Four 14(13 → 12)-Abeolanostane Triterpenoids with 6/6/5/6-Fused Ring System from the Roots of *Kadsura coccinea*

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Abstract

Four new rearranged 6/6/5/6-fused lanostane-type triterpenoids, kadcoccitanes A–D (1–4), were isolated from the roots of *Kadsura coccinea*, and their structures were mainly elucidated by comprehensive analysis of their spectroscopic data. Additionally, the structure of 1 was ambiguously verified by single-crystal X-ray diffraction, while the structure of 2, which features a novel 8,16-epoxy motif, was validated by quantum chemical calculation of NMR parameters and ECD spectrum. Moreover, 1 and 4 were found to exhibited anticoagulant activity, while 3 and 4 were found to possess anti-platelet aggregation activity.

Keywords *Kadsura coccinea* · Lanostane triterpenoid · NMR computation · Anti-platelet aggregation · Anticoagulant

1 Introduction

The plants of family Schisandraceae, including genera *Schisandra* and *Kadsura*, are invaluable sources of triterpenoids [1, 2]. *Kadsura coccinea* (Lem.) Smith is an economical fruit plant as well as a folk medicine usually employed for the treatment of gastropathy, rheumatic arthritis, postpartum abdominalgia with blood stasis, etc. [3]. Phytochemical investigations on the stems of *K. coccinea* collected from Guangxi and Yunnan Provinces have previously been undertaken by our research group, which revealed this species to be abundant with triterpenoids, especially lanostane triterpenoids [4–9]. Up to now, six kinds of unprecedented lanostane-type triterpenoid scaffolds have been isolated from the stems of *K. coccinea* by our group [4–7], namely, 14(13 → 12):13(17 → 16)-diabeolanostanes, 18(13 → 12)-abeo-13,17-secolanostanes, 14(13 → 12)-abeo-13,18-dinorlanostanes, 14(13 → 12)-abeo-12,13-secolanostanes, 14(13 → 12)-abeo-2,3-secolanostanes, and 13,14-secolanostanes. Besides, plenty of new triterpenoids belonging to conventional lanostanes [4–9], cycloartanes [10, 11] and schinortriterpenoids [10] have also been isolated from the stems of this species.

In the aforementioned research, the secondary metabolites of *K. coccinea* collected from different regions manifested significant differences. So, it was interesting to consider whether the secondary metabolites from different parts of the same plant differed or not. Thus, a phytochemical study on the roots of *K. coccinea* cultivated in Jingzhou Miao and Dong Autonomous County in Hunan Province was carried out. As a result, four new 14(13 → 12)-abeolanostane triterpenoids, kadcoccitaines A–D (1–4) were obtained (Fig. 1). Considering the folk medicinal value of *K. coccinea*, some of the compounds were tested for their antiplatelet aggregation and anticoagulant activities. Herein, the isolation, structure elucidation, and bioactivity screening of these compounds are described.
2 Results and Discussion

Kadcoccitane A (1) was obtained as colorless acicular crystals and possessed a molecular formula of C₃₀H₄₆O₅, which was determined by the HRESIMS ions at m/z 509.3251 [M + Na]⁺, calcd 509.3237), demonstrating eight degrees of unsaturation. The ¹H-NMR data of 1 (Table 1) indicated the existence of five singlet methyls (δH 1.06, 1.15, 1.25, 1.39 and 2.14), one doublet methyl (δH 1.16) and two olefinic protons (δH 5.29 and 6.07). Its ¹³C NMR and DEPT spectra revealed 30 carbon resonances (Table 2), including six methyls, nine methylenes (one hydroxymethyl carbon), seven methines (two olefinic carbons), and eight quaternary carbons (one carboxyl, one carbonyl and two olefinic carbons). Subtracting the four degrees of unsaturation generated by carboxyl, carbonyl, and olefinic groups, the remaining four degrees of unsaturation manifested that 1 had a tetracyclic structure.

