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Structure-based optimization of click-based histone deacetylase inhibitors

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ABSTRACT

Previously, we reported a click-chemistry based approach to the synthesis of a novel class of histone deacetylase (HDAC) inhibitors [1]. The lead compound NSC746457 was found to be as potent as SAHA (Vorinostat). Further optimization of NSC746457 by using the HDAC2-TSA crystal structure is described herein. Docking of NSC746457 into HDAC2 binding domain suggested that the hydrophobic residue Phe210 flanking the cap-group binding-motif could be exploited for structural optimization. Substitution on the methylene group of cinnamic cap region led to identification of more potent HDAC inhibitors: isopropyl derivative **5** and tert-butyl derivative **6**, with an IC_{50} value of 22 nM and 18 nM, respectively.

1. Introduction

Histone acetylation is believed to be associated with activation of gene transcription [2–8]. The acetylation status is dependent on the activities of both histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs acetylate histone lysine substrates and cause chromatin to relax, thus permitting various transcription factors to interact with DNA in order to allow for transcription to occur. HDACs deacetylate histone lysine substrates and condense chromatin, which prevents access of transcription factors and leads to transcriptional repression [9]. HATs deactivation and HDACs overexpression are associated with tumorigenesis [10]. Thus, HDAC inhibitors have emerged as therapeutic agents for the treatment of multiple human cancers. Several HDAC inhibitors are in clinical trials, both for use as a single-treatment or in combination therapy (Fig. 1). SAHA (Vorinostat) was approved by FDA in 2006 for the treatment of refractory cutaneous T-cell lymphoma (CTCL) [11,12], and Romidepsin (depsipeptide or FK-228) was approved in 2009 for treatment of CTCL in patients who have received at least one prior systemic therapy [13].

To date, eighteen HDAC isoforms have been identified in human. They can be divided into two categories, zinc-dependent enzymes (HDAC1-11) and NAD(+)-dependent enzymes (SIRT1-7). Among all HDAC isoforms, HDAC1 is the most well-studies since over-expression of HDAC1 is common to several cancer types. Numerous HDAC1 inhibitors have been discovered using structure-based design. Since no structure of human HDAC1 has been solved to date, several homology models of human HDAC1 have been constructed [14–17]. A series of HDAC inhibitors were docked into the models to understand the protein–ligand interactions in an effort to learn how to design more efficient HDAC inhibitors.

It was reported the crystal structure of human HDAC2 in complex with hydroxamates—TSA [18], which provides insight into the unique mode of action of hydroxamates in HDAC2 inhibition. The HDAC2 catalytic site consists of a narrow, tube-like pocket and a catalytic Zn^{2+} nestled 8 Å deep in the protein active site. The lipophilic tube is formed by Gly154, Phe155, His183, Phe210, and Leu276. The zinc ion is held by Asp181, His183, and Asp269 [19]. Previously, we reported a lead compound NSC746457 with an IC_{50} value of 104 nM. In this study, we describe efforts in optimization of NSC746457 using information gleaned from the crystal structure of HDAC2-TSA.

2. Docking and design

In order to understand the binding mode of protein–ligand interactions which might facilitate further optimization, NS746457

Abbreviations: HDAC, histone deacetylase; SAHA, suberoylanilide hydroxamic acid; TSA, trichostatin A.

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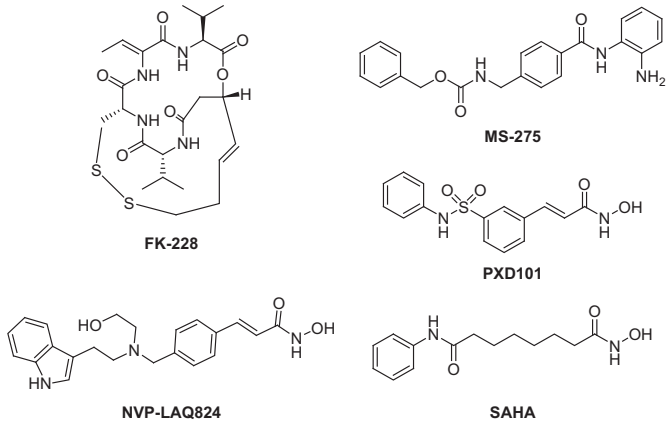


Fig. 1. Structures of representative HDAC inhibitors.

was docked into the active-site pocket of HDAC2 (Autodock 4.02). We chose HDAC2 for several reasons: deregulation of HDAC2 expression and activity has been linked to cancer development, and the HDAC2 crystal structure has been solved (whereas the HDAC1 structure has not been). HDAC2 (GenBank accession number: NP-001518) and HDAC1 (GenBank accession number: Q13547) are highly conserved, with 86% sequence identity and 94% sequence similarity (using BLAST). Both HDAC2 and HDAC1 have identical active sites and very small differences around the entrance of the channel. Therefore, we felt confident in using the HDAC2 crystal structure as a tool to use in the design and optimization of HDAC inhibitors.

The docking studies suggest two positive interactions accounting for the tight fit of NSC746457 in the active site of HDAC2 model (Fig. 2): (1) favorable pi–pi stacking interactions between the triazole and the Phe155 and Phe210 residues; (2) the active site Zn^{2+} ion, having Asp181, His183, and Asp269 as ligands, is coordinated with the two oxygen atoms of the hydroxamic acid group. Comparison of the binding mode of TSA and NS746457 with HDAC2 (Fig. 3) revealed that the hydrophobic residue Phe210 could be exploited to enhance the binding potency. Accordingly, we designed and synthesized a series of novel HDAC inhibitions by placing a variety of substituent groups on the methylene of cinnamyl scaffold of NSC746457.

3. Chemistry

The terminal alkyne precursor **F** was prepared as previously described [1] (Scheme 1). Propiolic acid was subjected to copper-catalyzed hydroiodination to give the *trans*-iodopropenoic acid **A** [20], which was then protected as MEM ester **B**. The Sonogashira coupling of **B** with TMS acetylene afforded intermediate **C**, which was then deprotected to provide the acid **D**. Conversion of acid **D** to the acid chloride using standard condition, followed by reaction with *O*-PMB-hydroxyamine, gave the PMB ester **E**. Desilylation provided the terminal alkyne **F**.

As illustrated in Scheme 2, compounds **1a**–**16a** can be prepared by three different routes using cinnamaldehyde as the starting material. Cinnamaldehyde was reacted with various Grignard reagents to give compounds **1a**–**11a** (route a). Deprotonation of the methyl group of several aromatic compounds, followed by reaction with cinnamaldehyde, gave the corresponding Adol products **13a**–**16a** (route c). Compound **12a** could not be obtained by the route c, since metalation of 2-methylthiazole with *n*-butyllithium proceed predominantly at the C-5 position. Accordingly, compound **12a** was prepared from cinnamaldehyde and 2-methylthiazole using $(Bu)_2BOTf$ (route b). Compounds **1a**–**16a** were converted to the azides **1b**–**16b**, which were then reacted with terminal alkyne **F** in the presence of $CuI{\cdot}P(OEt)_3$ to give the 1,4-disubstituted triazoles **1c**–**16c**. Deprotection of **1c**–**16c** with triisopropylsilane/TFA gave the final compounds **1**–**16**. The final compounds were purified using C18 reverse phase column chromatography to remove colored impurities.

4. Results and discussion

The novel HDAC inhibitors prepared above were screened using nuclei isolated from the cervical carcinoma cell line Hela, according to a modified method first described by Dignam et al. [21]. The HDAC enzyme activity assay developed by Wegener was used to determine IC_{50} values [22] (Table 1). The nucleus extracts were mixed with the prepared HDAC inhibitors diluted in various concentrations in assay buffer. The fluorescent substrate Ac-Arg-Gly-Lys(Ac)-AMC was added to the mixture, which was incubated at 37 °C for 20 min, then treated with a developer containing TSA to stop the reaction. Fluorescence was monitored after 20 min at excitation and emission wavelengths of 390 nm and 460 nm, respectively. TSA and SAHA were used as the positive controls.

To exploit the hydrophobic microdomain nearby Phe210 for structure optimization, we focused on the exploration of the methylene group on the cinnamyl moiety of NSC746457. To our gratification, substitution of the methylene group proved to be very important for HDAC activity in the enzymatic assay (Table 1). Among the eighteen analogs prepared, isopropyl and tert-butyl substituted cinnamyl derivatives (**5** and **6**) were the most potent, with an IC_{50} value of 22 nM and 18 nM, respectively. We found that shorter-chain groups, such as methyl (**1**) and ethyl (**2**), can increase potency, whereas straight chain groups larger than ethyl (**3** and **4**) lead to a decrease in potency. Branching of alkyl chain led to about a 5-fold increase in the potency relative to NSC746457 in the case of

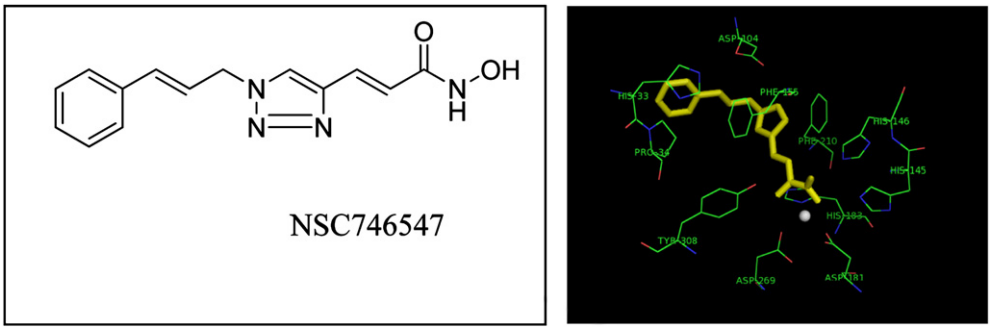


Fig. 2. Structure of docked NSC746547 (yellow capped sticks) into HDAC2 active site. The zinc is shown as a white sphere. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

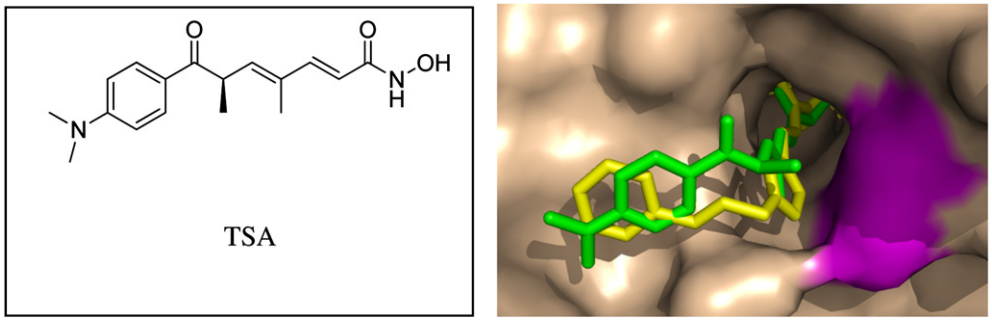


Fig. 3. Superposition of the docked conformation of NSC746457 (yellow capped sticks) and TSA in HDAC2 active site. Phe210 is shown in magenta. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the isopropyl and tert-butyl analogs, presumably since the bulkier group can enhance the interaction with the hydrophobic microdomain observed in the crystal structure of HDAC2. While cyclobutyl substitution was found to slightly increase the potency, cyclopentyl slightly decreased the potency. Most of the aromatic group resulted in some loss of potency, whereas the benzyl group was observed to slightly improve the potency.

