Inhibition of capsaicin and dihydrocapsaicin derivatives towards histone deacetylase and molecular docking studies

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Abstract

The natural products, capsaicin and dihydrocapsaicin, were modified at double-bond and phenolic moieties to provide twelve capsaicin and dihydrocapsaicin derivatives. The natural products and synthesized compounds were evaluated as histone deacetylase inhibitors via in vitro fluorometric assay at 500 mM concentrations. The results revealed that a methyl ester derivative and a silyl-protected dihydrocapsaicin were the best histone deacetylase inhibitors among the tested compounds with 87% and 85% inhibitions, respectively. Molecular docking experiments were conducted on the obtained compounds with the human HDAC8 enzyme. These data show a new method for providing putative histone deacetylase inhibitors from common natural products.

Keywords: red chili pepper, Capsicum annuum, histone deacetylase, HeLa cell, anticancer

1. Introduction

Anti-cancer drugs have become an essential part of global health care. Although various anti-cancer chemotherapies are widely used, an urgent need for novel anti-cancer agents remains. Plants have played a dominant role in providing herbal drugs for the treatment of a broad spectrum of diseases (Cragg et al., 2009). As a part of our ongoing research program aimed at the evaluation and structural modification of bioactive secondary metabolites from plants grown widely in Thailand, we have focused our research on the phytochemicals from Capsicum annuum. Red chili spur pepper (C. annuum), or hot pepper, is regularly used as a spice in Thai cuisine. Several experiments have been conducted to explore the clinical applications of natural products from this plant species (Laohavechvanich et al., 2006; Hayman et al., 2008). Capsaicin and dihydrocapsaicin, the spicy agents in chili peppers, partially mimic the structure of the FDA-approved histone deacetylase (HDAC) inhibitors suberoylanilide hydroxamic acid (1, SAHA, Vorinostat®; Zolinza™ (Paris et al., 2008) and trichostatin A (2, TSA). Therefore, capsaicin derivatives may also inhibit histone deacetylase. SAHA is approved for the treatment of a rare cancer, cutaneous T-cell lymphoma, in patients who have progressive, persistent or recurrent disease.

Histone deacetylase inhibitors can affect differentiation, growth arrest, or apoptosis in transformed cell cultures by blocking substrate access to the histone deacetylase-active site (Bertrand et al., 2010). The results obtained from X-ray crystallography and the structure–activity relation-
Structural modifications of a CAP/DHC mixture were performed without further purification to obtain various derivatives as demonstrated in Scheme 1 and Figure 2 (Kumoonma et al., 2010). All compounds were characterized by using spectroscopic techniques including IR, NMR and MS.

\[ N-(4-hydroxy-3-methoxybenzyl)-5-(3-isopropoxyloxiran-2-yl) \]
\[ \text{pantanamidine (5)} \]
\[ R = 0.30 \text{ (1:1 EtOAc/hexane), IR (neat) 3333, 2932, 1741,} \]
\[ 1645, 1515, 1463, 1372, 1277, 1125, 1036 \text{ cm}^{-1}. \]
\[ ^1H \text{ NMR (400 MHz, CDCl}_3 \) \] \[ \delta \] \[ 6.84 (d, J = 8.4 \text{ Hz, } 1H), 6.80 (s, 1H), 6.72 (d, J = 8.4 \text{ Hz, } 1H), 5.86 (br s, OCH), 5.85 (s, 1H), 4.35 (d, J = 5.4 \text{ Hz, } 2H), 3.85 (s, OCH), 2.70 (m, 1H), 2.42 (dd, J = 2.2, 7.0 \text{ Hz, } 1H), 2.00 (t, J = 7.6 \text{ Hz, } 2H), 1.70 (m, 2H), 1.52 (m, 1H), 1.50 (m, 2H), 1.45 (m, 2H), 0.95 (d, J = 6.6 \text{ Hz, } 3H), 0.90 (d, J = 6.6 \text{ Hz, } 3H). \]
\[ ^13C \text{ NMR (100 MHz, CDCl}_3 \) \] \[ \delta \] \[ 172.69 (C), 146.75 (C), 145.13 (C), 130.20 (C), 120.73 (C), 114.44 (C), 110.77 (C), 64.24 (C), 57.61 (C), 55.91 (OCH), 43.53 (C), 36.46 (C), 31.74 (C), 30.47 (C), 25.74 (C), 25.41 (C), 19.00 (C), 18.37 (C). \]
\[ \text{HRMS-ESI (m/z) [M + Na]+} \text{ caleed for } \text{C}_{19}H_{28}NO_4^+. \]
\[ \text{Na 344.1838, found 344.1840.} \]

