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Dragmacidin F: A New Antiviral Bromoindole Alkaloid from the Mediterranean Sponge *Halicortex* sp.

Adele Cutignano, Giuseppe Bifulco, Ines Bruno, Agostino Casapullo, Luigi Gomez-Paloma and Raffaele Riccio*

Dipartimento di Scienze Farmaceutiche, Università di Salerno, via Ponte don Melillo, 84084 Fisciano (Salerno), Italy

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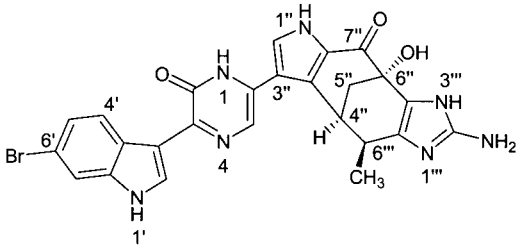
Abstract—A new bromoindole alkaloid, named dragmacidin F (**1**) and structurally related to other dragmacidins previously described, was isolated from a marine sponge of the genus *Halicortex* collected off the southern coast of Ustica Island (Italy). Structural elucidation of **1** was achieved by an extensive spectroscopic study (mainly by 1D/2D-NMR and MS techniques). Compound **1**, containing an unprecedented carbon skeleton that is very likely derived from cyclization of a partially oxidized form of dragmacidin D (**2**), showed in vitro antiviral activity against HSV-1 (EC_{50}=95.8 μM) and HIV-1 (EC_{50}=0.91 μM), thus proving to be responsible for the antiviral property exhibited by *Halicortex* extracts.

Introduction

Over the past few years several biologically interesting bromoindole alkaloids have been isolated from marine sponges of the genera *Topsentia*,[1,2] *Spongosorites*,[3–7] *Dragmacidon*,[8] *Hamacantha*,[9] *Hexadella*,[10] *Orina*,[11,12] and *Raphisia*.[13] A deep interest in this class of compounds is due both to their new molecular structures and their wide range of biological and pharmacological activities.

In this paper we wish to report the isolation and characterization of a new bioactive bromoindole alkaloid which we named dragmacidin F (**1**) on the basis of a chemical and biogenetic correlation to other known dragmacidins. Preliminary in vitro pharmacological screening on the ethanol extract of *Halicortex* sp., a sponge collected along the coast of Ustica Island (Italy), showed antiherpes activity. In order to isolate the metabolites responsible for the observed bioactivity, we submitted its crude acetone extract to extraction and solvent partition to yield an active *n*-butanol soluble fraction. The *n*-butanol extract was in turn subjected to silica gel chromatography (eluents: $CHCl_3$/MeOH, 9:1→1:1) and the Dragendorff active fractions were finally purified by gel filtration on a Sephadex LH-20 column (MeOH as eluent), affording the major bioactive dragmacidin F (**1**) as a pure compound.



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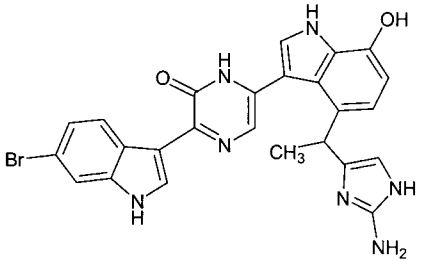

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Results and Discussion

Structurally, compound **1** can be added to the growing class of dragmacidins[3,4,8] and it is particularly related to dragmacidin D[4] (**2**); indeed, dragmacidin F (**1**) contains the same 3-[bromoindole-3-yl]-pyrazin-2-one portion of **2**. A novel polycyclic carbon framework, however, is present in the other molecular hemisphere, which conceivably might derive from cyclization of the 2-aminoimidazole containing

Keywords: alkaloids; antivirals; indoles; marine metabolites.

