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New stilbenoid with inhibitory activity on viral neuraminidases from *Erythrina addisoniae*

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ABSTRACT

Influenza occurs with seasonal variations and reaches the peak prevalence in winter causing the death of many people worldwide. A few inhibitors of viral neuraminidase, including amantadine, rimantadine, zanamivir, and oseltamivir, have been used as influenza therapy. However, as drug-resistant influenza viruses are generated rapidly, there is a need to identify new agents for chemotherapy against influenza. Therefore, research on more effective drugs has been given high priority. During the course of an anti-influenza screening program on natural products, two new compounds (**1** and **2**) along with seven known flavonoid derivatives (**3**–**9**) were isolated as active principles from an EtOAc-soluble extract of the root bark of *Erythrina addisoniae*. The stilbenoid (**2**) and chalcone (**3**, **4**, and **6**) compounds of the isolates exhibited stronger activity than the isoflavone ones. Compound **2**, which is a formylated stilbenoid derivative, exhibited strong inhibition of both influenza H1N1 and H9N2 neuraminidases with IC_{50} values of 8.80 ± 0.34 μg/mL and 7.19 ± 0.40 μg/mL, respectively.

Influenza is caused by a group of enveloped RNA viruses that belong to the *Orthomyxoviridae* family, and is an acute, highly contagious viral respiratory disease occurring seasonally in most areas of the world. Among the three types of influenza viruses: A, B, and C based on the antigenicity of nucleoprotein (NP) and matrix protein (M1) were reported,[1] the influenza A virus is responsible for annual epidemics or pandemic outbreaks. As influenza affects all age groups with the highest hospitalization rates found in children and the elderly, it causes an average of 110,000 hospitalizations and 20,000–50,000 deaths annually in the USA alone.[2–4]

The genome of the influenza A virus encodes two major surface glycoproteins, hemagglutinin (HA), and neuraminidase (NA), whose antigenicity defines 16 distinct HA and nine distinct NA subtypes. HA recognizes sialic acid on the surface of the host cell membranes, and virus particles penetrate the host cell surface by binding these residues. NA (also called sialidase) is a hydrolytic enzyme that catalyzes the cleavage of the terminal sialic acid residues attached to glycoproteins and glycolipids.[5] NA plays an important role in the final stage of an infection when NA cleaves sialic acid from the cell surface and progeny virions facilitate virus release from the infected cells.[6a,b] NA may also promote viral movement through respiratory tract mucus, thereby enhancing the viral infectivity. When the influenza virus is deficient in NA activity, the virus progeny can aggregate at the surface of an infected cell, which severely impairs the further spread of viruses to other cells.[6] Therefore, NA is a potential target for the development of agents against an influenza infection. However, although there are many licensed drugs available for influenza treatment, the occurrence of viral resistance with drug resistance by their high mutation rate suggests that the development of new therapeutic agents should be continued.[5,7]

During the course of our ongoing anti-influenza screening program on natural products, the extract of the root bark of *Erythrina addisoniae* was found to have significant inhibitory activity against influenza neuraminidases. The genus *Erythrina* (Leguminosae) is comprised of approximately 110 species of trees and shrubs that are distributed widely in tropical and subtropical regions with representative species being used in indigenous medicine.[8] Previous studies demonstrated that alkaloids, benzofurans, pterocarpans, and other flavonoids are constituents of this genus, which show PTP1B inhibition and cytotoxic activity.[9] This study investigated the antiviral properties of the plant *E. addisoniae*. The EtOAc-soluble extract of this plant exhibited inhibitory activity significantly on in vitro influenza neuraminidase assays.[10] Nine flavonoid derivatives were purified from the bioassay-directed isolation of the

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EtOAc-soluble extract of the root bark of *E. addisoniae*,[11] including one new isoflavone, 5,4′-dihydroxy-8-methylenehydroxy-[2″,2″-dimethyl-3″,4″-dehydro-pyrano-(1″,4″:-7,6)]isoflavone (**1**),[12] a new formylated stilbenoid derivative, (*Z*)-3-(7-hydroxy-5-methoxyphenyl)-2-(6′-hydroxy-4′-methoxyphenyl)acrylaldehyde (**2**),[12] and seven known compounds, licoagrochalcone A (**3**),[13] abyssinone VI (**4**),[14] erysenegalensein M (**5**),[15] 5′-prenylbutein (**6**),[16] corylin (**7**),[17] isowighteone (**8**), and wighteone (**9**).[18a] The structures of the known compounds were identified by 1D and 2D NMR analyses and confirmed by a comparison of the physicochemical and spectroscopic data with those published in the literature (Fig. 1).

