

# Syntheses, Protonation Constants and Antimicrobial Activity of 2-Substituted *N*-alkylimidazole Derivatives

Phumelele Kleyi,<sup>a</sup> Ryan S. Walmsley,<sup>a</sup> Isaac Z. Gundhla,<sup>a</sup> Tara A. Walmsley,<sup>b</sup> Tembisa I. Jauka,<sup>a</sup> Joanna Dames,<sup>b</sup> Roderick B. Walker,<sup>c</sup> Nelson Torto<sup>a</sup> and Zenixole R. Tshentu<sup>a,\*</sup>

<sup>a</sup>Department of Chemistry, Rhodes University, P.O. Box 94, Grahamstown, 6140, South Africa.

<sup>b</sup>Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, P.O. Box 94, Grahamstown, 6140, South Africa.

<sup>c</sup>Division of Pharmaceutics, Faculty of Pharmacy, Rhodes University, P.O. Box 94, Grahamstown, 6140, South Africa.

Received 31 May 2012, revised 16 July, accepted 3 October 2012.

## ABSTRACT

A series of *N*-alkylimidazole-2-carboxylic acid, *N*-alkylimidazole-2-carboxaldehyde and *N*-alkylimidazole-2-methanol derivatives [alkyl = benzyl, methyl, ethyl, propyl, butyl, heptyl, octyl and decyl] have been synthesized and the protonation constants determined. The antimicrobial properties of the compounds were tested against Gram-negative (*Escherichia coli*), Gram-positive (*Staphylococcus aureus* & *Bacillus subtilis* subsp. *spizizenii*) bacterial strains and yeast (*C. albicans*). Both the disk diffusion and broth microdilution methods for testing the antimicrobial activity showed that *N*-alkylation of imidazole with longer alkyl chains and the substitution with low  $pK_a$  group at 2-position resulted in enhanced antimicrobial activity. Particularly, the *N*-alkylimidazole-2-carboxylic acids exhibited the best antimicrobial activity due to the low  $pK_a$  of the carboxylic acid moiety. Generally, all the *N*-alkylimidazole derivatives were most active against the Gram-positive bacteria [*S. aureus* (MIC = 5–160  $\mu\text{g mL}^{-1}$ ) and *B. subtilis* subsp. *spizizenii* (5–20  $\mu\text{g mL}^{-1}$ )], with the latter more susceptible. All the compounds showed poor antimicrobial activity against both Gram-negative (*E. coli*, MIC = 0.15 to >2500  $\mu\text{g mL}^{-1}$ ) bacteria and all the compounds were inactive against the yeast (*Candida albicans*).

## KEYWORDS

*N*-alkylimidazoles, antimicrobial,  $pK_a$  effect.

## 1. Introduction

The use of imidazole compounds is well established in the field of medicinal chemistry, finding applications as anticancer<sup>1</sup>, antibacterial<sup>2</sup>, antifungal<sup>3</sup>, antiparasitic<sup>4</sup> and antidiabetic<sup>5</sup> drugs, to name a few. The discovery ofazole antibacterial and antifungal agents began with benzimidazole in 1944.<sup>6</sup> Since then there have been several reports noting the antifungal activity of imidazoles,<sup>6–8</sup> the action of which is suspected to be due to interference with ergosterol synthesis and membrane damage.<sup>9</sup> Azoles inhibit lanosteroldemethylase, a cytochrome P-450 dependent enzyme which is responsible for the synthesis of ergosterol. Ergosterol is a major sterol of the cytoplasmic membrane and is responsible for a variety of cellular functions. It is responsible for the fluidity and integrity of the cytoplasmic membrane, as well as the proper functioning of chitin synthetase. Chitin synthetase is in turn responsible for cell growth and division.<sup>9,10</sup> The mechanism of antibacterial action of azoles is believed to be through the inhibition of enoyl acyl carrier protein reductase (FabI), a novel antibacterial target.<sup>11</sup>

Further development and modification of antimicrobial agents remains crucial, since rapid mutation and subsequent drug resistance of new microbial strains continues.<sup>12</sup> Derivatizing the imidazole group with long alkyl chains has dramatically improved the antibacterial activity of simple imidazoles.<sup>2</sup> A few substituents at the 2-position of *N*-alkylimidazole derivatives such as the methyl group and the ether moiety have also been investigated for their effect on the antimicrobial activity.<sup>2,4</sup> In this study, several 2-substituted *N*-alkylimidazole derivatives were

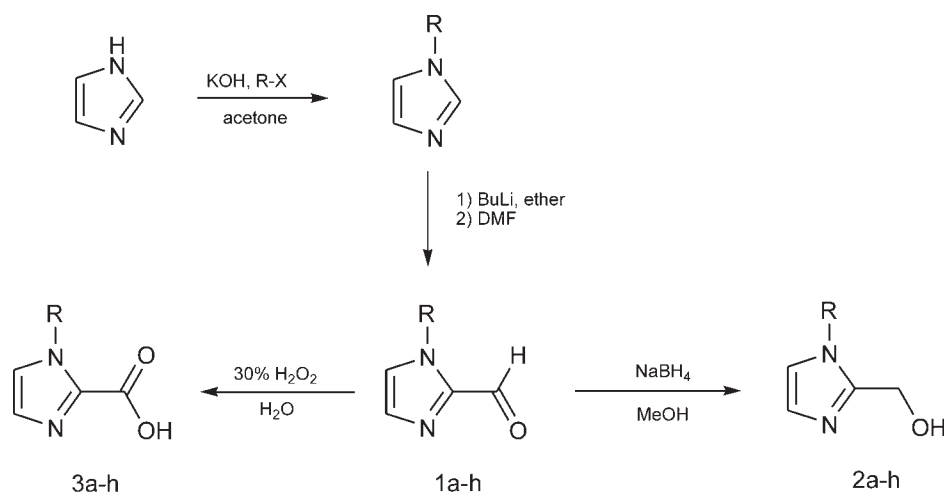
synthesized and the effect of the  $pK_a$  of the 2-substituent [aldehyde (CHO), alcohol (CH<sub>2</sub>OH), carboxylic acid (CO<sub>2</sub>H)] on the antimicrobial activity was investigated. The antimicrobial activity of 2-substituted *N*-alkylimidazole derivatives was tested against *Escherichia coli*, *Bacillus subtilis* subsp. *spizizenii*, *Staphylococcus aureus* and *Candida albicans*. The  $pK_a$  values, which may relate to drug solubility, permeability and protein binding,<sup>13</sup> were determined for all the synthesized imidazole compounds. The  $pK_a$  dependence of the activity of antimicrobial agents is well documented and, in general, a reduction in the  $pK_a$  of the antimicrobial agent enhances its penetration of the microorganism membrane which results in increased activity.<sup>14,15</sup> This study, therefore, essentially investigates the effect of the non-ionizable aldehyde group, the alcohol group which ionizes at high pH values, as well as the carboxylic acid group which ionizes at very low pH values on the antimicrobial activity of *N*-alkylimidazole derivatives.

## 2. Results and Discussion

### 2.1. Chemistry

The synthesis of *N*-alkylimidazole-2-carboxaldehydes (**1a–h**) and *N*-alkylimidazole-2-methanols (**2a–h**) was carried by previously reported methods.<sup>16–18</sup> Scheme 1 illustrates the synthesis steps of the 2-substituted *N*-alkylimidazole derivatives. The first step was the synthesis of *N*-alkylimidazole which was carried out by the reaction of imidazole and alkyl bromides in the presence of potassium hydroxide. The reaction of *N*-alkylimidazole and *n*-butyllithium at –78 °C (dry ice/acetone slurry) followed by the

\* To whom correspondence should be addressed. E-mail: z.tshentu@ru.ac.za



**Scheme 1**  
Synthesis of 2-substituted *N*-alkylimidazole derivatives

addition of dry DMF gave the desired *N*-alkylimidazole-2-carboxaldehydes (**1a–h**) in moderate to excellent yields. *N*-alkylimidazole-2-methanols (**2a–h**) were obtained by the reduction of *N*-alkylimidazole-2-carboxaldehydes (**1a–h**) with sodium borohydride at 0 °C in methanol. The novel synthesis of *N*-alkylimidazole-2-carboxylic acids (**3a–h**) was carried out by the oxidation of *N*-alkylimidazole-2-carboxaldehydes (**1a–h**) with 30 % hydrogen peroxide in water. This ‘green’ method was carried out at room temperature and it produced water as the only by-product and thus is an environmentally-friendly alternative to the current metal-catalyzed oxidation methods.

