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A New Oxoaporphine and Liriodenine's Anti-Neuroblastoma Potential from the Roots of *Polyalthia bullata* King

(Sebatian Oksoaporfina Baharu dan Potensi Anti-Neuroblastoma Liriodenin daripada Akar *Polyalthia bullata* King)

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ABSTRACT

Polyalthia bullata King's root yielded a new compound named 5-methylliridine (**1**) in addition to six previously identified compounds. These known compounds include liriodenine (**2**), 11-methoxyliriodenine (**3**), lysicamine (**4**), onychine (**5**), 5-hydroxy-6-methoxyonychine (**6**), and 8-methoxyeupolauridine (**7**). The structures of compounds **1**-**7** were determined through spectroscopic analysis. Liriodenine (**2**) exhibited a remarkable ability to decrease the cell viability of cancerous N2A cells to 22% within a 24 h timeframe, indicating its potential as an anti-neuroblastoma agent. Molecular docking results additionally suggested that oxoaporphines (**1**-**4**) have the potential to act as inhibitors of protein kinases. These findings highlight the therapeutic potential of *P. bullata* constituents in cancer treatment, particularly neuroblastoma, and contribute to understanding its medicinal properties.

Keywords: Anti-neuroblastoma; oxoaporphine; protein kinase; *Polyalthia bullata* King; Tongkat Ali Hitam

ABSTRAK

Akar *Polyalthia bullata* King menghasilkan satu sebatian baharu yang dikenali sebagai 5-metilliridine (**1**) bersama dengan enam sebatian yang sebelum ini telah dikenal pasti. Sebatian yang telah dikenali ini termasuk liriodenine (**2**), 11-metoksiliriodenine (**3**), lysicamine (**4**), onychine (**5**), 5-hidroksi-6-metoksionychine (**6**) dan 8-metoksieupolauridine (**7**). Struktur bagi sebatian **1-7** telah ditentukan melalui analisis spektroskopi. Liriodenine (2) menunjukkan keupayaan yang luar biasa dalam mengurangkan sel kanser N2A kepada 22% dalam jangka masa 24 jam, memberikan petanda tentang potensi sebagai agen anti-neuroblastoma. Hasil daripada dok molekul turut mencadangkan bahawa oksoaporfina (**1-4**) mempunyai potensi untuk bertindak sebagai perencat protein kinase. Penemuan ini menonjolkan potensi terapeutik bahan aktif *P. bullata* dalam rawatan kanser, khususnya neuroblastoma, serta menyumbang kepada pemahaman tentang sifat perubatannya.

Kata kunci: Anti-neuroblastoma; oksoaporfina; *Polyalthia bullata* King; protein kinase; Tongkat Ali Hitam

Introduction

Polyalthia bullata King, also known as 'Tongkat Ali Hitam' or 'Tongkat Ali Baginda' among the local community in Malaysia, belongs to the *Polyalthia* genus within the Annonaceae family (Ahmad et al. 2023; Alsailawi et al. 2023; Kamarul Zaman et al. 2020a, 2020b; Paarakh & Khosa 2009). This small tree is commonly found in the Indian subcontinent and the Malay peninsula (Connolly, Haque & Kadir 1996). The *Polyalthia* genus has been recognized to contain various compounds such as clerodane diterpenoids, flavonoids, acetogenin, and triterpenoids (Chen, Chia & Huang 2021; Paarakh & Khosa 2009; Yao et al. 2019). Previous research has demonstrated that *Polyalthia* species exhibit a wide

range of activities, including antibacterial, antimalarial, antiulcer, cytotoxic, and hypoglycemic and hypotensive effects (Faizi et al. 2003; Katkar, Suthar & Chauhan 2010; Panthama, Kanokmedhakul & Kanokmedhakul 2010; Prachayasittikul et al. 2009; Tekuri et al. 2019; Vishala et al. 2021; Wiart et al. 2004; Yoo et al. 2005). Despite the limited number of studies conducted on *P. bullata*, it has been discovered that this species shares the presence of aporphine compounds with other *Polyalthia* species (Ang & Lee 2006; Connolly, Haque & Kadir 1996; Din et al. 2005; Fun et al. 1996; Nantapap et al. 2017) (Figure 1).

EXPERIMENTAL DETAILS

GENERAL

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE III 600 MHz spectrometer. The UV measurements were conducted using the Agilent Cary 60 UV Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The IR spectra were acquired using a PerkinElmer 1760x FT-IR spectrophotometer. The HRMS data were obtained from the Agilent 6530 Q-TOF (Agilent Technologies, Santa Clara, CA, USA) mass spectrometer coupled with an Agilent 1200 series Rapid Resolution LC system (Agilent Technologies, Santa Clara, CA, USA). All solvents utilized in this study were sourced from Fisher Brand (AR grade, Loughborough, Leicestershire, UK).