Compound **1** was isolated as a white amorphous powder, and its UV spectrum exhibited the absorption maxima at 271 and 276 nm, respectively.[12] The ^{1}H and ^{13}C NMR spectra (Table 1) displayed the characteristic signals of H-2 at δ_H 8.25 (1H, s) with corresponding (C-2) olefinic oxymethine signals at δ_C 154.5, and a ketone carbon resonance (δ_C 182.2). These observations were indicative of an isoflavone skeleton.[19] The ^{1}H and ^{13}C NMR spectra exhibited a *para* disubstituted C-4′-hydroxy ring B, an oxymethylene at δ_H 4.76 (2H, d, J = 6.0 Hz, H-1‴), δ_C 52.4 (C-1‴), and δ_H 3.73 (1H, t, J = 6.0 Hz, 1‴-OH), and a 2,2-dimethylpyrano ring (Table 1).[18b,c] A single proton signal at δ_H 13.5 (1H, sh, s) in the ^{1}H NMR spectrum indicated a chelated hydroxy group at C-5.[9] The 2,2-dimethylpyrano ring was fused at C-6 and C-7, as indicated by the HMBC experiment (Fig. 2), revealing correlations between H-4″ (δ_H 6.70, d, 10.0 Hz) and C-5 (δ_C 157.2), C-6 (δ_C 106.5), and C-7 (δ_C 158.4). The position of the hydroxymethylene was also assigned using an HMBC experiment, where correlations of the oxymethylene protons (δ_H 4.76) with C-7 (δ_C 158.4), C-8 (δ_C 108.9), and C-9 (δ_C 156.5) suggested that this moiety is located at C-8. This observation was further supported by the molecular formula of $C_{21}H_{18}O_6$, which was assigned to this compound from the molecular ion peak at m/z 366.1103 $[M]^+$ obtained by HREIMS. Therefore, the structure of the new compound **1** was determined to be 5,4′-dihydroxy-8-methylenehydroxy-[2″,2″-dimethyl-3″,4″-dehydro-pyrano-(1″,4″:-7,6)]isoflavone, and named erythraddison A.[12]

Compound **2** was obtained as yellowish brown powder with absorption maxima at 229, 266, 365, and 383 nm in the UV spectrum. The IR spectrum suggested the presence of hydroxy groups at 3418 cm^{-1}, 2924, 2856 (C–C), 1708, 1622 (C=O), 1418, and 1160–1032 ascribable to aromatic ring.[12] The ^{1}H and ^{13}C NMR spectra of compound **2** exhibited characteristic signals for two sets of ABX aromatic spin systems (Table 1), two methoxy groups at δ_H 3.87, δ_C 56.2 (5-OCH$_3$), and δ_H 3.61, δ_C 55.8 (4′-OCH$_3$), and an aldehyde group at δ_H 9.60 (1H, s) and δ_C 194.2. This suggests a chemical structure similar to spinosan B for compound **2**.[20a] However, an additional proton singlet signal at δ_H 7.79 (H-3) was found in the ^{1}H NMR spectrum of compound **2**, which the molecular ion peak at m/z 300.0998 in the HREIMS also indicated a molecular formula of $C_{17}H_{16}O_5$. The correlations with the proton of H-3 to the aldehyde carbon at δ_C 194.2, and the quaternary carbon at δ_C 116.0 (C-1′) suggest that the arylbenzofuran ring in spinosan B was broken down at C-2 to form a stilbenoid derivative in compound

Table 1
^{1}H and ^{13}C NMR data of new compounds **1** and **2** from *Erythrina addisoniae*