6-Hydroxy-N-(4-hydroxy-3-methoxybenzyl)-8-methylnonanamide (6)

\[ R = 0.20 \text{ (1:1 EtOAc/hexane), IR (neat) 3334, 2933, 2867,} \]
\[ 1715, 1645, 1515, 1274, 1035, 820, 741 \text{ cm}^{-1}. \]
\[ ^1H \text{ NMR (400 MHz, CDCl}_3 \) \] \[ \delta \] \[ 6.84 (d, J = 8.0 \text{ Hz, } 1H), 6.80 (s, 1H), 6.75 (d, J = 8.0 \text{ Hz, } 1H), 5.70 (br s, 1H), 5.65 (br s, OCH), 4.35 (d, J = 5.4 \text{ Hz, } 2H), 3.85 (s, OCH), 3.65 (m, 1H), 2.20 (t, J = 7.8 \text{ Hz, } 2H), 1.70 (m, 2H), 1.62 (m, 2H), 1.50 (m, 2H), 1.45 (m, 2H), 1.40 (m, 1H), 0.90 (d, J = 6.6 \text{ Hz, } 3H), 0.90 (d, J = 6.6 \text{ Hz, } 3H). \]
\[ ^13C \text{ NMR (100 MHz, CDCl}_3 \) \] \[ \delta \] \[ 172.82 (C), 146.68 (C), 145.12 (C), 130.26 (C), 120.81 (C), 114.37 (C), 110.73 (C), 69.62 (C), 55.94 (OCH), 46.84 (C), 43.57 (C), 37.50 (C), 36.58 (C), 25.53 (C), 25.19 (C), 24.60 (C), 23.44 (C), 22.04 (C). \]
\[ \text{HRMS-ESI (m/z) [M + Na]+} \text{ caleed for } \text{C}_{19}H_{28}NO_4^+. \]
\[ \text{Na 346.1949, found 346.1999.} \]

(E)-9-(4-acetoxy-3-methoxybenzylamino)-2-methyl-9-oxonon-3-en-5-yl acetate (7)

\[ R = 0.60 \text{ (1:1 EtOAc/hexane), IR (neat) 3295, 3071, 2956,} \]
\[ 2869, 1768, 1731, 1646, 1514, 1464, 1422, 1372, 1247, 1153, 1020 \text{ cm}^{-1}. \]
\[ ^1H \text{ NMR (400 MHz, CDCl}_3 \) \] \[ \delta \] \[ 6.90 (s, 1H), 6.85 (d, J = 8.0 \text{ Hz, } 1H), 5.80 (s, 1H), 4.95 (m, 1H), 4.40 (d, J = 5.7 \text{ Hz, } 2H), 3.80 (s, OCH), 2.30 (s, 3H), 2.20 (t, J = 7.8 \text{ Hz, } 2H), 2.00 (s, 3H), 1.65 (m, 2H), 1.52 (m, 2H), 1.50 (m, 1H), 1.30 (m, 2H), 1.26 (m, 1H), 0.90 (d, J = 6.2 \text{ Hz, } 3H), 0.90 (d, J = 6.2 \text{ Hz, } 3H). \]
\[ ^13C \text{ NMR (100 MHz, CDCl}_3 \) \] \[ \delta \] \[ 172.59 (C), 170.94 (C), 169.10 (C), 151.19 (C), 139.07 (C), 137.32 (C), 122.85 (C), 120.01 (C), 112.19 (C), 103.40 (C). \]

2. Materials and Methods

2.1 Plant material

Dried red chili spur peppers \((\text{Capsicum annuum})\) were purchased from the local market in Khon Kaen Province, Thailand. One kilogram of plant materials was extracted as previously described to provide a mixture of Capsaicin (CAP, 3) and Dihydrocapsaicin (DHC, 4) (690 mg) as a yellow-orange oil (Kumoonma et al., 2009).

