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New Rubrolides from the Ascidian *Synoicum blochmanni*

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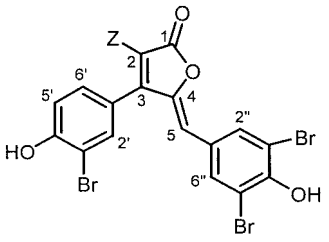
Abstract—The ascidian *Synoicum blochmanni* has been investigated as a part of our ongoing project directed towards the search for pharmacologically active marine natural products. The chemical study of *S. blochmanni* has afforded, in addition to four known compounds, six new members of the rubrolide family. Their structures were defined by spectroscopic methods with special emphasis in 1D and 2D NMR and MS. The cytotoxicities exhibited by the compounds of *S. blochmanni* against several cancer cell lines are presented.

Marine ascidians (tunicates) are widely recognized as a major source of bioactive natural products. A high number of nitrogen containing metabolites with significant biological activities have been described from ascidians, mainly peptides and aminoacid derived alkaloids. However, ascidians have also given rise to a smaller number of non-nitrogenous metabolites derived through diverse biosynthetic pathways.[1]

As a part of our ongoing project directed towards the search for pharmacologically active compounds from marine ascidians of the southern coast of Spain we have examined specimens of the red colonial tunicate *Synoicum blochmanni* collected off Tarifa Island (Cádiz, Spain). The methanol extract of *S. blochmanni* afforded six new non-nitrogenous metabolites (**1**–**6**) along with four related known compounds (**7**–**10**).[2–4]

Specimens of *Synoicum blochmanni* were collected by hand using SCUBA and immediately frozen. The Et_2O soluble fraction of a methanol extract was chromatographed on Silicagel. Further purifications using both normal and reversed phase HPLC of selected fractions led to the isolation of the new compounds (in order of elution): rubrolide I (**1**, 0.017% dry wt), rubrolide J (**2**, 0.003% dry wt), rubrolide K (**3**, 0.017% dry wt), rubrolide L (**4**, 0.018% dry wt), rubrolide M (**5**, 0.004% dry wt), and rubrolide N (**6**, 0.011% dry wt) together with the known compounds rubrolide A (**7**, 0.030% dry wt),[2] rubrolide B (**8**, 0.004% dry wt),[2] rubrolide C (**9**, 0.014% dry wt),[2,4] and prunolide A (**10**, 0.042% dry wt).[3]



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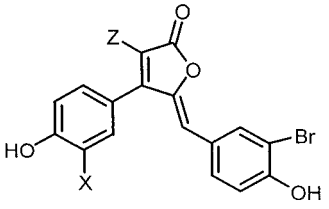
2 Z=H



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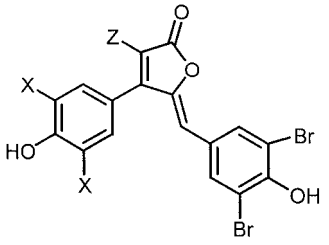
5 X=H Z=Cl
6 X=Cl Z=Br



4 X=H Z=Cl
7 X=Br Z=H
8 X=Br Z=Cl
9 X=H Z=H

Keywords: marine metabolites; biologically active compounds.

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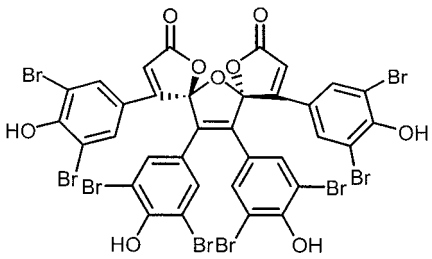

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Rubrolide K (**3**) was isolated as an amorphous orange powder. The molecular formula, $C_{17}H_9O_4ClBr_2$, was obtained from the high resolution mass measurement. The infrared spectrum exhibited absorptions at 3400 and 1747 cm^{-1} attributable to the presence of hydroxyl and carbonyl groups in the molecule.

