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Euphorbias of South Africa: Two New Phorbol Esters from *Euphorbia bothae*

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ABSTRACT

Two known phorbol esters, 12-deoxyphorbol-13-isobutyrate-20-acetate (1) and 12-deoxyphorbol-13-(2-methylbutyrate)-20-acetate (2), and two new phorbol esters, 12-deoxyphorbol-13-isobutyrate-16-angelate-20-acetate (3) and 12-deoxyphorbol-13-(2-methylbutyrate)-16-angelate-20-acetate (4), were isolated from the endemic South African plant *Euphorbia bothae*. Standard spectroscopic techniques were used to elucidate the structures of all four compounds. The interaction of 1–4 with opioid receptors was explored in an attempt to explain the unexplained stupor occasionally observed in herbivores browsing on *E. bothae*.

KEYWORDS

***Euphorbia bothae*, Euphorbiaceae, phorbol ester, opioid receptor.**

1. Introduction

Euphorbia species are prolific producers of secondary metabolites including sesqui-, di- and triterpenes.[1,2] Several *Euphorbia* species, including *E. ledienii* Berg, *E. coerulescens* Haw, *E. triangularis* Desf., *E. ingens* E. May, *E. tetragona* Haw and *E. cooperi* N. E. Br., are common to the arid regions of Southern Africa and are collectively referred to by the inhabitants of these areas as either 'noors' or 'noorsdoring'.[3] A further 'noors' species, *Euphorbia bothae* (Lotsy and Goddijn), is a succulent plant endemic to the thicket biome of the Eastern Cape province of South Africa.[4] Despite the irritant nature of latex exudates from this species, *E. bothae* has been shown to be a favoured browse species of the black rhinoceros (*Diceros bicornis)* in the Great Fish River valley of the Eastern Cape where this plant can comprise as much as 41% of the black rhino's diet.[5]

An earlier study of extractives of three South African 'noors' species *viz E. ledienii, E. coerulescens,* and *E. triangularis* attributed the irritant activity inherent in these plant species to a series of nine phorbol mono, di- and tri- esters differing in their esterification patterns around a common tigliane diterpene scaffold.[3] We therefore anticipated that phorbol ester secondary metabolites were similarly responsible for inducing the irritant contact dermatitis which we experienced on handling specimens of *E. bothae.* ^{1}H NMR analysis of extractives of *E. bothae* revealed a clearly resolved broad singlet at δ_H 7.60 reminiscent of the signal for the olefinic proton H-1 in the unsaturated tigliane skeleton of phorbol esters, and the presence of this proton signal in the various *E. bothae* chromatography fractions proved useful in successfully targeting fractions containing phorbol esters.

Interestingly, observations by wildlife field guides working in the Great Fish River valley attest to *E. bothae*'s ability to sporadically induce a state of stupor amongst herbivores e.g. kudu (*Tragelaphus strepsiceros*) and omnivores e.g. Chacma baboons (*Papio ursinus ursinus*).[6] Opiate and endogenous opioid neuropeptides induce their characteristic euphoric actions by stimulating in particular supraspinal mu-opioid receptors, with less prominent involvement of delta and kappa opioid receptors.[7] For the most part, biologically active phorbol esters are powerful activators of protein kinase C (PKC).[2] One of the many biological functions of this subcellular messenger is to up-regulate mu-opioid receptor expression.[8] This response it shares with cocaine, a widely recognized drug of abuse.[9] In fact, phorbol esters are implicated in autoregulation of the mu-opioid receptor and in regulating the perception of pleasure and euphoria.[10] This prompted the rationale to explore a link between possible opioid receptor binding by the phorbol ester metabolites in *E. bothae* and the dazed (almost trance-like) state occasionally observed in wildlife eating this plant.

