Glutathione *S*-Transferase Inhibiting Chemical Constituents of *Caesalpinia bonduc*

Chibuike C. UDENIGWE,[a,b] Athar ATA,*[,a] and Radhika SAMARASEKERA[c]

[a] *Department of Chemistry, The University of Winnipeg; 515 Portage Avenue, Winnipeg, Manitoba, R3B 2E9, Canada:* [b] *Department of Chemistry, The University of Manitoba; Winnipeg, MB, R3T 2N2, Canada: and* [c] *Natural Products Development Group, Industrial Technology Institute; Colombo–7, Sri Lanka.*

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Glutathione *S*-transferase inhibition assay-guided fractionations on the ethanolic extract of the bark of *Caesalpinia bonduc* resulted in the isolation of a new sterol, 17-hydroxy-campesta-4,6-dien-3-one (1) along with four known compounds, 13,14-*seco*-stigmasta-5,14-dien-3α-ol (2), 13,14-*seco*-stigmasta-9(11),14-dien-3α-ol (3), caesaldekarin J (4) and pipataline (5) as active constituents. Structures of compounds 1—5 were established on the basis of extensive NMR spectroscopic studies. The compounds (1—5) were isolated on the basis of their inhibitory activity against glutathione *S*-transferase, an enzyme that has been implicated in resistances during treatment of cancer and parasitic infections. Efforts to study structure–activity relationships of compounds 2 and 3 were also made by modifying their structures. The IC_{50} values of these compounds and their derivatives ranged from 57—380 μM and were compared to the inhibitory effects due to sodium taurocholate, an isoprene-derived GST inhibitor (IC_{50}=398 μM). A plausible biosynthesis of 13,14-*seco*-steroids has also been proposed.

Key words *Caesalpinia bonduc*; glutathione *S*-transferase; 13,14-*seco*-sterol; pipataline; caesaldekarin J

Caesalpinia bonduc L. (Fabaceae) is a medicinal plant predominantly distributed in the tropical and subtropical regions of Asia and the Caribbean. It is locally known as Nata Karanja (Hindi) in India and Kuburu in Sri Lanka, and possesses a lot of applications in folk medicines. For instance, its seed and bark extracts have been used as antihelmintic, anticancer, antimalarial, hypoglycemic, anti-inflammatory, antimicrobial, antirheumatic and antipyretic agents.[1,2] Previous phytochemical investigations on *C. bonduc* and some other members of the family Fabaceae have resulted in the isolation of several cassane and norcassane furanoditerpenes.[3—6] Some of these compounds were reported to exhibit inhibitory activity against interleukin-1 production[4] and growth inhibitory activity against malaria-causing *Plasmodium falciparum*.[5]

The glutathione *S*-transferase (GST) system is a primary cellular mechanism protecting against cytotoxic and genotoxic stress. GST isoenzymes are phase II detoxification enzymes that catalyze the conjugation of cytotoxic agents to glutathione producing a less reactive, water-soluble chemical species.[7] Physiological activities of these enzymes have been implicated in development of resistances by cancer cells and parasites towards chemotherapeutic agents and the body immune system, respectively.[8,9] Inhibition of GST has been proposed to improve prognosis in patients during cancer chemotherapy, and can be applied as adjuvant in antiparasitic chemotherapy and as antiparasitic drugs.[10,11] In our continuing effort to discover bioactive natural products from plant and marine sources, we discovered that the crude ethanolic extract of *C. bonduc* exhibited concentration-dependent GST inhibitory activity with an IC_{50} value of 83 μg/ml. Based on this activity, we performed bioassay-guided fractionations on this crude extract to isolate natural products exhibiting GST inhibitory activity. Consequently, we have successfully isolated a new sterol, 17-hydroxy-campesta-4,6-dien-3-one (**1**) as well as four known compounds, 13,14-*seco*-stigmasta-5,14-dien-3α-ol (**2**), 13,14-*seco*-stigmasta-9(11),14-dien-3α-ol (**3**), caesaldekarin J (**4**) and pipataline (**5**) as GST inhibitors. Spectroscopic methods were used to establish the structures of these new and known compounds. This paper describes the isolation and structure elucidation of these compounds as well as their GST inhibitory activity data. Three derivatives of compounds **2** and **3** were prepared to investigate the effects of the C-3 hydroxyl group and the C-5/C-6 double bond in 13,14-*seco*-steroids on GST inhibition. Inhibition of GST by compounds **1**—**5** and derivatives of **2** and **3** (**2a**, **3a**, **b**) was compared with activity of sodium taurocholate, a standard steroidal GST inhibitor under similar assay conditions. This indicated that the newly isolated compounds have more or less same activity as that of the standard inhibitor. Nearly half a dozen of 13,14-*seco*-steroids have been reported in the literature,[12—14] and no comments on their biosynthetic origin have been published. We hereby propose a plausible biogenetic pathway that could lead to the formation of the C/D *seco* ring of the 13,14-*seco*-steroids.