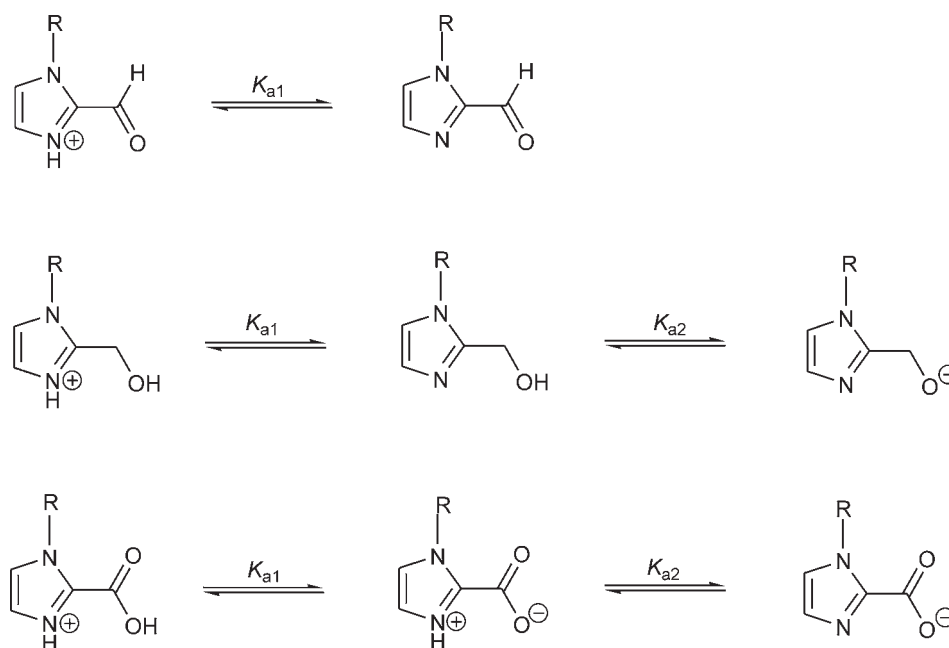
The characterization of the 2-substituted *N*-alkylimidazole derivatives was carried out using <sup>1</sup>H and <sup>13</sup>C NMR, IR and elemental analysis. <sup>1</sup>H NMR spectra of **1a–h** showed the appearance of a singlet in the region 9.7–9.9 ppm for one proton indicative of an aldehydic proton (CHO). Furthermore, the disappearance of the singlet in the region 7.5–8.0 ppm indicated the substitution of the imidazole proton at the 2-position. <sup>13</sup>C NMR spectra of **1a–h** showed the appearance of a signal in the region 180–185 ppm indicating the presence of the carbonyl carbon (CHO). For **2a–h**, the <sup>1</sup>H NMR spectra showed the disappearance of the aldehydic proton singlet, in **1a–h**, and the appearance of a new singlet in the region 4.5–4.7 ppm, indicative of the two methylene protons (CH<sub>2</sub>OH). <sup>13</sup>C NMR of **2a–h** exhibited the disappearance of the carbonyl carbon signal and the appearance of a new signal in the region 146–149 ppm for the methylene carbon (CH<sub>2</sub>OH). <sup>13</sup>C NMR showed a marked upfield shift of the carbonyl carbon to the region 156–159 ppm for the carboxylic carbon (COOH) compared with the aldehydic carbons in the range 180–185 ppm. IR spectra showed absorption bands as follows: 1680–1690 cm<sup>-1</sup> (C=O) confirming the formation of *N*-alkylimidazole-2-aldehydes **1a–h**, 3650–3480 cm<sup>-1</sup> (OH) which confirmed the formation of *N*-alkylimidazole-2-methanols **2a–h**, and 1639–1673 cm<sup>-1</sup> (C=O) and 3137–3190 cm<sup>-1</sup> (OH) which confirmed the formation of *N*-alkylimidazole-2-carboxylic acids **3a–h**. In our view, these compounds are a representation of a simple synthetic strategy through which the antimicrobial compounds based on azoles can be prepared. This approach is based on simply tuning the lipophilicity (alkyl chain)/hydrophilicity (2-substituent) balance, compared with current drugs which require specific configurations for the substituents.<sup>19</sup>

## 2.2. Protonation Constants

Scheme 2 depicts the protonation reaction equilibria for the 2-substituted *N*-alkylimidazole derivatives. The imidazole-nitrogen shows p*K*<sub>a</sub> values of 6.72–7.9, for the *N*-alkylimidazole-2-carboxylic acid derivatives, and similar values of 6.20–6.96 for the *N*-alkylimidazole-2-methanol derivatives (Table 1). For the *N*-alkylimidazole-2-carboxaldehyde derivatives, however, this constant shifts to significantly lower values (p*K*<sub>a</sub> = 5.19–5.37) due to the electron withdrawing effect of the aldehyde group. Similarly, the electron withdrawing benzyl substituent decreases the p*K*<sub>a</sub> values for the carboxylic acids, aldehydes and alcohols respectively. The p*K*<sub>a</sub> values for the carboxylic acid group are in the range 1.25–3.38, while for the alcohol group the values fall in the range 9.50–11.49. The p*K*<sub>s</sub> values of compounds (**3a**) and (**2d**) are in accordance with literature values,<sup>20,21</sup> while these values have not been determined for the other compounds before this account.

## 2.3. Antimicrobial Activity

A summary of the data appears in Table 2, while the full data can be found in the supplementary material section (Table S1). The *N*-alkylimidazole-2-aldehyde (**1e–g**), *N*-alkylimidazole-2-methanol (**2e–g**) and *N*-alkylimidazole-2-carboxylic acid (**3e–g**) derivatives showed excellent concentration dependent antibacterial activity against the Gram-positive bacteria, as evidenced by the zones of clearance. The activity was also highly dependent on the length of the alkyl chain, a trait previously observed.<sup>27</sup> Figure 1 illustrates the effect of the alkyl chain length on the antimicrobial activity of the imidazole derivatives. It can clearly be seen that the activity increased, irrespective of the substituent at the 2-position, as the alkyl chain length increased. This trend can be observed for all the bacterial strains (*S. aureus* and *B. subtilis* subsp. *spizizenii*) that the test compounds were active against. The antimicrobial activity also increased as the p*K*<sub>a</sub> of the 2-substituent decreased because the test compounds are fully ionized at the pH of the culture medium. However, it was also observed that, for *B. subtilis* subsp. *spizizenii*, compounds with the aldehyde substituent exhibited similar activities as those of the compounds with carboxylic acid substituent (Fig. 1B). This anomaly could probably be due to the oxidative environment in the *B. subtilis* subsp. *spizizenii* cell; resulting to the oxidation of the carboxaldehyde to carboxylic acid. At both



R = methyl, ethyl, propyl, butyl, heptyl, octyl, decyl

**Scheme 2**

The protonation reaction equilibria for the *N*-alkylimidazole derivatives. The protons are omitted for simplicity.

concentrations (50 & 100  $\mu\text{g}$ ), the compounds showed excellent activity against *B. subtilis* subsp. *spizizenii*, with the exception of **3e** which showed little activity (Table 2). Substitution of the alkyl chain with a benzyl group (**1h**, **2h**, **3h**) eliminates antibacterial activity completely (Table S1).

Generally, imidazole compounds are more active against Gram-positive bacteria,<sup>7</sup> as was the case in this study. The Gram-negative bacteria, *E. coli* proved to be the most resistant of all the bacteria tested. The resistance of *E. coli* has been attributed to it having an outer cell membrane which regulates the contents that enter or leave the cell.<sup>22</sup> Only compound **3g** showed slight activity at 50  $\mu\text{g}$  against *E. coli*, while all the other compounds showed slight activity only at 100  $\mu\text{g}$ . *C. albicans* was also highly resistant towards the compounds tested, with only compounds **1g** and **3g** showing a slight activity at 100  $\mu\text{g}$ . Generally, the active 2-substituted *N*-alkylimidazole derivatives exhibited excellent antibacterial activity compared to metronidazole, a commercial imidazole-containing antibacterial agent, which was used as a negative control in this study. It is well known that metronidazole possesses no activity against the chosen microorganisms but has been used for treatment of protozoan infections such as *Entamoeba histolytica*, *Giardia lamblia* and *Trichomonas vaginalis*.<sup>23</sup> Metronidazole, in this case, was chosen to illustrate how the effects of derivatization of imidazole can impact a wide spectrum of antimicrobial activity. Ampicillin, a commercial antimicrobial agent used as a positive control, however, showed superior antibacterial activity compared to the 2-substituted *N*-alkylimidazole derivatives.

The microdilution method displayed a similar trend (alkyl chain dependence and susceptibility of bacteria) to that observed in the disc diffusion method. Very low concentrations of **1e–g**, **2e–g** and **3e–g** (5–20  $\mu\text{g mL}^{-1}$ ) were required for the inhibition of the growth of Gram-positive *B. subtilis* subsp. *spizizenii*. These concentration ranges are better than those reported by Sharma *et al.*<sup>24</sup> for their imidazole derivatives. As expected, the minimum inhibitory concentrations decreased as the alkyl chain length on

imidazole was increased. Higher concentrations of compounds **1e–g** (>2500  $\mu\text{g mL}^{-1}$ ) were required to inhibit the growth of *E. coli* (Table 2). Compounds **2e–g** (200–500  $\mu\text{g mL}^{-1}$ ) and **3e–g**

**Table 1** Protonation constants ( $\text{pK}_a$ ) for 2-substituted *N*-alkylimidazole derivatives determined at  $25 \pm 0.1$  °C and  $I = 0.10$  M (TMACl).