2. Results and Discussion

The aerial parts of *E. bothae* were lyophilized and steeped in acetone overnight. The acetone extract was initially fractionated using polymeric (Diaion HP-20) reversed-phase separation and one of the semi-polar fractions (80:20 Me_2CO:H_2O) thus obtained was subjected to silica gel column chromatography followed by semi-preparative normal phase HPLC to afford two known phorbol esters, 12-deoxyphorbol-13-isobutyrate-20-acetate (**1**) and 12-deoxyphorbol-13-(2-methylbutyrate)-20-acetate (**2**) in addition to two new phorbol esters, 12-deoxyphorbol-13-isobutyrate-16-angelate-20-acetate (**3**) and 12-deoxyphorbol-13-(2-methylbutyrate)-16-angelate-20-acetate (**4**).

The structures of the known compounds **1** and **2** were determined by recourse to a combination of 1D and 2D NMR and HRESI mass spectral data. The 12-deoxyphorbol esters **1** and **2** have been previously reported to co-occur in four southern African *Euphorbia* species; *E. ledienii,*[2] *E. coerulescens,*[2] *E. triangularis*[2] and *E. fortissimo,*[11] and two west African *Euphorbia* species, *E. poisonii* Pax. and *E. unispina* N.E.[12] NOESY data confirmed the relative stereochemistry around the tigliane skeleton of **1** ($[\alpha]_D^{25}$ +55) and **2** ($[\alpha]_D^{25}$ +95) isolated from *E. bothae.* Surprisingly, given the ubiquitous occurrence of **1** in several species of *Euphorbia* collected across Africa,[2,11–17] only Fattoruso *et al.*[13] have

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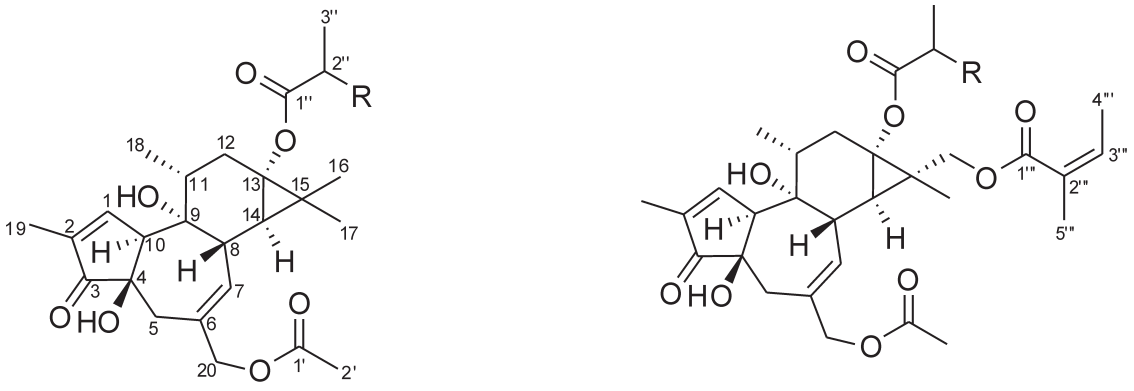

Scheme 1

reported an optical rotation ($[\alpha]_D^{25}$ +121) for this compound which they isolated from the Moroccan species, *E. resinifera*. The ^{1}H and ^{13}C NMR chemical shift data of **1**, isolated from *E. bothae* (Table 1), were in accordance with those reported previously for **1** by Fattoruso *et al.*[13] suggesting that, despite the difference in magnitude between their respective optical rotations, these compounds are identical. To the best of our knowledge neither an optical rotation nor a complete spectroscopic characterization has been previously reported for **2** and a fully assigned ^{1}H and ^{13}C NMR data set for **2** is presented in Table 1.

Compound **3** was isolated as a colourless oil and the positive-ion HRESI mass spectrum of this compound revealed a single pseudomolecular ion indicative of a molecular formula of $C_{31}H_{42}O_9$ (581.2705 $[M + Na]^+$, Δ 3.7 ppm). The ^{1}H and ^{13}C NMR

Table 1 ^{1}H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR data for **1–4**.

