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Synthesis and evaluation of antimycobacterial activity of new benzimidazole aminoesters

Yeong Keng Yoon [a], Mohamed Ashraf Ali [a,b,c,*], Ang Chee Wei [a], Tan Soo Choon [a], Rusli Ismail [d]

[a] Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Minden 11800, Penang, Malaysia
[b] New Drug Discovery Research, Department of Medicinal Chemistry, Alwar Pharmacy College, Alwar, Rajasthan 301030, India
[c] New Drug Discovery Research, Department of Medicinal Chemistry, Sunrise University, Alwar, Rajasthan 301030, India
[d] Centre of Excellence for Research in AIDS (CERiA), University Malaya, Minden 59990, Kuala Lumpur, Malaysia

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ABSTRACT

A total of 51 novel benzimidazoles were synthesized by a 4-step reaction starting from basic compound 4-fluoro-3-nitrobenzoic acid under relatively mild reaction conditions. The structure of the novel benzimidazoles was confirmed by mass spectra as well as ^{1}H NMR spectroscopic data. Out of the 51 novel synthesized compounds, 42 of them were screened for their antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ strain using BacTiter-Glo™ Microbial Cell Viability (BTG) method. Results of activity screened using Alamar Blue method was also provided for comparison purposes. Two of the novel benzimidazoles synthesized showed moderately good activity with IC_{50} of less than 15 μM. Compound **5g**, *ethyl 2-(4-(trifluoromethyl)phenyl)-1-(2-morpholinoethyl)-1H-benzo[d]imidazole-5-carboxylate*, was found to be the most active with IC_{50} of 11.52 μM.

1. Introduction

Tuberculosis (TB) is the oldest documented infectious disease. It is a chronic necrotizing bacterial infection with wide variety of manifestations caused by *Mycobacterium tuberculosis*, which has plagued humans throughout recorded and archeological history [1]. The primary site of infection is the lungs, followed by dissemination *via* the circulatory and lymphatic system to secondary sites including the bones, joints, liver and spleen.

In 2010, there were 8.8 million (range, 8.5–9.2 million) incident cases of TB, 1.1 million (range, 0.9–1.2 million) deaths from TB among HIV-negative people and an additional 0.35 million (range, 0.32–0.39 million) death from HIV-associated TB [2]. The introduction of the first line drugs like streptomycin, para-aminosalicylic acid and isoniazid for treatment some 50 years ago has witnessed in a remarkable decline in TB cases all over the world. The active TB is currently treated with a four first-line drug regimen comprising mainly isoniazid, rifampicin, pyrazinamide and ethambutol for a period of at least 6 months [3,4]. However, it did not take long for *M. tuberculosis* to find its way around these compounds, and since the mid of 1980s, the disease has been undergoing a resurgence driven by variety of changes in social, medical and economic factors as well as *M. tuberculosis*' resistance to the above-mentioned drugs itself. Despite extensive research in the last 40 years, no new anti-TB drugs have been introduced into the market by means of passing actual clinical trials [5]. Although a new TB drug, Bedaquiline, was released in USA [6] in December 2012, the trial was based on paradoxical surrogate measure to gain "fast track" approval by USFDA. However, decisions making under such time pressure may lead to unanticipated safety problems as shown by the higher chances of death by patients taking this drug, indicating treatment failure [7].

The benzimidazole nucleus is of significant importance in medicinal chemistry research and many benzimidazole-containing compounds exhibit important biological properties such as antiviral [8], anti-inflammatory [9] and anti-HIV [10]. In the light of the affinity they display toward a variety of enzymes and protein receptors, medicinal chemists thus classify them as "*privileged substructures*" for drug design [11].

Recently, there have been reported work done on utilizing benzimidazole derivatives to counter TB with relatively good results [12,13]; thus further reinforcing our belief that benzimidazole could potentially be a lead compound in our effort to discover new potent anti-TB agents.

* Corresponding author. New Drug Discovery Research, Department of Medicinal Chemistry, Alwar Pharmacy College, Alwar, Rajasthan 301030, India.
E-mail addresses: asraf80med@yahoo.com, asraf80med@rediffmail.com (M.A. Ali).

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In the present paper, we wish to report the synthesis and antimycobacterial activity of novel 2-substituted benzimidazole derivatives.

2. Results and discussion

2.1. Chemistry

The procedure to synthesize benzimidazole derivatives was adopted and modified from literature [14]. Our synthetic study into novel benzimidazoles started with 4-fluoro-3-nitro benzoic acid which was esterified in the presence of catalytic sulfuric acid in ethanol by refluxing for 8 h to afford the ethyl ester **1** in 75% yield. The ethylbenzoate **1** was then treated with various amines (see Experimental section) and DIPEA in dry dichloromethane at room temperature yielded amino compound **2**, which was reduced to the amine **3** using ammonium formate and 10% Pd/C for 3 h to give 60% yield. The structure of 3-amino ethylbenzoate **3** was confirmed by chromatographic analysis.

The phenylenediamine **3** was then refluxed with various substituted bisulfite adduct of aromatic aldehydes [15] in DMF overnight to afford benzimidazole derivatives **5**–**7** in moderate to good yields (38–90%). The structure of the novel benzimidazoles was confirmed by spectroscopic analysis and further unambiguously ascertained by single X-ray crystallographic analysis [16–19]. Among the literature reports available for the synthesis of benzimidazoles by the reaction of phenylenediamine with acid chloride [20], aldehyde [21] and acid [22], we found that access into benzimidazole derivatives *via* this metabisulfite route is efficient, environmental friendly and afforded good to excellent yield of the benzimidazoles.

The ^{1}H NMR spectrum of benzimidazole **5g** showed a singlet at δ 1.43 ppm due to the methyl group. The *N*-methylene protons from the piperazine appeared as a triplet at δ 2.33 ppm while the *O*-methylene showed a triplet at 3.55 ppm. Similar ^{1}H patterns were obtained for other substituted benzimidazoles derivatives. Proton NMR assignment for **5g** was shown as representation for the other compounds in the series (Fig. 1). The ^{13}C NMR spectrum of **5g** which resonated at δ 150.81 and 167.36 ppm is assigned to imine (C═N) and ester carbonyl carbon respectively (Tables 1 and 2).

The mechanism for the formation of the novel benzimidazole derivatives is proposed and summarized in Scheme 1.

2.2. Pharmacology

A total of 51 novel benzimidazole derivatives were synthesized and 42 of them were then analyzed for their antimycobacterial activities against *M. tuberculosis* $H_{37}R_V$ (MTB-H_{37}Rv). Another 9 compounds were obtained in gel-like form. As there might be ambiguity over the trace amount of solvent effect and the consistency of the weight from gel-like compounds, we omitted those compounds from further biological activity testing. Results are shown in Table 3.

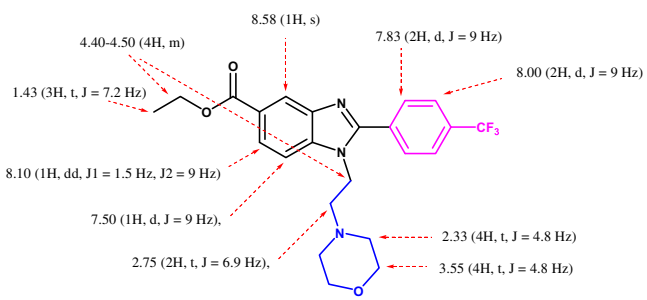


Fig. 1. ^{1}H and ^{13}C NMR chemical shifts of titled compound.

In vitro antimycobacterial activity of the compounds was evaluated against *M. tuberculosis* $H_{37}R_V$ (MTB-H_{37}Rv) in a HTS (High Throughput Screening) assay adapted from the microdilution AlamarBlue (AB) broth method as reported by Collins and Franzblau [23]. For comparison, an alternative method for end-point detection was assessed using the Promega reagent BacTiter-Glo™ Microbial Cell Viability (BTG). The BTG assay is a quantitative ATP assay for bacteria using luciferase production as an end-point detection point. Data was analyzed using the IDBS Activity Base software and the dose response result was analyzed using a four parameter logistic fit to the data (Excel Fit equation 205) with the maximum and minimum locked at 100 and 0. From these curves, EC_{90} and EC_{50} values were calculated. As references, six standard drugs used for TB treatment (Amikacin, Cycloserine, Ethambutol, Isoniazid, Pyrimethamine and Rifampicin) were also evaluated in the assays.

Comparing the three series of substitution at the 2-position of the benzimidazole core, it can be concluded that 4-(2-aminoethyl) morpholine gave the best activity. This could be due to the fact that heterocyclic moiety, such as morpholine group are biologically active. It also showed that electron donating groups somehow give rise to better antimycobacterial activities, which is consistent with those reported in literature [24,25]. Thus, a further modification of the 4-substitution on the sodium bisulfite adduct was carried out to further improve the activity. We synthesized compounds with a wide range of substitution including compounds with electron-donating as well as electron-withdrawing groups. Generally, we found that electron withdrawing group substituents at 4-position in the phenyl ring is important for good activities as shown by **5g**, **5b**, **5e** and **5p**. Of all 42 compounds which have been tested, compound **5g**, *ethyl 2-(4-(trifluoromethyl)phenyl)-1-(2-morpholinoethyl)-1H-benzo[d]imidazole-5-carboxylate* was found to be the most active with IC_{50} of 11.52 μM, IC_{90} of 16.53 μM and MIC of 50 μM respectively. It was followed closely by **5b**, *ethyl 2-(4-bromophenyl)-1-(2-morpholinoethyl)-1H-benzo[d]imidazole-5-carboxylate*, with IC_{50} of 12.54 μM, IC_{90} of 14.48 μM and MIC of 50 μM respectively.

