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Late-Stage C−H Coupling Enables Rapid Identification of HDAC Inhibitors: Synthesis and Evaluation of NCH-31 Analogues

Hiromi Sekizawa,[†] Kazuma Amaike,[†] Yukihiro Itoh,[‡] Takayoshi Suzuki,*[,‡,§] Kenichiro Itami,*[,†,∥] and Junichiro Yamaguchi*[,†]

[†]Institute of Transformative Bio-Molecules (WPI-ITbM) and Graduate School of Science, Nagoya University, Chikusa, Nagoya 464-8602, Japan

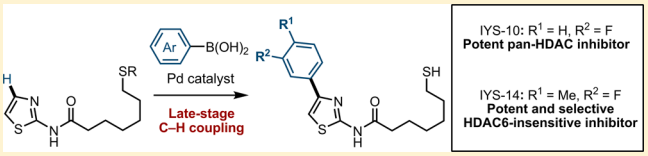
[‡]Graduate School of Medical Science, Kyoto Prefectural University of Medicine, 13 Taishogun Nishitakatsukasa-Cho, Kita-ku, Kyoto 603-8334, Japan

[§]JST, PRESTO, 4-1-8 Honcho Kawaguchi, Saitama 332-0012, Japan

[∥]JST, ERATO, Itami Molecular Nanocarbon Project, Nagoya University, Chikusa, Nagoya 464-8602, Japan

Supporting Information

ABSTRACT: We previously reported the discovery of NCH-31, a potent histone deacetylase (HDAC) inhibitor. By utilizing our C−H coupling reaction, we rapidly synthesized 16 analogues (IYS-1 through IYS-15 and IYS-Me) of NCH-31 with different aryl groups at the C4-position of 2-amino-thiazole core of NCH-31. Subsequent biological testing of these derivatives revealed that 3-fluorophenyl (IYS-10) and 4-fluorophenyl (IYS-15) derivatives act as potent pan-HDAC inhibitor. Additionally, 4-methylphenyl (IYS-1) and 3-fluoro-4-methylphenyl (IYS-14) derivatives acted as HDAC6-insensitive inhibitors. The present work clearly shows the power of the late-stage C−H coupling approach to rapidly identify novel and highly active/selective biofunctional molecules.



KEYWORDS: *Histone deacetylase, inhibitor, C−H coupling, HDAC6*

Histone deacetylases (HDACs) catalyze the removal of acetyl groups from *N*-acetylated lysine residues of various protein substrates such as histones and α-tubulin.[1,2] HDACs play important roles in various fundamental life processes including gene expression and cell cycle progression.[3] There are currently 18 known HDACs that are organized into several classes with regard to their DNA sequence similarity: class I HDACs (HDAC1−3 and HDAC8); class IIa HADCs (HDAC4, HDAC5, HDAC7, and HDAC10); class IIb HDACs (HDAC6 and HDAC10); class III HDACs (SIRT1−7); and class IV HDAC (HDAC11).[4] Class I, II, and IV HDACs (HDAC1−11) are zinc-dependent enzymes, whereas class III HDACs (SIRT1−7) are NAD^+-dependent enzymes.[4] Because HDACs are associated with various diseases, especially cancer, HDAC inhibitors have been developed as therapeutic agents such as anticancer drugs.[5] Indeed, two HDAC inhibitors vorinostat and romidepsin (Figure 1) have been approved by the FDA for the treatment of cutaneous T-cell lymphoma (CTCL).[6,7] However, the use of HDAC inhibitors is currently limited to CTCL. Therefore, there is a need to develop novel potent HDAC inhibitors as anticancer agents.

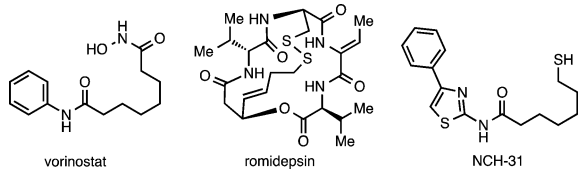


Figure 1. HDAC inhibitors: vorinostat, romidepsin, and NCH-31.

To date, a number of HDAC inhibitors have been identified,[8] including NCH-31 (Figure 1), which was discovered by one of us (T.S.).[9] Among the HDAC inhibitors reported so far, HDAC6-insensitive inhibitors[10] have been reported to show potent and selective cancer cell growth-inhibitory activity by reactive oxygen species-induced apoptosis.[11,12] Following these findings, we performed further investigation of NCH-31 derivatives, seeking to find potent HDAC inhibitors and selective HDAC6-insensitive inhibitors. Since the thiol group of NCH-31 is needed to coordinate the zinc ion in the active site of class I, II, and IV HDACs,[9] we decided to carry out the structural modification of the arylthiazole amide moiety of NCH-31 to obtain the desired HDAC inhibitors.

