ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Cytotoxicity And Structure-Activity Relationship Of Dammarane-Type Triterpenoids From Aglaia Species Against P-388 Murine Leukemia Cells.

Ace Tatang Hidayat[1,2], Desi Harneti[1], Nurlelasari[1], Rani Maharani[1], Tri Mayanti[1], Kindi Farabi[1], Julinton Sianturi[1], Khalijah Awang[3], and Unang Supratman[1,2,*].

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor 45363, Sumedang, Indonesia.
[2]Central Laboratory of Universitas Padjadjaran, Jatinangor 45363, Sumedang, Indonesia.
[3]Department of Chemistry, Faculty of Science, University of Malaya, Kualalumpur 50603, Malaysia.

ABSTRACT

The cytotoxic structure-activity relationships among a series of dammarane-type triterpenoids from Aglaia species were studied against P-388 murine leukimia cells. Twelve dammarane-type triterpenoids were identified as aglinone(**1**), aglinin A (**2**), aglininB(**3**), aglininC(**4**), aglininD(**5**), aglininE(**6**), 20S, 24S,25-Hydroxy-3-hydroxyepicotillol(**7**), eichlerianic acid (**8**), isoeiklerialakton(**9**), dammar-20,25-diene-3,24-diol (**10**), dammar-20S,5,24-en,3,20-diol (**11**), and 25-methoxy-5-dammar-20-en-3,24-diol (**12**) were isolated from the bark of three Aglaia species. Among the dammarane-type triterpenoids, dammar-20,25-diene-3,24-diol (**10**), showed strongest cytotoxic activity. The structure-activity relatiosnship study revealed that closed of A-ring and opened of side ring were important structural feature for cytotoxic activity.

Keywords: Aglaia;cytotoxicity;structure-activity relationship, dammarane, triterpenoids.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Dammarane-type triterpenoids have been gaining worldwide attention for a long time because of their potent bioactivities [1]. Dammarane-type triterpenoids are distributed in genera of Panax (Araliaceae), Gynostemma (Cucurbitaceae), Aralia (Araliaceae), Aglaia (Meliaceae), Bacopa (Scrophulariaceae), Copernicia (Arecaceae), Celastrus (Celastraceae), Forsythia (Oleaceae), Myrica (Myrica), Rhus (Anacardiaceae), Polyscias (Araliaceae) and Sapindus (Sapindus) [2-6].The basic molecular skeleton of dammarane-type triterpenoids comprises tetracyclic moiety and side chain moiety. The bioactivities and chemical properties of dammarane-type triterpenoids have been investigated, and several pharmacological effects have been disclosed, including antifatigue, antihyperglycemic, antiobesity, anticancer, anti-HIV, antioxidant, antiaging, immunostimulatory, antiatherosclerotic and antihypertensive effects [7-9].The genus Aglaia is the largest genus of the family of Meliaceae comprises more than 100 species distributed mainly in India, Indonesia, Malaysia and parts of the Western Pacific region[10]. Previous phytochemical studies on the genus Aglaia have revealed the presence of a variety of compounds with interesting biological activities, including recoglamides[11-13],bisamides [14],sterol [15,16], dammarane-type triterpenoids [16-19], and cycloartane-type triterpenoids [16,20]. We report here the cytotoxic activity for dammarane-type triterpenoids (**1**-**12**) isolated from three Aglaia species as well as structure-activity relationship for those compounds against P-388 murine leukemia cells.

MATERIALS AND METHODS

General

All the apparatus used for cell culture were sterilized and decontaminated using a Hirayama HICLAVE HVE-50. Cell culture handling was carried out in an ESCO Class II BSC Biosafety Cabinet. The healthy cells were spun down, adherent together and separated from unhealthy and dead cells by using Thermo Scientific Sorvall ST 16R centrifuge. All cultures were incubated in 5% CO_2 humidified incubator at 37 ^oC (ESCO Celculture CO_2 Incubator model number CCL-170B-8). Cell stocks were placed in a -86 ^oC ultra-low temperatur freezer (Scancool SCL 50 P) and preserved in a liguid nitrogen tank (Taylor-Wharton LS300).

Plant Material

The bark of A. glabrata was collected in Bogor Botanical garden, Bogor, West Java Province, Indonesia in August 2014. The plant was identified by the staff of the Bogoriense Herbarium, Bogor, Indonesia and a voucher speciemen (No. B0-1295315) was deposited at herbarium.