2.2 Structural modifications

Structural modifications of a CAP/DHC mixture were performed without further purification to obtain various derivatives as demonstrated in Scheme 1 and Figure 2.
N-(4-(tert-butyl dimethylsilyloxy)-3-methoxy benzyl) -6, 7-dihy droxy-8-methyl nonanamide (8)

R. 0.15 (1:1 EtOAc/hexanes. IR (neat) 3296, 3071, 2929, 2857, 2719, 1723, 1645, 1514, 1419, 1284, 1254, 1159, 1127, 1037, 902, 840, 783 cm⁻¹. ¹HNMR (400 MHz, CDCl₃) δ 9.75 (s, 1H), 7.05 (d, J = 7.9 Hz, 1H), 6.76 (s, 1H), 6.71 (d, J = 7.9 Hz, 1H), 5.93 (s, 1H), 4.00 (d, J = 10.7 Hz, 2H), 2.75 (m, 2H), 1.70 (m, 2H), 1.08 (m, 2H), 0.25 (m, 2H), 0.45 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 172.91 (C₂), 151.07 (C₁), 144.53 (C₄), 131.62 (C₁₃), 120.87 (C₂₁), 120.20 (Cl₂), 112.04 (C₇), 78.99 (C₈), 71.47 (C₉), 55.54 (OCH₃), 43.57 (C₁₀), 34.41 (C₁₁), 30.13 (C₁₂), 25.68 (C₁₃), 25.37 (C₁₄), 19.73 (C₁₅), 18.42 (C₁₆), 17.02 (C₁₇), -4.66 (C₁₈). HRMS-ESI (m/z) [M + H]⁺ caked for C₂₃H₂₈NO₃SiBr⁺ 544.2989, found 544.3000.

N-(4-(tert-butyl dimethylsilyloxy)-3-methoxy benzyl)-8-oxo nonanamide (9)

R. 0.50 (1:1 EtOAc/hexanes. IR (neat) 3296, 3071, 2929, 2857, 2719, 1723, 1645, 1514, 1419, 1284, 1254, 1159, 1127, 1037, 902, 840, 783 cm⁻¹. ¹HNMR (400 MHz, CDCl₃) δ 9.75 (s, 1H), 7.05 (d, J = 7.9 Hz, 1H), 6.76 (s, 1H), 6.71 (d, J = 7.9 Hz, 1H), 5.93 (s, 1H), 4.00 (d, J = 10.7 Hz, 2H), 2.75 (m, 2H), 1.70 (m, 2H), 1.08 (m, 2H), 0.25 (m, 2H), 0.45 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 202.15 (C₂), 172.12 (C₁₃), 151.07 (C₁), 144.52 (C₄), 131.60 (C₁₂), 120.86 (C₂₁), 120.19 (C₂), 111.98 (C₇), 55.48 (OCH₃), 43.54 (C₁₀), 43.55 (C₁₁), 36.28 (C₁₂), 25.68 (C₁₃), 25.04 (C₁₄), 21.54 (C₁₅), 18.41 (C₁₆), -4.67 (C₁₇). HRMS-ESI (m/z) [M + H]⁺ caked for C₂₃H₂₈NO₃SiBr⁺ 544.2989, found 544.3000.