* Corresponding author. Tel.: +390-89-962-818; fax: +390-89-962-652; e-mail: riccio@unisa.it

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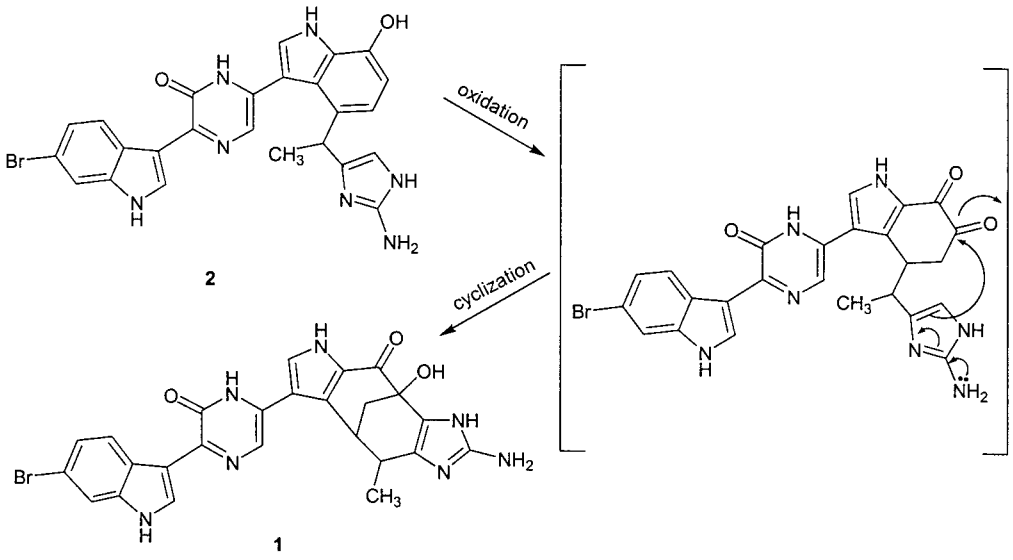

Figure 1. A plausible biogenetic pathway for **1**.

side-chain onto the 6″ position of the α-diketone form of the second indole system (Fig. 1) (for comparison purposes the numbering scheme of dragmacidin F (**1**) was assigned according to that of its possible biogenetic precursor dragmacidin D (**2**)).

The ESIMS spectrum of **1** contained an isotopic cluster of pseudomolecular ions at *m/z* 548/546 (ratio 1:1) ($M+H^+$) characteristic of a monobrominated compound. The composition $C_{25}H_{20}BrN_7O_3$ was deduced by HRESIMS ($[M+H]^+$ *m/z* 548.086722 ($C_{25}H_{21}{}^{81}BrN_7O_3$ requires 548.086878);

Table 1. NMR data for Dragmacidin F (CD_3OD)

	^{1}H δ, m, *J* (Hz)	^{13}C ppm	COSY	HMBC	ROESY
1-NH					
2		157.2			
3		149.6			
5	7.74, s	125.0		C3	H4″
6		128.1			
1′-NH					
2′	8.73, s	132.2		C3′; C3a′; C7a′	
3′		113.3			
3a′		126.4			
4′	8.61, d, *8.3*	125.3	H5′	C3′; C5′; C6′; C7a′;	H5′
5′	7.27, dd, *8.3,1.8*	124.6	H4′; H7′	C3a′; C6′; C7′	H4′
6′		116.8			
7′	7.65, s	115.3	H5′	C3a′; C6′; C5′	
7a′		138.8			
1″-NH					
2″	7.56, s	128.8		C3a″; C6; C7a″	
3″		113.3			
3a″		133.0			
4″	4.16, brs	36.8	H_25″; H6‴		H5; H5″B
5″A	2.49, brd, *11.8*	45.0	H5″B; H4″	C3a″; C4″; C6″; C7″; C4‴; C6‴	H5″B; H6‴
5″B	2.76, dd, *11.8, 3.50*		H5″A; H4″	C3a″; C4″; C6″; C7″; C4‴; C6‴	H5″A; H4″
6″		73.1			
6″-OH					
7″		189.0			
7a″		117.2			
1‴					
2‴		149.4			
2‴-NH_2					
3‴					
4‴		123.1			
5‴		125.9			
6‴	3.38, q, *7.0*	33.1	H7‴; H4″	C4″; C7‴; C7″; C4‴; C3a″; C5‴	H7‴; H5″A; H4″
7‴	0.93, d, *7.0*	15.8	H6‴	C4″; C5‴; C6‴	H6‴