No.	**1**[a]		**2**[a]	
	δ_H (J in Hz)	δ_C (ppm)	δ_H (J in Hz)	δ_C (ppm)
1			9.60, s	194.2
2	8.25, s	154.5		136.9
3		124.1	7.79, s	144.1
4		182.2		116.3
5		157.2		161.1
6		106.5	6.49, d, 2.0	99.6
7		158.4		162.2
8		108.9	6.17, dd, 2.0, 8.8	108.3
9		156.5	6.89, d, 8.8	131.7
10		105.9		
1′		123.0		116.0
2′	7.47, d, 8.4	131.3	6.75, d, 8.0	132.1
3′	6.92, d, 8.4	116.1	6.45, dd, 2.0, 8.0	108.4
4′		158.6		159.4
5′	6.92, d, 8.4	116.1	6.54, d, 2.0	100.4
6′	7.47, d, 8.4	131.3		159.8
1″				
2″		79.1		
3″	5.80, d, 10.0	129.5		
4″	6.70, d, 10.0	116.0		
5″	1.50, s	28.4		
6″	1.50, s	28.4		
1‴	4.76, d, 6.0	52.4		
5-OH	13.5, s			
5-OMe			3.87, s	56.2
4′-OH	8.51, s			
4′-OMe			3.61, s	55.8
1‴-OH	3.73, t, 6.0			

[a] Spectra were recorded at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) in acetone-d_6.

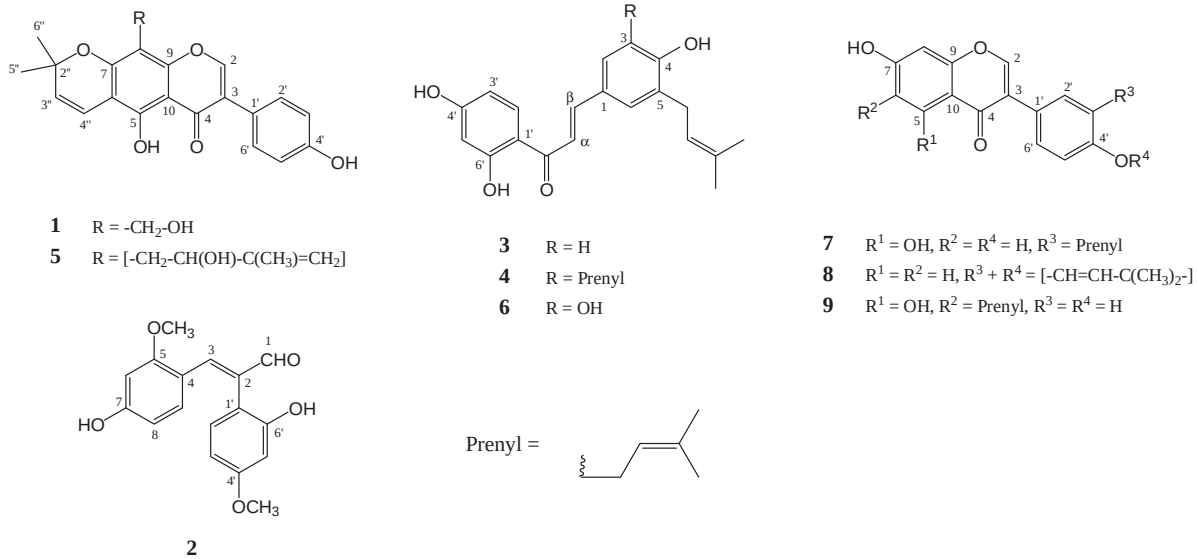
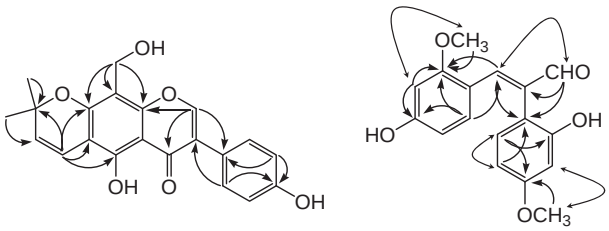


Figure 1. Chemical structures of compounds **1**–**9** isolated from *Erythrina addisoniae*.



1 2

Figure 2. Selected key HMBC (H → C) and NOESY (H ↔ H) correlations for new compounds (**1** and **2**).

