Asian Journal of Chemistry; Vol. 36, No. 1 (2024), 25-31

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2024.30551

Cytotoxicity and Molecular Docking to Histone Deacetylase of Phytochemicals from *Ventilago denticulata* Leaves

THIDATHEP MAHAAMNART[1], SIRIPIT PITCHUANCHOM[2], THANASET SENAWONG[3], SURAPON SAENSOUK[4] and MONGKOL NONTAKITTICHAROEN[1,*]

[1]Natural Products Research Unit, Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
[2]Multidisciplinary Research Unit of Pure and Applied Chemistry, Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahasarakham University, Maha Sarakham 44150, Thailand
[3]Natural Products Research Unit, Department of Biochemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
[4]Diversity of Family Zingiberaceae and Vascular Plant for Its Applications Research Unit, Walai Rukhavej Botanical Research Institute, Mahasarakham University, Maha Sarakham 44150, Thailand

*Corresponding author: E-mail: mongbu@kku.ac.th

Received: 2 September 2023; *Accepted*: 10 October 2023; *Published online*: 31 December 2023; AJC-21480

Phytochemical investigations of the ethyl acetate and methanol extracts of *Ventilago denticulata* leaves resulted in the isolation of five flavonoids (**1**-**5**) and three anthraquinones (**6**-**8**). Their structures were characterized using spectroscopic techniques including UV, IR, ^{1}H NMR, ^{13}C NMR and 2D NMR as well as mass spectrometry (MS). Among these, compounds **4** (rhamnetin) and **6** (frangulin B) emerged as potential HDAC inhibitors, displaying significant inhibitory activities against HeLa nuclear extract. Molecular docking with HDAC isoforms revealed their affinity, particularly for class II HDAC4 enzyme. Both compounds exhibited promising physico-chemical properties compliant with Lipinski's rule of five, indicative of their drug-like potential. *In vitro* antiproliferative assays demonstrated that compound **6** exhibited superior activity against HeLa, A549 and MCF-7 cancer cell lines with the IC_{50} values ranging from 8.35 to 20.93 µM, whereas compound **4** showed reduced cytotoxicity in non-cancerous Vero cells. Furthermore, compound **6** was isolated from *V. denticulata* for the first time.

Keywords: *Ventilago denticulata*, Cytotoxicity, Molecular docking, Flavonoid, Anthraquinone.

INTRODUCTION

Histone deacetylase enzymes (HDACs) govern a pivotal epigenetic modification, modulating chromatin architecture by removing acetyl moieties from lysine residues on histone tails. This enzymatic action often produces a more compact chromatin state, leading to transcriptional repression. Unbalanced HDAC activity has been observed in many carcinomas, primarily attributed to the epigenetic inhibition of critical tumor suppressor genes. Studying and searching for active compounds that inhibit HDACs is thus one of the essential directions for effective anticancer drug development [1,2].

Ventilago denticulata Willd. (Rhamnaceae) is a climbing shrub commonly found in tropical and subtropical forests, native to several countries in Southeast Asia. It can be found in Myanmar, Malaysia, Thailand, the Philippines and other Asian countries. In traditional Thai medicine, the plant is recognized for its multitude of pharmacological attributes and associated therapeutic advantages [3,4]. The leaves are used for their antihypertensive, hypocholesterolemic and hypoglycemic properties. The vines are employed for their efficacy in alleviating muscular pain, while the stem barks are utilized for analgesic purposes. Furthermore, the roots serve as an expectorant. Previous phytochemical studies on *V. denticulata*, also known as *V. calyculata*, have revealed a range of bioactive constituents such as anthraquinones, naphthalene derivatives, benzisochromanquinones and flavonoids [4-9]. These compounds have exhibited various bioactivities, including antioxidant, cytotoxic, antibacterial, antifungal and inhibitory actions on phosphodiesterase and acetylcholinesterase [4,7-9]. In our pursuit of bioactive compounds

This is an open access journal, and articles are distributed under the terms of the Attribution 4.0 International (CC BY 4.0) License. This license lets others distribute, remix, tweak, and build upon your work, even commercially, as long as they credit the author for the original creation. You must give appropriate credit, provide a link to the license, and indicate if changes were made.

from Thai medicinal plants, we present the isolation and characterization of phytochemicals from the leaves of *V. denticulata* by chromatographic methods. We further also investigated their potential cytotoxicity as histone deacetylase inhibitors.

EXPERIMENTAL

IR spectra were obtained from a Bruker Tensor 27 FT-IR spectrophotometer (Bruker, Germany) and the vibration bands were expressed in wavenumbers. The UV spectra were recorded on a Specord 200 plus double-beam spectrophotometer (Analytik Jena, Germany) and adsorptions were measured from 100 to 800 nm. NMR spectra were measured on a Bruker AVANCE NEO (400 MHz) spectrometer (Bruker, Germany) and the chemical shifts were reported in parts per million (ppm). HRESITOF mass spectra were recorded on a Finigan Mat INCOS 50 and Micromass LTC mass spectrometers. Melting points were determined using a Gallenkamp melting point apparatus (Sanyo Gallenkamp, UK) and reported without any corrections. Column chromatography (CC) was carried out over Merck silica gel (0.0063-0.200 nm or less than 0.0063 nm). Reverse phase CC was performed over LiChroprep RP-18 (40-63 μm). Size exclusion CC was carried out over Sephadex LH-20. Preparative TLC was performed on Merck silica gel PF_{254} (0.5 mm) plates. Commercial grade solvents were distilled at their boiling point ranges prior to use for extraction and chromatographic separation, whereas AR grade solvents were used for recrystallization.

The leaves of *Ventilago denticulata* were collected from the local conservation forest in Lampang province, Thailand. The plant was identified by Assoc. Prof. Dr. Surapon Saensouk, Mahasarakham University, Thailand. The voucher specimen (no. SPMSU002) was deposited at Mahasarakham University Herbarium, Thailand.