Detailed analysis of the 1D and 2D NMR spectra of 1 demonstrated its structure to be similar to those of kadcocine acids A–N [8], which had 14(13 → 12)-abeolanostane scaffolds featuring 6/6/5/6-fused ring systems. The planar structure of 1 could be confirmed by its ¹H-¹H COSY and HMBC data (Fig. 2). The HMBC correlations from H₂-27 to C-24 (δC 142.9), C-25 (δC 129.0) and C-26 (δC 170.9), and from H-24 (δH 6.07) to C-26 implied the existence of the C-24/C-25 double bond and carboxyl group at C-26. The COSY correlation of H-11 (δH 5.29)/H-12 and the HMBC correlations from H-12 and H₃-19 to C-9 (δC 154.4) suggested the presence of Δ⁹,¹¹ double bond. By analyzing its ROESY spectrum, the cross-peak of H-24/H₃-27 indicated that the Δ²₄,²₅ double bond took Z-geometry. However, the configuration of C-13 couldn’t be determined due to the lack of HO-13 signal in the ¹H NMR spectrum. Fortunately, the crystals of 1 were obtained and subjected to the X-ray diffraction analysis through CuKα radiation, which determined its absolute configuration to be 5R,8S,10S,12R,13R,14S,17R, and 20R (Flack parameter = 0.06(2)) (CCDC:1903695) (Fig. 2).

Kadcoccitane B (2) was obtained as white amorphous powder. The molecular formula C₃₀H₄₂O₆ was determined by HRESIMS ([M + Na]⁺ m/z 521.2872, calcd 521.2874), corresponding to ten indices of hydrogen deficiency. The ¹H-NMR spectrum showed proton signals which could be ascribed to six singlet methyls (δH 1.06, 1.15, 1.48, 1.73, 2.09 and 2.17), one doublet methyl (δH 0.92) and two olefinic protons (δH 5.94 and 6.15) (Table 1). Moreover, 30 carbon resonances (Table 2), including seven methyls, six methylenes, six methines (two olefinic carbons and two oxygen-bearing methines), and eleven quaternary carbons (one carboxyl, one carbonyl and four olefinic carbons) could be
Table 1 $^1$H NMR data for compounds 1–4 in pyridine-$d_5$ at 500 MHz ($\delta$ in ppm, $J$ in Hz)

| No. | 1     | 2     | 3     | 4     |
|-----|-------|-------|-------|-------|
| 1a  | 1.92 (overlap) | 2.00 (overlap) | 2.40 (overlap) | 1.86 (m) |
| 1b  | 1.79 (overlap) | 1.78 (overlap) | 1.59 (overlap) | 1.64 (overlap) |
| 2a  | 2.79 (overlap) | 2.65 (m) | 2.62 (overlap) | 1.97 (overlap) |
| 2b  | 2.49 (m) | 2.42 (m) | 2.42 (overlap) | 1.97 (overlap) |
| 3   |       |       |       | 3.49 (m) |
| 5   | 1.86 (overlap) | 1.54 (overlap) | 2.61 (overlap) | 0.97 (dd, 12.1, 2.0) |
| 6a  | 1.53 (m) | 2.06 (overlap) | 1.61 (overlap) | 1.75 (overlap) |
| 6b  | 1.44 (m) | 1.56 (overlap) | 1.33 (overlap) | 1.51 (m) |
| 7a  | 1.79 (overlap) | 2.77 (overlap) | 1.97 (dd, 12.2, 4.0) | 1.75 (overlap) |
| 7b  | 1.04 (overlap) | 1.80 (overlap) | 1.57 (overlap) | 1.39 (overlap) |
| 8   | 2.26 (br d, 12.9) |       | 1.52 (dd, 12.7, 3.4) | 2.48(m) |
| 11  |       |       |       |       |
| 12  |       |       |       |       |
| 15a | 1.67 (overlap) | 4.33 (s) | 1.61 (overlap) | 1.77 (overlap) |
| 15b | 1.67 (overlap) |       | 1.21 (overlap) | 1.43 (overlap) |
| 16a | 2.10 (overlap) | 4.70 (s) | 1.80 (overlap) | 1.77 (overlap) |
| 16b | 1.57 (overlap) |       | 1.80 (overlap) | 1.77 (overlap) |
| 17  | 2.03 (q, 5.8) |       | 2.57 (br s) | 1.64 (overlap) |
| 18a | 4.16 (2H, m) | 2.09 (3H, s) | 5.04 (d, 12.1) | 1.35 (3H, s) |
| 18b |       |       | 4.75 (d, 12.1) |       |
| 19  | 1.25 (3H, s) | 1.48 (3H, s) | 1.03 (3H, s) | 1.20 (3H, s) |
| 20  | 2.16 (overlap) | 2.81 (overlap) | 2.30 (br, s) | 2.42 (br s) |
| 21  | 1.16 (3H, d, 7.6) | 0.92 (3H, d, 6.8) | 1.11 (3H, d, 6.7) | 1.19 (3H, d, 7.1) |
| 22a | 1.96 (overlap) | 1.85 (overlap) | 1.70 (m) | 1.91 (overlap) |
| 22b | 1.24 (overlap) | 1.63 (br s) | 1.32 (overlap) | 1.31 (overlap) |
| 23a | 2.80 (overlap) | 3.05 (dq, 15.6, 7.9) | 2.81 (m) | 2.87 (overlap) |
| 23b | 2.80 (overlap) | 2.81 (overlap) | 1.28 (overlap) | 2.87 (overlap) |
| 24  | 6.07 (t, 7.5) | 6.15 (t, 6.9) | 6.12 (t, 7.5) | 6.13 (t, 7.4) |
| 27  | 2.14 (3H, s) | 2.17 (3H, s) | 2.12 (3H, s) | 2.15 (3H, s) |
| 28  | 1.15 (3H, s) | 1.73 (3H, s) | 1.37 (3H, s) | 1.10 (3H, s) |
| 29  | 1.39 (3H, s) | 1.15 (3H, s) | 1.20 (3H, s) | 1.26 (3H, s) |
| 30  | 1.06 (3H, s) | 1.06 (3H, s) | 1.03 (3H, s) | 1.12 (3H, s) |