2. The NOESY correlations between H-3 (δ_H 7.79, s) and aldehyde proton (δ_H 9.60, s) indicated a *cis* arrangement (*Z*-configuration) which two protons between H-3 and aldehyde are oriented on same side, and other substituents are opposite (Fig. 2 and S.2.15–S.2.18 in Supplementary data).[20b] The placement of the aldehyde moiety at C-2 was also confirmed by the HMBC experiment, showing correlations from the aldehyde proton to C-2 (δ_C 136.9) and C-1′ (δ_C 116.0). The arrangements of the rings (A and B), and the attachments of two methoxy groups to C-5 and C-4′ of ring A and B, respectively, were also supported by the HMBC experiment (Fig. 2) and the NOESY experiment (Fig. 2 and S.2.6–S.2.18 in Supplementary data). Accordingly, the structure of compound **2** was determined to be (*Z*)-3-(7-hydroxy-5-methoxyphenyl)-2-(6′-hydroxy-4′-methoxyphenyl)acrylaldehyde, which is a new natural formylated stilbenoid derivative called erythraddison B.[12] Interestingly, as compound **2** is a stillbenoid derivative with an aldehyde group attached to C-2, it was isolated from the *Erythrina* species for the first time.

All the compounds (**1**–**9**) isolated were tested for their inhibitory activity against both viral neuraminidases from influenza H1N1 and H9N2 using oseltamivir phosphate (Hoffiman-La Roche Ltd, Basel, Switzerland) as a positive control. The NA assay was performed as previously reported with a slight modification[10,21] by following the hydrolysis of 4-methylumbelliferyl-α-D-*N*-acetylneuraminic acid sodium salt hydrate (4-MU-NANA) (Sigma, M8639) via fluorescence. The initial velocity (v_i) was recorded over a range of concentrations and the data was analyzed using a nonlinear regression program [Sigma Plot 11.0 (SPCC Inc., Chicago, IL)]. As shown in Table 2 and Fig. 3, stilbenoid and chalcone compounds of the isolates exhibited dose-dependent inhibitory effects against both influenza neuraminidase activities. However, compound **2**, which is a stilbenoid derivative, had stronger effects against both influenza neuraminidases from H1N1 and H9N2 (IC_{50} values of 8.80 ± 0.34 and 7.19 ± 0.40 μg/mL, respectively) than the chalcone–type compounds (compounds **3**, **4**, and **6** with IC_{50} values ranging from 20.03 ± 0.35 to 26.44 ± 0.42 μg/mL), which in turn exhibited higher activity than the isoflavone ones (compounds **1**, **5**, and **7**–**9** with IC_{50} >50 μg/mL).

The inhibition pattern by compounds **2**–**4** and **6** was determined to evaluate the relative affinity of the compounds for both

Table 2
The inhibitory effects of isolated compounds **1**–**9** from *E. addisoniae* on the influenza neuraminidase activities

Compounds	H1N1		H9N2
	IC_{50}[a] (μg/mL)	Inhibition type (K_i, μg/mL)	IC_{50}[a] (μg/mL)
Erythraddison A (**1**)	>50	NT[b]	>50
Erythraddison B (**2**)	8.80 ± 0.34	Noncompetitive (9.83 ± 0.80)	7.19 ± 0.40
Licoagrochalcone A (**3**)	21.51 ± 0.25	Noncompetitive (22.34 ± 0.70)	20.03 ± 0.35
Abyssinone VI (**4**)	26.44 ± 0.42	Noncompetitive (27.49 ± 1.22)	24.56 ± 0.44
Erysenegalensein (**5**)	>50	NT[b]	>50
5′-Prenylbutein (**6**)	21.93 ± 0.44	Noncompetitive (22.71 ± 0.91)	20.47 ± 0.48
Corylin (**7**)	>50	NT[b]	>50
Isowighteone (**8**)	>50	NT[b]	>50
Wighteone (**9**)	>50	NT[b]	>50
Oseltamivir[c]	39.74 ± 1.54 (ng/mL)	NT[b]	4.94 ± 0.56 (ng/mL)

[a] IC_{50} values were determined by regression analyses and are expressed as the mean ± SD of three replicates.
[b] NT = not tested.
[c] Positive control.

