# Antimicrobial Rubrolides from a South African Species of *Synoicum* Tunicate

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No.	$\delta_{C}$ (mult)	$\delta_{\rm H}(J \text{ in Hz})$	COSY	HMBC	NOESY
2	169.5 (C)				
3	111.1 (CH)	6.23 (d, 0.6)		2, 4, 5, 1'	2'/6'
4	159.4 (C)				
5	146.7 (C)				
6	111.6 (CH)	6.28 (s)		4, 5, 2", 6"	2'/ 6', 2", 6"
1′	120.5 (C)				
2'	129.9 (CH)	7.47 (d, 8.6)	3'	4, 4', 6'	6, 3' <sup>a</sup>
3'	115.6 (CH)	6.95 (d, 8.7)	2'	1', 5', 4'	2′ <sup>a</sup>
4′	159.9 (C)				
5'	115.6 (CH)	6.95 (d, 8.7)	6'	1', 3', 4'	6′ª
6'	129.9 (CH)	7.47 (d, 8.6)	5'	4, 2', 4'	6, 5' <sup>a</sup>
1″	133.5 (C)				
2″	134.6 (CH)	8.10 (d, 2.1)		4", 5", 6"	6
3″	126.5 (C)				
4″	156.9 (C)				
5″	111.7 (CH)	7.10 (d, 8.6)	6″	6, 3″	7", 6" <sup>a</sup>
6″	131.2 (CH)	7.78 (dd, 8.6, 2.1)	5″	1", 4", 5"	6, 5″ <sup>a</sup>
7″	55.3 (CH <sub>3</sub> )	3.93 (s)		4″	5″

Table S1. NMR data for 3"-bromorubrolide F (1) in methanol- $d_4$ 

<sup>13</sup>C chemical shifts were measured from HMBC spectrum

HMBC correlations are presented from proton to indicated carbon. a - COSY artifacts observed in NOESY spectrum

Tuble 52. Think dua for 5° brombrubronde E (2) in methanor 44					
No.	$\delta_{C}$ (mult)	$\delta_{\rm H}(J \text{ in Hz})$	COSY	HMBC	NOESY
2	170.4 (C)				
3	111.9 (CH)	6.20 (d, 0.5)		2, 4, 5, 1'	2', 6'
4	158.3 (C)				
5	146.3 (C)				
6	114.5 (CH)	6.24 (s)		4, 5, 2"/ 6"	2', 6', 2"/ 6"
1'	123.2 (C)				
2'	133.6 (CH)	7.72 (d, 2.0)		3', 4', 6'	6, 6' <sup>a</sup>
3'	110.9 (C)				
4′	157.4 (C)				
5'	116.8 (CH)	7.03 (d, 8.3)	6'	1', 3'	6′ <sup>a</sup>
6'	129.5 (CH)	7.42 (dd, 8.3, 2.0)	5'	2', 4'	6, 2' <sup>a</sup> , 5' <sup>a</sup>
1″	125.2 (C)				
2″	133.2 (CH)	7.70 (d, 8.7)	3″	6, 4", 6"	6, 3"
3″	116.0 (CH)	6.83 (d, 8.7)	2″	1", 4", 5"	2"
4″	159.6 (C)				
5″	116.0 (CH)	6.83 (d, 8.7)	6″	1", 3", 4"	6″
6″	133.2 (CH)	7.70 (d, 8.7)	5″	6, 2", 4"	6, 5″

Table S2. NMR data for 3'-bromorubrolide E (2) in methanol- $d_4$ 

HMBC correlations are presented from proton to indicated carbon. a - COSY artifacts observed in NOESY spectrum