Extraction and isolation: Air-dried powdered leaves of *V. denticulata* (2.4 kg) were ground and extracted successively with organic solvents including *n*-hexane, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and methanol (MeOH) at room temperature. Solvents were evaporated under reduced pressure to give crude *n*-hexane (31.9 g), CH_2Cl_2 (46.8 g), EtOAc (45.3 g) and MeOH (580.0 g) extracts, respectively.

The EtOAc extract (38 g) was subjected to silica gel CC, gradient eluted with *n*-hexane:EtOAc (100:0-0:100 v/v) and EtOAc:MeOH (100:0-0:100 v/v) to obtain seven fractions (E_1-E_7). Fraction E_3 was further separated over RP-18 CC, eluted with MeOH:H_2O (60:40 v/v) to yield six fractions ($E_{3.1}$-$E_{3.6}$). Precipitates in fractions $E_{3.1}$ and E_4 were filtered out and recrystallized from MeOH to afford compounds 5 (13.2 mg) and 3 (1.3123 g), respectively. Filtrate of fraction E_4 was further separated over silica gel CC, gradient eluted with CH_2Cl_2:EtOAc (100:0-0:100 v/v) and EtOAc:MeOH (100:0-0:100 v/v) to yield six fractions ($E_{4.1}$-$E_{4.6}$). Precipitates in fractions $E_{4.3}$ and $E_{4.6}$ were filtered out and then recrystallized from MeOH to afford compounds **8** (128.5 mg) and **4** (21.1 mg), respectively.

The MeOH extract (100 g) was subjected to silica gel CC, gradient eluted with *n*-hexane:EtOAc (100:0-0:100 v/v) and EtOAc:MeOH (100:0-0:100 v/v) to give eight fractions (M_1-M_8). Fraction M_6 was separated over Sephadex LH-20 CC, eluted with CH_2Cl_2:MeOH (10:90) v/v) to yield three fractions ($M_{6.1}$-$M_{6.3}$). Precipitates in fractions $M_{6.2}$ and M_8 were filtered out and then recrystallized from MeOH to yield compound **6** (38.8 mg) and **1** (103.5 mg), respectively. Filtrate of M_8 was further separated over silica gel CC, gradient eluted with EtOAc:MeOH (100:0-0:100 v/v) to yield seven fractions ($M_{8.1}$-$M_{8.7}$). Fraction $M_{8.1}$ was separated over Sephadex LH-20 CC, eluted with MeOH to yield four fractions ($M_{8.1.1}$-$M_{8.1.4}$). The precipitate of $M_{8.1.2}$ was filtered out and then recrystallized from MeOH to afford compound 4 (13.7 mg). Fraction $M_{8.2}$ was separated over Sephadex LH-20 CC, eluted with MeOH to give six fractions ($M_{8.2.1}$-$M_{8.2.6}$). The precipitates of fractions $M_{8.2.2}$ and $M_{8.2.3}$ were filtered out and then recrystallized from MeOH to furnish compounds **2** (36.2 mg) and **7** (3.2 mg), respectively.

Rhamnazin-3-O-rhamninoside (1): Light yellow amorphous, m.p.: 233-234 ºC; UV (MeOH) λ_{max}, nm (log ε): 257 (3.79), 357 (3.67); IR (ATR, ν_{max}, cm^{-1}): 3334, 2906, 1655, 1596, 1037; HRESITOFMS *m/z* 785.2507 $[M + H]^+$ (calcd. for $C_{35}H_{45}O_{20}$, 785.2504); ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 12.49 (1H, s, OH-5), 9.18 (1H, s, OH-3′), 7.74 (1H, dd, *J* = 8.7, 2.2 Hz, H-6′), 7.52 (1H, d, *J* = 2.2 Hz, H-2′), 6.97 (1H, d, *J* = 8.7 Hz, H-5′), 6.65 (1H, d, *J* = 2.2 Hz, H-8), 6.34 (1H, d, *J* = 2.2 Hz, H-6), 5.31 (1H, d, *J* = 7.6 Hz, H-1″), 4.91 (1H, d, *J* = 5.4 Hz, H-1‴′), 4.59 (1H, d, *J* = 5.4 Hz, H-1‴), 3.86 (6H, s, OCH_3-7, OCH_3-4′), 1.02 (1H, d, *J* = 6.1 Hz, H-6‴), 0.92 (1H, d, *J* = 5.4 Hz, H-6‴′); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 177.6 (C-4), 165.2 (C-7), 160.9 (C-5), 156.4 (C-9), 156.6 (C-2), 150.2 (C-4), 146.0 (C-3′), 134.1 (C-3), 122.5 (C-6′), 121.7 (C-1′), 115.7 (C-5′), 111.3 (C-2′), 105.0 (C-10), 102.0 (C-1‴′), 100.2 (C-1‴), 100.7 (C-1′), 98.0 (C-6), 92.3 (C-8), 78.0 (C-3‴), 73.0 (C-3″), 72.1 (C-4‴′), 71.1 (C-2″), 71.0 (C-4‴), 70.5 (C-2‴′), 70.4 (C-3‴′), 70.1 (C-2‴), 68.6 (C-4″), 65.5 (C-6″), 17.9 (C-6‴), 17.7 (C-6‴′).

Ombuin-3-O-glucoside (2): Light yellow amorphous, m.p.: 294-295 ºC; IR (ATR, ν_{max}, cm^{-1}): 3325, 1662, 1606; HRESITOFMS *m/z* 493.1344 $[M + H]^+$ (calcd. for $C_{23}H_{25}O_{12}$, 493.1346); ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 12.60 (1H, s, OH-5), 9.27 (1H, s, OH-3′), 7.73 (1H, dd, *J* = 8.7, 2.0 Hz, H-6′), 7.61 (1H, d, *J* = 2.0 Hz, H-2′), 7.06 (1H, d, *J* = 8.7 Hz, H-5′), 6.73 (1H, d, *J* = 2.0 Hz, H-8), 6.39 (1H, d, *J* = 2.0 Hz, H-6), 5.51 (1H, d, *J* = 5.9 Hz, H-1″), 5.29 (1H, d, *J* = 3.4 Hz, H-2″), 4.29 (1H, t, *J* = 5.7 Hz, H-3″), 3.59 (1H, dd, *J* = 11.7, 5.6 Hz, H-4″), 3.09 (1H, brs, H-6″), 3.86 (6H, s, OCH_3-7, OCH_3-4′); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 177.7 (C-4), 165.2 (C-7), 160.9 (C-5), 156.4 (C-9), 156.2 (C-2), 150.2 (C-4′), 145.9 (C-3′), 133.8 (C-3), 122.5 (C-1′), 121.5 (C-6′), 115.8 (C-2′), 111.4 (C-5′), 105.1 (C-10), 100.7 (C-1′), 98.0 (C-6), 92.2 (C-8), 77.7 (C-5″), 76.5 (C-3″), 74.1 (C-2″), 69.4 (C-4″), 61.0 (C-6″), 56.5 (OCH_3-7), 56.1 (OCH_3-4′).

