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Marcy J. Balunas
University of Illinois

Bin Su
Cleveland State University, B.SU@csuohio.edu

Soedarsono Riswan
Indonesian Institute of Science

Harry H.S. Fong
University of Illinois

Robert W. Brueggemeier
Ohio State University

See next page for additional authors

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Authors

Marcy J. Balunas, Bin Su, Soedarsono Riswan, Harry H.S. Fong, Robert W. Brueggemeier, John M. Pezzuto, and A. Douglas Kinghorn

Isolation and characterization of aromatase inhibitors from *Brassaiopsis glomerulata* (Araliaceae)

Marcy J. Balunas, Bin Su, Soedarsono Riswan, Harry H.S. Fong, Robert W. Brueggemeier, John M. Pezzuto, A. Douglas Kinghorn

Introduction

Brassaiopsis glomerulata is a member of the Araliaceae that occurs in south and southeast Asia, including the Vietnamese peninsula (Van Kiem et al., 2003) and in Indonesia. *B. glomerulata* is a large shrub or small tree with thorns on the stems, palmate leaves with five to seven leaflets, pendulous panicles, and flowers in glomerulous heads (Regel, 1863). This species has several reported medicinal uses. In Vietnam, the plant is used to treat rheumatism and back pain (Van Kiem et al., 2003). In India, a group of indigenous tribes called the Nagas drink a juice extract of *B. glomerulata* bark to aid in digestion and to alleviate constipation.

The Nagas also use a paste of the bark of *B. glomerulata* to treat bone fractures and sprains (Changkija, 1999). This species is also used medicinally in China as one of several kinds of "tongcao" (unblocking herbs used to promote urination and assist in lactation) (Shen et al., 1998). Owing to its use as a "tongcao", *B. glomerulata* was tested using *in vivo* anti-inflammatory, anti-pyretic, and diuretic models (Shen et al., 1998). Moreover, *B. glomerulata* is reported to inhibit carrageenan induced swelling of rat paws (anti-inflammatory), and induce an antipyretic effect using a rat fever model induced by beer yeast or carrageenan, but no diuretic effect was reported (Shen et al., 1998).

Aromatase is the enzyme responsible for catalyzing the biosynthesis of estrogens (estrone and estradiol) from androgens (androstenedione and testosterone) (Johnston and Dowsett, 2003; Brueggemeier, 2006). Inhibition of aromatase has been shown to reduce estrogen production throughout the body to nearly undetectable levels and aromatase inhibitors are being used clinically to retard the development and progression of hormone responsive breast cancers [FDA approved aromatase inhibitors include anastrozole (Arimidex®), letrozole (Femara®), and exemestane (Aromasin®)]. As part of a research project directed

toward the study of new naturally occurring chemopreventive agents from plants (Kinghorn et al., 2004), the leaves of *Brassaiopsis glomerulata* (Blume) Regel (Araliaceae), collected in Indonesia, were found to inhibit the aromatase enzyme. Very limited phytochemical studies have been performed on plants in the genus *Brassaiopsis*, with the only isolates reported to date being three known triterpenes of the lupane subgroup (Van Kiem et al., 2003). Due to the strong aromatase activity and the lack of previous phytochemical research, bioassay guided fractionation of the leaves of *B. glomerulata* was initiated to isolate and identify compounds with potential aromatase inhibitory (AI) activity.

Results and discussion

A hexane soluble extract of the leaves of *B. glomerulata* exhibited significant aromatase inhibition with both enzyme based and cell based AI assays [6.9% control activity (PCA) at 20 $\mu\text{g/mL}$ and 7.2 PCA at 20 $\mu\text{g/mL}$, respectively]. Bioassay guided fractionation of the hexane extract led to the isolation of six compounds of the steroid and triterpenoid classes [spinasterone (**1**) (Wandji et al., 2002), stigmaterol (**2**) (Forgo and Kover, 2004), spinasterol (**3**) (Kojima et al., 1990), 7 β hydroxy 4,22 stigmastadien 3 one (**4**) (Ayyad, 2002), 6 β hydroxystigmastadien 3 one (**5**) (Kontiza et al., 2006), and oleanolic acid (**6**) (Seebacher et al., 2003)] (Fig. 1). The ethyl acetate extract of *B. glomerulata* was also found to exhibit moderate aromatase inhibition with the enzyme based and cell based AI assays (59.3 PCA at 20 $\mu\text{g/mL}$ and 37.0 PCA at 20 $\mu\text{g/mL}$, respectively).

