.t. |
| NSC 694622 | 3.42 ± 0.35 | 1.83 ± 0.47 |
| NSC 694623 | 15.9 ± 2.20 | n.t. |
| NSC 698599 | 15.3 ± 6.21 | 2.72 ± 0.60 |
| NSC 698600 | 6.51 ± 0.49 | n.t. |
| NSC 700864 | 49% @ 25 μM | n.t. |
| **4a** | 7.85 ± 0.46 | 88% @ 50 μM |
| **4b** | 3.53 ± 0.07 | 87% @ 50 μM |
| **4c** | 4.57 ± 0.23 | 91% @ 50 μM |
| **4d** | 1.64 ± 0.11 | 0.72 ± 0.05 |
| **4e** | 5.90 ± 0.85 | n.t. |
| **4f** | 4.63 ± 0.24 | 75% @ 50 μM |
| **4g** | 5.03 ± 0.14 | n.t. |
| **5** | 17% @ 50 μM | n.t. |
| **8a** | 22.96 ± 2.06 | n.t. |
| **8b** | 17.5 ± 1.27 | n.t. |
| **8c** | 6.94 ± 0.57 | n.t. |
| **8d** | 86.3 ± 3.40 | n.t. |
| **8e** | 101 ± 4.92 | n.t. |
| **8f** | 27.9 ± 4.50 | n.t. |
| **8g** | 22.2 ± 1.97 | n.t. |
| **8h** | 130 ± 8.49 | 25.5 ± 2.85 |
| **9** | 11% @ 50 μM | n.t. |
| **10** | 6% @ 50 μM | n.t. |
| V | 4.80 ± 0.20 | n.t. |
| NSC 145097 | 48% @ 300 μM | 20% @ 200 μM |
| NSC 279225 | n.i. @ 25 μM | n.t. |
| A 455691 (Ambinter) | 7.66 ± 1.29 | 51.3 ± 8.83 |
| A3737-0158368 (Chemical Block) | 3.69 ± 0.17 | n.t. |
| A4033-0171899 (Chemical Block) | 21% @ 50 μM | n.t. |
| T0504-9123 (Enamine) | 46% @ 50 μM | n.t. |
| K783-6791 (ChemDiverse) | 48% @ 50 μM | n.t. |
| K783-6798 (ChemDiverse) | 38% @ 50 μM | n.t. |

n.t.: not tested; n.i.: no enzyme inhibition (<5%) at the specified assay concentration.

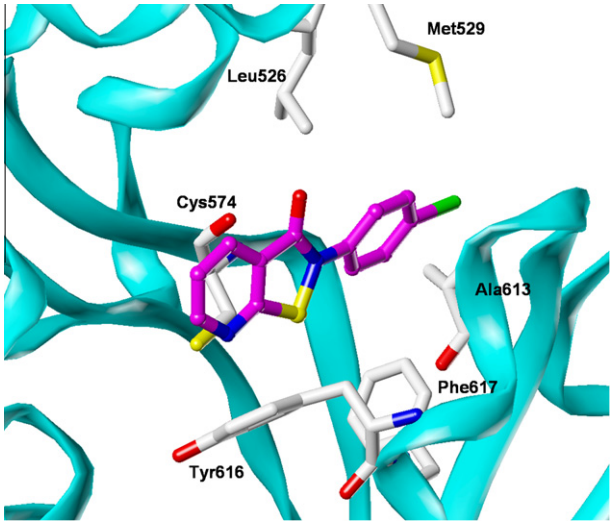



**Figure 3.** GOLD docking solution for pyridoisothiazolone **4d** (colored magenta) at the PCAF binding site. The sulphur of the isothiazolone inhibitor is in close proximity to the active site Cys574 thus enabling the formation of a covalent bond between the enzyme and the isothiazolone.

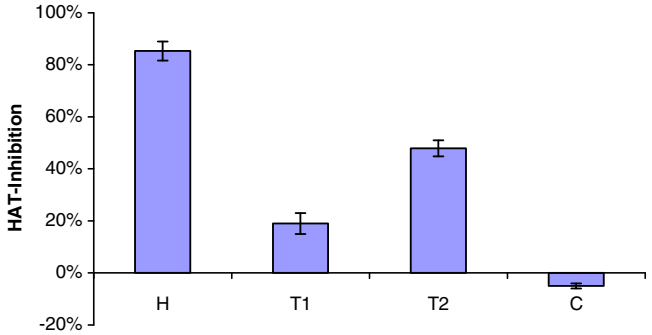



**Figure 4.** Reversibility of HAT inhibition by thiols (compound **NCS 694622**). DTT Influence on HAT inhibition by pyridoisothiazolones; Column H: 10 μl PCAF (0.01 mg/ml), 5 μM NSC 694622 (**5**), 100 μM AcCoA; Column T1: 10 μl PCAF (0.01 mg/ml), 5 μM NSC 694622 (**5**), 50 mM DTT added at preincubation step, 100 μM AcCoA; Column T2: 10 μl PCAF (0.01 mg/ml), NSC 694622 (**5**), 50 mM DTT added after preincubation step, 100 μM AcCoA; Column C: 10 μl PCAF (0.01 mg/ml), 50 mM DTT, 100 μM AcCoA.

**Table 3**
Antiproliferative effects in cancer cells ($GI_{50}$ value [μM] ± standard error [μM] or growth inhibition [%] at the specified concentration; SK-N-SH: human neuroblastoma cell line, HCT116: colorectal carcinoma cell line, MCF-7: breast adenocarcinoma cell line)

| Compound | SK-N-SH | HCT116 | MCF-7 |
|---|---|---|---|
| NSC 694614 | 21.3 ± 4.17 | n.i. @ 25 μM | n.t. |
| NSC 694615 | 15.2 ± 2.53 | 67% @ 25 μM | n.t. |
| NSC 694616 | 53.5 ± 2.23 | n.t. | 44.25 ± 3.55 |
| NSC 694617 | 15% @ 50 μM | n.t. | n.t. |
| NSC 694619 | 32% @ 25 μM | 29% @ 25 μM | n.t. |
| NSC 694620 | n.i. @ 25 μM | <10% @ 25 μM | n.t. |
| NSC 694621 | 19.2 ± 1.22 | 29% @ 25 μM | n.t. |
| NSC 694623 | 8.93 ± 0.20 | 21% @ 25 μM | n.t. |
| NSC 698600 | 12.2 ± 0.20 | 40% @ 25 μM | n.t. |
| NSC 700864 | 55% @ 25 μM | 33% @ 25 μM | n.t. |
| **4a** | 11.2 ± 1.88 | n.i. @ 25 μM | n.t. |
| **4b** | 16.1 ± 1.88 | 23% @ 25 μM | n.t. |
| **4c** | 37% @ 20 μM | 56% @ 20 μM | n.t. |
| **4d** | 10.3 ± 1.24 | 28% @ 25 μM | 37.2 ± 0.32 |
| **4f** | 6.20 ± 0.07 | 20% @ 25 μM | n.t. |
| **4g** | 6.99 ± 1.09 | 24% @ 25 μM | n.t. |
| **8a** | 18% @ 10 μM | 100% @ 25 μM | n.t. |
| **8b** | 20% @ 10 μM | 97% @ 25 μM | n.t. |
| **8c** | 9.53 ± 0.57 | 15.8 ± 1.67 | n.t. |
| **8f** | 31% @ 10 μM | 79% @ 25 μM | n.t. |
| **8g** | 26% @ 10 μM | 42% @ 25 μM | n.t. |
| **8h** | 0.48 ± 0.02 | 38% @ 25 μM | 30.2 ± 1.79 |
| V | 23.2 ± 1.52 | n.t. | n.t. |
| A3737-0158368 | 35% @ 100 μM | 49% @ 100 μM | n.t. |
| A4033-0171899 | n.i. @ 40 μM | n.i. @ 40 μM | n.t. |
| K783-6791 | 25.7 ± 5.52 | 22.6 ± 1.59 | n.t. |
| K783-6798 | 22.1 ± 0.71 | 35.8 ± 1.48 | n.t. |

n.t.: not tested; n.i.: no growth inhibition (<5%) at the specified assay concentration.

## 4.5. Enzyme preparation (PCAF)

The plasmid construct pQE80_P/CAF1.16 (5.2 kb, PCAF residues 493–658 (a gift of Professor John M. Denu, University of Wisconsin, Madison) was transformed for propagation and maintenance in competent *Escherichia coli* (TG1). The recombinant HAT domain of PCAF (residues 157–657) was overexpressed as *N*-terminal His-tagged fusion protein in ampicillin-sensitive *Escherichia coli* (BL21 D3) and 1 L LB medium. After IPTG induction at 18 °C overnight,[31–33] bacterial cells were centrifuged for 15 min (8000 rcf, 4 °C) and the supernatant was removed. The cell pellet was resuspended in 40 mL of lysis buffer pH 8.0 (1 mg/mL lysozyme, 0.1 mM phenylmethanesulfonyl fluoride, 1 mM β-mercaptoethanol, 300 mM NaCl, 50 mM Tris). After 30 min incubation and following

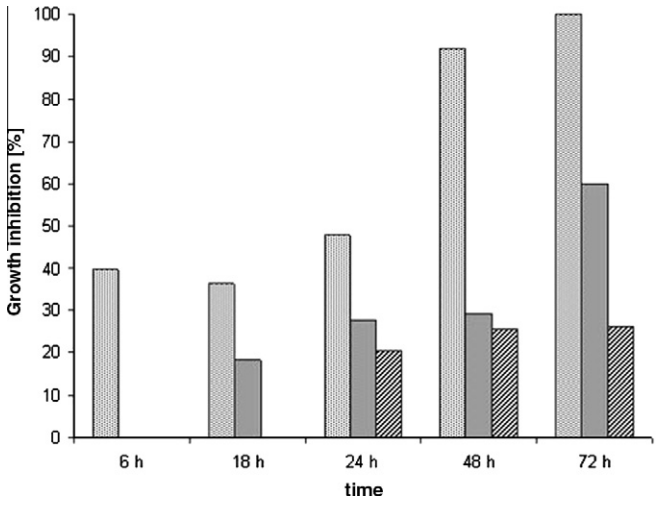



**Figure 5.** Time dependence of growth inhibition (compound **4d**). Growth inhibition of SK-N-SH neuroblastoma cells caused by different concentrations of compound **4d** was time-dependent up to 72 h of incubation time (dotted bars—**4d** 25 μM, grey bars—**4d** 10 μM, striped bars—**4d** 5 μM).

sonification (5-fold for 15 s, 60% output), the cell debris were removed by centrifugation (9000 rcf, 4 °C) in order to obtain clear supernatant. Protein purification was performed using Ni-NTA Superflow resin (Qiagen) according to the supplier's instructions. Prewashing of the beads with lysis buffer pH 8.0 (300 mM NaCl, 50 mM Tris) was repeated twice and subsequently the collected supernatant was placed on the resin. After washing with 40 mL of wash buffer pH 8.0 (1 mM β-mercaptoethanol, 10 mM imidazole, 300 mM NaCl, 50 mM Tris), the enzyme elution was performed according to a replacement from the resin with elution buffer pH 8.0 (1 mM β-mercaptoethanol, 500 mM imidazole, 300 mM NaCl, 50 mM Tris). Sephadex™ G-25 PD-10 Desalting column (Amersham Biosciences) was used according to the technical instructions for buffer exchange against storage buffer pH 6.0 (1 mg/mL BSA, 10% (v/v) glycerol, 20 mM trisodium citrate, 250 mM NaCl). In order to verify the purity and the presence of a 6xHis-tagged protein, SDS–PAGE and immunodetection with HRP mouse anti-6xHis antibody (BD Pharmingen, #552565), respectively, were performed under standard conditions. Protein quantification (12.6 mg enzyme from 1 L LB medium) was determined using Pierce® BCA Protein Assay Kit (Pierce Biotechnology). Samples with high specific histone acetyltransferase activity were stored in aliquots at −80 °C and used for the enzyme inhibition assay described below.

