RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2023.036

ScienceAsia 49 (2023): **421–427**

Cytotoxicity and molecular docking to DNA topoisomerase II of chalcone flavokawain B isolated from *Kaempferia elegans* rhizomes

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Received 7 Nov 2022, Accepted 26 Feb 2023
Available online 15 May 2023

ABSTRACT: Maceration of the air-dried rhizomes of *Kaempferia elegans* which were obtained from Chiang Mai, Thailand, using hexane, ethyl acetate, and methanol, was conducted. Isolation of hexane and methanol extracts yielded two known compounds: chalcone flavokawain B (1) and lactone 5,6-dehydrokawain (2), respectively. All extracts and two isolated compounds were tested for their cytotoxic activity against hepatocellular carcinoma (HepG2), acute lymphoblastic leukemia (MOLT-3), cholangiocarcinoma (HuCCA-1), and lung carcinoma (A549). Interestingly, compound 1 obtained from the crude hexane extract exhibited good cytotoxic activity against all 4 tested cancer cell lines with half maximal inhibitory concentration (IC_{50}) values ranging from 2.84 to 6.16 μg/ml, whereas compound 2 isolated from the crude methanol extract displayed cytotoxicity against only the MOLT-3 cell line with an IC_{50} value of 24.10 μg/ml and did not show cytotoxic effect against the other cell lines. This is the first report of the anticancer activities of *K. elegans in vitro*. To probe plausible targets, molecular docking studies of both compounds against a potential target, the DNA topoisomerase II-alpha complex, were conducted using an anticancer drug, etoposide as a reference. The results showed that the putative binding poses of both compounds were achieved by forming pi–pi interactions with DNA residue guanidine 13, similarly to what observed in etoposide. Therefore, the bioactive compound 1 has a potential to be an important marker of this plant for medical uses.

KEYWORDS: *Kaempferia elegans*, flavokawain B, human cancer cell, molecular docking, anticancer

INTRODUCTION

Kaempferia species are perennial rhizomic herbs, which belong to the Zingiberaceae family, and are commonly distributed in the tropical Asia including Thailand, China, India, Myanmar, Malaysia, Indonesia, Laos, Cambodia, and Vietnam [1]. There are approximately 60 species which are geographically found in various regions ranging from India to Southeast Asia [2, 3]. Thailand is one of the most biodiverse regions for *Kaempferia* species in the world, and there are more than 20 extant species collected from Thai forests. Several plants belonging to genus *Kaempferia*, for example *K. parviflora*, *K. pulchra*, and *K. galanga*, are widely used in folk medicine to treat different ailments such as cough, pain, infective diseases, wound infection, and digestion disorders [1]. Their rhizomes contain various chemical constituents such as isopimarane, abietane, labdane and clerodane diterpenoids, phenolic acids, phenyl-heptanoids, tetrahydropyrano-phenolic, phenolic glycosides, curcuminoids, steroids, monoterpenoids, diterpenoids, flavonoids, diarylheptanoids, cyclohexane oxide derivatives, and essential oils, which may be responsible for their therapeutic activities [1, 4].

Kaempferia elegans (Wall.), known as Peacock Ginger, is one of the Kaempferia species that showed very attractive patterned foliage and purple-colored flowers. Local Thai people usually call it by the name "Wan-kambungpai or Kambungpai" which is mostly used as an ornamental plant and thus is rarely exploited for its medicinal properties in folk medicine [5]. A few studies reported that isolation of bioactive compounds derived from the rhizomes of *K. elegans* gave flavokawain B, cardamonin, 5,6-dehydrokawain, crotepoxide, piconembrin, 5,7-dimethoxyflavanone, propadane A, propadane B, (+)-15,16-epoxy-8(17),13(16),14-labdatriene, (+)-pumiloxide, methylanti copalate, 13-oxo-14,15-bis-nor-labd-8(17)-ene, anticopalic

acid, anticopalol, 8(17)-labden-15-ol, labda-8(17), 13(14)-diene-15,16-olide, (+)-labda-8(17), 13(Z)-diene-15,16-diol, elegansin A, elegansin B, elegansin C, elegansin D, elegansin E, elegansol A, elegansol B, elegansol C, elegansol D, elegansol E, (-)-isopimara-8(14),15-diene, 8(14), 15-isopimara-dien-7α-ol, sandaracopimar-8(14),15-dien-11-one, 12-deoxyroyle anone, *ar*-abieta triene, abieta-8,11,13-trien-11-ol, and (-)-abieta-8,11,13-trien-7α-ol [6–8].