To further validate the utility of these HDAC inhibitors at the cellular level, compounds **1**, **2** and **5** were submitted to NCI for examination in 60 cancer cell lines (including leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma ovarian a, cancer, renal cancer, prostate cancer and breast cancer cells). The compounds were evaluated at five concentration levels (100, 10, 1.0, 0.1, 0.001 μM). The data for these novel compounds is provided in Table 2, along with comparison to NSC746457. No precise correlations were found between the inhibitory potency against HDACs and the antiproliferative effect in the tested cell lines. In general, compounds **1**, **2** and **5** showed excellent potency comparable to that observed for NSC746457. Additionally, these three compounds showed remarkable activity against leukemia cell lines (CCRF-CEM, HL-60(TB), K-562, MOLT-4, RPMI-8226, SR), with the GI_{50} value below 1 μM.

5. Conclusion

We have synthesized a novel series of cinnamyl derivatives using click chemistry, and have shown that they are potentially useful HDAC inhibitors. Among the compounds prepared, isopropyl derivative **5** and tert-butyl derivative **6** exhibited excellent potency against HDACs enzyme, with the IC_{50} value of 22 nM and 18 nM, respectively. The cellular assay showed that ethyl derivative **2**, isopropyl derivative **5** and benzyl derivative **11** were comparable in potency with the lead compound NSC746457. Interesting, the aforementioned three compounds also showed some selectivity among the various cell lines, with the best results seen in the Leukemia cell lines.

6. Experimental

6.1. Chemistry

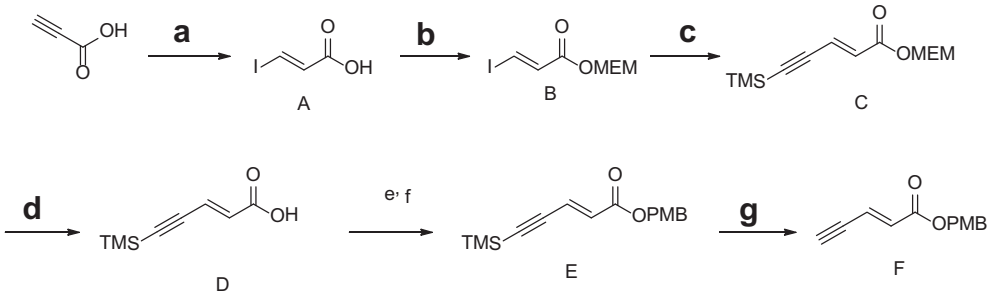
All reagents were purchased from Alfa Aesar, and used without further purification. Thin-layer chromatography (TLC) was carried out on silica GF254 plates (Qingdao). Column chromatography was performed on silica gel (200–300 mesh normal phase from Qingdao, or 200–400 mesh reverse phase from MED). ^1H and ^{13}C NMR spectra were obtained on Brucker Avance 300 or 400 spectrometer, using tetramethylsilane as an internal standard. High resolution mass spectra (HRMS) were obtained on a QFT-ESI mass spectrometer.

6.1.1. General procedure for synthesis of compound *1a*–*11a*

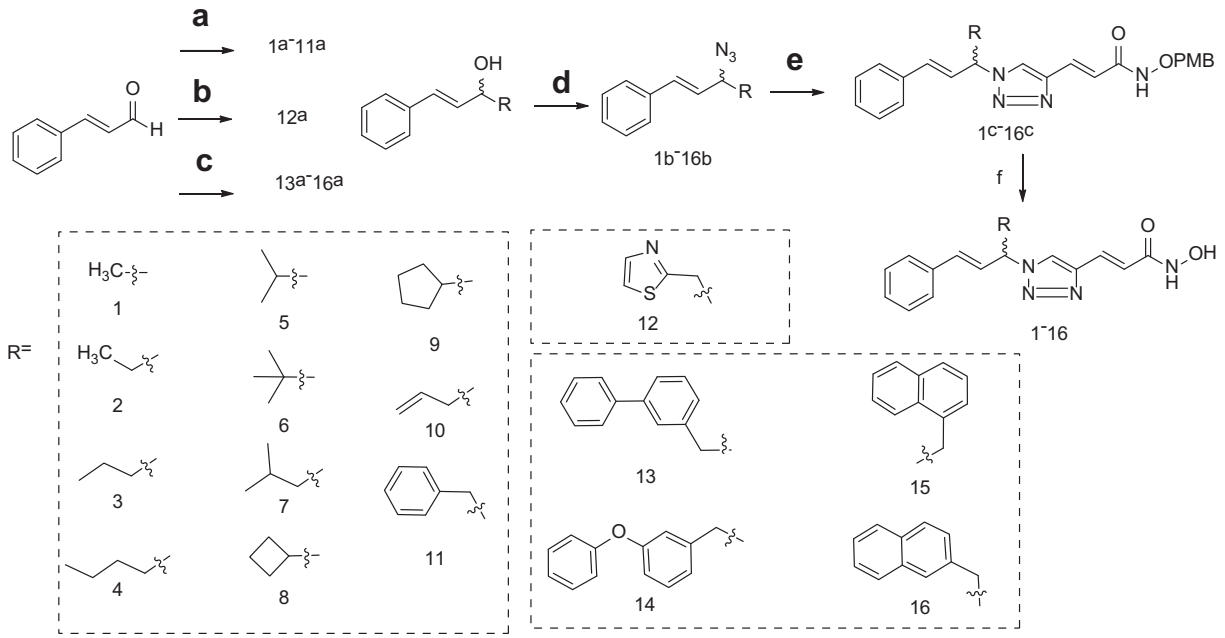
An oven-dried flask was charged with a solution of cinnamaldehyde (3.0 mmol in diethyl ether, 10 mL), and was cooled to 0 °C (ice water bath) under nitrogen. A solution of Grignard reagent in THF (2.0 equiv.) or freshly prepared in diethyl ether [23] was added dropwise, and the resulting suspension was gradually warm to rt and stirred overnight at rt. The reaction was cooled to 0 °C, and saturated aqueous ammonium chloride (10 mL) was added to the mixture. The organic layer was separated, and the aqueous layer was extracted with diethyl ether (2 × 15 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (ethyl acetate/petroleum: from 1:10 to 1:5).

*6.1.1.1. (E)-1-Phenyl-1-buten-3-ol (**1a**).* Colorless oil was obtained with yield of 92%; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.22–7.38 (m, 5H), 6.54 (d, J = 15.9 Hz, 1H), 6.24 (dd, J_1 = 6.3 Hz, J_2 = 15.9 Hz, 1H), 4.46 (apparent pent, J = 6.3 Hz), 2.00 (br, s, 1H), 1.35 (d, J = 6.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 136.9, 133.7, 129.4, 128.7, 127.7, 126.6, 68.9, 23.5.

*6.1.1.2. (E)-1-Phenyl-1-penten-3-ol (**2a**).* Colorless oil was obtained with yield of 88%; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.22–7.39 (m, 5H),



Scheme 1. Reagents and conditions: a) HI, CuI, 75%; b) MEMCl, K_2CO_3, 84%; c) $Pd(PPh_3)_4$, CuI, Et_3N, HC≡CTMS, 82%; d) HCl, quantitative; e) $(COCl)_2$, DCM, catalytic DMF; f) $H_2NOPMB \cdot HCl$. DIPEA, 0 °C to rt, 79%; g) CsF, quantitative.



Scheme 2. Reagents and conditions: a) for **1a**–**11a**: RMgX, anhydrous Et_2O, 0 °C to rt, overnight, 50–92%; b) for **12a**: $(Bu)_2BOTf$, *i*-Pr_2NEt_2, dry DCM, −78 °C to −20 °C, 23%; c) for **13a**–**16a**: *n*-BuLi, *t*-BuOK, R–CH_3, −15 °C to rt, overnight, 70–89%; d) DPPA, DBU, dry toluene, 45.6–80%; e) terminal alkyne precursor F, CuI·$P(OEt)_3$, DIPEA, 59.5–89%; f) TFA, Triisopropylsilane, DCM, 25.6–87.6%.

6.56 (d, $J = 15.9$ Hz, 1H), 6.20 (dd, $J_1 = 15.9$ Hz, $J_2 = 6.7$ Hz, 1H), 4.19 (q, $J = 6.2$ Hz, 1H), 1.83 (s, 1H), 1.60–1.71 (m, 2H), 0.96 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 136.8, 132.3, 130.4, 128.6, 128.5, 127.6, 126.5, 126.4, 74.4, 30.3.

*6.1.1.3. (E)-1-Phenylhex-1-en-3-ol (**3a**).* Colorless oil was obtained with yield of 78%; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.21–7.39 (m, 5H), 6.56 (d, $J = 15.9$ Hz, 1H), 6.22 (dd, $J_1 = 6.8$ Hz, $J_2 = 15.9$ Hz, 1H), 4.28 (q, $J = 6.6$ Hz, 1H), 1.35–1.64 (m, 4H), 0.95 (t, $J = 0.95$ Hz, 3H).

*6.1.1.4. (E)-1-Phenylhept-1-en-3-ol (**4a**).* Colorless oil was obtained with yield of 72%; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.21–7.41 (m, 5H), 6.56 (d, $J = 15.9$ Hz, 1H), 6.22 (dd, $J_1 = 6.8$ Hz, $J_2 = 15.9$ Hz, 1H), 4.27 (apparent q, $J = 6.5$ Hz, 1H), 1.61–1.66 (m, 1H), 1.33–1.41 (m, 4H), 0.89–0.96 (m, 3H).

*6.1.1.5. (E)-4-Methyl-1-phenyl-1-penten-3-ol (**5a**).* Colorless oil was obtained with yield of 82%; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.41 (d, $J = 7.1$ Hz, 2H), 7.34 (t, $J = 7.4$ Hz, 2H), 7.26 (t, $J = 7.3$ Hz, 1H), 6.59 (d, $J = 15.9$ Hz, 1H), 6.25 (dd, $J_1 = 7.0$ Hz, $J_2 = 15.9$ Hz, 1H), 4.04 (t, $J = 6.7$ Hz, 1H), 1.79–1.91 (m, 1H), 1.67 (br, 1H), 1.01 (d, $J = 6.8$ Hz, 3H), 0.97 (d, $J = 6.8$ Hz, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ: 136.9, 131.3, 131.0, 128.7, 127.7, 126.6, 78.3, 34.2, 18.4, 18.2.