Table 2. NMR data for Dragmacidin F (DMSO-d_6)

	^{1}H δ, m, *J* (Hz)	^{13}C ppm	COSY	HMBC	ROESY
1-NH	7.38			C5	H2″
2		156.9			
3		149.6			
5	7.52[a], s	128.7			H4″
6		132.3			
1′-NH	12.1, s		H2′;H7′	C2′; C3′; C3a′; C7a′	H2′; H7′
2′	8.71, s	132.9	1′-NH		1′-NH
3′		113.4			
3a′		126.7			
4′	8.54, d, *8.6*	126.0	H5′	C3′; C5′; C6′; C7a′;	H5′
5′	7.25, brd, *8.6*	124.8	H4′	C3a′; C6′; C4′	H4′
6′		116.2			
7′	7.68, s	116.5	1′-NH	C3a′; C6′; C5′; C7a′	1′-NH
7a′		139.0			
1″-NH	12.44, s			C2″; C3a″	H2″
2″	7.52[a], s	128.8		C6	1-NH; 1″-NH
3″		110.9			
3a″		132.3			
4″	4.01, brs	36.4			H6‴
5″A	2.26, brd, *10.9*	45.1	H5″B; H4″	C3a″; C4″; C6″; C7″; C4‴; C6‴	H5″B; H6‴; 6″-OH
5″B	2.53, brd, *10.9*		H5″A; H4″	C3a″; C4″; C6″; C7″; C4‴; C6‴	H5″A; H4″; 6″-OH
6″		72.9			
6″-OH	6.09			C5″; C6″	H5″A; H5″B
7″		188.9			
7a″		117.2			
1‴					
2‴		149.6			
2‴-NH$_2$	7.32, s				
3‴					
4‴		122.7			
5‴		125.1			
6‴	3.27, q, *7.0*	33.0	H7‴	C4″; C7‴; C7″; C4‴; C3a″; C5‴	H7‴; H5″A; H4″
7‴	0.78, d, *6.7*	16.8	H6‴	C4″; C5‴; C6‴	H6‴

[a] Overlapping signals.

m/z 546.087030 ($C_{25}H_{21}{}^{79}BrN_7O_3$ requires 546.088924)) and confirmed by NMR spectroscopic evidence (mainly ^{13}C- and ^{13}C-DEPT NMR data).

The presence of a 6-bromoindole-3-yl moiety was suggested by analysis of ^{1}H- and ^{13}C NMR resonances (CD_3OD; Table 1) which are superimposable to those observed for the same structural unit in dragmacidin D[4] (**2**). In the low-field region of the ^{1}H NMR spectrum four aromatic proton resonances (H2′ through H7′) could be confidently assigned to the 6-bromoindole residue by their characteristic chemical shifts and coupling constants. Moreover, ^{13}C NMR data assigned by {^{1}H–^{13}C}-gHSQC[14] measurements and long range ^{1}H–^{13}C connectivities, deduced through gHMBC[15] experiments (Table 1), gave definitive support to the structural assignment of this region of the molecule.

Further analogies between dragmacidin F (**1**) and D (**2**) were found in the NMR resonances (CD_3OD; Table 1) attributable to the 3,6-disubstituted-2(1H)-pyrazinone unit, featuring four sp^2-hybridized carbons observed at δ 157.2 (vs. 155.1) (C2), 149.6 (vs. 148.4) (C3), 125.0 (vs. 124.0) (C5), 128.1 (vs. 131.0) (C6) and an aromatic proton singlet at δ 7.74 (vs. 7.5) (H5). These assignments were also supported by spectral comparison with other molecules containing 2(1H)-pyrazinone ring[4] and by the observation of two low intensity correlations (1-NH to C5 and H5 to C3) in DMSO-d_6 (Table 2) and methanol-d_4 (Table 1) HMBC spectra, respectively. The proton NMR spectra of **1** also contained one additional aromatic resonance [H2″; δ 7.52 (s) in DMSO-d_6 and δ 7.56 (s) in methanol-d_4] which, in the DMSO-d_6 ROESY spectrum, showed dipolar interactions with two exchangeable protons at δ 12.44 (1″-NH) and δ 7.38 (1-NH). These data, together with those from ^{13}C NMR and HMBC spectra, are in good agreement with the presence of a trisubstituted pyrrole unit which is in turn connected to C6 of the 2(1H)-pyrazinone ring, as suggested by key connectivities, namely the H2″/C6 correlation (HMBC spectrum in d_4-methanol) and the 1-NH/H2″ dipolar contact (ROESY spectrum in DMSO-d_6). Accordingly, the 6-bromoindole-3-yl residue was linked to C3 of the central 3,6-disubstituted-2(1H)-pyrazinone ring, thus completing the characterization of the left side portion of