Bioassay guided fractionation of the ethyl acetate extract led to the isolation of seven compounds of the dipeptide, modified peptide, fatty acid, monoterpenoid, and benzenoid classes [*N* benzoyl ι phenylalaninyl *N* benzoyl ι phenylalaninate (**7**) (Catalan et al., 2003), *N* acetyl ι phenylalaninyl *N* benzoyl ι phenylalaninate (**8**) (Xiao et al., 2002), *N* benzoyl ι phenylalanine methyl ester (**9**) (Li et al., 2000), linoleic acid (**10**) (Ramsewak et al., 2001), () dihydroactinidiolide (**11**) (Mori and Khlebnikov, 1993), ()

dehydrololiolide (**12**) (Ravi et al., 1982), and 4 hydroxybenzaldehyde (**13**) (Levin and Du, 2002)] (Fig. 1).

Compound **9** was found to be a new natural product, not previously isolated from any organism. This compound was previously obtained as a synthetic peptide derivative, and was prepared using Lewis acid cleavage of a resin bond carbamate (Li et al., 2000). The stereochemistry of **9** at position C 8 was assigned tentatively as ι based on the comparison of the observed $[\alpha]_D$ of +75 $^\circ$ with the literature (Li et al., 2000). Following hydrolysis, a Marfey's analysis was undertaken to confirm the stereochemistry at this position. Marfey's analysis has become one of the standard methods for the determination of absolute configuration of compounds containing modified amino acid residues (Lang et al., 2006; Mitova et al., 2006). In the region of interest in the HPLC chromatogram, a peak appeared corresponding to the ι phe Marfey's derivative. To confirm this assignment, the Marfey's derivatives of ι phe and D phe were co injected separately with the Marfey's derivative of the hydrolysate of **9**. The ι phe derivative directly overlapped the peak of the hydrolysate derivative, while the D phe derivative eluted considerably after the hydrolysate derivative. The stereochemistry of **9** at position C 8 was therefore determined to be ι .

Marfey's analysis was also performed to confirm the stereochemistry of **7**, with positions C 8 and C 8' both being found to have the ι configuration, which was not determined at the time of its previous isolation (Catalan et al., 2003). Furthermore, the absolute stereochemistry of **8** at positions C 8 and C 8' was not assigned previously (Xiao et al., 2002). Using Marfey's analysis, the stereochemistry of positions C 8 and C 8' for compound **8** was determined to be ι at both positions.

The fatty acid, linoleic acid (**10**), was found to be significantly more active than the positive control, aminoglutethimide (AG) in the enzyme based AI assay (7.4 PCA at 20 $\mu\text{g/mL}$) ($P < 0.0001$) (Fig. 1). However, linoleic acid (**10**) was inactive in the cell based assay [147.6 PCA at 100 μM , the interference of fatty acids in the non cellular, enzyme based radiometric AI assay was previously

