Journal of Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • Publication Date (Web): 21 Aug 2015

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Identification of phenoxyalkylbenzimidazoles with anti-tubercular activity.

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KEYWORDS: anti-tubercular, drug discovery, structure-activity relationship, bactericidal activity, high throughput screening

ABSTRACT: We conducted an evaluation of the phenoxyalkylbenzimidazole series based on the exemplar 2-ethyl-1-(3-phenoxypropyl)-1*H*-benzo[*d*]imidazole for its anti-tubercular activity. Four segments of the molecule were examined systematically to define a structure-activity relationship with respect to biological activity. Compounds had sub-micromolar activity against *Mycobacterium tuberculosis*; the most potent compound had a minimum inhibitory concentration (MIC) of 52 nM and was not cytotoxic against eukaryotic cells (selectivity index = 523). Compounds were selective for *M. tuberculosis* over other bacterial species, including the closely related *Mycobacterium smegmatis.* Compounds had a bacteriostatic effect against aerobically-grown, replicating *M. tuberculosis*, but were bactericidal against non-replicating bacteria. Representative compounds had moderate to high permeability in MDCK cells, but were rapidly

metabolized in rodents and human liver microsomes suggesting the possibility of rapid *in vivo* hepatic clearance mediated by oxidative metabolism. These results indicate that the readily-synthesized phenoxyalkylbenzimidazoles are a promising class of potent and selective anti-tubercular agents, if the metabolic liability could be solved.

INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis,* is the second leading cause of death from an infectious disease and is a major global health problem. In 2013, according to the World Health Organization (WHO), 9 million new cases and 1.5 million deaths from the disease were reported, including 360 000 deaths among HIV-positive people[1]. In addition, one-third of the world's population is infected with latent TB, 10% of which is expected to develop active TB at some point in their lives[2]. Hence there is a desperate need for new TB drugs.

The benzimidazole core is a well-known privileged structure in medicinal chemistry as it is a versatile heterocycle, possessing a wide spectrum of biological activities including anti-bacterial[2-3], anti-parasitic[4-5], anti-fungal, and anti-viral activities[6-8]. The benzimidazole scaffold has also shown anti-mycobacterial activity in vitro[9-14]. Ojima *et al*[15] reported trisubstituted benzimidazoles with activity against *M. tuberculosis* and demonstrated that these are FtsZ inhibitors. Gong *et al*[16] also reported benzimidazole-based compounds with activity against *M. tuberculosis*. The benzimidazoles reported in these studies represent a variety of pharmacophores with most having extensive variation at the C-2 position for modulation of activity. The SAR of the series we report here has a particularly strict requirement for C-2 position with an ethyl group as the favored substituent. This could point to a novel mechanism of action or target distinct

from those already reported in the literature. Therefore it is an important pharmacophore for the discovery of new drugs.

We conducted an exploratory study to understand the structure-activity relationship of a benzimidazole series regarding anti-tubercular activity. We defined key features required for activity and determined that, importantly, the compounds lack significant cytotoxicity, are specific for *M. tuberculosis*, and are bactericidal against non-replicating bacteria, features which are desirable in a new therapeutic for tuberculosis.

RESULTS AND DISCUSSION

Confirmation of anti-tubercular activity

We were interested in the phenoxyalkylbenzimidazole (PAB) class of compounds since phenoxyalkylimidazoles and derivatives were previously identified as having activity against *M. tuberculosis*[17]. In this previous study 27/88 analogs tested had some activity, with the benzimidazole compound, 2-ethyl-1-(3-phenoxypropyl)-1*H*-benzo[*d*]imidazole being the most active[17]. We were interested in using this compound (Figure 1; **5)** as a starting point to explore the potential of the PAB class of compounds as a lead series for tuberculosis treatment.

We first wanted to confirm that the reference benzimidazole compound was active. We synthesized 2-ethyl-1-(3-phenoxypropyl)-1*H*-benzo[*d*]imidazole (**5**) and tested it for activity. We determined the minimum inhibitory concentration (MIC) against *M. tuberculosis* H37Rv (London Pride) [18]. We found the MIC of the synthesized compound (**5**) to be very similar to that previously reported - 5.2 μM (**Table 1**) as compared to 3.4 μM [17].

Structure-activity relationship (SAR) studies on 2-ethyl-1-(3-phenoxypropyl)-1*H*-benzo[*d*]imidazole (5)

We wanted to explore the structure-activity relationship of the PAB series to determine its potential for progression as a drug candidate. Therefore, we designed, synthesized and tested a large number of analogs in this process. PABs were synthesized according to **Scheme1**. Condensation of the 1,2-diaminobenzene with the appropriate carboxylic acid yielded the core benzimidazole intermediate (**1**) upon heating. Subsequent alkylation of **1** to generate the product was achieved by either of two methods. Either the benzimidazole (**1**) was deprotonated with sodium hydride and reacted with phenoxyalkyl bromide or it was reacted with a molar equivalent of dibromobutane and the resulting N-(bromoalkyl)benzimidazole treated with the appropriate phenol. Similarly, anilines were used in place of phenol to generate the corresponding aniline derivatives.

All compounds were tested for activity against *M. tuberculosis* in aerobic culture to determine the minimum inhibitory concentration (MIC). All active compounds were tested against eukaryotic cells to determine the cytotoxic concentration (TC_{50}). From these data we calculated the selectivity index ($SI = MIC/TC_{50}$).

Four segments of the reference molecule 2-ethyl-1-(3-phenoxypropyl)-1*H*-benzo[*d*]imidazole (**5, Fig 1**) were examined systematically to define the structure-activity relationships influencing potency. First we probed the consequence of alkyl chain length variation on anti-tubercular activity. Two sets of analogs were prepared bearing a 2-, 3- or 4-carbon linker with either a methyl group or an ethyl group as the substituent at the C-2 position of the benzimidazole moiety. The 4-carbon linked analog (**6**) was the most potent (MIC = 1.1 μM) and also had good

selectivity (SI = 19). The 2-carbon linked analog and analogs with branched spacer were not active (MIC > 20 μM) (**Table 1**). Interestingly, none of the analogs with methyl group as the C-2 substituent (**7 – 10**) were active (MIC >20 μM), suggesting the importance of ethyl group as the preferred substituent at C-2 position (**Table 1**).

Following up on the good activity of compound **6**, we investigated the effects of modulating the electronics of the phenyl ring region. Compounds bearing either electron-releasing groups or electron-donating groups on the phenyl ring were synthesized. Both weak electron-releasing groups (such as methyl) which inductively donate electrons to the phenyl ring, and strong electron-donating groups such as methoxy were investigated; *ortho*, *meta* and *para*-methylated phenyl analogs were synthesized and evaluated. All these compounds (**12-14**) displayed a slight increase in potency over **6** (**Table 2**). The p-methyl substituted analog (**14**, 0.15 μM) was the most potent and showed the highest selectivity (SI = 93). The addition of the bulkier isopropyl group at *ortho* positon (**16**, 20 μM) was detrimental to the anti-tubercular activity, relative to methyl group (**12**, 0.32 μM**)** suggesting that a sterically-hindered group at the *ortho* position of the phenyl ring has a negative impact on activity. An analog with a strong electron-donating group such as the methoxy group (**20**, 1.2 μM) had comparable activity to **6.** Replacement of the phenyl ether in **6** by benzyl ether as in **19** (3.6 μM) resulted in a three-fold decrease in potency (**Table 2**).

We synthesized a set of compounds incorporating strong electron-withdrawing substituents such as chloro-, nitro- and cyano-groups in the phenoxy region (**21-31**) (**Table 2**). These analogs were, in general, less potent compared to **6**. The mono- and di-halogenated compounds had similar activity to **6** except the dichloro compound (**24**, 20 μM) which was 20-fold less active.

The strongly electron-withdrawing cyano analog (**28**, 16 μM**)** and nitro analog (**31**, 7.9 μM) were much less potent relative to **6**.

We next investigated the influence of substituents on the benzo portion of the benzimidazole core (**Table 3**). The weak electron-donating methyl group at C-6 (**34**) and the weak electron-withdrawing chloride group at C-5 (**33**) resulted in the best potency and selectivity. The introduction of N-atoms (**38**, **39**), had a negative impact on activity (MIC > 20 μM). However, the aza group in combination with either a chloro (**35**, 6.1 μM) or a methyl (**40**, 1.5 μM) substitution resulted in active compounds. The introduction of strong electron-withdrawing group such as cyano (**41, 42**) was detrimental to the anti-tubercular activity. A methoxy substitution at C-6 (**36**, 0.9 μM) was favorable compared to C-5 (**37**) showing activity similar to that of compound **6**.

Next, we optimized the C-2 substitution on compound **6** (**Table 4**). Compounds were synthesized with a wide variety of C-2 substituents including electron-donating groups, electron-withdrawing groups, heterocyclic groups (with both saturated and unsaturated identities) and a sterically hindered group (*tert*-butyl). Interestingly, none of the compounds showed activity comparable to compound **6**. Three of the compounds, namely phenyl (**43**, 16 μM), ethanol-1-yl- (**45**, 20 μM and **46**, 11 μM) and acetamido (**47**, 11 μM) analogs, had a 10 -15-fold lower activity compared to compound **6**.

We then probed the effect of replacement of the linker oxygen on activity (**Table 5**). The amine analog (**54**, 0.47 μM) improved the selectivity index by a factor of 4.7 whereas a thioether analog (**53**, 1.4 μM) had no effect on activity and selectivity.

From these initial SAR studies based on **6**, we determined that the key substituents for activity enhancement are: a methyl group at the C-6 position, an ethyl group at the C-2 position of the benzimidazole, nitrogen as the hetero atom in the alkyl chain, and a methyl group at the *para* position of the phenyl ring. We used this knowledge to synthesize another set of analogs in an attempt to improve potency and selectivity further. This resulted in analogs with sub-micromolar potency (**Table 6**). The most potent compound (**58)** had a MIC of 0.11 μM and a SI of 109.

We also applied the same SAR lessons to improving the potency of compound **5 (Table 6)**. Again we obtained compounds with sub-micromolar activity and were able to improve potency 100-fold, as well selectivity by 55-fold. The substituted aniline analog **62** was the most potent with an MIC of 0.052 μM and a SI of 523.

Finally we explored the effect of replacing the phenyl ring with other heterocycles (**Table 7**). Replacement with the 3-pyridyl (**64)**, 4-pyridyl (**65**), 8-quinolinyl (**66**) or methyl (**69**) groups were all detrimental to the anti-tubercular activity (MIC > 20 μM). However, surprisingly a combination of chlorophenyloxadiazole and a thiother linker as in compound **68** was potent (MIC = 0.1 μM). Replacements of the benzimidazole moeity with an indole (**71–73**) or an indazole (**70**) resulted in loss of activity (**Table 8**).

***In silico* and in vitro ADME profile**

ADME was evaluated using predictive *in silico* models (data not shown). Based on these results, a representative set of compounds was tested in key *in vitro* assays including solubility, permeability and liver microsomal metabolic turnover (**Table 9**). Passive permeability across MDCK cells was moderate to high for the four compounds tested. Thermodynamic equilibrium solubility at pH 2 was >0.5 mg/mL for all compounds. This was in line with the expected

ionization of the basic benzimidazole group with pKa close to 6. However, at pH 6 and 7.4, the solubility was significantly lower, suggesting a potential impact on absorption, especially through the intestine. The compounds were rapidly metabolized with >90% lost in 30 min in rodents and human liver microsomes. These results suggested the possibility of rapid *in vivo* hepatic clearance mediated by oxidative metabolism. The relatively poor solubility at pH 6 and 7.4 as well as rapid microsomal turnover were not unexpected given relatively high lipophilicity with cLogP > 4 for most compounds.

The PAB series is bactericidal against non-replicating *M. tuberculosis*

We determined the microbiological profile of selected compounds from this series. We determined whether compounds were bactericidal or bacteriostatic under two conditions - aerobic growth (replicating conditions) and under nutrient starvation (non-replicating conditions).

We selected compounds, **5, 54**, **59**, **62**, and **68** based on potency and diversity and tested these for bactericidal activity in aerobic culture (**Figure 2**). *M. tuberculosis* was exposed to a range of compound concentrations (from 1-10X MIC) for 21 days and viable bacteria counted. None of the compounds was bactericidal (defined as >3 log kill in 21 days)[19] against *M. tuberculosis* under replicating conditions. An MBC (minimum bactericidal concentration) was not obtained for any compound. Only compound **5** showed any killing activity, with 2 logs of kill over 21 days. According to microbiological definition[19], all five compounds are bacteriostatic, since the MBC/MIC was >4. Compounds **5** and **68** were also tested for activity against non-replicating bacteria generated by nutrient starvation (**Figure 3**). In contrast to aerobic culture, compounds were clearly bactericidal against non-replicating bacteria, resulting in >3 logs kill over 21 days.

Even at 1x MIC, both compounds had sterilizing activity of more than 3 logs under these conditions. The bactericidal activity was time-dependent[19] i.e. the same rate of kill was noted at all concentrations.