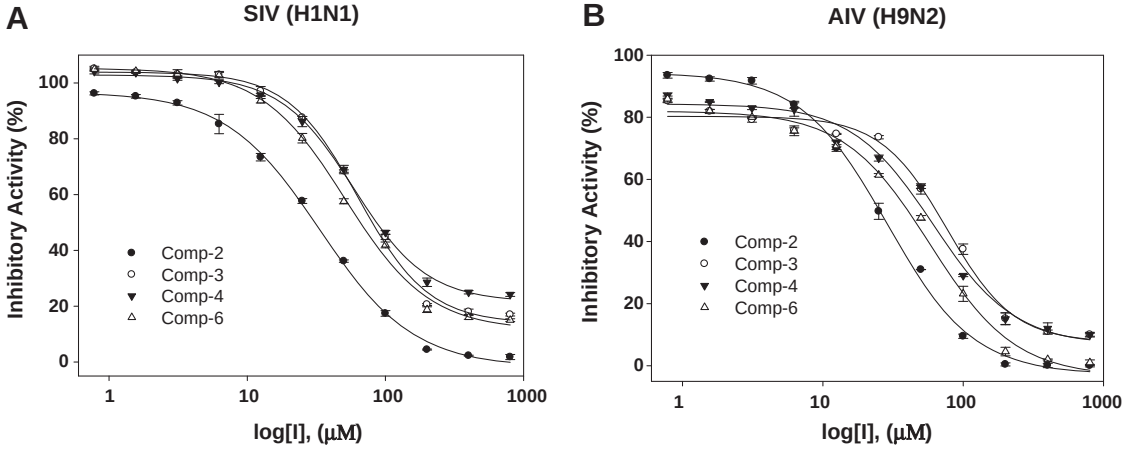


Figure 3. Effects of the isolated compounds (**1**–**9**) on the neuraminidase activities. (A) Concentration-dependent inhibition of neuraminidase (H1N1) by the isolated compounds **2**–**4** and **6**. (B) Concentration-dependent inhibition of neuraminidase (H9N2) by the isolated compounds **2**–**4** and **6**. Concentrations of the inhibitors are displayed on logarithmic scales. The IC_{50} was identified from the midpoint (neuraminidase activity = 50%) of the semilog plot.

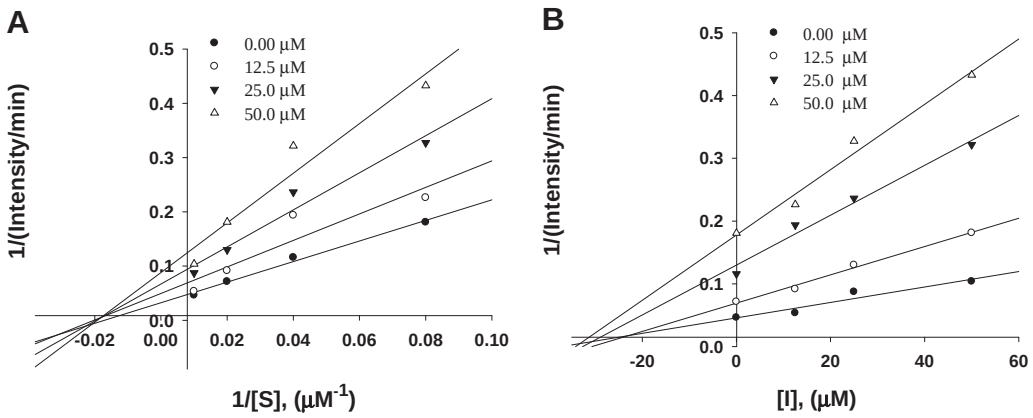


Figure 4. Graphical determination of the inhibition type for compound **2** on influenza neuraminidase H1N1. (A) Lineweaver–Burk plot for the inhibition of compound **2** on neuraminidase-catalyzed hydrolysis of the substrate was determined. The data is expressed as the mean reciprocal of the intensity/min for $n = 3$ replicates at each substrate concentration. (B) Dixon plot for the inhibition of compound **2** on the neuraminidase-catalyzed hydrolysis of the substrate was determined. The K_i value was determined from the negative *x*-axis value at the point of intersection of the four lines.

influenza neuraminidases from H1N1 and H9N2. The inhibition of the tested compounds was reversible because the enzyme activity decreased rapidly with increasing inhibitor concentration. As shown representatively in Fig. S.3B (Supplementary data), increasing the concentration of inhibitor **2** resulted in a decrease in the slope of the line, indicating that this compound is a reversible inhibitor. Therefore, the inhibition type of strong inhibitor **2** was analyzed using Lineweaver–Burk (Fig. 4A) and Dixon plots (Fig. 4B). Both experiments showed that compound **2** exhibited noncompetitive inhibition (for example, all lines in the Lineweaver–Burk plot met at a nonzero point on the *x*-axis). A replot of the slope versus the corresponding 1/[*S*] (inset Fig. 4B) yielded a straight line with a gradient of $K_m/V_{max}K_i$ and a *y*-axis intercept of $1/V_{max}K_i$, demonstrating the concordance of both graphs and affirming that compound **2** is a noncompetitive inhibitor.