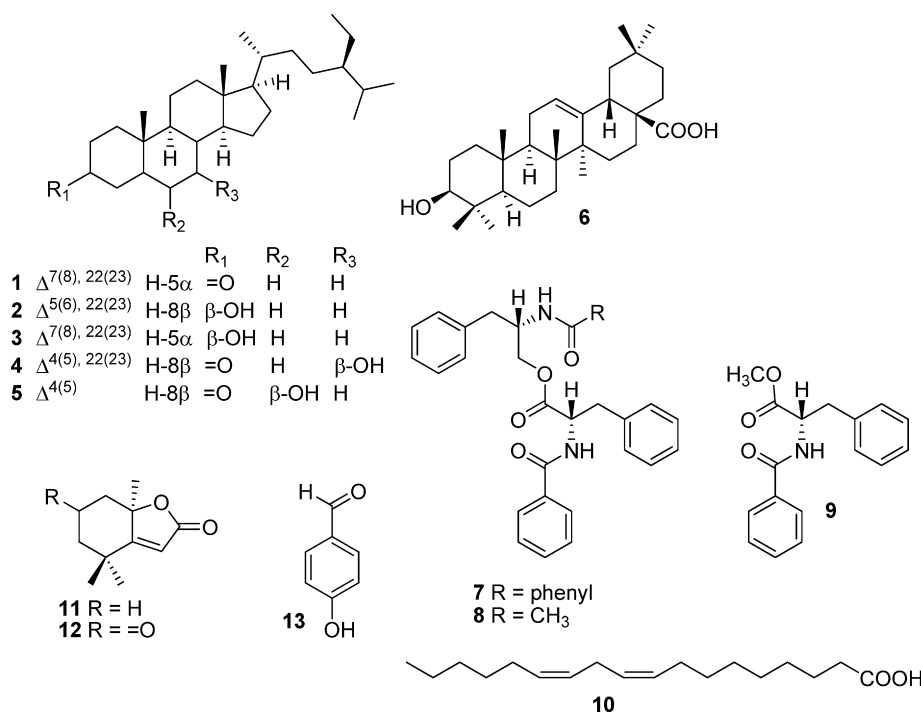


Fig. 1. Compounds isolated from *Brassaiopsis glomerulata*.

reported in Balunas et al., 2006]. Upon the isolation of linoleic acid from the ethyl acetate extract, the hexane extract was reexamined for the presence of this compound using comparative TLC patterns and trace amounts were found in the hexane extract also. Since unsaturated fatty acids can readily undergo lipid oxidation, forming hydroperoxides in the presence of oxygen (Boyd et al., 1992; Banni et al., 1996; Lee et al., 2005), it is possible that linoleic acid in the *B. glomerulata* extracts may have been oxidized during the course of bioassay guided fractionation, resulting in decreasing levels of activity of subsequent fractionation steps.

The monoterpeneoid () dehydrololiolide (**12**), was found to be active in the cell based AI bioassay (21.8 PCA at 50 μ M) (Fig. 2), with no statistical difference in aromatase inhibition activity between compound **12** and the positive control, letrozole ($P < 0.0001$). The other monoterpeneoid isolated from the ethyl acetate extract, () dihydroactinidiolide (**11**), was not able to be tested in either AI assay due to compound volatilization. Monoterpenoids commonly undergo volatilization and are thus often used for flavoring or as perfume ingredients. The decreasing levels of activity of *B. glomerulata* during the course of bioassay guided fractionation may be the result of other active and similarly volatile monoterpenoids. Two chlorinated monoterpeneoid pesticides, toxaphene and chlordane, have previously been reported to decrease aromatase activity in a SK BR 3 cell based AI assay, although the compounds were not direct inhibitors of aromatase but rather suppressed aromatase expression by antagonizing estrogen related receptor α 1 (ERR α 1) (Yang and Chen, 1999; Chen et al., 2001). The difference in the activity of **12** in the enzyme based AI assay (91.5 PCA at 20 μ g/mL) and in the cell based AI assay (21.8 PCA at 50 μ M) may be the result of indirect modulation of aromatase activity as was found with these chlorinated monoterpeneoid pesticides.

The new natural product, *N* benzoyl L phenylalanine methyl ester, **9**, was also found to be moderately active in the cell based AI bioassay (33.3 PCA at 50 μ M), although **9** was not active in the enzyme based AI assay (94.3 PCA at 20 μ g/mL). Some structural similarities exist between the chemical structure of **9** and letrozole, one of the AIs currently in clinical use, with two benzyl rings separated by an alkyl linker that contains a nitrogen. However, a detailed investigation of the structural similarities of **9** as