	1		**2**		**3**					**4**				
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C		^{1}H			^{13}C		^{1}H		
Pos	δ/ppm	δ/ppm	δ/ppm	δ/ppm	δ/ppm	$^1J_{CH}$/Hz	δ/ppm	mult	J/Hz	δ/ppm	$^1J_{CH}$ (Hz)	δ/ppm	mult	J/Hz
1	161.6	7.61	161.6	7.61	161.3	167	7.60	br.s		161.4	167	7.61	br.s	
2	133.0		133.0		133.1					133.2				
3	209.2		209.2		209.0					209.0				
4	73.8		73.8		73.7					73.7				
4-OH		2.12		2.08			2.11	br.s				2.15	br.s	
5a	39.2	2.51	39.3	2.51	39.3	129	2.52	d	19.6	39.3	127	2.52	d	18.9
5b		2.38		2.39		124	2.38	d	19.1		125	2.38	d	18.9
6	135.0		134.9		135.4					135.4				
7	134.2	5.73	134.2	5.73	133.0	156	5.71	d	4.9	133.1	157	5.71	d	4.6
8	39.7	3.00	39.7	3.00	39.0	128	3.07	t	5.1	39.1	128	3.07	t	5.0
9	76.1		76.1		76.2					76.2				
9-OH		5.62		5.65			5.61	s				5.65	s	
10	55.9	3.29	55.9	3.29	55.8	127	3.31	br.s		55.8	127	3.31	br.s	
11	36.6	1.96	36.6	1.96	36.5	130	1.99	m		36.5	130	1.99	m	
12a	31.9	2.06	32.0	2.07	31.8	132	2.10	dd	14.8, 6.9	31.8	134	2.10	dd	
	14.5,7.2													
12b		1.53		1.53		129	1.57	dd	14.6, 11.6		128	1.58	m	
13	63.2		63.2		63.0					63.0				
14	32.7	0.80	32.7	0.80	31.3	161	1.14	m		31.3	162	1.13	d	5.3
15	23.1		23.0		26.6					26.5				
16a	23.4	1.20	23.4	1.20	69.5	139	4.25	d	11.5	69.5	146	4.24	d	11.5
16b						145	4.06	d	11.3		149	4.06	d	11.4
17	15.5	1.07	15.5	1.07	11.7	127	1.20	s		11.7	127	1.20	s	
18	18.7	0.88	18.7	0.88	18.6	129	0.90	d	6.5	18.6	126	0.90	m	
19	10.3	1.79	10.3	1.79	10.3	128	1.79	br.s		10.3	128	1.79	s	
20a	69.9	4.47	70.0	4.47	69.6	150	4.46	d	12.4	69.7	148	4.46	d	12.5
20b		4.44		4.44		150	4.43	d	12.4		148	4.43	d	12.4
1′	171.0		171.0		170.9					170.9				
2′	21.1	2.05	21.1	2.05	21.1	130	2.05	s		21.1	130	2.05	s	
1″	179.2		178.9		179.1					178.9				
2″	34.5	2.54	41.4	2.35	34.4	129	2.52	sept	7.2	41.2	130	2.33	sex	7.0
3″a	18.9	1.16	26.7	1.69	18.7	129	1.13	d	6.9	26.6	121	1.65	sept	7.4
3″b				1.47							124	1.45	sept	7.2
4″	18.8	1.16	11.8	0.92	18.7	129	1.14	d	7.1	11.7	126	0.90	m	
5″	–	–	16.5	1.13	–	–	–	–	–	16.2	129	1.11	d	7.2
1‴	–	–	–	–	168.2					168.2				
2‴	–	–	–	–	128.1					128.1				
3‴	–	–	–	–	137.7	151	6.09	q	7.3	137.7	153	6.09	q	7.1
4‴	–	–	–	–	15.9	127	1.99	d	7.3	15.9	128	1.99	d	7.3
5‴	–	–	–	–	20.8	128	1.91	br.s		20.8	128	1.91	s	