Many biological activities and the phytochemical properties of crude extracts and isolated compounds of *K. elegans* have been reported [6–8]. Both anticopalic acid and anticopalol showed potent antimicrobial activity against Gram-positive bacteria (*Staphylococcus epidermidis, Enterococcus faecalis*, and *Bacillus cereus*) while 8(17)-labden-15-ol was selectively active against only *B. cereus* [6]. The abieta-8,11,13-trien-11-ol exhibited the potent inhibitory activity against aromatase with the IC_{50} value of 3.7 μM while 5 isolated compounds, including elegansin B, elegansin C, elegansol C, (-)-isopimara-8(14),15-diene, and sandaracopimar-8(14),15-dien-11-one, showed moderate inhibition of aromatase with IC_{50} values ranging from 7.2 to 11.8 μM [7]. Additionally, the isolated abieta-8,11,13-trien-11-ol showed the moderate to low cytotoxic activity against various cancer cell lines including T-cell acute lymphoblastic leukemia (MOLT-3), lung adenocarcinoma (A549), hepatocellular carcinoma (HepG2), hormone-dependent breast carcinoma (T47-D), multidrug-resistant small-cell lung carcinoma (H69AR), triple-negative breast cancer (MDA-MB-231), cervical carcinoma (Hela), cholangiocarcinoma (HuCCA-1), and hepatocellular carcinoma (S102) with IC_{50} values between 27.2 and 137.6 μM [7]. In 2021, the ethanol extract from *K. elegans* rhizome possessed UV A/B protection and antioxidant activities [8]. Notably, the isolated flavokawain B showed better UV A/B protection as compared to a commercial sunscreen. On the other hand, the 5,6-dehydrokawain isolated from *K. elegans* was reported as it exhibited only a few UV A/B protection and antioxidant activities [8]. Flavokawain B isolated from other medicinal plants has also been reported for its biological activities such as anti-inflammatory, antinociceptive, and anticancer properties. In addition, flavokawain B was the potent cytotoxic agent against various human cancer cell lines such as breast carcinoma (MCF-7) with IC_{50} value of 33.8 μM [9], A549 with an IC_{50} value of 11 μg/ml (38.7 μM) [10], leukemia (K562) with an IC_{50} value of 0.95 μg/ml (3.3 μM), ovarian tumor (A2780) with an IC_{50} value of 0.56 μg/ml (1.9 μM) [11], HepG2 with an IC_{50} value of 15.3 μM [12], human oral adenoid cystic cancer (ACC-2) with an IC_{50} value of 4.7 μM [13], mouth epidermal carcinoma (KB) with an IC_{50} value of 20.1 μM, and colorectal carcinoma (HCT116) with an IC_{50} value of ∼25 μM [14]. While the isolated 5,6-dehydrokawain showed the potent activities against K562 with an IC_{50} value of 2.88 μg/ml (12.6 μM), A2780 with an IC_{50} value of 3.79 μg/ml (16.6 μM) [11], MCF-7 with an IC_{50} value of 3.08 μg/ml (13.5 μM), HepG2 with an IC_{50} value of 6.8 μg/ml (29.8 μM), and larynx carcinoma (HEP-2) with an IC_{50} value of 8.7 μg/ml (38.1 μM) [15].

Based on the literature reviews of biologically active compounds from *K. elegans*, there are no reports of direct effects of *K. elegans* extract regarding its cytotoxicity against cancer cells. This led us to the investigation of medicinal properties of this plant by preparing the crude extracts from *K. elegans* rhizomes collected from Chiang Dao, Chiang Mai, Thailand for cytotoxic study against human cancer cell lines. We continuously explored the isolation of the bioactive constituents from rhizomes of *K. elegans* and structural elucidation of the isolated compounds using spectroscopic techniques. Additionally, *in vitro* cytotoxic activities of the isolated compounds and their molecular docking to DNA topoisomerase II-alpha complex, an anticancer target, were evaluated.

METHODOLOGY

Chemicals and instruments

All chemicals and reagents including AR-grade solvents and silica gels were obtained from Sigma Aldrich (USA) and Merck (Germany) companies. Melting points were measured on a Buchi B-540 melting point apparatus (Buchi, Switzerland). HRMS and IR spectra were obtained using a Bruker micro time-of-flight mass spectrometer (Bruker, USA) and a Perkin Elmer Spectrum ONE, respectively (Perkin Elmer, USA). NMR spectroscopic data were recorded on a Bruker Avance 300 spectrometer (^{1}H-NMR at 400 MHz and ^{13}C-NMR at 100 MHz). Silica gel for column chromatography employed Kieselgel 60 (70–230 mesh) (Merck, Germany). Kieselgel 60, F_{254} (230–400 mesh) was use for TLC. The chromatograms were visualized under 254 nm ultraviolet light (UV) after spraying with vanillin reagent, followed by heating in the oven.

Plant material collection and extraction

The rhizomes of *K. elegans* (Wall.) were collected during December 2019 in Chiang Dao district, Chiang Mai, Thailand. The plant materials were identified and authenticated by Mr. Thawatphong Boonma, a plant taxonomist at Brio Botanical Research Garden, Nakhon Nayok, Thailand. The voucher specimen (Savaspun No. 1) was deposited at the Khon Kaen University (KKU) herbarium, Thailand, before the extraction was conducted.

