Isolation of Acridone Alkaloids and *N*-[(4-Monoterpenyloxy)phenylethyl]-Substituted Sulfur-Containing Propanamide Derivatives from *Glycosmis parva* and Their Anti-herpes Simplex Virus Activity

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Six acridone alkaloids including a new glycosparvarine (1), three limonoids, and four *N*-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivatives including two new species, (+)-*S*-deoxydihydroglyparvin (10) and (+)-*S*-deoxytetrahydroglyparvin (11), were isolated from the branches and the leaves of *Glycosmis parva* Craib collected in the east of Thailand. Antiviral activity evaluation of isolates against herpes simplex virus (HSV) type 1 and 2 disclosed that two acridone alkaloids, glycosparvarine (1) and glycofolinine (4), showed moderate inhibitory activities with 50% effective concentration (EC_{50}) of 348 μM and 151 μM, respectively; as well, (+)-*S*-deoxydihydroglyparvin (10) exhibited anti-HSV activity at the lower concentration.

Key words *Glycosmis parva*; glycosparvarine; *S*-deoxydihydroglyparvin; *S*-deoxytetrahydroglyparvin; anti-herpes simplex virus activity

Glycosmis parva Craib (Rutaceae) is a wild small shrub distributed in Thailand.[1)] Some plants in the genus *Glycosmis* have been used as Thai traditional medicines for treatments of abscess, scabies, and snakebite. The genus *Glycosmis* is a rich source of acridone alkaloids and sulfur-containing propanamides especially in branches and leaves, respectively.[2)] Biological activities of acridone alkaloids were reported such as antimalarial activity,[3)] anti-herpes simplex virus (HSV) activity,[4)] tumor cell growth inhibition,[5)] suppression of human immunodeficiency virus type 1 (HIV-1) replication,[6)] induction of human promyelocytic leukemia (HL-60) cellular differentiation,[7)] antiproliferative activity,[8)] cancer chemopreventive activity,[9)] and modulation of P-glycoprotein activity.[10)] For sulfur-containing propanamides, antifungal activities were mainly reported.[11)] We examined the crude extracts of the branches and leaves of *G. parva* focused on anti-HSV activities against HSV-1 and HSV-2 and observed positive activities. In this paper, we report the structure elucidation of a new acridone alkaloid, glycosparvarine (**1**), two new *N*-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivatives, *S*-deoxydihydroglyparvin (**10**) and *S*-deoxytetrahydroglyparvin (**11**), which were isolated together with nine known compounds, and the anti-HSV activity of the isolates.

Results and Discussion

The MeOH extracts of the branches and the leaves of *G. parva* were partitioned with hexane. The remaining layers were further partitioned with EtOAc and *n*-BuOH after addition of water. Organic extracts were subjected to antiviral activity tests in plaque reduction assays against HSV-1 and HSV-2. The hexane and EtOAc extracts from branches, and the hexane extract from leaves displayed the anti-herpes virus activities in the inactivation inhibition, while the EtOAc extract from leaves exhibited the anti-herpes virus activities in both inactivation treatment and post-inhibition (Table 1).

Repeated chromatographies of the EtOAc extract of branches using the solvent systems of MeOH–$CHCl_3$, acetone–hexane, and acetone–$CHCl_3$ led to the isolation of a new acridone alkaloid, glycosparvarine (**1**), together with four known acridones [*N*-methylatalaphylline[12,13)] (**2**), *N*-methylcyclo-atalaphylline-A[14)] (**3**), glycofolinine[15)] (**4**), and citramine[16)] (**5**)] and three known limonoids [limonin[17—20)] (**7**) and a mixture of limonexic acid (**8**) and isolimonexic acid[21)] (**9**)]. In addition, examination of the EtOAc extract of leaves resulted in the isolation of two new *S*-deoxydihydroglyparvin (**10**) and *S*-deoxytetrahydroglyparvin (**11**) together with two known *N*-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivatives[22)] [glyparvin-A (**12**) and dihydroglyparvin (**13**)] and a known acridone [arborinine[23)] (**6**)] (Fig. 1).

