# The effect of structure on the electrochemical properties of 14 marine pyrroloquinoline metabolites

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The electrochemical properties of 14 structurally related pyrroloquinoline metabolites (compounds **1–14**) isolated from marine sponges were studied in pH-varied experiments using cyclic and square wave voltammetry. In general both substitution patterns and pH were observed to influence the reduction potentials of these molecules.

**Keywords:** pyrroloquinoline, marine, sponge, pH, voltammetry

Marine sponges of the family Latrunculiidae are prolific producers of bioactive alkaloid pigments possessing a characteristic pyrroloquinoline skeleton.[1-4] Pyrroloquinoline metabolites are strongly cytotoxic and were at one time considered as a potential source of new anti-tumour drugs.[3] Our recent isolation and identification of 21 pyrroloquinoline constituents from four species of South African latrunculid sponges[1] has provided us with an unique opportunity to compare the electrochemical properties of 14 of these metabolites (compounds **1–14**) representing four structural classes *i.e.* (a) tricyclic pyrroloquinolines, (b) discorhabdin C type metabolites, (c) discorhabdin D/V type metabolites, and (d) *bis* pyrroloiminoquinones (Fig. 1).

Details of the isolation and structure elucidation of these compounds are provided by Antunes *et al.*[1] Electrochemistry has often been used to describe and understand certain biological processes, as molecular electron transfer is proposed to be a crucial process in an organism's biological defence.[5] Physiological and biochemical processes associated with electrochemical reactions include photosynthesis, nerve excitation, blood coagulation, vision, smell and many enzymatic reactions, as well as the bioreductive activation of pro-drugs to a more toxic constituent.[5,6] Radisky *et al.*[4] have showed that the DNA reductive cleavage activity of selected marine sponge pyrroloquinoline metabolites [*e.g* damirone B (**2**) and discorhabdin A (**4**)] is mediated by redox chemistry. The results demonstrated that pyrroloquinoline mediated reductive cleavage of DNA (determined using a super coiled pBR322 DNA assay) is probably dependent on the capacity of the compound to intercalate into DNA in addition to its ability to produce a stable reactive semi-quinone radical. The stability of the radical is due to extended electron delocalisation over a vinyl coupled semiquinone species. Since quinones have been observed to produce semiquinones in reductive environments,[4,6-8] it may be assumed that this is one possible mechanism of reduction for these molecules.

Although the four classes of metabolites studied will be discussed separately, some common trends are evident. Generally, for the tricyclic pyrroloquinolines (Fig. 1), cyclic voltammetry (CV) revealed at least one clear reduction process as shown in Fig. 2 for compound **1**.

As expected, the ease of reduction decreased with an increase in pH for all compounds (Table 1).

In order to determine whether a diffusion-controlled process is the dominant mechanism at the electrode surface, the peak current (*i*a) *versus* the square root of the scan rate was monitored for selected compounds (Fig. 3).

Linear plots were obtained for scan rates ranging from 25 to 790 mV.s$^{-1}$ for all of the pyrroloquinolines at pH 5.5, which indicates that a diffusion-controlled process prevails at this pH. This trend was also observed at pH 7.5 for all compounds except **3** and **7**. The dynamics involved could include adsorption onto the electrode surface between scans or slow electron transfer between the compound and the electrode surface. The anodic to cathodic peak current ($i_a/i_c$) values obtained were also observed to be less than unity for these two compounds at pH 7.5, suggesting quasi-reversibility. No attempt was made to speculate about the exact sites of reduction on these complicated molecules but it is reasonable to assume that, in all probability, reduction takes place at the positively charged nitrogen atoms (N-18). Additional reduction may occur at the ketones (if present) at C-10, C-11 and perhaps C-3 (see Fig. 1 for atom numbering). These two types of functional groups were present on almost all the molecules studied (an exception being compound **2** which has a quaternary amine instead of an imine at N-18). The $\Delta E$ values for most of the compounds studied at both pH values ranged from 30 to 50 mV, while the standard potassium ferricyanide had $\Delta E$ values of 90 and 94 mV at pH 5.5 and pH 7.5 respectively. A study of the pH behaviour of these compounds from pH 3.0 to 8.0 revealed a linear plot with regards to the $E_{½}$ values with the gradient being ~30mV/division for most compounds. This suggests that most compounds underwent 2 electron reductions.