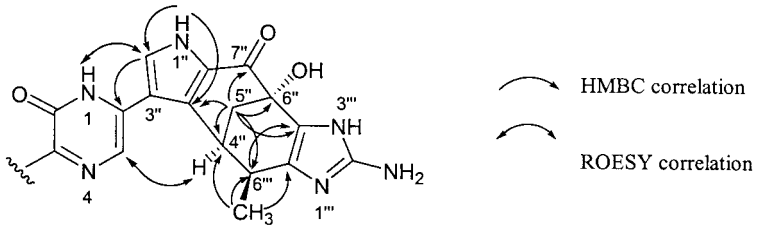

Figure 2. Key HMBC and ROESY correlations around the bicyclo[3.3.1]nonane moiety.

the structure **1**, already observed in dragmacidin D[4] (**2**) and accounting for $C_{12}H_7BrN_3O$.

The remaining signals in the ^{1}H- and ^{13}C NMR spectra indicated that the molecule still contained seven quaternary sp^2 carbons, one of which can be assigned to a carbonyl group (δ 189.0; C7″), one sp^2 methine, already assigned to the above-mentioned trisubstituted pyrrole unit, and five sp^3 carbons: one methyl, one methylene, two methines and a quaternary carbon bearing an oxygen (δ 73.1; C6″).

Moreover the ^{1}H NMR and COSY spectra showed a well-defined aliphatic spin system H_25''–H4″–H6‴–H_37''', which was shown to be part of a complex polycyclic structure with a central bicyclo[3.3.1]nonane skeleton on the basis of the following considerations. The large number of HMBC heterocorrelations observed for the H_25'' located this methylene on a bridge between two ring systems (Fig. 2).

The first six-membered ring includes carbons C4″ and C6‴ and two quaternary sp^2 carbons (C4‴ at δ 123.1 and C5‴ at δ 125.9), that exhibit correlations with H6‴ and H_37''', and is conceivably closed through the quaternary oxygen bearing carbon (C6″), as evidenced by the H_25''/C6″ and H_25''/C4‴ HMBC correlations. Furthermore, the HMBC correlations (DMSO-d_6; Table 2) of an exchangeable proton at δ 6.09 with C5″ and C6″, located a tertiary hydroxyl group at C6″. On the other side, a second six-membered ring is made up from an α,β-unsaturated carbonyl system (C3a″; C7a″; C7″) that was proven to be connected to C4″ and C6″ as shown in Fig. 2 by the following HMBC correlations: H_25''/C7″; H_25''/C3a″; H6‴/C3a″. The HMBC correlations of 1″-NH with C2″ and C3a″ and those of H_22'' with C3a″ and C7a″ defined the above-mentioned trisubstituted pyrrole unit as joined to the bicyclo[3.3.1]nonane framework through C3a″ and C7a″, as depicted in Fig. 1. This also led to the conclusion that, on the other edge of the bicyclo[3.3.1]nonane system, C5‴ and C4‴ had to be bridged through a guanidine residue to form a disubstituted 2-amminoimidazole ring. As a matter of fact, ^{13}C NMR analysis indicated that only a further sp^2 hybridized carbon (δ 149.4) remained to be placed in **1** and, according to mass spectral data, the presence of three additional nitrogen atoms bearing three exchangeable protons (two of which were evidenced at δ 7.32 in the DMSO-d_6 proton spectrum; Table 2) was the most reasonable hypothesis. Furthermore, the carbon resonances observed at δ 149.4 (C2‴), δ 123.1 (C4‴) and δ 125.9 (C5‴) are in good accordance with the expected values for a substituted 2-amino-imidazole moiety[16] and the ^{1}H NMR resonance observed at δ 7.32 (2‴-NH_2) in DMSO ^{1}H NMR spectrum (2H, brs, exchangeable) is reminiscent of that observed for the same guanidine functionality in dragmacidin D[4]. The presence of a 2-amino-imidazole moiety, joined to the C6‴ and C6″ positions of the bicyclic system was also in agreement with the biogenetic hypothesis of Fig. 1.

