JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Semisynthesis and Myocardial Activity of Thaliporphine N-Homologues

Chi-Ming Chiou,[†] Chin-Ting Lin,[†] Wei-Jang Huang,[§] Yu-Mei Chang,[‡] Yi-Jin Ho,[‡] Ming-Jai Su,[‡] and Shoei-Sheng Lee*,[†]

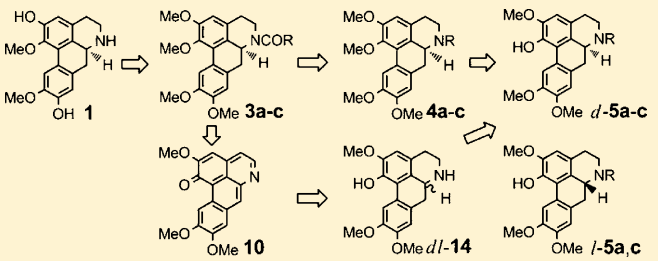
[†]School of Pharmacy, College of Medicine, National Taiwan University, Taipei 10051, Taiwan, Republic of China

[‡]Department of Pharmacology, College of Medicine, National Taiwan University, Taipei 10051, Taiwan, Republic of China

[§]Graduate Institute of Pharmacognosy, Taipei Medical University, Taipei 11031, Taiwan, Republic of China

Supporting Information

ABSTRACT: The N-homologues and optical isomers of thaliporphine (**5a**), a potent antiarrhythmic agent, were prepared starting from laurolitsine (**1**), an abundant aporphine present in *Phoebe formosana*. Treating *N*-propylnorglaucine with 90% H_2SO_4 yielded one additional product, an 11-sulfonyl-1,11-anhydroaporphine. Reaction of *N*-formylnorglaucine (**3a**) with 90% H_2SO_4, however, yielded the 9-sulfonyl-seco product as a major product. Treatment of **3a** with 98% H_2SO_4 yielded pancordine (**10**), which, upon catalytic hydrogenation, yielded (±)-wilsonirine. ^{1}H NMR spectroscopic analysis was applied successfully to monitor the optical purity of the crystalline salt while undertaking optical resolution. Thaliporphine (**5a**) was demonstrated to possess better positive inotropic and less negative chronotropic effects than the left-hand optical isomer and showed the best activity on rat cardiac tissue among the N-homologues prepared.



The aporphine alkaloid thaliporphine (**5a**) has been demonstrated to possess a potent antiarrhythmic effect with positive inotropic and slightly negative chronotropic effects in the rat heart.[1] This compound has been prepared via total synthesis[2] or by partial synthesis from selective 1-*O*-demethylation of glaucine (**4a**), by reacting with dibenzyldiselenide[3] or 90% H_2SO_4 for 13 days.[4] Total synthesis of aporphines usually starts from an appropriate catecholamine and phenylacetic acid, requiring about 10 reaction steps to achieve the products. Semisynthetic work requires the ready availability of glaucine (**4a**). Our group reported a facile preparation of this key intermediate by reacting boldine with phenyl trimethyl ammonium salt under strong alkaline conditions.[5] Norglaucine (**4d**), a key intermediate for the preparation of thaliporphine N-homologues, was also produced by reduction of glaucine *N*-oxide with ferrous sulfate, which gave secoglaucine, a B-ring-opening product.[5] To shorten the reaction time while preparing **5a** from **4a** by reacting with 90% H_2SO_4 and to avoid the formation of secoglaucine while preparing **4d**, which will serve as a direct intermediate for thaliporphine N-homologues (**5a**–**c**), several attempts have been made. The following report describes progress made in the semisynthesis of thaliporphine N-homologues and their optical isomers, starting from laurolitsine (**1**), and the cardiac effects of these compounds in a rat model.

RESULTS AND DISCUSSION

Laurolitsine (**1**), an abundant aporphine present in the Formosan lauraceous plant *Phoebe formosana* (Hayata) Hayata (Lauraceae) (0.8% w/w in the stems),[6,7] was isolated as a crude precipitate. Since *O*,*O*-dimethylation of crude **1** with phenyl trimethyl ammonium chloride under strong alkaline conditions gave several products, and the *N*-acyl products of **1** were crystallized easily, the first step for this approach was to react **1** with appropriate acid anhydrides. This step also served as the purification step for laurolitsine (**1**), and the result suggested the 25% purity of the crude precipitate. Reaction of **1** with ethyl formate in DMF yielded *N*-formyllaurolitsine (**2a**), $[M]^+$ 341, *N*-CHO at δ 8.61 (s) and 8.44 (s). Subsequent *O*-methylation of **2a** with MeI–K_2CO_3 yielded *N*-formylnorglaucine (**3a**) (87%), $[M]^+$ 359. Under such conditions, **1** yielded the corresponding quaternary ammonium salt. Reduction of the amide **3a** with lithium aluminum hydride afforded glaucine (**4a**) (84%) with identical physical data (NMR, $[\alpha]_D$) to those reported.[5] Selective 1-*O*-demethylation by 90% H_2SO_4 yielded the desired thaliporphine (**5a**) (63%),[4] as well as three minor products (Scheme 1). Of these, isoboldine (**6a**) and bracteoline (**7a**) have been reported.[4] The third compound (**8a**), $[M]^+$ and $[M + 2]^+$ at *m*/*z* 389 and 391 with a peak intensity ratio of 100:4, is reported for the first time, and the corresponding *N*-propyl homologue (**8c**) was obtained while using *N*-propylnorglaucine (**4c**) as starting material (Scheme 1). Compound **8c** gave a molecular formula of $C_{21}H_{23}O_6NS$ as

Special Issue: Special Issue in Honor of Lester A. Mitscher

Received: November 6, 2012

dx.doi.org/10.1021/np3007765 | *J. Nat. Prod.* XXXX, XXX, XXX–XXX

Scheme 1. Preparation of (+)-Thaliporphine Homologues (5a–5c) from Laurolitsine (1)

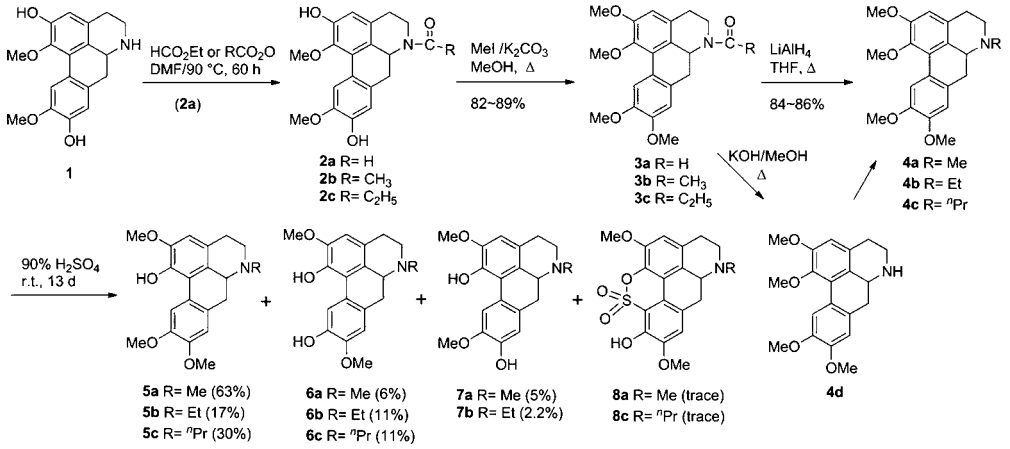


Table 1. ^{1}H and ^{13}C NMR Data and HMBC of Compounds 8c ($CDCl_3$) and 10 (Methanol-d_4)