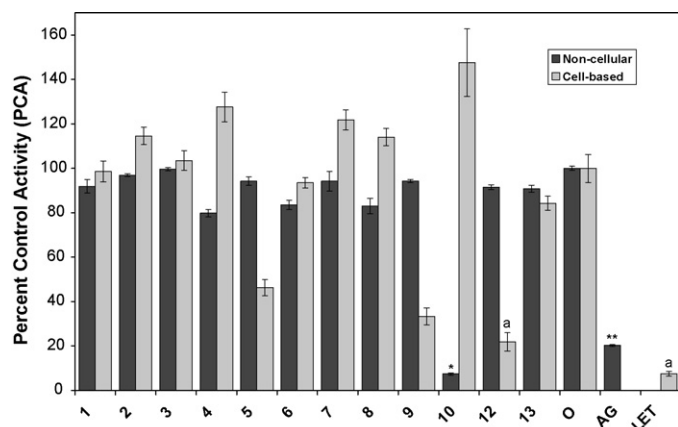


Fig. 2. Percent control activity (PCA) at 20 μ g/mL for compounds **1–13** isolated from *Brassaiopsis glomerulata* in the non-cellular, enzyme-based aromatase bioassay and in SK-BR-3 hormone-independent human breast cancer cells that overexpress aromatase (DMSO = dimethyl sulfoxide, blank/negative control; AG = aminoglutethimide, positive control in non-cellular assay, 50 μ M; LET = letrozole, positive control in cell-based assay, 10 nM). Each bar represents the mean and standard error of three replicates, with statistically significant differences indicated in the non-cellular assay by different numbers of stars and in the cell-based assay by different letters ($P < 0.0001$).

compared with anastrozole and letrozole was not possible during the course of the present study.

6 β Hydroxystigmasta 4 en 3 one (**5**) was found to be weakly active in the cell based AI bioassay. Interestingly, compound **5**, as well as compounds **9** and **12**, were not active in the enzyme based AI assay, perhaps indicating that they are acting through indirect regulation or modulation of aromatase activity rather than through direct aromatase inhibition (Yang and Chen, 1999; Chen et al., 2001; Díaz Cruz et al., 2005).

Brassaiopsis glomerulata has been reported to have various ethnobotanical uses, as mentioned earlier. The past history of medicinal use by human populations of *B. glomerulata* may be indicative of being safe when consumed. The strong aromatase inhibition of the hexane extract of *B. glomerulata* in both enzyme and cell based assays, coupled with the possibility of a favorable safety profile, may point to the potential for use of *B. glomerulata* for the chemoprevention of breast cancer. Further investigations of *B. glomerulata* are needed, including a recollection and subsequent bioassay guided compound isolation, as well as further biological studies on the mechanism of aromatase inhibition and *in vivo* testing.

Experimental

General experimental procedures

Enantiomerically pure standards L phenylalaninol (L phe ol), D phenylalaninol (D phe ol), L phenylalanine (L phe), and D phenylalanine (D phe) were purchased from Sigma Aldrich (St. Louis, MO). *N* α (2,4 Dinitro 5 fluorophenyl) L alaninamide (L FDAA, Marfey's reagent) was purchased from Sigma Aldrich (St. Louis, MO). HPLC of Marfey's derivatives was performed using two Waters 515 pumps and a Waters 2487 dual wavelength detector (Waters, Milford, MA). A preparative Waters SunFireTM C₁₈ column (19 \times 150 mm) was used for fractionation, while an analytical Waters SunFireTM C₁₈ column (4.6 mm \times 150 mm) was used for Marfey's analysis. Solvents were HPLC grade and used without further purification. Radiolabeled [1β ³H]androst 4 ene 3,17 dione, scintillation cocktail 3a70B, and SK BR 3 human breast cancer cells were obtained as described previously (Brueggemeier et al., 2005). Radioactivity was counted on an LS6800 liquid scintillation counter (Beckman, Palo Alto, CA). All other chemicals and reagents were purchased from Sigma Aldrich (St. Louis, MO).

Plant material

The leaves of *Brassaiopsis glomerulata* (Blume) Regel were collected by Dr. Soedarsono Riswan, Herbarium Bogoriense, Bogor, Indonesia, in 1996 (collection number BK 35). Dried plant material was stored at ambient temperature at the UIC Pharmacognosy Field Station in Downers Grove, Illinois until used for the present study. A voucher specimen (accession number P1750) has been deposited at the Field Museum of Natural History, Chicago, Illinois.

Extraction and isolation

Air dried leaves of *B. glomerulata* (1.1 kg) were ground and extracted with methanol overnight (3 \times 3 L). The macerate was concentrated *in vacuo* (46.1 g) and partitioned to afford a petroleum ether extract (11.6 g), an ethyl acetate extract (8.2 g), and an aqueous extract (13.3 g).

