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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

# Microwave synthesis, biological evaluation and docking studies of 2-substituted methyl 1-(4-fluorophenyl)-1*H*-benzimidazole-5-carboxylates

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**Abstract** A library of 22 novel 2-substituted fluorinated benzimidazoles (**5a-v**) was synthesized under microwave conditions in yields of between 85–96% and tested for their antimicrobial and antioxidant activity. Two trioxygenated derivatives **5p** and **5r** had minimum bactericidal concentration values ranging between 14.5–115.7 µM (**5p**) and 25.6–74.3 µM (**5r**) against *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumoniae*. The benzimidazole **5e** with a $CF_3$ substituent had the best antifungal activity at 94.3 µM against *C. albicans*. Compounds **5p** and **5r** also showed good antioxidant activities of 386.6 and 306.7 µM respectively, comparable to that of ascorbic acid. Docking studies of **5 h** and **5r** into the active site of topoisomerase II DNA-gyrase indicated that interaction with the $Mn^{2+}$ ion in the active site of the enzyme was crucial for antibacterial activity.

**Keywords** Benzimidazole · Microwave · Thermal · Antimicrobial · Antioxidant · Docking

## Introduction

Resistance to antibiotics is one of the major health concerns plaguing mankind in the 21st century. There is a constant need for new and improved antibiotics to fight of strains of bacteria that have become resistant to currently prescribed antibiotics. The benzimidazole moiety has gained importance in recent years having antibacterial, antifungal, antiviral, anticancer, antioxidant, anthelmintic, antiparasitic, antimycobacterial, antidiabetic, antihypertensive, analgesic, antipsychotic, anticoagulant, cardiovascular, and anti-inflammatory properties (Navarrete-Vázquez et al. 2001; Tomic et al. 2004; Biron 2006; Kuş et al. 2009; Narasimhan et al. 2010; Vyas and Ghate 2010; Arora 2011; Kalyankar et al. 2012; Barot et al. 2013; Gurvinder et al. 2013; Jain et al. 2013; Keri et al. 2015; Yoon et al. 2014; Singla et al. 2015). Synthesizing novel compounds with the benzimidazole moiety can therefore produce lead compounds for the pharmaceutical industry.

Benzimidazoles are generally synthesized by coupling reactions between *o*-phenylenediamines with carboxylic acids, carboxylic acid chlorides or aldehydes and in some cases esters and amides (Khanna et al. 2012; Panda et al. 2012; Keri et al. 2015; Prajapti et al. 2015; Azizian et al. 2016; Carvalho et al. 2015; Kattimani et al. 2015; Rithe et al. 2015; Saberi 2015; Saleh et al. 2015). In most cases, these reactions are carried out in the presence of acid catalysts containing Sn, Ti, Zr, Bi, In, Co, Ce, B, Zn, and Hf (Zhang et al. 2007), Ir (Tateyama et al. 2016), La (Kamal et al. 2014), acetic acid (Jain et al. 2013) and *p*-toluenesulfonic acid (Xiangming et al. 2007; Funel et al. 2014), basic catalysts such as NaOH (Rajasekhar et al. 2010), KOH (Al-Mohammed et al. 2013), or inorganic salts for example $NaHSO_3$ (Jain et al. 2013), and $Na_2S_2O_5$ (Yoon et al. 2015). There have been greener synthetic procedures

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reported recently, which made use of solvents such as methanol and water (Borhade et al. 2012; Chen et al. 2012; Rao et al. 2014), while other methods have been employed such as the grinding method (Banerjee et al. 2014), ultrasound (Patil et al. 2014) and visible light (Park et al. 2014). In recent years, there has been much interest in microwave syntheses (Das et al. 2012; Jacob 2012; Gawande et al. 2014). Using microwave synthesis, benzimidazoles were synthesized without the use of catalysts and organic solvents (Abdullah et al. 2012; Eren and Bekdemir 2014). Its advantages are, therefore, that it is a green technique, cheaper, carried out in shorter times and uses less energy than conventional synthesis.

In our study, we have synthesized fluorinated benzimidazole derivatives from *o*-phenyldiamine (methyl 3-amino-4-(4-fluoroaniline)benzoate) and various substituted aldehydes using three methods, conventional organic synthesis with a sodium metabisulphite catalyst and ethanol as solvent, the grinding method with iodine as a catalyst and under microwave conditions with no catalyst or solvent, and compared the yields and reaction times of these methods. We synthesized a total of 22 2-substituted fluorinated benzimidazoles (**5a–5v**) and tested them for their antimicrobial and antioxidant activity.

## Materials and methods

### General experimental procedures

All chemicals were supplied by Sigma-Aldrich via Capital Lab, South Africa. Organic solvents were redistilled and dried according to standard procedures. Silica gel 60 $F_{254}$ plates (Merck) were used for thin layer chromatography. Crude compounds were purified by column chromatography using silica gel (60–120 mesh) and a mobile phase of varying ratios of EtOAc:Hexane. Melting points were recorded using a Stuart Scientific SMP3 apparatus. UV spectra were obtained on a Varian Cary UV-vis spectrophotometer in MeOH. Infrared (IR) spectra were recorded on a Perkin Elmer 100 Fourier transform infrared spectrophotometer with universal attenuated total reflectance sampling accessory.

Microwave assisted reaction: All reactions which involved microwave irradiation were performed using a CEM Discover, Explorer-12 Hybrid microwave. $^{1}H$, $^{13}C$ and all two-dimensional nuclear magnetic resonance (2D NMR) spectra were recorded on a Bruker Avance instrument operating at 400 MHz. Chemical shifts are reported in $\delta$ values (ppm) relative to an internal standard of tetramethylsilane and referenced to the solvent line of $CDCl_3$ (7.24 ppm for $^{1}H$; 77.0 ppm for $^{13}C$), $CD_3OD$ (3.31 ppm for $^{1}H$; 49.0 ppm for $^{13}C$) or DMSO-$d_6$ (2.5 ppm for $^{1}H$; 39.5 ppm for $^{13}C$). High-resolution mass data were obtained using a Bruker micro TOF-Q II ESI instrument operating at ambient temperature. The purity of the compounds were determined by analytical high performance liquid chromatography on a Shimadzu-20A5 fitted with a C8 (150 mm × 5 µm) column using a mobile phase (A) of 0.1M $KHPO_4$ buffer and (B) methanol, with a linear gradient of 0 to 70% over a period of 60 min at a flow rate of 1 mL $min^{-1}$.

### General procedure for the preparation of methyl 4-fluoro-3-nitrobenzoate (2)

4-Fluoro-3-nitrobenzoic acid (0.5 g, 2.7 mmol) was dissolved in methanol (5 mL) and conc. $H_2SO_4$ (0.5 mL) at room temperature. The reaction mixture was heated with stirring in a 10 mL microwave process vial for 10 min at 80 °C. After completion of the reaction (as evident from thin layer chromatography (TLC)), the solvent was evaporated under reduced pressure, the reaction mixture basified by sodium bicarbonate and the aqueous layer extracted into ethyl acetate (3 × 5 mL). The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to yield **2** as a cream-colored powder (95%).

$^{1}H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 8.73 (1H, dd, $J = 7.2$, 2.2 Hz, H-2), 8.31 (1H, m, H-6), 7.38 (1H, dd, $J = 10.2$, 8.8 Hz, H-5), 3.95 (3H, s, H-8); $^{13}C$ NMR ($CDCl_3$, 100 MHz) $\delta$ 164.1 (C-7), 158.1 (d, $J_{CF} = 270.0$ Hz, C-4), 137.3 (C-3), 136.5 (d, $J = 9.7$ Hz, C-6), 127.8 (C-2), 127.2 (d, $J = 4.2$ Hz, C-1), 118.8 (d, $J = 21.2$ Hz, C-5), 52.9 (C-8); $^{19}F$ NMR ($CDCl_3$, 376.5 MHz) $\delta$ −110.55.

### General procedure for the preparation of methyl 4-(4-fluoroaniline)-3-nitrobenzoate (3)

Methyl-4-fluoro-3-nitrobenzoate **2** (0.5 g, 2.34 mmol) and 4-fluorophenyl aniline (0.36 mL, 2.34 mmol) were mixed in dimethylformamide (2 mL). The reaction mixture was subject to microwave irradiation with stirring in a 10 mL microwave process vial for 5 min at 80 °C. After completion of the reaction (monitored by TLC), the reaction mixture was washed with water (2 × 10 mL) followed by 10% $Na_2CO_3$ (10 mL). The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to afford **3** as a crude product. The solid was purified by column chromatography (30% ethyl acetate in hexane) to afford the desired product as a reddish solid in a yield of 90%.

$^{1}H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 9.67 (1H, s, NH-10), 8.90 (1H, d, $J = 2.0$ Hz, H-2), 7.95 (1H, dd, $J = 9.0$, 2.0 Hz, H-6), 7.29 (2H, dd, $J = 8.8$, 4.7 Hz, H-2a/6a), 7.18 (2H, *t*, $J = 8.8$ Hz, H-3a/5a), 7.03 (1H, d, $J = 9.0$ Hz, H-5), 3.89 (3H, s, H-8); $^{13}C$ NMR ($CDCl_3$, 100 MHz) δ 165.3 (C-7), 161.2

(d, $J_{CF}$ = 245.8 Hz, C-4a), 146.4 (C-4), 136.0 (C-2), 133.5 (d, $J$ = 3.3 Hz, C-1a), 132.1 (C-3), 129.24 (C-6), 127.5 (d, $J$ = 8.5 Hz, 2C, C-2a/6a), 119.2 (C-1), 116.9 (d, $J$ = 22.7 Hz, 2C, C-3a/5a), 115.3 (C-5), 52.2 (C-8); $^{19}$F NMR ($CDCl_3$, 376.5 MHz) $\delta$ −114.14.

### General procedure for the preparation of methyl 3-amino-4-(4-fluoroaniline) benzoate (4)

Methyl 4-(4-fluoroaniline)-3-nitrobenzoate **3** (0.5 g, 1 mmol), Zn (0.06 g, 0.5 mmol), and ammonium formate (0.54 g, 5 mmol) were added to methanol (5 mL). The reaction mixture was stirred at room temperature for 10 min. Upon completion of the reaction (monitored by TLC), it was filtered through Celite 545 to remove the zinc. The filtrate was evaporated under reduced pressure and the solid purified by column chromatography (30% ethyl acetate in hexane) to afford the desired product as a brown solid with a yield of 92%.

$^1$H NMR ($CDCl_3$, 400 MHz) δ 7.50 (1H, d, $J$ = 1.7 Hz, H-2), 7.47 (1H, dd, $J$ = 8.2, 1.7 Hz, H-6), 7.03 (1H, d, $J$ = 8.2 Hz, H-5), 6.99 (d, $J$ = 8.3 Hz, 2H, H-2a/6a), 6.95 (2H, dd, $J$ = 9.0, 8.3 Hz, H-3a/5a), 5.55 (s, 1H, NH-10), 3.85 (3H, s, H-8), 3.16 (2H, s, NH-9); $^{13}$C NMR ($CDCl_3$, 100 MHz) δ 167.0 (C-7), 158.3 (d, $J_{CF}$ = 239.4 Hz, C-4a), 138.3 (C-1a), 137.1 (C-4), 136.0 (C-3), 123.6 (C-1), 122.6 (C-6), 120.6 (d, $J$ = 7.7 Hz, 2C, C-2a/6a), 118.4 (C-2), 116.7 (C-5), 116.1 (d, $J$ = 22.5 Hz, 2C, C-3a/5a), 51.8 (C-8); $^{19}$F NMR ($CDCl_3$, 376.5 MHz) $\delta$ −121.46.