Extraction and isolation

The dried bark of A. glabrata(3.4 kg) was extracted with methanol (10 L) at room temperature for 2 days. After removal the solvent, the viscous concentrate of MeOH extract (180 g) was first suspended in H_2O and then partitioned with n-hexane and EtOAc, successively. The n-hexane soluble fraction (12.6 g) was fractionated by column chromatography on silica gel 60 using a gradient n-hexane- EtOAc to give seven fractions (A-G). Fraction C (310 mg) was chromatographed on a column of silica gel, eluted successively with a gradient of n-hexane-acetone (10:1-1:1) to give seven fractions (C01-07). Fraction C03 (67 mg) was chromatographed on a column of silica gel, eluted with n-hexane:acetone (1:1) to give **3** (20.6 mg). Fraction D (130 mg) was chromatographed on a column of silica gel, eluted successively with a gradient of n-hexane-EtOAc (10:1-7:3) to give fifteen fractions (D01-15). Fraction D08-10 were combined (84 mg) and was chromatographed on silica gel, eluted with n-hexane:EtOAc (3:2) to give **4** (17.4 mg). Fraction E (220 mg) was chromatographed on a column of silica gel, eluted successively with a gradient of n-hexane-acetone (10:1-1:1) to give six fractions (E01-06). Fraction E03-05 were combined (92 mg) and was chromatographed on silica gel, eluted with n-hexane:EtOAc (1:2) to give **5** (12.4 mg). The EtOAc soluble fraction (23.5 g) was fractionated by column chromatography on silica gel 60 using a gradient n-hexane-EtOAc to give six fractions (H-M). Fraction J (230 mg) was chromatographed on a column of silica gel, eluted successively with a gradient of n-hexane-acetone (20:1-1:1) to give five fractions (J01-05). Fraction J04 (78 mg) was chromatographed on a column of silica gel, eluted with gradient of n-hexane-acetone (10:1-1:1) to give **9** (15.3 mg). Fraction L (210 mg) was chromatographed on a column of silica gel, eluted successively with a gradient of n-hexane-acetone (10:1-1:1) to give eight fractions (L01-08). Fraction L06 (64 mg) was chromatographed on a column of silica gel, eluted with gradient of n-hexane-acetone (10:1-1:1) to give **12** (12.5 mg).

Cytotoxic Assay

Determination of the cytotoxic activities were conducted according to the methodology described in previous papers [14,15,21,22]. P-388 cells were seeded into 96-well plates at an initial cell density of approximately 3×10^4 cells cm^{-3}. After 24 h of incubation for cell attachment and growth, varying concentrations of samples were added. The compounds added were first dissolved in DMSO at therequired concentration. Subsequent six desirable concentrationswere prepared using PBS (phosphoric buffer solution, pH = 7.30–7.65). Control wells received only DMSO. The assay was terminatedafter a 48 h incubation period by adding MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; alsonamed as thiazol blue] and the incubation was continued foranother 4 h, in which the MTT-stop solution containing SDS(sodium dodecyl sulfate) was added and another 24 h incubation was conducted. Optical density was read by using a micro plate reader at 550 nm. IC_{50} values were taken from the plotted graph of percentage live cells compared to control (%), receiving only PBSand DMSO, versus the tested concentration of compounds (□M).The IC_{50} value is the concentration required for 50% growth inhibition. Each assay and analysis was run in triplicate and averaged.

RESULTS

Compounds **1**, **2**, **6**, **7** and **8** were isolated previously from the bark of A. smithii and identified as aglinone, aglinin A, aglinin E, 20S,24S,25-dihydroxy-3□-hydroxyepicotillol, and eichlerianic acid, respectively [23], whereas compounds **10** and **11** were isolated from the bark of A. eximia and identified as dammar-20,25-diene-3□,24-diol and dammar-20S,5□,24-en,3□,20-diol [15].The barkof A. glabrata were grounded and successively extracted with n-hexane, ethyl acetate and methanol. All of the extracts were evaluated cytotoxic activity against P-388 murine leukemia cells and the ethyl acetate showed strongest cytotoxic activity. Subsequent phytochemical analysis was therefore focused on the ethyl acetate extract. The ethyl acetate extract was chromatographer over a vacuum-liquid chromatographer (VLC) column packed with silica gel 60 by gradient elution. The VLC fractions were repeatedly subjected to normal-phase column chromatography and preparative TLC on silica gel GF_{254} to afford five cytotoxic compounds**3**, **4**, **5**, **9** and **12** (Figure 1). Cytotoxicity of all the isolated dammarane-type triterpenoids were evaluated against P-388 murine leukimia cells in vitro. All the dammarane-type triterpenoids isolated from A. smithii, A. eximia and A. glabarata are listed in Table 1. The IC_{50} values of P-388 murine leukemia cells with the isolated dammarane-type triterpenoids are summatized in Table 2. Artonin E was used as a standard in the experiments [24]. The structure-activity relationships among this series of dammarane-type triterpenoids (Table 1) were then comparatively predicted for P-388 murine leukimia cell lines.

Table 1: Name of dammarane-type triterpenoids isolated from Aglaia species.