	8c[a]			10[a]		
no.	δ_C multi.	δ_H multi. (*J*/Hz)	HMBC (C#)	δ_C multi.	δ_H multi. (*J*/Hz)	HMBC (C#)
1	135.8 C			180.2 C		
2	147.72 C			156.3 C		
3	112.6 CH	6.67 s	1, 2, 4, 3b	108.0 CH	6.27 s	1, 2, 3b, 4
3a	132.0 C			135.8 C		
3b	124.4 C			119.5 C		
4	28.9 CH_2	3.08 ddd (16.7, 11.3, 4.9) 2.69 br d (16.7)	5 3, 3a, 3b	121.9 CH	6.96 d (4.3)	3, 3b, 5
5	49.4 CH_2	3.21 br dd (11.5, 5.3) 2.53 dt (3.2, 11.9)	3a, 6a	149.7 CH	8.24 d (4.3)	4, 3a, 6a
6a	58.0 CH	3.55 br d (14.7)	3b	140.4 C		
7	33.1 CH_2	3.16 dd (14.7, 4.9) 2. 07 t (14.7)	6a, 3b, 6a, 7a, 8, 11a,	135.4 CH	7.33 s	3b, 6a, 8, 11a
7a	127.3 C			131.9 C		
8	115.6 CH	7.00 s	7, 9, 10, 11a	107.2 CH	6.30 s	7, 9, 10, 11a
9	147.67 C			150.6 C		
10	142.0 C			156.8 C		
11	116.6 C			104.6 CH	8.03 s	7a, 9, 10, 11b
11a	120.4 C			131.3 C		
11b	118.3 C			118.6 C		
MeO-2	56.4 CH_3	3.88 s	2	56.2 CH_3	3.72 s	2
MeO-9	56.7 CH_3	3.95 s	9	56.1 CH_3	3.71 s	9
MeO-10				55.9 CH_3	3.61 s	10

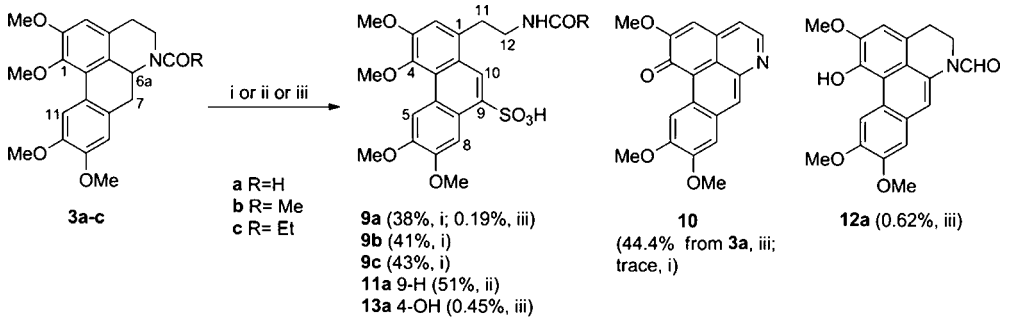
[a] ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data for the *N*-propyl group in **8c**: δ_H 2.89 (1H, ddd, *J* = 12.9, 10.0, 6.1 Hz) and 2.48 (1H, ddd, *J* = 12.5, 10.0, 6.0 Hz) (*N*-C<u>H</u>$_2$-C_2H_5), 1.60 (2H, m, *N*-CH_2-C<u>H</u>$_2$-CH_3), 0.96 (3H, t, *J* = 7.3 Hz, *N*-C_2H_4-C<u>H</u>$_3$); δ_C 56.1 (CH_2), 19.1 (CH_2), 12.0 (CH_3).

deduced by HRESIMS. Its IR spectrum showed strong absorptions at 1365 and 1175 cm^{-1}, characteristic for a sulfonate group,[8] and its ^{1}H NMR spectrum (Figure S1, Supporting Information) revealed two aryl methoxy singlets (δ 3.88 and 3.95) and two aromatic singlets (δ 7.00, H-8; δ 6.67, H-3) but lacked any signal for H-11 (ca. 8.00 ppm). In a NOED experiment, respective irradiation at the methoxy frequency enhanced the H-3 (δ 3.88) and H-8 singlets (δ 3.94), permitting the designation of the MeO-2 and MeO-9 signals. 2D-NMR spectroscopic analysis (HMBC and HETCOR) enabled the complete assignments of the ^{1}H and ^{13}C NMR data of **8c** to be made, as shown in Table 1. Of these, C-1 and C-9–11 were assigned by respective coupling to H-3 (C-1), H-8 [C-9, C-10, and C-11 (very weak)], and MeO-9 (C-9) in the HMBC spectrum (Figure S3, Supporting Information). The ^{13}C NMR spectrum of its *O*-acetylated product ($CDCl_3$) displayed an almost unchanged chemical shift for C-1, downfield shifts for C-9 (δ 151.9 vs 147.7), C-11 (δ 124.9 vs 116.7), and C-7a (δ 134.5 vs 127.3), and an upfield shift for C-10 (δ 134.5 vs 142.0) relative to the corresponding signals in **8c**, suggesting a phenolic functionality at C-10 in **8c**. Therefore, the sulfonate group could be located at C-11, and this formed a cyclic six-membered anhydride with the C-1 phenolic OH by condensation with the structure, as shown in Scheme 1.

Use of *N*-acetyl- (**2b**) and *N*-propionyllaurolitsine (**2c**) as key intermediates, obtained by reacting **1** with the corresponding acid anhydride, and following procedures similar to those for the preparation of **5a**, yielded *N*-ethylnorthaliporphine (*N*-ethylwilsonirine, **5b**) and *N*-propylnorthaliporphine (*N*-propylwilsonirine, **5c**) (Scheme 1), respectively. The ^{1}H NMR spectrum of **5b**, $[M]^+$ at *m*/*z* 355, revealed the signals of an *N*-ethyl group at δ 2.59 (2H, q) and 1.14 (3H, t), while that of **5c**,

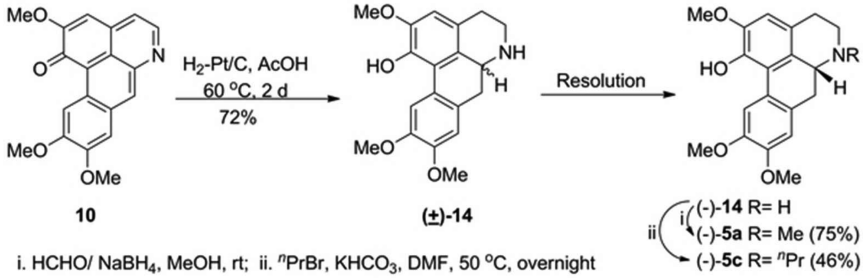
dx.doi.org/10.1021/np3007765 | J. Nat. Prod. XXXX, XXX, XXX–XXX

Scheme 2. Reaction of *N*-Acylaporphines (3a−3c) with 50, 90, or 98% H_2SO_4



i. 90% H_2SO_4, r.t., 4 d; ii. 50% H_2SO_4, 80 °C, 3.5h; iii. 98% H_2SO_4, r.t., 8 d.

Scheme 3. Preparation of (−)-Thaliporphine [(−)-5a] and (−)-*N*-Propylwilsonirine [(−)-5c] from Pancoridine (10)



i. HCHO/ $NaBH_4$, MeOH, rt; ii. ^{n}PrBr, $KHCO_3$, DMF, 50 °C, overnight

$[M]^+$ at *m/z* 369, revealed the signals of an *N*-propyl group at δ 2.60 (2H, m), 1.57 (2H, m), and 1.14 (3H).