Glycosparvarine (**1**) showed a molecular ion ($[M]^+$) at *m/z* 287.0799 in the HR-EI-MS, providing evidence for molecular formula of $C_{15}H_{13}NO_5$ (Calcd for $C_{15}H_{13}NO_5$, 287.0793). The IR spectrum showed absorption bands at 3500—3181

Table 1. Antiviral Activities of the Hexane, EtOAc, *n*-BuOH, and Aqueous Extracts from Branches and Leaves of *G. parva* against HSV-1 and HSV-2

Part/ Extract	Inactivation treatment			Post-treatment		
	Final conc.[a)] (μg/ml)	% inhibition[b)]		Final conc.[a)] (μg/ml)	% inhibition[b)]	
		HSV-1	HSV-2		HSV-1	HSV-2
Branches/						
Hexane	50	50	20	50	0	0
EtOAC	25	30	0	25	0	0
n-BuOH	100	0	0	100	0	0
Aqueous	100	0	0	100	0	0
Leaves/						
Hexane	12.5	20	20	25	0	0
EtOAC	12.5	50	30	25	70	60
n-BuOH	100	0	0	100	0	0
Aqueous	100	0	0	100	0	0

a) Concentration of extract that was not toxic to the cells used in assay. *b*) 0, no inhibition.

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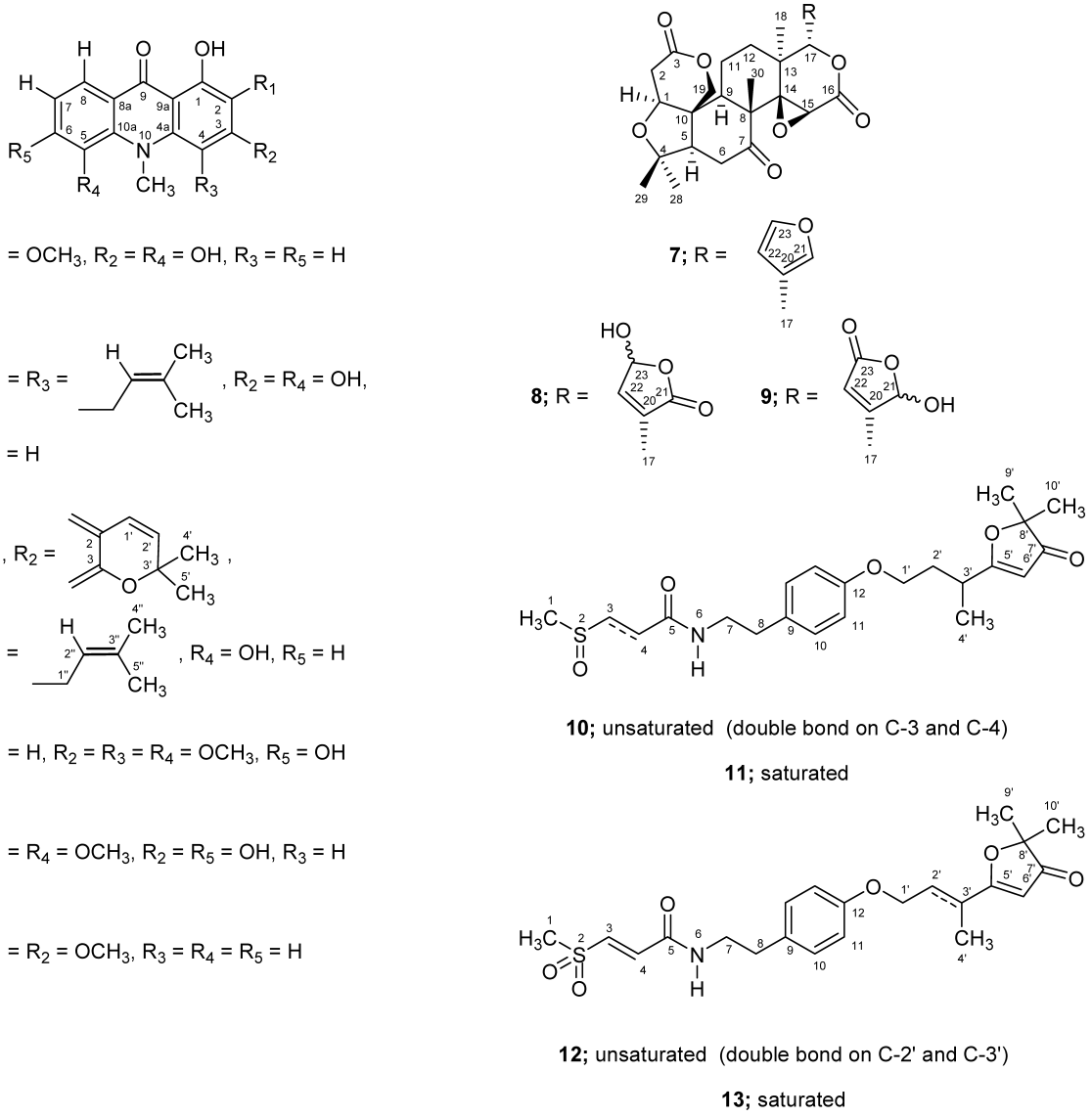