**Table 1** Electrochemical data ($E_{½}$ or $E_c$*) for compounds **1** to **14** in buffer solutions

| Compound (pH) | Process I (mV) | Process II (mV) | Process III (in mV) |
|---|---|---|---|
| **1** (5.5) | –350 | – | – |
| (7.5) | –422 | – | – |
| **2** (5.5) | –353 | – | – |
| (7.5) | –458 | – | – |
| **3** (5.5) | –251 | –450* | – |
| (7.5) | –334 | –469* | – |
| **4** (5.5) | –282 | – | – |
| (7.5) | –337 | – | – |
| **5** (5.5) | –307 | – | – |
| (7.5) | –358 | – | – |
| **6** (5.5) | –333 | –261* | – |
| (7.5) | –424 | –266 | – |
| **7** (5.5) | –346 | –222 | – |
| (7.5) | –435 | –594* | – |
| **8** (5.5) | –384 | –295* | – |
| (7.5) | –445 | – | – |
| **9** (5.5) | –253 | – | – |
| (7.5) | –309 | – | – |
| **10** (5.5) | –259 | – | – |
| (7.5) | –320 | – | – |
| **11** (5.5) | –438 | –333 | – |
| (7.5) | –478 | – | – |
| **12** (5.5) | –495 | –396 | –248 |
| (7.5) | –531 | –425 | –318 |
| **13** (5.5) | –525 | – | – |
| (7.5) | –637 | – | – |
| **14** (5.5) | –567 | – | – |
| (7.5) | –669 | – | – |

*No return wave therefore $E_c$

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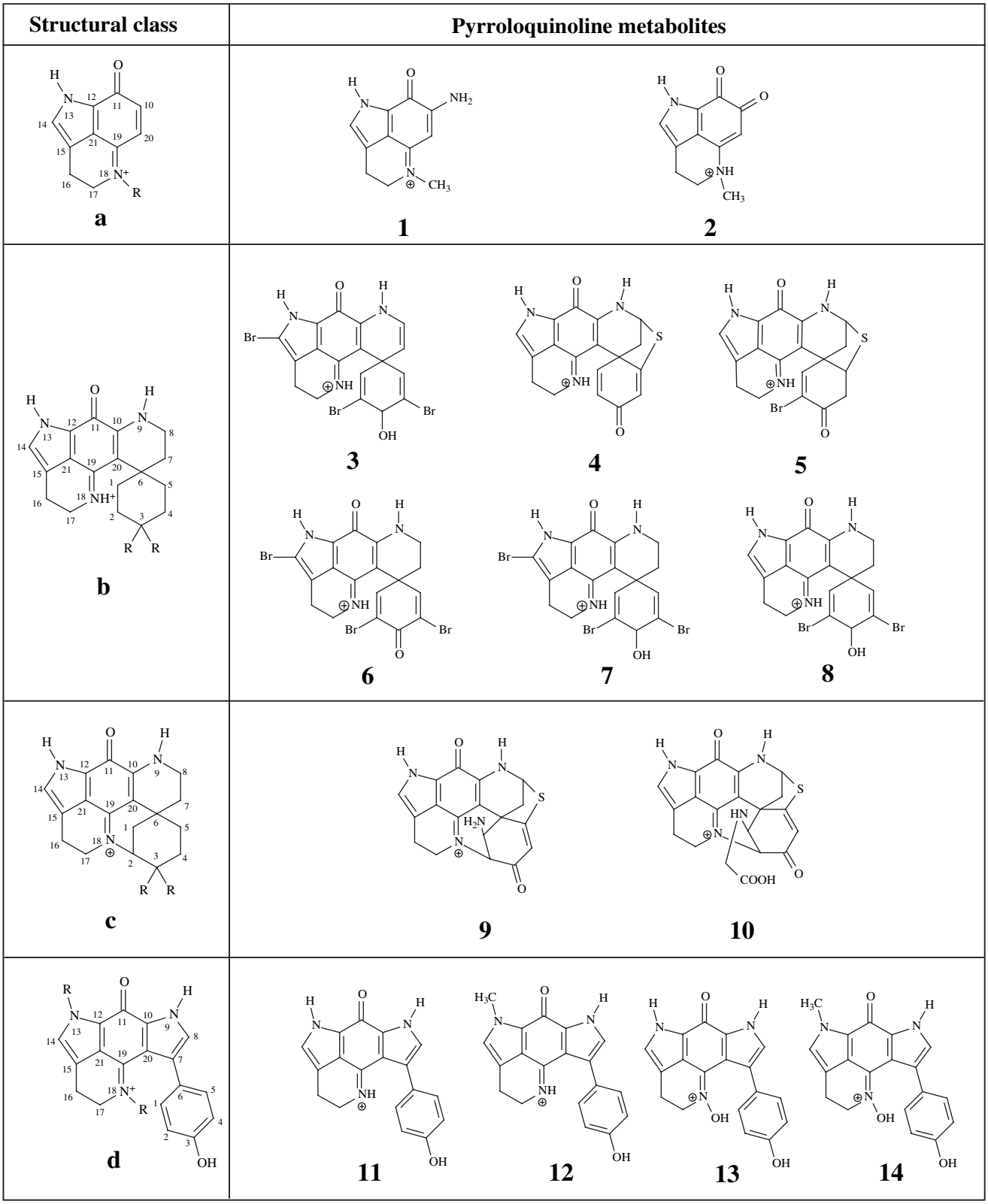