PAB series activity is specific for *M. tuberculosis*

To determine the spectrum of activity, we measured MICs on solid medium against a representative Gram negative species (*Escherichia coli*), a representative Gram positive species (*Staphylococcus aureus*) and a non-pathogenic mycobacterial species (*Mycobacterium smegmatis*). Compounds **6**, **53**, and **68** were inactive against all three species, with MIC_{99} > 100 μM on solid medium (**Table 10**), but were active against *M. tuberculosis* with MIC_{99} of 2.5-10 μM on solid medium. Thus the growth inhibitory activity of the PAB series is specific to *M tuberculosis*.

CONCLUSION

We conducted a systematic exploration of the phenoxyalkylbenzimidazole (PAB) series for its activity against *M. tuberculosis*. The compounds in this series show good activity and selectivity. The methyl group in the C-6 position of the benzimidazole and para position of the phenyl ring, ethyl group at the C-2 position of the benzimidazole core, 3 or 4 carbon atom linker, nitrogen as the hetero atom on the alkyl chain and benzimidazole as the core moiety are key determinants of the activity of the compounds in this series.

Interestingly, this series is bacteriostatic under replicating conditions but bactericidal under non-replicating conditions. The increased activity against non-replicating bacteria is of particular interest, since current therapeutic agents largely target replicating organisms. Thus development

of the PAB series has the potential to provide new agents which could shorten antibiotic therapy and treat latent infections. Based on these properties, we propose the PAB is a promising series for further exploration, if the physicochemical and ADME properties can be improved.

EXPERIMENTAL SECTION

Determination of minimum inhibitory concentration (MIC)

MIC were determined against *M. tuberculosis* H37Rv (London Pride), a laboratory-passaged derivative of H37Rv (ATCC 25618) which has been sequenced [18]. MICs were run as described [20]; briefly MICs were determined in Middlebrook 7H9 medium containing 10% OADC (oleic acid, albumin, dextrose, catalase) supplement (Becton Dickinson) and 0.05% w/v Tween 80 (7H9-Tw-OADC) under aerobic conditions. Compounds were prepared as 10-point two-fold serial dilutions in DMSO with a starting concentration of 20 μM. The final concentration of DMSO in the assay was 2%. Bacterial growth was measured by OD_{590} after 5 days of incubation at 37°C. Growth inhibition curves were plotted and fitted using the Gompertz model. The MIC was defined as the minimum concentration at which growth was completely inhibited and was calculated from the inflection point of the fitted curve to the lower asymptote (zero growth).

Cytotoxicity against eukaroytic cells

Cytotoxicity was determined against the African green monkey kidney cell line (Vero: ATCC CCL-81). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), High Glucose, GlutaMAX, 10% FBS, and 1x of penicillin-streptomycin solution (100 units/mL of penicillin, 100 μg/mL of streptomycin). Compounds were prepared as 10-point three-fold serial dilutions in DMSO with a starting concentration of 50 μM. CellTiter-Glo® Reagent (Promega) was added to

96-well plates after 2 days of incubation at 37°C, 5% CO_2 and relative luminescent units (RLU) measured. Inhibition curves were fitted using the Levenberg–Marquardt algorithm. Toxic concentration (TC_{50}) was defined as the concentration of compound that gave 50% inhibition of growth.

Bactericidal activity

For replicating conditions, a late log phase culture of *M. tuberculosis* was adjusted to $OD_{590=}0.1$ in 7H9-Tw-OADC and 50 µL used to inoculate 5 mL of 7H9-Tw-OADC containing compounds (final DMSO concentration of 2%). Cultures were incubated standing at 37°C and serial dilutions plated to determine colony forming units (CFUs) on Middlebrook 7H10 agar plus 10 % v/v OADC supplement. Plates were incubated for 4 weeks before colonies were counted.

For non-replicating conditions, late log phase bacterial cultures were grown in 7H9-Tw-OADC, harvested, resuspended in PBS-Ty (PBS + 0.05% w/v Tyloxapol) to an $OD_{590=}0.1$, and incubated at 37°C for 14 d prior to addition of compound. Compounds were added at indicated concentrations (final DMSO concentration of 2%). Cultures were incubated standing at 37°C and serial dilutions plated to determine colony forming units (CFUs) on Middlebrook 7H10 agar plus 10 % v/v OADC supplement. Plates were incubated for 6 weeks before colonies were counted.

Spectrum of activity

MICs were determined on solid medium using the serial proportion method [21]. LB agar was used for *E. coli* BL21 and *S. aureus* RN4220; 7H10-OADC was used for *M. smegmatis* mc^2155 and *M. tuberculosis*. Plates were incubated at 37°C for 1 day for *E. coli*, 2 days for *S. aureus,* 5-7

days for *M. smegmatis* and 21-28 days for *M. tuberculosis.* MIC was defined as the concentration of compound which yielded less than 1% CFUs.

ADME

MDCK Permeability - Test compound (10 μM) transport was measured across MDCK cell line in presence of a P-gp inhibitor in the absorptive direction and expressed as the percent transport over the incubation period. Benchmark compounds, atenolol and dexamethasone, were used to define three levels of transport to classify the test compounds as having low, medium, or high permeability. To determine microsomal turnover, test compounds were incubated (2μM) with liver microsomes in the presence of NADPH and loss of parent molecule was measured by LC/MS after 30 min. High throughput thermodynamic equilibrium solubility was measured utilizing 10 mM DMSO solutions. The DMSO was removed by drying before buffers at various pH were added. Fraction unbound in plasma was measured as described[22]. Briefly, test compound and mouse plasma were mixed together and placed into a dialysis block with plasma mixture on one side and buffer on the other. After 4.5 h incubation, samples taken from both sides. The fraction unbound was calculated by dividing the LC/MS/MS area of the buffer side by the LC/MS/MS area of the protein side.

Compound synthesis

General Methods

^{1}H and NMR spectral data were recorded in $CDCl_3$ or Acetone-d6 on a 300 MHz Bruker NMR spectrometer. Column chromatography was conducted on Revelaris flash chromatography system. Reactions were monitored using thin-layer chromatography (TLC) on silica gel plates. HPLC techniques and high resolution mass spectrometry were used to determine the purity of

compounds. Purity of all final products was >95% as determined by HPLC analysis conducted on an Agilent 1100 series LC system (Agilent ChemStation Rev.A.10.02; Phenomenex-Luna-C18, 4.8 mm × 150 mm, 5 μm, 1.0 mL/min, UV 254nm, room temperature) with MeCN/H_2O (0.05% TFA or HCOOH buffer) gradient elution. HPLC-HRMS was performed on a Gilson 321 HPLC with detection performed by a Gilson 170 DAD and a Finnigan AQA mass spectrometer operating in electrospray ionization mode using a Phenomenex Gemini C18 150x4.6mm column.

General procedure for synthesis of 1*H*-benzo[*d*]imidazole intermediates (1)

A mixture of diamine (1 eq) and acid (1 eq) was heated to the boiling point of the acid for 4 h. The reaction mixture was poured into a beaker containing ice water and neutralized with 2M NaOH. The resulting precipitate was filtered and dried under the vacuum. All of these intermediates were purchased from commercial sources except 2-ethyl-6-methyl-1H-imidazo[4,5-c]pyridine (**1a**)

General procedure for synthesis of compounds 2–10, 43–47, 51, 52, 53 and 70-71

To a solution of benzimidazole in anhydrous dimethylformamide was added 3 eq of sodium hydride and stirred for 0.5 h. Phenoxyalkylbromide (2 eq) was then added and stirred at R. T. until the disappearance of starting material monitored by TLC. The reaction was quenched with methanol. The organic layer was washed with water and dried with anhydrous sodium sulfate then concentrated *in vacuo*. The resulting crude mixture was purified by column chromatography.

General Procedure for synthesis of 1-(2-bromoalkyl)-1*H*-benzo[d]imidazole intermediates (11)

To a solution of benzimidazole in acetone was added 5 eq of sodium hydroxide, dibrimoalkane and a catalytic amount of sodium iodide. The reaction mixture was heated to 50 ^{o}C and stirred overnight. The acetone was then evaporated. The crude mixture was dissolved in ethyl acetate and washed with water. The organics were dried with anhydrous sodium sulfate and concentrated *in vacuo*. The crude reaction mixture was purified by column chromatography.

General procedure for synthesis of compounds 12–42, 59–61, 63–69, 71 and 72

To a solution of benzimidazole in dimethylformamide were added 5 eq of potassium carbonate and 2 eq of the phenol (or thiophenol). The reaction was stirred overnight at room temperature or until all of the starting material disappeared. The reaction mixture was washed with water and extracted with ethyl acetate. The organics were dried with anhydrous sodium sulfate and concentrated *in vacuo*. The crude mixture was purified by column chromatography.

General procedure for synthesis of compounds 54–58, 62 and 73

To a solution of benzimidazole in dimethylformamide were added 5 eq of potassium carbonate and 2 eq of the aniline. The reaction was stirred at 50 ^{o}C overnight (or until disappearance of the starting material). The reaction mixture was washed with water and extracted with ethyl acetate. The organics were dried with anhydrous sodium sulfate and concentrated *in vacuo*. The crude mixture was purified by column chromatography.

2-ethyl-6-methyl-1*H*-imidazo[4,5-*c*]pyridine (1a)

Yield **1a**: (0.7 g, 56%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.50 (t, J = 7.6 Hz, 3H), 2.73 (s, 3H), 3.06 (q, J = 7.5 Hz, 2H), 7.09 (d, J = 8.1 Hz, 1H), 7.92 (d, J = 8.0 Hz, 1H), 12.63 (s, 1H).

(98.1% purity).

2-ethyl-1-(2-phenoxyethyl)-1*H*-benzo[*d*]imidazole (2)

Yield **2**: (0.32 g, 89%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.49 (t, *J* = 7.5 Hz, 3H), 2.94 (q, *J* = 7.5 Hz, 2H), 4.13 (t, *J* = 5.3 Hz, 2H), 4.36 (t, *J* = 5.3 Hz, 2H), 6.60 – 7.04 (m, 3H), 7.09 – 7.51 (m, 5H), 7.70 – 7.86 (m, 1H).

LCMS – ESI $(M+H)^+$: 267.2 (99.3% purity). HRMS (ESI): calcd for $C_{17}H_{19}N_2O$, 267.1497; found, 267.1493.

1-(2-(4-chlorophenoxy)ethyl)-2-ethyl-1*H*-benzo[*d*]imidazole (3)

Yield **3**: (0.27 g, 70%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.49 (t, *J* = 7.5 Hz, 3H), 2.94 (q, *J* = 7.4 Hz, 2H), 4.12 (t, *J* = 5.4 Hz, 2H), 4.39 (t, *J* = 4.6 Hz, 2H), 6.63 (d, *J* = 8.9 Hz, 2H), 7.05 – 7.18 (m, 2H), 7.18 – 7.34 (m, 3H), 7.70 – 7.81 (m, 1H).LCMS – ESI $(M+H)^+$: 301.10 (98.8% purity). HRMS (ESI): calcd for $C_{17}H_{18}N_2OCl$, 301.1108; found, 301.1107.

1-((2,3-dihydrobenzo[*b*][1,4]dioxin-2-yl)methyl)-2-ethyl-1*H*-benzo[*d*]imidazole (4)

Yield **4**: (0.065 g, 16%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 – 1.56 (m, 3H), 2.81 – 2.98 (m, 2H), 3.89 – 4.02 (m, 1H), 4.15 – 4.26 (m, 1H), 4.29 – 4.41 (m, 2H), 4.49 – 4.62 (m, 1H), 6.75 – 6.99 (m, 4H), 7.14 – 7.38 (m, 3H), 7.67 – 7.88 (m, 1H).. LCMS – ESI $(M+H)^+$: 295.10 (99% purity). HRMS (ESI): calcd for $C_{18}H_{19}N_2O_2$, 295.1447; found, 295.1447.

2-ethyl-1-(3-phenoxypropyl)-1*H*-benzo[*d*]imidazole (5)

Yield **5 (1)**: (0.32 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (3H, CH_3, t, *J* = 7.5 Hz); 2.24 (m, 2H); 2.98 (2H, q, *J* = 7.5 Hz); 3.86 (2H, t, *J* = 5.3 Hz); 4.30 (2H, t, *J* = 6.7 Hz); 6.84 – 7.75

(m, 9H). LCMS – ESI $(M+H)^+$: 281.2 (99.5% purity). HRMS (ESI): calcd for $C_{18}H_{21}N_2O$, 281.1654; found, 281.1651.

2-ethyl-1-(4-phenoxybutyl)-1*H*-benzo[*d*]imidazole (6)

Yield **6**: (0.40 g, 99%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, CH_3, t, J = 7.4 Hz); 1.66-1.84 (m, 2H); 1.92 (m, 2H); 2.83 (2H, q, J = 2. 7.5 Hz); 3.88 (2H, t, J = 6.7 Hz); 4.06 (2H, t, J = 7.5 Hz);; 6.81 – 6.94 (m, 3H); 7.17 – 7.28 (m, 5H); 7.72 – 7.77 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 11.92, 20.85, 26.67, 26.90, 43.32, 67.10, 109.16, 114.41, 119.28, 120.90, 121.72, 121.99, 129.53, 135.10, 142.71, 155.91, 158.72. LCMS – ESI $(M+H)^+$: 295.2 (99.4% purity). HRMS (ESI): calcd for $C_{19}H_{23}N_2O$, 295.1810; found, 295.1806.

