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Short communication

Alkaloids from *Boophone disticha* with affinity to the serotonin transporter

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Abstract

Boophone disticha L. Herb (Amaryllidaceae) is used in traditional medicine for treatment of painful wounds, headaches, skin disorders, inflammatory conditions, rheumatic pains and anxiety. At least eight alkaloids have been characterized and reported in the literature. Of these buphanidrine and buphanamine have affinity to the serotonin transporter (SERT). Alkaloids from other Amaryllidaceae species have also shown affinity to SERT. In this study, an ethanol extract was prepared from dry bulbs. Through HPLC–UV separation five peaks were collected and characterized by LC-MS and ^{1}H NMR and led to the identification of crinine, buphanamine, buphanidrine, distichamine and buphanisine. The activity of these compounds was tested in a binding assay using [^{3}H]-citalopram as ligand and a functional SERT inhibition assay utilizing COS-7 cells expressing hSERT. The four active compounds, buphanamine, buphanidrine, buphanisine and distichamine, had IC_{50}-values of 55 μM, 62 μM, 199 μM and 65 μM respectively, in the binding assay. The alkaloids also showed activity in the functional assay, buphanidrine and distichamine being the most active with IC_{50}-values of 513 μM and 646 μM, respectively.

Keywords: *Boophone disticha*; Serotonin transporter; Traditional medicine

1. Introduction

Boophone disticha (L.f.) Herb. (Syn=*B. longepedicellata* Pax) is widely distributed in South Africa. It has been used in traditional medicine for centuries. The Khoi/San people once used a poison obtained from the bulb for their arrows. Decoctions and fresh scales from the bulbs are used against burns, painful wounds, eye conditions, pain, headaches, skin disorders, inflammatory conditions, rheumatic pains and anxiety (Steenkamp, 2005). Bulb decoctions are administered by the Zulu people, either orally or as enemas, to hysterical adolescent female (Gordon, 1947). Bulbs are used as a narcotic by Sotho and Xhosa (Watt and Breyer-Brandwijk, 1962; Jacot Guillarmod, 1971; Hutchings et al., 1996). The plant is also given to newly circumcised Sotho initiates, producing a stupor (Jacot Guillarmod, 1971).Unspecified groups use a weak decoction of the bulb scales which is commonly administered as a profound sedative to violent, psychotic patients (Van Wyk and Gericke, 2000). Bulb infusions are reported to cause hallucinations (Gelfand et al., 1985). Traditional healers and patients in South Africa drink bulb infusions to induce hallucinations for divinatory purposes, and also as a medicine to treat mental diseases. However, many injuries result from the toxic use of this plant (Sobiecki, 2002).

A number of biologically active alkaloids have been isolated from the bulbs. Several alkaloids have been characterized and reported. These include crinine, buphanidrine, buphanisine, buphanamine, lycorine, powelline, distichamine and 3-O-methylcrinamidine (Hauth and Stauffacher, 1961; Steenkamp, 2005). In a screening of plants used for anxiety and depression for affinity to the serotonin transporter in rat brain, leaf extracts of *B. disticha* had high affinity for the SSRI site (Nielsen et al., 2004). A range of Amaryllidaceae alkaloids has earlier been tested in a binding assay for affinity to the serotonin transporter (SERT). Several of these tested compounds showed high to moderate affinity for the transporter (Elgorashi et al., 2006). In

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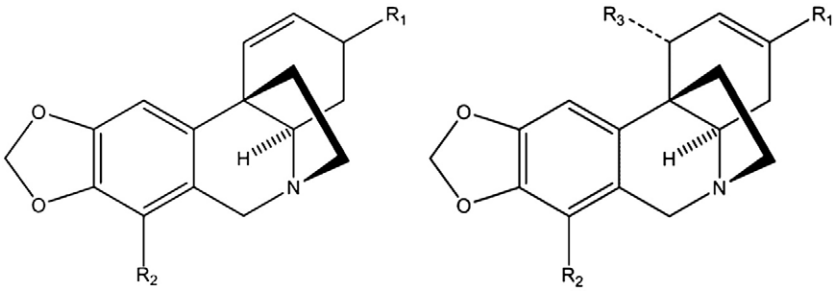


Fig. 1. Structures of (1) crinine, (2) buphanamine, (3) buphanidrine, (4) buphanisine and (5) distichamine.

another study buphanidrine and buphanamine have been reported to have affinity to SERT (Sandager et al., 2005). This affinity was attributed to the presence of a 1,3-dioxole moiety.

