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Synthesis and Structure Activity Relationship of 3-Hydroxypyridin-2-thione Based Histone Deacetylase Inhibitors

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Abstract

We have previously identified 3-hydroxypyridin-2-thione (3HPT) as a novel zinc binding group for histone deacetylase (HDAC) inhibition. Early structure activity relationship (SAR) studies led to various small molecules possessing selective inhibitory activity against HDAC6 or HDAC8 but are devoid of HDAC1 inhibition. To further delineate the depth of the SAR of 3HPT-derived HDAC inhibitors (HDACi), we have extended the SAR studies to include the linker region and the surface recognition group to optimize the HDAC inhibition. The current efforts resulted in the identification of two lead compounds **10d** and **14e** with potent HDAC6 and HDAC8 activities, but that are inactive against HDAC1. These new HDACi possess anti-cancer activities against various cancer cell lines including Jurkat J- γ 1 against which SAHA and the previously disclosed 3HPT-derived HDACi were inactive.

Introduction

Histone deacetylase (HDAC) inhibition is a promising epigenetic strategy for cancer treatment, and several distinct small molecule histone deacetylase inhibitors (HDACi) have been reported.¹ Currently, two of these HDACi, suberoylanilide hydroxamic acid (SAHA) (Figure 1) and cyclic peptide FK228 (Romidepsin), are approved for the treatment of cutaneous T-cell lymphoma (CTCL).² However, most HDACi, including the clinically approved agents, non-selectively inhibit the deacetylase activity of class I and II HDACs, and many suffer from metabolic instability. These shortcomings have been associated with reduced *in vivo* potency and toxic side effects.³ Currently, significant efforts are ongoing to

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Supporting Information Available: ¹H NMR and ¹³C NMR spectral information, solubility data and molecular modeling outputs. This material is available free of charge via the Internet at <http://pubs.acs.org>.

address these and other deficiencies of HDACi to bolster the potential of HDAC inhibition in cancer treatment.

Most HDACi fit a three-motif pharmacophoric model consisting of a zinc binding group (ZBG), a linker, and a surface recognition cap group. Hydroxamic acid (hydroxamate) is by far the most common ZBG moiety in HDACi owing to its ability to reliably chelate active site zinc ions.⁴ However, pharmacodynamic and pharmacokinetic liabilities of the hydroxamate moiety have prompted efforts to find better suited alternatives.⁵ Examples of non-hydroxamate ZBGs investigated include thiols, α -ketoesters, benzamide, trifluoromethylketone, oxime, phosphonates, and mercaptoacetamide.^{6, 7, 5, 8} However, most of these alternative ZBGs have thus far elicited reduced potency relative to hydroxamic acid.

Previous work from our lab has established 3-hydroxypyridin-2-thione (3HPT) as a non-hydroxamate ZBG for HDAC inhibition.⁹ Initial structure activity relationship (SAR) studies led to aryl- and diaryl-3HPT analogs possessing selective inhibitory activity against HDAC6 or HDAC8 but which were not active against HDAC1 (see Figure 1 for representative compounds **1**, **2** and **3**). In the same study, we observed that the replacement of the proximal phenyl ring of the lead biphenyl compound **2** with a 1,2,3-triazole ring resulted in the corresponding triazolyl analog **3** lacking HDAC6 inhibition activity but having improved HDAC8 inhibition (Figure 1). This observation suggests a possible divergence in the SARs of the triazole and biphenyl 3-HPT compounds.⁹ To further characterize such divergence, we have expanded the SAR studies on the 3-HPT compounds bearing triazole-linked cap groups. The current efforts identified two lead compounds, **10d** and **14e**, potent inhibitors of HDAC6 and HDAC8 but inactive against HDAC1. These new HDACi possess anti-cancer activities against various cancer cell lines including Jurkat J- γ 1 against which SAHA and the previously disclosed 3HPT-derived HDACi were inactive.

RESULTS AND DISCUSSION

SAR on the Linker Moiety

The hydrophobic linker moiety of most HDACi consists of flexible methylene spacer groups that separate the ZBG from the cap-group in order to tailor the intramolecular span between the active site Zn^{2+} ion and outer rim amino acid residues. Previous studies from our laboratory have shown that SAHA-like HDACi containing a 1, 2, 3-triazole ring within the linker region differentially inhibited HDAC's as a function of linker length.¹⁰ The lead compound **3** fits a description of an analog with one methylene spacer separating the triazole ring and the 3HPT ZBG. To probe the effect of the spacer length on HDAC inhibition activity, we initially synthesized and investigated the anti-HDAC activity of compounds **10a–f**, analogs of **3** with increasing methylene groups. The syntheses of target compounds are accomplished as shown in Scheme 1. The reaction of various bromoalkanols with sodium azide yielded their corresponding azidoalkanols **4a–f**. Subsequent mesylation of **4a–f**, followed by *N*-alkylation with *O*-methyl or *O*-benzyl protected 3-hydroxypyridin-2-one (3HP) gave the azido intermediates **6a–f**. The phenyl moiety, which serves as surface recognition group, was introduced via Cu(I)-catalyzed Huisgen cycloaddition reaction^{11, 12}

between phenylacetylene and the azido intermediates **6a–f** to afford compounds **7a–f**. The deprotection of the *O*-benzyl moiety of **7b–f** was accomplished using catalytic hydrogenation to afford compounds **8b–f** which upon treatment with P₄S₁₀ at 175°C gave the corresponding 3HPT compounds **10b–f** (scheme 1).¹³ While otherwise facile, this chemistry did not work for the 2 methylene linker compound because of an extensive degradation which resulted in an intractable mixture when the compound **8a** was exposed to P₄S₁₀ at 175°C. To obtain the requisite 3HPT compound **10a**, the *O*-methyl protected 3HP **7a** was first converted to its thione analog **9** using Lawesson's reagent.¹⁴ Subsequent BBr₃ deprotection of the methyl ether group yielded the desired compound **10a** (Scheme 1).

We then assayed compounds **10a–f**, as well as their 3HP congeners **8a–f**, against HDAC isoforms 1, 6 and 8, using SAMDI mass spectrometry as previously described.^{15, 16, 17} The 3HP compounds **8a–f** are inactive against all HDAC isoforms tested (data not shown), a result that is in agreement with our previous observation on aryl- and diaryl-3HP analogs.⁹ As previously reported diaryl-3HPT-based HDACi, compounds **10a–f** are not active against HDAC1. However, compounds **10a–f** do inhibit HDAC6 and HDAC8 (Table 1) and do so as a function of methylene linker length. Such apparent selectivity may result from active site structure differences between the isoforms. HDAC1 has a shallow active site as opposed to HDAC6 whose hydrophobic channel entrance appears large enough to accommodate the 3HPT group.¹⁸ Relative to the lead compound **3**, a progressive increase in the length of the methylene linker separating the triazole ring and the 3HPT group results in a corresponding increase in HDAC6 inhibition activity up to 5 methylene units (n=5) (Table 1, compare **10a–d**). A single methylene linker extension (from n = 5 to n = 6) isn't tolerated as the resulting analog **10f** is devoid of HDAC6 inhibition activity; however, a further extension of the methylene linker length (n = 7) fully restores anti-HDAC6 activity (Table 1, compare **10d**, **10e** and **10f**). Similar methylene linker length dependence in HDAC8 inhibition activity was observed. However unlike what was obtained with HDAC6, no complete loss and subsequent full restoration of anti-HDAC8 occurred when n = 6 and 7 respectively. This initial SAR study suggests that five methylene spacers (n = 5) are optimum for HDAC inhibition as the corresponding compound **10d** is the most potent in this series, with a markedly improved anti-HDAC activity relative to the starting lead compound **3**. Additionally, **10d** is equipotent against HDAC6 and HDAC8 with approximately 900 nM IC₅₀ (Table 1).

Molecular Docking Analyses

To understand the structural basis of dependence of methylene linker length on the HDAC inhibition by compounds **10a–f**, we docked selected analogs (**10a** and **10d**) against an HDAC6 homology model built from HDAC8 (PDB code: 3F0R) and HDAC8 (PDB code: 3SFF) using AutoDock 4.2.^{19, 20, 15} We observed that **10a** and **10d** adopt docked conformations on HDAC6 such that their 3HPT ZBG are able to maintain Zn²⁺ chelation irrespective of the methylene linker length. Unlike **10a**, however, the five methylene compound **10d** is further stabilized by a π -stacking interaction between its phenyl ring and that of Phe680 (Figure 2A). The docked poses found on HDAC8 have the phenyl ring of **10d** inserted into a solvent inaccessible hydrophobic pocket adjacent to the active site that is otherwise inaccessible to **10a** due to its shorter methylene spacers. Instead, the phenyl ring

of **10a** is positioned in a solvent exposed outer rim compartment of HDAC8 (Figure 2B). The favorable interactions of the phenyl ring with the active site amino acid side chains of HDAC6 and the sub-pocket of HDAC8 support the preference for the five methylene compound **10d**.

SAR on the Surface Recognition Group: optimization of the five methylene-linked compound

As demonstrated by *in vitro* studies and corroborated by docking analyses, the five methylene spacer groups induced optimal inhibition of both HDAC6 and HDAC8. This linker, along with the triazole ring, traverses the hydrophobic channel of the HDAC to present the 3- HPT to the active site for Zn^{2+} chelation. To further optimize the lead compound **10d**, we performed a SAR study on the cap group by investigating the effects of various phenyl ring substituents and substitution patterns on HDAC inhibition activity.

The synthesis of the desired phenyl substituted compounds followed a similar protocol described for the synthesis of compound **10** (Scheme 2). Briefly, Cu(I)-catalyzed Huisgen cycloaddition reaction^{11, 12} of appropriate substituted phenylacetylene with azido *O*-methyl protected 3-hydroxypyridin-2-one **11** gave the corresponding triazolyl intermediates **12a–g**. Subsequent treatment of **12a–g** with Lawessons' reagent followed by BBr_3 deprotection of the *O*-methyl group resulted in desired 3HPT compounds **14a–g**. Similarly, BBr_3 deprotection of the *O*-methyl group of **12a–g** afforded the corresponding 3HP compounds **15a–g**.

As expected, the 3HP compounds **15a–g** were inactive against all HDAC isoforms tested (data not shown). Similar to the 3HPT compounds **10a–f**, none of the substituted 3HPT compounds **14a–g** were active against HDAC1. Despite the diversity of the surface recognition groups tested, none was more potent than the unsubstituted lead compound **10d** against HDAC8 (Table 2). Against HDAC6, however, we observed an intriguing effect on inhibition activity. Among the methyl substituents (*para* **14a**, *meta* **14b**, *ortho* **14c**), only the *ortho*-substituted **14c** offered a potency enhancement against HDAC6 relative to **10d**. The inhibitory activity of the *para*-substituted compound **14a** is comparable to that of **10d** whereas the *meta*-substituted compound **14b** is somewhat less potent. Remarkably, substitution of methyl at the *meta*-position of **14b** with an electron withdrawing cyano group yielded the most potent HDAC6 inhibitor, compound **14e**, among the series. Compound **14e** is 2.5- and 3-fold more effective than the unsubstituted lead compound **10d** and the *meta*-methyl compound **14b** respectively (Table 2). The placement of the cyano group at the *para*-position conferred no additional benefit to HDAC inhibitory activity as the resulting compound **14d** is equipotent as the lead compound **10d** and the *para*-methyl compound **14a**. Moreover, switching to the electron donating *N,N*-dimethylamino group at the *para*-position (compound **14f**) did not enhance HDAC6 inhibition despite structural similarity to TSA. Addition of a different electron withdrawing group, trifluoromethyl moiety, to the *meta*-position in the presence of the cyano substituent at the *para*-position partially rescued HDAC6 inhibitory activity lost in the compound which has just only the cyano group at the *para*-position (Table 2, comparing **14d** and **14g**). This observation further supports the

influence of electron withdrawing groups at the *meta*-position position on the potency of the substituted 3-HPT compounds.

Molecular docking analysis on substituted 3HPT compounds

To gain insight into the binding interactions that underlie the SAR for HDAC inhibition, we docked each of the substituted 3HPT compounds against our HDAC6 homology model and HDAC8. For HDAC6, the phenyl ring of all compounds mediates a potential π -stacking interaction with the phenyl ring of Phe680 mimicking the interaction observed for compound **10d** (See Supplemental info Figure S1). Most striking, however, is the observation that the cyano group of compound **14e** is ideally positioned for a stabilizing H-bonding interaction with the hydroxyl side-chain and amide backbone of Ser568 and likely accounts for its enhanced potency against HDAC6 (Figure 3). Moreover, the concomitant reduction in electron density by the cyano group could further enhance stacking interaction with the phenyl ring of Phe680.