Results and Discussion

Compound **1** was purified as a colorless oil. The UV spec-

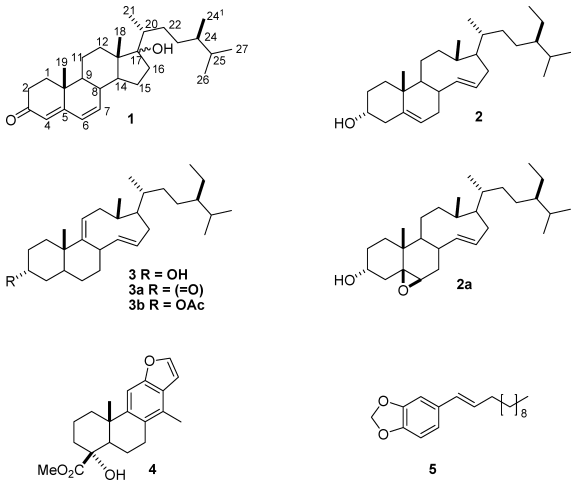

Fig. 1. Structures of Compounds **1**—**5**

* To whom correspondence should be addressed. e-mail: a.ata@uwinnipeg.ca

trum showed an absorption maximum at 284 nm indicating the presence of a six-membered enone with a double bond extended conjugation.[15] The IR spectrum displayed intense absorption bands at 3420 (OH), 2926 (CH), 1762 (C=O) and 1384 (C=C) cm^{-1}. HR-EI-MS showed a molecular ion peak at *m/z* 412.3138, corresponding to the molecular formula $C_{28}H_{44}O_2$ (Calcd 412.3141). This indicated the presence of seven double bond equivalents in compound **1**.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) of compound **1** showed two three-proton singlets at δ 0.72 and 1.16 due to the C-18 and C-19 methyl protons, respectively. Four 3H doublets at δ 0.71 (J=6.6 Hz), 0.80 (J=6.5 Hz), 0.82 (J=6.5 Hz) and 1.17 (J=6.6 Hz) were assigned to C-24^1, C-26, C-27 and C-21 methyl protons, respectively. The sp^2 hybridized C-6, C-4 and C-7 protons appeared at δ 5.76 (dd, J=7.4, 1.2 Hz), 5.72 (br s) and 5.92 (dd, J=7.4, 5.6 Hz), respectively. The ^{1}H–^{1}H correlation spectroscopy (COSY-45°) spectrum displayed the presence of three isolated spin systems "**1a**—**c**" (Fig. 2). The molecular formula suggested the presence of two oxygen atoms in compound **1** present as C-17 hydroxyl group and at C-3 carbonyl group. This was also supported by the ^{13}C-NMR data, which showed two resonances at δ 82.5 and 199.6, indicating the presence of oxygen functionalities at these carbon atoms. The ^{13}C-NMR chemical shift values of the C-17 side chain was found to be similar to values for synthetic campestane steroid of similar structure.[16] Complete ^{13}C-NMR chemical shift assignments of **1** and ^{1}H/^{13}C one-bond shift correlations of all protonated carbon atoms, as determined from HSQC spectrum, are presented in Table 1.