Ombuin (3): Light yellow amorphous, m.p.: 229-230 ºC; UV (MeOH) λ_{max}, nm (log ε): 257 (4.34), 367 (4.28); IR (ATR, ν_{max}, cm^{-1}): 3445, 3272, 2975, 2839, 1734, 1615, 1152; HRESITOFMS *m/z* 331.0818 $[M + H]^+$ (calcd. for $C_{17}H_{15}O_7$, 331.0818); ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 12.39 (1H, s, OH-5), 9.53 (1H, s, OH-3), 9.28 (1H, s, OH-3′), 7.67 (1H, d, *J* = 2.2 Hz, H-2′), 7.64 (1H, dd, *J* = 8.7, 2.2 Hz, H-6′), 7.04 (1H, d, *J* = 8.7 Hz, H-5′), 6.66 (1H, d, *J* = 2.2 Hz, H-8), 6.30 (1H, d, *J* = 2.2 Hz, H-6), 3.81 (3H, s, OCH_3-7), 3.80 (3H, s,

OCH_3-4′); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 176.5 (C-4), 165.4 (C-7), 160.8 (C-5), 156.6 (C-9), 149.9 (C-4′), 147.2 (C-2), 146.6 (C-3′), 136.9 (C-3), 123.8 (C-1′), 120.3 (C-6′), 115.2 (C-2′), 112.2 (C-5′), 104.5 (C-10), 98.0 (C-6), 92.4 (C-8), 56.5 (OCH_3-7), 56.1 (OCH_3-4′).

Rhamnetin (4): Yellow solid, m.p.: 294-295 ºC; UV (MeOH) λ_{max}, nm (log ε): 256 (2.41), 370 (2.38); IR (ATR, ν_{max}, cm^{-1}): 3322, 2942, 2831, 1739, 1612, 1021; HRESITOFMS *m/z* 315.0868 $[M–H]^-$ (calcd. for $C_{16}H_{11}O_7$, 315.0505); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.73 (1H, d, *J* = 2.2 Hz, H-2′), 7.58 (1H, dd, *J* = 8.5, 2.2 Hz, H-6′), 6.90 (1H, d, *J* = 8.5 Hz, H-5′), 6.71 (1H, d, *J* = 2.2 Hz, H-8), 6.36 (1H, d, *J* = 2.2 Hz, H-6), 3.87 (3H, s, OCH3-7); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 176.4 (C-4), 165.3 (C-7), 160.8 (C-9), 156.5 (C-5), 148.3 (C-4), 147.7 (C-2), 145.5 (C-3′), 136.5 (C-3), 122.3 (C-1′), 120.4 (C-6′), 116.0 (C-5′), 115.6 (C-2′), 104.4 (C-10), 97.9 (C-6), 92.3 (C-8), 56.4 (OCH_3-7).

Kaempferide (5): Yellow amorphous, m.p.: 229-230 ºC; UV (MeOH) λ_{max}, nm (log ε): 265 (3.24), 322 (3.05), 368 (3.31); IR (ATR, ν_{max}, cm^{-1}): 3322, 2942, 1739, 1612, 1505, 1322, 1021; HRESITOFMS *m/z* 301.0705 $[M + H]^+$ (calcd. for $C_{16}H_{13}O_6$, 301.0712); ^{1}H NMR (400 MHz, CD_3OD + $CDCl_3$) δ ppm: 8.30 (2H, d, *J* = 8.9 Hz, H-2′, H-6′), 7.12 (2H, d, *J* = 8.9 Hz, H-3′, H-5′), 6.70 (1H, d, *J* = 2.2 Hz, H-8), 6.50 (1H, d, *J* = 2.2 Hz, H-6), 4.07 (3H, s, OCH_3-4′); ^{13}C NMR (100 MHz, CD_3OD + $CDCl_3$) δ ppm: 176.6 (C-4), 166.1 (C-7), 161.3 (C-5), 159.7 (C-4′), 157.4 (C-9), 147.9 (C-2), 136.7 (C-3), 130.3 (C-2′, C-6′), 123.0 (C-1′), 116.0 (C-3′, C-5′), 104.9 (C-10), 98.2 (C-6), 92.4 (C-8), 56.1 (OCH_3-4′).

Frangulin B (6): Orange solid, m.p.: 195-196 ºC; UV (MeOH) λ_{max}, nm (log ε): 225 (2.88), 264 (2.63), 285 (2.52), 433 (2.34); IR (ATR, ν_{max}, cm^{-1}): 3389, 2882, 1681, 1630, 1479, 1386, 1041; HRESITOFMS *m/z* 403.1018 $[M + Na]^+$ (calcd. for $C_{20}H_{19}O_9$, 403.1029); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.02 (1H, s, OH-1), 11.90 (1H, s, OH-8), 7.49 (1H, d, *J* = 1.6 Hz, H-4), 7.23 (1H, d, *J* = 2.5 Hz, H-5), 7.16 (1H, m, H-2), 6.82 (1H, d, *J* = 2.5 Hz, H-7), 5.66 (1H, d, *J* = 3.6 Hz, H-1′), 4.18 (1H, d, *J* = 3.6 Hz, H-2′), 4.04 (1H, d, *J* = 9.5 Hz, H-4′a), 3.76 (1H, d, *J* = 9.5 Hz, H-4′b), 2.38 (3H, s, CH_3-3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 190.8 (C-9), 181.8 (C-10), 164.4 (C-8), 164.2 (C-6), 162.2 (C-1), 149.3 (C-3), 135.7 (C-10a), 133.5 (C-4a), 124.9 (C-2), 121.3 (C-4), 114.1 (C-9a), 111.4 (C-8a), 109.9 (C-7), 108.8 (C-5), 107.8 (C-1′), 79.4 (C-3′), 76.7 (C-2′), 75.4 (C-4′), 62.5 (C-3′′), 22.2 (CH_3-3).