In planning synthesis of NCH-31 derivatives, we realized that step-economical, late-stage C−H functionalization approach should be taken rather than following our previous synthesis of NCH-31 using Hantzsch thiazole synthesis (Figure 2). When following the classical condensation reaction to make NCH-31 derivatives, one needs to start from acetophenone derivatives to modify aryl groups; bromination of acetophenones, thiazole

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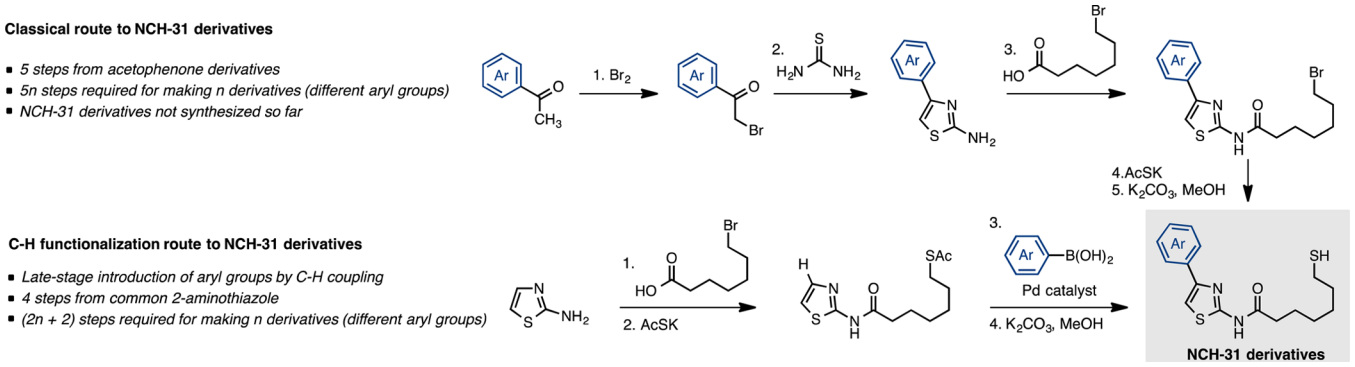

Figure 2. Synthesis of NCH-31 derivatives through classical and C−H functionalization routes.