Compound	R	$\text{pK}_{a1}$	$\text{pK}_{a2}$
<b>1a</b>	Methyl	5.12(2)	–
<b>1b</b>	Ethyl	5.11(3)	–
<b>1c</b>	Propyl	5.37(4)	–
<b>1d</b>	Butyl	5.32(4)	–
<b>1e</b>	Heptyl	5.36(8)	–
<b>1f</b>	Octyl	5.39(7)	–
<b>1g</b>	Decyl	5.50(3)	–
<b>1h</b>	Benzyl	5.06(9)	–
<b>2a</b>	Methyl	6.61(1)	10.91(3)
<b>2b</b>	Ethyl	6.94(9)	10.49(9)
<b>2c</b>	Propyl	6.92(9)	10.15(8)
<b>2d</b>	Butyl	6.95(6)	10.40(2)
<b>2e</b>	Heptyl	6.96(7)	10.22(7)
<b>2f</b>	Octyl	6.87(7)	11.32(6)
<b>2g</b>	Decyl	6.75(9)	11.49(8)
<b>2h</b>	Benzyl	6.20(5)	9.50(3)
<b>3a</b>	Methyl	1.25(6)	6.75(4)
<b>3b</b>	Ethyl	3.03 (6)	7.08(5)
<b>3c</b>	Propyl	3.00(5)	7.50(5)
<b>3d</b>	Butyl	2.90(1)	7.90(1)
<b>3e</b>	Heptyl	3.10(1)	7.50(1)
<b>3f</b>	Octyl	2.82(6)	7.77(6)
<b>3g</b>	Decyl	3.38(5)	7.68(9)
<b>3h</b>	Benzyl	2.37(6)	6.72(9)

**Table 2** Zones of inhibition (mm) of *N*-alkylimidazole derivatives using 50 and 100  $\mu\text{g}$  of compounds, and MIC values ( $\mu\text{g mL}^{-1}$ ).

Compound			<i>E. coli</i>			<i>S. aureus</i>			<i>B. subtilis</i> subsp. <i>spizizenii</i>			<i>C. albicans</i>	
2-substituent	R	No.	50	100	MIC	50	100	MIC	50	100	MIC	50	100
-CHO	Heptyl	<b>1e</b>	6.5	7.3	>2500	8.7	10.8	40	20.7	23.7	20	6.5	6.7
	Octyl	<b>1f</b>	6.5	7.2	>2500	9.7	11.5	10	22.0	25.7	5	6.5	8.7
	Decyl	<b>1g</b>	6.5	7.8	>2500	11.7	13.3	5	24.0	28.0	5	8.2	10.3
-CH <sub>2</sub> OH	Heptyl	<b>2e</b>	6.5	8.7	500	9.3	11.5	160	13.7	17.3	20	6.5	6.5
	Octyl	<b>2f</b>	6.5	9.0	400	8.7	12.0	80	15.7	17.7	10	8.3	8.7
	Decyl	<b>2g</b>	6.5	8.3	200	12.7	12.3	10	18.0	17.3	5	8.3	8.0
-COOH	Heptyl	<b>3e</b>	7.3	7.0	300	8.2	9.0	30	9.0	8.8	20	6.5	6.5
	Octyl	<b>3f</b>	8.2	8.5	150	11.7	19.2	20	18.0	23.7	5	6.5	8.0
	Decyl	<b>3g</b>	9.7	8.0	400	18.8	22.7	10	22.7	21.8	5	9.7	10.8
Metronidazole			6.5	7.3	650	6.5	8.0	300	6.5	11.3	300		
Ampicillin			29.7	40.0	40	33.3	38.7	>2500	29.3	32.7	5		
Ketoconazole												16.3	17.7

MIC means minimum inhibitory concentration ( $\mu\text{g mL}^{-1}$ ).

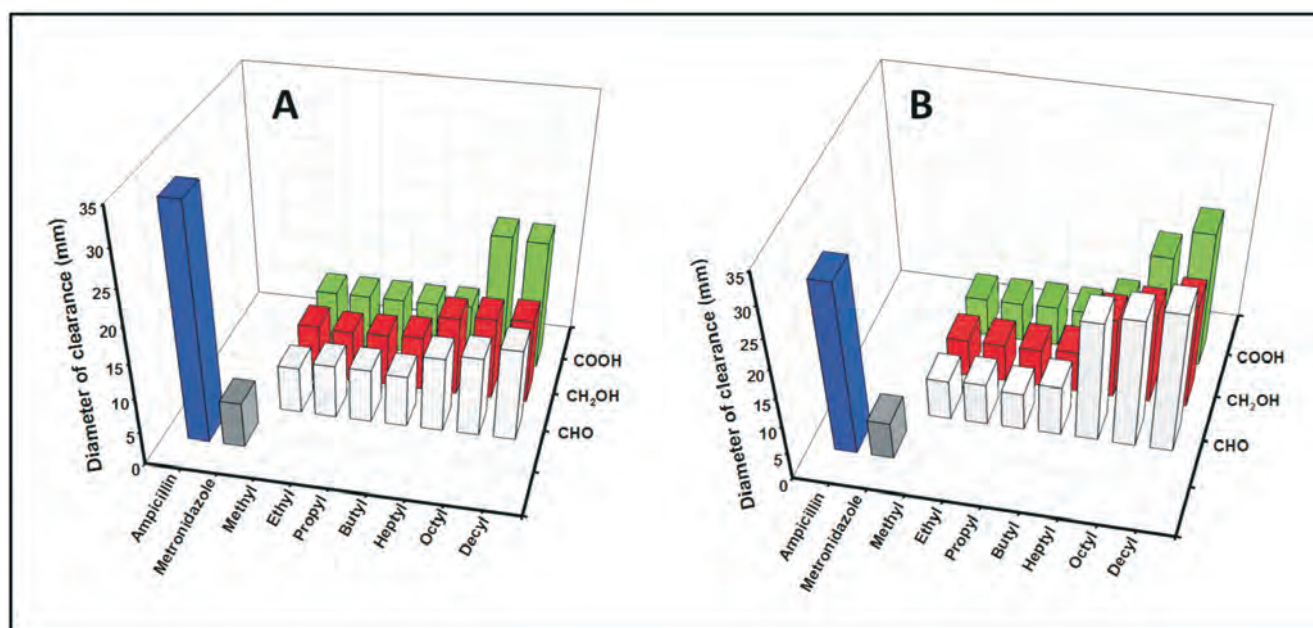
(40–300  $\mu\text{g mL}^{-1}$ ) showed lower minimum inhibitory concentrations against *E. coli*, compared to compounds **1e–g** (>2500  $\mu\text{g mL}^{-1}$ ). Since only compounds **1g** and **3g** showed a slight activity (at 100  $\mu\text{g}$ ) against *C. albicans*, in the disc diffusion method, the MICs were not determined.

In an attempt to establish the effect of the substituents at the 2-position of imidazole on the antimicrobial activity, *N*-alkylimidazole derivatives with the same alkyl chain but different substituent at the 2-position, were grouped together and their MICs compared (Fig. 2). It was observed that the carboxylic acid derivatives **3e–g** (with lowest  $\text{pK}_a$  values in the range 1.25–3.38) generally had the greatest activity at least for the octyl and decyl derivatives (Fig. 3) which was in agreement with the disc diffusion method. This could be because at the pH (~7.3) conditions of the culture medium the carboxylic acids are fully ionized, which probably allows for interaction with reactive residues on the surface of the cell membrane. Compounds with longer alkyl chain length and the carboxylic acid substituent exhibited enhanced antimicrobial activity compared

to compounds with the same alkyl chain length and the other substituents (carboxaldehyde and alcohols). Compounds with shorter chain length exhibited poor antimicrobial activity, even with the carboxylic acid at the 2-position. It was postulated that increasing the alkyl chain length increased the lipophilicity of the compounds, allowing for easy diffusion through the membrane. On the other hand, the low  $\text{pK}_a$  of the carboxylic acid substituent allows for easy ionizability which enhances binding to the proteins in the cell membrane. It has been reported previously that the activity of antimicrobial agents increases as their  $\text{pK}_a$  values are reduced.<sup>18,19,25</sup> On the other hand, the antimicrobial activities of the aldehyde and alcohol derivatives were comparable to each other and under the pH conditions employed these two derivatives would remain non-ionized.