Finely ground and dried powders of *K. elegans* rhizome (3.1 kg) were extracted with hexane, ethyl acetate, and methanol, respectively. Initially, the plant powders were macerated with 7.5 l hexane at room temperature for 3 days; the obtain extract was filtered

using cotton wools and was collected. The marc was repeatedly extracted with hexane 3 times until the extract became colorless. Next, the remaining marc was macerated with ethyl acetate and then methanol. Each extract solution was filtered and concentrated under a reduced pressure using a rotatory evaporator.

The dried rhizomes (3.1 kg) provided a crude hexane extract (17.3 g, 0.55% dry wt), a crude ethyl acetate extract (142.5 g, 4.60% dry wt), and a crude methanol extract (160.8 g, 5.19% dry wt). The viscous liquids obtained from the hexane and methanol extracts showed the formation of orange and yellow solids, respectively.

Isolation of pure compounds from *Kaempferia elegans* rhizomes

Purification of the solid from the hexane extract (1.4 g) using a column loaded siliga gel (50 g) with gradient elution solvents:hexane (100 ml), 1% EtOAc:hexane (100 ml), 2% EtOAc:hexane (100 ml), 4% EtOAc:hexane (500 ml), 10% EtOAc:hexane (500 ml), 20% EtOAc:hexane (250 ml), and lastly 40% EtOAc:hexane (100 ml) to obtain flavokawain B (1) (1.3 g, 0.042% yield) as orange needles.

The solid from the methanol extract (50 mg) was further subjected to chromatography on a silica gel (20 g) using gradient elution with CH_2Cl_2 (50 ml), 1% CH_2Cl_2:MeOH (100 ml), 2% CH_2Cl_2:MeOH (100 ml), 4% CH_2Cl_2:MeOH (500 ml), 10% CH_2Cl_2:MeOH (250 ml), and finally 20% CH_2Cl_2:MeOH (250 ml) to afford 5,6-dehydrokawain (2) (42 mg, 0.0014% yield) as a faint-yellow solid.

Cytotoxicity against human cancer cell lines

Crude rhizome extracts and pure compounds dissolved in DMSO were evaluated for their cytotoxic activity against adhesive cancer cell lines: HepG2, HuCCA-1, and A549 using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay [16]. Cytotoxicity against the non-adhesive MOLT-3 cell line was conducted using the 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assay [17]. Anticancer drugs: Doxorubicin and Etoposide were used as standard. The cytotoxicity was reported as an IC_{50} value in μg/ml.

Molecular docking

DNA topoisomerase II-alpha complexed with DNA and etoposide (PDB code 5GWK, resolution = 3.15 Å) was prepared using LePro software to remove water, metal ions, and ligands and add hydrogens [18]. The 3D structure of etoposide was taken from X-ray crystallographic structure (PDB code 5GWK), and hydrogen atoms were added using Openbabel software [19, 20]. For alternative conformations, ligand structures were obtained by conformational search using MMFF94 force field within −10 or −20 kcal/mol. The conformations with the largest root-mean-square deviations (RMSDs) compared with the input structure were selected for docking. Alternatively, these were manually generated and optimized in the gas phase using the MMFF94 force field.

Molecular docking was conducted using Ledock software based on simulated annealing and genetic algorithm optimization with physics/knowledge-based scoring function [18]. The charges were assigned as implemented in the software. The Cartesian coordinates of the binding pocket were set as follows: x min = 20.803, x max = 41.074, y min = −32.871, y max = −13.556, z min = −67.836, and z max = −48.463. The outputs of 20 poses whose RMSDs were within 1.0 Å were clustered to remove redundancy. The analysis of docking poses was done using Pymol and Maestro software [21, 22]. The RMSDs were calculated using the obrms tool within Openbabel [19, 20].

Statistical analysis

The data were presented as mean ± standard deviation (SD) of the results obtained from triplicate experiments. Significant levels were determined via one-way analysis of variance using the SPSS software (version 22, SPSS Inc., Chicago, IL, USA). Statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