2. Materials and methods

2.1. Extraction

Boophone disticha bulbs were obtained from near Mpompomeni, KwaZulu-Natal (S 29°33.296′ E 30°11.468′, Alt 1085 m). A voucher specimen has been deposited at the Bews Herbarium, University of KwaZulu-Natal, Pietermaritzburg (Stafford 53 NU). The bulbs were chopped and dried at 40 °C to constant weight and ground to a powder. One-hundred fifty g ground dry bulbs were extracted with 99.9% absolute ethanol (1 × 1500 ml, 1 × 1000 ml, and 1 × 500 ml). During each extraction the plant material was sonicated for 60 min. The extract was evaporated to dryness *in vacuo* and the residue was dissolved in 150 ml ethyl acetate. The solution was partitioned against 150 ml Milli-Q water (pH adjusted to 3 with anhydrous acetic acid) in a separation funnel. The ethyl acetate phase was washed with 2 × 75 ml Milli-Q water (pH 3). The pH was adjusted to 10 with NaOH and the aqueous phase was partitioned against ethyl acetate (3 × 100 ml). The ethyl acetate phase was evaporated to dryness and freeze dried for 24 h, yielding 240 mg residue.

2.2. Isolation — preparative HPLC

Preparative HPLC was carried out on a Waters 1525 Binary HPLC Pump, connected to a Waters 2996 Photodiode Array Detector. A Luna-C_{18} column was used (250 × 21.20 mm × 5 μm, Phenomenex) eluted with a mixture of acetonitrile (MeCN) and milli-Q water (MQ). The eluent composition was A: 100% MQ and B: 100% MeCN. The column was eluted with a linear gradient (A:B) from 100:0 (T_0) to 0:100 (T_{30}) to 0:100 (T_{35}) at a flow-rate of 10.0 ml/min. Samples (50 mg/ml, injection volume 450 μL) were filtered before each injection. Seven peaks were collected and analyzed by ^{1}H-NMR ($CDCl_3$) on a Mercury 300 MHz NMR instrument and LC-MS on an Agilent 6410 QQQ

Table 1
^{1}H NMR (300 MHz, $CDCl_3$) data of alkaloids isolated from *Boophone disticha*.