Recently, Liu reported that some stilbenoids from the lianas of *Gnetum pendulum* have anti-influenza viral activity with two different assays, neuraminidase (NA) activity assay and cytopathic effect (CPE) reduction assay.[22] Interestingly, our results also suggest that new stilbenoid and chalcone compounds from *E. addisoniae* have an inhibitory effect on the viral neuraminidases from H1N1 and H9N2. A study of the inhibition mode of these compounds revealed stilbenoid and chalcone compounds to inhibit viral NA in a noncompetitive pattern. Although the inhibition potency of its active principles was weak, chalcone and stilbenoids could be supplied in significant quantities from some natural sources. Generally, the combined use of two or more drugs, which have different mechanisms of action, may have several advantages over a single treatment with a NA inhibitor. Therefore, the stilbenoid and chalcones for the treatment of influenza infections can be considered a new lead compound for the development of NA agents.

Supplementary data

Supplementary data (^{1}H-, ^{13}C- and COSY-, HMBC-, and NOESY-NMR spectra of new compounds (**1** and **2**)) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.077.

References and notes

1. Sakudo, A.; Baba, K.; Tsukamoto, M.; Sugimoto, A.; Okada, T.; Kobayashi, T.; Kawashita, N.; Takagi, T.; Ikuta, K. *Bioorg. Med. Chem.* **2009**, *17*, 752.
2. (a) Glezen, W. P. *Epidemiol. Rev.* **1982**, *4*, 24; (b) Centers for Disease Control (CDC). ACIP. Prevention and control of influenza, recommendations of the Advisory Committee on Immunization Practices. *MMWR Morb. Mortal Wkly. Rep.* **2002**, *51*, 1.
3. Manon, M. J. C.; Anderson, D. K. *Influenza* **2007**, *1*, 35.
4. WHO. Available from: <http://www.who.int/mediacentre/factsheets/2003/fs211/en/, 2008>.
5. Forero, J. E.; Avila, L.; Taborda, N.; Tabares, P.; Lopez, A.; Torres, F.; Quinones, W.; Bucio, M. A.; Mora-Perez, W.; Rugeles, M. T.; Joseph-Nathan, P.; Echeverri, F. *Phytochemistry* **2008**, *69*, 2815.
6. (a) Air, G. M.; Laver, W. G. *Proteins* **1989**, *6*, 341; (b) Bucher, D. J.; Palese, P. *The Biologically Active Proteins of Influenza Virus: Neuraminidase*; Academic: New York, 1975; (c) Zhang, J.; Yu, K. Q.; Zhua, W.; Jiang, H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3009.
7. De Clercq, E. *Nat. Rev. Drug. Disc.* **2006**, *5*, 1015.
8. Oliver-Bever, B. *Medicinal Plants in Tropical West Africa*; Cambridge University Press: New York, 1981.
9. (a) Cui, L.; Thuong, P. T.; Fomum, Z. T.; Oh, W. K. *Arch. Pharmacol. Res.* **2009**, *32*, 325; (b) Na, M. K.; Jang, J. P.; Njamen, D.; Mbafor, J. T.; Fomum, Z. T.; Kim, B. Y.; Oh, W. K.; Ahn, J. S. *J. Nat. Prod.* **2006**, *69*, 1572; (c) Dao, T. T.; Nguyen, P. H.; Thuong, P. T.; Kang, K. W.; Na, M. K.; Ndinteh, D. T.; Mbafor, J. T.; Oh, W. K. *Phytochemistry* **2009**, *70*, 2053; (d) Nguyen, P. H.; Le, T. V. T.; Thuong, P. T.; Dao, T. T.; Ndinteh, D. T.; Mbafor, J. T.; Kang, K. W.; Oh, W. K. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6745.
10. *Influenza A (H1N1 and H9N2) neuraminidase inhibition assay*: The enzyme assay was performed as previously reported with a slight modification.[21] The large-scale influenza virus suspension was prepared from MDCK cells infected with the influenza virus H1N1 and H9N2. To inactivate the viral infectivity, the virus suspensions were treated with formaldehyde at a final concentration of 0.01% at 37 °C for 30 min. The NA activity was measured using 4-methylumbelliferyl-α-D-*N*-acetylneuraminic acid sodium salt hydrate (4-MU-NANA) (Sigma, M8639) in an acetate buffer as the substrate. All compounds were dissolved in DMSO and diluted to the corresponding concentrations in MES buffer (32.5 mM 2-(*N*-morpholino)-ethanesulfonic acid, 4 mM $CaCl_2$, pH 6.5). The enzyme inhibitory assay was carried out in 96-well plates containing 10 μL of the diluted virus supernatant (containing active influenza NA) and 10 μL of the isolated compound in the enzyme buffer. The mixture was incubated for 30 min at 37 °C. 30 μL of the 4-MU-NANA substrate per well was then added to the enzyme buffer. The enzymatic reactions were carried out for 2 h at 37 °C and terminated using 150 μL of a stop solution (25% ethanol, 0.1 M glycine, pH 10.7). The fluorescence intensity of the product (4-MU) was measured using a Spectramax M2^e spectrofluorometer with excitation and emission wavelengths of 360 and 440 nm, respectively. The IC_{50} for reducing the NA activity was then determined. For the enzyme kinetic study, 4-methylumbelliferone was quantified immediately without adding the stop solution. The data was analyzed using Sigmaplot 11.0 (SPCC Inc., Chicago, IL).