PLANT MATERIAL, EXTRACTION, AND ISOLATION

Polyalthia bullata King, collected from Alor Setar, Kedah, Malaysia in July 2016 is preserved as a voucher specimen (UM-072016b-B009) at the herbarium of the Chemistry Department, Universiti Malaya, Kuala Lumpur, Malaysia. Madam Najmiyah Mohamad Alias from Delima Jelita Herbs Sdn. Bhd. assisted in the plant identification. A total of 1 kg of air-dried root was sequentially extracted with hexane, chloroform, and 95% ethanol, resulting in crude extracts weighing 5.0 g, 6.0 g, and 40.1 g, respectively. The chloroform extract (6.0 g) was subjected to purification using column chromatography with silica gel 60 (0.040-0.063, Merck, Darmstadt, Germany). The solvent system employed was initially 100% chloroform, gradually transitioning to chloroform:methanol (90:10). The first column chromatography step yielded four fractions, namely A, B, C, and D. Fraction A was further purified using Chromatotron centrifugal thin-layer chromatography with a chloroform:hexane (6:4) solvent system, resulting in fraction A1. Subsequently, A1 underwent additional purification using Chromatotron centrifugal thin-layer chromatography with the same solvent system, leading to the isolation of compound **6**. Fraction B was subjected to column chromatography using 100% chloroform, with a gradual increase in polarity to chloroform:methanol (90:10), resulting in fractions B1, B2, and B3. Fraction B2 was purified using Chromatotron centrifugal thin-layer chromatography with 100% chloroform as the solvent, isolating compound **5**. Fraction C was subjected to column chromatography using 100% chloroform, gradually increasing the polarity to chloroform:methanol (90:10), yielding fractions C1, C2, and C3. Subfraction C2 was further fractionated using column chromatography with 100% chloroform, gradually transitioning to chloroform:methanol (90:10), resulting in

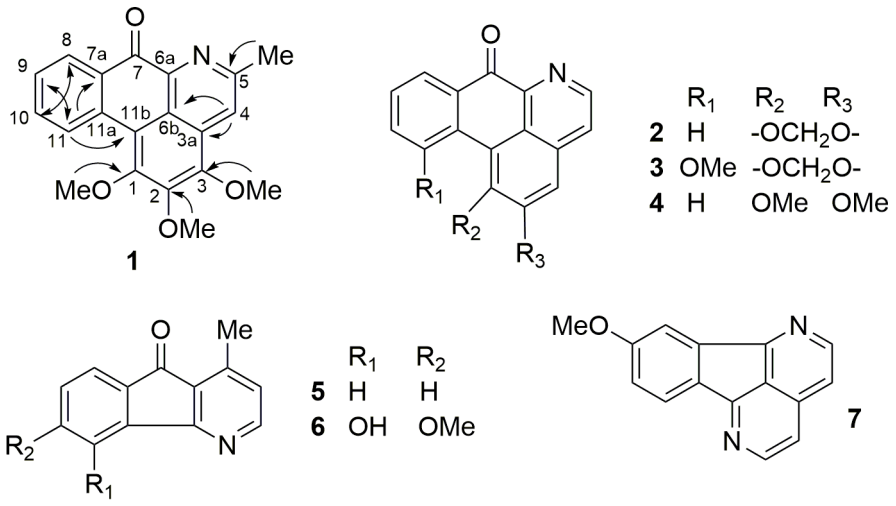

FIGURE 1. Compounds **1**-**7** and HMBC correlations (curved arrow; for **1** only)

six subfractions: C2-1, C2-2, C2-3, C2-4, C2-5, and C2-6. Chromatotron centrifugal thin-layer chromatography of subfraction C2-2, using a chloroform:methanol (98:2) solvent system, isolating compounds **1** and **2**. Subfraction C2-3 was purified using Chromatotron centrifugal thin-layer chromatography with a chloroform:methanol (98:2) solvent system, isolating compound **7**. Chromatotron centrifugal thin-layer chromatography of subfraction C2-4, using a chloroform:methanol (98:2) solvent system, yielded compound **4**. Lastly, subfraction C2-5 was purified using Chromatotron centrifugal thin-layer chromatography with a chloroform:methanol (98:2) solvent system, isolating compound **3**. The yields of the isolated compounds were as follows: compound **1** (4 mg), compound **2** (11 mg), compound **3** (2 mg), compound **4** (5 mg), compound **5** (3 mg), compound **6** (5 mg), and compound **7** (3 mg).