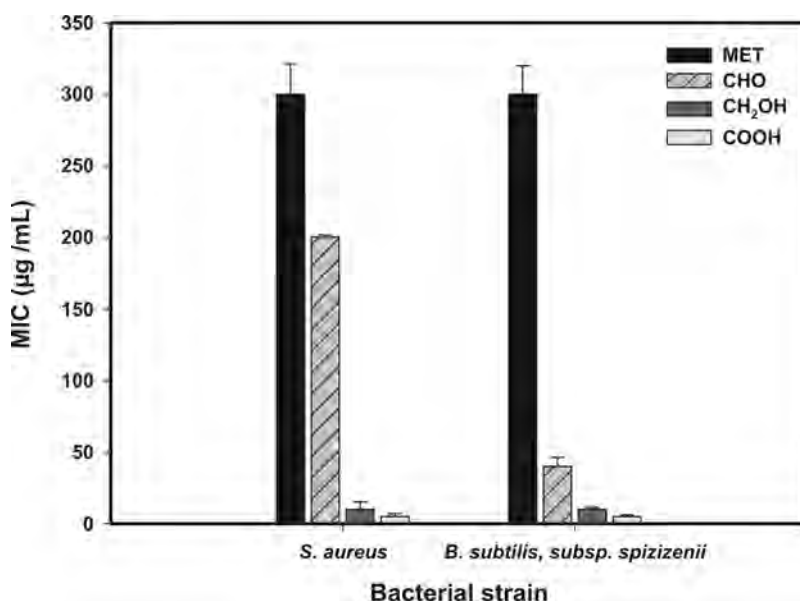
### 3. Conclusions

The 2-substituted *N*-alkylimidazole derivatives were prepared, characterized and their antimicrobial activity investigated. The compounds showed excellent activity against Gram-positive



**Figure 1** The effect of the alkyl chain length on the antimicrobial activity of 2 substituted *N* alkylimidazole derivatives at 50  $\mu\text{g}$  against *Staphylococcus aureus* (A) and *Bacillus subtilis* subsp. *spizizenii* (B). CHO = *N* alkylimidazole carboxaldehydes, CH<sub>2</sub>OH = *N* alkylimidazole 2 methanols and COOH = *N* alkylimidazole 2 carboxylic acids.





**Figure 2** The effect of the 2 substituent ( $pK_a$  effect) on antimicrobial activity of *N* alkylimidazole derivatives (*N* decylimidazole 2 carboxaldehyde (**1g**), *N* decylimidazole 2 methanol (**2g**) and *N* alkylimidazole 2 carboxylic acid (**3g**)).

bacterial strains (*S. aureus* and *B. subtilis* subsp. *spizizenii*), particularly *B. subtilis* subsp. *spizizenii*, at the tested concentrations. Poor antifungal activity of the compounds was also observed against *C. albicans* at the tested concentration. The antimicrobial activity of *N*-alkylimidazole derivatives increases with the increasing alkyl chain length. The *N*-alkylimidazole derivatives containing the carboxylic acid substituent at the 2-position also enhanced the antimicrobial activity, due to the compounds being fully ionized at the pH of the bacterial medium. These results clearly showed that simple *N*-alkylation of imidazoles with long alkyl chains at the 1-position, coupled with derivatization at the 2-position with low  $pK_a$  substituents result in an enhanced antimicrobial activity.

## 4. Experimental

### 4.1. Reagents and Instrumentation

Alkylbromides, *n*-butyllithium (2.5 M in hexane), imidazole (99.5 %) and acetone (laboratory reagent, >99.5 %) were obtained from Sigma Aldrich. Hydrogen peroxide (30 %), sodium borohydride, hydrochloric acid (32 %), potassium carbonate, sodium sulfate (anhydrous) and potassium hydroxide, diethyl-ether, dimethylformamide and chloroform were obtained from Merck Chemicals (SA) and were used as received. *E. coli* (AATC 8793), *S. aureus* (AATC 6538), *B. subtilis* subsp. *spizizenii* (AATC 6633) and *C. albicans* (AATC 2091) were obtained from Microbiologics. Mueller-Hinton, Nutrient (agar/broth) and Potato dextrose agar (Merck), metronidazole discs (50 µg) and blank discs (6.5 mm) were sourced from Davies Diagnostics. Metronidazole (for preparing 100 µg discs) was purchased from Changzhou Longcheng Medicine Raw Material Co., Ltd., Changzhou City, Jiangsu, China, and ketoconazole was purchased from Oman Chemicals and Pharmaceuticals, Al Buraimi, Sultanate of Oman.

The NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer. Infrared spectra were obtained with a Perkin Elmer Spectrum 400 FT-IR spectrometer. Microanalysis was carried using a Vario Elementar Microtube ELIII. The potentiometric titrations for the determination of protonation constants were carried out using a Metrohm 794 Titrino equipped with a Metrohm LL Ecotrode. The minimum inhibitory concentrations

were measured using LEDETECT96 microplate reader, equipped with CAPTURE96 software.

### 4.2. Synthesis of 2-Substituted *N*-alkylimidazole Derivatives

*N*-alkylimidazole-2-carboxaldehyde and *N*-alkylimidazole-2-methanol derivatives were prepared using literature methods (Scheme 1).<sup>16–18</sup> Several new *N*-alkylimidazole-2-carboxylic acids were prepared from the corresponding *N*-alkylimidazole-2-carboxaldehydes (Scheme 1), by the hydrogen peroxide facilitated oxidation in aqueous conditions at room temperature. The only purification necessary was the removal of residual water *in vacuo*, at room temperature. This afforded the *N*-alkylimidazole-2-carboxylic acid derivatives in quantitative yields. The details of the synthesis appear under experimental section with yields reported as well as the characterization data.

#### 4.2.1. General Procedure for the Preparation of 1-Alkylimidazoles

Imidazole (0.088 mol) was dissolved in 50 mL acetone and powdered KOH (0.10 mol) was added and the mixture allowed to stir for 2 h. Hereafter, alkylbromide (0.090 mol) was added dropwise and the solution stirred overnight. The white precipitate was removed by filtration and the filtrate evaporated. The resulting brown oil was distilled under vacuum (158 °C,  $4.7 \times 10^{-2}$  mbar) to afford a clear oil.

#### 4.2.2. General Procedure for the Preparation of 1-Alkylimidazole-2-aldehydes

To a suspension of 1-alkylimidazole (0.020 mol) in dry diethyl ether (50 mL) was added 2.5 M butyllithium (0.021 mol) at  $-78$  °C (dry ice/acetone slurry). After stirring for an hour, DMF (0.030 mol) was added and this solution stirred overnight. After completion of the reaction, water (2 mL) was added followed by 15 mL of 4N HCl. The aqueous layer was made basic by addition of potassium carbonate following which the product was extracted into chloroform. This organic layer was concentrated and the product was distilled at  $3.0 \times 10^{-1}$  mbar and 80 °C to yield a brown crystalline solid.

#### 1-Methylimidazole-2-aldehyde (**1a**)

Yield 76.1 %. Brown solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 4.03 (3H, s, NCH<sub>3</sub>), 7.16, 7.27 (2H, s, Im-H), 9.81 (1H, s, CHO); <sup>13</sup>C NMR

(400 MHz,  $\text{CDCl}_3$ ):  $\delta$  35.20, 127.69, 131.74, 144.00, 182.37. IR ( $\text{cm}^{-1}$ , KBr disk): 1686  $\nu(\text{C}=\text{O})$ . Anal. Calcd (found) for  $\text{C}_5\text{H}_6\text{N}_2\text{O}$ : C, 54.54 (54.25); H, 5.49 (5.65); N, 25.44 (25.19).

#### 1-Ethylimidazole-2-aldehyde (1b)

Yield 46.1 %. Brown solid.  $^1\text{H}$  NMR(400 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.44 (3H, t,  $J$  8.0,  $\text{NCH}_2\text{CH}_3$ ), 4.46 (2H, q,  $J$  8.0,  $\text{NCH}_2\text{CH}_3$ ), 7.25, 7.28 (2H s, Im-H), 9.81 (1H, s, CHO);  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.08, 42.60, 125.54, 131.37, 142.93, 181.60. IR ( $\text{cm}^{-1}$ , KBr disk): 1684  $\nu(\text{C}=\text{O})$ . Anal. Calcd (found) for  $\text{C}_6\text{H}_8\text{N}_2\text{O}$ : C, 58.05 (57.90); H, 6.50 (6.74); N, 22.57 (22.46).

#### 1-Propylimidazole-2-aldehyde.H<sub>2</sub>O (1c)

Yield 77.4 %. Brown oil.  $^1\text{H}$  NMR(400 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.93 (3H, t,  $J$  8.0,  $\text{N}(\text{CH}_2)_2\text{CH}_3$ ), 1.81 (2H, m,  $\text{NCH}_2\text{CH}_2$ ), 4.37 (2H, t,  $J$  8.0,  $\text{NCH}_2$ ), 7.21, 7.28 (2H, s, Im-H), 9.81 (1H, s, CHO);  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.30, 24.77, 49.71, 126.92, 131.96, 143.83, 182.38. IR ( $\text{cm}^{-1}$ , KBr disk): 1686  $\nu(\text{C}=\text{O})$ . Anal. Calcd (found) for  $\text{C}_7\text{H}_{12}\text{N}_2\text{O}_2$ : C, 58.83 (58.50); H, 7.74 (7.66); N, 17.94 (18.04).

#### 1-Butylimidazole-2-aldehyde.H<sub>2</sub>O (1d)

Yield 82.8 %. Brown oil.  $^1\text{H}$  NMR(400 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.94 (3H, t,  $J$  8.0,  $\text{N}(\text{CH}_2)_3\text{CH}_3$ ), 1.33 (2H, m,  $\text{CH}_2\text{CH}_2\text{CH}_2$ ), 1.77 (2H, m,  $\text{NCH}_2\text{CH}_2$ ), 4.40 (2H, t,  $J$  8.0,  $\text{NCH}_2$ ), 7.20, 7.28 (2H, s, Im-H), 9.81 (1H, s, CHO);  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  13.81, 19.85, 33.30, 47.77, 126.61, 131.78, 143.61, 182.19. IR ( $\text{cm}^{-1}$ , KBr disk): 1686  $\nu(\text{C}=\text{O})$ . Anal. Calcd (found) for  $\text{C}_8\text{H}_{14}\text{N}_2\text{O}_2$ : C, 56.45 (56.96); H, 8.29 (7.99); N, 16.46 (16.95).