2-methyl-1-(2-phenoxyethyl)-1*H*-benzo[*d*]imidazole (7)

Yield **7**: (0.31 g, 77%). ^1H NMR (300 MHz, $CDCl_3$): δ 2.68 (3H, CH_3, s); 4.23 (2H, t, J = 5.1 Hz); 4.47 (2H, t, J = 5.4 Hz); 6.72 – 6.84 (m, 2H); 6.86 – 7.01 (m, 1H); 7.19 – 7.26 (m, 4H); 7.31 – 7.36 (m, 1H); 7.66 – 7.72 (m, 1H). LCMS – ESI $(M+H)^+$: 253.2 (99% purity). HRMS (ESI): calcd for $C_{16}H_{17}N_2O$, 253.1341; found, 253.1340.

1-(2-(4-chlorophenoxy)ethyl)-2-methyl-1*H*-benzo[*d*]imidazole (8)

Yield **8**: (0.37 g, 85%). ^1H NMR (300 MHz, $CDCl_3$): δ 2.62 (3H, CH_3, s); 4.10 (2H, t, J = 5.1 Hz); 4.34 (2H, t, J = 5.7 Hz); 6.59 – 7.09 (m, 2H); 7.08 – 7.13 (m, 2H); 7.19 – 7.23 (m, 3H); 7.66 – 7.71 (m, 1H). LCMS – ESI $(M+H)^+$: 287.10 (99.1% purity). HRMS (ESI): calcd for $C_{16}H_{16}N_2OCl$, 287.0951; found, 287.0951.

2-methyl-1-(3-phenoxypropyl)-1*H*-benzo[*d*]imidazole (9)

Yield **9**: (0.36 g, 89%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.15-2.19 (m, 2H); 2.50 (3H, CH_3, s); 3.76 - 3.83 (m, 2H); 4.21 - 4.26 (m, 2H); 6.70 – 7.06 (m, 3H); 7.06 – 7.41 (m, 5H); 7.56 – 7.81 (m, 1H). LCMS – ESI $(M+H)^+$: 267.2 (97.1% purity). HRMS (ESI): calcd for $C_{17}H_{19}N_2O$, 267.1497; found, 267.1488.

2-methyl-1-(4-phenoxybutyl)-1*H*-benzo[*d*]imidazole (10)

Yield **10**: (0.36 g, 84%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.79 - 1.86 (m, 2H); 1.94 – 2.04 (m, 2H); 2.60 (3H, CH_3, s); 3.95 (2H, t, J = 5.9 Hz); 4.17 (2H, t, J = 7.9 Hz); 6.84 – 7.69 (m, 9H); 6.85 – 6.96 (m, 3H); 7.19 – 7.32 (m, 5H); 7.67 – 7.72 (m, 1H). LCMS – ESI $(M+H)^+$: 281.2 (98.3% purity). HRMS (ESI): calcd for $C_{18}H_{21}N_2O$, 281.1654; found, 281.1645.

1-(4-bromobutyl)-2-ethyl-1*H*-benzo[*d*]imidazole (11a)

Yield **11a**: (1.4 g, 49%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.48 (t, J = 7.7 Hz, 3H), 1.77 – 2.11 (m, 4H), 2.87 (q, J = 7.6 Hz, 2H), 3.36 (t, J = 6.0 Hz, 2H), 4.08 (t, J = 7.0 Hz, 2H), 7.10 – 7.33 (m, 3H), 7.59 – 7.86 (m, 1H). LCMS – ESI $(M+H)^+$: 281.1 (97.2% purity).

1-(4-bromobutyl)-2-ethyl-6-methyl-1*H*-benzo[*d*]imidazole (11b)

Yield **11b**: (1.39 g, 47%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.49 (t, J = 7.5 Hz, 3H), 1.86 – 2.08 (m, 4H), 2.50 (br. s, 3H), 2.89 (q, J = 7.5 Hz, 2H), 3.43 (t, J = 6.0 Hz, 2H), 3.96 – 4.28 (m, 2H), 6.97 – 7.24 (m, 2H), 7.46 – 7.70 (m, 1H).. LCMS – ESI $(M+H)^+$: 295.1 (98.2% purity).

1-(3-bromopropyl)-2-ethyl-6-methyl-1*H*-benzo[*d*]imidazole (11c)

Yield **11c**: (1.1 g, 63%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 – 1.59 (m, 3H), 2.13 – 2.39 (m, 2H), 2.37 – 2.54 (br. s, 3H), 2.77 – 3.04 (m, 2H), 3.17 – 3.51 (m, 2H), 3.94 – 4.45 (m, 2H), 6.81 – 7.26 (m, 2H), 7.42 – 7.67 (m, 1H).

. LCMS – ESI $(M+H)^+$: 281.1 (99.2% purity).

2-ethyl-1-(4-(o-tolyloxy)butyl)-1*H*-benzo[*d*]imidazole (12)

Yield **12**: (0.044 g, 79%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.51 (t, J = 7.0 Hz, 3H), 1.82 – 1.97 (m, 2H), 1.97 – 2.14 (m, 2H), 2.24 (s, 3H), 2.93 (q, J = 6.4, 7.0 Hz, 2H), 4.00 (t, J = 5.3 Hz, 2H), 4.21 (t, J = 7.4 Hz, 2H), 6.74 – 6.84 (m, 1H), 6.84 – 6.94 (m, 1H), 7.10 – 7.38 (m, 5H), 7.71 – 7.82 (m, 1H).LCMS – ESI $(M+H)^+$: 309.2 (99.4% purity). HRMS (ESI): calcd for $C_{20}H_{25}N_2O$, 309.1967; found, 309.1954

2-ethyl-1-(4-(m-tolyloxy)butyl)-1*H*-benzo[*d*]imidazole (13)

Yield **13**: (0.04 g, 72%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (t, 3H, CH_3, J = 7.0 Hz); 1.82 (m, 2H); 1.97 (m, 2H); 2.31 (s, 3H, CH_3); 2.84 (2H, q, J = 6.3, 7.1 Hz); 3.95 (2H, t, J = 5.3 Hz); 4.17 (2H, t, J = 6.7 Hz); 6.81 – 6.84 (m, 2H), 7.10 – 7.38 (m, 5H), 7.71 – 7.75 (m, 1H).. LCMS – ESI $(M+H)^+$: 309.2 (99.2% purity). HRMS (ESI): calcd for $C_{20}H_{25}N_2O$, 309.1967; found, 309.1958.

2-ethyl-1-(4-(*p*-tolyloxy)butyl)-1*H*-benzo[*d*]imidazole (14)

Yield **14**: (0.042 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, CH_3, t, J = 7.4 Hz); 1.82 - 1.91 (m, 2H,); 2.00 - 2.02 (m, 2H); 2.27 (s, 3H, CH_3); 2.90 (2H, q, J = 6.2, 7.5 Hz); 3.94 (2H, t, J = 5.3 Hz); 4.17 (2H, t, J = 6.7 Hz); 6.78 – 7.01 (m, 2H); 7.12-7.51 (m, 4H); 7.67 – 7.74 (m, 2H).

LCMS – ESI $(M+H)^+$: 309.2 (97.2% purity). HRMS (ESI): calcd for $C_{20}H_{25}N_2O$, 309.1967; found, 309.1962.

1-(4-(3,4-dimethylphenoxy)butyl)-2-ethyl-1*H*-benzo[*d*]imidazole (15)

Yield **15**: (0.055 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (t, *J* = 7.4 Hz, 3H), 1.73 – 1.91 (m, 2H), 1.92 – 2.09 (m, 2H), 2.18 (s, 3H), 2.22 (s, 3H), 2.90 (q, *J* = 7.3 Hz, 2H), 3.94 (t, *J* = 5.1 Hz, 2H), 4.19 (t, *J* = 6.0 Hz, 2H), 6.54 – 6.81 (m, 2H), 7.02 (d, *J* = 8.1 Hz, 1H), 7.12 – 7.41 (m, 3H), 7.74 (s, 1H).. LCMS – ESI $(M+H)^+$: 323.2 (99% purity). HRMS (ESI): calcd for $C_{21}H_{27}N_2O$, 323.2123; found, 323.2125.

2-ethyl-1-(4-(2-isopropylphenoxy)butyl)-1*H*-benzo[*d*]imidazole (16)

Yield **16**: (0.037 g, 61%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.11 – 1.26 (m, 6H), 1.37 – 1.56 (m, 3H), 1.77 – 1.94 (m, 2H), 1.94 – 2.15 (m, 2H), 2.80 – 3.00 (m, 2H), 3.19 – 3.38 (m, 1H), 3.90 – 4.05 (m, 2H), 4.10 – 4.27 (m, 2H), 6.72 – 6.85 (m, 1H), 6.85 – 7.00 (m, 1H), 7.06 – 7.36 (m, 5H), 7.68 – 7.80 (m, 1H).. LCMS – ESI $(M+H)^+$: 337.3 (99.5% purity). HRMS (ESI): calcd for $C_{22}H_{29}N_2O$, 337.2280; found, 337.2271.

4-(4-(4-(2-ethyl-1*H*-benzo[*d*]imidazol-1-yl)butoxy)-2,5-dimethylbenzyl)morpholine (17)

Yield **17**: (0.105 g, 92%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.48 (t, *J* = 7.5 Hz, 3H), 1.78 – 1.91 (m, 2H), 1.98 – 2.08 (m, 2H), 2.15 (s, 3H), 2.32 (s, 3H), 2.40 (t, *J* = 4.6 Hz, 4H), 2.90 (q, J = 6.0 Hz, 2H), 3.35 (s, 2H), 3.67 (t, *J* = 4.6 Hz, 4H), 3.96 (t, *J* = 5.8 Hz, 2H), 4.19 (t, *J* = 7.4 Hz, 2H), 6.58 (s, 1H), 6.98 (s, 1H), 7.18 – 7.25 (m, 2H), 7.27 – 7.34 (m, 1H), 7.69 – 7.79 (m, 1H).. LCMS – ESI $(M+H)^+$: 422.3 (98.7% purity). HRMS (ESI): calcd for $C_{26}H_{36}N_3O_2$, 422.2808; found, 422.2800.

4-(4-(4-(2-ethyl-1*H*-benzo[*d*]imidazol-1-yl)butoxy)phenyl)morpholine (18)

Yield **18**: (0.055 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.48 (3H, CH_3, t, *J* = 7.5 Hz); 1.79 - 1.88 (m, 2H); 2.01 - 2.04(m, 2H); 2.84 (2H, q, *J* = 6.0, 6.4 Hz); 3.13 (4H, t, *J*= 4.8); 3.83 (4H, t, *J* = 4.8 Hz); 3.96 (2H, t, *J* = 6.0 Hz); 4.16 (2H, t, *J* = 7.5 Hz); 6.26 – 6.65 (m, 3H); 7.04 – 7.41 (m, 4H); 7.71 – 7.75 (m, 1H). ^{13}C NMR ($CDCl_3$): δ ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.90, 11.92, 20.83, 26.70, 26.90, 43.30, 49.23, 66.87, 67.13, 76.68, 77.10, 77.53, 102.67, 105.15, 108.66, 109.20, 119.23, 121.74, 122.00, 129.93, 135.06, 142.61, 152.73, 155.90, 159.80.. LCMS – ESI $(M+H)^+$: 380.2 (99.2% purity). HRMS (ESI): calcd for $C_{23}H_{30}N_3O_2$, 380.2338; found, 380.2334.

1-(4-(benzyloxy)butyl)-2-ethyl-1*H*-benzo[*d*]imidazole (19)

Yield **19**: (0.010 g, 23%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, CH_3, t, *J* = 7.5 Hz); 1.66 (m, 2H); 1.90 (m, 2H); 2.41 (m, 4H); 2.89 (m, 2H); 3.50 (m, 2H); 4.10 (m, 2H); 4.44 (s, 2H, CH_2); 7.20 – 7.73 (m, 9H). LCMS – ESI $(M+H)^+$: 309.2 (97.9% purity). HRMS (ESI): calcd for $C_{20}H_{25}N_2O$, 309.1967; found, 309.1959.

2-ethyl-1-(4-(4-methoxyphenoxy)butyl)-1*H*-benzo[*d*]imidazole (20)

Yield **20**: (0.023 g, 50%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.50 (t, *J* = 7.5 Hz, 3H), 1.77 – 1.91 (m, 2H), 1.94 – 2.12 (m, 2H), 2.93 (q, *J* = 7.5 Hz, 2H), 3.79 (s, 3H), 3.96 (t, *J* = 6.0 Hz, 2H), 4.22 (t, *J* = 7.4 Hz, 2H), 6.72 – 6.93 (m, 4H), 7.17 – 7.27 (m, 2H), 7.30 – 7.38 (m, 1H), 7.67 – 7.91 (m, 1H). LCMS – ESI $(M+H)^+$: 325.0 (98.2% purity). HRMS (ESI): calcd for $C_{20}H_{25}N_2O_2$, 325.1916; found, 325.1913.

1-(4-(3-chlorophenoxy)butyl)-2-ethyl-1*H*-benzo[*d*]imidazole (21)

Yield **21**: (0.043 g, 73%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, CH_3, t, *J* = 7.4 Hz); 1.85 (m, 2H); 2.02 (m, 2H); 2.88 (2H, q, *J* = 7.4 Hz); 3.95 (2H, t, *J* = 5.3 Hz); 4.18 (2H, t, *J* = 6.7 Hz); 6.77 – 6.90 (m, 2H); 7.10 – 7.25 (m, 5H); 7.75 - 7.79 (m, 1H). LCMS – ESI $(M+H)^+$: 329.2 (99.2% purity). HRMS (ESI): calcd for $C_{19}H_{22}N_2OCl$, 329.1421; found, 329.1424.