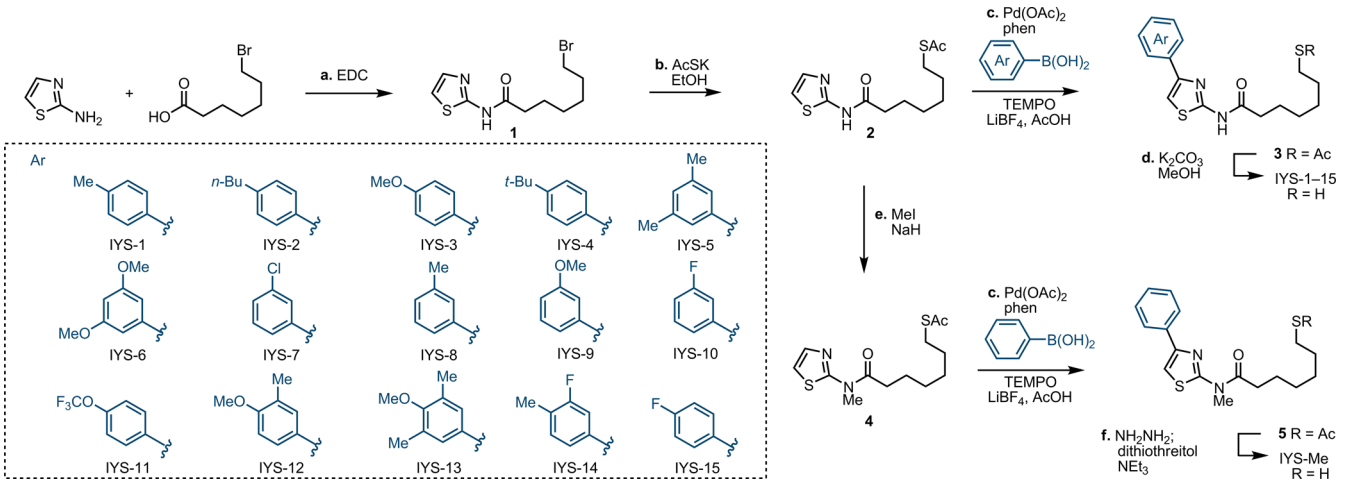

Figure 3. Synthesis of NCH-31 analogues (IYS-1−15 and IYS-Me) by C−H coupling. Reaction conditions: (a) EDC·HCl (1.4 equiv), CH_2Cl_2, 23 °C, 6 h, 80%; (b) AcSK (4.0 equiv), EtOH, 23 °C, 16 h, 98%; (c) $ArB(OH)_2$ (4.0 equiv), $Pd(OAc)_2$ (10 mol %), phen (10 mol %), $LiBF_4$ (1.5 euqiv), TEMPO (1.0 equiv), AcOH (1.0 equiv), DMAc, 100 °C, 10−29%; (d) K_2CO_3, MeOH, 23 °C; (e) MeI, NaH, DMF, 23 °C; (f) NH_2NH_2, CH_3CN; then dithiothreitol, NEt_3, 23 °C.

formation with thiourea, condensation with carboxylic acids, and introduction of the thiol unit.[13] An ideal, step-economical, diversity-oriented approach would be to introduce aryl groups onto a thiazole core of NCH-31 at the late stage of the synthesis. In doing so, we envisioned that the application of our recently developed unique Pd catalyst that promotes otherwise-difficult C4-selective C−H arylation of thiazoles with arylboronic acids should perfectly match this requirement (Figure 2).[14−16] Considering the preparation of the different C4-aryl substituents, the present C−H functionalization route only takes $2n + 2$ steps, whereas the classical route takes $5n$ steps (n = desired number of thiazole derivatives).

Herein, we demonstrate the synthesis of NCH-31 analogues by late-stage C−H coupling,[17−19] which lead to the rapid examination of the structure−activity and structure−selectivity relationships, and identification of new pan-HDAC inhibitors and HDAC6-insensitive inhibitors that are more potent and selective than NCH-31.

The synthesis of NCH-31 derivatives commenced with the condensation of 2-aminothiazole and 7-bromoheptanoic acid, which are both commercially available compounds, to provide bromide **1** in 80% yield (Figure 3). Thiolation of **1** by treatment with potassium thioacetate (AcSK) gave thiazole amide **2** in excellent yield. Thiazole **2** was then coupled with various arylboronic acids under our reported conditions for C4-selective C−H arylation of thiazoles,[15] which consists of $Pd(OAc)_2$ (10 mol %) and 1,10-phenanthroline (phen: 10 mol %) as a catalyst, 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO, 1.0 equiv) as an oxidant, AcOH (1.0 equiv), and $LiBF_4$ (1.5 equiv) in dimethylacetamide (DMAc) at 100 °C, to afford the corresponding coupling products. These products were then deacetylated to give IYS-1−15 with virtually complete C4-selectivity. Unfortunately, arylboronic acids with amino substituents, heteroaryl substituents, and ortho substituents did not work under the present conditions. Additionally, **2** was alkylated at the nitrogen atom of the amide by methyl iodide to afford **4** and was then C−H arylated at the C4-position and deacetylated to give IYS-Me. The synthesized NCH-31 analogues (IYS-1−15 and IYS-Me) were tested with an in vitro assay using human recombinant HDAC1, HDAC6, and HDAC9, a representative isozyme of Class I, IIb, and IIa HDACs, respectively (Figure 4). For HDAC1, IYS-1−15 (except IYS-5) showed moderate to excellent inhibition compared to NCH-31 at 0.1 μM, whereas IYS-Me did not show HDAC1 inhibition. In the case of HDAC6, a few compounds displayed moderate to good inhibition; particularly, IYS-9 and IYS-10 showed more than 70% inhibition at 1 μM, which is higher than NCH-31. However, IYS-1−5 and 11−14 were totally inactive against HDAC6. IYS-1, IYS-10, IYS-14, and IYS-15, which bear methyl or fluoro groups on the meta and/or para positions of the benzene ring, displayed HDAC9 inhibitory activity stronger than NCH-31 at 0.1 μM. These