The heteronuclear multiple bond connectivity (HMBC) spectrum of **1** was useful in the unambiguous determination of the position of the oxygenated quaternary carbon atom, and in connecting the partial structures of **1** (**1a**—**c**) as obtained from the COSY-45° spectrum. The HMBC spectrum showed ^{1}H/^{13}C long-range couplings of H-18 (δ 0.72) with C-12 (δ 39.6), C-14 (δ 55.8), C-17 (δ 82.5) and C-20 (δ 53.8). These HMBC interactions helped to decide the position of C-17 hydroxyl group. Important HMBC interactions of compound **1** are shown in Fig. 3. A combination of ^{1}H-, ^{13}C-NMR and mass spectral data suggested that compound **1** has a campestane skeleton. The presence of steroidal skeleton was also confirmed by a positive Liebermann–Burchard test.

After establishing a structure for compound **1**, the relative configuration of the chiral centers was established using the NOESY spectrum. It has been reported in the literature that H-9 and H-14 exist invariably in the α-orientation while H-8, H_3-18 and H_3-19 have β-stereochemistry in this class of natural products.[16] The NOESY spectral data of compound **1** indicated that all stereogenic centers have the same orientation as that of reported compounds in this class of steroids.[16] Our efforts to crystallize compound **1** to solve its structure by X-ray crystallographic studies were not successful and this is the reason that we were not able to establish the stereochemistry at C-17. Based on these spectroscopic evidences and comparison with literature data,[16,17] compound **1** was characterized as 17-hydroxycampesta-4,6-dien-3-one.

Compounds **2**—**5** were characterized as 13,14-*seco*-stigmasta-5,14-dien-3α-ol (**2**), 13,14-*seco*-stigmasta-9(11),14-dien-3α-ol (**3**), caesaldekarin J (**4**) and pipataline (**5**) by comparison of their ^{1}H-, ^{13}C-NMR and mass spectra data with those of reported compounds.[3,12—14,18] 2D NMR spectra (COSY, HSQC, HMBC, NOESY) were also used to confirm their structures. Our 2D NMR spectral data of **2** suggested that C-14 and C-15 protons resonated at δ 5.00 and 5.12, re-

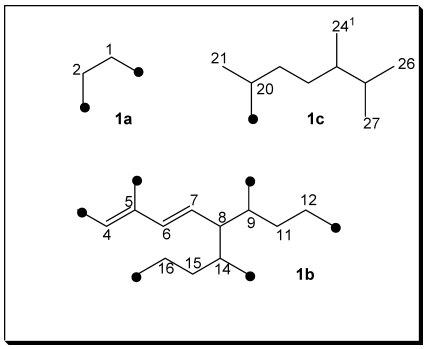

Fig. 2. Partial Structures of 17-Hydroxycampesta-4,6-dien-3-one (**1**) as Deduced from COSY-45° Spectrum

Table 1. ^{1}H- and ^{13}C-NMR Spectroscopic Data (300 and 75 MHz, Respectively in $CDCl_3$) for Bonducenone A (**1**)

Position	Bonducenone A (**1**)	
	δ_C, mult.[a]	δ_H (J in Hz)
1	35.7, CH_2	2.10, 1.65, m
2	32.0, CH_2	2.58, 2.38, m
3	199.6, qC	—
4	110.0, CH	5.72, br s
5	171.6, qC	—
6	123.7, CH	5.76, dd (7.4, 1.2)
7	123.7, CH	5.92, dd (7.4, 5.6)
8	35.3, CH	2.28, m
9	50.6, CH	1.53, m
10	38.8, qC	—
11	24.2, CH	1.55, 1.33
12	39.6, CH	2.02, 1.10
13	51.2, qC	—
14	55.8, CH	1.18, m
15	27.4, CH_2	1.61, 1.20, m
16	33.9, CH_2	2.09, 1.52, m
17	82.5, qC	—
18	11.9, CH_3	0.72, s
19	15.2, CH_3	1.16, s
20	53.8, CH	1.78, m
21	18.7, CH_3	1.17, d (6.6)
22	32.9, CH_2	1.28, 1.01, m
23	35.6, CH_2	1.30, 1.22, m
24	55.8, CH	1.12, m
25	56.0, CH	1.64, m
26	17.3, CH_3	0.80, d (6.5)
27	18.7, CH_3	0.82, d (6.5)
28 (24^1)	21.0, CH_3	0.71, d (6.6)

a) Multiplicities were determined by APT spectrum.