Since 90% sulfuric acid could cleave selectively the sterically hindered methyl group at MeO-1 in glaucine (**4a**), whether this reagent possessed a similar propensity to the corresponding *N*-acyl derivatives (**3a−3c**) was investigated. Reaction of *N*-formylnorglaucine (**3a**) with 90% H_2SO_4, however, yielded a secoaporphine, *N*-formyl-9-sulfonylseconorglaucine (**9a**) (38%), and a trace amount of an oxidized product (**10**) instead of the expected *N*-formylwilsonirine (*N*-formylnorthaliporphine) (Scheme 2). Compound **9a** gave a molecular formula of $C_{21}H_{23}O_8NS$ as deduced by HRFABMS. Its ^{1}H NMR spectrum exhibited four aromatic singlets, two methylenes as multiplets, four methoxy singlets, and one singlet for a formyl proton (δ 8.05). This structure was confirmed by two NOED experiments. Irradiation at the H-2 frequency (δ 7.14) enhanced the signals of MeO-3 (δ 3.99, 10.2%), H_2-12 (δ 3.30, 2.7%), and H_2-11 (δ 3.58, 1.2%). Irradiation of the H-10 singlet (δ 8.53) enhanced the latter two signals only, suggesting C-9 to be sulfonated. Compound **9a** was produced by two reaction steps. The first one was similar to the reaction of *N*-acylaporphines under refluxing HCl−MeOH conditions,[7,9] yielding the corresponding secoaporphines, and followed by sulfonation at the less hindered C-9. This was verified since reaction of **3a** with 50% H_2SO_4 yielded *N*-formylseconorglaucine (**11a**, 51%), for which the ^{1}H NMR spectrum showed a characteristic AB system for the H-9 and H-10 in the phenanthrene skeleton (δ 7.77 and 7.55, J_{AB} = 9.1 Hz) similar to that in *N*-formylsecolaurolitsine.[7] Compound **10** gave a molecular formula of $C_{19}H_{15}NO_4$ as deduced from the HREIMS and was identified as pancoridine by comparison of its ^{1}H NMR data with literature values,[10,11] showing four singlets and an AX system (δ 8.24 and 6.96, J_{AX} = 4.3 Hz) for aryl protons and three methoxy singlets (δ 3.72, 3.71, and 3.61) (Figure S4, Supporting Information). This structure was confirmed by analysis of the HMBC spectrum (Table 1; Figure S6, Supporting Information), which showed key correlations of H-3 (δ 6.27) to C-1 (δ 180.2), C-4 (δ 121.9), and C-3b (δ 119.5) and of H-5 (δ 8.24) and H-7 (δ 7.33) to C-6a (δ 140.4) (Table 1). This 2D-NMR analysis also led to the complete ^{1}H and ^{13}C NMR assignments shown in Table 1. Pancoridine (**10**) has been prepared from 6′-aminopapaverine by a two-step reaction, involving diazotization and skeletal rearrangement under warm 65% sulfuric acid,[10] and also has been isolated from plants such as *Popowia pisocarpa* (Bl.) Endl. (Annonaceae).[11] Since compound **10** is an oxidative product, it could be obtained in a better yield using a stronger oxidant such as 98% H_2SO_4. This was found to be the case, and up to a 44.4% yield of **10** was obtained by reacting **3a** with this reagent, accompanied by three minor products, **9a**, **12a**, and **13a** (Scheme 2). The ^{1}H NMR spectrum of **12a** showed one additional aryl proton signal but lacked the upfield-shifted MeO-1 singlet as compared to that of **3a**. Moreover, all the chemical shifts of the parent four aryl protons and three methoxy groups were downfield shifted, especially for H-11 (δ 9.21), accountable for a phenanthrene skeleton.[7] On the basis of these data, a selective *O*-demethylation at MeO-1 and didehydrogenation at C-6a and C-7 occurred while treating **3c** with 98% H_2SO_4. The structure proposed for **12a**, *N*-formyl-6a,7-didehydronorthaliporphine, was supported by NOED experiments upon irradiation at H-11 (δ 9.21), enhancing MeO-10 (δ 4.06, 9.1%), and irradiation at *N*C<u>H</u>O (δ 8.86), enhancing H_2-5 (δ 4.11, 1.5%) and H-7 (δ 7.19, 14.1%). Compound **13a** exhibited a molecular formula of $C_{20}H_{21}O_8NS$, as deduced from the HREIMS, representing a CH_2 unit less than **3a**. Its ^{1}H NMR spectrum was very similar to that of **3a** except for the absence of the MeO-4 signal (δ 3.84, **3a**). Therefore, **13a** was elucidated as 4-*O*-demethylated **9a**, i.e., *N*-formyl-9-sulfonylsecowilsonirine.

dx.doi.org/10.1021/np3007765 | J. Nat. Prod. XXXX, XXX, XXX−XXX

It has been reported that racemic wilsonirine (northaliporphine) [(±)-**14**] can be obtained by reduction of pancoridine (**10**) with Zn−Hg/HCl in 52% yield.[10] It was found that this product can be produced in a better yield (72%) by catalytic hydrogenation under the following conditions: 5 bar H_2, Pt−C, AcOH, 60 °C, 2 days (Scheme 3). Optical resolution of the racemate by (−)-di-*O*-toluoyltartaric acid and (+)-di-*O*-toluoyltartaric acid was accomplished to yield (−)-6a*R*- [(−)-**14**] and (+)-6a*S*-wilsonirine [(+)-**14**], respectively. Conventionally, the optical purity of the resolved compound was judged by the specific optical rotation of the crystalline acid−amine salt, obtained by fractional recrystallization. In this study, a convenient method for this purpose was found by examining the ^{1}H NMR spectrum of the crystalline salts (Figure 1; Figure S7, Supporting Information). While

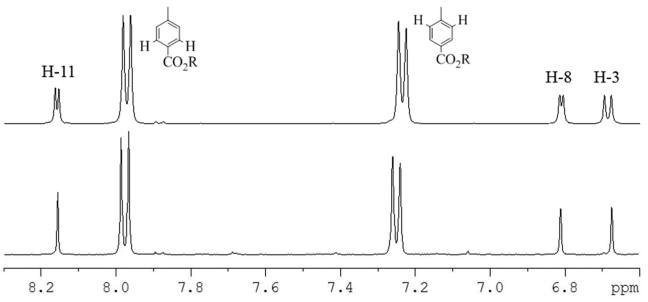


Figure 1. ^{1}H NMR spectra of the (−)-di-*O*-4-toluoyl-L-tartrates of (±)-**14** (top) and (−)-**14** in the aromatic region (methanol-d_4, 400 MHz).

adding the resolving agent, the ^{1}H NMR spectrum of the racemate **14** was found to split into two sets. Tracing the ^{1}H NMR spectra of the crystalline amine acid salts revealed that only one spectrum was observed for the product with constant specific optical rotation. This method is facile and is useful for tracing the optical purity of the resolved compounds.

The left-handed optical isomers of thaliporphine and *N*-propylwilsonirine [(−)-**5a** and (−)-**5c**] were prepared from (−)-wilsonirine [(−)-**14**] by reductive *N*-methylation and *N*-alkylation, respectively (Scheme 3).

The effects on the contractility and heart rate of rat cardiac tissues were evaluated for the thaliporphine homologues prepared and the corresponding optical isomers. The results shown in Table 2 indicate that all of the prepared thaliporphine homologues including both optical isomers (**5a**−**5c**, **14**) showed a negative chronotropic effect on the spontaneously beating rat right atrium at the concentration range of 10−30 μM. In right atria and electrically driven left atria and right ventricular strips, only thaliporphine [(+)-**5a**] showed a significant positive inotropic effect at a concentration between 3 and 30 μM. Since the positive inotropic effect of (+)-**5a** in the electrically driven left atrium and right ventricle was affected neither by prazosin (1 μM) nor by atenolol (3 μM), but partially by verapamil (1 μM) (unpublished observation), this effect caused by (+)-**5a** might be mediated by its partial calcium channel agonistic activity.[1] In contrast with (+)-**5a**, (−)-**5a** and (+)-*N*-propylwilsonirine [(+)-**5c**] showed negative inotropic effects in electrically driven right ventricular strips, which were similar to those of isoquinolines and bisbenzylisoquinolines.[14,15] The latter two types of compounds have been reported to exert a negative inotropic effect by inhibition of an L-type calcium inward current[14] or Bay K8644-stimulated ^{45}Ca uptake, probably via interaction with the diltiazem-binding sites.[15] Other compounds [(+)-**14**, (−)-**14**, (+)-**5b**, and (−)-**5c**] showed a significant negative chronotropic effect with an insignificant inotropic effect on right ventricular strips. In conclusion, the common observation of the negative chronotropic effects of most compounds shown in Table 2 may be mediated by their prolongation of action potential duration via inhibition of the potassium outward current. The differences in inotropic effects among these compounds may be due to their difference in activation or inhibition of L-type calcium currents. These speculations, however, need to be clarified by electrophysiological studies.