The petroleum ether extract was fractionated using Si gel vacuum liquid chromatography (Aldrich, Si gel 60, 63 200 mesh, 8.5 \times 18 cm) using 100% petroleum ether, followed by a gradient of increasing polarity of petroleum ether ethyl acetate, followed, in

turn, by ethyl acetate methanol. The column was then washed with 100% methanol. Altogether, 10 pooled fractions (F005–F014) were collected. Compound **1** (3.6 mg) was obtained as a precipitate from F006. A white solid precipitated from fraction F007, a mixture of two related compounds, and was chromatographed using silica gel (Aldrich, Si gel 60, 230–400 mesh, 4.5 × 46 cm), beginning with 8:1 hexane ethyl acetate, followed by a gradient of increasing polarity, and washed with 100% methanol to afford purified stigmasterol (**2**, 2.2 mg) and purified spinasterol (**3**, 1.9 mg).

Fractions F008, F009, and F010 were all considered active in the non cellular assay and were combined based on similar TLC profiles (for a total of 3.1 g). F008–F010 was fractionated using Diaion HP 20 sorbent (Aldrich, 4.0 × 21 cm), eluted by 1:1 MeOH:H₂O, 3:1 MeOH:H₂O, 100% MeOH, 3:1 MeOH:acetone, 1:1 MeOH:acetone, and 100% acetone. Compound **6** (2.7 mg) was obtained as a white precipitate from the 100% MeOH fraction. The 3:1 MeOH:acetone fraction (1.9 g) was further fractionated (Aldrich, Si gel 60, 230–400 mesh, 2.5 × 45 cm), starting with 9:1 hexane:acetone and continuing with increasing polarity until washing with 100% acetone, followed by 100% methanol. Five pooled fractions were obtained with the 3:2 hexane:acetone fraction (0.09 g) being further fractionated by preparative reversed phase HPLC (92% methanol in water, 8 mL/min, monitoring at 238 and 275 nm), yielding **4** (1.1 mg, *t_R* 36.8 min) and **5** (0.4 mg, *t_R* 50.4 min).

The ethyl acetate extract (8.1 g) was fractionated using Si gel vacuum liquid chromatography (Aldrich, Si gel 60, 63–200 mesh, 8.5 × 19 cm), beginning with 100% petroleum ether, followed by a gradient of petroleum ether:ethyl acetate, ethyl acetate:methanol, and 100% methanol. Altogether 17 pooled fractions (F029–F045) were collected. Compound **7** (19.8 mg) precipitated from fraction F034. F031 (0.01 g) was subjected to further fractionation (Aldrich, Si gel 60, 230–400 mesh, 1.0 × 15 cm) using an isocratic 5:1 hexane:ethyl acetate system, yielding pure compound **10** (0.1 mg). Fractions F032 and F033 were combined (0.1 g) and fractionated by preparative reversed phase HPLC separations (60% water in acetonitrile, 7 mL/min, monitoring at 220 and 254 nm) to afford **11** (0.9 mg, *t_R* 11.5 min).

Fraction F034 (0.3 g) was chromatographed using Sephadex LH 20 gel (Aldrich, 2.5 × 76 cm) in methanol, to afford 12 pooled fractions (F055–F066). The combination of F057–F058 (0.1 g) was worked up by preparative reversed phase HPLC separations (7 mL/min, monitoring at 220 and 254 nm). The solvent conditions for separation involved a 85:15 water:acetonitrile system for 10 min followed by a 60 min gradient to 1:9 water:acetonitrile, to afford compounds **12** (3.5 mg, *t_R* 29.5 min), **9** (0.8 mg, *t_R* 46.1 min), and **8** (3.7 mg, *t_R* 50.8 min). Fractions F059 and F060 were combined (0.02 g) and separated using preparative reversed phase HPLC separations (7 mL/min, monitoring at 220 and 254 nm). The solvent conditions for separation involved a 85:15 water:acetonitrile system for 15 min followed by a 45 min gradient to 1:9 water:acetonitrile, to afford compound **13** (1.9 mg, *t_R* 24.2 min).