Methyl-6-(2-bromo-4-(tert-butyl dimethylsilyloxy)-5-methoxy benzylamino)-6-oxo nonanamide (12)

R. 0.35 (1:1 EtOAc/hexanes. IR (neat) 3360, 2951, 1642, 1505, 1275, 1202, 1036 cm⁻¹. ¹HNMR (400 MHz, CDCl₃) δ 7.08 (s, 1H), 6.92 (s, 1H), 6.00 (br s, 1H), 4.40 (d, J = 6.0 Hz, 2H), 3.85 (s, OCH₃), 3.65 (s, 3H), 2.35 (t, J = 6.9 Hz, 2H), 2.20 (t, J = 6.9 Hz, 2H), 1.65 (m, 2H), 1.16 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 173.65, 172.42, 146.09, 145.92, 128.83, 118.44, 114.40, 113.15, 56.15 (OCH₃), 51.53 (C₁₂), 43.70 (C₉), 36.64 (C₈), 25.00 (C₇), 24.38 (C₆). HRMS-ESI (m/z) [M + H]⁺ caked for C₂₃H₂₈NO₂SBr⁺ 477.4060, found 477.4063.

N-(4-(tert-butyl dimethylsilyloxy)-3-methoxy benzyl)-8-methyl nonanamide (10)

R. 0.15 (EtOAc). IR (neat) 3435, 1634, 667 cm⁻¹. ¹HNMR (400 MHz, CDCl₃) δ 6.85 (d, J = 8.0 Hz, 1H), 6.80 (s, 1H), 6.75 (d, J = 8.0 Hz, 1H), 5.85 (br s, 1H), 4.63 (s, OH), 4.62 (s, OH), 4.35 (d, J = 5.3 Hz, 2H), 3.85 (s, OCH₃), 3.58 (q, J = 4.7 Hz, 1H), 3.12 (t, J = 5.1 Hz, 1H), 2.20 (m, 2H), 2.20 (m, 2H), 1.85 (m, 2H), 1.70 (m, 2H), 1.50 (m, 2H), 0.94 (d, J = 6.6 Hz, 3H), 0.25 (d, J = 6.6 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 167.29 (C₂), 146.17 (C₁₃), 145.10 (C₉), 130.23 (C₇), 120.78 (C₈), 114.43 (C₁₀), 110.76 (C₁₁), 78.97 (C₉), 71.47 (C₈), 55.95 (OCH₃), 43.54 (C₁₀), 36.41 (C₁₁), 33.36 (C₁₂), 30.09 (C₁₃), 25.33 (C₁₄), 25.11 (C₁₅), 19.71 (C₁₆), 16.96 (C₁₇). HRMS-ESI (m/z) [M + H]⁺ caked for C₁₅H₂₁NO₃ 340.2124, found 340.2100. Methyl-6-(2-bromo-4-(tert-butyl dimethylsilyloxy)-5-methoxy benzylamino)-6-oxo nonanamide (11)

R. 0.60 (1:1 EtOAc/hexanes. IR (neat) 3296, 3289, 3074, 2953, 2932, 2857, 1738, 1651, 1504, 1257, 1205, 1040, 969, 904, 840, 784 cm⁻¹. ¹HNMR (400 MHz, CDCl₃) δ 7.00 (s, 1H), 6.88 (s, 1H), 5.95 (br s, 1H), 4.40 (d, J = 5.9 Hz, 2H), 3.78 (s, OCH₃), 3.62 (s, 3H), 2.35 (t, J = 7.0 Hz, 2H), 2.20 (t, J = 7.0 Hz, 2H), 1.65 (m, 2H), 1.65 (m, 2H), 0.98 (s, 9H), 0.15 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 173.72 (C₂), 132.32, 150.50 (C₁₃), 145.29 (C₉), 124.59 (C₇), 114.10 (C₈), 113.42 (C₁₀), 55.62 (OCH₃), 51.15 (C₁₁), 43.65 (C₁₂), 36.17 (C₁₃), 33.64 (C₁₄), 25.60 (C₁₅), 25.03 (C₁₆), 24.39 (C₁₇), 18.39 (C₁₈), 4.69 (C₁₉). HRMS-ESI (m/z) [M + Na]⁺ caked for C₁₅H₂₁NO₃SiBr⁺ 488.1468, found 488.1468.
Histone deacetylase activity assay

Reagents and conditions: (a) XPS microplate spectrofluorometer (Molecular Devices, USA) was used as the positive available assay (Fluor de Lys assay system, Biomol, Enzo Life Sciences, USA). To calibrate for C26H35NO3, Na 420.2515, found 420.2518.