While phenyl ring substitution impaired HDAC8 inhibition, docking analyses offered useful insight into the putative hydrophobic pocket in which the inhibitor's phenyl group is expected to reside. Mono-substituted compounds fit within this sub-pocket while di-substituted compound **14g** is excluded, indicating a restrictive size limitation (see Figure 3 and supplementary info Figure S2). Space filling models of all the docked compounds reveal that the unsubstituted phenyl **10d** fit in the sub-pocket best, and any substituent slightly pushes the phenyl ring out of the hydrophobic pocket (Figure 3). Even though the size limitation may preclude the sub-pocket from affecting enhanced inhibition through lead development, it offers a distinctive feature not observed on HDAC6 that accounts for the pronounced isoform selectivity of compound **14e**. The lead candidates from the foregoing SAR studies are compounds **10d** and **14e**. Compound **10d** is equipotent against HDAC6 and HDAC8 while **14e** is 8-fold selective for HDAC6.

In Vitro Cell Growth Inhibition

We next tested the effect of lead compounds **10d** and **14e** and comparable compounds **10f** and **14c** on the proliferation of selected cancer cell lines. We investigated DU-145 (androgen independent prostate cancer), LNCaP (androgen dependent prostate cancer), the T-cell leukemia cell line Jurkat and Jurkat J.gam1, a mutant Jurkat cell line resistant to HDAC inhibition.^{21, 9}

Table 3 shows the IC₅₀ values of each compound against the cancer cell lines studied. The lead meta-cyano compound **14e** is about 2-fold more potent, relative to the unsubstituted congener **10d**, against DU145 and LNCaP prostate cancer cell lines. Such improved sensitivity with **14e** toward prostate cancer lines, particularly LNCaP, may be rationalized by its HDAC6 selectivity.²² LNCaP is an androgen dependent prostate cancer line whose viability is linked to the interaction between HSP90 and its client proteins including the androgen receptor.²³ Misregulation of HSP90-client protein interactions, following HDAC6 inhibition, is detrimental to cell viability.²² Inhibition of HSP90 has been shown to compromise the viability of DU145 offering a similar rationale for the enhanced cytotoxicity of **10d** against this cell line too.^{24, 25} Compounds **10d** and **14e** are equipotent against the

wild type Jurkat cell line, despite the enhanced HDAC8 inhibition activity of the former (Table 2) which is expected favor cytotoxicity to the Jurkat cell line.^{21, 26} It is reasonable that the compensation of the weaker HDAC8 inhibition activity of **14e** by the 3-fold increase in its anti-HDAC6, relative to that of **10d**, may contribute to its cytotoxicity in the Jurkat cell. The cytotoxicity of **10f**, analog containing a seven-methylene linker, and the *ortho*-methyl substituted five-methylene compound **14c** do not completely trend with the pattern of their HDAC inhibition activities. Against both prostate cancer lines investigated, **10f** is 2- and 3-fold less cytotoxic compared with lead compounds **10d** and **14e** respectively, while it is 3-fold less cytotoxic relative to both lead compounds against the wild type Jurkat cell line. The *ortho*-substituted compound **14c** is 2-fold less cytotoxic against DU145 while it is only marginally less cytotoxic against LNCaP and wild type Jurkat cell line compared with **14e**.

Although **10d** and **10f** have similar IC₅₀ against HDAC6, the latter has a significantly higher cellular IC₅₀ against LNCaP cells. Against Jurkat cell line, **14c** and **14e** also display similar incongruity, which we first attributed to solubility differences. To partially account for such pharmacokinetic liabilities, we estimated the solubility of each tested compound^{27, 28} but found that the four compounds had solubilities greater than the threshold proposed to adversely affect cell activity (Supplementary info Figure S3 and Table S1). This suggests that the observed discrepancies may be attributed to factors other than solubility.

We have shown previously that the Jurkat J γ cell line, a mutant Jurkat cell line lacking phospholipase C activity, is resistant to SAHA.⁹ To check if the 3HPT compounds would be similarly innocuous, we investigated the effect of the lead compounds **10d**, **10f** and **14e** on Jurkat J γ cell growth. We observed that compound **10f** is significantly less cytotoxic to Jurkat J γ cells versus their wild type counterpart. Surprisingly, both **10d** and **14e** are potently cytotoxic to Jurkat J γ cells with IC₅₀ values of 1 and 2 μ M respectively. Comparatively, the IC₅₀s of **10d** and **14e** against the healthy mammalian cell line Vero were estimated to be greater than 20 μ M, the highest tested concentration (Table 3). In order to indirectly evaluate the contribution of HDAC6 inhibition to the anticancer activity of the compounds, the anti-proliferative activity of the HDAC6 selective inhibitor tubastatin A was evaluated against these cell lines as well. Although tubastatin A was as potent as **10d** and **14e** in Jurkat cells, it was 2 to 3 fold less potent against LNCaP and inactive against Jurkat J γ (Table 3). Given that the pan-inhibitor SAHA and the HDAC6 selective tubastatin A are inactive against Jurkat J γ , the cytotoxic activity of lead compounds **10d** and **14e** against Jurkat J γ could be through inhibition of other yet to be identified cellular target(s).

Toward identifying other possible targets of the lead compounds **10d** and **14e**, we screened for their effect on the activity of a collection matrix metalloproteases (MMPs) which have been suggested to play roles in apoptosis.²⁹ The rationale for screening against MMPs is based on the fact that 3HPT has been previously used as ZBG in the design MMP inhibitors.³⁰ At the tested concentration of 10 μ M, **10d** and **14e** were inactive against MMP1, 2, 3, 7, 9 and 14, while the activity of MMP13 was halved at this very high concentration (Figure S4). Despite the marginal effect on MMP13, these proteins are not likely to be directly affected by the compounds tested.

We also tested lead compounds against HDAC2 which has been shown to be upregulated in certain malignancies and whose inhibition could result in apoptosis.^{31, 32} However, the IC₅₀ of **10d** and **14e** were 5840 ± 740 nM and 3680 ± 800 nM respectively suggesting that HDAC2 is not likely a direct target.

Intracellular target validation

The data presented above showed that the lead 3HPT compounds possess HDAC6 inhibition activities (Table 2); we nevertheless sought to confirm the involvement of HDAC6 inhibition in the mechanism of action of compounds **10d** and **14e**. Using Western blotting, we examined the level of tubulin acetylation, a common marker for intracellular HDAC 6 activity, in LNCaP cells following exposure to **10d**, **14e**.³³ We used SAHA as a positive control in this experiment. We observed that **10d** and **14e** increased tubulin acetylation but to a lesser extent than SAHA (Figure 4). These data propose that lead compounds **10d** and **14e** may derive their anti-proliferative effects from an HDAC6-dependent pathway.

Conclusion

We have previously reported 3HPT as an advantageous ZBG for HDAC inhibition. Early SAR studies led to aryl- and diaryl-3HPT compounds that are devoid of HDAC1 inhibition activity but possess inhibitory activity against HDAC6 or HDAC8.⁹ Herein, we have delineated the depth of the SAR of 3HPT-derived HDACi. The current efforts resulted in two lead compounds, **10d** and **14e**, demonstrating potent HDAC6 and HDAC8 activities without targeting HDAC1. Additionally, these new HDACi's enhanced tubulin acetylation in LNCaP cell line and possessed anti-proliferative activities against various cancer cell lines including Jurkat J-γ1 for which SAHA, Tubastatin A and the previously disclosed 3HPT-derived HDACi were inactive.

Experimental

Materials and methods

Bromoalkanoic acid, benzyl bromide, 4-bromobenzylbromide, 4-(bromomethyl)-1,1'-biphenyl, 3-methoxy-2(1H)-pyridone, propargyl bromide, phenylacetylene and representative boronic acids were purchased from either Sigma-Aldrich or Alfa-Aesar. Alkynes **16** and **18** that we could not obtain from commercial sources were synthesized using the Bestmann-Ohira reagent as described before (See Supplementary info).^{34, 10, 35} Anhydrous solvents and other reagents were purchased and used without further purification. Analtech silica gel plates (60 F₂₅₄) were used for analytical TLC, and Analtech preparative TLC plates (UV 254, 2000 Lm) were used for purification. UV light was used to examine the spots. Silica gel (200–400 Mesh) was used in column chromatography. NMR spectra were recorded on a Varian-Gemini 400 magnetic resonance spectrometer. ¹H NMR spectra were recorded in parts per million (ppm) relative to the peak of CDCl₃, (7.24 ppm), CD₃OD (3.31 ppm), or DMSO-*d*₆ (2.49 ppm). ¹³C spectra were recorded relative to the central peak of the CDCl₃ triplet (77.0 ppm), CD₃OD (49.0 ppm), or the DMSO-*d*₆ septet (39.7 ppm), and were recorded with complete heterodecoupling. Multiplicities are described using the abbreviation s, singlet; d, doublet, t, triplet; q, quartet; m, multiplet; and app,

apparent. High-resolution mass spectra were recorded at the Georgia Institute of Technology mass spectrometry facility in Atlanta. All final 3HPT-based compounds were established to be > 95% pure using HPLC. These HPLC analyses were done on a Beckman Coulter instrument with a Phenomenex RP C-18 column (250 mm × 4.6 mm), using water (solvent A) and acetonitrile (solvent B) gradient, starting from 40% to 80% of B over 20 min and constant at 80% for 5 min. The flow rate was 1mL/min and detection was at 379 nm. DU-145, LNCaP, Jurkat J.gam1 were obtained from ATCC (Manassas, VA, USA), Jurkat E6-1 cell line was kindly donated by Dr. John McDonald and grown on recommended medium supplemented with 10% fetal bovine serum (Global Cell Solutions, Charlottesville, VA, USA) and 1% pen/Strep (Cellgro, Manassas, VA) at 37°C in an incubator with 5% CO₂. Mouse anti-acetylated α -Tubulin antibody was obtained from Invitrogen (Life Technologies, Grand Island, NY, USA), rabbit anti-actin, rabbit anti-tubulin α antibodies and Tubastatin A were purchased from Sigma-Aldrich (St. Louis, MO, USA). Secondary antibodies, goat anti-rabbit conjugated to IRDye680 and goat anti-mouse conjugated to IRDye800 were purchased from LI-COR Biosciences (Lincoln, NE, USA). The CellTiter 96 AQueous One Solution Cell Proliferation assay (MTS) kit was purchased from Promega (Madison, WI, USA).

Histone deacetylase inhibition—The HDAC activity in presence of various compounds was assessed by SAMDI mass spectrometry. As a label-free technique, SAMDI is compatible with a broad range of native peptide substrates without requiring potentially disruptive fluorophores. To obtain IC₅₀ values, we incubated isoform-optimized substrates (50 μ M, detailed below) with enzyme (250nM, detailed below) and inhibitor (at concentrations ranging from 10nM to 1.0mM), in HDAC buffer (25.0 mM Tris-HCl pH 8.0, 140 mM NaCl, 3.0 mM KCl, 1.0 mM MgCl₂, .1 mg/mL BSA) in 96-well microtiter plates (60 min, 37°C). Solution-phase deacetylation reactions were quenched with trichostatin A (TSA) and transferred to SAMDI plates to immobilize the substrate components. SAMDI plates were composed of an array of self-assembled monolayers (SAMs) presenting maleimide in standard 384-well format for high-throughput handling capability. Following immobilization, plates were washed to remove buffer constituents, enzyme, inhibitor, and any unbound substrate and analyzed by MALDI mass spectrometry using automated protocols.¹⁷ Deacetylation yields in each triplicate sample were determined from the integrated peak intensities of the molecular ions for the substrate and the deacetylated product ion by taking the ratio of the former over the sum of both. Yields were plotted with respect to inhibitor concentration and fitted to obtain IC₅₀ values for each isoform-inhibitor pair.

Isoform-optimized substrates were prepared by traditional FMOC solid phase peptide synthesis (reagents supplied by Anaspec) and purified by semi preparative HPLC on a reverse phase C18 column (Waters). The peptide of sequence GRK^{ac}FGC was prepared for HDAC1 and HDAC8 experiments, while the peptide of sequence GRK^{ac}YGC was prepared for HDAC6 and HDAC2 experiments. Isoform preference for the indicated substrates was determined by earlier studies on peptide arrays.³⁶

HDAC1, HDAC6, and HDAC2 were purchased from BPS Biosciences. The catalytic domain of HDAC8 was expressed as previously reported.³⁶ Briefly, an amplicon was

prepared by PCR with the following primers: forward 5'-3' TATTCTCGAGGACCACATGCTTCA and reverse 5'-3' ATAAGCTAGCATGGAGGAGCCGGA. A pET21a construct bearing the genetic insert between NheI and XhoI restriction sites was transformed into *E. coli* BL21(DE3) (Lucigen) and expressed by standard protocols. Following purification by affinity chromatography, the His-tagged enzyme-containing fractions were purified by FPLC (AKTA) on a superdex size exclusion column (GE), spin concentrated, and stored at -80°C in HDAC buffer with 10% glycerol.

Molecular docking analysis—The docking studies were performed as previously reported with Autodock Vina through PyRx.^{37, 38} Following the 3D energy minimization of the ligand by ChemBioDraw 3D, the docking was run in a 25 Å cubic space encompassing the active site, the binding pocket and its surrounding.