5-METHYLLIRIDINE **(1)**

Yellowish oil; molecular formula $C_{20}H_{17}NO_4$; UV (EtOH) λ_{max} (log Ɛ) 205 (3.61), 208 (3.61), 244 (3.60), 270 (3.21), 304 (3.09), 317 (3.11), and 404 (2.92) nm; IR (NaCl) ν_{max} 2931, 1667, 1516, 1462, 1391, 1209, 1067, and 754 cm^{-1}; ^1H and ^{13}C NMR data: Table 1. HRMS *m/z* 358.0666 $[M+Na]^+$ (calcd. 358.1055) and 334.1270 $[M-H]^-$ (calcd. 334.1085).

NEUROBLASTOMA CELL VIABILITY ASSAY

Mouse N2A cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL, Life Technologies Inc., USA) supplemented with 10% fetal bovine serum, 100 μg mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin in a 5% CO_2 humidified atmosphere at 37 °C. Cells in 96-well plates (5.0×10^3 cells per well) were treated with compounds for 24 h. Cell viability was assessed using the Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Japan) following the manufacturer's protocol. At 24 h time points, 10 μL of CCK-8 solution was added to each well. After 3 h of incubation at 37 °C, the optical density (OD) at 450 nm was measured with a plate reader (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer). DMEM (blank sample; 100% growth) served as a negative control, while sodium dodecyl sulfate (SDS, 200 μg mL^{-1}; cell viability <2%) was used as a positive control. The cell survival rate was calculated using the formula: Cell Survival Rate % = (drug-stimulated cells OD / control group cells OD) × 100%.

MOLECULAR DOCKING EXPERIMENT

The structures of compounds **1**-**7** underwent optimization using the MM2 energy-minimized function within Chem3D Ultra version 16.0. Receptor protein crystal structures (AKT1: PDB ID 4EKK, coordinates: x-center = 13.685, y-center = 1.377, z-center = 19.389; PI3K: PDB ID 4FA6, coordinates: x-center = 44.555, y-center = 13.306, z-center = 31.313; ALK: PDB ID 3LCS, coordinates: x-center = -20.292, y-center = 11.084, z-center = -8.376; FAK: PDB ID 2IJM, coordinates: z-center = 2.031, y-center = 6.656, z-center = 6.756) were obtained from the Protein Data Bank. AutoDockTools version 1.5.6 prepared both the receptor proteins and ligands for the molecular docking experiment, utilizing a grid box parameter setting of grid box spacing = 1.0 Å and x-dimension = y-dimension = z-dimension = 20. Docking and binding affinity calculations were conducted using the AutoDock Vina program. Subsequently, the results were processed and analyzed through BIOVIA Discovery Studio Visualizer version 17.2.0.

RESULTS AND DISCUSSION

The roots of *P. bullata* yielded one new and six previously identified compounds (Figure 1). The newly discovered compound is 5-methylliridine (**1**). In contrast, the known compounds include liriodenine (**2**) (Tsai & Lee 2010), 11-methoxyliriodenine (**3**) (Guinaudeau, Leboeuf & Cavé 1979), lysicamine (**4**) (Tsai & Lee 2010), onychine (**5**) (Prachayasittikul et al. 2009), 5-hydroxy-6-methoxyonychine (**6**) (Mueller et al. 2009), and 8-methoxyeupolauridine (**7**) (Pan et al. 2011). Consistent with a previous investigation on the stem bark of *P. bullata*, the current study highlights the significant presence of aporphine alkaloids.

5-methylliridine (**1**) was obtained in the form of yellowish oil. HRMS showed the molecular formula $C_{20}H_{17}NO_4$, with *m/z* 358.0666 $[M+Na]^+$ and 334.1270 $[M-H]^-$, along with a DBE value 13. The IR spectrum exhibited characteristic peaks at 1667 cm^{-1} (indicative of carbonyl) and 1209 cm^{-1} (suggestive of ether). The ^1H NMR spectrum analysis identified five aromatic protons, one methyl group, and three methoxy groups. Based on the COSY spectrum, the aromatic protons at δ_H 7.92, 7.65 (2H), and 9.48 were connected, forming a partial structure of -CH-CH-CH-CH-, implying the presence of a 1,2-disubstituted benzene moiety. Consistent with the IR spectrum, the ^{13}C NMR spectrum displayed a ketone carbonyl signal at δ_C 179.1. Apart from the resonances associated with aromatic methine, methyl, and methoxy