The ^1H NMR spectrum of **3** contained two spin systems of three signals each at δ 8.04 (d, J=2.0 Hz, 1H), 7.72 (dd, J=8.8 and 2.0 Hz, 1H) and 7.08 (d, J=8.8 Hz, 1H) and at δ 7.78 (d, J=2.0 Hz, 1H), 7.48 (dd, J=8.4 and 2.0 Hz, 1H) and 7.22 (d, J=8.4 Hz, 1H) characteristics of two 1,3,4-trisubstituted aromatic rings. These two sets of proton signals were correlated in the HMQC experiment with the carbon doublets at δ 136.3, 132.5, and 117.5 and at δ 134.7, 130.9 and 117.5, respectively. The HMBC spectrum exhibited the three bond correlations between the proton signals at δ 8.04 and 7.72 with a carbon singlet at δ 156.2 assigned to a phenolic carbon, whilst the proton signal at δ 7.08 was correlated with the carbon signal at δ 127.2 (s), assigned to an aromatic carbon attached to a carbon residue, and with the signal at δ 110.9 (s) attributable to an aromatic carbon bearing bromine and adjacent to the phenolic carbon.[5] These observations suggested the presence of two 3-bromo-4-hydroxyphenyl residues in the structure of **3**.

The two aromatic rings accounted for twelve of the seventeen carbons of the molecular formula and eight of the twelve degrees of unsaturation. The remaining five carbons gave rise to the ^{13}C NMR signals at δ 164.5 (s), 149.6 (s), 146.1 (s), 117.8 (s), and 113.6 (d). The singlet at δ 164.5 together with the IR absorption at 1747 cm^{-1} indicated the presence of an ester group and the other four carbon signals above mentioned were attributed to two double bonds conjugated with the ester. Since there were four unsaturations remaining the ester must be cyclic. The spectroscopic data presented were consistent with an α,β-unsaturated butenolide bearing an exocyclic double bond. Therefore it was concluded that compound **3** must be a member of the series of the rubrolides, a group of non-nitrogenous metabolites isolated from the ascidian *Riterella rubra*.[2]

The ^1H NMR spectrum of **3** showed, in addition to the signals mentioned above, a singlet at δ 6.29 (s, 1H) due to an olefinic proton which was located at C-5 of the rubrolide skeleton upon observation in the HMBC spectrum of the three bond couplings between the signal at δ 6.29 and the two signals of the C-2″ and C-6″ carbons of one of the aromatic rings at δ 136.3 (d) and 132.5 (d). This olefinic proton signal was additionally correlated with the olefinic carbon signals at δ 146.1 (s) and 149.6 (s) assigned to C-4 and C-3, respectively, upon observation of the correlations between the δ 149.6 signal and the H-2′ and H-6′ aromatic proton signals at δ 7.78 and 7.48. The remaining chlorine substituent must be attached to the olefinic carbon C-2 that gave rise to the singlet at δ 117.8. The location of the chlorine substituent at C-2 was further verified by the observation in the electron impact mass spectrum of a multiple peak cluster at m/z 230,232,234 due to the $[C_8H_4OClBr]^{+\cdot}$ fragment arising by fragmentation type **a** of the rubrolide skeleton as shown in Fig. 1.

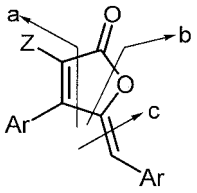

Figure 1. Characteristic fragmentations of the rubrolide skeleton using EIMS.

Finally, the configuration of the C-4,C-5 double bond was defined by a series of NOE difference spectroscopy experiments. Irradiation of the olefinic proton signal at δ 6.29 produced the expected enhancements of the H-2″ and H-6″ aromatic proton signals and, in addition, significant enhancements of the H-2′ and H-6′ aromatic proton signals were observed. These results required a *Z* geometry for the C-4,C-5 exocyclic double bond and confirmed the location of one of the bromohydroxyphenyl residues at C-3 of the butenolide ring. Structure **3** was therefore proposed for rubrolide K.

Rubrolide I (**1**) was obtained as an amorphous yellowish solid. The molecular formula, $C_{17}H_8O_4ClBr_3$, was obtained from the high resolution mass measurement. The ^1H and ^{13}C NMR spectra, together with the IR absorptions at 3270 and 1740 cm^{-1} clearly indicated that the structure of compound

Table 1. ^{13}C NMR data for rubrolides I (**1**), J (**2**), K (**3**), L (**4**), M (**5**), and N (**6**) (spectra recorded in CD_3COCD_3 excepting rubrolide N (**6**) run in CD_3OD)