No.	$\delta_{C}$ (mult)	$\delta_{\rm H}(J \text{ in Hz})$	COSY	HMBC	NOESY
2	171.5 (C)				
3	111.4 (CH)	6.16 (s)		2, 4, 5, 1'	2', 6'
4	159.5 (C)				
5	147.8 (C)				
6	115.0 (CH)	6.31 (s)		4, 5, 2"	2', 6', 2"/ 6"
1′	121.3 (C)				
2'	134.1 (CH)	7.70 (d, 2.2)	5'	4, 3', 4', 6'	6
3'	113.3 (C)				
4′	161.6 (C)				
5'	118.9 (CH)	6.94 (d, 8.3)	2'	1', 3'	6′ <sup>a</sup>
6'	130.2 (CH)	7.38 (dd, 8.3, 2.2)	2', 5'	2', 4'	6, 5′
1″	127.4 (C)				
2″	133.5 (CH)	7.79 (d, 8.6)	3″	3", 4", 6"	6, 3″ <sup>a</sup>
3″	115.2 (CH)	6.98 (d, 8.9)	2″	1", 4", 5"	2″ª, 7″
4″	162.1 (C)				
5″	115.2 (CH)	6.98 (d, 8.9)	6″	1", 3", 4"	6″ª, 7″
6″	133.5 (CH)	7.79 (d, 8.6)	5″	2", 4", 5"	6, 5″ <sup>a</sup>
7′	55.8 (CH <sub>3</sub> )	3.84 (s)		4″	3", 5"

Table S3. NMR data for 3'-bromorubrolide F (**3**) in methanol- $d_4$ 

<sup>13</sup>C chemical shifts were measured from HMBC spectrum

HMBC correlations are presented from proton to indicated carbon. a - COSY artifacts observed in NOESY spectrum

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No.	$\delta_{\rm C}$ (mult)	$\delta_{\rm H}(J \text{ in Hz})$	COSY	HMBC	NOESY
2	171.5 (C)				
3	111.7 (CH)	6.16 (s)		2, 4, 5, 1'	2', 6'
4	159.5 (C)				
5	147.5 (C)				
6	114.7 (CH)	6.22 (s)		4, 5, 2", 6"	2', 6', 2", 6"
1'	122.3 (C)				
2'	134.4 (CH)	7.70 (d, 2.2)	6'	4, 3', 4', 6'	3, 6, 6′ <sup>a</sup>
3'	113.1 (C)				
4′	161.2 (C)				
5'	118.8 (C)	6.97 (d, 8.4)	6'	1', 3', 4'	6′ª
6'	130.5 (CH)	7.39 (d, 8.4, 2.2)	2', 5'	4, 2', 4'	3, 6, 2 <sup>'a</sup> , 5 <sup>'a</sup>
1″	126.3 (C)				
2″	136.7 (CH)	8.03 (d, 2.1)	6″	6, 3", 4", 6"	6, 6″ <sup>a</sup>
3″	112.7 (C)				
4″	159.6 (C)				
5″	118.3 (CH)	6.87 (d, 8.6)	6″	1", 3", 4"	6″ <sup>a</sup>
6″	132.9 (CH)	7.59 (dd, 8.6, 2.1)	2", 5"	6, 2", 4"	6, 2″ <sup>a</sup> ,5″ <sup>a</sup>

Table S4. NMR data for 3', 3"-dibromorubrolide E (4) in methanol- $d_4$ 

HMBC correlations are presented from proton to indicated carbon. a - COSY artifacts observed in NOESY spectrum.

No.	$\delta_{C}$ (mult)	$\delta_{\rm H}(J \text{ in Hz})$	COSY	HMBC
2	171.5 (C)			
3	110.7 (CH)	6.11 (s)		2, 4, 5, 1'
4	161.0 (C)			
5	145.0 (C)			
6	115.9 (CH)	6.30 (s)		4, 5, 2"/ 6"
1'	120.6 (C)			
2'	131.2 (CH)	7.44 (d, 8.6)	3'	4
3'	117.2 (CH)	6.91 (d, 8.5)	2'	1', 4'
4′	159.7 (C)			
5'	117.2 (CH)	6.91 (d, 8.5)	6'	1', 4'
6'	131.2 (CH)	7.44 (d, 8.6)	5'	4
1″	125.0 (C)			
2″	133.7 (CH)	7.69 (d, 8.8)	3″	6, 4", 6"
3″	126.5 (CH)	6.81 (d, 8.8)	2″	1", 4"
4″	159.7 (C)			
5″	126.5 (CH)	6.81 (d, 8.8)	6″	1", 4"
6″	133.7 (CH)	7.69 (d, 8.8)	5″	6, 2", 4"

Table S5. NMR data for rubrolide E (5) in methanol- $d_4$ 

<sup>13</sup>C chemical shifts were measured from HMBC spectrum.