*6.1.1.6. (E)-4,4-Dimethyl-1-phenylpent-1-en-3-ol (**6a**).* Light yellow oil was obtained with yield of 50%. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.21–7.40 (m, 5H), 6.57 (d, $J = 15.9$ Hz, 1H), 6.28 (dd, $J_1 = 15.9$ Hz, $J_2 = 7.2$ Hz, 1H), 3.92 (d, $J = 7.2$ Hz, 1H), 1.62 (s, 1H), 0.97 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.9, 131.8, 129.6, 128.6, 127.6, 126.5, 81.0, 35.3, 25.8.

*6.1.1.7. (E)-5-Methyl-1-phenylhex-1-en-3-ol (**7a**).* Colorless oil was obtained with yield of 75%. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.39 (d, $J = 7.2$ Hz, 2H), 7.30–7.34 (m, 2H), 7.24–7.26 (m, 1H), 6.6 (d, $J = 15.9$ Hz, 1H), 6.22 (dd, $J_1 = 15.9$ Hz, $J_2 = 6.9$ Hz, 1H), 4.4 (q, $J = 6.8$ Hz, 1H), 1.73–1.83 (m, 1H), 1.54–1.61 (m, 2H), 1.39–1.46 (m, 1H), 0.959 (d, $J = 6.6$ Hz, 3H), 0.954 (d, $J = 6.6$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.7, 132.8, 130.1, 128.6, 127.6, 126.4, 71.3, 46.4, 24.6, 23.0, 22.5.

*6.1.1.8. (E)-1-Cyclobutyl-3-phenylprop-2-en-1-ol (**8a**).* Colorless oil was obtained with yield of 73%. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.23–7.38 (m, 5H), 6.58 (dd, $J = 15.9$ Hz, 1H), 6.15 (dd, $J_1 = 6.6$ Hz, $J_2 = 15.9$ Hz, 1H), 4.19 (t, $J = 7.2$ Hz, 1H), 2.44–2.53 (m, 1H), 1.81–2.11 (m, 6H), 1.69 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.8, 130.5, 130.2, 128.5, 127.5, 126.4, 76.8, 40.8, 24.4, 23.9, 17.9.

*6.1.1.9. (E)-1-Cyclopentyl-3-phenylprop-2-en-1-ol (**9a**).* Colorless oil was obtained with yield of 76%; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.22–7.39 (m, 5H), 6.58 (d, $J = 15.9$ Hz, 1H), 6.24 (dd, $J_1 = 7.0$ Hz, $J_2 = 15.9$ Hz, 1H), 4.08 (t, $J = 7.3$ Hz, 1H), 2.02–2.12 (m, 1H), 1.80–1.88 (m, 1H), 1.26–1.72 (m, 9H); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 137.0, 131.9, 130.7, 128.7, 127.7, 126.6, 77.3, 46.2, 29.1, 29.0, 25.83, 25.78.

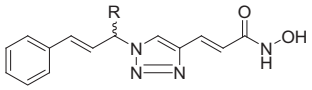
*6.1.1.10. (E)-1-Phenylpenta-1,4-dien-3-ol (**10a**).* Colorless oil was obtained with yield of 83%; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.22–7.39 (m, 5H), 6.61 (d, $J = 15.9$ Hz, 1H), 6.24 (dd, $J_1 = 15.9$ Hz, $J_2 = 6.3$ Hz, 1H), 5.81–5.91 (m, 1H), 5.15–5.21 (m, 2H), 4.36 (q, $J = 6.3$ Hz, 1H), 2.34–2.50 (m, 2H),1.86 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.7, 134.1, 131.6, 130.4, 128.6, 127.7, 126.5, 118.5, 71.8, 42.0.

*6.1.1.11. (E)-1,4-Diphenyl-3-buten-2-ol (**11a**).* Yellow oil was obtained with yield of 80%; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.24–7.38 (m, 10H), 6.6 (d, $J = 15.9$ Hz, 1H), 6.28 (dd, $J_1 = 6.3$ Hz, $J_2 = 15.9$ Hz, 1H), 4.55 (q, $J = 6.4$ Hz, 1H), 2.85–3.00 (m, 2H), 1.73 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 137.8, 136.8, 131.6, 130.5, 129.7, 128.7, 127.8, 126.8, 126.6, 73.6, 44.3 (one peak less due to overlap).

*6.1.2. (E)-4-Phenyl-1-(thiazol-2-yl)but-3-en-2-ol (**12a**)*

To a solution of 2-methylthiazole in dry DCM (10 mL) at −78 °C under nitrogen were successively added $(Bu)_2BTOf$ (0.45 mL), DIPEA (1.06 mL) and cinnamaldehyde (0.56 mL). After stirring at −20 °C overnight, pH 7 phosphate buffer (15 mL) was added and the mixture was warmed to 0 °C. The organic layer was separated, and the aqueous layer was extracted twice with DCM. The combined organic extracts were stirred with 30% H_2O_2 (5 mL) in methanol (15 mL) at rt. After the methanol was removed by concentration under reduced pressure, the mixture was extracted

Table 1
The inhibitory activity results of NSC746457 derivatives against HDACs in vitro.



Compound	R	HDACs IC50[a] (nM)
SAHA		67
TSA		3
NSC746457		104
1	H_3C-	62
2	H_3C	55
3		132
4		137
5		22
6		18
7		838
8		54
9		115
10		90
11		77
12		171
13		403
14		510
15		111
16		94

[a] Values are means of at least three independent experiments. IC_{50} values are calculated from respective concentration-effect curves.

with diethyl ether. The organic extracts were washed with saturated aqueous $NaHCO_3$ and brine, then were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by chromatography (ethyl acetate/petroleum: from 1:8 to1:3) to afford the compound **12a** as yellow oil with the yield of 23%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.74 (d, J = 3.4 Hz, 1H), 7.22–7.39 (m, 6H), 6.69 (d, J = 15.9 Hz, 1H), 6.29 (dd, J_1 = 6.1 Hz, J_2 = 15.9 Hz, 1H), 4.76–4.81 (m, 1H), 3.36 (dd, J_1 = 3.6 Hz, J_2 = 15.5 Hz, 1H), 3.24 (dd, J_1 = 8.3 Hz, J_2 = 15.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.4, 142.3, 136.6, 130.9, 130.3, 128.6, 127.7, 126.5, 118.5, 71.4, 40.0.

6.1.3. General procedure for preparation of compound *13a–16a*

A solution of aromatic compounds (1 mmol in 3 mL anhydrous THF) at −15 °C under nitrogen was added *t*-BuOK (1.5 equiv.) and *n*-BuLi (1.5 equiv.), which resulted in formation of a red reaction mixture. After 1 h, cinnamaldehyde (1.5 equiv.) was added dropwise and the mixture was gradually warmed to rt and stirred at rt overnight under nitrogen. The reaction mixture was poured into 40 mL ice water, and extracted three times with diethyl ether. The combined organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by chromatography (acetate/petroleum ether: from 1:10 to 1:5) to give the compounds **13a–16a**.

*6.1.3.1. (E)-1-(3-Diphenyl)-4-phenylbut-3-en-2-ol (**13a**).* Colorless oil was obtained from (3-methylphenyl)benzene with the yield of 68%; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.20–7.50 (m, 14H), 6.53 (d, J = 15.9 Hz, 1H), 6.23 (dd, J_1 = 6.3 Hz, J_2 = 15.9 Hz, 1H), 4.49 (q, J = 6.4 Hz, 1H), 2.82–2.98 (m, 2H), 1.66 (s, br, 1H).

*6.1.3.2. (E)-1-(3-Phenoxyphenyl)-3-phenylprop-2-en-1-ol (**14a**).* Colorless oil was obtained from 1-methyl-3-phenoxybenzene with the yield of 72%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.20–7.33 (m, 8H), 6.87–7.08 (m, 6H), 6.53 (d, J = 15.9 Hz, 1H), 6.21 (dd, J_1 = 6.3 Hz, J_2 = 15.9 Hz, 1H), 4.47 (q, J = 6.3 Hz, 1H), 2.85–2.88 (m, 2H), 1.92 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 157.4, 157.1, 139.7, 136.6, 131.2, 130.5, 129.7, 128.5, 127.6, 126.5, 124.4, 123.2, 119.9, 118.83, 118.78, 117.0, 73.3, 43.9.

*6.1.3.3. (E)-1-(Naphthalen-1-yl)-3-phenylprop-2-en-1-ol (**15a**).* Yellow oil was obtained from 1-methylnaphthalene with the yield of 73%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.09 (d, J = 8.3 Hz, 1H), 7.87 (d, J = 7.9 Hz, 1H), 7.76 (d, J = 7.3 Hz, 1H), 7.47–7.56 (m, 2H), 7.28–7.44 (m, 6H), 7.21–7.25 (m, 1H), 6.61 (d, J = 15.9 Hz, 1H), 6.34 (dd, J_1 = 15.9 Hz, J_2 = 6.3 Hz, 1H), 4.65–4.70 (m, 1H), 3.45 (dd, J_1 = 13.9 Hz, J_2 = 4.8 Hz, 1H), 3.30 (dd, J_1 = 13.8 Hz, J_2 = 8.1 Hz, 1H), 1.80 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.7, 134.0, 133.8, 132.2, 131.6, 130.3, 128.9, 128.6, 128.0, 127.7, 127.5, 126.5, 126.1, 125.7, 125.5, 123.8, 72.8, 41.3.

*6.1.3.4. (E)-1-(Naphthalen-2-yl)-3-phenylprop-2-en-1-ol (**16a**).* Yellow oil was obtained from 2-methylnaphthalene with the yield of 42%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.80–7.86 (m, 4H), 6.21–7.47 (m, 8H), 6.50 (d, J = 15.9 Hz, 1H), 6.36 (dd, J_1 = 5.8 Hz, J_2 = 15.9 Hz, 1H), 5.10 (d, J = 4.8 Hz, 1H), 4.46 (quint, J = 5.8 Hz, 1H), 2.97 (d, J = 6.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 136.7, 136.6, 133.7, 133.0, 131.6, 128.5, 128.3, 127.5, 127.3, 127.2, 127.1, 126.0, 125.7, 125.1, 71.9, 43.8 (two peaks less due to overlap).

6.1.4. General procedure for synthesis of compounds *1b–16b* from *1a–16a*

Alcohol **1a–16a** (1.0 equiv.) and diphenylphosphoryl azide (DPPA) (1.2 equiv.) were dissolved in dry toluene (0.5 mmol/mL) and cooled to 0 °C. Neat DBU (1.2 equiv.) was added, and the mixture was stirred at 0 °C for 2 h, and at rt overnight. The reaction was quenched by addition of water and the mixture was extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (from petroleum ether to 5% ethyl acetate in petroleum ether).