2-Methoxy-4-((8-methylnonanamido)methyl) phenylacetate (15)

Rf 0.70 (1:1 EtOAc/hexanes). IR (neat) 3296, 3071, 2927, 2855, 2227, 1767, 1646, 1607, 1541, 1416, 1421, 1360, 1271, 1199, 1122, 1036 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 6.95 (d, J = 8.4 Hz, 1H), 6.88 (s, 1H), 6.80 (d, J = 8.4 Hz, 1H), 5.95 (s, 1H), 4.35 (d, J = 5.4 Hz, 2H), 2.79 (s, OCH₃), 2.28 (s, 3H), 2.20 (t, J = 7.8 Hz, 2H), 1.98 (q, J = 7.2 Hz, 2H), 1.64 (m, 2H), 1.40 (m, 2H), 1.29 (m, 1H), 1.26 (m, 2H), 1.13 (m, 2H), 0.95 (d, J = 6.6 Hz, 3H), 0.95 (d, J = 6.6 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 173.21 (C), 169.12 (C), 151.15 (C), 139.01 (C), 137.37 (C), 122.78 (C), 119.95 (C), 112.10 (C), 55.85 (OCH₃), 43.38 (C), 38.72 (C), 36.53 (C), 32.19 (C), 29.60 (C), 29.27 (C), 27.21 (C), 25.25 (C), 22.62 (C), 22.62 (C), 20.61 (C). HRMS-ESI (m/z) [M + Na⁺] calculated for C26H35NO3 + Na 372.2151, found 372.2151.

Diethyl 2-methoxy-4-((8-methylnonanamido) methyl) phenylphosphonate (16)

Rf 0.45 (1:1 EtOAc/hexanes). IR (neat) 3202, 3073, 2929, 2867, 1651, 1514, 1464, 1274, 1213, 1157, 1126, 1034, 969, 820 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 7.19 (d, J = 8.4 Hz, 1H), 6.85 (s, 1H), 4.78 (d, J = 8.4 Hz, 1H), 5.95 (s, 1H), 4.35 (d, J = 5.4 Hz, 2H), 2.40 (q, J = 8.0 Hz, 4H), 3.79 (s, OCH₃), 2.20 (t, J = 7.8 Hz, 2H), 1.98 (q, J = 7.2 Hz, 2H), 1.64 (m, 2H), 1.38 (m, 2H), 1.35 (t, J = 8.0 Hz, 6H), 1.29 (m, 1H), 1.26 (m, 2H), 1.13 (m, 2H), 0.95 (d, J = 6.6 Hz, 3H), 0.95 (d, J = 6.6 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 173.08 (C), 150.75 (C), 139.16 (C), 112.28 (C), 119.89 (C), 112.38 (C), 64.57 (C), 55.92 (OCH₃), 43.24 (C), 38.62 (C), 36.49 (C), 32.19 (C), 29.59 (C), 29.26 (C), 27.21 (C), 25.25 (C), 22.59 (C), 22.59 (C), 15.99 (C). HRMS-ESI (m/z) [M + Na⁺] calculated for C₂₆H₃₈NO₇P + Na 466.2334, found 466.2341.

2.3 Histone deacetylase activity assay

Capsaicin (CAP, 3) was isolated from C. annuum fruits as a mixture with dihydrocapsaicin (DHC, 4). The separation of these two natural products was unsuccessfully attempted with different column chromatography methods. Therefore, structural modification of a CAP/DHC mixture was performed without further purification, as shown in Scheme 1. CAP (3) was reduced into DHC (4) in a good yield. The mixture of 3 and 4 was oxidized by mCPBA to produce the epoxide 5 along

![Diagram](image1)