Table 2 $^{13}$C NMR data for compounds 1–4 at 125 MHz in pyridine-$d_5$ ($\delta$ in ppm)

| No. | 1     | 2     | 3     | 4     |
|-----|-------|-------|-------|-------|
| 1   | 36.0 t | 36.3 t | 31.4 t | 36.2 t |
| 2   | 35.4 t | 34.7 t | 34.9 t | 28.6 t |
| 3   | 215.6 s | 215.6 s | 216.0 s | 78.3 d |
| 4   | 48.3 s | 47.7 s | 47.9 s | 39.7 s |
| 5   | 55.0 d | 53.0 d | 47.1 d | 53.3 d |
| 6   | 23.5 t | 20.3 t | 22.8 t | 21.8 t |
| 7   | 29.7 t | 35.2 t | 21.2 t | 21.4 t |
| 8   | 54.7 d | 92.8 s | 51.5 d | 49.0 d |
| 9   | 154.4 s | 153.3 s | 80.5 s | 156.0 s |
| 10  | 38.1 s | 37.5 s | 41.9 s | 38.3 s |
| 11  | 118.8 d | 130.4 d | 70.1 d | 119.2 d |
| 12  | 58.6 d | 88.2 s | 148.9 s | 66.0 d |
| 13  | 77.0 s | 137.7 s | 137.7 s | 76.7 s |
| 14  | 41.7 s | 59.6 s | 41.5 s | 43.5 s |
| 15  | 36.4 t | 78.1 d | 35.5 t | 35.3 t |
observed from its $^{13}$C NMR and DEPT spectra. All the afore-
mentioned carboxyl, carbonyl and olefinic groups accounted
for four out of nine degrees of unsaturation, indicative of a
pentacyclic structure for $2$.

Careful analysis of the HMBC and $^1$H-^1$H$ COSY spectra
(Fig. 3) of $2$ demonstrated that it shared the same structure
of side chain (C-20–C-27) as that of compound $1$, as well
as a similar A/B ring system except that C-8 ($\delta_C$ 92.8) was
oxygenated, as revealed by the $^1$H-^1$H$ COSY correlations of
H-5/H$_2$-6/H$_2$-7, together with the HMBC correlations from
H$_2$-6 and H$_2$-7 to C-8. Additionally, the HMBC corre-
lations from olefinic H-11 ($\delta_H$ 5.94) to C-12 ($\delta_C$ 88.2), from
H$_3$-28 to C-15 ($\delta_C$ 78.1), and from H-15 ($\delta_H$ 4.33) to C-16
($\delta_C$ 80.0) demonstrated that C-12, C-15, and C-16 were all
oxygenated. Besides, the correlations from H$_3$-18 and H-16
($\delta_H$ 4.70) to C-13 ($\delta_C$ 137.7) and C-17 ($\delta_C$ 136.8) suggested
the presence of the C-13/C-17 double bond. Most impor-
tantly, the correlation of H-16/C-8, the intensity of which
was nearly as strong as that of the H-15/C-8 correlation, was
observed in the HMBC spectrum. Hence, in consideration of
the 4-bond distance between H-16 and C-8 if following the
H-16/C-16/C-15/C-14/C-8 path, an epoxy was tentatively
constructed between C-8 and C-16.