1-(4-(2-chlorophenoxy)butyl)-2-ethyl-1*H*-benzo[*d*]imidazole (22)

Yield **22**: (0.04 g, 64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.49 (t, *J* = 7.5 Hz, 3H), 1.82 – 2.00 (m, 2H), 2.00 – 2.19 (m, 2H), 2.93 (q, *J* = 7.5 Hz, 2H), 4.06 (t, *J* = 5.7 Hz, 2H), 4.26 (t, *J* = 7.3 Hz, 2H), 6.81 – 7.02 (m, 2H), 7.17 – 7.31 (m, 3H), 7.31 – 7.45 (m, 2H), 7.70 – 7.87 (m, 1H).. LCMS – ESI $(M+H)^+$: 329.1 (99% purity). HRMS (ESI): calcd for $C_{19}H_{22}N_2OCl$, 329.1421; found, 329.1413.

1-(4-(4-chlorophenoxy)butyl)-2-ethyl-1*H*-benzo[*d*]imidazole (23)

Yield **23**: (0.036 g, 61%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, CH_3, t, *J* = 7.4 Hz); 1.85 (m, 2H); 2.02 (m, 2H); 2.88 (2H, q, *J* = 7.4 Hz); 3.95 (2H, t, *J* = 5.3 Hz); 4.18 (2H, t, *J* = 6.7 Hz); 6.77 – 6.90 (2m, 1H); 7.17 – 7.35 (m, 5H); 7.73 – 7.75 (m, 1H). LCMS – ESI $(M+H)^+$: 329.2 (97.5% purity). HRMS (ESI): calcd for $C_{19}H_{22}N_2OCl$, 329.1421; found, 329.1411.

1-(4-(2, 6-dichlorophenoxy)butyl)-2-ethyl-1*H*-benzo[*d*]imidazole (24)

Yield **24**: (0.057 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, CH_3, t, *J* = 7.4 Hz); 1.90 (m, 2H); 2.10 (m, 2H); 2.94 (2H, q, *J* = 7.4 Hz); 4.03 (2H, t, *J* = 5.3 Hz); 4.24 (2H, t, *J* = 6.7 Hz); 6.98 – 7.02 (m, 3H); 7.45 – 7.75 (m, 4H). LCMS – ESI $(M+H)^+$: 363.2 (98.2% purity). HRMS (ESI): calcd for $C_{19}H_{21}N_2OCl_2$, 363.1031; found, 363.1015.

1-(4-(2,4-dichlorophenoxy)butyl)-2-ethyl-1*H*-benzo[*d*]imidazole (25)

Yield **25**: (0.042 g, 64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (3H, CH_3, t, J = 7.5 Hz); 1.88 - 1.90 (m, 2H); 1.99 - 2.05 (m, 2H); 2.90 (2H, q, J = 7.5 Hz); 3.99 (2H, t, J = 5.3 Hz); 4.22 (2H, t, J = 6.7 Hz); 6.78 - 7.79 (m, 1H); 7.02 – 7.48 (m, 5H); 7.64 – 7.88 (m, 1H). LCMS – ESI $(M+H)^+$: 363.1 (99% purity). HRMS (ESI): calcd for $C_{19}H_{21}N_2OCl_2$, 363.1031; found, 363.1028.

1-(4-(4-chloro-3-fluorophenoxy)butyl)-2-ethyl-1*H*-benzo[*d*]imidazole (26)

Yield **26**: (0.053 g, 85%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.48 (t, J = 6.2 Hz, 3H), 1.73 – 1.90 (m, 2H), 1.90 – 2.09 (m, 2H), 2.89 (q, J = 7.8 Hz, 2H), 3.82 – 4.00 (m, 2H), 4.05 – 4.28 (m, 2H), 6.49 – 6.73 (m, 2H), 7.13 – 7.38 (m, 4H), 7.66 – 7.82 (m, 1H). LCMS – ESI $(M+H)^+$: 347.1 (98.7% purity). HRMS (ESI): calcd for $C_{19}H_{21}N_2OFCl$, 347.1326; found, 347.1316.

1-(4-(4-chloro-2-fluorophenoxy)butyl)-2-ethyl-1*H*-benzo[*d*]imidazole (27)

Yield **27**: (0.04 g, 64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, CH_3, t, J = 7.5 Hz); 1.85-1.93 (m, 2H); 2.02 - 2.04 (m, 2H); 2.88 (2H, q, J = 7.5 Hz); 3.98 (2H, t, J = 5.3 Hz); 4.21 (2H, t, J = 6.7 Hz); 6.59 – 6.98 (m, 2H); 6.97 – 7.20 (m, 2H); 7.21 – 7.52 (m, 2H); 7.72 – 7.77 (m, 1H). LCMS – ESI $(M+H)^+$: 347.1 (98.2% purity). HRMS (ESI): calcd for $C_{19}H_{21}N_2OClF$, 347.1326; found, 347.1325.

4-(4-(2-ethyl-1*H*-benzo[*d*]imidazol-1-yl)butoxy)benzonitrile (28)

Yield **28**: (0.046 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.36 – 1.58 (m, 3H), 1.76 – 1.92 (m, 2H), 1.94 – 2.11 (m, 2H), 2.79 – 2.99 (m, 2H), 3.89 – 4.08 (m, 2H), 4.09 – 4.29 (m, 2H), 6.79 –

6.98 (m, 2H), 7.13 – 7.34 (m, 3H), 7.44 – 7.63 (m, 2H), 7.65 – 7.83 (m, 1H). LCMS – ESI $(M+H)^+$: 320.2 (99.6% purity). HRMS (ESI): calcd for $C_{20}H_{22}N_3O$, 320.1763; found, 320.1754.

1-(4-(3-chloro-4-methylphenoxy)butyl)-2-ethyl-1*H*-benzo[*d*]imidazole (29)

Yield **29**: (0.051 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, CH_3, t, J = 7.5 Hz); 1.82 - 1.85 (m, 2H); 2.00 – 2.02 (m, 2H); 2.27 (3H, CH_3, s); 2.90 (2H, q, J = 7.5 Hz); 3.92 (2H, t, J = 5.3 Hz); 4.17 (2H, t, J = 6.7 Hz); 6.78 – 7.24 (m, 4H), 7.44 – 7.63 (m, 2H), 7.65 – 7.74 (m, 1H).. LCMS – ESI $(M+H)^+$: 343.2 (99.1% purity). HRMS (ESI): calcd for $C_{20}H_{24}N_2OCl$, 343.1577; found, 343.1572.

1-(4-(4-chloro-2-methylphenoxy)butyl)-2-ethyl-1*H*-benzo[*d*]imidazole (30)

Yield **30**: (0.038 g, 62%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, CH_3, t, J = 7.5 Hz); 1.85 – 1.87 (m, 2H); 2.02 – 2.04 (m, 2H); 2.15 (3H, CH_3, s); 2.84 (2H, q, J = 7.5 Hz); 3.93 (2H, t, J = 5.3 Hz); 4.19 (2H, t, J = 6.7 Hz); 6.62 – 6.68 (m, 1H); 7.06 – 7.09 (m, 2H); 7.17 – 7.40 (m, 3H); 7.63 – 7.84 (m, 1H). LCMS – ESI $(M+H)^+$: 343.2 (99.4% purity). HRMS (ESI): calcd for $C_{20}H_{24}N_2OCl$, 343.1577; found, 343.1566.

1-(4-(4-chloro-5-methyl-2-nitrophenoxy)butyl)-2-ethyl-1*H*-benzo[*d*]imidazole (31)

Yield **31**: (0.057 g, 82%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35 – 1.62 (m, 3H), 1.72 – 2.22 (m, 4H), 2.39 (s, 3H), 2.79 – 3.09 (m, 2H), 3.92 – 4.39 (m, 4H), 6.84 (s, 1H), 7.09 – 7.45 (m, 3H), 7.64 – 7.84 (m, 1H), 7.82 – 7.99 (m, 1H).LCMS – ESI $(M+H)^+$: 388.1 (95.1% purity). HRMS (ESI): calcd for $C_{20}H_{23}N_3O_3Cl$, 388.1428; found, 388.1411.

6-chloro-2-ethyl-1-(4-phenoxybutyl)-1*H*-benzo[*d*]imidazole (32)

Yield **32**: (0.023 g, 13%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (t, *J* = 7.3 Hz, 3H), 1.75 – 1.92 (m, 2H), 1.93 – 2.10 (m, 2H), 2.88 (q, *J* = 7.4 Hz, 2H), 3.97 (t, *J* = 5.8 Hz, 2H), 4.16 (t, *J* = 7.3 Hz, 2H), 6.82 – 7.06 (m, 3H), 7.14 – 7.40 (m, 4H), 7.70 (s, 1H).. LCMS – ESI $(M+H)^+$: 329.2 (97.2% purity). HRMS (ESI): calcd for $C_{19}H_{22}N_2OCl$, 329.1421; found, 329.1418.

5-chloro-2-ethyl-1-(4-phenoxybutyl)-1*H*-benzo[*d*]imidazole (33)

Yield **33**: (0.023 g, 13%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (t, *J* = 7.3 Hz, 3H), 1.75 – 1.92 (m, 2H), 1.93 – 2.10 (m, 2H), 2.88 (q, *J* = 7.4 Hz, 2H), 3.97 (t, *J* = 5.8 Hz, 2H), 4.16 (t, *J* = 7.3 Hz, 2H), 6.72 – 7.06 (m, 3H), 7.14 – 7.42 (m, 4H), 7.70 (s, 1H).. ^{13}C NMR ($CDCl_3$): δ 11.73, 20.84, 26.57, 26.80, 43.48, 66.99, 109.36, 114.41, 120.04, 120.97, 122.39, 127.80, 129.56, 135.72, 141.24, 156.84, 158.65. LCMS – ESI $(M+H)^+$: 329.2 (98.2% purity). HRMS (ESI): calcd for $C_{19}H_{22}N_2OCl$, 329.1421; found, 329.1407.

2-ethyl-6-methyl-1-(4-phenoxybutyl)-1*H*-benzo[*d*]imidazole (34)

Yield **34**: (0.125 g, 66%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, CH_3, t, *J* = 7.5 Hz); 1.84 – 186 (m, 2H); 1.99 – 203 (m, 2H); 2.47 (3H, CH_3, s); 2.88 (2H, q, *J* = 6.7 Hz); 3.96 (2H, t, *J* = 5.3 Hz); 4.15 (2H, t, *J* = 7.5 Hz); 6.88 – 7.05 (m, 3H), 7.14 – 7.31 (m, 4H), 7.28 (s, 1H). LCMS – ESI $(M+H)^+$: 309.2 (99% purity). HRMS (ESI): calcd for $C_{20}H_{25}N_2O$, 309.1967; found, 309.1964.

6-chloro-2-ethyl-3-(4-phenoxybutyl)-3*H*-imidazo[4,5-*b*]pyridine (35)

Yield **35**: (0.015 g, 17%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.48 (t, *J* = 7.5 Hz, 3H), 1.64 – 1.78 (m, 2H), 1.89 – 2.01 (m, 2H), 2.89 (q, *J* = 7.5 Hz, 2H), 3.18 (t, *J* = 6.9 Hz, 2H), 4.15 (t, *J* = 7.3 Hz, 2H), 6.40 (s, 1H), 6.49 (d, *J* = 7.4 Hz, 1H), 6.93 (d, *J* = 7.4 Hz, 1H), 7.19 – 7.26 (m, 2H), 7.26 – 7.32 (m, 1H), 7.70 – 7.78 (m, 1H).. LCMS – ESI $(M+H)^+$: 330.1 (98% purity). HRMS (ESI): calcd for $C_{18}H_{21}N_3OCl$, 330.1373; found, 330.1364.

2-ethyl-6-methoxy-1-(4-phenoxybutyl)-1*H*-benzo[*d*]imidazole (36)

Yield **36**: (0.025 g, 28%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (3H, CH_3, t, *J* = 7.5 Hz); 1.85 – 1.87 (m, 2H); 2.00 – 2.02 (m, 2H); 2.88 (2H, q, *J* = 2.1 Hz, 7.5 Hz); 3.98 (3H, CH_3, s); 4.14 (2H, t, *J* = 5.7 Hz); 4.23 (2H, t, *J* = 7.2 Hz); 6.86 – 7.06 (m, 3H), 7.11 – 7.21 (m, 4H), 7.31 (s, 1H). LCMS – ESI $(M+H)^+$: 325.2 (99.3% purity). HRMS (ESI): calcd for $C_{20}H_{25}N_2O_2$, 325.1916; found, 325.1916.