### 4.6. In vitro time-resolved fluorescence immunosorbent acetyltransferase assay for PCAF

The heterogeneous assay is performed in streptavidin-coated 96-well plates (Nunc). After each incubation step (1 h at 30 °C), six washing steps as well as a prewash step are necessary to remove the non bound fraction using wash buffer pH 7.5 (0.1% (v/v) Tween 20, 150 mM NaCl, 20 mM Tris base, 80 mM Tris–HCl) with 300 μL/step in an overflow mode (Tecan Columbus Plate washer). In the first incubation step, 20 pmol/well biotinylated histone peptide residues 1–21 of human histone H3 or residues 2–24 of human histone H4, respectively (Millipore, #12–403 respectively #12–372), diluted in the same buffer as mentioned above is bound to the wells. In the second step, the bound substrate is turned over in an enzymatic reaction. For this, preincubation of 10 μL/well enzyme PCAF, diluted in HAT-buffer pH 7.5 (0.1% (w/v) BSA (protease-free), 0.8% (v/v) Triton X-100, 100 mM HEPES), 2 μL of inhibitor DMSO-solution 18 μL of the HAT-buffer is performed for 10 min at 30 °C. Controls are treated in the same manner, using HAT-buffer instead of the inhibitor solution. Then, each mixture is transferred to the plate. To start the enzymatic reaction, 10 μL/well of a 400 μM acetyl-CoA solution in water is added to each well (final assay concentration 100 μM acetyl-CoA). The amount of the turnover is detected by a primary rabbit IgG antibody against the modification. 100 μL/well of anti-acetyl-histone H3 (Millipore, #06–599) or anti-acetyl-lysine (Abcam, #ab21623) respectively is added in appropriate dilutions in Tris-buffer pH 7.5 (0.1% (v/v) Tween 20, 0.5% (w/v) BSA (protease-free), 150 mM NaCl, 20 mM Tris base, 80 mM Tris–HCl). In the next step incubation with 5 pmol/well of a N1-Eu-labelled anti-rabbit secondary antibody (Perkin-Elmer, #AD0105), diluted in the same Tris-buffer as the first antibody, is followed. The europium label is cleaved off by adding of 100 μL/well of enhancement solution (Perkin-Elmer, #1244–105). After incubation for 10 min at room temperature, time-resolved fluorescence measurement at $\lambda_{ex}/\lambda_{em}$: 340/615 nm (BMG Polarstar) is performed for the quantification. Readout data were plotted as relative fluorescence units against logarithm of compound concentration using GraphPad Prism 4.00 Software in order to obtain $IC_{50}$ values.

### 4.7. Cell lines

SK-N-SH neuroblastoma cells were grown in DMEM low glucose (PAA) supplemented with 10% fetal bovine serum (PAA), 2 mM L-glutamine (Gibco Invitrogen) and 1% penicillin-streptomycin (Gibco Invitrogen). HCT116 colorectal cancer cells were grown in DMEM high glucose (PAA) supplemented with 10% fetal bovine serum (PAA), 2 mM L-glutamine (PAA), 1% non essential amino acids (PAA) and 1% penicillin-streptomycin (PAA), MCF-7 human breast adenocarcinoma cells were grown in RPMI-1640 (PAA) supplemented with 10% fetal bovine serum (PAA), 2 mM L-glutamine (Gibco Invitrogen) and 1% penicillin-streptomycin (Gibco Invitrogen).

### 4.8. Cell proliferation assay

Cells were seeded in 96-well tissue culture plates at a density of 5000 per well for SK-N-SH and MCF-7 and 4000 per well for HCT116. After 24 h of incubation, diluted compounds or DMSO vehicle were added to each well to a total volume of 100 μL; three replicates per concentration were used. Growth inhibition was determined using the CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay according to the manufacturer's instructions. Data was plotted as absorbance unit against logarithm of compound concentration using GraphPad Prism 4.02. 50% Growth inhibition ($GI_{50}$) was determined as compound concentration required to reduce the number of metabolic active cells by 50% compared to DMSO control.

### 4.9. Inhibitors

Standard chemicals were purchased from Acros, Sigma Aldrich, or Fluka. Solvents were dried before use according to standard procedures. Merck silica gel 60 was used for flash chromatography with the given eluents. Melting points were obtained after measurement with SMP2 (Stuart Scientific) and are uncorrected. NMR spectra were recorded on an Avance DRX 400 MHz spectrometer (Bruker) or an Unity 300 MHz spectrometer (Varian) respectively. Chemical shifts are reported in parts per million ($\delta$) relative to residual solvent peaks for $^1H$ and $^{13}C$ NMR spectra ($(CD_3)_2SO$: $^1H$ $\delta = 2.50$, $^{13}C$ $\delta = 39.92$; $CD_3OD$: $^1H$ $\delta = 3.34$, $^{13}C$ $\delta = 40.01$; $CDCl_3$: $^1H$ $\delta = 7.29$, $^{13}C$ $\delta = 76.98$) or in negative parts per million ($\delta$) relative to $CFCl_3$ for $^{19}F$ NMR spectra respectively. The coupling constants ($J$) are reported in Hertz. EI- and CI-mass

spectra were measured with a TSQ700 mass spectrometer (Thermoelectron), ESI- and PCI-mass spectra were recorded with a LCQ-Advantage mass spectrometer. Microanalysis were performed with Vario-EL (Elementaranalysensysteme). HPLC data for purity verification were obtained with Alliance Equipment (Waters, 2695 Separations Module, 2487 Dual $\lambda$ Absorbance Detector). Quantification was performed by determination of the peak area using the Millenium Software (Waters). HPLC analysis was performed under following conditions: LiChrospher® 60 RP—select B, 5 μm, 60Å, 250 × 4 mm (Merck); gradient mode elution ($CH_3CN$/$H_2O$:0.05% TFA); flow rate: 1 mL/min; detection mode: UV absorption: $\lambda$ = 254 nm). The samples for the HPLC analysis were solved in suitable solvent (acetonitrile or methanol, concentration: 1 mg/mL).

### 4.10. Synthetic procedure 1: amide preparation

Compound **1** (2-(benzylthio)nicotinic acid) (10 mmol, 2.5 g) was suspended in 50 mL of absolute dichloromethane and an equimolar amount of *N*,*N*-diisopropylethylamine (10 mmol, 1.3 g, 1.7 ml) was added. After stirring at room temperature for 5 min bis(2-oxo-3-oxazolidinyl)phosphonic chloride (10 mmol, 2.6 g) was added and the batch was stirred for further 5 min. Subsequently appropriate substituted aniline derivative (11 mmol) and *N*,*N*-diisopropylethylamine (20 mmol, 2.6 g, 3.3 ml) were added. The mixture was stirred at room temperature overnight. After solvent removal in vacuum, the residue was solved in ethyl acetate and washed consecutive three times with 2 M hydrochloric acid, water, 5% (w/v) sodium bicarbonate solution, water and brine. The organic phase was dried over sodium sulphate and evaporated to dryness. The crude product was recrystallized from ethyl acetate with petroleum ether (80–110 °C).

### 4.11. Synthetic procedure 2: phenylpyridoisothiazolones (4a–f)

Appropriate substituted amide (3 mmol) was dissolved in 30 mL dichloroethane and heated to 70 °C. Sulfuryl chloride (11.9 mmol) was added dropwise as 1 M solution in dichloroethane. After stirring for 45 min at 70 °C, the preparation was cooled to room temperature and washed then with 25 mL saturated solution of sodium bicarbonate. The organic layer was separated and the solvent was evaporated in vacuum. The products were purified using silica gel column chromatography with the reported eluents.

### 4.12. Synthetic procedure 3: benzylpyridoisothiazolones (8a–h)

Compound **7** (isothiazolo[5,4-*b*]pyridin-3(*2H*)-one) (10 mmol, 1.5 g) was dissolved in 40 mL of absolute methanol. After addition of 13 mmol of appropriate substituted benzyl bromide derivative and *N*,*N*-diisopropylethylamine (11 mmol, 1.4 g, 1.9 ml), the preparation was stirred at room temperature overnight. The reaction mixture was poured onto 150 mL 1 M hydrochloric acid and extracted three times with ethyl acetate. The combined organic layers were washed three times with water, once with brine and then dried over sodium sulphate and evaporated to dryness. The products were purified using silica gel column chromatography with ethyl acetate/cyclohexane 1:1 (v/v) as eluent.

#### 4.12.1. 2-(Benzylthio)nicotinic acid (1)

2-Mercaptonicotinic acid (32 mmol, 5.0 g) was dissolved together with potassium hydroxide (65 mmol, 3.6 g) in 40 mL of isopropyl alcohol and 8 mL of water. Benzyl chloride was added dropwise and the mixture was refluxed for 30 min. After cooling, the reaction mixture was concentrated to approximately 30 mL. The residue was treated with 30 mL of water and slightly acidified with acetic acid. The precipitate was filtered, washed with water and recrystallized from ethanol to give white crystals of **1** (2-(benzylthio)nicotinic acid) (yield: 26.7 mmol, 81%). Mp 194 °C; $^1$H NMR (400 MHz, $(CD_3)_2SO$): $\delta$ = 13.26 (bs 1H, COOH), 8.66 (dd, $^3J$ = 4.7 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 6-H), 8.22 (dd, $^3J$ = 7.8 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 4-H), 7.42–7.40 (m, $2H_{bn}$, 2′/6′-H), 7.32–7.23 (m, $4H_{bn/py}$, 3′/4′/5′/5-H), 4.37 (s, 2H, $CH_2$); $^{13}$C NMR (100 MHz, $(CD_3)_2SO$): $\delta$ = 166.76 (COOH), 161.05 ($C_q$, 2-$C_{py}$), 152.35 ($CH_{ar}$, 6-$C_{py}$), 139.47 ($CH_{ar}$, 4-$C_{py}$), 138.38 ($C_q$, 1′-$C_{bn}$), 129.62 (2 × $CH_{ar}$, $C_{bn}$), 128.74 (2 × $CH_{ar}$, $C_{bn}$), 127.28 ($CH_{ar}$, 4′-$C_{bn}$), 123.58 ($C_q$, 3-$C_{py}$), 119.51 ($CH_{ar}$, 5-$C_{py}$), 34.22 ($CH_2$); MS (CI $NH_3$ direct mode): *m*/*z* (%) 241.6 $[M+H]^+$ (100).