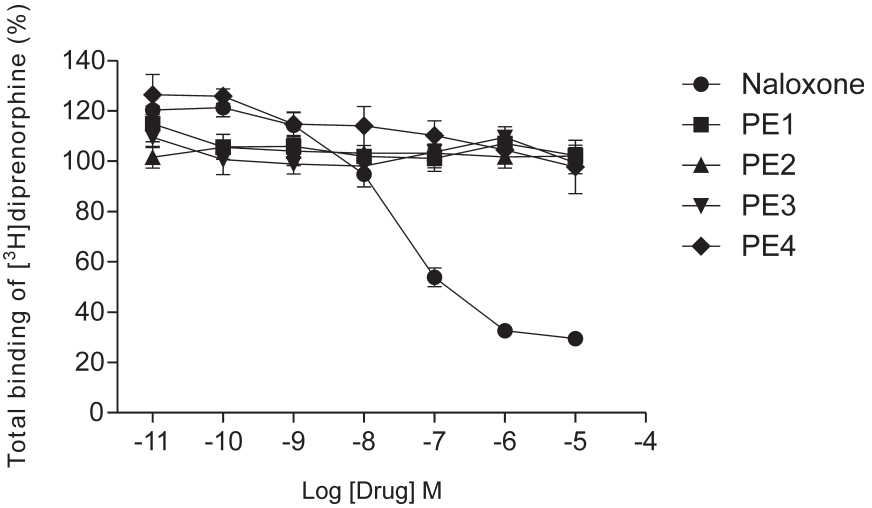

Figure 1 Competition with total [^{3}H]-diprenorphine (2 nM) binding by phorbol esters (PE) **1**–**4**. The results are represented as the mean standard error of duplicate determinations from three separate experiments for each data point.

chemical shift data of **3** (Table 1) showed signals consistent with the tigliane-type diterpene skeleton in **1** and **2** which thus accounted for seven of the 11 double bond equivalents required by the molecular formula of **3**. Comparison of the NMR data of **3** with those of **1** also suggested that both the C-13 isobutyryl and C-20 acetate ester moieties in **1** were conserved in **3**, thus accounting for a further two double bond equivalents. Further esterification of one of the two C-15 methyl groups in **3** was initially implied from the disappearance of one of the geminal methyl signals in the ^{1}H NMR spectrum of this compound. The ^{13}C NMR spectrum of **3** differed from the ^{13}C NMR spectrum of **1** through the appearance of six new, clearly delineated carbon resonances in the former spectrum which included: an additional ester carbonyl (δ_C 168.2), an olefinic methine (δ_C 137.7), an olefinic quaternary carbon (δ_C 128.1), a strongly deshielded methylene (δ_C 69.5), and two methyl carbons (δ_C 15.9 and 20.8).

An HMBC correlation from H-14 (δ_H 1.14) to the deshielded methylene carbon (C-16: δ_C 69.5) supported the esterification of one of the C-15 methyl groups with HMBC correlations from both methylene proton signals (H_2-16, δ_H 4.06 and 4.25) to the ester carbonyl (C-1‴ : δ_C 168.2) providing further unequivocal confirmation of the site of esterification. The structure of the unsaturated angelate ester functionality at C-15 followed from a series of HMBC correlations between the olefinic methine quartet (H_3-3‴, δ_H 6.09,) and the vinylic methyl carbon (C-4‴, δ_H 1.99) and from H_3-4‴ (δ_H 1.99) to both C-3‴ (δ_C 137.7) and the olefinic quaternary carbon C-2‴ (δ_C 128.1). Finally, HMBC correlations from the methyl singlet H_3-5‴ (δ_H 1.91) to both C-1‴ and C-3‴ positioned the remaining methyl group of the angelate ester. The geometry of the double bond was determined to be (*E*) from a NOESY correlation observed between H_3-5‴ and H-3‴. NOESY correlations between H-14 and both the methylene protons on C-16 placed the ester functionality in an α-position and defined the configuration at C-15. NOESY data also confirmed that **3** possessed the same relative configuration as **1** and **2**.