Both these compounds are more active than the standard drugs cycloserine and pyrimethamine. However none of the compounds screened in the present derivatives are found to be more potent than other used standard drugs. This clearly showed that the presence of electron donating group substitution at 4-position of the phenyl ring, caused marked improvement in antimycobacterial activity.

All the compounds were also tested for cytotoxicity (IC_{50}) in VERO cells at concentrations of 62.5 μg/mL. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 nonradioactive cell proliferation assay according to the manufacturer's protocol. All of the active compounds were found to be non-toxic till 62.5 μg/mL.

Encourage by the positive results we have reported here, further modification on the 2-susbstituted position on the benzimidazole core as well as 4-position on the bisulfite adducts as currently in progress in our laboratory.

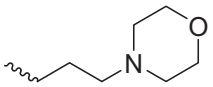
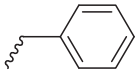
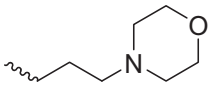
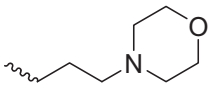
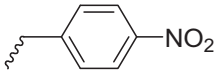
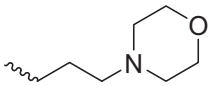
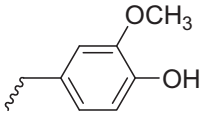
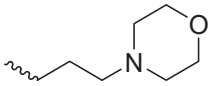
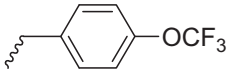
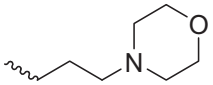
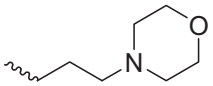
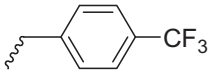
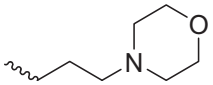
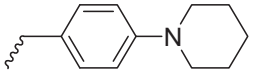
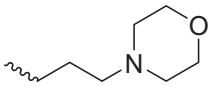
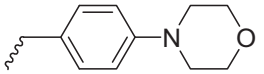
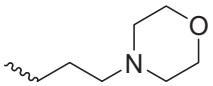
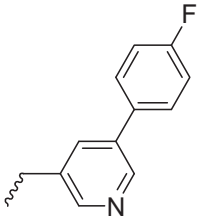
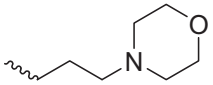
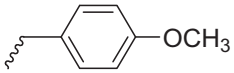
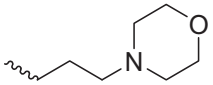
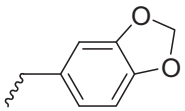
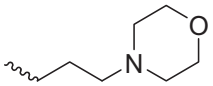
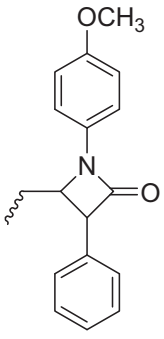
3. Experimental

3.1. Chemistry

All chemicals were supplied by Sigma–Aldrich (U.S.A) and Merck Chemicals (Germany). Purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G) in the

Please cite this article in press as: Y.K. Yoon, et al., Synthesis and evaluation of antimycobacterial activity of new benzimidazole aminoesters, European Journal of Medicinal Chemistry (2013), http://dx.doi.org/10.1016/j.ejmech.2013.06.025

Table 1
Physical properties and analytical results of compounds **5a**–**q**, **6a**–**q** and **7a**–**q**.

Compound	R1	R2	Formula	C/H/N calculated; (C/H/N found)	[M + H]	Yield (%)	M.p (°C)
5a			$C_{22}H_{25}N_3O_3$	69.56%/6.56%/11.11%; (69.42%/6.79%/11.25%)	380.3	50	–
5b			$C_{22}H_{24}N_3O_3Br$	57.67%/5.33%/9.21%; (57.80%/5.23%/9.40%)	458.2	65	205–206
5c			$C_{22}H_{24}N_4O_5$	62.22%/5.67%/13.20%; (62.40%/5.58%/13.22%)	425.2	78	210–211
5d			$C_{23}H_{27}N_3O_5$	64.89%/6.41%/9.90%; (65.01%/6.29%/10.02%)	427.2	70	201–202
5e			$C_{23}H_{24}N_3O_4F_3$	59.60%/5.20%/9.08%; (59.62%/5.20%/9.03%)	464.2	86	187–188
5f			$C_{24}H_{30}N_4O_3$	68.22%/7.21%/13.30%; (68.29%/7.27%/13.23%)	423.3	39	203–204
5g			$C_{23}H_{24}N_3O_3F_3$	61.67%/5.42%/9.37%; (61.66%/5.40%/9.37%)	448.2	85	183–184
5h			$C_{27}H_{34}N_4O_3$	70.12%/7.40%/12.11%; (70.25%/7.45%/12.82%)	463.3	73	–
5i			$C_{26}H_{32}N_4O_4$	67.20%/6.90%/12.21%; (67.20%/6.87%/12.24%)	465.3	77	186–187
5j			$C_{27}H_{27}N_4O_3F$	68.33%/5.67%/11.80%; (68.48%/6.83%/12.60%)	476.2	76	210–211
5k			$C_{23}H_{27}N_3O_4$	67.50%/6.74%/10.32%; (67.60%/6.67%/10.36%)	410.3	68	189–190
5l			$C_{23}H_{25}N_3O_5$	65.19%/6.03%/9.94%; (65.17%/6.08%/10.10%)	424.3	87	201–202
5m			$C_{32}H_{34}N_4O_5$	69.31%/6.20%/10.11%; (69.49%/6.39%/10.01%)	555.3	38	–

(*continued on next page*)

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Table 1 (*continued*)

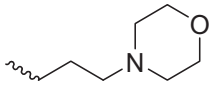
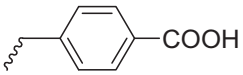
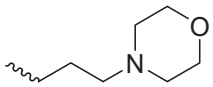
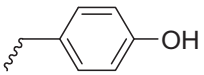
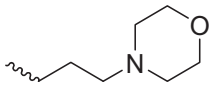
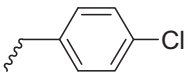
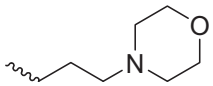
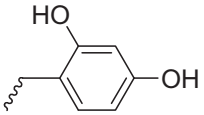
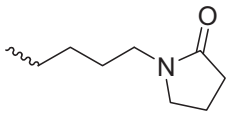
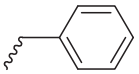
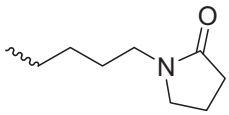
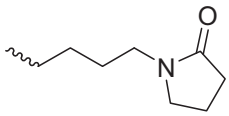
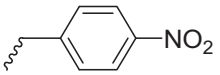
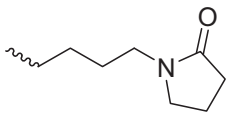
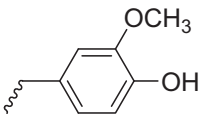
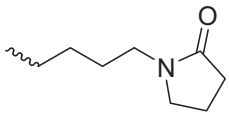
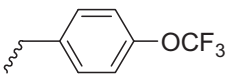
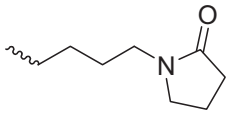
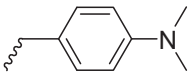
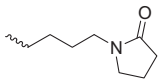
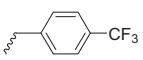
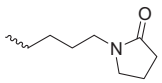
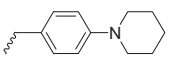
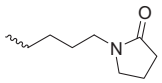
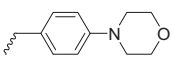
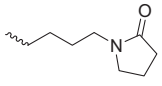
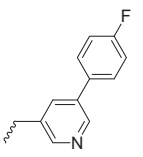
Compound	R1	R2	Formula	C/H/N calculated; (C/H/N found)	[M + H]	Yield (%)	M.p (°C)
5n			$C_{23}H_{25}N_3O_5$	65.19%/6.03%/9.94%; (65.24%/6.13%/10.00%)	424.2	89	215–216
5o			$C_{22}H_{25}N_3O_4$	66.78%/6.42%/10.61%; (66.51%/6.59%/10.53%)	397.2	55	193–194
5p			$C_{22}H_{24}N_3O_3Cl$	63.80%/5.81%/10.21%; (63.84%/5.65%/10.28%)	415.2	63	190–191
5q			$C_{22}H_{25}N_3O_5$	64.24%/6.13%/10.22%; (64.26%/6.15%/10.03%)	412.2	42	–
6a			$C_{23}H_{25}N_3O_3$	70.56%/6.46%/10.71%; (70.52%/6.41%/11.72%)	392.3	79	92–93
6b			$C_{23}H_{24}N_3O_3Br$	58.67%/5.13%/8.91%; (58.90%/5.25%/8.79%)	470.1	77	113–114
6c			$C_{23}H_{24}N_4O_5$	63.32%/5.54%/12.80%; (63.42%/5.57%/12.83%)	437.2	69	121–122
6d			$C_{24}H_{27}N_3O_5$	65.89%/6.21%/9.62%; (66.01%/6.27%/9.67%)	438.3	80	109–110
6e			$C_{24}H_{24}N_3O_4F_3$	60.67%/5.10%/8.78%; (60.69%/5.11%/8.74%)	476.2	90	100–101
6f			$C_{25}H_{30}N_4O_3$	69.12%/7.01%/12.90%; (69.16%/7.13%/12.92%)	435.3	72	112–113
6g			$C_{24}H_{24}N_3O_3F_3$	62.72%/5.33%/9.24%; (62.75%/5.36%/9.25%)	460.2	90	99–100
6h			$C_{28}H_{34}N_4O_3$	70.89%/7.20%/11.80%; (70.91%/7.08%/11.73%)	475.3	86	–
6i			$C_{27}H_{32}N_4O_4$	68.11%/6.82%/11.83%; (68.11%/6.80%/11.79%)	477.3	88	133–134
6j			$C_{28}H_{27}N_4O_3F$	69.11%/5.56%/11.20%; (69.08%/5.80%/11.14%)	487.2	90	140–141