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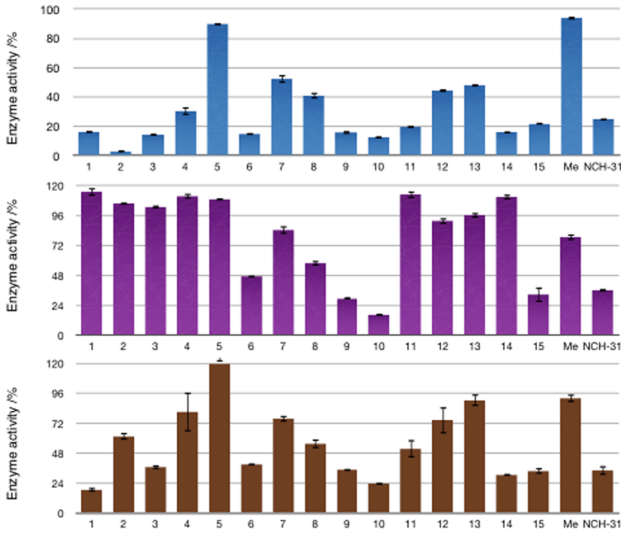
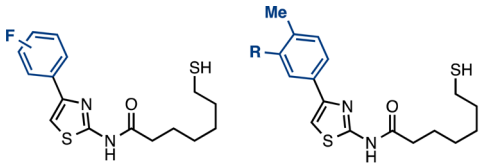


Figure 4. HDAC activity in the presence of IYS-1−15 and IYS-Me: blue bar for HDAC1 (enzyme activity % at 0.1 μM), purple bar for HDAC6 (enzyme activity % at 1 μM), and brown bar for HDAC9 (enzyme activity % at 0.1 μM).

results indicate that IYS-10 and IYS-15 might be a potent pan-HDAC inhibitor and that IYS-1 and IYS-14 might be potent HDAC6-insensitive inhibitors.

The IC_{50} values of IYS-1, IYS-10, IYS-14, and IYS-15 for HDAC1, HDAC6, and HDAC9 were also determined (Table 1). In these assays, NCH-31 inhibited HDAC1, HDAC6, and

Table 1. HDAC1, HDAC6, and HDAC9 Inhibition Data for NCH-31, IYS-1, IYS-10, IYS-14, and IYS-15



	HDAC1 IC_{50} (μM)	HDAC6 IC_{50} (μM)	HDAC9 IC_{50} (μM)
NCH-31	0.096	0.23	0.082
IYS-1	0.057	1.8	0.042
IYS-10	0.049	0.15	0.078
IYS-14	0.050	6.1	0.062
IYS-15	0.036	0.55	0.057

HDAC9 with IC_{50} values of 0.096, 0.23, and 0.082 μM, respectively. As shown in Table 1, IYS-1, IYS-10, IYS-14, and IYS-15 all showed HDAC1 and HDAC9 inhibitory activity more potent than NCH-31. As for HDAC6, IYS-10 displayed slightly more potent activity than NCH-31 (IC_{50} of IYS-10 = 0.15 μM; IC_{50} of NCH-31 = 0.23 μM), whereas IYS-1 and IYS-14 were less potent HDAC6 inhibitors (IC_{50} of IYS-1 = 1.8 μM; IC_{50} of IYS-14 = 6.1 μM). In particular, the HDAC6-inhibitory activity of IYS-14 was 27-fold weaker than that of NCH-31. Thus, IYS-10 and IYS-15 are potent pan-HDAC inhibitors and IYS-1 and IYS-14 are potent and selective HDAC6-insensitive inhibitors.

To explore the origin of the potent HDAC1-inhibitory activity of IYS-15 as compared to NCH-31, we initially performed a binding model study of the inhibitor (IYS-15 or NCH-31) with HDAC1 by using Molegro Virtual Docker 5.0. The simulations were performed based on the reported X-ray structure of HDAC1[20] and under the condition that the catalytic site was set as search space. As a result of these calculations, the thiolate group of both IYS-15 and NCH-31 is shown to coordinate to the zinc ion (Figure 5). However, the

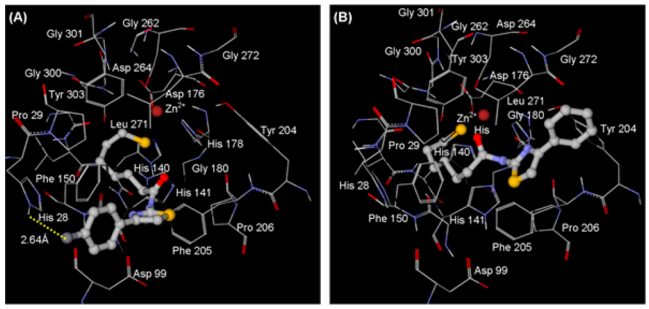


Figure 5. (A) View of the conformation of IYS-15 (ball and stick model) docked in the HDAC1 catalytic core. (B) View of the conformation of NCH-31 (ball and stick model) docked in the HDAC1 catalytic core.

lowest energy conformation of the aromatic ring portion of IYS-15 docked in HDAC1 was different from that of NCH-31. Specifically, the benzene ring of IYS-15 was positioned near His 28 and Phe 150, where the fluorine atom could form an N−H···F hydrogen bond with His 28 (Figure 5A). However, the benzene ring of NCH-31 was located adjacent to Tyr 204 where hydrogen bonds were not observed (Figure 5B). These results indicate that the hydrogen bond formation might contribute to the higher HDAC1-inhibitory activity of IYS-15.