carbons, the ^{13}C NMR spectrum exhibited signals corresponding to quaternary sp^2 carbons. The high DBE value and the abundance of quaternary sp^2 carbons, indicated the presence of a fused multiple aromatic ring structure in compound **1**. The NMR spectra of compound **1** closely resembled those of oxoaporphine alkaloids, specifically lysicamine (**3**) and liridine. Like lysicamine (**3**) and liridine (Sulaiman, Hamid & Awang 2003), the aromatic proton (δ_H 9.48) was assigned to H-11, and its unusually downfield chemical shift is attributed to the nearby methoxy substitution at position C-1. The methoxy group was assigned to positions C-1, C-2, and C-3 based on J^2 HMBC correlations from δ_H 4.18, 4.13, and 4.09 to the respective quaternary carbons at δ_C 160.2, 158.2, and 147.1. The methyl signal at δ_H 3.87 exhibited J^2 HMBC correlations to C-5 at δ_C 157.1, confirming its assignment as 5-Me. The downfield shift of the methyl group has resulted from the proximate presence of the neighbouring electron lone pair at N(6). Additional HMBC correlations are presented in Figure 1. Based on the collective observations, the structure of compound **1** was identified as 5-methylliridine.

TABLE 1. ^1H and ^{13}C NMR ($CDCl_3$, 600 and 150 MHz, respectively)

Position	^{13}C	^1H
1	160.2	-
2	158.2	-
3	147.1	-
3a	127.0	-
4	115.1	7.63 s
5	157.1	-
6a	131.8	-
6b	121.4	-
7	179.1	-
7a	131.9	-
8	128.7	7.92 dd (8, 2)
9	127.6	7.65 m
10	127.6	7.65 m
11	127.3	9.48 dd (8, 2)
11a	131.8	-
11b	n.d.	-
1-OMe	61.3	4.18 s
2-OMe	62.3	4.13 s
3-OMe	61.9	4.09 s
5-Me	31.0	3.87 s

n.d. – not detected

Neuroblastoma is a prevalent cancer in children, with a mortality rate of 15% among young patients (Greengard 2018; Kennedy et al. 2023; London et al. 2005; Maris et al. 2007; Wang, Chen & He 2023). Due to limited quantities of the isolated compounds, only compound **2** underwent a neuroblastoma cell viability assay. The results demonstrated that liriodenine (**2**) significantly decreased the viability of N2A cells to 22% within a 24-h period (Figure 2). This finding suggests that compound **2** holds promise as a potential anti-neuroblastoma agent. Previous studies have identified liriodenine (**2**) and other oxoaporphine alkaloids exhibiting various anticancer activities (Chen et al. 2013, 2012; Huang et al. 2022; Wei et al. 2014).

To explore potential targets in the neuroblastoma signalling pathway, particularly protein kinases (Bahmad et al. 2019; Gross et al. 2015; Jänne, Gray & Settleman 2009; Megison, Gillory & Beierle 2013), *in-silico* molecular docking experiments were conducted. The structures of compounds **1**-**7** were docked with four crucial protein kinases involved in regulating neuroblastoma cell survival: anaplastic lymphoma kinase (ALK) (Brenner & Gunnes, 2021; Del Grosso et al. 2011), focal adhesion kinase (FAK) (Gabarra-Niecko et al. 2003), phosphoinositide 3-kinase (PI3K) (Hennessy et al. 2005; Johnsen et al. 2008), and RAC-α serine/threonine-protein kinase (AKT1) (Hennessy et al. 2005; Johnsen et al. 2008). These protein kinases significantly affect cell proliferation, viability, and survival. Inhibiting their activation at the ATP-competitive binding domain has been demonstrated to reduce neuroblastoma cell survival. Table 2 presents the binding affinity of compounds **1**-**7**.

Compounds **1**-**4** classified as oxoaporphine-type compounds, exhibited favorable binding affinities to all four protein kinases compared to the native ligand (ATP), indicating their potential as protein kinase inhibitors. Liriodenine (**2**) demonstrated the strongest binding affinities to all protein kinases, reinforcing its potential as an anti-neuroblastoma agent following the in-vitro assay. On the other hand, compounds **5**-**7** displayed binding affinities comparable to ATP.

The conservation of the competitive ATP-binding domain among protein kinases is well established. In this study, the ATP-binding domain of the four protein kinases was superimposed, and the crucial amino acid residues involved in ligand-protein interactions between liriodenine (**2**) and the protein kinases were identified (Figure 3 and Table 2). The findings showed that other oxoaporphine compounds (**1**, **3**, and **4**) shared similar interactions with these amino acid residues. The favorable binding affinities exhibited by the oxoaporphine compounds can be attributed to two factors.