Thus the gross structure of the tetracyclic right end portion of dragmacidin F (**1**), accounting for $C_{13}H_{13}N_4O_2$ and leading to the molecular formula $C_{25}H_{20}BrN_7O_3$ was completed. Conclusive support came from the pattern of dipolar correlations. In this context, the two ROESY effects 1NH/H_2″ and H5/H4″ were particularly informative, assessing the orientation of the central 2(1H)-pyrazinone ring with respect to the tetracyclic core of **1** and also ruled out alternative possibilities of connecting the bicyclononane system to the two heterocyclic rings.

We also propose the relative stereochemistry of the bicyclo[3.3.1]nonane skeleton combining a series of data derived from inspection of Dreiding molecular models, ROESY responses and analysis of proton–proton coupling constants relative to the spin system H5″-H4″-H6‴ of the cyclohexene ring. Dreiding models and molecular mechanics calculations for the bicyclic moiety in the MM2 force field established that the *cis*-1,3-diaxial mode is the only possible way of closure, leaving the 6″OH and the H4″ in the equatorial orientation, as confirmed by the J-pattern of H4″–H_25''. The equatorial orientation of the CH_3 7‴ is based on a coupling constant analysis, in facts the H6‴, being adjacent to CH_3-7‴ and to H4″, appears in the ^{1}H NMR spectrum as a quintet with J=7 Hz, which is

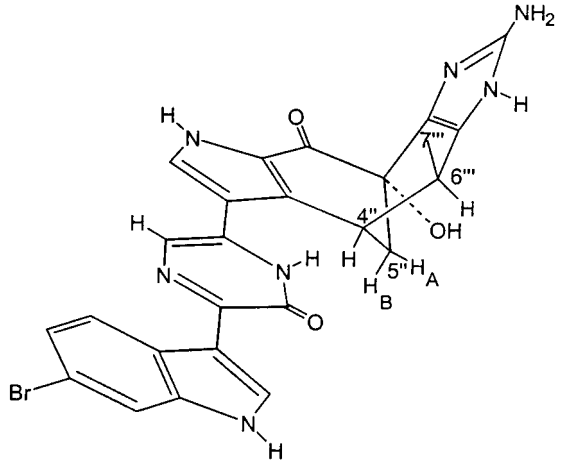

Figure 3. Spatial view of dragmacidin F (**1**), illustrating its relative stereochemistry and the orientation of groups around the bicyclic moiety.

what expected for the relative configuration of Fig. 3, on the basis of a calculated dihedral angle H4″/H6‴ of 50°. On the other hand, inverting the configuration at C6 leads to a model with a calculated dihedral angle H4″/H6‴ of 78° that, on the basis of the generalized Karplus curve, would give a coupling constant of 1.1 Hz, far away from the experimental value of 7 Hz. ROESY responses were very informative too, with a strong dipolar correlation between H6‴ and H5″A at δ 2.49 (the correlation between H5″A and CH_3-7‴ was absent) that suggested their 1,3-diaxial orientations. Moreover, in agreement with the calculated dihedral angle H4″/H6‴, we also observed a correlation peak between H4″ and H6‴ and not with CH_3-7‴, which corroborated our stereochemical hypothesis (Fig. 3).