Position namine[a]	δ_H multiplicity (*J* in Hz)				
	Bupha-	Buphanidrine[a]	Buphanisine[a]	Crinine[a]	Distichamine[a]
1	4.70 d (5.4)	6.58 d (9.2)	6.47 d (10.1)	6.57 d (9.9)	
2	6.06 dddd (10.1; 5.5; 2.7; 1.9)	5.96 ddd (10.0; 5.2; 1.3)	6.08 dd (5.2; 10.1)	5.96 dd (5.4; 9.9)	5.43 d (1.6)
3	5.89 ddd (2.8; 4.4; 10.0)	3.82 ddd (1.9; 4.0; 5.5)	3.86 ddd (1.9; 5.5; 6.9)	4.34 dd (1.8; 5.6)	
4α	2.99 m	2.11 m	2.11 m		2.53 dd (6.6; 17.5)
4β	2.31 m	1.60 dt (4.1; 13.5)	1.71 dt (3.9; 13.5)	1.74 dt (4.1; 13.5)	2.42 ddd (1.6; 11.0; 17.5)
4a	3.83 t (8.0)	3.31 dd (4.2; 13.5)	3.78 dd (3.8; 13.0)	3.40 dd (4.0; 13.6)	3.92 dd (6.2; 11.1)
6α	4.47 d (16.8)	4.25 d (17.4)	4.75 d (16.2)	4.39 d (16.8)	4.47 d (16.9)
6β	4.16 d (16.8)	3.81 d (17.4)	4.13 d (16.2)	3.77 d (16.8)	4,03 d (16.9)
7			6.56 s	6.47 s	
10	6.60 s	6.56 s	6.86 s	6.84 s	7.79 s
11endo	2.02 m	2.15 ddd (4.4; 9.3; 12.6)	2.18 ddd (4.4; 9.2; 13.0)	2.19 ddd (4.3; 9.1; 12.5)	2.35 ddd (4.2; 9.0; 12.8)
11exo	2.15 m	1.92 ddd (6.0; 10.6; 12.2)	1,94 ddd (6.0; 10.6; 12.2)	1.93 ddd (6.1; 10.6; 12.3)	2.20 ddd (6.6; 10.5; 12.8)
12endo	3.08 ddd (?; 8.7; 13.3)	2.88 ddd (6.0; 9.2; 13.5)	2.91 ddd (6.0; 9.2; 13.2)	2.90 ddd (6.1; 9.0; 13,0)	2.90 ddd (6.6; 9.0; 12.9)
12exo	3.94 m	3.37 m	3.43 m	3.34 m	3.81 m
OCH_2O	5.94 2xd (1.6)	5:86; 5.84 2xd (1.5)	5.97; 5.95 2xd (1.3)	5.90; 5.88 2xd (1.4)	5.91; 5.89 2xd (1.4)
3-OMe		3,36 s	3.37 s		3.74 s
7-OMe	4.01 s	3,96 s			4.00 s

a: The NMR data are comparable to previously published data (Viladomat et al., 1995; Sandager et al., 2005; Steenkamp, 2005).

instrument. Optical rotation was determined on a Perkin-Elmer 241 polarimeter.

2.3. [^{3}H]-Citalopram binding assay

The method described by Plenge et al. (1990) and Nielsen et al. (2004) was used with modifications. Briefly, whole rat brains, except cerebellum, were homogenized with an Ultra Turax homogenizer in 1:10 (w/v) buffer (Tris base 5 mM; NaCl 150 mM; EDTA 20 mM; pH 7.5). The homogenate was centrifuged at 14,000 *g* for 10 min and the homogenized tissue pellet washed with the same buffer 1:10 (w/v). The supernatant was discarded; the pellet was suspended in buffer (Tris base 5 mM; EDTA 5 mM; pH 7.5), left for 20 min at 0 °C and centrifuged at 14,000 *g* for 10 min. The supernatant was discarded and the pellet was suspended in buffer (Tris base 50 mM; NaCl 120 mM; KCl 5 mM; pH 7.5) and centrifuged at 14,000 *g* for 10 min. The supernatant was discarded and the protein pellet finally suspended in 1:10 (w/v) buffer (Tris base 500 mM; NaCl 1200 mM; KCl 50 mM; pH 7.5). The tissue homogenate was kept at −80 °C until use.

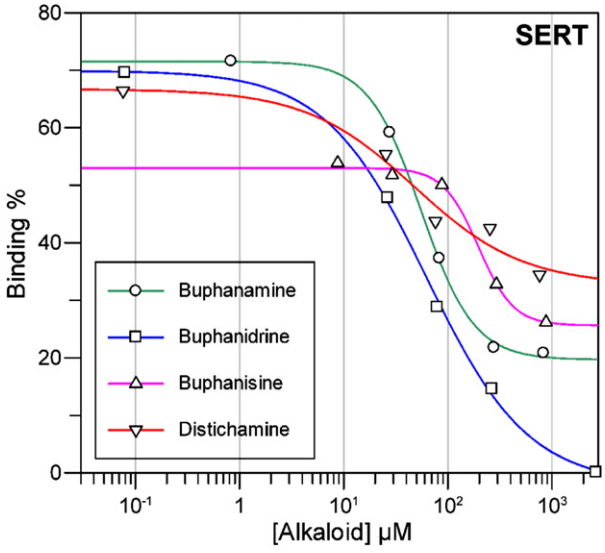
The compounds were tested in a minimum of five concentrations from 10 mg/ml (0.83 mg/ml in the assay) to 0.0003 mg/ml ($2.5 \cdot 10^{-5}$ mg/ml in the assay). Twenty-five µl of each dilution was mixed with 50 µl of [^{3}H]-citalopram (4 nM, 0.67 nM in the assay) and 225 µl of tissue suspension, respectively. The total binding of [^{3}H]-citalopram was determined with a solvent blank. Paroxetin (120 µM, 10 µM in the assay) was used for the determination of the non-specific binding. All samples were incubated for 2 h at 25 °C and then filtered under vacuum using Avantec GC50-25 mm glass fibre filters. The radioactivity on the filters was determined by liquid scintillation using Ultimo Gold XR as scintillation fluid. Specific binding was calculated as total binding minus unspecific binding. All experiments were done in triplicate.