Fig. 1. Acridone Alkaloids, Limonoids, and *N*-[(4-Monoterpenyloxy)phenylethyl]-Substituted Sulfur-Containing Propanamide Derivatives from *G. parva*

(broad) and 1644 cm^{-1} due to hydroxy and carbonyl groups, respectively. Characteristic absorptions of acridone alkaloid were observed in the UV spectrum (409, 321, 286, 266 nm).[24] The ^{1}H-NMR spectrum (see Experimental) showed the existence of methoxy (δ_H 3.88) and *N*-methyl (δ_H 4.08) groups. In aromatic region, the three among four protons displayed sequentially mutual coupling as ABX pattern [δ_H 7.14 (1H, dd, J=8.1, 7.7 Hz), 7.29 (1H, dd, J=7.7, 1.5 Hz), and 7.91 (1H, dd, J=8.1, 1.5 Hz)] and a remaining signal as singlet was observed at relatively higher field (δ_H 6.50). A lower-field shifted signal at δ_H 14.99 was assigned as an intramolecularly hydrogen-bonded proton to carbonyl oxygen and two signals at δ_H 8.95 and 9.31 as exchangeable protons. Appearances of the above intramolecularly hydrogen-bonded signal and ABX pattern signals including a deshielded proton (δ_H 7.91) indicated that **1** was constructed based on a 6,7,8-unsubstituted 1-hydroxy-10-methylacridone skeleton. In the ^{13}C-NMR spectrum (see Experimental) twelve signals were observed as aromatic carbons in addition to methoxy (δ_C 60.5), *N*-methyl (δ_C 41.3), and carbonyl (δ_C 181.6) functions. In HMBC experiment (Fig. 2), significant correlations between proton signal at δ_H 14.99 and carbon signals at δ_C

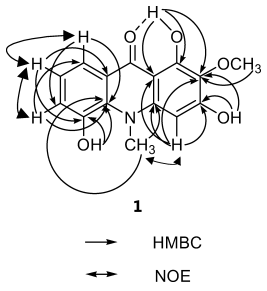

Fig. 2. Selected HMBC and NOE Correlations of **1**

105.8, 156.4, and 129.5, and between proton signal at δ_H 3.88 and carbon signal at δ_C 129.5 indicated the carbon signals at δ_C 105.8, 156.4, and 129.5 as C-9a, C-1, and C-2, respectively. *O*-Quaternary carbons were deduced by HMBC correlations of δ_H 8.95 (3-OH) with δ_C 158.4 (C-3) and 129.5 (C-2), and δ_H 9.31 (5-OH) with δ_C 147.8 (C-5) and 134.7 (C-10a). The strong HMBC cross-peaks of δ_H 6.50 with δ_C 105.8 (C-9a), 129.5 (C-2), and 158.4 (C-3) assigned the signal at δ_H 6.50 to be H-4. NOE experiments revealed to be within NOE distance of H-4 (δ_H 6.50) with the *N*-methyl

Table 2. ^{1}H- (600 MHz) and ^{13}C- (150 MHz) NMR Data of *S*-Deoxydihydroglyparvin (**10**) and *S*-Deoxytetrahydroglyparvin (**11**) in $CDCl_3$