#### 1-Heptylimidazole-2-aldehyde.H<sub>2</sub>O (1e)

Yield 74.2 %. Brown oil.  $^1\text{H}$  NMR(400 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.88 (3H, t,  $J$  8.0,  $\text{N}(\text{CH}_2)_6\text{CH}_3$ ), 1.28 (8H, m,  $\text{NCH}_2\text{CH}_2(\text{CH}_2)_4$ ), 1.78 (2H, m,  $\text{NCH}_2\text{CH}_2$ ), 4.38 (2H, t,  $J$  8.0,  $\text{NCH}_2$ ), 7.15, 7.27 (2H, s, Im-H), 9.81 (1H, s, CHO);  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  14.36, 22.88, 26.73, 29.11, 31.41, 31.99, 48.18, 126.55, 131.91, 143.70, 182.28. IR ( $\text{cm}^{-1}$ , KBr disk): 1685  $\nu(\text{C}=\text{O})$ . Anal. Calcd (found) for  $\text{C}_{11}\text{H}_{20}\text{N}_2\text{O}_2$ : C, 62.23 (62.01); H, 9.50 (9.56); N, 13.20 (13.29).

#### 1-Octylimidazole-2-aldehyde (1f)

Yield 53.3 %. Brown oil.  $^1\text{H}$  NMR(400 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.90 (3H, t,  $J$  8.0,  $\text{N}(\text{CH}_2)_7\text{CH}_3$ ), 1.30 (10H, m,  $\text{NCH}_2\text{CH}_2(\text{CH}_2)_5$ ), 1.79 (2H, m,  $\text{NCH}_2\text{CH}_2$ ), 4.40 (2H, t,  $J$  8.0,  $\text{NCH}_2$ ), 7.16, 7.29 (2H, s, Im-H), 9.83 (1H, s, CHO);  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  14.39, 22.96, 26.78, 29.41, 29.45, 48.19, 126.55, 131.91, 143.82, 182.39. IR ( $\text{cm}^{-1}$ , KBr disk): 1687  $\nu(\text{C}=\text{O})$ . Anal. Calcd (found) for  $\text{C}_{12}\text{H}_{20}\text{N}_2\text{O}$ : C, 69.19 (69.12); H, 9.68 (9.87); N, 13.45 (13.25).

#### 1-Decylimidazole-2-aldehyde (1g)

Yield 54.2 %. Brown oil.  $^1\text{H}$  NMR(400 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.85 (3H, t,  $J$  8.0,  $\text{N}(\text{CH}_2)_9\text{CH}_3$ ), 1.24 (14H, m,  $\text{NCH}_2\text{CH}_2(\text{CH}_2)_7$ ), 1.76 (2H, m,  $\text{NCH}_2\text{CH}_2$ ), 4.46 (2H, t,  $J$  8.0,  $\text{NCH}_2$ ), 7.14, 7.25 (2H, s, Im-H), 9.78 (1H, s, CHO);  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  14.50, 23.05, 26.78, 29.48, 29.65, 29.86, 31.42, 32.24, 48.22, 126.67, 131.90, 143.71, 182.38. IR ( $\text{cm}^{-1}$ , KBr disk): 1687  $\nu(\text{C}=\text{O})$ . Anal. Calcd (found) for  $\text{C}_{14}\text{H}_{24}\text{N}_2\text{O}$ : C, 71.14 (71.24); H, 10.23 (10.26); N, 11.85 (11.90).

#### 1-Benzylimidazole-2-aldehyde (1h)

Yield 75.1 %. Brown crystalline solid, 88–90 °C,  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  5.61 (2H, s,  $\text{NCH}_2\text{-Ph}$ ), 7.30–7.33 (4H, m, Ar-H), 7.21, 7.19 (2H, s, Im-H), 7.14 (1H, s, Ar-H), 9.84 (1H, s, CHO);  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  51.00, 126.41, 127.85, 128.48, 129.10, 132.02, 135.89, 143.39, 182.31. IR ( $\text{cm}^{-1}$ , KBr disk): 1685,  $\nu(\text{C}=\text{O})$ . Anal. Calcd (found) for  $\text{C}_{11}\text{H}_{10}\text{N}_2\text{O}$ : C, 70.95 (70.91); H, 5.41 (5.44); N, 15.04 (14.96).

#### 4.2.3. General Procedure for the Preparation of 1-Alkyl-imidazole-2-alcohols

A solution of the 1-alkylimidazole-2-aldehyde (3.4 mmol) in methanol (10 mL) was stirred at 0 °C while  $\text{NaBH}_4$  (0.34 g) was added. After stirring at this temperature for 2.5 h the solution was concentrated. The residue was taken up in  $\text{Et}_2\text{O}$  and extracted three times with  $\text{H}_2\text{O}$ . The ethereal solution was dried with  $\text{Na}_2\text{SO}_4$ , filtered and  $\text{Et}_2\text{O}$  removed *in vacuo* to obtain a pure product.

#### 1-Methylimidazole-2-methanol (2a)

Yield 56.1 %. White crystalline solid, m.p. 107–110 °C,  $^1\text{H}$  NMR(400 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.75 (3H, s,  $\text{NCH}_3$ ), 4.66 (2H, s,  $\text{CH}_2\text{OH}$ ), 6.84, 6.89 (2H, s, Im-H);  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  32.97, 55.60, 121.58, 126.65, 148.28. IR ( $\text{cm}^{-1}$ , KBr disk): 3417  $\nu(\text{O-H})$ ; 1637  $\nu(\text{C}=\text{N})$ ; 1499  $\nu(\text{C}=\text{N}-\text{C}=\text{N})$ . Anal. Calcd (found) for  $\text{C}_5\text{H}_8\text{N}_2\text{O}$ : C, 53.56 (53.19); H, 7.19 (7.10); N, 24.98 (24.52).

#### 1-Ethylimidazole-2-methanol (2b)

Yield 57.3 %. White crystalline solid, 83–86 °C,  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.44 (3H, t,  $J$  8.0,  $\text{NCH}_2\text{CH}_3$ ), 4.05 (2H, q,  $J$  8.0,  $\text{NCH}_2\text{CH}_3$ ), 4.66 (2H, s,  $\text{CH}_2\text{OH}$ ), 6.16 (1H, s,  $\text{CH}_2\text{OH}$ ), 6.87, 6.91 (2H, s, Im-H);  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.54, 41.08, 55.62, 119.40, 126.81, 147.77. IR ( $\text{cm}^{-1}$ , KBr disk): 3429  $\nu(\text{O-H})$ ; 1639  $\nu(\text{C}=\text{N})$ ; 1497  $\nu(\text{C}=\text{N}-\text{C}=\text{N})$ . Anal. Calcd (found) for  $\text{C}_6\text{H}_{10}\text{N}_2\text{O}$ : C, 57.12 (56.95); H, 7.99 (8.08); N, 22.21 (21.98).

#### 1-Propylimidazole-2-methanol (2c)

Yield 62.7 %. Clear oil.  $^1\text{H}$  NMR(400 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.93 (3H, t,  $J$  8.0,  $\text{N}(\text{CH}_2)_2\text{CH}_3$ ), 1.79 (2H, q,  $J$  8.0,  $\text{NCH}_2\text{CH}_2$ ), 3.96 (2H, t,  $J$  8.0,  $\text{NCH}_2$ ), 4.61 (2H, s,  $\text{CH}_2\text{OH}$ ), 6.82, 6.93 (2H, s, Im-H);  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.04, 23.68, 49.71, 53.69, 119.04, 121.61, 146.34. IR ( $\text{cm}^{-1}$ , KBr disk): 3417  $\nu(\text{O-H})$ ; 1635  $\nu(\text{C}=\text{N})$ ; 1497  $\nu(\text{C}=\text{N}-\text{C}=\text{N})$ . Anal. Calcd (found) for  $\text{C}_7\text{H}_{12}\text{N}_2\text{O}$ : C, 59.98 (59.55); H, 8.63 (8.97); N, 19.98 (19.37).

#### 1-Butylimidazole-2-methanol (2d)

Yield 52.6 %. Clear oil.  $^1\text{H}$  NMR(400 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.94 (3H, t,  $J$  8.0,  $\text{N}(\text{CH}_2)_3\text{CH}_3$ ), 1.36 (2H, m,  $\text{NCH}_2\text{CH}_2\text{CH}_2$ ), 1.76 (2H, m,  $\text{NCH}_2\text{CH}_2$ ), 3.99 (2H, t,  $J$  8.0,  $\text{NCH}_2$ ), 4.62 (2H, s,  $\text{CH}_2\text{OH}$ ), 6.85, 6.83 (2H, s, Im-H);  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  13.97, 20.22, 33.42, 46.25, 55.96, 120.24, 126.96, 148.05. IR ( $\text{cm}^{-1}$ , KBr disk): 3485  $\nu(\text{O-H})$ ; 1638  $\nu(\text{C}=\text{N})$ ; 1495  $\nu(\text{C}=\text{N}-\text{C}=\text{N})$ . Anal. Calcd (found) for  $\text{C}_8\text{H}_{14}\text{N}_2\text{O}$ : C, 62.31 (62.11); H, 9.15 (9.45); N, 18.17 (17.91).