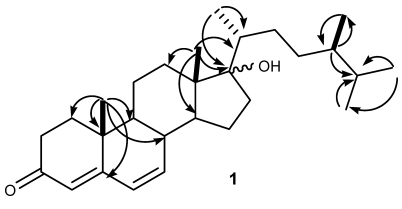

Fig. 3. Selected HMBC Interactions Observed in Compound **1**

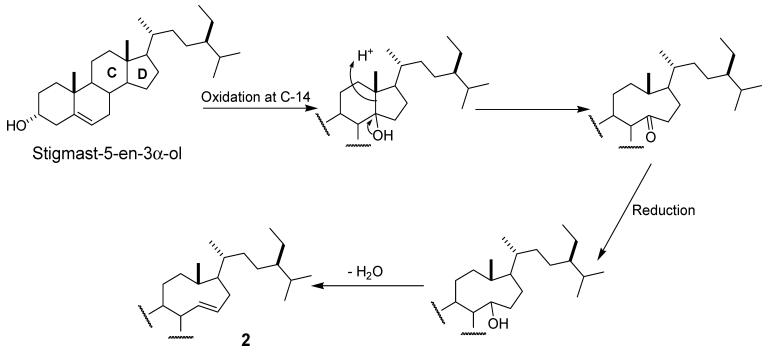

Chart 1. Proposed Biogenetic Pathway for the Formation of the C/D *seco* Ring of 13,14-*seco*-Stigmasta-5,14-dien-3α-ol (**2**) Starting from Stigmast-5-en-3α-ol

spectively. In the HSQC spectrum, H-14 (δ 5.00) and H-15 (δ 5.12) showed $^{1}H/^{13}C$ one-bond shift correlations with C-14 (δ 138.3) and C-15 (δ 129.3), respectively. Previous reports interchanged these ^{1}H- and ^{13}C-NMR chemical shift assignments at C-14 and C-15.[12,14] Our spectral data indicated that previously reported ^{1}H- and ^{13}C-NMR chemical shift assignments of C-14 and C-15 for compounds **2** and **3** need to be revised. Compounds **2**, **3** and **5** were isolated for the first time from this genus. To the best of our knowledge, there is no report available in the literature regarding the isolation of 13,14-*seco*-steroids from the genus *Caesalpinia*. Compound **2** was also isolated in our lab from *Barleria prionitis* (Acanthaceae). About half a dozen compounds have been previously reported in the class of 13,14-*seco*-steroids.[12—14] Biogenetically, the C/D *seco* ring may be produced in nature as illustrated in Chart 1.

Compound **2** was oxidized to its monooxirane derivative (**2a**) by reacting **2** with *m*-chloroperbenzoic acid using CH_2Cl_2 as solvent. The structure of **2a** was confirmed by MS, ^{1}H-NMR and 1D NOE. Compound **3** was oxidized to its C-3 keto derivative (**3a**) by treatment with PCC for 3 h at room temperature. An acetyl derivative (**3b**) of **3** was also prepared by reacting **3** with acetic anhydride using pyridine as a solvent. MS and ^{1}H-NMR data (Experimental) were used to confirm the structures of **3a** and **3b**.