This study provides a practical method to prepare glaucine (**4**) and establishes a method to prepare thaliporphine N-homologues, both starting from laurolitsine (**1**). A convenient method for examining the optical purity of the optically resolved isomers by ^{1}H NMR analysis is also disclosed. A preliminary investigation on the structure−activity relationships of the optical isomers of thaliporphine homologues revealed

Table 2. Effects of Thaliporphine N-Homologues on Contractile Tension and Heart Rate of Rat Cardiac Tissues[a]

assay	μM	(+)-**14**	(−)-**14**	(+)-**5a**	(−)-**5a**	(+)-**5b**	(+)-**5c**	(−)-**5c**
% heart rate (RA)	3	91 ± 6	100 ± 0	92 ± 8	92 ± 5	97 ± 3	91 ± 6	88 ± 4[c]
	10	78 ± 8[c]	96 ± 4	86 ± 5[c]	85 ± 7[c]	97 ± 3	80 ± 7[c]	79 ± 6[c]
	30	61 ± 15[c]	79 ± 6[c]	82 ± 7[c]	69 ± 10[c]	84 ± 4[c]	61 ± 8[c]	68 ± 7[c]
% tension								
1. RA	3	99 ± 3[d]	101 ± 1	104 ± 4	106 ± 17	93 ± 3	103 ± 6	121 ± 4[c]
	10	99 ± 3	103 ± 6	140 ± 12[c]	121 ± 32	127 ± 17	103 ± 6	131 ± 15[b]
	30	117 ± 13	105 ± 12	180 ± 12[d]	129 ± 41	162 ± 24[c]	103 ± 8	141 ± 24[b]
2. LA	3	94 ± 6[d]	103 ± 8	110 ± 7	106 ± 4	105 ± 6	99 ± 1	101 ± 1
	10	93 ± 8[b]	104 ± 9	180 ± 12[d]	103 ± 10	122 ± 9[b]	90 ± 4	105 ± 5
	30	89 ± 9	105 ± 5	243 ± 24[d]	100 ± 13	131 ± 10[c]	74 ± 7[c]	99 ± 1
3. RV	3	102 ± 4	108 ± 7	125 ± 6[c]	93 ± 4	100 ± 0	95 ± 1	100 ± 0
	10	96 ± 9	111 ± 10	154 ± 17[c]	80 ± 10	108 ± 8	85 ± 3[b]	100 ± 2
	30	90 ± 7	120 ± 10	163 ± 23[c]	72 ± 12[b]	111 ± 16	75 ± 2[d]	96 ± 9

[a]Contractions of left atrial (LA) and right ventricular (RV) strips were elicited by electrical stimulation driven at 2 Hz. Spontaneous contraction of the right atrium (RA) and heart rate were measured. Data are expressed as % of control values before chemical treatment. Mean values (control group: heart rate 242 ± 7 beats/min, twitch tension RA 121 ± 10, LA 140 ± 11, and RV 225 ± 17 mg) were obtained from four to five experiments. [b]$p < 0.05$. [c]$p < 0.01$. [d]$p < 0.001$, compared with the respective control by the Student's *t*-test.

dx.doi.org/10.1021/np3007765 | J. Nat. Prod. XXXX, XXX, XXX−XXX

thaliporphine (**5a**) to be the best cardiotonic agent, with a potent positive inotropic but a slightly negative chronotropic effect on rat cardiac tissues. These findings could be of value for the selection of lead compounds of the type studied herein for the potential development of cardiovascular drugs.

EXPERIMENTAL SECTION

General Experimental Procedures. The physical data of the prepared compounds were obtained using the following instruments: Fisher-Johns melting point apparatus (uncorrected); JASCO DIP-370 polarimeter; Perkin-Elmer 1760-X IR FT spectrometer (KBr); Hitachi 150-20 double-beam spectrophotometer (MeOH); Bruker AMX400 and AVIII600 NMR spectrometers using solvent peak as reference standard (methanol-d_4, δ_H 3.30 and δ_C 49.0; $CDCl_3$, δ_H 7.24 and δ_C 77.0) and standard pulse programs for 1D and 2D NMR measurements; JEOL JMX-HX110 (HREIMS), Finnigan TSQ-700 (EIMS), JEOL JMX-HX D100 (FABMS), and Bruker micrOTOF orthogonal ESI-TOF (HRESIMS) mass spectrometer.

*Preparation of N-Formyl- (**2a**), N-Acetyl- (**2b**), and N-Propionyllaurolitsine (**2c**).* Crude laurolitsine (**1**)[6] (50 g), DMF (112 mL), and ethyl formate (117 mL) were placed in a screw-tight sealed tube (500 mL). After degassing under a vacuum, the mixture was stirred at 90 °C for 60 h and was evaporated to give a residue. The residue was recrystallized from MeOH to afford **2a** (15.6 g). In a similar manner, reaction of crude **1** (10.0 g) with Ac_2O (2 mL) in DMF (25 mL) afforded **2b** (3.60 g), while reaction of crude **1** (30.0 g) with propionic anhydride (6.0 mL) in DMF (75 mL) yielded **2c** (8.02 g).

*N-Formyllaurolitsine (**2a**):* ^{1}H NMR (MeOH-d_4, 400 MHz) δ 8.36/8.22 (1:3) (1H, s, *N*CHO), 8.06/8.05 (3:1) (1H, s, H-11), 6.76/6.72 (1:3) (1H, s, H-8), 6.62/6/61 (1H, s, H-3), 4.72 (dd, J = 13.8, 4.2 Hz)/4.50 (dd, J = 14.4, 4.3 Hz) (3:1) (1H, H-6a),3.89 (3H, s, MeO-10), 3.59 (3H, s, MeO-1); EIMS (70 eV) m/z 341 (90, $[M]^+$), 296 (40), 283 (100), 240 (30), 58 (70).

*N-Acetyllaurolitsine (**2b**):* ^{1}H NMR (MeOH-d_4, 400 MHz) δ 8.06 (1H, s, H-11), 6.77/6.70 (1:2) (1H, s, H-8), 6.61 (1H, s, H-3), 3.89 (3H, s, MeO-10), 3.58 (3H, s, MeO-1), 2.21/2.19 (2:1) (3H, s, $NCOCH_3$); EIMS (70 eV) m/z 355 (88, $[M]^+$), 296 (74), 283 (100), 269 (42), 240 (18).

*N-Propionyllaurolitsine (**2c**):* ^{1}H NMR (MeOH-d_4, 400 MHz) δ 8.06 (1H, s, H-11), 6.71 (1H, s, H-8), 6.61 (1H, s, H-3), 3.88 (3H, s, MeO-10), 3.59 (3H, s, MeO-1), 2.52 (2H, q, $NCOCH_2CH_3$), 1.16 (3H, t, $NCOCH_2CH_3$); EIMS (70 eV) m/z 369 (100, $[M]^+$), 296 (87), 283 (85), 269 (44), 240 (16), 57 (34).

Preparation of *N*-Formyl- (3a), *N*-Acetyl- (3b), and *N*-Propionylnorglaucine (3c). *N*-Formylnorlaurolitsine (**2a**) (10.3 g, 29.4 mmol), K_2CO_3 (12.3 g), MeI (13 mL, 209 mmol), and MeOH (100 mL) were placed in a screw-tight sealed flask. After degassing under a vacuum, the mixture was stirred under reflux (70 °C) for 24 h. The reaction mixture was evaporated in vacuo, and the residue was partitioned between $CHCl_3$ (300 mL) and H_2O (pH 8.0, 150 mL × 2). The $CHCl_3$ layer was dried (Na_2SO_4) and evaporated to a residue, which was recrystallized from MeOH to afford **3a** (9.5 g, 87.0%). In a similar manner, reaction of **2b** (3.0 g, 7.83 mmol) with MeI (3.5 mL, 56.2 mmol)−K_2CO_3 (3.3 g) in MeOH (30 mL) gave **3b** (2.76 g, 89.0%), while reaction of **2c** (2.50 g, 6.78 mmol) with MeI (3.5 mL, 56.2 mmol)−K_2CO_3 (3.3 g) in MeOH (30 mL) yielded **3c** (2.21 g, 82.4%).

*N-Formylnorglaucine (**3a**):* ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.37/8.23 (1.0:2.2) (1H, s, *N*-CHO), 8.12/8.11 (1H, s, H-11), 6.78/6.74 (2.2:1.0) (1H, s, H-8), 6.63/6.60 (1.0:2.2) (1H, s, H-3), 4.89 (dd, J = 13.9, 4.2 Hz) and 4.46 (dd, J = 14.4, 4.3 Hz) (2.2:1.0) (1H, H-6a), 3.90 (9H, s, MeO-2, 9 and 10), 3.65 (3H, s, MeO-1); EIMS (70 eV) m/z 369 (5, $[M]^+$), 355 (100), 340 (40).