	1[a]	**2**[a]	**3**[b]	**4**[a]	**5**[a]	**6**[b]
1	164.3 (s)	168.6 (s)	164.5 (s)	164.5 (s)	164.7 (s)	165.6 (s)
2	118.5 (s)	114.1 (d)	117.8 (s)	117.5 (s)	116.9 (s)	119.0 (s)
3	149.4 (s)	148.3 (s)	149.6 (s)	150.8 (s)	151.0 (s)	149.8 (s)
4	146.9 (s)	141.7 (s)	146.1 (s)	147.1 (s)	146.3 (s)	146.2 (s)
5	112.0 (d)	110.9 (d)	113.6 (d)	111.9 (d)	113.5 (d)	114.8 (d)
1′	121.2 (s)	123.8 (s)	121.3 (s)	119.6 (s)	119.8 (s)	126.4 (s)
2′	134.7 (d)	134.2 (d)	134.7(d)	131.9 (d)	131.9 (d)	134.9 (d)
3′	110.9 (s)	112.4 (s)[c]	110.9 (s)	116.7 (d)	116.7 (d)	117.0 (s)
4′	157.1 (s)	157.6 (s)	157.0 (s)	160.6 (s)	160.5 (s)	153.1 (s)
5′	117.6 (d)	117.5 (d)	117.5 (d)	116.7 (d)	116.7 (d)	123.4 (d)
6′	131.0 (d)	130.3 (d)	130.9 (d)	131.9 (d)	131.9 (d)	130.5 (d)
1″	128.3 (s)	128.3 (s)	127.2 (s)	128.3 (s)	127.3 (s)	127.1 (s)
2″	135.3 (d)	135.2 (d)	136.3 (d)	135.2 (d)	136.3 (d)	136.7 (d)
3″	111.9 (s)	112.0 (s)[c]	110.9 (s)	111.9 (s)	110.8 (s)	111.5 (s)
4″	152.9 (s)	153.1 (s)	156.2 (s)	152.8 (s)	156.1 (s)	157.1 (s)
5″	111.9 (s)	112.0 (s)[c]	117.5 (d)	111.9 (s)	117.5 (d)	117.4 (d)
6″	135.3 (d)	135.2 (d)	132.5 (d)	135.2 (d)	132.4 (d)	133.0 (d)

[a] Assignments were aided by an HMQC experiment.
[b] Assignments were aided by HMQC and HMBC experiments.
[c] These assignments may be interchanged.

1 was closely related to that of rubrolide K (**3**) discussed above. Thus, the ^{13}C NMR spectrum exhibited three doublets at δ 134.7, 131.0, and 117.6 that were correlated in the HMQC spectrum with the ^{1}H NMR signals at δ 7.78 (d, J=2.0 Hz, 1H), 7.49 (dd, J=8.4 and 2.0 Hz, 1H), and 7.23 (d, J=8.4 Hz, 1H), respectively, attributable to a 3-bromo-4-hydroxyphenyl residue that, in addition, gave rise to the ^{13}C NMR singlets at δ 157.1, 121.2, and 110.9. Furthermore, the ^{13}C NMR spectrum also showed the signals at δ 164.3 (s), 149.4 (s), 146.9 (s), 118.5 (s), and 112.0 (d) suggesting that compound **1** contained, similarly to rubrolide K (**3**), the butenolide ring bearing an exocyclic trisubstituted double bond whose olefinic proton signal appeared at δ 6.31 (s, 1H) (Table 1).

The only remaining signal in the ^{1}H NMR spectrum of **1** was a singlet at δ 8.05 (s, 2H) assignable to two protons of the equivalent methine carbons on a symmetrically tetrasubstituted benzene ring which gave rise in the ^{13}C NMR spectrum to a doublet at δ 135.3 due to the two methine carbons mentioned, a singlet at δ 111.9 attributable to two carbons attached to bromine and two signals at δ 152.9 (s) and 128.3 (s) due to two carbons linked to oxygen and carbon, respectively. These chemical shifts fit better for a 3,5-dibromo-4-hydroxyphenyl substituent rather than the alternative 2,6-dibromo-4-hydroxyphenyl one.[5]

The location at C-3 of the 3-bromo-4-hydroxyphenyl residue was defined upon observation of the close similarities of the proton and C-1′ resonances of this ring in compound **1** with those exhibited by the same ring at C-3 in rubrolide K (**3**). The electron impact mass spectrum provided confirmation to these structural assignments upon observation of the multiple peak clusters at *m/z* 230,232,234 due to the $[C_8H_4OClBr]^{+\cdot}$ fragment and at *m/z* 290,292,294 due to the $[C_8H_4O_2Br_2]^{+\cdot}$ fragment arising by fragmentations type **a** and **b** of the rubrolide skeleton, respectively (Fig. 1).