*6.1.4.1. 1-((E)-3-Azidobut-1-enyl)-benzene (**1b**).* Light yellow oil was obtained from **1a** with the yield of 91%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40 (d, J = 7.7 Hz, 2H), 7.31–7.35 (m, 2H), 7.25–7.28 (m, 1H), 6.6 (d, J = 15.8 Hz, 1H), 6.14 (dd, J_1 = 7.4 Hz, J_2 = 15.8 Hz, 1H), 4.14–4.21 (m,1H), 1.38 (d, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.2, 132.3, 128.8, 128.5, 128.2, 126.8, 59.8, 20.4.

*6.1.4.2. [(1E)-3-Azido-1-penten-1-yl]-benzene (**2b**).* Light yellow oil was obtained from **2a** with the yield of 91%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40 (d, J = 7.5 Hz, 2H), 7.33 (dd, J_1 = 7.2 Hz, J_2 = 7.5 Hz, 2H), 7.26 (t, J = 7.2 Hz, 1H), 6.61 (d, J = 15.8 Hz, 1H), 6.10 (dd, J_1 = 15.8 Hz, J_2 = 8.0 Hz, 1H), 3.90–3.96 (m, 1H), 1.60–1.72 (m, 2H), 0.98 (t,

Table 2
In vitro testing expressed growth inhibition of cancer cell for compound NSC746457, compound **1**, **5** and **11**.[a]

Panel/Cell line	Compound							
	NSC746457		**1**		**5**		**11**	
	GI50[b]/μM	LC50[c]/μM	GI50[b]/μM	LC50[c]/μM	GI50[b]/μM	LC50[c]/μM	GI50[b]/μM	LC50[c]/μM
Leukemia								
CCRF-CEM	0.251	>100	0.442	>100	0.351	>100	0.320	>100
HL-60(TB)	0.249	>100	0.269	>100	0.728	>100	0.670	>100
K-562	0.550	>100	0.355	>100	0.705	>100	1.05	>100
MOLT-4	0.311	86.9	0.377	>100	0.427	>100	0.493	>100
RPMI-8226	0.523	>100	0.407	>100	0.317	>100	0.743	>100
SR	2.99	>100	0.385	>100	0.213	>100	0.624	>100
Non-small cell lung cancer								
A549/ATCC	1.09	94.6	1.47	>100	1.33	88.7	1.41	64.6
EKVX	1.75	>100	2.09	>100	1.81	>100	1.46	87.4
HOP-62	0.502	>100	1.34	48.2	1.24	23.4	1.44	18.3
HOP-92	0.199	68.2	0.677	7.53	1.73	22.1	1.08	11.6
NCI-H226	1.41	20.0	2.35	71.4	2.38	40.1	2.44	46.8
NCI-H23	1.50	>100	1.25	6.61	1.64	12.6	1.51	8.68
NCI-H322M	2.26	>100	1.28	>100	1.36	>100	1.37	46.7
NCI-H460	0.700	>100	0.612	42.7	0.761	42.9	1.04	27.2
NCI-H522	–	–	0.173	3.62	0.582	8.48	0.386	6.36
Colon cancer								
COLO 205	1.08	50.4	0.469	5.20	0.932	6.10	1.10	8.00
HCC2998	–	–	0.927	6.91	–	–	1.24	6.12
HCT-116	0.347	51.2	0.341	3.81	0.316	3.85	0.382	5.97
HCT-15	2.07	56.4	2.09	42.8	2.90	>100	1.59	74.5
HT29	0.566	>100	0.325	33.9	0.827	>100	0.547	>100
KM12	1.04	29.9	1.29	6.82	1.22	9.53	1.93	34.2
SW-620	0.478	81.3	0.383	5.15	0.649	9.22	0.892	13.6
CNS cancer								
SF-268	1.53	92.1	1.51	12.2	1.87	29.7	1.22	9.17
SF-295	0.743	6.91	1.06	>100	1.47	58.3	0.291	6.56
SF-539	0.965	57.1	0.934	4.61	1.19	8.21	1.14	6.91
SNB-19	1.53	31.7	1.87	37.6	2.41	66.6	2.00	18.8
SNB-75	0.318	>100	0.924	33.9	1.82	38.3	0.258	9.83
U251	0.773	5.82	0.777	4.50	1.13	5.66	0.948	5.84
Melanoma								
LOX IMVI	1.08	6.04	0.438	4.12	1.18	5.47	0.521	4.62
MALME−3M	0.461	82.0	0.279	51.9	0.311	68.4	0.305	28.6
M14	0.849	93.8	0.777	9.08	–	–	1.18	16.5
MDA-MB-435	–	–	0.440	30.8	0.719	31.6	0.866	15.7
SK-MEL-2	–	–	0.614	37.0	1.52	>100	1.89	49.2
SK-MEL-28	0.993	31.9	0.819	24.5	1.59	44.9	1.30	9.40
SK-MEL-5	0.271	4.02	0.573	4.52	0.730	5.17	0.879	5.30
UACC-257	0.393	44.8	1.35	>100	3.12	88.3	1.68	93.7
UACC-62	0.379	6.39	0.524	7.99	0.642	6.61	0.898	6.34
Ovarian cancer								
IGROV1	0.187	48.9	0.564	5.74	1.08	31.6	0.836	8.18
OVCAR-3	1.11	51.0	1.13	7.33	1.53	22.1	1.16	8.70
OVCAR-4	3.21	>100	0.636	18.3	4.06	71.4	1.75	61.6
OVCAR-5	0.260	38.9	0.488	>100			0.426	22.3
OVCAR-8	0.450	92.5	0.232	6.91	0.816	>100	0.630	>100
NCI/ADR-RES	–	–	–	–	–	–	0.342	>100
SK-OV-3	0.524	28.5	1.17	21.8	1.49	27.8	1.98	13.3
Renal cancer								
786−0	2.38	52.4	1.64	9.03	1.59	6.74	1.38	6.45
A498	1.11	5.88	0.472	4.83	1.51	8.63	1.16	5.46
ACHN	1.10	94.3	0.765		1.21	71.1	1.23	13.7
CAKI-1	0.619	5.79	0.401	5.86	0.487	6.30	0.440	6.25
RXF 393	<0.01	15.3	1.41	34.7	1.19	7.68	1.21	7.61
SN 12C	1.62	>100	1.29	>100	1.86	>100	2.34	>100
TK-10	0.495	>100	0.582	>100	0.531	>100	0.827	12.9
UO-31	1.12	52.8	0.549	31.9	1.48	40.9	0.650	6.57
Prostate cancer								
PC-3	1.30	73.5	0.890	66.7	1.65	46.5	1.52	>100
DU-145	0.580	>100	0.803	>100	1.09	>100	1.08	46.3
Breast cancer								
MCF7	0.730	>100	1.16	>100	1.32	>100	1.21	93.2
NCI/ADR-RES	0.180	>100	–	–	–	–	–	–
MDA-MB-231/ATCC	1.96	65.8	1.38	>100	1.97	73.4	2.21	53.9
HS 578T	0.619	>100	0.692	>100	–	–	0.934	>100
HAD-MB-435	0.537	28.8	–	–	–	–	–	–

(*continued on next page*)

Table 2 (*continued*)

Panel/Cell line	Compound							
	NSC746457		**1**		**5**		**11**	
	GI50[b]/μM	LC50[c]/μM	GI50[b]/μM	LC50[c]/μM	GI50[b]/μM	LC50[c]/μM	GI50[b]/μM	LC50[c]/μM
BT-549	3.92	85.6	2.11	>100	3.28	>100	1.62	74.6
T-47D	0.444	>100	0.667	>100	1.24	>100	0.411	>100
MDA-MB-468	0.452	94.3	0.299	7.63			1.52	61.7

[a] Data obtained from NIH's in vitro disease-oriented human tumor cell lines screen.
[b] GI50 is the drug concentration required to reduce the growth of treated cells to half that of control cells (as judged from the SRB signal) during the drug incubation. Determined at five concentration levels (100, 10, 1.0, 0.1 and 0.01 μM).
[c] LC50, which signifies a cytotoxic effect, is the molar concentration needed to kill 50% of the cells.

J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.2, 133.4, 128.8, 128.2, 127.2, 126.8, 66.4, 28.1, 10.5.

*6.1.4.3. (E)-(3-Azidohex-1-en-1-yl)-benzene (**3b**).* Yellow oil was obtained from **3a** with the yield of 59.7%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.24–7.41 (m, 5H), 6.60 (d, J = 15.8 Hz, 1H), 6.10 (dd, J_1 = 8.1 Hz, J_2 = 15.8 Hz, 1H), 4.00 (q, J = 7.3 Hz, 1H), 1.36–1.67 (m, 4H), 0.94 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 136.1, 133.1, 128.6, 128.1, 127.3, 126.6, 64.6, 36.7, 19.1, 13.7.

*6.1.4.4. (E)-(3-Azidohept-1-en-1-yl)-benzene (**4b**).* Yellow oil was obtained from **4a** with the yield of 81.4%; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.24–7.41 (m, 5H), 6.6 (d, J = 15.8 Hz, 1H), 6.34 (dd, J_1 = 8.0 Hz, J_2 = 15.8 Hz, 1H), 3.98 (apparent q, J = 7.3 Hz, 1H), 1.56–1.65 (m, 2H), 1.27–1.40 (m, 4H), 0.91 (t, J = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.1, 133.1, 128.7, 128.1, 127.3, 126.7, 65.0, 34.5, 28.1, 22.4, 14.0.

*6.1.4.5. [(1E)-3-Azido-4-methyl-1-penten-1-yl]-benzene (**5b**).* Light yellow oil was obtained from **5a** with the yield of 88%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41 (d, J = 7.7 Hz, 2H), 7.31–7.35 (m, 2H), 7.24–7.28 (m, 1H), 6.6 (d, J = 15.8 Hz, 1H), 6.13 (dd, J_1 = 8.5 Hz, J_2 = 15.8 Hz, 1H), 3.76–3.80 (m, 1H), 1.78–1.87 (m, 1H), 0.99 (d, J = 6.7 Hz, 2H), 0.95 (d, J = 6.7 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.3, 134.3, 128.8, 128.2, 126.8, 125.8, 71.4, 33.0, 19.1, 19.0.

*6.1.4.6. 1-((E)-3-Azido-4,4-dimethylpent-1-enyl)-benzene (**6b**).* Colorless oil was obtained from **6a** with the yield of 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.24–7.43 (m, 5H), 6.61 (d, J = 15.8 Hz, 1H), 6.19 (dd, J_1 = 15.8 Hz, J_2 = 8.9 Hz, 1H), 3.74 (d, J = 8.9 Hz, 1H), 0.96 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.2, 134.8, 128.7, 128.1, 126.7, 124.6, 75.2, 35.1, 26.4.