Emodin-8-O-β-D-glucoside (7): Orange solid, m.p.: 236-237 ºC; UV (MeOH) λ_{max}, nm (log ε): 224 (3.38), 282 (3.19), 422 (2.74); IR (ATR, ν_{max}, cm^{-1}): 3411, 3004, 1710, 1423, 1091; HRESITOFMS *m/z* 455.0955 $[M + H]^+$ (calcd. for $C_{21}H_{20}O_{10}$, 455.0954); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 13.15 (1H, s, OH-1), 11.34 (1H, s, OH-6, 7.45 (1H, d, *J* = 1.7 Hz, H-4), 7.28 (1H, d, *J* = 2.4 Hz, H-5), 7.15 (1H, d, *J* = 1.7 Hz, H-2), 6.99 (1H, d, *J* = 2.4 Hz, H-7), 5.05 (1H, d, *J* = 7.6 Hz, H-1′), 3.72 (1H, dd, *J* = 12.0, 2.2 Hz, H-6′a), 3.33 (1H, t, *J* = 8.9 Hz, H-3′), 3.24 (1H, d, *J* = 8.9 Hz, H-4′), 2.39 (3H, s, CH_3-3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 186.8 (C-9), 182.5 (C-10), 164.5 (C-6), 162.0 (C-1), 161.4 (C-8), 147.4 (C-3), 136.9 (C-10a), 132.4 (C-4a), 124.6 (C-2), 119.7 (C-4), 114.8 (C-9a), 113.7 (C-8a), 108.6 (C-5), 108.6 (C-7), 101.0 (C-1′), 77.6 (C-5′), 76.7 (C-3′), 73.6 (C-2′), 69.8 (C-4′), 60.9 (C-6′), 21.5 (CH_3-3).

Emodin (8): Orange solid, m.p.: 256-258 ºC; UV (MeOH) λ_{max}, nm (log ε): 221 (4.55), 253 (4.30), 289 (4.32), 437 (4.06); IR (ATR, ν_{max}, cm^{-1}): 3376, 2958, 2922, 2852, 1617, 1478, 1412, 1367, 1329, 1275, 1262; HRESITOFMS *m/z* 269.0464 $[M – H]^-$ (calcd. for $C_{15}H_9O_5$ 269.0455; ^{1}H NMR (400 MHz, CD_3OD + $CDCl_3$) δ ppm: 7.62 (1H, d, *J* = 1.0 Hz, H-4), 7.28 (1H, d, *J* = 2.4 Hz, H-5), 7.14 (1H, d, *J* = 1.0 Hz, H-2), 6.67 (1H, d, *J* = 2.4 Hz, H-7), 2.54 (3H, s, CH_3-3); ^{13}C NMR (100 MHz, CD_3OD + $CDCl_3$) δ ppm: 191.2 (C-9), 183.0 (C-10), 166.5 (C-8), 165.9 (C-6), 162.9 (C-1), 149.0 (C-3), 136.1 (C-4a), 134.0 (C-10a), 124.9 (C-2), 121.6 (C-4), 114.4 (C-9a), 110.1 (C-8a), 110.0 (C-7), 108.9 (C-5), 22.2 (CH_3-3).

Histone deacetylase enzymes (HDAC) inhibitory activity: The HDAC inhibitory activity was assessed using Fluor-de-Lys HDAC activity assay kit (Enzo Life Sciences International, Inc., USA). Following the manufacturer's instructions, briefly, a recombinant HeLa nuclear extract, diluted in a specified assay buffer, was dispensed into a microliter plate. The Fluor-de-Lys substrate, prepared in the same buffer, was then added to combine with the extract in the plate and incubated at 37 ºC for 10 min. The enzymatic reaction was halted by introducing a developer and the plate was left to stabilize at room temperature for an additional 10 min. Fluorescence intensity was quantified using a SpectraMax M5 analyzer (Molecular Devices, USA) at 360 nm excitation and 460 emission wavelengths. Trichostatin A (TSA) was used as the positive control. Each experiment was conducted in triplicate.

Molecular docking studies: Molecular docking studies was executed using AutoDock 4.2 to determine binding free energies and establish the best compound orientations with HDAC1, HDAC2, HDAC4, HDAC7 and HDAC8, corresponding to PDB codes: 4BKX [10], 3MAX [11], 2VQW [12], 3C0Z [13] and 1T64 [14]. The Lamarkian genetic algorithm (LGA) served as the primary computational method for all docking activities. Polar hydrogens were incorporated and Gasteiger charges were designated by AutoDockTools (ADT) [15]. All water molecules, non-participatory ions and ligands were omitted from the analysis. Adhering to the AutoDock force field [16,17], atomic solvation parameters were integrated, guided by the Stouten model and fragmental volumes. The grid box dimensions were set at 60 × 60 × 60 points, with a grid spacing of 0.375 Å. Grid map files were generated *via* the AutoGrid 4.2 software. Maximum energy evaluations were conducted over 2.5 × 106 steps, with a designated population comprising 200 ligand orientations. With 200 independent runs completed, the subsequent analysis centered on the final docked configuration, its RMSD with the crystalline structure, docking energy and projected binding free energy, elucidating interactions at the active site.

***In silico* physico-chemical properties:** SwissADME web server (http://swissadme.ch) was employed to analyze the physico-chemical profiles of the selected compounds [18]. This evaluation encompassed parameters such as molecular weight, topological polar surface area (TPSA), count of rotatable bonds, hydrogen-bond acceptors and hydrogen-bonds donors, as well

as lipophilicity. Furthermore, both Lipinski's and Veber's criteria were applied to validate their drug-likeness.