### General procedure for the preparation of fluorinated benzimidazoles (5a–5v)

*Microwave method*

The mixture of methyl 3-amino-4-(4-fluoroaniline) benzoate **4** (1 mmol) and various aldehydes (1 mmol) were heated in a 5 mL microwave process vial for 5–10 min at 110 °C to obtain compounds (**5a–5v**). After completion of the reaction (monitored by TLC), the reaction mixture was washed with water (2 × 5 mL) followed by brine solution (10 mL). The aqueous layer was extracted into ethyl acetate (3 × 5 mL). The organic layer was dried over anhydrous $Na_2SO_4$, concentrated under reduced pressure and the obtained solid purified by column chromatography (30–50% ethyl acetate in hexane) to afford the desired products in good yields (85–96%).

*Grinding (mechanochemical) method*

A mixture of methyl 3-amino-4-(4-fluoroaniline) benzoate **4** (1 mmol), various aldehydes (1 mmol), and iodine (10 mol %) were ground together using a mortar and pestle at room temperature between 5–45 min (Table 1). After completion of the reaction (confirmed by TLC), the mixture was treated with aqueous $Na_2S_2O_3$. The aqueous layer was extracted with ethyl acetate (3 × 5 mL), and the organic layer dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The solid obtained was purified by column chromatography (hexane/ethyl acetate) to afford the desired product (**5a–5v**) in moderate to good yields (10–56%).

*Conventional method*

3-Amino-4-(4-fluoroaniline) benzoate **4** (1 mmol), various aldehydes (1 mmol) and $Na_2S_2O_5$ (20 mol%) was refluxed in ethanol (20 mL) between 8–18 h. After completion of the reaction (as evident from TLC), the solvent was evaporated under reduced pressure and the crude mass washed with water (2 × 10 mL). The aqueous layer was extracted with ethyl acetate (3 × 25 mL), and the organic layer dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to afford **5a–5v** as crude products. The solid obtained was purified by column chromatography (hexane/ethyl acetate) to afford the desired product in good yields (60–90%).

*Spectral data*

Methyl 1-(4-fluorophenyl)-2-phenyl-1H-benzo[d]imidazole-5-carboxylate **(5a)** White solid (90% yield, 99% purity); mp 160–162 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 247 (2.63), 302 (2.19); IR $\upsilon_{max}$ 3069 (CH), 1713 (C=O), 1357 (C-F), 1185 (C-O) cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.58 (1H, bs, H-4), 7.98 (1H, d, $J$ = 8.6 Hz, H-6), 7.54 (2H, d, $J$ = 7.4 Hz, H-2b/6b), 7.38 (1H, m, H-4b), 7.32 (2H, m, H-2a/6a), 7.29 (2H, m, H-3b/5b), 7.23 (1H, m, H-7), 7.20 (2H, m, H-3a/5a), 3.94 (3H, s, H-11); $^{13}$C NMR ($CDCl_3$, 100 MHz) δ 167.4 (C-10), 162.4 (d, $J_{CF}$ = 248.6 Hz, C-4a), 154.0 (C-2), 142.2 (C-8), 140.2 (C-9), 132.2 (d, $J$ = 3.3 Hz, C-1a), 130.0 (C-4b), 129.2 (C-6b), 129.1 (d, $J$ = 8.7 Hz, 2C, C-2a/6a), 129.07 (C-2b), 129.0 (C-1b), 128.5 (2C, C-3b/5b), 125.4 (C-5), 125.1 (C-6), 122.2 (C-4), 117.1 (d, $J$ = 22.7 Hz, 2C, C-3a/5a), 109.3 (C-7), 52.1 (C-11); $^{19}$F NMR ($CDCl_3$, 376.5 MHz) $\delta$ −108.08; HRESIMS *m/z* (pos): 347.1198 $C_{21}H_{16}FN_2O_2$ (calcd. 347.1196).

Methyl 1,2-bis(4-fluorophenyl)-1H-benzo[d]imidazole-5-carboxylate (**5b**) Yellow solid (90% yield, 98% purity); mp 130–132 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 247 (2.7), 301 (2.4); IR $\upsilon_{max}$ 3071 (CH), 1711 (C=O), 1379 (C–F), 1157 (C-O) cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.56 (1H, bs, H-4), 7.99 (1H, dd, $J$ = 8.6, 1.4 Hz, H-6), 7.54 (2H, dd, $J$ = 8.8, 5.2 Hz, H-2b/6b), 7.29 (2H, dd, $J$ = 8.9, 4.8 Hz, H-2a/6a), 7.23 (2H, m, H-3a/5a), 7.19 (1H, d, $J$ = 8.6 Hz,

**Table 1** Comparison of the yields and duration of the final step in the benzimidazole synthesis using three different methods

| No. | $R_1$ | Microwave | | Grinding | | Conventional | |
|---|---|---|---|---|---|---|---|
| | | Time (min) | Yield[a] (%) | Time (min) | Yield[a] (%) | Time (h) | Yield[a] (%) |
| **5a** | Ph | 3 | 96 | 10 | 55 | 10 | 90 |
| **5b** | 4-F Ph | 3 | 93 | 10 | 53 | 8 | 85 |
| **5c** | 4-Cl Ph | 3 | 89 | 10 | 50 | 10 | 75 |
| **5d** | 4-Br Ph | 3 | 85 | 10 | 48 | 10 | 74 |
| **5e** | 4-$CF_3$ Ph | 5 | 90 | 10 | 45 | 8 | 70 |
| **5f** | 4-$NO_2$ Ph | 8 | 85 | 45 | 20 | 18 | 60 |
| **5g** | 4-$CH_3$ Ph | 6 | 88 | 20 | 30 | 12 | 72 |
| **5h** | 4-$OCH_3$ Ph | 6 | 90 | 25 | 25 | 16 | 60 |
| **5i** | 4-$NH_2$ Ph | 5 | 85 | 20 | 10 | 18 | 65 |
| **5j** | 4-OH Ph | 5 | 90 | 10 | 20 | 16 | 70 |
| **5k** | 4-$N(CH_3)_2$ Ph | 5 | 90 | 20 | 35 | 12 | 87 |
| **5l** | 4-$S(CH_3)$ Ph | 4 | 90 | 20 | 40 | 16 | 85 |
| **5m** | 2-Naphthyl | 5 | 95 | 20 | 56 | 12 | 80 |
| **5n** | α-(*E*)-prop-1-en-1-yl Ph | 5 | 97 | 25 | 50 | 12 | 75 |
| **5o** | 2-(6-Chloroquinolinyl) | 6 | 92 | 25 | 50 | 12 | 73 |
| **5p** | 2-OH-4,6-$(OCH_3)_2$ Ph | 5 | 90 | 30 | 30 | 16 | 60 |
| **5q** | 3,4-$(OH)_2$ Ph | 4 | 85 | 35 | 20 | 16 | 60 |
| **5r** | 2,3,4-$(OH)_3$ Ph | 8 | 85 | 40 | 20 | 16 | 76 |
| **5s** | 2-Thiophenyl | 3 | 95 | 30 | 50 | 15 | 75 |
| **5t** | 2-Furanyl | 3 | 95 | 30 | 50 | 15 | 75 |
| **5u** | n-Butyl | 6 | 90 | 25 | 30 | 18 | 70 |
| **5v** | n-Heptyl | 6 | 90 | 25 | 30 | 18 | 70 |

[a] Isolated yield after column chromatography

H-7), 7.02 (2H, *t*, $J = 8.8$ Hz, H-3b/5b), 3.95 (3H, s, H-11); $^{13}C$ NMR ($CDCl_3$, 100 MHz) $\delta$ 167.3 (C-10), 163.7 (d, $J_{CF} = 250.4$ Hz, C-4b), 162.5 (d, $J_{CF} = 249.2$ Hz, C-4a), 153.0 (C-2), 142.1 (C-8), 140.2 (C-9), 132.2 (d, $J = 3.2$ Hz, C-1a), 131.5 (d, $J = 8.6$ Hz, 2C, C-2b/6b), 129.2 (d, $J = 8.7$ Hz, 2C, C-2a/6a), 125.6 (C-5), 125.2 (C-6), 122.1 (C-4), 117.3 (d, $J = 23.0$ Hz, 2C, C-3a/5a), 115.8 (d, $J = 21.8$ Hz, C-3b/5b), 110.0 (C-7), 52.2 (C-11); $^{19}F$ NMR ($CDCl_3$, 376.5 MHz) $\delta$ −110.71, −109.46; HRESIMS *m/z* (pos): 365.1111 $C_{21}H_{15}F_2N_2O_2$ (calcd. 365.1102). * C-1b could not be detected.

Methyl 2-(4-chlorophenyl)-1-(4-fluorophenyl)-1H-benzo[d] imidazole-5-carboxylate (**5c**) Yellow solid (86% yield, 96% purity); mp 185–187 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 251 (3.23), 304 (2.85); IR $\upsilon_{max}$ 3059 (CH), 1708 (C=O), 1357 (C–F), 1181 (C–O), 729 (C–Cl ) $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 8.58 (1H, bs, H-4), 8.01 (1H, d, $J = 8.5$ Hz, H-6), 7.51 (2H, d, $J = 8.4$ Hz, H-2b/6b), 7.31 (4H, d, $J = 8.5$ Hz, H-2a/6a, H-3b/5b), 7.25 (2H, d, $J = 6.44$ Hz, H-3a/5a), 7.20 (1H, d, $J = 8.8$ Hz, H-7), 3.95 (3H, s, H-11); $^{13}C$ NMR ($CDCl_3$, 100 MHz) $\delta$ 167.3 (C-10), 162.5 (d, $J_{CF} = 249.2$ Hz, C-4a), 152.8 (C-2), 142.0 (C-8), 140.1 (C-9), 136.5 (C-4b), 132.1 (d, $J = 3.0$ Hz, C-1a), 130.7 (2C, C-2b/6b), 129.1 (d, $J = 8.7$ Hz, 2C, C-2a/6a), 128.9 (C-3b), 127.4 (C-1b), 125.7 (C-5), 125.3 (C-6), 122.2 (C-4), 117.3 (d, $J = 22.8$ Hz, 2C, C-3a/5a), 110.1 (C-7), 52.2 (C-11); $^{19}F$ NMR ($CDCl_3$, 376.5 MHz) $\delta$ −110.35; HRESIMS *m/z* (pos): 381.0818 $C_{21}H_{15}ClFN_2O_2$ (calcd. 381.0806).

