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Phytochemistry 69 (2008) 1750–1755
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Biologically active tetralones from New Caledonian *Zygogynum* spp.

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Received 17 December 2007; received in revised form 30 January 2008
Available online 10 March 2008

Abstract

Bioassay guided purification of the ethyl acetate extracts of the bark and leaves of five New Caledonian *Zygogynum* species (Winteraceae) led to the isolation and characterization of four phenyl-3-tetralones (3,4-dihydronaphthalen-1(2H)-one). Their structures were determined by various NMR techniques and chemical studies. The absolute configuration of the compounds was established by circular dichroism. The compounds showed binding affinity for peroxisome proliferator-activated receptor-γ (PPAR-γ) and significant inhibitory activity against KB cancer cell line.

Keywords: *Zygogynum stipitatum*; *Zygogynum pancheri*; *Zygogynum acsmithii*; *Zygogynum bailloni*; Winteraceae; Phenyl-3-tetralone; Zygolone; PPAR-γ; Cytotoxicity

1. Introduction

The genus *Zygogynum* belongs to the family Winteraceae, order Magnoliales, which includes the most primitive dicotyledons flowering plants. The flowers of Winteraceae have indeed often been considered as representative of the floral morphology of the earliest angiosperms (Doust, 2000). This genus consists of some 41 species distributed in the west pacific region including Australia, Papua-New-Guinea and New Caledonia. Of these, 18 species are endemic to New Caledonia (White and Vink, 1993).

In a systematic search for bioactive compounds in plants from New Caledonia, the ethyl acetate extracts of *Zygogynum stipitatum* Baillon showed potent binding activity on peroxisome proliferator-activated receptor-γ (100% at 10 μg/ml). This receptor is probably the isoform attracting most of the attention, since it has become clear that agonists to this isoform could play a therapeutic role in the treatment of diabetes, obesity, inflammation and cancer (Murphy and Holder, 2000). Biological activity was evaluated on fractions obtained from a preliminary standard HPLC fractionation. Subsequent bioactivity-directed fractionation using silica gel chromatography and preparative HPLC, resulted in the isolation of compounds **1** and **2**. Investigation of four other species of the *Zygogynum* genus, *Zygogynum pancheri* subsp. *elegans* Vink, *Zygogynum pancheri* subsp. *pancheri* (Baill.) Vink, *Zygogynum acsmithii* Vink and *Zygogynum bailloni* Tieghem led to the isolation of two other compounds **3** and **4** along with the previous ones. As far as we know, the chemical constituents of the genus *Zygogynum* are not widely studied except for three papers dealing with leaf essential oil content in three species of Australian *Zygogynum* (Brophy et al., 1994), alkaloids isolation from *Z. pauciflorum* (Ahond et al., 1990) and drimane sesquiterpenoids isolated

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doi:10.1016/j.phytochem.2008.01.025

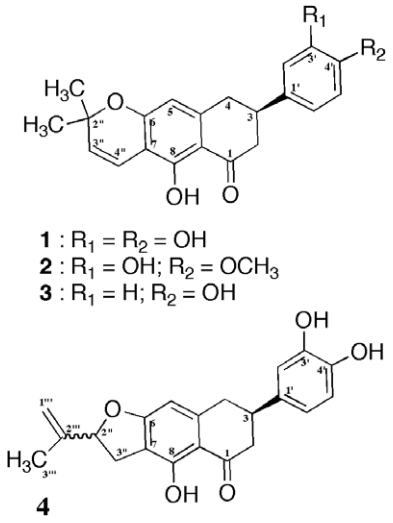

Fig. 1. Structures of compounds **1**–**4**.

from *Z. bailloni* in our team (Fomekong Fotsop et al., 2008).

In this report, we describe the isolation, structure elucidation and biological activities of four new phenyl-3-tetralones (3,4-dihydronaphthalen-1(2H)-one) named zygolone A (**1**), 4′-*O*–methyl zygolone A (**2**), 3′-deoxyzygolone A (**3**) and isozygolone A (**4**) from the bark and/or leaves of *Z. stipitatum, Z. pancheri, Z. acsmithii* and *Z. bailloni* (Fig. 1).