#### 1-Heptylimidazole-2-methanol.H<sub>2</sub>O (2e)

Yield 61.7 %. Clear oil.  $^1\text{H}$  NMR(400 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.88 (3H, t,  $J$  8.0,  $\text{N}(\text{CH}_2)_6\text{CH}_3$ ), 1.29 (8H, m,  $\text{NCH}_2\text{CH}_2(\text{CH}_2)_4$ ), 1.78 (2H, m,  $\text{NCH}_2\text{CH}_2$ ), 3.99 (2H, t,  $J$  8.0,  $\text{NCH}_2$ ), 4.62 (2H, s,  $\text{CH}_2\text{OH}$ ), 6.84, 6.82 (2H, s, Im-H);  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  14.10, 22.63, 26.73, 28.93, 31.14, 31.76, 46.25, 55.68, 119.93, 126.67, 147.86. IR ( $\text{cm}^{-1}$ , KBr disk): 3486  $\nu(\text{O-H})$ ; 1638  $\nu(\text{C}=\text{N})$ ; 1494  $\nu(\text{C}=\text{N}-\text{C}=\text{N})$ . Anal. Calcd (found) for  $\text{C}_{11}\text{H}_{22}\text{N}_2\text{O}_2$ : C, 61.65 (61.53); H, 10.35 (10.30); N, 13.07 (13.12).

#### 1-Octylimidazole-2-methanol.2H<sub>2</sub>O (2f)

Yield 68.6 %. Clear oil.  $^1\text{H}$  NMR(400 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.88 (3H, t,  $J$  8.0,  $\text{N}(\text{CH}_2)_7\text{CH}_3$ ), 1.29 (10H, m,  $\text{NCH}_2\text{CH}_2(\text{CH}_2)_5$ ), 1.78 (2H, m,  $\text{NCH}_2\text{CH}_2$ ), 3.99 (2H, t,  $J$  8.0,  $\text{NCH}_2$ ), 4.62 (2H, s,  $\text{CH}_2\text{OH}$ ), 6.85, 6.82 (2H, s, Im-H);  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  14.17, 22.74, 26.83, 29.26, 29.29, 31.88, 46.29, 55.74, 119.95, 126.77, 147.97. IR ( $\text{cm}^{-1}$ , KBr disk): 3602  $\nu(\text{O-H})$ ; 1638  $\nu(\text{C}=\text{N})$ ; 1494  $\nu(\text{C}=\text{N}-\text{C}=\text{N})$ . Anal. Calcd (found) for  $\text{C}_{12}\text{H}_{26}\text{N}_2\text{O}_3$ : C, 58.51 (58.55); H, 10.64 (10.63); N, 11.37 (11.59).

#### 1-Decylimidazole-2-methanol (2g)

Yield 79.2 %. Clear oil.  $^1\text{H NMR}$ (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.88 (3H, t,  $J$  8.0,  $\text{N}(\text{CH}_2)_9\text{CH}_3$ ), 1.22 (14H, m,  $\text{NCH}_2\text{CH}_2(\text{CH}_2)_7$ ), 1.78 (2H, m,  $\text{NCH}_2\text{CH}_2$ ), 3.99 (2H, t,  $J$  8.0,  $\text{NCH}_2$ ), 4.63 (2H, s,  $\text{CH}_2\text{OH}$ ), 6.83, 6.86 (2H, s, Im-H);  $^{13}\text{C NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  14.30, 22.86, 26.86, 29.37, 29.46, 29.66, 46.32, 55.17, 120.05, 126.80, 147.88. IR ( $\text{cm}^{-1}$ , KBr disk): 3636  $\nu(\text{O-H})$ ; 1637  $\nu(\text{C=N})$ ; 1494  $\nu(\text{C=N-C=N})$ . Anal. Calcd (found) for  $\text{C}_{14}\text{H}_{26}\text{N}_2\text{O}$ : C, 70.54 (69.96); H, 10.99 (10.43); N, 11.75 (11.48).

#### 1-Benzylimidazole-2-methanol (2h)

Yield 93.1 %. White powder, 85–88 °C,  $^1\text{H NMR}$ (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  4.62 (2H, s,  $\text{CH}_2\text{OH}$ ), 5.22 (2H, s, Ph- $\text{CH}_2$ ), 6.80, 6.88 (2H, s, Im-H), 7.14–7.34 (5H, m, Ar-H);  $^{13}\text{C NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  50.07, 56.26, 120.98, 127.37, 127.62, 128.45, 129.31, 136.78, 148.55. IR ( $\text{cm}^{-1}$ , KBr disk): 3485  $\nu(\text{O-H})$ ; 1638  $\nu(\text{C=N})$ ; 1495  $\nu(\text{C=N-C=N})$ . Anal. Calcd (found) for  $\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}$ : C, 70.19 (70.07); H, 6.43 (6.16); N, 14.88 (14.14).

#### 4.2.4. General Procedure for the Preparation of 1-Alkylimidazole-2-carboxylic Acids

1-Alkylimidazole-2-aldehyde (0.003 mol) was dissolved in water (1 mL). To this solution was added 30 %  $\text{H}_2\text{O}_2$  (1 mL). This was allowed to stir overnight following which, the water removed on a high vacuum pump to quantitatively yield a pure white crystalline product.

#### 1-Methylimidazole-2-carboxylic acid.H<sub>2</sub>O (3a)

Yield 99.9 %. White crystalline solid, m.p. 105–108 °C,  $^1\text{H NMR}$  (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  4.01 (3H, s,  $\text{NCH}_3$ ), 7.46, 7.41 (2H, s, Im-H);  $^{13}\text{C NMR}$  (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  36.73, 118.45, 125.83, 139.68, 158.67. IR ( $\text{cm}^{-1}$ , KBr disk): 3189  $\nu(\text{OH})$ ; 1655  $\nu(\text{C=O})$ ; 1517  $\nu(\text{C=C})$ ; 1464  $\nu(\text{C=N-C=N})$ . Anal. Calcd (found) for  $\text{C}_5\text{H}_8\text{N}_2\text{O}_3$ : C, 41.67 (41.58); H, 5.59 (5.53); N, 19.44 (19.42).

#### 1-Ethylimidazole-2-carboxylic acid.2H<sub>2</sub>O (3b)

Yield 99.9 %. White fatty solid, m.p. 68–70 °C,  $^1\text{H NMR}$  (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  1.45 (3H, t,  $J$  8.0,  $\text{NCH}_2\text{CH}_3$ ), 4.56 (2H, q,  $J$  8.0,  $\text{NCH}_2$ ), 7.40, 7.51 (2H, s, Im-H);  $^{13}\text{C NMR}$  (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  15.62, 45.05, 118.84, 124.05, 139.19, 158.59. IR ( $\text{cm}^{-1}$ , KBr disk): 3186  $\nu(\text{OH})$ ; 1657  $\nu(\text{C=O})$ ; 1508  $\nu(\text{C=C})$ ; 1465  $\nu(\text{C=N-C=N})$ . Anal. Calcd (found) for  $\text{C}_6\text{H}_{12}\text{N}_2\text{O}_4$ : C, 40.91 (40.82); H, 6.87 (6.82); N, 15.90 (15.89).

#### 1-Propylimidazole-2-carboxylic acid.2H<sub>2</sub>O (3c)

Yield 99.9 %. Clear oil.  $^1\text{H NMR}$  (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  0.84 (3H, t,  $J$  8.0,  $\text{NCH}_2\text{CH}_2\text{CH}_3$ ), 1.80 (2H, m,  $\text{NCH}_2\text{CH}_2$ ), 4.47 (2H, t,  $J$  8.0,  $\text{NCH}_2$ ), 7.38, 7.47 (2H, s, Im-H);  $^{13}\text{C NMR}$  (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  10.33, 23.95, 50.95, 118.75, 124.56, 139.39, 158.56. IR ( $\text{cm}^{-1}$ , KBr disk): 3143  $\nu(\text{OH})$ , 1658  $\nu(\text{C=O})$ , 1509  $\nu(\text{C=C})$ ; 1465  $\nu(\text{C=N-C=N})$ . Anal. Calcd (found) for  $\text{C}_7\text{H}_{14}\text{N}_2\text{O}_4$ : C, 44.20 (44.18); H, 7.42 (7.57); N, 14.73 (14.94).