2.4 Molecular docking studies

Using AutoDockTools 1.5.4 (ADT) and AutoDock 4.2 programs with a grid box size of 66×66×66 points and Lamarckian genetic algorithm search (Santer et al., 1999; Morris et al., 2009). The crystal structures of human histone deacetylase HDAC8 [PDB entry code: 1T64, complexed with the inhibitor, TSA, resolution: 1.90 Å] was obtained from the Protein Data Bank (available from http://www.rcsb.org, last accessed 30 November 2009). All water and non-interacting ions as well as TSA were removed. Then all missing hydrogen and sidechain atoms were added using the ADT program. Gasteiger charges were calculated for the system. For ligand setup, the molecular modeling program Hyperchem 8.0 was used to build the ligands (HyperChem Professional 8.0, HyperCube, Inc., Florida, USA, 2007). These ligands were optimized with the AM1 level.
with recovery of 4. Formation of the alcohol 6 was achieved by treating the 3 and 4 mixture with Hg(OAc)₂, and followed with NaBH₄. The structure of 6 was confirmed by 2D NMR experiments. The correlation between C₄ and H₄ could be observed from the CIGAR experiment. The racemic mixture of alcohol 6 was readily converted into diacetate 7, via reaction of 6 with acetic anhydride and pyridine. The diol 8 was obtained as a mixture of enantiomers after syn-dihydroxylation of the silyl protected 3 and 4 with NaIO₄ and K₂OsO₄. The aldehyde 9 was also gained as a minor product as well as the silyl ether derivative of DHC (13) which is shown in Figure 2. This oxidative cleavage condition was tried with 3 and 4, but it led to a decomposition of starting materials. Further oxidation of the aldehyde 9 was carried out with bromine in methanol to give methyl ester 11. Under this condition, the bromine atom replaced the hydrogen atom at the C₄ position in aromatic region of 11. The silyl group of 11 was removed by using TBAF to give methyl ester 12 in a good yield. Reaction between the previously obtained diol 8 and TBAF also provided the racemic mixture of the dihydroxy DHC 10 in a high yield. The spectroscopic data of 10 confirmed the structure of major regioisomer 6 which was gained from oxymercuration-demercuration of 3. The 'H NMR signals of H₁₄ which belonged to 6 and 10 appeared at 3.65 ppm and 3.58 ppm, respectively. The DHC synthetic derivative structures are depicted in Figure 2. The phenolic group of 4 was protected as silyl ether, benzyl ether, acetate ester and phosphate ester to provide 13, 14, 15 and 16, respectively.

CAP 3, DHC 4 and their synthetic derivatives were screened in vitro using a HeLa nuclear extract in a fluorometric assay at 500 μM concentrations. The inhibitory activities of all compounds against HDAC in vitro are presented in Table 1. Molecular docking studies were conducted with the human HDAC8 to gain more details on the binding mode and to obtain additional validation of the experimental results. All compounds were analyzed to allow comparisons of the calculated free energies of binding (∆G) and inhibition constants (Kᵢ), which are also shown in Table 1. The mixture of 3 and 4 showed no activity at 500 μM concentrations. Notably, compound 4 alone also exhibited no activity at the same concentration as the mixture of 3 and 4. These results indicate that no significant difference exists between a saturated side chain and one with a single double-bond. Modification of the double-bond side chain provided the epoxide 5 with no inhibitory activity. The results suggest that electron density of the epoxide functional group may not be enough for binding to the active site of HDAC. Six compounds (6, 7, 10, 11, 12 and 13) showed inhibitory activities against HDAC. Although the inhibitory activities were not comparable to that of TSA, these results provide valuable information regarding active-site binding to HDAC.

The incorporation of a hydroxy group and a dihydroxy group in the alkyl chains was conducted to gain compounds 6 and 8, which were expected to exhibit improved coordinating and chelating properties to the zinc ion. Surprisingly, only 6 showed inhibitory activity against HDAC with 80% inhibition. The HDAC inhibitory activity test of compound 7 was performed to examine whether the acetate ester in the alkyl side chain improved activity. Compound 7 exhibited 83% inhibition against HDAC. These data indicated that the hydroxy and acetyl groups can act as alternative zinc coordinating groups and provide better metal binding than the epoxide functional group. After the silyl group removal from 8, the dihydroxycapsaicin 10 was obtained and its inhibitory activity against HDAC increased dramatically. Therefore, the silyl group of 8 may be too bulky to allow ligand-enzyme