Glutathione *S*-Transferase Inhibition Compounds **1**—**5**, **2a**, **3a** and **3b** showed appreciable GST inhibitory activity relative to the standard inhibitor (Table 2). Compound **5** showed the best activity against GST with an IC_{50} value of 57 μM. **1** had an IC_{50} value of 380 μM whereas compounds **2** and **3** had IC_{50} values of 230 and 248 μM, respectively. Epoxidation of compound **2** resulted in about two-fold increase in GST inhibition. GST inhibition in this case may be attributed to a glutathione conjugate formed from compound **2a** *via* Michael addition. GSH adducts have been reported to posses enhanced GST inhibitory activity relative to the parent compounds from which they were formed.[19] Oxidation of compound **3** led to a 36% increase in GST inhibition whereas the acetylation of the C-3 hydroxyl group enhanced the activity by 38%. Most non-substrate GST inhibitors exhibit their activity at high concentrations. This is because inhibition occurs, with ligandin GST inhibitors, at concentrations high enough to populate the low affinity ligandin binding site on GST.[11] Compounds (**1**—**5**, **2a**, **3a**, **b**) showed activity comparably similar to the activity of the reference standard GST inhibitor under the assay conditions described in Experimental.

Table 2. IC_{50} Values of Compounds **1**—**5** and Their Derivatives in GST Inhibition

Compound	IC_{50} (μM)[a]
1	380
2	230
2a	118
3	248
3a	158
3b	153
4	250
5	57
Sodium taurocholate[b]	398

a) Results obtained in triplicates. *b*) Standard steroidal GST inhibitor.

Experimental

General Optical rotations were measured on a Hitachi Polatronic-D polarimeter. UV and IR spectra were recorded on Shimadzu UV-250 1 PC and Bomem Hartmann and Braun (MB Series) spectrophotometers, respectively. The ^{1}H-, ^{13}C-NMR, ^{1}H–^{1}H COSY, HSQC, HMBC and NOESY spectra were recorded on a Bruker Avance 300 spectrometer; chemical shifts are in ppm (δ) relative to $SiMe_4$ as internal standard, coupling constants *J* are in Hz. EI/CI and HR-EI-MS were measured on Hewlett Packard 5989B (*m*/*z*, rel. int. %) and INCOSSO, FINNIGA-MAT Mass spectrometers. Column chromatography was carried out on silica gel (200—400 mesh). Thin-layer chromatography was performed on Merck silica gel GF_{245} pre-coated plates. GST activity was measured on a thermostated HP 8452 Diode Array spectrophotometer. Equine liver GST was purchased from Sigma-Aldrich. Glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from MP Biomedicals.

Plant Material The bark of *C. bonduc* was peeled off from the stem with a knife and collected from Chalaw, Sri Lanka in December 2004. This was identified as *C. bonduc* by Dr. Radhika Samarasekera, and a voucher specimen deposited in the herbarium of Industrial Technology Institute, Colombo, Sri Lanka.

Extraction and Isolation The bark of *C. bonduc* (2 kg) was extracted with 98% ethanol at room temperature and evaporated under reduced pressure to yield a brownish gum. The crude extract (85 g) was loaded onto a silica gel column and eluted using 0—100% hexane–AcOEt and 0—100% AcOEt–MeOH, to afford several fractions. GST inhibition assay was carried out on the fractions, which provided fractions F to H as the most active fractions with about 44—60% GST inhibition at 41.66 μg/ml. Column chromatography (0—50% hexane–AcOEt) on fraction F yielded 23 fractions FFQ1—23. Preparatory TLC on FFQ9 (1 : 4 hexane/Et_2O) yielded compound **1**); 7.8 mg, 0.0092% yield, *Rf* 0.678 in 1 : 4 hexane/Et_2O) as a colorless oil and compound **4** (21 mg, 0.0247% yield, *Rf* 0.769 in 1 : 4 hexane/Et_2O) as a white solid. Column chromatography on fraction G (3 : 1 hexane/AcOEt) provided compound **2** as a white amorphous solid (28.3 mg, 0.033% yield, *Rf* 0.311 in 1 : 4 hexane/Et_2O) after crystallization in methanol. Preparatory TLC on FFQ11 (3 : 1 hexane/AcOEt) yielded compound **3** (9.6 mg, 0.0113% yield, *Rf* 0.396 in 1 : 4 hexane/Et_2O) as a white amorphous solid. Compound **5** (23.74 mg, 0.029% yield, *Rf* 0.76 in 80 : 20 : 0.1 hexane/Et_2O/AcOH) was isolated from preparatory TLC (3 : 1

hexane/AcOEt) of fraction FFQ1. Purity of these compounds was confirmed by the observation of homogenous spots on TLC in various solvent systems.