As for the stereochemistry of $2$, the H-15/H$_3$-28$\alpha$ corre-
lation in the ROESY spectrum of $2$ (Fig. 3) indicated that H-15 adopted $\alpha$-orientation. Moreover, the fact that
H-15 and H-16 both existed as singlets in the $^1$H-NMR
spectrum, together with the absence of the $^1$H-^1$H$ COSY
correlation of H-15/H-16, suggested that the dihedral
angle of H-15/C-15/C-16/H-16 was around 90°, thus
demanding H-16 to be $\alpha$-oriented. Noteworthily, though
there existed two possibilities for the orientation of HO-12
teoretically, the rigidity of the C/D ring system denied
the existence of the HO-12$\beta$ isomer. Moreover, the ste-
reochemistry of C-20 couldn’t be determined presently
since it was located on the flexible side chain. Thus, the
two possible C-20 stereoisomers of $2$, (5$R$,8$S$,10$S$,1
2$R$,14$R$,15$S$,16$R$,20$R$)-2 (2a) and (5$R$,8$S$,10$S$,1
2$R$,14$R$,15$S$,16$R$,20$S$)-2 (2b) (Fig. S71) were sub-
jected to quantum calculations of NMR chemical shifts

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**Fig. 2** Key $^1$H-^1$H$ COSY and HMBC correlations and X-ray crystallographic structure of $1$

**Fig. 3** Key $^1$H-^1$H$ COSY, HMBC and ROESY correlations of $2$
at MPW1PW91-SCRF/6-31 + G(d,p)//M06-2X-D3/def2-SVP level of theory in chloroform with IEFPCM solvent model (Tables S2 and S3). As a result, the calculated NMR shifts of 2b were found to be in better agreement with their experimental counterparts, as indicated by parameters including $R^2$, MAE, CMAE, as well as the DP4 + probability (Table 3). Then, quantum chemical calculation of spin–spin coupling constants (SSCC) of 2b was run at B97-2/pcJ-1 level with IEFPCM solvent model in chloroform (Table S5), the theoretical SSCC of $^3J_{H_{15}H_{16}}$ (0.5 Hz) could clearly explain the aforementioned splitting pattern of H-15 and H-16 in the $^1$H NMR spectrum, while the predicted SSCCs of $^3J_{H_{16}C_{8}}$ (7.0) could well account for the strong H-16/C-8 correlation in the HMBC spectrum. Thus, the planar structure as well as the relative configuration of 2 was established as depicted in Fig. 1, and subsequent TDDFT calculation at CAM-B3LYP-SCRF/def2-SVP level of theory in methanol with IEFPCM solvent model succeeded in establishing the absolute configuration of 2 as $5R, 8S, 10S, 12R, 14R, 15S, 16R$ and 20S (Fig. 4). Accordingly, kadococcitane B (2) represented the first example of 8,16-epoxy-14(13 → 12)-abeolanostane triterpenoid featuring an unusual 20S configuration.

Kadococcitane C (3), white amorphous powder, possessed a molecular formula of C$_{30}$H$_{48}$O$_6$ supported by the HRESIMS ([M + Na]$^+$ m/z 495.3439, calcd 495.3445) indicated that it possessed a molecular formula of C$_{30}$H$_{48}$O$_6$ suggesting seven degrees of unsaturation. The $^1$H NMR spectrum showed resonances for six singlet methyls, two olefinic protons, one hydroxymethyl methyl (δ$_H$ 1.26), and one olefinic proton (δ$_H$ 6.12) were observed from its $^1$H NMR spectrum (Table 1). The $^{13}$C NMR combined with DEPT spectra displayed 30 carbon signals (Table 2) which comprised six methyls, nine methylenes (one hydroxymethyl carbon), six methines (one olefinic and one oxygen-bearing carbons), and nine quaternary carbons (one carboxyl, one carbonyl and three olefinic carbons). Judging from the total degrees of unsaturation and those which was occupied by carbon–carbon and carbon–oxygen double bonds, a tetracyclic scaffold of 3 was conjectured to exist.