#### 1-Butylimidazole-2-carboxylic acid.3H<sub>2</sub>O (3d)

Yield 99.9 %. white paste.  $^1\text{H NMR}$ (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  0.86 (3H, t,  $J$  8.0  $\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ ), 1.25 (2H, m,  $\text{NCH}_2\text{CH}_2\text{CH}_2$ ), 1.78 (2H, m,  $\text{NCH}_2\text{CH}_2$ ), 4.51 (2H, t,  $J$  8.0,  $\text{NCH}_2$ ), 7.38, 7.47 (2H, s, Im-H), 14.53 (1H, s, COOH);  $^{13}\text{C NMR}$  (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  13.16, 19.34, 32.51, 49.27, 118.82, 124.53, 139.44, 158.63. IR ( $\text{cm}^{-1}$ , KBr disk): 3177  $\nu(\text{OH})$ ; 1660  $\nu(\text{C=O})$ ; 1506  $\nu(\text{C=C})$ ; 1462  $\nu(\text{C=N-C=N})$ . Anal. Calcd (found) for  $\text{C}_8\text{H}_{18}\text{N}_2\text{O}_5$ : C, 43.24 (43.34); H, 8.16 (7.94); N, 12.61 (12.62).

#### 1-Heptylimidazole-2-carboxylic acid.H<sub>2</sub>O (3e)

Yield 99.9 %. Colourless oil.  $^1\text{H NMR}$ (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  0.87

(3H, t,  $J$  8.0,  $\text{N}(\text{CH}_2)_6\text{CH}_3$ ), 1.27 (8H, m,  $\text{NCH}_2\text{CH}_2(\text{CH}_2)_4$ ), 1.87 (2H, m,  $\text{NCH}_2\text{CH}_2$ ), 4.66 (2H, t,  $J$  8.0,  $\text{NCH}_2$ ), 7.10, 7.47 (2H, s, Im-H), 9.87 (1H, s, COOH);  $^{13}\text{C NMR}$  (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  14.36, 22.87, 26.71, 29.09, 31.21, 31.95, 49.77, 119.89, 122.83, 140.76, 156.98. IR ( $\text{cm}^{-1}$ , KBr disk): 3148  $\nu(\text{OH})$ ; 1663  $\nu(\text{C=O})$ ; 1507  $\nu(\text{C=C})$ ; 1464  $\nu(\text{C=N-C=N})$ . Anal. Calcd (found) for  $\text{C}_{11}\text{H}_{20}\text{N}_2\text{O}_3$ : C, 57.87 (57.93); H, 8.83 (8.99); N, 12.27 (12.29).

#### 1-Octylimidazole-2-carboxylic acid.H<sub>2</sub>O (3f)

Yield 99.9 %. White fatty solid, m.p. <40 °C,  $^1\text{H NMR}$ (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  0.85 (3H, t,  $J$  8.0,  $\text{N}(\text{CH}_2)_7\text{CH}_3$ ), 1.25 (10H, m,  $\text{NCH}_2\text{CH}_2(\text{CH}_2)_5$ ), 1.82 (2H, m,  $\text{NCH}_2\text{CH}_2$ ), 4.60 (2H, t,  $J$  8.0,  $\text{NCH}_2$ ), 7.18, 7.49 (2H, s, Im-H), 8.29 (1H, s, COOH);  $^{13}\text{C NMR}$  (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  14.39, 22.95, 26.78, 29.43, 29.45, 31.13, 32.10, 49.77, 119.93, 123.36, 140.23, 157.59. IR ( $\text{cm}^{-1}$ , KBr disk): 3140  $\nu(\text{OH})$ ; 1639  $\nu(\text{C=O})$ ; 1508  $\nu(\text{C=C})$ ; 1464  $\nu(\text{C=N-C=N})$ . Anal. Calcd (found) for  $\text{C}_{12}\text{H}_{22}\text{N}_2\text{O}_3$ : C, 59.48 (59.71); H, 9.15 (9.51); N, 11.56 (11.51).

#### 1-Decylimidazole-2-carboxylic acid.H<sub>2</sub>O (3g)

Yield 99.9 %. White paste.  $^1\text{H NMR}$ (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.88 (3H, t,  $J$  8.0,  $\text{N}(\text{CH}_2)_9\text{CH}_3$ ), 1.25–1.33 (14H, m,  $\text{NCH}_2\text{CH}_2(\text{CH}_2)_7$ ), 1.88 (2H, m,  $\text{NCH}_2\text{CH}_2$ ), 4.71 (2H, t,  $J$  8.0,  $\text{NCH}_2$ ), 7.06, 7.41 (2H, s, Im-H), 9.34 (1H, s, COOH);  $^{13}\text{C NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  14.26, 22.83, 26.55, 29.27, 29.58, 29.79, 29.85, 31.07, 32.01, 49.44, 119.37, 122.23, 141.07, 156.05. IR ( $\text{cm}^{-1}$ , KBr disk): 3137  $\nu(\text{OH})$ ; 1672  $\nu(\text{C=O})$ ; 1508  $\nu(\text{C=C})$ ; 1464  $\nu(\text{C=N-C=N})$ . Anal. Calcd (found) for  $\text{C}_{14}\text{H}_{26}\text{N}_2\text{O}_3$ : C, 62.19 (62.15); H, 9.69 (9.64); N, 10.36 (10.33).

#### 1-Benzylimidazole-2-carboxylic acid (3h)

Yield 99.9 %. White powder, m.p. 72–73 °C,  $^1\text{H NMR}$ (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  5.89 (2H, s, Ph- $\text{CH}_2$ ), 7.34–7.51 (7H, m, Ar-H);  $^{13}\text{C NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  36.73, 118.45, 125.83, 139.68, 158.67. IR ( $\text{cm}^{-1}$ , KBr disk): 3137  $\nu(\text{OH})$ ; 1656  $\nu(\text{C=O})$ ; 1498  $\nu(\text{C=C})$ ; 1460  $\nu(\text{C=N-C=N})$ . Anal. Calcd (found) for  $\text{C}_{11}\text{H}_{10}\text{N}_2\text{O}_3$ : C, 65.34 (65.10); H, 4.98 (5.05); N, 13.85 (13.64).

### 4.3. Protonation Constants

The protonation constants of the synthesized compounds were determined by aqueous potentiometric titrations in a pH range of 2–11. The titrations were performed as 25 mL samples using tetramethylammonium hydroxide (TMAOH) and HCl with tetramethylammonium chloride (TMACl) as the ionic medium ( $I = 0.10\text{ M}$ ). These titration data were resolved using the program HYPERQUAD,<sup>26</sup> to solve for the protonation constants from an average of four titrations. The full details of this method appear in our previous publication.<sup>27</sup>

### 4.4. Antimicrobial Activity

The antimicrobial activity of the compounds was investigated against a Gram-negative (*E. coli* ATCC 8793), Gram-positive (*S. aureus* ATCC 6538 and *B. subtilis* subsp. *spizizenii* ATCC 6633) bacteria and yeast (*C. albicans* ATCC 2091) using the disk diffusion and the broth microdilution methods. To determine the zones of inhibition, blank disks (6.5 mm) were impregnated with 20  $\mu\text{L}$  of a methanolic solution containing the various compounds (including ketoconazole and ampicillin) such that 50  $\mu\text{g}$  and 100  $\mu\text{g}$  of the pure compound remained on the disk. Disks containing 100  $\mu\text{g}$  of metronidazole were prepared according to the method for the preparation of the 100  $\mu\text{g}$  test compounds. The disks were left overnight at room temperature to allow the methanol to evaporate, and then placed onto Mueller-Hinton agar plates streaked with the various bacteria. The plates were incubated at 37 °C for 18 h after which the zones of clearance were measured. For *C. albicans*, potato dextrose agar plates were



streaked and incubated at 30 °C for 48 h. A 0.5 McFarland standard ( $OD_{625} = 0.08\text{--}0.13$ ,  $1.5 \times 10^8$  CFU mL<sup>-1</sup>) was used to match the turbidity of the culture suspensions.

The minimum inhibitory concentrations were determined using the broth microdilution method. Single colonies were suspended in the Mueller-Hinton or Nutrient broth and incubated over a period of 2–6 h until an appropriate optical density ( $OD = 0.6\text{--}0.8$ ) at 625 nm was achieved. Methanolic solutions of test compounds (2.5 mg mL<sup>-1</sup>) were serially diluted in 96 well microplates using the broth. The bacterial suspension (5 µL) was added and the plates incubated at 37 °C for 18 h. Metronidazole was used as a negative control for antibacterial activity, and Ampicillin<sup>28</sup> and Ketoconazole<sup>29</sup> were used as positive controls for bacteria and yeast, respectively. Some of this data is reported in Table 2 and the full data can be obtained in the online supplement (Table S1).

#### Acknowledgements

We thank the SA National Research Foundation, Medical Research Council (MRC) and Water Research Commission (WRC) for funding.