Methyl 2-(4-bromophenyl)-1-(4-fluorophenyl)-1H-benzo[d] imidazole-5-carboxylate (**5d**) Cream colored solid (82% yield, 97% purity); mp 203–205 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 253 (3.28), 305 (2.92); IR $\upsilon_{max}$ 3072 (CH), 1707 (C=O), 1357 (C–F), 1153 (C–O), 523 (C–Br) $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) δ 8.57 (1H, s, H-4), 8.00 (1H, dd, $J = 8.6$, 1.4 Hz, H-6), 7.47 (2H, d, $J = 8.6$ Hz, H-3b/5b), 7.42 (2H, d, $J = 8.6$ Hz, H-2b/6b), 7.29 (2H, dd, $J = 9.0$, 4.8 Hz, H-2a/6a), 7.23 (2H, t, $J = 10.8$ Hz, H-3a/5a), 7.19 (1H, d, $J = 8.6$ Hz, H-7), 3.95 (3H, s, H-11); $^{13}C$ NMR ($CDCl_3$, 100 MHz) $\delta$ 167.3 (C-10), 162.6 (d, $J_{CF} = 249.2$ Hz, C-4a), 152.8 (C-2), 142.1 (C-8), 140.2 (C-9), 132.2 (d, $J = 3.3$ Hz, C-1a), 131.9 (2C, C-3b/5b), 130.9 (2C, C-2b/6b), 129.1 (d, $J = 8.7$ Hz, 2C, C-2a/6a), 127.9 (C-5), 125.7 (C-1b), 125.4 (C-6), 124.9 (C-4b), 122.2 (C-4), 117.4 (d, $J = 22.8$ Hz, 2C, C-3a/5a), 110.1 (C-7), 52.2 (C-11); $^{19}F$ NMR ($CDCl_3$,

376.5 MHz) $\delta$ −110.53; HRESIMS *m/z* (pos): 425.0310 $C_{21}H_{15}BrFN_2O_2$ (calcd. 425.0301).

Methyl 1-(4-fluorophenyl)-2-(4-(trifluoromethyl)phenyl)-1H-benzo[d]imidazole-5-carboxylate (**5e**) White solid (84% yield, 98% purity); mp 171–173 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 250 (2.83), 304 (2.5); IR $\upsilon_{max}$ 3081 (CH), 1712 (C=O), 1358 (C–F), 1196 (C–O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 8.59 (1H, bs, H-4), 8.01 (1H, dd, $J$ = 8.6, 1.4 Hz, H-6), 7.68 (2H, d, $J$ = 8.3 Hz, H-3b/5b), 7.58 (2H, d, $J$ = 8.3 Hz, H-2b/6b), 7.30 (2H, dd, $J$ = 8.9, 4.8 Hz, H-2a/6a), 7.24 (2H, *t*, $J$ = 10.5 Hz, H-3a/5a), 7.21 (1H, d, $J$ = 8.6 Hz, H-7), 3.95 (3H, s, H-11); $^{13}C$ NMR ($CDCl_3$, 100 MHz) δ 167.2 (C-10), 162.6 (d, $J_{CF}$ = 249.4 Hz, C-4a), 152.3 (C-2), 142.3 (C-8), 140.3 (C-9), 132.6 (C-1b), 132.1 (d, $J$ = 3.16 Hz, C-1a), 131.8 (q, $J_{CF}$ = 260.0 Hz, C-7b), 129.7 (2C, C-3b/5b), 129.1 (d, $J$ = 8.7 Hz, 2C, C-2a/6a), 125.8 (C-5), 125.6 (C-6), 125.5 (q, $J$ = 3.9 Hz, 2C, C-2b/6b), 122.5 (C-4), 117.5 (d, $J$ = 22.8 Hz, 2C, C-3a/5a), 110.2 (C-7), 52.2 (C-11); $^{19}F$ NMR ($CDCl_3$, 376.5 MHz) $\delta$ −62.98; HRESIMS *m/z* (pos): 415.1078 $C_{22}H_{15}F_4N_2O_2$ (calcd. 415.1070).

Methyl 1-(4-fluorophenyl)-2-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylate (**5f**) Yellow solid (70% yield; 98% purity); mp 214–216 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 229 (2.84), 320 (2.47); IR $\upsilon_{max}$ 3078 (CH), 1714 (C=O), 1510 (N–O), 1380 (C–F), 1204 (C–O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 8.60 (1H, bs, H-4), 8.18 (2H, d, $J$ = 8.1 Hz, H-3b/5b), 8.04 (1H, d, $J$ = 8.4 Hz, H-6), 7.76 (2H, d, $J$ = 8.1 Hz, H-2b/6b), 7.32 (2H, m, H-2a/6a), 7.27 (2H, m, H-3a/5a), 7.23 (1H, d, $J$ = 8.4 Hz, H-7), 3.96 (3H, s, H-11); $^{13}C$ NMR ($CDCl_3$, 100 MHz) $\delta$ 167.1 (C-10), 162.9 (d, $J_{CF}$ = 250.2 Hz, C-4a), 151.2 (C-4b), 148.4 (C-2), 142.0 (C-8), 140.3 (C-9), 134.9 (C-1b), 131.8 (d, $J$ = 3.1 Hz, C-1a), 130.3 (2C, C-2b/6b), 129.2 (d, $J$ = 8.7 Hz, 2C, C-2a/6a), 126.2 (C-5), 126.1 (C-6), 123.7 (2C, C-3b/5b), 122.6 (C-4), 117.7 (d, $J$ = 22.8 Hz, 2C, C-3a/5a), 110.4 (C-7), 52.3 (C-11); $^{19}F$ NMR ($CDCl_3$, 376.5 MHz) $\delta$ −109.63; HRESIMS *m/z* (pos): 392.1055 $C_{21}H_{15}FN_3O_4$ (calcd. 392.1047).

Methyl 1-(4-fluorophenyl)-2-p-tolyl-1H-benzo[d]imidazole-5-carboxylate (**5g**) White solid (80% yield, 96% purity); mp 156–158 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 214 (3.21), 250 (3.36), 304 (2.93); IR $\upsilon_{max}$ 3074 (CH), 1711 (C=O), 1381 (C–F), 1185 (C–O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 8.57 (1H, bs, H-4), 7.98 (1H, dd, $J$ = 8.5, 1.3 Hz, H-6), 7.43 (2H, d, $J$ = 8.1 Hz, H-2b/6b), 7.29 (2H, dd, $J$ = 8.7, 4.8 Hz, H-2a/6a), 7.21 (2H, t, $J$ = 8.7 Hz, H-3a/5a), 7.18 (1H, d, $J$ = 8.5 Hz, H-7), 7.13 (2H, d, $J$ = 8.1 Hz, H-3b/5b), 3.94 (3H, s, H-11), 2.34 (3H, s, H-7b); $^{13}C$ NMR ($CDCl_3$, 100 MHz) $\delta$ 167.3 (C-10), 162.5 (d, $J_{CF}$ = 248.7 Hz, C-4a), 154.0 (C-2), 140.6 (C-8), 140.1 (2C, C-4/4b), 132.4 (d, $J$ = 3.6 Hz, C-1a), 129.4 (2C, C-3b/5b), 129.3 (2C, C-2b/6b), 129.2 (d, $J$ = 8.7 Hz, 2C, C-2a/6a), 125.7 (C-1b), 125.5 (C-5), 125.1 (C-6), 121.8 (C-4), 117.2 (d, $J$ = 22.8 Hz, 2C, C-3a/5a), 110.0 (C-7), 52.2 (C-11), 21.4 (C-7b); $^{19}F$ NMR ($CDCl_3$, 376.5 MHz) $\delta$ −111.18; HRESIMS *m/z* (pos): 361.1354 $C_{22}H_{18}FN_2O_2$ (calcd. 361.1352).

Methyl 1-(4-fluorophenyl)-2-(4-methoxyphenyl)-1H-benzo[d]imidazole-5-carboxylate (**5h**) White solid (78% yield, 98% purity); mp 138–140 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 225 (2.90), 258 (3.00), 307 (2.75); IR $\upsilon_{max}$ 3080 (CH), 1710 (C=O), 1385 (C–F), 1180 (C–O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 8.54 (1H, d, $J$ = 1.5 Hz, H-4), 7.96 (1H, dd, $J$ = 8.5, 1.5 Hz, H-6), 7.48 (2H, d, $J$ = 8.9 Hz, H-2b/6b), 7.29 (2H, dd, $J$ = 8.7, 4.8 Hz, H-2a/6a), 7.21 (2H, *t*, $J$ = 8.7 Hz, H-3a/5a), 7.16 (1H, d, $J$ = 8.5 Hz, H-7), 6.83 (2H, d, $J$ = 8.9 Hz, H-3b/5b), 3.94 (3H, s, H-11), 3.79 (3H, s, H-7b); $^{13}C$ NMR ($CDCl_3$, 100 MHz) $\delta$ 167.6 (C-10), 162.5 (d, $J_{CF}$ = 248.6 Hz, C-4a), 161.0 (C-4b), 154.0 (C-2), 142.3 (C-8), 140.3 (C-9), 132.6 (d, $J$ = 3.3 Hz, C-1a), 131.0 (2C, C-2b/6b), 129.2 (d, $J$ = 8.7 Hz, 2C, C-2a/6a), 125.3 (C-5), 124.8 (C-6), 121.8 (C-4), 121.3 (C-1b), 117.2 (d, $J$ = 22.7 Hz, 2C, C-3a/5a), 114.1 (2C, C-3b/5b), 109.9 (C-7), 55.3 (C-7b), 52.1 (C-11); $^{19}F$ NMR ($CDCl_3$, 376.5 MHz) $\delta$ −111.23; HRESIMS *m/z* (pos): 377.1310 $C_{22}H_{18}FN_2O_3$ (calcd. 377.1301).

Methyl 2-(4-aminophenyl)-1-(4-fluorophenyl)-1H-benzo[d]imidazole-5-carboxylate (**5i**) Orange solid (75% yield, 97% purity); mp 225–227 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 233 (3.51), 327 (3.41); IR $\upsilon_{max}$ 3473 (NH), 3077 (CH), 1710 (C=O), 1382 (C–F), 1197 (C–O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 400 MHz) $\delta$ 8.26 (1H, d, $J$ = 1.3 Hz, H-4), 7.83 (1H, dd, $J$ = 8.5, 1.3 Hz, H-6), 7.52 (2H, dd, $J$ = 8.7, 5.0 Hz, H-2a/6a), 7.44 (2H, *t*, $J$ = 8.7 Hz, H-3a/5a), 7.21 (2H, d, $J$ = 8.6 Hz, H-2b/6b), 7.17 (1H, d, $J$ = 8.5 Hz, H-7), 6.49 (2H, d, $J$ = 8.6 Hz, H-3b/5b), 5.61 (2H, s, H-7b), 3.88 (3H, s, H-11); $^{13}C$ NMR (DMSO-$d_6$, 100 MHz) δ 166.7 (C-10), 161.8 (d, $J_{CF}$ = 244.9 Hz, C-4a), 155.1 (C-2), 150.6 (C-4b), 142.4 (C-8), 140.5 (C-9), 132.9 (d, $J$ = 3.0 Hz, C-1a), 130.4 (2C, C-2b/6b), 129.9 (d, $J$ = 8.9 Hz, 2C, C-2a/6a), 123.9 (C-5), 123.6 (C-6), 120.0 (C-4), 117.0 (d, $J$ = 22.9 Hz, 2C, C-3a/5a), 115.4 (C-1b), 113.1 (2C, C-3b/5b), 110.0 (C-7), 52.0 (C-11); $^{19}F$ NMR (DMSO-$d_6$, 376.5 MHz) $\delta$ −112.32; HRMS (*m/z*): 362.1307 $C_{21}H_{17}FN_3O_2$ (calcd. 362.1305).

Methyl 1-(4-fluorophenyl)-2-(4-hydroxyphenyl)-1H-benzo[d]imidazole-5-carboxylate (**5j**) White solid (80% yield, 96% purity); mp 252–254 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 226 (2.88), 259 (2.92), 308 (2.74); IR $\upsilon_{max}$ 3058 (CH), 1710 (C=O), 1384 (C-F), 1196 (C-O) $cm^{-1}$; $^1H$ NMR (MeOH-$d_4$, 400 MHz) δ 8.41 (1H, d, $J$ = 1.4 Hz, H-4), 7.97 (1H, dd, $J$ = 8.6, 1.4 Hz, H-6), 7.42 (2H, dd, $J$ = 8.7, 4.9 Hz,

H-2a/6a), 7.38 (2H, d, $J$ = 8.7 Hz, H-2b/6b), 7.33 (2H, *t*, $J$ = 8.7 Hz, H-3a/5a), 7.27 (1H, d, $J$ = 8.6 Hz, H-7), 6.76 (2H, d, $J$ = 8.7 Hz, H-3b/5b), 3.94 (3H, s, H-11); $^{13}$C NMR (MeOH– $d_4$, 100 MHz) $\delta$ 168.8 (C-10), 164.1 (d, $J_{CF}$ = 246.7 Hz, C-4a), 161.1 (C-4b), 156.6 (C-2), 143.0 (C-8), 141.5 (C-9), 133.8 (d, $J$ = 3.4 Hz, C-1a), 132.4 (2C, C-2b/6b), 130.9 (d, $J$ = 9.0 Hz, 2C, C-2a/6a), 126.5 (C-5), 125.9 (C-6), 121.7 (C-4), 120.8 (C-1b), 118.1 (d, $J$ = 23.2 Hz, 2C, C-3a/5a), 116.5 (2C, C-3b/5b), 111.5 (C-7), 52.7 (C-11); $^{19}$F NMR (MeOH- $d_4$, 376.5 MHz) $\delta$ −113.54; HRESIMS *m/z* (pos): 385.0970 $C_{21}H_{15}FN_2O_3Na$ (calcd. 385.0964).