In comparison with 1, two variations of compound 3 could be observed. One was that the double bond of C-9/C-11 of 3 was oxidized to a vicinal diol, which could be determined by the HMBC correlations from H$_2$-19 to C-9 (δ$_C$ 80.5), from H-11 (δ$_H$ 5.14) to C-10 and C-14, and from H-8 to C-11 (δ$_C$ 70.1) (Fig. 5). The other was that $\Delta^{12,13}$ double band was formed by the dehydration at C-13, which could be ascertained by the HMBC correlations from H-11, H$_2$-18 and H$_3$-28 to C-12 (δ$_C$ 148.9), and from H-11 and H$_2$-18 to C-13 (δ$_C$ 137.7) (Fig. 5). In the ROESY spectrum (Fig. 5), the cross peaks of HO-9/H$_3$-28α and H-11/H$_2$-19β suggested the α-orientation of HO-9 and HO-11, respectively.

Kadococcitane D (4) was isolated as white amorphous powder. Its HRESIMS data ([M + Na]$^+$ m/z 495.3439, calcd 495.3445) indicated that it possessed a molecular formula of C$_{30}$H$_{48}$O$_4$, suggesting seven degrees of unsaturation. The $^1$H NMR spectrum showed resonances for six singlet methyls (δ$_H$ 1.12, 1.10, 1.20, 1.26, 1.35 and 2.15), one doublet methyl (δ$_H$ 1.19) and two olefinic protons (δ$_H$ 5.56 and 6.13) (Table 1). The $^{13}$C NMR and DEPT spectra showed 30 carbon resonances (Table 2) including seven methyls, eight methylenes, eight methines (two olefinic and one oxygen-bearing carbons), and seven quaternary carbons (one carboxyl, and two olefinic carbons). Considering the three degrees of unsaturation generated by carboxyl and olefinic groups, compound 4 was a tetracyclic triterpenoid.

The structure of 4 also differed from that of 1 in two aspects. Firstly, the carbonyl at C-3 of compound 4 was reduced to hydroxy, which could be determined by the HMBC correlations from H$_2$-1, H$_2$-2, H$_3$-29 and H$_3$-30 to

### Table 3 Comparison of the key parameters of 2a and 2b in NMR computation

| Parameters | 2a | 2b |
|------------|----|----|
| $R^2 (^{13}$C) | 0.9983 | 0.9984 |
| MAE (^{13}$C) | 2.4 ppm | 2.2 ppm |
| CMAE (^{13}$C) | 1.7 ppm | 1.5 ppm |
| $R^2 (^1$H) | 0.9633 | 0.9845 |
| MAE (^1$H) | 0.23 ppm | 0.14 ppm |
| CMAE (^1$H) | 0.21 ppm | 0.13 ppm |
| DP4 + (^1$H data) | 0.00% | 100.00% |
| DP4 + (^13$C data) | 1.26% | 98.74% |
| DP4 + (all data) | 0.00% | 100.00% |

**Fig. 4** Experimental ECD spectrum of 2 (black); Calculated ECD spectra of (5R,8S,10S,12R,14R,15S,16R,20S)-2b (shift = −7 nm, blue) and its enantiomer (shift = −7 nm, blue dash).
Moreover, the HMBC correlations from H3-18 to C-12, C-13 (δC 76.7) and C-17 manifested that the hydroxymethyl of C-18 was replaced by a methyl (Fig. 6). The ROESY correlations of H-3/H-5α and H-8β/H-18/H-20β demonstrated the spatial α-orientation of H-3 and β-orientation of H3-18 (Fig. 6).

The phytochemical investigations on K. coccinea indicated that the secondary metabolites from the roots generally possess higher degrees of oxidation than those obtained from its stems, which may be mainly attributed to the abundant oxidases existing in the roots. However, to arrive at more definite conclusion, more in-depth research still needs to be undertaken. In addition, compounds 1, 3 and 4 were screened for the bioactivity against platelet aggregation induced by colloid. The result suggested that the inhibition ratios of 3 and 4 were 12.4 ± 12.5% and 19.4 ± 14.4% (p < 0.05), respectively, under the concentration of 100 µM.