*6.1.4.7. (E)-5-Methyl-1-phenylhex-1-en-3-ol (**7b**).* Colorless oil was obtained from **7a** with the yield of 82%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.24–7.4 (m, 5H), 6.61 (d, J = 15.8 Hz, 1H), 6.09 (dd, J_1 = 8.1 Hz, J_2 = 15.7 Hz, 1H), 4.03 (q, J = 7.5 Hz, 1H), 1.71–1.78 (m, 1H), 1.52–1.59 (m, 1H), 1.38–1.45 (m, 1H), 0.945 (d, J = 5.5 Hz, 3H), 0.931 (d, J = 5.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 135.9, 132.9, 128.5, 127.9, 127.2, 126.5, 63.0, 43.4, 24.7, 22.4, 22.2.

*6.1.4.8. (E)-(3-Azido-3-cyclobutylprop-1-en-1-yl)-benzene (**8b**).* Colorless oil was obtained from **8a** with the yield of 79%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.22–7.40 (m, 5H), 6.60 (d, J = 15.8 Hz, 1H), 6.04 (dd, J_1 = 8.0 Hz, J_2 = 15.8 Hz, 1H), 3.93 (t, J = 8.2 Hz, 1H), 2.47–2.57 (m, 1H), 1.79–2.14 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.2, 133.6, 128.7, 128.1, 126.7, 125.3, 69.4, 39.2, 25.4, 24.9, 18.0.

*6.1.4.9. (E)-(3-Azido-3-cyclopentylprop-1-en-1-yl)-benzene (**9b**).* Colorless oil was obtained from **9a** with the yield of 64%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.26–7.43 (m, 5H), 6.61 (d, J = 15.8 Hz, 1H), 6.149 (dd, J_1 = 8.5 Hz, J_2 = 15.8 Hz, 1H), 2.84 (t, J = 8.3 Hz, 1H), 2.03–2.13 (m, 1H), 1.81–1.89 (m, 1H), 1.52–1.73 (m, 5H), 1.38–1.47 (m, 1H), 1.25–1.34 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.1, 133.5, 128.6, 128.0, 126.6, 69.8, 44.3, 29.55, 29.46, 25.45, 25.36 (one peak less due to overlap).

*6.1.4.10. 1-((E)-3-Azidohexa-1,5-dienyl)-benzene (**10b**).* Colorless oil was obtained from **10a** with the yield of 80%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42 (d, J = 7.9 Hz, 1H), 7.33–7.37 (m, 2H), 7.27–7.31 (m, 2H), 6.64 (d, J = 15.8 Hz, 1H), 6.15 (dd, J_1 = 15.8 Hz, J_2 = 7.9 Hz, 1H), 5.78–5.88 (m, 1H), 5.19 (d, J = 15.6 Hz, 1H), 5.16 (d, J = 8.7 Hz, 1H), 4.11 (q, J = 7.1 Hz, 1H), 2.42 (t, J = 6.9 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 135.9, 133.5, 128.7, 128.2, 126.7, 126.5, 118.3, 64.1, 39.2 (one peak less due to overlap).

*6.1.4.11. (E)-3-Azido-1,4-diphenylbut-1-ene (**11b**).* Yellow oil was obtained from **11a** with the yield of 90%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.23–7.39 (m, 10H), 6.58 (d, J = 15.8 Hz, 1H), 6.15 (dd, J_1 = 7.9 Hz, J_2 = 15.8 Hz, 1H), 4.24–4.29 (m, 1H), 2.91 (d, J = 6.9 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 137.3, 136.1, 133.6, 129.6, 128.8, 128.6, 128.3, 127.0, 126.8, 126.6, 65.9, 41.6.

*6.1.4.12. 2-((E)-2-Azido-4-phenylbut-3-enyl)thiazole (**12b**).* Yellow oil was obtained from **12a** with the yield of 46%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.75 (d, J = 3.3 Hz, 1H), 7.19–7.41 (m, 6H), 6.68 (d, J = 15.8 Hz, 1H), 6.19 (dd, J_1 = 15.8 Hz, J_2 = 8.0 Hz, 1H), 4.58 (q, J = 7.3 Hz, 1H), 3.32 (d, J = 6.9 Hz, 2H); ^{13}C NMR(100 MHz, $CDCl_3$) δ: 165.4, 142.6, 134.4, 129.9, 128.7, 128.4, 126.8, 125.4, 119.2, 64.0, 38.6.

*6.1.4.13. 1-((E)-2-Azido-4-phenylbut-3-enyl)-3-phenylbenzene (**13b**).* Yellow oil was obtained from **13a** with the yield of 67%; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.30–7.41 (m, 8H), 6.94–7.13 (m, 6H), 6.61 (d, J = 15.8 Hz, 1H), 6.15 (dd, J_1 = 8.0 Hz, J_2 = 15.8 Hz, 1H), 4.28 (q, J = 7.3 Hz, 1H), 2.92 (d, J = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 157.4, 157.2, 139.2, 135.9, 133.7, 129.83, 129.80, 128.7, 128.3, 12.6.8, 126.3, 124.4, 123.3, 120.0, 118.9, 117.4, 65.6, 41.3.

*6.1.4.14. 1-((E)-2-Azido-4-phenylbut-3-enyl)-3-phenoxybenzene (**14b**).* Yellow oil was obtained from **14a** with the yield of 76%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.21–7.36 (m, 8H), 6.88–7.08 (m, 6H), 6.55 (d, J = 15.8 Hz, 1H), 6.10 (dd, J_1 = 8.0 Hz, J_2 = 15.8 Hz, 1H), 4.23 (q, J = 7.3 Hz, 1H), 2.82–2.88 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 157.3, 157.1, 139.1, 135.8, 133.6, 129.73, 129.69, 128.6, 128.2, 126.6, 126.2, 124.3, 123.2, 119.9, 118.8, 117.3, 65.5, 41.2.

*6.1.4.15. 1-((E)-2-Azido-4-phenylbut-3-enyl)naphthalene (**15b**).* Colorless oil was obtained from **15a** with the yield of 50%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.03 (d, J = 8.3 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.77 (d, J = 7.8 Hz, 1H), 7.48–7.57 (m, 2H), 7.24–7.44 (m, 7H), 6.56 (d, J = 15.8 Hz, 1H), 6.22 (dd, J_1 = 15.8 Hz, J_2 = 7.9 Hz, 1H), 4.45 (q, J = 7.2 Hz, 1H), 3.37 (d, J = 6.9 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 135.9, 134.0, 133.4, 133.2, 131.9, 129.1, 128.7, 128.2, 128.1, 127.8, 126.8, 126.7, 126.2, 125.7, 125.5, 123.4, 65.1, 38.6.

*6.1.4.16. 2-((E)-2-Azido-4-phenylbut-3-enyl)naphthalene (**16b**).* Yellow oil was obtained from **16a** with the yield of 51%; ^{1}H NMR

(300 MHz, $CDCl_3$) δ: 7.61–7.63 (m, 4H), 7.15–7.39 (m, 8H), 6.53 (d, J = 15.8 Hz, 1H), 6.11 (dd, J_1 = 7.9 Hz, J_2 = 15.8 Hz, 1H), 4.28 (q, J = 7.0 Hz, 1H), 3.00 (d, J = 6.9 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 135.9, 134.6, 133.5, 133.4, 132.4, 128.6, 128.2, 128.1, 128.0, 127.64, 127.59, 126.7, 126.4, 126.1, 125.6, 65.7, 41.5 (one peak less due to overlap).

*6.1.5. General procedure for synthesis of compounds **1c**–**16c** from **1b**–**16b***

The obtained white solid terminal alkyne **F** (1.0 equiv.) was dissolved in THF (20 mL). Azido compound **1b**–**16b** (1.2 equiv.), $CuI \cdot P(OEt)_3$ (1.0 equiv.) and DIPEA (2.0 equiv.) were added sequentially. The reaction was stirred overnight at rt, monitored by TLC. The solvent was removed under reduced pressure, and the residue was participated between DCM and saturated aqueous NH_4Cl. The aqueous layer was extracted twice with DCM, and the combined organic extracts were dried with Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified with flash chromatograph (MeOH/DCM: from 1% to 5%) to give the title product.

*6.1.5.1. (2E)-N-(4-Methoxybenzyloxy)-3-(1-((E)-4-phenylbut-3-en-2-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**1c**).* White solid was obtained from **1b** with the yield of 94%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.31 (s, 1H), 8.50 (s, 1H), 7.45–7.52 (m, 3H), 7.24–7.36 (m, 5H), 6.94 (d, J = 8.1, 2H), 6.53–6.65 (m, 3H), 5.50 (t, J = 6.3 Hz, 1H), 4.81 (s, 2H), 3.74 (s, 3H), 1.71 (d, J = 6.7, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.6, 159.4, 143.0, 135.7, 131.7, 130.7, 128.7, 128.3, 128.2, 127.9, 126.6, 123.4, 119.0, 113.7, 76.7, 58.0, 55.1, 20.6 (one peak less due to overlap).

*6.1.5.2. (2E)-N-(4-Methoxybenzyloxy)-3-(1-((E)-1-phenylpent-1-en-3-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**2c**).* White solid was obtained from **2b** with the yield of 92%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.28 (s, 1H), 8.52 (s, 1H), 7.44–7.48 (m, 3H), 7.26–7.26 (m, 5H), 6.94 (d, J = 8.4 Hz, 2H), 6.53–6.65 (m, 3H), 5.26 (apparent q, J = 7.3 Hz, 1H), 4.79 (s, 2H), 3.76 (s, 3H), 2..-2.13 (m, 2H), 0.83 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.6, 159.4, 142.9, 135.7, 132.7, 130.7, 128.7, 128.3, 128.2, 127.8, 127.5, 126.6, 123.7, 119.0, 113.7, 76.7, 64.1, 55.1, 27.9, 10.2.

*6.1.5.3. (2E)-N-(4-Methoxybenzyloxy)-3-(1-((E)-1-phenylhex-1-en-3-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**3c**).* White solid was obtained from **3b** with the yield of 62%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.27 (s, 1H), 8.52 (s, 1H), 7.26–7.48 (m, 8H), 6.94 (d, J = 8.4 Hz, 2H), 6.53–6.66 (m, 3H), 5.35 (q, J = 7.3 Hz, 1H), 4.79 (s, 2H), 3.76 (s, 3H), 1.94–2.11 (m, 2H), 1.14–1.29 (m, 2H), 0.90 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.5, 159.4, 142.8, 135.7, 132.5, 130.7, 128.6, 128.2, 128.1, 127.8, 127.6, 126.6, 123.5, 119.0, 113.7, 76.6, 62.4, 55.1, 36.5, 18.6, 13.3.

*6.1.5.4. (2E)-N-(4-Methoxybenzyloxy)-3-(1-((E)-1-phenylhept-1-en-3-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**4c**).* White solid was obtained from **4b** with the yield of 73%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.27 (s, 1H), 8.52 (s, 1H), 7.26–7.48 (m, 8H), 6.95 (d, J = 7.8 Hz, 2H), 6.54–6.66 (m, 3H), 5.34 (apparent q, J = 7.2 Hz, 1H), 4.79 (s, 2H), 3.76 (s, 3H), 2.01–2.10 (m, 2H), 1.10–1.37 (m, 4H), 0.85 (t, J = 7.1 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 162.7, 159.4, 142.9, 135.7, 132.5, 130.6, 128.6, 128.3, 128.1, 127.9, 127.7, 126.6, 123.5, 119.1, 113.7, 76.7, 62.7, 55.0, 34.3, 27.4, 21.6, 13.7; HRMS (ESI) calcd for $C_{26}H_{30}N_4O_3$ $[M + H]^+$ 447.2391 found 447.2385.