#### Supplementary Data

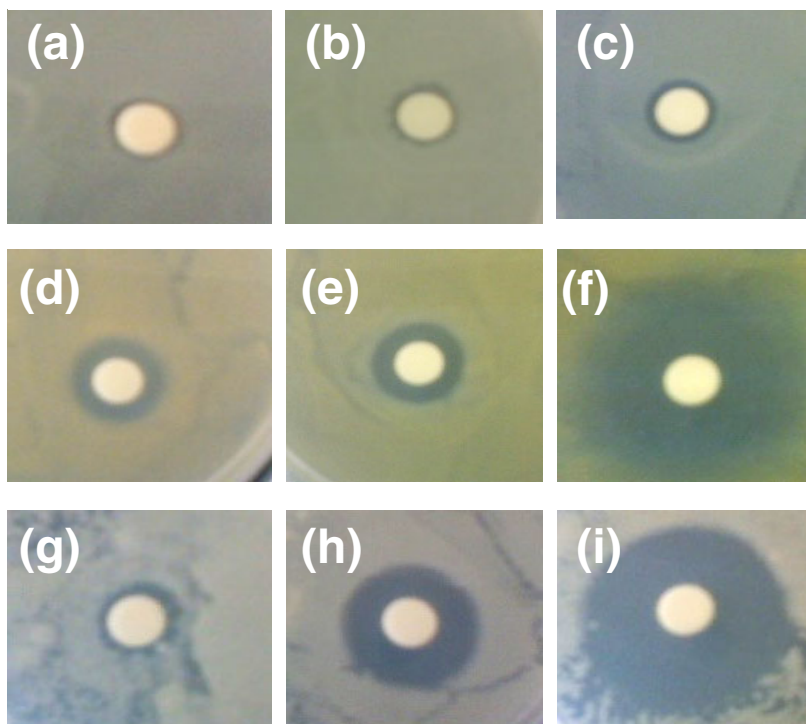
Supplementary information for the images showing the zones of inhibition (Fig. S1) and the table containing the full data of measured zone diameters (Table S1) can be found in the online supplement.

#### References

- 1 C. Congiu, M.T. Cocco and V. Onnis, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 989–993.
- 2 S. Khabnadideh, Z. Rezaei, A. Khalafi-Nezhad, R. Bahrinajafi, R. Mohamadi and A. Farrokhriz, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 2863–2865.
- 3 H. Göker, R. Ertan, H. Akgün and N. Yulug, *Arch. Pharm.*, 1991, **324**, 283–286.
- 4 B.S. Samant and M.G. Shukthankar, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 1015–1018.
- 5 L. Crane, M. Anastasiadou, S.E. Hage, J.L. Stigliani, G. Baziard-Mouysset, M. Payard, J.M. Leger, J.-G. Bizot-Espiard, A. Ktorza, D.-H. Caignard and P. Renard, *Bioorg. Med. Chem.*, 2006, **14**, 7419–7433.
- 6 D.W. Woolley, *J. Biol. Chem.*, 1944, **152**, 225–232.
- 7 M. Antolini, A. Bozzoli, C. Ghiron, G. Kennedy, T. Rossi and A. Ursini, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 1023–1028.
- 8 N. Khan, L. Soni, A. Gupta, S. Wakode, R. Wagh and S. Kaskhedikar, *Indian J. Pharm. Sci.*, 2006, **68**, 341–346.
- 9 D.J. Sheehan, C.A. Hitchcock and C.M. Sibley, *Clin. Microbiol. Rev.*, 1999, **12**, 40–49.
- 10 T.C. White, K.A. Marr and R.A. Bowden, *Clin. Microbiol. Rev.*, 1998, **11**, 382–402.
- 11 S.A.F. Rostom, H.M.A. Ashour, H.A.A. El Razik, A.E.F.H. Abd El Fattah and N.N. El-Din, *Bioorg. Med. Chem.*, 2009, **17**, 2410–2422.
- 12 D. Sharma, B. Narasimhan, P. Kumar, V. Judge, R. Narang, E. De Clercq and J. Balzarini, *Eur. J. Med. Chem.*, 2009, **44**, 2347–2353.
- 13 M. Meloun and S. Bordovska, *Anal. Bioanal. Chem.*, 2007, **389**, 1267–1281.
- 14 F. Jung, D. Boucherot, C. Delvare and A. Olivier, *J. Antibiot.*, 1993, **46**, 993–1012.
- 15 A. Dalhoff and S. Schubert, U. Ullmann, *Infection*, 2005, **33**, 36–43.
- 16 K.J. Oberhausen, J.F. Richardson and R.M. Buchanan, W. Pierce, *Polyhedron*, 1989, **8**, 659–668.
- 17 L.I. Kruse, C. Kaiser, W.E. DeWolf, J.A. Finkelstein, J.S. Frazee, E.L. Hilbert, S.T. Ross, K.E. Flaim and J.L. Sawyer, *J. Med. Chem.*, 1990, **33**, 781–789.
- 18 A.M. Roe, *J. Chem. Soc.*, 1963, 2195–2200.
- 19 C. Sheng, X. Che, W. Wang, S. Wang, Y. Cao, Z. Miao, J. Yao and W. Zhang, *Eur. J. Med. Chem.*, 2011, **46**, 5276–5282.
- 20 R.G. Franz, *AAPS J.*, 2001, **3**, 1–13.
- 21 B. Barszcz and J. Kulig, *J. Chem. Soc., Dalton Trans.*, 1993, **10**, 1559–1562.
- 22 S.P. Denyer, *Int. Biodeterior. Biodegrad.*, 1995, 227–245.
- 23 C.D. Freeman, N.E. Klutman and K.C. Lamp, *Drugs*, 1997, **54**, 679–708.
- 24 S. Sharma, S. Gangal and A. Rauf, *Eur. J. Med. Chem.*, 2009, **44**, 1751–1757.
- 25 C.M. Stiff, M. Zhong, R.W. Sarver, H. Gao, A.M. Ho, M.T. Sweeney, G.E. Zerunko and D.L. Romero, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 5479–5482.
- 26 P. Gans, A. Sabatini and A. Vacca, *Talanta*, 1996, **43**, 1739–1753.
- 27 R.S. Walmsley, Z.R. Tshentu, M.A. Fernandes and C.L. Frost, *Inorg. Chim. Acta*, 2010, **363**, 2215–2221.
- 28 O.O. Ayepola, *Adv. Nat. Appl. Sci.*, 2009, **3**, 188–191.
- 29 P. Tiwari, H. Rai, D.K. Upreti, S. Trivedi and P. Shukla, *AJPC*, 2011, **2**, 841–846.



**Supplementary data:**



**Figure S1:** Zones of clearance for 2-substituted *N*-alkylimidazoles. *E. coli*: (a) **3f** [50  $\mu$ g], (b) **2f** [100  $\mu$ g], **3g** [50  $\mu$ g]; *S. aureus*: (d) **3f** [50  $\mu$ g], (e) **2e** [100  $\mu$ g], (f) **3g** [100  $\mu$ g]; *B. spizizenii*: (g) **1d** [50  $\mu$ g], (h) **3g** [100  $\mu$ g], (i) **3f** [50  $\mu$ g]

**Table S1:** Zones of clearance for imidazole derivatives. Disks were 6.5 mm in diameter. The experiments were performed in triplicate and the reported results are the average of these values.

Conc	<i>E.coli</i>		<i>S. aureus</i>		<i>B. spizizenii</i>		<i>C. albicans</i>	
	50	100	50	100	50	100	50	100
1a	6.5	6.5	6.5	6.8	6.8	6.5	6.5	6.5
1b	6.5	6.7	6.5	7.8	7.2	8.0	6.5	6.5
1c	6.5	6.5	7.0	7.8	6.5	8.2	6.5	6.5
1d	6.5	7.0	7.3	7.5	8.7	9.0	6.5	6.5
1e	6.5	7.3	8.7	10.8	20.7	23.7	6.5	6.7
1f	6.5	7.2	9.7	11.5	22.0	25.7	6.5	8.7
1g	6.5	7.8	11.7	13.3	24.0	28.0	8.2	10.3
1h	6.5	6.5	6.7	8.5	9.3	11.3	6.5	6.5
2a	6.5	7.0	6.5	7.8	6.5	7.0	6.5	6.5
2b	6.5	7.8	8.0	8.5	6.5	6.5	6.5	6.5
2c	6.5	6.7	7.7	7.7	6.5	6.5	6.5	6.5
2d	6.5	7.0	8.5	8.0	7.0	7.7	6.5	6.5
2e	6.5	8.7	9.3	11.5	13.7	17.3	6.5	6.5
2f	6.5	9.0	8.7	12.0	15.7	17.7	8.3	8.7
2g	6.5	8.3	12.7	12.3	18.0	17.3	8.8	8.0
2h	6.5	6.8	6.7	8.5	6.5	6.5	6.5	6.5
3a	6.7	6.7	8.7	8.0	6.5	6.8	6.5	6.5
3b	8.0	7.2	9.0	8.0	6.5	8.2	6.5	6.5
3c	7.7	6.7	8.2	8.0	6.7	7.2	6.5	6.5
3d	8.0	6.7	8.7	8.0	6.5	8.0	6.5	6.5
3e	7.3	7.0	9.3	8.2	9.0	8.8	6.5	6.5
3f	8.2	8.5	11.7	19.2	18.0	23.7	6.5	8.0
3g	9.7	8.0	20.7	18.8	22.7	21.8	9.7	10.8
3h	7.8	7.2	8.3	8.0	6.8	7.3	6.5	6.5