**Fig. 1** Structural classes of pyrroloquinolines. (a) tricyclic pyrroloquinolines and pyrroloamino-ortho-quinones; (b) discorhabdin C type skeleton; (c) the discorhabdin D / V type skeleton; and (d) the *bis*-pyrroloiminoquinones.

The main reduction process for the molecules was assigned as process I and any additional reduction couples as process II or process III. Only the main reduction process, *i.e.* process I, was studied as a function of voltage sweep rate and switching potential, and the tabulated data (Table 1) summarises the main reduction processes for each of the pyrroloquinolines (**1** to **14**) studied. Where there was some uncertainty as to the presence of additional peaks in the data obtained from cyclic voltammetry (CV), Oster–Young square wave voltammetry (OSWV) was used. From the anodic to cathodic peak current values it was generally found that all molecules studied were stable to the first reduction process. No molecule was stable to the second or the third reduction process.

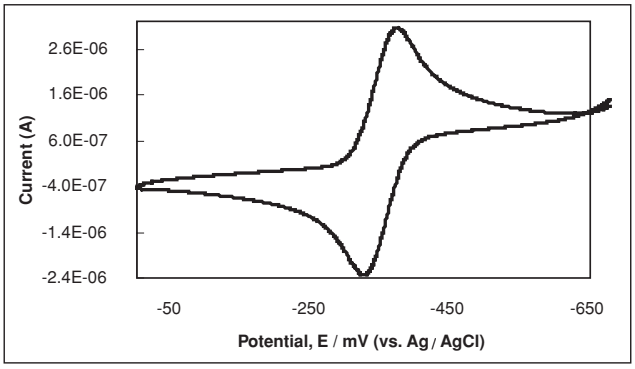


**Fig. 2** Cyclic voltammogram of compound **1** at pH 5.5. Scan rate: 25 mV/s.

*Tricyclic pyrroloquinolines (Compounds* **1** *and* **2***)*

The CV of compounds **1** (Fig. 2) and **2** showed reversible reduction behaviour as the anodic to cathodic peak currents ($i_a/i_c$) were near unity. The $E_{½}$ values (Table 1) showed that both compounds **1** and **2** were relatively more difficult to reduce than compounds **3–5**, **9** and **10**. Compound **1** has one ketone functionality at C-11 that may be reduced to a hydroxyl group in addition to an imine functionality at N-18 where reduction may occur readily resulting in saturation of the imine. Compound **2** has a positively charged quaternary amine at N-18 as well as two ketone functionalities at C-10 and C-11, which may be reduced to hydroxyls. The quaternary amine is still the most likely reduction site on compound **2** (Fig. 1a). Compounds **1** and **2** both have a methylated, charged N-18, with compound **2** also possessing an acidic proton on N-18. At pH 5.5 the $E_{½}$ potentials are similar for compound **1** and compound **2**. At pH 7.5 the easier reduction experienced by compound **1** ($E_{½}$ = -422 mV compared to $E_{½}$ = -458 mV for compound **2**) could be attributed to the more electron poor nitrogen at N-18 for compound **1**.