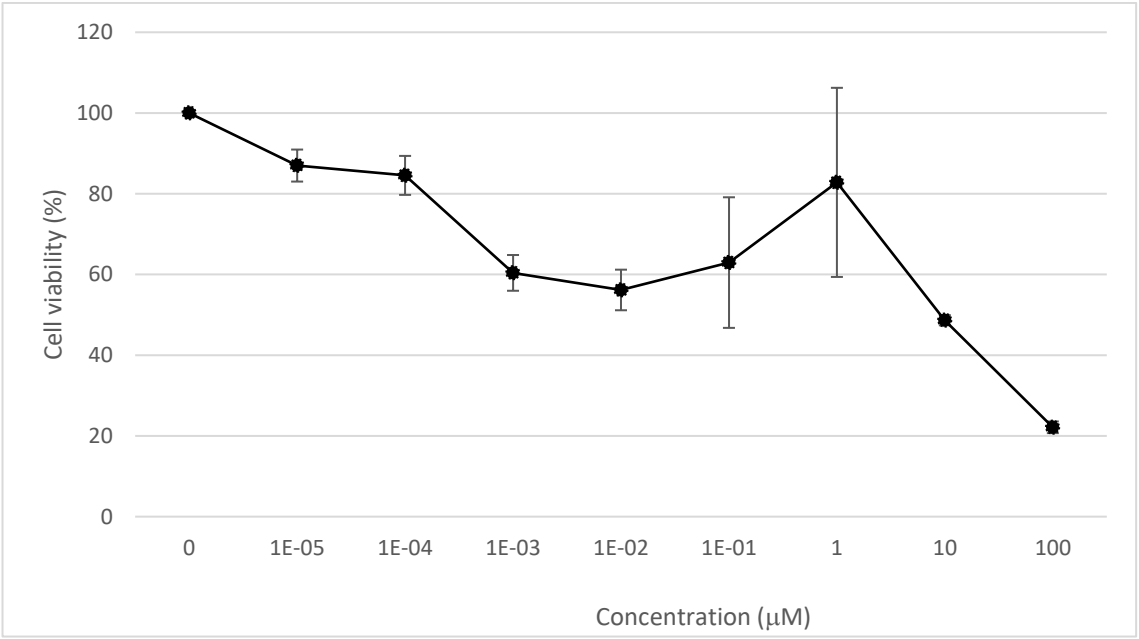

FIGURE 2. Compound **2** against N2A cell viability

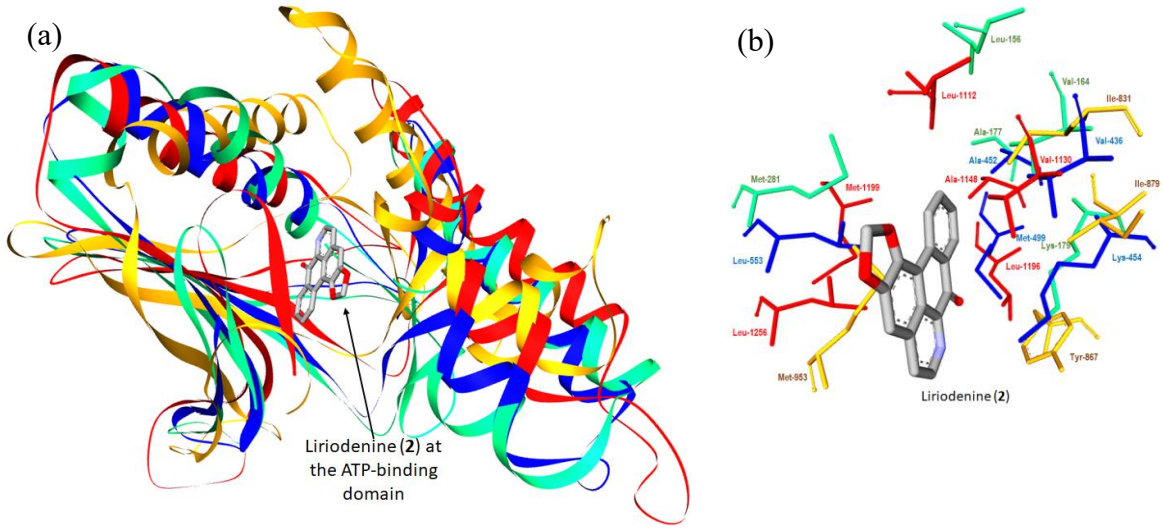

FIGURE 3. (a) ATP-binding domain of AKL (red), FAK (blue), PI3K (yellow), and AKT2 (green); (b) Interacting amino acid residues of AKL (red), FAK (blue), PI3K (yellow), and AKT2 (green)

TABLE 2. Binding affinity (kcal/mol) of compounds **1**-**7** and interacting amino acid residues

Compound	AKL	FAK	PI3K	AKT1
1	-9.4	-8.5	-8.5	-8
2	-10.3	-8.9	-9.7	-9.4
3	-10	-8.8	-9.4	-9.2
4	-9.6	-8.5	-8.5	-8.9
5	-8.3	-7.4	-7.8	-8.1
6	-8.3	-7.5	-7.5	-8
7	-8.4	-7.9	-8.3	-8.1
ATP	-8.3	-8.7	-7.1	-7.8
Amino acid residues	Leu-1122, Val-1130, Ala-1148, Leu-1196, Met-1199, Leu-1256	Val-436, Ala-452, Lys-454, Met-499, Leu-553	Ile-831, Tyr-867, Ile-879, Met-953	Leu-156, Val-164, Ala-177, Lys-179, Met-281