Microplastics abundance in shellfish

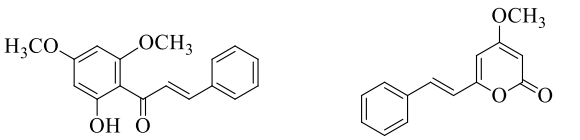
Air-dried and ground *K. elegans* rhizome powders were extracted by maceration using hexane, ethyl acetate, and then methanol to give the crude hexane extract (0.55% dry wt), the crude ethyl acetate extract (4.60% dry wt), and the crude methanol extract (5.19% dry wt), respectively. It was found that the crude methanol extract had the highest yield among those extracts. Furthermore, 2 pure compounds, flavokawain B (1) and 5,6-dehydrokawain (2) containing a kava chalcone and a kava lactone as a core structure, respectively, were isolated from the crude hexane extract and the crude methanol extract of the rhizomes of *K. elegans* by using a column chromatography technique. The melting point ranges of compounds 1 and 2 were obtained to be 92.5 °C–93.6 °C and 142.0 °C–143.9 °C, respectively. All the isolated compounds were verified by comparison of their mp, IR, NMR (^{1}H and ^{13}C) data, and ESI-MS with those reported in the literature identified as flavokawain B (1) [23] and 5,6-dehydrokawain (2) [24], respectively (Fig. 1, Fig. S1 and Fig. S2).

All the crude extracts and the isolated compounds (1 and 2) were tested for the cytotoxic activity against 4 human cancer cell lines (HepG2, MOLT-3, HuCCA-1, and A549), shown in Table 1. The result showed that both hexane and ethyl acetate extracts were active against all cancer cells with IC_{50} values in a range of

Table 1 Cytotoxicity of *K. elegans* extracts: flavokawain B (1), and 5,6-dehydrokawain (2) against human cancer and normal cell lines.

Extract/compound	Cytotoxicity (IC_{50}, μg/ml)				
	HepG2	MOLT-3	HuCCA-1	A549	MRC-5
Hexane	24.22 ± 7.37	nd	17.51 ± 0.73	48.33 ± 2.51	nd
Ethyl acetate	34.59 ± 1.64	nd	17.76 ± 4.37	57.57 ± 1.46	nd
Methanol	inactive	nd	inactive	inactive	nd
Flavokawain B (1)	5.87 ± 0.16	2.84 ± 0.71	5.56 ± 1.09	6.16 ± 0.13	6.96 ± 1.57
5,6-dehydrokawain (2)	inactive	24.10 ± 10.65	inactive	inactive	inactive
Doxorubicin*	0.36 ± 0.02	0.009 ± 0.001	0.58 ± 0.001	0.33 ± 0.049	1.32 ± 0.28
Etoposide*	36.71 ± 1.78	0.017 ± 0.001	–	–	–

* Positive control; inactive at 50 μg/ml for a pure compound and at 100 μg/ml for the extracts; nd = not determined.



flavokawain B (**1**) 5,6-dehydrokawain (**2**)

Fig. 1 Structures of flavokawain B (1) and 5,6-dehydrokawain (2) isolated from rhizome of *K. elegans*.

17–57 μg/ml, but the methanol extract was inactive. It is possibly due to high-polar small molecules existing in the methanol extract having less efficiency to penetrate the plasma membrane of cancerous cells [25]. Flavokawain B (1) inhibited all tested cancer cell lines with IC_{50} values between 2.84 and 6.16 μg/ml. Meanwhile, 5,6-dehydrokawain (2) showed activity only against MOLT-3 with an IC_{50} value of 24.10 μg/ml, but it was inactive against the other cell lines. It was found that flavokawain B (1) showed good cytotoxicity against HepG-2 cancer cells with an IC_{50} value of 5.87 μg/ml which is comparable to the previously reported value (IC_{50} = 8.3 μg/ml) [23]. Moreover, flavokawain B (1) was the most active against MOLT-3 with the IC_{50} value of 2.84 μg/ml, but this cytotoxic activity was 8.5-fold lower than that of 5,6-dehydrokawain (2) with the IC_{50} value of 24.10 μg/ml. Both isolated compounds were tested against MRC-5 as a normal lung fibroblast cell line. The results showed that 5,6-dehydrokawain (2) showed no cytotoxicity against MRC-5 while flavokawain B (1) had its IC_{50} value of 6.96 μg/ml which was higher compared to the cytotoxicity against 4 cancer cell lines.

In addition, both flavokawain B (1) and 5,6-dehydrokawain (2), which were isolated from *K. elegans* collected from Chiang Mai, Thailand, were reported as an UV A/B protecting agent excluded from the anticancer activity while these compounds were not found in *K. elegans* collected from Kanchanaburi, Thailand [6–8]. It was probably due to the different environmental stresses in both biotic and abiotic conditions such as light, temperature, soil water, soil fertility, salinity, and symbiotic microorganisms which can affect growth and development of the plants, and even their secondary metabolites [26].

Since flavokawain B (1) and 5,6-dehydrokawain (2) showed inhibitory effects against several cancer cell lines. This led us to the investigation of whether these compounds could interact with human DNA topoisomerase II-alpha (TOP2A), a prevalent enzyme that controls DNA topology. This enzyme is a target of recent anticancer drugs [27].

Before the docking experiments of compounds 1 and 2, the model was validated by redocking of etoposide by extracting the etoposide 3D structure from the drug-protein complex (PDB code 5GWK), followed by the docking of the etoposide back into the binding site of the protein. Generally acceptable models can reproduce the biologically active pose with RMSD of less than 2 Å.