*6.1.5.5. (2E)-N-(4-Methoxybenzyloxy)-3-(1-((E)-4-methyl-1-phenylpent-1-en-3-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**5c**).* White solid was obtained from **5b** with the yield of 79%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.28 (s, 1H), 8.52 (s, 1H), 7.43–7.48 (m, 3H), 7.25–7.3 5 (m, 5H), 6.93 (d, J = 8.3 Hz, 2H), 6.55–6.72 (m, 3H), 5.01 (t, J = 8.4 Hz, 1H), 4.78 (s, 2H), 3.74 (s, 3H), 2.74–2.36 (m, 1H), 0.95 (d, J = 6.7 Hz, 3H), 0.74 (d, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.6, 159.4, 142.8, 135.7, 134.2, 130.7, 128.7, 128.3,127.9, 126.7, 125.9, 123.8, 119.1, 113.7, 76.7, 69.0, 65.1, 32.9, 18.9, 18.8 (one peak less due to overlap).

*6.1.5.6. (2E)-N-(4-Methoxybenzyloxy)-3-(1-((E)-4,4-dimethyl-1-phenylpent-1en-3-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**6c**).* White solid was obtained from **6b** with the yield of 63%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.28 (s, 1H), 8.56 (s, 1H), 7.27–7.53 (m, 8H), 6.95 (d, J = 8.4 Hz, 2H), 6.83 (dd, J_1 = 9.2 Hz, J_2 = 15.7 Hz, 1H), 6.71 (d, J = 15.7 Hz, 1H), 6.59 (d, J = 15.7 Hz, 1H), 5.13 (d, J = 9.2 Hz, 1H), 4.80 (s, 2H), 3.76 (s, 3H), 0.95 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.5, 159.3, 142.2, 135.7, 134.9, 130.7, 128.6, 128.2, 127.8, 126.7, 124.7, 124.0, 119.0, 113.7, 76.6, 71.7, 55.1, 35.3, 26.4 (one peak less due to overlap).

*6.1.5.7. (2E)-N-(4-Methoxybenzoxy)-3-(1-((E)-5-methyl-1-phenylhex-1-en-3-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**7c**).* White solid was obtained from **7b** with the yield of 63%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.3 (s, 1H), 8.56 (s, 1H), 7.26–7.51 (m, 8H), 6.95 (d, J = 8.4 Hz, 2H), 6.53–6.69 (m, 3H), 5.41–5.47 (m, 1H), 4.82 (s, 2H), 3.77 (s, 3H), 2.01–2.08 (m, 1H), 1.84–1.91 (m, 1H), 1.31–1.38 (m, 1H), 0.95 (d, J = 6.6 Hz, 3H), 0.89 (d, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.6, 159.4, 142.9, 135.7, 132.4, 130.7, 128.6, 128.2, 128.1, 127.8, 126.6, 123.5, 119.1, 113.7, 76.6, 61.0, 55.o, 43.2, 24.3, 22.1, 21.8 (one peak less due to overlap).

*6.1.5.8. (2E)-N-(4-Methoxybenzyloxy)-3-(1-((E)-1-cyclobutyl-3-phenylallyl)-1H-1,2,3-triazol-4yl)acrylamide (**8c**).* White solid was obtained from **8b** with the yield of 81%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.26 (s, 1H), 8.48 (s, 1H), 7.26–7.48 (m, 8H), 6.94 (d, J = 8.3 Hz, 2H), 6.44–6.69 (m, 3H), 5.31 (t, J = 8.8 Hz, 1H), 4.79 (s, 2H), 3.76 (s, 3H), 2.95–3.05 (m, 1H), 1.70–2.09 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.5, 159.3, 142.7, 135.6, 133.1, 130.5, 128.5, 128.2, 128.0, 127.7, 126.5, 125.3, 123.4, 119.0, 113.6, 76.5, 67.2, 55.0, 38.2, 24.6, 24.5, 16.9.

*6.1.5.9. (2E)-N-(4-Methoxybenzyloxy)-3-(1-((E)-1-cyclopentyl-3-phenylallyl)-1H-1,2,3-triazol-4-yl)acrylamide (**9c**).* White solid was obtained from **9b** with the yield of 84%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.27 (s, 1H), 8.54 (s, 1H), 7.26–7.48 (m, 8H), 6.95 (d, J = 8.2 Hz, 1H), 6.56–6.70 (m, 3H), 5.12 (t, J = 8.4 Hz, 1H), 4.80 (s, 2H), 3.76 (s, 3H), 2.56–2.67 (m, 1H), 1.75–1.79 (m, 1H), 1.36–1.62 (m, 6H), 1.12–1.18 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.5, 159.3, 142.7, 135.6, 133.1, 130.6, 128.6, 128.2, 128.1, 127.8, 126.9, 126.6, 123.5, 119.0, 113.6, 76.6, 67.4, 55.0, 44.0, 29.24, 29.16, 24.9, 24.6.

*6.1.5.10. (2E)-N-(4-Methoxybenzyloxy)-3-(1-((E)-1-phenylhexa-1,5-dien-3-yl)-1H-1,2,3-triazole-4-yl)acrylamide (**10c**).* White solid was obtained from **10b** with the yield of 84%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.26 (s, 1H), 8.51 (s, 1H), 7.26–7.47 (m, 8H), 6.94 (d, J = 8.4 Hz, 2H), 6.54–6.65 (m, 3H), 5.65–5.75 (m, 1H), 5.44–5.50 (m, 1H), 5.01–5.10 (m, 2H), 4.78 (s, 2H), 3.76 (s, 3H), 2.82–2.87 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.5, 159.3, 142.8, 135.6, 133.2, 132.6, 130.7, 128.7, 128.2, 127.8, 127.1, 126.6, 123.7, 119.0, 118.6, 113.7, 76.6, 61.9, 55.1, 38.8 (one peak less due to overlap).

*6.1.5.11. (2E)-N-(4-Methoxybenzyloxy)-3-(1-((E)-1,4-diphenylbut-3-en-2-yl)-1H-1,2,3-triazol-4-yl) acrylamide (**11c**).* White solid was obtained from **11b** with the yield of 89%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.32 (br, 1H), 8.49 (s, 1H), 7.42–7.47 (m, 3H), 7.31–7.36 (m, 4H), 7.14–7.28 (m, 6H), 6.94 (d, J = 8.3, 2H), 6.56–6.68 (m, 3H), 5.67 (q, J = 7.3), 4.81 (s, 2H), 3.74 (s, 3H), 3.37–3.47 (m, one proton overlap with H_2O peak); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.5, 159.4, 142.7, 136.7, 135.6, 132.7, 130.7, 129.1, 128.7, 128.3, 128.2, 127.8,

127.4, 126.63, 126.57, 124.0, 119.0, 113.7, 76.6, 63.8, 55.1, 40.5 (one peak less due to overlap).

*6.1.5.12. (2E)-N-(4-Methoxybenzyloxy)-3-(1-((E)-4-phenyl-1-(thiazol-2-yl)but-3-en-2-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**12c**).* Yellow solid was obtained from **12b** with the yield of 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.27 (s, 1H), 8.55 (s, 1H), 7.72 (s, 1H), 7.57 (d, J = 3.0 Hz, 1H), 7.26–7.46 (m, 8H), 6.95 (d, J = 8.5 Hz, 2H), 6.54–6.66 (m, 3H), 5.85 (s, br, 1H), 4.78 (s, 2H), 3.85–3.95 (m, 2H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.4, 159.3, 142.8, 142.4, 135.4, 133.2, 130.7, 128.7, 128.3, 128.1, 127.8, 126.6, 126.5, 124.1, 120.5, 119.1, 113.7, 76.6, 62.1, 55.1, 37.4 (one peak less due to overlap).

*6.1.5.13. (2E)-N-(4-Methoxybenzyloxy)-3-(1-((E)-4-phenyl-1-m-diphenyl-3-en-2-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**13c**).* White solid was obtained from **13b** with the yield of 54%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.27 (s, 1H), 8.52 (s, 1H), 7.26–7.55 (m, 16H), 7.17 (d, J = 7.6 Hz, 1H), 6.94 (d, J = 8.4 Hz, 2H), 6.55–6.74 (m, 3H), 5.74 (q, J = 7.3 Hz, 1H), 4.8 (s, 2H), 3.76 (s, 3H), 3.48 (d, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.5, 159.4, 142.7, 140.1, 140.0, 137.3, 135.6, 132.7, 130.7, 128.8, 128.7, 128.2, 127.8, 127.5, 127.40, 127.35, 126.65, 126.58, 125.0, 124.1, 119.0, 113.7, 76.6, 63.7, 55.1, 40.6 (three peaks less due to overlap).

*6.1.5.14. (2E)-N-(4-Methoxybenzyloxy)-3-(1-((E)-1-(3-phenoxyphenyl)-4-phenylbut-3-en-2-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**14c**).* White solid was obtained from **14b** with the yield of 80%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.28 (s, 1H), 8.46 (s, 1H), 7.25–7.44 (m, 13H), 7.08 (t, J = 7.6 Hz, 1H), 7.02 (d, J = 7.6 Hz, 1H), 6.94 (d, J = 8.4 Hz, 2H), 6.83 (d, J = 8.4 Hz, 4H), 6.54–6.63 (m, 3H), 5.66 (q, J = 7.2 Hz, 1H), 4.80 (s, 2H), 3.76 (s, 3H), 3.40 (d, J = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.4, 159.3, 156.6, 156.1, 142.6, 138.8, 135.5, 132.7, 130.6, 129.8, 128.6, 128.1, 127.7, 127.0, 126.5, 124.4, 123.8, 123.0, 119.6, 118.9, 118.0, 117.2, 113.6, 76.5, 63.5, 55.0 (two peaks less due to overlap, one peak less due to overlap with DMSO-d_6).

*6.1.5.15. (2E)-N-(4-Methoxybenzyloxy)-3-(1-((E)-1-(naphthalen-1-yl)-4-phenylbut-3-en-2-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**15c**).* White solid was obtained from **15b** with the yield of 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.27 (s, 1H), 8.54 (s, 1H), 8.21 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 8.1 Hz, 1H), 7.78 (d, J = 8.2 Hz, 1H), 7.61 (pseudo t, J = 7.9 Hz, 1H), 7.54 (pseudo t, J = 7.5 Hz, 1H) 7.22–7.44 (m, 10H), 6.94 (d, J = 7.9 Hz, 2H), 6.78 (dd, J_1 = 7.5 Hz, J_2 = 15.9 Hz, 1H), 6.52–6.59 (m, 2H), 5.75 (q, J = 7.3 Hz, 1H), 4.79 (s, 2H), 3.84–3.95 (m, 2H), 3.76 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.5, 159.4, 142.7, 135.6, 133.4, 132.8, 132.6, 131.4, 130.7, 128.9, 128.7, 128.6, 128.2, 127.8, 127.50, 127.45, 127.3, 126.6, 126.3, 125.7, 125.3, 123.9, 123.6, 119.0, 113.7, 76.6, 63.1, 55.1, 37.7.