Firstly, the highly planar configuration of the oxoaporphine structure allows it to slide smoothly and fit within the binding domain (Figure 3). Secondly, four fully conjugated six-membered rings in the oxoaporphine structure enable the formation of multiple non-covalent π-interactions, such as π-σ, π-alkyl, and π-sulfur interactions, with the surrounding amino acid residues. Consequently, this enhances the stability and

strength of the binding. Additionally, the amine, carbonyl, and oxoalkyl functional groups in compounds **1**-**4** contribute to the binding affinity by forming hydrogen bonds between the oxygen or nitrogen atoms in the oxoaporphine and the adjacent amino acid residues. Based on these observations, it can be inferred that oxoaporphine alkaloids (**1**-**4**) hold potential as protein kinase inhibitors and anti-neuroblastoma agents.

Conclusions

In summary, this study successfully isolated and characterized seven compounds. Among them, a novel oxoaporphine alkaloid named 5-methylliridine (**1**) was identified. The remaining six compounds included three oxoaporphine alkaloids (liriodenine (**2**), 11-methoxyliriodenine (**3**), and lysicamine (**4**)), two 4-azafluorenone alkaloids (onychine (**5**) and 5-hydroxy-6-methoxyonychine (**6**)), and one naphthyridine alkaloid (8-methoxyeupolauridine (**7**)). Previous studies have highlighted various activities exhibited by these known compounds (**2**-**7**), such as antifungal, antibacterial, anticancer, and antimalarial properties.

The observed anti-neuroblastoma activity of liriodenine (**2**) aligns with previous reports on the anticancer effects of oxoaporphine alkaloids. Furthermore, molecular docking experiments indicated the potential of the oxoaporphines (**1**-**4**) as protein kinase inhibitors. Lastly, this study provides novel insights into the chemical constituents and potential activities of the less-studied plant species *P. bullata*. To the best of our knowledge, this is the first report documenting compounds **1**-**7** as chemical constituents of *P. bullata*.

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References

Ahmad, F., Said, S.A., Chakravarthi, S., Norhidayah, A., Mohamed, B., Edros, R.Z. & Vejayan, J. 2023. Comparison of three aphrodisiac plants *(Eurycoma longifolia*, *Polyalthia bullata* and *Stema tuberosa*) synonymous with Tongkat Ali. *Tropical J. Nat. Prod. Res.* 7(5): 3002-3008.

Alsailawi, H.A., Mudhafar, M., Hanan, A.H., Ayat, S.S., Dhahi, S.J., Ruaa, K.M. & Raheem Hussein, A. 2023. Phytochemical screening and antibacterial activities of *Antiaris toxicaria* stem, *Polyalthia rumphii* leaves and *Polyalthia bullata* stem extracts. *AIP Conf. Proc.* 2845(1): 020007.

Ang, H. & Lee, K. 2006. Contamination of mercury in tongkat Ali hitam herbal preparations. *Food and Chemical Toxicology* 44(8): 1245-1250.

Bahmad, H.F., Chamaa, F., Assi, S., Chalhoub, R.M., Abou-Antoun, T. & Abou-Kheir, W. 2019. Cancer stem cells in neuroblastoma: Expanding the therapeutic frontier. *Front Mol. Neurosci.* 12: 131.

Brenner, A.K. & Gunnes, M.W. 2021. Therapeutic targeting of the Anaplastic Lymphoma Kinase (ALK) in neuroblastoma-A comprehensive update. *Pharmaceutics* 13(9): 1427.

Chen, Y.C., Chia, Y.C. & Huang, B.M. 2021. Phytochemicals from *Polyalthia* species: Potential and implication on anti-oxidant, anti-inflammatory, anti-cancer, and chemoprevention activities. *Molecules* 26(17): 5369.

Chen, Z.F., Liu, Y.C., Huang, K.B. & Liang, H. 2013. Alkaloid-metal based anticancer agents. *Current Topics in Medicinal Chemistry* 13: 2104-2115.

Chen, Z.F., Liu, Y.C., Peng, Y., Hong, X., Wang, H.H., Zhang, M.M. & Liang, H. 2012. Synthesis, characterization, and *in vitro* antitumor properties of gold(III) compounds with the Traditional Chinese Medicine (TCM) active ingredient liriodenine. *Journal of Biological Inorganic Chemistry* 17: 247-261.