17-Hydroxycampesta-4,6-dien-3-one (**1**): 7.8 mg. Colorless oil. $[\alpha]_D^{25}$ +18° (c=0.14, $CHCl_3$). UV λ_{max} ($CHCl_3$): 284 nm; IR ν_{max} (KBr): 3332 (OH), 2918 (CH), 1662 (C=C), 1082 (C–O). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: see Table 1. ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: see Table 1. HR-EI-MS *m/z*: 412.3138 (M^+, $C_{28}H_{44}O_2$, Calcd 412.3141). CI-MS: 413 (M^+-H). EI-MS *m/z*: 412 (M^+), 394, 386, 370, 288, 271, 229, 147, 124, 43.

Epoxidation of 2 Five milligrams of **2** was dissolved in 4 ml of CH_2Cl_2 and 0.64 mM *m*-chloroperbenzoic acid. The mixture was stirred during 3 h at room temperature until the starting material was consumed as monitored by TLC. The mixture was later dried, reconstituted using CH_2Cl_2, and the resulting organic component purified using column chromatography (0—50% Hex/$CHCl_3$) to obtain **2a** in 72% yield.

5β,6β-Epoxy-13,14-*seco*-stigmast-14-en-3α-ol (**2a**): 3.6 mg. *Rf* 0.34. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.95 (dd) for H_α-6, 3.96 (m) for H-3. NOE ($CDCl_3$, 300 MHz) δ: 1.95 (dd) for H_α-4, 1.25 (dd) for H_α-7, 1.45 (dd) for H-8. CI-MS *m/z*: 431 (M^+-H). EI-MS *m/z*: 430 (M^+), 412, 398, 289, 253.

Oxidation of 3 Three milligrams of **3** (10 mM) was dissolved in CH_2Cl_2 and 15 mM pyridinium chlorochromate (PCC). The reaction mixture was stirred at room temperature for 3 h and reaction progress was monitored using TLC. Thereafter, the mixture was evaporated to dryness and extracted with CH_2Cl_2. The extract was washed twice using 2 N HCl and subsequently equal volume of aqueous $NaHCO_3$. The organic layer was dried over anhydrous $MgSO_4$ to afford **3a**.

3-Oxo-13,14-*seco*-stigmasta-9(11),14-diene (**3a**): 1.9 mg. *Rf* 0.89. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.40 (dd) and 2.47 (dd) for H_2-2, 3.24 (ddd) and 3.32 (ddd) for H_2-4, 2.33 (m) for H-5, 1.19 (s) for H_3-19, 0.70 (d) for H_3-18. CI-MS *m/z*: 413 (M^+-H). EI-MS *m/z*: 412 (M^+), 271.

Acetylation of 3 Three milligrams of **3** (3.62 mM) was dissolved in equal parts of pyridine and acetic anhydride. The mixture was stirred at room temperature for 3 h. On completion of reaction, the mixture was evaporated to dryness and reconstituted using CH_2Cl_2. The organic extract was washed using 2 N HCl and aq. $NaHCO_3$, and dried over anhydrous $MgSO_4$.

3α-Acetoxy-13,14-*seco*-stigmasta-9(11),14-diene (**3b**): 2.5 mg. *Rf* 0.91. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 4.62 (m) for H-3, 2.05 (s) for CH_3 (acetyl group). CI-MS *m/z*: 457 (M^+-H). EI-MS *m/z*: 456 (M^+).