Compounds 1 and 4 were tested for anticoagulant activity. The result showed that their IC50 values were 37.8 and 31.5 µM, respectively.

3 Experimental

3.1 General Experimental Procedures

1D and 2D NMR spectra were recorded on Bruker AV III 500 MHz spectrometer using TMS as the internal standard. Chemical shifts (δ) are expressed in ppm. HRESIMS was performed on an API QSTAR Pulsar i spectrometer. Melting point was recorded on an RDY-1B micro melting point apparatus. UV spectra were obtained on a Shimadzu UV-2401PC spectrophotometer. Optical rotations were measured in MeOH with a JASCO P-1020 polarimeter or
an Autopol VI, Serial #91058. IR spectra were obtained on a Bruker Tensor-27 FT-IR spectrometer using KBr pellets. Column chromatography (CC) was performed with silica gel (80–100 or 100–200 mesh; Qingdao Marine Chemical, Inc., Qingdao, People’s Republic of China). Analytical HPLC was performed on an Agilent 1260 liquid chromatograph with a Zorbax SB-C18 (4.6 mm × 250 mm) column. Semi-preparative HPLC was performed on an Agilent 1200 liquid chromatograph eluted by MeOH and then purified by repeated semi-preparative HPLC (MeCN/H$_2$O or MeOH/H$_2$O, 3 mL/min) to yield compound 2 (9.5 mg).

Fraction D6 (10 g) was chromatographed by silica gel with a gradient elution of PE/Me$_2$CO (1:0–0:1) to afford fractions D61–D65. Fr. D64 (1.5 g) was subjected to Sephadex LH-20 chromatography eluted by MeOH and then purified by repeated semi-preparative HPLC (MeCN/H$_2$O or MeOH/H$_2$O, 3 mL/min) to yield compound 3 (3.3 mg). Fr. D65 (980 mg) was chromatographed by Sephadex LH-20 eluted with MeOH to give fractions D651–D655. Fr. D65 (62 mg) was further purified by repeated semi-preparative HPLC (MeCN/H$_2$O or MeOH/H$_2$O, 3 mL/min) to yield compound 4 (7.4 mg).

3.4 Characteristic Data of Compounds 1–4

Kadoccitane A (1): Colorless acicular crystals, melting point: 213 °C. [α]$_{D}^{20}$ = 58.4 (c 0.214, MeOH). UV (MeOH) λ$_{max}$ (log ε) 206 (3.9) nm. IR (KBr) ν$_{max}$ 3440, 2936, 1703, 1638, 1421, 1241, 1035, 886, 647 cm$^{-1}$. CD (MeOH): 205 nm (δε = −0.75), 215 nm (δε = −1.03). Positive ESIMS m/z 509 [M + Na]$^+$.

1H and 13C NMR data in pyridine-$d_5$, see Tables 1 and 2. 1H and 13C NMR data in CDCl$_3$, see Table S1.

Crystal data for 1: C$_{30}$H$_{46}$O$_{5}$, M = 486.67, a = 10.6452(2) Å, b = 27.2411(5) Å, c = 28.4264(5) Å, α = 90°, β = 90°, γ = 90°. V = 8243.3(3) Å$^3$. T = 100 K, space group P2$_1$2$_1$2$_1$, Z = 12. μ(CuKα) = 0.618 mm$^{-1}$. 49684 reflections measured, 14281 independent reflections (R$_{int}$ = 0.0301). The final R$_1$ values were 0.0336 (I > 2σ(I)). The final wR(F$^2$) values were 0.0874 (I > 2σ(I)). The final R$_1$ values were 0.0338 (all data). The final wR(F$^2$) values were 0.0876 (all data). The goodness of fit on F$^2$ was 1.087. Flack parameter = 0.06(2).

Kadoccitane B (2): White amorphous powder. [α]$_{D}^{25}$ = −44.5 (c 0.163, MeOH). UV (MeOH) λ$_{max}$ (log ε) 216 (4.1) nm, 196 (4.1) nm, 206 (4.0) nm. IR (KBr) ν$_{max}$ 3445, 2967, 2934, 1693, 1640, 1458, 1383, 1048, 581 cm$^{-1}$. CD (MeOH): 222 nm (δε = 2.14), 244 nm (δε = −0.38). Positive ESIMS m/z 521 [M + Na]$^+$.