$$\%\text{Inhibition} = \frac{100}{1 + (IC_{50}/[I])}$$

11. *Bioassay-guided isolation of the isolated compounds* **1**–**9**: The root bark of *E. addisoniae* was collected in Cameroon. The botanical sample was identified and authenticated at the Cameroon National Herbarium (Yaoundé, Cameroon), where a voucher specimen (No. 41617/HNC) was deposited. The dried material (5 kg) was extracted with EtOAc at room temperature for 7 days. Removing the solvent under reduced pressure gave a dry product (155 g). A part of this (25 g) was subjected to silica gel column chromatography (10 × 60 cm; 63–200 μm particle size) using a gradient of *n*-hexane/acetone (from 20:1 to 0:1, each 3 L)

to yield five fractions (EA.1–EA.5) according to their TLC profiles. The inhibitory effects of each fraction on the neuraminidase activities were examined. The effects were concentrated in fractions 1 (EA-1), 2 (EA-2) and 3 (EA-3). Fraction 1 (EA-1, 3.8 g) was chromatographed by RP-C18 column chromatography (6.0 × 60 cm; 75 μm particle size) using a gradient of MeOH/H_2O (from 6:4 to 1:0), to yield compound **3** (54 mg) and five subfractions (EA-1.1–EA-1.5). Among these, subfractions EA-1.2 [eluted with MeOH/H_2O (7:3), 155 mg] exhibited the strongest activity and were purified by semi-preparative HPLC [RS Tech Optima Pak® C_{18} column (10 × 250 mm, 10 μm particle size); mobile phase MeCN/H_2O (65:35) over 40 min; flow rate 2 mL/min; UV detection at 254 nm] to give compounds **1** (5.2 mg; t_R = 21.4 min) and **4** (28.5 mg; t_R = 38.3 min). Fraction 2 (EA-2, 4.3 g) was also chromatographed over a RP-C18 column (6.0 × 60 cm; 150 μm particle size) and eluted with MeOH/H_2O (from 5:5 to 5:0, each 3 L), to yield four subfractions (EA-2.1–EA-2.4) and compound **5** (25 mg). Subfraction EA-2.3 [eluted with MeOH/H_2O (from 6.0:4.0 to 6.5:3.5), 957 mg] was purified by semi-preparative HPLC using an isocratic mixture of 55% MeCN in H_2O + 0.1% formic acid, over 50 min, flow rate 2 mL/min, UV detection at 254 nm afforded 13.4 mg of compound **8** (t_R = 27.2 min) and compound **6** (35.8 mg, t_R = 42.6 min), respectively. Fraction 3 (EA-3, 1.6 g) was subjected to RP-C18 column chromatography (5.0 × 60 cm; 150 μm particle size) using a gradient of MeOH/H_2O (from 4:6 to 4:0), separated into five subfractions (EA-3.1–EA-3.5). Among them, subfraction EA-3.2 exhibited potency to both neuraminidase assays. Purification of this subfraction by preparative HPLC [RS Tech Optima Pak® C_{18} column (10 × 250 mm, 10 μm particle size); mobile phase MeOH/H_2O (45:55) over 45 min; flow rate 2 mL/min; UV detection at 254 nm] resulted in the isolation of compounds **9** (12.1 mg; t_R = 16.4 min), **7** (15.7 mg; t_R = 23.5 min), and **2** (5.5 mg; t_R = 32.5 min).