Assay for Glutathione *S*-Transferase Inhibition The inhibitory activity of compounds **1**—**5**, **2a**, **3a** and **3b** against GST was assayed according to a modification of the spectrophotometric method of Habig *et al.*[20] Specific concentrations of the compounds were incubated with the enzyme at 22 °C for 30 min after which an assay was carried out. The final assay mixture contains final concentrations of the following, in a 3000 μl solution: 5 mM GSH, 1 mM CDNB, 100 mM phosphate buffer (pH 6.5) and GST (with initial activity of 0.12106 U/ml). The assay measures the activity of GST in conjugating CDNB to GSH, and the product of conjugation was measured at 340 nm using a HP 8452 Diode Array spectrophotometer equipped with a thermostated cell compartment (22 °C). The reaction was monitored for 40 s and GST inhibition was calculated with reference to a control assay. Basal coupling between the substrates was also analyzed, and was observed to be insignificant under these assay conditions. Substrate limitation was also taken into consideration, and was observed not to occur during the initial 60 s of the assay under the same assay conditions. The inhibitory activity of the compounds against GST was compared with the activity of sodium taurocholate, a standard GST inhibitor. All assays were carried out in triplicates.

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References

1) Chakrabarti S., Biswas T. K., Rokeya B., Ali L., Mosihuzzaman M., Nahar N., Khan-Azad A. K., Mukherjee B., *J. Ethnopharmacol.*, **84**, 41—46 (2003).
2) Gupta M., Mazumder U. K., Kumar R. S., Sivakumar T., Vamsi M. L. M., *J. Pharmacol. Sci.*, **94**, 177—184 (2004).
3) Lyder D. L., Peter S. R., Tinto W. F., Bissada S. M., McLean S., Reynolds W. F., *J. Nat. Prod.*, **61**, 1462—1465 (1998).
4) Kitagawa I., Simanjuntak P., Watano T., Shibuya H., Fujii S., Yamagata Y., Kobayashi M., *J. Nat. Prod.*, **42**, 1798—1802 (1994).
5) Linn T. Z., Awale S., Tezuka Y., Banskota A. H., Kalauni S. K., Attamimi F., Ueda J., Asih P. B. S., Syafruddin D., Tanaka K., Kadota S., *J. Nat. Prod.*, **68**, 706—710 (2005).
6) Kalauni S. K., Awale S., Tezuka Y., Banskota A. H., Linn T. Z., Kadota S., *Chem. Pharm. Bull.*, **53**, 1300—1304 (2005).
7) Chasseaud L. F., *Adv. Can. Res.*, **29**, 175—274 (1979).
8) Townsend D. M., Tew K. D., *Oncogene*, **22**, 7369—7375 (2003).
9) Brophy P. M., Pritchard D. I., *Exptl. Parasitol.*, **79**, 89—96 (1994).
10) Schultz M., Dutta S., Tew K. D., *Adv. Drug Delv. Rev.*, **26**, 91—104 (1997).
11) Mahajan S., Atkins W. M., *Cell. Mol. Life Sci.*, **62**, 1221—1233 (2005).
12) Ahmad B., Alam T., *Ind. J. Chem.*, **42B**, 1786—1790 (2003).
13) Sharma S. K., Ali M., Singh R., *J. Nat. Prod.*, **59**, 181—184 (1996).
14) Bhandari P., Kumar N., Singh B., Kaul V. K., *Chem. Pharm. Bull.*, **54**, 240—241 (2006).
15) Pavia D. L., Lampman G. M., Kriz G. S., "Introduction to Spectroscopy," 3rd ed., Brooks/Cole Thomson Learning, U.S.A., 2001.
16) Seto H., Fujioka S., Takatsuto S., Koshino H., Shimizu T., Yoshida S., *Steroids*, **65**, 443—449 (2000).
17) Mulheirn L. J., *Tetrahedron Lett.*, **14**, 3175—3178 (1973).
18) Koul S. K., Taneja S. C., Agarwal V. K., Dhar K. L., *Phytochemistry*, **27**, 3523—3527 (1988).
19) Schmidt T. J., *Planta Medica*, **66**, 106—109 (2000).
20) Habig W. H., Pabst M. J., Jakoby W. B., *J. Biol. Chem.*, **249**, 7130—7139 (1974).