Methyl 2-(4-(dimethylamino)phenyl)-1-(4-fluorophenyl)-1H-benzo[d]imidazole-5-carboxylate (**5k**) Brown solid (90% yield, 87% purity); mp 184–186 °C; UV $\lambda_{max}$ (MeOH) nm (log ε) 205 (3.18), 235 (3.30), 337 (3.23); IR $\upsilon_{max}$ 3049 (CH), 1707 (C=O), 1363 (C–F), 1196 (C–O) cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.54 (1H, bs, H-4), 7.94 (1H, dd, $J$ = 8.5, 1.4 Hz, H-6), 7.45 (2H, d, $J$ = 8.9 Hz, H-2b/6b), 7.34 (2H, dd, $J$ = 8.8, 4.8 Hz, H-2a/6a), 7.24 (2H, t, $J$ = 8.8 Hz, H-3a/5a), 7.13 (1H, d, $J$ = 8.5 Hz, H-7), 6.59 (2H, d, $J$ = 8.9 Hz, H-3b/5b), 3.95 (3H, s, H-11), 2.98 (6H, s, H-7b/8b); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 167.5 (C-10), 162.3 (d, $J_{CF}$ = 248.0 Hz, C-4a), 154.8 (C-4b), 151.1 (C-2), 142.4 (C-8), 140.4 (C-9), 133.1 (d, $J$ = 3.2 Hz, C-1a), 130.5 (2C, C-2b/6b), 129.3 (d, $J$ = 8.7 Hz, 2C, C-2a/6a), 124.9 (C-1b), 124.3 (C-6), 121.3 (C-4), 117.0 (d, $J$ = 22.8 Hz, 2C, C-3a/5a), 115.7 (C-5), 111.4 (C-3b/5b), 109.4 (C-7), 52.0 (C-11), 40.0 (2C, C-7b/8b); $^{19}$F NMR ($CDCl_3$, 376.5 MHz) $\delta$ −111.47; HRESIMS *m/z* (pos): 390.1620 $C_{23}H_{21}FN_3O_2$ (calcd. 390.1618).

Methyl 1-(4-fluorophenyl)-2-(4-(methylthio)phenyl)-1H-benzo[d]imidazole-5-carboxylate (**5l**) Yellow solid (90% yield, 90% purity); mp 182–184 °C; UV $\lambda_{max}$ (MeOH) nm (log ε) 207 (3.16), 238 (3.21), 277 (2.96), 316 (3.14); IR $\upsilon_{max}$ 3059 (CH), 1697 (C=O), 1377 (C–F), 1155 (C–O) cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.55 (1H, d, $J$ = 1.4 Hz, H-4), 7.97 (1H, dd, $J$ = 8.5, 1.4 Hz, H-6), 7.46 (2H, d, $J$ = 8.5 Hz, H-2b/6b), 7.30 (2H, dd, $J$ = 7.2, 4.8 Hz, H-2a/6a), 7.22 (2H, *t*, $J$ = 7.2 Hz, H-3a/5a), 7.19 (1H, d, $J$ = 7.3 Hz, H-7), 7.14 (2H, d, $J$ = 8.5 Hz, H-3b/5b), 3.94 (3H, s, H-11), 2.46 (3H, s, H-7b); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 167.3 (C-10), 162.5 (d, $J_{CF}$ = 249.1 Hz, C-4a), 153.5 (C-2), 142.2 (2C, C-9/4b), 140.1 (C-9), 132.3 (d, $J$ = 3.2 Hz, C-1a), 129.6 (2C, C-2b/6b), 129.2 (d, $J$ = 8.7 Hz, 2C, C-2a/6a), 125.6 (C-1b), 125.5 (2C, C-3b/5b), 125.2 (C-6), 124.8 (C-5), 121.8 (C-4), 117.3 (d, $J$ = 22.8 Hz, 2C, C-3a/5a), 109.9 (C-7), 52.2 (C-11), 14.9 (C-7b); $^{19}$F NMR ($CDCl_3$, 376.5 MHz) $\delta$ −110.83; HRESIMS *m/z* (pos): 415.0885 $C_{22}H_{17}FN_2O_2SNa$ (calcd. 415.0892).

Methyl 1-(4-fluorophenyl)-2-(naphthalen-2-yl)-1H-benzo[d]imidazole-5-carboxylate (**5m**) Yellowish solid (85% yield, 99% purity); mp 169–171 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 210 (3.33), 252 (3.62), 311 (3.10); IR $\upsilon_{max}$ 3064 (CH), 1701 (C=O), 1362 (C–F), 1156 (C–O) cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.61 (1H, bs, H-4), 8.09 (1H, s, H-2b), 8.01 (1H, dd, $J$ = 8.6, 1.2 Hz, H-6), 7.80 (1H, d, $J$ = 8.2 Hz, H-9b), 7.75 (2H, *t*, $J$ = 8.8 Hz, H-4b/7b), 7.58 (1H, dd, $J$ = 8.2, 1.6 Hz, H-10b), 7.51 (1H, t, $J$ = 7.0 Hz, H-5b*), 7.47 (1H, t, $J$ = 7.0 Hz, H-6b*), 7.33 (2H, dd, $J$ = 8.2, 4.7 Hz, H-2a/6a), 7.22 (1H, d, $J$ = 8.6 Hz, H-7), 7.20 (2H, *t*, $J$ = 8.2 Hz, H-3a/5a), 3.96 (3H, s, H-11); $^{13}$C NMR ($CDCl_3$, 100 MHz) δ 167.4 (C-10), 162.5 (d, $J_{CF}$ = 248.8 Hz, C-4a), 154.0 (C-2), 142.4 (C-8), 140.3 (C-9), 133.7 (C-3b), 132.8 (C-1b), 132.6 (d, $J$ = 3.2 Hz, C-1a), 130.0 (C-2b), 129.2 (d, $J$ = 8.7 Hz, 2C, C-2a/6a), 128.7 (C-7b), 128.2 (C-4b), 127.5 (C-9b$^{\#}$), 127.7 (C-6b$^{\#}$), 126.7 (C-5b), 126.4 (C-5), 125.8 (C-10b), 125.5 (C-8b), 125.2 (C-6), 122.2 (C-4), 117.2 (d, $J$ = 22.8 Hz, 2C, C-3a/5a), 110.0 (C-7), 52.2 (C-11); $^{19}$F NMR ($CDCl_3$, 376.5 MHz) $\delta$ −110.01; HRESIMS *m/z* (pos): 397.1349 $C_{25}H_{18}FN_2O_2$ (calcd. 397.1352). * and $^{\#}$ assignments may be interchanged.

(E)-Methyl 1-(4-fluorophenyl)-2-styryl-1H-benzo[d]imidazole-5-carboxylate (**5n**) White solid (87% yield, 97% purity); mp 210–212 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 227 (3.21), 268 (3.04), 332 (3.06); IR $\upsilon_{max}$ 3078 (CH), 1704 (C=O), 1635 (C=C), 1392 (C-F), 1154 (C-O) cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.52 (1H, bs, H-4), 8.05 (1H, d, $J$ = 16.0 Hz, H-1b), 7.95 (1H, dd, $J$ = 8.5, 1.0 Hz, H-6), 7.46 (2H, m, H-4b/8b), 7.43 (2H, m, H-2a/6a), 7.31–7.35 (5H, m, H-5b, H-6b, H-7b, H-3a/5a), 7.13 (1H, d, $J$ = 8.5 Hz, H-7), 6.74 (1H, d, $J$ = 16.0 Hz, H-2b), 3.94 (3H, s, H-11); $^{13}$C NMR ($CDCl_3$, 100 MHz) δ 167.4 (C-10), 162.8 (d, $J_{CF}$ = 249.1 Hz, C-4a), 152.5 (C-2), 142.3 (C-8), 139.6 (C-9), 138.9 (C-1b), 135.5 (C-3b), 130.9 (d, $J$ = 3.1 Hz, C-1a), 129.5 (2C, C-5b/7b), 129.4 (2C, d, $J$ = 8.8 Hz, C-2a/6a), 128.8 (C-6b), 127.4 (2C, C-4b/8b), 125.6 (C-5), 124.9 (C-6), 121.5 (C-4), 117.3 (d, $J$ = 23.9 Hz, 2C, C-3a/5a), 112.8 (C-2b), 109.6 (C-7), 52.2 (C-11); $^{19}$F NMR ($CDCl_3$, 376.5 MHz) $\delta$ −110.43; HRESIMS *m/z* (pos): 373.1355 $C_{23}H_{18}FN_2O_2$ (calcd. 373.1352).

Methyl 2-(6-chloroquinolin-2-yl)-1-(4-fluorophenyl)-1H-benzo[d]imidazole-5-carboxylate (**5o**) Yellowish solid (85% yield, 98% purity); Purity 98%; mp 228–230 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 257 (3.43), 321 (2.94), 336 (2.98); IR $\upsilon_{max}$ 3068 (CH), 1702 (C=O), 1386 (C–F), 1190 (C–O), 750 (C–Cl) cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.64 (1H, bs, H-4), 8.47 (1H, d, $J$ = 8.6 Hz, H-9b), 8.21 (1H, d, $J$ = 8.6 Hz, H-10b), 8.03 (1H, dd, $J$ = 8.6, 1.3 Hz, H-6), 7.72 (1H, d, $J$ = 9.2 Hz, H-4b), 7.46 (2H, m, H-5b/7b), 7.38 (2H, dd, $J$ = 9.2, 4.8 Hz, H-2a/6a), 7.24 (2H, t, $J$ = 9.2 Hz, H-3a/5a), 7.19

(1H, d, $J = 8.6$ Hz, H-7), 3.96 (3H, s, H-11); $^{13}$C NMR ($CDCl_3$, 100 MHz) δ 167.3 (C-10), 162.5 (d, $J_{CF} = 247.3$ Hz, C-4a), 151.2 (C-2), 149.2 (C-1b), 147.3 (C-8b), 141.9 (C-8), 141.2 (C-9), 136.5 (C-10b), 135.8 (C-6b), 133.7 (d, $J = 3.1$ Hz, C-1a), 129.6 (d, $J = 8.7$ Hz, 2C, C-2a/6a), 128.8 (C-7b*), 128.6 (C-5b*), 128.5 (C-4b), 126.2 (C-3b), 126.0 (C-6), 125.8 (C-5), 122.7 (C-4), 121.6 (C-9b), 116.3 (d, $J =$ 22.8 Hz, 2C, C-3a/5a), 110.7 (C-5), 52.2 (C-11); $^{19}$F NMR ($CDCl_3$, 376.5 MHz) $\delta$ −112.10; HRESIMS *m/z* (pos): 432.0931 $C_{24}H_{16}ClFN_3O_2$ (calcd. 432.0915). *assignments may be interchanged.