*6.1.5.16. (2E)-N-(4-Methoxybenzyloxy)-3-(1-((E)-1-(naphthalen-3-yl)-4-phenylbut-3-en-2-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**16c**).* White solid was obtained from **16b** with the yield of 46%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.70–7.78 (m, 3H), 7.42–7.58 (m, 5H), 7.23–7.32 (m, 6H), 7.14–7.18 (m, 2H), 6.86 (d J = 8.0 Hz, 2H), 6.45–6.67 (m, 3H), 5.47 (q, J = 6.4 Hz, 1H), 4.88 (s, 2H), 3.77 (s, 3H), 3.65 (dd, J_1 = 13.6 Hz, J_2 = 8.0 Hz, 1H), 3.53 (dd, J_1 = 13.2 Hz, J_2 = 6.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.3, 160.1, 135.4, 134.3, 133.4, 133.3, 132.5, 131.1, 129.0, 128.73, 128.68, 128.6, 128.4, 128.2, 128.1, 127.65, 127.60, 127.0, 126.7, 126.3, 125.9, 125.6, 125.3, 114.1, 65.0, 55.3, 42.0 (two peaks less due to overlap).

*6.1.6. General procedure for preparation of final compounds **1**–**16** from **1c**–**16c***

To a solution of the intermediate **1c**–**16c** (1 mmol, 1.0 equiv.) in DCM (40 mL) were sequentially added triisopropylsilane (3 mmol, 0.6 mL) and TFA (2 mL). The mixture was stirred at rt, monitoring by TLC. Upon completion, the reaction mixture was diluted with acetonitrile (100 mL), and neutralized with DOWEX® MARATHONE® WBA anion exchange resin (Aldrich). The mixture was filtered, and the resin was washed with acetonitrile (50 mL×2). The combined filtrate was concentrated under reduced pressure, and purified as described below.

*6.1.6.1. (2E)-N-Hydroxy-3-(1-((E)-4-phenylbut-3-en-2-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**1**).* The crude product was purified *via* C-18 reverse phase column chromatography (water/acetonitrile: from 5:1 3:1) to provide **1** as a white solid. Yield: 75%. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.78 (s, br, 1H), 9.02 (s, br, 1H), 8.44 (s, 1H), 7.44 (d, J = 7.3 Hz, 2H), 7.38 (d, J = 15.7 Hz, 1H), 7.32 (apparent t, J = 7.3 Hz, 2H), 7.25 (t, J = 7.3 Hz, 1H), 6.51–6.61 (m, 3H), 5.50 (apparent quart, J = 6.6 Hz, 1H), 1.69 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 162.4, 142.9135.6, 131.6, 128.7, 128.6, 128.0, 127.1, 126.5, 123.0, 119.4, 57.8, 20.6; HRMS (ESI) calcd for $C_{15}H_{15}N_4O_2$ $[M - H]^-$ 283.1200 found 283.1200.

*6.1.6.2. (2E)-N-Hydroxy-3-(1-((E)-1-phenylpent-1-en-3-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**2**).* The crude product was purified *via* C-18 reverse phase column chromatography (water/acetonitrile: from 5:1 to 3:1) to provide **2** as a white solid. Yield: 72%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.81 (s, br, 1H), 9.05 (s, br, 1H), 8.48 (s, 1H), 7.46 (d, J = 7.5 Hz, 2H), 7.40 (d, J = 15.7 Hz, 1H), 7.34 (t, J = 7.7 Hz, 2H), 7.27 (t, J = 7.3 Hz, 1H), 6.53–6.64 (m, 3H), 5.25 (q, J = 7.3 Hz, 1H), 2.01–2.12 (m, 2H), 0.83 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.4, 143.0, 135.6, 132.6, 128.6, 128.1, 127.4, 127.1, 126.5, 123.2, 119.5, 64.0, 27.8, 10.1; HRMS (ESI) calcd for $C_{16}H_{17}N_4O_2$ $[M - H]^-$ 297.1357 found 297.1358.

*6.1.6.3. (2E)-N-Hydroxy-3-(1-((E)-1-phenylhex-1-en-3-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**3**).* The crude product was purified *via* C-18 reverse phase column chromatography (water/acetonitrile from 5:1 to 3:1) to provide **3** as a white solid. Yield: 69%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.50 (s, 1H), 7.26–7.48 (m, 6H), 6.53–6.66 (m, 3H), 5.35 (apparent q, J = 7.3 Hz, 1H), 1.94–2.12 (m, 2H), 1.14–1.31 (m, 2H), 0.91 (t, J = 7.3 Hz, 3H) (two active protons less); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.4, 143.0, 135.7, 132.5, 128.6, 128.1, 127.7, 127.1, 126.6, 123.2, 119.5, 62.3, 36.5, 18.6, 13.3; HRMS (ESI) calcd for $C_{17}H_{19}N_4O_2$ $[M - H]^-$ 311.1513 found 311.1510.

*6.1.6.4. (2E)-N-Hydroxy-3-(1-((E)-1-phenylhept-1-en-3-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**4**).* The crude product was purified *via* C-18 reverse phase column chromatography (water/acetonitrile: from 5:1 to 3:1) to provide **4** as a white solid. Yield: 46%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.81 (s, 1H), 9.06 (s, 1H), 8.49 (s, 1H), 7.27–7.48 (m, 6H), 6.53–6.66 (m, 3H), 5.33 (q, J = 7.2 Hz, 1H), 2.00–2.13 (m, 2H), 1.10–1.32 (m, 4H), 0.85 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 162.4, 143.0, 135.7, 132.5, 128.6, 128.1, 127.7, 127.1, 126.6, 123.2, 119.6, 62.6, 34.2, 27.4, 21.6, 13.7; HRMS (ESI) calcd for $C_{18}H_{23}N_4O_2$ $[M + H]^+$ 327.1815 found 327.1821.

*6.1.6.5. (2E)-N-Hydroxy-3-(1-((E)-4-methyl-1-phenylpent-1-en-3-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**5**).* The crude product was purified *via* C-18 reverse phase column chromatography (water/acetonitrile: from 5:1 to 3:1) to provide **5** as a white solid. Yield: 48%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.79 (s, br, 1H), 9.03 (s, br, 1H), 8.47 (s, 1H), 7.46 (d, J = 7.5 Hz, 2H), 7.38 (d, J = 15.7 Hz, 1H), 7.34 (t, J = 7.4 Hz, 2H), 7.27 (t, J = 7.2 Hz, 1H), 6.56–6.70 (m, 3H), 5.00 (t, J = 8.5 Hz, 1H), 2.27–2.34 (m, 1H), 0.94 (d, J = 6.6 Hz, 3H), 0.74 (d, J = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 162.4, 142.9, 135.7, 134.1, 128.6, 128.2,

127.1, 126.6, 125.9, 123.4, 119.6, 68.9, 32.8, 18.84, 18.78; HRMS (ESI) calcd for $C_{17}H_{21}N_4O_2$ $[M + H]^+$ 313.1659 found 313.1665.

*6.1.6.6. (2E)-N-Hydroxy-3-(1-((E)-4,4-dimethyl-1-phenylpent-1-en-3-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**6**).* The crude product was purified *via* C-18 reverse phase column chromatography (water/acetonitrile: from 5:1 to 5:2) to provide **6** as a white solid. Yield: 37%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.51 (s, 1H), 7.51 (d, $J = 7.3$ Hz, 2H), 7.28–7.42 (m, 4H), 6.81 (dd, $J_1 = 8.2$ Hz, $J_2 = 15.7$ Hz, 1H), 6.70 (d, $J = 15.7$ Hz, 1H), 6.61 (d, $J = 15.7$ Hz, 1H), 5.11 (d, $J = 9.1$ Hz, 1H), 0.94 (s, 9H) (two active proton less); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.5, 142.4, 125.7, 134.9, 128.7, 128.2, 127.1, 126.6, 124.4, 123.9, 119.5, 71.8, 35.2, 26.4; HRMS (ESI) calcd for $C_{18}H_{21}N_4O_2$ $[M - H]^-$ 325.1670 found 325.1669.

*6.1.6.7. (2E)-N-Hydroxy-3-(1-((E)-5-methyl-1-phenylhex-1-en-3-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**7**).* The crude product was purified *via* C-18 reverse phase column chromatography (water/acetonitrile: from 5:1 to 5:2) to provide **7** as a white solid. Yield: 26%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.51 (s, 1H), 7.26–7.48 (m, 6H), 6.52–6.68 (m, 3H), 5.42 (dd, $J_1 = 7.1$ Hz, $J_2 = 8.4$ Hz, 1H), 1.99–2.07 (m, 1H), 1.83–1.90 (m, 1H), 1.29–1.36 (m, 1H), 0.94 (d, $J = 6.6$ Hz, 3H), 0.89 (d, $J = 6.6$ Hz, 3H) (two active proton less); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.4, 143.0, 125.6, 132.4, 128.7, 128.2, 127.8, 127.1, 126.6, 123.1, 119.5, 61.0, 43.2, 24.3, 22.1, 21.8; HRMS (ESI) calcd for $C_{18}H_{21}N_4O_2$ $[M - H]^-$ 325.1670 found 325.1667.

*6.1.6.8. (2E)-3-(1-((E)-1-Cyclobutyl-3-phenylallyl)-1H-1,2,3-triazol-4-yl)-N-hydroxyacrylamide (**8**).* The crude product was purified *via* C-18 reverse phase column chromatography (water/acetonitrile: from 5:1 to 5:2) to provide **8** as a white solid. Yield: 45%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.46 (s, 1H), 7.28–7.48 (m, 6H), 6.45–6.69 (m, 3H), 5.31 (t, $J = 8.7$ Hz, 1H), 2.06–3.06 (m, 1H), 1.72–2.08 (m, 6H) (two active protons less); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.3, 142.8, 135.6, 133.1, 128.5, 128.1, 126.9, 126.5, 125.4, 123.1, 119.5, 67.2, 38.2, 24.6, 24.5, 16.9; HRMS (ESI) calcd for $C_{18}H_{19}N_4O_2$ $[M - H]^-$ 323.1513 found 323.1508.