#### 4.12.2. 2-(Benzylthio)-*N*-phenylnicotinamide (2a)

Starting out from **1** (2-(benzylthio)nicotinic acid) (10 mmol, 2.5 g) and aniline (11 mmol, 1.0 g, 1.0 mL) according to synthetic procedure 1, **2a** (2-(benzylthio)-*N*-phenylnicotinamide) was obtained as white crystals (yield: 4.9 mmol, 49%). Mp 171 °C; $^1$H NMR (400 MHz, $(CD_3)_2SO$): $\delta$ = 10.39 (s, 1H, NH), 8.61 (dd, $^3J$ = 4.8 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 6-H), 7.92 (dd, $^3J$ = 7.6 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 4-H), 7.69 (d, $^3J$ = 8.2, $2H_{ar}$, 2″/6″-H), 7.41–7.37 (m, $2H_{bn}$, 2′/6′-H), 7.36–7.32 (m, $2H_{ar}$, 3″/5″-H), 7.31–7.26 (m, $3H_{bn/py}$, 3′/5′/5-H), 7.24–7.20 (m, $1H_{ar}$, 4″-H), 7.13–7.09 (m, $1H_{bn}$, 4′-H), 4.43 (s, 2H, $CH_2$); $^{13}$C NMR (100 MHz, $(CD_3)_2SO$): $\delta$ = 165.19 (C═O), 157.04 ($C_q$, 2-$C_{py}$), 150.54 ($CH_{ar}$, 6-$C_{py}$), 139.25 ($C_q$, 1′-$C_{bn}$), 138.51 ($C_q$, 1″-C), 136.12 ($CH_{ar}$, 4-$C_{py}$), 130.63 ($C_q$, 3-$C_{py}$), 129.48 (2 × $CH_{ar}$, 3″/5″-C), 129.16 (2 × $CH_{ar}$, $C_{bn}$), 128.74 (2 × $CH_{ar}$, $C_{bn}$), 127.32 ($CH_{ar}$, 4′-$C_{bn}$), 124.33 ($CH_{ar}$, 4″-C), 120.19 (2 × $CH_{ar}$, 2″/6″-C), 119.64 ($CH_{ar}$, 5-$C_{py}$), 33.84 ($CH_2$); MS (CI $NH_3$ direct mode): *m*/*z* (%) 321.0 $[M+H]^+$ (100).

#### 4.12.3. 2-(Benzylthio)-*N*-(4-chlorophenyl)nicotinamide (2b)

Starting out from **1** (2-(benzylthio)nicotinic acid) (10 mmol, 2.5 g) and 4-chloroaniline (11 mmol, 1.4 g) according to synthetic procedure 1, **2b** (2-(benzylthio)-*N*-(4-chlorophenyl)nicotinamide) was obtained as white crystals (yield: 5.4 mmol, 54%). Mp 155 °C; $^1$H NMR (400 MHz, $(CD_3)_2SO$): $\delta$ = 10.60 (s, 1H, NH), 8.62 (dd, $^3J$ = 4.8 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 6-H), 7.95 (dd, $^3J$ = 7.9 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 4-H), 7.72 (d, $^3J$ = 8.9 Hz, $2H_{ar}$, 2″/6″-H), 7.42–7.39 (m, $4H_{ar/bn}$, 2′/6′/3″/5″-H), 7.31–7.27 (m, $3H_{bn/py}$, 3′/5′/5-H), 7.24–7.22 (m, $1H_{bn}$, 4′-H), 4.42 (s, 2H, $CH_2$); $^{13}$C NMR (100 MHz, $(CD_3)_2SO$): $\delta$ = 165.27 (C═O), 157.11 ($C_q$, 2-$C_{py}$), 150.70 ($CH_{ar}$, 6-$C_{py}$), 138.46 ($C_q$, 1′-$C_{bn}$), 138.19 ($C_q$, 1″-C), 136.20 ($CH_{ar}$, 4-$C_{py}$), 130.31 ($C_q$, 3-$C_{py}$), 129.48 (2 × $CH_{ar}$, 3″/5″-C), 129.10 (2 × $CH_{ar}$, $C_{bn}$), 128.74 (2 × $CH_{ar}$, $C_{bn}$), 127.94 ($C_q$, 4″-C), 127.34 ($CH_{ar}$, 4′-$C_{bn}$), 121.74 (2 × $CH_{ar}$, 2″/6″-C), 119.64 ($CH_{ar}$, 5-$C_{py}$), 33.85 ($CH_2$); MS (CI $NH_3$ direct mode): *m*/*z* (%) 355.0 $[M^{35}Cl]^+$ (100).

#### 4.12.4. 2-(Benzylthio)-*N*-(3,4-dichlorophenyl)nicotinamide (2c)

Starting out from **1** (2-(benzylthio)nicotinic acid) (10 mmol, 2.5 g) and 3,4-dichloroaniline (11 mmol, 1.8 g) according to synthetic procedure 1, **2c** (2-(benzylthio)-*N*-(3,4-dichlorophenyl)nicotinamide) was obtained as white crystals (yield: 4.7 mmol, 47%). Mp 172 °C; $^1$H NMR (400 MHz, $(CD_3)_2SO$): $\delta$ = 10.75 (s, 1H, NH), 8.64 (dd, $^3J$ = 4.9 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 6-H), 8.07 (t, $J$ = 1.3 Hz, $1H_{ar}$, 2″-H), 7.97 (dd, $^3J$ = 7.6 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 4-H), 7.62 (d, $J$ = 1.3 Hz, $2H_{bn}$, 2′/6′-H), 7.41–7.39 (m, $2H_{ar}$, 5″/6″-H), 7.32–7.27 (m, $3H_{bn/py}$, 3′/5′/5-H), 7.24–7.22 (m, $1H_{bn}$, 4′-H), 4.42 (s, 2H, $CH_2$); $^{13}$C NMR (100 MHz, $(CD_3)_2SO$): $\delta$ = 165.51 (C═O), 157.28 ($C_q$, 2-$C_{py}$), 150.93 ($CH_{ar}$, 6-$C_{py}$), 139.31 ($C_q$, 1′-$C_{bn}$), 138.40 ($C_q$, 1″-C), 136.33 ($C_{ar}$, 4-$C_{py}$), 131.44 ($C_q$, 3″-C), 131.16 ($CH_{ar}$, 5″-C), 129.82 ($C_q$, 3-$C_{py}$), 129.49 (2 × $CH_{ar}$, $C_{bn}$), 128.75 (2 × $CH_{ar}$, $C_{bn}$), 127.36 ($CH_{ar}$, 4′-$C_{bn}$), 125.85 ($C_q$, 4″-C), 121.34 ($CH_{ar}$, 2″-C), 120.24 ($CH_{ar}$, 6″-C), 119.64 ($CH_{ar}$, 5-$C_{py}$), 33.88 ($CH_2$); MS (CI $NH_3$ direct mode): *m*/*z* (%) 389.0 $[M^{35}Cl^{35}Cl]^+$ (100).

#### 4.12.5. 2-(Benzylthio)-*N*-(4-fluorophenyl)nicotinamide (2d)

Starting out from **1** (2-(benzylthio)nicotinic acid) (10 mmol, 2.5 g) and 4-fluoroaniline (11 mmol, 0.82 g, 1.0 mL) according to synthetic procedure 1, **2d** (2-(benzylthio)-*N*-(4-fluorophenyl)nicotinamide) was obtained as white crystals (yield: 6.0 mmol, 60%). Mp 162 °C; $^1$H NMR (400 MHz, $(CD_3)_2SO$): $\delta$ = 10.52 (s, 1H, NH), 8.62 (dd, $^3J$ = 4.8 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 6-H), 7.94 (dd, $^3J$ = 7.6 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 4-H), 7.73–7.69 (m, $2H_{ar}$, 2″/6″-H), 7.41–7.39 (m, $2H_{bn}$, 2′/6′-H), 7.31–7.27 (m, $3H_{bn/py}$, 3′/5′/5-H), 7.24–7.17 (m, $3H_{ar/bn}$, 4′/3″/5″-H), 4.42 (s, 2H, $CH_2$); $^{13}$C NMR (100 MHz, $(CD_3)_2SO$): $\delta$ = 165.08 (C=O), 158.87 (d, $^1J_{C-F}$ = -238.9 Hz, $C_q$, 4″-C), 157.10 ($C_q$, 2-$C_{py}$), 150.61 ($CH_{ar}$, 6-$C_{py}$), 138.49 ($C_q$, 1′-$C_{bn}$), 136.13 ($CH_{ar}$, 4-$C_{py}$), 135.63 (d, $^4J_{C-F}$ = -2.5 Hz, $C_q$, 1″-C), 130.42 ($C_q$, 3-$C_{py}$), 129.49 (2 × $CH_{ar}$, $C_{bn}$), 128.74 (2 × $CH_{ar}$, $C_{bn}$), 127.33 ($CH_{ar}$, 4′-$C_{bn}$), 122.00 (d, $^3J_{C-F}$ = -7.7 Hz, 2 × $CH_{ar}$, 2″/6″-C), 119.64 ($CH_{ar}$, 5-$C_{py}$), 115.78 (d, $^2J_{C-F}$ = -22.1 Hz, 2 × $CH_{ar}$, 3″/5″-C), 33.85 ($CH_2$); $^{19}$F NMR (376 MHz, $(CD_3)_2SO$): $\delta$ = 118.38–118.45 (m, 1F, 4″-F); MS (CI $NH_3$ direct mode): *m*/*z* (%) 339.2 $[M+H]^+$ (100).