Finally, the *Z* geometry of exocyclic double bond was proposed by observation of the NOE enhancements produced on the aromatic proton signals H-2′, H-6′ and H-2″/H-6″ upon irradiation of the H-5 olefinic proton signal. These data are in agreement with structure **1** for rubrolide I.

Rubrolide J (**2**) was obtained as an orange amorphous solid. The molecular formula, $C_{17}H_9O_4Br_3$, was obtained from the high resolution mass measurement. The IR absorption together with the ^{1}H and ^{13}C NMR spectra of **2** indicated that this compound belonged to the rubrolide family and that its structure was closely related to that of rubrolide I (**1**) described above. Thus, the signals of the protons and carbons of both the 3,5-dibromo-4-hydroxyphenyl and the 3-bromo-4-hydroxyphenyl residues at C-5 and C-3 of the rubrolide skeleton, respectively, were present. However the ^{1}H NMR spectrum of **2** contained two olefinic proton signals at δ 6.34 (s, 1H) and 6.38 (s, 1H), suggesting that the structure of **2** was similar to that of rubrolide I (**1**) but lacked the chlorine substituent at C-2. The presence in the electron impact mass spectrum of the cluster of peaks at *m/z* 196,198 due to the fragment $[C_8H_6OBr]^{+\cdot}$ arising by fragmentation type **a** of the rubrolide skeleton (Fig. 1) confirmed the absence of chlorine at C-2.

The proposed structure and the *Z* geometry of the exocyclic double bond present in the rubrolide J (**2**) was confirmed by a series of NOEDS experiments, the most prominent of which were the enhancements observed on H-2′, H-6′ and H-2″/H-6″ signals upon irradiation of the olefinic proton signal of H-5 at δ 6.34.

Rubrolide L (**4**) was obtained as an amorphous orange solid. The molecular formula $C_{17}H_9O_4ClBr_2$, obtained from the high resolution mass measurement, indicated that compound **4** was an isomer of rubrolide K (**3**). The ^{13}C NMR spectrum of **4** exhibited the signals at δ 164.5 (s), 150.8 (s), 147.1 (s), 117.5 (s), and 111.9 (d) assigned to the α-chloro-β-lactone ring as in its isomer **3** and the ^{1}H NMR spectrum contained the singlet at δ 6.29 (s, 1H) characteristic of the H-5 proton of the exocyclic double bond of the rubrolides. Therefore the structural differences between both isomers had to be due to different substitution patterns on the aromatic rings.

The ^{1}H NMR spectrum of **4** showed a singlet at δ 8.05 (s, 2H) which together with the ^{13}C NMR signals at δ 152.8 (s), 135.2 (d), 128.3 (s), and 111.9 (s) were assigned to a 3,5-dibromo-4-hydroxyphenyl ring as that of rubrolide I (**1**). The remaining proton signals in the aromatic region were two doublets mutually coupled at δ 7.52 (d, J=8.8 Hz, 2H) and 7.06 (d, J=8.8 Hz, 2H), typical of a *p*-disubstituted benzene ring, which ascertained the presence of a 4-hydroxyphenyl substituent in the structure of **4**.

Similarly to the rubrolides (**1**–**3**) above discussed, the location of the aromatic rings as well as the stereochemistry of the exocyclic double bond were defined by a series of NOEDS experiments. Irradiation of the H-5 signal at δ 6.29 produced enhancements on the signal at δ 7.52 of H-2′/H-6′ of the *p*-hydroxyphenyl substituent and on the signal at δ 8.05 of the H-2″/H-6″ of the 3,5-dibromo-4-hydroxyphenyl substituent. These NOE results were consistent with the location of the aromatic rings either at C-3 or at C-5 and with a *Z* geometry of the exocyclic double bond. Furthermore, the general similarities in both ^{1}H and ^{13}C NMR data assigned to the 3,5-dibromo-4-hydroxyphenyl residue in compound **4** with those of the same ring in rubrolide I (**1**) suggested that compound **4** bore the 3,5-dibromo-4-hydroxyphenyl substituent at C-5. The electron impact mass spectrum provided confirmation to the proposed structural assignments showing the multiple peak clusters at *m/z* 262,264,266 assigned to the $[C_7H_5OBr_2]^{+\cdot}$ fragment and at *m/z* 152,154 assigned to the $[C_8H_4OCl]^{+\cdot}$ fragment due to the fragmentations type **c** and **a** of the rubrolide skeleton, respectively (Fig. 1). It was therefore proposed structure **4** for rubrolide L.