HMBC correlations are presented from proton to indicated carbon. a - COSY artifacts observed in NOESY spectrum

Table S6. <sup>1</sup>H and <sup>13</sup>C NMR data in methanol- $d_4$  for rubrolide F (**6a**), sodium salt of rubrolide F (**6b**), and originally identified rubrolide F (**6c**)

	6a		6b	6c
No.	$\delta_{C}$ (mult)	$\delta_{\rm H}(J \text{ in Hz})$	$\delta_{\rm H}(J \text{ in Hz})$	$\delta_{\rm H}(J \text{ in Hz})$
2	171.4 (C)			
3	114.9 (CH)	6.19 (d, 0.6)	5.99 (s)	6.07 (s)
4	160.6 (C)			
5	147.9 (C)			
6	112.0 (CH)	6.31 (s)	6.42 (s)	6.38 (s)
1′	122.7 (C)			
2'	131.3 (CH)	7.47 (d, 8.5)	7.33 (d, 8.8)	7.38 (d, 8.8)
3'	116.8 (CH)	6.95 (d, 8.5)	6.69 (d, 9.2)	6.80 (d, 8.5)
4′	161.4 (C)			
5'	116.8 (CH)	6.95 (d, 8.5)	6.69 (d, 9.2)	6.80 (d, 8.5)
6'	131.3 (CH)	7.47 (d, 8.5)	7.33 (d, 8.8)	7.38 (d, 8.8)
1″	127.2 (C)			
2″	133.7 (CH)	7.80 (d, 9.2)	7.79 (d, 8.7)	7.80 (d, 8.8)
3″	115.1 (CH)	6.98 (d, 8.9)	6.98 (d, 8.7)	6.97 (d, 8.8)
4″	162.1 (C)			
5″	115.1 (CH)	6.98 (d, 8.9)	6.98 (d, 8.7)	6.97 (d, 8.8)
6″	133.7 (CH)	7.80 (d, 9.2)	7.79 (d, 8.7)	7.80 (d, 8.8)
7″	55.5 (CH <sub>3</sub> )	3.85 (s)	3.84 (s)	3.84 (s)



Figure S1. <sup>1</sup>H NMR spectrum for 3"-bromorubrolide F (1; 700 MHz, methanol- $d_4$ )



Figure S2. COSY spectrum for 3"-bromorubrolide F (1; 700 MHz, methanol- $d_4$ )



Figure S3. HSQC spectrum for 3"-bromorubrolide F (1; 700 MHz, methanol- $d_4$ )



Figure S4. HMBC spectrum for 3"-bromorubrolide F (1; 700 MHz, methanol- $d_4$ )



Figure S5. NOESY spectrum for 3"-bromorubrolide F (1; 700 MHz, methanol- $d_4$ )



Figure S6. <sup>1</sup>H NMR spectrum for 3'-bromorubrolide E (**2**; 700 MHz, methanol- $d_4$ )



Figure S7. <sup>13</sup>C NMR spectrum for 3'-bromorubrolide E (**2**; 175 MHz, methanol- $d_4$ )



Figure S8. COSY spectrum for 3'-bromorubrolide E (2; 700 MHz, methanol- $d_4$ )



Figure S9. HSQC NMR spectrum for 3'-bromorubrolide E (2; 700 MHz, methanol- $d_4$ )



Figure S10. HMBC NMR spectrum for 3'-bromorubrolide E (2; 700 MHz, methanol- $d_4$ )



Figure S11. NOESY NMR spectrum for 3'-bromorubrolide E (2; 700 MHz, methanol- $d_4$ )



Figure S12. <sup>1</sup>H NMR spectrum for 3'-bromorubrolide F (**3**; 700 MHz, methanol- $d_4$ )



Figure S13. COSY NMR spectrum for 3'-bromorubrolide F (3; 700 MHz, methanol- $d_4$ )



Figure S14. HSQC NMR spectrum for 3'-bromorubrolide F (3; 700 MHz, methanol- $d_4$ )



Figure S15. HMBC NMR spectrum for 3'-bromorubrolide F (3; 700 MHz, methanol- $d_4$ )



Figure S16. NOESY NMR spectrum for 3'-bromorubrolide F (**3**; 700 MHz, methanol- $d_4$ )



Figure S17. <sup>1</sup>H NMR spectrum for 3', 3"-dibromorubrolide E (**4**; 700 MHz, methanol- $d_4$ )



Figure S18. <sup>13</sup>C NMR spectrum for 3', 3"-dibromorubrolide E (**4**; 175 MHz, methanol- $d_4$ )