*N-Acetyllnorglaucine (**3b**):* ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.15/8.12 (1.0:1.6) (1H, s, H-11), 6.76/6.74 (1H, s, H-8), 6.64/6.60 (1.0:1.6) (1H, s, H-3), 3.89 (3H, s, MeO-9), 3.87 (6H, s, MeO-2 and 10), 3.64 (3H, s, MeO-1), 2.20/2.16 (1.6:1.0) (3H, s, $NCOCH_3$); EIMS (70 eV) 383 (77, $[M]^+$), 324 (44), 311 (100), 297 (12), 265 (20).

*N-Propionylnorglaucine (**3c**):* ^{1}H NMR (MeOH-d_4, 400 MHz) δ 8.13 (1H, s, H-11), 6.76 (1H, s, H-8), 6.60 (1H, s, H-3), 3.89 (3H, s, MeO-9), 3.87 (6H, s, MeO-2 and 10), 3.59 (3H, s, MeO-1), 2.45 (2H, q, J = 7.2 Hz, $NCOCH_2CH_3$), 1.16 (3H, t, J = 7.2 Hz, $NCOCH_2CH_3$); EIMS (70 eV) m/z 397 (79, $[M]^+$), 324 (58), 311 (100), 265 (16), 57 (20).

Preparation of (+)-Glaucine (4a), (+)-*N*-Ethyl- (4b), and (+)-*N*-Propylnorglaucine (4c). To a suspension of $LiAlH_4$ (1.80 g, 47.4 mmol) in dry THF (20 mL) was added a solution of **3a** (8.0 g, 21.7 mmol) in dry THF (10 mL) dropwise at room temperature. The mixture was refluxed for 2 h under nitrogen. After quenching the excess $LiAlH_4$ with hydrated Na_2SO_4, the suspension was filtered with the aid of a Celite cake and the precipitate was washed with $CHCl_3$ (100 mL × 2). The filtrate and $CHCl_3$ washings were dried (Na_2SO_4) and evaporated to give a brown residue, which yielded **4a** (7.30 g, 84.0%) after recrystallization from diethyl ether. In a similar manner, reduction of **3b** (2.67 g, 6.97 mmol) by $LiAlH_4$ (380 mg, 10 mmol) in dry THF (30 mL in total) gave **4b** (2.22 g, 86.0% yield), while reduction of **3c** (4.01 g, 10.1 mmol) by $LiAlH_4$ (380 mg, 10 mmol) in dry THF (30 mL in total) gave **4c** (3.36 g, 86.0%).

*(+)-Glaucine (**4a**):* mp 112−114 °C (Et_2O); $[\alpha]^{24}_D$ +120 (c 0.30, MeOH); ^{1}H NMR ($CDCl_3$) δ 8.06 (1H, s, H-11), 6.75 (1H, s, H-8), 6.56 (1H, s, H-3), 3.91 (3H, s, MeO-9), 3.88 (3H, s, MeO-10), 3.86 (3H, s, MeO-2), 3.64 (3H, s, MeO-1), 2.53 (3H, s, NCH_3).

*(+)-N-Ethylnorglaucine (**4b**):* mp 94−96 °C (Et_2O); $[\alpha]^{24}_D$ +97 (c 0.31, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.05 (1H, s, H-11), 6.75 (1H, s, H-8), 6.56 (1H, s, H-3), 4.03 (3H, s, MeO-9), 3.90 (3H, s, MeO-2), 3.88 (3H, s, MeO-10), 3.59 (3H, s, MeO-1), 2.45 (2H, q, J = 7.2 Hz, NCH_2CH_3), 1.16 (3H, t, J = 7.2 Hz, NCH_2CH_3); EIMS (70 eV) m/z 369 (100, $[M]^+$), 354 (78), 338 (37), 312 (14), 281 (20).

*(+)-N-Propylnorglaucine (**4c**):* mp 95−97 °C (Et_2O); $[\alpha]^{24}_D$ +107 (c 0.33, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.03 (1H, s, H-11), 6.75 (1H, s, H-8), 6.54 (1H, s, H-3), 3.86 (3H, s, MeO-9), 3.83 (6H, s, MeO-2 and 10), 3.60 (3H, s, MeO-1), 2.88 (1H) and 2.43 (1H) (m, $NCH_2C_2H_5$), 1.50 (2H, m, $NCH_2CH_2CH_3$), 0.93 (3H, t, J = 7.2 Hz, $NC_2H_4CH_3$); EIMS (70 eV) m/z 383 (100, $[M]^+$), 368 (82), 354 (45), 352 (36), 281 (19).

Preparation of (+)-Norglaucine (4d). *N*-Formylnorglaucine (**3a**, 2.00 g, 5.42 mmol), potassium hydroxide (2.30 g, 41.5 mmol), and ethanol (50 mL) were placed in a 100 mL reaction bottle. The mixture was heated under reflux for 3 h. On cooling, the reaction mixture was concentrated under reduced pressure to give a residue that was partitioned between water (100 mL) and chloroform (300 mL × 3). The combined organic phase was dried (Na_2SO_4) and concentrated under reduced pressure to give a solid residue, which was purified by silica gel column chromatography, eluted with 0.2% MeOH−$CHCl_3$, to give **4d** (1.75 g, 95%): $[\alpha]^{24}_D$ +77.1 (c 0.35, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.09 (1H, s, H-11), 6.73 (1H, s, H-8), 6.58 (1H, s, H-3), 3.90 (3H, s, MeO-9), 3.88 (3H, s, MeO-10), 3.86 (3H, s, MeO-2), 3.65 (3H, s, MeO-1), 3.81 (1H, dd, J = 13.9, 4.2 Hz, H-6a); (+)-HRESIMS m/z 342.1682 $[M + H]^+$, calcd for $C_{20}H_{24}NO_4$, 342.1705.

Reaction of Glaucine (4a) with 90% Sulfuric Acid. Compound **4a** (3.0 g, 8.45 mmol) and 90% H_2SO_4(aq) (6.0 mL) were placed in a screw-tight sealed flask (100 mL).[4] The mixture after degassing under a vacuum was stirred for 13 days at room temperature. The reaction mixture was added dropwise into a beaker containing ice−water (100 mL), and the resultant suspension was adjusted to pH 8.0 with ammonia−water (25%) at 0 °C and partitioned against $CHCl_3$ (50 mL × 3). The combined $CHCl_3$ layers were dried (Na_2SO_4) and evaporated to give a brown residue, which was chromatographed over a silica gel column (160 g, 230−400 mesh), eluted with 1−3% MeOH in $CHCl_3$, to give **5a** (1.82 g, 63.0% yield), **6a** (148 mg, 5%), **7a** (167 mg, 6%), and **8a** (5 mg).

*11-Sulfonyl-1,11-anhydrobracteoline (**8a**):* mp 174−178 °C (MeOH); IR ν_{max} 3450 (s), 2750 (s), 1725 (m), 1600 (m), 1580 (s), 1500 (s), 1365 (s), 1280 (s), 1240 (s), 1165 (s), 1122 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.99 (1H, s, H-8), 6.67 (1H, s, H-3),

dx.doi.org/10.1021/np3007765 | J. Nat. Prod. XXXX, XXX, XXX−XXX

3.94 (3H, s, MeO-9), 3.88 (3H, s, MeO-2), 2.52 (3H, s, NCH_3); EIMS (20 eV) m/z 391 (4, $[M + 2]^+$), 389 (46, $[M]^+$), 388 (100), 346 (22), 267 (16).

Preparation of (+)-*N*-Ethyl- (5b) and (+)-*N*-Propylwilsonirine (5c). In a similar manner for the preparation of **5a** from **4a**, reaction of **4b** (1.50 g, 4.07 mmol) with 90% H_2SO_4(aq) (3.0 mL) yielded **5b** (247 mg, 17.0%), **6b** (152 mg, 11%), and **7b** (30 mg, 2.2%), while reaction of **4c** (1.20 g, 3.13 mmol) with 90% H_2SO_4(aq) (3.0 mL) gave (+)-**5c** (346 mg, 30.0%), **6c** (122 mg, 11.0%), and **8c** (10 mg).