Rubrolide M (**5**) was obtained as an amorphous orange solid. The molecular formula, $C_{17}H_{10}O_4ClBr$, was obtained from the high resolution mass measurement. The IR together with the ^{1}H and ^{13}C NMR spectra clearly indicated that compound **5** was a member of the rubrolide family bearing a 3-bromo-4-hydroxyphenyl residue at C-5 as rubrolide K (**3**) and a 4-hydroxyphenyl substituent at C-3 as rubrolide L (**4**). Both the NOEDS and MS experiments fully confirmed these structural assignments. Irradiation of the H-5 olefinic proton signal at δ 6.28 (s, 1H) produced

enhancements on the H-2′/H-6′ signal at δ 7.51 (d, J=8.6 Hz, 2H) and on the H-2″ and H-6″ signals at δ 8.05 (d, J=2.4 Hz, 1H) and 7.72 (ddd, J=8.6, 2.4 and 0.4 Hz, 1H), respectively. The electron impact mass spectrum showed a cluster of peaks at m/z 152,154 assigned to the $[C_8H_4OCl]^{+\cdot}$ fragment arising by fragmentation type **a** of the rubrolide skeleton (Fig. 1). These spectroscopic features together with the similarities mentioned above are in agreement with structure **5** for rubrolide M.

Rubrolide N (**6**) was isolated as an amorphous yellow solid. The molecular formula $C_{17}H_9O_4ClBr_2$, obtained from the high resolution mass measurement, indicated that **6** was an isomer of the rubrolides K (**3**) and L (**4**). Because the IR and ^{13}C NMR of **6** and of rubrolide K (**3**) were quite similar and the ^1H NMR signals shared the same multiplicities it was concluded that the difference in the structure of both isomers, **6** and **3**, had to be due to a different distribution of the halogen substituents on C-2, C-3′, and C-3″. A careful analysis of the carbon resonances aided by the HMBC and HMQC experiments allowed the structure elucidation of compound **6**. The presence of a 3-bromo-4-hydroxyphenyl substituent was evident upon comparison of the ^{13}C NMR signals of **6** with the data of other members of the rubrolides series bearing the same substituent. The H-2′, H-6′, and H-5′ of the second disubstituted phenyl ring gave rise to the ^1H NMR signals at δ 7.81 (d, J=2.0 Hz, 1H), 7.52 (dd, J=8.4 and 2.0 Hz, 1H), and 7.85 (d, J=8.4 Hz, 1H) and were correlated in the HMQC spectrum with the carbon signals at δ 134.9, 130.5, and 123.4, respectively. The H-2′ and H-6′ signals mentioned were correlated in the HMBC experiment with the signal of an aromatic carbon signal bearing oxygen at δ 153.1 (s), whilst the H-5′ signal was correlated with the signal of an aromatic carbon attached to carbon at δ 126.4 (s). Furthermore, this H-5′ signal exhibited and additional correlation with a signal at δ 117.0 attributable to an aromatic carbon bearing a chlorine substituent and adjacent to a phenolic carbon.[5] It was concluded that compound **6** possessed a 3-chloro-4-hydroxyphenyl substituent unusual in the rubrolide family.

The configuration of the C-4,C-5 double bond, the location of the aromatic rings, and of the remaining bromine substituent were defined with the aid of the three bond correlations showed in the HMBC spectrum and a series of NOEDS experiments. The presence in the HMBC experiment of a correlation between the C-2″ and C-6″ doublets at δ 136.7 and 133.0, respectively, with the olefinic proton signal at δ 6.12 (s, 1H) indicated that the 3-bromo-4-hydroxyphenyl ring was located at C-5 of the exocyclic double bond of the rubrolide skeleton. Furthermore, irradiation of the olefinic proton signal at δ 6.12 produced enhancements not only on the H-2″ and H-6″ signals at δ 7.99 (d, J=2.0 Hz, 1H) and 7.58 (dd, J=8.4 and 2.0 Hz, 1H), respectively, but also on the H-2′ and H-6′ signals of the 3-chloro-4-hydroxyphenyl residue, which must therefore be located at C-3. These NOE enhancements require a Z geometry for the C-4,C-5 double bond.