#### 4.12.6. 2-(Benzylthio)-*N*-(3,4-difluorophenyl)nicotinamide (2e)

Starting out from **1** (2-(benzylthio)nicotinic acid) (10 mmol, 2.55 g) and 3,4-difluoroaniline (11 mmol, 1.4 g, 1.1 mL) according to synthetic procedure 1, **2e** (2-(benzylthio)-*N*-(3.4-diflorophenyl)nicotinamide) was obtained as white crystals (yield: 6.9 mmol, 69%). Mp 149 °C; $^1$H NMR (400 MHz, $(CD_3)_2SO$): $\delta$ = 10.69 (s, 1H, NH), 8.63 (dd, System, $^3J$ = 4.8 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 6-H), 7.96 (dd, $^3J$ = 7.6 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 4-H), 7.78–7.82 (m, $1H_{ar}$, 2″-H), 7.45–7.39 (m, $4H_{ar/bn/py}$, 2′/6′/6″/5-H), 7.32–7.27 (m, $3H_{bn}$, 3′/4′/5′-H), 7.24–7.20 (m, $1H_{ar}$, 5″-H), 4.42 (s, 2H, $CH_2$); $^{13}$C NMR (100 MHz, $(CD_3)_2SO$): $\delta$ = 165.32 (C=O), 157.20 ($C_q$, 2-$C_{py}$), 150.83 ($CH_{ar}$, 6-$C_{py}$), 149.90 (dd, $^1J_{C-F}$ = -241.9 Hz, $^2J_{C-F}$ = -13.1 Hz, $C_q$, 3″-C), 146.06 (dd, $^1J_{C-F}$ = -188.7 Hz, $^2J_{C-F}$ = -12.8 Hz, $C_q$, 4″-C), 138.43 ($C_q$, 1′-$C_{bn}$), 136.30–136.19 (m, $C_q$, 1″-C), 129.99 ($CH_{ar}$, 4-$C_{py}$), 129.49 (2 × $CH_{ar}$, $C_{bn}$), 128.75 (2 × $CH_{ar}$, $C_{bn}$), 127.35 ($CH_{ar}$, 4′-$C_{bn}$), 127.45 ($C_q$, 3-$C_{py}$), 119.64 ($CH_{ar}$, 5-$C_{py}$), 117.88 (d, $^2J_{C-F}$ = -17.6 Hz, $CH_{ar}$, 5″-C), 116.57 (dd, $^3J_{C-F}$ = -5.9 Hz, $^4J_{C-F}$ = -3.4 Hz, $CH_{ar}$, 6″-C), 109.14 (d, $^2J_{C-F}$ = -21.5 Hz, $CH_{ar}$, 2″-C), 33.86 ($CH_2$); $^{19}$F NMR (376 MHz, $(CD_3)_2SO$): $\delta$ = 136.97–137.16 (m, 1F, 4″-F), 143.79–143.91 (m, 1F, 3″-F); MS (CI $NH_3$ direct mode): *m*/*z* (%) 357.1 $[M+H]^+$ (100).

#### 4.12.7. 2-(Benzylthio)-*N*-(4-methylphenyl)nicotinamide (2f)

Starting out from **1** (2-(benzylthio)nicotinic acid) (10 mmol, 2.5 g) and and *p*-toluidine (11 mmol, 1.2 g) according to synthetic procedure 1, **2f** (2-(benzylthio)-*N*-(4-methylphenyl)nicotinamide) was obtained as white powder (yield: 4.8 mmol, 48%). Mp 159 °C; $^1$H NMR (400 MHz, $(CD_3)_2SO$): $\delta$ = 10.37 (s, 1H, NH), 8.60 (dd, $^3J$ = 4.8 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 6-H), 7.92 (dd, $^3J$ = 7.6 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 4-H), 7.57 (d, $^3J$ = 8.3 Hz, $2H_{bn}$, 2′/6′-H), 7.41–7.38 (m, $2H_{ar}$, 2″/6″-H), 7.31–7.26 (m, $3H_{ar/py}$, 3″/5″/5-H), 7.22–7.20 (m, $1H_{bn}$, 4′-H), 7.15 (d, $^3J$ = 8.3 Hz, $2H_{bn}$, 3′/5′-H), 4.42 (s, 2H, $CH_2$), 2.27 (s, 3H, $CH_3$); $^{13}$C NMR (100 MHz, $(CD_3)_2SO$): $\delta$ = 164.97 (C=O), 157.06 ($C_q$, 2-$C_{py}$), 150.47 ($CH_{ar}$, 6-$C_{py}$), 138.52 ($C_q$, 1′-$C_{bn}$), 136.75 ($C_q$, 4″-C), 136.06 ($CH_{ar}$, 4-$C_{py}$), 133.32 ($C_q$, 1″-C), 130.68 ($C_q$, 3-$C_{py}$), 129.52 (2 × $CH_{ar}$, 3″/5″-C), 129.49 (2 × $CH_{ar}$, $C_{bn}$), 128.74 (2 × $CH_{ar}$, $C_{bn}$), 127.32 ($CH_{ar}$, 4′-$C_{bn}$), 120.19 (2 × $CH_{ar}$, 2″/6″-C), 119.62 ($CH_{ar}$, 5-$C_{py}$), 33.85 ($CH_2$), 20.92 ($CH_3$); MS (CI $NH_3$ direct mode): *m*/*z* (%) 335.1 $[M+H]^+$ (100).

#### 4.12.8. 2-(Benzylthio)-*N*-(3-methoxyphenyl)nicotinamide (2g)

Compound **1** (2-(benzylthio)nicotinic acid) (10. mmol, 2.5 g) was suspended in 100 mL of absolute dichloromethane and triethylamine (12 mmol, 1.2 g, 1.6 mL) was added to the stirred suspension. After 5 min, addition of isobutylchloroformiate (10 mmol, 1.4 g, 1.4 mL) was followed and the mixture was heated to 45 °C for 1 h. After cooling at room temperature, 3-methoxyaniline (12 mmol, 1.4 g, 1.3 mL) was added and the preparation was stirred overnight. The mixture was then treated with 50 mL of 1 M sodium hydroxide solution and the remaining aqueous phase was washed three times with dichloromethane. The combined organic layers were washed with brine, dried over sodium sulphate and evaporated in vacuum. The crude product was recrystallized from ethyl acetate with petroleum ether (80–110 °C) to give **2g** (2-(benzylthio)-*N*-(3-methoxyphenyl)nicotinamide) as white crystals (yield: 3.4 mmol, 33%). Mp 134 °C; $^1$H NMR (400 MHz, $(CD_3)_2SO$): $\delta$ = 10.44 (s, 1H, NH), 8.61 (dd, $^3J$ = 4.8 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 6-H), 7.92 (dd, $^3J$ = 7.6 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 4-H), 7.41–7.39 (m, $3H_{ar/bn}$, 2′/6′/6″-H), 7.31–7.22 (m, $6H_{ar/bn/py}$, 3′/4′/5′/2″/5″/5-H), 6.71–6.68 (m, $1H_{ar}$, 4″-H), 4.42 (s, 2H, $CH_2$), 3.34 (s, 3H, $CH_3$); $^{13}$C NMR (100 MHz, $(CD_3)_2SO$): $\delta$ = 165.22 (C=O), 159.89 ($C_q$, 2-$C_{py}$), 157.07 ($C_q$, 3″-C), 150.56 ($CH_{ar}$, 6-$C_{py}$), 140.43 ($C_q$, 1′-$C_{bn}$), 138.50 ($C_q$, 1″-C), 136.13 ($CH_{ar}$, 4-$C_{py}$), 130.57 ($C_q$, 3-$C_{py}$), 129.97 ($CH_{ar}$, 5″-C), 129.49 (2 × $CH_{ar}$, $C_{bn}$), 128.74 (2 × $CH_{ar}$, $C_{bn}$), 127.33 ($CH_{ar}$, 4′-$C_{bn}$), 119.62 ($CH_{ar}$, 5-$C_{py}$), 112.45 ($CH_{ar}$, 6″-C), 109.81 ($CH_{ar}$, 4″-C), 105.92 ($CH_{ar}$, 2″-C), 55.44 ($CH_3$), 33.85 ($CH_2$); MS (CI $NH_3$ direct mode): *m*/*z* (%) 351.2 $[M+H]^+$ (100).

#### 4.12.9. 2-(Benzylsulfinyl)-*N*-(3-methoxyphenyl)nicotinamide 3

Compound **2g** (2-(benzylthio)-*N*-(3-methoxyphenyl)nicotinamide) (2.9 mmol, 1.0 g) was dissolved in 25 mL of absolute dichloromethane and cooled to 0 °C. After addition of 70% *m*-chloroperbenzoic acid, the batch was stirred for 15 min at 0 °C. Then the reaction was quenched with 100 mL of 2 M sodium hydroxide solution and stirred intensively. The preparation was filtrated, the organic solvent was removed in vacuum and the residue after evaporation was combined with the precipitate to give compound **3** (2-(benzylsulfinyl)-*N*-(3-methoxyphenyl)nicotinamide) as white crystals (yield: 2.4 mmol, 83%). Mp 193 °C; $^1$H NMR (400 MHz, $(CD_3)_2SO$): $\delta$ = 10.73 (bs 1H, NH), 8.88 (dd, $^3J$ = 4.7 Hz, $^4J$ = 1.5 Hz, $1H_{py}$, 6-H), 8.29 (dd, $^3J$ = 7.7 Hz, $^4J$ = 1.3 Hz, $1H_{py}$, 4-H), 7.73 (dd, $^3J$ = 7.7 Hz, $^3J$ = 4.7 Hz, $1H_{py}$, 5-H), 7.38–7.33 (m, 4H, 3′/5′/2″/6″-$H_{ar/bn}$), 7.31–7.26 (m, $4H_{ar/bn}$, 2′/4′/6′/5″-H), 6.75–6.71 (m, $1H_{ar}$, 4″-H), 4.49 (d, $^2J$ = 12.5 Hz, 1H, $CH_2$), 4.15 (d, $^2J$ = 12.5 Hz, 1H, $CH_2$), 3.77 (s, 3H, $CH_3$); $^{13}$C NMR (100 MHz, $(CD_3)_2SO$): $\delta$ = 162.23 (C=O), 164.12 ($C_q$, 2-$C_{py}$), 159.91 ($C_q$, 3″-C), 151.72 ($CH_{ar}$, 6-$C_{py}$), 137.48 ($CH_{ar}$, 4-$C_{py}$), 132.48 ($C_q$, 1″-C), 131.89 ($C_q$, 1′-$C_{bn}$), 130.66 (2 × $CH_{ar}$, $C_{bn}$), 130.04 ($CH_{ar}$, 5″-C) 128.92 (2 × $CH_{ar}$, $C_{bn}$), 128.32 ($CH_{ar}$, 5-$C_{py}$), 125.72 ($CH_{ar}$, 4′-$C_{bn}$), 123.70 ($C_q$, 3-$C_{py}$), 112.91 ($CH_{ar}$, 6″-C), 109.93 ($CH_{ar}$, 4″-C), 106.46 ($CH_{ar}$, 2″-C), 60.99 ($CH_2$), 55.49 ($CH_3$); MS (CI $NH_3$ direct mode): *m*/*z* (%) 367.2 $[M+H]^+$ (100).