12. *Physical and spectroscopic data of new compounds*: (a) *Erythraddison A* (**1**): white amorphous powder; IR (KBr): ν_{max} cm^{-1}: 3425, 2973, 2926, 1599, 1450; UV (*c* 0.03, MeOH) λ_{max} nm: 210, 214, 276 nm; ^1H (400 MHz, acetone-d_6) and ^{13}C (100 MHz, acetone-d_6) NMR data, see Table 1; HREIMS *m*/*z* 366.1103 $[M]^+$, (calcd $C_{21}H_{18}O_6$ 366.1105). (b) *Erythraddison B* (**2**): yellowish brown powder; IR (KBr) ν_{max} cm^{-1}: 3418, 2924, 2856, 1708, 1622, 1418, 1379, 1265, 1160, 1032; UV (*c* 0.025, MeOH) λ_{max}: 209, 229, 266, 365, 383 nm; ^1H (400 MHz, acetone-d_6) and ^{13}C (100 MHz, acetone-d_6) NMR data, see Table 1; HREIMS *m*/*z* 300.0998 $[M]^+$ (calcd for $C_{17}H_{16}O_5$, 300.0998).
13. Asada, Y.; Li, W.; Yoshikawa, T. *Phytochemistry* **1998**, *47*, 389.
14. Nkengfack, A. E.; Kouam, J.; Vouffo, T. W.; Meyer, M.; Tempesta, M. S.; Fomum, Z. T. *Phytochemistry* **1994**, *35*, 521.
15. Wandji, J.; Awanchiri, S. S.; Fomum, Z. T.; Tillequin, F.; Libot, F. *Phytochemistry* **1995**, *39*, 677.
16. Yenesew, A.; Induli, M.; Derese, S.; Midiwo, J. O.; Heydenreich, M.; Peter, M. G.; Akala, H.; Wangui, J.; Liyala, P.; Waters, N. C. *Phytochemistry* **2004**, *65*, 3029.
17. Nkengfack, A. E.; Vouffo, T. W.; Fomum, Z. T.; Meyer, M.; Bergenforff, O.; Sterner, O. *Phytochemistry* **1994**, *36*, 1047.
18. (a) Gagnon, H.; Ibrahim, R. K. *Phytochemistry* **1997**, *44*, 1463; (b) Mizuno, M.; Matsuura, N.; Iinuma, M.; Tanaka, T.; Phengklai, C. *Phytochemistry* **1990**, *29*, 2675; (c) Mizuno, M.; Matsuura, N.; Tanaka, T.; Iinuma, M.; Ho, F. C. *Phytochemistry* **1991**, *30*, 3095.
19. (a) Mabry, T. J.; Markham, K. R.; Thomas, M. B. *The Systematic Identification of Flavonoids*; Springer: New York, 1970; (b) Agrawal, P. K. *Carbon-13 NMR of Flavonoids*; Elsevier: New York, 1989.
20. (a) Belofsky, G.; Carreno, R.; Lewis, K.; Ball, A.; Casadei, G.; Tegos, G. P. *J. Nat. Prod.* **2006**, *69*, 261; (b) Siles, R.; Ackley, J. F.; Hadimani, M. B.; Hall, J. J.; Mugabe, B. E.; Guddneppanavar, R.; Monk, K. A.; Chapuis, J. C.; Pettit, G. R.; Chaplin, D. J.; Edvardsen, K.; Trawick, M. L.; Garner, C. M.; Pinney, K. G. *J. Nat. Prod.* **2008**, *71*, 313.
21. Hung, H. C.; Tseng, C. P.; Yang, J. M.; Ju, Y. W.; Tseng, S. N.; Chen, Y. F.; Chao, Y. S.; Hsieh, H. P.; Shih, S. R.; Hsu, J. T. A. *Antiviral Res.* **2009**, *81*, 123.
22. Liu, A. L.; Yang, F.; Zhu, M.; Zhou, D.; Lin, M.; Lee, S. M.; Wang, Y. T.; Du, G. H. *Planta Med.* **2010**, [Epub ahead of print].