2. Results and discussion

Ground bark of *Z. stipitatum*, was extracted by ethyl acetate to give a crude extract. A small amount of this extract was submitted to a rapid filtration on a polyamide cartridge in order to remove polyphenols and tannins. The filtered extract was then submitted to a C-18 HPLC fractionation using a gradient mobile phase of acetonitrile/water 50:50 to 100:0 to give nine fractions as it was done in our previous studies (Bousserouel et al., 2005). Filtered extract and fractions were distributed into a 96 deep wells mother plate. Bioassay performed on daughter plate allowed to detect the active fraction. The filtered extract and fraction 4 exhibited 100% and 84% of binding activity at 10 μg/ml on PPAR-γ, respectively. A large amount of the ethyl acetate extract was then submitted to silica gel chromatography, followed by preparative HPLC (see experimental section) allowing to isolate compounds **1** and **2**. Using the same isolation procedure, the study of the bark of *Z. acsmithii* afforded compounds **1** and **4**, the bark of *Z. pancheri* subsp. *elegans* and *Z. pancheri* subsp. *pancheri* afforded compound **1**, and compound **3** was isolated from the leaves of *Z. bailloni* and *Z. pancheri* subsp. *pancheri*.

Compound **1**, obtained as a yellow oil, had the molecular formula $C_{21}H_{20}O_5$ supported by HRESIMS of the $[M+Na]^+$ ion peak at m/z 375.1237 (calcd. 375.1208). The IR spectral data of **1** suggested the presence of an aromatic ring (1520, 1446 cm^{-1}), as well as a conjugated carbonyl function (1660 cm^{-1}) and a hydroxy group (3328 cm^{-1}). The UV spectrum showed absorption bands at 275 nm and 303 nm. The ^1H and ^{13}C NMR spectra (Table 1) showed a low-field signal (δ_H12.95, 1H, *s*, OH-8) and a ketone carbonyl (δ_C 202.5, C-1). The signal of the C-1 atom appeared unusually down shielded at δ 202.5 because of a strong intramolecular H-bond with the OH group at C-8 as described by Kim et al. (1998). In the COSY spectrum of **1**, the correlations of the signal at δ 3.20 (1H, *m*, H-3) with those at δ 2.95 (2H, *m*, H-4) and two non-equivalent ketomethylene protons at δ 2.71 (1H, *dd*, $J = 17.5$, 12.1 Hz, H-2a) and δ 2.80 (1H, *dd*, $J = 17.5$, 2.4 Hz, H-2b) indicated the location of one saturated methine between two methylenes. In the aromatic part of the ^1H NMR spectrum of compound **1**, four aromatic protons appearing as one proton singlet at δ 6.16 (1H, *s*, H-5) and an AMX aromatic system pattern with one *meta*-coupled proton at δ 6.75 (1H, *d*, $J = 2$ Hz, H-2′), one *ortho*-coupled proton at δ 6.81 (1H, *d*, $J = 8$ Hz, H-5′) and one *ortho*- and *meta*-coupled proton at δ 6.65 (1H, *d*, $J = 8$, 2 Hz, H-6′) suggested the presence of a pentasubstituted and a 1,3,4-trisubstituted asymmetrical aromatic rings. Moreover, in the ^1H NMR spectrum, two *cis*-coupled olefinic protons at δ 6.65 (1H, *d*, $J_{3'',4''} = 10$ Hz, H-4″) and δ 5.53 (1H, *d*, $J_{3'',4''} = 10$ Hz, H-3″) and two methyl group singlet at δ 1.42 (6H, *s*, H-5″ and H-6″) indicated the presence of a 2,2-dimethylpyran ring. All these spectroscopic data suggested the presence of a trisubstituted tetralone moiety (Wang et al., 2006) along with a 2,2-dimethylpyran and a trisubstituted aromatic rings. This was also confirmed in the ^{13}C spectrum by the presence of eight quaternary aromatic carbons (four oxygenated) and four aromatic methines. In the HMBC spectrum, correlations observed between the proton at δ 6.75 (H-2′) and C-3, C-4′ and C-6′ (δ 39.9, 142.5 and 119.0, respectively), and the proton at δ 6.65 (H-6′) and C-3, C-2′ (δ 113.7) and C-4′, and the proton at δ 6.81 (H-5′) and C-1′ and C-3′ (δ 136.1 and 143.8, respectively) indicated the position of the 3′,4′-dihydroxyphenyl substituent at C-3. Other HMBC correlations observed between H-4″ (δ 6.65, *d*, $J_{3'',4''} = 10$ Hz) and carbons C-2″ (δ 77.9), C-6 (δ 160), C-7 (δ 107.3), C-8 (δ 159.3) and between the proton at δ 12.95 (OH-8) and C-7, C-8 and C-9 suggested that the dimethylpyran ring is fused to the first aromatic ring as depicted in Fig. 1. These data, together with other results of COSY and HMBC analysis, confirmed that compound **1** named zygolone A, was 8-hydroxy-3-(3′,4′-dihydroxyphenyl)-6,7-(2″, 2″-dimethylchromene)-tetralone.