#### 4.12.10. 2-Phenylisothiazolo[5,4-*b*]pyridin-3*(2H)*-one (4a)

Starting out from **2a** (2-(benzylthio)-*N*-phenylnicotinamide) (3 mmol, 1 g) using synthetic procedure 2, **4a** (2-phenylisothiazolo[5,4-*b*]pyridin-3*(2H)*-one) was obtained as white crystals after purification through silica gel column chromatography and cyclohexane/ethyl acetate 7:3 (v/v) (yield: 0.3 mmol, 10%). Mp 159 °C; $^1$H NMR (400 MHz, ($CDCl_3$): $\delta$ = 8.83–8.82 (m, $1H_{py}$, 6-H), 8.36 (d, *J* = 7.8 Hz, $1H_{py}$, 4-H), 7.72 (d, *J* = 7.7 Hz, $2H_{ar}$, 2′/6′-H), 7.52–7.49 (m, $2H_{ar}$, 3′/5′-H), 7.44–7.41 (m, $1H_{py}$, 5-H), 7.38–7.35 (m, $1H_{ar}$, 4′-H); $^{13}$C NMR (100 MHz, $(CDCl_3)$: $\delta$ = 162.44 (C=O), 161.86 ($C_q$, 2-$C_{py}$), 154.05 ($CH_{ar}$, 6-$C_{py}$), 136.59 ($C_q$, 1′-C), 135.25 ($CH_{ar}$, 4-$C_{py}$), 129.45 (2 × $CH_{ar}$, 3′/5′-C), 127.42 ($CH_{ar}$, 4′-C), 124.80 (2 × $CH_{ar}$, 2′/6′-C), 120.97 ($CH_{ar}$, 5-$C_{py}$), 119.75 ($C_q$, 3-$C_{py}$); MS (EI direct mode): *m*/*z* (%) 228.1 $[M]^+$ (100); microanalysis $[C_{12}H_8N_2OS]$: found/calculated C (63.19/63.14), H (2.99/3.53), N (11.86/12.27), S (14.09/14.05).

#### 4.12.11. 2-(4-Chlorophenyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one (4b)

Starting out from **2b** (2-(benzylthio)-*N*-(4-chlorophenyl)nicotinamide) (3 mmol, 1 g) using synthetic procedure 2, **4b** (2-(4-chlorophenyl)isothiazolo[5,4-*b*]pyridin-3(2*H*)-one) was obtained as white-yellowish crystals after purification through silica gel column chromatography and dichloromethane/methanol 100:6.25 (v/v) (yield: 0.5 mmol, 15%). Mp 196 °C; $^1$H NMR (400 MHz, ($CDCl_3$): $\delta$ = 8.84 (dd, $^3J$ = 4.7 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 6-H), 8.37 (dd, $^3J$ = 7.9 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 4-H), 7.69 (dt, $^3J$ = 5.2 Hz, $^4J$ = 3.0 Hz, $^5J$ = 2.2 Hz, 2H, 2′/6′-H), 7.49 (dt, $^3J$ = 5.2 Hz, $^4J$ = 3.0 Hz, $^5J$ = 2.2 Hz, 2H, 3′/5′-H), 7.45–7.43 (m, $1H_{py}$, 5-H); $^{13}$C NMR (100 MHz, ($CDCl_3$): $\delta$ = 162.46 (C=O), 161.65 ($C_q$, 2-$C_{py}$), 154.25 ($CH_{ar}$, 6-$C_{py}$), 135.32 ($CH_{ar}$, 4-$C_{py}$), 135.14 ($C_q$, 1′-C), 132.96 ($C_q$, 4′-C), 129.58 (2 × $CH_{ar}$, 3′/5′-C), 125.89 (2 × $CH_{ar}$, 2′/6′-C), 121.13 ($CH_{ar}$, 5-$C_{py}$), 119.53 ($C_q$, 3-$C_{py}$); MS (EI direct mode): *m*/*z* (%) 261.8 $[M^{35}Cl]^+$ (100); microanalysis [$C_{12}H_7ClN_2OS$]: found/calculated C (54.86/54.68), H (2.90/2.69), N (10.33/10.60), S (11.03/12.21).

#### 4.12.12. 2-(3,4-Dichlorophenyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one (4c)

Starting out from **2c** (2-(benzylthio)-*N*-(3,4-dichlorophenyl)nicotinamide) (3 mmol, 1 g) using synthetic procedure 2, **4c** (2-(3,4-dichlorophenyl)isothiazolo[5,4-*b*]pyridin-3(2*H*)-one) was obtained as fawn powder after purification through silica gel column chromatography and dichloromethane/methanol 100:6.25 (v/v) (yield: 1.6 mmol, 52%). Mp 252 °C; $^1$H NMR (400 MHz, ($CDCl_3$): $\delta$ = 8.85 (dd, $^3J$ = 4.7, $^4J$ = 1.7, $1H_{py}$, 6-H), 8.36 (dd, $^3J$ = 7.9, $^4J$ = 1.7, $1H_{py}$, 4-H), 7.94 (d, $^4J$ = 2.5, 1H, 2′-H), 7.62 (dd, $^3J$ = 8.7, $^4J$ = 2.5, 1H, 5′-H), 7.57 (d, $^3J$ = 8.7, 1H, 6′-H), 7.44 (dd, $^3J$ = 7.9, $^3J$ = 4.7, $1H_{py}$, 5-H); $^{13}$C NMR (100 MHz, ($CDCl_3$): $\delta$ = 162.44 (C=O), 161.54 ($C_q$, 2-$C_{py}$), 154.44 ($CH_{ar}$, 6-$C_{py}$), 136.11 ($C_q$, 1′-C), 135.33 ($CH_{ar}$, 4-$C_{py}$) 133.44 ($C_q$, 3′-C), 131.16 ($C_q$, 4′-C), 130.91 ($CH_{ar}$, 5′-C), 126.12 ($CH_{ar}$, 2′-C), 123.42 ($CH_{ar}$, 6′-C), 121.19 ($CH_{ar}$, 5-$C_{py}$), 119.37 ($C_q$, 3-$C_{py}$); MS (EI direct mode): *m*/*z* (%) 295.9 $[M^{35}Cl^{35}Cl]^+$ (100); HPLC purity analysis: 97.2%, retention time 25.71 min.

#### 4.12.13. 2-(4-Fluorophenyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one (4d)

Starting out from **2d** (2-(benzylthio)-*N*-(4-fluorophenyl)nicotinamide) (3 mmol, 1 g) using synthetic procedure 2, **4d** (2-(4-fluorophenyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one) was obtained as white-yellowish crystals after purification through silica gel column chromatography and cyclohexane/ethyl acetate 7:3 (v/v) (yield: 1.1 mmol, 38%). Mp 204 °C; $^1$H NMR (400 MHz, ($CDCl_3$): $\delta$ = 8.84 (dd, $^3J$ = 4.7 Hz, $^4J$ = 1.6 Hz, $1H_{py}$, 6-H), 8.37 (dd, $^3J$ = 8.1 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 4-H), 7.69–7.65 (m, 2H, 2′/6′-H), 7.44 (dd, $^3J$ = 7.8 Hz, $^3J$ = 4.8 Hz, $1H_{py}$, 5-H), 7.23–7.18 (m, 2H, 3′/5′-H); $^{13}$C NMR (100 MHz, ($CDCl_3$): $\delta$ = 162.65 (C=O), 161.37 (d, $^1J_{C-F}$ = -239.0 Hz, $C_q$, 4′-C), 161.79 ($C_q$, 2-$C_{py}$), 154.16 ($CH_{ar}$, 6-$C_{py}$), 135.30 ($CH_{ar}$, 4-$C_{py}$), 132.32 (d, $^4J_{C-F}$ = -3.0 Hz, $C_q$, 1′-C), 127.09 (d, $^3J_{C-F}$ = -8.5 Hz, 2 × $CH_{ar}$, 2′/6′-C), 121.08 ($CH_{ar}$, 5-$C_{py}$), 119.40 ($C_q$,3-$C_{py}$), 116.42 (d, $^2J_{C-F}$ = -22.8 Hz, 2 × $CH_{ar}$, 3′/5′-C); $^{19}$F NMR (376 MHz, ($CDCl_3$): $\delta$ = 112.99–113.06 (m, 1F, 4′-F); MS (EI direct mode): *m*/*z* (%) 246.0 $[M]^+$ (100); HPLC purity analysis: 99.8%, retention time 19.77 min.

#### 4.12.14. 2-(3,4-Difluorophenyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one (4e)

Starting out from **2e** (2-(benzylthio)-*N*-(3,4-difluorophenyl)nicotinamid) (3 mmol, 1 g) using synthetic procedure 2, **4e** (2-(3,4-difluorophenyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one) was obtained as white-yellowish crystals after purification through silica gel column chromatography and cyclohexane/ethyl acetate 7:3 (v/v) (yield: 0.4 mmol, 13%). Mp 219 °C; $^1$H NMR (400 MHz, ($CDCl_3$): $\delta$ = 8.85 (dd, $^3J$ = 4.7 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 6-H), 8.36 (dd, $^3J$ = 7.9 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 4-H), 7.73–7.68 (m, $1H_{py}$, 5-H), 7.46–7.41 (m, 2H, 2′/6′-H), 7.33–7.26 (m, 1H, 5′-H); $^{13}$C NMR (100 MHz, ($CDCl_3$): $\delta$ = 162.48 (C=O), 161.54 ($C_q$, 2-$C_{py}$), 154.40 ($CH_{ar}$, 6-$C_{py}$), 150.21 (dd, $^1J_{C-F}$ = -249.0 Hz, $^2J_{C-F}$ = -13.6 Hz, $C_q$, 3′-C), 149.16 (dd, $^1J_{C-F}$ = -248.5 Hz, $^2J_{C-F}$ = -12.5 Hz, $C_q$, 4′-C), 135.39 ($CH_{ar}$, 4-$C_{py}$), 132.76 (dd, $^3J_{C-F}$ = -8.0 Hz, $^4J_{C-F}$ = -3.9 Hz, $C_q$, 1′-C), 121.24 ($CH_{ar}$, 5-$C_{py}$), 120.75 (dd, $^3J_{C-F}$ = -6.4 Hz, $^4J_{C-F}$ = -3.7 Hz, $CH_{ar}$, 6′-C), 119.33 ($C_q$, 3-$C_{py}$), 117.83 (d, $^2J_{C-F}$ = -18.2 Hz, $CH_{ar}$, 5′-C), 114.62 (d, $^2J_{C-F}$ = -18.2 Hz, $CH_{ar}$, 2′-C); $^{19}$F NMR (376 MHz, ($CDCl_3$): $\delta$ = 134.00–134.11 (m, 1F, 4′-F), 137.52–137.64 (m, 1F, 3′-F); MS (EI direct mode): *m*/*z* (%) 264.0 $[M]^+$ (100); HPLC purity analysis: 97.0%, retention time 19.51 min.

#### 4.12.15. 2-(4-Methylphenyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one (4f)

Starting out from **2f** (2-(benzylthio)-*N*-(4-methylphenyl)nicotinamide) (3 mmol, 1 g) using synthetic procedure 2, **4f** (2-(4-methylphenyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one) was obtained as white-yellowish powder after purification through silica gel column chromatography and dichloromethane/methanol 100:6.25 (v/v) (yield: 1.8 mmol, 59%). Mp 143 °C; $^1$H NMR (400 MHz, ($CDCl_3$): $\delta$ = 8.82 (dd, $^3J$ = 4.7 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 6-H), 8.37 (dd, $^3J$ = 7.9 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 4-H), 7.59–7.56 (m, 2H, 2′/6′-H), 7.42 (dd, $^3J$ = 7.9 Hz, $^3J$ = 4.7 Hz, $1H_{py}$, 5-H), 7.32–7.28 (m, 2H, 3′/5′-H), 2.42 (s, 3H, $CH_3$); $^{13}$C NMR (100 MHz, ($CDCl_3$): $\delta$ = 162.49 (C=O), 161.91 ($C_q$, 2-$C_{py}$), 153.93 ($CH_{ar}$, 6-$C_{py}$), 137.66 ($C_q$, 4′-C), 135.19 ($CH_{ar}$, 4-$C_{py}$), 133.85 ($C_q$, 1′-C), 130.02 (2 × $CH_{ar}$, 3′/5′-C), 124.94 (2 × $CH_{ar}$, 2′/6′-C), 120.90 ($CH_{ar}$, 5-$C_{py}$), 119.73 ($C_q$, 3-$C_{py}$), 21.09 ($CH_3$); MS (EI direct mode): *m*/*z* (%) 241.8 $[M]^+$ (100); HPLC purity analysis: 98.9%, retention time 19.77 min.