This rationale defined the structure of rubrolide N (**6**) which displays an unprecedented halogenation pattern among the rubrolides since it contains an α-bromo-β-lactone ring and a chlorinated aromatic substituent that had not been previously reported in this family of compounds.

As a part of our investigations directed towards the search for new antitumor compounds from marine organisms, the compounds (**1**–**10**) isolated for *S. blochmanni* were tested against P-388 suspension culture of mouse lymphoid neoplasm and the monolayer cultures of human lung carcinoma (A-549), human colon carcinoma (HT-29) and human melanoma (MEL-28). The cytotoxicity assay results are detailed in the Experimental section. The new compounds rubrolides K(**3**), I (**1**), L (**4**) and M (**5**) showed significant cytotoxicities. Rubrolide M (**5**) was the most active compound tested with ED_{50}=1.2 μg/mL against the four tumor cell lines. Rubrolide K (**3**) showed a moderate activity against MEL-28 cell line (ED_{50}=5 μg/mL) and its highest activity against HT-29 cell lines (ED_{50}=1.2 μg/mL).

Experimental

General

IR and UV spectra were recorded on a Genesis Series FT IR Mattson and Phillips PU 8710 spectrophotometer, respectively. ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, on a Varian Unity 400 spectrometer using either CD_3OD or CD_3COCD_3 as solvents. Proton chemical shifts were referenced to the residual CD_3OD or CD_3COCD_3 signals at δ 3.30 and 2.04, respectively. ^{13}C NMR spectra were referenced to the central peak of CD_3OD and CD_3COCD_3 at δ 49.0 and 29.8, respectively. ^1H–^1H-COSY, HMQC and HMBC were performed using standard VARIAN pulse sequences. High resolution mass spectra were obtained by chemical ionization on a VG Autospec spectrometer. Low resolution mass spectra were recorded in a Finningan Voyager GC8000 instrument. Column chromatography was carried out using Merck Silica gel 60 (70–230 mesh). HPLC separations were performed on a LaChrom-Hitachi apparatus equipped with LiChrosorb Si 60 and LiChrosorb RP-18 columns using an UV detector. All solvents were spectral grade or were distilled from glass prior to use.

Collection, extraction, and isolation procedures. Specimens of *Synoicum blochmanni* (117 g dry weight) were collected by hand using SCUBA off Tarifa Island in May 1996 and immediately frozen. The frozen tissue was chopped and extracted with methanol at room temperature. After filtration the methanol solution was evaporated under reduced pressure yielding an aqueous residue that was exhaustively extracted with Et_2O. The organic layers were combined and the solvent evaporated to yield a yellow oily residue (1.1 g) which was chromatographed on a SiO_2 column eluting with mixtures of $CHCl_3$/MeOH of increasing polarity and, subsequently, MeOH. Selected non polar fractions were chromatographed by HPLC using mixtures of $CHCl_3$/MeOH yielding in order of elution: rubrolide B (**8**, 4.4 mg, 0.004% dry wt), rubrolide A (**7**, 35.3 mg, 0.030% dry wt), rubrolide I (**1**, 19.4 mg, 0.017% dry wt), rubrolide J (**2**, 3.2 mg, 0.003% dry wt), rubrolide K (**3**, 19.5 mg,

0.017% dry wt), rubrolide L (**4**, 21.2 mg, 0.018% dry wt), rubrolide C (**9**, 15.9 mg, 0.014% dry wt) and rubrolide M (**5**, 4.1 mg, 0.004% dry wt). More polar fractions yielded after separation on reversed phase HPLC with MeOH/H_2O (7:3) prunolide A (**10**, 48.8 mg, 0.042% dry wt) and rubrolide N (**6**, 12.8 mg, 0.011% dry wt). Final purification of each compound was accomplished by HPLC on reversed phase mode using mixtures of MeOH/H_2O.