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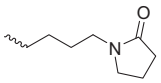
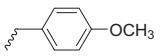
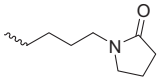
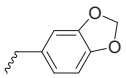
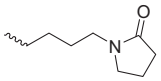
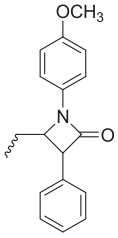
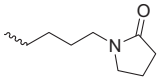
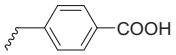
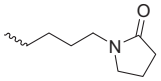
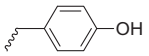
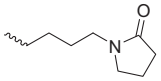
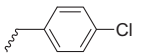
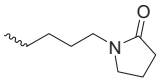
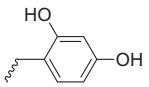
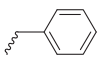
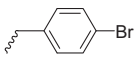
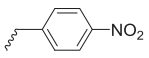
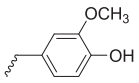
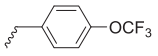
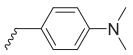
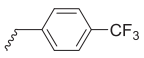
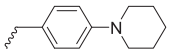
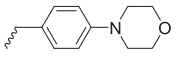
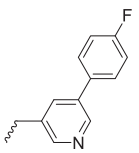
Table 1 (*continued*)

Compound	R1	R2	Formula	C/H/N calculated; (C/H/N found)	[M + H]	Yield (%)	M.p (°C)
6k			$C_{24}H_{27}N_3O_4$	68.40%/6.50%/10.00%; (68.35%/6.50%/10.10%)	422.3	81	100–101
6l			$C_{24}H_{25}N_3O_5$	66.22%/5.82%/9.71%; (66.20%/5.80%/9.81%)	436.2	89	–
6m			$C_{33}H_{34}N_4O_5$	70.03%/6.13%/9.89%; (70.28%/6.29%/9.65%)	567.4	40	–
6n			$C_{24}H_{25}N_3O_5$	66.22%/5.82%/9.71%; (66.21%/5.80%/9.76%)	434.2	82	–
6o			$C_{23}H_{25}N_3O_4$	67.81%/6.21%/10.34%; (67.72%/6.37%/10.33%)	408.2	66	128–129
6p			$C_{23}H_{24}N_3O_3Cl$	64.89%/5.72%/9.91%; (64.85%/5.82%/9.96%)	426.2	76	112–113
6q			$C_{23}H_{25}N_3O_5$	65.12%/6.03%/9.91%; (65.07%/6.15%/9.20%)	424.2	57	146–147
7a	–H		$C_{16}H_{14}N_2O_2$	72.20%/5.30%/10.50%; (72.16%/5.25%/10.58%)	267.1	79	177–178
7b	–H		$C_{16}H_{13}N_2O_2Br$	55.71%/3.80%/8.10%; (55.70%/3.79%/8.07%)	345.0	73	190–191
7c	–H		$C_{16}H_{13}N_3O_4$	61.70%/4.22%/13.53%; (61.70%/4.24%/13.50%)	312.0	75	204–205
7d	–H		$C_{17}H_{16}N_2O_4$	65.40%/5.21%/9.02%; (65.44%/5.23%/9.00%)	313.1	80	228–229
7e	–H		$C_{17}H_{13}N_2O_3F_3$	58.31%/3.71%/8.00%; (58.29%/3.72%/8.07%)	351.0	87	186–187
7f	–H		$C_{18}H_{19}N_3O_2$	69.90%/6.21%/13.62%; (69.95%/6.17%/13.59%)	310.1	84	220–221
7g	–H		$C_{17}H_{13}N_2O_2F_3$	61.11%/3.91%/8.43%; (61.16%/3.85%/8.38%)	335.1	90	183–184
7h	–H		$C_{21}H_{23}N_3O_2$	72.20%/6.61%/11.99%; (72.30%/6.69%/11.91%)	350.2	76	190–191
7i	–H		$C_{20}H_{21}N_3O_3$	68.42%/6.01%/11.98%; (68.41%/6.02%/11.96%)	352.2	77	204–205
7j	–H		$C_{21}H_{16}N_3O_2F$	69.81%/4.51%/11.61%; (69.79%/4.51%/11.60%)	362.2	66	>230

(*continued on next page*)

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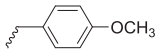
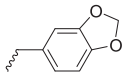
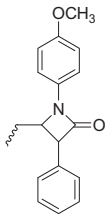
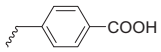
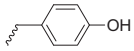
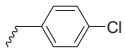
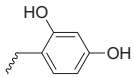
Table 1 (*continued*)

Compound	R1	R2	Formula	C/H/N calculated; (C/H/N found)	[M + H]	Yield (%)	M.p (°C)
7k	–H		$C_{17}H_{16}N_2O_3$	68.90%/5.41%/4.50%; (68.88%/5.50%/4.47%)	297.1	89	188–189
7l	–H		$C_{17}H_{14}N_2O_4$	65.81%/4.60%/9.02%; (65.85%/4.64%/8.91%)	311.0	89	210–211
7m	–H		$C_{26}H_{23}N_3O_4$	70.72%/5.30%/9.51%; (70.62%/5.53%/9.22%)	442.3	42	–
7n	–H		$C_{17}H_{14}N_2O_4$	65.81%/4.60%/9.02%; (65.84%/5.63%/9.08%)	311.0	71	>230
7o	–H		$C_{16}H_{14}N_2O_3$	68.11%/5.00%/9.91%; (68.19%/5.05%/9.82%)	283.1	82	209–210
7p	–H		$C_{16}H_{13}N_2O_2Cl$	63.89%/4.41%/9.32%; (63.90%/4.40%/9.30%)	301.1	85	182–183
7q	–H		$C_{16}H_{14}N_2O_4$	64.41%/4.30%/9.42%; (64.29%/4.39%/9.49%)	299.1	75	>230

solvent system chloroform–methanol (9:1). The spots were located under short (254 nm)/long (365 nm) UV light. Elemental analyses were performed on Perkin Elmer 2400 Series II CHN Elemental Analyzer and were within ±0.3% of the calculated values. ^1H and ^{13}C NMR were performed on Bruker Avance 300 (^1H: 300 MHz, ^{13}C: 75 MHz) spectrometer in $CDCl_3$ for (**5** and **6** series) and CD_3OD (**7** series) using TMS as internal standard. Mass spectra were recorded on Varian 320-MS TQ LC/MS using ESI. Crystal structure analysis was carried out using *Bruker* SMART APEXII CCD area-detector diffractometer. Melting point was performed on Gallenkamp MFB595.010M Melting Point apparatus. Column chromatography purification was done in solvent system chloroform–methanol (9:1) using Silica Gel 60 (0.063–0.200 mm).

3.1.1. Procedure for the preparation of ethyl-4-fluoro-3-nitrobenzoate (*1*)

4-Fluoro-3-nitrobenzoic acid (5 g, 27 mmol) was refluxed in ethanol (50 mL) and concentrated H_2SO_4 (2 mL) for 8 h. After completion of reaction (as evident from TLC), the solvent was evaporated under reduced pressure. The aqueous layer was extracted with ethyl acetate (25 mL × 3). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to yield **1** as cream-colored powder (75%).

3.1.2. General procedure for the preparation of 4-(2-substituted amino)-3-nitro-ethylbenzoate (*2*)

Ethyl-4-fluoro-3-nitrobenzoate, **1** (0.5 g, 2.34 mmol), amine [for **5**: 4-(2-Aminoethyl)morpholine; **6**: 1-(3-Aminopropyl)-2-pyrrolidinone; **7**: Aqueous ammonia solution, 38%] (2.58 mmol) and *N*,*N*-Diisopropylethylamine, DIPEA (0.49 mL, 2.78 mmol) were mixed in dichloromethane (10 mL). The reaction mixture was stirred overnight at room temperature. After completion of reaction (as evident from TLC), the reaction mixture was washed with water (10 mL × 2) followed by 10% Na_2CO_3 solution (10 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford **2** as brown oil/yellow solid.

3.1.3. General procedure for the preparation of ethyl-3-amino-4-(2-substituted amino)benzoate (*3*)

4-(2-Substituted amino)-3-nitro-ethylbenzoate, **2** (1 mmol), ammonium formate (3 mmol) and Pd/C (50 mg) were mixed in ethanol (10 mL). The reaction mixture was refluxed until completion (solution turned colorless). The reaction mixture was then filtered through Celite 545. The filtrate was evaporated under reduced pressure. It was resuspended in ethyl acetate and washed with water, dried over Na_2SO_4 and evaporated to dryness to yield **3** (60%).

3.1.4. General procedure for the preparation of sodium bisulfite adducts of 4-substituted benzaldehyde (*4*)

Appropriate benzaldehyde (10 mmol) was dissolved in ethanol (20 mL). Sodium metabisulfite (15 mmol) in 5 mL water was added in portion over 5 min. The reaction mixture was stirred at room temperature for 1 h and subsequently stirred at 4 °C overnight. The precipitate formed was filtered and dried to afford sodium bisulfite adducts (55%–90%).