#### 4.12.16. 2-(3-Methoxzphenyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one (4g)

A suspension of **3** (2-(benzylsulfunyl)-*N*-(3-methoxyphenyl) nicotinamide) (2 mmol, 0.7 g) in 50 mL dichloromethane was cooled to 0 °C and treated dropwise with trichloroacetic anhydride (2.3 mmol, 0.69 g, 0.41 mL). The preparation was stirred for 4 h while the temperature was rising to 25 °C. The reaction mixture was then quenched with 75 mL of 2 M sodium hydroxide solution and extracted. The aqueous phase was washed three times with dichloromethane. The combined organic layers were dried over sodium sulphate and the solvent was removed in vacuum. The residue was purified using silica gel column chromatography and dichloromethane/methanol 100:6.25 (v/v) as eluent to give **4g** (2-(3-methoxzphenyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one) as white crystals (yield: 0.9 mmol, 43%). Mp 114 °C; $^1$H NMR (400 MHz, ($CDCl_3$): $\delta$ = 8.83 (dd, $^3J$ = 4.7 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 6-H), 8.37 (dd, $^3J$ = 7.9 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 4-H), 7.45–7.26 (m, 4H, 2′/5′/6′/5-H), 6.92 (ddd, $^3J$ = 8.3 Hz, $^4J$ = 2.5 Hz, $^4J$ = 2.4 Hz, 1H, 4′-H), 3.88 (s, 3H, $CH_3$); $^{13}$C NMR (100 MHz, ($CDCl_3$): $\delta$ = 162.45 (C=O), 161.86 ($C_q$, 2-$C_{py}$), 160.26 ($C_q$, 3′-C), 154.08 ($CH_{ar}$, 6-$C_{py}$), 137.64 ($C_q$, 1′-C), 135.22 ($CH_{ar}$, 4-$C_{py}$), 130.14 ($CH_{ar}$, 5′-C), 120.97 ($CH_{ar}$, 5-$C_{py}$), 119.83 ($C_q$, 3-$C_{py}$), 116.76 ($CH_{ar}$, 6′-C), 113.40 ($CH_{ar}$, 4′-C), 110.44 ($CH_{ar}$, 2′-C), 55.48 ($CH_3$); MS (EI direct mode): *m*/*z* (%) 258.2 $[M]^+$ (100); microanalysis [$C_{13}H_{10}N_2O_2S$]: found/calculated (%) C (60.64/60.45), H (4.10/3.90), N (10.69/10.85), S (12.13/12.41).

#### 4.12.17. Isothiazolo[5,4-*b*]pyridin-3-ol (5)

To obtain **5** (isothiazolo[5,4-*b*]pyridin-3-ol), diphenyl phosphoryl azide (20 mmol, 5.5 g, 4.3 mL) was cooled to 0 °C in triethylamine (33 mmol, 3.3 g, 4.5 mL). Then, a suspension of 2-mercaptonicotinic (20 mmol, 3.10 g) acid in 10 mL pyridine was added dropwise and the mixture was stirred overnight at room temperature. The crude product was absorbed on $Al_2O_3$(basic, activate I) and washed first with methanol and

subsequently with 10% acetic acid methanol solution. The acid phase was neutralized with 5% (w/v) solution of sodium bicarbonate and extracted three times with dichlorometane. The combined organic layers were washed with diluted hydrochloric acid, treated with brine and dried over sodium sulphate. After solvent removal in vacuum, **5** (isothiazolo[5,4-*b*]pyridin-3-ol), was obtained as a yellow powder (yield: 4.2 mmol, 41%). Mp 243 °C; $^1$H NMR (400 MHz, $(CD_3)_2SO$): $\delta$ = 11.81 (bs 1H, OH), 8.82 (dd, $^3J$ = 4.6 Hz, $^4J$ = 1.6 Hz, 1H$_{py}$, 6-H), 8.31 (dd, $^3J$ = 8.0 Hz, $^4J$ = 1.6 Hz, 1H$_{py}$, 4-H), 7.51 (dd, $^3J$ = 8.0 Hz, $^3J$ = 4.6 Hz, 1H$_{py}$, 5-H); $^{13}$C NMR (100 MHz, $(CD_3)_2SO$): $\delta$ = 168.18 (C-OH), 163.51 (C$_q$, 2-C$_{py}$), 152.95 (CH$_{ar}$, 6-C$_{py}$), 133.55 (CH$_{ar}$, 4-C$_{py}$), 120.77 (CH$_{ar}$, 5-C$_{py}$), 118.92 (C$_q$, 3-C$_{py}$); MS (EI direct mode): *m*/*z* (%) 152.0 [M]$^+$ (100); HPLC purity analysis: 98.8%, retention time 9.35 min.

#### 4.12.18. 2-Mercaptonicotinamide (6)

A suspension of 2-mercaptonicotinic acid (77.3 mmol, 12.0 g) in 120 mL toluene and thionyl chloride (500 mmol, 58.9 g, 36.0 mL) was heated at reflux for 3 h. The preparation was cooled to room temperature and then concentrated in vacuum. The residue was resuspended in 80 mL toluene and concentrated again in vacuum. The obtained acid chloride was subsequently solved in a mixture of ammonium chloride (260 mmol, 14.0 g), concentrated ammonia (2.6 mmol, 100 mL) and 34 mL of water and stirred at room temperature overnight. After treatment with sodium borohydride (40 mmol, 1.51 g) for 1 h, the mixture was acidified with 3 M hydrochloric acid. The precipitate of **6** (2-mercaptonicotinamide) was obtained as brown powder (yield: 58.0 mmol, 75%). Mp 246 °C; $^1$H NMR (400 MHz, $(CD_3)_2SO$): $\delta$ = 14.00 (bs, 1H, SH), 10.07 (bs 1H, NH$_2$), 8.49 (dd, $^3J$ = 7.6 Hz, $^4J$ = 1.9 Hz, 1H$_{py}$, 6-H), 7.95 (dd, $^3J$ = 5.9 Hz, $^4J$ = 1.8 Hz, 2H, 4-H$_{py}$/1H-NH$_2$), 7.01 (dd, $^3J$ = 7.6 Hz, $^3J$ = 5.9 Hz, 1H$_{py}$, 5-H); $^{13}$C NMR (100 MHz, $(CD_3)_2SO$): $\delta$ = 174.49 (C=O), 165.52 (C$_q$, 2-C$_{py}$), 142.62 (CH$_{ar}$, 6-C$_{py}$), 141.70 (CH$_{ar}$, 4-C$_{py}$), 133.84 (C$_q$, 3-C$_{py}$), 114.05 (CH$_{ar}$, 5-C$_{py}$); MS (EI direct mode): *m*/*z* (%) 154.1 [M]$^+$ (100).

#### 4.12.19. Isothiazolo[5,4-*b*]pyridin-3(*2H*)-one (7)

A mixture of **6** (2-mercaptonicotinamide) (20 mmol, 3.1 g) with 20 mL concentrated sulphuric acid was refluxed at 100 °C until the amide was completely dissolved. The preparation was then cooled to room temperature, poured over 50 g ice and made alkaline (pH 11) with concentrated ammonia solution. Thereby, a small amount of precipitate was separated by filtration and the filtrate was heated to boiling. The hot aqueous phase was acidified with acidic acid (pH 4) and allowed to cool. The precipitate was isolated by filtration and washed with water to give **7** (isothiazolo[5,4-*b*]pyridin-3(*2H*)-on) as fawn-yellow powder (yield: 16.2 mmol, 81%). Mp 225 °C; $^1$H NMR (400 MHz, $CD_3OD$, -16 °C): $\delta$ = 8.82 (dd, $^3J$ = 4.7 Hz, $^4J$ = 1.7 Hz, 1H$_{py}$, 6-H), 8.37 (dd, $^3J$ = 8.0 Hz, $^4J$ = 1.7 Hz, 1H$_{py}$, 4-H), 7.52 (dd, $^3J$ = 8.0 Hz, $^3J$ = 4.7 Hz, 1H$_{py}$, 5-H); $^{13}$C NMR (100 MHz, $CD_3OD$, -16 °C): $\delta$ = 168.30 (C=O), 165.52 (C$_q$, 2-C$_{py}$), 152.76 (CH$_{ar}$, 6-C$_{py}$), 133.70 (CH$_{ar}$, 4-C$_{py}$), 120. (CH$_{ar}$, 5-C$_{py}$), 118.52 (C$_q$, 3-C$_{py}$); MS (EI direct mode): *m*/*z* (%) 152.0 [M]$^+$ (100).

#### 4.12.20. 2-Benzylisothiazolo[5,4-*b*]pyridin-3*(2H)*-one (8a)

Starting out from **7** (isothiazolo[5,4-*b*]pyridin-3(*2H*)-one) (10 mmol, 1.5 g) and benzyl bromide (13 mmol, 2.2 g, 1.5 mL) according to synthetic procedure 3, **8a** (2-benzylisothiazolo[5,4-*b*]pyridin-3*(2H)*-one) was obtained as white crystals (yield: 1.7 mmol, 17%). Mp 79 °C; $^1$H NMR (400 MHz, $(CDCl_3)$: $\delta$ = 8.74 (dd, $^3J$ = 4.7 Hz, $^4J$ = 1.7 Hz, 1H$_{py}$, 6-H), 8.31 (dd, $^3J$ = 7.9 Hz, $^4J$ = 1.7 Hz, 1H$_{py}$, 4-H), 7.39–7.35 (m, 6H, 2′/3′/4′/5′/6′/5-H), 5.09 (s, 2H, CH$_2$); $^{13}$C NMR (100 MHz, $(CDCl_3)$: $\delta$ = 163.62 (C=O), 162.37 (C$_q$, 2-C$_{py}$), 153.60 (CH$_{ar}$, 6-C$_{py}$), 135.71 (C$_q$, 1′-C), 134.84 (CH$_{ar}$, 4-C$_{py}$), 128.91 (2 × CH$_{ar}$), 128.46 (2 × CH$_{ar}$), 128.44 (CH$_{ar}$, 4′-C), 120.66 (CH$_{ar}$, 5-C$_{py}$), 119.28 (C$_q$, 3-C$_{py}$), 47.44 (CH$_2$); MS (EI direct mode): *m*/*z* (%)242.0 [M]$^+$ (100); microanalysis [$C_{13}H_{10}N_2OS$]: found/calculated (%) C (64.26/64.44), H (4.26/4.16), N (11.50/11.56), S (13.19/13.23).