*Discorhabdin C type compounds (***3** *to* **8***)*

The discorhabdin C type compounds (**3–8**) showed two reduction processes, I and II. The first process, I, was reversible at pH 5.5 for all compounds with $i_a/i_c$ ~ 1. The second process, II, was mainly irreversible (no return peak) except for compound **6** at pH 7.5 and compound **7** at pH 5.5, both of which had quasi-reversible peaks (there was a return peak but the anodic to cathodic currents were far less than unity). Table 1 presents the data obtained for the discorhabdin C type pyrroloquinolines. The ease of reduction at both pH 5.5 and 7.5 was observed as follows: **3** > **4** > **5** > **6** > **7** > **8** (Table 1). Compound **3** had the lowest reduction potential and was the only compound with a $\Delta^7$ olefin. This olefin was possibly responsible for the lowering of the potential as the analogous compound **7**, which differed only by the absence of the unsaturated bond, had far higher reduction potentials. The thioether functionalities on compounds **4** and **5** between C-5 and C-8 also resulted in a lower reduction potential of these molecules, with compound **4** containing an additional $\Delta^4$ olefin and having a lower reduction potential. Compound **6** differed from compound **7** only by oxidation at C-3 (*i.e.* ketone for compound **6** and hydroxyl for compound **7**). This functional group change leads to a slight increase in $E_{½}$, for compound **6** compared to compound **7**. Compounds **7** and **8** differ only in bromination at C-14 in the former compound. The presence of bromine at C-14 leads to a decrease in reduction potential. Bromine has a high electronegativity and could therefore draw electrons away from the ring, which should make compound **7** easier to reduce than compound **8**. Interestingly a plot of the $^{13}$C NMR chemical shifts for the C-19 carbon (C-19 was chosen because of its centrality in the two molecules) of the pyrroloquinoline skeleton, shown in Fig. 4, certainly gives an indication of the relationship between electron density at C-19 and the ease of reduction *i.e.* the more deshielded the C-19 carbon (*e.g.* compound **3**) the easier it is to reduce.

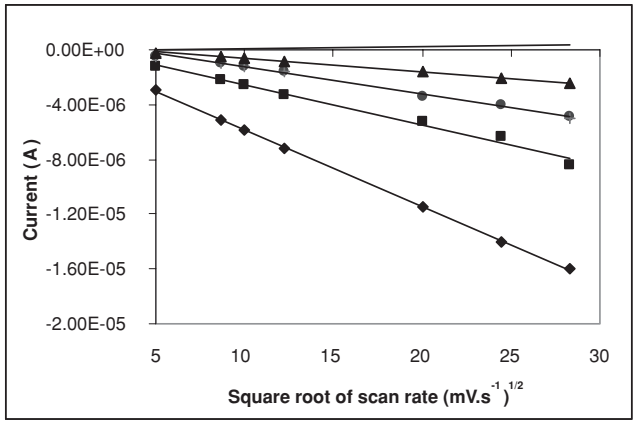


**Fig. 3** Plots of the peak current versus the square root of the scan rate for compounds 1(♦), 2(■), 6(●) and 13(▲) at pH 5.5.

*Discorhabdin D/V type compounds (compounds* **9** *and* **10***)*

The CV data for compounds **9** and **10** showed reversible behaviour with cathodic to anodic peak currents near unity except for compound **10** which showed quasi-reversible behaviour at pH 7.5 ($i_a/i_c$ ~ 0.80). Table 1 lists the electrochemical data obtained for the discorhabdin D/V type pyrroloquinolines (compounds **9** and **10**) and only one redox