3.1.5. General procedure for the preparation of 2-substituted benzimidazole derivatives (*5*–*7*)

Ethyl-3-amino-4-(2-substituted amino)benzoate, **3** (1 mmol) and various sodium bisulfite adducts, **4** (1.5 mmol) were dissolved in DMF (5 mL). The reaction mixture was stirred at 90 °C under N_2 atmosphere for 24–48 h. After completion of reaction (evident by TLC), the reaction mixture was diluted in ethyl acetate (25 mL) and washed with water (10 mL × 3). The organic layer was collected, dried over Na_2SO_4 and evaporated under reduced pressure to afford crude products. Final compounds **5**–**7** were obtained in 38–90% yields after recrystallization from ethanol or column purification.

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Table 2
^{1}H NMR and ^{13}C NMR results for compounds **5a**–**q**, **6a**–**q** and **7a**–**q**.

Compound	NMR (δ ppm)
5a	**^{1}H NMR**: 1.43 (3H, t, J = 7.2 Hz), 2.38 (4H, t, J = 4.8 Hz), 2.74 (2H, t, J = 6.9 Hz), 3.62 (4H, t, J = 4.8 Hz), 4.30–4.50 (4H, m), 6.90–7.10 (5H, m), 7.48 (1H, d, J = 8.4 Hz), 8.10 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 8.4 Hz), 8.55 (1H, s) **^{13}C NMR**: 14.81, 43.78, 54.33, 57.79, 61.26, 67.12, 110.09, 122.64, 124.70, 125.43, 128.70, 129.2, 129.18, 130.48, 139.12, 143.08, 156.18, 167.48
5b	**^{1}H NMR**: 1.44 (3H, t, J = 6.9 Hz), 2.25 (4H, t, J = 4.8 Hz), 2.66 (2H, t, J = 6.6 Hz), 3.45 (4H, t, J = 4.8 Hz), 4.44 (2H, q, J = 6.9 Hz), 4.54 (2H, t, J = 6.6), 7.66 (2H, d, J = 8.4 Hz), 7.75 (1H, d, J = 8.4 Hz), 7.90 (2H, d, J = 8.4 Hz), 8.08 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 8.4 Hz), 8.38 (1H, s) **^{13}C NMR**: 14.38, 42.79, 53.90, 57.49, 60.93, 66.69, 109.72, 122.44, 124.57, 124.73, 125.36, 129.15, 131.00, 132.08, 138.77, 142.73, 143.08, 154.62, 167.00
5c	**^{1}H NMR**: 1.45 (3H, t, J = 6.9 Hz), 2.34 (4H, t, J = 4.8 Hz), 2.78 (2H, t, J = 6.9 Hz), 3.55 (4H, t, J = 4.8 Hz), 4.40–4.50 (4H, m), 7.52 (1H, d, J = 9 Hz), 8.10–8.20 (3H, m), 8.44 (2H, d, J = 9 Hz), 8.59 (1H, s) **^{13}C NMR**: 14.38, 42.77, 53.86, 57.49, 60.51, 66.61, 109.96, 122.50, 125.56, 125.88, 126.18, 129.72, 129.99, 138.68, 142.51, 154.25, 167.03
5d	**^{1}H NMR**: 1.44 (3H, t, J = 7.2 Hz), 2.38 (4H, t, J = 4.8 Hz), 2.75 (2H, t, J = 6.9 Hz), 3.61 (4H, t, J = 4.8 Hz), 3.97 (3H, s), 4.40–4.50 (4H, m), 7.04 (1H, d, J = 8.1 Hz), 7.25 (1H, dd, $J1$ = 1.8 Hz, $J2$ = 8.1 Hz), 7.36 (1H, s), 7.46 (1H, d, J = 8.1 Hz), 8.06 (1H, dd, $J1$ = 1.8 Hz, $J2$ = 8.1 Hz), 8.55 (1H, s) **^{13}C NMR**: 14.21, 41.96, 54.30, 57.73, 59.20, 61.68, 67.27, 111.29, 116.37, 122.30, 125.73, 126.50, 131.76, 148.97, 150.05, 152.10, 167.52
5e	**^{1}H NMR**: 1.43 (3H, t, J = 7.2 Hz), 2.33 (4H, t, J = 4.8 Hz), 2.75 (2H, t, J = 6.9 Hz), 3.55 (4H, t, J = 4.8 Hz), 4.40–4.50 (4H, m), 7.60 (1H, d, J = 9 Hz), 7.82 (2H, d, J = 9 Hz), 7.99 (2H, d, J = 9 Hz), 8.12 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 9 Hz), 8.57 (1H, s) **^{13}C NMR**: 14.38, 42.79, 53.90, 57.49, 60.93, 66.69, 109.72, 122.44, 124.57, 124.73, 125.36, 129.15, 131.00, 132.08, 138.77, 142.73, 143.08, 154.62, 167.00
5f	**^{1}H NMR**: 1.44 (3H, t, J = 7.2 Hz), 2.36 (4H, t, J = 4.8 Hz), 2.80 (2H, t, J = 6.9 Hz), 2.90 (6H, s), 3.54 (4H, t, J = 4.8 Hz), 4.40–4.50 (4H, m), 6.65 (2H, d, J = 8.4 Hz), 7.25 (1H, d, J = 8.4 Hz), 7.50 (2H, d, J = 8.4 Hz), 8.00 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 8.4 Hz), 8.56 (1H, s) **^{13}C NMR**: 14.45, 40.20, 42.30, 54.11, 57.66, 60.79, 66.82, 109.09, 120.58, 122.70, 122.93, 123.19, 129.50, 130.75, 131.80, 135.74, 142.03, 153.68, 167.67
5g	**^{1}H NMR**: 1.43 (3H, t, J = 7.2 Hz), 2.33 (4H, t, J = 4.8 Hz), 2.75 (2H, t, J = 6.9 Hz), 3.55 (4H, t, J = 4.8 Hz), 4.40–4.50 (4H, m), 7.50 (1H, d, J = 9 Hz), 7.83 (2H, d, J = 9 Hz), 8.00 (2H, d, J = 9 Hz), 8.10 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 9 Hz), 8.58 (1H, s) **^{13}C NMR**: 14.80, 43.71, 54.22, 57.89, 61.28, 67.01, 110.17, 121.54, 122.80, 125.00, 125.73, 127.89, 129.33, 131.64, 139.05, 143.02, 150.81, 167.36
5h	**^{1}H NMR**: 1.40–1.50 (9H, m), 2.36 (4H, t, J = 4.8 Hz), 2.70 (2H, t, J = 6.6 Hz), 2.80 (4H, t, J = 6.6 Hz), 3.55 (4H, t, J = 4.8 Hz), 4.40–4.50 (4H, m), 6.65 (2H, d, J = 8.4 Hz), 7.25 (1H, d, J = 8.4 Hz), 7.50 (2H, d, J = 8.4 Hz), 8.02 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 8.4 Hz), 8.58 (1H, s) **^{13}C NMR**: 14.63, 25.35, 26.59, 42.75, 51.24, 53.40, 56.78, 60.89, 66.80, 109.18, 117.50, 121.69, 122.09, 129.48, 130.33, 131.72, 135.84, 142.56, 152.98, 167.50
5i	**^{1}H NMR**: 1.45 (3H, t, J = 6.9 Hz), 2.47 (4H, t, J = 4.8 Hz), 2.82 (2H, t, J = 6.6 Hz), 3.29 (4H, t, J = 4.5 Hz), 3.70 (4H, t, J = 4.8 Hz), 3.92 (4H, t, J = 4.5 Hz), 4.40–4.60 (4H, m), 7.04 (2H, d, J = 9 Hz), 7.49 (1H, d, J = 9 Hz), 7.75 (2H, d, J = 9 Hz), 8.07 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 9 Hz), 8.56 (1H, s) **^{13}C NMR**: 14.44, 45.46, 53.77, 56.75, 61.06, 63.40, 66.99, 110.20, 118.30, 122.04, 122.28, 123.05, 129.38, 130.56, 132.03, 135.97, 142.57, 153.03, 167.65
5j	**^{1}H NMR**: 1.45 (3H, t, J = 7.2 Hz), 2.36 (4H, t, J = 4.8 Hz), 2.77 (2H, t, J = 6.9 Hz), 3.57 (4H, t, J = 4.8 Hz), 4.40–4.50 (4H, m), 7.03 (2H, d, J = 9.6 Hz), 7.40–7.50 (3H, m), 8.10–8.20 (2H, m), 8.60 (1H, s), 8.77 (2H, s) **^{13}C NMR**: 14.50, 42.71, 54.08, 57.66, 61.05, 67.09, 111.13, 120.52, 122.24, 124.57, 125.63, 129.10, 132.05, 132.28, 133.40, 149.86, 153.86, 162.97, 167.85