Antiproliferative activity: The antiproliferative effects of the selected compounds on cancer cell lines, including HeLa (cervical cancer), A549 (lung carcinoma) and MCF-7 (breast adenocarcinoma), were evaluated using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) reduction assay. Cells were seeded at a density of 8×10^3 cells/well in 96-well plates and incubated at 37 ºC for 24 h. Subsequently, the cells were exposed to increasing concentrations of the compounds for 24, 48 and 72 h intervals. After each interval, the culture medium was replaced with 110 µL of MTT solution (0.5 mg/mL in PBS) (Sigma Chemical Co., USA) and incubated for an additional 2 h. The MTT formazan product was solubilized in DMSO and its absorbance was measured at the wavelength of 550 nm, using a microplate reader (Bio-Rad Laboratories, USA), with a reference wavelength of 655 nm. Cell viability was expressed as a percentage relative to control conditions and IC_{50} values were determined for each treatment group.

RESULTS AND DISCUSSION

Isolation of phytochemicals from the leaves of *Ventilago denticulata* by chromatographic methods provided eight compounds (Fig. 1). The structures of the isolated compounds were elucidated by UV, IR, 1D NMR (^{1}H and ^{13}C), 2D NMR (COSY, HSQC and HMBC), mass spectrometry (MS), as well as comparison of their spectral data with those reported in the literature. These compounds were characterized as five known flavonoids *viz*. rhamnazin-3-O-rhamninoside (**1**) [8], ombuin-3-O-glucoside (**2**) [19], ombuin (**3**) [20], rhamnetin (**4**) [21] and kaempferide (**5**) [22], together with three known anthraquinones *viz*. frangulin B (**6**) [23], emodin-8-O-β-D-glucoside (**7**) [24] and emodin (**8**) [9]. Notably, compound **6** was reported from *V. denticulata* for the first time.

Cytotoxic activity: The isolated compounds (**1**-**8**) were preliminary screened for total HDAC inhibitory activity against HeLa nuclear extract (Table-1) using the Fluor-de-Lys *in vitro*

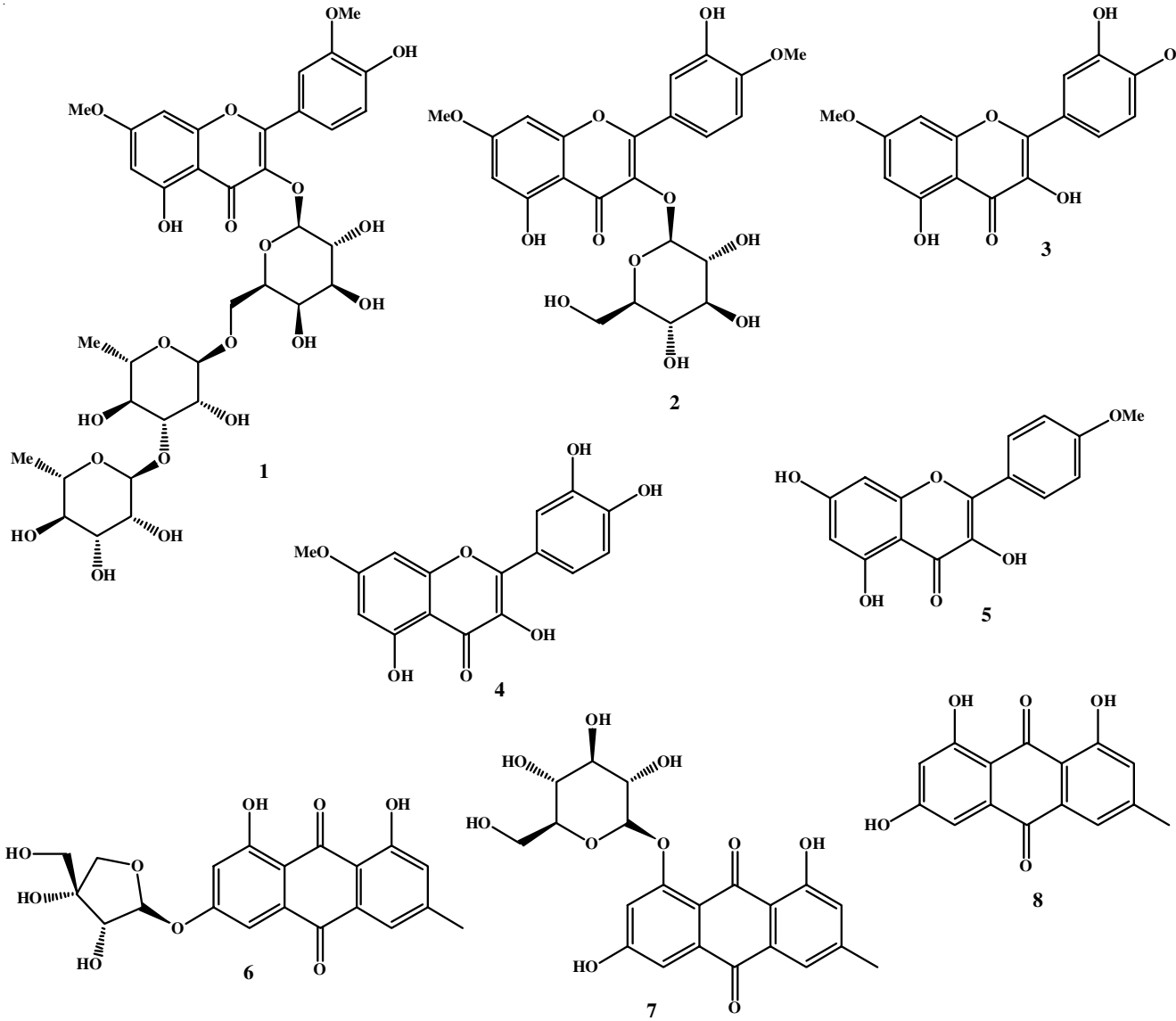

Fig. 1. Phytochemicals (**1**-**8**) isolated from leaves of *V. denticulata*

TABLE-1
HDAC INHIBITORY ACTIVITY OF
THE ISOLATED COMPOUNDS at 20 µg/mL

Compound	Inhibition (%)	Compound	Inhibition (%)
1	53.74	**6**	57.47
2	10.44	**7**	4268
3	17.96	**8**	17.30
4	55.09	TSA	86.10[a]
5	36.11	–	–

[a]At 2.5 µM

fluorescence activity assay kit. Compounds **4** and **6** displayed the highest inhibitory activities with percentage values of 55.09 and 57.74, respectively. The significant variance in the inhibitory performance across the compounds suggests potential structural or functional differences influencing their potency.