Figure S19. COSY NMR spectrum for 3', 3"-dibromorubrolide E (4; 700 MHz, methanol-*d*<sub>4</sub>)



Figure S20. HSQC NMR spectrum for 3', 3"-dibromorubrolide E (4; 700 MHz, methanol- $d_4$ )



Figure S21. HMBC NMR spectrum for 3', 3"-dibromorubrolide E (4; 700 MHz, methanol- $d_4$ )



Figure S22. NOESY NMR spectrum for 3', 3"-dibromorubrolide E (4; 700 MHz, methanol- $d_4$ )



Figure S23. <sup>1</sup>H NMR spectrum for rubrolide E (**5**; 300 MHz, methanol- $d_4$ )



Figure S24. HMBC NMR spectrum for rubrolide E (5; 300 MHz, methanol- $d_4$ )



Figure S25. <sup>1</sup>H NMR spectrum for rubrolide F (**6**; 700 MHz, methanol- $d_4$ )



Figure S26. <sup>1</sup>H NMR spectrum for rubrolide F + NaOH (6; 300 MHz, methanol- $d_4$ )



Figure S27. <sup>1</sup>H NMR spectrum for rubrolide F + NaOH + HCOOH (**6**; 300 MHz, methanol- $d_4$ )

**Detailed Taxonomic Description of** *Synoicum globosum* **Parker-Nance sp. nov.** (Ascidiacea, Aplousobranchia) from Algoa Bay, South Africa. (collection code SAF04-054)



Figure S28. Field collection SAF04-054 of Synoicum globosum Parker-Nance sp. nov.

## Distribution

**Type Locality**: South Africa, Eastern Cape Province, Algoa Bay, White Sands Reef, holotype SAF04-054 (TIC2004-643), 18 m, 20 July 2004, 33:59.916S, 25:42.573E; paratype TIC2004-398, 25 m, 15 July 2004, 34:0.436S, 25:43.296E.

## **Morphological Description**

The firm gelatinous colonies are divided in three parts (Figure S28), an oval to egg-shaped (5.0 x 3.5 cm in diameter and 4.5 cm high, Figure S29a) or slightly flattened heads (2.5 x 5.0 cm in diameter and 5.0 cm high, Figure S29b); a cone-shape stalk ( $3.0 \times 2.5 \text{ cm}$  in diameter by 1.5 cm high) and an irregular basal mass ( $3.0 \times 3.0 \text{ cm}$  in diameter by 2.5 cm high). The head may be shallowly subdivided or uneven. Externally the sand-free surface layer of the head forms a thin non-glossy, mat-finished skin over the internal test. A dense accumulation of fine sand particles is visible in the surface layer (1-3 mm thickness) of the short, sometimes slightly wrinkled or folded, tapering cone-shaped stalk, to give the stalk a fine sandpaper feel. The stalk gives way, when present, to a wider irregular basal attachment mass that is heavily encrusted (4-6 mm thickness) with larger sand particles and colonized by other organisms (Figure S29a).

The arrangement of the zooids is clearly visible in newly collected specimens where the red color of the zooids is in clear contrast to the mustard yellow of the external test. Little test material separates the zooids arranged in adjacent compact circular systems of 8 to 12 zooids (Figure S29a). The common cloacal aperture is centrally positioned, single and small. The diameter of the systems is between 1.6-2.7 mm. The distance between the common cloacal openings and of neighboring systems is between 1.7-3.7 mm. Although the position of the aperture of the zooids is discernible in preserved specimens, the arrangement of the zooids in distinct systems is not clear.

Internally the zooid, thoraxes and abdomens are closely packed together in recognizable layers with little test material between them. The internal test is transparent containing fine sand, shell and other foreign material (e.g. sponge megascleres and foraminiferan shells, possibly including the contents of incorporated faecal material; faecal pellets are not apparent). A denser layer of included material (1-3 mm thick) is visible under the layer that contains the zooid abdomens (2-3 mm under the surface of the colony). The test inclusion decreases in density towards the center of the colony as a whole.