Rubrolide I (1): orange–yellow powder; IR (film) 3270, 1740, 1604, 1500, 1297, 1156 cm^{-1}; UV (MeOH) λ_{max} 262 (ϵ=8800), 358 (ϵ=11800), 459 (ϵ=9000) nm; ^1H NMR (CD_3COCD_3) 8.05 (s, H-2″ and H-6″), 7.78 (d, *J*=2.0 Hz, H-2′), 7.49 (dd, *J*=8.4, 2.0 Hz, H-6′), 7.23 (d, *J*=8.4 Hz, H-5′), 6.31 (s, H-5); EIMS (70 eV) *m/z* (rel. int.) 556, 554, 552, 550, 548 (15:70:88:81:42), 463, 461, 459, 457 (12:37:37:13), 294, 292, 290 (32:66:35), 266, 264, 262 (11:21:11), 234, 232, 230 (14:51:40), 185, 183 (100:95); HREIMS *m/z* 551.7590, $C_{17}H_8O_4{}^{37}Cl^{79}Br_2{}^{81}Br$ requires *m/z* 551.7463; *m/z* 549.7630, $C_{17}H_8O_4{}^{37}Cl^{79}Br_3$ requires *m/z* 549.7634.

Rubrolide J (2): amorphous orange solid; IR (film) 3400, 1731, 1633, 1471, 1259, 1222 cm^{-1}; UV (MeOH) λ_{max} 257 (ϵ=4400), 358 (ϵ=5000), 446 (ϵ=3500) nm; ^1H NMR (CD_3COCD_3) 8.08 (s, H-2″ and H-6″), 7.80 (d, *J*=2.0 Hz, H-2′), 7.53 (dd, *J*=8.4, 2.4 Hz, H-6′), 7.20 (d, *J*=8.4 Hz, H-5′), 6.38 (s, H-2), 6.34 (s, H-5); EIMS (70 eV) *m/z* (rel. int.) 520, 518, 516, 514 (59:77:100:67), 464, 462, 460, 458 (3:7:7:3), 383, 381, 379 (49:73:55), 294, 292, 290 (34:75:46), 266, 264, 262 (11:22:13), 198, 196 (62:63), 185, 183 (78:75); HREIMS *m/z* 517.8018, $C_{17}H_9O_4{}^{79}Br^{81}Br_2$ requires *m/z* 517.8010; *m/z* 515.8042, $C_{17}H_9O_4{}^{79}Br_2{}^{81}Br$ requires *m/z* 515.8030; *m/z* 513.8059, $C_{17}H_9O_4{}^{79}Br_3$ requires *m/z* 513.8051.

Rubrolide K (3): amorphous orange powder; IR (film) 3400, 1747, 1598, 1495, 1414, 1290, 1188 cm^{-1}; UV (MeOH) λ_{max} 257 (ϵ=9900), 371 (ϵ=16000) nm; ^1H NMR (CD_3OCD_3) 8.04 (d, *J*=2.0 Hz, H-2″), 7.78 (d, *J*=2.0 Hz, H-2′), 7.72 (ddd, *J*=8.8, 2.0, 0.4 Hz, H-6″), 7.48 (dd, *J*=8.4, 2.0 Hz, H-6′), 7.22 (d, *J*=8.4 Hz, H-5′), 7.08 (d, *J*=8.8 Hz, H-5″), 6.29 (s, H-5); EIMS (70 eV) *m/z* (rel. int.) 476, 474, 472, 470 (15:68:98:42), 383, 381, 379 (14:28:14), 234, 232, 230 (3:14:11), 214, 212 (46:51), 186, 184 (24:26), 105 (100); HREIMS *m/z* 473.8525, $C_{17}H_9O_4{}^{37}Cl^{79}Br^{81}Br$ requires *m/z* 473.8506; *m/z* 471.8516, $C_{17}H_9O_4{}^{37}Cl^{79}Br_2$ requires *m/z* 471.8527; *m/z* 469.8549, $C_{17}H_9O_4{}^{35}Cl^{79}Br_2$ requires *m/z* 469.8556.