2.4. Functional SERT inhibition assay

The method used is described in details elsewhere (Kristensen et al., 2004). In short: COS-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum, 100 U/ml pencillin, 100 µg/ml streptomycin at 37 °C in a humidified 5% CO_2 environment. Cells were transfected with hSERT constructs with TransIT transfection reagent (Mirus Inc., Madison, WI), following the protocol supplied by the manufacturer. Subsequently, cells were dispensed into poly-D-lysine coated white 96-well plates at 50% confluence. Uptake inhibition assay was performed 48 h after transfection when cells were confluent. The media was removed and the cells were washed twice with phosphate-buffered saline (in mM: NaCl, 137; KCl, 2.7; Na_2HPO_4, 4.3; and KH_2PO_4, 1.4, pH 7.3) containing 0.5 mM $CaCl_2$ and 0.5 mM $MgCl_2$ (PBSCM). After washing, cells were incubated for 30 min in PBSCM containing 50 nM [^{3}H]-5-HT and increasing concentrations of the alkaloids. Uptake was terminated by washing twice with PBSCM. All washing steps were carried out with an automatic plate washer (ELx50 Microplate Strip Washer from Biotek). The amount of accumulated [^{3}H]-5-HT was determined by solubilizing cells in scintillant (MicroScint-20) followed by direct counting of plates in a Packard TopCounter. Resulting counts were converted to % inhibition of a control well that lacked alkaloids.

3. Results and discussion

The Amaryllidaceae alkaloids were partially purified through acidic-basic liquid partitioning. The LC-MS analysis of the alkaloid-enriched fraction showed eight peaks in the UV chromatogram (254 nm). By repeated preparative HPLC buphanamine, buphanidrine, buphanisine, crinine and distichamine were isolated (Fig. 1). The compounds were identified by comparison with previously published NMR data. The ^{1}H NMR data of the five compounds is listed in Table 1. However, with regards to distichamine not all NMR data were found in the literature. The optical rotation for the compounds were: Buphanamine: $[\alpha]_{539}^{28}$ −43.1 (*c* 0.174 mg/ml, $CHCl_3$); buphanidrine: $[\alpha]_{539}^{28}$ −6.2 (*c* 0.256 mg/ml, $CHCl_3$) Litt. value +4.2 (*c* 0.54, EtOH, 22 °C) (Viladomat et al., 1995); Buphanisine: $[\alpha]_{539}^{28}$ −35.2 (*c* 0.125 mg/ml, $CHCl_3$) Litt. value −28 (*c* 0.6, EtOH, 22 °C) (Viladomat et al., 1995); distichamine: $[\alpha]_{539}^{28}$ −41.6 (*c* 0.262 mg/ml, $CHCl_3$).