Cell viability assay—DU-145 and Vero cells were maintained in EMEM and DMEM respectively supplemented with 10 % FBS and 1% pen/strep while all other cell lines were maintained in RPMI 1640 supplemented with 10 % FBS and 1% pen/strep. DU-145, Vero and LNCaP cells were incubated on a 96-wells plate for 24 hours prior to a 72 hours drug treatment while Jurkat and Jurkat J.gammal cells were incubated in media containing the various compounds for 72 hours. Cell viability was measured using the MTS assay according to manufacturer protocol. The DMSO concentration in the cell media during the cell viability experiment was maintained at 0.1%.

Western blot analysis for tubulin acetylation—LNCaP cells were plated for 24 hours and treated with various concentrations of compounds for 4 hours. The cells were washed with PBS buffer and resuspended in CellLytic™ buffer containing a cocktail of protease inhibitor (Sigma-Aldrich, St. Louis, MO, USA). Following quantification through a Bradford protein assay, equal amount of protein was loaded onto an SDS-page gel (Bio-Rad, Hercules, CA, USA) and resolved by electrophoresis at a constant voltage of 100V for 2 hours. The gel was transfer onto a nitrocellulose membrane and probed for acetylated tubulin, tubulin and actin as loading control.

MMP assay—MMP inhibitor profiling kit was purchased from Enzo Life Sciences (Farmingdale, NY). The selection of the MMP tested was guided by the reported anti-proliferative of AG3340, a MMP inhibitor selective for isoforms 1, 2, 3, 7, 9, 13, 14.²⁹ Each MMP was incubated with the inhibitor (10µM) for 30 minutes at 37°C in the assay buffer. The OmniMMP™ fluorogenic substrate peptide was added and the reaction was allowed to proceed at 37°C for 30 minutes. The fluorescence was measured using a fluorescence plate reader with excitation at 328 nm and emission at 420nm.³⁹

Statistical analysis—The values reported as mean ± standard deviation from at least 2 independent triplicate experiments. A student's t-test was performed in Excel, and results with p value less than 5% were considered statistically different.

Representative Procedure for Conversion of Azidoalkanol to azidoalkyl methanesulfonate 5. Synthesis of 2-Azidoethyl methanesulfonate (5a)—To a

solution of compound 2- azidoethanol (1.00 g, 11.49 mmol) in THF (25 mL) and triethylamine (Et₃N) (2.418 mL, 17.24 mmol) was added mesyl chloride (1.328 mL, 17.24 mmol) at 0 °C, and the mixture was allowed to warm to room temperature. Stirring continued for 3 h, during which TLC revealed a quantitative conversion into a higher *R_f* product. CH₂Cl₂ (70 mL) and saturated sodium bicarbonate (50 mL) were added, and the two layers were separated. The organic layer was washed with sodium bicarbonate (2 × 50 mL), saturated brine (45 mL) and dried over Na₂SO₄. Solvent was evaporated off to give crude compound **5a** (1.70 g) as a colorless oil, which was used for next step without further purification. A similar procedure was used for the synthesis of **5b** through **5f**.

Representative procedure for synthesis of 1-(2-azidoalkyl)-3-methoxy pyridin-2-ones. Synthesis of 6 - 1-(2-Azidoethyl)-3-methoxy pyridin-2-one (6a)—A mixture of 3- methoxy pyridin-2-one (0.40 g, 3.20 mmol), **5a** (0.79 g, 4.80 mmol), and K₂CO₃ (1.32 g, 9.60 mmol) in THF was stirred at refluxing condition overnight. The reaction mixture was cooled down to room temperature, partitioned between CH₂Cl₂ (60 mL) and water (50 mL) and the two layers were separated. The organic layer was washed with water (2 × 35 mL), brine (1 × 30 mL), dried on Na₂SO₄ and evaporated *in vacuo*. The crude product was purified by flash chromatography eluting with a step gradient of acetone in CH₂Cl₂ (max 15%) to give 0.34 g of **6a** (55%) as colorless oil. ¹H NMR (400 MHz, CDCl₃) δ 6.77 (dd, *J* = 6.9, 1.6 Hz, 1H), 6.48 (dd, *J* = 7.5, 1.6 Hz, 1H), 5.96 (m, 1H), 3.91 (m, 2H), 3.62 (s, 3H), 3.52 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 157.39, 149.41, 128.61, 112.16, 104.37, 55.36, 49.00, 48.91. HRMS (EI) calcd for C₈H₁₀N₄O₂ [M]⁺ 194.0804 found 194.0816.

1-(3-Azidopropyl)-3-benzyloxypyridin-2-one (6b)—Reaction of 3-benzyloxypyridin-2-one (0.40 g, 1.99 mmol) and **5b** (0.53 g, 2.98 mmol) within 16 h as described for synthesis of **6a** gave compound **6b** (0.30 g, 53%) as a colorless oil. ¹H NMR (300 MHz, CDCl₃) δ 7.28 (m, 5H), 6.84 (dd, *J* = 6.9, 1.6 Hz, 1H), 6.60 (dd, *J* = 7.4, 1.5 Hz, 1H), 5.98 (t, *J* = 7.1 Hz, 1H), 4.96 (s, 2H), 3.97 (t, *J* = 6.8 Hz, 2H), 3.28 (t, *J* = 6.5 Hz, 2H), 1.98 (p, *J* = 6.7 Hz, 2H). ¹³C NMR (75 MHz, CDCl₃) δ 157.78, 148.65, 135.93, 128.85, 128.24, 127.69, 127.03, 115.16, 104.53, 70.39, 48.14, 47.06, 27.66. HRMS (EI) calcd for C₁₅H₁₆N₄O₂ [M]⁺ 284.1273 found 284.1271.

1-(4-Azidobutyl)-3-benzyloxypyridin-2-one (6c)—Reaction of 3-benzyloxypyridin-2-one (0.40 g, 1.99 mmol) and **5c** (0.58 g, 2.98 mmol) within 16 h as described for synthesis of **6a** gave compound **6c** (0.36 g, 62%) as a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ 7.22 (m, 5H), 6.78 (dd, *J* = 6.9, 1.7 Hz, 1H), 6.54 (dd, *J* = 7.4, 1.7 Hz, 1H), 5.91 (m, 1H), 4.96 (s, 2H), 3.85 (t, *J* = 7.2 Hz, 2H), 3.17 (t, *J* = 6.8 Hz, 2H), 1.71 (m, 2H), 1.48 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 157.52, 148.27, 135.78, 128.37, 127.98, 127.42, 126.82, 114.92, 104.32, 70.09, 50.43, 48.46, 25.83, 25.42. HRMS (EI) calcd for C₁₆H₁₈N₄O₂ [M]⁺ 298.1430 found 298.1446.

1-(5-Azidopentyl)-3-benzyloxypyridin-2-one (6d)—Reaction of 3-benzyloxypyridin-2-one (0.50 g, 2.49 mmol) and **5d** (0.62 g, 2.98 mmol) within 16 h as described for synthesis of **6a** gave compound **6d** (0.49 g, 63%) as a colorless oil. ¹H NMR

(400 MHz, CDCl_3) δ 7.35 (d, J = 8.0 Hz, 2H), 7.23 (m, 3H), 6.81 (m, 1H), 6.56 (m, 1H), 5.94 (m, 1H), 5.01 (s, 2H), 3.85 (m, 2H), 3.18 (m, 2H), 1.71 (m, 2H), 1.54 (m, 2H), 1.32 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ 157.68, 148.47, 135.98, 128.60, 128.12, 127.53, 126.93, 115.09, 104.32, 70.27, 50.77, 49.21, 28.19, 28.06, 23.37. HRMS (EI) calcd for $\text{C}_{17}\text{H}_{20}\text{N}_4\text{O}_2$ $[\text{M}]^+$ 312.1586 found 312.1589.

1-(6-Azidoethyl)-3-benzyloxypyridin-2-one (6e)—Reaction of 3-benzyloxypyridin-2-one (0.50 g, 2.49 mmol) and **5e** (0.66 g, 2.98 mmol) within 16 h as described for synthesis of **6a** gave compound **6e** (0.48 g, 60%) as a colorless oil. ^1H NMR (400 MHz, CDCl_3) δ 7.15 (m, 5H), 6.65 (dd, J = 7.4, 1.8 Hz, 1H), 6.45 (dd, J = 7.4, 1.8 Hz, 1H), 5.81 (t, J = 7.1 Hz, 1H), 4.92 (s, 2H), 3.76 (t, J = 6.8 Hz, 2H), 3.05 (t, J = 6.8 Hz, 2H), 1.58 (m, 2H), 1.40 (m, 2H), 1.90 (m, 4H). ^{13}C NMR (100 MHz, CDCl_3) δ 157.55, 148.25, 135.95, 128.60, 128.10, 126.95, 126.50, 115.05, 104.90, 70.50, 51.05, 49.20, 28.65, 28.50, 26.20, 25.80. HRMS (FAB) calcd for $\text{C}_{18}\text{H}_{23}\text{N}_4\text{O}_2$ $[\text{M}+\text{H}]^+$ 327.1821 found 327.1848.

1-(7-Azidoheptyl)-3-benzyloxypyridin-2-one (6f)—Reaction of 3-benzyloxypyridin-2-one (0.79 g, 3.38 mmol) and **5f** (0.81 g, 4.05 mmol) within 16 h as described for synthesis of **6a** gave compound **6f** (0.55 g, 48%) as a colorless oil. ^1H NMR (400 MHz, CDCl_3) δ 7.42 (dd, J = 7.8, 1.0 Hz, 2H), 7.31 (m, 3H), 6.85 (dd, J = 6.9, 1.7 Hz, 1H), 6.61 (dd, J = 7.4, 1.7 Hz, 1H), 5.99 (m, 1H), 5.09 (s, 2H), 3.93 (m, 2H), 3.23 (t, J = 6.9 Hz, 2H), 1.74 (m, 2H), 1.55 (m, 2H), 1.34 (m, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ 158.32, 149.19, 136.63, 129.15, 128.74, 128.13, 127.52, 115.66, 104.69, 94.66, 70.96, 51.62, 50.03, 29.22, 28.98, 26.77, 26.72.

Representative procedure for synthesis of 7. Synthesis of 1-

Phenyltriazolyethyl-3-methoxypyridin-2-one (7a)—Phenylacetylene (0.17 g, 1.67 mmol) and **6a** (0.27 g, 1.36 mmol) were dissolved in anhydrous THF (10 mL) and stirred under argon at room temperature. Copper (I) iodide (0.01 g, 0.07 mmol) and Hunig's base (0.1 mL) were added to the reaction mixture, and stirring continued for 4 h. The reaction mixture was diluted with CH_2Cl_2 (40 mL) and washed with 1:4 NH_4OH /saturated NH_4Cl (3 $\times$ 30 mL) and saturated NH_4Cl (30 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was triturated with hexanes to give 366 mg (91%) of white solid **7a**. ^1H NMR (400 MHz, CDCl_3) δ 7.68 (s, 1H), 7.47 (d, J = 7.5 Hz, 2H), 7.11 (m, 3H), 6.43 (d, J = 7.4 Hz, 1H), 6.35 (dd, J = 6.8, 0.9 Hz, 1H), 5.78 (t, J = 7.2 Hz, 1H), 4.57 (t, J = 5.7 Hz, 2H), 4.25 (t, J = 5.6 Hz, 2H), 3.53 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 157.70, 149.08, 147.21, 129.40, 128.30, 128.08, 127.80, 125.00, 120.91, 113.02, 105.44, 55.15, 49.72, 47.44. HRMS (EI) calcd for $\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_2$ $[\text{M}]^+$ 296.1273 found 296.1268.

1-Phenyltriazolylpropyl-3-benzyloxypyridin-2-one (7b)—Reaction of phenylacetylene (0.13 g, 1.25 mmol) and **6b** (0.30 g, 1.04 mmol) within 4 h as described for synthesis of **7a** gave compound **7b** (0.31 g, 77%) as a white solid. ^1H NMR (400 MHz, CDCl_3) δ 8.00 (s, 1H), 7.77 (d, J = 7.3 Hz, 2H), 7.27 (m, 8H), 6.88 (d, J = 5.7 Hz, 1H), 6.57 (d, J = 6.4 Hz, 1H), 5.95 (t, J = 7.1 Hz, 1H), 4.99 (s, 2H), 4.34 (t, J = 6.4 Hz, 2H), 3.94 (t, J = 6.4 Hz, 2H), 2.32 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ 157.92, 148.47, 147.30, 135.73, 130.26, 128.81, 128.49, 128.22, 127.76, 127.71, 127.01, 125.33, 120.16, 114.95,

104.83, 70.33, 47.00, 46.48, 29.30. HRMS (EI) calcd for $C_{23}H_{22}N_4O_2$ $[M]^+$ 386.1743 found 386.1734.