Compound **2**, obtained as a yellow oil, possesses a molecular formula $C_{22}H_{22}O_5$ supported by HRESIMS of the $[M+H]^+$ ion peak at m/z 367.1535 (calcd. 367.1545). The ^1H NMR and ^{13}C NMR spectra of compound **2** were almost similar to those of compound **1**. However, they showed signals characteristic of a methoxy group at δ_H

Table 1
^1H and ^{13}C spectroscopic data (500 and 125 MHz, $CDCl_3$) for zygolone A (**1**) and analogues (**2**–**4**)

	1		2		3		4	
	δ_H (*m*, *J* Hz)	δ_C	δ_H (*m*, *J* Hz)	δ_C	δ_H (*m*, *J* Hz)	δ_C	δ_H (*m*, *J* Hz)	δ_C
1	–	202.5	–	202.4	–	202.5	–	202.3
2a	2.71 (*dd*, 17.5, 12.1)	45.5	2.74 (*dd*, 17.0, 12.4)	45.4	2.64 (*dd*, 17.0, 12.4)	45.6	2.70 (*dd*, 17.1, 12.4)	45.5
2b	2.80 (*dd*, 17.5, 2.4)	–	2.83 (*dd*, 17.0, 2.3)	–	2.76 (*dd*, 17.0, 2.1)	–	2.80 (*dd*, 17.1, 2.5)	–
3	3.20 (*m*)	39.9	3.26 (*m*)	39.9	3.30 (*m*) 39.9	3.24 (*m*)	40.1	
4	2.95 (*m*)	37.9	2.96 (*d*, 8)	37.9	3.00 (*d*, 8)	38.3	3.00 (*m*)	38.4
5	6.16 (*s*)	107.9	6.17 (*s*)	107.8	6.17 (*s*)	107.0	6.23 (*s*)	101.7
6	–	160.0	–	159.8	–	160.0	–	167.0
7	–	107.3	–	107.2	–	107.2	–	111.6
8	–	159.3	–	159.7	–	160.0	–	143.8
9	–	110.9	–	110.9	–	110.9	–	110.7
10	–	145.8	–	145.4	–	146.0	–	147.2
1′	–	136.1	–	136.4	–	135.0	–	136.3
2′	6.75 (*d*, 2)	113.7	6.84 (*d*, 2)	112.8	7.12 (*d*, 8.3)	128.0	6.77 (*d*, 1.5)	113.7
3′	–	143.8	–	145.7	6.80 (*d*, 8.3)	115.0	–	143.2
4′	–	142.5	–	145.7	–	145.4	–	142.3
5′	6.81 (*d*, 8)	115.5	6.81 (*d*, 8.3)	110.7	6.80 (*d*, 8.3)	115.0	6.82 (*d*, 8.1)	115.5
6′	6.65 (*dd*, 8, 2)	119.0	6.72 (*dd*, 8.3, 2)	118.0	7.12 (*d*, 8.3)	128.0	6.68 (*dd*, 8.1, 1.5)	119.0
2″	–	77.9	–	77.8	–	77.8	5.28 (*dd*, 9.8, 8)	87.9
3″a	5.53 (*d*, 10)	127.5	5.54 (*d*, 10)	127.5	5.50 (*d*, 10)	127.6	2.95 (*dd*, 15.7, 8)	30.7
3″b	–	–	–	–	–	–	3.30 (*dd*, 15.7, 9.8)	30.7
4″	6.65 (*d*, 10)	115.5	6.67 (*d*, 10)	115.6	6.67 (*d*, 10)	115.5	–	–
5″	1.42 (*s*)	28.4	1.44 (*s*)	28.3	1.44 (*s*)	28.4	–	–
6″	1.42 (*s*)	28.4	1.44 (*s*)	28.3	1.43 (*s*)	28.4	–	–
1‴a	–	–	–	–	–	–	4.91 (*s*)	112.6
1‴b	–	–	–	–	–	–	5.06 (*s*)	112.6
2‴	–	–	–	–	–	–	–	143.2
3‴	–	–	–	–	–	–	1.74 (*s*)	16.9
OH-8	12.95 (*s*)	–	13.0 (*s*)	–	13.0 (*s*)	–	12.58 (*s*)	–
OH-3′	5.70 (*brs*)	–	5.70 (*brs*)	–	–	–	5.41 (*brs*)	–
OH-4′	–	–	–	–	4.70 (*brs*)	–	–	–
OCH_3	–	–	3.87 (*s*)	56.0	–	–	–	–