Marfey's analysis

Samples of **7–9** were independently hydrolyzed at 110 °C with 6N HCl for 1 h (Fujii et al., 2002). Hydrolysates were dried and Marfey's derivatives were prepared following a published procedure (Fujii et al., 2002). The dried hydrolysates of **7–9**, as well as the standards L-phe-ol, D-phe-ol, L-phe, and D-phe, were each dissolved in 100 µL of water. A 1 M sodium bicarbonate solution was prepared and 40 µL was added to each sample. A 1% (w/v) solution of L-FDAA in acetone was prepared and 100 µL was added to each vial. Each vial was then vortexed and incubated at 40 °C for 1 h. To quench reactions, 40 µL of 1N HCl were added. Each of the Marfey's

derivatives of the hydrolysates and standards were separately analyzed by HPLC. All four Marfey's derivatives of the standards were then analyzed concurrently. Each Marfey's derivative of the hydrolysates was then analyzed concurrently with each Marfey's derivative of the standards. The reaction mixtures were analyzed by HPLC using a 5 min isocratic mobile phase of 30% CH₃CN/70% 0.01N aqueous TFA followed by a gradient elution profile to 90% CH₃CN/10% 0.01N aqueous TFA over 60 min at a flow of 1 mL/min, monitoring at 340 and 256 nm.

Non cellular, enzyme based aromatase bioassay

This assay was performed as described in earlier publications (Kellis and Vickery, 1987; O'Reilly et al., 1995; Balunas et al., 2006). Human placental microsomes were obtained from human term placentas that were processed at 4 °C immediately after delivery from The Ohio State University Medical Center [OSU Institutional Review Board (IRB) protocol number 2002H0105, last approved in December 2006]. Extracts and compounds were originally screened at 20 µg/mL in DMSO using a non cellular microsomal radiometric aromatase assay. Samples [extracts or compounds, DMSO as negative control, or 50 µM (±) aminoglutethimide (AG) as positive control] were tested in triplicate. Each reaction mixture included sample, 100 nM [³H]androst 4-ene 3,17-dione (400,000–450,000 dpm), 0.1 M potassium phosphate buffer (pH 7.0), 5% propylene glycol, and an NADPH regenerating system (containing 2.85 mM glucose 6-phosphate, 1.8 mM NADP⁺, and 1.5 units glucose 6-phosphate dehydrogenase). Microsomal aromatase (50 µg) was added to initiate the reactions, which were then incubated in a shaking water bath at 37 °C, and quenched after 15 min using 2 mL CHCl₃. An aliquot of the aqueous layer was then added to 3a70B scintillation cocktail for quantitation of the formation of ³H₂O. Percent control activity (PCA) was determined as previously described (Kellis and Vickery, 1987; O'Reilly et al., 1995; Balunas et al., 2006).

Cell based aromatase bioassay

Samples found to be active using the non cellular assay were further tested at various concentrations in SK-BR-3 human breast cancer cells that overexpress aromatase, using a previously described method (Natarajan et al., 1994; Richards and Brueggemeier, 2003; Balunas et al., 2006). Cells were treated in triplicate with samples or 0.1% DMSO (negative control) or 10 nM letrozole (positive control). Results are initially determined as picomoles of ³H₂O formed per hour incubation per million live cells (pmol/h/10⁶ cells), with PCA calculated by comparison with the negative control, DMSO.

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References

- Ayyad, S.N., 2002. A new cytotoxic stigmastane steroid from *Pistia stratiotes*. *Pharmazie* 57, 212–214.
- Balunas, M.J., Su, B., Landini, S., Brueggemeier, R.W., Kinghorn, A.D., 2006. Interference by naturally occurring fatty acids in a non-cellular enzyme-based aromatase bioassay. *J. Nat. Prod.* 69, 700–703.