1-Phenyltriazolylbutyl-3-benzyloxypyridin-2-one (7c)—Reaction of phenylacetylene (0.12 g, 1.18 mmol) and **6c** (0.30 g, 1.01 mmol) within 4 h as described for synthesis of **7a** gave compound **7c** (0.31 g, 77%) as a white solid. 1H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.76 (m, 2H), 7.26 (m, 8H), 6.76 (dd, $J = 6.9, 1.5$ Hz, 1H), 6.56 (dd, $J = 7.4, 1.5$ Hz, 1H), 5.91 (t, $J = 7.1$ Hz, 1H), 4.99 (s, 2H), 4.31 (m, 2H), 3.89 (t, $J = 7.1$ Hz, 2H), 1.85 (m, 2H), 1.67 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.71, 148.38, 147.20, 135.83, 130.29, 128.45, 128.41, 128.16, 127.68, 127.63, 126.96, 125.25, 119.87, 114.99, 104.60, 70.26, 49.11, 48.02, 26.78, 25.65. HRMS (EI) calcd for $C_{24}H_{24}N_4O_2$ $[M]^+$ 400.1899 found 400.1888.

1-Phenyltriazolylpentyl-3-benzyloxypyridin-2-one (7d)—Reaction of phenylacetylene (0.04 g, 0.39 mmol) and **6d** (0.10 g, 0.32 mmol) within 4 h as described for synthesis of **7a** gave compound **7d** (0.09 g, 72%) as a white solid. 1H NMR (400 MHz, $CDCl_3$) δ 7.78 (m, 3H), 7.30 (m, 8H), 6.79 (d, $J = 6.8$ Hz, 1H), 6.57 (d, $J = 7.3$ Hz, 1H), 5.94 (t, $J = 7.1$ Hz, 1H), 5.02 (s, 2H), 4.31 (t, $J = 7.0$ Hz, 2H), 3.87 (t, $J = 7.2$ Hz, 2H), 1.91 (m, 2H), 1.74 (m, 2H), 1.30 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.80, 148.57, 147.39, 136.02, 130.44, 128.64, 128.56, 128.27, 127.81, 127.71, 127.05, 125.40, 119.57, 115.12, 104.49, 70.40, 49.71, 49.06, 29.42, 28.03, 23.05.

1-Phenyltriazolylhexyl-3-benzyloxypyridin-2-one (7e)—Reaction of phenylacetylene (0.08 g, 0.78 mmol) and **6e** (0.21 g, 0.65 mmol) within 4 h as described for synthesis of **7a** gave compound **7e** (0.16 g, 58%) as a white solid. NMR (400 MHz, $CDCl_3$) δ 7.66 (m, 3H), 7.20 (m, 8H), 6.68 (dd, $J = 6.9, 1.5$ Hz, 1H), 6.46 (dd, $J = 7.4, 1.5$ Hz, 1H), 5.82 (t, $J = 7.1$ Hz, 1H), 4.92 (s, 2H), 4.18 (t, $J = 6.8$ Hz, 2H), 3.75 (t, $J = 6.8$ Hz, 2H), 1.76 (m, 2H), 1.56 (m, 2H), 1.19 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.64, 148.46, 147.27, 135.95, 130.36, 128.57, 128.48, 128.19, 127.72, 127.60, 126.96, 125.32, 119.37, 115.02, 104.37, 70.45, 50.03, 49.35, 29.90, 28.66, 25.87, 25.78. HRMS (FAB) calcd for $C_{26}H_{29}N_4O_2$ $[M+H]^+$ 429.2290 found 429.2291.

1-Phenyltriazolylheptyl-3-benzyloxypyridin-2-one (7f)—Reaction of phenylacetylene (0.09 g, 0.88 mmol) and **6f** (0.25 g, 0.74 mmol) within 4 h as described for synthesis of **7a** gave compound **7f** (0.23 g, 70%) as a white solid. 1H NMR (400 MHz, $CDCl_3$) δ 7.79 (m, 3H), 7.31 (m, 8H), 6.83 (dd, $J = 6.9, 1.7$ Hz, 1H), 6.60 (dd, $J = 7.4, 1.7$ Hz, 1H), 5.97 (t, $J = 7.1$ Hz, 1H), 5.07 (s, 2H), 4.35 (t, $J = 7.1$ Hz, 2H), 3.91 (m, 2H), 1.88 (m, 2H), 1.71 (m, 2H), 1.32 (d, $J = 10.0$ Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.31, 149.13, 147.92, 136.57, 130.93, 129.15, 129.04, 128.74, 128.28, 128.15, 127.51, 125.90, 125.86, 119.75, 115.60, 104.79, 70.92, 50.52, 49.90, 30.42, 29.13, 28.72, 26.53, 26.46. HRMS (FAB) calcd for $C_{27}H_{31}N_4O_2$ $[M+H]^+$ 443.2447 found 443.2494.

1-Phenyltriazolylethyl-3-hydroxypyridin-2-one (8a)—To a solution of **7a** (0.10 g, 0.34 mmol) in dry CH_2Cl_2 (8 mL) was slowly added 1M BBr_3 (1.2 equiv) at $-30^\circ C$ under argon atmosphere. The reaction mixture was stirred for 48 h at room temperature. The

mixture was again cooled to $-30\text{ }^{\circ}\text{C}$ and the MeOH (5 mL) was slowly added to the mixture. After evaporation of solvent, the residue was adjusted to pH 7 with 1M NaOH and then extracted with CHCl_3 (30 mL $\times$ 3). The combined organic layer was dried over Na_2SO_4 to give 0.08 g (83%) of **8a** as slightly brownish solid without any further purification required. ^1H NMR (400 MHz, DMSO) δ 9.11 (s, 1H), 8.51 (s, 1H), 7.78 (d, $J = 7.3$ Hz, 2H), 7.43 (t, $J = 7.6$ Hz, 2H), 7.31 (t, $J = 7.3$ Hz, 1H), 6.75 (d, $J = 5.9$ Hz, 1H), 6.64 (d, $J = 6.0$ Hz, 1H), 5.95 (t, $J = 7.0$ Hz, 1H), 4.76 (t, $J = 5.5$ Hz, 2H), 4.42 (t, $J = 5.6$ Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ 158.25, 147.17, 146.77, 131.11, 129.34, 128.41, 128.31, 125.54, 122.23, 115.38, 105.76, 49.42, 48.33. HRMS (EI) calcd for $\text{C}_{15}\text{H}_{14}\text{N}_4\text{O}_2$ $[\text{M}]^+$ 282.1117 found 282.1120.

1-Phenyltriazolylpropyl-3-hydroxypyridin-2-one (8b)—To a solution of **7b** (0.29 g, 0.75 mmol) in CH_2Cl_2 :EtOAc:MeOH (2:2:1, 10 mL) was added 10% Pd on carbon (15 mg). Reaction was stirred under balloon hydrogen pressure for 5 h. Reaction mixture was filtered and solvent was evaporated. Column chromatography eluting with a step gradient of acetone in CH_2Cl_2 (max 20%) gave pure **8b** (0.19 g, 85%) as a slightly brownish solid. ^1H NMR (400 MHz, DMSO) δ 9.03 (s, 1H), 8.62 (s, 1H), 7.82 (d, $J = 7.6$ Hz, 2H), 7.38 (m, 3H), 7.15 (d, $J = 6.6$ Hz, 1H), 6.68 (d, $J = 6.4$ Hz, 1H), 6.11 (t, $J = 6.7$ Hz, 1H), 4.43 (t, $J = 6.4$ Hz, 2H), 3.99 (m, 2H), 2.27 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ 147.49, 129.71, 128.52, 127.99, 125.29, 120.42, 107.98, 66.61, 47.10, 29.27. HRMS (EI) calcd for $\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_2$ $[\text{M}]^+$ 296.1273 found 296.1274.

1-Phenyltriazolylbutyl-3-hydroxypyridin-2-one (8c)—Reaction of **7c** (0.25 g, 0.62 mmol) in anhydrous THF as described for synthesis of **8b** gave **8c** (0.12 g, 63%). ^1H NMR (400 MHz, CDCl_3) δ 7.79 (m, 3H), 7.41 (t, $J = 7.6$ Hz, 2H), 7.32 (t, $J = 7.4$ Hz, 1H), 6.76 (m, 3H), 6.13 (t, $J = 7.2$ Hz, 1H), 4.45 (t, $J = 6.8$ Hz, 2H), 4.02 (t, $J = 7.1$ Hz, 2H), 1.99 (m, 2H), 1.82 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ 158.47, 147.63, 146.62, 130.41, 128.69, 127.99, 126.60, 125.53, 119.80, 114.15, 107.10, 49.38, 48.57, 27.02, 26.01. HRMS (EI) calcd for $\text{C}_{17}\text{H}_{18}\text{N}_4\text{O}_2$ $[\text{M}]^+$ 310.1430 found 310.1425.

1-Phenyltriazolylpentyl-3-hydroxypyridin-2-one (8d)—Reaction of **7d** (0.09 g, 0.20 mmol) in anhydrous THF as described for synthesis of **8b** gave **8d** (0.05 g, 77%). ^1H NMR (400 MHz, DMSO) δ 8.56 (s, 1H), 7.82 (d, $J = 8.1$ Hz, 2H), 7.43 (t, $J = 7.7$ Hz, 2H), 7.32 (t, $J = 7.4$ Hz, 1H), 7.10 (dd, $J = 6.8, 1.6$ Hz, 1H), 6.65 (dd, $J = 7.2, 1.5$ Hz, 1H), 6.05 (t, $J = 7.0$ Hz, 1H), 4.38 (t, $J = 7.0$ Hz, 2H), 3.89 (t, $J = 7.2$ Hz, 2H), 1.89 (m, 2H), 1.67 (m, 2H), 1.25 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ 158.44, 147.67, 146.61, 130.51, 128.72, 128.01, 126.71, 125.57, 119.54, 113.81, 106.86, 49.86, 49.38, 29.58, 28.31, 23.19. HRMS (FAB) calcd for $\text{C}_{18}\text{H}_{21}\text{N}_4\text{O}_2$ $[\text{M}+\text{H}]^+$ 325.1664 found 325.1683.

1-Phenyltriazolylhexyl-3-hydroxypyridin-2-one (8e)—Reaction of **7e** (0.15 g, 0.35 mmol) in anhydrous THF as described for synthesis of **8b** gave **8e** (0.081 g, 69%). ^1H NMR (400 MHz, CDCl_3) δ 7.81 (m, 3H), 7.42 (m, 2H), 7.32 (m, 1H), 6.77 (m, 2H), 6.12 (t, $J = 7.1$ Hz, 1H), 4.38 (t, $J = 7.1$ Hz, 2H), 3.95 (m, 2H), 1.95 (m, 2H), 1.76 (d, $J = 7.0$ Hz, 2H), 1.39 (m, 4H). ^{13}C NMR (100 MHz, CDCl_3) δ 158.20, 147.25, 146.40, 130.30, 128.60,

127.85, 126.65, 125.40, 119.65, 113.90, 106.80, 50.20, 49.60, 30.05, 28.95, 26.00, 25.90. HRMS (FAB) calcd for $C_{19}H_{23}N_4O_2$ $[M+H]^+$ 339.1821 found 339.1814.

1-Phenyltriazolylheptyl-3-hydroxypyridin-2-one (8f)—Reaction of **7f** (0.22 g, 0.50 mmol) in anhydrous THF as described for synthesis of **8b** gave **8f** (0.13 g, 74%). 1H NMR (400 MHz, DMSO) δ 8.91 (s, 1H), 8.57 (s, 1H), 7.82 (d, J = 7.4 Hz, 2H), 7.42 (t, J = 7.6 Hz, 2H), 7.31 (t, J = 7.3 Hz, 1H), 7.09 (d, J = 6.6 Hz, 1H), 6.64 (d, J = 7.0 Hz, 1H), 6.04 (t, J = 6.9 Hz, 1H), 4.36 (t, J = 7.0 Hz, 2H), 3.86 (t, J = 7.2 Hz, 2H), 1.83 (m, 2H), 1.61 (m, 2H), 1.26 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.80, 147.89, 147.00, 130.88, 129.05, 128.30, 127.12, 125.87, 119.83, 114.13, 107.10, 50.48, 50.05, 30.37, 29.20, 28.67, 26.46, 26.42. HRMS (FAB) calcd for $C_{20}H_{25}N_4O_2$ $[M+H]^+$ 353.1977 found 353.1992.

1-Phenyltriazolylethyl-3-methoxypyridin-2-thione (9)—To a stirring solution of Lawesson's reagent (0.08 g, 0.19 mmol) in toluene (10 mL), was added starting material **7a** (0.10g, 0.33 mmol) and the reaction mixture was heated at refluxing temperature overnight. The reaction mixture was cooled down to room temperature and solvent was evaporated under reduced pressure. The crude product was purified by preparative-TLC, eluting with $CHCl_3$:Acetone:EtOH (10:1:0.2) to give **9** (0.07 g, 68%) as a yellow solid. 1H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, J = 7.4 Hz, 2H), 7.56 (s, 1H), 7.35 (t, J = 7.5 Hz, 2H), 7.28 (m, 1H), 6.97 (d, J = 6.6 Hz, 1H), 6.62 (d, J = 7.8 Hz, 1H), 6.37 (m, 1H), 5.13 (t, J = 5.6 Hz, 2H), 5.03 (t, J = 5.6 Hz, 2H), 3.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.92, 158.94, 147.52, 132.68, 129.92, 128.69, 128.14, 125.43, 120.96, 111.72, 110.22, 56.72, 56.63, 46.38. HRMS (EI) calcd for $C_{16}H_{16}N_4OS$ $[M]^+$ 312.1045 found 312.1039.