The *in vitro* antiproliferative activities of compounds **4** and **6** were evaluated using the MTT assay against HeLa, A549 and MCF-7 cancer cell lines (Table-2). Cisplatin served as the pharmacological control and Vero cells were used to assess non-cancer cell cytotoxicity. Over a 72 h period, compound **6** consistently demonstrated superior antiproliferative potency with IC_{50} values of 8.35, 17.37 and 17.27 µM for HeLa, A549 and MCF-7 cells, respectively, as compared to compound **4**. In contrast, compound **4** showed diminished cytotoxicity in non-cancerous Vero cells, indicating a potential selective profile.

TABLE-2
ANTIPROLIFERATIVE ACTIVITY OF COMPOUNDS **4**
AND **6** AGAINST CANCER CELL LINES

Cell lines	Time (h)	IC_{50} values (mean ± SD; n = 3; µM)		
		4	**6**	Cisplatin
HeLa	24	55.14 ± 1.12	10.58 ± 0.32	17.07 ± 1.00
	48	27.70 ± 1.50	9.36 ± 0.004	9.97 ± 0.34
	72	21.82 ± 0.30	8.35 ± 0.11	6.45 ± 0.13
A549	24	70.10 ± 0.35	24.80 ± 0.39	65.36 ± 8.11
	48	33.74 ± 0.27	19.43 ± 0.58	11.44 ± 1.99
	72	30.48 ± 0.28	17.37 ± 0.78	5.06 ± 0.01
MCF-7	24	175.45 ± 4.91	20.93 ± 0.34	29.17 ± 4.48
	48	135.39 ± 6.60	19.14 ± 0.35	13.75 ± 1.81
	72	30.92 ± 0.01	17.27 ± 0.31	10.42 ± 0.85
Vero cells	24	Inactive	4.77 ± 0.46	42.85 ± 2.38
	48	35.54 ± 1.67	4.37 ± 0.21	12.36 ± 0.63
	72	27.35 ± 0.36	3.98 ± 0.02	6.55 ± 0.81

Molecular docking: To determine the potential of compounds **4** and **6** as HDAC isoform-selective inhibitors, representative HDAC isoforms from classes I (HDAC1, HDAC2 and HDAC8) and II (HDAC4 and HDAC7) were selected for this computational exploration. The crystal structures of these HDAC isoforms were sourced from the Protein Data Bank (https://www.rcsb.org). In these *in silico* analyses (Table-3), compound **4** showed ΔG values across the HDAC isoforms ranging from -6.14 to -7.78 kcal/mol, with corresponding K_i values encompassing from 1.99 to 31.48 µM. Compound **6** exhibited ΔG values between -6.12 and -9.15 kcal/mol, with K_i values ranging from 0.94 to 32.66 µM. Notably, both compounds expressed remarkable affinity for HDACs class II, with the lowest binding energies observed for HDAC4, totaling to -9.65 kcal/mol for compound **4** and -7.78 kcal/mol for compound **6**. Furthermore, the *in silico* physico-chemical properties evaluation of compounds **4** and **6** (Table-3) revealed their compatibility with Lipinski's rule of five, which is a crucial predictor for the drug-likeness of a compound. Compound **4** has a molecular weight of 316.26 g/mol, comfortably within the advised limit of 500 g/mol. Moreover, it satisfies the other essential criteria: it has a number of hydrogen bond donors of 4 (maximum of 5 allowed), hydrogen bond acceptors count of 7 (maximum of 10 allowed), 2 rotatable bonds (well under the limit of 10) and a lipophilicity (log P) value of 2.23, which is below the recommended 5. Similarly, compound **6** exhibits promising attributes that meet all the parameters Lipinski's rule defines. These findings underscore the potential of both compounds **4** and **6** as viable candidates in drug development.

TABLE-3
In silico HDAC INHIBITORY ACTIVITY AND PHYSICO-CHEMICAL ANALYSIS OF COMPOUNDS **4** AND **6**

Parameters			Compound **4**	Compound **6**
Class I HDACs	HDAC1	ΔG (kcal/mol)	-6.39	-6.12
		K_i (µM)	20.76	32.66
	HDAC2	ΔG (kcal/mol)	-6.69	-6.53
		K_i (µM)	12.54	16.22
	HDAC8	ΔG (kcal/mol)	-6.92	-7.06
		K_i (µM)	8.49	6.66
Class II HDACs	HDAC4	ΔG (kcal/mol)	-7.78	-9.15
		K_i (µM)	1.99	0.94
	HDAC7	ΔG (kcal/mol)	-6.14	-7.17
		K_i (µM)	31.48	5.58
Physico-chemical properties	MW (g/mol)		316.26	404.37
	NRB[a]		2	3
	HBA[b]		7	9
	HBD[c]		4	5
	Log P[d]		2.23	1.95

[a]NRB: number of rotatable bonds, [b]HBA: number of hydrogen bond acceptors, [c]HBD: number of hydrogen bond donors, [d]Log P: logarithm of *n*-octanol/water partition coefficient.