Position	**10**		**11**	
	δ_H	δ_C	δ_H	δ_C
1	2.63 (3H, s)	39.9 q	2.57 (3H, s)	38.6 q
3/H-3a/H-3b	7.52 (1H, d, *J*=14.5 Hz)	146.3 d	3.11 (1H, ddd, *J*=13.2, 7.7, 7.7 Hz)	49.3 t
			2.85 (1H, ddd, *J*=13.2, 6.6, 6.6 Hz)	
4	6.69 (1H, dd, *J*=14.5, 1.6 Hz)	128.6 d	2.64—2.66 (2H, m)	28.8 t
5		162.6 s		169.9 s
6	6.80 (1H, overlapped signal)		6.13 (1H, br s)	
7	3.55 (2H, dt, *J*=6.8, 6.5 Hz)	41.2 t	3.46—3.48 (2H, m)	41.0 t
8	2.79 (2H, t, *J*=6.9 Hz)	34.6 t	2.75 (2H, t, *J*=6.9 Hz)	34.6 t
9		131.0 s		130.9 s
10	7.09 (2H, d, *J*=8.5 Hz)	129.6 d	7.08 (2H, d, *J*=8.5 Hz)	129.7 d
11	6.80 (2H, d, *J*=8.8 Hz)	114.8 d	6.80 (2H, d, *J*=8.5 Hz)	114.6 d
12		157.6 s		157.4 s
1′a	3.99 (1H, ddd, *J*=9.5, 6.3, 6.1 Hz)	65.3 t	3.98 (1H, ddd, *J*=9.4, 6.3, 6.3 Hz)	65.2 t
1′b	3.95 (1H, ddd, *J*=9.5, 6.3, 6.1 Hz)		3.96 (1H, ddd, *J*=9.4, 6.3, 6.3 Hz)	
2′a	2.14 (1H, m)	33.5 t	2.14 (1H, m)	33.4 t
	1.96 (1H, m)		1.97 (1H, m)	
3′	2.96 (1H, sextet, *J*=7.2 Hz)	32.7 d	2.96 (1H, sextet, *J*=7.1 Hz)	32.5 d
4′	1.28 (3H, d, *J*=7.2 Hz)	17.7 q	1.29 (3H, d, *J*=7.2 Hz)	17.6 q
5′		195.0 s		194.8 s
6′	5.35 (1H, s)	100.3 d	5.35 (1H, s)	100.1 d
7′		207.6 s		207.5 s
8′		88.6 s		88.5 s
9′	1.35 (3H, s)	23.0 q[a)]	1.36 (3H, s)	22.8 q[a)]
10′	1.36 (3H, s)	23.0 q[a)]	1.36 (3H, s)	22.8 q[a)]

a) Overlapped signals.

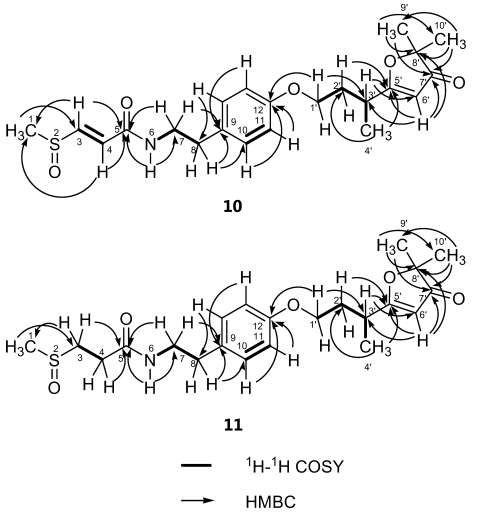

Fig. 3. Selected 2D NMR Correlations of **10** and **11**

group (δ_H 4.08), and that of H-7 (δ_H 7.14) with H-8 (δ_H 7.91) and H-6 (δ_H 7.29) (Fig. 2). These data allowed us to deduce the alignment of 1,3,5-trihydroxy-2-methoxy substituents. Thus, glycosparvarine (**1**) was reasonably deduced to be 1,3,5-trihydroxy-2-methoxy-*N*-methyl-9-acridone.

The pseudo molecular ion ($[M+H]^+$) peak at *m*/*z* 420.1831 in the HR-FAB-MS of the second new component was deduced to be the molecular formula of $C_{22}H_{29}NO_5S$ (Calcd for $C_{22}H_{30}NO_5S$, 420.1845). The UV spectrum showed three absorption maxima at 261, 225, and 204 nm. In the IR spectrum conjugated amide (3287, 1648 cm^{-1}) and ketone (1698 cm^{-1}) functions were observed in addition to a strong absorption due to sulfide stretching at 1039 cm^{-1}.[25] The ^{1}H-NMR spectrum (Table 2) showed three tertiary and one secondary methyl groups, and two ethylene units, one of which was connected to the root of the secondary methyl group, in the aliphatic proton region. In the lower field, the spectrum were observed seven protons, six of which were assignable to 1,4-disubstituted benzene [δ_H 6.80 (2H, d, *J*=8.8 Hz) and 7.09 (2H, d, *J*=8.5 Hz)] and *trans*-α,β-unsaturated carbonyl [δ_H 6.69 (1H, dd, *J*=14.5, 1.6 Hz) and 7.52 (1H, d, *J*=14.5 Hz)] systems. The ^{13}C-NMR spectrum (Table 2) supported the above assignments and showed the presence of one additional quaternary carbon (δ_C 88.6). These spectral data were similar to those of a known dihydroglyparvin,[22] which was formally composed of monoterpene, *p*-hydroxyphenethylamine, and *trans*-β-methylsulfonylacrylic acid subunits, except the surrounding of sulfur atom. The presence of 5-(1-methylpropyl)-3(2*H*)-furanone system as a monoterpene unit was suggested by HMBC correlations of δ_H 1.35 (H_3-9′) and 1.36 (H_3-10′) with δ_C 88.6 (C-8′) and 207.6 (C-7′); δ_H 5.35 (H-6′) with δ_C 32.7 (C-3′), 195.0 (C-5′), 207.6 (C-7′), and 88.6 (C-8′); δ_H 2.14 (H-2′a), 2.96 (H-3′), and 1.28 (H_3-4′) with δ_C 195.0 (C-5′); and δ_H 2.96 (H-3′) with δ_C 100.3 (C-6′) (Fig. 3). Additional HMBC correlations of δ_H 3.99 (H-1′a) with δ_C 157.6 (C-12) and δ_H 7.52 (H-3), 6.69 (H-4), 6.80 (N–H signal overlapped with the aromatic proton signal at δ_H 6.80 (H-11)), and 3.55 (H_2-7) with δ_C 162.6 (C-5) indicated the connection of the monoterpene part with *trans*-β-methylsulfinylacrylic acid one through *p*-hydroxyphenethylamine one. Thus, this could be deduced to be *S*-deoxydihydroglyparvin (**10**), which was supported by the appearances of characteristic fragment ions at *m*/*z* 64 and 167 in the EI-MS due to methylsulfide (H_3CSOH) and monoterpene ($C_{10}H_{15}O_2$) functions, respectively.