1-Phenyltriazolylethyl-3-hydroxypyridin-2-thione (10a)—To a solution of **9** (0.06 g, 0.19 mmol) in dry CH_2Cl_2 (8 mL) at $-30^\circ C$ was slowly added 1M BBr_3 in CH_2Cl_2 (0.22 mmol) under argon atmosphere and the reaction mixture was subsequently stirred for 48 h at room temperature. The mixture was cooled back to $-30^\circ C$ and MeOH (5 mL) was slowly added. The solvent was evaporated off, and the residue was adjusted to pH 7 with aqueous 1M NaOH and then extracted with $CHCl_3$ (3×30 mL). The combined organic layer was dried over Na_2SO_4 and solvent evaporated *in vacuo* to yield compound **10a** (0.06 g, 93%) as a dark violet solid. Retention time 8.1 min. 1H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.73 (d, J = 7.3 Hz, 2H), 7.51 (s, 1H), 7.40 (t, J = 7.5 Hz, 2H), 7.33 (t, J = 7.3 Hz, 1H), 6.95 (dd, J = 9.2, 7.2 Hz, 2H), 6.46 (m, 1H), 5.08 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.47, 155.10, 147.75, 132.00, 129.82, 128.77, 128.30, 125.53, 120.90, 113.66, 112.61, 57.45, 46.41. HRMS (EI) calcd for $C_{15}H_{14}N_4OS$ $[M]^+$ 298.0888 found 298.0888.

1-Phenyltriazolylpropyl-3-hydroxypyridin-2-thione (10b)—Compound **8b** (0.06 g, 0.20 mmol) was ground together with P_4S_{10} (0.05g, 0.11 mmol) in mortar and pestle to form grey powder. The powder was stirred under argon in a flask fitted with condenser and heated to $175^\circ C$ for 2 h. The reaction flask was covered with aluminium foil during reaction. After 2h of reaction, the flask was cooled down to room temperature, 10% MeOH in CH_2Cl_2 (25 mL) was added and stirring continued for another 15 min. The reaction mixture then washed with water (2×30 mL), the organic layer dried with Na_2SO_4 and solvent evaporated *in vacuo*. The crude product was purified by preparative TLC eluting with 8% MeOH in

CH₂Cl₂ to give **10b** (0.04 g, 64%) as olive green solid. Retention time 8.2 min. ¹H NMR (400 MHz, CDCl₃) δ 8.47 (s, 1H), 7.82 (m, 3H), 7.50 (d, *J* = 6.5 Hz, 1H), 7.43 (t, *J* = 7.5 Hz, 2H), 7.34 (t, *J* = 7.4 Hz, 1H), 6.97 (m, 1H), 6.66 (m, 1H), 4.62 (t, *J* = 6.9 Hz, 2H), 4.48 (m, 2H), 2.65 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 168.59, 155.22, 148.03, 131.85, 130.17, 128.84, 128.29, 125.61, 119.84, 113.87, 112.28, 54.99, 46.95, 28.02. HRMS (EI) calcd for C₁₆H₁₆N₄OS [M]⁺ 312.1045 found 312.1060.

1-Phenyltriazolylbutyl-3-hydroxypyridin-2-thione (10c)—Reaction of **8c** (0.06 g, 0.18 mmol) and P₄S₁₀ (0.05 g, 0.11 mmol) under neat conditions as described for synthesis of **10b** gave compound **10c** (0.03 g, 51%) as a olive green solid. Retention time 8.7 min. ¹H NMR (400 MHz, CDCl₃) δ 8.50 (s, 1H), 7.81 (m, 3H), 7.36 (m, 4H), 6.94 (d, *J* = 7.6 Hz, 1H), 6.62 (t, *J* = 6.8 Hz, 1H), 4.54 (t, *J* = 7.6 Hz, 2H), 4.46 (t, *J* = 6.4 Hz, 2H), 2.03 (m, 4H). ¹³C NMR (100 MHz, CDCl₃) δ 168.51, 155.08, 147.73, 131.02, 130.36, 128.79, 128.13, 125.58, 119.91, 113.87, 112.10, 56.96, 49.41, 27.02, 25.08. HRMS (EI) calcd for C₁₇H₁₈N₄OS [M]⁺ 326.1201 found 326.1200.

1-Phenyltriazolylpentyl-3-hydroxypyridin-2-thione (10d)—Reaction of **8d** (0.03 g, 0.08 mmol) and P₄S₁₀ (0.02 g, 0.05 mmol) under neat conditions as described for synthesis of **10b** gave compound **10d** (0.02 g, 73%) as a olive green solid. Retention time 9.6 min. ¹H NMR (400 MHz, DMSO) δ 8.55 (d, *J* = 2.9 Hz, 2H), 7.83 (t, *J* = 7.8 Hz, 3H), 7.43 (q, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 6.6 Hz, 1H), 7.08 (m, 1H), 6.92 (dd, *J* = 31.0, 6.8 Hz, 1H), 6.81 (m, 1H), 4.51 (m, 2H), 4.41 (t, *J* = 7.0 Hz, 2H), 1.89 (m, 4H), 1.32 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 168.37, 155.10, 147.66, 131.04, 130.46, 128.75, 128.05, 125.57, 119.68, 113.69, 111.96, 57.73, 49.73, 29.47, 27.12, 23.04. HRMS (EI) calcd for C₁₈H₂₀N₄OS [M]⁺ 340.1358 found 340.1364.

1-Phenyltriazolylhexyl-3-hydroxypyridin-2-thione (10e)—Reaction of **8e** (0.18 g, 0.53 mmol) and P₄S₁₀ (0.12 g, 0.27 mmol) under neat conditions as described for synthesis of **10b** gave compound **10e** (0.08 g, 43%) as a olive green semi-solid. Retention time 10.8 min. ¹H NMR (400 MHz, CDCl₃) δ 8.57 (s, 1H), 7.83 (d, *J* = 7.5 Hz, 2H), 7.76 (s, 1H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.32 (m, 2H), 6.94 (m, 1H), 6.72 (m, 1H), 4.49 (m, 2H), 4.40 (t, *J* = 6.9 Hz, 2H), 1.97 (m, 4H), 1.36 (m, 4H). ¹³C NMR (100 MHz, CDCl₃) δ 164.38, 162.90, 147.70, 130.54, 128.76, 128.03, 126.64, 125.61, 119.51, 118.75, 118.29, 59.74, 50.10, 29.91, 27.99, 25.82, 25.65. HRMS (ESI) calcd for C₁₉H₂₃N₄OS [M+H]⁺ 355.1587 found 355.1632.

1-Phenyltriazolylheptyl-3-hydroxypyridin-2-thione (10f)—Reaction of **8f** (0.12 g, 0.35 mmol) and P₄S₁₀ (0.08 g, 0.17 mmol) under neat conditions as described for synthesis of **10b** gave compound **10f** (0.05 g, 43%) as a olive green semi-solid. Retention time 12.1 min. ¹H NMR (400 MHz, DMSO) δ 8.55 (m, 1H), 7.81 (d, *J* = 8.2 Hz, 2H), 7.65 (d, *J* = 6.2 Hz, 1H), 7.42 (m, 2H), 7.30 (m, 1H), 7.02 (m, 1H), 6.85 (m, 1H), 4.50 (m, 2H), 4.36 (t, *J* = 7.1 Hz, 2H), 1.85 (d, *J* = 7.0 Hz, 4H), 1.33 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 147.76, 130.66, 128.76, 130.39, 128.98, 128.80, 128.06, 125.97, 125.67, 119.46, 60.10, 50.25, 30.11, 29.67, 28.33, 26.13, 26.10. HRMS (FAB) calcd for C₂₀H₂₅N₄OS [M+H]⁺ 369.1749 found 369.1762.

1-(5-azidopentyl)-3-methoxypyridin-2-one (11)—3-Methoxypyridin-2-one (1.8g, 14.1 mmol) and 5-azidopentyl methanesulfonate **5d** (3.5g, 16.9mmol) were dissolved in 2:1 (THF:MeOH) and stirred for 48 h at 80°C. Upon completion of the reaction, EtOAc was added and the organic layer was washed with H₂O, brine and dried to yield the crude product (2.42g, 73%). The crude product was washed with petroleum ether yielding (**11**) (1.96g, 59%). ¹H NMR (400 MHz, cdcl₃) δ 6.74 (dd, *J* = 6.9, 1.6 Hz, 1H), 6.46 (dd, *J* = 7.4, 1.6 Hz, 1H), 5.95 (t, *J* = 7.1 Hz, 1H), 3.81 (t, *J* = 7.3 Hz, 2H), 3.64 (s, 3H), 3.11 (t, *J* = 6.8 Hz, 2H), 1.67 – 1.57 (m, 2H), 1.51 – 1.41 (m, 2H), 1.31 – 1.20 (m, 2H). ¹³C NMR (101 MHz, cdcl₃) δ 157.46, 149.66, 127.87, 111.68, 104.34, 55.38, 50.72, 49.00, 28.14, 28.02, 23.28.

3-methoxy-1-(5-(4-(*p*-tolyl)-1H-1,2,3-triazol-1-yl) pentyl)pyridin-2-one (12a)—1-Ethynyl-4- methylbenzene (0.14 g, 1.22 mmol) and **11** (0.24 g, 1.02 mmol) were dissolved in a 1:1 anhydrous THF: DMSO 10 mL and stirred under argon at room temperature. Copper (I) iodide (0.01 g, 0.07 mmol) and Hunig's base (0.1 mL) were then added to the reaction mixture, and stirring continued for 4 h. The reaction mixture was diluted with CH₂Cl₂ (40 mL) and washed with 1:4 NH₄OH/saturated NH₄Cl (3 × 30 mL) and saturated NH₄Cl (30 mL). The organic layer was dried over Na₂SO₄ and concentrated *in vacuo*. The crude product was purified by preparative TLC with 12:1 CH₂Cl₂:MeOH, resulting in **12a** (0.39 g, quantitative yield). ¹H NMR (400 MHz, cdcl₃) δ 7.69 (s, 1H), 7.58 (d, *J* = 8.2 Hz, 2H), 7.07 (d, *J* = 8.4 Hz, 2H), 6.70 (dd, *J* = 6.9, 1.6 Hz, 1H), 6.43 (dd, *J* = 7.4, 1.6 Hz, 1H), 5.92 (t, *J* = 7.1 Hz, 1H), 4.20 (t, *J* = 7.1 Hz, 2H), 3.77 (t, *J* = 7.2 Hz, 2H), 3.62 (s, 3H), 2.22 (s, 3H), 1.87 – 1.72 (m, 2H), 1.68 – 1.52 (m, 2H), 1.29 – 0.89 (m, 2H). ¹³C NMR (101 MHz, cdcl₃) δ 157.77, 149.83, 147.52, 137.70, 129.38, 128.20, 127.80, 125.43, 119.54, 112.10, 104.80, 55.69, 49.81, 49.02, 29.57, 28.17, 23.14, 21.19.

3-methoxy-1-(5-(4-(*m*-tolyl)-1H-1,2,3-triazol-1-yl) pentyl)pyridin-2-one (12b)—1-Ethynyl-3- methylbenzene (0.12 g, 1.05 mmol) and **11** (0.21 g, 0.88 mmol) were reacted as described for the synthesis of **12a** to give compound **12b** (0.3 g, 97%) as a yellow oil. ¹H NMR (400 MHz, cdcl₃) δ 7.74 (s, 1H), 7.59 (s, 1H), 7.52 (d, *J* = 7.7 Hz, 1H), 7.20 (t, *J* = 7.6 Hz, 1H), 7.04 (d, *J* = 7.6 Hz, 1H), 6.74 (dd, *J* = 6.9, 1.7 Hz, 1H), 6.48 (dd, *J* = 7.5, 1.6 Hz, 1H), 6.05 – 5.90 (m, 1H), 4.27 (t, *J* = 7.1 Hz, 2H), 3.83 (t, *J* = 7.2 Hz, 2H), 3.67 (s, 3H), 2.29 (s, 3H), 1.92 – 1.79 (m, 2H), 1.74 – 1.62 (m, 2H), 1.34 – 1.17 (m, 2H). ¹³C NMR (101 MHz, cdcl₃) δ 157.53, 149.61, 147.34, 138.06, 130.18, 128.45, 128.34, 127.88, 125.94, 122.39, 119.49, 111.79, 104.55, 55.43, 49.58, 48.77, 29.31, 27.91, 22.87, 21.07.