1H and 13C NMR data in pyridine-$d_5$, see Tables 1 and 2. 1H and 13C NMR data in CDCl$_3$, see Table S1.

Kadoccitane C (3): White amorphous powder. [α]$_{D}^{25}$ = −37.7 (c 0.107, MeOH). UV (MeOH) λ$_{max}$ (log ε) 207 (4.0) nm. IR (KBr) ν$_{max}$ 3432, 2929, 2971, 1701, 1638, 1457, 1383, 1264, 1043, 893, 582 cm$^{-1}$. CD (MeOH): 196 nm (δε = −1.48), 207 nm (δε = +0.25), 225 nm (δε = −0.41). Negative ESIMS m/z 501 [M–H]$^−$. 1H and 13C NMR data in pyridine-$d_5$, see Tables 1 and 2.

Kadoccitane D (4): White amorphous powder. [α]$_{D}^{25}$ = −40.3 (c 0.120, MeOH). UV (MeOH) λ$_{max}$ (log ε) 208 (4.0) nm. IR (KBr) ν$_{max}$ 3433, 2936, 1695, 1383, 1252, 1037, 859, 580 cm$^{-1}$. CD (MeOH): 208 nm (δε = 0.15), 222 nm (δε = −0.25). Positive ESIMS m/z
495 [M + Na]⁺. ¹H and ¹³C NMR data in pyridine-d₅, see Tables 1 and 2.

3.5 Computational Method

Conformational analysis of 2a and 2b was initially performed in Spartan’16 (Wavenfunction, Irvine, CA, USA, 2016) using the Monte Carlo algorithm and Merck molecular force field (MMFF). To avoid losing relevant conformations during the conformational search stage, the “set torsions” function was used to give all rotatable bonds a six fold sampling, as well as to allow the atoms on the aliphatic ring to flip. Maximum 20000 conformers were examined for each diastereoisomer, and those obtained conformers within 20 kcal/mol were kept (1000 ones for each isomer).

These conformers were subjected to semiempirical geometry optimization using the GFN2-xTB method [12] implemented in the XTB code (version 6.1) in order to obtain conformers better correlating with DFT calculations. Subsequently, XTB geometries with a difference of distance geometry within 0.25 were clustered. Then, clustered XTB geometries within an energy window of 8 kcal/mol were subjected to a DFT energy calculation at M06-2X/def2-SVP level of theory with DFT-D3 dispersion correction [13] using Gaussian 09 program [14], and those conformers within an energy window of 5 kcal/mol were kept. The completion of step was hugely aided by the Molclus program [15] (and its “isostat” module).

The above screened conformers were subjected to DFT geometry optimization at M06-2X-D3/def2-SVP level of theory. Frequency analyses of all optimized conformers were undertaken at the same level of theory to ensure they were true local minima on the potential energy surface. Then, energies of all optimized conformers were evaluated at M06-2X-D3/def2-TZVP level of theory. Gibbs free energies of each conformers were calculated by adding “Thermal correction to Gibbs Free Energy” obtained by frequency analysis to electronic energies obtained at M06-2X-D3/def2-TZVP level of theory. Room-temperature (298.15 K) equilibrium populations were calculated according to Boltzmann distribution law:

\[
P_i = \frac{n_i e^{-\Delta G_i / RT}}{\sum_j n_j e^{-\Delta G_j / RT}} = \frac{Q_i}{Q_{(Relative)}},
\]

where \( P_i \) is the population of the \( i \)th conformer; \( n_i \) the number of molecules in \( i \)th conformer; \( \Delta G \) is the relative Gibbs free energy (kcal/mol); \( T \) is the temperature, usually the room temperature (298.15 K); \( R \) is the ideal gas constant (0.0019858895); \( Q \) is the partition function. Those conformers accounting for over 98% population were subjected to subsequent calculations.