<table>
<thead>
<tr>
<th>Compound</th>
<th>HDAC Inhibitory Activities (%)</th>
<th>∆G (kcal/mol)</th>
<th>Kᵢ (μM)</th>
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<tr>
<td>1</td>
<td>-6.23</td>
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<tr>
<td>2*</td>
<td>69 ± 1.09</td>
<td>-6.91</td>
<td>8.6</td>
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<tr>
<td>3 and 4</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
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<td>355.3</td>
</tr>
<tr>
<td>5</td>
<td>inactive</td>
<td>-4.39</td>
<td>610.49</td>
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<tr>
<td>6</td>
<td>80 ± 0.75</td>
<td>-5.41</td>
<td>108.7</td>
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<tr>
<td>7</td>
<td>83 ± 0.51</td>
<td>-5.46</td>
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<td>8</td>
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<td>16</td>
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</table>

* Positive control at 25 μM
binding or reinforce the dihydroxy group from chelating to the zinc ion. This hypothesis can be applied to explain the results obtained from the inactive aldehyde and the slightly active methyl ester against HDAC. The increasing inhibitory activity of the methyl ester may be mainly due to chelating of the methyl ester functional group to the zinc ion. Interestingly, the bromine atom at C10 of 12 did not restrict inhibitor-enzyme interaction. The biological data reported herein suggest that the highly polar functional groups play an important role in metal binding for HDAC. The HDAC inhibitory activities of compounds , , and were tested to examine whether the hydrophobic ether or ester groups of the aromatic region increased the activities. However, only modification of the hydroxyl group into tert-butylidimethyl silyl ether in resulted in increasing activity, with 85% inhibition. Apparently, the ester moieties did not provide enough electronic interaction between the enzyme and the inhibitor. The lack of activity with the ester moieties is most likely due to the polarized p-bonds. Of the two ether protecting groups (tert-butylidimethyl silyl ether in compound and benzyl ether in compound ) demonstrated a better interaction with the enzyme. The tert-butylidimethyl silyl ether can be assumed to be of compatible size with the hydrophobic pocket of the enzyme. As is evident from the results, the hydrophobic aromatic region is critical for binding, which is in agreement with the crystal structure. (, 2004).

Molecular docking studies were conducted to gain more details on the binding mode of the synthetic derivatives and to obtain additional validation of the experimental results. Visual inspection of the binding mode for SAHA () at HDAC8 binding site showed that its hydroxamic acid group approaches the zinc ion making ionic interaction with the zinc ion (Figure 3A). HIS180, HIS142, HIS143 and TYR306 are the other residues near the cofactor zinc ion along with the hydroxamic group of the inhibitor. There are four important interactions considered as hydrogen bonds. The hydrogen bond between the hydroxy group of TYR306 and the carbonyl moiety of the hydroxamic acid group was calculated as 2.6 Å. The imidazole moiety of the HIS143 is H-bonded to the amino part of the hydroxamic acid group (3.1 Å). The other hydrogen bond is between the hydroxy group of the hydroxamic acid and the imidazole moiety of HIS142 (2.7 Å). Finally, a hydrogen bond is observed between the hydroxy group of hydroxamic acid group and the imidazole moiety of HIS180 (2.3 Å). The high inhibition potency of SAHA () toward HDAC8 can be justified by these four strong hydrogen bonds as shown in Table 1. These results are relevant to that of the crystal structure of human HDAC8 complexed with the hydroxamic acid inhibitor (Finnin et al., 1999). Visual inspection of the position of 6 in the HDAC8 binding site shows that ASP87 and TYR100 are the residues near the aromatic and the amide regions of 6 (Figure 3B). Three important interactions are considered to be hydrogen bonds. One hydrogen bond occurs between the backbone carbonyl group of TYR100 and the hydrogen atom of the amide group that belongs to 6 (2.6 Å). Two hydrogen bonds are observed from coordinating between carboxylic group of ASP87 and the hydrogen atom at phenolic group of 6 (2.9 Å, 3.4 Å). Notably, the hydroxy group in the alkyl side chain of 6 forms a weak interaction with the zinc ion. This binding mode correlates well with the initially designed inhibitors. Moreover, a considerably lower inhibition constant is calculated for 6 compared to that of the previously mentioned capsaicin derivatives.