5k	**^{1}H NMR**: 1.44 (3H, t, J = 6.9 Hz), 2.36 (4H, t, J = 4.8 Hz), 2.74 (2H, t, J = 6.6 Hz), 3.59 (4H, t, J = 4.8 Hz), 3.91 (3H, s), 4.30–4.50 (4H, m), 7.06 (2H, d, J = 9 Hz), 7.45 (1H, d, J = 9 Hz), 7.76 (2H, d, J = 9 Hz), 8.04 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 9 Hz), 8.54 (1H, s) **^{13}C NMR**: 14.59, 42.61, 53.68, 57.47, 59.20, 61.67, 66.89, 108.15, 109.29, 120.12, 121.80, 150.04, 151.61, 167.68
5l	**^{1}H NMR**: 1.45 (3H, t, J = 7.2 Hz), 2.37 (4H, t, J = 4.8 Hz), 2.76 (2H, t, J = 6.9 Hz), 3.60 (4H, t, J = 4.8 Hz), 4.40–4.50 (4H, m), 6.10 (2H, s), 7.30 (1H, d, J = 9 Hz), 7.45–7.55 (3H, m), 8.06 (1H, d, J = 9 Hz), 8.53 (1H, s) **^{13}C NMR**: 14.60, 41.43, 54.30, 57.71, 61.05, 66.73, 102.83, 108.10, 108.64, 109.57, 109.85, 121.10, 122.80, 124.42, 125.86, 149.97, 151.45, 162.97, 167.67
5m	**^{1}H NMR**: 1.44 (3H, t, J = 7.2 Hz), 2.33 (4H, t, J = 4.8 Hz), 2.75 (2H, t, J = 6.9 Hz), 3.55 (4H, t, J = 4.8 Hz), 3.73 (3H, s), 4.40–4.50 (4H, m), 4.74 (1H, d, J = 6.6 Hz), 5.23 (1H, d, J = 6.6 Hz), 6.82 (2H, d, J = 9 Hz), 6.99 (2H, d, J = 9 Hz), 7.00–7.30 (5H, m), 7.50 (1H, d, J = 9 Hz), 8.10 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 9 Hz), 8.58 (1H, s) **^{13}C NMR**: 14.53, 42.92, 52.10, 53.89, 56.09, 57.88, 61.00, 61.74, 67.09, 112.35, 114.44, 125.30, 125.70, 128.81, 129.86, 143.95, 153.80, 167.79, 168.93
5n	**^{1}H NMR**: 1.44 (3H, t, J = 7.2 Hz), 2.68 (4H, t, J = 4.8 Hz), 3.01 (2H, t, J = 4.8 Hz), 3.79 (4H, t, J = 4.8 Hz), 4.46 (2H, q, J = 7.2 Hz), 4.64 (2H, t, J = 4.8 Hz), 7.50 (1H, d, J = 9 Hz), 7.82 (2H, d, J = 9 Hz), 8.10–8.20 (3H, m), 8.60 (1H, s) **^{13}C NMR**: 14.46, 42.78, 53.84, 57.49, 60.48, 66.60, 110.05, 112.51, 124.13, 125.09, 128.73, 129.13, 129.96, 130.17, 134.95, 143.85, 167.81, 169.04
5o	**^{1}H NMR**: 1.45 (3H, t, J = 7.2 Hz), 2.37 (4H, t, J = 4.8 Hz), 2.73 (2H, t, J = 6.9 Hz), 3.60 (4H, t, J = 4.8 Hz), 4.30–4.50 (4H, m), 6.88 (1H, d, J = 8.4 Hz), 7.47 (2H, d, J = 8.4 Hz), 7.59 (2H, d, J = 8.4 Hz), 8.08 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 8.4 Hz), 8.54 (1H, s) **^{13}C NMR**: 14.60, 42.62, 53.74, 57.50, 62.00, 67.03, 107.83, 109.87, 110.10, 111.04, 123.05, 126.59, 153.40, 157.70, 167.98
5p	**^{1}H NMR**: 1.44 (3H, t, J = 6.9 Hz), 2.26 (4H, t, J = 4.8 Hz), 2.68 (2H, t, J = 6.6 Hz), 3.46 (4H, t, J = 4.8 Hz), 4.42 (2H, q, J = 6.9 Hz), 4.54 (2H, t, J = 6.6), 7.66 (2H, d, J = 8.4 Hz), 7.73 (1H, d, J = 8.4 Hz), 7.84 (2H, d, J = 8.4 Hz), 8.07 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 8.4 Hz), 8.40 (1H, s) **^{13}C NMR**: 14.39, 42.70, 53.80, 57.11, 60.90, 65.69, 109.95, 122.54, 124.67, 125.92, 126.13, 127.45, 137.97, 142.72, 154.53, 167.50
5q	**^{1}H NMR**: 1.43 (3H, t, J = 7.2 Hz), 2.38 (4H, t, J = 4.8 Hz), 2.75 (2H, t, J = 6.9 Hz), 3.60 (4H, t, J = 4.8 Hz), 4.40–4.50 (4H, m), 6.66 (1H, s), 6.85 (1H, dd, $J1$ = 1.8 Hz, $J2$ = 8.1 Hz), 7.44 (1H, d, J = 8.1 Hz), 7.50 (1H, d, J = 8.1 Hz), 8.16 (1H, dd, $J1$ = 1.8 Hz, $J2$ = 8.1 Hz), 8.57 (1H, s) **^{13}C NMR**: 14.57, 42.60, 53.74, 57.45, 61.78, 67.62, 106.85, 108.95, 111.10, 112.83, 118.92, 120.89, 122.40, 125.33, 130.37, 153.60, 158.74, 168.00
6a	**^{1}H NMR**: 1.43 (3H, t, J = 7.2 Hz), 2.10–2.30 (4H, m), 2.55 (2H, t, J = 5.7 Hz), 3.40 (2H, t, J = 4.8 Hz), 3.50 (2H, t, J = 5.7 Hz), 4.30–4.50 (4H, m), 6.90–7.10 (5H, m), 7.48 (1H, d, J = 8.4 Hz), 8.10 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 8.4 Hz), 8.55 (1H, s) **^{13}C NMR**: 14.78, 18.24, 28.07, 31.15, 40.22, 42.93, 47.28, 61.28, 109.80, 122.78, 124.86, 125.53, 129.29, 129.63, 130.43, 130.59, 138.97, 143.13, 155.51, 167.44, 175.71
6b	**^{1}H NMR**: 1.45 (3H, t, J = 6.9 Hz), 2.10–2.30 (4H, m), 2.55 (2H, t, J = 5.7 Hz), 2.66 (2H, t, J = 6.6 Hz), 3.40 (2H, t, J = 4.8 Hz), 3.50 (2H, t, J = 5.7 Hz), 4.46 (2H, q, J = 6.9 Hz), 4.54 (2H, t, J = 6.6), 7.66 (2H, d, J = 8.4 Hz), 7.75 (1H, d, J = 8.4 Hz), 7.90 (2H, d, J = 8.4 Hz), 8.08 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 8.4 Hz), 8.40 (1H, s) **^{13}C NMR**: 14.58, 18.17, 28.04, 30.16, 40.23, 41.79, 46.4, 61.03, 108.10, 122.65, 124.78, 125.40, 126.19, 128.77, 129.21, 129.82, 130.56, 139.11, 143.13, 156.25, 167.57, 174.99
6c	**^{1}H NMR**: 1.45 (3H, t, J = 6.9 Hz), 2.10–2.30 (4H, m), 2.52 (2H, t, J = 5.7 Hz), 3.35 (2H, t, J = 4.8 Hz), 3.46 (2H, t, 5.7 Hz), 4.30–4.50 (4H, m), 7.52 (1H, d, J = 9 Hz), 8.10–8.20 (3H, m), 8.44 (2H, d, J = 9 Hz), 8.59 (1H, s) **^{13}C NMR**: 14.58, 18.23, 28.56, 28.68, 31.01, 38.55, 41.37, 46.79, 61.50, 109.96, 122.50, 125.56, 125.88, 126.18, 129.72, 129.99, 138.68, 142.51, 154.25, 167.03, 175.01
6d	**^{1}H NMR**: 1.44 (3H, t, J = 7.2 Hz), 2.10–2.30 (4H, m), 2.67 (2H, t, J = 5.7 Hz), 3.30 (2H, t, J = 4.8 Hz), 3.68 (2H, t, 5.7 Hz), 3.97 (3H, s), 4.30–4.50 (4H, m), 7.04 (1H, d, J = 8.1 Hz), 7.25 (1H, dd, $J1$ = 1.8 Hz, $J2$ = 8.1 Hz), 7.36 (1H, s), 7.46 (1H, d, J = 8.1 Hz), 8.06 (1H, dd, $J1$ = 1.8 Hz, $J2$ = 8.1 Hz), 8.60 (1H, s) **^{13}C NMR**: 14.70, 18.14, 28.17, 30.93, 40.05, 41.93, 47.22, 59.20, 61.68, 111.29, 116.37, 122.30, 125.73, 126.50, 131.76, 148.97, 150.05, 152.10, 167.50, 175.03
6e	**^{1}H NMR**: 1.44 (3H, t, J = 7.2 Hz), 2.10–2.30 (4H, m), 2.66 (2H, t, J = 5.7 Hz), 3.33 (2H, t, J = 4.8 Hz), 3.56 (2H, t, 5.7 Hz), 4.40–4.50 (4H, m), 7.60 (1H, d, J = 9 Hz), 7.82 (2H, d, J = 9 Hz), 7.99 (2H, d, J = 9 Hz), 8.12 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 9 Hz), 8.57 (1H, s) **^{13}C NMR**: 14.71, 18.03, 28.15, 31.00, 40.02, 42.80, 46.17, 61.74, 110.15, 121.18, 122.80, 124.99, 127.88, 129.26, 131.59, 139.06, 143.04, 150.82, 154.74, 167.37, 174.93

(*continued on next page*)

Table 2 (*continued*)