3-methoxy-1-(5-(4-(*o*-tolyl)-1H-1,2,3-triazol-1-yl) pentyl)pyridin-2-one (12c)—1-Ethynyl-2- methylbenzene (0.12 g, 1.06 mmol) and **11** (0.21 g, 0.89 mmol) were reacted as described for the synthesis of **12a** to give compound **12c** (0.21 g, 68%). ¹H NMR (400 MHz, CDCl₃) δ 7.72 – 7.62 (m, 1H), 7.61 (s, 1H), 7.16 (d, *J* = 3.1 Hz, 3H), 6.75 (dd, *J* = 6.9, 1.7 Hz, 1H), 6.48 (dd, *J* = 7.5, 1.6 Hz, 1H), 6.04 – 5.91 (m, 1H), 4.30 (t, *J* = 7.1 Hz, 2H), 3.85 (t, *J* = 7.2 Hz, 2H), 3.66 (s, 3H), 2.36 (s, 3H), 1.94 – 1.84 (m, 2H), 1.77 – 1.63 (m, 2H), 1.37 – 1.21 (m, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 157.89, 149.98, 146.89, 135.44, 130.81, 130.01, 128.78, 128.00, 125.99, 121.93, 112.14, 104.87, 55.76, 49.86, 49.12, 31.91, 29.67, 28.25, 23.26, 21.37.

4-(1-(5-(3-methoxy-2-oxopyridin-1(2H)-yl)pentyl)-1H-1, 2, 3-triazol-4-yl)benzonitrile (12d)—4-Ethynylbenzonitrile (0.13 g, 1.05 mmol) and **11** (0.21 g, 0.87 mmol) were reacted as described for the synthesis of **12a** to give compound **12d** (0.30 g, 95%). ¹H NMR (400 MHz, CDCl₃) δ 7.96 (s, 1H), 7.81 (d, *J* = 8.7 Hz, 2H), 7.53 (d, *J* = 8.7 Hz, 2H), 6.73 (dd, *J* = 6.9, 1.7 Hz, 1H), 6.46 (dd, *J* = 7.5, 1.6 Hz, 1H), 5.98 – 5.90 (m, 1H), 4.28 (t, *J* = 7.1 Hz, 2H), 3.80 (t, *J* = 7.2 Hz, 2H), 3.62 (s, 3H), 1.90 – 1.77 (m, 2H), 1.71 – 1.59 (m, 2H), 1.31 – 1.15 (m, 2H). ¹³C NMR (101 MHz, cdcl₃) δ 157.42, 149.48, 145.25, 134.76, 132.15, 127.79, 125.57, 120.99, 118.37, 111.83, 110.57, 104.52, 55.36, 49.63, 48.56, 29.04, 27.79, 22.71.

3-(1-(5-(3-methoxy-2-oxopyridin-1(2H)-yl)pentyl)-1H-1,2,3-triazol-4-yl)benzonitrile (12e)—3-Ethynylbenzonitrile **16** (see supporting info) (0.13 g, 1.05 mmol) and **11** (0.21 g, 0.87 mmol) were reacted as described for the synthesis of **12a** to give compound **12e** (0.31 g, 98%). ¹H NMR (400 MHz, CDCl₃) δ 8.01 – 7.97 (m, 1H), 7.96 (s, 1H), 7.94 (dt, *J* = 7.7, 1.5 Hz, 1H), 7.43 (dt, *J* = 7.7, 1.5 Hz, 1H), 7.38 (t, *J* = 7.5 Hz, 1H), 6.74 (dd, *J* = 6.9, 1.7 Hz, 1H), 6.46 (dd, *J* = 7.5, 1.6 Hz, 1H), 6.02 – 5.83 (m, 1H), 4.29 (t, *J* = 7.1 Hz, 2H), 3.80 (t, *J* = 7.2 Hz, 2H), 3.62 (s, 3H), 1.94 – 1.78 (m, 2H), 1.74 – 1.57 (m, 2H), 1.31 – 1.15 (m, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 157.74, 149.79, 145.29, 132.00, 131.09, 129.67, 129.60, 128.83, 128.14, 120.82, 118.50, 112.63, 112.14, 104.85, 55.68, 49.98, 48.93, 29.43, 28.14, 23.07.

1-(5-(4-(4-(dimethylamino)phenyl)-1H-1,2,3-triazol-1-yl)pentyl)-3-methoxypyridin-2-one (12f)—4-Ethynyl-N,N dimethylaniline (0.15 g, 1.02 mmol) and **11** (0.20 g, 0.85 mmol) were reacted as described for the synthesis of **12a** to give compound **12f** (0.27 g, 84%). ¹H NMR (400 MHz, CDCl₃) δ 7.55 (d, *J* = 2.4 Hz, 2H), 7.53 (s, 1H), 6.68 (d, *J* = 6.9 Hz, 1H), 6.59 (d, *J* = 9.0 Hz, 2H), 6.42 (d, *J* = 7.5 Hz, 1H), 5.93 – 5.87 (m, 1H), 4.15 (t, *J* = 7.1 Hz, 2H), 3.74 (t, *J* = 7.2 Hz, 2H), 3.60 (s, 3H), 2.80 (s, 6H), 1.81 – 1.71 (m, 2H), 1.64 – 1.53 (m, 2H), 1.22 – 1.07 (m, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 157.40, 149.82, 149.43, 147.58, 127.87, 126.10, 118.38, 117.97, 111.94, 111.81, 104.44, 55.32, 49.35, 48.68, 39.94, 29.19, 27.80, 22.78.

4-(1-(5-(3-methoxy-2-oxopyridin-1(2H)-yl)pentyl)-1H-1,2,3-triazol-4-yl)-2-(trifluoromethyl)benzonitrile (12g)—4-Ethynyl-2-(trifluoromethyl) benzonitrile (0.09 g, 0.45 mmol) **18** (see supporting info) and **11** (0.13 g, 0.54 mmol) were reacted as described for the synthesis of **12a** to give compound **12g** (0.19 g, 98%). ¹H NMR (400 MHz, CDCl₃) δ 8.25 (s, 1H), 8.12 (d, *J* = 5.2 Hz, 2H), 7.83 (d, *J* = 8.1 Hz, 1H), 6.81 (dd, *J* = 6.9, 1.5 Hz, 1H), 6.55 (dd, *J* = 7.4, 1.5 Hz, 1H), 6.06 (t, *J* = 7.1 Hz, 1H), 4.41 (t, *J* = 7.1 Hz, 2H), 3.91 (t, *J* = 7.2 Hz, 2H), 3.73 (d, *J* = 5.8 Hz, 3H), 2.07 – 1.91 (m, 2H), 1.83 – 1.68 (m, 2H), 1.41 – 1.27 (m, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 157.80, 149.88, 144.44, 135.56, 135.11, 128.51, 127.94, 123.40, 121.75, 120.79, 115.41, 111.99, 108.22, 104.87, 55.64, 50.05, 48.84, 29.25, 28.03, 23.55, 22.92.

1-(4-Tolyl)triazolylpentyl-3-methoxypyridin-2-thione (13a)—A suspension of **12a** (0.24 g, 0.67 mmol) and Lawesson's reagent (0.16 g, 0.40 mmol) in toluene (12 mL) was heated at reflux for 12 h. The reaction mixture was cooled to room temperature and solvent

was evaporated *in vacuo*. The crude solid was purified on preparative TLC, eluting with CH₂Cl₂: Acetone: MeOH (5:1:0.2), to give **13a** (0.23 g, 95%) as yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 7.77 (s, 1H), 7.73 (d, *J* = 8.2 Hz, 2H), 7.33 (m, 1H), 7.25 (dd, *J* = 11.4, 5.2 Hz, 2H), 6.66 (d, *J* = 7.8 Hz, 1H), 6.59 (dd, *J* = 7.8, 6.5 Hz, 1H), 4.60 (m, 2H), 4.43 (t, *J* = 6.9 Hz, 2H), 3.91 (s, 3H), 2.38 (s, 3H), 2.01 (m, 4H), 1.43 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 171.03, 158.50, 147.11, 137.37, 131.77, 129.02, 127.33, 125.03, 119.32, 111.60, 109.78, 56.27, 49.28, 30.48, 29.05, 26.51, 22.57, 20.82. HRMS (EI) calcd for C₂₀H₂₄N₄OS [M]⁺ 368.1671 found 368.1667.

1-(3-Tolyl)triazolypentyl-3-methoxypyridin-2-thione (13b)—Reaction of **12b** (0.14 g, 0.39 mmol) and Lawesson's reagent (0.09 g, 0.22 mmol) in toluene (10 mL) within 12 h as described for the synthesis of **13a** gave compound **13b** (0.10 g, 73%) as yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 7.78 (s, 1H), 7.62 (s, 1H), 7.54 (d, *J* = 7.7 Hz, 1H), 7.26 (m, 2H), 7.07 (d, *J* = 7.6 Hz, 1H), 6.59 (m, 1H), 6.52 (dd, *J* = 7.8, 6.5 Hz, 1H), 4.50 (m, 2H), 4.34 (t, *J* = 7.0 Hz, 2H), 3.81 (s, 3H), 2.32 (s, 3H), 1.90 (m, 4H), 1.33 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 171.47, 158.81, 147.45, 138.19, 131.83, 130.21, 128.58, 128.46, 126.03, 122.48, 119.68, 111.72, 109.80, 56.55, 56.47, 49.50, 29.24, 26.70, 22.77, 21.18. HRMS (EI) calcd for C₂₀H₂₄N₄OS [M]⁺ 368.1671 found 368.1682.

1-(2-Tolyl)triazolypentyl-3-methoxypyridin-2-thione (13c)—Reaction of **12c** (0.12 g, 0.35 mmol) and Lawesson's reagent (0.09 g, 0.21 mmol) in toluene (10 mL) within 12 h as described for the synthesis of **13a** gave compound **13c** (0.09 g, 72%) as yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 7.72 (m, 1H), 7.27 (m, 2H), 6.60 (m, 1H), 4.57 (m, 1H), 4.42 (t, *J* = 7.0 Hz, 1H), 3.86 (s, 1H), 2.44 (s, 1H), 1.99 (m, 2H), 1.41 (dt, *J* = 15.2, 7.6 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 171.81, 159.04, 146.84, 135.33, 131.86, 130.74, 129.81, 128.68, 127.94, 125.93, 121.86, 111.78, 109.79, 104.88, 56.75, 56.61, 49.60, 29.45, 26.85, 22.97, 21.36. HRMS (EI) calcd for C₂₀H₂₄N₄OS [M]⁺ 368.1671 found 368.1662.

1-(4-Benzonitrile)triazolypentyl-3-methoxypyridin-2-thione (13d)—Reaction of **12d** (0.21 g, 0.55 mmol) and Lawesson's reagent (0.13 g, 0.33 mmol) in toluene (12 mL) within 12 h as described for the synthesis of **13a** gave compound **13d** (0.15 g, 67%) as yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 7.64 (m, 3H), 7.28 (m, 1H), 6.73 (d, *J* = 8.3 Hz, 2H), 6.61 (d, *J* = 7.6 Hz, 1H), 6.52 (t, *J* = 7.1 Hz, 1H), 4.50 (m, 2H), 4.31 (t, *J* = 6.7 Hz, 2H), 3.81 (s, 3H), 2.92 (s, 6H), 1.89 (m, 4H), 1.32 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 171.44, 158.81, 149.78, 147.77, 131.91, 126.37, 119.05, 118.34, 112.52, 111.73, 109.84, 56.60, 56.49, 49.44, 40.45, 29.29, 26.74, 22.81. HRMS (EI) calcd for C₂₀H₂₁N₅OS [M]⁺ 379.1467 found 379.1469.

1-(3-Benzonitrile)triazolypentyl-3-methoxypyridin-2-thione (13e)—Reaction of **12e** (0.19 g, 0.52 mmol) and Lawesson's reagent (0.13 g, 0.31 mmol) in toluene (10 mL) within 12 h as described for the synthesis of **13a** gave compound **13e** (0.13 g, 67%) as yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 7.98 (s, 1H), 7.84 (d, *J* = 8.4 Hz, 2H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.30 (d, *J* = 6.5 Hz, 1H), 6.60 (d, *J* = 7.0 Hz, 1H), 6.52 (dd, *J* = 7.7, 6.6 Hz, 1H), 4.48 (m, 2H), 4.34 (t, *J* = 6.9 Hz, 2H), 3.76 (s, 3H), 1.88 (m, 4H), 1.31 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 171.23, 158.70, 145.36, 134.76, 132.30, 131.78, 125.68, 121.20,

118.49, 111.83, 110.73, 109.92, 56.43, 49.50, 29.01, 26.56, 22.56. HRMS (EI) calcd for $C_{20}H_{21}N_5OS$ $[M]^+$ 379.1467 found 379.1466.