Next, to understand why introducing a methyl group onto NCH-31 led to a decrease in HDAC6-inhibitory activity, we studied the binding mode of the inhibitor (IYS-14 or NCH-31) with a homology model of HDAC6. Inspection of the simulated HDAC6/NCH-31 complex showed that the phenyl group of NCH-31 was positioned in the region delineated by Arg 673, Phe 679, Phe 680, Leu 749, and Gly 751 (region 1 in Figure 6C), and the amide oxygen of NCH-31 could form a hydrogen bond with His 651 (Figure 6A). However, the 3-fluoro-4-methylphenyl group of IYS-14 was not located in region 1 because of a steric repulsion between the methyl group of IYS-14 and Arg 673, but it was located in region 2 (see Figure 6C) where it could interact only with Pro 681 (Figure 6B,C). The conformational change could cause the loss of a hydrogen bond between the amide oxygen of IYS-14 and His 651 (Figure 6B), which might lead to a decrease in HDAC6-inhibitory activity of IYS-14. In addition, the differences in activity between IYS-1 and IYS-8, IYS-5 and IYS-6, and NCH-31 and IYS-Me can be explained by similar binding model studies (see Figures S1−S6 in the Supporting Information).

In summary, we successfully prepared 16 NCH-31 analogues, which possess various aryl groups at the C4-position of the thiazole core, by late-stage C−H coupling. Screening of the NCH-31 analogues identified IYS-10 as a potent pan-HDAC inhibitor and IYS-14 as a potent and selective HDAC6-insensitive inhibitor. We believe that this methodology is of value in future studies for the development of more potent and selective HDAC inhibitors as well as other biologically active compounds containing an arylthiazole structure.[21−23] The late-stage C−H coupling approach will find use in many areas to

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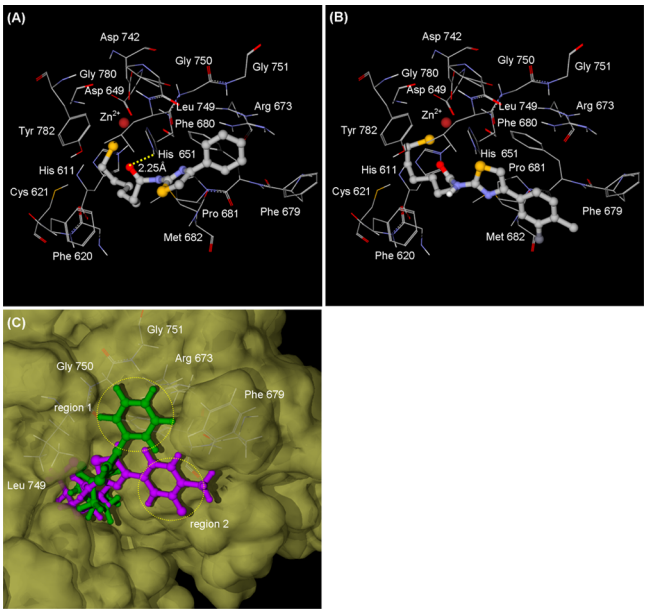

Figure 6. (A) View of the conformation of NCH-31 (ball and stick model) docked in the HDAC6 catalytic core. (B) View of the conformation of IYS-14 (ball and stick model) docked in the HDAC6 catalytic core. (C) Superimposition of IYS-14 (purple) and NCH-31 (green) in the active site of HDAC6.

rapidly identify novel and highly active/selective biofunctional molecules.[24,25]

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures for biological analysis and chemical synthesis, and characterization of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*(T.S.) E-mail: suzukit@koto.kpu-m.ac.jp.
*(K.I.) E-mail: itami@chem.nagoya-u.ac.jp.
*(J.Y.) E-mail: junichiro@chem.nagoya-u.ac.jp.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

HDAC, histone deacetylase; SIRT, sirtuin

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