The third new component was reasonably supposed to be *S*-deoxytetrahydroglyparvin (**11**) carrying a methylsulfinylpropanoic acid subunit instead of *trans*-β-methylsulfinylacrylic acid subunit in *S*-deoxydihydroglyparvin (**10**) by the following data: *m*/*z* 422.1985 (Calcd for $C_{22}H_{32}NO_5S$,

Table 3. Antiviral Activities of Isolated Compounds against HSV-1 and HSV-2 Determined by Plaque Reduction Assay

Compound	Final conc. (μg/ml)	EC_{50} [a] (inactivation treatment)		EC_{50} [a] (post-treatment)		TC_{50} [b]
		HSV-1	HSV-2	HSV-1	HSV-2	
1	—	100 μg/ml (348 μM)	>100 μg/ml (348 μM)	100 μg/ml (348 μM)	>100 μg/ml (348 μM)	>100 μg/ml (348 μM)
2	100	0	0	0	0	
3	100	0	0	0	0	
4	—	50 μg/ml (151 μM)	50 μg/ml (151 μM)	50 μg/ml (151 μM)	50 μg/ml (151 μM)	150 μg/ml (453 μM)
5	100	0	0	0	0	
6	100	0	0	0	0	
7	100	0	0	0	0	
Mixture of **8** and **9**	100	0	0	0	0	
10	—	12.5 μg/ml (29.8 μM)	18.7 μg/ml (44.6 μM)	12.5 μg/ml (29.8 μM)	18.7 μg/ml (44.6 μM)	37.5 μg/ml (89.4 μM)
11	100	0	0	0	0	
12	1.6	0	0	0	0	3.1 μg/ml (7.2 μM)
13	6.3	0	0	0	0	12.5 μg/ml (28.7 μM)

a) EC_{50} (50% effective concentration) was determined from three independent assays. Maximum concentration tested was 100 μg/ml. The EC_{50} of acyclovir against HSV-1 was 0.5 μg/ml, used as positive control. *b*) TC_{50} (50% cytotoxic concentration) was the concentration that was 50% cytotoxic to the cells used in assay, was determined from three independent assays.

422.2001) in the HR-FAB-MS; λ_{max} 261, 225, and 205 nm in the UV spectrum; ν_{max} 3291, 1695, 1662, and 1035 cm^{-1} in the IR spectrum; δ_H 2.57 (3H, s, SMe), 2.64—2.66 (2H, m, H_2-4), 2.85 (1H, ddd, J=13.2, 6.6, 6.6 Hz, H-3b), and 3.11 (1H, ddd, J=13.2, 7.7, 7.7 Hz, H-3a) in the ^{1}H-NMR spectrum (Table 2) δ_C: 38.6 (SMe), 28.8 (C-4), and 49.3 (C-3) in the ^{13}C-NMR spectrum (Table 2); and ^{1}H–^{1}H COSY and HMBC spectra (Fig. 3).