No of Compounds	Names
1	Aglinone
2	Aglinin A
3	Aglinin B
4	Aglinin C
5	Aglinin D
6	Aglinin E
7	20S,24S,25-dihydroxy-3□-hydroxyepicotillol
8	Eichlerianic acid
9	Isoeiklerialakton
10	Dammar-20,25-diene-3□,24-diol
11	Dammar-20S,5□,24-en,3□,20-diol
12	25-methoxy-5□-dammar-20-en-3□,24-diol

DISCUSSION

Repeated column chromatography of the methanolic extract of the bark of A. glabrata by bioassay-guided fractionation resulted in the isolation of five dammarane-type triterpenoids (Figure 1). Based on their

spectral evidence and a detailed comparison with published data, compounds**3**, **4**, **5**, **9** and **12**were identified as aglinin B [18,19], aglinin C [18,19], aglinin D [18,19], isoeiklerialakton [18,19] and 25-methoxy-5-dammar-20-en-3,24-diol [18,19]. The cytotoxic effects of five isolated compounds **3**, **4**, **5**, **9** and **12** along with seven previously isolated dammarane-type triterpenoids (**1**, **2**, **6**, **7** and **8**)and (**10** and **11**) against P-388 murine leukemia cells were conducted according to the method described in previous paper [14,15,22] and were used an artonin E (IC_{50} 0.3 g/mL) as a positive control [24].The cytotoxic activity of compounds **1-12** are shown in Table 2. Among the dammarane-type triterpenoids, dammar-20,25-diene-3,24-diol (**10**) and 25-methoxy-5-dammar-20-en-3,24-diol (**12**) having closed A-ring and opened side ring showed strong activity, whereas an opened A-ring and closed side ring like in aglinin A (**2**), aglinin B (**3**), eichlerianic acid (**8**), isoeiklerialakton (**9**) showed weak or no activity. These results suggested that the closed of A-ring and opened of side ring were important structural feature for cytotoxic activity. In addition, 25-methoxy-5-dammar-20-en-3,24-diol (**12**), having oxygenated substituent at side chain ring and olefinic moiety at C-20 showed the strongest activity among these compounds, indicated that oxygenated substituent at side chain ring and olefinic moieties enhanced the cytotoxic activity. The presence lactone in A-ring like in aglinone (**1**) showed stronger activity than an opened A-ring containing carboxylic acid and hydroxyl groups, while the presence lactone in side chain ring decrease activity, indicating that lactone ring in dammarane-type structure have slight cytotoxic effect. The presence of 3-hydroxy, 3-one and double bond moiety in A-ring showed no significant effect for cytotoxic activity.

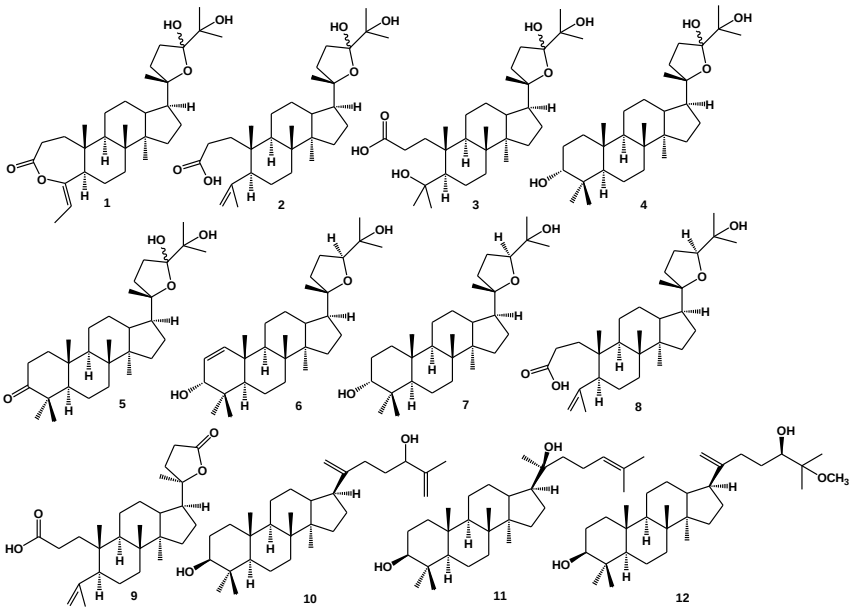

Figure 1: Chemical structures of compounds 1-12.

Table 2: Cytotoxic activity compounds 1-12 against P-388 murine leukimia cells

Compounds	IC_{50} (g/mL)
1	21.05
2	42.24
3	36.25
4	12.10
5	10.50
6	14.20
7	11.04
8	34.25
9	28.50
10	4.26
11	29.10
12	2.26

ISSN: 0975-8585

CONCLUSIONS

Preliminary insight towards the structure-activity relatioships among a series of dammarane-type triterpenoids against P-388 murine leukimia cell are proposed. A closed of A-ring and opened side ring in dammarane-type triterpenoids structure play important role for cytotoxic activity.