3.87 (3H, *s*) and δ_C 56.0. The location of the methoxy group on C-4′ was suggested by observation of a C–H long range correlations from H-2′ at δ 6.84 (1H, *d*, $J = 2$ Hz), H-6′ at δ 6.72 (1H, *dd*, $J = 8.3$, 2 Hz), and the methoxy protons to C-4′ at δ 145.7, in the HMBC spectrum. This location was confirmed by observation of correlation between the methoxy group and H-5′ at δ 6.81 (1H, *d*, $J = 8.3$ Hz) in the NOESY spectrum. Other COSY and HMBC correlations are comparable with those observed for compound **1**. Consequently, compound **2**, named 4′-*O*–methyl zygolone A, was identified as 8-hydroxy-3-(3′-hydroxy,4′-methoxyphenyl)-6,7-(2″, 2″-dimethylchromene)-tetralone.

Compound **3**, obtained as a yellow oil, possesses a molecular formula $C_{21}H_{20}O_4$ supported by HRESIMS of the $[M + H]^+$ ion peak at *m*/*z* 337.1442 (calcd. 337.1440) suggesting the presence of only two hydroxy groups instead of three in the case of compound **1**. The ^1H NMR and ^{13}C NMR spectra of compound **3** were almost similar to those of compound **1**. However, its ^1H NMR spectrum showed signals for the B-aromatic ring constituted by a characteristic AA′BB′ system of a *para*-disubstituted benzene ring at δ 7.12 (2H, *d*, $J = 8.3$ Hz, H-2′ and H-6′) and δ 6.8 (2H, *d*, $J = 8.3$ Hz, H-3′ and H-5′). These features suggested the structure 8-hydroxy-3-(4′-hydroxyphenyl)-6,7-(2″,2″- dimethylchromene)-tetralone for compound **3,** which was named 3″-deoxyzygolone A.