From the redocking, we found that the RMSD of the top-scoring pose of etoposide compared with the biologically active (X-ray) pose was 0.63 Å (docking score of −10.22 kcal/mol) when starting with the exact conformation as extracted from the X-ray crystallographic structure. When starting with alternative conformations, the RMSD were 0.73–0.91 Å (docking scores of −10.22 to −10.27 kcal/mol). This shows that the model can reproduce the correct binding pose of the etoposide within an acceptable RMSD threshold. The result is independent of the starting conformations.

By inspection of the native (X-ray) pose of the etoposide in the DNA topoisomerase II-alpha complexed with DNA (PDB code 5GWK), the ligand-protein and ligand-DNA interactions include the hydrogen bonding between the phenolic ring and Aspartic acid 463 residue and between the hydroxyl group adjacent to the anomeric carbon and DNA residue, Guanidine 13 (Fig. 2, pink lines). The middle aromatic ring of etoposide forms a pi–pi interaction with the same DNA residue, Guanidine 13 (Fig. 2, green lines).

We anticipate that, if similar interactions are found in the docked poses of compounds 1 and 2, it would indicate that the compounds can potentially

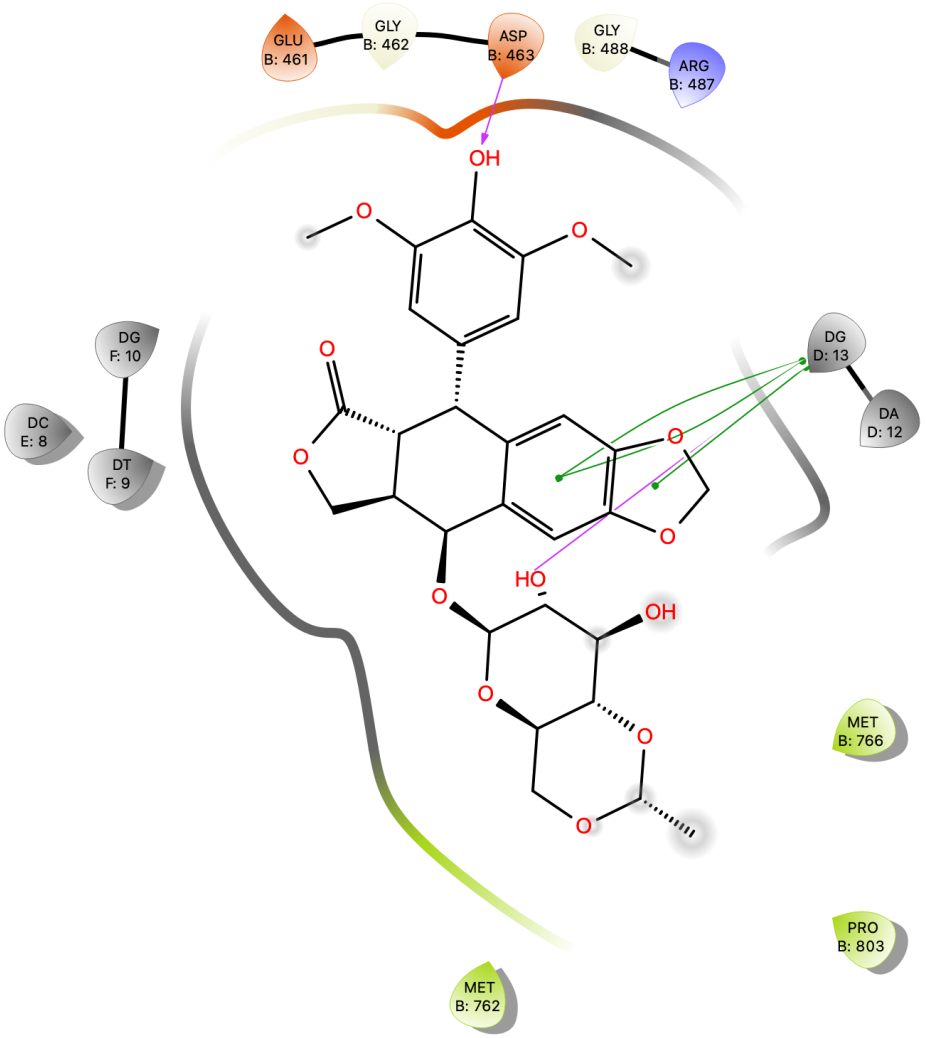


Fig. 2 Ligand-protein/DNA interactions in co-crystallized structure between Etoposide, DNA, and DNA topoisomerase II alpha: pink lines = H bond, green lines = pi–pi interactions (PDB code 5GWK, chain D).