The zooids are slender and between 8 and 20 mm in length. The thorax is between 1.0-2.7 mm, abdomen 0.9-2.0 mm, and the very long and slender posterior abdomen (6.0-14.8 mm in length) penetrates the inner and basal parts of the colony. The branchial opening has 6 small lobes and the atrial opening is small with a single ribbon-like tongue (Figure S29c, e). Small teeth or lobes are present on the flattened distal end of the tongue. This is less apparent in highly contracted zooids, in which it may appear pointed. The posterior rim of the opening may form a shallow pointed lobe and a dorsal papilla is generally visible just posterior of the atrial opening (Figure S29d). Twenty fine longitudinal muscles extend over the thorax. The branchial sac has 11-14 rows of stigmata with 10-11 stigmata in each of the rows. A narrow region of the branchial sac adjacent to the endostyle is unperforated (Figure S29c). The s-shaped oesophagus enters the stomach midway (Figure S29f). The smooth somewhat posteriorly flattened stomach is situated about half way down the abdomen. The posterior abdomen is long and thread-like, with immature ova and testes follicles evident in a few individuals. One or more vascular appendices varying in length extend from the posterior end of the posterior-abdomen.

One to two larvae are present in a developmental sequence in the atrial cavity of the zooids (Figure S29c). Immature larvae with a larval trunk length of 0.49 mm are characterized by two prominent media ampullae between the adhesive organs and four large lateral ampullae on each side (Figure S29g). As the larva matures (trunk length of 0.56-0.66 mm and width of 0.14-0.40 mm), the lateral ampullae divide to form 12 or more ampullae arranged in 2-3 rows on each side of the adhesive organs. Epidermal vesicles are present dorsally and ventrally. The larval tail is wound about halfway round the larval trunk (Figure S29h, i).

#### Synopsis of Synoicum Congeners

This species has a number of characteristics similar to that described for S. senegalense (Pérès, 1949) (from Senegal, Dakar, 8/7/48),<sup>1</sup> although the singular record and description of S. senegalense is short and wanting in many aspects. S. senegalense has no sand externally but sand is present internally. The zooids are 5-8 mm in length, 10-12 rows of stigmata with 9-11 stigmata per row, all zooids examined were immature and no larva were found.<sup>1</sup> The general species description,<sup>1</sup> as well as its accompanying illustration, highlights a number of differences in the zooid structure. This includes the shape of the atrial tongue, the absence of the dorsal papilla, the shape and characteristics entry point of the eosophagus into the stomach and the absence of vascular appendixes. In comparison Synoicum atlanticum Millar, 1968 from the Vema Seamount<sup>2</sup> has a similar number of rows of stigmata in the branchial sac, but has less sand internally and the surface of the stomach has a mulberry-like appearance. The shape of the colony of S. globosum differs from S. australe Millar, 1962 from Mossel Bay, South Africa<sup>3</sup> and the Vema Seamount,<sup>2</sup> and it has almost double the number of rows of stigmata in the branchial sac. Synoicum diaphanum Sluiter, 1927 from Morocco<sup>4</sup> and Senegal, West Africa<sup>5</sup> has more rows as well as more stigmata per row. The colonies of S. capense Millar, 1962 from False Bay, South Africa are heavily encrusted with sand and no sand in the interior of the colony<sup>3</sup> while S. floriferum Monniot, F. and Monniot, C., 2006<sup>5,6</sup> from Madagascar and Mozambique has delicate colonies free of sand externally and internally. The arrangement of the zooids in parallel rows radiating from a central common cloacal opening distinguish S. laboutei Monniot, F. and Monniot, C., 2006 from Madagascar<sup>6</sup> from S. globosum. Other species with more southern distributions, and from the Antarctic, that share characteristics with S. globosum include S. gairdi (Herdman, 1886),<sup>7,8</sup> which has similar larvae and rows of stigmata in the branchial sac but more stigmata per row. It further differs in the distribution of sand in the colony and the mulberry-like surface of the stomach. Finally, S. adareanum (Herdman, 1902) has a brood pouch,<sup>9</sup> S. georgianum (Sluiter, 1932) has almost three times the number of stigmata per row in the branchial sac<sup>7</sup> and *S. kerguelense* (Hartmeyer, 1911) forms encrusting sheets.<sup>10</sup>

#### References

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Figure S29. Morphology of *Synoicum globosum* Parker-Nance sp. nov. a) colony (TIC2004-398); b) colony (SAF04-054/TIC2004-643); c) zooid (thorax and abdomen only); d) part of thorax; e) atrial lip; f) stomach; g) immature larvae; h, i) mature larvae. Scales: a, b) 2 cm; c, f) 0.5 mm; d, e) 0.1 mm; g, h, i) 0.2 mm.