*(+)-N-Ethylwilsonirine (**5b**):* mp 94−96 °C (MeOH); $[\alpha]^{24}_D$ +58.5 (c 0.32, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.02 (1H, s, H-11), 6.76 (1H, s, H-8), 6.52 (1H, s, H-3), 3.90 (6H, s, MeO-2 and 9), 3.88 (3H, s, MeO-10), 2.59 (2H, q, J = 7.2 Hz, NCH_2CH_3), 1.14 (3H, t, J = 7.2 Hz, NCH_2CH_3); EIMS (20 eV) m/z 355 (100, $[M]^+$), 354 (78), 340 (42), 298 (30), 267 (25).

*(+)-N-Propylwilsonirine (**5c**):* mp 66−70 °C (MeOH); $[\alpha]^{24}_D$ +60.2 (c 0.33, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00 (1H, s, H-11), 6.75 (1H, s, H-8), 6.51 (1H, s, H-3), 3.90 (6H, s, MeO-2 and 9), 3.89 (3H, s, MeO-10), 2.60 (1H, m) and 2.43 (1H, m) ($NCH_2C_2H_5$), 1.57 (2H, m, $NCH_2CH_2CH_3$), 0.95 (3H, t, J = 7.2 Hz, $NC_2H_4CH_3$); EIMS (20 eV) m/z 369 (100, $[M]^+$), 354 (22), 340 (36), 298 (20), 267 (18).

*N-Ethylnorbracteoline (**6b**):* ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00 (1H, s, H-11), 6.74 (1H, s, H-8), 6.52 (1H, s, H-3), 3.90 (3H, s, MeO-9), 3.87 (3H, s, MeO-2), 2.59 (2H, q, J = 7.2 Hz, NCH_2CH_3), 1.15 (3H, t, J = 7.2 Hz, NCH_2CH_3); EIMS (20 eV) m/z 341 (100, $[M]^+$), 326 (18), 284 (15), 253 (4).

*N-Propylnorbracteoline (**6c**):* ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00 (1H, s, H-11), 6.75 (1H, s, H-8), 6.51 (1H, s, H-3), 3.90 (3H, s, MeO-10), 3.88 (3H, s, MeO-2), 2.83 (1H, m) and 2.47 (1H, m) ($NCH_2C_2H_5$), 1.56 (2H, m, $NCH_2CH_2CH_3$), 0.95 (3H, t, J = 7.2 Hz, $NC_2H_4CH_3$); EIMS (20 eV) m/z 355 (100, $[M]^+$), 340 (24), 326 (26), 284 (22), 265 (8), 253 (8).

*N-Ethylnorisoboldine (**7b**):* ^{1}H NMR (MeOH-d_4, 400 MHz) δ 7.99 (1H, s, H-11), 6.79 (1H, s, H-8), 6.52 (1H, s, H-3), 3.90 (3H, s, MeO-10), 3.88 (3H, s, MeO-2), 2.59 (2H, q, J = 7.2 Hz, NCH_2CH_3), 1.12 (3H, t, J = 7.2 Hz, NCH_2CH_3); EIMS (20 eV) m/z 341 (100, $[M]^+$), 326 (25), 284 (26), 253 (16).

*11-Sulfonyl-1,11-anhydrobracteoline (**8a**):* mp 174−178 °C (MeOH); IR ν_{max} 3450 (br s), 2960 (s), 2750 (s), 1580 (s), 1500 (s), 1365 (s), 1280 (s), 1240 (s), 1165 (s), 1122 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.99 (1H, s, H-8), 6.67 (1H,, H-3), 3.94 (3H, s, MeO-9), 3.88 (3H, s, MeO-2), 2.52 (3H, s, NCH_3); EIMS (20 eV) m/z 391 (4, $[M + 2]^+$), 390 (14), 389 (46, $[M]^+$), 388 (100), 346 (22).

*11-Sulfonyl-1,11-anhydro-N-propylnorbracteoline (**8c**):* mp 190−193 °C (MeOH); IR ν_{max} 3400 (br s), 2960 (s), 2940 (s), 1580 (s), 1505 (s), 1365 (s), 1270 (s), 1240 (s), 1175 (s), 1115 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.02 (1H, s, H-8), 6.67 (1H, s, H-3), 3.94 (3H, s, MeO-9), 3.88 (3H, s, MeO-2), 2.87 (1H, m) and 2.47 (1H, m) ($NCH_2C_2H_5$), 1.59 (2H, m, $NCH_2CH_2CH_3$), 0.95 (3H, t, J = 7.2 Hz, $NC_2H_4CH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 147.7 (s, C-2 and C-9), 142.0 (s, C-10), 135.8 (s, C-1), 127.1 (s, C-1a), 124.6 (s, C-3a), 118.3 (s, C-7a), 116.7 (s, C-1b), 115.6 (d, C-8), 112.5 (d, C-3), 58.0 (d, C-6a), 56.6 (q, MeO-9), 56.4 (q, MeO-2), 49.4 (t, C-5), 33.2 (t, C-7), 29.0 (t, C-4), 56.1 (t, $NCH_2C_2H_5$), 19.2 (t, $NCH_2CH_2CH_3$), 12.0 (q, $NC_2H_4CH_3$); EIMS (20 eV) m/z 419 (2, $[M + 2]^+$), 417 (50, $[M]^+$), 416 (100), 389 (18), 388 (85), 359 (35); HREIMS m/z 417.1207 $[M]^+$, calcd for $C_{21}H_{23}O_6NS$, 417.1246.

Reaction of *N*-Acylnorglaucines with 90% H_2SO_4. Compound **3a** (100 mg, 0.271 mmol) and 90% H_2SO_4(aq) (2.0 mL) were placed in a screw-tight sealed flask (25 mL). The mixture after degassing by vacuum was stirred for 4 days at room temperature. The reaction mixture was added dropwise into a beaker containing ice−water (30 mL), and the resultant suspension was adjusted to pH 8.0 with ammonia−water (25%) at 0 °C, passed through an Amberlite XAD-2 column (20 g), and eluted in sequence with H_2O (100 mL) and MeOH (100 mL) to give **9a** (47.1 mg, 37.5%) and a trace amount of **10** (1 mg) from the MeOH elution. In a similar manner, **9b** (50.4 mg, 41.2%) and **9c** (51.1 mg, 43%) were afforded from **3b** (101 mg, 0.264 mmol) and **3c** (100 mg, 0.252 mmol), respectively, by reacting with 90% H_2SO_4(aq) (each 2.0 mL).

*N-Formyl-9-sulfonylseconorglaucine (**9a**):* mp 205−208 °C (MeOH); IR ν_{max} 3410, 3210, 1665, 1530, 1470, 1400, 1250, 1125, 1050 cm^{-1}; ^{1}H NMR (MeOH-d_4, 400 MHz) δ 9.26 (1H, s, H-5), 8.53 (1H, s, H-10), 8.45 (1H, s, H-8), 8.05 (1H, s, NCHO), 7.14 (1H, s, H-2), 4.04 (3H, s, MeO-7), 3.99 (6H, s, MeO-6 and 3), 3.84 (3H, s, MeO-4), 3.58 (2H, m, H-11), 3.30 (2H, m, H-12); FABMS m/z 472 (33, $[M + Na]^+$), 451 (38, $[M + 2]^+$), 450 (100, $[M + H]^+$), 449 (95, $[M]^+$).

*N-Acetyl-9-sulfonylseconorglaucine (**9b**):* mp 216−220 °C (MeOH); IR ν_{max} 3450, 3200, 1630, 1525, 1470, 1400, 1270, 1120, 1050 cm^{-1}; ^{1}H NMR (MeOH-d_4, 400 MHz) δ 9.30 (1H, s, H-5), 8.54 (1H, s, H-10), 8.42 (1H, s, H-8), 7.31 (1H, s, H-2), 4.04 (3H, s, MeO-7), 3.99 (6H, s, MeO-6 and 3), 3.86 (3H, s, MeO-4), 3.51 (2H, m, H-11), 3.30 (2H, m, H-12), 1.91 (3H, s, $NCOCH_3$); FABMS m/z 486 (38, $[M + Na]^+$), 465 (38, $[M + 2]^+$), 464 (100, $[M + H]^+$), 463 (95, $[M]^+$).