Connolly, J.D., Haque, M.E. & Kadir, A. 1996. Two 7, 7′-bisdehydroaporphine alkaloids from *Polyalthia bullata*. *Phytochemistry* 43(1): 295-297.

Del Grosso, F., De Mariano, M., Passoni, L., Luksch, R., Tonini, G.P. & Longo, L. 2011. Inhibition of N-linked glycosylation impairs ALK phosphorylation and disrupts pro-survival signaling in neuroblastoma cell lines. *BMC Cancer* 11: 525.

Din, L.B., Zakaria, Z., Abdullah, A. & Yamin, B.M. 2005. 8H-Benzo[g][1,3]benzodioxolo[6,5,4-de]quinolin-8-one (oxocrebanine). *Acta Crystallographica Section E* 61(5): o1450-o1452.

Faizi, S., Khan, R.A., Azher, S., Khan, S.A., Tauseef, S. & Ahmad, A. 2003. New antimicrobial alkaloids from the roots of *Polyalthia longifolia* var. pendula. *Planta Medica* 69(4): 350-355.

Fun, H.K., Sivakumar, K., Yip, B.C., Othman, A.H. & Said, I.M. 1996. α,β,β,α-3,4-Dimethyl-2,5-bis(3,4,5-trimethoxyphenyl)tetrahydrofuran. *Acta Crystallographica Section C* 52(2): 414-416.

Gabarra-Niecko, V., Schaller, M.D. & Dunty, J.M. 2003. FAK regulates biological processes important for the pathogenesis of cancer. *Cancer Metastasis Rev.* 22(4): 359-374.

Greengard, E.G. 2018. Molecularly targeted therapy for neuroblastoma. *Children* 5(10): 142.

Gross, S., Rahal, R., Stransky, N., Lengauer, C. & Hoeflich, K.P. 2015. Targeting cancer with kinase inhibitors. *J. Clin. Invest.* 125(5): 1780-1789.

Guinaudeau, H., Leboeuf, M. & Cavé, A. 1979. Aporphine alkaloids. II. *Journal of Natural Products* 42(4): 325-360.

Hennessy, B.T., Smith, D.L., Ram, P.T., Lu, Y. & Mills, G.B. 2005. Exploiting the PI3K/AKT pathway for cancer drug discovery. *Nat. Rev. Drug Discov.* 4(12): 988-1004.

Huang, X., Hao, N., Wang, Q., Li, R., Zhang, G., Chen, G., Liu, S. & Che, Z. 2022. Non-food bioactive forest product liriodenine: Sources, chemistry, and bioactivities. *Industrial Crops and Products* 187(Part B): 115447.

Jänne, P.A., Gray, N. & Settleman, J. 2009. Factors underlying sensitivity of cancers to small-molecule kinase inhibitors. *Nat. Rev. Drug Discov.* 8(9): 709-723.

Johnsen, J.I., Segerström, L., Orrego, A., Elfman, L., Henriksson, M., Kågedal, B., Eksborg, S., Sveinbjörnsson, B. & Kogner, P. 2008. Inhibitors of mammalian target of rapamycin downregulate MYCN protein expression and inhibit neuroblastoma growth *in vitro* and *in vivo*. *Oncogene* 27(20): 2910-2922.

Kamarul Zaman, M.A., Azzeme, A.M., Ramle, I.K., Normanshah, N., Ramli, S.N., Shaharuddin, N.A., Ahmad, S. & Abdullah, S.N.A. 2020a. Induction, multiplication, and evaluation of antioxidant activity of *Polyalthia bullata* callus, a woody medicinal plant. *Plants* 9(12): 1772.

Kamarul Zaman, M.A., Azzeme, A.M., Ramli, S.N., Shaharuddin, N.A., Ahmad, S. & Abdullah, S.N.A. 2020b. Solvent extraction and its effect on phytochemical yield and antioxidant capacity of woody medicinal plant, *Polyalthia bullata*. *BioResources* 15(4): 9555-9568.

Katkar, K., Suthar, A. & Chauhan, V. 2010. The chemistry, pharmacologic, and therapeutic applications of *Polyalthia longifolia*. *Pharmacognosy Reviews* 4(7): 62-68.

Kennedy, P.T., Zannoupa, D., Son, M.H., Dahal, L.N. & Woolley, J.F. 2023. Neuroblastoma: An ongoing cold front for cancer immunotherapy. *J. Immunother. Cancer* 11(11): e007798.

London, W.B., Castleberry, R.P., Matthay, K.K., Look, A.T., Seeger, R.C., Shimada, H., Thorner, P., Brodeur, G., Maris, J.M., Reynolds, C.P. & Cohn, S.L. 2005. Evidence for an age cutoff greater than 365 days for neuroblastoma risk group stratification in the Children's Oncology Group. *J. Clin. Oncol.* 23(27): 6459-6465.