Methyl 1-(4-fluorophenyl)-2-(2-hydroxy-4,6-dimethoxyphenyl)-1H-benzo[d]imidazole-5-carboxylate (**5p**) White solid (65% yield, 99% purity); mp 68–70 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 229 (2.86), 267 (2.33), 297 (3.93); IR $\upsilon_{max}$ 3398 (O–H), 3074 (CH), 1712 (C=O), 1359 (C–F), 1153 (C–O) cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.51 (1H, bs, H-4), 8.00 (1H, dd, $J = 8.5$, 1.0 Hz, H-6), 7.31 (1H, d, $J = 8.5$ Hz, H-7), 7.26 (2H, dd, $J = 8.6$, 4.9 Hz, H-2a/6a), 7.16 (2H, t, $J = 8.6$ Hz, H-3a/5a), 6.32 (1H, d, $J = 1.9$ Hz, H-3b), 5.77 (1H, d, $J = 1.9$ Hz, H-5b), 3.94 (3H, s, H-11), 3.77 (3H, s, H-8b), 3.15 (3H, s, H-9b); $^{13}$C NMR ($CDCl_3$, 100 MHz) δ 167.2 (C-10), 163.1 (C-4b), 162.2 (d, $J_{CF} = 247.1$ Hz, C-4a), 160.1 (C-2b), 157.6 (C-6b), 151.8 (C-2), 140.4 (C-8), 138.5 (C-9), 133.8 (d, $J = 2.8$ Hz, C-1a), 126.9 (d, $J = 8.5$ Hz, 2C, C-2a/6a), 125.5 (C-5), 125.0 (C-6), 120.7 (C-4), 116.3 (d, $J = 22.8$ Hz, 2C, C-3a/5a), 110.0 (C-7), 96.2 (C-1b), 94.0 (C-3b), 90.8 (C-5b), 55.4 (C-8b), 54.3 (C-9b), 52.2 (C-11); $^{19}$F NMR ($CDCl_3$, 376.5 MHz) $\delta$ −112.41; HRESIMS *m/z* (pos): 445.1168 $C_{23}H_{19}FN_2O_5$ Na (calcd. 445.1176).

Methyl 2-(3,4-dihydroxyphenyl)-1-(4-fluorophenyl)-1H-benzo[d]imidazole-5-carboxylate (**5q**) Cream solid (65% yield, 97% purity); mp 230–232 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 226 (3.17), 264 (2.91), 313 (2.85); IR $\upsilon_{max}$ 3501 (O-H), 3062 (CH), 1712 (C=O), 1377 (C–F), 1177 (C–O) cm$^{-1}$; $^1$H NMR (MeOH– $d_4$, 400 MHz) $\delta$ 8.40 (1H, d, $J =$ 1.5 Hz, H-4), 7.97 (1H, dd, $J = 8.5$, 1.5 Hz, H-6), 7.42 (2H, dd, $J = 8.7$, 4.9 Hz, H-2a/6a), 7.33 (2H, t, $J = 8.7$ Hz, H-3a/5a), 7.26 (1H, d, $J = 8.5$ Hz, H-7), 7.01 (1H, d, $J = 2.1$ Hz, H-2b), 6.86 (1H, dd, $J = 8.2$, 2.2 Hz, H-6b), 6.7 (1H, d, $J =$ 8.2 Hz, H-5b), 3.95 (3H, s, H-11); $^{13}$C NMR (MeOH– $d_4$, 100 MHz) $\delta$ 168.8 (C-10), 164.1 (d, $J_{CF} = 246.8$ Hz, C-4a), 156.9 (C-2), 149.2 (C-3b), 146.6 (C-4b), 143.0 (C-8), 141.5 (C-9), 133.8 (d, $J = 2.8$ Hz, C-1a), 130.9 (d, $J = 8.9$ Hz, 2C, C-2a/6a), 126.5 (C-5), 125.9 (C-6), 123.1 (C-6b), 121.6 (C-4), 121.2 (C-1b), 118.1 (2C, d, $J = 23.2$ Hz, C-3a/5a), 117.7 (C-2b), 116.3 (C-5b), 111.5 (C-7), 52.7 (C-11); $^{19}$F NMR (MeOH- $d_4$, 376.5 MHz) $\delta$ −113.67; HRESIMS *m/z* (pos): 379.1094 $C_{21}H_{16}FN_2O_4$ (calcd. 379.1110).

Methyl 1-(4-fluorophenyl)-2-(2,3,4-trihydroxyphenyl)-1H-benzo[d]imidazole-5-carboxylate (**5r**) Green solid (85% yield, 99% purity); mp 214–216 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 230 (3.27), 322 (3.0); IR $\upsilon_{max}$ 3483 (O-H), 3069 (CH), 1709 (C=O), 1345 (C–F), 1152 (C–O) cm$^{-1}$; $^1$H NMR (MeOH– $d_4$, 400 MHz) $\delta$ 8.34 (1H, d, $J = 1.4$ Hz, H-4), 7.92 (1H, dd, $J = 8.5$, 1.4 Hz, H-6), 7.46 (2H, dd, $J = 8.7$, 4.9 Hz, H-2a/6a), 7.36 (2H, *t*, $J = 8.7$ Hz, H-3a/5a), 7.12 (1H, d, $J = 8.5$ Hz, H-7), 6.35 (1H, d, $J = 8.9$ Hz, H-6b), 6.15 (1H, d, $J = 8.9$ Hz, H-5b), 3.93 (3H, s, H-11); $^{13}$C NMR (MeOH– $d_4$, 100 MHz) $\delta$ 168.9 (C-10), 164.4 (d, $J_{CF} = 247.2$ Hz, C-4a), 155.2 (C-2), 149.5 (C-4b), 149.4 (C-2b), 141.5 (C-8), 141.0 (C-9), 134.6 (C-3b), 134.2 (d, $J = 3.3$ Hz, C-1a), 131.1 (d, $J = 8.9$ Hz, 2C, C-2a/6a), 126.5 (C-5), 125.8 (C-6), 121.0 (C-4), 120.4 (C-6b), 118.3 (d, $J = 23.3$ Hz, 2C, C-3a/5a), 111.0 (C-7), 107.8 (C-5b), 106.5 (C-1b), 52.7 (C-11); $^{19}$F NMR (MeOH– $d_4$, 376.5 MHz) $\delta$ −113.12; HRESIMS *m/z* (pos): 417.0874 $C_{21}H_{15}FN_2O_5Na$ (calcd. 417.0863).

Methyl 1-(4-fluorophenyl)-2-(thiophen-2-yl)-1H-benzo[d]imidazole-5-carboxylate (**5s**) White solid (74% yield, 90% purity); mp 156–158 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 219 (3.26), 254 (3.28), 322 (3.18); IR $\upsilon_{max}$ 3073 (CH), 1711 (C=O), 1386 (C-F), 1192 (C–O) cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.53 (1H, bs, H-4), 7.95 (1H, dd, $J = 8.5$, 1.0 Hz, H-6), 7.41 (2H, dd, $J = 8.2$, 4.8 Hz, H-2a/6a), 7.39 (1H, d, $J = 4.4$ Hz, H-3b), 7.30 (2H, *t*, $J = 8.2$ Hz, H-3a/5a), 7.03 (1H, d, $J = 8.5$ Hz, H-7), 7.02 (1H, bs, H-5b), 6.95 (1H, t, $J = 4.4$ Hz, H-4b), 3.93 (3H, s, H-11); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 167.3 (C-10), 163.2 (d, $J_{CF} = 249.6$ Hz, C-4a), 149.0 (C-2), 142.2 (C-8), 142.1 (C-1b), 140.6 (C-9), 131.7 (d, $J = 3.4$ Hz, C-1a), 130.2 (d, $J = 8.8$ Hz, 2C, C-2a/6a), 129.4 (C-3b), 129.2 (C-5b), 127.7 (C-4b), 125.5 (C-5), 125.1 (C-6), 121.7 (C-4), 117.5 (d, $J = 22.8$ Hz, 2C, C-3a/5a), 109.7 (C-7), 52.1 (C-11); $^{19}$F NMR ($CDCl_3$, 376.5 MHz) $\delta$ −109.62; HRESIMS *m/z* (pos): 353.0761 $C_{19}H_{14}FN_2O_2S$ (calcd. 353.0760).

Methyl 1-(4-fluorophenyl)-2-(furan-2-yl)-1H-benzo[d]imidazole-5-carboxylate (**5t**) Dark brown solid (76% yield, 98% purity); mp 139–141 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 258 (3.15), 313 (3.11), 326 (3.01); IR $\upsilon_{max}$ 3069 (CH), 1711 (C=O), 1359 (C–F), 1195 (C–O) cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.53 (1H, d, $J = 1.4$ Hz, H-4), 7.96 (1H, dd, $J =$ 8.5, 1.4 Hz, H-6), 7.48 (1H, bs, H-3b), 7.40 (2H, dd, $J = 8.5$, 4.7 Hz, H-2a/6a), 7.29 (2H, td, $J = 8.5$, 2.2 Hz, H-3a/5a), 7.06 (1H, d, $J = 8.5$ Hz, H-7), 6.39–6.40 (2H, m, H-4b/5b), 3.93 (3H, s, H-11); $^{13}$C NMR ($CDCl_3$, 100 MHz) δ 167.3 (C-10), 163.0 (d, $J_{CF} = 249.2$ Hz, C-4a), 145.7 (C-2), 144.9 (C-3b), 143.6 (C-8), 140.0 (C-9), 131.7 (d, $J = 3.2$ Hz, C-1a), 129.8 (d, $J = 8.8$ Hz, 2C, C-2a/6a), 125.7 (C-5), 125.4 (C-6), 122.0 (C-4), 117.2 (d, $J = 22.8$ Hz, 2C, C-3a/5a),

113.6 (C-5b), 111.8 (C-4b), 109.8 (C-7), 52.1 (C-11); $^{19}$F NMR ($CDCl_3$, 376.5 MHz) $\delta$ −110.00; HRESIMS *m/z* (pos): 337.0994 $C_{19}H_{14}FN_2O_3$ (calcd. 337.0988).

Methyl 1-(4-fluorophenyl)-2-propyl-1H-benzo[d]imidazole-5-carboxylate (**5u**) Cream solid (70% yield, 100% purity); mp 102–104 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 227 (3.16), 267 (2.40); IR $\upsilon_{max}$ 3083 (CH), 1715 (C=O), 1360 (C–F), 1166 (C–O) cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.49 (1H, d, $J$ = 1.4 Hz, H-4), 7.94 (1H, dd, $J$ = 8.5, 1.4 Hz, H-6), 7.34 (2H, dd, $J$ = 8.2, 5.2 Hz, H-2a/6a), 7.29 (2H, *t*, $J$ = 8.2 Hz, H-3a/5a), 7.05 (1H, d, $J$ = 8.5 Hz, H-7), 3.92 (3H, s, H-11), 2.76 (2H, t, $J$ = 7.5 Hz, H-1b), 1.76–1.86 (2H, sestet, H-2b), 0.94 (3H, t, $J$ = 7.5 Hz, H-3b); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 167.4 (C-10), 162.8 (d, $J_{CF}$ = 249.3 Hz, C-4a), 156.9 (C-2), 141.7 (C-8), 139.5 (C-9), 131.2 (d, $J$ = 2.8 Hz, C-1a), 129.2 (d, $J$ = 8.8 Hz, 2C, C-2a/6a), 125.1 (C-5), 124.8 (C-6), 121.2 (C-4), 117.3 (d, $J$ = 22.8 Hz, 2C, C-3a/5a), 109.6 (C-7), 52.1 (C-11), 29.4 (C-1b), 21.1 (C-2b), 13.9 (C-3b); $^{19}$F NMR ($CDCl_3$, 376.5 MHz) $\delta$ −110.53; HRESIMS *m/z* (pos): 335.1161 $C_{18}H_{17}FN_2O_2Na$ (calcd. 335.1172).