2-ethyl-5-methoxy-1-(4-phenoxybutyl)-1*H*-benzo[*d*]imidazole (37)

Yield **37**: (0.012 g, 13%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (3H, CH_3, t, *J* = 7.5 Hz); 1.83 – 1.86 (m, 2H); 2.01 – 2.04 (m, 2H); 2.88 (2H, q, *J* = 2.1 Hz, 7.5 Hz); 3.98 (3H, CH_3, s); 4.14 (2H, t, *J* = 6.0 Hz); 4.23 (2H, t, *J* = 7.2 Hz); 6.86 – 7.06 (m, 3H), 7.11 – 7.20 (m, 4H), 7.31 (s, 1H). LCMS – ESI $(M+H)^+$: 325.2 (99.4% purity). HRMS (ESI): calcd for $C_{20}H_{25}N_2O_2$, 325.1916; found, 325.1909.

2-ethyl-3-(4-phenoxybutyl)-3*H*-imidazo[4,5-*c*]pyridine (38)

Yield **38**: (0.075 g, 38%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.50 (t, *J* = 7.5 Hz, 3H), 1.77 – 1.97 (m, 2H), 1.95 – 2.18 (m, 2H), 2.94 (q, *J* = 7.5 Hz, 2H), 4.00 (t, *J* = 5.9 Hz, 2H), 4.28 (t, *J* = 7.4 Hz, 2H), 6.81 – 6.91 (m, 2H), 6.91 – 7.00 (m, 1H), 7.20 – 7.34 (m, 2H), 7.64 (d, *J* = 5.5 Hz, 1H),

8.41 (d, *J* = 5.6 Hz, 1H), 8.76 (s, 1H).. LCMS – ESI $(M+H)^+$: 296.0 (99% purity). HRMS (ESI): calcd for $C_{18}H_{22}N_3O$, 296.1763; found, 296.1760.

2-ethyl-1-(4-phenoxybutyl)-1*H*-imidazo[4,5-*c*]pyridine (39)

Yield **39**: (0.045 g, 23%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.51 (t, *J* = 7.5 Hz, 3H), 1.70 – 1.94 (m, 2H), 1.92 – 2.15 (m, 2H), 2.94 (q, *J* = 7.5 Hz, 2H), 4.01 (t, *J* = 5.8 Hz, 2H), 4.22 (t, *J* = 7.4 Hz, 2H), 6.82 – 6.92 (m, 2H), 6.92 – 7.02 (m, 1H), 7.22 – 7.37 (m, 3H), 8.38 (d, *J* = 5.6 Hz, 1H), 9.03 (s, 1H).. LCMS – ESI $(M+H)^+$: 296.0 (99.2% purity). HRMS (ESI): calcd for $C_{18}H_{22}N_3O$, 296.1763; found, 296.1758.

2-ethyl-6-methyl-1-(4-phenoxybutyl)-1*H*-imidazo[4,5-*c*]pyridine (40)

Yield **40**: (0.022 g, 28%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.50 (t, *J* = 7.5 Hz, 3H), 1.69 – 1.95 (m, 2H), 1.96 – 2.18 (m, 2H), 2.65 (s, 3H), 2.94 (q, *J* = 7.5 Hz, 2H), 4.03 (t, *J* = 6.1 Hz, 2H), 4.33 (t, *J* = 7.4 Hz, 2H), 6.66 – 7.11 (m, 4H), 7.18 – 7.40 (m, 2H), 7.85 (d, *J* = 8.1 Hz, 1H).

. LCMS – ESI $(M+H)^+$: 310.0 (99% purity). HRMS (ESI): calcd for $C_{19}H_{24}N_3O$, 310.1919; found, 310.1922.

2-ethyl-1-(4-phenoxybutyl)-1*H*-benzo[*d*]imidazole-6-carbonitrile (41)

Yield **41**: (0.022 g, 30%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (3H, CH_3, t, *J* = 7.5 Hz); 1.86 – 1.89 (m, 2H); 2.03 – 2.05 (m, 2H); 2.94 (2H, q, *J* = 2.1 Hz, 7.5 Hz); 4.04 (2H, t, *J* = 5.7 Hz); 4.23 (2H, t, *J* = 7.2 Hz); 6.86 – 7.02 (m, 3H), 7.22 – 7.37 (m, 2H), 7.48-7.77 (m, 2H), 8.04 (s, 1H) LCMS – ESI $(M+H)^+$: 320.2 (98.2% purity). HRMS (ESI): calcd for $C_{20}H_{22}N_3O$, 320.1763; found, 320.1764.

2-ethyl-1-(4-phenoxybutyl)-1*H*-benzo[*d*]imidazole-5-carbonitrile (42)

Yield **42**: (0.008 g, 11%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (3H, CH_3, t, J = 7.5 Hz); 1.86 – 1.89 (m, 2H); 2.03 – 2.04 (m, 2H); 2.94 (2H, q, J = 2.1 Hz, 7.5 Hz); 4.04 (2H, t, J = 5.7 Hz); 4.23 (2H, t, J = 7.2 Hz); 6.86 – 7.02 (m, 3H), 7.22 – 7.37 (m, 2H), 7.47-7.67 (m, 2H), 7.79 (s, 1H).LCMS – ESI $(M+H)^+$: 320.2 (98% purity). HRMS (ESI): calcd for $C_{20}H_{22}N_3O$, 320.1763; found, 320.1769.

1-(4-phenoxybutyl)-2-phenyl-1*H*-benzo[*d*]imidazole (43)

Yield **43**: (0.106 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.63 – 1.79 (m, 2H), 1.94 – 2.11 (m, 2H), 3.85 (t, J = 6.0 Hz, 2H), 4.32 (t, J = 6.0 Hz, 2H), 6.76 – 6.86 (m, 2H), 6.88 – 7.00 (m, 1H), 7.19 – 7.37 (m, 4H), 7.37 – 7.52 (m, 4H), 7.66 – 7.75 (m, 2H), 7.78 – 7.89 (m, 1H).. LCMS – ESI $(M+H)^+$: 343.2 (99.2% purity). HRMS (ESI): calcd for $C_{23}H_{23}N_2O$, 343.1810; found, 343.1804.

1-(4-phenoxybutyl)-2-(trifluoromethyl)-1*H*-benzo[*d*]imidazole (44)

Yield **44**: (0.070 g, 78%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.91 – 1.93 (m, 2H); 2.11 -2.14 (m, 2H); 4.00 (2H, t, J = 6.0 Hz); 4.44 (2H, t, J = 7.5 Hz); 6.89 – 7.17 (m, 3H), 7.37 – 7.62 (m, 4H), 7.67 – 7.92 (m, 2H). LCMS – ESI $(M+H)^+$: 335.0 (99.5% purity). HRMS (ESI): calcd for $C_{18}H_{18}N_2OF_3$, 335.1371; found, 335.1361.

1-(6-methyl-1-(4-phenoxybutyl)-1*H*-benzo[*d*]imidazol-2-yl)ethanol (45)

Yield **45**: (0.022 g, 24%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.57 (3H, CH_3, d, J = 6.6 Hz); 1.91 - 2.18 (m, 2H); 2.07 (m, 2H); 2.47 (3H, CH_3, s); 3.98 (2H, t, J = 6.3 Hz); 4.24 – 4.26 (2H, m); 5.09

(1H, q, *J* = 6.6 Hz); 6.79 – 7.00 (m, 4H); 7.00 – 7.11 (m, 1H); 7.19 (d, *J* = 8.2 Hz, 1H); 7.21 – 7.37 (m, 3H). LCMS – ESI $(M+H)^+$: 325.3 (97.2% purity). HRMS (ESI): calcd for $C_{20}H_{25}N_2O_2$, 325.1916; found, 325.1911.

1-(1-(4-(3-chloro-4-methylphenoxy)butyl)-6-methyl-1*H*-benzo[*d*]imidazol-2-yl)ethanol (46)

Yield **46**: (0.022 g, 23%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.57 (3H, CH_3, d, *J* = 6.6 Hz); 1.97 (2H, dd, *J* = 5.9 Hz, 11.7 Hz); 2.07 (2H, dd, *J* = 5.9 Hz, 11.7 Hz); 2.29 (3H, CH_3, s); 2.47 (3H, CH_3, s); 3.99 (2H, t, *J* = 6.3 Hz); 4.25 (2H, m); 5.09 (1H, q, *J* = 1.59 – 1.76 (m, 3H), 1.76 – 1.92 (m, 2H), 1.91 – 2.16 (m, 2H), 2.29 (s, 3H), 2.47 (d, *J* = 3.7 Hz, 3H), 3.94 (q, *J* = 6.4 Hz, 2H), 4.06 – 4.40 (m, 2H), 5.08 (q, *J* = 6.6 Hz, 1H), 6.52 – 6.79 (m, 1H), 6.81 – 6.96 (m, 1H), 7.01 – 7.14 (m, 2H), 7.51 (s, 1H), 7.60 (d, *J* = 8.1 Hz, 1H). (ESI): calcd for $C_{21}H_{26}N_2O_2Cl$, 373.1683; found, 373.1685.

N-(1-(5-(3-chloro-4-methylphenyl)pentyl)-1*H*-benzo[*d*]imidazol-2-yl)acetamide (47)

Yield **47**: (0.012 g, 41%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.75 – 1.91 (m, 2H), 1.94 – 2.08 (m, 2H), 2.24 (s, 3H), 2.29 (s, 3H), 4.00 (t, *J* = 6.1 Hz, 2H), 4.21 (t, *J* = 7.0 Hz, 2H), 6.62 – 6.77 (m, 1H), 6.89 (d, *J* = 2.6 Hz, 1H), 7.10 (d, *J* = 8.4 Hz, 1H), 7.20 – 7.33 (m, 4H).. LCMS – ESI $(M+H)^+$: 371.9 (99.5% purity). HRMS (ESI): calcd for $C_{25}H_{23}NCl$, 372.1479; found, 372.1485.

4-(1-(4-phenoxybutyl)-1*H*-benzo[*d*]imidazol-2-yl)morpholine (48)

Yield **48**: (0.029 g, 49%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.71 – 1.92 (m, 2H), 1.95 – 2.15 (m, 2H), 3.20 – 3.35 (m, 4H), 3.75 – 3.90 (m, 4H), 4.02 (t, *J* = 5.9 Hz, 2H), 4.13 (t, 2H), 6.89 (d, *J* = 8.1 Hz, 2H), 6.98 (t, *J* = 7.3 Hz, 1H), 7.12 – 7.24 (m, 2H), 7.25 – 7.36 (m, 3H), 7.54 – 7.72 (m,

1H).. LCMS – ESI $(M+H)^+$: 352.3 (96.4% purity). HRMS (ESI): calcd for $C_{25}H_{23}NCl$, 352.2025; found, 352.2020.

1-(4-(3-chloro-4-methylphenoxy)butyl)-1*H*-benzo[*d*]imidazol-2-amine (49)

Yield **49**: (0.07 g, 33%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.77 – 1.91 (m, 2H), 1.94 – 2.08 (m, 2H), 2.28 (s, 3H), 4.01 (t, *J* = 5.7 Hz, 2H), 4.21 (t, *J* = 7.0 Hz, 2H), 6.62 – 6.77 (m, 1H), 6.89 - 7.10 (m, 2H), 7.20 – 7.33 (m, 4H).. LCMS – ESI $(2M+H_2O)^+$: 578.1 (98.2% purity).

2-(4-methylpiperazin-1-yl)-1-(4-phenoxybutyl)-1*H*-benzo[*d*]imidazole (50)

Yield **50**: (0.047 g, 76%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.79 – 1.82 (m, 2H); 1.89 - 2.20 (m, 2H); 2.34 (3H, CH_3, s); 2.41 – 2.73 (4H, m); 3.21 – 3.41 (4H, m); 4.03 – 4.08 (4H, m); 6.88 – 6.97 (m, 3H); 7.08 – 7.42 (m, 5H); 7.50 – 7.72 (m, 1H). LCMS – ESI $(M+H)^+$: 365.3 (98.4% purity). HRMS (ESI): calcd for $C_{22}H_{29}N_4O$, 365.2341; found, 365.2344.

2-chloro-1-(4-phenoxybutyl)-1*H*-benzo[*d*]imidazole (51)

Yield **51**: (0.255 g, 65%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.64 – 1.91 (m, 2H); 1.97 - 2.15 (m, 2H); 3.97 (2H, t, *J* = 5.8 Hz); 4.27 (2H, t, *J* = 6.0 Hz); 6.88 – 6.97 (m, 3H); 7.15 – 7.46 (m, 5H); 7.74 – 7.76 (m, 1H). LCMS – ESI $(M+H)^+$: 300.9 (98.1% purity). HRMS (ESI): calcd for $C_{22}H_{29}N_4O$, 301.1108; found, 301.1103.

1-(4-phenoxybutyl)-1*H*-benzo[*d*]imidazole (52)

Yield **52**: (0.110 g, 98%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.68 – 1.98 (m, 2H), 1.93 – 2.21 (m, 2H), 3.98 (t, *J* = 5.9 Hz, 2H), 4.26 (t, *J* = 7.1 Hz, 2H), 6.78 – 7.04 (m, 3H), 7.21 – 7.38 (m, 3H),

7.37 – 7.46 (m, 1H), 7.75 – 7.89 (m, 1H), 7.97 (s, 1H).. LCMS – ESI $(M+H)^+$: 267.2 (99% purity).