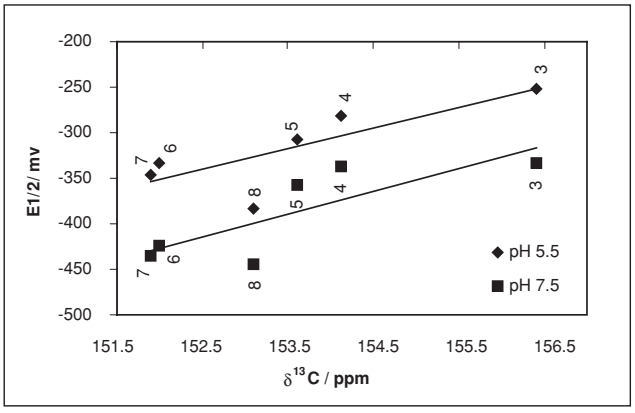


**Fig. 4** A plot of the C-19 $^{13}$C NMR shifts for the discorhabdin C type pyrroloiminoquinones.

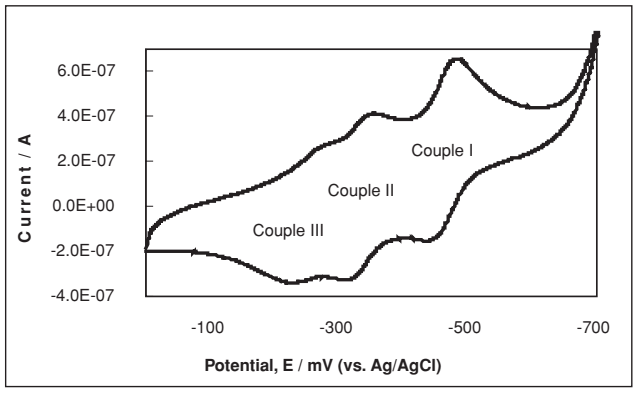


**Fig. 5** Cyclic voltammogram of compound 12 at pH 7.5. Scan rate: 25 mV/s.

couple was observed (similar to Fig. 2). In order to assess the effect of varying the substituent at C-1 in the discorhabdin D type compounds, the $E_{½}$ values of compounds **9** and **10** could be directly compared. Compound **9** (containing a primary amine at C-1) was easier to reduce than compound **10** which has a quaternary amine at C-1 as well as a carboxylic acid, which resulted in loss of reversible behaviour at pH 7.5, probably due to the deprotonation of the carboxylic acid. Surprisingly the primary amine at C-1 did not have as large an affect on the reduction potential as did the amine substituent at C-10 in compound **1** on changing from the protonated to the free form. This may indicate that the reductions do occur preferentially on the original pyrroloquinoline ring structure. These two compounds had relatively low reduction potentials probably due to the thioether moieties at C-5 to C-8. This same trend was observed for the discorhabdin C type compounds (**4** and **5**).

*bis-Pyrroloiminoquinones (compounds* **11** *to* **14***)*

The data obtained for the *bis*-pyrroloiminoquinone compounds (**11**–**14**) is presented in Table 1. The *bis*-pyrroloiminoquinones showed mainly one reversible process, I. Compound **11** showed two processes, I and II while compound **12** also showed three couples, I, II and III, (Fig. 5).

Table 1 shows that in general the *bis*- pyrroloiminoquinones were more difficult to reduce than the other molecules in this study (at both pH values), with $E_{½}$ (for couple I) ranging from –395 mV to –669 mV at both pH values. Structurally these molecules contain an additional fused pyrrole onto which is attached a phenol on C-7. This change in structure obviously leads to an increase in the reduction potential of the molecule. From the redox couples of both compounds **11** and **12** the $i_a/i_c$ was generally found to deviate from unity. The results indicate that these compounds exhibit either quasi-reversible or irreversible (no return peak) behaviour at both pH's. Slow electron transfer, or irreversible structural change, between the compound of interest and the working electrode is often responsible for quasi-reversible or irreversible systems. Hydrogen bonding may also result in inefficient or slow electron transfer. The cyclic voltammogram obtained for compound **12**, shown in Fig. 5, is the most complex obtained for any of the pyrroloiminoquinones (**1**–**14**) and shows three quasi-reversible couples, while the demethylated compound **11** shows only two reduction couples, implicating the possible involvement of the N-13 methyl group in the redox process, since the two compounds differ only in N-13 substitution. The electron donating methyl group in compound **12** possibly results in an increase in $E_{½}$ at both pH values. Compounds **13** and **14** are by far the most difficult to reduce, compared to the other molecules, and this may be ascribed to the presence of the electron donating N-18 oxime functionalities. The ease of reduction for *bis*-pyrroloiminoquinones **11**–**14** at pH 5.5 was as follows: **12** > **11** > **13** > **14**, while at pH 7.5 the sequence altered slightly to **11** > **12** > **13** > **14** (Table 1).