Methyl 1-(4-fluorophenyl)-2-hexyl-1H-benzo[d]imidazole-5-carboxylate (**5v**) Brown solid (70% yield, 61% purity); mp 66–68 °C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 227 (3.53), 264 (2.81); IR $\upsilon_{max}$ 3075 (CH), 1708 (C=O), 1399 (C–F), 1156 (C–O) cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.48 (1H, bs, H-4), 7.94 (1H, dd, $J$ = 8.5, 1.2 Hz, H-6), 7.36 (2H, dd, $J$ = 8.6, 5.0 Hz, H-2a/6a), 7.30 (2H, *t*, $J$ = 8.6 Hz, H-3a/5a), 7.06 (1H, d, $J$ = 8.5 Hz, H-7), 3.94 (3H, s, H-11), 2.76 (2H, *t*, $J$ = 7.5 Hz, H-1b), 1.77 (2H, quintet, $J$ = 7.5 Hz, H-2b), 1.25–1.35 (2H, m, H-3b), 1.23–1.26 (2H, m, H-5b), 0.88–0.90 (2H, m, H-4b), 0.84 (3H, *t*, $J$ = 6.8 Hz, H-6b); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 167.6 (C-10), 162.9 (d, $J_{CF}$ = 249.0 Hz, C-4a), 157.2 (C-2), 141.7 (C-8), 139.6 (C-9), 131.5 (d, $J$ = 3.1 Hz, C-1a), 129.2 (d, $J$ = 8.8 Hz, 2C, C-2a/6a), 124.8 (C-5), 124.5 (C-6), 121.3 (C-4), 117.2 (d, $J$ = 22.8 Hz, 2C, C-3a/5a), 109.4 (C-7), 52.0 (C-11), 31.3 (C-4b), 28.9 (C-3b), 27.6 (C-1b), 27.6 (C-2b), 22.4 (C-5b), 13.9 (C-6b); $^{19}$F NMR ($CDCl_3$, 376.5 MHz) $\delta$ −110.80; HRESIMS *m/z* (pos): 355.1816 $C_{21}H_{24}FN_2O_2$ (calcd. 355.1822).

## Single crystal X-ray diffraction analysis

Crystals suitable for X-ray diffraction were obtained by slow evaporation in a combination of ethyl acetate and n-hexane at room temperature. The crystal evaluation and data collection were performed on a Bruker Smart APEX II diffractometer with Mo Kα radiation ($\lambda$ = 0.71073 Å). The diffractometer to crystal distance was set at 4 cm. The initial cell matrix was obtained from three series of scans at different starting angles. Each series consisted of 12 frames collected at intervals of 0.5° in a 6° range with the exposure time of 10 s per frame. The final cell constants were calculated from a set of 4762 strong reflections from the actual data collection. Data collection method involved ω scans of width 0.5°. Data reduction was carried out using the program System Administrator's Integrated Network Tool+. The structure was solved by direct methods using SHELXS and refined. Non-H atoms were first refined isotropically and then by anisotropic refinement with full-matrix least-squares calculations based on $F^2$ using SHELXS. All H atoms were positioned geometrically and allowed to ride on their respective parent atoms. All H atoms were refined isotropically. The absorption correction was based on fitting a function to the empirical transmission surface as sampled by multiple equivalent measurements. The final least-squares refinement of 265 parameters against 4762 data resulted in residuals $R$ (based on $F^2$ for $I \geq 2\sigma$) and wR (based on $F^2$ for all data) of 0.0465 and 0.1048, respectively. The final difference Fourier map was featureless. The programs Olex-2 and Ortep-3 were used within the WinGX software package to prepare artwork representation (Spek 2003; Farrugia 2012). Crystallographic data (excluding structure factors) for the structure in this paper has been deposited with the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB21EZ, UK. Copies of the data can be obtained free of charge on quoting the depository number CCDC 1454700 (fax: +44-1223-336-033; E-mail: deposit@ccdc.cam.ac.uk, http://www.ccdc.cam.ac.uk).

## In vitro antimicrobial studies

The synthesized compounds were tested for their antibacterial and antifungal activity in vitro against the Gram +ve bacterial strains: *Staphylococcus aureus (*ATCC 25923*)* and methicillin resistant *S. aureus* (MRSA*)* (ATCC BAA-1683) and Gram -ve strains (*Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 31488), and *Pseudomonas aeruginosa (*ATCC 27853)) using ciprofloxacin and ampicillin as standards. For the antifungal assay *Candida albicans* (ATCC 10231) was used with tioconazole as the reference drug. A disc diffusion assay was used as an initial screening test to select potentially active compounds using a concentration of 10 mg mL$^{-1}$. Minimum bactericidal concentrations (MBCs) were only carried out on compounds that showed a zone of inhibition between 8–35 mm (**5b**, **5e**, **5h**, **5k–l,** and **5o–t**).

The microbial cultures were grown overnight at 37 °C in nutrient broth (UKZN Biolab, South Africa), adjusted to a 0.5 McFarland standard using distilled water and lawn inoculated onto Mueller-Hinton agar (MHA) plates. A volume of 10 µL of each sample (23.20–32.04 µM in 1 mL DMSO) was inoculated onto antibiotic assay discs

(6 mm diameter) and placed on the MHA plates which were incubated overnight at 37 °C for 24 h. After the incubation period, zones of inhibition were measured in mm. Compounds showing an inhibition zone of >9 mm were selected to determine their MBC values using the broth dilution assay with ampicillin and ciprofloxacin as the controls following the method in Andrews (2001).

For the broth dilution method the microbial cultures (adjusted to 0.5 McFarland) were prepared as described previously for the disc diffusion method. The test compounds were dissolved in DMSO (10 mg $mL^{-1}$) and subject to a 50% serial dilution in 1 mL Eppendorf tubes with Mueller-Hinton broth, inoculated with bacterial cultures (20 µL) and then incubated at 37 °C for 18 h. The total volume in each Eppendorf was 200 µL. A volume of 10 µL of each dilution was spotted on MHA plates and incubated at 37 °C for 18 h to determine the MBC (µM). Ampicillin, ciprofloxacin and tioconazole served as the standard drugs for the antimicrobial and antifungal studies, respectively. All experiments were performed in duplicate.

### In vitro antioxidant studies

The scavenging activity (antioxidant capacity) of the synthesized fluorinated benzimidazole compounds (**5a–5v**) on the stable radical, DPPH was evaluated according to a method by Murthy et al. (2012) with some modifications. A volume of 150 µL of methanolic solution of the test compounds at different concentrations (1000, 500, 200, 50, 20 and 10 µg $mL^{-1}$) was mixed with 2850 µL of the methanolic solution of DPPH (0.1 mM). An equal amount of MeOH and DPPH without sample served as a control. After 30 min of reaction at room temperature in the dark, the absorbance was measured at 517 nm against methanol as a blank using a UV spectrophotometer as mentioned above. The percentage free radical scavenging activity was calculated according to the following equation:

$$\%\ \text{Scavenging activity} = \left[\frac{\left(A_{\text{control}} - A_{\text{sample}}\right)}{A_{\text{blank}}}\right] \times 100$$

Where "$A_{control}$" is the absorbance of the control reaction (containing all reagents except the test compound) and "$A_{sample}$" is the absorbance of the reagents with a particular test compound.

### Molecular docking methodology

The "Prepare Protein" module was used to protonate the amino acid residues of the X-ray structure of topoisomerase II DNA-gyrase (PDB ID: 2XCT, resolution 3.35 Å). The native ligand and water molecules were removed from the protein, while the manganese ion ($Mn^{2+}$) present in the active site was retained. Different conformations of the representative compounds (RCs) (**5h** and **5r**) were generated at physiological pH using the "Prepare Ligands" module and subsequently minimized. The partial atomic charges on each atom were developed using the CHARMm force field. A binding sphere of diameter 7.6 Å was developed around the active site residues. The automated docking was performed using the CDocker algorithm (Wu et al. 2003) by generating new conformations of the ligands using the molecular dynamics method. The best pose of each RC was chosen based on the scoring function (-CDOCKER energy), and subjected further to binding energy calculations.

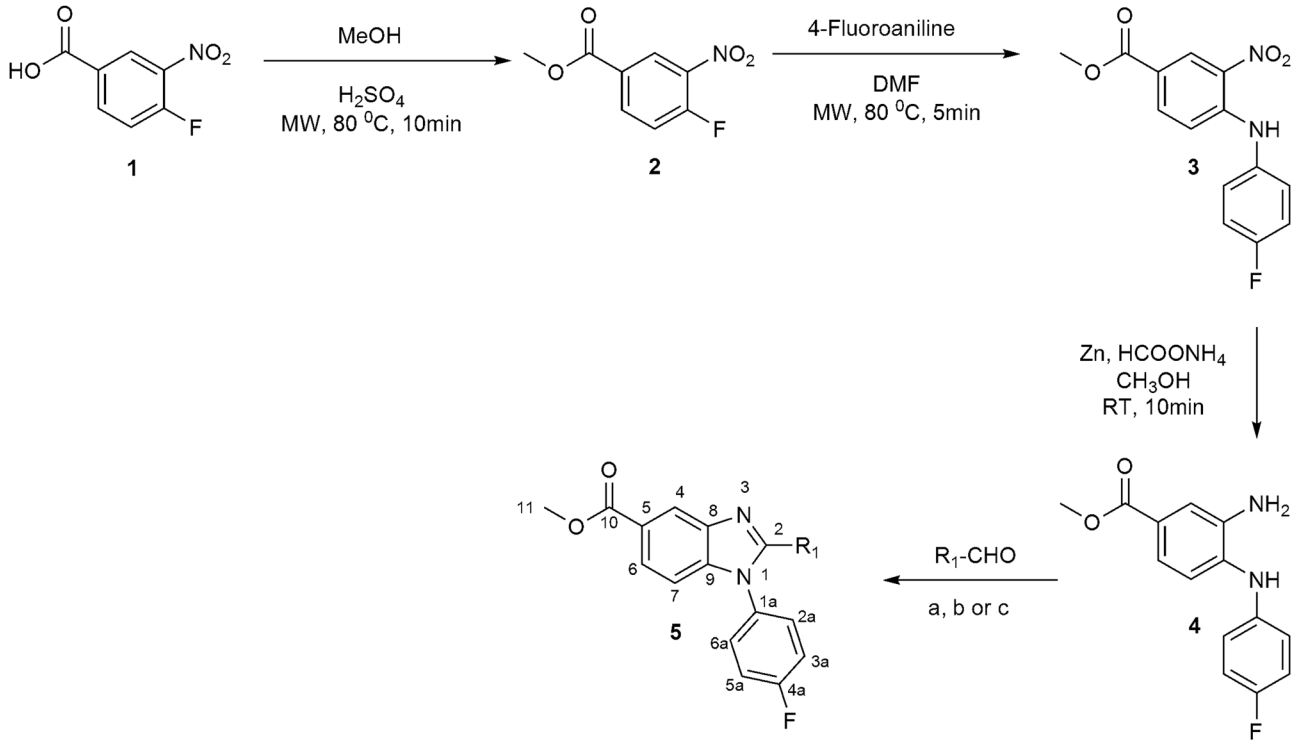
## Results and discussion

### Chemistry

Three fundamental methodologies were used in this study: microwave, conventional and mechanochemical synthesis. A series of 22 novel benzimidazole molecules (**5a–5v**) were synthesized employing these synthetic methods with different substituents at C-2 on the benzimidazole skeleton. The different substituents were all aldehyde derived (Table 1). These contained *para* halogenated phenyl groups, phenyl groups with *para* substituted electron donating and withdrawing groups as well as other heterocyclic ring moieties such as quinoline, furan, naphthalene and thiophene, and the alkyl chains butane and heptane. In addition to the different substituents present on the aromatic ring attached to C-2, an ester moiety was present at C-5 and a 4-fluorophenyl moiety at N-1 (Scheme 1).

The synthesis started with the esterification of 4-fluoro-3-nitrobenzoic acid (**1**), since the subsequent nucleophilic step is easier to perform with esters rather than acids due to the acidic nature of benzoic acids. The conversion to the ester (**2**) occurred in 95% yield. The fluoro substituent on the ester was then substituted with 4-fluoroanilne producing **3** in a 90% yield. Prior to the aldehyde being added, the nitro group of the methyl benzoate (**3**) was reduced (92% yield) with zinc and ammonium formate forming the *o*-diamino phenyl precursor (**4**) to the benzimidazoles (Scheme 1).