Positive-ion mode HRESIMS analysis of the colourless oil **4** gave rise to a single pseudomolecular ion suggesting a molecular formula of $C_{32}H_{44}O_9$ (595.2878 $[M + Na]^+$, Δ 0.8 ppm) requiring eleven double-bond equivalents and differing from the molecular formula of **3** by 14 a.m.u. Extensive congruency between the ^{1}H and ^{13}C NMR data of **3** and **4** was clearly evident (Table 1) and the only significant difference between these two compounds was confined to the structure of the ester functionality at C-13. This difference was consistent with the structural difference observed between the C-13 isobutyrate and 2-methylbutyrate ester moieties of **1** and **2** respectively. The structure of the C-13 2-methylbutyrate ester functionality in **4** was supported by the occurrence of similar correlations in the HMBC spectrum of **4** to those observed in the HMBC spectrum of **2**, and used to establish the structure of this ester functionality in the latter compound. Finally, NOESY data confirmed the α-orientation of the angelate ester substituted C-16 methylene moiety and indicated the same general relative configuration proposed for **1**–**3**.

To explore the possibility that opioid receptor binding may be the mechanism by which the phorbol ester metabolites exert their stupor state, competitive [^{3}H]diprenorphine (a non-selective opioid receptor antagonist) binding to opioid receptors in the presence of **1**–**4** was examined in an *ex vivo* competitive radioligand receptor assay using whole rat brain extract. Naloxone (an opioid receptor antagonist) was used as a reference substance. If the phorbol ester metabolites from *E. bothae* had affinity for any of the opioid receptors (mu, delta or kappa), a well-defined S-shaped displacement curve similar to that obtained with naloxone (Fig. 1) would have been evident, i.e. increasing phorbol ester concentrations would compete with and displace the [^{3}H]diprenorphine off the receptor. Kramer and Simon have clearly established that mu-opioid receptor agonists such as morphine increase [^{3}H]diprenorphine binding.[10] However, as depicted in Fig. 1, none of the four phorbol esters from *E. bothae* behaved as displacers of [^{3}H]diprenorphine from opioid receptors and none of them induced any concentration related inhibition of [^{3}H]diprenorphine binding over a wide concentration range of 100 nM – 1 μM. These data suggest that the opioid system is not an immediate biological target for **1**–**4**, and that possibly another signalling system, such as dopamine, may be responsible for the well-described central nervous system (CNS) effects of *E. bothae*. Phorbol ester-mediated activation of PKC is also known to phosphorylate the dopamine transporter leading to an increase in dopamine concentration in the synapse,[18] which in turn will lead to a reactive change in dopamine receptor binding. Since increased synaptic levels of dopamine are psychotomimetic,[7] dopamine receptor binding and/or dopamine release studies may represent an alternative mechanism worth investigating. There is also the possibility that other, as yet identified, secondary metabolites in *E. bothae* may be responsible for the CNS effects in the herbivores attracted to this species.

3. Experimental

3.1. General

The ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra were recorded on a Bruker 600 MHz Avance II spectrometer using standard pulse sequences. All directly-detected ^{1}H and ^{13}C chemical shifts (δ) were internally referenced to the residual solvent peak. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. HRESIMS were performed on a Waters API Q-TOF Ultima spectrometer by Dr Marietjie Stander at the Central Analytical Facility, Stellenbosch University. Diaion HP20 polystyrene divinylbenzene resin (supplied by Supelco) and Kieselgel 60 (230–400 mesh ASTM) were used for the initial chromatographic separations. HPLC was performed using a Whatman Magnum Partisil 10 Si semi-preparative column (10 × 500 mm) on a Spectra-Physics Spectra-Series P100 isocratic pump and a Waters 410 Differential Refractometer.

3.2. Plant Material

The aerial non flowering parts of *E. bothae* (Lotsy and Goddijn) were collected in September 2008 in the Great Fish River Reserve, Eastern Cape, South Africa (33°07′S, 26°38′E) where it dominates the 'short *Euphorbia* thicket' prevalent in the south-western region of this reserve.[5] Although mostly regarded as an independent species,[5] *E. bothae* has also been described as a hybrid of *E. coerulescens* Haw and *E. tetragona* Haw.[19] A voucher specimen of *E. bothae* was deposited in the Selmar Schonland Herbarium (GRA), Albany Museum, Grahamstown. Samples of *E. bothae* were frozen immediately after collection in the field and kept at –18 °C until extracted.