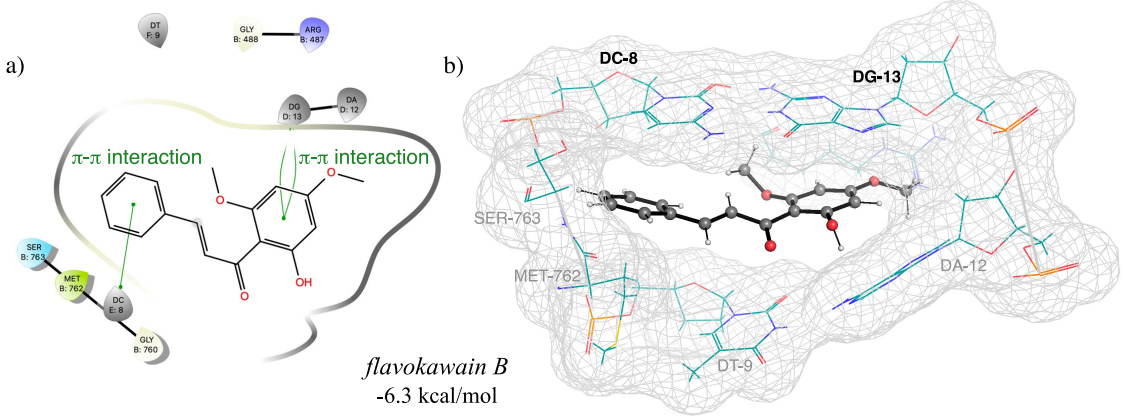


Fig. 3 Top scoring pose of flavokawain B (1): highlighting ligand-protein/DNA interactions (3a) and the binding pocket (3b).

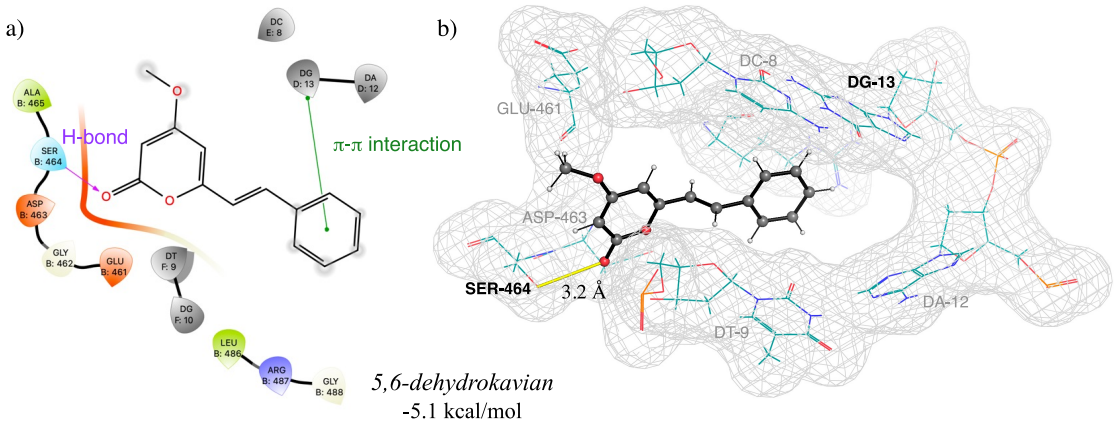


Fig. 4 Top scoring pose of 5,6-dehydrokawain (2): highlighting ligand-protein/DNA interactions (4a) and the binding pocket (4b).

form favorable interactions with the protein and the DNA, assisting in their roles as inhibitors in a similar fashion to etoposide. From the docking experiment of compound 1, the best scoring pose (−6.3 kcal/-mol) exhibited pi–pi interactions with DNA residues, Guanidine 13 (also seen in etoposide native pose) and Cytosine 8 (Fig. 3a, green lines). Fig. 3b illustrates the proximity and alignment between compound 1 and the Guanidine 13 and Cytosine 8 residues in the etoposide binding pocket.

For compound 2, the best scoring pose (−5.1 kcal/mol) showed a pi–pi interaction with the same DNA residue, Guanidine 13 (also found in etoposide native pose) and hydrogen bond to the protein residue, Serine 464 (Fig. 4a). The distance between heavy atoms was predicted to be 3.2 Å, coherent with a moderate H bonding interaction (Fig. 4b).

From the molecular docking results, compared with the X-ray crystallographic data, we found that all three molecules in this study: etoposide, compounds 1 and 2 exhibited one common feature, the pi–pi interaction with the DNA residue, Guanidine 13 (Fig. 2–Fig. 4). Compound 1 formed pi–pi interactions with Guanidine 13 at 2 regions whereas compound 2 formed pi–pi interaction at only one region (Fig. 3a and Fig. 4a, respectively). Assuming that the pi–pi interaction plays an important role in the binding, this result would be in agreement with our cytotoxicity test of these two compounds (IC_{50} = 2.84 and 24.10 μg/ml of compounds 1 and 2, respectively). The stronger pi–pi interactions of compound 1 could be owing to the three electron-donating groups (methoxy groups, $-OCH_3$) present in its structure (Fig. 3a) that increased electron density of the ring, leading to a relatively stronger pi–pi interaction [28]. In contrast, compound 2 without additional electron-donating group would form a relatively weaker pi–pi interaction with Guanidine 13 (Fig. 4a). However, further studies are required to prove that these interactions are indeed present or significant.