1-(4-(*N,N*-dimethylaniline))triazolypentyl-3-methoxypyridin-2-thione (13f)—

Reaction of **12f** (0.15 g, 0.40 mmol) and Lawesson's reagent (0.10 g, 0.24 mmol) in toluene (10 mL) within 12 h as described for the synthesis of **13a** gave compound **13f** (0.15 g, 98%) as yellow solid. 1H NMR (400 MHz, $CDCl_3$) δ 8.12 (t, J = 1.4 Hz, 1H), 8.06 (m, 1H), 7.90 (s, 1H), 7.59 (dt, J = 7.7, 1.4 Hz, 1H), 7.52 (t, J = 7.8 Hz, 1H), 7.33 (dd, J = 6.5, 1.4 Hz, 1H), 6.67 (dd, J = 7.9, 1.3 Hz, 1H), 6.60 (dd, J = 7.8, 6.5 Hz, 1H), 4.59 (m, 2H), 4.46 (t, J = 6.9 Hz, 2H), 3.89 (s, 3H), 2.02 (m, 4H), 1.43 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.30, 158.73, 145.13, 131.82, 131.71, 130.97, 129.52, 129.44, 128.64, 120.61, 118.28, 112.49, 111.81, 109.91, 56.44, 49.62, 29.11, 26.64, 22.68. HRMS (EI) calcd for $C_{21}H_{27}N_5OS$ $[M]^+$ 397.1936 found 397.1928.

1-(4-(3-Trifluoromethyl)-benzonitrile)triazolypentyl-3-methoxypyridin-2-thione (13g)—

Reaction of **12g** (0.09 g, 0.21 mmol) and Lawesson's reagent (0.05 g, 0.13 mmol) in toluene (10 mL) within 12 h as described for the synthesis of **13a** gave compound **13g** (0.08 g, 81%) as yellow solid. 1H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 8.12 (d, J = 6.7 Hz, 2H), 7.86 (d, J = 8.1 Hz, 1H), 7.36 (d, J = 6.5 Hz, 1H), 6.68 (d, J = 7.8 Hz, 1H), 6.61 (t, J = 7.2 Hz, 1H), 4.59 (m, 2H), 4.47 (m, 2H), 3.88 (s, 3H), 2.02 (m, 4H), 1.43 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.84, 159.14, 144.54, 135.55, 135.22, 133.51, 133.18, 131.81, 128.59, 121.86, 120.86, 115.46, 112.00, 109.97, 108.47, 77.31, 76.99, 76.67, 56.69, 49.87, 29.19, 26.77, 22.77. HRMS (EI) calcd for $C_{21}H_{20}N_5OSF_3$ $[M]^+$ 447.1341 found 447.1341.

1-(4-Tolyl)triazolypentyl-3-hydroxypyridin-2-thione (14a)—Reaction of **13a** (0.08 g, 0.20 mmol) and 1M BBr_3 (0.24 mL) in CH_2Cl_2 (8 mL) within 48 h as described for the synthesis of **10a** gave compound **14a** (0.04 g, 62%) as olive green solid. Retention time 11.0 min. 1H NMR (400 MHz, CD_3OD) δ 8.18 (s, 1H), 7.66 (d, J = 8.0 Hz, 2H), 7.55 (d, J = 5.7 Hz, 1H), 7.20 (d, J = 7.7 Hz, 2H), 6.97 (d, J = 6.7 Hz, 1H), 4.55 (m, 2H), 4.43 (t, J = 6.7 Hz, 2H), 2.34 (s, 3H), 2.01 (m, 4H), 1.39 (t, J = 22.5 Hz, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 149.25, 139.68, 130.93, 129.13, 127.01, 122.27, 51.41, 30.95, 24.51, 21.83. HRMS (EI) calcd for $C_{19}H_{22}N_4OS$ $[M]^+$ 354.1514 found 354.1512.

1-(3-Tolyl)triazolypentyl-3-hydroxypyridin-2-thione (14b)—Reaction of **13b** (0.10 g, 0.27 mmol) and 1M BBr_3 (0.33 mL) in CH_2Cl_2 (8 mL) within 48 h as described for the synthesis of **10a** gave compound **14b** (0.05 g, 56%) as olive green solid. Retention time 11.1 min. 1H NMR (400 MHz, CD_3OD) δ 8.20 (s, 1H), 7.61 (s, 1H), 7.55 (m, 2H), 7.26 (t, J = 7.6 Hz, 1H), 7.12 (d, J = 7.5 Hz, 1H), 6.92 (m, 2H), 4.55 (m, 2H), 4.44 (t, J = 6.7 Hz, 2H), 2.36 (s, 3H), 2.01 (m, 4H), 1.41 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 149.29, 140.05, 131.87, 131.78, 130.38, 130.20, 127.90, 127.63, 124.13, 122.51, 51.40, 31.07, 30.93, 28.97, 24.49, 22.02. HRMS (EI) calcd for $C_{19}H_{22}N_4OS$ $[M]^+$ 354.1514 found 354.1523.

1-(2-Tolyl)triazolypentyl-3-hydroxypyridin-2-thione (14c)—Reaction of **13c** (0.07 g, 0.20 mmol) and 1M BBr_3 (0.30 mL) in CH_2Cl_2 (8 mL) within 48 h as described for the synthesis of **10a** gave compound **14c** (0.05 g, 67%) as olive green solid. Retention time 10.6

min. ^1H NMR (400 MHz, CD_3OD) δ 7.94 (s, 1H), 7.61 (m, 1H), 7.47 (s, 1H), 7.21 (m, 1H), 6.97 (m, 1H), 6.76 (m, 1H), 4.52 (m, 1H), 4.45 (t, $J = 6.8$ Hz, 1H), 2.38 (s, 1H), 2.01 (m, 2H), 1.43 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 146.74, 135.53, 130.65, 129.46, 128.69, 128.21, 125.88, 122.79, 53.41, 49.83, 29.42, 27.32, 22.98, 20.55. HRMS (EI) calcd for $\text{C}_{19}\text{H}_{22}\text{N}_4\text{OS}$ $[\text{M}]^+$ 354.1514 found 354.1509.

1-(4-Benzonitrile)triazolypentyl-3-hydroxypyridin-2-thione (14d)—Reaction of **13d** (0.10 g, 0.271 mmol) and 1M BBr_3 (0.33 mL) in CH_2Cl_2 (8 mL) within 48 h as described for the synthesis of **10a** gave compound **14d** (0.053 g, 56%) as olive green solid. Retention time 9.4 min. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.53 (s, 1H), 8.27 (s, 1H), 7.82 (d, $J = 7.1$ Hz, 1H), 7.62 (d, $J = 8.8$ Hz, 2H), 7.06 (d, $J = 7.5$ Hz, 1H), 6.93 (m, 1H), 6.77 (m, 2H), 4.52 (d, $J = 6.9$ Hz, 2H), 4.36 (t, $J = 7.0$ Hz, 2H), 3.21 (s, 6H), 1.88 (m, 4H), 1.34 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 145.3, 132.0, 131.1, 129.7, 129.6, 128.6, 121.7, 112.7, 105.0, 49.8, 29.1, 27.0, 22.7. HRMS (EI) calcd for $\text{C}_{19}\text{H}_{19}\text{N}_5\text{OS}$ $[\text{M}]^+$ 365.1310 found 365.1310.

1-(3-Benzonitrile)triazolypentyl-3-hydroxypyridin-2-thione (14e)—Reaction of **13e** (0.10 g, 0.25 mmol) and 1M BBr_3 (0.30 mL) in CH_2Cl_2 (8 mL) within 48 h as described for the synthesis of **10a** gave compound **14e** (0.06 g, 60%) as olive green solid. Retention time 9.3 min. ^1H NMR (400 MHz, DMSO) δ 8.76 (s, 1H), 8.01 (d, $J = 8.1$ Hz, 2H), 7.87 (m, 2H), 7.65 (d, $J = 6.4$ Hz, 1H), 7.00 (m, 1H), 6.87 (d, $J = 8.2$ Hz, 1H), 6.70 (m, 1H), 4.44 (m, 4H), 1.89 (m, 4H), 1.31 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ 168.23, 155.01, 145.75, 134.86, 132.56, 131.00, 125.91, 121.05, 118.68, 113.76, 112.04, 111.17, 57.64, 49.81, 29.31, 27.06, 22.91. HRMS (EI) calcd for $\text{C}_{19}\text{H}_{19}\text{N}_5\text{OS}$ $[\text{M}]^+$ 365.1310 found 365.1313.

1-(4-(*N,N*-dimethylaniline)triazolypentyl-3-hydroxypyridin-2-thione (14f)—Reaction of **13f** (0.11 g, 0.25 mmol) and 1M BBr_3 (0.33 mL) in CH_2Cl_2 (8 mL) within 48 h as described for the synthesis of **10a** gave compound **14f** (0.07 g, 66%) as olive green solid. Retention time 4.6 min. ^1H NMR (400 MHz, CD_3OD) δ 8.38 (s, 1H), 8.15 (s, 1H), 8.10 (d, $J = 7.9$ Hz, 1H), 7.63 (m, 3H), 6.95 (d, $J = 7.5$ Hz, 1H), 6.67 (m, 1H), 4.57 (m, 2H), 4.48 (t, $J = 7.0$ Hz, 2H), 2.03 (m, 4H), 1.43 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 145.34, 132.04, 131.14, 129.72, 129.58, 128.63, 121.75, 112.74, 104.99, 49.79, 29.08, 27.04, 22.73. HRMS (EI) calcd for $\text{C}_{20}\text{H}_{25}\text{N}_5\text{OS}$ $[\text{M}]^+$ 383.1780 found 383.1776.

1-(4-(3-Trifluoromethyl)-benzonitrile)triazolypentyl-3-hydroxypyridin-2-thione (14g)—Reaction of **13g** (0.11 g, 0.25 mmol) and 1M BBr_3 (0.33 mL) in CH_2Cl_2 (8 mL) within 48 h as described for the synthesis of **10a** gave compound **14g** (0.072 g, 66%) as olive green solid. Retention time 12.4 min. ^1H NMR (400 MHz, CD_3OD) δ 8.59 (s, 1H), 8.35 (s, 1H), 8.21 (d, $J = 7.8$ Hz, 1H), 8.00 (t, $J = 9.5$ Hz, 1H), 7.58 (d, $J = 6.2$ Hz, 1H), 6.96 (d, $J = 7.5$ Hz, 1H), 6.72 (m, 1H), 4.57 (m, 4H), 2.04 (m, 4H), 1.42 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 146.09, 137.48, 137.24, 134.79, 130.46, 125.55, 124.87, 123.60, 122.77, 116.84, 109.75, 55.10, 51.63, 30.84, 28.90, 24.47. HRMS (EI) calcd for $\text{C}_{19}\text{H}_{19}\text{N}_5\text{OS}$ $[\text{M}]^+$ 365.1310 found 365.1310. HRMS (EI) calcd for $\text{C}_{20}\text{H}_{18}\text{N}_5\text{OS}$ $[\text{M}]^+$ 433.1184 found 433.1189.

1-(4-Tolyl)triazolypentyl-3-hydroxypyridin-2-one (15a)—Reaction of **12a** (0.10 g, 0.28 mmol) and 1M BBr₃ (0.34 mL) in CH₂Cl₂ (8 mL) within 48 h as described for the synthesis of **10a** gave compound **15a** (0.05 g, 52%) as light brown solid. ¹H NMR (400 MHz, CDCl₃) δ 7.71 (m, 3H), 7.24 (m, 2H), 6.76 (t, *J* = 6.4 Hz, 2H), 6.12 (t, *J* = 7.0 Hz, 1H), 4.40 (t, *J* = 6.9 Hz, 2H), 3.96 (t, *J* = 7.1 Hz, 2H), 2.38 (s, 3H), 2.00 (m, 2H), 1.82 (m, 2H), 1.39 (m, 2H); ¹³C NMR (100 MHz, DMSO) δ 171.80, 146.31, 137.02, 129.40, 128.08, 125.02, 120.79, 114.36, 105.20, 49.27, 48.22, 29.17, 28.01, 22.85, 20.82. HRMS (EI) calcd for C₁₉H₂₂N₄O₂ [M]⁺ 338.1743 found 338.1750.

1-(3-Tolyl)triazolypentyl-3-hydroxypyridin-2-one (15b)—Reaction of **12b** (0.07 g, 0.21 mmol) and 1M BBr₃ (0.24 mL) in CH₂Cl₂ (8 mL) within 48 h as described for the synthesis of **10a** gave compound **15b** (0.05 g, 74%) as light brown solid. ¹H NMR (400 MHz, CDCl₃) δ 7.74 (s, 1H), 7.67 (s, 1H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.30 (t, *J* = 7.6 Hz, 1H), 7.14 (d, *J* = 7.4 Hz, 1H), 6.76 (t, *J* = 6.2 Hz, 2H), 6.12 (t, *J* = 7.1 Hz, 1H), 4.39 (t, *J* = 6.9 Hz, 2H), 3.96 (t, *J* = 7.2 Hz, 2H), 2.39 (s, 3H), 1.99 (m, 2H), 1.80 (m, 2H), 1.38 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 158.58, 147.87, 146.52, 138.46, 130.38, 128.86, 128.67, 126.75, 126.31, 122.72, 119.48, 113.61, 106.85, 49.91, 49.45, 29.65, 28.38, 23.27, 21.38. HRMS (EI) calcd for C₁₉H₂₂N₄O₂ [M]⁺ 338.1743 found 338.1741.