Maris, J.M., Hogarty, M.D., Bagatell, R. & Cohn, S.L. 2007. Neuroblastoma. *Lancet* 369(9579): 2106-2120.

Megison, M.L., Gillory, L.A. & Beierle, E.A. 2013. Cell survival signaling in neuroblastoma. *Anticancer Agents Med. Chem.* 13(4): 563-575.

Mueller, D., Davis, R.A., Duffy, S., Avery, V.M., Camp, D. & Quinn, R.J. 2009. Antimalarial activity of azafluorenone alkaloids from the Australian tree *Mitrephora diversifolia*. *J. Nat. Prod.* 72(8): 1538-1540.

Nantapap, S., Punyanitya, S., Nuntasaen, N., Pompimon, W. & Meepowpan, P. 2017. Flavones from aerial parts of *Polyalthia bullata* and cytotoxicity against cancer cell lines. *Chemistry of Natural Compounds* 53(4): 762-763.

Paarakh, P.M. & Khosa, R. 2009. Phytoconstituents from the genus *Polyalthia* - A review. *Journal of Pharmacy Research* 2(4): 594-605.

Pan, E., Cao, S., Brodie, P.J., Callmander, M.W., Randrianaivo, R., Rakotonandrasana, S., Rakotobe, E., Rasamison, V.E., TenDyke, K., Shen, Y., Suh, E.M. & Kingston, D.G. 2011. Isolation and synthesis of antiproliferative eupolauridine alkaloids of *Ambavia gerrardii* from the Madagascar Dry Forest. *J. Nat. Prod.* 74(5): 1169-1174.

Panthama, N., Kanokmedhakul, S. & Kanokmedhakul, K. 2010. Polyacetylenes from the roots of *Polyalthia debilis*. *Journal of Natural Products* 73(8): 1366-1369.

Prachayasittikul, S., Manam, P., Chinworrungsee, M., Isarankura-Na-Ayudhya, C., Ruchirawat, S. & Prachayasittikul, V. 2009. Bioactive azafluorenone alkaloids from *Polyalthia debilis* (pierre) finet & Gagnep. *Molecules* 14(11): 4414-4424.

Sulaiman, M., Hamid, A.H.A. & Awang, K. 2003. Alkaloids and flavones from *Desmos dumosus*, Roxb. Saff. (annonaceae). *Malaysian Journal of Science* 22(1): 87-93.

Tekuri, S.K., Pasupuleti, S.K., Konala, K.K. & Pabbaraju, N. 2019. Pharmacological effects of *Polyalthia cerasoides* (Roxb.) Bedd.: A brief review. *Journal of Complementary Medicine Research* 10(1): 38-49.

Tsai, S.F. & Lee, S.S. 2010. Characterization of acetylcholinesterase inhibitory constituents from *Annona glabra* assisted by HPLC microfractionation. *J. Nat. Prod.* 73(10): 1632-1635.

Vishala, T.C., Hieu, H.V., Killari, K.N., Ranajit, S.K., Samanth, S., Polimati, H., Ketha, A., Annam, S.S.P., Nallapaty, S., Koneru, S.T. & Akula, A. 2021. A review on therapeutic benefits of active chemical moieties present in *Polyalthia longifolia. Indian Journal of Pharmaceutical Sciences* 83(4): 634-647.

Wang, H., Chen, X. & He, L. 2023. A narrative review of radiomics and deep learning advances in neuroblastoma: Updates and challenges. *Pediatr. Radiol.* 53(13): 2742-2755.

Wei, Y.B., Li, Y.X., Song, H. & Feng, X.J. 2014. Design, synthesis and anticancer activity of oxoaporphine alkaloid derivatives. *J. Enzyme Inhib. Med. Chem.* 29(5): 722-727.

Wiart, C., Mogana, S., Khalifah, S., Mahan, M., Ismail, S., Buckle, M., Narayana, A.K. & Sulaiman, M. 2004. Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia. *Fitoterapia* 75(1): 68-73.

Yao, L.J., Jalil, J., Attiq, A., Hui, C.C. & Zakaria, N.A. 2019. The medicinal uses, toxicities and anti-inflammatory activity of *Polyalthia* species (Annonaceae). *J. Ethnopharmacol.* 229: 303-325.

Yoo, H.D., Cremin, P.A., Zeng, L., Garo, E., Williams, C.T., Lee, C.M., Goering, M.G., O'Neil-Johnson, M., Eldridge, G.R. & Hu, J.F. 2005. Suaveolindole, a new mass-limited antibacterial indolosesquiterpene from *Greenwayodendron suaveolens* obtained via high-throughput natural products chemistry methods. *Journal of Natural Products* 68(1): 122-124.

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