Compound	NMR (δ ppm)
6f	^{1}**H NMR**: 1.44 (3H, t, J = 6.9 Hz), 2.10–2.30 (4H, m), 2.59 (2H, t, J = 5.4 Hz), 2.85 (6H, s), 3.40 (2H, t, J = 4.8 Hz), 3.55 (2H, t, 5.4 Hz), 4.40–4.50 (4H, m), 6.65 (2H, d, J = 8.4 Hz), 7.25 (1H, d, J = 8.4 Hz), 7.50 (2H, d, J = 8.4 Hz), 8.00 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 8.4 Hz), 8.60 (1H, s) ^{13}**C NMR**: 14.74, 17.99, 27.86, 31.11, 39.39, 40.25, 42.92, 47.68, 60.80, 109.09, 120.58, 122.70, 122.93, 123.19, 129.50, 130.75, 131.80, 135.74, 142.03, 153.68, 167.76, 175.05
6g	^{1}**H NMR**: 1.43 (3H, t, J = 7.2 Hz), 2.10–2.30 (4H, m), 2.73 (2H, t, J = 5.7 Hz), 3.55 (2H, t, J = 4.8 Hz), 3.70 (2H, t, 5.7 Hz), 4.40–4.50 (4H, m), 7.50 (1H, d, J = 9 Hz), 7.83 (2H, d, J = 9 Hz), 8.00 (2H, d, J = 9 Hz), 8.10 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 9 Hz), 8.89 (1H, s) ^{13}**C NMR**: 14.78, 18.01, 28.12, 31.00, 40.01, 42.76, 46.15, 61.27, 110.17, 121.54, 122.80, 125.00, 125.73, 127.89, 129.33, 131.64, 139.05, 143.02, 150.81, 167.36, 175.10
6h	^{1}**H NMR**: 1.45–1.55 (9H, m), 2.10–2.30 (4H, m), 2.60–2.80 (6H, m), 3.37 (2H, t, J = 4.8 Hz), 3.50 (2H, t, 5.4 Hz), 4.40–4.50 (4H, m), 6.65 (2H, d, J = 8.4 Hz), 7.25 (1H, d, J = 8.4 Hz), 7.50 (2H, d, J = 8.4 Hz), 8.02 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 8.4 Hz), 8.58 (1H, s) ^{13}**C NMR**: 14.64, 18.09, 25.35, 26.59, 27.85, 31.13, 39.28, 42.85, 47.67, 60.90, 109.18, 117.50, 121.69, 122.09, 129.48, 130.33, 131.72, 135.84, 142.56, 152.98, 167.52, 175.02
6i	^{1}**H NMR**: 1.44 (3H, t, J = 6.9 Hz), 2.10–2.30 (4H, m), 2.62 (2H, t, J = 5.7 Hz), 3.28 (4H, t, J = 4.8 Hz), 3.40 (2H, t, J = 5.7 Hz), 3.58 (2H, t, J = 5.7 Hz), 3.92 (4H, t, J = 4.5 Hz), 4.40–4.60 (4H, m), 7.04 (2H, d, J = 9 Hz), 7.49 (1H, d, J = 9 Hz), 7.75 (2H, d, J = 9 Hz), 8.07 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 9 Hz), 8.53 (1H, s) ^{13}**C NMR**: 14.54, 18.14, 28.07, 31.15, 40.61, 42.15, 47.70, 61.06, 63.40, 110.20, 118.30, 122.04, 122.28, 123.05, 129.38, 130.56, 132.03, 135.97, 142.57, 153.03, 167.66, 174.98
6j	^{1}**H NMR**: 1.44 (3H, t, J = 7.2 Hz), 2.10–2.30 (4H, m), 2.71 (2H, t, J = 5.7 Hz), 3.30 (4H, t, J = 5.7 Hz), 3.50 (2H, t, J = 5.7 Hz), 4.40–4.50 (4H, m), 7.03 (2H, d, J = 9.6 Hz), 7.40–7.50 (3H, m), 8.10–8.20 (2H, m), 8.60 (1H, s), 8.70 (2H, s) ^{13}**C NMR**: 14.50, 18.08, 27.98, 30.95, 40.11, 42.00, 48.03, 61.06, 111.14, 120.52, 122.24, 124.57, 125.63, 129.10, 132.05, 132.28, 133.40, 149.86, 153.86, 162.97, 167.85, 175.11
6k	^{1}**H NMR**: 1.45 (3H, t, J = 6.9 Hz), 2.10–2.30 (4H, m), 2.65 (2H, t, J = 5.4 Hz), 3.35 (2H, t, J = 5.4 Hz), 3.52 (2H, t, J = 5.4 Hz), 3.92 (3H, s), 4.30–4.50 (4H, m), 7.06 (2H, d, J = 9 Hz), 7.45 (1H, d, J = 9 Hz), 7.76 (2H, d, J = 9 Hz), 8.04 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 9 Hz), 8.54 (1H, s) ^{13}**C NMR**: 14.62, 18.11, 28.04, 30.99, 40.16, 41.98, 47.66, 59.21, 61.67, 108.15, 109.29, 120.12, 121.80, 150.04, 151.62, 167.70
6l	^{1}**H NMR**: 1.43 (3H, t, J = 7.2 Hz), 2.10–2.30 (4H, m), 2.60 (2H, t, J = 5.7 Hz), 3.67 (2H, t, J = 5.7 Hz), 3.87 (2H, t, 5.7 Hz), 4.40–4.60 (4H, m), 6.11 (2H, s), 7.30 (1H, d, J = 9 Hz), 7.45–7.55 (3H, m), 8.06 (1H, d, J = 9 Hz), 8.50 (1H, s) ^{13}**C NMR**: 14.60, 18.16, 28.07, 31.02, 39.22, 41.82, 46.90, 61.05, 102.83, 108.10, 108.64, 109.57, 109.85, 121.10, 122.80, 124.42, 125.86, 149.97, 151.45, 162.97, 167.67, 175.04
6m	^{1}**H NMR**: 1.44 (3H, t, J = 7.2 Hz), 2.10–2.30 (4H, m), 2.43 (2H, t, J = 5.7 Hz), 3.20 (2H, t, J = 5.7 Hz), 3.56 (4H, t, J = 5.7 Hz), 3.73 (3H, s), 4.30–4.50 (4H, m), 4.74 (1H, d, J = 6.6 Hz), 5.23 (1H, d, J = 6.6 Hz), 6.82 (2H, d, J = 9 Hz), 6.99 (2H, d, J = 9 Hz), 7.00–7.30 (5H, m), 7.50 (1H, d, J = 9 Hz), 8.10 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 9 Hz), 8.62 (1H, s) ^{13}**C NMR**: 14.59, 18.17, 27.99, 31.05, 39.94, 41.98, 47.17, 52.08, 56.09, 61.00, 61.74, 112.32, 114.11, 125.30, 125.70, 128.81, 129.86, 143.95, 153.80, 167.79, 168.93, 175.04
6n	^{1}**H NMR**: 1.45 (3H, t, J = 7.2 Hz), 2.10–2.30 (4H, m), 2.68 (2H, t, J = 5.7 Hz), 3.69 (2H, t, J = 5.7 Hz), 3.80 (2H, t, J = 5.7 Hz), 4.05 (2H, t, J = 5.7 Hz), 4.47 (2H, q, J = 7.2 Hz), 7.50 (1H, d, J = 9 Hz), 7.82 (2H, d, J = 9 Hz), 8.10–8.20 (3H, m), 8.62 (1H, s) ^{13}**C NMR**: 14.48, 18.20, 28.13, 31.10, 41.01, 42.93, 47.52, 60.60, 110.05, 112.51, 124.13, 125.09, 128.73, 129.13, 129.96, 130.17, 134.95, 143.85, 167.81, 169.04, 175.12
6o	^{1}**H NMR**: 1.45 (3H, t, J = 7.2 Hz), 2.10–2.30 (4H, m), 2.65 (2H, t, J = 5.4 Hz), 3.40 (4H, t, J = 5.4 Hz), 3.55 (2H, t, J = 5.4 Hz), 4.30–4.50 (4H, m), 6.88 (1H, d, J = 8.4 Hz), 7.47 (2H, d, J = 8.4 Hz), 7.59 (2H, d, J = 8.4 Hz), 8.08 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 8.4 Hz), 8.56 (1H, s) ^{13}**C NMR**: 14.62, 18.29, 28.75, 32.01, 41.23, 43.43, 48.28, 62.02, 107.83, 109.87, 110.10, 111.04, 123.05, 126.59, 153.40, 157.70, 167.99, 175.16
6p	^{1}**H NMR**: 1.45 (3H, t, J = 6.9 Hz), 2.10–2.30 (4H, m), 2.64 (2H, t, J = 5.7 Hz), 3.41 (2H, t, J = 5.7 Hz), 3.70 (2H, t, J = 5.7 Hz), 4.46 (2H, q, J = 6.9 Hz), 4.54 (2H, t, J = 6.6), 7.66 (2H, d, J = 8.4 Hz), 7.73 (1H, d, J = 8.4 Hz), 7.84 (2H, d, J = 8.4 Hz), 8.07 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 8.4 Hz), 8.40 (1H, s) ^{13}**C NMR**: 14.40, 18.13, 27.97, 30.75, 39.96, 42.03, 46.88, 61.01, 109.93, 122.44, 124.67, 125.92, 126.13, 127.45, 137.97, 142.72, 154.53, 167.50, 174.99
6q	^{1}**H NMR**: 1.43 (3H, t, J = 7.2 Hz), 2.10–2.30 (4H, m), 2.55 (2H, t, J = 5.7 Hz), 3.20 (4H, t, J = 5.7 Hz), 3.40 (2H, t, 5.7 Hz), 4.30–4.50 (4H, m), 6.66 (1H, s), 6.85 (1H, dd, $J1$ = 1.8 Hz, $J2$ = 8.1 Hz), 7.44 (1H, d, J = 8.1 Hz), 7.50 (1H, d, J = 8.1 Hz), 8.16 (1H, dd, $J1$ = 1.8 Hz, $J2$ = 8.1 Hz), 8.57 (1H, s) ^{13}**C NMR**: 14.58, 18.06, 27.85, 31.00, 40.01, 41.93, 46.97, 61.78, 106.85, 108.95, 111.10, 112.83, 118.92, 120.89, 122.40, 125.33, 130.37, 153.60, 158.74, 167.97, 175.00
7a	^{1}**H NMR**: 1.43 (3H, t, J = 7.2 Hz), 4.60 (2H, q, J = 7.2 Hz), 7.00–7.30 (5H, m), 7.48 (1H, d, J = 8.4 Hz), 8.10 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 8.4 Hz), 8.60 (1H, s) ^{13}**C NMR**: 14.05, 61.47, 124.60, 125.41, 127.39, 129.58, 129.78, 131.25, 154.20, 167.90
7b	^{1}**H NMR**: 1.44 (3H, t, J = 6.9 Hz), 4.46 (2H, q, J = 6.9 Hz), 7.68 (2H, d, J = 8.4 Hz), 7.76 (1H, d, J = 8.4 Hz), 7.90 (2H, d, J = 8.4 Hz), 8.08 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 8.4 Hz), 8.41 (1H, s) ^{13}**C NMR**: 14.68, 62.14, 125.43, 126.34, 128.64, 129.19, 129.51, 130.46, 137.94, 154.67, 168.45
7c	^{1}**H NMR**: 1.45 (3H, t, J = 6.9 Hz), 4.67 (2H, q, J = 6.9 Hz), 7.52 (1H, d, J = 9 Hz), 8.10–8.30 (3H, m), 8.44 (2H, d, J = 9 Hz), 8.62 (1H, s) ^{13}**C NMR**: 14.50, 61.54, 115.15, 117.60, 122.39, 124.90, 125.21, 128.60, 136.44, 149.82, 153.89, 168.53
7d	^{1}**H NMR**: 1.44 (3H, t, J = 7.2 Hz), 4.54 (2H, q, J = 7.2 Hz), 7.04 (1H, d, J = 8.1 Hz), 7.25 (1H, dd, $J1$ = 1.8 Hz, $J2$ = 8.1 Hz), 7.36 (1H, s), 7.46 (1H, d, J = 8.1 Hz), 8.06 (1H, dd, $J1$ = 1.8 Hz, $J2$ = 8.1 Hz), 8.55 (1H, s) ^{13}**C NMR**: 14.68, 56.18, 61.50, 110.85, 111.09, 112.70, 117.54, 122.20, 126.34, 149.57, 150.05, 151.62, 168.35
7e	^{1}**H NMR**: 1.43 (3H, t, J = 7.2 Hz), 4.61 (2H, q, J = 7.2 Hz), 7.60 (1H, d, J = 9 Hz), 7.82 (2H, d, J = 9 Hz), 7.99 (2H, d, J = 9 Hz), 8.12 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 9 Hz), 8.58 (1H, s) ^{13}**C NMR**: 14.69, 62.15, 115.54, 116.64, 128.00, 128.72, 128.91, 129.23, 129.52, 130.47, 154.75, 168.46
7f	^{1}**H NMR**: 1.44 (3H, t, J = 7.2 Hz), 2.85 (6H, s), 4.44 (2H, q, J = 7.2 Hz), 6.66 (2H, d, J = 8.4 Hz), 7.25 (1H, d, J = 8.4 Hz), 7.50 (2H, d, J = 8.4 Hz), 8.00 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 8.4 Hz), 8.56 (1H, s) ^{13}**C NMR**: 14.72, 40.30, 62.07, 113.06, 117.27, 114.80, 125.33, 129.24, 129.42, 153.69, 168.86
7g	^{1}**H NMR**: 1.44 (3H, t, J = 7.2 Hz), 4.60 (2H, t, J = 7.2 Hz), 7.50 (1H, d, J = 9 Hz), 7.83 (2H, d, J = 9 Hz), 8.00 (2H, d, J = 9 Hz), 8.13 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 9 Hz), 8.59 (1H, s) ^{13}**C NMR**: 14.59, 62.13, 115.46, 116.58, 127.27, 128.10, 129.50, 129.67, 130.13, 135.42, 156.50, 168.50
7h	^{1}**H NMR**: 1.30–1.50 (9H, m), 2.65 (2H, t, J = 6.6 Hz), 4.43 (2H, t, J = 6.9 Hz), 6.65 (2H, d, J = 8.4 Hz), 7.25 (1H, d, J = 8.4 Hz), 7.50 (2H, d, J = 8.4 Hz), 8.02 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 8.4 Hz), 8.58 (1H, s) ^{13}**C NMR**: 14.70, 25.39, 26.55, 50.33, 62.16, 114.90, 116.32, 117.38, 119.15, 125.46, 128.23, 143.58, 154.83, 155.49, 168.85
7i	^{1}**H NMR**: 1.45 (3H, t, J = 6.9 Hz), 3.29 (4H, t, J = 4.5 Hz), 3.92 (4H, t, J = 4.5 Hz), 4.73 (4H, t, J = 6.9 Hz), 7.04 (2H, d, J = 9 Hz), 7.49 (1H, d, J = 9 Hz), 7.75 (2H, d, J = 9 Hz), 8.07 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 9 Hz), 8.55 (1H, s) ^{13}**C NMR**: 14.69, 46.35, 62.17, 68.60, 114.40, 116.83, 117.00, 119.25, 126.10, 128.90, 154.59, 168.75
7j	^{1}**H NMR**: 1.45 (3H, t, J = 7.2 Hz), 4.70 (2H, t, J = 7.2 Hz), 7.03 (2H, d, J = 9.6 Hz), 7.40–7.50 (3H, m), 8.10–8.20 (2H, m), 8.61 (1H, s), 8.78 (2H, s) ^{13}**C NMR**: 14.51, 60.89, 118.60, 120.06, 120.25, 124.80, 125.29, 129.10, 132.00, 132.27, 133.42, 149.74, 153.85, 163.00, 168.55
7k	^{1}**H NMR**: 1.43 (3H, t, J = 6.9 Hz), 3.88 (3H, s), 4.53 (2H, t, J = 6.9 Hz), 7.06 (2H, d, J = 9 Hz), 7.47 (1H, d, J = 9 Hz), 7.75 (2H, d, J = 9 Hz), 8.04 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 9 Hz), 8.54 (1H, s) ^{13}**C NMR**: 14.69, 56.22, 61.45, 109.75, 109.98, 121.12, 122.18, 123.40, 150.05, 151.59, 168.67