*N-Propioyl-9-sulfonylseconorglaucine (**9c**):* mp 236−240 °C (MeOH); IR ν_{max} 3450, 3200, 1610, 1520, 1470, 1270, 1250, 1215, 1170, 1120, 1040 cm^{-1}; ^{1}H NMR (MeOH-d_4, 400 MHz) δ 9.29 (1H, s, H-5), 8.53 (1H, s, H-10), 8.41 (1H, s, H-8), 7.30 (1H, s, H-2), 4.03 (3H, s, MeO-7), 4.00 (6H, s, MeO-6 and 3), 3.85 (3H, s, MeO-4), 3.50 (2H, m, H-11), 3.29 (2H, m, H-12), 2.11 (2H, m, $NCOCH_2CH_3$), 1.07 (3H, t, $NCOCH_2CH_3$); FABMS m/z 500 (44, $[M + Na]^+$), 479 (38, $[M + 2]^+$), 478 (100, $[M + H]^+$), 477 (95, $[M]^+$).

Reaction of *N*-Formylnorglaucine with 50% H_2SO_4. Compound **3a** (101 mg, 0.27 mmol) and 50% H_2SO_4 (2.0 mL) were placed in a screw-tight sealed flask (25 mL). The mixture after degassing by a vacuum was stirred at 80 °C for 3.5 h. On cooling, the reaction mixture was added dropwise into a beaker containing ice−water (30 mL), and the resultant suspension was adjusted to pH 7.0 with ammonia−water at 0 °C and passed through an Amberlite XAD-2 column (20 g), eluted in sequence with H_2O (100 mL) and MeOH (100 mL), to give **11a** (52.0 mg, 51%) from the MeOH elution.

*N-Formylseconorglaucine (**11a**):* mp 134−135 °C (MeOH); R_f 0.51 (10% MeOH−$CHCl_3$); UV λ_{max} (log ε) 318 (4.01), 305 (3.96), 283 (4.38), 263 (4.87) nm; IR ν_{max} 3350, 2950, 1675, 1510, 1470, 1410, 1260, 1240, 1115, 1095 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.23 (1H, s, H-5), 8.17 (1H, s, NCHO), 7.77 (1H, d, J = 9.1 Hz, H-10), 7.55 (1H, d, J = 9.1 Hz, H-9), 7.18 (1H, s, H-8), 7.14 (1H, s, H-2), 4.05 (3H, s, MeO-7), 4.02 (3H, s, MeO-6), 4.01 (3H, s, MeO-3), 3.84 (3H, s, MeO-4), 3.69 (2H, m, H-11), 3.31 (2H, m, H-12); EIMS (20 eV) m/z 369 (100, $[M]^+$), 324 (20), 311 (42), 297 (30); HREIMS m/z 369.1573 $[M]^+$, calcd for $C_{21}H_{23}O_5N$, 369.1576.

*Reaction of N-Formylnorglaucine with 98% H_2SO_4: Preparation of Pancoridine (**10**).* Compound **3a** (4.04 g, 11.5 mmol) and 98% H_2SO_4 (8.1 mL) were placed in a screw-tight sealed flask (25 mL). The mixture after degassing by a vacuum was stirred for 8 days at room temperature. The reaction mixture was added dropwise into a beaker containing ice−water (100 mL), and the resultant suspension was adjusted to pH 7.0 with ammonia−water (25%) at 0 °C and extracted by $CHCl_3$ (200 mL × 3). The combined $CHCl_3$ layers were dried (Na_2SO_4) and evaporated to give a brown residue (2.56 g), which, on recrystallization from $CHCl_3$, yielded **10** (1.54 g, 44.4%): mp 211−213 °C; EIMS (70 eV) mlz 321 (100, $[M]^+$), 290 (100); HREIMS $[M]^+$ m/z 321.0992, calcd for $C_{19}H_{15}O_4N$, 321.1001. The mother liquor was chromatographed over a silica gel column (30 g, 70−230 mesh), eluted with 0−3% MeOH−$CHCl_3$, to give **12** (25 mg, 0.62%). The aqueous layer, after removal of the residual $CHCl_3$ left during partition, by condensation under reduced pressure, was passed through a Sephadex LH-20 column, eluted with MeOH−0.1% $HOAc_{aq.}$ (1:1), to give **9a** (10 mg, 0.19%) and **13a** (22.3 mg, 0.45%).

*N-Formyl-6a,7-didehydronorthaliporphine (**12a**):* ^{1}H NMR (MeOH-d_4, 400 MHz) δ 9.21 (1H, s, H-11), 8.86 (1H, s, NCHO), 8.41 (1H, s, H-8), 7.19 (1H, s, H-7), 7.14 (1H, s, H-8), 7.02 (1H, s, H-3), 4.11 (2H, m, H-5), 4.06 (3H, s, MeO-11), 4.03 (3H, s, MeO-9), 4.02 (3H, s, MeO-2), 3.18 (2H, m, H-4); NOEDs δ 9.21 (H-11) → 4.06 (MeO-10, 9.1%), δ 4.11 (H_2-5, 1.5%) ← 8.86 (NCHO) → 7.19

dx.doi.org/10.1021/np3007765 | J. Nat. Prod. XXXX, XXX, XXX−XXX

(H-7, 14.1%), δ 3.18 (H_2-4, 5.0%) ← 7.02 (H-3) → 4.02 (MeO-2, 9.1%); EIMS (20 eV) 353 (100, $[M]^+$), 338 (35), 311 (88), 297 (40).

*N-Formyl-9-sulfonylseconorthaliporphine (**13a**):* mp 193−195 °C (MeOH); IR ν_{max} 3500, 3200, 1680, 1525, 1480, 1385, 1260, 1180, 1050 cm^{-1}; ^1H NMR (MeOH-d_4, 400 MHz) δ 9.57 (1H, s, H-5), 8.54 (1H, s, H-10), 8.41 (1H, s, H-8), 8.04 (1H, s, *N*CHO), 7.28 (1H, s, H-2), 4.04 (3H, s, MeO-7), 4.01 (3H, s, MeO-3), 4.00 (3H, s, MeO-6), 3.57 (2H, m, H-11), 3.28 (2H, m, H-12); NOEDs δ 8.41 (H-8) → 4.04 (MeO-9, 11.0%), δ 3.28 (H_2-12, 3.3%) ← 8.54 (H-10) → 3.57 (H-11, 9.0%), δ 3.28 (H_2-11, 4.8%) ← 7.28 (H-2) → 4.01 (MeO-3, 11.6%); FABMS *m/z* 458 (48, $[M + Na]^+$), 436 (60, $[M + H]^+$), 435 (100, $[M]^+$); HREIMS *m/z* 435.1010 $[M]^+$, calcd for $C_{20}H_{21}O_8NS$, 435.0991.

Preparation of (±)-Wilsonirine [(±)-14]. Compound **10** (203 mg, 0.632 mmol), acetic acid (40 mL), and 10% Pt/C (150 mg) were placed in a 50 mL flask, in turn, and then the flask was placed in a Par hydrogenation apparatus. After degassing as usual for hydrogenation, H_2 (5 bar) was introduced and the mixture was stirred at 60 °C for 2 days. The reaction mixture was filtered with the aid of a Celite cake. After filtration, the Celite cake was washed with acetone (200 mL). The filtrate and acetone washings were evaporated to give a residue that yielded (±)-**14** (151 mg, 72%) after purification over a silica gel column (6 g, 70−230 mesh), eluted with 2−6% MeOH−$CHCl_3$.

*(±)-Wilsonirine [(±)-**14**]:* mp 210−212 °C (MeOH) (lit.[12] 202−204 °C); ^1H NMR (MeOH-d_4, 400 MHz) δ 8.18 (1H, s, H-11), 6.88 (1H, s, H-8), 6.72 (1H, s, H-3), 3.89 (3H, s, MeO-2), 3.85 (3H, s, MeO-9), 3.84 (3H, s, MeO-10); EIMS (70 eV) *m/z* 327 (70, $[M]^+$), 326 (100).