S-Deoxydihydroglyparvin (**10**) and *S*-deoxytetrahydroglyparvin (**11**) were isolated as optically active forms, $[\alpha]_D^{21}$ +68.2 ($CHCl_3$) in **10** and $[\alpha]_D^{18}$ +24.1 ($CHCl_3$) in **11**. Co-isolation of a known (+)-dihydroglyparvin (**13**), lacking the sulfur chiral center,[22] suggested that the carbon chiral center of the monoterpene units in these three might be the same absolute stereochemistry, even remaining unknown. (+)-Entadamide C had been isolated from *Entada phaseoloides* as a related and simple β-methylsufinylcarboxyamide derivative, and the absolute configuration of the sulfur atom had been reported to be an *R* configuration.[25] Thus, the positive sign of $[\alpha]_D$ in **10** and **11** could suggest the same *R*-configuration of the sulfur chiral center which is in (+)-entadamide C. We will independently carry out the asymmetric synthesis of these *S*-deoxyglyparvin derivatives for the establishment of the chiral centers.

Anti-HSV activities against HSV-1 and HSV-2 of the isolates (**1**—**13**) were assessed (Table 3). Glycofolinine (**4**) exhibited moderate activity with EC_{50} of 151 μM against both HSV-1 and HSV-2 in inactivation treatment and post-treatment, and the 50% cytotoxic concentration (TC_{50}) to the cells used in assay was 453 μM. A new alkaloid, glycosparvarine (**1**), also exhibited activity even with higher EC_{50} (348 μM). In 3-methylacridone series structure–activity relationship (SAR) for anti-herpes virus activity has been suggested the responsibility of the hydroxyl group at either the C-5 or C-6 positions such as citpressine-I and citrusinine-I.[4] Furthermore, potential anti-herpes virus activity has been observed in the 2,2-dimethylpyranoacridones.[4] Our studies showed that oxygen-substituted acridones could act as an additional acridone potential for anti-HSV activity.

In addition to *N*-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivatives, *S*-deoxydihydroglyparvin (**10**) exhibited antiviral activities against HSV-1 and HSV-2 with EC_{50} of 29.8 and 44.6 μM in activation treatment and post-treatment, respectively. However, the EC_{50} of **10** was close to the TC_{50} (89.4 μM). *S*-Deoxytetrahydroglyparvin (**11**) showed no activity at concentration tested (100 μg/ml) in inactivation treatment and post-treatment. In contrast, glyparvin-A (**12**) and dihydroglyparvin (**13**) showed no activity at the non-toxic concentration. The limonoid-type compounds (**7** and mixture of **8** and **9**) showed no activity against HSV-1 and HSV-2.

In conclusion, six acridone alkaloids including a new glycosparvarine (**1**), three limonoids, and four *N*-[(4-monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivatives including two new species, *S*-deoxydihydroglyparvin (**10**) and *S*-deoxytetrahydroglyparvin (**11**), were isolated from the branches and the leaves of *G. parva* collected in the east of Thailand. Antiviral activity evaluation of isolates against HSV-1 and HSV-2 disclosed the moderate activity of glycosparvarine (**1**) and glycofolinine (**4**). In addition, *S*-deoxydihydroglyparvin (**10**) exhibited anti-HSV activity at the lower concentration which was close to the cytotoxic concentration.

Experimental

General Procedures Melting points were measured on a micromelting point hot-stage apparatus (Yanagimoto) and are uncorrected. UV spectra were recorded on a JASCO V-530 UV spectrophotometer. IR spectra were recorded on a JASCO IR-230E spectrophotometer. Optical rotations were recorded on a JASCO P-1020 polarimeter. EI-MS and HR-EI-MS were measured on a JEOL GC-Mate spectrometer. FAB-MS and HR-FAB-MS were obtained by JEOL JMS-AX 500 and JEOL JMS-HX 110 spectrometers, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded with JEOL JNM ECP 600 spectrometer with TMS as an internal reference. For column chromatography (CC) and flash column chromatography (FCC) were used silica gel 60 (70—230 mesh ASTM; Merck) and silica gel 60 (230—400 mesh ASTM; Merck), respectively. Sephadex LH-20 and a recycling preparative HPLC machine (Japan Analytical Industry; model LC-9201) were also used for size exclusion chromatography.

Plant Material The branches and the leaves of *G. parva* were collected at Sakaerat, Wang Nam Khieo district, Nakorn Ratchasima province, Thai-

land in December 2007. A voucher (NSR 510209) has been deposited at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

Extraction and Isolation The dried branches (B) (12.35 kg) and leaves (L) (4.8 kg) were ground and macerated with MeOH (4×30 l for B; 4×10 l for L). The MeOH extracts (850 g for B; 650 g for L) were partitioned with hexane to obtain the hexane extracts (72 g for B; 135 g for L). The remaining layers were added with water and further partitioned with EtOAc to yield the extracts (321 g for B; 100 g for L). The aqueous layers were partitioned with *n*-BuOH to give the extracts (85 g for B; 93 g for L).