*6.1.6.9. (2E)-3-(1-((E)-1-Cyclopentyl-3-phenylallyl)-1H-1,2,3-triazol-4-yl)-N-hydroxyacrylamide (**9**).* The crude product was purified *via* C-18 reverse phase column chromatography (water/acetonitrile: from 5:1 to 5:2) to provide **9** as a white solid. Yield: 54%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.83 (s, 1H), 9.08 (s,1H), 8.51 (s, 1H), 7.26–7.48 (m, 6H), 6.57–6.70 (m, 3H), 5.11 (dd, $J_1 = 8.3$ Hz, $J_2 = 9.3$ Hz, 1H), 2.56–2.67 (m, 1H), 1.73–1.81 (m, 1H), 1.36–1.65 (m, 6H), 1.09–1.18 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 162.3, 142.8, 135.6, 133.0, 128.5, 128.0, 127.0, 126.9, 126.5, 123.1, 119.4, 67.2, 43.9, 29.2, 29.1, 24.8, 24.5; HRMS (ESI) calcd for $C_{19}H_{21}N_4O_2$ $[M - H]^-$ 337.1670 found 327.1669.

*6.1.6.10. (2E)-N-Hydroxy-3-(1-((E)-1-phenylhexa-1,5-dien-3-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**10**).* The crude product was purified *via* C-18 reverse phase column chromatography (water/acetonitrile: from 5:1 to 3:1) to provide **10** as a white solid. Yield: 61%. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.82 (s, 1H), 9.06 (s, 1H), 8.49 (s, 1H), 7.26–7.47 (m, 6H), 6.54–6.65 (m, 3H), 5.65–5.75 (m, 1H), 5.44–5.49 (m, 1H), 5.01–5.11 (m, 2H), 2.80–2.91 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 162.5, 142.9, 135.6, 133.2, 132.6, 128.6, 128.2, 127.2, 127.1, 126.6, 123.4, 119.6, 118.5, 61.9 (one peak less due to overlap with solvent DMSO-d_6); HRMS (ESI) calcd for $C_{17}H_{17}N_4O_2$ $[M - H]^-$ 309.1357 found 309.1352.

*6.1.6.11. (2E)-N-Hydroxy-3-(1-((E)-1,4-diphenylbut-3-en-2-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**11**).* The crude product was purified *via* C-18 reverse phase column chromatography (water/acetonitrile: from 5:1 to 3:1) to provide **11** as a white solid. Yield: 67%; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.22–7.39 (m, 5H), 6.56 (d, $J = 15.9$ Hz, 1H), 6.20 (dd, $J_1 = 15.9$ Hz, $J_2 = 6.7$ Hz, 1H), 4.19 (q, $J = 6.2$ Hz, 1H), 1.83 (s, 1H), 1.60–1.71 (m, 2H), 0.96 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$); δ: 136.8, 132.3, 130.4, 128.6, 128.5, 127.6, 126.5, 126.4, 74.4, 30.3; HRMS (ESI) calcd for $C_{21}H_{19}N_4O_2$ $[M - H]^-$ 359.1513 found 359.1512.

*6.1.6.12. (2E)-N-Hydroxy-3-(1-((E)-4-phenyl-1-(thiazol-2-yl)but-3-en-2-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**12**).* The crude product was purified *via* C-18 reverse phase column chromatography (water/acetonitrile: from 5:1 to 3:1) to provide **12** as a light yellow solid. Yield: 87%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.52 (s, 1H), 7.71 (d, $J = 3.3$ Hz, 1H), 7.56 (d, $J = 3.3$ Hz, 1H), 7.45 (d, $J = 7.2$ Hz, 2H), 7.27–7.37 (m, 4H), 6.58–6.66 (m, 3H), 5.82–5.87 (m,1H), 3.94 (dd, $J_1 = 8.8$ Hz, $J_2 = 15.1$ Hz, 1H), 3.84 (dd, $J_1 = 6.1$ Hz, $J_2 = 15.1$ Hz, 1H) (two active proton less); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.4, 162.3, 143.0, 142.4, 135.4, 133.2, 128.7, 128.3, 126.9126.6, 126.5, 123.8, 120.3, 119.7, 62.1, 37.4; HRMS (ESI) calcd for $C_{18}H_{16}N_5O_2S$ $[M - H]^-$ 366.1030 found 366.1029.

*6.1.6.13. (E)-3-(1-((E)-1-([1,1′-Biphenyl]-3-yl)-4-phenylbut-3-en-2-yl)-1H-1,2,3-triazol-4-yl)-N-hydroxyacrylamide (**13**).* The crude product was purified *via* C-18 reverse phase column chromatography (water/acetonitrile: from 5:1 to 3:1) to provide **13** as a white solid. Yield: 24%. ^{1}H NMR (400 MHz, DMF-d_7) δ: 8.75 (s, 1H), 7.27–7.64 (m, 15H), 6.63–6.86 (m, 3H), 5.88 (apparent q, $J = 5.88$Hz, 1H), 3.62 (d, $J = 6.1$ Hz); ^{13}C NMR (100 MHz, DMF-d_7) δ: 162.5, 143.4, 141.0, 140.8, 137.9, 136.4, 133.5, 132.8, 129.14, 129.08, 128.95, 128.6, 128.5, 128.1, 127.72, 127.66, 127.1, 127.0, 125.5, 124.4, 64.7, 41.3 (one peak less due to overlap); HRMS (ESI) calcd for $C_{27}H_{23}N_4O_2$ $[M - H]^-$ 435.1826 found 435.1828.

*6.1.6.14. (E)-3-(1-((E)-1-(3-Benzylphenyl)-4-phenylbut-3-en-2-yl)-1H-1,2,3-triazol-4-yl)-N-hydroxyacrylamide (**14**).* The crude product was purified *via* C-18 reverse phase column chromatography (water/acetonitrile: from 5:1 to 3:1) to provide **14** as a white solid. Yield: 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.43 (s, 1H), 7.25–7.44 (m, 9H),7.01–7.10 (m, 2H), 6.82 (s, 4H), 6.57–6,62 (m, 3H), 5.66 (q, $J = 7.2$ Hz, 1H), 3.40 (d, $J = 8$ Hz, 2H) (two active proton less); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.3, 156.7, 156.1, 142.8, 138.9, 135.6, 132.7, 129.9, 129.5, 128.7, 128.2, 127.2, 126.6, 124.5, 123.5, 123.1, 119.7, 119.5, 118.2, 117.3, 113.6, 63.6, 30.7; HRMS (ESI) calcd for $C_{27}H_{23}N_4O_3$ $[M - H]^-$ 451.1776 found 451.1782.

*6.1.6.15. (2E)-N-Hydroxy-3-(1-((E)-1-(naphthalen-1-yl)-4-phenylbut-3-en-2-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**15**).* The crude product was purified *via* C-18 reverse phase column chromatography (water/acetonitrile: from 5:1 to 3:1) to provide **15** as a white solid. Yield: 31%. ^{1}H NMR (400 MHz, DMF-d_7) δ: 11.13 (s, br, 1H), 9.51 (s, br, 1H), 8.81 (s, 1H), 8.51 (d, $J = 9.4$ Hz, 1H), 7.99–8.01 (m, 1H), 7.82 (t, $J = 7.1$ Hz, 1H), 7.75 (t, $J = 7.1$ Hz, 1H), 7.64–7.68 (m, 3H), 7.43–7.56 (m, 6H), 7.08 (dd, $J_1 = 7.6$ Hz, $J_2 = 15.9$ Hz, 1H), 6.93 (d, $J = 15.6$ Hz, 1H), 6.86 (d, $J = 15.9$ Hz, 1H), 6.07 (q, $J = 7.4$ Hz, 1H), 4.15–4.25 (m, 2H); ^{13}C NMR (100 MHz, DMF-d_7) δ: 162.5, 143.7, 136.4, 134.2, 133.4, 133.2, 132.1, 129.1, 128.9, 128.4, 128.0, 127.9, 127.7, 127.0, 126.6, 126.0, 125.6, 123.9, 123.7, 119.9, 63.9, 38.4 (one peak less due to overlap); HRMS (ESI) calcd for $C_{25}H_{21}N_4O_2$ $[M - H]^-$ 409.1670 found 409.1674.

*6.1.6.16. (2E)-N-Hydroxy-3-(1-((E)-1-(naphthalen-3-yl)-4-phenylbut-3-en-2-yl)-1H-1,2,3-triazol-4-yl)acrylamide (**16**).* The crude product was purified *via* C-18 reverse phase column chromatography (water/acetonitrile: from 5:1 to 3:1) to provide **16** as a white solid. Yield: 24%. ^{1}H NMR (400 MHz, DMF-d_7) δ: 11.13 (s, br, 1H),

9.51 (s, br, 1H), 8.81 (s, 1H), 8.51 (d, $J = 9.4$ Hz, 1H), 7.99–8.01 (m, 1H), 7.82 (t, $J = 7.1$ Hz, 1H), 7.75 (t, $J = 7.1$ Hz, 1H), 7.64–7.68 (m, 3H), 7.43–7.56 (m, 6H), 7.08 (dd, $J_1 = 7.6$ Hz, $J_2 = 15.9$ Hz, 1H), 6.93 (d, $J = 15.6$ Hz, 1H), 6.86 (d, $J = 15.9$ Hz, 1H), 6.07 (q, $J = 7.4$ Hz, 1H), 4.15–4.25 (m, 2H); ^{13}C NMR (100 MHz, DMF-d_7) δ: 162.5, 143.7, 136.4, 134.2, 133.4, 133.2, 132.1, 129.1, 128.9, 128.4, 128.0, 127.9, 127.7, 127.0, 126.6, 126.0, 125.6, 123.9, 123.7, 119.9, 63.9, 38.4 (one peak less due to overlap); HRMS (ESI) calcd for $C_{25}H_{21}N_4O_2$ $[M - H]^-$ 409.1670 found 409.1674.

6.2. Biology

6.2.1. Working reagents

TSA Stock: TSA was provided as a 2 mM stock solution in 100% dimethylsulfoxide (DMSO).

Assay Buffer: 50 mM Tris–HCl (pH 8.0), 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$.

Diluted Substrate Solution: The commercial Ac-Arg-Gly-Lys(Ac)-AMC substrate was dissolved in a little amount of DMSO solution, then diluted to 10 mM with PBS, finally diluted to 150 μM with HDAC assay buffer prior to each use. The final concentration in the assay was 60 μM.

Developer solution: 10 mg/mL trypsin in 50 mM Tris–HCl (pH 8.0), 100 mM NaCl, 2 μM TSA.

HDACs working solution: The HDAC enzymes were diluted in assay buffer prior to each use from a fresh aliquot of enzymes. The final concentration in the assay was 1–2 nM.

6.2.2. Experimental design

The reaction was performed in 96-well microplate in a final volume of 200 μL/well, as following: Compounds diluted in 50 μL HDAC buffer were mixed with 10 μL of diluted enzyme. The reaction was started by adding 40 μL substrate solution in HDAC buffer followed by 20 min of incubation at 37 °C. The reaction was stopped by adding 100 μL developer solution. After a 20 min incubation period at 37 °C, the release of AMC was monitored by measuring the fluorescence at Ex.390 nM and Em.460 nM. All experiments were carried out at least in triplicate.

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