The binding modes of compounds **4** and **6** with the HDAC4 template were examined. Key interactions include hydrogen bonds, π-π interactions, Zn^{2+} ion coordination and hydrophobic interactions (Fig. 2). Hydroxyl groups on the flavonoid B-ring of compound **4** form hydrogen bonds with His158 and Asp196; one of these groups also chelates with the zinc ion. Additionally, its aromatic ring systems engage in π-π stacking with His198 and Phe227 residues (Fig. 2a). In contrast, compound **6** shows high selectivity against HDAC4 (Fig. 2b). Hydroxyl groups on glycoside moiety of compound **6** form crucial interactions, including hydrogen bonds with His158 and His159 and zinc ion binding. The anthraquinone aglycone of compound **6** participates in π-π stacking interactions with Phe226 and Leu299. In summary, both compounds demonstrate hydrogen bonding, hydrophobic and zinc-binding interactions, characteristics of an effective HDAC inhibitor.

Conclusion

Five flavonoids and three anthraquinones were isolated from the leaves of *Ventilago denticulata*, of which compounds **4** (rhamnetin) and **6** (frangulin B) exhibited the most promising

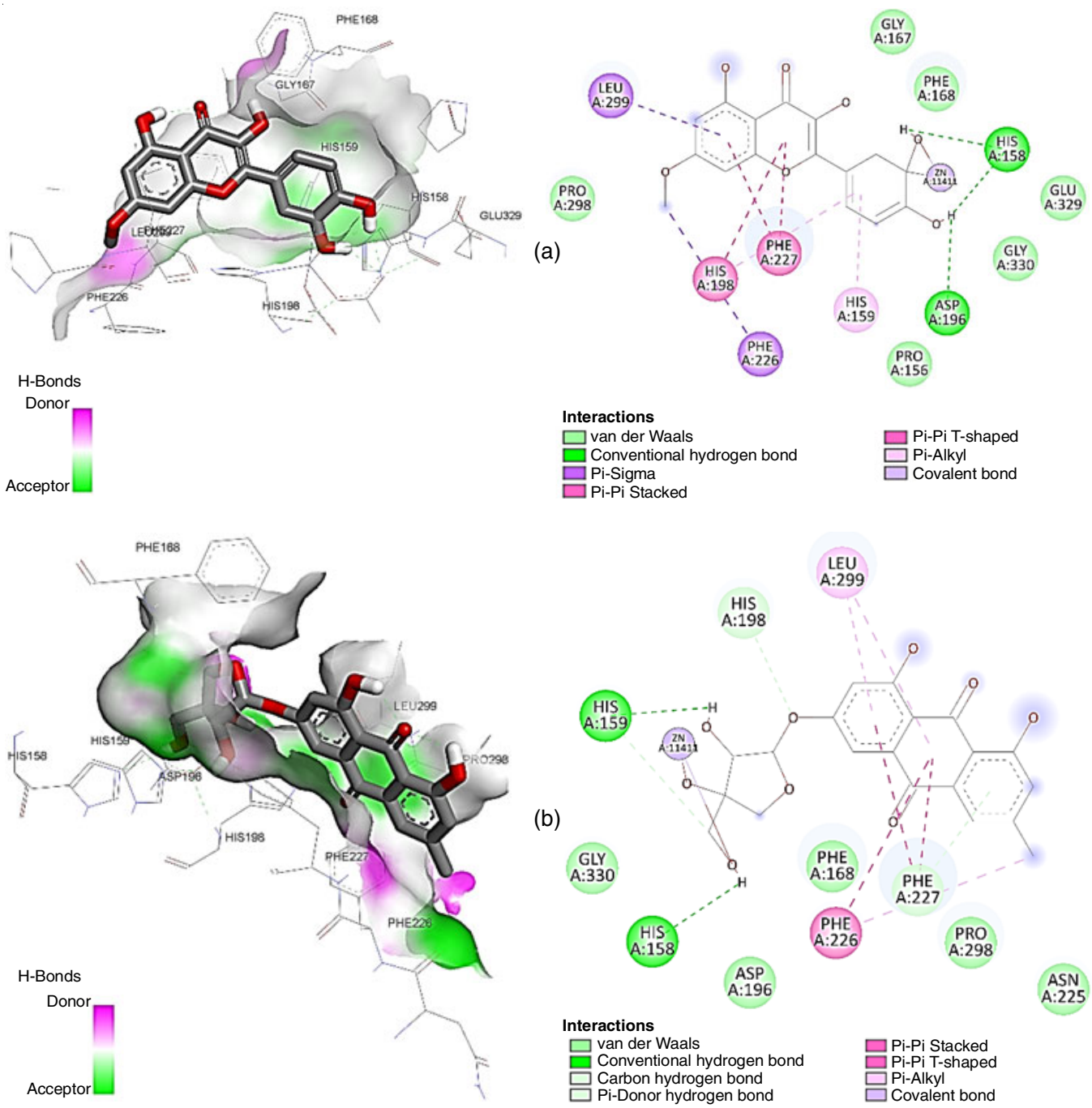

Fig. 2. Binding orientation between compounds **4** (a) and **6** (b) and HDAC4 template

HDAC inhibitory activity. Molecular docking studies revealed that both compounds have a high affinity for HDAC class II isoforms, particularly HDAC4, showing strong potential as HDAC isoform-selective inhibitors. Significantly, the binding interactions of these compounds with HDAC4 involved hydrogen bonds, π-π interactions, Zn^{2+} ion coordination and hydrophobic interactions. *In vitro* antiproliferative evaluations showed that compound **6** possessed superior potency against HeLa, A549 and MCF-7 cancer cell lines when compared to compound **4**. Meanwhile, compound **4** exhibited reduced cytotoxicity to non-cancerous Vero cells. Both compounds **4** and **6** align with Lipinski's rule of five, highlighting their potential as drug candidates.