#### 4.12.21. 2-(4-Chlorobenzyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one (8b)

Starting out from **7** (isothiazolo[5,4-*b*]pyridin-3(*2H*)-one) (10 mmol, 1.5 g) and 4-chlorobenzyl bromide (13 mmol, 2.7 g,) according to synthetic procedure 3, **8b** (2-(4-chlorobenzyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one) was obtained as white crystals (yield: 3.0 mmol, 30%). Mp 162 °C; $^1$H NMR (400 MHz, $(CDCl_3)$: $\delta$ = 8.77 (dd, $^3J$ = 4.7 Hz, $^4J$ = 1.7 Hz, 1H$_{py}$, 6-H), 8.32 (dd, $^3J$ = 8.0 Hz, $^4J$ = 1.7 Hz, 1H$_{py}$, 4-H), 7.41–7.32 (m, 5H, 2′/3′/5′/6′/5-H), 5.06 (s, 2H, CH$_2$); $^{13}$C NMR (100 MHz, $(CDCl_3)$: $\delta$ = 163.65 (C=O), 162.28 (C$_q$, 2-C$_{py}$), 153.73 (CH$_{ar}$, 6-C$_{py}$), 134.92 (CH$_{ar}$, 4-C$_{py}$), 134.41 (C$_q$, 1′-C), 134.21 (C$_q$, 4′-C), 129.78 (2 × CH$_{ar}$), 129.12 (2 × CH$_{ar}$), 120.79 (CH$_{ar}$, 5-C$_{py}$), 119.13 (C$_q$, 3-C$_{py}$), 46.73 (CH$_2$); MS (CI *NH*$_3$ direct mode): *m*/*z* (%) 222.0 [M$^{35}$Cl+H]$^+$ (100); microanalysis [$C_{13}H_9ClN_2OS$]: found/calculated (%) C (56.26/56.42), H (3.33/3.28), N (10.10/10.12), S (11.13/11.59).

#### 4.12.22. 2-(3,4-Dichlorobenzyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one (8c)

Starting out from **7** (isothiazolo[5,4-*b*]pyridin-3(*2H*)-one) (10 mmol, 1.5 g) and 3,4-dichlorobenzyl bromide (13 mmol, 3.1 g, 1.9 mL) according to synthetic procedure 3, **8c** (2-(3,4-dichlorobenzyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one) was obtained as white crystals (yield: 3.7 mmol, 37%). Mp 136 °C; $^1$H NMR (400 MHz, $(CDCl_3)$: $\delta$ = 8.79 (dd, $^3J$ = 4.7 Hz, $^4J$ = 1.7 Hz, 1H$_{py}$, 6-H), 8.33 (dd, $^3J$ = 7.9 Hz, $^4J$ = 1.7 Hz, 1H$_{py}$, 4-H), 7.48 (d, $^4J$ = 2.1 Hz, 1H, 2′-H), 7.45 (d, $^3J$ = 8.2 Hz, 1H, 5′-H), 7.41 (dd, $^4J$ = 7.9 Hz, $^4J$ = 4.7 Hz, 1H$_{py}$, 5-H), 7.23 (dd, $^3J$ = 8.2 Hz, $^4J$ = 2.1 Hz, 1H, 6′-H), 5.05 (s, 2H, CH$_2$); $^{13}$C NMR (100 MHz, $(CDCl_3)$: $\delta$ = 163.69 (C=O), 162.22 (C$_q$, 2-C$_{py}$), 153.90 (CH$_{ar}$, 6-C$_{py}$), 135.91 (C$_q$, 1′-C), 135.02 (CH$_{ar}$, 4-C$_{py}$), 133.08 (C$_q$, 3′-C), 132.68 (C$_q$, 4′-C), 130.90 (CH$_{ar}$, 5′-C), 130.21 (CH$_{ar}$, 2′-C), 127.59 (CH$_{ar}$, 6′-C), 120.90 (CH$_{ar}$, 5-C$_{py}$), 118.94 (C$_q$, 3-C$_{py}$), 46.22 (CH$_2$); MS (CI *NH*$_3$ direct mode): *m*/*z* (%) 311.0 [M$^{35}$Cl$^{35}$Cl+H]$^+$ (100); microanalysis [$C_{13}H_8Cl_2N_2OS$]: found/calculated (%) C (50.33/50.18), H (2.44/2.59), N (9.00/9.00), S (9.63/10.30).

#### 4.12.23. 2-(4-Fluorobenzyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one (8d)

Starting out from **7** (isothiazolo[5,4-*b*]pyridin-3(*2H*)-one) (10 mmol, 1.5 g) and 4-fluorobenzyl bromide (13 mmol, 2.5 g, 1.6 mL) according to synthetic procedure 3, **8d** (2-(4-fluorobenzyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one) was obtained as white crystals (yield: 2.0 mmol, 20%). Mp 152 °C; $^1$H NMR (400 MHz, $(CDCl_3)$: $\delta$ = 8.76 (dd, $^3J$ = 4.7 Hz, $^4J$ = 1.7 v, 1H$_{py}$, 6-H), 8.32 (dd, $^3J$ = 7.9 Hz, $^4J$ = 1.7 Hz, 1H$_{py}$, 4-H), 7.40–7.36 (m, 3H, 3′/5′/5-H), 7.10–7.06 (m, 2H, 2′/6′-H), 5.07 (s, 2H, CH$_2$); $^{13}$C NMR (100 MHz, $(CDCl_3)$: $\delta$ = 163.60 (C=O), 163.11 (d, $^1J_{C-F}$ = -163.6 Hz, C$_q$, 4′-C), 162.29 (C$_q$, 2-C$_{py}$), 153.70 (CH$_{ar}$, 6-C$_{py}$), 134.89 (CH$_{ar}$, 4-C$_{py}$), 131.56 (d, $^4J_{C-F}$ = -3.5 Hz, C$_q$, 1′-C), 130.31 (d, $^3J_{C-F}$ = -8.3 Hz, 2 × CH$_{ar}$, 2′/6′-C), 120.75 (CH$_{ar}$, 5-C$_{py}$), 119.23 (C$_q$, 3-C$_{py}$), 115.88 (d, $^2J_{C-F}$ = -21.7 Hz, 2 × CH$_{ar}$, 3′/5′-C), 46.72 (CH$_2$); $^{19}$F NMR (376 MHz, $(CDCl_3)$: $\delta$ = 113.14–113.21 (m, 1F, 4′-F); MS (CI *NH*$_3$ direct mode): *m*/*z* (%) 261.1 [M+H]$^+$ (100); HPLC purity analysis: 98.9%, retention time 18.87 min.

#### 4.12.24. 2-(3,4-Difluorobenzyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one (8e)

Starting out from **7** (isothiazolo[5,4-*b*]pyridin-3(*2H*)-one) (10 mmol, 1.5 g) and 3,4-difluorobenzyl bromide (13 mmol, 2.7 g, 1.7 mL) according to synthetic procedure 3, **8e** (2-(3,4-difluorobenzyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one) was obtained as white crystals (yield: 3.5 mmol, 35%). Mp 125 °C; $^1$H NMR (400 MHz,

($CDCl_3$): $\delta$ = 8.78 (dd, $^3J$ = 4.7 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 6-H), 8.33 (dd, $^3J$ = 7.9 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 4-H), 7.40 (dd, $^3J$ = 7.9 Hz, $^3J$ = 4.7 Hz, $1H_{py}$, 5-H), 7.24–7.13 (m, 3H, 2′/5′/6′-H), 5.04 (s, 2H, $CH_2$); $^{13}C$ NMR (100 MHz, ($CDCl_3$): $\delta$ = 163.65 (C=O), 162.22 ($C_q$, 2-$C_{py}$), 153.86 ($CH_{ar}$, 6-$C_{py}$), 150.39 (dpt, $^1J_{C-F}$ = -248.3 Hz, $^2J_{C-F}$ = -12.8 Hz, $^2J_{C-F}$ = -12.4 Hz, 2 × $C_q$, 3′/4′-C), 134.98 ($CH_{ar}$, 4-$C_{py}$), 132.71 (dd, $^3J_{C-F}$ = -5.2 Hz, $^4J_{C-F}$ = -4.0 Hz, $C_q$, 1′-C), 124.48 (dd, $^3J_{C-F}$ = -6.3 Hz, $^4J_{C-F}$ = -3.7 Hz, $CH_{ar}$, 6′-C), 120.87 ($CH_{ar}$, 5-$C_{py}$), 119.00 ($C_q$, 3-$C_{py}$), 117.74 (d, $^2J_{C-F}$ = -17.4 Hz, $CH_{ar}$, 5′-C), 117.47 (d, $^2J_{C-F}$ = -17.6 Hz, $CH_{ar}$, 2′-C), 46.38 ($CH_2$); $^{19}F$ NMR (376 MHz, ($CDCl_3$): $\delta$ = 136.21–136.31 (m, 1F, 4′-F), 137.61–137.73 (m, 1F, 3′-F); MS (CI $NH_3$ direct mode): *m*/*z* (%) 279.1 $[M+H]^+$ (100); HPLC purity analysis: 99.3%, retention time 19.44 min.

### 4.12.25. 2-(4-Methylbenzyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one (8f)

Starting out from **7** (isothiazolo[5,4-*b*]pyridin-3(*2H*)-one) (10 mmol, 1.5 g) and 4-methylbenzyl bromide (α-bromo-*p*-xylene, 13 mmol, 2.1 g,) according to synthetic procedure 3, **8f** (2-(4-methylbenzyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one) was obtained as white crystals (yield: 3.2 mmol, 32%). Mp 116 °C; $^1H$ NMR (400 MHz, ($CDCl_3$): $\delta$ = 8.75 (dd, $^3J$ = 4.7 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 6-H), 8.32 (dd, $^3J$ = 7.9 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 4-H), 7.37 (dd, $^4J$ = 7.9 Hz, $^4J$ = 4.7 Hz, $1H_{py}$, 5-H), 7.29 (d, $^3J$ = 8.0 Hz, 2H, 3′/5′-H), 7.20 (d, $^3J$ = 7.2 Hz, 2H, 2′/6′-H), 5.06 (s, 2H, $CH_2$), 2.37 (s, 3H, $CH_3$); $^{13}C$ NMR (100 MHz, ($CDCl_3$): $\delta$ = 163.87 (C=O), 162.40 ($C_q$, 2-$C_{py}$), 153.54 ($CH_{ar}$, 6-$C_{py}$), 138.37 ($C_q$, 1′-C), 134.81 ($CH_{ar}$, 4-$C_{py}$), 132.67 ($C_q$, 4′-C), 129.59 (2 × $CH_{ar}$), 128.53 (2 × $CH_{ar}$), 120.62 ($CH_{ar}$, 5-$C_{py}$), 119.41 ($C_q$, 3-$C_{py}$), 47.25 ($CH_2$), 21.17 ($CH_3$); MS (EI direct mode): *m*/*z* (%) 256.1 $[M]^+$ (96), 105.1 $[C_8H_9]^+$ (100); microanalysis [$C_{14}H_{12}N_2OS$]: *found/calculated (%)* C (65.45/65.60), H (4.81/4.72), N (10.94/10.93), S (12.57/12.51).