NMR shielding constants were calculated with the GIAO method at mPW1PW91-SCRF/6-31 + G(d,p) level with IEF-PCM solvent model in chloroform solvent. The shielding constants obtained were converted into chemical shifts by referencing to TMS at 0 ppm (\( \delta_{cal} = \sigma_{TMS} - \sigma_{cal} \)), where the \( \sigma_{TMS} \) was the shielding constant of TMS calculated at the same level. For each possible candidate, the parameters \( a \) and \( b \) of the linear regression \( \delta_{cal} = a\delta_{exp} + b \); the correlation coefficient, \( R^2 \); the mean absolute error (MAE) defined as \( \sum n_i |\delta_{cal} - \delta_{exp}|/\sum n_i \); the corrected mean absolute error, CMAE, defined as \( \sum n_i |\delta_{corr} - \delta_{exp}|/\sum n_i \), where \( \delta_{corr} = (\delta_{cal} - b)/a \), were calculated [16, 17]. The DP4 + probabilities of each possible candidate were calculated with the EXCEL spreadsheet provided by Sarotti et al. [18].

Spin–spin coupling constants were calculated at B97-2/pc1-1 [19] level with IEFPCM solvent model in chloroform.

TDDFT ECD calculations were run at CAM-B3LYP/def2-SVP level of theory in MeOH with IEFPCM solvent model. For each conformer, 30 excited states were calculated. The calculated ECD curves were generated using Multiwfn 3.6 software [20].

3.6 Activity Screening

3.6.1 Anti-platelet Aggregation Induced by Colloid

The experimental blood was extracted from the ears of Japanese white rabbits and conserved in vacuum blood-collection tubes with sodium citrate (whole blood: sodium citrate = 9:1). The blood-collection tubes were turned upside down gently to ensure homogeneous mixing of blood and anticoagulant and then centrifuged (200×g, 10 min). The supernatant was thus collected as platelet rich plasma (PRP). The residual blood was sequentially centrifuged (2400×g, 20 min) and supernatant was collected as platelet poor plasma (PPP). The platelet count of PRP was adjusted to 250 × 10⁹ L⁻¹ based on PPP [21–23]. The test samples were weighed accurately and dissolved with DMSO to 10 mM. The reference substance, Ticagrelor, was prepared to 0.5 mg/mL and 1 mmol/L, respectively, and conserved at same level. For each possible candidate, the parameters \( a \) and \( b \) of the linear regression \( \delta_{cal} = a\delta_{exp} + b \); the correlation coefficient, \( R^2 \); the mean absolute error (MAE) defined as \( \sum n_i |\delta_{cal} - \delta_{exp}|/\sum n_i \); the corrected mean absolute error, CMAE, defined as \( \sum n_i |\delta_{corr} - \delta_{exp}|/\sum n_i \), where \( \delta_{corr} = (\delta_{cal} - b)/a \), were calculated [16, 17]. The DP4 + probabilities of each possible candidate were calculated with the EXCEL spreadsheet provided by Sarotti et al. [18].

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Two cuvettes, one with stir bar and the other without stir bar, were put in the heater of the platelet aggregation apparatus, heated at 37 °C for 10 min. Then 250 μL of PRP and 2.5 μL of test sample were added into the cuvette with stir bar. 250 μL of PPP and 2.5 μL of DMSO were added into the cuvette without stir bar. After 5 min of heating, the two cuvettes were put at the test positions of PRP and PPP, respectively. After adjusting the baseline of recording curve, the inducer (1 μL of colloid) was added into the cuvettes. The curve of platelet aggregation was recorded and the maximum aggregation rate was accordingly calculated.

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The inhibition rate of the test samples against rabbit platelet aggregation induced by colloid could also be calculated. The formula is as follows: \( R = \frac{(A - B)}{A} \times 100\% \). \( R \): Inhibition rate, \( A \): The maximum aggregation of solvent, \( B \): The maximum aggregation of test sample (or reference substance).

### 3.6.2 Anticoagulant Activity

The test compounds were diluted with DMSO to 10 mM and then diluted with 0.02 M Tris-HCl (pH 7.4) with 5% Tween 80 to 1 Mm. The positive control was Low Molecular Weight Heparin (LMWH) and the blank control was 0.02 M Tris-HCl (pH 7.4) with 5% Tween 80 and 10% DMSO. The solution of the test sample or reference substance was added into the cuvette preheated at 37 °C and then the control plasma was also added into it. After heating at 37 °C for 2 min, the TT (thrombin time) reagent preheated at 37 °C was also added into it. The clotting time was recorded afterwards.

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**Compliance with Ethical Standards**

**Conflict of interest** The authors declare no conflict of interest.

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