Dragmacidin F exhibited interesting antiviral properties. It was tested for anti HSV-1 and anti HIV-1 activities; the concentration achieving 50% protection of virus infected cells from HSV-induced destruction was 95.8 μM. Furthermore, the concentration achieving 50% delay in formation of *syncythia* in HIV-1 virus infected cells was 0.91 μM.

Experimental

General

Melting point was measured on a Gallenkamp apparatus and is reported uncorrected. Spectral data were acquired on the following instruments: ESIMS-mass spectrometer LCQ Finnigan; HRESIMS-mass spectrometer VG Autospec; NMR-Bruker Avance DRX 600; $[\alpha]_D$-Perkin Elmer 161 polarimeter; UV-Beckman DU 640 spectrophotometer; IR-Bruker FT-IR model IFS 48.

Experimental biological material

A sample of *Halicortex* sp. was collected off the southern coast of Ustica (Italy) in the 'Grotta dei gamberi' at a depth of 45 m and taxonomically identified by Prof. M. Sarà group of Istituto di Zoologia—Università di Genova (Italy). Voucher specimen is available in the Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli 'Federico II'.

Isolation of dragmacidin F

The frozen organism (0.8 kg) was extracted exhaustively with acetone (3×1 L). This extract, filtered through paper and concentrated at low pressure, gave a brown oil. The oily residue mixed with H_2O (0.5 L) was partitioned with diethyl ether (3×1 L) and then with *n*-butanol (3×1 L). The BuOH soluble material (1.8 g) was fractionated by silica gel chromatography (Merck, Kieselgel 60, 230–400 mesh) at medium pressure using a gradient elution system $CHCl_3$/MeOH from 9:1 to 1:1 (20 ml/fraction). The Dragendorff positive fractions 190–200 (171.0 mg) were submitted to a further purification step on Sephadex LH-20 (Pharmacia) column at low pressure, using MeOH as eluting solvent (5 ml/fraction). The collected fractions were controlled by TLC on silica gel (Merck, kieselgel F_{254}, 0.25 mm) eluting with butanol/acetic acid/water (60:15:25) and revealed by exposure to UV radiation and by spraying with $Ce(SO_4)_2$ acid solution or the Dragendorff reagent. Homogeneous fractions were pooled into six groups. The group 12–18 gave dragmacidin F as a pure compound (20 mg).

Dragmacidin F

Pale yellow solid, mp >260°C. $[\alpha]_D^{25}=-159$ (c=0.4, MeOH); UV (MeOH) λ_{max}: 207 nm (ϵ=14 000), 274 nm (ϵ=6460), 280 nm (ϵ=6430), 388 nm (ϵ=9600); IR (KBr) ν_{max} 3308, 1645, 1595 cm^{-1}; ESIMS: $[M+H]^+$ m/z 548/546 (100,100); HRESIMS: $[M+H]^+$ m/z 548.086722 ($C_{25}H_{21}{}^{81}BrN_7O_3$ requires 548.086878); m/z 546.087030 ($C_{25}H_{21}{}^{79}BrN_7O_3$ requires 546.088924); ^1H and ^{13}C NMR (CD_3OD or DMSO-d6): see Tables 1–2.

Anti HSV-1 test

Tests for anti-herpes activity were performed by the group of Prof. S. Billaudel (University of La Rochelle, France). The activity was expressed as antiviral effective concentration that achieved 50% protection (EC_{50}) of virus-infected cells from the HSV-induced destruction.[17,18]

Anti HIV-1 test

Anti HIV-1 activity was monitored by the efficiency of the drug compound to inhibit *syncytia* formation after HIV-infection of MT_4, as described elsewhere.[19,20] The inhibitory concentration of drug compound was expressed as the concentration that caused 50% inhibition of *syncytia* formation (EC_{50}) without direct toxicity for the cells. Tests were performed by the group of Prof. J. C. Chermann (University of Marseille, France).

Molecular mechanics calculations

Minimization of the energy of **1** (Fig. 3) was carried out using the MM2 force field (CS Chem3D Pro, Cambridge Soft Corporation). A minimum RMS gradient of 0.010 was used for the convergence criteria of the gradient of the potential energy surface.

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