ACKNOWLEDGMENTS

The work was supported partially by Academic Leadership Grant of Universitas Padjadjaran (2016-2018 by US). We are grateful to Ms. Mrs. SuzanyDwiElita at Laboratory of Natural Products, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institute Technology Bandung, Indonesia for cytotoxicityassay.

.

REFERENCES

[1] Cao, J. Expert Opin. Ther. Patents., 2015, 25(7), 1-13.
[2] Kim, D.H. J. Ginseng. Res., 2012, 36(1), 1-15.
[3] Yang, W.Z; Hu, Y; Wu, W.Y; Ye, M; Guo, D., Phytochemistry, 2014, 106, 7-24.
[4] Connolly, J.D and Hill, R.A. Nat. Prod. Rep., 2003, 20(6), 640-659.
[5] Connolly, J.D and Hill, R.A. Nat. Prod. Rep., 2008, 25(4), 794-830.
[6] Kim, J.H.; Han, Y.N. Phytochemistry, 2011, 72 (11-12), 1453-1459.
[7] Jia, L., Zhao, Y.Q., Liang, X.J. Curr. Med. Chem., 2009, 16, 292-2942.
[8] Kuo, R.Y., Qian, K., Morris-Natschke, S.L., Lee, K.H. Nat. Prod. Rep., 2009, 26(10).
[9] Man, S.L., Gao, W.Y., Zhang, Y.J., Huang, L., Liu, C. Fitoterapia, 2010, 81(7), 703-714.
[10] Pannell, C.M., 1992.Kew Bulletin Additional Series XVI. HMSO, Kew, Richmond, Surrey, UK.
[11] Ishibashi, F., Satasook, C., Isman, M.B., Towers, G.H.N.,Phytochemistry. 1993, 32, 307–310.
[12] Wu, T.S., Liou, M.J., Kuoh, C.S., Teng, C.M., Nagao, T., Lee, K.H., J. Nat. Prod., 1997, 60,606–608.
[13] Nugroho, B.W., Edrada, R.A., Wray, V., Witte, L., Bringmann, G., Gehling, M., Proksch, P., Phytochemistry, 1999, 51, 367–376.
[14] Sianturi, J., Purnamasari, M., Darwati, Harneti, D., Mayanti, T., Supratman, U., Awang, K., Hayashi, H., Phytochemistry Letters, 2015, 13, 297–301.
[15] Harneti, D., Supriadin, A., Ulfah, M., Safari, A., Supratman, U., Awang K., Hayashi H., Phytochemistry Letters, 2014, 828–31.
[16] Leong, K,H., Looi, C,Y., Loong, X,M., Cheah, F,K., Supratman, U., Litaudon, L., Mustafa, M,R., Awang, K., PLOS ONE, 2016. 4, 1-17.
[17] Brader, G., Vajrodaya, S., Greger, H., Bacher, M., Kalchhauser, H., Hofer, O., J. Nat. Prod., 1998,61, 1482–1490.
[18] Roux, D., Martin, T., Adeline, T., Sevenet, T., Hadi, H., Pais, M.,Phytochemistry.1998, 49(6), 1745–1748.
[19] Mohamad, K., Martin, M.-T., Leroy, E., Tempete, C., Sevenet, T., Awang, K., Pais, M.,Phytochemistry, 1999, 51, 1031–1037.
[20] Zhang, F., Wang, J.S., Gu, Y.C., Kong, L.Y.,J. Nat. Prod, 2010, 73, 2042–2046.
[21] Alley, M.C., Scudiero, D.A., Monks, A., Hursey, M.L., Czerwinski, M.J., Fine, D.L.,Abbott, B.J., Mayo, J.G., Shoemaker, R.H., Boyd, M.R., Cancer Res., 1988, 48, 589–601.
[22] Farabi, K., Harneti, D., Nurlelasari., Maharani, R., Hidayat, A, C., Awang, K., Supratman, U., Shiono, Y. Phytochemistry Letters, 2017, 21, 211-215.
[23] Harneti, D., Tjokronegoro, R., Safari, A., Supratman, U., Loong, X., Mukhtar, M.M.,Mohamad, R., Awang, K., Hayashi, K.H., Phytochemistry Letters, 2012, .5, 496–499.
[24] Hakim, E.H., Achmad, S.A., Juliawaty, L.D., Makmur, L., Syah, Y.M., Aimi, A., Kitajima,M., Takayama, H., Ghisalberti, E.L.,J. Nat. Med., 2007, 61 (2)229–236.