### 4.12.26. 2-(3-Methoxybenzyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one (8g)

Starting out from **7** (isothiazolo[5,4-*b*]pyridin-3(*2H*)-one) (10 mmol, 1.5 g) and 3-methoxybenzyl bromide (13 mmol, 2.6 g, 1.8 mL) according to synthetic procedure 3, **8g** (2-(3-methoxybenzyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one) was obtained as white crystals (yield: 2.7 mmol, 27%). Mp 107 °C; $^1H$ NMR (400 MHz, ($CDCl_3$): δ =8.76 (dd, $^3J$ = 4.7 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 6-H), 8.32 (dd, $^3J$ = 7.9 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 4-H), 7.38 (dd, $^4J$ = 7.9 Hz, $^4J$ = 4.7 Hz, $1H_{py}$, 5-H), 7.33–7.29 (m, 1H, 5′-H), 6.98–6.96 (m, 1H, 6′-H), 6.92–6.88 (m, 2H, 2′/4′-H), 5.07 (s, 2H, $CH_2$), 3.82 (s, 3H, $CH_3$); $^{13}C$ NMR (100 MHz, ($CDCl_3$): $\delta$ = 163.63 (C=O), 162.41 ($C_q$, 2-$C_{py}$), 159.98 ($C_q$, 3′-C), 153.62 ($CH_{ar}$, 6-$C_{py}$), 137.17 ($C_q$, 1′-C), 134.86 ($CH_{ar}$, 4-$C_{py}$), 129.96 ($CH_{ar}$, 5′-C), 120.66 (2 × $CH_{ar}$, $5_{py}$/6′-C), 119.27 ($C_q$, 3-$C_{py}$), 113.97 ($CH_{ar}$, 4′-C), 113.94 ($CH_{ar}$, 2′-C), 55.25 ($CH_3$), 47.39 ($CH_2$); MS (EI direct mode): *m*/*z* (%) 272.1 $[M]^+$ (98), 121.1 $[C_8H_9O]^+$ (100); microanalysis [$C_{14}H_{12}N_2O_2S$]: *found/calculated (%)* C (61.36/61.75), H (4.52/4.44), N (10.16/10.29), S (11.66/11.77).

### 4.12.27. 2-(4-(Trifluoromethyl)benzyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one (8h)

Starting out from 7 (isothiazolo[5,4-*b*]pyridin-3(*2H*)-one) (10 mmol, 1.5 g) and 4-(trifluoromethyl)benzyl bromide (13 mmol, 2.4 g) according to synthetic procedure 3, 8 h (2-(4-(trifluoromethyl)benzyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one) was obtained as white-yellowish crystals (yield: 4.6 mmol, 46%). Mp 117 °C; $^1H$ NMR (400 MHz, ($CDCl_3$): $\delta$ = 8.78 (dd, $^3J$ = 4.7 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 6-H), 8.34 (dd, $^3J$ = 7.9 Hz, $^4J$ = 1.7 Hz, $1H_{py}$, 4-H), 7.65 (d, $^3J$ = 8.2 v, 2H, 3′/5′-H), 7.50 (d, $^3J$ = 8.1 Hz, 2H, 2′/6′-H), 7.41 (dd, $^3J$ = 7.9 Hz, $^3J$ = 4.7 Hz, $1H_{py}$, 5-H), 5.15 (s, 2H, $CH_2$); $^{13}C$ NMR (100 MHz, ($CDCl_3$): $\delta$ = 163.75 (C=O), 162.23 ($C_q$, 2-$C_{py}$), 153.88 ($CH_{ar}$, 6-$C_{py}$), 139.66 (d, $^5J_{C-F}$ = -1.0 Hz, $C_q$, 1′-C), 135.00 ($CH_{ar}$, 4-$C_{py}$), 130.62 (d, $^2J_{C-F}$ = -32.4 Hz, $C_q$, 4′-C), 128.55 (2 × $CH_{ar}$, 2′/6′-C), 125.90 (dd, $^3J_{C-F}$ = -7.5 Hz, $^3J_{C-F}$ = -3.7 Hz, 2 × $CH_{ar}$, 3′/5′-C), 123.85 (d, $^1J_{C-F}$ = -270.6 Hz, $C_q$, $CF_3$), 120.88 ($CH_{ar}$, 5-$C_{py}$), 118.95 ($C_q$, 3-$C_{py}$), 46.82 ($CH_2$); $^{19}F$ NMR (376 MHz, ($CDCl_3$): $\delta$ = 62.67 (2, 3F, $CF_3$); MS (EI direct mode): *m*/*z* (%) 310.1 $[M]^+$ (74), 159.0 $[C_8H_6F_3]^+$ (100); HPLC purity analysis: 98.4%, retention time 20.98 min.

### 4.12.28. 2-(3,4-Dichlorobenzyl)-1-oxoisothiazolo[5,4-*b*]pyridin-3*(2H)*-one (9)

Compound **8c** (2-(3,4-dichlorobenzyl)isothiazolo[5,4-*b*]pyridin-3*(2H)*-one) (13 mmol, 4.0 g) was solved at room temperature in 50% (v/v) methanol (21 mL). Potassium peroxomonosulphate (oxone®, 19.3 mmol, 11.9 g) was added in small portions to the stirred mixture. After consumption of the reactants (1 h), the batch was poured into 21 mL of water and extracted three times with dichloromethane. The collected organic layers were dried over sodium sulphate and evaporated. The residue was recrystallized from ethyl acetate to give **9** (2-(3,4-dichlorobenzyl)-1-oxoisothiazolo[5,4-*b*]pyridin-3*(2H)*-one) as white crystals (yield: 8.3 mmol, 64%). Mp 150 °C; $^1H$ NMR (400 MHz, ($CDCl_3$): $\delta$ = 8.98 (dd, $^3J$ = 4.8 Hz, $^4J$ = 1.6 Hz, $1H_{py}$, 6-H), 8.34 (dd, $^3J$ = 7.8 Hz, $^4J$ = 1.6 Hz, $1H_{py}$, 4-H), 7.74 (dd, $^4J$ = 7.8 Hz, $^4J$ = 4.8 Hz, $1H_{py}$, 5-H), 7.56 (d, $^4J$ = 2.0 Hz, 1H, 2′-H), 7.46 (d, $^3J$ = 8.2 Hz, 1H, 5′-H), 7.31 (dd, $^3J$ = 8.2 Hz, $^4J$ = 2.1 Hz, 1H, 6′-H), 5.22 (d, $^2J$ = 15.5 Hz, 1H, $CH_2$), 4.80 (d, $^2J$ = 15.6 Hz, 1H, $CH_2$); $^{13}C$ NMR (100 MHz, ($CDCl_3$): $\delta$ = 165.24 (C=O), 163.96 ($C_q$, 2-$C_{py}$), 155.17 ($CH_{ar}$, 6-$C_{py}$), 135.61 ($C_q$, 1′-C), 134.81 ($CH_{ar}$, 4-$C_{py}$), 133.08 ($C_q$, 3′-C), 132.69 ($C_q$, 4′-C), 130.91 ($CH_{ar}$, 5′-C), 130.58 ($CH_{ar}$, 2′-C), 127.98 ($CH_{ar}$, 6′-C), 127.44 ($CH_{ar}$, 5-$C_{py}$), 122.55 ($C_q$, 3-$C_{py}$), 46.61 ($CH_2$); MS (CI $NH_3$ direct mode): *m*/*z* (%) 344.0 $[M^{35}Cl^{35}Cl+NH_4]^+$ (100); microanalysis [$C_{13}H_8Cl_2N_2O_2S$]: found/calculated C (47.58/47.72), H (2.40/2.46), N (8.53/8.56), S (9.89/9.80).

### 4.12.29. 2-(3,4-Dichlorobenzyl)-1,1-dioxoisothiazolo[5,4-*b*]pyridin-3*(2H)*-one (10)

A mixture of **9** (2-(3,4-dichlorobenzyl)-1-oxoisothiazolo[5,4-*b*]pyridin-3*(2H)*-one) (6.0 mmol, 2.0 g) and tetrabutylammonium bromide (0.24 mmol, 0.77 g) in 40 mL ethyl acetate was treated with 5% (w/v) sodium hypochlorite solution (24 mmol, 36 g). The preparation was stirred intensively at room temperature and the reaction progress was monitored by thin layer chromatography. When the reaction had been completed (1 h), water (100 mL) was added to the mixture. The ethyl acetate phase was separated and washed twice with water. The organic layer was dried over sodium sulphate and evaporated. The crude product was purified using silica gel column chromatography and ethyl acetate/cyclohexane 2:1 (v/v) as eluent to obtain **10** (2-(3,4-dichlorobenzyl)-1,1-dioxoisothiazolo[5,4-*b*]pyridin-3*(2H)*-one) as white crystals (yield: 2.2 mmol, 36%). Mp 175 °C; $^1H$ NMR (400 MHz, ($CDCl_3$): $\delta$ = 9.04 (dd, $^3J$ = 4.9 Hz, $^4J$ = 1.6 Hz, $1H_{py}$, 6-H), 8.41 (dd, $^3J$ = 7.9 Hz, $^4J$ = 1.6 Hz, $1H_{py}$, 4-H), 7.81 (dd, $^4J$ = 7.9 Hz, $^4J$ = 4.9 Hz, $1H_{py}$, 5-H), 7.64 (d, $^4J$ = 2.1 Hz, 1H, 2′-H), 7.46 (d, $^3J$ = 8.2 Hz, 1H, 5′-H), 7.38 (dd, $^3J$ = 8.2 Hz, $^4J$ = 2.1 Hz, 1H, 6′-H), 4.90 (s, 2H, $CH_2$); $^{13}C$ NMR (100 MHz, ($CDCl_3$): $\delta$ = 156.85 (C=O), 155.93 ($C_q$, 2-$C_{py}$), 155.65 ($CH_{ar}$, 6-$C_{py}$), 134.19 ($C_q$, 1′-C), 133.86 ($CH_{ar}$, 4-$C_{py}$), 132.88 ($C_q$, 3′-C), 132.80 ($C_q$, 4′-C), 130.78 ($CH_{ar}$, 5′-C), 130.74 ($CH_{ar}$, 2′-C), 128.39 ($CH_{ar}$, 6′-C), 128.16 ($CH_{ar}$, 5-$C_{py}$), 122.23 ($C_q$, 3-$C_{py}$), 41.48 ($CH_2$); MS (CI $NH_3$ direct mode): *m*/*z* (%) 360.0 $[M^{35}Cl^{35}Cl+NH_4]^+$ (100); microanalysis [$C_{13}H_8Cl_2N_2O_3S$]: found/calculated (%) C (45.53/45.50), H (2.31/2.35), N (8.11/8.16), S (9.28/9.34).

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.01.063.

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