In the last step of the synthesis, different aldehydes were added to the precursor **4** using three methods: (i) without the use of solvents or catalysts in a microwave at 110 °C, (ii) grinding equimolar amounts of **4** and the aldehydes in the presence of iodine as a catalyst at room temperature and (iii) refluxing the amino benzoate (**4**) and various aldehydes with ethanol in the presence of $Na_2S_2O_5$ to produce 22 benzimidazoles (**5a–v**). The probable mechanism of the final step is imine formation of the aldehyde with the primary amino group followed by nucleophilic attack by the secondary amine to the imine carbon. Proton transfer from




**Scheme 1** Synthesis of fluorinated benzimidazoles **5a–5v**; **a** MW 110 °C, 3–8 min; **b** grinding, $I_2$ (10 mol%), 5–45 min; **c** $Na_2S_2O_5$ (20 mol%), EtOH, reflux, 8–18 hr

one nitrogen to the other followed by loss of $H_2$ resulted in the formation of the benzimidazoles **5a–5v** (Scheme 2). Their structures were confirmed by NMR spectroscopy (1D and 2D NMR) and single crystal X-ray diffraction. Of the three methods used for the synthesis, the best results were achieved under microwave conditions at 110 °C for 3–8 min with yields of between 85–96% (Table 1).

## Structural elucidation

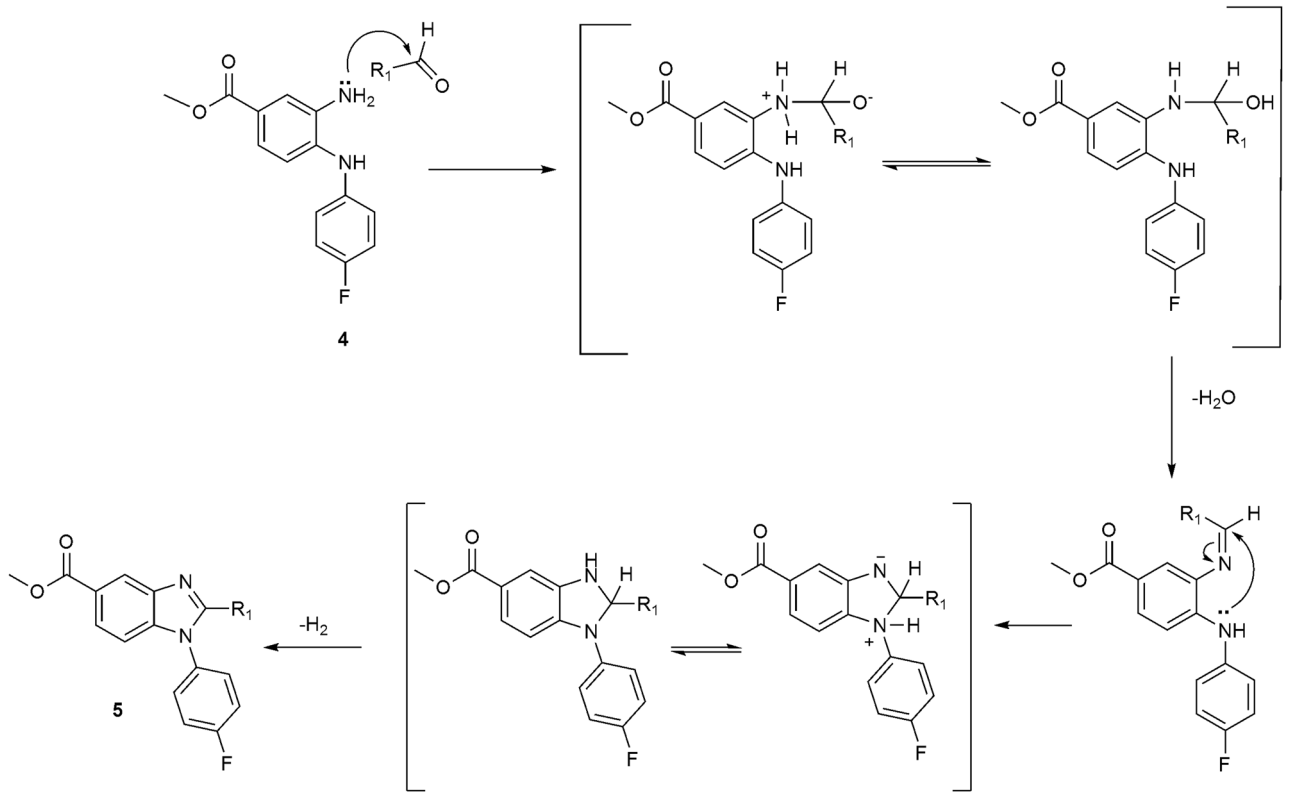
Using **5i** (methyl 2-(4-aminophenyl)-1-(4-fluorophenyl)-1*H*-benzo[*d*]imidazole-5-carboxylate) as a representative fluorinated benzimidazole, H-7, H-6 and H-4 on the benzimidazole core skeleton occurs at $\delta_H$ 7.17 (d, $J = 8.5$ Hz), 7.83 (dd, $J = 8.5$, 1.5 Hz) and 8.26 (d, $J = 1.3$ Hz) respectively. The ester methyl resonance (H-11) occurred as a singlet at $\delta_H$ 3.89. The ester carbonyl resonance (C-10) was identified from the HMBC correlation to H-11 and appeared at $\delta_C$ 166.7. The two aromatic C-N singlet resonances, C-8 and C-9 were assigned to $\delta_C$ 142.4 and 140.5. C-8 was distinguished from C-9 by a heteronuclear multiple bond correlation (HMBC) to H-6. C-5 to which the ester group was attached was assigned to $\delta_C$ 123.9 and showed an HMBC correlation to H-7. C-2 of the imidazole core skeleton was present at $\delta_C$ 155.1.

The aromatic protons of phenyl group H-2a/6a appeared as a dd at $\delta_H$ 7.52 ($J = 9.8$, 5.0 Hz), coupling to both F and H-3a/5a, the latter appearing as a triplet at $\delta_H$ 7.44, due to the same $J$ values (8.6 Hz) for both coupling to the F and to H-2a/6a. The C-4a fluorinated carbon resonance occurred as a doublet at $\delta_C$ 161.8 ($J = 244.9$ Hz). These assignments were supported by HMBC correlations between C-4a and H-2a/6a as well as H-3a/5a and C-1a, which occurred at $\delta$ 132.9 (d, $J = 3.0$ Hz). C-2a/6a appeared as a doublet at $\delta$ 129.9 ($J = 8.9$ Hz) and C-3a/5a appeared as a doublet at $\delta$ 117.0 ($J = 22.9$ Hz).

For the phenyl group (B) attached to C-2, the aromatic proton resonance of H-2b/6b appeared as a doublet at $\delta_H$ 7.21 ($J = 8.6$ Hz) and H-3b/5b appeared at $\delta_H$ 6.49 (d, $J =$ 8.6 Hz). An HMBC correlation between the corresponding C-3b/5b carbon resonance at $\delta$ 113.1 and the proton resonance at $\delta_H$ 5.61 ($NH_2$) confirmed the assignment of C-3b/5b. The H-2b/6b resonance was confirmed by a HMBC correlation with C-2. Selected HMBC correlations used in the structural elucidation of **5i** are provided in Fig. 1.

## Antimicrobial activity

The trioxygenated derivatives **5p** and **5r** showed broad spectrum activity against all strains of bacteria (*S. aureus*, *E. coli*, *K. pneumonia* and *P. aeruginosa*) with the




**Scheme 2** Proposed mechanism for the final step of the benzimidazole synthesis

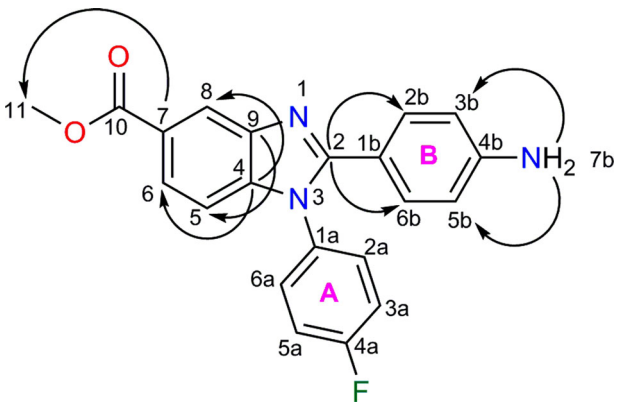


**Fig. 1** Selected HMBC correlations of compound **5i** (H→C)

exception of MRSA. Compound **5p** had MBC values of 115.7, 92.5 115.7 and 14.5 µM and **5r** MBC values of 74.3, 49.6, 37.2 and 25.6 µM against *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumoniae*, respectively. The activity of both these compounds were 10 fold greater than ampicillin in the same assay. Compound **5r** was 2b,3b,4b-trisubstituted with hydroxyl groups at each of the positions on the aromatic B ring and **5p** was 2b,4b,6b-trisubstituted with a hydroxy group at the 2b-position and methoxy groups at the 4b and 6b positions. Consequently, these were the only two trioxygenated molecules from the 22 compounds that was synthesized. Even the dihydroxy substituted compound **5q** showed poor antimicrobial activity in comparison to **5p** and **5r**. It is likely that this trioxygenation has something to do with its broad spectrum activity. However, the test compounds were not as active as ciprofloxacin, whose MBC was at least one order of magnitude better than the test compounds, including **5r** and **5p**.

The benzimidazole **5a** with an unsubstituted 2-phenyl group was relatively unreactive and when the halogens, F (**5b**), Cl (**5c**), and Br (**5d**) were placed at the *para* position of the 2-substituted phenyl group, only the fluorinated compound showed some form of antibacterial activity, showing the best activity against *K. pneumonia* at 107.3 µM. This activity was enhanced when the fluorine was replaced with a trifluoromethyl group (**5e**), which was active against MRSA with an MBC of 70.7 µM and against *K. pneumonia* (24.3 µM) and *C. albicans* (94.3 µM). Compounds **5f-5o**, **5q**, **5s**, and **5t** with various substituents at the *para* position of the 2-substituted phenyl group (Table 1) were all inactive as antimicrobial agents.

## Antioxidant activity

For antioxidant activity, the DPPH radical scavenging activity assay was used. The DPPH radical scavenging

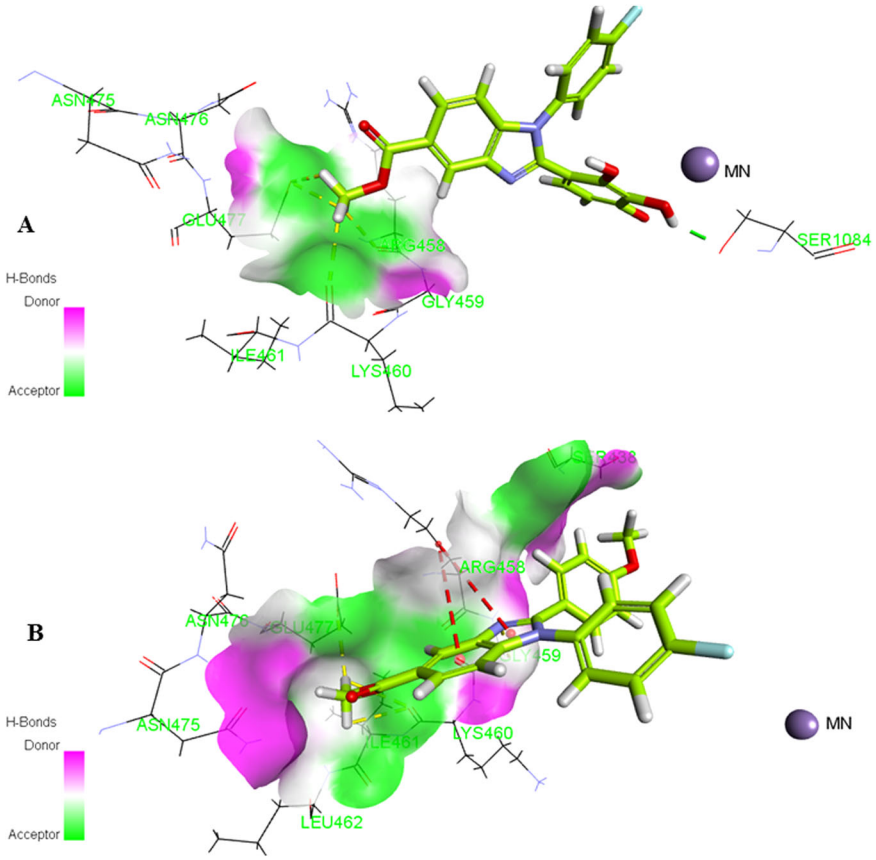



**Fig. 2** The orientation of **5r (a)** and **5h (b)** in the active site of topoisomerase II DNA-gyrase. Ligands are shown as sticks (*lemon color*) and amino acids of the protein as lines. *Green dotted lines* represent classical H-bonds, *yellow* dotted lines, non-classical H-bonds and *red dotted lines* hydrophobic interactions (color figure online)

assay measures the ability to scavenge free radicals in situ (Murthy et al. 2012). The trioxygenated compounds **5r** ($IC_{50}$ of 306.71 µM) and **5p** ($IC_{50}$ of 386.55 µM) with hydroxy and methoxy groups showed antioxidant activity comparable to ascorbic acid (264.50 µM). Two other compounds, **5q** ($IC_{50}$ of 1129.0 µM) with dihydroxy moieties and **5j** with a monohydroxy group ($IC_{50}$ of 1326.93 µM) was approximately four times worse than ascorbic acid. All the other compounds were five times or more, worse than ascorbic acid. Thus, the only compounds that could be considered as having antioxidant potential were **5r** and **5p**. Other methods to test for antioxidant activity was not carried out as this was only a preliminary antioxidant test for these compounds.