Compound **4** was obtained as a yellow oil. Its molecular formula was determined as $C_{21}H_{20}O_5$ from the pseudomolecular ion peak at *m*/*z* 375.1228 (calcd. 375.1208) in the HRESIMS. As in the case of compound **1**, the IR spectrum of compound **4** displayed absorption bands for hydroxyl group (3343 cm^{-1}), conjugated carbonyl function (1670 cm^{-1}) and aromatic ring (1520, 1480 cm^{-1}). The UV spectrum showed absorption bands at 243 nm and 293 nm. The ^1H and ^{13}C NMR spectra of compound **4** were nearly similar to those of compound **1**, showing the characteristic AMX aromatic system pattern of a trisubstituted B-ring and signals related to the tetralone moiety. The main difference observed in the ^1H NMR spectrum of **4** was the disappearance of the two olefinic protons at δ 5.53 and δ 6.65 as well as the two methyl groups at δ 1.42 indicating a noticeable modification in the chromene moiety. The ^1H NMR spectrum exhibited a signal for a vinyl methyl at δ 1.74 (3H, *s*, H-3‴), together with the resonances of two geminal vinyl protons at δ 4.91 (1H, *s*, H-1‴a) and δ 5.06 (1H, *s*, H-1‴b) suggesting an isopropenyl unit. In the ^1H-^1H COSY spectrum, the observation of correlations between protons at δ 5.28 (H-2″, *dd*, $J =$ 9.8,

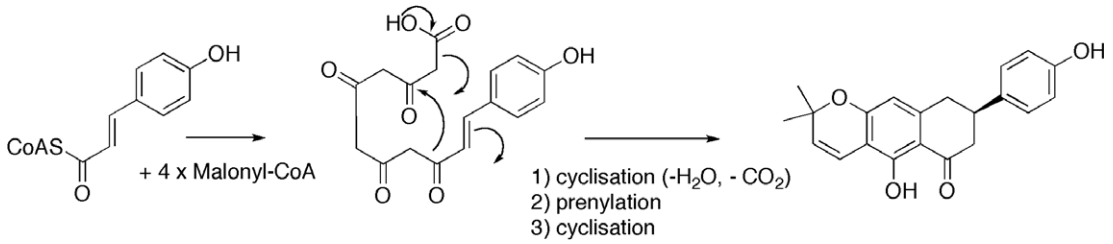

Fig. 2. Hypothetical biogenetic pathway proposed for compound **3**.

8 Hz), at δ 2.95 (H-3″a, *dd*, $J = 15.7$, 8 Hz) and at δ3.3 (H-3″b, *dd*, $J = 15.7$, 9.8 Hz) suggested the presence of a substituted dihydrofuran ring. In the HMBC spectrum, long range correlations from the methyl protons at δ 1.74 to C-1‴, C-2‴ and C-2″ and from the methylene protons H-3″ a and H-3″b to C-6, C-7, C8 and C-2‴ allowed to confirm that compound **4**, possessed a 2-(prop-1-en-2-yl)-2,3-dihydrofuran moiety fused to the tetralone ring as shown in Fig. 1. Therefore, compound **4**, named isozygolone A, was assigned the structure 8-hydroxy-3-(3′,4′-dihydroxyphenyl)-6,7-(2″(prop- 1‴-en-2‴-yl)-2″, 3″-dihydrofuran)-tetralone.

The CD spectra of compounds **1**–**4** showed five Cotton effects in $CHCl_3$ (see experimental section). The negative effects at 320–324 nm and the positive ones at 269–273 nm, recorded for compounds **1**–**4**, were attributed to the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions of the aryl-ketone chromophore of the molecules, respectively, as it was observed for flavanones (Slade et al., 2005). The absolute configuration of compounds **1**–**4** has been determined by the comparison of the Cotton effects between the phenyl-3-tetralone and the flavanone chemical series. Indeed, the presence of a methylene group on position 4 in the tetralones instead of an oxygen atom in the flavanones should not affect the sign of the Cotton effects observed. Since levorotatory flavanones with equatorial 2-aryl substituent exhibit a positive Cotton effect due to the $n \rightarrow \pi^*$ transitions (320–330 nm) and a negative Cotton effect in the $\pi \rightarrow \pi^*$ region (270–290 nm) (Gaffield, 1970), and due to the fact that the sign of the $n \rightarrow \pi^*$ band of CD is independent of the substitution pattern of the aromatic ring system (Snatzke et al., 1973), compounds **1**–**4**, which exhibited opposite Cotton effects, possess therefore the *R* configuration at C-3. The absolute configuration at C-2″ of compound **4** remain undetermined.