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Table 2 (*continued*)

Compound	NMR (δ ppm)
7l	**^{1}H NMR**: 1.45 (3H, t, J = 7.2 Hz), 4.47 (2H, t, J = 7.2 Hz), 6.10 (2H, s), 7.30 (1H, d, J = 9 Hz), 7.45–7.55 (3H, m), 8.06 (1H, d, J = 9 Hz), 8.53 (1H, s) **^{13}C NMR**: 14.70, 62.09, 103.31, 107.98, 108.24, 108.76, 109.79, 121.09, 122.82, 124.40, 125.96, 150.01, 151.50, 168.58
7m	**^{1}H NMR**: 1.44 (3H, t, J = 7.2 Hz), 3.73 (3H, s), 4.56 (2H, t, J = 7.2 Hz), 4.74 (1H, d, J = 6.6 Hz), 5.23 (1H, d, J = 6.6 Hz), 6.82 (2H, d, J = 9 Hz), 6.99 (2H, d, J = 9 Hz), 7.00–7.30 (5H, m), 7.50 (1H, d, J = 9 Hz), 8.10 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 9 Hz), 8.58 (1H, s) **^{13}C NMR**: 14.43, 52.00, 56.09, 60.90, 61.89, 111.45, 112.36, 125.29, 125.67, 128.77, 129.83, 143.95, 153.71, 168.50, 169.69
7n	**^{1}H NMR**: 1.45 (3H, t, J = 7.2 Hz), 4.47 (2H, q, J = 7.2 Hz), 4.64 (2H, t, J = 4.8 Hz), 7.50 (1H, d, J = 9 Hz), 7.82 (2H, d, J = 9 Hz), 8.10–8.20 (3H, m), 8.60 (1H, s) **^{13}C NMR**: 14.65, 61.62, 115.30, 116.80, 125.22, 128.74, 129.13, 133.59, 168.67, 170.94
7o	**^{1}H NMR**: 1.45 (3H, t, J = 7.2 Hz), 4.73 (2H, q, J = 7.2 Hz), 6.88 (1H, d, J = 8.4 Hz), 7.49 (2H, d, J = 8.4 Hz), 7.59 (2H, d, J = 8.4 Hz), 8.08 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 8.4 Hz), 8.56 (1H, s) **^{13}C NMR**: 14.69, 61.56, 105.68, 107.77, 109.20, 111.10, 122.95, 125.60, 153.29, 157.46, 168.58
7p	**^{1}H NMR**: 1.43 (3H, t, J = 6.9 Hz), 4.42 (2H, q, J = 6.9 Hz), 7.66 (2H, d, J = 8.4 Hz), 7.73 (1H, d, J = 8.4 Hz), 7.84 (2H, d, J = 8.4 Hz), 8.07 (1H, dd, $J1$ = 1.5 Hz, $J2$ = 8.4 Hz), 8.46 (1H, s) **^{13}C NMR**: 14.69, 62.15, 125.43, 126.35, 128.64, 129.20, 129.52, 130.47, 137.94, 154.68, 168.46
7q	**^{1}H NMR**: 1.44 (3H, t, J = 7.2 Hz), 4.35 (2H, q, J = 7.2 Hz), 6.66 (1H, s), 6.85 (1H, dd, $J1$ = 1.8 Hz, $J2$ = 8.1 Hz), 7.44 (1H, d, J = 8.1 Hz), 7.50 (1H, d, J = 8.1 Hz), 8.16 (1H, dd, $J1$ = 1.8 Hz, $J2$ = 8.1 Hz), 8.57 (1H, s) **^{13}C NMR**: 14.65, 61.78, 106.55, 108.35, 109.10, 112.15, 122.90, 126.10, 130.40, 153.87, 156.99, 168.75

3.2. Biology

The test samples were analyzed *in vitro* against *M. tuberculosis* $H_{37}Rv$ (*Mtb* $H_{37}Rv$) in a high throughput screen using an assay adapted from the microdilution AlamarBlue (AB) broth assay as reported by Collins and Franzblau [20]. All manipulations of *Mtb* $H_{37}Rv$ were conducted in accordance with the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition in Biosafety Level 3 containment laboratories.