3.3. Extraction and Isolation

Lyophilized plant material (158 g dry wt) of *Euphorbia bothae* was extracted with Me_2CO (2 × 2.0 l) for 18 h. The Me_2CO extracts were combined and decolorized with activated charcoal (140 g) and left to stir at RT for 1 h. The extract was filtered through celite and passed through a column of HP20 resin (150 mL). The eluent was diluted with H_2O (4.0 l) and passed through the column again. The resulting eluent was diluted with H_2O (17.0 L) and passed through the column one final time. The column was eluted successively with 500 mL volumes of H_2O, 20%, 40%, 60% and 80% Me_2CO in H_2O and Me_2CO. The 80% Me_2CO in H_2O was concentrated to dryness under reduced pressure to yield 413.1 mg of material. The fraction was divided into two equal portions which were each passed through a normal phase column (Si, 100 mL). The columns were eluted successively with 300 mL volumes of CH_2Cl_2, 1%, 2% and 10% MeOH in CH_2Cl_2. The 2% MeOH in CH_2Cl_2 fractions were combined and reduced to dryness under reduced pressure. The resulting material was separated on a semi-preparative Si normal phase HPLC column with 25% EtOAc in hexanes as the mobile phase at a flow rate of 4 mL/min to yield phorbol esters **2** (10.1 mg), **1** (9.9 mg), **4** (3.9 mg) and **3** (17.1 mg) with retention times of 31.8, 36.0, 42.8 and 46.0 min, respectively.

3.4 Characterization Data

3.4.1. *12-Deoxyphorbol-13-isobutyrate-20-acetate* (**1**)

Colourless oil; $[\alpha]_D^{25}$ +55 (*c* 0.27, CH_2Cl_2); ^{1}H and ^{13}C NMR data see Table 1; HRESIMS, obsd. *m/z* 483.2372 $[M + Na]^+$, $C_{26}H_{36}O_7Na$ requires 483.2359, Δ 1.3 ppm.

3.4.2. *12-Deoxyphorbol-13-(2-methylbutyrate)-20-acetate* (**2**)

Colourless oil; $[\alpha]_D^{25}$ +95 (*c* 0.10, CH_2Cl_2); ^{1}H and ^{13}C NMR data see Table 1; HRESIMS, obsd. *m/z* 497.2500 $[M + Na]^+$, $C_{27}H_{38}O_7Na$ requires 497.2515, Δ 3.1 ppm.

3.4.3. *12-Deoxyphorbol-13-isobutyrate-16-angelate-20-acetate* (**3**)

Colourless oil; $[\alpha]_D^{25}$ +65 (*c* 0.35, CH_2Cl_2); ^{1}H and ^{13}C NMR data see Table 1; HRESIMS, obsd. *m/z* 581.2705 $[M + Na]^+$, $C_{31}H_{42}O_9Na$ requires 581.2727, Δ 2.2 ppm.

3.4.4. *12-Deoxyphorbol-13-(2-methylbutyrate)-16-angelate-20-acetate* (**4**)

Colourless oil; $[\alpha]_D^{25}$ +70 (*c* 0.15, CH_2Cl_2); ^{1}H and ^{13}C NMR data see Table 1; HRESIMS, obsd. *m/z* 595.2878 $[M + Na]^+$, $C_{32}H_{44}O_9Na$ requires 595.2883, Δ 0.8 ppm.

3.5. Opioid Receptor Assay

Six male Sprague-Dawley rats (160–220 g) were decapitated to obtain membranes for the *ex vivo* opioid receptor assay. All animals were treated according to the code of ethics in research as laid down by the Animal Ethics Committee of the North-West University. Whole brains without cerebellum were quickly removed, weighed and the tissue was subsequently homogenized with an Ultra Turrax (5 s) in 40 vol ice-cold 50 mM Tris-HCl buffer containing 100 mM NaCl (pH 7,4). The homogenate was centrifuged (48 000 × *g*, 15 min, 4 °C) and the pelleted membrane fraction resuspended twice and centrifuged as above. The final pellet was resuspended in 100 volumes of the same buffer and rehomogenized with a Brinkman Polytron PT10 homogenizer (setting 6, 7 s). This membrane suspension was used immediately in the receptor assay.