The electrochemical behaviour of fourteen pyrroloquinolines was studied using cyclic voltammetry and square wave voltammetry. All compounds had negative reduction potentials within biologically relevant pH ranges and the pH-varied experiments demonstrated that the reduction potentials are pH dependent. Variation of the substituents on these molecules had a dramatic effect upon their reduction potentials. In this regard attachment of thioether functions between C-5 and C-8 led to lower reduction potentials, bromination on C-14 also lowered potentials. Fused pyridines containing phenol attachments led to higher reduction potentials and the presence of N-18 oximes increased reduction potentials dramatically. The molecules were generally found to be stable to the first reduction but less stable upon subsequent reductions. Interestingly, compounds **13** ($IC_{50}$ 128.2 μM) and **14** ($IC_{50}$ 16.5 μM) are significantly less cytotoxic to human colon tumor cells (HCT-116) than compounds **1**-**12** ($IC_{50}$ *ca* 0.1–3 μM) [1]. These data underline the importance of electrochemical processes in determining the cytotoxicity of pyrroloiminoquinone metabolites.

## Experimental

Voltammetric measurements were done in a conventional three-electrode system. Both cyclic voltammetry (CV) and osteryoung square wave voltammetry (OSWV) data were collected using a CV-50W Bio-Analytical System (BAS) voltammetric analyser. A gold electrode (0.8 mm in diameter) and a platinum electrode were used as the working and auxiliary electrodes, respectively. Before use in the electrochemical experiments, the surface of the gold electrode was hand polished on fine chamois leather containing γ–$Al_2O_3$ slurry. A Ag|AgCl (3 mol/dm$^3$ KCl) reference electrode was used for the aqueous solutions studied. The electrolyte buffer solutions were made up prior to data collection and the solutions saturated with nitrogen before running the experiment. Nitrogen gas was supplied by MG Fed gas and purified by passing through a Drierite self-indicating mesh 8 (anhydrous $CaSO_4$) from SAAR Chemicals. All experiments were carried out at ambient temperature and a dinitrogen atmosphere was maintained throughout. The buffer solutions were made up as follows:

pH 5.5: ($NaH_2PO_4.H_2O$ 6.22 mg/ml, $Na_2HPO_4$ (anhydrous) 0.232 mg/mL, $Na_2EDTA$ 0.375 mg/ml);

pH 7.5: ($NaH_2PO_4.H_2O$ 0.575 mg/ml, $Na_2HPO_4$ (anhydrous) 2.17 mg/mL, $Na_2EDTA$ 0.375 mg/ml).

Buffer solutions (pH 5.5 and 7.5) were used as the solvents and the potential swept between 800 mV and 0 mV for all experiments.

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## References

1 E.M. Antunes, D.R. Beukes, M. Kelly, T. Samaai, L.R. Barrows, K.M. Marshall, C. Sincich and M.T. Davies-Coleman, *J. Nat. Prod.*, 2004, **67**, 1268.
2 S. Sakemi, H.H Sun, C.W. Jefford and G. Bernardinelli, *Tetrahedron Lett*., 1989, **30**, 2517.
3 N.B. Perry, J.W. Blunt and H.M.G. Munro, *Tetrahedron,* 1988, **44**, 1727.
4 D.C. Radisky, E.S Radisky, L.R. Barrows, B.R. Copp, R.A Kramer and C.M. Ireland, *J. Am. Chem. Soc.,* 1993, **115**, 1632.
5 C. M. A. Brett and A. M. O. Brett, *Electrochemistry: Principles, Methods, Applications*, Oxford University Press, Oxford, 1993.
6 A. Monge, J.A. Palop, A. López de Cerain, V. Senador, F.J. Martínez-Crespo, Y. Sainz, S. Narro, E. García, C. de Miguel, M. González, E. Hamilton, A.J. Barker, E.D. Clarke and D.T. Greenhow, *J. Med. Chem*., 1995, **38**, 1786.
7 W.O. Foye, *Principles of Medicinal Chemistry*, 3rd edn., Lea and Febiger (UK) Ltd., U.K., 1989, Ch. 37.
8 B. Halliwell, J.M.C. Gutteridge, *Free Radicals in Biology and Medicine*, 2nd edn., Oxford University Press, Oxford, 1989.