Optical Resolution of (±)-Wilsonirine [(±)-14]. To a 500 mL round-bottomed flask were added (±)-**14** (2.0 g, 6.12 mmol), (−)-di-*O*-4-toluoyl-L-tartaric acid (2.6 g, 6.42 mmol), and MeOH (150 mL). The mixture was stirred under reflux until a solution was formed. After cooling to room temperature, the precipitate was filtered off and was recrystallized from MeOH three times to give white crystalline salts of (−)-**14** and (−)-di-*O*-4-toluoyl-L-tartaric acid [(−)-**15**] (680 mg) with $[\alpha]^{22}_D$ −90 (*c* 0.32, MeOH). The solution of (−)-**15** (680 mg) in H_2O (80 mL) was adjusted to pH 8.0 by ammonia−water (25%) and extracted with $CHCl_3$ (80 mL × 4). The combined $CHCl_3$ solution was dried (Na_2SO_4) and evaporated under reduced pressure to give a pinkish solid, which, upon recrystallization from MeOH, yielded (−)-**14** (281 mg): mp 111−113 °C, $[\alpha]^{25}_D$ −55 (*c* 0.31, MeOH) [lit.[13] mp 106−108 °C, $[\alpha]^{24}_D$ −53.9 (*c* 0.2, MeOH)]. The mother liquor of the crystalline (−)-**15** was evaporated under reduced pressure to give a residue, which was suspended in H_2O (100 mL), adjusted to pH 8.0 by ammonia−water (25%), and extracted with $CHCl_3$ (100 mL × 4). The combined $CHCl_3$ layers were dried (Na_2SO_4) and evaporated under reduced pressure to give a residue (1.22 g). Optical resolution of this residue with (+)-di-*O*-4-toluoyl-D-tartaric acid (1.5 g) was carried out by fractional recrystallization from MeOH three times to give (+)-**15** (580 mg): $[\alpha]^{25}_D$ +93.3 (*c* 0.31, MeOH), which yielded (+)-**14** (230 mg), mp 110−113 °C and $[\alpha]^{25}_D$ 56.7 (*c* 0.31, MeOH) [lit.[13] mp 107−109 °C, $[\alpha]^{24}_D$ +54.8 (*c* 0.1, MeOH)], from (+)-**15** (580 mg), following a similar procedure to that for the recovery of (−)-**14** from (−)-**15**.

Preparation of (−)-Thaliporphine [(−)-5a] and (−)-*N*-Propylwilsonirine [(−)-5c]. To the mixture of (−)-**14** (51 mg, 0.16 mmol), MeOH (4 mL), and 37% HCHO (0.1 mL) in a 50 mL round-bottomed flask was added $NaBH_4$ (50 mg) portionwise while stirring at room temperature. The reaction mixture was stirred for 1 h and was evaporated to give a residue, which was partitioned between H_2O (50 mL) and $CHCl_3$ (50 mL × 3). The combined $CHCl_3$ solution was dried (Na_2SO_4) and evaporated to give a residue (49.3 mg), which was chromatographed over a silica gel column (70−230 mesh, 5 g), eluted with 2% MeOH−$CHCl_3$, to give (−)-**5a** (39.3 mg, 75%): $[\alpha]^{25}_D$ −63.3 (*c* 0.40, MeOH), mp 185−187 °C (MeOH). Then, (−)-**14** (42.6 mg, 0.13 mmol) in DMF (4 mL) was reacted with *n*-propyl bromide (20 μL) in the presence of K_2CO_3 (60 mg) in a 2 mL round-bottomed flask at 50 °C overnight. After removal of DMF using an oil pump, the residue was purified in a similar manner as described for (−)-**5a** to give (−)-**5c** (20.1 mg, 46%): $[\alpha]^{25}_D$ −55.2 (*c* 0.32, MeOH), mp 66−69 °C (MeOH).

Mechanical Response of Thaliporphine Homologues on Rat Cardiac Tissues. The functions of the prepared thaliporphine homologues on rat cardiac tissues were evaluated following a reported method.[1] Strips of the right atrium, left atrium, and right ventricle, quickly dissected from the hearts of male Wistar-Kyoto rats, were put in an organ bath containing Tyrode's solution, bubbled with CO_2−O_2 (1:19), at 36 ± 0.2 °C. Contractions of spontaneously beating right atria and of electrically driven left atria and right ventricular strips were recorded by a force displacement transducer, connected to one end of the preparation by a fine silk thread. An optimal preload (0.5−1.0 g) on these preparations was set to obtain the maximum developed tension. The left atria and right ventricular strips were stimulated at a frequency of 2 Hz by a 1 ms rectangular pulse at supramaximal intensity via an isolated stimulator. Compounds were dissolved in DMSO, and the final concentration of DMSO in the bath did not exceed 0.1% (v/v), which caused no significant effect on the muscle contraction and heart rate.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

1D (^1H and ^{13}C NMR) and HMBC spectra of **8c** and **10** and ^1H NMR spectrum of the (−)-di-*O*-4-toluoyl-L-tartrates of (±)-**14** (top) and (−)-**14**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel/fax: +886 2 23916127. E-mail: shoeilee@ntu.edu.tw.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by National Science Council, Republic of China, under grants NSC 85-2331-B-002-241, NSC 97-2323-B-002-015, and NSC 100-2325-B-002-021.

■ DEDICATION

Dedicated to Dr. Lester A. Mitscher, of the University of Kansas, for his pioneering work on the discovery of bioactive natural products and their derivatives.

■ REFERENCES

(1) Su, M. J.; Chang, Y. M.; Chi, J. F.; Lee, S. S. *Eur. J. Pharmacol.* **1994**, *254*, 141−150.
(2) Teng, C. M.; Yu, S. M.; Lee, S. S.; Ko, F. N.; Su, M. J.; Huang, T. F. *Eur. J. Pharmacol.* **1993**, *233*, 7−12.
(3) Ahmad, R.; Saá, J. M.; Cava, M. P. *J. Org. Chem.* **1977**, *42*, 1228−1230.
(4) Castedo, L.; de Lera, A. R.; Saá, J. M.; Suau, R.; Villaverde, C. *Heterocycles* **1980**, *14*, 1135−1138.
(5) Huang, W. J.; Chen, C. H.; Singh, O. V.; Lee, S. L.; Lee, S. S. *Synth. Commun.* **2002**, *32*, 3681−3686.
(6) Lee, S. S.; Tsai, F. Y.; Chen, I. S.; Liu, K. C. S. *J. Chin. Chem. Soc. (Taipei, Taiwan)* **1993**, *40*, 209−212.
(7) Chiou, C. M.; Kang, J. J.; Lee, S. S. *J. Nat. Prod.* **1998**, *61*, 46−50.
(8) Nakanishi, K.; Solomon, P. H. *Infrared Absorption Spectroscopy*, 2nd ed.; Holden-Day, Inc.: San Francisco, 1981; p 51.
(9) Hufford, C. D.; Sharma, A. S.; Oguntimein, B. O. *J. Pharm. Sci.* **1980**, *69*, 1180−1183.
(10) Cava, M. P.; Noguchi, I.; Buck, K. T. *J. Org. Chem.* **1973**, *38*, 2394−2397.
(11) Jossang, A.; Leboeuf, M.; Cavé, A. *J. Nat. Prod.* **1986**, *49*, 1028−1035.

(12) Kupchan, S. M.; Liepa, A. J. *J. Am. Chem. Soc.* **1973**, *95*, 4062−4062.
(13) Hara, H.; Komoriya, S.; Miyashita, T.; Hoshino, O. *Tetrahedron: Asymmetry* **1995**, *6*, 1683−1692.
(14) Su, M. J.; Liu, P. J.; Tseng, C. D.; Tseng, Y. Z.; Lee, C. S. *J. Formosan Med. Assoc.* **1994**, *93*, 673−680.
(15) Felix, J. P.; King, F.; Shevell, J. L.; Garcia, M. L.; Kaczorowski, G. J.; Bick, I. R. C.; Slaughter, R. S. *Biochemistry* **1992**, *31*, 11793−11800.