CONCLUSION

Flavokawain B (1) and 5,6-dehydrokawain (2) isolated from *K. elegans* rhizomes demonstrated cytotoxic activity against human cancer cell lines. Compound 1 showed strong cytotoxicity against HepG2 cell line with an IC_{50} value of 5.87 μg/ml, whereas compound 2 was selectively active against MOLT-3 with the IC_{50} value of 24.10 μg/ml but not as good as compound 1 with the IC_{50} value of 2.84 μg/ml. Molecular docking analysis suggests putative binding poses where both compounds 1 and 2 formed pi–pi interactions with Guanidine 13, an interaction which is present in the crystallographic structure of the etoposide-DNA bound protein complex. Therefore, flavokawain B (1) may be a valuable potential inhibitor for the development of anticancer agent.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2023.036.

Acknowledgements: This work was financially supported by the Development Institute Pibulsongkram Rajabhat University under Grant Number [RDI-2-63-33], School of Science, University of Phayao under Grant Number [PBTSC65005], and the Grants for Development of New Faculty Staff, Ratchadaphiseksomphot Endowment Fund, Chulalongkorn University.

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Appendix A. Supplementary data

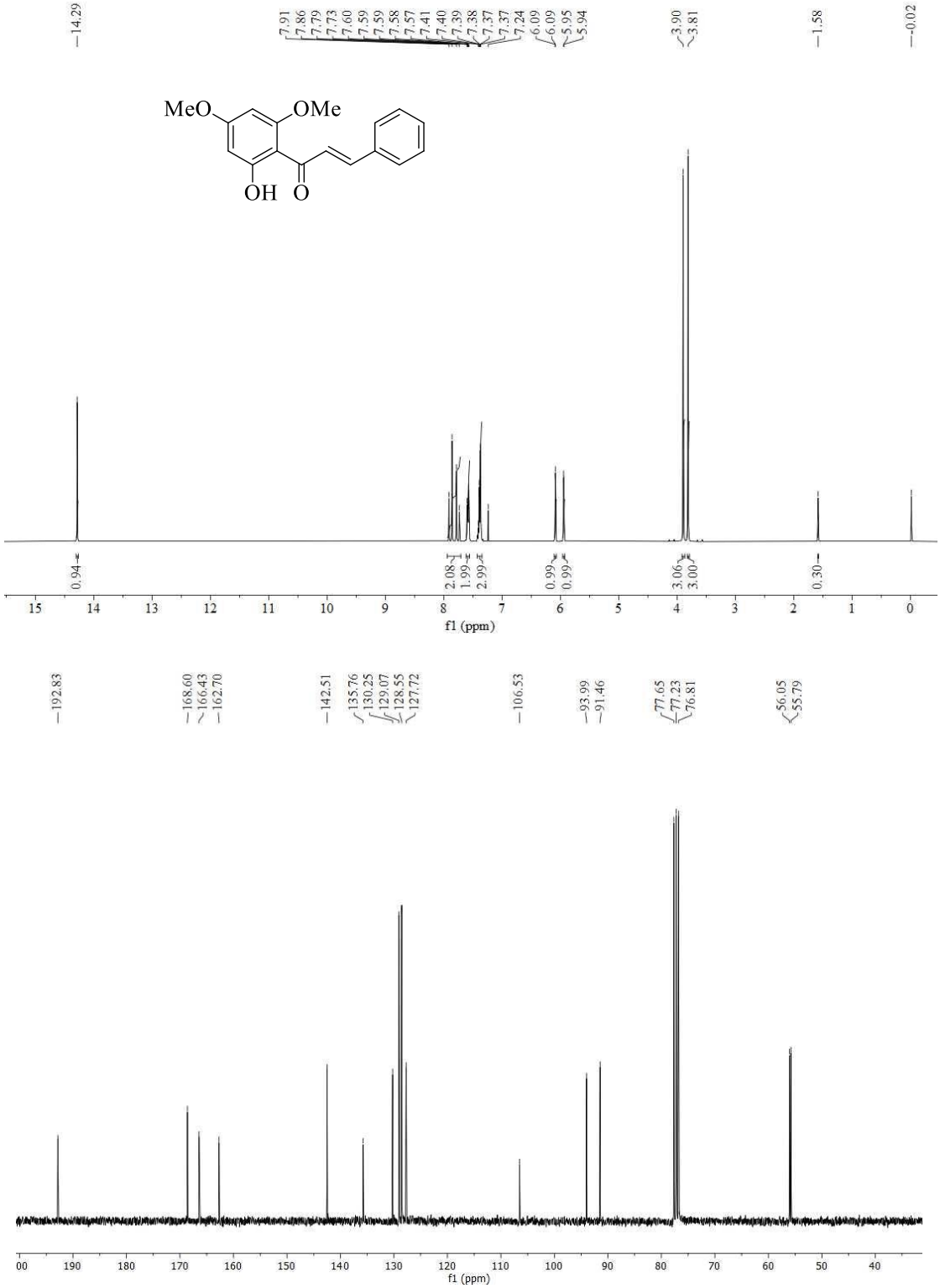

Fig. S1 ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) spectrum of flavokawain B (1).

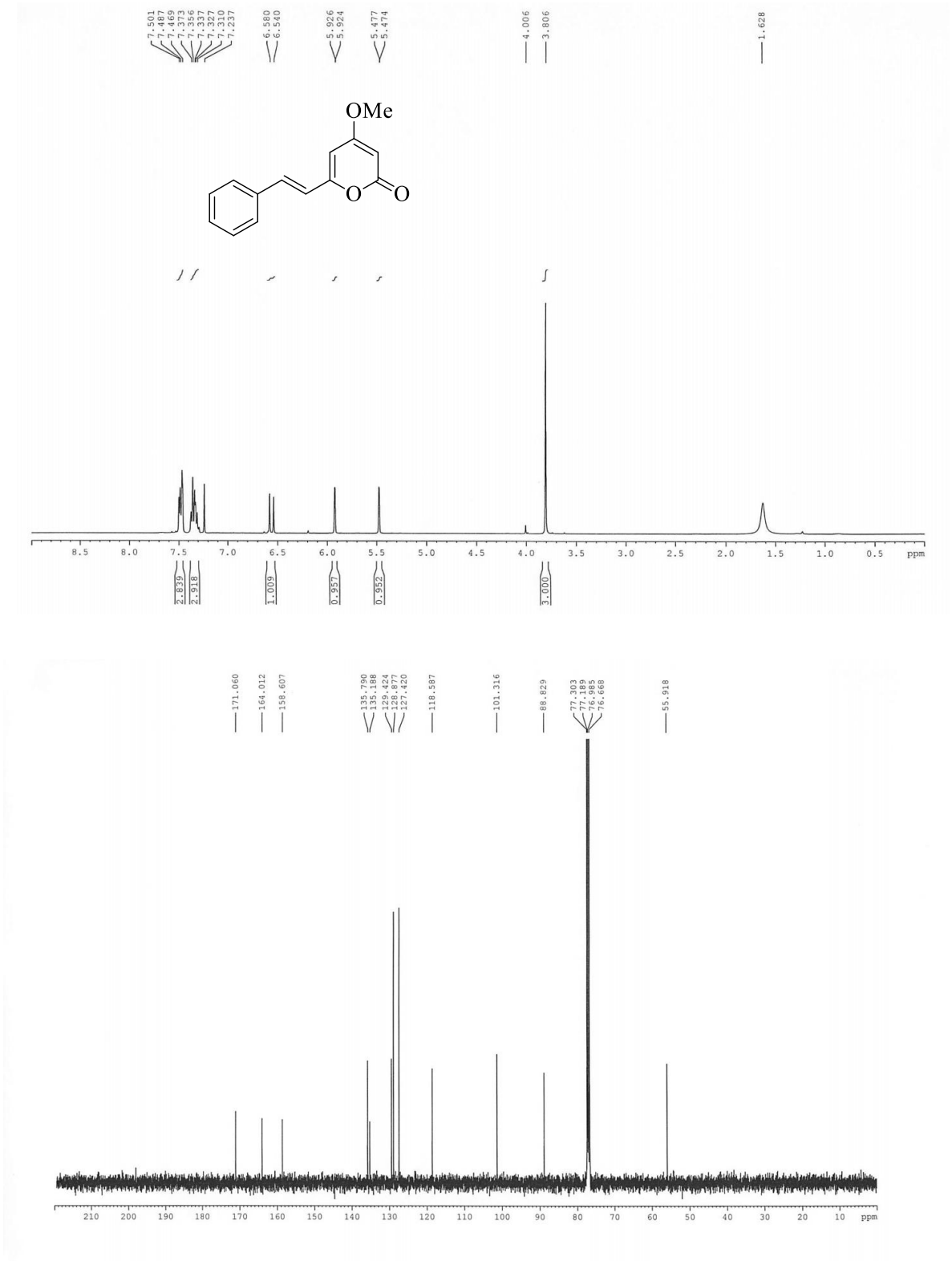

Fig. S2 ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) spectrum of 5,6-dehydrokawain (2).