ACKNOWLEDGEMENTS

The authors gratefully acknowledged to Khon Kaen University, Khon Kaen, Thailand for providing the financial support of this work (Grant No. 6200014001). One of the authors, Thidathep Mahaamnart, is also thankful to the Center of Excellence for Innovation in Chemistry (PERCH-CIC) for financial support. The authors extend their thanks to Mr. Kittisak Poopasith for his excellent NMR work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. A. Bouyahya, N. El Hachlafi, T. Aanniz, I. Bourais, H. Mechchate, T. Benali, M.A. Shariati, P. Burkov, J.M. Lorenzo, P. Wilairatana, M S. Mubarak and N.E. Omari, *Molecules*, **27**, 2568 (2022); https://10.3390/molecules27082568
2. M. Dacęko, B. de Pascual-Teresa, I. Ortín and A. Ramos, *Molecules*, **27**, 715 (2022); https://doi.org/10.3390/molecules27030715
3. A. Pongjanta, K. Pangjit and S. Srichairatanakul, *J. Med. Assoc. Thai.*, **99**, S51 (2016).
4. W. Molee, A. Phanumartwiwath, C. Kesornpun, N. Ngamrojanavanich, S. Surerum, K. Ingkaninan, C. Mahidol, S. Ruchirawat and P. Kittakoop, *Chem. Biodivers.*, **15**, e1700537 (2018); https://doi.org/10.1002/cbdv.201700537
5. B.K. Rao, T. Hanumaiah, C.P. Rao, G.R. Rao and K.J. Rao, *Phytochem.,* **22**, 2583 (1983); https://doi.org/10.1016/0031-9422(83)80170-8
6. B.K. Rao, T. Hanumaiah, J.U.M. Rao, K.V.J. Rao and R.H. Thomson, *Phytochem.,* **23**, 2104 (1984); https://doi.org/10.1016/S0031-9422(00)84996-1
7. N. Lomchoey, J. Nontakham, P. Suebsakwong and S. Suksamran, *KKU Sci. J.*, **45**, 701 (2017).
8. M. Azizah, P. Pripdeevech, T. Thongkongkaew, C. Mahidol, S. Ruchirawat and P. Kittakoop, *Antibiotics*, **9**, 606 (2020); https://doi.org/10.3390/antibiotics9090606
9. N. Hangsamai, T. Mahaamnart, S. Kanokmedhakul, K. Kanokmedhakul, K. Photai, T. Senawong, S. Pitchuanchom and M. Nontakitticharoen, *Molecules,* **27**, 1088 (2022); https://doi.org/10.3390/molecules27031088
10. C.J. Millard, P.J. Watson, I. Celardo, Y. Gordiyenko, S.M. Cowley, C.V. Robinson, L. Fairall and J.W.R. Schwabe, *Mol.Cell.*, **51**, 57 (2013); https://10.1016/j.molcel.2013.05.020
11. J.C. Bressi, A.J. Jennings, R. Skene, Y. Wu, R. Melkus, E.D. Jong, S. O'Connell, C.E. Grimshaw, M. Navre and A.R. Gangloff, *Bioorg. Med. Chem. Lett*., **20**, 3142 (2010); https://10.1016/j.bmcl.2010.03.091
12. M.J. Bottomley, P.L. Surdo, P.D. Giovine, A. Cirillo, R. Scarpelli, F. Ferrigno, P. Jones, P. Neddermann, R.D. Francesco, C. Steinkühler, P. Gallinari and A. Carfí, *J. Biol. Chem*., **283**, 26694 (2008); https://10.1074/jbc.M803514200
13. A. Schuetz, J. Min, A. Allali-Hassani, M. Schapira, M. Shuen, P. Loppnau, R. Mazitschek, N.P. Kwiatkowski, T.A. Lewis, R.L. Maglathin, T.H. McLean, A. Bochkarev, A.N. Plotnikov, M. Vedadi and C.H. Arrowsmith, *J. Biol. Chem.*, **283**, 11355 (2008); https://10.1074/jbc.M707362200
14. J.R. Somoza, R.J. Skene, B.A. Katz, C. Mol, J.D. Ho, A.J. Jennings, C. Luong, A. Arvai, J.J. Buggy, E. Chi, J. Tang, B.C. Sang, E. Verner, R. Wynands, E.M. Leahy, D.R. Dougan, G. Snell, M. Navre, K.W. Knuth, R.V. Swanson, D.E. McRee and L.W. Tari, *Structure*, **12**, 1325 (2004); https://10.1016/j.str.2004.04.012
15. S.J. Weiner, P.A. Kollman, D.A. Case, U.C. Singh, C. Ghio, G. Alahona, S. Profeta and P.A. Weiner, *J. Am. Chem. Soc*., **106**, 765 (1984); https://doi.org/10.1021/ja00315a051
16. G.M. Morris, D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew and A.J. Olson, *J. Comput. Chem*., **19**, 1639 (1998); https://doi.org/10.1002/(SICI)1096-987X(19981115)19:14<1639::AID-JCC10>3.0.CO;2-B
17. G.M. Morris, D. S. Goodsell, R. Huey and A.J. Olson, *J. Comput. Aided Mol. Des*., **10**, 293 (1996); https://10.1007/BF00124499
18. A. Daina, O. Michielin and V. Zoete, *Sci Rep*., **7**, 42717 (2017); https://10.1038/srep42717
19. J. Si, D. Chen, Q. Chang, L. Shen and Z. Zhu, *Acta Bot. Sin.*, **36**, 239 (1994).
20. M.M. Salem, S.R. Hussein, R. Sharawy, A. Khateeb, E.A. Ragab, K.M. Dawood and S. Negoumy, *Egypt. Pharma. J*., **15**, 1 (2016); https://10.4103/1687-4315.184025
21. H. Itokawa, K. Suto and K. Takeya, *Chem. Pharm. Bull.*, **29**, 254 (1981); https://doi.org/10.1248/cpb.29.254
22. E.J. Lee, B.H. Moon, Y. Park, S.W. Hong, S.H. Lee and Y.G. Lee, *Bull. Korean Chem. Soc.*, **29**, 507 (2008); https://doi.org/10.5012/bkcs.2008.29.2.507
23. G.W. Francis, D. Aksnes and Ø. Holt, *Magn. Reson. Chem.,* **36**, 769 (1998); https://doi.org/10.1002/(SICI)1097-458X(1998100)36:10<769::AID-OMR361>3.0.CO;2-E
24. A.K. Khalil, K.M. Akter, H.J. Kim, W.S. Park, D. M. Kang, K.A. Koo and M.J. Ahn, *Plants*, **9**, 222 (2020); https://doi.org/10.3390/plants9020222