- Banni, S., Contini, M.S., Angioni, E., DeLana, M., Dessi, M.A., Melis, M.P., Carta, G., Corongiu, F.P., 1996. A novel approach to study linoleic acid autoxidation: Importance of simultaneous detection of the substrate and its derivative oxidation products. *Free Radic. Res.* 25, 43–53.
- Boyd, L.C., King, M.F., Sheldon, B., 1992. A rapid method for determining the oxidation of *n*-3 fatty acids. *J. Am. Oil Chem. Soc.* 69, 325–330.
- Brueggemeier, R.W., 2006. Update on the use of aromatase inhibitors in breast cancer. *Expert Opin. Pharmacother.* 7, 1919–1930.
- Brueggemeier, R.W., Diaz-Cruz, E.S., Li, P.K., Sugimoto, Y., Lin, Y.C., Shapiro, C.L., 2005. Translational studies on aromatase, cyclooxygenases, and enzyme inhibitors in breast cancer. *J. Steroid Biochem. Mol. Biol.* 95, 129–136.
- Catalan, C.A., de Heluani, C.S., Kotowicz, C., Gedris, T.E., Herz, W., 2003. A linear sesterterpene, two squalene derivatives and two peptide derivatives from *Croton hieronymi*. *Phytochemistry* 64, 625–629.
- Changkija, S., 1999. Folk medicinal plants of the Nagas in India. *Asian Folk. Stud.* 58, 205–230.
- Chen, S.U., Zhou, D.J., Yang, C., Okubo, T., Kinoshita, Y., Yu, B., Kao, Y.C., Itoh, T., 2001. Modulation of aromatase expression in human breast tissue. *J. Steroid Biochem. Mol. Biol.* 79, 35–40.
- Diaz-Cruz, E.S., Shapiro, C.L., Brueggemeier, R.W., 2005. Cyclooxygenase inhibitors suppress aromatase expression and activity in breast cancer cells. *J. Clin. Endocrinol. Metab.* 90, 2563–2570.
- Forgo, P., Kover, K.E., 2004. Gradient enhanced selective experiments in the ¹H NMR chemical shift assignment of the skeleton and side-chain resonances of stigmasterol, a phytosterol derivative. *Steroids* 69, 43–50.
- Fujii, K., Yahashi, Y., Nakano, T., Imanishi, S., Baldia, S.F., Harada, K., 2002. Simultaneous detection and determination of the absolute configuration of thiazole-containing amino acids in a peptide. *Tetrahedron* 58, 6873–6879.
- Johnston, S.R., Dowsett, M., 2003. Aromatase inhibitors for breast cancer: lessons from the laboratory. *Nat. Rev. Cancer* 3, 821–831.
- Kellis Jr., J.T., Vickery, L.E., 1987. Purification and characterization of human placental aromatase cytochrome P-450. *J. Biol. Chem.* 262, 4413–4420.
- Kinghorn, A.D., Su, B.-N., Jang, D.S., Chang, L.C., Lee, D., Gu, J.Q., Carcache-Blanco, E.J., Pawlus, A.D., Lee, S.K., Park, E.J., Cuendet, M., Gills, J.J., Bhat, K., Park, H.S., Mata-Greenwood, E., Song, L.L., Jang, M., Pezzuto, J.M., 2004. Natural inhibitors of carcinogenesis. *Planta Med.* 70, 691–705.
- Kojima, H., Sato, N., Hatano, A., Ogura, H., 1990. Sterol glucosides from *Prunella vulgaris*. *Phytochemistry* 29, 2351–2355.
- Kontiza, I., Abatis, D., Malakate, K., Vagias, C., Roussis, V., 2006. 3-Keto steroids from the marine organisms *Dendrophyllia cornigera* and *Cymodocea nodosa*. *Steroids* 71, 177–181.
- Lang, G., Mitova, M.I., Cole, A.L.J., Din, L.B., Vikineswary, S., Abdullah, N., Blunt, J.W., Munro, M.H.G., 2006. Pterulamides I–VI, linear peptides from a Malaysian *Pterula* sp. *J. Nat. Prod.* 69, 1389–1393.
- Lee, S.H., Oe, T., Arora, J.S., Blair, I.A., 2005. Analysis of Fe^{II}-mediated decomposition of a linoleic acid-derived lipid hydroperoxide by liquid chromatography/mass spectrometry. *J. Mass Spectrom.* 40, 661–668.