The major interaction between HDAC8 and compound is the hydrogen bond between the hydroxy group of TYR100 and the carbonyl moiety of the acetyl group on the side chain of TYR100 (2.9 Å) as shown in Figure 4A. Surprisingly, the amide moiety of 7 has the potential to act in metal binding, whereas the carbonyl of the acetyl group on the aromatic ring appears to serve as the surface-recognition area. These interactions contribute to the low inhibition constant for 7. Compound 10 binds to HDAC8 with two hydrogen bonds (Figure 4B). The hydroxy group at C10 of 10 forms the hydrogen bond to ASP101 (2.9 Å), whereas another hydroxy group at C11 and the amide group chelate to the zinc ion. The other hydrogen bond occurs between the methoxy group and LYS33 (2.7 Å). The lower inhibitory activity of 10 than that of 7 also correlates well with the calculated binding energy as shown in Table 1. The binding mode of 11 shows no hydrogen bond between the ligand and the enzyme (Figure 4C). However, there is a weak coordination of the methoxy group towards the zinc ion. This weak
interaction decreases the in vitro inhibitory activity of 11 as well. Figure 4D shows the binding mode of 12 in the active site cavity of HDAC, which illustrates that two hydrogen bonds are important for the inhibitor–enzyme binding. The amino group of MET274 (2.8 Å) accepts the hydrogen bond from the phenolic group of 12. The methoxy group of 12 also binds to the amino group of PRO273 (3.0 Å). The molecular docking results of 13 in the complex with HDAC8 suggest that the tert-butyl dimethyl silyl group and the aromatic ring of the ligand are oriented toward the zinc ion (Figure 5). TYR306 makes the close π–π interaction with the phenyl moiety of the ligand. Much of the low free binding energy results from these strong interactions. Analysis of the molecular docking results of compound 13 also revealed that the methoxy and the silyloxy groups in the aromatic region may interact weakly with the zinc ion. Therefore, the phenolic portion of 13 played a critical role for zinc ion binding, not as the surface recognition element. These results correlate with the inhibitory activity of 13 from the in vitro experiments.

An independent report from our group showed that 6 possessed a good HDAC inhibitory activity with an IC50 value of 72 μM, whereas a commercially available capsaicin acted as only a weak inhibitor (IC50 > 13.2 mM) (Senawong et al., 2015). In addition, 6 could also induce apoptosis in HCT116 colon cancer cell lines more efficiently than capsaicin. Toxicity against normal cells of 6 was lower than that of capsaicin as well. The highly potent hydroxamic acids such as TSA were reported to have poor pharmacokinetics and high toxicities (Hahnen et al., 2008; Suzuki et al., 2005). Several non-hydroxamic acids, for instance, curcumin, (E)-resveratrol, sinapinic acid and auroxins have been developed as HDAC inhibitors to solve these problems (Tatar et al., 2009; Erden et al., 2009; Senawong et al., 2013; Zwick et al., 2014).

4. Conclusions

In conclusion, a series of novel capsaicin and dihydrocapsaicin derivatives were designed and synthesized. All of the previously discussed procedures confirm that capsaicin and dihydrocapsaicin can be utilized as lead compounds for the preparation of various derivatives for biological activity tests. Six derivatives exhibited inhibitory activity against HDAC in the micromolar concentration range. A conventional modification of the phenolic group into a silyl ether resulted in improved activity compared to that of dihydrocapsaicin. Incorporation of hydroxy and dihydroxy groups into the alkyl side chain provided alternative coordination of the zinc ion and resulted in the increased activity. Although their inhibitory activities were not comparable to that of TSA, one compound was already proved to be a potential and safe anticancer agent. The molecular docking studies provided valuable information and allowed us to estimate the free energy of binding, the binding modes, and the inhibition constants. These obtained data show that minor structural changes in capsaicin and dihydrocapsaicin can significantly improve both HDAC inhibitory and anticancer activities.

These novel non-hydroxamic acid HDAC inhibitors should be further studied and developed as anticancer drugs.

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