2-ethyl-1-(4-(phenylthio)butyl)-1*H*-benzo[*d*]imidazole (53)

Yield **53**: (0.055 g, 53%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (3H, CH_3, t, J = 7.5 Hz); 1.85 – 2.06 (m, 2H); 2.84 – 2.88 (m, 4H); 2.89 (2H, q, J = 7.5 Hz); 4.09 (2H, t, J = 6.9 Hz); 6.81 – 7.01 (m, 3H); 7.12 – 7.30 (m, 4H); 7.67 - 7.75 (m, 2H). LCMS – ESI $(M+H)^+$: 311.2 (99.3% purity). HRMS (ESI): calcd for $C_{19}H_{23}N_2S$, 311.1582; found, 311.1588.

N-(4-(2-ethyl-1*H*-benzo[*d*]imidazol-1-yl)butyl)aniline (54)

Yield **54**: (0.014 g, 34%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, CH_3, t, J = 7.4 Hz); 1.65 – 1.68 (m, 2H); 1.90 - 195 (m, 2H); 2.84 (2H, q, J = 7.4 Hz); 3.14 (2H, t, J = 5.3 Hz); 3.41 (1H, NH, s); 4.13 (2H, t, J = 6.7 Hz); 6.56 – 6.59 (m, 2H); 6.73 – 6.78 (m, 1H); 7.10 – 7.35 (m, 4H); 7.59 – 7.74 (m, 2H). LCMS – ESI $(M+H)^+$: 294.0 (99% purity). HRMS (ESI): calcd for $C_{19}H_{23}N_2S$, 294.1970; found, 294.1969.

N-(4-(2-ethyl-6-methyl-1*H*-benzo[*d*]imidazol-1-yl)butyl)aniline (55)

Yield **55**: (0.022 g, 42%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, CH_3, t, J = 7.5 Hz); 1.66 – 1.94 (m, 2H); 2.47 (3H, CH_3, s); 2.75 – 3.03 (m, 4H); 3.14 (2H, t, J = 6.5 Hz); 3.70 (1H, NH, s); 4.11 (m, 2H); 6.46 – 6.83 (m, 3H); 6.85 – 7.43 (m, 4H); 7.52(s, 1H). LCMS – ESI $(M+H)^+$: 308.2 (97.9% purity). HRMS (ESI): calcd for $C_{20}H_{26}N_3$, 308.2127; found, 308.2131.

N-(4-(2-ethyl-6-methyl-1*H*-benzo[*d*]imidazol-1-yl)butyl)-4-methylaniline (56)

Yield **56**: (0.022 g, 40%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, CH_3, t, *J* = 7.4 Hz); 1.64 – 1.68 (m, 2H); 1.88 – 1.92 (m, 2H); 2.23 (CH_3, s); 2.47 (3H, CH_3, s); 2.86 (2H, q, *J* = 7.5 Hz); 3.12 (2H, t, *J* = 6.7 Hz); 3.50 (1H, NH, s); 4.10 (2H, t, *J* = 7.4 Hz); 6.52 - 6.54 (m, 2H); 6.91 – 7.11 (m, 4H); 7.44– 7.71 (m, 1H). LCMS – ESI $(M+H)^+$: 322.2 (99% purity). HRMS (ESI): calcd for $C_{21}H_{28}N_3$, 322.2283; found, 322.2286.

N-(4-(2-ethyl-6-methyl-1*H*-benzo[*d*]imidazol-1-yl)butyl)-2,4-dimethylaniline (57)

Yield **57**: (0.018 g, 32%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (3H, CH_3, t, *J* = 7.5 Hz); 1.66 – 1.73 (m, 2H); 1.88 – 1.95 (m, 2H); 2.07 (s, 3H); 2.24 (CH_3, s); 2.47 (3H, CH_3, s); 2.72 – 2.99 (m, 2H); 3.17 (2H, q, *J* = 6.4 Hz); 4.11 (2H, t, *J* = 7.3 Hz); 6.51 (m, 1H); 6.79 – 7.11 (m, 4H); 7.12 – 7.33 (m, 1H); 7.43 – 7.72 (m, 1H). LCMS – ESI $(M+H)^+$: 336.3 (98.3% purity). HRMS (ESI): calcd for $C_{22}H_{30}N_3$, 336.2440; found, 336.2442.

3-bromo-N-(4-(2-ethyl-6-methyl-1*H*-benzo[*d*]imidazol-1-yl)butyl)-4-methylaniline (58)

Yield **58**: (0.037 g, 54%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (3H, CH_3, t, *J* = 7.5 Hz); 1.56 – 1.82 (m, 2H); 1.88 – 2.00 (m, 2H); 2.28 (3H, CH_3, s); 2.47 (3H, CH_3, s); 2.86 (2H, q, *J* = 7.5 Hz); 3.08 (2H, t, *J* = 6.9 Hz); 3.50 (1H, NH, s); 4.10 (2H, t, *J* = 5.7 Hz); 6.34 – 6.59 (m, 1H); 6.77 (s, 1H); 6.88 – 7.37 (m, 3H); 7.36 – 7.76 (m, 1H). LCMS – ESI $(M+H)^+$: 400.2 (99% purity). HRMS (ESI): calcd for $C_{21}H_{27}N_3Br$, 400.1388; found, 400.1389.

1-(3-(3-chloro-4-methylphenoxy)propyl)-2-ethyl-6-methyl-1*H*-benzo[*d*]imidazole (59)

Yield **59**: (0.015 g, 31%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (3H, CH_3, t, *J* = 7.5 Hz); 2.19 - 2.31 (m, 5H); 2.38 (3H, CH_3, s); 2.45 (3H, CH_3, s); 2.86 (2H, q, *J* = 7.5 Hz); 3.87 (2H, t, *J* = 5.5 Hz); 4.31 (2H, t, *J* = 7.5 Hz); 6.67 – 7.69 (m, 1H); 6.89 – 6.91 (m, 1H); 6.95 – 7.16 (m, 3H); 7.44

– 7.72 (m, 1H). LCMS – ESI $(M+H)^+$: 343.2 (98% purity). HRMS (ESI): calcd for $C_{20}H_{24}N_2OCl$, 343.1577; found, 343.1576.

2-ethyl-6-methyl-1-(3-(*p*-tolyloxy)propyl)-1*H*-benzo[*d*]imidazole (60)

Yield **60**: (0.025 g, 58%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, CH_3, t, J = 7.5 Hz); 2.25 (m, 2H); 2.30 (3H, CH_3, s); 2.38 (3H, CH_3, s); 2.88 (2H, q, J = 6.7 Hz); 3.86 (2H, t, J = 5.3 Hz); 4.31 (2H, t, J = 7.5 Hz); 6.76 – 6.89 (m, 2H); 6.91 – 7.16 (m, 3H); 7.44 – 7.62 (m, 2H). LCMS – ESI $(M+H)^+$: 309.2 (99.1% purity). HRMS (ESI): calcd for $C_{20}H_{25}N_2O$, 309.1967; found, 309.1959.

1-(3-(3,4-dimethylphenoxy)propyl)-2-ethyl-6-methyl-1*H*-benzo[*d*]imidazole (61)

Yield **61**: (0.025 g, 55%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.41 (3H, CH_3, t, J = 7.5 Hz); 2.25 (m, 5H, CH_3, CH_2); 2.38 (3H, CH_3, s); 2.47 (3H, CH_3, s); 2.88 (2H, q, J = 6.7 Hz); 3.86 (2H, t, J = 5.3 Hz); 4.31 (2H, t, J = 7.5 Hz); 6.51 – 6.76 (m, 2H); 6.91 – 7.12 (m, 3H); 7.43 – 7.65 (m, 1H). LCMS – ESI $(M+H)^+$: 323.2 (98.8% purity). HRMS (ESI): calcd for $C_{21}H_{27}N_2O$, 323.2123; found, 323.2119.

4-bromo-N-(3-(2-ethyl-6-methyl-1*H*-benzo[*d*]imidazol-1-yl)propyl)-3-methylaniline (62)

Yield **62**: (0.013 g, 24%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.43 (3H, CH_3, t, J = 7.5 Hz); 2.10 (m, 2H); 2.25 (3H, CH_3, s); 2.47 (3H, CH_3, s); 2.86 (2H, q, J = 7.5 Hz); 3.14 (2H, t, J = 6.6 Hz); 4.22 (2H, t, J = 7.2 Hz); 6.49 – 6.52 (m, 2H);); 6.90 – 7.22 (m, 3H); 7.46 – 7.70 (m, 1H).. LCMS – ESI $(M+H)^+$: 388.2 (99% purity). HRMS (ESI): calcd for $C_{20}H_{25}N_3Br$, 386.1232; found, 386.1221.

2-ethyl-6-methyl-1-(3-(*p*-tolylthio)propyl)-1*H*-benzo[*d*]imidazole (63)

Yield **63**: (0.030 g, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, CH_3, t, *J* = 7.4 Hz); 1.64 (m, 2H); 1.95 (m, 2H); 2.32 (3H, CH_3, s); 2.47 (3H, CH_3, s); 2.88 (2H, q, *J* = 6.7 Hz); 4.03 (2H, t, *J* = 7.5 Hz); 7.07 – 7.11 (m, 2H); 7.14 – 7.26 (m, 3H); 7.44 – 7.61 (m, 2H). LCMS – ESI $(M+H)^+$: 339.2 (99.3% purity). HRMS (ESI): calcd for $C_{21}H_{27}N_2S$, 339.1895; found, 339.1901.

2-ethyl-1-(4-(pyridin-3-yloxy)butyl)-1*H*-benzo[*d*]imidazole (64)

Yield **64**: (0.027 g, 47%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, CH_3, t, *J* = 7.4 Hz); 1.85 (m, 2H\); 2.02 (m, 2H); 2.88 (2H, q, *J* = 7.5 Hz); 4.02 (2H, t, *J* = 5.3 Hz); 4.20 (2H, t, *J* = 6.7 Hz); 7.14 – 7.40 (m, 5H); 7.74 (s, 1H); 8.23 – 8.27 (m, 2H). LCMS – ESI $(M+H)^+$: 296.2 (99.1% purity). HRMS (ESI): calcd for $C_{18}H_{22}N_3O$, 296.1763; found, 296.1764.

2-ethyl-1-(4-(pyridin-4-yloxy)butyl)-1*H*-benzo[*d*]imidazole (65)

Yield **65**: (0.013 g, 24%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.49 (3H, CH_3, t, *J* = 7.4 Hz); 1.87 (m, 2H); 1.94 - 2.20 (m, 2H); 2.91 (2H, q, *J* = 7.5 Hz); 4.01 (2H, t, *J* = 5.9 Hz); 4.21 (2H, t, *J* = 7.2 Hz); 6.77 (d, *J* = 5.5 Hz, 2H);7.10 – 7.44 (m, 3H); 7.73 – 7.77 (m 2H); 8.42 (d, *J* = 5.4 Hz, 2H). LCMS – ESI $(M+H)^+$: 296.2 (97.3% purity). HRMS (ESI): calcd for $C_{18}H_{22}N_3O$, 296.1763; found, 296.1767.

4-(4-(2-ethyl-1*H*-benzo[*d*]imidazol-1-yl)butoxy)quinoline (66)

Yield **66**: (0.008 g, 13%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, CH_3, t, *J* = 7.4 Hz); 1.90 (4H, s); 2.83 (2H, q, *J* = 7.5 Hz); 4.02 (2H, t, *J* = 5.3 Hz); 4.14 (2H, t, *J* = 6.7 Hz); 6.22 (1H, d, *J* = 7.8 Hz); 7.12 – 7.49 (m, 6H); 7.57 – 7.89 (m, 2H); 8.46 (1H, d, *J* = 6.9 Hz). LCMS – ESI $(M+H)^+$: 346.2 (98.9% purity). HRMS (ESI): calcd for $C_{22}H_{24}N_3O$, 346.1919; found, 346.1921

5-(4-(2-ethyl-1*H*-benzo[*d*]imidazol-1-yl)butoxy)-2-methylbenzo[d]thiazole (67)

Yield **67**: (0.037 g, 56%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, CH_3, t, *J* = 7.4 Hz); 1.89 (m, 2H); 2.02 (m, 2H); 2.79 (3H, CH_3, s); 2.90 (2H, q, *J* = 7.5 Hz); 4.04 (2H, t, *J* = 5.3 Hz); 4.19 (2H, t, *J* = 6.7 Hz); 6.95 (m, 1H); 7.08 – 7.53 (m, 4H); 7.55 – 7.87 (m, 2H). LCMS – ESI $(M+H)^+$: 366.2 (99.3% purity). HRMS (ESI): calcd for $C_{21}H_{24}N_3O$, 366.1640; found, 366.1644.

2-(4-chlorophenyl)-5-((4-(2-ethyl-1*H*-benzo[*d*]imidazol-1-yl)butyl)thio)-1,3,4-oxadiazole (68)

Yield **68**: (0.050 g, 67%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.48 (3H, CH_3, t, *J* = 7.5 Hz); 1.97 (4H, m); 2.88 (2H, q, *J* = 7.8 Hz); 3.28 (2H, t, *J* = 6.9 Hz); 4.18 (2H, t, *J* = 6.9 Hz); 7.08 – 7.42 (m, 3H); 7.42 – 7.58 (m, 2H); 7.71 – 7.73 (m, 1H); 7.83 – 8.0 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 11.87, 20.86, 26.79, 28.76, 31.99, 42.94, 109.04, 119.36, 121.83, 122.00, 122.11, 127.92, 129.48, 134.98, 138.01, 142.68, 155.81, 164.34, 165.08. LCMS – ESI $(M+H)^+$: 413.1 (99.4% purity).