The binding affinity of compounds **1**–**4** for PPAR-γ was evaluated by competition against an isotopically labeled reference compound (rosiglitazone), as described earlier (Ferry et al., 2001). Results are given by the K_i value which is the inhibition constant of a compound determined at equilibrium in competition with a reference ligand. Compounds **1**–**4** exhibited K_i values of 5.8, 17.9, 4.9 and 4.7 μM, respectively, indicating a strong binding activity on PPAR-γ. However, compounds **1**–**4** showed significant cytotoxic activity on KB cancer cell line with IC_{50} values of 1.4, 6.5, 2.6 and 3.5 μM, respectively.

3. Conclusions

In the present investigation, chemical studies of *Z. stipitatum*, *Z. pancheri* subsp. *elegans, Z. pancheri* subsp. *pancheri, Z. bailloni* and *Z. acsmithii* have led to the isolation and identification of four new phenyl-3-tetralones. To the best of our knowledge, this is the first time that some natural phenyl-3-tetralones were isolated and identified from higher plants. From a biogenetic point of view, these compounds could result from sequential condensations of 4-coumaroyl-CoA with four C_2 units coming from malonyl-CoA followed by a Claisen-type reaction, leading to the formation of an aromatic polyketide (Abe et al., 2004). Simultaneous decarboxylation and cyclisations of this intermediate would lead to the formation of the phenyl-3-tetralones. A subsequent prenylation followed by a final cyclisation would lead to the formation of zygolones (Fig. 2). These compounds, present in five different *Zygogynum* spp., could be seen as a chemical marker of this genus. Their isolation is a consequence of a random screening of New Caledonian plant extracts on a given receptor associated with the use of a new strategy of search for new active natural products. The four compounds showed binding activity on peroxisome proliferator-actived receptor-γ and exhibited significant cytotoxicity on KB cells.

4. Experimental

4.1. General experimental procedures

The NMR spectra were recorded on a Bruker 500 MHz (advance 500) spectrometer. Samples dissolved in $CDCl_3$ were reported in δ (ppm) downfield from TMS. ESIMS was obtained on a Navigator mass Thermoquest. HRESIMS were run on a MALDI-TOF spectrometer (Voyager-De STR; Perspective Biosystems). Kromasil semi-preparative and preparative C_{18} columns (250 × 10 mm and 250 × 21.2 mm, I.D, 5 μm, Thermo®) were used for preparative HPLC separations using a Waters autopurification system equipped with a binary pump (Waters 2525), a UV–Vis diode array detector (190–600 nm, Waters 2996) and a PL–ELS 1000 ELS detector Polymer Laboratory. IR spectra were obtained on a Nicolet FTIR 205 spectrophotometer. The UV spectra were recorded on a Perkin–Elmer Lambda 5 spectropho-

tometer. Optical rotations and CD spectra at 22 °C were measured on a JASCO P-1010 polarimeter and a JASCO J-810 spectrophotometer, respectively. Silica gel 60 (35–70 μm) and analytical TLC plates (Si gel 60 F 254) were purchased from SDS (France). All other chemicals and solvents were analytical grade and used without further purification.

4.2. Plant material

The bark and leaves of *Z. stipitatum* Baillon, *Z. pancheri* subsp. *elegans* Vink, *Z. pancheri* subsp. *pancheri* (Baill.) Vink, *Z. acsmithii* Vink and *Z. bailloni* Tieghem, were collected in different ecological formations of the main island of New Caledonia; dense rain forest (Tchamba, Tchingou and Nodela, North Province) for the three first, maquis flora (Goro and Monts Dzumacs, South Province) for the two last, by two of us (ML and VD). The corresponding voucher specimens (LIT-0175, DUM-220, DUM-578, DUM-197 and LIT-1275, respectively) are kept at the Herbarium of the Botanical and Tropical Ecology Department of the IRD Center, Noumea, New Caledonia.