## Molecular docking

Several drugs including ciprofloxacin and lomefloxacin exhibit antibacterial action by inhibiting bacterial type II topoisomerases (topoisomerase IV and DNA-gyrase) (Mitscher 2005). In order to support the antibacterial results and gain some insight into the binding characteristics of the compounds with bacterial proteins, two representative compounds (RCs), **5r** (most potent) and **5h** (least potent) were docked into the empty active site of topoisomerase II DNA-gyrase. 3D-coordinates of the enzyme co-crystallized with its inhibitor, ciprofloxacin (pdb id: 2XCT (Bax et al. 2010)), were downloaded from the RCSB protein data bank. A binding sphere covering the active site was generated and docking of the RCs was performed using the CDOCKER module (Wu et al. 2003) implemented in Discovery Studio *ver* 4.0 (Accelrys) along with ciprofloxacin.

The binding affinity of the docked compounds was assessed by computing their binding energies (BEs) [$E_{complex}$-($E_{protein}$+$E_{ligand}$)]. The more negative the BE, the stronger the interaction with the enzyme. Compound **5r** exhibited stronger interaction (BE = −124.2 kcal $mol^{-1}$) with the enzyme in comparison to **5h** (BE = −24.3 kcal $mol^{-1}$). This consequently supports the experimental results as **5r** showed the highest MBC in the experimental assay performed. The BE of **5r** had a difference of approximately 30 kcal $mol^{-1}$ from the computed BE of ciprofloxacin (−156 kcal $mol^{-1}$).

The best docked pose of **5r** and **5h** in the active site of topoisomerase II DNA-gyrase (Fig. 2) showed that both the RCs interacted with similar amino acid residues (Arg458, Lys460 and Glu477). This was also observed for ciprofloxacin (Fig. 3). Compound **5r** (Fig. 2a), the most active antibacterial agent, formed a classical hydrogen bond with Ser 1084 (1.98 Å) and four non-classical hydrogen bonds

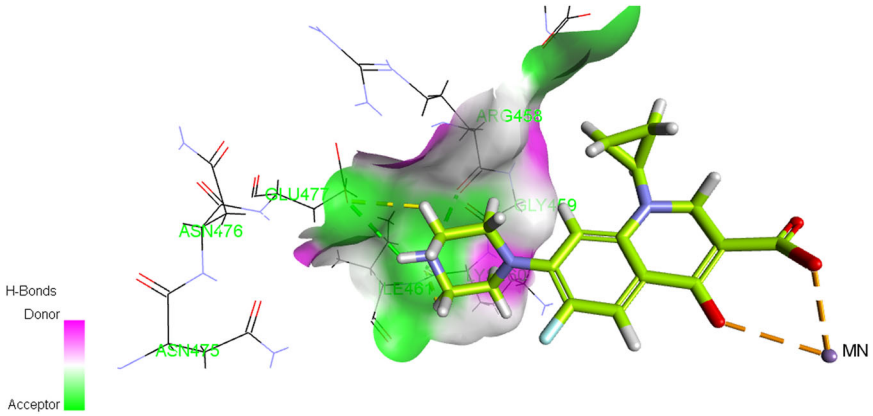



**Fig. 3** The orientation of ciprofloxacin in the active site of topoisomerase II DNA-gyrase. The ligand is shown in sticks (*lemon*) and amino acids of the protein are depicted in line format. Classical H-bonds (*green dotted*), non-classical H-bonds (*yellow dotted*) and co-ordinate bonds (*golden dotted*) (color figure online)

**Table 2** Antimicrobial activity of the synthesized compounds, MBCs in μM

| No. | Gram-positive bacteria | | Gram-negative bacteria | | | Fungus |
|---|---|---|---|---|---|---|
| | S.aureus | MRSA | E.coli | P.aeruginosa | K.pneumoniae | C.albicans |
| **5b** | 268.19 | 536.42 | 321.83 | 536.42 | 107.28 | 214.56 |
| **5e** | 141.49 | 70.73 | 282.98 | 565.98 | 24.32 | 94.33 |
| **5h** | 2492.54 | 1661.69 | 103.85 | 623.12 | 830.85 | 1246.27 |
| **5k** | 803.02 | 401.51 | 200.76 | 250.93 | 301.11 | 803.02 |
| **5l** | 398.18 | 1992.48 | 149.43 | 199.24 | 149.43 | 398.18 |
| **5o** | 1087.38 | 906.13 | 407.76 | 362.46 | 724.92 | 362.46 |
| **5p** | 115.65 | 555.21 | 92.53 | 115.65 | 14.45 | 369.85 |
| **5q** | 206.61 | 439.06 | 232.45 | – | – | 3305.99 |
| **5r** | 74.32 | 446.03 | 49.56 | 37.15 | 25.55 | 421.24 |
| **5s** | – | – | 1109.50 | – | – | 443.81 |
| **5t** | – | – | 348.66 | – | – | 1859.62 |
| **Ampicilin** | 55.89 | 894.36 | 447.18 | 1788.73 | 447.18 | – |
| **Ciprofloxacin** | 1.84 | 7.36 | 1.84 | 1.84 | 3.68 | – |
| **Tioconazole** | – | – | – | – | – | 100.84 |

**Table 3** Antioxidant activity of the synthesized compounds by the DPPH assay ($IC_{50}$ in μM)

| No. | $IC_{50}$ (μM) |
|---|---|
| **5j** | 1326.93 |
| **5p** | 386.55 |
| **5q** | 1129.01 |
| **5r** | 306.71 |
| **Ascorbic Acid** | 264.50 |

**Table 4** Hydrogen bond interactions for **5k**

| D-H…A | d(D-H) | d(H…A) | d(D…A) | <(DHA) |
|---|---|---|---|---|
| C(5b)-H(5b)…O(2)#1 | 0.95 | 2.49 | 3.4101(14) | 162.3 |
| C(8b)-H(8bi)…O(2)#1 | 0.98 | 2.63 | 3.3278(15) | 128.3 |
| C(7)-H(7)…F(1)#2 | 0.95 | 2.59 | 3.1002(13) | 113.6 |
| C(2a)-H(2a)…N(3)#3 | 0.95 | 2.46 | 3.2737(14) | 143.3 |
| C(6a)-H(6a)…N(3)#4 | 0.95 | 2.52 | 3.4611(14) | 171.9 |

with Arg458 (2.61 Å), Lys460 (2.45 Å) and Glu477 (2.71 Å, 2.76 Å). The least active compound (**5h**) displayed three hydrogen bonds with Glu477 (2.61 Å) and Lys460 (3.03 Å, 3.05 Å) in addition to hydrophobic interactions with Arg458 (Fig. 2b). The fewer and relatively longer hydrogen bond distances in **5h** could have led to its weaker binding with the enzyme and in turn lower activity. Ciprofloxacin (the native ligand) fitted nicely into the active site of the enzyme through five hydrogen bonds with Glu477 (2.78 Å, 2.80 Å), Lys460 (2.34 Å, 2.40 Å) and Arg458 (2.14 Å) amino residues and formed another two co-ordinate bonds with $Mn^{2+}$ (Fig. 3). However, both compounds failed to show any interaction with $Mn^{2+}$, which may be responsible for them not being as active as ciprofloxacin. (Tables 2 and 3)

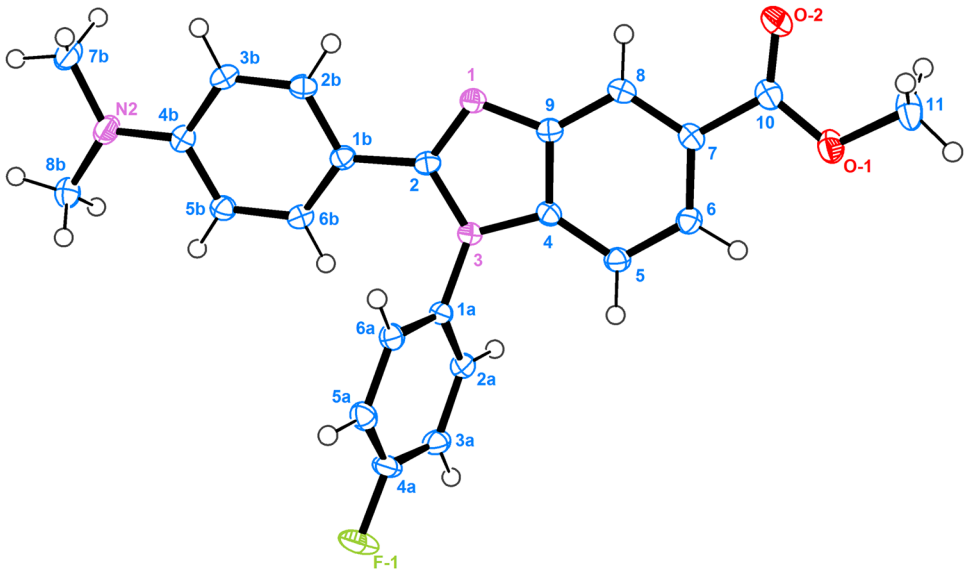



**Fig. 4** Ortep diagram for **5k**

## Crystal structure

The crystal structure of the parent compound, methyl 2-(4-(dimethylamino)phenyl)-1-(4-fluorophenyl)-1*H*-benzo[*d*] imidazole-5-carboxylate (**5k**) was obtained and the refinement data are contained in the Supplementary file (Table S1). Compound **5k** crystallized in the monoclinic P 21/c space group with two molecules in the asymmetric unit. The ester group and *N*-dimethyl group point away from each other and all groups are in the plane except the 4-fluoro phenyl group, which is perpendicular to the benzimidazole moiety on N-1. Weak hydrogen bond interactions (C–H…O, C–H…F and C–H…N) were observed in the crystal structure (Table 4). Further selected bond lengths were described in the supplementary file (Table S2). An ortep diagram of **5k** is given in Fig. 4.

## Conclusion

We have developed an efficient, cost effective and faster route for 2-substituted fluorinated benzimidazoles via a solvent and catalyst free microwave method. Compounds **5p** and **5r** with trioxygenated substitution showed better activity than ampicillin against the microbial strains used in the assays. These two compounds could be good leads for antimicrobial agents and will lead to further trioxygenated substituted benzimidazoles being synthesized in order to improve on the antibacterial activity and possibly find an excellent candidate to be developed into an antiobiotic.

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### Compliance with ethical standards

**Conflict of interest** The authors declare no competing financial interests.

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