Buphanamine

Parameter	Value	Std. Error
Y Range	51,8863	1,8135
IC 50	55,3046	3,8046
Slope factor	1,7187	0,1739
Background	19,6492	1,1941

Buphanidrine

Parameter	Value	Std. Error
Y Range	71,9681	3,5404
IC 50	62,3968	8,7732
Slope factor	0,8897	0,1164
Background	-2,0471	2,7441

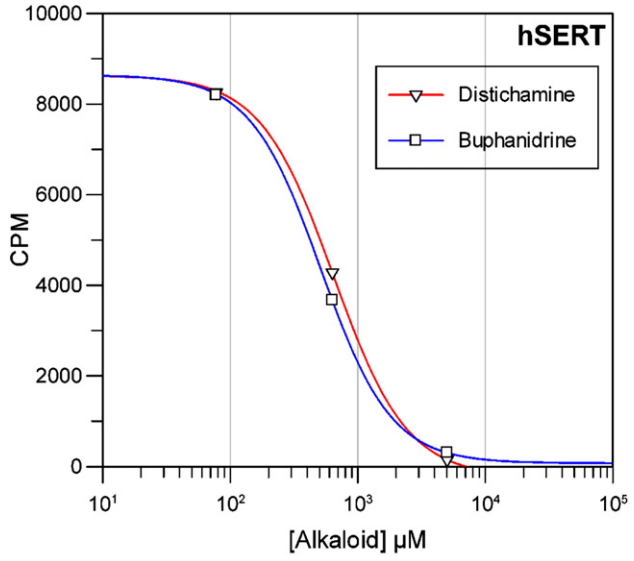
Buphanisine

Parameter	Value	Std. Error
Y Range	27,4024	2,0834
IC 50	196,0030	24,8662
Slope factor	2,5763	0,5876
Background	25,6144	1,6721

Distichamine

Parameter	Value	Std. Error
Y Range	34,0496	14,4650
IC 50	47,8744	52,9639
Slope factor	0,8332	0,8426
Background	32,6736	12,6793

Fig. 2. IC_{50} determinations for buphanamine (○), buphanidrine (□), buphanisine (Δ) and distichamine (▽) in the binding assay.



Distichamine			Buphanidrine		
Parameter	Value	Std. Error	Parameter	Value	Std. Error
Y Range	8863,6137	818,9607	Y Range	8569,8891	291,4239
IC 50	646,0695	117,0363	IC 50	512,9888	37,6036
Slope factor	1,5130	0,6354	Slope factor	1,5718	0,2669
Background	-229,8529	747,7120	Background	73,2136	261,0379

Fig. 3. IC_{50} determinations for distichamine (▽) and buphanidrine (□) in the functional SERT assay.

The activity of the alkaloids observed in the SERT binding assay (Fig. 2) was used to calculate IC_{50} values for the active compounds using Grafit 5© (Erithacus Software Limited). Buphanamine, buphanidrine and distichamine were the most active with IC_{50} values of 55±4 µM (K_i=23 µM), 63±9 µM (K_i=26 µM) and 65±7 µM (K_i=27 µM), respectively. Buphanisine showed a relative lower potency giving an IC_{50} value of 199±25 µM (K_i=82 µM). Crinine showed only weak activity in the SERT assay. This corresponds to earlier findings by Elgorashi et al. (2006).

Only buphanidrine and distichamine were tested in the functional SERT inhibition assay. Both buphanidrine and distichamine showed activity in the functional assay (Fig. 3), with IC_{50}-values of 513±38 µM and 646±117 µM respectively.

The presence of the 1,3-dioxole moiety is suspected to be responsible for the SERT affinity of these Amaryllidaceae alkaloids (Sandager et al., 2005). It has been suggested that some of these alkaloids could account for a dual effect in the treatment of depression and Alzheimer's disease (Elgorashi et al., 2006). However the crinine-type alkaloids which show affinity for SERT has only weak acetylcholinesterase inhibitory effects (Elgorashi et al., 2004). On the other hand, lycorine-type alkaloids have demonstrated activity as acetylcholinesterase inhibitors, but they showed no or only weak affinity for SERT (Elgorashi et al., 2004, 2006).

The results from this study provide a rationale for the use of *B. disticha* against anxiety and other central nervous system diseases in traditional medicine and enhance our understanding of the effects of this plant on the central nervous system.

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