4.3. Extraction and isolation

The milled and dried bark of *Z. stipitatum* (890 g) were extracted sequentially by maceration in hexane (3 × 2.0 l), EtOAc (3 × 4.5 l) and MeOH (3 × 4.5 l) at room temperature and concentrated under *vacuum* at 40 °C to afford 10, 23.4 and 29.5 g, respectively. The EtOAc extract (4.5 g) was submitted to a silica gel column chromatography using a gradient of *n*-hexane-CH_2Cl_2 (80:20 to 0:100) and CH_2Cl_2–MeOH (100:0 to 80:20) to give 13 fractions (fractions 1–13) according to their TLC profile. Fraction 7 (2.67 g) was submitted to a second silica gel column chromatography using a gradient of CH_2Cl_2–MeOH (100:0 to 50:50) to give 11 fractions (fractions 7–1 to 7–11). From fraction 7–4 (211 mg), a semi-preparative C-18 Kromasil column, (250 × 10 mm, I.D, 5 μm) using an isocratic mobile phase consisting of MeCN–H_2O 60:40 at 4.7 ml min^{-1} was used to purify zygolone A (**1**) (t_R 11.2 min, 35 mg). The same method was applied to fractions 4 + 5 (217 mg) to give 4′-*O*-methyl zygolone A **2** (t_R 22.5 min, 31 mg). Extraction of bark (200 g) with EtOAc (3 × 1 l, 1 h each) of *Z. pancheri* subsp. *elegans* yield 4.2 g of crude extract. The EtOAc extract was submitted to a silica gel column chromatography using a gradient of *n*-heptane/AcOEt (80:20 to 0:100) and washed with MeOH to give 19 fractions (fractions 1–19) according to their TLC profile. Fraction 9 was submitted to a preparative HPLC (C-18 Kromasil column, 250 × 21.2 mm, I.D, 5 μm) using an isocratic mobile phase MeCN–H_2O 50:50 at a flow rate of 21.2 ml min^{-1} to give **1** (t_R 25 min, 11.8 mg). Extraction of bark (200 g) with EtOAc (3 × 1 l, 1 h each) of *Z. acsmithii* yield 3.3 g of crude extract. The EtOAc extract was submitted to a silica gel column chromatography using a gradient of *n*-heptane/AcOEt (80:20 to 0:100) and washed with MeOH to give 14 fractions (fractions 1–14) according to their TLC profile. Fraction 9 was submitted to a preparative HPLC (C-18 Kromasil column, 250 × 21.2 mm, I.D, 5 μm) using an isocratic mobile phase MeCN–H_2O 50:50 at a flow rate of 21.2 ml min^{-1} to give **1** (t_R 25.2 min, 4 mg) and isozygolone A **4** (t_R 19.8 min, 4 mg). The leaves of *Z. bailloni* (600 g) were extracted sequentially by maceration in *n*-hexane (3 × 2.0 l), EtOAc (3 × 4.0 l), and MeOH (3 × 4.0 l) at room temperature and concentrated under vacuum at 40 °C to afford 4.4, 17.9 and 34.5 g, respectively. The EtOAc extract (9.3 g) was submitted to a silica gel column chromatography using a gradient of *n*-heptane-AcOEt (80:20 to 0:100) and AcOEt–MeOH (100:0 to 80:20) to give 15 fractions (fractions 1–15) according to their TLC profile. Preparative HPLC purification performed on fraction 2 (962 mg/1.22 g) (C-18 Kromasil column, 250 × 21.2 mm, I.D, 5 μm) using an isocratic mobile phase consisting of MeCN–H_2O 65:35 at a flow rate of 21.2 ml min^{-1} afforded 3′-deoxyzygolone A **3** (t_R 17.8 min, 31 mg). Using the same procedure, the purification of the EtOAc extracts obtained from leaves (580 g) and bark (490 g) of *Z. pancheri* subsp. *pancheri*, afforded compounds **3** (9 mg) and **1** (47 mg), respectively.