Binding assay. The assay for [^{3}H]diprenorphine binding to opioid receptors was performed according to Gutierrez *et al.*[20] Aliquots of 0.9 mL of the whole brain membrane preparation, containing 100–300 μg protein, were incubated with 2 nM [^{3}H]diprenorphine (50.9 Ci mmol^{-1}, Amersham International) and various concentrations of competing drugs (naloxone and phorbol esters PE 1, 2, 3 and 4) in a final volume of 1 mL for 60 min at 25 °C. The assay was terminated by rapid vacuum filtration through Whatman GF/B filters presoaked for 30 min in 0.1 % bovine serum albumin incubation buffer. The filters were washed rapidly with 2 × 5 mL ice-cold buffer. Radioactivity trapped on the filters was determined by liquid scintillation counting (Tri-Carb 4660, Packard). All experiments were conducted in duplicate and repeated three times. Data were graphically presented with Graphpad Prism® (Graphpad software, version 5.0 for Windows®, San Diego, USA) as the percentage of total binding obtained in relation to the control (total [^{3}H]diprenorphine binding in the absence of drug).

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References

1 Q-W. Shi, X-H. Su and H. Kiyota, *Chem. Rev.,* 2008, **108**, 4295–4327.
2 G. Goel, H P.S. Makkar, G. Francis and K. Becker, *Int. J. Toxicol.,* 2007, **26**, 279–288.
3 S. Sosath, H.H. Ott and E. Hecker, *J. Nat. Prod.* 1988, **51**, 1062–74.

4 C.F. Johnson, R.M. Cowling and P.B. Phillipson, *Biodivers. Conserv.,* 1999, **8**, 1447–1456.
5. B.L. Luske, T. Mertens, P.C. Lent, W.F. de Boer and H.H.T. Prins, *Afr. J.* Ecol., 2009, **47**, 509–517.
6 D. Hood, *Africa Geographic,* 2005, **13**, 25.
7 B.E. Leonard *Fundamentals of Psychopharmacology,* 3rd edn, John Wiley and Sons, Chichester, UK, pp 375–416. 2003.
8 C. Börner, V. Höllt and J. Kraus, *Mol. Pharmacol.*, 2002, **61**, 800–805.
9 A.V. Azaryan, L.J. Coughlin, B. Buzas, B.J. Clock and B.M. Cox *J. Neurochem.* 1996, **66**, 443–448.
10 H.K. Kramer and E.J. Simon, *J. Neurochem.*, 1999, **72**, 585–593.
11 A.D. Kinghorn and F.J. Evans *J. Pharm. Pharmacol.* 1975, **27**, 329–3339.
12 R.J. Schmidt and F.J. Evans, *Lloydia* 1977, **40**, 225–229.
13 E. Fattorusso, V. Lanzotti, T. Taglialatela-Scafati, C. Gian and G. Appendino, *Eur. J. Org. Chem.,* 2002, **1**, 71–78.
14 M. Gschwendt, and E. Hecker, *Tetrahedron Lett.,* 1969, **40**, 3509–3512.
15 R. K Baslas, and N.C. Gupta, *Herba Hungarica,* 1983, **22**, 35–38.
16 M. Hergenhahn, W. Adolf and E. Hecker, *Tetrahedron Lett.* 1975, 1595–1598.
17 M. Hergenhahn, S. Kusumoto and E. Hecker, *J. Cancer Res. Clin.,* 1984, **108**, 98–109.
18 M.A. Cervinski, J.D. Foster and R.A. Vaughan *J. Biol. Chem.,* 2005, **280**, 40442–40449.
19 A. Rodriguez *New Plantsman,* 1993, **14**, 204–233.
20 M. Guiterrez, L. Menendez, R. Brieva, A. Hidalgo and A. Baamonde, *General Pharmacology,* 1998, **31**, 747–751.