The EtOAc extract (29.34 g) of branches was subjected to CC eluted with a gradient solvent system of MeOH–$CHCl_3$ (0%, 1%, 2%, 5%, 10%, 20%, 30%, and 100% MeOH/$CHCl_3$) to give thirteen fractions (BE1—BE13). BE4 (7.00 g) was washed with Et_2O to obtain **2** (0.85 g) and the soluble part (4.04 g) was purified by CC (20—100% Me_2CO/hexane) to give ten sub-fractions (BE4-1—BE4-10). Washing of BE4-3 (0.30 g) with Et_2O and separation of BE4-5 (0.39 g) by CC (20% Me_2CO/hexane) afforded **3** (35 mg) and **4** (19 mg), respectively. Fraction BE5 (0.50 g) was separated into twelve sub-fractions (BE5-1—BE5-12) by CC (20—30% Me_2CO/hexane). Further purification of BE5-9 (0.11 g) by CC (20—30% Me_2CO/hexane) gave **7** (6.5 mg) and a mixture of **8** and **9** (8 mg). BE6 (0.15 g) was purified by CC (30—40% Me_2CO/hexane) to give **5** (10.6 mg). After washing of BE9 (0.59 g) with Et_2O, the soluble part (0.28 g) was subjected to CC (2% Me_2CO/$CHCl_3$) to afford **1** (6.8 mg).

The EtOAc extract (11.60 g) of leaves was washed with CH_2Cl_2. After removal of the insoluble part, a soluble part (5.38 g) was purified by CC (0%, 0.5%, 1%, 2%, 5%, 10%, 20%, 30%, and 100% MeOH/$CHCl_3$) to afford twelve fractions (LE1—LE12). Washing of LE3 (1.02 g) with Me_2CO followed by recrystallization from CH_2Cl_2–Me_2CO gave **6** (122.7 mg) as yellow needles, mp 181 °C (lit.[16] mp 177—178 °C).

LE5 (550 mg) was separated to five fractions (LE5-1—LE5-5) by Sephadex LH-20 column chromatography using 50% MeOH/$CHCl_3$. A part (79 mg) of LE5-3 (260 mg) was washed with Et_2O, followed by recrystallization from CH_2Cl_2–Et_2O, to afford **12** (15 mg) as colorless needles, mp 136—138 °C (lit.[15] mp 134—137 °C). The evaporated supernatant (44 mg) was purified by repeated CC using different solvent systems (4% MeOH/$CHCl_3$, 60—70% EtOAc/hexane, and 45% EtOAc/$CHCl_3$) to afford **13** (8 mg). LE6 (1.13 g) was separated to nine fractions (LE6-1—LE6-9) by Sephadex LH-20 column chromatography using 50% MeOH/$CHCl_3$. After removal of the soluble fraction of LE6-5 (184 mg) in CH_2Cl_2–Et_2O, the solid (60 mg) was subjected to flash CC (40% Me_2CO/hexane) to give **10** (28 mg).

A part (357 mg) of LE7 (669 mg) was separated to six fractions (LE7-1—LE7-6) by Sephadex LH-20 column chromatography using 50% MeOH/$CHCl_3$. LE7-3 (255 mg) was purified by CC (2% MeOH/$CHCl_3$) to afford six fractions (LE7-3-1 to LE7-3-6). LE8 (244 mg) was separated to six fractions (LE8-1—LE8-6) by Sephadex LH-20 column chromatography using 50% MeOH/$CHCl_3$. After LE7-3-4 (70 mg) and LE8-4 (187 mg) were combined, the whole was separated by CC (3%, 4%, 10%, 20%, 30%, and 100% MeOH/$CHCl_3$) to afford eight fractions, the third fraction (94 mg) of which was subjected to recycling preparative HPLC followed by double CC (2% MeOH/$CHCl_3$) to afford **11** (15 mg).