- Levin, J.I., Du, M.T., 2002. Rapid, one-pot conversion of aryl fluorides into phenols with 2-butyn-1-ol and potassium *t*-butoxide in DMSO. *Synth. Commun.* 32, 1401–1406.
- Li, W.R., Yo, Y.C., Lin, Y.S., 2000. Efficient one-pot formation of amides from benzyl carbamates: application to solid-phase synthesis. *Tetrahedron* 56, 8867–8875.
- Mitova, M.I., Stuart, B.G., Cao, G.H., Blunt, J.W., Cole, A.L.J., Munro, M.H.G., 2006. Chrysosporide, a cyclic pentapeptide from a New Zealand sample of the fungus *Sepedonium chrysospermum*. *J. Nat. Prod.* 69, 1481–1484.
- Mori, K., Khlebnikov, V., 1993. Carotenoids and degraded carotenoids. 8. Synthesis of (+)-dihydroactinidiolide, (+)-actinidiolide and (–)-actinidiolide, (+)-loliolide and (–)-loliolide as well as (+)-epiloliolide and (–)-epiloliolide. *Liebigs Ann. Chem.* 77–82.
- Natarajan, N., Shambaugh 3rd, G.E., Elseth, K.M., Haines, G.K., Radosevich, J.A., 1994. Adaptation of the diphenylamine (DPA) assay to a 96-well plate tissue culture format and comparison with the MTT assay. *Biotechniques* 17, 166–171.
- O'Reilly, J.M., Li, N., Duax, W.L., Brueggemeier, R.W., 1995. Synthesis, structure elucidation, and biochemical evaluation of 7 α - and 7 β -arylaliphatic-substituted androst-4-ene-3,17-diones as inhibitors of aromatase. *J. Med. Chem.* 38, 2842–2850.
- Ramsewak, R.S., Nair, M.G., Murugesan, S., Mattson, W.J., Zasada, J., 2001. Insecticidal fatty acids and triglycerides from *Dirca palustris*. *J. Agric. Food Chem.* 49, 5852–5856.
- Ravi, B.N., Murphy, P.T., Lidgard, R.O., Warren, R.G., Wells, R.J., 1982. C-18 terpenoid metabolites of the brown alga *Cystophora moniliformis*. *Aust. J. Chem.* 35, 171–182.
- Regel, E., 1863. *Brassaiopsis glomerulata* Blume (t. 411) Araliaceae. *Gartenflora* 12, 275–276.
- Richards, J.A., Brueggemeier, R.W., 2003. Prostaglandin E₂ regulates aromatase activity and expression in human adipose stromal cells via two distinct receptor subtypes. *J. Clin. Endocrinol. Metab.* 88, 2810–2816.
- Seebacher, W., Simic, N., Weis, R., Saf, R., Kunert, O., 2003. Complete assignments of H-1 and C-13 NMR resonances of oleanolic acid, 18 α -oleanolic acid, ursolic acid and their 11-oxo derivatives. *Magn. Reson. Chem.* 41, 636–638.
- Shen, Y., Zeng, N., Jia, M., Zhang, Y., Wei, Y., Ma, Y., 1998. Experimental studies on anti-inflammatory, antipyretic and diuretic effects of several species of Tongcao and Xiao-tongcao. *Zhongguo Zhongyao Zazhi* 23, 687–690.
- Van Kiem, P., Dat, N.T., Van Minh, C., Lee, J.J., Kim, Y.H., 2003. Lupane triterpenes from the leaves of *Brassaiopsis glomerulata*. *Arch. Pharm. Res.* 26, 594–596.
- Wandji, J., Tillequin, F., Mulholland, D.A., Wansi, J.D., Fomum, T.Z., Fuendjiepi, V., Libot, F., Tsabang, N., 2002. Fatty acid esters of triterpenoids and steroid glycosides from *Gambeya africana*. *Planta Med.* 68, 822–826.
- Xiao, Y.Q., Li, L., You, X.L., Bian, B.L., Liang, X.M., Wang, Y.T., 2002. A new compound from *Gastrodia elata* Blume. *J. Asian Nat. Prod. Res.* 4, 73–79.
- Yang, C., Chen, S., 1999. Two organochlorine pesticides, toxaphene and chlordane, are antagonists for estrogen-related receptor α -1 orphan receptor. *Cancer Res.* 59, 4519–4524.