2-ethyl-1-(4-methoxybutyl)-*1H*-benzo[d]imidazole (69)

Yield **69**: (0.63 g, 38%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (3H, CH_3, t, *J* = 7.7 Hz); 1.60 (m, 2H); 1.82 (m, 2H); 2.85 (2H, q, *J* = 7.5 Hz); 3.30 (3H, CH_3, s); 3.34 (2H, t, *J* = 6.0 Hz); 4.07 (2H, t, *J* = 6.9 Hz); 7.16 – 7.34 (m, 3H); 7.72 – 7.75 (m, 1H). LCMS – ESI $(M+H)^+$: 233.0 (99% purity).

1-(4-phenoxybutyl)-1*H*-indazole (70)

Yield **70**: (0.050 g, 22%). ^{1}H NMR (300 MHz, $CDCl_3$): 1.78 (2H, m); 2.20 (2H, m); 3.90 (2H, t, *J* = 6.3 Hz); 4.48 (2H, t, *J* = 7.2 Hz); 6.87-7.14 (m, 3H); 7.24 – 7.35 (m, 4H); 7.53 – 7.69 (m,

2H); 8.29 (s, 1H). LCMS – ESI $(M+H)^+$: 267.2 (98.8% purity). HRMS (ESI): calcd for $C_{17}H_{19}N_2O$, 267.1497; found, 267.1491.

2-(4-chlorophenyl)-5-((4-(2-ethyl-1*H*-indol-1-yl)butyl)thio)-1,3,4-oxadiazole (71)

Yield **71**: (0.043 g, 73%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (3H, CH_3, t, *J* = 7.5 Hz); 1.95 (m, 4H); 2.78 (2H, q, *J* = 7.5 Hz); 3.30 (2H, t, *J* = 6.0 Hz); 4.14 (2H, t, *J* = 7.2 Hz); 6.29 (1H, s); 6.99 – 7.23 (m, 2H); 7.26 -7.30 (m, 2H); 7.38 – 7.66 (m, 3H); 7.82 – 8.12 (m, 2H). LCMS – ESI $(M+H)^+$: 412.0 (98.2% purity). HRMS (ESI): calcd for $C_{22}H_{23}N_3OSCl$, 412.1250; found, 412.1265.

1-(4-(3-chloro-4-methylphenoxy)butyl)-2-ethyl-1*H*-indole (72)

Yield **72**: (0.037 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (3H, CH_3, t, *J* = 7.5 Hz); 1.84 (m, 2H); 1.95 (m, 2H); 2.32 (3H, CH_3, s); 2.81 (2H, q, *J* = 7.5 Hz); 3.93 (2H, t, *J* = 6.0 Hz); 4.17 (2H, t, *J* = 7.2 Hz); 6.31 (1H, s); 6.69 – 6.71 (m, 1H); 6.92 – 6.94 (m, 1H); 7.01 – 7.23 (m, 3H); 7.22 – 7.40 (m, 1H); 7.57 – 7.60 (m, 1H). LCMS – ESI $(M+H)^+$: 342.0 (99% purity). HRMS (ESI): calcd for $C_{21}H_{25}NOCl$, 342.1625; found, 342.1622.

3-bromo-N-(4-(2-ethyl-1*H*-indol-1-yl)butyl)-4-methylaniline (73)

Yield **73**: (0.032 g, 58%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.38 (3H, CH_3, t, *J* = 7.5 Hz); 1.63 (m, 2H); 1.85 (m, 2H); 2.28 (3H, CH_3, s); 2.76 (2H, q, *J* = 7.5 Hz); 3.08 (2H, t, *J* = 7.5 Hz); 4.13 (2H, t, *J* = 7.5 Hz); 6.30 (1H, S); 6.43 – 7.45 (m, 1H); 6.79 (s, 1H); 6.92 – 7.22 (m, 3H); 7.27 – 7.80 (m, 2H); 5.57 – 5.59 (m, 1H). LCMS – ESI $(M+H)^+$: 384.9 (99.5% purity). HRMS (ESI): calcd for $C_{21}H_{26}N_2Br$, 385.1272; found, 385.1279.

ACKNOWLEDGEMENTS

This research was funded in part by grant OPP1024038 from the Bill & Melinda Gates Foundation and in part by Eli Lilly and Company in support of the mission of the Lilly TB Drug Discovery Initiative. We thank James Ahn, Bjorn Sunde, Alfredo Blakeley, Lindsay Flint and Aaron Korkegian for technical assistance.

ABBREVIATIONS USED.

PAB, phenoxyalkylbenzimidazole; DAD, diode array detector; TC_{50}, concentration required to inhibit growth of eukaryotic cells by 50%; SI, selectivity index, a ratio of MIC to TC_{50}; CFU, colony forming units; RLU, relative luminescent units; OADC, oleic acid, albumin, dextrose, catalase.

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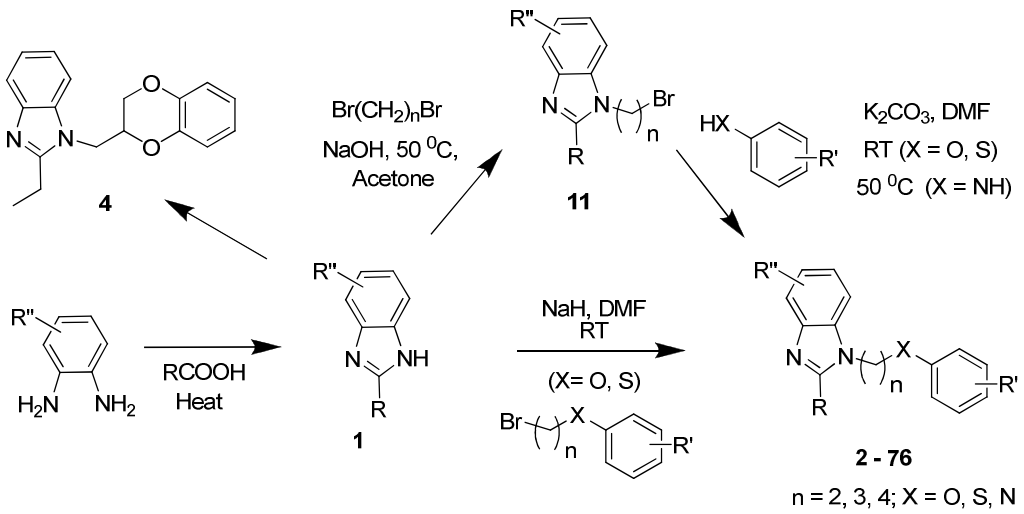
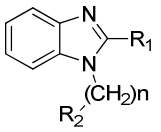


Scheme 1: Synthesis of phenoxyalkylbenzimidazoles



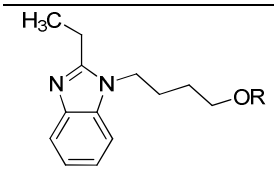
Compound	R_1	n	R_2	MIC[a] (μM)	TC_{50}[b] (μM)	SI[c]
2	Ethyl	2	Phenoxy	>20	ND	NC
3	Ethyl	2	4-Chlorophenoxy	>20	ND	NC
4	Ethyl	1	2,3-Dihydrobenzo[*b*][1,4]dioxin-2-yl	>20	ND	NC
5	Ethyl	3	Phenoxy	5.2 ± 1.9	>50	>9.6
6	Ethyl	4	Phenoxy	1.1 ± 0.4	21 ± 7.1	19
7	Methyl	2	Phenoxy	>20	ND	NC
8	Methyl	2	4-Chlorophenoxy	>20	ND	NC
9	Methyl	3	Phenoxy	>20	>50	NC
10	Methyl	4	Phenoxy	>20	>50	NC

Table 1: Effect of N-1 alkyl linker length on the biological activity of 2-ethyl and 2-methyl benzimidazoles.

[a] MIC is the minimum concentration required to inhibit the growth of *M. tuberculosis* completely in liquid culture [20]. MICs are the average of two independent experiments ± standard deviation. [b]

TC_{50} is the concentration required to inhibit growth of eukaryotic cells (Vero cell line) by 50%. TC_{50} is the average of two independent experiments ± standard deviation. [c] Selectivity index (SI) is calculated as MIC/ TC_{50}.

ND – not determined. NC- not calculated.

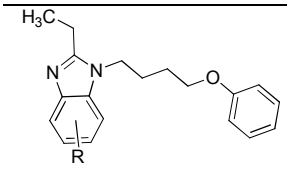


Compound	R - group	MIC[a] (μM)	TC_{50}[b] (μM)	SI[c]
6	Phenyl	1.1 ± 0.4	21 ± 7.1	19
12	2-Methylphenyl	0.32 ± 0.06	16 ± 3.5	50
13	3-Methylphenyl	0.42 ± 0	19 ± 0.7	45
14	4-Methylphenyl	0.15 ± 0.07	14 ± 0.7	93
15	3, 4-Dimethylphenyl	0.30 ± 0.1	14 ± 2.0	53
16	2-Isopropylphenyl	20	19 ± 1.0	0.7
17	4-(2,5-dimethylbenzyl)morpholine	1.3 ± 0.6	48 ± 9.9	15
18	4-Morpholinophenyl	>20	32 ± 2.5	NC
19	Benzyl	3.6 ± 0.3	32 ± 11	8.9
20	4-Methoxyphenyl	1.2 ± 0.4	21 ± 7.1	27

21	3-Chlorophenyl	0.88 ± 0.3	22 ± 0.7	25
22	2-Chlorophenyl	0.68 ± 0.08	16 ± 9.0	23
23	4-Chlorophenyl	1.5 ± 0.5	14 ± 0.7	9.3
24	2,6-Dichloropheny	20	13 ± 1.4	1.5
25	2,4-Dichloropheny	1.8 ± 0.8	23 ± 1.4	13
26	4-Chloro-3-fluorophenyl	1.6 ± 0.4	16 ± 1.4	10
27	2-Fluoro-4-chlorophenyl	1.5 ± 0.4	5.7 ± 1.5	3.8
28	4-Cyanophenyl	16 ± 6.0	27 ± 6.4	1.7
29	3-Chloro-4-methylphenyl	0.23 ± 0.08	14 ± 2.1	61
30	4-Chloro-2-methylphenyl	1.5 ± 0.6	20 ± 2.1	2.3
31	4-Chloro-3-methyl-5-nitrophenyl	7.9 ± 0	18 ± 0.7	19

Table 2: Effect of aryl ether substitutent on biological activity.

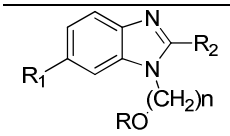
[a] MIC is the minimum concentration required to inhibit the growth of *M. tuberculosis* completely in liquid culture [20]. MICs are the average of two independent experiments ± standard deviation. [b] TC_{50} is the concentration required to inhibit growth of eukaryotic cells (Vero cell line) by 50%. TC_{50} is the average of two independent experiments ± standard deviation. [c] Selectivity index (SI) is calculated as MIC/ TC_{50}. ND – not determined. NC- not calculated.



Compound	R-group	MIC[a] (μM)	TC_{50}[b] (μM)	SI[c]
6	H	1.1 ± 0.4	21 ± 7.1	19
32	6-Chloro	7.1 ± 3.0	25 ± 7.8	3.5
33	5-Chloro	0.30 ± 0.1	16 ± 1.5	53
34	6-Methyl	0.27 ± 0.09	18 ± 2.5	67
35	7-Aza-5-chloro	6.1 ± 0.5	38 ± 8.4	6.2
36	6-Methoxy	0.90 ± 0.1	27 ± 2.1	30
37	5-Methoxy	>20	ND	NC
38	6-Aza	>20	ND	NC
39	5-Aza	>20	ND	NC
40	5-Aza-6-methyl	1.5 ± 0.6	71 ± 17	47
41	6-Cyano	>20	>20	>20
42	5-Cyano	>20	>20	>20

Table 3: Effect of benzo substitutions on biological activity.

[a] MIC is the minimum concentration required to inhibit the growth of *M. tuberculosis* completely in liquid culture [20]. MICs are the average of two independent experiments ± standard deviation. [b] TC_{50} is the concentration required to inhibit growth of eukaryotic cells (Vero cell line) by 50%. TC_{50} is the average of two independent experiments ± standard deviation. [c] Selectivity index (SI) is calculated as MIC/ TC_{50}. ND – not determined. NC- not calculated.



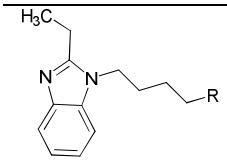
Compound	R_2	R_1	n	R	MIC[a] (μM)	TC_{50}[b] (μM)	SI[c]
6	Ethyl	H	4	Phenyl	1.1 ± 0.4	21 ± 7.1	19.1
43	Phenyl	H	4	Phenyl	16 ± 5.9	19 ± 2.1	1.2
44	Trifluoromethyl	H	4	Phenyl	>20	ND	NC
45	Ethanol-1-yl	Me	4	Phenyl	20	ND	NC
46	Ethanol-1-yl	Me	4	3-Cl-4-MePhenyl	11 ± 0.1	8.6 ± 8.1	0.8
47	Acetamido	Me	4	3-Cl-4-MePhenyl	11 ± 4.5	8.3 ± 3.4	0.8
48	Morpholino	H	4	Phenyl	20	20	20
49	Amino	H	4	Phenyl	>20	ND	NC
50	*N*-Methylpiperazino	H	4	Phenyl	>20	ND	NC
51	Chloro	H	4	Phenyl	>20	ND	NC

52	H	H	4	Phenyl	>20	ND	NC

Table 4: Effect of benzimidazole's C-2 substitution on biological activity.