1-(2-Tolyl)triazolypentyl-3-hydroxypyridin-2-one (15c)—Reaction of **12c** (0.07 g, 0.18 mmol) and 1M BBr₃ (0.27 mL) in CH₂Cl₂ (8 mL) within 48 h as described for the synthesis of **10a** gave compound **15c** (0.05 g, 87%) as light brown solid. ¹H NMR (400 MHz, dmsO) δ 8.92 (s, 1H), 8.36 (s, 1H), 7.71 (m, 1H), 7.25 (m, 1H), 7.09 (d, *J* = 6.8 Hz, 1H), 6.64 (d, *J* = 6.9 Hz, 1H), 6.04 (t, *J* = 7.0 Hz, 1H), 4.39 (t, *J* = 6.9 Hz, 1H), 3.88 (t, *J* = 7.0 Hz, 1H), 2.41 (s, 1H), 1.90 (m, 1H), 1.67 (m, 1H), 1.25 (m, 1H). ¹³C NMR (100 MHz, CD₃OD) δ 146.28, 135.00, 130.15, 128.96, 128.21, 127.72, 125.38, 122.09, 106.72, 49.43, 48.98, 29.05, 27.78, 22.69, 20.09. HRMS (EI) calcd for C₁₉H₂₂N₄O₂ [M]⁺ 338.1743 found 338.1741.

1-(4-Benzonitrile)triazolypentyl-3-hydroxypyridin-2-one (15d)—Reaction of **12d** (0.08 g, 0.20 mmol) and 1M BBr₃ (0.24 mL) in CH₂Cl₂ (8 mL) within 48 h as described for the synthesis of **10a** gave compound **15d** (0.04 g, 60%) as light brown solid. ¹H NMR (400 MHz, CDCl₃) δ 7.69 (d, *J* = 8.8 Hz, 2H), 7.61 (s, 1H), 6.77 (m, 4H), 6.11 (t, *J* = 7.1 Hz, 1H), 4.37 (t, *J* = 6.9 Hz, 2H), 3.96 (t, *J* = 7.2 Hz, 2H), 2.99 (s, 6H), 1.99 (m, 2H), 1.81 (m, 2H), 1.39 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 158.57, 150.35, 148.26, 146.61, 126.84, 126.61, 118.87, 118.11, 113.72, 112.49, 106.86, 49.83, 49.52, 40.46, 29.70, 28.41, 23.34. HRMS (EI) calcd for C₁₉H₁₉N₅O₂ [M]⁺ 349.1539 found 349.1539.

1-(3-Benzonitrile)triazolypentyl-3-hydroxypyridin-2-one (15e)—Reaction of **12e** (0.09 g, 0.25 mmol) and 1M BBr₃ (0.30 mL) in CH₂Cl₂ (8 mL) within 48 h as described for the synthesis of **10a** gave compound **15e** (0.06 g, 72%) as light brown solid. ¹H NMR (400 MHz, DMSO) δ 8.93 (s, 1H), 8.76 (s, 1H), 8.01 (d, *J* = 7.7 Hz, 2H), 7.90 (d, *J* = 8.0 Hz, 2H), 7.09 (d, *J* = 6.4 Hz, 1H), 6.64 (d, *J* = 7.1 Hz, 1H), 6.04 (t, *J* = 7.0 Hz, 1H), 4.41 (t, *J* = 6.5 Hz, 2H), 3.88 (t, *J* = 6.9 Hz, 2H), 1.89 (m, 2H), 1.67 (m, 2H), 1.24 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 146.62, 145.80, 134.92, 132.57, 126.69, 125.93, 120.88, 118.70,

114.06, 111.19, 107.06, 50.00, 49.29, 29.43, 28.28, 23.08. HRMS (EI) calcd for $C_{19}H_{19}N_5O_2$ $[M]^+$ 349.1539 found 349.1544.

1-(4-(*N,N*-dimethylaniline))triazolylpentyl-3-hydroxypyridin-2-one (15f)—

Reaction of **12f** (0.08 g, 0.22 mmol) and 1M BBr₃ (0.26 mL) in CH₂Cl₂ (6 mL) within 48 h as described for the synthesis of **10a** gave compound **15f** (0.05 g, 64%) as light brown solid. ¹H NMR (400 MHz, CD₃OD) δ 8.39 (s, 1H), 8.16 (s, 1H), 8.12 (d, *J* = 7.5 Hz, 1H), 7.68 (d, *J* = 7.5 Hz, 1H), 7.61 (t, *J* = 7.6 Hz, 1H), 7.04 (m, 1H), 6.76 (m, 1H), 6.21 (m, 1H), 4.45 (m, 2H), 3.99 (m, 2H), 2.01 (m, 2H), 1.78 (m, 2H), 1.33 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 145.61, 131.97, 131.31, 129.74, 129.65, 129.10, 120.30, 118.49, 112.99, 104.90, 50.07, 49.40, 29.52, 28.37, 23.17. HRMS (EI) calcd for $C_{20}H_{25}N_5O_2$ $[M]^+$ 367.2008 found 367.2007.

1-(4-(3-Trifluoromethyl)-benzonitrile)triazolylpentyl-3-hydroxypyridin-2-one (15g)

—Reaction of **12g** (0.04 g, 0.10 mmol) and 1M BBr₃ (0.17 mL) in CH₂Cl₂ (6 mL) within 48 h as described for the synthesis of **10a** gave compound **15g** (0.04 g, 85%) as light brown solid. ¹H NMR (400 MHz, CDCl₃) δ 8.24 (s, 1H), 8.12 (d, *J* = 7.9 Hz, 1H), 8.00 (s, 1H), 7.86 (d, *J* = 7.9 Hz, 1H), 6.75 (t, *J* = 5.8 Hz, 2H), 6.12 (t, *J* = 7.0 Hz, 1H), 4.44 (t, *J* = 6.9 Hz, 2H), 3.96 (t, *J* = 7.1 Hz, 2H), 2.02 (m, 2H), 1.81 (m, 2H), 1.37 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 158.58, 146.54, 144.71, 135.49, 135.26, 128.57, 126.70, 123.59, 123.54, 121.46, 115.47, 113.82, 108.63, 107.02, 50.19, 49.31, 29.45, 28.30, 23.12. HRMS (EI) calcd for $C_{20}H_{18}N_5O_2F_3$ $[M]^+$ 417.1413 found 417.1425. HRMS (EI) calcd for $C_{20}H_{18}N_5O_2F_3$ $[M]^+$ 417.1413 found 417.1425.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

HDAC	Histone deacetylase
HDACi	Histone deacetylase inhibitors
ZBG	Zinc binding group
TSA	Trichostatin A
3HPT	3-hydroxypyridin-2-thione
MMP	Matrix metalloproteins
CTCL	Cutaneous T-cell lymphoma

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a)

b)

Figure 1.

(a) Representative examples of HDACi. (b) Representative aryl- and diaryl-3HPT based HDACi with their HDAC inhibition activities (IC_{50}). ^a% inhibition of the compounds at 10 μ M.⁹

A

i)

ii)

B

i)

ii)

Figure 2.

Molecular docking studies on **10a** and **10d**. A) HDAC6 homology model built from HDAC8 (PDB code: 3F0R). i) Zn^{2+} chelation is observed for both **10a** (green) and **10d** (orange). ii) π -stacking interaction of the phenyl ring of **10d** with Phe680. B) HDAC8 (PDB code: 3SFF). i) Zn^{2+} chelation of **10a** (yellow) and **10d** (green). ii) The phenyl ring of **10d** (green) is inserted into a hydrophobic pocket adjacent to the active site whereas that of **10a** (yellow) is in a solvent exposed HDAC8 outer rim.

Figure 3.

Molecular docking studies on substituted 3HPT compounds. i) HDAC6 homology model built from HDAC8 (PDB code: 3F0R). The cyano group of compound **14e** is positioned for a potential H-bonding interactions with the hydroxyl side-chain and amide backbone of Ser568. ii) HDAC8 (PDB code: 3SFF). The cap groups of mono-substituted compounds are inserted within HDAC8 sub-pocket while that of di-substituted compound **14g** is excluded. iii) HDAC8. Space filling model reveals a better fit of the unsubstituted phenyl **10d** (green)

within HDAC8 sub-pocket, relative to a representative mono-substituted compound **14b** (orange).

Figure 4.

Western blot analysis of tubulin acetylation (HDAC6 inhibition) in LNCaP cell line. Lanes - 1. **10d** (20 μ M); 2. **10d** (5.43 μ M – IC₅₀); 3. **14e** (20 μ M); 4. **14e** (3.49 μ M – IC₅₀); 5. **SAHA** (20 μ M); 6. **SAHA** (2.31 μ M – IC₅₀); 7. Control.

Scheme 1.

Synthesis of 3-HPT based HDACi **10** for SAR studies. Conditions: (a) NaN_3 , DMF, 75°C . (b) methanesulfonyl chloride, Et_3N , THF; (c) 3-methoxypyridin-2-one or 3-benzyloxypyridin-2-one, K_2CO_3 , THF/DMF, reflux. (d) Phenylacetylene, CuI, DIPEA, THF. (e) H_2 , Pd/C, THF for $R = \text{Bn}$; BBr_3 , DCM for $R = \text{Me}$. (f) for **7a** only: Lawesson's reagent, toluene, reflux; (g) P_4S_{10} , 175°C , neat. (h) BBr_3 , DCM

Scheme 2.

Optimization of the surface recognition group – synthesis of the five methylene-linked 3HPT HDACi. a) 3-methoxypyridin-2-one, K_2CO_3 , THF:MeOH, reflux. b) Substituted phenylacetylenes (**a–g**), CuI, DIPEA, DMSO:THF. c) Lawesson's reagent, Toluene, reflux. d) BBr_3 , DCM.

Table 1

SAR on linker of triazolic compounds

<p>$n = 1, 2, 3, 4, 5, 6, 7$</p>				
Compound	n	HDAC1 IC ₅₀ (nM)	HDAC6 IC ₅₀ (nM)	HDAC8 IC ₅₀ (nM)
3a	1	NI [*]	41% [†]	1570±1067
10a	2	NI	14%	56%
10b	3	NI	3628±1363	63%
10c	4	NI	1085±333	3303±260
10d	5	NI	911± 173	917±139
10e	6	NI	22%	6751±910
10f	7	NI	955 ±150	1377±205
SAHA	-	38±2	144±23	232±19

^{*} No significant Inhibition (below 20% Inhibition at 10 μ M concentration)

[†] % inhibition of the compounds at 10 μ M are given if the IC₅₀ was above 10 μ M

Table 2

HDAC inhibition profile of the substituted 3HPT compounds.

Compound	R	HDAC1 IC ₅₀ (nM)	HDAC6 IC ₅₀ (nM)	HDAC8 IC ₅₀ (nM)
10d		NI*	911 ± 173	917 ± 139
14a		NI	807 ± 207	2533 ± 823
14b		NI	1100 ± 443	1660 ± 416
14c		NI	637 ± 160	2402 ± 263
14d		NI	905 ± 249	1465 ± 217
14e		NI	356 ± 72	2831 ± 520
14f		NI	1006 ± 425	1482 ± 389
14g		NI	661 ± 121	2258 ± 1005
SAHA	-	38 ± 2	144 ± 23	232 ± 19

* No significant Inhibition (below 20% Inhibition at 10 µM concentration))

† % inhibition of the compounds at 10 µM are given if the IC₅₀ was above 10 µM

Table 3

Cell Viability Assay. IC₅₀ of selected compounds against various cancer cell lines.

Compound	Cellular IC ₅₀ (μ M)				
	DU-145	LNCaP	Jurkat	Jurkat J- γ	Vero
10d	9.33 $\pm$ 0.96	5.43 $\pm$ 0.47	3.27 $\pm$ 0.60	1.86 $\pm$ 0.21	>20
10f	17.72 $\pm$ 3.28	10.95 $\pm$ 1.92	9.04 $\pm$ 1.31	>20	NT
14c	11.08 $\pm$ 2.38	4.95 $\pm$ 0.43	5.18 $\pm$ 1.18	NT	NT
14e	5.03 $\pm$ 1.13	3.49 $\pm$ 0.34	3.44 $\pm$ 0.57	0.90 $\pm$ 0.12	>20
SAHA	2.49 $\pm$ 0.2	2.31 $\pm$ 0.74	1.49 $\pm$ 0.10	NI	5.20 $\pm$ 0.96*
Tubastatin A	NT	10.88 $\pm$ 1.49	3.38 $\pm$ 0.26	NI	>20

NT = Not Tested

NI = No inhibition at 20 μ M;

* reference 15