4.4. Compound *1* (*zygolone A*)

Yellow oil; $[\alpha]_D^{22^\circ}$ +22.4° (*c* 0.1, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3328, 2920, 2358, 1660, 1600, 1520, 1305, 1135, 1087, 812 cm^{-1}; UV ($CHCl_3$) λ_{max}(log ε) 275 nm (4.46), 303 nm (3.98); CD ($CHCl_3$, *c* 1.42 mmol/l) $\lambda_{ext}(\Delta\varepsilon)$ 226 (−5.6), 239 (−4.6), 273 (7.9), 295 (−1.2), 320 (−2.2); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR, ($CDCl_3$, 125 MHz), see Table 1. HRESIMS of the $[M + Na]^+$ ion peak at *m*/*z* 375.1237 (calcd. 375.1208).

4.5. Compound *2* (*4′-O-methyl zygolone A*)

Yellow oil; $[\alpha]_D^{22^\circ}$ +20° (*c* 0.1, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3454, 2910, 1615, 1520, 1300, 1140, 720 cm^{-1}; UV ($CHCl_3$) λ_{max} (logε) 270 nm (4.36), 302 nm (3.90); CD ($CHCl_3$, *c* 1.45 mmol/l) $\lambda_{ext}(\Delta\varepsilon)$ 227 (−5.9), 235 (−4.6), 272 (7.3), 295 (−1.2), 321 (−2.5); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1. HRESIMS $[M + H]^+$ *m*/*z* 367.1535 (calcd. for 367.1545).

4.6. Compound *3* (*3′-deoxyzygolone A*)

Yellow oil; $[\alpha]_D^{22^\circ}$ +21° (*c* 0.1, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3450, 2930, 2340, 1614, 1500, 1410, 1320, 1135, 920 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 270 nm (4.15), 300 nm (3.85); CD ($CHCl_3$, *c* 1.59 mmol/l) $\lambda_{ext}(\Delta\varepsilon)$ 225 (−5.4), 238 (−4.2), 270 (7.6), 293 (−1.4), 321 (−2.4); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1. HRESIMS $[M + H]^+$ *m*/*z* 337.1442 (calcd. 337.1440).

4.7. Compound **4** (isozygolone A)

Yellow oil; $[\alpha]_D^{22^\circ}$ +69.6° (*c* 0.1, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3343, 2920, 2358, 1670, 1601, 1520, 1480, 1444, 1323, 1273, 1124, 1087, 814 cm^{-1}; UV ($CHCl_3$) $\lambda_{max}(\log \varepsilon)$ 243 nm (4.19), 293 nm (4.35); CD ($CHCl_3$, *c* 1.42 mmol/l) $\lambda_{ext}(\Delta\varepsilon)$ 223 (4.1), 232 (−3.7), 269 (7.6), 293 (−1.8), 324 (−2.2); ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1. HRESIMS of the $[M + Na]^+$ ion peak at *m*/*z* 375.1228 (calcd. 375.1208).

4.8. Biological activity

The binding affinity of compounds **1**–**4** was evaluated on PPAR-γ by competition against an isotopically labeled reference compound (rosiglitazone), as described by Ferry et al. (2001). Results are expressed as binding activity, i.e. percentage of inhibition of the binding of labeled reference compound, or as K_i, the concentration corresponding to 50% of such inhibition, and corrected for experimental conditions according to Cheng and Prusoff (1973). The human KB tumor cell line, mouth epidermoid carcinoma was originally obtained from the ATCC. The cytotoxicity assays were performed according to a published procedure (Tempete et al., 1995). Taxotere was used as reference compound.

Acknowledgement

The authors are very grateful to North and South Provinces of New Caledonia who facilitated our field investigation. The authors are very grateful to Dr. Tanguy Jaffré and Dr. Jean-Marie Veillon of the Botany and Plant Ecology Department, Research Institute for the Development, Nouméa, for their help in the identification of the plants, Bruno Fogliani and Thomas Pouplin, for technical assistance in the plant collection, and Geneviève Aubert, (ICSN) who performed the cytotoxic assays on KB cells.

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