[a] MIC is the minimum concentration required to inhibit the growth of *M. tuberculosis* completely in liquid culture [20]. MICs are the average of two independent experiments ± standard deviation. [b] TC_{50} is the concentration required to inhibit growth of eukaryotic cells (Vero cell line) by 50%. TC_{50} is the average of two independent experiments ± standard deviation. [c] Selectivity index (SI) is calculated as MIC/ TC_{50}.

ND – not determined. NC- not calculated.

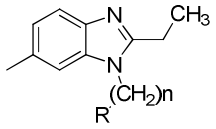


Compound	R-group	MIC[a] (μM)	TC_{50}[b] (μM)	SI[c]
6	phenoxy	1.1 + 0.4	21 ± 7.1	19
53	benzenesulfenyl	1.4 ± 0.3	31 ± 8.3	22
54	Anilinyl	0.47 ± 0.09	42 ± 7.8	89

Table 5: Effect of hetero atoms of the alkyl chain on biological activity.

[a] MIC is the minimum concentration required to inhibit the growth of *M. tuberculosis* completely in liquid culture [20]. MICs are the average of two independent experiments ± standard deviation. [b] TC_{50} is the concentration required to inhibit growth of eukaryotic cells (Vero cell line) by 50%. TC_{50} is the average of two independent experiments ± standard deviation. [c] Selectivity index (SI) is calculated as MIC/ TC_{50}.

ND – not determined. NC- not calculated.

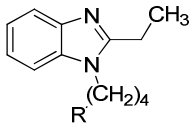


Compound	R-group	n	MIC[a] (μM)	TC_{50}[b] (μM)	SI[c]
55	Anilinyl	4	0.32 ± 0.2	21 ± 2.1	66
56	4-Methylanilinyl	4	0.15 ± 0.05	20 ± 0.7	133
57	2,4-Dimethylanilinyl	4	0.31 ± 0.2	20 ± 4.9	65
58	3-Bromo-4-methylanilinyl	4	0.11 ± 0.06	12 ± 7.1	109

59	3-Chloro-4-methylphenoxy	3	0.22 ± 0.1	17 ± 2.1	77
60	4-Methylphenoxy	3	0.27 ± 0.09	15 ± 2.1	56
61	3,4-Dimethylphenoxy	3	0.22 ± 0.1	7.3 ± 2.1	33
62	4-Bromo-3-methylanilinyl	3	0.052 ± 0.2	27±12.3	523
63	(p-tolyl)thio	3	3.3 ± 0.4	12 ± 0.7	3.6

Table 6: Optimization of compound 6 for biological activity

[a] MIC is the minimum concentration required to inhibit the growth of *M. tuberculosis* completely in liquid culture [20]. MICs are the average of two independent experiments ± standard deviation. [b] TC_{50} is the concentration required to inhibit growth of eukaryotic cells (Vero cell line) by 50%. TC_{50} is the average of two independent experiments ± standard deviation. [c] Selectivity index (SI) is calculated as MIC/ TC_{50}. ND – not determined. NC- not calculated.



Compound	R-group	MIC[a] (μM)	TC_{50}[b] (μM)	SI[c]

6	Phenoxy	1.1 ± 0.4	21 ± 7.1	19
64	3-Azaphenoxy	>20	>50	NC
65	4-Azaphenoxy	>20	>50	NC
66	Quinolin-4-oxy	>20	>50	NC
67	2-Methylbenzothiazol-5-oxy	1.5 ± 0.9	21 ± 10.2	14
68	2-(4-Chlorophenyl)oxa-3,4-diazol-5-thio	0.10 ± 0.07	22 ± 9.2	220
69	Methoxy	>20	>50	NC

Table 7: Effect of aryl ether replacements on biological activity

[a] MIC is the minimum concentration required to inhibit the growth of *M. tuberculosis* completely in liquid culture [20]. MICs are the average of two independent experiments ± standard deviation. [b] TC_{50} is the concentration required to inhibit growth of eukaryotic cells (Vero cell line) by 50%. TC_{50} is the average of two independent experiments ± standard deviation. [c] Selectivity index (SI) is calculated as MIC/ TC_{50}. ND – not determined. NC- not calculated.

Compound	Name	MIC[a] (μM)	TC_{50}[b] (μM)	SI[c]
70	1-(4-phenoxybutyl)-1H-indazole	>20	>50	NC
71	2-(4-chlorophenyl)-5-(4-(2-ethyl-1H-indol-1-yl)butylthio)-1,3,4-oxadiazole	>20	>50	NC
72	1-(4-(3-chloro-4-methylphenoxy)butyl)-2-ethyl-1H-indole	>20	ND	NC
73	3-bromo-N-(4-(2-ethyl-1H-indol-1-yl)butyl)-4-methylaniline	>20	ND	NC

Table 8: The effect of benzimidazole core variation on biological activity

[a] MIC is the minimum concentration required to inhibit the growth of *M. tuberculosis* completely in liquid culture [20]. MICs are the average of two independent experiments ± standard deviation. [b] TC_{50} is the concentration required to inhibit growth of eukaryotic cells (Vero cell line) by 50%. TC_{50} is the average of two independent experiments ± standard deviation. [c] Selectivity index (SI) is calculated as MIC/ TC_{50}. ND – not determined. NC- not calculated.

	MDCK Passive Permeability - % A→B transport	% Turnover by liver microsomes in 30 min.			Equilibrium thermodynamic solubility (mg/mL)			Mouse Fu,pl[a]	Predicted Fu,pl[b]	clogP[c]
		Mouse	Rat	Human	pH 2	pH 6	pH 7.4			
34	ND	99.9	99.9	98.9	0.63	0.04	0.01	ND	0.01	5.04
68	4.4 (High)	99.8	99.9	99.9	0.83	<0.001	<0.001	0.006	0.005	5.42
33	3.6 (High)	99.8	99.7	98.5	0.67	<0.001	<0.001	0.004	0.004	5.13
54	21.5 (High)	99.9	99.9	90.6	0.61	0.01	<0.001	0.045	0.028	4.16

Table 9: *In vitro* and *in silico* ADME data

[a]Fu,pl – Fraction unbound in plasma; [b]Predicted using a QSAR model built based on data generated for >3000 compounds measured internally (unpublished); [c]clogP predicted by Chemaxon model (www.chemaxon.com)

Compound	*Mycobacterium tuberculosis*	*Mycobacterium smegmatis*	*Escherichia coli*	*Staphylococcus aureus*
53	10	>100	>100	>100
68	2.5	>100	>100	>100
6	5	>100	>100	>100

Table 10: Spectrum of activity.

The activity of compounds against four bacterial species was tested on solid medium. MIC_{99} was determined using the serial proportion method[21]. The MIC_{99} (μM) was defined as the concentration of compound which yielded less than 1% CFUs.

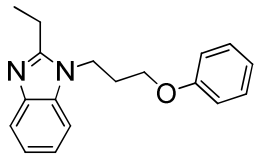


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Figure 1: Structure of 2-ethyl-1-(3-phenoxypropyl)-1*H*-benzo[*d*]imidazole

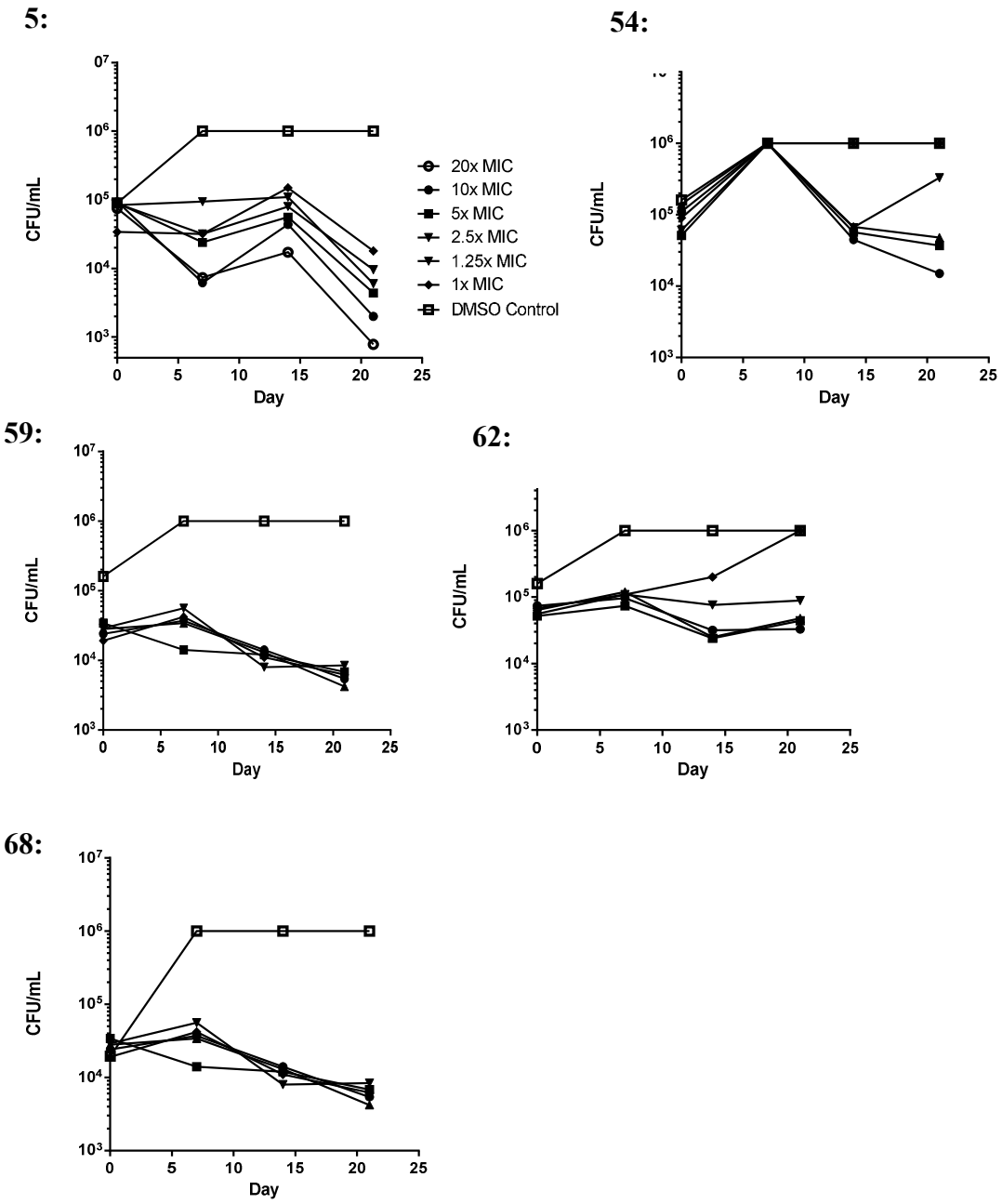

Figure 2: Activity of compounds against replicating *M. tuberculosis*. *M. tuberculosis* was exposed to varying concentrations of compound under aerobic conditions in standard growth medium (7H9-Tw-OADC). Viability was monitored by determining colony forming units (CFU). The lower limit of detection was 100 CFU/mL. The upper limit of detection was 10^6 CFU/mL.

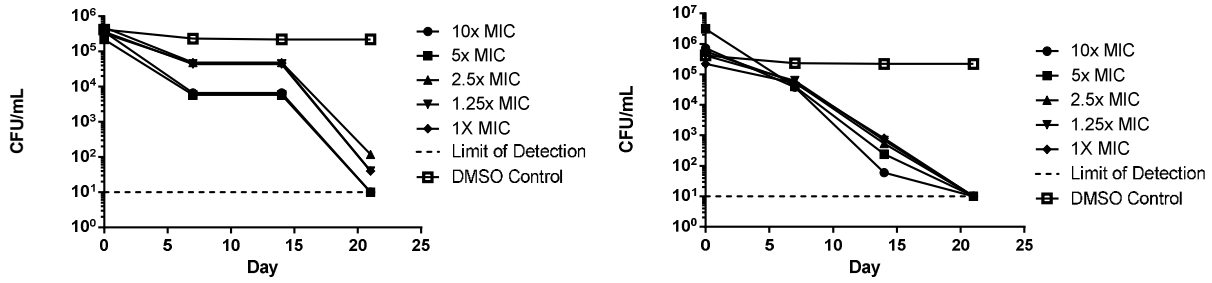

Figure 3: **Activity of compounds against non-replicating *M. tuberculosis***

M. tuberculosis was subjected to complete starvation in PBS-Ty for 14 days prior to inoculation into PBS-Ty containing compounds and incubated standing at 37°C. Viability was monitored by determining colony forming units (CFU). The lower limit of detection is 10 CFU/mL. Left panel - compound **68** MIC = 0.10 μM); right panel – **5,** MIC = 5.2 μM.

Graphical abstract

190x254mm (96 x 96 DPI)