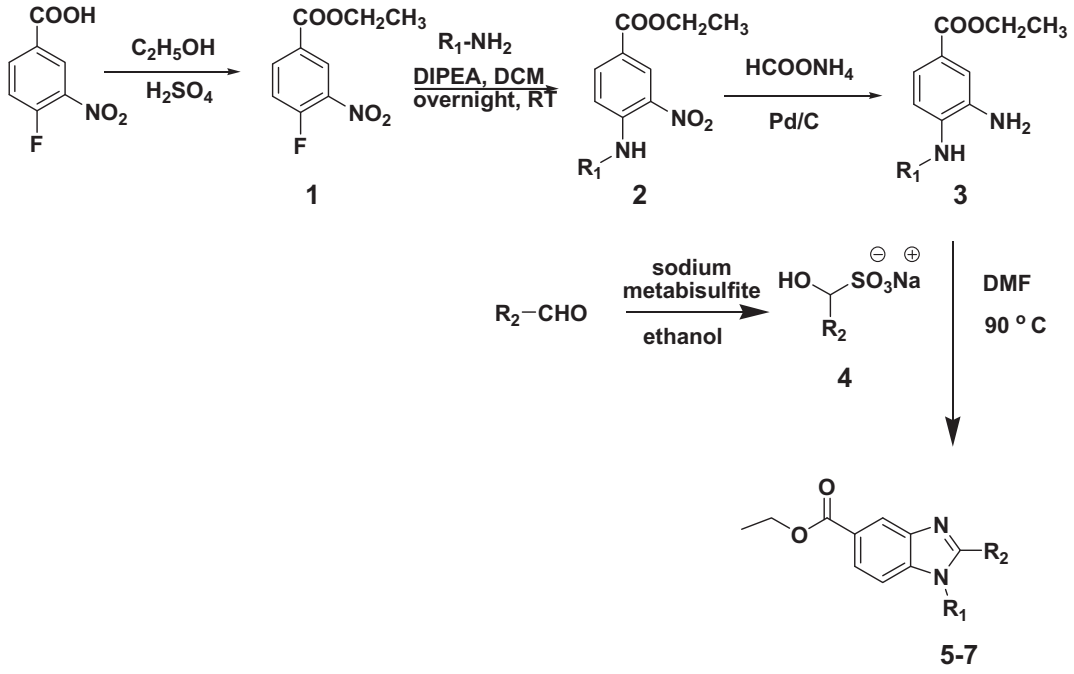
In brief, our assay uses *Mtb* $H_{37}Rv$ in a 384-well plate format with in-plate DMSO carrier controls, 3.198 μM amikacin, 0.17 μM amikacin controls and 320 compounds. The *Mtb* $H_{37}Rv$ + DMSO carrier control provides a 100% growth control for each plate. The 3.198 μM amikacin completely inhibits growth of the bacteria and is used in place of uninoculated media as the background control; whereas, the 0.17 μM amikacin control approximates the MIC of amikacin ranging from 30 to 80% inhibition indicative of a positive growth inhibition and proper assay performance. The media used for both compound preparation and *Mtb* $H_{37}Rv$ plating was assessed for contamination by plating two 384 well plates with media alone. The plates were checked for contamination by visual inspection and end-point detection. The compounds were evaluated in 10-point stacked plate dose response method. Compounds were serially diluted 1:2 from 100 μM through 0.195 μM. The plates were read fluorometrically after incubation with the compounds and addition of AB. Tests were performed in triplicate.

An alternative method for end-point detection was assessed using the Promega reagent BacTiter-Glo™ Microbial Cell Viability (BTG). The BTG plates were briefly incubated for 20 min at room temperature, sealed with Perkin Elmer clear TopSeal A and read from the top using luminescence on an Perkin Elmer Envision. Tests were performed in triplicate.

Results from both types of assays were presented in this paper for comparison purposes.

3.2.1. Autofluorescence

Compounds in media were pre-read from the high concentration plate with no AlamarBlue or bacteria added. Fold increase was



Scheme 1. Protocol for synthesis of titled compounds.

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Table 3
Antimycobacterial activity of 2-substituted benzimidazole derivatives against *M. tuberculosis* $H_{37}Rv$.

Compound	Alamar blue			BTG			Cytotoxicity (μg/mL)
	IC_{50} (μM)	IC_{90} (μM)	MIC (μM)	IC_{50} (μM)	IC_{90} (μM)	MIC (μM)	
5a	NT	NT	NT	NT	NT	NT	NT
5b	16.88	>100	NA	12.54	14.48	50	>62.5
5c	>100	>100	NA	>100	>100	NA	>62.5
5d	>100	>100	NA	>100	>100	NA	>62.5
5e	38.08	55.82	100	26.94	51.91	100	>62.5
5f	>100	>100	NA	>100	>100	NA	>62.5
5g	16.14	44.46	100	11.52	16.53	50	>62.5
5h	NT	NT	NT	NT	NT	NT	NT
5i	>100	>100	NA	>100	>100	NA	>62.5
5j	>100	>100	NA	>100	>100	NA	>62.5
5k	99.79	>100	NA	95.52	>100	NA	>62.5
5l	>100	>100	NA	>100	>100	NA	>62.5
5m	NT	NT	NT	NT	NT	NT	NT
5n	>100	>100	NA	>100	>100	NA	>62.5
5o	>100	>100	NA	>100	>100	NA	>62.5
5p	44.75	87.52	100	39.07	70.72	100	>62.5
5q	NT	NT	NT	NT	NT	NT	NT
6a	48.97	63.26	100	50.04	71.29	100	>62.5
6b	>100	>100	NA	>100	>100	NA	>62.5
6c	>100	>100	NA	>100	>100	NA	>62.5
6d	>100	>100	NA	>100	>100	NA	>62.5
6e	>100	>100	NA	>100	>100	NA	>62.5
6f	>100	>100	NA	97.77	>100	NA	>62.5
6g	>100	>100	NA	>100	>100	NA	>62.5
6h	NT	NT	NT	NT	NT	NT	NT
6i	>100	>100	NA	>100	>100	NA	>62.5
6j	61.45	83.63	100	54.36	80.17	100	>62.5
6k	>100	>100	NA	>100	>100	NA	>62.5
6l	NT	NT	NT	NT	NT	NT	NT
6m	NT	NT	NT	NT	NT	NT	NT
6n	NT	NT	NT	NT	NT	NT	NT
6o	>100	>100	NA	>100	>100	NA	>62.5
6p	>100	>100	NA	>100	>100	NA	>62.5
6q	>100	>100	NA	>100	>100	NA	>62.5
7a	>100	>100	NA	>100	>100	NA	>62.5
7b	>100	>100	NA	61.99	>100	NA	>62.5
7c	>100	>100	NA	>100	>100	NA	>62.5
7d	83.66	>100	NA	51.89	74.82	100	>62.5
7e	>100	>100	NA	90.45	>100	NA	>62.5
7f	70.50	92.68	100	60.78	83.80	100	>62.5
7g	>100	>100	NA	>100	>100	NA	>62.5
7h	33.83	>100	NA	38.19	77.87	100	>62.5
7i	>100	>100	NA	>100	>100	NA	>62.5
7j	>100	>100	NA	>100	>100	NA	>62.5
7k	>100	>100	NA	>100	>100	NA	>62.5
7l	>100	>100	NA	>100	>100	NA	>62.5
7m	NT	NT	NT	NT	NT	NT	NT
7n	>100	>100	NA	>100	>100	NA	>62.5
7o	92.30	>100	NA	10.68	67.40	100	>62.5
7p	83.66	>100	NA	51.89	74.82	100	>62.5
7q	>100	>100	NA	>100	>100	NA	>62.5
Amikacin	0.12	0.14	0.16	0.07	0.12	0.16	>62.5
Cycloserine	24.76	28.01	100	23.55	26.38	100	>62.5
Ethambutol	3.45	>200	NA	1.50	1.64	6.25	>62.5
Isoniazid	0.19	>5	NA	0.13	0.20	0.31	>62.5
Pyrimethamine	25.09	28.00	100	24.27	46.37	100	>62.5
Rifampicin	0.02	0.02	0.16	0.02	0.03	0.04	>62.5

calculated using the median of the positive control wells from the AlamarBlue *Mtb* $H_{37}Rv$ assay for the *Mtb* $H_{37}Rv$ control wells. The criteria for a compound being considered autofluorescent was defined as having >50% fluorescence of the *Mtb* $H_{37}Rv$ control wells. None of the analyzed compounds were found to be autofluorescent.

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