Rubrolide L (4): amorphous reddish–orange solid; IR (film) 3390, 1760, 1608, 1470, 1259, 1174 cm^{-1}; UV (MeOH) λ_{max} 265 (ϵ=8400), 353 (ϵ=11000), 454 (ϵ=15800) nm; ^1H NMR (CD_3COCD_3) 8.05 (s, H-2″ and H-6″), 7.52 (d, *J*=8.8 Hz, H-2′ and H-6′), 7.06 (d, *J*=8.8 Hz, H-3′ and H-5′), 6.29 (s, H-5); EIMS (70 eV) *m/z* (rel. int.) 476, 474, 472, 470 (15:70:100:49), 383, 381, 379 (19:38:20), 294, 292, 290 (16:32:17), 266, 264, 262 (6:11:6), 185, 183 (48:55), 154, 152 (30:80); HREIMS *m/z* 473.8523, $C_{17}H_9O_4{}^{37}Cl^{79}Br^{81}Br$ requires *m/z* 473.8506; *m/z* 471.8500, $C_{17}H_9O_4{}^{37}Cl^{79}Br_2$ requires *m/z* 471.8527; *m/z* 469.8549, $C_{17}H_9O_4{}^{35}Cl^{79}Br_2$ requires *m/z* 469.8556.

Rubrolide M (5): amorphous orange solid; IR (film) 3400, 1750, 1600, 1505, 1260, 1174 cm^{-1}; UV (MeOH) λ_{max} 252 (ϵ=10000), 366 (ϵ=19000), 446 (ϵ=3200) nm; ^1H NMR (CD_3COCD_3) 8.05 (d, *J*=2.4 Hz, H-2″), 7.72 (ddd, *J*=8.6, 2.4, 0.4 Hz, H-6″), 7.51 (d, *J*=8.6 Hz, H-2′ and H-6′), 7.08 (d, *J*=8.4 Hz, H-5″), 7.06 (d, *J*=8.6 Hz, H-3′ and H-5′), 6.28 (s, H-5); EIMS (70 eV) *m/z* (rel. int.) 396, 394, 392 (33:100:86), 303, 301, (42:40), 214, 212 (59:62), 186, 184 (30:27), 154, 152 (15:48); HREIMS *m/z* 393.9453, $C_{17}H_{10}O_4{}^{37}Cl^{81}Br$ requires *m/z* 393.9430; *m/z* 391.9474, $C_{17}H_{10}O_4{}^{35}Cl^{79}Br$ requires *m/z* 391.9451.

Rubrolide N (6): amorphous yellow solid; IR (film) 3470, 1760, 1598, 1484, 1239, 1040 cm^{-1}; UV (MeOH) λ_{max} 259 (ϵ=8100), 378 (ϵ=13200) nm; ^1H NMR (CD_3OD) 7.99 (d, *J*=2.0 Hz, H-2″), 7.85 (d, *J*=8.4 Hz, H-5′), 7.81 (d, *J*=2.0 Hz, H-2′), 7.58 (dd, *J*=8.4, 2.0 Hz, H-6″), 7.52 (dd, *J*=8.4, 2.0 Hz, H-6′), 6.90 (d, *J*=8.4 Hz, H-5″), 6.12 (s, H-5); EIMS (70 eV) *m/z* (rel. int.) 476, 474, 472, 470 (14:73:100:42), 383, 381 (9:18), 214, 212 (22:23); HREIMS *m/z* 471.8553, $C_{17}H_9O_4{}^{35}Cl^{79}Br^{81}Br$ requires *m/z* 471.8536; *m/z* 469.8568, $C_{17}H_9O_4{}^{35}Cl^{79}Br_2$ requires *m/z* 469.8556.

Cytotoxicity assays. Both the new and the known metabolites (**1**–**10**) isolated from *S. blochmanni* were tested against the four tumor cell lines mentioned. The individual cell lines identifiers are given along with the corresponding ED_{50} (μg/mL) values for each of the compounds tested. Values of ED_{50} over 5 μg/mL are not reported. **Rubrolide I (1):** HT-29 (5); r**ubrolide K (3):** P-388 (2.5), A-549 (2.5), HT-29 (1.2), MEL-28 (5); **rubrolide L (4):** P-388 (5), A-549 (5), HT-29 (2.5), MEL-28 (5); **rubrolide M (5):** P-388 (1.2), A-549 (1.2), HT-29 (1.2), MEL-28 (1.2); **rubrolide B (8):** P-388 (5), A-549 (5), HT-29 (5), MEL-28 (5).

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