Glycosparvarine (**1**): Orange amorphous mass. ^{1}H-NMR (acetone-d_6, 600 MHz) δ: 3.88 (3H, s, 2-OC<u>H</u>$_3$), 4.08 (3H, s, NC<u>H</u>$_3$), 6.50 (1H, s, H-4), 7.14 (1H, dd, J=8.1, 7.7 Hz, H-7), 7.29 (1H, dd, J=7.7, 1.5 Hz, H-6), 7.91 (1H, dd, J=8.1, 1.5 Hz, H-8), 8.95 (1H, s, 3-O<u>H</u>), 9.31 (1H, s, 5-O<u>H</u>), 14.99 (1H, s, 1-O<u>H</u>). ^{13}C-NMR (acetone-d_6, 150 MHz) δ: 41.3 (q, NMe), 60.5 (q, OMe), 91.8 (d, C-4), 105.8 (s, C-9a), 117.6 (d, C-8), 120.6 (d, C-6), 122.8 (d, C-7), 124.1 (s, C-8a), 129.5 (s, C-2), 134.7 (s, C-10a), 144.3 (s, C-4a), 147.8 (s, C-5), 156.4 (s, C-1), 158.4 (s, C-3), 181.6 (s, C-9). IR (ATR) cm^{-1}: 3522, 3181, 1644, 1595, 1557, 1452, 1287, 1223, 1134, 991, 833. UV λ_{max} (MeOH) nm (log ε): 204 (4.36), 232 (4.29), 266 (4.60), 286 (4.49), 321 (sh) (4.11), 409 (3.79). EI-MS *m/z*: 288, 287 [M]$^+$, 272, 244, 130, 58. FAB-MS *m/z*: 288 [M+H]$^+$, 287 [M]$^+$. HR-EI-MS *m/z*: 287.0799 (Calcd for $C_{15}H_{13}NO_5$, 287.0793).

(+)-*S*-Deoxydihydroglyparvin (**10**): Colorless amorphous mass. ^{1}H- and ^{13}C-NMR: see Table 2. IR (ATR) cm^{-1}: 3287, 3051, 2930, 1698, 1648, 1616, 1584, 1548, 1511, 1456, 1379, 1362, 1328, 1240, 1174, 1039, 984, 803. UV λ_{max} (MeOH) nm (log ε): 204 (4.31), 225 (4.29), 261 (4.30). EI-MS *m/z*: 187, 167, 139, 103, 91, 77, 64. FAB-MS *m/z*: 420 [M+H]$^+$, 442 [M+Na]$^+$. HR-FAB-MS *m/z*: 420.1831 [M+H]$^+$ (Calcd for $C_{22}H_{30}NO_5S$, 420.1845). $[\alpha]_D^{21}$ +68.2 (c=0.10, $CHCl_3$).

(+)-*S*-Deoxytetrahydroglyparvin (**11**): Colorless oil. ^{1}H- and ^{13}C-NMR: see Table 2. IR (ATR) cm^{-1}: 3291, 3075, 2975, 2929, 1695, 1662, 1581, 1511, 1457, 1379, 1362, 1300, 1241, 1176, 1035, 933, 806. UV λ_{max} (MeOH) nm (log ε): 205 (4.15), 225 (4.16), 261 (4.16). EI-MS *m/z*: 286, 167, 146, 140, 139, 120, 107, 69. FAB-MS *m/z*: 422 [M+H]$^+$, 444 [M+Na]$^+$, 460 [M+K]$^+$. HR-FAB-MS *m/z*: 422.1985 [M+H]$^+$ (Calcd for $C_{22}H_{32}NO_5S$, 422.2001). $[\alpha]_D^{18}$ +24.1 (c=0.04, $CHCl_3$).

Viruses and Cells HSV strains used were HSV-1 (KOS) and HSV-2 (Baylor186). Vero cells (ATCC CCL81) were grown and maintained in Eagle's minimum medium supplemented with 10% fetal bovine serum.

Plaque Reduction Assay Anti-HSV activity of the compound was determined by plaque reduction assay modified from the reported method.[26] Briefly, in post-treatment assay, Vero cells, in 96-well tissue culture plate, were infected with 30 plaque forming units of HSV-1 (KOS) or HSV-2 (Baylor186). After 1 h incubation at room temperature for virus adsorption, the cells were added with overlay media containing various concentrations of the compound. The infected cultures were incubated at 37 °C for 2 d. The infected cells were fixed and stained, and then the number of plaques was counted. The 50% effective concentration (EC_{50}) was determined from the curve relating the plaque number to the concentration of the compound. Acyclovir was used as a positive control. In inactivation assay, each of 30 plaque forming units of HSV-1 (KOS) or HSV-2 (Baylor 186) was mixed with various concentrations of compound and incubated for 1 h, then the mixture was added to Vero cells in 96-well tissue culture plate. After 1 h incubation for virus adsorption, the overlay media were added. The infected cultures were incubated at 37 °C for 2 d. The infected cells were fixed, stained, and the plaques were counted. The 50% effective concentration (EC_{50}) was determined.

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